PN-AAD-031

ANNUAL REPORT OF A GRANT TO STRENGTHEN THE COMPETENCE OF THE UNIVERSITY OF PUERTO RICO

IN DISEASE CONTROL AND RELATED INSECTS AND CULTURAL PRACTICES OF SOYBEANS AND OTHER FOOD LEGUMES FOR TROPICAL AND SUBTROPICAL AREAS

October 1, 1975 to September 30, 1976

Submitted to

THE AGENCY FOR INTERNATIONAL DEVELOPMENT
DEPARTMENT OF STATE
WASHINGTON D.C.

BY

UNIVERSITY OF PUERTO RICO
COLLEGE OF AGRICULTURAL SCIENCES
MAYAGUEZ CAMPUS

211(d) Annual Report of the University

of Puerto Rico

Date due: December 15, 1976

Date: December 16, 1976

Grant Title: A Grant to Strengthen the Competence of the University of Puerto Rico in Disease Control and Related Insects and Cultural Practices of Soybeans and Other Food Legumes for Tropical and Subtropical Areas.

Grantee: University of Puerto Rico, Mayaguez Campus

Grant Program Director: Dr. Raúl Abrams

AID Sponsoring Technical Office: Bureau of Technical Assistance
Office of Agriculture

Statistical Summary:

1- Period of Grant: October 1, 1973 to October 1, 1978

2- Amount of Grant: $500,000.00

3- Expenditures

3.1 Report Period: $101,502.27

3.2 Accumulated: $267,951.48

3.3 Anticipated for next year: $125,500.00

II. Narrative Summary

During the grant period the University of Puerto Rico - Mayaguez Campus has significantly expanded its knowledge base relative to crop protection of the tropical food legumes, particularly soybeans, and has developed competence in the field of plant pathology, weed control, nematology and entomology of the tropical food legumes.

The grant has had a marked effect on the Plant Sciences curriculum of the Agronomy Department. Nine new courses relevant to crop protection have been developed during the grant period. A program has been established for a Bachelor of Science and Master of Science degrees in Plant Sciences with options in crop protection.

Five graduate students contributed to grant objectives during the report period. Four of them received their Master of Science degree during the report period and the other will complete the requirements in the near future. Two of the students that received their Master of Science degree were from Nicaragua and have returned to their country and are presently teaching at the University level. The other two, from El Salvador are also teaching at the University level. Graduate student research has involved studies on bacterial and fungal diseases of soybeans in the tropics, nematodes and their control and variety, planting date, plant population studies at two different ecological locations.

Research activities in the area of soybean pathology, including seed pathology and weed control have increased significantly during the reporting period. Much research has been coordinated in cooperation with the University of Illinois in order to achieve the grant objectives of both universities.

The graduate student exchange program between both universities continues to be a complete success. Two graduate students from the University of Illinois have completed their Ph. D. thesis research work at Puerto Rico and have returned to Illinois to complete their degree requirements. A new graduate student, Mr. Steve Foor, is presently conducting his research in seed pathology under tropical conditions. Five staff members of the College of Agricultural Sciences, UPR-Mayaguez, are working toward advanced degrees in crop protection at the University of Illinois.

A new staff member, Dr. Michael Ellis, Plant Pathologist, joined the program replacing Dr. Victoro Garcia, who returned to Peru.

Research activities have increased significantly and at the present time we have a vigorous program in the area of plant breeding and crop protection for soybeans for the tropics which we consider unique in the world and will provide the much needed information and expertise for the less developed countries of the world in the tropics.

III. Detailed Report

A. General Background and Description of the Problem

In the past two years hunger and famine has ravaged hundreds of millions of the poorest citizens in at least 40 nations. The world food picture does not present a pleasant prospect for many regions, especially the over-populated areas in the tropical regions of the world, despite great advances like in cereal crop production. The world reserves of grain have reached a 22-year low. The main cause of the increase in food demand is, of course, the population explosion which is far greater in the tropics than elsewhere in the world. A major lag in research and production exists in the tropics for the more extensively grown legume crops, many of which are excellent sources of cheap high quality vegetable protein.

Soybeans is one of the world's principal legume crops. It may become increasingly important in the tropical and subtropical parts of the world where there is great need for the improvement of the human diet. Recent research in Puerto Rico by ARS and the University of Illinois, indicates that soybean production is feasible in the tropical world. However, further information is needed in order to obtain higher yields through the full use of year-round growing weather, combined with high yielding adapted cultivars and intensive cultural and management practices.

To this end, AID awarded a five-year grant to the University of Puerto Rico in order to strengthen the existing competence to provide needed training, research and informational linkages, technical assistance and consultation on major problems related to limiting diseases and associated insects and cultural practices of soybeans and other food legumes for the

tropical and subtropical areas. The developed competencies will be used to increase significantly soybean production and utilization to improve the human diet of the low income areas of the world.

It will be a collaborative effort with the University of Illinois and will provide the University of Puerto Rico with two senior and one junior staff members, short term visiting lecturers, technicians and with financial support for three graduate students. It will also provide for staff and student travelling strengthen library and increase University of Puerto Rico; capacity to do research in control of soybean and other food legume diseases and insects and cultural practices.

In coordination with the University of Illinois, it will provide facilities to graduate students and staff members from Illinois to do their research work in the tropics and for graduate students and staff members from Puerto Rico to pursue advanced studies toward a Ph. D. at Illinois.

B. Purpose of the Grant

The purpose of this grant is to foster the development of University of Puerto Rico competence and response capability in disease control and related insects and cultural practices of soybean and other food legumes for tropical and subtropical areas of the world. A cooperative and coordinated program with the University of Illinois will be developed to create a joint competence in soybean research, training, linkages with international and national centers and direct assistance.

C. Objectives of the Grant

1. Objectives Restated. The major objectives of this grant are to increase and improve the capabilities of the University of Puerto Rico

to provide background knowledge and research, training and informational linkages, technical assistance and consultation on major problems of disease control and related insects and cultural practices of soybeans and related legumes for tropical and subtropical areas in a collaborative effort with the University of Illinois.

The primary thrusts in strengthening U.P.R.-Mayaguez competency will be as follows:

(1) Improvement of U.P.R.-Mayaguez understanding of the current status of present and proposed practices in the area of crop protection for soybean and other food legumes and their applicability to LDC's in the tropics. This will include a coordinated and teamwork effort between U.P.R.-Mayaguez and U. of Illinois to have a better understanding of the disease, pest and weed control of soybeans and food legumes for the tropics and utilize and capitalize these experiences with the breeding program.

(2) Development of mechanisms which make possible effective interdisciplinary research; advisory and extension capabilities relating to crop protection of soybeans and other food legumes of the tropics.

(3) Improvement of educational capabilities at U.P.R.-Mayaguez for students and staff to acquire greater knowledge of the principles and practices of crop protection of soybean and other food legumes for the tropics.

2. Review of Objectives. The major objectives of this grant are to create at the University of Puerto Rico a comprehensive knowledge,

degree of infestation. To evaluate different methods of control and also develop control systems for specific situations of climatic, soil, crops, weeds and farming systems that would be recommended to growers.

(6) Initiation and expansion of an undergraduate and graduate training program in soybean and other food legumes protection. (Crop Protection).

(7) Establishment of research and informational linkages with LDC's and International Centers.

Grant activities have in general been consistant with original objectives.

D. Review of Critical Assumptions

Assumptions were made that will have a negative impact on the productivity of the proposed program if they should prove to be incorrect.

These assumptions are as follows:

(1) That this redisigned 211(d) grant will be supported by AID for three years from October 1, 1975, the effective date of the initiation of the redesigned grant program.

(2) That AID will support a complementary companion 211(d) grant to the University of Illinois to develop a response in the improvement of soybeans for tropical and subtropical areas.

(3) It is assumed that AID will provide assistance in the initiation of linkages with tropical LDC's in identifying training needs of institutions and individuals and in exchange of information as part of the linkage network.

(4) It is assumed that U. S. and national LDC institutions will cooperate with UPR-Mayaguez in the initiation and strengthening of linkages, exchanges and visits by staff and students, information exchange and other cooperative activities.

(5) It is assumed that increasing numbers of students, both from U.S. and LDC's will be interested in graduate programs at UPR-Mayaguez and the educational offerings that are relevant to crop protection of soybeans and other food legumes for the tropical and subtropical areas of the world.

E. Accomplishments

Accomplishments are discussed under five headings: (1) expanded knowledge base, (2) education and training, (3) advisory capacity, (4) information capacity, and (5) linkages.

1. Expanded Knowledge Base. The University of Puerto Rico-Mayaguez Campus research activities carried out under the grant and supported with funds from it may be grouped as follows: agronomic field research, thesis research,and cooperative field and laboratory research.

a. Agronomic field research

Most of the work published and research conducted in soybean diseases, storage and weed control is mainly from the temperate zones. Relatively little is known about diseases and weeds affecting leguminous crops, particularly soybeans in the tropics. In many instances basic information relating to tropical diseases, the predisposing factors, the vectors, if any, etc. are little known. Rarely are specific economic control measures available to the farmer. Thus, the information base which supports production of these crops is entirely inadequate.

On the basis of this rationale, research activities have been conducted in the following areas:

(1) Chemical Control of Internally Seed-borne fungi.

The use of fungicides in deveoping countries is generally considered to be highly limited due to economic factors. In contrast with foliar applications of fungicides, the use of fungicides for seed treatment is very inexpensive. The general recommended rate for seed treatment fungicides is 2 to 4 ounces per bushel or 100 pounds of seed. When the total cost per acre or hectare is calculated, the result is very low, therefore, fungicide seed treatment is economically feasible even at the small farmer level.

In cooperation with the University of Illinois (Dr. J. B. Sinclair and P. R. Hepperly), experiments were established at both universities using poor seed quality lot of wells soybeans produced in Illinois. A total of 30 chemical seed treatments were used which included several fungicides, combinations of fungicides, antibiotics, and combinations of antibiotics and fungicides. All treatments were established in replicated trials at both universities.

Based on the results of the above mentioned trial, the 14 best treatments were applied to poor quality seeds of the cultivar Jupiter which were produced in Puerto Rico.

The emergence percentage at Illinois and Puerto Rico of non-treated control seeds (wells) was 33 and 28, respectively. The best seed treatment resulted from the application of Penicillin G + Arasan 50 red, with a germination percentage of 65 and 63 at Illinois and Puerto Rico, respectively. This represents an increase of 100% in germination at both locations (Table 1, in appendix).

In the second set of experiments, non-treated seeds of the cultivor Jupiter had a 60 percent germination at Puerto Rico. Seeds treated with Penicillin G + Arasan 50 red emerged at the rate of 85 percent. All chemical seed treatments had a significantly higher germination percentage than the untreated control (Table 2 , in appendix).

These results indicate that the use of fungicides can be beneficial in improving the germination of poor seed quality lots. A very interesting and applied result is that the best treatment resulted from a combination of an antibiotic and fungicide. This tends to indicate that internally seed-borne bacteria may also play a role in decreasing field emergence and stands of soybean under field conditions.

(2) Seed Pathology Culture Collection.

The soybean seed pathology culture collection was initiated in 1975. The purpose of developing a tropical soybean culture collection is to provide a means to maintain a semi-permanent record of fungal and bacterial organisms that have been found in association with soybeans under tropical conditions.

Laboratory cultures of fungi and bacteria are maintained on Potato Dextrose Agar culture tube plants. Cultures are covered with sterile mineral oil for preservation. Duplicates of each culture are made and ready for distribution to other soybean researchers around the world and for further laboratory and field investigations.

The collection will be maintained and increased and at present it consists of the following genera of fungi: Corynespora, Cladosporium, Alternaria, Fusarium, Syncephalastrum, Epicoccum, Chaetomium,

Nodulosporium, Arthrinium, Helminthosporium, Curvularia, Phoma, Trichoderma, Sclerotium, Nigrospora, Leptosphaerulina, Penicillium, Pestalotia, Drechslera, Xylaria, Diaporthe, Hormodendrum, Glomerella Rhizoctonia, Botryodiplodia, Aspergillus, Colletotrichum, Cercospora, Rhizopus, and Vermicularia.

The following genera of bacteria are also included: Xanthomonas and Bacillus.

a. Pathogenicity Testing of Seed-borne Fungi.

All of these microorganisms were recovered from inside soybean seeds (internally seed-borne) grown in Puerto Rico. It is apparent that the microflora of soybeans produced under tropical conditions is composed of a large variety of microorganisms. At the present time, pathogenicity tests are being conducted with all microorganisms which are currently in the collection. The procedure used for pathogenicity testing is as follows: Three replications of 100 seeds of the soybean variety Boosier were surface sterilized and placed in petri dishes containing 15 day old (sporulating) cultures of each fungus. Seeds were shaken inside the petri dishes until covered with spores, then plated on sterile PDA. Three replications of 100 non-inoculated seeds were planted as controls. Percent seed germination in culture dishes was recorded after seven days at 25°C.

Results of the tests conducted thus far are as follows: Control seed (non-inoculated) germinated 95 percent. The most pathogenic fungi appeared to be Vermicularia, Colletotrichum, Phomopsis and Botryodiplodia. The percent germination for inoculated seeds was: Vermicularia, 9; Colletotrichum, 10; Phomopsis, 16 and Botrydiplodia, 31. Phoma and

Glomerella appeared to be slightly pathogenic with percent germinations of 75 and 85, respectively. Additional tests are currently being conducted and will be summarized in next years report.

(3) Disease Nurseries - Screening Tropically Adapted Germplasm for Disease Resistance.

a. 1975-76 Disease Nurseries

Soybean disease nurseries were established at the Agricultural Experimental Substations of Isabela, Fortuna, Adjuntas and Lajas. At each experimental site seven soybean cultivars were grown in a replicated experiment. The cultivars were: "Improved Pelican," "Williams," "Bonus," "Clark 63," "Hardee," "Pickett 71," and "Biloxi." Each variety had been selected because of differing susceptibility to known major diseases of soybeans. The overall goal of this investigation was to obtain a basic understanding of the various pathological problems that might be encountered at any one of the four experimental sites.

Throughout the experiment visual observations were made of each soybean variety at each site. Soybeans of each variety were harvested at physiological maturity and at a 30 day delayed harvest. Laboratory bioassasy showed that there were high percentages of seedborne organisms with soybeans produced in the substations of Adjuntas and Isabela. The most predominant fungus isolated from seeds harvested at Isabela was Cercospora which causes the disease purple seed stain. The most predominant organism found in association from seeds from the Adjuntas substation was Diaporthe, causal agent of pod and stem blight of soybeans. Soybeans tested from the Fortuna substation were almost entirely free from seedborne organisms. This can be attributed to low rainfall and dry weather conditions during the experiment.

The disease nursery at Lajas was lost due to insect damage. In general, the most common fungi recovered from this study were Fusarium, Alternaria, Botrydiplodia; Cercospora and Diaporthe. Soybeans collected in the delayed harvest situation at both Isabela and Adjuntas were totally deteriorated. Daily rains, insects, disease pressure, and high humidity were the cause for this deterioration. At the Fortuna substation soybeans harvested in the delayed harvest still maintained good seed health.

The continuation of soybean disease nurseries to assess present and potential pathological problems is important. Through these investigations good educated selections of disease problems to be dealt with can be made.

b. 1976-77 Disease Nurseries

Rather than plant disease nurseries of a few lines at two or three locations, we recently decided to plant nurseries which contain the entire germplasm collection for maturity groups 8, 9 and 10 at one location (Isabela). This represents 400 soybean lines which are planted in at least 4 replications. By planting nurseries of this type, we can more closely observe the disease reactions of each line to several different diseases under natural conditions.

It would be desirable to plant large nurseries at several locations on the island, however; the number of man-hours required to maintain and take disease ratings at several locations is (at present) extremely limiting.

At present, the disease nurseries appear to have great potential. They are extremely important as a means of detecting potential sources of disease resistance. By observing disease reactions in the field to specific diseases, the number of lines which would be included for more specific screening methods to a particular disease would be greatly reduced.

The importance and need for discovering and incorporating resistance to plant disease into our promising tropically adapted lines cannot be over-emphasized. - The development of a strong association and cooperation between plant pathology and breeding is underway. In cooperation with Dr. E. H. Paschal II (plant breeder) the screening of tropically adapted germplasm for disease resistance will play a major role in Intsoy plant pathology activities at the University of Puerto Rico.

1. Disease Nursery I - July, 1976.

In a cooperative experiment between the University of Illinois (Dr. R. M. Goodman and Mr. Glen Bowers) and the University of Puerto Rico, six randomized replications of the 400 soybean lines (U.S.D.A. germplasm collection for groups 8, 9 and 10) were planted in the field at Isabela. Five replications were hand inoculated with soybean mosaic virus (SMV) in an attempt to study seed transmission of the virus in soybean. This same experiment was also used as our first large scale disease nursery.

Natural infections of various plant diseases were observed and recorded at regular intervals. The disease which appeared to infect almost all plants, in addition to SMV, was bacterial pustule (*Xanthomonas phaseoli* var. *sojensis*). Of the 400 lines observed, 7 lines showed no infection by bacterial pustule in all 6 replications, and, therefore; appeared to be immune to pustule. In addition, 8 other lines appeared to have high levels of resistance to pustule but were not immune. Seven lines (not the same) also appeared to be immune to SMV. The following lines were apparently immune to SMV and bacterial pustule:

SMV	Bacterial Pustule
Majos	PI 323.580
Nela	PI 164.884
PI 341.242	PI 205.915
PI 324.068	PI 222.550
PI 341.245	PI 259.540
Buffalo	PI 341.250
PI 274.453	Hardee (late selection)

Even though these results are preliminary, it appears highly possible that we have good sources of resistance or tolerance to bacterial pustule and possibly Soybean mosaic virus.

More work is needed before actual resistance can be confirmed.

2. Disease Nursery II - October, 1976.

In the second large scale disease nursery, 440 lines were planted in four randomized replications at the Isabela Experiment Station.

In order to obtain the full potential of the nursery, it was decided that two of the replications would be protected from insect pests through the application of insecticide. The other two replications are not being protected with insecticide. In this manner, disease readings can be made on all four replications and readings for damage due to various insect pests can be made on the two non-protected replications. Thus, we have a combined disease-insect nursery.

At present, disease incidence is increasing in the nursery, however, the first disease reading has not yet been made.

The first reading for insect damage has been recorded. Many lines were completely defoliated by the leaf roller or pega-pega (*Hydolepta* sp.). The data has not yet been summarized, however, it appears that early

maturing lines that were completely defoliated have been essentially destroyed by this insect pest. The results of disease nursery - II will be summarized in next years report.

(4) Weed Science.

Research in this field was initiated in January 1975 by establishing herbicide screening trials at the Isabela, Lajas and Fortuna substations. These substations are located in regions which have different soil and climatic conditions. The predominant weed species also differ from one region to another. The variety Improved Pelican has been used in most trials because to its adaptation to tropical environmental conditions.

a. Chemical Control -

Individual replicated trials for Pre-plant incorporated (PPI) and Pre-emergent (PRE) herbicides were performed at the Isabela and Lajas Substations. The trials at Isabela were started in October 1975 and harvested in March 1976.

In the PPI trial, nine herbicides were tested in 21 treatments. Weed population in the experimental area consisted of *Euphorbia heterophylla*, (52%); *Ipomoea* sp., (14%); *Rhynchosia minima*, (11%); and *Bidens pilosa*, (21%).

Adequate control of these weeds at 5 weeks after application was obtained only with the treatments in which metribuzin was included, namely trifluralin + metribuzin (0.75 + 0.5)[1/] and A-820 + metribuzin (1.0 + 0.5). Control ratings of 65% were recorded 9 weeks after treatment in the plots which had received these treatments.

Although good control was obtained, soybeans were damaged by metribuzin. The damage was manifested by loss of stand, stunting and

---

[1/] The numbers following the name of the products refer to the rate in Kg active ingredient per hectare.

reduced yields as compared to the unweeded checks.

In the PRE trials, twenty-three compounds were tested. Adequate control of the prevalent weed species was obtained with metribuzin (0.75 & 1.0), oxadiazon (1.0 & 1.5), RP 20810 (0.75 & 1.0) and RP 20630 (0.75 & 1.0). All of these compounds caused some toxicity to soybeans, especially at the higher rates of application. Most plants appeared to recover and provided good yield. Metribuzin was less toxic applied at pre-emergence than when incorporated.

The evaluations of PPI and PRE treatments in Lajas was started on May 25, 1976 and harvested on October 4. The field in which the trials were established was heavily infested with Sorghum halepense. For this reason, it was necessary to manually remove this weed from all plots in order to observe herbicide performance toward the rest of the weed population. Predominant annual weeds were Ipomoea sp. (40%), Phyllanthus niruri (30%), Grasses (20%), Cyperus rotundus (5%) and about 5% other species.

Twenty-five products were tested in sixty treatments. The best controls with the PPI treatments at 4 weeks after application were obtained with combinations of products. The combinations vernolate (2.0) + trifluralin (0.75) + metribuzin (0.5); vernolate (2.0) + trifluralin (1.0); vernolate (2.0) + A-820 (1.0) + metribuzin (0.5) and vernolate (2.0) + AC 92553 (0.75) provided over 80% control. Trifluralin (2.0) and oryzalin (1.5) also provided 80% control. Over 70% control was obtained with EPTC (2.0) + AC 92553 (0.75); trifluralin (1.0); trifluralin (0.75) + metribuzin (0.5); oryzalin (1.0) and with fluchloralin (1.25).

Good controls at 6 weeks were observed with all of the combinations mentioned and also with trifluralin (2.0); fluchloralin (1.25) and oryzalin (1.5). In general, higher yields were provided by the treatments giving better weed control.

Weed population in the area of the PRE trial was slightly different from the PSI and consisted of Ipomoea spp. (50%), Grasses (Johnson grass not included), Portulaca olenaceae (15%) and of other species (10%).

The majority of the chemicals effectively controlled annual grasses until 4 weeks after application but gave poor control of dicotyledoneous weeds. Broad leafed weeds were controlled consistantly up to 6 weeks only by metribuzin (0.75 & 1.0) and RP 20630 (1.0). Since both of these compounds also provided satisfactory control of grasses they gave the best control up to 6 weeks after planting.

Future work in this station will stress the evaluation of combination of compounds to get a broader control spectrum.

The results of all chemical control trials point out the necessity of considering the weed populations of a given zone before making herbicide recommendations. which are usually made on the basis of selectivity of a certain compound to the crop, with no consideration given to the susceptibility of specific weeds to that compound. As a result, money and time are wasted and the crop is not properly protected against weeds.

A new trial was established in May 18, 1976 at Isabela using plots of larger size (10 m x 3.0 m), in which the best treatments from the previous trials in this sub-station were included.

Larger plots were used in order to have a better view of how the treatments would behave in large scale operations. Weed population consisted of Euphorbia heterophylla (71%); Ipomoea sp. (18%); narrow leafed weeds (9%) and other species mainly Phynchosia minima and Bidens pilosa (2%). The narrow leafed species present were Digitaria sanguinalis, Echinochloa colonum, Eleusine indica and Cyperus rotundus.

Under the conditions of this trial, the PPI treatments, especially those in which vernolate was included, were phytotoxic to soybeans. It is possible that too deep incorporation, abundance of water and light textured soil had a combined effect in causing toxicity to the crop. Injury was observed as stunted growth and reduction of stand.

Trifluralin (1.0) effectively controlled narrow leafed weeds but had no effect on broad leafed weeds. This treatment yielded 21% higher than the unweeded control, but 11% less than the hand weeded control. Vernolate (2.5) gave similar control than trifluralin (1.0) but was highly toxic to soybeans. Vernolate (2.0) + trifluralin (1.0) resulted in severe loss of stand, stunting and a marked reduction in yield. Weed control was not markedly improved over either of the products used alone. Alachlor (1.5) appeared to cause very little damage to the crop, and provided good control of grasses; however, the control of broadleaf weeds was poor. Yield was 16% higher than unweeded control but 15% lower than handweeded control. Oryzalin (1.0) was slightly toxic to soybeans and also gave poor control of broadleafs. Yield was lower than the unweeded control. Metribuzin (0.75) caused some toxicity to soybean. Toxicity was observed as reduced plant height and stand. Weed weights, especially of broadleafs were also reduced. Yields were only 5% lower than the handweeded plots. Alachlon (1.0) + metribuzin (0.75) gave good control of Ipomoea sp.. This combination also caused slight phytotoxicity to soybeans; however, total weed control was excellent, reducing both population and weight of grasses and broadleafed weeds. This treatment also provided the highest yields, out yielding the handweeded plots by 8%. RP 20810 (1.0), oxadiazon (1.0) and RP 20630 (1.0) constitute a group of chemically related compounds which

show promise for use on soybeans as pre-emergence treatments. The most active of the three appears to be RP 20630 which provided excellent control of grasses and broadleafed weeds and produced yields similar to those obtained with the combination of alachlor and metribuzin. Yield was 7% higher than the handweeded plots. RP 20630 and RP 20810 controlled Euphorbia heterophylla more efficiently than oxadiazon. Ipomoea sp. was not controlled by any of the three products.

Linuron (1.0 and 1.5) did not effectively control Euphorbia heterophylla which was the most abundant weed in the field. Broadleafs in general were poorly controlled. Yields, obtained with this treatment, were lower than those of the unweeded control. When mixed with alachlor performance was improved.

Future work at Isabela will be conducted in fields which contain different weed species in order to arrive at specific recommendations for different situations.

A similar trial was planted on July 14 at Lajas and is being harvested at present. The results of this trial will be presented in next years report.

b. Weed Competition Studies -

Two competition experiments were carried out during 1975-76 at Isabela. Plots were handweeded at different time intervals so as to provide competition at different stages of plant development. Weed populations were similar to those previously reported for Isabela.

Results from the two studies indicate that weed competition lasting for 1, 2, 3 or 4 weeks after crop emergence had little effect on yield. When weeds were allowed to compete with soybeans for five or more weeks, yields

were markedly reduced. The longer the period of weed competition the larger the reduction of yield. These results seem to indicate that weed competition during the period between week 4 and 5 after emergence is critical and that soybeans must be free of weeds during this period to obtain the potential yields of the variety.

Plots kept clean only up to 1, 2, 3 or 4 weeks after emergence, allowing weeds to develop afterwards, yielded 25, 19, 14 and 10% less, respectively, than plots kept clean up to the fifth week and unweeded afterwards. These plots in turn yielded only 6% less than plots kept clean until the 10th week. It appears, from these results that weeds that appear after the fifth week of emergence of the soybean crop had little adverse effect on yield, if the crop has been kept free of weeds up to this time. Yields from plots receiving only one handweeding were higher when the weeding was performed at 4 weeks than at either 2, 3, 5 or 6 weeks.

The previous results indicate that herbicides to be recommended for soybeans should effectively protect the crop from weed competition at least during the first five weeks following emergence. They also point out the importance of timing mechanical control at around the forth week after emergence of the crop.

In cooperation with Dr. E. H. Paschal II (plant breeder) another experiment was planted at Isabela on July 12, 1976 in order to evaluate the behavior of 8 soybean cultivars toward weed competition and to observe if there were varietal differences in susceptibility to metribuzin. Cultivars differing in growth habits, morphology and maturity date were selected. The varieties included were: Jupiter, Improved Pelican, Hardee, a selection of Hardee of later maturity, Williams, SJ-2, PI 341241 and CH #3. Each

variety received 3 treatments: No weeding, handweeding at 4 weeks after emergence and 0.75 Kg metribuzin applied as a pre-emergent treatment.

Injury ratings taken at 2 and 4 weeks after application of metribuzin showed only slight differences in sensitivity to this chemical among the varities tested. Williams, Hardee and Jupiter gave higher injury scores, and CH #3 the lowest. There was more injury from metribuzin in locations where water accumulated.

Predominant weeds in the experimental plots were Digitaria sanguinalis, Echinochloa colonum, Eleusine indica, Cyperus rotundus and Amarauthus dubius. Metribuzine provided excellent control of most of these species, with the exception of Cyperus rotundus. Total weed control was over 80% at 30 days after application.

Comparisons of the fresh and dry weights of weeds in the non-weeded plots taken at 4 and 8 weeks after planting, showed greater development of weeds in plots of the variety Williams which, of all varieties used, had the shortest plant height.

b. Cooperative Field and Laboratory Research

Much of the research initiated within the last two years deals directly or indirectly with soybean seed quality in the tropics. Traditionally, small farmers in the tropics use seed which has been saved from the previous crop. In general, this practice leads to the use of poor quality seed (low percentage germination and field emergence), as well as, the transmission of diesease producing microorganisms which are carried on or inside of the seed. In order for soybean production to increase in the tropics, a source of high quality seed for planting must be established.

The soybean seed quality program which as been initiated at the

University of Puerto Rico is interdisciplinary. The program includes individual and cooperative research between plant breeding, plant pathology, agricultural engineering, mycology and weed science. The program stresses the following phases of soybean seed production: the discovery of tropically adapted germplasm which has good seed quality characteristics; production of high quality seeds; storage of soybean seeds under tropical conditions, chemical control of seed-borne pathogens and studying the effects of internally seed-borne microorganisms on soybean seed quality in the tropics.

(1) Screening tropically adapted germplasm for seed quality characteristics. (Cooperative project between plant breeding and plant pathology.)

The cooperative study between soybean breeding-plant pathology is directed at finding those germplasm entries that show genetic resistance to internally seedborne microorganisms. Studies are being conducted on the world germplasm collection of maturity groups VIII, IX, and X which have been grown at the Agricultural Substation of Isabela. The plant pathology laboratory has been given a 100 seed sample for each of the 397 germplasm entries of the collection. These samples undergo a bioassay to determine the percentage of internally seedborne microorganisms. This bioassay consists of planting 100 surfaced sterilized seeds of each entry on 25 petri dishes containing Potato Dextrose Agar (Difco) (4 seeds/plate) and incubating them at 25°C for 5-7 days. After the appropriate incubation period data is then recorded pertaining to percentage germination, fungi and bacteria.

The goals of this cooperative study may be summarized as:

1. To identify genotypes with resistance to seedborne microorganisms.
2. To study the relationship of seedborne microorganisms and tropical germplasm.

3. To develop techniques for assaying "seed quality attributes" of ttropical germplasm and breeding lines.
4. To incorporate "seed quality attributes" into tropically adapted cultivars.

Figure 1 shows the range of germination percentage of the 259 genotypes of groups VIII and IX. The range of germination percentage was from 0-100% with more than 70% of the accessions germinating 80% or better. The group VIII and IX plantings matured under reasonably favorable conditions. Although temperature and relative humidity were high by temperate standards, there was not an excess of rainfall during seed maturation. The group X plots, on the other hand, received excessive rainfall as the plants were maturing, resulting in lower quality seed and lower germination percentages (Figure 2). The range of germination percentage in group X was from 1-100%, with a mean of of 68% compared to a mean germination percentage of 85% for groups VIII and IX. Percent of internally seedborne fungi exhibited an inverse relationship with germination percentage (Figures 3 and 4). More than 60% of the accessions of groups VIII and IX had 20% or less total fungi, while only 12% of the group X material had 20% or less total fungi. Although more than 30 genera of fungi were found in association with the seed of this study, probably the most important seed quality affecting pathogens is of the genus Phomopsis, causal organism of pod and stem blight of soybeans. The seed samples of groups VIII and IX ranged from 0-51% Phomopsis with a mean of 7% (Figure 5). Group X (Figure 6) ranged from 0-48% Phomopsis, with a mean of 13%.

In a second series of experiments, twenty-four lines were choosen from the original 397 lines which were assayed. Of the lines selected, 21

were selected because of favorable results from the first experiment (low percentage of internally seed-borne fungi and high percentage germination in-vitro). Three lines which showed poor seed quality characteristics (high percentage of internally seed-borne fungi and low percentage germination in-vitro) were selected as checks. The 24 lines were planted in a randomized replicated field trial at the Isabela Experiment Station. Each plot consisted of 3 rows (6 meters). One row was harvested at physiological maturity. The second and third rows were harvested at 2 and 4 weeks after maturity, respectively. Fifty seeds from each replication and harvest date were surface sterilized and plated on Potato dextrose agar (PDA). The recovery of internally seed-borne fungi and germination in-vitro were recorded after 7 days at 25°C. One hundred seeds from each replication and harvest date were planted in the sand bench and in the field at Isabela. Percentage field emergence was recorded after 10 days in sand and 15 days in the field.

The major goals of this experiment may be defined as:

1. To test if our laboratory evaluations are reliable interpretations of the expected performance of these germplasm entries under field conditions.
2. To test if the apparent resistance from the preliminary field trials is maintained throughout this experiment and a second laboratory evaluation.
3. To identify genotypes that maintain "good seed quality attributes" when the harvest is delayed after physiological maturity.

4. To evaluate agronomic characteristics of selected germplasm entries.

Figure 7 shows the effects of delayed harvest on sand emergence, in-vitro germination, total fungi, and Phomopsis averaged over all 24 genotypes of the study. Delaying harvest after maturity date decreased sand emergence and in-vitro germination and increased the incidence of Phomopsis and other fungi. To demonstrate the genetic differences present, the sand emergence of 6 genotypes is depicted in Figure 8. PI 204.331, PI 205.912, and PI 205.908 were selected from the preliminary screening on the basis of their high germination potential and apparent resistance to seedborne microorganisms. Jupiter and Hardee are commercial cultivars reasonably well adapted to tropical conditions. PI 240.672 was included as a control because of its susceptibility to seedborne microorganisms. In the first harvest emergence of all six lines was above 90%. At the second harvest emergence had fallen off to less than 90% for the two commercial cultivars and PI 240.672. The three apparently resistant genotypes emerged at an average rate of 95% when harvested 4 weeks after maturity. Jupiter, PI 240.672, and Hardee, however, emerged at the rate of 67, 54, and 49%, respectively, in the third harvest. Percent total fungi of the six selected lines is summarized in Figure 9. Delaying harvest 2 and 4 weeks after maturity did not appreciably increase percent total fungi in the three resistant lines. Mean percent total fungi was 17% for these genotypes. The less resistant lines, however, averaged 38, 51, and 76% total fungi in the first, second, and third harvests, respectively. Percent Phomopsis followed the same trend as total fungi with the resistant lines averaging 1% in the first

and second harvest and 7% in the third harvest. Percent Phomopsis increased from 23 to 50% from the first to the third harvest of the susceptible lines.

After confirming the apparent resistance to seedborne microorganisms and the increased seed quality of the selected lines over the commercial cultivars in the other environments, research will be initiated to study the mode of inheritance of these properties and the feasibility of transferring them to otherwise tropically adapted cultivars.

(2) Production of high quality seed.

(Plant pathology)

The production of high quality seed involves areas of investigation such as the use of fungicides to control seedborne microorganisms and the incorporation of genetic characteristics which increase seed quality. These areas are discussed in other sections of this report and are an integral portion of the research involved with seed production.

In research which has been conducted, the use of certain agronomic practices such as harvesting seeds immediately at maturity has been observed to be extremely important in the production of high quality seeds. A one or two week delay in harvest has been shown to result in an increase in internally seedborne fungi and a decrease in seed germination and field emergence. The importance and practice of harvesting immediately at maturity must be emphasized. Care and punctuality with time of harvest can be directly used by the small farmer. It appears that the practice alone could greatly increase seed quality in the tropics.

(a) Soybean seed quality trial.

(Plant pathology)

The goal of this investigation was to assess the "seed health" of soybeans produced in Puerto Rico as compared to certified soybeans produced in the U.S.A. (mainland).

This trial involved the growing of seeds from the soybean varieties that had been produced in the Isabela Disease Nursery. The purpose was to compare the performance of the original certified seed to physiologically mature (1st) and delayed harvested (2nd) seeds of the same variety under field conditions. In a second portion of the experiment we wanted to test the "seed health" of soybeans produced from the fungicide spray field trial in comparison with the original certified seed and non-sprayed checks.

The results from this experiment show that generally the percent emergence and stand at physiological maturity was greater for plants that had been grown from original certified seed than from seed of first or second harvested seed from the disease nursery. In all cases both emergence and final stand were the lowest in plants grown from seeds from delayed harvests. Plants grown from original seed produced higher yields than first and second harvested plants. Plant heights, number of pods per plant, and seed weight also differed. A laboratory bioassay of the seed showed no significant differences in the amount of seedborne microorganisms between any of the treatments.

Plants grown from seeds which had a previous history of being treated with fungicides were taller and yielded better than original certified seed or plants grown from the non-sprayed control. All soybeans were harvested at physiological maturity. Laboratory bioassay for the presence of internally seedborne microorganisms showed no differences between any of the treatments.

(b) Germination potential of second generation seeds.

(Plant pathology)

Objectives:

1. To determine the germination potential and growth rate of seeds from plants sprayed with foliar fungicides.
2. To study seed quality in terms of seed microflora of second generation seeds from plants sprayed with folier fungicides.

Variables include:

1. Seeds from plants sprayed with the following foliar fungicides:
   a. Benlate
   b. DuTer
   c. Dithane M-45
   d. Bravo-6F
   e. Merteck 340-F
2. Seeds from the above sprayed plants were harvested at maturity or three weeks after maturity.
3. Seeds were either nontreated or treated with the following chemical seed treatment:
   a. Benlate - surface applied
   b. Arasan - surface applied
   c. Benlate - applied with an organic solvent Methylene chloride
   d. K Penicillin G - applied with Methylene chloride
   e. K Penicillin G - applied with Methylene chloride and Arasan - surface applied
4. Seeds from the above plants will be bioassayed and germination in sand recorded. Also, the seeds will be treated with Arasan and germination in sand recorded.

Parameters measured:

1. Field emergence.
2. Germination and the amount and type of microorganism recorded (Bioassay).
3. Germination in sand.

Results: Data from this experiment is still being collected. It would appear that seeds from plants sprayed with fungicides have a much higher potential to germinate than seed from nonsprayed plants. This potential, however, cannot be measured unless seed treatment fungicides are applied.

(3) Soybean seed storage under tropical conditions.

(Cooperative project between UI Agricultural Engineering Department and UPR-Plant Pathology)

The availability of high quality seed at planting is indispensable for profitable soybean production. The hot and humid environment in the tropics makes it difficult to produce and maintain seed viability up to planting time from the production date. This is a serious problem for an emerging crop like soybean where high quality seeds are not readily available in the local market. To improve yield and quality of product a high quality seed is an essential requirement. Small farmers are generally greatly handicapped by their limited resources to depend on sophisticated facilities for storing and maintaining seed quality under controlled enrironment conditions. Therefore, simple and inexpensive methods for producing and preserving high quality seed can be of great help in improving soybean production by small farmers. Such improvements can be achieved through plant breeding, agronomic

practices, and simple engineering techniques. It is envisaged that the small farmers particularly in underdeveloped areas will be the most direct beneficiary of these studies.

General Work Plan:

The activities in this research area were divided into three phases as follows:

a) Phase 1 - Seed storage studies using Illinois certified seed to establish a base of reference for comparison.

Current status: Completed experimental data being analyzed.

b) Phase 2 - Seed storage studies using seed produced in Puerto Rico under tropical conditions. Seeds were also stored either with or without fungicide seed treatment.

Current status: Still in progress with Arason (Thiram).

c) Phase 3 - Integrated seed improvement at production, drying and storage levels.

Current status: Planned pending availability of funds and personnel.

a) Phase I

Seed storage studies using Illinois certified seed were completed. Emphasis was directed towards using resources generally available to the farmer in the tropics. 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 carton box with about six-inch rice hull insulation; three initial moisture

levels: M_1 - 12.5%, M_2 - 10.7%, M_3 - 8.6% moisture content dry basis; four containers: C_1 - sealed metal can, C_2 - metal can with plastic 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 determination, and pathological studies at the start and the end of the storage period were conducted.

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 weight basis. The seeds were reconditioned and were allowed to absorb moisture from the surrounding. This was done by spreading the seed in about one inch layer in a cold storage room for several weeks until moisture content reached to about 13-14 percent. The seeds were then dried using slightly heated air of about 90-95°F until the three desired moisture levels were attained. The drying process was done by placing all the seeds in the laboratory dryer and withdrawing about one-third of the seeds when the appropriate moisture level was reached. The dried seeds were then sealed in double layer plastic bags. At the end of the drying period the seed lots were divided into three approximately equal parts each of different moisture level. Then the seeds were sub-divided 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 seed. The percentage of broken seed was about 1-2 percent. Mechanically and insect damaged seeds were separated by hand picking from the whole seed. 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 sub-divided into 8 groups consisting of 50 seeds each.

Preparation of the Sandbed for Germination Tests

Sandbed germination test was used for determining the percent of germination of the seed at the end of the storage period. The sandbeds were first fumigated using Methyl Bromide one week before the seeding. One hundred forty-four germination tests could be performed on the sandbed at one time. The remaining samples for germination tests were made after completing the first set of germination tests. In each case the sandbeds were first fumigated at least one week prior to seeding.

Summary of Results

The summary of the results of tropical storage studies of U.S. soybean certified seed is presented in Table 3. Statistical analysis indicates that all the storage variables studied except storage location showed highly significant effect on germination of U.S. Woodworth soybean certified seed stored in the tropics.

The apparent effect of storage container on germination after three months storage is as follows: Germination deteriorates very rapidly after

three months storage for C_4 with germination decreasing 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 occured 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 appeared after six months with germination decreasing from 92 percent to around 72 percent. 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 period. It appeared that containers which minimize changes in moisture content of the seed in storage maintain seed viability stored up to nine months to a desirable level. The commercial fertilizer jute bag with plastic lining is relatively air tight. However, in this experiment the bag was cut into 12 pieces and then sewed using commercial needle. This greatly reduced the moisture insulating property of the bag. The containers which maintain the seed initial moisture content and minimize moisture exchange between the seed and atmosphere tend to produce relatively high germination. This is especially evident for six and nine months storage periods. These results suggest that the ability of the container to minimize moisture changes during storage is as important as the low initial moisture content of the seed in preserving seed viability in the tropics where wide daily fluctuations of relative humidity exist. Generally, for a typical day, it is lowest in the afternoon having a value of around 60 percent and highest in the early morning with a value close to 100 percent.

The influence of the seed initial moisture content on germination with respect to containers and storage periods indicate the following results:

Significant effect of the seed initial moisture content with respect to storage periods on germination was strikingly visible 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 reaching a value of 12 percent after nine months storage; for C_2 about 3 percent and 36 percent for six and none months storage respectively; for C_3 about 14 percent and 29 percent for six and nine months respectively; for C_4 about 5 percent and 0 percent for six and nine months storage respectively. The seed with low initial moisture content yields significantly better germination than the seed high initial moisture content after nine months storage for all the containers except C_4 where germination was practically zero for all the three initial moisture levels after nine months storage. These results corroborate findings of similar studies which showed that seed with low initial moisture moisture content is a key factor for maintaining seed viability in storage.

Results of statistical analysis confirmed the highly significant effect of storage periods on germination for various containers and initial moisture levels. Germination remained above 90 percent for all the containers for three months storage period. With lower storage periods divergence on the ability of the various containers to maintain seed viability is clearly evident with C_1 and C_2 being able to keep germination close to 90 percent, C_3 around 80 percent, and C_4 slightly above 50 percent for six months storage period. For nine months storage period only C_1 was able to maintain germination close to 90 percent, C_2 slightly above 70 percent, C_3 and C_4 produced an

unacceptably low germination of about 15 percent and practically 0 percent germination respectively.

The influence of storage condition on germination with respect to containers and storage periods indicate that: 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. Containers with marked increase of moisture content in storage were relatively more affected by insulation than containers with less moisture gain in storage. The effect of insulation tends to increase with the length of storage time. However, in terms of percent gain in germination of insulated over ambient storage conditions, the difference was relatively small. Results of statistical analysis demonstrate 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 considered practically insignificant and probably not worth the additional expense for storing the seed in insulated box. This apparent contradiction simply implies that a difference between the two storage conditions exists. However, caution should be exercised in applying this finding to ascertain that the benefits far exceed the additional expense.

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 favorable than in Mayaguez in terms of temperature and relative humidity. However, this has no significant effect on germination.

Considerable insect damage developed while the samples were in storage. While no actual count was made to determine the percent of seed affected, visual observation indicated that the damage was negligible for three months storage for all the containers but for the six and especially after the nine months storage the damage was clearly evident. The cloth bags revealed 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 for the sealed metal can treatment were probably contaminated during the preparation of the samples. Because of the proximity of the samples the ambient and insulated treatments were almost equally affected. The rice hull for insulation was intentionally used without chemical treatment to more truly represent the actual way of which the farmer will use it. But it turned out to be the likely source of the damaging insects.

Effect of Internally Seed-borne Microorganisms on Seed Deterioration in Storage - Phase I

The purpose of the plant pathology cooperation in this study is to relate the association of internally seedborne microorganisms to their effect on seed storage. A considerable amount of work has been conducted at the University of Illinois in an attempt to show the importance of seedborne organisms in seed quality deterioration. The majority of the work has been with a bacterium *Bacillus subtilis* which has been shown to reduce soybean seed quality drastically under poor storage conditions. Little work has been done showing the relationship of internally seedborne fungi to seed deterioration in storage.

Seed samples from 3, 6, and 9 month harvest periods of the storage experiment were given to the plant pathologist for determination of internally

seedborne microorganisms. A bioassay was conducted on each sample to obtain data concerning percent germination, fungi and bacteria.

The experiment was modeled as a factorial design. The factors of the experiment were (1) geographical storage location, (2) storage in ambient or insulated conditions, (3) initial seed moisture content, (4) storage container, and (5) time of beans in storage. The variety of soybeans used during these investigations was certified "Woodworth." The analysis of results involved the combination of both agronomic and plant pathological laboratory testing.

Previous investigations completed at the University of Illinois has shown the importance of the bacterium Bacillus subtilis as being a detrimental seed-borne bacterium associated with soybeans. The expression of this organism on soybeans has been found to be enhanced by poor storage conditions which include high moisture and temperature storage.

Seed samples from this experiment were both tested for germination in sandbench and in the laboratory for internally seedborne microorganisms. From studies in the laboratory, it was found that B. subtilis appeared to cause the major deterioration of the soybeans.

Of the four containers used, the sealed metal can was the best, second best was the unsealed metal can with a plastic lining, third best was the fertilizer bag with a plastic lining, and the cloth bag proved to be the worst. Seeds stored better with initially lower moisture levels. Results showed that the longer the soybeans were in storage the more they would deteriorate. This meant the loss in germination and the increase in occurrence of B. subtilis. Soybeans tested at the onset of the experiment expressed low percentages of internally seedborne fungi. Fungi subsequently during the experiment and analysis in the laboratory did not play a major role in the deterioration of the soybean seed.

This study clearly showed the importance of good storage practices in the tropics. Regardless of the quality of seed from either a good genetic background or from good agronomic practices, the inability to store such seed effectively will cancel all benefits. Storage problems of soybeans at the small farm level will hinder the development of soybean production and usage.

b) Phase II

Phase II of the seed storage experiments is currently being conducted. This experiment is similar to Phase I only it uses seed produced in Puerto Rico. In addition, seeds were placed into storage either treated or non-treated with fungicides.

The objectives of these experiments are as follows:

1. Determine moisture content of seeds.
2. Measure sandbench germination.
3. Measure germination, number and type of fungi and bacteria associated with seed on potato dextrose agar (DPA), bioassay.

The following variables were included:

1. Noninsulated and insulated storage conditions.
2. Nontreatment or treatment with Arasan (Thiram).
3. Three initial moisture levels
    a. 7%
    b. 10%
    c. 13%
4. Four container types
    a. sealed tin can
    b. nonsealed tin can
    c. plastic-lined fertilizer bag
    d. cloth bag

5. Three storage periods

   a. 3 months
   b. 6 months
   c. 9 months

This study is at present in the fifth month so only results from the first storage period are available. However, there appears to be a very large reduction in germination potential from some of the initial samples after three months. This reduction is closely associated with the initial moisture content of the seeds. All container types except the cloth bag seem to have an effect on maintaining germination potential.

Chaetomium sp. was the only fungus that increased noticeably during the first bioassay. However, no large reduction in germination was noted between seeds chemically treated and those not treated. Bascillus sp. was also observed on a large portion of the dead seed.

The final results of Phase II will be summarized in next years report.

c) Phase III

Phase III of the seed storage experiments will be initiated sometime this year. Information concerning Phase III will be presented in next years report.

(4) Chemical control of seedborne microorganisms during storage in order to measure germination potential.

(Plant Pathology)

The following variables were included in this experiment:

1. Five varieties

   a. Jupiter
   b. Improved Pelican
   c. Wells
   d. Amsoy-71
   e. Bonus

2. Chemical treatment of seeds before storage or after storage.

3. Chemical treatments

   a. Arasan (Thiram) - surface applied
   b. Benlate (Benomyl) - surface applied
   c. Benlate - applied using the organic solvent carrier Methylene chloride
   d. K Penicillin G - applied using Methylene chloride
   e. K Penicillin G and Arasan - applied using Methylene chloride and surface respectively

The storage time was ten weeks and all treatment variety combinations were stored in cloth bags in the greenhouse. The following parameters were measured:

1. Germination in sand

2. Bioassay; amount and type of seedborne microorganisms

3. Viability using a tetrazolium test

4. Field emergence

Results: The data from this experiment is at present being collected. It appears that the actual germination of seeds stored under the above conditions is reduced 0-15% by seedborne microorganisms depending upon the variety used. This is only a small portion of the overall loss in germination potential. The remainder can be attributed to seed deterioration and subsequent death.

There were no predominant fungi in this study. Very low percentages of *Aspergillus*, *Penicillium* and *Alternaria* were recorded. The most frequently encountered organism was *Bascillus sp.*

It was noted that Arasan (Thiram) appears to reduce the amount of *Bacillus sp.* on agar plates.

When surface sterilants were used in this study the germination of seeds were much higher than was recorded in the field or sandbench. It would

appear that seeds placed under this type of tropical storage condition deteriorate rapidly (10 weeks) and although not dead, are in a weakened condition. These conditions are favorable for invasion of soil pathogens in the field. Sandbench germination was much higher than field emergence.

(5) Chemical control of seedborne pathogens - Foliar applications of fungicides.

In most crop production programs chemical controls of pathogens have played a major role. This is also beginning to become the case with soybeans. A considerable amount of research conducted at the University of Illinois has shown the excellent potential of using fungicides to both control diseases in soybeans and increase yields. It was from this foundation that these investigations were initiated. An identical replicated fungicide spray field experiment was established at the Agricultural Experimental Substations of Adjuntas, Fortuna, and Isabela to test the effectiveness of the fungicides Benlate and Dithane M-45. The variety used during these investigations was "Bonus." The chemicals were applied to experimental plots of soybeans at the rates of either 1/4, 1/2, or 1 lb. per acre. The timing period of application also varied being either at early pod, early pod and two weeks later, or only two weeks later. Two years of similar studies had been conducted at the University of Illinois. It was the goal of this experiment to see if this spray program would work under tropical conditions.

At the Isabela substation Cercospora was the most predominant fungal pathogen in the non-sprayed plots. This fungus was controlled effectively by both of the fungicides tested. The effectiveness of the fungicides on controlling seedborne pathogens of soybeans that kill the seed could not be

assessed since in the spray fields of Isabela and Fortuna there was only 5-6% dead seed assayed in any treatment including non-sprayed plots due to seed-borne microorganisms. In such low percentages of disease, it is difficult to measure statistical differences in disease control. It is usually under delayed harvest situations that these chemicals express their true potential of protecting the seed against the invasion of seedborne diseases. Situations such as this often develop when machinery cannot go into the field for harvesting during periods of heavy prolonged rains.

(6) Effect of germination temperature on the composition of internally borne microflora recovered from soybean seeds.

The service goal of the mycology section is to provide fungal identifications and ecological data for storage, breeding, and plant pathological investigations. The research goal of the mycology section is to test the applicability of the concepts of general ecology to plan pathological problems.

A study is currently underway to analyze the community structure of soybean fungi along a temperature gradient. The three, immediately practical results of the experiments are: 1) determination of the best temperature for seed quality testing of seeds produced in the tropical environment of Puerto Rico (this information is needed in the breeding project). 2) to discover whether each seed harbors several internally borne fungi only one of which is activated under a particular set of conditions; and 3) to determine the optimum temperature for pathogenesis by each of the common seedborne fungi. The latter two results are important to the development of seed storage practices in the tropics.

Of more general interest is the study of the ecological diversity of these fungal communities. This information will lay the theoretical groundwork for possible biological control of plant pathogens. By analyzing the eveness of distribution of the individual among the species present, dominance patterns for each species can be determined. These patterns indicate how well a species can compete with other microorganisms across a range of conditions (in this case temperature). They will also show whether dominant fungal species tend to be generalist with the capacity to occur over a broad range of conditions as has been suggested by studies with higher plants. Preliminary results indicate that the expected decrease in species richness and ecological diversity and increase in dominance to indeed occur in fungal communities as harshness of the environment increases.

Additional temperature experiments with insolates of the dominant species from the different temperatures will reveal the degree of correspondence between fundamental and realized niche widths and will indicate whether the breath of the fundamental niche is due to genetic heterogeneity or phenotypic plasticity. (The investigators are aware of the possible complications of heterokaryosis).

The final result of these experiments will tell us whether those fungi best able to compete with the plant pathogens are generalists or are specialized for the limiting factor in each particular set of conditions. We must know this in order to plan a strategy for the microbiological control of plant pathogenic fungi.

Preliminary Results:

More than 25 species of fungi were isolated (Table 4, see appendix), most of which are considered to be saprofites or weak secondary pathogens. Of the 10 species not previously reported from soybeans only Botryodiplodia theobromae and Chaetomium globosum, the second and third most abundant species, are of potential pathological importance. B. theobrome is one of the most common secondary pathogens in the tropics and in this study was consistantly associated with seed infected with Diaporthe phaseolorum.

D. phaseolorum, by far the most abundant fungus, appeared to have a temperature optimum of about 25°C and its frequency was drastically reduced at 35°C and above. The relative abundance of minor species increased at 35°C. The optimum temperature for total fungi was 25°C.

Perhaps the most interesting results occured at 35° and 40°C where the number of bacteria and % seed germination increased while the number of fungi decreased (Table 5, see appendix). Though no attempt was made to identify the bacteria, virtually all of the colonies resembled those of Bacillus subtilus identified in other studies.

A complete report of this investigation is being prepared for publication.

c. Thesis Research

Five graduate students, including four from Latin America LDC's were partially supported with grant funds to conduct their research for the M.S. thesis during the reporting period. The name of the students and their respective thesis titles and objectives are shown as follows:

Ernesto Leypón

Title: Etiology and control of the most important bacterial diseases of soybeans in Puerto Rico.

Objectives: To isolate, characterize and identify the pathogenic bacteria occurring on soybeans under different ecologic and climatic conditions in Puerto Rico. Conduct pathogenicity trials with the most prevalent and pathogenic bacteria and to determine the effectiveness of some bactericides in controlling these diseases.

Summary of Results: Diseased material for isolation purposes was collected from the foliage of experimental plantings established at the Isabela, Corozal and Adjuntas Substations. A total of 13 pathogenic cultures were identified, eight belonging to *Xanthomonas phaseoli* var. *sojensis*, the bacterial pustule organism, and five to the genus *Pseudomonas*. Four of the *Pseudomonas* were isolated from lesions induced by the pustule organism.

These investigations demonstrated that in Puerto Rico exist two bacterial diseases of soybean namely bacterial pustule caused by *X. phaseoli* var. *sojensis* and wildfire caused by

Pseudomonas tabaci. The first disease represents a menace to the successful cultivation of soybean in Puerto Rico and in other tropical areas if resistant material is not used to establish field plantings. On the other hand, wildfire is not common, occurring sporadically in field plantings. However, it was shown that damage to the foliage was far greater when both Xanthomonas and Pseudomonas interacted in the same lesion. This occurred both in the greenhouse and in the field. It is suspected that a symbiotic association of the metabolic type occurs between these two organisms where X. phaseoli modifies the substrate for subsequent invasion of the tissues by Pseudomonas tabaci. The latter organism contributes to the syndrome by increasing the severity of the disease.

Based on the results on chemical control, where the copper compounds effectively protected the foliage of soybean against the disease, we can suggest the use of these chemicals when resistant cultivars are not readily available. Moreover, a combination of resistance and chemical control would prove ideal in securing higher yields in areas where the disease occurs.

José A. González

Title: Effects of the amount of inoculum on the epidemiology of purple seed stain disease of soybean.

Objectives: Study the effect of levels of inoculum present on soybean seeds on the incidence of the disease and the relationship between climate and disease prevalence.

Summary of Results: Field plots were planted with seed lots of soybean (Glycine max (L.) Merrill) cultivar Amsoy 71 with 0, 10, 25, 50, 75, and 100% seeds infected with Cercospora kikuchii. Emergence, plant size, and yield were significantly reduced when 10% or more of the seeds used were stained. Percentage of stained seeds recorded at harvest was significantly greater for plots planted with lots containing 25% infected seeds than for those established with disease-free seeds. Daily amount of C. kikuchii inoculum present in the field was monitored with a Burkard volumetric, continuosly sampling spore trap. Higher concentrations of inoculum were detected at mid morning and mid afternoon with the highest around 11:00 A.M.. Higher inoculum levels were recorded during September through November when the mean diurnal temperature ranged from 24-26C and rainfall reached a maximum of 209 mm. A positive correlation ($r = 0.248$), significant at the 5% level, was obtained between daily average temperatures and the amounts of spores detected in the field. These levels coincided with stages of pod setting and seed development of the soybean plants.

Ricardo León

Title: Effect of date of planting and locations on yield, yield components and chemical composition of three soybean cultivars.

Objectives: To study the effect of date of planting and locations on the yield, yield components and chemical composition of the seed on early, intermediate and late varieties of soybeans.

Summary of Results: Soybean cultivars Williams, Davis and Hardee, considered as early, intermediate and late maturing for Puerto Rico were planted at two ecological locations; namely Isabela and Lajas, during the four seasons of the year in replicated trials. Date of planting, location and varieties influenced significantly yield and the yield components. The variety x date of planting interaction was highly significant for most characteristics studied and of greater magnitude than the variety x location interaction. This tends to indicate that date of planting has a higher influence in varieties performance than locations. Highest yields were obtained during the spring and summer plantings. Highest yielding variety for all planting dates was Hardee, followed by Davis and Williams, respectively. Seed quality was superior in the winter and spring plantings because of the lower temperatures and rainfall. Varieties and locations had no significant effect on oil and protein percentages but they were affected somewhat by date of planting.

César Hananía

Title: Control of the root-knot nematode (*Meloidogyne* spp.) on soybeans.

Objectives: To screen varieties for resistance to the root-knot nematode and to evaluate and determine the optimum dose of systemic nematicides for the control of the nematodes.

Summary of Results: Greenhouse studies were conducted where 19 varieties of soybeans were screened for resistance to different isolates of the root-knot nematode Meloidogyne incognita. Fresh and dry weights of stem and leaves, including immature pods, stem length, root length and nematode populations in the roots and soil were made 60 days after inoculation. Varieties Bonus, Improved Pelican and Tracy were moderately susceptible; Williams and Bragg were highly susceptible to the Urena lobata isolate of M. incognita. For the sweet potato isolate; Bonus, Semmes, and Otootan showed a low degree of susceptibility; Jupiter was moderately susceptible and Hill and Forrest were highly susceptible. For the isolate pepper I, Ramson was resistant. Davis, Hardee, Improved Pelican and Clark-63 were moderately susceptible and Bragg was highly susceptible. For isolate pepper II, Colland and Cocker-Hampton were resistant, Lee-68 and Williams were moderately susceptible and Hill and Adolfia highly susceptible.

Three different dosages of three systemic nematicides (Furadan, Temik and Nemacur) were applied at planting time at two locations, Isabela and Adjuntas, using Bonus soybean as test variety. At Isabela the best results were obtained with the application of 12.5 kg/ha of Nemacur, followed by the same dose of Furadan. At Adjuntas, Furadam at 20 kg/ha was significantly better than the other treatments.

Federico Cuebas

Title: Mutagenic specificity of 5-bromodeoxyoridine in germinating soybean (Glycine Max (L) Merrill) seeds.

Objectives: To study (1) the efficiency of BUDR as a mutagen in soybeans and (2) to study the mutagenic specificity of BUDR as a function of treatment period during seed soaking.

Summary of Results: Soybean seeds, in the second cell cycle during germination, were treated with 5-bromodeoxyuridine (BUDR) at the concentration of 150 ug/ml. Seeds were soaked for 1, 5, 7.5, 10, 12.5 and 15 hours at 25°C. The treated materials were grown in the field and M_2 progenies were screened for mutations at seedling stage. It was found that BUDR was an effective mutagen for inducing genetic alterations in soybean seeds. The highest overall mutation frequency was reached at the peak of the S-period. Four types of mutations were recovered with the unidirectionally oriented first leaf pair type being the most frequent, the twisted first leaf pair type next, the twisted central leaflet type, the third and the twisted lateral leaflets type the least. The latter two types which affected the first trifoliate leaves were found most abundant in the treatment where the seeds were treated at 15 hrs. in the post cold treatment soaking, indicating clearly the dependence of induction of specific types of mutations by BUDR on treatment period. On the other hand, the former two types were induced most readily

BEST AVAILABLE COPY

when the seeds were treated at 12.5 hrs. in the post cold treatment soaking. But these two types also tended to spread over other treatment periods.

The DNA synthetic period was determined by the ^{3}H-thymidine labeling study in soaked soybean seeds. The S-period of the first cell cycle in the germinating seeds was found to peak at 16 hrs. of seed soaking at 25°C and that of the second cell cycle at 12.5 hrs. during the post cold treatment soaking at 25°C.

The above mentioned graduate students, two from Nicaragua, two from El Salvador and one from Santo Domingo, have completed their requirements for the MS degree and returned to their countries.

Miguel A. López

Title: Identification of the races of field bean rust (*Uromyces appendiculatus* Pers. Unger) present in Puerto Rico.

Objectives: To study the prevalence of different and most virulent races of bean rust in Puerto Rico and their control with chemical fungicides.

Three new students; from Ecuador, Columbia and Puerto Rico started graduate work toward an MS degree in Crop Protection during the first semester of academic year 1976-77. They will be doing their thesis research with the food legumes program.

(7) Summary of cooperative field and laboratory research.

Although the University of Puerto Rico does not have a research contract for soybeans, staff members are actively engaged in research funded by AID for the Soybean Resource Base. Most of the staff and facilities cooperation has been on research such as variety trials, variety date of planting - plant population studies, seed storage studies, plant pathology and entomology studies and seed quality studies. The on-going research work in these areas has been intensified considerably during the reporting year. New laboratory facilities at the College Farm (la Finca) are being developed and the laboratory research work in the area of seed pathology has developed into a very strong program for research and training for both universities. These accomplishments are reported in the previous sections.

2. Education and Training. In addition to the new courses which were previously incorporated into the teaching program, the following new courses were offered during the academic year:

(a) Plant Science 560 - Insects of Tropical Crops. Three credit hours. Two one-hour lecture and one three-hour laboratory per week.

(b) Plant Science 581 - Nematode Diseases of Plants. Three credit hours. Two one-hour lectures per week and one three-hour laboratory per week.

(c) Plant Science 601 - Properties and Actions of Herbicides. Three credit hours. Two one-hour lectures per week and one three-hour laboratory per week.

(d) Plant Science 608 - Advanced Phytopathology of Tropical Crops. Four credit hours. Two two-hour lectures per week.

(e) Plant Science 648 - Plant Physiology and Crop Productivity. Three credit hours. Two lectures and one three-hour laboratory per week.

Four graduate students from the University of Illinois have been stationed at our Campus to conduct their thesis research problems under tropical environment. Arrangements are underway for two more students. In a similar way five staff members of our College of Agricultural Sciences are working toward their Ph. D.'s in the area of crop protection at the University of Illinois.

Dr. Michael Ellis, Plant Pathologist, was appointed Assistant Professor on a 50 percent basis of his time and salary from the grant. Dr. Ellis will be teaching an undergraduate course in Plant Pathology and conducting research in the area of plant pathology and seed pathology of food legumes, i. e., soybeans, field beans, cowpeas and pigeon peas.

3. Advisory Capacity. Staff members participating in grant activities have acquired new advisory capacity through research in relevant areas by participation in workshops, seminars, dialogue with colleagues from U.S. and LDC institutions or in international centers and on-site experience in tropical countries. Their names, field of specialization and advisory capacity functions are tabulated below:

Name	Specialization	Functions*
Dr. Pedro Melendêz	Plant Pathology	1, 2, 5
Dr. Guillermo Riveros	Weed Scientist	1, 2, 4, 5
Dr. Michael Ellis	Plant Pathologist	1, 2, 4, 5
Dr. Raúl Abrams	Plant Breeding	3, 4, 5
Dr. A. Ayala	Nematology	1, 2, 5
Dr. J. Bird	Virology	3, 4, 5
Mrs. A. Cortes	Bacteriology	1, 2
Dr. C. Cruz	Entomology	1, 2, 5
Dr. H. Paschal	Plant Breeding	3, 4, 5

*Functions
1 - Problem identification
2 - Problem analysis
3 - Project design
4 - Resource evaluation and planning
5 - Education and training

4. Information Capacity. On account of a strong staff field research program and student thesis research program, a large amount of information, data and materials relative to soybean breeding, weed control, insect control, plant pathology, nematology and seed pathology pertaining to the tropics and with practical applications to the LDC's of the tropics is becoming available. This information and data is being published in scientific journals of U.S. and Puerto Rico and will be distributed among the different LDC's of the tropics and international centers that have an interest in the production of soybeans and other food legumes for human consumption. A technological package of recommendations for soybean production, protection, and utilization for the tropics is to be prepared for the near future for distribution.

5. Linkages. During the grant period linkages have been developed with the following institutions:

(a) International Crops Research Institute for the Semi-Arid Tropics, Hyberadad, India.

(b) Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

(c) Instituto Colombiano Agropecuario (ICA), Colombia.

(d) Instituto Interamericano Ciencias Agricolas, (IICA), El Salvador, San Salvador.

(e) Instituto Nacional de Investigaciones Agropecuarias (INIAP), Quito, Ecuador.

(f) Universidad de Panamá, Panamá.

(g) International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

(h) Centro Internacional de la Papa (CIP), Lima, Perú.

(i) Ministerio de Alimentación, Lima, Perú.

(j) Asian Vegetable Research and Development Center (AVRDC), Taiwan.

At the national level linkages have been established with the Mayaguez Institute of Tropical Agriculture, Agricultural Research Service, U.S.D.A., Beltsville, Iowa State University, North Carolina State University, Mississippi State University, University of Hawaii and other U.S. universities.

These linkages were established in part through the 211(d) grant and the INTSOY program.

Some of these linkages have been very fruitful resulting in joint research and cooperative projects with the food legumes. At the present time we have joint research projects with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Cooperative projects with the University of the West Indies, Trinidad, University of Panamá, IITA and ARS, Plant Introductions, were conducted during the reporting period. Relationships

are maintained with many scientists and institutions as well as exchange of information and materials with domestic and foreign scientists.

F. Impact of Grant Supported Activities in Achieving Grant Purpose

The grant supported activities are considered an integral part of the overall effort of the Agronomy Department. As a result of this, the majority of the staff members and also a significant group of undergraduate and graduate students have shown a keen interest in contributing or getting involved in the grant program, regardless of the source of individual funding.

The grant has strongly influenced the teaching program since it has stimulated the offering of a new option in the department in the area of Crop Protection. This new option has been already approved by the Faculty and consists of thirteen courses at the undergraduate and graduate levels, respectively. In our revised curriculum four of these courses are required for all students majoring in our department. The majority of the departments in our Faculty have also included at least two of these Crop Protection courses as requisites. By providing improved laboratory facilities, audiovisual equipment and increased library resources, the grant has favorably enhanced the quality of teaching. As a direct result of this, the number of graduate students in the area of Crop Protection in legumes and other crops is fifteen, a fivefold increase in three years.

Teaching and research laboratory and field facilities have been improved significantly since the implementation of the grant. New laboratory facilities, including five greenhouses, have been made available for students and staff members at the College Farm for research and teaching. The laboratory

facilities for plant pathology and nematology in our main building have been redesigned and are already available for our students. As a direct result of this we have had a significant increase in the number of students, both graduate and undergraduate majoring in Crop Protection. It has also provided a firm base in support of the student exchange program with the University of Illinois.

The grant enabled the appointment of three new staff members. At the termination of the grant all these staff members will have tenure with the University of Puerto Rico and the experience gained in the performance of grant activities will continue to be available to the institution. Two technical assistants also appointed with grant funds will remain with the institution and their experience will be an asset for the field and laboratory research program of the department.

Grant funds have been used to provide travel support for staff members. This provides a two fold benefit for the institution and the program: (1) by broadening the professional experience and establishment of professional contacts with scientists from other countries and universities and (2) new linkages and establishment of new joint research projects in Central America, ICRISAT and IITA, as well as exchange of information and materials with domestic and foreign scientists.

G. Other Resources for Grant-Related Activities

The grant contributes to the overall teaching commitments of the Department of Agronomy of the University of Puerto Rico. As the chief goal of the grant is to strengthen the existing competency, the funds provided by this

grant cannot replace existing funds for current projects. On the contrary, activities carried under this grant have been additives to existing programs at the University.

In direct support to the grant, the University has been providing and will continue to provide:

1. Administrative costs including salaries and utilities in all administrative officers and facilities. The basic salary of the project leader, Dr. Raúl Abrams, $23,664.00 comes from the University budget. No funds from the grant are used to cover costs of services of senior administrative officers in the College of Agricultural Sciences.

2. Access to all persons concerned or related to the grant to relevant laboratories, field research facilities, and libraries. The facilities of the Agricultural Experiment Station and Substations are also available. The field research under the grant is using field plots at Ponce, Adjuntas, Lajas and Isabela Substations. This land is provided free of charges for the grant program. The services of the Central Analytical Laboratory of the Agricultural Experiment Station, the soil testing laboratory at Mayaguez and of the Nuclear Center are also available free of charge. Money wise, these contributions are very difficult to assess and they are not shown in Table 6.

3. Office, classrooms, laboratories, and other space for faculty and students and special meetings related to the new program. In addition the University provides to all persons concerned or related to the grant all services and facilities that are normally provided to the regular staff and to the regular students. These contributions are also very difficult to assess and are not shown in Table 6.

4. The members of the faculty in the Agronomy Department not directly funded by the grant are considered an integral part of the grant program. These salaries, are shown in Table 6 as university support to grant supported activities.

5. The sub-professional personnel of the Department who are under university, state funding are considered also an integral part of the grant program and take part in related activities. Their salaries are also shown in Table 1.

H. Utilization of Institutional Response Capabilities in Development Programs

(1) At the request of Dr. Fred Beinroth, Project Director of the Soils Benchmark Project; Drs. Guillermo Riveros, H. Paschal and Michael Ellis, participated in the planning and development of soybean trials for this project to be established in Puerto Rico, Brazil and Venezula for technology transfer studies.

Drs. Michael Ellis and Pedro Meléndez have been participating and cooperating with ARS personnel in studies and planning for a workshop in soybean rust to be held in Puerto Rico.

At the request of the University of Illinois, Dr. R. Abrams participated in a short orientation and training program on soybean production in the U.S. for a group of six professionals of the Ministry of Food of Perú .

Mr. Albert Zumbiehl, advisor for the Republic of Gabon, visited our university and requested our services for information on soybeans and for the establishment of an ISVEX variety trial in Gabon. These requests have been accomplished.

A joint research program in the area of pigeon pea variety selection and production between the University of Puerto Rico and the Universities of Panamá and El Salvador in Central America has been continued during the reporting period. The National Agricultural Institute from Venezuela has also requested our cooperation in this same area.

(2) During the academic year 1975-76, the College of Agricultural Sciences had an enrollment close to 650 students of which approximately 10 percent came from the following foreign countries: Belize, Bolivia, Chile, Colombia, Dominican Republic, Haiti, El Salvador, Panamá, Nicaragua, Guatemala and Venezuela.

At the graduate level the total number of students was 58 of which 24 or approximately 41 percent were from LDC's. They came from the following countries: El Salvador 4; Nicaragua 3; Cuba 1; Guatemala 2; Dominican Republic 3; Brazil 1; Colombia 5; Perú 1; Panamá 2; and Ecuador 2.

During the reporting period two of the graduate students funded by the grant, M. E. Leypón and Mr. Ricardo León, from Nicaragua and El Salvador, respectively, received their M.S. degree in Crop Protection and Crop Breeding from our College of Agricultural Sciences. Their thesis title was: Etiology and Control of the Most Important Bacterial Diseases of Soybeans in Puerto Rico and The Effect of Date of Planting and Locations on the Yield and Yield Components of Three Soybean Cultivars in Puerto Rico.

Three more graduate students will finish their degrees in Crop Protection during the next academic year.

The following distinguished scientists visited the Mayaguez Campus during the reporting period:

Dr. D. Sharma
Plant Breeder
International Crops Research Institute for
the Semi-Arid Tropics (ICRISAT)
Hyderabad, India

Miss Djaliba Bruce
Plant Breeder
Tropical Institute of Agricultural Research (IRAT)
Agoueve, Togo

Mr. Albert Zumbiehl
Industrialist
Advisor for Republic of Gabon

Dr. Richard L. Cooper
Plant Breeder
University of Illinois

Dr. Harry Minor
Agronomist
University of Illinois

Dr. James Sinclair
Plant Pathologist
University of Illinois

Mr. Heleodoro Miranda
Principal Investigator
Interamerican Institute of Agricultural Sciences
San Salvador, El Salvador

Drs. Cooper, Minor and Sinclair presented seminars during their visit for the benefit of our students and staff members.

I. Next Year's Plan of Work and Anticipated Expenditures.

The cooperative work with the University of Illinois, in all areas, i.e., breeding, pathology and entomology will continue very strong. This program has broadened considerably during the reporting year and we feel very optimistic and enthusiastic with the results obtained thus far. This is a unique program, particularly in the aspect of seed quality and pathology of soybeans and other food legumes for the tropics. This program is interdisciplinary and involves a plant breeder, two agricultural engineers, a mycologist, a weed control man, two plant pathologists and graduate students.

Other areas that will be broadened are 1) entomology, specially insect vectors of virus diseases, 2) utilization in the plant breeding program of the accomplishments obtained from the seed pathology research work and 3) the possibility of including the utilization aspect in the program. The Experiment Station has excellent facilities and personnel for food technology and it is our thinking that the utilization portion as applied to tropical LDC's is very important and should be considered as part of our future plans with soybeans.

The weed control research work has been expanded and the results obtained already are very promising. Further studies will be conducted with chemical and complementary mechanical weed control and frequency of treatments for test control.

The new curriculum has already been approved by the Faculty and is pending of approval by the Academic Senate. This new curriculum included an option in Crop Protection both at the undergraduate and graduate level. Most probable it will be in effect during the next academic year. Many of the courses, developed by the grant have been approved by the Office of the

Dean of Studies and have become an integral part of the department's teaching program. They will continue to be offered during the academic year 1976-77 by grant-supported staff and will be evaluated and modified if necessary and appropriate.

The University of Puerto Rico will continue and broaden the cooperation on joint projects with the University of Illinois during 1976-77. The graduate student exchange program between the two universities will be continued and expanded. The results and experiences from all these joint programs with the University of Illinois have been very fruitful and rewarding for both universities.

The grant will continue to support the research conducted by graduate students for their theses.

In addition the following effort will be continued:

1- New courses on the subject of crop protection will be offered.

2- Physical layouts and facilities will be added or modified.

3- More graduate students will be recruited.

4- New laboratory facilities and equipment will be developed to support teaching and research activities of the program.

It is intended that staff members related to the program will attend scientific matters of mutual interest thus increasing their capabilities to work toward the development of a soybean and other food legumes for the tropical and subtropical regions of the world.

It is estimated that the anticipated expenditures related to the above mentioned activities will be as follows:

Salaries:	
Professional	45,000.00
Graduate Assistants	6,000.00
Technical Aids	12,330.00
Clerical	5,265.00
Stipends	
Fringe benefits	3,000.00
Travel	
Domestic	3,000.00
International	10,000.00
Departamental Support	
Equipment	5,000.00
Library and teaching aids	2,000.00
Supplies, Materials and Services	10,000.00

Research

Laboratory and field trials in the area of plant pathology, nematology and weed control will be conducted at Mayaguez, Isabela, Lajas, Fortuna and Adjuntas Substations.

The close collaborative and cooperative projects in the area of breeding, entomology, pathology and agricultural engineering with the University of Illinois will be continued at the above mentioned sites.

It is estimated that the expenditures related to the above activities will be as follows:

Wages (Field laborers)	12,000.00
Supplies, materials and services	5,000.00

J. Involvement of Minority Personnel and Women.

The grant has provided opportunities of employment for one woman in a clerical position at the department, two graduate assistants majoring in plant pathology and a part time technical aid in seed pathology. Our future plans related to the graduate assistant are to incorporate her as part of our staff in the department as soon as she obtains her Ph. D.

Table I

Distribution of 211(d) Grant Funds and Contributions From Other Sources Funding*

Reporting Period October 1, 1975 to September 30, 1976

Grant Objectives/Outputs	Period Under Review	211(d) Expenditures		Projected to end of Grant	Non 211(d) Funding* Amount
		Cumulative Total	Projected Next Year		
Knowledge Base	60,901.36	137,513.77	65,000.00	280,000.00	32,000.00
Teaching	33,495.75	107,421.18	45,500.00	170,000.00	25,000.00
Information Capacity	2,350.43	3,761.31	4,000.00	15,000.00	
Linkages	4,754.73	19,255.22	11,000.00	35,000.00	
Total	101,502.27	267,951.48	125,500.00	500,000.00	57,000.00

* These figures are our best estimates.

Table II-A

211(d) Expenditure Report

Actual and Projected Summary

Under Institutional Grant #AID/ta-G-50

	Expenditures to date		Projected Expenditures YEAR		Total
	Reporting Period	Cumulative Total	4	5	
Salaries	61,862.64	160,267.46	75,000.00	80,000.00	315,267.46
Employees Benefit	7,182.55	10,418.71	5,000.00	7,000.00	22,418.71
Travel & Subsistence	22,727.85	37,228.40	12,000.00	15,000.00	64,228.40
Supplies & Materials	7,035.11	23,582.39	8,000.00	8,000.00	39,582.39
Research Support	2,694.12	36,454.52	10,000.00	12,049.02	58,503.04
					500,000.00

Table II-B

211(d) Expenditure Report

Reporting Year Detail

Under Institutional Grant #AID/ta-G-50

Reporting Period Oct. 1, 1974 to Sept. 30, 1975

I. A. Salaries		
Academic	Percentage Time	Amount
R. Abrams		2,400.00
P. Meléndez	50	10,792.00
M. Ellis	50	2,133.00
G. Riveros	100	18,000.00
C. Cruz	25	1,500.00
B. Clerical	100	4,680.00
Technical Aids (2)	100	11,160.00
Field laborers		5,925.60
C. Fringe Benefits		7,182.55
II. Student Support		
Ivonne Shattuck	U.S.A.	1,500.00
Ernesto Leypón	Nicaragua	750.00
R. Leon	El Salvador	1,500.00
A. González	Nicaragua	1,500.00
III. Guest Lecturer		
One		825.76

Cont. Table II-B

	Percentage Time	Amount
IV. Travel		
A. Domestic		7,900.49
B. Foreign		14,827.36
V. Equipment		
VI. Library Acquisitions		875.00
VII. Publications		None
VIII. Other		8,051.00

Table III-A

Requests for Assistance Received During Reporting Period October 1, 1975 to September 30, 1976

A. Requests Attended

Description of Request for Assistance	Whom did you Assist?	Who Requested Assistance	Who Funded Assistance	Size of Effort		Result of Assistance
				Dollar	Man Days	
Technical assistance: To evaluate soybean production areas in Peru	U.I.-AID	AID Mission Peru	BOA-1109 AID-Peru	1,900.00	10	Report and recommendations to AID Mission-Peru
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	ICRISAT	ICRISAT	---	---	30	Joint Research Program in Pigeon Peas
Evaluation of Pigeon Pea Germplasm Collection	ARS-USDA	ARS-USDA	ARS-USDA	18,000.00	90	Evaluation & increase of 4,200 accessions of Pigeon Pea
International Institute of Agricultural Sciences (IICA) of the Organization of American States (OAE)	IICA-Panama Costa Rica and El Salvador	IICA-OAE	---	---	5	Continue Joint Research Program in Food Legumes

Appendix

Table 1

Results of the Cooperative (UPR-UIUC)
Soybean Seed Treatment Trial 1976

Treatment[1/]	Rate	Mean Stand Count (21 days) Urbana	Mayaguez
Penicillin G + PEG + Arasan 50 Red	1,000 units + 0.45 g/ml water + 2 oz/100 lbs	65 A	63 AB
UBI 1127	2 oz/100 lbs	61 AB	50 FG
Mertect LSP 30	4 oz/bu	61 AB	60 ABCD
Zinc Omadine	4 oz/bu	58 ABC	62 ABCD
UBI 1127	4 oz/100 lbs	58 ABC	63 ABC
Mertect LSP 30	2 oz/bu	56 ABCD	55 DEF
Penicillin-G + DCM + Arasan 50 Red	1,000 units/ml DCM + 2 oz/100 lbs	56 ABCD	65 A
Penicillin-G + DCM	1,000 units/ml DCM	56 ABCD	49 FGH
Demosan 65W + Arasan 50 Red	4 + 2 oz/100 lbs	54 ABCDE	55 BCDEF
Vitavax Flowable (34%)	2 oz/100 lbs	52 ABCDEF	51 FG
Arasan 50 Red	2 oz/100 lbs	51 BCDEFGH	53 EF
Soybean Seed Protectant (MO) (HB)	4 oz/bu	49 BCDEFGH	52 F
Soybean Seed Protectant (MO) Exp. (HB)	2 oz/bu	48 BCDEFGHI	51 F
Terra-Coat L-205 + zinc Omadine	4 + 2 oz/bu	48 CDEFGHI	62 ABCD

Table 1 (continued)

Treatment[1/]	Rate	Mean Stand Count (21 days) Urbana	Mayaguez
Terra-Coat ZN 2055	5 oz/bu	46 DEFGHIJ	53 EF
Demosan 65W + Arasan 50 Red	2 + 2 oz/100 lbs	45 EFGHIJ	50 FG
UBI 1109	4 oz/100 lbs	43 EFGHIJK	55 CDEF
Orthocide 75 WP	1 oz/bu	42 EFGHIJK	63 ABC
BAS 317 Tech 97%	40 g a. i. /bu	42 EFGHIJK	42 HI
UBI 1109	2 oz/bu	41 FGHIJK	43 GHI
Difolatan 4 FL	1.5 oz/bu	40 FGHIJK	51 F
Penicillin-G + PEG	1,000 units + 0.45 PEG/ml water	39 GHIJK	39 IJ
BASF 317 Tech 97%	20 g a. i. /bu	39 GHIJK	39 IJ
UBI 1109	1 oz/bu	39 GHIJK	40 IJ
Vitavax 25 DB	2 oz/bu	38 GHIJK	39 IJ
CGA-1-82 (50 WP)	0.75 oz/100 lbs	37 HIJK	39 IJ
BASF 317 Tech 97%	60 g a. i. /bu	36 HIJK	40 IJ
Terra-Coat L-205	4 oz/bu	35 IJK	15 L
Nontreated (control)	---	33 JK	28 K
Demosan 65W	4 oz/100 lbs	32 JK	32 JK
CGA-38140 (50WP)	0.75 oz/100 lbs	31 K	33 JK

1/ PEG = polyethyleneglycol; DCM = dichloromethane;

Table 2

Results of the UPR
Soybean Seed Treatment Trial 1976

Treatment[1] and Rate	Mean Stand Count (20 days)[2]
Penicillin-G + DCM 1,000 units/ml DCM + Arasan 50 Red 2 oz/100 lbs	84 a
Arasan 50 Red 2 oz/100 lbs	84 a
Vitavax Flowable (34%) 2 oz/100 lbs	83 a
Zinc Omadine 4 oz/bu	83 a
UBI 1127 4 oz/100 lbs	83 a
Penicillin-G + PEG 1,000 units + 0.45 g/ml water + Arasan 50 Red 2 oz/110 lbs	82 ab
Orthocide 75WP 1 oz/bu	81 ab
UBI 1109 4 oz/100 lbs	80 ab
Soybean Seed Protectant (MO) (HB) 4 oz/bu	78 b
Mertect LSP 30 4 oz/bu	74 c
Demosan 65W 4 oz/100 lbs	74 c
Penicillin-G + DCM 1,000 units/ml DCM	70 d
Penicillin-G + PEG 1,000 units + 0.45 g/ml water	68 de
Terra-Coat ZN 2055 5 oz/bu	66 e
Nontreated (control)	60 f

[1]PEG = polyethyleneglycol; DCM = dichloromethane.

[2]Letters indicate Duncan's Multiple Range groupings of treatments which do not differ significantly at the .05 level.

Table 3. Effect of Different Storage Variables on Germination of U. S. Soybean Certified Seed Stored in the Tropics

Variable Code	Description of Variable	Mean Germination Percent	Mean Moistu Content, perc dry basis
Container			
C_1	Sealed metal container	91.9	10.6
C_2	Metal can with one mil plastic lining	85.9	11.5
C_3	Fertilizer jute bag 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 box 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

BEST AVAILABLE COPY

Table 4. Fungal Species Recovered from Soybean Seeds, Ranked by their Total Abundance At All Temperatures

1. Diaporthe phaseolorum
* 2. Botryodiplodia theobromae
* 3. Chaetomium globosum
4. Cercospora kikuchi
5. Fusarium semitectum
* 6. Penicillium notatum
* 7. Aspergillus oryzae
* 8. Aspergillus chevaliere
9. Aspergillus flavus var. columna
* 10. Cladosporium cladosporioides
11. Penicillium spp.
* 12. Chaetomium cuprinum
13. Alternaria alternata
14. Aspergillus niger
* 15. Phoma herbarum
16. Nigrospora oryzae
17. Macrophomina phaseolina
18. Mucor sp.
* 19. Eremascus sp.
* 20. Periconia minutissima
21. Unidentified steril mycelium
22. Aspergillus sidowii
23. Curvularia lunata
24. Acremonium sp.

* Species not previously reported from soybeans

Table 5. Abundance of Principle Fungal Species, Total Fungi and Bacteria, Number of Fungal Species, and % Seed Germination

Species	20°	25°	30°	35°	40°
1.*	45.6**	49.2	40.0	16.0	0.6
2.	8.0	4.4	4.8	9.8	2.2
3.	1.6	4.2	2.8	2.6	1.0
4.	2.8	2.0	1.8	1.0	0.2
5.	1.4	1.6	1.8	1.0	0.0
6.	1.4	1.6	1.2	0.6	0.2
% seeds w fungi	60.0	73.0	56.4	34.6	3.6
% seeds w bacteria	12.8	14.5	27.8	36.8	57.8
% seed germ.***	6.5	6.5	4.2	11.2	16.2
No. fungal sp.	19	17	15	15	5

* by number as in Table 4

** % of seeds yielding fungal species

*** based on 4 replications

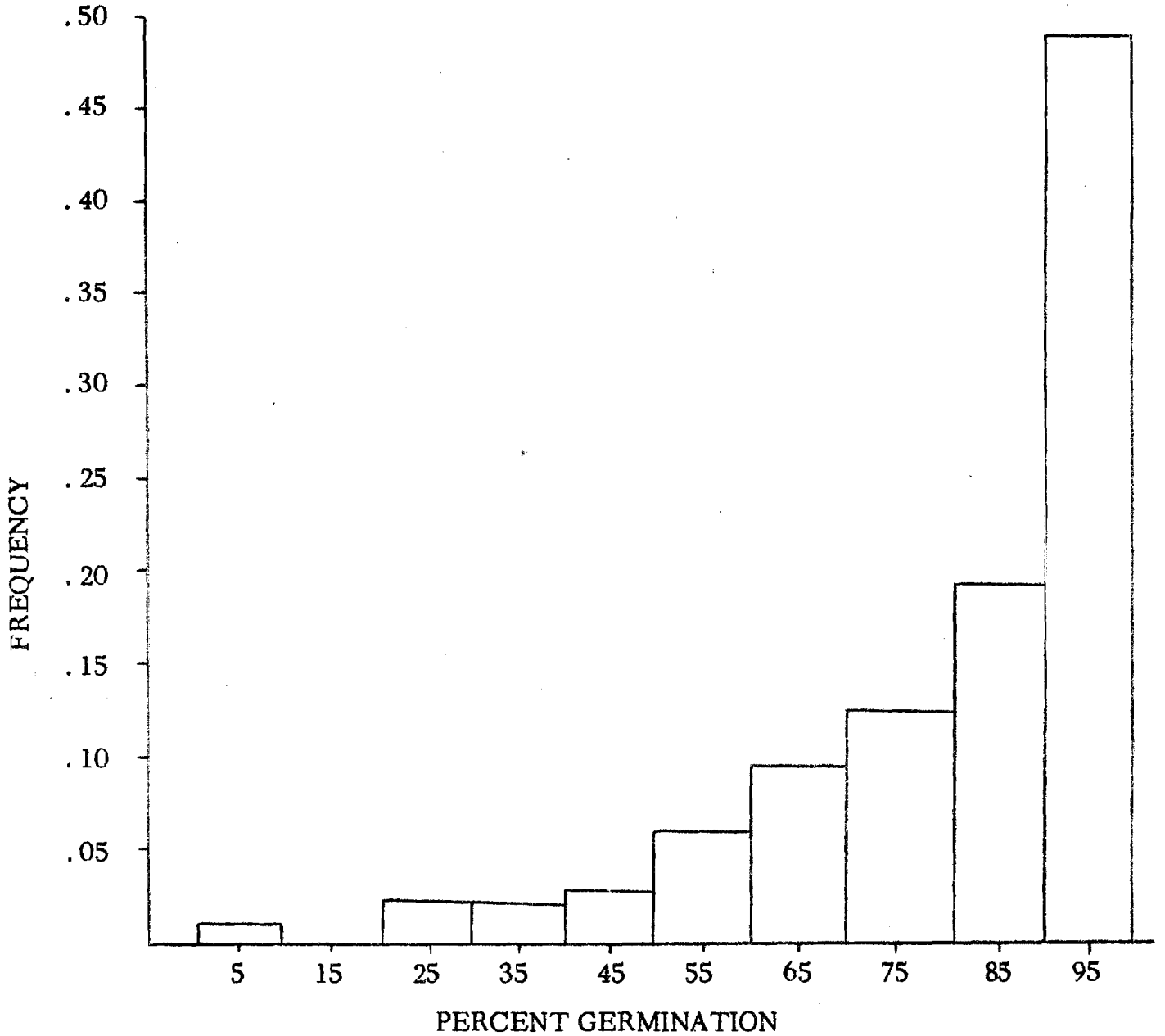

Figure 1. Frequency distribution of germination percentage of maturity groups VIII and IX.

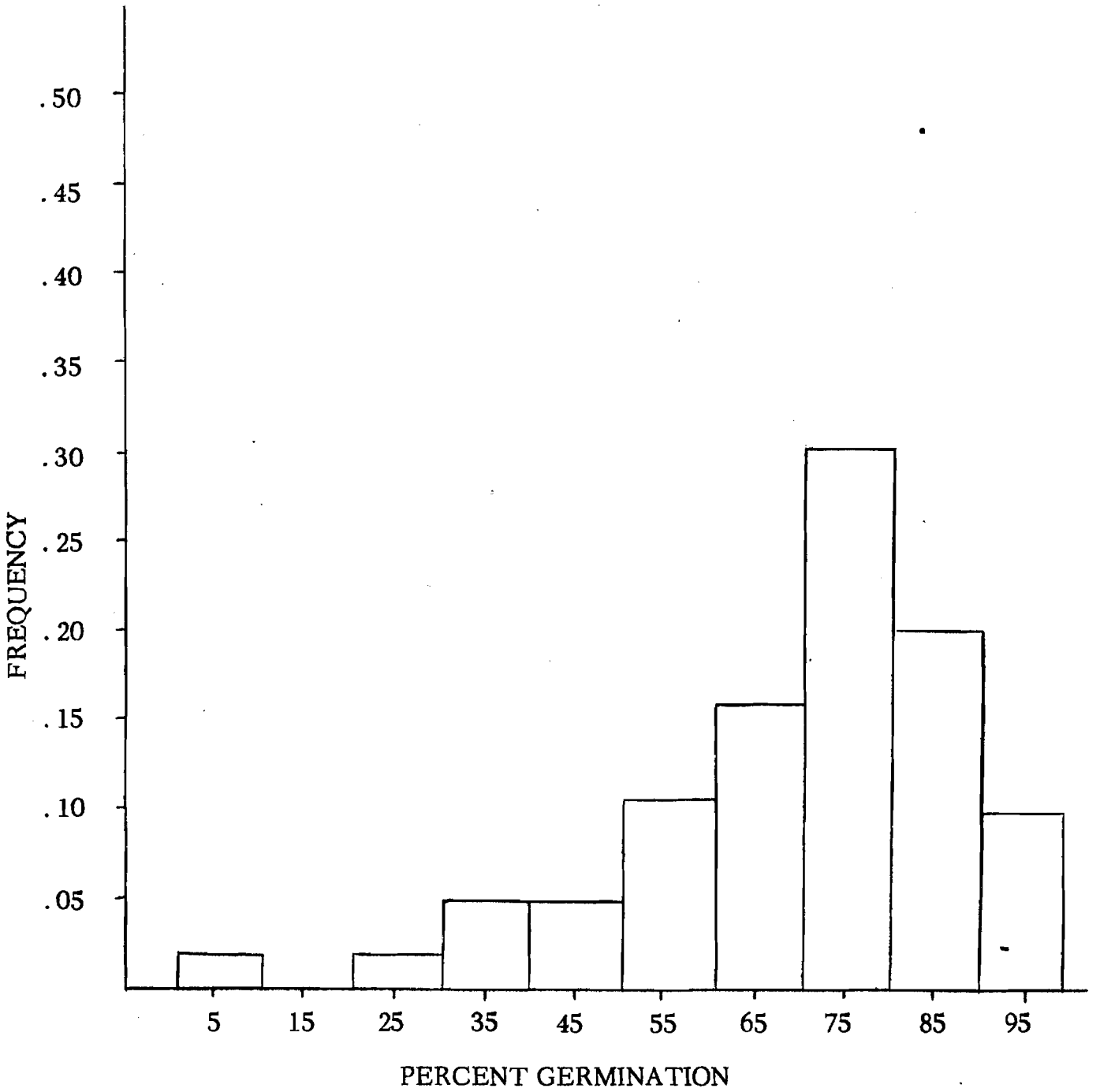

Figure 2. Frequency distribution of germination percentage of maturity group X.

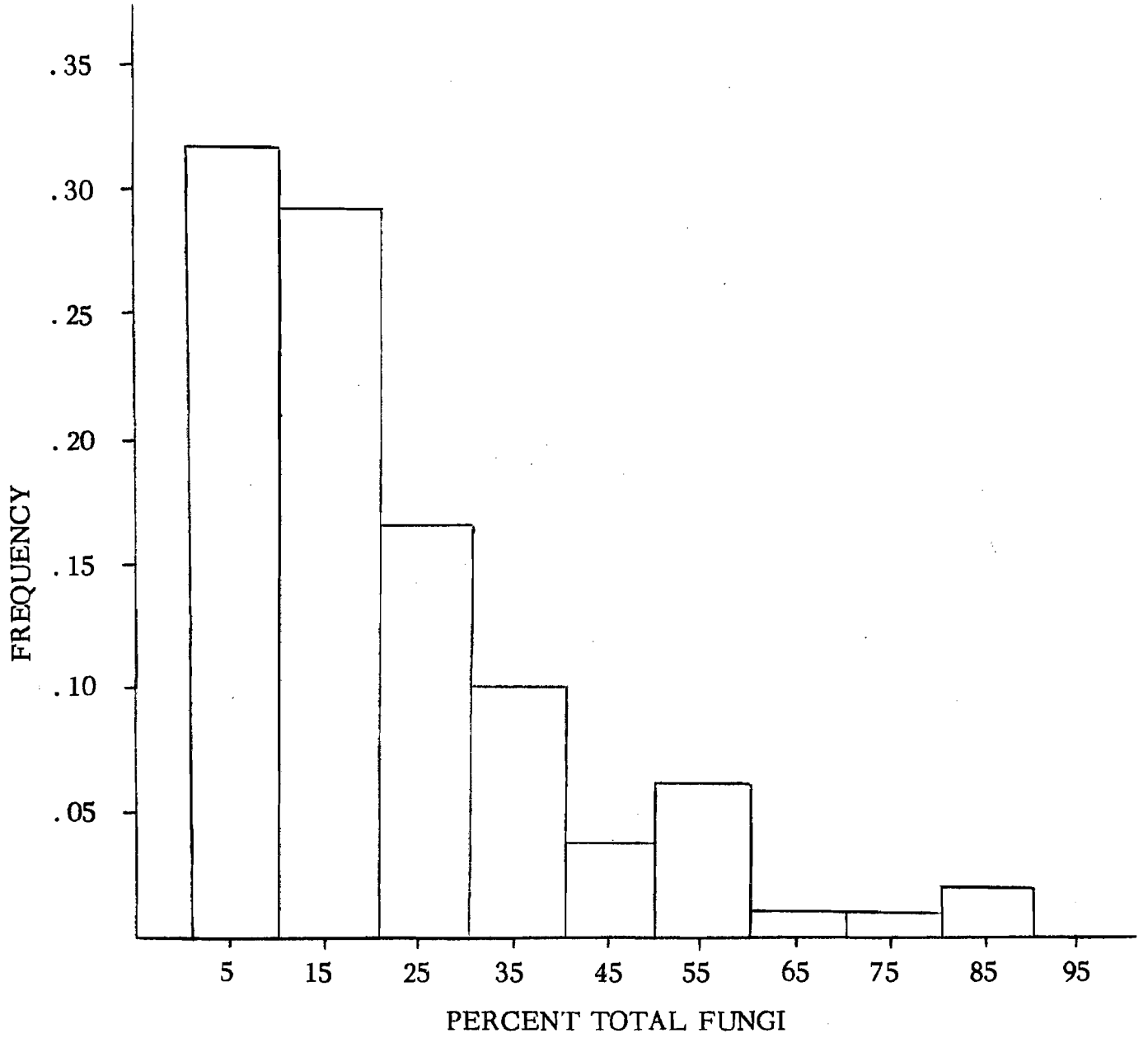

Figure 3. Frequency distribution of percent total fungi of maturity groups VIII and IX.

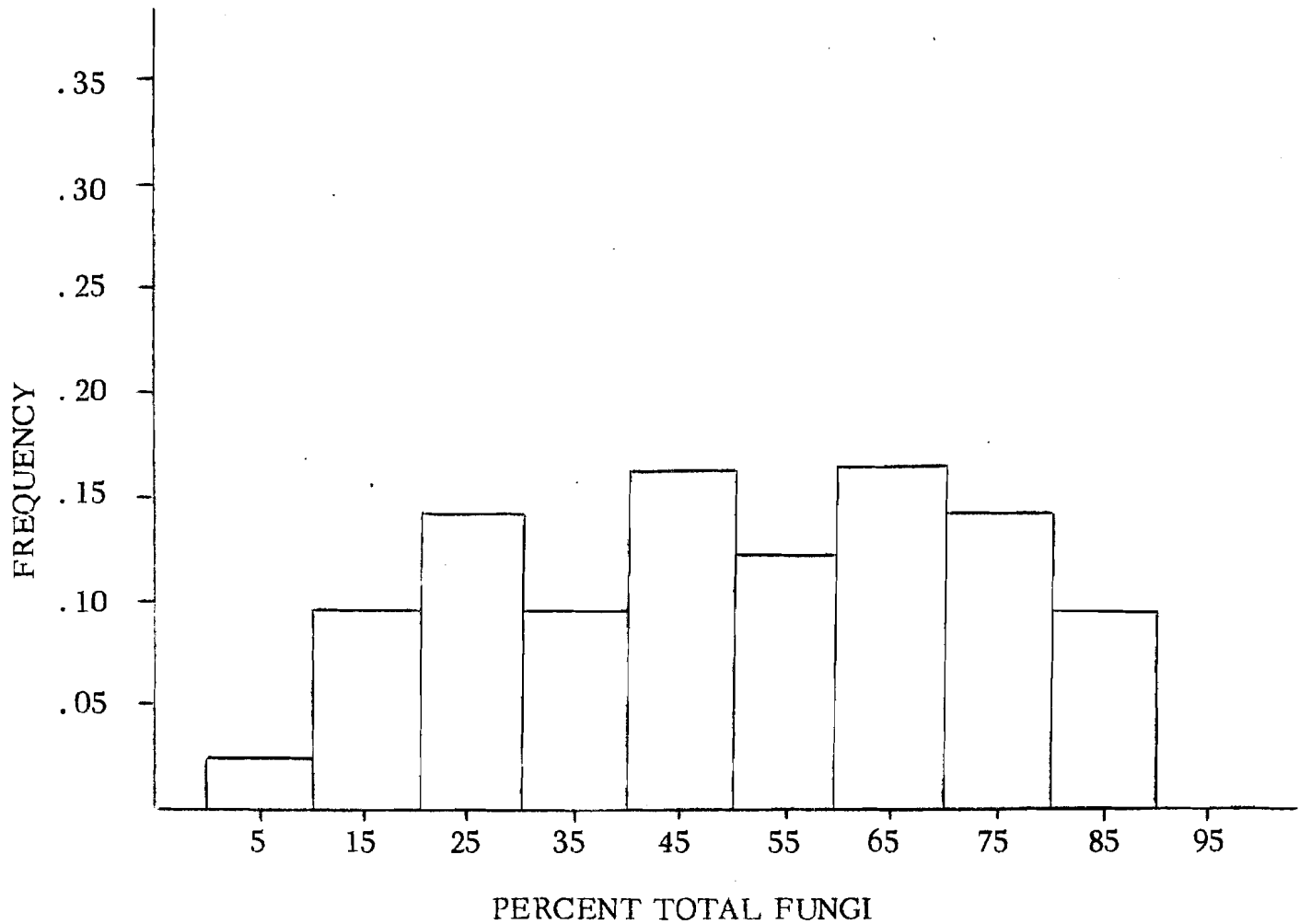

Figure 4. Frequency distribution of percent total fungi of maturity group X.

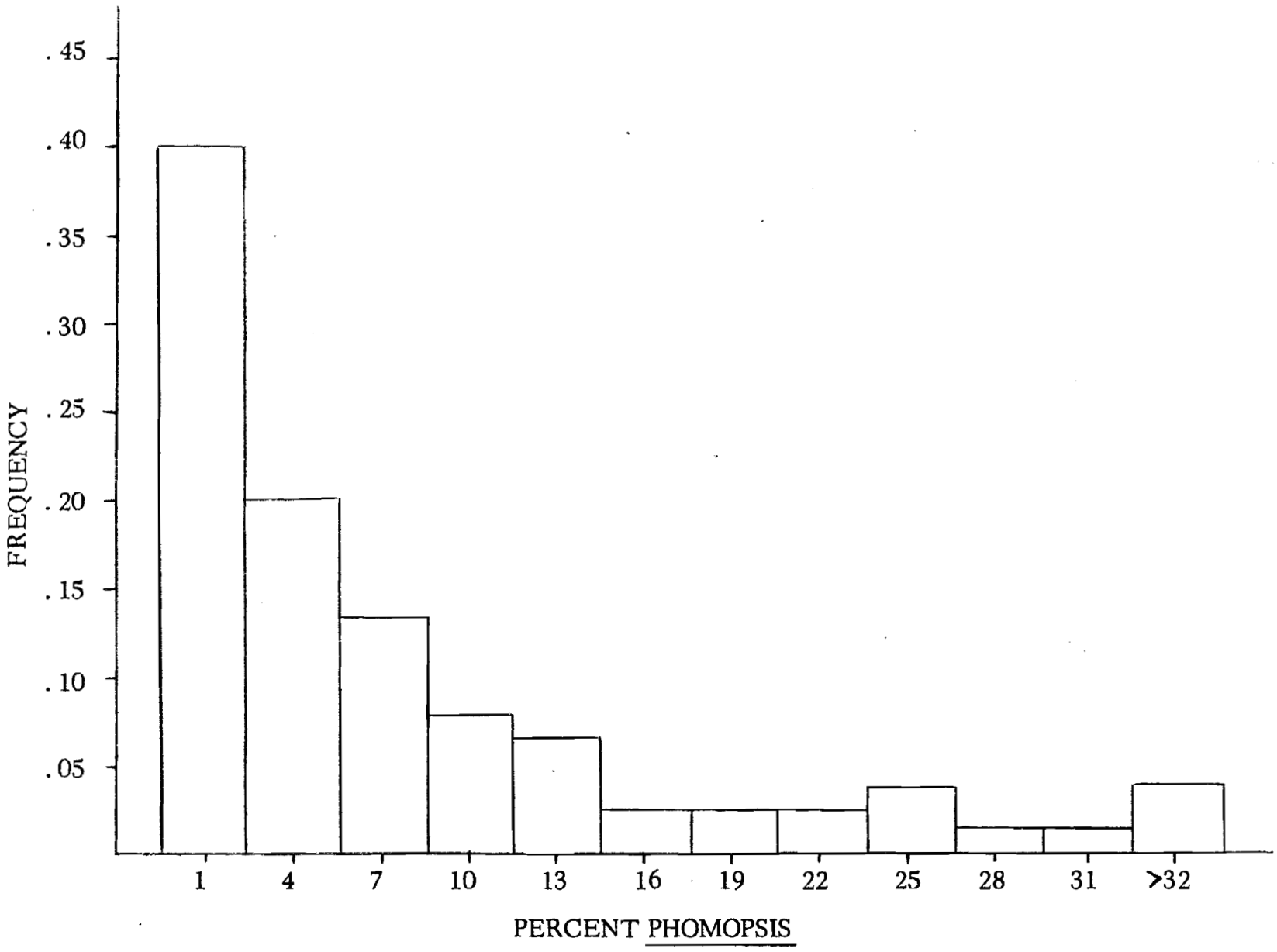

Figure 5. Frequency distribution of percent Phomopsis of maturity groups VIII and IX.

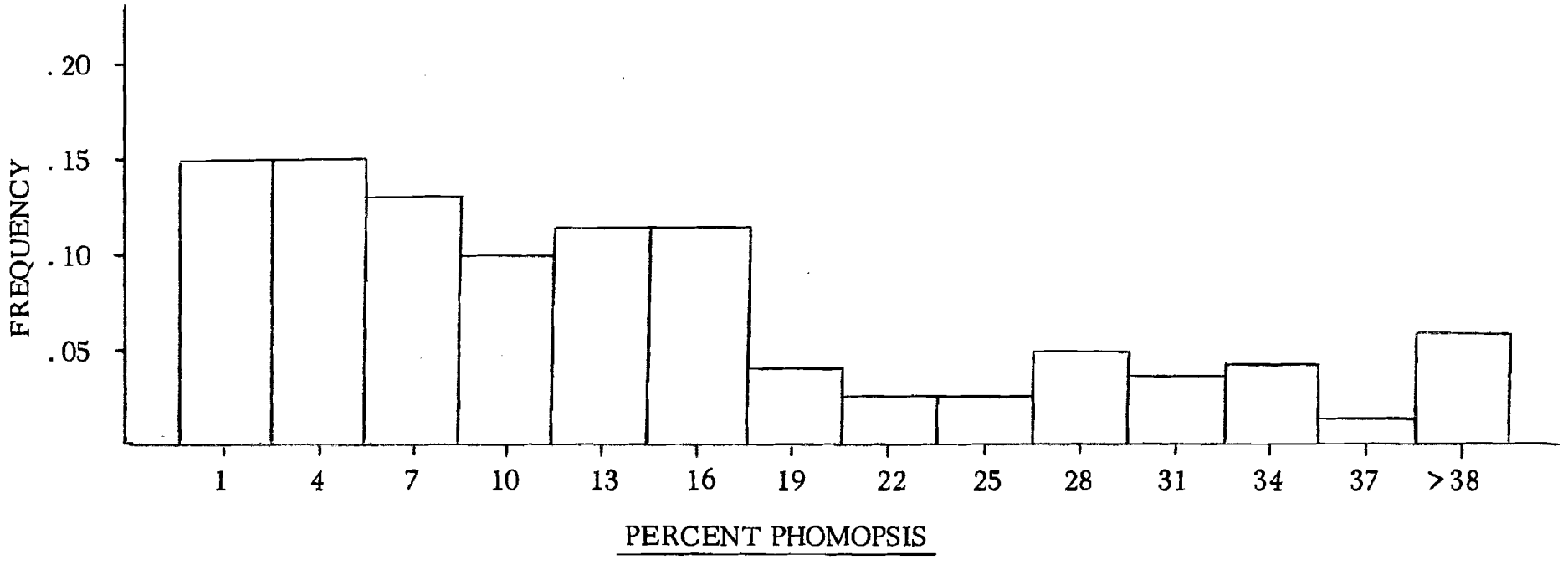

Figure 6. Frequency distribution of percent Phomopsis of maturity group X.

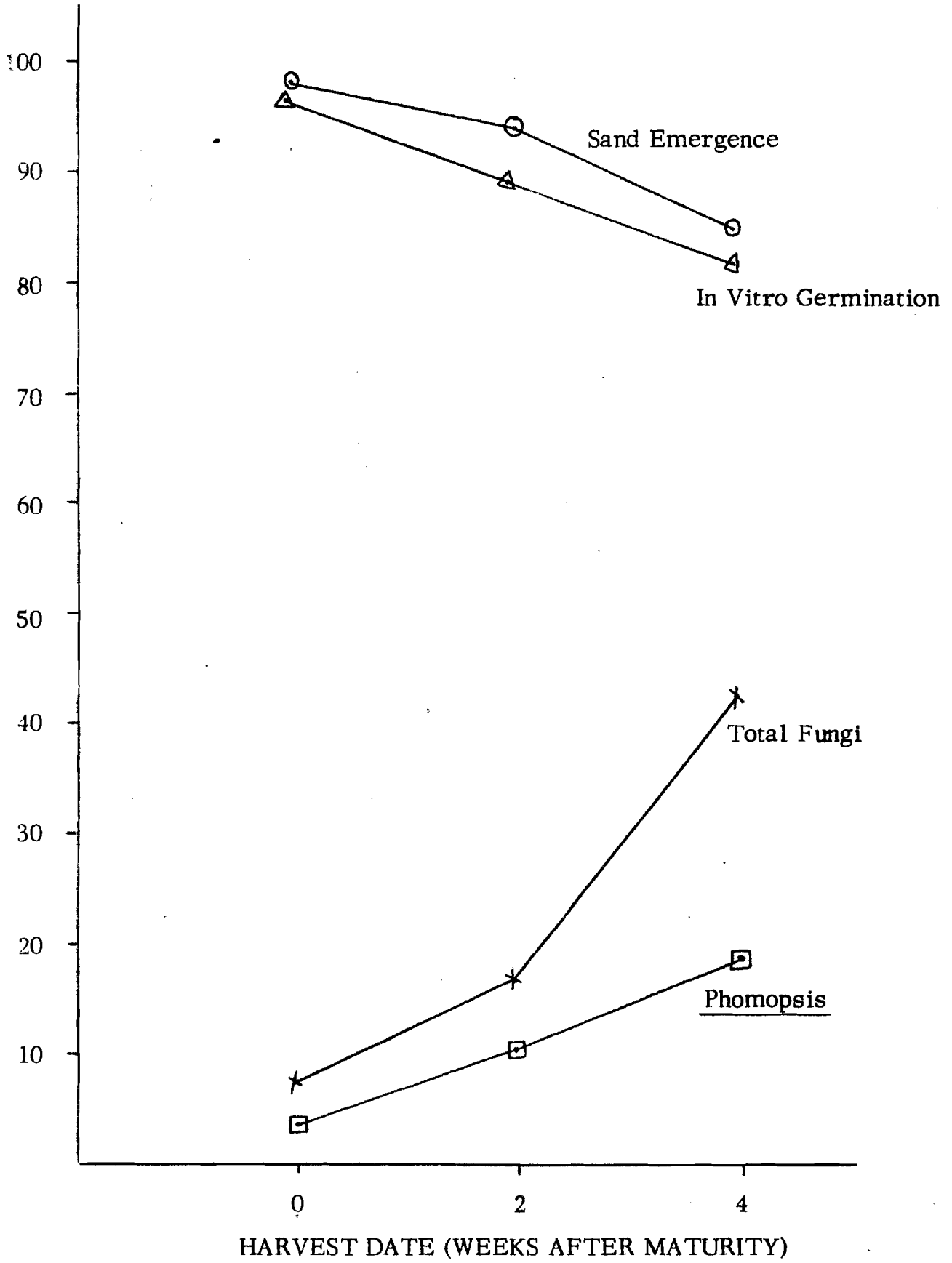

Figure 7. Effect of delayed harvest on sand emergence, in vitro germination, total fungi, and Phomopsis of 24 selected cultivars.

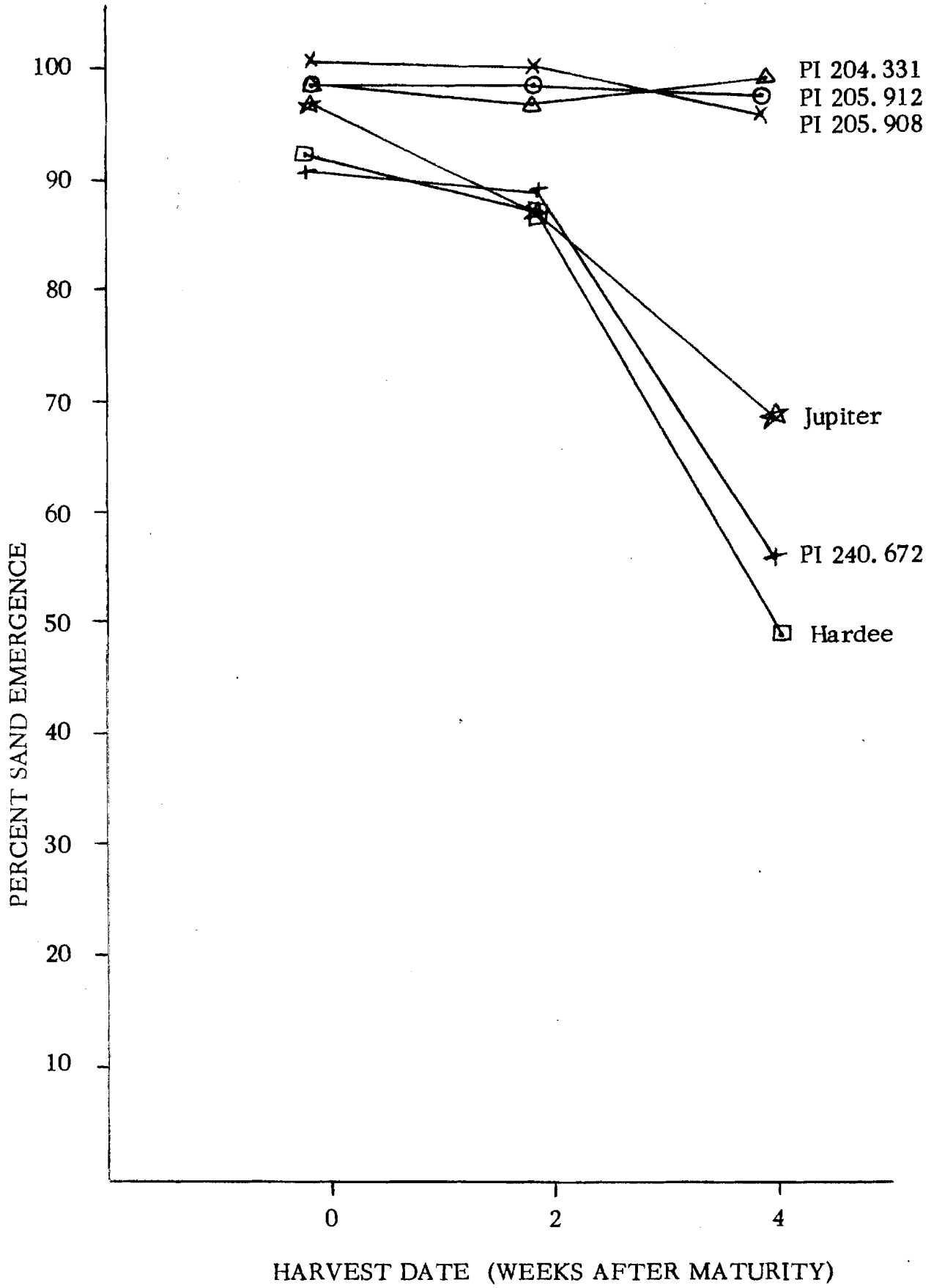

Figure 8. Effect of delayed harvest on sand emergence of 6 selected cultivars.

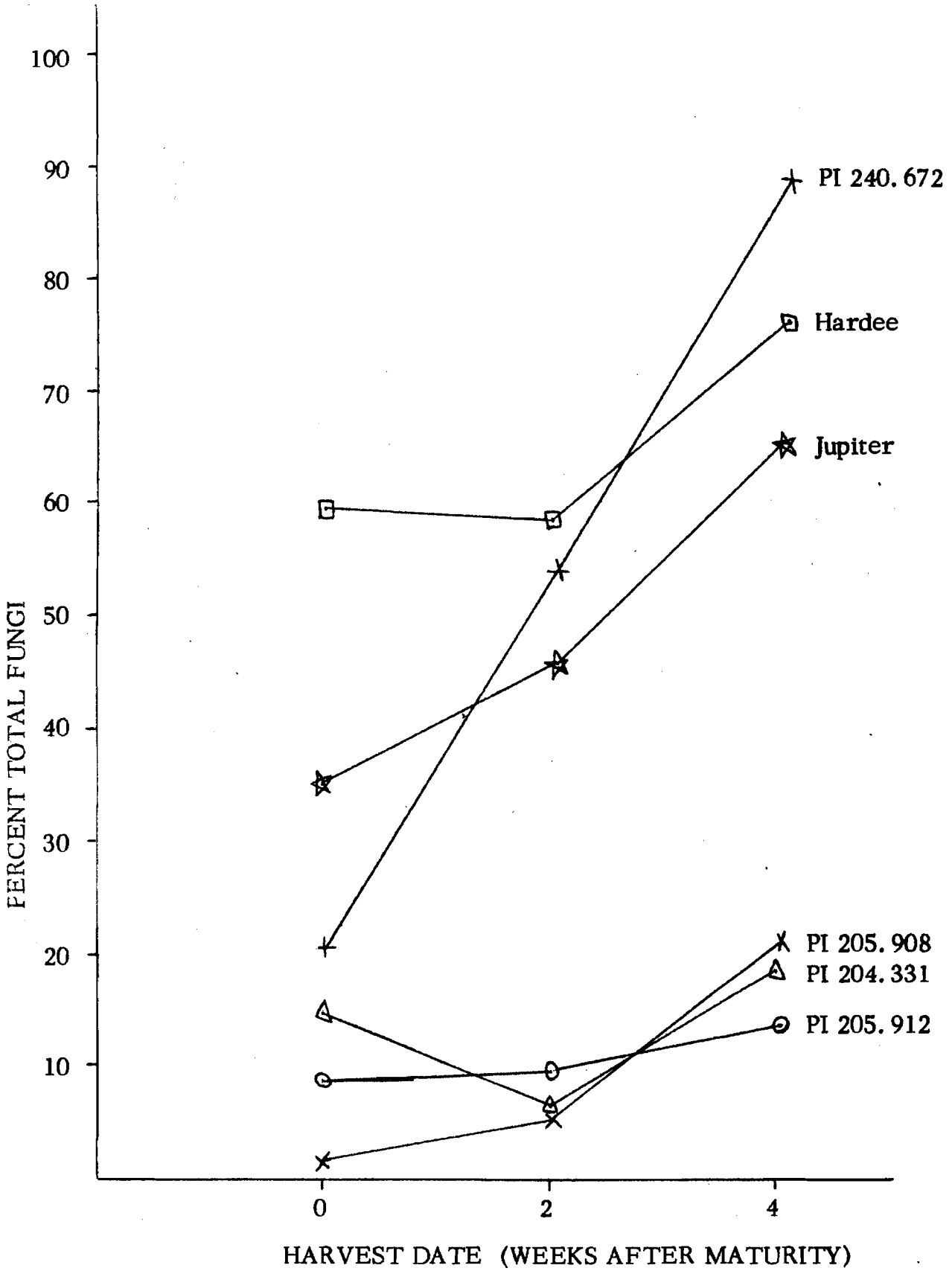

Figure 9. Effect of delayed harvest on percent total fungi of 6 selected cultivars.