Cowpea Mottle Virus: A Seed-Borne Virus with Distinctive Properties Infecting Cowpeas in Nigeria

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The senior author is grateful to the Director, Institute of Agricultural Research and Training, for providing funds to carry out part of the investigations and for permission to publish the results. Portions of the research were carried out at the Boyce Thompson Institute, Yonkers, NY 10701, while the second and third authors were virologist and post-doctorate research associate, respectively; and at the Institute of Agricultural Research, Ahmadu Bello University, Samaru, Zaria, Nigeria, by the fourth author. Travel of the second author to Nigeria and Central America was sponsored by the National Science Foundation's SEED program Grant No. INT 76-10717.

The authors are indebted to L. Bos and D. Z. Maat, Institute for Phytopathological Research, Wageningen, The Netherlands, and to H. Waterworth, U.S. Department of Agriculture, Plant Introduction Station, Glen Dale, MD 20769, USA, for serological tests with cowpea mottle virus and its antiserum; and to R.S. Ochieng, International Institute of Tropical Agriculture, Ibadan, Nigeria, for supplying adult *Ootheca mutabilis*.

Accepted for publication 24 October 1977.

ABSTRACT

SHOYINKA, S. A., R. F. BOZARTH, J. REESE, and H.W. ROSSEL. 1978. Cowpea mottle virus: a seed-borne virus with distinctive properties infecting cowpeas in Nigeria. Phytopathology 68: 693-699.

A single-component, 30-nm diameter isometric, plant virus with 20% RNA, $S_{20,w}$ = 122, and protein subunit molecular weight of 44,500 daltons was isolated from Nigerian cowpeas. These properties are distinct from any previously described virus. On the basis of host range, geographic distribution of isolates, and distinctive serological properties, the virus was considered to be the cowpea mottle virus which was previously described in terms of its biological properties. The virus was transmitted in the seed of all varieties of cowpeas tested and by the beetle *Ootheca mutabilis* in which it was retained for 5 days. The virus infects many species of Leguminosae, Chenopodiaceae, and Solanaceae and appears to be confined to Nigeria.

Additional key words: buoyant density, cowpea yellow mosaic virus, cowpea severe mosaic virus, southern bean mosaic virus.

Robertson (18) reported the occurrence of cowpea mottle virus (CMeV) infecting cowpeas (*Vigna unguiculata*) in western, eastern, and southern provinces of northern Nigeria. He described the host range and properties in crude juice of the virus and concluded through serological studies that it was unrelated to cowpea yellow mosaic virus (CYMV) (7, 10). No physicochemical description was attempted, and apart from being listed in *Plant Virus Names* (17), no further reference occurred until Rossel (19) reported the isolation of a virus from *Voandzeia subterranea* which he tentatively identified as CMeV. This research is part of a systematic effort to identify and characterize the grain legume viruses of Nigeria. We describe here the host range, distribution, symptomatology, vector transmission, purification, morphology, physicochemical properties, and serology of a virus which is considered to be Robertson's CMeV.

MATERIALS AND METHODS

Virus isolates, inoculation, and cultivation.—One isolate of CMeV, YB-I, was collected from a naturally infected plant at Ilora Crop Research Station (72 kilometers northwest of Ibadan in the derived savanna zone of southwestern Nigeria). The second isolate, 64692, was obtained from cowpea Accession No. 64692 (hereafter abbreviated Acc. No.), during a routine screening of local and exotic cowpea cultivars at Moor Plantation (Ibadan, Nigeria).

Inoculum of each isolate was prepared by grinding infected cowpea leaves in 0.1 M (pH 7.0) potassium phosphate buffer. Then these inocula were rubbed onto primary leaves of healthy, 7- to 10-day-old seedlings of cowpeas which had been previously dusted with 22-μm particle size (600-mesh) Carborundum. In Nigeria, cowpea cultivars Brabham 892A and Ife Brown were used. In the USA cultivar Calif. No. 5 Blackeye (1975 crop, George Taite Seed Co., Norfolk, VA 23504) was used. The Calif. No. 5 seeds were considered to be free of seed-transmitted viruses following the observation of 2,000 test seedlings, 400 of which were indexed to cowpea to test for seed-transmitted viruses. All subsequent investigations were made from systemically infected leaves of one of these hosts.

Host range.—Host range studies were conducted in Nigeria. Five to 10 plants of each species or cultivar were inoculated, along with five plants of cowpea cultivar Prima to demonstrate infectivity of inoculum. Return inoculations were made from inoculated and newly

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developed leaves of test plants 1 to 2 wk after inoculation. For such reisolations, leaf samples were pooled from many inoculated plants of the same species or cultivar to make inoculum.

Properties in crude juice were determined using filtered homogenates of systemically infected leaves.

All test plants were maintained in an insect-proof screenhouse (Nigeria) or glasshouse (USA) at temperatures between 22 C (average minimum) and 32 C (average maximum). In Nigeria, Dimethoate (Rogor 40) was applied at weekly intervals to reduce chances of insect transmission. In the USA, glasshouses were fumigated weekly with Nicofume and dusted weekly with Karathane.

Virus purification.—Systemically infected Calif. No. 5 cowpea leaves were harvested 2 to 3 wk after inoculation of primary leaves, blended in a Waring Blendor in 0.1 M potassium phosphate buffer pH 7.0, and subjected to three cycles of differential centrifugation. All experiments were done in this buffer.

Further purification for chemical and physical analysis and for antiserum production was carried out in a Beckman (Beckman Instruments, Inc., Spinco Division, Palo Alto, CA 94304) Ti-15 zonal rotor using a 10-40% radially linear gradient of sucrose (3). The virus sample, approximately 25 ml, was made 5% sucrose, layered over the gradient, and overlayered with 110 ml of buffer. Following centrifugation for 4 hr at 30,000 rpm in a Beckman Model L centrifuge, the sample was fractionated by pumping the gradient through an ISCO (ISCO, Lincoln, NE 68505) UA-2 ultraviolet monitor at the rate of about 30 ml/min. Fractions that exhibited ultraviolet absorbancy were centrifuged at 30,000 rpm in a Beckman 30 rotor for 18 hr and resuspended in buffer.

For chemical and physical analysis, the density gradient-purified virus was dialyzed for 48 hr against 1 liter of buffer. The buffer was changed three times and the final dialyzate was used as the blank in subsequent tests.

Analytical sucrose density gradient analysis.—Sucrose density gradients (10-40%) were centrifuged 2.0 hr at 39,000 rpm in a Beckman SW-41 rotor at 5 C (15). Following centrifugation, analysis and fractionation were carried out on ISCO equipment. Samples consisted of 0.6-1.0 OD_{260} absorbancy units in a volume of .05-0.2 ml.

Analytical ultracentrifugation.—Sedimentation velocity and density in CsCl were determined in a Beckman Analytical Ultracentrifuge equipped with electronic speed control and scanner. An ANF-Ti rotor was used and the methods described by Chervenka (8) were generally followed. For the sedimentation velocity experiment, the instrument was run at 20,000 rpm (20.0 C) and pictures were taken at 4 min intervals. Samples contained virus at approximately $A_{265} = 0.8$ which was considered to be at infinite dilution. Viscosity and density of the dialyzate were determined in a Cannon Ubbelohde (Cannon Instrument Co., State College, PA 16801) viscometer and a 50.00-ml pycnometer at 25.00 C, respectively. The results reported are the average of two experiments.

Density in CsCl was determined by the hinge-point method as previously reported for studies of the density of RNA in Cs_2SO_4 (2). The Beta factor was taken from Chervenka (8).

Ultraviolet absorbancy spectra.—Dialyzate was used as a blank in determining the ultraviolet absorbancy spectra and the light-scattering correction was applied by the method of Englander and Epstein (13). A Gilford 2400S (Gilford Instrument Laboratories, Oberlin, OH 44074) ultraviolet spectrophotometer was used to scan samples from 220-400 nm.

Nucleic acid.—Orcinol and diphenylamine colorimetric tests for nucleic acid were by the method of Dische (11).

Protein analysis.—The molecular weight of the protein subunits was determined by digesting the purified virus with 6 M urea and 2% sodium lauryl sulfate (SLS), and electrophoresis on 6% polyacrylamide gels (PAGE). Standard proteins used were bovine serum albumin, mol wt 67,500 (12); ovalbumin, mol wt 44,500 ± 900 (5, 6); and turnip yellow mosaic virus protein, mol wt 20,000 (4). Electrophoresis was carried out by the method of Dunker and Rueckert (12).

Electron microscopy.—Carbon-coated-Formvar grids [74-μm (200-mesh)] were floated on drops containing samples for 1-3 min, rinsed in H_2O, and negatively stained with 1% uranyl acetate in H_2O. Grids were examined on a Zeiss EM9-2S electron microscope, and pictures were taken at a magnification of ×27,000. The electron microscope was calibrated with a grid replica and catalase (22).

Serology.—Antiserum was prepared by intramuscular injection of rabbits with purified virus and Freund's complete adjuvant (Difco Laboratories, Detroit, MI 48232). Normal serum of each rabbit was taken prior to the first injection. Serological tests were made by Ouchterlony double diffusion in 1% agar on glass slides using Gellman equipment (Scientific Products, Obetz, OH 43207). Sodium azide (0.1%) was used as a preservative. In tests performed by the senior author, the methods of Crowley (9) were followed.

The virus was tested serologically against the following antisera: cowpea yellow mosaic virus (CYMV) (1), cowpea severe mosaic virus (CSMV) (1), okra mosaic virus (OkMV) (4), tobacco ringspot virus (TRV) (15), the virus isolated from *V. subterranea* (19), and antisera from the American Type Culture Collection (Rockville, MD 20852): southern bean mosaic virus (SBMV, PVAS-2, and PVAS-11), bean pod mottle virus (BPMV, PVAS-9), and squash mosaic virus (SMV, PVAS-14). Because CYMV, CSMV, OkMV, and SBMV were also under study, serological tests against these viruses using reciprocal controls were made often.

In addition to the serology tests carried out by the authors, the antiserum was tested against a number of viruses by L. Bos and D. Z. Maat and the antigen was tested against a number of virus antisera by H. Waterworth.

Insect transmission.—Nonviruliferous individual adults of *Ootheca mutabilis* (Chrysomelidae) were obtained from R. S. Ochieng who recently developed a method for rearing and culturing this species in the laboratory (R.S. Ochieng, *personal communication*). Beetles were starved for 12 hr before acquisition feeding. Cowpea cultivar Brabham 892A was used as both the source and test plant for CMeV. An acquisition feeding time of between 1 hr and 24 hr was allowed before

transferring the beetles to fresh test plants for periods ranging from 2 hr to 1 day. The retention of the virus following a 24-hr acquisition feeding period was tested by serial transfer to healthy test plants every 24 hr for 10 or more days.

Seed transmission.—To determine percentage infection in naturally infected plants, 100 seeds each of cowpea cultivar Ibadan White and Moor Plantation Acc. No. 64692 were sown in trays. The number of seedlings showing symptoms at the 3-wk stage was expressed as a percentage of germinated seedlings. Three to five lots of 100 seeds each per cultivar were germinated in steam-sterilized compost for this assessment.

Healthy seedlings of cowpea cultivar Ibadan White and cultivar French bean were inoculated manually with sap from infected seedlings. After the pods were harvested, 20 seeds from each of five infected plants were sown for seed infection assessment.

To test transmission in seeds obtained from inoculated plants of cultivar Calif. No. 5, three groups of about 60 plants each were inoculated at the primary leaf stage and 1 and 2 wk thereafter. When seed pods were mature, all seeds were harvested and allowed to dry for 7 days before planting. After 3 wk, plants were observed for evidence of virus infection.

Infectivity of leaves and various reproductive parts of naturally infected and manually inoculated plants of cowpea cultivar Ibadan White were determined by grinding the appropriate detached vegetative or reproductive parts in buffer and indexing the inoculum on healthy seedlings of the same variety.

RESULTS

Sedimentation analysis and electron microscopy.—Sucrose density gradient analyses of samples purified only by differential centrifugation are shown in Fig. 1-A and samples further purified by zonal centrifugation (B) are shown in Fig. 1-B. Zonal purification removed a broad band (f) which had the sedimentation, absorbancy, and electron micrographic properties of phytoferritin (15). The sample contained a large band (V) which sedimented at $S_{20,w} = 122 \pm 1.50$ in the analytical ultracentrifuge. A smaller, faster-moving band (d) was not discernible at the concentrations used for analytical ultracentrifugation (Fig. 2-A). Its approximate sedimentation velocity in sucrose density gradients and its appearance in negatively stained electron micrographs indicated that it was a dimer band. When the sample from the major band (V) and the dimer band (d) were diluted to the same optical density and bioassayed on cowpeas, both were infective. The ferritin band (f) was not infective. Measurement of 200 negatively stained particles from four different electron micrographs resulted in a measured particle diameter of 30 ± 1.5 nm (Fig. 3).

Estimates of percentage nucleic acid.—A positive orcinol test and negative diphenylamine test indicated that the virus contains ribonucleic acid. A typical ultraviolet absorbancy spectrum with a maximum and minimum absorbancy at 262 and 242 nm, respectively, and $A_{260/280} = 1.66$ was obtained. In CsCl, density gradient centrifugation, a buoyant density of $1.3492 \pm .0040$ (Fig. 2-B) was obtained. Both values are consistent with a nucleic acid percentage of approximately 20% (20).

Protein subunit molecular weight.—Following degradation of the protein with SLS-Urea, a single peptide was obtained which co-electrophoresed with ovalbumin in PAGE (Fig. 4). The molecular weight of ovalbumin has been determined by many workers. Those reported prior to 1941 were reviewed by Bull (5) who considered 45,000 daltons to be the best estimate. A more recent study of Castellino and Barker (6) reported values obtained by membrane osmometry and by sedimentation equilibrium measurements of native and guanidine hydrochloride-dissociated proteins. If the average of the values obtained by the two methods is taken as the best estimate, the molecular weight of ovalbumin and the peptides of CMeV is $44{,}500 \pm 1{,}500$ daltons.

Serology.—Antiserum prepared against CMeV was specific to the virus and did not react with normal serum or juice of healthy plants (Fig. 5). The virus did not react with CYMV, CSMV, SBMV, OkMV, BPMV, SMV, or TRV antisera.

The virus did react with antiserum to the virus previously isolated from *V. subterranea* and there was no spur formation.

Serological tests between CMeV and antisera to the following viruses were made by H. E. Waterworth: hibiscus chlorotic ringspot, a hibiscus virus from G. M. Behncken (Indooroopilly, Queensland, Australia); Arabis mosaic; broad bean mottle; sowbane mosaic; southern bean mosaic; bean rugose mosaic; bean mild mosaic; carnation ringspot; *Pelargonium* ringspot; brome mosaic; elm mosaic; almond mosaic; artichoke crinkle; tobacco streak; a pear virus from S. H. Smith (Pennsylvania State University, University Park, PA 16802); apple mosaic; eggplant mosaic; tomato ringspot; squash mosaic; lolium mosaic; and cucumber necrosis. In these tests the virus did not react with any heterologous antisera, but did react with its homologous antiserum.

No reactions were obtained between CMeV antiserum and the following viruses by L. Bos and D. Z. Maat: Arabis mosaic, strawberry latent ringspot, tomato black ring, raspberry ringspot, cherry leaf roll, carnation ringspot, tobacco streak, and cucumber mosaic.

Properties in crude sap.—In crude sap of cowpeas CMeV was infective at a dilution of 2×10^{-5} after heating 10 min at 60 C but not 65 C and lost infectivity after 24 hr incubation at 22 C.

Symptomatology.—The primary leaves of many cultivars of cowpeas developed inconspicuous chlorotic lesions 4-5 days after inoculation. This was followed by intense chlorosis covering the entire lamina of young, fully expanded trifoliolate leaves. In some cultivars this chlorosis was occasionally interspersed with green islands (Fig. 6). This reaction was generally displayed by many cowpea cultivars to different isolates of the virus. Sometimes, however, cultivar Brabham 892A produced necrotic local lesions on being inoculated with a seed-borne isolate from cultivar Ibadan White. Some cultivars, such as Ife Brown, showed vein-clearing symptoms on inoculated primary leaves, followed by a bright yellow mosaic and a slight leaf distortion of systemically infected leaves.

Variations of these symptoms sometimes were obtained under glasshouse conditions. In some cases, inoculated plants of both Prima and Brabham 892A

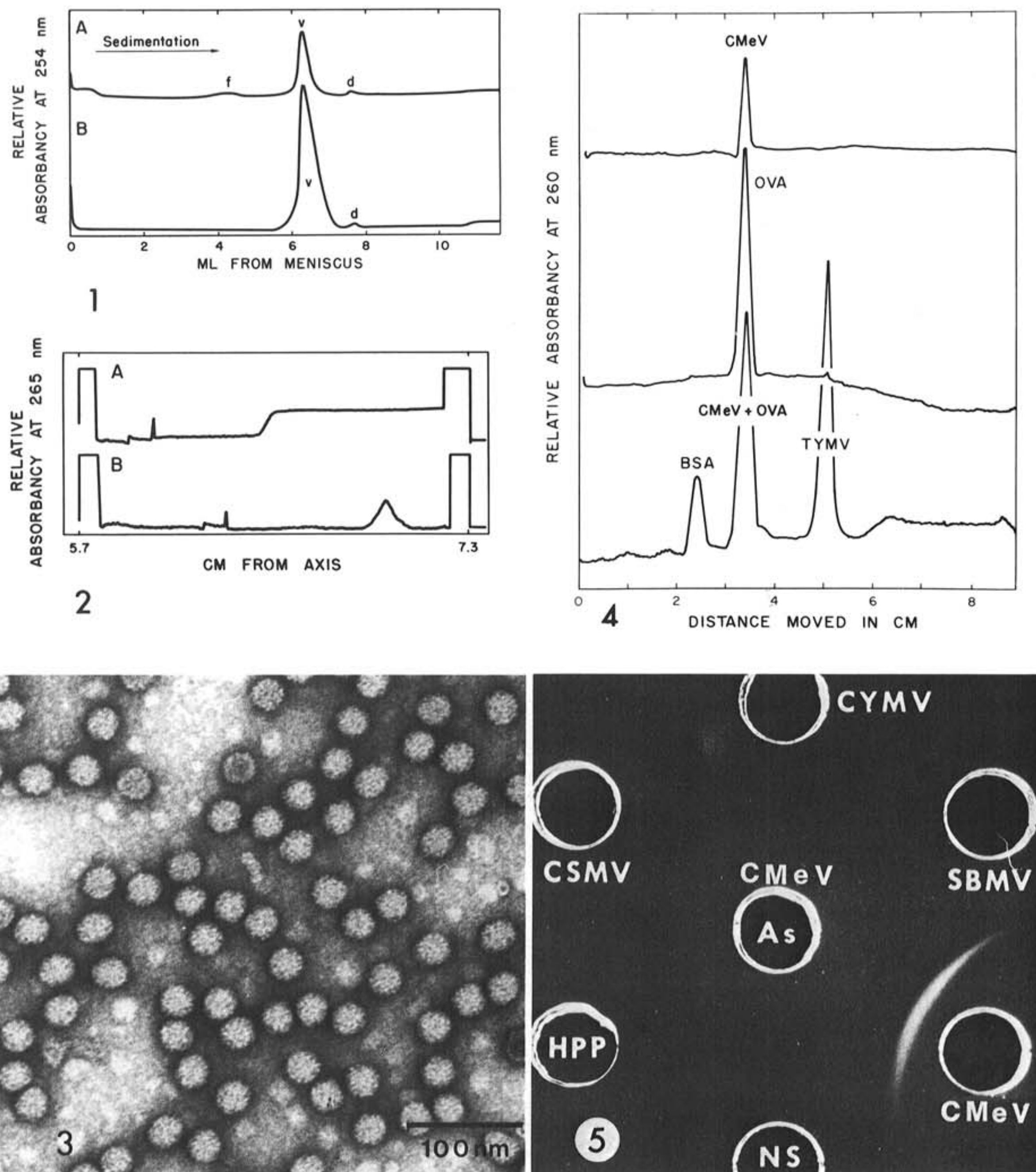

Fig. 1-5. Properties of cowpea mottle virus (CMeV). **1)** Ultraviolet absorbancy at 254 nm of 10-40% sucrose density gradients following centrifugation at 39,000 rpm for 2.0 hr at 5 C in a Beckman SW-41 rotor and scanning in an ISCO density gradient fractionator system. (Curve A) Cowpea mottle virus preparation after purification by three cycles of differential centrifugation. (Curve B) The virus peak of the same preparation further purified by sucrose density gradient centrifugation in the zonal rotor. Virus band (V), viruses aggregated into dimers (d), and phytoferritin band (f). **2)** Analytical ultracentrifugation scanning patterns of sedimentation velocity run of (Curve A) CMeV 24 min from zero time at 20,000 rpm and (Curve B) CsCl density gradient centrifugation after 18 hr at 44,000 rpm. **3)** Electron micrograph of CMeV negatively stained with 1% uranyl acetate. **4)** Scanning pattern of stained gels following electrophoresis on 7% polyacrylamide gels for 5 hr at 5 mA per tube. Standard proteins used in this analysis were bovine serum albumin (BSA), ovalbumin (OVA), and turnip yellow mosaic virus (TYMV). Migration in the gel is shown as movement from left to right. **5.** Double-diffusion serological reaction. The antiserum to CMeV (CMeV-AS) is contained in the center well. Outer wells contain cowpea yellow mosaic virus (CYMV), cowpea severe mosaic virus (CSMV), healthy cowpea extract (HPP), normal sera (NS), CMeV homologus antigen (CMeV),and southern bean mosaic virus—cowpea strain (SBMV).

reacted by showing only mild systemic mottle without any distortion or reduction in leaf size (Fig. 7). Field symptoms were usually similar to those obtained under glass where singly infected cowpeas (cultivar Ife Brown) showed yellow blotching which was sometimes intermingled with whitish spots. Plants infected by a mixture of CYMV and CMeV often reacted with severe mosaic, leaf distortion, reduction in leaf size, and early senescence. Flower abortion and witches broom symptoms were common. Symptoms on Calif. No. 5 are relevant to seed transmission tests and discussed below.

Host range.—In general, susceptible hosts reacted with one, or a combination of, the following types of symptoms: severe chlorosis, uniform mosaic or mosaic-mottle, local lesions with or without systemic spread, vein necrosis, and symptomless.

Phaseolus vulgaris 'French Dwarf Prince', 'Prince', and 'UK Commercial'; *P. lunatus* (Acc. No. 64009); *P. acutifolius* 'latifolius'; *P. aureus*; *P. mungo*; *P. lathyroides*; *Glycine max* 'Hampton Soya'; and *Cajanus cajan* developed primary and systemic symptoms similar to those described for *Vigna unguiculata. Phaseolus mungo* exhibited veinal necrosis on inoculated primary leaves followed by systemic uniform mosaic on subsequent trifoliolate leaves.Chlorotic lesions developed on inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa* without systemic spread. *Canavalia ensiformis* reacted with systemic mottle symptoms. Systemic chlorotic blotching and enations were produced on infected plants of *Solanum macrocarpon* and *S. aethiopicum*. Virus was recovered from the following symptomless hosts that were inoculated and observed for 4-6 wk: *Physalis angulata*, *Nicotiana tabacum* 'Turkish', *N. glutinosa*, *Lycopersicon peruvianum*, *Tetragonia expansa*, and *Petunia hybrida*. Cowpea mottle virus was not recovered from the following symptomless test species: *Atriplex hortensis*, *Datura stramonium*, *Nicotiana clevelandi*, *Zinnia elegans*, *Vicia faba*, *Chenopodium murale*, *Lycopersicon esculentum*, *Cucumis sativus*, *Cassia obtusifolia*, and *Nicotiana tabacum* 'White Burley'.

Insect transmission.—Cowpea mottle virus was transmitted readily from infected to healthy cowpea plants (cultivars Brabham 892A, Ife Brown, and Prima) by *Ootheca mutabilis*. After acquisition feeding periods of 1 hr and 24 hr, beetles transmitted the virus to 6/10 and 8/10 plants, respectively. After 24 hr of acquisition, CMeV was retained for 5 days (Table 1).

Seed transmission.—Seeds were collected from naturally infected cultivar Ibadan White and Acc. No. 64692 grown at Moor plantation and germinated. In three tests with Ibadan White, 9.6, 10.3, and 6.0% were infected. In three tests with Acc. No. 64692, 3.8, 9.6, and 6.0% were infected.

When 20 seeds from each of five manually inoculated plants of cultivar Ibadan White and cultivar French Bean were germinated, 7 and 6% (respectively) contained seed-transmitted CMeV. With each cultivar, seed from four of five plants tested produced infected seeds.

In seed transmission tests with cultivar Calif. No. 5 inoculated at three different growth stages, plants inoculated at the primary leaf stage produced the most severe disease reaction, but those inoculated 2 wk later produced the most seed transmission. Plants inoculated at the primary leaf stage were affected with extensive veinal necrosis and severe mosaic. For several days it appeared that they would not survive. Plants inoculated 1

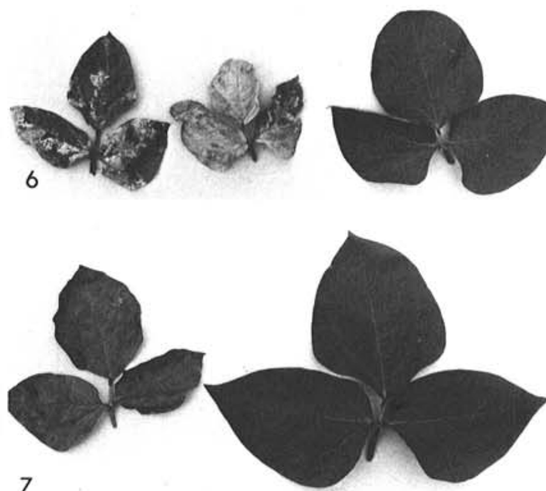

Fig. 6-7. Symptoms of cowpea mottle virus (CMeV) on cowpea leaves. **6)** Systemically infected leaves of cowpea cultivar Brabham 892A showing chlorotic and mottle symptoms (left). and severe chlorosis (center) following inoculation with CMeV. A comparable leaf from a noninoculated plant is shown on the right. **7)** Systemically infected leaves of cowpea cultivar Brabham 892A showing pure mottle symptoms (left) following inoculation with CMeV. A comparable leaf from a noninoculated plant is shown on the right.

TABLE 1. Retention of cowpea mottle virus after a 24-hr acquisition feeding by *Ootheca mutabilis* followed by a 24-hr serial transfer test feeding

Beetle No.	Transfer Day									
	1[a]	2	3	4	5	6	7	8	9	10
1	+	+	+	+	+	–	–	–	–	–
2	+	+	+	+	–	–	–	–	–	–
3	+	+	+	–	+	–	–	–	–	–
4	+	+	+	+	–	–	–	–	–	–
5	+	+	+	D	–	–	–	–	–	–

[a]Symbols: + = positive transmission obtained; – = no transmission obtained; and D = beetle died.

wk after the primary leaf stage produced a mosaic in all subsequently developed leaves. Plants inoculated 2 wk after the primary leaf stage were very slow to develop symptoms compared to those of the other groups of plants. Mosaic first appeared 2 to 3 wk after inoculation. Eventually all plants in all groups produced the typical severe mosaic characteristic of this disease.

Seed transmission occurred in seed from plants inoculated at all three stages. Without exception, all seedlings showing mosaic symptoms germinated at least 1 wk later than normal seedlings and were very weak in appearance. The juice of all seedlings showing symptoms reacted positively with CMeV antiserum and negatively with CYMV, CSMV, and SBMV antiserum. None of the healthy-appearing test seedlings reacted positively with CMeV antiserum. The number of seed transmissions obtained from plants inoculated at three different stages was: youngest test plants, 1/79; older test plants, 1/93; and oldest test plants, 7/111. This striking difference in seed transmission among these three groups of test plants is highly significant within this test.

In tests to determine the location of virus in flower parts, CMeV was recovered by mechanical transmission from whole flowers, anthers and pistils, whole immature pods, and milk-stage seed, but not from the keel of systemically infected plants.

Geographic distribution.—Cowpea mottle virus was collected from 14 farms in southwestern Nigeria. It also was detected serologically from two of 14 cowpea plants showing mosaic symptoms on the University Experimental Farm at Nsukka. It was not detected serologically from any of 10 cowpea plants showing mosaic symptoms on the experimental farm at Samaru.

Separation from mixed infections.—Cowpea mottle virus often was found in mixed infections with CYMV in different locations throughout southwestern Nigeria. They were separated by inoculating young seedlings of *Chenopodium amaranticolor*. These developed chlorotic lesions on inoculated leaves and systemic necrotic lesions. Cowpea yellow mosaic virus was obtained by punching out individual necrotic lesions, grinding these in buffer, and then inoculating susceptible cowpea cultivars. Similarly, CMeV was isolated by grinding single chlorotic local lesions excised with a small punch from inoculated leaves of *C. amaranticolor* in buffer. The inoculum was rubbed on the primary leaves of test cowpea cultivars which developed typical CMeV symptoms 4-5 days after inoculation.

An alternate procedure for separating mixed infections of CYMV and CMeV was to react the juice of a plant showing mixed infection with two successive lots of antiserum against one of the viruses, centrifuge to remove the precipitate, and inoculate cowpea plants with serial dilutions of the juice containing the remaining virus. This procedure resulted in elimination of the virus whose antiserum was used to absorb it.

DISCUSSION

Robertson (18) first recorded and described CMeV. Based on symptomatology, host range, physical properties in crude sap, and serology, he concluded that it was different from other known viruses infecting cowpeas.

The virus was not serologically related to Trinidad cowpea mosaic virus (10), Nigerian cowpea yellow mosaic virus (7), or to an unnamed cowpea strain of southern bean mosaic virus (SBMV-CS). The cowpea isolate was serologically related to an isolate of the virus obtained from *Voandzeia subterranea*, although the two isolates differed slightly in host range and physical properties (18).

The virus which we call CMeV is a small spherical, single-component virus which sediments at 122 S, has about 20% RNA, and a protein subunit molecular weight of 44,500 ± 1,500. No other spherical plant virus cataloged in the *Descriptions of Plant Viruses* (14) has this combination of properties, and exhaustive tests did not indicate a serological relationship with any virus except the isolate from *V. subterranea* (19). Cowpea mottle virus is distinct from any virus whose physicochemical properties are known.

Its designation as CMeV, the same virus described by Robertson (18), is based on similar host range, geographic distribution, and the serological relationship to a *Voandzeia* isolate. Since Robertson recognized cowpea and *Voandzeia* isolates of CMeV, designation of these isolates as CMeV appears warranted, but it is not impossible that the host range of this virus and the original CMeV is merely coincidental.

The identification of CMeV without the use of serology is difficult. In sucrose density gradient centrifugation, it sediments as a fast-moving shoulder on the bottom component of cowpea mosaic virus and is therefore difficult to detect in mixed infections. It is not distinguishable by electron microscopy from other plant viruses of this size. Its general symptomatology in cowpeas overlaps the symptoms of other cowpea viruses, especially CYMV (1), CSMV (1), and cowpea chlorotic mottle virus (CCMV) (16). Multiple infections with CYMV and SBMV-CS (21) are common throughout its known geographic distribution.

Besides the characteristic brilliant chlorotic symptoms induced by CMeV in cowpeas and other susceptible legumes, reactions of certain hosts can be used to distinguish it. Whereas CMeV infected *Phaseolus lunatus*, *P. vulgaris*, *Cajanus cajan*, and *Canavalia ensiformis* systemically, these hosts were either symptomless or unsusceptible to infection by CCMV. Similarly, our isolates of CMeV did not systemically infect *Chenopodium amaranticolor* as did CYMV, a reaction which consistently aided the separation of the two viruses when found in mixed infections.

The known natural distribution of CMeV is limited to Nigeria. The virus was not found on experimental farms in El Salvador, Costa Rica, and Colombia during the summer of 1976 (Bozarth, *unpublished*). It is difficult to envision a virus as virulent to cowpeas and as easy to purify as CMeV occurring in the USA or Europe without having been previously described.

Cowpea mottle virus was previously reported to be seed borne in *V. subterranea* and its vector was unknown (18). Our investigations showed that CMeV is seed-borne in cowpeas and transmitted by *Ootheca mutabilis*.

The seed-borne nature of CMeV, its virulence to cowpeas, its transmissibility by beetles which also transmit other cowpea viruses, and the tendency to find the virus in multiple infections with CYMV illustrate the

complexity of the problem of controlling cowpea viruses in Nigeria. Cowpea mottle virus is potentially a serious threat to cowpeas grown throughout the world. Every effort should be made to confine it to its natural environment which at present appears to be Nigeria.

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