

A virus causing ringspot of *Passiflora edulis* in the Ivory Coast

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SUMMARY

A mechanically transmissible virus causing leaf mottling and ringspotting of *Passiflora edulis* var. *flavicarpa* in the Ivory Coast is described. Its particles are flexuous rods 810-830 nm long and 15 nm wide. It infects mainly species of Passifloraceae and Leguminosae; *Passiflora foetida* is a good diagnostic host. *Aphis gossypii* and *Aphis spiraecola* transmit the virus after brief acquisition feeds. Seed transmission was not detected. In crude sap of *P. edulis*, infectivity was lost after 10 min at 65-70 °C, 12-14 days at 24 °C or dilution to 10⁻⁷. A purification method is described, using Triton-X-100 as clarifying agent. The virus is serologically related but not identical to passionfruit woodiness virus from Queensland. The virus seems to belong to the potato virus Y group and has the cryptogram */*:*/(6):E/E:S/Ap. It is designated passionfruit ringspot virus.

INTRODUCTION

In 1968, viruslike symptoms were observed on leaves of *Passiflora edulis* var. *flavicarpa* in the Ivory Coast. Shrivelled fruits with a reduced pulp cavity were also found, however without 'woodiness'. 'Woodiness' is an economically important disease of the passionfruit elsewhere, and several different viruses have been reported to cause this symptom. Magee (1948) found a strain of cucumber mosaic virus causing woodiness in New South Wales (Australia). Taylor & Kimble (1964) confirmed this finding, but also found an elongated virus, the passionfruit woodiness virus - Queensland (PWV-Q) causing the disease in Queensland (Australia). McKnight (1953) studied the symptomatology of PWV-Q, a mechanically transmissible virus; Greber (1966) showed that it was also the cause of the passion vine tip blight disease and Teakle & Wildermuth (1967) did host range studies. Another elongated virus causing 'woodiness' in Nigeria, passionfruit mosaic virus, was described by Martini (1962). Nattrass (1939, 1940, 1944) reported two types of passionfruit woodiness disease in Kenya; the virus of one type was readily transmitted by mechanical inoculation, but that of the other could only be transmitted by graft inoculation.

Other passionfruit viruses causing diseases lacking the 'woodiness' symptom have been reported, namely a strain of cucumber mosaic virus which could be easily transmitted by mechanical inoculation from *P. edulis* in Hawaii (Ishii & Pascual,

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1964); a strain of cucumber mosaic virus in *P. caerulea* (Teakle, Gill, Raabe & Taylor, 1963; Zschau, 1964) and passiflora latent, a virus of the potato virus S group, in *P. caerulea* (Schnepf & Brandes, 1961; Brandes & Wetter, 1963). In the Philippines, Del Rosario, Benigno & Libed (1964) studied an elongated virus from *P. foetida* and called it *Passiflora foetida* chlorotic spot virus and in New Guinea Van Velsen (1961) described another virus from this species.

The work described here was done to determine the identity of the virus, or viruses, causing the disease of yellow passionfruit in the Ivory Coast.

MATERIALS AND METHODS

Growth conditions. All test plants were grown in screenhouses, where temperatures varied from 28 to 35 °C during the day. Relative humidity was always 95–100% and day-length *c.* 12 h.

Chenopodium species were given 4 h extra light, provided by fluorescent tubes.

Inoculations. Inocula were prepared by grinding infected leaves of *P. edulis* in 0.05 M potassium phosphate buffer pH 7, containing 1% Na₂SO₃. All plants used in host range studies were inoculated when young and growing vigorously. Two to four wk later, back inoculations were made to *P. foetida* to detect symptomless infections.

Virus and antiserum gifts. PWV-Q, mild strain, was kindly provided by D. S. Teakle (Australia) and propagated in *P. edulis*. Antiserum against passiflora latent virus was obtained from R. Bercks (Braunschweig), but its homologous titre was not quoted.

Infectivity assay. No suitable local lesion host was found for the virus, so a systemic host, *P. foetida*, was used for infectivity assay. *P. foetida* is also a good diagnostic species.

Determination of in vitro properties. The standard procedures described by Bos, Hagedorn & Quantz (1960) were used with crude sap from young leaves of *P. edulis* plants, infected at least three months, as the source of the virus.

Aphid transmission. Aphid transmission experiments were done in the laboratory with *Aphis gossypii* reared on okra (*Hibiscus esculentus*; Malvaceae), and *A. spiraeicola* collected from *Eupatorium conyzoides* (Compositae), a commonly occurring herb. Adult and late instar apterous aphids were starved for 3–5 h, then allowed an acquisition feed of about 1 min on diseased *P. edulis* and an inoculation access period of 16–20 h on healthy seedlings of *P. edulis* and *P. foetida*.

Centrifugations. High-speed centrifugations were done in a Beckman L 50 centrifuge. A Sorvall RC 2B refrigerated centrifuge was used for the low-speed centrifugations. The sucrose density gradients were prepared by layering 4, 7, 7 and 7 ml of a 0.1 M citrate buffer pH 6.6, containing 10, 20, 30 and 40 g sucrose respectively in 100 ml solution. Centrifugations were made in the rotor SW 25.1 for 150 min at 24 000 rev/min (83 000 g). Gradients were fractionated by piercing the bottom of the tube and collecting the drops in about 50 fractions. The optical density of each fraction, diluted with water to 2.5 ml, was determined at 260 nm in a Zeiss PMQ II spectrophotometer.

Electron microscopy. Virus preparations were examined in a Siemens Elmiskop 1a electron microscope after negative staining with 1% uranyl acetate or 1% uranyl formate. Electron micrographs were taken at a magnification of 20000. Length measurements were made on prints with a final magnification of 56000. No independent magnification standard was available.

Serology. Antiserum to an Ivory Coast isolate was prepared in a rabbit injected intramuscularly with purified virus, emulsified with the same volume of Freund's incomplete adjuvant. A total of 18 mg virus was injected at weekly intervals over a period of 8 wk. Serum was obtained 5 days after the last injection and stored at 4 °C with an equal volume of glycerol. The microprecipitation reaction under paraffin oil in Petri dishes was used (van Slogteren, 1954). For antigen, plant juice was clarified with an equal volume of chloroform. Results were observed with a binocular microscope after incubation for about 16 h.

RESULTS

Symptomatology in passionfruit

In passionfruit plantations two types of diseased plants were recognized: *a*. A few plants had malformed leaves with a severe mosaic (plate, figs 1, 4) and other leaves of normal shape showing spotting or mottling (plate, fig. 6). The plants with an advanced stage of the disease appeared stunted and bore little or no fruit. Such fruit remained small, immature and, in many instances, shrivelled. Typical 'woodiness', i.e. abnormal thickening and hardening of the pericarp tissue and reduction of the pulp cavity, was not found. *b*. More commonly the plants were not stunted and the leaves were not malformed but were spotted or mottled (plate, fig. 6) with ringspots on the younger leaves (plate, fig. 5), especially where these were shaded. Fruits were symptomless.

When sap from plants with either type of symptom was inoculated mechanically to young *P. edulis* seedlings, spotting, mottling and ringspots developed but not malformation or severe mosaic, and the fruit remained symptomless. It seems therefore that the agent causing symptoms of type *b* can be transmitted mechanically from both type *a* and type *b* plants, but not that causing stunting and leaf and fruit malformation.

Although the symptoms of cucumber mosaic virus in *P. edulis* leaves as described by Taylor & Kimble (1964) resemble the malformation and severe mosaic found in the Ivory Coast, cucumber mosaic virus could not be detected by transmission to the indicator species *Nicotiana glutinosa*, *Cucumis sativus* and *Vigna sinensis*. Furthermore, crude sap from *P. edulis* with the severe symptoms did not react with cucumber mosaic virus antiserum and attempts failed at transmission by aphids, *Aphis gossypii*, to *C. sativus* and *N. glutinosa*. Thus cucumber mosaic virus seems not to be involved in the production of the severe symptoms and the cause of the severe disease is not clear. The virus causing the milder type of symptoms is the subject of this paper.

*Susceptible plants and symptomatology**Passifloraceae*

Passiflora edulis cv. *flavicarpa*. Young developing leaves showed vein yellowing and epinasty 5–20 days after inoculation. Mottling and rugosity sometimes followed soon afterwards. About two months after inoculation, spotting, mottling and ringspotting developed (plate, figs 5, 6). Fruits and flowers remained symptomless.

P. foetida. Young leaves developed vein yellowing and epinasty in 4–7 days. These primary symptoms could disappear completely before the secondary symptoms of dotting and mosaic appeared on the now well-developed leaves (plate, figs 2, 3). Some inoculated leaves reacted with vein yellowing, or vein banding and mottling. Neither flowers nor fruits showed symptoms. All inoculated plants became infected.

P. quadrangularis, Giant grenadilla. Local lesions, 1–2 mm in diameter, developed in 4–6 days, followed by leaf necrosis, stem necrosis and death of the young plant. Older plants were not infected. This plant is not suitable as a local lesion host.

Systemic symptoms were also shown by: *P. alba*, *P. caerulea*, *P. cincinnata*, *P. eichleriana*, *P. ligularis*, *P. seemannii*, *P. suberosa*, *P. vespertilio*, *Adenia glauca* and *A. lobata*.

P. adenopoda remained symptomless, but virus could be recovered from systemically infected leaves.

Chenopodiaceae

Chenopodium amaranticolor and *C. album*. Faint spotting on inoculated leaves.

C. ambrosioides, *C. botrys* and *C. quinoa*. Symptomless, but virus recovered from inoculated leaves.

C. foetidum. Faint spotting on the inoculated leaf when grown at 24 °C. No systemic infection could be detected in the *Chenopodium* species tested.

Leguminosae

Phaseolus vulgaris (French bean), cv. Triomphe de Farcy. Inoculated leaves reacted with faint chlorotic spots, a light green vein banding and slight necrosis of the veins; cv. Beka reacted with necrotic local lesions followed by vein necrosis.

Cassia occidentalis. Systemically infected leaves developed ringspots.

Species remaining symptomless although systemically infected: *Cassia hirsuta*, *Crotalaria retusa*, *Dolichos axillarius*, *Indigofera hirsuta*, *I. trita*, *Lathyrus odoratus*, *Phaseolus calcaratus*, *P. lathyroides*, *P. limensis*, *P. vulgaris* cvs Bountiful and Nain fin de Bagnol, *Sesbania sesban* and *Tephrosia vogelii*.

Symptomless species whose inoculated leaves were infected: *Canavallia ensiformis*, *Dolichos lablab*, *P. vulgaris* cvs Burpee's stringless green pod, Torrent d'or and Widusa and *Vigna sinensis* cv. Deschine.

Other families

Symptomless but virus recovered from systemically infected leaves: *Nicotiana benthamiana*, *N. megalosiphon* and *Portulaca oleracea*.

Symptomless but virus recovered from inoculated leaves: *Cucurbita pepo*, *Helichrysum bracteatum*, *Gomphrena globosa*, *Vinca rosea* and *Zinnia elegans*.

Species not infected

Centrosema pubescens, *Chenopodium bonus-henricus*, *C. rubrum*, *Crotalaria usura-moensis*, *Cucumis sativus*, *Datura stramonium*, *Desmodium discolor*, *D. lasiocarpum*, *Glycine max* cvs *Grandifolia*, *Gracilis* and *Communis*, *Lycopersicon esculentum* cv. *Moneymaker*, *Medicago sativa*, *Melilotus alba*, *Nicotiana clevelandii*, *N. glutinosa*, *N. tabacum* cvs *Samsun* and *Xanthi-nc*, *Passiflora incarnata*, *P. mollissima*, *Phaseolus lunatus* cv. *Pole runner*, *P. mungo*, *P. vulgaris* cvs *Mangetout* *Princesse*, *Immuna*, *Pinto*, *Prelude*, *Processor*, *Scotia* and *Wagenaars dubbele zonder draad*, *Physalis alkekengi*, *P. floridana*, *P. peruviana*, *Pisum sativum* cvs *Zuckererbse* and *Saveur fin*, *Pueraria phaseloides*, *Solanum dulcamara*, *S. nigrum*, *Sonchus oleraceus*, *Tephrosia candida*, *Tetragonia expansa*, *Vicia faba*, *V. sepium*, *Vigna cylindrica*, *V. sinensis* cvs *Ramshorn*, *Blackeye*, *Beltsville* and *Whippoorville*.

Means of spread

Aphids. The isolate was transmitted after brief acquisition feeding periods by the two aphid species tested. Using the formula of Swenson (1967) it was calculated that 3-5% of the aphids transmitted.

Seed. Two months after germination, the isolate was detected in none of 100 seedlings of *P. edulis* and none of 300 seedlings of *P. foetida*, grown from seed collected from systemically infected plants.

Properties in vitro

The dilution end-point of the virus was found to be 10^{-6} - 10^{-7} , but the greatest change in infectivity occurred at 10^{-3} - 10^{-4} . The thermal inactivation point (10 min heating) was 65-70 °C and at c. 24 °C the longevity *in vitro* was 12-14 days.

Purification

Fresh leaves of *P. edulis* and *Adenia lobata* were homogenized in a Waring Blendor with twice the amount (w/v) of 0.5 M potassium phosphate buffer, pH 7.4, containing 1% 2-mercaptoethanol and 1 M urea. Urea was used to break up viral aggregates and to solubilize virus particles (Damirdagh & Shepherd, 1970). The sap was strained through several layers of aseptic gauze, chilled in an ice bath and clarified by low-speed centrifugation (16300 g for 10 min). To dissolve chloroplast fragments, Triton-X-100 detergent was added dropwise with stirring to a concentration of 5% by volume (van Oosten, 1972). After stirring for 30 min the preparation was centrifuged at 105000 g for 150 min. In a second cycle of differential centrifugation, the virus was sedimented through a 2 cm deep layer of 20% sucrose in the SW 25.1 rotor (83000 g for 150 min). The pellets were resuspended in 0.025 M potassium phosphate buffer, pH 7.4, containing 0.1% 2-mercaptoethanol and 1 M urea. The virus was further purified by sucrose density gradient centrifugation. Opalescent virus bands were found at 20-24 mm beneath the meniscus. Virus yields, calculated

on the basis of the virus collected from the gradients and using the extinction coefficient $OD_{260}^{1\text{cm}}$ for the morphologically similar tobacco etch virus of 2.4 for 1 mg/ml (Purcifull, 1966), were between 5 and 10 mg of virus/kg leaf for *P. edulis* and between 12 and 42 mg of virus/kg leaf for *A. lobata*. Virus was collected, diluted with distilled water and concentrated by ultracentrifugation (105 000 g for 150 min). The resulting pellets were resuspended in 0.05 M borate buffer, pH 8. These virus preparations were characterized by the following u.v. absorption spectrum: maximum at 260 nm, minimum at 247 nm, max/min ratio of 1.13–1.18, on a 260/280 ratio of 1.22–1.25 and an A 260/230 ratio of 0.28–0.34. The A 260/280 ratio corresponds to a nucleoprotein containing 5.5–6.5% RNA (Layne, 1957).

Aggregation occurs, as shown by the pelleting of the virus through the sucrose solution, but the amount of sedimented virus was less when citrate buffer was used as solvent for the gradient than when the urea-containing suspension buffer was used.

Crude sap could also be clarified by adding *n*-butanol to make 10% of the final volume. The virus could then be concentrated and further purified by two cycles of differential centrifugation or by precipitation with 10% polyethyleneglycol 6000 during one hour at 4 °C (Albrechtova & Klir, 1970), followed by centrifugation in the SW 25.1 rotor as described. In the sucrose gradient the virus was present at the same level as before but yields were considerably smaller.

Electron microscopy

Flexuous rods were found in infective virus preparations, purified by the Triton-X-100 procedure (Plate, fig. 7). Of 232 particles measured from a freshly prepared virus suspension, purified from *A. lobata*, 189 (80%) were 15 × 750–950 nm with a modal length of 810–830 nm. A virus suspension purified from *P. edulis* and stored for two months at 4 °C contained more broken particles: of 303 particles measured, 159 (52%) were 15 × 650–850 nm and their modal length was 740–760 nm. Virus particles were not aggregated.

Serology

The antiserum, after absorption with the clarified sap of healthy plants, reacted to a dilution of 1/4096 with clarified sap of *P. edulis*, infected with the Ivory Coast isolate, and to a dilution of 1/256 with *P. edulis* sap containing PWV-Q mild strain. Neither of the two passionfruit viruses reacted with an antiserum against passiflora latent virus.

DISCUSSION

P. foetida is a good diagnostic species, *P. edulis* a good host for maintaining virus cultures and *A. lobata* a good source of virus for purification. *A. lobata* seems also to be the most important natural source of the described isolate, which I call passionfruit ringspot virus (PRV).

Of the four elongated viruses reported to infect *Passiflora* species (Martini, 1962; Schnepf & Brandes, 1961; Brandes & Wetter, 1963; Del Rosario *et al.* 1964; Taylor & Kimble, 1964; Teakle & Wildermuth, 1967) only two, passiflora latent virus and PWV-Q are sufficiently well studied to allow a valid comparison with PRV. PRV can

easily be distinguished from passiflora latent virus on the basis of particle length and host range. Attempts to transmit passiflora latent virus by aphids, *Myzus persicae*, failed. PRV has a modal length and a host range very similar to PWV-Q: they infect most members of the Passifloraceae, many species of the Leguminosae and Chenopodiaceae and a few species in other plant families, such as the Solanaceae and Cucurbitaceae. Moreover, they are serologically related, though not closely. However, their symptomatology on *P. edulis*, *P. foetida* and *P. quadrangularis* is quite different, and it seems best to delay a decision on whether they should be considered strains of the same virus until more detailed tests for serological relationship of PRV to bean common and bean yellow mosaic viruses have been made, as they have for PWV-Q (Taylor & Kimble, 1964; Teakle & Wildermuth, 1967), and the extent of biological differences in host range, symptomatology, etc. (Bos, 1970) assessed more fully.

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EXPLANATION OF PLATE

- Fig. 1. Naturally infected shoot of *P. edulis* with leaf malformation (left) and mild symptoms (right).
- Fig. 2. Mosaic on old leaf of experimentally infected *P. foetida*.
- Fig. 3. Dotting on old leaf of experimentally infected *P. foetida*.
- Fig. 4. Naturally infected leaf of *P. edulis* with leaf malformation and mosaic.
- Fig. 5. Mottling and ringspotting on young leaf of experimentally infected *P. edulis*.
- Fig. 6. Spotting on old leaf of experimentally infected *P. edulis*.
- Fig. 7. Electron micrograph of a purified virus preparation, stained with uranyl formate. Bar represents 180 nm.

