Southern Bean Mosaic Virus Isolated from Cowpea (*Vigna unguiculata*) in the Ivory Coast

L. GIVORD, Laboratoire de Virologie, Centre ORSTOM d'Adiopodoumé, B. P. V 51, Abidjan, Côte d'Ivoire

ABSTRACT

Givord, L. 1981. Southern bean mosaic virus isolated from cowpea (*Vigna unguiculata*) in the Ivory Coast. Plant Disease 65:755-756.

A disease characterized by vein clearing, vein banding, and distortion of leaves and growth reduction of cowpea plants was observed in the northern Ivory Coast. The host range of virus isolated from diseased plants was almost totally restricted to leguminous species and was seed-transmitted in cowpea. Electron microscopy of purified preparations showed isometric particles 30 nm in diameter. In cowpea mechanically inoculated with purified virus, symptoms were identical to those observed in the field. The virus was identified as southern bean mosaic virus by host range, physical properties, and serologic tests. No other viruses were associated with the disease. The virus reduced seed weight of cultivars California Blackeye and Edible Blackeye 11 and 59%, respectively. This is the first report of this disease in the Ivory Coast.

During a survey for viruses in the northern region of the Ivory Coast, tissue was collected from stunted cowpea plants, *Vigna unguiculata* L. (Walp.), showing vein banding and distortion of leaves. From this material, I isolated a virus with isometric particles that was readily transmissible by mechanical inoculation of sap and that induced in cowpea in the screenhouse the same symptoms as seen in the field. The virus appeared to be identical with the cowpea strain of southern bean mosaic virus (SBMV). The following study was done to confirm this identification.

MATERIALS AND METHODS

Virus propagation and properties. The virus was maintained by sap transmission in cowpea cultivars Early Ramshorn and Edible Blackeye in the screenhouse. The temperature ranged from 28 to 35 C during the day, and relative humidity was always 90–100%.

Crude extracts were obtained 18–25 days after inoculation by grinding infected leaves (1:1, w/v) with 0.01 M sodium phosphate buffer, pH 7.0. The thermal inactivation point, dilution end point, and longevity in vitro were determined according to the methods of Bos et al (2). Six Early Ramshorn (7) plants for each temperature, dilution, and day were used to determine if the sap was infectious.

Purification, centrifugation, and electron microscopy. The virus was purified from cowpea by Steere's method (14). The sedimentation coefficient (S) was evaluated by boundary sedimentation in a Beckman Spinco Model E analytical centrifuge equipped with Schlieren optics. S at each concentration was corrected to $S_{20,w}$ as described by Schachman (10). $S^{\circ}_{20,w}$ was determined by extrapolation to zero concentrations of the $S_{20,w}$ values measured at different concentrations. Leaf dip preparations and purified virus were examined in a Siemens 101 electron microscope at a nominal magnification of 12,000. Negative and positive staining with uranyl acetate were done.

Serology. Rabbits were injected weekly with 1 or 2 mg of virus in 0.5 ml of 0.01 M sodium phosphate buffer emulsified with 0.5 ml of Freund's incomplete adjuvant. Rabbits were bled at weekly intervals starting 1 wk after the second injection.

Double-diffusion tests were performed in plates with 3-mm diameter wells, 4 mm apart in 0.7% agar (Agar Noble, Difco) with 0.9% NaCl and 0.1% NaN_3. Relationships with other viruses were determined by the intragel cross-absorption test (15).

Transmission. Healthy aphids, *Aphis gossypii* Glover and *A. spiraecola* Patch, were starved for 2 hr and then allowed an acquisition feeding of 2 min or 2 days on infected cowpea plants and an inoculation access period of 48 hr on healthy cowpea plants. Flea beetles, *Podagrica decolorata* Duvivier, were given 24-hr acquisition and 48-hr inoculation periods (16).

Seeds from infected Black Syste cowpeas that were recently harvested or stored as long as 2 mo at 24 C were planted in a screenhouse.

Effect on yield. The number and size of pods and the number and weight of seed produced by 50 healthy plants and 50 plants infected with the Ivory Coast isolate were determined in the greenhouse for California Blackeye and Edible Blackeye cowpeas.

RESULTS

Symptoms. The description of symptoms is based on the criteria proposed by Bos (1). Except for Black Syste, every cowpea cultivar inoculated reacted with chlorotic local lesions. In Edible Blackeye cowpeas, the systemic symptoms were leaf chlorosis and vein clearing 7 days after inoculation; growth reduction, chlorosis, and dark green blotches on some leaves 13 days after inoculation; and vein banding, coarse mosaic, distortion, puckering, and blistering of leaves and stunting of plants 16 days after inoculation (Fig. 1). Symptoms on other cowpea cultivars were similar.

Host range. The virus was readily transmitted by mechanical inoculation with sap from cowpea leaves or pods. Cowpea cultivars Black Syste, California Blackeye, Edible Blackeye, and Early Ramshorn showed systemic symptoms. Virus was recovered from the following hosts, which reacted with systemic symptoms only: *Glycine max*, *Phaseolus atropurpureus*, and *Vigna sesquipedalis* (Leguminosae); and *Gomphrena globosa* (Amaranthaceae). Other hosts including *Phaseolus vulgaris* 'nain mangetout Contender,' *Pisum sativum* 'Wando,' and *Voandzeia subterranea* (Leguminosae) were symptomless.

Virus was not recovered in back inoculation tests to cowpea from *Arachis hypogaea*, *Cajanus cajan*, *Dolichos lablab* 'd'Egypte' and 'Soudan Pourpre,' *Phaseolus vulgaris* 'Tendercrop,' 'nain fin de Bagnoles,' 'nain Triomphe de Farcy,' and 'nain mangetout Top Crop,' *Pisum sativum* 'Douce Provence,' *Psophocarpus tetragonolobus*, *Vicia faba* 'd'Aguadulce' (Leguminosae); *Tetragonia expansa* (Aizoaceae); *Vinca rosea* (Apocynaceae); *Brassica pekinensis* 'PeTsai' (Cruciferae); *Citrullus vulgaris*, *Cucumis melo* 'Cantaloup charentais,' *C. sativus* 'Burpee Poinsett' and 'Straight Eight,' *Cucurbita pepo* 'Kürhii Diamant' (Cucurbitaceae); *Zea mays* (Gramineae); *Hibiscus esculentus* (Malvaceae); *Passiflora edulis* (Passifloraceae); *Capsicum annuum* 'Carré doux d'Amérique,' *Datura stramonium*, *Nicotiana benthamiana*, *N. glutinosa*, *N. tabacum* 'Samsun' and 'White Burley,' *N. Xedwardsonii* Christie & D. W. Hall, *Physalis angulata* (Solanaceae).

Virus properties. In crude extract, the

Present address of author: Laboratoire de Virologie, Institut de Biologie Moléculaire et Cellulaire du C.N.R.S., 15, rue Descartes, 67084 - Strasbourg Cedex - France.

Accepted for publication 19 May 1981.

0191-2917/81/09075502/$03.00/0

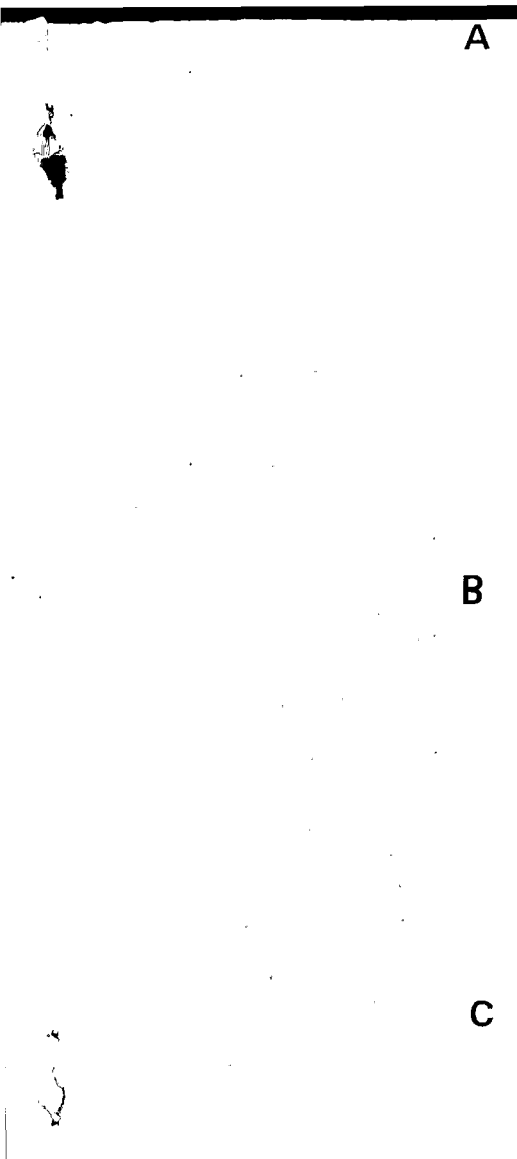

Fig. 1. Symptoms on cowpea infected with an Ivory Coast isolate of southern bean mosaic virus: **(A)** Chlorotic local lesions on primary leaf. **(B)** Line pattern mosaic. **(C)** Distortion of trifoliolate leaf.

virus had a dilution end point between 10^{-7} and 10^{-8}, a thermal inactivation point above 95 C, and a longevity in vitro greater than 31 days at 24–26 C. Crude extract frozen and thawed 43 times remained infective. Purified virus resuspended in 0.01 M sodium phosphate buffer, pH 7.0, was highly infectious and produced characteristic symptoms on inoculated cowpeas. Virus particles were degraded when 0.01 M ethylenediaminetetraacetic acid was used in resuspending the pellets (6). The ultraviolet absorption spectrum of the virus had a maximum at 260 nm and a minimum at 242 nm; the 260:280 absorption ratio was 1.62 and the E_{max}/E_{min} ratio was 1.37.

Electron microscopy of leaf-dip and purified preparations showed particles with a 30-nm diameter.

Analytical ultracentrifugation revealed a single component with an extrapolated sedimentation coefficient of $S^{\circ}_{20,w} = 110$ S.

Serologic relationships. In double-immunodiffusion tests, antisera to the Ivory Coast isolate reacted strongly with the homologous antigen up to a dilution of 1/2,048 or 1/4,096. Homologous and heterologous reactions of the Ivory Coast isolate with the cowpea strain of SBMV (5,12) (type strain) and SBMV from Ghana (8) revealed no differences among the three strains. Intragel cross-absorption tests confirmed these results.

No relationship was found between the Ivory Coast isolate and probable and possible members of the SBMV group (9) of turnip rosette, cocksfoot mottle, rice yellow mottle, and sowbane mosaic viruses, using antiserum to each virus and the Ivory Coast isolate as antigen. Similarly, no relationship was apparent using antisera to the Ivory Coast isolate and any heterologous antigen except sowbane mosaic virus.

Transmission. All attempts to transmit the Ivory Coast isolate with aphids or flea beetles were unsuccessful.

In the two tests involving 100–150 seedlings, the Ivory Coast isolate was transmitted through seed at rates of 15 and 44%. Identity of the virus in a random selection of infected seedlings was confirmed by serology. No virus was detected in control lots grown at the same time and under the same conditions.

Effect on yield. In two tests, numbers of seeds and pods were reduced an average of 55 and 61%, respectively, in Edible Blackeye plants infected with the Ivory Coast isolate, compared with virus-free plants. Average reductions in seed weights were 11 and 59% for infected plants of California Blackeye and Edible Blackeye, respectively.

DISCUSSION

SBMV was first described in 1943 by Zaumeyer and Harter (18) in the U.S.A. Later, a severe bean mosaic strain (17) and a cowpea strain (12) that were serologically distinct were described (5). Cowpea strains of SBMV were also reported from Ghana (8) and Nigeria (13). An isolate has been found in bean in France (3).

In vitro properties of SBMV have some diagnostic value (4) and, when considered with data on symptoms, host range, seed transmission, physicochemical properties, and serologic relationships, indicate that the Ivory Coast virus from cowpea is an isolate of SBMV (11).

Unlike other isolates of SBMV, the Ivory Coast isolate infected a non-Leguminous host, *G. globosa*. Unlike the cowpea strain of SBMV (12), it infected bean, which is also a symptomless host of the Ghana strain (8). The Ivory Coast isolate and the Ghana strain have very similar host ranges, and it would be interesting to know if the Ghana strain infects *G. globosa*. The preliminary serologic tests using antisera to the Ivory Coast isolate showed no differences among the three cowpea strains.

ACKNOWLEDGMENTS

I wish to acknowledge the help of A. Nicolaieff with the electron microscopy and G. de Marcillac with analytical ultracentrifugation. I thank L. Bos, P. Catherall, R. I. Hamilton, C. I. Kado, N. Larroque, D. Peters, and R. Richins for gifts of antigen and antisera and M. Hollings for performing the serologic tests with turnip rosette virus. I thank A. C. Durham for the cowpea strain of SBMV provided to him by R. Hull from an original isolate provided by R. J. Shepherd.

LITERATURE CITED

1. Bos, L. 1964. Symptoms of Virus Diseases in Plants. Centre for Agricultural Publications and Documentation, Wageningen, the Netherlands.
2. Bos, L., Hagedorn, D. J., and Quantz, L. 1960. Suggested procedures for international identification of Legume viruses. Tijdschr. Plantenzieckten 66:328-343.
3. Ferault, A. C., Spire, D., Bannerot, H., Bertrandy, J., and LeTan, T. 1969. Identification dans la région parisienne d'une marbrure du Haricot comparable au Southern bean mosaic virus (Zaumeyer et Harter). Ann. Phytopathol. 1(4):619-629.
4. Francki, R. I. B. 1980. Limited value of the thermal inactivation point, longevity in vitro and dilution end point as criteria for the characterization, identification and classification of plant viruses. Intervirology 13:91-98.
5. Grogan, R. G., and Kimble, K. A. 1964. The relationship of severe bean mosaic virus from Mexico to southern bean mosaic virus and its related strain in cowpea. Phytopathology 54:75-78.
6. Hsu, C. H., Sehgal, O. P., and Pickett, E. E. 1976. Stabilizing effect of divalent metal ions on virions of southern bean mosaic virus. Virology 69:587-595.
7. Kuhn, C. W., and Brantley, B. B. 1963. Cowpea resistance to the cowpea strain of southern bean mosaic virus. Plant Dis. Rep. 47(12):1094-1096.
8. Lamptey, P. N. L., and Hamilton, P. I. 1974. A new cowpea strain of southern bean mosaic virus from Ghana. Phytopathology 64:1100-1104.
9. Matthews, R. E. F. 1979. Third report of the international committee on taxonomy of viruses. Classification and nomenclature of viruses. Intervirology 12:130-296.
10. Schachman, H. K. 1959. Sedimentation velocity. Chapter 4 in: Ultracentrifugation in Biochemistry. Academic Press, New York. 272 pp.
11. Shepherd, R. J. 1971. Southern bean mosaic virus. No. 57. Descriptions of plant viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., London.
12. Shepherd, R. J., and Fulton, R. W. 1962. Identity of a seed-borne virus of cowpea. Phytopathology 52:489-493.
13. Shoyinka, S. A., and Okusanya, B. A. O. 1975. Field occurrence and identification of southern bean mosaic virus (cowpea strain) in Nigeria. (Abstr.) Niger. J. Plant Prot. Occas. Publ. 1:27.
14. Steere, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.
15. van Regenmortel, M. H. V. 1966. Plant virus serology. Adv. Virus Res. 12:207-271.
16. Walters, H. J. 1964. Transmission of southern bean mosaic virus by the bean leaf beetle. Plant Dis. Rep. 48:935.
17. Yerkes, W. D., and Patino, G. 1960. The severe bean mosaic virus, a new bean virus from Mexico. Phytopathology 50:334-338.
18. Zaumeyer, W. J., and Harter, L. L. 1943. Two new virus diseases of beans. J. Agric. Res. 67:305-328.

A
Fig 1 A. PD 1564 GIVORD
80% 150 line screen
TE INC. 522-4381

C
Fig 1 C PD 1564 GIVORD
75% 150 line screen
TE INC. 522-4381

B