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DEVELOPMENT OF IMPROVED VARIETIES OF SOYBEANS SUPPORTING CULTURAL AND MARKETING PRACTICES FOR PRODUCTION IN THE TROPICS AND INFORMATION DELIVERY SYSTEMS

Annual Report on
Contract AID/ta-c-1294
April 1, 1976 to March 31, 1977

Submitted to
The U.S. Agency for International Development
Department of State
Washington, D.C.

by
The University of Illinois at Urbana-Champaign
College of Agriculture
International Soybean Program, INTSOY

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REPORT SUMMARY

A. Statistical Summary

1. Project Title and Contract Number: Development of Improved Varieties of Soybeans and Supporting Cultural and Marketing Practices for Production in the Tropics and Information Delivery Systems - AID/ta-c-1294.

2. Principal Investigator, Contractor and mailing address:

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3. Contract Period: April 1, 1976 - March 31, 1979

4. Period Covered by Report: April 1, 1976 - March 31, 1977

5. Total AID Funding of Contract to Date: $2,085,855

6. Total Expenditures for Report Period: $479,715

B. Narrative Summary of Accomplishments

Substantive progress was made toward the development and exploitation of the inherent potential of the soybean as an efficient source of high quality edible oil and high quality protein for the diets of both the rural and urban poor in developing countries.

Work continued on developing improved genetic materials for use in LDC breeding programs. The International Soybean Variety Evaluation Experiment (ISVEX) is the focal point of this effort and has provided significant data on the adaptability of temperate zone cultivars in the tropics. Studies on factors limiting the production of high quality seed were initiated in Thailand, Sri Lanka, Ecuador, Puerto Rico, California and Illinois. Research continued on cooking qualities of different varieties of soybeans. Experiments were conducted on the identification of virus resistant tropically adapted soybean germplasm, especially resistance to soybean mosaic virus (SMV). Work on the development of insect resistant genetic material focused on the physiology and interaction of host-plant resistance and insect pests, predation and insecticides as well as on the screening of breeding lines.

Recruitment, planning and site selection were the principal activities in the area of rhizobium production and management.

Three information systems, TAXIR, SIRIC and IRCSA were utilized in the management and dissemination of knowledge on soybean production,

protection, and utilization. The INTSOY Newsletter continued to be a useful means of informing a general audience about the several aspects of the overall program, including research specific to this project. Report No. 11 in the INTSOY Publications Series "International Soybean Variety Experiment, Second Report of Results," D. K. Whigham, Editor was released in December 1976. Plans were made to publish the proceedings of the March 1977 workshop on soybean rust held in Manila in cooperation with the Asian Vegetable Research and Development Center and the Philippines Council for Agriculture and Resources Research.

Studies on disease control were addressed on an interdisciplinary basis with plant pathology and entomology as the lead disciplines. Fungal and viral diseases that limit soybean production were studied and potential control methods were developed. Charcoal rot, various seedborne fungi, soybean mosaic virus and cowpea mosaic virus received intense scrutiny.

Progress continued in the development of model insect, weed and disease control management systems suitable for adaptation or modification for specific environments. The pilot pest management program initiated in Southern Brasil in 1974/75 was officially recommended by the state of Parana in 1976 and a similar program was adopted on a national scale for 1977. Leadership on weed control studies is being exerted by colleagues at the University of Puerto Rico, Mayaguez Campus. Disease control studies were alluded to in the above paragraph.

Cooperative work between UIUC and UPR/MC agricultural engineers resulted in publication of a study on improved soybean storage technology Reducing seed moisture content and maintaining it during storage were shown to be essential ingredients for preserving seed quality at an acceptable level for nine month's storage in the tropics. Sealed metal containers or plastic bags placed in metal containers with covers offered adequate storage protection for seed with a low initial moisture content.

Activities associated with home processing of soybeans were phased out in the first six months of the report period. A final report on home processing activities was prepared for the Agency.

INTSOY research staff engaged in a number of ancillary activities relevant to the general objectives of the research project and contributory to the development of response capability in soybean production, protection and use. These activities included participation in sponsored training courses, conduct of seminars, and advisory service to LDC's under Agency task orders.

REPORT OF PROGRESS

A. Background

In April 1973 a contract was awarded to the University of Illinois at Urbana-Champaign (UIUC) by the U.S. Agency for International Development calling on the University to "perform a research and development program directed toward the development of improved variety of soybeans." The contract expired March 31, 1976, by which time sufficient progress had been made to warrant the award of a contract to extend and expand efforts begun under the previous contract to give greater attention to necessary efforts in pest management, storage and handling of soybean seed, marketing economics, rhizobium technology and dissemination of information.

Work under the previous contract focused on soybean production, protection and food processing. Results of the International Soybean Variety Evaluation Experiment (ISVEX) showed that the soybean plant has wide adaptation. Plant protection work featured close cooperation between departments of UIUC and the University of Puerto Rico, Mayaguez Campus (UPR/MC) focusing on pest management techniques, identifying soybean diseases, generating basic information about soybean pathogens that can be used to predict their spread and importance, devising methods to control or reduce the impact of diseases and assisting in breeding tropically adopted soybean cultivars with resistance to important pathogens. Progress in food science and soybean food processing showed sufficient promise to warrant continued support. Soybean tenderization experiments demonstrated that ammonium bicarbonate, as well as sodium bicarbonate, is an effective tenderizing agent. Prototype soybean foods were developed and underwent nutritional evaluation. Initial studies were made on development of concepts and equipment for village and home level simple processing of soy-based foods. Unfortunately, support could not be generated within the Agency to permit continuation of the latter two lines of study under a renewed contract and these activities were necessarily phased out during the past year.

Three major regional conferences, in Puerto Rico, Ethiopia and Thailand were held for soybean scientists of Central America, South America and the Caribbean, Africa and the Middle East and Asia and Oceania respectively. Participating were 393 people from 57 countries considering together the potential of soybeans as a major world food crop. Information and research findings were exchanged and field trips to experiment stations were features of each conference.

Significant progress was made in the area of linkages and networks. The ISVEX program provides direct linkages to soybean programs in over 90 countries and is supplemented by site visits by INTSOY staff in other disciplines. International agricultural research centers, research institutes and other institutions executed memoranda of understanding with INTSOY to take up work of mutual interest. The beginnings of an international soybean research network were made.

The INTSOY Newsletter proved to be a useful vehicle for dissemination of information. From an initial issue of 500 copies, the Newsletter mailing list expanded to over 1,600, with over half the issues going to overseas addresses. The INTSOY Publications Series contributed to the joint UIUC and UPR/MC soybean response capability as well as providing a means of making research results readily available to those interested in tropical and subtropical production. Of the 10 publications prepared during the period of the previous research contract three dealt with the economics of marketing or production of soybeans, two with identification of insects or diseases, one with a description of soybean cultivars released in the U.S. and Canada, one with the results of the first ISVEX and set of proceedings for each of the three regional soybean conferences.

Training courses in "Soybean Processing for Food Uses" and "Technical and Economic Aspects of Soybean Production" were developed and conducted. Country programs, chiefly under aegis of task orders written under an AID Basic Ordering Agreement strengthened the development of advisory response capability.

With the foregoing record of accomplishment, the tasks remaining to be addressed, and the level of competence exhibited by the INTSOY staff the Agency issued a new contract, entitled "Development of Improved Varieties of Soybeans and Supporting Cultural and Marketing Practices for Production in the Tropics and Information Delivery Systems" with effect for three years commencing April 1, 1976.

B. Project Objectives as Stated in Contract

The purpose to which this contract contributes is the development and exploitation of the inherent potential of the soybean as an efficient source of high quality edible oil and high quality protein for diets of both the rural and urban poor. Six continuing objectives address the development of technology and generation of knowledge. They include:

1. Development of improved genetic materials for use in LDC soybean breeding programs.
2. Improvement of technologies for rhizobium production and management in tropical ecologies.
3. Management and expansion of the knowledge base in soybean production, protection and utilization.
4. Improvement of the knowledge, useful for disease control in soybeans.
5. Development of model insect, weed and disease control management systems which can be adapted or modified for specific environments.
6. Development of improved seed storage technology.

A seventh objective was to be completed during the first year of operation, namely "Phasing out of previous activities on home processing of soybeans by October 31, 1976." This objective was realized and a final report entitled "Soybean Processing Using Low Level Technology" was submitted to the Agency in December, 1976.

C. Accomplishment by Objectives

The interdisciplinary approach utilized in INTSOY programs is well illustrated in the following reports of progress on each of the six continuing objectives. Individual researchers cooperate in addressing problems associated with interrelated objectives in a method of operation that maximizes the limited personnel, facilities and fiscal resources available to the program. Summaries of progress under each continuing objective are discussed below. Complete reports by individual scientists and their collaborators detailing their contributions over several objectives are presented in Appendices I-VI.

Objective 1. Development of improved genetic materials for use in LDC breeding programs.

Substantial assistance in work toward this objective is provided by the INTSOY plant breeder stationed at the UPR/MC under 211(d) grant AID/CM/ta-G-73-49. Results of the superior work of Dr. E. Hamer Paschal, II, are discussed in the annual reports of that grant and will not be covered in this report. Work in soybean variety development, cooking quality, disease and insect control as they relate to identifying characteristics appropriate for incorporation in a breeding program is discussed below.

Soybean Variety Development. The major objectives of soybean variety development are high yield and high protein per unit of land area, suitable photoperiod response, resistance to yield and quality limiting diseases and insect pests and wide adaptability in tropical and subtropical environments. The primary thrust in the soybean variety development work has been through the International Soybean Variety Evaluation Experiment (ISVEX). This comprehensive variety testing program in which collaboration is by voluntary association, has grown from 33 countries and 90 locations in the first year of the research contract to over 100 countries and 290 locations at the completion of 1976. In this experiment, a standard variety trial is grown at each location and data are collected and returned to the trial coordinator at Urbana-Champaign for analysis. Initially, the trial included 20 varieties in U.S. maturity groups I to IX. During the second year, the number of varieties was reduced from 20 to 15 and included the 12 best performing varieties in ISVEX-1. Some cooperating LDCs desired short-season soybean varieties to fit local environmental conditions or cropping systems. In response to these interests, two sets of varieties were established, an early set of varieties representing U.S. maturity groups 00 and V and a later maturing set of varieties representing U.S. maturity groups III to IX. In 1976, this was expanded to include six different sets made up from 50 cultivars. These sets are organized by zones which are based on latitude and altitude. Cooperators provided the land, labor, fertilizer, and management necessary for the experiment. Instructions for management and data collection were sent to each cooperator to standardize methods among sites. General information about the site and the experiment was returned to INTSOY. This information included details of latitude, elevation, and soil conditions of the site plus date of planting and harvesting, amount of moisture, fertilizer used, local varieties tested, and diseases and insects of the environment. Data reported for each plot by cooperators included yield, seed weight, days to flower, days to maturity,

canopy height at flower, plant height at maturity, and estimates of lodging and shattering. Seed samples were also returned to INTSOY for protein and oil content analysis.

The high grain yield achieved in experimental plots with good management indicates that soybeans do have production potential in many new areas of the world. Temperate environment cultivars do not produce ideal plant types when grown in the tropics but extensive breeding programs can improve these characteristics. The ISVEX has provided answers to some questions about the adaptability of temperate zone cultivars in the tropics and questions relating to seed quality and growth at high altitudes.

Data is being received from the fourth ISVEX conducted during 1976. To date, data has been received from 55 sites in 31 countries which tested ISVEX-4. ISVEX-5 is now under distribution. Four sets of cultivars are being used in ISVEX-5. These sets of cultivars are organized by environmental zones utilizing cultivars that have performed well in past experiments and new cultivars with potential in each environment. At this time ISVEX-5 has been requested by 143 sites in 80 countries. Eighty trials have already been distributed.

In 1976, new material increased in Puerto Rico was distributed to selected locations. The purpose was to identify potential areas of adaptation for these new cultivars and measure their performance relative to established cultivars with proven potential for high yields. This Soybean Preliminary Observation Trial (SPOT) has been designed to serve as a screening test for newly developed cultivars. In 1976, SPOT was conducted at six sites which represent the range of environments where ISVEX has been successfully grown. These sites were in Ecuador, India, the Philippines, Puerto Rico, Rhodesia, and Sri Lanka. Data was received from these sites and is presently being analyzed. SPOT-2 is presently being organized.

SPOT-2 will include six new entries. These will replace those entries in SPOT that showed the poorest performance. The number of locations for SPOT-2 is also being increased. This will allow for a better evaluation of the SPOT material. Ten locations have already been contacted. These locations are in Puerto Rico, Ecuador, Peru, Ghana, Rhodesia, Sri Lanka, India, Thailand, Indonesia, and the Philippines.

Joint experiments were organized and carried out with the International Institute of Tropical Agriculture (IITA) and the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) in 1976 to eliminate duplication of testing the same material at the same research stations. In 1977, joint experiments are to be carried out with IITA. These joint experiments originally carried out in the 20°S - 20°N region of Africa have now expanded. Due to cooperator interest in other areas, 14 sites have requested joint INTSOY/IITA experiments outside of Africa. As in the past, the Food and Agriculture Organizations of the United Nations (FAO) and the French Institut de Recherches Agronomiques Tropicales et des Cultures Vivrieres (IRAT) have requested trials. These trials are primarily requested from Africa and Asia.

Three other studies in variety development illustrate the complementarity of work between the research contract and the INTSOY 211(d) grant AID-CM/ta-g-73-49. Two of these studies mentioned in previous reports have been completed. Articles are being prepared for publication. One study concerned itself with the effects of storage conditions and environment during maturation on soybean seed quality and crop performance. The findings may permit the identification of steps to slow down the decline in vigor and viability in soybean seeds stored in tropical environments. This will be important in breaking the barrier of seed storage problems under tropical conditions. The other study identified a genotype which possesses unique photoperiod insensitive properties which will be useful in the breeding of soybean lines with wide latitudinal adaptation.

Another student conducted research which tested three soybean varieties planted at three planting dates for differences in seasonal nitrogen fixation activities. The research is helping to determine when during the growing season nodule activity is most necessary, and when nodule activity is not used by the soybean. Results will be helpful in determining the best planting date (if the grower is forced into planting at a particular time) as regards seasonal nitrogen fixation. A M.S. dissertation on the subject will be completed by August 1977.

Soybean Performance: Effects of Environmental and Management

The extensive soybean testing network (ISVEX) established by INTSOY has shown that the yield potential of soybeans in tropical areas is similar to that in temperate zones. However, little is known about how environmental and management variables influence yield under tropical conditions. Therefore, data collected in 1974 through ISVEX were used to separate and quantify the effects of environment and management on soybean performance in the tropics.

The ISVEX project included 86 sites in 1974, but cooperating researchers at only 25 sites returned information on all the variables pertinent to our analysis. Countries represented in the study were: El Salvador, Guyana, Indonesia, Ivory Coast, Malaysia, Philippines, Puerto Rico, Rhodesia, Sri Lanka, and Thailand.

At each site, data were collected on agronomic, environmental, and management variables. Agronomic variables included yield and other characteristics indicative of plant growth and development. Environmental variables were those determined by geographic features and weather. Management variables were those directly or indirectly controlled by the cooperator. Plant population, nodule number, and nodule dry weight were included among the management variables since they depend to a considerable extent on management decisions made by the cooperator.

Simple correlation coefficients were computed to determine linear associations between various individual agronomic, environmental, and management variables. In addition, multiple regression analyses were performed to examine the relationships between the individual agronomic variables and the groups of variables designated as environmental and management.

Simple correlation coefficients between the individual agronomic, environmental, and management variables are presented in Table 1. Coefficients of determination for regression models relating agronomic variables to environmental and management variables are given in Table 2.

Agronomic characteristics significantly correlated with yield were seed weight and plant height. Highest yields were produced under conditions which resulted in the tallest plants and/or heaviest seeds.

The range of observed mean site yields in this study was 519 to 4,450 kg/ha. Differences in yield among sites were not significantly correlated with any of the individual environmental or management variables measured (Table 1). The coefficient of determination for the multiple linear regression model including only the environmental variables [R^2(E)] was not significant (Table 2). The coefficient of determination for the multiple linear regression model including only the management variables [R^2(M)] was significant. After adjustment for management effects, the five environmental variables did account for a significant proportion of the variability [R^2(E')] in yield. However, the effects of the six management variables, after adjustment for environmental effects, accounted for more than 2.7 times as much of the variation in yield as did environment [R^2(M')]. This result strongly suggests that management practices may be of greater importance than altitude, latitude, daylength, maximum temperature, and minimum temperature as determinants of soybean productivity in the tropics. Also, the high mean yield for the 25 sites (2034 kg/ha) and the lack of a strong association between environmental variables and yield suggests that the group of cultivars tested may be grown satisfactorily in tropical environments.

Yield was positively associated with plant height. Under photoperiodic conditions which stimulate early flowering, such as those encountered in this study, the final performance of the crop can be expected to be quite dependent on the conditions during early growth. While yield did not correlate well with individual environmental and management variables, the combination of management practices which interacted favorably with environmental variables to produce taller plants apparently also tended to produce higher yields.

An increase in total N availability, as inferred by an increase in the number of nodules and nodule dry weight, was associated with a significatn increase in plant height but not yield. Since soybeans are known to respond to applied nutrients when these are deficient in the soil, the lack of a significant association in this study is interpreted as an indicator that P and K were generally available in adequate amounts. The relationship between plant stand and yield was also probably not significant because of the high mean plant population achieved at most sites in this study.

Results are available which show that soybeans have produced acceptable yields in the tropics. This study included only U.S. cultivars which are commercially available. Therefore, cultivars currently exist which can be used to establish soybean production projects in tropical countries. Breeding programs for the development of better adapted

cultivars should follow the establishment of soybean production. Cooperation between countries and international institutes, through the sharing of research results and newly developed material, should minimize costs involved in establishing new production programs.

This study has shown that management variables can be more important than environment in explaining among site variation in yield. Therefore, considerable effort must be made in training farmers in the proper management of soybeans when they are introduced as a new crop in a region. Management practices differ among regions, and research should be conducted to determine appropriate practices for each region. Without an adequate understanding of the crop, farm yields will probably be low and soybeans may be rejected as a cash or food crop.

Additional information is needed to determine the effect of the different variables on individual soybean cultivars. Knowledge of cultivar response to management and environmental variables will help in the selection of cultivars for evaluation in new areas with soybean production potential.

Soybean Seed Quality Studies: Production Aspects

Variable soybean seed quality frequently necessitates the use of excessive seeding rates to compensate for poor emergence. In tropical regions, where yield of early maturing cultivars is particularly dependent on establishment of adequate plant stands, seed quality problems may limit the commercial use of such varieties.

In 1976, a study was initiated to 1) monitor seed quality of introduced, early maturing cultivars in different tropical environments, 2) determine the effects of variations in seed quality on stand establishment and productivity of the subsequent soybean crop and 3) evaluate the usefulness of a foliar applied fungicide in improving soybean seed quality in the tropics.

Trial plans, seeds, inoculant, and fungicide for foliar application were supplied for installation of the study in Thailand, Sri Lanka, Ecuador, Puerto Rico, California, and Illinois. Management instructions were similar to those provided with the International Soybean Variety Experiment except for details of treatment application. Treated plots rereceived two applications of mixture of Benlate and Captan, the first at early pod set and the second 18-20 days later. The few carefully selected locations chosen for the study represented a broad range of environment conditions.

Agronomic data and seed samples have been received from six of the eight locations where the trial was planted. The seed samples were forwarded to Mike Ellis, INTSOY pathologist, University of Puerto Rico for bioassay. Results of this phase of the study are not yet available.

Table 1. Simple correlation coefficients between individual agronomic, environmental, and management variables measured at 25 locations in 1974.

	Agronomic Variables 1/				
Agronomic Variables	Yield	Seed Weight	No. days to flower	No. days from flowering to maturity	Plant height
Seed weight	.49*				
No. days to flower	-.10	.00			
No. days from flowering to maturity	.22	.46*	.27		
Plant height	.46*	-.13	.31	.27	
Environmental Variables					
Altitude	-.12	-.01	.81**	.32	.31
Latitude	.07	-.46*	.22	.10	.57**
Daylength	-.16	-.35	.36	.38	.44*
Max. temperature	-.03	-.21	-.60**	-.20	-.08
Min. temperature	-.01	-.16	-.68**	-.18	-.28
Management Variables					
Nodule number	.19	-.18	.20	.21	.67**
Nodule dry weight	.37	.13	.06	.09	.47*
Nitrogen	-.02	-.43*	.00	-.08	.22
Phosphorus	.05	-.26	-.03	.14	.09
Potassium	-.38	-.33	-.14	-.29	-.35
Plant Stand	.33	.06	.03	-.06	.13

1/ Agronomic variable are based on the mean performance of 10 cultivars common to each site.

* significant at the 5% level

** significant at the 1% level

Table 2. Coefficients of determination from regression analyses relating agronomic variables to environmental and management variables.†

Dependent Variable	$R^2(E)$	$R^2(M)$	$R^2(E+M)$	$R^2(E')$	$R^2(M')$
Yield	.08	.53*	.79**	.26*	.71**
Seed weight	.35	.29	.72*	.43*	.37
No. days to flower	.69**	.11	.76*	.65**	.07
No. days from flowering to maturity	.20	.45	.70*	.25	.50*
Plant height	.42*	.66**	.71*	.05	.29

† $R^2(E)$, $R^2(M)$, $R^2(E')$, and $R^2(M')$ are defined in the text. $R^2(E+M)$ is the total variability accounted for by the management and environment variables measured.

* significant at the 5% level

** significant at the 1% level

Table 3. Effects of foliarly applied fungicides on yield and seed quality of soybeans, 1976

	Locations					
				Illinois		
	Puerto Rico	Ecuador	California	Urbana	Brownstown	Dixon Springs
		Yield, kg/ha				
Treated	1868	2554	3150	3322	2170	4048
Non-treated	1564	2698	3223	3235	1926	3763
Difference 1/	304*1/	-144*	-73	87	244*	285
		Quality 2/				
Treated	2.42	2.50	-	1.58	2.08	2.42
Non-treated	3.58	2.75	-	2.00	2.00	2.17
Difference	-1.16*	-0.25		-0.42*	0.08	0.25

1/ An asterisk indicates that treated and non-treated are statistically different at the 5% level of probability.

2/ Quality was noted on a scale of 1 to 5, with 1 indicating very good quality and 5 very poor quality.

Effects of the foliarly applied mixture of fungicides on soybean yield and seed quality are presented in Table 3. Yield was not increased by treatment at either of the two locations, Boliche, Ecuador or Davis, California, where the growing season was dry and irrigation required for proper development of the crop. At the other locations, yield was increased by from 3 to 18 percent. Seed quality, as visually rated, was significantly improved at Isabela, Puerto Rico and Urbana, Illinois only.

The most important results of this study will relate to the effects of treatment on seed quality. Several seedborne pathogens have been shown to reduce seed viability and seedling vigor. Control of these through appropriate management of the crop may provide a short-term solution to pathogen related seed quality problems in the tropics. Incorporation of improved seed quality characteristics - including resistance to seedborne pathogens - through breeding is considered to be a more valuable long-term goal and is a primary focus in the INTSOY program in Puerto Rico.

Soybean Cooking Quality and Nutritional Studies of Prepared Foods.

1. Research was continued on varieties of soybeans which become the most tender upon cooking. Thirty varieties of soybeans were tested for tenderness after cooking by one of four methods. The objective tenderness of each variety was compared to its chemical composition and physical characteristics in a search for factors which correlated with the tenderness of cooked whole soybeans.

Data from varieties harvested in 1974 were reported last year. These experiments were repeated with the 1975 crops in order to test consistency from one crop year to the next. The data indicated that similar factors influenced the tenderness of cooked whole soybeans in both crop years.

a. The most important factor affecting objective tenderness of cooked soybeans was the composition of soaking and cooking solution. Use of bicarbonate solutions instead of tap water increased the tenderness of soaked and cooked soybeans nearly 3-fold.
b. Soaking before cooking increased tenderness about 1.8-fold compared to cooking without soaking.
c. The relative tenderness among varieties varied with soaking and cooking treatment. A given variety was not always more tender than another given variety under all four soaking and cooking conditions. However, this variation was not as great as reported last year. This year, three varieties, Amsoy '71, Hark and Kanrich were among the six most tender varieties in all four treatments.
d. The tenderness of each variety of cooked whole soybeans was not consistent from one crop to the next. Only Bonus and Cobb soybeans were among the six most tender varieties in both years.
e. Several physical characteristics of the dry raw whole soybean, but no chemical characteristics, correlated with the tenderness of cooked, whole soybeans. Factors which correlated well with tenderness included weight/bean, volume/bean, density/bean, rehydrated weight/bean and rehydrated volume/bean.

2. A study was completed on the effects of using different sieve fractions of milled corn and different preparation techniques on the nutritional quality (protein efficiency ration) of drum dried corn:soy flakes.

The study was begun during the last contract period on the effects of different sieve fractions of dry milled corn and different preparation procedures on the nutritional quality of drum dried corn:soy flakes. It was completed early in this contract year. Corn fractions included ground whole corn, commercial cornmeal, and three fractions from ground whole corn viz., over 22 wire mesh screen, over 32 wire mesh screen and through 32 wire mesh screen. Different corn fractions were used for preparation of drum dried corn:soy flours and the PER's of the flours were measured. PER's were affected by the fraction of corn used but they were not significantly affected by gelatinization of the corn:soy before drum drying. Increasing the amount of corn from 67% to 80% of the blend improved the PER for only one of the three corn fractions tested. These effects could not be accounted for by the balance of essential amino acids in the corn:soy flours.

Disease Control

Experiments were conducted on the identification of virus resistant tropically adapted soybean germplasm. Soybean mosaic virus (SMV) is the most widespread soybean virus. It is transmitted to healthy plants in the field by aphids that feed on infected plants arising from SMV-infected seeds. Several lines and a few named varieties have been reported by others as having a degree of resistance to SMV, but experience has shown that all resistance available in named soybean varieties can be broken by SMV isolates other than those used to develop the resistant variety. No SMV resistance has been reported previously in tropically adapted soybean germ plasm. Plantings of germ plasm in the INTSOY collecting were made at Isabela, P.R. in 1976, some of which were inoculated with the Illinois severe isolate of SMV. (see Appendix III for details) Four P.I. lines and the varieties Majos, Nela, Yelnada and Buffalo were apparently resistant to infection by the SMV isolate used. While results are incomplete with the P.I. lines and three varieties, there is some indication that Buffalo may represent a new source of resistance to a wide range of SMV isolates. Further tests are in progress to confirm this tentative conclusion, as are tests with the other seven apparently resistant lines.

Two experiments have been conducted to screen for possible sources of resistance to cowpea mosaic virus (CPMV). One experiment was done in the greenhouse at Urbana and the other in the field at Isabela, P.R. In the greenhouse, 121 selected named varieties in the maturity group 00 to IV were mechanically inoculated with one isolate of CPMV and the reactions were observed. There was no indication of resistance in any of the varieties tested. Under field conditions at Isabela, 100 more varieties including experimental lines randomly selected from maturity groups V to X were tested. Results from this experiment indicated that among the varieties and lines tested, none were completely free of symptoms. However, there were several lines showing consideration variations in reactions to the virus (Table 1), with some plants remaining uninfected even though they were inoculated twice, while others showed CPMV symptoms. The experiment has not yet been completed. Seeds from inoculated plants that remained healthy are now being harvested and will be tested again in the greenhouse to confirm the reactions to CPMV.

Resistance to soybean yellow mosaic virus in India was found by Dr. B. B. Singh in the P.I.171,443. This line has been increased at Isabela for future testing with the whitefly-transmitted viruses that affect soybeans in tropical America. Experiments will begin in the next contract year.

Tests were also conducted to observe the rate of SMV seed transmission in both tropically adopted germ plasm and northern germ plasm. Preliminary results indicate that several of the tropically adopted germ plasm lines have low rates of seed transmission (less than 1%) from fully infected plants.

Seed was also obtained from the U.S. Regional Soybean Laboratory in Urbana for selected lines of maturity groups II and III. These were grown near Urbana and inoculated with the Illinois severe isolate of SMV. Leaves from plants expressing no symptoms were tested using techniques developed at UIUC by Soong and Milbrath (see Appendix III for details). Many of the soybean lines tested had relatively low rates of SMV seed transmission. Thirty-five lines were chosen for further testing.

Insect Control

Contributions toward the development of insect resistant genetic material focused on four areas: 1) physiology of host-plant resistance to insect pests; 2) interaction of host-plant resistance and predation; 3) interactions of host-plant resistance and insecticides; and 4) screening of breeding lines.

The physiological studies, seek to isolate compounds in soybean plants responsible for insect resistance phenomena and to determine physiological reactions of the pests to these compounds. When completed, profiles of the major components (aminoacids, lipids, etc.) will permit detailed characterization of the resistant and susceptible soybean lines.

Studies on the interaction of host-plant resistance and predation were conducted using caged soybean plants on which were established colonies of Mexican bean beetle larvae. Injury to the susceptible plants was lower than to resistant plants at low predation levels because larvae on the latter ate more to complete development. At high predation levels injury to resistant lines was lower because the larvae were exposed to the predators for a longer period of time and were billed as third visitors before most of the feeding injury occurred.

The hypothesis that insects feeding on resistant lines may become more susceptible to lower doses of insecticides was tested on soybean looper and Mexican bean beetle larvae. Preliminary results indicate a substantially (8 to 10-fold lower dosage of insecticide is required to kill the same population of larvae reared on resistant as opposed to susceptible soybean plants. If these preliminary results are confirmed, the use of partially resistant varieties could be tested in control of such hard to kill pests as the soybean looper.

Progenies of the UIUC soybean breeding program were screened for resistance against the Mexican bean beetle and other defoliators at four locations in the U.S. Lines that performed best at all four locations will be included in preliminary yield tests in 1977. Ten of the best selections were sent to Puerto Rico and included in a test on transmission of cowpea mosaic virus by Cerotomic ruficorius. Although C. ruficorius populations were low these entries showed some of the lowest levels of damage and also some indication of reduced virus infection.

Objective 2. Improvement of technologies for rhizobium production and management in tropical ecologies.

Site selection and recruitment of personnel for the newly authorized position of rhizobium microbiologist were the principal activities addressed under this objective in the report period.

After considerable discussion and review of possible tropically oriented locations at which the INTSOY microbiologist might be stationed, the decision was made to base the position at the University of Puerto Rico, Mayaguez Campus. Several factors influenced the decision. The island of Puerto Rico, with its variety of soil types and rainfall patterns, has a subtropical climate and is, itself, an excellent laboratory for soil and crops research. The INTSOY plant breeder, funded by a companion 211(d) grant

is located at UPR/MC and interaction between these two scientists is vital. The University of Puerto Rico added a soil microbiologist to its staff to serve on a 211(d) grant in tropical soils. The stationing of the INTSOY rhizobium microbiologist in the same laboratory offered each man a colleague-to-colleague relationship. Finally, UPR/MC made available substantial laboratory space for both microbiologists. Total equipment needs are reduced through sharing of facilities.

Intensive recruitment efforts attracted applications from several highly qualified microbiologists from among whom Dr. R. Stewart Smith, Agricultural Laboratories, Inc., Columbus, Ohio, was selected. Dr. Smith reports for duty in Puerto Rico in June 1977.

Dr. Smith has prepared an outline of rhizobial research that, in broad terms, will serve as his work plan, consistent with the objectives of the contract. A number of individual research projects may develop into large scale activities, utilizing the use of graduate students and/or cooperating INTSOY staff members. Priorities for research will be given to those studies listed in the research contract Output #2.

The overall plan of work addresses both basic rhizobial research and the production and use of rhizobium inoculants to give the program balance between the basic and applied aspects. The workplan is as follows:

I. Basic Rhizobial Research

A. Strain evaluation and selection

1. Soybean variety - Rhizobium strain interaction and fixation efficiency. Cooperate with Dr. Paschal in testing the most promising varieties for the tropics and sub-tropics.

2. The influence of soil environmental factors on rhizobial strain survival and infectiveness. Factors including soil temperature, soil moisture, pH, aluminum, organic matter and soil type.

3. Examine and identify reliable parameters and procedures for the evaluation of nodulation by knowledgeable cooperators.

4. ISVEX or SPOT type of strain testing and evaluation. Initial work in Puerto Rico and later with selected tropical cooperators.

B. Factors influencing rhizobial growth, survival and soybean nodulation in tropical and sub-tropical soils.

1. The influence or the various soil types and environmental factors on the performance of Rhizobium japonicum in the tropics.

2. The influence of seed quality and vigor on the quantative establishment of nodulation. Cooperate with Dr. Ellis in the establishment and surveillance of the variability in seed quality.

3. The influence of certain insect pests (Cerotoma) which in the larvae stage feed upon soybean roots and nodules. Cooperate with Dr. Irwin and Cathy Eastman in evaluating the damage this pest may do to the nitrogen fixing system of soybeans in the tropics.

4. The influence of herbicides and weed competition to the overall soybean available nitrogen supply and also the influence on nodulation establishment. Cooperate with Dr. Riveros in the establishment, maintenance and evaluation of this study.

5. Investigate the various soil types and environmental factors available at the several Experimental Stations in Puerto Rico with respect to rhizobial establishment and survival longivity.

II. Rhizobia Inoculants - Production and Use

A. Rhizobial populations necessary to establish nodulation.

1. Determine indigenous rhizobial populations at the various Experimental Stations and correlate populations to time of application and method of use.

2. Determine the minimum number of Rhizobium japonicum necessary to achieve maximum nodulation and yield at various Experimental Stations. Expand similar studies to LDC field trials.

B. Inoculant carriers and method of inoculant application.

1. Evaluate inoculant carriers for the tropics (peat, coconut, water, etc.).

2. Evaluate inoculant forms and their feasibility in the tropics (seed applied powder, granular, seed applied liquid, frozen soil applied liquid).

3. Evaluate inoculation methods and their feasibility in the tropics.

a. seed application - at planting, pelleting, preinoculation.
b. soil application - granular and liquid.

4. Consider inoculant carriers and application methods suitable for both the small hand-planting soybean farmer and also the larger mechanized soybean farmer.

5. Study inoculant storage and transport conditions in the tropics and evaluate the performance of various inoculant carriers, forms and methods under these conditions. (Output 2a)

C. Reinoculation of established soybeans without nodulation.

1. Examine techniques for the inoculation of established soybeans.

2. Determine the number of rhizobia necessary with various soil types and environmental conditions.

3. Evaluate the influence of soil type on the reinoculation of established soybeans.

4. Determine the time period within which successful reinoculation can be achieved.

Objective 3. Management and expansion of the knowledge base in soybean production, protection and utilization.

Management of information on the production and protection of soybeans is accomplished primarily through three basic systems. They are TAXIR (Taxonomic Information Retrieval System), SIRIC (Soybean Insect Research Information Center) and IRCSA (International Reference Collection of Soybean Arthropods), respectively. These systems have been featured in previous INTSOY Newsletter and on site visits to the University of Illinois by soybean research and extension workers from abroad. Brief accounts of each system are described below.

TAXIR

The soybean germplasm data bank is a storage bank of information concerning soybeans. Information is obtained from five sources of germplasm:

1) Named soybean varieties
2) Plant Introduction lines
3) Genetic Type collection lines
4) Forage collections
5) Collections of wild species related to the cultivated soybean

The data are entered and stored according to TAXIR, the taxonomic information retrieval system which facilitates rapid retrieval of data from the bank.

The data bank is continually expanded as new courses of soybean germplasm become available. Addition of approximately 800 new Plant Introduction lines collected from several countries is in progress, bringing the total number of entries in the bank to approximately 4,500. The number of countries from which soybean germplasm has been obtained now stands at 108. A total of 175 descriptive bits of information can be recorded for each entry. These break down into 39 morphological and chemical characters, 40 genetic characters, 79 disease and insect pest characters covering 26 separate diseases, and 17 general pieces of information. Information on each entry is spread across 9 computer cards.

With each major addition of entries and descriptions, both the data bank and the TAXIR vocabulary which controls it are updated. Information for all 175 descriptions has not been recorded for all entries, but as the data become available from screening tests for disease resistance and other characteristics, it is entered into the bank. For example, chemical attributes of seed proteins analyzed over the past year by visiting scholars have been added to the bank and these can serve as useful indicators for the dissemination of soybean germplasm throughout the world. A bibliography of sources of information is maintained for future reference.

In early 1976 INTSOY Publication No. 9 was released, a booklet containing tables of morphological descriptions and responses to foliar, stem, and root diseases of 331 soybean cultivars released in the United States and Canada. Work is now in progress on revising pedigree descriptions for the 331 named varieties, and this is planned for publication in the near future. Many requests for such a pedigree listing have been received. Other requests for printouts of information from investigators both within the University and outside have been filled throughout the year.

The possibility of utilizing a visual display system in connection with the data bank has been discussed. This system would simplify correction and addition of information by providing direct access into the data bank, thereby circumventing the necessity of handling large numbers of computer cards.

SIRIC

The Soybean Insect Research Information Center (SIRIC) is a computerized information storage and retrieval system for the worldwide literature of arthropods associated with soybeans. SIRIC is primarily a service oriented unit, operating in close cooperation with the Soybean Entomology Research Team of the Illinois Natural History Survey and the University of Illinois.

Created in 1969, SIRIC was originally designed as a manually operated system capable of handling a few thousand citations. In the late 1960's there was a sharp increase in the number of papers published in the area of soybean entomology. The establishment of this Information Center was, therefore, a very timely decision.

In 1969 there was only a small number of entomologists working full time on soybean insect pests in the United States. In 1976 more than a dozen different centers, especially in the midwest and southeast, had entomologists working in multidisciplinary soybean research programs. The increase in the numbers of published papers is a direct result of the research being conducted in these institutions.

Basically these objectives are:

1. Compilation of the literature of soybean related arthropods and the establishment of a data bank for this literature.

2. Assistance to researchers in an international scale in the form of computer searches.

3. Assistance in special searches (usually manually done for types of information not covered by the data base).

4. Assistance to researchers in securing copies of documents in SIRIC's holdings.

5. Compilation and publication of comprehensive bibliographies on key soybean insect pests.

6. Assistance to researchers by providing literature surveys leading to the production of review articles and monographs covering areas of key importance in soybean entomology.

Basic operations of SIRIC consist of: a) updating, b) computer searches, c) special searches, and d) system upgrading.

During the time covered by this report 165 requests for service were filled in the form of computer printouts, manual compilations, copies of documents, and referral services. Requests outside the scope of SIRIC were routed to the proper institutions and specialists.

Sixty percent (98) of all requests for information or services came from researchers at the University of Illinois or their graduate students.

The remaining forty percent (67) were received from individuals in other institutions in the United States and abroad.

SIRIC Librarians were asked to help in training students and researchers in the use of reference tools and development of search strategies. In the spring of 1976 the use of SIRIC was the topic of a graduate seminar presented by students of Entomology 426 who were instructed by SIRIC staff.

Following the four bibliographies already published on the literature of arthropods associated with soybeans, SIRIC is working on the compilation of a fifth bibliography of two Heliothis species. Heliothis zea and Heliothis virescens (the corn earworm and tobacco budworm) are major pests of soybeans, corn, cotton, and tobacco and are considered the pests of greatest economic importance in the western hemisphere. The literature of these species comprises a large number of citations and studies are underway to use the computer for self-indexing the bibliography. If the self-indexing system proves successful this feature will be incorporated in future computer searches.

SIRIC provided literature searches to investigators in the following countries: Brazil, Colombia, El Salvador, Upper-Volta, India, Indonesia, Israel, Philippines, Puerto Rico, Rhodesia, Saudi Arabi, and Sweden.

The information center was visited by soybean specialists and administrators from the following countries: Brazil, Canada, Iran, Israel, Mexico, Pakistan, Peru, Rhodesia, Sri Lanka, Sweden, Taiwan, Thailand, and by the participants of the 1976 training program in soybean production. A lecture on the activities of SIRIC is presented on these occasions to familiarize potential users with the services offered by the center.

Most of these visitors manifest great interest in the published bibliographies since reference services are seldom adequate in many developing countries.

IRCSA

A reduction in personnel from 2.5 fte to 1.0 fte since 1975/76 has slowed up the progress of processing material. Nevertheless, during the last year a large amount of work was completed. Consolidation of workload and reorganization of priorities followed the staff reduction. The reorganization and consolidation phase is now complete.

Acquisitions of soybean-associated arthropods came mostly from El Salvador, Ecuador, Nigeria, Sri Lanka, Philippines, Mexico and Puerto Rico. A large backlog of material from Brazil has been processed, but much

still remains from Brazil to process. It is felt that an adequate knowledge of soybean-associated arthropods has been gained, that the collection has represented a large number of the total species composition of soybean fields from most areas of the Americas. What is needed is an expansion of material emphasizing Africa and Asia.

Over one hundred systematists are currently collaborating with IRCSA, some 30 percent of which are from outside the United States. This is a 10 percent increase over last year's collaborators. This network of collaborating systematists is extremely important to IRCSA if IRCSA is to maintain its high standard of accuracy in determinations of specimens.

Work is nearing completion on a treatise of the Cerotoma beetles attacking soybeans in the western hemisphere. These beetles are important because as larvae they attack the nodules containg nitrogen-fixing bacteria, as adults they feed on leaves, often causing severe defoliation and also as adults, they are the prime vectors of several important viruses of soybeans. Among them bean pod mottle virus and cowpea mosaic virus are two of the worst in the western hemisphere. A clear, concise description of each species, biologies, distributions, natural enemies and other important facts will be summarized in this publication.

The complex of stinkbugs in soybeans is large and often confusing. Certain species are of economic importance as pests, others are important predators, still others apparently are inconsequential to the production of soybeans. It is important for research agronomists, extension personnel and farmers to be able to separate and categorize these species that form complexes. In order to alleviate these perplexing identification problems, a manual on the stinkbugs of soybean in the western hemisphere has been initiated.

To date, literature has been searched; IRCSA houses a rich collection of stinkbugs especially from the Americas. Drawings of the various species will be initiated soon. Distribution records are already computer tabulated.

More complete information on the SIRIC and IRCSA systems, and their integration into the overall pest management area, may be found in Appendix V.

Results from the ISVEX program are also being adapted for computer use and retrieval. Three computer programs have now been developed to handle the ISVEX raw data:

1) A program to statistically analyze data from individual ISVEX sites.

2) A program to statistically analyze combinations of ISVEX sites which can then be used in the ISVEX annual reports.

3) A program to printout randomizations of the required number of treatments and replicates to be carried out in the following year.

Data from ISVEX results in 1973 and 1974 have been combined and analyzed statistically.

Expansion of the knowledge base is advanced by means of 1) the INTSOY Publications Series, 2) publication in referred journals and other scientific publications by individual staff and 3) conferences and workshops.

The INTSOY Publications Series has been adequately discussed in previous reports to the Agency. Eleven publications have been issued to date. They include the proceedings of the regional conferences for soybean research and extension workers from Latin America, Africa, the Middle East, Asia and Oceania, the results of the first two years of ISVEX, three papers on the economics of producing and processing soybeans in India, a selection of useful literature on soybean entomology, an annotated bibliography of soybean diseases and a morphological description of over 300 soybean cultivars released in the United States and Canada.

INTSOY staff, cooperatively and individually, have compiled a substantial list of publications. These were detailed in Appendix V of the final report on contract AID/CM/ta-c-73-9. Additional publications by INTSOY staff during the present report period are included in their detailed reports.

In March 1977 a workshop on soybean rust was held in Manila, the Philippines. Cooperating with INTSOY were the Asian Vegetable Research and Development Center (AVRDC) the Philippines Council for Agriculture and Resources Research (PCARR) and other Philippine government agencies. Contract funds were made available to invite eminent scientists from Asian countries where soybean rust is a major problem to Manila for intensive sessions on strategies to attack the problem. Areas of cooperative interest were established and plans were made to develop a coordinated research effort on soybean rust and to seek the funds necessary for its implementation. The papers presented at the workshop constitute a useful "state of the arts". These papers are being edited and will be published as an edition of the INTSOY Publication Series during the latter part of 1977.

The INTSOY Newsletter, while not intended to be a major vehicle for distribution of detailed knowledge on soybean production, protection and utilization, is an effective means of bringing such information to the attention of interested readers. From an initial printing run of 500, the Newsletter now reaches approximately 1600, over half of whom are overseas.

Objective 4. Improvement of the knowledge useful for disease control in soybeans.

Disease control is addressed on an interdisciplinary basis utilizing scientists in plant pathology and entomology as the lead disciplines. Work concentrates on fungicidal, viral and vector control approaches.

A major thrust of the fungal and bacterial research specialist is to better understand the effect of various microorganisms on soybean seed and seed quality and develop means of control.

Studies on nonchemical control methods on charcoal rot were continued. The competitive sophrophyte colonization of Machrophomina phaseolina was reduced by increasing organic matter, and hence the microbial population of the soil, and was also influenced by soil type, source of inoculum and subotrate.

Seed borne fungi on common bean was observed. Mature seeds were removed from dry bean pods that had been, or had not been, in contact with soil in the field. Fungi representing 14 genera were recovered. Significantly more internally seed-borne fungi, and less germination and field emergence, were recorded among seeds from pods in contact with the soil than from pods not in contact with soil. Field emergence of seeds from pods treated with Thiram was singificantly higher than for non-treated seeds. A tetrazolium test showed that the percentage of viable seeds was higher than indicated from the germination studies for seeds from pods in contact with soil, but not for seeds from pods not in contact with soil.

Seed-borne microorganisms in soybean were studied. Fungicide seed treatment of soybeans can reduce the quantity of seed-borne fungi and increase the emergence of low-quality seeds, but may have little or no effect on high-quality seeds. New methods for treating soybean seeds are being studied. One promising method is the use of solvents such as dichloromethane and polyethylene glycol for incorporating antibiotics into soybean seeds, plus conventional seed treatment fungicides. Antibiotics control seed-borne bacteria and fungicides control the gungi. When penicillin G was combined with Arasan 50 Red, the mean stand count in the field was near the percent germination measured by a warm test in the laboratory.

Fungicide sprays were studied for control of internally seed-borne fungi: D. phaseolorum var. sojae (Phomopsis sp.) is seed-borne in soybeans and causes food and stem blight as well as seed deterioration. This fungus increases in occurrence in seeds and, concommitantly, germination decreases as harvest dates are delayed. The occurrence of this pathogen in soybean seeds can be reduced with foliar applications of benonyl, a systemic fungicide which is taken up by germinating soybean seeds and is systemic in soybean seedlings. The amount of seed-borne D. phaseolorum var. sojae was significantly reduced below that of non-sprayed when soybean plants were sprayed with one of the following fungicides or fungicide combinations: benonyl, benonyl plus zinc-iron, coptofol, chlorothodonil, moncozeb, thiabendazole, and thiophonate-methyl. The occurrence of D. phaseolorum var. sojae was always less in seeds from plants sprayed with benonyl than those from the other fungicides. Soybean plants sprayed with benonyl remained green longer than nonsprayed plants. D. phaseolarum var. sojae may cause early defoliation and death of soybean plants. It is also possible that benonyl may be phototoxic in delaying maturity.

For details on these studies, and on those conducted on common beans, cowpea and Stylosouthes scabru, as well as assessment of factors that influence diseases of soybeans and cowpea, please refer to Appendix IV.

Studies by the soybean virologist focused on soybean mosaic virus (SMV), purification and characterization of whitefly-transmitted viruses, virulance of SMV isolates from soybean germplasm, detection of seedborne SMV in soybean seed lots and the characterization of cowpea mosaic virus (CPMV) isolates.

Studies on the mechanism of seed transmission of SMV

a. Effect of time of inoculation. Soybean plants were inoculated at different stages of reproductive development to determine the effect of the time of inoculation upon rates of mottling and seed transmission. Two ten-plant hills of the cultivar

Williams were mechanically inoculated with SMV (Illinois severe isolate) two weeks after planting. Twenty plants were similarly inoculated each week for eight consecutive weeks. The first two inoculations were done before flowering. The inoculations of the remaining six weeks were done during the various reproductive stages of the plant according to the developmental descriptions proposed by Fehr et al. (1971). The third inoculation was done at the R1 stage where there is one flower at any node. The plants were in stage R2 during the fourth week where there is a flower at the node immediately below the uppermost node with a completely unrolled leaf. In the fifth week the plants were in R4 where there is a pod two cm long at one of the four uppermost nodes with a completely unrolled leaf. The next significant stage of development was during the seventh week of inoculation when the plants were in R6. During this stage there is a pod containing full size beans at one of the four uppermost nodes with a completely unrolled leaf.

At the end of August all of the symptomless plants were removed. When the plants reached maturity each of the eight inoculation sets were harvested and threshed separately. Two samples of 200 seeds were counted out from each set, determining the percentage mottling in the process. Two hundred seeds from each set were germinated and 100 randomly selected seedlings were individually indexed with the detached Top Crop bean method for the presence of SMV.

b. Presence of SMV in reproductive tissue during plant development. Another study was done to determine at what points in development virus could be detected in the reproductive parts of a plant. Again the development descriptions of Fehr et al. will be used with the addition of stages R7 and R8. R7 is when the plant is at physiological maturity with the pods yellowing and 10 percent of the leaves yellow. Stage R8 is harvest maturity with 95 percent of the pods being brown. Two different cultivars of soybeans were used, Merit and Midwest. Merit was selected because it was reported by Kennedy and Cooper to have a nonmottling character and not to transmit SMV through the seeds. Midwest was selected because Kendrick and Gardner reported it to have a high rate of seed transmission (29 percent).

The plants were mechanically inoculated with the Illinois severe isolate of SMV well before either cultivar flowered. Ten plants from each cultivar were examined during each of the following stages, R1, R2, R4, R6, R7 and R8. They were also checked after the harvested seeds were germinated and again after six months storage in the cold room. A sample from every node from each of the plants was indexed for the presence of SMV. In Merit 8, 10, 12, 12, 12, 12, 12 and 12 nodes were examined respectively during R1, R2, R4, R6, R7 and R8 germination and storage-germination. In Midwest 6, 11,

12, 12, 12, 12, 12 and 12 nodes were checked from each plant during the same respective stages. When each stage was checked ten plants were randomly selected from both lines. In stages and R2 entire flowers were indexed. In stages R4 and R6 entire pods were indexed. During R7 and R8 the pods and seeds were separately indexed. During the germination stage two seeds from each node-plant position were placed on moistened cellulose pads and placed in a germinator. When the seedlings had fully expanded primary leaves, one plant from each node-plant position was indexed individually. Seeds were stored in the cold room for six months. These were then germinated and indexed as above. The percentage seed mottling from each node-plant position was also determined from the seeds used in the germination stage.

The node position on the plant did not affect the seed transmission or mottling rates in either or the cultivars tested. In the early stages of both cultivars the virus was present in 100 percent of the material tested. As the material began to mature, the presence of the virus began to decrease. This decrease was more rapid in Merit, which fell to 0 percent, than in Midwest. This may be explained by the fact that Midwest matured much slower than Merit. The pods lost virus slightly faster than the seeds. Germination immediately after harvest or after six months storage did not affect the presence of virus in the seeds.

c. Mottling of the seed coat in the variety Merit. In a third study, each of 87 SMV symptom expressing plants of a population of the cultivar Merit were harvested individually. These plants had been inoculated with the Illinois severe isolate of SMV during the first trifoliolate stage. The seeds from each plant were checked for mottling percentage to determine if this trait was homogeneously expressed in this cultivar.

When Merit was inoculated with the Illinois severe isolate of SMV, the seeds produced by infected plants exhibited mottling. This is contrary to the work reported by Kennedy and Cooper in which Merit was reported to have a gene conferring nonmottling. When the mottling rate was determined for 87 individual plants a bimodal distribution appeared. This suggests that this variety is not homogenous for expression of seed coat mottling. The mottling rates ranged from 0 to 100 percent.

Purification and characterization of whitefly transmitted viruses

a. Bean golden yellow mosaic viruses. One of the most important unsolved problems in tropical agriculture is the cause and control of leaf curl and yellow mosaic diseases associated with pathogens transmitted by the ubiguitous tropical whitefly, *Bemisia tabaci*. Soybeans in India and tropical America are affected, sometimes seriously, by such diseases as are other legumes and many other crops.

A major breakthrough in the understanding of the causes of this important group of tropical plant diseases has been made in the past year by the INTSOY plant virology group. Whitefly-transmitted pathogens, long presumed to be viruses, have been difficult to work with because they were not characterized and they were not mechanically transmissible. Results obtained by the virologist at the University of Puerto Rico showed that the whitefly-transmitted pathogen causing bean golden yellow mosaic disease was mechanically transmissible. Joint research by UPR and UIUC virologists resulted in a method for purification of a novel virus from infected plants and proof that the virus was the cause of the disease. Subsequent results at Urbana and in cooperation with an electron microscopist at the University of Arkansas have extended knowledge about this unprecedented plant virus. We have learned that the amount of virus in infected tissues rises and then falls drastically, so that successful transmission by mechanical means occurs only during the time when the virus concentration is high. The virus has an unusual genome molecule; it is the first plant virus ever shown to contain single-stranded DNA. The virus causes cytopathological effects in infected cells that are unlike those caused by any other plant virus, and are somewhat similar to those caused by a group of single-stranded DNA animal viruses.

There are several ways in which these results are of practical significance. We know now why mechanical transmission of the virus was difficult, and we know better how to go about attempting transmission of other similar agents such as that causing soybean yellows or mung bean yellow mosaic in India, Sri Lanka and Pakistan. Mechanical transmissibility allows for efficient screening of germ plasm lines for resistance to disease. Identification of a virus causing these diseases also opens the way for studies on relationships of the pathogens causing diseases of unrelated crops that may be grown in the same area. Such studies could be conducted before only with vectors; now more critical and rapid studies will be possible using antisera produced against the various purified viruses. The possible relationships between viruses with similar properties that infect animals and plants always raises important questions about cross-infection. Identification and characterization of this new virus will allow such studies to be conducted.

Technical details of the methods and results of studies on the purification and properties of bean golden yellow mosaic virus are given in Appendix III.

Future plans for the next contract year include preparation of specific antisera to this virus, further characterization of the pathological alterations in infected plants, and further studies on the morphology and biochemical components of the virus itself.

b. Whitefly-transmitted viruses of soybeans. Studies on the whitefly-transmitted virus of beans described above were possible because the virus was readily mechanically transmissible. With methods developed in the above work, we are now in a position to conduct experiments on presumably similar viruses that infect soybeans. Preliminary research at the end of the contract year conducted in the laboratory of Dr. Julio Bird, Rio Piedras, P.R., indicated that these methods will probably work well for two whitefly-transmitted viruses occurring in Puerto Rico that infect soybeans.

These studies will be extended. In addition, a survey will be made in Sri Lanka, in cooperation with the INTSOY entomologist, with a view to setting up a research program to study means of controlling spread of whitefly-transmitted viruses into the soybean crop.

Many types of studies need to be done. Etiological research on other whitefly-transmitted pathogens should be conducted to determine if they are similar to the golden yellow mosaic virus. Antisera should be prepared to various viruses of this group and native vegetation tested to determine when the viruses of interest in soybeans come from (i.e., what are the important sources of primary inoculum). Epidemiological studies on the timing and pattern of virus spread in soybeans should be conducted in fields of different sizes under monocropping and multiple cropping schemes with and without effective weed control. Breeding studies should be conducted to identify and incorporate whitefly or virus resistance into adapted cultivars for areas where the disease is important. Experiments on the biology and behavior of the whitefly must be conducted and a major effort to devise economically feasible, environmentally sound methods for whitefly control should be started.

These experiments must be conducted in Asia under conditions where the disease of soybeans caused by a whitefly-transmitted virus is important. The choices currently are India or Sri Lanka. Considerable work has been done in the past in India, but the principle workers have retired or moved to other problems. Any regional research program on this problem should be closely coordinated with and jointly advised by colleagues at G. B. Pant University of Agriculture and Technology and ICRISAT. The developing interest in soybeans in Sri Lanka and the availability of entomology and virology counterparts engaged in soybean research may make Sri Lanka the location of choice for the proposed research. Reports indicate that whitefly-transmitted yellows in soybeans is increasingly prevalent there, and it is conceivable that the disease could threaten soyben production in Sri Lanka as it did in India in the late 1960's.

Whitefly-transmitted viruses may be controllable in the field by controlling the vector populations. This might be done with low-dosage insecticides, with parasites that infect and kill the whiteflies, or by breeding plant types resistant to whitefly feeding. There is a tremendous opportunity to conduct studies in tropical areas where whitefly-transmitted viruses and whiteflies are prevalent and important. Nothing, for example, is known about the epidemiology of these viruses. Little is known about the biology of the whitefly vector, Benisia tabaci, and less about the behavior of the vector. Since this vector transmits the pathogens that cause severe diseases in many tropical crops (cassava, tobacco, tomato, soybeans, mung beans, common beans, cotton pigeon peas, sweet potato, etc.), and since the pathogens are probably similar to the one we have worked with from Phaseolus, we are in an outstanding position to make a major contribution to disease control in tropical crops by concentrating a major effort on whiteflies and the pathogens they transmit.

Studies on the virulence of SMV from soybean germ plasm

Soybean mosaic virus (SMV) is seed transmitted, and is widely distributed in germ plasm collections. Little is known about the biological aggressiveness or virulence of SMV isolates. Experience shows, however, that putatively SMV-resistant soybean lines developed with the use of one SMV isolate often become infected when tested in another environment with a different SMV isolate. It thus seems important to obtain an understanding of the range of virulence of SMV isolates for the use of breeders who could then use several differnt SMV isolates for selection of resistant material in breeding programs.

A preliminary study is now underway in which recently introduced PI lines obtained from the USDA germ plasm collections (Urbana and Stoneville) are being used as sources of virus isolates. These are being tested for virulence by inoculation to several putatively SMV resistant varieties (including the variety Buffalo recently discovered by INTSOY to be resistant to SMV). The determinations are at an early stage, and no results are yet available.

Detection of seedborne SMV in soybean seed lots

A cooperative study funded in part by the contract and in part by the contract and in part by the Illinois Crop Improvement Association, is designed to develop a method for detection of SMV in seeds. Under investigation is a method employing an enzyme linked chemically to SMV-specific antibodies. When the virus is present in an unknown sample, it sticks to a surface which is then treated with virus-specific enzyme-labeled antibody. The presence of the antibody, and thus of the virus, can then be revealed by covering the surface with a substrate solution in which the enzyme acts to cause a color reaction.

This method should allow for the convenient testing of several hundred individual seeds of a seed lot for seed certification purposes, and this is of potential use to breeders, seedsmen, and pathologists.

Characterization of cowpea mosaic virus isolates

a. Properties of CPMV isolates from Puerto Rico. CPMV isolates used in this work were originally obtained from naturally infected soybeans from Puerto Rico. Symptoms observed in soybeans were striking. Both systemic mosaic and budblight were observed under field and greenhouse conditions. These isolates of CPMV belonged to the same subgroup (severe) as the Arkansas isolate of the virus (CPMV-Ark). The identity of these isolates were confirmed by symptomatology, serology, electrophoretic property, and sedimentation and analysis study.

CPMV demonstrated heterogeniety in velocity sedimentation. Three components with different nucleic acid composition were observed when the virus was subjected to centrifugation. The two components containing nucleic acid are necessary for infection. Attempts are now being made to obtain an *in vitro* "hybrid" of CPMV by mixing one component of one strain with the complementary component of another strain. Although true genetic recombination has not been observed with viruses, results from this experiment could be a model as to how new isolates of CPMV are derived under natural conditions.

b. Isolation of CPMV from a beetle collected in an Illinois soybean field. Cooperative studies were conducted on a new CPMV isolate recovered from a striped cucumber beetle trapped in a soybean field in southern Illinois during a routine survey. The virus, which is similar but not identical to CPMV isolates originating in Africa, infects soybeans but causes only mild symptoms. No significant yield loss was caused by this isolate in a test conducted at Urbana in the summer of 1976.

Significant interreaction between the plant pathologists and entomologists produced collaborative studies on virus-vector relationships.

All soybean viruses are transmitted by insect vectors. Some viruses are aphid-transmitted, others whitefly-transmitted, still others are beetle-transmitted. The virus-vector interaction and relationship is fairly specific. One group, the leafhopper-transmitted viruses, are not yet known to attack soybean. In studying the field spread of viruses, it is crucial to understand vector behavior, biology, and population dynamics. It is also essential to understand the relationship between the virus and the vector.

Soybean viruses are particularly severe and damaging in the tropics and subtropics. Through a team approach, the INTSOY virologist and INTSOY entomologist have begun a program to understand the spread phenomenon of soybean viruses. To date studies on three such viruses have begun: soybean mosaic virus, cowpea mosaic virus and tobacco ringspot virus.

Soybean mosaic virus (SMV) and aphid vectors

Soybean mosaic virus (SMV) is the most widespread of the viruses attacking soybean. It is seedborne and is transmitted in the field by

certain species of aphids in a non-persistent, stylet borne manner. Reports from Ecuador, Indonesia, and Spain indicate that SMV could be a limiting constraint to soybean production in several parts of the world. Since over 30 percent and 20 percent of the lines held in the northern and southern germ plasm collections respectively; contain a significant degree seed export.

Except for a few species mentioned under II below, aphids are not known to colonize soybeans in the western hemisphere. There are, however, at least two species known to colonize in Asia: Aphis glycine and Aphis craccivora. In the western hemisphere SMV is spread in the field by transient alate aphids. To better understand the role aphids play in the spread of SMV, a study was begun on aphid diversity and abundance in Illinois soybeans. About 60 species of aphids were collected in soybean fields. Of these species, the following are known vectors of SMV, either from literature accounts or from our field experiments; those discovered to be SMV vectors for the first time are preceeded by an asterisk*:

Acyrthosiphon pisum
Aphis craccivora
Aphis fabae
Aphis gosspii
*Aphis spiraecola
*Capitophorus elaegni (questionable)
Macrosiphum euphorbiae
Myzus persicae
Rhopalosiphum maidis
*Rhopalosiphum padi
Rhopalosiphum rufiabdominalis

Details on this study and on the studies below may be found in Appendix V.

Seasonal flight activity and abundance differed greatly between the 1975 and 1976 growing season. Flight activity occurred late in the 1975 growing season with one peak in late July-early August and a second peak in September. In the 1976 growing season flight activity occurred early with the major peak occurring in June and early July. This fluctuation in flight activity timing could have profound effects on SMV spread and on SMV invasion into developing seeds of infected soybeans. If SMV infection occurs prior to or during early bloom, virus can move into and infect developing seeds resulting in relatively high rates of seed transmission. If infection occurs after early bloom the rate of seed transmission is low presumably because virus particles are blocked from entering developing seeds. Therefore, late season flight activity is not as detrimental to seed production as is early flight activity.

That the pattern of spread is directly related to wind direction is demonstrated from the 1976 experiment. Aphid flight activity is affected by wind speed and direction. Aphids tend to fly downwind, as would be expected from the resultant SMV spread pattern.

Although it is not yet known how far an aphid can carry viable virus, the SMV spread pattern obtained this past growing season suggests that it can be carried at least 150 feet.

There was no observable secondary spread in the field by aphids; all spread was from a primary inoculum source. There are two sources of evidence for this: field spread was not clumped, but occurred in a radiating

series of concentric circles, biased by wind direction, and decreasing in intensity as the distance of the primary inoculum source. The second source of evidence came from field experiments in which aphids were collected upwind and downwind from an inoculum source and then tested for transmission ability on healthy soybean plants. Of the downwind aphids, 7 percent carried SMV; of the upwind aphids, none carried SMV.

Identification of field vectors of SMV

SMV has proven to be a problem in certain developing countries particularly with respect to seed production. A means of identifying important vectors is essential. Although many potential vectors have been identified by means of transmission experiments conducted under laboratory conditions (see above), the importance of each of these in the field has not been assessed.

Living aphids were trapped upwind and downwind of a 92' x 92' field of mechanically inoculated soybeans. Collecting was done twice per week for 1-2 hours on nets 15 ft. long and 4 ft. high. Each aphid was allowed to probe a healthy seedling, and was then recaptured and preserved for identification. The plants were appropriately labelled and grown in an insect-free greenhouse to determine those that became infected. Positive identification of infected plants was obtained by infectivity indexing. In order to identify the large number of field collected aphids the scientist working on this problem spent one week with Dr. Manya Stoetzel in the USDA/ARS Systematic Entomology Laboratory, Beltsville, Maryland.

During the growing season, 939 aphids of at least 28 species were collected. Specimens of four of these species, which comprised 60 percent of the yearly total catch, proved capable of transmitting SMV. These four species were Rhopalosiphum maidis (28 percent), Myzus persicae (14 percent), Aphis craccivora (9 percent) and Macrosiphum euphorbiae (9 percent). Aphids of the subfamily Pemphiginae comprised an additional 6 percent of the catch. This figure may be underestimating the time populations since Pemhiginae often burrow and are therefore difficult to recapture. In these experiments Pemphiginae never transmitted SMV. There were no positive transmission resulting from feeding by any of the 266 aphids trapped upwind of the infected soybeans, indicating that SMV was not being carried into the field. Of the 673 aphids collected downwind of the field, 7 percent (48) transmitted SMV. These 48 aphids include 17 M. persicae, 13 R. maidis, 16 M. euphorbiae, 2 A. craccivora, and 10 specimens awaiting positive identification by Dr. Manya Stoetzel, USDA/ARS Systematics Entomology Laboratory, Beltsville, Maryland. In total 115 M. persicae, 219 R. maidis, 63 M. euphorbiae, and 40 A. craccivora were trapped downwind of the infected field; therefore, the rate of transmission of each species is approximately 15 percent, 6 percent 10 percent and 5 percent, respectively.

The transmission rates given above are not necessarily the same as would be obtained from laboratory transmission experiments since there is no way of knowing that an aphid collected downwind of infected soybeans had actually probed an infected plant; however, they do indicate how many aphids of a given species at a given location downwind of an inoculum source are

of a given species which transmit regularly under laboratory conditions might not have the behavioral characteristics to do so in the field; and the field is the real world. The experiment needs to be repeated to establish the importance of annual variation in aphid populations. In 1976 sampling began in late June. In the future, sampling should be initiated earlier, before spring aphids, notably A. craccivora, reach peak flight abundance. After SMV has been positively identified, one of the first steps in controlling it is to determine the principal vectors. The method used in these studies provides this information. Such a technique would be useful for research on SMV in developing countries where SMV has been shown to be a production problem.

Yield loss threshold in soybeans caused by cowpea mosaic virus. CPMV is a potentially serious virus in soybeans, causing severe symptoms. The virus is transmitted by several species of beetles that feed on both cowpea and soybeans. Natural infection of soybeans with CPMV has been noted in Puerto Rico since 1974, but the virus has not become widespread.

In 1976 an experiment was conducted to determine the yield loss caused by CPMV infection at different stages of plant development and in different proportions of the plant population. Soybeans (Improved Pelican) were planted at Isabela, P.R. and inoculated mechanically (5 m rows) at either the primary leaf or bloom stage. The experiment was replicated 4 times. Treatments were 0, 25, 50, 75, or 100 percent of plants inoculated. Results showed a serious reduction in yield caused by CPMV inoculation at the primary leaf stage and a significant reduction when all plants were inoculated at the bloom stage. The yield loss threshold appeared to be at less than 25 percent of the plant population if infection occurred early and 75 percent if it occurred at bloom. More refined statistical analyses will be made of these data and the experiment will be repeated in 1977.

Sericothrips variabilis (Beach) and its potential to transmit tobacco ringspot virus (TRSV). Bud blight occurs throughout the soybean growing areas and the soybean thrips occurs in much of Latin America. Similar types of thrips occupy similar niches in other parts of the world. A series of laboratory experiments was conducted during the winters of 1975 and 1976 to determine the potential for Sericothrips variabilis, the soybean thrips, to transmit TRSV, the causal organism of bud blight. During this period of time about 320 adult transfers and 90 pupal transfers were performed. Adult transfers consisted of allowing thrips to develop on a soybean plant that was infected with TRSV. The adults, having fed for some time on the plant, were then transferred to healthy soybean plants. Pupal transfers were slightly different. Again, the thrips were allowed to develop on infected plants. Just prior to pupating, this species of thrips drops to the soil and therein pupates. The thrips were transferred to healthy soybeans during the pupal stage. In no cases were there positive reactions when the plants were tested for the presence of TRSV. One further test is currently underway. To a large cage containing a single TRSV infected plant and several healthy plants were added several hundred adult thrips. These are being allowed to develop and multiply in the cage. At the end of a month all of the plants in the cage will be tested for the presence of TRSV. If none have the virus, other than the source plant, it may be assumed that this species is incapable of transmitting TRSV.

Objective 5. Development of Model Insect, Weed and Disease Control Management Systems Which can be Adapted or Modified for Specific Environments.

During the 1974-75 soybean growing season a pilot pest management program was tested in Southern Brazil. Nine fields, varying in size from 10 to 30 hectares were selected in commercial farms. Paired fields within each farm were used in the experiment. One field of the pair was managed following a simple pest management strategy and the other field was managed following the farmers own criteria. The strategy consisted of scouting fields once a week to monitor populations of defoliating lepidopterous caterpillars and pod sucking stink bugs, which were the target insect pests. Damage thresholds and treatment decisions were based on assessment of defoliation levels, pest populations, and presence of pathogens, mainly the fungus Nomuraea rileyi (Farlow) Samson. Minimal dosages of non-persistent pesticides were prescribed in fields reaching damage thresholds.

Although treatment decisions by farmers were probably influenced by the pest management program they made a total of 9 treatments (average one per field) as opposed to only 2 treatments made in the pest management fields, a reduction of 78 percent in the number of insecticide applications in the experimental program.

The program was repeated in 1976 and was officially recommended by the state of Parana (2nd largest producer in Brazil). In 1977 a similar program was adopted on a national scale. The program allowed testing under normal field conditions some parameters of soybean pest management systems developed in the United States of America. It demonstrated that simple scouting procedures and adequate economic damage thresholds can be used in preliminary pest management strategies even if phenology of key pests is incompletely known.

Soybean production in Brazil underwent explosive growth in the past 6 years. Production increased from about 1.5 million metric tons in 1970 to nearly 10 million tons in 1975. Area planted to soybean expanded from 1.3 million ha in 1970 to 5.7 million ha in 1975.

Many insect problems in Brazilian soybean are similar to those encountered in the soybean producing areas of Southern U.S. The main defoliating species are the velvetbean caterpillar, Anticarsia gemmatalis Hubner, and several Plusiinae, with the predominance of Pseudoplusia includens Walker, the soybean looper. The main pod-feeding pests are the southern green stinkbug, Nezara viridula (L.) and Piezodorus guildinii (Westwood).

Additional studies on thrips, aphids, spider mites, potato leaf hopper and bean leaf beetle are being conducted. These are summarized below. For details please refer to Appendix V.

Bionomics and spatial distribution of soybean-associated thrips. Thrips represent a group of insects for which very little biological information is available. Yet, in any given soybean ecosystem thrips are more abundant than any other single group of insects. Thrips can cause damage

to soybean plants and some species are vectors of certain soybean virus (see above). Thus a program was initiated to attempt to understand the importance of thrips in a soybean ecosystem and how best to sample thrips populations.

In order to perfect a reliable sampling method, experiments were carried out in Urbana and Lexington, Kentucky, to determine the spatial and temperal distribution of thrips in soybean. Three main species of thrips occur in soybean: Sericothrips variabilis (Beach), Frankliniella tritici (Fitch), and F. fusca (Hinds). A fourth, Thrips tabaci Lind, was present in small numbers. Results of experiments indicated that no significant differences occurred between plants or between plots sampled. However, highly significant differences occurred in the vertical distribution of the species. For F. tritici, the most preferred parts were the flowers and growing terminals. This species reached its peak abundance during the blooming period of soybeans and disappeared from soybean fields as flowering ceased. Adult flight activity continued throughout the major part of the growing season, however. Frankliniella fusca was found on soybeans in relatively low numbers, and only adults occurred on soybean. This species occurred on soybean in the early growing season and could be found indiscriminately on terminals and leaves. Sericothrips variabilis reached its peak abundance during early pod set, after F. tritici had disappeared from samples. This was true of the adult flight activity as well. Both F. tritici and S. varibilis were more abundant within a large (300 x 600') soybean field than in areas immediately next to the field; the reverse was true for F. fusca.

From the study, it seems reasonable to attempt to sample Sericothrips variabilis populations by concentrating on the first instar larva, and by concentrating the sampling method to center leaflets of the fifth or sixth trifoliate from the top of the plant. Variation between samples was fairly low, and five such center leaflets from scattered plants in the field would give a good index of population density.

Aphid species colonizing soybeans in the Americas. Aphids are important as pests of soybean because they transmit several important viruses. However, in the western hemisphere they are not known to colonize (i.e. produce progeny) on soybeans. There are a few species in Asia and Africa which do colonize soybeans, and there, they cause considerable damage to the plant stand.

There are also a few species in the western hemisphere that are capable of colonizing soybeans. One species was found throughout the soybean-growing area of Ecuador. That species has not yet been identified. At Urbana two additional species have been observed, one Aphis gossypii, on soybeans in the greenhouse, and a second, Aphis craccivora, on young soybean seedlings in the field. We have subsequently been able to rear species on detached soybean cotyledons in petri dishes in the laboratory. When either species was placed on healthy soybean seedlings, it would multiply initially, especially on the cotyledons, but would soon die as the plants developed. Malformation of the soybean plant was observed where several aphid specimens were allowed to feed on young seedlings in the laboratory. Among the various malformations the most serious was a killing of the growing point. This caused very bushy growth patterns. In a few cases,

the entire plant was killed. In all cases leaves were severely malformed and yellow.

Recently, we discovered that Myzus persicae can also develop on soybean cotyledons.

Spider mites. Spider mites are very sensitive to nutritional factors. In diets differing in nitrogen levels mites grow and reproduce at different rates. Reports are somewhat conflicting in regard to the optimal N levels. We have hypothesized that the increase in mite populations on soybean may result from a more favorable nutritional environment in plants under water stress. Sugars and free amino acids in leaves increase under conditions of water deficiency, both conditions being highly favorable for mite development. The effect of soybean nutrition and water balance on mite development is being investigated in our laboratory, in cooperation with Dr. J. G. Rodriquez, University of Kentucky, Lexington. The objective of this investigation is to test the hypothesis that spider mite outbreaks result from nutritional effects via metabolic changes in soybean plants under conditions of water deficiency.

Non-nodulating 'Clark' soybeans were planted in a hydroponic system. Three nutrient solutions were used differing in the level of nitrate supplied. These solutions contained .75, 7.5, and 15.0 nMol of NO_3. Plants in the .75 mMol regime were obviously chlorotic. After plants became well established in the system about 60 mites were placed on the upper 3 leaves. Colonies became well established after a few weeks and plants are being sampled using a paper print technique. This technique is based on laying a leaflet on a sheet of white filter paper and rolling a cylinder over the leaf. Mites on the leaf surface against the paper and are crushed leaving a permanent and identifiable mark or print on the paper.

The number of mites on the leaves from plants in the various nutritional regimes is being counted. Preliminary observations suggest that higher populations are developing on the 15.0 mMol plants than on either the 7.5 or the .75 mMol plants. If this trend holds this experiment will lend some support to our hypothesis although we still need to determine the relationship between N-levels and water stress.

Potato leafhopper. The potato leafhopper, Empoasca fabae migrates from the south every year and becomes established in soybean fields in rather large numbers. The species is consistently present in soybean fields but its impact on growth and production is not clear. Studies conducted in Iowa resulted in rather low economic thresholds. We completed a two year study on the economic thresholds of the potato leafhopper using near isogenic lines of 'Clark' soybeans differing in the characteristics of their pubescence. This or closely related species are abundant on soybeans around the world.

In 1976 different blocks of plants were selectively sprayed to produce various levels of leafhopper populations during three different stages of plant growth. A control block was sprayed weekly throughout the entire season.

Preliminary analysis of the data has shown a strong effect of pubescence type on yield ($P<.001$). However, the single effect of treatments (suspended sprays) and the interactions of treatments and pubescence were not statistically significant. Although additional analyses of correlation are being performed there is an indication that infestation at all stages of development had similar effects on yield at the population levels that were present.

Bean leaf beetle. Much of the work concentrated on improving sampling procedures for larvae and adults.

Larval extraction from soil samples was made using a simple flotation method adapted from the egg separation procedure previously developed in our laboratory. The method uses a separatory funnel with air bubbling in a saturated aqueous solution of NaCl. Soil samples containing known numbers of larvae yielded over 90 percent recovery of third instars. Experiments were also conducted to determine how long soil samples could be stored without losing precision of recovery. It was observed that soil samples can be stored in a freezer at about -10°C, without significant loss in recovery.

A study in adult population distribution patterns was conducted using the beat-cloth method. This sampling method was compared with the sweep-net and to an absolute method based on caging and harvesting all plants in a 1.8 x 1.8 m area. Samples were taken under two field situations--lodged plants, and erect plants. In both cases the beat cloth yielded the best estimates of beetle populations and the sweep-net was particularly inefficient with lodged plants.

Studies are being conducted on the rates of growth and development of bean leaf beetle larvae reared at various temperature regimes. The effect of various food plants on adult survival and fecundity are also being measured. These data will help improve preliminary models for the bean leaf beetle population dynamics.

Objective 6. Development of Improved Storage Technology.

Staff from the Departments of Agronomy and Agricultural Engineering, UIUC, and the Department of Agricultural Engineering, UPR/MC devoted considerable attention to this area.

Favorable results from varietal and agronomic trials at many locations around the world have resulted in wider production of soybeans in tropical and subtropical areas. The maintenance of soybean seed quality during storage in these hot and humid environments has been recognized as a limiting factor in soybean production. High quality seed is required to obtain adequate stands for profitable soybean production. Soybean seed must often be imported in the initial stages of production development in a new area. As local seed production becomes possible, a storage technology suitable for the environment and scale of operations must be available.

A project was developed to study practical and inexpensive methods for storing seed under tropical conditions. The initial phase of the study was to evaluate tropical storage properties of certified seed produced in

the continental U.S. This work was done to establish a basis for subsequent storage studies with seed produced in the tropics under different sets of production conditions. In addition, published data on the storage properties of U.S. certified seed under ambient tropical conditions appeared to be unavailable.

Soybean seed does not store well as compared to other legumes. The major environmental factors influencing seed deterioration are temperature and moisture. Moisture is usually considered in terms of seed moisture content. Since seeds lose or gain moisture from the surrounding air until reaching equilibrium at a given set of conditions, it would also be appropriate to think of moisture in terms of the relative humidity of the storage environment. Storage fungi are a major cause of reduced germination in soybeans, especially when soybean seed is stored at moisture contents above 12.5 percent. The results, detailed in Appendix VI are for storage under ambient tropical conditions of Woodworth certified seed from Illinois.

The results showed that the viability of high quality seed declined rapidly to almost zero after nine months of improper storage in a tropical environment. The loss of seed viability appeared to be closely associated with a marked gain in seed moisture during storage. Containers resistant to moisture vapor transmission tended to preserve seed quality. Drying soybean seed to a safe moisture content was shown to be of little value if no effort was made to prevent moisture gain during storage. The moisture content of unprotected seed approached equilibrium with the humid atmosphere of the tropical environment. Reducing the seed moisture content and maintaining it during storage were shown to be essential ingredients for preserving seed quality at an acceptable level for nine months storage in the tropics. Sealed metal containers or plastic bags placed in metal containers with covers offered adequate storage protection for seed with a low initial moisture content.

Objective 7. Phasing Out of Previous Activities on Home Processing of Soybeans by October 31, 1976.

This objective was satisfied. A final report on home processing activities was submitted to the Agency.

During the six month phase out period four major projects were completed:

1. The development of a procedure for preparation of a palatable and generally acceptable soy beverage for home and village industry was completed. This work was carried out by an INTSOY supported graduate student working under faculty direction.

2. The filtering devices which are required for preparation of the soy beverage at the village level were evaluated.

3. The low cost ($704.00) Decker hammermill for grinding of dry soy, cereals and soy:cereal combinations was evaluated for performance.

4. A simple device was designed, constructed, and evaluated for separation of cracked soybeans into the hull and cotyledon fractions.

Because these projects are fully discussed in the final report, brief summaries on each are given below.

Development of a Procedure for Preparation of a Palatable Soy Beverage for Home and Village Use. The study on developing the concept for preparation of a palatable soy beverage for home and village industry was completed. Work during this contract period covered final development of the preparation concepts as well as a comprehensive organoleptic evaluation of soy beverages prepared from whole raw dry soybeans, cracked or broken raw dry soybeans.

This concept involved very low level technology and a brief description of the process follows. Whole soybeans were dropped directly into boiling water and the beans were boiled for ten minutes. After boiling, the weight of beans and water was adjusted to the starting level and the bean-water mixture was ground in a Waring blender. The ground slurry was simmered for an additional 20 minutes after which sufficient water was added to replace that lost by boiling and the slurry was filtered. The filtrate or milk was flavored by the addition of sugar, salt and diacetyl or vanilla.

Cracked or broken raw dry beans and powdered raw dry soybeans were also used to prepare the soymilk. A comprehensive organoleptic evaluation of the soy milks prepared from the three types of soy raw materials showed no significant differences in organoleptic properties with these exceptions. Color scores were significantly (5 percent level) higher for the soy milk prepared from the powdered dry raw soybeans. Flavor and mouth drying characteristics of beverage prepared from powdered soybeans were significantly (5 percent level) inferior to beverages prepared from whole or cracked beans. However, all scores were in the range of 7 on a hedonic scale of 9 which indicated good acceptance for all products. The procedure is considered ready for further testing and improvement under conditions in developing countries.

Evaluation of Filtering Concepts for Village Level Preparation of Soy Beverage. Three systems were developed for filtering soy slurries in preparation of soy milk. These were a simple filter sock or bag, a cradle filter and a basket centrifuge. These filtering devices were thoroughly tested using soybean slurries made from whole, cracked and powdered soybean. These tests showed that soy milks prepared from the three different raw soybean types were quite similar with respect to yield, percent protein, solids and sediment. Comparison of the soy milks prepared with the different filtering devices showed that the centrifuge produced the highest yield of milk, followed by the sock and cradle methods. However, the sediment content of the milk filtered with the sock method was invariably much lower than that from the centrifuge or cradle. When all factors were considered the sock filter was judged to be the most desirable method. This method employs the lowest level of technology and is by far the cheapest and easiest to manufacture.

Evaluation of Mill for Grinding Dry Soybeans and Various Cereal Products. A Decker Grainmaster hammermill was purchased for $704.00. This price included three mill screens and was the lowest priced hammermill in about sixty quotations from suppliers. This mill was tested using dry soybeans, corn and soybean-corn mixtures. The mill performed acceptably and was found to be satisfactory for grinding dry raw soybeans for soy beverage preparation. This mill is recommended for installation as a cooperative utility for village use.

Development of a Device for Separation of Hulls from Cotyledons. A device was designed, constructed and tested for the separation of hulls and cotyledons from dehulled whole dry soybeans. Thorough testing showed that this equipment would separate virtually 100 percent of the hulls and approximately 64 percent of the hypocotyls from the cotyledons. This unit offers a simple, low cost system that can be readily constructed in developing countries.

D. Ancillary Activities

INTSOY is a program which derives support from multiple sources through a variety of instruments. The professional staff of senior and younger scientists is uncommonly gifted and imbued with a spirit of cooperation and collaboration with soybean research and extension workers worldwide. The development of an adequate support staff, still in process, is one of the more important objectives of the program to increase the scope and effectiveness of staff scientists. The willingness of the Agency to share in the attainment of an adequate support staff is evidence of the Agency's sophistication as a research supporting body. The directorate staff is lean and comprised of senior level personnel with substantial experience in international development activities.

Because INTSOY is a total program, and because its staff are encouraged to participate in all aspects of the program, research contract personnel also contribute their expertise to ancillary activities. Past reports have included mention of these activities in such a way as to cause some confusion as to where the activities under report, e.g. research activities, and ancillary activities joined and where they were separate. This confusion was not intentional on the part of INTSOY, and in this report only those activities directly related to the objectives of the research contract have been reported to this point.

Research contract staff have participated in a number of areas, all consistent with the overall goal of increasing the program's institutional capacity to serve in a research, advisory and educational capacity to LDC institutions committed to solving protein-caloric deficiency problems among their populace, and improving the lot of their rural and urban poor through the production and utilization of soybeans. Advisory service has been provided under Agency supported task orders. International organizations such as the Food and Agriculture Organization of the United Nations have requested and received assistance from INTSOY staff on identification missions, technical consultations and country programs. International agricultural research centers interact with INTSOY and its professional staff on matters of mutual interest.

Contract staff have made significant contribution to two highly successful short courses sponsored cooperatively by the Agency and the U.S. Department of Agriculture. These courses, "Soybean Processing for Food Uses" and "Technical and Economic Aspects of Soybean Production" have been offered for the past three years. Participation by 85 persons from 40 countries have been sponsored by USAID, FAO, foundations, cooperating governments and institutions and private industry. These training programs are a cornerstone of the total INTSOY effort.

Workshops, seminars and conferences have been enriched by the contributions of INTSOY research staff. Collaborative programs under various Memoranda of Understanding have also benefitted from the participation of contract staff.

These ancillary activities are supportive of both the total INTSOY program and of the research contract under report. Participation in these activities enriches and broadens the capacities of the research staff and will continue to be encouraged.

E. Administration, Staff and Budget

The project is administered by a director and assistant director located at UIUC and an associate director located at UPR/MC. The directorate staff receives policy guidance from an executive committee comprised of heads of departments involved in the program and representatives from the Experiment Station, the Cooperative Extension Service, International Agricultural Programs and the Office of International Programs and Studies.

The extremely capable professional and support staff who contributed to the success of predecessor contracts was retained to address the objectives of this project. Staff turnover was minimal and excellent replacement staff was identified as necessary. The quantity and quality of responses to positions advertised is an indication of the reputation for response capability INTSOY is developing.

By the end of the report period the directorate and professional group numbered 6.0 fulltime equivalent staff. They were assisted by 11.75 fulltime equivalent support personnel. The significant contribution of the various support personnel to the overall success in progress toward project goals is greatfully acknowledged. A listing of all personnel who served during the report period is presented on page 53.

Funds for project operations were generally adequate and available. A summary of expenditures for the first year of the project and estimate of requirements to the end of the project is given below.

Program Budget

Line Item	Actual Exp. 4/1/76-3/31/77	Est. Exp. 4/1/77-3/31/79	Total Est. Budget
1. Salaries	$214,225	$675,265	$889,490
A. Professional	(103,250)		
B. Support	(110,975)		
2. Fringe Benefits	21,740	94,040	115,780
3. Indirect Costs	141,390	445,670	587,060
4. Travel & Transportation	42,280	157,720	200,000
5. Other Direct Costs	31,205	99,470	130,675
6. Equip. and Supplies	28,875	133,975	162,850
	$479,715	$1,606,140	$2,085,855

INTSOY Project Staff

April 1, 1976 - March 31, 1977

Name	Rank/Title	Department	FTE
A. Professional Staff			
1. Dunker, R. E.	Asst. Agronomist	Agronomy (to 10-20-76)	1.0
2. Ferrier, L. K.	Asst. Professor	Food Science	.75
3. Goodman, R. M.	Asst. Professor	Plant Pathology	.75
4. Hill, H. J.	Asst. Agronomist	Agronomy (from 12-1-76)	1.0
5. Irwin, M. E.	Asst. Professor	Ag. Entomology	1.0
6. McCowen, T. A.	Asst. Director	INTSOY Administration	.25
7. Minor, H. C.	Asst. Professor	Agronomy	.75
8. Rodda, E. D.	Professor	Ag. Engineering	.25
9. Sinclair, J. B.	Professor	Plant Pathology	.25
10. Thompson, W. N.	Director	INTSOY Administration	.25
11. Whigham, D. K.	Asst. Professor	Agronomy	.75
B. Support Staff			
1. Appleman, J. D.	Res. Assistant	Food Science (to 11-23-76)	1.0
2. Baer, K. D.	Res. Assistant	Food Science (to 8-31-76)	1.0
3. Bowers, G. R., Jr.	Res. Assistant	Plant Pathology (from 5-21-76)	.50
4. Burnsmier, Helen	Clerk III	Agronomy (to 8-20-76)	1.0
5. Cunningham, Patricia	Clerk-Typist III	INTSOY Administration	1.0

INTSOY Project Staff
(continued)

6.	Eckenback, Susan L.	Res. Assistant	Agronomy (from 7-21-76)	1.0
7.	Endsley, Margaret R.	Clerk-Typist III	Agronomy	1.0
8.	Halbert, Susan E.	Res. Assistant	Ag. Entomology (from 5-21-76)	.50
9.	Kanthamani, S.	Res. Assistant	Food Science (to 11-23-76)	.25
10.	McCune, T. D.	Res. Assistant	Food Science	1.0
11.	Newell, Christine A.	Res. Associate	Agronomy	1.0
12.	Primmer, R. R.	Assistant I	Ag. Engineering	.50
13.	Schaefer, Elizabeth	Res. Assistant	Ag. Entomology (from 6-7-76)	1.0
14.	Shock, Teresa L.	Asst. Plant Pathologist	Plant Patholody (from 9-7-76)	1.0
15.	Taeger, Deanna	Secretary	INTSOY Administration	1.0
16.	Thongmeearkom, P.	Res. Assistant	Plant Pathology	.25
17.	Toews, Vicki L.	Clerk-Typist III	Plant Pathology	.50
18.	Tucker, Margaret E.	Clerk-Typist III	Food Science	.50

APPENDIX I

Report of Harry C. Minor
Assistant Professor of Agronomy

Soybean Variety Development

The major objectives of soybean variety development are high yield and high protein per unit of land area, suitable photoperiod response, resistance to yield and quality limiting diseases and insect pests and wide adaptability in tropical and subtropical environments. The primary thrust in the soybean variety development work has been through the International Soybean Variety Evaluation Experiment (ISVEX). This comprehensive variety testing program in which collaboration is by voluntary association, has grown from 33 countries and 90 locations in the first year of the research contract to over 100 countries and 290 locations at the completion of 1976. In this experiment, a standard variety trial is grown at each location and data are collected and returned to the trial coordinator at Urbana-Champaign for analysis. Initially, the trial included 20 varieties in U.S. maturity groups I and IX. During the second year, the number of varieties was reduced from 20 to 15 and included the 12 best performing varieties in ISVEX-1. Some cooperating LDCs desired short-season soybean varieties to fit local environmental conditions or cropping systems. In response to these interests, two sets of varieties were established, an early set of varieties representing U.S. maturity groups 00 through V and a later maturing set of varieties representing U.S. maturity groups III through IX. In 1976, this was expanded to include six different sets made up from 50 cultivars. These sets are organized by zones which are based on latitude and altitude. Cooperators provided the land, labor, fertilizer, and management necessary for the experiment. Instructions for management and data collection were sent to each cooperator to standardize methods among sites. General information about the site and the experiment was returned to INTSOY. This information included details of latitude, elevation, and soil conditions of the site plus date of planting and harvesting, amount of moisture, fertilizer used, local varieties tested, and diseases and insects of the environment. Data reported for each plot by cooperators included yield, seed weight, days to flower, days to maturity, canopy height at flower, plant height at maturity, and estimates of lodging and shattering. Seed samples were also returned to INTSOY for protein and oil content analysis.

The results of the first and second ISVEX have been published as numbers 8 and 11 in the INTSOY publication series. Data for the third ISVEX is under analysis and results will be published later in 1977. The preliminary analysis of these data is found in appendix 1-A.

The high grain yield achieved in experimental plots with good management indicates that soybeans do have production potential in many new areas of the world. Temperate environment cultivars do not produce ideal plant types when grown in the tropics but extensive breeding programs can

improve these characteristics. The ISVEX has provided answers to some questions about the adaptability of temperate zone cultivars in the tropics and questions relating to seed quality and growth at high altitudes.

Data is being received from the fourth ISVEX conducted during 1976. To date, data has been received from 55 sites in 31 countries which tested ISVEX-4. ISVEX-5 is now under distribution. Four sets of cultivars are being used in ISVEX-5. These sets of cultivars are organized by environmental zones utilizing cultivars that have performed well in past experiments and new cultivars with potential in each environment. At this time ISVEX-5 has been requested by 143 sites in 80 countries. Eighty trials have already been distributed.

In 1976, new material increased in Puerto Rico was distributed to selected locations. The purpose was to identify potential areas of adaptation for these new cultivars and measure their performance relative to established cultivars with proven potential for high yields. This Soybean Preliminary Observation Trial (SPOT) has been designed to serve as a screening test for newly developed cultivars. In 1976, SPOT was conducted at six sites which represent the range of environments where ISVEX has been successfully grown. These sites were in Ecuador, India, the Philippines, Puerto Rico, Rhodesia, and Sri Lanka. Data was received from these sites and is presently being analyzed. SPOT-2 is presently being organized.

SPOT-2 will include six new entries. These will replace those entries in SPOT that showed the poorest performance. The number of locations for SPOT-2 is also being increased. This will allow for a better evaluation of the SPOT material. Ten locations have already been contacted. These locations are in Puerto Rico, Ecuador, Peru, Ghana, Rhodesia, Sri Lanka, India, Thailand, Indonesia, and the Philippines.

Joint experiments were organized and carried out with the International Institute of Tropical Agriculture (IITA) and the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) in 1976 to eliminate duplication of testing the same material at the same research stations. In 1977 joint experiments are to be carried out with IITA. These joint experiments originally carried out in the 20° S - 20° N region of Africa have now expanded. Due to cooperator interest in other areas, 14 sites have requested joint INTSOY/IITA experiments outside of Africa. As in the past, the Food and Agriculture Organizations of the United Nations (FAO) and the French Institut de Recherches Agronomiques Tropicales et des Cultures Vivrieres (IRAT) have requested trials. These trials are primarily requested from Africa and Asia.

Three other studies in variety development illustrate the complementarity of work between the research contract and the INTSOY 211(d) grant AID/CM/ta-g-73-49. Two of these studies mentioned in previous reports have been completed. Articles are being prepared for publication. One study concerned itself with the effects of storage conditions and environment during maturation on soybean seed quality and crop performance. The findings may permit the identification of steps to slow down the decline in vigor and viability in soybean seeds stored in tropical environments. This will be important in breaking the barrier of seed storage problems under

tropical conditions. The other study identified a genotype which possesses unique photoperiod insensitive properties which will be useful in the breeding of soybean lines with wide latitudinal adaptation.

Another student conducted research which tested three soybean varieties planted at three planting dates for differences in seasonal nitrogen fixation activities. The research is helping to determine when during the growing season nodule activity is most necessary, and when nodule activity is not used by the soybean. Results will be helpful in determing the best planting date (if the grower is forced into planting at a particular time) as regards seasonal nitrogen fixation. A M.S. dissertation on the subject will be completed by August 1977.

Soybean Performance: Effects of Environmental and Management

The extensive soybean testing network (ISVEX) established by INTSOY has shown that the yield potential of soybeans in tropical areas is similar to that in temperate zones. However, little is known about how environmental and management variables influence yield under tropical conditions. Therefore, data collected in 1974 through ISVEX were used to separate and quantify the effects of environment and management on soybean performance in the tropics.

The ISVEX project included 86 sites in 1974, but cooperating researchers at only 25 sites returned information on all the variables pertinent to our analysis. Countries represented in the study were: El Salvador, Guyana, Indonesia, Ivory Coast, Malaysia, Philippines, Puerto Rico, Rhodesia, Sri Lanka, and Thailand.

At each site, data were collected on agronomic, environmental, and management variables. Agronomic variables included yield and other characteristics indicative of plant growth and development. Environmental variables were those determined by geographic features and weather. Management variables were those directly or indirectly controlled by the cooperator. Plant population, nodule number, and nodule dry weight were included among the management variables since they depend to a considerable extent on management decisions made by the cooperator.

Simple correlation coefficients were computed to determine linear associations between various individual agronomic, environmental, and management variables. In addition, multiple regression analyses were performed to examine the relationships between the individual agronomic variables and the groups of variables designated as environmental and management. Simple correlation coefficients between the individual agronomic, environmental, and management variables are presented in Table 1. Coefficients of determination for regression models relating agronomic variables to environmental and management variables are given in Table 2.

Agronomic characteristics significantly correlated with yield were seed weight and plant height. Highest yields were produced under conditions which resulted in the tallest plants and/or heaviest seeds. The range of observed mean site yields in this study was 519 to 4,450 kg/ha. Differences

in yield among sites were not significantly correlated with any of the individual environmental or management variables measured (Table 1). The coefficient of determination for the multiple linear regression model including only the environmental variables [$R^2(E)$] was not significant (Table 2). The coefficient of determination for the multiple linear regression model including only the management variables [$R^2(M)$] was significant. After adjustment for management effects, the five environmental variables did account for a significant proportion of the variability [$R^2(E')$] in yield. However, the effects of the six management variables, after adjustment for environmental effects, accounted for more than 2.7 times as much of the variation in yield as did environment [$R^2(M')$]. This result strongly suggests that management practices may be of greater importance than altitude, latitude, daylength, maximum temperature, and minimum temperature as determinants of soybean productivity in the tropics. Also, the high mean yield for the 25 sites (2034 kg/ha) and the lack of a strong association between environmental variables and yield suggests that the group of cultivars tested may be grown satisfactorily in tropical environments.

Yield was positively associated with plant height. Under photoperiodic conditions which stimulate early flowering, such as those encountered in this study, the final performance of the crop can be expected to be quite dependent on the conditions during early growth. While yield did not correlate well with individual environmental and management variables, the combination of management practices which interacted favorably with environmental variables to produce taller plants apparently also tended to produce higher yields.

An increase in total N availability, as inferred by an increase in the number of nodules and nodule dry weight, was associated with a significant increase in plant height but not yield. Since soybeans are known to respond to applied nutrients when these are deficient in the soil, the lack of a significant association in this study is interpreted as an indicator that P and K were generally available in adequate amounts. The relationship between plant stand and yield was also probably not significant because of the high mean plant population achieved at most sites in this study.

Results are available which show that soybeans have produced acceptable yields in the tropics. This study included only U.S. cultivars which are commercially available. Therefore, cultivars currently exist which can be used to establish soybean production projects in tropical countries. Breeding programs for the development of better adapted cultivars should follow the establishment of soybean production. Cooperation between countries and international institutes, through the sharing of research results and newly developed material, should minimize costs involved in establishing new production programs.

This study has shown that management variables can be more important than environment in explaining among site variation in yield. Therefore, considerable effort must be made in training farmers in the proper management of soybeans when they are introduced as a new crop in a region. Management practices differ among regions, and research should be conducted to determine appropriate practices for each region. Without an adequate

understanding of the crop, farm yields will probably be low and soybeans may be rejected as a cash or food crop.

Additional information is needed to determine the effect of the different variables on individual soybean cultivars. Knowledge of cultivar response to management and environmental variables will help in the selection of cultivars for evaluation in new areas with soybean production potential.

Soybean Seed Quality Studies

A. Production Aspects

Variable soybean seed quality frequently necessitates the use of excessive seeding rates to compensate for poor emergence. In tropical regions, where yield of early maturing cultivars is particularly dependent on establishment of adequate plant stands, seed quality problems may limit the commercial use of such varieties.

In 1976, a study was initiated to 1) monitor seed quality of introduced, early maturing cultivars in different tropical environments, 2) determine the effects of variations in seed quality on stand establishment and productivity of the subsequent soybean crop and 3) evaluate the usefulness of a foliar applied fungicide in improving soybean seed quality in the tropics.

Trial plans, seeds, inoculant, and fungicide for foliar application were supplied for installation of the study in Thailand, Sri Lanka, Ecuador, Puerto Rico, California, and Illinois. Management instructions were similar to those provided with the International Soybean Variety Experiment except for details of treatment application. Treated plots received two applications of mixture of Benlate and Captan, the first at early pod set and the second 18-20 days later. The few carefully selected locations chosen for the study represented a broad range of environment conditions.

Agronomic data and seed samples have been received from six of the eight locations where the trial was planted. The seed samples were forwarded to Mike Ellis, INTSOY pathologist, University of Puerto Rico for bioassay. Results of this phase of the study are not yet available.

Effects of the foliarly applied mixture of fungicides on soybean yield and seed quality are presented in Table 3. Yield was not increased by treatment at either of the two locations, Boliche, Ecuador or Davis, California, where the growing season was dry and irrigation required for proper development of the crop. At the other locations, yield was increased by from 3 to 18 percent. Seed quality, as visually rated, was significantly improved at Isabela, Puerto Rico and Urbana, Illinois only.

The most important results of this study will relate to the effects of treatment on seed quality. Several seedborne pathogens have been shown to reduce seed viability and seedling vigor. Control of these through appropriate management of the crop may provide a short-term solution to pathogen related seed quality problems in the tropics. Incorporation of

Table 1. Simple correlation coefficients between individual agronomic, environmental, and management variables measured at 25 locations in 1974.

	Agronomic Variables 1/				
	Yield	Seed Weight	No. days to flower	No. days from flowering to maturity	Plant height
Agronomic Variables					
Seed weight	.49*				
No. days to flower	-.10	.00			
No. days from flowering to maturity	.22	.46*	.27		
Plant height	.46*	-.13	.31	.27	
Environmental Variables					
Altitude	-.12	-.01	.81**	.32	.31
Latitude	.07	-.46*	.22	.10	.57**
Daylength	-.16	-.35	.36	.38	.44*
Max. temperature	-.03	-.21	-.60**	-.20	-.08
Min. temperature	-.01	-.16	-.68**	-.18	-.28
Management Variables					
Nodule number	.19	-.18	.20	.21	.67**
Nodule dry weight	.37	.13	.06	.09	.47*
Nitrogen	-.02	-.43*	.00	-.08	.22
Phosphorus	.05	-.26	-.03	.14	.09
Potassium	-.38	-.33	-.14	-.29	-.35
Plant stand	.33	.06	.03	-.06	.13

1/ Agronomic variable are based on the mean performance of 10 cultivars common to each site.
* significant at the 5% level
** significant at the 1% level

Table 2. Coefficients of determination from regression analyses relating agronomic variables to environmental and management variables.†

Dependent Variable	$R^2(E)$	$R^2(M)$	$R^2(E+M)$	$R^2(E')$	$R^2(M')$
Yield	.08	.53*	.79**	.26*	.71**
Seed weight	.35	.29	.72*	.43*	.37
No. days to flower	.69**	.11	.76*	.65**	.07
No. days from flowering to maturity	.20	.45	.70*	.25	.50*
Plant height	.42*	.66**	.71*	.05	.29

† $R^2(E)$, $R^2(M)$, $R^2(E')$, and $R^2(M')$ are defined in the text. $R^2E+M)$ is the total variability accounted for by the management and environment variables measured.
* significant at the 5% level
**significant at the 1% level

Table 3. Effects of foliarly applied fungicides on yield and seed quality of soybeans, 1976

	Locations					
	Puerto Rico	Ecuador	California	Illinois Urbana	Illinois Brownstown	Illinois Dixon Springs
	Yield, kg/ha					
Treated	1868	2554	3150	3322	2170	4048
Non-treated	1564	2698	3223	3235	1926	3763
Difference 1/	304* 1/	-144*	-73	87	244*	285
	Quality 2/					
Treated	2.42	2.50	-	1.58	2.08	2.42
Non-treated	3.58	2.75	-	2.00	2.00	2.17
Difference	-1.16*	-0.25		0.42*	0.08	0.25

1/ An asterisk indicates that treated and non-treated are statistically different at the 5% level of probability.

2/ Quality was noted on a scale of 1 to 5, with 1 indicating very good quality and 5 very poor quality.

improved seed quality characteristics--including resistance to seedborne pathogens--through breeding is considered to be a more valuable long-term goal and is a primary focus in the INTSOY program in Puerto Rico.

B. Soybean Seed Storability

A series of experiments to determine specific changes in viability and vigor of soybeans as influenced by environmental conditions during maturation and subsequent storage were reported tin the Final Report on contract AID/CM/ta-c-73-19. The effect of seed source and planting date on seedling emergence, yield, and subsequent seed viability was also reported.

Seed viability and vigor were impared by humidity treatments during the maturation stage of growth. The high incidence of Phomopsis was associated with a decline in seed vigor before physiological maturity. Williams cultivar tolerated adverse high humid conditions and had better seed quality than cultivars of comparable maturity, i.e., Wayne and Woodworth. Studies of effects of storage conditions on soybean viability, however, involved only one cultivar so did not permit the measurement of differential effects. Because differential effects may exist, a new research program was initiated in 1976 to study genetic differences in the tropical germplasm for storability under adverse conditions. Three hundred ninety-six seed production plots were established at Isabela, Puerto Rico in February 1976. Many of the later lines did not mature because of the effects of increasing daily photoperiods after the vernal equinox. The plants were treated twice with a systemic fungicide, Benlate, in an attempt to control seedborne fungal pathogens. Seed produced from the lots were also treated with Penicillin to control seedborne bacteria. The principal objective of the study was to separate disease-related seed quality attributes from physical attributes inherent in a given variety. Seed samples of each of the 235 genotypes were placed in storage conditions simulating ambient gropical environments. Subsamples of each genotype were removed at biweekly intervals and tested for germination after being subjected to a rapid aging process.

Most of the genotypes initially had germination percentages in excess of 80 percent. However, in as little as six weeks, several lines had less than ten percent of their initial germination, and separation of genotypes as to storability became obvious. Table 1 illustrates the performance of several of the lines with better than average storability in comparison with the cultivars Hardee and Jupiter. The only genotype which maintained an appreciable level of germinability over the length of the study was Barchet, a genotype having a hard seedcoat. All seeds having this characteristic at the end of 12 weeks of storage germinated upon scarification. Subsequent screening of a large number of genotypes has resulted in the identification of 19 genotypes having more than 30 percent "hardseed". These lines will be planted in summer 1977 to assess the usefulness of the hard seedcoat characteristic in improving seed quality, to make crosses for a study of inheritance of "hardseed" and to produce sufficient material for evaluation of the storability of such seed under a range of storage environments.

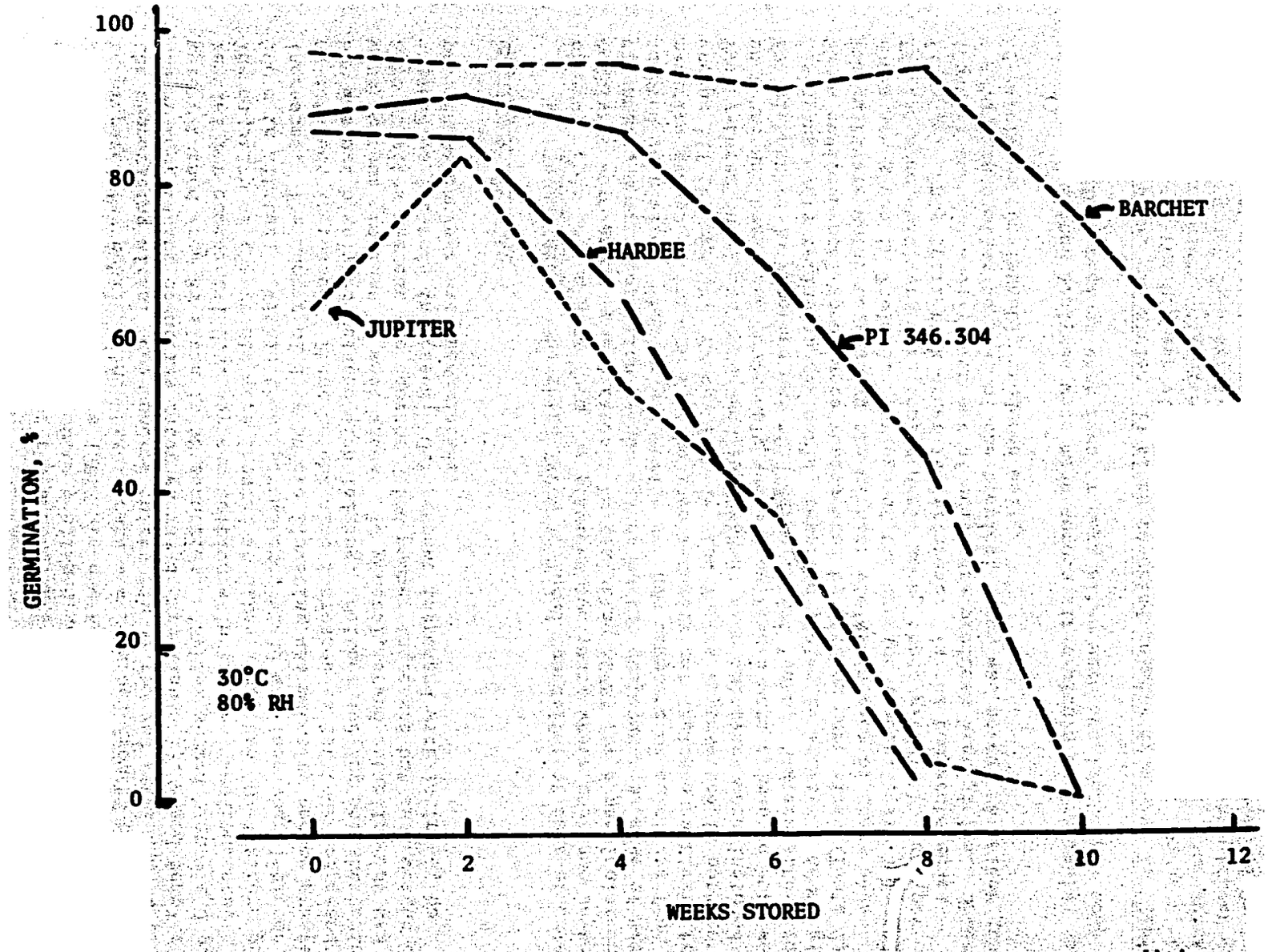

Figure 1. Germination of four genotypes under simulated tropical storage conditions.

International Soybean Variety Evaluation Experiment (ISVEX)

The 1974 results, for the second International Soybean Variety Evaluation Experiment (ISVEX), were published and distributed to interested parties. Results for the third ISVEX are now being analyzed and will soon be prepared for publication. Usable data was returned from 53 percent of the cooperating sites. One hundred thirty six of the 259 sites and 64 of the 90 cooperating countries returned valid data (Table 1). Seed samples were returned from 89 sites in 48 countries. ISVEX-2 showed high yield potential for commercial U.S. soybean cultivars in tropical environments. The majority of the trials were located at sites less than 30° latitude and 500 m in altitude. Within these parameters, yield was less when latitude increased. The number of days before flowering and maturity became greater as latitude increased. Plant height also increased as latitude increased but was not highly correlated with lodging. Neither lodging nor shattering were serious problems at most sites. In general, stand establishment was best at sites nearest the equator. Pod number increased but seed weight decreased as latitude increased. Oil percent increased with latitude, but protein percentage was inconsistent.

Summary of Results

A. Third ISVEX. The third ISVEX included two different sets of cultivars. This is a change from the previous two years where the same soybean cultivars were grown on all sites. By tailoring ISVEX for a particular environment, more information may be gained by the cooperator because of bette adapted cultivars. The early maturity set was composed of cultivars from maturity groups 00 through V. The late maturity set included cultivars from groups III through IV. Groups III, IV, and V were represented in both sets. Twenty-three cultivars were used in ISVEX-3; nine cultivars were included that had not been used previously and six cultivars were common to both sets The highest yield reported was 6053 kg/ha in Sri Lanka. Sixteen sites, representing every region in the world, reported yields in excess of 4000 kg/ha Seven environmental zones were represented by five or more sites (Table 2). The cultivars which have shown the highest yields in each of these seven zones are given in Table 3. Throughout the tropics (zones I, II, III, and IV) the same cultivars were consistently among those giving the best performance. Only their rank order is different. In the temperate zones (zones X and XI) a different set of cultivars produced high yields. However a situation similar to that of the tropical zones was noted. A select group of cultivars consistently produced well.

Combined analyses were made across sites within environmental zone and geographical regions when five or more sites were available in a zone.

Africa. In Africa, 13 sites reported results from zone I. Jupite was reported as the highest yielding cultivar with 1821 kg/ha. However, thi was not significantly different from Hardee, Forrest, or Davis. The average yield reported was 1422 kg/ha. Jupiter was the latest maturing cultivar witl 103 days to maturity as compared to the mean of 89 days. Hardee and Davis did not differ significantly from the grand mean of 89 days. Jupiter with its late maturity had a plant height of 51 cm as compared to the grand mean of 30 cm. Hardee and Davis developed heights not significantly different from the grand mean.

Ten sites reported results from zone III in Africa. Zone III has the same latitude as zone I, but is higher in altitude. Yields at these higher altitude sites were less than those reported at the lower altitude sites. The mean was 1158 kg/ha. Only three cultivars were common to all sites. These three cultivars, Forrest, Williams, and Columbus, did not differ significantly in yield. They did take longer to mature at the higher elevation. The mean for maturity was 125 days which is 40 days longer than zone I. Plant height was also higher in zone III; 33 cm was the average for these varieties in zone I and 42 cm was the average in zone III.

Thirteen sites reported results from zone IV in Africa. Jupiter, the highest yielding cultivar, produced a yield of 1610 kg/ha. However, none of the varieties were significantly different in yield although the range went from 1277 kg/ha for Clark 63 to 1610 kg/ha for Jupiter. Plant height for these cultivars was reported as 36 cm which is not very different than the 33 cm reported in zone I. Days to maturity were less than in zone I with an average of 92 days.

Asia. In Asia, 17 sites reported data from zone I. However, only two varieties were reported from all stations. Forrest and Williams had yields of 1877 kg/ha and 1837 kg/ha, respectively. Forrest and Williams had plant heights of 33 cm and 40 cm and maturity days of 89 and 87 days, respectively. Five sites reported results from zone VII. These results showed Columbus (1472 kg/ha) producing the highest yield. However, the yield was not significantly different from Forrest, Williams, Calland, Clark 63, and Woodworth. The average yield was 1271 kg/ha. Forrest took an average of 32 days to flower and 97 days to mature compared to the average of 30 and 94 days, respectively. Calland was the tallest cultivar in these trials; it attained a height of 44 cm. The rest of the cultivars were not significantly different from the mean of 39 cm. In the latitudes between 31°- 40° (zone X) five sites reported results. The average yield in this zone was 897 kg/ha. Williams produced the highest yield (984 kg/ha) but this was not significantly different from the other cultivars tested (same cultivars as reported in zone VII). Days to maturity ranged between 121 days for Forrest to 105 days for Woodworth. Plant height ranged from 84 cm for Calland to 72 cm for Woodworth.

Mesoamerica. All the Mesoamerica sites (8) that reported results were in zone IV. Columbus produced the highest yields, but was not significantly different from Williams, Hardee, and Forrest. The yields ranged from 2120 kg/ha for Columbus to 1349 kg/ha for Woodworth. Columbus was reported to flower in 26 days and mature in 87. The average days to flowering for all varieties was 27 with maturity at 90. Plant height ranged from 44 cm for Columbus to 30 cm for Tracy.

South America. Sixteen sites in zone I reported results from South America. Jupiter was the highest yielding cultivar at 2673 kg/ha. Jupiter was significantly taller (62 cm) and later maturing (107 days) than the other cultivars reported. The mean days to flower and maturity were 33 and 97 days, respectively, for the varieties reported. Plant height averaged 37 cm. The earliest maturing cultivar was Bossier at 89 days. The shortest cultivar was Cobb at 26 cm and produced the lowest yield.

B. Fourth ISVEX. A total of 290 trials were distributed to 82 countries in 1976 (Table 4). Six sets of cultivars composed the fourth ISVEX. The distribution of the different sets were determined by the latitude and altitude of the sets where the trial was to be grown. Two sets of cultivars were organized on a cooperative basis with other international institutions. These were the International Institute for Tropical Agriculture in Ibadan, Nigeria and the Southeast Asia Regional Center for Graduate Study and Research in Agriculture at Laguna, the Philippines.

Fifty different cultivars comprised these six sets. Out of these 50 cultivars 34 were developed in the United States. The rest were developed by breeders in Asia and Africa. The cultivars Calland, Williams, Woodworth, Clark 63, and Forrest were included in all six sets. Data has been received from 54 sites in 31 countries that tested ISVEX-4.

C. Fifth ISVEX. Four sets of cultivars are presently being distributed in 1977. The cooperative trial with IITA is being continued for its second year. However, the cooperative trial with SEARCA has been discontinued. The sets are organized by environmental zones utilizing cultivars which have performed well in past experiments and new entries with potential in each environment. Two cultivars, Cobb and Williams, are included in all four sets. As of April 20, 1976, ISVEX-5 has been requested by 143 sites in 80 countries. Eighty trials have already been distributed. Several new cultivars are being included in ISVEX-5. Among them Kahala, a large seeded vegetable type. Another cultivar is Rillito, a release from Arizona. This cultivar may show greatest potential under arid conditions. This year a blend, designated B-1, is also included. The blend is made up of equal proportions of three soybean cultivars Bossier, Cobb, and Davis. Test results, by private companies, have shown the effectiveness of blends in attaining high stable yields in the United States.

D. SPOT-1. The Soybean Preliminary Observation Trial (SPOT) was developed in cooperation with the 211(d) activities of the University of Illinois and the University of Puerto Rico. This trial is set up to serve as a screening test for new material. SPOT can identify cultivars which have high yield potential and can identify the environment to which these cultivars are best adapted. Cultivars which do show high yield response will then be included as new entries in ISVEX. The rank of cultivars tested in SPOT-1 for yield is presented in Table 5. Four out of the six sites conducted successful trials. The varieties Jupiter and Bossier, which have previously demonstrated their high yield potential in ISVEX, were used as checks. These cultivars gave a reference point to which the performance of new cultivars could be evaluated.

E. SPOT-2. This year ten sites have been suggested for SPOT. At present, these sites are being contacted. Although, many of these sites have expressed an interest in SPOT, not all of them have agreed formally. The sites which conducted SPOT last year (Puerto Rico, Ecuador, India, Philippines, Rhodesia, and Sri Lanka) along with new proposed sites (Indonesia, Ghana, Peru and Thailand) will give the cultivars a thorough testing. The cultivars included in this year's test are:

CH-3	Santa Rosa
Buffalo	GH23-2-1 (1)
SJ-2	UFV-1
Jupiter	IAC-73-4013
Bossier	UFV-1 (BP-20)
M-79	Local
HLS	Orba

Discussion

The soybean cultivars which consistently produce high yields in the tropics have originated from the southern United States. Davis, Jupiter, Forrest, Hardee, Improved Pelican, and Bossier have now completed their third year of testing. Over these years they have consistently produced the highest yields. In ISVEX we call on the individual cooperator to substitute his own locally adapted cultivar. Seldom do these cultivars produce as well as those provided by INTSOY. The soybean breeding program in the United States has developed high yielding cultivars, which produce well under tropical environments even though they were not selected specifically for tropical conditions. We hope to encourage breeders in the tropics to develop their own high yielding soybean cultivars. SPOT will serve as the preliminary testing ground for their efforts.

TABLE 1. Sites which completed the Third International Soybean Variety Experiment (ISVEX-3)

Region	Country	Site	Protein & Oil Analysis
Africa	Algeria	Khemis-Miliana	
	Burundi	Bujumbura	*
		Mosso	
	Cameroon	Wum	*
	Congo	Lekana	*
	Dahomey	Ina	*
	Egypt	Bahteem	*
		Seds	*
	Ethiopia	Arussie Negellie	*
		Bako	*
		Debra Zeir	*
		Jimma	*
		Dembere Kella	*
	The Gambia	Yundum	*
	Ghana	Legon	
	Ivory Coast	Abidjan	
		Badikaha	
		Dekokaha	
		Gagna	
		Odiene	
		Seguela	
		Sirasso	
	Lesotho	Mejametalana	*
	Mali	Koulikoro	*
		Sikasso	*
	Mauritis	Pamplemousses	*
		Reduit	*
	Niger	Maradi	*
		Gaya	*
	Reunion	St. Denis	*
		St. Denis	*
		St. Pierre	*

Region	Country	Site	Protein & Oil Analysis
Africa	Rhodesia	Salisbury	*
	Rwanda	Rubona	
		Karama	*
	Senegal	Sefa	
	Sierra Leone	Njala	
	Swaziland	Malkerns	*
	Tanzania	Ilonga	
		Lyamungu	
		Mbeya	
		Ukiriguru	
	Togo	Amoutchou	*
		Davie	*
		Kitangbo	*
	Upper Volta	Bobo-Dioulasso	*
		Farako-Ba	*
		Saria	*
	Zambia	Magoye	*
		Mufulira	*
Asia	Bangladesh	Joydevpur	
	India	Jabalpur	*
	Indonesia	Bogor	*
		Kebun Jeruk	*
	Korea	Jeju	
		Suweon	
		Suweon	
		Suweon	
	Nepal	Kumaltar	*
	Pakistan	Islamabad	
		Lahore	
		Lyallpur	
		Rawalpindi	
		Swat	
		Tandojam	
		Tandojam	

Region	Country	Site	Protein & Oil Analysis
Asia	Philippines	La Carlota	*
		La Carlota	*
		Los Banos	
	Sri Lanka	Alutharama	*
		Alutharama	
		Angunjkolapalessa	*
		Gannoruwa	*
		Gannoruwa	*
		Kilinochichi	
		Okkempitiya	*
		Maha Illuppallama	*
		Moneragala	
		Balbalogoda	*
		Puttalam	
		Ratmalogara	*
		Thirunelvely	*
		Thirunelvely	
	Taiwan	Shanhua	
	Thailand	Farm Suwan	
		Khon Kaen	*
		Surat Thani	*
Europe	Hungary	Godollo	*
	Italy	Sassari	
	Spain	Madrid	*
	Yugoslavia	Novi Sad	*
MesoAmerica	Bahamas	San Andros	*
	Belize	Central Farm	*
	Costa Rica	Canas	
		Guanacaste	
	Honduras	La Lima	*
	Jamaica	Caymanas	
	Martinique	Le Lamentin	*
		Managua	*
		Nagarote	*
		Posoltega	*

Region	Country	Site	Protein & Oil Analysis
MesoAmerica	Panama	Chichebre	
		Rio Hato	
	Trinidad and Tobago	Port-of-Spain	
Middle East	Iran	Karaj	*
		Rezaiyeh	*
	Israel	Bet Dagan	*
	Jordan	Wadi Dulail	
	Lebanon	Beqa'a	
	Saudi Arabia	Al-Hassa	*
		Wadi Jizan	*
North America	U.S.A.	Urbana, Illinois	
Oceania	Fiji	Legalega	*
		Seaqaqa	*
	Tahiti	Papeete	
South America	Argentina	Parana	*
	Bolivia	Adapo-Izozoz	
		Palometillas	*
		Santa Cruz	*
		Santa Cruz	*
	Brazil	Manaus	*
	Chile	La Plantina	
	Colombia	Espinol Tolima	
		Motilonia	*
		Turipana	*
	Ecuador	Boliche	*
		Pallatanga	
		Pichilingue	*
		Portoviejo	*
	French Guiana	Cabassou	*
	Guyana	Enmore	
		Enmore	
		La Bonne Intention	
		Mon Repos	*

Region	Country	Site	Protein & Oil Analysis
South America	Peru	Chiclayo	
		El Porvenir	
		El Porvenir	
		Piura	
	Venezuela	Maracay	

* More than one experiment during year

TABLE 2. Description of environmental zones for the third International Soybean Variety Evaluation Experiment.

Zone	Latitude	Altitude	Number of Sites
I	≤ *10°59'	≤ *500	50
II	≤ *10°59'	501 - 1000	5
III	≤ *10°59'	≥ **1000	12
IV	11° - 20°59	≤ *500	32
V	11° - 20°59	501 - 1000	1
VI	11° - 20°59	≥ **1000	3
VII	21° - 30°59	≤ *500	11
VIII	21° - 30°59	501 - 1000	0
IX	21° - 30°59	≥ **1000	2
X	31° - 40°59	≤ *500	9
XI	31° - 40°59	501 - 1000	7
XII	31° - 40°59	≥ **1000	2
XIII	≥ **41°	≤ *500	2

*≤ = less than or equal to.

**≥ = greater than.

TABLE 3. Rank of cultivars for high yield within zones with five or more sites for the third International Soybean Variety Experiment.

Zone I	Zone II	Zone III	Zone IV	Zone VII	Zone X	Zone XI
(50 sites)	(5 sites)	(12 sites)	(32 sites)	(11 sites)	(10 sites)	(7 sites)
Jupiter	Jupiter	Williams	Davis	Forrest	Williams	Woodworth
Davis	Improved Pelican	Davis	Jupiter	Jupiter	Calland	Williams
Hardee	Hardee	Forrest	Forrest	Bossier	Clark 63	Columbus
Improved Pelican	Davis	Bossier	Columbus	Davis	Woodworth	Calland
Forrest	Forrest	Hardee	Hampton 266A	Hardee	Columbus	Clark 63

Table 4. List of cooperators for the fourth International Variety Evaluation Experiment, 1976

Region	Country	Name	Address
AFRICA	Algeria	Officer-in-Charge	Station Regionale I.D.C.I. Khemis-Miliana El-Asnam, ALGERIA
	Botswana	Mr. Warren Wagner	Manager Farmers Brigade Box 208 Mochudi, BOTSWANA
	Burundi	Mr. E. Sebatutsi	Director General ISABU B. P. 795 Bujumbura, BURUNDI
	Cameroon	Monsieur le Director de l'I.C.V.T.	B.P. 2123 Yaounde Republique Unie du CAMEROON
		Monsieur le Chef da la Station I.C.V.T.	B. P. No. 44 Dschang, CAMEROON
		Director	Wum Area Development Authority Wum via Bamenda P. O. Box 13 CAMEROON
	Central Africa Republic	Mr. Emilein Lefort	Project Manager FAO, CAF/72/006 c/o Resident Representative UNDP P. O. Box 872 Bangui, REPUBLIC OF CENTRAL AFRICA
	Congo	Ing. Ivetic Obrad	Expert de la FAO BT. 3 Lekana Brazzaville, CONGO
	Dahomey	Monsieur le Directeur IRAT/BENIN	B. P. 422 Cotonou - Republique Populaire Du Benin (DAHOMEY)
		Mr. E. Limburg	Action Rurale B. P. 5 Save Benin (DAHOMEY)
	Egypt	Samir Ali Mahmoud	Agricultural Research Centre Field Crops Institute Legumes Section Giza, A.R.E., EGYPT

Region	Country	Name	Address
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	Ethiopia	Dr. Alemu Mengistu	Agricultural Experiment Station P. O. Box 32 Debre Zeit ETHIOPIA
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		Dr. Bob Dadson	Department of Crop Science Faculty of Agriculture University of Ghana Legon, GHANA
		Mr. Hector Mercer-Quarshie	Crops Research Institute P. O. Box 3785 Kumasi, GHANA
	Ivory Coast	Dr. A. D. Assa	Ministry of Agriculture B. P. 4322 Abidjan, IVORY COAST
	Lesotho	Mr. Chu Chen-Kien	P. O. Box 789 Maseru LESOTHO
		Project Manager	Senqua Project P. O. Box 301 Maseru, LESOTHO
	Liberia	Mr. Gunnar Barnes	c/o United Methodist Church P. O. Box 1010 Monrovia, LIBERIA
		Mr. James Kokro	Central Agriculture Experiment Station Ministry of Agriculture Monrovia, LIBERIA

Region	Country	Name	Address
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		Mr. Y. O. Tzou	Central Agricultural Experiment Station Suakoko, Bong County Ministry of Agriculture, LIBERIA
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	Mali	Monsieur le Chef de la Mission IRAT au Mali	Boite Postale 438 Bamako, REPUBLIC OF MALI
	Mauritius	Dr. A. L. Owadally	Divisional Scientific Officer Government of Mauritius Ministry of Agriculture and Natural Resources Reduit, MAURITIUS
	Niger	Monsieur le Directeur General de l/INRAN	Ministere de l'Economie et du Climat B. P. 429 Niamey, REPUBLIQUE DU NIGER
	Rhodesia	Dr. J. R. Tattersfield	Head Crop Breeding Institute Causeway Salisbury, RHODESIA
	Rwanda	ISAR Karama	B. P. 121 Kigali, RWANDA
		Mr. F. Iyamuremye	Director General ISAR Station de Rubona B. P. 138 Butare REPUBLIQUE RWANDAISE
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Region	Country	Name	Address
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	Togo	Mr. J. Marquette	Le Chef de la Mission IRAT au TOGO a B. P. 1163 Lome, TOGO
	Uganda	Mr. C. K. Bulungu	Department of Crop Science Makerere University P. O. Box 7062 Kampala, UGANDA
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Region	Country	Name	Address
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	India	Dr. S. M. Sharma	Botanist (Soybean) Department of Plant Breeding and Genetics J. M. Krishi Vishwa Vidyalay Jabalpur - 4 (M.P.) INDIA
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Region	Country	Name	Address
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	Poland	Prof. dr. Jerzy Szyrmer	Instytut Hodowli I Aklimatyzacji Roslin Radzikow 05-870 Blonie K/Warszawy POLAND
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	Costa Rica	Mr. Rodrigo Alfaro Monge	In-Charge of the Soybean Progam Ministerio de Agricultura y Ganaderia San Jose, COSTA RICA
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Region	Country	Name	Address
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	Mexico	Ing. Benito Cazares Enriquez	INIA Campo Agricola Experimental Apdo. Postal No. 40 Apatzingan, Mich., MEXICO
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NORTH AMERICA	United States	Mr. R. E. Dunker	Department of Agronomy University of Illinois Urbana, ILLINOIS 61801
SOUTH AMERICA	Argentina	Dr. Raul Vicentini	INTA Estacion Experimental Regional Agropecuaria Casilla Correo 128 3100 Parana, Entre Rios, ARGENTINA
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Region	Country	Name	Address
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	Colombia	Ing. Gilberto Bastidas R.	Director of Nacional Programa Leguminosas Anuales I.C.A. Palmira, COLOMBIA
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Region	Country	Name	Address
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	Saudi Arabia	Mr. Ziad Adham	Acting Director General of Agricultural Research and Development Department Ministry of Agriculture and Water Riyadh, SAUDI ARABIA

Region	Country	Name	Address
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	New Caledonia	Monsieur le Chef du Service le l'Agriculture	B. P. 43 Noumea, NEW CALEDONIA
	New Hebrides	Mr. B. L. Weightman	Head of Section Experimentation, Training, and Demonstration Department of Agriculture Port Villa, NEW HEBRIDES

Table 5. Rank of cultivars for high yield in the Soybean Preliminary Observation Trial at their respective sites.

Experimental Zones	IV	I	IV	IV
Location	Isabella Puerto Rico	Boliche Ecuador	Pantnagar India	Laguna Philippines
	Hardee - LS	Jupiter	Bossier	UPL SY-2
	Santa Rosa	M-79	PK-71-21	SJ-1
	SJ-2	Hardee LS	Hardee LS	Clark 63
	SJ-1	Buffalo	SJ-1	SJ-2
	Jupiter	CH-3	M-98	Bossier

APPENDIX II

Report of Leslie K. Ferrier
Assistant Professor of Food Science

Research was Conducted in the Following Areas

1. The search was continued for varieties of soybeans which become the most tender upon cooking. Thirty varieties of soybeans were tested for tenderness after cooking by one of four methods. The objective tenderness of each variety was compared to its chemical composition and physical characteristics in a search for factors which correlated with the tenderness of cooked whole soybeans.

2. A study was completed on the effects of using different sieve fractions of milled corn and different preparation techniques on the nutritional quality (protein efficiency ratio) of drum dried corn:soy flakes.

3. The development of a procedure for preparation of a palatable and generally acceptable soy beverage for home and village industry was completed. This work was carried out by an INTSOY supported graduate student working under faculty direction.

4. The filtering devices which are required for preparation of the soy beverage at the village level were evaluated.

5. The low cost ($704.00) Decker hammermill for grinding of dry soy, cereals and soy:cereal combinations was evaluated for performance.

6. A simple device was designed, constructed, and evaluated for separation of cracked soybeans into the hull and cotyledon fractions.

Summary

1. Effect of Variety on Tenderness of Cooked Whole Soybeans.

Data from varieties harvested in 1974 were reported last year. These experiments were repeated with the 1975 crops in order to test consistency from one crop year to the next. The data indicated that similar factors influenced the tenderness of cooked whole soybeans in both crop years.

a. The most important factor affecting objective tenderness of cooked soybeans was the composition of soaking and cooking solution. Use of bicarbonate solutions instead of tap water increased the tenderness of soaked and cooked soybeans nearly 3-fold.

b. Soaking before cooking increased tenderness about 1.8-fold compared to cooking without soaking.

c. The tenderness of cooked soybeans varied about 2-fold with variety.

d. The relative tenderness among varieties varied with soaking and cooking treatment. A given variety was not always more tender than another given variety under all four soaking and cooking conditions. However, this variation was not as great as reported last year. This year, three varieties, Amsoy '71, Hark and Kanrich were among the six most tender varieties in all four treatments.

e. The tenderness of each variety of cooked whole soybeans was not consistent from one crop to the next. Only Bonus and Cobb soybeans were among the six most tender varieties in both years.

f. Several physical characteristics of the dry raw whole soybean, but no chemical characteristics, correlated with the tenderness of cooked, whole soybeans. Factors which correlated well with tenderness included weight/bean, volume/bean, density/bean, rehydrated weight/bean and rehydrated volume/bean.

2. Nutritional Quality of Drum-dried Corn:soy Flours.

A study was begun during the last contract period on the effects of different sieve fractions of dry milled corn and different preparation procedures on the nutritional quality of drum dried corn:soy flakes. It was completed early in this contract year. Corn fractions included ground whole corn, commercial cornmeal, and three fractions from ground whole corn viz., over 22 wire mesh screen, over 32 wire mesh screen and through 32 wire mesh screen. Different corn fractions were used for preparation of drum dried corn:soy flours and the PER's of the flours were measured. PER's were affected by the fraction of corn used but they were not significantly affected by gelatinization of the corn:soy before drum drying. Increasing the amount of corn from 67% to 80% of the blend improved the PER for only one of the three corn fractions tested. These effects could not be accounted for by the balance of essential amino acids in the corn:soy flours.

3. Development of a Procedure for Preparation of a Palatable Soy Beverage for Home and Village Use.

The study on developing the concept for preparation of a palatable soy beverage for home and village industry was completed. Work during this contract period covered final development of the preparation concepts as well as a comprehensive organoleptic evaluation of soy beverages prepared from whole raw dry soybeans, cracked or broken raw dry soybeans.

This concept involved very low level technology and a brief description of the process follows. Whole soybeans were dropped directly into boiling water and the beans were boiled for ten minutes. After boiling, the weight of beans and water was adjusted to the starting level and the bean-water mixture was ground in a Waring blender. The ground slurry was simmered for an additional 20 minutes after which sufficient water was added

to replace that lost by boiling and the slurry was filtered. The filtrate or milk was flavored by the addition of sugar, salt and diacetyl or vanilla.

Cracked or broken raw dry beans and powdered raw dry soybeans were also used to prepare the soymilk. A comprehensive organoleptic evaluation of the soy milks prepared from the three types of soy raw materials showed no significant differences in organoleptic properties with these exceptions. Color scores were significantly (5% level) higher for the soy milk prepared from the powdered dry raw soybeans. Flavor and mouth drying characteristics of beverage prepared from powdered soybeans were significantly (5% level) inferior to beverages prepared from whole or cracked beans. However, all scores were in the range of 7 on a hedonic scale of 9 which indicated good acceptance for all products. The procedure is considered ready for further testing and improvement under conditions in developing countries.

4. Evaluation of Filtering Concepts for Village Level Preparation of Soy Beverage.

Three systems were developed for filtering soy slurries in preparation of soy milk. These were a simple filter sock or bag, a cradle filter and a basket centrifuge. These filtering devices were thoroughly tested using soybean slurries made from whole, cracked and powdered soybean. These tests showed that soy milks prepared from the three different raw soybean types were quite similar with respect to yield, percent protein, solids and sediment. Comparison of the soy milks prepared with the different filtering devices showed that the centrifuge produced the highest yield of milk, followed by the sock and cradle methods. However, the sediment content of the milk filtered with the sock method was invariably much lower than that from the centrifuge or cradle. When all factors were considered the sock filter was judged to be the most desirable method. This method employs the lowest level of technology and is by far the cheapest and easiest to manufacture.

5. Evaluation of Mill for Grinding Dry Soybeans and Various Cereal Products.

A Decker Grainmaster hammermill was purchased for $704.00. This price included three mill screens and was the lowest priced hammermill in about sixty quotations from suppliers. This mill was tested using dry soybeans, corn and soybean-corn mixtures. The mill performed acceptably and was found to be satisfactory for grinding dry raw soybeans for soy beverage preparation. This mill is recommended for installation as a cooperative utility for village use.

6. Development of a Device for Separation of Hulls from Cotyledons.

A device was designed, constructed and tested for the separation of hulls and cotyledons from dehulled whole dry soybeans. Thorough testing showed that this equipment would separate virtually 100% of the hulls and approximately 64% of the hypocotyls from the cotyledons. This unit offers a simple, low cost system that can be readily constructed in developing countries.

Items 3 to 6 were reported in detail earlier in a terminal report (Nov. 1976) and will not be discussed further.

1. Effect of Variety on Tenderness of Cooked Whole Soybeans.

During FY 1976, cooking trials were conducted on 28 varieties of soybeans from the 1974 crop. Most of these were U.S. varieties used in the INTSOY trials. Those trials demonstrated that:

a. The tenderness of each variety after cooking depended on the soaking and cooking treatments given.

b. The tenderness of any given variety of soybeans relative to the other varieties depended on the soaking and cooking treatment. No variety was consistently most tender under all soaking and cooking conditions.

c. Varieties which had large seed size and the greatest change in size during soaking and cooking tended to yield the most tender cooked soybeans.

During FY 1977, these variety tenderness experiments were repeated using soybeans from the 1975 crop. Complete repetition of the experiment was necessary since it is well known that crop characteristics vary from one year to the next.

As described last year, four combinations of soaking and cooking were tested since the different processing methods greatly affect tenderness. Twelve physical and chemical measurements were made on each soybean variety in order to find factors which may be used to predict the tenderness of cooked whole soybeans.

Methods

Thirty varieties of soybeans from the 1975 crop (Table 1) were soaked and cooked using four methods as outlined in Table 2. Triplicate 100 g samples of mature, whole soybeans were soaked and cooked for studies on their rehydration, tenderness measurements and organoleptic trials. U.S. varieties of soybeans were supplied by Dr. D. K. Whigham, INTSOY. All solutions, including tap water, were adjusted to pH 7.5 with NaOH or HCl. After soaking, the soybeans were drained and fresh solutions were added for blanching. The soybeans were cooked for 60 minutes and tenderness was determined objectively using a LEE-Kramer shear press and subjectively with a taste panel of 6-10 members. Tenderness data was evaluated statistically using an analysis of variance.

The following physical measurements were made: volume/bean; (by displacement of water in a sealed flask); weight/bean (average of 300 beans); density before treatment; and density after soaking and cooking. Amount and rate of water uptake by the beans was measured by placing 60 g samples in a water bath at 25°C and measuring weight difference at 6, 13, 20, and 36 minutes, and at 1, 1.5, 3, 4.5, and 6 hours. Each variety was analyzed for nitrogen, fat, moisture, fiber, ash, and carbohydrate (by difference).

TABLE 1. SOYBEAN VARIETIES TESTED AND PRODUCTION AREA

Variety	State Produced	Variety	State Produced
Amsoy 71	Illinois	Hodgson	Minnesota
Bonus	Illinois	Improved Pelican	Louisiana
Beeson	Illinois	Kanrich	Illinois
Bossier	Louisiana	Ozonoshiki	Japan
Bragg	Arkansas	Pampeira	Brazil
Calland	Illinois	Perola	Brazil
Clark 63	Illinois	Pickett	Arkansas
Cobb	Alabama	Planalto	Guaiba, Brazil
Corsoy	Illinois	Prata	Guaiba, Brazil
Columbus	Kansas	Prize	Illinois
Davis	Arkansas	Ransom	Arkansas
Essex	Tennessee	Steele	Uncertain
Forrest	Arkansas	Swift	Minnesota
Hark	Illinois	Williams	Illinois
Hill	Arkansas	Woodworth	Illinois

TABLE 2. EXPERIMENTAL DESIGN FOR SOAKING AND BLANCHING SOYBEANS

Combination	Soak Conditions	Blanch Conditions
1.	None	Tap water, 60 min. 100°C.*
2.	None	0.5% $NaHCO_3$, 60 min. 100°C.
3.	Tap water, 20 hr, room temp.	Tap water, 60 min, 100°C.
4.	0.5% $NaHCO_3$, 20 hr. room temp.	0.5% $NaHCO_3$, 60 min. 100°C.

* Tap water; partially softened water as supplied by the city of Urbana, Illinois, 80 ppm hardness as Ca^{++}, 80 ppm Na^{+}.

Mean values for physical and chemical data for each variety were correlated with the tenderness values determined for each variety by a regression analysis.

Results

Tenderness (softness) values obtained for cooked samples of each variety of soybean are listed in Table 3. Varieties are listed in order of increasing firmness of soybeans soaked in 0.5% $NaHCO_3$ and cooked in 0.5% $NaHCO_3$. The six most tender varieties are underlined for each treatment. It is important to note that the different soak and cook solutions substantially altered the relative position of the varieties (with respect to tenderness).

A comparison of tenderness data from the two years show considerable differences. No statistical correlation was found in values obtained from the same variety in the two crop years tested (Table 4).

The four soak and cook treatments resulted in different mean tenderness values of the cooked whole soybeans (Table 3). This confirmed previous results (FY 1976) using 28 varieties of soybeans. Soaking increased the mean tenderness for all varieties by roughly 90%. Use of bicarbonate increased tenderness about 300% if the soybeans were soaked and cooked, and about 50% if the soybeans were cooked without prior soaking. The range of tenderness values depended on whether the soybeans were soaked or not. Soaked and cooked soybeans varied about 2.5-fold in tenderness from the softest to the firmest variety. The same varieties, when not soaked, varied about 1.7-fold in tenderness.

Chemical and physical measurements were made on each variety of soybean (Tables 5 and 6). Again this year, few of the physical, and none of the chemical factors tested seemed to correlate with the tenderness values of the cooked soybeans. Those factors that did were primarily related to the size of the bean; the larger beans were more tender after cooking (Tables 3, 7 and 8). As shown in Table 8, the density of the dry soybeans had the greatest correlation with cooked soybean tenderness. Other factors which correlated well were weight/bean, volume/bean, rehydrated weight and rehydrated volume (Figures 1-5). Most of these factors were correlated with tenderness in the previous year's tests.

Each sample was evaluated for tenderness by trained taste panelists. Samples were evaluated on a 9 point scale with 9 being extremely soft, 5 being ideal softness and 1 being extremely firm. An analysis of variance showed that significant differences in tenderness (P=0.0001) was caused by presence or absence of soaking, the composition of the solution used for soaking and cooking, the variety, the interaction of solution with the presence or absence of soaking, and the interaction of variety with the presence or absence of soaking (Table 9).

TABLE 3. EFFECT OF VARIETY, SOAK, AND BLANCH SOLUTION ON TENDERNESS OF COOKED WHOLE SOYBEANS

VARIETY	Soak Sol'n	0.5% $NaHCO_3$	Tap Water	None	None
	Blanch Sol'n	0.5% $NaHCO_3$	Tap Water	0.5% $NaHCO_3$	Tap Water
		Mean Shear Press Peak Height (psi)			
Amsoy 71		11.7	43.0	28.0	52.6
Kanrich		12.3	36.2	30.7	47.8
Prize		13.7	59.4	38.2	70.3
Hark		13.7	44.4	34.8	57.4
Bonus		15.0	45.7	44.4	69.0
Swift		15.5	48.8	44.2	63.6
Improved Pelican		16.4	46.4	36.7	57.3
Steele		16.7	46.1	47.6	70.9
Ozonoshiki		17.0	40.3	45.5	55.5
Williams		17.0	35.5	43.0	60.8
Hodgson		19.1	59.7	52.7	77.0
Woodworth		19.1	58.8	49.7	76.1
Pickett		20.3	66.7	40.3	66.7
Planalto		20.9	46.4	45.5	64.6
Cobb		21.5	48.8	33.3	51.2
Forrest		21.5	50.0	54.5	68.5
Ransom		21.5	64.2	55.8	77.3
Clark 63		21.8	60.0	57.0	72.7
Davis		21.8	60.0	57.0	72.7
Perola		22.1	56.1	61.5	67.9
Hill		22.1	61.5	40.6	61.8
Prata		23.6	51.5	42.4	61.5
Columbus		23.6	63.9	48.2	78.8
Essex		24.2	61.8	57.3	82.1
Bragg		26.1	48.8	50.9	72.4
Bossier		26.7	62.7	54.2	86.0
Calland		27.0	60.6	55.8	87.3
Corsoy		27.0	53.6	50.9	75.8
Beeson		27.9	64.6	55.8	91.0
Pampeira		28.2	63.0	57.9	80.6
Treatment Mean		20.5	53.3	46.8	69.2
Treatment Range		16.5	23.7	33.5	43.2

*Six most tender varieties for each treatment are underlined. Values are means for triplicate, 100 gm, cooked samples obtained using a Lee-Kramer shear press.

TABLE 4. COMPARISON OF TENDERNESS VALUES FOR SOYBEAN VARIETIES FROM THE 1974 and 1975 CROPS

Variety	Soak/Blanch Sol'n	Shear Press Value (psi)			
		0.5% $NaHCO_3$		Tap Water	
		1976	1977	1976	1977
Bonus		14.70	15.0	41.06	45.7
Calland		14.85	27.0	52.88	60.6
Hodgson		14.85	19.1	40.30	59.7
Woodworth		15.46	19.1	56.55	58.8
Amsoy 71		16.14	11.7	61.06	43.0
Hank		16.36	13.7	52.27	44.4
Williams		16.51	17.0	58.97	35.5
Improved Pelican		16.51	16.4	48.33	46.4
Clark 63		16.82	21.8	22.43	50.6
Corsoy (74)		17.27	27.0	59.09	53.6
Swift		18.79	15.5	58.33	48.8
Columbus		18.94	23.6	57.27	63.9
Kanrich		19.69	12.3	70.61	36.2
Beeson		20.76	27.9	68.79	64.6
Cobb		25.45	21.5	69.09	48.8
Pickett 71		25.76	20.3	63.03	66.7
Forrest		27.42	21.5	75.46	50.0
Davis		31.89	21.8	75.15	60.0
Bossier		32.58	26.7	77.81	62.7
Mean		20.04	19.94	60.97	52.63
Std. Dev.		5.74	5.09	11.11	9.51
r		.36		.10	

TABLE 5. COMPOSITION OF 30 VARIETIES OF DRY, RAW, WHOLE SOYBEANS

VARIETY	MOISTURE %	OIL %	FIBER %	PROTEIN %	ASH %	NFE* %
Amsoy 71	7.3	25.1	5.0	39.7	4.48	18.42
Bonus	6.3	24.9	4.6	45.2	4.36	14.64
Beeson	6.7	25.2	5.5	40.1	4.37	18.13
Bossier	6.7	23.6	4.8	41.8	4.63	18.47
Bragg	6.3	21.5	5.1	40.3	3.84	22.96
Calland	6.7	22.8	4.6	42.1	4.46	19.34
Clark 63	6.4	24.5	5.3	41.3	4.77	17.73
Cobb	6.3	23.5	4.9	40.1	4.45	20.75
Corsoy	6.6	23.7	6.4	42.2	4.70	16.40
Columbus	6.7	23.6	5.9	41.3	4.06	18.44
Davis	6.3	21.3	5.6	40.8	4.96	22.04
Essex	6.6	23.1	4.9	43.0	4.61	17.79
Forrest	6.5	22.4	5.0	41.3	4.54	20.26
Hark	7.2	24.3	5.3	39.8	4.48	18.92
Hill	6.5	21.8	5.0	44.3	4.36	18.04
Hodgson	6.3	25.7	4.8	38.8	4.48	19.92
Improved Pelican	6.5	22.8	5.1	42.2	4.65	18.75
Kanrich	8.1	21.3	4.0	42.8	4.40	19.40
Ozonoshiki	6.7	21.5	4.1	42.1	5.10	20.50
Pampeira	6.1	25.6	4.8	38.3	3.88	21.32
Perola	6.2	24.7	4.9	38.3	4.09	21.81
Pickett	6.2	22.4	5.6	39.9	4.09	21.81
Planalto	6.3	27.8	4.9	35.6	4.18	21.22
Prata	6.7	23.6	4.7	40.0	4.32	20.68
Prize	8.4	21.5	4.8	39.5	4.24	21.56
Ransom	6.3	26.2	4.6	38.8	3.74	20.36
Steele	6.3	23.7	4.8	39.1	4.44	21.66
Swift	6.1	24.9	5.2	35.4	4.73	23.67
Williams	6.7	24.8	5.1	41.0	4.60	17.80
Woodworth	6.4	25.5	4.7	39.9	4.61	18.89

*Nitrogen free extract. All analyses reported on an "as is" basis.

TABLE 6. PHYSICAL CHARACTERISTICS OF 30 VARIETIES OF WHOLE SOYBEANS

VARIETY	DRY			REHYDRATED[a]		
	WT/BEAN (grams)	VOL/BEAN (cm^3)	DENSITY (g/cc)	WT BEAN (grams)	VOL BEAN (cm^3)	DENSITY (g/cc)
Amsoy 71	.170	.138	1.232	.402	.372	1.081
Bonus	.152	.126	1.206	.361	.332	1.087
Beeson	.169	.141	1.195	.413	.379	1.089
Bossier	.144	.123	1.173	.330	.302	1.092
Bragg	.137	.115	1.193	.329	.303	1.084
Calland	.169	.141	1.199	.399	.368	1.083
Clark 63	.155	.129	1.204	.347	.319	1.087
Cobb	.140	.119	1.175	.328	.303	1.084
Corsoy	.143	.118	1.208	.352	.326	1.080
Columbus	.122	.103	1.178	.282	.260	1.087
Davis	.134	.113	1.188	.321	.295	1.088
Essex	.146	.120	1.217	.318	.292	1.091
Forrest	.159	.135	1.176	.365	.335	1.090
Hark	.139	.115	1.205	.353	.326	1.083
Hill	.094	.078	1.205	.253	.236	1.072
Hodgson	.193	.161	1.199	.442	.370	1.194
Improved Pelican	.101	.084	1.202	.236	.217	1.115
Kanrich	.305	.242	1.260	.724	.667	1.086
Ozonoshiki	.389	.332	1.172	.957	.893	1.072
Pampeira	.191	.163	1.175	.441	.405	1.088
Perola	.161	.138	1.170	.373	.342	1.090
Pickett	.126	.107	1.179	.303	.279	1.088
Planalto	.162	.137	1.182	.403	.373	1.080
Prata	.137	.115	1.194	.319	.294	1.084
Prize	.288	.237	1.215	.686	.636	1.079
Ransom	.172	.143	1.200	.403	.372	1.085
Steele	.172	.142	1.210	.397	.366	1.084
Swift	.161	.133	1.211	.376	.347	1.084
Williams	.159	.129	1.233	.392	.362	1.083
Woodworth	.160	.132	1.212	.365	.335	1.090

[a]Rehydrated in tap water at room temperature (about 22°C) for 24 hours.

TABLE 7. PERCENT INCREASE IN WEIGHT OF SOYBEANS DURING SOAKING AND BLANCHING

VARIETY	% CHANGE IN WEIGHT (DRY TO COOKED STATE)	% CHANGE IN WEIGHT DURING SOAKING IN 0.5% $NaHCO_3$ at 25°C.	
		6 Hr.	24 Hr.
Amsoy 71	157.4	106.1	137.4
Bonus	147.2	113.2	129.2
Beeson	153.3	104.2	140.3
Bossier	146.8	106.6	128.9
Bragg	149.4	96.9	136.6
Calland	159.0	97.6	137.4
Clark 63	152.1	111.0	128.3
Cobb	154.9	110.9	128.0
Corsoy	155.8	101.4	140.6
Columbus	153.7	112.0	129.7
Davis	153.0	106.5	132.5
Essex	149.8	112.9	119.3
Forrest	153.5	109.4	128.3
Hark	155.5	108.0	139.6
Hill	178.4	145.2	152.3
Hodgson	145.3	111.1	123.9
Improved Pelican	159.0	106.5	135.5
Kanrich	150.3	103.7	136.6
Ozonoshiki	164.4	95.7	144.7
Pampeira	141.7	108.5	127.9
Perola	142.2	106.2	128.3
Pickett	149.0	110.6	135.6
Planalto	147.6	106.2	135.8
Prata	147.1	106.2	128.0
Prize	148.9	123.6	135.4
Ransom	153.1	114.8	132.7
Steele	151.6	111.9	131.0
Swift	155.3	112.4	131.9
Williams	150.4	110.5	132.2
Woodworth	153.8	113.9	127.7

TABLE 8. SIGNIFICANCE OF FACTORS AFFECTING TENDERNESS[1]

Factor	Regression Coefficient (r)	Probability[2] (P)
Density	.542	.002
Weight/bean	.350	.053
Volume/bean	.327	.078
Rehydrated wt/bean	.365	.048
Rehydrated vol/bean	.364	.048

[1] Data from varieties soaked and blanched in 0.5% $NaHCO_3$.

[2] Significance was determined by doing a linear regression analysis.

P = Probably that a greater F value would be obtained.

FIGURE 1. CORRELATION OF DRY SOYBEAN DENSITY WITH TENDERNESS OF DIFFERENT VARIETIES OF COOKED WHOLE SOYBEANS

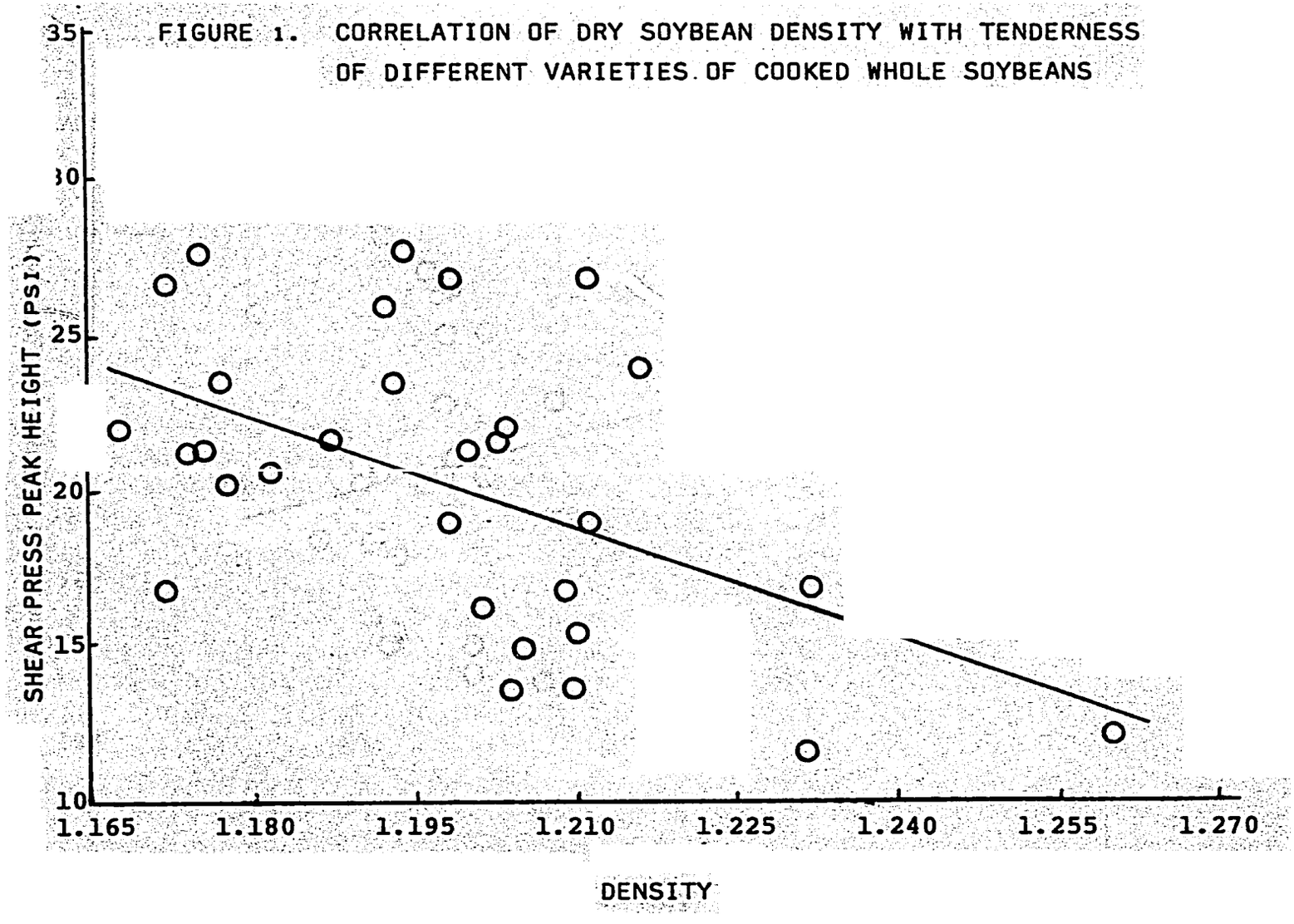


FIGURE 2. CORRELATION OF DRY BEAN WEIGHT AND TENDERNESS OF COOKED WHOLE SOYBEANS

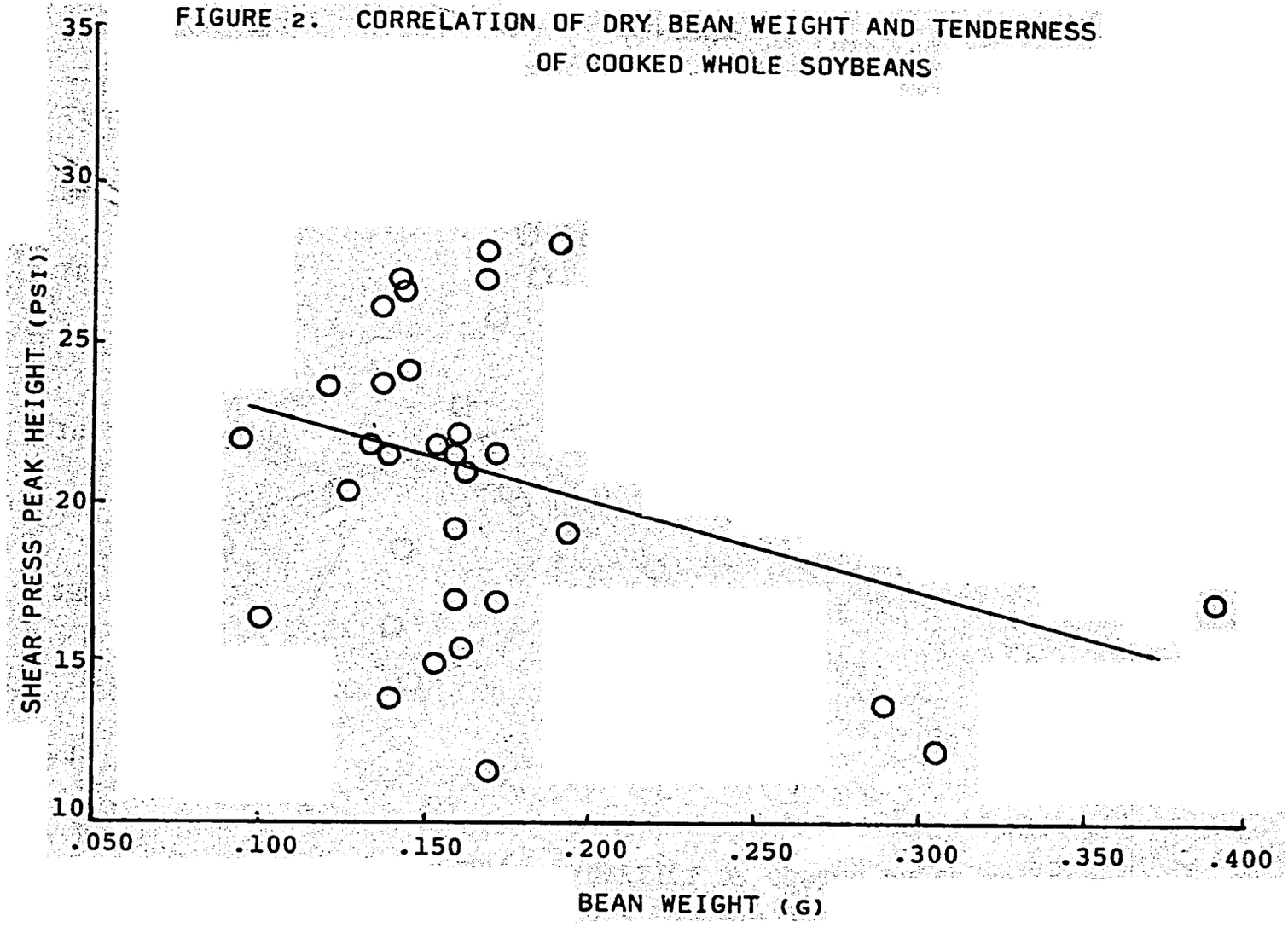


FIGURE 3. CORRELATION OF BEAN VOLUMN ON FIRMNESS OF DIFFERENT VARIETIES OF COOKED WHOLE SOYBEANS

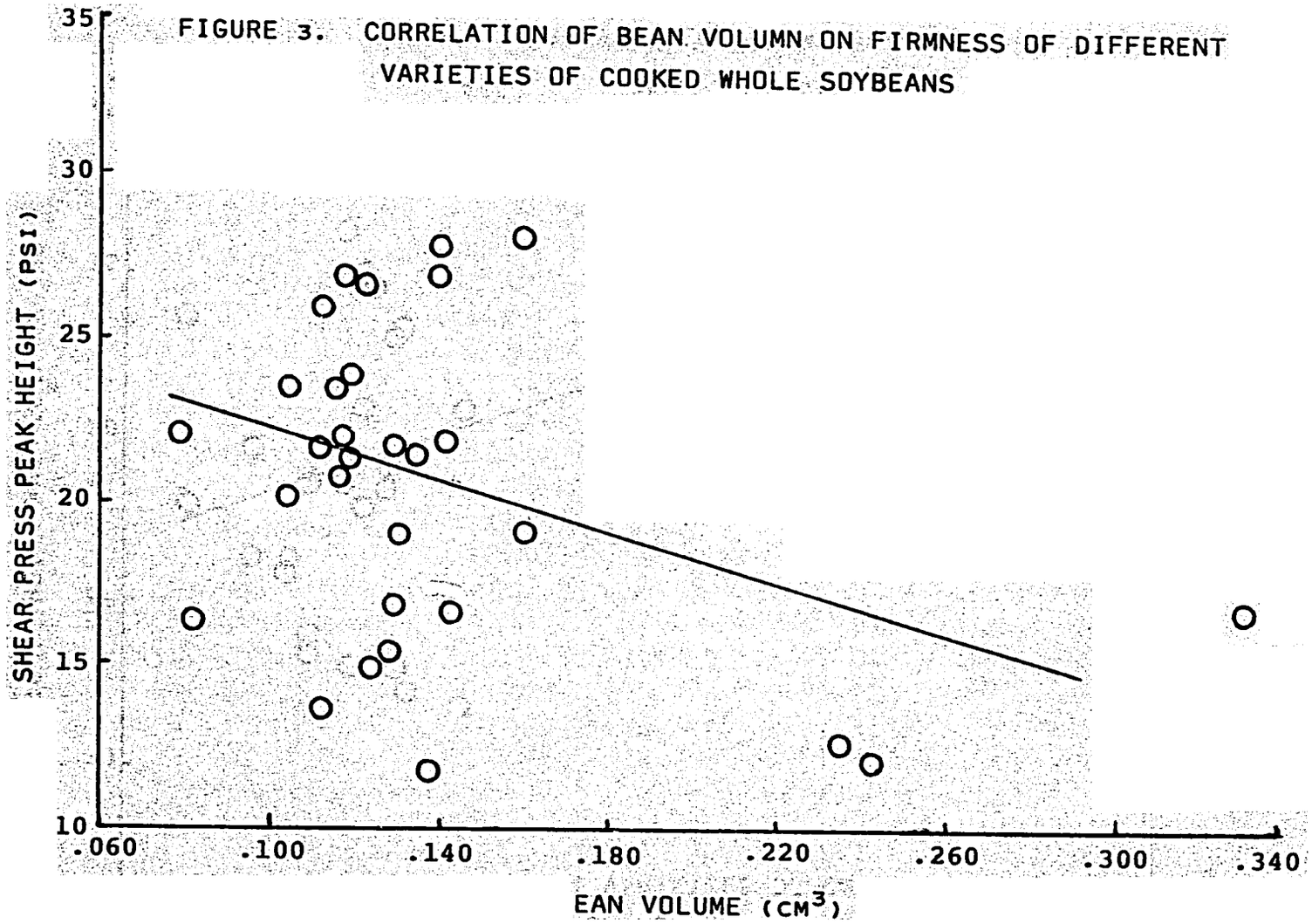


FIGURE 4. CORRELATION OF REHYDRATED BEAN WEIGHT ON TENDERNESS OF DIFFERENT VARIETIES OF COOKED WHOLE SOYBEANS

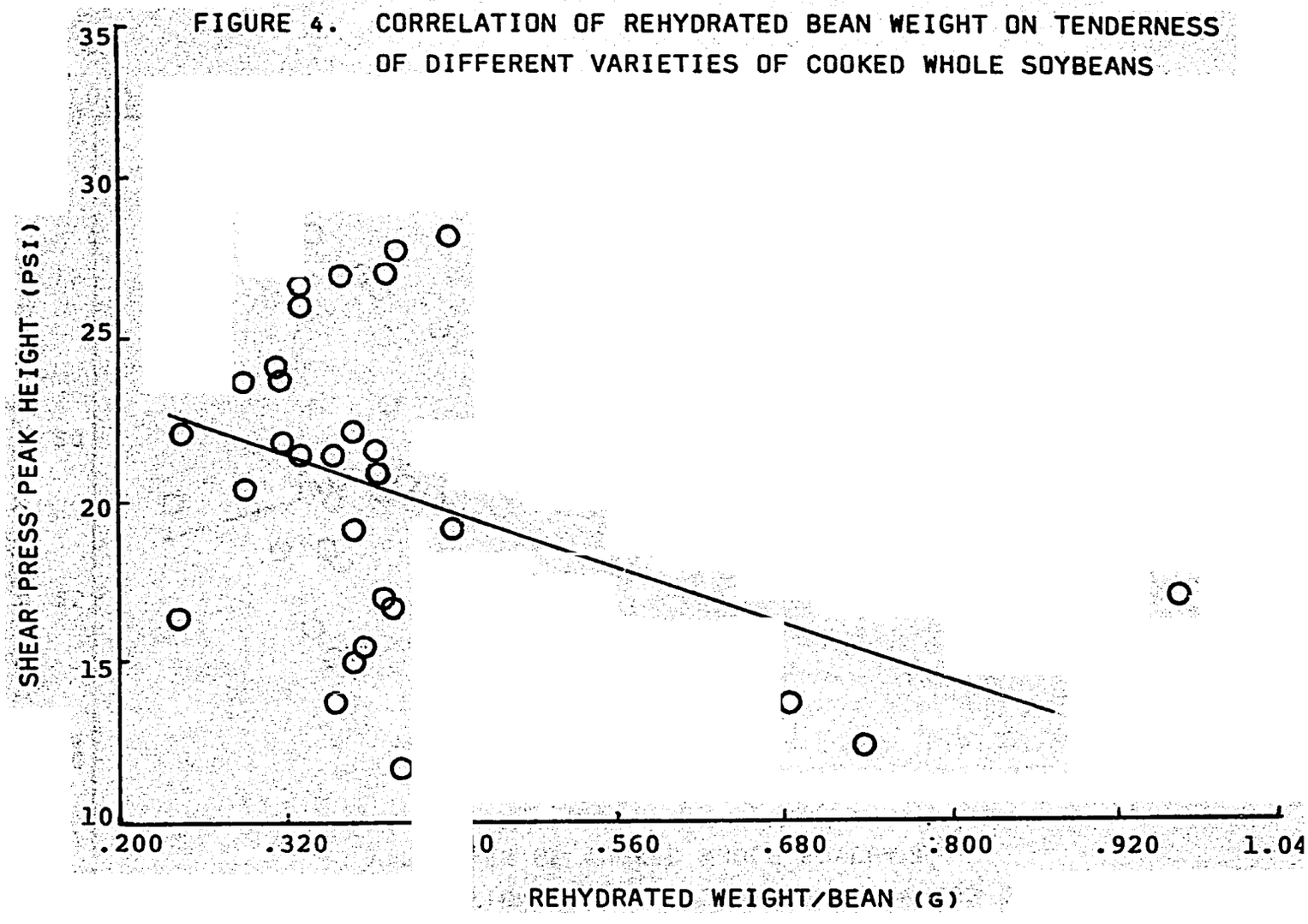


FIGURE 5. CORRELATION OF REHYDRATED BEAN VOLUMN ON TENDERNESS OF DIFFERENT VARIETIES OF COOKED WHOLE SOYBEANS

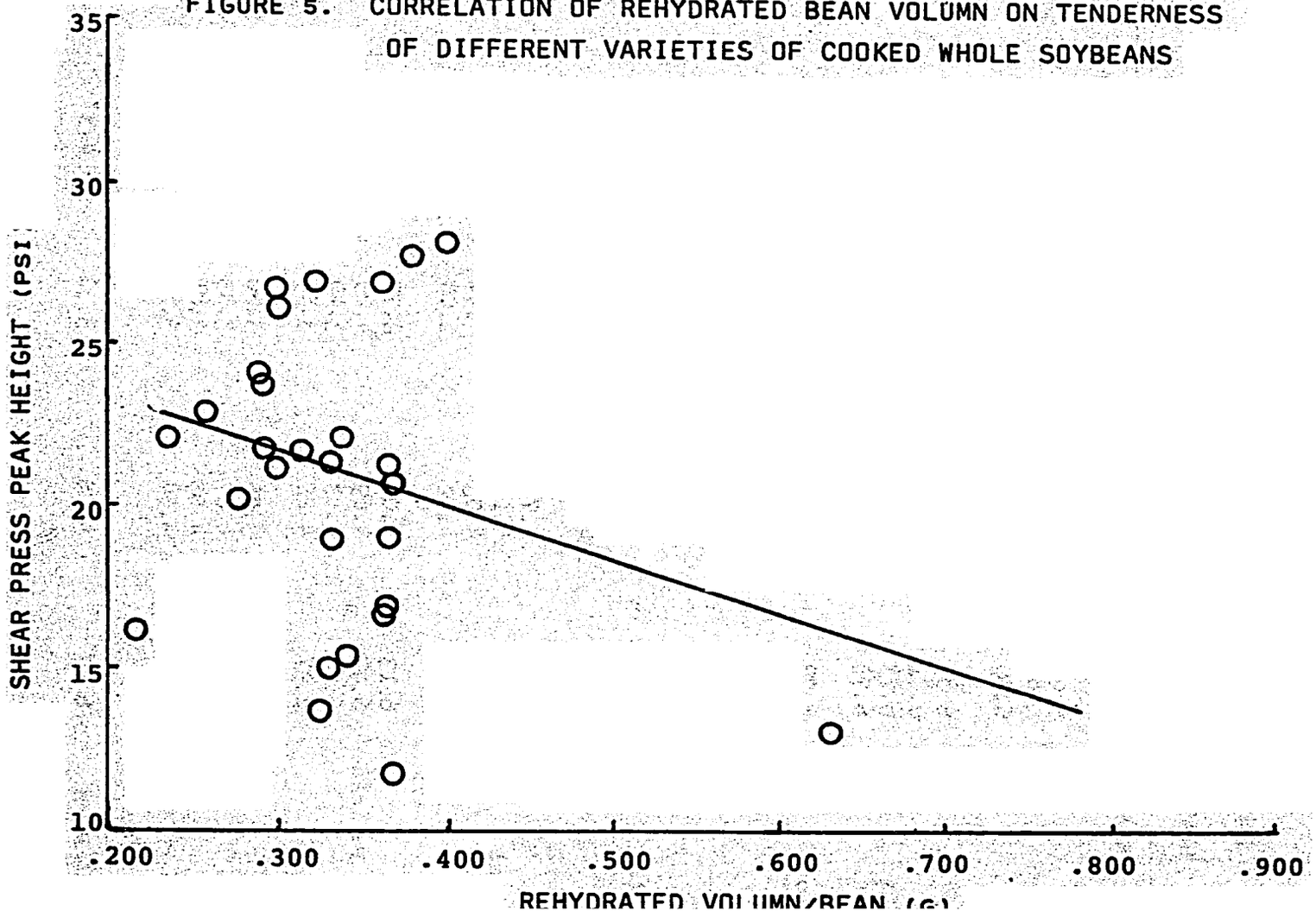


TABLE 9. MEAN ORGANOLEPTIC TENDERNESS SCORES FOR SOYBEAN VARIETIES AFTER DIFFERENT SOAK AND BLANCH TREATMENTS

VARIETY	MEAN TENDERNESS SCORES				
SOAK	0.5% $NaHCO_3$	TAP WATER	NONE	NONE	OVERALL
BLANCH	0.05% $NaHCO_3$	TAP WATER	0.5% $NaHCO_3$	TAP WATER	MEAN
Columbus	7.00	3.89	3.[illegible]0	4.52	4.59
Bragg	6.94	4.77	4.16	4.67	5.14
Hill	6.92	4.23	3.73	4.58	4.87
Davis	6.88	4.19	3.31	4.27	4.66
Forrest	6.85	4.50	3.65	4.27	4.82
Corsoy	6.81	4.18	3.04	4.00	4.50
Clark 63	6.73	4.59	3.46	4.44	4.80
Pickett	6.73	3.79	3.30	4.69	4.69
Planalto	6.72	4.30	3.65	4.07	4.70
Essex	6.69	4.15	3.35	4.23	4.61
Swift	6.66	4.42	3.72	4.45	4.82
Perola	6.66	3.75	3.21	4.48	4.53
Cobb	6.58	4.78	4.31	5.56	5.30
Woodworth	6.47	4.13	3.22	4.39	4.56
Beeson	6.39	3.63	3.03	3.93	4.25
Hodgson	6.30	4.07	3.27	4.07	4.43
Kanrich	6.29	4.76	4.33	4.81	5.05
Ozonoshiki	6.25	5.13	5.00	4.55	5.24
Bossier	6.23	4.03	2.94	3.97	4.30
Bonus	6.17	4.12	3.00	3.58	4.22
Calland	6.13	4.00	2.77	3.70	4.16
Improved Pelican	6.07	4.69	4.23	5.31	5.08
Ransom	6.07	4.03	3.40	4.24	4.44
Prata	5.97	4.25	3.52	4.04	4.45
Pampeira	5.97	3.75	3.20	3.89	4.21
Williams	5.90	4.67	3.43	4.10	4.52
Prize	5.86	4.05	3.81	3.90	4.40
Steele	5.69	4.58	3.53	4.54	4.59
Treatment Mean	6.47	4.25	3.50	4.37	

Nutritional Evaluation of Corn:soy Flours

Section II.

During the previous contract year, experiments were conducted to simplify the preparation process and improve the organoleptic acceptability of drum dried corn:soy flakes. Data from these experiments was presented in last year's report. Nutritional analyses on these products were begun during the latter part of that contract year and the data was received and evaluated early during this contract year. These studies were designed to evaluate the effect of a) the corn fraction, b) gelatinization of the corn: soy slurry before drying, and c) the effect of corn:soy ratio on PER of the corn:soy flakes.

Methods

Whole corn was milled and fractionated as outlined last year to produce four corn fractions, a) ground whole corn, b) the fraction retained on a 22 wire mesh screen, c) the fraction retained on a 32 wire mesh screen, and d) the fraction passing through 32 wire mesh screen. Each fraction was further ground through a hammermill in order to equalize particle size. Corn:soy flours were prepared as described in the previous report. The processing flow diagram is shown in Figure 6. Eleven corn:soy flours were prepared using different corn fractions, and different corn:soy ratios. Some of the corn:soy slurries were gelatinized before drum drying.

The proximate composition, amino acid profile and PER of the samples were determined. These analyses were performed by the Ralston Purina Company, St. Louis, Mo., using standard procedures.

Results

The proximate composition of each corn fraction is given in Table 10. The more finely ground particles were lower in protein, fat, fiber and ash. This reflects the removal of the hull and germ which are more difficult to grind finely. The proximate composition of each corn:soy blend is given in Table 11. Changing the ratio of corn:soy from 4:1 to 2:1 increased the protein content by about 4.7%. The protein content of the corn fraction used ranged from 6.8 to 10.2% and this affected the protein content of the blends which ranged from 13.3 to 16.3%. The fat and fiber contents also varied with the corn:soy ratio and corn fraction used (Table 11). Thus, the corn fraction used affects composition and nutritional characteristics of the corn:soy blend. As reported last year, consumer acceptability was also affected by corn fraction.

The PER's for each sample are given in Table 12 and were significantly different among samples. The overall experiment was divided into 3 sub experiments to help identify the causes of the variation in PER. The effect of corn fraction on PER is given in Table 13 for samples which were gelatinized before drum drying and had a corn:soy ratio of 4:1. Blends containing whole corn and "through 35 mesh screen" received the highest PER's whereas commercial meal had the lowest PER. The corn:soy ratio affected the

FIGURE 6. PREPARATION OF DRUM-DRIED CORN:SOY FLAKES

Clean whole soybeans

↓

Blanch in
boiling tap water

↓

Drain. Grind in
Waring blender with
tap water or bicarbonate
solution in soft water

↓

Add corn
flour and blend

↓

Gelatinize
(optional)

↓

Grind to smooth slurry
(20% solids) in Reitz mill using
0.023" screen

↓

Drum dry (4-5% Moisture)

TABLE 10. PROXIMATE COMPOSITION OF CORN FRACTIONS[1]

Corn Fraction	Moisture %	Protein %	Fat %	Fiber %	Ash %	NFE[2] %
Whole	11.52	8.84	4.60	2.30	1.62	82.62
Over 22 WMS[3]	11.67	10.21	7.74	3.09	2.18	76.81
Over 32 WMS	12.50	9.20	1.94	1.60	1.32	85.90
Thru 32 WMS	13.83	6.82	1.19	0.71	0.90	90.38

[1] Protein, fat, fiber, ash and NFE calculated on a dry weight basis.

[2] NFE (Nitrogen free extract) obtained by difference

[3] WMS = Wire mesh screen; 22 or 32 wires/linear inch

TABLE 11. PROXIMATE COMPOSITION OF DRUM DRIED CORN:SOY FLAKES (AS IS BASIS)

Corn fraction	Gelatinized	Corn:soy ratio	Protein %	Fat %	Fiber %	Ash %	Moisture %
Whole	+	4:1	15.00	4.01	2.61	1.80	8.36
Thru 32 mesh screen	+	4:1	13.45	2.84	1.66	1.40	8.58
On 32 mesh screen	+	4:1	15.45	4.08	2.22	1.82	9.60
On 22 mesh screen	+	4:1	16.25	5.89	3.11	2.10	7.83
Com'l meal	+	4:1	16.25	2.99	1.63	1.06	10.20
Whole	-	4:1	14.85	4.88	2.38	1.83	8.60
Thru 32 mesh screen	-	4:1	13.25	3.26	1.45	1.29	11.95
Com'l meal	-	4:1	15.35	2.93	1.61	1.31	10.45
Whole	+	2:1	19.40	6.82	2.84	2.14	9.17
Thru 32 mesh screen	+	2:1	18.45	4.72	2.40	1.76	7.52
Com'l meal	+	2:1	18.45	3.73	1.99	1.54	10.15

TABLE 12. PER'S OF DRUM DRIED CORN:SOY FLAKES.

Corn Fraction	Corn:soy Ratio	Gelatinized	PER (casein = 2.50)
Thru 32 WMS[1]	4:1	-	2.52[2]
Thru 32 WMS	4:1	+	2.40
Whole corn	4:1	+	2.35
Whole corn	2:1	+	2.31
Com'l meal	4:1	-	2.28
Com'l meal	2:1	+	2.27
Whole corn	4:1	-	2.27
Over 32 WMS	4:1	+	2.23
Over 22 WMS	4:1	+	2.21
Thru 32 WMS	2:1	+	2.20
Com'l meal	4:1	+	2.12

[1] WMS = Wire mesh screen. 22 or 32 wires/linear inch.

[2] Vertical lines indicate groups of corn:soy flakes which had statistically similar PER's based on Duncan's multiple range test ($P < 0.05$).

PER significantly only when "through 32 WMS" corn was used (Table 14). There was no significant effect on the PER when whole corn or commercial cornmeal was blended with soy in the two ratios tested. This may indicate that it is desirable to base soy fortification recommendations on protein content rather than to adhere too strictly to the corn:soy ratio which in theory gives the maximum PER. This may be particularly true when the corn is not highly refined as would be the case in poor rural areas. Gelatinization did not significantly affect PER (Table 15). This suggests that this step may be omitted. However, as discussed in last year's report, gelatinization may improve consumer acceptance and it improves the drum drying characteristic of the product.

As reported last year, the corn fraction, corn:soy ratio, and processing method affected the organoleptic scores of the products. This suggests that consumer preference, processing method and possibly other factors such as costs of raw ingredients and processing, are at least as important as the PER in determining the optimum level of added soy.

Data for the amino acid analyses of the soy:corn samples above are given in Table 16. There appears to be no correlation of essential amino acids with the PER of the corn:soy flour. A linear regression analysis comparing the PER with each essential amino acid showed no positive correlations ($r<0.26$ in all cases).

TABLE 13. EFFECT OF CORN FRACTION ON PER'S OF DRUM DRIED CORN:SOY FLOUR[3].

Corn Fraction [1]	PER
Thru 32 WMS [2]	2.40
Whole corn	2.35
32 WMS	2.23
22 WMS	2.21
Com'l meal	2.12

[1] Corn:soy ratio = 4:1
All samples gelatinized before drum drying

[2] WMS = Wire mesh screen. 22 or 32 wires/linear inch.

[3] Vertical lines indicate groups of corn:soy flakes which had statistically similar PER's based on Duncan's multiple range test ($P < 0.05$).

TABLE 14. EFFECT OF CORN:SOY RATIO AND CORN FRACTION ON PER'S OF DRUM DRIED CORN:SOY FLOURS.

Corn Fraction	PER Ratio 2:1	PER 4:1	Mean
Thru 32 WMS[1]	2.20 ab[2]	2.40 cd	2.31
Whole corn	2.27 abc	2.35 bcd	2.30
Com'l meal	2.27 abc	2.12 a	2.20
Mean	2.25	2.29	

[1] WMS = Wire mesh screen. 22 or 32 wires/linear inch.

[2] Samples with the sample letter after the PER value are not significantly different.

TABLE 15. EFFECT OF GELATINIZATION ON PER OF DRUM DRIED CORN:SOY FLOURS

Corn Fraction[1]	PER		
	Gelatinized	Not Gelatinized	Mean
Thru 32 WMS[2]	2.40 cd[3]	2.52 d	2.46
Whole corn	2.35 bcd	2.27 abc	2.20
Com'l meal	2.12 a	2.28 abc	2.31
Mean	2.29	2.36	

[1]Corn:soy ratio = 4:1
All samples gelatinized before drum drying

[2]WMS = Wire mesh screen. 22 or 32 wires/linear inch.

[3]Samples with the sample letter after the PER value are not significantly different.

TABLE 16. AMINO ACID PROFILE OF DRUM DRIED CORN:SOY FLOURS.

Corn Fraction	Whole Corn	On 32 WMS[1]	On 22 WMS	Com'l Meal	Whole Corn	Thru 32 WMS	Com'l Meal	Whole Corn	Thru 32 WMS	Com'l Meal
Gelatinized	+	+	+	+	-	-	-	+	+	+
Corn:soy Ratio	4:1	4:1	4:1	4:1	4:1	4:1	4:1	2:1	2:1	2:1
Asp	8.27	8.16	9.17	8.80	9.02	9.51	9.71	9.95	10.51	9.97
Glu	22.33	22.91	18.89	19.45	18.79	19.40	21.17	18.87	19.78	20.22
Ala	4.16	4.17	5.81	5.78	5.70	5.40	6.07	5.21	5.11	5.58
Ile	3.84	3.81	3.90	4.04	4.05	4.14	4.33	4.20	4.36	4.34
Phe	4.85	4.96	4.76	4.90	4.78	4.75	5.25	4.91	5.04	5.13
NH_3	1.35	1.40	2.06	2.19	2.16	2.11	2.23	1.97	2.03	2.16
Thr	2.91	2.86	3.80	3.37	3.62	3.72	3.85	3.61	3.89	3.65
Pro	9.00	9.32	5.08	4.55	4.62	4.35	5.64	3.06	4.64	4.50
Val	2.69	2.52	4.58	4.47	4.69	4.68	4.81	4.64	4.75	4.75
Leu	9.40	9.77	9.35	10.09	9.43	9.21	10.68	8.66	8.89	9.97
His	1.71	1.70	2.73	2.64	2.79	2.79	2.78	2.74	2.80	2.75
Arg	3.02	2.87	5.29	4.50	5.35	5.06	4.62	5.52	5.75	5.25
Ser	4.65	4.74	5.24	5.02	5.07	5.14	5.43	5.21	5.33	5.31
Gly	3.03	2.91	4.22	3.76	4.22	4.08	3.99	4.20	4.24	4.07
Met	0.81	0.83	1.00	1.16	1.09	1.09	1.17	0.95	1.07	1.05
Tyr	2.75	2.61	2.19	1.78	2.26	1.93	2.07	2.19	2.04	2.13
Lys	3.85	3.75	4.78	4.38	4.76	4.98	4.50	5.46	5.64	5.11

[1] Wire mesh screen. 22 and 32 wire/linear inch.

APPENDIX III

Report of Robert M. Goodman
Assistant Professor of Plant Pathology

Contract Objective 1

Identification of virus-resistant tropically-adapted soybean germ plasm

a. Soybean mosaic virus (SMV) is the most widespread soybean virus. It is transmitted to healthy plants in the field by aphids that feed on infected plants arising from SMV-infected seeds. Several lines and a few named varieties have been reported by others as having a degree of resistance to SMV, but experience has shown that all resistance available in named soybean varieties can be broken by SMV isolates other than those used to develop the resistant variety. No SMV resistance has been reported previously in tropically adapted soybean germ plasm.

All tropically adapted soybean germ plasm in the INTSOY collection was planted in randomized hills (6 replications) at Isabela, P.R. in July 1976, and the plants in five replications were inoculated with the Illinois severe isolate of SMV when the plants were at the primary leaf stage. Inoculation was by hand with the sap from infected leaves homogenized in 50 mM potassium phosphate buffer, pH 7.0. In September 1976, the entire field was examined and data taken on the prevalence of SMV symptoms. In hills where some plants were infected and others not, those showing no symptoms were removed. Hills in which no plants appeared infected were not rogued. The noninoculated replication was examined for SMV prevalence, but no plants were removed. At the end of the growing season all hills were harvested and the seed bagged separately. Seeds from entries free of virus symptoms in all replications were selected for later testing in the greenhouse. Four P.I. lines (341.242, 324.068, 374.193, 238.109) and the varieties Majos, Nela, Yelnanda, and Buffalo were apparently resistant to infection by the SMV isolate used. Results are incomplete with the P.I. lines and 3 varieties, but the variety Buffalo has now been tested with 12 widely varying SMV isolates. Over 200 plants were inoculated at least twice and only one plant became infected. Absence of infection of symptomless plants was tested by back-inoculation to susceptible soybean varieties and by indexing on detached Top Crop bean leaves. Results of all such tests were negative.

We therefore believe that Buffalo may represent a new source of resistance to a wide range of SMV isolates. Further tests are in progress to confirm this tentative conclusion, as are tests with the other seven apparently-resistant lines. Buffalo was also planted at Isabela in December 1976 and May 1977 for use in the INTSOY crossing program.

b. Cowpea mosaic virus. Two experiments have been conducted to screen for possible sources of resistance to cowpea mosaic virus (CPMV). One experiment was done in the greenhouse at Urbana and the other in the field at Isabela, P.R. In the greenhouse, 121 selected named varieties in the maturity group 00 to IV were mechanically inoculated with one isolate of CPMV and the reactions were observed. There was no indication of resistance in any of the varieties tested. Under field conditions at Isabela, 100 more varieties including experimental lines randomly selected from maturity groups V to X were tested. Results from this experiment indicated that among the varieties and lines tested, none were completely free of symptoms. However, there were several lines showing considerable variations in reactions to the virus (Table 1), with some plants remaining uninfected even though they were inoculated twice, while others showed CPMV symptoms. The experiment has not yet been completed. Seeds from inoculated plants that remained healthy are now being harvested and will be tested again in the greenhouse to confirm the reactions to CPMV.

c. Whitefly-transmitted viruses. Resistance to soybean yellow mosaic virus in India was found by Dr. B. B. Singh in the P.I. 171,443. This line has been increased at Isabela for future testing with the whitefly-transmitted viruses that affect soybeans in tropical America. Experiments will begin in the next contract year.

Table 1. Soybean germ plasm lines with apparent resistance to CPMV in a field trial at Isabela, P.R.

	No. healthy plants/total no. plants in replication shown				
	1	2	3	4	5
GH31-8-2(1)	0/6	0/6	1/6	3/6	1/5
GH31-8-4(2)	0/6	0/6	2/6	1/6	1/5
PI259,538	0/6	0/6	1/5	4/6	1/6
PI381,657	0/6	0/6	1/6	4/6	3/5
L76-12-14[a/]	0/6	1/6	2/6	4/7	1/6

a/ Lines so identified are Mexican bean beetle resistant lines obtained from Dr. M. Kogan, Illinois Natural History Survey and University of Illinois.

Identification of soybean germ plasm with low rates of SMV seed transmission

a. Tropically adapted soybean germ plasm. Another purpose for which the tropical germ plasm planting was inoculated with SMV (see

above) was to test for the rates of SMV seed transmission. The seeds harvested from SMV infected plants were examined for seed coat mottling. Two hundred seeds from each line were planted in greenhouse sand benches and the seedlings that emerged were inspected for evidence of SMV infection. In cases where no seedlings had symptoms, they were tested by indexing on detached Top Crop bean leaves to determine if the virus was present.

Preliminary results of these tests, which are as yet incomplete, indicate that several of the tropically-adapted germ plasm lines have low rates of seed transmission (less than 1%) from fully infected plants These studies will be continued, with plantings of larger numbers of seeds and retesting of candidate lines that appear to be free of virus transmission. Eventually, promising lines will be incorporated into the INTSOY breeding program in an attempt to develop soybean varieties that do not produce SMV infected seeds.

b. Northern germ plasm. Seed was obtained from the U.S. Regional Soybean Laboratory in Urbana for selected lines of maturity groups II and III. All 101 named varieties, two pairs of isolines for the Rm gene, all nineteen F.C. lines, all nine wild lines and 364 randomly selected plant introductions were used. Each of the lines was assigned a code number and ten seeds from each were planted in each of the five separately randomized replications. Each replicated line was planted in a hill on a two foot grid in a field near Urbana, Illinois, during late May 1976.

Inoculum was prepared by growing the cultivar Kanrich in a sand bench in the greenhouse. When at the unifoliate stage these plants were mechanically inoculated with the Illinois severe isolate of SMV. Three weeks after inoculation this material was harvested and .05 M sodium phosphate, pH 7.0, was added in a 1:5 ratio (w/v). Carborundum was added for an abrasive and, using a gauze pad, the seedlings in the field were mechanically inoculated on June 21-23. The number of plants emerging at each hill was recorded. The number of plants expressing symptoms in each hill was recorded on two occasions after inoculation (7/14 and 7/28). All of the plants were inoculated several weeks before flowering. In late August all of the sumptomless plants were rogued out. In the few instances where none of the plants in a hill expressed symptoms, these plants were checked for the presence of SMV by indexing. The virus free plants were rogued out of these hills. As the hills reached maturity, from mid-September until the end of October, they were harvested, threshed and bagged individually. The replicates were later bulked into their respective coded lines. This seed was then stored in a cold room.

A 200 seed sample from each line was counted out, and the coat, hilum, and mottle color, the degree of mottling (light, moderate or severe) and the percentage of mottled seed were determined. During ten consecutive weeks these 200 seed samples from fifty lines were planted out in rows one inch apart in sand benches in the greenhouse. When the seedlings had fully expanded primary leaves, ten to fourteen days after planting, they were observed for SMV symptoms (rugosity and mosaic). The percentage of plants expressing symptoms in each line was recorded. The lines not expressing any symptoms were checked for the presence of SMV by indexing. It has been determined in our lab that the index technique is sensitive enough to detect one SMV infected plant out of a sample of ten. One primary leaf from each plant of a symptomless line was harvested. Ten of these leaves were stacked up and a thin section (several mm) was cut from all ten leaves. These sections are ground up in one milliliter of .05 M sodium phosphate, pH 7.0 buffer. Carborundum is then added and a cotton-tipped applicator is used to mechanically inoculate fully expanded primary leaves of the French bean (Phaseolus vulgaris) cultivar Top Crop that had been given a 12 hour dark treatment prior to inoculation. Detached leaves were placed on moist filter paper in a petri dish and incubated under continuous light at 30°C. If SMV is present in the sample, a local lesion response occurs after 48 hours. The lesions initially formed as discrete local lesions followed by necrosis spreading along small veins. This technique was developed at the University of Illinois by M. M. Soon and G. M. Milbrath.

Many of the soybean lines tested had relatively low rates of SMV seed transmission. Since low rates of seed transmission and nonmottling are both desirable, we selected for further testing the 35 lines that showed no SMV seed transmission in the 200 seed test described above and that showed light mottling only if less than 10% of the seeds. These lines are listed in Table 2.

Contract Objective 3

The third of three regional soybean conferences, cosponsored by INTSOY, the Government of Thailand and the Asian Vegetable Research and Development Center, was held in Chiang Mai, Thailand in February 1976. In late March 1976, the assembly and editing of the proceedings of that conference began. Over 600 manuscript pages were submitted for publication. Many required extensive editing or rewriting. The final publication, entitled "Expanding the Use of Soybeans," is 261 type-set pages long in 8 1/2 x 11 inch format. It contains 29 general session papers, the proceedings of five symposia, and 12 country reports. The INTSOY plant virologist was the editor of this proceedings.

Also published during the current contract year was a comprehensive review of soybean viruses, a review article on the epidemiology of soybean viruses, and several research articles (see Annexure A).

Table 2. Thirty-five lines having both low levels of mottling (≤10% and light) and not seed transmitting the virus (according to Top Crop indexing).

A. K. (Harrow)	P.I.60.279
Cloud	P.I.68.398
Funman	P.I.68.680
Granger	P.I.70.019
Hawkey 63	P.I.70.036
Magna	P.I.70.199
Manchu 606,Wis	P.I.88.30[illegible]
Manchu 2204	P.I.88.305
Mansoy	P.I.88.351
Mukden	P.I.91.115
Seneca	P.I.91.159
Virginia	P.I.92.718-2
F.C.02.002B	P.I.92.718-2
F.C.04.007A	P.I.153.280
F.C.31.678	P.I.153.292
F.C.31.684	P.I.198.929
P.I.54.859	P.I.360.835
	P.I.378.561

The collection and card filing of soybean virus literature, begun during the preceeding contract, was continued.

Contract Objective 4

Studies on the mechanism of seed transmission of SMV

a. Effect of time of inoculation. Soybean plants were inoculated at different stages of reproductive development to determine the effect of the time of inoculation upon rates of mottling and seed transmission. Two ten-plant hills of the cultivar Williams were mechanically inoculated with SMV (Illinois severe isolate) two weeks after planting. Twenty plants were similarly inoculated each week for eight consecutive weeks. The first two inoculations were done before flowering. The inoculations of the remaining six weeks were done during the various reproductive stages of the plant according to the developmental descriptions proposed by Fehr et al. (1971). The third inoculation was done at the R1 stage where there is one flower at any node. The plants were in stage R2 during the fourth week where there is a flower at the node immediately below the uppermost node with a completely unrolled leaf. In the fifth week the plants were in R4 where there is a pod two cm long at one of the four uppermost nodes with a completely unrolled leaf. The next significant stage of development was during the seventh week of inoculation when the plants were in R6. During this stage there is a pod containing full size beans at one of the four uppermost nodes with a completely unrolled leaf.

At the end of August all of the sumptomless plants were removed When the plants reached maturity each of the eight inoculation sets were harvested and threshed separately. Two samples of 200 seeds were counted out from each set, determining the percentage nottling in the process. Two hundred seeds from each set were germinated and 100 randomly selected seedlings were individually indexed with the detached Top Crop bean method for the presence of SMV. The results are shown in Table 3.

b. Presence of SMV in reproductive tissue during plant development. Another study was done to determine at what points in development virus could be detected in the reproductive parts of a plant. Again the development descriptions of Fehr et al. will be used with the addition of stages R7 and R8. R7 is when the plant is at physiological maturity with the pods yellowing and 10% of the leaves yellow. Stage R8 is harvest maturity with 95% of the pods being brown. Two different cultivars of soybeans were used, Merit and Midwest. Merit was selected because it was reported by Kennedy and Cooper to have a nonmottling character and not to transmit SMV through the seeds. Midwest was selected because Kendrick and Gardner reported it to have a high rate of seed transmission (29%).

Table 3. Effect of time of inoculation on seed mottling, germination, and SMV seed transmission of Williams soybeans.

Week	Developmental Stage[a]	Seed Mottling[b] (%)	Germination[c] (%)	Seed Transmission[d] (%)
1		69.0	90.5	18.0
2		65.8	91.5	19.0
3	R1	80.5	89.5	11.0
4	R2	72.3	85.0	2.0
5	R4	80.0	88.0	4.0
6		69.8	87.0	4.0
7	R6	77.0	85.0	3.0
8		78.5	92.0	4.0

a/ According to Fehr et al. 1971.

b/ 400 seed samples.

c/ 200 seed samples.

d/ 100 seed samples.

The plants were mechanically inoculated with the Illinois severe isolate of SMV well before either cultivar flowered. Ten plants from each cultivar were examined during each of the following stages, R1, R2, R4, R6, R7, and R8. They were also checked after the harvested seeds were germinated and again after six months storage in the cold room. A sample from every node from each of the plants was indexed for the presence of SMV. In Merit 8, 10, 12, 12, 12, 12, 12, and 12 nodes were examined respectively during R1, R2, R4, R6, R7, R8 germination and storage-germination. In Midwest 6, 11, 12, 12, 12, 12, 12, and 12 nodes were checked from each plant during the same respective stages. When each stage was checked ten plants were randomly selected from both lines. In stages R1 and R2 entire flowers were indexed. In stages R4 and R6 entire pods were indexed. During R7 and R8 the pods and seeds were separately indexed. During the germination stage two seeds from each node-plant position were placed on moistened cellulose pads and placed in a germinator. When the seedlings had fully expanded primary leaves, one plant from

each node-plant position was indexed individually. Seeds were stored in the cold room for six months. These were then germinated and indexed as above. The percentage seed mottling from each node-plant position was also determined from the seeds used in the germination stage. Results are given in Table 4, 5 and Fig. 1.

The node position on the plant did not affect the seed transmission or mottling rates in either of the cultivars tested. In the early stages of both cultivars the virus was present in 100% of the material tested. As the material began to mature, the presence of the virus began to decrease. This decrease was more rapid in Merit, which fell to 0%, than in Midwest. This may be explained by the fact that Midwest matured much slower than Merit. The pods lost virus slightly faster than the seeds. Germination immediately after harvest or after six months storage did not affect the presence of virus in the seeds (Fig. 1).

c. Mottling of the seed coat in the variety Merit. In a third study, each of 87 SMV symptom expressing plants of a population of the cultivar Merit were harvested individually. These plants had been inoculated with the Illinois severe isolate of SMV during the first trifoliolate stage. The seeds from each plant were checked for mottling percentage to determine if this trait was homogeneously expressed in this cultivar.

When Merit was inoculated with the Illinois severe isolate of SMV, the seeds produced by infected plants exhibited mottling. This is contrary to the work reported by Kennedy and Cooper in which Merit was reported to have a gene conferring no mottling. When the mottling rate was determined for 87 individual plants a bimodal distribution appeared (Fig. 2). This suggests that this variety is not homogenous for expression of seed coat mottling. The mottling rates ranged from 0 to 100%.

Purification and Characterization of whitefly transmitted viruses.

a. Bean golden yellow mosaic viruses. One of the most important unsolved problems in tropical agriculture is the cause and control of leaf curl and yellow mosaic diseases associated with pathogens transmitted by the ubiguitous tropical whitefly, *Bemisia tabaci*. Soybeans in India and tropical America are affected, sometimes seriously, by such diseases as are other legumes and many other crops.

A major breakthrough in our understanding of the causes of this important group of tropical plant diseases has been made in the past year by the INTSOY plant virology group. Whitefly-transmitted pathogens, long presumed to be viruses, have been difficult to work with because they were not characterized and they were not mechanically transmissible. Results obtained by the virologist at the University of Puerto Rico showed that the whitefly-transmitted pathogen causing bean golden yellow mosaic

Table 4. SMV infection of Merit soybean reproductive tissues at various stages of plant development.

Node	R1	R2	R4	R6	R7	R8	Germination
1	10	10	10	9(10)*	6(6)	0(0)	0/8@
2	10	10	10	8(10)	7(5)	0(0)	0/9
3	10	10	10	9(10)	6(4)	0(0)	0/8
4	10	10	10	8(10)	4(4)	0(0)	0/9
5	10	10	10	10(10)	6(6)	0(0)	0/8
6	10	10	10	10(10)	3(3)	0(0)	0/9
7	10	10	10	9(10)	4(5)	1(0)	0/10
8	10	10	10	9(10)	7(5)	0(0)	0/10
9	--	10	10	9(10)	6(6)	0(0)	0/7
10	--	10	10	9(10)	9(5)	0(0)	0/8
11	--	--	10	9(10)	7(6)	0(0)	0/9
12	--	--	10	9(10)	6(5)	0(0)	0/9

Numbers indicate the number of positive reactions for SMV using the detached Top Crop index method.

* Indicates seed (pods).

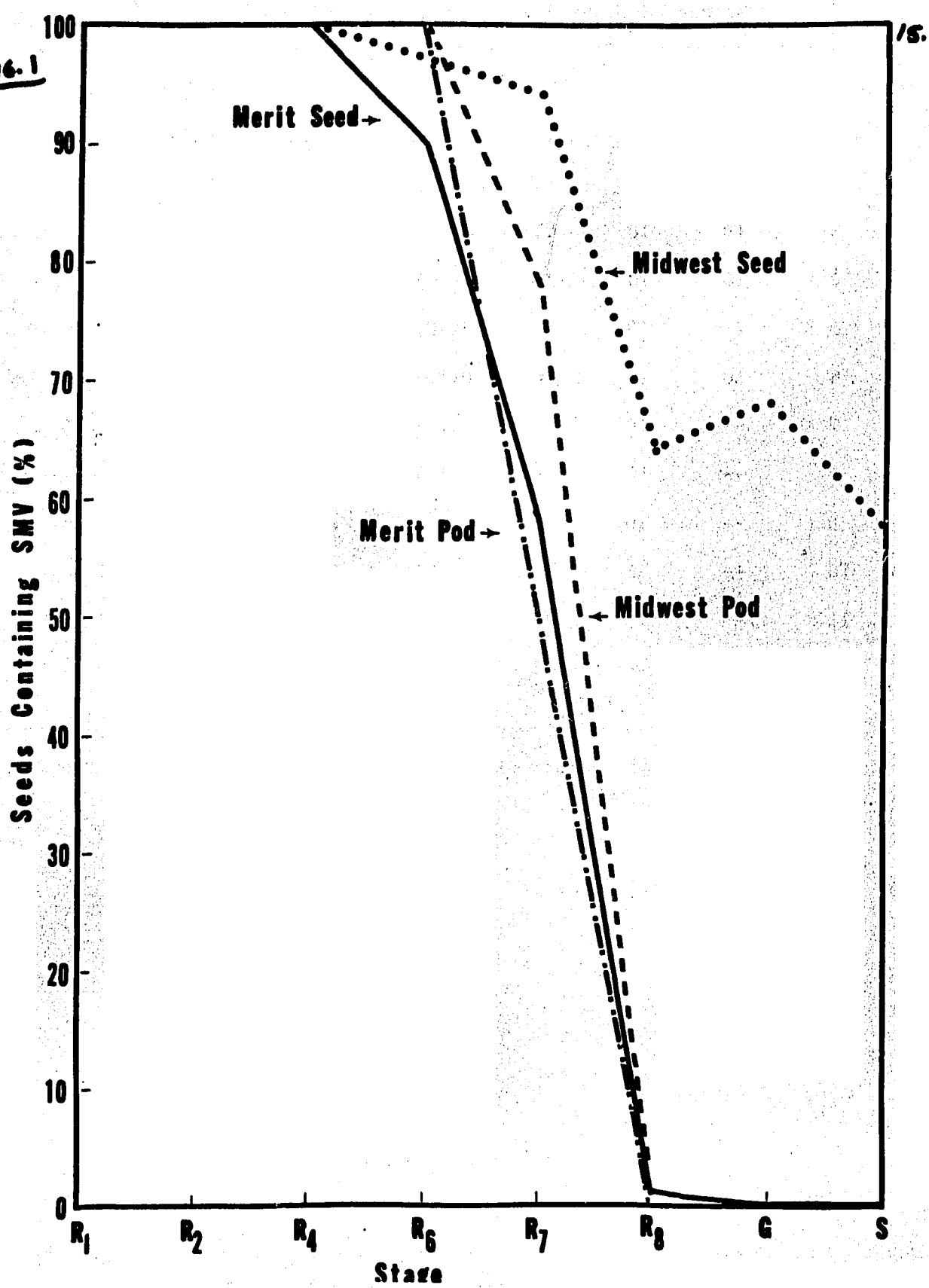
@ Indicates the number of positives/the number of plants tested, since not all of the plants produced seeds that germinated.

Table 5. SMV infection of Midwest soybean reproductive tissues at various stages of plant development.

Node	R1	R2	R4	R6	R7	R8	Germination
1	10	10	10	10(10)	10(5)	6(0)	6/10
2	10	10	10	9(10)	9(8)	6(0)	6/10
3	10	10	10	10(10)	10(8)	8(0)	8/10
4	10	10	10	9(10)	10(8)	5(0)	8/10
5	10	10	10	9(10)	9(8)	9(0)	6/10
6	10	10	10	10(10)	9(10)	8(0)	10/10
7	--	10	10	9(10)	8(7)	8(0)	4/8
8	--	10	10	10(10)	9(8)	5(0)	1/6
9	--	10	10	10(10)	10(7)	6(0)	6/8
10	--	10	10	10(10)	10(7)	4(0)	4/6
11	--	10	10	10(10)	9(9)	7(0)	8/8
12	--	--	10	10(10)	10(9)	8(0)	4/8

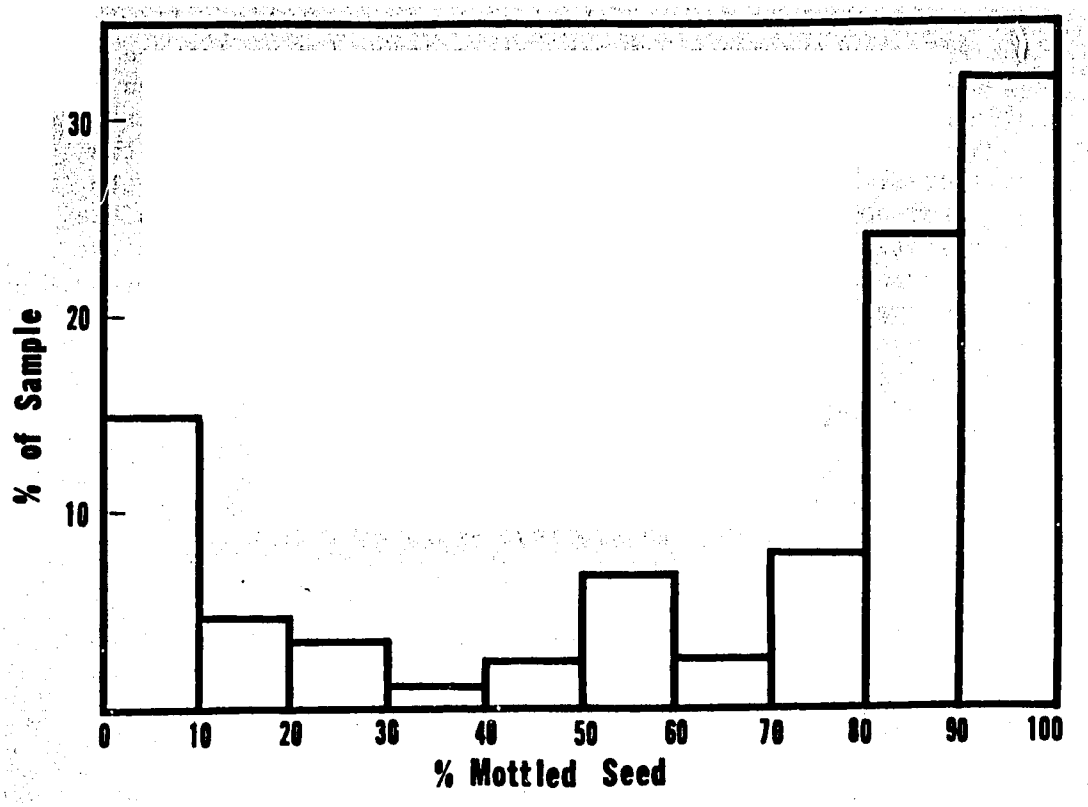
Numbers indicate the number of positive reactions for SMV using the detached Top Crop index method.

FIG. 1


Merit Seed
Midwest Seed
Merit Pod
Midwest Pod
Seeds Containing SMV (%)
100
90
80
70
60
50
40
30
20
10
0
R1
R2
R4
R6
R7
R8
G
S
Stage

/5.

Fig. 2



disease was mechanically transmissible. Joint research by UPR and UIUC virologists resulted in a method for purification of a novel virus from infected plants and proof that the virus was the cause of the disease. Subsequent results at Urbana and in cooperation with an electron microscopist at the University of Arkansas have extended knowledge about this unprecedented plant virus. We have learned that the amount of virus in infected tissues rises and then falls drastically, so that successful transmission by mechanical means occurs only during the time when the virus concentration is high. The virus has an unusual genome molecule; it is the first plant virus ever shown to contain single-stranded DNA. The virus causes cytopathological effects in infected cells that are unlike those caused by any other plant virus, and are somewhat similar to those caused by a group of single-stranded DNA animal viruses.

There are several ways in which these results are of practical significance. We now know why mechanical transmission of the virus was difficult, and we know better how to go about attempting transmission of other similar agents such as that causing soybean yellows or mung bean yellow mosaic in India, Sri Lanka and Pakistan. Mechanical transmissibility allows for efficient screening of germ plasm lines for resistance to disease. Identification of a virus causing these diseases also opens the way for studies on relationships of the pathogens causing diseases of unrelated crops that may be grown in the same area. Such studies could be conducted before only with vectors; now more critical and rapid studies will be possible using antisera produced against the various purified viruses. The possible relationships between viruses with similar properties that infect animals and plants always raises important questions about cross-infection. Identification and characterization of this new virus will allow such studies to be conducted.

Technical details of the methods and results of studies on the purification and properties of bean golden yellow mosaic virus were published in the January 1977 issue of Phytopathology, V.67, No.1.

Future plans for the next contract year include preparation of specific antisera to this virus, further characterization of the pathological alterations in infected plants, and further studies on the morphology and biochemical components of the virus itself.

b. Whitefly-transmitted viruses of soybeans. Studies on the whitefly-transmitted virus of beans described above were possible because the virus was readily mechanically transmissible. With methods developed in the above work, we are now in a position to conduct experiments on presumably similar viruses that infect soybeans. Preliminary research at the end

of the contract year conducted in the laboratory of Dr. Julio Bird, Rio Piedras, P.R., indicated that these methods will probably work well for two whitefly-transmitted viruses occurring in Puerto Rico that infect soybeans.

These studies will be extended. In addition, a survey will be made in Sri Lanka, in cooperation with the INTSOY entomologist, with a view to setting up a research program to study means of controlling spread of whitefly-transmitted viruses into the soybean crop.

Many types of studies need to be done. Etiological research on other whitefly-transmitted pathogens should be conducted to determine if they are similar to the golden yellow mosaic virus. Antisera should be prepared to various viruses of this group and native vegetation tested to determine when the viruses of interest in soybeans come from (i.e., what are the important sources of primary inoculum). Epidemiological studies on the timing and pattern of virus spread in soybeans should be conducted in fields of different sizes under mono-cropping and multiple cropping schemes with and without effective weed control. Breeding studies should be conducted to identify and incorporate whitefly or virus resistance into adapted cultivars for areas where the disease is important. Experiments on the biology and behavior of the whitefly must be conducted and a major effort to devise economically feasible, environmentally sound methods for whitefly control should be started.

These experiments must be conducted in Asia under conditions where the disease of soybeans caused by a whitefly-transmitted virus is important. The choices currently are India or Sri Lanka. Considerable work has been done in the past in India, but the principle workers have retired or moved to other problems. Any regional research program on this problem should be closely coordinated with and jointly advised by colleagues at G. B. Pant University of Agriculture and Technology and ICRISAT. The developing interest in soybeans in Sri Lanka and the availability of entomology and virology counterparts engaged in soybean research may make Sri Lanka the location of choice for the proposed research. Reports indicate that whitefly-transmitted yellows in soybeans is increasingly prevalent there, and it is conceivable that the disease could threaten soybean production in Sri Lanka as it did in India in the late 1960's.

Whitefly-transmitted viruses may be controllable in the field by controlling the vector populations. This might be done with low-dosage insecticides, with parasites that infect and kill the whiteflies, or by breeding plant types resistant to white fly feeding. There is a tremendous opportunity to conduct studies in tropical areas where whitefly-transmitted viruses and whiteflies are prevalent and important. Nothing, for example, is known about the epidemiology of these viruses.

Little is known about the biology of the whitefly vector, Bemisia tabaci, and less about the behavior of the vector. Since this vector transmits the pathogens that cause severe diseases in many tropical crops (cassava, tobacco, tomato, soybeans, mung beans, common beans, cotton, pigeon peas, sweet potato, etc.), and since the pathogens are probably similar to the one we have worked with from Phaseolus, we are in an outstanding position to make a major contribution to disease control in tropical crops by concentrating a major effort on whiteflies and the pathogens they transmit.

Studies on the virulence of SMV from soybean germ plasm. Soybean mosaic virus (SMV) is seed transmitted, and is widely distributed in germ plasm collections. Little is known about the biological agressiveness or virulence of SMV isolates. Experience shows, however, that putatively SMV-resistant soybean-lines developed with the use of one SMV isolate often become infected when tested in another envirnoment with a different SMV isolate. It thus seems important to obtain an understanding of the range of virulence of SMV isolates for the use of breeders who could then use several different SMV isolates for selection of resistant material in breeding programs.

A preliminaty study is now underway in which recently introduced PI lines obtained from the USDA germ plasm collections (Urbana and Stoneville) are being used as sources of virus isolates. These are being tested for virulence by inoculation to several putatively SMV resistant varieties (including the variety Buffalo recently discovered by INTSOY to be resistant to SMV). The determinations are at an early stage, and no results are yet available.

Detection of seedborne SMV in soybean seed lots. A cooperative study funded in part by the contract and in part by the Illinois Cooperative Improvement Association, is designed to develop a method for detection of SMV in seeds. Under investigation is a method employing an enzyme linked chemically to SMV-specific antibodies. When the virus is present in an unknown sample, it sticks to a surface which is then treated with virus-specific enzyme-labeled antibody. The presence of the antibody, and thus of the virus, can then be revealed by covering the surface with a substrate solution in which the enzyme acts to cause a color reaction.

This method should allow for the convenient testing of several hundred individual seeds of a seed lot for seed certification purposes, and this is of potential use to breeders, seedsmen, and pathologists.

Characterization of cowpea mosaic virus isolates.

a. Properties of CPMV isolates from Puerto Rico. CPMV isolates used in this work were originally obtained from naturally infected soybeans from Puerto Rico. Symptoms observed in soybeans were striking. Both systemic mosaic and budblight were observed under field and greenhouse conditions. These isolates of CPMV belonged to the same subgroup (severe) as the Arkansas

isolate of the virus (CPMV-Ark). The identity of these isolates were confirmed by symptomatology, serology, electrophoretic property, and sedimentation analysis study.

CPMV demonstrated heterogeniety in velocity sedimentation. Three components with different nucleic acid composition were observed when the virus was subjected to centrifugation. The two components containing nucleic acid are necessary for infection. Attempts are now being made to obtain an in vitro "hybrid" of CPMV by mixing one component of one strain with the complementary component of another strain. Although true genetic recombination has not been observed with viruses, results from this experiment could be a model as to how new isolates of CPMV are derived under natural conditions.

b. Isolation of CPMV from a beetle collected in an Illinois soybean field. Cooperative studies were conducted on a new CPMV isolate recovered from a striped cucumber beetle trapped in a soybean field in southern Illinois during a routine survey. The virus, which is similar but not identical to CPMV isolates originating in Africa, infects soybeans but causes only mild symptoms. No significant yield loss was caused by this isolate in a test conducted at Urbana in the summer of 1976 (Table 6).

Table 6. Effect of CPMV infection on yield of Williams soybeans grown at Urbana, summer 1976.

Treatment	Yield (kg/ha)
Healthy	3573
CPMV-Illinois	3330
CPMV-Arkansas	1773
CPMV-Puerto Rico	1852

Contract Objective 5

Yield loss threshold in soybeans caused by cowpea mosaic virus. CPMV is a potentially serious virus in soybeans, causing severe symptoms. The virus is transmitted by several species of beetles that feed on both cowpea and soybeans. Natural infection of soybeans with CPMV has been noted in Puerto Rico since 1974, but the virus has not become widespread.

In 1976 an experiment was conducted to determine the yield loss caused CPMV infection at different stages of plant development and in different proportions of the plant population. Soybeans (Improved Pelican) were planted at Isabela, P.R. and inoculated

mechanically (5 m rows) at either the primary leaf or bloom stage. The experiment was replicated 4 times. Treatments were 0, 25, 50, 75, or 100% of plants inoculated. Results showed a serious reduction in yield caused by CPMV inoculation at the primary leaf stage and a significant reduction when all plants were inoculated at the bloom stage. The yield loss threshold appeared to be at less than 25% of the plant population if infection occurred early and 75% if it occurred at bloom (Table 7). More refined statistical analyses will be made of these data and the experiment will be repeated in 1977.

Table 7. Effect of inoculation time and proportion of infected plants on yield of Improved Pelican soybeans inoculated with cowpea mosaic virus.

Stage of plant development at inoculation	Mean yield (kg/ha) following inoculation of percentage of plants shown				
	0	25	50	75	100
Primary leaf	1430	955	796	533	29
Bloom	1406	1114	857	809	597

ANNEXURE A

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APPENDIX IV

Report of James B. Sinclair
Professor of Plant Pathology

I. Introduction

The research in this portion of the program involved interdisciplinary, interdepartmental and international activities, and included studies on soybean, common bean, cowpea and Stylosanthes scabra. Publications during this period include shared authorship with scientists from Brazil (C. C. Machado), Colombia (G. E. Galvez, J. E. Ferguson, and B. Grof), India (O. D. Dhingra), Nigeria (R. J. Williams), Pakistan (M. B. Ilyas), and Thailand (C. Prasartsee). Co-authors in the U.S. include scientists from Mississippi State University Seed Technology Laboratory (C. H. Andrews), University of Puerto Rico, Mayaguez (M. A. Ellis and A. Rodriguez-Marcano), University of California, Berkeley (R. W. Schneider) and the University of Illinois Agricultural Extension Service (M. C. Shurtleff and B. J. Jacobsen). These articles appeared in international journals (Transactions of the British Mycological Society, Acta Botanica India and Proceedings of the Fourth Reinhardsbrunner Symposium, DDR), as well as domestic U.S. (Phytopathology) Plant Disease Reporter, Fungicide and Nematicide Tests, Results of 1975) and those published in Illinois (Transactions of the Illinois Academy of Sciences and Illinois Research).

The major thrust of the research was to better understand the effect of various microorganisms on soybean seed and seed quality and develop means of control. Other research has included similar studies on common bean and cowpea. In addition, studies were done on charcoal rot of soybean roots and stems, and developing methods of control. The studies on factors influencing soybean seed quality and their control in Illinois has been supported in part by the Illinois Crop Improvement Association and the Illinois Agricultural Experiment Station.

II. Control

A. Nonchemical (see references 1, 4):

Charcoal rot. The competitive saprophytic colonization (CSC) of Macrophomina phaseolina on stem pieces (substrate units) of soybean and maize was estimated from the calculated inoculum density required to colonize 50% of the substrate units (EID_{50}). The EID_{50} was found to be an inverse function of CSC. EID_{50} values for M. phaseolina were low when inoculum, prepared using the Cambridge Method was used to determine CSC, suggesting that M. phaseolina had a high CSC. However, when sclerotia or infected plant material were added to soil as inoculum, EIC_{50} values were high, suggesting a low CSC. The Cambridge Method is considered invalid for estimating the CSC of a plant-pathogenic, soil-borne fungus. The CSC of M. phaseolina was

reduced by increasing the organic matter and hence the microbial population of the soil and also was influenced by soil type, source of inoculum and substrate.

Soybean and maize stem pieces colonized by Fusarium roseum were not colonized by M. phaseolina. A substance was produced by F. roseum in the substrate that caused inhibition of M. phaseolina. M. phaseolina grew from colonized stem pieces in soil infected roots of soybean seedlings.

Seed-borne fungi on common bean. Mature seeds were removed from dry bean (Phaseolus vulgaris cvs. Calima, Guali, Huasano, Porrillo Sintetico and Tui) pods that had been, or had not been, in contact with soil in the field. Fungi representing 14 genera were recovered from seeds bioassayed on potato-dextrose agar (PDA) (22-24°C) after surface sterilization (Table 1). Significantly more internally seed-borne fungi, and less germination and field emergence, were recorded among seeds from pods in contact with soil than from pods not in contact with soil. Field emergence of seeds from pods in contact with the soil treated with thiram (2 g/1000 g seeds) was significantly higher than for nontreated seeds (Table 2). A tetrazolium test showed that the percentage of viable seeds was higher than indicated from the germination studies for seeds from pods in contact with soil, but not for seeds from pods not in contact with soil.

Table 1. Percentage recovery of fungi dry bean (Phaseolus vulgaris) seeds of five cultivars removed from pods either in contact with soil (C) or not in contact with soil (NC) in the field at Cali, Colombia.

	Mean percentage recovery[a/]											
			Rhizoctonia		Phomopsis		Macrophomina		Alternaria		Other	
Cultivar	Fusarium spp.		solani		spp.		phaseolina		spp.		fungi	
	C	NC	C	NC	C	NC	C	NC	C	NC	C	NC
Calima	31	2	5	0	5	0	1	0	8	1	14	0
Guali	45	22	9	2	4	0	6	0	4	1	22	5
Huasano	51	14	6	0	6	0	3	0	8	5	14	7
Parrillo Sintectico	56	10	10	0	10	0	3	0	6	7	7	2
Tui	34	8	2	1	4	0	2	0	9	3	27	2

a/ Based on three replications of 100 seeds per each replicate planted on potato-dextrose agar (22-24°C) for 7 days.

Table 2. Mean percentage germination in culture, total internally seed-borne fungi (total fungi), and field emergence of nontreated and thiram-tested (T) seeds from pods of five dry bean *Phaseolus vulgaris*) cultivars either in contact (C) or not in contact (NC) with soil in the field at Cali, Colombia

Treatment[a/]		Cultivar				
		Calima	Guali	Huasano	Parrillo Sintetico	Tui
Germination	C	57	46	50	47	59
	NC	100	92	95	97	99
	LSD .05	17.9	9.3	16.9	10.5	11.6
	LSD .01	41.2	2.4	38.6	24.2	26.9
Total fungi	C	64	90	88	92	78
	NC	3	30	26	19	14
	LSD .05	22.2	21.1	13.6	6.1	6.9
	LSD .01	34.0	44.4	31.3	13.9	15.9
Field emergence	C	63	47	58	51	69
	CT	82	75	69	68	85
	NC	92	86	84	83	95
	NCT	95	90	86	86	94
	LSD = .05	18.7	13.4	13.0	12.4	14.8
	LSD = .01	28.9	20.7	19.7	19.0	22.3

a/ Means based on three replications of 100 seeds each, except for viability test which was based on 100 seeds. Viability measured using tetrazolium test. Seeds treated with thiram at a rate of 2 g per 1,000 g of seed.

B. Chemical Control (see references 2, 3, 5, 6, 8, 11, 12, 13, 17, 18, 19, 21).

Seed-borne microorganisms in soybean. Fungicide seed treatment of soybeans can reduce the quantity of seed-borne fungi and increase the emergence of low-quality seeds (germination below 70 percent and a high incidence of seed-borne organisms), but may have little or no effect on high quality seeds (germination above 70 percent and low incidence of seed-borne organisms).

Table 3. Mean (three replications of 100 seeds each) stand counts from Beeson (poor) and Amsoy 71 (good) soybean seeds treated with various fungicides

Treatment and Rates	Mean Stand Counts			
	Poor Quality Seed		Good Quality Seed	
	15 days	30 days	15 days	30 days
Thylate 65 WP 1.23 oz/bu	88 a*	92 a	85 bc	85 a
Captan 80 WP 1.0 oz/bu	85 ab	87 ab	91 a	88 a
UniRoyal 1109 4.0 oz/bu	83 abc	85 abc	82 bcd	89 a
UniRoyal 1127 2.0 fl oz/100 lbs	83 abc	85 abc	84 abcd	83 a
Demosan T 4.0 oz/bu	81 abc	84 abcd	87 abc	85 a
UniRoyal 1127 4.0 fl oz/100 lbs	78 abcde	80 bcdef	81 cd	81 a
UniRoyal 1109 2.0 oz/bu	77 abcde	81 bcdefg	91 a	89 a
Vitavax SP 4.0 oz/bu	77 abcde	81 bcde	85 abc	85 a
UniRoyal H719 2.0 oz/100 lbs	74 bcde	73 efgh	83 abcd	82 a
Nontreated (Control)	72 bcde	76 bcdefg	84 abcd	85 a
Celemerck 20EC 1.0 oz/bu	71 bcde	75 bcdefg	88 abc	87 a
Benlate 50 WP 4.0 oz/bu	68 cde	74 defgh	81 cd	86 a
UniRoyal P368 0.25 oz a.i./100 lbs	65 de	69 gh	76 d	82 a
UniRoyal P368 0.50 oz a.i./100 lbs	65 de	70 fgh	90	86 a
UniRoyal H719 75% 4.0 oz/100 lbs	64 de	69 gh	90 ab	86 a
Benlate 50 WP 2.0 oz/bu	64 de	64 h	80 cd	81 a

* Numbers followed by the same letter are not significantly different at the 5 percent level as determined by Duncan's New Multiple Range Test.

New methods for treating soybean seeds are being studied. One promising method is the use of solvents such as dichloromethane and polyethylene glycol for incorporating antibiotics into soybean seeds, plus conventional seed treatment fungicides (Table 4). Antibiotics control seed-borne bacteria and fungicides control the fungi. When penicillin G was combined with Arasan 50 Red, the mean stand count in the field was near the percent germination measured by a warm test in the laboratory.

Table 4. Mean stand counts of soybean seeds (Wells cultivar) 21 days after planting untreated or treated with various fungicides and antibiotics[a/]

Treatment[b/]	Rate	Stand count[c/]
Penicillin G in PEG + Arasan 50 Red	1,000 units in 0.45 g./ml. water + 2 oz./100 lb.	64 a
Penicillin G in DCM + Arasan 50 Red	1,000 units/ml. + 2 oz./100 lb.	61 a
Penicillin G in DCM	1,000 units/ml. DCM	53 b
Orthocide 75W (captan)	1 oz./bu.	53 b
Arasan 50 Red (thiram)	2 oz./100 lb.	52 b
Penicillin G in PEG	1,000 units in 0.45g./ml. water	39 c
Control (untreated)	. . .	31 d

Table 4 (continued)

a/ Warm test germination = 68 percent.

b/ PEG = polyethylene glycol; DCM = dichloromethane.

c/ Selected from combined data from two tests conducted at the Agronomy South Farm, Urbana, and Isabela Station, University of Puerto Rico, Mayaguez. Numbers followed by the same letters are not significantly different at the 5-percent level as determined by the Duncan's New Multiple Range Test.

Fungicide activity was detected in dormant soybean (Glycine max 'Wells') seeds after soaking for 0.5, 1.5, 4, or 24 hours in 25, 50, 100, 200, 400, 800, or 1,600 µg/ml methyl2-benzimidazolecarbamate (MBC) or [2-(4'-thiazolyl)benzimidazole] (thiabendazole) in dichloromethane (methylene chloride) (DCM), but not when captan, thiram, and carboxin were used. Carboxin, but not captan or thiram, lost fungicidal activity when mixed with DCM. Dichloromethane facilitated the movement of MBC and thiabendazole into dormant soybean seeds in the absence of water. Zones of inhibition in agar plates around treated seeds increased in size with increased concentration of fungicide and soaking time (Table 5). Soybean (cultivars Hill and Wells) seeds treated with MBC in DCM and thiabendazole in DCM had decreased incidence of internally-borne fungi (Phomopsis spp.), higher germination in vitro, and emergence in vermiculite and soil than control seeds treated with DCM alone (Table 6). Dichloromethane appeared to have some antifungal activity.

Table 5. Inhibition of Penicillium expansum on potato-dextrose agar plates by soybean (Glycine max) seed halves treated with various concentrations of methyl 2-benzimidazolecarbamate (MBC) or [2-(4'-thiazolyl) benzimidazole] (thiabendazole) (TBZ) in dichloromethane (DCM)

Concentration	Inhbition zones (mm) around seeds soaked for time in hours a/							
	0.5		1.5		4		24	
(µg/ml)	MBC	TBZ	MBC	TBZ	MBC	TBZ	MBC	TBZ
25	2.9	0.0	4.3	2.6	5.2	4.3	6.6	6.4
50	4.7	2.0	5.7	3.6	7.0	6.0	8.3	7.5
100	7.1	3.0	9.3	4.8	10.9	7.2	12.4	7.7
200	9.0	3.8	10.1	6.0	11.5	7.9	13.3	9.3
400	10.9	5.4	11.5	7.7	12.3	9.8	14.7	11.9
800	11.8	7.5	13.5	10.0	14.6	11.8	15.3	13.7
1,600	13.5	10.2	14.6	12.0	15.6	13.5	17.0	15.6

	MBC	TBZ
LSD (P=0.05) =	0.46	0.31
LSD (P=0.01) =	0.66	0.92

a/ Means based on 10 seeds in each of three replications. Control seeds soaked in DCM alone produced no zones of inhibition, for any treatment time.

Table 6. Germination, total fungi, and Phomopsis spp. at 25 C, and emergence in vermiculite and soil in the greenhouse from soybean (Glycine max) seeds of two cultivars soaked for 5 hours in 800 µg/ml methyl 2-benzimidazole carbamate (MBC) or [2-(4'-thiazolyl)benzimidazole] (thiabendazole) (TBZ) in dichloromethane (DCM)

	Percent means for cultivar[a/]									
	Hill					Wells				
Treatment	PG	TF	PHO	EV	ES	PG	TF	PHO	EV	ES
MBC in DCM	93	8	5	95	86	91	10	1	94	87
TBZ in DCM	93	10	5	96	83	91	8	1	92	86
DCM	63	31	26	72	57	80	24	4	76	72
Control	60	90	33	66	52	74	48	8	75	65
LSD (P=0.05)	8	6	6	7	6	3	10	3	9	6
LSD (P=0.01)	12	9	9	11	9	5	14	4	13	9

[a/] Based on three replications of 100 seeds per replication and treatment. PG = percent germination; TF = total fungi; PHO = Phomopsis spp.; EV = emergence in vermiculite; and ES = emergence in soil.

Use of fungicide sprays for control of internally seed-borne fungi. D. phaseolorum var. sojae (Phomopsis sp.), is seed-borne in soybeans and causes pod and stem blight as well as seed deterioration. This fungus increases in occurrence in seeds and, concommitantly, germination decreases as harvest dates are delayed. The occurrence of D. phaseolorum var. sojae in seeds will vary between different cultivars and among seed lots of the same cultivar grown in different regions. The occurrence of this pathogen in soybean seeds can be reduced with foliar applications of benomyl.

Benomyl, a systemic fungicide, is taken up by germinating soybean seeds and is systemic in soybean seedlings. Benomyl is rapidly hydrolyzed into methyl 2-benzimidazole-carbamate (MBC) in solution and in plants and is absorbed through the wall of developing soybean pods and entered into the seeds within 23 hours after application of benomyl in the greenhouse. MBC moves through pod walls and into seeds and was absorbed through stem tissues and translocated upward into seeds of plants sprayed with benomyl in the field.

We found that the amount of seed-borne D. phaseolorum var. sojae was significantly reduced below that of nonsprayed plants when soybean plants were sprayed with one of the following fungicides or fungicide combinations: benomyl, benomyl plus zinc-ion, captafol, chlorothalonil, mancozeb, thiabendazole, and thiophanate-methyl (Table 7). The occurrence of D. phaseolorum var. sojae was always less in seeds from plants sprayed with benomyl than those from the other fungicides. Soybean plants sprayed with benomyl remained green longer than nonsprayed plants. D. phaseolorum var. sojae may cause early defoliation and death of soybean plants. However, it is also possible that benomyl may be phytotoxic in delaying maturity.

Cultivars may differ in their susceptibility to seed infection by D. phaseolorum var. sojae. This difference may be due to: differences in maturity groupings; in susceptibility to seed infection by fungi; differences in the amount of seed-borne inoculum and/or to the number of seeds infected with fungi at the time of planting; or differences in the number of weeks between harvests. These differences

may require different spray regimes regarding time of spray and number of applications of fungicides.

Delayed harvest results in the increase of the occurrence of internally seed-borne D. phaseolorum var. sojae and other fungi in soybean. Spraying of soybean plants before maturity with benomyl can significantly reduce seed-borne fungi and even with a delay in harvest date, the fungicide will control the amount of seed-borne fungi.

Table 7. Effect of fungicides and two harvest dates on percentage soybean seed germination in vitro and percentage occurrence of internally seed-borne Diaporthe phaseolorum var. sojae (Phomopsis sp.) (Dps) and total fungi

		Cultivar											
		'Lee 68'						'Hill'					
	Number of	Germ[b]		Dps[b]		Total fungi		Germ[b]		Dps[b]		Total fungi	
Treatment[a]	Sprays	FH	DH	FH	DH	FH	DH	FH	DH	FH	DH	FH	DH
Benomyl	1	92.5	96.0	1.5	1.0	2.5	2.5	85.5	31.0	14.0	64.0	15.5	86.0
	2	96.5	97.5	0.0	1.0	0.5	3.0	91.5	76.0	5.5	3.5	12.5	41.5
	3	92.5	94.5	1.0	0.5	1.0	1.5	95.5	77.0	3.5	1.0	13.5	40.0
Chloro-thalonil	1	87.5	83.0	5.5	13.0	6.0	14.5	86.0	10.0	13.5	88.5	14.0	93.0
	2	91.0	81.0	5.0	14.5	7.3	16.0	89.5	11.0	9.0	82.5	9.5	87.0
	3	91.5	82.5	4.5	9.5	5.0	11.5	93.5	18.5	6.5	77.5	7.5	77.5
Captofol	1	90.0	87.5	6.0	7.0	6.5	12.0	53.5	14.5	46.0	74.5	47.0	78.0
	2	91.2	85.5	3.0	7.0	2.0	8.5	73.0	16.5	25.5	75.5	27.0	80.0
	3	86.5	85.0	4.0	7.5	5.5	9.0	78.0	4.0	21.0	94.0	22.0	94.0
Mancozeb	1	91.5	86.5	4.0	10.0	4.0	12.0	79.5	7.0	20.5	90.5	21.5	93.0
	2	86.0	82.5	6.0	9.5	6.2	14.0	71.0	8.5	28.5	81.0	30.0	84.5
	3	91.5	71.0	4.0	14.0	5.0	16.0	72.0	9.0	26.0	81.0	26.0	87.0
Control		90.5	75.5	3.5	19.0	6.0	26.0	75.5	6.0	24.0	87.5	25.5	88.5
$FLSD_{05}$ (harvest)		1.1		1.9		0.9		2.4		3.0		3.8	
$FLSD_{01}$		2.1		3.6		1.8		4.3		7.2		7.1	
$FLSD_{05}$		4.0		2.5		2.2		3.5		3.6		12.9	
$FLSD_{01}$		5.4		3.3		2.9		4.7		4.7		17.1	

a/ Applied at a rate of 1/2 pound formulated material per acre.

b/ Percentage based on 200 seeds from each first harvest (FH) and delayed harvest (DH). There were almost five weeks between harvests for 'Lee 68' and eight weeks for 'Hill'.

Charcoal rot of soybean. The effectiveness of 10 soil fungicides in reducing viability of *Macrophomina phaseolina* sclerotia in soil depended upon the concentration of the fungicide used and exposure time. The most effective fungicides in laboratory tests in descending order were: benomyl, thiophanate-methyl, thiram, thiabendazole, triforine, and captan. There was greater reduction of sclerotium viability by all fungicides in low than in high organic-matter-containing soils. In laboratory tests, benomyl, thiophanate-methyl, thiabendazole, and thiram were effective in reducing sclerotium viability in soybean stem pieces placed in fungicide-treated soil (Table 8). The reduction in viability was greater when free sclerotia were used, least when sclerotia were in fresh stem pieces, and intermediate when sclerotia were in partially decomposed stem pieces.

Table 8. Recovery of *Macrophomina phaseolina* from infected soybean stem pieces at various days after treatment with various fungicides at 200 µg/g of soil, in two soil types

	Recovery of *M. phaseolina* (%)[b]					
	4 days		8 days		14 days	
Fungicide[a]	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
Benomyl	78	88	48	62	7	33
Thiophanate-methyl	93	100	67	82	32	47
Thiabendazole	100	100	77	85	48	62
Thiram	100	100	100	100	82	92

[a] The following fungicides were ineffective: captan, triforine, Hymexazole, ethazol, pentachloronitrobenzene, and carboxin. A = silty-sand-loam, B = silty-loam.

[b] Percentage of stem pieces giving rise to *M. phaseolina* when plated on a selective agar medium. A = silty-sand-loam, B = silty-loam.

B. Common bean (*Phaseolus vulgaris*):

Dry bean (Tui) seeds were harvested at maturity and 1, 2, 3 or 4 weeks after maturity (late harvest) from nonsprayed plants or plants sprayed with the systemic fungicides benomyl and oxcarboxin alone or in combination at four 9-day intervals beginning 40 days after planting. The percentage seed germination of internally seed-borne fungi increased with each harvest date for all treatments (Table 9). The decrease in percentage germination and emergence and the increase in seed-borne fungi was, in general, significantly less for seeds from fungicide-sprayed plants than the controls for the first four harvest dates (Table 10). All seeds harvested after 4 weeks were invaded nearly 100% by fungi.

C. *Stylosanthes scabra*:

Colletotrichum gloeosporioides and fungi of nine other genera were recovered from surface sterilized pods and seeds of *Stylosanthes scabra* grown in Colombia, South America. This is the first report of *C. gloeosporioides* being seed-borne in *Stylosanthes* spp. The number of fungi recovered from pods was greater than

from seeds. Isolates of C. gloeosporioides produced anthracnose symptoms and killed seedlings of Stylosanthes guyanensis cv. CIAT-1198. Recovery of C. gloeosporioides and other fungi was reduced or was not possible when pods and seeds were scarified by using a sulfuric acid soak for 3 minutes.

Table 1. Alternaria, Fusarium and Rhizoctonia species recovery in mean percent from dry bean (Phaseolus vulgaris cv. Tui) seeds from plants nontreated or sprayed with two systemic fungicides and harvested at maturity and 4 weekly intervals thereafter

Treatment	Harvest in weeks after maturity	Percent Recovery[1/]		
		Alternaria	Fusarium	Rhizoctonia
Benomyl	0	1	1	1
	1	12	2	0
	2	22	1	0
	3	33	4	1
	4	85	4	4
Oxycarboxin	0	1	2	1
	1	5	5	0
	2	8	14	0
	3	1	45	3
	4	7	79	4
Benomyl plus oxycarboxin	0	2	1	1
	1	4	9	1
	2	11	9	1
	3	39	8	4
	4	84	4	5
Nontreated	0	2	9	1
	1	2	32	6
	2	2	63	5
	3	8	69	6
	4	3	84	7

1/ Based on three replications of 100 seeds/replication for each treatment and harvest date.

Table 10. Mean percentage germination in culture plates (Germ.), field emergence (15 days) (Emerg.), total internally seed-borne fungi (Fungi), and 1,000-seed weights of seeds from dry bean (Phaseolus vulgaris cv. Tui) plants nontreated or sprayed with two systemic fungicides and harvested at maturity and 4 weekly intervals

Treatment	Harvest after maturity in weeks	Mean percentage[1/] Germ.	Emerg.	Fungi.	1,000 seed mean weight in grams
Benomyl	0	95	87	4	201
	1	92	84	15	205
	2	92	87	26	201
	3	93	82	38	205
	4	76	71	96	206
Oxycarboxin	0	98	86	5	194
	1	97	86	13	207
	2	91	84	26	204
	3	82	82	56	198
	4	75	67	99	197
Benomyl plus oxycarboxin	0	94	85	4	201
	1	95	84	17	208
	2	92	86	27	203
	3	86	84	54	203
	4	74	72	98	205
Nontreated	0	87	86	13	152
	1	79	75	48	149
	2	63	69	86	147
	3	64	67	100	150
	4	60	61	100	154
$LSD_{.05}$ =		3.71	3.43	8.87	13.86
$LSD_{.01}$ =		4.94	4.57	11.82	15.15

1/ Based on three replications of 100 seeds per replication for each treatment and harvest date.

III. Assessment of factors that influence diseases

A. Soybean (see references 7, 9, 10, 14, 15, 20, 22):

The concept of microorganisms being internally seed-borne in soybeans has heretofore suggested that the organism would primarily be found between the seedcoat and the cotyledons or in the cotyledonary tissues.

The seedcoat of a healthy soybean seed consists of a cuticle and three different cell layers: the palisade (exodermis), hourglass (hypodermis), and parenchyma (parenchyma and endosperm), which would provide a nutrient source

for microorganisms. When the seedcoat cuticle and palisade layer are damaged, the hourglass cell layer is exposed with the parenchyma layer usually remaining intact but provides potential sites for entrance and colonization by fungi.

The hourglass cell layer is the site of initial colonization by Cercospora kikuchii, Colletotrichum dematium var. truncata, and Diaporthe phaseolorum var. sojae. Other fungi isolated from the seedcoats of surface-sterilized soybean seeds are species of: Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, and Rhizopus.

The mycelium of C. kikuchii was found equally abundant in the hourglass and parenchyma cell layers. The mycelium appeared to grow parallel to the longitudinal axis of the parenchyma cells, irregularly in the hourglass layer, but sparsely in the palisade layer. Within the hourglass cell layer, the mycelium was more abundant in the hilum region. The mycelium was found in cotyledonary tissues (epidermis and parenchyma) showing purple stain symptoms about the lesion.

C. dematium var. truncata mycelium was found only in the hourglass cell layer and never observed in the other two cell layers of the seedcoat or in cotyledonary or embryo tissues.

D. phaseolorum var. sojae mycelium was observed to grow abundantly in the hourglass cell layer, less so in the parenchyma layer, and least in the palisade layer. The mycelium grew parallel to the long axis of the palisade cells, irregularly in the hourglass layer, and longitudinally in the parenchyma layer. Mycelial mats were found between the seedcoat and the cotyledons. In the cotyledons, mycelium was found only in the epidermis and the first one or two layers of the parenchyma cells beneath such mats.

Trichothecium sp. was found associated with decayed soybean seeds incubated on moist cellulose pads at 26 C. Such seeds were covered with the pink fungal growth. Trichothecium sp. was isolated in pure culture and pathogenicity studies made. Two-hundred seeds of each of the cultivars Amsoy 71, Beeson, Wayne, Wells and Williams were treated as follows: (i) nontreated, dried seeds; (ii) soaked in 0.1% Tween-80 solution for 1 hour without vacuum infiltration; or (iii) soaked as in (ii) and placed under vacuum (25-30 psi) for 1 hour. The inoculated treatments consisted of (iv) soaking the seeds in a Tween-80 solution containing a conidial suspension (2.5×10^5 spores/ml) of Trichothecium sp. followed by vacuum infiltration for 1 hour or (v) as (iv) with no vacuum infiltration. All seeds were air dried to 11% moisture, placed at 35 C for 24 hours, then placed on moist cellulose pads at 26 C for 5 days. The percent germination per cultivar per treatment (i - v), respectively, were: Amsoy 71, 66, 68, 40, 30, 36; Beeson, 85, 83, 71, 28, 28; Wayne, 90, 93, 87, 78, 69; Wells, 86, 88, 84, 56, 54; and Williams, 95, 95, 90, 68, 62. Similar results on seeds not placed at 35 C yielded similar results. This was the first report for the U.S. of Trichothecium sp. being pathogenic to soybean seeds.

Bacillus subtilis has been associated with the aerial parts of soybeans as well as with the seeds. We found B. subtilis in the parenchyma and hourglass cell layers of surface-sterilized seeds. The hourglass cell layer of the soybean seedcoat appears to be the initial site of colonization by B. subtilis. Amsoy 71 seeds exhibited 10% seed decay associated with B. subtilis at 25 C. Soybean seeds were surface disinfected with 0.25% NaOCl, 70% ethanol and washed in sterile distilled water. Seeds were then treated with 500 μg/ml K penicillin G in dichloromethane; or nontreated. Seeds were placed on moist cellulose pads (Kimpac) and incubated at 40 C. At 5-hr intervals plates of nontreated and treated seeds were

at 25 C and 4 C and incubated for a total of 60 hours. Germination, hypocotyl and radicle lengths, and seed deterioration using Tetrazolium chloride were determined. Treated seeds had longer radicles and hypocotyls than nontreated seeds at each temperature regime. Increased exposure time to 40 C reduced hypocotyl and radicle length of both treated and nontreated seeds. Tetrazolium chloride tests indicated that nontreated seeds showed greater amounts of dead tissue than did treated seeds.

Effect on Illinois-grown seeds. Seed lots of twelve soybean (*Glycine max*) cultivars were harvested from growing regions in northern, central or southern Illinois in 1973, 1974 and 1975. There were 39 lots of Amsoy 71, Bonus, or Wayne harvested in 1973; 64 lots of Amsoy 71, Beeson, Clark 63, Corsoy, Cutler, Kent, Wayne, Wells, or Williams harvested in 1974; and 48 lots of Amsoy 71, Beeson, Hark, Wayne, Wells, Williams, or Woodworth harvested in 1975. All seed lots were bioassayed in culture plates after surface disinfection for occurrence of seed-borne microorganisms and percentage germination. Germination differed significantly between but not within years and in occurrence of seed-borne microorganisms between and within regions and years (Table 11). The occurrence of *Phomopsis* spp. was related to year and growing regions, but not to cultivar. Cultivar interactions were noted in germination, occurrence of total fungi and *Bacillus subtilis* regardless of growing region and year. Germination alone was not sufficient for determining soybean seed health; the occurrence of seed-borne microorganisms must also be measured.

Table 11. Germination and occurrence of Phomopsis spp., total fungi, and Bacillus subtilis for various soybean cultivars grown in three regions in Illinois in 1973, 1974, and 1975.

Region or year	No. Lots	Mean Percentage[a/] Germination	Phomopsis	Total fungi	Bacillus subtilis
			1973		
Region					
North	8	72.3 a	5.5 a	21.1 a	26.4 a
Central	18	77.1 a	5.5 a	11.7 a	15.5 a
South	13	73.9 a	4.5 a	16.2 a	23.8 a
			1974		
Region					
North	18	94.1 a	0.2 a	6.6 a	10.2 a
Central	25	91.1 a	1.8 b	9.1 a	8.4 a
South	21	90.2 a	4.5 c	11.6 a	18.4 b
			1975		
Region					
North	16	71.9 a	3.1 a	6.5 a	16.8 a
Central	16	76.8 a	7.8 b	13.6 b	8.2 a
South	16	71.6 a	9.6 b	16.9 b	12.2 a
			Combined regions		
Year					
1973	39	75.0 a	5.19 a	15.2 b	20.5 b
1974	64	91.6 b	2.25 b	9.2 a	12.2 a
1975	48	73.4 a	6.84 a	12.3 ab	12.4 a
			Combined years		
Region					
North	42	81.5 a	2.3 a	9.3 a	15.8 b
Central	59	82.2 a	4.5 ab	11.1 ab	10.5 a
South	50	80.0 a	6.1 b	14.4 b	17.8 b

a/ Means based on 400 seeds per lot in 1973 and 1974, and 200 seeds per lot in 1975. Numbers followed by the same letter are not significantly different (P = .05) based on Duncan's New Multiple Range Test.

The concept of "germination potential". Present germination systems determine soybean seed viability by placing soybean seeds in an artificial medium at selected temperatures. This is assumed to measure the actual number of viable seeds in a lot. We propose a system for evaluating soybean seed germination based on the highest attainable number of seeds that germinate and which we term "germination potential". Germination potential is a measure of the actual number of viable seeds in any sample which we determine by reducing to a minimum the effect of seed-borne microorganisms on germination in an artificial medium or in the field. Wells soybean seeds were nontreated or treated with 500 μg/ml K penicillin G in dichloromethane and 0.5 g/100 g seeds thiram as Arasan 50 Red. Nontreated seeds germinated in a range of 20 to 78%, treated seeds in a range of 28 to 85%. The difference between treated and nontreated seeds was due to the fungistatic and bacteriacidal effects of the seed treatment. The measured viability of a treated seed lot is the "germination potential" of that seed lot. Germination was not "increased" with seed treatmnet. Comparison of the germination of nontreated with the germination potential of treated seed gives a measure of the effect of

seed-borne microorganisms on germination. By reducing the effect of seed-borne microorganisms, other factors affecting seed germination can be studied.

B. Cowpea (see reference 16).

Yield loss of cowpea was correlated with Cercospora leaf spot disease severity from artifically-simulated, chemically-regulated, and naturally-occurring epidemics at Ibadan, Nigeria. Areas under disease progress curves were correlated with yield loss regardless of the curves (Fig. 1). The yield-loss-estimation model was not accurate for low disease levels. A critical-point model was constructed and tested that related time of occurrence of specific disease severities to percent yield loss (Fig. 2). However, the model could be used only for uninterrupted disease progressions. A third model that integrates these two methods is discussed in relation to chemical control programs (Figs. 3, 4).

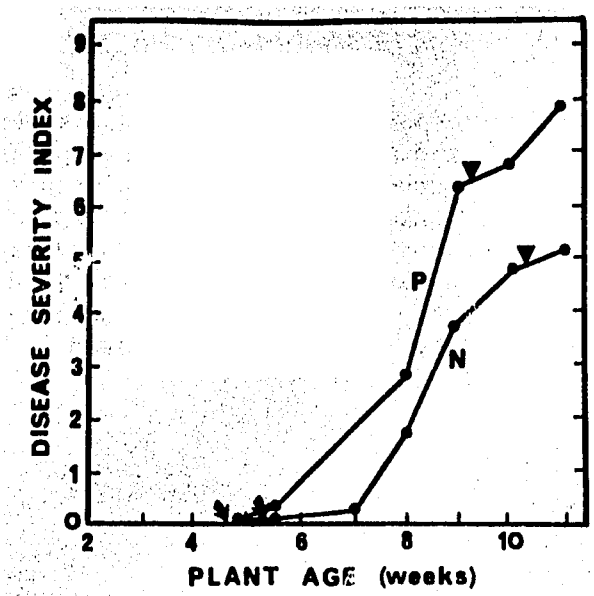

Fig. 1. Disease progress curves for Cercospora leaf spot of the cowpea [*Vigna unguiculata* 'Frima' (P) and 'New Era' (N)]. The curves are based on a disease severity scale of 0-9, where 0 = no disease and 9 = complete defoliation, at given plant ages. Arrows and triangles indicate times of flowering and first harvest, respectively.

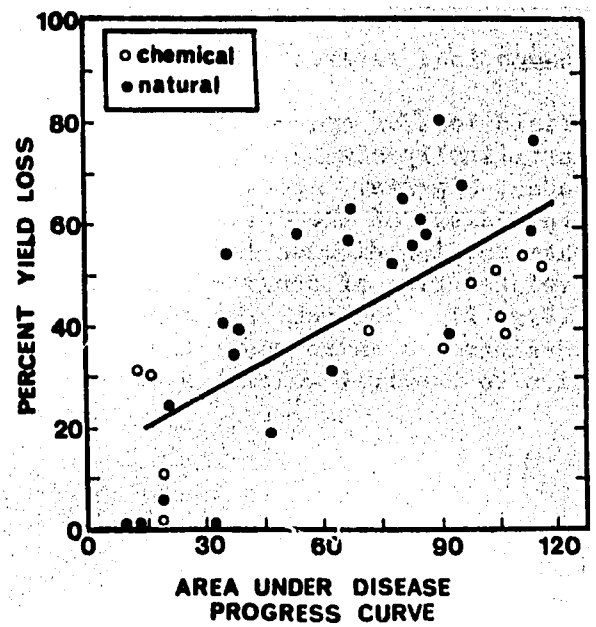

Fig. 3. Relationship of area under disease progress curves and percent yield losses for the combined data of 24 natural and 12 chemically-regulated Cercospora leaf spot epidemics of cowpea (*Vigna unguiculata*). The regression equation is Y = 0.43 X + 14.95 with a coefficient of determination of 0.702 which is significant at $P = 0.01$.

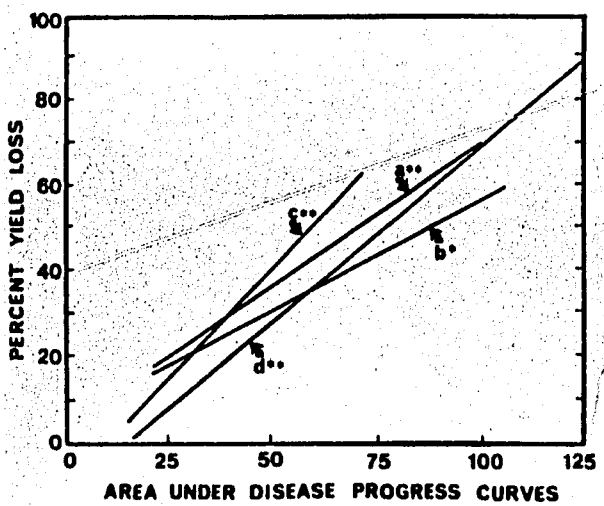

Fig. 2. Relationships of areas under disease progress curves to percent yield losses for: natural (a), chemically regulated (b), and two artifically simulated (c and d) Cercospora leaf spot epidemics of cowpea (*Vigna unguiculata*). Coefficients of determination for a, b, c, and d are 0.826, 0.684, 0.846, and 0.689, respectively, which are significant at $P = 0.01$ (**) and $P = 0.05$ (*).

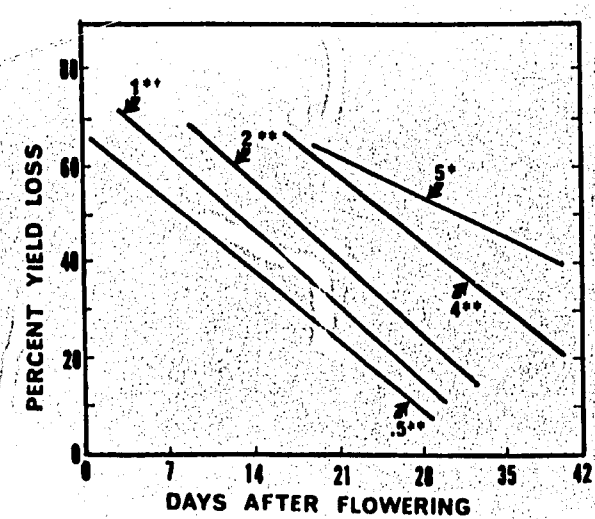

Fig. 4. Relationship of percent yield loss of cowpea (*Vigna unguiculata*) to time of occurrence (days after flowering) of Cercospora leaf spot at five disease severity indexes (DSI). Coefficients of determination for DSI's 0.5, 1, 2, 4, and 5 are −0.680, −0.746, −0.725, −0.697, and −0.485, respectively. Correlations are significant at $P = 0.01$ (**) and $P = 0.05$ (*).

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APPENDIX V

Report of Michael E. Irwin
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Within the entomology component of INTSOY, work has focused on three major areas: 1) service oriented activities, 2) virus-vector relationships, and 3) pest management systems. The virus-vector work and the service oriented activities are integral parts of the larger pest management component. In order to establish reliable pest management programs with viable options, a series of facts is needed: what are the key pests, the minor and potential pests, the vectors of important viruses, the natural enemy complexes? What are the ways that these insects can best be sampled? What are the population levels above which the yield declines? What are economically viable options for the small farmer?

I. DEVELOPMENT OF IMPROVED GENETIC MATERIALS FOR USE IN LDC SOYBEAN BREEDING PROGRAMS.

Physiology of host-plan resistance to insect pests (M. Kogan, C. Eastman): Grants supported in part by CSRS and the Illinois Soybean Program Operating Board are financing this program. The purpose is to isolate compounds in soybean plants responsible for the resistance phenomenon and then to determine the physiological reactions of the pests to these compounds.

Eight plant types used in these investigations were planted in the vegetable crop farm, Urbana, in a 3-replication, randomized block design. Leaves from each plot were harvested at three different stages of development: full vegetative growth, full bloom, and pod-set. Leaves were dipped in liquid nitrogen in the field, crushed and stored in a deep-freezer at -60°C. These materials are being used in phytochemical analyses. To date the sterol and trace metal (including Al, B, Cu, Fe, Mg, P, Si, Zn, Na, Ca, and K) analyses have been completed. Complete profiles of the major components (aminoacids, lipids, carbohydrates, etc.) will permit a detailed characterization of the resistant and susceptible soybean lines. Bioassays for attractants, feeding excitants and nutrient efficiency are being improved for use in connection with the systematic fractionation of the soybean samples.

Interaction of host-plant resistance and predation (C. Boulton, M. Kogan, P. Price): Certain partially resistant soybean lines were shown to cause an elongation of the larval stage of the Mexican bean beetle and the corn earworm. Since these lines seem to be less nutritious than other more susceptible ones, the larvae consume more foliage to complete development. This apparent paradox of a plant character with no obvious adaptive advantage can be explained if a predator is superimposed on the plant/herbivore system.

Experiments were conducted using caged soybean plants (susceptible and partially resistant lines) on which were established colonies of Mexican

bean beetle larvae. Three levels of predation were established with nymphs of the predatory bug Podisus maculiventris. Injury to the susceptible plants was lower than to resistant plants at low predation levels because larvae on the latter ate more to complete development. However, at high predation levels, injury to resistant lines was lower because the larvae were exposed to the predators for a longer period of time and were killed as third instars before most of the feeding injury occurred. These experiments may provide some interesting insights into the evolution of insect/plant interactions because the action of predators may represent a type of selection pressure favoring an otherwise undersirable plant character. From the practical standpoint this opens some interesting possibilities of integrating host-plant resistance with biological control by means of parasites and predators.

Interactions of host-plant resistance and insecticides (M. Kogan): The hypothesis that insects feeding on resistant lines may become more susceptible to lower dosages of insecticides was tested using topical applications of toxaphene and carbaryl on soybean looper and Mexican bean beetle larvae reared on susceptible and on resistant soybean plants. Preliminary tests resulted in an LD50 of .8 ug of carbaryl for the Mexican bean beetle reared on 'Bragg' (susceptible), .36 ug on PI 274.507 (very susceptible), .11 ug on PI 171.451 (resistant), and .10 ug on PI 229.358 (highly resistant). With the soybean looper results of only two tests are available and show that LD50 with toxaphene was 27.9 ug on PI 274.507 (very susceptible) as opposed to 2.58 ug on PI 171.451 (resistant); or more than a tenfold reduction in the dosage necessary to kill the same population reared on the resistant plants. If these preliminary results are confirmed, the use of partially resistant varieties could be tested in control of so-called hard to kill pests such as the soybean looper.

Screening of breeding lines (R. L. Bernard, M. Kogan in cooperation with R. Edwards - Purdue University, and T. Elden USDA-Beltsville): Progenies of the Illinois breeding program were screened for resistance against the Mexican bean beetle and other defoliators. Concurrent plantings were made in Illinois, Indiana (2 locations), and Maryland. Level of resistance and agronomic characteristics were rated in the three locations outside Illinois, and agronomic characteristics only where evaluated in Illinois. The lines that performed best in all four locations were harvested. Most of the 52 advanced lines that were selected are F6 generation progenies of the varieties 'Williams', 'Wayne', 'Amsoy 71', 'Beeson', 'Cutler 71', and 'Steele' with either PI 171.451 or 229.359 as sources of resistance. These selections will be included in preliminary yield tests in 1977.

Ten of the best selections were sent to Puerto Rico and included in a test on transmission of cowpea mosaic virus by Cerotoma ruficornis. The tests were conducted by Dr. R. Goodman and Mr. Thongmeearkom. Although C. ruficornis populations were low our entries showed some of the lowest levels of damage and also some indication of reduced virus infection.

II. MANAGEMENT AND EXPANSION OF THE KNOWLEDGE IN SOYBEAN PRODUCTION, PROTECTION AND UTILIZATION.

Soybean Insect Research Information Center (J. Kogan, B. Irwin): The Soybean Insect Research Information Center (SIRIC) is a computerized

information storage and retrieval system for the world-wide literature of arthropods associated with soybeans. SIRIC is primarily a service oriented unit, operating in close cooperation with the Soybean Entomology Research Team of the Illinois Natural History Survey and the University of Illinois.

Created in 1969 SIRIC was originally designed as a manually operated system capable of handling a few thousand citations. The attached graph shows that in the late 1960's there was a sharp increase in the number of papers published in the area of soybean entomology. The establishment of this Information Center was, therefore, a very timely decision.

In 1969 there was only a small number of entomologists working full time on soybean insect pests in the United States. In 1976 more than a dozen different centers, especially in the midwest and southeast, had entomologists working in multidisciplinary soybean research programs. The increase in the numbers of published papers is a direct result of the research being conducted in these institutions (fig. 1).

The main objectives of the center are periodically reassessed in response to changing information needs of researchers.

Basically these objectives are:

1. Compilation of the literature of soybean related arthropods and the establishment of a data bank for this literature.

2. Assistance to researchers in an international scale in the form of computer searches.

3. Assistance in special searches (usually manually done for types of information not covered by the data base).

4. Assistance to researchers in securing copies of documents in SIRIC's holdings.

5. Compilation and publication of comprehensive bibliographies on key soybean insect pests.

6. Assistance to researchers by providing literature surveys leading to the production of review articles and monographs covering areas of key importance in soybean entomology.

Basic operations of SIRIC consist of: a) updating, b) computer searches, c) special searches, and d) system upgrading.

Updating is a continuous process done by routinely searching major indexing and abstracting journals, such as Review of Applied Entomology and Bibliography of Agriculture; current awareness journals, such as Current Contents; and computerized bibliographic data bases to which SIRIC has access. Since July, 1976 SIRIC has been receiving assistance in accessing several data bases such as CAIN (National Agricultural Library Catalog), Biological Abstracts and Bioresearch Index, Chemical Abstracts, etc. through cooperators of the USDA Regional Soybean Laboratory in Urbana.

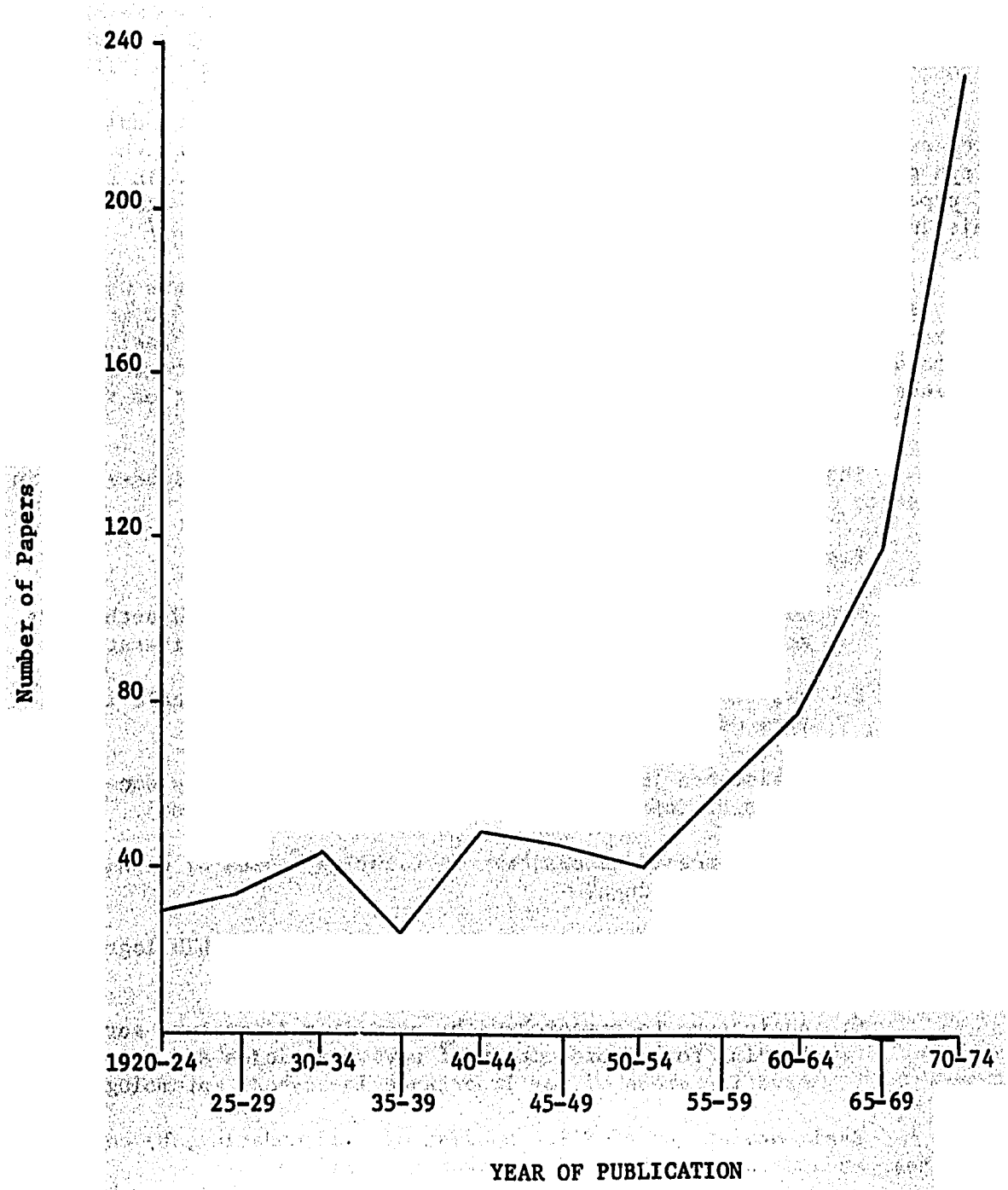


Figure 1. Publication rate of papers in soybean entomology at five-year intervals. After J. Kogan, 1977.

The relevant citations found by any one of these three means are processed for computerized storage. An unique feature of this system is the collection of reprints of all citations stored in the data base. This feature allows SIRIC to provide copies of documents in addition to the printouts of bibliographies. Duplication is limited to documents that can be legally reproduced.

Computer searches are done upon request for any researcher or student interested in information on soybean arthropod pests in the United States or abroad and are free of charge. Searches are done within certain parameters at the same time reflecting the information needs of users and the subject contents of documents in the data base.

Special searches not covered by SIRIC's data base, but necessary for the development of research projects are welcomed because they often reflect new aspects of soybean investigations in countries where the crop has been recently introduced and, therefore, unfamiliar to U.S. researchers. Insect pests associated with soybeans have been reported from other countries and this has led to a better understanding of soybean pests in general. SIRIC maintains close working relationships with a large number of researchers in institutions abroad, sending printouts of requested searches, copies of documents, and receiving in turn, copies of articles published in soybean producing countries. These articles are often published in foreign journals not easily located in the U.S. libraries making SIRIC a valuable depository of foreign soybean entomology literature.

Upgrading the system. As with any information storage and retrieval system, SIRIC is continually being reevaluated for efficiency of performance and for its objectives.

Bibliographical references are still being stored on tape/disk combinations using Fortran IV language for retrieval on the IBM 360/75, but a new version (IV) of SIRIC computer programs is being developed for the new Cyber 175 computer of the University of Illinois and should be in full operation by mid-1977.

A key component of the system is its controlled vocabulary. The Hierarchical Code Descriptors (HCD) thesaurus is used in input and output operations. The rapid growth of the literature of soybean associated arthropods makes it necessary to continually revise its taxonomic section by adding new insect species reported to be found in this association. Because this literature is becoming increasingly complex the same revision process is followed in all sections of the thesaurus.

During the time covered by this report 165 requests for service were filled in the form of computer printouts, manual compilations, copies of documents, and referral services. Requests outside the scope of SIRIC were routed to the proper institutions and specialists.

Sixty percent (98) of all requests for information or services came from researchers at the University of Illinois or their graduate students.

The remaining forty percent (67) were received from individuals in other institutions in the United States and abroad.

SIRIC performed comprehensive bibliographic searches used in the production of review articles and special reports. A comprehensive survey of the literature of Orius spp. was done in support of Dr. M. Irwin's research project. The bibliography of Diabrotica longicornis and D. virgifera was updated and it will soon be published. A bibliography on the potato leafhopper, an important pest of soybeans, was prepared through alfalfa project funds. A special literature search was done for Dr. W. H. Luckmann on the chemical control of Circulifer tenellus. The literature of sampling methods for 18 soybean insect species was searched and sent to cooperators in the United States and abroad participating in the publication of a book on sampling methods edited by Drs. M. Kogan and M. Irwin.

SIRIC Librarians were asked to help in training students and researchers in the use of reference tools and development of search strategies. In the spring of 1976 the use of SIRIC was the topic of a graduate seminar presented by students of Entomology 426 who were instructed by SIRIC staff.

SIRIC was contacted by the Empresa Brasileira de Pesquisa Agropecuaria - Departamento de Informacrao e Documentacrao (Brazil) with the purpose of establishing a formal exchange of information services. We were also asked to help in the literature search of Epinotia aporema, a serious soybean pest in Brazil and elsewhere in Latin America.

Following the four bibliographies already published on the literature of arthropods associated with soybeans, SIRIC is working on the compilation of a fifth bibliography of two Heliothis species. Heliothis zea and Heliothis virescens (the corn earworm and tobacco budworm) are major pests of soybeans, corn, cotton, and tobacco and are considered the pests of greatest economic importance in the western hemisphere. The literature of these species comprises a large number of citations and studies are under way to use the computer for self indexing the bibliography. If the self indexing system proves successful this feature will be incorporated in future computer searches.

SIRIC provided literature searches to investigators in the following countries: Brazil, Colombia, El Salvador, Upper-Volta, India, Indonesia, Israel, Philippines, Puerto Rico, Rhodesia, Saudi Arabi, and Sweden.

The information center was visited by soybean specialists and administrators from the following countries: Brazil, Canada, Iran, Israel, Mexico, Pakistan, Peru, Rhodesia, Sri Lanka, Sweden, Taiwan, Thailand, and by the participants of the 1976 training program in soybean production. A lecture on the activities of SIRIC is presented on these occasions to familiarize potential users with the services offered by the center.

Most of these visitors manifest great interest in the published bibliographies since reference services are seldom adequate in many developing countries.

The brochure What is SIRIC?, published in 1975, was widely distributed during the World Soybean Research Conference held in August, 1975 and at the INTSOY Regional Conference held in February, 1976, at Chiangmai, Thailand.

Throughout its seven years of existance SIRIC has operated with various levels of staffing. The quality and amount of service provided to researchers has been directly proportional to the man power available to the system. The needs of SIRIC are rather modest, if we exclude the work towards publication of bibliographies. Despite its small staff SIRIC has accomplished a great deal. A moderate expansion of the clerical staff would greatly increase the efficiency of the system.

International Reference Collection of Soybean-Associated Arthropods (IRCSA) (John Bouseman): In 1975-76 there were two-and-a-half FTE's involved with IRCSA; now there is one. This drastic reduction in personnel has slowed up the progress of processing material. Nevertheless, during the last year a large amount of work was completed. Consolidation of workload and reorganization of priorities followed the staff reduction. The reorganization consolidation phase is now complete.

Acquisitions of soybean-associated arthropods came mostly from El Salvador, Ecuador, Nigeria, Sri Lanka, Philippines, Mexico and Puerto Rico. A large backlog of material from Brazil has been processed, but much still remains from Brazil to process. It is felt that an adequate knowledge of soybean-associated arthropods has been gained, that the collection has represented a large number of the total species composition of soybean fields from most areas of the Americas. What is needed is an expansion of material emphasizing Africa and Asia.

Over one hundred systematists are currently collaborating with IRCSA, some 30% of which are from outside the United States. This is a 10% increase over last year's collaborators. This network of collaborating systematists is extremely important to IRCSA if IRCSA is to maintain its high standard of accuracy in determinations of specimens.

Work is nearing completion on a treatise of the Cerotoma beetles attacking soybeans in the western hemisphere. These beetles are important because as larvae they attack the nodules containing nitrogen-fixing bacteria, as adults they feed on leaves, often causing severe defoliation and also as adults, they are the prime vectors of several important viruses of soybeans. Among them bean pod mottle virus and cowpea mosaic virus are two of the worst in the western hemisphere. A clear, concise description of each species, biologies, distributions, natural enemies and other important facts will be summarized in this publication.

The complex of stinkbugs in soybeans is large and often confusing. Certain species are of economic importance as pests, others are important predators, still others apparently are inconsequential to the production of soybeans. It is important for research agronomists, extension personnel and farmers to be able to separate and categorize these species that form complexes. In order to alleviate these perplexing identification problems a manual on the stinkbugs of soybean in the western hemisphere has been initiated.

To date, literature has been searched; IRCSA houses a rich collection of stinkbugs especially from the Americas. Drawings of the various species will be initiated soon. Distribution records are already computer tabulated.

Recent requests for assistance by IRCSA follow:

Mr. Oswald Andrade Soybean Research Institute University of Maryland Princess Anne, Maryland	Determination of soybean-associate Lepidoptera from Maryland.
Dr. Andrej Bertels Empresa Brasiliera de Pesquisa Agropecuária (EMBRAPA) Pelotas, BRAZIL	Determination of stored product in from soybeans in in Brazil.
Mr. S. Cathiresapillai Department of Agricultural Biology Faculty of Agriculture University of Sri Lanka Peradeniya, SRI LANKA	Determination of soybean-associatec insects from Sri Lanka.
Ing. Sóstenes Delgado G. Centro de Investigaciones Agricolas del Noroeste Campo Agricola Experimental Ciudad Delicias, Chihuahua, MEXICO	Determination of soybean-associated insects from Chihuahua, Mexico.
Dr. C. Eastman Department of Entomology Louisiana State University Baton Rouge, Louisiana	Distributional information on soybean-association leaf beetles and a fly from Louisiana.
Dr. Y. Kobayashi Tohoku National Agriculture Experiment Station Akahira, Morioku, JAPAN	Exchange of museum specimens of Asiatic stink bugs for the same from the New World.
Dr. Charles LeSar Department of Entomology University of Illinois Urbana, Illinois	Determination of insect prey taken from soybean-associated spiders in Illinois.
Dr. Mark Mayse Department of Entomology University of Illinois Urbana, Illinois	Determination of soybean-associated insects and mites from soybeans in Illinois.
Dr. Francisco Pacheco Centro de Investigaciones Agricolas del Noroeste Ciudad Obregón, Sonora, MEXICO	Determinations and distributional information on soybean-associated insects from Sonora and Sinaloa.

Dr. Henry N. Pitre Department of Entomology Mississippi State University Mississippi State, Mississippi	Determination of dipterous parasites of soybean-associated leaf beetles from Mississippi.
Dr. José R. Quezada CENTA Santa Tecla El Salvador	Identification of soybean-associated insects from El Salvador.
Ing. Oswaldo Valarezo INIAP Boliche Agricultural Experiment Station ECUADOR	Identification of soybean-associated insects from Ecuador
Dr. Gilbert Waldbauer Department of Entomology University of Illinois Urbana, Illinois	Determinations and distributional information on soybean-associated stink bugs from Colombia.
Dr. Charles R. Ward Consortium for International Development Santa Cruz, BOLIVIA	Determination of soybean-associated insects from Bolivia.

III. SOYBEAN DISEASE CONTROL.

All soybean viruses are transmitted by insect vectors. Some viruses are aphid-transmitted, others whitefly-transmitted, still others are beetle-transmitted. The virus-vector interaction and relationship is fairly specific. One group, the leafhopper-transmitted viruses, are not yet known to attack soybean. In studying the field spread of viruses, it is crucial to understand vector behavior, biology, and population dynamics. It is also essential to understand the relationship between the virus and the vector.

Soybean viruses are particularly severe and damaging in the tropics and subtropics. Through a team approach, the INTSOY virologist and INTSOY entomologist have begun a program to understand the spread phenomenon of soybean viruses. To date studies on two such viruses have begun: soybean mosaic virus and tobacco ringspot virus.

Soybean mosaic virus (SMV) and aphid vectors (M. E. Irwin, R. M. Goodman, S. Halbert, E. A. Schaeffer, T. Shock): Soybean mosaic virus (SMV) is the most widespread of the viruses attacking soybean. It is seedborne and is transmitted in the field by certain species of aphids in a non-persistent, stylet borne manner. Reports from Ecuador, Indonesia, and Spain indicate that SMV could be a limiting constraint to soybean production in several parts of the world. Since over 30 percent and 20 percent of the lines held in the northern and southern germ plasm collections respectively; contain a significant degree of seedborne SMV it limits and interferes with breeding programs domestically and internationally. It also has potential ramifications for seed quality control and seed export.

Except for a few species mentioned under II below, aphids are not known to colonize soybeans in the western Hemisphere. There are, however, at least two species known to colonize in Asia: Aphis glycine and Aphis craccivora. In the western hemisphere SMV is spread in the field by transient alate aphids. To better understand the role aphids play in the spread of SMV, a study was begun on aphid diversity and abundance in Illinois soybeans. About 60 species of aphids were collected in soybean fields, the most abundant of which are listed below:

Acyrthosiphon pisum (Harris)
Amphorophora sp.
Anoecia querci (Fitch)
Anoecia setariae Gillette & Palmer
Aphis armoraciae (Cowen)
Aphis craccivora Koch
Aphis fabae Scopoli
Aphis gossypii Glov.
Aphis nerii (Fonsc.)
Aphis rumicis L.
Aphis spiraecola Patch
Brachycaudus helichrysi (Kaltenbach)
Capitophorus elaegni (del Guer.)
Capitophorus hippophaes (Wlk.)
Chaetosiphon fragaefolii (Ckll.)
Chaitophorus pusillus H. & F.
Dactynotus rosae
Dactynotus tarde (H. & F.)
Drepanaphis acerifoliae (Thomas)
Dysaphis tulipae (Forsc.)
Eriosoma rileyi Thomas
Hyadaphis erysimi (Kltb.)
Hyadaphis foeniculi
Iziphya flabella (Sanborn)
Myzocallis asclepiadis
Macrosiphoniella sanborni
Macrosiphum avenae (F.)
Macrisiphum euphorbiae (Thomas)
Masonaphis sp.
Myzus persicae (Sulzer)
Nearctaphis bakeri (Cowen)
Nearctaphis crataegifoliae (Fitch)
Remphigus spp.
Periphyllus sp.
Phorodon sp.
Pleotricophorus sp.
Rhopalosiphum maidis (Fitch)
Rhopalosiphum nymphaeae (L.)
Rhopalosiphum padi (L.)
Rhopalosiphum rufiabdominalis (Sasaki)
Schizaphis graminum (Rondani)
Therioaphis maculata (T.)

Of these species, the following are known vectors of SMV, either from literature accounts or from our field experiments; those discovered to be SMV vectors for the first time are preceeded by an asterisk*:

Acyrthosiphon pisum
Aphis craccivora
Aphis fabae
Aphis gossypii
*Aphis spiraecola
*Capitophorus elaegni (questionable)
Macrosiphum euphorbiae
Myzus persicae
Rhopalosiphum maidis
*Rhopalosiphum padi
Rhopalosiphum rufiabdominalis

Seasonal flight activity and abundance differed greatly between the 1975 and 1976 growing season (fig. 2). Flight activity occurred late in the 1975 growing season with one peak in late July-early August and a second peak in September. In the 1976 growing season flight activity occurred early with the major peak occurring in June and early July. This fluctuation in flight activity timing could have profound effects on SMV spread and on SMV invasion into developing seeds of infected soybeans. If SMV infection occurs prior to or during early bloom, virus can move into and infect developing seeds resulting in relatively high rates of seed transmission. If infections occurs after early bloom the rate of seed transmission is low presumably because virus particles are blocked from entering developing seeds. Therefore, late season flight activity is not as detrimental to seed production as is early flight activity.

The pattern of spread is directly related to wind direction as is demonstrated from our 1976 experiment (fig. 3). Aphid flight activity is affected by wind speed and direction. Aphids tend to fly downwind, as would be expected from the resultant SMV spread pattern.

Although we do not yet know how far an aphid can carry viable virus, the SMV spread pattern obtained this past growing season suggests that it can be carried at least 150 feet.

There was no observable secondary spread in the field by aphids; all spread was from a primary inoculum source. There are two sources of evidence for this: field spread was not clumped, but occurred in a radiating series of concentric circles, biased by wind direction, and decreasing in intensity as the distance to the primary inoculum source. The second source of evidence came from field experiments in which aphids were collected upwind and downwind from an inoculum source and then tested for transmission ability on healthy soybean plants. Of the downwind aphids, 7% carried SMV; of the upwind aphids, none carried SMV.

Laboratory experiments are currently underway to determine transmission efficiency of the various aphid species identified as potential vectors. We are using Myzus persicae apterae as a standard upon which to base other species. We are using alatae of other species for the most part in these laboratory tests since it is alatae which are important in the field spread of SMV. Preliminary results indicate that Myzus persicae apterae tend to transmit better if they feed on a source plant for about 30 seconds. Longer or shorter acquisition probes are less effective. Also, in preliminary retention tests, the species looses its ability to transmit if isolated for more than 30 minutes after acquisition probes. These laboratory tests are giving us first approximations of what might occur in the field.

Identification of field vectors of SMV (S. Halbert): SMV has proven to be a problem in certain developing countries such as Ecuador, particularly with respect to seed production. A means of identifying important vectors is essential. Although many potential vectors have been identified by means of transmission experiments conducted under laboratory conditions (see above), the importance of each of these in the field has not been assessed.

Living aphids were trapped upwind and downwind of a 92' x 92' field of mechanically inoculated soybeans. Collecting was done twice per week for 1-2 hours on nets 15 ft. long and 4 ft. high. Each aphid was allowed to probe a healthy seedling, and was then recaptured and preserved for identification. The plants were appropriately labelled and grown in an insect-free greenhouse to determine those that became infected. Positive identification of infected plants was obtained by infectivity indexing. In order to identify the large number of field collected aphids Ms. Halbert spent one week with Dr. Manya Stoetzel in the USDA/ARS Systematic Entomology Laboratory, Beltsville, Maryland.

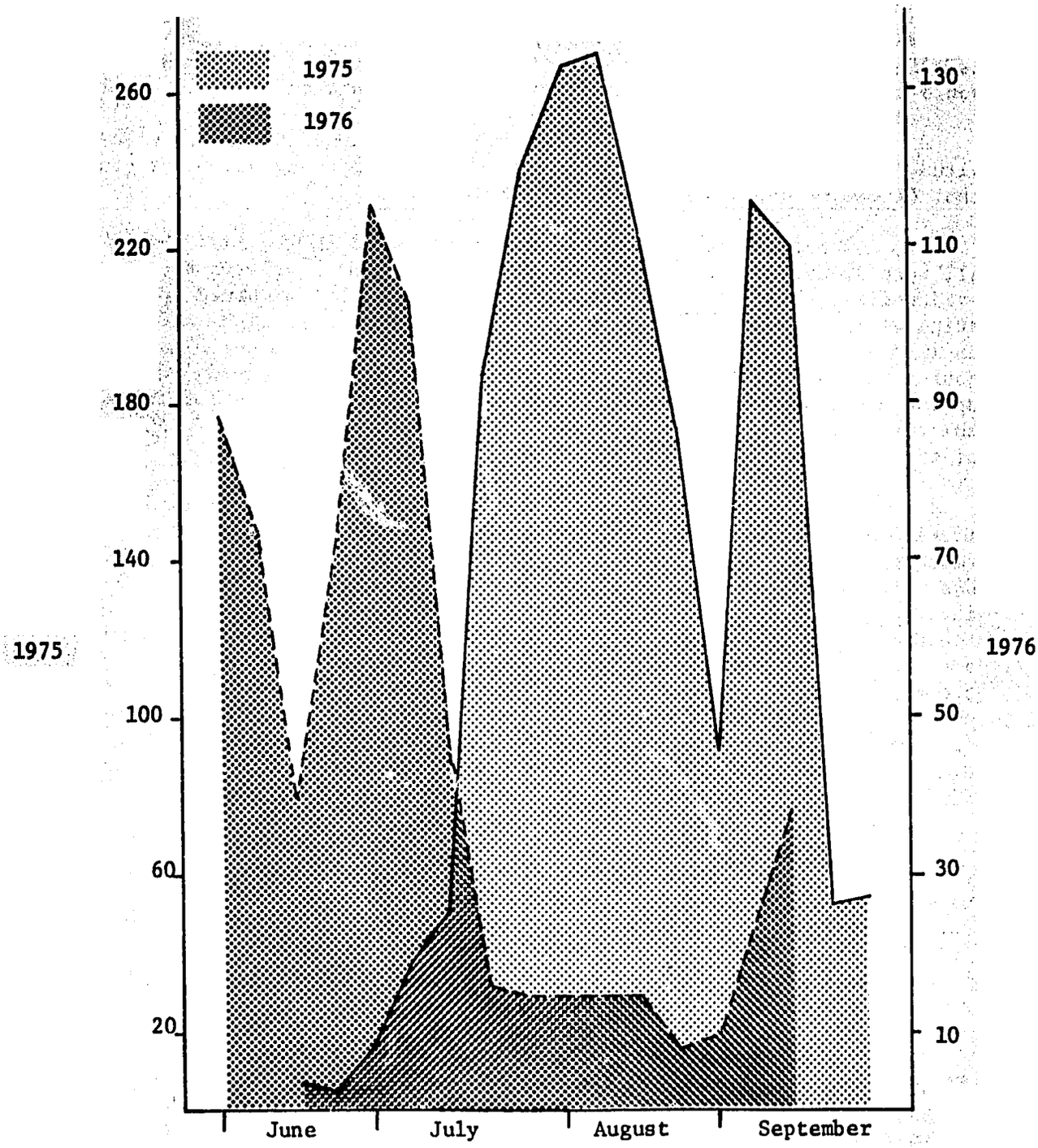


Figure 2. Flight phenology and relative abundance of SMV-transmitting aphids, Tolono, 1975 and 1976 seasons.

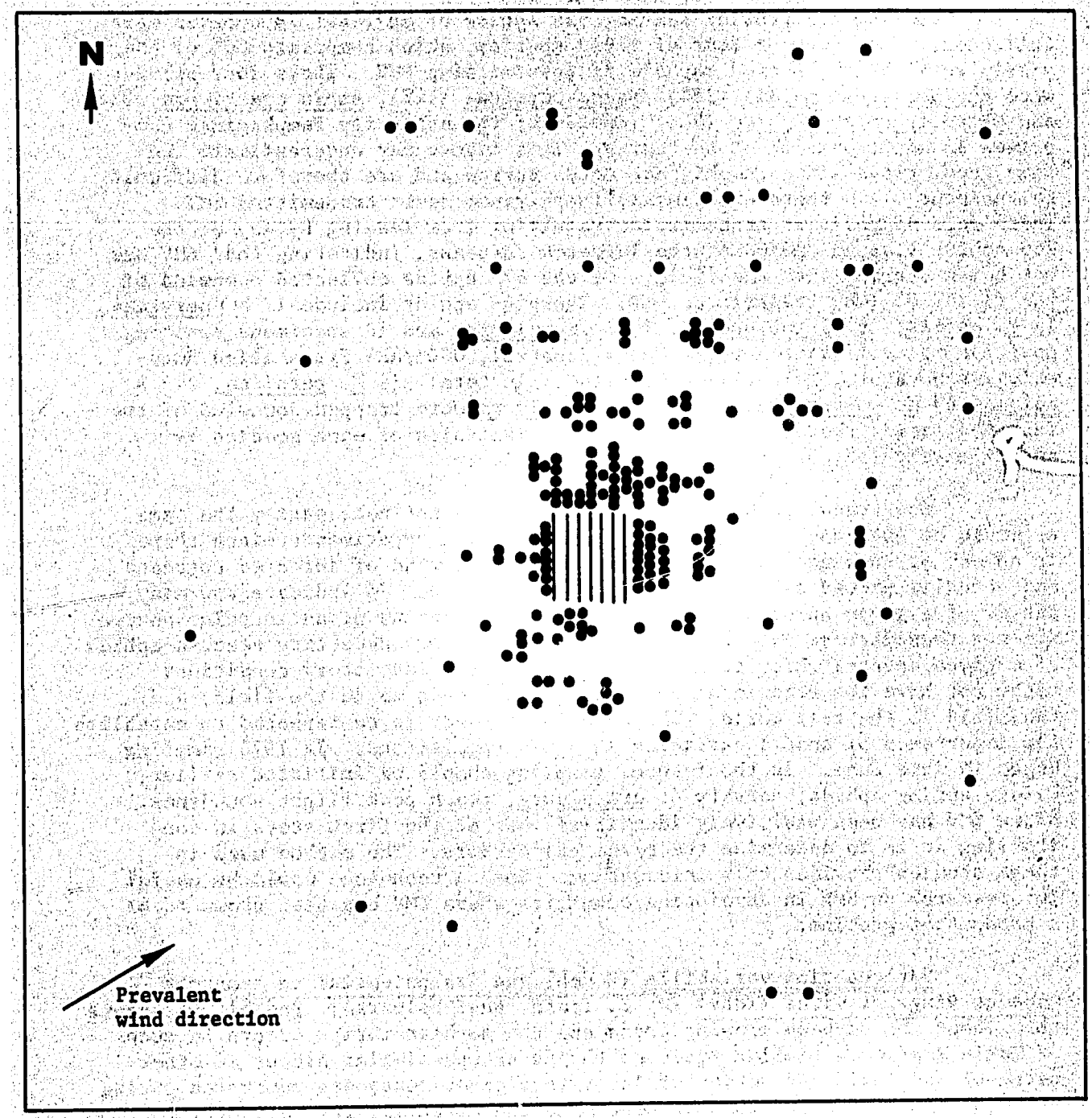


Figure 3. Pattern of spread of SMV from primary inoculum source (solid lines in center). Field 300' x 300' Williams soybeans, Tolono, Illinois, 1976.

During the growing season, 939 aphids of at least 28 species were collected. Specimens of four of these species, which comprised 60% of the yearly total catch, proved capable of transmitting SMV. These four species were Rhopalosiphum maidis (28%), Myzus persicae (14%), Aphis craccivora (9%), and Macrosiphum euphorbiae (9%). Aphids of the subfamily Pemphiginae comprised an additional 6% of the catch. This figure may underestimate the time populations since Pemphiginae often burrow and are therefore difficult to recapture. In these experiments Pemphiginae never transmitted SMV. There were no positive transmission resulting from feeding by any of the 266 aphids trapped upwind of the infected soybeans, indicating that SMV was not being carried into the field. Of the 673 aphids collected downwind of the field, 7% (48) transmitted SMV. These 48 aphids include 17 M. persicae, 13 R. maidis, 16 M. euphorbiae, 2 A. craccivora, and 10 specimens awaiting positive identification by Dr. Manya Stoetzel, USDA/ARS Systematics Entomology Laboratory, Beltsville, Maryland. In total 115 M. persicae, 219 R. maidis, 63 M. euphorbiae, and 40 A. craccivora were trapped downwind of the infected field; therefore, the rate of transmission of each species is approximately 15%, 6%, 10%, and 5%, respectively.

The transmission rates given above are not necessarily the same as would be obtained from laboratory transmission experiments since there is no way of knowing that an aphid collected downwind of infected soybeans had actually probed an infected plant; however, they do indicate how many aphids of a given species at a given location downwind of an inoculum source are not transmitters. Both types of experiments are necessary because aphids of a given species which transmit regularly under laboratory conditions might not have the behavioral characteristics to do so in the field; and the field is the real world. The experiment needs to be repeated to establish the importance of annual variation in aphid populations. In 1976 sampling began in late June. In the future, sampling should be initiated earlier, before spring aphids, notably A. craccivora, reach peak flight abundance. After SMV has been positively identified, one of the first steps in controlling it is to determine the principal vectors. The method used in these studies provides this information. Such a technique would be useful for research on SMV in developing countries where SMV has been shown to be a production problem.

Sericothrips variabilis (Beach) and its potential to transmit tobacco ringspot virus (TRSV) (M. E. Irwin, Suzi Halbert): Bud blight occurs throughout the soybean growing areas and the soybean thrips occurs in much of Latin America.. Similar types of thrips occupy similar niches in other parts of the world. A series of laboratory experiments was conducted during the winters of 1975 and 1976 to determine the potential for Sericothrips variabilis, the soybean thrips, to transmit TRSV, the causal organism of bud blight. During this period of time about 320 adult transfers and 90 pupal transfers were performed. Adult transfers consisted of allowing thrips to develop on a soybean plant that was infected with TRSV. The adults, having fed for some time on the plant, were then transferred to healthy soybean plants. Pupal transfers were slightly different. Again, the thrips were allowed to develop on infected plants. Just prior to pupating, this species of thrips drops to the soil and therein pupates. The thrips were transferred to healthy soybeans during the pupal stage. In no cases were there positive reactions when the plants were tested for the presence of

TRSV. One further test is currently underway. To a large cage containing a single TRSV infected plant and several healthy plants were added several hundred adult thrips. These are being allowed to develop and multiply in the cage. At the end of a month all of the plants in the cage will be tested for the presence of TRSV. If none have the virus, other than the source plant, then I feel we will be safe in assuming that this species is incapable of tansmitting TRSV.

IV. DEVELOPMENT OF PEST MANAGEMENT SYSTEMS.

Bionomics and spatial distribution of soybean-associated thrips (M. E. Irwin, K. Yeargan (University of Kentucky) L. J. Stannard, E. A. Schaeffer): Thrips represent a group of insects for which very little biological information is available. Yet, in any given soybean ecosystem thrips are more abundant than any other single group of insects. Thrips can cause damage to soybean plants and some species are vectors of certain soybean virus (see above). Thus a program was initiated to attempt to understand the importance of thrips in a soybean ecosystem and how best to sample thrips populations.

In order to perfect a reliable sampling method, experiments were carried out in Urbana and Lexington to determine the spatial and temperal distribution of thrips in soybean. Three main species of thrips occur in soybean: Sericothrips variabilis (Beach), Frankliniella tritici (Fitch), and F. fusca (Hinds). A fourth, Thrips tabaci Lind. was present in small numbers. Results of experiments indicated that no significant differences occurred between plants or between plots sampled. However, highly significant differences occurred in the vertical distribution of the species (fig. 4). For F. tritici, the most preferred parts were the flowers and growing terminals. This species reached its peak abundance during the blooming period of soybeans and disappeared from soybean fields as flowering ceased. Adult flight activity continued throughout the major part of the growing season, however. Frankliniella fusca was found on soybeans in relatively low numbers, and only adults occurred on soybean. This species occurred on soybean in the early growing season and could be found indiscriminately on terminals and leaves. Sericothrips variabilis reached its peak abundance during early pod set, after F. tritici had disappeared from samples. This was true of the adult flight activity as well (fig. 5). Both F. tritici and S. varibilis were more abundant within a large (300 x 600') soybean field than in areas immediately next to the field; the reverse was true for F. fusca (fig. 6). This leads me to believe that F. fusca is incidental in soybean fields and does not colonize.

From our study, it seems reasonable to attempt to sample Sericothrips variabilis populations by concentrating on the first instar larva, and by concentrating the sampling method to center leaflets of the fifth or sixth trifoliate from the top of the plant. Variation between samples was fairly low, and five such center leaflets from scattered plants in the field would give a good index of population density.

In order to develop sampling methods for Sericothrips variabilis, several parameters had to be investigated. In our method the center leaflet of each trifoliate was sampled rather than the whole trifoliate. An

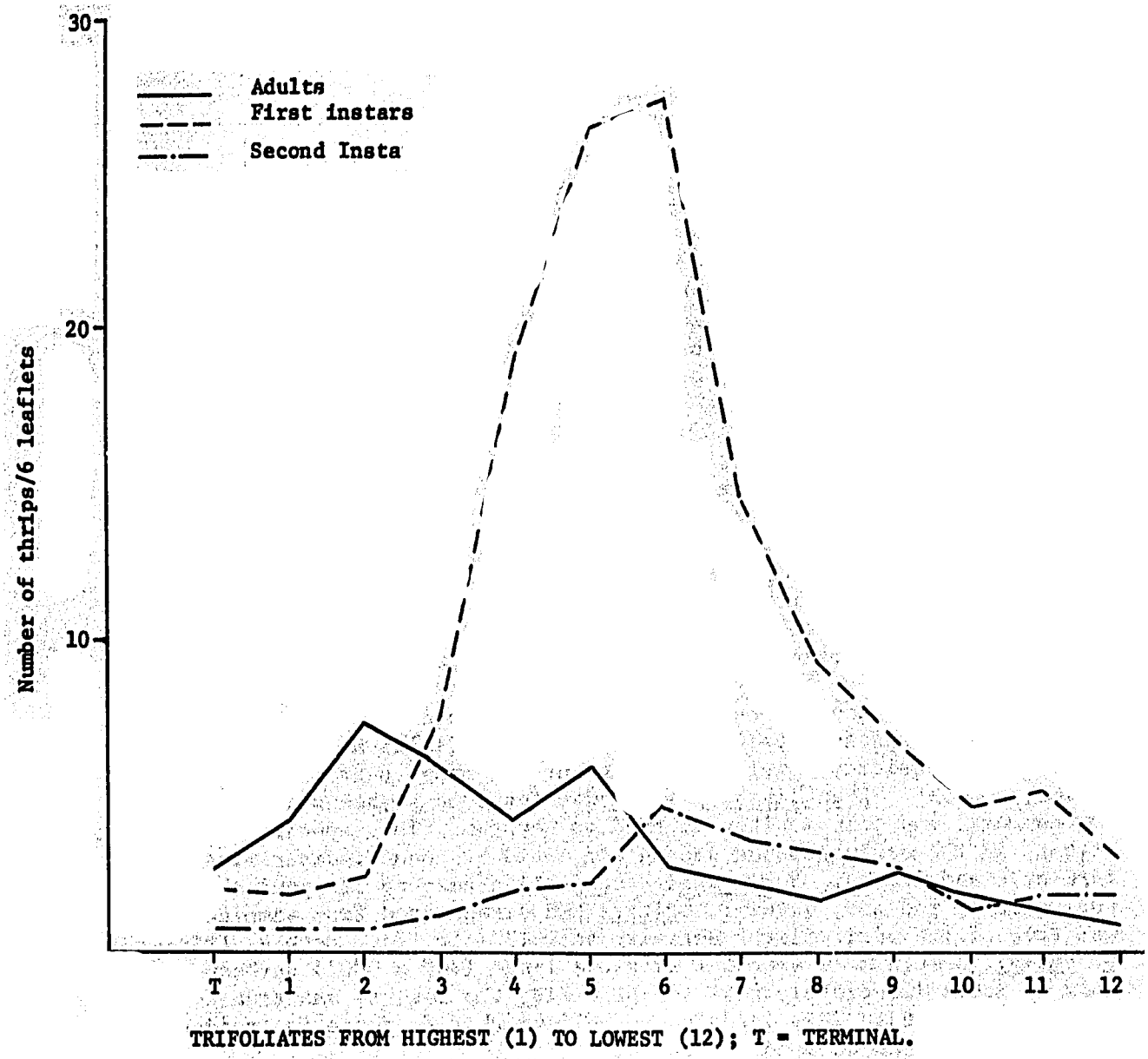


Figure 4. Vertical distribution of Sericothrips variabilis in Williams soybean, pod-fill stage, August,17, 1976, Urbana, Illinois.

experiment was conducted to determine whether the center leaflet differed significantly from the lateral leaflet in the number of specimens. Results showed that even though the center leaflet was significantly larger than each lateral leaflet, there was no significant difference between the two with respect to thrips abundance. This might best be explained by the fact that female thrips oviposit in the trifoliate before it is expanded. The area available for oviposition on the center and lateral leaflets probably is very similar prior to expanding.

Two different methods for counting the specimens were used. One method employed direct counting under the microscope of individual leaflets. The other used a washing technique when several leaflets were pooled. An experiment was conducted which showed that there was no significant differences between the two sampling methods for comparable samples.

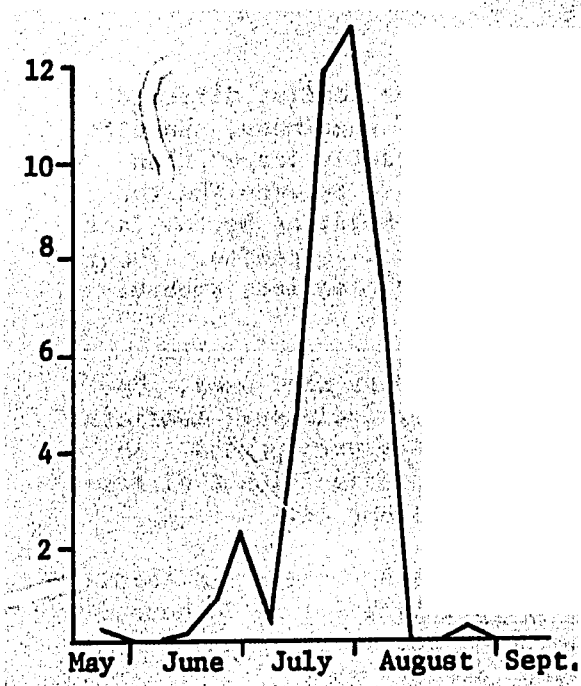
A third experiment was performed to test whether there was significant movement of various instars of Sericothrips variabilis in 24 hours. We expected no movement of first or second instars, but we thought adults might move. The experiment was performed twice, a month apart, and the results are identical: there is significant movement of first and second instars, but not of adults (fig. 7). It is hoped that further testing next summer will reveal the reason for this currently puzzling result.

Aphid species colonizing soybeans in the Americas (M. E. Irwin, E. A. Schaeffer, S. Halbert, M. Stoetzel (USDA/ARS/SEL, Beltsville, MD)): Aphids are important as pests of soybean because they transmit several important viruses. However, in the western hemisphere they are not known to colonize (i.e. produce progeny) on soybeans. There are a few species in Asia and Africa which do colonize soybeans, and there, they cause considerable damage to the plant stand.

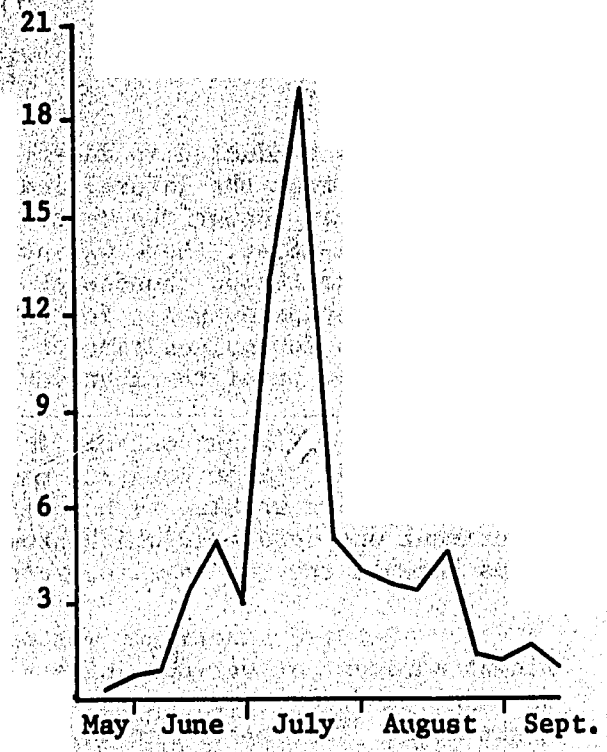
During the past year I have noticed that there indeed are a few species in the western hemisphere that are capable of colonizing soybeans. One species was found throughout the soybean-growing area of Ecuador. That species has not yet been identified. At Urbana two additional species have been observed, one Aphis gossypii, on soybeans in the greenhouse, and a second, Aphis craccivora, on young soybean seedlings in the field (fig. 8). We have subsequently been able to rear species on detached soybean cotyledons in petri dishes in the laboratory. When either species was placed on healthy soybean seedlings, it would multiply initially, especially on the cotyledons, but would soon die as the plants developed. Malformation of the soybean plant was observed where several aphid specimens were allowed to feed on young seedlings in the laboratory. Among the various malformations, the most serious was a killing of the growing point. This caused very bushy growth patterns. In a few cases, the entire plant was killed. In all cases leaves were severely malformed and yellowed.

Recently, we discovered that Myzus persicae can also develop on soybean cotyledons.

Spider mites (M. Kogan, C. Helm): Spider mites are very sensitive to nutritional factors. In diets differing in nitrogen levels mites grow and reproduce at different rates. Reports are somewhat conflicting in regard to the optimal N levels. We have hypothesized that the increase in



THRIPS TABACI



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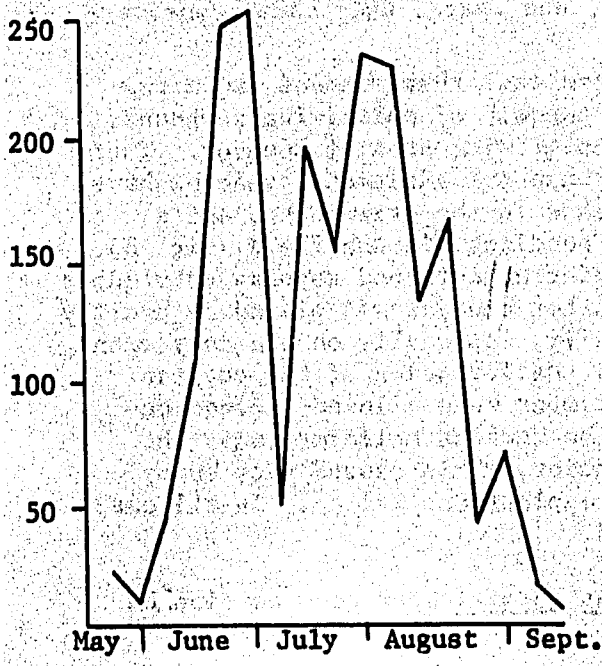

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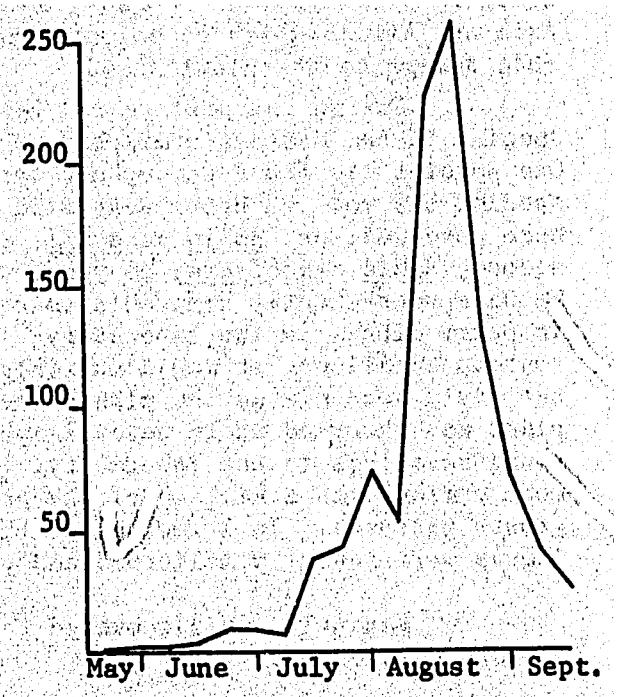

SERICOTHRIPS VARIABILIS

Figure 5. Seasonal distribution of adults of four species of thrips at weekly intervals in a soybean field, Tolono, Illinois. Mean weekly catch on ten 6" x 6" sticky traps during the 1976 season.

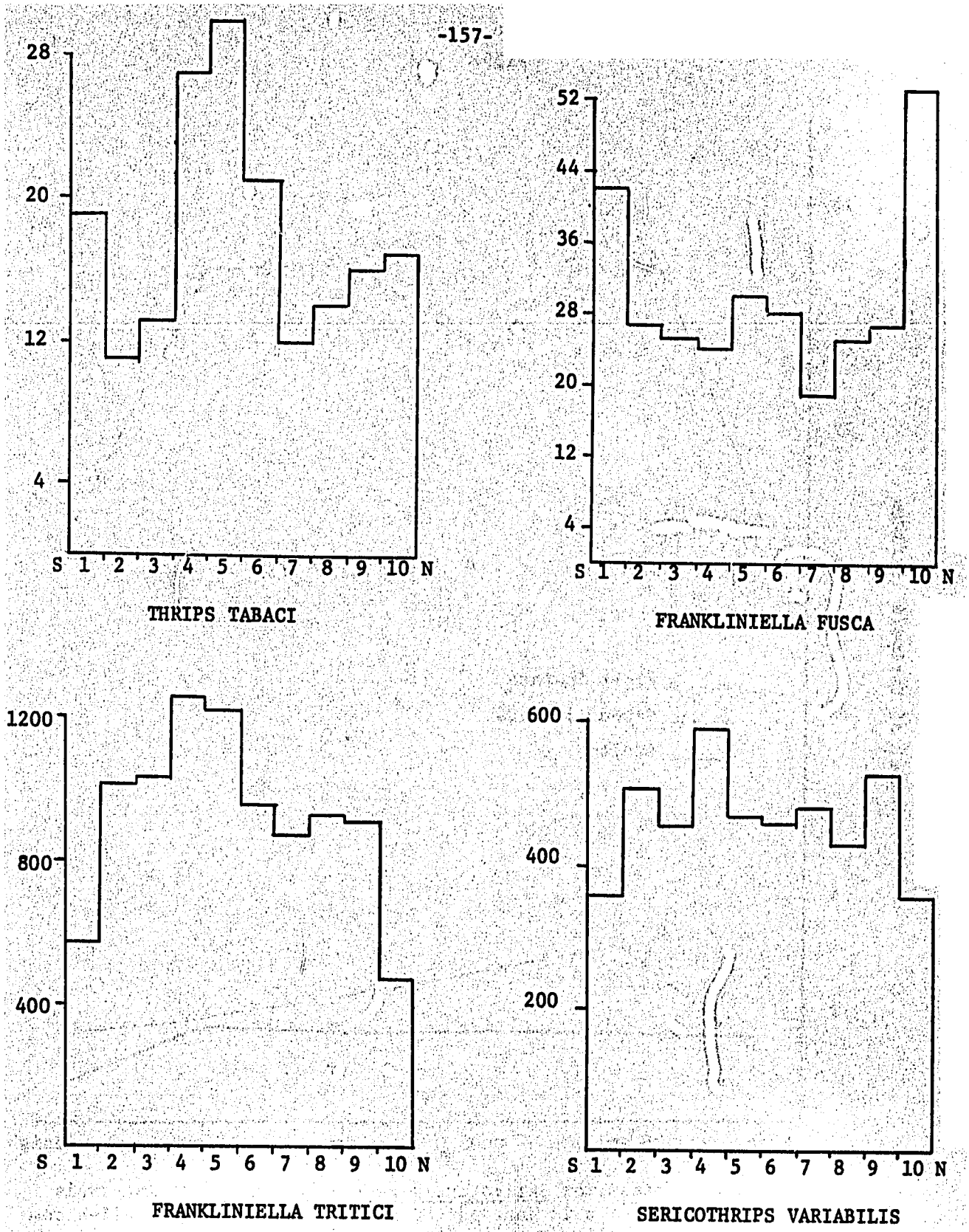

Figure 6. Distribution of four species of thrips in a south (S) to north (N) transect of a soybean field, Urbana, Illinois. Total specimens captured during the 1976 season. Bars represent 6" x 6" sticky traps, 70' apart in field. Traps 1 and 10 outside field.

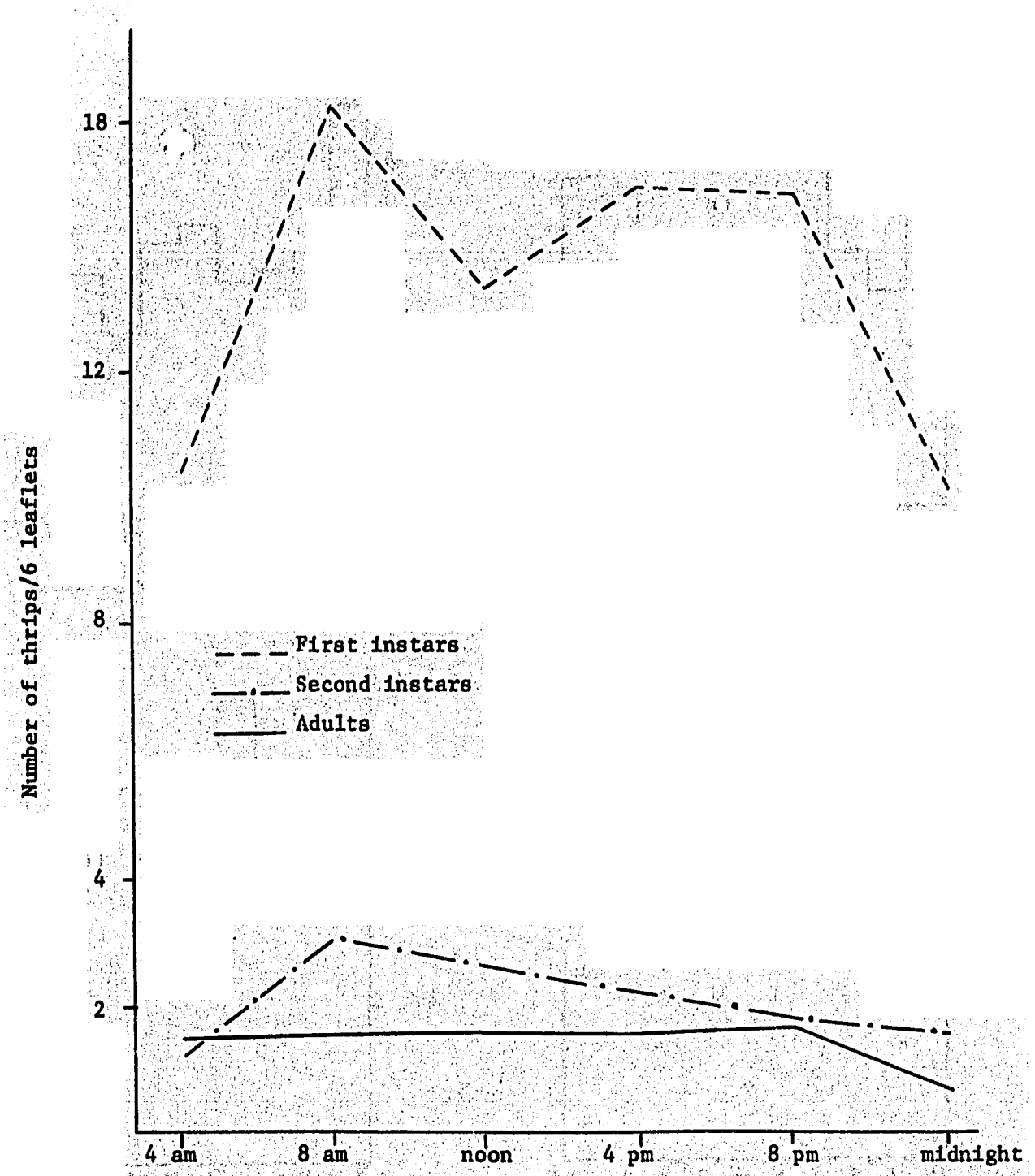

Figure 7. Totals for Sericothrips variabilis stadia at intervals of four hours for a 24 hour period in Williams soybeans, full-bloom stage, July 27, 1976, Urbana, Illinois.

Figure 8. Aphis craccivora colonizing young Williams soybean in the field in July, 1976. Urbana, Illinois.

mite populations on soybean may result from a more favorable nutritional environment in plants under water stress. Sugars and free amino acids in leaves increase under conditions of water deficiency, both conditions being highly favorable for mite development. The effect of soybean nutrition and water balance on mite development is being investigated in our laboratory, in cooperation with Dr. J. G. Rodriguez, University of Kentucky, Lexington. The objective of this investigation is to test the hypothesis that spider mite outbreaks result from nutritional effects via metabolic changes in soybean plants under conditions of water deficiency.

Non-nodulating 'Clark' soybeans were planted in a hydroponic system. Three nutrient solutions were used differing in the level of nitrate supplied. These solutions contained .75, 7.5, and 15.0 mMol of NO_3. Plants in the .75 mMol regime were obviously chlorotic. After plants became well established in the system about 60 mites were placed on the upper 3 leaves. Colonies became well established after a few weeks and plants are being sampled using a paper print technique. This technique is based on laying a leaflet on a sheet of white filter paper and rolling a cylinder over the leaf. Mites on the leaf surface against the paper are crushed leaving a permanent and identifiable mark or print on the paper.

The number of mites on the leaves from plants in the various nutritional regimes is being counted. Preliminary observations suggest that higher populations are developing on the 15.0 mMol plants than on either the 7.5 or the .75 mMol plants. If this trend holds this experiment will lend some support to our hypothesis although we still need to determine the relationship between N-levels and water stress.

The sampling technique used in this experiment will be adapted for field sampling because it is much easier to visually count mite populations on the paper "print" than on the leaves.

Potato leafhopper (M. Kogan and C. Helm): The potato leafhopper, *Empoasca fabae* migrates from the south every year and becomes established in soybean fields in rather large numbers. The species is consistently present in soybean fields but its impact on growth and production is not clear. Studies conducted in Iowa resulted in rather low economic thresholds. We completed a two year study on the economic thresholds of the potato leafhopper using near isogenic lines of 'Clark' soybeans differing in the characterisitcs of their pubescence. This or closely related species are abundant on soybeans around the world.

In 1976 different blocks of plants were selectively sprayed to produce various levels of leafhopper populations during three different stages of plant growth. A control block was sprayed weekly throughout the entire season.

Preliminary analysis of the data has shown a strong effect of pubescence type on yield ($P<.001$). However, the single effect of treatments (suspended sprays) and the interactions of treatments and pubescence were not statistically significant. Although additional analyses of correlation are being performed there is an indication that infestation at all stages of development had similar effects on yield at the population levels that were present.

Bean leaf beetle (M. Kogan, G. P. Waldbauer, C. Helm, LeeAnn Turner, and T. Anderson): Much of our work concentrated on improving sampling procedures for larvae and adults.

Larval extraction from soil samples was made using a simple flotation method adapted from the egg separation procedure previously developed in our laboratory. The method uses a separatory funnel with air bubbling in a saturated aqueous solution of NaCl. Soil samples containing known numbers of larvae yielded over 90 percent recovery of third instars. Experiments were also conducted to determine how long soil samples could be stored without loosing precision of recovery. It was observed that soil samples can be stored in a freezer at about -10°C, without significant loss in recovery.

A study in adult population distribution patterns was conducted using the beat-cloth method. This sampling method was compared with the sweep-net and to an absolute method based on caging and harvesting all plants in a 1.8 x 1.8 m area. Samples were taken under two field situations--lodged plants, and erect plants. In both cases the beat cloth yielded the best estimates of beetle populations and the sweep-net was particularly inefficient with lodged plants.

Studies are being conducted on the rates of growth and development of bean leaf beetle larvae reared at various temperature regimes. The effect of various food plants on adult survival and fecundity are also being measured. These data will help improve preliminary models for the bean leaf beetle population dynamics.

Results of a pilot pest management program in southern Brazil (M. Kogan, S. G. Turnipseed (Clemson Univ., SC), M. Shepard (Clemson Univ., SC)): During the 1974-75 soybean growing season a pilot pest management program was tested in Southern Brazil. Nine fields, varying in size from 10 to 30 hectares were selected in commercial farms. Paired fields within each farm were used in the experiment. One field of the pair was managed following a simple pest management strategy and the other field was managed following the farmers own criteria. The strategy consisted of scouting fields once a week to monitor populations of defoliating lepidopterous caterpillars and pod sucking stink bugs, which were the target insect pests. Damage thresholds and treatment decisions were based on assessment of defoliation levels, pest populations, and presence of pathogens, mainly the fungus *Nomuraea rileyi* (Farlow) Samson. Minimal dosages of non-persistent pesticides were prescribed in fields reaching damage thresholds.

Although treatment decisions by farmers were probably influenced by the pest management program they made a total of 9 treatments (average one per field) as opposed to only 2 treatments made in the pest management fields, a reduction of 78 percent in the number of insecticide applications in the experimental program.

The program was repeated in 1976 and was officially recommended by the state of Paraná (2nd largest producer in Brazil). In 1977 a similar program was adopted on a national scale. The program allowed us to test under normal field conditions some parameters of soybean pest management

systems developed in the United States of America. It demonstrated that simple scouting procedures and adequate economic damage thresholds can be used in preliminary pest management strategies even if phenology of key pests is incompletely known.

Soybean production in Brazil underwent explosive growth in the past 6 years. Production increased from about 1.5 million metric tons in 1970 to nearly 10 million tons in 1975. Area planted to soybean expanded from 1.3 million ha in 1970 to 5.7 million ha in 1975.

Many insect problems in Brazilian soybean are similar to those encountered in the soybean producing areas of Southern U.S. The main defoliating species are the velvetbean caterpillar, Anticarsia gemmatalis Hübner, and several Plusiinae, with the predominance of Pseudoplusia includens Walker, the soybean looper. The main pod-feeding pests are the southern green stinkbug, Nezara viridula (L.) and Piezodorus guildinii (Westwood).

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APPENDIX VI

Report of Errol D. Rodda
Professor of Agricultural Engineering[1]

Favorable results from varietal and agronomic trials at many locations around the world have resulted in wider production of soybeans in tropical and subtropical areas. The maintenance of soybean seed quality during storage in these hot and humid environments has been recognized as a limiting factor in soybean production. High quality seed is required to obtain adequate stands for profitable soybean production. Soybean seed must often be imported in the initial stages of production development in a new area. As local seed production becomes possible, a storage technology suitable for the environment and scale of operations must be available.

The primary objective of this project was to study practical and inexpensive methods for storing seed under tropical conditions. The initial phase of the study was to evaluate tropical storage properties of certified seed produced in the continental U.S. This work was done to establish a basis for subsequent storage studies with seed produced in the tropics under different sets of production conditions. In addition, published data on the storage properties of U.S. certified seed under ambient tropical conditions appeared to be unavailable.

Soybean seed does not store well as compared to other legumes. The major environmental factors influencing seed deterioration are temperature and moisture (1, 2, 4). Moisture is usually considered in terms of seed moisture content. Since seeds lose or gain moisture from the surrounding air until reaching equilibrium at a given set of conditions, it would also be appropriate to think of moisture in terms of the relative humidity of the storage environment. Storage fungi are a major cause of reduced germination in soybeans, especially when soybean seed is stored at moisture contents above 12.5 percent (3). The results presented in this paper are for storage under ambient tropical conditions of Woodworth certified seed from Illinois.

MATERIALS AND METHODS

Experimental Variables

The experimental variables investigated were; two storage locations: L_1 - Mayaguez Campus, L_2 - Isabela Substation; two storage conditions: S_1 - shelf-stored in ambient conditions, S_2 - stored in cardboard carton with about six-inches of rice hull for insulation; three initial moisture levels, dry basis: M_1 - 12.5%, M_2 - 10.7%, M_3 - 8.6%; four

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1 With cooperation of Eliodoro J. Ravalo, Assistant Professor of Agricultural Engineering, University of Puerto Rico, Mayaguez Campus.

containers: C_1 - sealed metal can[2], C_2 - metal can with one mil plastic lining, C_3 - fertilizer jute bag with one mil plastic lining, C_4 - cotton cloth bag; three storage periods: T_1 - three months, T_2 - six months, T_3 - nine months. Sealed samples in can were also stored in a controlled environment of 3° C and 25° C at Urbana, Illinois. The samples were placed in storage on February 26, 1975 with three replications. Germination tests, moisture content determinations, and pathological studies were conducted at the start and the end of the storage period. Results of the pathological study and the controlled environment tests will be published in separate papers reporting further work.

Preparation of Samples for Storage Tests

Four hundred thirty two 200-gram samples were prepared using certified Woodworth seed from Illinois. The initial moisture content of the seed was about 10-11 percent, dry basis. The seeds were reconditioned and were allowed to absorb moisture from the surrounding air. This was done by spreading the seed in a one-inch layer in a cold storage room for several weeks until the moisture content reached 13 to 14 percent. The seeds were then dried to the three desired moisture levels with air heated to about 33° C. The drying was done by placing all the seeds in the laboratory dryer, withdrawing about one-third of the seeds when the appropriate moisture level was reached. The dried seeds were then sealed in double plastic bags. At the end of the drying period the seed lots of each moisture content were divided into three approximately equal parts. The seeds were then subdivided into 200-gram samples and placed in the different containers.

Preparation of Samples for Germination Tests

The seed samples placed in storage were a mixture of mechanically broken and whole seeds. The percentage of broken seed was 1 to 2 percent. Mechanically damaged seeds and seeds damaged by insects were separated from the whole seed by hand. Samples for germination tests were taken at random from the whole seeds. Four hundred seeds were used for germination tests for each treatment. The seeds were further subdivided into 8 groups of 50 seeds each.

Preparation of Sandbed for Germination Tests

Sandbed germination tests were used for determining the germination percentage of the seed at the end of the storage period. The sandbeds were fumigated again at least one week prior to seeding.

RESULTS AND DISCUSSION

The germination test results are presented in Table 1. Statistical analysis indicates that all the storage variables studied except

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[2] A special acknowledgement is due Mr. Larry Gold, Manager of Star Can, Star-Kist Caribe, Inc. for supplying and sealing the cans used in this project.

storage location had highly significant effects on the germination of U.S. certified Woodworth soybean seed stored in the tropics.

The apparent effect of the storage container on germination after three months storage is clearly evident in Figure 1. The germination decreased very rapidly after three months storage for C_4, with germination dropping from about 92 percent to around 22 percent for six months and to 0 percent for nine months. The rapid deterioration of germination for C_3 occurred after six months storage, decreasing from about 81 percent to 14 percent after nine months of storage. For C_2, the significant drop in germination also appeared after six months, with germination decreasing from 92 percent to around 72 percent. The sealed metal can, C_1, exhibited the least reduction in seed viability with germination dropping from 94 percent to 89 percent after nine months storage. It appears that containers which minimize moisture content change during storage (Table 1 and Figure 2) maintain seed viability at a desirable level for up to nine months. The commercial fertilizer jute bag with a plastic lining is relatively air tight. However, in this experiment the bag was cut into 12 pieces and then sewed. This greatly reduced its effectiveness as a moisture barrier. Figure 2 shows the change of moisture content during storage for the four containers with soybean seed at an initial moisture content of about 8.6 percent, dry basis. The containers which maintain the initial seed moisture content (minimize moisture exchange between the seed and atmosphere) tend to maintain relatively high germination. This is especially evident for the six and nine months storage periods. These results suggest that the ability of the container to minimize moisture change during storage is as important as a low initial seed moisture content in preserving seed viability in the tropics where wide daily fluctuations of relative humidity exist. On a typical day the relative humidity may reach a low of around 60 percent in the afternoon and a high of close to 100 percent in the early morning.

The influence of the initial seed moisture content on germination with respect to containers and storage periods is shown in Figure 3. The significant effect of the initial seed moisture content on germination with respect to storage period was strikingly evident after six months storage for C_3 and C_4 and after nine months storage for C_1 and C_2. The difference in germination between the seed with the lowest and highest initial moisture content for C_1 was negligible for the first six months and reached a value of 12 percent after nine months storage. For C_2 the difference was about 3 percent and 36 percent for six and nine months storage, respectively; for C_3, about 14 percent and 29 percent, and for C_4, about 5 percent and 0 percent. The seed with a low initial moisture content germinated significantly better than the high initial moisture content seed after nine months storage for all the containers except C_4. For C_4, the germination was practically zero after nine months storage at all three initial seed moisture contents. The results of this study under ambient conditions are similar to those of earlier studies under constant conditions which showed that low moisture content is a key factor for maintaining seed viability in storage.

Figure 3 also shows the effect of storage time with respect to containers and initial seed moisture content. Statistical analysis confirmed the obviously significant effect of storage period on germination

for various containers and initial moisture levels. Germination remained about 90 percent for all the containers for three months storage. With longer storage periods, divergence of seed viability between containers is clearly evident, with C_1 and C_2 maintaining germination close to 90 percent, C_3 around 80 percent, and C_4 slightly above 50 percent for the six months storage. For nine months storage, only C_1 was able to maintain germination close to 90 percent, with C_2 slightly above 70 percent. C_3 and C_4 results were unacceptably low at about 15 percent and practically 0 percent germination, respectively.

The influence of storage condition on germination with respect to containers and storage periods is shown in Figure 4. The insulated storage condition exhibited higher germination than ambient storage for all the containers for all the storage periods except for C_1 for six months storage. Improvement in germination due to insulation was evidently associated with its ability to reduce moisture changes in storage (Figure 2). Containers with a marked increase in seed moisture content during storage were relatively more affected by insulation than containers with less moisture gain during storage. The effect of insulation tended to increase with storage time for the duration of this study. However, in terms of percent gain in germination of insulated over ambient storage conditions, the difference was relatively small. The statistical analysis indicated that a difference in germination between ambient and insulated storage conditions of about 2.5 percent was highly significant. This relatively small difference, while statistically significant, is not considered worth the additional expense of storing seed in an insulated container. The analysis simply indicates that there is a difference between the two storage conditions. Therefore, caution should be exercised in interpreting this result.

The mean germination for seed stored in Isabela and Mayaguez was 70 percent and 69 percent respectively. The overall storage environment in Isabela was slightly more favorable than in Mayaguez for both temperature and relative humidity. However, there was no significant effect on germination.

Considerable insect damage was done while the samples were in storage. No actual count was made to determine the percent of seed affected, but visual observation indicated that the damage was negligible for three months storage for all the containers. After six months storage, and even more after nine months, the damage was clearly evident. The cloth bag samples had the most insect damage at the end of the nine months storage period. The sealed metal can treatment showed the least insect damage. The seeds in the sealed metal can treatment were probably contaminated during the preparation of the samples. Probably because of the proximity of the samples, the ambient and insulated treatments were almost equally affected. The rice hulls for insulation were intentionally used without chemical treatment to represent the way a farmer would use them. However, the rice hulls appeared to offer a favorable environment for insects.

SUMMARY

Storage studies using U.S. certified soybean seed were conducted in Puerto Rico with the aim of developing simple and inexpensive methods for maintaining seed quality during storage in the tropics. The results showed that the viability of high quality seed declined rapidly to almost zero after nine months of improper storage in a tropical environment. The loss of seed viability appeared to be closely associated with a marked gain in seed moisture during storage. Containers resistant to moisture vapor transmission tended to preserve seed quality. Drying soybean seed to a safe moisture content was shown to be of little value if no effort was made to prevent moisture gain during storage. The moisture content of unprotected seed approached equilibrium with the humid atmosphere of the tropical environment. Reducing the seed moisture content and maintaining it during storage were shown to be essential ingredients for preserving seed quality at an acceptable level for nine months storage in the tropics. Sealed metal containers or plastic bags placed in metal containers with covers offered adequate storage protection for seed with a low initial moisture content.

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Table 1. Effect of Different Storage Variables on Germination of U.S. Certified Soybean Seed Stored in the Tropics

Variable Code	Description of Variable	Mean Germination, Percent	Mean Moisture Content, percent dry basis
Container			
C_1	Sealed metal can	91.9	10.6
C_2	Metal can with one mil plastic bag	85.9	11.5
C_3	Fertilizer bag, jute with plastic lining	62.9	13.3
C_4	Cotton cloth bag	37.9	13.9
Initial Moisture Content			
M_1	12.5 percent, dry basis	65.2	13.3
M_2	10.7 percent, dry basis	70.1	12.3
M_3	8.6 percent, dry basis	73.6	11.4
Storage Period			
T_1	Three months	93.1	11.8
T_2	Six months	72.0	12.4
T_3	Nine months	43.9	12.9
Storage Condition			
S_1	Ambient	68.4	12.7
S_2	Carton with rice hull insulation	70.9	12.0
Location			
L_1	Mayaguez Campus	69.2	12.5
L_2	Isabela Substation	70.1	12.2

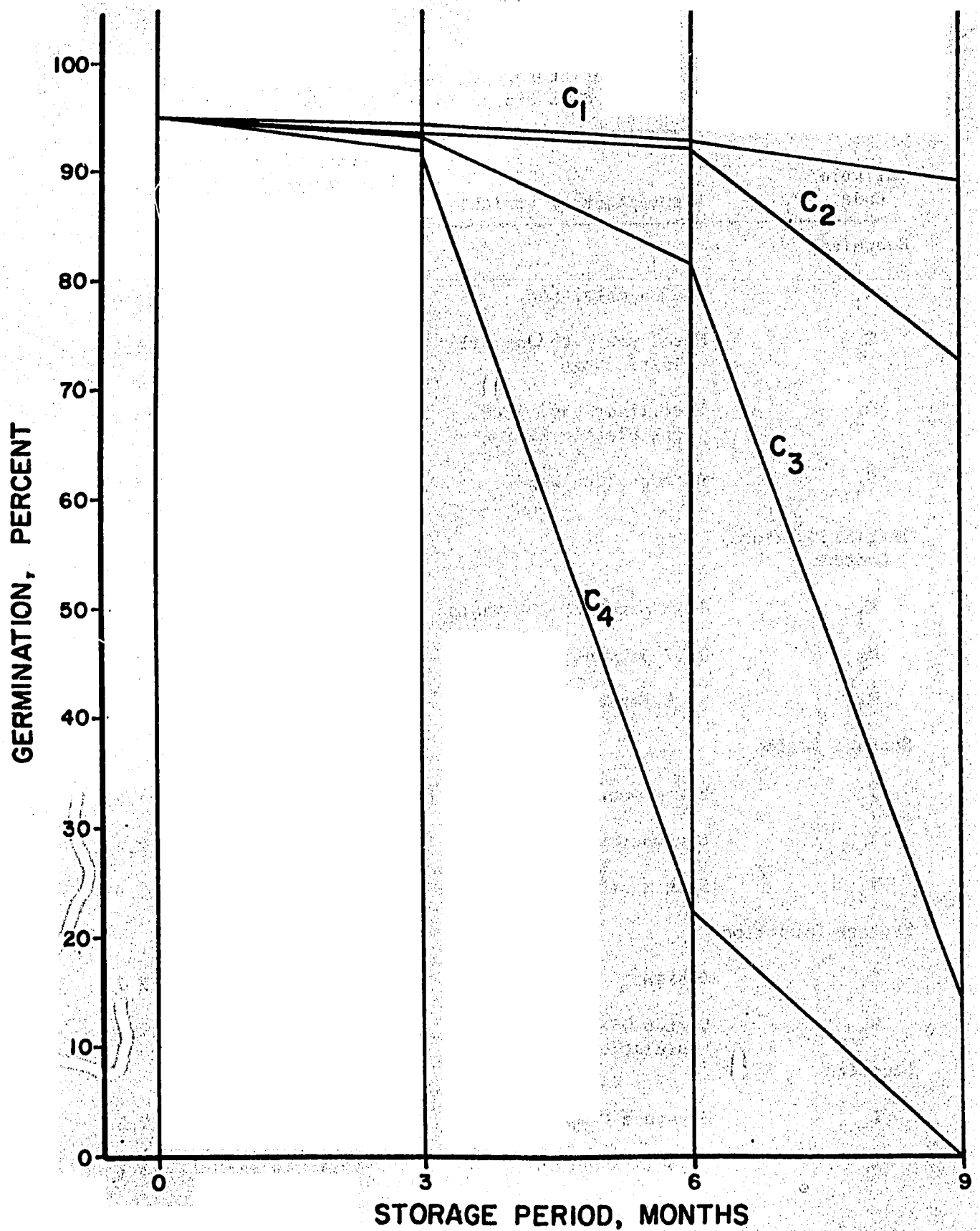

Fig. 1 Variation of germination with respect to containers and storage period

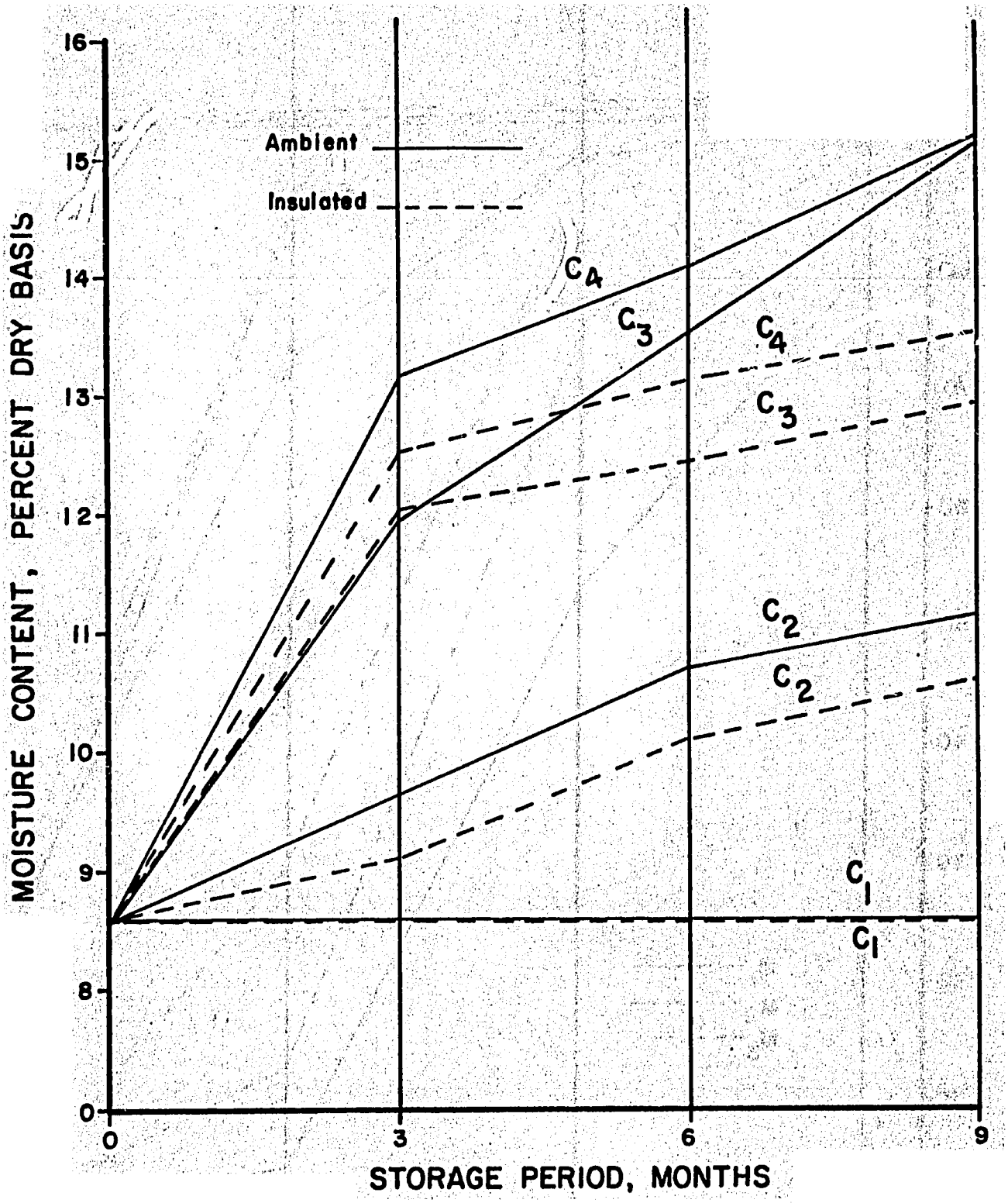

Fig. 2 Change in moisture in storage

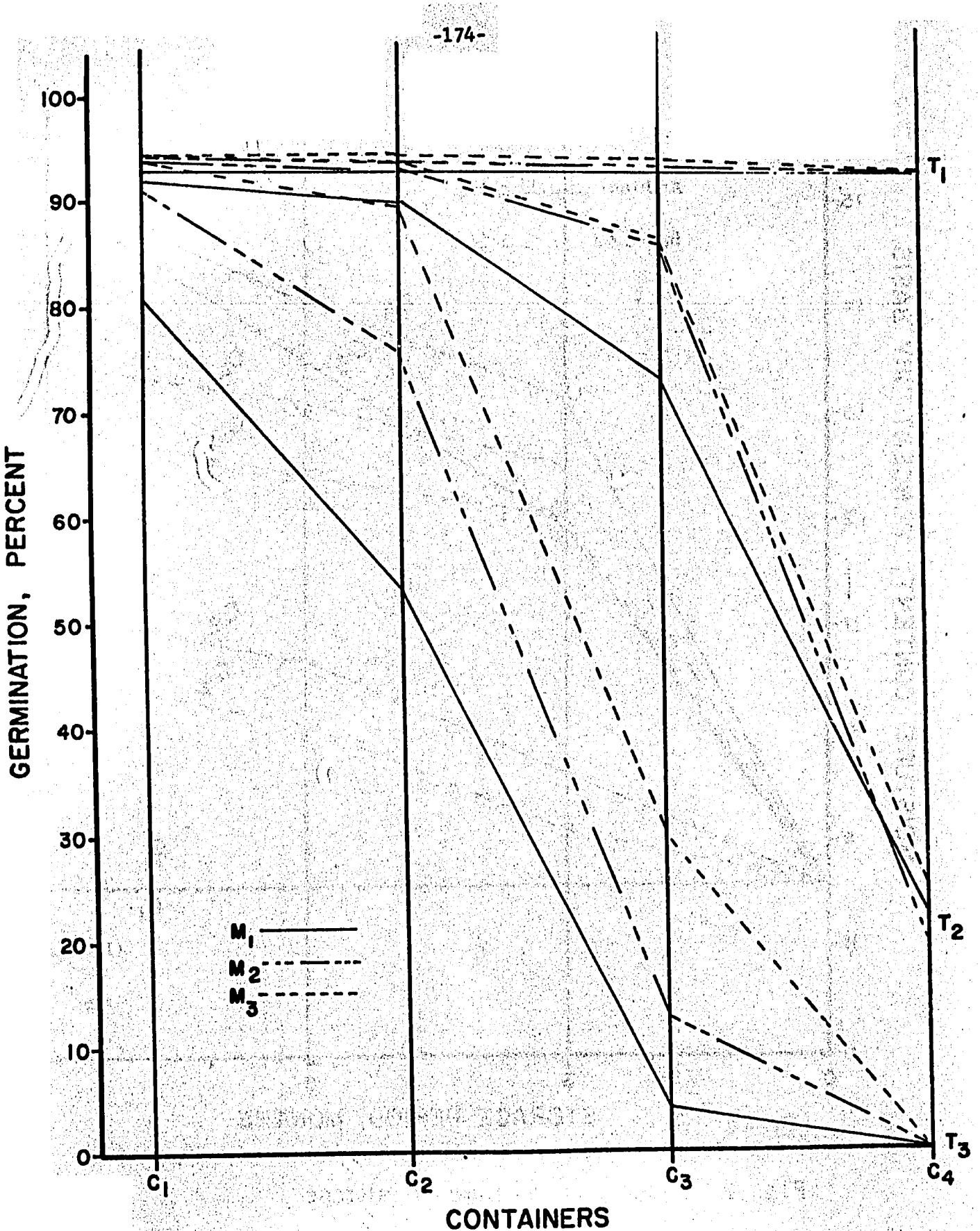

Fig. 3 Variation of germination for different storage conditions

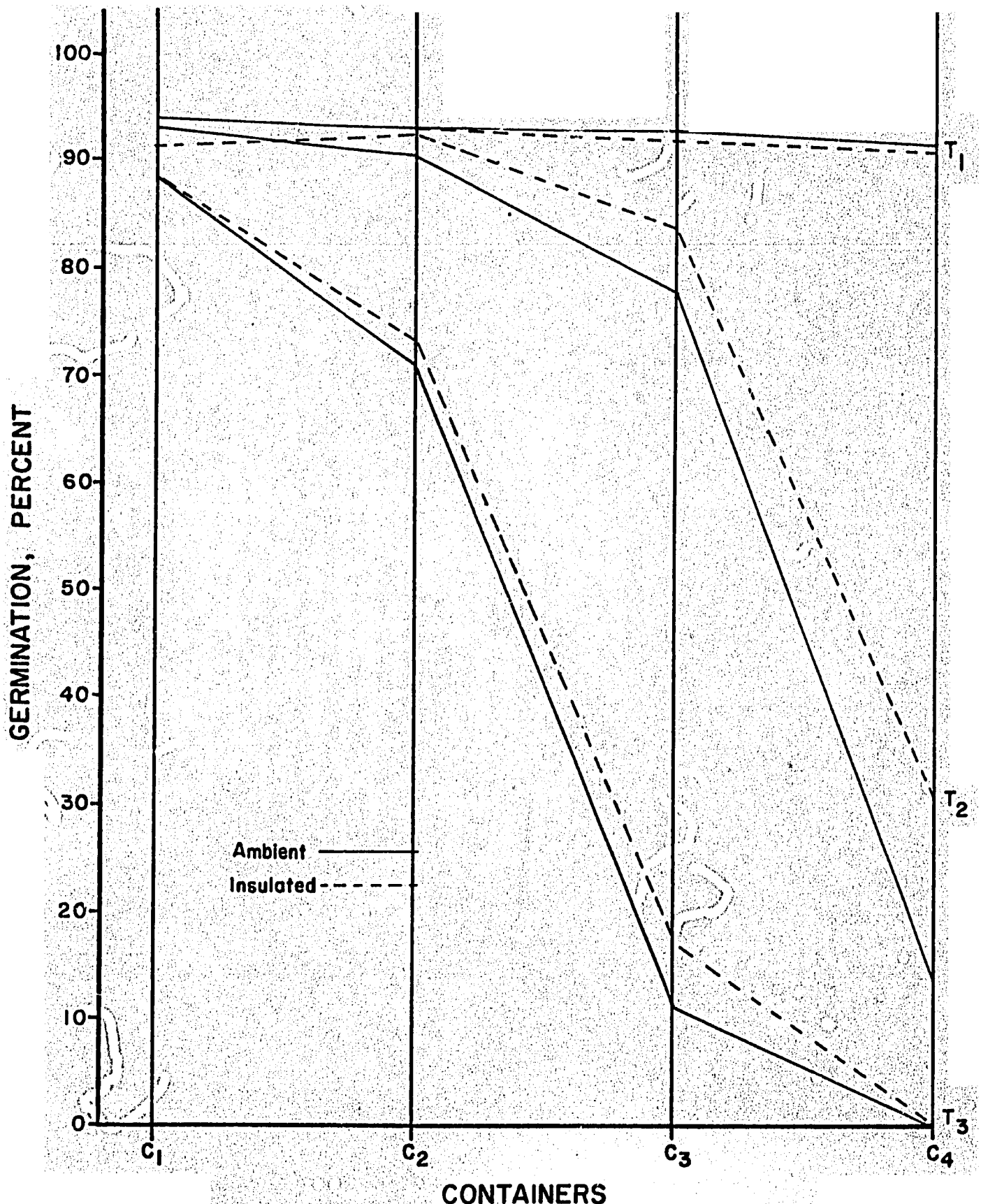

Fig. 4 Effect of insulation on germination