10 Years of Collaborative Research

on Beans and Cowpeas

# FY 90 Annual Report

Bean/Cowpea Collaborative

Research Support Program

10 YEARS OF COLLABORATIVE RESEARCH

ON BEANS AND COWPEAS

□□□

# FY 90 ANNUAL REPORT

□□□

Bean/Cowpea Collaborative

Research Support Program

# THE BEAN/COWPEA CRSP

**An international community of persons, institutions, agencies, and governments committed to collectively strengthening health and nutrition in developing countries by improving the availability and utilization of beans and cowpeas**

MICHIGAN STATE UNIVERSITY
December 1990

Grant No. DAN-1310-G-SS-6008-00
Agency for International Development
Washington, DC

## TABLE OF CONTENTS

INTRODUCTION . . . . . 1

PROJECT HIGHLIGHTS
- CRSP Management . . . . . 2
- Management Office Annual Report . . . . . 6
- Training Summary . . . . . 11
- Women in Development Annual Report . . . . . 13
- Roster of Projects . . . . . 18

BRAZIL/BOYCE THOMPSON INSTITUTE . . . . . 19

CAMEROON/PURDUE UNIVERSITY . . . . . 63

DOMINICAN REPUBLIC/UNIVERSITY OF NEBRASKA . . . . . 82

DOMINICAN REPUBLIC/UNIVERSITY OF WISCONSIN . . . . . 96

ECUADOR/UNIVERSITY OF MINNESOTA . . . . . 111

GUATEMALA/CORNELL UNIVERSITY . . . . . 122

HONDURAS/UNIVERSITY OF PUERTO RICO . . . . . 135

INCAP/WASHINGTON STATE UNIVERSITY . . . . . 154

MALAWI/UNIVERSITY OF CALIFORNIA-Davis . . . . . 174

MEXICO/MICHIGAN STATE UNIVERSITY . . . . . 190

NIGERIA/UNIVERSITY OF GEORGIA . . . . . 218

SENEGAL/UNIVERSITY OF CALIFORNIA-Riverside . . . . . 235

TANZANIA/WASHINGTON STATE UNIVERSITY . . . . . 247

List of Abbreviations and Acronyms . . . . . 263

Cover design by Chris Altese

# INTRODUCTION

When Hubert Humphrey was sold on the idea of bringing into a three-way partnership the Agency for International Development (A.I.D.), developing countries, and the U.S. Land-Grant college system, it was an exciting notion that caught the attention of many in the development community. A.I.D., with funds for U.S. development efforts, was losing much of its scientific and technical staff. The U.S. Land-Grant system, while rich in scientific and technical staff and physical infrastructure important for the conduct of research, was facing limited availability of funds for participation in development. Developing countries were short on staff, physical infrastructure and financial resources but had indigenous knowledge, germplasm, ecologies, and the epidemics to study, needed by the research system. In addition, as demographers pointed to the aging of the baby boomers with the resulting drop in university enrollments, many universities saw developing countries as a source of new student clientele. Identified as an initiative of Title XII of the U.S. Foreign Assistance Act, it was a partnership made in Heaven.

The Bean/Cowpea CRSP was the third Collaborative Research Support Program to evolve from this initiative. It is a coordinated effort which addresses hunger and malnutrition in Africa, Latin America, and the Caribbean through research on beans (*Phaseolus vulgaris*) and cowpeas (*Vigna unguiculata*). The focus of the CRSP is collaboration, a characteristic which distinguishes it from more traditional agricultural research. The collaborative approach includes financial, as well as operation, collaboration in the conduct of the projects. That is, U.S. and Host Country (HC) institutions, as well as A.I.D., provide monetary or in-kind support necessary for the scientific undertakings.

Among the unique aspects of the CRSP are the extensive linkages which are forged over time and which support the collegial, multiple-way flow of information, innovative ideas, and scientific resources. These linkages are composed of diverse disciplines, nationalities, and cultures. As the partners exchange data with one another, the benefits of their research enrich both the U.S. and HCs and contribute to the development of sustainability in the agricultural production systems around the world.

In the research projects of the CRSP, attention is focused on particular aspects of bean and cowpea production (e.g., improved varieties with resistance to diseases and/or insects) and utilization (e.g., issues of storage, processing or nutrition) as identified in the Global Plan. Working together, the research teams are generating responses beneficial for U.S. farmers as well as small-scale producers and the rural and urban poor in developing countries. Since the initiation of this program in 1980, major successes have been achieved well beyond that envisioned in the original scopes of work. Indeed, in the most recent review by the External Evaluation Panel, this CRSP was judged to be outstanding. This report of the tenth year of Bean/Cowpea CRSP research and training documents the validity of that assessment.

# CRSP MANAGEMENT

## *THE MANAGEMENT OFFICE*

The Bean/Cowpea CRSP Management Office is the operational component of the Management Entity, Michigan State University. The ME is represented by the MSU Vice-President for Finance, the official recipient of the prime grant from A.I.D., and the Dean of the College of Agriculture and Natural Resources, in whose unit the CRSP is organizationally located. The MO, on behalf of the ME, is the link between A.I.D. and CRSP projects organized through subcontracts to ten U.S. lead universities and one private research institute. These institutions in turn support participating HC institutions in Africa, Latin America, and the Caribbean. The MO encourages the development of active, well-coordinated research teams and is the prime support of the management support groups (e.g., Board of Directors) which undergird the overall administration of the CRSP. The major responsibilities of the MO are to:

1. Generate and maintain a Global Plan with U.S. and HC input to give direction and order to the conduct of the CRSP.

2. Receive and reallocate funds against that plan, documenting usage of all A.I.D. funds to the CRSP;

3. Establish a system for effective program management, fiscal accountability and the accounting of funds, including the required institutional match;

4. Monitor project fiscal, research and training activity in the U.S. and HCs;

5. Provide fiscal/administrative support (e.g., travel clearances, equipment purchase approvals from A.I.D.) and guidance to the lead institutions;

6. Coordinate and guide project work plans, research and training to keep them compatible with the goals of the prime grant and the Global Plan;

7. Keep the basic orientation of the CRSP (i.e., small-scale farmers and women) central in program operations;

8. Encourage project networking and complementarity;

9. Facilitate communications throughout the CRSP and with external groups;

10. Provide staff and administrative support to the Board of Directors, the Technical Committee, and the External Evaluation Panel;

11. Enforce the policies and approved recommendations of these groups and A.I.D.;

12. Assist in developing and executing legal documents and agreements between CRSP institutions;

13. Organize and carry out national and international meetings, conferences and workshops in support of CRSP goals;

14. Represent the CRSP throughout the national and international agriculture and development community;

15. Provide documentation as requested by A.I.D., BIFAD, special review teams, and the offices of the Management Entity.

Although CRSP management is complex, this management arrangement makes it possible for a broad array of scientific and technological expertise and facilities to be engaged in a coordinated way in addressing critical problems of world hunger. Smooth and efficient operation of the MO is essential to the functioning and success of the overall CRSP.

In 1990, the MO staff included:

Dr. Pat Barnes-McConnell, Director
Dr. Russell Freed, Deputy Director
Ms. Sue Bengry, Administrative Officer
Ms. Bonnie Zell, Program Secretary

*MANAGEMENT SUPPORT GROUPS*

Three advisory groups support the management of the CRSP: A Board of Directors (BOD), a Technical Committee (TC), and an External Evaluation Panel (EEP). These groups work closely with the MO to guide the CRSP through policy decisions, budget allocations, research strategy, review, and evaluation. In addition, the CRSP enjoys important support both from an A.I.D. Program Officer, Dr. Harvey Hortik, and a liaison to the Board for International Food and Agricultural Development (BIFAD), Mr. W. Fred Johnson.

***The Board of Directors:***

The BOD is the executive committee for CRSP policy and budget. It consists of five institutional representatives (IRs) from the U.S. lead institutions. Members serve for three years. IRs are designated by the chief executives of their institutions to represent them in CRSP policy and administrative matters. These IRs are typically administrators of international agriculture programs, deans of agriculture or experiment station directors. The BOD elects its own chairperson and secretary. The Program Director is the MO liaison to the BOD. The A.I.D. Program Officer (Dr. Hortik) is the A.I.D. liaison to the BOD.

Members of the BOD for FY 90 were:

Dr. Louis J. Boyd
Coordinator, Internat'l Agriculture
201 Conner Hall, Cedar Street
University of Georgia
Athens, GA 30602

Dr. Carlos Cruz, Director
Department of Crop Protection
University of Puerto Rico
Pinero Annex 313
Mayagüez, PR 00708

Dr. Eldor A. Paul, Chair
Department of Crop and Soil Sciences
286 Plant and Soil Sciences Building
Michigan State University
East Lansing, MI 48824

Dr. D. Woods Thomas*
Director, Internat'l Programs in Agr
26 Agricultural Administration Building
State and Marsetellar Streets
Purdue University
West Lafayette, IN 47907

Dr. Dale Vanderholm**
Associate Dean
Agricultural Research Division
109 Agricultural Hall
University of Nebraska-Lincoln
Lincoln, NE 68583-0704

*BOD Secretary
**BOD Chair

The BOD held three meetings and conference calls during the year. Action taken at those meetings included:

1. Review of the EEP report and enactment of policy decisions as appropriate in response to the EEP findings.

2. Guidance in developing grant extension proposal.

3. Guidance and support in the organization and functioning of CRSP management.

4. Review and approval of project and MO budgets for FY 91.

***The Technical Committee:***

The TC advises the BOD and MO in areas of project management, technical research strategy, and technology development. It has specific responsibility for technical monitoring of the CRSP projects, review/revision of the CRSP Global Plan, establishment of priorities for new research, evaluation of new proposals, reviewing budgets, and development of criteria for evaluation of existing projects. The TC consists of five investigators engaged in CRSP projects from U.S. institutions plus a scientist from a participating HC institution. TC members are appointed to three-year terms by the BOD. Representatives of the relevant international research centers (CIAT and IITA) also meet with the Technical Committee.

The members of the TC for FY 90 were:

Dr. James Beaver
Department of Agronomy & Soils
University of Puerto Rico
Pinero 221
Mayagüez, PR 00708

Dr. Peter H. Graham
Department of Soil Science
University of Minnesota
256 Borlaug Hall
1991 Upper Buford Circle
St. Paul, MN 55108

Dr. Douglas P. Maxwell*
Department of Plant Pathology
University of Wisconsin
284 Russell Labs, 1630 Linden Dr.
Madison, WI 53706

Dr. Larry Murdock**
Professor and Coordinator, RIISP
Entomology Hall, S. University Dr.
Purdue University
West Lafayette, IN 47907

Dr. Douglas Pachico
Coordinator, Bean Program
CIAT
Apartado Aereo 6713
Cali, Colombia

Dr. R. Dixon Phillips
Department of Food Science
University of Georgia
Melton Bldg., Woodruff Dr. at Hwy 3
Griffin, GA 30223-1797

Dr. Shiv R. Singh
IITA Nigeria
c/o Mrs. M. Larkin
L. W. Lambourn & Co.
Carolyn House, 26 Dingwall Rd.
Croydon CR9 3EE England

Dr. James Teri
Sokoine University of Agriculture
Box 3005 Subpost Office, Chuo Kikuu
Morogoro, Tanzania

*TC Secretary
**TC Chair

The WID Specialist is an ex-officio member of this committee. The Deputy Director is the MO liaison to the Technical Committee. The A.I.D. Program Officer for the CRSP (Dr. Hortik) is the A.I.D. liaison.

The TC held three meetings and conference calls during the year. Actions taken included:

1. Review of project annual report evaluations and follow-up of the EEP recommendations, with appropriate action taken, including recommendations for FY 91 workplans and budgets.

2. Recommendations for project adjustments.

3. Review and recommendation of log frame modifications for extension grant proposal.

4. Review and recommendations of extension proposals for FY 89-92.

5. Initiation of CRSP-wide germplasm conservation activities for both beans and cowpeas.

*External Evaluation Panel:*

The EEP, responsible directly to A.I.D. and BIFAD, reviews and evaluates the CRSP management and the progress of project research and training. Annual Reports of each project are used in the review. In addition, each year the HC or U.S. components of some projects are reviewed in the field. A fiscal and administrative management evaluation is made from data provided by the MO. The report of the Panel is published by the MO each year following their annual meeting with the BOD.

For FY 90, panel members, nominated by the BOD and approved by A.I.D. and BIFAD, were:

Dr. Jack Robins**
6707 55th Street, Ct. W.
Tacoma, WA 98467

Dr. Edna McBreen, Director
Office of International Programs
2112 Agricultural Sciences Building
West Virginia University
Morgantown, WV 26506

Dr. Kenneth Rachie
13 Coronado Trace
Hot Spring Village, AR 71909

Dr. Art Siedler
Department of Food Science
College of Agriculture
University of Illinois
Urbana, IL 61801

**EEP Chair

A schedule of EEP site visits to eight HCs was executed during the August-December 1990 period, usually involving two EEP/MO members/staff. EEP members McBreen, Rachie and Siedler were assigned the four projects not covered by HC site visits and the Women in Development and training programs.

# MANAGEMENT OFFICE ANNUAL REPORT

Fiscal year 1990 was an exceptionally demanding year for the Management Office (MO). In addition to the regular management duties which support the effective functioning of the individual projects, there were many new activities taken on by the MO, the major ones of which are listed below. These and other activities were spread throughout the year but because of their complexity, constant demands were maintained on the four person MO staff (3.65 FTE). The Program Director and the Deputy Director, both of whom recently had been promoted to full professor, maintain tenured positions in their respective departments in the MSU College of Agriculture and Natural Resources (.35 FTE). Major areas of activity in the MO included:

1. Maintaining regular programmatic support to, and communication with, the CRSP projects (e.g., all international travel--approvals, dates and whereabouts; equipment--purchase approvals processed through A.I.D.; troubleshoot any difficulties or misunderstandings in the HC or in the U.S.; new or changed A.I.D. rules and regulations).

2. Staying current with regular fiscal and contract responsibilities (e.g., receiving and posting quarterly reports from projects, processing the quarterly reimbursements, compiling monthly fiscal status reports for internal management, reconciling accounts against approved budgets).

3. Hosting the FY 89 EEP meeting coordinated with the annual meeting of the IRs and BOD, preparing and publishing the FY 89 EEP Report; for FY 90, preparing the materials for, and the reports of, the FY 90 EEP; organizing the FY 90 EEP HC site visits, travel clearances, travel reservations and arrangements; making preparations for the FY 90 EEP meeting coordinated with the annual meeting of the IRs and BOD.

4. Hosting TC meetings; preparing materials for, and reports from, the TC; organizing travel clearances and arrangements; organizing conference calls.

5. Hosting BOD meetings; preparing materials for, and reports from, the BOD; making travel arrangements; organizing conference calls.

6. Initiating documentation and approval procedures for the next extension (to be a five-year extension) because this was the second year of the current three-year extension (May, 1989 to March, 1892).

7. Facilitating the phaseout of three projects (Brazil, Guatemala, and INCAP).

8. Facilitating the reorganization of two projects (Nigeria--new HC to collaborate with University of Georgia; Malawi--new U.S. lead institution to replace Michigan State: University of California-Davis identified with input from TC and collaborators from the HC and U.S. social science teams).

9. Working closely with the newly formed CRSP Council, which is an organization of leaders from the eight existing CRSPs convened to generate greater political and financial support, facilitate inter-CRSP research and training, and to provide a focal point for A.I.D./CRSP interactions.

10. Concluding, and reporting the results of, the Senegal economic impact study carried out through the MO.

11. Maintaining training records, reporting training activities and providing support to HC students regarding visas, income taxes, etc.

12. Concluding, and reporting the results of, the Bean/Cowpea CRSP-wide training study from the returned training survey forms sent to all CRSP alumni who could be located (return rate 53 percent).

13. Negotiating, and implementing buy-ins to the Bean/Cowpea CRSP for additional activity in support of related goals (i.e., Economic Impact Studies for all CRSPs with technology ready for evaluation for A.I.D./S&T/AGR--$200,000; Purdue post-doc for storage research technical assistance to Cameroon--$80,500; three-year, cowpea improvement research collaboration with the Ministry of Agriculture in Egypt, negotiated in FY 90 but funding actually not received until FY 91--$230,000).

14. Organizing and hosting a major international researchers meeting (see publication of proceedings) with the participation of U.S. and HC PIs and their colleagues and students, CRSP IRs, and representatives from IARCs, the CRSP Council, A.I.D. and non-CRSP developing countries interested in beans and cowpeas (during this time period, the MO hosted and participated in concurrent meetings organized for the TC and the CRSP Council).

15. Maintaining information storage, retrieval and dissemination systems through up-to-date office computerization, also important for maintaining fiscal accountability and reporting.

Many of the CRSP responsibilities reflected above required MO travel, domestic and international. A schedule of this travel is listed below.

*FY 90 MO TRAVEL*

| | | |
|---|---|---|
| October 1989 | 10/10-13 | Freed, OIT Contractors' Conference, Washington, D.C. |
| | 10/12-13 | Bengry, OIT Contractors' Conference, Washington, D.C. |
| | 10/29-11/3 | Barnes-McConnell, Centers' Week, Washington, DC |
| November 1989 | 11/5-9 | Barnes-McConnell, Bean Improvement Cooperative, Toronto |
| | 11/15-18 | Freed, Participate in EEP Evaluation of INCAP, Pullman, WA |
| | 11/28-30 | Barnes-McConnell, Cross-CRSP Meeting, Washington, DC |
| | 11/30 | Bengry, meet with project staff re accounting procedures, Minneapolis, MN |
| December 1989 | 12/5-6 | Barnes-McConnell, participate in EEP review, Madison, WI |
| | 12/7-8 | Freed, participate in EEP review Ecuador project, St. Paul, MN |
| January 1990 | 1/10-13 | Freed, TC meeting, Phoenix, AZ |
| | 1/5-9 | Barnes-McConnell, meet with USAID & IRA officials, Cameroon |
| | 1/10-13 | Barnes-McConnell, TC meeting, Phoenix, AZ |
| | 1/20-26 | Barnes-McConnell, BOD Meeting, Orlando, FL |
| | 1/20-26 | Bengry, BOD Meeting, Orlando, FL |

| | | |
|---|---|---|
| February 1990 | 2/6-9 | Barnes-McConnell, CRSP Council Presentations, Washington, DC |
| | 2/26-3/23 | Barnes-McConnell, discuss buy-ins, Malawi, Tanzania, Zimbabwe |
| | 2/22-3/12 | Bengry, meetings with project personnel re reimbursement process, Malawi |
| June 1990 | 6/1-15 | Barnes-McConnell, discuss buy-in, Egypt |
| | 6/18 | Freed, discuss Bean/Cowpea CRSP and Cameroon National Cereals Research and Extension Project (NCR/E), Fort Wayne, IN |
| July 1990 | 7/14-17 | Barnes-McConnell, BOD meeting, Riverside, CA |
| | 7/14-17 | Bengry, BOD meeting, Riverside, CA |
| | 7/20-21 | Barnes-McConnell, SANREM Meeting, Washington, DC |
| August 1990 | 8/2-5 | Bengry, meet with project personnel regarding budget practices, UC-Davis |
| September 1990 | 9/4-5 | Barnes-McConnell, Board on Agriculture, Washington, DC |
| | 9/13-26 | Freed, attend Sokoine Bean Workshop, Tanzania; meet with personnel of newly reorganized project, Malawi |
| | 9/22-10/13 | Barnes-McConnell, discuss buy-ins with administrators in Niger and Zimbabwe |

The complexity of the CRSP is demonstrated by the fact that the multiple projects involve women and men from twelve developing countries and twelve U.S. institutions representing different disciplines, languages and cultures. In addition, the diverse institutions require acknowledgement of a significant number of sometimes conflicting bureaucratic regulations emanating from A.I.D., the ME university, each participating U.S. institution, and the HC institutions. The dedication of the MO staff and the cooperation of all of the relevant contracts officers at the ME, the participating universities and A.I.D. have been crucial for successful CRSP management.

In this regard, it is with sadness that the MO acknowledged in FY 90, the retirement of the MSU Contracts Officer, Mr. Jerry Jacobs. The amicable and extremely competent Mr. Jacobs helped the CRSP through the Planning Grant in the late 1970s and worked closely with the Contracts Office at A.I.D. in negotiating a way to make this new CRSP mode implementable. The subsequent grant language and the agreed-upon language interpretation have been critical to the success of the Bean/Cowpea CRSP. The new contracts officers in both of those offices, as well as their counterparts at the other collaborating institutions, most fortunately, are carrying on in that tradition.

For the MO, the year was organized according to responsibility needs and activity deadlines—the staff met each of these in turn. The extensive monthly output by the MO, as reflected below, highlights the noteworthy level of achievement in managing the intricacies of this program.

OCTOBER—Prepared paper for Bean Improvement Cooperative meeting in Toronto; to support increased coordination with IARCs, attended Centers' Week in Washington and concurrent CRSP Council meetings to plan for spring 1990 Congressional presentation; worked with EEP for project reviews; projects' encumbrances received and processed.

NOVEMBER—Received annual reports from the projects—organized, duplicated and distributed them to the TC, EEP and BOD; accompanied EEP on site review to Washington State; finalized arrangements for EEP meeting in conjunction with annual IRs meeting hosted by the BOD to be held in Orlando, Florida in January; received last of FY 89 Quarterly reports, reconciled accounts and constructed final FY 89 financial report; received information and slides from other CRSPs for spring Congressional presentation and constructed multi-media presentation; wrote and distributed draft script for suggestions; engaged MSU media center in making velcro poster board display for that event; Bean/Cowpea CRSP Budget Process Book published and distributed.

DECEMBER—Accompanied EEP on site review to Wisconsin and Minnesota; prepared final materials for EEP, IRs, BOD and TC meetings and sent out to them; for spring Congressional presentation, continued polishing script and working with other CRSPs to get necessary input for two-projector, two-speaker, Cross-CRSP slide show; put together information needed by Small Ruminant CRSP for the Cross-CRSP booklet they were doing for the same event; PulseBeat, the CRSP newsletter, produced and prepared for publication.

JANUARY—Hosted Technical Committee meeting in Phoenix; negotiated Cameroon BOA (Basic Ordering Agreement) buy-in with the Cameroon A.I.D. Mission and took negotiated agreements to TC meeting for input prior to submission to BOD for approval; in Orlando, hosted EEP meeting and annual meeting of IRs with BOD; continued polishing two-projector slide show integrating input from other CRSPs into script and coordinating contributed slides; PulseBeat published and distributed.

FEBRUARY—Distributed final two-projector slide show script to CRSP Council and second speaker and convened day before presentation to practice; made presentation to Congress, the Environmental Community, the World Bank and A.I.D. administrators; began development of Cross-CRSP Socio-Economic Impact Study proposal; started preparing materials for April International Researchers Meeting; EEP Report published; distributed Malawi project preproposals to TC in preparation for conference call.

MARCH—Negotiated local currency buy-in by Zimbabwe Ministry of Agriculture and worked with their cowpea team to develop scope of work for cowpea farming systems research and identify responsibilities; discussed potential buy-in with Tanzania Mission; conducted internal audit in Malawi regarding close-out of MSU project account (in preparation for new U.S. lead institution), organized 20-page list of receipts needed from Malawi with instructions to Malawi as to how to proceed, and received commitment from Malawi to cooperate with the first receipts submitted promptly; hosted TC conference call to screen preproposals for Malawi project; communicated with the final four institutional candidates for the Malawi project regarding submission of full proposals; continued preparations for April International Researchers Meeting.

APRIL--Made final travel arrangements, logistical support, and materials preparations for International Researchers Meeting, TC meeting, and CRSP Council meeting; held meeting with A.I.D. representative regarding development of new Sustainable Agriculture CRSP; discussed schedule for up-coming EEP HC reviews for next CRSP extension with project personnel; discussed Economic Impact Study with other CRSPs and developed plan.

MAY--Concluded International Researchers Meeting and current meetings of the CRSP Council (to plan Impact Studies and other business) and the TC (to review FY 91 proposed workplans and budgets, the Global Plan and new U.S. institution for the Malawi project); contacted CRSP researchers and, based on their input, wrote five proposals for Egypt per A.I.D. instructions for subsequent buy-in negotiations; worked with Tanzania PI, in the U.S. for the TC and Researchers Meeting, to write Tanzania Mission buy-in proposal; Cameroon buy-in received.

JUNE--Negotiated with Egypt Mission and Ministry of Agriculture regarding buy-in, with Egyptian counterparts rewrote proposal to meet circumstances and developed budget; Cameroon sub-grant to Purdue sent out and funding put in place; held cooperation discussions in Ft. Wayne with representative of Cameroon NCR/E project and U.S. PI for CRSP/Cameroon project; processed subcontracts amendments to obligate the second half of each projects' FY 90 budget minus their FY 89 carry-forward.

JULY--Prepared and sent out BOD materials; held BOD meeting at University of California-Riverside; received materials on SANREM (Sustainable Agriculture and Natural Resource Management CRSP) and attended first SANREM planning meeting and presented paper; distributed, received back and returned to A.I.D., the A.I.D./BIFAD directory questionnaires.

AUGUST--Prepared first draft of Researchers Meeting Proceedings; organized and coordinated first HC review trips of EEP; began preparations for the December EEP meeting to be held concurrent with the annual meeting of the IRs hosted by the BOD; reconciled newest stack of Malawi receipts received.

SEPTEMBER--Received signed ten column budget sheets for FY 91; funds finally received for Economic Impact Study; project extension proposals received, organized and distributed to TC, BOD and EEP; began work on latest returns from training survey mailed worldwide to CRSP graduates; Processed subcontract amendments to obligate to each project the first half of their FY 91 funds; participated in CRSP Bean Workshop in Tanzania co-sponsored with CIAT and gave MSTAT training; in Malawi, assisted in further introduction of new U.S. and Malawi teams.

This overview of the monthly activities of the MO suggest the pressure on the staff but nonetheless the level of accomplishment generated. The EEP, in this year's review, has acknowledged the excellent performance of the MO in spite of the load it has assumed. Undoubtedly, this performance is a product of the dedication, professionalism, and patience of all of the participants of this CRSP, U.S. and HC, who at all levels, scientific and administrative, contribute time, energy and cooperation far exceeding the call of duty. Because of this, we all reap great benefits--U.S. farmers, small-scale producers in developing countries and, ultimately, consumers--we all profit from the scientific and interpersonal achievements of the successfully functioning initiative known as the Bean/Cowpea CRSP.

# TRAINING SUMMARY

The collaboration among CRSP scientists is significantly strengthened by the CRSP training component. Training research scientists through degree and non-degree programs has increased the number and efficiency of bean and cowpea workers around the world. These linkages, established through graduate study at the various CRSP institutions, extend collaboration among CRSP scientists well beyond the life of any project. Life-long student/teacher relationships provide innumerable opportunities for the exchange of ideas and information.

Twelve students received advanced degrees in FY 90 (see chart). Currently 71 students are enrolled in degree programs. To date the CRSP has trained 204 students (60 Bachelors, 91 Masters and 53 PhDs). The MO has published a Training Report book which covers the ten-year period 1980-90 and lists all the degree and non-degree students.

DEGREE TRAINING COMPLETED IN FY 90

| *NAME* | *CITIZENSHIP* | *GENDER* | *DEGREE* | *UNIVERSITY* | *DEPT.* | *CRSP PROJECT* |
|---|---|---|---|---|---|---|
| Abdulla, M. | Sudan | M | MSc | UC-Rvrside | Plant Sci. | Hall |
| Carranaza, O. | Guatemala | M | BSc | INCAP | Food Sci. | Swanson |
| Dessert, K. | USA | F | PhD | MSU | Crop Sci. | Swanson |
| Godoy, G. | Dom. Rep. | F | PhD | UNL | Plant Path. | Coyne |
| Herrera, B. | Guatemala | M | BSc | INCAP | Food Sci. | Swanson |
| Hoyos, R. | Colombia | M | MSc | MSU | Crop Sci. | Swanson |
| Hsieh, H. | Taiwan | F | MSc | WSU | Fd Sci/Tech | Swanson |
| Khairallah, M. | Lebanon | F | PhD | MSU | Crop Sci. | Harpstead |
| Mafuleka, M. | Malawi | F | PhD | MSU | Food Sci. | Isleib |
| Magalhaes, B. | Brazil | M | PhD | Cornell | Entomology | Roberts |
| Nzuzi, L. | Zaire | M | PhD | WSU | Fd Sci/Hm Nt | Swanson |
| Tuan, Y. | | F | MSc | UGA | Food Sci. | McWatters |

In June of 1987 the Technical Committee developed guidelines concerning degree training which were distributed again to the PIs. This document served to remind the PIs of the importance of training as well as the realities of operating a training program through a research grant.

The Management Office has initiated several studies related to training under the direction of Dr. James Oehmke, MSU agricultural economist, and several graduate students. In 1990 they completed the "Impact Study of the Bean/Cowpea CRSP in Senegal." This study lists several impacts of the CRSP training:

1. For each returned degree candidate, Senegal saved up to $200,000 which is the cost per year of an expatriate scientist.

2. Because of the returned graduates, a multidisciplinary team has been assembled to conduct research on the major aspects of cowpea production.

3. There appears to be a lower turnover rate of CRSP-trained Senegalese scientists as compared with those trained in other programs.

Dr. Oehmke's group has finished a draft paper on "Returns to Education: The Impacts of MSU Training of West African Scientists." This study showed a 22 percent rate of return (monetary only) to training and a 31 percent rate of return when both monetary and non-monetary benefits are considered. The paper concludes that "the investments made by the CRSP in educational and training have had positive returns."

Their third study concerns the impact of CRSP training in Malawi and Tanzania. This study is still in process, but should show some very impressive impacts in eastern and southern Africa. Sokoine University has one of the most impressive HC multidisciplinary bean teams in Africa. Bunda College is a very close second. In addition to their research efforts, the CRSP trainees, through their teaching assignments, are making significant contributions to the training of other African scientists.

The CRSP has been very effective in using training to advance the research goals of the CRSP as well as building strong research and teaching institutions in the HCs. In the extension proposals each project is required to outline their training schedule for the extension period. The training plan is developed in collaboration with the HC PI and the research and personnel directors in the HC. These plans include both degree and non-degree programs. The chart below summarizes each project's training plan for the 1989-92 extension period. It also outlines the current status of those plans.

# WOMEN-IN-DEVELOPMENT (WID) ANNUAL REPORT

The WID Program provides support to CRSP projects on gender and social science concerns and assists PIs in identifying researchers to meet project needs in these areas. In addition to this support function, the WID specialist, Dr. Anne Ferguson, conducts primary research on women's and other small-scale farmers' roles in bean pro- duction in Malawi as a Co-PI on the UC-Davis (previously MSU) project. Both functions are reviewed in this report which is divided into four sections: I., WID support to the CRSP projects; II., WID-related primary research; III., Linkages; and IV., WID-related publications.

I. WID Support to the CRSP Projects: In this capacity Dr. Ferguson participated as an ex-officio member of the TC in its evaluation of project annual reports and extension proposals, acted as a resource person for the PIs on WID and social science issues, and represented the CRSP WID program at workshops and conferences. Each function is briefly described:

A. Support to the Technical Committee:

1. The 1992-97 extension process got underway during 1990 with projects writing and submitting their extension proposals. Dr. Ferguson participated in these efforts in two ways:

**Preparation of the WID extension proposal.** In addition to the functions currently performed and pending additional financial support (level 3 budget request), the WID program proposes to develop a model of socioeconomic impact evaluation and to develop participatory research strategies which can be used by CRSP PIs and others. The social impact analysis model will present ways projects can monitor the effects of their technology development process in the HCs. The participatory research strategies will provide means for women and other farmers and consumers to have input into the early stages of technology development (for both production oriented and food technology projects).

**Review of project extension proposals.** Together with the TC, Dr. Ferguson reviewed the 1992-97 project extension proposals. Written suggestions regarding WID and social science were included with the TC reviews of each of the projects and copies were sent to all PIs.

2. Also, Dr. Ferguson reviewed the 1989 project annual reports and participated in the TC discussion and evaluations of these reports.

B. Direct Support to PIs: Dr. Ferguson worked closely with the following projects during FY 1990:

1. Ecuador/UMN. This project, which is just getting underway, focuses on biological nitrogen fixation. Dr. Ferguson sent the U.S. PI, Dr. Peter Graham, copies of research reports and articles on bean production and farming systems which were generated under the auspices of the previous Ecuador/Cornell project. Dr. Patricia Garrett, the U.S. Co-PI together with Dr. Jorge Uquillas, HC Co-PI, conducted surveys of bean production in a number of provinces in Ecuador where Dr. Graham is working. These could be useful to him in his research.

   Dr. Ferguson has also been in contact with two graduate students at the UMN who are working with the project gathering information on soils and farming practices in Ecuador, and she has recently begun efforts to identify resource people within Ecuador who could be of assistance to the project.

2. Nigeria/UGA. This food technology project is undergoing reorganization as part of the extension process. A new HC is being sought and a new research agenda will be established. Dr. Ferguson has worked with the U.S. PIs, Dr. R. Phillips and Ms. McWatters, on the draft of the extension proposal in which key constraints to cowpea processing and use were identified, and she will continue to assist them in institutionalizing WID/social science once they identify a country and specific research topics.

3. Senegal/UC-Riverside. Progress has been made in identifying someone to examine the impact of project technologies in Senegal. During 1990, an ISRA researcher, Madame Seynabou Tall, was engaged to examine adoption of the cowpea minikit. She conducted a survey which is currently being analyzed. It is anticipated that Madame Tall will also explore the socio-economics of improved cowpea storage methods and of fresh pod production, considering in both cases class and gender issues. Dr. Ferguson has made arrangements to meet with Madame Tall and discuss her research when she visits Riverside for the annual project meeting in January 1991.

4. Tanzania/WSU. The new U.S. Co-PI, Dr. Lorna Butler, began work with the Tanzania Project this year. Dr. Butler, who will take over direction of the project when Dr. Silbernagel retires in 1992, is an anthropologist with strong interests in participatory research and training. During 1990, Dr. Ferguson worked closely with Dr. Butler in a number of ways. First, she provided Dr. Butler with project-related reports and articles written by the previous U.S. Co-PI, Dr. Jean Due, so that she would have an understanding of the socio-economic and WID research undertaken by the CRSP. Second, Drs. Ferguson and Butler sponsored a participatory research workshop for agricultural scientists from Tanzania and Malawi at the end of September. This is described in greater detail in Section I.C.

5. Other Projects: Dr. Ferguson continued to provide assistance to the Cameroon/Purdue investigators who are conducting surveys of cowpea storage practices and forming a panel of farmers to evaluate project technologies. Also, she and Dr. Jim Beaver shared information on various artisanal seed production projects underway in the Central American region which could be of interest to other CRSP projects.

C. Represent the CRSP/WID Program in Training Workshops and Conferences:

1. Participatory Research Workshop. Drs. Butler and Ferguson gave a mini-workshop on participatory research techniques at Sokoine University of Agriculture in Morogoro, Tanzania, September 24-25, 1990. It was held at the close of the joint Bean/Cowpea CRSP-Sokoine-CIAT-SADCC Bean Workshop held September 17-22. The mini-workshop, attended by 18 researchers from Tanzania and Malawi, included principally biological scientists but also some agricultural extension and social science researchers. They presented a general introduction to participatory research (contrasting it with other paradigms of agricultural research such as transfer of technology and farming systems), discussed three participatory research case studies, and provided a brief field experience for participants using some of the research techniques discussed.

   The workshop was well received, and they anticipate holding a more extensive one during 1991 for CRSP researchers from Tanzania and Malawi. A workshop agenda is available as is a copy of the presentation on participatory research (part of a larger paper delivered at the CRSP-CIAT-SADCC workshop). A more extensive discussion of the workshop is presented in the Tanzania/WSU annual report in this book.

2. American Anthropological Association Symposium: Agriculture and Nutrition: Exploring the Links, co-organized with Ms. M. Graham and Dr. A. Millard, held at the American Anthropological Association Meetings, Washington, D.C., November 15-19, 1989. They organized a panel of eight researchers to discuss the relationship between agricultural change and nutritional status in various African and Latin American countries. Dr. Ferguson presented a paper, "Agriculture and Nutrition in Malawi: Exploring the Links," co-authored with Ann V. Millard and Stanley Khaila.

3. In addition, the following papers on CRSP/WID related research were presented at workshops and professional conferences: (A list of WID-related articles/books which were published or accepted for publication during 1989-90 is included in Section IV.)

   "So the Grandparents May Survive: Farmer Participation in Bean Improvement in Malawi," presented at the Ninth Sokoine University of Agriculture Bean/Cowpea CRSP and Second CIAT/SADCC Bean Research Workshop, Morogoro, Tanzania, September 17-22, 1990.

   "Component Breeding: A Strategy for Bean Improvement in Eastern Africa and Other Regions Where Beans are Grown as Mixtures," co-authored with S. Sprecher, presented at First SADCC Regional Bean Research Workshop, Mbabane, Swaziland, October 4-7, 1989.

II. WID-Related Primary Research: In addition to the CRSP-wide support functions, the WID specialist is a Co-PI on the Malawi/MSU project (now the UC-Davis project with a sub-contract to MSU). The research examines

smallholder bean production and seed handling practices with a special focus on the role of women in these areas. It is carried out in collaboration with Dr. Richard Mkandawire, a rural sociologist in the Department of Crop Production at Bunda College of Agriculture. An extended discussion of the 1990 research is found in the Malawi/MSU annual report below. A list of activities undertaken during 1990 is provided here.

A. A survey of bean production and seed handling practices was carried out in the Southern Region--in Matapwata EPA, Blantyre/Shire Highlands Agricultural Development District--during 1990. The south was the only part of the country not yet surveyed in the CRSP socioeconomic research. This study took place in April and involved a team of twelve Bunda College students, four of them women. A total of 300 farmers was interviewed. A summary of the research findings is in the Malawi report below.

B. Papers on component breeding, a plant improvement strategy proposed to maintain crop genetic diversity and meet smallholders needs, were presented at two conferences.

C. Considerable time and effort were spent in identifying a new U.S. lead institution for the Malawi project, assisting with the transition between lead institutions and in writing the 1992-97 Malawi project extension proposal.

III. Linkages:

A. During 1990, Drs. Butler and Ferguson worked to establish collaborative ties between their respective projects in Malawi and Tanzania and between the CRSP and the CIAT/SADCC/Great Lakes participatory research program. They discussed CRSP participation in a participatory research seminar Dr. Louise Sperling (CIAT/Great Lakes Program) is proposing to hold in June or July 1991. In addition, researchers from Dr. Todo Edje's CIAT/SADCC participatory research program took part in the September mini-workshop in Morogoro, Tanzania.

B. Dr. Ferguson also began exploring the possibility of collaboration with Dr. J. Ashby at CIAT, Cali, Colombia to meet the needs of CRSP Latin American projects for training in participatory research methodologies.

C. Dr. Ferguson routinely shares information generated by the CRSP projects with other CRSP projects for which the information is relevant.

D. Dr. Ferguson also maintains ties with the Rockefeller Southern Africa program, and with numerous WID organizations both in the U.S and abroad.

IV. PUBLICATIONS AND PRESENTATIONS

Ferguson, A., Gallin, R.S., and M. Aronoff (Eds.). 1989. The Women and International Development Annual, Vol.1. Boulder, CO: Westview Press.

Ferguson, A., Gallin, R.S., and M. Aronoff. 1989. Women and International Development: Creating an Agenda. In R.S. Gallin, M. Aronoff and A. Ferguson (Eds.), The Women and International Development Annual, Vol.1. Boulder, CO: Westview Press, 1989, pp. 1-24.

Ferguson, A. and S. Whiteford (Eds). Harvest of Want: Struggles for Food Security in Mexico and Central America Accepted for publication. Boulder, CO: Westview Press.

Ferguson, A. and S. Whiteford (Eds). Social Dimensions of Food Security and Hunger: An Overview. In Harvest of Want: Struggles for Food Security in Mexico and Central America. Accepted for publication. Boulder, CO: Westview Press.

Ferguson, A. and R.S. Gallin (Eds). The Women and International Development Annual, Vol.2. Accepted for publication. Boulder, CO: Westview Press.

Ferguson, A. and R.S. Gallin (Eds). Gender, Class and Ethnicity: Exploring the Links. In The Women and International Development Annual, Vol.2. Accepted for publication. Boulder, CO: Westview Press.

Ferguson, A., Millard A., and S. Khaila. Crop Improvement Programs and Nutrition in Malawi: Exploring the Links. Accepted for publication. Food and Nutrition Bulletin.

# BEAN/COWPEA CRSP PROJECTS, FY 90

| Title of Project | Host Country/U.S. Institution |
|---|---|
| Insect Pathogens in Cowpea Pest Management Systems for Developing Nations | Brazil/Boyce Thompson Institute |
| Preservation of Post Harvest Cowpeas by Subsistence Farmers in Cameroon | Cameroon/Purdue University |
| Biology, Epidemiology, Genetics and Breeding for Resistance to Pathogens of Beans with Emphasis on Those Causing Bacterial and Rust Diseases | Dominican Republic/University of Nebraska-Lincoln |
| Molecular Approaches for the Control of Bean Golden Mosaic Virus | Dominican Republic/University of Wisconsin-Madison |
| Improving the Productivity of Phaseolus Beans Under Conditions of Low-Input Agriculture Through Genetic Selection of Host Cultivars and Rhizobium Strains for Enhanced Symbiotic Efficiency | Ecuador/University of Minnesota |
| Agronomic, Sociological, and Genetic Aspects of Bean Yield and Adaptation | Guatemala/Cornell University |
| Improvement of Bean Production in Honduras Through Breeding for Multiple Disease Resistance | Honduras/University of Puerto Rico |
| Improving of Dry Bean Nutritional Quality and Acceptability | INCAP/Washington State University |
| Improvement and Host Pathogen Co-Adaptation in Malawi, a Secondary Center of Diversity | Malawi/University of California-Davis |
| Improving Resistance of Environmental Stress in Beans Through Genetic Selection for Carbohydrate Partitioning and Efficiency of Biological Nitrogen Fixation | Mexico/Michigan State University |
| Appropriate Technology for Cowpea Preservation and Processing and a Study of Its Socioeconomic Impact on Rural Populations in Nigeria | Nigeria/University of Georgia |
| A Program to Develop Improved Cowpea Cultivars, Management Methods and Storage Practices for Semiarid Zones | Senegal/University of California-Riverside |
| Breeding Beans (*Phaseolus vulgaris* L.) for Disease, Insect and Stress Resistance and Determination of Socioeconomic Impact on Smallholder Farm Families | Tanzania/Washington State University |

# BRAZIL - BOYCE THOMPSON INSTITUTE

*INSECT PATHOGENS IN COWPEA PEST MANAGEMENT SYSTEMS FOR DEVELOPING NATIONS*


**Principal Investigators:**

| | | |
|---|---|---|
| Donald W. Roberts (U.S.) | Biological Control Program | Boyce Thompson Institute |
| Eliane Quintela (HC) | Entomology Department | EMBRAPA/CNPAF |

**Co-Investigators:**

| | | |
|---|---|---|
| Leslie I. Allee | Plant Protection Program | Boyce Thompson Institute |
| Jose Emilson Cardoso | Plant Pathology | EMBRAPA/CNPAF |
| Raymond Carruthers | Plant Protection Unit | USDA/ARS |
| Heidi Firstencel | Plant Protection Unit | USDA/ARS |
| Steven Kreuger | Biological Control Program | Boyce Thompson Institute |
| Raymond St. Leger | Plant Protection Program | Boyce Thompson Institute |
| Claudio L. Messias | Genetics Department | University Campinas |
| Stephen P. Wraight | Biological Control Program | Boyce Thompson Institute |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

Host Country

**Control of *Empoasca* leafhoppers with *Zoophthora radicans*.** We have been investigating this pathogen since the beginning of the project and found considerable early success in developing mass production, formulation, and application techniques. Introductions of this fungus into alfalfa and beans (*Phaseolus*) in New York in 1985, in cowpeas in Goias in 1986, and in beans in São Paulo in 1990 (see below) resulted in spectacular field-wide epizootics. However, our inability to duplicate these successes in cowpeas in northeastern Brazil over the past three field seasons indicate that this pathogen is not adapted to the environmental conditions of that region (especially the equatorial temperatures). Moreover, even within its natural range, the fungus is limited by moisture conditions; in 1989, field tests in Goias failed in dry, windy weather. Nevertheless, the successes we have encountered, especially this past season in southern Brazil, indicate that use of this pathogen could constitute an important component of integrated pest management programs (IPM) both in Brazil and the U.S. (in this country, USDA scientists are also currently studying this pathogen for leafhopper control in alfalfa and potatoes).

Laboratory tests initiated in 1989 to determine the effects of temperature on spore germination and the host invasion process (S. Galaini-Wraight, Ph.D. thesis) were completed and the data analyzed. Observations of spores inoculated onto leafhopper nymphs indicated that the optimal temperature for spore germination, appressorium development, and host infection is ca. 25°C (Tables 1-4). The time required for infection of 50 percent of the leafhoppers decreased from 35.2 hours at 10°C to less than six hours at 25°C. The extended period of time required for penetration at low temperatures is partially explained by the fact that low temperatures stimulate production of secondary capilloconidia which must, in turn, germinate to effect host penetration (Fig. 1). We have observed a maximum of 14-15 hours of overnight moisture (dew) in bean and cowpea fields in Brazil. Thus, the data in Table 4 showing 10 percent infection after 13.6 hours at 10°C indicate that this temperature may be close to the threshold for *Zoophthora radicans* transmission in leafhopper populations. The most critical phase in the invasion process appears to be spore germination, since at 30°C only 6 percent of the spores germinated (Table 1), but the germlings were nearly as successful at invading the host as were germlings produced within the range of optimum temperatures (Table 4). These results suggest that one might initiate an attempt to identify strains with a greater potential for application in the warm tropics simply by selecting for germinability at high temperatures.

Studies of temperature and instar effects on *Z. radicans* spore production on leafhopper cadavers was also completed during FY 90 (L. Leite, M.S. thesis). Total spore production increased nearly geometrically with instar and was optimal at 20-25°C (Tables 5, 6).

Discussions with Dr. R. Freed during the 1989 project review resolved that we should conduct field trials of *Z. radicans* in beans (*Phaseolus*) in southern Brazil (on the assumption that we would encounter environmental conditions more favorable to disease transmission than in the northeast). Applications of a dry mycelium of *Z. radicans* strain 2282 (originally isolated from *Empoasca vitis* in Yugoslavia) were made in a 30-day-old 0.5 ha planting of beans owned by a small farmer in Capo Bonito, São Paulo on April 15. The leafhopper infestation was rapidly increasing in the field; and the small plants, with a mean of less than five trifoliates, were showing signs of severe stress. Pretrial observations revealed that a native strain of Z. radicans was present in the field, but infection rates were extremely low, and no infected leafhoppers were found in samples collected the morning of April 16. Four days after application, mean percent infection in the test plots treated at a rate of 0.25-0.5 g fungus per m2 was 16.2 percent while in plots that received 1-2 g per m2 infection was 33.5 percent. At the same time, untreated plots located 5 m from the treated plots showed a mean infection rate of 6.8 percent while more distant control plots (18 and 36 m) showed 3.4 percent (Table 7). Subsequent samples collected at three to four day intervals revealed that the treatments had apparently initiated a field-wide epizootic which, on April 30, culminated in infection levels of 55-70 percent over all sample plots (treated and untreated). Epizootic development was clearly stimulated by near optimal conditions of moisture and temperature beginning April 23 (Figs. 2, 3).

The trial comprised a non-random array of treatment and control plots designed to provide evidence that infections observed outside the treatment plots resulted from dispersal of the applied fungus rather than an outbreak of the native pathogen. Each dose of fungus was applied in a different area of the field (the areas separated by ca. 20 m). Within each treatment area, fungus was applied to three replicated 2 x 2 m plots, and two control (untreated) plots were established ca. 5 m from treatment plots. The April 26 data in Table 7 show that infection in the untreated plots associated with the 0.25, 0.5, 1.0 and 2.0 g/m2 treatment plots was 23.1, 36.8, 41.1, and 41.9 percent, respectively, providing strong evidence that the fungus we applied actually caused the observed epizootic. Isozyme analysis of strains isolated from the field are planned to verify this conclusion.

The overall results of the trial were extremely impressive, demonstrating the explosive epizootic potential of *Z. radicans* in leafhopper populations. Infection increased from nearly 0 percent on April 16 to 60-70 percent within 14 days. Over the total three-week monitoring period, leafhopper densities decreased from approximately 6/trifoliate to less than 1/trifoliate, representing an approximate 85 percent reduction in the pest population to a level below the economic threshold. This was accomplished through application of dry mycelium at a rate of only 100 g per ha.

These results demonstrate that the *Z. radicans* dry mycelium technology developed over the long course of the project has considerable potential for control of *Empoasca* leafhoppers in beans in southern Brazil. We have transferred all aspects of the technology to the collaborating scientists of the Instituto Biologico of São Paulo state (primarily via project-trained M.S. student, Luis Leite, who is employed by the institute). They now have the expertise to independently conduct the final stages of evaluation and pilot testing of our project.

We have, unfortunately, not been successful in applying this technology to cowpeas in northeastern Brazil (see 1989 report). In 1990 we made a third attempt at field applications. On the nights of the trials, temperatures remained high until 22:00 hours, inhibiting dew formation. Ultimately, wet conditions did not prevail long enough during the night to support sporulation of the mycelium, and no infection occurred. These results, combined with the failures of the previous two field seasons, strongly suggest that *Z. radicans* is not adapted to the harsh environmental conditions of northeastern Brazil and will have little potential for control of leafhoppers in that region unless strains can be developed that are effective under high temperature--low moisture conditions.

**Control of the cowpea curculio with *Metarhizium anisopliae* and *Beauveria bassiana*.** A low-cost, low-technology method has been developed for biological control of *Chalcodermus* weevils, the principal field pest of cowpea in Brazil. Field trials of this methodology (spray application of conidia to the soil at the base of plants) in experimental plots at CNPAF (central cerrado) and EPACE (arid serto) in 1988 and in subsistence-level cultures in northern

Ceara (humid litoral) in 1989, have consistently demonstrated 30-50 percent control of weevil larvae. Our project scientists and collaborating EPACE researchers consider these results encouraging, but recognize that, to prove economically feasible, this marginal level of control will need to be augmented by cultural and other control measures in an integrated management system. Accordingly, the ongoing research in Ceara includes damage evaluations needed to establish economic thresholds, and at one of the study sites, all pods are being removed from the fields after the principal harvest period to prevent them from serving as pest reservoirs, and the harvested pods are being sprayed during drying and storage to infect emerging larvae and prevent their development to the adult stage. A major effort was put into this research in FY 90 by CNPAF, EPACE, and BTI staff, but abnormally low weevil infestations near Fortaleza this year will delay data collection until the FY 91 season.

**Control of bruchid beetles in stored cowpeas with *Beauveria bassiana* and *Metarhizium anisopliae*:** Treatment of stored cowpea seeds with dry spores of *B. bassiana* and *M. anisopliae* was identified last year as a promising new low-technology method for control of the highly destructive cowpea weevil, *Callosobruchus maculatus*. We determined that initiation of this research at such a late point in the project was justified because of the great potential benefits from success and ease of conduct of the experiments. In recently completed tests, fungal treatments provided levels of control nearly equal to Vertimec--a commercially produced avermectin-based pesticide (Table 8). These highly encouraging results indicate that continued CRSP support of this work is warranted--perhaps at an increased level. Moreover, if our results continue to show promise, studies could be readily initiated in Africa at minimal cost to the CRSP.

**Biology of *Cerotoma arcuata*.** Chrysomelid beetles of the genus *Cerotoma* are among the most important cowpea and bean defoliators in Brazil and throughout Latin America. However, at the time this project was initiated, virtually nothing was known of the biology of the principal Brazilian species, and no laboratory colonies existed. An important achievement of this project has been the successful colonization of *C. arcuata* (related in last year's report), and the elucidation of various aspects of the biology of this pest. During 1990, laboratory studies revealed that the larval stage has three instars (Fig. 5), and that females produce a mean of 1291.6 eggs (Fig. 6). Mean longevity of females and males is 68 and 69 days, respectively, under laboratory conditions. Our success in this area of research was realized through a long-term commitment to basic research made possible by the Bean/Cowpea CRSP.

**Compatibility of entomopathogenic fungi with chemical insecticides.** Since there are some occasions when insecticides may be used in conjunction with entomopathogenic fungi in integrated pest management programs, the effect of several insecticides on two of the most likely fungal candidates for insect control, viz., *Metarhizium anisopliae* and *Beauveria bassiana*, was examined in the laboratory. Two dosages were tested: the dosage per ha recommended by the producer of the product and half of this dose in potato dextrose agar based on 200 liters of water sprayed per hectare. The

products were Cypermethrin, Deltamethrin, Diflubenzuron, Trichlorfon, Methyl Parathion, Demeton-S-Methyl, Fenvalerate, and Endosulfan. The chemicals were mixed into the culture medium when it was approximately 50° ± 2°C and immediately poured into petri dishes. Each plate was inoculated in three places with a suspension containing 104 spores. After 12 days, the diameter of the fungal colonies, and the number and viability of spores on a disc cut from the colony was evaluated (Tables 9, 10). Each treatment had ten replications. Pathogenicity of *B. bassiana* and *M. anisopliae* was evaluated using adult *Cerotoma arcuata* and fifth instar larvae of *Chalcodermus bimaculatus*, respectively, at a dosage of 107 conidia per ml with 40 insects per treatment and four repetitions. At the high dose of Methyl Parathion there was no growth of the fungi, and with Endosulfan the diameter of the colonies was 0.7 and 0.2 cm while the control was 3.7 and 3.6 cm for *B. bassiana* and *M. anisopliae* respectively. The pathogenicity of *B. bassiana* to *C. arcuata* was not changed by exposure to chemical pesticides. *M. anisopliae*, treated with Trichlorfon at the half dose and Diflubenzuron at the normal dose killed 95 and 92 percent of *C. bimaculatus* larvae, respectively, whereas untreated fungus produced only 47 percent mortality (Table 11).

**Control of cowpea/cotton intercrop pests with *Beauveria bassiana*.** Approximately 80 percent of cowpea production in northeastern Brazil is done through intercropping. The most common intercropped plants are cassava, watermelon, corn, and cotton. The small amount of cotton produced by subsistence farmers in consortium with cowpea is important to the family economies. The cotton boll weevil did not occur in Brazil until about five years ago when it was introduced into southern Brazil. It has recently arrived in northeastern Brazil where it presents a hazard to cotton production. As part of our subsistence farmer program, we were asked by our collaborating group in northeastern Brazil, EPACE, to evaluate the susceptibility of this insect to some of the pathogens we are using against another weevil pest, viz. the *Chalcodermus bimaculatus* weevil of cowpea. Laboratory tests indicated the insect was susceptible to *Beauveria bassiana*. In small scale field plots the fungus alone was not effective against the boll worm, whereas one of the insecticides tested, Deltamethrin at a dose of 10 kg/ha, did yield approximately 60 percent reduction in insect attack of cotton squares four days after the fourth pesticide application (Table 12). The same level of control was attained when the fungus was applied in combination with a reduced rate of Deltamethrin (1 kg/ha). This is a very promising result since a 90 percent reduction of chemical insecticides would be of great financial and health value to subsistence farmers.

**Control of the whitefly, *Bemisia tabaci*, with *Verticillium lecanii*.** The whitefly has recently emerged as the single most important pest of common beans (*Phaseolus*) in Brazil, due to its role as a plant disease vector. The present critical situation has lead most Brazilian bean entomologists to begin studying the problem. During the past year, the project HC PI (bean entomologist E.D. Quintela) initiated research on the biological control of whitefly, and these investigations were naturally integrated into the CRSP project.

Whiteflies, being members of the order Homoptera (as are leafhoppers), are significantly affected by only one group of insect pathogens, the fungi. Among the fungal pathogens of whiteflies, the hyphomycete *V. lecanii* is one of the most commonly encountered in the field. Laboratory screening of five isolates of this fungus during FY 90 revealed substantial differences in virulence (Table 13) and identified at least one promising candidate for further investigation (stain VL-6).

U.S.

**Control of *Diabrotica* larvae with *Beauveria* and *Metarhizium*.** In Brazil, the larvae of *Diabrotica* beetles develop on the roots of corn that is commonly intercropped with cowpea. When adults emerge from the soil they frequently defoliate young cowpea plants. In the U.S., *Diabrotica* is comprised of a complex of species that are major pests of corn and vegetable crops in which damage and treatment costs exceed $1 billion per year. Improvements were made during FY 1990 on bioassay techniques for applying *M. anisopliae* and *B. bassiana* conidia to *Diabrotica* larvae. These techniques allow for accurate determination of dosage rates in terms of viable conidia applied per surface area. Data pooled from six bioassays of *B. bassiana* (ARSEF 731) against mid-second instar larvae (eight to nine days old) showed that at eight days post-application mortality was equal to, or greater than 60 percent in all dosage groups (Fig. 1). A significant increase in daily larval mortality appeared to occur as dosage rates exceeded 6 x $10^3$ conidia/$mm^2$ and again as rates exceeded 10 x $10^3$ conidia/$mm^2$. The $LD_{50}$ at day five of the bioassay was determined to be 9.02 x $10^3$ conidia/$mm^2$. The mean times to death ranged between 5.45 days (standard deviation = 1.44 days) for larvae treated with 4-6 x $10^3$ conidia/$mm^2$ and 4.42 days (standard deviation = 1.83 days) for larvae treated with 10-12 x $10^3$ conidia/$mm^2$. Scanning electron micrographs of larvae inoculated with ca. 7.3 x $10^3$ conidia/$mm^2$ revealed that conidia do not remain attached to raised, exposed portions of the cuticle. Instead, they may be scraped off and/or pushed into lower, intersegmental regions of the cuticle by the movements of larvae. The high dosage rates of conidia required to kill *Diabrotica* larvae support our work with mycelial particles as the best form of fungal inoculum to apply to soil for control of the larval life stage. Mycelial particles are capable of producing and transferring large quantities of conidia to the host which, by "mass action," more effectively penetrate the cuticle and kill the host (see control of scarab grubs below).

**Storage of dry mycelial particles.** A study on the long-term (one year) storage of *M. anisopliae* (ARSEF 925) and *B. bassiana* (ARSEF 731) mycelial particles was initiated. Included as treatments were two particle sizes (125-250µm and 250-500µm), two particle types (standard [unlyophilized] and lyophilized), and four storage temperatures (4°, 11°, 21°, and 30°C). After 14 weeks of storage, mean conidia production (per milligram of mycelium) of both fungi was not significantly affected by particle size, type, or storage temperature when compared to initial levels of conidia production (Figs. 8 and 9). Although not yet carried to term, these extremely positive results underscore the potential for industrial-scale development of mycelial particles as an practical form of inoculum.

**Control of scarab grubs with *Metarhizium* and *Beauveria* mycelial particles.** Investigations were completed that confirmed preliminary results of earlier studies of factors that may influence the efficacy of entomopathogenic fungi against white grubs. Bioassay of different types of *M. anisopliae* inoculum (conidia vs. various sizes of standard and lyophilized mycelial particles) against Japanese beetle grubs showed that mortality occurred significantly quicker in mycelium compared to conidia-treated soil (Fig. 10). These were surprising results considering it may take several days for the full complement of infectious conidia to be formed on mycelial particles in soil. In addition, dosage was not a factor because initial levels of inoculum in conidia-treated soil were equal to, or higher than, inoculum levels detected 21 days post-treatment in mycelium treated soil. One explanation for the greater efficacy of mycelial particles may be their potential to transfer conidia to the host cuticle in high density clusters. Conidia, in close proximity to each other, have been shown to interact during the invasion process (fusion of germ tubes and appressoria) which results in more effective entry to the host body cavity.

Bioassays were also conducted that demonstrated soil temperature and moisture may significantly impact the effectiveness of fungal applications against grubs. In soil inoculated with *M. anisopliae* mycelial particles, Japanese beetle grubs died more quickly in the high temperature/low moisture environment and survived longest in the low temperature/high moisture environment (Fig. 11). The difference in mortality rates may be attributed to better sporulation and survival of fungal inoculum and higher levels of grub stress in the low moisture environments. In contrast to the *M. anisopliae*/Japanese beetle relationship, European chafer mortality in *B. brongniartii*-treated soil was inversely related to soil temperature and not affected by soil moisture (Fig. 12). The relatively low temperature optimum for growth and infectivity of *B. brongniartii* (generally assumed to be 23°C) was probably the driving factor that determined host mortality.

***In vitro* studies of the nutritional, environmental, and molecular biology of the germination and infection processes of *Zoophthora radicans*.** The basic studies summarized here comprise the Ph.D. thesis of Bonifacio Magalhaes, who has now returned to Brazil and resumed his former position as HC PI. This work commenced with the discovery of a novel *in vitro* system for induction of appressorium formation that opened the door to investigations of key factors regulating this critical step in the host infection process. A poster presentation of a part of this work was recognized as the best student contribution at the 1989 annual meeting of the Society for Invertebrate Pathology.

Conidium germination and differentiation of *Zoophthora radicans* on water agar were investigated to identify requirements for the production of appressoria. Appressorium formation was more sensitive to environmental and nutritional changes than conidium germination. Appressorium formation was optimal when a large volume of liquid medium (3 ml/962 $mm^2$) was used (Fig. 13). Temperature affected both formation of germ tubes and appressoria, and an

optimal temperature-nutrient interaction was found for appressorium formation at 25-30°C and 1 percent yeast extract (Fig. 14). At 15°C, conidium germination was depressed independent of the nutrient concentration and appressoria did not form. Of 12 nitrogen sources tested, the best for germination and appressorium formation were yeast extract and Bacto-Soytone (Table 14). Glucose, maltose, and starch were the best of the 12 carbon sources tested (Table 15). Increasing osmotic pressure caused by different concentrations of maltose and polyethylene glycol adversely affected formation of germ tubes and appressoria (Fig. 15). Subculturing did not affect formation of germ tubes, but the frequency of appressorium formation decreased after 12 transfers (Fig. 16). The optimal pH for appressorium formation was between 7.0 and 7.2 (Fig. 17). *Z. radicans* produced appressoria on cuticles of dead *Empoasca fabae* (Harris) nymphs.

The fungus *Zoophthora radicans* requires external $Ca^{2+}$ for appressorium formation but not for conidium germination. The number of appressoria formed is dependent on the $Ca^{2+}$ concentration of the medium (Fig. 18). At low $Ca^{2+}$ concentration (100 pM), nuclear division and germ tube growth were significantly reduced compared to higher $Ca^{2+}$ concentrations (10 and 1000 µM) (Table 16). Treatment of conidia with a $Ca^{2+}$-antagonist ($Nd^{3+}$) and a $Ca^{2+}$-channel blocker (nifedipine) inhibited differentiation, showing that a $Ca^{2+}$ influx is required for appressorium formation (Fig. 19, 20). Furthermore, the partial yet saturating inhibition by nifedipine and complete inhibition by $Nd^{3+}$ indicated that two kinds of $Ca^{2+}$ channels are involved in differentiation. A contribution of intracellular $Ca^{2+}$ in the signal transduction chain for the formation of appressoria was demonstrated by the inhibitory effect of the intracellular $Ca^{2+}$ antagonist TMB-8 (Fig. 21). The calmodulin antagonists R24571, TFP, W-7, and W-5 inhibited appressoria at concentrations which had no effect on germination (Fig. 22), indicating that a $Ca^{2+}$/calmodulin system was involved in regulating appressorium formation.

2. Other research-related results

**Technical research publications.** The project published or had in press 11 research publications for FY 90. An additional four manuscripts were submitted for publication. Also, 12 reports on project research were presented at scientific meetings.

**Germplasm conservation and use.** Ten strains of entomopathogenic fungi were added to the IPRC/Brazil germplasm collection during FY 90 (Table 17). The culture collection now contains nearly 300 isolates. More than 100 of these have been provided to Brazilian university and government laboratories where their potential for biological control of a wide variety of field crop, garden, and pasture pests is being investigated. In addition, the USDA repository in Ithaca met numerous requests worldwide for Brazilian isolates collected by our CRSP project.

**CRSP-produced or -recommended technology available for use in the HC and other developing countries.** Methods for mass culture and formulation of entomopathogenic fungi developed at BTI and other institutions have been successfully adapted for mass production of

our most promising fungal pathogens of cowpea insect pests using low-cost materials and equipment available in developing countries. The resultant technology is adequate for production of sufficient quantities of fungal inoculum required for small- to moderate-scale field trials and could readily be modified for mass production at the cottage industry or grower-cooperative level.

**CRSP-produced or -recommended technology available for use worldwide.** A spray-application chamber designed specifically for uniform topical inoculation of test insects with aqueous suspensions of fungal conidia was constructed at BTI. Shortly after the project's inception, a similar tower was built at the IPRC/Brazil laboratory where it is currently utilized for screening and bioassay of candidate pathogens. The schematic for the chamber and the modified spray nozzle were requested by and provided to other insect pathologists in Brazil. At present, five of these chambers are in use or under construction in two government and two university research centers.

**Socio-economic impact of biological control technologies for cowpea in northeastern Brazil—A rapid rural appraisal in Ceara.** Cowpeas (*Vigna unguiculata* [L.] Walp.) are the most important legume crop in Ceara state in northeastern Brazil. Ceara is, in turn, the largest cowpea production area in Brazil. Cowpeas, on average, comprise 51.5 percent of bean consumption in the diet of the Ceara population. Nearly 62 percent of the cowpea consumed in the region is unpurchased. Cowpea production is scattered and restricted to small land areas throughout the state and production yield is highly variable, often depending on rainfall. The level of technological input, use of labor, and methods of pest control also varies considerably.

**Project aim.** The rural appraisal covered nine cities in major producing regions of Ceara state. The research group was composed of an agricultural economist of the socio-economic research area, an entomologist of EPACE (the state agricultural research enterprise) and an M.Sc. student from the Agricultural Economics Department at the University of Ceara.

Three cowpea production systems were formerly identified for sample classification: rainfed, usually under consortium; swamp land (varzeas) with a certain degree of moisture and higher yields; and channel or sprinkler irrigated systems. An attempt was made to cover the three production systems even though stratified appropriate samples could not be anticipated.

The data collected comprised a series of socio-economic variables in a three page questionnaire which aimed at general social information (education levels, family size), use of land and other production factors, production costs and detailed information on the common knowledge of pests and control.

A total of 83 families in 41 communities were visited and information collected. Of these, 45 farmers own the land they farm, while three have no title to the land and the other 35 farmers are related to cooperatives in irrigated perimeters.

Another questionnaire was discussed and developed in Fortaleza with the technician (Iara Oliveira) working for BTI, in the hope that she would be able to apply it to obtain a more detailed information set for cost analysis and technology follow-up. She will be working with a farm family evaluating results of biological control techniques and will participate in the socioeconomic study.

We hope to be able, from this experience, to discuss four points based on the visited areas:

- Can we extrapolate general socio-economic aspects of cowpea production from aggregate data to the micro level, with detail on farmers, families, role of women and characterization?

- What is the potential impact of biological control techniques on the basis of incidence of pests and farmers' reaction to alternative control measures?

- What is the cost analysis for different systems analyzed?

- How should we ensure farmer follow-up for technology implementation and future tests?

The FY 90 research has generated considerable baseline data on the economics of cowpea production in Ceara (Tables 18-23). These data indicate that use of chemical pesticides increases cowpea yields by 93.5 percent (Table 19), but at a 64.9 percent increase in total production costs (Table 20). This information will ultimately provide the basis for determining the economic feasibility of our proposed *Chalcodermus* weevil control technology. Answers to the questions outlined above as well as an assessment of the potential impact of these new technologies on small-scale farmers and women should be available by the end of FY 91.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Small-scale field trials conducted in farmers' fields indicate that the technologies we have developed for control of *Chalcodermus* weevils and *Empoasca* leafhoppers may be useful. Additional field trials and economic viability studies are planned for 1991. Since the proposed technologies are still in the experimental stage, they have not yet affected production at the national level.

B. Institutional Development and Training

1. Changes since FY 89. The state of Ceara is the major producer of cowpea in Brazil. For the past three years, our project has collaborated with the state agricultural research agency of that state (EPACE). Two of their scientists have made major commitments to our microbial control efforts. As an important part of the institutionalization of our project in Brazil, we undertook in FY 90 an upgrading of the research laboratory of this team at Pitaguari near Fortaleza. The director of the station made available to us a room approximately four times the size of the facility previously used for the insect pathology work. We have provided the materials

for renovation of this space. The work was commenced in mid-1990 and will be completed before the end of 1990. The new facility is adjacent to that of the plant pathology section of the station, and the two groups have agreed to share the previously existing plant pathology equipment such as autoclaves and ovens. Both the HC PI and the U.S. PI were personally involved in design of the facility and in procurement of supplies. With the completion of this laboratory, we will have two microbial control units in Brazil as the result of Bean/Cowpea CRSP activity.

An important final step in the institutionalization of the project at CNPAF, Goiania, was realized in 1990 when EMBRAPA scientist Bonifacio Magalhaes completed his doctoral program at Cornell/BTI and returned to Brazil to resume his former position as HC PI.

2. Over the life of the project. It can be judged at this time that virtually all of our efforts in the area of project institutionalization have been fully successful. The insect pathology laboratory we established in Brazil has been fully integrated into CNPAF's newly established Integrated Pest Management (IPM) Program. The laboratory is fully equipped and staffed by two project-trained scientists, Dr. Bonifacio Magalhaes and M.S.-level entomologist Eliane Quintela (FY 90, HC PI). Both are full-time, permanent EMBRAPA scientists with the expertise to continue the work initiated by the project and attract external support after the CRSP phase-out in October 1991. Establishment of the research facility in Ceara (described above) will make possible the continuation of project studies in the principal cowpea producing region of Latin America.

   Project training has had, and continues to have, a considerable impact on the Latin American scientific and technical community. The training of more than 100 agronomists in the project's five short courses (one to two weeks) and four undergraduate student interns (one month to one year) has served well to expand and update the knowledge base in the fields of insect pathology and integrated pest management. The project's four Brazilian graduate students (trained at the M.S. level in Brazilian universities) have been highly successful in securing government research and university faculty positions. Project-trained entomologist Luis Leite of the Instituto Biologico, São Paulo, is currently collaborating with project scientists in assessing the potential of *Z. radicans* for leafhopper control in southern Brazil. The project has achieved its goal to promote the professional development of women. One of the project's two Ph.D. students, two of five M.S. students, three of four student interns, and approximately half of the agronomists trained in the short courses are women.

3. Project training to be completed by the end of the project period. Our two remaining CRSP-supported graduate student, U.S. Ph.D. candidate Sandra Galaini-Wraight and Brazilian M.S. candidate Luis Leite will complete their degree requirements in FY 91.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

Our project has been engaged in the current lines of research addressing the objectives outlined in the log frame since the inception of the Bean/Cowpea CRSP in 1982. The project objectives were originally planned to be achievable by the end of the FY 89-91 extension period, and thus approximately one year remains for achievement of the objectives.

It is our judgment that the project is essentially on schedule. The log frame lists four project purposes: (1) Develop microbial agents for control of cowpea pests and assist Brazilian institutions in implementing this technology at the small farm level. A methodology based on soil applications of entomopathogenic hyphomycetes has been developed and is being tested in concert with cultural control measures for low-technology management of *Chalcodermus* weevils in the field, and applications of the same pathogens show promise for control of cowpea storage weevils. A third method employing an entomophthoralean fungus has been developed for control of *Empoasca* leafhoppers. The highly successful FY 90 field trial described in this report indicates that this method may find application in common beans (*Phaseolus*) in southern Brazil. Additional field and laboratory tests and socioeconomic analyses of these technologies are planned for 1991 in collaboration with Brazilian scientists. If the indications from these final CRSP-supported evaluations are positive, we have confidence that the scientists trained by our project possess the expertise and dedication to carry on toward the ultimate goal of incorporating these technologies into the IPM systems that are currently being developed for bean and cowpea culture. (2) Establish a permanent insect pathology laboratory and resource center at CNPAF, and (3) Train Latin American scientists in insect pathology. These goals have been achieved as reported in section I.B. (4) Create a database on cowpea insects and pathogens. Considerable literature is being produced by this project and many talks presented. Its team members are compiling existing information and providing new information. In summary, the goals and timetables of the project are being satisfactorily fulfilled.

To our knowledge, we are the only group in the world working on the microbial control of cowpea pests.

The likely contributions of the lines of research reported here are reductions in cowpea and bean insect pest populations and consequent increases in yield or grain quality. The potential benefits are difficult to quantify at this juncture, because none of the pest control technologies we have developed have yet been implemented at the small-farm level. We have demonstrated that entomopathogenic fungi can significantly reduce pest populations in grain legume cultures. However, these pathogens function in delicate balance with the myriad other biotic and abiotic components of the field environment and cannot alone provide high levels of pest control over all growing seasons. In particular, fungal pathogens may be ineffective under drought conditions. Today, however, especially as environmental and pesticide resistance concerns continue to grow worldwide, the emphasis in applied entomology is toward implementation of multiple technology IPM strategies within sustainable agricultural systems. This trend is evidenced by the 1989 decision by CNPAF/EMBRAPA to reorganize its

entomology department into an IPM unit. In this context, we have developed biological control methods possessing the potential to serve as important components of IPM systems currently being conceived for bean and cowpea pest control in Latin America and elsewhere.

We believe that our project has made a significant contribution to the field of biological crop protection in Brazil. As a direct result of our project, a well trained and highly motivated team of microbial control specialists and technicians has been assembled at CNPAF. These CRSP-trained scientists are now members of a newly established IPM unit with an administrative mandate to develop IPM strategies for beans and cowpeas. Development of biological, environmentally safe methods for control of insect pests of short-cycle field crops like cowpeas is a difficult undertaking that will succeed only through long-term investment in basic and applied research, such as has been made by the Bean Cowpea/CRSP.

D. Evidence of Biological/Social Sciences Integration

(See section I.A.)

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

Our principal collaboration continues to be with the USDA Plant Protection Research Unit located on the Cornell campus. This unit is also studying the potential of *Z. radicans* for control of *Empoasca* leafhoppers. USDA systems ecologists are currently developing simulation models of the *E. fabae-Z. radicans* host-pathogen system, and are utilizing data from our studies in Brazil. Collaboration was also realized in the mass production of *Z. radicans* dry mycelium formulation for field applications in the U.S. against leafhoppers on alfalfa. Scientists from this USDA unit discovered and provided us with the Yugoslavian strain of *Z. radicans* (ARSEF 2282) which was successfully released in São Paulo (Section I.A.1.).

Closer relations with the International Center for Tropical Agriculture (CIAT) were established in FY 90 when HC PI Eliane Quintela was invited to the center for a working visit in which she exchanged ideas with CIAT bean entomologists and studied the isoenzyme analysis techniques used at the center.

Our close collaborations with the Ceara state agricultural research unit (EPACE) and the Instituto Biologico of São Paulo were related in the foregoing discussions of research results.

## II. FUNDING/FISCAL MANAGEMENT

A. Problems Regarding Funding

The project encountered no significant problems in this area during FY 90.

B. Adequacy of Current Management System

In Brazil, the CNPAF business office prepares a bill shortly after the end of each month which is submitted to the HC PI. This document is copiously documented with copies of all receipts. It is examined by the HC PI, returned to the CNPAF business office if corrections are needed, and then transmitted to the U.S. PI who examines the documentation in detail before signing it off for payment by the BTI business office. The latter also examines the bills and its documentation before paying the bill. The time from billing to receipt of payment is usually one month or less. Because of the exceptional staff involved at BTI and in Brazil, and because of the detailed accounting procedures required by BTI management, Bean/Cowpea CRSP funds and commodities have been, and will continue to be, properly utilized for the benefit of the CRSP.

C. Activity Towards Buy-Ins or Other Funding

There have been no possibilities for buy-ins in Brazil because no USAID Mission exists in that country.

III. PROJECT STATUS

A. Appropriateness of Activities to Goals of the Global Plan

Insects are a major constraint to cowpea production worldwide. Our Brazil entomology project is currently the only Bean/Cowpea CRSP project committed to both field and storage insect studies. The major thrust of the project is development of insect disease agents for use in integrated pest management programs, especially in concert with cultural control measures and as alternatives or supplements to synthetic chemical insecticides. Their safety and their low cost, technologically-simple mass production methodologies make insect pathogens very attractive insect control agents for growers with limited resources. Since women actively participate in cowpea and bean production on small farms in Brazil, increased yields and increased production reliability will benefit both men and women. The project, although emphasizing cowpeas, also conducts studies in common beans; thereby increasing the global relevance of the project. Because of long standing interest by Brazilians in using insect pathogens to control some of their major pests, this country is a particularly appropriate setting for our biological control study. We are succeeding in developing a strong core of well trained microbial control specialists and, if we develop reproducible methods to control bean and cowpea pests with microorganisms, other nations with large subsistence farmer populations can call upon this resource in attacking their insect problems.

B. Balance Between Research and Training

1. Expenditures for research and training. The strong emphasis on training that has characterized the project from its inception was continued in FY 90. This training, viewed as essential to institutionalization, has taken the form of (a) annual short courses with approximately 15 students each--many of these being M.S.-level agronomists, (b) intern training of up to one year for

post-baccalaureate students, and (c) formal degree training at U.S. and Brazilian universities (five M.S., two Ph.D.). The training activities have been balanced by a very strong research program. In some cases, the training and research are combined, as in the thesis research of students. During FY 90, expenditures in Brazil were made for the salaries and research of the HC PI and US-RA, research of a collaborating Brazilian economist, technicians' salaries, research of one Ph.D. student, and for the stipends and research of three student interns (two in Goiania and one in Ceara). Expenditures in the U.S. were made for the salary and research of one postdoctoral associate, technicians' salaries, research (supplies) for two visiting Brazilian scientists, stipend for one Ph.D. student, and for the dissertation research (supplies) of a second Ph.D. student (returning HC PI, Bonifacio Magalhaes).

2. Expenditures related to research and training plans. Expenditures in FY 90 were made without major deviation from the FY 89 plan.

C. Balance of Domestic vs. Overseas Activities

Because of the commitment of the Title XII Program to subsistence farmers, our project has been focused on research and training which will aid this part of the world population. Accordingly, the numbers of people supported by the project has been higher in Brazil--where at least 20 million people in the cowpea-consuming region have incomes of less than $500 per year--than in the United States. A significant amount of research has been conducted in the United States, and this work was so designed that it can be related to both third world and U.S. farmers. Over the life of the project, considerably more than one-half of the USAID-provided funds were expended for Brazil.

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

This project has enjoyed a very high level of collaborative effort between Brazilian scientists in many parts of the country, but especially at CNPAF (the HC Institution), and scientists at BTI (the U.S. Institution). This has been especially true in the past five years. An extremely compatible team has developed in which most of the people have been members of our group for three years or longer. Both the Brazilian and U.S. personnel have been involved with all aspects of project planning, budgeting, and training, and have contributed (as principal authors) to the many research papers that have been published by the project. EMBRAPA has expressed, at the highest level, a desire to collaborate fully in our project; and, as far as financially possible, they have held true to this promise.

E. Relative Contributions of Collaborating Institutions and Individuals

The commitment and contributions of each of the sponsoring institutions, viz. EMBRAPA/CNPAF and BTI, have been clearly stated and implemented by administrators, scientists and students in both locations. The collaboration has been such between the institutions that it cannot be fairly stated that the commitment of one has been more than the other. The new chief of CNPAF, Homero Aidar, has been particularly supportive of our collaborative project.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

USAID is represented in Brazil by a single individual stationed at the U.S. Embassy in Brasilia. His specialty is not agriculture, and accordingly, his involvement with the Bean/Cowpea CRSP is not within his area of primary responsibility. Nevertheless, he has been very cooperative.

G. Evidence of Institutionalization

1. Faculty recognition. The U.S. PI, Donald Roberts, was awarded the L.O. Howard Distinguished Achievement Award by the Eastern Branch of the Entomological Society of America in October 1989. The award was made largely on the basis of Dr. Roberts' accomplishments at the international level.

2. Integration with international and domestic commodity research programs. Integration of our research program with that of the USDA Plant Protection Research Unit was discussed in section I.E. The insect pathology laboratory we established in Brazil and its CRSP-trained staff have been fully integrated into the CNPAF Integrated Pest Management Program which is an important arm of the Brazilian national cowpea program. The Brazilian and U.S. Principal Investigators were invited to give presentations at the two international biological control symposia that have been held in Brazil (the 2nd Simposio de Controle Biologico was held in Brasilia during FY 90).

3. Internal project management. Project support from BTI and CNPAF management has been, and continues to be, extremely high.

4. Student/Professor interactions. In our judgment, student-professor interaction has been excellent. The reputation of our project has always been such as to elicit an overwhelming number of applications to our short courses and to our intern and degree-training programs both at CNPAF and BTI. We have, in most instances, been able to select our students from among many well qualified applicants.

IV. PUBLICATIONS AND PRESENTATIONS

U.S. papers and reviews

Allee, L. L., M. S. Goettel, A. Gol'berg, H. S. Whitney and D. W. Roberts. 1990. Infection by *Beauveria bassiana* of *Leptinotarsa decemlineata* Larvae as a Consequence of Fecal Contamination of the Integument Following *per os* Inoculation. Mycopathologia 111: 17-24.

Galaini-Wraight, S., S. Wraight, R. I. Carruthers, B. P. Magalhaes, and D. W. Roberts. Description of a *Zoophthora radicans* (Zygomycetes: Entomophthorales) Epizootic in a Population of *Empoasca kraemeri* (Homoptera: Cicadellidae) on Beans in Central Brazil. J. Invertebr. Pathol., submitted.

Goettel, M.S., R.J. St. Leger, S. Bhairi, M.K. Jung, B.R. Oakley, D.W. Roberts, and R.C. Staples. 1990. Pathogenicity and growth of *Metarhizium anisopliae* stably transformed to benomyl resistance. Curr. Genet. 17: 129-132.

Krueger, S.R., M.G. Villani, J.P. Nyrop, and D.W. Roberts. 1991. Effect of soil environment on the efficacy of fungal pathogens against scarab grubs in laboratory bioassays. Biological Control, submitted.

Krueger, S.R., M.G. Villani, A.S. Martins, and D.W. Roberts. 1991. Efficacy of soil applications of *Metarhizium anisopliae* (Metsch.) Sorokin conidia, and standard and lyophilized mycelial particles against scarab grubs. J. Invertebr. Pathol., submitted.

Roberts, D.W. 1989. World picture of biological control of insects by fungi. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 84, Supp. III: 89-100.

Roberts, D.W., J. Fuxa, R. Gaugler, M. Goettel, R. Jaques, J. Maddox. 1990. Use of pathogens in insect control. In Pimentel, D. (ed.), Handbook of Pest Managemenet in Agriculture. CRC Press, Boca Raton, Florida. (in press).

Wraight, S.P., T.M. Butt, S. Galiani-Wraight, L.L. Allee, R.S. Soper, and D.W. Roberts. 1990. Germination and infection processes of the entomophthoralean fungus *Erynia radicans* on the potato leafhopper, *Empoasca fabae*. J. Invertebr. Pathol. 56: 157-174.

Abstracts and presentations

Krueger, S.R., M.G. Villani, A.S. Martins, and D.W. Roberts. Efficacy of *Metarhizium anisopliae* conidia and dry mycelium in soil against scarabeid larvae. Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia. August 20-24, 1990.

Krueger, S.R. Interaction of scarab grubs, entomopathogenic fungi, and the soil environment. Eastern Branch Meeting of the Entomological Society of America, Baltimore, Maryland. October, 1990.

Roberts, D.W., and A.E. Hajek. Entomopathogenic fungi as bioinsecticides. Entotech Symposium on Industrial Mycology--IInd Annual Meeting of the Mycology Society of America, University of Wisconsin, Wisconsin. June 24-28, 1990.

Roberts, D.W. Practical applications of fungi against scarabs. International Workshop: Pathogens in Scarab Pest Management, Canterbury Agricultural and Science Centre, Lincoln, New Zealand. August 15-17, 1990.

Roberts, D.W. Presidential Address. Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia. August 20-24, 1990.

Roberts, D.W. Metabolite production by entomopathogenic fungi. 2nd Symposium of Biological Control (SICONBIOL), Instituto Israel Pinheiro, Brasilia, Brazil. October 14-18, 1990.

Roberts, D.W. Biotechnology and biological control: Fungi. 2nd Symposium on Biological Control (SICONBIOL), Instituto Israel Pinheiro, Brasilia, Brazil. October 14-18, 1990.

St. Leger, R.J., R.C. Staples, and D.W. Roberts. A model to explain differentiation of appressoria by the entomopathogen, *Metarhizium anisopliae*. Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia. August 20-24, 1990. Poster session.

HC papers and reviews

Magalhaes, B.P., T.M. Butt, R.A. Humber, E.J. Shields, and D.W. Roberts. 1990. Formation of appressoria *in vitro* by the entomopathogenic fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales). J. Invertebr. Pathol. 55: 284-288.

Magalhaes, B.P., R. Wayne, R.A. Humber, E.J. Shields, and D.W. Roberts. 1990. Calcium regulated appressorium formation of the entomopathogenic fungus *Zoophthora radicans*. Protoplasma (in press).

Magalhaes, B.P., R.J. St. Leger, R.A. Humber, L.L. Allee, E.J. Shields, and D.W. Roberts. 1990. Nuclear events during germination and appressorial formation of the entomopathogenic fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales). J. Invertebr. Pathol. (in press).

Magalhaes, B.P., R.A. Humber, E.J. Shields, and D.W. Roberts. 1991. Effects of environment and nutrition on conidial germination and appressorium formation by *Zoophthora radicans* (Zygomycetes: Entomophthorales): A pathogen of the potato leafhopper (Homoptera: Cicadellidae). Environ. Entomol. (in press).

Pereira, R.M., and D.W. Roberts. 1990. Dry mycelium preparations of the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. J. Invertebr. Pathol. 56: 39-46.

Quintela, E.D., J.C. Lord, S.P. Wraight, S.B. Alves, and D.W. Roberts. 1990. Pathogenicity of *Beauveria bassiana* (Hyphomycetes: Moniliales) to larval and adult *Chalcodermus bimaculatus* (Coleoptera: Curculionidae). J. Econ. Entomol. 83: 1276-1279.

Quintela, E.D., J.C. Lord, S.B. Alves, and D.W. Roberts. Persistência de *Beauveria bassiana* em solo do cerrado e sua interaces com microrganismos dosolo. Anais Socied. Entomol. do Brasil (submitted).

Abstracts and presentations

Alves, H.M., S.E.M. Sanchez, and E.D. Quintela. 1990. Inimigos naturais de espécies prejudiciais ao feijo e caupi. III Reunio Nacional de Pesquisa de Feijo, Vitoria, Es. May 13-18. p. 40.

Magalhaes, B.P., R.A. Humber, E.J. Shields, and D.W. Roberts. Effects of environment and nutrition on conidial germination and appressorial formation by *Zoophthora radicans* (Zygomycetes: Entomophthorales): A pathogen of the potato leafhopper (Homoptera: Cicadellidae). Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia. August 20-24, 1990.

Quintela, E.D., S.P. Wraight, S. Galiani-Wraight, and D.W. Roberts. 1990. Patogenicidade de *Beauveria bassiana* e *Metarhizium anisopliae* a *Cerotoma arcuata* Olivier (Coleoptera: Chrysomelidae) e *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae). III Reunio Nacional de Pesquisa de Feijo, Vitoria, Es. May 13-18. p. 39.

Quintela, E.D., and D.W. Roberts. 1990. Controle de *Chalcodermus bimaculatus* (Coleoptera: Curculionidae) no solo com fungos entomopatogênicos. II Simposio de Controle Biologico, Brasilia, D.F. October 14-18. pp. 67-8.

Table 1. Temperature-dependent germination of *Zoophthora radicans* oval primary and secondary conidia on *Empoasca kraemeri*.

| Temperature (°C) | % germination[a] (95% CI) | Time (hr) to germination of 50% of germinable spores (95% CI) | Probit line slope ± SE |
|---|---|---|---|
| 0.5 | 0.0 | ------ | ------ |
| 5 | 81.7 ± 0.85 | 3.4 (3.3-3.6) | 8.5 ± 0.58 |
| 10 | 93.4 ± 0.79 | 3.2 (1.2-5.2) | 4.2 ± 0.93 |
| 15 | 94.4 ± 0.99 | 2.0 (0.8-3.0) | 3.5 ± 0.67 |
| 20 | 91.9 ± 1.93 | 1.1 (0.8-1.3) | 1.7 ± 0.21 |
| 25 | 94.3 ± 2.52 | 1.6 (0.7-2.4) | 1.6 ± 0.19 |
| 28 | 70.5 ± 0.99 | 1.5 (0.2-2.7) | 1.8 ± 0.48 |
| 30 | 41.5 ± 3.66 | 2.9 (1.3-4.0) | 4.0 ± 0.56 |
| 32 | 6.4 ± 1.13 | ------ | ------ |

[a] Total germination observed within 72 hr at 0.5-10°C, 24 hr at 15°C, and 18 hr at 20-32°C.

Table 2. Effect of temperature on the formation of *Zoophthora radicans* appressoria on *Empoasca kraemeri*

| Temperature (°C) | Percent of total inoculum producing appressoria ± SE | Percent of germinated inoculum producing appressoria ± SE | Time (hr) to formation of 50% of appressoria (95% CI)[a] | Probit line slope ± SE |
|---|---|---|---|---|
| 5 | 7.3 ± 0.36 | 9.1 ± 0.52 | 32.8 (18.2-56.4) | 2.4 ± 0.80 |
| 10 | 12.2 ± 1.76 | 12.8 ± 1.92 | 22.3 (12.4-29.9) | 4.8 ± 1.31 |
| 15 | 13.0 ± 4.86 | 13.7 ± 4.99 | 9.0 (7.7-10.5) | 4.4 ± 0.44 |
| 20 | 12.3 ± 0.86 | 13.5 ± 0.90 | 5.7 (3.7-7.4) | 3.7 ± 0.80 |
| 25 | 14.7 ± 1.46 | 15.5 ± 1.55 | 5.4 (4.5-6.3) | 7.5 ± 1.42 |
| 28 | 9.6 ± 1.28 | 14.5 ± 1.97 | 5.5 (4.3–6.7) | 7.0 ± 1.55 |
| 30 | 4.1 ± 0.52 | 10.8 ± 1.51 | 4.9 (4.3-5.3) | 8.8 ± 1.59 |
| 32 | 0.0 | 0.0 | ------ | ------ |

[a] 95% confidence intervals reported for all treatments except 5°C, in which case, the limits presented are at the 90% confidence level

Table 3. Temperature-dependent penetration of *Empoasca kraemeri* by *Zoophthora radicans*

| Temperature (°C) | % of total inoculum producing penetrations ± SE | % of germinated inoculum producing penetration ± SE | % of appressoria producing penetrations ± SE | % of penetrations from oval conidia: capilliconidia (SE) | Time (hr) to formation of 50% of penetrations (95% CI) | Probit line slope ± SE |
|---|---|---|---|---|---|---|
| 5 | 3.1 ± 0.20 | 3.8 ± 0.21 | 42.6 ± 3.32 | 89.5:10.5 (1.02) | 50.0 (39.6-67.0) | 6.9 ± 1.82 |
| 10 | 7.0 ± 0.78 | 7.4 ± 0.82 | 58.1 ± 2.09 | 60.7:39.3 (3.59) | 25.1 (19.2-30.5) | 6.0 ± 1.26 |
| 15 | 8.0 ± 2.65 | 8.4 ± 2.65 | 64.1 ± 4.11 | 72.0:28.0 (10.1) | 13.8 (11.1-19.0) | 5.2 ± 1.05 |
| 20 | 7.3 ± 0.51 | 8.0 ± 0.68 | 59.7 ± 4.66 | 77.9:22.1 (1.35) | 6.6 (4.7-8.3) | 6.3 ± 1.51 |
| 25 | 10.1 ± 2.52 | 10.6 ± 2.66 | 63.6 ± 5.42 | 96.7:3.3 (1.80) | 6.1 (5.8-6.4) | 8.5 ± 0.86 |
| 28 | 6.1 ± 0.72 | 8.1 ± 1.26 | 68.9 ± 6.04 | 98.0:2.0 (2.00) | 6.0 (4.8-7.3) | 9.4 ± 2.16 |
| 30 | 2.3 ± 0.40 | 5.7 ± 1.16 | 55.0 ± 5.85 | 100:0 (-) | 6.6 (5.7-8.6) | 6.1 ± 1.83 |
| 32 | 0.0 | 0.0 | ------- | ------- | ------- | ------- |

Table 4. Temperature-dependent infection of *Empoasca kraemeri* by *Zoophthora radicans*

| Temperature (°C) | Dose-spores per leafhopper ± standard deviation | Total percent infection[a] | Time (hr) to infection of 10% of leafhoppers (95% CI) | Time (hr) to infection of 50% of leafhoppers (95% CI) | Probit line slope ± SE |
|---|---|---|---|---|---|
| 5 | 22.2 ± 6.78 | 42.6 ± 2.14 | 32.5 (22.2-38.3) | ------ | 4.0 ± 0.99 |
| 10 | 20.5 ± 7.17 | 73.9 ± 7.15 | 13.6 (8.9-17.4) | 35.2 (30.1-41.9) | 3.1 ± 0.49 |
| 15 | 25.9 ± 7.78 | 78.2 ± 7.55 | 6.1 (4.7-7.2) | 13.8 (12.3-15.8) | 3.6 ± 0.45 |
| 20 | 20.4 ± 6.69 | 80.2 ± 1.60 | 4.0 (3.1-4.6) | 7.3 (6.7-8.1) | 4.8 ± 0.62 |
| 25 | 22.0 ± 7.02 | 83.8 ± 1.95 | 3.4 (1.5-4.4) | 5.8 (4.6-7.1) | 5.4 ± 0.77 |
| 28 | 21.7 ± 6.85 | 62.8 ± 4.50 | 3.0 (1.1-4.2) | 8.1 (6.4-10.8) | 2.9 ± 0.51 |
| 30 | 20.1 ± 7.36 | 33.3 ± 2.95 | 5.0 (3.3-6.0) | ------ | 3.0 ± 0.74 |
| 32 | 22.3 ± 8.50 | 0.0 | ------ | ------ | ------ |

[a] Percent of leafhoppers with at least one *Z. radicans* penetration observed within 72 hr at 5-10°C, 24 hr at 15°C, and 18 hr at 20-32°C.

Table 5. Influence of temperature on the production of conidia of *Zoophthora radicans* on cadavers of fifth-instar *Empoasca kraemeri*

| Temperature (°C) | Number of cadavers monitored | Total conidia produced (x $10^4$) | Mean time (hr) until production of 2.5, 50, and 97.5% of total conidia following death of leafhoppers[a] | | | Meantime of peak production of conidia[a] |
|---|---|---|---|---|---|---|
| | | | 2.5% | 50% | 97.5% | |
| 5 | 9 | 1.3 | 39.1 | 81.6 | 171.8 | 70.6 |
| 10 | 13 | 1.4 | 26.7 | 55.0 | 116.4 | 43.7 |
| 15 | 15 | 2.5 | 17.9 | 32.4 | 62.4 | 29.0 |
| 20 | 21 | 2.6 | 9.1 | 19.4 | 42.6 | 16.6 |
| 25 | 17 | 2.5 | 9.5 | 18.0 | 34.4 | 16.1 |

[a] Mean of times estimated by log normal curves fitted to the sporulation data from each cadaver.

Table 6. Production of conidia of *Zoophthora radicans* on cadavers of different instars of *Empoasca kraemeri* at 20°C

| Leafhopper instar | Number of cadavers monitored | Total conidia produced (x $10^3$) | Mean time (hr) until production of 2.5, 50, and 97.5% of total conidia following death of leafhoppers[a] | | | Meantime of peak production of conidia[a] |
|---|---|---|---|---|---|---|
| | | | 2.5% | 50% | 97.5% | |
| First | 4 | 2.2 | 6.0 | 15.1 | 41.1 | 11.8 |
| Second | 14 | 3.8 | 6.1 | 15.1 | 40.0 | 11.9 |
| Third | 14 | 7.8 | 6.2 | 16.2 | 47.9 | 12.3 |
| Fourth | 14 | 12.7 | 7.0 | 17.8 | 46.8 | 14.0 |
| Fifth | 21 | 26.4 | 9.1 | 19.4 | 42.6 | 16.6 |
| Adult | 9 | 20.7 | 13.5 | 24.3 | 44.3 | 22.1 |

[a] Mean of times estimated by log normal curves fitted to the sporulation data from each cadaver.

Table 7. Percent infection of *Empoasca kraemeri* in treated and untreated areas of a bean field (*Phaseolus*) in Capão Bonito, São Paulo, following application of dry mycelium of *Zoophthora radicans* on the night of 15 April, 1990.

| | Sample dates | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16 April | 20 April | 23 April | 26 April | 30 April | 4 May | 7 May |
| Mean number of leafhoppers collected per plot ± SD | 7.2 ± 3.5 | 15.7 ± 7.1 | 11.3 ± 6.5 | 11.5 ± 4.5 | 7.8 ± 4.5 | 4.5 ± 3.0 | 2.0 ± 1.8 |
| Treated plots (dose)[a] | | | | | | | |
| 2 g/m² | 0.0 | 29.3 | 53.0 | 18.0 | 67.6 | 4.8 | 0.0 |
| 1 g/m² | 0.0 | 37.6 | 28.0 | 16.5 | 42.1 | 80.0 | 80.0 |
| 0.5 g/m² | 0.0 | 18.0 | 23.3 | 12.5 | 55.0 | 54.2 | 9.5 |
| 0.25 g/m² | 0.0 | 14.4 | 31.7 | 14.9 | 73.7 | 94.4 | 77.8 |
| means | 0.0 | 24.8 | 34.0 | 15.5 | 59.6 | 58.4 | 41.8 |
| Untreated plots[b] (location relative to treatment plots) | | | | | | | |
| 5 m from 2 g/m² plots | 0.0 | 6.3 | 4.5 | 41.9 | 69.4 | 25.0 | 33.3 |
| 5 m from 1 g/m² plots | 0.0 | 6.7 | 39.4 | 41.1 | 78.6 | 61.3 | 37.5 |
| 5 m from 0.5 g/m² plots | 0.0 | 6.3 | 10.5 | 36.8 | 69.5 | 37.5 | 41.7 |
| 5 m from 0.25 g/m² plots | 0.0 | 7.7 | 13.4 | 23.1 | 59.5 | 58.3 | 58.3 |
| means | 0.0 | 6.8 | 17.0 | 35.7 | 69.3 | 45.5 | 42.7 |
| 18 m from 2 g/m² plot | --- | 0.0 | 13.3 | 8.5 | 40.4 | 35.6 | 75.0 |
| 36 m from 2 g/m² plot | --- | 6.7 | 28.3 | 10.3 | 66.7 | 40.5 | 45.0 |
| means | --- | 3.4 | 20.8 | 9.4 | 53.6 | 38.1 | 60.0 |

[a] Samples collected from three 2 x 2 m plots per dose.

[b] Samples collected from two untreated 2 x 2 m plots located approximately 5 m from each group of treatment plots and from three untreated plots located 18 m and three located 36 m from the 2 g/m² treatment plots.

Table 8. Number of cowpea seeds damaged by *Callosobruchus maculatus* following treatment of the seeds with *Beauveria bassiai* *Metarhizium anisopliae* and Vertimec, and storage for 112 and 137 days.

| Dose g/60 kg Treatment[a] | After 112 days of storage: Number of seeds of seeds | Number of perforated sampled | Number of holes seeds | Number of seeds total | After 137 days of storage: Number of perforated sampled | Number of holes seeds | total |
|---|---|---|---|---|---|---|---|
| *M. anisopliae* | 100 | 200 | 6.0 | 7.0 | 100 | 3.7 | 4.5 |
| 400 | 200 | 2.0 | 3.0 | 100 | 4.0 | 4.0 | |
| *B. bassiana* | 100 | 200 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Avermectin | 60 | 200 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| 120 | 200 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | |
| Control | 0 | 200 | 200.0 | 562.0 | 100 | 100.0 | 462.0 |

[a] Treatments conducted in 24 cm high x 13 cm diameter containers with 1000 g of cowpea seeds naturally infested.

Table 9. Growth of *Beauveria bassiana* or *Metarhizium anisopliae* on potato dextrose agar treated with two concentrations each of eight insecticides after 12 days.

| Insecticide | Dose[a] | Colony diameter (cm)[b]: *B. bassiana* | % Inhibition | *M. anisopliae* | % Inhibition |
|---|---|---|---|---|---|
| Cypermethrin | 1 | 1.8 | 51.0 | 2.0 | 44.4 |
| | 2 | 1.8 | 51.0 | 2.1 | 41.7 |
| Deltametrin | 1 | 2.9 | 21.6 | 2.8 | 22.2 |
| | 2 | 3.5 | 5.4 | 3.3 | 8.3 |
| Diflubenzuron | 1 | 3.6 | 2.7 | 3.0 | 16.7 |
| | 2 | 3.3 | 10.8 | 3.4 | 5.5 |
| Trichlorfon | 1 | 3.5 | 5.4 | 2.8 | 22.2 |
| | 2 | 2.9 | 21.6 | 2.2 | 39.0 |
| Methyl Parathion | 1 | 0.0 | 100.0 | 0.0 | 100.0 |
| | 2 | 0.3 | 92.0 | 0.0 | 100.0 |
| Methyl Dimeton | 1 | 1.7 | 54.0 | 2.0 | 44.4 |
| | 2 | 1.7 | 54.0 | 2.1 | 41.7 |
| Fenvaralate | 1 | 1.7 | 54.0 | 1.5 | 58.3 |
| | 2 | 1.9 | 48.6 | 1.5 | 58.3 |
| Endosulfan | 1 | 0.7 | 81.1 | 0.2 | 94.4 |
| | 2 | 1.0 | 73.0 | 0.8 | 77.8 |
| Control | — | 3.7 | 0.0 | 3.6 | 0.0 |

[a] Dose 1: the lowest dose recommended of the commercial product at the concentration attained when prepared for spraying at 200 liters of water/ha. Dose 2: half of the lowest dose recommended.

[b] Average of ten colonies.

Table 10. Number of conidia of *Beauveria bassiana* or *Metarhizium anisopliae* produced on potato dextrose agar treated with two concentration each of eight insecticides after 12 days.

| Insecticide | Dose[a] | Number of conidia[b] *B. bassiana* x $10^8$ | % Produced | *M. anisopliae* x $10^8$ | % Produced |
|---|---|---|---|---|---|
| Cypermethrin | 1 | 86.0 | 110.0 | 4.4 | 183.0 |
| | 2 | 49.0 | 62.8 | 1.5 | 62.5 |
| Deltametrin | 1 | 43.0 | 55.1 | 4.8 | 200.0 |
| | 2 | 47.0 | 60.2 | 6.8 | 283.3 |
| Diflubenzuron | 1 | 42.0 | 53.8 | 4.5 | 187.5 |
| | 2 | 63.0 | 80.8 | 1.7 | 70.8 |
| Trichlorfon | 1 | 6.5 | 8.3 | 0.3 | 12.5 |
| | 2 | 1.9 | 2.4 | 0.07 | 2.9 |
| Methyl Parathion | 1 | 0.0 | 0.0 | 0 | 0.0 |
| | 2 | 0.15 | 0.19 | 0 | 0.0 |
| Methyl Dimeton | 1 | 65.0 | 83 | 1.0 | 41.7 |
| | 2 | 45.0 | 57.7 | 0.6 | 25.0 |
| Fenvaralate | 1 | 72.0 | 92.3 | 1.6 | 66.7 |
| | 2 | 58.0 | 74.3 | 2.0 | 83.3 |
| Endosulfan | 1 | 0.12 | 0.15 | 1.4 | 58.3 |
| | 2 | 0.4 | 0.51 | 0.9 | 37.5 |
| Control | --- | 78.0 | 100.0 | 2.4 | 100.0 |

[a] Dose 1: the lowest dose recommended of the commercial product to 200 liters of water/ha. Dose 2: half of the lowest dose recommended.

[b] Average of ten colonies. Sampled by cutting a disc of the colony with a cork borer.

Table 11. Percent mortality of *Cerotoma arcuata* adults and *Chalcodermus bimaculatus* larvae spray-inoculated with *Beauveria bassiana* and *Metarhizium anisopliae* respectively, produced on potato detrose agar treated with two concentrations each of eight insecticides.

| Insecticide | Dose[a] | % Mortality of *Cerotoma* adults by *B. bassiana*[b] | % Mortality of *Chalcodermus* larvae by *M. anisopliae*[b] |
|---|---|---|---|
| Cypermethrin | 1 | 47.5 | 59.4 |
| | 2 | 57.5 | 36.8 |
| Deltamethrin | 1 | 62.5 | 73.7 |
| | 2 | 62.5 | 65.0 |
| Diflubenzuron | 1 | 40.0 | 92.1 |
| | 2 | 67.5 | 40.5 |
| Trichlorfon | 1 | 40.0 | 60.5 |
| | 2 | 47.5 | 94.6 |
| Methyl Parathion | 1 | --- | ---NG |
| | 2 | --- | ---NG |
| Methyl Dimeton | 1 | 27.6 | 28.2 |
| | 2 | 52.5 | 79.5 |
| Fenvaralate | 1 | 62.5 | 50.0 |
| | 2 | 60.0 | 56.4 |
| Endosulfan | 1 | 52.5 | 44.7 |
| | 2 | --- | ---NR |
| Control (with fungi) | | 67.5 | 47.4 |
| Control (no fungi) | | 2.5 | 13.5 |

[a] Dose 1: the lowest dose recommended of the commercial product to 200 liters of water/ha. Dose 2: half of the lowest dose recommended.

[b] Values are means determined for four replications of 10 larvae. Spray-inoculated at a dosage of $10^7$ conidia/ml.

Table 12. Effect of the fungus *Beauveria bassiana* isolate #280 on *Anthonomus grandis* in field conditions. Maracanaú, Ceará, Brazil, 1990.

| Treatments | Kg a.i./ha[1] G.C./ha[2] | % SA[3] PT[4] | 7DA2S[5] | 4DA4S | Efficiency[6] 7DA2S | 4DA4S |
|---|---|---|---|---|---|---|
| Control | 0 | 0 | 22 | 43 | - | - |
| Deltamethrin + *B. bassiana* | 1.0 kg + 352.5 g | 7 | 19 | 18 | 13.6 | 58.1 |
| Deltamethrin | 10.0 kg | 17 | 4 | 17 | 81.8 | 60.5 |
| Cypermethrin | 8.3 kg | 15 | 22 | 51 | 0.0 | 0.0 |
| *B. bassiana* | 352.5 g | 14 | 40 | 40 | 9.0 | 7.0 |

D.A.E. Phenolology[7] 1st flower = 43

D.A.E. sprays = 50, 54, 61, and 65.

[1] Kg of active ingredient per hectare

[2] Grams of conidia per hectare

[3] Percentage floral squares attacked by boll weevil

[4] Counts before spraying

[5] Seven days after the second spraying

[6] Corrected mortality by Abbott's formula

[7] Days after emergence of plants

Table 13. Pathogenicity of the five strains of the fungus *Verticillium lecanii* to the whitefly, *Bemisia tabaci*.

| Strain | Number of insects tested | Percent mortality Days after treatment[a] 3 | 6 |
|---|---|---|---|
| VL-3 | 40 | 7.5 | 20.0 |
| VL-4 | 28 | 17.8 | 28.6 |
| VL-5 | 34 | 26.5 | 50.0 |
| VL-6 | 45 | 28.9 | 73.3 |
| VL-7 | 43 | 23.3 | 55.8 |
| Control | 38 | 0.0 | 13.1 |

[a] Test insects exposed to bean leaves spray-inoculated with aqueous suspensions of spores. The inocula of each isolate contained $10^6$ conidia/ml except VL-3 which contained $10^7$ conidia/ml.

Table 14. The effect of nitrogen sources on formation of germ tubes and appressoria (percentage; mean ± SD)[1] of Z. radicans 24 hr postinoculation in the dark at 25°C.

| Nitrogen source (0.3%)[2] | Conidia forming germ tubes | Germlings forming appressoria |
|---|---|---|
| Nitrate | 13.8 ± 1.2 a | 0.0 |
| Urea | 16.7 ± 7.0 a | 0.0 |
| L-Glutamic acid | 16.9 ± 3.6 a | 0.0 |
| L-Phenylalanine | 24.8 ± 0.2 a b | 0.0 |
| Ammonium sulfate | 24.9 ± 7.6 a b c | 0.0 |
| DL-Alanine | 36.9 ± 1.3 b c | 0.0 |
| L-Methionine | 36.9 ± 13.2 b c | 0.0 |
| L-Asparagine | 43.7 ± 7.6 c | 0.0 |
| Ammonium chloride | 72.0 ± 8.4 d | 0.0 |
| Casein | 75.3 ± 0.8 d e | 6.5 ± 2.0 a |
| Soytone | 77.6 ± 4.3 d e | 29.8 ± 6.1 b |
| Lactalbumin hydrolysate | 80.8 ± 9.7 d e | 16.9 ± 5.9 b |
| Water | 88.0 ± 2.5 e f | 0.0 |
| Control (1% YE) | 95.5 ± 1.3 f | 54.0 ± 3.9 c |

[1]Means within columns followed by the same letter are not significantly different by Tukey's test ($\alpha = 0.05$) using the transformation arc sine (square root of %). From 3 replicates of 100 conidia or germlings each.

[2]In a basal solution containing (w/v) 0.1% $K_2HPO_4$, 0.05% $MgSO_4.7H_2O$, and 1% glucose.

Table 15. Effect of carbon sources on formation of germ tubes and appressoria (percentage; mean ± SD)[1] of Z. radicans 24 hr postinoculation in the dark 25°C.

| Carbon source (1%)[2] | Conidia forming germ tubes | Germlings forming appressoria |
|---|---|---|
| Glycerol | 35.4 ± 0.7 a | 11.2 ± 2.3 a b c d |
| Ethyl alcohol | 43.4 ± 2.2 a b | 6.4 ± 2.0 a b |
| Lactose | 46.3 ± 4.1 a b | 2.0 ± 1.8 a |
| Sorbitol | 62.7 ± 17.2 b c | 8.0 ± 3.0 b c d e |
| Mannitol | 72.5 ± 2.5 c d | 18.9 ± 3.1 b c d e |
| Cellobiose | 75.8 ± 2.1 c d | 14.6 ± 6.9 a b c d e |
| Starch | 76.4 ± 5.4 c d | 33.5 ± 14.9 d e f |
| Glucose | 78.9 ± 6.6 c d | 36.9 ± 10.9 e f |
| Sucrose | 81.9 ± 5.6 d e f | 16.7 ± 8.4 b c d e |
| Trehalose | 88.8 ± 2.0 e f | 10.9 ± 4.3 a b c |
| Maltose | 88.9 ± 3.4 e f | 34.5 ± 10.5 e f |
| Fructose | 93.8 ± 2.5 f | 26.1 ± 14.6 c d e |
| Control (1% YE) | 88.3 ± 3.2 e f | 52.4 ± 2.8 f |

[1]Means within columns followed by the same letter are not significantly different by Tukey's test ($\alpha = 0.05$) using the transformation arc sine (square root of %). From 3 replicates of 100 conidia or germlings each.

[2]In a basal solution containing (w/v) 0.1% $K_2HPO_4$, 0.05 % $MgSO_4.7H_2O$, and 0.3% Soytone.

Table 16. The effect of $Ca^{2+}$ on nuclear division, hyphal length and hyphal width of Z. radicans 24 hr after inoculation[1]

| $[Ca^{2+}]$ | Nuclear division (%)[2] | Hyphal length ($m \times 10^{-4}$) | Hyphal width ($m \times 10^{-4}$) | Hyphal volume[3] ($m^3 \times 10^{-10}$) |
|---|---|---|---|---|
| pCa 10 (1 pM) | 0.5±0.9a | 101.71±23.78a | 5.83±1.1a | 2,710 |
| pCa 5 (10μM) | 34.9±4.2 b | 239.71±87.5 b | 6.93±1.5a | 9,040[4] |
| pCa 3 (1000 μM) | 10.4±2.5 c | 230.36±88.8 b | 6.20±1.3a | 6,950[4] |

[1]Numbers indicate mean ± SD. Means within columns followed by the same letter are not significantly different by orthogonal comparisons ($\alpha = 0.05$; $n = 300$).

[2]Percent data transformed to arc sine (square root of %) for analysis and retranslated.

[3]Assuming hyphae are cylindrical.

[4]Due to the spatulate form of the appressoria these values are underestimates.

Table 17. Isolates of entomopathogenic fungi added to the IPRC/Brazil culture collection during FY 1990.

CP 285 - *Metarhizium anisopliae*
Acc: 89-11-9
Date: 9 November 1989
Site: CNPAF, Goiânia, Goiás
Host: Queen Isoptera (termite)
Coll: Cecília Czepak

Cl 286 - *Verticillium lecanii*
Acc: VL-1
Date: 1990
Site: Colômbia
Host: *Erinnyis ello* - Yuca
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 287 - *Verticillium lecanii*
Acc: VL-2
Date: 1990
Site: England
Host: *Trialeurodes vaporariorum*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 288 - *Verticillium lecanii*
Acc: VL-3
Date: 1990
Site: Colômbia
Host: *Trialeurodes vaporariorum*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 289 - *Verticillium lecanii*
Acc: VL-4
Date: 1990
Site: Colômbia
Host: *Trialeurodes vaporariorum*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 290 - *Verticillium lecanii*
Acc: VL-5
Date: 1990
Site: Colômbia
Host: *Trialeurodes vaporariorum*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 291 - *Verticillium lecanii*
Acc: VL-6
Date: 1990
Site: Colômbia
Host: *Spodoptera frugiperda*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 292 - *Verticillium lecanii*
Acc: VL-7
Date: 1990
Site: Colômbia
Host: *Mysus persicae* Crisantemo
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 293 - *Beauveria bassiana*
Acc: VL-8
Date: 1990
Site: Colômbia
Host: *Diaspididae* (Scale)
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

CP 294 - *Beauveria bassiana*
Acc: VL-9
Date: 1990
Site: Colômbia
Host: *Saissetia caffeae*
Coll: Alex E.B. Pardey - E.E. Túlio Ospica, ICA

Table 18. Cowpea production, area, and yields by farm size (ha) (on average) by stratum - Ceará, July, 1990.

| Strata (in ha) | No. | Average production in stratum (kg) | Average cowpea area in stratum (ha) | Average yields in stratum (kg/ha) |
|---|---|---|---|---|
| I (<50 ha) | 34 | 1063.9 | 1.9 | 582.7 |
| II (6-10 ha) | 15 | 1678.9 | 3.2 | 557.1 |
| III (11-50 ha) | 26 | 1185.2 | 3.6 | 390.9 |
| IV (51-100 ha) | 5 | 3196.0 | 6.3 | 479.6 |
| V (101-500) | 2 | 3805.0 | 15.0 | 661.0 |

Table 19. Mean productivity under various conditions of technology use in culture of cowpea in the state of Ceará.

| Method | Yes | | No | | Relative Gain (%) |
|---|---|---|---|---|---|
| | No. | Mean (kg/ha) | No. | Mean (kg/ha) | |
| Mixed cropping | 16 | 325 | 62 | 548 | 68.6 |
| Irrigation | 36 | 734 | 42 | 303 | 142.2 |
| Pesticide spraying | 52 | 598 | 26 | 309 | 93.5 |
| Mechanized soil preparation | 45 | 635 | 33 | 321 | 97.8 |
| Fertilizer | 27 | 766 | 55 | 401 | 90.8 |

Table 20. Average variable cost of production using different technologies in culture of cowpea in the state of Ceará.

| Method | Cost of labor | | | Cost of other factors | | | Total cost | | |
|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | % | Yes | No | % | Yes | No | % |
| Mixed cropping | 9382 | 6885 | 36.3 | 3653 | 1665 | 19.4 | 13034 | 8550 | 52.4 |
| Irrigation | 10287 | 7571 | 35.9 | 5413 | 1252 | 33.2 | 15701 | 8823 | 77.9 |
| Pesticide spraying | 9704 | 7067 | 37.9 | 4105 | 1308 | 21.4 | 13808 | 8375 | 64.9 |
| Mechanized soil preparation | 9633 | 7722 | 24.7 | 4687 | 1107 | 23.4 | 14321 | 8830 | 62.2 |
| Fertilizer | 9988 | 8673 | 15.2 | 7094 | 2661 | 16.7 | 17082 | 11334 | 50.7 |

Table 21. Median variable costs of production per hectare of cowpea (*Vigna unguiculata*) on farms of different sizes.

| Size class | No. of farms | % | Cost of labor (A) (per ha) | Other cost factors (B) | Total cost (A+B) Cr$ | US$ |
|---|---|---|---|---|---|---|
| I (<50 ha) | 33 | 42.3 | 9012 | 3719 | 12731 | 153.40 |
| II (6-10 ha) | 15 | 19.2 | 7415 | 3873 | 12287 | 136.00 |
| III (11-50 ha) | 25 | 32.0 | 9062 | 1929 | 10991 | 132.42 |
| IV (51-100 ha) | 5 | 6.4 | 10630 | 3693 | 14323 | 172.57 |
| Total or avg. | 78 | 99.9 | 8825 | 3173 | 11998 | 144.55 |

Table 22. Matrix of correlations (standard error) of economic variables associated with cowpea production.

| | Production returns | Yield from irrigation | Cost of spraying pesticides | Cost of fertilizing | Cost of preparing soil | Cost without labor | Cost of labor | Total costs |
|---|---|---|---|---|---|---|---|---|
| Production returns | 1.00 | 0.52 (0.0001) | 0.267 (0.055) | 0.907 (0.0007) | 0.400 (0.007) | 0.664 (0.0001) | 0.325 (0.004) | 0.551 (0.0001) |
| Yield from irrigation | | 1.0 | 0.099 (0.48) | 0.508 (0.16) | 0.411 (0.005) | 0.757 (0.0001) | 0.255 (0.024) | 0.534 (0.0001) |
| Cost of spraying pesticides | | | 1.0 | 0.863 (0.006) | 0.338 (0.038) | 0.398 (0.0035) | 0.341 (0.013) | 0.459 (0.0006) |
| Cost of fertilizing | | | | 1.0 | 0.706 (0.05) | 0.847 (0.004) | -0.43 (0.244) | 0.511 (0.16) |
| Cost of soil preparation | | | | | 1.0 | 0.742 (0.0001) | -0.12 (0.436) | 0.172 (0.265) |
| Cost without labor | | | | | | 1.0 | 0.195 (0.09) | 0.587 (0.001) |
| Cost of labor | | | | | | | 1.0 | 0.908 (0.0001) |
| Total costs (labor + machinery) | | | | | | | | 1.0 |

Table 23. Productivity (kg/ha) of different cultivars of cowpea in Ceará.

| Name of cultivar | No. of fields | Productivity (kg/ha) |
|---|---|---|
| BR-1 Poty[a] | 33 | 623.4 |
| Coruja[b] | 30 | 464.0 |
| EPACE[b] | 11 | 466.0 |
| Vinagre[b] | 2 | 838.0 |
| Barrigudo[b] | 2 | 234.2 |
| Canapum[b] | 3 | 214.2 |
| Quarentenha[b] | 2 | 443.3 |
| Pitiuba[a] | 12 | 573.3 |
| Casca Puba[b] | 5 | 234.3 |
| Sempre Verde[a] | 1 | 800.0 |
| Carioca (*P. vulgaris*)[a] | 2 | 1086.7 |

[a] Breeder's line

[b] Native line

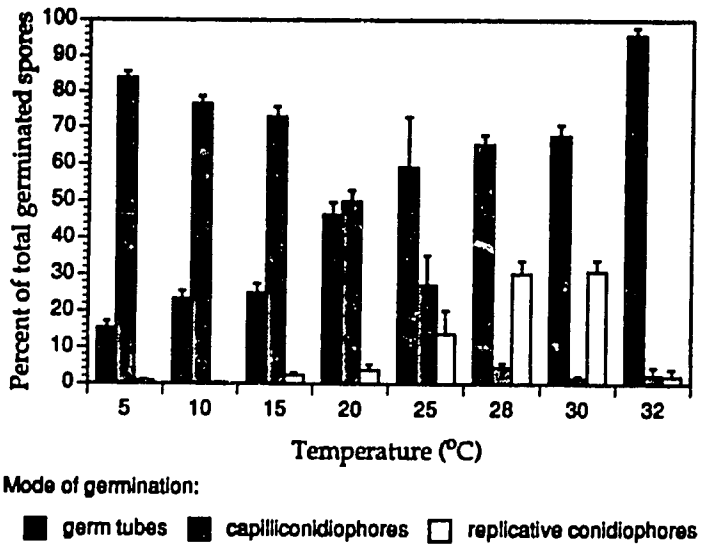



Fig. 1. Influence of temperature on the mode of germination of oval primary and secondary spores of *Zoophthora radicans* on *Empoasca kraemeri*.

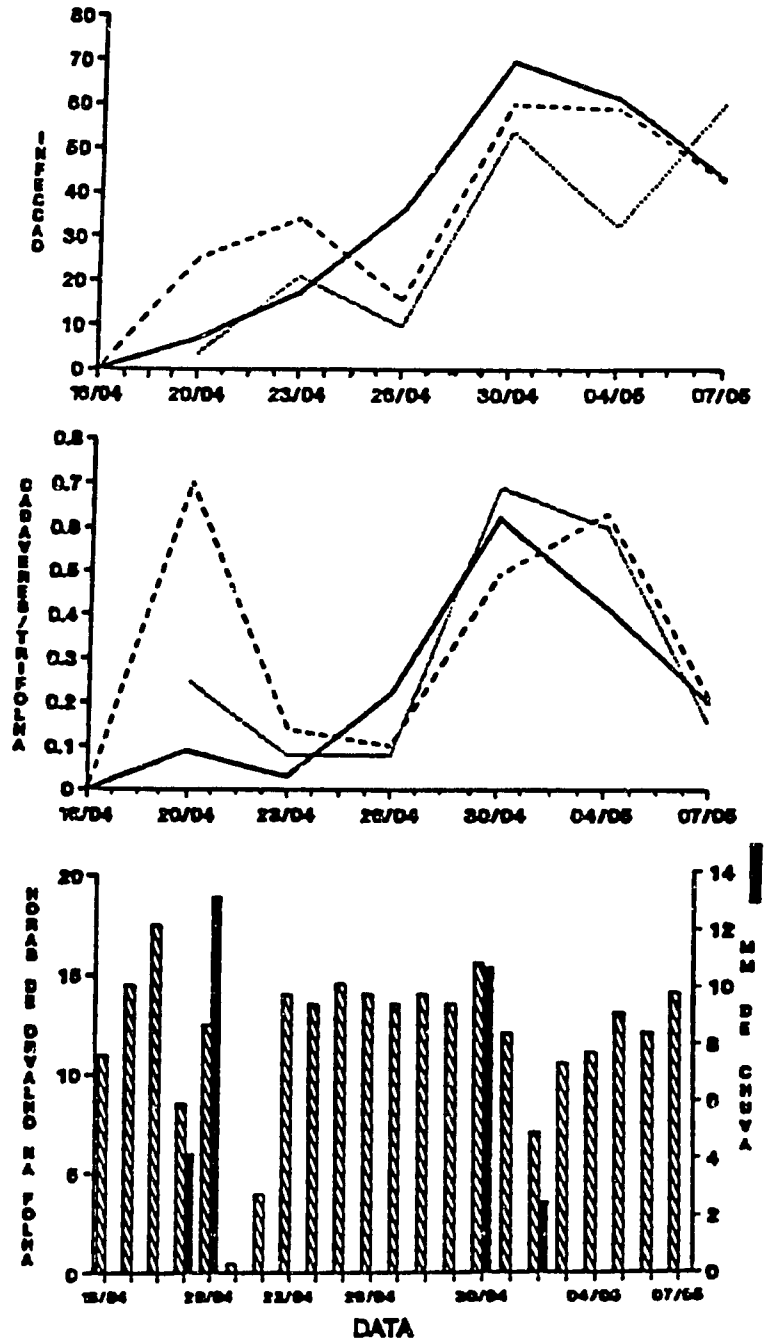



Fig. 2. Percent infection of *Empoasca kraemeri* by *Zoophthora radicans* following applications of dry mycelium in a bean field in Sao Paulo, Brazil with associated inoculum and environmental moisture data. Mean percent infection is reported for fungus-treated plots (---), untreated plots located approximately 5 m from the treated plots (—), and untreated plots located 18 and 36 m from the treated plots (·····). Inoculum levels are expressed as the number of leafhopper cadavers supporting active (sporulating) fungus per trifoliate and moisture is recorded as the number of hours during which foliage was wet each night and and daily rainfall in mm.

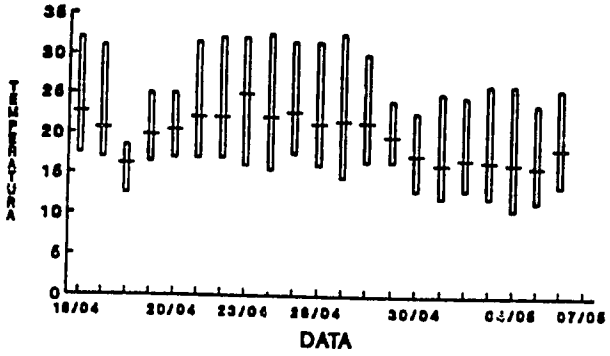



Fig. 3. Temperature expressed as the daily maximum, minimum, and hourly mean recorded within the canopy of a bean field in Sao Paulo, Brazil following applications of *Zoophthora radicans* for leafhopper control.

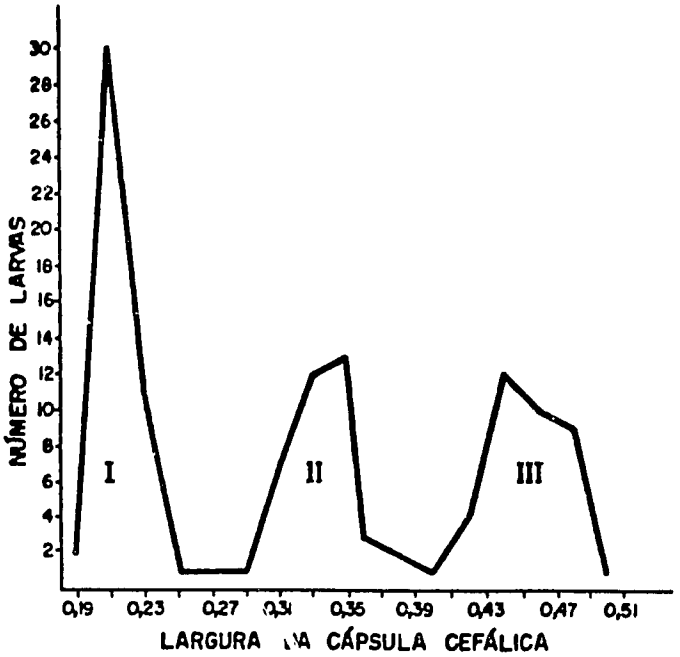



Fig. 5. Curva de distribuição de frequências de larguras de Cápsulas Cefálicas de C. arcuata em Caupi (Vigna unguiculata).

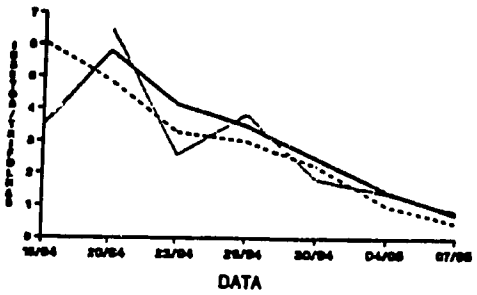



Fig. 4. *Empoasca kraemeri* population densities in a bean field in Sao Paulo, Brazil following applications of *Zoophthora radicans*. Numbers of leafhoppers per trifoliate are reported for fungus-treated plots (---), untreated plots located approximately 5 m from the treated plots (—), and untreated plots located 18 and 36 m from the treated plots (·····).

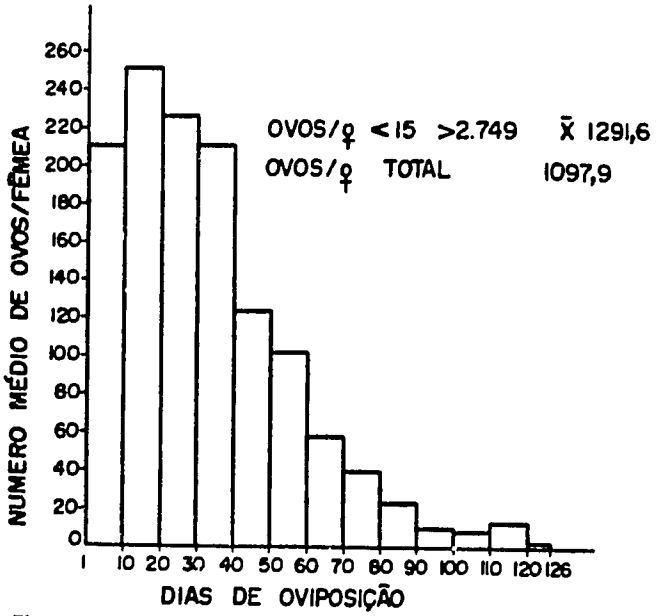



Fig. 6. Número médio de ovos por fêmea de C. arcuata a intervalos de 10 dias.

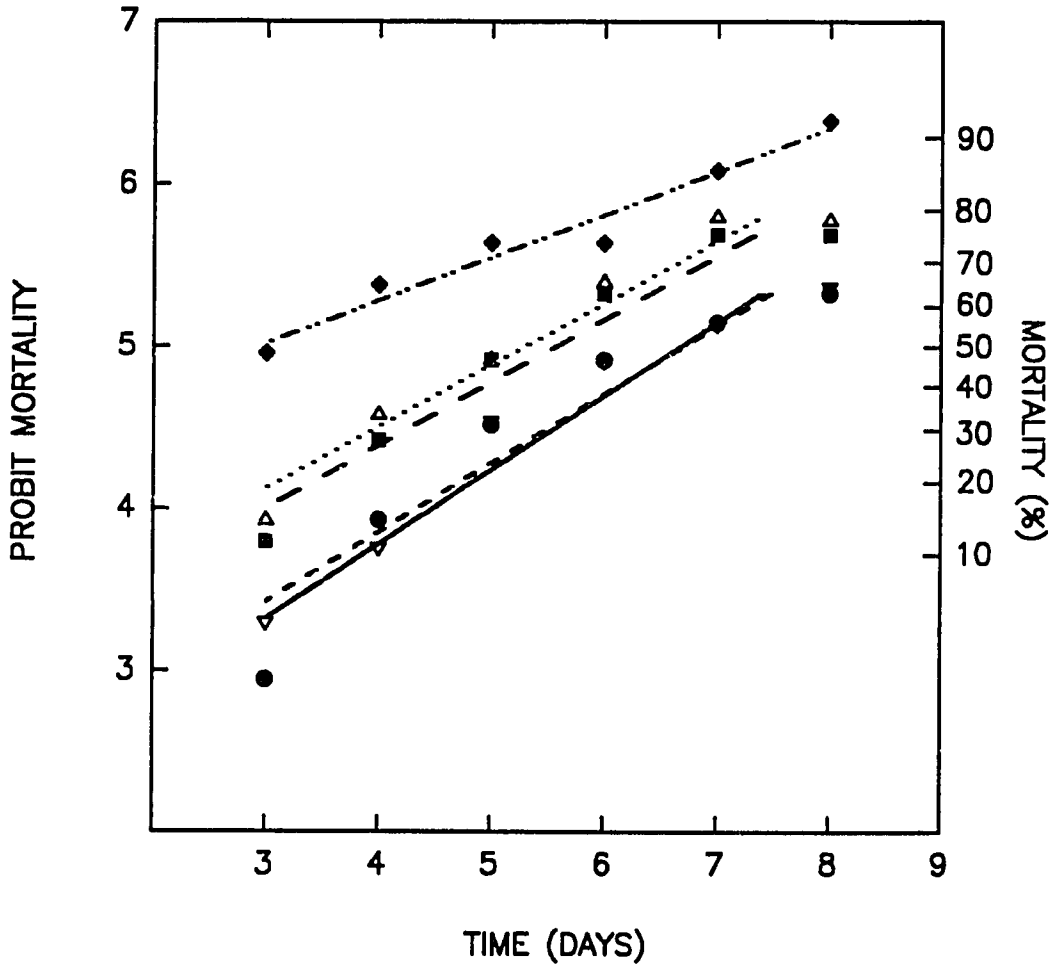



Figure 7. Bioassay of *Beauveria bassiana* (ARSEF 731) conidia against second instar *Diabrotica undecimpunctata* larvae.

Symbol and line legend:

● ——— 2-4 x $10^3$ conidia/$mm^2$; ▽ - - - 4-6 x $10^3$ conidia/$mm^2$;
■ — — 6-8 x $10^3$ conidia/$mm^2$; △ · · · 8-10 x $10^3$ conidia/$mm^2$;
◆ —··— 10-12 x $10^3$ conidia/$mm^2$

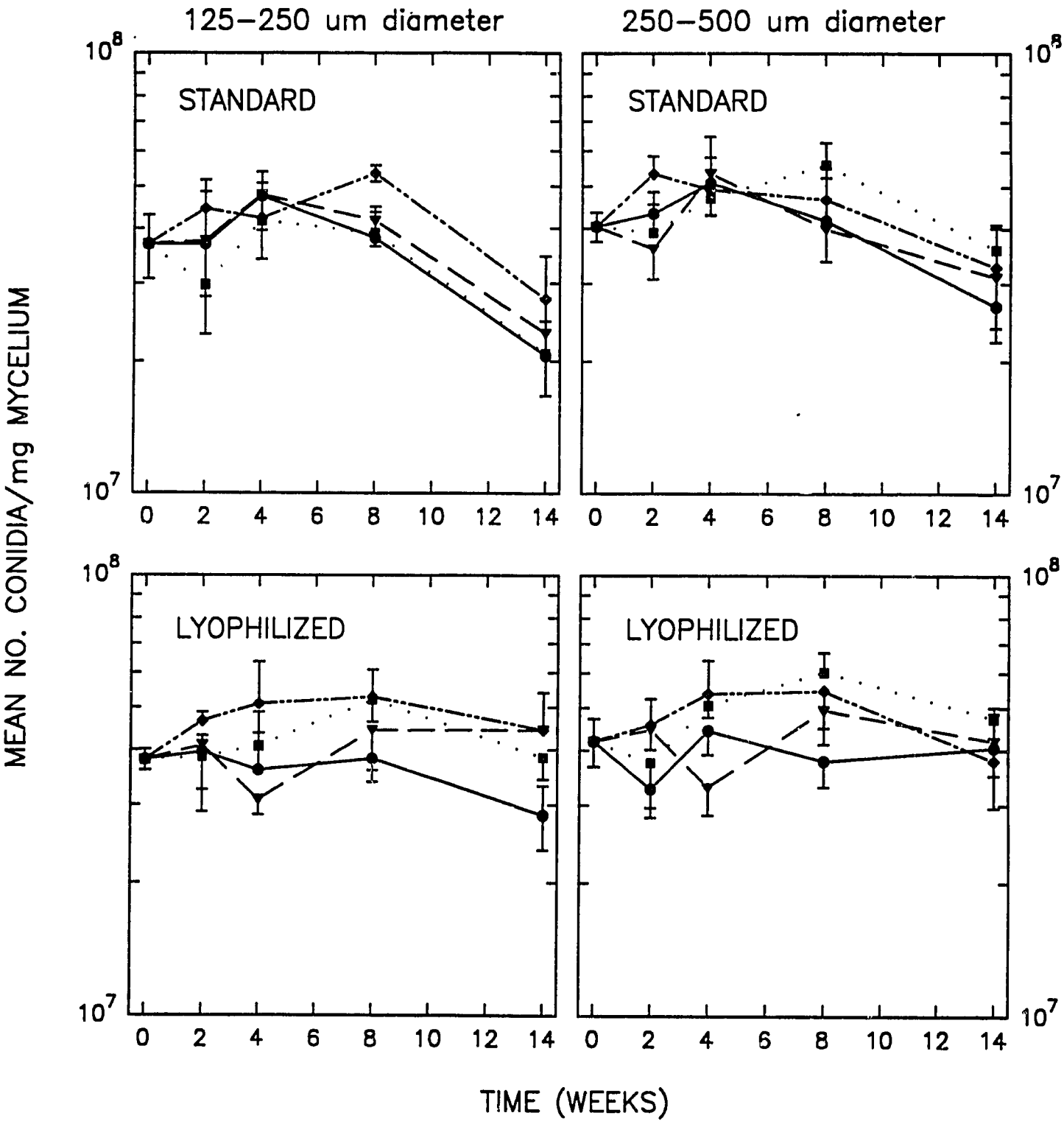



Figure 8. Conidia production from two sizes of *B. bassiana* (ARSEF 731) standard and lyophilized mycelium particles stored at different temperatures.

Symbol and line legend:

● ——— 4°C; ▼ - - - 11°C; ■ ····· 21°C; ◆ —··— 30°C

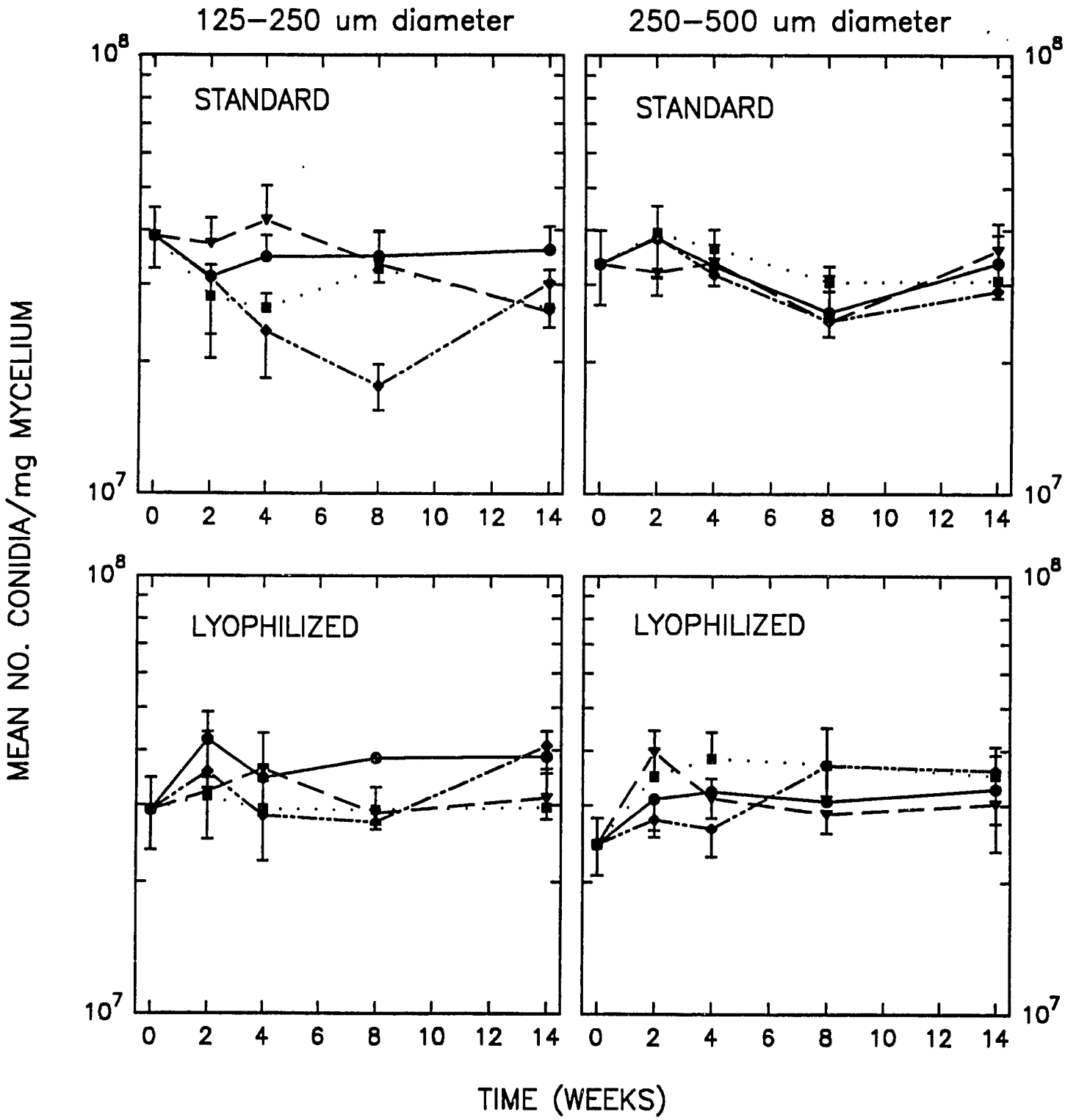



Figure 9. Conidia production from two sizes of *M. anisopliae* (ARSEF 925) standard and lyophilized mycelium particles stored at different temperatures.

Symbol and line legend:

● ——— 4°C; ▼ - - - 11°C; ■ ····· 21°C; ◆ —··— 30°C

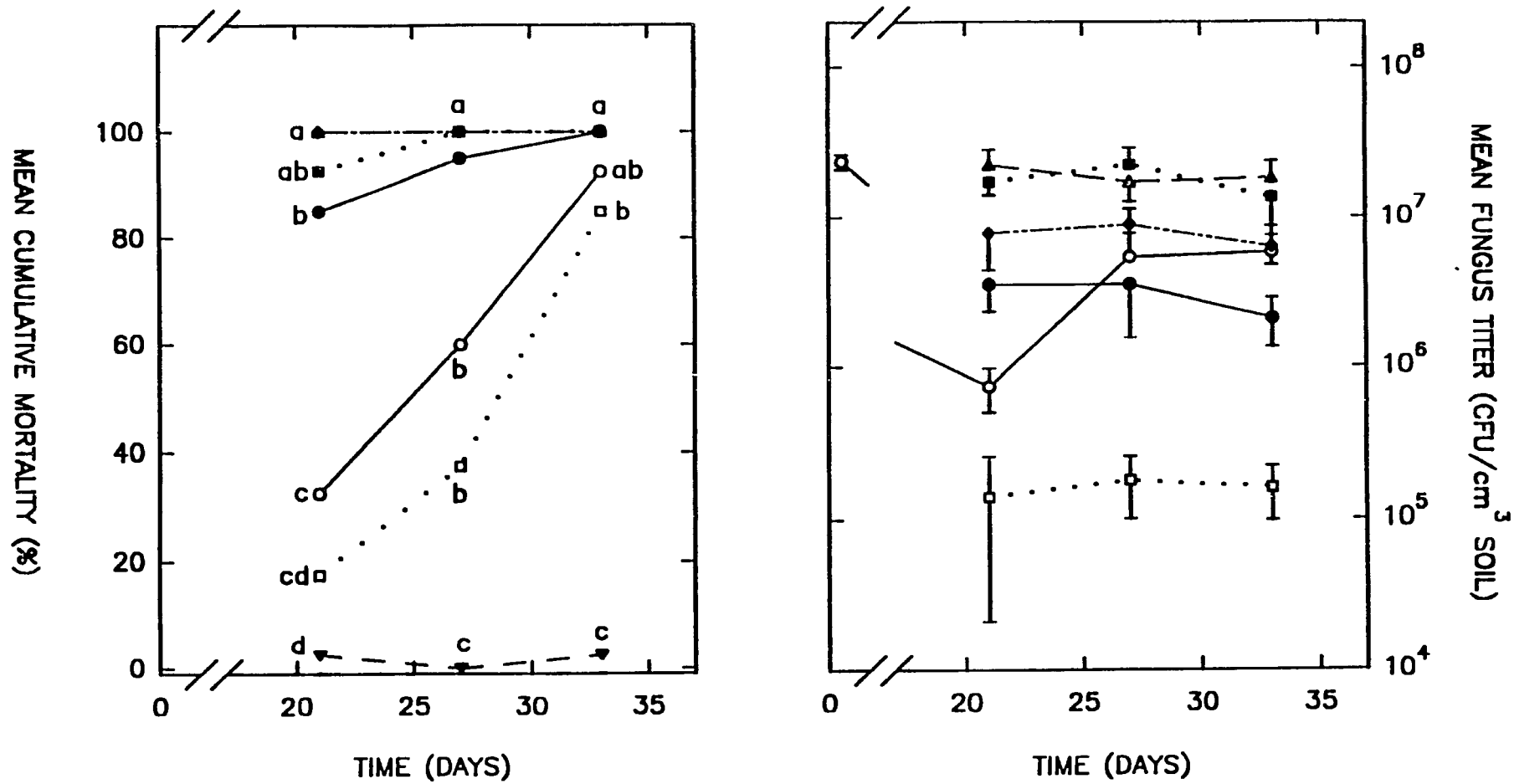



Figure 10. Bioassay number 1: Mean Japanese beetle mortality and mean *M. anisopliae* soil titer. Means within each sample date with the same letter are not significantly different (P<0.05; LSMEANS Procedure, [SAS, 1985]). Error bars denote standard deviation.

Symbol and line legend:

▼ - - - control; ○ ——— conidia; □ ····· S<125; ● ——— S125;
■ ····· S250; ◆ —··— L125; ▲ - - - L250

Mortality in the L125 and L250 treatments was 100% for all sample dates.

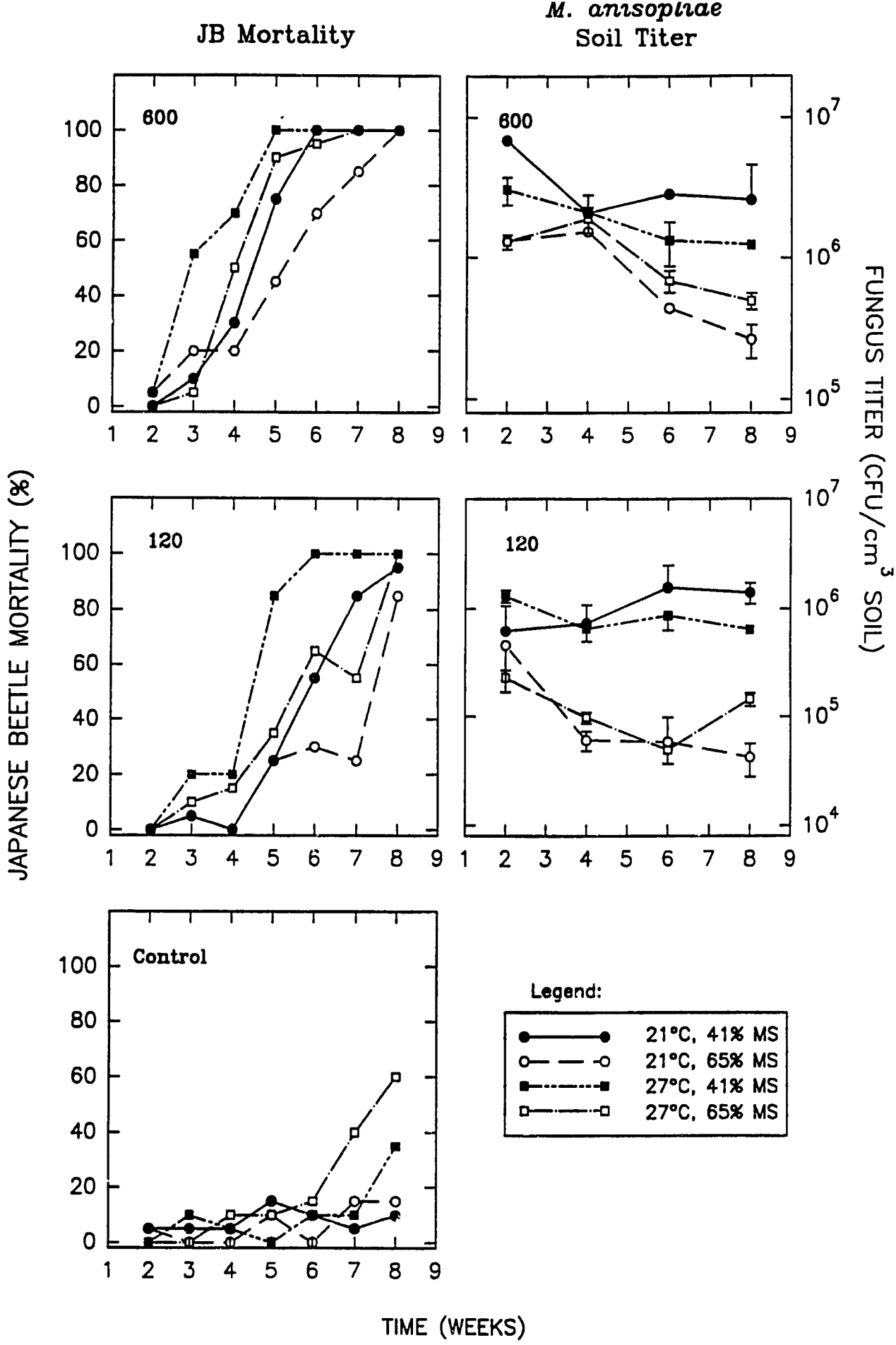



Figure 11. Effect of soil environment on mean Japanese beetle grub mortality and mean *M. anisopliae* titer in soil that was inoculated with either 600 or 120 PCM plus an uninoculated control soil. Error bars denote standard error of the mean.

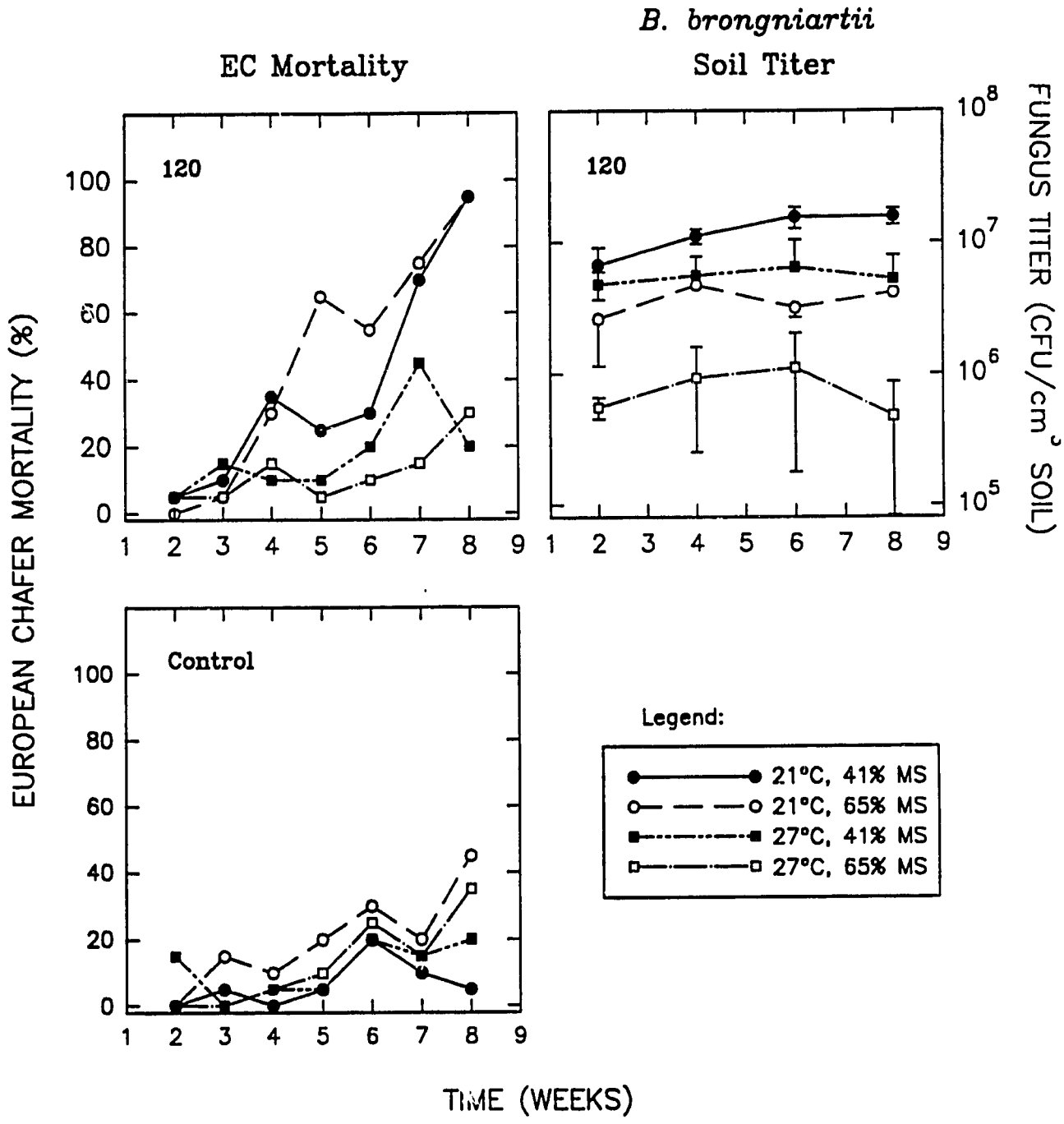



Figure 12. Effect of soil environment on mean European chafer grub mortality and mean *B. brongniartii* titer in soil that was inoculated with 120 PCM plus an uninoculated control soil. Error bars denote standard error of the mean.

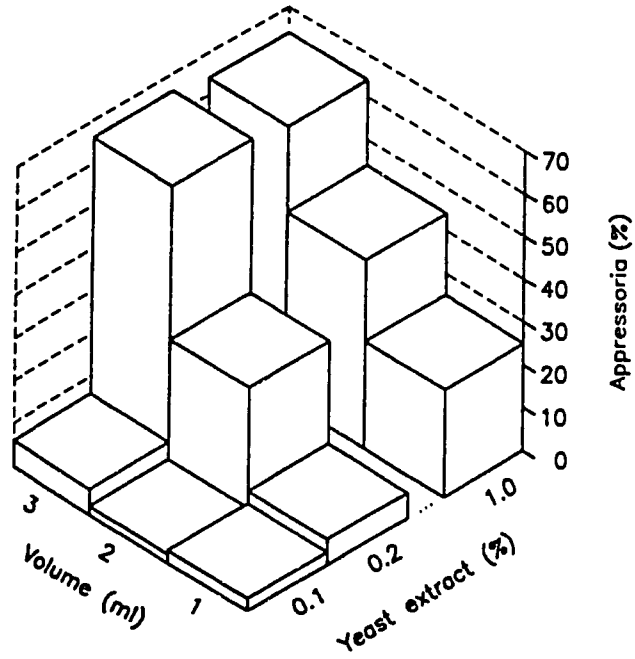



**Fig. 13.** Appressorium formation by Z. radicans using three yeast extract concentrations and three volumes of liquid medium 24 hr postinoculation at 25°C in the dark.

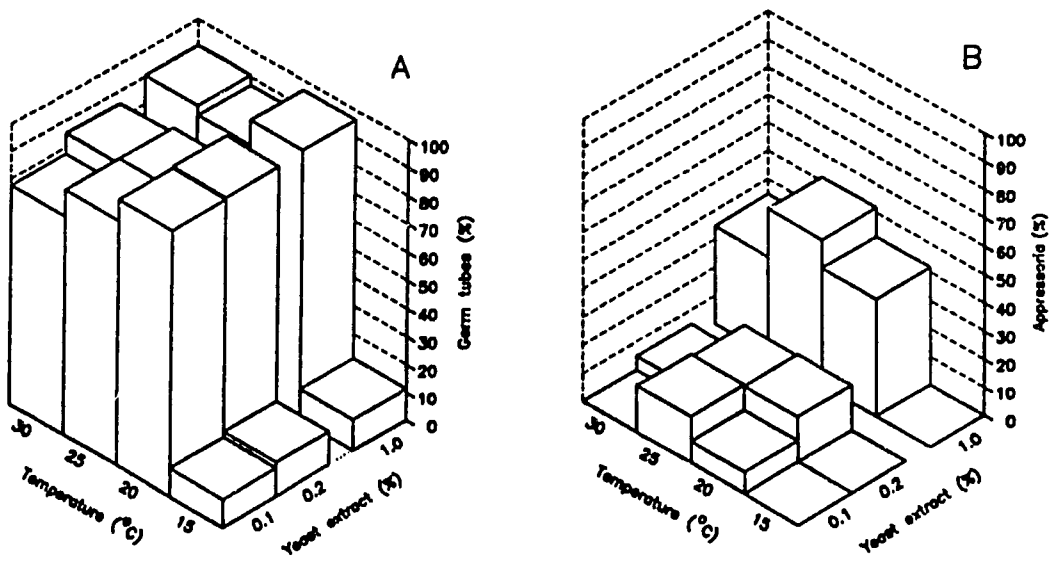



**Fig. 14.** (A) Formation of germ tubes and (B) appressoria by Z. radicans at five temperatures and three yeast extract concentrations, 15 hr postinoculation on water agar.

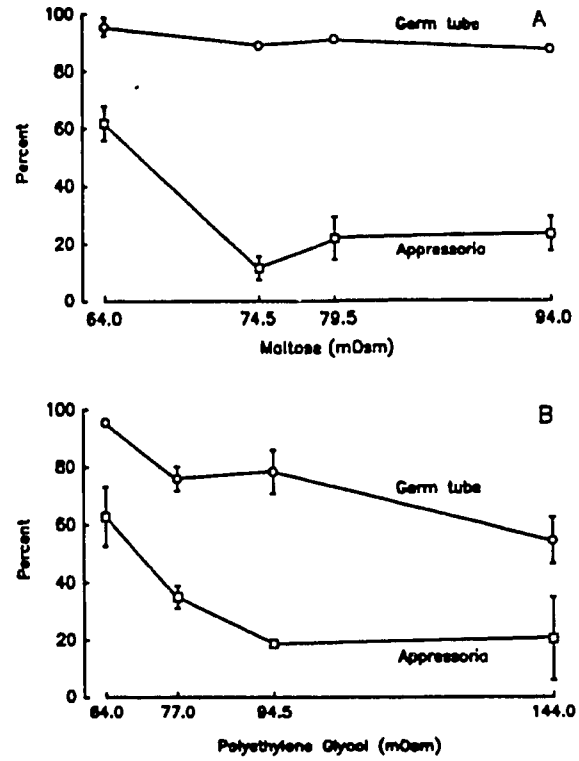



**Fig. 15.** Formation of germ tubes and appressoria of Z. radicans under different concentrations of (A) maltose and (B) polyethylene glycol 24 hr postinoculation at 25°C.

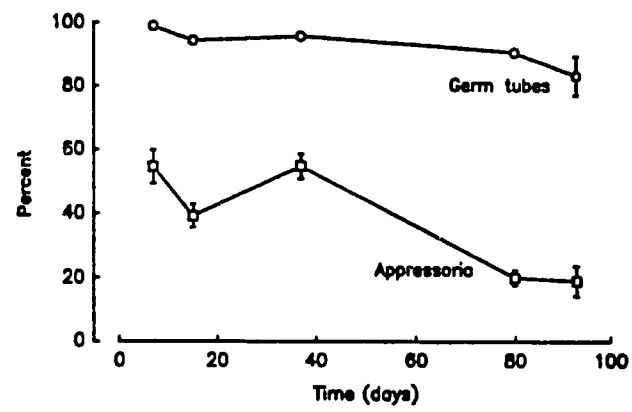



**Fig. 16.** Percent formation of germ tubes and appressoria by Z. radicans 24 hr postinoculation as affected by subculturing weekly at 25°C. Fungal cultures maintained at 20°C on SDAY.

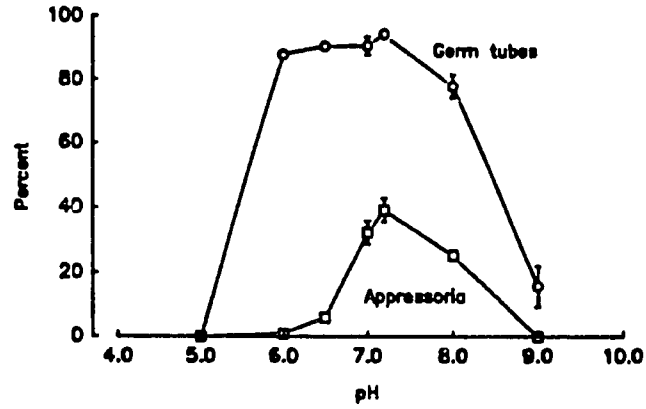



**Fig. 17.** Formation of germ tubes and appressoria by Z. radicans in 1% yeast extract at different pHs 24 hr postinoculation at 25°C.

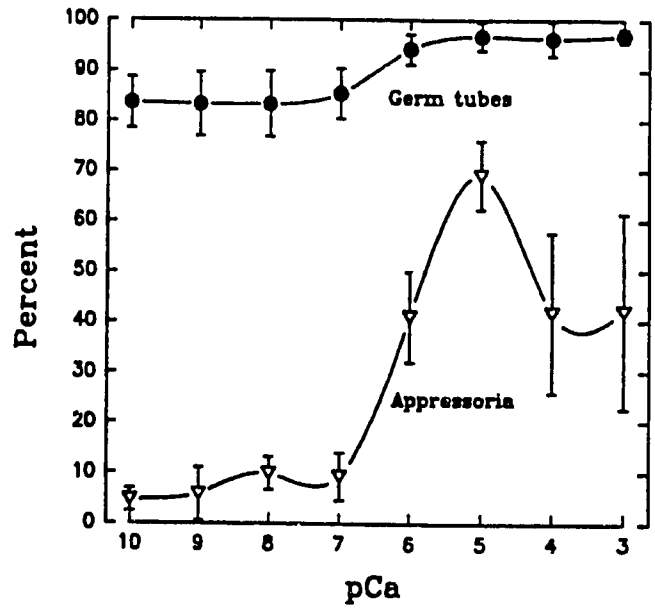



**Fig. 18.** The effect of external $Ca^{2+}$ concentration on germination and appressorium formation. Cells were maintained in each concentration for 24 hr in the dark. pCa = -log[$Ca^{2+}$].

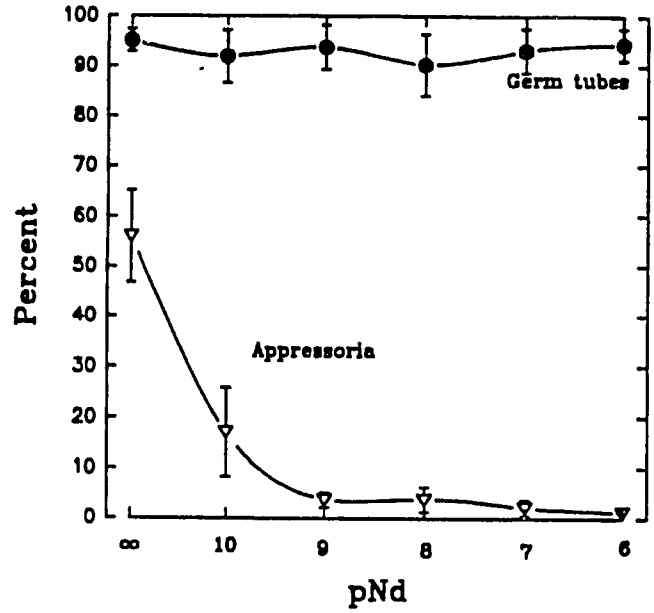



**Fig.** 19. The effect of concentration of external $NdCl_3$ on germination and appressorium formation. Cells were maintained in each concentration for 24 hr in the dark. pNd = -log[$Nd^{3+}$].

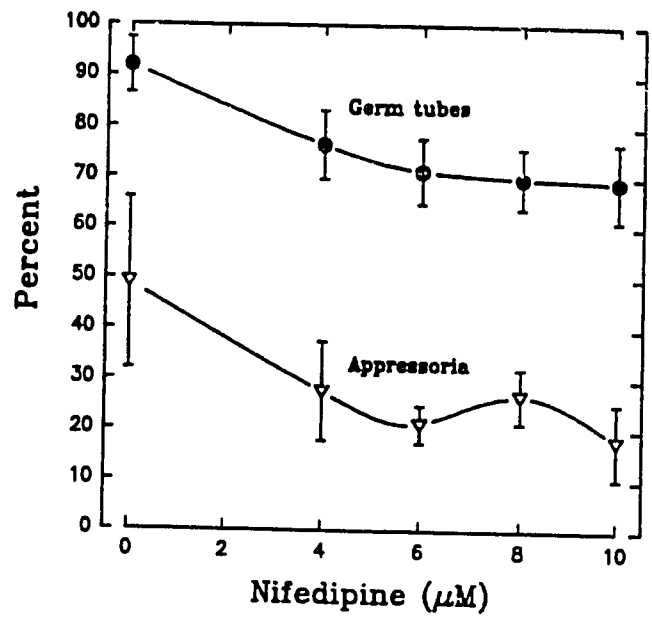



**Fig.** 20. The effect of nifedipine on formation of germ tubes and appressoria determined 24 hr after inoculation. All treatments included 0.25% DMSO.

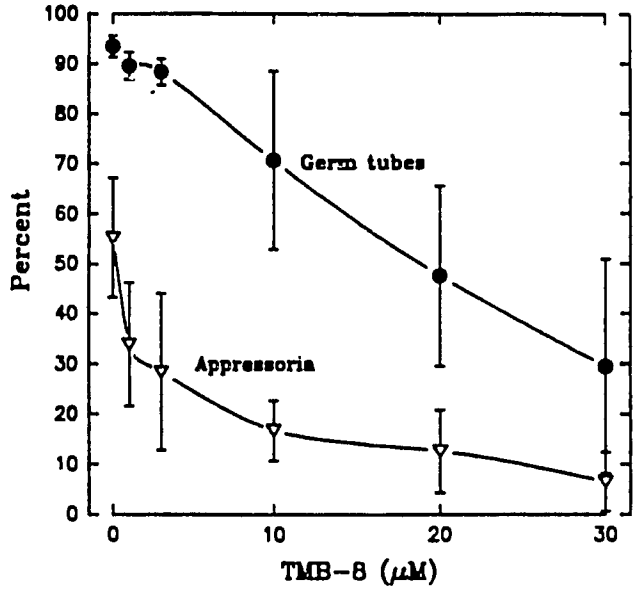



**Fig. 21.** The effect of TMB-8 on germination and differentiation. Differentiation was determined 24 hr postinoculation.

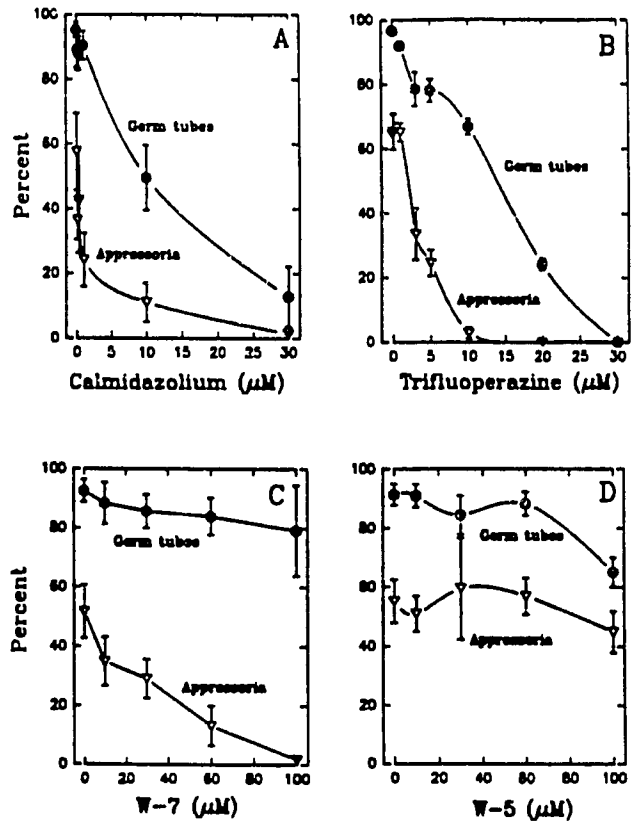



**Fig. 22.** The effect of $Ca^{2+}$/calmodulin complex inhibitors on formation of germ tubes and appressoria formation. a) R24571. b) Trifluoperazine. c) W-7. d) W-5. Differentiation was determined 24 hr postinoculation. The experiment with W-5 was repeated two times with three replicates per experiment.

# CAMEROON - PURDUE UNIVERSITY

*PRESERVATION OF POST HARVEST COWPEAS BY SUBSISTENCE FARMERS IN CAMEROON*


Principal Investigators:

| | | |
|---|---|---|
| Larry Murdock (U.S.) | Department of Entomology | Purdue University |
| Zachee Boli (HC) | Experiment Station/Maroua | Institut de la Recherche Agro. |

Co-Investigators:

| | | |
|---|---|---|
| Ousman Boukar | Agronomy/Garoua | Institut de la Recherche |
| Endondo Chevalier | Department of Agronomy | Institut de la Recherche |
| Laurie W. Kitch | Department of Entomology | Purdue University |
| Georges Ntoukam | Entomology/Maroua | Institut de la Recherche |
| Richard E. Shade | Department of Entomology | Purdue University |
| Moffi E. Ta'Ama | Entomology/Maroua | Institut de la Recherche |
| Jane L. Wolfson | Department of Entomology | Purdue University |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Third-year survey of farmer storage practices.** When our project in north Cameroon was initiated in 1987, we recognized that we didn't know enough about peoples' awareness of and attitudes about losses of cowpeas to storage pests, about the insect species involved, or about postharvest storage practices. Reliable knowledge about these components of the system was essential if we were to (1) address the most important insect constraint first, and not a secondary, local, or sporadic pest (2) develop effective technologies to relieve the constraint and (3) devise technologies which would ultimately be implementable. We believed that such knowledge would also serve as a baseline to help us assess the impact of our project-developed technologies after they began to be disseminated.

We obtained the required information by sending Dr. Jane Wolfson to Cameroon for three to five weeks during the storage season of each of the first three full years of the project. In Cameroon, she worked with IRA scientists and technicians and traveled extensively through the region, observing cowpea storage among low resource farms and interviewing women and men farmers. During the first year she worked in Cameroon in November, during the second in January, and during the third, in March. Following is a summary of her observations made in March 1990, a time well into the storage season. Her goals, as laid out in the FY 90 Research Workplan, were: (1) to complete the description of cowpea storage methodologies and of attendant insect infestations on low resource

farms in areas previously covered in northern Cameroon; (2) to initiate the survey in previously unsurveyed area of Waza and further north and (3) to seek additional information on economics of cowpea storage, particularly losses to bruchids.

In addition to interviews with 117 farmer households, from which cowpea samples were collected, 29 cowpea samples were purchased at 11 local markets. Localities visited in past seasons were again visited (areas around Maroua, Mokolo, Koza, Mora and Bogo). For the first time, the region around Waza and north of Waza was visited. The farmers interviewed belonged to 21 different ethnic groups. Twenty-five percent of the farmers visited in 1990 were re-visits from previous years in an attempt to confirm earlier responses. The information gathered confirmed and extended that gathered in previous years. Information was collected on (i) household demographics, (ii) cowpea agronomic practices, (iii) field size and (iv) cowpea yields, (v) cowpea storage practices, (vi) farmer perceptions of cowpea storage losses, (vii) farmer's familiarity with bruchid weevils, (viii) cowpea consumption patterns, (ix) insecticide usage patterns and the factors influencing those patterns. Samples (50 pods or 100 g of seeds) obtained from farmers and markets were analyzed for bruchids and bruchid damage. Samples stored in pods were assessed for breakage. In addition, seed samples were brought back to Purdue for seed increase and testing for bruchid resistance (cf. section I.A.2.).

This year's data strengthens many of the conclusions drawn last year. Women were found, as before, to have primary responsibility for storage in most households. Further, they were involved in cowpea production in 92 percent of the households, although they often shared this responsibility with the men. Gender-specific responsibilities depended upon ethnic group, e.g., among the Kapski and Mandara only males stored cowpeas, whereas among the Mafa, Guidar, Guiziga and Moufou most storage was done by women.

Confirming earlier observations, it was clear that cowpea storage was generally a process; the crop is moved at least once at some time during the storage season. The "danki" (pole platform) was used as the primary storage site by all but 15 farmers. The length of storage on the danki ranged from less than one week to the entire dry season (until the rains began the following year); 11 percent stored on the danki less than one month, 39 percent one to two months, 22 percent three to five months, 28 percent six months or longer. The reasons people gave for moving the crop off the danki and into the second storage site and--in those cases where the danki was not used at all--the reason for not storing on the danki, were usually the same: insects. Eighty seven percent of farmers who did not use a danki treated their cowpeas immediately after harvest to control insects. Eighty two percent of farmers who used a danki indicated that insects were the key factor determining the time at which they moved and/or threshed and/or treated their stored cowpeas. Farmers were quite aware of the presence of weevils in the field and in storage; only eight of 115 farmers indicated that they did not see them.

Our sampling in past years indicated that there are two important bruchids infesting cowpeas in this region; *Bruchidius atrolineatus* and *Callosobruchus maculatus*. These insects have different patterns of seasonal abundance, *B. atrolineatus* is common at harvest and then disappears while *C. maculatus* is uncommon at harvest but increases in numbers during storage. Some farmers spoke of two different weevils and indicated that they moved their cowpeas during the time when the first insect had decreased in number and before the second one began to increase. Farmers often explained that they moved their cowpeas off of the danki at the time of year when they did because, "if they moved them earlier they had greater losses and if they moved them later they had greater losses." This year, the logic of this explanation and the implied understanding of the insects dynamics that underlie it became clear.

In an attempt to gain a fuller understanding of the importance of cowpeas to the farmers in the region under study, farmers were asked about their cowpea consumption patterns. The results indicate that cowpeas are even more important to these farmers than we had imagined. Most farmers (62 percent) consume cowpeas every single day and 91 percent of these eat cowpeas at both daily meals. Less than 20 percent consumed cowpeas less than four times a week. Farmers have different strategies for replacing dietary cowpeas when their harvest has been consumed; 59 percent indicated that they purchase more cowpeas at the market; 24 percent indicated that they purchase if they have the funds to do so; 9 percent budget their cowpeas so that they do not run out and 7 percent do without when their stores are depleted, saying, "the sauce is thin."

Mean cowpea hectarage per household (mean number of household persons 11.9, mode 9) for cowpea cultivation is about 1 ha but in fact only 20 households grew cowpeas on over 1 ha (field size in the mountainous regions were not reported in hectares and are excluded from this discussion). The mean hectarage farmed per cowpea producer was only 0.25 ha. Cowpeas were grown as a monocrop by 27 percent of farmers, and intercropped with sorghum, millet or cotton by 67 percent of farmers; only 7 percent of farmers used both methods. All but 15 farmers rotated either their fields or rows within the fields.

Thirty one percent of farmers applied insecticides to their cowpeas in the field with the numbers of treatments numbering from 2-10. There were five reasons given for not applying insecticides. The most common reason (57 percent) was that insecticides were not available for the farmers to use. Farmers who do not have access to Sodecoton agents cannot obtain field insecticides but even those who do live in cotton growing regions often report that Sodecoton will not sell insecticides for non-cotton crops. Other reasons for not using insecticides are: (1) a general lack of information regarding insecticides (even that they exist and can be useful); (2) lack of money; (3) and that insecticides burn sorghum leaves which is important to farmers who intercrop. An even smaller proportion of the farmers interviewed use insecticides on their stored food grain (16 percent). Insecticides for storage are apparently more easily available than insecticides for field application; only 17 percent

of farmers who use them report access problems. The most common reasons for not using insecticides on food grain were lack of cash (32 percent), no information regarding utility (18 percent), fear of toxicity ((16 percent), and satisfaction with the current storage method (16 percent).

A little over half of the cowpea samples collected in March, 1990 (four to five months into the storage season) were grain samples (53 percent), the remaining samples were still in pods. At the time of collection, some samples were very heavily damaged (97 percent of the grains in the samples had holes), while other samples were undamaged. The numbers of insects emerging from the cowpeas within 35 days of sampling was monitored and ranged from 0 to over 2000. Storage methods associated with those samples having the smallest numbers of emerging insects were storage in drums, with ash, with herbs, and with insecticides although, some samples with few emerging insects were from untreated danki-stored pods that had been grown without insecticides.

We believe that the survey component of the project has successfully completed the goals set forth in the log frame. We now have a good understanding of (i) the storage practices that are used in northern Cameroon, (ii) the persons in the household responsible for production and storage, (iii) the methods currently used in attempts to mitigate losses. This information has strongly influenced our choice of research objectives and should assist the Cameroon national programs in targeting their extension activities. We also have baseline data on the insect infestation levels that farmers experience. We have not yet been able to sort out those components of the farmers production methods that contribute most significantly to reducing losses, and work needs to continue in this arena. Numerous herbs have been identified by farmers as protectants and should be evaluated.

During FY 91, it is our intention to complete the analysis of the data gathered during the three year survey, formulate the conclusions, and to publish the results as a formal report.

**Assessment of farmer cowpea preferences and growing practices.** Little or no published data exists concerning the types of cowpeas (plant and seed types) preferred by farmers in northern Cameroon. Because our project is seeking to breed cowpea types incorporating combined seed and pod resistance and thus suitable for storage, we began exploratory work to develop a better understanding of farmer preferences with respect to cowpea varieties. We conducted farmer interviews in 17 villages in two major farming system zones with the major objective to understand cowpea preferences and growing practices preferences among farmers in the region. This survey was conducted by Dr. Laurie Kitch and Mr. Georges Ntoukam.

Eight of the villages where interviews were conducted were selected from a list of representative villages developed by Dr. Jane Wolfson, based on her storage methods survey (i.e., representative of larger regions where particular storage practices predominate). Selection of other villages was based on discussions with NCRE/TLU

(National Cereals Research and Extension Project/Testing and Liaison Unit) researchers who helped guide us to areas where cowpeas were considered an important crop. The 17 villages visited were found in two major farming system zones. One zone (Farming System Zone 1) was in the Mandara Mountains, northwest of Maroua, the other covered Farming System Zone 3, a large area of plains primarily south and west of Maroua. A total of 131 farmers were interviewed, many of whom were men. In each village, the chief was contacted and purpose of the visit explained. He was asked to help identify important cowpea farmers from the village to see their crop and discuss with them their cowpea cultivation practices. Interviews were normally conducted on-site, at the farmers' field, and were usually one-on-one where possible. Except for the responses to the question about constraints to production, the percentages given below are expressed as the percentage of responses given for each question since in some cases an individual farmer could respond more than once (for example, some farmers planted both in association and had another intercropped field of cowpeas, therefore the total number of responses was 138 rather than 131). Some of the principal observations are summarized below.

Mean area planted to cowpeas in this survey group was slightly more than one-half hectare. Nearly three-fifths of the interviewed farmers grew cowpea in association rather than as a monocrop. When cowpea was grown as a monocrop, the variety used was almost without exception a variety released by IRA (IRA varieties, developed as a result of the IRA/CRSP project, are VYA--a local, BR-1 [IITA cultivar IT81D-985] and BR-2 [IITA cultivar [IT81D-994]. The mean yield was 260 kg/ha. The farmers got their seed primarily from the marketplace (43 percent), saved it themselves (32 percent) or got it from IRA or Sodecoton, the cotton parastatal (18 percent). Two farmers out of five regularly used insecticides. They say insects are the chief constraints to production or availability of cowpeas (89 percent said field insects are a problem, 55 percent said storage insects are a problem); other problems paled by comparison. Two farmers out of three store their cowpeas for at least one month after harvest; nine out of ten use some sort of treatment to preserve their store from insects (34 percent use insecticides, 34 percent ash, and 19 percent herbs). Farmers from the two zones strongly prefer large, white seeds, with brown and red seeds preferred by smaller but significant numbers. Favored pod characteristics are long and large, non-dehiscent, and white. Nearly four out of five people interviewed use cowpea leaves for "sauce," and nine out of ten use cowpeas as forage.

The results of the Kitch survey and the Wolfson survey summarized above are not formally comparable for geographical, methodological, socio-economic, ethnic, and seasonal reasons as well as the fact that the studies differed in objectives, approach, and personnel (male interviewer in one study, female in the other). Nevertheless, where there is overlap, our initial impression is that there is good overall agreement regarding (i) the importance of cowpea to low resource farmers, (ii) farmers' awareness of postharvest losses to insects, (iii) need for preventive measures (iv) actual preventive measures taken, (v) amounts of cowpeas stored after harvest in a

typical farming unit, and (vi) importance of leaves as food and as fodder. From the outset, our project has tried hard to understand the views, attitudes, and remedies used by the eventual users of project-developed technologies, and to gain first-hand experience through observation. We saw these sources of information as the keys to developing effective and implementable technologies. The results of our surveys need yet to be more fully analyzed and summarized, but we feel that they have already benefitted our project enormously by increasing our understanding of the complexity of the human and agricultural systems in which we hope to intervene usefully.

**Bases of seed and pod resistance to cowpea weevil.** From the outset, our project strategy was to devise or adapt a variety of effective, implementable technologies which can protect cowpeas from cowpea weevil and other postharvest insect pests. North Cameroon has numerous ethnic groups, among whom there is a variety of postharvest cowpea storage and processing practices. To meet the needs of this variety of peoples who live in a range of agroecologies, we felt it was essential to provide a range of storage technologies which could be combined in different ways to promote cowpea storage. By doing so, we would also better serve the broader needs of the CRSP because the variety of technologies would likely include forms which could be exported to other regions or even applied to common bean as well.

One technology we are convinced will be useful and acceptable involves developing cowpea types which store well. Our idea was to breed cowpeas which combine seed and pod resistance traits. This would take into account the fact that farmers currently store cowpeas in pod form for extended periods before they thresh and subsequently store the cowpeas as loose grain. Cowpea types with combined seed and pod resistance should therefore be readily acceptable in current postharvest storage and processing systems. IITA researchers have laid an excellent foundation for our work by: (1) developing cowpea lines with seed resistance to cowpea weevil; and (2) by recognizing that the cowpea pod can provide a substantial degree of protection against cowpea weevil as long as the pod wall remains intact.

Our approach was to build upon this knowledge by systematically screening cowpeas in pod form for overall resistance to cowpea weevil. Promising lines were then studied to ascertain the basis of combined seed/pod resistance. During the past year, we screened 30 cultivars as intact pods against cowpea weevil. Pod resistance was measured as pre-establishment larval mortality (PreM); those larvae dying after egg hatch but before penetrating into the seeds, and as post-establishment within-seed mortality (PostM). Among the 30 varieties examined, PreM ranged from 58 percent to 99.4 percent and PostM from 6.7 to 82.6 percent. Ten varieties exhibited total intact pod mortality greater than 95 percent.

In searching for the basis of intact pod resistance, we explored whether it was due to seed resistance factors, pod-wall factors, or to interactions between pod and seed. It was clear that resistance

to breakage was a necessary characteristic for overall protection against the cowpea weevil, but that other factors are also important. In this connection we note parenthetically that we have observed that farmer-selected local cultivars from the region are invariably tough and non-dehiscent. Seed coat thickness was the factor among several tested (pod wall thickness, breakage index, pod strength, pod:seed ratio, seed cavity space, and seed coat thickness) which correlated highly with total mortality and pre-establishment larval mortality. Our conclusion is that it is the interactions between seed and pod which are decisive in PreM, not pod wall or seed characters alone. The methods and hypotheses developed during the last year will give much momentum to our goal of breeding for combined seed and pod resistance. As part of the process of developing this effort, Dr. Laurie Kitch has prepared a long term breeding plan.

**Initial hybridizations to combine seed and pod resistance.** Crosses have been made between/among the following varieties: IT81D-985, IT81D-994, IT81D-1137, IT87S-1393, IT81D-897, VYA, and Maroua 16. The objectives are to transfer disease- and insect-resistant characteristics into local Cameroon lines (VYA and Maroua 16) as well as improving the pod resistance of materials such as IT81D-994 and IT81D-985. $F_1$ plants of these crosses are presently (November 1990) growing under irrigation for projected harvest of $F_2$ seeds in December.

Crosses have been made between pod resistant lines TVu 1890, Tars 36 and pod susceptible lines IT81D-975 and TVx 3236. $F_1$ plants from these crosses are currently growing in an off-season nursery. They will be analyzed using methods developed in the past year to assess the inheritance of pod resistance.

Several highly pod-dehiscent lines have also been selected this year (from lines in the pod screening trial) which will be of potential use in inheritance studies of dehiscence.

**Ash for postharvest preservation of cowpeas—laboratory studies.** Our surveys and observations among low resource farmers in north Cameroon had revealed that the use of ash for preservation of cowpeas was widespread, but that some users were not confident that the method was effective. The survey had also revealed that the amount of ash used and the details of the technology varied among farmers. We have continued our efforts to understand the conditions under which ash can protect cowpeas against cowpea weevils. Studies at Purdue by Jane Wolfson, Dick Shade, Paul Menzer, and Larry Murdock and by Georges Ntoukam in Cameroon demonstrated that: (1) co-storage of cowpeas and ash arrests the development of cowpea weevil infestations; (2) a minimum ratio of three volumes of ash to four volumes of cowpea completely arrests weevil population growth; and (3) a 3 cm layer of ash on top of a container of stored seeds will prevent reinfestation by adult weevils. On the basis of these observations, demonstration tests of this technology have begun in the early postharvest storage season of 1990 (cf. Section I.A.1. below).

**Solar disinfestation of cowpeas.** During the past two years we have shown that cowpeas infested with cowpea weevil can be disinfested by heating them to 57°C. for a few minutes, that all stages of this insect are killed, that germination and cooking time are little affected, at least with California Blackeye 5 (CB5) seeds, and that a simple, cheap solar heater will serve to produce the desired temperatures. During the past year we have continued our efforts to develop this methodology, understand its limitations, and bring it to farmers in demonstration tests. The results of the experiments of the past year have not been fully analyzed and are not yet available.

At Purdue, we have begun to examine more closely the effects on cowpea germination of exposure of cowpeas to elevated temperatures. In experiments carried out by Mr. Endondo Chevalier while he was studying for his M.S. degree in Agronomy at Purdue, exposure of CB5 seeds to temperatures as high as 80°C for one hour did not significantly reduce germination, while at 85°C and above germination was substantially reduced. Plans are for Mr. Endondo to continue these experiments in Cameroon using the three cultivars released by IRA (VYA, BR-1, and BR-2).

In Cameroon, the solar treatment procedure has been improved and expanded. While data are not yet available, preliminary experiments indicate that the solar heater can be assembled using 3 m x 3 m squares of black and translucent plastic sheeting. With this expanded area, it is possible to treat 50 kg of cowpea seeds at a time, and to reach within seed temperatures of 70°C.

**Varietal adaptation trials.** A new approach has been adopted this year to simplify and bring more focus to the varietal screening trials procedure. In the past, a great deal of energy has been expended to take very precise yield data on large numbers of lines which are very clearly not adapted to the far north of Cameroon. This has been very demanding with regards to time as well as project resources. In view of the initiation of the breeding work and the upcoming need to evaluate larger number of breeding lines we adopted a new approach to evaluating exotic lines from IITA or from other sources.

**Evidence of extent of use to date.** In the fall of 1990 we have just begun to test our project-developed solar technology and our ash storage technology, working with the TLU and individual cowpea farmers. Results of our work are thus not yet disseminated, but are getting closer to the farmers. Our demonstration/testing plan follows: Tests will be carried out in cooperation with the Maroua TLU at four villages with ten farmers per village. Four storage techniques which are in the developmental stage will be demonstrated to obtain farmer feedback on the potential usefulness of each technology and possible changes to make the technology more practical or effective.

The tests will be conducted with two farmers assigned to each technique. Since farmers were earlier given seeds of either BR-2 or VYA to plant, their own harvest will be used for the tests. (It

will be important to insure that tests are carried out with VYA only when possible.) If not, care must be taken that BR-2 is not used as the local control (since it has seed resistance). We observed however, that in most fields farmers had mixed either locals or VYA with BR-2, so that for most tests we will probably be using a mixture of susceptible and resistant seeds.

Farmers will be asked to store their cowpeas unthreshed, in pod form, until the tests are conducted. Four technologies will be evaluated: Ash storage, solar heater treatment followed by storage in double bag (clear), double bagging (*sac en ecaille* covered with clear bag on outside), and long-term storage in pods on the danki of BR-2, which has a breakage-resistant pod and seed resistance. In each village, a local control treatment assigned to two farmers will consist of storing cowpea seeds in sacks or granaries untreated. The quantity of seed treated in the demonstrations will be 40kg.

An important aspect of the tests will be the artificial infestation of the farmers' seeds used in each treatment with bruchid infested seeds from the laboratory. This infestation will be done at the rate of 1 percent, i.e. 1 percent of the seeds used in each demonstration will be infested seeds from our Maroua laboratory. Each infested seed will have two to three bruchid larvae within which will be expected to emerge within one week after setting up the demonstration. This level of initial infestation is sufficient to ensure that nearly every seed would be damaged by the end of three to four months.

Based upon the results of these tests we shall develop technical bulletins and recommendations to disseminate the information through nation programs.

Information generated in the project is being made available through publications submitted or in preparation (see below). Here it should be pointed out that, thanks to the efforts of Mr. Georges Ntoukam, our project has been identified as a lead center for cowpea storage in the SAFGRAD RENACO network (RENACO is the West and Central Africa Cowpea Research Network). Mr. Ntoukam has participated as a board member of the network and has used this mechanism to begin alerting other National Agricultural Scientists in the region (cf. Section IV, Publications, below).

2. Other research-related results

**Germplasm conservation and evaluation.** Seed Resistance Bioassays. A total of 166 cowpea accessions collected from individual farmers or purchased in local markets by Dr. Jane Wolfson were brought to Purdue and assayed for resistance to cowpea weevil. Five lines showed significant resistance as measured by delays in developmental time—the most reliable measure of resistance—in a single blind assay. However, all five lines proved to be improved types derived from IITA's TVu 2027. The materials collected are also being grown out in Cameroon to assay for combined seed/pod resistance.

Dr. Laurie Kitch and Mr. Georges Ntoukam also made a large collection of local varieties during 1990 and conducted preliminary bioassays for seed resistance. Eight promising lines were sent to Purdue for further evaluation. These lines are being grown out at the time of writing for subsequent assay. Preliminary indications are that one line may exhibit non-preference resistance, but confirmation is necessary.

Collections were also made this year of some locals near Mayo-Oldeme which appear to possess resistance or tolerance to flower thrips, in that under high infestation pressure (10 + thrips per flower) they continue to set four to five pods per raceme. This is a remote area in the Mandara Mountains where insecticides are not used, and this local variety is well known in the area for its tendency to set four to five pods per raceme while others typically only have one or at most two pods.

Pod Resistance Bioassays. The general objective of the pod resistance screening is to identify additional sources of pod resistance, especially among rough-seeded cowpea types. Approximately 300 exotic cowpea lines in addition to the above mentioned 150 locals (#2) have been grown out this year and pods harvested from all materials. We are presently multiplying the laboratory bruchid culture to begin the pod resistance screening.

Materials collected will be shared with Dr. Tony Hall of the UC Riverside CRSP project as well as made available to the IITA germplasm unit.

**Seed production.** While seed production is not a part of our project, the IRA CRSP activity has in the past assisted Project Semencier, the seed production activity in the north, by supplying foundation seed. Because of concerns about virus infestations on our research plots, we have not provided the seed production unit with seed during the past year. However, we are currently attempting to grow sufficient virus-free seed of three IRA-released varieties and make them available for seed increase next year.

**Impact of other CRSP-produced technology.** None arising directly from our project as yet, but interest has been expressed to project participants concerning using solar heating for other crops, including common bean and maize . Interest has also been expressed by USDA scientists in developing applications of the Purdue-developed biomonitor device to study other stored products insect species—the biomonitor being developed before we began participating in the CRSP.

Impact on production/storage of cowpeas. While systematic figures are not available, our surveys during the past year indicate that varieties identified as adapted to north Cameroon by the Georgia phase of the IRA-CRSP project and released by IRA are being accepted and disseminated in the region. Dr. Laurie Kitch's initial survey indicates that one farmer in four in the two Farming System Zones examined uses IRA-released varieties. It is premature to expect impact of our storage project, but initial impressions growing out

of the first demonstration-tests of project developed storage technologies indicate a high degree of interest among extension agents and farmers. Our project continues to test, on a small scale, insecticides available in the north for efficacy in controlling cowpea insects in the field. Information on treatment schedules, application rates, sprayer types and methods are shared with Sodecoton, a principal source of insecticides in the region.

**How research findings address needs of small-scale farmers and women.** Our surveys (cf Section I.A.1.) show that women are heavily involved in cowpea production and storage in most regions and ethnic groups in north Cameroon. The surveys by Dr. Wolfson and by Dr. Kitch as well as observations by other CRSP team members have served to sensitize us to the real problems and needs of small farmers, including women. Because of this awareness, all of the technology developed has the ultimate users of it in mind. We have learned from the people extensively, for they have served as sources of researchable ideas. Among their contributions are: (1) the notion of ash storage; (2) solar exposure of grain for drying--which led us to the idea of a solar heater for cowpea disinfestation (although we have learned subsequently that this idea of using solar heating for disinfestation is not novel with us and acknowledge the prior publication of solar heating for insect control by our colleagues at the University of Georgia); (3) the use of local cultivars which universally have tough, non-dehiscent pods; and (4) the widespread use of storage of cowpeas in pod form.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. No data available.

B. Institutional Development and Training

The project is on track with regards to its long-term plan of developing a team of IRA researchers (entomologist/agronomist/breeder) capable of dealing with cowpea and related legumes. Under this plan, Mr. Chevalier Endondo completed his degree in production agronomy at Purdue University, West Lafayette, IN, in August 1990. Plans are for Mr. Georges Ntoukam, IRA entomologist, to begin work toward his Ph.D early in 1991. Mr. Boukar Ousman, IRA cowpea breeder, will work with Dr. Laurie Kitch on cowpea breeding for storage resistance and thereby gain experience and develop his capacity as a breeder. In due course, he will go on for training as a cowpea breeder.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

We have made considerable progress toward developing cowpea storage technologies suitable for low resource farmers in Cameroon to minimize losses to storage insects. We have far greater understanding of the role of cowpea in the farm/family/nutritional economy of low resource farmers in the north, the role of women, and the nature of post-harvest processing. We have convincing evidence that ash storage is an effective method for storing small to medium amounts of cowpeas and we are currently preparing a technical bulletin describing how ash can be

used by low resource people for cowpea storage. As part of the process of preparing that bulletin, we are (autumn 1990) testing the methods with groups of cooperating farmers in four villages in north Cameroon. We have made substantial strides toward developing a disinfestation technique using solar heating and cheap materials, and are likewise testing the method with cooperating farmers. With further experience, we believe it may be possible to treat as much as 50 kg of cowpeas at a time—roughly one third of an average family's yield. Other technologies are at various stages of development and further exploration, including double bagging, drum storage with insecticide, and co-storage with botanicals. We have laid the basis for breeding cowpea types which will store well, and have initiated a breeding effort which will lead to types adapted to the region, which have combined seed and pod resistance and which will "fit in" to the cowpea processing systems people now use.

We have likewise made excellent progress toward developing an institutional capacity in IRA to deal with cowpeas, as outlined above.

Considering that our project is has just completed its third full year, and that we are already virtually on the threshold of beginning to disseminate project-developed technology, we feel we have fulfilled or exceeded every reasonable expectation.

D. Evidence of Biological/Social Sciences Integration

From the outset our project sought to develop technology which will be implementable. We felt that to succeed in doing this it would be absolutely essential that we understand the sociological and biological milieux in which we are working. Accordingly, we have expended considerable resources in each of the first three years to sensitively assess the roles of both women and men in the growing, processing, and marketing of cowpeas—primarily the work of Dr. Jane Wolfson, who frequently obtained excellent guidance from Dr. Ann Ferguson at MSU. While not yet completely analyzed, Dr. Wolfson's data and input (plus those of all team members in or visiting Cameroon) have profoundly affected decisions about the kinds of research we should undertake (ash, solar, bagging, plant breeding) both at Purdue and in Cameroon.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

Our project has enjoyed good relationships with other CRSP projects, particularly those which deal with cowpeas. We have shared germplasm with the Hall/Riverside project—have grown our some 150 lines of cowpeas from Senegal in Cameroon during the last year, looking for pod resistant types, given entomological advice, traded visits at various times, etc. We have also enjoyed cordial relationships with the McWatters/UGA project. The IRA/Purdue project has likewise collaborated with Judith Hall, who was associated with the Roberts/BTI project, assessing the efficacy of various oil treatments—devised for common bean protection against common bean weevil—on cowpeas weevils. Leaders of the three ongoing CRSP cowpea projects have agreed to meet sometime in the coming year—and perhaps annually after that—to increase the level understanding among the projects, to open new channels for communication, and to explore specific opportunities for collaboration.

The Purdue CRSP teams collaboration with IITA is excellent. All of the CRSP team members, plus many other Purdue scientists from four departments are participating in a concerted effort to bring biotechnology to bear on cowpea improvement. Principal objectives are to introduce insect resistance into cowpea (1) through interspecific hybridization and (2) through specific gene transfer. Ongoing collaborative work involves scientists from the IITA Grain Legume Improvement Program led by Dr. S.R. Singh and a team of scientists from several Italian Institutes, led by Professor Luigi Monti of the University of Naples. Scientists from several additional U.S. universities are beginning to participate in the work, including Professors Oyette Chambliss (cowpea breeder) and Narendra Singh (molecular biology) of Auburn University, Prof. Nevin Young of the University of Minnesota (RFLP analysis), and Prof. Richard Pratt, of Ohio State University (interspecific hybridization). These researchers plus nine Italians (including Board Member Gerardo Perlsasca), and eight from IITA (including GLIP Director S. R. Singh and Deputy Director General--Research Ken Fischer), convened at Purdue University, West Lafayette, IN, July 16-19, 1990 to discuss research related to the use of biotechnology for cowpea improvement. The intention of the group is to seek major funding for cowpea improvement for insect resistance through biotechnology under a collaborative program that would encompass a Purdue-led consortium of American universities, IITA-GLIP, and the Italian Institutes led by Professor Monti.

Cordial relations continue with Dr. B.B. Singh, IITA cowpea breeder and officer-in-charge of IITA's Kano Substation, where a large effort is being mounted to breed cowpeas adapted for intercropping. The CRSP team has kept up a steady collaboration with Dr. B.B. Singh for more than five years; most recently we are completing a study of the impact of locality on the expression of the cowpea weevil resistance character. Dr. B.B. Singh visited Purdue to discuss this and related matters in early October 1990.

The Purdue CRSP team is also maintaining good relations with CIAT. Many of the techniques and technologies developed for cowpea weevil are potentially applicable to common bean bruchids as well. Most recently, Larry Murdock was an invited participant to CIAT's week-long meeting in Cali, Colombia, to form an "Advanced *Phaseolus* network."

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

We continue to experience some degree of delay in funds reaching the project in Maroua. Revolving fund expenses are reimbursed to the IRA/CRSP team in Maroua upon arrival of receipts at Purdue University. Turn-around time at Purdue is about one or two weeks. The reimbursement is then sent to IRA/Yaounde by wire or by DHL for subsequent transfer to Maroua. While funds have sometimes been passed on rapidly, at other times there have been substantial delays in their reaching Maroua. In those cases, project activities have been hindered due to lack of funds,

sometimes seriously. Consequently, project personnel have sometimes found it necessary to use personal funds to keep project activities going while awaiting reimbursement—in effect making personal loans to the project.

This is not only bad for morale, it is a drag on project performance.

B. Adequacy of Current Management System

The most serious problem relates to procurement of capital items that are not of American source origin. We applied for permission to purchase a Toyota or Nissan truck for the project in November of 1989 and we are still, in November 1990, awaiting approval from USAID/Washington. Our reasons for wanting a Toyota or Nissan are remarkably simple--dealers of each and service for each are available in the area, while dealers and service for American origin vehicles are not. Fortunately, we have been able to maintain project activities during the interim for a simple reason indeed--the USAID Mission/Yaounde made available to us for the time being a serviceable vehicle--a Toyota Landcruiser, incidentally. Why each purchase of each non-American source origin capital item takes a year or more is hard to understand. These delays damage morale and project performance.

C. Activity Towards Buy-Ins or Other Funding

Working with the USAID Mission in Yaounde, our project requested and obtained a buy-in for the sum of $81,000. These funds served to keep our project viable during the period of transition between Phase II and Phase III of the NCRE project. During Phase II of the NCRE project, resources had been made available to support an ex-patriate scientist assigned to the project. Because IRA project scientists are trained only at the Ing. Agronome or M.S. degree level, and require guidance, mentoring, as well as further training, the FY 90 Mission buy-in served to support the expenses of a Purdue CRSP scientist who spent six months in Cameroon providing administrative guidance to the project, serving as mentor and collaborator to the IRA scientists, as well as pursuing specific research objectives related to the 1990 Project Workplan. Outputs of the activity will include solar heater kits ready for extensive on-farm testing, a technical bulletin on ash storage of cowpeas, a technical bulletin on drum storage, a technical bulletin on double bagging storage techniques, identified adapted cowpea lines possessing seed, pod, and combined seed/pod resistance, production of virus-free seed of BR-1 for Project Semencier, and completion of M.S. training for one IRA scientist, and initiation of Ph.D training for an IRA entomologist.

Funding to support or enhance project activities was also made available from other sources: (i) from a French project in Maroua coordinated by Mr. Rene Billaz, approximately $8,000 to cover all costs of cowpea work by IRA cowpea breeder Mr. Boukar Ousman, who is based at the IRA Sanguere Antenna, Garoua; (ii) from the EEC, $6,000 to cover costs of pre-extension trials of IRA/CRSP project-developed technology (iii) from SAFGRAD/RENACO, $2,000 for general project support.

In October 1990, Larry Murdock and Laurie Kitch prepared, at the request of Mr. Ernest Gibson, ADO at USAID/Yaounde, a draft preproposal for additional buy-in activity covering the years 1991-1994.

The Purdue Bean/Cowpea CRSP team is the core of a larger Purdue group called RIISP (Research Initiative: Insects of Stored Pulses). RIISP scientists are from four different academic departments, and work on a variety of aspects of improvement of beans and cowpeas in international agriculture. While the primary focus is on insect resistance, we employ traditional and biotechnology-based approaches to seed and plant improvement. The success of the group is reflected in the funding it has received--this funding does not impinge directly on the goals of the CRSP cowpea storage project, but it is extremely beneficial to the project nevertheless. Current funding of non-CRSP RIISP activities include: USDA Competitive Grant, PI L.L. Murdock, Co-PIs R.E. Shade and J. Huesing, funding $85,000 for 1990--1992, to carry out structure/activity and mode of action studies of lectins on insects, including the cowpea weevil--lectin genes may prove useful in introducing insect resistance into cowpea through gene transfer.

USDA Competitive Grant, PI L.L. Murdock, Co.-PI Jane Wolfson, funding $100,000 for 1987--June 1991, to assess the impact on Mexican bean beetle of feeding on foliar cysteine proteinase inhibitors--genes coding for cysteine proteinase inhibitors may prove useful for introducing resistance into cowpea through genetic engineering.

USAID Title XII Program Support Grant Funds, L.L. Murdock and R.E. Shade, Co-P.I's, $20,000 for FY 1991, to support activities related to international work of the RIISP group, including evaluation of effects of lectins on cowpea weevil.

Purdue/IITA Memorandum of Understanding, PI L.L. Murdock, Co-PIs R. A. Bressan (Horticulture), R.E. Shade, S.S. Nielsen (Food Science), P.M. Hasegawa (Horticulture), P.E. Dunn (Entomology), $450,000 total for January 1989 through December 1991), to lay the basis for introducing insect resistance into cowpea using biotechnology (interspecific hybridization and genetic transformation using recombinant DNA).

USAID Special Centers Constraints grant, PI P.M. Hasegawa, Co-PIs L.L. Murdock, R.A. Bressan, L.W. Kitch, and R.E. Shade. $90,000 for 1989-1991, to lay the basis for introducing resistance to pod bug and pod borers into cowpea, focusing mainly on interspecific hybridization, in collaboration with IITA.

Purdue University "Crossroads '90" funding, to develop a program for alternative insect management strategies (AIMS) for Indiana, focusing on biotechnology and biological control for insect pest management. L.L. Murdock will be Director of the program, participants include R.A. Bressan, P.M. Hasegawa, P.E. Dunn, R.E. Shade, J. Stuart, S.S. Nielsen, and J. Neal. Funding is $82,500 for three years, July 1990 through July 1993. Activities will include laying the basis for biotechnological improvements of maize for insect resistance using tools, concepts and techniques developed under RIISP.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

We feel that our goals are on target. Our strategy is to develop a repertoire of technologies which can be used by low resource farmers to store cowpeas from harvest to harvest. These technologies will include ash storage, cowpea lines with seed and pod resistance, solar heating, special storage containers, in drums, and with fumigants or insecticides. In addition, we are in hot pursuit of biotechnological improvements of cowpea--using other sources of funds. Any given low resource farmer (and more affluent farmer as well) in Cameroon--woman or man--who has cowpeas to store, should find among this project-developed variety of technologies forms which they can use, combine in different ways, and afford.

If we indeed succeed in developing and helping disseminate these technologies to the people of Cameroon and if, as a result, they can store their cowpeas for much longer periods without loss to insects (or with mitigated loss, at least), we will have succeeded in improving the lot of small farmers in an LDC and contributing to the solution of a persistent problem of cowpea production and utilization in an important area of production.

It may be useful to note that most of the technologies we are developing are not site- or culture -specific, but can fairly easily be adapted to other sites or cultures, other commodities, and other storage pests. Thus we can be confident that the solar technology could be used to disinfest maize (on the cob or in loose grain form) infested with maize or rice weevils. The same is probably true for ash storage, and even for insect resistance genes we identify. Thus, the outputs of our project developed in Cameroon should have far broader application elsewhere.

### B. Balance Between Research and Training

We are committed to training one HC scientist with CRSP funds on a continuing basis until we will have trained a team of three IRA researchers, two with M.S. degrees, one in production agronomy, and one in plant breeding, and a Ph.D. in entomology. This is as far as our resources can stretch if we are to maintain a viable research effort in Cameroon: with one of the project scientists out of the country for training, only two are left to maintain project momentum. We understand that there is a possibility that the NCRE project may provide training for one additional IRA/CRSP scientist in Phase III.

### C. Balance of Domestic vs. Overseas Activities

We feel that the balance is about right. The more basic aspects of technology development are initiated at Purdue and the more applied aspects carried forward in the Host Country.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

The Purdue and IRA scientists plan their research collaboratively in annual two to four week-long review and planning sessions, developing a detailed workplan which is the basis of the following season's work. Individuals have clear responsibilities under the plan. Purdue

scientists have visited Cameroon frequently, in some cases for periods lasting several months, to carry out research, conduct surveys, and to assist in project administration as well as day-to-day management (during the period since the ex-patriate departed the project). We feel we are a highly collaborative team, and that the HC team is getting steadily stronger as a result of training and shared experience.

E. Relative Contributions of Collaborating Institutions and Individuals

The Purdue team has contributed project leadership and guidance and has led the effort to develop the more basic aspects of the work. The Cameroon side has contributed greatly to adapting the methodologies to Cameroon materials and needs, e.g. in developing, from the prototype solar heater which could handle one or two kg of seeds, a practical, improved heater technology that can treat 40-50 kg of seeds at a time. The same is true for ash storage technology development. Both sides have contributed substantially and importantly to project progress. IRA has supported the project very well in providing scientists, technicians, and facilities. A further dissection of relative contributions is hardly useful in a project which is still very new compared to most other Bean/Cowpea CRSP projects.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

The USAID Mission/Yaounde has been very supportive of the project. This is evident by the encouragement the project gained from A.I.D. officers during the difficult transition year, 1989, by the $81,000 buy-in provided by the Mission during 1990, and by the request of the new ADO, Mr. Ernest Gibson, for a preproposal for an additional buy-in.

G. Evidence of Institutionalization

IRA's assignment of three scientists, plus other resources available to it through a French contribution, reflects a mature, long-term commitment to develop a research capacity capable of dealing with this important crop over the long term.

The IRA/CRSP facilities (laboratories, offices, and storage room) at Djarengol (in Maroua) now available to the project reflect a further commitment of IRA to this project and activity. It is heartening that when space recently became available in the main building at Djarengol, it was assigned to the project for use as a storage research space.

When this project started in 1987, technicians and scientists knew little about storage insects, their culture, biology, handling procedures, and bioassay techniques. After a bit more than three years, there is a fully functioning storage entomology laboratory at Djarengol, to which is now being added an important new storage area where project experiments and research can be stored under controlled and secure conditions. Technicians have been trained in many aspects of storage entomology as well as in collection of survey data, record keeping, and other procedures.

H. Other Comments

While our project has made much progress, we are currently in a critical situation as regards continuing our work in Cameroon. To keep project activities going in Cameroon while one or more IRA scientists are away for training, it is absolutely essential that a more experienced scientist be available in Maroua to (i) to carry out numerous project management duties on site, assisting the younger IRA scientists (ii) carry forward the breeding work (hybridizations, pod screening), to which the project is committed (iii) to collaborate in continuing entomological studies and to push forward storage technology development (iv) coordinate and collaborate with the NCRE phase II project and with the TLU units to develop needed technological packages needed by small farmers. If this buy-in is funded, our project will be in excellent condition to pursue its goals over the next four years. If the buy-in is not funded, we will face the prospect of severely reduced output of research on the Cameroon side. Considering how far we have come in three years, we question whether we could accept a severely curtailed research output in Cameroon--while being forced to abandon our breeding effort because we would not be financially able to maintain our Purdue CRSP scientist in Cameroon for the required length of time--and we feel that our notion of combining seed and pod resistance through breeding is an innovative and exciting one and we wish not to abandon it.

Lacking a buy-in (1) we may have to leave Cameroon and seek a site elsewhere where the needs are similar but where the national program can commit additional and more-experienced scientists to the effort; (2) we may try to find resources elsewhere--though there seem to be few sources at present.

IV. PUBLICATIONS AND PRESENTATIONS

L.W. Kitch, R.E. Shade, and L.L. Murdock. 1991. Resistance to the cowpea weevil, *Callosobruchus maculatus*, in pods of cowpea, *Vigna unguiculata*. Entomologia exp. appl, accepted pending revision.

J.L. Wolfson, R.E. Shade, P. Menzer, and L.L. Murdock. Ash storage for postharvest preservation of cowpeas. in preparation.

L.L. Murdock and R.E. Shade. Eradication of cowpea weevil infestations using solar heating. American Entomologist, accepted pending revision.

G. Ntoukam. 1990. Searching for suitable storage methods of cowpea. SAFGRAD/Newsletter. No. 24, 8-9, June.

L.W. Kitch, L.L. Murdock, and R.E. Shade. 1990. Resistance to *Callosobruchus maculatus* (F.) in pods of cowpea *Vigna unguiculata*. Plant Resistance to Insects Newsletter 16: 50-51.

G. Ntoukam. 1989. L'Utilisation de l'energie solaire dans la lutte contre les predateurs du niebe en stockage (*Callosobruchus maculatus*). Symposium volume: Reunion Biannuelle de Presentation des Resultats de la Recherche. Yaounde, Cameroon, 6-10 Fevrier, 1989.

L.L. Murdock. 1990. "Research + Solar Energy = Lower Crop Losses" Note in the Information Memorandum for the Administrator, USAID, July 20, 1990.

Related group publications during 1990 which enhanced or supported project activities but are funded by other sources:

Murdock, L.L. Huesing, J.H., Nielsen, S.S., Pratt, R.C., and Shade, R.E. 1990. Biological effects of plant lectins on the cowpea weevil. Phytochem. 29: 85-89.

Shade, R.E., Furgason, E.S., and Murdock, L.L. 1990. Detection of hidden insect infestations by feeding-generated ultrasonic signals. American Entomologist 36: 231-234.

Wolfson, J.L. and Murdock, L.L. 1990. Diversity of digestive proteinase activity among insects. J. Chem. Ecol. 16: 1089-1102.

Wolfson, J.L. and Murdock, L.L. 1990. Growth of *Manduca sexta* on wounded tomato plants: role of induced proteinase inhibitors. Entomol. exp. appl. 54: 257-264.

Pratt, R.C., Singh, N.K., Shade, R.E., Murdock, L.L. and Bressan, R.A. 1990. Isolation and partial characterization of a seed lectin from tepary bean that delays bruchid beetle development. Plant Physiol. 93: 1453-1459

Huesing, J.H., Murdock, L.L., and Shade, R.E. 1991. Effect of wheat germ isolectins on development of cowpea weevil. Phytochemistry, in press.

Wolfson, J.L., and Murdock, L.L. Colorado potato beetle: Influence of hostplant on midgut digestive proteases. Oecologia, submitted.

Huesing, J.E., Shade, R.E., Chrispeels, M.J., and Murdock, L.L. a-Amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Science (Wash.), submitted.

Huesing, J.L., Murdock, L.L. and Shade, R.E. Rice and stinging nettle lectins: insecticidal activity similar to wheat germ agglutinin. Entomol. exp. appl, submitted.

# DOMINICAN REPUBLIC - UNIVERSITY OF NEBRASKA-LINCOLN

*BIOLOGY, EPIDEMIOLOGY, GENETICS, AND BREEDING FOR RESISTANCE TO PATHOGENS OF BEANS WITH EMPHASIS ON THOSE CAUSING BACTERIAL AND RUST DISEASES*


Principal Investigators:

| | | |
|---|---|---|
| Dermot P. Coyne (U.S.) | Department of Horticulture | University of Nebraska |
| Freddy Safadin Garcia (HC) | CESDA | Secretaria de Estado de Agric. |

Co-Principal Investigators:

| | | |
|---|---|---|
| James S. Beaver (U.S.) | Department of Agronomy & Soils | University of Puerto Rico |
| James R. Steadman (U.S.) | Department of Plant Pathology | University of Nebraska |
| Graciela Godoy (HC) | CESDA | SEA |

Investigators:

| | | |
|---|---|---|
| Cristobal Adames | EEAL | SEA |
| Rodrigo Echavez-Badel | Crop Protection | SEA |
| Aridia Figueroa | CESDA | SEA |
| Adalgisa Mora Garcia | EEAL | SEA |
| Miguel Herrera | CESDA | SEA |
| Margaret Mmbaga | Plant Pathology | University of Nebraska |
| Orlando Bido Montero | EEAL | SEA |
| Mohamed Mohamed | Horticulture | University of Nebraska |
| Julio Cesar Nin | EEAL | SEA |
| Mercedes Rodriguez | EEAL | SEA |
| Eladio Arnaud Santana | EEAL (Head) | SEA |
| Fernando Oviedo Terrero | EEAL | SEA |
| Samuel Concepcion Tio | CENDA | SEA |
| Anne K. Vidaver | Plant Pathology | University of Nebraska |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Cultivars.** The recently introduced PC-50 cultivar has been increased, grown, and well received in the Dominican Republic (see below). Great Northern dry bean cultivar "Starlight" (formerly GN NE-85-43) was released in Nebraska. This cultivar has larger and brighter white seed than any of the currently grown cultivars and should give Nebraska a competitive edge in foreign markets where improved seed quality is desired. The cultivar has resistance to rust and its upright open plant canopy provides an avoidance mechanism to the white mold fungus. The pods of this cultivar express high resistance to the bacterial diseases common blight and halo blight.

**Genetic Traits.** Information on the traits nonspecific resistance to rust (leaf pubescence) and leaf and seed pod resistance to the common blight bacterium in dry beans was disseminated and is in use (see below).

**Methods (Cell and Tissue Culture).** No previous reports indicating regeneration of explants from cotyledons and embryonic axes or from callus in common dry beans (*Phaseolus vulgaris* L.) existed. However, we have produced viable and fertile plants from both cotyledonary and explants of four common dry bean genotypes on Gamborg's B5 medium supplemented with benzyladenine (BH) (see papers by Mohamed et al. in Section IV). Both of these techniques can be used in genetic transformation systems. Also regeneration from embryonic axes may be used to increase the efficiency of hybrid plant recovery from embryo cultures in interspecific crosses. This research was reported to peers at the national annual meetings of the Plant Growth Regulator Society of America and the American Society for Horticultural Science during 1990. Success also was achieved (a first report) with two bean genotypes in regeneration of embryonic callus in culture. The plantlets have not yet been transferred to pots.

**Technical papers, research notes, reports.** Project publications during 1989-1990 are categorized as follows: 20 refereed technical articles, five nonrefereed research notes, and two nonrefereed reports (see Section IV). Nine presentations were made at various types of meetings (see Section IV).

**Evidence of extent of use to date.**

Cultivars: Freddy Saladin reported that enough seed of the new Pompadour cultivar "PC-50" was produced during the past year to plant 60 percent of the bean crop in the Dominican Republic. A survey of the acceptance of "PC-50" by growers in the DR indicated that 92 percent of growers surveyed liked the new cultivar and would like to plant it again (Diaz and Almanzor, 1990; see Section IV for citation). Popularity of the variety was due to high yields, seed purity, large seed size and attractive color, wide adaptation, and type of plant architecture (DR).

Four strip tests of the new GN Starlight were grown on farmers' fields in western Nebraska in 1990. Five growers produced certified and/or foundation seed of the new cultivar while approximately 3,000 lbs. of foundation seed were produced in Idaho. Grower response was favorable.

Genetic Traits: Germplasm possessing nonspecific resistance (pubescence) to rust and high tolerance to common blight were distributed for use in breeding programs in Tanzania, CIAT, Puerto Rico, Dominican Republic, North Dakota, and Michigan.

2. Other research-related results

**Germplasm conservation and use.** Accessions: A Pompadour landrace collection was deposited in the Plant Introduction Station, Pullman, WA. An additional collection of Pompadour landraces was made in the DR during 1990 and stored in the seed bank at the Arroyo Loro Experiment Station, San Juan de la Maguana.

International exchange: The Caribbean Adaptation Nursery (CAN) was sent by UPR to cooperators in the Dominican Republic, Haiti, Jamaica, Guatemala (to evaluate for golden bean mosaic virus resistance) and Panama (for evaluation of web blight resistance). A common blight nursery (CIAT) was evaluated at the Fortuna Substation, Puerto Rico and Andean origin CIAT lines were tested for rust and hairiness.

**Seed production.** Seed of the new "PC-50" was increased and distributed to farmers by the Seed Department, Ministry of Agriculture, Dominican Republic. About 60 percent of the dry bean crop was planted to this cultivar. Seed of two breeding lines were increased for release in 1991. V-030 (UPR) is a white seeded, early maturing, high yielding line with resistance to rust. A black seeded line, H-270 (derived from the MSU breeding program), has an erect plant architecture, good yield potential, and rust resistance. H-270 will replace Venezuela-44 (susceptible to rust in the DR).

XAN 174, XAN 176, and XAN 178 (CIAT) and Belneb #1 (USDA and Nebraska) were found to have high levels of resistance to common bacterial blight (CBB) (Puerto Rico). Preliminary tests indicate that Belneb #1 also has resistance to BGMV. Resistance to common bacterial blight in these lines is derived from Nebraska developed common bacterial blight resistant germplasm. Indeterminate Pompadour type landraces were found to have higher tolerance to common bacterial blight than the determinate landraces (tested at UPR).

Seed of the NE breeding lines Pinto EP-1 (resistant to common bacterial blight and BCMV) and GN 85-55 (resistant to common bacterial blight, rust, and BCMV) has been increased and release of the lines is planned for 1991 (NE). EP-1 is the first Pinto breeding line with high resistance to common bacterial blight. GN breeding lines ND6-89-7 and ND6-89-15 also look promising for possible release.

**Impact of CRSP recommended technology.** At the recommendation of the bean research group in the DR, the planting of beans and other crops that are hosts to the whitefly was suspended in the San Juan de Maguana valley for a period of several weeks. This fallow period appeared to have reduced whitefly populations and, consequently, the incidence of BGMV.

**Project impact on production and consumption.** It is expected that the introduction of PC-50 will contribute to increased as well as more stable yields and increased bean consumption provided there are price incentives to grow the crop and that the price of beans is affordable to poor people. At the moment because of the deteriorating economy, the poor can only afford to buy limited amounts of beans.

**How the research findings address the needs of small-scale farmers and women.** Improved yield potential and stability and greater levels of disease resistance should increase the income of small-scale farmers and result in a greater availability of beans to the DR consumer. Disease resistance will reduce the need to use pesticides.

3. Changes in national production/consumption of beans/cowpeas in the Host Country

**Commercial.** Government statistics on bean production in the DR are unreliable, so data is not presented here.

**Bean seed.** It is estimated that 60 percent of the bean crop in the next year in the DR was planted to the Title XII developed PC-50. The mean yield of the new cultivar PC-50 obtained from a survey of 52 growers (random sample) from across the country was 987 kg/hectare this past year compared to the mean country yield of 505 kg/hectare for the period (1978-1988).

B. Institutional Development and Training

1. Changes since FY 89. Graciela Godoy was awarded her Ph.D. (Plant Pathology), UNL, and returned to the DR project. She was appointed Co-PI in October 1990, in order to assume increased responsibility for research. It is expected that Eladio Arnaud will be a Co-PI when he returns after completing his Ph.D. Dr. Haytham Zaiter (Syria) completed his postdoctoral training and accepted a Department of Agronomy position, American University of Beirut, Lebanon. Fernando Oviedo and Mercedes Rodriguez were assigned to permanent positions in SEA, and the former participated in a plant breeding course sponsored by CIAT. Miguel Herrera received informal training in bean breeding at UPR. Alfonsina Sanchez continues to receive partial support from the project to pursue her M.S. degree in Crop Protection (UPR). Matthew Blair traveled to the DR in March, 1990 to evaluate bean genotypes for BGMV resistance. Mr. Blair plans to conduct a major portion of his M.S. thesis research in the DR during 1991. Bean researchers from Panama, Guatemala, Costa Rica, Mexico, and the Dominican Republic visited the research plots at the Isabela Substation in February, 1990 as part of a PROFRIJOL tour of the Caribbean. Cristobal Adames participated in this tour. Samuel Concepcion spent four months at the Escuela Agricola Panamericana assisting the Honduras Bean/Cowpea CRSP project conduct research with rust. Mohamed Meskine (Morocco) (funded by MIAC) started his M.S. program in Plant Pathology (bean rust) Fall, 1990 under the direction of J.R. Steadman.

2. Over the life of the project. When we first started the cooperative project (1981-82), the DR bean program lacked sufficient trained personnel, had insufficient facilities, vehicles, and little equipment, and lacked financial support to conduct a bean research program. Nine students have since received M.S. or Ph.D. degrees. Now a viable bean research program has been developed in the DR. Facilities have been improved, particularly at Arroyo Loro Experiment Station, where screenhouses, work rooms, a seed storage room (controlled environment), and a plant pathology laboratory have been established. Basic laboratory equipment, two vehicles, and two motor bikes were purchased. The bean (legume) team has earned a good reputation for conducting bean research and, consequently, has begun to attract support from other donor agencies such as PROFRIJOL. During the past few years the DR Ministry of Agriculture (SEA) has supported a greater portion of the salaries of eight researchers comprising the bean research group. Long-term

assistance, however, will likely be necessary. The economic outlook in the DR and other developing countries has continued to worsen, especially due to the Gulf crisis. At present salary levels and with increasing inflation, it is difficult for SEA to maintain staff members with advanced degrees. All those receiving M.S. degrees are now employed outside the project in the DR, except one who is at the Arroyo Loro Experiment Station (F. Oviedo), one in Puerto Rico, and one who is pursuing a Ph.D. degree at UNL.

3. Project training to be completed by the end of the project period. It is expected that Eladio Arnaud (non-Title XII funds) and Debbie Fujimoto (Canada) (some research supported by Title XII funds) will complete their Ph.D. degrees in 1991. E. Arnaud plans to return to the Title XII project in the DR. Dr. Mohamed will be hired (on Title XII funds) as a postdoctoral in Plant Breeding and Genetics in 1991 when he receives an appropriate visa. Expected to complete M.S. degrees are: Guen Hwa Jung (Korea) (UNL Title XII funding), Alfonsina Sanchez (DR) (UPR partial Title XII funding), and Matthew Blair (UPR-partial Title XII funding). Margaret Mmbaga will complete her postdoctoral assignment in Plant Pathology within this period.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

Research on various aspects of CBB and rust (as outlined in Log Frame FY 89-92) has been conducted since the extension of the project in 1985. When the project was merged with UPR in 1987, attention was also given to other disease constraints, particularly web blight and BGMV (see Log Frame 1989-92) along with improved yield and adaptation of beans.

It was considered that ten years would be required to develop and introduce new disease resistant dry bean varieties. The project is on schedule. Several varieties have been released and some promising new lines are being prepared for release.

We have the only Title XII bean project involved with diseases of beans in the lowland tropics. The research conducted by project personnel is well integrated into the national DR legume program. The research also benefits (both complementary and supplementary) dry bean research projects throughout the Third World including East Africa, and assists CIAT and U.S. programs since we have done fundamental research on resistance to and epidemiology of diseases affecting bean production. Project members also cooperate with CIAT in planting dry bean nurseries (Caribbean Adaptation nursery, common bacterial blight and rust nurseries). Also CIAT materials are evaluated for reaction to web blight and BGMV. Germplasm (sources of nonspecific resistance to rust and resistance to common bacterial blight), and information (see publication list) derived from the project are being utilized around the world in bean producing countries and by CIAT.

A bean breeding program has been established in the DR. Bean germplasm has been identified which can serve as sources of resistance to the most important diseases (rust, common bacterial blight, BGMV, and web blight) in a number of developing countries, CIAT, and U.S. Improved cultivars have been released in the DR ('PC-50' is now grown on 60 percent of DR bean acreage), in Nebraska (GN Starlight released in 1990), and in Puerto Rico. The DR breeding program will release two new cultivars in

1991 (a white seeded and a black seeded line). The DR program also has developed new lines with greater levels of disease resistance than traditional varieties, More testing is needed in order to determine the performance of these lines on small farms. Limited quantities of seed of recently released varieties have been multiplied by SEA. Disease management schemes, especially for CBB, have been improved by research on survival of the bacteria on weed hosts and on bean leaves and debris. Rust genetic management has made progress through knowledge of leaf pubescence and virulence, but more epidemiological data is needed.

D. Evidence of Biological/Social Sciences Integration

Women have played an important role in the UNL/UPR/DR project. Dr. Anne Vidaver, internationally renowned bacteriologist and Head of the Department of Plant Pathology at UNL, is an investigator on this project. Dr. Graciela Godoy received her Ph.D. in Plant Pathology at UNL in 1990 and returned to the DR to become a project Co-PI. Dr. Mildred Zapata received her Ph.D. in Plant Pathology at UNL two years ago and is now an assistant professor at UPR, Mayaguez, Puerto Rico, working partially on Title XII with Dr. J. Beaver. Dr. Margaret Mmbaga currently holds a visiting scientist position with J.R. Steadman. Lisa Sutton (M.S.) is a half-time assistant to D.P. Coyne. Three out of eight technical personnel on the project at the Arroyo Loro Experiment Station are women while the former assistant to the PI (DR) was a woman. In addition, four women (DR) have been sent overseas for training (CIAT, Costa Rica, UPR, and UNL).

The development of beans with improved seed qualities and yield will contribute to improved nutrition of women and families provided economic incentives continue to encourage bean production.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

The Bean/Cowpea CRSP projects in the DR and Honduras continue to be closely linked. Both countries benefit from an exchange of germplasm. A red mottled line from the DR proved to be a source for both the "I" gene for resistance to bean common mosaic virus and red seed color. The dense pubescence found in the Pompadour germplasm collection has been transferred to small red breeding lines and may provide race nonspecific resistance to rust. Small red lines are being used as parents in an attempt to improve the heat tolerance of red mottled beans. S. Concepcion provided assistance to the Honduras CRSP project on pubescence and nonspecific rust resistance in beans. Small red lines from the Honduran project were also evaluated for resistance to BGMV in the DR this past year.

The project also collaborates with the other Bean/Cowpea CRSP projects which deal with bean diseases. The project has sent bean lines to Dr. Silbernagel for use in East Africa and he has increased breeding lines in a winter nursery in PR for use in the CRSP project in Tanzania. The project has trained a scientist from Uganda (CBB) and given training in Africa. The project also collaborates with D.P. Maxwell, UWI, in the collection of plant samples for his molecular studies on bean golden mosaic virus, supplying germplasm and field sites, identifying trainees, and overall has integrated well with this project.

The DR project works closely with the CIAT bean research program. A common bacterial blight nursery was planted in PR in collaboration with CIAT. The Caribbean Adaptation dry bean nursery was planted in several countries in the basin in 1990. Several other cooperative nurseries are conducted both in the DR and PR. Travel is planned so that trips to the DR by CIAT and Bean/Cowpea CRSP personnel (UPR, UNL, UWI) can coincide.

Results from research conducted in the DR will be useful now in Jamaica and in the future in Haiti. Puerto Rico is a member of PROFRIJOL which provides an opportunity for the exchange of information among bean researchers in the region. A Midwest regional dry bean nursery was planted in Nebraska (organized by K. Grafton, North Dakota) to evaluate publicly developed varieties of GN, Pinto, and navy beans.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

A major problem that has been difficult to solve is transportation. Because of limits on funding and the need to use budgeted funds for materials and supplies, supplemental salaries, and operating costs to support ongoing research, a new vehicle purchase is not possible. Even if money were available, a non-U.S. source release would be needed. Purchase of a used vehicle, leasing, and renting have all been investigated and are nonviable options. The government has no vehicles to give the project and the U.S. Mission has not been able to help. Motor bikes are considered death sentences by some workers. Thus, procurement of adequate transportation remains a major limitation in the DR with a steady-state A.I.D. budget.

Loss of trained researchers to private industry has continued as purchasing power of the Dominican peso has lost over 20 percent in recent months. The government has increased salaries and has instituted a system of rewarding researchers with advanced degrees but recent petroleum prices and other inflationary trends have negated these gains.

### B. Adequacy of Current Management System

The use of advance funding and reimbursement on the basis of valid receipts has worked well for both the U.S. and DR accounting systems. Visits and phone calls keep everyone up-to-date on use of funds and equipment. A new grant proposal system has been introduced in the DR whereby a short proposal listing objectives, procedures, and funding needs is prepared by investigators. Proposals are reviewed by PIs and Co-PIs from UNL, UPR, and DR with recommendations forwarded to the project initiators. For the 1990-1991 season, 23 proposals were submitted. A few projects were rejected for marginal relevance to CRSP objectives or the need to focus research at one location (Arroyo Loro Station), some projects were modified, and some were deferred for future consideration. The process was considered worthwhile, and the project will attempt to incorporate UWI, UNL, and UPR proposals in the future.

C. Activity Towards Buy-Ins or Other Funding

The research program at UNL has utilized a USDA competitive grant, local industry grants, company grants, USAID scholarships, and regional research funds to expand UNL bean research. UPR has utilized local industry support and other USAID funded legume projects for the bean program. In the DR, PROFRIJOL (COSUDE-CIAT) (funding from the UNL, USAID, and Swiss Bank) has contributed to bean research. The PNUD project (UNL funding) helps with on-farm testing. A PL480 seed production project has been approved by USAID but still needs final approval by the DR government. We plan to pursue the USAID project MUCIA now with Instituto Superior Agricultura (ISA) to find opportunities to interact on training. A private DR foundation (USAID supported partially) also offers individual grant opportunities.

III. PROJECT STATUS

A. Appropriateness of Activities to Goals of the Global Plan

1. Research results. This project is one of three remaining Bean/Cowpea CRSP projects dealing with bean diseases. In the recently published CIAT publication on bean production problems, it states: "of the major world crops, beans are probably one of the most susceptible to disease and insect attacks . . . diseases and pests constitute the major factor that significantly lowers on-farm yield." The project is attempting to improve the resistance of red mottled beans. This seed type is grown in the Caribbean, the Andes, and in East Africa. In addition, pinto, and large and small white beans are being improved for multiple disease resistance.

2. Other research-related results. The project in the Dominican Republic is the only project in the Bean/Cowpea CRSP attempting to develop beans for altitudes less than 1000 m. Diseases such as CBB, BGMV, and web blight are important factors limiting bean production in lower altitudes. Puerto Rico is the only U.S. location where whiteflies and BGMV can be studied in the field. There should be more attention paid to the ecological consequence of increased cultivation of beans in the mountainous regions of the tropics. Increased population pressure has resulted in areas being cultivated which cannot sustain bean production over a number of years. Cultivation of beans on the coastal plains during the cool season provides an alternative to increased production in the mountains. This system can be applied to other countries.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. This DR project is the only one located in the Caribbean. Germplasm and research information developed by the project should be useful throughout the region including needy countries such as Haiti and Jamaica.

Resistance strategies, race nonspecific rust resistance, common bacterial blight, and bean golden mosaic resistance sources, and general disease management strategies as well as improved germplasm will be available for the bean community in Africa, Central and South America, as well as North America.

B. Balance Between Research and Training

Training costs in FY 90 = $6,000; Research costs = $180,000
Training costs projected, FY 91 = $15,600; Research costs = $167,400

In order to maximize research output with an initial decline in our budget and now steady state funding, project resources were diverted from graduate training to technical assistance. However, the need for continued training resulted in the use of other options. The project has had three LASPAU scholars, two departmental assistantships (UNL and UPR), a shared assistantship with a BNF project, CIAT training grants, USAID Morocco project assistantship, and visiting scientists. Short-term training at CIAT, UNL, and UPR for breeding and pathology has been conducted. Thus, although our CRSP project resources heavily favor research support, we have balanced training by using other financial sources. There will be a continuing need to train DR researchers as the DR economic problems create legume program turnovers. We are planning to pursue the USAID sponsored project MUCIA for future training opportunities in the DR. The FY 91 plans have taken into account CRSP-sponsored training as well as the many other training opportunities.

C. Balance of Domestic vs. Overseas Activities

The activities on the project involve basic research being done primarily at UNL and some at UPR while applied research is underway at all institutions. This year the level of basic studies in the DR will increase as some other sources of funding are found (i.e., private foundation) and more highly trained researchers such as Dr. Godoy and F. Oviedo are now conducting experiments. The research, especially basic, will be more collaborative.

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Research planning as well as budget development for FY 91 was done at the Bean/Cowpea CRSP PI meeting held at Michigan State University in May 1990. Research progress was first discussed and then specific plans for the next year were formulated based on the overall project objectives. Training needs in the DR were defined and potential students/trainees identified. Non-CRSP funding possibilities were also explored. Budget plans were formulated at this time as the MO had been given the FY 91 amount by then. Other trips to DR, PR, or international meetings were determined and these trips attempted to have UNL, UPR, DR, UWI, and CIAT representatives available each time. During these trips for research data collection or presentations, further interactions on budget, research, training, and publications occurred.

For FY 90 the project instituted a research proposal format. For this year DR/UNL investigators drafted a brief (one to two pages) write up that covered objectives, procedures, and budget and addressed overall project objectives. These proposals were reviewed by UPR, UWI, UNL, and DR Co-PIs resulting in elimination of some projects while others were modified and approved. CIAT also was sent a copy of the research proposals. We feel this approach has improved collaborative interactions and we plan to continue it next year.

E. Relative Contributions of Collaborating Institutions and Individuals

1. U.S. and HC contributions. U.S. contributions for FY 90 were $71,190. These contributions were based on percent time of PI, Co-PIs, and investigators devoted to the project plus benefits and overhead. HC contributions were U.S. $32,200 derived from salary and benefits of PI and investigators paid by SEA.

2. Other funding. As in most projects, there are contributions such as laboratory supplies, secretarial assistance, greenhouse supplies, labor, etc., that cannot be conveniently documented. Both U.S. and HC have coordinated integrated bean improvement programs that rely on many contributions to be successful.

   Regional Research (USDA) funding for project W-150 has given the UNL/UPR bean program support for increased involvement in national and international nurseries, germplasm exchange, and coordination of our project with other domestic bean improvement programs. The Nebraska Dry Bean Commission funds some travel to western Nebraska breeding and disease management nurseries, technical assistance for plot maintenance, and data acquisition and a few supplies. A small grant program from chemical and seed industry is used for travel to meetings and hourly technical assistance. The dollar amount estimates for these sources in FY 91 are $13,000 for W-150, $10,000 NDBC, and $3,000 from industry. In FY 90 $7,500 was available from a CSRS competitive grant on bean rust nonspecific resistance. A renewal effort for a CSRS grant in FY 91-92 is contemplated. The DR also has PROFRIJOL (Swiss-funded) support for specific bean research objectives in the HC.

   A.I.D. provides funding that allows the basic bean or legume programs of the DR, PR, and UNL to interact collaboratively on objectives that have local, regional, and international impact. The money A.I.D. provides would not be enough to fund, for example, a breeding program per se with a breeder, pathologist, entomologist, and agronomist along with the laboratory, greenhouse, and field facilities needed. The institutional bean programs have highly qualified personnel as well as facilities but lack adequate operating expenses and technical support personnel to assist in the research. The system of mutual contributions works well and is an excellent concept.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

The Mission continues to support the project. It has been suggested by them that additional support for bean research may be obtained from a research foundation that has been established in the DR. The existence of the general problem of core funding vs. mission funding means that the DR Mission cannot spend much time on our project. They have been as helpful as they can be given their goals and demands on their time. We wish they could help us with the transportation problems.

G. Evidence of Institutionalization

1. Faculty recognition. Dermot P. Coyne received the Nebraska Chapter of Gamma Sigma Delta Award of Merit in November 1989. The Nebraska bean improvement research and extension program (includes CRSP) has been nominated for the UNL-ARD-IANR Team Effort Award. F. Saladin was selected as president of PROFRIJOL project. A.K. Vidaver and J.R. Steadman were invited to participate in Biotechnology in developing countries panels convened at CIAT in Cali, Colombia and the National Research Council in Washington, DC, respectively. Dr. A.K. Vidaver was recognized with a UNL award for her efforts as Interim Director of the Biotechnology Center at UNL.

2. Integration of commodity research programs with CRSP. The UNL and UPR breeding and bean improvement programs, as well as the DR legume program, are closely involved with the Honduras and DR as well as the UWI CRSP projects. In addition, there is cooperation with USDA, Tanzanian, and MSU projects. The integration of rust resistance from the DR Pompadour landrace materials into U.S. and Honduran breeding programs and the integration of UNL derived common bacterial blight resistance into the Honduras and DR programs are examples of program integration. Disease management strategies have also been derived in the DR from basic information formulated in the U.S. and applied in the DR. Grad students from HCs have helped further basic research while in the U.S., but have taken the knowledge "home" and have begun to use it to improve HC research.

3. Internal project management. The UPR and UNL administrative support has been excellent. Both Grants and Contracts Offices have given time and expertise for the project. DR administrative support is also very good despite difficult economic conditions.

4. Student/Professor relationships. Contact between student/professor has remained after return of students to HC duties. As an example, G. Godoy returned to work with J.R. Steadman on collaborative research in July, 1990.

IV. PUBLICATIONS AND PRESENTATIONS

Aggour, A.R., D.P. Coyne, A.K. Vidaver, and K. Eskridge. 1989. Transmission of the common blight pathogen in bean seed. Journal American Society for Horticultural Science 114(6):1002-1008.

Angeles-Ramos, R., A.K. Vidaver, P. Heise, and D.P. Coyne. 1990. Isolation and properties of epiphytic *Xanthomonas campestris* pv. *phaseoli* and pectolytic *Xanthomonads* from weeds. Phytopathology (accepted, in press).

Anonymous. 1990. Bean/Cowpea CRSP. Global View, Special Edition (p. 3). Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE.

Arnaud Santana, Eladio, Estela Pena Matos, D.P. Coyne, and Anne Vidaver. 1991. Longevity of *Xanthomonas campestris* pv. *phaseoli* in naturally infested dry bean debris (*Phaseolus vulgaris*). Plant Disease (submitted).

Coyne, D.P., J.R. Steadman, D.T. Lindgren, and D.S. Nuland. 1991. Starlight, Great Northern dry bean. HortScience (accepted, in press).

Coyne, D.P., J.R. Steadman, D.T. Lindgren, D.S. Nuland, J.S. Beaver, F. Saladin, and E.A. Santana. 1990. Genetics and breeding for resistance to pathogens of beans with emphasis on those causing bacterial and rust diseases in the Dominican Republic and Nebraska. Abstract: 87th Annual Meeting of the North Central Region, American Society for Horticultural Science, Lincoln, July 25, 1990.

Coyne, D.P. 1990. Report to the Nebraska Dry Bean Commission on dry bean breeding research during 1988.

Coyne, D., J.R. Steadman, Nuland, D., and Lindgren, D. 1990. Evaluation of dry bean cultivars grown in common blight inoculated and natural infectednurseries. Biological and Cultural Tests for Control of Plant Diseases 5:77.

Diaz, Vianela, and Ramon Almanzor. 1990. Resultado de la encuesta aplicada a los productores de habichuela de la variedad "PC-50." Report. p. 1-91.

Echavez-Badel, R. 1990. *Choanephora cucurbitarum* foliar blight of dry beans (*Phaseolus vulgaris* L.). J. of Agric. of the Univ. of Puerto Rico 74(2):203-205.

Godoy, G., J.R. Steadman, and G. Yuen. 1990. Bean blossom bacteria have potential for biological control of white mold disease caused by *Sclerotinia sclerotiorum*. Ann. Rep. Bean Improv. Coop. BIC/NDBC Meeting 33:45.

Godoy, G., J.R. Steadman, M. Dickman, and R. Dam. 1990. Proof that oxalic acid is the primary determinant of pathogenicity for *Sclerotinia sclerotiorum* on bean and implications for white mold disease control. Ann. Rep. Bean Improv. Coop. Proceedings BIC/NDBC Meeting 33:47.

Godoy, G., J.R. Steadman, R. Dam, and M. Dickman. 1989. Selection and use of oxalate minus mutants to study pathogenicity of *Sclerotinia sclerotiorum* cause of bean white mold disease. Phytopathology 79:1145.

Godoy, G., J.R. Steadman, and G. Yuen. 1990. *Erwinia herbicola* and *Bacillus polymyxa*: Two blossom-resident bacterial antagonists of *Sclerotinia sclerotiorum* cause of white mold disease of bean. Annual meeting of the American Phytopathological Society, Caribbean Division, Mayaguez, Puerto Rico, May 27-31.

Godoy, G., J.R. Steadman, M.B. Dickman, and R. Dam. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. Physiol. and Mol. Plant Pathol. (in press).

Ishimaru, C., K.M. Eskridge, and A.K. Vidaver. 1990. Distribution analyses of nationally occurring epiphyte populations of *Xanthomonas campestris* pv. *phaseoli* on dry beans. Phytopathology (accepted, in press).

Jimenez, J.A., D.P. Coyne, and F. Saladin. 1989. Imbibition, germination and cooking time of seeds of dry beans (*Phaseolus vulgaris* L.) stored in different containers. Journal of Agriculture of the University of Puerto Rico 73(4):327-337.

Lindgren, D., D.P. Coyne, J.R. Steadman, and D. Nuland. 1990. Evaluation of dry bean cultivars for reactions to rust and bronzing. Biological and Cultural Tests for Control of Plant Diseases 5:78.

Mateo Solano, M., J.S. Beaver, and F.S. Saladin Garcia. 1989. Heritabilities and phenotypic correlations of morphological traits of beans. J. of Agric. of the Univ. of Puerto Rico 73(4):349-359.

Nimbaga, M. and J.R. Steadman. 1990. Adult plant rust resistance and leaf pubescence on dry beans. Ann. Rep. Bean Improv. Coop. Proceedings IC/NDBC Meeting 33:61.

Mohamed, Mohamed F., Paul E. Read, and Dermot P. Coyne. 1990. In vitro response of bean (*Phaseolus vulgaris* L.) cotyledonary explants to benzyladenine in the medium. Ann. Meeting Plant Growth Regulator Society of America, St. Paul, MN.

Mohamed, M.F., P.E. Read, and D.P. Coyne. 1990. Plant regeneration in vitro from the embryonic axis of common and tepary bean. Abstract: 87th Annual Meeting of the American Society for Horticultural Science, Tucson, AZ.

Nuland, D.S., D.T. Lindgren, D.P. Coyne, and J.R. Steadman. 1990. A cooperative system for screening dry beans for multiple disease resistance, yield, and performance in Nebraska. Abstract: North Central Region, American Society for Horticultural Science, Annual Meeting, July 25, University of Nebraska, Lincoln, NE.

Nuland, D., D. Lindgren, D. Coyne, and J.R. Steadman. 1990. Evaluation of dry bean cultivars grown in a white mold nursery. Biological and Cultural Tests for Control of Plant Diseases 5:79.

Oviedo, F., J.S. Beaver, and J.R. Steadman. 1990. Caracterizacion de la pubescencia acicular en las hojas de genotipos de habichuela. J. of Agric. of the Univ. of Puerto Rico 74(2):111-119.

Oviedo, F., J.S. Beaver, and J.R. Steadman. 1990. Characterization of trichomes on the lower surface of bean leaves. Ann. Rep. Bean Improv. Coop. Proceedings BIC/NDBC Meeting 33:90-91.

Rodriguez, Mercedes, Alfonsina Sanchez, and Estela Pena. 1990. Transmision de Mustia hilachosa (*Trianatephorus cucumeris*) frank sonk a traves de la semilla de *Phaseolus vulgaris* L. Trabajo a presentar en la reunion anual de PCCMCA XXXVI, El Salvador, Marzo, 1990.

Sanchez, A., P.R. Hepperly, R. Echavez-Badel y J.S. Beaver. 1990. Avance en la identificacion de la microflora en semillas de habichuela (*Phaseolus vulgaris* L.) de la Republica Dominicana. Paper presented at the XXX Anual Meeting of the American Phytopathological Society, Caribbean Division held in Mayaguez, Puerto Rico, May 28-31, 1990.

Shaik, M., T.A. Dickinson, and J.R. Steadman. 1989. Variation in rust susceptibility in beans: Predicting lesion size from leaf developmental stage measured by leaf age, length, and plastochron index. Phytopathology 79:1035-1042.

Shaik, M. and J.R. Steadman. 1989. The effect of leaf developmental stage on the variation of resistant and susceptible reactions of *Phaseolus vulgaris* to *Uromyces appendiculatus*. Phytopathology 79:1028-1035.

Yuen, G.Y., G. Godoy, and J.R. Steadman. 1990. Biocontrol of white mold disease of dry bean with *Erwinia herbicola*. Joint Annual Meeting of the American and Canadian Phytopathological Societies. August 8-9, Grand Rapids, Michigan.

Zaiter, H.Z., D.P. Coyne, R.B. Clark, and J.R. Steadman. 1990. Soil type and potting medium influence on rust pustule grade (size) on dry bean leaves (*Phaseolus vulgaris* L.). Ann. Rep. Bean Improv. Coop. Proceedings BIC/NDBC Meeting 33:65.

Zaiter, H.Z., D.P. Coyne, and J.R. Steadman. 1990. Inheritance of abaxial leaf pubescence in beans. Journal American Society for Horticultural Science 115(1):158-160.

Zaiter, H.Z., D.P. Coyne, and J.R. Steadman. 1990. Rust reaction and pubescence in Alubia beans. HortScience 25(6):664-665.

Zaiter, H.Z., D.P. Coyne, and J.R. Steadman. 1990. Coinoculation effects of the pathogens causing common bacterial blight, rust, and bean common mosaic in *Phaseolus vulgaris* L. Journal American Society for Horticultural Science 115(2):319-323.

Zaiter, H.Z., D.P. Coyne, R.B. Clark, and J.R. Steadman. 1990. Soil type and potting medium influence on rust pustule grade (size) on dry bean leaves (*Phaseolus vulgaris*). Abstract: 87th Annual Meeting of the American Society for Horticultural Science, Tucson, AZ.

Zaiter, H.Z., D.P. Coyne, R.B. Clark, and J.R. Steadman. 1991. Growth media and leaf nutrient concentration influence on rust pustule diameter of leaves of dry beans. Horticultural Science (accepted, in press).

# DOMINICAN REPUBLIC - UNIVERSITY OF WISCONSIN-MADISON

*MOLECULAR APPROACHES FOR THE CONTROL OF BEAN GOLDEN MOSAIC VIRUS*


**Principal Investigators:**

| | | |
|---|---|---|
| Douglas P. Maxwell (U.S.) | Department of Plant Pathology | University of Wisconsin |
| Freddy Saladin (HC) | Bean Program | CESDA/SEA |

**Co-investigators:**

| | | |
|---|---|---|
| Dermot P. Coyne | Department of Horticulture | University of Nebraska |
| James S. Beaver | Department of Agronomy & Soils | University of Puerto Rico |

**Investigators:**

| | | |
|---|---|---|
| Paul G. Ahlquist | Pathology | University of Wisconsin |
| Lee Calvert | Virology Unit | CIAT (Colombia) |
| Aridia Figueroa | Vegetable Virology | CESDA/SEA |
| Stephen F. Hanson | Pathology | University of Wisconsin |
| F. J. Morales | Virology Unit | CIAT (Colombia) |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

1. Research results. Molecular characterization of four isolates of bean-infecting geminiviruses has shown that the golden mosaic inducing isolates in the Caribbean and Central America are distinct from those in Brazil and that separate breeding programs for disease resistance should be developed for these two regions.

   Bean dwarf mosaic geminivirus (BDMV) DNAs A and B were sequenced and sequence comparisons with other geminiviruses showed that BDMV is a distinct bean-infecting geminiviruses and is not closely related to other geminiviruses.

   General and isolate-specific geminiviral DNA probes have been developed. These probes were used to detect geminiviruses in bean, weeds, and other crop plants from Argentina, Brazil, Costa Rica, Dominican Republic, Egypt, El Salvador, Honduras, Puerto Rico, South Africa, and U.S. Agdia, Inc., a diagnostic laboratory in Indiana, has recently made arrangements with the Wisconsin Alumni Research Foundation to evaluate our general geminiviral probe for use in their commercial laboratory. Our results indicate that weeds are not a major source of BGMV inoculum in the Dominican Republic. Use of our general probe showed that tomatoes in Florida and Costa Rica were infected with a whitefly-transmitted geminivirus. Also, we found that several ornamentals were infected with geminiviruses in Florida and Utah.

In collaboration with Agracetus, Inc., clones of the four geminiviral isolates were found to be infectious by inoculating bean radicles with cloned DNA using electric discharge particle acceleration methods (particle gun). It was not possible to infect beans with cloned DNA by standard mechanical inoculation methods. This makes it possible to initiate mutational analysis studies to develop virus-derived schemes for the creation of transgenic plants with resistance to BGMV.

Sequence alignments of the putative replicative gene for the sequenced geminiviruses indicated that there may be an ATP binding site in this protein; and thus, this catalytic site is an appropriate target for evaluation of the dominant lethal scheme for creation of transgenic beans resistant to BGMV and other geminiviruses, e.g., the tomato geminivirus causing problems in Florida, Middle-East and Central America.

Scientists at Agracetus, Inc. have had some initial success transforming bean tissue using a modification of the methods they employ for soybean transformation.

Polymerase chain reaction methods were used to show that the cloned BGMV-DR DNAs are representative of the BGMV in the Dominican Republic.

Clones of BGMV-GA were used by Agracetus, Inc. to explore the possibility of constructing a minichromosome to assist in the transformation of soybeans.

Bean germplasm was evaluated for the Dominican Republic and CIAT. It was found that two landraces (Pompadour G and Pomedadour J) from the Dominican Republic, which were thought to be resistant, were susceptible and did not give typical golden mosaic symptoms when infected with BGMV-DR. Also, there was a correlation between symptom development to BGMV-DR and moderate levels of field resistance to BGMV.

Several bean cultivars immune to BDMV were detected and these will be used in an inheritance study of factors controlling disease resistance to geminiviruses.

2. Other research-related results

**Germplasm conservation and use.** Pompadour J and Pompadour G were collected by F. Saladin as part of the University of Nebraska CRSP and these were evaluated in the Dominican Republic, Puerto Rico and Wisconsin. This seed has been provided to CIAT.

**Impact of other CRSP-produced technologies.** Some interest has been expressed by researchers in the use of the pathovar specific DNA probes for the detection of *Xanthomonas campestris* pathovar *phaseoli*. These probes will be provided to CIAT , University of Nebraska, and University of Puerto Rico for future studies on variability in *X. c.* pathovar *phaseoli*.

**How results address needs of small-scale farmers and women.** Technology is being developed which will provide better control strategies for one of the major constraints to bean production in Latin America. This will have a positive impact on yield and quality of beans. Every effort is being made to provide technical training for women. Nine women have received training in biotechnology during FY 90.

**Other research results of importance.** The coat protein region of the bean common mosaic potyvirus, an RNA virus, was cloned.

Restriction fragment length polymorphism analysis of 26 isolates of *Xanthomonas campestris* pv. *phaseoli* and xcp var. fuscans indicated that considerable genetic variability exists in this pathovar. This should be considered when cultivar are evaluated for disease resistance.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. The University of Nebraska CRSP project is coordinating a baseline survey of bean production in the Dominican Republic to use in an assessment of the impact of PC 50, which is being increased for future release to growers.

B. Institutional Development and Training

It should be noted that this project has not had funds for training of HC personnel and funds have been available for one postdoctoral fellow or scientist. Other funds have been sought for training personnel and nine individuals have received training this year. Also, there has not been a host country budget for this project.

1. Changes since FY 89. Since FY 89, Ms. Maria Rojas (funded on a USAID scholarship from Costa Rica) has started an M.Sc. degree program, Ms. Ann Batista, a Ph.D. student in Molecular Biology did a rotation in our laboratory, Ms. Eunice Zambolim (funded by the Brazilian Government) is receiving special training in molecular virology of viruses, Ms. Mercedes Otoya from CIAT received three-months training on RFLP analysis, and Ms. Mary Bett (funded by a State research assistantship) is a Ph. D. graduate student who started a rotation in our laboratory in August. Considerable effort was devoted to bring Lic. Aridia Figueora from the Dominican Republic to our laboratory for three-months, but she could not get a visa because she was on the immigration list.

2. Over the life of the project. A PSTC/USAID grant provides funds for Ms. R. Teresa Martinez from the Dominican Republic to receive training in virology and vegetable crop pest management. Efforts are underway as indicated by a letter from Director Metz to have her hired at CESDA. She is funded by a PSTC/USAID grant until December 1991.

3. Project training to be completed by the end of the project period. Ms. R. Teresa Martinez from the Dominican Republic (2.5 years, funded by PSTC/USAID). Ms. Mercedes Otoya from CIAT (3 months, funded by CIAT). Ms. Denise Smith from the U.S. will complete an M.Sc. (funded by State and Hatch). Ms. Elisabeth S. Hidayat from Indonesia will complete an M.Sc. (funded by the World Bank). Ms. Eunice Zambolim from Brazil (one year funding by Brazilian government) will have received training in molecular virology of RNA plant viruses. Dr. Robert L. Gilbertson (funded by CRSP) from the U.S. will have received two years training and he has accepted a faculty position at University of California-Davis. He is also Co-PI on the Malawi CRSP project. It is expected that Dr. Wayne McLaughlin from the University of West Indies will receive three months training in the summer of 1991.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

An intensive research program on BGMV was initiated in January 1987 when Dr. Gilbertson went to the University of Florida to receive training on methods for mechanical transmission of geminiviruses.

Cloning and sequencing BGMV-BZ, BGMV-GA, BGMV-DR and BDMV-CO: This has been achieved. Development of general and isolate-specific probes for above listed bean-infecting geminiviruses (four): This has been achieved and manuscript has been accepted for publication in Plant Disease. Develop a BGMV/protoplast system for virus replication studies: This has not been achieved and may not be necessary since the full-length clones can be used to infect plants using the particle gun. BGMV DNA modified by site-directed mutagenesis to form a dominant lethal: These experiments are underway for site direct mutagenesis of the putative ATP binding site in the AL1 gene product.

The research is on schedule as a result of an extremely hard effort by personnel involved in the project and the expenditure of non-CRSP funds at about 300 percent of the CRSP funds. It is estimated that the first dominant lethal scheme for plant virus resistance will be evaluated in a model system by the end of this extension period. Transgenic bean plants with the viral coat protein gene will be available for evaluation in the five-year extension period.

Our project is the major research project in the world on bean-infecting geminiviruses and it is not duplicated anywhere. Our efforts compliment those at CIAT as CIAT scientists they have done the initial characterization of the geminiviral isolates, BGMV-GA, BGMV-DR, and BDMV-CO, and we have then done the molecular characterization of these isolates. All our research efforts are coordinated with Dr. Francisco Morales, CIAT virologist. He collects plant samples on his trips to Africa and South American and then we probe these samples with our isolate-specific geminiviral DNA probes. This allows CIAT personnel to better understand the range of genetic diversity in bean-infecting geminiviruses and allows them to plan their breeding programs. Our Wisconsin team also works closely with Dr. Judy Brown, University of Arizona in the characterization of bean calico mosaic geminivirus. She completed the host range and transmission studies and our group cloned part of the virus. Our studies on germplasm evaluation and inheritance

studies are closely coordinated with the University of Nebraska/DR CRSP project and University of Puerto Rico/Honduras CRSP project. We have also provided geminivirus identification for scientists in Egypt, Costa Rica, and South Africa. These scientists did not have access to someone who was willing to help them with geminivirus identification.

The most closely related geminiviruses research project is at Washington University, St. Louis and directed by Dr. R. Beachy. His research team has recently obtained a detectable level of resistance to tomato yellow leaf curl geminiviruses in transgenic Nicotiana benthamiana plants expressing viral coat protein.

Our characterization of bean-infecting geminivirus has provided clear evidence that breeding programs need to be designed for specific regions, e.g., Brazil or the Caribbean and Central America. Also, the use of our isolate-specific probes have shown that weeds may not be the main source of inoculum in the Dominican Republic. Also, our general probe for whitefly-transmitted geminiviruses is being developed commercially by Agdia, Inc. The research on dominant lethals may provide the basis for a new approach for the control of geminiviruses and other viruses. Stimulation of research on the development of a bean transformation system using biolistic approaches may have considerable impact on future development of beans. Once transgenic beans are available with resistance to geminiviruses, it is expected that this technology could be applied to cultivars for many regions and these resistant cultivars will have a significant impact on bean yields in Latin America (potentially increasing yields by 10-20 percent).

D. Evidence of Biological/Social Sciences Integration

Our project has made a major effort in the training of women in biotechnology and this past year, nine women have received training on this project. Only two men have received training.

In our field trip to the Dominican Republic, R. Teresa Martinez discussed the growing of beans which were developed by transformation technology with several individuals at various levels, e.g., seedsman, experimental station workers, growers, and administrators. There was not a concern as long as the transgenic plants were better than current beans.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

Since we started working on molecular characterization of bean common mosaic potyvirus (an RNA virus), we received virus isolates from Dr. M. Silbernagel (Washington State CRSP) and Dr. J. Kelly (Michigan State CRSP). Also, Dr. Roy French, USDA scientist at the University of Nebraska, has been very helpful. Our efforts on RFLPs of the common bacterial blight pathogen involved Dr. M. Pastor-Corrales, bean pathologist from CIAT. He supplied the isolates for the study and a paper will be published jointly with him and his technician, M. Otoya. We also have started to process samples for Ing. Roger Meneses, CATIE, Costa Rica for the detection of geminiviruses in vegetables.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

Since no HC funds have been available, this has created a research program in which very little responsibility has been associated with the HC. Funding level by CRSP has been extremely low for the expected output in the area of molecular biology research and training.

### B. Adequacy of Current Management System

Last November, a three person review panel visited our geminivirus team at the University of Wisconsin and reviewed all aspects of the project. It is pleasing to note that our project received the highest rating along with several other projects by the EEP.

### C. Activity Towards Buy-Ins or Other Funding

Dr. Maxwell participated in the CRSP effort to establish a research project on beans with the Agricultural Research Center, Giza, Egypt. This is likely to result in a project funded by USAID with emphasis on biological nitrogen fixation. Recently, a trip to Costa Rica was made to discussion geminivirus research with people at CATIE, the Universidad de Costa Rica, and the USAID Mission. It is hoped that Costa Rica will become an HC in the next extension period. Additional funding for this project was obtained from the following sources in FY 90: Hatch (support for half-time specialists, $10,000), University of Wisconsin Graduate School (three months of a visiting scientist, $5,000), State funds (one research assistantship, $12,500, and one half-time specialist, $10,000), USAID Scholarship from Costa Rica (one research assistantship, $12,000), PSTC/USDA grant ($39,000 for visiting scientists, and trainee), CNPQ from Brazil (one trainee, about $6,000), and World Bank (one research assistantship, $18,000). It is also important to note that Agracetus, Inc. donated all the time and resources for sequencing the component A of BDMV and for the particle gun experiments with the viral cloned DNAs and they have make a major commitment to bean transformation (estimated contribution over $20,000). This is a total of $132,500 in direct costs or $190,800 including indirect costs. The CRSP budget for last year was about $78,000 for direct and indirect. Thus, there is about 2.5 times as much support from non-CRSP sources and this does not include faculty salaries.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

The Global Plan addresses the need to improve the living conditions of small farm producers in LDC's and to increase the availability of low cost nutritious foodstuffs for rural and urban poor in LDC's. Our effort involves the collaboration of scientists from Brazil, Dominican Republic, CIAT, Agracetus, Inc., University of Puerto Rico, University of Nebraska, and University of Wisconsin-Madison. The focus of the project is on the molecular characterization of the geminiviral pathogens causing diseases of bean. From our sequence data, it is

evident that two different strains of BGMV occur in Latin America and that other bean-infecting geminiviruses are present in Colombia and Mexico. This would indicate that breeding programs for resistance need to be continued for these different strains and geminiviruses. The general geminiviral DNA probe has been used to detect geminiviruses in weeds and other plants, e.g., tomatoes.

The isolate-specific probes for the different geminiviruses can be used to determine if weeds are a main source of inoculum. This information can then be used in designing better control strategies.

Major effort will be devoted to using recombinant DNA technology to develop beans resistant to BGMV. Considerable progress was made when we found that the full-length clones of the four geminiviral isolates were infectious. This will allow studies on genome function and the development of virus-derived schemes for creation of transgenic beans. Additionally, Agracetus, Inc. is developing a bean transformation using modifications of the soybean technology which they have developed. If successful, these methods could dramatically increase the availability of adapted bean lines with resistance to a major constraint to production in Latin America.

Through the efforts of CRSP, PSTC, CIAT, CESDA-Dominican Republic, Agracetus, Inc., University of Puerto Rico, University of Nebraska, and University of Wisconsin, the largest research program devoted to basic studies on this bean golden mosaic viral complex has been organized and will lead to new understandings of this disease and improvements in disease control.

B. Balance Between Research and Training

If funds for Dr. Robert L. Gilbertson, an Assistant Scientist, are assigned to training, then 60 percent of the direct costs of the CRSP are for training. Our project has never had an adequate training component and other resources have been sought. Thus for FY 90, funds for three M.Sc. students, three trainees and one visiting scientist were obtained from other sources. See section II.C. for additional details.

The training program for FY 90 was essentially as expected in FY 89 except Maria Rojas joined our program form Costa Rica and we didn't know for certain if this was going to work out.

C. Balance of Domestic vs. Overseas Activities

Because of the highly technical nature of the research, which involves techniques associated with molecular biology and special resources, the major research effort has been at the University of Wisconsin and Agracetus, Inc. This year more effort was devoted to collecting field samples in the Dominican Republic than in the past; and it is expected that additional experiments on whitefly transmission of geminiviruses in weeds to beans can be undertaken in the coming year. The field research for the evaluation of germplasm is conducted in the Dominican Republic in cooperation with the University of Nebraska and University of Puerto Rico.

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Drs. Maxwell and Gilbertson and Ing. Teresa Martinez visited the Dominican Republic in February to collect samples and arrange for future research. In April, Ing. Freddy Saladin, HC PI, visited the University of Wisconsin and plans were discussed for future research and budgets. Since funds for a HC budget have not been available, only minimal effort has been devoted to this process. Our project serves as the major research project on bean-infecting geminiviruses for Latin America and thus research priorities are decided by the team which includes CIAT personnel. In July, Dr. James Beaver, Dr. Morales (CIAT), Dr. Silbernagel (WSU), and Dr. Pastor-Corrales (CIAT) visited our laboratory to discuss future research. These visits have provided opportunities for the international scientists to interact directly with the geminivirus team and scientists from Agracetus, Inc.

E. Relative Contributions of Collaborating Institutions and Individuals

| | |
|---|---|
| USAID/CRSP: | |
| Direct costs | $53,884 |
| (Funds for assistant scientist, supplies, and travel) | |
| Indirect costs | $23,709 |
| TOTAL | $78,000 |
| | |
| U.S. - University of Wisconsin: | |
| Specialist | $10,000 |
| Research Assistantship | $12,500 |
| Graduate School | $ 5,000 |
| TOTAL | $27,500 |

Salary for Drs. Maxwell and Ahlquist provided by the University of Wisconsin and are part of the matching funds ($18,877). Other sources of funds discussed in section II.C.

HC: HC funds were used for support of HC PI and Aridia Figueroa (about 5-10 percent of their time was devoted to CRSP activities for this project).

Other funding:

| | |
|---|---|
| Funds spent at UW-Madison (direct costs): | |
| Hatch (used for a half-time specialist) | $10,000 |
| | |
| PSTC/USAID (used for support of trainee, supplies and travel) | $36,000 |
| CIAT (used for support for Ms. M, Otoya, CIAT for 3 months) | $ 6,000 |
| USAID Scholarship from Costa Rica (M.Sc. graduate student) | $12,000 |
| CNPQ, Brazil (trainee from Brazil) | $ 6,000 |
| World Bank-Indonesia program | $18,000 |
| Gift funds (travel) | $ 2,000 |
| TOTAL | $90,000 |

Estimate of funds provided by Agracetus, Inc.: Scientists at Agracetus sequenced BDMV component A and provided the primers needed for sequencing component B. They also provided the resources that we used to show that our viral clones from four isolates were infectious.

Estimated value: $20,000

Importance of these funds: Non CRSP funds provide about 65 percent of the financial resources needed for us to achieve the current level of training and research activities. The contribution of Agracetus, Inc. can not be over emphasized. Without their help, we would not have known that our viral clones were infectious and a considerable effort (one to two years) may have been devoted to this activity rather than four months. We are now in a position to start a mutational analysis of the viral genome.

Also, Agracetus, Inc. has started an effort to transform beans with the coat protein gene from BGMV-GA. They were not interested in bean transformation until we started working with them in August 1989.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

We have always visited USAID mission personnel during our trips to the Dominican Republic and had a useful exchange of information. Unfortunately, the USAID personnel had to refuse permission for Lic. A. Figueroa to come to our lab for training because she is on an immigration list. This is a USAID policy.

G. Evidence of Institutionalization

1. Faculty recognition. Dr. Maxwell has been appointed to serve on the College's International Agricultural Program Committee and his CRSP project activities will be featured in the International Ag Newsletter from UW-Madison. Ag administration and departmental faculty have been very understanding of Dr. Maxwell's need to devote time to this CRSP project. As Departmental Chair, this has caused some stress on other faculty and office staff.

2. Integration of commodity research programs with CRSP. Dr. Maxwell had approved a new HATCH project on "Development of transgenic *Phaseolus vulgaris* plants with resistance to geminiviruses" and he also contributed to the preparation of the new W-150 regional project on Genetics and Breeding Beans for Improved Disease Resistance and Yield.

   Internationally, Dr. Maxwell was appointed to the newly formed Steering Committee of the Advanced Bean Research Network in September 1990 at an international workshop at CIAT, Cali, Colombia. He will be representing the CRSP Technical Committee.

3. Internal project management. Dr. Maxwell does all the administrative management of the project and with Drs. Gilbertson and Ahlquist, has formulated research direction policies. Dr. Gilbertson has been the day-to-day advisor for the research on BCMV, Xcp and PCR technologies.

Dr. K. Shapiro, Director of International Programs for the College, was recently assigned the position of Institutional Representative. He has been extremely supportive and has provided some administrative personnel to help with paperwork associated with USAID trainees. He also visited our research facilities and discussed the CRSP research program in considerable detail. He has also been supportive of Dr. Maxwell's travels to Egypt and Costa Rica.

4. Student/Professor relationships. Dr. Maxwell has had a challenging year as "mentor" to so many Latin women and U.S. women students. He devotes as much time as he can to their special educational and social needs. An undergraduate student, Amy O. Loniello, supported by CRSP won a research award scholarship for her research proposal on characterization of bean calico mosaic geminivirus and she also received the first D. J. Hagedorn Fellowship in plant pathology. (Dr. Hagedorn started this Bean/Cowpea CRSP project in Brazil in 1982 and he established a scholarship fund when he retired.)

H. Other Comments

Dr. Maxwell will resign as Departmental Chair on June 30, 1991 so that he can devote more time to the research and training activities associated with this CRSP project.

Much of the success of this project has been the result of Dr. Robert L. Gilbertson's efforts. He departed October 1, 1990 for UC-Davis and he will be a Co-PI on the Malawi CRSP project. Our team will miss him in many ways.

## IV. PUBLICATIONS AND PRESENTATIONS

Publications

Gilbertson, R. L., S. H. Hidayat, R. T. Martinez, S. A. Leong, J. C. Faria, F. J. Morales, and D. P. Maxwell. 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Disease (In Press).

Gilbertson, R. L., J. C. Faria, S. F. Hanson, F. J. Morales, P. Ahlquist, D. P. Maxwell, and D. R. Russell. 1990. Infection of beans (*Phaseolus vulgaris*) with cloned geminiviral DNA mediated by electric discharge particle acceleration. Phytopathology (submitted for publication).

Gilbertson, R. L., R. E. Rand, and D. J. Hagedorn. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. Plant Dis. 74:322-327.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, P. G. Ahlquist, F. J. Morales, D. P. Maxwell, and D. R. Russell. 1990. Infection of beans (*Phaseolus vulgaris*) with cloned geminiviral DNA mediated by electric discharge particle acceleration. Ann. Report Bean Improvement Cooperative (BIC) 33:165-166.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, E. Hendrastuti, P. G. Ahlquist, F. J. Morales, and D. P. Maxwell. 1990. Relationships among bean-infecting geminiviruses. Ann. Report BIC 33:72-73.

Gilbertson, R. L., E. Hendrastuti, R. T. Martinez, and D. P. Maxwell. 1990. The application of nucleic acid probes for the detection of plant pathogens. Proceedings of the 82nd Annual Quebec Society for Plant Protection (In Press).

Gilbertson, R. L., and D. P. Maxwell. 1990. Common bacterial blight of bean. Chapter for Plant Diseases of International Importance. (In Press).

Presentations

Faria, J. C., R. L. Gilbertson, S. F. Hanson, P. Ahlquist, and D. P. Maxwell. 1990. Variabilidade do virus do mosaico dourado do feijoeiro e uso de sondas para a sua characterizacao. Brazilian Bean Research Meeting, May 1990.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, E. Hendrastuti, P. G. Ahlquist, F. J. Morales, and D. P. Maxwell. 1989. Relationships among bean-infecting geminiviruses. Bean Improvement Cooperative Meeting, November 1989, Toronto, Ontario, Canada.

Gilbertson, R. L., E. Hendrastuti, R. T. Martinez, and D. P. Maxwell. 1990. The application of nucleic acid probes for the detection of plant pathogens. 82nd Annual Quebec Society for Plant Protection, June, 1990, Quebec, Canada.

Gilbertson, R. L., E. H. Hidayat, and D. P. Maxwell. 1990. DNA probes for detection of plant pathogens. Workshop at the American Phytopathological Society, August, Grand Rapids, MI.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, F. J. Morales, P. Ahlquist, D. P. Maxwell, and D. R. Russell. 1990. Infection of beans with cloned geminiviral DNA mediated by electric discharge particle acceleration. Annual Meeting of the American Phytopathological Society, August, Grand Rapids, MI.

Faria, J. C., R. L. Gilbertson, F. J. Morales, D. R. Russell, P. G. Ahlquist, S. F. Hanson, and D. P. Maxwell. 1990. Sequence of infectious clones of two mechanically transmissible isolates of bean golden mosaic geminiviruses. Annual Meeting of the American Phytopathological Society, August, Grand Rapids, MI.

Maxwell, D. P. 1990. Biotechnology and International Agriculture. Biotechnology Workshop for Industry, March 1990,. Milwaukee, WI.

Maxwell, D. P. 1990. Molecular biology of bean-infecting geminiviruses. Advanced Research Workshop, September 1990, CIAT, Cali, Colombia.

Gilbertson, R. L. 1990. Biotechnology and implications for research on bean diseases of beans. Advanced Research Workshop, September 1990, CIAT, Cali, Colombia.

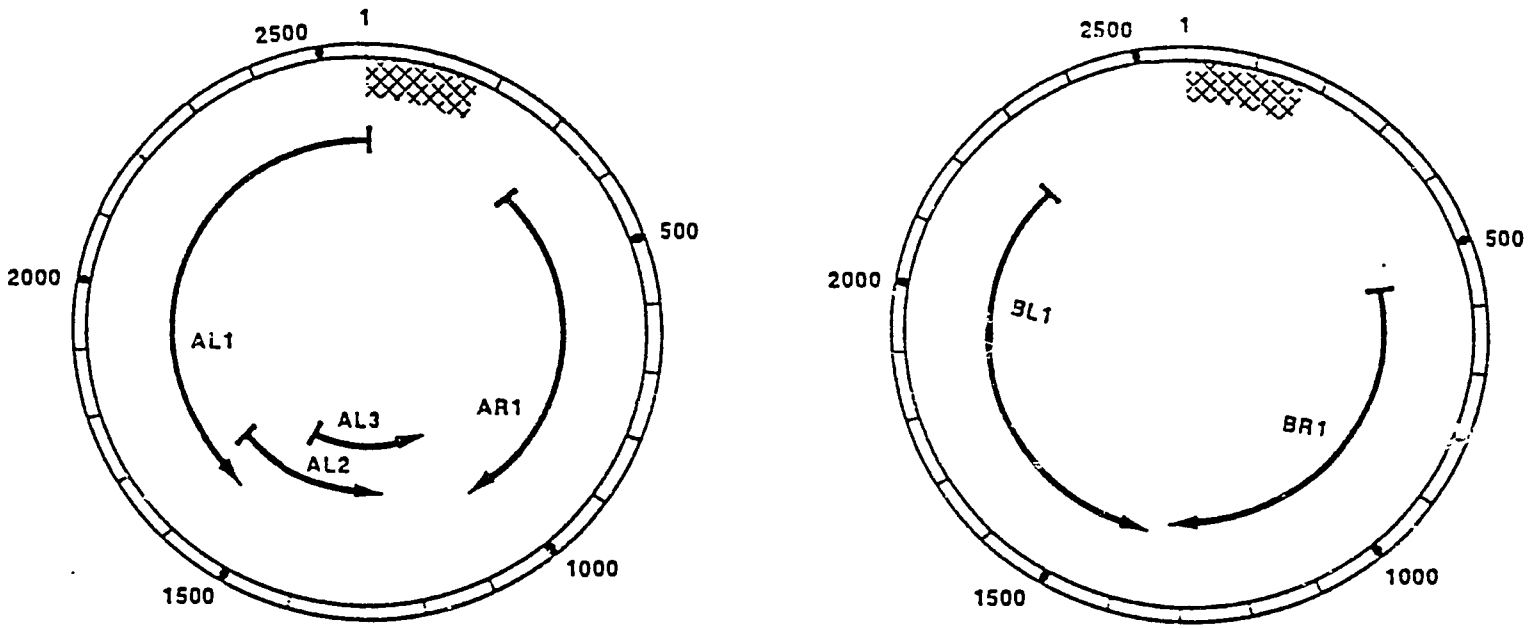



Fig. 1. Schematic map showing common region (cross hatched region starting in position number 1), open reading frames (genes, e.g. AR1), for the replicative forms (component DNA A, left circular DNA molecule, and component DNA B, right circular DNA molecule) of bean dwarf mosaic geminivirus isolate from Colombia. This map is based on computer asisted analysis of sequence data.

Fig. 2. Sequence of bean dwarf mosaic geminivirus DNA A.

```
   1  TGGCATTTTT GTAATAAGAG CTGGTACTCC AGTTGAGTTA CTCCAATTCC
  51  CCCCTCTCAA AACTATCTCA TTCTATTGGA GTATTGGAGT TACTTATATA
 101  CTAGAACCCT CAATCTGGTT TCGGAACACG TGGCGGCCAT CCCTATAATA
 151  TTACCGGATG GCCGCGCTTC GGAGTACGCT CTCTCTCTCC CTTTAATTTG
 201  AATTAAAGCG CACTGCTTTC GTCTCAGCCA ATCATATTGC GCCTGACGAG
 251  CTTAGATATT TATAACAACT TGGGCCCTAA GTTGTTGGTT GTACGATATA
 301  AATTAAAGCT AACCCGGCCC ACTGTCTTTA ACTCAAAATG CCTAAGCGCG
 351  ATGCCCCATG GCGCTCTATG GCGGGAACGA CAAAGGTCAG TCGCAATGCC
 401  AATTACTCTC CCCGTGGGGG AATTGGGCCA AAGATGACAA GGGCCGCAGA
 451  GTGGGTTAAC AGGCCCATGT ACAGGAAGCC CAGGATCTAT CGAACGCTAA
 501  GGACGCCTGA CGTCCCACGA GGTTGTGAAG GCCCATGTAA GGTGCAGTCT
 551  TATGAACAGC GTCACGATAT TTCACATGTT GGGAAGGTAA TGTGTATCTC
 601  TGATGTCACA CGTGGTAATG GCATTACTCA CCGTGTTGGC AAGCGTTTTT
 651  GTGTTAAGTC TGTGTATATT CTAGGAAAGA TTTGGATGGA TGAGAATATC
 701  AAGCTCAAGA ACCACACGAA CAGTGTTATG TTCTGGTTGG TCAGGGACCG
 751  TAGACCGTAT GGAACGCCCA TGGATTTCGG CCAGTTGTTC AACATGTTTG
 801  ACAACGAGCC CAGCACTGCC ACGGTTAAGA ACGATCTTCG CGATCGTTTT
 851  CAAGTTATGC ATAAGTTCTA TGGGAAAGTC ACAGGTGGAC AGTATGCGAG
 901  CAATGAACAG GCAATCGTCA AGCGTTTTTG GAAGGTCAAC AATCATGTGG
 951  TTTACAATCA TCAAGAGGCT GGCAAGTATG AGAATCATAC GGAGAACGCC
1001  TTATTATTGT ATATGGCATG TACACATGCC TCTAATTCTG TGTATGCAAC
1051  TCTGAAAATT CGGATCTATT TTTACGATTC GATCATGAAT TAATAAAGTT
1101  TGAATTTTAT TGAATGATAT TCTATTACAT GAGTTACATA CGATCTGTCT
1151  GTTGCGAATC GAACACCTCT AATGACATTG TTAATGGATA TAATTCCTAA
1201  TTGATCTAAA TACATAATAA CTAAATGTTT AAATCTATTT AAATAAGTCG
```

Sequenc of BDMV DNA A continued.

```
1251  ACCCAGAAGC TGTCGTCGAT ATCGTCCAGA CTTGGAAGTT CAGGAAGGCT
1301  TTGTGGAGAT GCAACGCTCT CCTCAGGTTG TGGTTGAACC GTATCTGTAC
1351  GCTGTATACC CTGCTGTTGG TGTACAACGG TTCCTCTACT CTGTATATCT
1401  TGAAATAGAG GGGATTTTCT ATCTCCCAGA TATACACGCC ATTCTCTGCT
1451  TGAAGTGCAG TGATGAGCTC CCCTGTGCGT GAATCCATGT CCCGTACAAC
1501  CTATGTGTAT GTAGATGGAG CAACCCCATT CCAGGTCAAT GCGGCGTCTC
1551  CTGATCGCCC GCCGCTTGGC TTGCCTGTGT GCCTTCTTGA TAGAGGGTGG
1601  AGTCGAGAGT GATGAAGATT GCATTCTTCA ACGTCCAATT CCTTAGCGCT
1651  GTATTTTCCT CTTTGTTTAG GAAATCTTTA TAGCTGGCAC CCTCACCAGG
1701  ATTGCATAGC ACGATTGATG GGATCCCTCC TTTAATTTGA ACAGGCTTGC
1751  CGTACTTGCA ATTTGACTGC CAATTCTTTT GGGCCCCAAT CAATTCCTTC
1801  CAGTGCTTTA ACTTTAGATA ATGCGGTGCG ACGTCATCAA TGACGTTATA
1851  CTCCACTTCG TCTGTGAACA CTTTGGAATT GAAGTCTAGG TGTCCACTTA
1901  AATAATTATG TGGGCCTAGT GCTCGAGCCC ACATCGTCTT CCCTGTTCTC
1951  GAATCACCTT CTACTATGAG ACTTACTGGT CTTTCTGGCC GCGCAGCGGA
2001  ACCTCTCCCG AAATAATCGT CTGCCCACTC TTGCATCTCG TCTGGAACGT
2051  TAGTAGACGA AGAGAGGGGA AACGGAGGAA CCCATGGTTC CGGAGCCTTT
2101  TTGAATATGC TGGTGGCGTT AGCGACCAGG TTGTGATGCT GAAGGAAGAA
2151  ATGTTGCGGT TGTTCTTCCT TTATTATGCG CAGAGCTTGC TCTGCGGATC
2201  CTGCATTTAA CGCCTTTGCG TATGTATCGT TAGCAGACTG CTGACCTCCT
2251  CTAGCAGATC TGCCGTCGAT TTGAAACACT CCCCATTCGA CGGTGTCGCC
2301  GTCTTTGTCG ATGTAGGACT TGACGTCGGA GCTGGATTTA GCTCCCTGAA
2351  TGTTCGGATG GAAATGTGCT GACCTGGTTG GGGAAACCAG ATCGAAGAAT
2401  CTGTTATTCG TGCATTGGTA TTTACCTTCG AACTGGATGA GGACGTGCAG
2451  ATGAGGTTCC CCATCTTCGT GTAATTCCCT GCAAATCTTG ATGAATTTCT
2501  TGTTAACTGG AGTTTTTAGG TTTTGGATTT GGGAAAGTGC TTCCTCTTTA
2551  GTAAGAGAGC ACTGTGGATA TCTGAGGAAA TAGTTCCTCG ATTGAACTCT
2601  AAATTTCTTA GGCGG
```

Fig. 3. Sequence of bean dwarf mosaic geminivirus DNA B.

```
   1  TGGCATTTTT GTAATAAGAG ATGGTACTCC AGTTGAGTTA CTCCAATTCC
  51  CTCCCCTCAA AACTATCTCA TTCTATTGGA GTATTGGAGT TACTTAAATA
 101  CTAGAACCCT CAATCTGGTT TCGGAACACG TGGCGGTCAT CCGTATAATA
 151  TTACCGGATG GCCGCGCTTC GGAGTACGCT CTCTCTCCTC TCCCCTGGTG
 201  CGTTCCTGGT CCCCTGCCAC CTGCCACTCT CCTAGTGGAT GGTCGCTCGT
 251  CTTTTCCTGC GAGTTGTGGG CCGTAGTTTG AATGATTAAT CTTTAATTTA
 301  AATTAAAGAT GA TTTTTACA TGTCGCGCGA TCTCATTTGA ATCTTGAATA
 351  ATTGTCTCGC GGTTCATGAC TACGGCCCAC TGTACTATAT AATGGACGTG
 401  GCTGATTTTA GACCATGCTG CTATGTCTAT TTATTCAATT TGAACAACCA
 451  TTTTCTATAA ATGAGGACGG ACCATACATT CCATTCAAGC TGACTCAGCT
 501  GTCAACGACG TGAAAATATC TATTTGTATT GGTATTATCG CCTAATAATG
 551  TATGGTTTGC GGAATAAACG TGGTTCATCG TTCAGCCATC GCCGATTTTA
 601  TTCACGTAGC AGTTTTTTAA ATCGCTTGTC CGCTAATAAG CGTCATGATG
 651  GCAAACGTCG AGCTATGAAT CCTAGTAAGC CCATTGACGA GCCCAAGATG
 701  TCAGCCCAAC GCATACATGA GAACCAGTAT GGGCCTGAAT TTGTAATGGC
 751  CCATAATTCA GCCATTTCTA CGTTTATCAG CTACCCCAGC AAGGGCAAGA
 801  TGGAACCCAA CCGATCGAGG TCCTATATTA AGTTGAAACG ACTTCGTTTC
 851  AAAGGGACTG TCAAGATTGA TCGTGTTCAA CCAGATATGA ACATTGACGG
 901  TTCTGCCCCA AAAGTGGAAG GAGTGTTCTC TCTGGTGGTT GTTGTGGATC
 951  GTAAACCCCA CTTGGGTGCG TCTGGATGCC TGCATACATT CGACGAGCTG
1001  TTCGGTGCAA GGATCCATAG CCATGGTAAT CTCAGCATAA CACCCTCTTT
1051  GAAAGACCGA TTCTACATAA GACACGTGTT CAAACGTGTA TTGTCCGTGG
1101  AGAAGGATAC GATGATGGTT GACGTGGAAG GATCTACATC GCTCTCTAAC
1151  AGGCGATATA ATTGTTGGTC CACTTTTAAG GATCTTGACC ATGAGTCATG
1201  CAAGGGTGTT TATGACAACA TCAGCAAGAA CGCCCTCCTA GTATATTACT
```

Sequence of BDMV DNA B continued.

```
1251  GTTGGATGTC AGATACTATG TCAAAGGCAT CTACTTTTGT ATCGTTTGAC
1301  CTTGATTATA TCGGTTGATT AATGATAATT GTAATAAAAA GCTATTATTG
1351  AACTTTCAAT TCCTCAACAA AGAAATTATT GCAACGATTT GGGCTGATAA
1401  GCTTACAGTT ACTATTTATA CACTCCTGGA CAGTGTTTTT CACTAGCTCG
1451  TTTAATTGCC CCATCGACAT AGTAATGTTG GATTCCGCTC TCTGGGCCCC
1501  TACAATTGAG GCAGACTCCC CTGGGTCTAA GACGCTTGTT CCAAGCCTGC
1551  TGAGATGCCT ATATGGATGC ATTGCGTTTT CCACCTCTGA GTCGGCATCG
1601  GAGTTGCTGA GCCCAATTGT ACTCCGTGAA GCCCATGATT CACCCGGCTT
1651  GATCTCTATT GGGCCTGGTA GTCCAATCCT TGACATGGAT GCGCATCTTA
1701  TGGGTTTCCT TTCCCATCTT CCGTAGTCGA CATGTGAAAA GTCGACATCT
1751  TTATCTGTGA ACTGTTTCGA CAGGATCTTT ACTGTCGGTG CCCGGAAGGG
1801  TATATCCACG GAGTGTTTCG CCGTCGATAG TTTCAGTTTC CCTTTGAACT
1851  TGGCGAAGTG GGTCCTCTGA TGAACATTCG TATCGCAAAC CCTATAATAG
1901  AGTTTCCATG GAATTGGGTC TTTCAACGAG AAGAACGAAG CCGAGAAATA
1951  ATGTAGATCT ATGTTGCATC TGATTGGAAA TGTCCAGGAA GCCTGCAGCG
2001  ATTCGTTGTC TGTCATTCGC TTGTCGTGGA TCTCGACAAT GACCGACCCT
2051  GTCGCGTTAA TCGGCACTTG TTGCCTGTAC TCTATGACGC AGTGGTCGAT
2101  CTTCATGCAG CTACGGCTCA GCCTAGCTGT TAACTGCGAC GCCGTGGAAG
2151  GGAATTGCAG AATTATCTCA GTGAGGTCAT GAGAAAGTTG ATATTCGTCC
2201  CGATGGGACT CTATGTAGTT AAATGCGTTG GGAGGATTGA CCAATTGAGA
2251  ATCCATATAA AGAATAATGG CCGCGCAGCG GAACCGATTG CTGAAGTTGA
2301  ACTGGTGAAG AAGAATTTAG GC TGTAATT GAAGAACAAA TGATGAACTG
2351  TTCTTGAATG TGGAGAGGGT TTCTGGGAAA CTCAGAAAGT TTGTAAAGAA
2401  ATTGATGAAC AGTTGTTGAA CTTCTGCTGA ATATGAGATG TTTTTTGAGA
2451  AAGAGTAGAA AGCTGAAGAG GAATTACTTG TTTATACTCT GCTAGATCTG
2501  TTAGGGTTTA TATAGAAGGT TAGAATCTGT GTTTATAGTT GAGAGCTTCC
2551  ACGAGAAGTC TATAATAGAA GAGAA
```

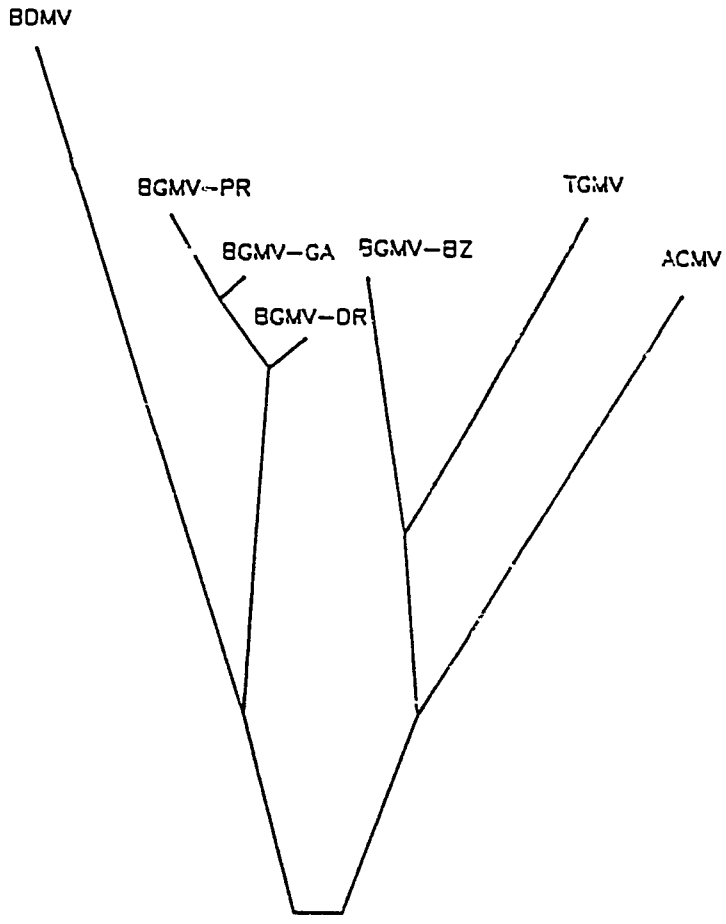



Fig. 4. Phylogenetic bree derived from computer comparions of the common region for BDMV, BGMV-PR, BGMV-DR, BGMV-GA, BGMV-BZ, tomato golden mosaic geminivirus (TGMV), and African cassava mosic geminivirus (ACMV). Clearly, BGMV-BZ, BDMV, and BGMV-GA/BGMV-DR form three distinct bean-infecting geminiviruses.

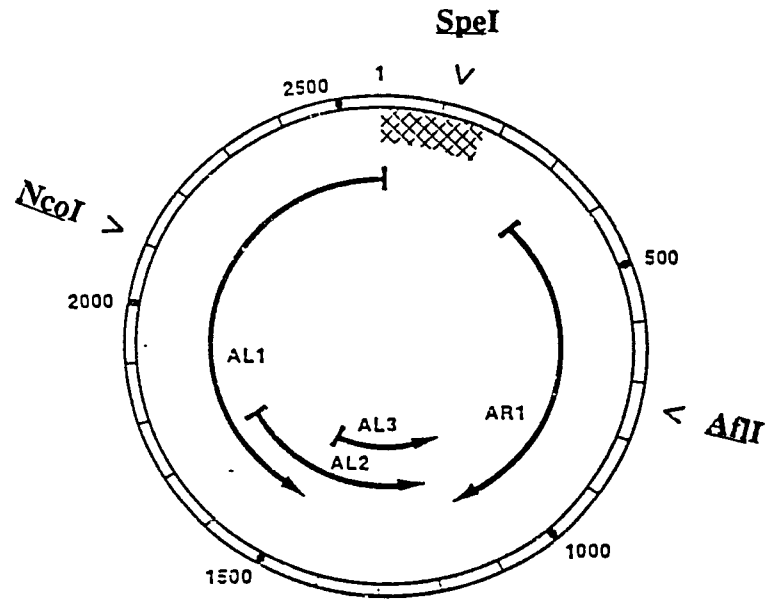



Fig. 5. Mutational analysis for BGMV-GA DNA A mutations at three restriction sites, SpeI (common region), AflI (AR1, coat protein gene), and NcoI (AL1, replicative product gene). Mutant DNA A molecules were inoculated with wild-type DNA B molecules by electric discharge particle acceleration methods. With the common region mutant for DNA A (added one nucleotide), symptoms were delayed and attenuated. The mutation in the coat protein changed one amino acid and this resulted in delayed symptom expression. The frame shift mutation in the replicative gene resulted in no symptoms and no viral DNAs were detected. Wild-type DNA A and B molecules resulted in typical symptoms in 10-14 days.

# ECUADOR - UNIVERSITY OF MINNESOTA

*IMPROVING THE PRODUCTIVITY OF PHASEOLUS BEANS UNDER CONDITIONS OF LOW-INPUT AGRICULTURE THROUGH GENETIC SELECTION OF HOST CULTIVARS AND RHIZOBIUM STRAINS FOR ENHANCED SYMBIOTIC EFFICIENCY*


Principal Investigators:

| | | |
|---|---|---|
| Peter H. Graham (U.S.) | Department of Soil Science | University of Minnesota |
| Gustavo Bernal (HC) | Phytopathology | INIAP |

Investigators:

| | | |
|---|---|---|
| Deborah Allan | Soil Science | University of Minnesota |
| David Davis | Horticulture | University of Minnesota |
| Consuelo Estevez | Pathology | INIAP |
| Carl Rosen | Soil Science | University of Minnesota |
| B. Henson | Pathology | INIAP |
| David Hubbell | Pathology | INIAP |
| E. Lopez | Pathology | INIAP |
| J. Wong | Pathology | INIAP |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

1. Research results. This project has only been underway since May 1989. No results obtained to date have been disseminated in HC or U.S.

2. Other research-related results

Host Country

A baseline study has been initiated on soil properties in three regions of bean production in Ecuador. In Imbaburra province 65 percent of soils tested proved deficient in zinc, 71 percent deficient in manganese, and more than 30 percent either iron or nitrogen deficient. Data on the extend to which foliar fertilization is used to control these microelement limitations is conflicting, and will need to be resolved. Micronutrient fertility trials will also need to be undertaken as confirmation of the importance of these constraints to symbiotically grown plants. Soil analysis for the Chimboraso and Loja regions is still in process.

Six high organic matter soils were collected and tested for their suitability as legume inoculant carriers. Only one soil, from Lago Agrio, had an organic matter content greater than 30 percent. This will be further tested and compared to sterile filter mud as an inoculant carrier.

Three inoculation studies have been undertaken. At Ibarra and Chaltura, twelve strains of *R. leguminosarum* bv *phaseoli* from the UMR collection were evaluated. There was a strong response to N-fertilization and *Rhizobium* inoculation in each experiment, with biological yield increased 38 percent with N-fertilization at Chaltura, and 40 percent at Ibarra (Table 1). CIAT152 and UMR1632 were the best strains tested, each giving biological yields similar to, or greater than, the +N treatment. These strains differed somewhat in nodulation pattern, with CIAT 151 showing limited primary root nodulation, but heavy nodulation on secondary roots, while UMR1632 tended to more uniform nodule distribution. Reasons for this difference will be evaluated. While biological yield responded strongly to inoculation, yield differences between inoculated and uninoculated treatments were not significant. This suggests either low N harvest index in the Ecuadorian variety used in these trials, or severe disease problems late in the growth cycle. Both points are being studied. In a third trial using N15, even uninoculated control plants were reasonably nodulated (presumably through infection by native soil strains), and there was no response to inoculation. Even so, nitrogen fixation rates were significant with the percent of plant nitrogen derived from fixation estimated at from 31.74 to 41.44 percent.

MPN counts of soil rhizobia were taken for 22 soils in the Imbaburra region. Counts averaged more than 10,000 rhizobia per gram of soil, a level at which competition between inoculant strains and those already in level at which competition between inoculant strains and those already in soil is to be expected. *Rhizobium* strain isolations have been made from each region of bean production, and will be used to determine the relative efficiency of the native soil strains. This will determine the need for studies on host variety restriction of nodulation in *Phaseolus* beans.

Eighty varieties selected from CIAT's 1989 IBYAN and EP nurseries for Ecuadorian grain type, high yield and disease resistance, as well as varieties identified in various studies as active in nitrogen fixation, are being multiplied in Ecuador prior to field testing. A further 60 varieties which combine high yield with a long vegetative phase but rapid reproductive development, have been requested from CIAT.

U.S.

Studies to evaluate cultivar variation in traits affecting nodule formation and development have been continued. In the FY 89 report, differences in speed of nodulation among 80 cultivars of *Phaseolus vulgaris* were evaluated. Eight cultivars selected from this study were inoculated with *Rhizobium* then grown for 3-25 days prior to the determination of number of infection sites, nodule number and mass and plant dry weight. A modified haemotoxylin stain was used to determine infection sites per root (Fig. 1). Cultivars differed significantly in number of nodule initials produced per plant (Fig. 2), in the percentage of infections giving rise to nodules, and in the rate at which nodule mass increased (Fig. 3). The latter trait appears closely correlated to shoot growth rate. Cultivar variation

in this trait could be of considerable significance. As Phia and Munns (1987) have reported, one reason for the weaker nitrogen fixation found in beans is their poor early shoot development. It appears from this data that a number of traits contributing to early initiation of nitrogen fixation have been identified, and could be tested for in routine screenings. Studies to evaluate a wider range of cultivars and to initiate crosses between them are underway.

Eighty varieties of beans have been evaluated for tolerance to low levels of soil P. Phosphate charged aluminum/sand mixtures were used to control phosphate availability, with three levels of P supply (30,8 and 1.4 μMP) tested. All plants were dependent on *Rhizobium* for nitrogen supply. Cultivar variation in growth at 1.4 μM was evident, with the Ecuadorian cultivar E838 apparently particularly tolerant to low soil P levels (Fig. 4). Studies to detail cultivar differences in response have been planted, as have studies on the response of efficient and inefficient cultivars to mycorrhizal infection.

Studies on the response of bean rhizobia to acidity have continued. Acid-shock proteins have been identified following the exposure of *Rhizobium* cells to acid pH, and in one case at least, are affected by phosphorus levels in the cell. Exposure to acid conditions have been shown to cause reduced extracellular polysaccharide production and at least partial breakdown in the synthesis of cell wall components (Fig. 5). Cells exposed to acidity modify cell metabolism to reduce acid loading, with glucose metabolism markedly reduced at acid pH. Studies to determine whether acid-shocked cells switch to the utilization of amino compounds as a means of generating ammonium for the control of cytoplasmic acidity are underway.

Since most bean soils in the Imbaburra region seem to contain abundant rhizobia (HC see above), experiments have been undertaken to develop a dot blot method for the identification of bean rhizobia. This will be of importance in studies on competition for nodulation sites in Ecuador, but will also have value in determining which strains should be used in restriction studies with cultivars of *Phaseolus vulgaris*. In an initial study with nodules from Ecuador, the most frequent serological reactions were with antisera to strains 413.2 and CIAT676 (57 and 48 percent of nodules), respectively. If this is confirmed in subsequent evaluations, one or another of these strains will be used to identify cultivars of bean which are limited in their ability to nodulate with the predominant soil strains, and so more likely to respond to inoculation.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Since this project was only initiated in 1989, it is too early for it to have had effects on statistics of national production, or to have documented how research results address the needs of women/small scale farmers.

B. Institutional Development and Training

1. Changes since FY 89. INIAP is in the process of receiving independence from the Ministry of Agriculture. This is likely to happen in 1991 and is expected to result in budget increases for

INIAP researches in Ecuador. Talks have been held with INIAP management over the development of a national bean program, and with the HC PI over the possibilities of collaboration with other centers/universities for bean research in Loja.

Ing. Consuelo Estevez will complete her M.S. degree in November 1990 and return to Ecuador as HC PI. Ing. Gustavo Bernal has recently initiated English language classes at Minnesota, and is working part time on his M.S. research project. Two "egresados" from the national university are completing undergraduate thesis research with funding provided by the project, and two additional students have been identified. A difficulty at the moment is in the identification of suitable students for graduate training in the fields of plant breeding, soil fertility and agronomy. This is the reason emphasis has been given to attracting undergraduate students into this area.

The age of this project (18 months) means that institutional development and training of HC personnel has only just begun. On the other hand, we are perhaps slightly ahead of schedule in identifying personnel for training, and in their progress.

In addition to HC personnel, a number of other students are receiving at least partial support form this project. These include Martha Chaverra (Colombia), Sharon Aarons (Jamaica), Patrick Jjemba (Uganda) and Kathy Draeger (U.S.). It is anticipated that by the end of this extension period two third-world students will have completed Ph.D.s and two completed M.S. degrees. Ms. Draeger and Ing. Bernal should be within one year of completing M.S. degrees.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

Again, given the short time this project has been in operation, it is difficult for it to have strayed very far from the initial guidelines and log frame. The research appears to be on schedule in the U.S., but slightly delayed in the HC through the difficulty associated with the naming of the post-doctoral fellow. With the return of Ing. Estevez to Ecuador in November we anticipate that opportunities for collaboration with other centers in areas where INIAP does not have research personnel will open up, and more than make up for this delay. Contacts have already been established with Dr. Rogelio Lepiz, the CIAT bean agronomist in Ecuador, and should facilitate future research.

This is the only project in the CRSP with a major emphasis on nitrogen fixation and one of the few to emphasize large-seeded grain types. INIAP has a relatively small research program, with little emphasis to date on varietal development, so this project represents a major force for varietal improvement in this country. INIAP is also limited in funding for training, and so will be markedly strengthened by the training activities proposed.

At the international level, the U.S. PI is one of very few scientists working on enhancement of nitrogen fixation in beans, and the person who first identified Puebla 152 as superior in nitrogen-fixing ability. Many of the strains of *Rhizobium* currently recommended for beans also derive from the U.S. laboratory. The only serious research on similar

lines is that being conducted by CIAT, and both PIs maintain open communications with Dr. Kipe-Nolt. Since much of the CIAT research is for other grain types and adaptation conditions we see it as a source of promising lines, complementary to our own program, and are careful to involve CIAT personnel (Rogelio Lepiz in Ecuador, Judy Kipe-Nolt in Cali) in project planning. Both U.S. and HC PIs are aware of the need for regionalization of research focus in this project.

Biological nitrogen fixation is a means of maintaining plant growth and development without the need for expensive (and in third-world countries, often unavailable) fertilizer nitrogen. It is also a way to reduce production costs and better regulate the fluxes of nitrogen in soil and ground water. All of these are important factors in a world where the oil and natural gas supply needed for fertilizer production is precarious and environmental pollution an ever-increasing problem. Because of these points the research undertaken in this project is likely to contribute significantly to bean improvement in both the HC and U.S., and should lead the way to advances with their crop legumes.

D. Evidence of Biological/Social Sciences Integration

WID issues *per se* have not been a major focus of the program to this time; greater emphasis in the short-term having been given to the initiation of biological research activities and training. Budget also has been a limiting factor, though WID issues will form part of the study to be initiated by Kathryn Draeger in Ecuador in January (Ms. Draeger is a McArthur Fellow whose course work includes both biological and sociological components). While WID issues have been stressed, it should be pointed out that the HC PI from November will be female, and that five of the six graduate students involved in this project to date have also been female. The participation of Dr. Ferguson in the project in 1991 will be of considerable help in better defining WID issues in Ecuador.

The project can build on the previous activities of the Cornell group in Ecuador, but will have to use considerable diplomacy in so doing. It is apparent from conversations with INIAP management that this group left ruffled feathers in Ecuador, and for this reason social and culinary issues have been downplayed to date. Again, even were this not so, budget limitations and the range of activities being initiated would have limited the emphasis given this area.

Because of his contacts with the Mexico CRSP, the U.S. PI has maintained strong contacts within this group, and is working in collaboration with Javier Castellanos in the field testing of some high nitrogen-fixing lines. He has also collaborated with the molecular genetics group at Cuernava, this research leading to the identification of acid-tolerant strains identified by the PI as members of a unique sub-species of bean rhizobia, favored by their acid-tolerance, but limited in their competitive ability with Mexican bean cultivars.

Linkage with the Tanzania project has also been strong. The U.S. PI visited Tanzania in September to participate in the SADCC/SUA/WSU meeting and to discuss aspects of BNF research with Susan Nchimbi. The HC PI also visited WSU to discuss breeding strategies associated with root-rot and to collect bean differentials and promising root-rot resistant lines.

Through Dr. D. Hubbell the project has received support from faculty members at the University of Florida. Dr. Mary Collins participated in the evaluation of peat sources for possible use as legume inoculants, Dr. F. Sylvia will visit Ecuador shortly to examine the diversity of mycorrhizal fungi in bean soils. Neither activity was a charge on the project budget.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

This project was the least well funded of any of the CRSP projects in FY 90, limiting to some degree the scope of initiatives and the rate at which different activities could be undertaken. Budgetary restrictions severely limited the salary of the post-doctoral fellow in Ecuador, and are likely to shorten the time he will stay with the project.

Current financial reporting in Ecuador does limit the turnover of receipts and the availability of funds for HC purchases. This is being overcome by maintaining significant funds in the hands of the post-doctoral fellow.

### B. Adequacy of Current Management System

No problem in this area. UMN ORTTA personnel initiate few support services but respond reasonably to communications from the U.S. PI.

### C. Activity Towards Buy-Ins or Other Funding

A buy-in for bean research in Egypt is being negotiated. Areas where buy-ins could have value in Ecuador include the development of an inoculant industry capability in that country for grain and pasture legumes, and in institution building related to agronomic education. To date USAID, Quito has not responded to either initiative.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

Activities undertaken in this project are all directly related to specific constraints identified in the Global Plan, and address major problem areas for bean production in Ecuador, other areas of Latin America and the U.S.

### B. Balance Between Research and Training

In that most of the research in both the U.S. and HC constituted part of a graduate degree program, or will be included in such a program (i.e., the work done by Gustavo Bernal), it is difficult to separate these components. We estimate a 50:50 division, with most of the research costs associated with the activities of Drs. Hubbell and Henson. Even a considerable part of their work involved training of "egresados" and lecturing at INIAP and in local universities (see below).

C. Balance of Domestic vs. Overseas Activities

Because of the delay in naming Dr. Henson as post-doctoral fellow, because Ing. Estevez has been delayed in the completion of her M.S. thesis, and because it has taken some time to develop procedures for the new project in Ecuador, activities currently are stronger in the U.S. than in the HC. Balance will be restored in the new financial year, with none of these points likely to be a constraint.

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Collaboration has been excellent. The U.S. PI discussed plans of research and budget proposals with INIAP management and Ing. Estevez prior to their implementation, met in June with Ing. Cevallos (Director-General, INIAP), and his staff to determine activities and budget for the extension document, and together with Ing. Estevez will hold a review meeting to evaluate progress and decide future research strategies and collaborative activities in December. Included in this meeting will be a discussion of social and food science needs in the project. The presence of Ing. Estevez in the U.S. during this period has been a big plus. Not only did it facilitate decision making, it enabled her to see some of the bottlenecks in both institutions and in the CRSP, and made her aware of the responsibilities of both collaborating parties.

E. Relative Contributions of Collaborating Institutions and Individuals

U.S. and HC institution support of the research activities were as stated in the budget, and mainly constituted support for salary of project PIs. At UNM, Miss Draeger and Miss Chaverra received funding from the work-study program, Mr. Jjemba was separately funded through the Uganda project, and Ms. Aarons was supported through a grant from the University experiment station. As mentioned above, the University of Florida supported visits to Ecuador from Drs. Collins and Sylvia.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

Mr. Peters, the USAID project officer in Quito is trained in the field of nitrogen fixation, and so has taken some interest in the project. He has been instrumental in solving visa problems, but has not been involved in direct project activities.

G. Evidence of Institutionalization

The project is too new to have achieved significant institutionalization in either HC or U.S. institutions, but in the U.S. has been effective in drawing together faculty members from different departments and disciplines. Gene Allen has been strongly supportive of project activities, and as Vice-president for Agriculture, certainly has the position for institutionalization of the project. We expect similar integration both within INIAP and with other institutions once Ing. Estevez returns to Ecuador.

While no awards for international activities have come from UMN, the U.S. PI has been a member of the Technical Committee during FY 90, and

has been invited to make keynote speeches at the tenth anniversary celebration of the Molecular Genetics Group in Cuernavaca (January 1991) and at the Thirteenth North American *Rhizobium* meetings at Banff (August 1991).

Student/professor interactions have been excellent. The diversity of nationalities involved (Australian, Colombian, Ecuadorian, Jamaican, Ugandan and U.S.) has facilitated interaction and made for a tight and coherent group.

## IV. PUBLICATIONS AND PRESENTATIONS

### Publications


Vargas, A. A. T. and Graham, P. H. 1989. Cultivar and pH Effects on Competition for Nodule Sites Between Isolates of *Rhizobium* in Beans. Plant and Soil 117, 195-200.

Aaron, S. A. and Graham, P. H. 1990. Response of *Rhizobium Leguminosarum* bv *Phaseoli* to Acidity. Paper presented to the Third International Congress, "Plant and Soil Interaction at Acid pH." Plant and Soil. In Press.

Oliveira, L. A. and Graham, P. H. 1990a. Evaluation of strain competitiveness in Rhizobium Leguminosarum bv Phaseoli Using a Nod+Fix-Natural Mutant. Archives of Microbiology. 153, 305-310.

Oliveira, L. A. and Graham, P. H. 1990b. Speed of Nodulation and Competitive Ability Among Strains of Rhizobium Leguminosarum bv Phaseoli. Archives of Microbiology. 153, 311-315.


### Presentations


Graham, P. H. 1990. Breeding for Enhanced Nitrogen Fixation in *Phaseolus vulgaris*. In SADCC/SUA/WSU Regional workshop, Morogoro, Tanzania. September 1990. In press.

Martinez, E., Pardo, M. A., Pinero, F. D., Graham, P. H. Franco, A. A., Palacios, R. and Segovia, L. 1990. Genetic Relatedness and Taxonomic Considerations of *Rhizobium* Strains that Nodulate *Phaseolus vulgaris*. In: Nitrogen Fixation, Achievements and Objectives. P. M. Gresshoff, et al. (Eds.) Chapman & Hall, 1990. In press.

Hubbell, D. H. 1990. La asociacion symbiotica de *Rhizobium* y leguminosa. Seminar given to final year students in Agronomy at the Universidad Nacional de Quito; at the Universidad Catolica de Quito, and to the First Grain Legume Workshop, Quito, Ecuador.

Table 1: Response to inoculation of Phaseolus vulgaris in trials at Ibarra and Chaltura, Ecuador.

| Inoculation treatment | Nodule No. | | Nodule Mass (mg/pl) | | Yield (g/pl) | |
|---|---|---|---|---|---|---|
| | Primary | Secondary | Primary | Secondary | Biological | Seed |
| a) Ibarra | | | | | | |
| -N | 19.2 | 57.5 | 28.5 | 89.6 | 98.3 | 28.54 |
| +N | ND | | ND | | 131.5 | 34.64 |
| UMR 1173 | 33.2 | 141.7 | 37.5 | 111.9 | 113.9 | 39.92 |
| UMR 1384 | 4.0 | 13.5 | 53.0 | 360.5 | 125.0 | 37.75 |
| UMR 1632 | 56.5 | 333.1 | 60.5 | 297.0 | 131.6 | 37.01 |
| UMR 1640 | 34.5 | 126.4 | 44.5 | 151.8 | 127.8 | 31.12 |
| CIAT 151 | 4.0 | 20.8 | 63.0 | 286.5 | 134.5 | 36.18 |
| b) Chaltura | | | | | | |
| -N | 35.5 | 85.9 | 25.0 | 56.2 | 90.0 | 23.16 |
| +N | 11.0 | 39.6 | 16.8 | 48.6 | 124.4 | 24.54 |
| UMR 1173 | 32.3 | 144.4 | 77.3 | 202.9 | 88.3 | 21.42 |
| UMR 1385 | 24.3 | 59.8 | 135.0 | 335.9 | 100.3 | 22.66 |
| UMR 1632 | 57.0 | 233.8 | 143.0 | 409.9 | 130.8 | 21.70 |
| UMR 1640 | 73.2 | 284.2 | 85.0 | 248.6 | 103.7 | 19.75 |
| CIAT 151 | 33.7 | 123.9 | 127.2 | 433.0 | 142.2 | 19.70 |

Fig. 1: Roots of Phaseolus vulgaris five and seven days after inoculation. The roots have been cleared with lactophenol and stained with haemotoxylin using the method of Dudley et al (1987) to show root initials and developing root nodules.

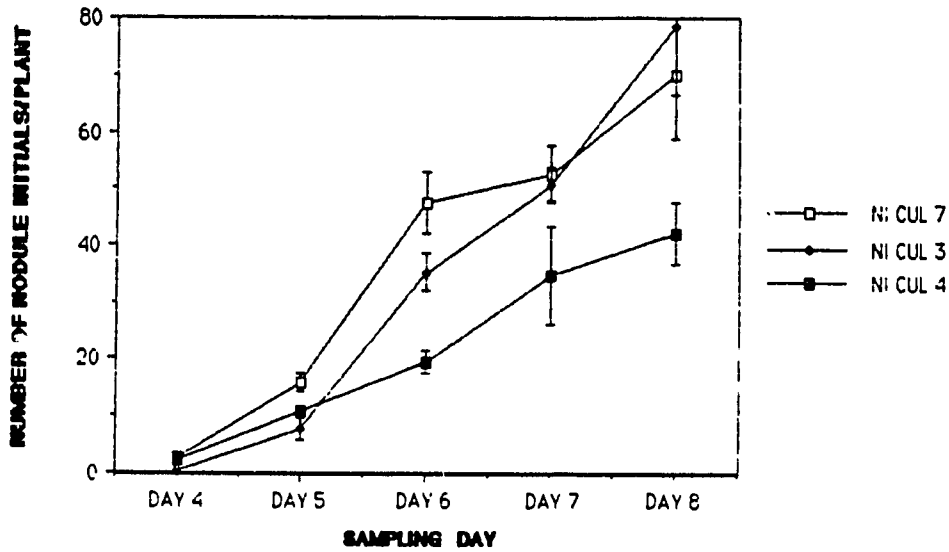



Fig 2: Variation in the number of nodule initials produced per plant with time after planting. Nodule initials were detected following the staining of roots with haemotoxylin, with 20 plants evaluated per cultivar. Cultivar 7 = RIZ21, Cultivar 3 = Bico de Ouro, and Cultivar 4 = CIAT 82EP 13.

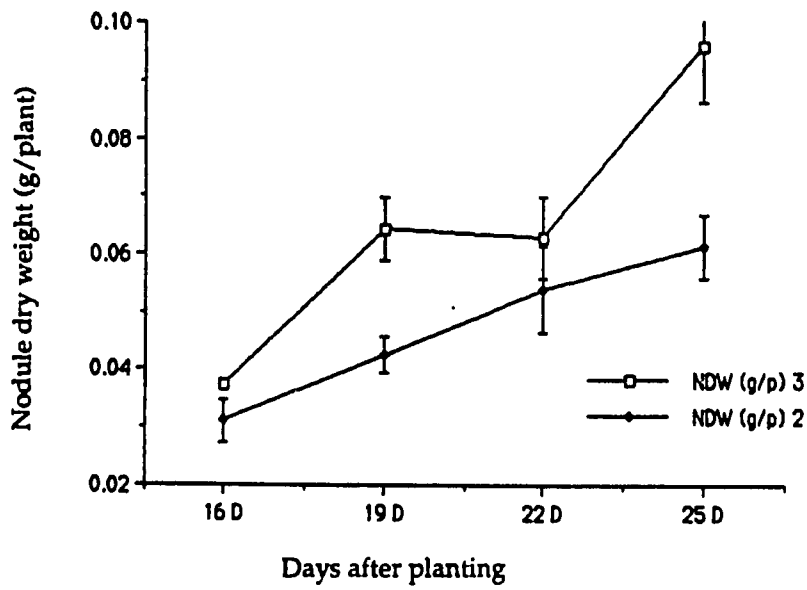



Fig. 3: Cultivar variation in rate of nodule dry matter development as a function of time (days) after inoculation. Cultivar 3 = Bico de Ouro, and Cultivar 2 = CIAT 83 EP 186.

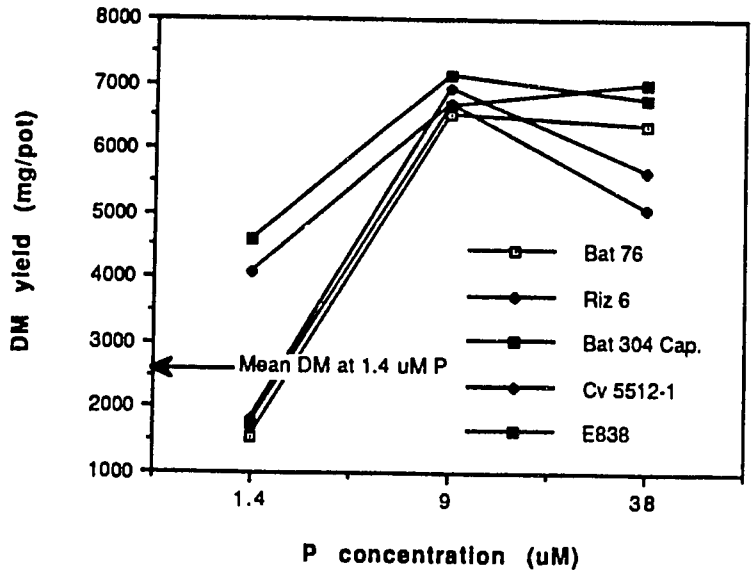



Fig. 4: Effect of level of available phosphorus on the dry matter produced by cultivars of Phaseolus vulgaris. P level was regulated using phosphate-charged aluminum/ sand mixtures prepared according to the methods of Schettini et al. (1987).

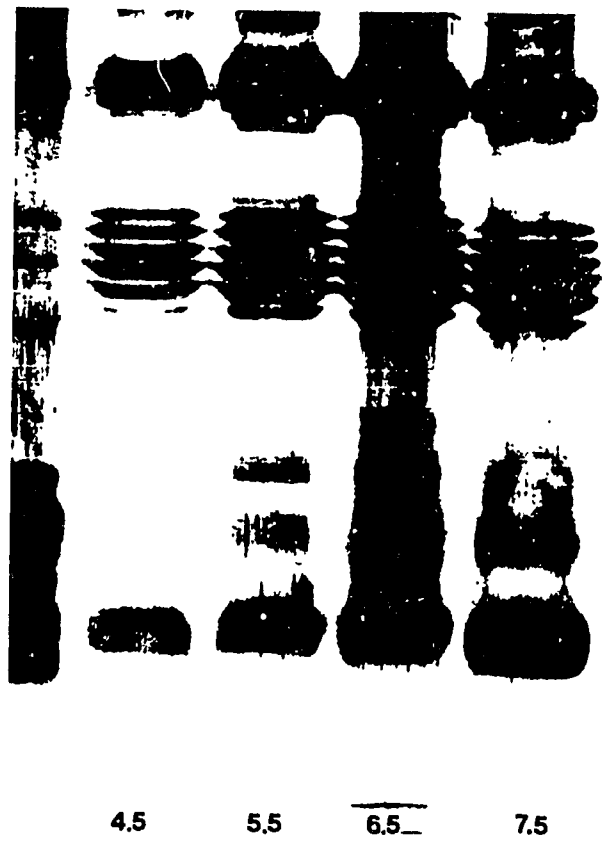



Fig. 5: Influence of acid pH on the composition of lipopolysaccharides produced by Rhizobium leguminosarum bv phaseoli. Cells were grown at pH 4.5 to 7.5, centrifuged and washed, then suspended in sample buffer and subject to SDS-PAGE. Gels were silver stained using the method of Cava et al. (1989) to reveal lipopolysaccharide banding patterns.

# GUATEMALA - CORNELL UNIVERSITY

*AGRONOMIC, SOCIOLOGICAL AND GENETIC ASPECTS OF BEAN YIELD AND ADAPTATION*


Principal Investigators:

| | | |
|---|---|---|
| Donald Wallace (U.S.) | Department of Plant Breeding | Cornell University |
| Porfirio Masaya* (HC) | Bean Program | ICTA |

Co-Investigators:

| | | |
|---|---|---|
| Rafael Rodriguez | Bean Program | ICTA |
| Samuel Ajquejay | Bean Program | ICTA |

*Left March 1990.


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Background.** We have found that the many physiological-genetic components of yield are integrated into three major components. Each is controlled by both genetics and environment (Fig. 1). They are: 1) the total accumulated biomass, with control by virtually all genes; 2) the proportion of the biomass partitioned to the yield (the harvest index), which is controlled by fewer genes; 3) the time the cultivar requires to develop to harvest maturity.

The growing season is not genetically controlled, but high yield requires correspondence of its duration with the time the genotype needs to develop to harvest maturity. Additionally this time (component 3) is modified by the environment within the growing season, as are the biomass and harvest index. The growing season duration plus its environment, therefore, become the 4th physiological component (Fig. 1, 2) of yield.

Negative correlations among the three genetically controlled components have suggested a hypothesis. It is that control over days to flowering and maturity by maturity genes is a consequence of prior control over partitioning of the photosynthate to current yield accumulation vs. to continued vegetative growth (Fig. 2). Competition for the photosynthate (Fig. 2) causes the negative correlations. Because of the negative correlations, efficient breeding requires simultaneous selection for all three genetically controlled components. The only positive correlation among them is that the total biomass becomes positively correlated with the days to harvest maturity (the duration of growth).

Variations of daylength, temperature and moisture (and other environmental factors within component 4; see Fig. 1, 2) cause quantitative variation of all three of the genetically controlled components of yield. The genotype x environment interactions that result must be understood and accounted for if breeding is to be efficient.

**Elucidation of genotype x environment interactions.** Days to flowering expressed by 119 $F_8$ progenies were measured in three environments. They were a summer-time greenhouse (15.5 hours & 28°), a lowland site in Guatemala (29° & 13.5 hours) and a highland site (19° & 13.5 hours) (Fig. 4). For 58 of the 119 the days were also measured in a winter-time greenhouse with ten-hour daylength. The progenies were from Rabia de Gato x San Martin, two Guatemalan cultivars with differential changes by temperature of their time to flowering and maturity (Fig. 3). Randomly derived progenies from the eighth generation after the cross were compared because 99 percent will be homozygous for each gene difference between the parents. Also, half will have each parent's genotype.

The $F_8$ progenies were divided equally between early and late flowering in the 15.5 hour environment (Fig 4B, E). This indicated control by one gene. The late progenies changed to early under short daylength (Fig 4A), indicating a photoperiod gene. Delay of flowering by the photoperiod sensitive genotype was expressed under 29° in association with 13.5 hour daylength (Fig. 4C, G). This control was masked, however, by a proportion of the insensitive genotype shifting toward later flowering, plus a larger proportion of the sensitive progenies becoming relatively earlier. These shifts represent alterations by the high temperature of activities of other genes. Delay by the photoperiod gene did not occur at 19° (Fig. 4D).

Additive Main effects and Multiplicative Interaction effects analysis (AMMI statistical analysis) quantified the relative effects by each genotype, daylength and temperature (Fig. 5). The ten-hour daylength caused the lowest average days to flowering. The 19° caused the largest average days. Respectively, these two environments also caused the largest and second largest negative effect on the deviation from the grand mean days to flowering. The early, photoperiod insensitive genotype caused a low average days to flowering and caused a negative effect on the deviation from the grand mean. In contrast, the photoperiod sensitive genotype caused a larger average days to flowering and a more positive effect on the deviation.

AMMI analysis considers a treatment to be one progeny grown in one environment. The effect by the specific genotype on the deviation from the grand mean days to flowering of the treatment and the effect by the specific environment are the two components of the genotype x environment interaction. The effect by the genotype multiplied by the effect by the environment is the effect on the days to flowering of the treatment that arises through the G x E interaction. The negative effect by the early (photoperiod insensitive) genotype times the positive effect by 15.5 hours or by

29° (Fig. 5) will result in a negative deviation from the grand mean. This indicates that the insensitive genotype will flower slightly earlier on average in the 15.5 hours and 29° environments than in the ten-hour environment (Fig. 5 A, B, C, D; compare Fig. 5B & E with Fig. 5C & F). The negative effect by the insensitive genotype x the large negative effect by 19° indicates a large positive deviation from the grand mean days to flowering. That is, the insensitive genotype will be caused to flower much later by 19° (Fig. 5D & F). On the contrary, the sensitive genotype will flower earlier at 19° than at 29° (in agreement with Fig. 5C, D & F). Earlier flowering is indicated at 19° than 29°, because the positive effect on deviation caused by the sensitive genotype x the negative effect by the 19° environment constitutes negative deviation from the grand mean days to flowering. The sensitive genotype with its positive effect on the deviation, in contrast, will express later flowering in the 15.5 hours and 29° environments because both environments cause a positive effect on the deviation.

For each AMMI analysis the average of a progeny is measured across all tested environments, the average for an environment is measured across all tested genotypes, and the grand mean is measured across all the data. Therefore, the averages and the grand mean were altered by both adding the 4th (10 hours) environment but reducing the $F_8$ progenies from 119 to 58 (Fig. 5 A, B, C). Nevertheless, the order of averages of the insensitive and sensitive genotypes and of the 15.5 h, 29° and 19° environments was the same. The generalized interpretation was not altered.

Interpretation above was based on the largest effect through the G x E interaction upon the deviation from the grand mean days to flowering. This was axis 1 of a principal component analysis (Fig. 5A, D), but PCA axis 2 was also significant (Fig. 5B, E). Across the four environments, 29° vs. 19° caused the largest effect on the deviation (Fig. 5A), while 15.5 hours vs. ten-hour caused the second largest effect.

Data are presented for but one of six crosses possible among four cultivars with different adaptations to temperature (Fig. 3). Days to flowering of $F_8$ progenies from all six crosses was recorded in 1989. For all six progenies, distributions of the days to flowering in either or both of 15.5 hours and 19° indicated genetic control by one or else two genes. Control by a few major genes was never clearly indicated at 29°. At 19° where photoperiod gene activity did not occur, control by one or two genes over days to flowering is probably via differences in either number of nodes on the shoots, in the lowest node to flowering, and/or in rate of node development. Control of days to flowering by other than photoperiod gene activity caused the early and late flowering genotypes to have separate but parallel regression lines (data not presented) for the effect on the deviation plotted against the average days. This contrasts with the single regression line for control by photoperiod gene activity (as in Fig. 5).

Recording of days to flowering, days to maturity, total biomass and yield (a complete yield system analysis) was completed by October for the first 1990 planting season in Guatemala. A second season was planted in September. Data for complete yield system analyses will also be collected during both 1991 seasons. All four physiological components of yield plus the associated rates will be analyzed with AMMI. The 1990 and 1991 plantings include a third elevation. Its intermediate temperature (25°) was not used in 1989, because 25° had caused the smallest differences in days to flowering and maturity among the four parents (Fig. 3). Yields and yields per day should be high at 25°.

**Evidence of use.** Both Guatemala and New York use yield system analysis in breeding for higher yield. More U.S. states and Canadian provinces that participate in the Cooperative Dry Bean Nursery, which is coordinated by the University of Idaho, are collecting data for yield system analysis. Some are basing crosses and breeding objectives on data revealed by YSA.

2. Other research-related results

**Guatemala.** A red bean has been released as cultivar DorICTA. Its adaptation is from the moist and hot environment near sea level to the 25° of 1200 m elevation. DorICTA is tolerant of golden mosaic. Its yield is far above any commercial check. It is the first improved cultivar for commercial production in the lowlands. Being a red bean, it will be grown for export.

A small seeded black bean is in on-farm trials and may be released in 1991. It has adaptation to lowland and marginal sites. It has the highest level of resistance to golden mosaic and stiff non-lodging stems with few branches and indeterminate growth habit.

Negro Cuyuta is being tested for mechanized production in the lowlands. It is determinate with uniform maturity, and a plant habit that is not altered by high temperature. It is not very tolerant to golden mosaic. It has no yield advantage at standard plant densities but is being tested at higher densities.

Cultivar releases for tropical lowlands are due to CRSP research. Lowland research began only after the CRSP started to use 29° for elucidating bases of adaptation to daylength and temperature.

Highland farmers with 19° or cooler temperature are beginning to grow two plantings of early cultivars in monocrop, rather than one in multicropping of cultivars that require the full eight to nine month growing season. Breeding lines are outyielding the standard, cultivar Texel. Farmers want yet earlier maturity for three plantings per year. Evidence suggests yield per year has at least doubled, in agreement with our evidence that earlier maturity results from more rapid growth of the yield organs (a higher daily rate of yield accumulation, see Figs 1,2).

**New York.** One line had among the highest yields for each of the three years. A yield advantage of 30 to 40 percent is suggested. A previously released line with 20 percent higher yield was not commercially accepted because of a poor canned product. Thus, the most important commercial merit of the line with 30 to 40 percent higher yield is its superior canned product. It will be released if it shows superior canning again in 1990.

The Salvadoran indeterminate climbing cultivar Rojo 70 will not flower in daylength longer than 12 hours. Homozygous, determinate-bush segregates from Rojo 70 crossed with the insensitive New York cultivar Redkloud are either: (1) photoperiod insensitive like Redkloud with flowering at about 35 days after planting; (2) moderately sensitive like Redkote with flowering at about 40 days; or (3) never flower until the September daylength is shortened to 12 hours (80 to 90 days after planting). In long daylength the days to flowering is controlled by the photoperiod gene activity. Our hypothesis is that a second gene enhances activity of the major photoperiod gene. On the contrary, under short daylength days to flowering of these lines spans only 35 to 45 days, and the days are highly correlated with the number of nodes on the plant shoots.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Acreage has been maintained in spite of more golden mosaic. Farmers are planting an out of season crop, to compensate for losses. New cultivar releases are popular. Estimated production was 3.2 million hundred-weight. The second season should yield about one third as much.

B. Institutional Development and Training

1. Changes since FY 89. In March 1990, Ing. R Rodriguez became the principal investigator.

2. Over the life of the project. ICTA's bean program was weakened by Dr. Masaya's acceptance of other employment, followed by acceptance by Victor Monterroso (M.S. degree) of other employment. Earlier, Fernando Aldana (M.S. degree) was transferred to leadership of ICTA's cereal program. These changes have removed all English-speaking scientists.

3. Project training to be completed by the end of the project period. All five ICTA scientists now working on the project are participating in English training.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

The numerous processes and traits that interact to give yield have been conceptualized as being integrated into four highly integrated components. Yield System Analysis has been developed. Applied to yield trials, YSA measures the level of each of the four components of yield for each cultivar. The endpoint of the component is quantified plus its average daily rate of progress toward this endpoint (Fig. 2). The AMMI model has been shown to quantify the genotype x environment interaction effect by each individual cultivar (genotype) and the effect by each

different environment. Variations of days to flowering across multiple environments plus AMMI analysis have: 1) revealed that relatively few maturity genes control adaptation to the growing season duration and its environment; 2) quantified the effects by different daylengths and temperatures on the expression of these genes.

Funding is requested to allow ICTA to complete collection of data on flowering time, maturity time, biomass and yield for the second season of 1991. This will extend beyond the September 30 end of the fiscal year. Data collection for all theoretical and basic objectives can be achieved by December 1991.

This research has back-stopped and strengthened the Guatemalan bean breeding program on black beans, the only black bean breeding in Central America. ICTA participates in a network for sharing methodology and cultivars with Mexico and Caribbean and Central American countries. ICTA collaborates with CIAT on improving disease resistance.

Methodologies and knowledge developed have enabled the ICTA Bean Research Team to: a) more efficiently develop bean varieties for diverse cropping systems, and for different environments. b) more efficiently breed for higher yields. c) exercise leadership in breeding early maturing bean varieties for Central America and the Caribbean in close collaboration with CIAT.

With modifications, the knowledge and methodologies developed for improved efficiency of breeding for adaptation and higher yield of bean can be applied to most crops in the U.S.

More efficient breeding for adaptation and higher yield at each different geographical site can maximize yield on a regional and world wide bases.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

USAID supports testing and validation by ICTA of models that simulate bean growth and yield, in collaboration with IBSNAT. Cornell is introducing yield system analysis and AMMI analysis to U.S. bean researchers, in conjunction with IBSNAT's introduction of modeling. The introductions are through the W-150 regional project of the U.S., to both U.S. and Canadian participants in the Cooperative Dry Bean Nursery. One Canadian bean researcher applied yield system analysis in 1985; this site plus three U.S. states have done so annually since 1987. The CDBN annual report summarizes the yield system analyses.

Collaboration has begun with biotechnologists at Cornell and at CIAT to characterize any molecular genetic associations with the genotypes for adaptation described in this report.

## II. FUNDING/FISCAL MANAGEMENT

Continuing devaluation of the Quetzal has reduced dollar expenditures by ICTA.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

One global objective is improvement of genetic potential for yields.

### B. Balance Between Research and Training

A Ph.D. candidate from Costa Rica was supported with 28 percent of the $52,394 budgeted for Cornell. A Ph.D. candidate from Botswana performed the genetic and G x E analyses presented in this report, at no cost to the CRSP. About 4 percent of the $47,000 budgeted for ICTA was spent for English training and computation training (see section III.B.2). ICTA's bean program has recently developed need for advanced degree training.

### C. Balance of Domestic vs. Overseas Activities

Not addressed.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Results reported used data from environments in Guatemala plus from environments in the temperate zone of New York.

### E. Relative Contributions of Collaborating Institutions and Individuals

Cornell contribution was $38,208. CIAT funding for Ph.D. training of Seja Mmopi facilitated data collection in the temperate zone of New York, plus analysis and interpretation of all data. ICTA contribution was $20,500 (QI03,069)

### F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

USAID in Guatemala remains supportive but not actively involved.

### G. Evidence of Institutionalization

Cornell fully recognizes international activities by faculty. The CRSP research is fully integrated with ongoing genetic and bean breeding research. The CRSP developed yield system analysis and the application of AMMI analysis are likewise fully integrated into ICTA's bean program. ICTA credits CRSP research and procedures for recent successes in breeding for improved adaptation and high yield. Almost all research by this CRSP project has been through student/professor interactions.

### H. Other Comments

As the ICTA-Cornell project is finalized in 1991, ICTA is contacting Dr. Garrell Long about the possibility of a continuing project. It would be concerned with breeding for resistance to insects. ICTA also wants to collaborate with Dr. Maxwell and his project on golden mosaic. ICTA wants to have a continuing relationship with the Bean/Cowpea CRSP.

## IV. PUBLICATIONS AND PRESENTATIONS


Masaya, P. Efecto del fotoperiodo y la temperatura sobre la adaptacion y el rendimiento de frijol comun, (*Phaseolus vulgaris* L.) Trabajo presentado en el I Congreso Guatemalteco de Agrometeorologia Guatemala, 2-6 de Octubre, 1989.

Scully, B.T. and D.H. Wallace. 1990. Variation in and relationship of biomass, growth rate, harvest index and phenology to yield of common bean. J. Amer. Soc. Hort. Sci. 115:218-225.

Yourstone, K.S. and D.H. Wallace. Application of plastochron index to common bean grown in controlled environments. J. Amer. Soc. Hort. Sci. 115:820-823.

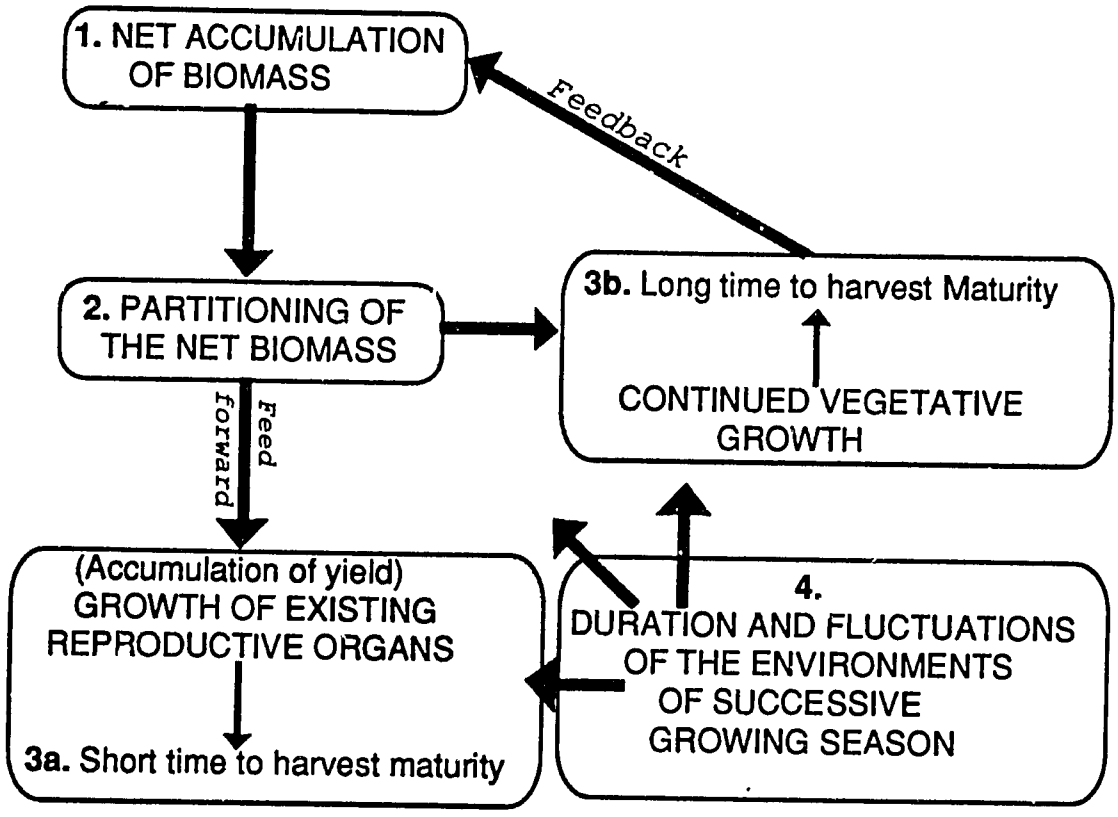
Yourstone, K.S. and D.H. Wallace. Effects of photoperiod and temperature on rate of node development in indeterminate bean. J. Amer. Soc. Hort. Sci. 115:824-828.

Wallace, D.H., K.S. Yourstone, and P.N. Masaya. 1991. Photoperiod gene control over partitioning between reproductive vs. vegetative growth. Submitted tc Crop Sci.

Wallace, D.H., P.N. Masaya and R.W. Zobel. 1991. Photoperiod, temperature and genotype interaction controls over days and node to flower of bean (*Phaseolus vulgaris* L.). Submitted to J. Amer. Soc. Hort. Sci.; Minor changes are being made as suggested by reviewers.

Wallace, D.H. (with about ten co-authors) 1991. Improving efficiency of breeding for higher crop yield. Manuscript being reviewed by co-authors and by Theoretical and Applied Genetics.

**Fig. 1. Four physiological components of yield. Three are controlled by genetics and environment. The fourth is the duration of the growing season plus the attendant environment.** *All effects on yield by the duration and environment of the growing season are implemented through the genetic activities. These effects are symbolized by the grey fill of boxes representing the three genetically controlled components.*



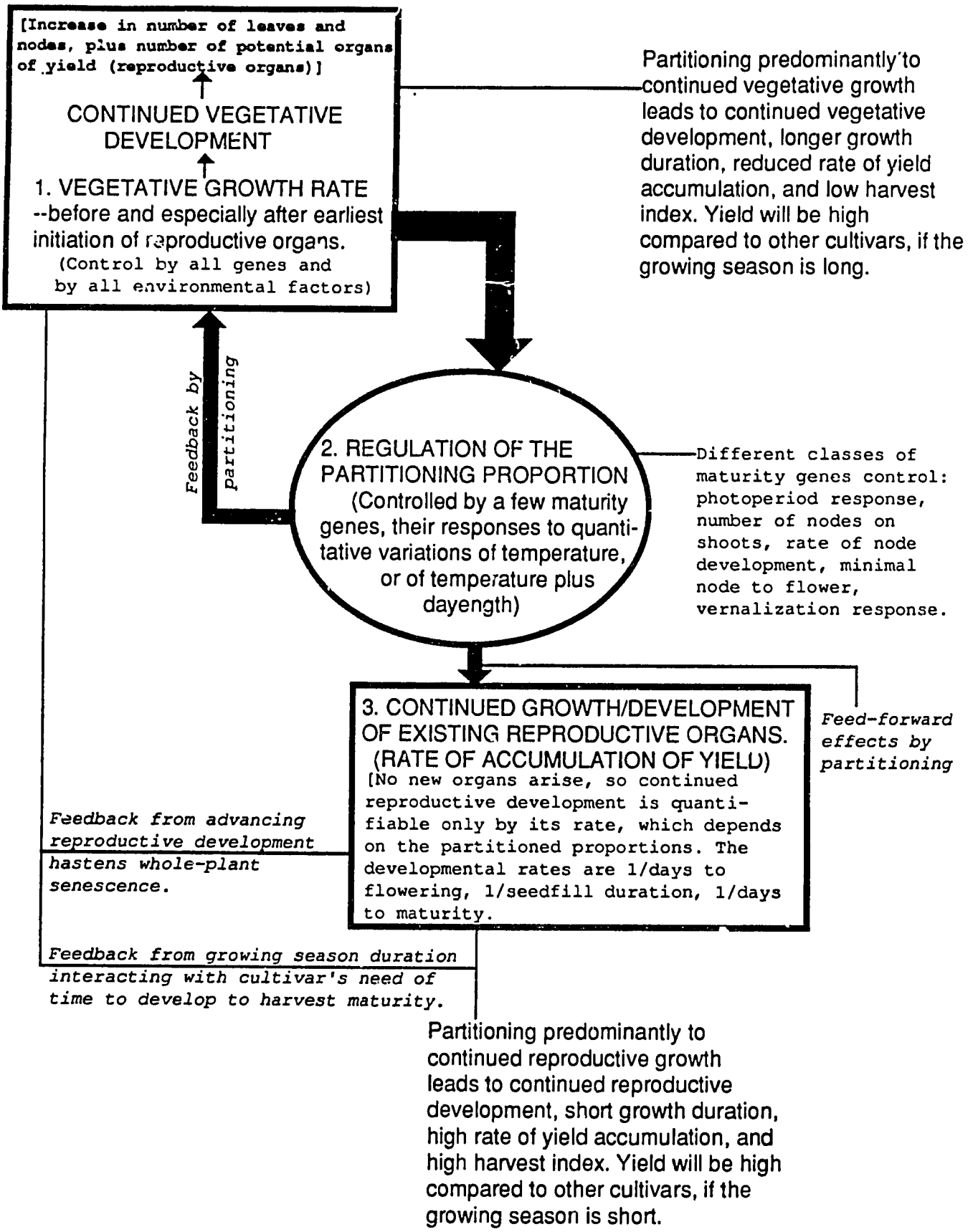
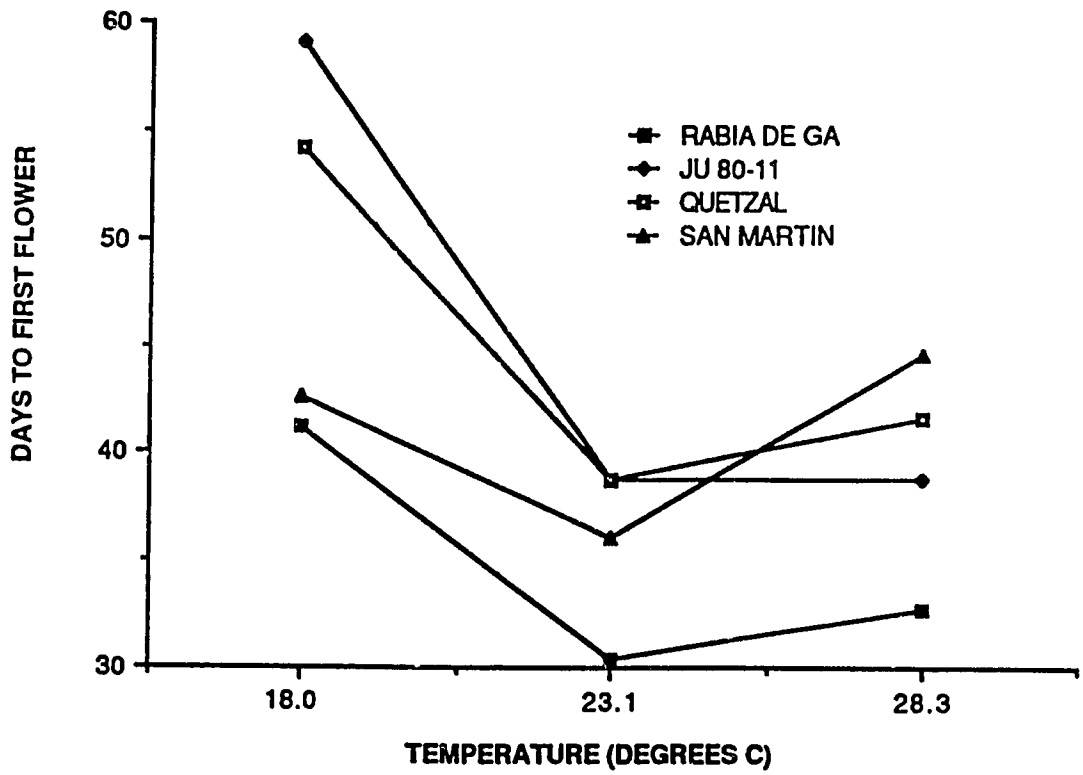



**Fig. 2. Feedback and feed-forward effects among the three genetically and environmentally controlled major components of yield.**

Figure 3. Days to first flower of four indeterminate bush genotypes grown at mean temperature of 18.0, 23.1 and 28.3° C.



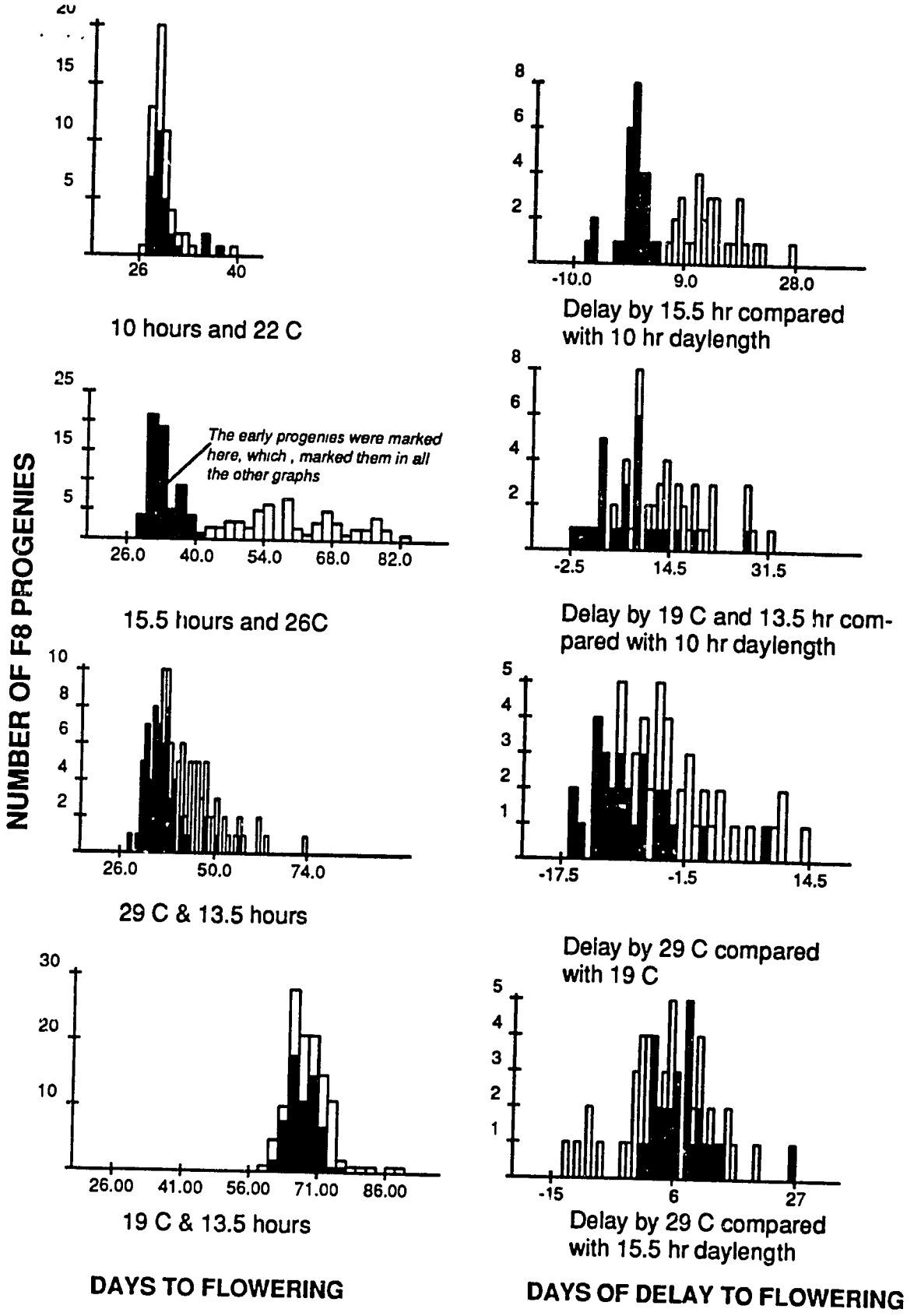
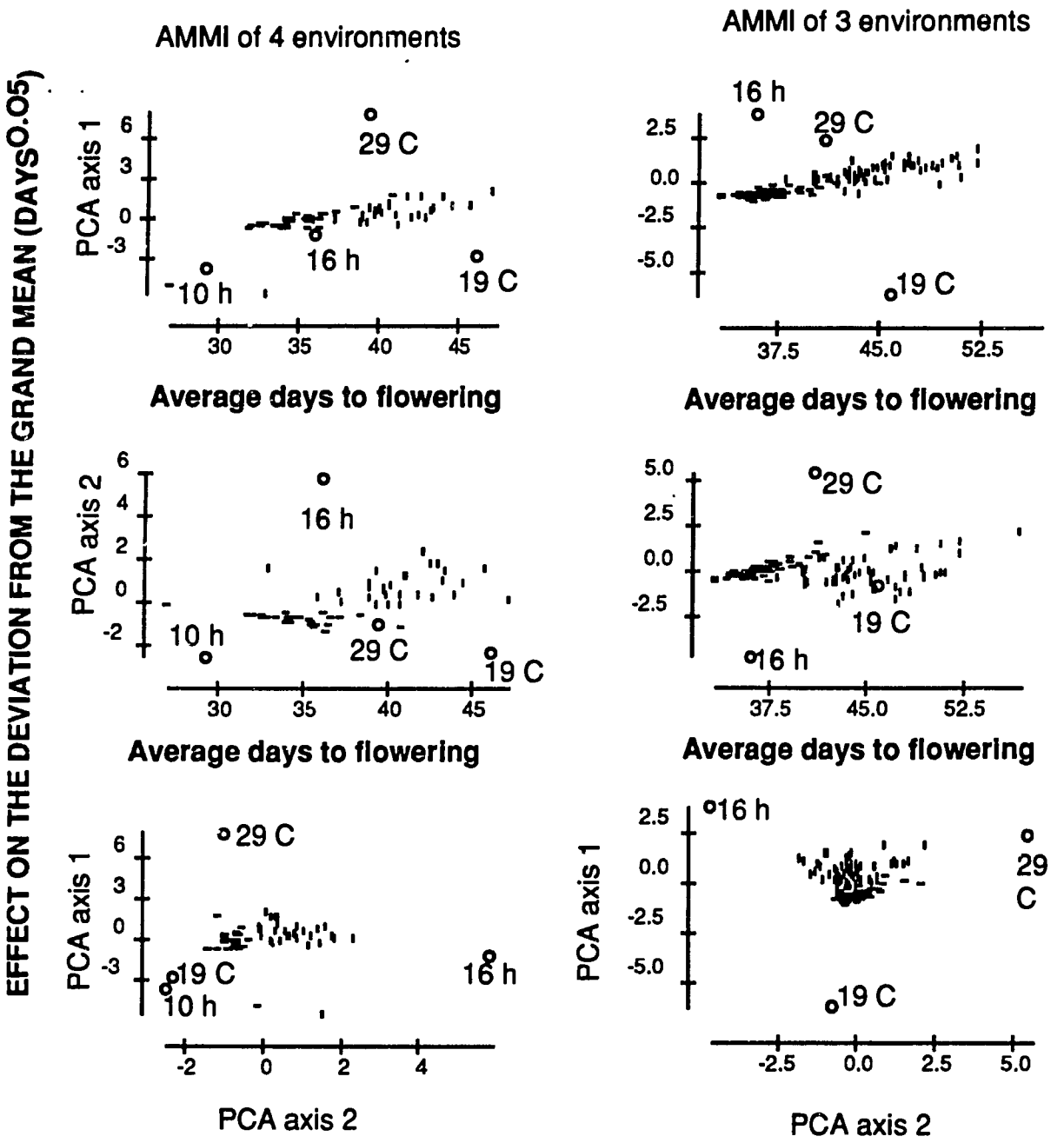



**Fig. 4. Distributions of days to flowering in four daylength-temperatures and the delays of days by long daylength, high temperature and low temperature.**




**EFFECT ON THE DEVIATION FROM THE GRAND MEAN DAYS TO FLOWERING BY PCA AXIS 2 (DAYS$^{0.05}$)**

Fig. 5. Effects by insensitive vs. photoperiod sensitive genotype, by long vs. short daylength, and by high vs. low temperature on the average days to flowering plotted against the largest (PCA axis 1) and the second largest (PCA axis 2) effect on the deviation from the grand mean days to flowering caused by the genotype x environment interaction.
ALSO (IN THE BOTTOM TWO GRAPHS)
The largest effect on the deviation from the grand mean days to flowering through the genotype x environment interaction plotted against the second largest effect through the genotype x environment interaction.

Insensitive genotype is shown as -. Sensitive genotype is shown as ı.

# HONDURAS - UNIVERSITY OF PUERTO RICO

*IMPROVEMENT OF BEAN PRODUCTION IN HONDURAS THROUGH BREEDING FOR MULTIPLE DISEASE RESISTANCE*


Principal Investigators:

| | | |
|---|---|---|
| James S. Beaver (U.S.) | Agronomy and Soils | University of Puerto Rico |
| Juan Carlos Rosas (HC) | Agronomy | Escuela Agricola Panamericana |

Co-Principal Investigator:

| | | |
|---|---|---|
| James R. Steadman | Plant Pathology | University of Nebraska |

Co-Investigators:

| | | |
|---|---|---|
| Rodrigo Echavez-Badel | Crop Protection | University of Puerto Rico |
| Roberto Young | Agronomy | Escuela Agricola Panamericana |

Investigator:

| | | |
|---|---|---|
| Margaret Mmbaga | Plant Pathology | University of Nebraska |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Breeding for disease resistance.** Small red breeding lines having both the I gene and the $bc_3$ genes for resistance to bean common mosaic virus (BCMV) were crossed with a group of promising small red breeding lines and advanced to the $F_2$ generation. The combination of the I gene and the $bc_3$ gene provides protection against a wide range of BCMV strains including the necrotic strain. Resistance to the necrotic strain will be of particular importance if bean production expands to lower altitude regions where higher temperatures prevail. Crosses between these lines and small red lines with dense pubescence are planned in order to attempt to combine resistance to bean common mosaic virus with race non-specific resistance to rust. Small red breeding lines EAP12-88 and HND43-40 have been found to have resistance to bean common mosaic and anthracnose and have moderate levels of resistance to web blight and common bacterial blight. Several crosses were made between small red breeding lines and common blight tolerant genotypes XAN174, XAN176, XAN186, and BelNeb. A study of the pedigrees of these genotypes indicate that some of their common blight resistance originated from tepary beans. Bean golden mosaic virus (BGMV) has been reported in Honduras with increasing frequency. In recognition of this threat to bean production, populations derived from crosses with tolerant lines such as DOR303, A429, and DOR364 have been developed. Samples of what appeared to be BGMV were collected from beans in Honduras and sent to the University

of Wisconsin for DNA hybridization with BGMV isolated from other countries. A population derived from a cross between the small red variety Catrachita and DOR303 will be used in a study of the inheritance of field resistance to BGMV which will be conducted in the Dominican Republic (DR) in 1991.

Improved small red varieties such as Danli 46, Desarrural 1R, Chingo R, and Catrachita have been used as sources of earliness, greater yield potential, adaptation and/or seed quality. These varieties have been crossed with sources of resistance to common bacterial blight, anthracnose, web blight, and BGMV. Some of the most promising breeding lines such as HND43-40 and EAP12-88 are derived from crosses between local small red varieties and sources of disease resistance. A group of $F_5$ lines from crosses between Honduran varieties and disease resistant breeding lines has been planted at the EAP during the 1990 postrera growing season. A group of 70 advanced generation breeding lines developed at the UPR was evaluated at the EAP. Six lines were identified as having greater seed yield and more common bacterial blight and rust resistance than the local check variety Catrachita. These lines were released to the National Bean Research Program (BRP) and PROFRIJOL for testing in national and regional nurseries.

**Recurrent selection program.** Although the primary objective of the project is to develop small red bean germplasm with greater levels of disease resistance, the breeding programs in both Puerto Rico (PR) and Honduras are designed to combine enhanced disease resistance with other important traits such as early maturity, drought tolerance, greater biological nitrogen fixation (BNF), and higher yield potential. In order to move toward this long range goal, a recurrent selection program was initiated in Honduras. A total of 28 populations was created by intermating eight promising small red lines. The $F_3$ generation of these populations was evaluated at the Escuela Agricola Panamericana (EAP) during the 1990 primera growing season for resistance to common bacterial blight, yield, and adaptation. Seed of sixteen lines selected from each population was planted during the 1990 postrera growing season. These lines will be evaluated for resistance to common bacterial blight, rust, and bean golden mosaic and for adaptation, yield, maturity, and seed type. Six to eight of the lines from each population will be selected for further evaluation in a nursery planted in February, 1991. Advanced generation lines derived from each cycle of selection will be released to the BRP of the Ministry of Natural Resources (MNR) for regional testing.

**Rust research conducted in the field.** Rust differentials were used to study rust incidence at various locations in Honduras. Pustule size and intensity of foliar damage were recorded at 15, 30, and 45 days after planting. The severity of rust and variation in pustule size were greater in El Paraiso than in the Olancho valley. Another study conducted at the EAP using planting dates ranging from September to November found inoculum pressure to be greater during the earlier planting dates. A mobile nursery was tested as a means of monitoring variability in virulence on farms. These trials were conducted by a plant pathologist from the DR, Samuel Concepcion, during the 1989 postrera growing season.

A group of advanced generation sister lines derived from crosses between dense pubescent red mottled lines from the DR and glabrous small red lines have been developed. Lines within each population show much variation in density of pubescence on the lower surface of trifoliate leaves above the third node. These populations were planted in replicated field trials in Honduras and PR to study the relationship within a common genetic background between pubescence density and the severity of infection to rust. Results from this research should help to determine if the race non-specific resistance to rust is only related to pubescence density or if other factors are also involved. During the past year the trials at both locations were affected by other diseases. Bean golden mosaic infected the plots in Honduras whereas root rot reduced plant populations in PR. Seed of the lines is being multiplied so that the trial can be repeated in FY 91.

**Yield loss due to common bacterial blight.** common bacterial blight (CBB) is considered to be one of the most important diseases limiting bean production in the region. The second year of a study to estimate the yield loss caused by common blight was conducted in Honduras using genotype and chemical control treatments. Yield loss in unprotected plots was correlated with the severity of infection and ranged from 22 percent for the resistant genotype XAN155 to 42 percent for the susceptible genotype Catrachita. Chemical control (streptomycin + oxytetracycline) was effective but uneconomical. Weekly applications were necessary in order to reduce yield loss to less than 10 percent. A manuscript describing this research has been submitted to the Journal of Agriculture of the UPR. The project will continue to develop germplasm with greater resistance to leaf and pod infection and reduced seed transmission. Crop rotation, the use of disease-free seed and practices that promote the decomposition of plant residue should complement resistance to CBB.

**Inheritance of resistance to common bacterial blight.** An inheritance study of the foliar reaction to common bacterial blight was conducted using three populations derived from crosses between susceptible and resistant lines. Fifty $F_3$ and $F_4$ lines from each cross were evaluated for field reaction using artificial inoculation of *Xanthamonas campestris* pv. *phaseoli*. Results for this research will help to refine breeding and selections methods used to develop beans with greater common blight resistance.

**Bean Golden Mosaic Virus.** Bean golden mosaic (BGMV) has become a major constraint for bean production in Central America and the Caribbean. In Honduras, commercial bean varieties are susceptible to this disease. Although yield loss has been observed in the postrera growing season, the degree of yield loss needs to be quantified. Ana Bohorquez, a fourth year student at the EAP, plans to conduct a study which will quantify the yield loss due to BGMV at the farm level. During the next two years, Ms. Bohorquez will study the effect of genotype, planting date, chemical control, bean/maize intercropping and weed control on the development of BGMV. Most of this research will be supported by a scholarship from a Federal Republic of Germany (GTZ) project at the EAP.

BGMV tolerant genotypes DOR364 and DOR391 were selected from PROFRIJOL bean yield and adaptation trials and used by the breeding program as parents. Red mottled landrace varieties Pompadour G and Pompadour J from the Dominican Republic were also used as sources of tolerance to BGMV. Early generation selection of breeding lines for BGMV will be conducted in Honduras whereas the tolerance of advanced lines to BGMV will be confirmed in field trials conducted in the Dominican Republic. Since BGMV is expected to become a more important bean disease in the region, future releases of small red varieties will need to have some tolerance to BGMV.

Squash blot samples collected from bean plants and weeds were sent to the University of Wisconsin for the detection of geminiviruses using the DNA probe hybridization technique. The Bean Golden Mosaic Virus (BGMV) collected from bean plants in Honduras was found to be closely related to BGMV from the Dominican Republic. Seed of several small red genotypes were sent to the University of Wisconsin to be used for inoculation studies under controlled conditions.

**Early maturity.** A manuscript describing the inheritance of early maturity found in indeterminate small red varieties has been accepted for publication in Crop Science. Narrow sense heritabilities (NSH) for days to first flower (DFF) were large, suggesting that early generation selection for DFF in these populations would be successful. On the other hand, NSH estimates for days to physiological maturity (DPM) ranged from intermediate to large suggesting that it may be more effective to select for DPM in replicated advanced generation nurseries. Since the genetic correlations between DFF and DPM were positive and significant, selection to modify one of the traits will likely result in a change in the other. The early maturity Honduran lines, Cuarenteno and Cincuenteno, have been crossed with several of the most promising small red breeding lines and earlier maturity lines will be selected from these populations.

**Reproductive period.** In the inheritance of early maturity study, the number of days between DFF and DPM was considered the length of the reproductive period (RP). The NSH for RP were intermediate to low indicating that selection for RP would be most effective in replicated advanced generation nurseries. With one exception, genetic correlations between DFF and RP were negative and significant. This suggests that a greater RP could be selected in small red beans by reducing DFF rather than increasing DPM. Large differences in the RP were found among the $F_3$ lines produced from the crosses between early and later maturity genotypes. In fact, a few of the lines were found to have RP more than ten days longer than either parent. A randomly selected group of lines from each population were advanced to the $F_6$ generation. These lines were planted in replicated nurseries in PR in order to study the relationship between the length of the RP and traits such as yield, yield components and harvest index. Preliminary results indicate that there may be an optimum length of the reproductive period for high yielding small red bean genotypes.

**Heat tolerance.** The tolerance of bean lines to the high day and night temperatures which prevail during the summer months in the lowland humid tropics was studied in PR. Small red bean genotypes were identified which produced a near-normal number of pods (10 to 12) per plant. Greater tolerance to heat may permit an expansion of the range of adaptation of beans in Honduras. Expansion into the lower altitude of valleys and coastal plains would permit the production of annual crops such as beans in regions where the agriculture would be more sustainable. A six parent diallel among heat tolerant small red lines was created and advanced to the $F_2$ generation. A replicated trial containing the $F_2$ populations and heat sensitive and tolerant check genotypes was planted at the Isabela Substation in May 1990. Some populations produced plants with very good seed yields. This study is part of the M.S. thesis research of Mr. Roldan Echeverria. A group of small red $F_4$ lines derived from crosses between promising breeding lines and heat tolerant lines were also evaluated at the Isabela Substation.

**Value of maintaining heterogeneity in crosses having similar agronomic and seed characteristics.** Heterogeneity is generally considered to be desirable for varieties that will likely be grown in a wide range of environmental conditions. In "good x good" crosses breeders can maintain genetic variability by early generation bulking of seed. Thirteen promising small red lines were recombined in a crossing block and advanced to the $F_3$ generation in PR. A bulk and a random sample of lines from each population will be advanced to the $F_6$ generation during the upcoming year. A study designed to compare the performance of the bulk populations with the single seed descent lines will be conducted in Honduras and PR during FY 91.

**Social-economic studies in a typical bean/maize agricultural community.** During the past year, efforts were made to establish collaboration with a group of social workers from the National University of Honduras (UNAH). Ms. Margarita Oseguera, professor, and Mr. David Erazo, a graduate student in an M.S. program on Latin American social work, have agreed to conduct studies in Moreceli, a typical rural community in which the principal economic activity is the bean/maize production system. The Bean/Cowpea CRSP project has supported the social scientists by providing transportation and a scholarship for Mr. Erazo. Collaboration with this group will provide a means of measuring the acceptance of bean varieties developed by the project.

**Breeding lines.** Ten advanced generation lines developed by the Bean/Cowpea CRSP project were released to the NBP and PROFRIJOL for multi-site testing in the VIDAC and VAN nurseries during the primera growing season. In the VIDAC89 nurseries, genotypes developed by the project were identified to have resistance to anthracnose (HND43-40 and EAP12-88) and tolerance to web blight (HND43-40, EAP10-88, EAP12-88 and HND62-9). In addition, UPR64-1 was among the ten best of 134 lines evaluated in the VIDAC nursery for yield, adaptation and resistance to CBB and rust. UPR75-14 was selected by NBP researchers to be included in the VINAR nursery which will be conducted in several locations in Honduras.

A major effort has been undertaken by the project to accelerate the release of the BGMV tolerant variety DOR364. Seed of DOR364 was multiplied at the EAP under irrigation during the dry season. More than 15 bushels of seed of DOR364 was provided to the NBP for on-farm trials which are required for varietal release in Honduras. Project personnel also provided assistance in preparing a description of the variety. Using foundation seed provided by the Bean/Cowpea CRSP project, the Seed Production Unit at the EAP produced 200 bushels of certified seed of DOR364 which was made available to the NBP, extensionists and farmers. Basic seed of the new small red variety Oriente was also produced at the EAP.

Ms. Sonia Fortin, NBP researcher stationed at the EAP, has collaborated with extensionists from the EAP Rural Development Program in conducting on-farm trials to obtain information that will be used to support the release of DOR364. The on-farm trials will also be used to study how practices such as early planting, multiple cropping, the elimination of alternate hosts and the judicious use of insecticides may contribute to the control of bean golden mosaic.

CIAT bean researchers backcrossed the dominant I gene for resistance to BCMV into the Honduran small red varieties Desarrural and Chingo. Basic seed of the BCMV resistant versions of these varieties was multiplied at the EAP and provided to the National Bean Program for additional increase. A program to replace the susceptible varieties with the resistant versions will be initiated next year in Olancho and Yoro where the varieties Desarrural and Chingo are preferred by farmers.

The Bean/Cowpea CRSP project maintains breeder's seed of commercial varieties such as Catrachita and new varieties such as DOR364, Oriente, Desarrural 1R and Chingo R. In addition, foundation and certified seed is produced by the Seed Production Unit at the EAP. In 1990, 400 bushels of high quality bean seed was made available to the NBP, extensionists and farmers.

During 1990, the Bean/Cowpea CRSP project in collaboration with the NBP has prepared bulletins describing the causal organisms, symptoms and methods of control of six bean diseases (anthracnose, CBB, rust, web blight, angular leaf spot and BGMV) and four pests (leafhopper, *Apion godami*, slugs and *Diabrotica* spp.) that limit bean production in Honduras. These bulletins have been distributed to extensionists, technicians and farmers. The PDR Communication Section from the EAP contributed to the design and editorial production of the bulletins. Bulletins describing DOR364, Oriente, Desarrural 1R, and Chingo R will be prepared upon their release.

**Publications and presentations.** Eight articles describing research supported by the project were published in the 1989 Annual Research Report of the EAP Department of Agronomy. This report is distributed to various institutions in Honduras and Central America involved in bean research. Three presentations were made at the XXXVI Annual PCCMCA Meeting held in San Salvador, El Salvador, one presentation was made at the BIC Meeting held in Toronto, Canada and two presentations were made at the 9th SUA/CIAT Workshop held in

Morogoro, Tanzania. Mr. José Serracin from Panama and Mr. Ramiro Moncada from Honduras completed thesis research as part of their requirement for an Ingeniero Agronomo degree at the EAP. A total of 11 manuscripts were prepared for publication in refereed journals.

2. Other research-related results

**Conservation and exchange of germplasm.** Nearly 500 accessions of beans originally collected in Honduras were received from CIAT during the past year. These accessions were part of a collection which existed at the EAP but was lost many years ago. A seed increase and a preliminary evaluation of these accessions will be conducted during the upcoming year. The collection will be maintained at the EAP by Germplasm Bank Unit. The number of bean accessions at the EAP will be increased by collections supported by the IBPGR.

The project continues to cooperate with CIAT, PROFRIJOL, and the NBP in conducting cooperative nurseries. Evaluation of breeding lines from CIAT was conducted in VIDAC and ECAR trials conducted at the EAP. The EAP is also used by the NBP as a site for evaluating advanced lines. Common bacterial blight, rust, and BGMV Disease Nurseries from CIAT were conducted.

A group of tepary from the USDA/ARS/TARS were evaluated at the EAP for common bacterial blight resistance. These lines appear to be excellent candidates for interspecific hybridization since they combine high levels of resistance to common bacterial blight with adaptation to Honduras and a relatively large seed size.

Both common bean and tepary bean genotypes have been sent by project personnel to bean researchers at PROFRIJOL, CIAT, University of Wisconsin, University of Minnesota, University of Nebraska, Rutgers University, Chapingo-Mexico, and to Bean/Cowpea CRSP projects in Ecuador and the Dominican Republic.

**Seed production.** Breeder's seed of advanced generation lines, commercial varieties and breeding lines is maintained by the project. Foundation seed of released or soon to be released varieties is produced at the EAP and distributed to the NBP. More than 400 bushels of certified seed of Catrachita and DOR364 were produced at the EAP. The certified seed of DOR364 was provided to the NBP and EAP extension projects for on-farm testing or sold directly to farmers.

**Impact of CRSP produced technology.** Recommendations for an integrated control of the major bean diseases in Honduras using disease resistance, appropriate cultural practices, "clean" seed and the judicious application of pesticides has been promoted. Studies supported by the project to quantify the yield loss caused by common bacterial blight and bean golden mosaic were used to develop these recommendations. Many of the recommendations are described in the bean disease and insect pest bulletins which project personnel helped to prepare.

It is expected that the use of DOR364 and the adoption of cultural practices such as early planting during the postrera growing season will significantly reduce yield loss due to BGMV. Results from on-farm trials will be used to measure the impact of the use of DOR364 and other practices on the development of BGMV.

The release of BCMV resistant lines Desarrural 1R, Chingo R, and Oriente should help to reduce yield loss due to seed-transmitted BCMV in Yoro and Olancho. These varieties should be of particular value to small-holder farmers who grow their own seed.

**Project impact on production and consumption of beans.** Bean production has declined in Honduras since the late 1970's due to diseases, pests, drought, and low soil fertility. The recent release of varieties with resistance to BCMV and BGMV has the potential to significantly increase bean production over the current national average of 500 kg/ha. The potential impact of project research will be even greater when the project releases small red varieties that combine resistance to several diseases such as common bacterial blight, rust, web blight, anthracnose, BGMV, and BCMV.

**Needs of small-scale farmers and women.** Since most bean production in Honduras is on small-scale farms, the development of small red bean varieties with greater levels of disease resistance will be of direct benefit to small-scale farmers and their families. Disease resistance should result in a greater and/or more stable supply of beans which should benefit the Honduran consumer. Because farmers can grow their own seed, the continued use of a disease resistant variety is independent of the scale of production. Greater levels of disease resistance should also reduce the need to use pesticides to control diseases or vectors of diseases.

3. Changes in national production/consumption of beans/cowpeas in the Host Country

| | Bean production | | | |
|---|---|---|---|---|
| | 1978-1988 | 1987-88 | 1990 | 1991 |
| Hectares planted | 73,100 | 62,200 | | |
| Yield (kg/ha) | 500 | 540 | | |
| Total production (tons) | 36,600 | 36,000 | 45,654* | 51,016* |

Source: National Basic Grains Program (1989 and 1990) *=Estimates

During the past ten years, yields and the area of production of beans in Honduras have not changed greatly. At present, local production supplies approximately 60 percent of national demand for beans which is estimated to be 57,800 tons. The projected increases in production in 1990 and 1991 are expected to be due to an increase in yield rather than an increase in area of production.

B. Institutional Development and Training

1. Changes since FY 89. Mr. Jonathan Cerna completed an M.S. degree at the UPR and went to Iowa State University to pursue a Ph.D. in plant breeding and genetics. The project has supported the research of several fourth year students at the EAP who received B.S. degrees. During the upcoming year, two of the assistants of Dr. Juan Carlos Rosas are expected to begin graduate training. Mr. Oswaldo Varela plans to come to the UPR to pursue an M.S. degree and Mr. Roberto Young plans to begin work toward a Ph.D. at Michigan State University. This turnover of personnel at the EAP will require some short term training of assistants in bean research techniques.

   Dr. Jacobo Caceres, the EAP plant pathologist, is expected to resign during the upcoming year. A replacement has not been identified and it has not been defined how a replacement will cooperate with the project. In order to strengthen the plant pathology capabilities at the EAP, one of the assistants that Dr. Rosas plans to recruit will have a strong background in plant pathology. In addition, Mr. Roldan Echeverria, a Ministry of Natural Resources employee, will complete requirements for an M.S. degree in plant breeding at the UPR and will return to Honduras. It is expected that he will be stationed at the EAP to assist the project in plant breeding and plant pathology research.

   In August 1990, Mr. Frederico Rodriguez replaced Mr. Jose Jimenez as Head of the BRP of the Ministry of Natural Resources. Mr. Rodriguez has agreed to continue the close collaboration established between the EAP and the Ministry of Natural Resources. Project personnel participate in the annual meeting of the BRP.

2. Over the life of the project. The bean research capability within Honduras has been greatly enhanced over the life of the project. Dr. Rosas has established an impressive bean research program at the EAP which has been successful in obtaining support from many donors in addition to the Bean/Cowpea CRSP. The project has increased collaboration with the BRP of the Ministry of Natural Resources. Ms. Sonia Fortin, a BRP member, has been stationed at the EAP to conduct field experiments of mutual interest. The EAP has also increased involvement in informal training in Honduras through the preparation of bulletins and by participating in workshops for extensionists and farmers.

3. Project training to be completed by the end of the project period. Two Hondurans will have completed M.S. degrees from the UPR and another two will have initiated graduate studies at the UPR and MSU. Approximately six undergraduate students at the EAP will have received support by the project to conduct their thesis research. Research assistants will have received short term training at CIAT and the University of Nebraska in bean research techniques. Project personnel will have collaborated with the BRP in preparing bulletins and by participating in workshops.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

Very satisfactory progress has been achieved toward the objectives stated in the log frame. The bean breeding program supported by the project has produced small red breeding lines that have greater yield potential and enhanced levels of resistance to several of the most important diseases in Central America. A few of the most promising breeding lines are being tested in national and regional performance trials. Four improved varieties, DOR364, Oriente, Desarrural 1R, and Chingo R, will be released in collaboration with the NBP before the end of 1991. Results from research dealing with rust, heat tolerance, early maturity, reproductive period, and the inheritance of resistance to common blight and bean golden mosaic should have global impact. Project personnel have been active in preparing manuscripts and making presentations at scientific meetings that describe research achievements.

Most of the research is on schedule. Due to the increased importance of bean golden mosaic, the project has had to place greater emphasis on the development of breeding lines with greater levels of resistance to this disease.

The research conducted by the project is complementary to the efforts of the BRP of the Ministry of Natural Resources. Project personnel at the UPR and EAP conduct research directed toward the development of disease resistant bean germplasm. The research in Puerto Rico is focused on the development of small red bean populations with enhanced levels of disease resistance and improved agronomic traits whereas researchers at the EAP have emphasized the development of breeding lines that combine disease resistance with yield potential, adaptation, drought tolerance and/or enhanced BNF. The breeding program at the EAP supported by the Bean/Cowpea CRSP project also provides a mechanism for useful traits identified by other bean research programs at the EAP to be incorporated into an adapted small red bean genetic background. Most of the BRP activities are directed toward performance testing of promising breeding lines. The NBP also collaborates with CIAT in the development of beans with resistance to *Apion godmani*. Bean lines developed by the Bean/Cowpea CRSP project are tested in nurseries that are conducted in cooperation with the BRP, PROFRIJOL, and CIAT. An increasing portion of the lines tested in these cooperative nurseries were developed by the Bean/Cowpea CRSP project.

The Honduras project is the only project in the Bean/Cowpea CRSP attempting to develop small red bean germplasm with enhanced levels of disease resistance. In addition to Honduras, small red beans are produced and consumed in Nicaragua, El Salvador, and Costa Rica. Although diseases continue to be one of the most important factors limiting bean production in Central America, CIAT, in recent years, has reduced bean pathology research in the region. The web blight project in Costa Rica has been terminated and the future of the bean golden mosaic research in Guatemala is uncertain. Project personnel also collaborates with the PROFRIJOL project which supports bean pathology research conducted by the national bean research programs in Central America and Caribbean.

The EAP is one of the few locations in the region with adequate trained personnel and facilities to support research that will lead to the development of multiple disease resistant bean germplasm. Research at the EAP should result in the development of disease resistant bean germplasm having other useful traits such as bruchid resistance, enhanced biological nitrogen fixation and/or drought tolerance.

During the upcoming year the project plans to participate in the release in Honduras of three bean varieties having common mosaic resistance. The project has also been active in helping to accelerate the release of bean golden mosaic resistant line DOR364. The release of these varieties should have in the near future a beneficial impact on bean production in Honduras. Since the most promising breeding lines developed by the project are tested in regional performance trials, bean research programs in other Central American countries have access to germplasm developed by the Honduras Bean/Cowpea CRSP project. The prospect of releasing small red bean germplasm during the next extension period with even greater levels of disease resistance appears to be good.

The rust research supported by the project is unique within the Bean/Cowpea CRSP. The development of bean germplasm and management strategies that will provide race non-specific resistance to rust would have global application. Bean researchers in Africa and the U.S. are already using dense leaf pubescence to develop beans with greater rust resistance.

The projected increased demand for beans in Central America and the Caribbean may require increased production of beans at lower altitudes. This trend may be accelerated as marginal areas for bean production on hillsides lose productivity. The extension of the range of bean production into lower altitude regions or during planting dates not considered traditional for beans will require germplasm with greater heat tolerance. In addition, greater levels of resistance to common bacterial blight, web blight, and bean golden mosaic will be needed. Heat tolerant bean germplasm with resistance to these diseases would be useful throughout Central America and the Caribbean.

Although the Honduras project is directed toward the development of small red bean germplasm, breeding lines developed by the project may be useful for improving the disease resistance of other seed types such as navy, black, and red Mexican beans. Bean breeders in the U.S. have frequently used Central American germplasm as a source of disease resistance.

D. Evidence of Biological/Social Sciences Integration

Although the project does not have a social science objective, we have established collaboration with a group of social scientists from the National University of Honduras. This collaboration has provided the project with an opportunity to measure the acceptance of bean varieties developed by the project in a typical rural community. Results from this collaboration should be useful in determining how well the project research is addressing the needs of bean growers and bean consumers.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

The great amount of collaboration that has been established among the HC and U.S. PIs is one of the strengths of this project. The PIs from the EAP, UPR, and UNL all participate in the development of work plans, budgets, travel plans, and annual reports. Travel is planned so that the PIs can visit the U.S. and HC sites during periods when disease readings and/or evaluation of germplasm can be conducted. In recent years, the project has had an increased level of interaction with the Dominican Bean/Cowpea project. Last year, Mr. Samuel Concepcion traveled to Honduras to assist the project with rust research. In addition, the Dominican Republic was used as a site for screening small red beans for resistance to bean golden mosaic. Honduras has been used to screen the rust resistance of Dominican red mottled beans having dense leaf pubescence. The HC and U.S. PIs from the UPR, UNL, and Wisconsin Bean/Cowpea CRSP projects met in the Dominican Republic last year to discuss the status of bean golden mosaic research. Squash blot samples were sent to the University of Honduras. The Honduras Bean/Cowpea CRSP project has germplasm and expertise in BNF which may be useful to the Ecuador Bean/Cowpea CRSP project. The project also has the potential to participate in cross-CRSPing activities. Dr. Rosas will represent the Bean/Cowpea CRSP in a meeting that will be held in Honduras to discuss cross-CRSPing.

During the past year collaboration with USDA-ARS researchers has permitted the evaluation of bean germplasm in Honduras for resistance to rust and common bacterial blight. Collaboration with CIAT and the BRP of the Ministry of Natural Resources has already been mentioned. During the past year the exchange of germplasm and information has been active. Contact with bean researchers in other Central American and Caribbean countries is maintained by participation in the annual meetings of PROFRIJOL and the PCCMCA. The U.S. researchers have an opportunity to report results from project research through Regional Hatch Project W-150 meetings and biennial meetings of the Bean Improvement Cooperative. One of the long term benefits of the Bean/Cowpea CRSP is the increased level of communication that now exists among bean researchers working in both developed and developing countries.

II. FUNDING/FISCAL MANAGEMENT

A. Problems Regarding Funding

Audit/Project Management Reviews. The most recent UPR audit was performed by Mr. Jaime Hernandez Vega in 1985. He made several recommendations which were adopted by the EAP Finance Office. Dr. Russell Freed and Dr. Harvey Hortik visited the EAP in 1988 and Dr. Ken Rachie and Dr. Art Siedler reviewed HC activities in August 1990. Dr. Rachie and Dr. Gray reviewed U.S. activities in November 1989. The comments and suggestions of the reviewers were very constructive.

The EAP adopted a new accounting procedure that resulted in considerable delays in the preparation of fiscal reports. This prevented the UPR from providing the EAP with rapid reimbursement for their expenses. In addition, the HC PI has experienced some difficulty in monitoring the

rate of spending. Ms. Erla de Caceres, Finance Officer, is currently responsible for reporting project expenses. The new procedure has begun to function and fewer delays are expected during FY 91. However, the exchange rate, prices, and wages are expected to be subject to more change than in the past which may make reporting HC expenses more difficult.

During the past year there have been delays of several months for obtaining permission to purchase equipment (microcomputer, motorcycle). These delays reduce the amount of time that the equipment can be used by the project.

The current policies and procedures for managing funds have proven to be acceptable. The finance officers at both the U.S. and HC institutions are familiar with the present policies and procedures. The advent of the FAX machine has improved communication between the U.S. and HC.

B. Adequacy of Current Management System

Funds were adequate to conduct the research and training activities outlined in the FY 90 work plan. In March 1990, the rate of exchange of lempiras:dollars changed from 2:1 to 4:1. Prices for materials and supplies increased immediately whereas salaries were not adjusted. Consequently, the project realized a temporary savings in HC personnel expenses. Some of the savings was used to purchase a motorcycle and to support collaboration with social scientists from the National University of Honduras. It should be emphasized, however, that salary increases are expected in the near future and that the rate of spending for personnel during FY 91 will return to previous levels. The Ford Bronco purchased by the project five years ago has been retired. At present, the project is paying $100/month to lease a vehicle. In order to accomplish the research during the next extension period, another motorcycle and a pickup truck will need to be purchased.

C. Activity Towards Buy-Ins or Other Funding

Support for the USAID Mission in Honduras has been reduced in recent years. At present, there appears to be no opportunity to obtain a significant buy-in from the local Mission. Nevertheless, there may be opportunities to use funds from existing programs such as the LUPE project to train HC personnel.

Honduras was selected as one of four sites where the possibility of cross-CRSPing activities is being explored. During FY 91, Honduras will receive $25,00 for this purpose. Dr. Juan Carlos Rosas will represent the Bean/Cowpea CRSP in the cross-CRSP planning meeting.

III. PROJECT STATUS

A. Appropriateness of Activities to Goals of the Global Plan

The major focus of the project is to develop small red bean germplasm with enhanced levels of disease resistance. Rust, common bacterial blight, bean common mosaic, bean golden mosaic virus, and anthracnose

limit bean yield in Honduras and in many other bean growing regions of the world. Small red beans are consumed in many Central American countries such as Honduras, El Salvador, Nicaragua, and Costa Rica. Moreover, multiple disease resistant small red genotypes would be useful as breeding lines to improve the disease resistance of other seed types such as navy, black, and red Mexican beans. The project is also developing strategies for the deployment of rust resistance genes that will result in more durable resistance. Disease resistance should result in greater and more stable seed yields and should reduce the need to use pesticides. Other traits such as enhanced biological nitrogen fixation and reduced cooking time will only be useful in the tropics when introduced into adapted, disease resistant bean genotypes.

Greater heat tolerance will permit beans to be grown in lower altitude regions in the tropics. Heat tolerant beans would permit many countries to expand the area of production and/or permit beans to be grown during seasons not considered traditional for bean production. Heat tolerance would also reduce the need to produce beans on hillsides where production of annual crops is not sustainable.

B. Balance Between Research and Training

1. Expenditures for research and training. The balance between research and training was appropriate to address the objectives in the workplan.

Expenditures in FY 90
Research: $119,512
Graduate training: $15,000

It should be noted that these values do not include expenditures to support fourth year students at the EAP and the informal training of Samuel Concepcion and Roberto Young. The experience that Dr. Margaret Mmbaga has gained as a Research Associate at the UNL should be considered as training. Participation of project personnel in BRP workshops and assistance in the preparation of bulletins should also be considered training activities. The need for institution building at the EAP is less than in many developing countries since the EAP already has good facilities and a well trained staff.

The balance between training and research has remained fairly constant during FY 89 and FY 90. The project budgeted support for one graduate student at the UPR and supports the thesis research of two undergraduate students at the EAP.

C. Balance of Domestic vs. Overseas Activities

The present distribution between U.S. and HC activities has been appropriate to address the program constraints. Each institution makes a unique contribution to the project. Researchers at the UNL provide the expertise to conduct basic research with rust and other bean pathogens. Researchers at the UPR develop bean populations with disease resistance and heat tolerance and conduct research to study the

inheritance of useful traits. Most of the plant breeding is conducted at the EAP. Several field experiments have been conducted in both Puerto Rico and Honduras. These activities will result in publications in which HC and U.S. researchers are co-authors. Breeding lines and research results are frequently exchanged among project personnel.

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

The level of collaboration among project personnel is excellent. Researchers from the U.S. and HC meet often. Travel to the U.S. and HC is planned so that ongoing research can be observed. Work plans, budgets, training, and travel activities are prepared after thorough consultation among U.S. and HC personnel. The well trained staff and the good facilities at the EAP facilitate the development of collaborative research.

E. Relative Contributions of Collaborating Institutions and Individuals

1. U.S. and HC contributions. Host country in-kind contributions to the project during FY 90 was estimated to be more than $43,000. The time that Juan Carlos Rosas and Jacobo Caceres dedicate to the project is donated by the EAP. The EAP also provides the project with land, use of machinery and irrigation equipment, a greenhouse and screenhouse, a computer, and a laboratory and germplasm storage facility. Transportation, communication, publication, and administrative services are also provided by the EAP.

   The time James Beaver and James Steadman dedicate to the project is donated by the Universities of Puerto Rico and Nebraska. Both U.S. universities provide project personnel with good facilities for conducting bean research. The UPR charges the project a reduced rate of indirect costs which results in a savings of at least $5,000 per year. The contributions made by the U.S. and HC institution exceed the 25 percent minimum established by the Bean/Cowpea CRSP.

2. Other contributions. Additional contributions to the project include cooperation and services provided by the Germplasm and Seed Production Units at the EAP. The BRP of the Ministry of Agriculture provides significant support to the project by stationing an agronomist at the EAP to help conduct research of mutual interest. The BRP also tests the performance of advanced lines in trials that are conducted on farms throughout Honduras.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

Mr. Craig Anderson, the current CRSP Liaison Officer at the Honduras USAID Mission, has expressed great interest in cooperating with the project. Although budget restrictions create an environment not favorable for a buy-in, Mr. Anderson indicated that the Mission may be able to support some activities such as training through existing projects. The project has provided Mr. Anderson with reports and publications and he has plans to visit the EAP to review ongoing research.

G. Evidence of Institutionalization

1. Faculty recognition. The EAP administration has supported the development of a bean research program that has gained international recognition for their achievements. A significant portion of the time of the HC PI is dedicated to bean research. The success that the EAP bean program has had in obtaining external funding indicates the research capabilities of the HC personnel. The Seed Production Unit at the EAP insures that seed of the varieties released by the project can be multiplied. The collaboration that has been established with the BRP of the Ministry of Natural Resources provides a mechanism for breeding lines developed by the Bean/Cowpea CRSP project to be tested on small farms in Honduras. Collaboration with PROFRIJOL permits genotypes developed by the project to be tested in several countries in Central America and the Caribbean. Participation in the Bean/Cowpea CRSP has not proven to be detrimental to the careers of the U.S. PIs since both have been promoted during the present extension period.

2. Integration of domestic and international commodity research programs. Collaboration with the BRP of the Ministry of Natural Resources and the CIAT coordinated PROFRIJOL program has resulted in a great amount of integration of activities. Project personnel participate in the annual meeting of the BRP. Trials of mutual interest are conducted and EAP researchers have been very active in obtaining information needed to release promising small red breeding lines as varieties. Several cooperative nurseries from CIAT are conducted by the project. An increasing number of the entries in the regional advanced line performance trials were developed by the Bean/Cowpea CRSP project. BRP personnel and CIAT researchers frequently visit the EAP to review ongoing research. Research results are shared with other bean researchers at the annual meetings of the PCCMCA and PROFRIJOL.

3. Internal project management. Preparation of workplans, budgets, and reports are made on a timely basis. This is possible due to the good working relationship that exists among the HC and U.S. PIs. Fax messages have proved an opportunity to exchange information on a frequent basis. There have been some delays in preparation of fiscal reports but this problem is expected to be resolved in the near future. The administrators at the EAP, UPR, and UNL support project activities. These factors have contributed to the favorable evaluations that the project has received.

4. Student/Professor interactions. Graduate students at the UPR are considered part of the bean research team. The HC students are expected to work with project personnel in conducting a wide range of activities related to plant breeding. This breadth of experience is usually needed when the student returns to the HC to initiate a research program. The fourth year students at the EAP conduct thesis research on topics related to the project. Students at both the UPR and EAP have frequent interaction with the PIs.

## IV. PUBLICATIONS AND PRESENTATIONS


Allen, E. A., H. Hoch, J. R. Steadman and R. J. Stavely. 1990. Influcne of leaf surface featuers on spore deposition and epiphytic growth of fungi. chapter 7 in Microbiology of the Phyllosphere. (In press).

Castano, J., J. Zepeda y S. Zuluaga. 1990. Epidemiologia y control de la roya de frijol (*Uromyces appendiculatus* [Pers.] *Ung.*) mediante mezclas en Cuarenteno, variedad endemica de Honduras. CEIBA (In press).

Cerna, J. and J. S. Beaver. 1989. A visual indicator of the physiological maturity stage of indeterminate dry bean. Journal of Agriculture of the University of Puerto Rico. 73(4):362-365.

Cerna, J. and J. S. Beaver. 1990. Inheritance of early maturity in indeterminate dry beans. Crop Science. (In press).

Del Rio, L., P. Hepperly and J. S. Beaver. Internally seedborne microorganisms and their association with dry bean seed quality in Honduras. Journal of Agriculture of the University of Puerto Rico (In press).

Del Rio, L. 1990. Caracterizacion de microflora de semilla de frijol en dos regiones de Honduras. CEIBA (In press).

Rosas, J. C., J. D. Erazo y J. R. Moncada. 1991. Tolerencia a sequia en germoplasma de frijol commun y frijol tepari. CEIBA (In press).

Serracin, J., R. A. Young, J. C. Rosas and J. Caceres. Danos causados por *Xanthomonas campestris* pv. *phaseoli* y su effecto en el rendimiento de frijol comun. Journal of Agriculture of the University of Puerto Rico (In review).

Unander, D. W., R. Diaz-Donaire, J. S. Beaver, J. Cerna and D. Rueda. 1989. Yield stability of dry bean genotypes in Honduras. Journal of Agriculture of the University of Puerto Rico 73(4):340-347.

Zaiter, H. Z., D. P. Coyne and J. R. Steadman. 1990. Rust reaction and pubescence in Allubia beans. HortScience 25:664-665.

Zaiter, H. Z., D. P. Coyne and J. R. Steadman. 1990. Coinoculation effects of the pathogens causing common blight, rust and bean common mosaic in *Phaseolus vulgaris* L. Journal of American Society for Horticulture Science 115:319-323.


Presentations


Cerna, J. and J. S. Beaver. 1989. Inheritance of early maturity of indeterminate dry beans. Presentation made at the Biennial Meeting of the Bean Improvement Cooperative held in Toronto, Canada. November.

Concepcion, S., J. R. Steadman, R. A. Young, J. C. Rosas, O. I. Varela, S. Fortin y J. Caceres. 1990. Monitoreo preliminar de la incidencia de roya (*Uromyces appendiculatus*) en frijol comun en Honduras. 1 Presentation made at the XXXVI Annual Meeting of the PCCMCA held in San Salvador, El Salvador, March.

Fortin, S., J. C. Rosas, R. A. Young y O. I. Varela. 1990. Evaluacion de la resistencia a enfermedades y rendimiento de frijol rojo pequeno en el VICAR89 en Honduras. Presentation made at the XXXVI Annual Meeting of the PCCMCA held in San Salvador, El Salvador, March.

Mmbaga, M. T. and J. R. Steadman. 1990. Adult plant resistance and leaf pubescence in *Phaseolus vulgaris* L. Paper presented at the 9th SUA/CRSP Bean Research Workshop held at Sokoine University of Agriculture, Morogoroo, Tanzania, September.

Mmbaga, M. T. and J. R. Steadman. 1990. Epidemiological significance of rust urendiniospore production and release in relation to leaf pubescence in dry beans (*Phaseolus vulgaris* L.). Paper presented at the 9th SUA/CRSP Workshop held at Sokoine University of Agriculture. Morogoro, Tanzania, September.

Pena, E., A. Pantoja and J. S. Beaver. 1990. Determinacion de pubescencia de cuatro materiales geneticos de frijol y su efecto sobre el insecto *Bemisia tabaci* Gennadius. Presentation made at the XVII Congress of the Colombian Entomological Society held in Cartagena, Colombia, July.

Serracin, J., R. A. Young, J. C. Rosas y J. Caceres. 1990. Incidencia de la enfermedad causada por *Xanthomonas campestris* pv. *phaseoli* y su efecto en el rendimiento de frijol commun en Honduras. Presentation made at the XXXVI Annual Meeting of the PCCMCA held in San Salvador, El Salvador, March.

Other Publications

Freytag, G. F., M. Zapata, R. E. Wilkinson, and J. H. Lopez Rosa. 1990. Release of five common bean germplasm lines resistant to common blight (*Xanthamonas campestris* pv. *phaseoli*). Announcement of release of germplasm distributed to bean researchers.

Moncada, J. R. 1990. compartamiento agaronomico del frijol comun (*Phaseolus vulgaris* L.) bajo estres de sequia impuesta en diferentes etapas de crecimiento. Ing. Agron., thesis. EAP. El Zamorano, Honduras. 51 p.

Serracin, J. Cuantificacion de danos causados por *Xanthomonas campestris* pv. *phaseoli* en tres cultivares de frijol commun (*Phaseolus vulgaris* L.) Ing. Agron. Thesis. EAP, El Zamorano, Honduras, 50 p.

D. The Annual Research Report of the EAP Department of Agrnomy contains six articles that describe other research supported by the project

Concepcion, S., J. S. Beaver, J. R. Steadman, J. C. Rosas and R. A. Young. 1990. Evaluacion de la incidencia de *Uromyces appendiculatus* en genotipos de frijol con pubescencia foliar.

Fortin, S., J. C. Rosas, R. A. Young y O. I. Varela. 1990. Evaluacion de resistencia a enfermedades y rendimiento de frijol rojo pequeno en el VICAR89 en Honduras.

Fortin, S., J. C. Rosas y R. A. Young. 1990. Evaluacion de la resistencia a *Xanthomonas campestris* pv. *phaseoli* en el vivero regional de bacterosis comun del frijol.

Fortin, S., J. S. Rosas, R. A. Young. 1990. Evaluacion de genotipos de frijol rojo pequeno del VIDAC89 por su rendimiento, adaptacion y resistencia a emfermedades.

Rosas, J. C. and R. A. Young. 1990. Incremento del rendimiento de germoplasma de frijol rojo pequeno brillante mediante mejoramiento intra-poblacional.

Varela, O. I. y J. C. Rosas. 1990. Caracteristicas agronomicas, resistencia a enfermedades y rendimiento de lineas avanzadas de frijol comun.

# INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA (INCAP) - WASHINGTON STATE UNIVERSITY

*IMPROVEMENT OF DRY BEAN NUTRITIONAL QUALITY AND ACCEPTABILITY*


**Principal Investigators:**

| | | |
|---|---|---|
| Barry G. Swanson (U.S.) | Food Science & Human Nutrition | Washington State University |
| Ricardo Bressani (HC) | Division of Agriculture | INCAP |

**Co-Investigators:**

| | | |
|---|---|---|
| E. Acevedo | Nutrition Science | INCAP |
| M. R. Bennink | Food Science & Human Nutrition | Michigan State University |
| E. Calderon | Agriculture & Food Science | INCAP |
| C. De Bosque | Agriculture & Food Science | INCAP |
| L. Elias | Nutrition Science | INCAP |
| A. Garcia-Sota | Nutrition Science | INCAP |
| R. C. Hoseney | Grain Science | Kansas State University |
| G. Hosfield | USDA | Michigan State University |
| G. Huertas | Agriculture & Food Science | INCAP |
| P. Seib | Grain Science | Kansas State University |
| J. Sosa | Nutrition Science | INCAP |
| M. Uebersax | Food Science & Human Nutrition | Michigan State University |
| L. Velasquez | Agriculture & Food Science | INCAP |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Digestibility and electrophoretic characteristics of bean (*Phaseolus vulgaris*) protein fractions.** *In vitro* digestibility of water and salt soluble bean protein fractions was 68-82 percent at pH 8.0 using a multienzyme system of trypsin, chymotrypsin and peptidase. *In vitro* digestibility was inversely related to trypsin inhibitor activity of the bean protein fractions. The digestibility of protein fractions from three cultivars of beans were studied. Electrophoretic techniques demonstrated that the water soluble protein fraction of beans was markedly more resistant to multienzyme *in vitro* digestibility than the salt soluble bean proteins. SDS-PAGE electrophoresis patterns indicated that heated water and salt soluble bean proteins from the three varieties of beans were effectively digested by the multienzyme system.

**Digestibility of phaseolin after deglycosylation with trifluoromethane sulfonic acid (TFMSA).** Native phaseolin, the major storage protein of beans was deglycosylated with TFMSA at 23°C for one hour. TFMSA removed 89 percent of the carbohydrate from phaseolin. Purified native and deglycosylated phaseolin were

digested by proteases for 30 minutes. Carbohydrate removal resulted in improved proteolysis (digestibility) as evidenced by low molecular weight polypeptide subunits on SDS-PAGE. At an enzyme:phaseolin ratio of 1:10 the largest polypeptide subunit from deglycosylated phaseolin had an MW less than 14 kilodaltons. The results suggest that improved enzymic digestion of phaseolin is related to conformational changes induced by deglycosylation. The carbohydrate moiety of phaseolin presumably exerts a definite effect on the conformation of phaseolin and an phaseolin digestibility.

***In vitro* protein digestibility of selected canned Navy beans.** *In vitro* assay for the measurement of protein digestibility was used in this experiment because it is inexpensive, requires a short time and only a small amount of protein. Protein digestibility indicates the availability of the amino acids contained in foods. The nitrogen contents and *in vitro* protein digestibility of canned products from various cultivars and experimental lines are presented in Table 25. The nitrogen contents of studied beans varied from 4.25 to 4.73 percent(db), probably due to genetic background, growing environments, cultural practices, and post-harvest handling conditions. There was no significant difference ($P>0.05$) in *in vitro* protein digestibility of canned dry beans obtained from the Cooperative Elevator Co. However, protein digestibility values of canned C20 and Seafarer beans from the Cooperative Elevator Co. were significantly higher ($P<0.05$) than those from Michigan Dry Edible Bean Research. Growing locations can exert a large influence on processing quality of navy beans (Hosfield et. al., 1984) and, in this case, had a greater effect on canned bean protein digestibility than did cultivar influence.

In general, canning process is applied to cook beans to obtain a palatable product, using substantial amount of heat which excess the requirement for microbiological safety.

Appropriate heat treatments were also reported to improve the digestibility of protein due to inactivation of anti-nutrients such as phytohemagglutinin and heat-labile protease inhibitors (Liener and Thompson, 1980 and Deshpande and Nielsen, 1987).

At present, there are limited research findings in protein digestibility of canned navy beans, thus, we are unable to compare the obtained digestibility results with previous research. Two other factors which may cause difficulty in result comparison are the differences in methods for sample preparation and *in vitro* protein digestibility determination. The protein digestibility (percent) of these canned bean samples are higher than those values found in most literature (70-77 percent) (FAO/UN, 1970; Tobin and Carpenter, 1978; Marquez and Lajolo, 1981; Wolzak et al., 1981 and Wolzak et al., 1981). These higher digestibility values may be due to the method of sample preparation used in this study, washed canned beans were freeze-dried and ground through 20 mesh screen before *in vitro* protein digestibility determination. This preparation treatment, by reducing bean particle size, should significantly improve the protein digestibility of canned bean samples. However, the protein digestibility of canned beans is still lower than that of casein (90 percent-92 percent), thus, suggesting that other factors limit digestibility.

***In vitro* protein digestibility of cotyledon flours and isolated protein fractions.** In this study, the cotyledon portions of C-20 and Seafarer beans were separately ground through 20 mesh screen to obtain cotyledon flours. These flours were subsequently extracted to yield the following protein fractions: albumin, GI and GII. There were significant differences ($P<0.05$) in nitrogen content among these flours and protein fractions within each cultivar, in an ascending order as follows: cotyledon flour, albumin, GI and GII (Table 26). Among isolated protein fractions, the GII fractions possess the highest purity as demonstrated by their highest nitrogen content.

The AOAC *in vitro* protein digestibility method requires 10 mg of nitrogen in 10 ml distilled-deionized water.

Casein (standard) and albumin were completely solubilized in aqueous solution where as GI, GII and cotyledon flour formed an aqueous suspension. However, upon autoclaving at 121°C for 20 minutes, albumin and GI fractions formed precipitates at the bottom of the test tubes. When adjusting pH to 8.0 ± 0.03, GII fractions of both C-20 and Seafarer were completely solubilized whereas the other samples were partially solubilized. The protein digestibility of all samples, both uncooked and autoclaved, are presented in Table 27. Without heat treatment, GI of both C-20 and Seafarer were the most digestible (88.5 - 91.2 percent) fractions, following with GII (83.35 and 85.38 percent), cotyledon flour (72.75 and 73.09 percent) and albumin 69.82 and 71.74 percent). Phytohemagglutinin (PHA) has been found in GII fraction and thus, may contribute to low protein digestibility of this fraction. The least digestible fractions, albumins, from C-20 (69.8 percent) and Seafarer (71.7 percent) may be due to the natural protease inhibitor activity such as trypsin inhibitor. Marquez and Lajolo (1981) have published that large amount (73 percent) of total) trypsin inhibitor activity is associated with the albumin fraction isolated from Brazilian beans (*Phaseolus vulgaris*). However, they reported that albumin was more digestible than GI and GII in a raw state using three separate enzyme systems: trypsin, pancreatin and pepsin-pancreatin. The use of multi-enzyme system (trypsin, peptidase, chymotrypsin and protease) in this study may improve the digestion of GI and GII over albumin. The applicability of multi-enzyme digestion is preferable since it is similar to the human complex proteolytic enzyme system.

The protein digestibility values of cooked (autoclaved) samples are summarized in Table 27. Comparison between the protein digestibility of raw and cooked samples were illustrated in Figures 24 and 25 for C-20 and Seafarer, respectively. Heat treatment by autoclaving at 121°C for 20 minutes greatly improved the protein digestibility of cotyledon flour and GII fraction. Slight increases in protein digestibility of albumins were observed after heat treatment. In contrast, the lowering of digestibility of bean albumin when heat treated was claimed by Marquez and Lajolo (1981). They suggested that digestibility was due to the aggregation of autoclaved albumins and the presence of heat stable trypsin inhibitor. In this study, all samples (raw and cooked) were vortex-mixed for one minute prior to enzyme digestion, since all solid foods are usually chewed before passing to the stomach and further mixed inside the stomach and along the digestive tract. The partial breakdown of heated albumin

aggregates may facilitate enzyme-substrate interaction. The relative order of percent digestibility ranked from high to low is GII, GI, cotyledon flours for both Seafarer and C-20 cultivars. Many previous investigators (Marquez and Lajolo, 1981; Bradbear and Boulter, 1984 and Desphpande and Nielsen, 1987) have also reported the improvement of protein digestibility by heat treatments. The ANOVA analysis of raw and heated bean protein samples is presented in Table 28. Bean protein fraction and heating (autoclaving at 121°C, 20 minutes) have a slightly significant ($P<0.001$) effect on protein digestibility. Heating improves the bean protein digestion but to different degrees depending on protein fraction/form.

Protein digestibility assessment of beans comprised of different cultivars, crop locations and/or conditions resulted in significant differences. Greater differences were demonstrated between growing locations than between cultivars. The agronomic environmental influence on protein digestibility warrants additional research.

Fractionation and evaluation of specific cotyledon proteins demonstrated that the Globulin I (GI) possesses the highest percent digestibility in contrast to albumin and Globulin II (GII) which had lower digestibility.

Heat treatment (autoclave: 121°C for 20 minutes) of the primary protein fractions resulted in enhance digestibility of GII fraction to that equivalent of GI and casein. Heat induced improvement was not substantially demonstrated for albumin, suggesting the presence of a heat stable protein coagulation structure and/or active protease inhibitor.

**Evaluation of digestibility and protein quality.** Previous studies provided very useful information on the nutritional limitations in beans, however, since those characteristics may be genetically controlled, it becomes necessary to use beans with different genetic background to tie the nutritional effects with particular genetic traits. Using this approach and with beans provided by CIAT (Dr. Steve Bibbee), chemical and biological tests have been conducted on eight pairs of beans with contrasting genetic characteristics. These were plus or minus tannins, or plus or minus arcelin, plus or minus lectins, and plus or minus resistance to BCMV. Two additional commercial bean cultivars were used as references. So far, the results indicate significant differences in protein digestibility for all beans, but particularly between the plus and minus tannin beans, higher for the beans with no tannins. Differences were also found in protein quality, higher for the beans with different degrees of resistance to BCMV. Detailed protein fractionations and amino acid content is being conducted to relate these characteristics to the biological parameters measured.

In other studies, beans from CIAT (Dr. C. Cardona), with and without arcelin were evaluated. Arcelin is the lectin fraction which provides resistance to *Zebrotus* attack on beans. The results obtained clearly indicated processing to destroy arcelin as judged by its disappearance in cooked beans and from animal performance when beans with and without arcelin were fed at high levels of intake. These results were interpreted to mean beans containing arcelin are safe for human consumption.

An additional set of beans with diverse genetic background provided by Dr. G. Hosfield are being studied chemically and nutritionally, but results are not yet available.

Following the same approach, a Guatemalan bean collection from the U.S. is being screened for differences in sulfur amino acid (met + cys) and in trypsin inhibitors content. A large variation was found in sulfur amino acid content, both in the original beans and in the new seed. Variability in TI content was lower about three-fold between beans. TI are supposed to be rich sources of sulfur-containing amino acids. Thus, the idea is to test both, low and high TI-containing beans for protein digestibility and protein quality, as well as high and low total sulfur containing beans. The TI of beans have been isolated and its susceptibility to heat destruction confirmed. A larger sample is being isolated for amino acid analysis, particularly S-amino acids. Beans with high and low levels of TI have been identified and will be planted.

**Acceptability and cooking quality.** Beans are known to require relatively long periods of cooking, more so, if they happen to be hard. Due to the cost of energy, beans are a relatively expensive food. As an approach to study the problem, a variety (Ostua) recently released by ICTA for commercial production, was subjected to cooking at eight different altitudes over sea level (25 to 7500 ft). Cooking conditions, source of energy (propane), containers for cooking and evaluation of cooking were kept as equal as possible. The results indicated cooking time to vary from 77 minutes at 25 ft to 275 minutes at 7500 ft over sea level. At all altitudes, moisture at the end of cooking in the cooked beans was 62 percent, and the solids in the cooking broth were equivalent to 11 percent of the weight of cooked beans. Trypsin inhibitors, hemagglutinins and amylase inhibitors were absent in all beans. Protein content decreased with altitude, probably increasing in the cooking solids. Water absorption index increased, as well as soluble solids from the cooked flours. Available lysine did not change, nor the protein quality. Other analyses are underway. Pressure is probably playing a significant role in influencing the rate of reactions taking place, which are faster at low altitudes as compared to rates at high altitudes. This, as well as other factors will be studied in the future. Other studies of cooking of beans, with both, soft and hard-to-cook beans processed in one locality are beginning to provide information on the physical and chemical changes which take place upon cooking. Water soaking of soft and hard-to-cook reduces cooking time, as well as bean grain hardness. For both types of beans, K concentration released into the soaking water increased, but more solids were found from the hard-to-cook beans.

On the other hand, the color of the broth from soft beans is darker than that from hard-to-cook. So far, no other major changes have been detected.

**Cooking quality of beans in Africa.** The most common method of cooking beans in much of Africa is over an open wood fire. Beans take longer to cook than other foods and require a good supply of firewood. In Rwanda, the demand for firewood exceeds the supply, and causes a depletion of human and physical resources. The purpose

of this study was to ascertain whether fast cooking beans and novel bean cooking methods could reduce the demand for firewood, and allow women more time for income generating activities. The experiments were conducted on 15 homesteads in southern Rwanda. Beans were cooked on the homestead with a measured quantity of firewood. "Calima" and "Rubona 5" were evaluated as pure lines and as components of market mixtures. The traditional open wood fire cooking method was compared to soaking prior to cooking, and cooking in a modified crock pot cooker also known as a haybasket cooker. The haybasket cooker conserved firewood because the beans cooked for four hours in a pot over a hot stone placed inside the insulated haybasket. Results indicated that the haybasket cooker and "Calima," a fast cooking cultivar, significantly reduced fuelwood consumption and the amount of labor needed to prepare beans for eating. When data were averaged over three experiments, "Calima" required 16 percent less firewood and 8 percent less time to cook than the slow cooking cultivar, "Rubona 5." The haybasket cooker used 40 percent less firewood than the traditional open fire cooking method. Cooking time in beans is variable and has no relationship to agronomic yield, hence fast cooking bean types with high yields can be selected by bean breeders. Fast cooking beans and the haybasket cooker are sustainable technologies easily adapted because the haybasket cooker is easily constructed and the fast cooking beans satisfy acceptability criteria at a minimum cost to consumers.

**Haybasket cooking of beans in Rwanda.** Follow-up studies in Rwanda have indicated that 90 percent of the households on which the haybasket cooker was tested are still using this technology. The Rwandan Ministry of Energy is interested in overseeing the use of the haybasket through their extension offices and nutrition centers and privately sponsored programs.

**Bean callus and embryoid culture techniques.** Opaque globules formed on bean callus induced on primary leaf explants cultured on induction media (IM) containing 10 to 30 mg/l 2,4-D. Calli with globules produce structures reminiscent of somatic embryos (embryoids) after subculture in a liquid challenge medium (LCM). Calli maintained on IM for two, three, four and five weeks produced significantly more (26 to 34/callus) embryoids in LCM than calli maintained on IM for one week (12/callus). Well developed embryoids only occurred after calli were subcultured in liquid B5 with 0.1 to 1.0 mg/l IBA. Calli subcultured in LCM with > 10 mg/l IBA turned necrotic and died. Embryoids produced in B5 with 2,4-D and NAA (0.1 to 1.0 mg/l) proliferated roots and formed "frosty" appearing structures, respectively. No differences were detected in number or quality of embryoids produced in LCM from callus maintained on IM in continuous light or darkness regardless of the induction time. Ethylene accumulation in IM cultures inhibited globule formation.

**Hard-to-cook development in beans.** The development of the hard-to-cook condition in beans is well known and studies have been conducted by various groups to understand the biochemical and physical events taking place. Likewise, attempts have been made to find simple technologies to inhibit the hardening process or reduce its rate of development. Although some have been effective, they are difficult to implement at field level. In the present study,

the effect of covering the seed coat of beans with an oil film was tested. This technology has been shown to be effective in the control of insect attack to beans. The rationale was that the oil film would reduce water exchange from the environment to the cotyledons and vice versa. Black and red beans were used, and stored with and without oil covering at 4, 22 and 37°C, the latter at a higher relative humidity than at 22°C. Samples were evaluated up to 12 w. The results showed a much lower development of the hard-to-cook conditions for treated beans at 37°C, particularly for black beans. The study will be repeated using saturated vegetable oils at a concentration of 0.6 percent to insure a better film covering of the whole surface of the beans.

**Dietary fiber analyses of hard-to-cook (HTC) beans.** The cotyledons of HTC beans contained increased levels of soluble dietary fiber, but constant levels of insoluble fiber compared to normal cooking beans. The total level of fiber increased in the HTC cotyledons, possibly indicating that phenolics have reacted with hemicellulose or pectic substances in the cell wall.

HTC cotyledons can be conveniently separated from bean hulls by pearling followed by hand-removal of hull contamination. Normal cooking beans were placed in a proofing cabinet (22°C, 100 percent RH, 24 hours to 18 percent moisture content) dried and then pearled. The yields of pure cotyledons were 68 percent for HTC beans and 56 percent for the normal beans.

**New product development, to diversify uses of beans.** The most common way beans are prepared for consumption is as whole beans, from which strained and fried beans are also prepared. These products are also available commercially as produced by the food industry. It is important to diversify the uses of beans to increase both production and consumption. In this respect and as a follow-up of other studies conducted previously, both recently harvested and hard-to-cook beans were milled and fractionated mechanically, to obtain seed coat-free white bean flour fractions. The results from both, hard-to-cook and dry-recently-harvested beans, confirmed previous findings, in that all fractions had a similar protein content (20.4 - 24.1 percent), crude fat (2.3 - 2.6 percent), crude fiber (1.9 - 2.8 percent) and ash (4.4 - 4.5 percent). These fractions are being analyzed for lysine and sulfur amino acids and functional properties for diverse applications. Studies have continued on the improvement of common bean nutritive value in mixtures with soybeans or TSP. Mixtures of 58 percent red kidney beans and 42 percent whole soybeans and of 68 percent red kidney beans with 32 percent TSP gave protein quality values equivalent to 70 and 77 percent of the value of casein as compared to 52 percent for beans alone.

2. Other research-related results

**Germplasm conservation and use.** Received 15 packets (100 seeds) of large seeded African accessions for cooking rate studies. Obtained from CIAT 14 accessions (50-100 seeds) of COS Corron and Manteca type beans of Chilean origin for flavonoid control gene studies.

Sent 17 different genetic stocks of seed (500 g lots) to INCAP, Guatemala for digestibility studies. The same 17 stocks were sent to Washington State University to conduct *in vitro* digestibility studies and corroborate results with INCAP.

**Seed production and distribution.** Two hundred thirty-one $F_2$ bean populations with good red seed coat color and 100 seed weights of 40-60 g were grown in the field and represent the $C_2S_2$ cycle of recurrent selection for improved architecture and consumer acceptance. Twenty isoline pairs of beans differing in seed color and isogeneic for the J gene controlling shiny seed coat and after darkening. Six white-seeded (navy) genetic stocks differing for starch and fiber content were grown to study bean seed indigestibility. A bean collection native of Guatemala, representing materials produced and consumed in the country, is being screened for nutritive value. Amount of seed is being increased. Bean samples produced in Puerto Rico and obtained from Dr. James Beaver, have been received for cooking tests.

**Use of haybasket cooker and Mattson penetrometer.** Technology and fast cooking beans allows more time for women to seek income generating actives; soil erosion and consequences are reduced because fewer trees are required for fuelwood; afforestation-deforestation is more balanced; cottage industry established through manufacture and marketing of haybaskets. Use and distribution of 25 pin Mattson penetrometer bean cooker for objective cooking time analyses.

Use of haybasket cooker technology and fast cooking beans can save 85 hours of labor and reduce fuelwood usage by 224 Kg per year. A 2 percent increase in family income can be expected because of the conservation of labor using the haybasket and fast cooking bean technologies.

Improved cookability reduces the inconvenience of cooking beans by women and alleviates the frustration that accompanies the inability of preparing nutritious meals because of discarding long cooking or uncookable beans. Women have more time for income generating activities with the use of fast cooking cultivars. In East Africa, small children accompany their mothers to the work site during the work day. This procedure complicates feeding schedules for children and families. By using haybasket cooker technology, accompaniments are cooked with beans during the day and a warm meal is ready for family consumption in the evening. Food stuffs are cooked overnight in the haybasket, thus, providing ample warm food throughout the day for children. The planting of fast cooking cultivars by small farmers limits the deforestation of family farms, requires less family income for purchasing fuel, and improves soil fertility because of the increased conservation of crops used for fuel and the reduction of soil erosion.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. The consumption of dry beans is increasing in the United States, according to the popular press. The increase in consumption is attributed to relationship developed between consumption of beans, dietary fiber and cholesterol reduction.

B. Institutional Development and Training

1. Changes since FY 89. The relationship between INCAP and both WSU and MSU, has been very good, in terms of collaboration and exchange of ideas, beans and research tools. Likewise, WSU has helped INCAP's research efforts in equipment and reagents. At least four students will have been trained at INCAP by the end of the period from October 1, 1990 to September 30, 1991. Efforts will be made to have INCAP's staff to receive training at WSU and/or MSU on chemical techniques and processing.

2. Over the life of the project. Over life of project, training component has been outstanding. Training of students among WSU, MSU, INCAP, CIAT and the Universidade Sao Paulo has constantly improved.

3. Project training to be completed by the end of the project period.

Krista Shellie, Ph.D. (MSU)
Rodrigo Hoyos, M.S. (MSU)
Anup Chilukuri, Ph.D. (WSU)
Gasinee Trakoontivakorn, Ph.D. (WSU)
Li Ma, M.S. (WSU)
Adeline Cheong, M.S. (WSU)
Huei Hsieh, M.S. (WSU)

Students who will not complete degree being pursued by end of FY 92:

Frank Elia, Ph.D. (MSU)
Jose Berrios, Ph.D. (WSU)

C. Progress Achieved in Relation to the Extension Proposal Log Frame

The WSU, MSU, KSU, and INCAP project has been active for approximately nine years. Original time estimates were 10-15 years in duration. Extension project objectives require an additional three to five years; however, many objectives have been completed or partially completed. Significant amounts of information have been produced, both innovative and confirmatory in nature. The main result is that a closer cooperation has been established between plant breeders and geneticists and biochemical/nutritional activities. The concept of grain quality for beans is now an accepted area of research for national and international agricultural research centers. With this collaboration and a refined awareness in grain quality contributing to desired bean quality characteristics, practical advances will be faster.

Stronger relationships have been established with national bean programs, but particularly with CIAT; also with IITA and ICRISAT.

Specifically we have characterized the constraints to cooking quality of beans, the post-harvest conditions that result in hard-to-cook beans. We have developed tests and equipment to analyze and screen hard-cooking beans. MSU has determined the inheritance and genetic variability of cooking quality of beans.

Incomplete objectives include evaluating the stability and risks associated with natural toxicants in beans. We also proposed evaluating the protein quality of beans and developing ways to enhance protein

digestibility and quality. We proposed to develop dry bean genotypes with improved cooking quality and protein digestibility. We proposed development and publication of an analytical manual to provide methods to screen and assay cooking and nutritional quality of beans.

Additional genetic selection studies and development of germplasm are long term objectives requiring constant reevaluation of genetic materials used as parents.

Most of the research objectives of this project are unique. MSU, WSU and INCAP are internationally recognized research laboratories for assessing dry bean quality. This reputation is substantiated by numerous visitors, requests for information and invitations extended to CRSP involved scientists. MSU is taking the lead in organization and publication of a book containing food quality standards for beans and standard experimental protocols for use by bean breeders and other scientists, and principal investigators are participating in authoring and editing two texts on the nutritional quality of beans (*Phaseolus vulgaris*).

Contribution of the proposed research to HC and U.S., as well as to amelioration of global constraints. To the HC: Germplasm with improved digestibility and concomitant nutritional advantages will be combined with the seed characters preferred (color, size, shape, etc.) by consumers and released for use by national and federal breeding programs in Latin America and East Africa.

Joint publications will result from collaborative work in the areas of methods development, a procedures manual for assessing food quality, inheritance strategies, and germplasm releases.

Graduate and post-doctoral training will directly improve research capability and strengthen the technological base of Guatemala and other developing countries. Women will become a significant part of the professional and scientific network at institutions and universities of developing countries and be fairly represented and utilized at all levels of science and technology. Training through workshops and short courses will provide for practical implementation of solutions to constraints which limit nutrient bioavailability. Training received by the principal and co-investigators in biotechnology, cereal chemistry, and food technology will permit a more efficient utilization of all techniques, instruments, and approaches to attain the long range goal of the proposed research.

To the U.S.:

**Assess the value of identified genetic traits and new breeding methods.** Using improved genetic stocks, measure the contribution of recently identified traits related to indigestibility and nutritional value toward the fulfillment of stated goals for varietal improvement. In a parallel sense, the contribution of novel breeding approaches derived from theoretical studies such as the inbred-backcross and congruity backcross methods can be determined.

**Identify the interaction of recently introduced traits with other important agronomic characters.** The introduction of newly improved nutritional traits interacts with many important traits, influencing

production and disease/insect responses. The identification of second-order effects through cooperative testing is essential for the timely adjustment of background genotypes and breeding strategies for deployment of the new characters.

**Determine the probable stability of newly introduced traits.** Widespread testing of new breeding lines and cultivars gives public and private breeders and other researchers insight into the spatial and temporal stability of a preferred trait, and suggests strategies for recombining the new trait with appropriate genetic backgrounds and other complementary mechanisms to produce durable resistance.

**Identify production implications of newly introduced traits.** Cooperative evaluation of elite germplasm often reveals production opportunities or requirements not rapidly apparent in one or a few test locations such as plant/row spacing, planting/harvest dates, use and timing of external inputs, and equipment. Optimal production practices are recognized as quickly as possible, which benefits producer, consumer, researcher, and industry.

**Recommend priorities for germplasm collection, maintenance, and development by public institutions.** It is largely through cooperative testing of new germplasm that the bean research community (public and private) has identified priorities and proposed improvement strategies. The Bean/Cowpea CRSP has been a major network for stimulating collaboration among bean researchers in the U.S.

**Provide a forum for scholarly interaction.** The exchange of information and ideas that accompanies the evaluation of advanced lines and new cultivars is a fertile environment for public and private researchers, for student and teacher, and for professionals with a variety of the disciplinary skills necessary to accurately assess the value of a bean genotype in production agriculture.

Knowledge acquired, concepts developed, and germplasm identified will be incorporated into useful breeding and selection procedures and cultivars or cultigens. These aspects of the science will be applied toward developing adapted bean cultivars, according to the needs in different production areas and the classes of bean utilized.

D. Evidence of Biological/Social Sciences Integration

Women will be provided with a weanling food for infants, farmer self-sufficiency in bean production will increase and there will be a larger demand for beans in the marketplace. Women in Guatemala and Central America most often are the primary vendors at market thus they will be the prime recipients of increased family incomes. Prolonged shelf-life of beans will permit a continuous supply of beans in the year around diet. Improved cookability will reduce the inconvenience of cooking beans by women and alleviate the frustration that accompanies the inability of preparing nutritious meals because of discarding uncookable beans. Women will have more time for income generating activities with the use of fast cooking cultivars.

Project emphasis is on recruitment of female graduate students from HC and developing countries. Women will become a significant part of the professional and scientific network at institutions and universities of developing countries and be fairly represented and utilized at all levels of science and technology.

Other social science issues addressed include: improved health and well-being of rural people; better country ecology and natural resource conservation.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

- Tahzania/WSU Project and Sokoine University
- Malawi/MSU CRSP Project
- Nigeria/Georgia CRSP Project
- University São Paulo
- CIAT Bean quality program
- ICRISAT legume quality
- IITA Cowpea quality program
- American University, Beirut
- New South Wales bean quality program
- University of Guelph
- University of Alberta

II. FUNDING/FISCAL MANAGEMENT

A. Problems Regarding Funding

Limited funding is a research constraint. An unexpected 15 percent reduction in FY 91 will impair proposed research. Declining budgets have limited graduate student research through reductions in supplies, equipment and manuscript preparation funding.

Preliminary talks are underway to prepare a joint proposal on protein/energy/mineral availability of beans among HC institutions.

B. Adequacy of Current Management System

No activity towards buy-ins in Guatemala. Additional funding has been successful in past and efforts will be continued. Reporting of research conducted with integrated funding has been questioned, received and reviewed with less than complimentary comments in the past.

III. PROJECT STATUS

A. Appropriateness of Activities to Goals of the Global Plan

A major goal for small-scale producers in lesser developed countries is to improve the ability to provide sufficient dietary protein and energy to prevent protein-calorie malnutrition for all age groups and to allow good growth in children. Providing adequate protein and energy requires sufficient production of high quality, readily acceptable foods which meet nutritional requirements. Quality implies that essential nutrients are present in adequate amounts and are readily digested and absorbed. The WSU/INCAP project personnel are cognizant of the need to be

concerned with protein and energy production per unit of land and with food acceptability; however, the main focus of FY 90 research was directed towards improving the bioavailability of protein, carbohydrate, and vitamins in readily consumed foods (beans and cowpeas). Grain quality characteristics and utilization of beans are important and appropriate activities to goals of the Global Plan.

B. Balance Between Research and Training

1. Expenditures for research and training. MSU separates research ($13,383) and training ($9,875). WSU believes research and training are integrated since primary expenditures ($45,093) are for graduate students conducting research with $3,500 worth of supplies. INCAP provided no separation.

2. Expenditures related to research and training plans. Research and training plans are similar for FY 89 and FY 91.

C. Balance of Domestic vs. Overseas Activities

Overseas activities included:

OAS supported PI travel to Brazil
WSU student in Brazil (CRSP support)
Brazil student at WSU (University São Paulo/OAS support)
International Program Development Office (IPDO), WSU supported travel to Tanzania
AFGRAD Postdoctoral studies in conjunction with Ghana Food Science Institute

D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

- research planning in Anaheim, CA, IFT Annual Meeting
- writing, revising extension proposal via telephone
- PI meeting at MSU
- standard beans analyzed in collaborative study--MSU, WSU and INCAP
- participation in Western Regional Research Project 150

E. Relative Contributions of Collaborating Institutions and Individuals

HC: $32,075. Salaries of INCAP investigators have been provided by the institution as a contribution to the project.

U.S.: $55,391. In kind contributions.

Other funding:

WSU College of Agriculture, Research Assistantship + tuition waiver
W-150 Regional Research, Travel + State Research project
International Program Development Office, Travel to Bean Workshop in Tanzania

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

No enthusiastic interest in involvement or support by USAID Mission or Consulate.

G. Evidence of Institutionalization

1. Faculty recognition. Several invitations received to present papers at Foreign institutions; invitations to write book chapters in foreign publications. Presented invited paper at International Symposium entitled, "Legumes--production, processing, food value, and Health Benefits"--June, 1990. Contacted by student from the University of Dar es Salaam, Tanzania to work on the genetics of cookability in pure lines and/or races of beans.

2. Integration of domestic and international commodity research programs. Ongoing with integration of USDA/ARS and CRSP projects

3. Internal project managment. Support from administrative assistants in University Departments; support of Department Chairpersons; support of University contracts and grants personnel

4. Student/Professor interactions. Advisors to 16 graduate students working on CRSP project; serving on graduate committees of graduate students working on WSU/INCAP and Malawi CRSP projects.

IV. PUBLICATIONS AND PRESENTATIONS

Bressani, R. 1989. Revision sobre la calidad del grano de frijol. Arch. Latinoamer. Nutr. 39.

Uebersax, M.A., Reungsakulrach, Songyos, and Hosfield, G.L. Uses of common dry field beans. 1989. Chapt. 16, pp. 231-252. In Lusas, E.W., Erickson, Dr.R., and Nip Wai-Kit (Eds.). Food uses of whole oil and protein seeds. Amer. Oil Chem. Soc., Champaign, IL (Book Chapter).

Watt, E.E., Hosfield, G.L., Hoyos, R.A., and Saunders, J. The effects of calcium, iron, and EDTA on rooting and callus weight in common bean. 1989. Annu. Rep. of Bean Improvement Coop. 32:34-36.

Wassimi, N.N., Hosfield, G.L., and Uebersax, M.A. Inheritance of physico-chemical seed characters related to culinary quality in dry bean. 1990. J. Amer. Soc., Hort. Sci. 115:492-499.

Kohnhorst, A.L., Uebersax, M.A., and Zabik, M.E. 1990 Production and functional characteristics of protein concentrates. JAOCS, May. 67:285.

Lorimer, N.L, Zabik, M.E., Harte, N.C., Stachiw, N.C., and Uebersax, M.A. 1990. Navy Bean Flour Fractions in Composite Doughs: Effect of bean grade on Wheat Dough Rheology Parameters and Microstructure: Composite Doughs Using Prime and Cull Navy Bean Flour Fractions. Cereal Chem.

Bakker, J.L, Uebersax, M.A., Kowal, A.P., McGinnis, R.K., Williams, D. 1990. Process for making frozen rehydrated legumes. U.S. Patent No. 4,900,578.

Uebersax, M.A. and V. Khader. 1989. Legumes in Indian Diets. MI Dry Bean Digest. 13(4):10-13.

Occena, L.G., Uebersax, M.A., Long, R.A., and Middleton, J. 1990. Quality assessment of dark red kidney beans grown under deer exclusion conditions. MI Dry Bean Digest p. 14-17.

N.L. Lorimer, M.E. Zabik, J.B. Harte, N.C. Stachiw and M.A. Uebersax. 1990. Navy Bean Protein Effects on Composite Flour Rheology. Chemical Bonding and Microstructure: Navy Bean Protein Effect on Wheat Flour. Cereal Chem.

Hosfield, G.L. and Uebersax, M.A. 1990. The definition and measurement of culinary quality in dry bean. MI Dry Bean Digest. Vol. 14 no. 4. pp. 20-22,27.

Swanson, B.G. 1990. Pea and lentil protein extraction and functionality. JAOCS 67(5):276-280.

Swanson, B.G. and W.E. Artz. 1989. Protein interactions with procyanidins. Proceedings of the 3rd Symposium on Food Proteins. Abhandlungen der Akademie der Wissenschaften der DDR. pp. 115-127.

Extudio comparativo de la composicion quimica y valor nutritivo del *Phaseolus coccineus* (piloy) y del *Phaseolus vulgaris* (Frijol Comun). E. Calderon: L. Veasquez; R. Bressani. Arch. Latinoamer. Nutr.

Changes in dietary fiber content and its composition as affected by processing of black beans (*P. vulgaris* var. Tamazulapa). E.Acevedo; L. Velasquez; R. Bressani J. Agr. Food Chem.

Some chemical components and nutritive value of bean cooking broth. R. Bressani; R. Gomez-Brenes; J.E. Braham. J. Food Sci.

Apparent digestibility of bean protein evaluated in humans, rats and *in vitro* assays. C. De Godinez; R. Bressani; M. Melgar. Nutr. Rept. Internat.

Srisuma, N., Ruengsakulrach, S., Uebersax, M.A., Bennink, M.R. and Hammerschmidt, R. 1990. Cell Wall Polysaccarides of Navy Bans (*Phaseolus vulgaris*). Submitted for Journal of Agricultural and Food Chemistry.

Shellie-Dessert, K.C., and Hosfield, G.L. Implications of genetic variability for dry bean cooking time and novel cooking methods for fuelwood conservation in Rwanda. 1990. Ecology of Food and Nutrition. (In press).

Silverstein, Lori J., Barry G. Swanson and David F. Moffett. 1991. Procyanidin from black bean (*Phaseolus vulgaris*) inhibits glucose and alanine transport in rat ileum. Submitted to Amer. J. Physiol.

Silverstein, Lori J., Barry G. Swanson and David F. Moffett, 1991. Black bean procyanidin and electrolyte transport in the rat ileum. Submitted to Amer. J. Physiol.

Chilukuri, Anup and Barry G. Swanson. 1991. Microstructure of adzuki beans (*Vigna angularis* cv. Express). Submitted to Food Structure.

Plahar, Wisdom and Barry G. Swanson. 1991. Pasting properties, tannin content and protein digestibility and quality of cowpeas (*Vigna unguiculata*. Submitted to J. Food Sci.

Kohnhorst, A., D.M. Smith and M.A. Uebersax. 1990. Compositional, Nutritional and Functional Properties of Meals, Flours and Concentrates from Navy and Kidney Beans (*Phaseolus vulgaris*). Journal of Food quality. (Accepted for Press: Vol. 14).

Kohnhorst, A., D.M. Smith, M.A. Uebersax. 1990. Production and characterization of a protein concentrate from navy beans. Food Chemistry. (In press).

Abstracts

Beaumarchais, J.M., Uebersax, M.A., and Hosfield, G.L. Feasibility and quality evaluation of fully cooked individually quick frozen (IQF) dry beans. Abstr. 1989 Bienn. Meeting of Bean Improvement Coop. (Abstract)

Ruengsakulrach, S., Sirsuma, N., Uebersax, M.A., and Hosfield, G.L. Complex carbohydrates of navy beans (*Phaseolus vulgaris*): Starch. Abstr. 1989 Bienn. Meeting of Bean Improvement Coop. (Abstract)

Srisuma, N., Ruengsakulrach, S., Uebersax, M.A., and Hosfield, G.L. Complex carbohydrates of navy beans (*Phaseolus vulgaris*): Cell wall polysaccharides. Abstr. 1989. Bienn. Meeting of Bean Improvement Coop. (Abstract)

Hoyos, R.A., and Hosfield, G.L. The use of somatic tissues in liquid and solid media for evaluating embryogenesis in common bean (*Phaseolus vulgaris* L.). Abstr. 1989 Bienn. Meeting of Bean Improvement Coop. (Abstract)

Hosfield, G.L., and Uebersax, M.A. Quality in dry bean--can it be improved? Abstr. 1989 Bienn. Meeting of Bean Improvement Coop. (Abstract)

Presentations

Purificacion de inhibidores de tripsina de frijol negro. J. Orgiz. Asociacion de Nutricionistas y Dietistas de Guatemala. Memorias del XII Congreso de Nutricion de Centro América y Pamama. Junio 11-15, 1990. Guatemala.

Tercer Seminario y Primer Simposium Nacional de Biotechnologia, abril 19-20, 1990, Guatemala.

Caracterizacion fisico-quimica-nutritional del Piloy (*P. coccineus*) en comparacion con frijol comun (*P. vulgaris*). E. Calderon. Asociacio de Nutriciionistas y Dietistas de Guatemala. Memorias XII Congreso de Nutricion de Centro América y Pamama. Junio 11-15, 1990. Guatemala--PCCMCA--1990, El Salvador, C.A.

Impact of starchy legumes in developing countries. R. Bressani. AACC Meeting, Washington, D.C., October 29-November 2, 1989.

Analisis de la cantidad y composicion de la fibra dietética del frijol negro (*P. vulgaris* var. Tamazulapa) preparado en forma tradicional. L.A. Velasquez. Asociacion de Nutricionistas y Dietistas de Guatemala. Melorias del XII Congreso de Nutricion de Centro América y Pamama. Junio 11-15, 1990.

Hosfield, G. Quality in dry bean-can it be improved? 1989. Presented at 1989 Biennial meeting of the Bean Improvement Cooperative, Toronto, Ontario, Canada, November 5-10, 1989.

Hosfield, G. The genetic control of production and food quality factors in dry beans. Presented at the International Symposium, "Legumes-Production, Processing, Food Value, and Health Benefits Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 18, 1990.

Swanson, Barry G. 1990. The dry bean-Its many food values. Agripac, Inc. Customer Workshop. May 8. Los Angeles.

Swanson, Barry G. 1990. Chairman and organizer of Symposium on "Legumes-Production, Processing, Food Value and Health Benefits. Annual Meeting of the Institute of Food technologists. June 18. Anaheim.

Uebersax, M. and S. Ruengsakulrach. 1990. Strategies and procedures for processing dry beans. Symposium on Legumes-Production, Processing, Food Value and Health Benefits. Annual Meeting of the Institute of Food Technologists. June 18. Anaheim.

Phillips, R.D. and K.H. McWatters. 1990. Contribution of cowpeas (*Vigna unguiculata*) to nutrition and health. Symposium on Legumes-Production, Processing, Food Value and Health Benefits. Annual Meeting of the Institute of Food Technologists, June 18, Anaheim.

Hughes, J.S. 1990. Potential contribution of dry bean dietary fiber to health. Symposium on Legumes-Production, Processing, Food Value and Health Benefits. Annual Meeting of the Institute of Food Technologists, June 18, Anaheim.

Swanson, Barry G. 1990. Proteins in dry beans--quality and quantity. Meeting of Dry Bean Research Committee, National Food Processors Association. February. San Francisco.

Swanson, Barry G. 1989. Lectures on dry bean protein digestibility. Departmento de Alimentos e Nutricao Experimental, Univesidade de Sao Paulo, November, Sao Paulo, Brazil.

Swanson, Barry G. 1990. Bean nutritive quality, digestibility and ways of integrating food quality factors into a breeding program. Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. "Progress in improvement of common beans in eastern and southern Africa." Sokoine University of Agriculture, September, Morogoro, Tanzania.

**Table 25. Nitrogen Content (%,db) and In-vitro Protein Digestibility of Canned Navy Beans from Various Cultivars and Breeding Lines**

| Bean Cultivar | %N | %Protein Digestibility |
|---|---|---|
| Cooperative Elevator Co. | | |
| C-20 | 4.65 ± 0.03 | 86.28 ± 0.48 |
| Seafarer | 4.51 ± 0.04 | 86.29 ± 0.16 |
| Fleetwood | 4.42 ± 0.01 | 86.40 ± 0.32 |
| 84004 | 4.73 ± 0.04 | 86.85 ± 0.64 |
| Michigan Dry Edible Bean Research | | |
| C-20 | 4.68 ± 0.45 | 83.91 ± 0.64 |
| Seafarer | 4.24 ± 0.12 | 83.01 ± 1.28 |
| Mayflower | 4.53 ± 0.39 | 82.90 ± 0.16 |
| Bunsi | 4.37 ± 0.18 | 84.72 ± 0.83 |
| N85027 | 4.44 ± 0.00 | 83.80 ± 2.07 |
| N87007 | 4.58 ± 0.04 | 84.14 ± 0.00 |
| N85006 | 4.38 ± 0.02 | 85.15 ± 1.12 |
| N84032 | 4.43 ± 0.00 | 85.83 ± 0.16 |
| N85606 | 4.24 ± 0.03 | 85.38 ± 0.16 |
| $LSD_{(0.05)}$ | 0.38 | 1.81 |

**Table 26. Nitrogen Content [1] (%,db) of Cotyledon Flours and Isolated Bean Protein Fractions**

| | Bean Cultivar | |
|---|---|---|
| Flour/Protein Fraction | C-20 | Seafarer |
| Cotyledon Flour | 4.80 ± 0.02a | 4.12 ± 0.03a |
| Albumin | 9.86 ± 0.35b | 9.90 ± 0.04b |
| Globulin I | 14.04 ± 0.18c | 13.76 ± 0.22c |
| Globulin II | 15.71 ± 0.21d | 15.50 ± 0.82d |

[1]n-3,Means in a column followed by different letters are significantly different ($P<0.05$).

**Table 27. The Effect of Heating (Autoclave, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from C-20 and Seafarer**

| Heating Effect/ Protein Fraction | % Protein Digestibility[1] | |
|---|---|---|
| | C-20 | Seafarer |
| **Raw** | | |
| Cotyledonary Flour | 72.75 ± 0.16b | 73.09 ± 0.32a |
| Albumin | 69.82 ± 0.80a | 71.74 ± 0.98a |
| GI | 88.54 ± 0.16d | 91.21 ± 0.48b |
| GII | 83.35 ± 0.79c | 85.38 ± 0.48b |
| **Autoclaved** | | |
| Cotyledonary Flour | 80.98 ± 0.96b | 81.66 ± 0.32b |
| Albumin | 73.20 ± 0.48a | 73.54 ± 0.00a |
| GI | 91.59 ± 1.59c | 91.58 ± 0.00c |
| GII | 92.60 ± 0.48c | 92.94 ± 0.32d |

[1]n-3, Means in a column followed by different letters are significantly different ($P<0.05$).

**Table 28. Analysis of Variance for the Effect of Isolated Bean Protein Fractions and Heat Treatment on Protein Digestibility**

| Treatment | df | Mean Square | |
|---|---|---|---|
| | | C-20 | Seafarer |
| Bean | 3 | 315.41*** | 329.84*** |
| Cooking Effect (Autoclave) | 1 | 142.97*** | 83.48*** |
| Bean Protein Fraction x Cooking Effect | 3 | 10.37*** | 16.83*** |
| Error | 8 | | |

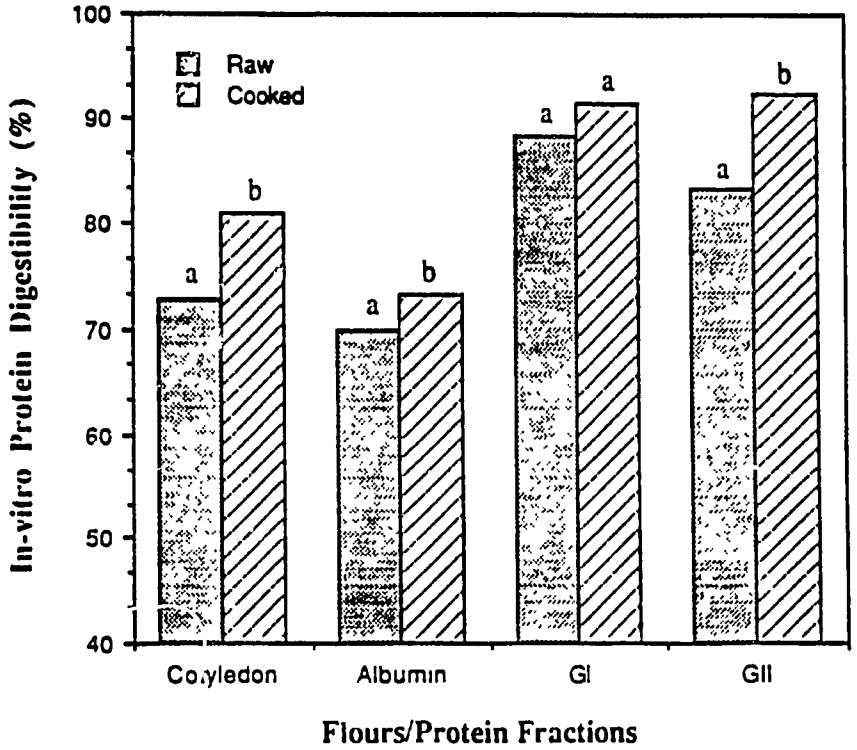



Figure 24. Effect of Heating (Autoclaved, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from Navy Bean "C-20"

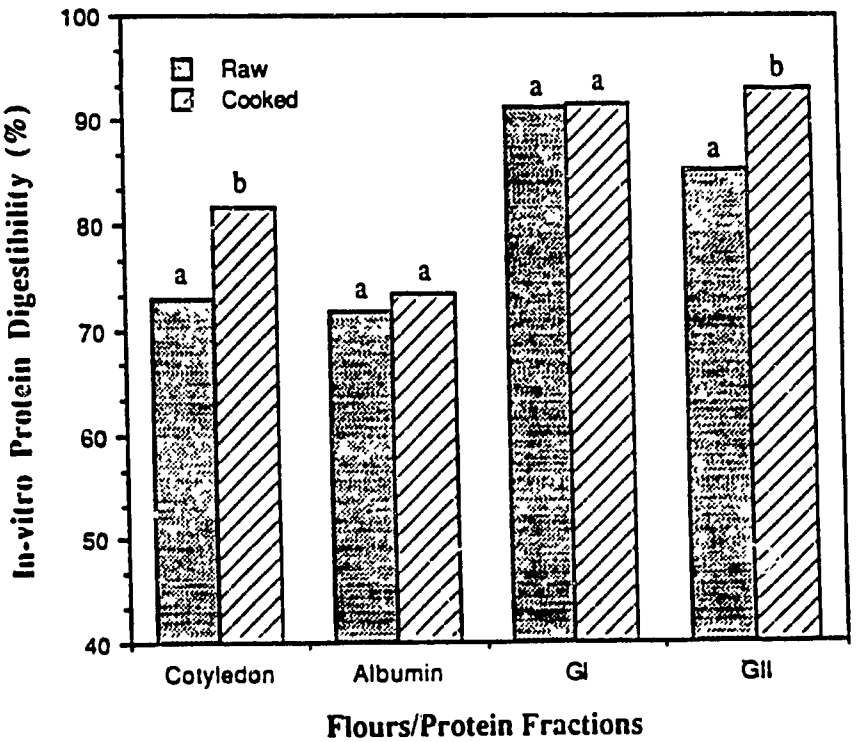



Figure 25. Effect of Heating (Autoclaved, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from Navy Bean "Seafarer"

# MALAWI - UNIVERSITY OF CALIFORNIA-DAVIS

*IMPROVEMENT AND HOST PATHOGEN CO-ADAPTATION IN MALAWI, A SECONDARY CENTER OF DIVERSITY*


**Principal Investigators:**

| | | |
|---|---|---|
| Paul Gepts (U.S.) | Agronomy and Range Science | University of California-Davis |
| A. B. C. Mkandawire (HC) | Crop Production Department | Bunda College of Agriculture |

**Co-Principal Investigators:**

| | | |
|---|---|---|
| Anne Ferguson | Department of Anthropology | Michigan State University |
| Robert Gilbertson | Department of Plant Pathology | University of California-Davis |
| Richard Mkandawire | Department of Rural Development | Bunda College of Agriculture |
| Steven Temple | Agronomy and Range Science | University of California-Davis |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

U.S.: A study which provides evidence that the cytoplasms of the two major gene pools of *P. vulgaris* are distinct was completed as a Ph.D. dissertation by M. Khairallah. Part I of the dissertation represented a continuation of the genetic diversity research on Malawian bean germplasm. The objectives were to examine the diversity of a sample of the Malawian lines at the mitochondrial DNA (mtDNA) level, and to compare and contrast it to the morphoagronomic (Martin 1984) and allozyme variation (Sprecher 1988). Twenty lines were selected to include both "related" as well as "distant" lines; three pure-bred cultivars were included as controls. Despite the use of eight restriction endonucleases to digest the mtDNA, and cosmid clones covering approximately 200 kb of the bean mitochondrial genome, the results showed a high level of mtDNA homogeneity among the 23 bean lines examined. However, five restriction fragment length polymorphisms (RFLPs) were detected that differentiated the Malawian lines into the Mesoamerican and the Andean gene pools, providing the first evidence that the cytoplasms of the two major gene pools of *P. vulgaris* are distinct.

In the second part of the dissertation, the mtDNA diversity was examined in extant wild bean populations to understand how and when the mitochondrial genomes of the two gene pools of cultivated beans became distinct. The mtDNA of six wild bean accessions from Central and South America were digested with nine endonucleases and analyzed by Southern hybridization. Observation of 20 RFLPs clearly pointed to a higher amount of mtDNA variability in wild than in cultivated beans. The distribution of mtDNA RFLPs among the wild beans indicated that in four out of the five inter-gene pool RFLPs, the

mutations arose soon after domestication, two in the Mesoamerican and two in the Andean gene pool. The observed stability of the mtDNA restriction patterns makes it an attractive system to study bean origin and evolution. The analysis of a larger number of wild bean accessions is essential before definite answers about bean origin and domestication can be presented. The identification of four endonucleases that detected significantly more mtDNA RFLPs than did other enzymes will facilitate this task.

In the third part of the dissertation, mtDNA restriction patterns from three *Phaseolus* species were examined to estimate their relative genome sizes and determine the level of interspecific variability. The mitochondrial genome size estimates for *P. vulgaris*, *P. coccineus*, and *P. acutifolius* were 456, 325, and 400 kb, respectively. The high level of mtDNA variation between the species contrasted with the homogeneity with *P. vulgaris*. Indices of relatedness between species were computed and demonstrated that *P. vulgaris* and *P. coccineus* are more related to each other than either is to *P. acutifolius*, which was equally distant from the other two species.

Four hundred and two Malawian landraces were characterized on the basis of isozyme patterns for 23 enzymes in work carried out by Dr. G. Acquaah. Electrophoretic separation of protein molecules was effected in 10 percent starch gels using a tris-borate and a morphoine-citrate electrophoresis buffer system. The results, as presented in Table 1, show that a very limited number of enzymes are polymorphic in the Malawian landraces. Further analysis involving enzymes in category 1 (Table 1) corroborated a previous report (Sprecher 1988) that the two bean gene pools (Andean and Mesoamerican) were marked by alternate alleles at the six enzyme loci. Of the new enzymes investigated in addition to the six, significant tentative polymorphism was found only for MDH. Furthermore, principal factor analysis showed the MDH polymorphism to be independent of the gene pool association described above.

Not only does the paucity of polymorphism limit the use of isozymes as markers in dry bean, but also the gene pool allozyme specificity and the partial reproductive isolation of the gene pools further limit the application of allozymes in bean breeding.

A study of the mechanisms of dry bean hardening in storage, with special reference to the role of pectin methylesterase, phytase and lignin was carried out by M. Mafuleka, forming the research for her Ph.D. dissertation in Food Technology at MSU.

The phytic acid degradation and lignin formation mechanisms in the hard-to-cook phenomena of the hard (red) and soft (white) decorticated Malawian beans were examined. Samples stored under varying temperatures, humidities and time periods (60°F or 15.6°C; 95°F or 35°C; 55% RH, 85% RH; four and eight months respectively) were compared to the control groups (35°F or 2°C, 30% RH, zero months). Phytase activities (inorganic phosphate), phytic acid, total pectic and water soluble pectic substances, calcium and magnesium ions, and lignin levels were determined

spectrophotometrically. Titrimetric, micro-kjeldahl and Kramer Shear Press procedures were used to determine total pectic substances' degree of esterification (pectin methylestrase [PME] activities), protein contents and cooked bean firmness, respectively.

Increased cooked hardness, elevated phytase and PME activities and slight increases in lignin levels existed in both the hard and soft beans stored under adverse conditions for the extended period (eight months). Positive correlations between cooked bean hardness and phytase activities (r = 0.83), among legume seed hardness and lignin levels (r = 0.71), and between the hardness of seeds stored for eight months and PME activities (r = 0.67) r = 0.67) were found. The phytic acid degradation mechanism appeared to be the dominant system directing the hard-to-cook defect of the beans under adverse conditions over the eight months storage period.

A total of 650 bean landraces collected from about three quarters of Malawi was investigated for seed storage protein (phaseolin) type in work carried out by G. Aquaah. Of the over a dozen phaseolin types known (including A, B, C, CH, S, Sb, Sd, T, To, Tcaj, I, J, K and M), only four, S, T, C, and H, molecular forms were observed in the Malawian collection. The most predominant phenotype was the T phaseolin pattern which occurred in 49 percent of the genotypes, while the least frequent type was H (2.5 percent) (see Table 2).

The seed size ranges and means are presented in Table 3. The "S" phaseolin type is associated with small seed size and the Mesoamerican center of domestication, with the T, C, and H, types are generally large-seeded and associated with the Andean center of domestication (Gepts and Bliss 1986). All the phaseolin types were found in a wide range of seed size categories (see Table 3). This is attributable to natural crossing and selection among genotypes. The phaseolin types observed indicates that the Malawian landraces were introduced from both centers of domestication.

Host Country

Host/pathogen coadaptation studies: The disease pathogen samples of angular leafspot (ALS) were collected in the Southern Region, and single spore cultures were isolated at Bunda College. At maturity the seed was collected from the bagged diseased plants from farmers' fields. A single batch of duplicate samples of pathogens was shipped to Michigan State University for isozyme analysis. Due to a change in U.S. lead institution, these samples have been shipped to Dr. Paul Gepts of University of California at Davis. The seed was multiplied at the Bunda Farm and is currently being harvested. This seed will be sent to University of California, Davis for further research.

ALS is one of the most important diseases in Malawi. In an attempt to screen both the local germplasm and introductions, up to 1248 of the 2778 accessions of the Malawian Germplasm Collection and a few from outside Malawi were evaluated for their reaction to ALS during the past two cropping seasons at Bunda College. Screening was done both in the field and in the screenhouse. Almost 33 percent of the lines showed resistance to ALS while about 22 percent had an intermediate reaction.

Ten isolates of *Phaeoisariopsis griseola* (the ALS pathogen) from different bean growing areas of the country were grown and inoculated on a set of differential varieties. Results showed that the pathogen is highly variable, supporting earlier results (1988/89) and confirming the need for developing bean varieties that possess general rather than specific resistance. The existence of this pathogenic variability reinforces the need for collecting more isolates from the different bean growing areas to ascertain the extent of the variability. The results also indicated that PI167399, Coco ala Creme and G05434 were resistant to all the isolates. G05434 had smallest lesions when infected by all isolates while Evolutie had the largest.

Sociological survey of bean production and use practices: A social science survey, under the direction of CRSP researchers R. Mkandawire and A. Ferguson, was conducted in the Southern Region (Thyolo) during 1989/90 cropping season. This was the only region of the country where there had been no socioeconomic or cultural surveys of bean production conducted by the CRSP.

This survey of 350 small-scale farmers demonstrated that beans are an important cash as well as food crop in this area. In fact, beans ranked first as a crop providing income for most households. (Matapwata EPA, where the study was carried out, is located approximately 30 kms south of Blantyre, the largest city in Malawi.) The farmers face constraints to bean production caused by factors such as diseases, pests, drought, lack of seed, lack of preferred varieties, lack of extension support and lack of land. In this area, farmers indicated a strong preference for bush beans over climbers, and among the bush types they preferred Chimbamba (a red kidney) and Kaulesi (small purple speckled) for their culinary aspects, marketability and tolerance to diseases.

The survey revealed that the majority of households were headed by women many of whose husbands were away working in the nearby cities of Blantyre/Limbe or on estates. Land holdings were very small, with many farms being under half a ha. In part because of the small size of farms and also because of market pressures from the nearby towns and estates, farmers in this area grew a limited number of bean varieties (range between 1-6) as compared to farmers in the Central and Northern regions. Data analysis of this survey is not yet completed although a preliminary report has been prepared.

2. <u>Other research-related results</u>. Germplasm conservation, screening and use: A major thrust of the CRSP program at Bunda College is to screen local and introduced germplasm. Bunda College has the largest collection of *Phaseolus* germplasm in eastern and southern Africa. A large portion of this material was collected as the result of CRSP research.

**Yield trials.** During 1989/90 the National Bean Variety Trial was placed at seven sites spanning the different agro-ecological zones in Malawi. In the Northern Region these included: Sokola (Misuku), Chitipa; Ng'onga Extension Planning Area, Rumphi;

Lunyangwa, Mzuzu; Champhira Extension Planning Area, Mzimba. In the Central Region trials were placed at: Bunda, Lilongwe; Dedza Hills, Dedza; and in the Southern Region the trial was situated at Matapwata Extension Planning Area, Thyolo.

Twelve genotypes comprising introductions, landrace selections, hybrid materials and a released variety (Nasaka) as a check, were included in the trials. Seed yields were significantly different among genotypes except at Champhira and Sokola. In general, yields were higher than those obtained last year. CIAT bean lines A344 and A286 were among the highest yielders at these locations and showed marked resistance to diseases. We need to determine if these would be acceptable to Malawian farmers. The alternative would be to use them in crosses for disease resistance. The trial will be repeated in the 1990/91 cropping season.

The Adaptation Trial is a component of the Breeding Scheme. It is comprised of introduced materials that have shown promise for a number of attributes. Results indicate a wide range of adaptational variation and significant yield differences among entries at Bunda, thus selections could be made from these entries. The local entries, Nasaka and 25-2, gave close to average yield but were far out-yielded by A286. The top three yielders, A286, A344, and BAT477, also showed resistance to ALS, CBB, BCMV and WB. However, they are small-seeded, a character that most small-scale farmers in Malawi rarely like. G05434, which yielded higher than the two local entries, is large-seeded and may be more acceptable to farmers. The same would be true for Perry Marrow.

**Disease resistance screening.** Selection for disease resistance in the segregating $F_3$ populations from crosses initiated in 1987 commenced in 1989/90. In these crosses some parental lines were the CIAT genotypes discussed above. Unfortunately, entries for Thyolo and Dedza were planted late and were subsequently lost. However, 50 test lines have been selected to enter into a Preliminary Variety Trial for further evaluation.

**Agronomic trials.** An experiment was conducted to determine the effects of nitrogen fertilizer application on the uptake of nitrogen by maize and beans in an intercropping system. Two contrasting bean varieties in terms of growth habit, Nasaka (dwarf) and Kanzama (climber), were grown in sole stands or intercropped with NSCM41 maize hybrid under four N-fertilizer levels: 0, 40, 80 and 120 kg N/ha. There were significant maize grain yield differences among N-fertilizer rates and between cropping systems. The maize/climbing bean system gave the highest maize yield. The content of nitrogen differed significantly between bean varieties with the climber having greater rates of uptake of N. Bean yields increased significantly only upon increasing nitrogen application to 40 kg N/ha except in the monoculture stand of Nasaka.

The effect of phosphorus on biological nitrogen fixation was investigated in an experiment with three bean varieties: Nasaka, P402, and 13-3, and three phosphorus fertilizer rates: 0, 25, 50 kg P205/ha; and inoculation or no inoculation. Higher phosphorus level

significantly increased effective nodulation. However, there were no significant interactions in mean number of effective nodules due to the effect of P fertilizer, bean variety, or inoculation. The bean variety P402 gave the highest response to inoculation and produced highest yields.

**Drought trials.** Two experiments were conducted during the 1989/90 cropping season to determine the response of bean genotypes to moisture stress at two stages of growth. One experiment was at Kasinthula Agricultural Research Station in the Shire Valley (80m above sea level) and the other was at Bunda College (118m above sea level) during the off-season under irrigation.

At Kasinthula, the time taken by beans to reach flowering did not differ among moisture regimes. However, the time to end of flowering was significantly reduced when drought was imposed during pod-filling. The size of canopy was significantly reduced by moisture-stress at flowering. The number of pods per plant was significantly reduced by moisture-stress at both flowering and pod-filling stages. However, 100-seed weight was significantly reduced only by moisture-stress at both flowering and pod-filling. The reduction at the former stage was a result of reduction in number of pods/plant whereas the reduction at the latter stage was a result of reduction in 100-seed weight.

The best yielding genotypes under drought were Domino when drought was imposed at flowering and Sapelekedwa, A268, A286 and 8-7 when imposed at pod-filling.

At Bunda, drought did not seem to have been well-imposed because there were no significant seed yield reductions. However, drought at flowering reduced the number of days to physiological maturity, leaf area index and canopy height of beans.

Due to better canopy characteristics and yielding ability as seen above, seven genotypes were selected for further evaluation: 25-2x8-7, Sapelekedwa, 2-10, 25-2, 8-7, 6-1, and 5-2.

**Seed production and distribution of CRSP produced cultivars.** Seed of Nasaka and other released varieties was multiplied under irrigation at Bunda College and at Lifuwu Rice scheme during 1989-90. This seed will be made available to selected Agricultural Development Districts for further multiplication and release to smallholders.

**Impact of other CRSP produced technology.** Information on the component breeding methodology, a bean breeding strategy for areas of the world where beans are grown as mixtures, is being disseminated and discussed at workshops in the eastern and southern African regions.

**Project impact on production and consumption of beans and cowpeas (on-farm trial results etc.).** During the 1989/90 cropping season, a pilot on-farm trial was conducted in the Lilongwe Rural Development Project of LADD at Kapini. Five farmers were issued seed of

Sapelekedwa (bush red kidney), Nasaka (bush khaki kidney), 8-7 (purple kidney), 5-2 (olive green medium roundish Nyauzembe), and 25-2x8-7 (white kidney). Farmers grew these beans following the recommendations of the Bean Program and were monitored throughout the season. The highest yield was obtained from the farmer who grew Sapelekedwa (900 kg/ha). This could represent a substantial increase over the general average of 300 kg/ha and comes closer to the government requirement of at least 1000 kg/ha. The Bean Program has, therefore, multiplied seed of released varieties which is currently being harvested for distribution to smallholder farmers for further increase.

**How the research findings specifically address the needs of small-scale farmers and women.** Our surveys have revealed that in many areas of Malawi beans are an important food and cash crop grown principally by women. Bean production and use surveys and the farmer participatory research methodologies we are developing provide information to breeders and other biological scientists, allowing them to direct their research toward meeting the needs of women and other small-scale farmers in the different regions of the country. For example, we are able to identify preferred growth habits, seed types, maturity dates, and other characteristics important to farmers in the three regions of Malawi.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Using FAO statistics and information from various Ministries of Agriculture in eastern and southern Africa, Dr. W. Grisley, CIAT economist in the Great Lakes Bean Program, recently presented a paper which indicated that Malawi was one of the few countries in the area where bean production per ha. has been increasing over the last decade. A copy of this paper has been requested. While such information is interesting, we recognize that increased yields can be due to a large number of factors in addition to the bean breeding program (for example, weather, more use of fertilizer, fewer diseases, and market factors).

Data presented above indicate that materials developed and being tested in the bean breeding program can substantially increase yields under researcher managed conditions. We also have some very preliminary information (from farmer managed on-farm trials) which suggests that some of the varieties being developed also perform well under farmer managed conditions.

B. Institutional Development and Training

1. Changes since FY 89. During 1990 a new U.S. lead institution was selected to head the Malawi CRSP Project. Dr. Paul Gepts, Department of Agronomy, University of California Davis, is the new U.S. PI. Co-PIs at the same institution are Dr. Steve Temple (Dept. of Agronomy) and Dr. Bob Gilbertson (Dept. of Plant Pathology). The U.S. social science component of the project remains at MSU under the direction of Dr. Anne Ferguson as Co-PI.

We are very saddened to report that the Chairperson of the Crop Production Department at Bunda College, Dr. S. Chienda, who has always been very supportive of the CRSP project, passed away in early October 1990.

2. Over the life of the project. U.S.: Currently there are no U.S students being supported by this project. Malawi: One student, Mercy Mafuleka, supported by the project, completed her Ph.D. degree during 1990 in Food Technology and Nutrition at MSU.

   Two students, supported by the project and in their first year of study, are currently enrolled (September 1990) in M.S. programs at Bunda College of Agriculture: Mr. P. Kambewa (Department of Rural Development) and Mr. D. Mandala (Crop Production Department). We anticipate these students will complete their degrees in late 1992.

   Other countries: One student, Mireille Khairallah from Lebanon, completed her Ph.D. degree in the Department of Crop and Soil Science, Michigan State University.

   Non-degree training: A Participatory Research Workshop was held for Malawian and Tanzanian researchers at Sokoine University, Tanzania in late September 1990.

3. Project training to be completed by the end of the project period. With the return of Dr. Mafuleka to Bunda College, the CRSP project will have trained two Ph.D. students (one in plant physiology and the other in food technology), one M.S. student (agronomy) and provided partial financial support to one other M.S. student (plant breeding). Another Ph.D. student (pathology) worked closely with CRSP scientists although he was supported by funding from other sources. These researchers, together with those receiving support from other sources who have recently returned to Bunda College, form the nucleus of a strong bean improvement program. As discussed in the 1992-97 project extension proposal, important gaps in the program remain to be filled in plant breeding, entomology and sociology/anthropology.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

This project has undergone a gradual evolution since its inception in 1982. The original project goals (1982-88) were to characterize the genetic diversity present in Malawian beans and to examine the biological and sociocultural factors which accounted for this diversity. This phase was essentially completed by 1988, except for the Ph.D. dissertation of M. Khairallah.

During the 1989-92 extension period, the goals of the project were to examine host pathogen coadaptation with a focus on angular leafspot and anthracnose, to study farmers' perceptions and management of bean diseases, to develop innovative plant breeding strategies for bean improvement programs in areas where farmer's grow mixtures, and to further the development of the bean breeding and improvement program at Bunda College by screening introduced and indigenous germplasm.

It was anticipated that research on the first two objectives in the 1989-92 workplan would be well advanced by the end of the 1989-92 extension period. The third and forth objectives are of an ongoing, long-term nature.

While work toward achievement of these objectives has advanced, it has done so more slowly than anticipated. In part this is explained by the resignation of the newly appointed PI at Michigan State University, Dr. T. Isleib, and the search for a new U.S. lead institution--all of which slowed research progress at the U.S. institution during 1989/90. (The PI at Bunda College also changed during this extension period--from Dr. W. Msuku to Dr. A. Mkandawire--although this did not hinder the research.)

Other factors which did delay progress were the loss of the CRSP vehicle (totaled in an accident) during 1989, delays in obtaining clearance to purchase a new vehicle, and the amount of time needed to identify and get graduate students admitted at Bunda College. This latter delay has slowed realization of the study of farmer's perception and management of bean diseases, as much of the field work for this investigation as to be undertaken by these students.

On the other hand, other aspects of the work have progressed well, as information presented in I.A. indicates.

At Bunda College, the CRSP research dovetails with the interests of the National Bean Improvement Program. The National Program is interested in exploring strategies for bean improvement that meet the diverse needs of small-scale farmers in Malawi, and in screening germplasm for improvement purposes. Angular leaf spot, the principal focus of the host/pathogen co-adaptation studies, is a major disease problem in Malawi. Researchers in the national program are also interested in learning what farmers' perceptions of bean diseases are and what measures they take to combat them since this information can be used to design cultural and other low input control measures.

CRSP project research in Malawi is coordinated with that of other projects aimed at improving bean production in the country. For example, close links exist between our CRSP program and the newly funded Rockefeller Grain Legume Improvement Project. Also, the bean program is developing close working relationships with other research groups in the Ministry of Agriculture--particularly with the maize commodity researchers and with the Adaptive Research Unit.

Collaboration exists between the CRSP project and the CIAT/SADCC Bean Program headquartered in Arusha, Tanzania, and between the Malawi CRSP and Tanzania CRSP projects. Researchers from Malawi now regularly attend the CRSP Annual Bean Workshop at Sokoine University.

The study of co-adaptation is important to Malawi because beans from both gene pools are often grown in the same field. Understanding the plant/pathogen interactions will help set breeding objectives for the two gene pools in Malawi and the U.S. The germplasm screening and cultivar development undertaken in Malawi under CRSP auspices is directly relevant to the National Bean Program objectives.

The information gathered on bean production practices, the role of women in agriculture and seed preferences in the three regions of Malawi is useful to breeders and other plant scientists. This information also provides anthropological insights into the extent, causes and role of diversity in beans. Now that social science information is available from the three regions, we can prepare a monograph on the socioeconomic underpinnings of bean diversity in Malawi which will be of interest to scientists concerned with the maintenance of crop genetic diversity and food security issues.

The dissertations Khairallah and Sprecher corroborated the organization of *P. vulgaris* diversity into two major gene pools. The identification of mtDNA markers could be used to study inter-gene pool crosses and mitochondrial inheritance in beans. In addition, the observed stability of the mtDNA RFLPs makes it a good system to study bean origin(s) and domestication.

The co-adaptation study will also contribute to basic knowledge of *P. vulgaris* in terms of evolution and adaptation.

D. Evidence of Biological/Social Sciences Integration

Since its inception, this project has made efforts to understand the gender division of labor and decision-making with regard to bean production and use in Malawi. Researchers have used these insights to advantage in the bean improvement effort. Information on farmer bean varietal preferences and rationales for these preferences allow researchers to better assure that the new varieties, techniques and technologies will be accepted and used by women and other bean farmers.

In addition to the applied use of this research, we have also gained a better understanding of women's roles in the maintenance of genetic diversity in beans and have come to appreciate the role such diversity plays in food security and survival strategies.

M. Mafuleka recently completed a Ph.D. degree in Food Technology, studying some of the factors responsible for the hard-to-cook phenomenon in beans. Hard-to-cook beans are a major constraint to bean consumption in Malawi because of the scarcity and high cost of fuel wood, a problem which continues to grow. Dr. Mafuleka will work with the CRSP project and the Bean Improvement Program to screen bean cultivars for this and other culinary and food quality characteristics.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

See I.C. above.

II. FUNDING/FISCAL MANAGEMENT

A. Problems Regarding Funding

A number of problems were encountered this year in Malawi and the U.S. which together are producing a financial crisis (see following items 1 through 4). We realize many of these problems are not unique to the

Malawi project, and we urge the TC and MO to consider these general issues when they meet to consider funding levels for the 1992-97 extension period. Business as usual (or even a 5 percent annual increase in project budgets) will mean a severe reduction in research and training efforts.

1. Depletion of the advance of funds ($25,000) given to Bunda College so that no operating funds were available to cover the period between the time the College submitted its receipts to MSU and when it received its quarterly reimbursement. We discovered that the project at Bunda College had not been submitting all its receipts to MSU. The costs of some items and services turned in directly to the administration building at Bunda College, rather than to the CRSP secretary, went unrecorded--but not unpaid. Consequently the advance of funds was nearly exhausted. We have asked Bunda College to submit receipts for these expenses for reimbursement purposes.

2. With a new U.S lead institution identified and three new U.S. PIs added--all of whom have research agendas--the project does not have adequate funds to pursue its objectives. (See repeated requests by P. Gepts to MO for additional/supplemental funding.)

3. Airfares to Malawi and costs of travel within the country continue to skyrocket. (For example, the cost of petrol was nearly $3.50 a gallon in August, 1990 before the significant increases caused by the Mid-east crisis, and a round trip excursion fare ticket to Malawi from California costs approximately $4,000.00). These increases in prices together with the increase in the number of PIs who need to visit Malawi on an annual basis place serious financial burdens on the project.

4. The administration at Bunda College has instituted a policy of charging indirect costs. While we support this measure, the funds for the indirect costs (estimated to be $1,500 in 1991, for example) will have to be taken out of the already inadequate research budget.

B. Adequacy of Current Management System

The project has been in a state of transition between lead institutions in 1990--MSU is phasing out in its role as lead institution, (although it retains a subcontract for the project's socioeconomic research) and UCD is taking over.

C. Activity Towards Buy-Ins or Other Funding

Dr. P. Barnes McConnell visited the USAID mission in Malawi in February to discuss the possibility of a buy-in. At that time, the Mission was in a state of transition with a new agricultural officer, deputy director and other personnel scheduled to arrive in the next six months. The Chief Agricultural Research Officer, Dr. Mkamanga, was supportive of the CRSP project, accorded beans a high research priority and was eager to encourage the Mission to support the buy-in, but he has since taken a new job outside the country.

The CRSP works closely with the newly established Rockefeller Grain Legume Project. We coordinate equipment purchases, training and research efforts.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

This is the only project in the CRSP which examines the role of bean genetic diversity in cropping systems and small-scale farmer food security strategies. One of the project's aims is to develop innovative plant breeding methods to meet the needs of women and other farmers who grow beans as mixtures. The maintenance and use of plant genetic diversity is a key focus of the newly evolving area of sustainable agriculture and accords with the CRSP mandate to develop low-input crop improvement strategies for poor farmers in developing countries.

The research on host/pathogen co-adaptation may result in findings which will be of benefit to breeding for disease resistance world-wide.

Biological and social science researchers in this project share the same research objectives and work in a collaborative fashion. The integration of the biological and social science research agendas may serve as a model for other projects. We anticipate that this close collaboration among scientists will result in improved varieties, techniques and technologies which are readily accepted by farmers.

### B. Balance Between Research and Training

During 1990, the project supported the final year of the Ph.D. training of Mireille Khairallah (Crop and Soil Sciences, MSU) and Mercy Mafuleka (Food Technology, MSU), at an estimated cost of $35,000 representing approximately 20 percent of the project's annual budget.

Expenditures for training were as we anticipated. Over the last three years, with the decline in funds, we have curtailed training efforts in the U.S and have elected to support two M.S. students at Bunda College. These students began their programs in September 1990.

### C. Balance of Domestic vs. Overseas Activities

During 1990, research in the U.S was somewhat curtailed by the departure of the U.S. PI, Dr. T. Isleib. Outside of the completion of the two doctoral dissertations and allozyme analysis of the Dedza germplasm collection, most of the research was conducted in Malawi by CRSP investigators there. Dr. Ferguson's field research is normally carried out in Malawi in collaboration with Dr. Richard Mkandawire, while analysis and write-up are done in the U.S. With the identification of the new U.S. team of researchers at UCD, we expect to have a strong U.S. biological program underway once again.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

A high level of collaboration between the U.S and Malawi has existed in research, training and administrative efforts. All workplans and budgets are developed collaboratively. The Malawian PI, Dr. Alex Mkandawire, and the social science Co-PI, Dr. Richard Mkandawire, participated in the selection of the new U.S. lead institution.

E. Relative Contributions of Collaborating Institutions and Individuals

1. U.S. and H.C. contributions to the FY 90 budget. Outside of U.S. institution indirect costs, the project's research and training funds are nearly equally divided between the U.S. and Malawi. Until recently when a decision was reached to charge overhead, Bunda College has assumed the costs of administration of the project there. The U.S. institution has traditionally contributed the time of the P.I.

2. Other funding above that by A.I.D. and participating HC institutions. None this year.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

We have maintained good relations with the USAID mission in Lilongwe, keeping its personnel up-to-date with our research and inviting them to Bunda College field days and other functions. During 1990, there were a number of changes at the mission--for example, both the Deputy Director and the Agricultural Development Officer are new to Malawi. This change in personnel, plus the changes at the Ministry of Agriculture in Malawi, has necessarily slowed efforts toward a buy-in.

G. Evidence of Institutionalization

1. Faculty recognition. In Malawi, researchers' time is necessarily oriented toward teaching duties, although the administration is supportive of participation of its faculty in international work. The administration at Bunda College remains supportive of faculty involvement in the CRSP research efforts since these dovetail with the interests of the National Bean Improvement Program headquartered at the College.

2. Integration of domestic and international commodity research programs. As noted, Bunda College has the mandate for bean improvement in Malawi, with the National Bean Improvement Program situated at the College. The National Bean Program and the CRSP project also maintain close ties with the CIAT/SADCC Bean Program. A plant breeder from this program (Dr. Aggarwal) will shortly take up a position at the College working with the National Bean Improvement program, in addition to his regional duties. Thus, there is a high degree of integration among the CRSP, the National Bean Improvement and the CIAT/SADCC programs, with many researchers simultaneously involved in these programs.

   In addition, the CRSP program and the National Bean Improvement Program in Malawi maintain close collaboration with other commodity research groups in the country including the maize program and the Rockefeller Grain Legume Improvement Project.

3. Student/Professor interactions. The CRSP project has provided Bunda College undergraduate students with the opportunity to gain valuable field research experience in both the biological and social sciences. Most recently during 1990, Drs. Mkandawire and Ferguson carried out a survey of bean production practices in

southern Malawi, involving twelve students (eight men and four women) as research assistants. These students participated in the data analysis and write up of results from this survey, all of these activities representing valuable training experiences. Similar experiences are routinely provided to students in the Crop Production Department.

## IV. PUBLICATIONS AND PRESENTATIONS


Ferguson, A. E. 1990. So the grandparents may survive: Farmer participation in bean improvement in Malawi. Paper presented at Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. Sokoine University of Agriculture, Morogoro, Tanzania, September 17-22.

Ferguson, A. E., A. V. Millard and S. Khaila. 1991. Crop improvement programs and nutrition in Malawi: Exploring the links. Food and Nutrition Bulletin (in press). (Also presented as a paper at the American Anthropological Association Meetings, Washington, D.C., November 15-19.)

Ferguson, A. E. and S. Sprecher. 1989. Component breeding: A strategy for bean improvement in eastern Africa and other regions where beans are grown as mixtures, paper presented at the First SADCC Regional Bean Research Workshop, Mbabane, Swaziland, October 4-7, 1989 and at the Workshop on Bean Research at the Sokoine University of Agriculture, Morogoro, Tanzania, September 27-29.

Gepts, P. 1990. Genetic resources available in *Phaseolus vulgaris*. Paper presented at the Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. Sokoine University of Agriculture, Morogoro, Tanzania, September 17-22.

Kambewa, P. S., R. M. Mkandawire and A. E. Ferguson. 1990. Bean production practices and bottlenecks among smallholder farmers in Blantyre Agricultural Development Division, Malawi. Paper presented at the Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. Sokoine University of Agriculture, Morogoro, Tanzania, September 17-22.

Khairallah M. M. 1990. Mitochondrial genome diversity in *Phaseolus*, with special reference to *P. vulgaris* L. Ph.D. Dissertation, Michigan State Univesity, East Lansing, MI.

Khairallah M. M., M. W. Adams, B. B. Sears. 1990. Mesoamerican and Andean gene pools of beans: Evidence from mitochondrial RFLPs of bean lines from Malawi. Annu. Rep. Bean Improv. Coop. 33:84-85. (Also, poster presented at the Biennial BIC Meeting, Nov. 7-9 1989, Toronto, Canada, and at the Bean/Cowpea CRSP Research Meeting, April 30-May 3, 1990, East Lansing, MI.)

Khairallah M. M., M. W. Adams, B. B. Sears. 1990. Mitochondrial DNA polymorphisms of Malawian bean lines: Further evidence for two major gene pools. Theor. Appl. Genet. 80: (in press).

Mafuleka, Mercy. 1990. Mechanisms of dry bean (*Phaseolus vulgaris*) hardening in storage: Role of pectin methylesterase, phytase and lignin in decorticated hard and soft Malawian bean landraces. Ph.D. Dissertation, Department of Food Science and Human Nutrition, Michigan State University.

Mkandawire, A. B. C. and F. V. P. Gundo. 1990. Progress with SADCC/CIAT drought sub-project. Paper presented at Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop, Sokoine University of Agriculture, morogoro, Tanzania, September 17-22.

Mkandawire, A. B. C. and M. W. Adams. 1990. Water-use efficiency and its role in drought tolerance of the common bean (*Phaseolus vulgaris* L.). Paper presented at Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. Sokoine University of Agriculture, Morogoro, Tanzania, September 17-22.

Msuku, W. A. B. and J. Bokosi. 1990. Evaluation of the Malawi germplasm for resistance to angular leafspot. SADCC/CIAT Progress Report for 1988/89 and 1989/90. Paper presented at Ninth SUA/CRSP Bean Research Workshop, Sokoine University of Agriculture, Morogoro, Tanzania, September 17-22.

Mughogho, S. K., K. F. D. Nyirenda and A. B. C. Mkandawire. 1990. The Effect of Applied Phosphorus on Biological Nitrogen Fixation by Beans. Paper presented at Ninth SUA/CRSP Bean Research Workshop and Second SADCC/CIAT Regional Bean Research Workshop. Sokoine University of Agriculutre, Morogoro, Tanzania, September 17-22.

Sprecher S. L., and M. M. Khairallah. 1989. Association of male sterility with gene pool recombination in bean. Annu. Rep. Bean Improv. Coop. 32:56-57.

References

Gepts, P. L. and Bliss, F. A. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris* L). Economic Botany 40: 469-478.

Martin, G. B. 1984. Genetic diversity of bean landraces in Northern Malawi. M.S. Thesis, Michigan State Univesity, East Lansing, MI.

Sprecher, S. L. 1988. Allozyme differentiation between gene pools in common bean (*Phaseolus vulgaris* L.), with special reference to Malawian germplasm. Ph.D. Dissertation, Michigan State University, E. Lansing, MI.

Table 1. Classification of enzymes investigated on Malawian landraces for plymorphism and electrophoretic results.

| Category | Enzyme |
|---|---|
| 1. Consistent, scorable, polymorphism (1) | ME, SKD, DIA, PRX, ACP, RUB |
| 2. Consistent, scorable, polymorphism (2) | MDH, GOT |
| 3. Consistent, scorable, no polymorphism | PGI, ENP, PGM, ADH, PGD, G2D, G1P, CAT, G-6-PDH |
| 4. Consistent, scorable, rare polymorphism | LAP |
| 5. Inconsistent, scorable, polymorphism (2) | IDH, ACO, FLE, SOD |
| 6. Not scorable | MPI |

Key: Malic enzyme (ME), Shikimate dehydrogenase (SKD), Diaphorase (DIA), Peroxidase (PRX), Acid phosphatase (ACP), Ribulosebisphosphate carboxylase (RUB), Malate dehydrogenase (MDH), Glutamate oxaloacetate transaminase (GOT), Glucose phosphate isomerase (PGI), Endopeptidase (ENP), Phosphoglucomutase (PGM), Alcohol dehydrogenase (ADH), 6-Phosphogluconate dehydrogenase (PDG), Glucose-2-phosphate dehydrogenase, Glucose-1-Phosphate (G1P), Catalase (CAT), Glucose-6-phosphate dehydrogenase (G-6-PDH), Leucine aminopeptidase (LAP), Isocitrate deydrogenase (IDH), Aconitate dehydrogenase (ACO), Fluorescent esterase (FLE), Superoxide dismutase (SOD), Manose-6-phosphate isomerase (MPI).

Table 2. Geographic distribution of dry bean phaseolin types present in Malawian landraces.

| Site | Phaseolin type | | | | Total |
|---|---|---|---|---|---|
| | S | T | C | H | |
| Chitipa North | 4 | 41 | 25 | 0 | 70 |
| Misuku Hills | 16 | 22 | 5 | 4 | 47 |
| Livingstonia Hills | 25 | 31 | 13 | 2 | 71 |
| South Raku Plain | 4 | 9 | 2 | 2 | 17 |
| Mabulabo Trad. Authority | 1 | 41 | 10 | 1 | 53 |
| Lilongwe | 33 | 87 | 40 | 1 | 163 |
| Dedza Hills | 70 | 89 | 66 | 3 | 229 |
| Total | 153 | 320 | 161 | 16 | 650 |
| Percentage | 23.5 | 49.23 | 24.8 | 2.5 | 100% |

Table 3. Average single seed weights and ranges (in grams) associated with various phaseolin types in Malawian landraces.

| Phaseolin type | Seed weight range | | Avr. weight |
|---|---|---|---|
| S | .11 | .60 | .30 |
| T | .21 | .92 | .44 |
| C | .16 | .95 | .46 |
| H | .23 | .52 | .40 |

# MEXICO - MICHIGAN STATE UNIVERSITY

*IMPROVING RESISTANCE OF ENVIRONMENTAL STRESS IN BEANS THROUGH GENETIC SELECTION FOR CARBOHYDRATE PARTITIONING AND EFFICIENCY OF BIOLOGICAL NITROGEN FIXATION*


**Principal Investigators:**

| | | |
|---|---|---|
| Dale Harpstead (U.S.) | Dept. of Crop and Soil Science | Michigan State University |
| Jorge Acosta Gallegos (HC) | Legume Program | INIFAP |

**Co-Investigators:**

| | | |
|---|---|---|
| George Acquaah | Dept. of Crop and Soil Science | Michigan State University |
| Dora Maria Aquilera | Durango Experimental Station | INIFAP, Durango |
| Maria de la Paz-Arieta | Durango Experimental Station | INIFAP, Durango |
| Ronald Ferrera | Post-Graduate School | C.P., Chapingo |
| Eunice F. Foster | Dept. of Crop and Soil Science | Michigan State University |
| Peter Graham | Department of Soil Science | University of Minnesota |
| Rigoberto Rosales | Durango Experimental Station | INIFAP, Durango |
| Susan L. Sprecher | Dept. of Crop and Soil Science | Michigan State University |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

Host Country

In 1990, "Negro Durango" was registered as a new black-seeded cultivar and along with previously released cultivars, Pinto Villa and Bayo Victoria, has been extensively evaluated in farmers' fields. One-hectare lots of each of these cultivars is maintained as Basic Seed by the National Seed Organization of Mexico. In the case of Negro Durango, the National Seed Organization will certify production from 130 ha, grown under dryland conditions, for sale as Certified Seed in 1991. The best available evidence for the success of these genotypes has been the increased demand for seed by the public and the interest of the private sector seed producers to obtain the basic stocks of these cultivars for Certified Seed production.

**Breeding for adaptation and tolerance to drought.** Since the area served by the bean breeding program of Mexico is vast and extends more than a thousand kilometers north of the main plant breeding station, special protocols have been taken to provide seed of early selection materials for observation at outlying regional research sites. This will provide opportunities for station scientists to select genotypes that have the potential to be increased as varieties and released for regional use. Since materials in these early generation collections will vary for maturity, plant type, seed

size, shape and color, local preferences can become a significant factor in variety selection. This strategy works well within theprograms of recurrent selection which are in place. For example, genotypic recombinants can be selected for a high frequency of types resistant to root-rot diseases but relatively unselected for major agronomic characteristics. Local selection and evaluation programs can be employed to make final selections based on regional needs and preferences.

The basic focus in this program therefore is the identification and recombination of bean genotypes which show useful production characteristics under the prevailing rainfall conditions of the Mexican semi-arid plateau. During the summer of 1989, 175 $F_2$ populations involving different numbers of parents, were grown under rainfed conditions at Durango experimental station. Visual selection was carried out on the $F_2$ populations for highly heritable and easy to score traits, such as disease resistance, days to flower, seed size, and color traits. In addition, the plant's general vigor was taken into account to further facilitate individual plant selection.

More than 2600 individual $F_2$ plant selections were made among populations derived from biparental crosses (Tables 1 and 2, Appendix A). Populations involving three and four parents were bulk harvested (one pod/plant). Individual plant selections and bulks were advanced and increased in a winter nursery at Los Mochis, Sinaloa. This increase provided seed for early generation testing of $F_4$ families at several locations throughout the semi-arid plateau. Selected plants came most frequently from crosses made between genotypes from different breeding programs in Mexico and the U.S. where $F_2$ populations showed larger phenotypic variation.

During the 1990 summer season the same set of 2600 $F_4$ families were established at: Fco. I. Madero, Dgo., Sandovales, Ags., and Tepatitlan, Jal. A partial set of 1300 black seeded families was established at Calera, Zac. and approximately 800 pinto families were planted at Chihuahua. The current growing season has been excessively wet with rainfall amounts of 500 mm in Sandovales Ags., where the average rainfall for the previous 15 years was 300 mm during the growing season. However, the segregating families are being challenged by several diseases at each location: anthracnose, common blight and root rot at Durango; halo blight, rust, anthracnose and root rot in Aguascalientes and sclerotinia white mold in Jalisco. In this last location, the entire set of families is being tested under controlled conditions against all available anthracnose isolates from the area, which will delay the screening for drought resistance one or two generations.

**Phenological escape mechanism for drought tolerance in beans.** In beans, one of the effects of drought expressed during the reproductive stage is the hastening of physiological maturity. Since there is evidence for genotypic variation for this trait, six cultivars were grown under rainfed conditions, and phenology and yield data were recorded (Tables 1 and 2, Appendix B). These cultivars were chosen on the basis of dissimilar phenological behavior in the face of terminal drought. The experiment was established at two sites in the semi-arid region, Sandovales, Ags.,

and Durango, Dgo., on July 21 and 29, 1989, respectively. At Sandovales, all cultivars reached the 50 percent flowering stage at the same time, however, there were differences in physiological maturity and thus differences in length of total reproductive phase (RP). Bayo Dgo., a mid-season cultivar, and Pinto Nacional-1, an early cultivar, reached physiological maturity in the shortest time, showed the shortest reproductive period (RP) and the highest yields. In Durango, the same cultivars including the early Bayo Madero cultivar, showed the highest yields. At this site, cultivar phenology was affected by a mild early frost with subsequent favorable environmental conditions. For several years, a strong negative correlation (Table 3, Appendix B) has been noticed between the length of the RP and seed yield. No advantage was found for cultivars previously classified as possessing developmental plasticity in the face of terminal drought. However, this year top yielding cultivars at Sandovales exhibited this trait, possibly in response to low night temperatures during pod filling.

**Capacity for nitrogen fixation in 12 bean cultivars.** Twelve bean cultivars were grown at "Bajio" experimental station in Guanajuato under two nitrogen fertilizer treatments (0 and 80 kg/ha). Prior to planting, the seed was inoculated with a Rhizobium multi-strain available from CINVESTAV-Irapuato, Mexico. Among the early cultivars, Chih.51 and Mich.89-A showed the highest numbers of nodules, while UW.21-58 and Negro Argel were the best nodule producers in the later maturity group (Table 1, Appendix C). Comparing both total dry matter and total nitrogen, early cultivars were generally better than late cultivars at both, 40 and 80 DAP. However, late cultivars were superior to early cultivars for the relative amount of nitrogen fixed at physiological maturity. Cultivars 83-VEF-MXA-283, L-1-1-1-3-CM and Bayocel were among the best late maturing cultivars while Mich.89-A and Flor de Mayo Bajio were the best early cultivars. Top yielders in the nitrogen fertilized treatment were: Flor do Mayo Bajio, Chih.51 and Mich.89-A among the early and Bayocel and L-1-1-1-3-CM among the late cultivars. Top yielding cultivars without added nitrogen fertilizer were Bayocel and Negro Argel (Table 2, Appendix C). The results indicate that many plant characteristics are involved in the plant's ability to fix and utilize atmospheric nitrogen.

The three best Rhizobium strains from previous evaluations were tested under rainfed conditions using two bean cultivars and two nitrogen rates (0 and 20 kg/ha at three sites in the semi-arid highlands: Fco. I. Madero, Dgo., Calera, Zac. and Pabellon, Ags in 1990. Preliminary data indicates a low response to inoculation alone and a better response to the addition of nitrogen fertilizer in comparison to the control with no inoculant and no N fertilizer.

**Protein content and cooking time of 154 bean genotypes from the semi-arid highlands.** In order to be more interactive with concerns which may emanate from culinary and organoleptic preferences of consumers, a laboratory has been established to measure these and other food quality traits. Bean varieties have traditionally been classified on the basis of color, size, and cooking characteristics of the seed. Pintos, black, and reds are phenotypic expression in

seed coats with what has been associated, over time, with perceptions of flavor and/or food desirability. The failure of a bean variety to achieve a favorable perception in the view of the consumer may result in a market discrimination which can render the variety useless as a commercial product.

Recognizing that cooking time is both an economic factor and a convenience factor in food preparation, the CRSP project has become concerned with the food quality of bean cultivars. Although food quality in beans depends on a multiplicity of factors the major concern is the hard-to-cook defect (HTC). In order to evaluate genetic and environmental effects on HTC, seeds of 20 cultivars grown at five sites in the semi-arid highland of Mexico were uniformly handled after physiological maturity to eliminate variability for harvest differences. The beans were tested for water uptake capacity, percentage of seed protein and cooking time. The group of genotypes included six early, eight mid-season and six full-season cultivars. A wide range of variability was observed for cooking time among lines and within locations. Averaged over all cultivars, the shortest cooking time was observed at Calera, Zac., while the maximum cooking time was noted at Los Chacon, Chih. Genotypes Mayocoba and A 252 showed the shortest cooking time, of 63 and 82 minutes, respectively across locations. (Table 1, Appendix D). Soil and climatic data have been taken into order to help interpret the observed results. Since the results indicated a large genotype by environment interaction this study will be repeated with more cultivars over several years. In another preliminary study, seed quality traits were assessed in 154 bean genotypes. Genotypic variability was found for time to cook, seed protein content and seed water absorption. Cultivars with high protein content in the seed were: Ags.19, CIAT 326/85, BAT 477, V8025 and Chis.12-B. Cultivars Oax.48-A, Ver.167, CIAS72 X RR L-5-1-1, Oax.110, CIAS72 X RR L-7-1-2, CIAT 369/85, CIAS72 X RR L- 5-1-2 and UW.21-58 exhibited short cooking time, ranging from 39 to 45 minutes for 75 percent of seed cooked (Table 2, Appendix D). Cream, bayo, brown, and pinto genotypes showed the longest average cooking time, while small whites were easiest to cook. In general, in all seed classes both hard and easy to cook genotypes were identified. In addition, it was observed that large seeded cultivars exhibited harder to cook phenomenon than small seeded cultivars. These studies will be especially important for final variety evaluation and will directly affect the ultimate consumer.

U.S.

**Isozyme markers as predictors of drought resistance factors in beans.** Marker assisted selection offers an attractive alternative to direct selection of difficult to determine traits. The genetic complexity of drought tolerance makes it a logical choice for isozyme analysis. Fifty genotypes differing in the reaction to drought but belonging to the Meso-American gene pool were subjected to analysis with 23 enzymes. This group of enzymes includes the six enzymes previously designated as characterizing through alternate alleles the two major gene pools in beans. The isozymes are listed on Table 1, Appendix E, and have been classified in four major groups depending on the different isozyme patterns.

One group included the polymorphic enzymes of known genetic basis and gene pool specificity including the major seed storage protein phaseolin. Six enzymes showed potential polymorphisms but will need verification as to their genetic basis. The final group of ten enzymes exhibited no polymorphisms among the genotypes tested. A rare allelic form Diap-1 was detected in one line A-70 (Table 2, Appendix E).

A representative sample of the results of electophoresis is presented in Table 2, Appendix E. Alleles for the first six enzymes/proteins have been known to be gene pool specific (Sprecher 1988). Occasionally, alleles characteristic of the Andean gene pool were observed in genotypes like Bayo Madero from the Meso-American gene pool which were used in the drought breeding program. Malic enzyme exhibited more variability than could be attributed to the breeding pedigree of the genotypes while rubisco and diaphorase showed no polymorphism. No definite pattern emerged from the isozyme profiles to indicate a correlation between response to drought and isozyme patterns. The fact that, for example BAT-477 (drought tolerant) and BAT-1224 (drought susceptible) only differed in isozyme allele for diaphorase (Diap-2) does not mean the enzyme necessarily has a functional role in the response to drought of dry bean. A sufficiently large number of polymorphic isozymes need to be detected to be useful for mapping. Additionally these polymorphisms need to be scattered across the genome since drought resistance is a classic quantitative trait under polygenic control from factors distributed throughout the genome. The current isozyme polymorphisms are too specific to the major gene pool variability in beans and the lack of sufficient polymorphisms within the Meso-American gene pool from where the majority of germplasm of interest comes, makes the use of isozymes as markers redundant. The recommendation from the present study is to pursue more elaborate and sensitive techniques such as PCR and RFLPs for the purpose of identifying sufficient numbers of molecular markers that might be associated with drought prediction.

**Physiological mechanisms of drought tolerance, nitrogen, partitioning and remobilization.** Previous work in Mexico indicated that two cultivars Bayo Madero and Pinto Nacional are early maturing and may be drought evaders. However, since moisture stress decreases the days to physiological maturity in Pinto Nacional but not in Bayo Madero and Bayo Madero has lower N concentration than does Pinto Nacional, this suggests that both genotypes have different forms of adaptation to drought.

During the summer of 1989, a greenhouse study of N partitioning and remobilization was conducted utilizing the same two bean cultivars. The moisture stress treatment was initiated at 27 days after planting (DAP). In stressed plants, the water status of the soil was allowed to decrease to -1.0 MPa before rewatering occurred. At that point, the soil was rewatered to field capacity as previously determined by a soil desorption curve. This procedure continued from stress induction until harvest. N partitioning and remobilization were determined by inoculating plants; growing them in a soil low in N; sampling at five different times during the growing season with subdivision of plants into leaves, stems, reproductive tissue and roots; and total N analysis of each plant part.

Although the cultivars did not differ in percent stem N, Bayo Madero had a significantly greater stem dry weight throughout the experiment (Table 1, Appendix F). As expected stem dry weight was less under water stress and N content (Table 2) was significantly greater. The lack of significant difference in leaf dry weight under moisture stress is mainly due to the high degree of leaf senescence induced by the stress and the subsequent variability in N concentration in leaves of stressed plants. The N content within reproductive parts of stressed plants at harvest (Table 3) compared to non stress may indicate that the stress exceeded the point at which remobilization of photosynthesates or N can occur to help sustain yield. This fairly severe stress of -1.0 MPa resulted in increased leaf senescence and pod abortion. The results suggest that the previously observed adaptation to drought noted in field experiments of Pinto Nacional and Bayo Madero is due to evasion via earliness rather than resistance. This is suggested by the genotypes' inability to remobilize N from stems to leaves during stress or to minimize yield reduction during stress.

**Effect of root systems on N partitioning.** Previous work has provided evidence that the adaptation of breeding line Lef-2-RB to drought is mainly due to increased root growth. Lef-2-RB is also the parent of a genotype that has indicated increased N remobilization to reproductive sinks during drought. In contrast genotype 9-39-1 was previously identified as being drought susceptible and as exhibiting less N remobilization during drought. This study was designed to compare N partitioning in the various plant organs after plants were grafted onto a different root or shoot with N partitioning in the original plant grown under both moisture limiting and non-limiting conditions.

During the fall of 1989, a reciprocal grafting experiment was conducted utilizing genotypes 9-39-1 and Lef-2-RB. Reciprocal grafting involved grafting the shoot of 9-39-1 onto the root of Lef-2-RB and vice versa. In order to negate the differences due to grafting, the controls consisted of Lef-2-RB grafted onto itself and 9-39-1 grafted onto itself. Moisture stress was initiated four weeks after the grafts were made and continued until sampling, six weeks later. Pots were watered by weight. At sampling, plants were subdivided into leaves, stems, roots, and reproductive structures and analyzed for total N.

When the susceptible genotype (9-39-1) provided both the shoot and root, plants accumulated more leaf area, total dry matter, and N under non-limiting moisture conditions and less under moisture stress (Table 1, Appendix G). Stem N concentration was greatest under stress and less under non-stress, which would indicate an inability to remobilize N from stems to leaves during moisture stress (Table 2). Smaller differences for leaf N content, stem N concentration, leaf area, and total dry weight were observed between stressed and non-stressed plants combining both shoots and roots of Lef-2-RB, indicating greater stability under stress and continued ability to translocate N during stress (Table 1). The shoot response of both genotypes was similar to the combined shoot and root responses which would indicate that the root of the genotype has less effect on N partitioning than the shoot. Lef-2-RB exhibited greater efficiency of N utilization than 9-39-1.

2. Other research-related results

**Host Country germplasm collection.** In addition to the preservation and use of bean genotype collections from Central and South America, wild and weedy species of beans in central Mexico are of special interest to this project. These species have survived and propagated for long periods of time, and perhaps even evolved, under conditions of water stress. Genotypes surviving under these conditions may have accumulated genes giving adaptation to drought stress. No known barrier exists to the intercrossing of wild and cultivated forms of *P. vulgaris*. It will be advantageous to identify the drought responses of selected wild and weedy types and to include the best in the project breeding program. To date, sixty collections of wild beans have been made and are being included in future crossing programs to increase genetic variability.

If hybrids are successfully made between the wild collections and cultivated bean genotypes, seed will be shared with curators of the CIAT and East Africa bean germplasm collections some of which are currently in use in Mexico and at other bean improvement locations.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Bean production and consumption in Mexico is almost an omni-present and deeply engrained cultural trait. It is unlikely that the impact of new technology in the form of new varieties can be measured over a short period of time. It may require decades. What can be measured, however, is the degree of public acceptance of new variety releases and the response of consumers to these as food products. To date, there has been a steadily increasing demand for the seed of new varieties which is a positive indicator of their acceptance. Field days conducted on farms in Durango have focused attention upon these new cultivars and have resulted in increased demand for seed in that region. A future measure of the usefulness will be their contributions to yield stability. It is anticipated that this will be reflected primarily in price stability, especially in years of adverse growing conditions.

B. Institutional Development and Training

In March 1989, Dr. Jorge Acosta G. who completed his Ph.D. studies as a CRSP scholar was promoted to the position of National Coordinator of Legume Programs in Mexico. This is symbolic of the degree of maturation that has occurred within the family of programs of which the Bean/Cowpea CRSP is a part and the potential for even more effective program integration in the future.

The decision was made in 1988 to invest all of the economic resources of the Mexico portion of the CRSP project in direct research activities. Candidates for advanced training were required to apply for scholarship support from sources other than the CRSP. Several students from Mexico are being awarded scholarship support from the National Bean Program of INIFAP and CONACYT. Porfirio Ramirez and A. Nunez-Barrios, both Ph.D. candidates at Michigan State University, Franciso Ibarra at University of California-Riverside, and A. Pajarito-Ravelero, an M.S. student at

Salitillo, Mexico, will complete their studies in 1991, with funding from INIFAP. S. Padilla-Ramirez, from the bean research program at the Aguascalientes Station, began his Ph.D. studies at New Mexico State University in 1990. He is jointly funded from the Fulbright Foundation and CONACYT (Mexico). Specific training programs in the highly technical areas of plant physiology and/or biotechnology will be implemented as the specific need for this training is identified and, if required, may be supported from CRSP resources.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

No major problems were encountered in the management and utilization of allocated funds in FY 90. Approval procedures and the auditing of expenditures was carried out with a minimum of delay or inconvenience.

### B. Adequacy of Current Management System

Current management procedures are adequate. The mechanics of management due to deficiencies in the rural telephone, fax and mail services of Mexico resulted in delays and inconveniances.

### C. Activity Towards Buy-Ins or Other Funding

No activity toward buying funding occurred in 1990.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

The activities in both the Host Country and the U.S. institution are appropriate relative to the goals and objectives of the Global Plan.

### B. Balance Between Research and Training

In the Mexico/MSU project there has been steady progress toward increases in research investments and a decrease in the percentage of CRSP funds dedicated to training. The training activities which are directly related to this project but supported by non-CRSP funding may be interpreted as further support and endorsement of the project.

### C. Balance of Domestic vs. Overseas Activities

An effective balance exists between Host Country and U.S. level of activities. The respective programs are complementary.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Joint planning is effective. Cooperation and exchanges of both products and data are proceeding without problem. Drs. Foster and Harpstead participated in Annual INIFAP bean reporting and planning conferences during March 1989 and 1990.

### E. Relative Contributions of Collaborating Institutions and Individuals

In the cases of both the U.S. and Host Country institutions contributions to the project activities were "in kind" through salary support and the indirect contributions of services and facilities.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

On March 14, 1990, Harpstead made an appointment with the Agriculture Attache in Mexico, Mr. Grant Petrie to brief him on the CRSP activities. Appropriate technical publications, activity reports and pictures were left for his review and use. He expressed deep appreciation for the opportunity to become informed relative to bean support project activities in Mexico.

G. Evidence of Institutionalization

Dr. Jorge Acosta, Host Country PI actively participates in Central and South American bean improvement activities. A part of this involvement takes place under the auspices of CIAT, Cali, Colombia.

IV. PUBLICATIONS AND PRESENTATIONS

Acosta-Gallegos, et al. 1990. Biomasa y sus componentes en variedades indeterminadas de frijol. Presented at the I annual meeting of the Sociedad Mexicana de Genetica, held at Tlaxcala, Tlax. 8-12 Oct., 1990.

Acosta-Gallegos, et al., 1990. Mecanismo fenologico de escape en relacion a la adaptacion a temporal. Presented at the XIII annual meeting of the Sociedad Mexicana de Fitogenetica, held at Cd. Juarez, Chic., Mex. 3-7 Sept., 1990. p.508.

Ochoa-Marquez, et al., 1990. Adaptacion de variedades de frijol de ciclo intermedio en la region semiarida de Mexico. Presented at the XIII annual meeting of the Sociedad Mexicana de Fitogenetica, held at Cd. Juarez, Chih., Mex. 3-7 Sept., 1990. p.509.

Ochoa-Marquez, et al., 1990. Adaptacion de variedades de frijol de ciclo precoz en la region semiarida de Mexico. Presented at the XIII annual meeting of the Sociedad Mexicana de Fitogetica, held at Cd. Juarez, Chih., Mex. 3-7 Sept., 1990. p.510.

Padilla-Ramirez, J.S. y J.A. Acosta-Gallegos. 1990. Evaluacion de la capacidad de ajuste osmotico en dos variedades de frijol (*Phaseolus vulgaris* L.). Presented at the XIII annual meeting of the Sociedad Mexicana de Fitogenetica, held at Cd. Juarex, Chic., Mex. 3-7 Sept., 1990. p.116.

Acosta-Gallegos, J.A. 1990. Procedimientos y avances de selecion para resistencia a sequia en frijol. Talk delivered at the symposia on stress factors during the XIII annual meeting of the Sociedad Mexicana de Fitogenetica, held at Cd. Juarez, Chih., Mex. 3-7 Sept., 1990.

Acosta-Gallegos, et al., 1990. Interacion simbiotica entre cepas de Rhizobium y cultivares de frijol (*Phaseolus vulgaris*) bajo condiciones de temporal. Submitted to be presented at the XV meeting of the Sociedad Latino Americana de Rhizobiologia, to be held at Guatemala, Guatemala. 19-23 Nov., 1990.

Benitez, et al., 1990. Efecto de la inoculacion con Rhizobium en dos variedades de frijol sometidas a riego y sequia ciclica. Submitted to be presented at the XV meeting of the Sociedad Latino Americana de Rhizobiologia, to be held at Guatemala, Guatemala. 19-23 Nov., 1990.

Vega-Segovia, et al., 1990. Competencia entre poblaciones de Rhizobium leguminosarum biovar phaseoli en frijol (*Phaseolus vulgaris* L.). Submitted to be presented at the XV meeting of the Sociedad Latino Americana de Rhizobiologia, to be held at Guatemala, Guatemala. 19-23 Nov., 1990.

Foster, E. F. and A. Nunez-Barrios. 1990. Effects of drought on N utilization in beans. 1990 Agronomy Abstracts.

Foster, E. F. and A. Nunez-Barros. (In Preparation) Effects of drought on N utilization in beans.

Foster, E.F. and A. Nunez-Barrios. 1990. Effects of drought on N utilization in beans. Presented at the American Society of Agronomy Meetings, October 1990.

Appendix A

Table 1. Parental and origin ( ) of biparental population selected for resistance to *Fusarium* spp.

| | | |
|---|---|---|
| PINTO NAL.1 (INIFAP)* | X | OLATHE (CSU) |
| OLATHE (CSU) | X | CHIH.11-D (INIFAP) |
| BAYO MADERO (INIFAP) | X | P86275 (MSU) |
| PINTO SIERRA (MSU) | X | OLATHE (CSU) |
| P86275 (MSU) | X | BAYO MADERO (INIFAP) |
| PINTO SIERRA (MSU) | X | PINTO NAL. 1 (INIFAP) |
| PINTO SIERRA (MSU) | X | CHIH.11-D (INIFAP) |
| BAYO MADERO (INIFAP) | X | P86295 (MSU) |
| BAYO VICTORIA (INIFAP) | X | OLATHE (CSU) |

Table 2. Parents and origin ( ) of additional biparental crosses grouped by seed color

| | | |
|---|---|---|
| ----------BLACKS---------- | | |
| BAT 260 (CIAT)* | X | NEGRO DGO. (INIFAP) |
| OAX.112 (INIFAP) | X | BAT 5208 (CIAT) |
| PUE.152 (INIFAP) | X | BAT 67 (CIAT) |
| PUE.152 (INIFAP) | X | BAT 5208 (CIAT) |
| A 212 (CIAT) | X | OAX.112 (INIFAP) |
| BAT 67 (CIAT) | X | NEGRO DGO. (INIFAP) |
| BAT 76 (CIAT) | X | NEGRO QRO. (INIFAP) |
| NEGRO DGO. (INIFAP) | X | XAN 116 (CITA) |
| NEGRO DGO. (INIFAP) | X | OAX.112 (INIFAP) |
| A 212 (CIAT) | X | PUE.152 (INIFAP) |
| A 212 (CIAT) | X | NEGRO DGO. (INIFAP) |
| ----------PINTOS---------- | | |
| PINTO SIERRA (MSU) | X | PINTO NAL. 1 (INIFAP) |
| PINTO NAL. 1 (INIFAP) | X | OLATHE (CSU) |
| BAYO VICTORIA (INIFAP) | X | OLATHE (CSU) |
| BAYO VICTORIA (INIFAP) | X | P86295 (MSU) |
| DGO.222 (INIFAP) | X | OLATHE (CSU) |
| PINTO MEX. 80 (INIFAP) | X | P86295 (MSU) |
| ----------COLORED---------- | | |
| F. MAYO-44 (INIFAP) | X | P86270 (MSU) |
| CHIH.11-D (INIFAP) | X | BAYO DGO. (INIFAP) |
| CHIH.11-D (INIFAP) | X | BAYO MADERO (INIFAP) |
| D.C. STA.RITA (INIFAP) | X | BAYO MADERO (INIFAP) |
| F. MAYO 44 (INIFAP) | X | PINTO CARMELO (INIFAP) |

* CIAT = Centro Internacional de Agricultura Tropical, Colombia; INIFAP = Instituto de Investigaciones Forestales y Agropecuarias, Mexico; MSU = Michigan State University, USA; CSU = Colorado State University, USA.

Appendix B

Table 1. Agronomic characteristics of six bean varieties grown under drought at Sandovoles Ags. Mexico.

| Variety | Days to Flower | Days to Maturity | TRP* | Yield kg/ha | Yield g/m2/day |
|---|---|---|---|---|---|
| Pinto Nal-1 | 35 | 73 | 38 | 844 | 2.22 |
| Bayo Madero | 37 | 81 | 44 | 839 | 1.91 |
| Mich. 91-A | 38 | 85 | 47 | 713 | 1.52 |
| Bayo Dgo. | 37 | 75 | 38 | 826 | 2.17 |
| Qro. 3-B-1 | 39 | 85 | 46 | 760 | 1.65 |
| Tlax. 475 | 39 | 88 | 49 | 788 | 1.61 |
| Mean | 38 | 81 | 44 | 795 | 1.85 |
| LSD 0.05 | 1.0 | 2.6 | 2.5 | 154 | 0.36 |

*Total Reproductive Period

Table 2. Agronomic characteristics of six varieties of bean grown under drought at F. I Madero Dgo Mexico.

| Variety | Days to Flower | Days to Maturity | TRP* | Yield kg/ha | Yield g/m2/day |
|---|---|---|---|---|---|
| Pinto Nal-1 | 36 | 88 | 52 | 1378 | 2.65 |
| Bayo Madero | 38 | 96 | 60 | 1015 | 1.69 |
| Mich. 91-A | 46 | 98 | 52 | 1092 | 2.10 |
| Bayo Dgo. | 43 | 95 | 52 | 1488 | 2.86 |
| Qro. 3-B-1 | 47 | 99 | 52 | 1177 | 2.26 |
| Tlax. 475 | 49 | 100 | 51 | 1063 | 2.08 |
| Mean | 43 | 96 | 53 | 1202 | 2.27 |
| LSD 0.05 | 1.2 | 1.7 | 2.4 | 402 | 0.69 |

*Total Reproductive Period

Table 3. Simple correlation between agronomic characteritics of six bean varieties grown at two locations in the semi arid region.

| Charater | | Character: Flowering | Maturity | TRP@ | Yield | g/m2/day |
|---|---|---|---|---|---|---|
| Flowering | (1) | ----- | 0.76 | - 0.56 | - 0.33 | - 0.13 |
| | (2) | ----- | 0.94** | 0.90* | - 0.75 | - 0.92** |
| Maturity | (1) | ----- | ----- | 0.12 | - 0.71 | - 0.64 |
| | (2) | ----- | ----- | 0.99** | - 0.71 | - 0.97** |
| TRP | (1) | ----- | ----- | ----- | - 0.40 | - 0.61 |
| | (2) | ----- | ----- | ----- | - 0.68 | - 0.96** |
| Yield | (1) | ----- | ----- | ----- | ----- | 0.97** |
| | (2) | ----- | ----- | ----- | ----- | 0.85* |

@ Total Reproductive Period
(1) F.I. Madero, Dgo. (2) Sandovales, Ags.
*** Significant at P > 0.05 y 0.01, respectively

Appendix C

Table 1. Phenological traits of number of Nodules at two growth stages for 12 bean varieties grown at two nitrogen levels.

| Variety | Days to Flower | Days to Maturity | Nodules/$m^2$ 40 DAP N+** | 40 DAP N- | 80 DAP N+ | 80 DAP N- | Average no.nod. |
|---|---|---|---|---|---|---|---|
| Pinto Amer. | 44 | 95 | 100 | 130 | 54 | 84 | 94 |
| O.C.S. Rita 83 | 44 | 97 | 162 | 232 | 92 | 178 | 166 |
| Chih.51 | 45 | 100 | 192 | 434 | 296 | 292 | 303 |
| F.M. Bajio | 52 | 96 | 386 | 160 | 160 | 296 | 250 |
| Mich.89-A | 53 | 99 | 446 | 462 | 230 | 346 | 371 |
| BAT 477 | 52 | 109 | 226 | 232 | 288 | 494 | 310 |
| 453-3-2-CM | 58 | 108 | 266 | 248 | 136 | 284 | 233 |
| Bayocel | 60 | 111 | 266 | 174 | 210 | 258 | 252 |
| 83-VEF-MXA-238 | 64 | 107 | 316 | 200 | 244 | 316 | 269 |
| Negro Argel | 64 | 112 | 302 | 424 | 186 | 404 | 329 |
| L-1-1-1-3-CM | 67 | 134 | 234 | 272 | 136 | 238 | 220 |
| UW. 21-58 | 69 | 113 | 208 | 356 | 380 | 538 | 370 |
| Mean | 56 | 107 | 259 | 285 | 201 | 311 | |

* DAP - Days after planting
** N+ 80kg N/ha applied
N- No N applied

(Appendix C continued)

Table 2. Dry matter and nitrogen concentration in 12 bean varieties grown under two levels of N fertilizer.

| Variety | Treatment | 40 DAP | | | 80 DAP | | |
|---|---|---|---|---|---|---|---|
| | | DM** | N | Total N | DM | N | Total N |
| | | $g/m^2$ | ---%--- | $g/m^2$ | $g/m^2$ | ---%--- | $g/m^2$ |
| Pinto Amer. | N+ | 218 | 2.59 | 5.6 | 324 | 2.28 | 7.4 |
| | N- | 154 | 2.45 | 3.8 | 260 | 2.09 | 5.4 |
| O.C.S. Rita | N+ | 204 | 2.00 | 4.1 | 262 | 2.28 | 6.0 |
| | N- | 130 | 2.57 | 3.3 | 144 | 2.23 | 3.2 |
| Chih.51 | N+ | 128 | 1.96 | 2.5 | 300 | 2.43 | 7.3 |
| | N- | 126 | 2.12 | 2.7 | 236 | 2.20 | 5.2 |
| F.M. Bajio | N+ | 152 | 1.75 | 2.7 | 298 | 2.20 | 6.6 |
| | N- | 140 | 2.03 | 2.8 | 222 | 2.28 | 5.1 |
| Mich.89-A | N+ | 184 | 2.57 | 4.7 | 238 | 1.89 | 4.5 |
| | N- | 126 | 2.28 | 2.9 | 236 | 2.34 | 5.5 |
| BAT 477 | N+ | 126 | 2.52 | 3.2 | 256 | 2.37 | 6.1 |
| | N- | 108 | 2.53 | 2.7 | 204 | 2.43 | 5.0 |
| 453-3-2-CM | N+ | 158 | 2.43 | 3.8 | 3.8 | 1.92 | 5.9 |
| | N- | 124 | 2.28 | 2.8 | 218 | 2.06 | 4.5 |
| Bayocel | N+ | 114 | 2.65 | 3.0 | 298 | 2.69 | 8.0 |
| | N- | 128 | 1.21 | 1.5 | 238 | 2.43 | 5.8 |
| 83VEF-MXA238 | N+ | 144 | 2.65 | 3.8 | 306 | 1.97 | 6.0 |
| | N- | 106 | 1.61 | 1.7 | 180 | 2.17 | 3.9 |
| Negro Argel | N+ | 134 | 2.61 | 3.4 | 240 | 2.30 | 5.5 |
| | N- | 110 | 2.59 | 2.8 | 234 | 2.45 | 5.7 |
| L-1-1-1-3-CM | N+ | 118 | 1.96 | 2.3 | 242 | 1.83 | 4.4 |
| | N- | 110 | 2.26 | 2.5 | 196 | 2.23 | 4.4 |
| UW. 21-58 | N+ | 110 | 2.76 | 3.0 | 216 | 1.12 | 4.6 |
| | N- | 110 | 2.68 | 2.9 | 170 | 2.58 | 4.3 |
| Mean | N+ | 149 | 2.34 | 3.5 | 274 | 2.19 | 6.0 |
| | N- | 123 | 2.22 | 2.7 | 212 | 2.29 | 4.8 |

*DAP - Days After Planning
** DM Dry Matter
@ N+ = 80 kg N/ha applied
N- = No Nitrogen applied

(Appendix C continued)

Table 3 Seed yield, total dry matter, and total N at physiological maturity in 12 bean varieties grown at two levels of Nitrogen fertilization.

| Variety | Treatment | Yield | DM* | Total N | Yield ratio -N/+N | HI** | NHI@ |
|---|---|---|---|---|---|---|---|
| | | kg/ha | ----g/m$^2$---- | | % | | |
| Pinto Amer. | N+ | 3574 | 595 | 12.1 | 89 | 0.60 | 0.82 |
| | N- | 2857 | 476 | 10.8 | | 0.60 | 0.80 |
| O.C.S. Rita | N+ | 3037 | 578 | 11.8 | 94 | 0.53 | 0.76 |
| | N- | 2781 | 527 | 11.1 | | 0.53 | 0.72 |
| Chih.51 | N+ | 3036 | 516 | 11.3 | 100 | 0.59 | 0.77 |
| | N- | 3055 | 532 | 11.4 | | 0.58 | 0.76 |
| F.M. Bajio | N+ | 3369 | 595 | 10.6 | 85 | 0.51 | 0.79 |
| | N- | 2479 | 484 | 9.1 | | 0.51 | 0.75 |
| Mich.89-A | N+ | 3385 | 652 | 13.6 | 75 | 0.52 | 0.69 |
| | N- | 2768 | 497 | 10.2 | | 0.56 | 0.71 |
| BAT 477 | N+ | 3729 | 662 | 15.5 | 80 | 0.56 | 0.77 |
| | N- | 3108 | 493 | 12.4 | | 0.63 | 0.83 |
| 453-3-2-CM | N+ | 3494 | 662 | 13.3 | 91 | 0.56 | 0.74 |
| | N- | 2354 | 609 | 12.2 | | 0.53 | 0.75 |
| Bayocel | N+ | 4698 | 820 | 14.7 | 78 | 0.57 | 0.80 |
| | N- | 4063 | 634 | 11.6 | | 0.64 | 0.79 |
| 83VEF-MXA238 | N+ | 4120 | 765 | 21.2 | 61 | 0.54 | 0.77 |
| | N- | 3391 | 616 | 13.0 | | 0.55 | 0.75 |
| Negro Argel | N+ | 3352 | 693 | 12.8 | 113 | 0.48 | 0.71 |
| | N- | 4031 | 665 | 14.5 | | 0.61 | 0.82 |
| L-1-1-1-3-CM | N+ | 4254 | 754 | 15.5 | 74 | 0.56 | 0.77 |
| | N- | 3549 | 570 | 11.6 | | 0.62 | 0.79 |
| UW. 21-58 | N+ | 3400 | 712 | 13.0 | 110 | 0.48 | 0.75 |
| | N- | 3419 | 650 | 14.4 | | 0.53 | 0.77 |
| Mean | N+ | 3621 | 661 | 13.8 | 88 | 0.55 | 0.76 |
| | N- | 3229 | 563 | 11.9 | | 0.57 | 0.77 |

*DM - Dry Matter
**HI - Harvest Index
@NHI - Nitrogen Harvest Index
N+ = 80 kgN/ha applied
N- = No nitrogen applied

Appendix D

Table 1 Cooking time of twenty bean cultivars grown under rainfed conditions at five locations from the semiarid highlands of Mexico. 1989.

| Cultivar | Madero Dgo. | Durango Dgo. | Sandovales Ags. | Calera Zac. | Chacon Chih. | Ave. |
|---|---|---|---|---|---|---|
| | minutes | | | | | |
| Early | | | | | | |
| Bayo Madero | 75* | 113 | 96 | 82 | 153 | 103 |
| C101XDgo225 | 99 | 131 | 90 | 85 | 95 | 100 |
| Chuh.51 | 111 | 115 | 111 | 83 | 133 | 110 |
| Mayocoba | 72 | 47 | 62 | 72 | 61 | 63 |
| Pinto Nal.1 | 79 | 81 | 88 | 71 | 116 | 87 |
| O. Cabra S.R. | 113 | 134 | 106 | 106 | 135 | 119 |
| Mid-season | | | | | | |
| Zac. 86 | 92 | 107 | 89 | 77 | 149 | 103 |
| Pinto Villa | 107 | 103 | 82 | 86 | 137 | 103 |
| Mich. 91-A | 76 | 118 | 72 | 72 | 141 | 96 |
| 83-VEF-MXA-238 | 79 | 104 | 49 | 67 | 118 | 84 |
| Bayo Zac. | 96 | 134 | 112 | 80 | 162 | 117 |
| Bayo los Llanos | 130 | 144 | 90 | -- | 159 | 131 |
| Gto. 157 | 93 | 93 | 93 | 59 | 130 | 94 |
| Manzano | 86 | 123 | 118 | 55 | 110 | 98 |
| Late | | | | | | |
| A 322 | 100 | 92 | 73 | 68 | 106 | 88 |
| MAM 10 | 89 | 94 | 79 | 70 | 112 | 89 |
| A 424 | 109 | 109 | 94 | 71 | 174 | 111 |
| Ags. 49 | 106 | 112 | 135 | 84 | 169 | 121 |
| Rio Grande | 89 | 107 | 78 | 54 | 112 | 88 |
| A 252 | 89 | 86 | 85 | 54 | 96 | 82 |
| Average | 95 | 112 | 90 | 73 | 128 | 99 |

* Cooking time (minutes) of 75% of sample, determined with a modified Matson cooker.

(Appendix D continued)

Table 2. Cooking time of 154 bean genotypes grouped by seed color

| COLOR | Cooking Time Range | Cooking Time Average | Cooking Time S.D.* | 100 Seed weight |
|---|---|---|---|---|
| | ---------- minutes | | --------- | g |
| Bayo (51) | 55-170 | 96 | 29 | 28 |
| Black (18) | 49-100 | 73 | 14 | 20 |
| O de Cabra (16) | 48-129 | 77 | 21 | 29 |
| Pinto (14) | 49-131 | 83 | 26 | 28 |
| Flor de Mayo (8) | 53-150 | 82 | 31 | 25 |
| Red (9) | 43-145 | 80 | 37 | 22 |
| Purple (7) | 35-89 | 65 | 17 | 20 |
| White (7) | 42-88 | 57 | 18 | 17 |
| Brown (5) | 55-111 | 83 | 21 | 22 |
| Brown (5) | 52-72 | 64 | 9 | 28 |
| Yellow (10) | 50-135 | | | |

(#) Number of genotypes
* S.D. - Standard Deviation

Table 3. Cooking Time of 154 bean varieties grouped by maturity

| Group* | Days to Flower | Days to Maturity | Cooking Time Min. | Cooking Time Max. | Cooking Time Mean | Cooking Time S.D.* | Seed Weight |
|---|---|---|---|---|---|---|---|
| | | | ----------Minutes | | | ---------- | --g-- |
| P1 | <37 | 79-85 | 57 | 129 | 83.0 | 31.59 | 28 |
| P2 | >38 | 79.85 | 55 | 129 | 78.0 | 26.46 | 26 |
| I1 | <41 | 86.91 | 54 | 158 | 80.4 | 30.80 | 27 |
| I2 | >41 | 86.91 | 70 | 121 | 89.2 | 16.95 | 30 |
| T1 | <43 | 92.99 | 50 | 170 | 82.6 | 31.43 | 28 |
| T2 | >43 | 92.99 | 43 | 119 | 74.7 | 21.39 | 22 |
| MT | N.D. | 100.11 | 42 | 145 | 91.6 | 32.98 | 23 |
| MPR | N.D. | 79.00 | 54 | 131 | 76.0 | 29.51 | 31 |
| PR | N.D. | 79.85 | 35 | 128 | 73.6 | 21.00 | 24 |
| IR | N.D. | 86.91 | 74 | 170 | 108.6 | 31.04 | 24 |
| TR | N.D. | 92.99 | 82 | 136 | 110.7 | 17.35 | 24 |

* P1 = Early group 1, P2 = Early group 2, I1 = Intermediate group 1, I2 = intermediate group 2, T1 = Late group 1, T2 = Late group 2, MT = Very late, MRP = Very early susceptable to rust, PR = early susceptible to rust, IR = Intermediate susceptible to rust, TR = late susceptible to rust
* S.D. - Standard deviation

(Appendix D continued)

Table 4. Relationship between seed size and cooking time in different bean classes separated by seed color.

| Seed Color | n | Regression Equation | Correlation | F Value | Signif. |
|---|---|---|---|---|---|
| Bayo | 51 | Y=56+1.45x | 0.35 | 6.68 | ** |
| Black | 18 | Y=68+0.29x | 0.09 | 0.14 | |
| Ojo de Cabra | 16 | Y=29+1.76x | 0.36 | 2.17 | ** |
| Bayo Brown | 5 | Y=69+0.62x | 0.13 | 0.05 | |
| Pinto | 14 | Y=114-1.01x | 0.18 | 0.41 | |
| Purple | 7 | Y=86-1.01x | 0.18 | 0.18 | |
| Red | 9 | Y=-25+4.69 | 0.61 | 4.05 | ** |
| Brown | 5 | Y=148-2.79x | 0.46 | 0.82 | |
| Flor de Mayo | 8 | Y=205-4.95x | 0.37 | 0.92 | |
| Yellow | 10 | Y=119-0.81x | 0.13 | 0.15 | |
| Analysis | | | | | |
| General | 143 | Y=55+1.24x | 0.28 | 12.26 | ** |

n = Number of genotypes

Table 5. Correlation between cooking time and different traits.

| Traits | Cooking Time |
|---|---|
| 100 seed weight | 0.29* |
| Water Absorption | -0.29* |
| % Imbibed Seed | -0.33* |
| Color | -0.23* |
| Earliness | 0.14 N.S. |
| Days to Maturity | 0.11 N.S. |
| Protein Content | 0.02 N.S. |

* = Significant
N.S. = Non significant

(Appendix D continued)

Table 6. Quality characteristic of 154 bean varieties grown in Celaya Gto.

| Variety | Seed Color | 100 Seed Weight | Protein | Cooking Time | Water** Absorption | Imbibed Seed | Days to Maturity |
|---|---|---|---|---|---|---|---|
| | ----- | g ----- | % | minutes | % | % | |
| Desc.20 | BR* | 33 | 22.5 | 72 | 114 | 100 | 77 |
| FM-51-CM | FM | 23 | 22.0 | 74 | 38 | 100 | 76 |
| O.C.S.Rita 83 | OC | 28 | 21.4 | 129 | 74 | 86 | 74 |
| P. Americano | P | 28 | 22.0 | 57 | 114 | 100 | 75 |
| A 59 | CF | 23 | 21.5 | 55 | 106 | 100 | 83 |
| B. los Llanos | B | 32 | 22.4 | 126 | 49 | 66 | 86 |
| CIAT 139/85 | B | 25 | 22.0 | 90 | 106 | 100 | 83 |
| CIAT 318 | N | 25 | 20.6 | 64 | 106 | 100 | 82 |
| CIAT 326/85 | N | 24 | 25.5 | 67 | 119 | 100 | 79 |
| CIAT 367/84 | P | 29 | 21.3 | 65 | 102 | 100 | 85 |
| BAT 477 | B | 20 | 25.3 | 59 | 97 | 100 | 85 |
| B. Victoria | B | 42 | 17.8 | 158 | 93 | 94 | 84 |
| Chis.12-B | R | 18 | 23.0 | 54 | 105 | 100 | 84 |
| Chis.254 | N | 18 | 25.0 | 67 | 110 | 100 | 79 |
| CIAT 130/85 | B | 22 | 23.7 | 82 | 113 | 100 | 83 |
| CIAT 226 | B | 22 | 24.7 | 75 | 109 | 98 | 84 |
| FM Bajio | FM | 24 | 20.7 | 66 | 110 | 100 | 84 |
| F. Mayo 307 | FM | 24 | 20.8 | 64 | 101 | 100 | 83 |
| 1984-14-3 | OC | 33 | 21.2 | 92 | 103 | 100 | 84 |
| 1984-18-3 | OC | 32 | 23.3 | 110 | 115 | 100 | 82 |
| Tam.8 | B | 43 | 21.2 | 55 | 101 | 100 | 81 |
| C101XDgo225 | OC | 33 | 21.4 | 107 | 102 | 96 | 79 |
| Chih.51 | OC | 37 | 22.3 | 86 | 93 | 96 | 79 |
| Chih.86 | OC | 29 | 21.9 | 90 | 102 | 100 | 82 |
| Chis.273 | P | 41 | 22.6 | 75 | 110 | 100 | 86 |
| CIAT 192 | B | 28 | 22.8 | 70 | 113 | 98 | 80 |
| Desc. 19 | OC | 29 | 21.0 | 87 | 105 | 96 | 82 |
| DGO.227 | B | 34 | 22.3 | 103 | 118 | 100 | 85 |
| F.Mayo RMC | FM | 25 | 21.2 | 72 | 108 | 100 | 80 |
| G - 13695 | P | 28 | 23.4 | 74 | 111 | 100 | 86 |
| G - 12747 | P | 27 | 19.9 | 82 | 42 | 75 | 84 |
| L - 1213-2 | P | 26 | 24.6 | 95 | 115 | 98 | 80 |
| MAM - 10 | B | 27 | 22.1 | 82 | 108 | 98 | 84 |
| Pinto Nal-1 | P | 27 | 23.1 | 71 | 103 | 94 | 79 |
| Pinto Villa | P | 22 | 22.0 | 121 | 103 | 60 | 84 |
| 1984-14-4 | B | 25 | 23.8 | 120 | 80 | 86 | 87 |
| Bayo Madero | B | 39 | 20.4 | 103 | 81 | 82 | 91 |
| B.LL.G-2IND. | P | 28 | 21.1 | 112 | 98 | 96 | 87 |
| Bayo Zac. | B | 41 | 19.0 | 170 | 105 | 100 | 86 |
| Chis.52-2 | M | 18 | 22.0 | 58 | 104 | 100 | 86 |
| CIAT 42/85 | B | 34 | 23.0 | 72 | 108 | 98 | 91 |
| CIAT 138/85 | B | 24 | 22.0 | 60 | 97 | 100 | 87 |
| CIAT 250/84 | A | 30 | 19.5 | 50 | 106 | 100 | 88 |
| CIAT 330 | B | 14 | 19.0 | 67 | 113 | 98 | 91 |
| CIAT 332 | B | 28 | 19.6 | 76 | 102 | 100 | 86 |
| Negro Dgo. | N | 31 | 22.0 | 80 | 105 | 100 | 87 |

(Continuation of Table 6, Appendix D.)

| Variety | Seed Color | 100 Seed Weight | Protein | Cooking Time | Water** Absorption | Imbibed Seed | Days to Maturity |
|---|---|---|---|---|---|---|---|
| | ----- | g ----- | % | minutes | % | % | |
| 1984-16-4 | M* | 26 | 18.6 | 59 | 95 | 96 | 89 |
| 1984-16-5 | M | 23 | 17.2 | 77 | 105 | 100 | 88 |
| 1984-17-3 | M | 22 | 22.1 | 89 | 96 | 94 | 87 |
| Pue.483 | A | 18 | 21.1 | 82 | 11 | 98 | 91 |
| Pue.493 | N | 22 | 22.0 | 62 | 109 | 100 | 91 |
| II-11-18-M-M | B | 39 | 18.9 | 151 | 57 | 100 | 86 |
| II231-M-5-M | OC | 34 | 23.1 | 91 | 92 | 86 | 86 |
| II933-M-52-2 | B | 26 | 20.1 | 80 | 106 | 100 | 87 |
| 453-3-2-CM | FM | 30 | 17.3 | 53 | 102 | 100 | 87 |
| CIAT-333-85 | B | 28 | ---- | 56 | 106 | 100 | 87 |
| A 252 | OC | 24 | 21.8 | 72 | 70 | 64 | 91 |
| AB 136 | M | 21 | 12.0 | 61 | 106 | 100 | 87 |
| Ags.62 | PR | 34 | 19.8 | 70 | 111 | 100 | 88 |
| 72X1424RRL5 | R | 22 | 24.5 | 43 | 95 | 96 | 90 |
| 72X1424RRL5 | R | 23 | 23.6 | 48 | 105 | 98 | 90 |
| 72X1424RRL7 | R | 22 | 22.8 | 45 | 92 | 88 | 92 |
| Chis.265 | M | 15 | 24.1 | 71 | 108 | 100 | 86 |
| CIAT 9/85 | B | 26 | 17.8 | 60 | 102 | 100 | 91 |
| CIAT 12 | B | 29 | 19.6 | 77 | 106 | 100 | 89 |
| CIAT 133/85 | B | 24 | 22.0 | 91 | 23 | 84 | 88 |
| CIAT 220 | B | 25 | 19.8 | 119 | 28 | 100 | 91 |
| CIAT 269/85 | OC | 2 | 20.3 | 51 | 26 | 100 | 92 |
| CIAT 335 | B | 21 | 24.0 | 69 | 111 | 100 | 88 |
| CIAT 369/85 | OC | 25 | 19.4 | 48 | 105 | 100 | 91 |
| F.Mayo 240 | FM | 23 | 19.3 | 76 | 18 | 100 | 88 |
| JAYATECO | N | 11 | 20.6 | 91 | 104 | 100 | 87 |
| MANZANO | R | 23 | 20.8 | 115 | 90 | 94 | 91 |
| Mex.226-E | A | 17 | 21.6 | 90 | 106 | 98 | 88 |
| Mich.91-A | R | 21 | 23.1 | 105 | 99 | 100 | 92 |
| Oax.37 | N | 18 | 21.8 | 64 | 52 | 63 | 92 |
| Oax.112 | N | 11 | 21.1 | 65 | 106 | 96 | 88 |
| 1984-9-1 | B | 32 | 19.6 | 95 | 89 | 80 | 90 |
| 1984-13-1 | OC N | 23 | 22.9 | 90 | 100 | 94 | 91 |
| 1984-23-1 | B | 29 | 22.6 | 103 | 89 | 76 | 89 |
| Pue.286 | BC | 13 | 23.0 | 54 | 92 | 86 | 91 |
| 83VEF-MXA238 | P | 28 | 21.0 | 68 | 106 | 100 | 91 |
| V8025 | N | 18 | 25.3 | 90 | 106 | 100 | 92 |
| Ags.49 | B | 36 | 22.0 | 88 | 106 | 98 | 87 |
| BAYOCEL | B | 20 | 19.6 | 103 | --- | 94 | 88 |
| BAYOCEL-1 | B | 21 | 21.3 | 82 | 108 | 100 | 88 |
| B. Dgo. 012 | B | 39 | 23.5 | 133 | 121 | 100 | 85 |
| CIAT 352 | B | 29 | 18.3 | 100 | 85 | 84 | 89 |
| Col.9 | B | 15 | 21.5 | 87 | 96 | 86 | 88 |
| Dgo.5 | B | 31 | 22.0 | 118 | 79 | 80 | 38 |
| Dgo.69 | A | 25 | 20.8 | 124 | 81 | 84 | 90 |
| Gto.157 | B R | 21 | 22.8 | 130 | 92 | 90 | 91 |
| Hgo-80 | R | 34 | 23.5 | 145 | 100 | 80 | 88 |

(Continuation of Table 6, Appendix D.)

| Variety | Seed Color | 100 Seed Weight | Protein | Cooking Time | Water Absorption | Imbibed Seed | Days to Maturity |
|---|---|---|---|---|---|---|---|
| | ----- | g ----- | % | minutes | % | % | |
| Mex.265 | N* | 18 | 21.8 | 72 | 20 | 30 | 90 |
| Mich.1-B-1 | B | 23 | 21.9 | 90 | 91 | 84 | 88 |
| Mich.89 | R | 19 | 22.3 | 67 | 65 | 20 | 86 |
| Oax.110 | BC | 11 | 22.5 | 45 | 105 | 100 | 88 |
| Pue.288 | BC | 12 | 23.8 | 44 | 121 | 98 | 91 |
| Ver.167 | BC | 14 | 22.8 | 42 | 105 | 100 | 91 |
| A 193 | PR | 49 | 22.6 | 54 | 108 | 100 | 93 |
| CIAT 272/85 | M | 20 | 20.0 | 65 | 106 | 100 | 95 |
| CIAT 297/85 | OC | 34 | 21.6 | 60 | 90 | 92 | 95 |
| GTO.297-85 | BC | 34 | 20.5 | 88 | 107 | 96 | 97 |
| Oax.53 | P | 24 | 18.3 | 131 | 61 | 60 | 93 |
| URG 1769 | B | 25 | 20.5 | 57 | 99 | 96 | 98 |
| A 322 | B | 28 | 20.6 | 94 | 89 | 90 | 95 |
| Ags.19 | B | 19 | 25.6 | 74 | 100 | 100 | 96 |
| A64X48RR-L-2 | BR | 24 | 21.3 | 69 | 107 | 100 | 95 |
| A64X48RR-L-3 | BR | 28 | 20.8 | 63 | 112 | 100 | 96 |
| A64X48RR-L-4 | BR | 27 | 23.1 | 52 | 111 | 98 | 97 |
| Chis.3-A | N | 20 | 20.6 | 100 | 100 | 98 | 96 |
| CIAT-116-85 | B | 26 | 18.8 | 74 | 70 | 68 | 96 |
| CIAT-146-84 | B | 27 | 18.8 | 83 | 23 | 88 | 95 |
| CIAT-334-85 | A | 23 | 23.3 | 84 | 111 | 96 | 91 |
| CIAT-339 | OC | 24 | 21.1 | 57 | 97 | 100 | 95 |
| CIAT-359 | R | 19 | 24.0 | 98 | 120 | 100 | 97 |
| CIAT-393-84 | OC | 27 | 20.3 | 76 | 100 | 96 | 97 |
| CIAT-408-85 | FM RJ | 22 | 22.5 | 60 | 89 | 92 | 97 |
| Jal.142 | BC | 19 | 22.5 | 70 | 100 | 100 | 97 |
| L-1-1-1-2-CM | OC | 27 | 20.0 | 75 | 74 | 82 | 93 |
| L-3-1-1-1-CM | OC | 27 | 20.1 | 60 | 99 | 100 | 95 |
| L-3-1-1-2-CM | P | 25 | 20.0 | 49 | 102 | 100 | 95 |
| L-3-1-1-14CM | P | 26 | 19.9 | 52 | 100 | 100 | 94 |
| Negro Argel | N | 18 | 20.7 | 57 | 91 | 14 | 96 |
| Negro Qro.78 | N | 21 | 20.0 | 61 | 93 | 96 | 93 |
| Oax.48-A | M | 24 | 18.8 | 35 | 94 | 90 | 98 |
| 1984-9-3 | B | 31 | 20.5 | 79 | 82 | 88 | 96 |
| 1984-26-1 | B | 35 | 22.3 | 128 | 85 | 96 | 95 |
| URG-2034 | A | 23 | 24.0 | 116 | 85 | 82 | 99 |
| URG-2063 | B N | 26 | 20.5 | 83 | 110 | 98 | 100 |
| URG-2183 | N | 21 | 21.1 | 90 | 90 | 90 | 97 |
| Ver.175 | N | 18 | 22.4 | 69 | 86 | 88 | 95 |
| UW.21-58 | N | 19 | 21.6 | 49 | 106 | 100 | 91 |
| CIAT 60/85 | B | 27 | 19.4 | 125 | 57 | 62 | 96 |
| CIAT 343/84 | FM | 23 | 19.8 | 150 | 31 | 32 | 95 |
| G-11949 | FM | 26 | 20.1 | 105 | 28 | 34 | 94 |
| Qro.27-C | CF | 18 | 19.6 | 97 | 85 | 84 | 95 |
| Rio Grande | B | 19 | 19.2 | 125 | 44 | 48 | 96 |
| URG-57 | A | 21 | 19.8 | 101 | 53 | 66 | 98 |
| URG-1408 | CF | 28 | 23.7 | 74 | 100 | 98 | 98 |

(Continuation of Table 6, Appendix D.)

| Variety | Seed Color | 100 Seed Weight | Protein | Cooking Time | Water Absorption | Imbibed Seed | Days to Maturity |
|---|---|---|---|---|---|---|---|
| | ----- | g ----- | % | minutes | % | % | |
| URG-1424 | N* | 24 | 22.2 | 93 | 86 | 88 | 96 |
| URG-1714 | B | 24 | 23.3 | 170 | 75 | 76 | 98 |
| URG-2093 | N | 25 | 21.0 | 78 | 83 | 90 | 98 |
| URG-2174 | B | 25 | 21.8 | 76 | 70 | 72 | 100 |
| CIAT 57/85 | B | 23 | 19.6 | 120 | 70 | 70 | 106 |
| Garbancillo | B | 28 | 24.0 | 136 | 51 | 48 | 97 |
| URG-1696 | G | 24 | 21.3 | 107 | 100 | 98 | 112 |
| URG-1755 | A | 24 | 23.1 | 135 | 36 | 48 | 111 |
| URG-1757 | A | 30 | 23.1 | 98 | 92 | 88 | 102 |
| URG-2130 | CF | 23 | 21.9 | 82 | 43 | 56 | 112 |
| URG-2204 | B | 20 | 21.3 | 100 | 43 | 52 | 100 |
| URG-2298 | CF | 22 | 21.7 | 111 | 126 | 50 | 102 |
| 500 Criollo CP | B | 23 | 21.7 | 106 | 42 | 46 | 112 |
| 1984-23-1 | OC CF | 23 | 22.9 | 55 | 102 | 98 | 91 |

*B = bayo and white, N = black, OC = ojo de cabra, P = pinto, FM = Flor de mayo, R = Red, M = purple, C = brown, BR = bayo brown, A = yellow

**Percent Water Absorption = $100\ (W_2-W_1)/W_1$
$W_1$ = Original dry seed weight of 50 g
$W_2$ = Weight after soaking in 75 mls of distilled water for 16 hours.

Appendix E

Table 1. Group 1. Enzymes/proteins showing polymorphism with known genetic basis, gene pool specificity and consistent electrophoretic results.

| Enzyme | Abbreviation | Gene pool specificity | |
|---|---|---|---|
| | | Andean | Meso-American |
| Phaseolin | PHA | F | S |
| Acid phosphatase | ACP | F | S |
| Diaphorase | DIA-1 | F | S |
| Malic enzyme | ME | S | F |
| Peroxidase | PRX | S | F |
| Rubisco | RUB | S | F |
| Shikimate dehydrogenase | SKD | S | F |
| Diaphorase | DIA-2 | S | S |

Group 2. Enzymes showing possible polymorphism but without genetic basis and sometimes inconsistent electrophoretic results.

| Name | Abbreviation |
|---|---|
| Aconitase | ACO |
| Fluorescent esterase | FLE |
| Glutamate oxaloacetate transaminase | GOT |
| Isocitrate dehydrogenase | IDH |
| Malate dehydrogenase | MDH |
| Superoxide dismutase | SOD |

Group 3. Enzymes with no polymorphism

| Name | Abbreviation |
|---|---|
| Alcohol dehydrogenase | ADH |
| Catalase | CAT |
| Endopeptidase | ENP |
| Glucose-1-phosphate | G1P |
| Glucose-2-phosphate dehydrogenase | G2D |
| Glucose-6-phosphate dehydrogenase | G6PD |
| Glucose-6-phosphate isomerase | PGI |
| Leycine aminopeptidase | LAP |
| Phosphoglucomutase | PGM |
| 6-Phosphogluconate dehydrogenase | 6PGD |

Appendix E

Table 2. Isozyme profiles of forty three genotypes investigated and their drought response.

| Genotype | Enzyme locus | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Drought | Me | Skd | Rub | Dia-1 | Acp | Pha | Dia-2 |
| Gene pool specificity: | | | | | | | | |
| Andean | | S | S | S | F | F | F | S |
| Meso-American | | F | F | F | S | S | S | S |
| BAT-477 | T | F | F | F | S | S | S | F |
| BAT-1224 | S | F | F | F | S | S | S | S |
| BAT-67 | S | F | F | F | S | S | S | S |
| BAT-260 | - | F | F | F | S | S | S | S |
| BAT-85 | T | F | F | F | S | S | S | S |
| A-70 | S | F | F | F | R | F | S | S |
| Negro-Qro | T | F | F | F | S | S | S | F |
| Pinto NAL-1 | T | F | F | F | S | S | S | S |
| Bayo Madero | T | S | F | S | S | F | S | S |
| Durango 222 | T | F | F | F | S | S | S | S |
| L-1213-2 | - | F | F | F | S | S | S | S |
| Red Mexican | T | F | F | F | S | S | S | S |
| 4-7 Mezathan | T | F | F | F | S | S | S | S |
| OAX-14-J-C | T | F | F | F | S | S | S | S |
| Negro San Luis | - | F | F | F | S | F | S | S |
| Bayo Victoria | T | F | F | F | S | S | S | S |
| 83-VEF-MXA-238 | - | F | F | F | S | S | S | S |
| G-6342 | T | F | F | F | S | S | S | F |
| AGS 77 | T | S | F | F | S | S | S | S |
| A-322 | - | F | F | F | S | S | S | S |
| O.C. Sta. Rita 83 | T | F | F | F | S | S | S | S |
| Pinto Mex 80 | T | F | F | F | S | S | F | S |
| CHIH. 11D | T | F | F | F | S | S | S | S |
| Pinto Villa | T | S | F | F | S | S | S | S |
| OA 112 | T | F | F | F | S | S | S | S |
| N11 9-7 | - | F | F | F | S | S | F | S |
| PUE 152 | | S | F | F | S | S | F | F |
| Katolika | T | S | F | F | S | S | S | H |
| 8-17 | T | S | F | F | S | S | F | S |
| 48-66 | T | S | F | F | S | S | S | S |
| 8-3 | S | F | F | F | S | S | S | S |
| N81017 | T | S | F | F | S | S | S | H |
| 8-25-2 | S | F | F | F | S | S | S | H |
| 9-39-1 | S | S | F | F | S | S | S | S |
| 8-47 | T | F | F | F | S | S | S | S |

(Table 2, Appendix E continued)

| Genotype | Enzyme locus | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Drought | Me | Skd | Rub | Dia-1 | Acp | Pha | Dia-2 |
| 8-4 | S | F | F | F | S | S | S | H |
| 39-17-1 | S | S | F | F | S | S | F | S |
| 42-M-1 | T | F | F | F | S | S | S | H |
| Lef-2-RB | T | F | F | F | S | S | S | S |
| II-900-5-M-45 | T | F | F | F | S | S | S | S |
| 8-42-M-2 | T | F | F | F | S | S | S | S |
| AC 1028 | T | F | F | F | S | S | S | S |
| Sierra | T | S | F | F | S | S | S | F |

F = fast (more anodal) allele, S = slow (less anodal) allele, R = rare faster than F allele, H = a heterozygote between F and S for Dia-2.

Appendix F

Table 1. Dry weight of different plant organs obtained at five sampling dates for a 1989 greenhouse study utilizing Bayo Madero and Pinto Nacional under two moisture regimes.

| Cultivar | Days after Planting | | | | |
|---|---|---|---|---|---|
| | 27 | 34 | 46 | 61 | 81 |
| | g | | | | |
| | | | STEM | | |
| Bayo Madero | 0.56 a* | 1.00 a+ | 1.23** | 1.17 | 1.48 a+ |
| Pinto Nacional | 0.44 b | 0.90 b | 0.93 b | 1.04 | 1.37 b |
| Water | N.S. | * | ** | ** | ** |
| Water x Cult. | N.S. | N.S. | N.S. | N.S. | N.S. |
| C.V. (%) | 13 | 10 | 9 | 14 | 8 |
| | | | LEAF | | |
| Bayo Madero | 1.06 | 1.10 b* | 1.17 a** | 0.85 | 1.20 |
| Pinto Nacional | 1.05 | 1.34 a | 1.04 b | 0.91 | 1.19 |
| Water | N.S. | ** | ** | ** | ** |
| Water x Cult. | N.S. | N.S. | ** | N.S. | N.S. |
| C.V. (%) | 9 | 13 | 6 | 41 | 25 |
| | | | ROOT | | |
| Bayo Madero | 0.43 b+ | 0.70 b* | 0.75 | 0.66 | 0.79 |
| Pinto Nacional | 0.58 a | 1.10 a | 0.87 | 0.65 | 0.82 |
| Water | N.S. | N.S. | ** | ** | ** |
| Water x Cult. | N.S. | N.S. | N.S. | N.S. | N.S. |
| C.V. (%) | 26 | 27 | 23 | 24 | 20 |
| | | | REPRODUCTIVE | | |
| Bayo Madero | 0.38 | 0.13 | 1.28 b** | 2.14 | 1.09 b+ |
| Pinto Nacional | 0.38 | 0.14 | 1.71 a | 1.76 | 1.57 a |
| Water | N.S. | * | ** | * | ** |
| Water x Cult. | N.S. | N.S. | **. | N.S. | N.S. |
| C.V. (%) | 30 | 28 | 11 | 24 | 30 |

**,*,+ Indicate significant difference at $P < 0.01$, 0.05, and 0.10, respectively.

(Appendix F continued)

Table 2. N concentration (% N) in different plant organs obtained at five sampling dates for a 1989 greenhouse study utilizing Bayo Madero and Pinto Nacional under two moisture regimes.

| Cultivar | Days After Planting | | | | |
|---|---|---|---|---|---|
| | 27 | 34 | 46 | 61 | 81 |
| | g N/100 g dry matter | | | | |
| | | | STEM | | |
| Bayo Madero | 2.4 | 2.1 | 2.0 | 1.9 b+ | 1.9 |
| Pinto Nacional | 2.5 | 2.3 | 2.3 | 2.3 a | 2.0 |
| Water | N.S. | ** | ** | ** | ** |
| Water x Cult. | N.S. | N.S. | N.S. | N.S. | N.S. |
| C.V. (%) | 8 | 8 | 12 | 19 | 16 |
| | | | LEAF | | |
| Bayo Madero | 4.1 b* | 4.1 | 3.4 | 3.6 | 2.4 |
| Pinto Nacional | 4.3 a | 4.5 | 3.0 | 2.7 | 3.3 |
| Water | N.S. | N.S. | N.S. | * | N.S. |
| Water x Cult. | N.S. | N.S. | N.S. | N.S. | N.S. |
| C.V. (%) | 5 | 30 | 56 | 44 | 78 |
| | | | ROOT | | |
| Bayo Madero | 2.7 | 2.6 a* | 2.5 | 2.0 | 2.3 a* |
| Pinto Nacional | 2.8 | 2.1 b | 2.3 | 2.0 | 2.1 b |
| Water | N.S. | N.S. | * | N.S. | N.S. |
| Water x Cult. | N.S. | N.S. | N.S. | N.S. | N.S. |
| C.V. (%) | 14 | 9 | 11 | 7 | 6 |

**,*,+ Indicate significant difference at $P < 0.01$, 0.05, and 0.10, respectively.

Table 3. Percentage of total plant nitrogen located in different plant organs at harvest.

| Variety | Treatment | Reproductive | Stem |
|---|---|---|---|
| | | % | |
| Bayo Madero | Stress | 13.6 | 40.8 |
| Pinto Nacional | Stress | 4.8 | 45.9 |
| Bayo Madero | Non-Stress | 21.1 | 14.1 |
| Pinto Nacional | Non-Stress | 24.0 | 14.8 |

Appendix G

Table 1. N content (mg N/plant), leaf area, and total dry weight/plant (g) for reciprocal grats of the genotypes 9-39-1 and Lef-2-RB under stress and non-stress conditions.

| Grafting Treatment | Stress Level | mg N/plant |
|---|---|---|
| Shoot x Root | | |
| 9-39-1 x 9-39-1 | N | 153.5 a* |
| 9-39-1 x Lef-2-RB | N | 144.7 ab |
| Lef-2-RB x 9-39-1 | N | 143.3 ab |
| Lef-2-RB x Lef-2-RB | N | 127.6 b |
| Lef-2-RB x 9-39-1 | S | 110.6 c |
| Lef-2-RB x Lef-2-RB | S | 110.4 c |
| 9-39-1 x 9-39-1 | S | 96.82 c |

| Grafting Treatment | Stress Level | Leaf area |
|---|---|---|
| Shoot x Root | | $dm^2$ |
| 9-39-1 x 9-39-1 | N | 870.7 a* |
| 9-39-1 x Lef-2-RB | N | 855.0 a |
| Lef-2-RB x 9-39-1 | N | 803.6 ab |
| Lef-2-RB x Lef-2-RB | N | 733.9 b |
| Lef-2-RB x Lef-2-RB | S | 413.5 c |
| Lef-2-RB x 9-39-1 | S | 378.2 c |
| 9-39-1 x 9-39-1 | S | 348.4 c |

| Grafting Treatment | Stress Level | g dw/plant |
|---|---|---|
| Shoot x Root | | |
| Lef-2-RB x 9-39-1 | N | 10.83 |
| 9-39-1 x 9-39-1 | N | 9.84 |
| 9-39-1 x Lef-2-RB | N | 9.46 |
| Lef-2-RB x Lef-2-RB | N | 9.40 |
| Lef-2-RB x 9-39-1 | S | 6.44 |
| Lef-2-RB x Lef-2-RB | S | 6.17 |
| 9-39-1 x 9-39-1 | S | 4.79 |

*Indicates significant difference at $P < 0.05$ according to DMRT.

Table 2. N concentration (g N/100 g dry wt.) for reciprocal grafts of the genotpes 9-39-1 and Lef-2-RB under stress and non-stress conditions.

| Grafting Treatment | Stress Level | % N in stem |
|---|---|---|
| Shoot x Root | | |
| 9-39-1 x 9-39-1 | S | 1.63 a* |
| Lef-2-RB x 9-39-1 | S | 1.20 b |
| Lef-2-RB x Lef-2-RB | S | 1.12 bc |
| Lef-2-RB x Lef-2-RB | N | 1.03 bc |
| Lef-2-RB x 9-39-1 | N | 1.00 bc |
| 9-39-1 x Lef-2-RB | N | 0.91 bc |
| 9-39-1 x 9-39-1 | N | 0.78 c |

*Indicate significant difference at $P < 0.05$ according to DMRT.

# NIGERIA - UNIVERSITY OF GEORGIA

*APPROPRIATE TECHNOLOGY FOR COWPEA PRESERVATION AND PROCESSING AND A STUDY OF ITS SOCIOECONOMIC IMPACT ON RURAL POPULATIONS IN NIGERIA*


**Principal Investigators:**

| | | |
|---|---|---|
| Kay McWatters (U.S.) | Food Science and Technology | University of Georgia |
| Dickson O. Nnanyelugo (HC) | Home Science and Nutrition | University of Nigeria-Nsukka |

**Co-Investigators:**

| | | |
|---|---|---|
| Larry R. Beuchat | Food Science and Technology | University of Georgia |
| Angus S. D. Chidebelu | Home Science and Nutrition | University of Nigeria-Nsukka |
| Manjeet S. Chinnan | Food Science and Technology | University of Georgia |
| Henrietta Ene-Obong | Home Science and Nutrition | University of Nigeria-Nsukka |
| Yen-Con Hung | Food Science and Technology | University of Georgia |
| Alfred I. Ihekoronye | Food Science and Technology | University of Nigeria-Nsukka |
| Patrick O. Ngoddy | Food Science and Technology | University of Nigeria-Nsukka |
| Ikemefuna C. Obizoba | Home Science and Nutrition | University of Nigeria-Nsukka |
| E. Chinwe Okeke | Home Science and Nutrition | University of Nigeria-Nsukka |
| Bridget N. Onah | Agricultural Economics | University of Nigeria-Nsukka |
| Veronica I. Onuorah | Home Science and Nutrition | University of Nigeria-Nsukka |
| Nwachukwu D. Onwuka | Food Science and Technology | University of Nigeria-Nsukka |
| R. Dixon Phillips | Food Science and Technology | University of Georgia |
| Adaoha C. Uwaegbute | Home Science and Nutrition | University of Nigeria-Nsukka |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Examples of research results disseminated and in use.** Economics of Village Mill Operation at Isiala-Ngwa and Ogbodu-Aba: During FY 90, the Africare project at Isiala-Ngwa was able to cover its variable costs for all products manufactured. These include cowpea supplement (100 percent cowpea flour), corn meal, and soybean flour. None of the products covered the assigned fixed costs at the level of production indicated in Tables 1 and 2. Reasons for poor performance appear to be due to uneconomic pricing of sales and low overall output. In 1990, the mill operated at about 20 percent of its installed capacity as a result of engagement of mill staff in the process of starting up three new mills in two other local government areas of Imo State.

At Ogbodu-Aba, six tons of grain were processed during the period averaging about half a ton per month. Four tons of assorted flour (maize, sorghum and cowpea) and blends were produced giving a mean extraction rate of 60 percent. Of the total output, approximately 50 percent was supplied to the project as test samples, and the remaining half was sold primarily as the weaning food, Cerebabe. Total income from sales was N9,500 which covers about half of the operating expenditure.

Impact Assessment Activities: Impact deriving from the introduction of cowpea-based products is being monitored and assessed at four levels.

**Workshops and field tests:** During FY 90, six workshops were conducted at Nsukka, Adani, Onitsha and Okigwi and involved 300 Home Economics teachers, nurses, and housewives. Testing of different cowpea products was also carried out in six village markets in the neighborhoods of Nsukka, Adani and Onitsha. High acceptability ratings were recorded in all tests with dehulled cowpea products scoring better than the hulled. In response to growing inquiries by the public for additional workshops, a recipe book has been written for general application.

**Village-household activities:** At Ogbodu-Aba, a household survey was conducted to determine the usage of cowpea products by 300 village families in the communities of Onicha, Agushire, Ikeagwu and Amagunze. The price of cowpea products remains a primary impediment to its widespread adoption as a prime weaning food.

**Acceptability of dehulled cowpea splits in Nigerian cooking:** Dehulled cowpea splits were prepared as pottage and cowpea-rice mixture to determine consumer reactions to the use of dehulled splits in traditional cooking. Consumer tests involving 500 subjects were conducted in markets at Onitsha, Nsukka and Okigwi. The flavor, color and overall acceptability of dehulled cowpea dishes were significantly superior to the hulled counterparts among high, middle and low-income brackets. One hundred percent of the consumers would buy dehulled cowpea splits if they were available in the market at a price which is at par with the conventional hulled cowpeas. Dehulled cowpea splits have the extra advantage of reduced cooking time and reduced incidence of flatulence in consumers.

Thirty nine vendors were used to quantify margins derived from the sale of akara and moin-moin prepared from cowpea splits and dehulled cowpea flour. Profit margins were found to be similar.

**Energy expenditure patterns relating to cowpea products:** During FY 90, energy expenditure measurements were carried out, using the "diary of activities" method with rural housewives and vendors who produce moin-moin and akara via traditional as contrasted from flour pasting process for cowpeas. In FY 90, a series of measurements using a portable oxylog machine confirmed the earlier finding that rural women spend 1.5 times more energy to prepare akara via the traditional pasting process than from flour. They spend three times more energy to prepare moin-moin by the traditional route also.

**Advances in moin-moin processing:** Paul Okechukwu's work at Cornell University revealed that firmness uniformity of canned moin-moin is maximized and phase separation is minimized by mixing cowpea flour and all ingredients with water at 70°C and holding for 15 minutes. Addition of fat increases caloric density of the product and functions as a shortener to decrease gel strength and enhance firmness uniformity. Moin-moin gel firmness increases rapidly above 72°C, attains a peak at 87°C, then decreases progressively at higher

temperatures. Heat transfer in moin-moin production is conduction controlled. The slowest heating zone in canned moin-moin is located at a point 14 percent off-center along the central axis of the can. For this reason, $F_0$ values based on the can's geometric center may be underestimated by as much as 30 percent. A safety margin of >30 percent is recommended in the design of thermal processes for canned moin-moin.

**Effect of pre-decortication drying temperature on cowpea flour components in akara and moin-moin preparations:** Joyce Enwere's fractionation and electrophoretic analysis of proteins from large brown eye Kano white cowpeas wetted and then dried at 30, 80 and 120°C revealed that heat treatment at > 80°C reduced solubility of the albumin but not the globulin fraction. These results suggest that heat denaturation and insolubilization of the albumin fraction may be responsible for the inability of pastes from high temperature pretreatments to foam. Poor foam development, compact microstructure, and textural hardness increased as drying temperature increased. Protein changes at 100°C also significantly affected moin-moin texture but had only a minor effect on the microstructure.

**Nutritional and chemical evaluation of Cerebabe weaning food:** Data from a nutritional evaluation of weaning foods involving four-to six-year-old children are being analyzed. Sixteen children, eleven in the experimental group and five controls, were involved in the study. The experimental group was fed Cerebabe as the sole source of protein, and the following parameters were assessed: weight gain, blood profile, nitrogen balance, acceptability of the food as judged by presence/absence of abdominal problems, willingness to consume the food and quantity of food consumed. Cerebabe was made into different recipes such as pancake, foo-foo pap, and pudding (moin-moin) to increase the variety of products offered. Acceptability was quite good and enthusiastic.

**Hard-to-cook (HTC) defect:** A method for determining extent of cowpea hardening without the necessity of cooking the seeds was developed. Seeds stored at -18°C and 30°C/64 percent RH for 15 months were studied. The hardness of cooked 30°C/64 percent RH seeds measured by an Instron was four times that of control seeds stored at -18°C. Mechanical properties of individual uncooked seeds were determined with an Instron equipped with a Warner-Bratzler shear blade at different crosshead speeds. Peak force of normal and HTC seeds recorded from force-deformation curves were not significantly different. However, HTC cowpeas required higher shear-compression energy than normal cowpeas. Thus, it is possible to detect the HTC defect in uncooked seeds using this method.

The interaction of $Ca^{2+}$ with demethylated pectin is commonly considered responsible for the HTC defect in legume seeds. Pectin methylesterase is known to be activated by temperatures between 45 and 70°C. HTC defect was induced by incubating fresh cowpea seeds at elevated temperatures followed by soaking in calcium ion solution. Degree of hardness increased as incubation temperature increased up to 70°C, then declined at higher temperatures.

However, free methanol released, pectin esterase activity, and degree of cell wall pectin methylation did not follow the same trend. Nevertheless, this model shows promise for studying the HTC phenomenon, and further studies are underway.

Disseminating Project Technology to U.S. Industry: Technologies developed for producing cowpea flour and akara from it were demonstrated to ten individuals representing the California Dry Bean Advisory Board, Blackeye Varietal Council, manufacturers of cowpea-based foods, and academic researchers. The response of this group was enthusiastic with a consensus that akara made either from whole or decorticated seeds would probably be successful in the U.S. market. This contact has produced a gift of 1,000 lbs of cowpeas for ongoing research at the U.S. institution.

**Evidence of extent of use to date.** Team members have continued to publish research findings in scientific journals, present papers at professional and scientific meetings, and participate actively in seminars and workshops. Five thousand copies of a recipe booklet on cowpea flour-derived products emanating from HC research have been published. Awareness of village mill activities at Ogbodu-Aba and Isiala-Ngwa is becoming widespread in Anambra and Imo States. Imo State alone now has three village mill projects. Project findings (particularly improved cowpea flour and splits, infant weaning foods, and supplements formulated and tested by the HC team) are increasingly being used by roadside vendors of akara and moin-moin and in maternity and child rehabilitation clinics in the two States mentioned above. Commercial processors typified by such organizations as Silver Spoon Breakfast Service at Enugu and Lisabi Mills in Lagos are growing in size and number and continue to benefit from innovations as they evolve from the project.

2. Other research-related results

**Project impact on production and consumption of beans and cowpeas.** The Nigerian government's ban on wheat import since 1987 and the effects of inflation on the Nigerian economy continue to encourage the use of cowpea flour in baking and other applications. Weaning foods based on dehulled cowpea splits have been introduced and are gaining in acceptance. One highly popular delicacy for older children, "Baby Dinner," is a mixture of cooked dehulled cowpea splits, steamed dices of ripe plantain, steamed flaked fish, and carrots. Other developments include fortifying cassava and maize meals with cowpea flour during the preparation of traditional Nigerian foo-foo, pancakes, and baked goods such as cakes, cookies and corn bread.

**How the research findings specifically address the needs of small-scale farmers and women.** Extensive acceptability tests involving rural women and low-income earners were conducted to determine the adaptability of dehulled cowpeas in Nigerian cooking. Results showed that cooking fuel and the time and energy expended in traditional cowpea food preparation were significantly higher than when flour or splits were used. Cowpea splits cook in only 20-45 minutes as compared to hulled cowpeas which cook in 60-140 minutes.

Savings of fuel to rural women whose predominant source of fuel is firewood simply cannot be overestimated. Use of cowpea flour for such foods as akara and moin-moin would be of considerable value in ameliorating hardships endemic in the lives of rural women. Six out of twenty vendors producing and marketing akara and moin-moin who were involved in one study now routinely use cowpea flour in their production.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Since this project focuses on utilization rather than production of cowpea, it is unlikely that an impact on national production has yet been made. Increasing demand for cowpeas through facilitated preparation techniques would be expected to eventually lead to increased production.

B. Institutional Development and Training

1. Changes since FY 89. Five B.S. students will graduate from the HC institution by December 1990. Miss J. Atti and Miss H. Egbuna are M.S. students in nutrition working on sprouted and fermented cowpea products. Mr. K. Ugwuja is doing his M.S. project in nutrition on clinical evaluation of weaning foods. Mrs. Miriam Awachie has completed her M.S. project in food science on the effect of enzymes on the functional properties of cowpea flour. Mrs. Chika Ndiokwelu, a Ph.D. candidate, is working on the use of cowpea in a high energy formula for use in hospital malnutrition. Mrs. H. N. Ene-Obong is doing her Ph.D. project on the effect of sprouting on nutrients in cowpea. Mrs. P. O. Ikeme has commenced her Ph.D. program at Cambridge University on energy expenditure patterns in cowpea production, processing, preparation and consumption. Mrs. Joyce Enwere and Mr. Paul Okechukwu are at the final stages of cowpea-related Ph.D. research in Food Science and Technology. At UGA, Ms. Shona Holt completed research on modeling and optimization of products from composite flour blends by mixture response surface methodology and will receive the M.S. degree in December 1990.

2. Over the life of the project. Facilities at the HC institution have grown, and the capabilities of staff for independent research on cowpea-related interests have been enhanced. Both HC departments have become significantly better equipped and staffed as a result of the project.

3. Project training to be completed by the end of the project period. During the remaining portion of the project (1990-1992), the HC institution expects the completion of three Ph.D., three M.S. and six B.S. programs. These training targets exceed those anticipated in the extension proposal when originally conceived. This is because enhanced all-round progress has enabled the HC to perform better than anticipated in this respect.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

This CRSP project has been engaged in the lines of research addressing these objectives for two years during this extension proposal. The original time estimate for achieving the objectives was October 1991.

The estimated time remaining to achieve the objectives is one calendar year. The objectives anticipated in the FY 89-92 extension log frame are being met on schedule.

Although work on germination, fermentation and the application of cowpea flour is reported from other countries, the HC's line of research is unique because of the high consumption of cowpea and its diverse use in Nigeria. HC research is addressing simultaneously: primary utilization constraints such as monotony of cowpea in the diet, incidence of antinutritional factors and flatulence, wasting energy-intensity of preparative methods, and excessive demand on the time of cowpea users.

Several likely benefits of the research are apparent for the amelioration of global constraints. Increased contribution of cowpeas will enhance human nutrition by reducing storage losses, improving convenience-of-use, and enhancing nutritional quality. Innovations in cowpea-based products and the technology for processing them are making a profound impact on village life in Nigeria by showing ways in which the status of maternal and child health can be enhanced in rural areas. Small-scale farming is being stimulated by the presence of village mills in rural areas which demand cowpeas and cereals as raw materials and provide a dependable market outlet for small growers. Farmers can organize themselves into cooperatives to supply raw materials and market products. The Africare/Imo State Child Survival Project at Isiala-Ngwa provides an ongoing case study of this model of rural development. Reduction of oligosaccharide content of cowpea products will enhance utilization by improving digestibility. Knowledge of the microflora naturally present in cowpea paste and meal and the study of fermentation of cowpea products by lactic acid bacteria can contribute to the development of procedures to prevent microbiological and health hazards (e.g., diarrhea) associated with product contamination and/or deterioration. In the U.S., the food industry is being introduced to akara which could help increase the level of protein intake from plant rather than animal sources.

D. Evidence of Biological/Social Sciences Integration

Identification of and attention to relevant WID issues: Research in this project has been directed toward developing convenient and more nutritious uses of cowpea that are less labor-intensive and less energy-(human as well as fossil) and time-demanding so that rural and urban women can spend less time and energy in food preparation and have more time for other activities such as child care, income generation, and education.

Identification of and attention to other social and/or production issues: Workshops being conducted in urban and rural areas expose women to novel ways and techniques of cowpea utilization which hold vast potential for profitable commercial activities for them.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

Linkages with national institutions/organizations include IITA programs on cowpea, soybean, and cassava; University of Ibadan; village women groups; and government agencies such as Imo State Africare project.

Linkages with private groups include Silver Spoon Breakfast Service, Enugu; Francis Foods, Nsukka; Lisabi Mills, Lagos. International linkages include University of Nairobi, Kenya; University of Ghana, Legon; Noguchi Memorial Institute, Ghana; and Agricultural University, Wageningen. As a result of a Cowpea Workshop organized by the HC PI at an international nutrition congress in Seoul, Korea, the HC team is corresponding with parties in Sri Lanka, Thailand, Indonesia, and Denmark.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

The reimbursement procedure adopted by USAID for HC institutions continues to be counterproductive. As in past years, the practice of transferring funds in two allotments has been costing the project six months of wasted time every year. Each fund transfer takes three months to effect. The first allotment is authorized at the beginning of the new fiscal year when the preceding year's funds have been exhausted. The second allotment is only authorized after the first allotment has been encumbered. In the absence of any form of bridging finance, the HC is left with long intervals of waiting and unproductive time between fund transfers.

The process for getting approvals through USAID for international travel and purchase of equipment continues to be painfully slow. Source-origin waivers are even slower. The ordering and acquisition of equipment requiring these waivers are terribly delayed and often negated. The HC has been waiting for one year for approval to purchase a vehicle that is absolutely necessary for the completion of planned research.

### B. Adequacy of Current Management System

This project recommends, as in the FY 89 report, that policy reform be initiated that would enable USAID to vest powers in the Management Office to approve prebudgeted equipment purchases from both U.S. and other sources.

### C. Activity Towards Buy-Ins or Other Funding

As a result of hosting a postgraduate student from the Netherlands for two months at no direct cost to the CRSP, the HC team has been invited to collaborate on a project on weaning food development and field-testing via a linkage agreement with Dr. Robert Nout of the Department of Food Science, Agricultural University, Wageningen, the Netherlands. Project funds in the amount of $12,000 per year for an initial period of two years will be provided by IFS (International Foundation for Science, an agency of Swedish foreign aid program). In addition, the HC team is collaborating with IITA on projects pertaining to the impact of cowpea intercropping with cassava at no cost to the CRSP. The HC team has also received a grant of $10,000 for further improvement on cowpea milling equipment from UNU. At the request of the Management Office, the U.S. team wrote a proposal for a cowpea utilization buy-in project in Egypt which was not funded. Efforts to initiate a cross-CRSP effort continue with informal cooperation between project team members and colleagues

from the Peanut and INTSORMIL CRSPs. Discussions with Dr. Norge Jerome, Director of the Office of Nutrition, USAID, have suggested the possibility of additional project funding from that source.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

Activities on the project are germane to goals of the Global Plan (see section I.C.).

### B. Balance Between Research and Training

1. Expenditures for research and training

| | Research | Training |
|---|---|---|
| Direct costs | $ 82,569.35 | $21,500.04 |
| Indirect costs | 17,419.50 | 4,586.94 |
| Total | $ 99,988.85 | $26,086.98 |

2. Expenditures related to research and training plans. The project is continuing to maintain a fair balance between research and training components. There was a slight decrease in the training component between FY 89 and FY 90. At least 20 percent of the HC's FY 90 budget was spent on various forms of assistantships, with additional funds used to provide supplies and specialized equipment for student research and other forms of training. Student training has become an institutionalized and integral part of the research programs. At the U.S. institution, direct costs for M.S. student assistantships in FY 90 came to $6,434.47. In addition, although accounted as a personnel cost, the salary plus fringe benefits for a postdoctoral associate supported by the CRSP amounted to $12,251.68 (direct cost).

### C. Balance of Domestic vs. Overseas Activities

Both teams have continued, as in the past, to coordinate and harmonize activities in such a way as to take maximum advantage of the complementary strengths and resources of the cooperating institutions. Product development activities, impact assessment involving econo-socio-cultural and nutritional surveys and acceptance-testing of cowpea-based products are carried out in the HC. Questions of a more basic nature are addressed at the U.S. institution. HC Ph.D. students in search of fundamental answers to intricate questions which cannot be satisfactorily addressed with the HC's modest facilities spend part of their time working with the advanced facilities of the U.S. institution under the guidance of the U.S. team. This arrangement has been mutually supportive of the work program of the project.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Research planning for FY 89 was initiated at the September 1988 coordination meeting held at Nsukka. Co-investigators McWatters and Phillips visited Nigeria and met with the HC team of researchers

including students and project staff to review progress during FY 88 and the overall goals of the project-extension proposal for FY 89-92. Following these reviews, two other meetings were held between McWatters, Phillips, Nnanyelugo (HC PI), and Ngoddy. Broad research goals for FY 89 were developed bearing in mind achievements and gaps identified in 1988 and the projected goals of the 1989/92 extension proposal. Once these guidelines were agreed upon, general budget plans were made. Following this meeting, each group developed detailed workplans and budgets which were then combined and transmitted to the MO.

Personnel training is integral to the research program with students engaged in various aspects of the work. Thus, decisions regarding training and its cost were made during the planning meeting in September. Details of training and associated costs were developed by each group as part of the final workplan and budget.

Publications/presentations are initiated by individual scientists on either side. In cases of joint publications between U.S. and HC scientists, coauthored drafts are reviewed within each institution prior to submission to the desired publication media.

E. Relative Contributions of Collaborating Institutions and Individuals

U.S. and HC Contributions to FY 90 Budget: Cost sharing contribution by the University of Georgia was 37 percent of the USAID contribution to the U.S. institution. This amounted to a cash contribution of $30,485.79 ($22,878.53 direct plus $7,607.26 indirect). The University of Nigeria contributed staff time of HC PI and other co-investigators on the project. All facilities of the departments of Food Science and Technology and Home Science and Nutrition and the services of the technical staff of both departments were also made available to the project at no overhead cost to the CRSP. These contributions are in-kind.

Other Funding Over and Above That by A.I.D. and Participating U.S. and HC Institutions: Other contributions by the HC institution included administrative support both within the project departments and at the University Bursary which handles project accounts and assists with facilitating staff travels. Facilities of other departments of the University of Nigeria such as the Mechanical Engineering Workshops and the Biochemistry Laboratories were available to the project at no overhead cost. At the University of Georgia, CRSP activities are well integrated into state and USDA-supported programs. University rather than USAID funds often support CRSP-related publications, presentations, and activities at meetings. UGA faculty not formally associated with the project contribute technical expertise and valuable ideas.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

There is no USAID mission in Nigeria. However, Ms. Keys McManus is the USAID liaison at the U.S. Embassy in Lagos and has consistently showed a positive disposition to be of assistance to the CRSP.

G. Evidence of Institutionalization

1. Faculty recognition. From the HC institutional point of view, there is growing evidence to suggest that the University of Nigeria, as a result of her CRSP linkage with the University of Georgia, has become a focal point in Africa for cowpea-related research. There is a growing demand for scientific/technical scholarly publications from HC scientists from around the globe. Over the last three years, such requests have averaged over 300 per year. Staff members of the HC institution are increasingly called upon to review articles for international journals on cowpea-related topics. In 1989, not less than 15 such requests were received. Applications by aspiring postgraduate students especially from African countries who wish to work on legume-related research at the University of Nigeria are on the increase. In 1989, both departments could only admit 5 percent of qualified applicants. In 1989, the HC institution received and obliged a request from the Netherlands to host an M.S. student from the Department of Food Science, Agricultural University, Wageningen for two months of research on cowpea-based weaning foods. The student successfully completed the program and has returned to the Netherlands.

   From the U.S. institutional point of view, team members have frequently been called upon to consult with private and governmental agencies on development issues and provide technical advice to industry. Publications from CRSP research are requested from international researchers, and team members are regularly invited to organize and participate in national and international symposia. As a result of CRSP participation, the University of Georgia is now recognized as the principal U.S. site of cowpea utilization research. Requests to host visiting scientists and students, many sponsored by Fulbright or national government programs, are numerous and usually accommodated. Within the University of Georgia College of Agriculture, the Department of Food Science and Technology at the Griffin location enjoys a reputation of being the preeminent site for international research.

2. Integration of domestic and international commodity research programs. Various linkages have been forged with external organizations including IITA, Africare/Imo State Child Survival Project, private companies, and other academic institutions in Ghana, the Netherlands and elsewhere. As a result, the HC institution has considerably expanded its capacity to integrate domestic with international commodity research programs in a manner of which CRSP projects provide a veritable lesson in "learning by doing." As discussed above, CRSP activities are well integrated into overall research at the U.S. institution. Although no formal commodity research program in Georgia is directed toward cowpeas, they are an important crop in the state. The U.S. team has had extensive contact with a major processor of frozen cowpeas in Georgia and has evaluated use of a byproduct of its operation as the basis for industrial akara making. Evaluations with dry Green Acre cowpeas provided by the company showed that satisfactory akara could be made from this cultivar. Discussions with California processors of cowpea-based foods are generating interest in akara as a possible retail or institutional food.

3. Internal project management. The rigorous accounting and reporting system demanded by the CRSP has generated and fostered the establishment of an internal project management discipline which can only thrive at the HC institution if it is able to mobilize the required institutional management support. Over the nine years of the project, the HC has learned to bring to bear on the activities of its work program a growing degree of internal management discipline and complementary institutional support from the bursary. This has enabled the HC team to respond determinedly to the obligations of achieving both targeted research focus and accountability. These are essential cornerstones on which to build a lasting institutional research culture in the HC institution. The Bean/Cowpea CRSP project was one of the first of its kind at the University of Georgia and has provided valuable experience at all levels in administering international research projects and their budgets.

4. Student/Professor interactions. In view of extensive and growing staff and student involvement in cowpea research and training activities at the HC institution, the CRSP program of work must be seen as having served an important catalytic role in the institutionalization of the essential elements of research culture. The fundamental framework of CRSP activities engender student/professor interaction because student training is made an integral part of the research program. Staff and students work hand-in-hand in pursuit of CRSP goals. The demonstration effect of the substantial achievement in research and training outputs attained by this method provides a beach-head from which to chart a lasting tradition of collaborative, mission-oriented and targeted research thrusts at the HC institution. At the U.S. institution, the CRSP has supported a number of students allowing an overall increase in the training effort. Student/professor interactions have been mutually beneficial and resulted in well qualified graduates being placed in a variety of industry and academic positions.

H. Other Comments

Two students at the U.S. institution received special recognition during FY 90. Ms. Shona Holt, M.S. candidate, received several awards for papers she presented at annual meetings of professional scientific societies. These were the Student Scientist Award from the Food Science and Human Nutrition Section of the Southern Association of Agricultural Scientists, the Rita Waters Scholarship ($500) from the Georgia Nutrition Council, and the Rosemary Pangborn Graduate Paper Award ($300) from the Sensory Evaluation Division of the Institute of Food Technologists. Ms. Yu-Hui Tuan, M.S. student, received a travel scholarship from the American Association of Cereal Chemists to attend the annual AACC meeting and present a paper.

## IV. PUBLICATIONS AND PRESENTATIONS

Abbey, B. W., R. D. Phillips and K. H. McWatters. 1989. Preparation and Uses of Legumes and Oilseeds in Africa. In E. W. Lusas, D. Erickson and W.-K. Nip (eds.), Food Uses of Whole Oil and Protein Seeds. Champaign, IL: American Oil Chemists' Society, Chapter 18, 281-304.

Bulgarelli, M. A. and L. R. Beuchat. 1990. Influence of Indigenous Microflora on Some Chemical Properties of Cowpea Paste. Journal of Food Science 55:141-145.

Bulgarelli, M. A. and L. R. Beuchat. 1990. Functional Properties of Cowpea Paste as Influenced by Indigenous Microflora. Journal of Food Science 55:146-150.

Ene-Obong, H. N. 1989. Coping with Hard Times with Cowpea. Paper presented to Christ Church Women's Association, University of Nigeria, Nsukka, Nigeria, June 13.

Fletcher, S. M., K. H. McWatters and A.V.A Resurreccion. 1989. Analysis of Consumer's Willingness to Pay for New Fried Food Prepared from Cowpea Flour. Paper presented at Annual Meeting of the American Agricultural Economics Association, Baton Rouge, LA, July 30-August 2 (Abstract, American Journal of Agricultural Economics 71(5):1348).

Holt, Shona D. 1990. Modeling and Optimization of Products Utilizing Composite Flour Blends by Mixture Response Surface Methodology. M. S. Thesis, University of Georgia, Athens.

Holt, S., A.V.A. Resurreccion and K. H. McWatters. 1990. Formulation and Evaluation of Muffins Containing Wheat, Cowpea, Peanut, Sorghum and Cassava Flours. Paper presented at 87th Annual Meeting, Southern Association of Agricultural Scientists, Little Rock, AR, February 4-7 (Abstract 3, Food Science and Human Nutrition Section Abstracts 27:2).

Holt, S. D., A.V.A. Resurreccion and K. H. McWatters. 1990. A Response Surface Approach to Optimize Quality of Muffins Containing Peanut and Other Nonwheat Flours. Paper presented at 22nd Annual MeetingAmerican Peanut Research and Education Society, Stone Mountain, GA, July 10-12.

Holt, S. D., A.V.A. Resurreccion and K. H. McWatters. 1990. Optimization of Muffin Formulations Utilizing Wheat, Cowpea, Peanut, Sorghum and Cassava Flours Using Mixture Response Surface Methodology. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 682, p. 234).

Hung, Y.-C. and K. H. McWatters. 1990. Effect of Holding Time on the Functionality of Cowpea Paste and Quality of Akara. Journal of Food Science 55:558-559.

Hung, Y.-C. and K. H. McWatters. 1990. Whippability and Foam Stability of Cowpea Paste. In W.E.L. Spies and H. Schubert (eds.). Engineering and Food, Volume 1, Physical Properties and Process Control. New York: Elsevier Science Publishing Co., 257-263. Proceedings of the Fifth International Congress on Engineering and Food, Cologne, Federal Republicrmany, May 28-June 3, 1989.

Hung, Y.-C., K. H. McWatters, R. D. Phillips and M. S. Chinnan. 1990. Effects of Pre-decortication Drying Treatment on the Microstructure of Cowpea Products. Journal of Food Science 55:774-776, 807.

Hung, Y.-C., R. D. Phillips and K. H. McWatters. 1990. Effect of Prolonged Storage on Development of the Hard-to-Cook Condition in Cowpeas. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 642, p. 226).

Ikeme, P. O. and D. O. Nnanyelugo. 1988. Compositional Studies on "Akidi Ani" (*Vigna unguiculata*) and Its Contribution to the Diets of People in Anambra State. Paper presented at Annual Conference of Nutrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 19).

Ikeme, P. O. and D. O. Nnanyelugo. 1988. Usage Patterns of Bambara Groundnut (*Voandzeia subterranea*) and Akidi Ani (*Vigna unguiculata*) in Anambra State. Paper presented at Annual Conference of Nutrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 42).

Mbah, B. N. and D. O. Nnanyelugo (eds.). 1990. Food Crops Production, Utilization and Nutrition. Ibadan, Nigeria: Dotham Publishers Ltd. Proceedings of a Course Held at the University of Nigeria, Nsukka, April 10-23, 1988. 161 pages.

McWatters, K. H. 1990. Functional Characteristics of Cowpea Flours in Foods. Journal of the American Oil Chemists' Society 67:272-275.

McWatters, K. H. and A. L. Branch. 1989. Effect of Storage on Functional Properties of Cowpea Meal and Sensory Quality of Akara, a Meal-based Food. In A. H. Ghee, T. C. Yam and C. Tan (eds.). Trends in Food Product Development. Singapore: Singapore Institute of Food Science and Technology, 220-223. Proceedings of 7th World Congress of Food Science and Technology, Singapore,8-October 2, 1987.

McWatters, K. H., N. J. Enwere and S. M. Fletcher. 1990. Consumer Response to Akara (Fried Cowpea Paste) with or without Various Sauce Flavors. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 275, p. 155).

Ndubuaku, V. O., A. C. Uwaegbute and D. O. Nnanyelugo. 1988. Flatulence Factors and Other Discomforts Associated with Consumption of Cowpeas (Vigna unguiculata). Paper prd at Annual Conference of Nutrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 22).

Ndubuaku, V. O., A. C. Uwaegbute and D. O. Nnanyelugo. 1989. Flatulence and Other Discomforts Associated with Consumption of Cowpea (*Vigna unguiculata*). Appetite 13:171-181.

Ngoddy, P. O. 1989. Cereal and Legume Processing and Storage as Illustrated with Maize and Cowpea. In B. N. Mbah and D. O. Nnanyelugo (eds.). Food Crops Pron, Utilization and Nutrition. Ibadan, Nigeria: Dotam Publications Ltd., 58-62. Proceedings of a Course Held at the University of Nigeria, Nsukka, April 10-23, 1988.

Nnanna, I. A. and R. D. Phillips. 1990. Protein and Starch Digestibility and Flatulence Potential of Germinated Cowpeas (*Vigna unguiculata*). Journal of Food Science 55:151-153, 183.

Nnanna, I. A., R. D. Phillips, K. H. McWatters and Y.-C. Hung. 1990. Effect of Germination on the Physical, Chemical, and Sensory Characteristics of Cowpea Products: Flour, Paste, and Akara. Journal of Agricultural and Food Chemistry 38:812-816.

Nnanyelugo, D. O., V. Ndubuaku and A. C. Uwaegbute. 1989. Metabolic Effects of Processed Cowpea (*Vigna unguiculata*) in Adolescent Boys and Girls. Proceedings of the 14th International Congress of Nutrition, Seoul, Korea, August 30-September 4 (Abstract F30-3, p. 441).

Nnanyelugo, D. O., E. K. Ngwu, Asinobi, H.C. Ezumah and F. I. Nweke. 1989. Biological Processes of "Wasting" and "Stunting" and Subsequent "Catch Up" Growth in Predominantly Cassava Producing Areas and Their Relationship to Farming Systems. Paper presented at 14th International Congress of Nutrition, Seoul, Korea, August 30-September 4 (Abstract P2-144).

Nnanyelugo, D. O., A. C. Uwaegbute and V. Ndubuaku. 1989. Abdominal Discomforts Following the Consumption of Cowpeas (*Vigna unguiculata*) Proceedings of the 14th International Congress of Nutrition, Seoul, Korea, August 30-September 4 (Abstract P4-132, p. 798).

Nnanyelugo, D. O., A. C. Uwaegbute, V. O. Ndubuaku and P. O. Ngoddy. 1989. Cowpea Utilization in Developing Countries. Workshop No. W23, organized at the 14th International Congress of Nutrition, Seoul, Korea, September 2.

Nnanyelugo, D. O., A. C. Uwaegbute and E. K. Ngwu. 1989. Nutrient Requirements and Nutritional Status in Nigeria and Policy Considerations. Invited paper presented at National Workshop on Nutrition Policy for Nigeria, National Institute for Policy and Strategic Studies, Kuru, Jos, Nigeria, January 16-19 (pp. 1-58).

Nnanyelugo, D. O., A. C. Uwaegbute and V. O. Ndubuaku. 1989. Medical and Health Aspects of Cowpeas (*Vigna unguiculata*). In A. H. Ghee, K. W. Lu and K. A. Kim (eds.). Trends in Nutrition and Food Policy. Singapore: Singapore Institute of Food Science and Technology, 27-34. Proceedings of 7th World Congress of Food Science and Technology, Singapore, September 28-October 2, 1987.

Nwagbara, C. A., D. O. Nnanyelugo, E. K. Ngwu and C. O. Asinobi. 1990. Impact of Intercropping and Utilization of Cowpea and Soybean in Predominantly Cassava Producing Areas of Bendel State. Paper presented at Cassava Forum of Resource and Crop Management Program, International Institute of Tropical Agriculture, Ibadan, a, June 11.

Obizoba, I. C. 1990. Nutritive Quality of Blends of Corn with Germinated Cowpeas (*Vigna unguiculata*), Pigeon Pea (*Cajanus cajan*), and Bambarra groundnut (*Voandzeia subterranea*). Cereal Chemistry 67:230-232.

Okechukwu, P. E., P. O. Ngoddy, K. H. McWatters and M. A. Rao. 1990. Thermal Processing and Texture of Moin-Moin. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 632, p. 224).

Okeke, E. C. and D. O. Nnanyelugo. 1988. Energy and Nutrient Intake Adequacy of Rural Population in Anambra State—Implications for the Formulation of Development Policies for Nigeria. Paper presented at Annual Conference of Nutrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 53).

Okeke, E. C. and D. O. Nnanyelugo. 1988. Food Habits and Consumption Pattern of Low Income Households in Rural Nigerian Communities. Paper presented at Annual Conference of Nutrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 46).

Okeke, E. C. and D. O. Nnanyelugo. 1989. Intrafamilial Distribution of Food and Nutrients in a Rural Nigerian Population. Ecology of Food and Nutrition 23:109-123.

Okeke, E. C. and D. O. Nnanyelugo. 1989. Nutritional Status of Farm Families in the Derived Savannah of Anambra State, Nigeria. Proceedings of the 14th International Congreson, Seoul, Korea, August 30-September 4 (Abstract P1-106, p. 522).

Okeke, E. C. and U. A. Okafor. 1989. Current Breast-feeding and Weaning Practices in Anambra State. Nigerian Journal of Nutritional Sciences 10(1):21-30.

Okeke, E. C., F. I. Nweke and D. O. Nnanyelugo. 1988. The Consequences of Absence of Adult Males on the Nutritional Status of Members of Rural Farm Households and Implications for Rural Development Programmes: A Case Study. Journal of Institute of African Studies 3:69-75.

Onah, B. N. 1989. Cowpea Utilization in Isi-Uzo Local Government Area of Anambra State. Paper presented at Annual Conference of Nutrition Society of Nigeria, Lagos, Nigeria, October 16-18 (Abstract, p. 6).

Onuoha, E. M., E. C. Okoli and I. C. Obizoba. 1988. Mineral Balance and Liver Composition Studies of Rats Fed Cowpea (*Vigna unguiculata*) Supplemented with Rice. Paper presented at Annual Conferencetrition Society of Nigeria, Ibadan, Nigeria, November 1-4 (Abstract, p. 34).

Onuorah, V. I., A. C. Uwaegbute and N. J. Enwere. 1989. Cowpea Utilization and Nutrition (Practical). In B. N. Mbah and D. O. Nnanyelugo (eds.). Food Crops Production, Utilization and Nutrition. Ibadan, Nigeria: Dotam Publications Ltd, 121-126. Proceedings of a Course Held at the University of Nigeria, Nsukka, April 10-23, 1988.

Onwuka, N. D. 1989. Improved Cowpea Processing and Storage. Paper presented at Workshop on Cowpea Utilization, Processing and Storage, University of Nigeria, Nsukka, July.

Onwuka, N. D. 1989. Practical Cowpea Storage. In B. N. Mbah and D. O. Nnanyelugo (eds.). Food Crops Production, Utilization and Nutrition. Ibadan, Nigeria: Dotam Publications Ltd., 63-67. Proceedings of a Course Held at the University of Nigeria, Nsukka, April 10-23, 1988.

Phillips, R. D. and K. H. McWatters. 1990. Contribution of Cowpeas (*Vigna unguiculata*) to Nutrition and Health. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 321, p. 164).

Phillips, R. D., C. R. Santerre and Y.-C. Hung. 1990. Physical-Textural Characteristics of a Wheat-Free, Bread-Like Product Made by Extrusion Cooking. Paper presented at 75th Annual Meeting, American Association of Cereal Chemists, Dallas, TX, October 14-18 (Abstract 157, Cereal Foods World 35(8):833).

Shewfelt, R. L., P. C. Ezell, P. A. Mitchem, M. E. Erickson and K. H. McWatters. 1990. Effect of Phospholipid Hydrolysis on Peroxidation of Cowpea Seed Microsomes. Paper presented at 87th Annual Meeting, Southern Association of Agricultural Scientists, Little Rock, AR, February 4-7 (Abstract 22, Food Science and Human Nutrition Section Abstracts 27:8).

Tuan, Yu-Hui. 1989. Effect of the Hard-to-Cook Defessing on the Nutritional Quality of Cowpea. M. S. Thesis, University of Georgia, Athens.

Tuan, Y.-H. and R. D. Phillips. 1990. Effect of Hard-to-Cook Defect and Processing on the Nutritional Quality of Cowpea. Paper presented at 1990 Annual Meeting, Institute of Food Technologists, Anaheim, CA, June 16-20 (Abstract 97, p. 122).

Uwaegbute, A. C. 1988. Cowpeas for Infant Feeding, Problems and Solutions. Paper presented at Cowpea Workshop, Univeof Nigeria, Nsukka, Nigeria, April 24.

Uwaegbute, A. C. 1989. Chemical and Organoleptic Evaluation of Germinated Cowpea Flours. Paper presented at Annual Conference of Nutrition Society of Nigeria, Lagos, Nigeria, October 16-18 (Abstract, p. 8).

Uwaegbute, A. C. and D. O. Nnanyelugo. 1989. Towards Improving the Nutritional Value of Nigerian Weaning Foods. Invited paper presented at National Workshop on Weaning Foods, Federal Institute of Industrial ResearRRO), Lagos, Nigeria, August 24. In Proceedings of Weaning Foods in Nigeria, pp. 63-85.

Uwaegbute, A. C. and D. O. Nnanyelugo. 1989. Cowpea Utilization and Nutrition. In B. N. Mbah and D. O. Nnanyelugo (eds.), Food Crops Production, Utilization and Nutrition. Ibadan, Nigeria: Dotam Publications Ltd., 111-120. Proceedings of a Course Held at the University of Nigeria, Nsukka, April 10-23, 1988.

Uwaegbute, A. C. and D. O. Nnanyelugo. 1989. Usage Patterns of Cowpeas (*Vigna unguiculata*) for Infant Feeding in Nigeria. In A. H. Ghee, K. W. Lu and K. A. Kim (eds.). Trends in Nutrition and Food Policy. Singapore: Singapore Institute of Food Science and Technology, 201-205. Proceedings of 7th World Congress of Food Science and Technology, Singapore, September 28, - October 2, 1987.

Table 1. Approximate Costs and Returns for the Different Products at Isiala-Ngwa Mill, 1988 - July 1990 in Naira (N)

| Product | 1988 | | | | | 1989 | | | | | 1990 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TVC | TFC | TR | GM | Profit | TVC | TFC | TR | GM | Profit | TVC | TFC | TR | GM | Profit |
| Supplement (100% Cowpea) | 14605 | 3969 | 12495 | (2110) | (6079) | 7400 | 2250 | 8602 | 1202 | (1048) | 4390 | 2983 | 4568 | 178 | (2805) |
| Weaning Food* (33% Cowpea, 67% Maize) | 501 | 300 | 936 | 435 | 135 | 80 | 37 | 181 | 101 | 64 | - | - | - | - | - |
| Corn Meal | 16308 | 9534 | 10985 | (5323) | (14857) | 17907 | 11921 | 14862 | (3045) | (14966) | 5030 | 10036 | 6612 | 1582 | (8454) |
| Bulk Maize Flour** | - | - | - | - | - | 9821 | 7427 | 9765 | (55) | (7483) | 621 | 1420 | 630 | 9 | (1411) |
| Soybean Flour*** | - | - | - | - | - | - | - | - | - | - | 1428 | 2208 | 2212 | 784 | (1424) |

*Currently produced only on request
**Not produced in 1988
***Produced only in 1990

TVC = Total variable cost
TFC = Total fixed cost
TR = Total revenue
GM = Gross margin = (TR - TVC)
Profit = TR - (TVC + TFC)

Table 2. Break-Even Quantities for Different Outputs at the Relevant Selling Prices, 1988-1990, and Sales Figures in kg.

| Product | 1988 | | 1989 | | 1990 | |
|---|---|---|---|---|---|---|
| | B-E QTY* | SALES | B-E QTY* | SALES | B-E QTY* | SALES |
| Supplement (100% Cowpea) | ** | 1688 | 1731 | 955 | 7458 | 508 |
| Weaning Food | 88 | 126 | 9 | 24 | - | - |
| Corn Meal | ** | 3923 | ** | 5308 | 11670 | 1837 |
| Bulk Maize Flour | - | - | ** | 3255 | 35500 | 210 |
| Soybean Flour | - | - | - | - | 561 | 177 |

*B-E Qty = Break-Even Quantity (i.e. amount at the given costs and selling price required to break-even).

**Not achievable because products are sold at prices below production costs.

# SENEGAL - UNIVERSITY OF CALIFORNIA-RIVERSIDE

*A PROGRAM TO DEVELOP IMPROVED COWPEA CULTIVARS, MANAGEMENT METHODS AND STORAGE PRACTICES FOR SEMIARID ZONES*


Principal Investigator:

| | | |
|---|---|---|
| Anthony E. Hall (U.S.) | Botany and Plant Sciences | Univ. of California-Riverside |
| Limamoulaye Cisse (HC) | Crop Production | ISRA |

Co-Investigators:

| | | |
|---|---|---|
| Muborak Abdalla | Botany and Plant Sciences | Univ. of California-Riverside |
| Faisal Ahmed | Botany and Plant Sciences | Univ. of California-Riverside |
| Ndiaga Cisse | Crop Production | ISRA |
| Madiagne Diagne | Farming Systems | ISRA |
| Alan N. Eckard | Botany and Plant Sciences | Univ. of California-Riverside |
| Seynabou Gereye Tall | Crop Production | ISRA |
| C. Owen Gwathmey | Botany and Plant Scinces | Univ. of California-Riverside |
| Mbaye Ndiaye | Crop Production | ISRA |
| Prabodh N. Patel | Botany and Plant Sciences | Univ. of California-Riverside |
| Dogo Seck | Crop Production | ISRA |
| Moctar Wade | Crop Production | ISRA |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

The ISRA team has developed an advanced line (#275) in which they have incorporated resistance to cowpea weevil (at the seed level), bacterial blight, and mosaic viruses. This line has now been subjected to four years of multilocation testing on research stations, and two years of multilocation on-farm testing. The advanced line has equivalent or higher grain yields than the best available varieties over the full range of environmental conditions where cowpea are grown in central and northern Senegal. Tests by the Food Technology Institute (ITA) in Dakar indicate that this line has grain quality that is equivalent to some local varieties (e.g., 58-57), but not as good as some less widely used varieties (e.g., Bambey 21 and CB5). Evaluations conducted in two on-farm nurseries in 1990 designed to screen for resistance to the parasitic weed, *Striga gesnerioides*, indicate that #275 may have strong resistance to *Striga*, confirming results obtained in 1989. Storage experiments demonstrated that K-Othrine PP2 at 50g/100kg grain can effectively store cowpea grain for more than six months, whereas Actellic and Actellic Super were only effective for four months, and that sealed drums can be effective for more than six months. Scientific papers were published showing that measurements of carbon isotope composition may be useful in breeding cowpeas for adaptation to drought (Hall et al., 1990), classifying cowpea accessions for their

tolerance to heat (Patel and Hall, 1990), showing that yield potential may be increased by selecting for higher harvest index (Kwapata and Hall, 1990b), and describing and comparing models for breeding for tolerance to heat and adaptation to drought (Hall 1990).

Evidence of the extent of use of new technology is being obtained by surveys conducted by Dr. Seynabou Tall, who joined the project in 1990. She has made studies in seven villages where on-farm "Mini-Kit" trials have been or are being conducted. She interviewed 15 people in each village, including: five men and five women from "Mini-Kit" families, and five cultivators of cowpea who had not been directly involved in the "Mini-Kit" experiments. A complete report is not yet available, but some preliminary information is presented here. A majority of the Mini-Kit farmers (55 percent) and the non-Mini-Kit farmers (67 percent) have used chemical products to aid the storage of cowpeas, but most of them use Actellic and only a few have started to use K-Othrine (Note: the project has not conducted extensive Mini-Kit tests with K-Othrine). The reasons for the use of Actellic are that it has been commercially available for many years, and has been distributed to farmers by the extension agency (probably for use with storing seed of other crops, such as millet and peanut, where it is more effective than with cowpea). Most farmers wives cannot read, but they listen to agricultural programs on the radio which are presented in a local language. This indicates that a radio program to advise extension agents, farmers, and farmers wives on the use of K-Othrine and sealed drums in the storage of cowpea could be beneficial and an effective means for disseminating this technology. Dr. Tall is also conducting on-farm tests of the cooking quality of the advanced line #275. As a consequence of two years of on-farm testing, this line is becoming adopted by farmers, and ISRA has requested the responsible agency to officially release it as a new variety in Senegal.

2. Other research-related results

**Germplasm conservation and use.** Under funding from USDA and this project, 600 lines from IITA were propagated in a glasshouse and are now being multiplied in the field at Riverside together with 400 other accessions from the UCR collection. We are cooperating with Dr. R. Hampton, Oregon State University, to characterize the seed-borne viruses present in these materials and in California to minimize the possibility of importing new diseases into California. Drs. R. O. Hampton and P. N. Patel have established that substantial variation is present in cowpea response to different isolates of potyvirus, including representatives of both cowpea aphid-borne mosaic and blackeye cowpea mosaic virus from Senegal and elsewhere. We have developed a set of cowpea genotypes that is useful for differentiating among and diagnosing these potyvirus isolates.

This project now has a collection of 2,500 accessions of cowpea with descriptor information on a computerized data base. We have provided the following seed to cooperators in 1990. We sent 500g each of seed of 328 new accessions to the USDA PI Station in Georgia

to enhance the U.S. collection. We provided the Purdue/Cameroon CRSP project with 200 accessions for screening for pod resistance to bruchids. We provided seeds of 30 accessions to Dr. Nevin Young for his RFLP genome mapping project on cowpea, and the same accessions to Dr. Norman Weeden for complementary studies of isozyme variation. We provided cowpea seed to the CRSP projects in Puerto Rico and the Dominican Republic; a Peace Corps Project in Belize; Bunda College, Malawi; El Obeid Research Station, Sudan; South Africa; a heat shock protein project at Texas Tech University; the Research Institute for Irrigation, Hungary; the International Atomic Energy Agency, Austria; and projects screening for resistance to *Lygus*, root knot nematodes, and *Fusarium solani* at the University of California. The UCR cowpea germplasm collection and data base has become a valuable resource for scientists throughout the world. Unfortunately, our funding support from USDA for cowpea germplasm multiplication and characterization ended on September 30, 1990, and we are continuing this activity at a modest level using our CRSP funds.

**Seed production.** The project is producing breeders seed of all varieties used in Senegal, and the advanced line #275, and assisting the ISRA seed multiplication service to produce one to two tons of disease-free foundation seed per year. P. N. Patel inspected the seed producing fields in Senegal in 1990 and advised the new ISRA plant pathologist, Mbaye Ndiaye, concerning the inspection of fields for diseases and methods for producing disease-free seed.

**Impact of other CRSP-produced or recommended technology.** The farmers involved in the "Mini-Kit" experiments are producing the equivalent of certified seed and through the use of the improved storage techniques are making available high quality seed to farmers. They produced five tons on experimental plot and many more tons on their own fields for the 1990 season. The "Mini-Kit" farmers are using modified plates provided by the project to permit the seeding of cowpeas in rows using their peanut planters. During the 1990 season there were many non-Mini-Kit fields of cowpea in Senegal that had been planted with peanut planters.

**Project impact on production and consumption of cowpeas.** The statistics for cowpea production are described in Section III. They indicate that the production of cowpea as dry grain during the last three years of the project was 30 percent greater than the production during the ten years prior to the project. Most of this increase was due to improved efficiency of production with higher yields/area. These statistics only consider cowpea production of dry grain and a substantial portion of the early cowpeas are consumed on the farm or sold as fresh "southern peas." Discussions with farmers indicate that selling fresh cowpeas can be extremely profitable, and Dr. Tall has initiated studies to obtain information on this aspect of cowpea production and consumption.

**How the research findings specifically address the needs of small-scale farmers and women.** This project is developing varieties of cowpea and management methods that have greater and more stable production, while requiring only minimal inputs that are available

to small-scale farmers. With one exception, the "Mini-Kit" farmers chosen for cooperative research have small farms. In early 1990, the project hired a Senegalese sociologist, Dr. Seynabou Tall, to study women's roles in cowpea production, storage, and utilization. Madame Tall has expertise studying the role of women in development in Senegal, and she has an established commitment to women's rights. We are hoping that she will provide guidance to the project so that the technology being developed and extended will be beneficial to all members of farm families, and indirectly to the urban poor.

3. Changes in national production/consumption of beans/cowpeas in the Host Country.

   See Table at end of this report

B. Institutional Development and Training

1. Changes since FY 89. Plant pathologist, Mbaye Ndiaye, was hired by the project in May 1990 to replace the expatriot scientist, D. G. Gaikwad, who had returned to India. Dr. P. N. Patel worked with Mbaye Ndiaye in Senegal in August and September 1990 to train him in methods of screening plants for resistance to bacterial blight and mosaic viruses. Field entomologist, Amadou Bal, left Senegal in May 1990 to be a professor in Niger. The project does not yet have another entomologist. Khady Diop, who obtained a B.S. in entomology at UCR and returned to Senegal on February 18, 1990, has not yet established a research program due to health problems. Storage entomologist, Dogo Seck, left Senegal in September 1990 to begin a Ph.D. program in Belgium. The seed technologist, Famara Massaly, left Senegal in January 1990 to begin an M.S. program at Mississippi State University. The seed multiplication project is now directed by Artur Da Silva. Sociologist, Dr. Seynabou Tall, was hired by the project in January 1990. Economist, Moustapha Gaye, from the Farming Systems Department of ISRA is providing a part-time contribution to the project working with Dr. Tall. Four technicians were terminated in June 1990 in response to austerity measures taken by the Government of Senegal. At UCR, Mubarak Abdalla transferred from the M.S. to the Ph.D. program. Since July 1, 1990, the project has been providing substantial financial support to three Ph.D. students from the Sudan: Mubarak Abdalla, Faisal Ahmed, and Abdel Ismail. These students had been conducting research for the CRSP project and in recent months the government of the Sudan has been unable to fund their present scholarships or extend them.

2. Over the life of the project. The extent of research on field and storage entomology will be substantially reduced due to the shortage of scientists in Senegal. For storage research this is not a problem because the project is emphasizing the extension of storage technologies developed by the project, in cooperation with the extension agencies. The agronomist, Samba Thiaw, will return to Senegal in January 1990 and will conduct some high priority research on the use of seed dressings to control hairy caterpillar. Screening breeding lines for resistance to cowpea aphid will be

conducted by technicians supervised by the plant breeder or the agronomist. ISRA has promised to hire a replacement field entomologist as soon as this is possible. The cost of conducting the project in Senegal will be increased because the project is paying the salaries of the plant pathologist and the sociologist, and will have to hire more temporary laborers to compensate for the technicians who were terminated due to shortages of Government funds. For FY 91, a majority of the funds for UCR will be spent on the training costs of the three Sudanese students. Support is being sought from UCR and to date the project has obtained a tuition waiver for one quarter for one of the Sudanese students, and elimination of indirect costs for these training expenses. The extreme shortage of funds by the project at UCR will result in a reduction in the research that can be accomplished compared with previous years.

3. Project training to be completed by the end of the project period. Mbaye Ndiaye will undertake a general training course on cowpeas at IITA, Ibadan, in October and November 1990, and gain experience in the virology of cowpea diseases at Oregon State University in 1992 under informal training with Dr. R. O. Hampton. The following students are projected to complete their degrees by the following dates: Samba Thiaw, M.S. Plant Science, December 1990; Owen Gwathmey, Ph.D. Botany, January 1991; Claudia Petrie and Faisal Ahmed, Ph.D.s in Botany, Fall 1991; Abdel Ismail, Ph.D. Botany, Winter 1992; and Mubarak Abdalla, Ph.D. Botany (Plant Genetics), Spring 1992. Ndiaga Cisse is projected to begin a Ph.D. program in Plant Breeding in the U.S. in August 1991 if the five-year extension is granted.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

The program goal, since the project was initiated in August 1981, was to increase grain production and yield stability of cowpea grown on small farms in Senegal and other semiarid regions. For Senegal, this was achieved during the period 1985-1989 with total grain production and yield/area that were 108 percent and 28 percent, respectively, greater than the base-line period of 1970-79. The impact of the project on production in other semiarid regions is unknown and was probably negligible.

The project purpose was to develop improved cowpea varieties, management methods, and storage practices for small farms in Senegal. We anticipated making significant progress on the project purpose by the end of this phase in 1992.

The project has made significant progress. Advanced breeding line #275 has resistance to cowpea weevil, bacterial blight, and mosaic viruses, and probably some useful resistance to Striga and greater yield stability than available varieties. If this line is released by the Government of Senegal in 1991, the project will have achieved more than was planned, originally, because the resistance to Striga is a consequence of serendipity. Unfortunately, the agency responsible for varietal release in Senegal is inexperienced and the release may take more time than is warranted (the line is clearly superior to available

material). Sowing cowpeas in rows at high densities using a modified peanut planter is proving to be effective and is being accepted by farmers. Methods for extending the improved storage techniques, involving K-Othrine treatment or sealed-drum storage, are being developed at this time. Cowpea varietal intercropping was shown to increase the stability of cowpea grain and hay production under dry infertile soil conditions on experiment stations. The next step is to evaluate cowpea varietal intercrops on farm conditions in cooperation with farmers and this will be initiated in 1991.

This project conducts most of the research on cowpea in Senegal, and is the national cowpea research program. SAFGRAD has designated the program as one of the leading centers for cowpea research with responsibility for exporting technology to other countries in Africa. The focus of the program on cowpea sole cropping and varietal intercropping complements the program of IITA which, in recent years, has begun to emphasize the intercropping of cowpea with cereals.

Senegal should plan on increasing cowpea production to 100,000 tons/year by the year 2010 to meet the food needs of a population that is projected to double within 20 years, which will more than double the demand for cowpea due to shortages of other foods. The basis for achieving a four- to five-fold increase in national production within 20 years is to develop cowpea production systems that are two to three times more productive (yield/ha) but require only modest inputs, and double the total area of cowpea under cultivation. This increased production of cowpea would enhance nitrogen fixation and improve the fertility of the soil in the Peanut Basin contributing to the yields of millet and peanut. Continuation of the project, with progress similar to that achieved since 1985, would enable Senegal to meet the production goal of 100,000 tons/year by 2010. The project is contributing to the development of varieties for California which will help this industry to continue as the major world-wide exporter of cowpea. The basic research at UCR on methods for breeding crops with improved heat tolerance and adaptation to drought has the potential to make long-term contributions to increasing the stability of production on a global scale of several crop species in addition to both cowpeas and beans.

D. Evidence of Biological/Social Sciences Integration

Structural problems within the Government of Senegal and funding constraints have impeded the integration of the agricultural sciences research of this project with complementary research on social sciences. The secondment of Economist, Moustapha Gaye, from the Farming Systems Department in 1989 and the hiring of Sociologist and specialist in Women-in-Development, Dr. Seynabou Tall, in 1990 have enabled this project to initiate linkages between agricultural and social sciences research. In addition, the project has collaborated since 1989 with Food Technology Institute (ITA) in Senegal on the evaluation of the food quality of the advanced lines and varieties.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

This project assisted the University of Georgia/Nigeria project to plan a presentation on the food processing of cowpea and "akara" which was made in southern California in June 1990. The organization of cowpea farmers in California appreciated the presentations of Kay McWatters and the Georgia Team because of their potential contribution to increasing the consumption and demand for cowpeas in the U.S. and elsewhere. We also held discussions with the Food Technology Institute (ITA) and USAID Mission in Senegal to facilitate the possible establishment of a University of Georgia/ITA project on cowpea food processing in Senegal. We contributed to the Purdue/Cameroon project by providing seed of 200 cowpea accessions for screening for pod resistance to bruchids. Purdue has provided us with cowpea accessions from Cameroon which we will multiply to enhance the U.S. cowpea germplasm collection. We are planning even closer collaboration with the Purdue and Georgia projects in the future because there are so few cowpea CRSP projects that we must coordinate our efforts.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

The Government of Senegal faced a budget crisis in 1990 which required the termination of many ISRA technicians (more than 300) and decreased the likelihood of their hiring new scientific staff. Fortunately, the cowpea project only lost four technicians. ISRA hired two Senegalese scientists paid by the project to keep the project operational. These are temporary positions, but one of the positions will be considered for a permanent position when and if new Government positions become authorized. The U.S. dollar fell to an all-time low, making less money available in Senegal. The Government of the Sudan defaulted on student support, necessitating the use of UCR project funds to continue the support of three Sudanese students. The Agricultural Experiment Station of the University of California has been subjected to a major budget cut since July 1, 1990, whose magnitude has not yet been determined. The external review of the project in Senegal in 1990 necessitated the expenditure of extra costs for the travel of the Principal Investigator, which had not been included in the budget. The project is presently being conducted in California with instructions to minimize expenditures in all sectors and the project technician will be terminated in a few months if financial conditions do not improve.

### B. Adequacy of Current Management System

Financial reporting by ISRA has been more prompt than in earlier years.

### C. Activity Towards Buy-Ins or Other Funding

The USAID Mission provided ISRA with some funds to support an extension of the cowpea research to the Senegal River and to pay some of the operational expenses of the socio-economic studies conducted during 1990.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

SAFGRAD and A.I.D. have designated Senegal as a technology producing country. This project has helped ISRA to achieve this status for cowpea, and further assistance would help ISRA achieve the goal of exporting cowpea technology to neighboring countries. The more basic research being conducted at UCR is useful to crop production research projects throughout the world.

### B. Balance Between Research and Training

1. Expenditures for research and training. Expenditures on training in FY 90 were $22,890 for Senegal and $14,070 at UCR, and on research (excluding overhead) were $66,441 for Senegal and $65,295 at UCR.

2. Expenditures related to research and training plans. Budgeted expenditures on training in FY 90 were $32,782 for Senegal and $-0- at UCR, and on research (excluding overhead) were $56,375 for Senegal and $77,965 at UCR. The differences between actual and budgeted expenses on training are due to the unexpected return of Khady Diop to Senegal and the need for the project to support the Sudanese students. This enhanced the funds available for research in Senegal and decreased the research funds available at UCR.

### C. Balance of Domestic vs. Overseas Activities

Appropriate, considering the philosophy of the project which is to promote the evolution of independent, but collaborating, programs in Senegal and California with minimal long-term use of expatriates.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

The ISRA team met with A. E. Hall in Senegal in August and September 1989 to finalize the workplan and budget for FY 90. A. E. Hall and Ndiaga Cisse jointly presented the research report for the project at the PI's meeting at MSU in May 1990. The UCR and ISRA teams met at UCR following the May meeting to evaluate the 1989 summer season research and plan the 1990 summer season research. In September 1990, A. E. Hall and P. N. Patel met with the ISRA team in Senegal, helped with the organization of the external review, finalized the workplan and budget for FY 91, and finalized planning for the five-year extension.

### E. Relative Contributions of Collaborating Institutions and Individuals

The A.I.D. contribution to the project in FY 89/90 was $230,500 with direct costs of $93,657 for ISRA and $85,965 for UCR. The ISRA contributions were approximately $22,770 for the salaries of scientists, $44,275 for the salaries of technicians, and $95,450 for the costs at four experimental stations giving a total of $211,243, including indirect costs. The UCR contributions were $26,499 for salaries and $40,000 for the costs at two experimental stations giving a total of $95,051, including indirect costs.

ISRA received approximately $8,000 from the USAID Mission to extend the research into the Senegal River region and to support costs of socio-economic studies. Two U.S. students working on the project at UCR received research assistantships from UCR totalling $22,200. Three Sudanese students working on the project while studying for graduate degrees received a total of approximately $45,000 for the year from the Government of the Sudan.

F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

The USAID Mission has been using this project as a model in its plans to assist ISRA to strengthen the other crop research programs in Senegal.

G. Evidence of Institutionalization

The Dean, College of Agricultural and Natural Sciences at UCR, has actively supported this project.

The project continues to work with the cowpea farmers of California and receive funding from the Blackeye Varietal Council ($18,500 in 1990). The cowpea germplasm obtained by the project has benefitted the cowpea breeding programs for California.

The Management Services Officer and her staff at UCR have continued to provide excellent administrative support for the project, but they find that it takes considerable time, and the project PI has spent far too much time on project administration this year.

The support from professors at UCR in Botany and Plant Sciences, Soil and Environmental Sciences, and Entomology for Samba Thiaw, Mubarak Abdalla, Faisal Ahmed, Abdel Ismail, Claudia Petrie, and Owen Gwathmey has been excellent. The difficulties that developed for Khady Diop during her stay at UCR were due to her personal, psychological problems, and in retrospect were not solvable despite the sincere and sensitive efforts of the PI and several personnel at UCR to provide her with assistance.

H. Other Comments

Previous annual reports have been useful to the project because they enabled us to analyze the scientific progress being made. The format required for this report did not permit much discussion of scientific results, and in relation to the time spent to prepare it is of limited value to the project. I recommend that consideration be given to changing the format of annual reports so that they are more useful to scientists.

## IV. PUBLICATIONS AND PRESENTATIONS

Gwathmey, C. O. 1990. Summary of 1989 Cowpea Stay-Green Research Results. Report to Project Annual Meeting, UCR, May 7-11, 1990, Manuscript, Riverside, CA: University of California.

Hall, A. E. 1990. Breeding for heat tolerance—an approach based on whole-plant physiology. HortScience 25:17-19.

Hall, A. E. 1990. Plant adaptation to hot and dry stresses in relation to horticultural plant breeding. Plenary Lecture, XXIII International Horticultural Congress, Florence, Italy, August 27-September 1, 1990.

Hall, A. E. and Limamoulaye Cisse. 1990. A Program to Develop Improved Cowpea Cultivars, Management Methods, and Storage Practices for Semiarid Zones. PI's Meeting, East Lansing, Michigan, May 1990.

Hall, A. E., R. G. Mutters, K. T. Hubick, and G. D. Farquhar. 1990. Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci. 30:300-305.

Ismail, A. 1990. Correlation between carbon isotope discrimination and water use efficiency in cowpea. Report to Project Annual Meeting, UCR, May 7-11, 1990, Manuscript, Riverside, CA: University of California.

Kwapata, M. B. and A. E. Hall. 1990a. Response of contrasting vegetable-cowpea cultivars to plant density and harvesting of young green pods. I. Pod production. Field Crops Res. 24:1-10.

Kwapata, M. B., A. E. Hall and M. A. Madore. 1990. Response of contrasting vegetable-cowpea cultivars to plant density and harvesting of young green pods. II. Dry-matter production and photosynthesis. Field Crops Res. 24:11-21.

Kwapata, M. B. and A. E. Hall. 1990b. Determinants of cowpea (*Vigna unguiculata*) seed yield at extremely high plant density. Field Crops Res. 24:23-32.

Patel, P. N. and A. E. Hall. 1990. Genotypic variation and classification of cowpea for reproductive responses to high temperature under long photoperiods. Crop Sci. 30:614-621.

Petrie, C. 1990. Research Report—Comparison of Water Relations of Cowpea and Millet under Drought. Report to Project Annual Meeting, UCR, May 7-11, 1990, Manuscript, Riverside, CA: University of California.

Bal Amadou, 1990. Criblage du niebe contre less thrips a Bambey et Nioro en 1989. CNRA, Bambey, Senegal. Institut Senegalais de Recherches Agricoles.

Cisse Ndiaga. 1990. Cowpea breeding research in Senegal for the ISRA/UCR CRSP project. Presentation at the PI's Meeting, East Lansing, Michigan, May 1990.

Cisse Ndiaga, Waly Ndiaye, Assane Sene and Mbaye Diagne. 1990. Amelioration du niebe—Project CRSP-niebe, rapport annuel 1989. CNRA, Bambey, Senegal. Institut Senegalais de Recherches Agricoles.

Diagne Madiagne. 1990. Report. CNRA Bambey, Senegal. Institut Senegalaise de Recherches Agricoles.

Diop Abou. 1990. Minimum de traitements insecticide sur le niebe a Bambey at Louga en 1989. CNRA, Bambey, Senegal. Institut Senegalaise de Recherches Agricoles.

Faye Ndeye, Ngor Diagne and Ousseynou Cisse. 1990. Pathologie due niebe, rapport annuel 1989. CNRA, Bambey, Senegal. Institut Senegalaise de Recherches Agricoles.

Seck Dogo. 1990. Programme de recherches sur la protection des stocks de niebe. Compagne 88/89. Station de Nioro du Rip. Institut Senegalaise de Recherches Agricoles.

Thiaw Samba, Adiouma Niane and Ibou Sane. 1990. Phytotechnie du niebe-project CRSP niebe, rapport annuel--1989. CNRA, Bambey, Senegal. Institut Senegalais de Recherches Agricoles.

Table. Changes in Cowpea Production in Senegal (Source: Ministry of Rural Development, Senegal)

| Year | Hectares Planted | | Yield/Hectare | | Total Production | |
|---|---|---|---|---|---|---|
| | Louga* | Total | Louga | Total | Louga | Total |
| | —— hectares —— | | — kg/ha — | | ——— tons ——— | |
| 1970-79 | 25,203 | 63,300 | 295 | 293 | 7,746 | 18,568 |
| 1980 | 28,516 | 54,247 | 370 | 315 | 10,539 | 17,080 |
| 1981 | 30,218 | 68,484 | 401 | 420 | 12,110 | 28,777 |
| 1982 | 28,627 | 47,930 | 233 | 276 | 6,671 | 13,245 |
| 1983 | 12,658 | 40,000 | 371 | 325 | 4,696 | 13,000 |
| 1984 | 8,400 | 53,000 | -0- | 302 | -0- | 16,000 |
| 1985 | 63,557 | 121,000 | 434 | 545 | 27,588 | 66,000 |
| 1986 | 53,733 | 117,607 | 451 | 466 | 24,001 | 54,863 |
| 1987 | 35,114 | 71,480 | 325 | 400 | 11,412 | 28,625 |
| 1988 | 29,067 | 69,121 | 235 | 251 | 6,820 | 17,320 |
| 1989 | 23,123 | 64,809 | 255 | 407 | 5,903 | 26,350 |

*Louga and Saint-Louis regions

# TANZANIA - WASHINGTON STATE UNIVERSITY

*BREEDING BEANS (PHASEOLUS VULGARIS L.) FOR DISEASE, INSECT AND STRESS RESISTANCE AND DETERMINATION OF SOCIOECONOMIC IMPACT ON SMALLHOLDER FARM FAMILIES*


Principal Investigators:

| | | |
|---|---|---|
| M. J. Silbernagel (U.S.) | Dept. of Plant Pathology/USDA | Washington State University |
| James M. Teri (HC) | Department of Crop Science | Sokoine Univ. of Agriculture |

Co-Investigators:

| | | |
|---|---|---|
| L. Butler | Department of Rural Sociology | Washington State University |
| P. R. Dimoso | Department of Crop Science | Sokoine Univ. of Agriculture |
| A. Femi Lana | Department of Crop Science | Sokoine Univ. of Agriculture |
| E. E. Maeda | Food Science and Technology | Sokoine Univ. of Agriculture |
| F. T. B. Magayane | Ag. Education and Extension | Sokoine Univ. of Agriculture |
| A. N. Minjas | Department of Crop Science | Sokoine Univ. of Agriculture |
| G. I. Mink | Department of Plant Pathology | Washington State University |
| R. N. Misangu | Department of Crop Science | Sokoine Univ. of Agriculture |
| S. Nchimbi | Department of Crop Science | Sokoine Univ. of Agriculture |
| J. J. B. Rugambisa | Department of Rural Economy | Sokoine Univ. of Agriculture |
| C. L. Rweyemamu | Department of Crop Science | Sokoine Univ. of Agriculture |
| K. P. Sibuga | Department of Crop Science | Sokoine Univ. of Agriculture |
| B. G. Swanson | Food Science & Human Nutrition | Washington State University |


• • • • • • •

## I. PROGRESS DURING FY 90

### A. Specific Research Contributions

#### 1. Research results

**Use of BCMV monoclonal antisera by U.S. seed industry and breeding programs.** BCMV diagnosis and serotype identification of suspect vegetative tissues and seed lots has become routine in the Washington State Crop Improvement Association and Washington State Department of Agriculture Phytosanitary Certification Programs, and is becoming more accepted by Idaho regulatory agencies as well.

Annually hundreds of specimens of diseased and/or suspect bean plant and seed materials are sent to the Prosser serodiagnosis lab by public and private breeders and seed companies from all over the U.S. and abroad.

Monoclonal antibodies (MCABs) against the standard (serotype B) and necrotic (serotype A) strains of BCMV are routinely sent to collaborators who want to set their own virus testing programs.

Prof. Mink is helping coordinate an international effort to standardize the identification and detection of potyviruses in general and BCMV in particular. The Prosser developed MCABs, the

bean host differentials and our large collection of well characterized strains of BCMV are crucial to this effort. The identification of the NL-8 strain of BCMV (1989 and 1990) in the nations largest certified bean seed producing state (Idaho) would not have been possible without the Prosser MCABs and bean host differentials (see list of publications).

The BCMV differential hosts (564 seed samples) for biological strain identification and inoculum of known strains (83 virus samples) of all seven BCMV pathotypes were sent to 26 public or private collaborators in FY 90. In addition, six fungal cultures were also provided.

**Other U.S. research in progress.** Continued disease screening for BCMV and halo blight (HB) of crosses between drought tolerant TM0101 X advanced disease resistant lines from the SUA and Prosser programs. Sent 95 populations of the above materials to SUA for local selection and screening. Continued work on characterization of adzuki strain of BCMV. Continued (second year) testing (10 cvs) of Beangro computerized growth model developed at Univeristy of Florida (Hooganboom). Grew out the National Dry Bean Cooperative Nursery (coordinated by University of Idaho Dr. Myers) of 32 cvs in various dry bean classes (plus local controls) to help generate validation data for Wallace's Yield System Analysis approach to improved breeding strategies. Began screening of $F_2$ populations to develop near isogenic lines in a common background of "Stringless Green Refugee" that differ by each of the seven known single genes for BCMV resistance. Lines resistant to the test BCMV strains are backcrossed to "SGR." These populations will be turned over to Jim Myers soon in anticipation of Dr Silbernagel's retirement. Dr. Myers will complete this work in the FY 92-97 extension period. The root rot differential (20 cvs) were tested for fusarium wilt resistance. Several lines are highly resistant including A55 which has been crossed extensively with commercial U.S. cvs to produce more upright plant habit in future U.S. cvs. Fusarium wilt (= Fusarium yellows disease) is becoming more of a problem in the past several years in our Midwestern bean producing states (North Dakota, Colorado, and Nebraska) and has been found in Tanzania. BCMV (necrosis resistant) and/or halo blight resistant advanced snap bean, kidney, navy, and great northern breeding lines developed at Prosser were field tested in multiple sites across the U.S. including CRSP bean collaborators at MSU, Universities of Idaho, and Nebraska, plus North Dakaota and Colorado State Universities.

**United States—Puyallup.** Dr. Lorna Butler, WSU agricultural anthropologist, has been given a 25 percent research appointment so she can participate in the WSU/SUA Bean CRSP program. She made two trips to Tanzania in 1990 (Feb. and Sept.) to organize and help set up an on-station participatory research program at SUA. She has made extensive contacts with other international researchers (CIAT and ISNAR) involved in similar research. Her activities are discussed in the HC section.

Host Country: Farmer Participatory Research/On-Farm Trials--Mollel/Nchimbi/Butler/Sibuga.

**Improved cultivar.** TMO 216 (being increased for release) distributed to selected farmers in Kilosa district. All indicated strong acceptance for the following reasons: (1) This year beans were planted later than usual because of heavy rains during the early part of the season which caused flooding. The later planted crops experienced some drought during the latter part of the season (see weather data, Table 4). TMO 216 withstood drought and yielded more than the farmers' local varieties; (2) TMO 216 cooked faster and tested just as good as the other local varieties. The khaki color of TMO 216 (instead of the reddish Canadian Wonder type) was not mentioned. This was surprising because it had earlier been thought that the khaki color might not be acceptable. Farmers in Mgeta who are more market oriented rated TMO 216 very high in every characteristic except marketability because bean merchants are used to mostly red type beans.

**Social Science Studies.** A smallholder farmer on-station PR program was initiated at SUA in 1990. The major objective was to involve small holder farmers in bean characteristic and varietal evaluation. The on-station trials will complement the on-farm trials.

A team of two male and three female researchers were involved: an extension specialist, an agronomist, a weed scientist, and a technician breeder. Farmers from two villages (Dumila and Magole) participated. Bean "experts" from these two villages were identified using simple questionnaires. The bean "experts" evaluated plants at two growth stages, i.e., at podding and at maturity. Sixteen farmers participated in evaluation during podding stage and nine farmers at maturity.

Lines HB 1-1, EP 10-4 and EP 4-4 were identified by farmers as the best lines and the ones which they would like to try in their fields. The criteria farmers used for selection were number of pods per plants, apparent resistance to drought and seed size.

This trial will be repeated before conclusions can be made about these lines. On-farm trial using the same lines in the fields of the same farmers will be conducted next season.

**Breeding—Nchimbi/Dimoso/Liwenga.** Evaluation of 25 advanced breeding lines identified three bean breeding lines EP 3-3, EP 3-9 and EP 6-3 which out performed TMO 216. These three lines are local crosses between the best Prosser hybrids and TMO 216. These lines were also resistant to BCMV, rust and ALS (Table 1).

**Agronomy—Nchimbi/Rweyemamu/Hatibu/Kayombo/Simalenga/Tesha.** Response to P: Under Morogoro conditions, some bean lines had significant response to P indicating that P could be a limiting factor in bean production at Morogoro (Table 2).

Tillage Methods: Effects of tillage methods on soil physical properties and on yield performance of three cvs: Canadian Wonder, Selian Wonder and TMO 216, were investigated for a sandy loam soil at Morogoro, Tanzania. Nine tillage treatments were compared: hand

hoeing (HH), minimum tillage (MT), minimum tillage with mulch (MTM), mouldboard ploughing (M), mouldboard ploughing followed by disc harrowing once (MH1), mouldboard ploughing followed by disc harrowing thrice (MH3), disc ploughing (D), disc ploughing followed by disc harrowing once (DH1) and disc ploughing followed by disc harrowing thrice (DH3). In the second year MTM was withdrawn and two tillage treatments were added, namely animal ploughing (AP) and single axle tractor ploughing (SA).

DH3 was found to be the best overall tillage method in relation to bean yield of all three test varieties. TMO 216 variety excelled in yield performance irrespective of the tillage method used (Table 3).

To sustain bean production by tillage methods which involve tractor ploughing followed by harrowing, periodic subsoiling or chiselling and use of rotations may be necessary to minimize soil compacting hazards.

Rainfall which deviated significantly from normal affected the yields (Table 4).

Drought Tolerance: Two common bean cultivars, Lyamungu 85 and TMO 216, were tested in the greenhouse to find out whether or not they are drought resistant. Lyamungu 85 has recently been released and TMO 216 is expected to be released soon. A comparison was made in the greenhouse between these two cultivars and a fairly well known resistant cultivar TMO 101 and also a tepary bean (*P. acutifolius* Gray).

On the basis of their flowering response, seed and biomass production, stomatal frequency, harvest index, drought susceptibility index, and chlorophyll content under simulated drought conditions, it was found that both Lyamungu 85 and TMO 216 are quite sensitive to drought and therefore should be recommended only for high and for medium rainfall areas. However, TMO 216 was found to be more productive under drought conditions than local cvs in on-farm trials in Kilosa.

Bean Mixtures: Pure stands and mixtures differed significantly for percent germination, plant stands at flowering and at harvest, number of pods per plant, seeds per pod and seed yield in grams per plant, but there was no significant difference for seed yield (in kilograms) per hectare.

Based on the performance of the cultivars when grown in pure stands, some of the mixtures had higher than the expected yields and some were less than their respective expected yields. This suggests that not all mixtures are superior over pure stands and that for the mixture to perform better than expected from the pure stands, it depends on the type and proportions of the component cultivars and perhaps the degree of plant competition in the mixed stands (Table 5).

Sources of Nitrogen (N): The conventional inorganic sources of N are in most cases beyond the easy reach of most small scale bean producers. Organic fertilizers (mulches) and possibly the use of

artificial inoculation and/or supplementary inorganic N as a starter in the bean production systems seems to be the most convenient alternative in dealing with the current fertilizer problem.

The performance of four common bean lines was used to determine the effects of five alternative sources of N in bean production. Among the sources of N, NITROSUA ($S_4$) and NITROSUA plus a starter N ($S_5$) gave the highest nodulation on all the varieties. The highest N-yield and dry matter production at 50 percent flowering were obtained where sulphate of ammonia ($S_2$) and $S_5$ were used.

The data obtained for components of seed yield showed that the three sources viz. sulphate of ammonia, poultry manure and NITROSUA plus a starter N dose had the highest potential for improving the common bean seed yield under the prevailing agro-ecological conditions.

**Plant Pathology—Teri, Nchimbi, Lyimo, Mmbaga.** Screening for multiple disease resistance: Screening of segregating populations was continued at SUA as a routine part of the program to develop high yielding varieties with multiple disease (BCMV, rust, ALS) resistance. This was the major effort during the year in line with our global responsibility. Mmbaga continued her work at University of Nebraska with Dr. Steadman on adult plant resistance and leaf pubescence.

Effect of Nitrogen source on diseases: The effects of calcium ammonium nitrate (CAN), sulphate of ammonia (SA), urea and poultry manure on development of angular leaf spot, rust, BCMV and root rots in beans grown in lower and higher densities were studied using split-split plot design.

Plots receiving 60 kg N/ha had significantly higher levels of BCMV, rust and angular leaf spot than in 30 kg N/ha. The effect of fertilizer types on severity of diseases and on yield differed significantly. Plots fertilized with urea developed low levels of BCMV, angular leafspot, rust and root rots, contributing to higher grain yield. Generally plots fertilized with excess of calcium ammonium nitrate and sulphate of ammonia developed high levels of diseases with low grain yields. Significantly higher incidences of root rot were recorded in plots fertilized with poultry manure.

These results suggest that there may be advantages in the use of urea N in beans. The apparent bonus is a reduction in the incidence of diseases and high grain yield. More studies are needed.

Bean Root Rot by Bean Fly Interaction: Bean fly infested plants at Morogoro and Mbeya showed vascular discoloration typical of Fusarium wilt. *Fusarium oxysporum* was consistently isolated from all such bean fly infested plants. Pathogenicity tests are planned to determine if synergism exists for this bean fly/Fusarium association.

**Food Science—Maeda/Samwel/Mgowe:** Only modest support was provided for this work. Soaking of high tannin bean varieties for four hours is associated with over 60 percent reduction in tannin levels and maximum moisture imbition. The latter variable is critical in influencing bean cooking times. It would therefore be advantageous to soak high tannin bean varieties in alkaline media for four hours so as to tap the combined effects on enhanced cookability and tannin reduction. In Tanzania, for example, local "Magadi" which is alkaline is added to soaking water to enhance cookability. It would therefore be appropriate to investigate the most optimum concentration of Magadi, a soaking media which will bring the pH to 9.0 so as to enhance maximum leaching of tannins from beans.

Information was obtained on some of the chemical transformations in the course of sprouting beans. It is probable that increases in total acidity and reducing sugars have opposing effects in enhancing acceptability of bean sprouts, although this technique of bean processing has advantages such as increases in the *in vitro* digestibility and vitamin C, and reduction of tannins. It is thus important to optimize the sprouting duration so as to prevent reduced acceptability due to excessive accumulation of organic acids. Should the acidity increase to levels which would not cause a pH drop below 6.6, there will be an increase in protein solubility (Sosulki and McCurd, 1987).

**Evidence of extent of use.** Difficult to determine for the varieties at this stage. The annual SUA/WSU Bean CRSP workshops have grown as shown by this year's joint workshop. The proceedings have continued to be in high demand, including the exchange of information and germplasm. TMO 101, which was identified in our program as being drought tolerant, is being used in the region by CIAT and other national programs in breeding work for drought tolerance as reported in this year's workshop.

2. Other research-related results

**Germplasm conservation and use.** Accession: We continued to receive segregating materials from Prosser and accessions from Zambia and Uganda which are being selected for various characters in our breeding program. International exchange: Our collections were distributed to Zambia, Uganda and SADCC/CIAT on request.

**Seed production and distribution of CRSP produced cvs.** Modest progress was made in seed multiplication of promising cultivars because of weather (floods and/or drought) and lack of appropriate equipment (tractor and implements). Request has been made to purchase tractors and implements to overcome this constraint. Additionally, we are exploring possibilities of entering into contract with private seed producers to multiply our promising lines for OFT and distribution to growers.

**Impact of CRSP recommended technology.** Trials with inoculant NITROSUA in other parts of the country continued to show promising results. Inoculum is now being produced in a pilot plant at SUA and is being distributed to growers at a minimal cost.

**Project impact on production.** Too early to determine.

**How do the research findings specifically address the needs of small-scale farmers and women?** The goal of the project from the start has been to address the problems of small-scale producers and the needs of women.

3. Changes in national production/consumption of beans/cowpeas in the Host Country. Too early to determine.

B. Institutional Development and Training

1. Changes since FY 89. Cornel Rweyemamu was admitted for Ph.D. studies in agronomy at MSU starting August 1990.

2. Over the life of the project. Degree and non-degree training has been an important feature of the Bean CRSP in Tanzania. Five Tanzanians have completed Ph.D. training in the USA. Three more are about to complete their Ph.D. training. Many undergraduate students have done their final year search projects under the project.

3. Project training to be completed by the end of the project period. Martha Quentin, Robert Mabagala and Flavianus Magayane are expected to complete their Ph.D.s during the period.

C. Progress Achieved in Relation to the Extension Proposal Log Frame

The project has been operating for ten years. During that time, the U.S. contribution has been focused on bean diseases (BCMV, halo blight and root rot). We have identified sources of resistance effective breeding strategies, means of biological and serological strain identification, and field testing techniques suitable for HC conditions. All of these capabilities (except serodiagnosis) are in place in Tanzania. So we have accomplished about 95 percent of what we had planned. In the future, SUA will need a trained Tanzanian virologist to be self-sufficient in this area because there are many other virus problems of beans and other crops in serious need of research attention. Since we have essentially completed most of what we intended to do, our BCMV research priorities have recently been expanded to initiate a program (see I.A.1. above) to develop a set of seven near-isogenic bean lines in a common (SGR) genetic background, each with a single isolated gene for resistance to BCMV. The completion of this project in the FY92-97 extension period will greatly simplify and accelerate the breeding of resistant cvs (especially in DCs) and the identification of new strains of the virus. (Only about half of the theoretically possible strains can now be identified for lack of cvs with specific combinations of resistance factors).

Host Country

This CRSP has been involved in this line of research since its inception in 1981.

Breeding programs are essentially of long term nature and it was estimated that by now preliminary high yielding varieties with multiple disease resistance should be available to growers. TMO 216 is one such variety. TMO 101 is now being used as a source of drought tolerance. Lines EP 3-3, EP 3-6 and EP 3-9 are also potentially high yielding lines due for future release (two to three years).

The project is generally on schedule. A bottleneck experienced has been in rapid seed multiplication of the promising lines which has been alluded to above. Also, progress has been slow in development of insect resistant cvs. Insect resistance will require another ten years. Lack of resistant germplasm has been a major restraint.

Relation of research elsewhere: Our Bean CRSP is both unique and complementary of research being carried out elsewhere in that it combines a strong multidisciplinary research approach including not only the biological aspects of bean improvement but also the socio-cultural aspects and addressing the specific needs of small-scale producers and women. The farmer participatory research approach is being institutionalized.

The bean lines with multiple resistance to BCMV, ALS, rust and HB are likely to be useful to other bean improvement programs in the region. Some of these lines have already been shared with other national bean programs. TMO 101 is already being used as a source of drought tolerance in the CIAT drought nurseries in Eastern and Southern Africa.

D. Evidence of Biological/Social Sciences Integration

The (25 percent research) appointment of Dr. Lorna Butler (WSU agricultural anthropologist) to the WSU/SUA Bean CRSP team is evidence of this project's recognition of the importance of small-holder farm families and the role of women in development.

Dr. Butler has made recognizance-organizational visits to SUA in Feb and Sept 90 to consult with co-workers Dr. Nchimbi and Mollel on setting up an on-station participatory research (PR) program to provide a communication link between smallholder women bean specialists and SUA biological scientists.

Butler, Nchimbi, and Mollel have visited and consulted with PR specialists Dr. Louise Sperling (CIAT-Rwanda) and Dr. Anne Ferguson (CRSP-Malawi); and Butler has visited ISNAR representatives in the Netherlands.

Butler and Ferguson provided a two-day training session to 18 PR workers after the 1990 SUA-CIAT-CRSP workshop.

Dr. Maeda (SUA) continues to assist the SUA breeder to evaluate the advanced bean lines for consumer acceptability and nutritional characteristics.

E. Collaboration with Other Bean/Cowpea CRSP Projects; Linkages with Other CRSPs, and Other External Groups

The Ecuador/UMN/Graham project has a root rot component on which the Tanzania/WSU U.S. PI has advised, and reciprocally the UM-U.S. PI has advised the SUA breeder on breeding for enhanced BNF, and he attended the 1990 SUA bean workshop at which he presented two papers.

The DR/UNL project continues to keep the SUA rust workers updated on the relationship of long dense pubescence to rust resistance. Tanzanian pathologist Dr. M. Mmbaga is working with Dr. Steadman in U.S. as a post-doc. Dr. Mmbaga presented two papers at the 1990 workshop.

Dr. Anne Ferguson, Dr. Paul Gepts and Dr. Alex Mkandawire (Malawi/UCD CRSP) attended the 1990 SUA bean workshops and presented papers. Dr. Ferguson stayed two additional days to help Dr. Butler put on a training session for PR workers.

Dr. Barry Swanson, U.S. PI of Guatemala/WSU, attended the 1990 SUA Bean Workshop and presented a food science paper on improving the nutritional quality of beans.

Dr. James Myers, University of Idaho bean breeder and designated Tanzania/WSU bean breeder, attended the 1990 Bean Workshop to present two papers and to consult extensively with the SUA bean team.

Dr. Russ Freed, assistant director, Bean/Cowpea CRSP-MO, represented the CRSP at the 1990 SUA bean workshop and presented a paper on data management and analysis via the MSTAT software.

The 9th Annual SUA Bean Workshop held in Morogoro, Tanzania, from 17-21 September 1990, was jointly sponsored by the WSU/SUA Bean CRSP and the SADCC/CIAT regional bean teams in eastern and southern Africa. Eighty registered attendees from 13 countries (plus about 15 SUA students) presented a series of papers reviewing the progress in African bean improvement in the past ten years, and establishing strategies and priorities for the coming decade. The jointly published proceeding will serve as a reference point for years to come for present and future researchers, administrators and sponsors.

## II. FUNDING/FISCAL MANAGEMENT

### A. Problems Regarding Funding

As in FY 89

### B. Adequacy of Current Management System

As in FY 89

### C. Activity Towards Buy-Ins or Other Funding

A proposal was written and submitted to USAID-Tanzania. It is still under discussion. See Section III.F. below.

## III. PROJECT STATUS

### A. Appropriateness of Activities to Goals of the Global Plan

Very appropriate--most efforts have addressed the goal of developing widely adapted bean cultivars with multiple disease resistance that are acceptable to smallholder bean producers and to ensure that the new production technologies do not affect women adversely.

### B. Balance Between Research and Training

1. Expenditures for research and training. Expenditures for training (55 percent) were more than research, which was justified at the beginning, but will be changed to favor more funds for research than training in the future.

2. Expenditures related to research and training plans. About on target.

### C. Balance of Domestic vs. Overseas Activities

Same as in FY 89, about 50:50.

### D. Level of Collaboration/Cooperation Between U.S. and HC Institutions

Has remained the same over the years with joint planning, preparation of budgets etc. during annual workshops at SUA.

### E. Relative Contributions of Collaborating Institutions and Individuals

Contributions from A.I.D. $194,750; from WSU (in kind) $29,187; and from SUA (in kind) $20,000.

USDA (in kind) 25 percent of U.S. PIs time $13,000; use of USDA facilities $2,000 light and heating oil (25 percent)

### F. Interest, Involvement, and Support of USAID Mission and/or U.S. Embassy

The USAID-Dar es Salaam office has been very cooperative in: (1) assisting with student travel and training clearances; (2) letting us use pouch mailing privileges; (3) supporting the 9th Annual Bean Workshop ($15,000); and (4) discussing the possibility of a three to five year buy-in at $100,000/year to expand/complement the SUA/WSU Bean CRSP activities.

### G. Evidence of Institutionalization

1. Faculty recognition (Kellog Foundation fellowship for Prof. Teri). Collaborative efforts exist with some of the IARC's and donor agencies in research related matters in which the HC PI has been involved from time to time.

2. Integration of domestic and international commodity research programs. This has been excellent. The Ministry of Agriculture (Lyamungu), the Uyole Agricultural Centre, and SUA teams, including the SADCC/CIAT program, have worked very closely over the years. This year's annual SUA/WSU bean research workshop was very well attended by all parties. The farmer participatory training workshop was also well attended by all teams.

3. Internal project management. Excellent

4. Student/Professor interaction. Excellent.

## IV. PUBLICATIONS AND PRESENTATIONS

The 1989 proceedings of the SUA/WSU Bean Research Workshop were edited by Maeda and Nchimbi. They could not be printed because of difficulties experienced by our Roman Catholic printer who had to handle excessive work due to the Papal visit to Tanzania in September. The proceedings will now be printed in the U.S.

Several papers by the SUA/WSU CRSP team were presented in the 1990 joint workshop with CIAT.

Rweyemamu, C. L. 1990. Common Bean (*Phaseolus vulgaris* L.) Response to Minjingu Rock Phosphate (MRP) in Tanzania. Bean Improvement Cooperative Annual Report 33:147-148.

Mohamed, R. A. and Teri, J. M. 1989. Farmers Strategies of Insect Pest and Disease Management in Small Scale Production Systems in Megeta, Tanzania. Summary presented at Symposium on Pest Management and the African Farmer. Full text in Insect Science and Its Applications 10(6):1989.

Forester, R. L., Myers, J. R., Mink, G. I. and Silbernagel, M. J. 1991. NL-8 Strain of Bean Common Mosaic Virus Detected in Idaho Bean Seed Fields. Disease note submitted to Plant Disease.

Table 1. Promising High Yielding Bean Cultivars with Multiple Disease Resistance from Crosses Made at Prosser and SUA, Screened at Morogoro.

| Bean line | Seed colour | Days to 50% flowering | Days to maturity | 100 Seed weight | Yield (Kg/ha) |
|---|---|---|---|---|---|
| EP 9-3 | Dark purple | 34 | 64 | 39.5 | 3399 |
| EP 3-9 | Tan (Khaki) | 37 | 66 | 36.6 | 2966 |
| HB 3-2 | Dark purple mottled | 37 | 66 | 34.8 | 2965 |
| EP 3-1 | Dark purple | 34 | 64 | 34.6 | 2916 |
| HB 2-1 | Tan (Khaki) | 35 | 65 | 38.8 | 2467 |
| EP 5-2 | Tan (Khaki) | 35 | 65 | 34.4 | 2390 |
| EP 6-6 | Cream | 33 | 65 | 38.2 | 2383 |
| EP 5-1 | Tan (Khaki) | 33 | 66 | 32.6 | 2331 |
| EP 9-1 | Cream | 35 | 65 | 36.1 | 2291 |
| HB 3-4B | Cream | 35 | 63 | 29.3 | 2290 |
| EP 3-2 | Cream | 36 | 66 | 37.3 | 2275 |
| EP 7-5 | Cream | 35 | 65 | 39.9 | 2158 |
| EP 4-4 | Red | 36 | 67 | 39.6 | 2151 |
| EP 3-7 | Cream | 35 | 65 | 33.5 | 2001 |
| EP 8-3 | Cream | 34 | 64 | 39.0 | 1921 |
| EP 10-4 | Tan (Khaki) | 35 | 68 | 33.0 | 1920 |
| EP 10-4 | Tan (Khaki) | 35 | 68 | 33.0 | 1920 |
| EP 7-3 | Red | 37 | 66 | 34.5 | 1911 |
| HB 1-5 | Tan (Khaki) | 35 | 65 | 37.1 | 1850 |
| HB 3-3 | Tan (Khaki) | 38 | 67 | 38.1 | 1843 |
| EP 3-6B | Red | 37 | 67 | 30.3 | 1886 |
| EP 7-4 | Tan (Khaki) | 32 | 63 | 25.6 | 1761 |
| EP 1-1 | Dark purple, red mottled | 35 | 68 | 32.3 | 1743 |
| EP 3-10 | Dark purple | 37 | 64 | 28.1 | 1686 |
| EP 7-6 | Red | 34 | 63 | 39.4 | 1628 |
| EP 6-4 | Cream | 35 | 67 | 32.7 | 1587 |
| EP 3-3 | Tan Khaki | 35 | 64 | 32.5 | 1582 |
| EP 6-3 | Red | 34 | 66 | 37.6 | 1555 |
| EP 6-5 | Khaki mottled | 35 | 65 | 32.1 | 1536 |
| HB 3-5 | Purple, red speckles | 34 | 65 | 32.7 | 1514 |
| EP 3-5 | Dark purple red speckles | 37 | 67 | 32.7 | 1469 |
| EP 5-3 | Cream | 35 | 67 | 35.1 | 1466 |
| EP 10-1 | Red | 33 | 64 | 31.8 | 1405 |
| EP 4-3 | Tan (Khaki) | 35 | 65 | 28.6 | 1384 |
| EP 3-4 | Dark purple | 35 | 64 | 46.1 | 1347 |
| EP 7-2 | Cream | 36 | 68 | 31.9 | 1302 |
| EP 8-2 | Cream | 34 | 71 | 36.1 | 1278 |
| EP 10-3 | White | 32 | 63 | 32.8 | 1240 |
| HB 1-1 | Light purple mottled | 33 | 63 | 29.4 | 1231 |
| EP 3-6A | Light red | 36 | 70 | 30.8 | 1206 |
| EP 4-2 | Dark purple | 39 | 75 | 24.2 | 1202 |
| EP 7-1 | Dark purple | 39 | 71 | 29.9 | 1072 |

Table 2. Response of Bean Lines to P at Morogoro.

| | FERTILIZER LEVELS | | | |
|---|---|---|---|---|
| BEAN LINE | $P_1$ | $P_2$ | $P_3$ | $P_4$ |
| TMO 333 | 986.667 a | 1002.00 a | 1088.667 a | 1432.667 a |
| TMO 101 | 861.333 a | 899.33 ab | 1068.00 a | 1072.00 b |
| TMO 216 | 756.667 a | 785.33 ab | 940.667 a | 1160.00 b |
| KENYA | 805.667 a | 858.66 ab | 901.667 ab | 1113.667 b |
| A 55 | 485.667 b | 725.00 b | 668.00 b | 698.667 c |

Values which are followed by the same letters in each column are not significantly different from each other according to Duncan's Multiple Range Test (P = 0.05)

Table 3. Effect of Tillage Treatments on Bean Grain Yield ($kgha^{-1}$)

| | MARCH - JUNE 1989 | | | MARCH - JUNE 1990 *1 | | |
|---|---|---|---|---|---|---|
| TREATMENT | Selian Wonder | Canadian Wonder | TMO 216 | Selian Wonder | Canadian Wonder | TMO 216 |
| HH | 500 bc | 379 d | 518 cd | 148 cd | 118 ab | 214 ab |
| MT | 491 bc | 378 d | 480 d | 107 d | 67 b | 104 b |
| MTM | 462 c | 550 ab | 502 cd | - | - | - |
| M | 560 abc | 512 bc | 700 ab | 226 ab | 139 ab | 235 ab |
| MH1 | 501 bc | 520 abc | 801 a | 185 abc | 158 a | 260 a |
| MH3 | 672 a | 609 a | 690 abc | 206 abc | 176 a | 310 a |
| D | 539 abc | 428 cd | 512 bcd | 234 a | 157 ab | 237 ab |
| DH1 | 611 ab | 570 ab | 660 abcd | 223 abc | 189 a | 324 a |
| DH3 | 680 a | 560 ab | 710 a | 242 a | 201 a | 345 a |
| SA | - | - | - | 156 bcd | 128 ab | 159 b |
| AP | - | - | - | 165 abcd | 130 ab | 164 b |

Letters a-d denote significance at the 5% level using Duncan's new multiple range test. Means with the same letter are not significantly different.

HH - hand hoeing; MT - minimum tillage; MTM - minimum tillage with mulch; M - mouldboard ploughing; MH1 - mouldboard ploughing followed by disc harrowing once; MH3 - mouldboard ploughing followed by disc harrowing thrice; D - disc ploughing; DH1 - disc ploughing followed by disc harrowing once; DH3 - disc ploughing followed by disc harrowing thrice; AP - animal ploughing; SA - single axle tractor ploughing.

*1 See Table 4 for weather records. In 1990 bad floods in March, then drought rest of season.

Table 4. Growing Season Rainfall, Open Pan Evaporation and Air Temperature at SUA, 1989 and 1990 Growing Seasons

| Growing Season 1989 | March | April | May | June | Total |
|---|---|---|---|---|---|
| Rainfall (mm) | 146.4 | 250.4 | 112.7 | 11.7 | 521.2 |
| departure[1] | 21.7 | 56.6 | 16.8 | -8.5 | 86.6 |
| Open pan evaporation (mm) | 160 | 96 | 16.8 | 78 | 408 |
| Mean air temperature (°C) | 25.9 | 24.9 | 23.9 | 21.9 | - |

| Growing Season 1990 | March | April | May | June | Total |
|---|---|---|---|---|---|
| Rainfall (mm) | 229.1 | 193.6 | 56.8 | 10.2 | 489.7 |
| departure[1] | 98.6 | -0.2 | -37.0 | -9.4 | 52 |
| Open pan evaporation (mm) | 119 | 110 | 112 | 92 | 433 |
| Mean air temperature (°C) | 25.2 | 25.4 | 24.0 | 22.3 | - |

[1]Departure from a 17-year average

Table 5. Grain Yields of Pure Lines and Mixtures.

| | Grain yield grams/plant* | Grams/$M^2$ | Kg/ha | 100 Seeds wt.** |
|---|---|---|---|---|
| Kabanima | 3.36 c | 45.58 | 455.8 | 29.63 bc |
| TMO 216 | 5.40 abc | 83.08 | 530.8 | 18.13 a |
| Selian Wonder | 5.70 abc | 65.68 | 656.8 | 34.74 a |
| TMO 333 | 7.88 a | 92.23 | 922.3 | 28.50 bc |
| M1 | 6.38 abc | 73.20 | 732.0 | 27.48 bc |
| M3 | 4.58 bc | 59.95 | 599.5 | 21.88 de |
| M4 | 4.80 bc | 66.17 | 661.7 | 28.85 bc |
| M5 | 5.48 abc | 64.6 | 646.0 | 25.78 cd |
| M6 | 6.38 abc | 86.17 | 861.7 | 31.08 ab |
| C.V (%) | 28.41 | 29.09 | 29.09 | 10.80 |
| S.E | 0.82 | 10.48 | 104.8 | 1.47 |

N.B * Significantly different at P = 0.05
** Significantly different at P = 0.01
*** Significantly different at P = 0.001

Means followed by a common letter within a column do not differ significantly according to DNMRT ($P \leq 0.05$)

| | | | |
|---|---|---|---|
| M1 - 25% K, | 25% TMO 216, | 25% S.W, | 25% TMO 333 |
| M2 - 40% K, | 20% TMO 216, | 20% S.W, | 20% TMO 333 |
| M3 - 20% K, | 40% TMO 216, | 20% S.W, | 20% TMO 333 |
| M4 - 20% K, | 20% TMO 216, | 40% S.W, | 20% TMO 333 |
| M5 - 20% K, | 20% TMO 216, | 20% S.W, | 40% TMO 333 |
| M6 - 30% K, | 10% TMO 216, | 30% S.W, | 30% TMO 333 |

**Project/University Abbreviations:**

BTI - Boyce Thompson Institute
INCAP - Instituto de Nutricion de Centro-america y Panama
KSU - Kentucky State University
MSU - Michigan State University
UCD - University of California-Davis
UCR - University of California-Riverside
UGA - University of Georgia
UMN - University of Minnesota
UNL - University of Nebraska-Lincoln
UPR - University of Puerto Rico
UWI - University of Wisconsin-Madison
WSU - Washington State University

**State Abbreviations:**

AR - Arkansas
AZ - Arizona
CA - California
GA - Georgia
IL - Illinois
IN - Indiana
MI - Michigan
MN - Minnesota
ND - North Dakota
NE - Nebraska
PR - Puerto Rico
WA - Washington
WI - Wisconsin
TX - Texas

## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| A.I.D. | Agency for International Development |
| AACC | American Association of Cereal Chemists |
| AFGRAD | African American Institute program |
| AIMS | Alternative insect management strategies |
| ALS | Angular Leaf Spot |
| AMMI | Additive Main Effects and Multiple Interaction Effects Analysis |
| ANOVA | An analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| ATP | Adenosine Triphosphate |
| BCMV | Bean Common Mosaic Virus |
| BDMV | Bean Dwarf Mosaic Virus |
| BGMV | Bean Golden Mosaic Virus |
| BH | Benzyladenine |
| BIC | Bean Improvement Cooperative |
| BIFAD | Board for International Food and Agricultural Development |
| BNF | Biological Nitrogen Fixation |
| BOA | Basic Ordering Agreement |
| BOD | Board of Directors |
| BRP | Bean Research Program |
| C | Centigrade |
| CAN | Caribbean Adaptation Nursery |
| CATIE | Centro Agrónomico Tropical de Investigación y Enseñanza (Tropical Agricultural Center for Investigation and Teaching) |
| CBB | Common bacterial blight |
| CDBN | Cooperative Dry Bean Nursery |
| CEIBA | Journal of the Escuela Agricola Panamericana |
| CESDA | Centro Sur de Desarrollo Agropecuario (South Center for Agricultural Development) |
| CIAT | Centro Internacional de Agricultura Tropical (International Center of Tropical Agriculture) |
| CNPQ | Brazilian National Science Foundation |
| CNRA | Centre National de Recherches Agronomiques |
| CONACYT | Consejo Nacional de Ciencia y Technologia (Agency responsible for support of students) |
| COSUDE | UN, USAID, and Swiss funding program coordinated by CIAT |
| CRSP | Collaborative Research Support Program |
| DNA | Deoxyribonucleic Acid |
| DR | Dominican Republic |
| EAP | Escuela Agricola Panamericana |
| ECAR | Trial for breeding lines in Honduras |
| EEAL | Experimental Estacion Arroyo Loro |
| FEC | European economic community |
| EEP | External Evaluation Panel |
| EMBRAPA | Empresa Brazileira de Pesquisa Agropecuaria (Brazilian Enterprise for Agricultural Investigations) |
| EPACE | The Ceara State Agricultural Extension Service |
| FAO/UN | Food and Agricultural Organization of the United Nations |
| FTE | Full Time Equivalent |
| FY | Fiscal Year |
| GLIP | Grain Legume Improvement Program |
| GN | Great Northern |
| GTZ | Deutsche Gesellschaft fuer Technische Zusammenarbeit (German Agency for Technical Cooperation) |
| HB | Halo blight |
| HC | Host Country |
| HTC | Hard to cook |

| | |
|---|---|
| IARC | International Agricultural Research Center |
| IBA | Indole Butyric Acid |
| IBPGR | International Board of Plant Genetic Resources |
| IBSNAT | International Benchmark Site Network for Agro Technology Transfer |
| ICRISAT | International Crops Research Institute for the Semi Arid Tropics |
| ICTA | Instituto de Ciencias y Tecnologia Agricola (Institute of Agricultural Science and Technology) |
| IFS | International Foundation for Science (Swedish foreign aid program) |
| IFT | International Food Technology |
| IITA | International Institute of Tropical Agriculture |
| INCAP | Instituto de Nutricion de Centroamerica y Panama (Institute of Nutrition of Central America & Panama) |
| INIAP | Instituto Nacional de Investigaciones Agropecuarias (National Institute of Agricultural Investigations) |
| INIFAP | Instituto Nacional de Investigaciones Forestales y Agropecuarias (National Institute of Forestry and Agricultural Investigations) |
| INTSORMIL | Sorghum/Millet CRSP |
| IPDO | International Project Development Office |
| IPM | Integrative Pest Management |
| IPRC | Insect Pathology Resource Center |
| IR | Institutional Representative |
| IRA | Institut de la Recherché Agronomique (Institute of Agronomic Research) |
| ISA | Instituto Superior Agricultura |
| ISNAR | International Service for National Agricultural Research |
| ISRA | Institut Sénégalais de Recherches Agricoles (Senegalese Institute Agricultural Research) |
| ITA | Institut de Technologie Alimentaire (Food Technology Institute in Senegal) |
| LASPAU | Latin American Scholarshipo Program for American Universities |
| MCAB | Monoclonal antibodies |
| ME | Management Entity |
| MIAC | MidAmerica International Agriculture Consortium |
| MNR | Ministry of Natural Resources |
| MO | Management Office |
| MSTAT | Microcomputer Statistical and Data Management Package |
| MSU | Michigan State University |
| MUCIA | Midwest Universities Consortium for International Activities |
| MW | Molecular weight |
| N | Nitrogen |
| NBP | National Bean Program |
| NCRE | National Cereals Research and Extension Project |
| NSH | Narrow sense heritabilities |
| OAS | Organization of American States |
| OIT | Office of International Training (A.I.D.) |
| ORTTA | Office of Research Technology Transfer Administration |
| PCCMCA | Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios (Central American Cooperative Program for the Improvement of Food Crops) |
| PCR | Polymerase Chain Reaction |
| PHA | Phytohemagglutinin |
| PI | Principal Investigator |
| PROFRIJOL | Research network of Latin American & Caribbean countries |
| PSTC | Program in Science and Technology Cooperation |
| RENACO | West and Central Africa cowpea research network |
| RFLP | Restriction Fragment Length Polymorphism |
| RIISP | Research Initiative: Insects of Stored Pulses |
| RNA | Ribonucleic Acid |
| S&T/AGR | Science and Technology/Agriculture |
| SADCC | Southern African Development Coordinating Committee |
| SAFGRAD | Semiarid Food Grain Research & Developm't Project |
| SANREM | Sustainable Agriculture and Natural Resource Management CRSP |
| SEA | Secretaria de Estado de Agricultura (Secretary of State for Agiculture) |
| SGR | Stringless Green Refugee |
| SUA | Sokoine University of Agriculture |
| TARS | Tropical Agriculture Research Station |
| TC | Technical Committee |
| TFMSA | Trifluoromethane sulfonic acid |
| TLU | Training Liaison Unit |
| TSP | Texturized Soy Protein |
| UNAH | National University of Honduras |
| UNU | United Nations (UN) University |
| U.S. | United States |
| USDA/ARS | U.S. Department of Agriculture/Agricultural Research Station |
| VAN | Variety trial |
| VIDAC | Vivero de Adaptacion Centroamericano |
| VINAR | Variety trial |
| WB | Web blight |
| WID | Women in International Development |
| YSA | Yield System Analysis |