Neth. J. Pl. Path. 81 (1975) 152–154 SHORT COMMUNICATION

Passionfruit ringspot virus isolated from Adenia lobata in Ivory Coast

J. J. DE WIJS and J. D. MOBACH

Office de la Recherche Scientifique et Technique Outre Mer (ORSTOM), Abidjan, Côte d'Ivoire

Accepted 22 April 1975

During a study on passionfruit viruses in Ivory Coast, a survey for wild Passifloraceae in the neighbourhood of the ORSTOM, revealed that *Adenia lobata* was very often present in het forest border. Plants showing mosaic symptoms in their leaves were first found in 1970. Check inoculations on *Passiflora edulis* var. *flavicarpa* and *P. foetida* caused the same symptoms in these test plants as passionfruit ringspot virus (PRV) did (De Wijs, 1974). *A. lobata* plants with less obvious symptoms and even without symptoms gave the same reaction. One plant was chosen for further indentification of the virus involved. From the beginning of the study PRV was used for comparison with the *Adenia* virus (AV).

The preparation of inoculum, determination of the in vitro properties, aphid transmission experiments, purification of the AV from *A. lobata*, length measurements of the virus particles and serological testing of the crude juice of *P. edulis* were done as described earlier (De Wijs, 1974). Purified virus preparations were examined in an electron microscope after contrasting with neutral 2% (w/v) phosphotungstate. For antigen from *A. lobata* the crude sap had to be clarified more thoroughly to avoid aspecific reactions with the available antiserum (homologous titre 4096) against PRV. Crude sap of *A. lobata* had to be emulsified with an equal volume of chloroform for 30 min and clarified by low-speed centrifugation, 10 min. at 12,000 *g*, in the SS 34 rotor of a Sorvall RC2B refrigerated centrifuge. The supernatant was deep frozen at −18 °C for 18 h and once more clarified by low-speed centrifugation after thawing to get rid of fraction 1 protein (Van Regenmortel, 1964). Antigen was then concentrated by high-speed centrifugation in a Spinco L50 preparative centrifuge: 150 min at 54,000 g in the R 30 rotor. The pellets were resuspended in a 0.9% NaCl solution and clarified by low-speed centrifugation.

Host range and symptoms. Different lots of test plants were simultaneously inoculated with PRV or AV. Most test plants reacted similarly on inoculation with each of the isolates. Seven species of the Passifloraceae, sixteen species of the Leguminosae and 12 species of nine other families were retested. Five species and one cultivar reacted in a different way to PRV as found earlier: No PRV could be recovered this time from *Chenopodium album*, *Dolichos lablab*, *Sesbania sesban* and *Phaselus vulgaris* 'Widusa'. However *D. lablab* and *S. sesban* could get infected locally by AV. *Indigofera hirsuta* and *Phaseolus calcaratus* were only infected locally and not systemically. Not infected by AV, although by PRV, was *Canavallia ensiformis*. Additionally tested were the

following twelve species and seven cultivars. *Passiflora warmingii* reacted with systemic symptoms, *P. gracilis* and *Lathyrus odoratus* showed no symptoms although systemically infected. The inoculated leaves of *Cucurbita pepo* 'Nicaise' and 'Medullosa' were infected but showed no symptoms. Not infected by neither of the two isolates were: *Aster grandifloris*, *Citrullus vulgaris*, *Cucumis melo* 'Ananas d'Amérique', *Cucurbita pepo* 'Blanche non coureuse', 'Citrouille de Touraine', 'Jaune gros de Paris', *Lupinus* sp., *Petunia* sp., *Phaseolus atropurpureus*, *P. vulgaris* 'Cordine' and 'Internor', *Taraxacum officinale* and *Theobroma cacao*.

In all cases where different reactions of the host plants were found on either AV and PRV inoculation or between the PRV inoculated plants of this study and the earlier work on PRV by De Wijs (1974), locally reacting plants were involved, from which the virus could only be recovered with difficulty, suggesting that these plants are poor hosts for the virus isolates. The differences in reaction might therefore depend more on the condition of the host than on its susceptibility for the virus isolates.

Properties in vitro. These were determined for AV in sap of *A. lobata* and for PRV in sap of *P. edulis*. The dilution end points were found to be 10^7–10^8 for PRV and 10^5–10^6 for AV but the greatest change in infectivity occurred at 10^5–10^6 resp. 10^4–10^5. The thermal inactivation (10 min heating) was 65–70 °C for the two isolates and the longivity in vitro 12–14 days for PRV and 38–40 days for AV, the two at 24 °C. The differences in dilution end point and longivity in vitro reflect rather the difference in host plant involved than differences between the two isolates.

Aphid transmission. AV was transmitted after brief acquisition feeding periods by *Aphis spiraecola*. The transmission rate of a single aphid was 6%, which is in good agreement with the transmission rate of 3–5% found for PRV.

Purification and electron microscopy. Flexuous rods were found in purified infective AV preparations. Of 832 particles measured, 347 (41%) were 15 × 650–800 nm with a modal length of 775–800 nm. PRV, purified from *A. lobata*, had a modal length of 810–830 nm (De Wijs, 1974).

Serology. Saturated PRV antiserum reacted to a dilution of 1/4096 with clarified sap of *P. edulis* diseased with PRV, and to the same dilution with clarified sap of *A. lobata* diseased with AV.

No major differences were found in host range, aphid transmission, particle form and length and serology between the two isolates. The virus isolated from *A. lobata* is therefore considered as being passionfruit ringspot virus (PRV) (De Wijs, 1974).

Samenvatting

'Passionfruit ringspot virus' geïsoleerd uit Adenia lobata in Ivoorkust

Een virus, geïsoleerd uit *Adenia lobata*, een wilde Passifloracee die zeer algemeen voorkomt in bosranden in de omgeving van het ORSTOM, bleek identiek te zijn aan het eerder beschreven 'passionfruit ringspot virus', een lid van de potyvirusgroep.

References

Regenmortel, M. H. V. van, 1964. Separation of an antigenic plant protein from preparations of plant viruses. Phytopathology 54: 282–289.
Wijs, J. J. de, 1974. A virus causing ringspot of *Passiflora edulis* isolated in Ivory Coast. Ann. appl. Biol. 77: 33–40.

Addresses

J. J. de Wijs: Ciba-Geigy Ltd., Agrochemicals Division, R-1207, 4002 Basle, Switzerland.
J. D. Mobach: Laboratorium voor Virologie, Binnenhaven 11, Wageningen, the Netherlands.

Neth. J. Pl. Path. 81 (1975) 154–156 SHORT COMMUNICATION

A device for the incubation of Fusarium-inoculated tulip bulbs in a constant air stream

B. H. H. BERGMAN

Bulb Research Centre, Lisse

Accepted 13 May 1975

Inoculation experiments in tulip bulbs with *Fusarium oxysporum* Schlecht f. sp. *tulipae* Apt gave frequently inconsistent results when performed in stagnant air, even under strictly standardized conditions. This variability was thought to be due to the accumulation of ethylene produced in large quantities by the inoculum and by infected bulbs (Kamerbeek and de Munk, 1968; de Munk, 1972). Ethylene influences the metabolic processes in tulips, e.g. demonstrated by the induction of gum formation (Kamerbeek et al., 1971) and by the prevention of the de novo synthesis of tuliposid A in the outer scale after lifting (Beijersbergen and Bergman, 1973). The last phenomenon is of special interest, since on the presence of this compound the concept is based of a defence mechanism of the bulb against infection by *F. oxysporum* (Bergman and Beijersbergen, 1971).

The fungus grows well under anaerobic conditions, but was proved to produce hardly any ethylene (Kamerbeek and Swart, in prep.). Preliminary experiments showed that incubation of inoculated bulbs in an atmosphere without oxygen resulted in a considerable reduction of infections, while addition of ethylene had a reverse effect. Since bulbs and inoculum consume oxygen, it was probable that fluctuations in oxygen pressure may add to the inconsistency of results of experiments in stagnant air.

It was therefore felt necessary to avoid these disturbing influences in inoculation experiments meant to judge variations in susceptibility of bulbs during the growth period and storage, or to compare the susceptibility of various cultivars. For this reason a device was made and tested (Fig. 1) to incubate bulbs in a reproducably constant and