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A MOSAIC DISEASE OF CENTROSEMA PUBESCENS BENTH. CAUSED BY PASSIONFRUIT WOODINESS VIRUS

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SUMMARY

A mosaic disease of centro (*Centrosema pubescens*) in Queensland was shown by physical, morphological and serological comparisons of the causal virus to be due to infection by a strain of passionfruit woodiness virus (PWV). This strain produced an almost latent infection of passionfruit (*Passiflora edulis*) and although it had a similar leguminous host range to PWV, symptoms were sometimes distinguishable. Naturally infected centro and *Passiflora suberosa*, growing in close association, each carried a characteristic and easily distinguishable strain of PWV.

I. INTRODUCTION

A virus disease of centro (*Centrosema pubescens* Benth.) from Papua-New Guinea was described by van Velsen and Crowley (1961, 1962). This virus caused natural infection of a number of tropical legumes, including several *Crotalaria* species, *Calopogonium mucunoides* Desv. and *Desmodium distortum* (Aubl.) Macbride.

Taylor and Kimble (1964) suggested that passionfruit woodiness virus (PWV) could possibly cause natural infection of subtropical legumes. Teakle and Wildermuth (1967) showed that *Crotalaria usaramoensis* Bak. and centro are hosts of PWV.

Several records of mosaic diseases of centro have been made in Queensland. G. M. Behncken (personal communication) collected a virus which he showed to be aphid transmissible and to have a dilution end-point of 10^{-4} and a thermal inactivation temperature between 55 and 60°C. He also found that this isolate produced symptoms on eight leguminous hosts. J. H. Simmonds in 1967, from a different location, collected a virus on centro showing similar symptoms. Mosaic-infected material collected from this latter area provided the isolate used in most of the present work.

II. MATERIALS AND METHODS

Isolate (A) was one of several isolates collected in a small area near Brisbane where much of the centro showed mosaic symptoms. Another centro isolate, obtained from material submitted from North Queensland by W. Pont (Department of Primary Industries, Cairns) was also tested.

Isolates were first transferred by mechanical inoculation from field material to bean (*Phaseolus vulgaris* L.), phasey bean (*Phaseolus lathyroides* L.) and centro. In all mechanical inoculations leaf material was ground with 0·1M, pH 7 phosphate buffer to which 0·1% sodium sulphite had been added. Inoculum was rubbed by finger onto carborundum-dusted leaves and then washed off with tap water.

Partial purification of isolate (A) from inoculated bean primary leaves was accomplished by a method similar to that described by Taylor and Kimble (1964). Preparations were examined in a Siemens Elmiskop 1A electron microscope. Tobacco mosaic virus (TMV) for particle length comparisons was included in the preparations before negative staining with potassium phosphotungstate.

An antiserum was prepared by three intramuscular injections of this partially purified virus, each 1 ml with 1 ml of Freund's adjuvant, into a rabbit which was bled from the ear 1 week after the last injection. Drop precipitin serology (van Slogteren 1954) was carried out in 9 cm plastic petri dishes using this antiserum and a PWV antiserum.

Cross-protection experiments with a tip blight strain of PWV and aphid transmission tests were carried out using methods similar to those previously described (Greber, 1966). Three South Queensland centro isolates and the North Queensland centro isolate were used in conjunction with a South Queensland tip blight PWV isolate from *Passiflora suberosa* L. in cross-protection tests. Systemic penetration by the protecting virus had to be confirmed by indexing to phasey bean when the "terminal reaction" (McKnight 1953) was absent. Ten *Myzus persicae* (Sulz.) were transferred to each plant in aphid transmission tests using isolate (A).

III. RESULTS

All isolates produced similar symptoms in centro (Figure 1). The leaves formed just after inoculation showed a chlorotic mottle which later gave way to distortion and mosaic. Symptoms appeared to be more severe under lower light levels than in full sunlight.

Host reactions on various legumes using isolate (A) were found to resemble those of PWV, and all isolates were then inoculated to passionfruit (*Passiflora edulis* Sims) and *P. subpeltata* Ortega. The resulting infection in *P. edulis* produced very mild symptoms and sometimes was apparently symptomless. However, all isolates could be recovered readily by inoculation back to phasey bean.

On *P. subpeltata,* the symptoms (Figure 2) were more prominent, with the dark green areas characteristically associated with the leaf veins. The virus could be consistently reisolated to produce the typical mosaic disease when mechanically inoculated back to centro.

When a severe tip blight strain of PWV (Greber 1966) was grafted to passionfruit carrying the centro virus, no severe disease resulted and the latter virus evidenly protected well against the severe strain.

The virus was transmitted by *M. persicae* from infected *P. subpeltata* leaves to healthy seedlings of the same species. All five of the test plants were infected.

In microprecipitin drop tests, partially purified isolate (A) reacted well with a PWV antiserum up to a dilution of 1/64. This isolate also reacted well with its homologous antiserum at a 1/128 dilution.

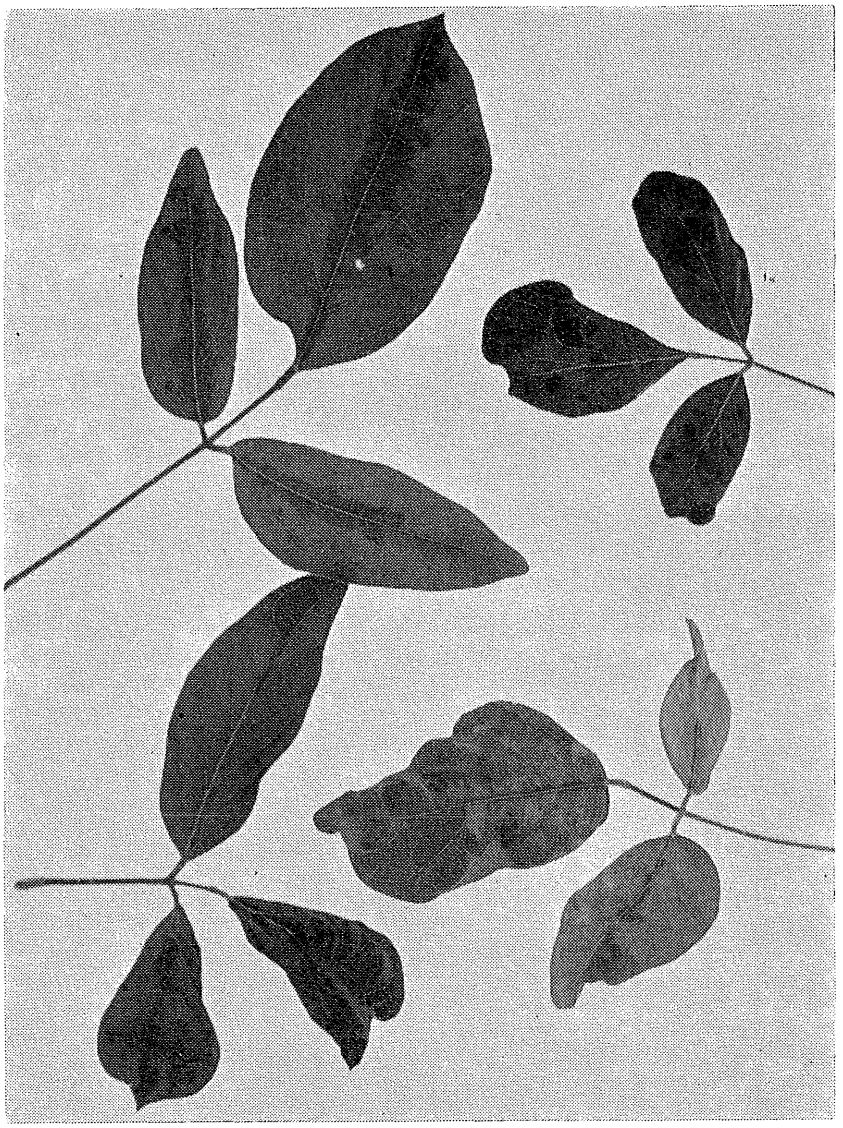

Fig. 1.—Symptoms on leaves of *Centrosema pubescens* caused by natural infection with a strain of passionfruit woodiness virus.

When partially purified isolate (A) was mixed with an equal volume of 2% potassium phosphotungstate and examined on electron microscope grids, numerous flexuous rod particles characteristic of the PVY group (Brandes 1964) were seen. By comparison with the normal length of TMV particles at 300 nm, it was calculated that the most frequent length of 50 measured centro isolate particles in preparations obtained from bean leaves, was approximately 720 nm.

Examination of the locality from which isolate (A) was obtained showed that the centro vines were extensively intertwined with *Passiflora suberosa.* Several isolations from the latter, however, yielded only the severe tip blight

strain of PWV which commonly infects this species (Greber 1966). When the PWV isolates from these *P. suberosa* vines were inoculated to centro seedlings, necrotic lesions were produced on inoculated leaves but no apparent systemic symptoms resulted. No plants of *P. subpeltata* were found in the area, but a few vines of *P. edulis* showing typical mottle PWV symptoms were in the vicinity.

The centro virus isolate (A) in phasey bean sap survived a 10 min exposure to 55°C but not 60°C, when inoculated back to phasey bean.

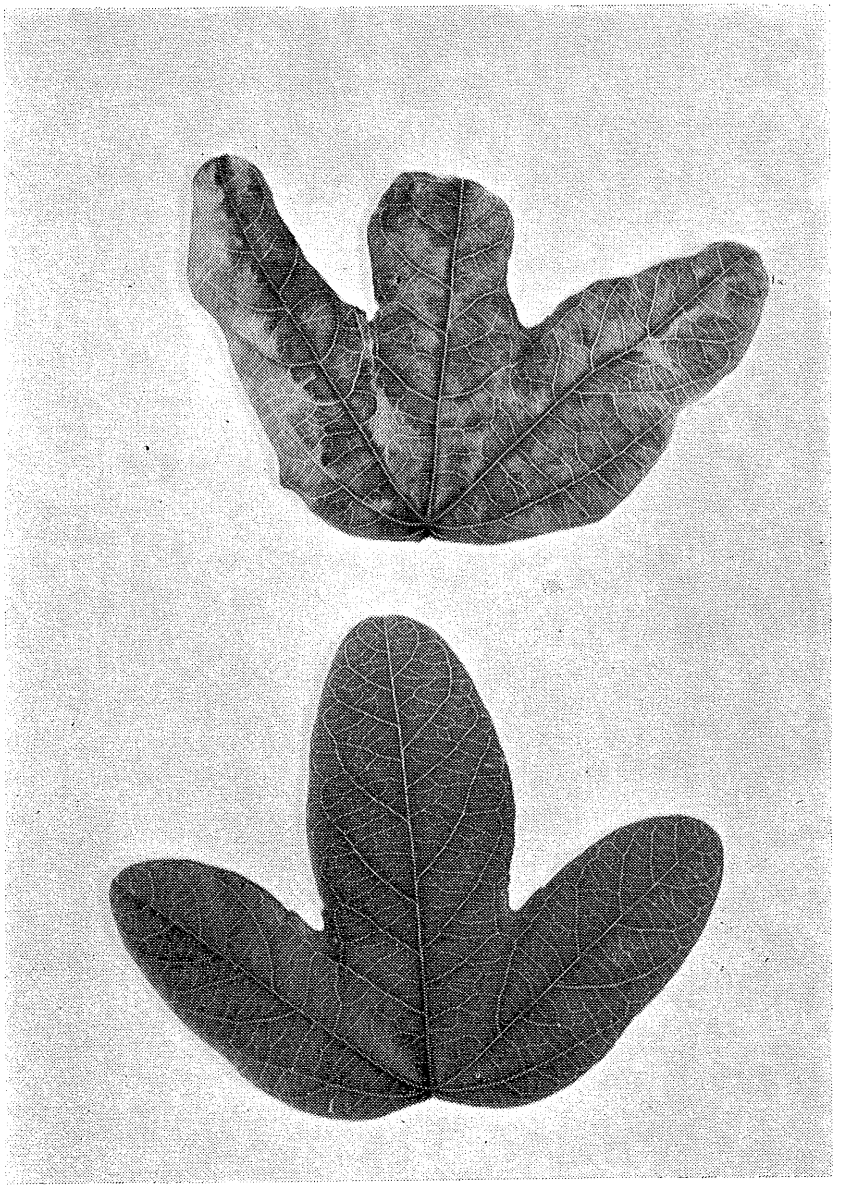

Fig. 2.—Leaf of *Passiflora subpeltata* infected with an isolate of passionfruit woodiness virus from *Centrosema pubescens,* compared to a healthy leaf.

IV. DISCUSSION

The infection of species of *Passiflora* and other host range similarities, together with the serological relationship and similarity in particle morphology and physical properties, indicate that this virus infecting centro in Queensland is a strain of PWV. Symptomological differences, however, exist between these centro isolates and other PWV isolates obtained from species of *Passiflora.* Since PWV occurs in many different strains (McKnight 1953; Simmonds 1959; Greber 1966) it seems reasonable to suppose that the centro isolates are host selected variants of this virus.

McKnight (1953) showed that the "terminal reaction" of PWV in passion-fruit was associated with a severe, mottle-producing disease, while a milder "fern leaf" disease did not produce the terminal reaction. These observations can be correlated with subsequent experiments reported here and elsewhere (Greber 1966), where the centro isolates produced an almost latent infection in passion-fruit while the very severe isolates associated with *P. suberosa* caused a severe mottle and highly necrotic terminal reaction. Both these types of isolates were shown to occur naturally in close association, but appear to be confined to their ecologically preferred hosts.

V. ACKNOWLEDGEMENTS

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