

## HOST RANGE AND PARTICLE LENGTH OF PASSIONFRUIT WOODINESS VIRUS

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### SUMMARY

Passionfruit woodiness virus (PWV) was found to have a wider natural and experimental host range than previously reported, particularly in the *Leguminosae*. Additional species in the *Passifloraceae* and certain members of the *Amaranthaceae*, *Chenopodiaceae*, *Cucurbitaceae* and *Solanaceae* were also infected. The symptoms induced in the newly-discovered susceptible plants usually resembled those induced by bean common mosaic virus or bean yellow mosaic virus. However, neither bean common mosaic virus nor bean yellow mosaic virus infected species of *Passiflora*.

The normal particle length of PWV was determined to be 730–745m $\mu$ , close to the 750 m $\mu$  reported for bean common mosaic virus and bean yellow mosaic virus. On the basis of particle length and other properties, it is concluded that PWV should be placed with bean common mosaic virus and bean yellow mosaic virus in Brandes's "Potato virus Y—group".

### I. INTRODUCTION

Passionfruit woodiness virus (PWV), which is common in Queensland, was recently characterized by Taylor and Kimble (1964). These workers found that in French bean (*Phaseolus vulgaris* L.), PWV caused symptoms resembling those of bean common mosaic virus (BCMV) or bean yellow mosaic virus (BYMV), depending on the variety of bean infected. Serological tests showed, however, no relationship between PWV and BCMV or BYMV. Further, PWV was found to have flexible, rod-shaped particles, with a normal length of 670 m $\mu$ , i.e. 80m $\mu$  shorter than those of BCMV or BYMV (Brandes 1964). Because of the differences in biological and physical properties from these and other viruses, Taylor and Kimble (1964) considered PWV to be a previously undescribed virus.

The present paper reports on further investigations into the characteristics of PWV. In general, the results agree closely with those obtained by Taylor and Kimble (1964), except that the particle length of PWV was found to be greater, approximating that of BYMV and BCMV, and the experimental host range of PWV was wider; also approximating that of BYMV and BCMV. In addition, three natural leguminous hosts of PWV were discovered.

## II. MATERIALS AND METHODS

*Virus sources.*—The isolate of PWV used in most tests was a “severe” isolate derived by graft propagation from that used by Simmonds (1959), as was one of those used by Taylor and Kimble (1964). The virus was isolated for the work reported in the present paper by rubbing the sap of infected passion vine leaves onto French bean leaves. Another isolate was obtained by R. S. Greber, Queensland Department of Primary Industries, from naturally infected soybean (*Glycine max* (L.) Merr.). It differed from the other isolate in causing a more severe mosaic disease of soybean (Figure 1).

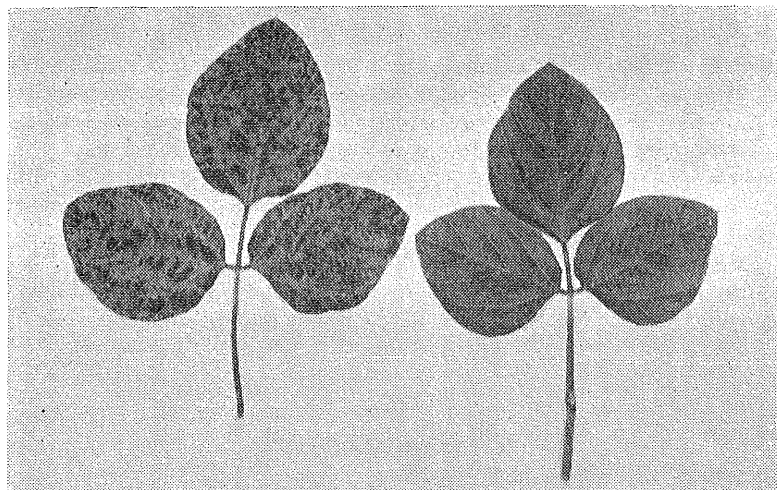


Fig. 1.—Soybean with yellow mosaic caused by passionfruit woodiness virus (left). Healthy leaf on right.

The BCMV was obtained from a French bean plant grown from infected seed by J. C. Johnson, Queensland Department of Primary Industries. Another isolate, which caused similar symptoms so far as it was tested, was obtained from infected French bean plants in the field.

The BYMV was obtained from a plant of *Medicago polymorpha* L. Another isolate, BYMV-Pea, which caused somewhat different symptoms in a range of hosts, was isolated by R. S. Greber from pea (*Pisum sativum* L.).

All the viruses were maintained in French bean var. Bountiful.

*Host-range.*—All tests were done at temperatures of 60°-80°F in a glasshouse provided with evaporative cooling. In transfers, infectious sap was diluted approximately fivefold with 1%  $K_2HPO_4$  and was brushed onto carborundum-dusted leaves. The inoculated leaves of plants were assayed after 7-14 days and the uninoculated leaves after 14-21 days, using French bean, phasey bean (*Phaseolus lathyroides* L.) or *Dolichos biflorus* L. as assay plants. Host range tests were done at least three times.

*Electron microscopy.*—PWV preparations were examined in a Siemens Elmiskop 1 electron microscope. Using a leaf dip method, a heavily infected primary leaf of Bountiful bean was cut, the cut edge dipped momentarily in a drop of 1% neutral phosphotungstic acid on a cellulose nitrate-coated grid, and the drop partially blotted, and air-dried. Only occasional flexible, rod-shaped particles were observed in using this method. However, numerous particles were observed in preparations of PWV partially purified from bean leaves by the butanol-centrifugation method (Taylor and Kimble 1964). These preparations were prepared for electron microscopy by two methods. In the first, tobacco mosaic virus in clarified tobacco sap was added to the virus suspension, and an equal volume of 1% neutral phosphotungstic acid was added before atomizing onto the grids. In the second, polystyrene latex particles (264 m $\mu$  diameter) were added to the virus suspension, which was atomized onto the grids and then shadowed with platinum-paladium.

### III. RESULTS

#### (a) Host Range Investigations

In glasshouse tests, 16 new leguminous hosts of PWV and 9 new non-leguminous hosts of PWV were found. The names and authorities of the hosts and the symptoms induced are given in Table 1. There was a relatively close coincidence in the host ranges of PWV, BYMV and BCMV, and in the symptoms induced. The similarity was closer between PWV and BYMV than between PWV and BCMV. PWV was, however, readily distinguished from BYMV and BCMV by its ability to infect *Passiflora edulis*, *P. seemannii* and *P. subpeltata* (*P. alba*).

New hosts of diagnostic value were phasey bean and *Dolichos biflorus*. Especially distinctive symptoms were the tip blight reaction of young phasey bean seedlings infected with PWV (Figure 2), the necrotic local lesions caused by PWV and BYMV on *D. biflorus* (Figure 3), and the rapid stem necrosis and death of *D. biflorus* seedlings infected with BCMV. The yellow vein banding caused by PWV on *Medicago scutellata* (Figure 4) resembled that caused by BYMV.

TABLE 1

HOST RANGE AND SYMPTOMS OF PASSIONFRUIT WOODINESS VIRUS (PWV), BEAN YELLOW MOSAIC VIRUS (BYMV)  
AND BEAN COMMON MOSAIC VIRUS (BCMV)

Asterisk \* indicates irregular recovery of virus

Host	Symptoms		
	PWV	BYMV	BCMV
<i>Amaranthaceae</i> — <i>Gomphrena globosa</i> L. . . .	Symptomless local infection.	As for PWV.	As for PWV.
<i>Chenopodiaceae</i> — <i>Chenopodium album</i> L. . . .	*Chlorotic local lesions on inoculated leaves.	*As for PWV, but becoming necrotic; tending to run along veins.	*As for PWV, tending to run along veins.
<i>C. amaranticolor</i> Coste & Reyn . . .	*Chlorotic local lesions on inoculated leaves. (Sometimes indistinct.)	*As for BYMV on <i>C. album</i> .	*As for BCMV on <i>C. album</i> .
<i>Cucurbitaceae</i> — <i>Cucumis sativus</i> L. (long green cucumber)	*Symptomless local infection.	Not tested.	*Symptomless local infection.
<i>Leguminosae</i> — <i>Arachis hypogaea</i> L. (peanut) . . .	Symptomless local infection.	Not tested.	Not tested.
<i>Calopogonium mucunoides</i> Desv. (calopo)	Systemic mottle; downwards curving of leaf margins.	As for PWV.	As for PWV.
<i>Centrosema pubescens</i> Benth. (centro)	Systemic mottle.	Not infected.	Not infected.
<i>Crotalaria usaramoensis</i> Bak. f., (a rattlepod)	Systemic yellow mosaic.	Not tested.	Not tested.
<i>Dolichos biflorus</i> L. (horse gram) . . .	Large reddish-brown local lesions in inoculated primary leaves; necrotic spots and vein necrosis developing in upper leaves; stem and petiole necrosis; plants dying prematurely.	As for PWV.	Vein necrosis, diffuse necrotic spots or rings in inoculated primary leaves; stem and petiole necrosis; rapid death of plants.
<i>D. lablab</i> L. (lablab bean) . . . .	Symptomless local infection.	Not tested.	As for PWV.
<i>Glycine javanica</i> L. . . . .	Symptomless local infection.	Not infected.	Chlorotic local lesions.

<i>G. max</i> (L.) Merr. (Nanda soybean)	Chlorotic or necrotic spots in inoculated primary leaves; vein chlorosis and mosaic developing in upper leaves. (PWV-Soybean produced a more prominent mosaic than the passionfruit isolate.)	As for PWV.	Indistinct chlorotic or necrotic spots in inoculated primary leaves; local infection only.
<i>Lathyrus odoratus</i> L. (sweetpea) ..	Symptomless systemic infection.	Stem necrosis and systemic leaf mottle; sometimes premature death.	Symptomless local infection.
<i>Medicago scutellata</i> Mill. (snail medic)	Systemic yellow vein banding.	As for PWV.	As for PWV, but less yellow.
<i>Phaseolus atropurpureus</i> DC. (siratro)	Symptomless systemic infection.	Not infected.	As for PWV.
<i>P. lathyroides</i> L. (phasey bean) ..	Chlorotic or necrotic local lesions in inoculated primary leaves; vein necrosis in upper leaves; leaf fall; bud blight.	Chlorotic local lesions in inoculated primary leaves, vein clearing and yellow mosaic.	Symptomless in inoculated primary leaves; vein clearing followed by mild and then blister mosaic of new growth.
<i>P. vulgaris</i> L. (French bean) ..	Chlorotic or necrotic local lesions in inoculated primary leaves, followed by vein necrosis; necrotic areas developing along veins of upper leaves; systemic mosaic developing in new growth.	Chlorotic local lesions in inoculated primary leaves; rugosity and downward cupping of leaf margins in upper leaves; necrosis of leaf veins petioles, and stems; yellow mosaic developing in new growth; sometimes premature death of plants. (BYMV-Pea less necrotic.)	Nil or faint chlorotic lesions in inoculated leaves; downward curving of leaf margins, leaf distortion and mild mottle. Sometimes new growth becoming symptomless.
<i>Pisum sativum</i> L. (Greenfeast or B27 pea)	Symptomless systemic infection.	Systemic mottle; stem necrosis and premature death. (BYMV-Pea systemic mottle only.)	Symptomless local infection.
<i>Trifolium subterraneum</i> L. (subterranean clover)	Systemic yellow vein banding.	As for PWV.	Not infected.
<i>Vicia faba</i> L. (broad bean) ..	Symptomless local infection.	Symptomless systemic infection (BYMV-Pea caused a systemic mottle.)	As for BYMV.
<i>Vigna sinensis</i> (Torner) Savi (Black-eye cowpea)	Not infected.	Not infected.	*Symptomless systemic infection.

TABLE 1—continued

Host	Symptoms		
	PWV	BYMV	BCMV
<i>V. vexillata</i> Benth. . . . .	Symptomless systemic infection.	Symptomless local infection.	Mild systemic downward curving of leaf margins.
<i>Passifloraceae</i> —			
<i>Passiflora edulis</i> Sims (passionfruit)	Vein clearing; leaf rugosity; leaf mottle.	Not infected.	Not infected.
<i>P. herbertiana</i> Lindl. . . . .	Symptomless systemic infection.	Not tested.	Not tested.
<i>P. seemanni</i> Griseb. . . . .	As for <i>P. edulis</i> .	Not infected.	Not infected.
<i>P. subpeltata</i> Ortega (white passion flower)	As for <i>P. edulis</i> .	Not infected.	Not infected.
<i>Solanaceae</i> —			
<i>Nicotiana clevelandii</i> Grey . . . . .	Symptomless systemic infection.	As for PWV.	As for PWV.
<i>N. tabacum</i> L. (Turkish or Xanthi tobacco)	*Symptomless local infection.	*As for PWV.	*As for PWV.
<i>Petunia hybrida</i> Vilm. (petunia) . . . . .	Symptomless systemic infection.	Not tested.	Not tested.

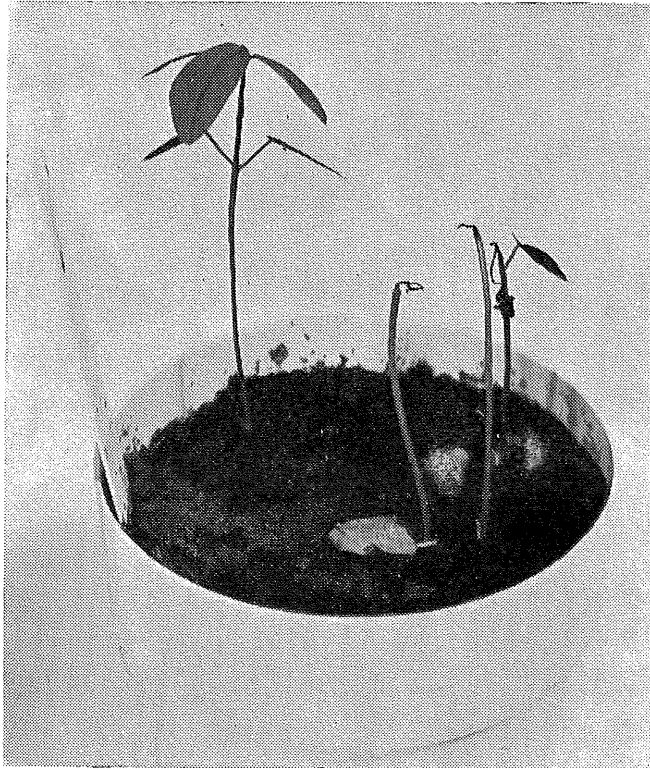


Fig. 2.—*Phaseolus lathyroides* with tip blight and leaf drop caused by passionfruit woodiness virus (right). Healthy plant on left.

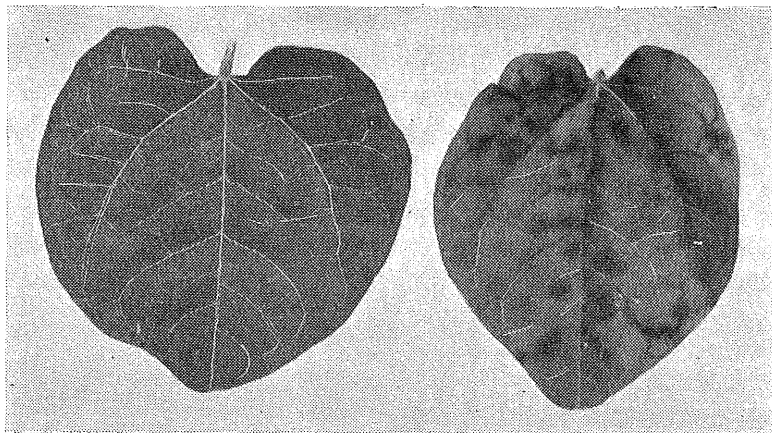


Fig. 3.—*Dolichos biflorus* with necrotic local lesions and vein necrosis caused by passionfruit woodiness virus (right). Healthy leaf on left.

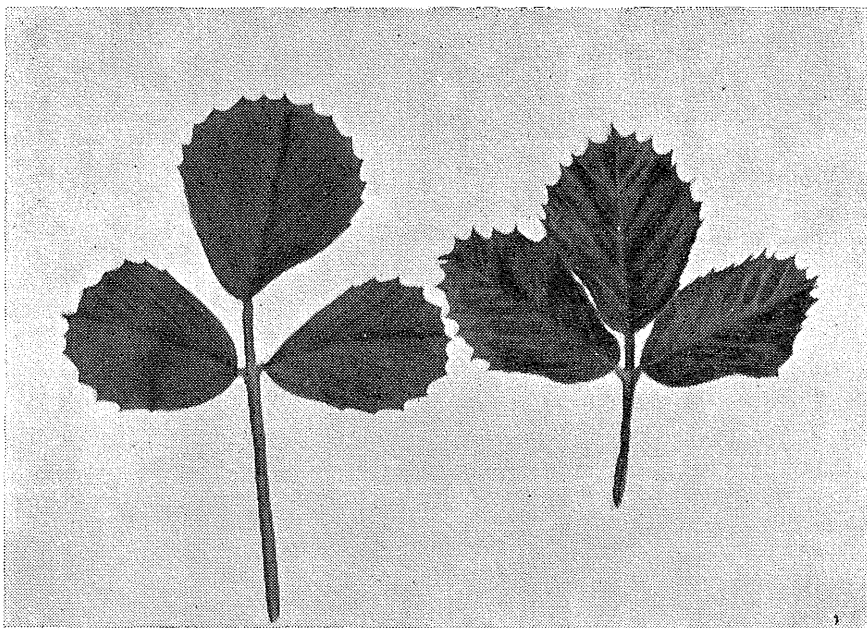


Fig. 4.—*Medicago scutellata* with yellow vein banding caused by passionfruit woodiness virus (right). Healthy leaf on left.

Plants from which none of the viruses was recovered were *Carica papaya* L. (papaw), *Datura stramonium* L., *Lycopersicon esculentum* Mill. (tomato), *Nicotiana glutinosa* L. and *Trifolium repens* L. (white clover).

In field surveys, three new hosts of PWV were found, viz. peanut, soybean and *Crotalaria usaramoensis*. The infected peanut and soybean plants were growing in quarantine plots at the Redlands Horticultural Research Station, near Brisbane. In peanut, the virus concentration was low and no symptoms were detected, whereas in soybean the virus concentration was higher and a prominent mottle was caused. The symptoms in soybean at Redlands bore some resemblance to those caused by soybean mosaic virus and the virus was transmitted by *Myzus persicae* Sulz. in a non-persistent manner (R. S. Greber unpublished data). It was also established that the virus caused typical PWV symptoms in passion seedlings and in other hosts, but that viruses of the soybean mosaic virus-type from soybean growing in other localities did not infect passion seedlings. Further, in a cross protection test it was shown that the soybean virus from Redlands resembled the severe strain of PWV in protecting passion vine seedlings from infection with the tip blight strain of PWV recently described by Greber (1966).

The infected *Crotalaria* plants were found in two localities in the Nambour district, about 75 miles north of Brisbane. The symptoms included a prominent yellow mosaic (Figure 5). Incidence in this weed species was low in 1965, but in one locality revisited in 1966 exceeded 50%.



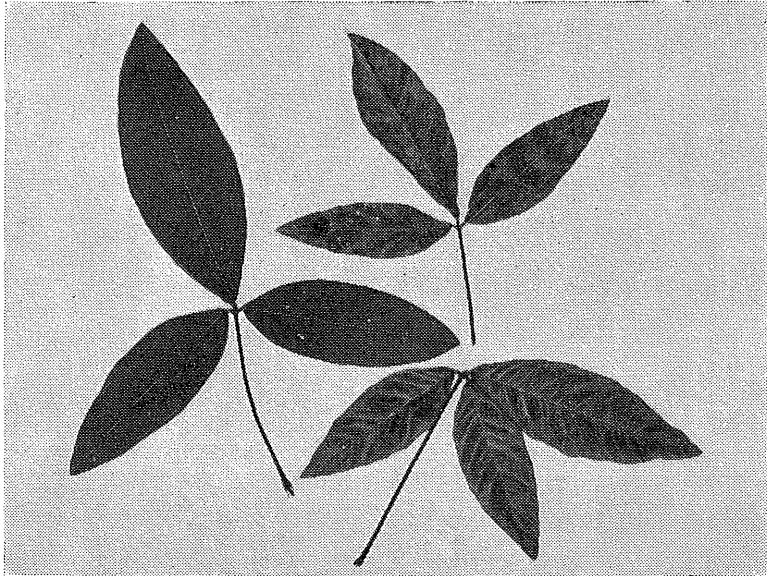


Fig. 5.—*Crotalaria usaramoensis* with yellow mosaic caused by passionfruit woodiness virus (natural infection). Healthy leaf on left.

In all three cases, the naturally infected legumes were growing adjacent to PWV-infected passion vines, which presumably were the original source of the virus.

#### (b) Electron Microscopy

In photographic plates of negatively stained preparations (Figure 6), 16 out of 20 (80%) tobacco mosaic virus (TMV) particles measured 13.0–13.8  $\mu$ m (mean 13.3  $\mu$ m). Assuming that the normal particle length of TMV is 300  $\mu$ m (Brandes 1964), it was calculated that 111 out of 195 (57%) PWV particles measured 630–810  $\mu$ m, with a normal length of 745  $\mu$ m (Figure 7). Allowing for variability both in the TMV and in the PWV, it was calculated that the standard deviation was 49.5  $\mu$ m.

In photographic plates of the shadowed preparations, all of 14 polystyrene latex particles (264  $\mu$ m diameter) measured 4.8–5.1  $\mu$ m diameter with a mean diameter of 4.9  $\mu$ m. Taking the latex particles as a standard, it was calculated that 23 out of 53 (43%) PWV particles measured 700–750  $\mu$ m, with a mean length of 730  $\mu$ m. In this case the standard deviation was calculated to be 48  $\mu$ m.

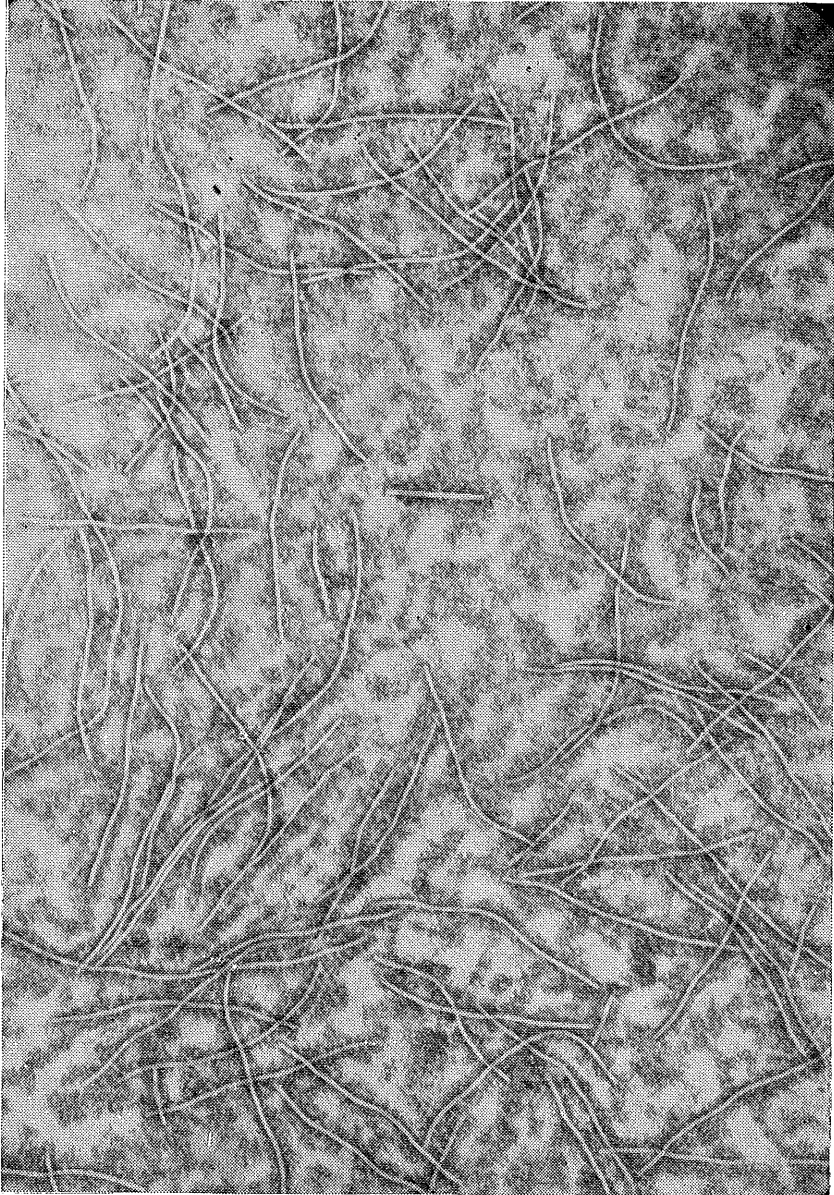


Fig. 6.—Purified preparation of passionfruit woodiness virus with a tobacco mosaic virus particle in centre. (Negatively stained with phosphotungstic acid. Approx. X 40,000).

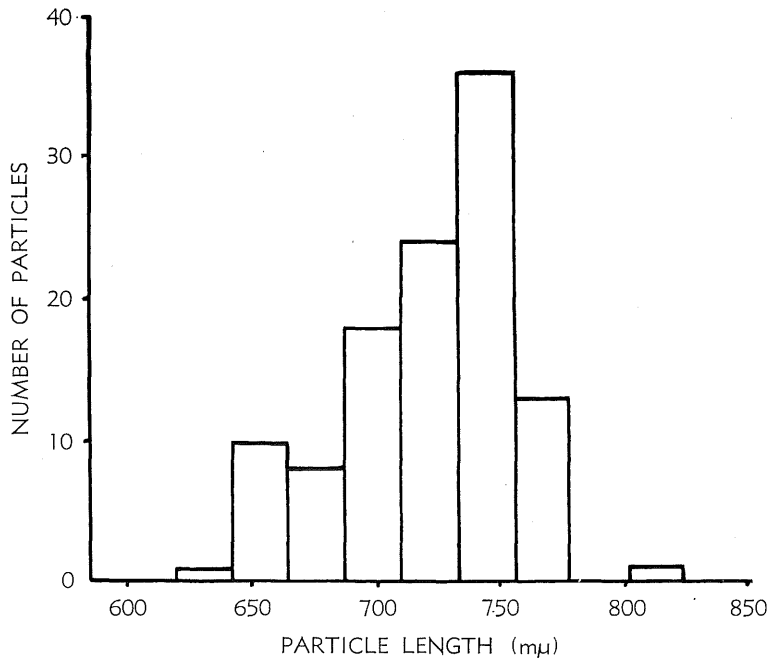


Fig. 7.—Histogram of particle length-frequency distribution of passionfruit woodiness virus in a negatively stained preparation when tobacco mosaic virus was used as the standard. Note that the normal particle length is approximately 745 m $\mu$ .

#### IV. DISCUSSION

For many years, the known host range of the Queensland PWV was restricted to the *Passifloraceae*. The eight species known to be susceptible were *Passiflora caerulea* L., *P. edulis* Sims, *P. foetida* L., *P. laurifolia* L., *P. maliformis* L., *P. quadrangularis* L., *P. suberosa* L. and *P. subpeltata* Ortega (Simmonds 1966). Two additional species of *Passiflora* found susceptible in the present investigation were *P. herbertiana* and *P. seemannii*. Recently Taylor and Kimble (1964), transmitted PWV to two species in the *Leguminosae*, viz. French bean and *Sesbania exaltata* Rof. Sixteen additional leguminous host species were found in the present investigation (Table 1). Further, PWV was transmitted to four other families, viz. *Amaranthaceae* (1 species), *Chenopodiaceae* (2 species), *Cucurbitaceae* (1 species) and *Solanaceae* (3 species).

Although all but one of the species listed in Table 1 were found to be susceptible to PWV, they were not all equally susceptible. For instance, cucumber and tobacco were infected only irregularly, when highly infectious inoculum was brushed over the leaves of young, vigorously-growing test plants. However, since recovery of viruses was achieved in at least three tests, they are considered to be susceptible. Cucumber and tobacco were not found susceptible by Taylor and Kimble (1964).

PWV caused local lesions in *Chenopodium album* in three out of three tests, and in *C. amaranticolor* in four out of six tests. Two tests with *C. quinoa* Willd. were negative. PWV was recovered from local lesions from *Chenopodium album* and *C. amaranticolor* only once each. Since these plants are known to contain a strong inhibitor of virus infection, and since the chlorotic local lesions did not resemble mere plant damage, these also are considered to be valid hosts. It is of interest that Hollings (1957) reported that *C. amaranticolor* reacted to BYMV with "Few, faint, chlorotic local lesions about  $\frac{1}{2}$  mm in diameter after about 2 weeks, later tending to become necrotic. An unreliable reaction, usually occurring only with inoculations from young infected tissues". The situation with PWV and *C. amaranticolor* appears to be similar.

Of the new hosts, only the two species of *Passiflora*, 12 of the 16 legumes, *Nicotiana clevelandii* and *Petunia hybrida* became systemically infected under the conditions of the tests and hence could be considered potential natural sources of infection. With some of these, it is possible that systemic infection followed heavy infection of the rubbed leaves. Whether systemic infection would result following aphid transmission has yet to be determined.

Taylor and Kimble (1964) suggested that in Queensland a reservoir of PWV might exist in a subtropical leguminous species and that it might be spread by aphids from this source to passion vines. However, although peanut, soybean and *Crotalaria usaramoensis* were found to be naturally infected with PWV in the present study, there was no evidence that they constituted a virus reservoir for infection of passion vines. Indeed, the reverse appeared to be the case. In each instance the infected leguminous plants were growing adjacent to infected passion vines, which were considered to be the source of PWV. Passion vines are a major source of PWV (McKnight 1953). Further, wild species of *Passiflora*, such as *P. subpeltata*, are often found infected with PWV, although distant from passion vines (McKnight 1953; Teakle, unpublished data), and probably serve as sources of infection. On the other hand, the high incidence of PWV in soybean in 1963 (R. S. Greber unpublished data) and in *Crotalaria usaramoensis* in 1966 suggested that some soybean to soybean and *Crotalaria* to *Crotalaria* spread was taking place. Thus, the suggestion of Taylor and Kimble of PWV spread from a legume to passion vines is feasible.

Brandes (1964) and Brandes and Bercks (1965) have found the normal particle lengths of rod-shaped viruses to be relatively constant, and have erected a number of virus groups based on normal particle length and other properties, such as serological relationships. If PWV particles have a normal length of 670 m $\mu$ , as found by Taylor and Kimble (1964), PWV would be placed in the "Potato virus S-group" (620-700m $\mu$ ). On the other hand, if PWV particles have a normal length of 730-745 m $\mu$ , as found in the present investigation, PWV would be placed with BCMV and BYMV in the "Potato virus Y-group" (730-790 m $\mu$ ).

Consideration of the properties of PWV, such as high particle flexibility, low thermal inactivation point and low to medium concentration, indicates a closer relationship of PWV with the "Potato virus Y-group" than with the "Potato virus S-group" (Table 2).

TABLE 2

COMPARISON OF SOME CHARACTERS OF PASSIONFRUIT WOODINESS VIRUS (PWV) WITH THOSE OF THE POTATO VIRUS S-GROUP AND OF THE POTATO VIRUS Y-GROUP

Character	Potato Virus S-group†	Potato Virus Y-group†	PWV
Normal particle length ..	620-700 m $\mu$	730-790 m $\mu$	730-745 m $\mu$ (1) (670 m $\mu$ *)
Particle flexibility .. ..	Rigid to slightly flexible	Flexible	Flexible (2)
Thermal inactivation point ..	Usually 60-70°C	Usually 50-60°C	55-60°C*
Concentration .. .. .	Medium to high	Low to medium	Low to medium (3)

† Data from Brandes (1964) and Brandes and Bercks (1965).

(1) Data from present study.

\* Data from Taylor and Kimble (1964). Thermal inactivation point confirmed by present authors (unpublished data).

(2) See Fig. 5.

(3) Few particles present in leaf dip preparations observed in the electron microscope.

Taylor and Kimble (1964) detected no precipitin reaction between PWV-antiserum and BCMV or BYMV. In similar tests, we detected no precipitin reaction at high dilutions of PWV-antiserum but the technique was not sufficiently refined to detect reaction at low dilutions (unpublished data). So far as they went, these tests supported the conclusion of Taylor and Kimble that PWV was an undescribed virus distinct from BCMV and BYMV.

The relationship of PWV with other viruses with rod-shaped particles described from species of *Passiflora* is uncertain, except that PWV would appear to be distinct from the passiflora latent virus which has a particle length of only 650 m $\mu$  (Brandes 1964). PWV appears to have somewhat similar properties to the virus causing chlorotic spot of *P. foetida* in New Guinea (van Velsen 1961), but it differs in infecting French bean and *P. edulis*. PWV differs from the virus infecting *P. foetida* in the Philippines (Rosario, Benigno, and Libed 1964) in its lower thermal inactivation point (55-60° v. 70-75°), in being transmitted by *Aphis gossypii* and in infecting French bean.

PWV differs from the virus studied by Martini (1962) in Nigeria in infecting French bean and in symptoms in *Chenopodium amaranticolor*.

Whether these differences reflect basic differences between the viruses or merely differences at the strain level or in technique is uncertain. It is unlikely that the problem will be elucidated until normal particle lengths and serological interrelationships are determined.

## V. ACKNOWLEDGEMENTS

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## REFERENCES

- BRANDES, J. (1964).—Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. *Mitt. Biol. BundAnst. Ld-u. Forstw.* 110.
- BRANDES, J., and BERCKS, R. (1965).—Gross morphology and serology as a basis for classification of elongated plant viruses. *Adv. Virus Res.* 11:1-24.
- GREBER, R. S. (1966).—Passion vine tip blight disease. *Qd J. Agric. Anim. Sci.* 23:533-8.
- HOLLINGS, M. (1957).—Reactions of some additional plant viruses on *Chenopodium amaranticolor*. *Pl. Path.* 6:133-5.
- MARTINI, C. K. H. (1962).—Some properties of the virus causing woodiness of passion fruit in Western Nigeria. *Ann. Appl. Biol.* 50:163-8.
- McKNIGHT, T. (1953).—The woodiness virus of the passion vine. *Qd J. Agric. Sci.* 10:4-35.
- ROSARIO, MARIA S. DEL, BENIGNO, D. A., and LIBED, L. P. (1964).—Virus diseases of weeds in the Philippines. I. *Passiflora foetida* Linn. *Philipp. Agric.* 48:95-112.
- SIMMONDS, J. H. (1959).—Mild strain protection as a means of reducing losses from the Queensland woodiness virus in the passion vine. *Qd J. Agric. Sci.* 16:371-80.
- SIMMONDS, J. H. (1966).—"Host Index of Plant Diseases in Queensland." (Dept. of Primary Industries: Brisbane).
- TAYLOR, R. H., and KIMBLE, K. A. (1964).—Two unrelated viruses which cause woodiness of passion fruit (*Passiflora edulis* Sims). *Aust. J. Agric. Res.* 15:560-70.
- VELSEN, R. J. VAN (1961).—Chlorotic spot, a virus disease of *Passiflora foetida* in New Guinea. *Papua New Guin. Agric. J.* 13:160-6.

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