

Cowpea Mosaic, a Virus Disease of *Vigna Sinensis* in New Guinea.

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SUMMARY.

A STUDY has been made of a mosaic virus disease of *Vigna sinensis* (Linn.) Savi ex Hassk. located on the Gazelle Peninsula of New Britain in the Territory of Papua and New Guinea. Field observations on its occurrence and symptoms, together with experimental data on host range, mechanical, insect and other modes of transmission are recorded.

The virus was found naturally in the field on *V. sinensis* and *V. sesquipedalis* Fruwirth. The host range is restricted mainly to the Leguminosae, although *Nicotiana rustica* L. and *Sesbania speciosa* Taub., ex Engl. were artificially infected.

The virus is mechanically transmissible, has a thermal inactivation point between 60 and 62 degrees C. for an exposure of ten minutes, a dilution end point of 1:400 and a longevity in vitro of less than 84 hours. Cowpea mosaic virus is transmitted by *Aphis gossypii* (Glover) and *Myzus persicae* (Sulzer) in a non-persistent manner, but not by *Toxoptera aurantii* (B.D.F.), *A. craccivora* Koch., *Halticus tibialis* Reut. nor *Planococcus citri* (Risso). It is not seed transmitted. Although no serological investigations were carried out, the virus appears to be related to other mosaic viruses of cowpea investigated by McLean (1941), Snyder (1942) and Yu (1946).

Introduction.

Virus infected cowpea, var. "De Groite" plants were first observed by Mr. C. Brooks at the educational centre at Raval on the Gazelle Peninsula in November, 1960. Nearby *V. sesquipedalis* F. plants were also found with mosaic symptoms. This disease was investigated at the Lowlands Agricultural Experiment Station, Kerevat, New Britain, to determine the identity of the virus and its host range.

Mosaic viruses of cowpeas have been recorded in U.S.A. (McLean 1941, Anderson 1955b, Warid and Plakidas 1952), Trinidad (Dale 1949), Nigeria (Chant 1959), South Africa (Klessner 1960), and China (Yu 1946).

Cucumber mosaic (Klessner 1960, Anderson 1955a) and tobacco mosaic (Chant 1959), viruses also readily infect *V. sinensis* (Linn.) Savi ex. Hassk.

EXPERIMENTAL RESULTS.

Mechanical Transmission.

The virus is mechanically transmissible with ease and in host range tests and the determina-

tion of physical properties, sap was obtained from artificially infected plants of *V. sinensis* var. "De Groite".

Host Range.

Experiments were carried out to determine the host range of the virus. Infective sap was obtained from infected *V. sinensis* var. "De Groite" and plants were inoculated in the glass house with the sap with the aid of 500 grit carborundum. The plants were maintained for 28 days and then ground up with a mortar and pestle. *V. sinensis* var. "De Groite" seedlings were inoculated and maintained for a further 28 days. The results of the host range tests are given in Appendix I. The host range appears to be mainly restricted to Leguminosae, although *Nicotiana rustica* L. was found to be a symptomless carrier and *Sesbania speciosa* Taub ex. Engl. was also infected.

Symptoms produced on susceptible hosts.

Vigna sinensis var. "De Groite"—

Mosaic symptoms appeared on inoculated plants 5-7 days after inoculation and were persistent. The first trifoliate leaves were normal



Plate I.—Mosaic and leaf distortion on *Vigna sinensis* variety "De Groite". (x 1/3)

apart from vein banding and mosaic symptoms. Subsequent leaves had severe mosaic symptoms and were severely blistered and puckered and were greatly reduced in size (Plate I). There was a marked reduction in yield and severe stunting of the diseased mature plant.

Vigna sinensis varieties—

Cowpea varieties "Poona Pea", "Black Eye", "Xape", "Witzenberg Upright", "Newera Gray", "Training White" and "Dr. Saunder's Upright" all exhibited mosaic symptoms 5-7 days after the inoculation of the first two leaves. The symptoms were persistent, but the infections produced no reduction in yield or vigour.

Phaseolus vulgaris L. var. "Pinto Bean"—

Mosaic symptoms appeared on the first set of trifoliate leaves which emerged 7-10 days after the inoculation of the first two leaves. Subsequent leaves all bore mosaic symptoms with severe leaf curling, and distortion (Plate II). The plants were reduced in size compared with healthy plants. The symptoms were persistent.

Phaseolus mungo L.—

Mosaic symptoms and leaf distortion appeared on the first set of trifoliate leaves which emerged 8-10 days after the inoculation of the first two

leaves. The symptoms were persistent and the vigour of the plant was reduced. The infected leaves were reduced in size.

Phaseolus aureus Roxb.—

Severe mosaic symptoms with vein banding appeared on the first set of trifoliate leaves developed after the inoculation of the first true leaves. The leaves were not reduced, but the plants were stunted. The symptoms were persistent.

Phaseolus calcaratus Roxb.—

Small discrete pale green swellings appeared on the upper surface of the inoculated leaves 2-3 days after inoculation (Plate III). Subsequent leaves bore typical mosaic symptoms which were persistent. The plants were not reduced in vigour.

Vigna sesquipedalis Fruith—

Typical mosaic symptoms appeared 7-10 days after inoculation, and were persistent. The plants were not reduced in vigour.

Mucuna deerigianum Small—

Pale chlorotic circular spots appeared on the first set of trifoliate leaves which emerged after inoculation. Subsequent leaves also showed dis-



Plate II.—Mosaic and leaf distortion pattern on *Phaseolus vulgaris* variety "Pinto Bean" following inoculation with cowpea mosaic virus. (x 1/3)

crete circular spots, but there were fewer than on the first leaves. Large chlorotic areas appeared on subsequent leaves and when near the leaf margin they caused leaf distortion (Plate IV). Subsequent leaves showed chlorotic spotting and severe leaf distortion. The diseased plants were greatly reduced in yield and vigour. The symptoms were persistent.

Canavalia ensiformis DC.—

The leaves which emerged after inoculation exhibited a dark green mosaic pattern with some leaf distortion. The leaves became rolled at the margin and were often misshapen. The mosaic and leaf distortion were systemic, but as the leaves matured, the symptoms became indistinct.

Nicotiana rustica L.—

This host was a symptomless carrier.

Sesbania speciosa Taub ex Engl.—

Discrete pale green swellings appeared on the first true leaf 2 days after inoculation. The pinnate leaves exhibited mosaic symptoms and small circular pale green swellings on the upper leaf surface. On the fourth and subsequent

pinnate leaves only mosaic symptoms were observed. The mosaic symptoms were persistent (Plate III).

Physical Properties.

The resistance of the virus to exposure at various temperatures for 10 minutes, to ageing *in vitro* at 1 degree C. and to dilution were studied. *Vigna sinensis* var. "De Groite" was used for the test plant. The virus was active after an exposure to 60 degrees C. for 10 minutes, but inactive after an exposure of 62 degrees (Table I). It was inactive after an exposure of 84 hours at 1 degree C. (Table II), and was inactivated when diluted to 1:400 but active at a dilution of 1:300 when diluted with distilled water (Table III).

Insect Transmission.

Klesser (1960) recorded *Aphis craccivora* Koch. capable of transmitting cowpea mosaic virus A and cowpea mosaic virus cucumber



Plate III.—On right side of photograph, two leaves of *Phaseolus calcaratus* showing small discrete swellings following inoculation. On the left, *Sesbania speciosa*, showing swellings on the first true leaf two days after inoculation with cowpea mosaic virus. (x 1/3)

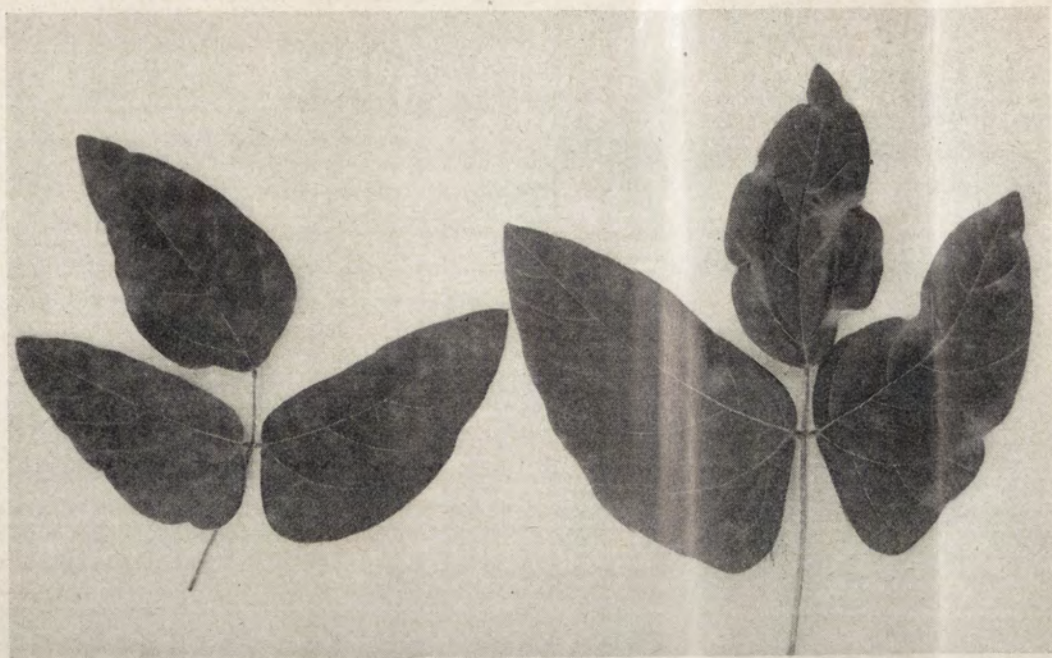


Plate IV.—Chlorotic spotting on *mucuna deeringianum* following inoculation with cowpea mosaic virus. Leaf distortion on the leaflet on the right hand side. (x $\frac{1}{4}$)

strain. *A. gossypii* (Glover) and *Macrosiphum solanifolii* Ashm. were recorded by Anderson (1955b) and McLean (1941) to be capable of transmitting cowpea mosaic virus. McLean (1941) also recorded *Macrosiphum bisi* Kalt. capable of transmitting cowpea mosaic virus. In Trinidad, Dale (1953) was able to transmit cowpea mosaic by a beetle *Ceratoma ruficornis* (Oliv.), and Chant (1959) recorded a beetle *Ootheca mutabilis* Sahlb. in Ghana capable of transmitting cowpea mosaic.

At Raval, the aphid *A. craccivora*, shield bugs (identity not known) and *Planococcus citri* (Risso) were the only insects found feeding on *Vigna sinensis* in the field. In the glass house, tests were carried out to determine the insect vectors using the above insects. *A. gossypii*, *Toxoptera aurantii* (B.d.F.) and *Myzus persicae* were also included in the test. The insects were starved for two hours, allowed an access feed for two hours and test fed until the end of 28 days or until they died. The results are given in Table IV. Only *A. gossypii* and *Myzus persicae* were found capable of transmitting the virus. Further attempts were made with

A. craccivora in an endeavour to transmit the virus with access feeding times ranging from two minutes to 24 hours, and test feeds from two hours to six days. However no transmission was obtained.

Persistency of virus in Aphis gossypii and Myzus persicae.—

Fourth instar nymphs of *A. gossypii* were collected from stock colonies maintained on *Citrullus vulgare* Schrad. in the glass house. The aphids were starved for two hours and then fed diseased cowpea leaves for 15 minutes. The nymphs were then transferred by brush to healthy seedlings of *Vigna sinensis* one per plant, for a test feed of 24 hours. Every 24 hours, the aphids were shifted to correspondingly numbered fresh seedlings. The aphids were maintained for ten days or until they died. After 28 days the results were recorded (Table V). Of the 20 aphids tested, 12 were able to transmit the virus to *V. sinensis* within the first 24 hours of feeding, but none transmitted the virus after this period. Thus the virus appears to be transmitted by *A. gossypii* in a non-persistent manner.

A similar experiment was carried out using *M. persicae*. Fourteen aphids transmitted the virus within the first 24 hours of the test feed, but no transmission was recorded in subsequent 24 hourly test feed periods. Thus *M. persicae* transmits the virus in a non-persistent manner. *Effect of starvation on efficiency of transmission by Aphis gossypii and Myzus persicae.*

Fourth instar nymphs of *A. gossypii* and *M. persicae* were collected from stock colonies and divided into two lots. One lot was starved for two hours, then allowed an access test feed of two hours and then transferred to test seedlings for a test feed of 24 hours: only one nymph per plant. The plants were maintained for 28 days and the results are recorded in Table VI.

It is evident that pre-access feed starvation increases the efficiency of transmission by *A. gossypii* and *M. persicae*.

The effect of access feeding period on transmission.

Fourth instar nymphs of *A. gossypii* and *M. persicae* were collected from stock colonies and starved for two hours. Lots of twenty nymphs were allowed access feeds of 30 seconds, five minutes, and 15 minutes and two hours. The aphids were then transferred to test plants of *Vigna sinensis* for a test feed of 24 hours. After 28 days, the results were recorded (Table VII).

It is evident that *A. gossypii* and *M. persicae* are capable of transmitting the virus after an access feeding period of 30 seconds. This supports the previous evidence that the virus is transmitted in a non-persistent manner by *A. gossypii* and *M. persicae*.

Attempted Seed Transmission.

Seed was collected from artificially and field infected plants of *Vigna sinensis*, planted in the glass house and allowed to grow to maturity. A thousand seeds were collected from each of these and a thousand from healthy plants: 941 plants were established from seed from artificially inoculated plants, 929 from field infected plants and 931 from healthy plants. At maturity all the plants under observation were healthy. Five hundred seeds were collected from artificially infected *Vigna sesquipedalis* and 463 were established. At maturity, all plants were healthy. Thus it is evident that the virus is not transmitted by seed.

DISCUSSION AND CONCLUSION.

In Appendix II all available references of cowpea mosaic viruses have been collected for comparison with the virus investigated. Since no serological investigations could be conducted the main criteria for identification are the host range and mode for transmission. The Kerevat cowpea mosaic virus differs widely in host range to cowpea mosaic viruses A and B and cucumber mosaic viruses investigated by Chant (1959), Dale (1954) and Anderson (1955a). Also the virus is not transmitted by *Aphis craccivora*.

The virus investigated has similar host range, physical properties and host symptoms to the cowpea mosaic viruses investigated by Snyder (1942), McLean (1941) and Yu (1946). However the virus is not seed borne in asparagus-bean or cowpea var. "De Groite", whereas the above three viruses are all seed transmitted.

The virus is transmitted in a non-persistent manner by *Aphis gossypii* which was also found to transmit cowpea mosaic virus (McLean 1941; Yu 1946).

Since the symptoms and the properties of the virus *in vitro* vary due to climatic conditions, different reactions in host varieties etc., it is reasonable to consider the virus is related to the viruses investigated by Snyder (1942) McLean (1941) and Yu (1946). Thus the virus is considered to be a strain of cowpea mosaic virus.

In the literature cited there is no mention of the mode of transmission of cowpea mosaic virus by its aphid vectors. The virus investigated is readily transmitted by *Aphis gossypii* and *Myzus persicae*, but in all tests carried out, *A. craccivora* and *Toxoptera aurantii* failed to transmit the virus. Nymphs of *A. gossypii* and *M. persicae* were able to transmit cowpea mosaic virus after an access feeding time of 30 seconds and within the first 24 hours of test feeding. No transmission was obtained with test feeding periods greater than 24 hours. Pre-access feeding starvation increased the efficiency of transmission in both *A. gossypii* and *M. persicae*.

Thus it is concluded that the Kerevat strain of cowpea mosaic virus is transmitted in a non-persistent manner by the aphids *Aphis gossypii* and *Myzus persicae*.

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The photographs were taken by Mr. A. E. Charles.

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Table I.

The thermal inactivation of cowpea mosaic virus using *Vigna sinensis* var. "De Groite" as the test plant.

Temperature in degrees Centigrade.	Proportion of Infected Plants.
29	20/20
40	18/20
48	18/20
50	15/20
55	12/20
58	4/20
60	1/20
62	0/20
64	0/20
65	0/20

Table II.

Longevity in vitro at 1 degree C. of cowpea mosaic virus using *Vigna sinensis* var. "De Groite" as the test plant.

Time in hours of Exposure.	Proportion of Plants Infected.
0	20/20
24	16/20
48	10/20
60	8/20
72	3/20
84	0/20
96	0/20
144	0/20

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Table III.

Dilution end point of cowpea mosaic virus using *Vigna sinensis* var. "De Groite" as the test plant.

Dilution.	Proportion of Plants Infected.
Undiluted	20/20
1: 10	20/20
1: 100	3/20
1: 200	2/20
1: 300	1/20
1: 400	0/20
1: 500	0/20
1: 1,000	0/20

Table IV.

Transmission of cowpea mosaic virus by insects found infesting *Vigna sinensis* in the field.

Insect Species.	No. Insects/plants.	Proportion of Plants Infected.
<i>Aphis craccivora</i> *	10	0/20
<i>A. gossypii</i> *	10	14/20
<i>Planococcus citri</i> *	10	0/20
<i>Halictus tibialis</i> *	10	0/20
Shield bugs	10	0/20
x <i>Toxoptera aurantii</i> *	10	0/20
x Sweet Potato aphid	10	15/20

* identified by Dr. V. F. Eastop of the Commonwealth Institute of Entomology.

x not found on *Vigna sinensis* in the field.

Table V.

The longevity of cowpea mosaic virus in apterous forms of *Aphis gossypii*, using *Vigna sinensis* var. "De Groite" as the test plant.

Days	1	2	3	4	5	6	7	8
Aphid Number								
2	x	—	—	—	—	—	—	—
3	x	—	—	—	—	—	D	
4	—	—	—	D				
5	x	—	—	—	—	—	—	—
6	x	—	—	—	D			
7	x	—	—	—	—	D		
8	—	—	—	—	—	—	—	—
9	—	—	—	—	D			
10	x	—	—	D				

x Virus transmitted.

— Virus not transmitted.

D Aphid died.

Table VI.

The effect of pre-access starvation on the efficiency of transmission of cowpea mosaic virus by *Aphis gossypii* to *Vigna sinensis* var. "De Groite".

Treatment.	Proportion of Plants Infected.
Starved	12/20
Non-starved	4/20

Table VII.

The effect of variations in access feeding periods on the transmission of cowpea mosaic virus by *Aphis gossypii* to *Vigna sinensis* var. "De Groite".

Access Feeding Period.	Proportion of Plants Infected.
30 seconds	5/20
5 minutes	8/20
15 minutes	16/20
2 hours	12/20

Appendix I.

The Host Range of Cowpea Mosaic Virus.

Host.	Reaction.	Proportion of Plants Infected.
<i>Vigna sinensis</i> var.—		
"De Groite"	Mosaic	20/20
"Poona Pea"	Mosaic	12/20
"Black Eye"	Mosaic	13/20
"Xape"	Mosaic	4/20
"Witzenberg Upright"	Mosaic	10/20
"Newera Gray"	Mosaic	12/20
"Training White"	Mosaic	19/20
"Dr. Saunder's Upright"	Mosaic	16/20
<i>V. sesquipedalis</i> Fruwirth	Mosaic	2/20
<i>Canavalia ensiformis</i> DC.	Mosaic	12/20
<i>Phaseolus mungo</i> L.	Mosaic distortion	14/20
<i>P. aureus</i> Roxb.	Mosaic vein-banding	16/20
<i>P. calcaratus</i> Roxb.	Swelling mosaic	20/20
<i>Mucuna deeringiana</i> (Small)	Chlorotic spotting	18/20
<i>Sesbania speciosa</i>	Swellings mosaic	20/20
<i>Nicotiana rustica</i> L.	Symptomless	3/20
<i>Phaseolus vulgaris</i> L. var.—		
"Pinto Bean"	Mosaic	12/20
"Brown Beauty"		
"Top Crop"		0/20
"Bountiful"		0/20
"Genfer"		0/20
"Saxa"		0/20
"Beka"		0/20

Appendix I.—continued.
The Host Range of Cowpea Mosaic Virus—continued.

Host.	Reaction.	Proportion of Plants Infected.
<i>Pisum sativum</i> L. var.		
" Earlicrop "		0/20
" Pluck Market "		0/20
" Wisconsin "		0/20
" Dippes Foli "		0/20
" Perfection "		0/20
<i>Crotalaria anagyroides</i> H.B. et K.		0/20
<i>C. juncea</i> L.		0/20
<i>C. spectabilis</i> Roth.		0/20
<i>C. mucronata</i> Desv.		0/20
<i>Dolichos lablab</i> L.		0/20
<i>Medicago sativa</i> L.		0/20
<i>M. orbicularis</i>		0/20
<i>Trifolium pratense</i> L.		0/20
<i>T. repens</i> L.		0/20
<i>T. hybridum</i> L.		0/20
<i>T. incarnatum</i> L.		0/20
<i>Melilotus alba</i> Desv.		0/20
<i>Lupinus albus</i> L.		0/20
<i>Cajanus cajan</i> Millsp.		0/20
<i>Calapogonium mucunoides</i>		0/20
<i>Flemingia congesta</i>		0/20
<i>Vicia faba</i> L.		0/20
<i>V. villosa</i> Roth.		0/20
<i>Glycine max</i> Mer.		0/20
<i>Cassia tora</i> L.		0/20
<i>C. occidentalis</i> L.		0/20
<i>Leucaena glauca</i>		0/20
<i>Lathyrus odoratus</i> L.		0/20
<i>Arachis hypogaea</i> L.		0/20
<i>Zinnia elegans</i> Jacq.		0/20
<i>Lycopersicum esculentum</i> Mill var.		
" Grosse Lisse "		0/20
<i>Datura stramonium</i> L.		0/20
<i>Chenopodium amaranticolor</i> Coste et Reyn.		0/20
<i>Petunia hybrida</i> Vilm. var.— " Rosy Morn "		0/20
<i>Physalis floridana</i>		0/20
<i>Cucumis calivus</i> L. var.— " Palmetto "		0/20
<i>Nicotiana tabacum</i> L. var.— " White Burley "		0/20
<i>N. glutinosa</i> L.		0/20

Appendix II.

Comparison of host range, physical properties and aphid vectors of cowpea mosaic viruses reported in literature.

	Keravat cowpea mosaic virus.	Cowpea mosaic Yu (1946).	Cowpea mosaic Snyder (1942).	Cowpea mosaic McLean (1941).	Cowpea mosaic A Klesser (1960).	Cowpea mosaic B Klesser (1960).	Cucumber mosaic strain Klesser (1960).	Cowpea Tobacco mosaic strain STR. Chant (1959).	Cowpea yellow mosaic Chant (1959).	Cowpea mosaic Dale (1949).	Cowpea mosaic Anderson (1955b).	Cowpea mosaic cucumber Anderson (1955a).
<i>Vigna sinensis</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>V. Sesquipedalis</i>	x		x		x	x	x					
<i>Phaseolus vulgaris</i>	x	—		—	x	x	x	x	x	—	—	x
<i>P. mungo</i>	x				x	x	x			x		
<i>P. calcaratus</i>	x									x		
<i>P. aureus</i>	x									x		
<i>Sesbania speciosa</i>	x									x		
<i>Mucuna</i> sp.	x							x	x			
<i>Vicia faba</i>	—	—		—	x	x	x				x	x
<i>Arachis hypogaea</i>	—	—			x	—	x		—			
<i>Pisum sativum</i>	—	—	—	—	x	x	—					x
<i>Nicotiana tabacum</i>	—	—		—	—	—	x	x	—	—		x
<i>N. rustica</i>	x											
<i>N. glutinosa</i>	—		—	—	—	—	x	—	—	—		x
<i>Cucumis sativus</i>	—	—		—	—	—	x	—	—	—	—	x
<i>Zinnia elegans</i>	—	—		—	—	—	x	—	—			x
<i>Lycopersicon esculentum</i>	—	—		—					—	—		x
<i>Petunia hybrida</i>	—	—		—								
<i>Chenopodium amaranticolor</i>	—											
<i>Datura stramonium</i>	—	—		—								
<i>Crotalaria juncea</i>	—				x	x	x	x	—	x		
<i>C. spectabilis</i>	—				x	x	x				x	—
<i>Dolichos lablab</i>	—	—		x	x	x	x	x	—	x	x	x
<i>Glycine max.</i>	—	—	—	—	x	x	x			x	x	—
<i>Lathyrus odoratus</i>	—	—	—	—	x	x	—					
<i>Lupinus albus</i>	—	—			x	x	x					
<i>Medicago sativa</i>	—	—			x	—	x					
<i>Trifolium repens</i>	—	—	—		x	—	—					
<i>T. pratense</i>	—	—	—		x	x	x					
<i>T. hybridum</i>	—				x	—	x					
<i>T. incarnatum</i>	—				x	x	x					
<i>Centrosema</i> sp.	—							x	—			—
<i>Canavalia ensiformis</i>	x	x	—	x								
<i>Aphis gossypii</i>	x	x	—	x								
<i>A. craccivora</i>	—				x	?	x					
Dilution end pt.	1:400											
Thermal In. Pt. (°C)												
Longevity (days)	3-4	3-4	9-15	2-3	2-4	2-3	4-5	4-6		20	1-2	

x susceptible.

— resistant

.... no results given.

