THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued quarterly by the Agricultural Experiment Station of the University of Puerto Rico, for the publication of articles by members of its personnel, or others, dealing with any of the more technical aspects of scientific agriculture in Puerto Rico or the Caribbean Area.

Vol. LVIII APRIL 1974 No. 2

A Mosaic of Beans (*Phaseolus vulgaris* L.) Caused by a Strain of Common Cucumber Mosaic Virus[1]

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INTRODUCTION

Virus diseases significantly reduce the yield of beans in Tropical America. Until recently few virus diseases of beans and other legumes had been recognized in Puerto Rico (2,11). Lately several such diseases have been characterized on the Island (1,6,7,8,17,18,19) and the discovery of others is highly probable.

The present study is part of a major cooperative effort directed to the identification and characterization of the diseases of beans and cowpeas in the American Tropics. This work deals specifically with an aphid-borne mosaic virus (BVBMV) of unknown provenance, found on beans (*Phaseolus vulgaris* L. var. La Vega) in plots located at the Agricultural Experiment Station in Río Piedras, Puerto Rico. The disease has been denominated vein-banding mosaic of beans (BVBM). Its characteristic symptoms have not been observed, as far as we know, on commercial bean plantings in Puerto Rico. The mildness and at times the ephemeral nature of its symptoms (remission followed by recrudescence) on the aforementioned host makes the disease a hard one to recognize.

MATERIALS AND METHODS

The materials and methods employed for these studies were as described previously (19). Plant species unknown to the writers were identified by

[1] Manuscript submitted to Editorial Board September 7, 1973.

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[3] Research supported in part by the United States Agency for International Development under contract entitled "Improvement of Tropical Production of Beans and Cowpeas Through Disease and Insect Control".

Mr. Roy Woodbury, Taxonomist, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P.R. Aphids were identified by Mr. Silverio Medina-Gaud, Entomologist of the same Station.

HOST RANGE

Inoculation by Mechanical Means

Actively growing young, healthy plants (in the cotyledon stage in many cases) were dusted with carborundum (600 mesh) and inoculated using swabs or dental buds (depending on the size of the leaves) impregnated with sap expressed from diseased bean, cucumber, or Virginia 12 tobacco plants. Tobacco leaves gave the highest virus titer. The main stock of the virus was maintained on *P. vulgaris* var. La Vega. These virus-affected plants were kept in a presumably insect-proof mini-greenhouse provided with a filtered air ventilating system. At frequent intervals the older virus stock plants were replaced with freshly inoculated ones.

Inoculation Via Aphids and Host Preferences

Aphis gossypii Glover, *A. craccivora* Koch, and *Dactynotus ambrosiae* (Thomas) were used in the transmission trials. Strict adherence to the simple methods described previously was observed in tests with aphids (17). The following plants were inoculated: *P. vulgaris* var. La Vega, *P. vulgaris* var. Diablo, *Vigna sinensis* (L.) Savi ex Hassk. var. Black, *Cucumis sativus* L. var. Black Diamond and *Musa* sp. (plantain var. Maricongo). The preferences of the various aphid species for the stated host plants were also recorded.

PHYSICAL PROPERTIES

Thermal inactivation and dilution end points were determined as described earlier (16). 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 & Reyn. were used as indicators, but in subsequent tests *C. quinoa* was employed solely as it was found to be a superior local lesion indicator.

SEROLOGY

The serological relationships of BVBMV were determined using the Hennisch (13) double-diffusion test as well as the Ouchterlony gel-diffusion

test (4). Specific cucumber mosaic virus (CMV) antisera were obtained from Dr. Walter Kaiser who acquired them originally from Drs. D. Z. Maat, R. J. Shepherd, and H. A. Scott. The antisera had been stored in a freezer since acquisition. Several tests were made. Some included heterologous as well as homologous antigens.

RESULTS

HOST RANGE

Transmission by Mechanical Means

During the early phases of the work the virus was repeatedly transmitted by mechanical means to bean, cucumber, tobacco and other plants. It was reisolated from such plants and from single lesions on *C. quinoa*. Isolates from these secondary hosts consistently elicited the characteristic symptoms of the disease on the primary host as well as on tobacco and cucumber.

A series of plant species and varieties belonging to several families were successfully inoculated by mechanical means. Table 1 indicates the plants that developed symptoms as well as those that failed to develop them under the conditions of our tests. Symptoms on *P. vulgaris* var. La Vega were, in order of appearance, vein clearing, inward curling of the leaf tips (trifoliates), and mild mosaic (fig. 1). The most prominent symptom appeared later and consisted of a banding of the veins which was evident also on other hosts (fig. 2).

On cucumber, symptomatology was reminiscent of that elicited by the local cucumber strain of CMV (fig. 3) 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 (9).

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. 4). 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 always high even when *C. quinoa* was used as an indicator. Several factors including high ambient temperatures probably are responsible for the stated behavior of this differential host. The problem with infectivity could be surmounted in most instances by employment of Hildebrand's (14) rolled leaf method of inoculation. It seemed that the strain of CMV studied by us is extremely heat labile. Thermal inactivation tests revealed extreme heat sensitivity.

TABLE 1.—*Symptoms incited by the vein-banding mosaic virus of beans on a series of selected hosts inoculated mechanically*

Family	Plant species tested	Symptoms[1]
Amaranthaceae	*Gomphrena globosa* L.	—
Apocynaceae	*Vinca rosea* L.	—
Carduaceae	*Bidens pilosa* L.	—
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.	—
	Phaseolus aborigeneus Burk.	VB
	P. aconitifolius Jacq.	—
	P. acutifolius Gray, Wright	—
	P. acutifolius Gray, Wright var. latifolius Freeman	—
	P. aureus Roxb.	—
	P. calcaratus Roxb.	—
	P. lathyroides L.	M, VB, P
	P. lunatus L. var. Sieva	VC, M
	P. mungo L.	—
	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 sinensis (L.) Savi ex. Hassk. var. Black	R, M
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 torvum Sw.	OL

[1] Key to symptoms: 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.

Transmission via Aphids and Host Preferences

The results of transmission trials with aphids are presented in table 2. Of the aphids tested, *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. 5).

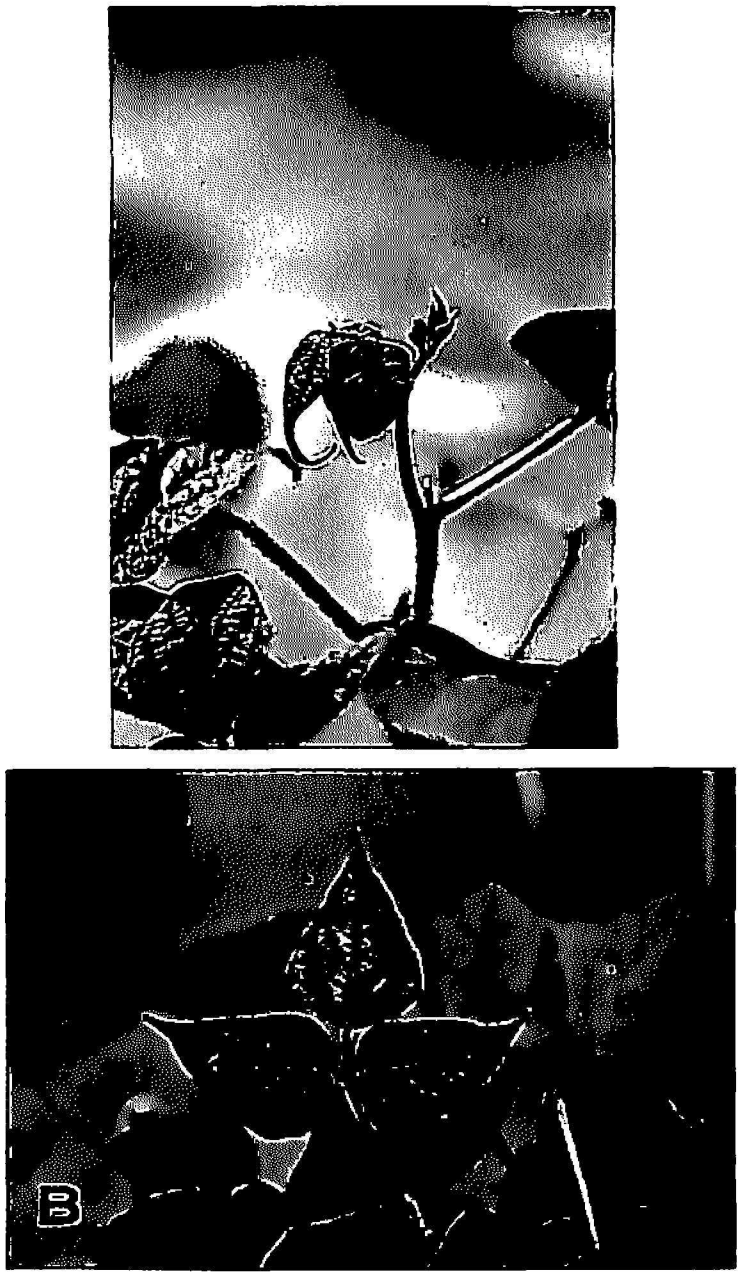

FIG. 1.—Primary symptoms (vein-clearing and inward curling of the leaf tips) elicited by BVBMV on a bean plant of the variety La Vega, A, and mild mosaic on a plant of the same variety, B.

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 other cucurbits and legumes. It fed fairly well even on plantains (*Musa* sp.), although no colonies were secured on this host.

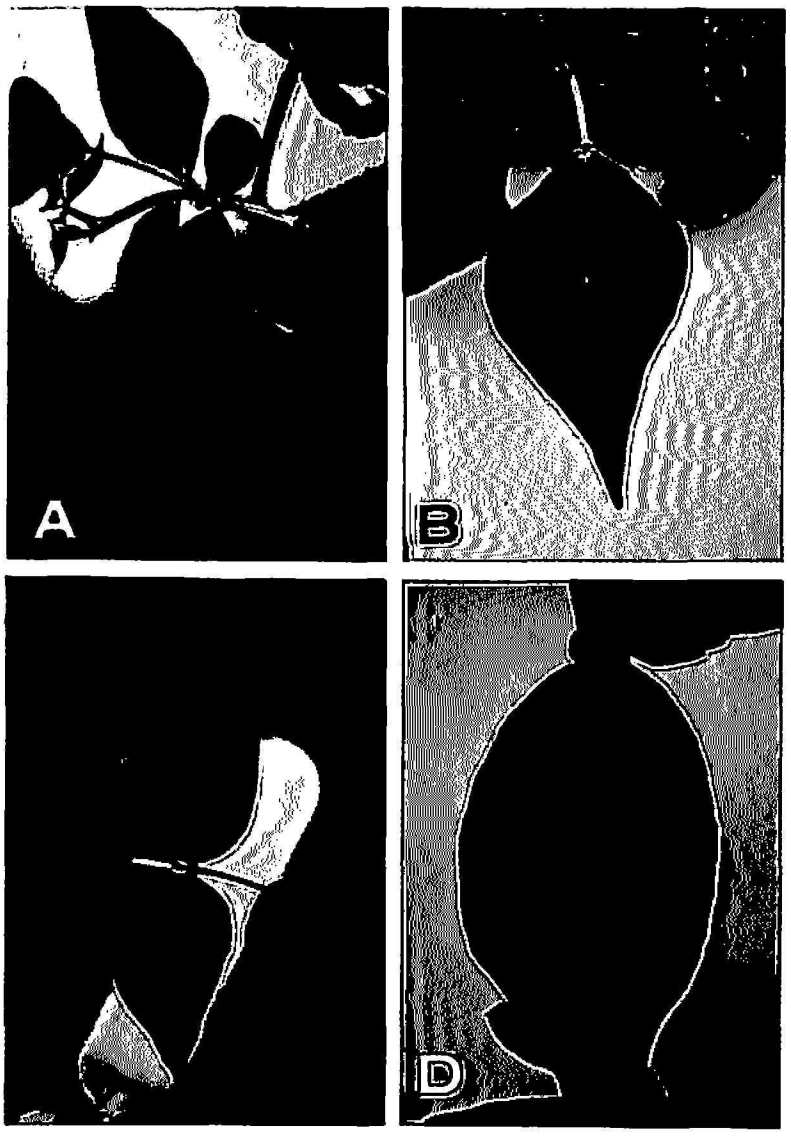

FIG. 2.—Vein-banding symptoms on various hosts, i.e. bean var. Diablo, A, bean var. Diablo (very mild symptoms), B, *Phaseolus aborigeneus*, C, and *Nicotiana tabacum* var. V-12, D.

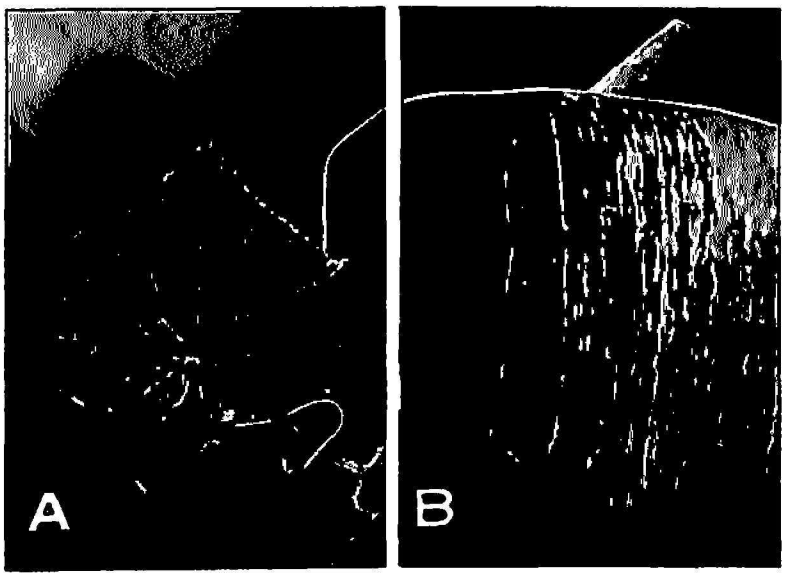

FIG. 3.—Symptoms elicited on cucumber, variety Black Diamond by BVBMD, A, and generalized mosaic caused by the same virus on plantain, B.

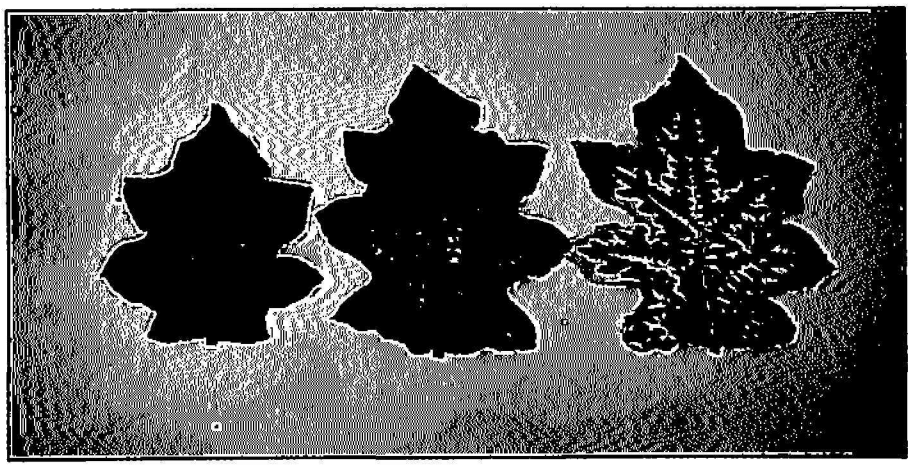

FIG. 4.—Oak-leaf symptoms on leaves detached from a BVBMV affected *Solanum torvum* plant.

TABLE 2.—*Transmission of the vein-banding virus from various sources to different hosts via aphids*

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

[1] 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.

[2] 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.

PHYSICAL PROPERTIES

Several thermal inactivation trials were carried out. The first test was effected using *C. sativus* var. Black Diamond as an indicator. In this preliminary test the virus became inactive at 45° to 50° C. In two subsequent trials the virus was found to be capable of withstanding, similarly, temperatures of up to but not beyond 50° C. The virus did not stand dilutions of more than 10^{-1}. When the untreated expressed sap was left standing for 45 minutes at an air temperature of 40° C the virus became inactive (evidenced by the results of inoculation tests using *C. quinoa* as indicator).

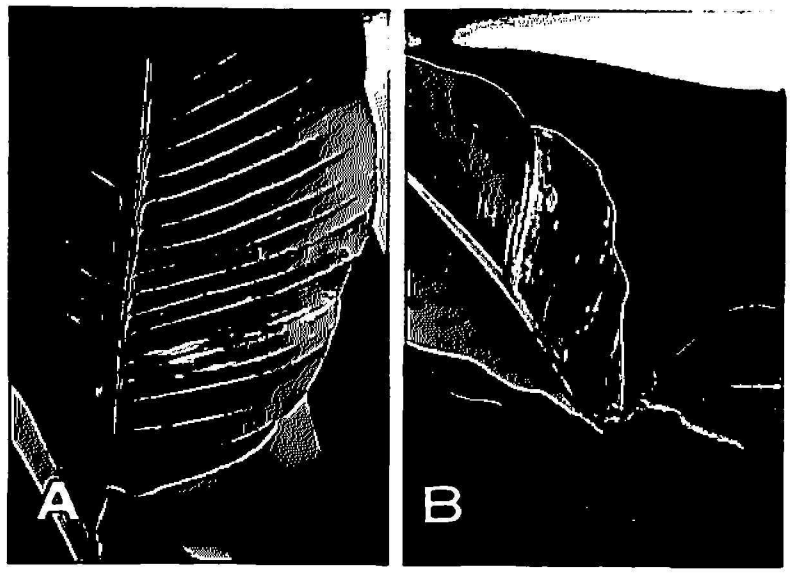

FIG. 5.—Primary symptoms induced on plantain by the bean virus, A, and by a common strain of CMV, B.

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 (fig. 6). 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.

DISCUSSION

The host range of the vein-banding virus of beans resembles in general that of the CMV studied by Wellman (21). It is also quite similar to the host range of the virus studied in Puerto Rico by Adsuar and Cruz-Miret (2). Reactions obtained on selected differential hosts such as *C. quinoa*, *Luffa cylindrica* (L.) Roemerer, *S. torvum* and plantain (*Musa* sp.) of the variety Maricongo indicated that the studied local bean virus is related to CMV. The results of serological and physical property studies confirmed the aforementioned findings regarding the identity of the bean virus. At

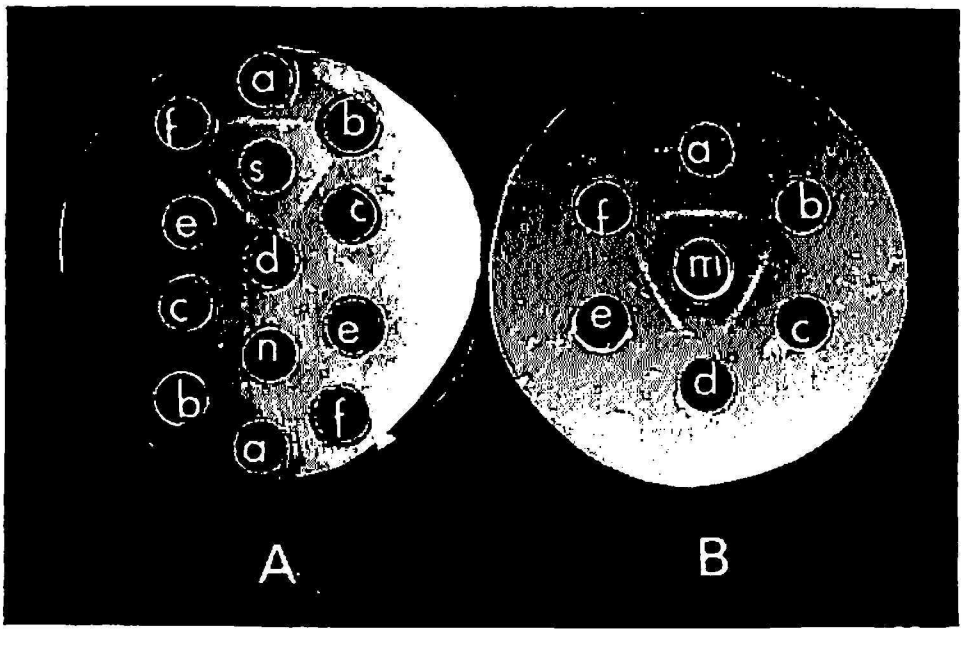

FIG. 6.—Reaction of various cucumber mosaic virus antisera with BVBMV: A, Wells a,c,e—antigen (BVBMV); wells b,d,f—healthy plant sap; well s—Scott's CMV antiserum; well n—Scott's CMV normal antiserum. B, Wells a,c,e—antigen (BVBMV); wells b,d,f—healthy plant sap; well m—Maat's CMV antiserum.

first the authors were under the impression that they were working with the local *Commelina* strain of CMV which causes shrivelling and necrosis of the stems of cucumber plants. However, this belief was abandoned when efforts to infect beans with the said strain of CMV failed. At any rate it seems that the strain involved is closely related, in some respects, to the *Commelina* one. Of the aphid species tested, *A. gossypii* seemed to be the most efficient vector. This aphid prevails in Puerto Rico on many wild cucurbitaceous hosts (often affected by CMV) and also occurs on *Commelina* as a race distinct from the one that normally colonizes *C. sativus*. *A. gossypii* also occurs on plantains and bananas and is involved in the transmission of the oak-leaf mosaic virus of *S. torvum*, another strain of CMV.

Bean plants inoculated mechanically or via aphids become moderately affected a few days after inoculation. These symptoms tend to lose their severity and may disappear eventually although the virus can be recovered. Pods are produced by affected plants and may be mildly mottled but not distorted. Tests are being conducted in an attempt to determine the effect of the virus on the yield of bean plants of the variety La Vega. Trials are also being carried out in an effort to find out whether the etiologic agent is seed transmitted. The results of a preliminary trial indicated that the virus is not seed transmitted. A more critical test is being carried out to determine whether the virus is present in seed collected from plants inoculated by mechanical means. Not many instances have been disclosed by a preliminary survey of the literature in which CMV is implicated as the causal agent of bean mosaic, or rather where CMV has been isolated from mosaic-affected *P. vulgaris* field plants. Kaiser et al. (15) listed beans as being a natural host of CMV in Iran. Anderson (3) recently isolated a CMV strain from cowpeas. He was able to inoculate successfully, by artificial means, quite a number of plant species including beans. Others (5,12,20) have isolated at one time or another CMV strains that are capable of affecting beans. The virus isolated by Bhargava (5) would not affect beans systemically although it could be recovered from the inoculated leaves. Bos (10) has just 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 incited by our local bean virus.

SUMMARY

A virus (BVBMV) capable of inciting mild mosaic and vein-banding symptoms on various hosts was isolated from field beans (*Phaseolus vulgaris* L.) growing in experimental plots. The causal agent was transmitted by mechanical means as well as via aphids. The results of transmissibility, serology, host range and physical property studies indicate that the virus is a strain, possibly a new one, of the common cucumber mosaic virus (CMV) complex.

RESUMEN

Un virus (BVBMV) capaz de provocar ligeros síntomas de mosaico y el bandeado de las nervaduras en varias especies de plantas fue aislado de habichuelas (*Phaseolus vulgaris* L.) cultivadas en un predio experimental. El virus se transmitió por medios mecánicos y a través de varias especies de áfidos. Los resultados obtenidos de estudios realizados sobre la transmisibilidad, gama de plantas hospedadoras, serología y propiedades físicas del referido agente viroso indican que se trata de una cepa, posiblemente nueva, del mosaico común del pepinillo (CMV).

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