Curly Dwarf Mosaic Disease of Beans from El Salvador

J. P. Meiners, H. E. Waterworth, R. H. Lawson, and F. F. Smith

First, second, and third authors, Research Plant Pathologists, and fourth author, Research Entomologist (Retired), Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705; and second author, U. S. Plant Introduction Station, Glenn Dale, MD 20769.

Appreciation is extended to J. Kaper for use of certain equipment, to H. Scott for critical review of the manuscript, and to W. R. Povish, C. W. Rogers, and S. S. Hearon for technical assistance.

Accepted for publication 20 August 1976.

ABSTRACT

MEINERS, J. P., H. E. WATERWORTH, R. H. LAWSON, and F. F. SMITH, 1977. Curly dwarf mosaic disease of beans from El Salvador. Phytopathology 67: 163-168.

A virus related serologically to squash mosaic virus and quail pea mosaic virus was associated with a disease of *Phaseolus vulgaris* widely distributed in experimental plots and commercial fields in El Salvador. Prominent field symptoms were mosaic, dwarfing, downward curling of leaves, and rugosity. Field symptoms were reproduced in some bean cultivars mechanically inoculated with extracts from naturally infected leaves. However, symptoms ranged from mild mosaic to lethal top necrosis among the 30 cultivars tested. Fourteen other species of legumes were susceptible, and seven were immune. Of 41 nonleguminous genera tested, *Gomphrena globosa* and *Chenopodium quinoa* were the only susceptible species. A polyhedral virus, measuring 23 to 25 nm in diameter, designated bean curly dwarf mosaic virus (BCDMV), was associated with the disease. Antiserum to this virus reacted only with the homologous antigen and with quail pea mosaic virus. However, antisera to quail pea mosaic, squash mosaic, and Echtes Ackerböhnemosaik viruses reacted with BCDMV. None of the 47 other antisera reacted with purified BCDMV. Mexican bean beetles and spotted cucumber beetles transmitted BCDMV. Properties of BCDMV most closely resemble those of viruses in the comovirus group, but it is distinct from bean rugose mosaic virus of Costa Rica, which also is a comovirus. Bean curly dwarf mosaic virus should be considered to be a strain of quail pea mosaic virus.

Additional key words: serology, electron microscopy.

In November 1971, bean (*Phaseolus vulgaris* L.) plants showing extreme dwarfing, mosaic, leaf rugosity, and curling were observed in the Central American Uniform Bean Varietal Nursery at San Andres, El Salvador. All cultivars and experimental lines in the nursery showed symptoms. Plants with the typical bright yellow symptoms characteristic of the golden mosaic disease (3), as well as symptoms characteristic of bean common mosaic disease also were present.

Plants with symptoms were brought to the Agricultural Research Center, Beltsville, Maryland, where an infectious agent was transferred mechanically to several bean cultivars. Symptoms produced on *P. vulgaris* 'Topcrop' were different from other virus-induced symptoms previously described in beans. Subsequently, two undescribed viruses were detected in the source material. One of the viruses, bean mild mosaic virus (BMMV) is described in a separate paper (11). This paper describes host range, purification, and properties of the second virus, which has been named bean curly dwarf mosaic virus (BCDMV). A preliminary report has been published. (12).

MATERIALS AND METHODS

Bean curly dwarf mosaic virus was mechanically transmitted from the diseased plants of several bean cultivars collected at San Andres, El Salvador, to Topcrop, Stringless Green Refugee, and Columbia Pinto bean cultivars. It was separated from BMMV by inoculating mung bean [*Vigna radiata* (L.) Wilczek] or pea (*Pisum sativum* L.). Both of these species are immune to BMMV. BCDMV was maintained in Topcrop, Stringless Green Refugee, and Columbia Pinto. It was tested with antisera to 45 viruses (10). Five additional antisera used in this study were obtained from J. Fulton (squash mosaic and quail per mosaic); R. Campbell (radish mosaic); H. Paul (Echtes Ackerböhnemosaik); and A. Granett (plantago mottle). Squash mosaic virus (SqMV) (Wisconsin strain) was provided by R. Webb. Quail pea mosaic virus (QPMV) from J. Fulton was isolated from *Strophostyles helvola* (L.) Ell. Sources of spotted cucumber beetles (*Diabrotica undecimpunctata howardi* Barber) and Mexican bean beetles (*Epilachna varivestis* Mulsant), of specialized chemicals and descriptions of equipment are described elsewhere (11).

In vitro properties were determined following procedures described elsewhere (6). In host range and

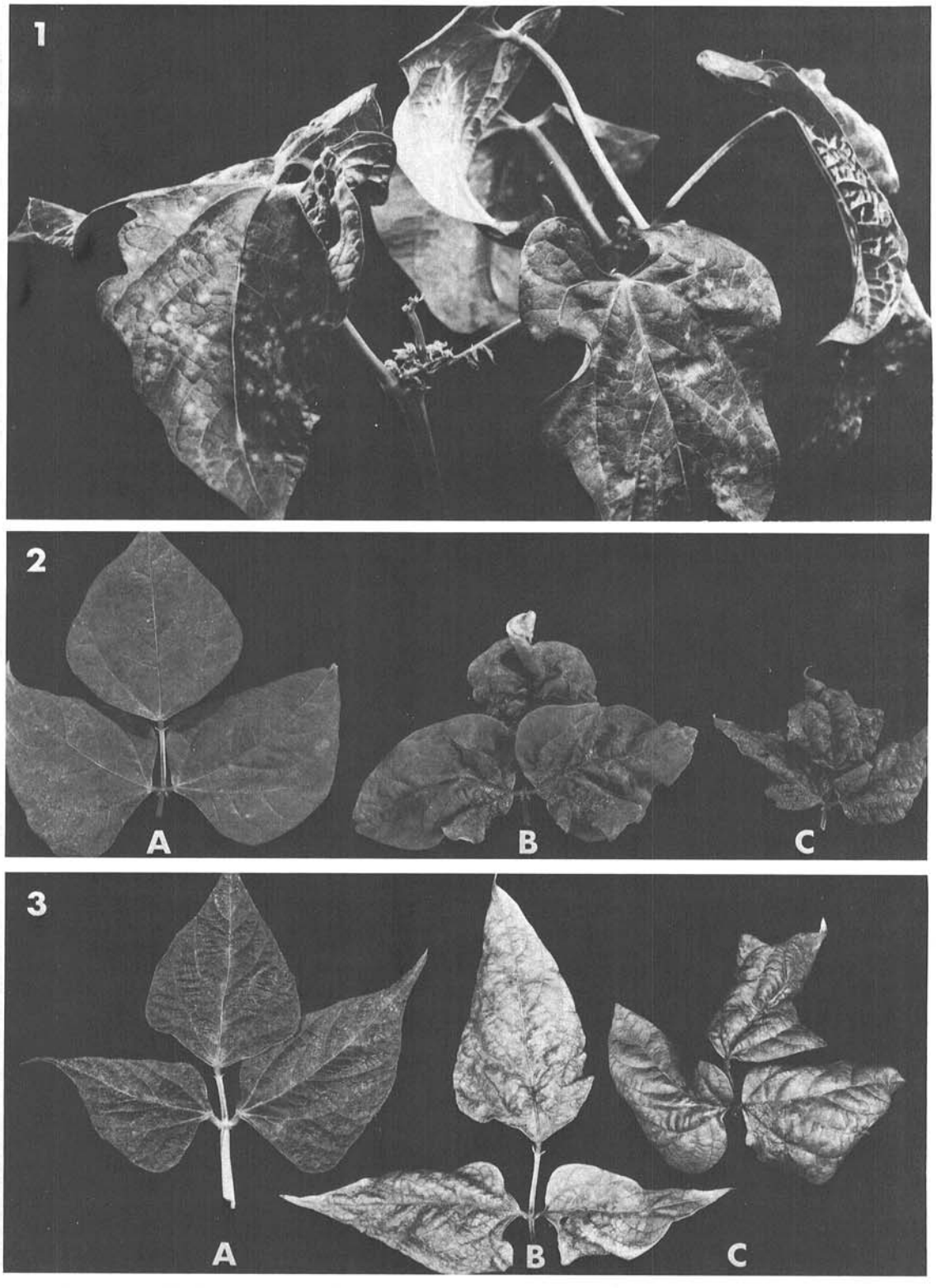


Fig. 1-3. 1) Bean (cultivar Topcrop) plant infected by mechanical inoculation with bean curly dwarf (BCDMV) and bean mild mosaic (BMMV) viruses. **2)** (A to C) El Salvador 184 bean infected with: (A) BMMV; (B) BCDMV; and (C) both viruses. **3)** (A to C) Bean cultivars infected with BCDMV: (A) 27-R; (B) Porillo #1; and (C) El Salvador 184.

TABLE 1. Reaction of leguminous species to mechanical inoculation with bean curly dwarf mosaic virus (BCDMV)[a]

Genus, species, and cultivar	Reaction[b]
Cajanus cajan Millsp.	L, S, TN
Cicer arietinum L. 'WCH #1, 'No. 74'	S
Crotalaria juncea L.	S
Dolichos lablab L.	L
Glycine max (L.) Merr. 'Kanrich', 'Scott'	S
Lathyrus sativus L.	N
Lens culinaris Medic.	L, S
Macroptillium lathyroides (L.) Urb	S
Phaseolus acutifolius Gray 'Red Tepary'	L, S
Phaseolus lunatus L.'Henderson Bush',	N
'Dixie Butterpea', 'Jackson Wonder'	S
Phaseolus vulgaris L. 30 cultivars	L, S, TN
Pisum sativum L	
'Alaska', 'Perfected Wales'	S
Sesbania exaltata (Raf.) Cory	L,S
Vicia faba L. 'Bell'	S
Vigna radiata (L.) Wilczek	
'Thailen', 'Berken', 'Moren'	S

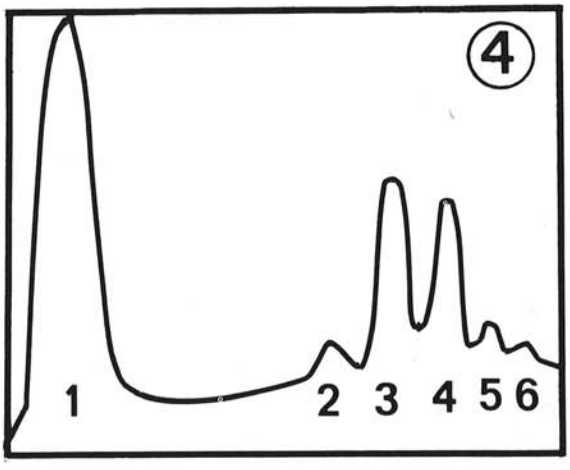
[a]The following species/cultivars were not infected by BCDMV: *Arachis hypogaea* L., 'Florunner' and 'Tifspan'; *Canavalia ensiformis* DC.; *Dolichos biflorus* L.; *Macroptillium atropurpureum* (DC.) Urb 'Siratro'; *Phaseolus coccineus* L., 'Kelevedon Marvel', 'Harrison's Tenderpod', and 'Achievement'; *P. lunatus* L., 'Fordhook 242'; *Tephrosia vogelii* Hook; and *Vigna unguiculata* (L.) Walp., 'No. 5 Blackeye' and 'Yardlong'.

[b]Abbreviations defined: S = systemic infection; N = systemic infection without symptoms; L = local infection; and TN = top necrosis.

insect transmission studies, inoculated species were back-indexed to Topcrop and *Dolichos lablab* L. Procedures for insect transmission studies are described elsewhere (11).

Purification.—Bean curly dwarf mosaic virus was purified from fresh Topcrop leaves which had been inoculated 2 wk earlier. The virus was extracted and concentrated by ultracentrifugation (11). The tan-colored resuspended high speed pellets were clarified by adding activated charcoal (Merck 18351) at 0.05 g/ml and centrifuging 15 min later at 8,000 *g* for 5 min. Virus in this now nearly colorless supernatant was further purified by density gradient centrifugation and reconcentrated as previously described (11). In some experiments gradients were 5-30% sucrose. These procedures were repeated with healthy bean tissue. A portion of each gradient fraction was bioassayed on Topcrop bean, and the balance of each was dialyzed, reconcentrated, and used for RNA studies or observed with the electron microscope. Samples of virus in buffer were placed on Formvar-coated grids and stained with 2% potassium phosphotungstic acid (KPTA) adjusted to pH 7.0 with KOH.

Immunization and serology.—Antiserum to BCDMV was produced by immunizing a rabbit intramuscularly with 3-4 mg of purified virus in Freund's incomplete



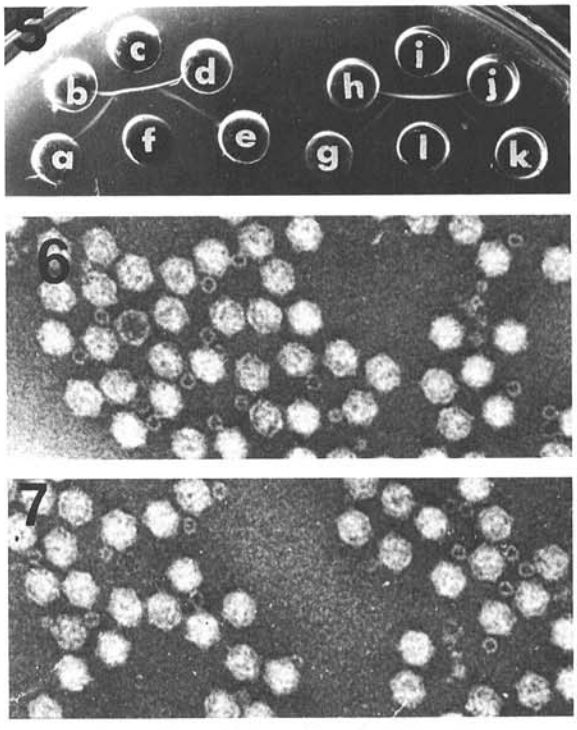


Fig. 4-7. 4) Typical ultraviolet scanning profile of partially purified bean curly dwarf mosaic virus (BCDMV) centrifuged into 5-30% sucrose density gradients [absorbance–ordinate: sedimentation (left to right)-abcissa]. Fractions from peaks three through six were highly infectious. Virus from peaks two, three, and four represent top, middle, and bottom components of BCDMV. **5)** Agar double-diffusion serology showing spur-type reactions between quail pea mosaic virus (QPMV) antiserum diluted 1:10 (well F) and QPMV (well C), BCDMV (well B), and squash mosaic virus (well D). There is no reaction with bean mild mosaic virus (wells A and E) (11). The second set shows the reaction between squash mosaic virus antiserum diluted 1:10 (well L), with QPMV (well H), squash mosaic virus (well I), and BCDMV (well J). There is no reaction with either bean pod mottle virus (well G) or cowpea mosaic virus (well K). All viruses were partially purified. **6-7)** Electronmicrographs of BCDMV from density gradient fractions from: **6)** peak three and, **7)** peak five. Virions from peaks three through six viewed via the electron microscope were indistinguishable.

adjuvant. Serological procedures were described previously (11).

RESULTS

Symptoms and host range.—Bean plants from El Salvador from which BCDMV was isolated showed dwarfing, leaf rugosity and curling, mosaic, moderate leaf epinasty, and twisting of the upper leaves. Primary leaves of Topcrop inoculated mechanically with sap from these infected plants exhibited epinasty and chlorotic lesions. The growth of the buds and trifoliolate leaves was almost completely suppressed. The stems and petioles developed internal necrosis, and the plants died (Fig. 1). Subsequently, we found that two viruses were present in these plants—BCDMV and BMMV (11).

Following separation of the viruses, we found that BMMV produces a very mild mosaic on bean cultivars (Fig. 2-A), whereas BCDMV causes symptoms closely resembling those of plants observed in El Salvador (Fig. 2-B). Inoculated primary leaves of plants infected with BCDMV exhibited epinasty, chlorotic and/or necrotic local sesions, and/or vein necrosis in cultivars Bountiful, Columbia Pinto, Kentucky Wonder, Plentiful, Potomac, Stringless Green Refugee, and Topcrop. Inoculated primary leaves of the remaining cultivars remained symptomless.

All of the 30 bean cultivars inoculated with BCDMV developed specific systemic symptoms (Fig. 3) which varied among cultivars. Top necrosis was prominent in Bountiful, Commodore, Ideal Market, Kentucky Wonder, Mexico 309, Potomac, Tendergreen, Tenderlong 15, and Topcrop, followed by death of plants or a systemic mosaic characterized by extreme stunting of top growth with accompanying necrosis. This was in sharp contrast with QPMV which incited no systemic symptoms in topcrop or Pinto beans, which confirmed previous work (8). Other cultivars infected with BCDMV showed mosaic accompanied by dwarfing, leaf rugosity, curling, and vein necrosis but no top necrosis. These included Beka, Columbia Pinto, Diablo, El Salvador #184, Great Northern U. I. 59, Guatamala 416, ICA Tui, La Vega, Michelite, Pinto U. I. 111, Pinto U. I. 114, Plentiful, Porillo #1, Sanilac, Santa Ana, Sensuntepeque, Stringless Green Refugee, Surecrop Wax, Sutter Pink, 27- cultivars were similar to those observed in the field.

In combination, BCDMV and BMMV, produce severe effects on many bean cultivars (Fig. 2). The combination of the two viruses usually is lethal on Topcrop, Tendergreen, and other cultivars originating in the United States. Alone, BCDMV produces relatively mild symptoms on cultivars such as Guatamala 416, ICA Tui, Sensuntepeque, and 27-R, but when combined with BMMV, produces typical curly dwarf mosaic symptoms similar to those found in the field. Other cultivars such as El Salvador #184 and Porillo #1 show symptoms typical of BCDMV that are intensified when BMMV is added to the inoculum. Symptoms produced on Diablo, La Vega, Santa Ana, and Villa Gro are mild when inoculated with BCDMV alone or combined with BMMV, and these must be regarded as resistant to both viruses. All resistant cultivars are of Latin American origin.

Fifteen species in 13 genera of legumes were susceptible to BCDMV and seven species in six genera were immune (Table 1). Systemic symptoms ranged from mild mosaic, veinbanding and vein-clearing with no stunting to severe mosaic, top necrosis, and stunting. Local lesions also were produced on six species. One of these, *Dolichos lablab* L., showed distinct necrotic local lesions about 1 wk after inoculation and proved to be a useful assay host.

The only nonleguminous hosts susceptible to BCDMV were *Chenopodim quinoa* Willd. and *Gomphrena globosa* L. Each of the species which were resistant to infection by BCDMV. Although BCDMV was serolgically related to SqMV, BCDMV did not infect any of the five cucurbit species tested and SqMV did not infect any of the leguminous species.

Purification and electron microscopy.—Yields of purified BCDMV were 10-15 μg/100 g of tissue or about 10% of the yields obtained with BMMV (11). Following rate zonal density gradient centrifugation, BCDMV was observed as a single 16 mm-wide mildly opalescent band 2.5 cm below the meniscus; but when fractionated and scanned with ultraviolet light, at least three distinct viral

TABLE 2. Transmission of bean curly dwarf mosaic virus by Mexican bean beetle and spotted cucumber beetle

Kind of beetle and period of acquisition feeding (hr)	Period of inoculation feeding (hr)	Larvae per plant (avg. no.)	Ratio of exposed plants/ infected plants
Mexican bean beetle			
24	24	0.1	9/18
24	24	1.0	12/27
24	0-16	1.0	23/54
24	16-24	1.0	15/54
24	24	1.5	22/36
24	48	1.5	8/36
24	72	1.5	2/36
Spotted cucumber beetle			
18	24	1.0	4/57
6	24	1.3	10/26
24	24	1.0	0/18
24	48	1.0	1/18
24	72	1.0	0/18

components were resolved especially in the 5-30% gradients (Zones 2, 3, and 4, in Fig. 4.). These were not present in gradients with healthy plant preparations. In most experiments with large amounts of virus, two additional zones were resolved in UV absorbance profiles. Virions from each of the zones 3 through 6 were infectious, measured 23-25 nm in diameter, and were indistinguishable with the electron microscope (Fig. 6 and 7). Most of the virions in zone two were empty. Material in zone one was nonviral. Maximum: minimum ratios and the 260:280 ratio of virus from zone 3 averaged 1.26 and 1.71, respectively. Zones 2, 3, and 4 were regarded as corresponding with top, middle, and bottom components, respectively, of other comoviruses.

Serology and relationships to other viruses.—Antiserum to BCDMV reacted to a titer of 1:128 in agar double diffusion tests. It did not give a detectable reaction with healthy plant proteins. Antiserum of BCDMV diluted no more than 1:10 reacted with QPMV but not with BMMV, SqMV, Echtes Ackerböhnemosaik virus (EABMV), and several other legume viruses. However, antisera to QPMV, SqMV, and EABMV each reacted with BCDMV at 1 mg/ml when they were diluted as much as 1:128, 1:32, and 1:8, respectively. In crude juice or in purified form, BCDMV did not react with any of 47 other antisera including bean rugose mosaic (4) and BMMV (11). Spur formation was observed when BCDMV and QPMV, in adjacent wells, were allowed to react with QPMV antiserum diluted 1:16 (Fig. 5) and when BCDMV and SqMV were in adjacent wells and allowed to react with SqMV antiserum (Fig. 5). Hence, BCDMV was related, but not identical, to three viruses. Gel diffusion serology was a reliable technique to detect BCDMV in juice of diseased bean plants.

Properties in vitro.—Bean curly dwarf mosaic virus in sap extracted from and bioassayed on Topcrop plants was infectious after diluting 1:10 with 0.025 M phosphate buffer and heating at 50 C for 10 min but not at 55 C, after incubating at room temperature for 3 wk but not after 4 wk, and after further dilution to 1×10^{-5} but not after diluting 1×10^{-6}.

Beetle transmission. —Results of transmission tests with Mexican bean beetles and spotted cucumber beetles are presented in Table 2. Transmission of BCDMV by beetles resulted in development of typical symptoms in Topcrop, El Salvador 184, 27-R, Sensuntepeque, Columbia Pinto, and Stringless Green Refugee. Infection of BCDMV was confirmed by subinoculations to Topcrop and by serological tests. Both beetles transmitted the virus, but the former was a more efficient vector than the latter. The rate of initial transmission in the tests with Mexican bean beetle is similar to results obtained with cowpea mosaic by Jansen and Staples (5); however, in our tests, the Mexican bean beetle retained inoculativity of BCDMV for 3 days, whereas cowpea mosaic was retained for only 2 days. The spotted cucumber beetle appeared to lose inoculativity more rapidly than did the Mexican bean beetle.

Female leafminers (*Liriomyza satizae* Blanchard) and adult greenhouse whiteflies [*Trialeurodes vaporariorum* (Westwood)] did not transmit the virus.

Seed transmission.—Approximately 400 plants were grown from seed harvested from 27 BCDMV-infected plants representing nine cultivars of *P. vulgaris* and one cultivar of *P. acutifolius*. No transmission of the virus through the seed was detected.

DISCUSSION

Bean curly dwarf mosaic has been observed in bean fields in various parts of El Salvador during the wet season and at all seasons in San Andres where irrigated beans are grown during the dry season. The virus was isolated from plants collected in El Salvador in 1971, 1973, and in 1975. Surveys show that as many as 15% of the plants at the edge of fields show symptoms, while symptoms are present in less than 1% of the plants near the center. Distribution suggests that vectors introduce the virus from wild plants growing in the periphery of fields. Plants near the periphery become infected early, are extremely stunted, and produce no yield. Those toward the center of the fields and infected at progressively later stages of growth are proportionately less severely affected and produce limited yields. Symptoms in some plants are observed only near the terminal growth, particularly in the indeterminate pole-type cultivars.

Our greenhouse tests show that both the Mexican bean beetle and the spotted cucumber beetle can transmit BCDMV. Low populations of the Mexican bean beetle occur in El Salvador in localized areas, and it could transmit curly dwarf in the rainy season. The relatively low incidence of bean curly dwarf in El Salvador bean fields may be due to the low efficiency of indigenous vectors or the infrequent movement of vectors from wild hosts into bean fields. Although beetle vectors of this disease have not been investigated in El Salvador, the prime suspects are the banded cucumber beetle (*Diabrotica balteata* Le Conte) and a flea beetle [*Cerotoma ruficornis* (Oliver)] because of their general distribution and their known capacity to transmit related viruses.

Symptoms in *P. vulgaris* and in other species of legumes resemble those of bean rugose mosaic (BRMV) described by Gamez (2, 4). However, BCDMV differs from BRMV in symptoms produced on specific bean cultivars, in host range, properties in vitro, size of the virions, and in its serological relations to other viruses. Furthermore, BRMV and BCDMV are not serologically related to each other.

We conclude that BCDMV is not the cause of any of the described virus diseases of bean (1, 9, 13). It and QPMV (8) from Arkansas should be considered as strains of the same virus. In our studies, each was serologically related to SqMV. However, neither should be considered as a strain of SqMV in view of host range and properties in vitro. One serological difference is that QPMV was reciprocally serologically related to bean pod mottle virus, confirming the results of Moore (8), whereas BCDMV was not.

We observed four-to-six ultraviolet light-absorbing components in centrifuged sucrose density gradients with BCDMV. All but the top two were infectious. The top, middle, and bottom components are presumably represented in peaks two, three, and four (Fig. 4). The most rapid-sedimenting virions in zones 5 and 6 may have represented dimers or trimers of virus in zone 4; however,

aggregation was not observed in electron microscopic examinations. It is also possible that the bottom component of BCDMV became separated into two or three groups of particles with slightly different buoyant densities. Mazzone et al. (7) reported that the bottom component of serologically related SqMV separated into two zones when centrifuged in CsCl gradients.

LITERATURE CITED

1. BIRD, J., and K. MARAMOROSCH. Ed. 1975. Tropical diseases of legumes. Academic Press, New York. 171 p.
2. GAMEZ, R. 1968. Enfermedades virosas del frijol en Costa Rica. I. Mosaico Rugoso. Pages 129-131 *in* XIV Reunión Anual. Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios: Tegucigalpa, Honduras. 27 Febero-1 Marzo 1968. IICA Publicatión Miscelánea No. 67.
3. GAMEZ, R. 1971. Los virus del frijol en Centroamérica. I. Transmisión por moscas blancas y plantas hospedantes del virus del mosaico dorado. Turrialba 21:22-27.
4. GAMEZ, R. 1972. Los virus del frijol en Centroamérica II. Algunas propiedades y transmisión par crisomélidos del virus del mosaico rugoso del frijol. Turrialba 22:249-257.
5. JANSEN, W. P., and R. STAPLES. 1970. Transmission of cowpea mosaic virus by the Mexican bean beetle. J. Econ. Entomol. 63:1719-1720.
6. KAPER, J. M., and H. E. WATERWORTH. 1973. Comparison of molecular weights of single-stranded viral RNAs by two empirical methods. Virology 51:183-190.
7. MAZZONE, H. M., N. L. INCORDONA, and J. KAESBERG. 1962. Biochemical and biophysical properties of squash mosaic virus and related macromolecules. Biochem. Biophys. Acta 55:164-175.
8. MOORE, B. J. 1973. Quail pea mosaic virus: A new member of the comovirus group. Plant Dis. Rep. 57:311-315.
9. SMITH, K. M. 1972. A textbook of plant virus diseases. Academic Press, New York and London. 684 p.
10. WATERWORTH, H. E., R. H. LAWSON, and R. L. MONROE. 1976. Purification and properties of Hibiscus chlorotic ringspot virus. Phytopathology 66:570-575.
11. WATERWORTH, H. E., J. P. MEINERS, R. H. LAWSON, and F. F. SMITH. 1976. Purification and properties of a virus from El Salvador which causes mild mosaic in bean cultivars. Phytopathology 67:169-173.
12. WATERWORTH, H. E., J. P. MEINERS, and F. F. SMITH. 1975. Bean curly dwarf mosaic disease caused by a virus serologically related to squash mosaic virus. Proc. Am. Phytopathol. Soc. 1:49.
13. ZAUMEYER, W. J., and R. H. THOMAS. 1957. A monographic study of bean diseases and methods for their control. U. S. Dept. Agric. Tech. Bull. 868. 255 p.