Rothamsted Repository Download

A - Papers appearing in refereed journals

Brunt, A. A., Kenten, R. H., Gibbs, A. J. and Nixon, H. L. 1965. Further studies on cocoa yellow mosaic virus. *Journal of General Microbiology.* 38, pp. 81-90.

The publisher's version can be accessed at:

- https://dx.doi.org/10.1099/00221287-38-1-81

The output can be accessed at: https://repository.rothamsted.ac.uk/item/95z0z/further-studies-on-cocoa-yellow-mosaic-virus.

Further Studies on Cocoa Yellow Mosaic Virus

By A. A. BRUNT* and R. H. KENTEN

West African Cocoa Research Institute, Tafo, Ghana†

and A. J. GIBBS and H. L. NIXON

Rothamsted Experimental Station, Harpenden, Hertfordshire

SUMMARY

Cocoa yellow mosaic virus from Sierra Leone is readily transmitted by sap to many woody and herbaceous dicotyledonous plants. It is serologically related to wild cucumber mosaic virus and to turnip yellow mosaic virus, and has similar chemical and physical properties. It is stable when frozen or lyophilized, inactivated when kept for 10 min. at 65°, and is precipitated without loss of infectivity in a half-saturated ammonium sulphate solution. The particles appear to be 25 mμ across when mounted in neutral phosphotungstate, and 29 mμ when shadowed. All preparations contained infective nucleoprotein particles which sedimented at 108 S, and non-infective protein shells which sedimented at 49 S.

INTRODUCTION

In 1958 cocoa plants with unusual symptoms were found in Sierra Leone, from which Blencowe, Brunt, Kenten & Lovi (1963) obtained a virus apparently different from any virus previously recognized in cocoa in West Africa. In the present paper we report the results of further experiments with this virus, which we propose shall be called cocoa yellow mosaic virus (CYMV).

METHODS

A stock culture of CYMV from Giehun, Sierra Leone, was kept in graft-infected cocoa seedlings (*Theobroma cacao* L.) and in *Chenopodium amaranticolor* Coste & Reyn and *C. quinoa* Willd. seedlings infected originally by inoculation with cocoa leaf extracts.

The infectivity of different virus suspensions was compared by counting either the numbers of lesions that developed on the inoculated leaves of *Chenopodium amaranticolor* or the proportion of inoculated cocoa beans which later produced seedlings showing symptoms (after about 80 days). Cocoa beans were used in preference to cocoa seedlings, not only for convenience, but also because they showed symptoms sooner, usually on the first leaves produced. In the first experiments one cotyledon was removed from each bean before it was inoculated, but later whole beans were used because, as with cocoa swollen shoot virus (Brunt, Kenten & Nixon, 1964), they were more susceptible: in nine tests a total of 149

---

* Present address: Glasshouse Crops Research Institute, Littlehampton, Sussex, England.
† Since renamed: Cocoa Research Station, Ghana Academy of Sciences, Tafo, Ghana.

out of 197 (75 %) inoculated whole beans, but only 76 out of 192 (40 %) inoculated half beans, were infected. Cocoa beans and plants were inoculated by using a small hog's bristle brush (Reeve's no. 3) that had been dipped in inoculum; *C. amaranticolor* and other plants were inoculated by finger. An abrasive was usually added to the inocula: Celite 545 (Johns Manville Ltd.) for cocoa beans and hard-leaved plants and Hyflo Super Cell (Johns Manville Ltd.) or 600-mesh carborundum for other plants.

Some purified preparations of the virus were made from infected cocoa leaves. The leaves were mechanically macerated in twenty times their weight of water or a neutral buffer containing either 0·05 M-Na_2HPO_4+0·005 M-diethydithiocarbamate (DIECA) or 0·05 M-Na_2HPO_4+0·01 M-thioglycollic acid+0·005 M-DIECA. The slurry was then filtered through muslin, and the resulting extract clarified by centrifugation for 20 min. at 8000 *g*. The virus was then either sedimented from the clarified extract by centrifugation at 75,000 *g* for 2 hr or precipitated by half-saturating the extract with ammonium sulphate and centrifuging at 8000 *g* for 20 min. The pellet was then resuspended in $\frac{1}{50}$th to $\frac{1}{200}$th of the original volume of water, or neutral 0·03 M-phosphate, or in normal saline when used to inject rabbits. Other preparations of the virus were made from leaves of infected *Chenopodium amaranticolor*. Infected leaves were macerated mechanically in at least five times their weight of a solution containing 0·05 M-ascorbic acid+0·1 M-disodium hydrogen phosphate (pH 7·0). Chloroform (a quarter of the volume of buffer) was then added, and, after the mixture had been macerated further to form an emulsion, it was centrifuged at 8000 *g* for 10 min. and the aqueous phase removed. The virus was then purified and concentrated from the aqueous extract by two or more cycles of differential centrifugation, with 0·03 M-phosphate (pH 7·5) as the suspending fluid. Finally the preparations were dialysed against 0·03 M-phosphate to remove traces of ascorbic acid. The other viruses used in the serological tests were purified in the same way.

Preparations were fractionated by centrifuging them in sucrose density gradients, and were examined in a Siemens Elmiskop I electron microscope by the methods described by Nixon & Harrison (1959).

Antisera were prepared by injecting rabbits intravenously with partially purified virus preparations obtained from artificially infected cocoa seedlings by ultra-centrifugation. The virus was injected into the rabbits either intravenously or intramuscularly, after being emulsified in an equal volume of Freund's complete adjuvant. The rabbits were bled about 10 days after the last injections. One rabbit produced a serum with a titre of 1/512 after three intravenous injections at 3-day intervals, whereas another gave a titre of 1/1280 after one intravenous injection followed by two intramuscular injections at fortnightly intervals, with a final intravenous injection 1 week later. Serological tests were done by the tube precipitation method (Bawden, 1958).

RESULTS

Host-range and symptoms

The two plant species we found to be most useful for work with cocoa yellow mosaic virus (CYMV) were cocoa and *Chenopodium amaranticolor*. Blencowe *et al.* (1963) described the mosaic symptoms shown by infected cocoa (Pl. 1, fig. 2).

C. amaranticolor was readily infected by inoculation with water extracts of infected cocoa or *C. amaranticolor*; numerous necrotic lesions developed on inoculated leaves 1–2 weeks after inoculation, and tip leaves produced later showed chlorotic and necrotic flecks and many were distorted (Pl. 1, fig. 3).

The susceptibility of other plant species to CYMV was tested. Most species were

Table 1. *Hosts of cocoa yellow mosaic virus*

(*a*) Species susceptible to CYMV (*those infected systemically)

Apocynaceae	*Vinca rosea* L.*
Begoniaceae	*Begonia*
Bombacaceae	*Adansonia digitata* L.*, *Ceiba pentandra* (L.) Gaertn.*, *Pachira oleagina* Decne.
Chenopodiaceae	*Beta vulgaris* L. (sugar beet var. Klein E.)†, *Chenopodium amaranticolor* Coste & Reyn.*†, *C. quinoa* Willd.‡
Cucurbitaceae	*Cucurbita ficifolia* Bouche, *C. melo-pepo* L. (Delicious Golden), *C. pepo* L. (Orange Gourd), *Cucumis melo* L. (Cantaloup Charantais), *C. sativus* L. (Lockies Perfection, Tender and True, Gherkin), *Luffa cylindrica* Roem., *Momordica charantia* L.
Papillionaceae	*Voandezia subterranea*, *Vigna sinensis* L.†
Solanaceae	*Nicotiana tabacum* L. (White Burley), *N. clevelandii* Gray†, *Nicandra physaloides* L.*
Sterculiaceae	*Abroma augusta* L.*, *Cola lateritia* K. Schum. var. *maclaudi* (A. Chev.) Brenan & Keay*, *Leptonychia pubescens* Keay, *Sterculia foetida*, *Theobroma cacao* L.*, *T. bicolor* Humb. & Bonpl.*, *T. grandiflora* K. Schum.*, *T. speciosa* Spreng*

(*b*) Species inoculated with CYMV, but which showed no symptoms, and from which CYMV was not recovered

Amaranthaceae	*Gomphrena globosa* L.†
Bombacaceae	*Bombax brevicuspe* Sprague
Caesalpiniaceae	*Caesalpinia pulcherrima* Sw.
Compositae	*Calendula officinalis* L., *Lactuca taraxifolia*, *Zinnia elegans* Jacq.
Cucurbitaceae	*Momordica foetida*
Cruciferae	*Brassica chinensis* L.‡, *Raphanus sativus* L.
Euphorbiaceae	*Ricinodendron heudelotii* (Baille) Pierre ex Pax.
Gramineae	*Avena sativa* L. (var. Blenda)†, *Dactylis glomerata* L.†, *Hordeum vulgare* L. (var. Proctor)†, *Lolium perenne* L. (var. S22)†, *Zea mays* L.
Musaceae	*Musa balbisiana* L.†
Nyctaginaceae	*Boerhavia diffusa*
Papillionaceae	*Arachis hypogea* L., *Phaseolus lunatus* L., *P. vulgaris* L. (Canadian Wonder Improved, Kentucky Wonder, Masterpiece, Prince‡), *Pisum sativum* L. (Laxton's Wonder Superb), *Soja max*†, *Trifolium incarnatum* L.†
Polemoniaceae	*Phlox drummondii* Hook
Portulaccaceae	*Montia perfoliata* (Willd.) Howell.†
Solanaceae	*Capsicum frutescens* L. (Tabasco), *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicotiana glutinosa* L., *Petunia hybrida* Villm.‡, *Physalis floridana* L.†, *P. peruvia* L., *P. pruinosa* L., *Solanum melongea* L., *S. verbascifolium*
Sterculiaceae	*Cola gigantea* A. Chev., *Guazama ulmifolia* Lam., *Sterculia tragacantha* Lindl.
Tiliaceae	*Corchorus aestuans* L.
Umbelliferae	*Daucus carota* L.†

All plants inoculated with CYMV from, and back tested to *Theobroma cacao*; except those marked †, which were inoculated with partially purified CYMV and back tested to *Chenopodium amaranticolor*, and those marked ‡, which were tested in both ways.

inoculated with infective cocoa leaf extracts but some were inoculated with pre-
parations of the virus purified from *Chenopodium amaranticolor*. All were tested
for infection after about 1 month by inoculating sap from the leaves which had been
inoculated and from the uninoculated tip leaves to either cocoa beans or *C. amar-
anticolor*. Table 1 shows that CYMV has many hosts, which include woody and
herbaceous species of different dicotyledonous families. The failure to infect some
species may have been caused by inhibitors of infection in the cocoa leaf extract
or preparations from *C. amaranticolor*; however, some species known to be influenced
by such inhibitors, including *Cucumis* and *Nicotiana* spp., were readily infected in
these tests.

Properties of the virus

In these tests water extracts of the first flush leaves of cocoa seedlings, infected
as beans, were used. Since cocoa leaf extracts are very mucilaginous, they were
diluted to contain the equivalent of 1 g. leaf in 20 ml. distilled water. Such diluted
extracts were at about pH 6·3. Extracts were made only from leaves showing
clear symptoms, for these were more infective than extracts of symptomless leaves
or other parts of infected plants. Such extracts sometimes caused infection when
diluted to 10^{-4} with water and inoculated with Celite (Table 2) which increased the
infection end-point a thousand-fold.

Table 2. *Thermal inactivation of cocoa yellow mosaic virus*

Dilution of extract	Cocoa leaf extracts heated for 10 min. at					
	65°	60°	55°	50°	45°	Unheated
	% infected beans (about 25 beans per treatment)					
1/1	0*	5	25	80	67	74
1/10	—	0	3	41	63	87
1/100	—	0	0	3	23	30
1/1000	—	0	0	0	0	7

* 1 g. leaf extracted in 20 ml. distilled water.

Stability at different temperatures. When 2 ml. samples of extracts were heated to
different temperatures, some infectivity remained after 10 min. at 60°, but not at
65° (Table 2). At 25–30° the extracts were infective after 16 days, but not after
32 days; at 0–4° they were still infective after 76 days; other extracts containing
0·2 M-phosphate buffer (pH 8·0) were highly infective after 100 days at 0–4°.
Infected cocoa leaves and water extracts of the leaves were frozen and thawed with
no apparent effect on the infectivity of the virus they contained. Infectivity was
also retained in cocoa leaves dried and stored over anhydrous alumina at 25°, and
in lyophilized cocoa leaf extracts or *Chenopodium amaranticolor* sap.

Precipitation of the virus. CYMV precipitated from water extracts when these
were half saturated with ammonium sulphate. Precipitates were suspended in
distilled water, dialysed for 24 hr against 0·003 M-phosphate (pH 8·0), and centri-
fuged briefly at 8000 *g* to sediment insoluble material. The resulting suspensions
were almost colourless, slightly turbid, and contained most of the infectivity of the
original extract. By contrast, when extracts of infected cocoa leaves were mixed
with different amounts of ethanol or acetone in the cold to give concentrations of

30–75 % and 25–60 % (v/v), respectively, the sparse precipitates which formed were not infective when resuspended in water.

The virus was not precipitated when extracts of infected cocoa leaves were adjusted to different pH values between pH 3 and pH 6, though infectivity was apparently destroyed when the extract was acidified to pH 3·0.

Partially purified preparations

Partially purified preparations made by differential centrifugation were slightly turbid and usually colourless or slightly yellow. The virus crystallized when ammonium sulphate was added slowly to these preparations as described by Markham & Smith (1949) for crystallizing turnip yellow mosaic virus (TYMV); these crystals were similar to but smaller than those of TYMV. They were kept in the ammonium sulphate solution for 12 months at 4°, without increasing in size; they dissolved when the ammonium sulphate was removed by dialysis.

Electron microscopy. Extracts of infected cocoa or *Chenopodium amaranticolor* leaves contained many approximately spherical particles, not found in comparable preparations from uninfected plants. The particles appeared to be 25·2 mμ across when negatively stained (Pl. 1, fig. 5) and 29·0 mμ when shadowcast after fixation with 2 % (v/v) formalin for 1 hr. The first figure is presumably an underestimate because stain penetrates into the particles, and the second an overestimate because the particles flatten on drying. The apparent size of negatively stained CYMV particles is almost the same as that recorded by Nixon & Gibbs (1960) for similarly treated turnip yellow mosaic virus particles, but fixed and shadowed CYMV particles appear to be slightly larger than untreated shadowed TYMV at 26 mμ (Cosentino, Paigen & Steere, 1956). CYMV particles shadowcast without fixation with formalin always appear very flattened, unlike TYMV particles, which retain their shape well. Some of the particles in negatively stained preparations of CYMV seemed empty because they were penetrated by the phosphotungstate and were visible only as outlines (Pl. 1, fig. 4). Others were partly penetrated by the stain and were an intermediate grey tone on the plate (Pl. 1, fig. 5, near the upper edge).

In the best electron micrographs, particles were indistinguishable from those of turnip yellow mosaic virus (Huxley & Zubay, 1960; Nixon & Gibbs, 1960), and showed the same arrangement of morphological subunits.

Centrifugation. Purified CYMV preparations, suspended in neutral 0·1 M-KCl, were examined in a Spinco Model E analytical centrifuge. All showed two boundaries, with sedimentation coefficients of 49S and 108S (corrected to infinite dilution in water at 20°), which we have called the top component and whole virus, respectively (Markham & Smith, 1949). The top component absorbed much less ultraviolet (u.v.) radiation than the whole virus, and during preparation by differential centrifugation some of it was inevitably lost. When the virus in extracts from infected *Chenopodium amaranticolor* leaves was concentrated and purified by precipitation with ammonium sulphate and examined in the analytical centrifuge the area of the schlieren peak for the top component was about two-thirds of that from whole virus.

The two components were separated by centrifugation in sucrose density gradients. Electron microscopy showed that the whole virus fraction contained mostly intact virus particles, unpenetrated by phosphotungstate. It had the u.v.-absorption spectrum of a nucleoprotein (Fig. 1), and was highly infective. By

contrast, the top component contained mostly particles penetrated by phospho-
tungstate, had the u.v.-absorption spectrum of a protein (Fig. 1), and was only
slightly infective. We have assumed that the few intact particles and slight in-
fectivity of the top component was due to incomplete separation, and that
apparently empty particles seen in the whole virus fraction were either due to in-
complete separation or to damage during mounting for electron microscopy. The
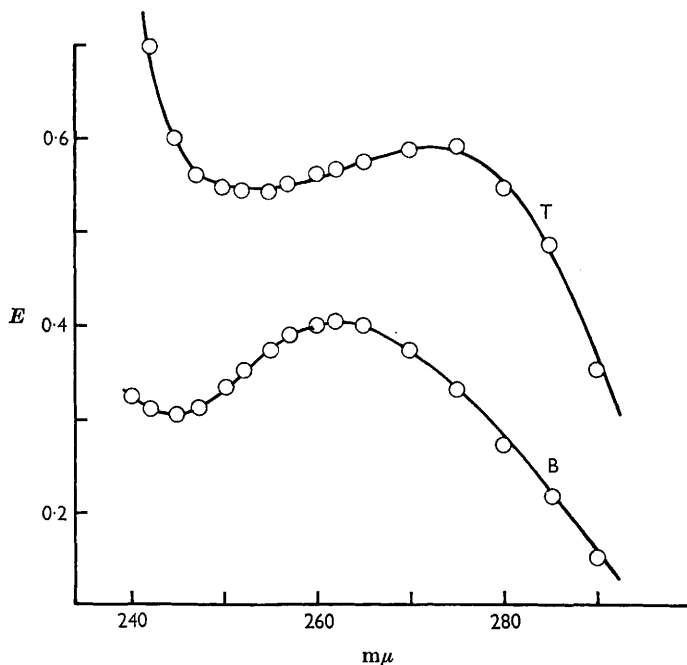

Fig. 1. Ultraviolet absorption spectra of separated cocoa yellow mosaic virus components.
Upper line is spectrum of top component, lower line that of the bottom component
(whole virus).

separated components each reacted with an antiserum made by injecting a rabbit
with an unfractionated CYMV preparation. In gel diffusion tests each component
gave only one line of precipitate, and in suitable tests these lines were confluent.

Thus CYMV preparations contain both fast sedimenting infective nucleoprotein
particles and more slowly sedimenting non-infective protein particles, which have
the shape and size of and are antigenically similar to the nucleoprotein particles.

Electrophoresis. CYMV preparations were dialysed against neutral 0·06 M-
phosphate buffer and then examined in a Perkin-Elmer Model 38 A electrophoresis
apparatus with a schlieren optical system.

All the preparations examined separated into two components, the major one
migrating at $-11\cdot8 \times 10^{-5}$ cm./sec./V./cm. and the minor at $-11\cdot3 \times 10^{-5}$ cm./
sec./V./cm. Because of the small difference in the mobilities of these electrophoretic
components it was impossible to separate them and study their composition. They
seem not to correspond to the protein and nucleoprotein components of the virus
preparation, for in one experiment the separated whole virus contained both electro-

phoretic components in the same relative amounts as in the unfractionated preparations.

Serology. Our results show that CYMV resembles turnip yellow mosaic (TYMV) and wild cucumber mosaic (WCMV) viruses in many respects. TYMV and WCMV are serologically related (MacLeod & Markham, 1963), so tests were made to find whether CYMV is serologically related to them and to cowpea mosaic virus, which is also transmitted by beetles (Dale, 1949; Chant, 1959). Table 3 shows the sources of the viruses and antisera used in the tests.

Table 3. *Hosts and sources of virus antigens*

Virus	Host species for virus for injection	Host species for virus used as antigen	Source
Cocoa yellow mosaic	*Theobroma cacao*	*Chenopodium amaranticolor*	Sierra Leone
Wild cucumber mosaic	*Cucurbita pepo*	*C. pepo*	MacLeod & Markham
Turnip yellow mosaic	*Brassica chinensis*	*B. chinensis*	Scotland
Cowpea mosaic			
Nigeria	*Phaseolus vulgaris*	*P. vulgaris*	Nigeria
Trinidad	*Phaseolus vulgaris*	*P. vulgaris*	Trinidad
Surinam	?	—	Hari Agrawal

Table 4. *Antiserum titrations*

Antiserum titre (reciprocal of dilution end-point) after 4-hr incubation at 37°. In parentheses least dilution tested, when no reaction.

				Antigen			
				Cowpea mosaic virus		Healthy sap	
Antiserum	Cocoa yellow mosaic virus	Wild cucumber mosaic virus	Turnip yellow mosaic virus	Nigeria	Trinidad	*C. amaranticolor*	*P. vulgaris*
Cocoa yellow mosaic	512	0 (2)	0 (2)	0 (2)	—	0 (2)	—
Wild cucumber mosaic	8–16	1024	16	0 (2)	—	—	—
Turnip yellow mosaic	0 (2)	8	1024	0 (2)	—	—	—
Cowpea mosaic							
Nigerian	0 (1)	0 (2)	0 (2)	64	64	—	0 (2)
Surinam	—	—	—	0 (16)	128	—	—

The serological relationships between the viruses were estimated by determining the precipitation end-points of the antisera when titrated against purified preparations of the different viruses. First the precipitation end-point of each virus preparation was found using the homologous antiserum, then the preparation was used as antigen in the antiserum titration tests at approximately four times the concentration at the precipitation end-points.

The tests (Table 4) confirmed the results of MacLeod & Markham (1963) and showed ʀ distant serological relationship between TYMV and WCMV. The WCMV antiserum, but not the TYMV antiserum, precipitated CYMV, whereas the CYMV antiserum did not precipitate either TYMV or WCMV. The failure of CYMV antiserum to precipitate WCMV, when the reciprocal test was positive, may be because it had a lower titre than the WCMV antiserum.

Thus CYMV is apparently serologically related to WCMV but not to TYMV, even though TYMV and WCMV are related. However, further tests showed that WCMV has some antigenic groups common to both TYMV and CYMV, for when

WCMV antiserum was mixed with excess CYMV it did not precipitate TYMV, and when mixed with excess TYMV it did not precipitate CYMV.

Neither CYMV, WCMV, TYMV nor their antisera gave precipitates in tests with the cowpea mosaic viruses or their sera. Limited tests showed that the different cowpea mosaic viruses were serologically related.

All the precipitates formed in these tests were white and granular, as usually obtained with isometric virus antigens, and unlike the less-dense microgranular and often coloured precipitates formed when plant proteins react with their antisera.

Dorner, Kahn & Wildman (1958) and van Regenmortel (1963) found that the 'Fraction 1' proteins of different plant species were serologically related. Van Regenmortel (1963) has suggested that reactions with such proteins, which are often present in plant-virus preparations, might be responsible for the reports of distant serological relationships between viruses, such as those reported by MacLeod & Markham (1963). However, our results confirm those of MacLeod & Markham (1963) and provide further evidence to suggest that plant proteins are not responsible for the observed serological reactions. Dorner et al. (1958) and van Regenmortel (1963) apparently found one cross-reacting plant protein antigen in all species. However, our results (Tables 3, 4) show the presence of at least three distinct antigens; one in *Phaseolus vulgaris* only, one in both *Brassica chinensis* and *Cucurbita pepo* only, and another in *C. pepo* and *Chenopodium amaranticolor* only. Furthermore, the preparations of CYMV, WCMV, and TYMV used as antigens in our tests were highly purified and concentrated, and they were therefore diluted 1/32, 1/100, and 1/150 respectively for use in serological tests. The WCMV and TYMV preparations showed no components attributable to plant proteins when examined in the analytical centrifuge. By contrast, the cowpea mosaic preparation was relatively impure. It was diluted only to 1/10 for serological tests, and contained more plant protein than the other virus preparations. Out of all those tested, it should therefore have been the most readily precipitated by antibodies to plant proteins, and yet it reacted only with its homologous antiserum. Dorner et al. (1958) found that the precipitation end-point of untreated tobacco sap was only 1/320 when tested in ring interface tests with an antiserum specifically prepared against 'Fraction 1' protein. Thus it is unlikely that enough plant protein to give a precipitate was present in the diluted antigen preparations we used except with cowpea mosaic virus preparations.

DISCUSSION

An interesting feature of cocoa yellow mosaic virus (CYMV) is its similarity to turnip yellow mosaic virus (TYMV) and wild cucumber mosaic virus (WCMV). Their particles have similar shape, size, external morphology, and sedimentation rates; moreover, MacLeod & Markham (1963) and MacLeod (personal communication) found that the nucleoprotein particles of all three viruses contain similar amounts of nucleic acid, of similar composition (with 37–40 % cytidylic acid). Therefore it is perhaps not surprising that these viruses are serologically related; however, the relationship is unusual in that WCMV antiserum reacted with both CYMV and TYMV, though there was no reaction between TYMV and CYMV antiserum or CYMV and TYMV antiserum.

The natural vector of CYMV in Sierra Leone is not known. Blencowe et al. (1963)

failed to transmit the virus by three species of mealy-bug, and attempts in Sierra Leone to transmit the virus by soil and seed also failed; 60 pots of soil collected from around the roots of naturally infected cocoa plants were each sown with 5 cocoa beans, none became infected, and 150 beans from naturally infected cocoa plants all produced apparently healthy seedlings. However, the similarity of CYMV to TYMV and WCMV, both of which are transmitted by chrysomelid beetles, suggests that CYMV may also have a beetle vector.

Other viruses have been found to be beetle transmitted, including cowpea mosaic viruses in Trinidad (Dale, 1949) and in Nigeria (Chant, 1959). Our tests, and those of Shepherd (1963) and Agrawal & Maat (1964) have shown that the two cowpea viruses are closely serologically related, but that they are apparently unrelated to CYMV, TYMV or WCMV. Cowpea mosaic virus also differs from CYMV in other ways, for although preparations of it have two components, like those in CYMV preparations, the particles appear different in the electron microscope (Chant, 1959) and do not show clearly defined subunits when mounted in phosphotungstate. Furthermore, bean pod mottle virus, which has recently been shown to be beetle-transmitted (Ross, 1963; Walters, 1964) and is serologically closely related to cowpea mosaic virus (Shepherd, 1963), has a nucleic acid composition (Semancik & Bancroft, 1964) quite different from that of CYMV.

REFERENCES

AGRAWAL, H. & MAAT, D. Z. (1964). Serological relationships among polyhedral plant viruses and production of high titred antisera. *Nature, Lond.* **202**, 674.

BAWDEN, F. C. (1958). Reversible changes in strains of tobacco mosaic virus from leguminous plants. *J. gen. Microbiol.* **18**, 751.

BLENCOWE, J. W., BRUNT, A. A., KENTEN, R. H. & LOVI, N. K. (1963). A new virus disease of cocoa in Sierra Leone. *Tropical Agriculture*, **40**, 233.

BRUNT, A. A., KENTEN, R. H. & NIXON, H. L. (1964). Some properties of cocoa swollen shoot virus. *J. gen. Microbiol.* **36**, 303.

CHANT, S. R. (1959). Viruses of Cowpea, *Vigna unguiculata* L. (Walp.) in Nigeria. *Ann. appl. Biol.* **47**, 565.

COSENTINO, V., PAIGEN, K. & STEERE, R. L. (1956). Electron microscopy of turnip yellow mosaic virus and the associated abnormal protein. *Virology*, **2**, 139.

DALE, W. T. (1949). Observations on a virus disease of cowpea in Trinidad. *Ann. appl. Biol.* **36**, 327.

DORNER, R. W., KAHN, A. & WILDMAN, S. G. (1958). Proteins in green leaves. VIII. The distribution of 'Fraction 1' protein in the plant kingdom as detected by ultracentrifugal analyses. *Biochim. biophys. Acta*, **29**, 240.

HUXLEY, H. E. & ZUBAY, G. (1960). The structure of the protein shell of turnip yellow mosaic virus. *J. mol. Biol.* **2**, 180.

MACLEOD, R. & MARKHAM, R. (1963). Experimental evidence of a relationship between turnip yellow mosaic virus and wild cucumber mosaic virus. *Virology*, **19**, 190.

MARKHAM, R. & SMITH, K. M. (1949). Studies on the virus of turnip yellow mosaic. *Parasitology*, **39**, 330.

NIXON, H. L. & GIBBS, A. J. (1960). Electron microscope observations on the structure of turnip yellow mosaic virus. *J. mol. Biol.* **2**, 197.

NIXON, H. L. & HARRISON, B. D. (1959). Electron microscopic evidence on the structure of tobacco rattle virus. *J. gen. Microbiol.* **21**, 582.

ROSS, J. P. (1963). Transmission of bean pod mottle virus in soybeans by beetles. *Plant Dis. Reptr.* **47**, 1049.

SEMANCIK, J. S. & BANCROFT, J. B. (1964). Further characterization of the nucleoprotein components of bean pod mottle virus. *Virology*, **22**, 33.

SHEPHERD, R. J. (1963). Serological relationship between bean pod mottle virus and cowpea mosaic virus from Arkansas and Trinidad. *Phytopathology*, **53**, 865.

VAN REGENMORTEL, M. H. V. (1963). Serologically related plant contaminants in preparations of partly purified plant viruses. *Virology*, **21**, 657.

WALTERS, H. J. (1964). Transmission of bean pod mottle virus by bean leaf beetles. *Phytopathology*, **54**, 240.

EXPLANATION OF PLATE 1

Fig. 1. Necrotic local lesions on leaf of *Chenopodium amaranticolor* inoculated with a purified cocoa yellow mosaic virus (CYMV) preparation.

Fig. 2. Tip leaf of *C. amaranticolor* plant systemically infected with CYMV, showing distortion and necrotic flecks.

Fig. 3. Chlorotic patterns on systemically infected leaf from a mechanically inoculated cocoa seedling.

Fig. 4. Electron micrographs showing empty protein particles of the top component of CYMV, separated from a purified preparation in a sucrose density gradient, and mounted in phosphotungstate; about × 240,000.

Fig. 5. Nucleoprotein particles from the bottom component. Details as for fig. 4.

Fig. 6. Schlieren pattern from a purified preparation of CYMV sedimenting in the analytical ultracentrifuge. Sedimentation is from left to right.