Characterization of a Blackeye Cowpea Mosaic Virus Strain from South Carolina

J. F. MURPHY, Former Graduate Assistant, and O. W. BARNETT and W. WITCHER, Professors, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377

ABSTRACT

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A blackeye cowpea mosaic virus isolate (BlCMV-NR) recovered from a cowpea plant grown in Greenville County, South Carolina, differed from BlCMV-FS (a Florida isolate like the type strain) in its biological properties (symptomatology and host range) but not in its physiochemical properties. On cowpea, BlCMV-NR induced reddish necrotic ringspots on inoculated leaves that spread into a network of veinal necrosis. A systemic mosaic developed with distortion of leaflets and stunting of the plant. BlCMV-NR but not BlCMV-FS infected *Ocimum basilicum*, *Phaseolus lunatus* 'Henderson-Bush,' *P. vulgaris* 'Bountiful,' and *Vicia faba*. Both BlCMV isolates were transmitted in a nonpersistent manner by the aphid *Macrosiphum euphorbiae*. Purified preparations of BlCMV-NR averaged 9.8 mg/100 g of tissue, whereas yields of BlCMV-FS averaged 4.9 mg/100 g of tissue. The estimated molecular weight of protein structural units of both BlCMV isolates were similar on 5 and 8% acrylamide gels, and the RNA molecular weight of each isolate averaged 2.9×10^6. The two BlCMV isolates induced similar cytoplasmic inclusions with pinwheels and scrolls. The isolates were indistinguishable by serology tests and were very similar by cDNA hybridization tests.

Virus diseases are a major limiting factor in the production of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) in the southeastern United States. One of the viruses, blackeye cowpea mosaic virus (BlCMV), is a seedborne potyvirus originally described by Anderson (1) as causing a mosaic disease of cowpeas in Florida. Previous reports of BlCMV (15,18,27) from various geographic locations described isolates that resemble the type strain characterized by Lima et al (16). BlCMV, like other potyviruses, is transmitted by aphids in a nonpersistent manner. They typically consist of flexuous rod-shaped particles constructed from molecules of a single protein component and a single strand of RNA.

A recent survey of cowpeas in South Carolina (6) showed that BlCMV was distributed throughout the state with incidence of infection in 13 counties varying from <0.1 to 56.5%. In 1982, a field of cowpeas in Greenville County was visited where many plants had virus-like symptoms. Plants were severely affected (stunted with blistered and malformed leaves). Extracts from leaf samples reacted positively to BlCMV-FS antiserum. However, when cowpea plants were inoculated, resulting symptoms were atypical of those described for BlCMV.

Here we compare this Greenville County isolate, designated as the necrotic ringspot strain of BlCMV (BlCMV-NR), with an isolate from Florida (BlCMV-FS) that resembles BlCMV-type strain (16); the isolates differed in biological but not physiochemical properties. No survey has been conducted to determine the prevalence of BlCMV-NR. A preliminary report has been published (23).

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MATERIALS AND METHODS

Isolation. A virus isolate derived from cowpea samples collected in Greenville County caused symptoms atypical for BlCMV on Knuckle Purple Hull cowpea under greenhouse conditions. The isolate was transferred to *Chenopodium amaranticolor* Coste & Reyn., in which three successive single local lesion transfers were made. Then a single local lesion from *C. amaranticolor* was used to inoculate Knuckle Purple Hull cowpea. This virus isolate, BlCMV-NR, was propagated and maintained in this cultivar. BlCMV-FS was from cowpea seed (supplied by F. W. Zettler, University of Florida, Gainesville) from the seed lot from which BlCMV-type strain originated.

Host range. Young primary leaves of test plants were dusted with corundum (aluminum oxide, 600-mesh) and sap-inoculated with BlCMV-NR or BlCMV-FS after grinding systemically infected cowpea tissue in 0.03 M sodium phosphate buffer, pH 8.0 (about 1 g of tissue to 5 ml of buffer). Test plants were assayed for virus infection serologically and/or by inoculation of cowpea indicator plants.

Aphid transmission. Aphid transmission studies were performed as described by Mali and Kulthe (18) with the aphid *Macrosiphum euphorbiae* (Thomas). Plants were maintained in the greenhouse at 23 ± 3 C for symptom expression. Aphids not exposed to virus source plants and fed on healthy cowpea were used as controls.

Stability in vitro. The infectivity in sap was determined for each BlCMV isolate (2). Extracts of systemically infected cowpea leaves ground in 0.03 M sodium phosphate buffer, pH 8.0 (1 g of tissue to 5 ml of buffer), were treated and inoculated to cowpea to determine virus viability. Longevity in vitro was tested at 24-hr intervals.

Purification. Both BlCMV-NR and BlCMV-FS were purified by the butanol purification method of Lima et al (16). Systemically infected cowpea leaves were harvested about 15 days postinoculation for BlCMV-NR and 20 days for BlCMV-FS. Tissue was homogenized in 0.5 M potassium phosphate buffer, pH 7.5, containing 0.01 M disodium ethylenediamine tetraacetate (Na_2 EDTA), 1% sodium sulfite (w/v), and 1 M urea (2 ml of buffer per gram of tissue). Homogenized tissue was clarified by adding *n*-butanol to a final concentration of 8.5% (v/v) and stirred for 4 hr at 4 C. Before use, *n*-butanol was clarified (to reduce aldehydes that may be harmful to virions) by two washings for 5–10 min in 1.6 M sodium bisulfite (25 ml of sodium bisulfite to 200 ml of *n*-butanol). This procedure was repeated but with 50 ml of distilled water, first for 30 min, then overnight. Virus concentration was determined spectrophotometrically with an extinction coefficient of 2.4 $(mg/ml)^{-1}$ cm^{-1} at 260 nm after correction for light scattering (24). An A_{260nm}/A_{280nm} of 1.2 was used as a measure of purity (16).

RNA extraction and relative molecular weight. Viral RNA extraction was done by the method of Brakke and Van Pelt (3) as modified by Reddick and Barnett (25). Viral RNA was collected and precipitated in cold absolute ethanol overnight at −20 C with the addition of 3 M sodium acetate, pH 5.6, added to a final concentration of 0.1 M. The RNA was centrifuged at 12,100 *g* for 40 min and

resuspended in 300 μl of triple-distilled water. Concentration was determined spectrophotometrically using an extinction coefficient of 25 $(mg/ml)^{-1}$ cm^{-1} at 260 nm (12).

Addition of DNase was by the method of Makkouk and Gumpf (17). Virus preparations were resuspended in 1 ml of 0.1 M Tris-Cl buffer, pH 7.8, 4 mM magnesium chloride, and DNase (Boehringer Mannheim Biochemicals, Indianapolis, IN) at 60 μg/ml and incubated 30 min at room temperature. After incubation, the RNA was extracted and centrifuged as described.

Viral RNA molecular weight was estimated on cylindrical tube gels (5). RNA from each BlCMV isolate was subjected to electrophoresis at 5 mA per gel for about 3.5 hr and analyzed at 260 nm with a GCA/McPherson spectrophotometer and gel scanner. The molecular weights of BlCMV-NR and BlCMV-FS RNAs were determined by comparison with the relative migrations of tobacco mosaic virus RNA (2.05×10^6) and with *Escherichia coli* 23 S RNA (1.07×10^6) and 16 S RNA (0.55×10^6).

Protein structural unit molecular weight. The protein structural unit molecular weight of each BlCMV isolate was estimated by a modification of the method of Weber and Osborn (30). Viral protein was dissociated in 0.02 M sodium phosphate buffer, pH 7.1, with 2% sodium dodecyl sulfate (SDS) and 2% dithioerythritol and heated at 100 C for 5 min. An electrophoresis calibration kit (Pharmacia Fine Chemicals, Piscataway, NJ), used to provide low molecular weight protein standards, included phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400). Protein samples were layered onto 5 or 8% acrylamide gels polymerized in 0.1 M sodium phosphate buffer, pH 7.1, containing 0.1% SDS and subjected to electrophoresis at 5 mA per gel for 8–10 hr. Gels were stained with 0.2% Coomassie Brilliant Blue R-250 dissolved in a solution of acetic acid, methanol, and distilled water (7:46.5:46.5, v/v) for at least 2 hr, and destained with a solution of methanol, acetic acid, and distilled water (7:10:83, v/v). Some gels were analyzed with a spectrophotometer and gel scanner at 280 nm.

Molecular hybridization. Synthesis of ^{3}H-complementary DNA (cDNA) was according to Reddick and Barnett (25) modified by using 5 μg of viral RNA per 50 μl reaction volume with 20 mM dithioerythritol and ^{3}H-TTP with a specific activity of 17.6 Ci/mmole (New England Nuclear, Boston, MA).

Hybridization conditions were as described by Gould and Symons (11) and modified by Reddick and Barnett (25). Viral RNA was appropriately diluted to give a specific R_0t value in "low salt" hybridization buffer (0.01 M Tris-Cl buffer, pH 7.0, 0.18 M sodium chloride, 1 mM EDTA, and 0.05% SDS) with the addition of 2 μl of ^{3}H-cDNA at 2,000 cpm/μl. The hybridization solution was placed in a boiling water bath for 2–7 min, then incubated at 60 C for the appropriate length of time. Hybridization was stopped by chilling the mixtures on ice. The extent of hybrid formation was determined by the described method (25). The single-strand-specific S1 nuclease (Sigma) was used at a concentration of three units per microliter (15 units total). The percent hybridization values were corrected for S1 nuclease resistance (10).

Electron microscopy. Virus particle morphology and modal length for each BlCMV isolate were determined on nitrocellulose-coated, carbon-fronted grids (200-mesh) exposed to an ultraviolet light source for 10–15 sec. Grids were floated on a purified virus preparation (concentration 0.01 mg/ml in 0.1 M potassium phosphate buffer, pH 7.0) for 1 hr at room temperature. Grids were then floated on distilled water for 1–2 min and stained with 1% uranyl acetate, pH 6.8, for 5–10 min at room temperature in the dark. Particles were measured from photographs at a magnification of 39,600. The calibration standard was a carbon replica with 2,160 lines per millimeter.

Inclusion body morphology was determined for each BlCMV isolate. Cowpea tissue systemically infected with either isolate was fixed in 3.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 6.4, postfixed in a 1:1 solution of 2% osmium tetroxide and cacodylate buffer, dehydrated in a graded ethanol series, and embedded in Spurr's medium. Sections were cut at about 60–90 nm and examined in an electron microscope.

Serology. Antiserum to BlCMV-NR was produced by injecting a New Zealand white rabbit. Multiple injections were given, each emulsified in an equal volume of Freund's complete (first injection) or incomplete adjuvant. The immunized rabbits were bled every week beginning 3 wk after the first injection. Antiserum was diluted 1:1 with glycerol, brought to 1% sodium azide, and stored at −20 C. Antisera titers were determined by SDS-gel double diffusion in 0.75% agarose with 0.2% SDS, 0.1% sodium azide, and 0.7% sodium chloride dissolved in 0.1 M Tris-Cl buffer, pH 9.0 (13).

Homologous-heterologous titers were compared using SDS-gel diffusion with three parallel rows of wells, six wells per row, 5 mm from edge to edge. Antisera (all bleedings were individually tested) were placed in the center row of wells in a twofold dilution series (diluted in saline, 8.5 g sodium chloride per liter of distilled water). One of the BlCMV isolates was added to wells along one side of the antiserum while wells on the opposite

Table 1. Partial host ranges for the type (FS) and necrotic ringspot (NR) isolates of blackeye cowpea mosaic virus (BlCMV)

Host[a]	Symptoms[b] BlCMV-FS	Symptoms[b] BlCMV-NR
Chenopodium quinoa Willd.	CCL (4/4)[c]	CCL (4/4)
C. amaranticolor Coste & Reyn.	CCL (8/8)	CCL red borders (8/8)
Glycine max (L.) Merr. 'Bragg'	+ (2/5)	+ (5/7)
Ocimum basilicum L.	− (0/8)	M (6/8)
Vicia faba L.	− (0/10)	MR, Mt (8/12)
Phaseolus lunatus L. 'Henderson-Bush'	− (0/4)	VC, Mt, D (4/4)
Arachis hypogeae L. 'Florunner'	+ (2/8)	+ (1/8)
Vigna unguiculata (L.) Walp. subsp. *unguiculata*		
'Knuckle Purple Hull'	VC, M, GVB (8/8)	NR, VC, M, D (8/8)
'California Blackeye'	VC, M, GVB (8/8)	NR, VC, M, D (8/8)
'Dixielee'	VC, M, GVB (8/8)	NR, VC, M, D (8/8)
'Worthmore'	− (0/8)	− (0/8)
Crotolaria spectabilis Roth.	M (4/8)	M, D, GVB (8/8)
P. vulgaris L.		
'Improved Tendergreen'	− (0/4)	− (0/4)
'Topcrop'	− (0/6)	− (0/8)
'Royal Burgandy'	− (0/4)	− (0/4)
'Bountiful'	− (0/8)	E, M (2/8)
Canavalia gladiata (Jacq.) DC.	− (0/4)	− (0/4)
Capsicum annuum L. 'Early Calwonder'	− (0/4)	− (0/4)
Cucurbita pepo L. 'Small Sugar'	− (0/4)	− (0/4)
Nicotiana tabacum L. 'Burley 21'	− (0/2)	− (0/4)
Dolichos lablab L.	− (0/2)	− (0/3)

[a] Plants sap-inoculated with infected tissue ground in 0.03 M sodium phosphate buffer, pH 8.0, and maintained under greenhouse conditions.

[b] CCL = chlorotic local lesions, NR = necrotic ringspot local lesions, VC = veinclearing, M = mosaic, Mt = mottling, D = distortion, GVB = green veinbanding, and E = epinasty; + = symptomless, virus detected by ELISA and/or back-inoculation to cowpea, and − = no virus infection detected.

[c] Number of plants infected/number of plants inoculated.

side received the other BlCMV isolate. Antigen was obtained from infected cowpea tissue ground (1 g in 5 ml) in 0.03 M sodium phosphate buffer, pH 8.0.

Intragel absorption experiments (28) were carried out using SDS-gel diffusion.

Preparation of immunoglobulin (Ig) for enzyme-linked immunosorbent assay (ELISA) and the procedures for the double-antibody sandwich method of direct ELISA were according to McLaughlin et al (22). Purified BlCMV-NR and BlCMV-FS at concentrations of 150, 250, 500, 750, 1,000, and 2,000 ng/ml in PBS-Tween (0.02 M phosphate, 0.15 M sodium chloride, 0.003 M potassium chloride, and 0.02% sodium azide, pH 7.3, with the addition of 0.05% Tween 20), healthy cowpea sap (undiluted, diluted 1:10 and 1:100), and PBS-Tween alone were tested against Ig to both virus strains. Treatments were arranged in a 4 × 4 lattice square design with five replicates per Ig. The top half of two polystyrene ELISA plates (Dynatech Laboratories, Inc., Chantilly, VA) was charged with Ig to one BlCMV isolate while the other half of the two ELISA plates was charged with Ig to the other BlCMV isolate. Substrate reactions were stopped by adding 50 μl of 3 M sodium hydroxide per well.

RESULTS

Host range. A partial host range for the two BlCMV isolates is listed in Table 1. In *V. unguiculata* subsp. *unguiculata*, BlCMV-NR caused reddish necrotic ringspots on inoculated leaves (Fig. 1) that later spread into a network of reddish veinal necrosis. Systemic vein-clearing was followed by mosaic, stunting, and distortion (Fig. 2), often with the entire leaf surface turning reddish. The BlCMV-FS isolate caused systemic veinclearing followed by mosaic and green veinbanding (Fig. 3). In *Crotolaria spectabilis* Roth., both BlCMV isolates caused a systemic mosaic; however, the mosaic caused by BlCMV-NR was in the form of a large dark green oak-leaf-shaped area covering the midvein with intermittent veinal chlorosis and some distortion. BlCMV-NR caused a systemic mosaic in *Ocimum basilicum* L., and in *Phaseolus vulgaris* L. 'Bountiful,' primary leaves became epinastic and a faint mosaic developed systemically. In *Vicia faba* L., dark red necrotic local lesions were followed by systemic mottling, and *P. lunatus* L. 'Henderson-Bush' developed systemic veinclearing, mosaic, and distortion with blistering. The BlCMV-FS isolate caused no symptoms in *O. basilicum, P. vulgaris* 'Bountiful,' *V. faba,* or *P. lunatus* 'Henderson-Bush,' and no infection was detected by back-inoculations to cowpeas or by ELISA.

Aphid transmission. Both BlCMV isolates were transmitted in a non-persistent manner by *M. euphorbiae.* Plants inoculated with BlCMV-NR by aphids developed a veinal necrosis on inoculated leaves after about 10–15 days, followed by a systemic mosaic. BlCMV-FS caused typical systemic mosaic symptoms about 26 days after inoculation.

Stability in vitro. Both BlCMV isolates remained infective after being heated for 10 min at 60 but not 65 C. BlCMV-NR remained infective after dilution to 10^{-4} but not 10^{-5}, whereas BlCMV-FS remained infective after dilution to 10^{-3} but not 10^{-4}. Both BlCMV isolates were infective after storage of sap from infected cowpeas for 1 but not 2 days at room temperature.

Purification. Virus yields for BlCMV-NR averaged 9.8 mg/100 g of tissue when plants were harvested about 13–15 days postinoculation but only 3.8 mg/100 g of tissue when harvested 16–18 days postinoculation. The A_{260nm}/A_{280nm} ratio for BlCMV-NR purified preparations, when corrected for light scattering, averaged 1.27 ± 0.09 (11 virus preparations). Virus yields for BlCMV-FS averaged 4.9 mg/100 g of tissue when plants were harvested about 20 days postinoculation. The A_{260nm}/A_{280nm} ratio for BlCMV-FS purified preparations, when corrected for light scattering, averaged 1.30 ± 0.17 (five virus preparations).

RNA isolation and molecular weight. After centrifugation on sucrose gradients, RNA preparations of BlCMV-NR or BlCMV-FS contained three peaks (Fig. 4) designated top, middle, and bottom. The top peak probably contained dissociated capsid protein and host components. The middle peak dissociated in the presence of 60 μg/ml of DNase (Fig. 4). The top and middle peaks were also observed in extracts of healthy cowpea tissue. The bottom peak, but not the top or middle peak, was infectious to cowpea. The viral RNA from both isolates had an average estimated (1.8%

Fig. 1. Local necrotic ringspots with red-brown borders on Knuckle Purple Hull cowpea leaf after inoculation with blackeye cowpea mosaic virus isolate NR.

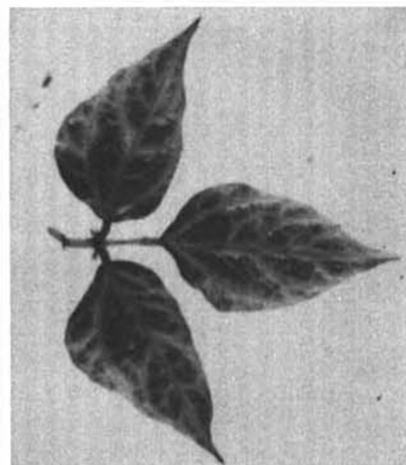

Fig. 2. Severe systemic symptoms of yellow veinbanding, blistering, and dwarfing on Knuckle Purple Hull cowpea leaf after inoculation with blackeye cowpea mosaic virus isolate NR.

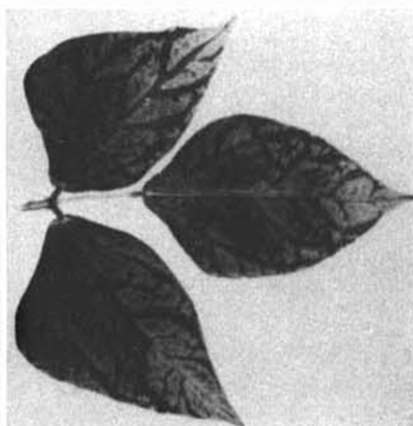

Fig. 3. Systemic symptoms of green vein-banding and mosaic on Knuckle Purple Hull cowpea leaf after inoculation with blackeye cowpea mosaic virus isolate FS.

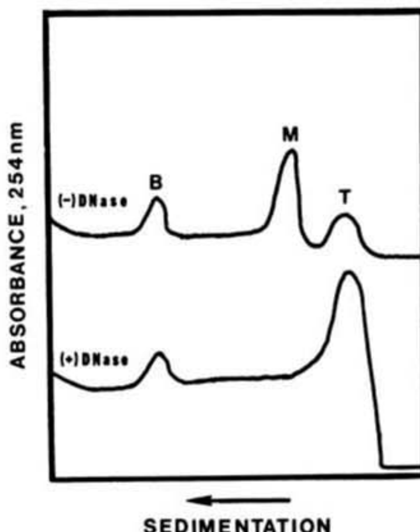

Fig. 4. Ultraviolet absorbance profiles of BlCMV-NR RNA migration through 7.5–30.0% modified sucrose step gradients. (−)DNase represents an untreated sample, (+)DNase represents a sample treated with 60 μg/ml DNase. T = top peak, probably containing dissociated capsid protein and host components; M = DNA of host (cowpea) origin, and B = BlCMV-NR RNA.

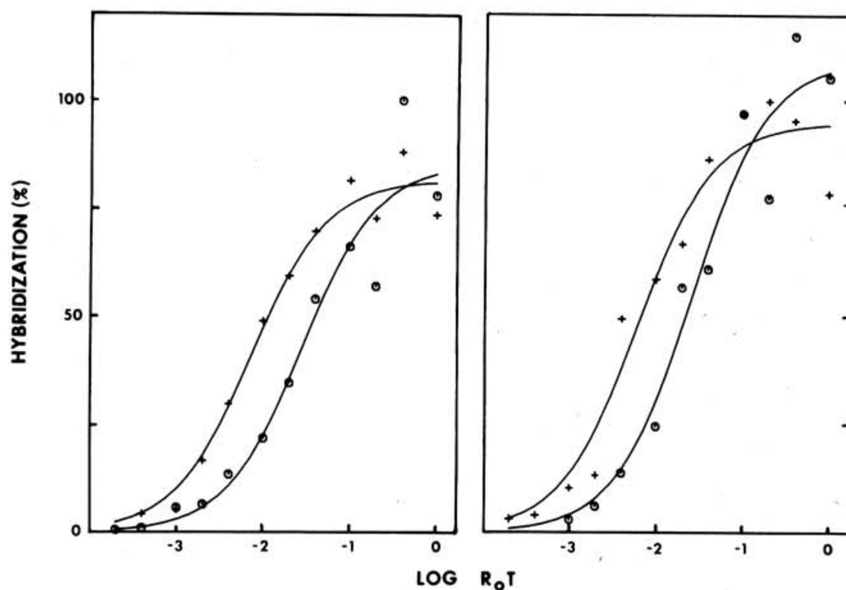

Fig. 5. R_0t curves showing hybridization of blackeye cowpea mosaic virus (BlCMV) isolates **(left)** FS cDNA and **(right)** NR cDNA with BlCMV-FS (circles) and BlCMV-NR (pluses) RNAs.

Table 2. Hybridization kinetic data for the type (FS) and necrotic ringspot (NR) isolates of blackeye cowpea mosaic virus (BlCMV)

cDNA	RNA	Maximum hybridization[a] (%)	R_ot 1/2 (10^{-2} mol s^{-1})	R_ot 1/2/MW[b] of (10^{-9})
BlCMV-FS	BlCMV-F	85.3 ± 6.2 (2)	2.7 ± 0.008	9.3
	BlCMV-NR	81.5 ± 2.6 (4)	0.7 ± 0.001	2.4
	Healthy	0.00 (2)	···	
BlCMV-NR	BlCMV-NR	95.1 ± 4.2 (4)	0.6 ± 0.001	2.1
	BlCMV-FS	109.7 ± 6.9 (2)	2.6 ± 0.007	9.0
	Healthy	2.77 (2)	···	

[a] Upper asymptote ± standard error. Replications per R_ot determination in parentheses.
[b] Molecular weight of BlCMV (FS and NR) RNA was 2.9×10^6.

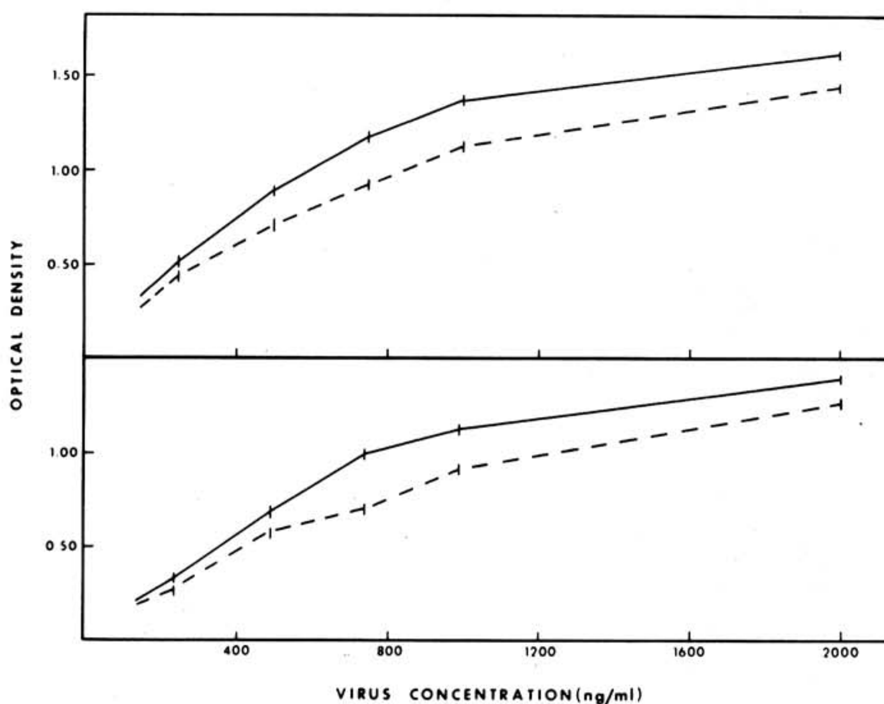

Fig. 6. Dose response curves by direct ELISA with blackeye cowpea mosaic virus (BlCMV) isolate **(top)** NR and **(bottom)** FS antisera-purified BlCMV-NR (solid line) and BlCMV-FS (dashed line). Standard errors of 0.0194 and 0.0418, respectively.

polyacrylamide gels) molecular weight of 2.9×10^6.

Protein subunit relative molecular weight. In 5% polyacrylamide gels, BlCMV-NR protein had an estimated molecular weight of 33,000, whereas that of BlCMV-FS was 32,500. In 8% polyacrylamide gels, molecular weight estimations were 35,800 ± 552 and 35,800 ± 1,650 for BlCMV-NR and BlCMV-FS, respectively.

Molecular hybridization. Hybridization kinetics of BlCMV-NR cDNA and BlCMV-FS cDNA to homologous and heterologous RNAs were similar for cDNA to each BlCMV isolate (Fig. 5). The results are represented adequately by the curve $f/g = 1 - 1(1 + R_0t/h)$, where f = the fraction of nucleic acid strands renatured at time t, g = the upper asymptote, and h = the $R_0t1/2$ (25). A higher percentage of hybridization occurred with BlCMV-FS RNA to either of the cDNAs when compared with that observed for BlCMV-NR RNA (Fig. 5, Table 2). The rate of hybridization for BlCMV-NR RNA to either of the cDNAs was greater than that for BlCMV-FS RNA.

Electron microscopy. Flexuous rod-shaped particles were observed from purified preparations of BlCMV-NR and BlCMV-FS. The modal length of at least 100 virus particles of each BlCMV isolate was 765 nm. Cytoplasmic inclusion bodies in the form of pinwheels and scrolls were identified in sections of leaf tissue infected with BlCMV-NR and BlCMV-FS (7).

Serology. Antisera to BlCMV-NR and BlCMV-FS had titers, determined in SDS-gel double-diffusion tests, of 1:4 and 1:8, respectively. Confluent bands of precipitin consistently developed in SDS-gels between BlCMV-NR and BlCMV-FS when either serum was used. No reaction to healthy cowpea sap occurred. Individual bleedings of antisera to each BlCMV isolate reacted equally to homologous and heterologous antigen. Sera did not react with their homologous antigen after intragel absorption with heterologous antigen.

Dose-response curves by ELISA with purified preparations of antigen showed BlCMV-NR to have higher optical density readings (405 nm) than BlCMV-FS when reacted with either BlCMV-NR or BlCMV-FS Ig (Fig. 6). In both tests, the slopes of homologous and heterologous reactions were similar, with the greatest difference in optical density reading being only 0.245 (1,000 ng/ml) for tests using BlCMV-NR Ig and 0.293 (750 ng/ml) for tests using BlCMV-FS Ig.

DISCUSSION

The BlCMV-NR isolate recovered from the cowpea sample in South Carolina was shown to differ biologically from BlCMV-FS. These biological

differences included host range, symptom type, and in cowpea, symptom severity. Although the BlCMV isolates differed biologically, they were similar in most of their physiochemical characteristics. Of special interest is the similarity of each of the BlCMV isolates studied here to those in previous reports (1,16). The host range and symptomatology of BlCMV-NR are very similar to those of the isolate studied by Anderson (1) in the original description of BlCMV, especially in cowpea, *V. faba, C. spectabilis,* and *P. lunatus* 'Henderson-Bush.' This differs from the host range and symptomatology of BlCMV-FS, which closely resembles that of the BlCMV isolate characterized as the type strain by Lima et al (16). Direct comparisons involving Anderson's isolate, the isolate described by Lima et al, and the necrotic ringspot isolate could not be carried out because Anderson's original isolate is unavailable (8).

Field samples showing viruslike symptoms are often found to be infected with two or more viruses. Kuhn et al (15) reported such occurrences in cowpea and indicated four methods of separating mixed infections. Single-lesion transfer through a local lesion host is a generally accepted method of obtaining "pure" virus strains. However, reports (9,19,21) have indicated that mutants can arise within a single lesion, with both subsequently being transferred. Single-lesion transfer of BlCMV-NR through *C. amaranticolor*, with subsequent inoculation into cowpea, resulted in symptom development typical of BlCMV-NR. Purified virus preparations from such cowpea tissue, when used as inoculum to cowpea, also induced symptoms typical of BlCMV-NR.

Comparative tests of the two BlCMV isolates studied here showed similarities in their mode of transmission, stability in vitro, particle morphology and modal length, and cytoplasmic inclusion body morphology. Estimates of protein subunit molecular weight for each isolate were similar to reported values (16,27), as were the estimates of RNA molecular weight (20).

Different strains of a virus often require different periods of time to reach maximum concentration in systemically infected tissue (26). BlCMV-NR reached maximum concentration in cowpea about 5 days before BlCMV-FS and caused a more severe reaction than BlCMV-FS. The absorbance ratio, A_{260nm}/A_{280nm}, for both BlCMV isolates from purified preparations was consistently higher than the reported value of 1.2 (16). Extracts of viral RNA from such preparations always yielded an additional fraction (middle peak, Fig. 4) that absorbed at 254 nm. Makkouk and Gumpf (17) observed a similar occurrence with potato virus Y and found that the material corresponding to such a peak was degraded in the presence of DNase. They concluded that the middle fraction was DNA of host origin. Such was the case with BlCMV-NR and BlCMV-FS. Furthermore, the middle fraction was obtained from healthy cowpea tissue subjected to the BlCMV purification procedure.

Direct double-antibody sandwich (DAS) and indirect ELISA are sensitive techniques for detecting plant viruses. DAS-ELISA is more specific than indirect ELISA (29) and often allows differentiation of closely related strains (14). A range of concentrations of purified preparations of each BlCMV isolate gave DAS-ELISA reactions that were very similar in both the slope of their respective dose-response curves and optical density readings at 405 nm. BlCMV-NR had higher optical density readings than BlCMV-FS when reacted with BlCMV-NR Ig and BlCMV-FS Ig. Chen et al (5) described a similar situation with DAS-ELISA with soybean mosaic virus isolates, which they attributed to lateral aggregation of virus particles at the antigen binding sites of the antibody molecules. This would allow for an increase in the binding of conjugated antibody with subsequently increased substrate reactions. If this were the cause of the higher optical density readings for the heterologous reactions (BlCMV-NR antigen and BlCMV-FS Ig), the same could be said for homologous reactions (BlCMV-NR antigen and BlCMV-NR Ig). Based on SDS-gel diffusion and DAS-ELISA results, we are therefore reluctant to suggest that a serological distinction exists between BlCMV-NR and BlCMV-FS.

Molecular hybridization results did not reveal large differences in RNA homologies but suggested that the RNA from each BlCMV isolate had certain characteristics peculiar to that isolate. Thus, hybridization kinetic curves showed that the rate of hybridization of BlCMV-NR RNA was faster than that of BlCMV-FS RNA with both cDNAs. This subtle difference could be due to small changes in nucleotide sequence that result in slightly altered secondary structures of the RNAs.

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LITERATURE CITED

1. Anderson, C. W. 1955. *Vigna* and *Crotalaria* viruses in Florida. II. Notations concerning cowpea mosaic virus (*Marmor vignae*). Plant Dis. Rep. 39:349-353.
2. Bos, L. 1983. Order out of chaos. Pages 93-107 in: Introduction to Plant Virology. Longman Inc., New York. 160 pp.
3. Brakke, M. K., and Van Pelt, N. 1970. Properties of infectious ribonucleic acid from wheat streak mosaic virus. Virology 42:699-706.
4. Bruening, G., Beachy, R. N., Scalla, R., and Zaitlin, M. 1976. *In vitro* and *in vivo* translation of the ribonucleic acids of a cowpea strain of tobacco mosaic virus. Virology 71:498-517.
5. Chen, L. C., Durand, D. P., and Hill, J. H. 1982. Detection of pathogenic strains of soybean mosaic virus by enzyme-linked immunosorbent assay with polystyrene plates and beads as the solid phase. Phytopathology 72:1177-1181.
6. Collins, M. H., Murphy, J. F., Witcher, W., and Barnett, O. W. 1984. Survey of cowpeas in South Carolina for six viruses. Plant Dis. 68:561-563.
7. Edwardson, J. R. 1974. Some Properties of the Potato Virus Y Group. Fla. Agric. Exp. Stn. Monogr. 4. 398 pp.
8. Edwardson, J. R., Zettler, F. W., Christie, R. G., and Evans, I. R. 1972. A cytological comparison of inclusions as a basis for distinguishing two filamentous legume viruses. J. Gen. Virol. 15:113-118.
9. Garcia-Arenal, F., Palukaitis, P., and Zaitlin, M. 1984. Strains and mutants of tobacco mosaic virus are both found in virus derived from single-lesion-passage inoculum. Virology 132:131-137.
10. Gonda, T. J., and Symons, R. H. 1978. The use of hybridization analysis with complementary DNA to determine the RNA sequence homology between strains of plant viruses: Its application to several strains of cucumoviruses. Virology 88:361-370.
11. Gould, A. R., and Symons, R. H. 1977. Determination of the sequence homology between the four RNA species of cucumber mosaic virus by hybridization analysis with complementary DNA. Nucleic Acids Res. 4:3787-3802.
12. Hill, J. H., and Benner, H. I. 1980. Properties of soybean mosaic virus ribonucleic acid. Phytopathology 70:236-239.
13. Jones, R. T., and Diachun, S. 1977. Serologically and biologically distinct bean yellow mosaic virus strains. Phytopathology 67:831-838.
14. Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol. 40:309-318.
15. Kuhn, C. W., Brantley, B. B., and Sowell, G. 1966. Southern pea viruses: Identification, symptomatology and source of resistance. Ga. Agric. Stn. Bull. 157. 22 pp.
16. Lima, J. A. A., Purcifull, D. E., and Hiebert, E. 1979. Purification, partial characterization and serology of blackeye cowpea mosaic virus. Phytopathology 69:1252-1258.
17. Makkouk, K. M., and Gumpf, D. J. 1974. Isolation and properties of potato virus Y ribonucleic acid. Phytopathology 64:1115-1118.
18. Mali, V. R., and Kulthe, K. S. 1980. A seedborne potyvirus causing mosaic of cowpea in India. Plant Dis. 64:925-928.
19. Matthews, R. E. F. 1981. Variability. Pages 454-525 in: Plant Virology. 2nd ed. Academic Press, New York. 897 pp.
20. Matthews, R. E. F. 1981. The groups of plant viruses. Pages 715-732 in: Plant Virology. 2nd ed. Academic Press, New York. 897 pp.
21. McGovern, M. H., and Kuhn, C. W. 1984. A new strain of southern bean mosaic virus derived at low temperatures. Phytopathology 74:95-99.
22. McLaughlin, M. R., Barnett, O. W., Burrows, P. M., and Baum, R. H. 1981. Improved ELISA conditions for the detection of plant viruses. J. Virol. Meth. 3:13-25.
23. Murphy, J. F., Barnett, O. W., and Witcher, W. 1984. A blackeye cowpea mosaic virus strain from South Carolina. (Abstr.) Phytopathology 74:632.
24. Purcifull, D. E. 1966. Some properties of tobacco etch virus and its alkaline degradation products. Virology 29:8-14.
25. Reddick. B. B., and Barnett, O. W. 1983. A comparison of three potyviruses by direct hybridization analysis. Phytopathology 73:1506-1510.
26. Steere, R. L. 1959. The purification of plant viruses. Adv. Virus Res. 6:1-73.
27. Taiwo, M. A., Gonsalves, D., Provvidenti, R., and Thurston, H. D. 1982. Partial characterization and grouping of isolates of blackeye cowpea mosaic and cowpea aphidborne mosaic viruses. Phytopathology 72:590-596.
28. Van Regenmortel, M. H. V. 1982. Serological techniques. Pages 73-133 in: Serology and Immunochemistry of Plant Viruses. Academic

Press, New York. 302 pp.
29. Van Regenmortel, M. H. V., and Burckard, J. 1980. Detection of a wide spectrum of tobacco mosaic virus strains by indirect enzyme-linked immunosorbent assays (ELISA). Virology 106:327-334.
30. Weber, K., and Osborn, M. 1975. Protein and sodium dodecyl sulfate: Molecular weight determination on polyacrylamide gels and related procedures. Pages 179-223 in: The Proteins. Vol. 1. H. Neurath and R. L. Hill, eds. Academic Press, New York. 547 pp.