CHLOROTIC SPOT, A VIRUS DISEASE OF PASSIFLORA FOETIDA IN NEW GUINEA

By R. J. van Velsen *

SUMMARY

STUDIES on the symptomatology, identity and transmission of passion fruit chlorotic spot virus, a hitherto undescribed virus, are recorded. The virus can be mechanically transmitted to *Passiflora foetida, P. quadrangularis* L., *P. edulis* Sims. var. *flavicarpa, P. alba* Link and Otto., *Crotalaria anagyroides* H.B. et K., and *Nicotiana sylvestris* Spegazzini and Comes., but not to *Passiflora edulis* Sims., nor *P. suberosa* L.

The thermal inactivation point of the virus lies between 60 and 65 degrees C. for an exposure of 10 minutes ; the dilution end point lies between 10^{-6} and 10^{-7} and longevity in vitro is between 48 and 72 hours at a room temperature of 28 degrees C.

The virus is transmitted in the laboratory by wingless adults of *Aphis gossypii* Glover in a non-persistent manner, and occurs naturally in the field on *Passiflora foetida, P. quadrangularis,* and *P. edulis* var. *flavicarpa.*

INTRODUCTION

Virus diseases of the passion vine have been recorded in Australia (Cobb, 1901), Kenya (McDonald, 1937), South Africa (Storey, 1940), England (Bewley, 1923), Sumatra (Palm, 1922) and New Zealand (Chamberlain, 1954).

Species of *Passiflora* occur throughout the Territory of Papua and New Guinea. *P. edulis* is mainly grown, sometimes commercially, in the Highlands at Goroka, Kainantu, and Wau. *P. quadrangularis, P. edulis* var. *flavicarpa* and *P. quadrangularis* var. *macrocarpa* are grown occasionally in backyard gardens at lower levels. *P. foetida* grows wild in many cleared areas throughout the Territory, but has not been found at Wau, Goroka or Kainantu where *P. edulis* is grown. Woodiness virus has not been recorded in the Territory of Papua and New Guinea to date, and the following investigations were carried out to determine the host range, identity and method of field transmission of the virus causing a chlorotic spotting on *P. foetida* growing wild at the Lowlands Agricultural Experiment Station at Keravat, New Britain.

Passiflora foetida, P. quadrangularis, and *P. edulis* var. *flavicarpa* are found naturally infected in the field with the chlorotic spot virus.

EXPERIMENTAL STUDIES

Throughout the investigations, *P. foetida* was used as the indicator plant unless otherwise stated, and field-infected plants were used as the source of inoculum. Infectious sap was obtained by grinding infected leaves of *P. foetida* in a mortar and straining through muslin. The test plants were inoculated when 21 days old, the filtrate being applied by a cotton pad with the aid of 500 grit carborundum. The plants were maintained in insect-proof cages for 28 days following inoculation, where the air temperature varied from 65 to 93 degrees F.

The test plants listed in Table 1 were inoculated with infectious sap at various ages as indicated. Twenty-eight days after inoculation, the leaves from the test plants above the point of inoculation were removed, ground up and the filtrate inoculated onto *P. foetida* seedlings. Of the plants tested, only *P. foetida, P. alba, P. quadrangularis, P. edulis* var. *flavicarpa, Crotalaria anagyroides,* and *Nicotiana sylvestris* are susceptible. It is important to note that *Passiflora edulis* was not infected in three separate tests.

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TABLE 1.—THE HOST RANGE OF CHLOROTIC SPOT OF PASSION FRUIT

Test Plant.	Reaction.	Proportion of Plants Infected.*
† *Passiflora alba* (b)	Mottle	14/21
P. edulis (b)	Nil	0/60a
P. edulis var. *flavicarpa* (b)	Mottle	13/20
P. foetida (b)	Chlorotic spot	20/20
P. quadrangularis (b)	Mottle	15/20
† *P. suberosa* (b)	Nil	0/20
Cucumis sativus var. "palmetto" (b)	Nil	0/20
C. melo (b)	Nil	0/20
Cucurbita moschata (b)	Nil	0/20
C. pepo (b)	Nil	0/20
C. pepo var. *medullosa* (b)	Nil	0/20
C. melopepo (b)	Nil	0/20
Citrullus vulgaris (b)	Nil	0/20
Nicotiana tabacum var. "White Burley" (c)	Nil	0/20
N. glutinosa (c)	Nil	0/20
N. rustica (c)	Nil	0/20
N. sylvestris (c)	Vein clearing	4/20
Lycopersicon esculentum (d)	Nil	0/20
Petunia hybrida var. "Rosy Morn" (c)	Nil	0/20
Solanum melongena (c)	Nil	0/20
S. nigrum (c)	Nil	0/20
S. dulcamara (c)	Nil	0/20
Vigna sinensis var. "Cowpea" (d)	Nil	0/20
V. sinensis var. "Black Eye" (d)	Nil	0/20
Phaseolis vulgaris var. "Brown Beauty" (d)	Nil	0/20
P. vulgaris var. "Pinto bean" (d)	Nil	0/20
P. mungo (d)	Nil	0/20
Seshania speciosa (e)	Nil	0/20
Crotalaria anagyroides (b)	Mosaic	10/20
Stizolobium deeringianum (d)	Nil	0/20
Centrosema pubescens (d)	Nil	0/20
Chenopodium amaranticolor (c)	Nil	0/20

* In all fractions, the numerator indicates the number of plants developing symptoms, and denominator indicates the number of plants inoculated.

† Seed kindly supplied by the Queensland Department of Agriculture, Brisbane.

a. results of three tests.

b. indicates test plants inoculated when cotyledons present.

c. inoculated when six leaves present.

d. inoculated when first true leaves present.

e. inoculated when seven days old.

Symptoms Induced by the Virus

Passiflora foetida

In the field, the first three terminal leaves of the vines show no symptoms. The fourth and subsequent leaves, however, bear numerous irregular-shaped yellow spots scattered over the leaf surface, giving the leaves a distinct spotted appearance (Plate A). The leaves are normal in shape and size and the plants are not stunted and flower and set seed normally.

In the laboratory, chlorotic leaf symptoms appear seven to 14 days after inoculation.

Passiflora quadrangularis

The young leaves at the tip of the tendrils on field-infected vines appear healthy, but the third and subsequent leaves show a distinct dark-green/light-green mottle (Plate B). The mature leaves, however, are dark-green and appear normal, although the virus has been isolated from them. The fruit is reduced in size and is slightly malformed.

Leaf symptoms, as described above, appear 14 to 21 days after inoculation in the laboratory.

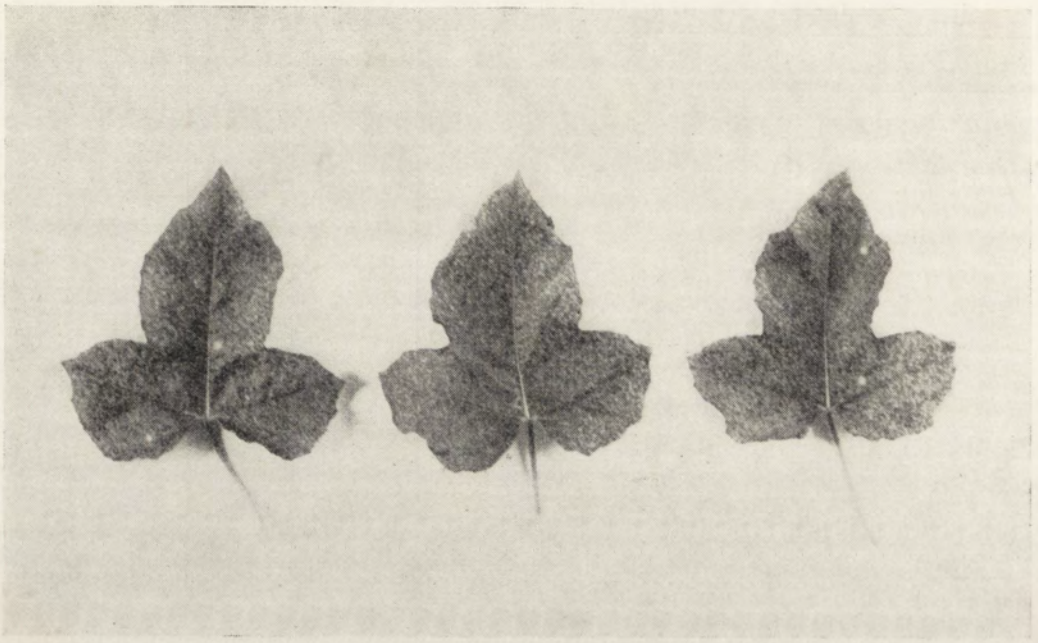

PLATE A.—*Leaves from field-infected* Passiflora foetida *L. showing chlorotic spotting.*

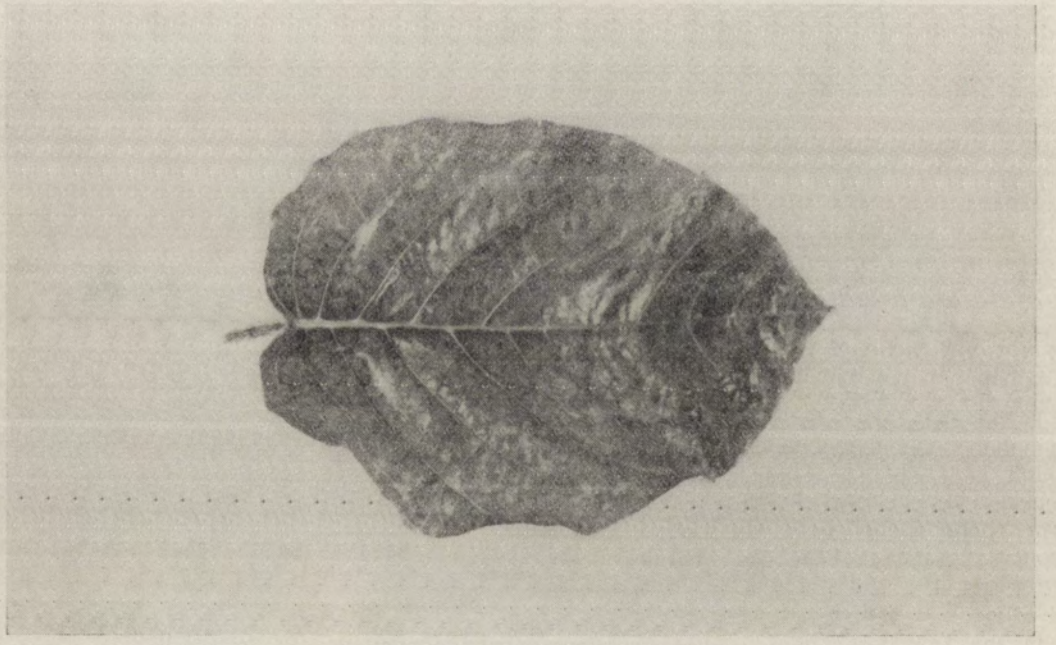

PLATE B.—*Leaf from diseased* Passiflora quadrangu'aris *L. showing mottle symptoms.*

P. edulis var. *flavicarpa*

The foliar symptoms are similar to those recorded on *P. quadrangularis*. However, infected vines bear normal edible fruits.

P. alba

This species of *Passiflora* has not been found on the Gazelle Peninsula, and thus no field symptoms are available. In laboratory tests, foliar symptoms were recorded seven to nine days following inoculation. The leaves emerging develop a distinct yellow-green mottle, which is not accompanied by vein clearing, wilting of the petioles or curling of the leaves as occurs with woodiness virus (McKnight, 1953). As the diseased leaves mature they become darker and the mottle is less distinct. The leaves are not stunted or malformed. When the plants are pruned heavily, the emerging leaves exhibit a distinct mosaic pattern and the leaves are severely stunted and malformed (Plate C).

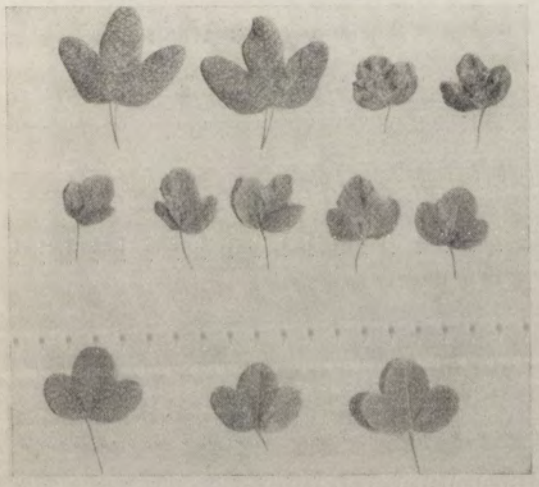

PLATE C.—*Leaves from* Passiflora alba *Link & Otto. Top two rows of leaves taken from plants inoculated with chlorotic spot virus, showing mottle and leaf distortion. Bottom row showing healthy leaves.*

Crotalaria anagyroides

C. anagyroides develops leaf symptoms seven to 14 days after inoculation. The first leaves emerging after inoculation bear no symptoms, but the following leaves show a light-green mosaic. The plants are slightly stunted, but flower and set seed normally.

Nicotiana sylvestris

A distinct vein clearing appears 40 to 50 days after inoculation. The leaves are reduced in size when compared with healthy leaves (Plate D).

PLATE D.—Nicotiana sylvestris *Spegazzini & Comes. Leaf on left from a healthy plant. Leaf on right showing vein clearing and reduction in leaf size following inoculation with passionfruit chlorotic spot virus.*

Physical Properties

The usual methods for the determination of the physical properties were used. *Passiflora foetida* was used throughout as the indicator plant. The dilution end point of the virus lies between 10^{-6} and 10^{-7} (Appendix 1), using distilled water as the diluent. The virus is inactivated in extracted sap following a 10-minute exposure at 65 degrees C., but not at 60 degrees C. (Appendix 2). The longevity in vitro of the virus lies between 48 and 72 hours when the solution is kept at room temperature of 28 degrees C. (Appendix 3).

Attempted Seed Transmission

From field observations carried out at Keravat, 45 to 100 per cent of the plants of *Passiflora foetida* showed chlorotic spot symptoms. The possibility of the virus being seed-transmitted was investigated. Seeds were collected from field-infected plants, washed to remove pulp, sorted, counted, and then planted out into sterilized forest soil. The seedlings were kept under observation for eight weeks. Seeds were

also collected from disease-free plants grown in the laboratory. The results as recorded in Appendix 4 illustrate that the virus is not seed-transmitted.

Insect Transmission

A collection was made of insects found feeding on diseased *Passiflora foetida* in the field at Keravat and these were released in the laboratory onto healthy test plants of *P. foetida.* Specimens were kept and were forwarded to Dr. J. J. H. Szent-Ivany for identification. Of the insects tested (Appendix 5), only *Aphis gossypii* was found to transmit the virus.

Further transmission tests using *Aphis gossypii* as the vector were carried out to determine whether the virus is transmitted in a persistent manner or not. Aphids were collected from laboratory colonies maintained on *Cucurbita moschata* Duchesne, starved for two hours and then allowed an access feed of 10 minutes on diseased leaves of *Passiflora foetida.* The aphids were then removed and placed onto healthy plants of *Passiflora foetida* with the aid of a camel's-hair brush. Single apterous aphids were used. The aphids were then shifted to fresh plants every 24 hours or until they died. The results in Appendix 6 indicate that the virus is transmitted in a non-persistent manner. A further test was carried out in which the access-feeding time was less than 30 seconds. Of the 20 aphids tested, 11 were able to transmit the virus in a test-feeding period of two hours, preceded by an access-feeding period of 30 seconds. Thus, it is evident that the virus is transmitted in a non-persistent manner by *Aphids gossypii.*

DISCUSSION

The only other virus recorded on *Passiflora* spp. is passion fruit woodiness, which Magee (1948) states is a strain of cucumber mosaic virus. Passion fruit woodiness readily infects *P. edulis.* McKnight (1953) was unsuccessful in transmitting passion fruit woodiness to tomato, *Datura stramonium* L., cucumber, *Lupinus mutabilis* Sweet, and tobacco. The virus investigated at Keravat could not be transmitted to the above hosts or to *P. edulis.*

Since no serological tests could be carried out at Keravat, the host range and symptom pattern are used as the critical criteria in identifying the virus. The symptom patterns of the virus on *Passiflora foetida, P. alba, and P. edulis* var. *flavicarpa* are distinct from those recorded by McKnight induced by passion fruit woodiness. In Appendix 7, a comparison is made between cucumber mosaic (Smith, 1957), passion fruit woodiness (McKnight, 1953) and passion fruit chlorotic spot. From this comparison it is evident that the host range of passion fruit woodiness (McKnight, 1953) is similar to that of passion fruit chlorotic spot where results are available, but it must be noted that the virus is not transmitted to *P. edulis* or *P. suberosa.* It is essential to note that passion fruit chlorotic spot differs from cucumber mosaic virus in its host range. Thus, it is not valid to term passion fruit chlorotic spot a strain of cucumber mosaic virus. The mean maximum temperature at which the tests were carried out was 92 degrees F. The author is of the opinion that the virus investigated is distinct from cucumber mosaic and passion fruit woodiness virus and is a new virus hitherto undescribed. It is termed chlorotic spot virus of passion fruit.

ACKNOWLEDGEMENTS

The author wishes to acknowledge that the insect identifications were carried out by Dr. J. J. H. Szent-Ivany and the photographs are the work of Mr. A. E. Charles.

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APPENDIX 1.—The dilution end point of chlorotic spot of passion fruit, using *Passiflora foetida* as the test plant.

Dilution.	Proportion of Plants Infected.
Undiluted	20/20
1 : 10	20/20
1 : 100	20/20
1 : 1,000	20/20
1 : 10,000	20/20
1 : 100,000	12/20
1 : 1,000,000	3/20
1 : 10,000,000	0/20
1 : 100,000,000	0/20

APPENDIX 2.—The thermal inactivation point of chlorotic spot of passion fruit, at an exposure of 10 minutes, using *Passiflora foetida* as the test plant.

Temperature in Degrees Centigrade.	Proportion of Plants Infected.
28	20/20
35	20/20
40	16/20
45	11/20
50	9/10
55	6/20
60	2/20
65	0/20
70	0/20

APPENDIX 3.—The longevity in vitro of chlorotic spot of passion fruit, at a room temperature of 28 degrees Centigrade, using *Passiflora foetida* as the test plant.

Time of Exposure in Hours.	Proportion of Plants Infected.
0	20/20
24	16/20
48	2/20
72	0/20
96	0/20
120	0/20
144	0/20

APPENDIX 4.—The percentage of seed transmission of chlorotic spot virus in *Passiflora foetida*.

Plant.	No. Seeds Planted.	No. Plants.	Per Cent. Germination.	Per Cent. Diseased.
Healthy	2,000	1,973	98.7	Nil
Diseased	2,000	1,968	98.4	Nil

APPENDIX 5.—Transmission of chlorotic spot of passion fruit by insects found infesting *Passiflora foetida* in the field.

Insect Species	No. Insect per plant.	Proportion of Plants Infected.
Aphis gossypii (a)	20	6/20
Red Spider	20	0/20
Euricania splendida F. (b)	20	0/20
Nisia atrovenosa Leth. (c)	20	0/20

a. Identified by Dr. V. F. Eastop.
b. Identified by Dr. J. J. H. Szent-Ivany.
c. Identified by Mr. R. G. Fennah.

APPENDIX 6.—Persistency test of chlorotic spot virus using *Aphis gossypii* as the vector.

Aphid Number	Time after access feed in days. 1	2	3	4	5	6	7	8	9	10	11
1	+	—	—	—	D						
2	+	—	—	—	—	D					
3	+	—	—	—	—	—	—	—	—	D	
4	+	—	—	—	—	D					
5	—	—	—	—	—	—	—	—	D		
6	—	—	—	—	—	—	—	—	—	—	—
7	+	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	D					
9	+	—	—	D							
10	—	—	—	—	—	—	—	D			
11	—	—	—	—	—	—	—	—	—	—	D
12	—	—	—	—	—	—	—	—	—	—	—

D death of aphid.
\+ infection.
— non-infective.

APPENDIX 7.—Comparison of cucumber mosaic virus (Smith, 1957), passion fruit woodiness (McKnight, 1953) and chlorotic spot virus (van Velsen).

Property.	Cucumber Mosaic.	Passion Fruit Woodiness.	Chlorotic Spot
Host range—			
Passiflora alba	N.R.	+	+
P. edulis	+	+	—
P. edulis var. *flavicarpa*	N.R.	+	+
P. quadrangularis	N.R.	N.R.	+
P. suberosa	N.R.	+	—
P. foetida	N.R.	+	+
Cucumis sativus	+	—	—
Tobacco	+	—	—
Tomato	+	—	—
Nicotiana glutinosa	+	—	—
Petunia hybrida	+	—	—
Dilution end point	1 : 10,000	N.R.	10^{-7}
Thermal In. Pt.	60-70	N.R.	65 degrees C.
Longevity	3-4 days	N.R.	3 days
Insect vectors	*Aphis gossypii*	*A. gossypii*	*A. gossypii*
	Myzus persicae	*M. persicae*	N.R.
	Macrosiphum euphorbiae	*Macrosiphum gei*	N.R.
	Aulacorthum solani	N.R.	N.R.
Persistency	*Non-persistent*	N.R.	*Non-persistent*

+ susceptible.

— not susceptible.

N.R. No results given.

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