*Ann. appl. Biol.* (1981), **97**, 99–107
*With 1 plate*
*Printed in Great Britain*



# Further properties of peanut clump virus and studies on its natural transmission

BY J. C. THOUVENEL AND C. FAUQUET

*Laboratoire de Virologie, Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), B.P. V 51, Abidjan, République de Côte d'Ivoire, Afrique de l'Ouest*

(*Accepted 12 August 1980*)

SUMMARY

Purified preparations of particles of peanut clump virus (PCV) had $A_{260}/A_{280}$ values (corrected for light scattering) of 1·00. They contained rod-shaped particles with sedimentation coefficients of 183 *S* and 224 *S*, and a density in CsCl of 1·32 g/ml. PCV infected 36 species in 8 plant families. No serological relationship was detected between PCV and barley stripe mosaic, beet necrotic yellow vein, *Nicotiana velutina* mosaic and tobacco mosaic viruses.

PCV was seed-borne for two generations in groundnut (*Arachis hypogaea*) but was not seed-borne in great millet (*Sorghum arundinaceum*), *Phaseolus mungo* or *Nicotiana benthamiana*.

Seedlings of groundnut, great millet and wheat (*Triticum aestivum*) became infected when grown in soil from groundnut fields with outbreaks of clump disease, and the infectivity of soil survived air-drying at 25 °C for 3 months. Groundnut seedlings became infected when grown in sterilised soil contaminated with washed roots of naturally-infected *S. arundinaceum* but not in soil to which roots of naturally infected groundnut or shoots of infected groundnut were added, or in which mechanically inoculated groundnut seedlings were grown at the same time. The patchy distribution of PCV in a crop was related to the infectivity of the soil for groundnut and to the presence of *Polymyxa graminis* resting spores which could be detected in the roots of *S. arundinaceum* bait seedlings, but not in those of groundnut.

The results indicate that PCV is transmitted by a vector that is resistant to air-drying and closely associated with *S. arundinaceum* roots. For these reasons *P. graminis* is thought to be the vector of PCV.

## INTRODUCTION

Peanut clump virus (PCV) is a virus with rod-shaped particles of two predominant lengths (190 and 245 nm) and causes a disease of groundnut (*Arachis hypogaea*) in Upper-Volta and Senegal (Thouvenel, Germani & Pfeiffer, 1974). Some of the biological and biochemical properties of PCV have been described by Thouvenel, Dollet & Fauquet (1976), including a method of purification by acidification of clarified leaf extracts (Thouvenel, Fauquet & Dollet, 1978).

A natural host (*Sorghum arundinaceum*) of the virus was found in Upper-Volta (Dollet, Fauquet & Thouvenel, 1976), and seed-transmission in groundnut was demonstrated by Thouvenel, Fauquet & Lamy (1978).

In this paper further details are described of the *in vitro* properties of purified PCV particles and of the host range and transmission of the virus in seed and from the soil.

## MATERIALS AND METHODS

The virus isolate used was originally obtained from Saria, Upper-Volta, by sap transmission from a clump-affected groundnut plant to *A. hypogaea* cv. TE 3 and *Chenopodium amaranticolor*.

Plants were grown in sterilised soil in glasshouses where the average temperature was 30 °C, but occasionally reached 35 °C. The relative humidity varied from 80 to 98%. PCV was purified from infected *C. amaranticolor* leaves, harvested 5 days after inoculation and stored frozen at −30 °C. The virus was purified by acid clarification of leaf extracts using the method of Thouvenel, Fauquet & Dollet (1978). The host range was determined by inoculating various plant species using purified virus with $A_{260}$ values of 0·1. Symptomless infection was confirmed by back inoculation to the indicator species *C. amaranticolor* or *A. hypogaea* as follows. Frozen leaves were ground (1 g/2 ml) in 0·1 M potassium phosphate buffer pH 7·1 containing 0·02 M cysteine hydrochloride and 2·5 mg/ml bentonite and the extract manually inoculated to Carborundum-dusted leaves of indicator plants.

Virus concentration and ultra-violet absorption spectra were determined with a Beckman model 5230 u.v. spectrophotometer. Corrections for light-scattering were made by the graphical method of Noordam (1973). Absorbance between 320–600 nm was plotted on log/log graph paper. Values for absorbance between 320 and 600 nm gave a linear relationship. Light-scattering was determined by extrapolation between 320 and 230 nm. The values obtained were subtracted from the measured absorbance of PCV preparations.

For equilibrium sedimentation, virus particles were suspended in 4 ml 38% caesium chloride in 0·05 M borate buffer, pH 8. The suspension was centrifuged in a Beckman SW50 rotor at 115 000 *g* for 18 h. The gradients were collected through a needle from the bottom of each tube in 3 drop fractions (*c.* 60 µl). The optical density was determined with 50 µl of each fraction diluted to 1 ml; the density being calculated from the refractive index, measured with an Abbe refractometer.

For analytical ultracentrifugation, virus preparations were centrifuged in a Beckman model E analytical ultracentrifuge using Schlieren optics.

Antisera to the following viruses were kindly supplied as follows: *Nicotiana velutina* mosaic (B. D. Harrison), beet necrotic yellow vein (C. Putz) and barley stripe mosaic virus (J. G. Atabekov).

## RESULTS

### *Properties of purified PCV*

*Density in caesium chloride.* After equilibrium sedimentation in a caesium chloride gradient, a zone of light-scattering material occurred slightly above the zone produced by a tobacco mosaic virus marker (Text-fig. 1). The virus was very concentrated, but virus particles neither precipitated nor aggregated. In 10 experiments, the average density of the PCV peak was 1·32 ± 0·01. The average density of the tobacco mosaic virus marker was 1·33 ± 0·01.

*Analytical ultracentrifugation.* The Schlieren pattern obtained using preparations of PCV showed three components with sedimentation coefficients ($S_{20,w}$) of 183 *S*, 224 *S* and an average of 330 *S*. The two first peaks corresponded to the two types of PCV particles; the third, very broad peak, corresponding to different types of aggregated particles.

*UV absorption spectrum.* After correcting for light-scattering, purified PCV had an absorption maximum of 270 nm and a minimum absorption of 249 nm. The ratios, corrected for light-scattering, were: $A_{max}/A_{min} = 1{\cdot}32$ and $A_{260}/A_{280} = 1{\cdot}00$ (Text-fig. 2).

### *Host range*

It was found previously (Thouvenel, Dollet & Fauquet, 1976) that PCV could not be sap-transmitted from *C. amaranticolor* to groundnut, probably because of the presence of

inhibitors in *C. amaranticolor* sap. Later, it was found that groundnut could be infected by inoculation with virus purified from *C. amaranticolor* (Dollet, Fauquet & Thouvenel, 1976). Accordingly, a range of plant species were then tested with purified PCV. The results (Table 1) showed that 36 species of eight plant families became infected. The host range was wide and included species of *Aizoaceae, Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Gramineae, Leguminosae, Scrophulariaceae,* and *Solanaceae.*

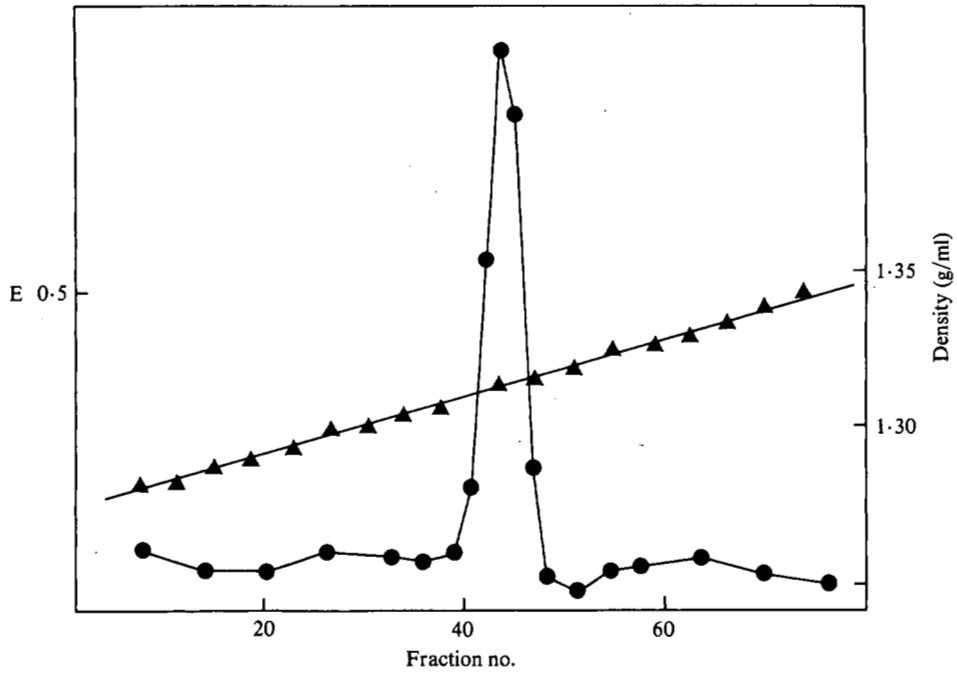



Fig. 1. U.v.-absorption profile at 254 nm (●—●—●) of a caesium gradient. Purified particles of PCV (2 mg) were mixed with the caesium chloride solution, before centrifugation for 18 h at 115 000 *g* in a Beckman SW50 rotor. The density of each fraction (▲—▲—▲) was calculated from its refractive index.

Tests were also made to groundnut cultivars commonly grown in West Africa, using seeds provided by IRHO (Institut de Recherche des Huiles et Oléagineux, and to two cultivars Jumbo and Spanish (Burpee Seeds) from the USA. Plants were infected as follows: cv. TE 3 (4/24), cv. 55 437 (17/22), cv. 57 422 (7/18), cv. 48 115 (14/22), cv. 59 127 (8/15), cv. 28 206 (9/17), cv. 149 A (2/13), cv. Jumbo (5/15) and cv. Spanish (6/26). In all instances the inoculated leaves were symptomless but infected plants became clumped and systemic symptoms, such as chlorotic spots and eye-spots, were observed on the young leaves (Plate, fig. 1).

### *Serological tests*

Serological tests were done using the micro-precipitin technique (Van Slogteren, 1955). There was no reaction between purified preparations of PCV and antisera diluted 1/4 and 1/16 to beet necrotic yellow vein – French strain (homologous titre, 1/1024), *Nicotiana velutina* mosaic (1/512) or tobacco mosaic (1/1024) viruses. There was no reaction between PCV antiserum (titre, 1/2048) and tobacco mosaic and barley stripe mosaic viruses.

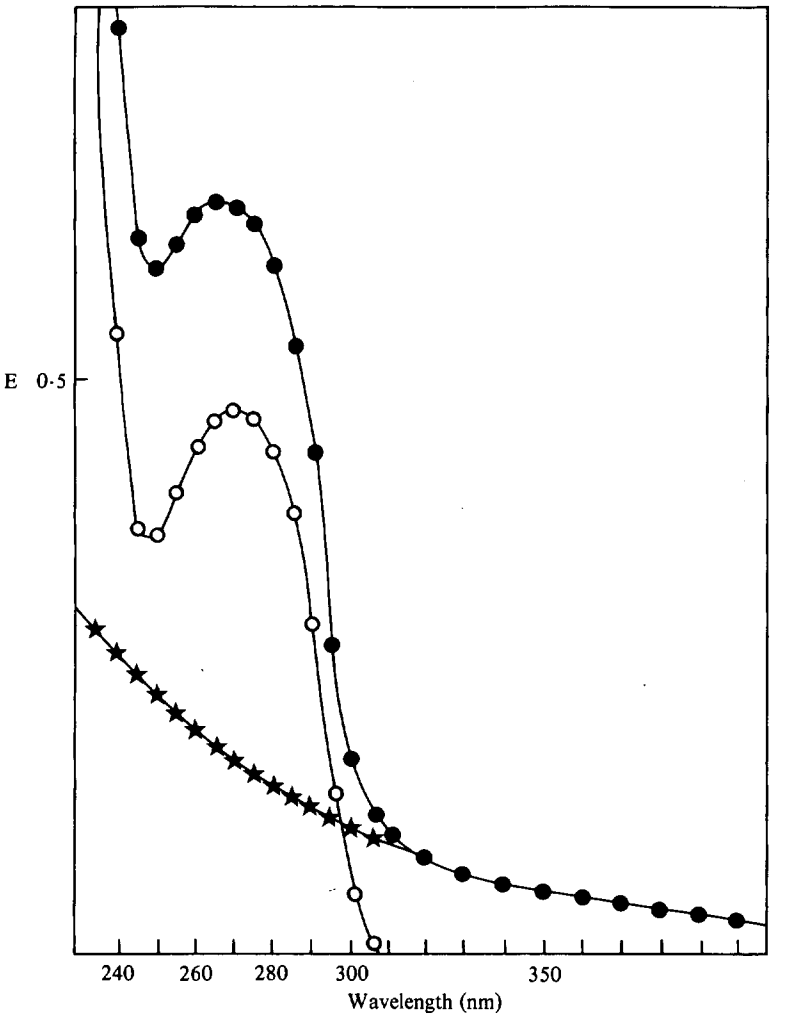



Fig. 2. U.v.-absorption spectrum of purified PCV (0·2 mg/ml) before (●—●—●) and after (○—○—○) correction for light scattering. The calculated correction (★—★—★) is also plotted.

### *Seed transmission*

Clump disease of groundnuts is not common but has been found in widely separated places including Bambey, Senegal (Bouhot, 1967); Saria, Upper-Volta (Germani & Dhéry, 1973) and Bouaké, Ivory Coast (Portères & Legleu, 1937). This suggested that PCV might be seed-transmitted, despite the negative results obtained by Bouhot (1967). Subsequently, it was found to be seed-transmitted at a rate of 24% in seed from artificially infected plants and at a rate of 14% in seed of naturally-infected plants (Thouvenel, Fauquet & Lamy, 1978).

In further tests with other naturally-infected groundnut cultivars an average of 5·9% seed infection was found to occur (Table 2).

Seeds were collected from the diseased plants (second generation seeds) and, of the seedlings tested, 19·2% (34/177) were infected with PCV.

Seed transmission of PCV was not detected in *Sorghum arundinaceum* (0/122), *Phaseolus mungo* (0/150) or *Nicotiana benthamiana* (0/280), using seed harvested and planted in the same conditions as used for groundnut.

Table 1. *Species infected by mechanical inoculation with purified PCV*

| Species | Reaction | | | |
|---|---|---|---|---|
| | Inoculated leaves | | Non-inoculated leaves | |
| Aizoaceae | | | | |
| *Tetragonia expansa* | Concentric RS | (+) | No symptom | (–) |
| Amaranthaceae | | | | |
| *Celosia argentea* | No symptom | (+) | Mottle | (+) |
| *Gomphrena globosa* | Spots | (+) | No symptom | (–) |
| Chenopodiaceae | | | | |
| *Beta vulgaris* | Spots | (+) | No symptom | (–) |
| *C. amaranticolor* | Concentric RS | (+) | No symptom | (–) |
| *C. quinoa* | Concentric RS | (+) | No symptom | (–) |
| *Spinacia oleracea* | Yellowing | (+) | No symptom | (–) |
| Cucurbitaceae | | | | |
| *Cucumis sativus* | Necrotic spots | (+) | No symptom | (–) |
| *Cucurbita pepo* var. medullosa | Necrotic spots | (+) | No symptom | (–) |
| Gramineae | | | | |
| *Bromus arvensis* | No symptom | (+) | Slight mottle | (+) |
| *B. commutatus* | No symptom | (+) | Slight mottle | (+) |
| *B. macrostachys* | No symptom | (+) | Slight mottle | (+) |
| *B. racemosus* | No symptom | (+) | Slight mottle | (+) |
| *B. sterilis* | No symptom | (+) | Slight mottle | (+) |
| *Sorghum arundinaceum* | No symptom | (+) | No symptom | (+) |
| *Triticum aestivum* | No symptom | (+) | Stunt and mottle | (+) |
| *T. durum* | No symptom | (+) | Stunt and mottle | (+) |
| Leguminosae | | | | |
| *Arachis hypogoea* | No symptom | (+) | RS and clump | (+) |
| *Crotalaria usaramoensis* | No symptom | (+) | Mottle and clump | (+) |
| *Lathyrus odoratus* | No symptom | (+) | No symptom | (+) |
| *Melilotus alba* | No symptom | (+) | No symptom | (+) |
| *Phaseolus mungo* | No symptom | (+) | Line pattern | (+) |
| *P. vulgaris* | No symptom | (+) | Mosaic | (+) |
| *Vigna sinensis* cv. Black | Concentric RS | (+) | No symptom | (–) |
| *V. sesquipedalis* | Concentric RS | (+) | No symptom | (–) |
| *Voandzeia subterranea* | Concentric RS | (+) | No symptom | (+) |
| Scrophulariaceae | | | | |
| *Antirrhinum majus* | No symptom | (+) | No symptom | (+) |
| *Torenia fournieri* | Concentric RS | (+) | Line pattern | (+) |
| Solanaceae | | | | |
| *Nicotiana benthamiana* | No symptom | (+) | Mosaic and clump | (+) |
| *N. clevelandii* | No symptom | (+) | Mosaic and clump | (+) |
| *N. clevelandii* × *N. glutinosa* | No symptom | (+) | Mosaic | (+) |
| *N. glutinosa* | No symptom | (+) | Mosaic | (+) |
| *N. megalosiphon* | Yellow spots | (+) | No symptom | (–) |
| *N. tabacum* cv. Samsun | No symptom | (+) | Vein banding | (+) |
| *N. tabacum* cv. Samsun NN | No symptom | (+) | Vein banding | (+) |
| *N. tabacum* cv. Xanthi-nc | Yellowing | (+) | No symptom | (–) |
| *N. tabacum* cv. White Burley | Yellowing | (+) | No symptom | (–) |
| *Petunia hybrida* | No symptom | (+) | Mottle | (+) |
| *Physalis floridana* | No symptom | (+) | Mottle | (+) |

(+) = virus detected by inoculation of sap to *C. amaranticolor*.
(–) = virus not detected by similar test.
RS = ringspot.

Table 2. *Seed transmission of PCV in groundnut*

| Cultivars | Tested 5 months after harvest | | | | Tested 7 months after harvest | | | | Totals | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % germination | No. of plants tested | No. of plants diseased | % diseased plants | % germination | No. of plants tested | No. of plants diseased | % diseased plants | % germination | No. of plants tested | No. of plants diseased | % diseased plants |
| TE 3 | 80·5 | 260 | 27 | 10·4 | 75·4 | 248 | 11 | 4·4 | 77·9 | 508 | 38 | 7·5 |
| 149 A | 24·8 | 82 | 8 | 9·8 | 56·8 | 197 | 8 | 4·1 | 41·2 | 279 | 16 | 5·7 |
| TS 32-1 | 68·9 | 217 | 5 | 2·3 | 69·4 | 220 | 11 | 5·0 | 69·1 | 437 | 16 | 3·7 |
| KH 241 D | 42·7 | 153 | 9 | 5·9 | 63·1 | 219 | 14 | 6·4 | 52·8 | 372 | 23 | 6·2 |
| Reported for cv. TE 3 tested 1 month after harvest* | | | | | | | | | 80·2 | 607 | 26 | 4·3 |
| Reported for cv. 73–33 tested 2 months after harvest* | | | | | | | | | 85·2 | 133 | 18 | 13·5 |
| Total for all cultivars | | | | | | | | | 65·2 | 2336 | 137 | 5·9 |

*Thouvenel, Fauquet & Lamy, 1976.

### *Soil transmission*

Thouvenel, Dollet & Fauquet (1976) confirmed the suggestion made by Germani & Dhéry (1973) that PCV was soil-borne by sowing seeds of healthy groundnut plants in soil in which diseased plants had grown. Subsequently, the groundnut seedlings developed typical symptoms of the disease.

In further experiments, infective field soil from Upper-Volta was sown with groundnut, great millet (*S. arundinaceum*) and wheat (*Triticum aestivum*). After 3 wk, symptoms appeared in groundnut (5/12) and wheat (9/12) plants only. However, crude sap from the leaves of great millet (6/12) induced the typical symptons of PCV when used to inoculate *C. amaranticolor* plants. The results confirmed that groundnut, great millet and wheat all became infected by sowing them in infective soil. When the seed was sown in sterilised soil as controls, all the seedlings remained virus-free.

In a further experiment in which groundnuts were sown in infective field soil, previously air-dried at 25 °C for 3 months, 8% of the seedlings (5/60) developed clump disease symptoms.

Failure to find nematodes of virus-vector genera such as *Trichodorus* in infective field soil, together with the fact that the soil remained infective after air-drying for 3 months, suggested that the vector was a fungus. When the roots of groundnut and great millet plants from the field were examined for the presence of a fungus by staining with acid fuchsin in lactophenol (Britton & Rogers, 1963), clusters of resting spores similar to those of a plasmodiophoromycete fungus were observed, but only in the great millet roots (Plate, fig. 5). Observations on the different stages of the life cycle of this fungus in culture, particularly the multinucleate plasmodia that develop into sporangia with exit tubes, indicated that it was *Polymyxa graminis* Ledingham (Thouvenel & Fauquet, 1980).

Resting spores of *P. graminis* were also found in roots of *Sorghum cernuum*, *Triticum aestivum* and *T. durum* and all other graminaceous plants grown in infective soil became infected with PCV. In contrast, however, no stage of the life cycle of *P. graminis* was found in groundnut roots.

The frequency of soil transmission of PCV varied at different times of year. It was greatest during the dry windy period (the Harmatan) in December when *P. graminis* grew most abundantly in glasshouse in the Ivory Coast (Thouvenel & Fauquet, 1980). During the other periods of the year, the rate of transmission was low and sometimes transmission was not possible.

To obtain further evidence of the relationship between the clump disease and *P. graminis*, soil samples were taken from the centre, at the edge and outside an area of clump-affected plants of a

Table 3. *Relationship between PCV infection of groundnut seedlings and occurrence of* Polymyxa graminis *in soil samples from inside or outside a groundnut clump disease-affected area*

| Soil sample | | Transmission of PCV | | Occurrence of *P. graminis** |
|---|---|---|---|---|
| Site of affected area | Depth (cm) | No. plants tested | % | |
| Centre | 0–15 | 29/89 | 32·6 | + |
| Centre | 15–30 | 20/94 | 21·3 | + |
| Edge | 0–15 | 2/76 | 2·6 | + |
| Edge | 15–30 | 5/106 | 4·7 | – |
| Outside the area | 0–15 | 0/96 | 0·0 | – |
| Outside the area | 15–30 | 0/69 | 0·0 | – |

**P. graminis* was detected by growing *Sorghum arundinaceum* seedlings in the soil samples and examining their roots after 6 wk.

crop in Upper-Volta. Groundnut seedlings were planted in the soils and the symptoms which developed in them were recorded. The presence of PCV in plants was confirmed by inoculation of sap to *C. amaranticolor*. Seeds of great millet were sown in the same soil samples and their roots examined for resting spores of *P. graminis* 6 wk later. The results (Table 3) indicated that soil from the centre of the affected area was more infective and contained more *P. graminis* than soil from the edge, whereas soil from outside the area was not infective and did not contain *P. graminis*.

Because *P. graminis* was *not* found in groundnut roots, the possibility was tested that they contained a vector other than *P. graminis*. Diseased groundnut and great millet seedlings grown in infective soil were removed from the soil and their roots washed copiously in running water for 24 h. The seedlings were then replanted in sterilised soil and seeds of groundnut and great millet were sown in the same soil. PCV was transmitted from diseased great millet to groundnut (3/12 plants) and great millet (10/12 plants), but the virus was not transmitted from diseased groundnut to bait seedlings of groundnut (0/12 plants) or great millet (0/12 plants). *Polymyxa graminis* was found in the roots of all great millet plants that became infected in these tests. In a further test, roots of PCV-infected great millet from the field in Upper-Volta, stored dry at 25 °C for 2 yr, were used to contaminate sterilised soil. None of the great millet seedlings (0/24 plants) when grown in this soil became infected although *P. graminis* was found in their roots.

In contrast to the successful transmission of PCV using washed roots of naturally-infected great millet as the inoculum, no transmission occurred when 24 healthy groundnut plants and 24 groundnut plants infected with PCV by mechanical inoculation were grown in sterilised soil in the same container. In addition, no transmission occurred when groundnut seeds were grown in sterilised soil contaminated with lyophilised and crushed pieces of infected groundnut leaves.

## DISCUSSION

In a previous study (Thouvenel, Dollet & Fauquet, 1976), the failure to transmit PCV from *C. amaranticolor* to groundnut was attributed to inhibitors in *C. amaranticolor* sap. This was confirmed in the present investigation, because PCV was transmitted to a wide range of plants, including groundnut, using purified virus as inoculum. In groundnut, the typical field symptoms of clump disease were reproduced. Among the cultivars tested, none was completely resistant but cv. 149 A was more difficult to infect by inoculation than other cultivars. The host range of PCV was extensive and included species of several different families.

PCV had a density in CsCl of 1·32 g/ml, which was slightly lower than that of tobacco mosaic virus particles. This value and those of the sedimentation coefficients (183 *S* and 224 *S*) and u.v. absorption values ($A_{max}$ at 270 nm, and $A_{260}/A_{280}$ of 1·00) corresponded closely with those of barley stripe mosaic virus (Atabekov & Novikov, 1971). However, no serological relationship was found with any previously described rod-shaped virus. PCV was not related to tobraviruses, tobamoviruses (Thouvenel, Dollet & Fauquet, 1976), barley stripe mosaic virus, beet necrotic yellow vein virus (Putz, 1977), soil-borne wheat mosaic virus or *Nicotiana velutina* mosaic virus. Dr B. D. Harrison found no reaction between PCV antiserum and potato mop-top or *Nicotiana velutina* mosaic viruses (personal communication) and beet necrotic yellow vein virus did not react with PCV antiserum (Putz, 1977).

The results of transmission tests indicated that the natural vector of PCV is *Polymyxa graminis*. This fungus occurs in Upper-Volta and Senegal in the same areas as the groundnut clump disease, and is known to be the vector of other viruses with elongated particles (Brakke, 1971). Our experiments demonstrated a correlation between the presence of *P. graminis* and the spread of PCV, and soil transmission rarely occurred in plants in the glasshouse unless *P. graminis* was also found in the roots of the infected plants. In the few instances where resting spores of *P. graminis* were not observed, the fungus may have occurred in vegetative stages not stained by fuchsin. Alternatively, resting spores may have been so few that they escaped detection.

Although *P. graminis* was not found in groundnut, it is possible that the fungus infected groundnut roots without the fungus developing in the roots. This hypothesis of incomplete development is supported by the fact that groundnut roots that became infected naturally with PCV were unable to provide sources of infection to other plants, whereas comparable roots of great millet were able to do so.

Roots dried for 2 yr did not cause infection, possibly because PCV was inactivated in the roots when preserved in the laboratory in Ivory Coast. However, field observations in Upper-Volta indicated that soil remains infective for over two years in the absence of a crop (Dollet, Fauquet & Thouvenel, 1976). The results of our tests, therefore, may simply reflect the unsuitability of the climatic conditions in the Ivory Coast for transmission of the virus. Experiments were made in the Ivory Coast which has a humid tropical climate, a relatively constant temperature (27 °C $\pm$ 5 °C) and two rainy seasons; whereas Upper-Volta has a large day and night fluctuation in temperature (more than 20 °C) and one rainy season. Clump disease did not develop well at temperatures below 30 °C (Germani, Thouvenel & Dhéry, 1975), whereas *P. graminis* is most active at a lower temperature.

In morphological and physico-chemical properties, PCV differed from other multi-component viruses, and no serological relationship with other viruses was established. Nevertheless, PCV resembled hordeiviruses and *Nicotiana velutina* mosaic virus in its seed transmission, and had the same spectrophotometric properties as barley stripe mosaic virus. PCV, therefore, may be intermediate between hordeivirus and tobamovirus groups.

Further studies on the characterisation of the virus are now in progress.

Thanks are due to various workers for providing samples of antisera and virus and to Dr S. R. Christie and the Institut de Recherche sur les Huiles et Oléagineux (IRHO) for seeds. Thanks are also due to Professor L. Hirth and Dr B. D. Harrison for helpful suggestions.

## REFERENCES

ATABEKOV, J. G. & NOVIKOV, V. K. (1971). Barley stripe mosaic virus. *CMI/AAB Descriptions of Plant Viruses* No. 68, 4 pp.

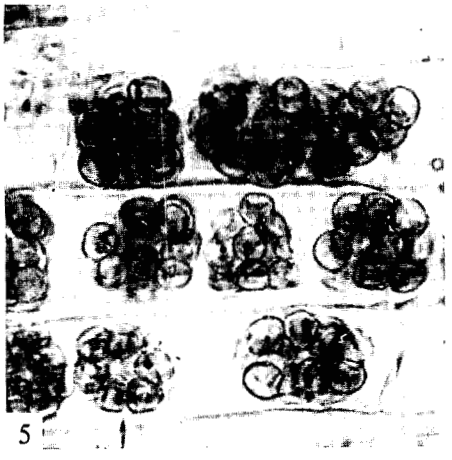
BOUHOT, D. (1967). Observations sur quelques affections des plantes cultivées au Sénégal. *Agronomie tropicale, Nogent* **22**, 888–890.

1

2

3

4



5

BRAKKE, M. K. (1971). Soil-borne wheat mosaic virus. *CMI/AAB Descriptions of Plant Viruses* No. 77, 4 pp.

BRITTON, M. P. & ROGERS, D. P. (1963). *Olpidium brassicae* and *Polymyxa graminis* in roots of creeping bent in golf putting greens. *Mycologia* **55**, 758–763.

DOLLET, M., FAUQUET, C. & THOUVENEL, J.-C. (1976). *Sorghum arundinaceum*, a natural host of peanut clump virus in Upper-Volta. *Plant Disease Reporter* **60**, 1076–1080.

GERMANI, G. & DHERY, M. (1973). Observations et expérimentations concernant le rôle des nématodes dans deux affections de l'arachide en Haute-Volta: la chlorose et le clump. *Oléagineux* **28**, 235–242.

GERMANI, G., THOUVENEL, J.-C. & DHERY, M. (1975). Le rabougrissement de l'arachide: une maladie à virus au Sénégal et en Haute-Volta. *Oléagineux* **30**, 259–266.

NOORDAM, D. (1973). Spectrophotometry. In: *Identification of plant viruses*, pp. 88–102. Ed. Pudoc. Wageningen.

PORTERES, R. & LEGLEU, R. (1937). La rosette de l'arachide. Connaissances actuelles, relation avec la date des semis dans le pays du Baoulé-Nord, méthodes prophylactiques à appliquer. *Annales agricoles de l'Afrique Occidentale* **1**, 332–355.

PUTZ, C. (1977). Composition and structure of beet necrotic yellow vein virus. *Journal of General Virology* **35**, 397–401.

THOUVENEL, J.-C., DOLLET, M. & FAUQUET, C. (1976). Some properties of peanut clump, a newly discovered virus. *Annals of Applied Biology* **84**, 311–320.

THOUVENEL, J.-C. & FAUQUET, C. (1980). First report of *Polymyxa graminis* L. in Africa, and its occurrence on new *Sorghum* Moench host species. *Plant Disease* **64** (In press).

THOUVENEL, J.-C., FAUQUET, C. & DOLLET, M. (1978). Influence du pH sur le virus du clump de l'arachide (peanut clump virus) et nouvelle méthode rapide de purification. *Annales de Phytopathologie* **10**, 93–99.

THOUVENEL, J.-C., FAUQUET, C. & LAMY, D. (1978). Transmission par la graine du virus du clump de l'arachide. *Oléagineux* **33**, 503–504.

THOUVENEL, J.-C., GERMANI, G. & PFEIFFER, P. (1974). Preuve de l'origine virale du rabougrissement ou 'clump' de l'arachide en Haute-Volta et au Sénégal. *Comptes-rendus de l'Académie des Sciences, Paris* **278**, 2847–2849.

VAN SLOGTEREN, D. H. M. (1955). Serological micro-reactions with plant viruses under paraffin oil. *Proceedings of the 2nd Conference on Potato Virus Disease, Lisse-Wageningen*, 1954, pp. 51–54.

(*Received 7 May 1980*)

## EXPLANATION OF PLATE

Fig. 1. Symptoms of clump disease in a young groundnut leaf (right), healthy leaf (left).
Fig. 2. Symptoms of PCV in *Nicotiana benthamiana.*
Fig. 3. Symptoms of PCV in *Nicotiana clevelandii.*
Fig. 4. Symptoms of PCV in *Triticum aestivum* (left), healthy plant (right).
Fig. 5. Cystosori of *Polymyxa graminis* in great millet roots.