Characterization of cowpea mottle virus on cowpea (*Vigna unguiculata*) in the Ivory Coast and the identification of a new vector

J.-C. Thouvenel, E. Tia and L.D.C. Fishpool

Laboratoire de Phytovirologie d'Adiopodoumé, ORSTOM, B.P. V51, Abidjan, Ivory Coast, West Africa

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In Ivory Coast, a mechanically transmissible virus, with isometric particles 30 nm in diameter, was isolated from cowpea (*Vigna unguiculata*) that showed stunt and severe mosaic. This Paper describes the characterization of the virus as an isolate of cowpea mottle virus, previously known only from Nigeria, and reports a new chrysomelid beetle vector, *Monolepta tenuicornis* Jacoby.

Keywords: Cowpea mottle virus; Vector; Chrysomelidae; *Monolepta tenuicornis*

Cowpea (*Vigna unguiculata* (L.) Walp.) is a widely distributed grain legume in West Africa, particularly common in the sahelian zone. As a result of demographic shifts provoked by severe droughts to the north, this crop is now widely grown in the forest area in southern Ivory Coast. Only a few diseased plants were observed in 1982. By 1986, however, cowpea plants with conspicuous symptoms of bright mosaic, vein-banding, distortion of leaves and often stunting of the whole plant were widely found during the rainy season around Abidjan. The virus is easily mechanically transmissible, and it has been identified as an isolate of cowpea mottle virus (CMeV) (Thouvenel, 1988), previously described by Shoyinka *et al.* (1978) in Nigeria and, until now, only known from that country, some 3000 km to the east of Ivory Coast. The following is a report of our observations on this disease.

Materials and methods

Cowpea leaves gathered from a single plant with severe symptoms in the field were used as the original inoculum. The virus was maintained in *V. unguiculata* and easily transmitted by mechanical inoculation after grinding in 0.1 M phosphate buffer, pH 7.1, containing 0.02 M cysteine hydrochloride. Test plants were grown in insect-proof glasshouses under local climatic conditions (mean temperature 28°C, average relative humidity 90%). Inoculated plants were checked for virus infection by back-inoculation to *V. unguiculata* or by serology.

Virus transmission tests were undertaken, using potential insect vector species collected from the cowpea crop. The species concerned are listed in the Results. They were tested, species by species, by placing them on healthy cowpea seedlings for 24 h in an insect-proof glasshouse. The insect-inoculated seedlings were maintained in a glasshouse and observed for symptom development over a three-week period. After the tests, insects were prepared, mounted and sent to the Museum National d'Histoire Naturelle, Paris, for identification. In a second experiment, the insects were allowed to feed for 24 h on diseased cowpea, and subsequently transferred sequentially to a series of healthy seedlings for access periods of 24 h each.

Thermal inactivation point, dilution end point, and ageing *in vitro* were determined according to the methods of Bos *et al.* (1960) using crude sap of leaves from diseased *V. unguiculata* and seedlings of *V. unguiculata* as test plants to be inoculated.

To purify the virus, leaves of infected *V. unguiculata* were ground in 0.2 M sodium borate, pH 8, containing 1% mercaptoethanol and 1% polyvinylpyrrolidone (MW 30 000) (1 g 3 ml^{-1}); chloroform was added during the grinding (1/2 vol).

The mixture was centrifuged for 15 min at 15 000*g* and the supernatant was treated with 6% PEG (MW 6000). The precipitate was pelleted by 15 min of centrifugation at 15 000*g*. Pellets were re-suspended in 0.05 M borate buffer, pH 8, containing 1.0% mercaptoethanol. After removal of the insoluble material by centrifugation for 10 min at 10 000*g*, the virus was sedimented through a 2-cm deep layer of 20% sucrose (95 000*g* for 150 min). The pellets were re-suspended in the same borate buffer without mercaptoethanol and further purified by sucrose density-gradient centrifugation (10–40% in 0.1 M borate buffer, pH 8).

An opalescent virus band was collected, diluted with water, and centrifuged 150 min at 78 000*g* before being re-suspended in borate buffer, pH 8. The ultraviolet absorption spectrum of the virus preparation was determined with a Beckman 5230 spectrophotometer.

The molecular weight of viral protein was determined by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulphate. An electrophoresis calibration kit (Pharmacia, Uppsala, Sweden) was used as reference (phosphorylase b, MW 94 000; bovine serum albumin, MW 67 000; ovalbumin, MW 43 000; carbonic anhydrase, MW 30 000; soybean trypsin inhibitor, MW 20 100; and δ lactalbumin, MW 14 400).

For electron microscopy, virus preparations were stained with 2% aqueous uranyl acetate and

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observed using a Siemens Elmiskop 102 at the Groupe d'Etude et de Recherches en Microscopie Electronique (GERME) at Adiopodoumé in Ivory Coast.

An antiserum was prepared in rabbits by two intramuscular injections of about 3 mg of purified virus. Immunodiffusion in 1% agarose was used for serological tests, using purified virus preparations or crude extracts of leaves.

Results

Symptomatology and host range

The primary leaves of cowpea developed diffuse chlorotic lesions 3–5 days after inoculation, often followed by veinal necrosis and detachment of inoculated leaves. Systemic symptoms which appeared 7–9 days after inoculation on young leaves included chlorosis, veinal mottle, yellow mosaic, and sometimes distortion. The entire plant was stunted.

The virus was easily transmitted by mechanical inoculation. The following plants developed systemic symptoms, from which the virus could be recovered by back-inoculation to cowpea: *Cajanus cajan* Millsp., *Canavalia ensiformis* DC., *Desmodium lasiocarpum* D.C., *Dolichos lab-lab* L., *Glycine max* Merr., *Phaseolus mungo* L., *Phaseolus vulgaris* L. and *Vigna sesquipedalis* Fruwirth. Diffuse chlorotic lesions developed on inoculated leaves of *Chenopodium amaranticolor* Coste et Reyn. and *C. quinoa* Willd.

Properties in crude sap

In crude cowpea sap, diluted in water, CMeV was infective at a dilution of 10^{-5} but not at 10^{-6}; thermal-inactivation point was between 65° and 70°C. The infectivity of *V. unguiculata* sap was lost after 5 days at about 27°C, but remained infectious after 1 month at 4°C. Purified virus was still infectious after 3 months at 4°C.

Purification and physical properties

The purified virus was highly infectious and produced typical symptoms on CMeV hosts. The ultraviolet absorption spectrum of the purified virus had a maximum at 260 nm and a minimum at 242 nm. The A_{260}/A_{280} ratio was 1.65 and the A_{260}/A_{242} ratio was 1.25 (without correction for light scattering). Based on the extinction coefficient $\epsilon_{260}^{0.1\%} = 5.0$ (Bozarth and Shoyinka, 1979), the average yield of purified virus was 300 mg kg^{-1} leaves.

Electron micrographs of purified virus preparations showed isometric particles with a diameter of 30 nm.

Molecular weight of protein subunit

The molecular weight of protein subunits was found to be slightly greater than that of ovalbumin, 45 000 ± 500 Da.

Serology

In agarose gel double immuno-diffusion tests, homologous antiserum reacted with purified CMeV (Ivory Coast isolate) when diluted to 1/1024.

In double immuno-diffusion tests, purified virus reacted strongly with antisera prepared against cowpea mottle virus-Nigeria (two batches, gifts from Dr Ladipo and from Dr Shoyinka).

Purified CMeV failed to react in agarose gel double immuno-diffusion tests with antisera prepared against the following viruses (donors in parentheses): alfalfa mosaic (Musil), broad bean true mosaic (Cockbain), broad bean wilt (Smith), cocksfoot mild mosaic (Paul), cowpea chlorotic mottle (Bancroft), cowpea mosaic (Van Kammen), cowpea yellow mosaic (Ladipo), cucumber mosaic, desmodium yellow mottle (Scott), hibiscus chlorotic ringspot (Fauquet), molinia streak (Paul), panicum mosaic (Toler), pea enation (Musil), peanut stunt (Mink), rice yellow mottle virus, scrophularia mottle (Koenig), southern bean mosaic-Ivory Coast strain, sowbane mosaic (Kado), tephrosia latent (Bock) and voandzeia necrotic mosaic (Fauquet).

Transmission

Amongst the insects collected from the cowpea crops were two species of beetle, present in large numbers. These were subsequently identified as *Monolepta tenuicornis* Jacoby and *Medythia quaterna* Fairmaire (Coleoptera; Chrysomelidae). Both of these proved capable of transmitting the virus to cowpea: 11 positive results from 247 attempts using *M. tenuicornis,* six out of 74 for *M. quaterna.*

Artificial transmission tests, with acquisition and inoculation times both of 24 h, gave 61/120 and 34/47 positive results, respectively, for these two species. Retention time of the virus was found to be seven days for *M. tenuicornis* and six days for *M. quaterna.*

All attempts to transmit the Ivory-Coast isolate of CMeV from *Vigna* sp. to *Vigna* sp. using other insect species collected from cowpea (listed below) were unsuccessful, irrespective of acquisition times and inoculation periods.

The species tested included *Asbecesta cyanipennis* Harold, *Exosoma dalmani* Jacoby, *Lema* sp. and *Smaragdina* sp. (Coleoptera; Chrysomelidae), *Apalachrus azureus* Er (Coleoptera; Malacodermoidea), *Dryadocoris* sp. (Heteroptera; Pentatomidae), *Dysdercus* sp. (Heteroptera; Pyrrhocoridae), *Myla* sp. (Heteroptera; Coreidae) and *Zonocerus variegatus* Linnaeus (Orthoptera; Pyrgomorphidae).

The virus was found not to be seed-borne in 100 seeds harvested from naturally infected cowpea.

Effect on yield

On the basis of visual symptoms only, within the experimental plot of 50 plants, the yield of the naturally infected cowpea plants was reduced by about 65% compared with that of apparently healthy plants.

Discussion

Data on host range, virus properties and serological relationships of the virus isolated from cowpea in the Ivory Coast are similar to those previously reported for the Nigerian isolate of cowpea mottle virus (CMeV) (Shoyinka *et al.*, 1978).

This indicates strong similarities between the Nigerian and Ivory Coast isolates, and it is surprising

that CMeV has not been reported in neighbouring countries, for example Burkina Faso and Ghana, where cowpea is widely cultivated. Although the seed-transmission test proved negative, this result is perhaps not surprising in view of the observations of Allen *et al.* (1982) who found a very low level of seed-borne infection (0.4%). Thus, it is possible that this apparent spread of CMeV from Nigeria to Ivory Coast could have resulted from seed-transmission (Allen *et al., ibid.*), followed by dissemination of the virus to wild plants in areas surrounding cultivation sites to constitute a permanent virus reservoir from which cowpea crops could be re-infected during the rainy season. This, however, requires the presence of an effective vector. The two main vectors reported in Nigeria are the chrysomelids *Ootheca mutabilis* Sahlb. and *Medythia quaterna* (Allen *et al.*, 1981). In our trials in southern Ivory Coast we have found *M. quaterna*, but not *O. mutabilis*. We did find however that another chrysomelid, *Monolepta tenuicornis*, was capable of transmitting the disease. This species was not recorded by Allen *et al.* (1981); and indeed, to our knowledge, this is the first record of this species as a virus vector. Of the three species, *O. mutabilis* at least is definitely known to occur in Ghana (Marfo, 1985), so the disease may yet be found there.

Widely distributed in the Ivory Coast during the rainy season (May–October), the virus is difficult to find in the dry season (December–March). This observation agrees with those of Ochieng (1978) on the biology of *O. mutabilis* in Nigeria where a similar climatic regime occurs. He found that there are two distinct generations, with peaks in number of adults from April to June and again from August to October. It is possible that the other two vector species have similar life cycles.

Other viruses have been found on cowpea in Ivory Coast in previous surveys: southern bean mosaic (Givord, 1981) and cucumber mosaic and cowpea mild mottle viruses (Fauquet and Thouvenel, 1987), but this is the first time a disease of economic importance has been reported. As a result of natural infection, CMeV appears now to have become a significant disease, as judged by its effects on yield; control will probably be best achieved by the selection and development of resistant cultivars, as recognized by Allen *et al.* (1982).

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