MILD STRAIN PROTECTION AS A MEANS OF REDUCING LOSSES FROM THE QUEENSLAND WOODINESS VIRUS IN THE PASSION VINE.

and the state of the second

By J. H. SIMMONDS, M.Sc., Government Plant Pathologist,

Division of Plant Industry.

SUMMARY.

The need for a means of reducing loss from the woodiness disease of passion vine (Passiflora edulis Sims) is briefly stated and a practical attempt to achieve this object by making use of mild strain protection is described.

In replicated trials in their second and third years, vines carrying a mild strain of the woodiness virus showed a marked superiority over those carrying a severe strain in number, weight and size of fruit free from woody symptoms.

I. INTRODUCTION.

Diseases of one sort or another have always made passion vine growing in Queensland an uncertain venture. In the early days brown spot was serious; later, woodiness came into prominence, with Fusarium wilt important in more restricted localities. Satisfactory control measures have been devised for brown spot and wilt (Simmonds 1930; Purss 1958) but woodiness is still a limiting factor. Fig. 1 shows various stages of severity of the disorder.

Woodiness was first recorded in Queensland in August 1927. At that time the disease was of rare occurrence and vines could be expected to remain in production for eight years or more. By 1932 woodiness had become comparatively common and it was not long before the expected commercial life of a plantation had dropped to as low as two years.

II. THE WOODINESS VIRUS IN QUEENSLAND.

In 1928 R. J. Noble described a woodiness disease of the passion vine in New South Wales which he showed was due to a sap transmissible virus (Noble 1928). This disease was further described in 1938 (Noble and Noble 1938) and shown to be transmissible by four species of aphids. Later Magee (1948) stated the virus concerned to be cucumber virus 1.

Because of the similarity of symptoms it has been generally assumed that the disease in Queensland is the same as that described for New South Wales. However, McKnight (1953) was unable to produce any evidence that the virus causing the Queensland disease was cucumber mosaic.

The position has recently been clarified by Taylor (1959), who showed that two distinct viruses cause woody symptoms in passion fruit. The disease occurring in Victoria is caused by a strain of cucumber mosaic virus, while the Queensland disease is due to a distinct and so far unidentified virus. The

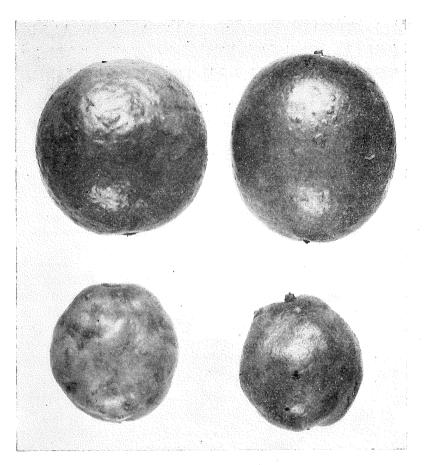


Fig. 1.

Passion Fruit Woodiness. Above: Severe and mild form of the pimpled condition. Below: Severe woody symptoms.

production of "woody" symptoms in the rind of passion fruit is apparently a common reaction to more than one stimulus. For example, fruit fly stings will produce an appearance difficult at times to distinguish from virus effects.

The Victorian woodiness disease has been known in Queensland on imported nursery plants consisting of *Passiflora edulis* scions grafted on to *P. caerulea* stock. It is characterised by a broad yellow or yellow brown mosaic of the leaves, which are markedly deformed, while the whole vine remains stunted almost to a cessation of growth. So far there has been no evidence of the Victorian virus infecting *P. edulis* plants raised in Queensland. It is likely that the Queensland woodiness disease occurs also in New South Wales, at least in the northern part of that State.

MILD STRAIN PROTECTION AND WOODINESS VIRUS

III. MILD STRAIN PROTECTION AS A POSSIBILITY.

There is no evidence of any worthwhile resistance to woodiness disease in individuals of P. *edulis* and the fact that infection frequently comes from outside the plantation (McKnight 1953) gives little hope for control through the vector.

McKnight (1953) put forward a strain complex hypothesis for the Queensland woodiness virus and suggested the possibility of obtaining mild strain immunity.

The theory of strain protection whereby two strains of the same virus are mutually exclusive once they have colonised a host is now a recognised tool for determining the relationship of viruses affecting a common host (Price 1940). However, the use of this theory in reducing economic losses from virus diseases has been by no means so successful. For a short-term crop the work involved is seldom justified, while for a long-term perennial one there is the risk that a severe strain will eventually superimpose itself on the milder form or that complications will arise over reactions with other viruses (Posnette and Todd 1955; Grant and Higgins 1957). The passion vine lies between these two extremes. A cropping cycle of three to four years would be economically sound and an alteration to the virus complement after this time of less importance. In an endemic area, too, natural infection is so rapid and universal that there can be few qualms about deliberately infecting the vines with a mild form of the disease.

The work described below was essentially a practical attempt to apply the mild strain protection theory to obtaining relief from the ravages of woodiness. Many interesting aspects of the fundamental relationship between the strains of the Queensland woodiness virus and its host remain to be investigated.

(1) Selection of Mild Strains.

For the preliminary collection of virus strains, plant material was obtained in three commercial plantations in south-eastern Queensland from individual vines which appeared to have some distinct symptoms associated with their virus complement. Transfers to potted seedlings were made in the glasshouse, using four methods. The majority were made by the petiole graft or tissue insertion method described by McKnight (1953). Sap transmission and tip grafts were used to a lesser extent. A few transmissions were made from *P. edulis* var. *flavicarpa*, *P. laurifolia* and a *P. edulis* hybrid in addition to those from the purple passion vine.

Fifty plants were inoculated in this way on May 5 and July 31, 1953. Of these 32 were planted in the field at the Redlands Experiment Station on Sept. 17. Periodical recordings were made of the virus symptoms displayed until the first crop of fruit matured in October 1954. Unfortunately, an unexpected visitation of Fusarium wilt caused the death of a number of vines before maturity.

Reviewing the observations made, there seemed little definite correlation between the origin and symptoms displayed by the graft material selected and the type of symptoms developed subsequently by the grafted plant. At the time the latter were fruiting it was observed that severe bullate mottle was associated with moderate to excessive numbers of woody fruit, whereas in the few cases where exceptionally few woody fruit developed, the bullate mottle was inconspicuous and the virus symptoms in general more moderate. In one vine which displayed almost pure fern-leaf symptoms, woody fruit were in excess.

This project was carried a step further in 1954, when from the trial just described three plants were selected which produced a much below average number of woody fruit and had a milder virus record generally. Leaf and tip material from these was grafted on to P. edulis seedlings in the glasshouse on Sept. 30 and Oct. 21, 1954. At the same time graft transmissions were made from two plants naturally infected with woodiness.

This material was used for a 6 x 3 replicated field trial planted in Border F7 at the Redlands Experiment Station. The mild strains used were 443P, 443S and 523D and the normal strains C8 and P3. One treatment without prior inoculation was included.

Although the trial was planted in new land where it was hoped Fusarium wilt would not be a factor, so many vines were affected by this disease that statistical analyses was impossible. However, a count of woody fruit in the spring crop harvested on Nov. 25, 1955, was definitely in favour of the mild strains, there being fewer than half the number of woody fruit in these as in the vines inoculated with the normal strain.

Fortunately, about this time a means of overcoming the wilt problem through the use of resistant stocks became available (Purss 1958). The opportunity was taken to incorporate some of the vines bearing mild strains as scions in a stock-scion trial laid down in Border C6 in 1955 and further observations on their cropping capacity were made.

(2) Replicated Field Trials, 1956 and 1957.

Early in 1956 tips from selected vines carrying mild strains and one with the normal severe strain were tip and side grafted onto a range of Fusariumresistant stocks then available. On Mar. 28, 1956, these were planted in Border F7 at the Redlands Experiment Station. Two series infected with mild strains of woodiness (10 and 15) were compared with a normal woody strain (16) on two types of stock (*P. edulis* var. *flavicarpa* and a *P. edulis* hybrid). Each stock-scion combination was replicated three times. As the stocks appeared to have no influence on the amount of woodiness developed, the harvest figures were subsequently analysed as a $3 \ge 6$ trial without reference to the stock factor.

The two mild strains originated from 443P and comprised "10" from plant 10 in the 1955 stock-scion trial Border C6 and "15" from plant 15E

MILD STRAIN PROTECTION AND WOODINESS VIRUS

ladie I.	Та	ble	1.
----------	----	-----	----

Replicated Trial, 1956. Mean Percentage Fruit per Vine, Spring Crop, 1956.

Virus Strain	n.	Non-woody.	Woody.
10 (mild)		 95.7	• 4•3
15 (mild)		 81.0	19.0
16 (severe)		 32.0	68.0

Table 2.

Replicated Trial, 1956. MEAN NUMBER AND WEIGHT OF FRUIT PER VINE, Spring Crop, 1957.

Virus Strain.	Numl	ber and	Weight (lb.).		
virus Strain.	Non-woody.	Woody.	Non-woody.	Woody.	
10 (mild)	501	114	41.3	7.8	
15 (mild)	453 -	122	33.6	8.3	
16 (severe)	103	373	6.7	22.2	

in the 1954 replicated field trial in Border F7. The severe strain "16" originated from P3 and was obtained from plant 16E in the latter trial.

A light spring crop was harvested in 1956 and normal crops in 1957 and 1958. The fruit were mostly mature green when picked. They were hand-graded for size and the presence of woodiness symptoms. A small number of fruit exhibiting a mild pimpled condition of the skin (Fig. 1) of no commercial significance was classed with the non-woody. Results appear in Tables 1–4 and are illustrated in Figs. 2–5.

Table	3.
-------	----

Replicated Trial, 1956. Mean Number and Weight of Fruit per Vine, Spring Crop, 1958.

Virus s	Strain			Number	umber Weight (lb.).			
VILUS N			Large.	Small.	Woody.	Large.	Small.	Woody.
10 (mild)	••		242	289	81	20.4	16.2	4.8
15 (mild)	••		195	267	98	15.4	14-3	5.4
16 (severe)	••	•••	89	147	270	7.0	8.0	12.9

As a result of the method of propagating the virus (at first by leaf insertion and later by tip grafting), the basic P. *edulis* plant material had been largely restricted to two lines. The inclusion of some additional material seemed desirable to ensure that the results obtained were not linked with host reaction. Accordingly, a further trial was laid down in 1957 along the lines of that of the previous year but including one treatment in which tips from

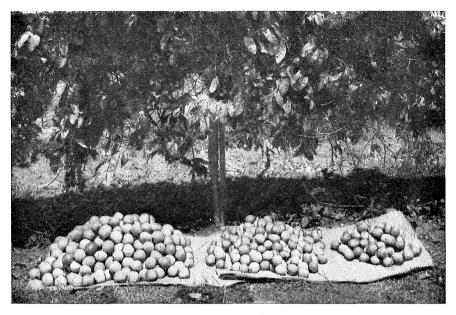


Fig. 2.

Field Trial, 1956. Spring Crop, 1957. Vine No. 5, Mild Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

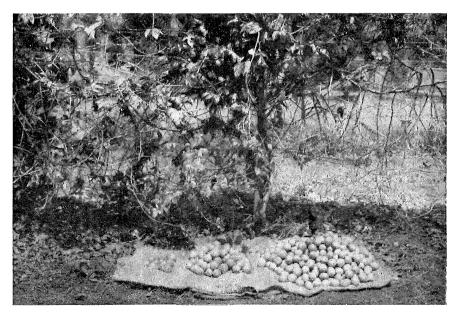


Fig. 3.

Field Trial, 1956. Spring Crop, 1957. Vine No. 4, Severe Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

376

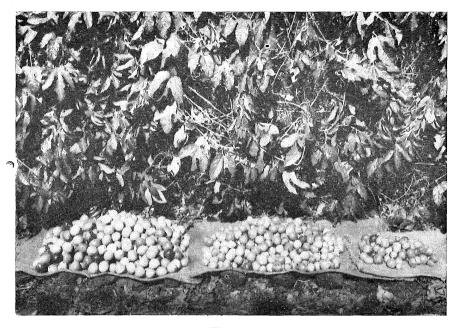


Fig. 4.

Field Trial, 1956. Spring Crop, 1958. Vine No. 5, Mild Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

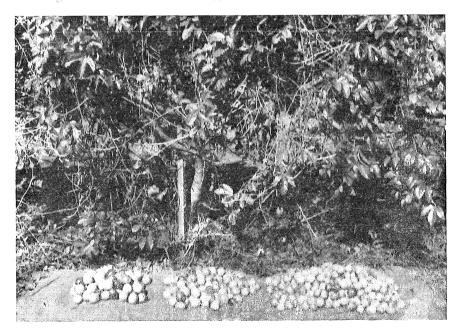


Fig. 5.

Field Trial, 1956. Spring Crop, 1958. Vine No. 4, Severe Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

Table 4.

			Spring Cre	ор, 1958.			
		Total Numb	tal Number of Fruit. Percentage Large.		Percentage Woody.		
Virus Strain.		Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.
A. 10 (mild)		2.76	573	38.3	38.4	20.7	12.5
B. 15 (mild)		2.71	513	36.3	35.0	23.8	16.2
C. 16 (severe)		2.69	493	24.4	17.1	46.7	53.0
S.E. mean of 6		·070 No significant differences		1·23 A, B >> C		1·63 A, B << C	

Replicated Trial, 1956. Summary-Mean Values and Significant Differences, Spring Crop, 1958.

Prior to analysis, the log transformation was used on Total Number and the inverse sine transformation was used on Percentage Large and Percentage Woody in order to equalise the variances.

seedlings grown from commercial seed were tip-grafted onto flavicarpa-edulis combinations already infected with mild strain virus. The mild strain used was "10" and was obtained from the original plant 10 in the stock-scion trial and vine 5 in the 1956 replicated trial. The grafted plants were set out alongside the previous 1956 trial on Aug. 8, 1957. The first crop was harvested in November 1958. The results appear in Tables 5 and 6.

Table 5.

Replicated Trial, 1957. Mean Number and Weight of Fruit per Vine, Spring Crop, 1958.

		Number.		Weight (lb.).		
Virus Strain.	Large.	Small.	Woody.	Large.	Small.	Woody.
Mild	168	171	39	15.0	10.2	2.4
Mild (commercial scion)	196	192.	43	15.8	11.0	2.7
Severe	19	156	265	1.5	8.6	12.9

Table 6.

Replicated Trial, 1957. Summary—Mean Values and Significant Diferrences, Spring Crop, 1958.

	Total Number.		Percentage Large Fruit.		Percentage Woody Fruit.	
Virus Strain.	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.
A. Mild	2.54	350	40.1	41.5	19.6	11.3
B. Mild (commercial scion)	$2 \cdot 61$	408	40.4	42.0	18.8	10.4
C. Severe	$2 \cdot 62$	412	11.2	3.7	$51 \cdot 6$	61.5
S.E. Scion mean	ean ·044 No significant differences		1.58		2.09	
			A, B >> C		A, B << C	

Prior to analysis the log transformation was used on Total Number and the inverse sine transformation was used on Percentage Large Fruit and Percentage Woody Fruit in order to equalise the variances.

MILD STRAIN PROTECTION AND WOODINESS VIRUS

Periodic vine-to-vine examinations for leaf symptoms in the 1956 and 1957 field trials showed that in addition to the increased number of woody fruit the vines infected with a severe strain in general exhibited a more pronounced chlorotic mottle and bullate mottle than those bearing a mild strain.

IV. DISCUSSION.

It will be seen from the tables that it is possible to inoculate passion vines with selected strains of the Queensland woodiness virus which will protect them under natural conditions of infection from more severe strains. The mild strain has apparently remained uncontaminated through successive transfers over five years.

In the replicated field trials, vines bearing severe strains were interspersed among those inoculated with the mild form. Owing to close planting, much interweaving of runners took place. Although reasonable precautions were taken during pruning operations, opportunity for mechanical transfer must have occurred. In spite of this no instance was noted of a vine showing symptoms suggesting invasion by the severe strain. Differences from vine to vine in yield and proportion of woody fruit occurred throughout the experiments but the variation remained within the mild or severe category, depending on the nature of origin.

It is becoming increasingly necessary in south-eastern Queensland to work passion vines onto a Fusarium-resistant stock. This makes the use of mild strain protection a practical proposition as the only additional work entailed is the selection of scion material from a parent vine bearing the required strain of the woodiness virus.

In this investigation, because little was known regarding possible strain selection by mechanical transfer and because severe woody symptoms are at a minimum during warm weather, propagation has been by tip-grafting during the summer months. Mechanical sap transfer at this or other times might yield equally satisfactory results and eliminate the need for grafting where wilt was not a problem.

A demonstration plot using both methods has now been planted and it remains to be seen how far commercial practice is prepared to accept the avenue for control suggested by these investigations.

V. ACKNOWLEDGEMENTS.

Grateful acknowledgement is made to the staff of the Redlands Experiment Station for assistance in cultural operations connected with the field trials and to Mr. P. McGovern, Senior Biometrician, for statistical analyses and advice.

REFERENCES.

- GRANT, T. J., and HIGGINS, R. P. 1957. Occurrence of mixtures of Tristeza virus strains in citrus. Phytopathology 47:5:272-276.
- MAGEE, C. J. 1948. Woodiness or mosaic disease of passion fruit. Agric. Gaz. N.S.W. 59:199-202.
- McKNIGHT, T. 1953. The woodiness virus of the passion vine (*Passiflora edulis* Sims). Qd J. Agric. Sci. 10:4-35.
- NOBLE, R. J. 1928. Some observations on the woodiness or bullet disease of passion fruit. Proc. Roy. Soc. N.S.W. 62:79-98.
- NOBLE, R. J., and NOBLE, N. S. 1938. Aphid vectors of the virus of woodiness or bullet disease in passion fruit (*Passiflora edulis* Sims). Proc. Roy. Soc. N.S.W. 72:293-317.
- POSNETTE, A. F., and TODD, J. MCA. 1955. Virus diseases of cacao in West Africa. IX. Strain variation and interference in virus 1A. Ann. Appl. Biol. 43:433-453.
- PRICE, W. C. 1940. Acquired immunity from plant virus diseases. Quart. Rev. Biol. 15:338-361.
- PURSS, G. S. 1958. Studies of the resistance of species of *Passiflora* to Fusarium wilt (F. oxysporum f. passiflorae). Qd J. Agric. Sci. 15:95-99.

SIMMONDS, J. H. 1930. Brown spot of the passion vine. Qd Agric. J. 34:564-585.

TAYLOR, R. H. 1959. An investigation of the viruses which cause woodiness of passion fruit. J. Aust. Inst. Agric. Sci. 25:71.

(Received for publication June 30, 1959.)