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ON

IMPROVEMENT OF TROPICAL PRODUCTION OF BEANS AND COWPEAS THROUGH DISEASE AND INSECT CONTROL

Contract AID/CM/ta-C-73-26

January 1 - December 31 , 1975

Submitted to

Agency for International Development
Department of State
Washington, D.C.

ANUAL REPORT

on

IMPROVEMENT OF TROPICAL PRODUCTION OF BEANS AND COWPEAS THROUGH DISEASE AND INSECT CONTROL

(CONTRACT NO. AID/CM/ta-C-73-26)

JANUARY 1 - DECEMBER 31, 1975

Prepared by

Project Senior Staff

at

Agricultural Experiment Station
College of Agricultural Sciences
University of Puerto Rico, Mayaguez Campus
Mayaguez, Puerto Rico

and

Mayaguez Institute of Tropical Agriculture
Mayaguez, Puerto Rico

Submitted to

Agency for International Development
Department of State
Washington, D. C.

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PROPOSED BUDGET 100

I. ANNUAL REPORT SUMMARY

Improvement of Tropical Production of Beans and Cowpeas thru Disease and Insect Control, Contract AID/CM/ta-C-73-26
Project Title and Contract Number

Julio H. López-Rosa, University of Puerto Rico, Mayaguez Campus
Principal Investigator and Contractor

University of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico 00708
Contractor's Address

May 14, 1973 to April 30, 1976 Contract Period (as amended) From - To	1 January - 31 December 1975 Reporting Period From - To
Total Expenditures and Obligations Through Previous Contract Year	$ 414,833.00
Total Expenditures and Obligations For Current Contract Year	$ 385,426.00

Narrative Summary of Accomplishments and Utilization:

A. ACCOMPLISHMENTS

1. VIROLOGY

The work on the virology phase of the project continued to be focused on rugaceous (whitefly-transmitted) legume viruses. The work covered the study of various aspects of the bean golden yellow mosaic, *Euphorbia prunifolia*, *Rhynchosia* and *Malachra* mosaic viruses.

The bean golden yellow mosaic virus (BGYMV) is the most important rugaceous virus of beans (*Phaseolus vulgaris*) and lima beans (*P. lunatus*) in Puerto Rico and Central America. The virus does not affect cowpeas (*Vigna unguiculata*), nor most Asiatic and African edible legumes of the

genera Vigna and Phaseolus. The results of host range studies indicated that the Puerto Rican virus is closely related to the Central American and northern South American forms of BGYMV. Excellent infection rates (up to 100%) were obtained through the use of new methodology developed to mechanically inoculate bean seedlings. The virus withstands desiccation and was partially concentrated by high speed centrifugation. The particles are strikingly similar to the ones recently visualized by workers in Colombia.

The local Euphorbia prunifolia virus was fully characterized, concentrated and visualized through the electron microscope with the collaboration of members of the Waksman Institute of Microbiology, Rutgers University. The particles are generally 30 nanometers in diameter. Visualization of this agent is perhaps one of the first instances where a spherical virus is definitely found associated with a rugaceous disease.

The Rhynchosia minima virus was isolated from soybeans. Incidence was low, but the virus seems to be potentially dangerous to this crop. The Malachra capitata agent is a newly discovered rugaceous virus. The virus causes a dark-yellow, mildly distorting mosaic on beans, but unlike the Rhynchosia virus, if fails to produce enations on Malachra and V-12 tobacco.

2. CONTROL OF FUNGUS AND BACTERIAL DISEASES

Evaluation of chemicals for control of fungus and bacterial diseases of beans and cowpeas was continued. The fungicides Macuprax and mancozeb,

applied weekly to the foliage, controlled powdery mildew of cowpea, caused by Erysiphe polygoni. Mancozeb, at a dosage of 1.14 kg/ha, was as effective in controlling this disease as it was at 4.56 kg/ha, the highest dosage tested. Yields from the Macuprax treatments almost doubled those for the non-sprayed plots. The systemic fungicide benomyl failed to control powdery mildew, even when high dosages of the chemical were employed. This suggests that races of the pathogen resistant to the fungicide were present in the field. Oxycarboxin and chlorothalonil, at 2.28 and 1.14 kg/ha, respectively, protected bean cultivar Bountiful against rust (Uromyces appendiculatus), but failed to protect cultivar Blanca del País. The fungicides copper hydroxide, basic copper sulphate, Macuprax and chlorothalonil failed to control common bacterial blight of beans, caused by Xanthomonas phaseoli. EL 273, a new fungicide, was very effective in vitro against Corynespora cassiicola, causal agent of target spot of cowpea. Dosages as low as 60 ppm drastically reduced radial spread of the fungus.

3. ENTOMOLOGY

Research in the entomology phase included work in various fields. The most important noxious insects of beans, cowpeas and lima beans were identified. Evidence of resistance to several of the major insect pests of beans and cowpeas, e.g., leafhoppers (Empoasca spp), leafminer (Liriomyza sativae), bean beetle (Cerotoma ruficornis), bean weevil (Acanthoscelides obtectus), cowpea curculio (Chalcodermus ebeninus), and cowpea weevil (Callosobruchus chinensis) was found. Studies on the leafminer made possible an understanding of the host range, biology, and natural control of this insect. Methods of chemical control were developed for some of the insects of economic importance.

4. EVALUATION OF GERMPLASM FOR RESISTANCE TO DISEASES

Work on the phase of germplasm evaluation for resistance to diseases covered an ample variety of host plant materials, pathogens and localities.

Field screening of recurrent selection populations for rust resistance was performed. Eight hundred-twelve lines consisting of single F_1, bulk of 3 - 10 segregant plants, and bulk from advanced lines which had been selected for yield and multiple disease resistance, were screened for resistance to a single rust cultivar. These selections displayed two main types of responses, expressed as resistance and tolerance. Resistance in different F_1 selections was conditioned by either a dominant or a recessive factor. Only one F_2 progeny, out of 37, had a segregation indicative of a two-factor interaction. The qualitative resistance was not affected by the age of the host plants. Tolerance was expressed as a) the effect of the host age on pustule size, and infection intensity; b) host characteristics which allow low intensity of rust infection; c) compatibility, expressed by foliage tissue as absence of chlorosis after infection, and pedicular indehiscence; and d) hypophyllous rust development. The data on rust response of the recurrent population indicated that tolerance is a complex of characteristics which are independent from a rust-host resistance interaction, but when present in conjunction with resistance, it is superimposed on and modifies the resistance interaction.

The First International Bean Rust Nursery, which originated at the Bean Rust Research Workshop held at CIAT, Cali, Colombia in October 1974,

provided an opportunity to compare the performance of entries from various countries, not only against rust but also against a number of diseases, adaptability and yield. Fourteen cultivars were selected for having the highest resistance against rust in Puerto Rico.

Beans seeds from the UPR-MITA Program were exchanged with seeds from the Michigan State University Program for screening against bacterial blight. Of 81 Michigan accessions tested for resistance to a single strain of Xanthomonas (Col. #113), three were highly tolerant and 13 were tolerant. This suggested that tolerance encountered under Michigan conditions is also functional under the tropical environment in Mayaguez. From the 62 Puerto Rican lines tested in Michigan, 29 were rated as tolerant to highly tolerant. The widespread presence of tolerance among lines suggested that a) in the tropics plants are exposed, during screening, to greater disease stress than in temperate zones; and b) Xanthomonas strains are more numerous and more virulent in the tropics than in temperate zones.

Four hundred thirty-six hybrid lines from Prosser, Washington, were screened for rust resistance at Isabela. None were resistant, but 94 lines showed varying degrees of tolerance. Hybrid 3R-1315 had the highest tolerance. This same hybrid had a high level of tolerance to bacterial blight in a field screening test at Fortuna. Three-fourths of the hybrids died due to a combination of rust and rhizoctonia root rot. Lines 3R-7, 3R-210, -320, -321, 120-2 and 1519 were highly tolerant to rhizoctonia root rot. Considering the wide spectrum of disease resistance that had been bred into these hybrids, a greater frequency

of survivability than what was encountered was expected. The predominance of susceptibility at F_3 and F_4 stages of segregation in such a large number of crosses suggests that under tropical conditions disease intensity and pressure are much higher than those encountered at Prosser, Washington. This relationship holds true for cultivars and hybrids lines received from other areas of the temperate zone. It could be that under our screening conditions plants are exposed to excessive disease pressure levels. However, within the same trial there were many other selections and hybrid lines from the tropics that grew free of diseases and yielded well. On the other hand, the seed form, plant habit, earliness, and the general appearance of the surviving hybrids were much more refined than those the tropical lines with multiple disease resistance. Hybridization of the two groups should provide, at least for the tropics, cultivars with excellent seed characteristics, high yield, multiple resistance to diseases, and adaptability to severe conditions.

Fifty-one Ecuadorian bean accessions, screened for resistance to diseases, had, as a group, a high degree of overall resistance. Several of these materials were found excellent for inclusion in multiple disease resistance breeding.

Twelve bean accessions from the Instituto Colombiano Agropecuario (ICA), Palmira, evaluated for disease resistance, were found to have a high level of overall resistance to rust, rhizoctonia root rot, and charcoal rot (Macrophomina phaseoli), and also yielded well. Some of these cultivars can serve as parental material for the improvement of beans for tropical cultivation.

Evaluation of scarlet runner bean (Phaseolus coccineus) germplasm was initiated. Accessions were classified according to flower, raceme, foliage, cotyledon position and habit. Ninety-four accessions had hypogeous cotyledons and 124 had epigeous. However, 61 out of the 124 entries with epigeous cotyledons were P. coccineus and the remainder were classified as P. vulgaris. Observation of the materials suggested the presence of putative hybrids between P. coccineus and P. vulgaris. These hybrids exhibit various levels of caryotypic differences, which depend upon gene dosage received from either parental species and the direction of their selection. The putative hybrids indicated that P. coccineus and P. vulgaris are subspecies rather than species. If this assumption is correct, then the transfer of disease resistance characteristics should prove much less complicated than if they were distinct species. Twelve accessions contained individual plants highly resistant to the soil-borne complex of diseases. High levels of bacterial blight resistance were encountered in a number of individual plants from different accessions. Forty-nine percent of the Pc-E and only 5-6 percent of the Pc-H accessions were susceptible to rust. A number of plants had single or multiple resistance against angular leaf spot, cercospora leaf spot, anthracnose, powdery mildew, common bean mosaic virus (CBMV) and cowpea mosaic virus (CPMV).

Preliminary screening of tepary beans (Phaseolus acutifolius) suggested that before germplasm from this species can be used in crosses for introducing bacterial blight resistance into common bean, it should possess resistance to rhizoctonia root rot, common bean mosaic virus, rust and possibly powdery mildew. Otherwise, more undesirable characteristics

will be added to hybrids while being screened for bacterial blight resistance. The results of this initial trial indicated that among the tepary accessions available it is not difficult to find multiple disease resistance.

Seven hundred nineteen lima bean (Phaseolus lunatus) PI accessions were grown at Isabela. Thirteen accessions yielded over 1,000 grams per 5-meter row. The seed size varied between 15-91 grams per 100 seed. Bacterial blight, bacterial pustule, powdery mildew, downy mildew, anthracnose, angular leaf spot, cercospora leaf spot, diaporthe leaf spot, rhizoctonia root rot, charcoal rot, golden yellow mosaic, and rugose mosaic were some of the diseases that affected these accessions. Sixty-one accessions were either resistant or tolerant to some or many of the diseases, and survived 11 months of growth in the field, until the trial was discontinued. Nine accessions were tolerant to golden yellow mosaic virus, while accession PI-224713 could be considered as resistant.

Sixty accessions were received from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and planted for evaluation against Puerto Rican cowpea mosaic virus (PRCPMV), strain X-19. Accessions Tvu-2331 and Tvu-4544 were resistant, and Tvu-1190 and Tvu-6666 segregated for resistance.

5. BREEDING

The first cycle of crossing, involving 11 base parents for the formation of an improved population of disease-resistant bean (Phaseolus vulgaris L.) germplasm was accomplished under greenhouse conditions. From this popu-

lation 81 F_1 hybrids have been field tested and 16 superior hybrids are being recrossed for the 2nd cycle of recombination. Multiple location testing of disease resistant parental lines of Phaseolus showed a considerable location effect. The highest yields obtained in these tests in Puerto Rico ranged around 2,000 kg/ha. Insects and diseases were significantly responsible for yield reduction only in specific locations which emphasizes the importance of multiple disease resistance for yield stability. Similar testing is being undertaken on a cooperative basis in various countries in the American tropics.

B. UTILIZATION

Information developed by the Project was disseminated via eight papers given at technical meetings in developing American countries, an article published in a scientific journal and the book Tropical Diseases of Legumes. Personal contacts with legume workers from Central America and Colombia was another effective means of disseminating information.

Findings on chemical control of bean and cowpea diseases were transferred to soybean in Panamá and utilized in commercial bean production in Puerto Rico.

Multiple disease resistant germplasm developed by the Project was utilized in seven Asiatic, African and European countries and Australia, and in twelve research institutions in the United States.

Cooperative testing of promising Project bean lines and selections was initiated in Central America.

Plans were made during the Bean Protection Workshop at CIAT to assemble a collection of bean cultivars resistant to leafhoppers (*Empoasca* spp) to be tested in developing American countries.

A Nicaraguan graduate student is conducting thesis research on physiologic races of the bean rust fungus.

II. STATEMENT OF PROJECT OBJECTIVES AS DESCRIBED IN THE CONTRACT

The major objective of the Project is to investigate and develop methods for controlling diseases and insects of selected food legumes adapted to the tropics, namely, beans and cowpeas. The primary focus will be on improvement of production through control of diseases (caused by viruses, bacteria, fungi and mycoplasma) and insects. The program will receive complementary inputs from work carried out by the University of Puerto Rico in biology, nutrition, genetics, and other sciences in the tropical environment.

III. ACCOMPLISHMENTS TO DATE

A. FINDINGS

1. VIROLOGY

The work on the virology phase of the project continued to be directed to locate, identify, determine the range of, and characterize legume viruses, with particular emphasis on rugaceous (whitefly-transmitted) ones. These studies were aimed at enabling project personnel to a) gauge the potential of the viruses as inciters of bean and cowpea diseases and b) test germplasm for resistance to specific viruses, and also provide adequate control means. Accordingly, the work covered the study of various aspects of the bean golden yellow mosaic (BGYM), *Euphorbia prunifolia*, *Rhynchosia* and *Malachra* mosaic viruses. Biological transmissions were effected, in all cases, via the whitefly *Bemisia tabaci* Genn. The research findings for this period follow.

Bean Golden Yellow Mosaic Virus (BGYMV)

This is the most important rugaceous virus of beans (*Phaseolus vulgaris*) and lima beans (*Phaseolus lunatus*) in Puerto Rico and Central America. It will not affect cowpeas (*Vigna unguiculata*) nor most Asiatic and African edible legumes of the genera *Vigna* and *Phaseolus*. The results of recent host range studies indicate that the Puerto Rican virus is closely related to the Central American and northern South American forms of BGYMV. Symptoms incited by the local virus closely resemble those provoked by the Central American strains (visual observations while surveying in El Salvador). The properties of the local agent were determined and, in general, it can be stated that it withstands:

a) Dessication (via calcium chloride or freeze dryer) for at least 2 weeks. Infectivity is gradually lost with passage of time.

b) Dilutions of up to, but no more than 1:100 (16.6% infection against 83% infection in dilution 1:10).

c) Temperatures of up to, but not more than 50°C, but with low infection rates (4.5%). At 30°C the infection rate was 81.8%.

d) Freezing (undiluted crude sap kept in freezer at -8°C for at least 4 weeks). Frozen in leaves at same temperature the agent became inactive.

Inoculum was obtained from young bean plants only, using the tenderest topmost expanding diseased leaves (obtained 14-20 days after inoculation). In these tests the most infective inoculum was found to be that of bean cultivar Top-Crop. Thus, as a general practice, the aforementioned cultivar was used as source of virus. Regardless of conditions, inoculum from Phaseolus lunatus should not be used because it lacks infectivity. Sap was extracted employing chilled utensils and icebaths and using cold 0.1 M K_2HPO_4 (1:4 to 1:10 dilution of inoculum). For best results, inoculations were practiced in greenhouses during daytime (full sunlight) with ambient temperatures in the 27-35°C range. Carborundum (600 mesh), at the rate of 1 gram per 100 ml of diluted inoculum, was added to the inoculum. The cotton swab, as well as the airbrush methods of inoculation were equally adequate for inoculation.

The method developed by us, or rather the conglomerate of known practices employed by us, to mechanically inoculate bean plants with BGYMV should greatly facilitate the screening of cultivars for resistance to this virus which causes one of the most important diseases of beans in the American Tropics.

The local BGYMV disease agent can be partially concentrated by high speed centrifugation (as evidenced by increased infectivity of the pellet). In all probability the Puerto Rican BGYMV disease agent is a virus similar in properties to the Central American BGYMV purified and visualized recently by Gálvez and associates in Colombia (personal communication).

Euphorbia prunifolia virus

The local rugaceous Euphorbia prunifolia virus was fully characterized, concentrated and visualized through the electron microscope with the cooperation of members of the Waksman Institute of Microbiology, Rutgers University. The agent was isolated and the particles vary in size although generally they are about 30 nanometers in diameter. The Euphorbia virus withstands dessication (calcium chloride and freeze dryer) for periods of up to 12 weeks or more. The thermal inactivation point of this virus was found to lie between 55 and 60°C. Very low infection rates were obtained (6%) when infective sap was diluted to 1:1000. Sap ceases to be infective at 21°C if left standing for more than 24 hours.

Visualization of the causal agent of the Euphorbia mosaic through the electron microscope is perhaps one of the very first instances where a spherical virus is definitely found to be associated with a rugaceous disease. Recently, Gálvez and cooperators in Colombia (personal communication) found that the Central American bean golden yellow mosaic (BGYM) disease, another rugaceous malady, is caused by a spherical virus. Thus two spherical viruses transmitted by the whitefly Bemisia tabaci, and typically causing leaf curl, stunting and yellow mosaics have been implicated as the cause of rugaceous diseases.

Probably most of these diseases will be found eventually to be caused by similar entities. In all probability they will be found to comprise a separate and definite group of plant viruses.

Rhynchosia minima virus

The Rhynchosia virus was again isolated from soybeans. This time it was recovered via whiteflies from a field in the Gurabo Substation. Incidence was low, but the virus seems to be potentially dangerous to soybeans. Characteristic cup-like and leaf-like enations became evident on indicator plants (V-12 tobacco) when inoculations were made via the whitefly Bemisia tabaci.

Malachra capitata virus

This newly discovered rugaceous virus produces a vivid yellow-green mottle on Malachra plants. The disease is distinct from the one incited on the same host by the Rhynchosia minima virus. The Malachra virus causes a dark green-yellow, mildly distorting mosaic of beans, but unlike the Rhynchosia virus, it fails to produce enations on Malachra and V-12 tobacco. It also fails to incite symptoms on Sida carpinifolia and other members of the same genus.

2. CONTROL OF FUNGUS AND BACTERIAL DISEASES

Powdery Mildew of Cowpea

The fungicides Macuprax and mancozeb, at the rates of 1.12, 2.28 and 4.56 kg/ha, protected the foliage of cowpea plants against powdery mildew. This is evidenced by the low disease indices, and the high yields from plots treated with these fungicides. Yields from plots treated with Macuprax at the rate of 2.28 kg/ha almost doubled those obtained from plots not sprayed with this protectant.

In previous experiments, the systemic fungicide benomyl protected cowpea plants against mildew. However, in this test it failed to control the disease. These results could be due either to inactivation of the chemical in storage, or to the presence in the field of races of the fungus resistant to the fungicide.

Rust of Beans

Severe rust infection occurred early in the season, mainly on cultivar Bountiful. However, the high disease indices recorded may not be totally attributable to rust damage, since during a period of heavy rains an outbreak of bacterial blight completely defoliated the plants. As blight attack took place late in the season, differences in yields of cultivar Bountiful were not influenced by the presence of this disease. The only two chemicals which increased yields of Bountiful significantly, when compared to the non-sprayed plots, were oxycarboxin (2.28 kg/ha) and chlorothalonil (1.14 kg/ha). Triforine did not protect this cultivar against rust. None of the fungicides tested protected the foliage of cultivar Blanca del País against rust. However, yields were high for all the treatments included, thus suggesting that rust was not a limiting factor in production.

Common Bacterial Blight of Beans

In a trial to evaluate copper hydroxide, basic copper sulfate, Macuprax and chlorothalonil for control of common bacterial blight of beans (*Xanthomonas phaseoli*), none of the treatments protected cultivar R-19 against the disease. This is the third experiment in which both copper hydroxide and basic copper sulfate either fail to control bacterial blight, or the chemicals provide just partial protection against the pathogen.

In Vitro Tests with EL 273

Results of *in vitro* tests with the new fungicide EL 273 showed that a dosage as low as 60 ppm was sufficient to reduce radial growth of *Corynespora cassiicola*, causal agent of target spot of cowpea, down to only 12.6 mm, compared to growth on PDA free of the fungicide, where radial growth averaged 67.4 mm. Growth of the fungus was even more drastically reduced when a concentration of 180 ppm was employed. Increasing the dosages beyond this point did not result in further appreciable reduction in fungal growth.

3. ENTOMOLOGY

Survey of Noxious Insects

Surveys of noxious insects were conducted on beans and cowpeas. Observations on the presence of insect/mite pests were made also on lima beans and scarlet runner beans planted at Isabela and Mayaguez, respectively, for evaluation of germplasm. The noxious insects/mites recorded on plantings of these four species are listed below, in descending order of importance for each species.

Beans (*Phaseolus vulgaris*)

Leafhoppers	*Empoasca kraemeri* Ross and Moore, and *E. Millsi* Ross*
Dry-seed weevil	*Acanthoscelides obtectus* (Say)
Bean leaf beetle	*Cerotoma ruficornis* Olivier
Leafminer	*Liriomyza sativae* Blanchard
Leaftier	*Hedylepta indicata* (Fabricius)
Leaf-roller	*Urbanus proteus* (Linnaeus)
Stem-borer	*Elasmopalpus lignosellus* (Zeller)
Sugarcane weevil	*Diaprepes abbreviatus* Linnaeus

* Determined by J.P. Kramer, Systematic Entomology Laboratory, IIBIII.

Cowpeas (Vigna unguiculata)

Cowpea curculio	Chalcodermus ebeninus Boheman
Dry-seed weevil	Callosobruchus chinensis Linnaeus
Bean beetle	Cerotoma ruficornis Olivier
Leafminer	Liriomyza sativae Blanchard
Leafhoppers	Empoasca kraemeri Ross and Moore, and E. Millsi Ross
Pod-borers	Maruca testulalis (Geyer) and Etiella zinckenella (Treitschke)
Aphids and cutworms	

Lima Beans (Phaseolus lunatus)

Leaftier	Hedylepta indicata
Red spider	Tetranychus telarius
Leafhoppers	Empoasca spp.
Bean leaf beetle	Cerotoma ruficornis
Leafminer	Liriomyza sativae
Lace bug	Corythucha gossypii
Pod weevil	Chalcodermus ebeninus
Sugarcane weevil	Diaprepes abbreviatus
Leafminer (very similar to coffee leafminer)	Leucoptera sp.
Pod-borers	Maruca testulalis and Fundella pellucens

Scarlet Runner Beans (Phaseolus coccineus)

Bean beetle	Cerotoma graminea
Pod-borer	Maruca testulalis
Lace bug	Corythucha gossypii
Leafhoppers	Empoasca spp.

Resistance Studies

Screening for resistance to insect damage involved 97 cowpea and 88 bean cultivars in several plantings throughout the year. Bean cultivars were evaluated for resistance to the most common pests in the area, such as the leafhopper, the bean weevil, the bean beetle and the vegetable leafminer. Cowpeas were evaluated for resistance to the cowpea curculio, the cowpea weevil and preliminarily, to the bean beetle, the leafhopper and the leafminer. Most of the effort was devoted to the leafhopper on beans and to the cowpea curculio on cowpeas.

Beans

Leafhopper - *Empoasca* spp. (Homoptera: Cicadellidae)

Evaluation of bean cultivars for resistance to leafhoppers was based on the relationship of plant symptoms and populations of nymphs of the insect. Forty-one cultivars showed evidence of resistance to the leafhopper, 25 were moderately resistant, and 14 were highly susceptible. Among the beans most resistant to leafhopper attack were some local cultivars, e.g., Bonita Sel. 1-8, Colorada del País and Naranjito.

Other leafhopper resistant cultivars were: 71-1R-101, 15R-194, Mexico 235, PCCMCA-50600, Mexico 309, 15R-57, 15R-52, Ven. 36, 15R-55, 15R-87, 15R-149, Porrillo No. 1, I-2-2-2, Criollo Sto. Domingo, Jamapa, 71-1R-136, Pompadour, K-2-1-2, Col. 163-A, Mexico 1403, SPK-155212, SB-31 (PCCMCA-R63), 70-10R-19 (PCCMCA-R2), PCCMCA-50613, PCCMCA-51052, Mexico 1225, Preto (2449), PCCMCA-R96, Rojo Garán and PCCMCA-Jamapa. Cultivars Gold Crop, 71-1R-113, 15R-42, 15R-193, 15R-287, Galana, 27R, Big Bend Red Mexican, Col. 1-63-A, La Vega, Santa Ana, Nicaragua, 51057, 51052, Ven. 63 and Borinquen were moderately affected by *Empoasca*. Jamaica, Diablo, French Horticultural,

15R-277, Royal Red Kidney, Colombia Pinto (USDA), 15R-224, 51051, SB-34 (PCCMCA-R84), Porrillo No. 1 (PCCMCA-R100), I-2-2-2-Y-Test Sel. A, SB-68 (PCCMCA-R89), H-2-5-1 and Rayada were the cultivars most seriously affected by this insect.

Eight leafhopper resistant bean cultivars from CIAT, Cali, Colombia were planted along with cultivars Bonita (resistant) and Rayada (susceptible). All CIAT cultivars were more resistant than Rayada. Only one cultivar (73-Vul. 3128) was slightly superior and 2 cultivars (B-343 and PI-208-769) were similar to Bonita. Five cultivars (72-Vul. 25096, PI-200974, B-1087, B-1096 and 73-Vul. 3624) were more affected than Bonita. None of them compared with Bonita in yield.

Based on the results available to date, we may conclude that all bean cultivars are subject to leafhopper attack and that the populations of Empoasca present on different cultivars are not indicative of differences in susceptibility. Susceptibility is expressed by plant symptoms and reduction in yield. Resistant cultivars may support, without marked symptoms and reduction in yield, higher leafhopper populations than susceptible ones. This indicates the resistance involved should be a type of tolerance of the plant to the insect attack. A field test to determine the level of leafhopper population causing economic damage was performed by using a susceptible and a resistant cultivar. Different dosages of the insecticides disulfoton (Di-syston) and carbofuran (Furadan) were applied to promote different leafhopper population levels on the experimental plants. Leafhoppers did not appear in considerable numbers until a month after planting, when the plants were already developed. By that time, populations on plots without insecticides were 6 and 3 nymphs per 10 leaflets

on Rayada and Bonita, respectively. Forty-four days after planting leafhopper populations were at about 30 nymphs per 10 leaflets on both cultivars. None of the leafhopper population levels evident in this test affected yield in any of the cultivars. Covariance analyses were performed with population levels and bean yield and none of the comparisons were significant, indicating that such populations levels were not detrimental to bean yield at that stage of plant development. Regulation of Empoasca populations via granular systemic insecticides applied at different dosages, may facilitate future management studies with this insect pest.

Vegetable leafminer - Liriomyza sativae (Diptera: Agromyzidae)

For the past four years, progress in screening for resistance to the vegetable leafminer in the Isabela area, has been limited, due to very low populations levels of this insect. Only one group of 32 cultivars was subjected to an infestation high enough for a meaningful evaluation. This evaluation was performed by examining the primary and subsequent trifoliate leaves of the bean plant. The percentage of mined leaves plus a visual estimate of the area consumed by the larvae were recorded. Of the 32 cultivars evaluated, Ecuador 299 and SB-31 (PCCMCA-R63) were highly resistant to leafminer attack.

Fundamental biological studies of the leafminer on beans were performed at Río Piedras. Leaf samples were collected at random from each of 32 bean cultivars grown at the Isabela Substation. The material was taken to the laboratory in Río Piedras. Emerging pupae were collected at 6-day intervals and placed in clear plastic ice cups (1.5 x 1.6 x 1.1 inches) and incubated at 26°C. Counts were made of the number of a) Liriomyza sativae adults, b) parasites which had emerged, and c) nonviable pupae.

The emerging pupae varied from 1-8 per bean leaf. The least infested cultivars (1 to 2.75 pupae per leaf) were Mexico, 71-15R-42, Big Bend Red Mexican, 71-15R-57, 51057, 71-15R-194, Pompadour, 61-1R-101, 71-15R-87 and Royal Red Kidney. Cultivars 71-1R-136, 71-15R-55, 71-15R-193, Bonita, Rayada, 71-15R-149, Jamapa, Porrillo No. 1, 71-1R-113, Colorada del País and Galana were moderately infested (2.76 to 3.75 pupae per leaf). Cultivars 71-15R-52, La Vega, 71-15R-287, French Horticultural, Jamaica, 71-15R-277, Naranjito, 27-R, Diablo, Criollo Sto. Domingo, Santa Ana and Nicaragua had the highest infestations (3.76 to 8.00 pupae per leaf).

From a total of 962 pupae collected, 33% were parasitized, 47% were nonviable for unknown reasons and only 20% emerged as adult leafminers. Apparently, there is an effective natural control of this insect in the Isabela area. Even in an instance of low infestation, there was 80% natural control on the pupal stage. The fungus Nigrospora, in addition to the insect parasite complex, may play a significant role in the regulation of L. sativae populations. This could possibly explain the low population levels observed at the Isabela Substation for the past 4 years.

Ten bean cultivars were used to evaluate several methods to determine leafminer infestation. Plants of all cultivars were similarly infested with the leafminer. The most accurate method, from the standpoint of determining the undamaged leaf area, was the one based on the percentage area consumed by the larvae. The fasted method of estimation, with little possibility of attributing leaves to the wrong category was the mined or unmined categorization of leaves. The most accurate method, for purposes of making projections of future population levels, was that of counting each mine occurring on a leaf.

On February 1975, the bean variety, Galana, was planted in the shadehouse at Río Piedras to determine the duration of the life cycle of L. sativae, in Puerto Rico. The plants were observed daily to determine when the first mine appeared. It was assumed that oviposition occurred the day flies were first observed on a leaf or leaflet.

The average duration in days of the different life stages of L. sativae was: egg, 5.8; larva, 3.7; prepupa and pupa, 9.9; and adult, 7.2. Under conditions of high relative humidity, i.e., 100%, emergence from the pupae was only 25% of that observed under conditions of lower humidity. The prepupa emerged and pupated on the surface of the soil or on the abaxial host plant leaf surface. If pupation occurred on leaf surfaces wind eventually dislodged the pupae. The newly emerged prepupae and pupae were bright yellow in color. After 2 days, the color turned to dark brown. This colorations made the puparium extremely inconspicuous on the surface of the soil.

In cowpeas grown in the shadehouse at Río Piedras, L. sativae prepupae emerged from the adaxial surface of the leaflets as well as the abaxial. Emergence from the adaxial surface was much more prevalent in cowpeas than in beans. In all other host plants observed, i.e., tomato, pepper and cucumber, prepupae emerged from the abaxial surface. Less than 10% of the prepupae emerged from the adaxial surface of bean leaves, as opposed to 90% from the cowpea leaves.

The larval stage of development of L. sativae is the stage during which the primary damage to the host plant is inflicted. The exact amount of leaf area consumed was measured by tracing the white pattern of the mine on tracing paper. This paper was then superimposed on Keuffel and Esser Co.

12-5285 sq mm graph paper. The sq mm's were then counted and an estimate of the damaged area necessary for the completion of development was obtained. It was found that the amount of effective photosynthetic tissue consumed during the larval stage of development ranged from 40 to 69 sq mm, with an average consumption level of 50.7 sq mm.

An experiment, to determine the critical wind velocity at which L. sativae adults would not fly, was conducted at the Fortuna Substation on May 1975. Flies which were not ovipositing were observed, the assumption being that nonovipositing flies could fly from the leaf at any moment when they were so compelled. A pencil was used to stimulate the flies to fly. A wind meter was used to estimate the wind velocity. The temperature at the time of these observations was approximately 29°C. These observations indicated that adults of L. sativae are not able to fly when wind speeds are greater than 8 km/hr. At speeds below this they fly readily.

L. sativae may be found in all stages of development throughout the year. There might be approximately 12 generations a year under the climatic conditions existing in Puerto Rico. Therefore, this insect has the potential of reaching pest levels in a very short period of time, assuming environmental checks, such as parasites, are not as effective as they are in the Isabela area.

A host range study of L. sativae was conducted in the shadehouse at Río Piedras. Plants representing 10 families and a total of 62 different varieties were established and examined for infestation by L. sativae. Datura stramonium, Amaranthus dubius, Cleome speciosa and Ipomoea tiliacea are non-cultivated hosts of L. sativae. Among the cultivated host plants of this insect are cucumber (Cucumis sativus), cantaloupe (Cucumis melo),

cabbage (Brassica chinensis), broccoli (Brassica oleracea italica), radish (Raphanus sativus), head lettuce (Lactuca sativa capitata), leaf lettuce (Lactuca sativa crispa), sunflower (Helianthus annus), pea (Pisum sativum), cowpea (Vigna unguiculata), bean (Phaseolus vulgaris), bushbean (Phaseolus vulgaris humilis), lima bean (Phaseolus lunatus), okra (Hibiscus esculentus), pepper (Capsicum annuum), tomato (Lycopersicon esculentum), eggplant (Solanum melongena), watermelon (Citrullus vulgaris), and pumpkin (Cucurbita pepo). Larvae of L. sativae were not observed on carrot (Daucus carota), anise (Pimpinella anisum), collard (Brassica oleracea acephala), cauliflower (Brassica oleracea botrytis), cabbage var. Copenhagen Market (Brassica oleracea capitata), endive (Cichorium endivia), beet (Beta vulgaris), spinach (Spinacia oleracea), sweet corn (Zea mays), and onion (Allium cepa).

Bean weevil - Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae)

Screening of bean cultivars for resistance to the dry-seed weevil have been based on natural field infestation. Samples of 100 seeds from 4 replicates of each cultivar were set at room temperature for 2-3 weeks, after which time all the infested seeds and holes per sample were recorded. Seed infestation percentages ranged from 0 to 87. The least affected cultivars in the test were Preto Garuará, Bonita, 50600, R-88 (SB-51), R-84 (SB-5), 51052, Guatemala 401, Porrillo No. 1, R-94, Preto (2449), Jamapa, Ven. 36, H-2-4-A, 15R-149, 15R-193, 15R-194, 15R-57, 15R-42, 71-1R-101, 15R-87, 15R-52, 15R-55 and Col. I-63-A. Eight cultivars from CIAT, tested along with Rayada and Bonita, had one per cent or less infested seeds, while in Bonita and Rayada 3.1 and 8.5% of the seeds, respectively, were infested. Several cultivars were moderately attacked, while others, namely, Diablo, 27-R, Jamaica, 15R-277, Colorada del País, Pompadour, 15R-224, Apollo,

H-2-5-1, Galana, I-2-2-2, Ecuador 299, Rayada, Mexico 235, Rojo Garán, Col. 163-A, Borinquen and K-2-1-2 were seriously damaged. This is preliminary information based on two planting dates. Additional tests are needed to continue the evaluation process in order to obtain conclusive results. Eventually, laboratory tests will be performed to confirm resistance.

Bean leaf beetle - *Cerotoma ruficornis* Olivier (Coleoptera· Chrysomelidae)

Forty-six bean cultivars were evaluated for resistance to the bean leaf beetle. Based on a visual estimate, an index from 1-5 was assigned to each plot, where one represented the least and five the most affected cultivars. Twenty-four cultivars showed some measure of resistance. The least affected cultivars were Diablo, Royal Red Kidney, Apollo, Big Bend Red Mexican, Gold Crop, I-2-2-2, K-2-1-2, Borinquen, Naranjito, 15R-277, Jamaica, 27R, Colombia Pinto, 15R-194, Colorada del País, Rayada and H-2-4-A. Most of the cultivars, however, were rated as susceptible to leafhopper attack. Resistance to *C. ruficornis* has been difficult to detect in the field. Tests to determine resistance to this insect should be conducted in controlled environments.

Cowpeas

Cowpea curculio - *Chalcodermus ebeninus* Boheman (Coleoptera: Curculionidae)

Results of resistance studies with the cowpea curculio on cowpea cultivars has shown a wide range of variation. While all cultivars were attacked, some of them showed evidence of resistance. Data was obtained from fresh pod samples on the number of punctured pods (for feeding or for ovipositing), total number of punctures and total number of seed perforations per sample, emergence of larvae, and punctures on dry seeds. Data on dry seed punctures and on emergence of larvae per cultivar have not correlated with the data on fresh pods and will not be considered on subsequent trials.

Cowpea cultivars 10R-61, 4R-173, 4R-421, 4R-338, 4R-109, 4R-271, PI-170869, PI-162699, PI-170867, PI-171891, PI-163448, PI-167024, PI-179124, PI-163143, PI-292871, PI-164974, 7805 and 7806 have shown resistance to the cowpea curculio. Moderate resistance was observed in cultivars 7807, PR-V-70-4R-28, PR-V-70-4R-38, PR-V-70-4R-2, PR-V-70-4R-18, C-105, Zipper Cream, Lentejas, PI-293460, PI-170871 and in some others which under a moderate insect population will be lightly affected, but which under a heavy population pressure will be seriously damaged. Cultivars PR-V-70-4R-22, Early Ramshorn, and a native variety were highly susceptible. One or more of the latter cultivars is being used as susceptible check in every planting.

Results indicate that possibly two types of resistance to the cowpea curculio are involved. These are postulated as antibiosis and nonpreference. Further trials and selections are needed to clarify the picture. The most resistant cultivars will be tested under controlled conditions to confirm resistance and to identify the factors involved.

Cowpea weevil - *Callosobruchus chinensis* Linnaeus (Coleoptera: Bruchidae)

Data on resistance in cowpea to the dry seed weevil was based on a 100-seed sample from 4 replicates from each cultivar. Cultivars 10R-61-7806, 7805, C-105, 4R-222, 4R-137, 4R-445, 10R-58, 10R-3, 4R-339, 4R-180-1, 4R-145, 4R-141, 4R-421, 4R-169, 4R-173, PR-V-70-4R-1, PR-V-70-4R-2, PR-V-70-4R-22, Zipper Cream, PR-V-70-4R-38, PR-V-70-4R-28, PR-V-70-4R-138, Lentejas, PI-162699, PI-171891, PI-185466, PI-293449, PI-163448, PI-189378, PI-182317, PI-183250 and PI-175963 showed evidence of resistance. Early Ramshorn, Gordo and a native cultivar were highly susceptible. Further selection will be made and the most resistant cultivars will be tested under controlled conditions.

Leafhopper - *Empoasca kraemeri* and *E. Millsi* Ross (Homoptera: Cicadellidae)

Fifty-eight cowpea cultivars subjected to heavy leafhopper attack were evaluated for resistance to leafhopper. Nymph counts and visual ratings of symptoms were recorded. The prevalent main symptom was a yellowing of the leaves. Based on these data, cultivars Lentejas, 4R-283, PR-V-70-4R-28, 4R-277, PR-V-70-4R-22, 4R-289 and 4R-373 were rated as resistant. Cultivars 4R-176, 4R-9, PR-V-70-4R-2, 4R-91, 4R-178, 4R-349, 4R-105, 4R-343, 4R-200 and 4R-179 were moderately resistant. Some others, e.g., 10R-61, 4R-93 and 4R-412, were highly susceptible. As on beans, the number of leafhopper nymphs per leaflet could be misleading, since the most resistant cultivars are able to support higher populations of the insect than susceptible ones.

Bean beetle - *Cerotoma ruficornis*

A preliminary evaluation for bean beetle damage was performed on several cowpea cultivars by assigning an index to each plot according to the amount of leaf area consumed. Cultivars 4R-111, 4R-271, 4R-396, 4R-150, 4R-169, 4R-180, 4R-253, 4R-258, 4R-417 showed evidence of resistance to this insect. Other cultivars planted in different seasons were not severely attacked and evaluation could not be performed.

Vegetable leafminer - *Liriomyza sativae*

Preliminary observations on leafminer infestation were performed at the Isabela Substation on a group of 33 cowpea cultivars. The emerging pupae from random leaf samples from each cultivar were collected. The number of emerging pupae from the different cultivars varied from 0.33 to 3.67 per leaf. Emergence of less than one pupa per leaf was considered as preliminary evidence of resistance. Cultivars 4R-222, 4R-173, 4R-176, 4R-200 and 10R-61 were rated in this category. Cultivars 4R-178, 4R-445,

4R-429, 4R-169, 4R-258, 4R-171, 4R-343, 4R-179, 4R-396, 4R-202, 4R-283, 4R-327 and 10R-3, which had 1.0-1.5 pupae per leaf, were considered as moderately resistant.

From a total of 415 leafminer pupae collected, 25% were parasitized, 52% were non-viable for unknown reasons, and from 23%, adult flies emerged.

Another 40 cultivars were evaluated in Río Piedras, using a categorization system based on the percentage area consumed by the larvae. The categories were 25 percentage points in length, i.e., 0 to 25, 26 to 50, 51 to 75 and 76 to 100% area consumed. The percentages of mined area ranged from 10.47 to 31.13. The less affected cultivars were PR-71-10R (Nos. 173, 1, 111, 14, 91, 105, 95 and 445), and PI (Nos. 255776, 255815, 227827 and 255788). Leafminer infestation in both groups was considered low. Further tests are needed to reach conclusions.

Chemical Control

Several field tests have been performed with insecticides for the control of the most important insects of cowpeas. Early Ramshorn and a native cultivar, both susceptible to the cowpea weevil and to the cowpea curculio, were used in the trials. Twenty-five fresh pod samples, from 4 replicates, were collected and examined for incidence of the cowpea curculio. The most effective control for the cowpea weevil and for the cowpea curculio was Thiodan 2E (endosulfan), at the rate of 2 pints in 100 gals of water per acre, applied weekly during pod development.

Other effective chemicals were Moxie 50 (methoxychlor), at the rate of 4 lbs per acre, and Lannate 1.8L (methomyl), at the rate of 2 pints per acre. Toxaphene and Cygon 2.67E (dimethoate) also effected some control.

4. EVALUATION OF GERMPLASM FOR RESISTANCE TO DISEASES

Screening of a Recurrent Selection Population for Resistance to Rust

Background

For the past 5 years this project has reported on the occurrence of cross pollination of beans (*Phaseolus vulgaris*) in the field by the Carpenter bee, *Xylocopa brasilianarum*. Due to natural cross pollination, which generally varies from 5-12.5 percent of the observable characteristics in the F_1 progenies, a recurrent selection scheme was initiated. The recurrent selection consisted of a population that included lines selected for multiple disease resistance. During the successive screening trials the susceptible lines were excluded and new resistant entries were added to the population. Resistance against rust, caused by *Uromyces appendiculatus*, is a major goal of this project. The present rust resistance trial, planted at Isabela, was intended for locating sources of multiple, as well as monogenic resistances.

The population under study included 375 lines of beans. Each line consisted of seeds of either a) a single F_1 hybrid with multiple resistance, or b) a bulk of 3 to 10 segregants selected for yield and resistance, or c) a bulk from an advanced line or a cultivar which was selected for yield and multiple resistance. The majority of the selections were previously evaluated in a field screening trial at Mayaguez.

Results

Response to Rust (Field Inoculation) of a Recurrent Selection Population of Beans

The rust population which was introduced as inoculum into the bean plot had originated from a few virulent pustules appearing on cultivar La Vega in the greenhouse. Hence, the rust population in the plot should

have been predominantly homogenous and the bean responses should have been relatively uniform, distinct and without many variants. However, the data indicated that the bean population displayed a wide range of responses to rust.

Resistance

A resistance response is the result of a) the absence of interactions between rust and beans which is expressed as immunity; b) the absence of disease development, expressed by necrosis of the host tissue at the time of infection, which is designated as resistance; and c) the formation of minute and sparsely sporulating pustules of less than 100 microns in diameter and intensities of less than 30 percent, which is designated as moderate resistance. Environmental conditions and aging of the bean tissues have either slight or no effect on a resistance response.

A susceptibility response is the result of a) the development of large pustules (400 microns to 2 millimeters), which are usually bordered with chlorotic tissues; b) high infection intensity per unit of leaf area; and c) a relatively short incubation period. These factors cause reduction in leaf size, retard the growth of the plant and finally cause defoliation.

At 60 days, the rust resistant bean lines had an average height of about 40 centimeters (growth index of 4.2) and had retained 82 percent of their foliage, whereas the susceptible lines had an average height of 10 centimeters (growth index of 1.5) and had retained only about 12 percent of their normal foliage. Ninety percent of the leaf surface of the remaining 12 percent foliage of the susceptible lines was covered with '5' type pustules. Other data (tables omitted) afforded a more uniform genetic background for the comparison of the effect of rust on resistant and susceptible beans than the data just presented. The segregants in each

row had originated from a single F_1 hybrid. These F_1 hybrids were chosen for their resistance to rust. Hence a number of progenies apparently possessed tolerance as well as resistance. In spite of the tolerance of some progenies, the resistant progenies were twice as tall and retained almost twice as much foliage than the susceptible ones. Again it should be kept in mind that 73.5 percent of the remaining 52.0 percent foliage on the susceptible progenies was covered with '4.6' type pustules. This loss to rust of healthy foliage area will no doubt affect the development of seeds and reduce yield.

When the response of the recurrent bean population to rust is being recorded in the field, a range of expressions is at first confronted. However, repeated field observations and the analysis of the data indicated that there were two major types of bean response to rust, which were designated as a) qualitative resistance, and b) tolerance. These two types of response and the data pertaining to them are discussed in the following paragraphs.

Qualitative Resistance

In general, two types of resistance were observed in segregating progenies. In one group resistance was conditioned by dominant factor(s), while in another, it was governed by recessive factor(s). Also, the data show that besides the gene-for-gene type of interaction between the beans and the rust, there were other ratios that suggested gene interaction. These segregation ratios suggest that within the bean gene-pool, resistance to the rust population is conditioned by different, distinct factors.

Levels of Qualitative Resistance

Indexing of the bean response for rust interaction indicated that there were gradations within each of the qualitative types of resistance. The

segregant progenies show the range of interactions that were encountered within the bean population and give the corresponding levels of responses within both the dominant and the recessive modes of inheritance. Whether bean response to rust was conditioned by either dominant or recessive factors, it consisted of a range of expressions that included resistance (R), moderate resistance (MR), moderate susceptibility (MS), susceptibility (S), and high susceptibility (HS). These responses suggest that within the two types of resistance, different genes condition a range of interactions with their corresponding counterparts in the rust population. Hence, in beans, qualitative factors with various levels of expression are responsible for conditioning responses against rust.

F_2 progenies from only one F_1 hybrid, out of 37 observed, displayed more than two groups of segregants. The 38 progenies within Row-492 segregated into 28 plants with 5,85 to six plants with 1,0 to four plants with 3,50 response, which closely approximates a ratio of 12:HS to 3:S to 1:MS.

Tolerance

What is tolerance, and how can it be measured? Perhaps the host-pathogen interaction between P. vulgaris and U. appendiculatus can provide an answer to these questions.

Tolerance of a host to a disease is intimately associated with the environment. The growth of the host, the development of the disease, and the effect of the environment are the interrelated and variable components of a tolerance interaction. Hence, in order to measure tolerance it is necessary to record the sum total of these interactions.

Perhaps this is an opportune time to state what is not tolerance. Quite often an intermediate grade response to rust is considered as a tolerance reaction. However, as segregation data indicate, an intermediate response could be due to a single gene which has a moderately high threshold for expression of resistance. Also qualitative responses either do not vary or vary only slightly with the age of the host. Hence, different levels of resistance should not be confused with tolerance. Tolerance, then is a characteristic which by superimposing upon, and modifying the normal host-parasite interaction, alters the host response. According to this definition the following characteristics were identified as components of a complex which governs tolerance in P. vulgaris.

a) Host age

An often repeated statement in the bean growing areas of the temperate zone is that rust will not attack beans until flowering or pod set stage. In the tropics rust attacks seedlings as well as mature plants. Actually, in our plots, seedling-resistance/mature-plant-susceptibility has been an exception, as evidenced by the occurrence of only nine lines with such a relationship among the 375 lines of the population. Plants with this type of host response are designated as "late rusters".

One form of tolerance is conditioned by the aging of the host which modifies the rust response that was expressed at the seedling stage. The data at hand indicate that this type of tolerance is not a fixed reaction, but constitutes a gradation of modifications. In general, an age-influenced tolerance modifies either pustule size, or rust intensity, or a combination of both.

(1) Grade or Pustule Size

The majority of rust responses include either seedling-susceptibility/mature-plant-susceptibility or seedling-resistance/mature-plant-resistance combinations in which there is no change in pustule grade. However, in the case of "slow rusters", the plant becomes more susceptible as it matures. On the other hand, in mature plant tolerance, the size of the pustules decreases as the age of the host increases.

(2) Intensity or Frequency of Pustule Formation

A common expression of tolerance is the reduction in pustule frequency as plants advance in age. In other words, rust intensity decreases as the host age increases. This reduction in intensity is often associated with a reduction in pustule grade. In a few instances a decrease in rust intensity was not associated with the corresponding changes in pustule grade.

(3) "Low Rusters"

Occasionally a bean line is encountered which supports a low intensity of rust pustules throughout its growing period. Among the "low rusters", disease diminishes as plants increase in age. However, as plants mature, the reduction in intensity is much less than the corresponding decrease in pustule grade. The low intensity rust infection seems to be associated with '3' to '4' type grades at seedling stage. None of the seedling lines displaying '5' type pustules supported a low intensity of rust incidence.

b) Compatibility

Bean plants that do not display a clearly defined physiologic reaction to rust infection are called rust compatible. Two types of rust compatibility were identified in beans. These included foliar compatibility and pedicular indehiscence.

(1) Foliage Compatibility

Leaves of a number of bean lines do not develop chlorotic halos around the rust pustules. The lack of chlorosis or hypersensitive reaction to infection is termed compatible interaction. The leaf surface of the compatible plants supports a large number of pustules (60 to 90 percent). The main problem with pustule intensities of over 50 to 60 percent of the leaf surface is that as soon as the plant is under moisture stress, the diseased leaves desiccate and die. An important advantage of the rust compatible over the susceptible-incompatible is that the latter interaction causes rapid defoliation when rust intensity reaches 30 to 50 percent of the leaf surface, while at these intensities the compatible interaction permits leaves to carry on photosynthetic and respiration functions. Cultivar La Vega is a good example of a rust compatible bean.

(2) Pedicular Indehiscence

An outstanding feature of the tolerance complex in bean is resistance against defoliation. A number of highly susceptible bean lines, in spite of rust intensities of 70 to 95 percent, retain their foliage for a long period. The difference between the susceptible-defoliating and tolerant non-defoliating beans is the indehiscence characteristic of the petiole. Cultivar La Vega shows, in addition to foliage compatibility, resistance to defoliation. As the average values for this cultivar indicate, it is as susceptible to rust as the 10 selected lines. However, plants of La Vega are three times larger and have about six times more foliage than the susceptible-defoliating type plants. This ability to retain leaves while heavily rusted allows the plants to produce a moderate yield. The

resistance to pedicular dehiscence seems to be due to delay in corky tissue formation at the abscission layer between the pedicle and the branches. Field observations suggest that there are grades of indehiscence characteristics.

c) Hypophyllus Rusters

On certain beans, rust pustules develop only primarily on the lower leaf surface. Up to date, this characteristic has been observed on bush beans, or beans with determinate habit. Also, hypophyllus rust has a grade '3' limit. When pustule sizes are greater than '3' rust appears on the upper leaf surface as well. Often the intensity of rust incidence on the lower surface is about 50 to 70 percent, while on the upper surface it may be 5 to 10 percent. In the hypophyllus rusters leaf morphology may be the reason for pustule formation in the parenchymatous tissue. The hypersensitive reaction to rust may result in chlorosis and defoliation. However, when observing hypophyllus rusters from above, the reason for defoliation is not readily apparent.

Other Diseases

In tropical soils, rhizoctonia root rot and stem canker of beans (*Rhizoctonia solani*) causes yield reduction by infecting either the root system or the branches. Few cultivated fields, especially in the lowland tropics, are free from *R. solani*. The soil infestation increases by repeated planting of beans in the same plot. Our field plots, both at Mayaguez and at Isabela, have been under continuous cultivation of beans and cowpeas for the past 5 to 7 years. Hence, these plots are heavily infested with soil-borne pathogens, especially *R. solani*.

The majority of the population, 244 out of 375 lines had been selected for high tolerance or resistance to the soil-borne diseases endemic in our lowland field plots. These selections included rows R-437 to R-658 and R-790 to R-812. About one-third of the population, or 131 lines, which included rows R-659 to R-789, had not been sufficiently screened for resistance against rhizoctonia root rot and stem canker. Some of these lines were either susceptible or segregated for resistance against this disease.

The primary criterion on our screening program is resistance to soil-borne diseases. If a bean cultivar cannot withstand the onslaught of soil-borne diseases in the tropical soil, it shall be of little value to a farmer no matter how many other foliage resistances it possesses. It is for this reason that screening against foliage diseases is conducted in plots which are heavily infested with soil-borne pathogens. As our data indi ate, rust resistance alone is of little value if selected bean lines are susceptible to rhizoctonia root rot. On the other hand, resistance to soil-borne diseases only provides leaf surface for the rust to attack, if beans possess no resistance against rust. However, when resistance against both diseases occur in the same plants, the result is lush growth and subsequent high yields.

Lines R-437, 468, 481, 511, 527, 545, 548, 582, 594, 703 and 736 were susceptible to common bean mosaic virus (CBMV). Nineteen lines were susceptible to southern blight (Sclerotium rolfsii). These lines were R-464, 509, 525, 576, 656, 657, 658, 702, 703, 704, 711, 716, 717, 729, 738, 739, 740 and 751. At least 50 percent of the plants in each row were affected. Other diseases appeared at very low frequency and did not cause any significant mortality.

Level of Segregation

One hundred twenty-four of the 375 lines placed in the recurrent selection population segregated either for disease response or for agronomic characteristics or for both.

Discussion

Previous studies have indicated that at a given period of the year different rust populations occurred at geographically isolated locations on the Island. Also, that at a given location the race make-up of the population might change with time. Hence, in order to overcome the effect of the endemic rust population on this field inoculation test, a large quantity of inoculum was introduced into the field from the greenhouse. The rust culture that was introduced into the field was highly pathogenic to cultivar La Vega. The overall response of the beans indicated that the rust population was homogeneous, i.e., only one rust strain was predominantly present. There is no doubt that during the 3-month period of the trial spores of other rust races blew into the plot. In very few instances, however, more than one type of interaction on a bean leaf was encountered.

The range of responses expressed by the bean selections to the single rust culture suggested that the rust virulence was governed by a number of different factors which interacted with corresponding bean genotypes. An interesting aspect of the F_2 progenies was segregation for intermediate grade interactions. This suggested that qualitative inheritance of resistance is not necessarily the presence or absence of reaction. Factors with variable thresholds for resistance may interact with corresponding genes for virulence in the rust to express segregations for, e.g., moderate and

severe susceptibility. Hence, it is important to realize that there are levels of qualitative resistance and not to confuse this characteristics with those that express tolerance.

One of the F_2 progenies segregated into a ratio approximating 12 susceptible to 3 resistant to one moderately susceptible. The possible explanations for this ratio are: a) a seed mixture, which is possible; b) two rust races (but then why should only this line respond to it in such a ratio); c) the progenies with the intermediate susceptibility could have been expressing mature-plant resistance (this suggests segregation for tolerance as well as resistance in the same line); and d) two sets of factors governed the interaction of the R-492 progenies with the rust. In order to obtain a 12:3:1 ratio it is necessary to assume that: a) factor "S" is dominant for conditioning susceptibility, b) factor "R" is dominant for conditioning resistance, and c) S is epistatic to R. The result of such a segregation will be a ratio of 12(SS,Ss): 3(ss+RR,Rr): 1(ss+rr). This ratio suggests that the ss+rr combination conditions moderate susceptibility interaction with the rust genotype. It is realized that this type of hypothesis does not fit with Flor's "gene-for-gene" hypothesis. Although Flor's hypothesis provides an excellent working rule for the study of the genetics of host-parasite interactions, it is felt that, as is the case with the majority of biological rules, that there are always exceptions.

Many bean genotypes, whether resistant, moderately susceptible or susceptible to rust, show a hypersensitivity response expressed as chlorotic leaf tissue. The characters that condition formation of the chlorotic tissue are undesirable features of these genotypes. The reason for

it is that, at times, there are more chlorotic leaf tissues than the leaf surface which is covered by the rust pustules. When breeding for rust resistance the genotypes which develop chlorotic tissue should be omitted from future recombinations.

One of the problems encountered in recording rust interaction in screening trials is how often readings should be performed. This concern arises, for example, when one encounters a seedling-susceptibility response which changes to a mature-plant-resistance response. When such a change occurs then the questions are: a) at what stage of plant growth the change began?, and b) was the change in pustule size a gradual one? The only way to find out the details of these responses is to test the tolerant lines. However, two recordings are the minimum, and the whole field should be observed, at least, four times during the test period.

How to distinguish between resistance and tolerance to rust has been a major concern during our screening trials. The data extracted from the screening of the recurrent selection bean population for rust resistance suggested that tolerance is not the result of a gene-for-gene interaction. The data suggested that tolerance is a host characteristic which is independent from resistance, but when it occurs in conjunction with resistance it superimposes and modifies the resistance interaction.

Tolerance does not consist of only a single characteristic but includes a complex of characteristics. These characteristics include: a) interrelation between host age and pustule size, b) interrelation between host age and rust intensity, c) host ability to allow only a low intensity infection, d) compatibility between leaf tissue and rust fungus, e) ability of plants

to retain their leaves in spite of severe rust intensity, and f) hypophyllus development of the rust pustule. Tolerance, in general, is the sum expression of at least two of the above mentioned characteristics in a plant.

The most desirable type of rust resistance is one that combines qualitative resistance with tolerance characteristics. A complaint that plant breeders express about this type of combination is that the qualitative resistance masks the tolerance response and hence it is difficult to ascertain that the latter is present. In the screening of the recurrent selection population it was observed that the segregation of the F_2 progenies revealed whether an F_1 hybrid contained both types of responses. However, in order to ascertain the presence of tolerance in the F_2 progenies, the response of the segregants should be recorded at least twice. Also, it is necessary to record the percentage of normal foliage borne by the plants at the final recording.

Mature-plant resistance is an important factor against rust and it should be incorporated into the bean population as a basic characteristic. The combination of mature-plant resistance plus foliage compatibility and pedicular indehiscence should provide a bean plant with a high degree of rust tolerance. Inclusion of qualitative resistance on top of this will most likely result in a universal rust resistant line such as cultivar Ecuador-229.

Observation has suggested that high temperatures cause the breakdown of the tolerance response, while cool weather shifts the tolerance response toward resistance. These observations need to be studied further. If the temperature-rust tolerance relationship holds true, then lines which

show tolerance under tropical conditions should be mostly rust resistant in the temperate zone. The behavior of cultivar La Vega suggests that temperature relationships do exist. The relationship between bean response to temperature variation and its effect on rust interaction could be due to still another type of tolerance.

One of the reasons for defoliation of the rusted plants is the desiccation of the diseased leaves. Field observations indicate that rust susceptible plants affected by root rots defoliate rapidly, while root rot resistant plants keep producing foliage, which the fungus infects. If the latter type encounters water stress, then its diseased leaves desiccate. Hence any characteristic that prevents desiccation should prolong the life of the rusted plant. Therefore, if such characteristics are identified, they would comprise another factor in bean tolerance to rust.

Susceptibility to root rots seems to be associated with earliness in beans. Among the root rot resistant lines, pod maturity is not associated with plant senescence. The determinate (bush type) beans are more senescence-sensitive that the indeterminate types. However, among the root rot resistant bush types the continuation of growth after pods have matured depended upon the presence of nutrients. In general, a long growing period in the field is indicative of resistance against soil-borne diseases. It seems that if beans with high root-rot and foliage-disease resistance are developed, then it would be necessary to chemically defoliate the crop prior to harvest.

It is interesting to note here that one-third of the population was visibly designated as segregating while not a single flower of the previous generations had been hand-pollinated.

International Bean Rust Nursery Trials

Rust (*Uromyces appendiculatus*) is one of the most prevalent diseases of beans in the tropics. It may attack susceptible seedlings at the primary leaf stage causing defoliation and death of the plants. By 1973 a number of rust resistant bean cultivars were developed by researchers in tropical America, and were being reevaluated at various geographical locations. At the Bean Rust Research Workshop held at CIAT, in October 1974, it was decided to start an International Bean Rust Nursery (IBRN). At that gathering representatives from 10 countries, including Puerto Rico, expressed interest in planting the IBRN trials. Trials were planted at Isabela and Adjuntas.

Rust incidence was severe at Isabela. The overall rust intensity for the IBRN entries at 30 days after sowing was 40%. The rust intensity for the whole plot dropped to 28% within 10 days after the first recording. Defoliation of the severely infected cultivars was the primary reason for the lower intensity. At this stage, some cultivars supported only immature leaves.

At Adjuntas, IBRN entries had a longer vegetative growth and rust intensity was 23% at 50 days after sowing, and reached an overall intensity of 38% at 80 days after sowing. The application of Plantvax at 70 days reduced rust incidence for about 2 weeks, but by 30 days later rust intensity reached 44%.

The fourteen cultivars listed below were rated as resistant to moderately resistant at both locations. These cultivars are likely candidates for further IBRN trials.

IBRN No.	Name	Overall Response in Puerto Rico
4	California Small White #643	MR
6	Diacol-Calima	R
7	Compuesto Chimaltenango-3	R
9	Cuilapa-72	MR
10	70-15R-277-Bk (PR)	R
13	Mexico-309	R
15	Ica-Guali	MR
19	Turrialba-4	MR
25	Linea-37 (Colombia)	MR
26	Ecuador-299	R
34	Compuesto Chimaltenango-2	R
45	Negro San Remo	R
47	71-1R-63 (Puerto Rico)	R
55	Redland Green Leaf B	R

The following seven cultivars are suggested as candidates for differentials due to their response to rust populations in Puerto Rico and CIAT.

IBRN No.	Name	Rust Reaction		
		CIAT 1974	PR 1975	
			Isabela	Adjuntas
6	Diacol-Calima	5,99	2,30	---
10	70-15R-277-Bk (PR)	4,50	1,10	4,30
21	PI-319549	1,20	5,50	---
50	Diacol-Nima	5,99	1,0	5,20
54	PI-199044	4,50	1,0	5,80
55	Redland Green Leaf B	4,50	1,10	---
56	PI-313664	4,80	1,0	4,80

Cultivars Pinto No. 650 (IBRN No. 8), Porrillo Sintético (IBRN No. 35), and Jamapa (IBRN No. 58) are suggested as IBRN susceptible standards. Cultivars Mexico-309 (IBRN No. 13), Ecuador-299 (IBRN No. 26), and Compuesto Chimaltenango-2 (IBRN No. 34) are suggested as IBRN resistant standards.

It is suggested that the eighteen cultivars listed below be removed from future IBRN trials due to their high degree of susceptibility, both at CIAT and Puerto Rico.

IBRN No.	Name	IBRN No.	Name
2	Cuva-168-N	24	Ky Wonder No. 780
5	27R	27	Porrillo-70
12	Ky Wonder No. 814	28	Bountiful No. 181
14	Turrialba-1 (S-19-N)	29	ICA-Tui
17	Negro Jalpatagua	48	PI-163372
18	San Pedro Pinula	49	NEP-2
20	U.S. No. 3	51	PI-165426 (Black)
22	Porrillo-1	53	Rico-23
23	Rico Padro-896	57	PI-165426 (White)

The remaining 18 cultivars should be reviewed by the IBRN collaborators, and their merits for being retained in the trials should be decided upon by their consensus. It is also suggested that the following six cultivars, which had a resistance response to the rust races at CIAT in their 1974 trial, should be included in the IBRN trials for further observation.

CIAT 1974 No.	Name	Rust Reaction
32	15R-148 (PR)	2,50
42-129	Frijol Trigo - E	2,5
57-143	51052	1f,5
65-135	Ipala-72	1f,5
67-133	IAN-5091	1,0
-148	PI-289400	1f,20

Bacterial Blight Trials

During 1975 bean seeds were exchanged between Drs. Alfred W. Saettler (Michigan State University) and Nader G. Vakili for screening against bacterial blight. The purpose of this exchange was to expose bean lines selected for tolerance to bacterial blight to a wider range of pathogenic strains under different environmental conditions.

The January field trial of the Michigan accessions at Isabela was a failure due to dry winds. Even daily sprinkler irrigation could not counter the desiccation caused by the winds. However, the September greenhouse tests at Mayaguez, indicated that a number of lines were tolerant to bacterial blight.

Bean lines selected for their field tolerance to bacterial blight in Puerto Rico were sent to Dr. Saettler for screening at East Lansing. Twenty-nine out of 62 Puerto Rican lines sent to Michigan, were considered tolerant to highly tolerant (less than 1.0 disease index) to Xanthomonas strains prevalent in Michigan. The high incidence of tolerance among the Puerto Rican beans suggests that a) in the tropics plants are exposed, during screening, to greater disease stress than in the temperate zone; b) there are more Xanthomonas strains with greater virulence in the tropics than in the temperate zone; c) different criteria were used in recording tolerance. The third possibility does not apply because methods of recording the disease were very similar.

Of the 81 Michigan accessions tested in Mayaguez for resistance against a single strain of Xanthomonas (Col. #113), three were highly tolerant and 13 were tolerant. This indicates that the tolerance encountered under Michigan conditions is also functional under the tropical environment of Mayaguez. At the December 1975 CIAT Bean Program Meeting, Dr. Saettler stated that some of his lines, which were highly tolerant in Michigan, were susceptible in Mayaguez and vice versa. These findings are very important in seeking tolerance to bacterial blight in beans because they point to the existence of pathogenic strains of Xanthomonas.

Screening of Bean Hybrids from Prosser, Washington, for Resistance to Diseases

Arrangements were made with Dr. D. W. Burke to screen the hybrids developed by the bean project at Prosser, Washington, against the endemic diseases in Puerto Rico. Through this cooperation the Puerto Rico bean program received potentially high yielding and commercially acceptable bean lines for use in its breeding efforts, while the Prosser program received information on disease resistance and adaptability of their hybrid beans and also seeds from the selected progenies.

Isabela Trial

Rust

Rust spreaded with such rapidity among the test lines that there was risk of losing most of the foliage before disease response could be rated. At 30 days, immediately after the recording of rust response, and again 15 days later, all test lines were treated with soluble foliage fertilizer. These treatments helped the majority of the 436 test lines to reach flowering and pod set. However, in some lines rust was so severe that before leaflets could reach full expansion they were covered with pustules. At rust intensities of 90% or higher, the green color of the leaf tissue was not visible and in some lines, stems, petioles and young pods were infected. Seventy-two days after sowing only 94 out of the 436 test lines planted still maintained some foliage.

Rhizoctonia Root Rot and Stem Canker

Seventy days after sowing, 301 lines out of the 436 lines sown, or roughly three-fourths of the F_3 and F_4 hybrids, had died to a combination of rhizoctonia root rot and rust. Of the remaining lines, 42 had some

plants with green stems, but with no foliage. The remaining 94 lines showed different levels of resistance to rhizoctonia stem canker. Some lines, such as 3R-7, 3R-29, 3R-67, 3R-317, 3R-320 and 3R-321, had tolerance to both rhizoctonia stem canker and rust. The high percentage of foliage retention of these lines at 71 days after planting indicated adaptability to environment and tolerance to disease pressure in the field.

Selection of F_3 and F_4 Lines for Future Tests

The first 10 groups of hybrids were selected for their tolerance to diseases and their relatively high yield. The second group was selected from 9 hybrids lines that contained a high percentage of plants with rhizoctonia stem canker resistance. The remaining 10 lines were selected for their seed form characteristics. Altogether, 45 bulk selections were made from 27 hybrid lines. These selections were planted at the Fortuna Substation for seed increase and further disease screening.

Fortuna Trial

Selected hybrids from Dr. D. W. Burke's crosses were planted at the Fortuna Substation on June and harvested on September 1975, 97 days after sowing. All data, except on germination and bacterial blight rating at 60 days, were recorded between 95 to 97 days after sowing.

Bacterial Blight

The bacterial blight rating at 60 days was made at the start of the rainy season. In the period from mid-August to mid-September rainfall was on the increase. Hence the mid-September recording reflected the true response of these hybrids to this disease. All hybrids, except Fort-R-23, which is Burke's 3R-1315, were either susceptible, or highly susceptible. Hybrid 3R-1315 is a pinto, which was the most rust resistant line in the Isabela trial.

Soil-borne Diseases

There was very little incidence of rhizoctonia stem canker. However, fusarium root rot (*Fusarium solani*) and charcoal rot (*Macrophomina phaseoli*) caused some mortality. Three of the hybrid lines that had a high incidence of charcoal rot also had macrophomina stem canker. The lesions of this disease generally occur around stem nodes. Cultivars with a stand of 20 green plants, or higher, at harvest time were rated as tolerant to soil-borne diseases. Hybrid Fort-48 had 88% of green plants at harvest. This cultivar is Burke's hybrid number 3R-583, which has small white pea beans. Seventeen of the selections had 20 (40% stand) or more green plants at harvest. These selections will be tested in replicated yield trials and compared with standard white cultivars such as Bonita, U.S. No. 643, and NEP-2.

Conclusions

Considering the wide spectrum of disease resistance that had been bred into these hybrids, a greater frequency of survivability than what was encountered was expected. The predominance of susceptibility at F_3 and F_4 stages of segregation in such a large number of crosses suggested that under tropical conditions disease intensity and pressure are much higher than those encountered at Prosser, Washington. This relationship holds true for cultivars and hybrid lines received from other areas of the temperate zone. It could be that under our screening conditions plants are exposed to excessive levels of disease pressure. However, within the same trial there were many other selections and hybrid lines from the tropics that grew free of disease and yielded well. On the other hand, the seed form, plant habit, earliness, and the general

appearance of the surviving hybrids were much more refined than the tropical lines with multiple disease resistance. Hybridization of the two groups should provide, at least for the tropics, cultivars with excellent seed characteristics, high yields, multiple resistance to diseases, and adaptability to severe conditions.

Resistance Trials with Ecuadorian Beans

Fifty-one bean accessions from Ecuador, selected as a result of a visit to the Boliche and Pichilingue Agricultural Experiment Stations, located in the Pacific lowlands, were screened for resistance to diseases. These Ecuadorian materials consisted on nine cultivars sold at the town market at Quevedo, and 42 accessions found free from common bean mosaic virus (CBMV) and bean yellow mosaic virus (BYMV) at the Boliche Station.

These Ecuadorian cultivars had, as a group, a high degree of resistance to rhizoctonia root rot and stem canker. Cultivar Canario had maintained over 50% stand after 130 days of growth in soil highly infested with *R. solani* and was also free from rust and bacterial blight.

The virus resistant cultivars had a high degree of resistance to CBMV strains endemic in Puerto Rico. However, they were generally susceptible to cowpea mosaic virus (CPMV), bacterial blight (*Xanthomonas* strains) and rust. Their response to cucumber mosaic virus (CMV) was variable. Accessions MITA-7771, 7775, 7776, 7792, and 7795 could be used as sources of virus resistance in breeding programs.

The Ecuadorian selections maintained excellent stands both at Isabela and Adjuntas, which suggested a high degree of tolerance to soil-borne diseases. Accession MITA-7792 had a high percentage of viable plants four months after sowing at Adjuntas. Accessions MITA-7766, 7767, and

7790 became naturally infected with CBMV at Adjuntas even though they were resistant when inoculated with strains available at Mayaguez. Accessions MITA-7773 and 7777 were resistant to both rust and angular leaf spot (ALS). Accessions MITA-7767, 7768, 7770, and 7775 were resistant to ALS, while Accession 7758 was extremely susceptible to this disease. Stem lesions of ALS caused death and defoliation of Accession 7758 within 75 days after sowing. Accession MITA-7773, 7777, 7779, 7780, 7799, 7800, and 7801 were resistant to rust races present at Mayaguez, Isabela and Adjuntas. Accessions MITA-7779 and 7780 had, besides resistance to rust, erect bush habit, with small leaves pointing upward instead of spreading horizontally. The bush Accession MITA-7800 was resistant to CBMV, CMV, CPMV and rust; moderately susceptible to bacterial pustule and cercospora pod blotch; tolerant to soil-borne diseases; and susceptible to bacterial blight, ALS and phytophthora blight. Several of these Ecuadorian accessions were found excellent for inclusion in multiple disease resistance breeding.

Colombian Advanced Bean Cultivars

Background

The Colombian Grain Legumes and Oil Seeds Improvement Program, Instituto Colombiano Agropecuario (ICA), Palmira, Colombia, has been involved in breeding for high-yielding bean cultivars adaptable to the climate of the Cauca Valley. A number of outstanding cultivars, such as Nima and ICA-Guali, were developed. The climate of the Cauca Valley, 3° North and 1,000 meters elevation, is somewhat similar to that of western Puerto Rico, 18° North and near sea level. This similarity was one of the reasons for our interest to observe ICA's advanced cultivars against the endemic diseases in Puerto Rico and to assess their response.

Results

Growth Index and Earliness

There was very little difference in the vigor of plants whether cultivated in Isabela or in Mayaguez. As would be expected, the four bush types were early, the three semi-vine types were mid-season, and the semi-vine to low-vine types varied between mid and late. The exception was ICA-Tui that appeared as a tall bush, but was late in pod set.

Bacterial Blight

The Las Gómez field at Mayaguez is heavily infested with *Xanthomonas* strains. There was sufficient rain and overhead irrigation to incite a heavy incidence of bacterial blight. However, as data indicate, six cultivars were slightly susceptible (SS) or tolerant to the disease. These data are the summary of four field observations. The fact that there is such a high degree of tolerance to bacterial blight in the ICA cultivars is very interesting and warrant further study.

Rust

The ICA varieties resisted defoliation even under severe rust infection. Variety ICA-Guali was immune to rust races endemic in Puerto Rico, while Calima, Nima, L-17, and 10119 were resistant. Variety L-35 appeared to be rust tolerant.

Rhizoctonia Root Rot

All of the 12 varieties had a high degree of resistance to rhizoctonia root rot. Twenty-five seeds were planted per variety. The germination percentage was high and the varieties maintained excellent stands even after their pods were harvested. Our experience with root rot diseases has shown that varieties which persist in the field generally possess a

high degree of resistance to charcoal rot (Macrophomina phaseoli). Hence, except variety L-35, the rest had varying degrees of resistance. Five of the varieties retained sufficiently strong root systems to have green foliage 130 days after sowing.

The combined characteristics of a) retaining foliage against rust, and b) resistance to soil-borne diseases, were the main reasons for the high vigour of these varieties in the two field screening trials. It should be added here that these cultivars were not grown isolated from other entries in the screening trial. In the same trial there were lines that had lost 100% foliage within 25 days after sowing; and others that had 90-95% seedling mortality due to soil-borne diseases.

Even though this is a preliminary trial, ICA's cultivars show an appreciable amount of resistance to diseases. Perhaps one reason for such a high amount of resistance in the ICA cultivars is that environmental conditions in the Cauca Valley are similar to those in Puerto Rico.

Scarlet Runner Bean Trials

The scarlet runner bean (Phaseolus coccineus) study was initiated in 1975 with two objectives in mind. Firstly, the investigation is concerned with the disease and insect pest resistance potentials of this species. It is hoped that P. coccineus may contribute to the establishment of a wider germplasm base which could be added to that of the common bean, P. vulgaris. The second objective is to develop high yielding disease and pest resistant scarlet runner bean cultivars for tropical highlands.

Background

Scarlet runner beans are cultivated on highlands of tropical America from Mexico to Perú and Chile. In their upland habitat many of the cultivars show vigorous growth, freedom from diseases and pest, and yield well. Due to their general stoutness and high yield this species has often attracted the attention of breeders involved in the improvement of the common bean. Besides the possibility of transferring new factors for resistance into *P. vulgaris* germplasm, the flower of the scarlet runner bean is morphologically self-sterile, which may provide a possible scheme for future development of hybrids for commercial cultivation.

A total of 256 P.I. accessions were received from the W-6 Plant Introduction Station, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington. Nine commercial cultivars were received, in addition, from Dr. J. P. Meiners, Agricultural Research Service, Beltsville, Maryland.

Results

Taxonomic Evaluations

Cotyledon Position

As soon as seeds germinated it was noticed that some accessions had hypogeous (H) and other had epigeous (E) cotyledonal position on the seedling axis. Of the 256 accessions, 94 had hypogeous and 124 had epigeous cotyledons. The remaining 38 accessions were not classified.

Species and Caryotypes

Taxonomic evaluations were performed independently by two investigators, Drs. N. G. Vakili and G. F. Freytag. On the basis of overall foliage and inflorescence appearance, pod shape, seed form, and cotyledon

position, Vakili divided the accessions into 83 P. coccineus-hypogeous (Pc-H), and 61 P. coccineus-epigeous (Pc-E). Freytag recorded flower and agronomic characteristics of 193 accessions and divided them into P. vulgaris and P. coccineus, which consisted of the caryotypes: 'Pueblo', 'Botil', and 'Cubás'.

Freytag classified 49 of the 53 P. vulgaris as belonging to this species. Of the remaining 4 he designated one as Botil and 3 as Cubás. Data from both ratings agreed in that all hypogeous types belonged to P. coccineus. Only one Pc-E was graded as Pueblo type by Freytag. There was a complete agreement on the Pc-E types between the two classifications. Only four (3 Cubás and 1 Botil, as mentioned earlier) out of 61 Pc-E were regarded by Vakili as Pv-E. This close agreement indicated that there were distinct recognizable characteristics that could be applied in indexing the caryotypes of the interspecific hybrids.

Vakili's observation, and the data on morphologic and agronomic characteristics by Freytag suggested that Pc-H-Pueblo caryotype is at one end of the scale, while the Pv-E common bean caryotype is on the opposite end. The Pc-H-Botil caryotype is closely associated with Pc-H Pueblo. However, according to Freytag, the Botil caryotype also includes epigeous characteristics which are closer to P. vulgaris than to P. coccineus. The Pc-E Cubás caryotype is either intermediate between Pc-H and Pv-E or closer to Pv-E. Freytag placed only three of the 52 Cubás caryotype group into the Pc-H group. Also, among the Pv-E group accessions there were many individual plants which did not possess complete P. vulgaris caryotypes, but had some P. coccineus components. The above observations suggested the presence of putative hybrids between P. coccineus

and P. vulgaris. These hybrids exhibited various levels of caryotypic differences which depend upon gene dosage received from either parental species and the direction of their selection. The putative hybrids indicate that P. coccineus and P. vulgaris are subspecies rather than species. If this assumption is correct, then the transfer of disease resistance characteristics should prove much less complicated than if they were distinct species.

Disease Resistance

During the survey of edible legume diseases in tropical America, conducted from 1970-1973 lushly growing scarlet runner beans, were commonly encountered in the highlands. This raised the question of how adaptable and resistant these cultivars would be in the lowlands. The 1975 trial provided a partial answer to this question.

Soil-borne Diseases

Rhizoctonia root rot was, by far, the most important soil-borne disease of Phaseolus coccineus in this screening trial. Fifty-two of the 256 accessions were susceptible, while another 52 accessions consisted of both resistant and susceptible plants by three months after sowing. At five months after sowing only 71 accessions had survived and two months later only 39 accessions had viable plants. Eight months after sowing all accessions were dead due to overall unfavorable environmental conditions. Accessions Pc-H-Pueblo 201299, 201309, 201312, 224711, 273448; Pc-H-Botil 201447, 307663, 311180; Pc-E-Botil 311205, 312035; and Pc-E-Cubás 201338, 317583 were resistant to rhizoctonia roo' rot. In general, it appeared that the Pc-H-Pueblo caryotype contained the largest frequency of rhizoctonia root rot resistance.

Charcoal rot, caused by Macrophomina phaseoli, was the second most important soil-borne disease. Sixteen accessions had plants with either charcoal rot or ashy stem blight. However, both phases of this disease occurred after plants have been weakened by either rhizoctonia root rot or by other diseases. Fifteen of the 16 Macrophomina-infected accessions had hypogeous cotyledons.

Southern blight (Sclerotium rolfsii) occurred sparsely in the field. Only accessions (190074 and 311174), both with Cubâs caryotypes, were highly susceptible to this disease.

In the tropics resistance to soil-borne diseases is an essential characteristic of any edible legume. In the following screening trials resistant selections will be further subjected to soil-borne diseases under different environmental conditions in the Island.

Bacterial Diseases

Bacterial blight (Xanthomonas phaseoli) was present, but it did not restrict plant growth. Incidence of the disease was recorded seven times. One hundred and forty-eight P. coccineus accessions were rated as susceptible and 26 were rated as resistant. A number of accessions consisted of different response types. In these cases the seeds of the resistant or tolerant plants were selected for further screening. The response of some accessions varied between SS, MS and S, which was most likely due to climatic influence during the trial. Eighty-seven accessions were not positively rated and will be screened further.

Thirty-one samples of tissues affected by bacterial blight were collected for isolation. Four of the isolates developed pigmentation on nutrient agar. One isolate had light yellow colonies, and the rest

had yellow, smooth and butterous colonies. Inoculation of P. vulgaris and Vigna unguiculata with a number of these isolates indicated that they were pathogenic to both species.

The field observations and summation of the data suggest that in general there is more tolerance and resistance to bacterial blight among P. coccineus germplasm than is encountered within P. vulgaris germplasm.

Viruses

Due to limited application of insecticides, there was a gradual increase in the frequency of virus incidence in the field. Ninety days after sowing 49 accessions had virus symptoms; at 110 days there were 66 with affected plants, at 120 days, 100; and at 145 days after sowing there were 132 diseased accessions. Both cowpea mosaic virus (CPMV) and common bean mosaic virus (CBMV) were present in the field. Out of 20 virus infected samples, 4 contained only CPMV, 8 contained only CBMV, and 8 contained both viruses. Both viruses were also identified against their antisera. The bean beetle (Ceratoma ruficornis) was the vector of CPMV, while field tools, blustery winds and some aphids were responsible for the natural spread of CBMV.

Thirty-seven accessions, out of the 56 with Pc-H caryotype and 42 of the 94 with Pc-E caryotype, were infected with viruses. Since P. coccineus has a high frequency of natural cross pollination, it would be difficult to distinguish, at this stage of our knowledge, to what extent the P. vulgaris genotype is included in the 42 epigeous accessions.

The incidence of golden yellow mosaic (GYM) was very low in the field. Only four accessions had GYM affected plants. However, on the highlands of Guatemala this species becomes severely affected by this disease.

Foliage Diseases

P. coccineus accessions had, in general, a different range of susceptibility than do those of P. vulgaris. The difference was more in the degree of susceptibility than in types of pathogens. The foliage diseases, in descending order of importance, were: powdery mildew (Erisyphe polygoni), rust (Uromyces appendiculatus), cercospora leaf spot (Cercospora cruenta), angular leaf spot (Isariopsis griseola), anthracnose (Colletotrichum lindemuthianum), ascochyta leaf spot (Ascochyta boltshauser) and alternaria leaf spot (Alternaria tenuis).

Powdery Mildew

Only Phaseolus acutifolius surpassed P. coccineus in degree of susceptibility to powdery mildew. The hypogeous was more susceptible than the epigeous caryotype, with 32.6 and 16.9 percent of the plants infected, respectively.

Rust

Urediospores of U. appendiculatus were collected by means of a cyclone collector attached to a heavy duty vaccum cleaner. The spores were gathered from the P. vulgaris spreader rows from the 1975 rust trials. About one gram of spores was suspended in a liter of water (sticker-spreader liquid was used to reduce surface tension on urediospores), which produced a concentration of about 105-106 spores per milliliter of water. Eight liters of spore suspension were applied as inoculum to the plot. Forty-nine and a half percent of the epigeous and 6.5 percent of the hypogeous accessions were susceptible to rust. The susceptibility of the Pc-E caryotypes to the same rust populations to which P. vulgaris cultivars are susceptible is again suggestive of the very close genotypic relationships between P. vulgaris and the Pc-E-Cubás caryotype.

Cercospora Leaf Spot

Many P. coccineus accessions were highly susceptible to cercospora leaf spot. In P. vulgaris, cercospora leaf spot follows other foliage diseases, insect damage to leaves, or appears when plants have reached senescence. However, in P. coccineus young leaves were infected. The Pc-H caryotype was more susceptible than the Pc-E caryotype. The percentages of infection were 30.5 to 13.0, respectively. The higher resistance of the Pc-E caryotype to CLS suggested a close genotypic relationship to P. vulgaris which is epigeous.

Angular Leaf Spot

Eighteen Pc-H caryotype accessions and seven Pc-E caryotype accessions were susceptible to angular leaf spot (ALS). During warm summer days ALS is not a prevalent disease of P. vulgaris at Mayaguez (near sea level). However, these P. coccineus accessions were more susceptible to this disease than any P. vulgaris line screened in the same field during the same period.

Anthracnose

The climatic conditions in the warm lowland tropics are not optimum for the anthracnose disease of beans, or of Phaseolus spp. in general. However, if infected seed is imported from the cool temperate zone or from tropical highlands, the disease appears on the seedlings. By the time the crop matures only a very few anthracnose lesions can be detected on the foliage. The seed of this crop is free from anthracnose. The 50 P. coccineus accessions which were susceptible at seedling stage responded in the above mentioned manner.

Other Foliage Diseases

Nine accessions had ascochyta leaf spot. The disease appeared early in the plot and no pod lesions were encountered.

Five accessions were susceptible to alternaria leaf spot disease. This disease, under the warm and humid tropical conditions, causes lesions on the foliage. High rainfall and humidity enhance its spread. Generally, only a very few susceptible host genotypes were encountered during the summer screening trials.

Potentials of *P. coccineus* as a Source of Disease Resistance in Intersubspecific Crosses

The preliminary data from the scarlet runner bean trial suggested that the Pc-H caryotype is a good source of resistance to rust, bacterial blight, and rhizoctonia root rot. However, accessions in this group may be weak donors due to their susceptibility to powdery mildew, cercospora leaf spot, angular leaf spot, and charcoal rot.

The material at hand, although not extensive, shows that there is a large number of putative hybrids with varied combinations of resistances. Screening and selection for multiple disease resistance may produce a superior *P. coccineus* line in a short time.

Tepary Bean Trials

The short term objective for the tepary (*Phaseolus acutifolius*) trials is to develop cultivars that are adapted to the hot and humid tropical environment, and possess resistance against bacterial blight, common bean mosaic virus, cowpea mosaic virus, rust, powdery mildew and soil-borne diseases. The long term objective is to introduce the above sources of resistance into the *P. vulgaris* germplasm.

Background

Phaseolus acutifolius, or tepary beans, has interested bean (*P. vulgaris*) breeders for years. The reason for this interest, especially in the United States, has been the presence in tepary of resistance against bacterial blight, caused by *Xanthomonas phaseoli*. In the tropics, bacterial blight is a major disease of beans. In the tropical environment the disease is enhanced by a favorable combination of year-round presence of inoculum in perennial edible and wild legumes and high temperatures and humidity. Throughout our disease resistance trials no *P. vulgaris* cultivar or hybrid lines has been encountered that could be regarded as highly tolerant to this disease. Dr. Max Schuster, University of Nebraska, provided the project about 3 years ago, with his bacterial blight resistant tepary beans, which included his Tep. 2, Tep. 10 and Tep. 11. However, due to high susceptibility to soil-borne diseases, especially rhizoctonia root rot and southern blight, these tepary lines succumbed before reaching flowering stage. Hence in 1974 it was decided to observe a larger number of tepary accessions and to select those which are adaptable to hot and humid tropical soils before concentrating on their bacterial blight resistance. A total of 23 *P. acutifolius* accessions were received for this purpose from the W-6 Regional Plant Introduction Station, Pullman, Washington. The Nebraska Tepary 10 and 11 were added as standards to this group.

Results

Sixty-five days after sowing accessions PI-312197, 310440, 331181, 310802, 321637, 319439, and 263590 appeared to be very poorly adapted. The first four accessions were lost without producing seed. The fifth

to the eighth accessions consisted of mixed populations and the best individuals were subsequently selected. Eleven of the 23 had vine habit and two accessions included wild P. vulgaris type plants. Eight, or 1/3 of the accessions, appeared variable or mixed at this date.

Diseases

Soil-borne diseases, mainly rhizoctonia root rot and southern blight, were one of the main limiting factors in the growth of the tepary accessions. Common bean mosaic virus reduced plant size and pod set, but increased viability of the root rot resistant plants as a result of retaining juvenile habit. Only one accession was field resistant to common bean mosaic virus. Both powdery mildew and rust were so severe on some accessions that the systemic fungicide Plantvax was applied in order to prevent complete defoliation and death of the plants.

There was a range of response, both within and among the accessions, to bacterial blight. Accessions 200749, 200902, 201268, 239056, 263590, 310800, 310803, 319438, 419441, 319488, and 321638 displayed a resistance response or were free from lesions during their growth.

The results of this preliminary screening suggested that it should not be a difficult task to assemble resistance against rhizoctonia root rot, common bean mosaic virus, rust and possibly powdery mildew. However, it appears at this point that before a tepary line is used in a cross for introducing bacterial blight resistance it should possess resistance to the other diseases mentioned above. Otherwise more undesirable characters would be added to hybrids while these are screened for resistance to bacterial blight.

Lima Bean Trials

The objectives of the lima bean (*Phaseolus lunatus*) trials are to a) find sources of disease resistance in this species, b) combine these resistances in vining types of this species, c) distribute the resistant cultivars, d) provide sources of disease resistance to lima bean breeders in the temperate zone, and e) transfer the factors for resistance from *Phaseolus lunatus* to *P. vulgaris*.

Background

Lima bean is the most widely cultivated edible legume throughout tropical America. It covers the fences around backyards or is planted in small patches. The majority of the indigenous cultivars are biennial or even perennial. The same diseases and insect pests attack both *P. lunatus* and *P. vulgaris*. Hence, lima beans serve as a reservoir of pathogens, especially, the whitefly-transmitted golden yellow mosaic virus. During fall plantings diseases are spread from farmers backyards to common bean plots. Either eradication of lima beans from the backyards or introduction of disease resistance into cultivars of this species could reduce the impact of this disease dissemination cycle. Seven hundred and thirty-three lima bean P.I. accessions received from the Plant Introduction Station, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington, were planted at Isabela. Prior to planting, seeds were examined for uniformity of color and shape.

Results

Agronomic Characteristics

Eleven of the P.I. accessions were *Phaseolus vulgaris*, three were *P. coccineus* and the remaining 719 were *P. lunatus*. Twenty accessions

had bush habit, 695 were vines and four were a mixture of bush and vine. Vine habit included determinate to very long indeterminate habit, that in some cases continued growth for 11 months, until the end of the trial. Eighteen accessions had strap or lanceolate leaves, 690 had normal ovate-acuminate leaves and the remaining 11 contained a mixture of the two types of plants.

Maturation and Days to Harvest

Generally, in edible legumes the higher the susceptibility to a given disease, the earlier is the maturation date of the cultivar. For this reason during this trial all accessions were observed for the duration of their pod production. Nine of the accessions which survived 9 months of growth, not only bore mature pods but also continued to flower. The early cultivars began flowering within 40 days and their dry pods were harvested by 88 to 100 days after sowing. The mid-season cultivars began to flower between 40 to 60 days. The late accessions began to flower within 60 to 80 days and their pods could be harvested within 100 to 150 days after sowing. The very late accessions began to flower about 90 to 100 days after sowing and their pods were ready for harvest from 130 days onward. Some accessions like the primitive types, PI-264603 and 264605, grew vegetatively and failed to flower through the 11 months of the trial.

Yield

Yield is affected by adaptability and resistance to diseases. The accessions were divided into the following five groups according to yield capacity: 1) low, 1 to 500 grams; 2) moderate, 501 to 750 grams; 3) good, 751 to 1,000 grams; 4) high, 1,001 to more; and 5) no yield information.

There were 441 accessions in group 1; 102 accessions in group 2; 44 accessions in group 3; 13 accessions in group 4; and there was no yield information on the 121 remaining accessions. The 13 accessions that yielded over 1,000 grams were PI-249047, 256386, 256400, 256405, 256423, 362748, 362757, 362765, 362774, 362795, 3628 , 362809 and 362820.

Seed Size

The smallest seeds in the Phaseolus lunatus germplasm trial were those of cultivar Chilipuca, from Guatemala. Their weight per 100 seeds was six grams. The heaviest seeds corresponded to PI-363015, which weighed 93 grams per 50 seeds. Within this range the weight per seed was as follows: 1) four accessions with less than 15 grams per 100, 2) 106 accessions between 15 to 30 grams per 100, 3) 458 accessions between 31 to 50 grams per 100, 4) 44 accessions between 51 to 70 grams per 100, 5) 26 accessions between 71 to 90 grams per 100, and 6) 9 accessions weighed over 91 grams per 100 seeds. The majority of the accessions with heavy seeds were among the low yielding group.

Diseases

Foliage Diseases

Although several foliage diseases were evident, the intensity of each disease was not as severe as to cause widespread defoliation and death of any accession. The major diseases were bacterial blight and golden yellow mosaic, followed by bacterial pustule, powdery mildew, scab and angular leaf spot. The minor diseases were lima bean viruses, downy mildew, anthracnose, cercospora leaf spot and diaporthe leaf spot.

Bacterial Blight

Lesions of bacterial blight, caused by Xanthomonas, began to appear in the field 3 months after sowing and by the fourth month (mid-April) the disease was well established and had caused defoliation in some accessions. The response of the accessions to the disease at four months after sowing was as follows: sixty-two accessions were highly susceptible, 124 were susceptible, and 147 were rated as moderately susceptible, hence, in this preliminary trial a total of 333 of the 719 accessions were susceptible to natural field infection. Two hundred-ten accessions were slightly susceptible or moderately resistant, while 176 showed no infection. Therefore 386 accessions would be selected for further bacterial blight screening.

Golden Yellow Mosaic

Golden yellow mosaic started to appear in the plot at about seven months after sowing. The delayed infestation by the whitefly (Bemisia tabaci) was due to the application of insecticides for control of the leaftier (Hedylepta indicata). Insecticide application was discontinued 6 months after sowing and golden yellow mosaic started to appear in the field one month later. Nine months after sowing, some surviving accessions were 100% infected. An interesting finding was that a number of accessions with very low disease incidence and intensity were located next to accessions which were severely affected. At 9 months after sowing, accessions 224713 and 257387 had 100% of their normal foliage and no GYMV infection; accession 347847 had 70% foliage and 5% GYM; accessions 224712, 257386 and 362916 had 100% foliage and 10% GYM affected leaves. Eleven months after sowing accession 224713 was growing vigorously and had a diffused light yellow GYM pattern on 10% of its foliage.

Bacterial Pustule

One hundred and forty-nine accessions developed symptoms that were best described as those of bacterial pustule. At first glance the disease resembled bacterial spot, caused by *Pseudomonas syringae*, but closer examination showed the presence of pustules. A description of bacterial pustule symptoms as observed in lima beans in the field follows:

> Small white lesions, one millimeter in diameter, appear mainly near the primary leaf veins and the leaf margin. The white spot defuses into a pale green lesion about 2-4 mm in diameter, while at the same time its center becomes watersoaked and forms a bubble on the leaf. During high precipitation and humidity, the pustules remain expanded and surrounded by the difused pale green halo. As soon as air humidity decreases, the bubble collapses and the pale halo disappears, leaving a depression with a light brown center. Within a day, the margin of the depression turns purple. The leaf tissue surrounding the spot becomes watersoaked and turns necrotic. Heavy infection results in defoliation. Bacterial pustules with similar symptoms as on the leaves, also appear on pods. Isolation from single lesions resulted in yellow butterous colonies on nutrient agar.

Pathogenicity tests with the organisms isolated from single lesions of bacterial pustule on four different *P. lunatus* accessions indicated that both *P. lunatus* and *P. vulgaris* were susceptible, while *Vigna unguiculata* was resistant to moderately susceptible.

The bacterial isolates from *P. lunatus* pustules were similar in cultural characteristics to those of the standard *Xanthomonas* species. Hence, it is suggested that the causal organism of bacterial pustule of lima beans in the humid tropics is a strain of *Xanthomonas*.

Powdery Mildew

Powdery mildew, caused by *Erysiphe polygoni*, appeared in the plot by the third month and was present throughout the 11 months of the trial.

Six months after sowing 93 accesions were susceptible to this disease. As in the case of Vigna unguiculata, disease incidence was lower during periods of high humidity and higher during dry periods. The reason for reduction during wet periods appeared to be infection of E. polygoni hyphae by the hyperparasite Cincinobolus sp.

Scab

A combination of stem, foliage and pod diseases made it difficult at the beginning, to recognize which was the predominant disease. Sixty-seven accessions had either leaf or stem or pod lesions 6 months after sowing. The fungi which were present in one or other of these spots were Elsinoe phaseoli, Colletotrichum sp., Isariopsis griseola, Peronospora, Cercospora and Volutella.

The scab disease, caused by Elsinoe phaseoli, was especially severe on pods and stems of a number of accessions which were growing in the field from June-November. Some pods were so severely infected that no seed was produced. The majority of the 67 accessions with stem and leaf spots were susceptible to scab.

Anthracnose

Previous experience had shown that anthracnose (Colletotrichum lindemuthianum) did not occur on edible legumes planted in the hot tropical lowlands. Usually anthracnose appeared as a seed-borne disease on seedlings and after 3-4 weeks it disappeared without spreading. The seed source of these infected seedlings could be traced to either cool tropical highlands or to the temperate zone. However, in the lima bean trial, anthracnose appeared in the field 5-6 months after sowing and in the midst of warm rainy weather. This was our first observation of an out-

break of anthracnose on a Phaseolus species in lowland tropical climate. The problem of identifying anthracnose as such was complicated by the presence of scab and angular leaf spot lesions on leaves and stems and of Volutella sp. acervuli on infected pods.

Angular Leaf Spot

Angular leaf spot is caused by Isariopsis griseola. The name of the malady is actually a partial misnomer when it is applied to the lesions caused by this fungus on lima beans. Some accessions developed round spots, resembling target spot, on leaves located high up on the vine. Other lesions extended down the secondary veins of the lower surface of the leaf - a symptom very similar to that of anthracnose. The stem lesions were elongated and could easily be mistaken for either scab or anthracnose. The round concentric lesions on the leaves could be mistaken, from a distance, with diaporthe leaf spot. The response of P. lunatus to angular leaf spot in the lowlands is similar to that of P. vulgaris, i.e., neither species is severely affected by this disease in the hot lowland tropics. P. coccineus, in contrast, is much more susceptible to the disease under the same conditions.

Downy Mildew

The downy mildew encountered in the field was not the lima bean mildew caused by Phytophthora phaseoli. In this instance the pathogen was Peronospora sp. Incidence of the malady was low. Both leaves and pods were affected. Foliar lesions were round to irregular in shape, 5-15 millimeters wide, with hypertrophy of the vascular bundles, causing the lesions to appear distorted. In the early stages of lesion development, both the upper and the lower leaf surfaces were cream-colored.

However, as the lesions matured the upper surface became tan, while the lower turned grayish. The velvety gray underside of the lesions suggested that sporangiophores, if and when produced, occur on the lower leaf surface. During the trial no sporangia were encountered on these lesions. No profuse mycelial growth, as shown by Zaumeyer and Thomas (USDA Tech. Bul. No. 868, pages 142-145), was observed, even though infection occurred during periods of high humidity. Pods lesions were circular, 5-10 millimeters in diameter, and while young had raised translucent to watersoaked centers. Small raised spots on the surface of the lesion indicated the location of the oospores under the epidermis. When lesions matured these small spots were the first to become grayish and later the whole center became depressed and gray. Oospores were obovate to spherical, with a long axis of 20 and a short axis of 15 microns. The oospore wall was not uniformly thick, varying from 1.5-3.0 microns.

Cercospora Leaf Spot

Small lesions with gray centers and purple margins, with characteristic sporodochia and conidia of *Cercospora*, occurred on the leaves during the rainy season. The symptoms of cercospora leaf spot added to the problem of identifying, at first glance in the field, diseases present on a given accession.

Volutella

Acervuli of a *Volutella* sp. occurred on pod lesions, often in connection with different diseases. At times the fungus occurred alone as a very weak pathogen. The only problem with *Volutella* is that its acervuli resemble those of *C. lindemuthianum*, hence its presence may cause mistakes in recording anthracnose in the field.

Diaporthe Leaf Spot

Only one case of diaporthe leaf spot was recorded during the trial. The disease, incited by *Diaporthe phaseolorum*, caused large leaf spots with purple margins and drab centers with concentric rings. Black perithecia were imbedded in the necrotic tissue of the lesion. No pod infection by the fungus was recorded.

Soil-borne Diseases

In our preliminary screening trials priorities are set in seeking sources of multiple resistance. The first item is the capacity to survive and outlast the majority of the germplasm in the same trial. The ability of a cultivar to survive and yield well under a given environmental condition is mainly, a function of its agronomic and morphological characteristics on one side and of its resistance against endemic diseases and pests on the other. If foliage diseases were not a limiting factor, then plant mortality could be due to soil-borne diseases. Conversely, a long and productive growth could be equated with resistance to soil-borne diseases. Resistance is often expressed by the vigor and the percentage of the plants growing in the field after a given period. The vigor under survivability is expressed as a combination of growth index and the percentage of normal foliage at the recording date. Six months after sowing, the 719 accessions were divided, accordingly, into: 1) dead - 122; 2) very weak - 111 with poor growth, index 1-2, and 0-5% foliage; 3) weak - 189 had poor to moderate growth, index 1-3, and 10-30% foliage; 4) fair - 181 had moderate to very good growth, index 3-5, and 40-60% foliage; 5) good - 84 had good to very good growth, index 4-5, and 70-90% foliage; and 6) very good - 28 had a growth index of 5 and had retained all their normal foliage.

Insecticide applications were discontinued after 6 months of growth, at which time seed from the majority of the accessions had been harvested. Thirty-eight accessions had excellent stand 9 months after sowing. At this time these accessions had survived the main portion of the rainy season. Twelve of the accessions still retained their normal foliage, even though some, like PI-241780, were totally infected with GYM virus. The trial was discontinued after 11 months of growth, when each of 61 accessions still had ten or more plants thriving in the field.

Root rot, incited by Rhizoctonia solani, and charcoal rot and ashy stem blight, incited by Macrophomina phaseoli, were the main causes of mortality among the soil-borne maladies. Root knot nematodes (Meloidogyne incognita) caused formation of large galls on a number of accessions. No selection for resistance to nematodes was attempted.

International Cowpea Mosaic Virus Nursery

During 1974 and 1975 attempts were made by the International Institute of Tropical Agriculture (IITA) and the Puerto Rico Edible Legumes Project to locate cowpea, Vigna unguiculata, germplasm resistant to diverse strains of cowpea mosaic virus (CPMV) from different geographical regions. The results of these regional tests indicated that there were a number of strains which possessed unrelated pathogenicities. For example, cowpea selections resistant to the Puerto Rican strain of CPMV were susceptible to the Nigerian strain and conversely, the cowpea selections resistant to the Nigerian strain were susceptible to the Puerto Rican strain. This was further complicated by the occurrence of a third virulent strain in India. In this same period Drs. H. A. Scott of the University of Arkansas and R. F. Bozarth of the Boyce Thompson Institute found

that the Nigerian CPMV belongs to the 'sb' group while the Puerto Rican strain was similar to the Arkansas strain of bean mosaic. Since it became evident that different strains of the virus were involved and that regional resistance to one strain did not convey the same to another strain, it was decided to combine resistances and to screen in the three geographical areas. This phase of the work was initiated. The first group of 60 hybrids from the International Cowpea Mosaic Virus Nursery, received from IITA, was evaluated in the greenhouse against Puerto Rican cowpea mosaic virus (PRCPMV), strain X-19. Accessions Tvu 2321 and Tvu 4544 were resistant and Tvu 1190 and Tvu 6666 segregated for resistance.

5. BREEDING

Bean Breeding for High Levels of Multiple Disease Resistance

The objectives of this phase of the work on bean breeding for high levels of multiple disease resistance were 1) to form an improved population by manual crossing using as base parents the best sources of multiple disease resistance available and to incorporate new germplasm at each cycle of crossing, and 2) to test first generation hybrids at each cycle selecting base parents for the next intercrossing and to test and select outstanding families and plants in the segregating F_2 and F_3.

Crossing Program

Procedure

The procedure being followed for the formation of improved populations, which is summarized in diagram 1, consists of crossing selected materials in all possible combinations in the greenhouse. The F_1 and subsequently segregating progenies of all of the superior individuals

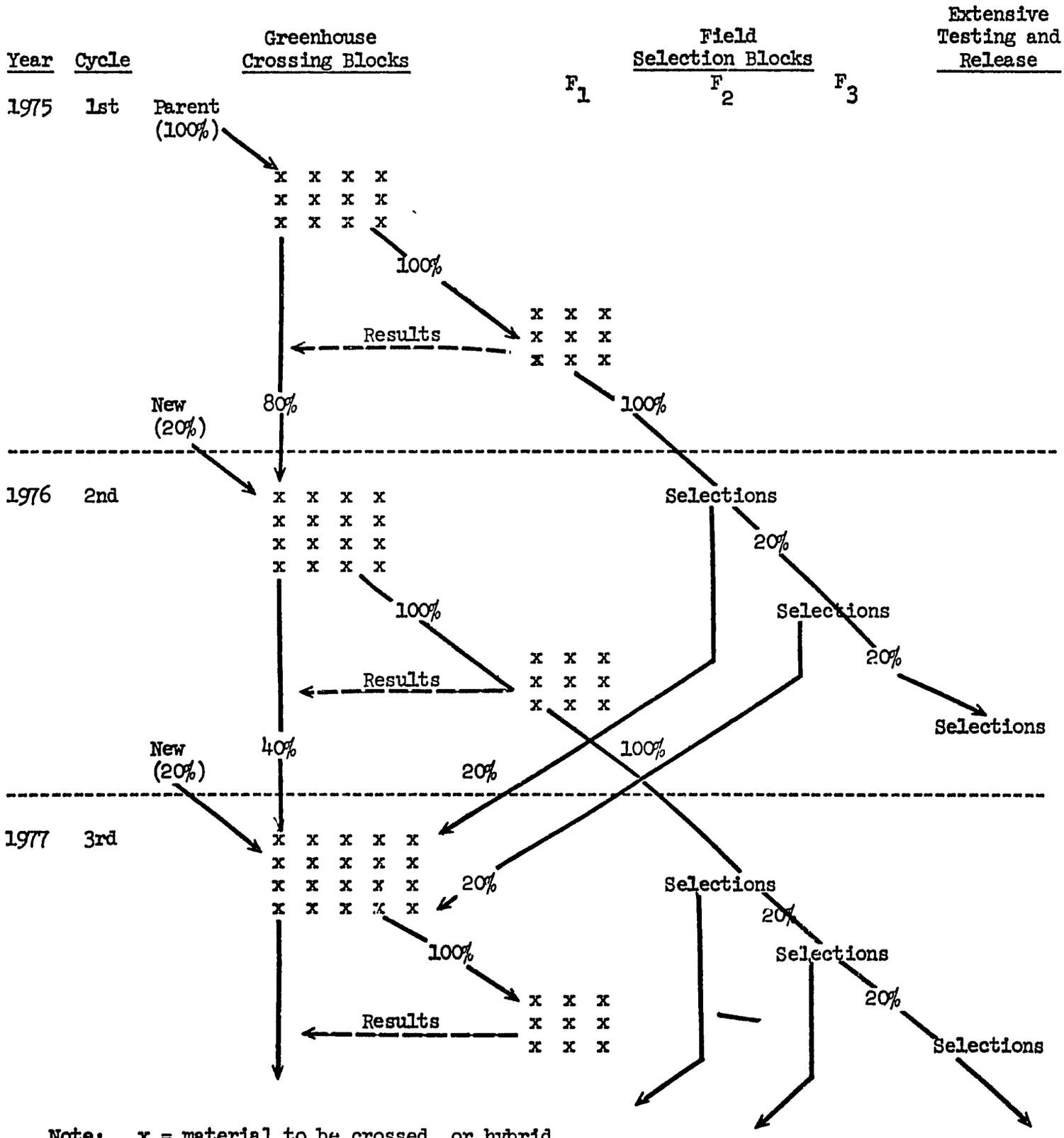
from the various generations are then again crossed in all possible combinations to form a new cycle. These cross combinations are to be field and greenhouse tested to select the superior crosses of progeny containing new combinations or higher levels of resistance. At each cycle, new disease resistance or other improved germplasm may be introduced into the manually crossed population. The procedure is repeated as long as it yields appreciably higher levels or new combinations of disease resistance, yield, agronomic or quality characteristics.

First Cycle

A group of 11 materials from the Project multiple resistant bean lines were selected for the 1st cycle of population improvement on the basis of their disease resistance, yield, plant type, uniformity and availability of seed. These materials were placed in the Adaptability and Yield Trials for planting in Puerto Rico and Central and South America to establish base data on which to judge future improvement.

At the same time as these trials were being carried out, additional seeds were planted in a screened greenhouse in Mayaguez for the 1st cycle of crossing. Using 10 to 12 plants of each of the 11 lines selected, a diallel crossing procedure was undertaken. No flowers were allowed to self until after the crosses desired had been made on each plant. Flowers were selected at random each day among those at an appropriate stage of development and were emasculated in the bud and pollinated using opened flowers, and were sometimes repollinated on subsequent days. All pollinations were made in the morning with the most favorable period from 9:00 to 11:00 a.m. Crossing began December 17, 1974, and terminated March 4, 1975, with the majority of the successful crosses

Diagram 1. Diagram of procedure for population improvement of Phaseolus.



Note: x = material to be crossed, or hybrid
( ) = composition of following block
--- = information used in selecting parent materials

made during the month of January. This coincides with the cooler part of the year in Mayaguez with normal daily maximum temperatures around 32-35°C and the minimum temperatures at 6:00-7:00 a.m. at about 16°C. Greenhouse temperatures at which the crosses were actually made were approximately 4°C above the outside temperatures.

All pods were harvested as they matured and in practically all cases this occurred 30 days after pollination. At harvest the pods were placed in individual coin envelopes and stored in an air conditioned room.

All F_1 hybrid seeds produced by the manual crosses on the parent plant in the 1st cycle of crossing were measured for the 3 seed dimensions, perpendicular and parallel to the seed hilum by the use of a machinist's micrometer with vernier to the nearest 0.1 mm. Subsequently samples of approximately 1 kg size of the parental materials were extracted from the seed harvested in the Fortuna field trials and hand cleaned of immature and damaged seed. These samples were further subdivided to 100 gram size and 10 seeds were extracted at random for dimension measurements. The data from these seed measurements were subjected to discriminant, regression and correlation analysis to study the variability of seed size and shape in the F_1's and parents.

Second Cycle

Sufficient seeds were obtained for 81 of the 110 possible cross combinations of the 1st cycle to permit field testing in Fortuna. The results of the field evaluation were used to select 16 of the F_1's deemed most appropriate for the 2nd cycle of crossing (double crossing) on the basis of plant vigor and type, disease and insect resistance, and yield.

Results and Discussion

Ninety-four of the 110 possible cross combinations with the 11 base parent materials resulted in seed formation during the 1st cycle of crossing. A total of 846 crosses were made of which 44.2% set some seed. Differences in fertility levels among varieties were probably caused in large part from bud, flower and pod drop as a response to the high greenhouse temperatures. Particularly vulnerable to bud abscission were the varieties French Horticultural and Ecuador 299. Ecuador 299 was observed to drop buds and flowers during early growth in December and to maintain buds and set pods later during February. This was suspected as being a response to temperature and day length interaction and was later observed more strikingly during the May-September field plantings when this parent variety took more than 100 days to initiate flowering.

A peculiar type of sterility response was observed in La Vega, which often set pods that continued to develop for 15 to 20 days without embryo or seed, and frequently set one-seeded pods. Selfed pods, however, usually contained 4-5 seeds per pod.

The best maternal parents were 15R-148 and French Horticultural with successful crossing levels of 71% and 55%, respectively, while as pollen parents the best were Jamapa and Ecuador 299 with 54% and 48% successful crosses. Mex. 27-R was considered a good parent with numerous flowers and with large and copious pollen. French Horticultural was considered a poor parent, in large part because it did not flower well under the hot greenhouse conditions. The best overall parent was 15R-148 which flowered copiously and continually and its pods frequently contained 5-6 seeds.

A total of 866 seeds were produced from the 846 crosses made and were obtained from the various crosses and their reciprocals. Some idea of relative fertilities may be obtained from these data, although it must be borne in mind that these are the results from differing numbers of crosses made at different times. On this basis the most successful crosses were: 15R-87 x Jamapa, La Vega x 15R-55 and Bonita x Mex. 27-R.

Information from Seed Measurements

Seed measurements were taken to see how constant these were for parent varieties and to what extent the hybrid seed (embryos) might resemble their parents in size and shape, in view of the large maternal influence reported in the literature.

In order to classify the parent material according to shape and volume of the seed, a discriminate function of the form

$$I = C_1 + C_2 \cdot L \cdot W \cdot T + C_3 \cdot L/W + C_4 \cdot L/T + C_5 \cdot W/T$$

where C_1, $C_2 \ldots\ldots C_5$ are the parameters of the equation used to maximize the differences between the indices of the parent material, L W T is the product of the three measurements and represents the volume or size of the seed, the ratios L/W, L/T, W/T of the measurements represent the shape and I stands for the index. Once the parameters of the equation were calculated, statistical comparisons of the indices were made which showed that the equation is useful to discriminate between most of the groups. The calculated values of C were as follows:

$C_1 = -3.39067$

$C_2 = 0.001837$

$C_3 = 1.350284$

$C_4 = -0.360443$

$C_5 = 0.999290$

These values indicate that the ratio of seed length to width is the most useful in maximizing differences due to seed shape (viz. C_3), while the least useful is volume (viz. C_2), which is equivalent to seed size or weight and has commonly been used for classification purpose with varying degrees of success.

Statistical comparisons of the index values using the standard error of the difference and the appropriate 't' values showed that the equation is useful to discriminate between all varieties, with the exception of 15R-87, La Vega, Jamapa, 50600 and Bonita. These are all fairly small, black seeded varieties, with the exception of the white seeded Bonita, and seem to form a fairly uniform group. On the basis of seed similarities Bonita is possibly more closely related to these blacks than would be apparent from the seed color alone.

A mean index was also calculated from the F_1 greenhouse grown seed for each of the crosses made. Comparisons of these F_1 mean indexes with those of the field grown parents showed that in most cases the F_1 index was larger, with the largest values being obtained from the crosses of 15R-87, 50600, Mex. 309, La Vega and Bonita with Mex. 27-R.

From the considerable increase of the F_1 index value over the female parent index value, one would expect a similar effect in the reciprocals, that is, a large positive effect on seed index values. However, such is not the case since in these reciprocals the effect is variable from a negative effect with 15R-87 to a large positive effect with La Vega; and these results, if caused by maternal control, are not at all consistent for these same parents in other crosses.

Study of correlation coefficients indicate a very strong maternal control of seed dimensions in hybrid F_1 seed produced on the parent plant (from 0.78 to 0.59) and a very low influence from the pollen parent (from -0.17 to -0.06).

At this time the seed measurements and index values, though of considerable use in discriminating between relatively pure parent lines, cannot be used to distinguish or to predict expected results in F_1 hybrid seed. Additional study will be needed to help clarify this situation and to separate the effects of locality and growing conditions which may be confounding factors involved in these present comparisons.

Field and Greenhouse Testing of Hybrids

Procedure

Since the amount of seed produced from each manual cross varied widely it was divided among the following tests as far as it would go and in the order indicated:

a) Two seeds or more are retained to be grown in the greenhouse for crossing (if selected for this purpose from field test results) and for selfed seed production.

b) Two seeds are placed in a field trial under favorable conditions for good yields to obtain selfed seed increase and field information including yield comparison.

c) Four to ten seeds are tested in the greenhouse for disease resistance.

d) Remaining seeds are greenhouse grown for selfed seed increase.

It was hoped that each of these tests could be carried out before the subsequent crossing cycle would take place, so that the information

could be used to keep the number of lines involved in the crossing block to a minimum of those with the best levels and combinations of desired characteristics, especially yield and disease resistance. It is now apparent that a simple field testing can be carried out rapidly for this purpose but that the greenhouse inoculation testing will be impossible to accomplish in time for this use. This latter testing should, nevertheless, supply good basic information on types of inheritance involved that will be of great value in the breeding program.

Greenhouse Crossing and F_2 Seed Increase

At least two seeds of each F_1 selected for crossing were planted individually in pots to facilitate moving them about for easy access to all plants on the bench. All residual F_1 seeds were planted similarly, one seed per pot for production of F_2 seed. First flowers appeared about 1st October and manual crosses were made as for the first cycle.

Greenhouse Disease Testing

From two-ten F_1 seeds of each of the crosses were separated for planting in the greenhouse in small groups with the parent materials as checks for a series of multiple inoculations under controlled conditions.

Field Testing

Eighty-one F_1 hybrids were selected from among the crosses producing sufficient seed to test two seeds of each in a 9 x 9 simple lattice design, planted at Fortuna on May 1975. Each hybrid seed was planted as the center plant of a hill plot composed of 9 plants in 3 rows with a uniform 20 x 20 cm spacing between plants. The 8 border seeds were planted from the maternal parent material. A 1 x 1 meter spacing was

used between hill plots. Dry pods were harvested at about weekly intervals as the plants entered maturity until mortality, with the last harvest on October 10, 1975.

Results and Discussion

Greenhouse Crossing

Only preliminary results of the greenhouse crossing in the second cycle are available since these plants were still growing at year's end and some crosses are still being attempted. Nevertheless it is possible to say that it has been much more difficult to obtain seed set this year than last and it is believed that this has been caused by trying to do the crossing earlier in the year (beginning in October, 1975, as compared to December, 1974), when daylengths were slightly longer and greenhouse temperatures slightly higher. If temperatures can be kept low with evaporative coolers, daylength will become less important in causing flower and pod dehiscence.

Field Testing

Up to initiation of flowering there was little interplant competition in spite of very large differences in apparently superior F_1 plant vigor over the maternal border parent plants. At this time staking was required to keep the large vining type F_1 hybrids from covering the border plants since in some cases these hybrids became large plant masses 1 to 1 1/2 meters in height and about a 1/2 meter in diameter while the parent plants were often less than a third this size. The continuous moderate winds required frequent attention to maintaining the plants tied to the stakes and the stakes upright. The parents, Mex. 309 and Ecuador 299, were mainly responsible for the excessive vegetative growth of their F_1 hybrids.

During mid-season considerable attack by loopers and pod and bud worms caused heavy defoliation and required frequent application of insecticides. Red spider mite entered the trial during the latter part of the harvest period, but was not severe enough at this growth stage to merit chemical control. All of these pests showed marked preferences for certain bean lines over others and in some cases caused severe damage while other lines seemed to be almost completely unaffected.

Disease incidence was fairly low due to the hot, dry climate prevailing during the trial. Nevertheless a large number of the F_1 hybrids were affected by a virus, either common bean mosaic and/or cucumber mosaic. In general symptoms appeared late during the blooming period and were not always very pronounced. Though the maternal lines Bonita, 15R-148 and Mex. 309 frequently showed symptoms, there were plots apparently free and their F_1 hybrids varied in virus incidence. The parent, Ecuador 299 became 100% infected with rugose mosaic virus, which became more apparent as the plant passed 2-3 months of vegetative growth and did not bloom until past 100 days. No seed was set. This virus was very infrequent in other lines. No rust was observed in any of these plants. Common bacterial blight incidence was moderate during the later growth period and no appreciable level of resistance was observed.

Many of the F_1 hybrids closely resembled their parents in all aspects including yield, especially those crosses between similar black-seeded types. When crossed with widely divergent types the hybrids tended towards large vining plants with considerable apparent vigor and in some cases this resulted in increased yields. Within these large vegetative types, daylength sensitivity was contributed by Ecuador 299, though these were considerably variable in response.

Adaptability and Yield Trials of Disease Resistant Beans and Cowpeas in Puerto Rico

The objectives of the adaptability and yield trials with disease resistant beans and cowpeas were to a) establish base information on the adaptability and yield potential at several locations in Puerto Rico of selected disease resistant lines for use in breeding of beans and cowpeas; b) establish, insofar as possible, variety yield interactions with disease and locality; and c) develop a simple procedure for efficient testing of new materials with cooperators.

Procedure

Two simple experimental designs were selected which would satisfy the criteria judged basic to the work: a) use of a minimum of land and labor, b) require a minimum of seed, and c) provide adequate levels of significance in the data. The most important factor from the breeding standpoint was the desire to be able to test first generation hybrids where seed might be limited to 5 or 10 or to 30 seeds at most.

The more traditional design selected, randomized blocks of short drilled rows, has been used with success in Central America for some years. However, it was decided to reduce the plot size to an absolute minimum of a single 2-meter long row because of limited seed supply for the more advanced lines and to increase the replications, from the usual 4, to 6. It was decided to use a seed spacing of 10 cm as appropriate for the majority of the commercial bush types. This would require 120 seeds of 100% viability for each locality. In this design it seemed appropriate to plant to stand by adjusting the amount of seed required per meter in accordance with the germinability, as determined by green-

house tests in sterile sand, since it was wished to include the effect of soil-borne diseases on stand and yield at each locality. Shortly after germination, stand population counts were made and then thinned to 10 plants per meter, with the exception that no thinning was done at Adjuntas.

The other design selected consisted of hill plots of 9 plants in 3 rows of 3 plants each at uniform spacings of 20 cm. The center plant was intended to be harvested separately from the border plants so that the useful plot may consist of a single plant with most of the border effect removed, or the yield of the 9 plants may be combined. This design should permit testing first generation hybrids (as the center plant) against their maternal parents (as the border plants) and it is for this purpose that the hill plot technique has been used. In order to increase efficiency when large numbers of hybrids are to be tested, a lattice design is used which may be analyzed either as randomized blocks or as a lattice if there is a need for increased precision. A balanced lattice square for 16 entries with 5 replications was actually used. In testing the parents two seeds were planted at each plant position to be thinned to one shortly after germination since it was felt that a full stand would be more important than the soil-borne disease affect at germination.

Since the plantings coincided with the cooler, dryer season deemed most appropriate for these legume crops, sporadic seasonal rainfall was supplemented by irrigation as required. Very little sprinkler irrigation was required at Adjuntas because generally adequate moisture levels were maintained by rainfall. Increasingly greater amounts of irrigation water were required in Mayaguez, Fortuna, Lajas and Isabela, in part due to inadequate rainfall, but also due to high temperatures and wind movement.

Results and Discussion

Row Plot Yield Trials

The statistical analysis of the data indicated that there were highly significant varietal mean differences at all localities, with the exception of Lajas for the Phaseolus test and Mayaguez for the Vigna test. However, the combined average means for varieties were highly significant. It was also apparent that fairly reasonable variety mean differences could be detected where the coefficient of variability was about 30% or less.

The data for the combined experiments indicated that for the cultivars tested, Vigna will double the yields of Phaseolus when averaged over locations. Nevertheless yield potentials seem to be about equal at the better locations. Since a wide row spacing has been used for both crops with the consequent inclusion of a large degree of border effect, it would be very interesting to see how the varieties would respond to increased population density. Nevertheless this has not been deemed practical at present due to the many plant types and consequently different spacings required. Some type of stratification will be most likely required in the future.

Phaseolus

The best 4 or 5 lines were considerably better than the lower yielding lines. Cultivars tentatively identified to be retained as a repeat base for future testing were: 50600, La Vega, Mex. 309, Porrillo, Jamapa, 15R-55 for the black seeded types, Bonita as the only white, and 15R-148 as the only red.

Since these tests were conducted during the most favorable season, disease and insect incidences were generally low and no consistent effects on yields could be determined. For instance, Bonita and 15R-148, which were heavily infected by viruses at Fortuna and Adjuntas, gave fairly low yields at Adjuntas. However, Bonita yielded well at Fortuna in spite of the virus infection. No other cultivars were consistently affected by viruses.

Soil-borne diseases, present on the seed or in the soil, should have a direct effect on yields by: a) reducting stand, especially during germination; and b) reducting plant vigor or by early mortality after flowering. Both of these effects are measured in the field by stand counts and plant longevity, though the part played by each specific pathogen is not so easily determined, especially when several are involved simultaneously. No consistent significant differences could be determined between varieties or localities in these trials. Nevertheless it was obvious that a major problem existed in Lajas due to the heavy soils and difficulty with irrigation causing water-logging of the soil, which, combined with the high prevalence of Sclerotium and Macrophomina, certainly contributed to great loss and irregular distribution of stand. Phaseolus was considerably more affected throughout the growing season than Vigna, though no consistent varietal reactions could be determined.

Because of the common occurrence of soil-borne diseases in the tropics wherever legumes are repeatedly grown, a major effort will continue to be made to include resistant lines in these adaptability trials and to improve experimental field testing techniques.

The varietal response of La Vega over locations is of particular interest since it ranked first in yield in 3 out of 5 localities, but dropped to 11th and 12th place in Isabela and Mayaguez, respectively, which may be related to the similar soil type of the two locations since climate and disease incidence were quite different.

In Fortuna, where the highest overall yields were obtained, the best cultivar yielded about double that of the lowest yielding cultivar. On a per hectare base all yields could be considered satisfactory from a commercial standpoint and could very likely be increased considerably by reducing row spacing.

No consistent differences could be found with seedling diseases, viruses, population or longevity. Cultivar 71-1R-113, which had the highest incidence of seedling disease, also had the best longevity and maintained a good stand, while Mex. 27-R, with an intermediate level of seedling disease, was reduced in stand and longevity, probably through the effect of the disease. Similar results were observed with La Vega. There seemed to be no effect of virus (probably cucumber mosaic) on yield at this locality, perhaps because of the relatively late attack, since the two main cultivars affected (Bonita and 15R-148) nearly always yield well.

Vigna

Average location yields appeared to be fairly comparable other than at Mayaguez, but there was a great difference between high and low yielding cultivars at all locations. The variety differences may have been due, in large part, to loss of stand, which was most noticeable in the cultivars of U.S. origin, characterized by determinate plant type

and with large, light colored seed. By observation, these materials seemed to be most affected by the heavy soils and root rots, but the yield trial data were not consistent in this regard and did not show statistical significance.

The highest yielding material was all of indeterminate plant type and with small, highly pigmented seeds. The best of these, 4R-373, a black seeded type, seemed to be the most adaptable, giving good yields at all locations, though fairly lower at Adjuntas. Of the other high yielding cultivars, 10R-3, a red, gave low yields at both Adjuntas and Mayaguez; 10R-61, a black, ranked low at Fortuna; and 4R-482, a pinto, placed low at Adjuntas. The best large seeded U.S. variety, 70-F-51, gave about the same yield at all locations, thus placing first in Mayaguez, second in Isabela, and fourth in Adjuntas. However, at the other two locations, where Vigna seemed particularly well adapted, this cultivar ranked comparatively low. This cultivar, as well as the generally higher yielding ones, will be retained for continued testing and for breeding work.

A closer look at the results from Lajas, where Vigna gave the best overall yield, showed that the best cultivar gave nearly twice the average yield and the poorest cultivars less than half the average. Neither soil-borne diseases, as measured by seedling plants affected, nor virus diseases, seemed to be correlated with yield. Stand, as well as longevity, were obviously reduced and responsible in part for low yields of the U.S. varieties previously mentioned. Yield per plant however, did not uniformly increase with reduced population as one would expect. The higher yielding varieties seemed to have both increased longevity (in

part due to disease resistance) and increased yield per plant. The five highest yielding varieties, as well as 70-F-51 as previously explained, will be tentatively retained as a repeat base for future testing.

Hill Plot Yield Trials

Since at the spacing used (20 x 20 cm) the population per hectare would be 250,000 plants, a gram of yield per plant would be equivalent to 250 kg per hectare. Therefore the only yields to surpass 2,000 kg/ha were at Adjuntas where the average yield was a little over 1,000 kg/ha. All yields at the other localities were considerably lower and were probably due to low soil fertility and extremely poor utilization of the small amount of fertilizer applied. Reduced stand from disease and water logged soils also was in part responsible for the very low yields at Lajas.

Although one would expect the center plants to yield less, due to interplant competition, such was not the case in general, perhaps due to the overall reduction of growth. The border effect was very sporadic, noticed only with a few cultivars at each location.

The variability for the border plants seemed to be reduced at all locations in comparison with the center plants, perhaps due to the increased populations composing the average for the plot, and this effect was great enough to cause a statistical significance in the data, with the exception of the data from Lajas. There were no significant differences between cultivars for the center plant data except for the combined analysis, and this is probably due to the considerable plant to plant variation within variety.

The hill plot results generally agreed with the row plot results. The best varieties changed position from location to location but the poorest yields were obtained with the same varieties in both types of trials. Due to the considerably increased labor required to plant, takes notes and harvest the hill plot design it is felt that this system will be appropriate only when a very limited amount of seed is available, such as with the first generation hybrids.

The analysis of the data from the adaptability and yield trials with disease resistant beans and cowpeas suggested the following conclusions: 1) superior yielding lines with good general adaptability were identified within the disease resistant Phaseolus and Vigna materials; 2) best results were obtained with Phaseolus at Fortuna and Adjuntas and with Vigna at Lajas and Fortuna; 3) no consistent significant disease response was found over location, probably caused by the relatively favorable growing season and low disease incidence; and 4) results from both the row plot and hill plot trials were similar, and because the row plots were much easier to manage they were deemed more satisfactory as field plots for variety comparison at several localities.

Comparative Yield Trials of Advanced Materials from Other Programs

Comparative yield trials with advanced materials from other programs were established with the purpose of 1) obtaining advanced lines from other bean improvement programs which may be outstanding in disease resistance, yield, quality factors such as protein, or other agronomic characteristics; and 2) testing these advanced lines under favorable conditions in Puerto Rico in order to select the outstanding ones for use in Project population improvement work.

Procedure

Through cooperative arrangements, 12 materials were received from the University of Wisconsin, 9 from CIAT, and 29 from Michigan State University. All of these were planted in Fortuna along with 14 Project breeding materials. The design used as a 9 x 9 simple lattice with individual plots of a single 2 meter row, at 1 meter spacing, with 10 seeds per meter of row. Dry pods were harvested as they matured at approximately weekly intervals and threshed and cleaned by hand. All harvested grain was fumigated with fosamine and allowed to come to equilibrium moisture content in an air conditioned room at about 50% R.H. before weighing. Final yields were calculated by regression analysis.

Results and Discussion

A summary of the principal information obtained was returned to the cooperators for use by them to evaluate their materials. For Puerto Rican conditions this test indicated that the Project breeding material is, in general, on a par or superior when yield is used as the criterion to judge performance. Apparently our material is well adapted and yields well in these growing conditions, in spite of disease and insect incidence. However, there is great variability in general performance and responses between lines from all programs.

There were among the Wisconsin improved materials several fairly promising populations, with fair disease resistance, good plant type, longevity, and yield. Population 74-36 was selected to cross into our populations. Other Wisconsin materials will be planted also for possible reselection.

The Michigan advanced lines contained a number of bush and snap types and generally flowered profusely. They seemed to have a high resistance to viruses and a marked susceptibility to bacterial blight, with little longevity or resistance to soil-borne diseases. Lines 4044-12-1 and 4210-12 blk were selected for crossing into our populations. Others will continue to be studied for possible use at a later date.

Several of the CIAT materials were of similar origins to some of our breeding materials. Accession P5o8-A is our 15R-87, which has been distributed as PR-5. CIAT numbers P488-B and P566-A are Porrillo 70 and Porrillo Sintético, respectively, which were most certainly derived from the original Porrillo No. 1, and since this variety was developed in El Salvador they may all be somewhat related to P5-1-h, which is PI-307824 from El Salvador. In regards to this group of Porrillo types only our Porrillo and the P5-1-h were attacked by rust, while P488-B and P566-A showed a relatively high incidence of common bean and Rhynchosia mosaic virus and no rust. This seems to indicate that the improved Porrillos (P488-B and P566-A) have gained some rust resistance at the expense of losing some virus resistance. We have selected P459-C-L (originally cultivar Jamapa reselected in Venezuela) for our breeding work because it seems to offer slightly better plant type, earliness and resistance than our Jamapa.

Daylength sensitivity may be responsible for the late flowering of the Wisconsin numbers 74-15 and 74-19 and the Michigan line 41177-3 blk, all of which required at least a week longer than the normal 40 days to flower. Ecuador 299 showed a strong photoperiodic response,

taking several months longer to flower at this planting season that would be the case in December. As this variety became completely infected with Rhynchosia mosaic virus, even after flowering began, practically no seeds were set. Nevertheless this variety is known to have one of the highest levels of foliage disease resistance so that it will be a prime objective in our population breeding to obtain segregates with resistances to the virus and foliage diseases and with daylength insensitivity.

Cooperative Work in Progress

The cooperative work in progress is aimed at: 1) establishing close working relationships with other scientists whose work with grain legumes is related to, or closely parallels that of the UPR-MITA Bean-Cowpea Project sponsored by USAID; 2) distributing the Project's improved disease resistant germplasm into geographical areas of need and to interested scientists; and 3) obtaining useful information on adaptability and yield of the Project's improved materials from cooperative testing to assist in guiding the breeding program.

International Cooperation Initiated

Seven proposed cooperative research plans have been drawn up after visits and discussions with the people involved, and are presently in various stages of development with Costa Rica, El Salvador, Honduras, Guatemala, Mexico, and the United States.

Additionally, two sets of the Adaptability and Yield Trials for beans and cowpeas have been sent to Ing. G. Bastidas, Instituto Colombiano Agropecuario (ICA), Palmira, Colombia.

Colombia

Results from one trial with beans at CIAT were received and indicated a somewhat different disease picture than is usual in Puerto Rico, though yields were similar. A delay of nearly 6 months was experienced in the receipt of trials sent to the ICA program in Colombia, so that the results of two additional bean trials and the cowpea trial have not yet been received for study. The principal CIAT parent materials have been received and tested in Puerto Rico and are in general quite similar to some of the Project materials.

Costa Rica

No germplasm has yet been received from Costa Rica. However, the plots with the Project lines planted near Alajuela were visited during the early growth stage and a considerable incidence of root rots was observed. Final results have not yet been received.

Honduras

Of the two seed lots sent to Honduras on the same day, one has not yet arrived after 6 months. The varieties sent to the Escuela Agrícola Panamericana for seed increase arrived on time and were observed in the field at flower initiation. Due to the high moisture levels caused by frequent rainfall, local varieties were severely infected with anthracnose and root rots. Neither were in evidence in the seed increase plots, though a foliage and stem rot (possibly Pythium or Phytophthora) was present on all varieties, except Ecuador 299. Ecuador 299 has also since been reported as free of rust. Seed should now be available from this planting for some extensive local testing in Honduras. No improved disease resistant material is available from Honduras for trial in Puerto Rico.

El Salvador

Though slow in arriving through the mails this trial was planted in San Andrés at about the normal season and was observed in the field shortly after germination. Soil-borne diseases were causing considerable stand reduction in the more susceptible lines. As El Salvador has a very high incidence of golden yellow mosaic virus it is possible there will be no adequate resistance in the common bean lines while the cowpea lines should show a considerable advantage in this respect. Seed of El Salvador's varieties, such as the improved cowpea CENTA 105, which has a very acceptable seed and plant type, but has been seen to be susceptible to mildew and cowpea mosaic virus in San Andrés, has not yet been received in Puerto Rico for testing.

Guatemala

Bean variety trials have been observed in Jutiapa, among which were identified some lines (especially II 32) apparently with variable degrees of tolerance or resistance to golden yellow mosaic virus. These are to be reselected in Guatemala and will later be screened for additional diseases in Puerto Rico.

Mexico

Germplasm collections of cultivated races and wild types of *Phaseolus coccineus* were recently received from Mexico for study and disease screening. These have been germinated in the greenhouse at the Adjuntas Substation. Seed of disease resistant material of *Phaseolus* and *Vigna* for the tropical lowlands is being prepared to be sent for trial by the Mexican cooperators.

Wisconsin

All materials have been exchanged as intended. Project materials sent to Wisconsin are being used in various research projects on protein content and type. Field tests in Wisconsin have been postponed due to the possibility of seed-borne diseases and clean seed is being increased in the greenhouse. Line 15R-148 and Bonita have shown fairly high levels of total protein at over 24%.

Wisconsin materials received in Puerto Rico have been field tested and one has been selected for Project's population breeding. Wisconsin materials from the second cycle of crossing are on hand and will be planted in early 1976 at Isabela and Adjuntas.

Germplasm Collection and Maintenance, and Seed Stock Increase

One-hundred thirty-nine new materials were accessioned this year, and though a fairly small number, some very promising improved cultivars and selected lines have been received from Boliche, Ecuador and from CIAT in Colombia. We have also received several interesting collections of native varieties and wild types of the *Phaseolus coccineus* complex from the Graduate School of Agriculture at Chapingo, Mexico.

Enlargement of the cold storage facilities was completed during the year, which has permitted removing the relatively large working collections from the environmental chamber used for germplasm storage to the new permanent walk-in room. The germplasm is now held at 10°C, 30% R.H. and the working collection at 6°C, 50% R.H.

Eighty-nine disease resistant bean materials were increased in short rows to approximately 1/2 kg size and 19 beans and 15 cowpeas were increased to larger amounts varying up to 20 kg of seed to reno-

vate and maintain appreciable amounts of basic seed stock on hand for distribution. Increase of seed in this tropical climate has required careful management, a great deal of roguing and intensive seed cleaning and grading to maintain our fairly high quality seed standards.

B. OPERATIONAL SIGNIFICANCE

In the case of some rugaceous viruses (whitefly-transmitted) it was possible to achieve a 100 per cent infection rate via mechanical inoculation of expressed sap. These findings suggest that such procedures might be applied for determining relative susceptibility to the aforementioned viruses. Then the next step should be to determine whether the results of mechanical inoculation are comparable to those obtained when the natural vector, i.e., Bemisia tabaci, is employed to secure infection. Should this be the case then it would be possible to appropriately screen large seedling populations for resistance to the bean golden yellow mosaic virus.

Races of the fungus Erysiphe polygoni apparently resistant to benomyl are already present in the field, since the chemical did not protect cowpea plants against powdery mildew this year. However, other fungicides, of the preventive type (contact) namely Macuprax (copper sulfate + ethylene bisdithiocarbamate), mancozeb, chlorothalonil, and dinocap, have proved to be very effective in controlling the disease. These contact fungicides can be combined in sprays with benomyl, thus reducing the chances for the development of resistance against this chemical. Resistance to benomyl has been reported throughout the world in such pathogens as Cercospora, Erysiphe, Fusarium, Monilinia, Septoria,

Sphaerotheca, Venturia and Colletotrichum. Mixing the systemic with the nonsystemics, and reducing both the concentration and number of applications for a given systemic are among the practices recommended by scientists dealing with the problem of resistance. Alternate chemicals were suggested for the control of early blight of celery (Cercospora apii) in Florida, and for the control of leaf spot of peanuts, apple scab, and powdery mildew of cucurbits in Florida, as well as in other states.

Erratic results were obtained in tests to evaluate the systemic fungicides oxycarboxin (Plantvax) and triforine (Saprol) for the control of bean rust (Uromyces appendiculatus). However, these fungicides, due their systemic properties (longer residual effects), are highly recommended by workers engaged in chemical control of this rust. Therefore, more research with these systemics is needed in order to establish their role in controlling rust of beans in the tropics. Additional research is needed with contact fungicides due to the fact that systemics alone eventually fail to provide good protection, since the pathogen develops resistance against this type of chemicals. The fungicide chlorothalonil (contact), used for the first time in our experiments to control rust, was effective at the rate of 1.14 kg/ha. Fungicides such as this should be used in combination with the systemics in order to prevent pathogens from mutating to races tolerant or resistant to systemics.

The valuable information on resistance to insect attack evolved from this project complements the work in progress in other countries. Bean cultivars with apparent resistance to leafhoppers are being tested at CIAT, Colombia, and eventually will be evaluated in other countries of Latin America and elsewhere.

A basic multiple disease resistant (MDR) stock of *Phaseolus vulgaris* is being developed that not only has high levels of resistance to the prevalent diseases, but also outyields commercial cultivars when grown under stress. This basic MDR stock is necessary in order to increase bean production in the tropics, as well as to protect, through incorporation of resistance, the bean crop in the temperate zone from pathogens of tropical origin.

The findings in the breeding phase of the project tend to agree with those of other investigators in many respects. It was found, for instance, that beans are particularly well adapted to the lowland, dry, fertile loam soils, conducive to low disease incidence, and also to the highland, humid, sandy mineral soils which favor high disease incidence. Other points of agreement are that 1) certain diseases such as root rots are, apparently, more correlated with yield losses than are others, such as the foliage diseases and 2) some parental materials are outstanding in certain disease resistances, though they may be very susceptible to others. Examples of the latter are the cultivars listed below:

Cultivar	Reaction to disease	
	Resistance	Susceptibility
Mexico	rust, root rots	viruses
Ecuador 299	rust, root rots	Cowpea mosaic virus
La Vega	viruses	rust
Bonita	root rots	viruses, rust

C. SIDE EFFECTS OF THE WORK

Research experience in the entomological aspect of this project has shown that insect problems on edible legumes are more complex than was originally anticipated. Several insect pests attack different organs (leaves, pods, seeds) of beans and cowpeas and each has to be studied separately. Sampling methodology to ascertain infestation and damage is different for each species of insect and thus time consuming.

Research results up to date have shown that it is neither a difficult nor a highly time consuming task to develop bean lines resistant to a complex of root rot pathogens, common bean mosaic virus, and rust. However, Phaseolus vulgaris germplasm has its limitations. For example no resistance or high tolerance to common bacterial blight, angular leaf spot, soybean rust and web blight has been encountered. Under high temperatures this species has a tendency for low pod set plus a greater sensitivity to root rots, factors which together result in very low yield. Because of these shortcomings it has been necessary to search in other species of Phaseolus for higher levels of disease resistance.

D. RESEARCH DESIGN

No modifications in research design appear to be necessary, except for some insect sampling methods that will no longer be used, and others that will be modified in order to optimize the quality of the information obtained.

The concept of improved population development as a germplasm pool from which new and improved recombinants may be selected is relatively old, though now it is just beginning to come into wide usage. The main

problem has been with the management of the population and the early testing and selection of parents for subsequent crossing cycles. Our preliminary data seem to indicate that effective selection can be accomplished in the early generations (F_1 and F_2) for parents for subsequent cycles. We have also utilized progeny from these selections, carried through F_3 and F_4 as family bulks, for selection of relatively homozygous multiple disease resistant cultivars. Hence, no modifications of techniques are presently foreseen in the breeding aspect of the project.

IV. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

A. PROJECT OUTPUT: LIST OF PAPERS AND PUBLICATIONS

1. Bird, J. and K. Maramorosch (Eds.). 1975. Tropical Diseases of Legumes, Academic Press, N.Y., 182 pp.

2. Bird, J., M. Kimura, Amelia C. Monllor, Rita L. Rodríguez y K. Maramorosch. 1975. Mosaico de Euphorbia prunifolia Jacq. en Puerto Rico: transmisión, hospederas y etiología. Memoria XXI Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, Vol. 1: 233-234.

3. Bird, J., Josefina Sánchez, Rita L. Rodríguez y Amelia C. Monllor. 1975. Gama de hospederas de varios virus rugáceos de Puerto Rico. Memoria XXI Reunion Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, Vol. 1: 259-260.

4. Bird, J., A. Cortés Monllor, J. Sánchez y R. L. Rodríguez. 1975. Propiedades de dos virus transmitidos por la mosca blanca Bemisia tabaci Genn. en Puerto Rico. Paper given at the Bean Plant Protection Workshop, CIAT, Cali, Colombia.

5. Bird, J., R. L. Rodríguez, A. Cortés Monllor y J. Sánchez. 1975. Transmisión del mosaico dorado de la habichuela (Phaseolus vulgaris) en Puerto Rico por medios mecánicos. Paper given at the Bean Plant Protection Workshop, CIAT, Cali, Colombia.

6. Cruz, Carlos. 1975. Daño y combate de insectos en el frijol común, Phaseolus vulgaris, y en el frijol de costa, Vigna unguiculata. Memoria XXI Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, Vol. 1: 241-249.

7. Freytag, George F. 1975. Breeding beans for the tropics. Memoria XXI Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, Vol. 1: 97-104.

8. Meléndez, Pedro L. y Rocío del Pilar Rodríguez. 1975. Control químico de las enfermedades del frijol (Phaseolus vulgaris, Vigna unguiculata). Memoria XXI Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios. (Summary of paper was omitted, by mistake, from the Proceedings.)

9. Rodríguez, Rita L., J. Bird y Amelia C. Monllor. 1975. Mosaico de Canavalia maritima (haba de playa) en Puerto Rico. Memoria XXI Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, Vol. 1: 237-239.

10. Vakili, N. G., W. J. Kaiser, J. E. Pérez and Amelia Cortés-Monllor. 1975. Bacterial blight of beans caused by Xanthomonas pathogenic types from Puerto Rico. Phytopathology 65: 401-403.

B. DISSEMINATION

Besides the papers listed in IV.A., research findings were disseminated via personal contact with 1) representatives from Central American and Caribbean countries attending: a) the XXI Annual Meeting of the Programa Cooperativo Centroamericano para el Mejoramiento de los Cultivos Alimenticios, held at San Salvador, El Salvador, and b) the Bean Plant Protection Workshop held at CIAT, Cali, Colombia, and with 2) personnel from the USAID sponsored project engaged in the development of a soybean industry in Panamá. In addition, information was disseminated through visits to establish cooperative field testing made to the following collaborators:

1. Dr. Guillermo Hernández Bravo, CIAT, Colombia
2. Ing. Gilberto Bastidas, ICA, Colombia
3. Dr. Eduardo Jiménez, MAG, Costa Rica
4. Prof. Víctor Muñoz, EAP, Honduras
5. Ing. Víctor Daccarett, DESARURAL, Honduras
6. Ing. Bernardo Patiño, CENTA, El Salvador
7. Dr. Alfonso Crispín, INIA, México

C. UTILIZATION

Findings on chemical control of diseases of beans and cowpeas are being applied in Panamá to soybeans, since several pathogens of beans and cowpeas also attack this grain legume. Results from chemical control trials are being applied also by some bean growers in Puerto Rico.

Two bean cultivars rated in our trials as resistant to insect damage were evaluated at CIAT. Cultivar Bonita maintained its resistance to leafhoppers (Empoasca spp) when tested in Colombia.

Multiple disease resistant germplasm developed by the Project was utilized in agronomic, disease resistance, breeding, and nutritional studies by researchers from Australia, Austria, Republic of China, Costa Rica, Netherlands, Kenya, France, Philippines and St. Lucia. Personnel from 12 research institutions in the United States also utilized materials developed by the Project.

The multiple disease resistant red bean cultivar Ecuador 299 (seed supplied by the Project), is being utilized in Honduras in a crossing program initiated to transfer these resistances to local cultivars.

D. FEEDBACK

Requests to continue to receive promising advanced lines for evaluation were received from all our Central American cooperators attending the XXI PCCMCA Meeting in El Salvador. Requests for seed were also received from the University of Florida AID Contract in El Salvador. Copies of papers given at meetings were also requested by Central American and Colombian research workers.

E. DEVELOPING COUNTRIES INVOLVEMENT

During the XXI Annual PCCMCA Meeting in El Salvador discussions were held with many Central American participants to ascertain the interest and needs of their countries in participating in a cooperative testing of the Project disease resistant materials. Because of the interest expressed, a visit in the field at the institution concerned was made between 6-22 April. Planting materials were subsequently sent and a visit to the field to observe these plantings was made between 12-29 October. In several locations materials were being tested and data

obtained, while poor delivery through the mails had caused the delay of other shipments. Because of this the report of these materials will be considerably delayed. However, considerable enthusiasm has been generated and helpful disease resistance is being provided to interested persons in country.

Fruitful exchange of materials and ideas aimed at the improvement of edible legumes was initiated with personnel at the Boliche Experiment Station, Guayaquil, Ecuador.

It was decided at the Bean Protection Workshop held at CIAT to assemble a collection of bean cultivars resistant to Empoasca to be tested in each of the developing American countries represented.

A graduate student from Nicaragua is conducting thesis research related to the identification of physiologic races of the bean rust fungus, *Uromyces appendiculatus*, in Puerto Rico.

V. WORK PLAN FOR THE COMING YEAR

A. ANTICIPATED ACCOMPLISHMENTS

1. Further significant progress in the characterization of rugaceous (whitefly transmitted) viruses of edible legumes.

2. Accelerated progress in the screening, via mechanical inoculation with the agent, of parental germplasm and hybrids of *Phaseolus* for resistance to the whitefly-transmitted bean golden yellow mosaic virus (BGYMV).

3. Further advances in technology to protect beans and cowpeas from diseases and noxious insects via chemical control practices, management and host resistance.

4. Results of evaluation of F_3 bean hybrids for combined resistance to rust and to soil-borne and virus diseases, as well as tolerance to leafhoppers.

5. Scarlet runner beans (*Phaseolus coccineus*) and tepary beans (*Phaseolus acutifolius*) selected for multiple disease resistance.

B. PROCEDURES AND ACTIVITIES

The procedures to be used and the activities to be carried out in order to attain the anticipated results are those currently used in researches of this type. Procedures and activities for the coming year will be essentially in line with methodology used throughout the life of the Project.

C. SIGNIFICANT FACTORS PROMOTING OR IMPEDING ACCOMPLISHMENTS

It is anticipated that positive factors, such as availability of staff and physical facilities, favorable climate, and a developing network of collaborators will promote accomplishments.

D. PLANS FOR ADDITIONAL INVOLVEMENT OF DEVELOPING COUNTRIES

1. Intensified efforts to form an effective network of collaborators for cooperative testing of promising Project materials.

2. Continued participation by the staff in meetings and workshops where representatives from developing countries interested in edible grain legume improvement can be contacted.

3. Publication of a field manual of diseases and insects of tropical grain legumes for distribution to interested personnel and institutions in developing countries.

4. Increased participation of graduate students (M S level) from developing countries in thesis research related to grain legume improvement.

E. PROPOSED BUDGET

The estimated total amount of funds needed to support these plans is $470,000.