Tropical Diseases of Legumes

Edited by

Julio Bird

Karl Maramorosch

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Contents

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Preface

Tropical Diseases of Legumes is oriented toward the needs of tropical countries, primarily those of Central and South America, the Caribbean area, and West Africa. However, the problems presented are also pertinent to the developing countries of Asia and the Pacific islands. There are at present very few books dealing specifically with tropical plant diseases and none dealing with the important subject covered in this treatise. This is the first book devoted entirely to tropical diseases of legumes, that group of plants that provide an important and often sole source of protein in the diet of millions of people. In an increasingly hungry world there is an immediate need to raise the production of legumes through better knowledge of plant diseases, by ultimate prevention of these diseases, and through improved crop production.

The contributors to this volume participated in a workshop held in June 1974 at the Río Piedras Agricultural Experiment Station of the University of Puerto Rico (Mayagüez Campus). Following the workshop, the decision was made to edit and publish the papers presented at the workshop. Some of the chapters deal with as yet unpublished experimental data, others with recent literature, and still others are historical or speculative in nature. All contribute some unpublished information and personal interpretations and conclusions. It is hoped that the purpose of the book will be achieved, namely to provide a stimulating forum for discussion of new findings and observations in tropical legume disease research.

The Editors wish to pay special tribute to the Agency for International Development, U.S. Department of State in Washington, D.C., and to the University of Puerto Rico, for sponsoring the workshop, and to all of the authors for their excellent contributions. Sincere gratitude is expressed to Dr. Arturo Morales Carrión, President of the University of Puerto Rico, to Prof. Rafael Pietri Oms, Chancellor of the Mayagüez Campus, Prof. Salvador Alemañy, former Dean of the College of Agricultural Sciences, Dr. Russell Desrosiers of the U.S. Department of State, Dr. Mario E. Pérez, Acting Director, Agricultural Experiment Station, Río Piedras as well as to many others who gave moral support to the idea of organizing the workshop that resulted in the preparation of this book. Academic Press deserves special credit for its part in producing the volume in record time.

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Rugaceous Diseases

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RUGACEOUS (WHITEFLY-TRANSMITTED) VIRUSES IN PUERTO RICO

Julio Bird, Josefina Sánchez, Rita L. Rodríguez, and Frank J. Juliá

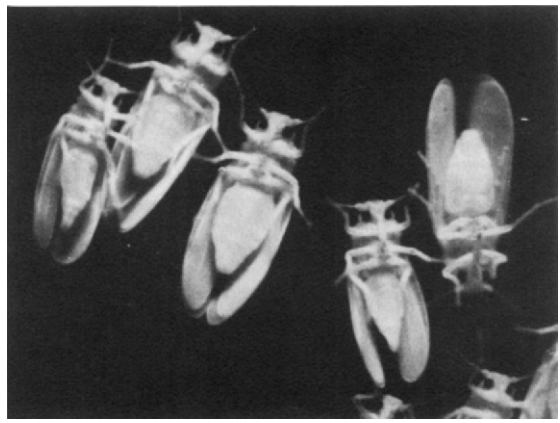
I. Introduction

Not much is known about the etiology of the Puerto Rican rugaceous* diseases of *Jatropha gossypifolia* (L.) Pohl.**, *Sida carpinifolia* L., *Rhynchosia minima* DC., *Merremia quinquefolia* Hall., *Jacquemontia tamnifolia* Griseb., *Phaseolus lunatus* L., and *Euphorbia prunifolia* Jacq. Their causal agents are apparently quite small, enough so to be acquired and spread in the feeding process by *Bemisia tabaci* Genn., a tiny whitefly, (Fig. 1) provided with minute sucking mouth parts (Russell, 1957). The etiologic agents of these diseases have not been viewed with the light or electron microscopes.*** Three of the causal agents of these local maladies *(R. minima, P. lunatus* and *E. prunifolia* mosaics) have been transmitted by mechanical means although the rates of infection obtained in all but one host have never exceeded 18% (Lot *et al*, 1973 and Bird *et al.*, 1973). Little is known, also, about the etiology of similar diseases propagated by the whitefly *B. tabaci* in other parts of the world (Costa, 1969). These maladies are presumed to be caused by either viruses or viroids. In the present article we will use the term "viruses" when referring to the etiologic agents of the aforementioned diseases. While this is done for convenience, it is recognized that the designation may be inappropriate (at least for some of the agents).

*This term is used to denominate diseases of uncertain etiology propagated by *Bemisia tabaci* Genn. and characterized by symptoms on their hosts such as malformation, leaf curl, enations and yellow mosaic.

**Many of the specific names used in this article were extracted from Britton and Wilson's "Botany of Porto Rico and the Virgin Islands", Vol. V and VI of the Scientific Survey of Porto Rico and the Virgin Islands published by the New York Academy of Sciences, 1925.

***Recently the mosaic of *E. prunifolia* was found to be associated with a spherical virus.



(Fig. 1). Enlarged ventral view of male (tapered caudally) and female (blunt) specimens of the whitefly *Bemisia tabaci*.

(Fig. 2). *Jatropha gossypifolia* plant affected by the *Jatropha* mosaic.

The local viruses treated in this article will be discussed in the same sequence in which they were found and studied in Puerto Rico. For quick reference a series of short descriptions of the treated diseases will be included as an addendum to this paper.

II. The Viruses

A. MOSAIC VIRUS OF *JATROPHA GOSSYPIFOLIA* (L.) POHL.

The mosaic (Fig. 2) of *J. gossypifolia* was the first rugaceous disease subjected to detailed studies in Puerto Rico (Bird, 1957). Its host range seemed at first to be restricted to species within the genus *Jatropha* such as *J. podagrica* Hook. and *J. multifida* L. Certain solanaceous species were later found to be susceptible although the disease incited in them by the *Jatropha* virus was rather mild. The *Jatropha* virus was thought to be similar to the agent studied on *E. prunifolia* in Brazil (Costa and Bennett, 1950) but the result of host range studies indicated that the two diseases were not related. *Croton lobatus* L., *Jacquemontia tamnifolia* and *Phaseolus vulgaris* L. were recently shown to be extremely susceptible to the *Jatropha* mosaic virus although the vector, a race of the whitefly *B. tabaci* which is restricted mostly to certain species within the genus *Jatropha,* could not properly colonize these three hosts. The disease resulting on *J. tamnifolia* by infection with the *Jatropha* virus is not very similar, from the standpoint of symptomatology, to the one provoked by another of the viruses studied in Puerto Rico and which will be discussed later, i. e., the mosaic virus of *J. tamnifolia.* The results of a preliminary trial indicated that soybeans could also be infected by the *Jatropha* virus via the *Jatropha* race of *B. tabaci.* The *Jatropha* mosaic virus is presently receiving much of the writers' attention since it was shown that some legumes are very susceptible to this agent.

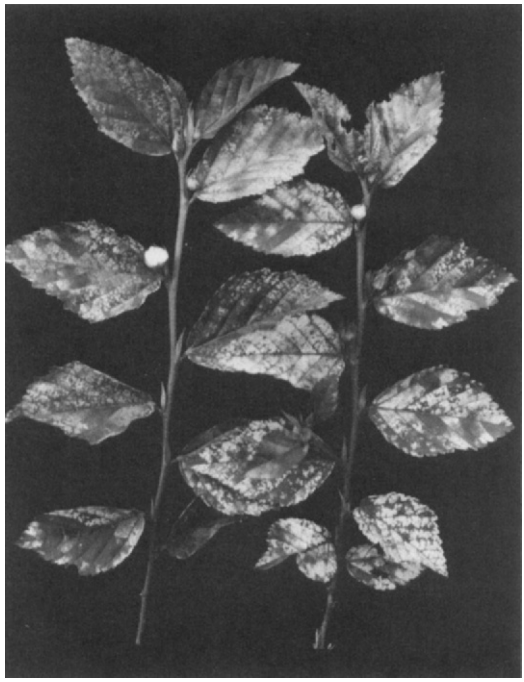
B. MOSAIC VIRUS OF *SIDA CARPINIFOLIA* L.

A mosaic virus of *S. carpinifolia* (Fig. 3) similar in some respects to one reported from Brazil (Costa, 1954; Orlando and Silberschmidt, 1946, and Silberschmidt and Tommasi, 1956) was studied in Puerto Rico (Bird, 1958).

Several of the species of *Sida* known in Puerto Rico have been shown lately (Bird and Sánchez, 1966) to be susceptible to the mosaic virus of *S. carpinifolia* which is perhaps the commonest mosaic in the Island. The causal agent, persistently (Watson and Roberts, 1936) transmitted via the race of *B. tabaci* that is normally associated with malvaceous hosts, is capable of infecting tobacco producing, as in the case of the *Jatropha* virus, a rather mild form of leaf curl. *S. carpinifolia* and *S. rhombifolia* L. are excellent hosts for the *Sida* race of the whitefly vector although both are subject to attack by mites which at times ruin the insect stock cultures. Attempts made in Puerto Rico to transmit the *Sida* mosaic virus to *P. vulgaris* and other legumes have not been successful. Thus it seems that this virus is not closely related to its Brazilian counterpart.

C. MOSAIC VIRUS OF *RHYNCHOSIA MINIMA* DC.

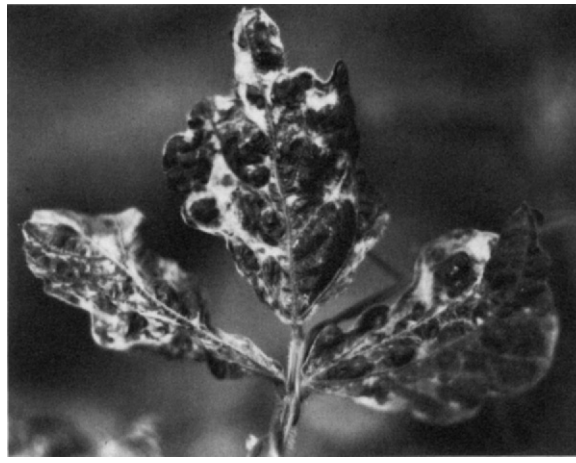
The *Rhynchosia* virus affects many wild hosts in the Leguminosae and also infects some species in the Malvaceae and Solanaceae (Figs. 4 and 5). Its main reservoir on the Island seems to be *R. minina* although it commonly affects *Macroptilium lathyroides* (L.) Urban *(Phaseolus lathyroides* L.). Recently,



(Fig. 3). Mosaic of *Sida* on *Sida carpinifolia.*

(Fig. 4). *Rhynchosia* mosaic on *R. minima* (A) and *Phaseolus vulgaris* (B).

(Fig. 5). Enations incited by the *Rhynchosia* virus on tobacco (A) and *Malachra capitata* (B).



(Fig. 6). Trifoliolate leaf of *Glycine max* affected by the *Rhynchosia* mosaic.

Malachra capitata L., a well distributed and abundant malvaceous weed in Puerto Rico, was found to be an excellent host for the *Rhynchosia* virus. At times, the incidence of mosaic symptoms on *M. capitata* seems to be universal in some localities of the western and southern sections of Puerto Rico. On beans the *Rhynchosia* virus causes leaf malformation, yellowing, witches broom and stunting. Plants affected early in their development fail to produce seed although they may flower and branch profusely. The *Rhynchosia* virus has primary affinity for leguminous hosts and is transmitted by *B. tabaci* race *sidae.* It can be distinguished from all other Puerto Rican whitefly-transmitted viruses by the fact that it provokes the formation of leaf and cup-like enations on tobacco and *M. capitata.*

Many varieties and lines of beans have been tested for resistance to the *Rhynchosia* mosaic virus but few were found to possess resistance. Two varieties, La Vega and Santa Ana, have shown some degree of resistance when subjected to viruliferous whiteflies (large populations) in the insectary. They were also found to possess resistance under field conditions. In this particular area of varietal resistance our work was restricted to the insectary phase of the screening. All other credit in this phase of the work on resistance is due to our USDA, AID cooperator in Mayagüez, Puerto Rico, Dr. Nader Vakili who collected, improved and field tested the aforementioned bean varieties.

In the insectary phase of the screening process test plants were placed in a rather large insectary containing viruliferous stock cultures of the insect vector. Potted virus source plants were randomly distributed on the tables in the insectary and the test plants dispersed among the virus-source plants. The insects were not disturbed nor the plants shaken (to avoid forceful migration). If the test plants were preferred hosts the insects would migrate and colonize them of their own accord. At the same time and within the same premises test plants from the same batch distributed on the tables were covered with small cylindrical lumite topped cages (Bird, 1958). Viruliferous insects were then released, at the rate of 50 per plant in each cage. Thus restricted, the insects would either feed and survive, feed and die, or fail to feed and die. Similarly they would either colonize or fail to colonize the test plants depending, of course, on the relative preference of the insect for the particular plant species. Concomitantly employing these two methods denominated free and forced-feeding, respectively, valuable data on relative resistance of the various test plants was gained. Plants were also exposed to the virus under field conditions.

Employing these methods marked resistance to the *Rhynchosia* virus has been located by the authors on high-yielding lines of pigeon peas *(Cajanus indicus* Spreng.) developed by Dr. Raúl Abrams, Plant Breeder, Agricultural Experiment Station of the University of Puerto Rico. Soybeans, *Glycine max* (L.) Merril, are quite susceptible to the *Rhynchosia* mosaic virus (Bird *et al.*, 1974) and are not likely to produce a crop once affected (Fig. 6). This malady would certainly pose a problem to soybeans in areas where the vector and the etiologic agent are prevalent. Certainly this has happened in some experimental fields at the Fortuna and Isabela Agricultural Experiment Substations in Puerto Rico.

D. MOSAIC VIRUS OF *MERREMIA QUINQUEFOLIA* HALL.

This mosaic has been known to occur in Puerto Rico for quite a number of

years (Bird and Sánchez, 1971). Affected *Merremia* plants, due to the vivid yellow and green foliar blotches are often used as ornamentals (Fig. 7). Vines grow well even when their foliage is severely mosaiced. The vector, again *B. tabaci* race *sidae,* is extremely efficient and capable of spreading the disease to a good number of convolvulaceous species not including *Ipomoea batatas* L. Recently it was shown that the *Merremia* virus could be transmitted via the whitefly vector to tomatoes, *Datura stramonium* L. and beans. The causal agent also causes a mild mosaic on *Nicotiana tabacum* L. and *M. capitata.* However symptoms on these last two hosts tend to fade away with time although they may recurr if the plants are pruned and fertilized. In contrast with the *Rhynchosia* virus the *Merremia* virus fails to incite the formation of enations on the aforementioned hosts. The *Merremia* virus will not confer protection to tobacco against the *Rhynchosia* virus, that is, tobacco plants affected by the *Merremia* virus will develop enations and a markedly severe disease if inoculated via grafting, with *Rhynchosia* mosaic virus.

E. MOSAIC VIRUS OF *JACQUEMONTIA TAMNIFOLIA* GRISEB.

The virus causing this mosaic (Fig. 8) primarily affects *J. tamnifolia,* a densely pubescent convolvulaceous weed occurring on sandy plains, banks, and slopes at lower elevations in the northern and western districts of Puerto Rico. The foliage of affected plants exposed to full sunlight will develop a vivid golden yellow mosaic; plants failing to receive enough sunlight usually bear rather pale yellow mosaic symptoms. This is true, in general, of all the rugaceous diseases known to exist in Puerto Rico. Besides the primary host other plant species including *J. pentantha* (Jacq.) G. Don. and beans can be efficiently inoculated via the whitefly vector *B. tabaci* race *sidae.* Tobacco is susceptible and usually develops moderately severe leaf curl symptoms after inoculation.

A seemingly different mosaic perhaps provoked by a strain of the same virus and also transmitted by *B. tabaci* race *sidae* was recently found affecting *J. pentantha* (Fig. 9), a glabrous species occurring on thickets, banks and hillsides at lower and middle elevations. This particular agent incites rather striking symptoms on tobacco. This has led us to denominate the malady crumpling disease of tobacco (Fig. 10). The virus also causes leaf distortion and mosaic of beans and *D. stramonium* but fails to protect tobacco from invasion by the *Rhynchosia* mosaic virus.

F. MOSAIC VIRUS OF *PHASEOLUS LUNATUS* L. (BEAN GOLDEN YELLOW MOSAIC VIRUS)

From the standpoint of incidence the *Phaseolus lunatus* virus = bean golden yellow mosaic (BGYMV) (Fig. 11) is perhaps the most important bean virus occurring on the island of Puerto Rico (Bird *et al.,* 1972). This virus was first discovered on wild plants of *P. lunatus* on the northern coast of Puerto Rico and later identified on *P. vulgaris* in the dryer areas. The vector is *B. tabaci* race *sidae.* The causal agent is transmitted persistently and acquired and inoculated by the vector rather easily. The range of this unique virus is seemingly restricted to legumes. The symptoms (golden yellow mosaic and golden yellow veins) induced on beans by the causal agent are distinct, in color intensity, from those provoked by the other rugaceous viruses. The presence of this virus on beans will not confer protection against the other Puerto Rican whitefly-transmitted

(Fig. 7). *Merremia aegyptia* vine affected by the *M. quinquefolia* mosaic virus.

(Fig. 8). Young plant of *Jacquemontia tamnifolia* affected by the *J. tamnifolia* mosaic virus.

(Fig. 9). *Jacquemontia pentantha* plant affected by the *J. pentantha* mosaic virus.

(Fig. 10). V-12 tobacco plant affected by the *Jacquemontia pentantha* virus.

11a

11b

(Fig. 11). Symptoms incited by the Bean Golden Yellow mosaic virus: Mosaic (A) and yellow veins (B).

viruses of beans. Definitely it will not affect tobacco. On beans and *P. lunatus* its presence is accused, even in mixed infections, by the appearance of a bright golden yellow color component. *M. lathyroides* (Fig. 12) seems to be the main wild reservoir of the virus in Puerto Rico. No resistance has been located so far in any of the many bean varieties tested locally. Plants affected by the malady fail to produce seed. The golden yellow mosaic virus of beans, or a closely related entity, seems to occur in some of the Caribbean Islands as well as in Central and Northern South America. Our disease resembles, at least from the standpoint of symptoms, the golden yellow mosaic studied in Brazil (Costa, 1965). It also bears some resemblance to the bean golden yellow mosaic studied in Costa Rica (Gámez, 1969, 1970). Not until cross inoculation, host range, cross protection, and other tests are made in one locality with the various isolates will the kinship question be elucidated. Electron microscopy studies might bring forth rewarding evidence regarding the nature and relationships of the golden yellow mosaic diseases of bean.

G. MOSAIC VIRUS OF *EUPHORBIA PRUNIFOLIA* JACQ.

This mosaic, characterized by yellow-green mottling and yellow veins, was discovered very recently in Puerto Rico affecting *E. prunifolia* (Fig. 13) in a small area surrounding an experimental soybean field in the Isabela Agricultural Experiment Substation. For more than 15 years the writers had been on the lookout for the *Euphorbia* mosaic after studying a report on the existence of such a disease in Brazil (Costa and Bennet, 1950). In Isabela most of the cases of mosaic were concentrated in a small area receiving artificial light during the night. Most of the *E. prunifolia* plants growing 100 feet or more away from the artificially illuminated ones bore no symptoms of mosaic and were devoid of whiteflies. The fact that the mosaic was nowhere to be encountered but on that particular farm raised questions regarding its possible origin. Lately the mosaic has spread to all the Isabela Substation grounds. Was the causal agent brought in with some Brazilian cassava, *Manihot utilissima* Pohl., introductions (vegetatively propagated) or was the virus transmitted by whiteflies from a local host to *E. prunifolia*? Tests conducted under controlled conditions in the greenhouse indicated that the virus was efficiently transmitted by *B. tabaci* race *sidae* and that the agent was capable of infecting *D. stramonium* (Fig. 14), *P. vulgaris, N. tabacum* (Fig. 15), and *Glycine max* as well as seedling plants of the primary host. In preliminary trials the causal agent was transmitted (31% infection) by mechanical means from *E. prunifolia* to *D. stramonium.* Better results (50% infection) were obtained when the agent was secured from *D. stramonium.* The virus appears to be closely related to the one reported from Brazil by Costa and Bennett (1950).

III. Addendum

A. MOSAIC VIRUS OF *JATROPHA GOSSYPIFOLIA* (L.) POHL. (PUERTO RICO)

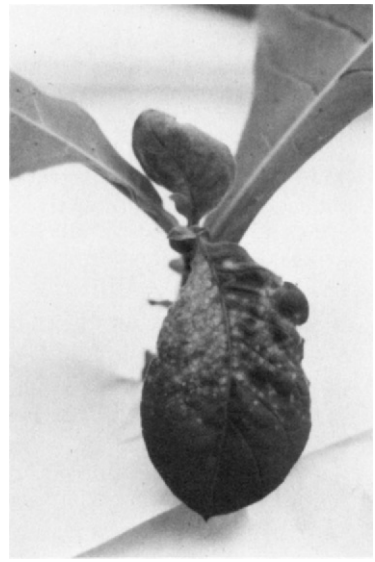
Main diseases:

Yellow mosaic of *Jatropha gossypifolia* and related species. Enations, curl, and distortion of leaves of *J. gossypifolia.* Leaf curl and stunting of *Phaseolus vulgaris* L. Mild leaf curl of tobacco.

(Fig. 12). Field plant of *Macroptilium lathyroides* affected by Bean Golden Yellow mosaic virus.

(Fig, 13). *Euphorbia prunifolia* plant with symptoms incited by the *Euphorbia* mosaic.

(Fig. 14). *Datura stramonium* leaf with symptoms (rings and mosaic) incited by the *Euphorbia prunifolia* virus (inoculated by mechanical means).



(Fig. 15). Symptoms provoked by the *Euphorbia* virus on a tobacco plant of the V-12 variety.

Geographical distribution:

Similar diseases known to occur on *J. gossypifolia* in Jamaica, Santo Domingo, and the Virgin Islands.

Host range and symptomatology:

Affects species within the Euphorbiaceae, Convolvulaceae, Leguminosae and Solanaceae.

Susceptible species:

Euphorbiaceae:

Croton lobatus L.; *Jatropha gossypifolia* L.; *J. multifida* L.; *J. podagrica* Hook.

Convolvulaceae:

Jacquemontia tamnifolia Griseb.

Leguminosae:

P. vulgaris L.; *Glycine max* (L.) Merril.

Solanaceae:

Nicotiana tabacum L.

Propagation species:

Primary host excellent for maintenance of causal agent as well as vector.

Assay species:

None.

Strains:

Not studied.

Transmission by vectors:

Persistently transmitted (4 days) by the *Jatropha* race of the whitefly *Bemisia tabaci* Genn. Agent acquired and transmitted after short feeding periods (2 hours and 10 minutes, respectively). Single whiteflies are capable of transmitting causal agent.

Transmission through seed:

Not seed transmitted.

Transmission by dodder:

No transmission by *Cuscuta campestris* nor by *Cassytha filiformis*.

Serology:

Not studied.

Relationships:

Unknown.

Stability in sap:

Presumed to be unstable - not sap transmissible.

Purification:

Not attempted.

Particle structure:

Causal agent has not been visualized with aid of electron microscope.

B. MOSAIC VIRUS OF *SIDA CARPINIFOLIA* L. (PUERTO RICO)

Main diseases:

Yellow mosaic of *Sida carpinifolia* and other *Sida* species in Puerto Rico. Mild leaf curl of tobacco.

Geographical distribution:

In Puerto Rico wherever *Sida* is found. Similar diseases seem to be well distributed in the West Indies and Florida.

Host range and symptomatology:

In Puerto Rico restricted to Malvaceae and Solanaceae producing a yellow mosaic.

Susceptible species:

Malvaceae:

Altheae rosea (L.) Cav.; *Bastardia viscosa* (L.) H.B.K.; *Corchorus aestuans* L.; *Hibiscus brasilensis* L.; *Malvaviscus* sp.; *Sida acuminata* D.C.; *S. aggregata* Presl.; *S. carpinifolia* L.; *S. cordifolia* L.; *S. glabra* Mill, *S. glomerata* Cav., *S. humilis* Cav.; *S. procumbens* Sw.; *S. rhombifolia* L.; *S. urens* L.

Solanaceae:

Nicotiana tabacum L.

Propagation species:

Vector and infectious agent can be maintained on *S. carpinifolia.*

Assay species:

None.

Strains:

Not studied.

Transmission by vectors:

Persistently transmitted (7 days) by the *Sida* race of the whitefly *Bemisia tabaci* Genn. Acquired and transmitted in short feeding periods (15 and 20 minutes, respectively). Single insects are efficient vectors.

Transmission through seed:

Not transmitted through seed.

Transmission by dodder:

Not attempted.

Serology:

Not studied.

Relationships:

Perhaps related to *Sida* viruses studied by Orlando and Silberschmidt (1946) and by Costa (1954) in Brazil. However, attempts made to transmit the Puerto Rican virus by mechanical means or via *B. tabaci* to *Phaseolus vulgaris* have not been successful.

Stability in sap:

Not studied.

Purification:

Not attempted.

Particle structure:

Not studied.

C. MOSAIC VIRUS *RHYNCHOSIA MINIMA* DC. (PUERTO RICO)

Main diseases:

Yellow mosaic symptoms on most hosts, leaf distortion, beading of veins, enations on tobacco and *Malachra capitata*, stunting, restriction of leaf lamina, etc.

Geographical distribution:

In all areas where *R. minima* is found in Puerto Rico. Not much is known about its distribution elsewhere although similar diseases seem to exist in Tropical America.

Host range and symptomatology:

Affects species within Lamiaceae, Leguminosae, Malvaceae, Solanaceae and Oxalidaceae producing mosaic and leaf curl.

Susceptible species:

Lamiaceae:

Salvia splendens Sellow.

Leguminosae:

Cajanus indicus Spreng.; *Canavalia ensiformis* (L.) DC.; *C. maritima* (Aubl.) Thou.; *Crotalaria juncea* L.; *Glycine max* (L.) Merril; *Macroptilium lathyroides* (L.) Urban; *Pachyrrhizus erosus* (L.) Urban; *Phaseolus aborigineus* Burk., *P. acutifolius* A. Gray, P.I. Wright; *P. acutifolius* A. gray var.*latifolius; P. coccineus* L.; *P. lunatus* L.; *P. trichocarpus* C. Wright; *P. vulgaris* L.; *Rhynchosia minima* DC.; *R. reticulata* DC.; *Vigna aconitifolia* (Jacq.) Maréchal; *V. angularis* (Willd.) Ohwi and Ohashi.

Malvaceae:

Abelmoschus esculentus (L.) Moench; *Gossypium hirsutum* L.; *Malachra capitata* L.

Oxalidaceae:

Oxalis barrelieri L.; *Oxalis* sp.

Solanaceae:

Nicotiana acuminata Hook; *N. alata* Link and Otto; *N. bonariensis* Lehmann; *N. clevelandii* Gray; *N. glauca* Grah.; *N. glutinosa* L.; *N. knightiana* Goodspeed; *N. maritima* Wheeler; *N. paniculata* L.; *N. tabacum* L.

Propagation species:

Rhynchosia minima to maintain virus and stock colonies of insect vector. *Nicotiana tabacum* to maintain virus for infective sap (mechanical inoculation).

Assay species:

None.

Strains:

Several strains seem to exist.

Transmission by vectors:

Persistently transmitted (7 days) by the *Sida* race of *Bemisia tabaci.* Single insects are efficient vectors. Minimum acquisition and minimum inoculation feeding periods - 24 hrs.

Transmission through seed:

Not transmitted through seed.

Transmission by dodder:

Not tested.

Serology:

Negative results.

Relationships:

Seemingly unrelated to other locally studied rugaceous entities.

Stability in sap:

Seems to be rather unstable. Under optimum conditions not more than 18% rate of infection obtained when inoculated by mechanical means using adjuvants. Tobacco (Virginia 12) is best source of infective sap for mechanical inoculation.

Purification:

Not attempted.

Particle structure:

Causal agent has not been visualized with aid of electron microscope.

D. MOSAIC VIRUS OF *MERREMIA QUINQUEFOLIA* HALL. (PUERTO RICO)

Main diseases:

Causal agent incites a brilliant yellow mosaic of *M. quinquefolia, M. aegyptia* Urban and other convolvulaceous weeds.

Geographical distribution:

As far as known only reported from Puerto Rico.

Host range and symptomatology:

Affects species within the Convolvulaceae, Leguminosae, Malvaceae and Solanaceae producing brilliant yellow mosaic diseases.

Susceptible species:

Convolvulaceae:

Calonyction aculeatum (L.) House; *I. angustifolia Jacq. I. crassucaulis* (Benth.) B.L. Robinson; *I. dissecta* (Jacq.) Pursh; *I. tiliaceae* (Willd.) Choisy; *I. triloba* L.; *I. tuberosa* (L.) Meissn.; *Merremia aegyptia* Urban; *M. quinquefolia* Hall.

Leguminosae:

Pachyrrhizus erosus (L.) Urb.; *Phaseolus aborigineus* Burk.; *P. acutifolius* A. Gray, Wright; *P. coccineus* L.; *P. lunatus* L.; *P. vulgaris* L.; *Vigna umbellata* (Thumb.) Ohwi and Ohashi.

Malvaceae:

Malachra capitata L.

Solanaceae:

Datura stramonium L.; *Lycopersicon esculentum* Mill.; *Nicotiana tabacum* L.

Propagation species:

M. quinquefolia and *M. aegyptia* are excellent hosts for the virus and the vector.

Assay species:

None.

Strains:

Not studied.

Transmission by vectors:

Transmitted by the *Sida* race of the whitefly *Bemisia tabaci*. Twenty-four hours feeding period will fully charge whiteflies with causal agent. Single insects (male or female) are capable of transmitting the etiologic agent. Fifty viruliferous individuals per plant will insure good rate of infection.

Transmission through seed:

Not seed transmitted.

Transmission by dodder:

Not attempted.

Serology:

Not studied.

Relationships:

Seemingly unrelated to other locally studied rugaceous entities.

Stability in sap:

Preliminary attempts made to transmit agent mechanically using sap with adjuvants were unsuccessful.

Purification:

Not attempted.

Particle structure:

Attempts made to visualize agent with aid of electron microscope were unsuccessful.

E. MOSAIC VIRUS OF *JACQUEMONTIA TAMNIFOLIA* GRISEB. (PUERTO RICO)

Main diseases:

Causes bright yellow mosaic of *Jacquemontia tamnifolia*. Also incites stunting and curling diseases of beans *(Phaseolus vulgaris* L.) and a moderately severe leaf curl of tobacco.

Geographical distribution:

Found in Puerto Rico on sandy plains, banks and slopes at lower elevations at northern and western districts. Not known to occur elsewhere.

Host range and symptomatology:

Affects species within the Convolvulaceae, Leguminosae and Solanaceae producing moderately severe leaf curl.

Susceptible species:

Convolvulaceae:

Jacquemontia pentantha (Jacq.) G. Don; *J. tamnifolia* Griseb.

Leguminosae:

Phaseolus aborigineus Burk.; *P. acutifolius* A. Gray var. *latifolius; P. coccineus* L.; *P. vulgaris* L.; *Vigna aconitifolia* Jacq.) Maréchal; *V. angularis* (Willd.) Ohwi Ohashi.

Solanaceae:

Nicotiana tabacum L.

Propagation species:

Jacquemontia tamnifolia is an excellent host for the causal agent and the insect vector.

Assay species:

None.

Strains:

A seemingly related agent has been isolated from *Jacquemontia pentantha* via the whitefly *Bemisia tabaci*. This isolate causes a severe crumpling disease of tobacco.

Transmission by vectors:

Transmitted by the *Sida* race of *Bemisia tabaci*. It is relevant at this point to mention the fact that the causal agent of the mosaic of *Jatropha gossypifolia* which is transmitted by the *Jatropha* race of *B. tabaci*, is capable of infecting plants of *Jacquemontia tamnifolia*. Symptoms on this last host are not as virulent as those incited by the field isolate of *J. tamnifolia* virus.

Transmission through seed:

Not seed transmitted.

Transmission by dodder:

Not studied.

Serology:

No serological studies have been carried out.

Relationships:

Not known.

Stability in sap:

Not studied.

Purification:

Not attempted.

Particle structure:

No particles have been visualized via the electron microscope.

F. MOSAIC VIRUS OF *PHASEOLUS LUNATUS* L. (PUERTO RICO)

Main diseases:

On Beans *(Phaseolus vulgaris* L.) and lima beans *(P. lunatus* L.) bright golden yellow mosaic. At times only golden yellow veins giving leaves a reticulated appearance, the veinal system yellow, backgrounded by the green color of the leaves.

Geographical distribution:

Similar diseases occur in the West Indies, Central and South America.

Host range and symptomatology:

Restricted to legumes (mostly to species within the genus *Phaseolus*).

Susceptible species:

Leguminosae:

Macroptilium lathyroides (L.) Urban; *Phaseolus aborigineus* Burk.; *P. coccineus* L.; *P. lunatus* L.; *P. vulgaris* L.; *Vigna radiata* (L.) Wilczek.

Propagation species:

Infectious agent can be maintained on *P. lunatus*.

Assay species:

None known.

Strains:

Various strains seem to exist.

Transmission by vectors:

Persistently transmitted (5 days) by the *Sida* race of the whitefly *Bemisia tabaci* Genn. Acquired and transmitted in short feeding periods (5 minutes in both cases) and efficiently transmitted by single males or females.

Transmission through seed:

Not transmitted through seed.

Transmission by dodder:

Not tested.

Serology:

Not studied.

Relationships:

Possibly related to viruses studied by Gámez (1969, 1970) in El Salvador and by Costa (1965) in Brazil.

Stability in sap:

Seemingly unstable. The causal agent has been transmitted by mechanical means, using adjuvants.

Purification:

Not attempted.

Particle structure:

Causal agent has not been visualized with the aid of electron microscope.

G. MOSAIC VIRUS OF *EUPHORBIA PRUNIFOLIA* JACQ. (PUERTO RICO)

Main diseases:

Striking yellow mosaic of *E. prunifolia.* At times only yellow vein netting is evident. Yellow leaf curl of *Datura stramonium* L., *Nicotiana tabacum* L. and *Glycine max* (L.) Merril. Yellow leaf curl, necrotic blotching and witches broom of *Phaseolus vulgaris* L.

Geographical distribution:

The mosaic virus of *E. prunifolia* seems to have been introduced recently into Puerto Rico. So far it is restricted to a small area (municipality of Isabela) in the northern coast.

Host range and symptomatoloty:

Affects species within Euphorbiaceae, Leguminosae and Solanaceae.

Susceptible species:

Euphorbiaceae:

Euphorbia prunifolia Jacq.

Leguminosae:

Phaseolus vulgaris L.; *Glycine max* (L) Merril.

Solanaceae:

Nicotiana tababum L.; *Datura stramonium* L.

Propagation species:

D. stramonium is an excellent host for the causal agent but a poor one for the insect vector.

Assay species:

D. stramonium

Strains:

Not studied.

Transmission by vectors:

Specifically transmitted with great ease by the whitefly *Bemisia tabaci* race *sidae*.

Transmission through seed:

Not studied.

Transmission by dodder:

Not attempted.

Serology:

Not studied.

Relationships:

Apparently not related to other local rugaceous entities studied in Puerto Rico. Fails to infect *Rhynchosia minima* DC. and will not produce enations on tobacco.

Stability in sap:

The agent has been transmitted by mechanical means employing carborundum and phosphate buffer (0.1 M).

Agent remains active after infected leaves are dehydrated with calcium chloride at ambient temperature (75°F).

Purification:

Can be partially concentrated by differential and density gradient centrifugation.

Particle structure:

Very recently spherical particles (viral) were visualized via electron microscope.

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INCREASE IN THE POPULATIONAL DENSITY OF *BEMISIA TABACI*, A THREAT OF WIDESPREAD VIRUS INFECTION OF LEGUME CROPS IN BRAZIL

A. S. Costa

I. Introduction

Whitefly-transmitted diseases attributed to viruses have been known to be quite widespread among species of the natural weed stands in cultivated and pasture areas of the states of Minas Gerais, Paraná, Sao Paulo, and other parts of Brazil. Representatives of Compositae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Solanaceae, and other families are frequently found infected with the golden type of mosaic or curl diseases usually associated with transmission by the whitefly, *Bemisia tabaci* Genn.

Some of the crop plants raised in Brazil become infected with whitefly-transmitted diseases. Among these, beans *(Phaseolus vulgaris* L.) and soybeans, *Glycine* max (L.) Merr., cotton *(Gossypium hirsutum* L.), tobacco *(Nicotiana tabacum* L.), and tomatoes *(Lycopersicon esculentum* Mill.) might suffer losses of varying importance.

In some instances, infection in the plantings represents occasional passage of the disease agent from the natural weed reservoirs to the crop plants, little or no transmission taking place within the crop. In other cases, host-adapted strains of

the viruses have evolved that are easily spread by the vector within the field crops. Incidence of the disease in the field crops, in the first instance, is generally light and bears a relationship to the presence of infected weeds in the field borders. In the second case, the incidence may attain high levels within the plantings if the vector breeds well on the varieties planted.

Whitefly-transmitted diseases of crop plants in Brazil were considered of minor importance in the past, although severe outbreaks had been known to occur in restricted areas. In the last few years, the populational density of *Bemisia tabaci* has reached such high levels in cultivated areas in the states of Minas Gerais, Paraná, and Sao Paulo, that the diseases transmitted by this vector have become of major importance to such crops as beans (mostly dry bean) and tomatoes (unstaked).

II. Whitefly-transmitted Diseases of the Bean and Soybean Crops in Brazil

The bean and soybean plants can be infected with the *Abutilon* and *Euphorbia* mosaic agents (Costa, 1955). In addition, the bean plant is susceptible to the golden mosaic disease, to which the soybean seems to be resistant in Brazil (Costa, 1962). These diseases of the bean and soybean plants in Brazil have already been described (Costa, 1955, 1965; Costa *et al.* 1970). However, a short description will be included in this paper for comparative purposes.

A. BEAN CRUMPLING

This disease is rarely encountered in the field and only when outbreaks of the *Euphorbia* mosaic virus occur in the natural stands of the susceptible weed *(E. prunifolia* Jacq.) and these happen to be in the immediate vicinity of the bean plantings. The economic importance of crumpling is nihil.

Symptoms

Crumpling (Fig. 1) is mostly a local lesion disease, generally necrotic. Symptoms appear at the feeding punctures of the viruliferous vector. Systemic invasion seldom develops in inoculated plants, and when this occurs, takes the form of a few scattered necrotic or sometimes chlorotic lesions in the trifoliolate leaves. As the leaflets expand, they become crumpled or twisted, because of the uneven growth of the areas near the lesions and the rest of the leaflets. Abnormal axillary growth is developed and might also be crumpled. The plant becomes stunted.

Etiology

The *Euphorbia* mosaic virus (EMV), responsible for the crumpling of the bean plants, was described by Costa and Bennett (1950) and later was used to inoculate bean and other legume plants in comparative tests with the *Abutilon* mosaic virus (Costa and Carvalho, 1960b). The EMV is less infectious to beans than the latter. *Phaseolus angularis* (Willd.) Wright, *P. aureus* Roxb., *P. calcaratus* Roxb., and *P. trinervius* Heyne are resistant. The virus is not seed-transmitted. It may be transmitted mechanically to some host plants, but not to beans. Its physical properties, as determined with the crude sap, were: thermal inactivation between 55°C and 60°C; dilution end point about 5^{-5}; and resistance to aging *in vitro* longer than 48 hours (Costa and Carvalho, 1960b).

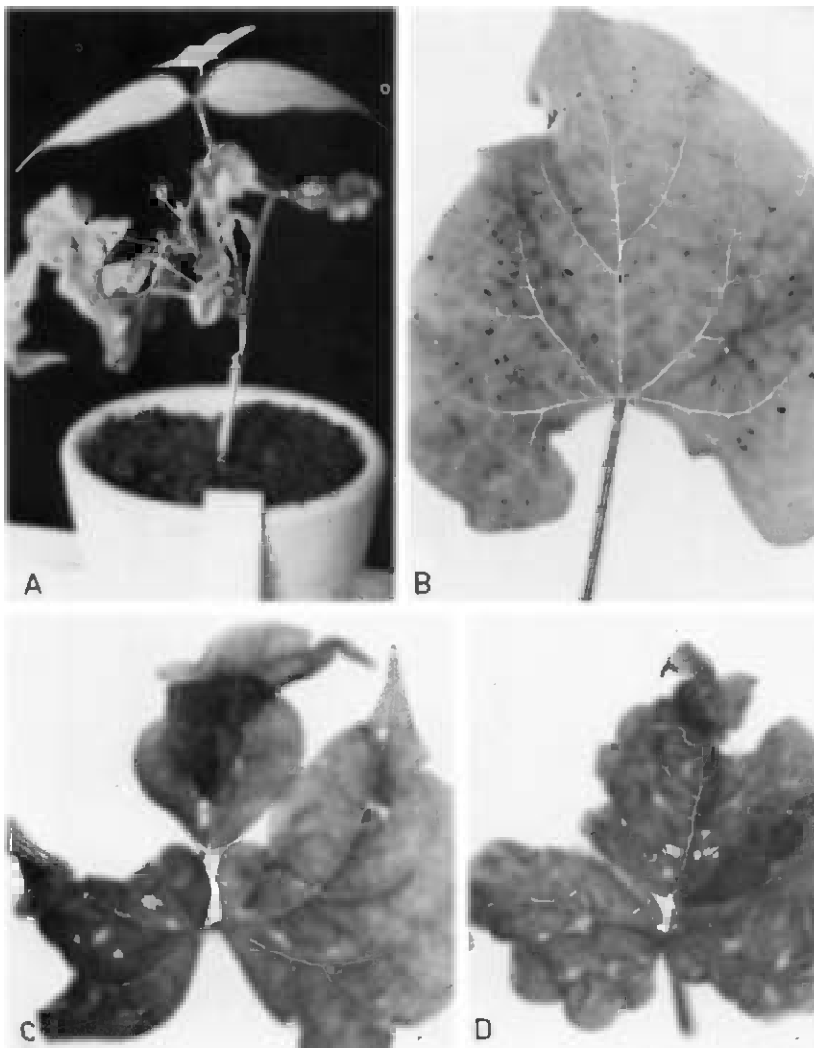

Fig. 1. Bean crumpling. A. Infected plant. B. Necrotic local lesions at the feeding punctures of the vector. C and D. Crumpled leaves showing chlorotic or necrotic lesions and the crumpling effect.

B. BEAN DWARF MOSAIC

This bean disease results from infection of the plants by the common strains of the *Abutilon* mosaic virus from malvaceous species that are usually infected among the weed vegetation in cultivated areas and mostly in pastures in Brazil. These weeds, species of *Sida,* have been recorded in all of Brazil, from Rio Grande do Sul to the Amazonas states (Silberschmidt and Tommasi, 1955). It is, thus, to be expected that infection of bean plants with the virus will occur anywhere they are planted in the Country.

Although the incidence of *Abutilon* mosaic among the weeds may be very high, the presence of dwarf mosaic is generally low and rarely will exceed 5% in bean plantings. Even in 1972, 1973, and early in 1974, a period in which there was an enormous increase in the vector population in cultivated areas in Southern Brazil, incidence of bean dwarf mosaic remained relatively low.

In a field test, a comparison of the yield of healthy and infected plants of 5 varieties was made. Potted plants started in the greenhouse were separated in 2 groups, one of them being exposed to a viruliferous whitefly population for 72 hours. The insects were killed and the inoculated plants and controls were set in the field. The total yield of 2 control plots of 10 plants each per variety was: Chumbinho, 687 g; Douradinho, 271 g; Miudo, 319 g, 109-3568, 724 g; and Pintado, 363 g. The yield of the same number of plants infected with dwarf mosaic was nihil.

Symptoms

Infected plants (Fig. 2 and 3) are generally dwarfed and tend to become bunchy or rosetted. The yellow spots in the older leaves are few and accompanied by a rolling downward of the leaflets. If the plant is infected after making some growth, the symptoms will be evident only in the new growth, it may have short and zig zag internodes and small leaves with greenish yellow mottle. Leaves well developed before infection remain normal or might even be larger than normal. Plants at this stage may resemble bean plants infected with the curly top virus in Western United States, except for the yellow mosaic spots in the younger leaves. Infected plants usually do not produce pods, but if these are present they may be malformed.

Etiology

Dwarf mosaic of beans is caused by the *Abutilon* mosaic virus found in native Malvaceae in Brazil. Attempts to determine the particle morphology of this virus, as well as that of other whitefly-transmitted viruses, have been made over a period of years in the Electron Microscopy Laboratory of the Virus Department at the Instituto Agronómico, Campinas. In partially purified preparations or in leaf dips, no particle could be consistently detected that was associated with infected material and not present in control preparations (E. W. Kitajima, unpublished; I. J. B. Camargo, unpublished). More recently, in ultrathin sections of *Sida* spp. leaves showing the yellow mosaic induced by *Abutilon* mosaic virus, the presence of isometric particles, 20-25nm in diatemer, was observed in the sieve tube elements, (Fig. 4), but not in those of normal plants (Kitajima and Costa, 1974).

Fig. 2. Bean dwarf mosaic. A. Field infected dwarf and normal plant. B and C. Abnormal sprouting of infected plants. C. Yellow mosaic spots on leaves.

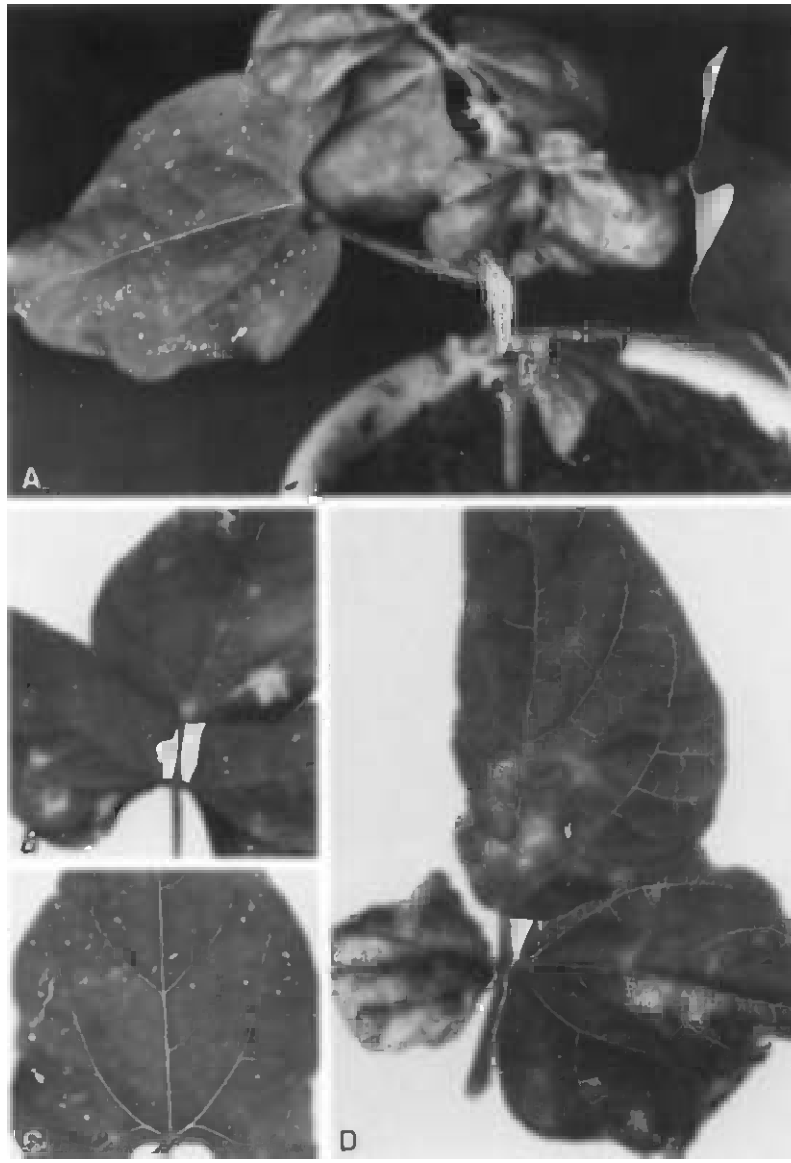

Fig. 3. Bean dwarf mosaic. A. Experimentally infected plant showing stunting. B and C. Chlorotic lesions at feeding points of the vector. D. Systemic yellow mosaic.

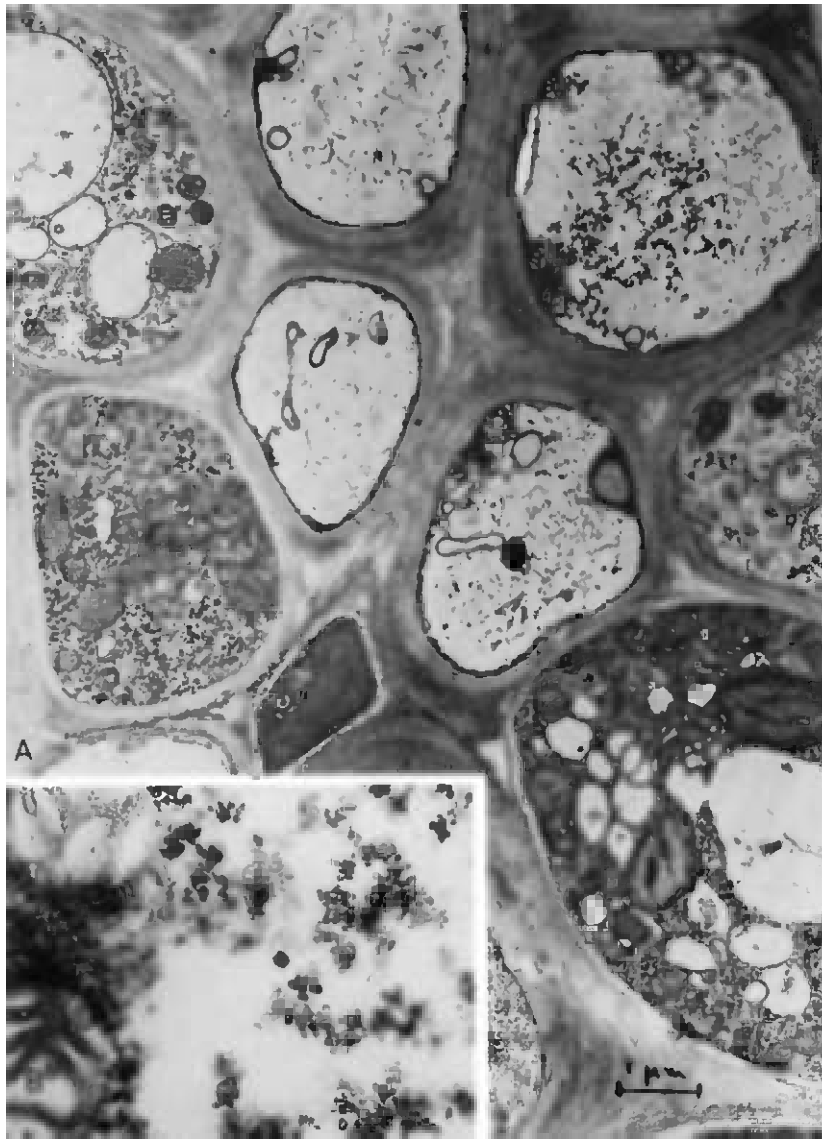

Fig. 4. Ultrathin sections of leaves of *Sida micrantha* infected with the *Abutilon* mosaic virus. A. Section of a leaf vein, showing virus-like isometric particles in a sieve tube. B. Enlarged detail of the sieve tube with particles (Kitajima and Costa, 1974).

Dwarf mosaic of beans can be reproduced easily when young plants are exposed to a *Bemisia tabaci* population bred in insectaries on diseased *Sida* plants. Inoculation by confining virus-carrying whiteflies on the bean test plants is generally less successful, unless a great number of insects per plant is used. Acquisition of the virus by non-viruliferous whiteflies fed on diseased bean plants is generally negative (Costa, 1965). Thus, field infection of bean plants is totally dependent on the presence of vectors that acquired the virus from infected malvaceous weeds as no spread from bean to bean occurs within the plantings.

Mechanical transmission of dwarf mosaic virus from bean to bean is practically negative. The virus, however, has been transmitted mechanically from *Sida micrantha* St. Hil. and *Malva parviflora* L. when the latter is used as a test plant (Costa and Carvalho, 1960a). This permitted the determination of the physical properties of the virus in crude preparations. Activity was still present in preparations heated for 10 min at 55°C, but not in those heated at 60°C; some preparations were still active at dilutions of 5^{-5}, but not when the dilution was 5^{-6}; preparations aged for 48 hr kept some activity, but none was detected in preparations aged for 72 hr (Costa and Carvalho, 1960b).

Practically all tested varieties of *Phaseolus vulgaris* behaved as susceptible to dwarf mosaic. Resistance was found in other species of the genus: *P. angularis, P. aureus, P. calcaratus,* and *P. trinervius* (Costa, 1965).

C. BEAN GOLDEN MOSAIC

This disease was first recorded in Brazil in 1961 and reported a few years later (Costa, 1965) as a minor disease of the bean crop in the State of Sao Paulo. It is now known to be present in practically all important bean growing areas of the Country (Minas Gerais, Paraná, and Sao Paulo) and was recorded also in the arid lands along the Sao Francisco River Valley (Costa, 1972). In the last few years, it has become a major disease of the bean crop and is now a limiting factor in the dry season plantings made in certain areas of Southern Brazil where they are started at the end of the growing season of most annual crops.

Symptoms

The disease is not generally found in very young plantings, unless viruliferous whiteflies are present in high numbers. Symptoms (Fig. 5) of infection become more noticeable when the plants have already developed 3 or 4 trifoliolate leaves. The first symptoms may be noticed as rolling downward of young leaves which later show the golden mosaic symptoms. These may be predominantly on the veins or involve large areas of the leaf parenchyma. For most bean varieties, there is little reduction of the leaf and plant sizes resulting from infection. Some varieties such as Chumbinho-Opaco, Opaco-Paraná, Opacao, Cara-Suja and others might show definite stunting and reduction in size of their parts. A certain degree of recovery might be shown by some plants after the initial shock symptoms. Pods develop on diseased plants and may present golden mosaic spots and be malformed. There is a definite reduction in yield of the infected plants, but the amount of losses has not yet been critically evaluated under field conditions. Observations indicate that the losses vary with the bean variety, age of the plant when infected, and strain of the virus.

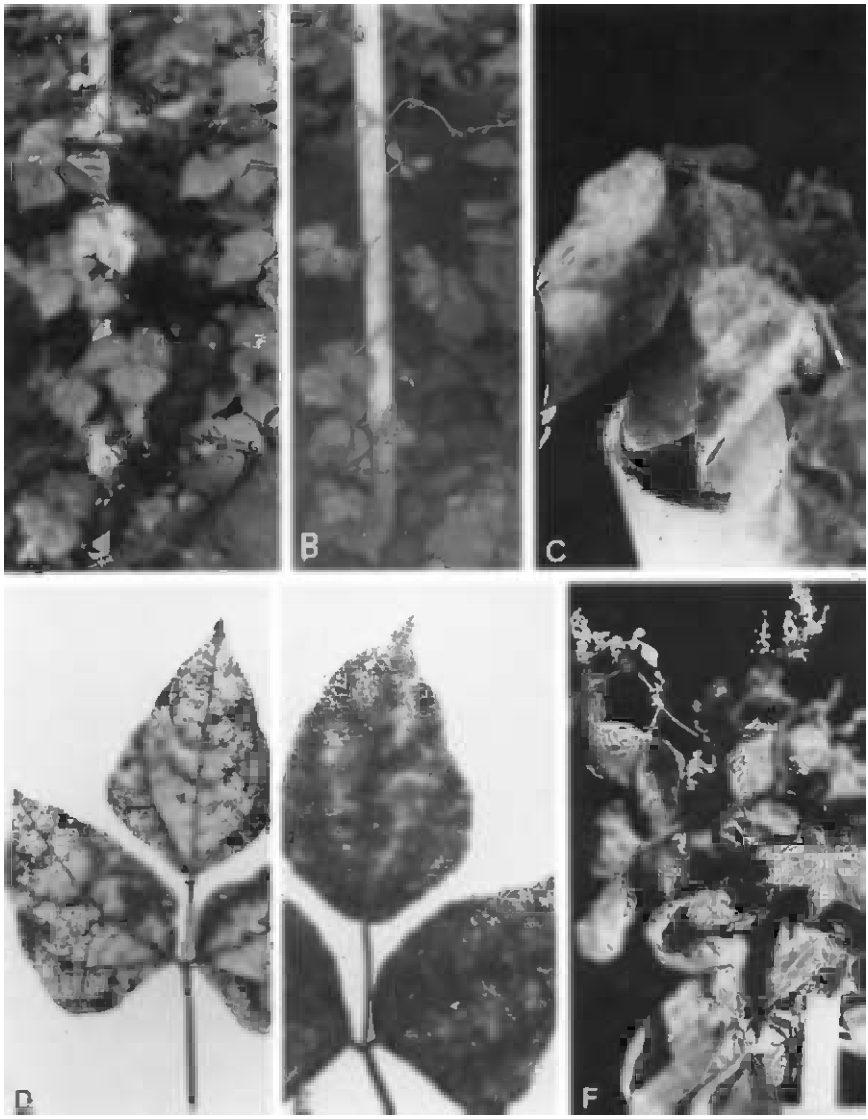

Fig. 5. Bean golden mosaic. A and B. Infected field pole bean plants. C. Dry bean variety infected experimentally. D and E. Leaves showing two patterns of golden mosaic. F. Bean plant infected with the mixture, golden mosaic and line pattern viruses.

Etiology

The causal agent of the golden mosaic disease of beans is assumed to be a virus and is considered to belong to the *Abutilon* mosaic virus complex. It probably represents an evolved variant of this complex that became adapted epidemiologically to certain species of the legume family.

Bean golden mosaic virus was described as causing yellow mosaic of *Phaseolus longepedunculatus* Mart. (Flores and Silberschmidt, 1966), a plant that had already been determined as one of the potential weed reservoirs of bean golden mosaic virus (Alves Lima, 1967). Those authors reported several additional species as host plants of the virus. Among the legume plants: jackbean *(Canavalia ensiformis* DC.), guar, *Cyamopsis tetragonolobus* (L.) Taub, *Desmodium aculeatum, Teramnus uncinatus,* and soybean (referred to as *Glycine hispida* Maxim.); in the Solanaceae, only tomatoes and *Nicandra physaloides* (L.) Gaert. were found susceptible. Some of these findings need confirmation, as the writer has so far been unable to infect soybeans or tomatoes with different isolates of the virus.

Species of the genus *Sida,* such as *S. rhombifolia* L. and *S. micrantha,* that are extremely susceptible to the *Abutilon* mosaic virus, behave as very resistant to golden mosaic virus. *Malva parviflora* is a host plant of the two viruses, reacting to bean golden mosaic virus with symptoms that may differ from those induced by the other virus (Costa, 1965; Flores and Silberschmidt, 1966).

Attempts to determine the particle morphology of this virus in partially purified preparations or in leaf dips gave negative results. In ultrathin sections of bean leaves infected with golden mosaic, the presence of isometric particles 20-25 nm in diameter was observed in the lumen of the sieve tube elements, but not in those of normal plants (Kitajima and Costa, 1974).

Bean golden mosaic from Brazil is not seed-transmitted. The virus probably remains in infested areas in such reservoirs as lima bean *(Phaseolus lunatus* L.), *P. longepedunculatus,* and probably other legume weeds. In areas where bean crops overlap, infected old plants are important reservoirs. Other sources are small back yard vegetable gardens in which dry or pole beans are grown several times a year. Attempts to transmit the virus by mechanical inoculation, using different techniques, buffers, and activators have failed to help transmission. Negative results were also reported by Flores and Silberschmidt (1966) in their attempts to transmit the same virus from *P. longepedunculatus* by mechanical methods.

Mixed Infection of Bean Plants with Golden Mosaic and Other Viruses.

In bean plantings where golden mosaic is very prevalent, the occurrence of mixed infections is obviously common. Two types of mixed infections have been recorded, the other virus components being common bean mosaic and line pattern mosaic (Costa *et al.*, 1972).

Bean plants infected with the mixture of golden mosaic and common bean mosaic viruses are very stunted and resemble plants infected with dwarf mosaic, with the difference that they may be much more frequent in the plantings. Those infected by the line pattern mosaic and golden mosaic viruses are not much reduced in size, but form more branches than normal with short internodes and small leaves and flowers (Fig. 5 F).

D. SOYBEAN CRINKLE MOSAIC

Crinkle mosaic is a minor soybean disease. Its incidence in soybean plantings in Sao Paulo and Paraná has not been determined accurately because of the difficulties involved in diagnosing the disease in the field or testing for the virus experimentally. Potentially, crinkle mosaic may become important in areas where the presence of *Abutilon* mosaic virus in Malvaceae is abundant and there is a high density of the vector.

Losses resulting from crinkle mosaic infection were determined experimentally, comparing the yields of infected plants and comparable controls of 13 varieties. Potted plants were infected at an early stage by exposing them to populations of viruliferous whiteflies and then were transplanted along with the controls to experimental plots. When compared with the non-infected controls, yields of infected plots with 100% infection were 13 to 87% lower (Table I). These results probably represent a maximum loss never equalled in the plantings because all test plants were infected when very young.

TABLE I
YIELD LOSSES INDUCED BY INFECTION[1] OF SOYBEAN PLANTS WITH CRINKLE MOSAIC

Soybean variety	Total yield of 10-15 plants (g)		Percentage yield reduction
	Healthy controls	Infected plants	
1. Abura	215	150	30.2
2. Abura (flor branca)	385	116	69.9
3. Acadian	205	130	36.6
4. Aliança	245	52	78.8
5. Aliança Preta	285	35	87.7
6. Cotia 14	255	95	62.7
7. I. A. 75	192	166	13.5
8. I. A. 220	250	194	22.4
9. I. A. 455	83	65	21.7
10. L. 411	380	75	80.3
11. L. A. 411219	505	83	83.6
12. Paraná tardia	114	80	29.8
13. Yelnando	400	215	46.2
TOTAL	3,514	1,456	58.6

[1]Soybean plants infected by exposing them to a viruliferous population of *Bemisia tabaci* bred on *Sida* spp. plants infected with the *Abutilon* mosaic virus.

Symptoms

Affected plants are reduced in size in varying degrees, depending upon variety. The leaves may be smaller and show mosaic and crinkle symptoms. Soybean plants infected with crinkle mosaic at late stages are practically impossible to be distinguished from those infected with common soybean mosaic or by the yellow bean mosaic virus, in the field.

Etiology

Crinkle mosaic results from infection of soybean plants with the *Abutilon* mosaic virus as in the case of bean dwarf mosaic virus. Of several legume crop plants that were exposed to infection in insectaries where a population of viruliferous whiteflies was present, soybeans were the most susceptible and gave 100% infection (Table II). Although soybean plants are easily infected by the vector, recovery of the virus from soybean is difficult and transmission from soybean to soybean is generally negative. Attempts to infect soybeans by mechanical inoculation have failed, as well as tests to recover the virus from vector infected soybean plants by mechanical inoculation on adequate test plants.

TABLE II
EFFECT OF INOCULATING[1] VARIOUS LEGUMES WITH THE *ABUTILON* MOSAIC VIRUS

Species of legume crop	Number of plants		Percentage of infection
	Inoculated	Infected	
	No.	No.	%
1. Peanut *(Arachis hypogea L.)*	227	9	4.0
2. Bean *(Phaseolus vulgaris)*	272	260	95.6
3. Lima bean *(P. lunatus)*	20	8	40.0
4. Jackbean *(Canavalia ensiformis)*	36	21	58.3
5. Guar *(Cyamopsis tetragonolobus)*	30	19	63.3
6. Lentil *(Lens culinaria* Medik)	45	38	84.4
7. Soybean *(Glycine max)*	330	330	100
8. Lupine *(Lupinus albus* L.)	20	4	20.0

[1]Test plants were inoculated using viruliferous whiteflies bred on diseased *Sida* spp.

E. SOYBEAN DWARF MOSAIC

Soybean plants are less susceptible to dwarf mosaic than to crinkle mosaic. Data are not available on its occurrence in different soybean growing areas of Brazil, but the incidence seems lower than that of crinkle mosaic in Sao Paulo. Its economic importance at present is negligible.

Symptoms

The symptoms induced by this whitefly-transmitted disease resemble those of crinkle mosaic. However, the mosaic symptoms and the stunting effect of dwarf mosaic on soybeans are stronger than in the case of crinkle mosaic.

Etiology

Dwarf mosaic of soybeans is produced by infection with the *Euphorbia* mosaic virus. As in the case of crinkle mosaic, the vector has to acquire the virus from infected *E. prunifolia* to be effective in transmitting it to soybeans. Acquisition from soybeans is practically always negative. The virus is not seed-borne. It passes mechanically with great difficulty when the inocolum is obtained from or used to inoculate the soybean plant.

Since the disease is of little importance no attempt has been made to screen varieties for resistance to dwarf mosaic.

III. Relationship Between Whitefly-transmitted Bean and Soybean Diseases from Different Countries

Bean golden mosaic virus from Brazil was considered by Costa (1965) to be possibly related to the virus that causes yellow mosaic of lima beans in India, described by Capoor and Varma (1948). Mung bean yellow mosaic virus, as studied by Nene (1972), seems to be a different virus, as it is not infectious to most varieties of beans and does not infect lima beans. Yellow mosaic of *Dolichos lab-lab* (Ramakrishnan *et al.*, 1973), also caused by a whitefly-transmitted virus, seems to be different from bean golden mosaic virus.

A golden mosaic of beans reported from El Salvador, C.A. by Gámez (1971) was considered by this investigator as similar to the Brazilian disease. Bird *et al.*, (1972) also pointed out that golden yellow mosaic of beans in Puerto Rico, a disease caused by a virus that produces the same type of symptoms on lima beans, resembles golden mosaic of Brazil. The relative similarity in host range of these viruses, their virus-vector-relationships, and other properties, as they have been studied, also indicate that they are probably related. An important difference, however, has been reported recently concerning the golden mosaic virus from El Salvador that possibly makes it distinct from the other viruses mentioned above. Golden mosaic has not been transmitted mechanically in Brazil so far (Costa, 1965; Flores and Silberschmidt, 1966; Costa, 1974). Bird *et al.*, (1972) also failed in transmitting mechanically the lima bean golden yellow mosaic virus from Puerto Rico. However, the bean golden mosaic disease from El Salvador is apparently transmissible by mechanical inoculation (Meiners *et al.*, 1973). Of course, this difference may be merely due to different techniques employed by the various investigators that studied the diseases.

The group of whitefly-transmitted disease agents seems to be very plastic in nature (Costa and Kitajima, 1974). Thus, the presence of similar diseases in Brazil, El Salvador, and Puerto Rico might not represent the same virus that was distributed to these areas. They could be strains evolved in a parallel manner from natural complexes that already existed in these areas, that became host-adapted to beans, lima beans, and other legume species. Of course, if seed transmission of the golden mosaic virus could be demonstrated to occur in case of one of the susceptible crops or weeds, and especially in the former instance,

then the possibility of all these viruses being identical or closely related would be greater because of its possible distribution to different countries in this manner. Seed transmission, however, has been negative in the tests reported so far (Costa, 1955).

Bean dwarf mosaic in Brazil is induced by virus strains of the *Abutilon* mosaic virus complex commonly found on *Sida* species. In Puerto Rico, species of this genus are also infected with a whitefly-transmitted virus that possibly belongs to the same complex. However, a bean disease similar to dwarf mosaic of Brazil that occurs in Puerto Rico is not caused by the virus strains from *Sida* spp., but by another whitefly-transmitted virus from *Rhynchosia minima* (L.) DC. (Bird and Sánchez, 1971; Bird *et al.*, 1972). In Puerto Rico, beans seem to be resistant to the virus from *Sida* and in Brazil, a non-identified species of *Rhynchosia*, possibly *R. minima* could not be infected with the *Abutilon* mosaic virus from *Sida*. These comparison would suggest that dwarf mosaic of beans in Brazil is different from the similar bean disease of Puerto Rico. This is also supported by the fact that the bean disease in Puerto Rico seems to reach a higher incidence in the plantings than the Brazilian disease. This fact would indicate that the causal virus in Puerto Rico may pass from bean to bean by means of the vector. This does not occur in Brazil to any appreciable extent.

Abutilon and *Euphorbia* mosaic viruses infect the soybean in Brazil, inducing crinkle and dwarf mosaics, respectively. Neither disease is similar to a yellow mottle of soybean reported from India, caused by the mung bean yellow mosaic virus (Nene, 1972). This last virus can be acquired by the vector from infected soybean plants (Nene, 1972). They are also different from a soybean yellow mosaic transmitted by *Bemisia tabaci* in Colombia that passes from soybean to soybean (G. E. Galvez, personal communication). Acquisition of the viruses from soybean in the case of the two diseases in Brazil is extremely poor.

IV. Increase in Populational Density of *Bemisia tabaci* in Brazil

Although quite widespread among weeds and cultivated plants in Brazil, populations of this whitefly never attained high densities in the past. An exception was the high infestation of cotton plants recorded in the end of 1967 and early in 1968 in the localities of Sao Pedro do Ivai and Monte Castelo in the State of Paraná (G. B. Gonçalves, personal communication). An outbreak of bean golden mosaic also occurred in Cruzeiro-do-Sul, PR. in 1971, associated to an increase of the whitefly populations (H. F. G. Sauer, personal communication). In the last few years, and especially in the first quarter of 1973 and 1974, extremely high populations of *Bemisia tabaci* were recorded among cultivated plants and weeds in Northern Paraná and neighboring parts of Southern Sao Paulo. The whitefly populations were particularly dense in soybean plantings, especially in the late ones. The enormous population of the insect bred on soybeans led to a general infestation of many other crops and weeds.

Bemisia tabaci is presently considered an important pest for the soybean crop in some areas of Paraná and Sao Paulo. Damage induced by the insect is of various types: (a) as phloem feeders, the young forms and adults compete with the plant for synthates; (b) the great number of insects feeding on the leaves must destroy or impair numerous cells at the feeding puncture sites and in the trajectory of their stylets in the leaf tissues; (c) the insect may be slightly toxicogenic to soybean, inducing a generalized vein clearing; (d) they act as

vectors of viruses and may even spread bacteria and fungi; (e) the excretions of great number of insects permits the development of sooty mold.

The rather rapid increase in the soybean acreage that took place in areas of Paraná and Sao Paulo in the last few years is considered as the main factor that led to the abnormal increase in population density of *Bemisia tabaci* in the same areas. The soybean plant is an excellent feeding and breeding host of the insect and the rather wide planting period from September to January permitted the breeding of various successive generations of the whitefly.

Evidence that the soybean was the main factor responsible for the increase in the whitefly population is based mostly on sampling of the crops and visual observations. Leaves of late planted soybeans were so heavily infested on their underside with the nymphal instars of the insect (Fig. 6 A) that practically no free space could be found by many of the crawling first instar insects that in many instances were found fixed on the upper side of the leaf, a fact never recorded when the population of the insect was low. Observations showed a good correlation between the presence of soybeans and high whitefly populations on other crops and weeds. Also, diseases transmitted by them were more prevalent on weeds or on crops planted in areas where the soybean was present than on the same plants in other areas where it was not.

It is also recognized that environmental factors must have played a favorable role for whitefly increase in that last few years in Brazil, as the population of other species, other than *Bemisia tabaci*, became also more dense. This was the case of one of the citrus whiteflies, *(Aleurothrixus floccosus* Mask.), that became prevalent in some citrus orchards, a fact never recorded before. Also, some of the cassava whiteflies, *A. aepim* (Goeldi) and *B. tuberculata* Bondar, have been more prevalent in the plantings in recent years.

The increase in the whitefly population brought about by soybean plantings in the case of *Bemisia tabaci* has been particularly bad for the bean crop planted in January, February or March, called the dry season planting. These get started when the soybean plantings are approaching maturity or turning senescent, becoming thus an excellent food and breeding host for the migrating whiteflies that leave the soybean fields. *B. tabaci* feeds and breeds well on beans (Fig. 6 A and B).

V. Migration of *Bemisia tabaci* in Areas of High Population Density

This whitefly species was reported as an insect that does not migrate far, although it could be transported to long distances by wind currents (Varma, 1963). Nene (1972) reported results that indicate movement of this vector to distances of at least 300 feet and that it can ascend 40 feet in the air.

Migration habits of *Bemisia tabaci* are not expected to be greatly modified by population density, but they are easier to follow when the insect is abundant. The high population density reached by this species in areas entirely planted to soybeans in Paraná permitted the observation of some migrating facts concerning the insect. Flocks of this whitefly were noticed flying in the soybean fields and vicinities during the warm hours of the day. In small towns surrounderd by soybean fields (Sertanópolis and others), flocks of the insect could be seen moving along the streets when they passed in front of a dark background, such as that represented by an open store door not well lighted inside. Since the distance that these insects had to travel from the plantings to reach the towns

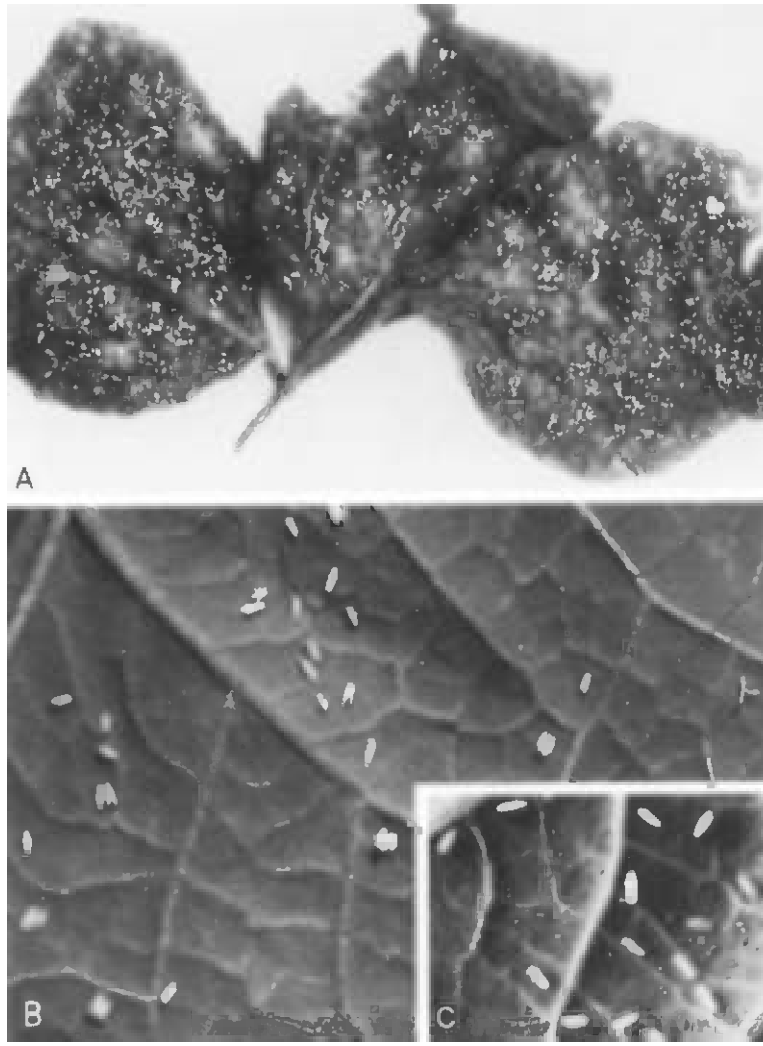

Fig. 6. *Bemisia tabaci* on soybean and beans. A. Soybean leaf viewed on the underside, showing heavy infestation of nymphs and adults, representative of late plantings in the State of Paraná. B. Paired whiteflies on bean leaves. C. Female whiteflies laying eggs on bean leaves.

amounted to a few kilometers, it may be inferred that flying on their own or helped by wind currents, this important vector moves farther than is generally recognized.

VI. Pairing-off of Male and Female Whiteflies

Observations of whitefly colonies at Campinas, have shown that male and female *Bemisia tabaci* have a tendency to pair off when feeding or sitting on leaves (Fig. 6 B). Male insects are generally smaller than females, thus it is easy to distinguish the different pairs.

An attempt has been made to mark different pairs of the whiteflies in order to release them together in a cage for further observation as to whether or not the same components of the pair would pair off again. Some of the food dyes were tried as markers, but none colored the insects in an adequate manner to permit identification.

VII. Control of Whitefly-transmitted Virus Diseases

Control of bean golden mosaic by the use of resistant varieties, highly productive and suitable to the various market preferences, seems still far away. All varieties of *Phaseolus vulgaris* that have been tested in Brazil (over 350) were found susceptible. Gámez (1971) reported testing more than 4,000 bean varieties and found all susceptible to the golden mosaic of El Salvador. He points out that resistance to golden mosaic has not been located in species of *Phaseolus* from the Western Hemisphere that are easily crossed with *P. vulgaris*. Only in Asiatic species of the genus was he able to find resistance, but these are incompatible in crosses with *P. vulgaris*.

Until varieties resistant to golden mosaic and other whitefly-transmitted virus diseases can be developed, control measures for them have to depend on isolation of plantings, eradication of plant virus reservoirs, and destruction or repellency of the vector. Resistance to the vector has also to be considered.

Field observations have indicated that in Brazil, dry season bean plantings located far from soybean fields suffered only slight losses from golden mosaic, whereas those near areas where this crop was very extensive were practically 100% infected. Soybeans act only as breeding host plants for the vector, since no golden mosaic virus has been recovered from them so far. Golden mosaic is also less important in the rainy season planting, because the whitefly population that becomes very low during the dry and cool winter months does not reach high levels during the relatively short growing period of the crop in the spring.

Delaying the dry season bean planting may reduce golden mosaic infection in Southern Brazil, as the whitefly population decreases rapidly when the average temperature falls. However, these delayed plantings might run into problems such as low soil water content and danger of frost. In some of the bean growing areas of Sao Paulo, growers plant beans several times a year, so that been plantings overlap. This practice is conducive to a carry over of the vector and virus, resulting in most cases in a higher incidence of bean golden mosaic. A bean free period, between two successive plantings, would eliminate the virus reservoirs represented by infected plants in the older planting.

Eradication of plant virus reservoirs is usually a difficult control measure to be applied. In many cases, the plant that act as virus reservoirs are quite wide-

spread in the crop land areas, making such eradication unfeasible. Besides *Phaseolus longepedunculatus*, no other plant of the weed vegetation has been found by the writer that is susceptible to bean golden mosaic virus. *Desmodium acculeatum* and *Teramnus uncinatus* were found susceptible by Flores and Silberschmidt (1966). *P. lunatus* is usually infected when growing in vegetable gardens or fences and might be one of the species that carry over the virus between periods when there are no bean plantings. The writer is, however, of the opinion that there must be another bean or weeds that are widespread in the crop land areas and serve as golden been mosaic virus reservoirs.

Chemical control of the vector by the application of insecticides might be of little value in cases where viruliferous vectors move in great numbers from outside into the plantings. In case of golden mosaic in Brazil, most of the disease spread is within the planting. A reduction of the whitefly population in the planting might lend to a decrease in the incidence of this disease, but experimental field data are not available yet.

Adult whiteflies are easily killed by insecticides. Eggs and nymphs of the insect are more difficult to destroy. In a series of tests, various types of insecticides were tried on nymphal instars and adults of *Bemisia tabaci* reared on potted soybean plants raised in insectaries (Suplicy *et al.*, 1974). The insecticidal effect of the different treatments on the young forms of the whitefly was calculated by the number of live nymphs in relation to total number present on leaf samples of treated plants and controls. The best insecticides are listed in Table III.

TABLE III
MORTALITY RATES OF PRE-ADULT INSTARS OF *BEMISIA TABACI* FOLLOWING ONE SPRAY APPLICATION OF DIFFERENT INSECTICIDES (SUPLICY *ET AL.*, 1974).

Common name	Trade name	Percentage of commercial insecticide in spray	Percentage mortality rates
Azimphos ethyl	Gusathion A 40 EC	0.1	93.6
Cytrolane[1]	Cytrolane 250 E	0.5	94.3
Dicrotophos	Bidrin 50 S	0.15	98.8
Dimethoate[1]	Rogor 50 EC	0.2	89.6
Malathion	Malatol 50 EC	0.2	86.5
Methidation	Supracide 40 EC	0.15	98.3
Methomyl.	Lannate 90 WP	0.1	98.3
Mevinphos	Fosdrin EC 24	0.15	97.3
Monocrotophos	Azodrin 60 EC	0.125	92.4
Monocrotophos (10%) + Camphechlor (40%)	Azodrin - Toxafeno EC	0.5	99.8
Monocrotophos (17%) + Endrin (8%)	Afidrin 25 EC	0.25	93.0
Monocrotophos (15%) + Endrin (20%)	Azodrex 35 EC	0.375	95.3
Ortho Hamidop	Monitor 50 EC	0.1	90.9
Protheate	Fostion 60 EC	0.125	99.3

[1]Toxic effect on test plants.

Control of virus diseases transmitted by *Bemisia tabaci* by the application of insecticides have been tried in India by Nene (1972) and by Ramakrishnan *et al.*, (1973). The later investigators carried out several trials to test different insecticides for the control of *Bemisia tabaci* in attempts to reduce the incidence of yellow mosaic in *Dolichos lab-lab* plantings. They were unable to assess the comparative value of the insecticides tried because the vector populations in the experiments were extremely low.

Nene (1972) tried systemic and non-systemic insecticides including mineral oil, to control *Bemisia tabaci* on urd bean plants. This investigator was particularly interested in determining the effectiveness of the insecticides in killing rapidly the vector, reasoning that fast killing would reduce the chances of acquisition and inoculation of mung bean yellow mosaic virus. Testing was done by applying the insecticides on 3-week old urd bean plants, waiting for the preparation film to dry on the plant (about 1 hr.) and then confining 50 adult whiteflies per plant and making mortality observations at intervals. Apparently his best results were obtained with mineral oil (an emulsifiable mineral oil called "Orchard Oil", applied at a 2% rate). This oil killed 100% of the adult population between 15 and 30 minutes after application. Mineral oil was also very effective for killing (100%) whitefly eggs on leaves of caged cotton plants. For the control of *B. tabaci* on soybeans, urd beans and other plants, Nene (1972) recommends two spray formulations:

1. 0.1% Thiodan (endosulfan) + 0.1% Metasystox (oxydemeton - methyl) + 2% orchard oil;
2. 0.1% Malathion + 0.1% Metasystox + 2% orchard oil.

Studies on the olfactory and color reactions of *Bemisia tabaci* were made by Mound (1962). He determined that this insect is attracted by the blue/ultraviolet and yellow parts of the spectrum. He also found that this whitefly has no easily detectable olfactory reaction.

Mound's results suggest two possible lines of approach to control *Bemisia tabaci*: (1) the use of attractive color traps or barriers; (2) the use of backgrounds or barriers of a color that is repellent to the insect.

Attractive color traps or trap plant barriers sprayed with insecticides that would collect and kill winged whiteflies do not offer great hopes in areas where there is a dense population, but might help in controlling the insect when in low numbers. The possibility of using backgrounds of repellent colors might promote some whitefly control, reducing the number of insects that alight on the plants and, consequently, reducing virus infection as in the case of aphids (Smith *et al.*, 1964; Johnson *et al.*, 1967; Smith and Webb, 1969; Costa, C. L., 1972). Barriers (artificial or natural) of repellent colors placed around plantings could also be tested.

A saw dust mulch reduced considerably the population of *Bemisia tabaci* on tomato seedlings in the seed bed (Avidov, 1957). Oviposition on the two cotyledons of control plants in unmulched seed-beds, 8 days after germination, averaged 62.2 per seedling and was nihil on seedlings in treated plots. This investigator attributed the controlling effect of the sawdust mulch on the whitefly population to an increase in air temperature in a 10 cm layer immediately

above the mulch, where the seedlings were. The temperature in this layer reached at times 47-51°C, a range that is higher than the lethal temperature for the whitefly (46-47°C). The temperature in the same air layer above the untreated plots did not exceed 42-45°C.

Nitzany *et al.*, (1964) reported that straw-mulching areas planted to cucumbers had a repellent effect on *Bemisia tabaci* and delayed the appearance of a disease of this crop caused by the bottle gourd mosaic virus, vectored in a non-persistent manner by this whitefly. The effect of the straw mulch on the whitefly is not discussed by these authors who only called it a repellent one. Apparently, other investigators misunderstood from the paper, that quotes results and interpretations of Avidov (1956), that Nitzany *et al.*, (1964) also attributed the effect of the straw mulch to the increase in air temperature above it (Smith and Webb, 1969; Costa, 1972). In both papers, these authors suggested, what is very likely to be the case, that the effect of mulch on the whitefly could be a matter of reflected color repellency. It seems to the writer, however, that Nitzany *et al.* (1964) did not commit themselves as to what the effect of the mulch on the whitefly was.

VIII. Discussion

Whitefly-transmitted viruses have been known to cause important diseases in Asia for many years (Thung, 1932; Pal and Tandon, 1937; Capoor and Varma, 1948) and also in Africa (Kirkpatrick, 1931; Storey and Nichols, 1938; McClean, 1940). They seem to be of economic importance for various legume crops particularly in India (Nariani, 1960; Varma, 1963; Nene, 1972; Ramakrishnan *et al.*, 1973). In the Western Hemisphere, they have been increasing in importance for legume crops and other economic plants in recent years. In Brazil this has also been true and this fact has been attributed to an abnormal increase in the populational density of the whitefly vector, *Bemisia tabaci*.

The main reason advanced to explain this sudden populational increase of the whitefly vector in Brazil has been the greatly augumented soybean acreage and especially the extended sowing period that has been used by growers, from September to January. Since the soybean is an excellent food and breeding plant for *Bemisia tabaci* under Brazilian environmental conditions, this expanded planting period in the same areas permits the breeding of several successive generations of the vector. The possibility that new and more prolific races of the insect have developed has been considered.

The whitefly-transmitted diseases of soybeans in Brazil have been of little importance so far. The two described diseases, crinkle mosaic and dwarf mosaic, are caused by viruses transmitted only by vectors that acquire the virus from the natural weed reservoirs and are mostly unable to do it efficiently when feeding on infected soybean plants. This greatly reduces virus spread within soybean plantings, in spite of the fact that the insect reaches high population density on this crop. It is to be expected that the increased soybean acreage, the high density of *Bemisia tabaci* in soybean fields and weeds, and the high plasticity of the whitefly-transmitted viruses will sooner or later lead to the appearance of a variant strain of one of the natural virus complexes that might become epidemiologically adapted to infect the soybean plant, be easily acquired by the

vector from it, and remain from season to season in weed reservoirs or other crop plants. In India, a whitefly-transmitted yellow mosaic of soybean, apparently of economic importance, is known to occur. It is caused by the same virus that infects mung and urd beans in the country (Nariani, 1960; Nene, 1972). This virus can be acquired by *Bemisia tabaci* from soybeans and also infects the plant with relative facility.

If a new virus variant, transmitted efficiently from soybean to soybean by the vector appears and becomes established in this crop in Brazil, then losses to growers could become staggering as in the case of golden mosaic in dry season plantings of beans.

Chemical whitefly control in soybean plantings when the population is high has been rather difficult and expensive. The writer has been advising as one of the contributing control measures the narrowing of the sowing period, as the observational evidence has indicated that it is mostly on the late plantings that the whitefly population reaches high density. If the growers were also to sow all the soybean within a period of 6 to 8 weeks, it would be highly probable that the problems associated with the whitefly as a pest or vector would be considerably minimized, with further benefit for the next dry season bean crop. It is obvious that the application of insecticides might become a necessary complement as a control measure, but the number of applications could be reduced and the efficiency of the treatments would perhaps be greater.

Some natural enemies of *Bemisia tabaci* have been reported elsewhere (Nene, 1972) and have also been noticed in Brazil. It is possible that some of the natural enemies of this whitefly in Brazil are not so widespread yet, but will eventually become so and help in reducing the abnormally great populations that have been present in recent years. Studies along this line would be of great interest for certain areas of Brazil.

Whitefly-transmitted diseases are considered by many to be of a viral nature. However, conclusive evidence is still lacking. The writer has used the name virus in the present paper, as it would be difficult and cumbersome to avoid it by the use of terminology such as disease agent, and so forth.

The morphology of the disease agent transmitted by whiteflies has not been established. The subject was reviewed briefly by the writer (Costa, 1969) and more recently by Kitajima and Costa (1974). Reports on possible types of particles that might represent viruses transmitted by whiteflies are those of Sharp and Wolf (1949) that claimed having purified tobacco leaf-curl virus from Venezuela and determined that it was an isometric particle 39 nm in diameter. Sun (1964) examined ultrathin sections of *Abutilon striatum* var. *thompsonii* leaves infected with the *Abutilon* mosaic virus and reported the presence of spheroidal particles 80 nm in diameter in the cytoplasm. They were considered to represent the virus particle and consisted of an inner core about 16 nm in diameter, sorrounded by an outer envelope. Kitajima and Costa (1974) reported that isometric particles 20-25 nm in diameter could be found in the lumen of the sieve tube elements in ultrathin sections of several species of plants infected with whitefly-transmitted viruses. The particles were not seen in parenchyma cells, but this might be the result of their being difficult to distinguish from normal cell particles, especially if they occur in low concentration in them.

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DISEASES TRANSMITTED BY *BEMISIA TABACI* IN EL SALVADOR

Carlos Granillo, A. Díaz,
M. Anaya, and L. A. Bermúdez de Paz

I. Introduction

Whitefly-transmitted viruses cause serious problems for crops in El Salvador. Differential emphasis has been given in recent years to diseases spread by the whitefly *Bemisia tabaci* Genn. Little was known about these diseases before 1960 and published reports were few. About 1960 there were heavy epidemics in the coastal areas of the country mainly in cotton *(Gossypium hirsutum* L.), kenaf (*Hibiscus cannabinus* L.), and beans (*Phaseolus vulgaris* L.). Observed symptoms on cotton are: poorly developed leaves (small, and sometimes exhibiting an intense mosaic), deformed buds, shortened stems, and in most cases stunting of the plants.

Kenaf is a fiber utilized in the manufacture of sacks. With the worldwide paper shortage, this plant is becoming very important since the stems can be utilized in the paper industry at a cost lower than that for trees. Since the time commercial kenaf culture was initiated, a disease which we have called kenaf mosaic has been observed. Diseased plants exhibit a vivid golden mosaic, the effect on yield is not known.

Beans *(Phaseolus vulgaris)* are not widely grown in the coastal areas, but in variety trials performed at the Santa Cruz Porrillo Experiment Station a high incidence of golden mosaic was observed. In *P. acutifolius,* known as white sweet bean, we have observed a high incidence of a disease which is probably golden mosaic.

Some wild plants found in the areas surrounding kenaf, cotton and sweet bean fields showed symptoms similar to those described above, these plants were *Sida* sp., *Wissadula amplissima, Malachra* sp., *Calopogonium* sp., and others.

The development of epidemics coincide with the resurgence of high populations of *B. tabaci,* a whitefly reported as an excellent vector of plant viruses. In order to study the relationship of the above diseases and compare them with those described from other places, a series of investigations were initiated. The results are given below.

II. Establishment of Colonies

Colonies of whiteflies used for this study were reared on healthy sweet potato plants *(Ipomoea batatas).* The colonies were kept in an insectary separate from the greenhouse. Plants used in all of the experiments were grown in cans, using soil treated with methyl bromide. Temperatures in the greenhouse ranged from 19° to 29°C. The host plants were placed in cages lined with organdy cloth to isolate them from arthropods other than whiteflies. Leaves from apparently healthy cotton plants, with nymphs in the last instar stage, were collected and placed on damp paper inside the cages. Good emergence of adults was obtained and virus-free colonies secured. In order to further ensure that colonies were free of virus, groups of flies were transferred to healthy plants of cotton, kenaf, and *Sida* and maintained on them for 48 hours. None of the plants developed symptoms of disease.

III. Isolation and Transmission of the *Sida* and Kenaf Viruses

Virus isolates for this study were obtained from *Sida* and kenaf plants infected naturally in the area of Santa Cruz Porrillo. The plants were transplanted to clay pots and maintained in the greenhouse.

Extracts from diseased kenaf leaves were prepared in a mortar with 0.05 M phosphate buffer, pH 7.1. The plants were dusted with carborundum, 400 mesh, prior to inoculation. Mosaic of kenaf was not successfully transmitted by mechanical means.

Seed secured from infected plants were germinated in the greehouse and the resulting progeny observed for a period of 60 days. None of the plants developed symptoms of mosaic. This indicates that the causal agent is not seed-transmitted.

In the kenaf mosaic virus transmission trials with the vector *B. tabaci,* acquisition and inoculation feeding periods ranged between 24 and 28 hours. The virus was transmitted by the vector to kenaf "Guatemala 51", cotton "Stoneville 7A", *Datura stramonium, Wissadula amplissima,* and *Malva parviflora.* Other inoculated plants which showed no symptoms, nor from which the virus could be recovered were: *Petunia hybrida, Nicotiana tabacum, N. glutinosa, Chenopodium amaranticolor, Rhynchosia* sp., *Capsicum annuum, Helianthus annuus, Phaseolus vulgaris* and *Sida* sp.

Several affected *Sida* plants were tested as sources of virus, using the acquisition and inoculation periods described above. Kenaf plants failed to develop mosaic symptoms. Some of the inoculated plants developed a few yellow dots, which appeared 15 days after colonization. It was possible to transmit the pathogen from *Sida* to *Sida* and to cotton. The symptoms observed on cotton were different from those observed on the plants inoculated with the kenaf agent. It was possible to transmit the causal agent to *P. vulgaris* "27-R", although the rate of infection was low.

IV. Discussion

It has been demonstrated in this study that the whitefly *Bemisia tabaci* transmits the etiologic agents which cause diseases of cotton, kenaf, *Sida* and beans. Results suggest the causal agent of the disease which we call "kenaf mosaic" is the same which causes the disease known as "cotton mosaic".

However, this kenaf agent is apparently different from the one which causes infectious chlorosis of Malvaceae and bean golden yellow mosaic. The symptoms incited by the infectious chlorosis agent on cotton and beans in the greenhouse are not frequently encountered in the field. More studies are needed to properly characterize these whitefly-transmitted disease agents.

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OBSERVATIONS ON THE GOLDEN MOSAIC OF BEAN *(Phaseolus vulgaris* L.) IN JAMAICA

R. E. Pierre

I. Introduction

Whiteflies, as vectors of plant viruses and virus-like agents, have assumed prominence only within relatively recent times but the diseases which they transmit appear to be of tremendous importance particularly in tropical areas. Varma (1963), for example, has briefly described some 28 whitefly-transmitted diseases most of which are from various parts of the tropics. Beans are susceptible to a number of whitefly-transmitted diseases. Costa (1965) reported the occurrence in Brazil of three whitefly-transmitted bean virus diseases known as golden mosaic, crumpling and mottled dwarf. Schieber (1970) noted the presence of golden mosaic in the Dominican Republic and this disease also has been reported to be widespread in the coastal pacific plains of Central America (Gámez, 1971).

Golden mosaic undoubtedly is the most important limiting factor in bean production in the lowland areas of Jamaica and attempts to solve this problem are reported herein.

II. Symptoms of Golden Mosaic

Costa (1965) reported the lack of occurrence of symptoms of golden mosaic on young bean plants in Brazil. In Jamaica, however, symptoms have been observed on young plants in the field as early as 14 days after planting. On these plants the first trifoliolate leaves generally are bright yellow in colour, somewhat reduced in size and often cupped. When early infection occurs, plants generally are stunted.

On older plants, a range of symptoms may appear on a single plant ranging from predominantly green with blotches of yellow on older leaves to an almost

completely bright yellow colour of the younger leaves. Periodically leaves with a yellow veinal network are observed but this is much more pronounced on lima bean *(Phaseolus lunatus).*

Infected plants set fewer pods and these generally are mal-formed with yellow blotches on the surface and contain poorly developed seeds.

III. Transmission and Host Range

Several unsuccessful attempts to mechanically transmit this disease from bean to bean, with or without phosphate buffer (0.005M at pH 7.0), have been made in our laboratory. In addition, disease failed to develop in over 300 plants that were grown under insect-free conditions from seeds collected from infected plants. Admittedly, the number of plants was small but Costa (1965) also grew over 500 bean and 300 lima bean plants with negative results, so it appears unlikely that seed-borne infection could be of major significance in this disease.

Golden mosaic is very easily transmitted by the white-fly *(Bemisia tabaci* Genn.) and in Jamaica, this insect breeds rather profusely on poinsettia *(Euphorbia pulcherrima)* a very common ornamental plant.

In greenhouse studies, we have bred large numbers of whiteflies on poinsettia and exposed them to golden mosaic infected beans and other species which showed bright, yellow mosaic symptoms for acquisition periods of 12-24 hours. A number of healthy plants of species on which these bright yellow mosaic symptoms have been observed then were exposed to these viruliferous whiteflies. Using this technique, we have been able to transmit golden mosaic from bean to bean, bean to lima bean and bean to *Macroptilium lathyroides (Phaseolus lathyroides),* the latter being an extremely common weed. Reciprocal transmissions also have been made. In these tests first symptoms became evident in the range 8-11 days on bean and lima bean, and 6-7 days on *M. lathyroides.*

Attempts to transmit what appears to be *Abutilon* mosaic from *Malvastrum coromandelianum* to bean or lima bean with this whitefly have been unsuccessful. Similarly, we have been unable to transmit the bright yellow mosaic disease of *Rhynchosia minima* - another extremely common leguminous weed - to bean, but were successful in transmitting it to soybean *(Glycine max).*

IV. Distribution and Seasonal Occurrence

Observations in Jamaica indicate that golden mosaic is prevalent mainly at lower elevations. In the more elevated bean growing regions e.g. Christiana Area Land Authority and Yallahs Valley Area Land Authority, golden mosaic is rarely found. This possibly is attributable to a temperature effect on the whitefly vector.

In monthly plantings in small plots over a period of two years, a lower percentage of infected plants has been obtained in the cooler months of November through March (Table I.)

TABLE I
INFLUENCE OF TIME OF PLANTING ON INCIDENCE OF
GOLDEN MOSAIC ASSESSED FOUR WEEKS AFTER MID-MONTHLY PLANTINGS

Month	% Infected Plants	Mean Temperature (°C)
Jan.	22.3	23.3
Feb.	24.9	23.9
Mar.	16.8	23.9
Apr.	91.5	24.4
May	45.3	24.7
June	82.7	26.1
July	81.7	26.7
Aug.	67.2	26.1
Sept.	54.1	25.6
Oct.	60.5	25.0
Nov.	25.8	24.4
Dec.	8.5	23.9

V. Influence of Time of Infection on Yield

In an effort to determine the effect on yield of virus infection at various stages of plant growth, a large plot of the bean variety Charleroi was planted. Infected plants (25-50) were randomly selected and tagged weekly from 17 to 44 days after planting. Untagged infected plants were rogued weekly. At harvest time data on number of pods, number of seeds and dry weight of seeds were collected from tagged plants plus 50 randomly selected healthy plants.

The results, shown in Table II, indicate that the earlier infection occurred, the greater was the reduction in the number of pods per plant and the number and weight of seeds produced in comparison with healthy plants. Plants on which symptoms developed within 17 days after planting produced 33% less pods and 51% less seeds which weighed 57% less than that produced by healthy plants.

TABLE II
INFLUENCE OF TIME IN INFECTION OF THE BEAN VARIETY
CHARLEROI ON POD AND SEED PRODUCTION

Age of Symptom Occurrence	No. pods/pl	No. Seeds/pl	Seed Wt/pl (gm)
Within 17 days	5.0	9.0	3.1
18-24	6.5	10.9	3.7
25-31	6.0	11.4	3.9
32-38	7.5	14.5	5.6
39-44	7.5	13.9	5.7
Healthy	7.5	18.4	7.3

VI. Evaluation of Varieties for Resistance to Golden Mosaic

Gámez (1971) has evaluated over 4,000 bean varieties in Costa Rica and failed to find resistant varieties. In Jamaica, we have tested over 100 varieties and similarly found them all to be susceptible to this disease.

In two recent tests, 25 varieties were planted on March 30 and May 11, 1973 and assessed 50-55 days later.

In general, the incidence of golden mosaic was lower in the first planting and levels of infection ranged from 29% in the variety Redkote to 90% in the variety Sanilac. In the second planting, infection ranged from 41% in the variety Pinto UI 114 to 100% in the variety 6R-25. Seventeen varieties had over 90% infected plants in the second test.

Although there was a fair amount of variation in the infection of different varieties, this does not appear to be due to any inherent tolerance to the disease-causing agent by these varieties. The varieties Redkote and Miss Kelly, for example, had 29 and 37% infection respectively in the first planting, but this increased to 96 and 98% respectively in the second planting. However, there appears to be some varietal preference by the whiteflies as indicated by the variable period taken by different varieties to reach a high percentage of infection. In this respect, the small white-seeded pea bean types (Michelite, Sanilac) appear to be favoured hosts.

VII. Effect of Insecticides on Incidence of Golden Mosaic

A total of 15 insecticides have been applied, generally with a 10 day interval between applications beginning 10 days after planting. In general, we have failed to obtain significant reduction in the incidence ot this disease in small plots, although some of the insecticides e.g. gardona, formothion, perfekthion, metasystox and diazinon, appear to be promising.

VIII. Discussion

Several observations of practical significance were made and these have resulted in effective control measures for golden mosaic in Jamaica. The disease rarely occurs at high elevations and the incidence generally is low in the cooler months of the year. This possibly is attributable to a temperature effect on the vector. Avidov (1956) working in Israel, found that excessively hot, cold or rainy weather conditions were lethal to this whitefly. He showed further that the highest average longevity occurred during periods when the average temperature was within the range of 14-21°C. He also noted that the development cycle was shorter at high temperatures, ranging from 85 days at 14°C to 14 days at 30°C. Although temperatures in the lowland areas of Jamaica are not as extreme as in Israel, the differences apparently are sufficient to exert a marked effect on the whitefly population, and so far, all plantings that have been made during the cooler months (November through February) according to our recommendations, have been almost totally free of this disease.

The two alternative hosts that have been found so far are very important sources of inoculum. *M. lathyroides* is very widely distributed and the pole types of lima bean persist, especially in backyard gardens, for very long periods. In addition, we have pointed out that poinsettia which apparently is the preferred

breeding host for the whitefly, is a very common ornamental plant. Although elimination of these species may be an effective control measure this procedure is, nevertheless, impractical. Further, it is quite likely that additional hosts exist since this whitefly is known to feed on many plant species.

Several insecticides are lethal to whiteflies. In a recent paper, Nene (1973) reported that mineral oil instantly immobilizes *B. tabaci* and he obtained good control of mung yellow mosaic virus with mixtures of mineral oil plus malathion, endosulfan or oxydemeton-methyl. Gámez (1971) has indicated that the virus borders on the persistent type with minimum acquisition and transmission periods of three hours in each case. It appears, therefore, that there would be ample time for a good insecticide to exert its lethal effects on the vector. Unfortunately we have not obtained effective control by using insecticides on small plots but it is expected that insecticide treatments would be much more effective in large fields. For reasons of economy, one should aim at obtaining effective control during the first 5-6 weeks since it is within this period that infection results in the greatest yield reduction. It may even be possible to obtain effective control in large fields by alternating overall and peripheral spray applications. These measures may be particularly useful if bean varieties became available that can produce reasonably high yields from plantings made during the hot season. At present it undoubtedly is more economic to obtain control by insect dodging especially since this period corresponds with that which is most favourable for growth of the bean plant in the lowland areas.

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MECHANICAL TRANSMISSION OF WHITEFLY *(Bemisia tabaci)*-BORNE DISEASE AGENTS OF BEANS IN EL SALVADOR

J. P. Meiners, R. H. Lawson, F. F. Smith, and A. J. Díaz

I. Introduction

Beans *(Phaseolus vulgaris* L.) are an important food crop in El Salvador, as well as in other countries in Latin America. In tropical and semitropical bean-growing areas, whitefly-borne diseases are widespread and often destructive. Some of the most economically important diseases of beans are those in which the causal agents are transmitted by the sweetpotato whitefly *(Bemisia tabaci* Gennadius). One of these, common in a complex with other diseases on beans in El Salvador, has been identified as golden mosaic (Gámez, 1971, Meiners *et al.*, 1973, Zaumeyer and Smith, 1966).

Golden mosaic disease (GMD) of beans was first described in Brazil and was reported to be restricted to *P. vulgaris* and *P. lunatus* L. (Costa, 1965). More recently, Gámez (1971) has reported transmission of the disease agent by *B. tabaci* to *Vigna radiata* (L.) Wilczek (*P. aureaus* Roxb.), *Phaseolus acutifolius* A. Gray, *P. lathyroides* L., *P. coccineus* L., and *P. vulgaris* subsp. *aborigineus* (Burk.) Burk. Diseases of lima bean *(P. lunatus)* caused by whitefly-transmitted agents have been reported from India (Capoor and Varma, 1948) and Puerto Rico (Bird and Sánchez, 1971, Bird *et al.*, 1972). Meiner *et al.*, (1973) reported transmission of the disease agent to bean with *B. tabaci* from the wild host *Calopogonium mucunoides* Desv. Costa (1965) transmitted the agent readily from bean to bean with the whitefly vector, but was unable to transmit it mechanically.

Costa (1965) also described other whitefly-transmitted diseases of beans, one of which was associated with *Euphorbia* mosaic disease (EMD) agent from

Euphorbia prunifolia Jacq. Similarly, a mosaic disease on *E. prunifolia* also occurs in El Salvador. Efforts by Costa (1965) to transmit the EMD agent mechanically to bean were unsuccessful.

Certain whitefly-transmitted disease agents might be mechanically transmitted by modified inoculation techniques or use of suitable test plants (Costa, 1969). Mechanical transmission would facilitate research with such agents, particularly in screening for disease resistance and in the attempt to identify and characterize the pathogen. Therefore, we attempted to transmit the GMD and the EMD agents by mechanical techniques. A summary of a part of the results has been published (Meiners *et al.*, 1973).

II. Materials and Methods

A. SOURCES OF DISEASE AGENT

Inoculum for the mechanical transmission studies was infected *P. vulgaris* cultivar 27-R from El Salvador. Plants were infected by exposure to whiteflies that had fed for 1 day on *C. mucunoides* or *E. prunifolia* (Costa, 1965). Plants also were infected by our grafting bean plants from El Salvador field plantings to stems of young 27-R potted bean plants or by our inserting infected stem tissue into diagonal cuts made in stems of healthy plants.

B. INOCULATION PROCEDURE

In tests at Santa Tecla, El Salvador, adults from colonies of agent-free *Bemisia tabaci* were maintained on caged healthy plants of *Sida carpinifolia* L., *Euphorbia prunifolia*, or sweetpotato, *Ipomoea batatas* (L.) Lam. The whiteflies were fed for 1 day on an infected source plants in a plastic cage 20 cm tall and 100 cm diam. With fine mesh cloth covering the openings. Inoculations were made by our caging 10 adults per test plant in cotyledon stage and feeding them for 1 day, after which they were removed and destroyed. By suction pipette, all transfers were made in a dark room with a single light source for controlling movement of whiteflies that left the plant. The plants were grown in a screened greenhouse sprayed often to control any free insects. Insect colonies were maintained in a separate greenhouse. Transmission tests were made in a third greenhouse.

Leaves showing symptoms were triturated in 0.1 M phosphate buffer, pH 6.8. The sap was rubbed on primary leaves of test plants that had been dusted with 320-mesh silicon carbide. Three plants per 4-inch pot were inoculated 7 to 10 days after planting, when the primary leaves were partially expanded. After inoculation, the plants were placed in the greenhouse or in growth chambers at 21°, 24°, 27°, 32°, and 35°C. The chambers had a diurnal light cycle of 1,500 lux for 12 or 16 hours. The plants were kept in the chambers for about 2 weeks and then placed in the greenhouse, where the temperature varied from a minimum of 18°C at night to a maximum of 30°C during the day, with a natural photoperiod and sunlight intensity of about 1,900 lux maximum. Bean cultivars Topcrop, Stringless Green Refugee, Columbia Pinto, La Vega, Santa Ana, El Salvador No. 184, and Porrillo No. 1, and the lima been cultivar Henderson Bush were inoculated.

III. Results

A. GOLDEN MOSAIC

Whiteflies readily transmitted the causal agent of GMD from infected *C. mucunoides* (Fig. 1) to bean cultivar 27-R (Fig. 2). After mechanical inoculation, symptoms typical of those described for GMD (4) developed on all the bean and lima bean cultivars. The symptoms on Stringless Green Refugee are shown in Fig. 3. At 21°, 24°, and 27°C fewer test plants were infected than those exposed to 32° and 34°C (Table I). Furthermore, symptoms usually developed at 32°C in less than 2 weeks after inoculation, at 27°C in about 3 weeks, and at 21°C in about 4 weeks (Table II). Columbia Pinto, La Vega, and Santa Ana plants showed no symptoms when incubated at 21° or 27°C after inoculation. A few La Vega and Santa Ana plants showed symptoms only when incubated at 32°C. Both of these varieties were selected for resistance to GMD. We tried to recover the agent from selected symptomless plants by mechanically inoculating Topcrop plants and incubating them at 32°C; however, no symptoms developed. Parallel attempts to recover the agent from plants showing symptoms succeeded.

TABLE I
EFFECT OFF INCUBATION TEMPERATURE ON THE MECHANICAL TRANSMISSION OF GOLDEN MOSAIC AGENT TO BEAN AND LIMA BEAN CULTIVARS

	Temperature of incubation (°C)					
Cultivar	21	24	27	32	35	Greenhouse [1]
Topcrop	1/3 [2]	1/3	3/3	3/3	3/3	2/15
Stringless Green Refugee	3/3	1/3	2/3	3/3	3/3	0/15
Columbia Pinto	0/3		0/3	3/3		
La Vega	0/3		0/3	1/6		0/6
Santa Ana	0/3		0/3	2/6		2/6
El Salvador No. 184		1/3			2/3	0/6
Porrillo No. 1		1/3			0/3	1/6
Henderson Bush	1/6		6/6	6/6		15/18
TOTAL	5/21	4/12	11/21	18/27	8/12	20/72
Plants showing symptoms (%)	24	33	52	67	67	28

[1] Temperatures varied from 18°C minimum at night to 30°C maximum in the daytime.

[2] Numerator is the number of plants showing characteristic golden mosaic symptoms; denominator indicates number of plants inoculated.

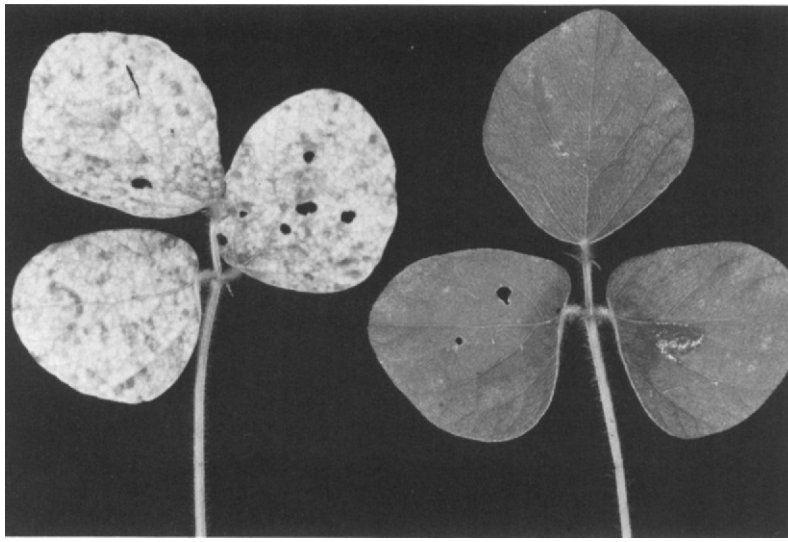

Fig. 1. Left - symptoms of golden mosaic disease on leaf of *Calopogonium mucunoides;* right - symptom-free leaf.

Fig. 2. Symptoms caused by the causal agent of golden mosaic disease on 27-R bean cultivar following transmission by whitefly *(Bemisia tabaci).*

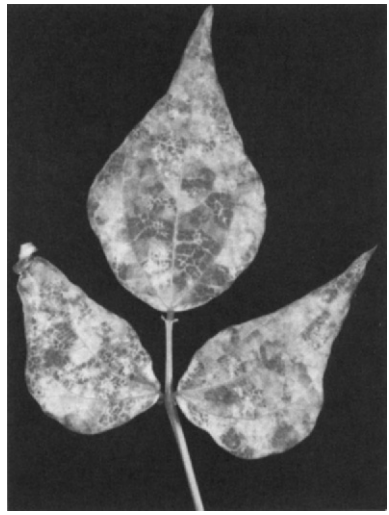

Fig. 3. Symptoms caused by the causal agent of golden mosaic disease on Stringless Green Refugee bean cultivar following mechanical inoculation.

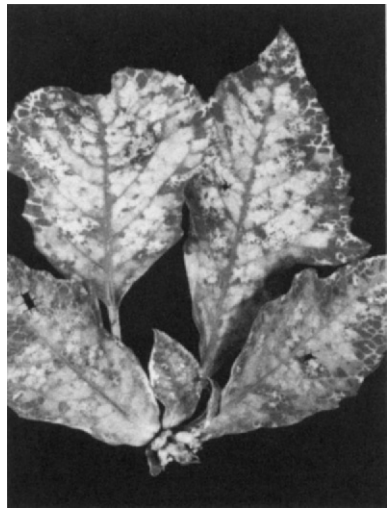

Fig. 4. Mosaic caused by whitefly-borne agent on *Euphorbia prunifolia*.

TABLE II
EFFECT OF INCUBATION TEMPERATURE ON SYMPTOM DEVELOPMENT IN BEANS MECHANICALLY INOCULATED WITH GOLDEN MOSAIC DISEASE AGENT

Days after	Temperature of incubation (°C)			Total
inoculation	21	27	32	
13	0/6[1/]	0/6	6/6	6/18
23	2/6	5/6	6/6	13/18
28	4/6	5/6	6/6	15/18

[1/] Numerator is the number of plants showing characteristic golden mosaic symptoms; denominator indicates number of plants inoculated.

Several plants inoculated mechanically and showing typical symptoms were selected for transmission tests with the whitefly vector. Juice squeezed from the leaves of diseased plants was stained with 2% potassium phosphotungstate (K-PTA) (pH 7.0) and examined with the electron miscroscope. No virus-like particles were detected. The infected plants were returned to El Salvador. There, whiteflies free of the agent were allowed to feed on the infected bean plants for 1 day and then were caged for 1 day on healthy seedlings of 27-R beans in the cotyledonary and first trifoliate leaf stage. Freed from the whiteflies and soil, these plants were returned to Beltsville, replanted in pots, and incubated at 32°C in a growth chamber. After 2 weeks, these plants developed symptoms typical of golden mosaic. Sap extracts negatively stained in 2% K-PTA failed to reveal any virus-like particles, and no evidence of virus or mycoplasma-like organisms was found in ultrasections of infected bean leaves.

Three plants each of Topcrop and Stringless Green Refugee were inoculated mechanically with leaf tissue from 27-R plants with symptoms and incubated at 32°C. Typical golden mosaic symptoms developed in one plant of each cultivar. Later, bean plants infected mechanically with the same culture of the agent and showing typical symptoms were again returned to El Salvador, where the disease agent was again transmitted to bean plants by whiteflies. These plants were returned to Beltsville, where mechanical transmission to the bean cultivar Stringless Green Refugee again succeeded. Two of three plants inoculated and incubated at 32°C produced typical symptoms of GMD. In all mechanical and insect transmission tests, uninoculated and unexposed control plants developed no symptoms.

B. EUPHORBIA MOSAIC

Results of transmission tests with the agent from infected leaves of *E. prunifolia* (Fig. 4) were similar to those of GMD agent from *C. mucunoides.* After transmission by whitefly from *E. prunifolia* to 27-R cultivar of bean in El Salvador, the infected plants were brought to Beltsville. Two of three Topcrop plants and one of three Stringless Green Refugee plants inoculated mechanically and incubated at 32°C showed symptoms identical to those in the beans inocu-

lated by whitefly. Symptoms on Stringless Green Refugee (Fig. 5) included a bright-yellow mosaic pattern, as with GMD. However, leaves showed considerable distortion, as contrasted with GMD, with which little or no distortion occurs (Fig. 3).

Infected Topcrop bean plants were returned to El Salvador, where El Salvador No. 184 plants inoculated by whiteflies developed typical symptoms (Fig. 6). Infected plants were again brought to Beltsville and inoculated mechanically to Topcrop bean. One of three plants inoculated showed typical symptoms when incubated at 35°C, whereas plants incubated at 24°C and in the greenhouse showed no symptoms. Control plants developed no symptoms in any of the tests.

Throughout the tests, samples of infected plant tissue were examined with the electron microscope. As with GMD, no virus-like particles were detected.

IV. Discussion

This is believed to be the first report of the mechanical transmission of a whitefly-transmitted disease agent producing golden mosaic of bean. The disease agent was transmitted at 21°, 24°, and 27°C temperatures, but transmission succeeded better and symptoms developed faster at temperatures above 30°C. Likewise, this is the first report of mechanical transmission from bean to bean of a whitefly-transmittable agent originating in *E. prunifolia.*

Many attempts were made to purify a virus by conventional procedures from bean plants with golden mosaic disease.* With one exception, we have not succeeded in reproducing the disease using inoculum from density gradient fractions or even from high-speed pellets. Likewise, we have not been able to find virus-like particles with the electron microscope in any of scores of variously prepared high-speed preparations. In the one successful instance, typical symptoms were observed in Topcrop beans rubbed with inoculum from a gradient fraction. The causal agent was later transferred to other Topcrop bean plants with whiteflies, where it again produced typical golden yellow mosaic symptoms. We did not try to purify the *Euphorbia* mosaic agent.

Because no virus-like particles were detected, possibly these whitefly-transmitted agents are not associated with a typical nucleoprotein virus particle. If the agent is a nucleoprotein, it may be very small and not distinguishable from normal host cell constituents in the electron microscope.

The agents studied in this paper originated in a wild host and were transmitted by the whitefly vector to bean. Later, they were alternately transmitted mechanically and by the whitefly from bean to bean. Repeated efforts to transmit the agent of GMD from bean to *C. mucunoides* and other wild legumes by mechanical methods have failed. However, the whitefly transmitted the agent from bean back to *C. mucunoides* in 1973 in El Salvador.

Costa (1965) reported mechanical transmission of the disease agent of *Euphorbia* mosaic to *Datura stramonium* L. from *Euphorbia* or *Datura* plants. We have been unable to transmit this agent mechanically to either *D. stramonium* or *E. prunifolia* from *E. prunifolia* or bean.

*We are indebted to H. E. Waterworth for the attempts to purify the agent of GMD.

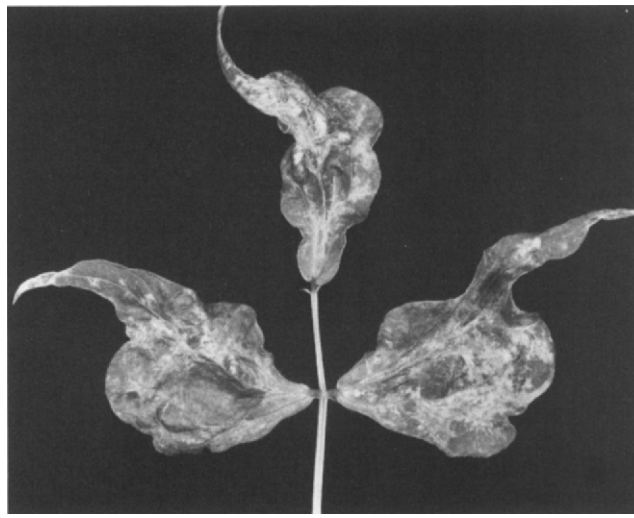

Fig. 5. Symptoms caused by the causal agent of *Euphorbia* mosaic disease on Stringless Green Refugee bean following mechanical inoculation.

Fig. 6. Symptoms caused by the causal agent of Euphorbia mosaic on El Salvador No. 184 bean following transmission by whitefly *(Bemisia tabaci).*

On several occasions, we tried at Beltsville to mechanically transmit agents from diseased plant material obtained in Puerto Rico to beans. These have included mosaics on bean, *Rhynchosia minima* (L.) DC, *Sida carpinifolia* L., and *Phaseolus lathyroides*, all showing symptoms typically associated with whitefly-transmitted diseases (Bird *et al.*, 1972). All attempts have failed. Two whitefly-transmitted diseases from Puerto Rico have recently been transmitted mechanically by Bird and coworkers only by special methods. These results with whitefly-transmitted agents in Brazil and Puerto Rico, as contrasted with our results obtained with agents from El Salvador, raise a question of the existence of local strains of whitefly-transmitted disease agents that differ in several properties, including mechanical transmissibility, host range, and symptoms produced on plant hosts.

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ETIOLOGY OF WHITEFLY-BORNE DISEASES

Karl Maramorosch

I. Introduction

The importance of whitefly transmitted disease agents becomes apparent if one considers the crops of economic importance that are affected. Cassava mosaic disease in Africa and other parts of the world, tobacco leaf curl in Indonesia, Africa, India and American tropics, cotton leaf curl in Africa, and several bean diseases in tropical and subtropical areas are among the major diseases caused by whitefly-borne agents. Transmission by whiteflies occurs also in subtropical and even in temperate zones. In the Mediterranean areas, such as Israel (Loebenstein and Harpaz, 1960) and in the United States in California, Florida, Georgia and Maryland outbreaks caused by whitefly vectors have been reported (Costa, 1969). Varma (1963) listed 28 different diseases ascribed to whitefly vector transmission, and while some of these might have been caused by the same or related strains of the same agent, there are certainly more than 30 different agents known today, all transmitted by the same species, *Bemisia tabaci*. Until recently, no adequate serological or electron microscopy diagnostic methods were available for proper identification of these diseases. Most reports were based on vector transmission (Maramorosch, 1963, 1969) and on symptomatology (Holmes, 1964).

Until now, whitefly-borne disease agents have always been considered as viruses (Corbett and Sisler, 1964; Carter, 1973). This has been based merely on an assumption as there was no direct chemical or morphological evidence to characterize the various disease agents as viruses. The present review has been prepared so as to point out the uncertain status of the whitefly-transmitted agents. The evidence supporting viral etiology of the respective diseases will be reviewed in the hope that critical evaluation will stimulate further research and eventually characterize, properly, different agents transmitted to plants by *B. tabaci*.

II. Historical

More than 70 years ago the infectious nature of the spotting of *Abutilon striatum* var. *thompsoni* was demonstrated by Baur (1904). This classic example of ingenuity and careful experimentation, eventually lead to important basic and applied discoveries concerning vector transmission and control of whitefly-borne disease agents. Baur demonstrated that the agent causing the spotting of *Abutilon* was graft transmissible, hence infectious. Thus originated the name of the disease, "infectious chlorosis." Baur noticed that the severity of the spotting increased in bright sunlight. This observation led him to the first cure of a plant disease, by means of prolonged shading. Another remarkable cure was developed by Baur (1906a; 1906b) when he obtained healthy plants by surgery, that is, by removing all leaves with spots. After repeated excising of spots and spotted leaves, no further chlorosis appeared and even in intense sunlight the plants remained healthy.

Although the infectious nature of the *Abutilon* disease was clearly demonstrated by Baur, neither the nature of the infectious agent, nor the natural means of transmission were established by him. Nevertheless, plant pathologists assumed that the disease was caused by a virus. This assumption was based on the failure to isolate fungi or bacteria from diseased tissues, and on the graft transmissibility of the disease agent.

Natural transmission of infectious chlorosis did not occur in Europe or North America and the only means of infecting plants was by grafting pieces of spotted plants onto healthy ones. This gave the geneticist Darlington (1944) the ill-conceived idea that the infectious agent of *Abutilon thompsoni,* a plant virus, arises spontaneously from a plasmagene, whenever the incompatible plant proteins of two *Abutilon* species are brought together by a graft. Darlington thought that since grafting is an artificial invention of man that does not occur in nature, a virus could not depend on mere grafting for its only means of survival. What this hypothesis overlooked was first of all, that it was never proven that the disease agent was a virus. Besides, grafting is not necessarily an artificial invention of man, since it also occurs naturally, in the form of root grafts, as well as by means of certain parasitic plants, such as various species of dodder *(Cuscuta* sp.). The latter are widely used by plant pathologists for the transmission of plant pathogenic viruses. Darlington's hypothesis was critically reviewed by Bawden (1964) who presented numerous examples of viruses surviving in nature in the absence of known vectors. The *de novo* origin of viruses has long since been abandoned. It might be of interest to point out that even though grafting is a very efficient method for the transmission of whitefly-borne agents, various species of *Cuscuta* did not transmit these agents experimentally, despite repeated efforts (Capoor and Varma, 1950; Bird, 1957; Clerk, 1960; Erwin and Meyer, 1961; Kunkel, personal communication).

III. Insect Transmission

Orlando and Silberschmidt (1946) published their classic experiments in which they demonstrated that a disease that resembled the infectious chlorosis of *Abutilon* and that was spreading in Sao Paulo, Brazil, was caused by a whitefly-borne agent. The discovery was made through serendipity. The fortunate accident occurred when a glass cage, covering a control plant, broke

and the plant became invaded by whiteflies. Although the cage was replaced the next day and the invading whiteflies destroyed, the exposed plant became diseased with infectious chlorosis. The remaining caged plants, exposed to different species of aphids and leafhoppers, remained free from the disease. The demonstration that plants can become experimentally infected by using *B. tabaci* revealed the importance of whitefly-borne disease agents in the tropics of America, Africa, and Asia (Maramorosch, 1963; Silberschmidt *et al.*, 1957; Bird, 1958; Costa and Bennett, 1953; Flores and Silberschmidt, 1958; Flores *et al.*, 1960). Numerous tropical diseases with symptoms resembling infectious chlorosis were found to have disease agents transmitted by *B. tabaci.* The interactions between whitefly vectors and the disease agents were first described by Costa and Bennett (1950). The time of acquisition, transmission, and retention were accurately determined and no questions were raised about the determination of the agent as a plant-pathogenic virus. For the following quarter of a century just as during the earlier years, all reports, reviews, and textbooks listed whitefly transmitted agents as viruses, and whiteflies as virus vectors (Corbett and Sisler, 1964; Maramorosch, 1969; Carter, 1973).

Symptomatology, although based on rather vague criteria, has been used widely for the identification of virus diseases for many years (Holmes, 1964). As already pointed out, graft transmissibility was also considered to support the hypothesis that viruses were involved. Additional evidence quoted in support of viral etiology was the transmission by insects, even though disease agents other than viruses were known to be transmissible by such vectors (Carter, 1973). Since no disease agents were visualized for isolated from plants affected by the respective diseases, viruses were consistently incriminated in all instances in which whitefly transmission could be established.

IV. Mechanical Transmission

Mechanical transmission of whitefly-borne disease agents was attempted for many years. Lack of mechanical transmission was reported by Baur (1904), Lindemuth (1907), Hertzsch (1928), Kunkel (1930), Klebahn (1932), Costa (1937; 1955; 1965), Silberschmidt (1943), Hollings (1959) and others. Crandall (1954) reported successful mechanical transmission, but his results were not confirmed by Costa (1955). Most of the above tests were with *Abutilon* infectious chlorosis. The first successful mechanical transmission of a whitefly-borne agent was reported by Costa and Bennett (1950) with the agent causing *Euphorbia* mosaic. Sheffield (1957; 1958) transmitted an agent of sweet potato disease in East Africa to petunia plants. Cohen and Nitzany (1960) mechanically transmitted the whitefly-borne agent of cucumber yellow vein. In the same year Costa and Carvalho (1960) obtained mechanical transmission with a high degree of success with the agent of infectious chlorosis from Malvaceae, introduced into *Malva parviflora.* Mechanical transmission of the same agent from *Abutilon* directly to *M. parviflora* was unsuccessful, but when the agent was first introduced by grafting from *Abutilon* to *Sida micrantha,* mechanical transmission with the inoculum from this plant to *M. parviflora* was successful. Flores and Silberschmidt (1967) were able to transmit the infectious chlorosis agent directly from *Abutilon* to *M. parviflora.* Mechanical transmission of whitefly-borne agents of bean diseases in El Salvador has been carried out successfully by Meiners *et al.*, (1973) and a detailed account of this work is presented for the

first time in this volume. Recently the agents of bean golden mosaic and of *Rhynchosia* mosaic have been transmitted mechanically by Bird and co-workers in Puerto Rico, as described in the present volume.

Direct evidence of viral involvement requires the demonstration that viruses are present and cause the respective disease. Recently such evidence has been provided by Bird *et al.*, (1974). Their study revealed, by electron microscopy techniques, the presence of icosahedral virions in one of the plants infected via whiteflies. Morphologically similar virions were present in the infectious, partially purified extract used to inoculate plants mechanically. The same disease was induced, and thin sections revealed the presence of icosahedral virions in the experimentally inoculated plants.

The finding that some viruses are transmitted by whiteflies does not, by itself, permit the generalization that all whitefly-borne agents are viruses. In the case of leafhopper vectors, we know of several instances where the same species can transmit a virus and a mycoplasmalike agent, or a rickettsialike agent. It is therefore conceivable that whiteflies carry more than one type of disease agent and in each instance it will be necessary to establish, by proper methods, the actual nature of these plant pathogens.

Even if particles, such as those illustrated by Sun (1964) would be found in association with whitefly-borne disease agents, mere association would not provide sufficient evidence for their etiologic role, unless supported by infectivity and the reproduction of the same disease with purified particles introduced into test plants. Artifacts frequently have been reported as so-called viruslike particles but they certainly do not provide evidence that a virus is involved as a causative agent (Dalton and Haguenau, 1973).

V. Mycoplasmalike and Rickettsialike Organisms

Evidence against viral etiology is indirect. Repeated attempts have been made in several laboratories to find viruses in extracts as well as to visualize such viruses in thin sections of plant tissues derived from whitefly-inoculated plants with typical symptoms of infectious chlorosis. Until recently, all such attempts were futile and neither viruses, nor "viruslike particles" were localized in *Abutilon, Sida, Phaseolus,* or other plants. This might have been due to either the absence of virions, or due to their low concentration in infected plants. The latter possibility cannot be excluded.

The first questioning of the established assumption that all whitefly-borne diseases are caused by viruses came in the wake of the breakthrough in Japan in 1967, when several plant diseases, previously ascribed to viral causes, were found associated with, and, most likely caused by mycoplasmalike organisms (MLO) (Doi *et al.*, 1967; Ishiie *et al.*, 1967; Nasu *et al.*, 1967). Treatment with tetracycline antibiotics caused temporary remission of MLO disease symptoms and the wall-less microorganisms deteriorated and disappeared as ascertained by electron microscopy (Ishiie *et al.*, 1967; Wolanski, 1973). Renewed attempts were made to find MLO in thin sections of plants with infectious chlorosis or leaf curl symptoms, especially in Brasil and in Puerto Rico. Until now, no MLO have been detected in such plants and no MLO have been linked with whitefly-borne etiology. The small bacteria or rickettsialike organisms (RLO), recently recognized as etiologic agents of certain plant diseases (Maramorosch, 1974) have not been detected in any of the leaf curl or infectious chlorosis-type

diseases and there is no evidence at present to support the hypothesis that whiteflies transmit such microorganisms.

VI. Viroids

Before the realization that agents, smaller than viruses, now identified as viroids (Diener, 1974) can cause plant diseases and can be mechanically transmitted to plants, the mere mechanical transmission of a filterable agent to plants often sufficed to define such as agent as a virus. This is no longer acceptable and thus the demonstration that some whitefly-borne agents can be transmitted mechanically no longer proves their viral nature.

The term viroid designates a newly recognized class of pathogens, devoid of a protein coat and consisting merely of a small nucleic acid genome (Diener, 1974). No conventional virus particles are found in diseases caused by viroids. The RNA of viroids, such as the causative agents of potato spindle tuber, chrysanthemum stunt, or citrus exocortis disease is of very low molecular weight. The agent thus differs basically from conventional viruses. Visualization by electron microscopy of purified viroids of potato spindle turber has been achieved recently (Diener, 1974). Molecular weight estimates obtained by electron microscopy were in agreement with the values obtained by other methods. The molecular weight is suffient to code for 70 to 80 amino acids. The 3 known viroids infecting plants are all mechanically transmissible and no insect vectors are known.

The above information on viroids does not preclude the possibility that such disease agents could have whitefly vectors, but there is no evidence to support this hypothesis at this moment. The inclination of some plant pathologists to incriminate viroids when no virus particles, MLO, or RLO can be detected in a diseased plant (Randles, 1975) is not justified in the absence of positive evidence.

VII. Possible Diversity of Agents

There is no *a priori* reason to believe that whiteflies can transmit only one kind of disease agent, and that, if one agent is recognized, all other whitefly-borne disease agents would necessarily be of the same type. It was believed at one time that leafhoppers transmit only plant viruses, but now it is known that leafhoppers also transmit MLO and RLO; the same species that can transmit a virus biologically, may also transmit an MLO or RLO agent. Thus the finding that one of the whitefly-borne agents is a virus does not necessarily imply that all of the agents transmitted by *B. tabaci* are viruses. In fact, there is good evidence to support the viral etiology of 2 diseases associated with whitefly vectors, but the etiology of all other diseases with *B. tabaci*-transmitted agents remains unsolved.

The growing awareness that some other plant disease agents, customarily referred to as viruses, but not properly characterized, might represent other disease agents is apparent from a recent report in which the curly top disease of sugar beets in California no longer was referred to as caused by the curly top virus, but as caused by the "curly top agent" (Mygyarosy *et al.*, 1975).

VII. Conclusions

Whitefly-borne agents of plant diseases have been classified as viruses merely on the basis of negative evidence, that is, inability to detect fungi or bacteria as causative agents. The graft transmissibility and vector transmission in no way preclude the possibility that other etiologic agents might be involved. In several instances mechanical transmission have been achieved, and in two instances there is evicence that viruses are the causative agents of the respective diseases. The possibility that some of the whitefly-borne agents are MLO or RLO is not supported by electron microscopy evidence. Although viroids of 3 known plant diseases are mechanically transmissible, this does not necessarily mean that viroids might not have natural insect vectors. There is no evidence at present to support the contention that any of the whitefly-borne agents are viroids. The etiology of all but 2 diseases associated with whitefly vectors remains unsolved.

Control measures of diseases of uncertain etiology are usually very difficult. However, in instances where the mode of transmission is known, as is the case with whitefly-borne disease agents, control measures can be applied against the vectors. The difficulties in controlling *B. tabaci* are enormous and it appears more feasible to use standard plant breeding techniques, to obtain resistant varieties or varieties that would not be frequented by *B. tabaci*. Larvae of whiteflies are killed much more slowly than the adults, as pointed out by Van der Laan (1961).

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A NEW TYPE OF WHITEFLY-TRANSMITTED DISEASE-- A LINK TO THE APHID-TRANSMITTED VIRUSES

James E. Duffus

I. Introduction

The yellowing virus diseases, characterized by stunting of infected plants, accompanied by rolling, yellowing, reddening, and brittleness of affected leaves, are emerging as the most important artificial group of plant virus diseases.

Studies of these diseases have been hampered by the almost total lack of transmission by means other than grafting or by the insect vectors themselves and by the similarity of the symptoms to early ripening, drought, excessive moisture, nutritional deficiencies, or soil conditions.

Despite the difficulties involved in assessing damage and etiology of these diseases, they are listed among the most damaging diseases of barley, oats, grapes, potatoes, spinach, and sugar beets. (Anonymous, 1965). In addition, viruses of these yellowing groups have been recovered from, or have been shown to be capable of seriously reducing yields of such diverse crops as pea (Quantz and Volk, 1954), bean (Tinsley, 1959), cotton (Costa, 1956), flax (Muskett and Colhoun, 1948), groundnut (Storey and Bottomley, 1928), strawberry (Horne, 1922), table beet (McLean, 1953), broccoli (Duffus, 1960a), cauliflower (Duffus, 1960a), cabbage (Duffus, 1964), cantaloupe (Duffus, 1965), carrot (Stubbs, 1948), cucumber (Duffus, 1965), clover (Grylls

and Butler, 1959), and lettuce (Duffus, 1960b).

The viruses inducing these diseases fall into three currently recognized virus groups (Gibbs, 1969); persistent aphid-transmitted viruses with isometric particles, 23-30 nm; semipersistent aphid-transmitted viruses with flexuous, filamentous particles; and a virus transmitted by whiteflies, beet pseudo yellows virus.

II. Background

Studies of the yellows complex of sugarbeet involve constant concern over the possibility of contamination in the greenhouse. Nonviruliferous aphid colonies are checked on healthy indicator plants each time aphids are used in transmission; noninfested plants are used also as a check against spread of the yellow viruses in the greenhouse. Yellowing contaminations became numerous and seriously threatened the beet indexing and strain differentiation work, despite the lack of viruliferous aphids in insectary compartments or in the greenhouse but following a slow build-up of the common greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). Transmission tests in the laboratory have shown that this insect transmits a yellowing virus to sugarbeet and other crop and weed plants (Duffus, 1965).

The disease on these hosts, although unrelated, closely resembles in symptoms the other beet yellows diseases and was designated as beet pseudo-yellows.

III. Materials and Methods

The greenhouse whitefly, *T. vaporariorum*, used in these tests and collected in the greenhouse was identified by Louise M. Russell, Entomology Research Division, USDA. Colonies were reared in rectangular cloth and glass-sided cages. Host and vector relationships were conducted using a virus isolate collected in the greenhouse. Other isolates, transmitted by the greenhouse whitefly from field plants, produced similar symptoms on common host plants but were not studied further. Viruliferous colonies were reared on virus-infected plants *of Malva parviflora* L. Nonviruliferous whiteflies were reared on *Solanum dulcamara* L. Insects were moved with an aspirator tube connected to a water suction apparatus. Cloth-covered cylindrical cages were used to enclose plants that were to be infested with large numbers of insects. In tests in which smaller numbers of insects were confined to plants or in tests in which the insects were moved from plant to plant, small clip-type leaf cages as described by McLean (1962) were used.

After routine tests with whiteflies, all plants were sprayed with malathion. After treatment, plants were placed in greenhouses which were fumigated weekly with nicotine and tetraethyl pyrophosphate (TEPP). The gradual build-up of whiteflies in the greenhouse, which preceded the discovery of beet pseudo-yellows, followed a program of fumigation with only nicotine. Since the discovery of the relationship of the greenhouse whitefly to the virus, addition of TEPP to the weekly fumigation and occasional spraying with malathion has practically eliminated the insect from the greenhouse. Noninfected plants were placed in the greenhouse with each series of plants inoculated as a check for contamination by viruliferous whiteflies. Since the start of the intensified whitefly control program, no obvious contaminations have been evident.

IV. Economic Importance

Thus far, the beet pseudo-yellows virus has been isolated from the Salinas greenhouse and from two weed species *(Conium maculatum* L. and *Taraxacum officinale* Web.) from various locations in the Salinas Valley. However, few attempts have been made to determine the incidence and distribution of the virus in the field. The virus is potentially important. The vector *T. vaporariorum* is an abundant and destructive species in greenhouses and in the field in warmer climates. It has numerous host plants and worldwide distribution (Russell, 1963).

In the greenhouse, the virus caused yellowing-type diseases of sugarbeet *(Beta vulgaris* L.), spinach *(Spinacia oleracea* L.), cucumber *(Cucumis sativus* L.), squash *(Cucurbita moschata* Dcne.), muskmelon *(Cucumis melo* L.), flax *(Linum usitatissimum* L.), lettuce *(Lactuca sativa* L.), carrot *(Daucus carota* L.), and several ornamentals, and it may occur in these species in nature.

The disease is probably not economically significant on sugarbeet in the field since the insect is not commonly found on this plant. The disease can be confusing, however, in a greenhouse research program on strains and vectors of the sugarbeet yellows viruses, because of the close similarity of symptoms induced on common host plants.

V. Host Range

The host range was determined by infesting at least six seedlings of each species tested with 30-50 viruliferous whiteflies for 48 hr. The presence of virus in each plant species tested for susceptibility was determined by whitefly transfer to shepherd's-purse *(Capsella bursa-pastoris)* seedlings about 60 days after inoculation.

Plants susceptible to beet pseudo-yellows virus are listed in alphabetical order:

Amaranthaceae--*Gomphrena globosa* L.

Caryophyllaceae--Spergula arvensis L.

Chenopodiaceae--*Beta macrocarpa* Guss., *B. vulgaris* L., *Chenopodium album* L., *C. amaranticolor* Coste & Reyn., *C. capitatum* (L.) Asch., *C. murale L.*, *Spinacia oleracea* L.

Compositae--*Callistephus chinensis* (L.) Nees, *Cichorium endiva* L., *Lactuca sativa* L., *L. serriola* L., *Senecio vulgaris* L., *Sonchus oleraceus* L., *Taraxacum officinale* Webber, *Zinnia elegans* Jacq.

Cruciferae--*Capsella bursa-pastoris* (L.) Medic.

Cucurbitaceae--*Cucumis sativus* L., *C. melo* L., *Cucurbita moschata* Dcne.

Geraniaceae--*Erodium cicutarium* (L.) L'Her., *Geranium dissectum* L.

Linaceae–*Linum grandiflorum* Desf., *L. usitatissimum* L.

Malvaceae--*Malva parviflora* L.

Portulacaceae--*Claytonia perfoliata* Donn.

Ranunculaceae--*Aquilegia* sp.

Solanaceae--*Nicotiana clevelandii* Gray, *N. glutinosa* L., *N. tabacum* L., *Physalis ixocarpa* Brot., *P. wrightii* Gray, *Solanum dulcamara* L.

Umbelliferae--*Conium maculatum* L., *Daucus carota* L.

Urticaceae--*Urtica californica* Green.

Plants showing no indication of infection include:

Aizoaceae--*Tetragonia expansa* Murr.

Compositae--*Helianthus annuus* L.

Convolvulaceae--*Ipomea nil* (L.) Roth, *I. purpurea* (L.) Roth., *I. tricolor* Cav.

Cruciferae--*Raphanus sativus* L., *Sisymbrium irio* L., *Thlaspi arvence* L.

Cucurbitaceae--*Citrullus lanatus* (Thunb.) Mansf., *Cucurbita pepo* L.

Leguminosae--*Medicago sativa* L., *Phaseolus vulgaris* L.

Malvaceae--*Althaea rosea* (L.) Cav., *Gossypium hirsutum* L., *Hibiscus esculentus* L., *Lavatera assurgentiflora* Kellogg, *Malva sylvestris* L.

Solanaceae--*Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicandra physaloides* (L.) Gaertn., *Physalis floridana* Rybd.

VI. Symptoms

Species infected by the beet pseudo-yellows virus showed, in general, stunting, interveinal yellowing, and/or chlorotic spotting. Species that naturally have red pigment tended to show intensification of red color in interveinal areas when infected. Symptoms on many of the common host plants were very similar to symptoms induced by beet yellows, beet western yellows, and malva yellows viruses, all of which are aphid-transmitted. Descriptions of symptoms on a selected group of host plants follow.

1) *Beta vulgaris*

Infected sugarbeet plants showed chlorotic spotting or splotching uniformly on the older and intermediate leaves. As the disease progressed, the yellowing became more intense and more general. Older infected leaves were chlorotic except for scattered small islands of green tissue. In older leaves, there were also irregular, bright-yellow areas 1-1.5 cm in diam. Leaves were thickened and brittle. The yellowing symptoms were more uniform with less tendency toward sectoring and green veins than with beet and western yellows.

2) *Capsella bursa-pastoris*

Plants of shepherd's-purse inoculated in the greenhouse showed initial symptoms 15-20 days after inoculation. Lower leaves developed severe chlorosis and moderate leaf curl. As the disease progressed, the yellowing developed acropetally. Yellow leaves were thickened and brittle. The symptoms were similar to symptoms induced by the beet western yellows virus on this host.

3) *Lactuca sativa*

Infected lettuce plants exhibited severe interveinal yellowing symptoms on the older and intermediate leaves. Symptoms were similar to those induced by the beet western and malva yellows viruses.

4) *Linum usitatissimum*

Flax infected in the greenhouse showed marked interveinal yellowing, especially near the leaf margins and base of the leaves on the lower two-thirds of

the plant. Beet pseudo-yellows symptoms on this host were indistinguishable from symptoms induced by beet western and and malva yellows viruses.

5) *Chenopodium capitatum*

Infected plants showed symptoms similar to those induced by mild isolates of the beet yellows virus. Striking interveinal reddening of the older leaves was characteristic. Beet pseudo-yellows virus-infected plants, however, showed slightly more purple coloration and a sharper contrast between the interveinal areas and the green veins than beet yellows virus-infected plants.

6) *Taraxacum officinale*

Older leaves of infected plants showed reddening and chlorosis of interveinal areas which, at times, were sharply delimited by the veins.

7) *Nicotiana glutinosa*

Bright interveinal yellowing symptoms with dark-green veins were characteristic of beet pseudo-yellows virus in this host.

8) *Cucumis melo*

Small (1-mm diam), orange-yellow, raised areas appeared on the intermediate and older leaves of affected muskmelon. Later, these areas coalesced to form large thickened areas on the leaf surface. Irregular necrotic areas then developed and the leaves died prematurely. Plants were rather severely stunted.

VII. Transmission Tests

A) MECHANICAL

Numerous attempts were made to transmit the beet pseudo-yellows virus mechanically by techniques that included the use of abrasives, phosphate buffer, and sodium sulfite. Virus sources used included sugarbeet, shepherd's-purse, and *Nicotiana clevelandii.* The plants inoculated included these and a number of other species found to be susceptible when inoculated by the whitefly vector. The results were negative in all tests.

B) INSECTS

Preliminary studies had indicated that the beet pseudo-yellows virus was readily transmitted by the greenhouse whitefly (*T. vaporariorum).* However, because of the similarity in symptoms to the aphid-transmitted yellows viruses of beet, it was desirable to determine whether common aphid vectors of these viruses could transmit the whitefly-transmitted virus. Tests to determine whether some of the common aphid species are vectors of beet pseudo-yellows virus were carried out with beet, shepherd's purse, or sowthistle as the virus source and test plants. Non-viruliferous aphids of the various species tested were placed on the source plants for 24 hr, and then about 25 individuals were transferred to each of a number of test plants for an infection feeding period of 48 hr. Under these conditions none of the aphid species used was capable of transmitting the beet pseudo-yellows virus. These species included *Amphorophora lactucae* (Linnaeus), *Aphis fabae* Scopoli, *Acyrthosiphon barri* (Essig), and *Myzus persicae* (Sulzer).

VIII. Virus-vector Relationships

A) RELATION OF NUMBERS OF INSECTS TO VIRUS TRANSMISSION

Viruliferous whiteflies reared on diseased plants of *M. parviflora* were used in tests to determine the relative efficiency of different numbers of insects in securing infection with the beet pseudo-yellows virus. The whiteflies, singly or in groups of 5, 10, 20, or 40, were allowed a 48-hr. infection feeding period on shepherd's-purse tests plants. The results (Table I) indicate that single greenhouse whiteflies are capable of transmitting the beet pseudo-yellows virus. Transmission increased markedly, however, when larger numbers of insects were used. In tests to determine other properties of the virus, groups of 20 insects were used.

TABLE I
RELATION OF NUMBERS OF GREENHOUSE WHITEFLIES TO TRANSMISSION OF BEET PSEUDO-YELLOW VIRUS

Test no.	Shepherd's-purse seedlings infected out of eight inoculated when colonized with the indicated no. of viruliferous insects/plant				
	1	5	10	20	40
	no.	no.	no.	no.	no.
1	2	7	5	6	8
2	0	1	2	3	5
3	0	4	4	5	5
4	1	7	7	8	8
5	0	2	2	5	6
6	2	7	8	8	8
Transmission (%)	10.4	58.3	58.3	72.9	83.3

B. ACQUISITION FEEDING PERIOD

The feeding time on a virus source plant required for nonviruliferous greenhouse whiteflies to become infective was studied over a feeding period range of 1 to 48 hr. After the feeding period on diseased shepherd's purse, the insects were removed in groups of 20 and placed on shepherd's-purse seedlings for a 48-hr infection feeding. The results (Table II) indicate that the insects may become viruliferous in a 1-hr feeding period. The transmission efficiency of the vectors increased with an increase in feeding time on the virus source.

TABLE II
RESULTS OF TESTS TO DETERMINE THE TIME REQUIRED FOR NON-VIRULIFEROUS GREENHOUSE WHITEFLIES TO BECOME INFECTIVE WITH THE BEET PSEUDO-YELLOWS VIRUS

Test no.	Shepherd's-purse seedlings infected out of eight inoculated with groups of 20 insects fed on the virus source for the indicated period in hr					
	1	3	6	12	24	48
	no.	no.	no.	no.	no.	no.
1	0	2	2	3	6	6
2	1	4	5	5	8	7
3	1	2	1	1	4	2
4	0	2	1	4	6	8
5	0	1	2	2	5	4
6	0	0	2	4	6	7
Transmission (%)	4.2	22.9	27.1	39.6	72.9	70.8

C. INFECTION FEEDING PERIOD

Tests designed to determine the time required for viruliferous whiteflies to transmit the beet pseudo-yellows virus were conducted using feeding periods of 1 to 48 hr with insects reared on diseased cheeseweed. Groups of 20 insects were placed on each of the shepherd's-purse test plants and were permitted to feed for designated periods. The results (Table III) indicate that viruliferous whiteflies are capable of inducing infection within a 1-hr feeding interval. Viruliferous insects induced infection after a 6-hr feeding period at a high level of efficiency.

TABLE III
RESULTS OF TESTS TO DETERMINE THE FEEDING TIME REQUIRED BY 20 VIRULIFEROUS GREENHOUSE WHITEFLIES TO INFECT SHEPHERD'S-PURSE SEEDLINGS WITH THE BEET PSEUDO-YELLOWS VIRUS

Test no.	Plants infected out of eight on which the whiteflies were allowed to feed for the indicated period in hr					
	1	3	6	12	24	48
	no.	no.	no.	no.	no.	no.
1	1	3	8	8	8	8
2	0	2	3	3	4	7
3	0	4	7	7	5	8
4	2	3	7	8	8	8
5	0	4	3	5	6	8
6	1	2	6	6	7	7
Transmission (%)	8.3	37.5	70.8	77.1	79.2	95.8

D. LATENT PERIOD OF THE VIRUS IN THE VECTOR

The latent period of the beet pseudo-yellows virus in the vector was studied by allowing nonviruliferous whiteflies to feed on a virus source for 3, 6, or 12 hr and then transferring them in groups of 20/plant to healthy shepherd's-purse for the necessary time intervals to permit testings for latent periods of 6, 12, 24, and 48 hr. The results (Table IV) indicate that the latent period in the vector, if any, is less than 6 hr.

TABLE IV
RESULTS OF TESTS TO DETERMINE THE LATENT PERIOD OF THE BEET PSEUDO-YELLOWS VIRUS IN THE GREENHOUSE WHITELY

Test no.	Feeding period on virus source	Shepherd's-purse seedlings infected out of eight inoculated with 20 insects in which the virus had the indicated latent period in hr			
		6	12	24	48
	hr	no.	no.	no.	no.
1	3	1	3	2	2
	6		4	5	2
	12			4	6
2	3	0	0	0	0
	6		1	3	1
	12			7	6
3	3	1	4	0	7
	6		6	8	5
	12			7	8

E. PERSISTENCE

The ability of viruliferous house whiteflies to retain the beet pseudo-yellows virus was determined by two methods. Whiteflies reared on diseased cheeseweed plants were transferred in groups of 20 in daily serial transfers on healthy shepherd's-purse seedlings. The results (Table V) show that all groups of insects lost transmitting ability in 6 days or less. Although insects in the different groups died at various times at the termination of the experiment (15 days) there was still an average of approximately four individuals/group.

The ability of viruliferous whiteflies to retain the beet pseudo-yellows virus when feeding on an immune host was determined by placing insects reared on diseased cheeseweed on immune cotton *(Gossypium hirsutum)* or tomato *(Lycopersicon esculentum)* plants and then testing the whiteflies at intervals by transferring them to shepherd's-purse seedlings. Under these conditions, the virus was retained by the vector for a maximum of 4 days.

TABLE V
SHEPHERD'S-PURSE SEEDLING INFECTED (+) AND NONIFECTED (-) IN DAILY SERIAL TRANSFERS USING GROUPS OF 20 VIRULIFEROUS GREENHOUSE WHITEFLIES REARED ON A BEET PSEUDO-YELLOWS SOURCE PLANT

Whitefly colony no.	Successive daily transfers[a]						
	1	2	3	4	5	6	7
1	+	+	+	+	-	-	-
2	+	+	+	+	+	-	-
3	+	+	+	+	+	+	+
4	+	+	-	-	-	-	-
5	+	+	+	+	-	-	-
6	+	+	+	+	+	-	-
7	+	+	+	+	+	+	+
8	+	+	+	+	-	-	-
9	+	+	+	-	-	-	-
10	+	+	+	-	-	-	-
11	+	+	+	+	+	-	+
12	+	+	+	-	-	-	-
13	+	+	-	+	-	-	-
14	+	+	+	+	+	-	-
15	+	+	+	-	-	-	-
16	+	+	+	+	+	-	+

[a]No seedlings were infected by the whitefly colonies in daily transfers from the eighth to the 15th day, inclusive.

IX. Discussion

Viruses transmitted by whiteflies are heterogenous. One of the two major groups of whitefly-transmitted viruses induces color deviations of the variegation type on affected leaves. The other major group induces malformation (leaf and vein thickening, or enations) on affected plants. All of the some 23 whitefly-transmitted viruses are transmitted by *Bemisia* sp. except for BPSV (transmitted by *Trialeurodes vaporariorum)* and sweet potato yellow dwarf virus (Hildebrand, 1960) (transmitted by *Trialeurodes abutilonea).*

Symptoms induced by BPYV on common host plants are quite distinct from symptoms of the other whitefly-transmitted viruses and seem to be much more like those induced by the aphid-transmitted yellowing viruses. Excluding the fact that BPYV is transmitted by *T. vaporariorum,* the virus appears to be more closely related to the aphid-transmitted viruses of the yellows group than to the typical whitefly-transmitted viruses.

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Mosaic Diseases

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A MOSAIC VIRUS OF *CANAVALIA MARITIMA* (BAY-BEAN) IN PUERTO RICO

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I. Introduction

Canavalia maritima (Aubl.) Thou. is a littoral, fabaceous weed generally found in Puerto Rico in association with *Ipomoea pes-caprae* (L.) Roth. and at times with *Vigna repens* (L.) Kuntze (Vélez and van Overbeek, 1950). This weed occurs in Florida, the Virgin Islands, Bermuda, the West Indies, continental tropical America and the Old World tropics (Britton and Wilson, 1924).

In Puerto Rico this plant species is often found affected by a mosaic virus which produces mottling, vein banding and distortion of the leaves. Due to its high incidence and widespread distribution along the coastal belt of Puerto Rico, the *Canavalia* mosaic was classed as a potentially dangerous malady. It was subjected to careful study after beans, cowpeas and soybeans were found to be particularly susceptible hosts. These researches were supported in part by the United States Agency for International Development under a contract entitled "Improvement of Tropical Production of Beans and Cowpeas Through Disease and Insect Control" and were directed to the identification of major diseases of beans and cowpeas.

II. Materials and Methods

A. HOST RANGE

Materials and methods employed in these studies, unless otherwise stated, were essentially similar to those described previously (Pérez and Bird, 1971). Affected as well as healthy plants and seeds were originally obtained from various beaches in the Municipality of Dorado, Puerto Rico. Seeds from severely affected as well as from unaffected plants were used indiscriminately since no transmission had been observed in the case of hundred of seedlings obtained from the seeds of severely mosaiced *Canavalia* vines.

1. Mechanical Inoculation

The host range was determined, for the major part, employing mechanical inoculation. A series of plants species, mainly within the legume family, were inoculated with dental cotton buds drenched in undiluted expressed sap containing carborundum (600 mesh). Test plants, at the time of inoculation, were mostly in the 4-6 leaf stage. However, legumes were generally inoculated when the primary leaves were fully expanded. Test plants were always paired to healthy controls. Success of inoculation was judged on the basis of appearance of symptoms within a reasonable period (6-20 days).

2. Inoculation Via Aphids

Aphis craccivora Koch, *Myzus persicae* Sulzer and *Dactynotus ambrosiae* Thomas were used in transmission tests. In Puerto Rico only *A. craccivora* has been found under natural conditions to occur in association with *C. maritima* (Smith *et al.*, 1963). The other aphids were tried in transmission tests although they were not presumed to be important vectors in nature. Aviruliferous stock cultures of the various aphids were established by colonizing healthy seedlings with insects obtained from presumably virus-free field plants. *D. ambrosiae* was cultured on *Bidens pilosa* L., *A. craccivora* on *Vigna unguiculata* L. (Walp.) var. Black and var. Early Ramshorn, *A. gossypii* on *Crotalaria striata* DC. and *Cucumis sativus* L., var. Black Diamond and *M. persicae* on *Nicotiana Tabacum* L., and *Datura stramonium* L. At least 20 plants each of *C. maritima, C. ensiformis* (L.) DC., *V. unguiculata* and *Phaseolus vulgaris* L. were colonized (50 aphids/plant) in a series of tests. The aphids were fasted for 15 minutes prior to being transferred to the test plants where they were allowed to feed for 24 hours before being killed with a nicotine sulfate spray. As in the case of mechanical inoculation an equal number of control plants were included in the various trials. However, uninoculated controls were maintained in separate cages. Only apterous forms were used in the aforementioned trials.

B. PHYSICAL PROPERTIES

Thermal inactivation and dilution end points were determined using techniques described by Pérez and Cortés-Monllor (1971).

C. SEROLOGY

Methods employed for serology were in general similar to those described by Pérez and Cortés-Monllor (1971).

A series of microprecipitin (van Slogteren, 1955) and gel diffusion (Ball, 1961) tests were effected. In view of the fact that the *Canavalia* virus is rod shaped, additives like free ammonia and detergent were employed in the gel diffusion tests. The final pH of the agar in the plates was 9.1. Polyethylene glycol (PEG) MW6000 was used to precipitate the virus for the microprecipitin trials (Hebert, 1963). The virus was tested against the following antisera: bean common mosaic virus, bean yellow mosaic virus, bean southern mosaic virus (cowpea strain), soybean mosaic virus, broad bean mottle, cowpea mosaic virus (Puerto Rico), cowpea mosaic virus, (Trinidad), cowpea mosaic virus (Arkansas), clover white mosaic virus, clover yellow mosaic virus and tobacco ringspot virus. The aforementioned sera were obtained from various sources but mostly from the American Type Culture Collection.

D. ELECTRON MICROSCOPY

The electron micrographs were taken with a JEOL 120 electron microscope at 80 KV. The mosaic affected *Canavalia* leaf samples were fixed with 1.5 per cent glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 2 hours at 4°C, post-fixed in a graded ethanol series and embedded in an epoxy resin. The sections, made on an MT-2 Porter-Blum microtome, were stained with uranyl magnesium acetate and lead citrate.

III. Results and Discussion

A. HOST RANGE

1. Mechanical Transmission

The virus was transmitted by mechanical means with relative ease from diseased to healthy *C. maritima* plants. Rate of infection was never less than 80 per cent. Greater percentage of infection (up to 100 per cent) was obtained when plants were inoculated at the first trifoliolate stage. Those inoculated at the primary leaf stage took more time to develop symptoms and percentage infection was less (never more than 80 per cent).

On *C. maritima* vein clearing usually became evident 7 days after inoculation. This symptom was followed by a very conspicuous dark green vein banding, puckering and various degrees of mottling and distortion of the foliage. Generally, plants had developed the whole range of mosaic symptoms 20 days after artificial inoculation (Fig. 1).

Table I shows the results of studies on the host range of the *Canavalia* virus as determined by mechanical inoculation. As a rule the aforementioned symptoms became prevalent on most of the leguminous hosts tested. Several plant species, particularly *P. acutifolius* Gray, Wright, *P. acutifolius* Gray, Wright var. latifolius, *Canavalia ensiformis*, *C. gladiata* DC., and *P. lunatus* L. var. Haba de Tocón were extremely susceptible and developed epinasty followed in some instances by acronecrosis. *P. acutifolius* var. *latifolius* developed chlorosis followed by defoliation and eventual acronecrosis. *C. ensiformis* suffered from defoliation and acronecrosis later developing a characteristic witches broom.

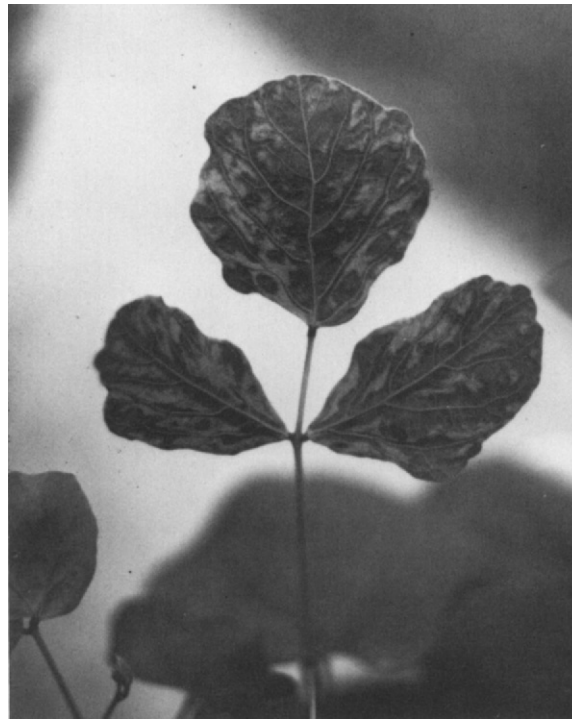

Fig. 1. Trifoliolate leaf of *Canavalia maritima* with mosaic symptoms.

TABLE I
HOST RANGE OF THE *CANAVALIA* MOSAIC VIRUS AS DETERMINED BY MECHANICAL INOCULATION

Family	Plant species treated	Number of plants successfully inoculated over plants treated	Symptoms[1/]
Amaranthaceae	*Gomphrena globosa* L.	0/15	–
Carduaceae	*Bidens pilosa* L.	0/15	–
Chenopodiaceae	*Chenopodium album* L.	4/8	NL
	C. amaranticolor Coste and Reyn.	20/20	NL
	C. quinoa Willd.	20/20	NL
	Spinacia oleracea L.	4/8	CS
Cucurbitaceae	*Cucumis sativus* L. var. Black Diamond	0/48	–
Leguminosae	*Cajanus indicus* Spreng.	8/20	M, NVN, S
	Canavalia ensiformis (L.) DC.	20/20	Ep, NVN, Ac
	C. gladiata DC.	20/20	Ep, NVN, Ac
	C. maritima (Aubl.) Thou.	40/50	M
	Cassia occidentalis L.	2/8	M
	Crotalaria striata DC.	0/10	–
	Glycine max (L.) Merr. var. Kanrich	25/43	M
	Lupinus luteus L.	4/8	M
	Medicago sativa L.	0/30	–
	Phaseolus aborigeneus Burk.	15/24	M, NVN
	P. acutifolius Gray, Wright	6/17	Ep, Ac
	P. acutifolius Gray, Wright var. *latifolius*	40/60	Ep, Ac
	P. lunatus L. var. Haba de Tocón	8/16	Ep, Ac
	P. lunatus L. var. Henderson Bush Lima	6/18	M
	P. mungo L.	4/32	M
	P. ricciardianus Tenore	0/40	–
	P. vulgaris L. var. Bountiful	22/39	Y, M
	P. vulgaris L. var. Diablo	13/56	Y, M
	P. vulgaris L. var. La Vega	0/46	–

TABLE I continued

Family	Plant species treated	Number of plants successfully inoculated over plants treated	Symptoms[1/]
	P. vulgaris L. var. Topcrop	9/30	NL
	P. vulgaris L. var. U.S. Green Refugee	0/14	–
	Pisum sativum L. var. Perfected Wales	0/25	–
	P. sativum L. var. Perfection	0/25	–
	Trifolium pratense L.	0/30	–
	T. repens L. var. Ladino	0/30	–
	Vicia faba L.	10/90	M
	Vigna aconitifolia (Jacq.) Maréchal	0/35	–
	V. angularis (Willd.) Ohwi and Ohashi	12/28	M
	V. radiata (L.) Wilczek	8/15	M, NVN
	V. umbellata (Thunb.) Ohwi and Ohashi	0/23	–
	V. unguiculata (L.) Walp. var. Black	0/20	–
	V. unguiculata (L.) Walp. var. Early Ramshorn	48/50	M
Solanaceae	*Capsicum annuum* L. var. California Wonder	0/15	–
	Datura stramonium L.	0/8	–
	Nicotiana glauca Grah.	0/8	–
	N. glutinosa L.	0/8	–
	N. tabacum L. var. Virginia 12	0/8	–
	Solanum torvum Sw.	0/10	–

[1/] Key to symptoms: NL-necrotic lesions; CS-chlorotic spots; M-mosaic; NVN-net vein necrosis; S-stunting; Ep-epinasty; Ac-acronecrosis; Y-yellowing.

Extremely striking symptoms were evident on affected bean (epinasty, chlorosis, mosaic) and cowpea (severe mosaic) plants (Fig. 2). The virus was also capable of affecting soybeans of the variety Kanrich (Fig. 3).

2. Transmission Via Aphids

The results of transmission trials are given in Table II. Of the aphids tested, *D. ambrosiae* was found to be incapable of transmitting the causal agent of the *Canavalia* mosaic. *A. craccivora, M. persicae* and *A. gossypii* proved to be fairly efficient vectors from *Canavalia* to *Canavalia.*

Aphis craccivora was not only a fairly good vector but the only species capable of colonizing *C. maritima* and *C. ensiformis* under the conditions of the tests. This aphid species is also known to breed and feed on many of the legume hosts of the *Canavalia* virus in Puerto Rico.

B. PHYSICAL PROPERTIES

In two tests conducted the virus became inactive at 55-60°C. The results of two trials on the dilution end point showed that the virus could stand dilutions of up to but not more than 10^{-3}.

C. SEROLOGY

Repeated microprecipitin and gel diffusion tests failed to reveal serological relationships between the *Canavalia* virus and BYMV and BCMV. Likewise no serological relationship was found to exist between the causal agent of the *Canavalia* mosaic and the other viruses tested.

D. ELECTRON MICROSCOPY

Figures 4 and 5 depict the *Canavalia* virus in the different formations, i.e., brush aggregates, circular lamellae and pinwheels. According to the 5th author the straw brush formations were common; however, pinwheels were rather scarce. The various inclusions were observed to be similar to those found associated with an aphid-borne cowpea mosaic virus (CMV) in Florida (Zettler *et al.*, 1967; Edwardson *et al.*, 1972; and Zettler and Evans, 1972).

IV. Conclusion

On the basis of evidence obtained from the initial host range studies the writers thought at first that they were dealing with either a strain of bean yellow mosaic virus (BYMV) or of bean common mosaic virus (BCMV). However, as studies progressed it became evident that the host range of the *Canavalia* virus was wider than that of BCMV. As shown in Table I our virus has failed to infect other species that are recognized hosts of BYMV. Serological tests have failed repeatedly to reveal relationship between the *Canavalia* virus and both BCMV and BYMV. Certainly, the evidence secured from the physical property studies is not sufficient without supporting data to establish relationships between the *Canavalia* virus and the common and yellow mosaics of bean. Evidence secured through host range and electron microscopy studies indicates that the *Canavalia* virus might be related to the Florida cowpea mosaic virus. Both the *Canavalia* and Florida viruses are capable of infecting beans. In view of the above and

Fig. 2. Bean (A) and cowpea (B) leaves from plants affected by the *Canavalia* mosaic.

Fig. 3. Soybean plant of the variety Kanrich affected by the *Canavalia* virus.

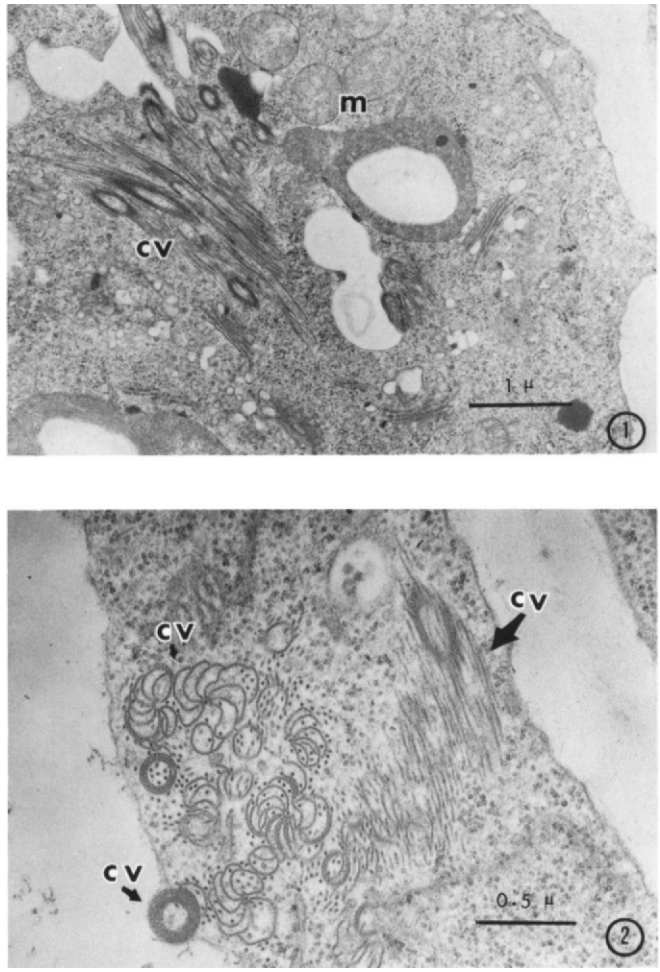

Fig 4. 1. Part of a mesophyll cell of a diseased *Canavalia* plant *(Canavalia* virus = CV). The virus appears in the form of straw brush aggregations.

m = mitochondria. Magnification X 21,400 Bar = 1 u

2. The *Canavalia* virus particles in this electron micrograph appear in three different formations: large arrow points to straw brush aggregates; medium size arrow indicates circular lamellae; small arrow marks pinwheels. The straw brush formations were most commonly observed and the pinwheels were the least common. Magnification X 43,700. Bar = 0.5 u

TABLE II
TRANSMISSION OF THE *CANAVALIA* VIRUS TO THREE LEGUMINOUS HOSTS VIA SEVERAL APHID SPECIES

Aphid species tested	Plant species tested and number of plants infected over number of plants inoculated		
	C. maritima	*C. ensiformis*	*V. unguiculata*
Aphis craccivora	4/24, 6/24, 1/6	2/3, 0/9	10/20
Myzus persicae	11/15, 2/6	0/9	
Aphis gossypii	6/28		
Dactynotus ambrosiae	0/6	0/9	

considering the ultrastructural similarities between the *Canavalia* and the Florida cowpea viruses, we are inclined to believe that these two entities are related. However to what extent they are related is not known with certainty particularly when a recent trial involving hundreds of cowpea seedlings failed to indicate seed transmission in the case of the *Canavalia* virus. In contrast, the Florida cowpea virus is transmitted through the seed of cowpea plants.

The *Canavalia* mosaic is easily propagated by aphids to beans, cowpeas, and other valuable legumes and thus should be treated as a potentially dangerous malady.

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A VEIN BANDING MOSAIC OF BEANS INCITED BY A STRAIN OF CUCUMBER MOSAIC VIRUS

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and Roger Lawson

I. Introduction

In preliminary studies a virus resembling cucumber mosaic virus (CMV) was isolated from mosaic-affected plants of the bean variety La Vega. The symptoms were as a rule extremely mild, mainly an evanescent foliar mottling accompanied by a not too well defined dark green banding of the main and secondary veins. The mosaic had a tendency to become more severe during cool spells (24°-27°C) and to disappear when the diurnal temperatures were around 32°C. Since the characteristic symptoms of this mosaic had not been observed by us in Puerto Rico and in view of the fact that a preliminary survey of the literature revealed few instances (Anderson, 1955; Ball, 1961; Hagedorn, 1959; Kaiser *et al.*, 1972; Silberschmidt, 1955) where CMV was implicated as a causal agent of bean mosaic it was thought advisable to study this disease. The malady was denominated bean vein banding mosaic virus (BVBMV) on account of its salient symptom on bean plants.

II. Materials and Methods

A. VIRUS TRANSMISSION STUDIES

1. Transmission by Mechanical Means

Materials and methods used in these particular studies were essentially similar to those described previously (Pérez and Bird, 1971, 1973). Most plants were inoculated either in the cotyledon or 4-6 leaf stage and either swabs or dental buds impregnated with sap expressed from affected plants were used for inoculation. Diseased bean, cucumber or tobacco plants were used as sources of inoculum. Tobacco provided the highest titer but the main stock of the causal agent was kept on *Phaseolus vulgaris* L. var. La Vega in order to avoid some of the viruses that infect tobacco. Virus stock plants were maintained in an insect-proòf minigreenhouse provided with a filtered air ventilating system. As virus source plants became old they were replaced with freshly inoculated ones. Plants species were identified by Mr. Roy Woodbury, taxonomist, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, Puerto Rico.

2. Transmission Via Aphids

Methods similar to those described previously (Pérez and Bird, 1973) were employed in our endeavours with aphids. *Aphis gossypii* Glover, *A. craccivora*, Koch, and *Dactynotus ambrosiae* Thomas were used in the transmission trials. *P. vulgaris* var. La Vega, *P. vulgaris* var. Diablo, *Vigna sinensis* Endl. Hassk. = *V. unguiculata* (L.) Walp., *Cucumis sativus* L. var. Black Diamond and *Musa* sp. (plantain var. Maricongo*) were inoculated via aphids. The preferences of the various aphids species for the stated hosts were recorded.

In the case of plantains, aphids collected with camel hair brushes and released on the site of inoculation (cone formed at the base of the newest partially opened plantain leaf) had a tendency to migrate without successfully inoculating the plants. The problem was surmounted by detaching tender, heavily colonized, virus-affected cucumber leaves and placing them in the aforementioned cones assuring that the undersides of the cucumber leaves (with the feeding insects) were facing inwards. Plantain leaves thus treated were lightly tied above the plug formed by the aphid-colonized cucumber leaves. It took about 24 hours for most of the aphids to migrate to the basal cone from the gradually wilting detached cucumber leaves. Good rates of infection were obtained in the test reported in Table II as well as in subsequent trials.

B. PHYSICAL PROPERTIES

The methods utilized previously by Pérez and Bird (1971) were employed to determine the thermal inactivation and dilution end points. Virginia-12 tobacco plants were used as virus source plants. To avoid long delays, three experienced persons made the inoculations during May and June 1973 in the greenhouse at an ambient temperature of approximately 38°C. The sap was expressed in an air conditioned room (22°C). Saps, once having received the differential heat treatments, were held at a temperature of approximately 5°C by partial immersion of the containers (serological tubes) in cooled tap water. In preliminary trials both *Chenopodium quinoa* Willd. as well as *C. amaranticolor* Coste &

**Musa acuminata* X *Musa balbisiana hybrid 3N* = 3X (AAB-type) variety Maricongo.

TABLE I
RANGE OF BEAN VEIN BANDING MOSAIC VIRUS ON A SERIES OF SELECTED HOSTS INOCULATED MECHANICALLY

Family	Plant species tested	Symptoms*
Amaranthaceae	*Gomphrena globosa* L.	--
Apocynaceae	*Vinca rosea* L.	--
Carduaceae	*Bidens pilosa* L.	--
Chenopodiaceae	*Chenopodium quinoa* Willd.	--
	C. amaranticolor Coste & Reyn.	LL
Cucurbitaceae	*Cucumis sativus* L. var. Black Diamond	VC, M
	Luffa cylindrica (L.) Roemerer	VC, CS, R
	Momordica charantia L.	CS, M
Leguminosae	*Cassia occidentalis* L.	--
	Glycine max (L.) Merr.	--
	Macroptilium lathyroides (L.) Urban	M, VB, P
	P. acutifolius Gray, Wright var. *latifolius*	--
	P. lunatus L. var. Sieva	VC, M
	P. ricciardianus Tenore	--
	P. vulgaris L. var. Columbia	VC, ICT, M, VB
	P. vulgaris L. var. Diablo	VC, ICT, M, VB
	P. vulgaris L. var. La Vega	VC, ICT, M. VB
	P. vulgaris L. var. Santa Ana	VC, ICT, M, VB
	Vigna aconitifolia (Jacq.) Maréchal	--
	V. mungo (L.) Hepper	--
	V. radiata (L.) Wilczek	--
	V. umbellata (Thunb.) Ohwi and Ohashi	--
	V. unguiculata (L.) Walp.	RM
Solanaceae	*Capsicum annuum* L. Var. Large Bell Hot	VC, M
	Datura metel L.	LCB
	D. stramonium L.	LCB
	Lycopersicon esculentum Mill. var. Floradel	M
	Nicotiana glutinosa L.	VC, R, M, VB
	N. tabacum L. var. Virginia 12	VC, R, M, VB
	Solanum tovurm Sw.	OL

*Key to symptoms: LL--local lesions; VC--vein clearing; M--mosaic; CS--chlorotic spots; R--ringspots; VB--vein banding; P--puckering; ICT--inward curling of leaf tips; LCB--large chlorotic blotches; OL--oak leaf; --no symptoms.

Reyn. were used as indicators, but in later tests *C. quinoa* was employed solely as it was found to be a superior local lesion indicator for the virus under study.

C. SEROLOGY

The serological relationships of BVBMV were determined via the Hennisch double diffusion, the Ouchterlony gel-diffusion (Ball, 1961) and the micro-precipitin (Slogteren, 1955) tests. Specific cucumber mosaic virus (CMV) antisera were obtained from the fifth author who acquired them originally from Drs. D. Z. Maat, R. J. Shepherd, and H. A. Scott. Several tests were made. Heterologous as well as homologous antigens were represented in some of them.

D. ELECTRON MICROSCOPY

The virus samples intended for electron microscopy were secured from the original isolate which was maintained on bean plants of the variety La Vega and frequently passed through tobacco and cucumber and back-inoculated to beans. This was done to assure that the isolate was capable of infecting and producing typical symptoms on the aforementioned hosts. The "purity" of the isolate was also checked frequently by inoculating *C. quinoa.* For visualization under the electron microscope purified BVBMV was mixed with an equal volume of 4% glutaraldehyde and stained with 2% phosphotungstic acid neutralized with KOH.

III. Results

A. VIRUS TRANSMISSION STUDIES

1. Transmission by Mechanical Means

Initially the virus was transmitted repeatedly by mechanical means to various hosts which included bean, cucumber, tobacco and other species. It was recovered from the mentioned plants as well as from single lesions on *C. quinoa.* Isolates from these hosts consistently incited the characteristic symptoms of the disease on the primary host as well as on tobacco and cucumber (which were considered by us as the diagnostic species). As shown by Table I, a number of plant species and varieties belonging to several families were successfully inoculated by mechanical means. Symptoms on the primary host, *P. vulgaris* var. La Vega were, in order of appearance, vein clearing, inward curling of the leaf tips (trifoliolates), and mild mosaic. The salient symptom, as mentioned before, was a dark green banding of the main and secondary veins which was often evident on other hosts.

On cucumber, symptomatology was reminiscent of that elicited by the local cucumber strain of CMV although at times, and depending on the temperature, the virus behaved on this host like the *Commelina* strain of CMV. The local *Commelina* strain causes shrivelling and necrosis of the stem at temperatures in the vicinity of 24°C (Bird *et al.*, 1971).

On tobacco, banding of the veins and oak-leaf pattern were the most prominent symptoms. Typical oak-leaf symptoms appeared late in the development of *Solanum torvum* Sw. plants inoculated with the virus (Fig. 1). This symptom was known to be associated specifically with various local strains of CMV. As stated previously, *C. quinoa* was found superior to *C. amaranticolor* as a local lesion indicator for the virus. However, the number of local lesions was not

Fig. 1. Oak leaf symptoms on a *Solanum torvum* leaf affected by BVBMV.

TABLE II
TRANSMISSION OF THE VEIN BANDING VIRUS FROM BEAN AND CUCUMBER PLANTS TO DIFFERENT HOSTS VIA APHIDS

Virus source plant / Test plant	Aphid species tested and plants infected over plants inoculated			Symptoms** on infected test plants
	A. *craccivora*	A. *gossypii*	A. *ambrosiae*	
Phaseolus vulgaris L. var. La Vega / *P. vulgaris* var. La Vega	0/8*	5/6	0/8	VC, ICT, M, VB
Cucumis sativus L. var. Black Diamond / *P. vulgaris* L. var. La Vega	3/8	14/14	1/6	VC, ICT, M, VB
Cucumis sativus L. var. Black Diamond / *C. sativus* var. Black Diamond	2/4	14/14	0/9	VC, CS, R
Cucumis sativus L. var. Black Diamond / *Vigna unguiculata* (L.) Walp. var. Black	0/12			
Cucumis sativus L. var. Black Diamond / *P. vulgaris* L. var. Diablo		4/4		VC, ICT, M, VB
Cucumis sativus L. var. Black Diamond / Plantain var. Maricongo (*Musa* sp.)		5/6		LS, M, Ac

*Number of plants infected per number of plants inoculated; an equal number of uninoculated control plants were included in each test. None of the control plants developed symptoms of disease.

**Key to symptoms: VC--vein clearing; M--mosaic; CS--chlorotic spots; R--ringspots; VB--vein banding; ICT--inward curling of leaf tips; LS--lenticular spots; Ac--acronecrosis.

always high even when *C. quinoa* was used as an indicator. Several factors including high ambient temperatures probably were responsible for the stated behavior of this differential host. The problem with infectivity could be surmounted in most instances by employment of the rolled leaf method of inoculation (Hildebrand, 1956). It seemed that the strain of CMV studied by us was extremely heat labile. Thermal inactivation tests revealed extreme heat sensitivity.

2. Transmission Via Aphids

The results of transmission trials with aphids are presented in Table II. Of the aphids tested in these and similar tests, *A. gossypii* was the most efficient vector. *D. ambrosiae* and *A. craccivora* proved to be poor vectors. Symptoms obtained on plantains of the variety Maricongo were quite similar to those normally induced by common CMV (Fig. 2).

In general, varieties of *V. sinensis* were excellent hosts for *A. craccivora*. Beans were disliked by this aphid, contrary to what was expected, to the extent that they would not survive for more than 24 hours after colonization. *P. vulgaris*, *V. sinensis* and *C. sativus* were not agreeable (preferred) hosts for *D. ambrosiae*. *A. gossypii* showed marked preference for *C. sativus*, *P. vulgaris* and the other cucurbits and legumes. It fed fairly well even on plantains *(Musa* sp.), although no colonies were secured on this host.

B. PHYSICAL PROPERTIES

The result of one early trial on thermal inactivation using *C. sativus* var. Black Diamond as an indicator showed that the virus became inactive at 45° to 50°C. Data obtained from subsequent trials using *C. amaranticolor* and *C. quinoa* as test plants confirmed the initial findings. The virus did not stand dilutions of more than 10^{-1} and became inactive if left standing (virus-containing, untreated sap) for 45 minutes at an air temperature of 40°C.

C. SEROLOGICAL RELATIONSHIPS

The results of a series of immunodiffusion tests were quite similar and indicated that the virus under study pertained to the common cucumber mosaic virus complex. A positive reaction was obtained when the antigen was reacted with the three CMV antisera. No reaction, as expected, occurred when the antigen was tested against the normal antisera. Very distinct precipitation bands were formed in the areas between the antigens and the corresponding CMV antisera 2 days after incubation at room temperature (24°C). Double precipitation lines were formed in the case of Shepherd's CMV antiserum. The results of subsequent tests where homologous (local CMV) as well as heterologous antigens (tobacco ringspot virus) were reacted simultaneously with the various antisera also indicated that BVBMV was serologically related to CMV.

Evidence obtained from repeated trials employing the microprecipitin test also indicated that the local bean virus was a strain of CMV.

D. ELECTRON MICROSCOPY

Particles like those which characterize normal CMV were detected upon studying the local bean virus under the electron microscope. Figure 3 shows the typical spherical particles of cucumber mosaic virus.

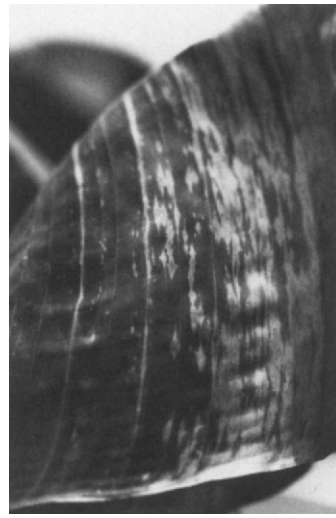

Fig. 2. Mosaic of plantains (variety Maricongo) induced by BVBMV.

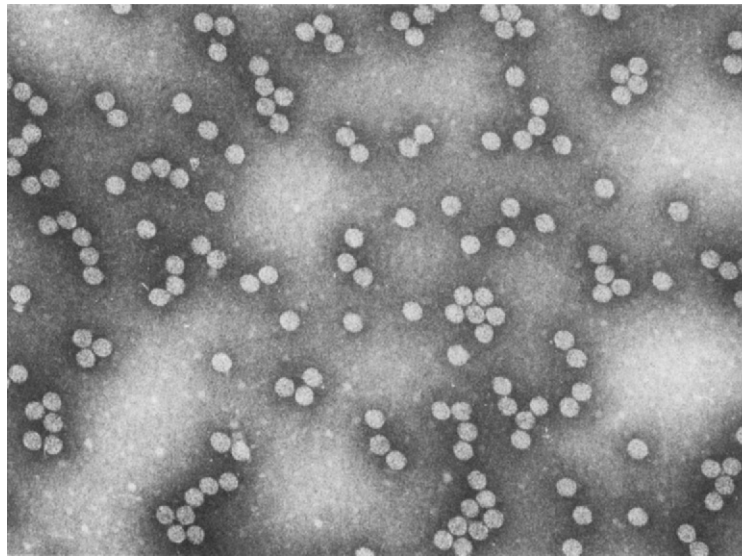

Fig. 3. Purified CMV mixed with an equal volume of 4% glutaraldehyde and stained with 2% phosphotungstic acid neutralized with KOH. Magnification – 181,000 X.

IV. Discussion

The host range of the vein-banding virus of beans resembles in general that of the celery strain of CMV (Wellman, 1934). It is also similar to the host range of the cucumber mosaic virus studied in Puerto Rico (Adsuar and Cruz-Miret, 1950). Reactions obtained on hosts such as *C. quinoa*, *Luffa cylindrica* (L.) Roemerer, *S. torvum* and plantain (*Musa* sp) of the variety Maricongo indicated that the local virus is related to CMV. The results of serological, physical property and electron microscopy studies confirmed the above findings regarding the identity of the local bean virus.

The writers believe that *S. torvum* and the aforementioned variety of plantain are excellent indicators for the presence of CMV. So far as we have been able to ascertain, the oak leaf reaction on *S. torvum* can be considered unequivocal. The fact that infection of this host can be accomplished with ease by mechanical inoculation makes the process of identification a simple one. The telltale symptom (lenticular spots) incited by CMV on plantains is diagnostic but good rates of infection can only be obtained employing aphids. So far all the isolates of CMV tested on these two hosts including the bean strain recently isolated in Illinois by Milbrath (refer to article in this volume) as well as the local *Commelina*, *Crotalaria* and *Musa* strains (mild and severe) of CMV have incited the characteristic symptoms.

Bos (1973) recently terminated studies on a new south European bean virus that is, as ours, serologically closely related to normal CMV and which, in certain hosts, elicits symptoms similar to those provoked by our local bean virus.

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ISOLATION OF A STRAIN OF CUCUMBER MOSAIC VIRUS FROM BEANS IN ILLINOIS

G. M. Milbrath, J. Bird and Josefina Sánchez

I. Introduction

Cucumber mosaic virus has a wide host range among dicotyledons and monocotyledons (Gibbs and Harrison, 1970). There are previous reports of cucumber mosaic virus (CMV) infecting beans, but the occurrences are limited to a few geographical locations (Hagedorn, 1950). Last year a virus isolate resembling CMV was found infecting beans in Illinois and has been under investigation in cooperation with Dr. Bird and associates.

II. Materials and Methods

The virus was isolated originally from *Phaseolus vulgaris* (garden bean) in a local planting. Actively growing young plants were mechanically inoculated by rubbing with cotton swabs impregnated with sap expressed from virus infected tissue. Carborundum (600 mesh) was added to the inoculum just prior to inoculation.

Aphis gossypii Glover was also used in the transmission trials. The aphids were fasted for 10 minutes, subsequently fed on infected source plants and then transferred to the basal cone of the newest unfurling leaf of a plantain *(Musa* sp.) cultivar. The plants were observed for symptoms for 2-3 weeks.

For purification and electron microscopy infected tobacco tissue was homogenized on 0.5 M phosphate buffer, pH 8.0, and clarified with diethyl ether at 5,000 g for 15 minutes. The clarified sap was then centrifuged at 100,000 g for 20 minutes and the pellet was resuspended in 0.005 M borate buffer pH 9.0 (containing 0.005 Na_2 EDTA) and clarified at 5,000 g. The supernatant was again centrifuged at 100,000 g for 90 minutes, the pellet was resuspended in a minimal amount of borate buffer and layered on to 10-40% sucrose gradients and centrifuged for 3 hr at 24,000 rpm in a S 27.1 rotor.

III. Results and Discussion

The virus induced an initial mosaic, followed by epinasty and apical necrosis in several bean cultivars such as 'Bountiful', 'Great Northern', 'Pinto', 'Red Kidney', 'Stringless Refugee', and 'Top Crop'. The virus induced a bright yellow

mosaic in systemically infected leaves of tobacco *(Nicotiana tabacum* L. 'Virginia 12', 'Xanthi'). In *Chenopodium amaranticolor* and *C. quinoa* the virus induced necrotic local lesions 5-7 days after inoculation. *Vicia faba* exhibited necrosis of the inoculated leaves, and later a severe stem necrosis that spread throughout the aerial portion of the plant. Infected cucumbers *(Cucumis sativus* L. 'Black Diamond') showed initially a mild vein-clearing followed by a generalized mosaic. Local lesions were produced on the inoculated cotyledons of *Luffa cylindrica* (L.) Roem. 4-6 days after inoculation. An oak leaf pattern developed in systemically infected *Solanum torvum.* Cowpeas, *Vigna unguiculata* (L.) Walp. 'Lalita', 'New Era', 'Prima', 'Blackeye', were susceptible to the virus and developed a generalized mosaic followed by leaf and stem necrosis. Plantain shoots inoculated via aphids developed lenticular chlorotic spots 5-10 days after inoculation. The chlorotic spots eventually coalesced and large areas of the leaf became necrotic.

Preliminary attempts to purify the virus from systemically infected tobacco were successful. After the last centrifugation (3 hr. at 24,000 rpm) a diffuse band was observed 15-25 mm below the meniscus; a comparable band was not obtained from healthy tissue prepared in the same manner. When the band was removed and stained with 1% uranyl acetate and examined in the electron microscope, icosahedral virus particles were observed. They were about 20-22 nm in diameter.

The specific symptoms incited on *Luffa cylindrica, Solanum torvum* and plantain *(Musa* sp.) by the garden bean isolate as well as the results of electron microscopy studies indicate that a strain of CMV is the cause of the new mosaic of beans in Illinois. The virus is very similar in host range and physical properties to the CMV strain isolated by Bird *et al.* (1974) in Puerto Rico which they designated bean vein-banding mosaic virus (BVBMV). The agent is also transmitted by the same aphid. It is possible that in Illinois the virus spreads in summertime to beans and cucumbers from perennial weeds. Studies are underway to characterize the virus further and to compare it with other CMV strains.

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A NEW VIRUS DISEASE OF BEANS TRANSMITTED BY CHRYSOMELID BEETLES

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I. Introduction

Symptoms of virus diseases were observed on field beans in El Salvador. Variations in symptomatology led us to suspect the presence of several viruses. Four viruses have been described in Central America: southern mosaic, common mosaic, golden mosaic, and rugose mosaic virus (Gámez, 1971, 1972; Moreno *et al.*, 1968; Murillo, 1967). All of these viruses are probably present under our conditions.

II. Isolation of the Virus

Virus isolates (presumably the same agent) were obtained from bean plants *(Phaseolus vulgaris* "27 R") infected naturally at the San Andres Experiment Station. The virus was maintained on the same variety, which was mechanically inoculated when the first true leaves were completely expanded. The extracts used for mechanical inoculations were prepared by grinding diseased leaves in a mortar with 0.05 M. phosphate buffer, pH 7.1. Prior to inoculation the leaves were dusted with carborundum (400 mesh). After inoculation, plants were rinsed with water and maintained in the greenhouse at temperatures ranging from 19° to 29°C.

The virus was transmitted mechanically and inoculated plants developed symptoms of severe infection, with deformity of the veins, blistering of the leaves and formation of enations.

III. Host Plants

Plants of different genera and families were inoculated. The virus produced local lesions on the inoculated leaves of the following varieties of *Phaseolus vulgaris:* Mex 29-N, Kentucky Wonder, Bountiful, S-182-N, Jamapa. The fol-

lowing varieties developed systemic symptoms in addition to local lesions: Stringless Green Refugee, Plentiful, Turrialba 2, and Sanilac. *P. lunatus* var. Henderson Bush also became infected. Sure Crop Wax, Col 109-R, Mex 80-R, Top Crop, Red Kidney and soybean *(Glycine max)* developed systemic symptoms only. *Vigna sinensis* var. Black eye failed to develop symptoms and the agent could not be recovered from inoculated plants.

IV. Seed Transmission

Young plants of the variety 27-R were artificially infected and used in seed transmission trials. The plants were maintained in the greenhouse until harvest. A total of 480 seedlings obtained from such seed failed to develop symptoms of disease, indicating that the virus is not seed-transmitted.

V. Transmission by Insects

The insects used were the aphid *Myzus persicae* and the chrysomelid beetles *Diabrotica balteata* Lec. and *Cerotoma ruficornis* (Olivier). Colonies of *M. persicae* were maintained on radish plants. The host plants were placed in cages covered with fine organdy cloth. Short and long periods of acquisition were used with aphids. In the short periods the aphids were starved in a petri dish for no less than one hour. Afterwards, the aphids were transferred with a fine brush to a leaf exhibiting severe symptoms, and allowed an uninterrupted acquisition feed ranging from 30 to 60 seconds. Once the acquisition period was completed, five aphids were transferred to each healthy test plant and allowed to remain on it for 24 hours.

For long periods of acquisition the aphids were transferred to the virus source without previous starvation. After 24 hours on the virus source the aphids were moved to healthy plants for 24 hours, using 5 aphids per plant. When the inoculation periods were completed, the aphids were killed with an insecticide. The virus could not be transmitted by aphids.

In transmission tests with the chrysomelids *D. balteata* and *C. ruficornis* presumably virus-free insects were collected from wild populations on corn and beans. Healthy bean plants were colonized with the beetles to make sure that they were free of the virus. For virus acquisition the insects were fed on diseased plants for 24 hours. After the acquisition feed the insects were transferred to healthy test plants at a rate of three insects per plant and maintained on these last for 24 hours. The test plants were then observed for appearance of symptoms. The virus was transmitted by both chrysomelids.

VI. Physical Properties

Sap from diseased plants was submitted to aging in vitro. The thermal inactivation and dilution end points were determined employing standard methods. The virus was infective after being held for three days at an approximate temperature of 23°C. It was infective after 10 minutes at 70°C, but inactive at 75°C. The dilution end point was between 10^{-4} and 10^{-5}. All trials were carried out using variety Mex 29-N, which gave well-defined local lesions.

VII. Discussion

The virus studied is somewhat different (symptomatology and host range) from others reported from Central America. The importance of this virus in El Salvador is, at present, minimal. It has been observed sporadically in recent years, although, it should be noted that lately its incidence seems to be increasing. The virus may become a serious problem in the future due to the abundance of its vectors in the Zapotitan Valley. Further studies are necessary to properly characterize the virus.

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SOME OBSERVATIONS ON THE SEED-TRANSMISSION OF BEETLE-TRANSMITTED COWPEA MOSAIC VIRUS

Syed Q. Haque and Geeta C. Persad

I. Introduction

The beetle transmissible cowpea mosaic virus (CpMV) is an important and widespread disease of *Vigna* spp. in Trinidad (Dale, 1949, 1953) and the Southern Caribbean (Phelps and Haque, 1973). This disease has been reported from Nigeria (Chant, 1959), Surinam (Van Hoof, 1963) and the United States of America (Smith, 1924). The Trinidad isolate of cowpea mosaic virus is transmitted by the beetle *Cerotoma ruficornis* which happens to be an important pest of *Vigna* spp. as well (Dale, 1953). Dale (1949) reported this virus to be seed-borne in asparagus bean but not in the three varieties of cowpea that he tested. It is, however, not clear from Dale's (1949) report as to whether the seeds were collected from the plants inoculated with CpMV and grown under glasshouse conditions. There are several viruses which produce more or less similar symptoms and are known to be seed-borne in *Vigna unguiculata* (McLean, 1941; Yu, 1946; Capoor *et al.*, 1947; Haque and Chenulu, 1972; Dale, 1949). The present investigations were carried out to re-examine the seed-transmissibility of CpMV in cowpea and other selections of *V. unguiculata.*

II. Materials and Methods

The virus used in these studies was obtained from the culture of the CpMV maintained in the glasshouse on *V. unguiculata* for which the physical properties, host range and transmission were initially checked and found to agree with those reported by Dale (1949). This is believed to be a representative of a severe strain of CpMV described by Agrawal (1964). The entire experiment was conducted in an insect-proof glasshouse. Seed-borne infection was tested according to the methods used by Haque and Chenulu (1972) for the aphid-transmitted mosaic disease of cowpea. Healthy plants of several selections of *V. unguiculata* were inoculated with CpMV and allowed to grow and form pods. Seeds from these pods were collected and sown in small pots filled with sterilized potting mixture. Observations were recorded as the percentage of plants showing virus symptoms. The plants were kept under observation for a period of 40 days.

III. Results and Discussion

Seeds from 7 selections of *V. unguiculata* plants affected by cowpea mosaic virus were tested for seed-borne infection according to the methods described earlier. The results are presented in Table I.

TABLE I
SEED-BORNE INFECTION OF COWPEA MOSAIC VIRUS IN DIFFERENT SELECTIONS OF *V. UNGUICULATA*

Selections of *V. unguiculata*	Number of seeds germinated	Seed transmission %
530*	80	0.0
530/1*	68	0.0
530/2*	90	3.3
530/3*	52	0.0
72*	75	4.0
Cowpea black-eye	95	5.3
Cowpea local	85	5.8

*Selections from International Institute of Tropical Agriculture, Ibadan, Nigeria.

The results presented in Table I show that seed-borne infection of cowpea mosaic virus was evident in 4 out of 7 selections of *V. unguiculata.* The percentage of transmission ranged from 3.3 to 5.8. Since the number of seeds tested was not uniformly large, the percentage of infection should not be regarded as the comparative potential of different selections tested. These results confirm Dale's (1949) finding that the Trinidad isolate of cowpea mosaic virus is seed-borne in *V. unguiculata.* Dale (1949) tested only 3 varieties of cowpea and opinioned that the seed-borne infection in untested varieties of cowpea should not be discounted. The present findings that CpMV is seed-borne in some varieties of cowpea is, therefore, not in contradiction with Dale's findings.

Since both asparagus bean and cowpea (erstwhile *V. sinensis)* are now regarded as *V. unguiculata* (Verdcourt, 1970), Dale's expression that the CpMV is not seed-borne in cowpea does not carry much significance. Zink *et al.* (1956) in their studies with lettuce mosaic virus observed that even a small percentage of seed infection could give heavy infection within the crop if the vector was active. The seed-borne infection of CpMV in *V. unguiculata* assumes special significance since the vector *(Cerotoma ruficornis)* of this virus is also an important pest of *V. unguiculata* under Trinidad conditions.

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BEETLE TRANSMISSION OF LEGUME VIRUSES

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I. Introduction

Leaf-feeding beetles have been recognized as vectors of certain plant viruses. Walters (1969) and Selman (1973) have reviewed general aspects of beetle transmission of viruses. Those beetle-transmitted viruses infecting legumes which may be of importance in the tropics will be considered in this paper. We will emphasize the associations between viruses and beetles and will suggest that a poorly understood biological phenomenon is involved in transmission. We hope this paper will help dispel the commonly held notion (Smith, 1965) that, within this group of vectors, transmission is accomplished by a simple mechanical process involving contamination of the insect's mouthparts.

II. The Viruses

Beetle-transmitted viruses all have polyhedral particles approximately 28 nm in diameter. They are easily transmitted mechanically, are very stable, and are highly antigenic. No vector other than beetles has been found for any of these viruses. They fall into several groups distinguishable on the bases of their serological reactions and the number of centrifugal components which can be demonstrated in purified preparations.

A. THE COWPEA MOSAIC VIRUS GROUP
(COMOVIRUS, HARRISON *ET AL.*, 1971).

This virus group contains the most numerous and widely distributed beetle-transmitted viruses (Table I) in such legumes as bean, cowpea, and soybean. Seed transmission has been reported for some members in some hosts. Three centrifugal components, usually referred to as top, middle, and bottom components, are characteristic. The group is composed of many viruses or strains which are related serologically. Serological differences, however, as well as host reactions are used to distinguish strains and viruses. In the literature the terms "isolate," "virus," and "strain" have been randomly used in this group and the terms do not denote degrees of differences or similarities.

B. THE SOUTHERN BEAN MOSAIC VIRUS GROUP (SHEPHERD, 1971).

One centrifugal component in purified preparations is characteristic of viruses in this group. The viruses are typically very stable and have a high thermal inactivation point (near 90°C). Seed transmission has been reported in bean and cowpea.

C. THE COWPEA CHLOROTIC MOTTLE VIRUS GROUP
(BROMOVIRUS, HARRISON *ET AL.*, 1971).

The viruses in this group also have one centrifugal component in purified preparations, but they are much less stable than southern bean mosaic virus (SBMV) and have thermal inactivation points near 70°C. Our recent studies have shown that cowpea chlorotic mottle (CCMV) and bean yellow stipple (BYSV) viruses are serologically related. BYSV was described from bean by Zaumeyer and Thomas (1950). The virus was also isolated from soybean (Walters, 1958) and from bean in Central America (Gámez, 1972a).

III. The Beetles

Several families of beetles contain members which have been recognized as vectors of plant viruses (Table I). The most widespread and important vectors of most of the legume viruses mentioned in Section II are the leaf beetles, which are classified in the subfamily Galerucinae of the family Chrysomelidae. The genus *Cerotoma* contains two species which attack agriculturally important legumes (Nichols *et al.*, 1974). *C. ruficornis* occurs in Florida and Texas, throughout Central America and the Caribbean islands, and into northern Venezuela. *C. trifurcata* is present in large numbers in southern United States and also in Puerto Rico. Species of *Diabrotica*, commonly referred to as cucumber beetles, are widely distributed throughout the United States, Central America, and South America on many different hosts including legumes (Smith, 1966). Species of *Acalymma*, *Ootheca*, and *Colaspis* also vector certain viruses.

The flea beetles (subfamily Halticinae, family Chrysomelidae) have been shown to transmit several viruses in hosts other than legumes. Only limited information is available about transmission of legume viruses by this group of beetles. *Systena sp.* is a vector of cowpea mosaic virus (CPMV) in Costa Rica.

A recent addition to the list of vectors from Coleoptera is *Epilachna varivestis*, the Mexican bean beetle, which is a member of the Coccinellidae. This

species is widespread in North America. Other leaf-feeding members of this genus are found in various areas of the world.

Epicauta vittata in the family Meloidae transmitted bean pod mottle virus (BPMV) at low levels.

IV. Virus-Beetle Relationships

The beetle-transmitted viruses can be acquired by their vectors during acquisition feeding periods of 24 hours or less and can be transmitted immediately and efficiently. With many of the virus-vector associations a high percentage of beetles transmit virus for 2 days (Walters, 1969). The rate of transmission thereafter drops rapidly to a low percentage, but some beetles occasionally transmit virus to several plants over an extended period of time. In the case of the cowpea strain of southern bean mosaic virus (CP-SBMV) and *C. trifurcata.* virus retention and transmission have been recorded for as long as 19 days (Walters and Henry, 1970).

It has often been assumed that virus transmission is accomplished by contamination of the mouthparts of a beetle (Smith, 1965). The mechanism of transmission, however, is not explained this easily. We do not know why beetles can transmit certain viruses and why they cannot transmit certain other viruses such as tobacco ringspot virus (TRSV) or the cowpea strain of tobacco mosaic virus (CP-TMV). Viruses have been detected in the hemolymph, regurgitant, and fecal material of viruliferous beetles but none of this information defines the mechanism of virus transmission.

A. RETENTION OF TRANSMISSIBILITY

Retention of ability to transmit virus is probably related to a variety of factors, many of which are incompletely understood. Both Walters, (1969) and Selman (1973) suggest that retention time is a characteristic of each particular virus. Those viruses which are transmitted for only 1 or 2 days fall into one group and the other group contains those viruses transmitted for longer periods of time. Recent studies (Fulton and Scott, 1974) indicate that the retention period can be related to the particular beetle involved. When *E. varivestis* was compared with *C. trifurcata* it was found that CPMV, BPMV, SBMV and CP-SBMV were rarely transmitted beyond one day by *E. varivestis* whereas *C. trifurcata* transmitted these viruses for longer periods of time. Gámez (1972b) showed that bean rugose mosaic virus (BRMV) was transmitted by *C. ruficornis* for 7 to 9 days but *D. balteata* and *D. adelpha* transmitted the virus only 1 to 3 days.

Environmental factors may be involved in virus retention. Walters *et al.*, (1972) have shown that BPMV is retained by a low percentage of beetles *(C. trifurcata)* during an overwintering period. This period of several months far exceeds the usual retention of approximately 7 days (Sanderlin, 1973) under normal feeding conditions.

Under active feeding conditions the time or amount of acquisition feeding may affect the length of time the virus is transmitted, but the degree of effect apparently varies with different viruses and vectors. Dale (1953) found only a slight increase in the length of retention time of CPMV by *C. ruficornis* when a 24 hour acquisition feed was compared with a 5 minute acquisition feed. Jansen

and Staples (1971) also found a correlation between virus retention and the extent of feeding of *C. trifurcata* and *Diabrotica* spp. on CPMV-infected hosts. A much greater effect on retention was observed by Walters and Henry (1970) using CP-SBMV and *C. trifurcata.* After a 30 minute acquisition feed the ability to transmit virus was retained for only 5 days as compared with 19 days retention after a 24 hour acquisition feed.

The acquisition host, the transmission host, or both may affect the length of time a beetle will transmit virus. Jansen and Staples (1970a) found that *C. trifurcata* retained CPMV much longer when the virus was acquired from a cowpea source than from a soybean source. They further demonstrated that retention of ability to transmit virus was much greater using cowpea rather than soybean as a transmission host.

B. VECTOR EFFICIENCY

Efficiency in transmitting virus during the first day after acquisition is not necessarily correlated with retention time. For example, *C. trifurcata* often transmits CPMV, BPMV, SBMV, and CP-SBMV for several days while *E. varivestis* rarely transmits these viruses beyond one day. However, the relative amount of initial transmission with either species of beetle is essentially the same.

In other associations different species of beetles may differ in relative amounts of initial tranmission. Jansen and Staples (1971) obtained a higher initial rate of transmission of CPMV with *C. trifurcata* and *D. balteata* than with *D. undecimpunctata, D. virgifera* and *A. vittatum*, Gámez (1972b) found that *C. ruficornis* had a much higher rate of transmission of BRMV the first day than did *D. balteata* or *D. adelpha.*

The species of plants involved, either as a virus acquisition source or as a transmission host, may be responsible for differences in transmission the first day. Jansen and Staples (1970a) showed that with soybean as the acquisition host there was much less initial transmission of CPMV by *C. trifurcata* than when cowpea was the source. The rate of initial transmission was also low when soybean was the transmission host even though acquisition was by feeding on the more favorable host, cowpea. Our studies indicate that, with CCMV and *E. varivestis,* beetles become viruliferous from feeding on infected bean but not from infected cowpea although viruliferous beetles can transmit CCMV to cowpea as well as to bean. Jansen and Staples (1970a) obtained no transmission of CPMV by *D. undecimpunctata* when soybean was the test host.

C. VIRUS ASSOCIATION WITH HEMOLYMPH

Freitag (1956) first demonstrated that squash mosaic virus could be detected in the hemolymph of viruliferous cucumber beetles. Additional studies by Slack and Scott (1971), Slack and Fulton (1971), and Sanderlin (1973) showed that other viruses in the CPMV and SBMV groups could be detected in hemolymph. We have recently shown that viruses in the CCMV group also appear in the hemolymph of viruliferous beetles.

The presence of virus in the hemolymph occurs regardless of whether a long or a short retention period is involved. With *E. varivestis,* which has a short retention period for CPMV, BPMV, SBMV, and CP-SBMV, virus can be detected

in the hemolymph (Fulton and Scott, 1974). Slack and Fulton (1971) suggested, and expanded studies on the association of virus with beetle hemolymph seem to confirm, that virus in the hemolymph is characteristic of beetle-transmitted viruses.

Injection of purified virus into the hemocoel results in transmission to plants on which the beetles subsequently feed (Slack and Scott, 1971; Sanderlin, 1973; Fulton and Scott, 1974). From our studies it appears that beetles become viruliferous following injection of virus into the hemocoel in all virus-vector combinations.

Viruses which are not transmitted by beetles such as TRSV or CP-TMV cannot be detected in the hemolymph of beetles which have fed on infected plants nor do beetles become viruliferous following injection of purified virus into hemocoels (Slack and Fulton, 1971, and unpublished). Perhaps if we could determine why these viruses are not transmitted it would lead us to an understanding of the mechanisms involved in beetle transmission of plant viruses.

In spite of the demonstrated correlation of viruses in the hemolymph of beetles with virus vectoring, the significance of this phenomenon is not understood. Our observations indicate that the virus appears in the hemolymph very quickly after feeding is initiated on an infected host. We know from the injection studies that virus in the hemolymph can be involved in transmission, but the method by which this is accomplished is puzzling. It might be suggested that virus moves from the hemocoel into the midgut and that mouthparts are contaminated by regurgitation but, as we will indicate later, virus in the regurgitant is not indicative of transmission. It is also known that many beetles leak hemolymph from articulations of mandibles and legs, a process known as reflexive bleeding (Happ and Eisner, 1961). We have detected CPMV in hemolymph of *E. varivestis* collected from reflexive bleeding. Techniques, however, have not been devised to effectively evaluate the significance of reflexive bleeding from mandibles or legs in transmission.

The fate of virus in hemolymph of beetle vectors has also been studied. Slack and Scott (1971) showed that virus could be detected in the hemolymph of bean leaf beetles for extended periods of time after they acquired CP-SBMV by feeding. The percentage of beetles with detectable virus decreased with time similar to the reduction of transmission by beetles. A similar reduction of detectable virus with time was apparent when virus was injected into beetles. Sanderlin (1973) demonstrated that CPMV injected into *C. trifurcata* could be detected for longer periods of time than injected BPMV. He postulated that a selective response of the beetle might account for a faster elimination of BPMV.

In the studies mentioned above viruses in the hemolymph were detected by inoculation of systemic hosts. When local lesion hosts were used to quantify virus in the hemolymph, virus was rarely detected. When hemolymph was diluted, it was apparent that an inhibitor was affecting the detection of virus. It was necessary to dilute the hemolymph approximately 1:100 with buffer to get beyond the effect of the inhibitor upon CPMV and SBMV. Virus was then detected in hemolymph of viruliferous beetles at dilutions of as great as 1:1000. The amount of virus in the hemolymph has not yet been effectively determined for any virus-vector association but since it can be detected at these high dilutions, it must be greater than we anticipated.

D. VIRUS ASSOCIATION WITH REGURGITANT

Markham and Smith (1949) pointed out that beetles which vector viruses lack salivary glands and regurgitate during feeding. Since that report it has often been accepted that virus in beetle regurgitant satisfactorily explains the mechanism of transmission of virus through contamination of mouthparts. Selman (1973) contends that beetles do have 'salivary' glands and that he has never seen chrysomelid beetles regurgitate while feeding naturally. Jansen and Staples (1970a, 1971), however, observed copious amounts of regurgitated fluid during feeding by *C. trifurcata, D. balteata,* and *D. undecimpunctata.* Our observations with *E. varivestis* and *C. trifurcata* indicate that these beetles regurgitate during feeding. *E. varivestis* were induced to drink from an aqueous solution of bromophenol blue and then allowed to feed on bean leaves. Regurgitation of the dye was readily observed at many feeding sites.

Beetles fed on infected plants contain virus in regurgitant. These observations have been made with induced regurgitation. This was accomplished either by placing a heated scalpel behind the beetle or, more commonly, by teasing the mouthparts with a needle. Regurgitant was easily collected in a capillary tube. Virus could be detected when regurgitant was inoculated to susceptible hosts. Slack and Fulton (1971) detected BPMV and TRSV in regurgitant of *C. trifurcata* fed on infected plants. We have since detected SBMV, CP-SBMV, TRSV, and CP-TMV in regurgitant of *E. varivestis* fed on infected plants. When virus in the regurgitant was quantified on local lesion hosts, it was found that the infectivity was equal to or greater than the infectivity of an equal volume of sap extracted from the acquisition host of the beetles. Regurgitant is not a hostile medium for virus, for we have stored regurgitant containing SBMV or CP-TMV at room temperature for seven days and have found that virus activity is maintained at a level equal to virus in plant sap stored under identical conditions.

Virus can be detected in regurgitant for several days following feeding on an infected plant. Virus disappears with time but appears again when the beetle is given another acquisition feeding. Virus in the regurgitant, however, cannot be correlated with the ability of a beetle to transmit virus. In studies with *E. varivestis,* CP-SBMV and SBMV were detected in the regurgitant for several days after the beetle ceased to transmit virus.

Selman (1973) states, "That beetles can be made to regurgitate fluid which is infective is no proof that this is the natural mode of transmission". We agree with this, especially when we consider that non-vectored viruses such as TRSV and CP-TMV are detectable in regurgitant. Slack and Fulton (1971) showed that both BPMV and TRSV were present in regurgitant of *C. trifurcata* fed on infected plants but only BPMV was transmitted by the beetles. We have attempted many times to transmit CP-TMV with *E. varivestis* and *C. trifurcata* with no success. Furthermore, beetles were unable to transmit CP-TMV when purified virus was injected into the hemocoel or when mouthparts were intentionally contaminated with purified virus or infectious regurgitant.

E. VIRUS ASSOCIATION WITH FECES

Freitag (1956) demonstrated that virus could be detected in fecal material of cucumber beetles fed on squash mosaic virus-infected plants. Slack and Fulton (1971) detected BPMV in feces of viruliferous beetles. We have detected

CPMV in feces of *C. trifurcata* for six days and *E. varivestis* for four days following feeding on infected plants. Non-vectored viruses such as TRSV (Slack and Fulton, 1971) and CP-TMV are also readily detected in feces of beetles feeding on infected plants. Virus in feces apparently is not correlated with any mechanism of transmission.

V. Projections

An evaluation of all the information on vectoring of legume viruses by beetles does not present a clear picture of the mechanisms which are involved. A seemingly simple process which has been assumed to only involve contamination of beetle mouthparts (Smith, 1965) is in reality a complex biological phenomenon. An understanding of the way in which virus in the hemolymph of beetles is involved in the transmission process seems to be the key to understanding this phenomenon.

Under field conditions factors related to occurrence and spread of beetle-transmitted viruses present a complexity of variables. In temperate areas in southern United States there is a period of overwintering for both beetles and viruses. *C. trifurcata* overwinters as an adult and it has been shown that BPMV can overwinter in adult beetles (Walters *et al.*, 1972). The viruses may also overwinter in perennial legumes (Moore *et al.*, 1969), but the relative importance of the methods of overwintering in different situations is not known. The seed-borne nature of some of the beetle-transmitted viruses may be a factor. Little information is available on effects of migratory habits of beetles (Smith, 1966; Smith and Allen, 1932) on occurrence and spread of viruses.

An entirely different situation is encountered in tropical areas where beetles are active throughout the year. Furthermore, the small farmers in the tropics grow multiple crops each year and the land is cropped continously. A variety of different crops are grown in sequence and usually two or more crops are in the ground simultaneously. Obviously, cropping associations and sequences may have a striking effect on beetle populations, relative abundance of different beetle species, and beetle movement, and consequently may affect virus amount, type, and spread.

In both the temperate and tropical areas a lack of information on the variables involved hampers efforts to control the problems. The damage caused by beetles is a factor by itself. Such damage is exaggerated by the presence of viruses. Various species of beetles may exhibit a host preference. In addition, there is evidence that, with some viruses, certain legumes are poor sources for virus acquisition and some are poor transmission hosts. The complex relationships that exist among the viruses, the beetle species, and the various cropping situations will have a bearing on methods used to improve the yield of legumes, including breeding and selection for resistance to viruses or beetles.

TABLE I

BEETLE VECTORS OF LEGUME VIRUSES

Virus	Vector	References
Cowpea Mosaic (Comovirus) Group		
Bean pod mottle	*Cerotoma trifurcata*	Ross, 1963; Walters, 1964a
	Diabrotica balteata	Horn *et al.*, 1970
	Diabrotica undecimpunctata	ibid.
	Colaspis flavida	ibid.
	Colaspis lata	ibid.
	Epicauta vittata	Patel and Pitre, 1971
Bean pod mottle, J-10	*Cerotoma trifurcata*	Moore and Scott, 1971
Bean rugose mosaic	*Cerotoma ruficornis*	Gámez, 1972b
	Diabrotica balteata	ibid.
	Diabrotica adelpha	ibid.
Cowpea mosaic	*Cerotoma trifurcata*	Smith, 1924
	Systena sp	Gamez, unpublished
Cowpea mosaic, Arkansas	*Cerotoma trifurcata*	Walters and Barnett, 1964
	Epilachna varivestis	Fulton and Scott, 1974
Cowpea mosaic, severe and yellow strains	*Cerotoma trifurcata*	Jansen and Staples, 1971
	Diabrotica balteata	ibid.
	Diabrotica undecimpunctata	ibid.
	Diabrotica virgifera	ibid.
	Acalymma vittatum	ibid.
	Epilachna varivestis	Jansen and Staples, 1970b
Cowpea mosaic, Trinidad	*Cerotoma ruficornis*	Dale, 1949
Cowpea mosaic, Cuba	*Cerotoma ruficornis*	Kvicala *et al.*, 1970
Cowpea mosaic, Nigeria	*Ootheca mutabilis*	Chant, 1959
Quail pea mosaic	*Cerotoma trifurcata*	Moore, 1973
Southern Bean Mosaic Virus Group		
Southern bean mosaic	*Cerotoma trifurcata*	Walters, 1964b
	Epilachna varivestis	Fulton and Scott, 1974
Southern bean mosaic, cowpea	*Cerotoma trifurcata*	Walters, 1965
	Epilachna varivestis	Fulton and Scott, 1974
Cowpea Chlorotic Mottle (Bromovirus) Group		
Cowpea chlorotic mottle, *Desmodium* isolate	*Cerotoma trifurcata*	Walters and Dodd, 1969
	Diabrotica undecimpunctata	ibid.
Bean yellow stipples	*Cerotoma ruficornis*	Gámez, 1972a
	Diabrotica balteata	ibid.
Cowpea chlorotic mottle	*Epilachna varivestis*	Fulton, unpublished
Broad bean mottle	*Acalymma trivittata*	Walters & Surin, 1973
	Diabrotica undecimpunctata	ibid.
	Colaspis flavida	ibid.

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Bacterial Diseases, Chemical Control, and Ecology of Pathogens

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EFFECT OF SEED-BORNE BACTERIA IN SOYBEAN ON GERMINATION AND EMERGENCE

James B. Sinclair

I. Introduction

There are at least five genera of bacteria reported to be seed-borne in soybean (Noble and Richardson, 1968). Among these is *Pseudomonas glycinea,* causal agent of bacterial blight of soybean *(Glycine max). Bacillus* spp. were reported to be associated with soybean seeds in India (Singh *et. al.,* 1973) and *Bacillus* sp. and *B. lathyri* were isolated from soybean leaves showing symptoms of a bacterial blight (Adams, 1923; Clinton, 1916; Pethybridge, 1926). Dadson and Hume (personal communication), University of Ghana, reported a major problem of getting poor emergence of soybeans at high soil temperatures. R. J. Williams (personal communication), IITA, Nigeria, reported a complete kill of soybean seeds in field trials at Ibadan when seeds were planted in soil with temperatures between 33°-37°C. He found a bacterium associated with each deteriorated seed examined. During routine examination for seed-borne organisms in soybean, we found that a bacterial growth commonly occurred over the surface of some seeds when surface-sterilized and plated on synthetic media (Nicholson and Sinclair, 1971; Nicholson, *et al.,* 1973a; Nicholson, *et al.,* 1973b; White, *et al.,* 1972). We have considered the bacterium to be *P. glycinea,* but *Bacillus* spp. may also play an important role.

II. Results

P. glycinea (and/or *Bacillus* spp.) inhibited germination of soybean seeds (Nicholson and Sinclair, 1971; Nicholson, *et al.,* 1972a, Nicholson, *et al.,* 1973b; White, *et al.,* 1972). Two isolates of the bacterium were recovered from infected seeds and were distinguished *in vitro* in that one had a smooth surface and margin; and the other, a rough surface and margin. Both isolates were tentatively identified as *P. glycinea* by their identical reaction to a series of microbiological tests as that of a known culture of *P. glycinea.* When suspensions of our two isolates were infiltrated by vacuum into sterilized 'Amsoy' seeds, germination was significantly inhibited (death of seed) to 45% by the rough-

margined isolates, and was significantly delayed to 84% by the smooth-margined isolates, as compared to 90% germination of the control seeds. The known culture of *P. glycinea* inhibited germination 68%. The bacterium was isolated from 17 seed lots of 'Lee 68' collected from five states. Recovery ranged from 4 to 54% among the individual lots (Table I). The incidence of the bacterium was correlated with the inhibition of germination both in naturally infested seeds and artificially inoculated seeds. Similar results were obtained from studies on soybean seeds grown in India (Nicholson, *et al.*, 1973a).

TABLE I
CORRELATION BETWEEN INCIDENCE OF *PSEUDOMONAS GLYCINEA* IN 17 SEED LOTS OF LEE 68 SOYBEANS WITH PERCENT GERMINATION IN VITRO AND PERCENT FIELD EMERGENCE AT THREE LOCATIONS, 1970.

Lot No.[a]	In vitro tests[b]		% Field Emergence				
	% Seed with *P. glycinea*	% Germination	La.[c] April	La.[c] Oct.	Ky.[d] May	Miss.[e] May	Miss.[e] June
1	22	64	54	13	59	84	27
2	41	63	48	11	54	80	36
3	55	34	11	1	18	38	11
4	52	19	12	5	19	29	12
5	8	90	80	32	85	98	72
6	14	74	73	57	62	85	80
7	35	68	78	69	65	83	78
8	4	96	83	61	88	96	88
9	3	87	83	67	78	95	86
10	11	84	90	56	80	95	88
11	9	81	78	52	71	93	75
12	20	68	70	43	73	88	78
13	8	96	92	69	84	98	87
14	9	91	83	66	81	97	83
15	9	87	72	30	86	96	73
16	64	82	85	38	78	96	77
17	17	89	85	75	86	99	88
Avg.	23	75	69	44	69	85	67
Correlation coefficient with % p[f]		-.72	-.64	-.61	-.69	-.67	-.65

a/ Lots 1-4 from Louisiana, 5-7 from Mississippi, 8-12 from South Carolina, 13-16 from Texas, and 17 from Illinois.
b/ Based on 108 surface-sterilized seeds/lot assayed for internally borne micro-organisms on nine differential media.
c/ Based on four replications of 100 seeds each. Stand counts taken 28 days after planting at Louisiana State University.

d/ Based on six replication of 200 seeds each. Stand counts taken 5 days after planting at University of Kentucky.
e/ Based on six replications of 50 seeds each. Stand counts taken 18 and 22 days after planting for May and June, respectively, at Mississippi State University.
f/ % level of significance = -.590.

Thirty lots of 'Lee 68' (36,000 seeds) were bioassayed for the presence of the bacterium by plating 100 seeds/replicate on moist Kimpac cellulose pads in 15-cm diameter culture plates. The plates were incubated for 3 days at either 20°, 25°, 30°, or 35°C and examined for the presence of bacterial growth. All seeds from which colonies resembling *Pseudomonas* spp. (and/or *Bacillus* spp.) developed, did not germinate. There were the two colony types, rough and smooth, described by Nycholson and Sinclair (1971) as variants of *P. glycinea.* The bacterium was recovered from a significantly greater percentage of seeds incubated at 35°C than at the other three temperatures. Occurrence of the bacterial colonies in all seed lots significantly decreased with decrease in incubation temperature.

In studies using constant soil-temperature tanks, White *et al.* (1972), showed that the emergence of soybean seedlings inoculated with the rough colony isolate was significantly reduced at 20°, 25°, 30°, 35°, and 40°C. Emergence from inoculated seeds was greater at 30°C than at either 25 or 35°C. Emergence occurred at 40°C, but seedlings did not develop beyond the cotyledonary stage.

Studies (unpublished data) from our laboratory showed that streptomycin sulfate controls these bacteria on treated seeds, but is phytotoxic. Also, hexachlorophene showed some promise, but it is toxic to humans and may be of restricted use. R. W. Williams (personal communication) suggested that placing light-colored straw over newly planted soybean beds would reduce losses due to these bacteria in high temperature soils.

III. Conclusion

The bacteria associated with soybean seeds can cause severe losses in stands and emergence of seedlings in the tropics, particularly when planted in soils with temperatures of 30°C or more. Our studies have shown that these bacteria can significantly reduce stands in soils of the temperate region. We have found that there are several bacteria involved in the seed deterioration, probably spp. of *Pseudomonas* and *Bacillus.* The exact role of each bacterium and their interaction in causing this condition is being studied.

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THE CONTROL OF COWPEA DISEASES IN THE IITA GRAIN LEGUME IMPROVEMENT PROGRAM

R. J. Williams

I. Introduction

The cowpea *Vigna unguiculata* (L) Walp., an important supplier of protein to the people in tropical regions particularly in Africa, is subject to attack by pathogens throughout its seedling and adult life. Members of the major groups of plant pathogens, fungi, bacteria, viruses and nematodes, can all cause disease in the cowpea, and all parts of the plant can be affected. The Grain Legume Improvement Program (GLIP) of the International Institute of Tropical Agriculture (IITA) has taken the cowpea as its major species in its effort to increase in quantity and quality the availability of protein for the people of the humid tropics. The control of diseases is one of the essential requirements for the development and maintenance of a high level of cowpea production. The major emphasis in GLIP pathology is on the identification and utilization of host plant resistance. However, the possibilities of chemical control of some of the diseases have also been examined.

II. Disease Control by Fungicides

A. SEEDLING MORTALITY

Seedling mortalities of up to 75% with both pre- and post-emergence deaths have been observed at IITA in cowpeas planted during cool wet weather. The two major pathogens involved are *Rhizoctonia solani* Kühn and *Pythium aphanidermatum* (Edson.) Fitz. As an immediate control measure was required and as it was unlikely that resistance would be found in such young seedlings to these unspecialized soil-borne pathogens a major effort was made to find suitable fungicide seed treatments. After preliminary greenhouse pot tests with pure cultures of the pathogens four fungicides were selected for use in field trials. In 1972 the fungicides benomyl (Benlate), chloroned (Demosan 65W), carboxin

(Vitavax), and thiram (Arasan - 50 - Red) were tested singly and in various combinations as seed dressings at the rate of 2g product/kg seed in ten field plantings from March through October. The most effective treatments were Demosan and Vitavax (Table I). However, Vitavax caused a yellowing of primary leaves and a slight stunting of growth and for this reason was not included in the final two trials in late September and October in which the greatest amount of seedling mortality occurred. Treatment with Benlate was ineffective, and seed thus treated gave a lower mean and minimum establishment than untreated seed.

TABLE I
MEAN AND MINIMUM PERCENT ESTABLISHMENT OF PRIMA COWPEA 22 DAYS AFTER PLANTING SEED TREATED WITH VARIOUS FUNGICIDES IN TEN FIELD PLANTINGS IN 1972

Treatment*	Mean	Minimum
Demosan	86	79
Demosan + Arasan	86	75
Arasan	84	68
Vitavax**	89	77
Vitavax + Arasan	87	77
Benlate + Arasan	81	69
Benlate	64	30
Untreated	69	46

*Fungicides used as dry dressings at 2g product(s)/kg seed.
**Vitavax data from only eight trials.

In 1973 the fungicides Demosan and Arasan-75 were tested at two rates, singly and in combination, at four locations in southern Nigeria. The mean and minimum establishment data (Table II) indicate that Demosan provided the most effective and stable control of seedling mortality.

TABLE II
OVERALL MEAN AND MINIMUM PERCENT ESTABLISHMENT OF PRIMA COWPEA 22 DAP SEED TREATED WITH VARIOUS FUNGICIDES IN PLANTINGS AT FOUR LOCATIONS IN SOUTHERN NIGERIA, OCTOBER 1973

Treatment		Percent Establishment	
Fungicide(s)	Rate (g/kg seed)	Minimum	Mean (s. d.)
Demosan	4	83.9	86.6 (2.3)
Demosan	2	81.2	83.8 (2.2)
Demosan + Arasan-75	4 + 2	81.2	86.3 (3.7)
Demosan + Arasan-75	2 + 4	80.1	86.3 (3.6)
Demosan + Arasan-75	4 + 4	79.5	84.9 (4.7)
Demosan + Arasan-75	2 + 2	79.5	85.4 (5.3)
Arasan-75	4	76.4	84.9 (5.6)
Arasan-75	2	73.2	78.9 (5.5)
Untreated	-	49.5	68.0 (14.5)

Demosan was also successful, alone and in combination with the insecticide carbofuran (Furadan), as a seed treatment for two cowpea varieties in eight plantings (March through October) in GLIP agronomy trials in 1973 (Table III).

TABLE III
OVERALL MEAN AND MINIMUM PERCENT HILL ESTABLISHMENT OF PRIMA AND PALE GREEN COWPEA, 22 DAP SEED TREATED WITH DEMOSAN AND FURADAN, IN EIGHT PLANTINGS (MARCH THROUGH OCTOBER, 1973) AT IITA

Treatment		Prima		Pale Green	
Chemical(s)	Rate(s)	Minimum	Mean	Minimum	Mean
Demosan	0.2% a.i.	92.4	95.8	84.3	94.5
Demosan + Furadan	0.2% a.i. + 1.0% a.i.	84.3	91.2	68.1	83.3
Furadan	1.0% a.i.	63.1	75.2	47.2	78.0
Untreated	-	59.6	74.7	47.2	84.3

Thus in 22 field plantings in southern Nigeria during 1972 and 1973, the fungicide Demosan used as a dry seed dressing at rates of 2g and 4g/kg seed gave a high-level and stable control of seedling mortality in cowpea.

B. WET STEM ROT

Cowpea wet stem rot, caused by *Pythium aphanidermatum*, is characterized by a grey-green water-soaked girdle of the stem extending from soil level up to and sometimes including the lower portions of the lower branches. Infected plants quickly wilt and die. Field incidence normally ranges between 0.5-10.0% although occasional incidences of 30% have been observed. The occurrence of 5-10% stand reduction due to wet stem rot is unlikely to cause significant yield reduction in cowpea but is important in plots of valuable breeding materials and experiments such as population and spacing studies. In an attempt to determine a control method for this disease several fungicides were tested on Prima cowpea in two seasons in 1973. None of the fungicide treatments gave a wet stem rot incidence significantly lower than the untreated plots, and the benzimidazole fungicides, as had been observed in previous years, gave greatly increased wet stem rot incidence compared with non-treated plots (Table IV).

TABLE IV
MEAN NO. OF PLANTS PER PLOT OF PRIMA COWPEA WITH WET STEM ROT IN PLOTS TREATED WITH VARIOUS FUNGICIDES, OR UNTREATED WITH FUNGICIDES AND DAMAGED AT BASE OR NON-DAMAGED

Treatment	1st Season 1973	Second Season 1973	$\bar{X}$
Benlate*	26.7 a***	24.0 a	25.4
BASF 2460F	21.3 ab	17.3 ab	19.3
Tecto-40	14.7 bc	14.3 bc	14.5
Perenox	8.7 cd	9.0 c	8.9
Dithane M-45	8.3 cd	8.3 c	8.3
Arasan-75	7.0 cd	8.0 cd	7.5
Untreated non-damaged	8.3 cd	6.0 d	7.2
Untreated, damaged**	8.0 cd	5.7 d	6.9
Daconil 2787	3.3 d	8.7 cd	6.0
Difolatan	3.3 d	5.7 d	4.5
LSD P = 0.05	10.5	7.7	

*All fungicides used at 0.2% a.i. and sprayed weekly onto stem bases.
**Stems scraped with knife at and below soil surface weekly.
***Values within season followed by the same letter are not statistically significant P = 0.05.

Difolatan was the fungicide treatment with the least wet stem rot in both seasons and in both the incidence was lower than in the untreated plots. The two best fungicides in this study, Daconil 2787 and Difolatan, are being tested again for wet stem rot control and are being compared with Benlate for the control of *Cercospora* leaf spot and anthracnose in cowpea. In addition a preliminary trial is in progress to determine a rapid method for screening for resistance to wet stem rot in cowpea.

C. ANTHRACNOSE

Cowpea anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. is characterized by tan lenticular lesions on stems, petioles, leaf veins, peduncles and pods, and can cause up to 50% yield reduction. In the determination of incidence/yield loss relationships for cowpea anthracnose several fungicides have been tested. The results from two field trials in 1972 (Table V and VI) indicate that anthracnose incidence was least in plots treated with Benlate (0.2% a.i.). Dithane-M-45 also effected good control (not significantly different from Benlate).

TABLE V
FUNGICIDE TREATMENTS AND ANTHRACNOSE INCIDENCE IN TVu 91 COWPEA 48 DAP, JUNE 1972

Fungicide	% a. i.	Frequency* of application (days)	% Incidence Anthracnose 48 DAP
Perenox	0.1	7	70
Perenox	0.1	14	78
Perenox	0.2	7	70
Perenox	0.2	14	75
Kocide 101	0.2	7	75
Kocide 101	0.2	14	77
Dithane M-45	0.2	7	48
Dithane M-45	0.2	14	63
Dithane Z-78	0.2	7	61
Dithane Z-78	0.2	14	62
Benlate	0.2	7	37
Benlate	0.2	14	41
Untreated	-	-	98

*Spray schedule began 14 DAP and ended 56 DAP.

TABLE VI
ANTHRACNOSE INCIDENCE IN TVu 91 COWPEA 51 DAP AFTER TREATMENT WITH BENLATE AND DITHANE – M-45, OCTOBER 1972

Treatment	% Anthracnose Incidence
Benlate at 14 day intervals from 14 DAP	31.5
Dithane-M-45 at 14 day invervals from 14 DAP	35.9
Dithane-M-45 at 14, 28 and 42 DAP	45.5
Dithane-M-45 at 14 and 28 DAP	72.8
Untreated	78.7
No fungicide and inoculum introduced 7 DAP	80.2

*Fungicides applied at 0.2% a.i.

The results on fungicidal control of cowpea anthracnose have enabled an assessment of yield loss/incidence relationships, and have provided information on a means of control for experiment stations. However, the use of fungicides to control cowpea anthracnose at the peasant farmer level is unlikely to be an economic feasibility. Therefore a major effort has been made to identify sources of host plant resistance to this disease, and a summary of the progress made is given below in section III on Host Plant Resistance.

D. CERCOSPORA LEAF SPOT

Two species of *Cercospora, C. canescens* and *C. cruenta* can cause severe leaf spotting and defoliation in cowpea at Ibadan. *C. cruenta* is the most serious for it occurs severely in susceptible varieties at any time of the year, while *C. canescens* is only occasionally severe. In 1972, in an attempt to determine the yield reduction potential of *Cercospora* leaf spot, two field trials were made with varieties known to be highly susceptible to leaf spot, and the fungicides Benlate and Kocide 101. *C. canescens* did not occur severely in either experiment. *C. cruenta* was severe in untreated plots in both experiments, and was almost completely controlled by biweekly application of 0.2% a.i. Benlate. The yield in untreated plots was 57% of that in treated plots in the first season's experiment and 75% of yield in treated plots in the second experiment. The yield reduction was lower in the second experiment as the rains finished during pod fill period and the plants in the treated plots were not able to fully utilize their greater leaf area. Though it is possible to effect almost complete control of *Cercospora* leaf spot by using the fungicide Benlate a major effort has been made to identify host plant resistance to this disease and the progress made is reported below in section III on Host Plant Resistance.

III. Disease Control by Host Plant Resistance

Apart from the possible use of seed dressings the peasant farmer in tropical regions is unlikely to adopt the use of fungicides and bactericides for disease control in food crops for some considerable time. Hence the major emphasis for disease control in GLIP is through the utilization of host plant resistance (HPR). Of course, before HPR can be utilized it has to be identified and a massive effort is being made to screen the cowpea germplasm collection at IITA (some 6,800 entries as of May 1974) for sources of HPR to the major cowpea pathogens occurring in the region.

Figure 1 indicates the basic plan and flow of materials in the program for identification and utilization of HPR. As the germplasm is not collected all at one time different materials are at different stages of development.

The basic key factors in any screening program are i. to have large variable genetic pool and ii. to have techniques whereby the desired characters can be selected. The first factor is amply provided by our huge germplasm collection. The second factor, i.e. the most desirable screening technique, is one which is subject to variability dependent upon the nature of the disease, and is a subject for which there is a considerable difference of opinion amongst plant pathologists. The basic philosophy of the IITA GLIP is that as the resistance must of necessity be effective in the field, then the screening for resistance should, wherever feasable, be conducted in the field. Also the techniques used should allow for a quantitative assessment of resistance and not just give a + or − answer. To this end a disease nursery system has been developed whereby test lines are evenly exposed to sources of inoculum of the major bacterial and fungal pathogens but are not directly inoculated. In Figure 2 the basic design of the disease nursery is illustrated. The susceptible spreader lines are planted 14 days before the test lines, and are inoculated with field grown inoculum from constantly maintained disease gardens. Initially single disease nurseries were attempted but as it was impossible to keep all other diseases away, the nurseries

soon became multiple disease nurseries. At the present time spreader lines for rust, anthracnose, *Cercospora* leaf spot and bacterial pustule are all planted together at each end of test rows so that there is multiple exposure to inoculum and thus an opportunity to select for multiple disease resistance. Irrigation is used to supplement rainfall to ensure good conditions for disease spread, and the validity of the system is checked by placement of known susceptibles to the diseases as test rows at strategic points in the nursery. In 1973 using this system 578 cowpea lines, selected on their performance in 1972 disease nurseries and germplasm planting, were exposed to inoculum of anthracnose, *Cercospora* leaf spot, bacterial pustule and rust. Other diseases that occurred naturally in the nursery were *Corynespora* leaf spot, bacterial blight, web blight and cowpea mosaic. Quantitative data was taken for anthracnose, bacterial pustule and *Cercospora* leaf spot incidence and qualitative data was taken on the incidence of the other diseases. Results (Table VII) indicate the availability of a large pool of both single and multiple resistance. The 140 + lines with resistance to the major bacterial and fungal pathogens represent diverse plant types with wide range of seed color, size and texture. In 1974 100 of the multiple resistant lines have been sent to cooperators in Philippines, India, Tanzania, Puerto Rico, Colombia and Brazil for testing against the important cowpea pathogens in those regions. Through this International Cowpea Disease Nursery (ICDN) seed of multiple resistant lines will be distributed around the world.and sources of broad spectrum stable resistance can be identified.

TABLE VII
NO. OF COWPEA LINES FREE FROM VARIOUS DISEASES OR WITH VARIOUS CATEGORIES OF DISEASE INCIDENCE AFTER EXPOSURE OF 578 LINES TO INOCULUM OF THE DISEASES IN FIELD NURSERIES AT IITA IN 1973

Disease(s)	Free	Disease Category			
		Low Susceptible	Mod. Susceptible	High Susceptible	Segregating
Anthracnose (anth)	275	61	12	67	163
Cercospora leaf spot *(C. cr.)*	454	31	14	37	41
Bacterial pustule (B.P.)	451	10	51	36	28
Rust	526	-	34*	-	-
Virus	433	-	144*	-	-
Corynespora leaf spot	305	-	273*	-	-
Bacterial blight	540	-	38*	-	-
Cercospora leaf spot *(C. ca.)*	574	-	4*	-	-
All diseases	32	-	-	-	-
All but *Sclerotium* stem rot	39	-	-	-	-
Anth. *C. cr.*, B.P. and Rust	141	-	-	-	-
Anth. *C. cr.*, and B.P.	162	-	-	-	-

*Degree of susceptibility not recorded.

The program for the identification of virus resistance in cowpea is only just beginning at IITA. Much of the developmental work at IITA has been conducted by Dr. R. M. Gilmer, visiting Professor at the University of Ibadan on a two year appointment from Cornell University. Also we are fortunate to have Dr. S. O. Soyinka at the University of Ife Institute for Agricultural Research and Training, Moor Plantation, Ibadan who has a deep interest in the virus diseases of cowpea and is identifying the various viruses in cowpea in the region. The major virus problem appears to be cowpea mosaic virus (CPMV) which is closely related to CPMV isolate from Arkansas (USA) and El Salvador. The virus, which is an isodiametric particle about 27-29 nm, can cause a range of symptoms in different cowpea cultivars, varying from a mild hardly discernible mosaic or mottle to a severe mosaic and blistering of leaves with severe stunting of plants and even death of the plant. The methods for large scale screening to CPMV are still being examined. In a recent field trial with 157 Cowpea lines, seedlings at the primary leaf stage were sap inoculated with two isolates of CPMV known to produce severe reactions in several varieties. Test rows were 3m long. One isolate was used on five plants at one end of the test row, the other isolate was used on five plants at the other end of the row, and the plants between were left uninoculated. The plots were not sprayed with insecticides and the plots were naturally infested with an active population of *Ootheca mutabilis* a known vector of CPMV. A great variability in reaction was observed, and allowed the identification of immune, resistant and tolerant (marked symptoms but no check in growth) lines. The field screening method is likely to be superior to a screening under greenhouse conditions for light intensity and temperature in the greenhouse are hard to maintain at normal levels, and in the field the opportunity is available to examine the capacity of the plant to tolerate infection and produce a reasonable yield. As a result of this preliminary work, large scale field screening for resistance to CPMV will be undertaken. Lines immune, resistant and tolerant to the two CPMV isolates will be tested further against a wider range of local isolates and the successful lines from these tests will be compiled into an International Cowpea Mosaic Virus Nursery for distribution and testing on a world wide basis.

In the above I have covered our approaches and progress in the identification of disease resistance in cowpea. I have not touched upon the major issue of utilization of the identified resistance. This is the next major step in our program. We are doing some conventional pedigree breeding and also are beginning to move into population improvement through the use of male sterility identified by our breeding staff. Using male sterile populations it is envisaged that massive gene flow can be generated that will enable rapid utilization of the identified sources of resistance.

EVALUATION OF NEMATODE POPULATION IN PIGEON PEA

N. D. Singh

I. Introduction

Pigeon pea, *Cajanus cajan* (L.) Millsp. is an important food legume in the Caribbean (Spence and Williams, 1972). Within recent years, considerable amount of research is being conducted in selection and breeding, cultural practices, mechanical harvesting, pest and disease control and processing to improve yield and quality of pigeon pea (Spence, 1974). Preliminary findings have shown a number of plant parasitic nematodes associated with pigeon pea (Singh and Farrell, 1972; Singh, 1973). Many of these nematode species have been reported to attack pigeon pea roots in Puerto Rico (Ayala, 1962; Ayala and Ramírez, 1964). Because of increasing importance of the crop it becomes necessary to obtain basic information on the behaviour of plant parasitic nematodes in its cultivation.

The purpose of this study was to evaluate nematode populations in a pigeon pea cultivation. The cultivation used was in fact an experiment being conducted by Miss A. Edwards (U.W.I.) on three pigeon pea varieties at varying plant densities.

II. Materials and Methods

The experiment used in this study was on River Estate sandy clay loam soil located at the University Field Station. A sample of the top 15 cm of soil comprised approximately 55% sand, 22% silt and 23% clay and had a pH of 5.2 and a cation exchange capacity of 9 mequiv/100 gm soil (Edwards, personal communication). The site was infested predominantly with mixed population of *Meloidogyne incognita* (Kofoid and White) Chitwood; *Rotylenchulus reniformis* Linford and Oliveira; *Pratylenchus* spp.; *Tylenchorhynchus* sp. and *Helicotylenchus dihystera* (Cobb) Sher. Also present in lesser numbers were *Rotylenchus* sp., *Aphelenchus avenae* Bastian; *Tylenchus* sp. and *Xiphinema* sp.

The area had previously been under fallow with *Paspalum conjugatum* as the dominant weed.

TABLE I
INFLUENCE OF PLANT POPULATION DENSITY OF THREE PIGEON PEA VARIETIES ON NEMATODE POPULATIONS PER 200 cc^3 SOIL
(MEAN OF THREE REPLICATES)

Variety	Nematode populations as affected by planting distances (cm)														
	Pratylenchus spp.			*Helicotylenchus dihystera*			*Rotylenchulus reniformis*			*Tylenchorhynchus* sp.			*Meloidogyne incognita*		
	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45
Indian	180[a]	73	153	53	20	53	3207	1440	4267	73	93	113	120	13	53
U.W.I. Dwarf (27/4A)	253	73	153	113	7	60	4253	1220	2767	67	93	113	87	80	47
Trinidad Tall	207	53	233	73	47	60	4807	2687	6427	47	80	33	27	60	60

a = Average number per 200 cc^3 soil sample.

Pigeon pea varieties were Indian, University of the West Indies Dwarf (27/4A) and Trinidad Tall (Tall). Seeds were sown by hand on January 16, 1974 at spacing of 15 x 15 cm, 30 x 30 cm, and 45 x 45 cm apart, resulting in plant populations of 75, 382; 18, 076 and 8, 016 plants per hectare respectively. The size of the experimental plot was 728 x 728 cm. A split plot design with three replicates was used (Edwards, personal communication). Soil samples were taken from each plot five months after sowing (Kleyburg and Oostenbrink, 1959). Each sample (200 cc^3 was processed by modified Cobb's decanting and sieving method (s'Jacob and Bezooijen, 1970). Duplicate samples consisting of 10% of each suspension were examined under the stereo-microscope and generic counts made.

Pod borer *(Ancylostoma stercora)* was controlled with application of "Gardona" during early pod development (Parasram, 1973). The insecticide was applied with a low volume mist-blower sprayer. The plants were watered by natural rainfall and hand weeding was done as needed (Edwards, personal communication). Logarithmic transformation of population data was used to stabilize variance.

III. Results and Discussion

The results showed significant differences (P = 0.05) existed in the populations of *Pratylenchus* spp.; *Helicotylenchus dihystera* and *Rotylenchulus reniformis* for all pigeon pea varieties at the various planting distances (Table I). The nematode populations were significant lower at spacing 30 x 30 cm apart than at spacing 15 x 15 cm or 45 x 45 cm apart. Further work is needed for elucidating these results as no clear explanation is possible on the basis of the present data. At the same time, *Pratylenchus* spp.; *H. dihystera; R. reniformis* and *M. incognita* were found to be highest in the U.W.I. Dwarf variety at spacing 15 x 15 cm. Except for *R. reniformis,* similar results were obtained for the Indian variety. The Trinidad Tall variety gave the highest count of *R. reniformis* at the different planting distances. There were no significant differences in *Tylenchorhynchus* sp. and *Meloidogyne incognita* as affected by the variety or planting distances.

In general, the high nematode counts for *R. reniformis* and *Pratylenchus* spp., in particular, that were observed in all three varieties suggest a high degree of susceptibility to nematode attack. In Puerto Rico, pigeon pea has also been reported susceptible to *R. reniformis* and *Helicotylenchus* sp. (Ayala, 1962). In this study, the relationships between the nematode populations and growth response of pigeon pea were not evaluated.

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THE IMPORTANCE OF DISEASES IN RELATION TO THE GRAIN LEGUME RESEARCH PROGRAM IN THE EASTERN CARIBBEAN

J. A. Spence

I. Introduction

The Grain Legume Program in the Eastern Caribbean is currently engaged in studies on pigeon peas *(Cajanus cajan)*. The Program is a multidisciplinary one and is aimed at developing an intensive row crop system of cultivation.

Traditionally, indeterminate varieties have been used and planting has been with the first rains in May or June. Since most pigeon pea varieties are short-day plants, flowering does not take place until the shorter days of November or December, by which time the plants have become large woody shrubs and harvesting is both difficult and costly and can only be carried out manually.

Determinate varieties have been produced in breeding programs at the University of the West Indies and at the University of Puerto Rico which, if planted in December or January, will flower early on young plants which can be cropped at a high population density (Spence and Williams, 1972). By this means the time from planting to cropping can be reduced from some 200 days to 110 days and mechanical harvesting from the "physiological dwarfs" so produced, becomes feasible.

The multidisciplinary program (Spence, 1974) aimed at further development of the row crop system includes studies on:

(1) Breeding and Selection; (2) Physiology and Biochemistry; (3) Microbiology; (4) Microclimatology; (5) Crop Protection; (6) Agronomy and Mechanization; (7) Food Technology and (8) Economics.

(1) Breeding and Selection

New germ plasm has been introduced in which a wide range of vegetative and flowering characteristics are exhibited. In addition to the search for types well adapted to the intensive row crop system, the program aims at trying to find an early day-neutral variety which would allow year around production. Resistance to diseases, particularly pigeon pea rust, which will be discussed later, is also a major aspect of the breeding program.

(2) Phisiology and Biochemistry

These studies are aimed at understanding the processes which control yield, resistance to drought and resistance to diseases. Thus it has become apparent early in these studies that the harvest index (proportion of economic yield to biological yield) is low for pigeon peas and that the flowering and pod setting processes need detailed study.

Further, a system whereby the day-length is extended with incandescent lights in the field is being used in a search for day-neutral types. Initial results indicate that the day-neutral characteristic is present in the material now available.

(3) Microbiology

In the Eastern Caribbean pigeon peas become nodulated by naturally occurring *Rhizobia* but the effectiveness of the inoculation process, and efficiency of the resulting nodules in fixing nitrogen, have hitherto not been studied. The program now in progress involves a comparison of naturally ocurring *Rhizobia* strains with strains imported from other regions, and a study of the reaction of different varieties to these strains.

(4) Microclimatology

Pigeon peas have been reported to be drought tolerant (Purseglove, 1968) but there is no published information on the nature of this characteristic. The present studies are aimed at relating the reaction of different varieties to moisture stress while detailed assessment of the degree of stress is made by microclimate studies in the crop canopy.

(5) Crop Protection

The main concern in insect pests is a pod borer, which is effectively controlled with insecticides. The disease problems will be discussed later.

(6) Agronomy and Mechanization

An agronomic system based on full mechanization is being devised and to this end a mechanical harvester has been designed and a prototype has been fabricated. This is still under test, but early results are promising. With the high cost of manual harvesting, the development of this harvester is critical for the commercial viability of the pigeon pea industry.

(7) Food Technology

At present split peas are imported into the Eastern Caribbean in substantial quantities, mainly as chick peas *(Cicer arietinum).* The characteristics of available pigeon pea varieties are being studied to determine their suitability for use as split peas. This would be an additional use for pigeon peas in the region which are now used only as green (vegetable) peas.

(8) Economics

It is vital that any new practices introduced for the production of pigeon peas are economically viable. Further, the market potential must be fully explored. Both aspects of the pigeon pea industry are under study in the Eastern Caribbean Program.

II. Diseases of Pigeon Peas

There is very little published information on diseases of pigeon peas in the Caribbean. A recent bibliography of published papers on diseases of pigeon peas (Barnes, 1973) indicates 6 fungus diseases and 2 virus diseases in reports from three countries in the region - Puerto Rico, Bermuda and Trinidad and Tobago. These diseases (by country in which they have been reported to occur) are:-

PUERTO RICO

Fungal Diseases:

1. Canker (*Phoma* sp.); 2. *Colletotrichum cajani;* 3. *Pellicularia filamentosa.*

Virus Diseases

1. Cowpea mosaic; 2. *Rhynchosia* mosaic.

BERMUDA

Fungal Diseases

1. *Uredo cajani* (rust); 2. *Sclerotinia sclerotiorum.*

TRINIDAD AND TOBAGO

Fungal Disease

Canker (*Physalospora* sp., *Phoma* sp.).

Virus Disease

Cowpea mosaic

The total number of diseases reported to affect pigeon peas as indicated in the bibliography by Barnes (1973), which is a survey of the world-wide literature, is:- 20 of fungal origin, 6 caused by viruses, 2 bacterial diseases and 2 of unknown cause.

The world list taken from Barnes is as follow:-

Fungal Diseases

1. *Armillaria mellea;* 2. *Ascochyta imperfecta;* 3. *Cercospora cajani;* 4. *Chaetoseptoria wellmanii;* 5. *Collectotrichum cajani;* 6. *Diplodia cajani;* 7. *Fusarium udum;* 8. *Gibberella fujikuroi subglutinans;* 9. *Macrophoma cajani;* 10. *Oidiopsis taurica;* 11. *Phoma* sp.; 12. *Phyllostica cajani;* 13. *Physalospora* sp.; 14. *Pythium aphanidermatum;* 15. *Rhizoctonia* sp.; 16. *Stemphyllium* sp.; 17. *Synchytrium phaseolus radicta;* 18. *Synchytrium umbilicatum;* 19. *Uredo cajani*; 20. *Vellosiella* sp.

Virus Diseases

1. Cowpea mosaic; 2. Mosaic; 3. Pale mosaic; 4. *Rhynchosia* mosaic; 5. Sterility disease; 6. Yellow mosaic.

Bacterial Diseases

1. *Pseudomonas* sp.; 2. *Xanthomonas cajani.*

Diseases of Unkown Cause

1. Gall disease; 2. Witches' broom.

This list includes only those organisms which have been reported to cause diseases but it does not include organisms which appear to occur as secondary infections.

III. Diseases of Importance to the Caribbean

An assessment of the available information would seem to indicate that the following diseases are of potential importance in the Caribbean region:-

Fungal Diseases

1. *Uredo cajani* (rust); 2. Canker *(Phoma* sp.); 3. *Sclerotium rolfsii;* 4. *Cercospora* sp.; 5. *Fusarium* sp.

Virus Diseases

1. *Rhynchosia* mosaic; 2. Sterility.

The literature on these diseases is limited indicating the need for expanded research if production of pigeon peas is to be developed as a major enterprise in the region.

Recent publications indicate some investigations on canker in Puerto Rico and on *Sclerotium rolfsii* in Trinidad. Investigations on rust particularly on chemical control, are being conducted in Jamaica by R. Pierre.

Canker

Investigations by Alvarez García (1960) have shown that in Puerto Rico infections of canker of epidemic proportions can occur. During such an epidemic in 1954, in a commercial planting of the variety Kaki, Alvarez García isolated a *Phoma* species which was successfully inoculated into healthy plants. The disease had been previously reported from Trinidad by Leach and Wright (1930) who associated it with *Physalospora* the imperfect stage of which these workers reported to be either *Phoma* or *Macrophoma.*

Sclerotium rolfsii

This disease has recently been found in Trinidad severely attacking a University of the West Indies dwarf variety of pigeon peas planted after a grass *(Paspalum fasciculatum).* Investigations by Phelps (1974) have shown that susceptibility varies with age, young seedling being very susceptible but resistance increasing with age, mature plants being apparently highly resistant.

Phelps has further shown that while *Sclerotium rolfsii* did not attack the grass *(Paspalum fasciculatum),* it will colonise dead clippings when the grass is cut and from these foci it will attack pigeon peas. He also found varietal differences in susceptibility, with the determinate University of the West Indies varieties being most susceptible and the semi-determinate Indian varieties showing resistance.

Rust Disease

There are little or no published data on rust *(Uredo cajani)* but observations

in Jamaica and Trinidad indicate that severe attack can occur followed by leaf fall, which would be expected to significantly reduce yields. The University of the West Indies dwarf varieties are on the whole very susceptible. There is one variety which shows resistance in Trinidad but which has been reported recently to have lost its resistance in Jamaica (R. Pierre, personal communication). This could imply that different strains of the rust exist.

The Grain Legume Program in the Eastern Caribbean has recently given priority to the problem of rust and a Plant Pathologist, R. Leather, is now working full time on the epidemiology of the disease in relation to the microclimate of the crop canopy. He will devise a suitable system for testing resistance to the disease. It is hoped by collaboration with workers in other parts of the Caribbean to designate the position with respect to strains of the organisms.

IV. Conclusions

It is clear that there is urgent need for:-

1. Surveys for occurrence of diseases of pigeon peas in the various Caribbean countries.
2. Surveys to indicate crop losses due to the disease.
3. Assessment of the potential danger from some of the diseases which at present appear to be of minor importance.
4. More intensive studies of those diseases which have already been shown to be of importance.

It is only when the above information has been obtained and documented that effective quarantine measures can be taken to minimize spread of the diseases to new areas; and only then can control measures be included in pigeon pea production systems to minimize economic losses.

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Origin, Improvement, and Prospects of the Common Bean

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RESEARCH RELATED TO THE ORIGIN AND IMPROVEMENT OF THE COMMON BEAN *(Phaseolus vulgaris* L.)

George F. Freytag

I. Introduction

Historically the common bean has been one of the most important food crops of the American tropics and sub-tropics; along with corn, rice or other principally starchy crops it has been one of the staples in the diet providing a good proportion of the protein since pre-colonial days.

With the present foreseeable world food shortages and our increased knowledge of balanced diets and nutritional values, we are newly focusing attention on the vegetable proteins, especially those of the legumes both American and Asian. Most of this attention is understandably on increasing yield or providing resistance. However, more recently it is on providing a "balanced" protein efficient in sustaining human growth without supplementation, (Anonymous, 1955, 1965, 1970, 1971, 1973; Aykroyd and Doughty, 1964), and the control of factors such as climate and soil which may affect the protein (Silbernagel, 1971).

To undertake these principally plant breeding tasks, the germplasm on hand is being investigated as well as searches made for new sources often located in the centers of crop origins. Notable among these efforts are those collections made by Plant Introduction personnel in Central America and subsequently the screening underway at the Caribbean Pulse Improvement Project in Puerto Rico, both of the USDA. Thus we should find and increased interest in these 'centers of plant origin' and the inter-relationships of the cultivars and species found there.

This presentation will be a selected review of some of the research from different fields which may have a bearing on the origins of the common bean.

II. Research on the Origins of the Common Bean

A. BOTANICAL

There are possibly over 200 species of *Phaseolus* with indications that the great majority of these, at least 126, are American. (Willis, 1966; Ditmer *et al.*, 1937). The 4 principal cultivated species and their centers of origin as indicated by number of species and distribution, are:

Lunatus - Guatemala highlands - Mackie (1943).

Acutifolius - southwestern U.S. - Freeman (1912).

Vulgaris - México and Guatemala - Bukasov (1930), Ditmer *et al.*, (1937). C. America to Argentina - Burkart (1943).

Coccineus - México and Guatemala highlands - Bukasov (1930), Ditmer *et al.*, (1937).

The Asiatic species can usually be differentiated by their yellow flowers and small seeds as in the rice bean *(P. calcaratus)* and the mung, urd, or gram beans *(P. aureus, mungo,* etc.). Seed sometimes confused with *vulgaris* are the Asian soybean *(Glycine max)* and the African cowpea *(Vigna* spp.).

Morphological studies of bean variation indicate from 2 to 3 species, one of which was theorized to be *P. coccineus,* as hybridizing to form the cultivated bean complex (Freytag, 1955). Later work indicated two principal races of *P. coccineus* as domesticates of the wild species, (Freytag, 1965) one of which had been raised to subspecies rank (Hernández *et al.*, 1959) and by some thought to be species (Smartt, 1974).

The other introgressive species was first found in 1941-42 in Argentina and named *P. aborigineus* (Burkart, 1952) and later was found to extend into Venezuela, in México and Central America (Burkart and Brucher, 1953). Comprehensive collections have been made in México and Central America and 3 main types of the wild species proposed as producing the principal bean cultivars, i.e. brown, spotted, and black seeded types (Gentry, 1969).

The taxonomy of the genus remains confused with the mojority of the specimens in the herbaria either unnamed or incorrectly identified. The only important treatise on the genus (Piper, 1926) does not even supply a key to the principal section of *Euphaseolus.*

B. GENETIC

Early crosses showed limited compatibility between cultivated varieties of *vulgaris* and *coccineus* (Mendel, 1866). The most successful crosses are obtained using *vulgaris* as the female parent, though a certain number of these produce dwarf and misshapen plants and sterility (Lamprecht, 1939, 1948). These crosses have been investigated by others since with similar results (Smartt, 1970; Thomas, 1964; Hawkins and Evans, 1973; Al-Yasiri and Coyne, 1966; Honma, 1962) and cytoplasmic incompatibility is retained through the BC_2 (Wall, 1970).

Wild *vulgaris (aborigineus)* crosses readily with cultivated *coccineus* (Klotz *et al.*, 1966), however, dwarf plants are obtained from the cross of cultivated Tropical Black x wild *vulgaris* (Freytag, unpublished).

The subspecies *darwinianus* was shown to be highly fertile in crosses with cultivated *vulgaris* (Smartt, 1974).

Other species crosses with *vulgaris* have shown high degrees of incompatibility due in large part to embryo abortion. Some have been obtained by embryo culture or through use of heterozygous parents:

Vulgaris x *acutifolius* Honma (1956), Coyn (1964), Smartt (1970).

Vulgaris x *lunatus* Lorz (1952), Honma (1956), Honma and Heeckt (1959).

Others working in this field are Oliver Norwell and F. Bliss, though much of this work has not yet been published.

C. ARCHEOLOGIC

Corn and beans were probably domesticated concurrently in Central México. Early material dates from 7,000+ years ago (B.P.) both in Tehuacan and Ocampo and spreads northward at later dates: 1,000 B.P. in the southwest U.S. to 600 B.P. in northern areas. The oldest South American material is about 2,500 B.P. from Nazca, Perú. There has been no apparent change from the moderately large seed size during this time. The bean has only become of importance since the development of agriculture and pot cooking about 1,800 years ago. Other species indicate an origin from 500-700 B.P. for the cultivated lima bean, 5,000 B.P. for the tepary, and a limited record of *coccineus* cultivars since about 2,200 B.P. (Kaplan, 1956, 1967).

D. BACTERIOLOGIC

The specificity and cross-inoculation groups for *Rhizobium* nodule bacteria have been known for some time (Fred *et al.*, 1932) as well as the efficiency, or lack thereof, in fixing atmospheric nitrogen (Burton *et al.*, 1954). *Rhizobium* from *P. vulgaris* are peculiar in that:

1) They will only inoculate *vulgaris* (Barua and Bhaduri, 1967).

2) No nodules are produced in acid and aluminum-toxic soils such as those of Brazil)Freire, 1969; Dobereiner, 1961).

There seems to be a closer relationship of Asiatic *Phaseolus* species to the cowpea than to the common bean since they can be inoculated with bacteria from the cowpea while the common bean cannot (Barua and Bhaduri, 1967). According to O.N. Allen (personal communication) there should be a difference in the specificity of bacteria found on the open-pollenated and self-pollenated species.

E. BIOCHEMICAL

Isoenzyme analysis has been used successfully to indicate the origin of some cultivated crops such as wheat and cotton (Johnson and Thein, 1970; Johnson, 1972). The basic technique (Boulter *et al.*, 1966) depends on the separation of large molecules by differences in their molecular size and polarity which can be influenced by such things as substrate, solvent(s) and pH. The techniques used may be: paper, column, or gas chromatography, electrophoresis, and density centrifugation.

Paper chromatography has been used to separate flavonoids of the normal leaf for distinguishing bush types from vining types in greenhouse conditions (Brown *et al.*, 1971).

Column (disc) electrophoresis has been used at the University of Wisconsin by Dr. T. Hall and co-workers to separate albumins (isoenzymes) as well as seed storage proteins (globulins) (McLeester *et al.*, 1973). The globulin fraction of bean protein consists of two principal types differentiated by their solubility in salt solutions:

G-1 fraction (legumin) soluble in 0.5M NaCl - approx. 53% of total protein.

G-2 fraction (vicilin) soluble in 0.06M NaCl - approx. 19% of total protein.

These have been further identified in polymer and dissociable peptide forms by centrifugation (Sun *et al.*, 1974). Using SDS acrylamide gels at a near neutral pH (6.5 to 7.2) they have found 2– and 3–banded G-1 globulin bean types, the latter being correlated with significantly higher methionine content.

Additionally work is being done to incorporate these protein types into improved hybrid populations at the same time improvement work is being accomplished for yield and disease resistance (Blis, personal communication).

III. Future Prospects

Bean researchers at this moment now have at their fingertips a number of exciting situations never before available to them which should assist in making rapid advances in bean improvement. Among these are:

1) The improved image of the directly consumed protein crops as the energy crisis and food shortages become more pronounced - this will cause a great change in our basic thinking and policies and should direct more resources towards support of bean improvement on an international scale.

2) The availability of a living and highly variable germplasm from the original native species; a practically unlimited gene pool including a self-fertilized and a cross-fertilized source.

3) Improved research techniques such as some of those mentioned in this paper (plant breeding, laboratory analysis, and computer assistance).

4) Trained individuals and educational and research institutions which have the capacity to train graduate students in research techniques and in the phylosophy of the practical application of results to production problems in the field.

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BEAN *(PHASEOLUS VULGARIS)* DISEASES IN THE TROPICAL AMERICAS

Eddie Echandi

The discovery of the New World marked the beginning of the recorded history of beans *(Phaseolus vulgaris).* Beans were cultivated in the Americas long before the arrival of Columbus, for cultivated forms are found in pre-Colombian Inca tombs in the desert coast of Peru. Also indications of their cultivation in Mexico and Central America are found in the archives of ancient Mayan history. Beans originated in Southern Mexico, Central and South America. Today we find many wild types particularly in the Andean section of South America and also in Mexico and Central America.

Practically all beans cultivated in the American tropics are indigenous and have suffered little or no change with the exception of those imposed by natural selection and occasional man-made selections and crosses. It is only logical that through many centuries of cultivation many types have evolved and have adapted to the selection pressure imposed by diseases and pests, consumers and growers. Some of these types can be found today in the Central American countries particularly in Guatemala where a great variety of disease resistant types, seed colors, sizes, plant types or architecture and other characteristics are present.

With the exception of certain areas of Peru, Colombia and Mexico beans are produced by small farmers of low economic resources that own or rent the land. In Central America, for instance, beans are produced on farms that range from 1 to 5 acres and in many cases bean farming is a subsistence operation.

Bean seed production and distribution in the American Tropics is not well-developed as it is in the temperate zone. With the possible exception of some areas of Mexico and Colombia, the majority of farmers plant seed from the previous crop produced on the same farm or use grain purchased on the local market. The reason for this is that in many countries, disease-free seed is not available, or if it is available, it is produced in small quantities, insufficient to satisfy the demand.

Planting systems in the tropical Americas have been adapted to the multiple ecological conditions of the area and to the incidence of diseases. The same phenomenon has occurred in the temperate zone, but adaptation here has been to fewer ecological zones and fewer diseases. In the tropics, planting systems vary from the most primitive in which the brush is burned or cut and the seed is broadcasted, to the highly mechanized systems where advanced technology is utilized. The primitive planting systems are typical of climates with heavy rainfall and high temperature, where diseases and insects are the limiting factors. Such conditions occur in Costa Rica, the Pacific coasts of Nicaragua and Panamá and the coast of Ecuador. Modern planting methods are used in most coastal valleys of Peru and some regions of Colombia and Mexico, where climatic conditions are adequate for the crop to develop properly and where damage from diseases and pests is tolerable.

Compared with the temperate zone, there are more bean diseases in the tropics; Wellman (1969) indicated 52 diseases for the temperate zone and 253-280 for the tropics. However, with a few exceptions, the important diseases in the temperate zone are also important in the tropics, but many times more destructive (Echandi, 1973). Some of the reasons for their being more destructive in the tropics have already been mentioned, infested or infected seed, no seed treatment, no rotation, no use of resistant varieties and no chemical control.

Economically important bean diseases of the American tropics are already known. With few exceptions, it can also be said that methods and tools for their control are known. Consequently, it would appear that steps to increase bean production in the American tropics should emphasize the use of the available information and materials to the best advantage -- the reader should not be left with the impression that all the pathological problems of beans are solved. Nevertheless it should be emphasized that there is a great deal of phytopathological information applicable to the tropics that is not being used at the moment and that if used adequately would result in a great increase in bean production to this part of the world.

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Subject Index

P

R

S

T

U

V

W

A 5
B 6
C 7
D 8
E 9
F 0
G 1
H 2
I 3
J 4