APHID VECTORS OF THE VIRUS OF WOODINESS OR BULLET DISEASE IN PASSION FRUIT (*PASSIFLORA EDULIS* SIMS).

By R. J. NOBLE, Ph.D., M.Sc., B.Sc.Agr.,
Biologist,
and N. S. NOBLE, D.Sc.Agr., M.Sc., D.I.C.
Assistant Entomologist, New South Wales Department of Agriculture.

(With Plates VI, VII and one text figure.)

(*Manuscript received, November 16, 1938. Read, December 7, 1938.*)

INTRODUCTION.

The records of the N.S.W. Bureau of Statistics and Economics on production of passion fruit in New South Wales indicate a definite decline in the average yield per vine in recent years. The position in respect of the period 1913-1927 was reported in a previous paper[6] and subsequent production figures are shown in Table I.

TABLE 1.—*Passion Fruit Production in New South Wales.*

Year Ending 30th June.	Vines in Bearing.	Yield in Bushels.	Average Yield per Vine.
1928	168,649	73,230	0·43
1929	215,425	40,211	0·18
1930	203,895	51,051	0·25
1931	203,035	57,595	0·28
1932	243,454	57,226	0·23
1933	256,471	59,558	0·23
1934	289,242	68,050	0·23
1935	209,007	40,144	0·19
1936	155,336	29,832	0·19
1937	174,940	31,550	0·18

Data in respect of vines in bearing refer only to plantations of one acre and upwards, whereas total yield figures include production from smaller areas also. The average yields computed from available records thus are slightly higher than is actually the case but the figures provide a useful index of the position.

On this basis, the average annual yields per vine for each five-year period from 1913 to 1937 are 0·40, 0·46, 0·31, 0·27 and 0·20 bushels respectively, and when it is recalled that individual vines in commercial production in the past have produced annually yields in excess of two bushels, an average of less than one-fifth of a bushel per vine, as in recent years, is indicative of a very low level of production.

Fungous diseases may cause serious losses from time to time but the most important of these diseases, Brown Spot (*Alternaria passiflorœ* Simmonds) may be controlled by the application of Bordeaux mixture at appropriate periods, and there can be little doubt that the widespread incidence of the woodiness or bullet disease is mainly responsible for the present unsatisfactory position.

History and Distribution of the Disease.

As previously reported,[6] the disease has long been known to occur in New South Wales; Simmonds[10] records that since 1931 it has assumed serious proportions in Queensland and is now the most important disease of passion vines in that State. The disease is known to occur also in Victoria. Elsewhere there are records of the occurrence of a mosaic or virus disease affecting species of *Passiflora* in England,[1] Sumatra[8] and South Africa.[3] In Kenya[5] the record is that of the occurrence of the woodiness virus in passion fruit.

Simmonds[10] reported the occurrence of the woodiness virus in *Passiflora alba* in Queensland as determined by mechanical transmission experiments. Mosaic symptoms on *Passiflora alba* and on *P. cœrulea* as well as on *Tacsonia mollissima* have been noted in N.S.W. Most of the *Passiflora* species introduced for possible plant improvement purposes have also proved susceptible to the woodiness virus disease.

Symptoms.

The main features of the disease include an abnormal thickening and hardening of the tissues of the pericarp of the fruit of *P. edulis*. This was demonstrated[6] to be due to the development of sclerenchymatous cells which replaced a considerable portion of the parenchymatous tissue on the inner section of the pericarp. In a previous description[6] of symptoms on the fruit, reference was made also to the presence, on some fruits, of small scabs or eruptions which appeared to have burst through the skin.

It has since been demonstrated by Simmonds[9] that this feature is a symptom of the scab disease of passion fruit caused by *Cladosporium* sp. Scab infections may occasionally occur in fruits which are also affected with woodiness. The phellogen discussed by Butler,[2] (p. 207) refers only to occasional scab lesions present in sections of woody fruit tissue in the slides which he examined.

Fruits on diseased vines are occasionally stippled or blotched and may show small ring-like markings instead of a uniform green or purplish coloration, but it is not known whether such markings are characteristic of the passion fruit woodiness virus under certain environmental conditions or whether they are manifestations of another virus infection.

The foliage of diseased vines is also distinctive, and is briefly described in a previous paper.[6] Under mild temperature conditions, terminal leaves are markedly down-curled along the axis of the main veins. In some cases the upper surfaces of the tips of the leaves may press against the under surfaces of their petioles or main veins so that the laminæ are curled in cylindrical fashion. Marked clearing of the veins in these young leaves has been observed. Upper leaves just below the youngest leaves are frequently dipped or pointed downwards more strongly than is the case in normal plants.

An upper immature but unfolded leaf on each of a number of inoculated test plants has been noted as apparently normal during recording of results, and at a subsequent examination, less than 8 hours later, has been observed to be markedly down-curled. The progressive development of the leaf curl symptom in three plants after removal from the glasshouse, is illustrated in Pl. VI, figs. A-D.

The leaf curl symptoms are most evident when growth of the terminal leaves occurs at temperatures below 80° F. Under higher temperature conditions there is less curling, but puckering and mosaic mottling are the first manifestations of the presence of infection. In the present series of studies it was noted that frequently after the development of leaf curl symptoms and while temperatures did not exceed 80° F., there was a tendency for the upper leaves to become chlorotic and subsequently to fall from the plant.

Growth was always checked for the time being and, although terminal growth might be resumed, infected

plants were always shorter than the uninfected controls. Secondary symptoms in the nature of yellowish spots or blotches were frequently observed in leaves which were fully formed at the time of inoculation.

It was apparent that, as in the case of so many other virus diseases, development of symptoms was dependent largely on the nature of the environmental conditions under which the plants were growing. It appeared that reactions occurred most readily in vigorously growing plants exposed to relatively high humidities and to temperatures which did not exceed 70–75° F. Masking was again observed when temperatures exceeded 85° F., although disease symptoms again developed in the new growth of such plants when lower temperature conditions were experienced. It was noted, however, that some infected plants continued to produce diseased foliage under conditions which resulted in the masking of symptoms in most other infected plants.

Although the leaf curl symptom is frequently observed in field plantations, the most commonly noted foliage symptom is that of a malformation, stunting and puckering of the leaves as previously described and illustrated.[6] This development has always followed the leaf curl symptom in mechanical and vector transmission experiments. Less frequently the malformation of leaves took the form of a reduced development of the lamina. Such leaves were proportionately longer and narrower than normal juvenile leaves or on occasion showed a type of " fern leaf " formation somewhat resembling the type of leaf growth observed occasionally in tomatoes affected with tobacco mosaic (Pl. VII, fig. 1).

It cannot yet be stated whether the various symptoms observed on the virus-infected passion plants result from the action of one or more plant viruses. Assuming that one virus only is concerned it may be indicated at this stage, however, that although complete host range studies of the passion fruit woodiness virus have not been completed, it was not found possible to transmit this virus mechanically to certain Solanaceous plants (*Datura stramonium*, *Lycopersicum esculentum*, *Nicotiana tabacum*, and *Nicotiana glutinosa*) under conditions which permitted ready expression of the common tobacco mosaic virus disease symptoms in such plants.

In the field, the development of woody fruits is recorded only on passion vines showing some of the abovementioned

foliage abnormalities. On completion of the 1928 experiments on mechanical transmission of the disease as previously recorded,[6] three healthy and three diseased plants were retained under insect-proof conditions in a glasshouse until flowers and fruits were developed in 1929. The flowers were artificially pollinated and fruits were obtained from all vines under observation. Diseased (woody) fruits developed on the vines which had shown and were still showing evidence of mosaic or virus infected leaves and normal fruits were obtained from the healthy vines.[7]

Studies on Insect Vectors.

After demonstration that the virus of woodiness could be transferred by mechanical means it was but natural to suggest that insects may be of importance also in the spread of the disease under field conditions.[6] It is recalled, however, that such an assumption may not be entirely justified by analogy with other virus diseases. Tobacco mosaic, for instance, although readily transferred mechanically or by means of aphid vectors in a tomato crop, presumably only may be transferred mechanically in tobacco crops. All available evidence suggests most strongly that insect vectors are not of importance in dispersal of tobacco mosaic in tobacco plantations, but the disease may be spread most readily in the crop by other means.

Passion vines are subjected to a good deal of handling under commercial conditions. The vines are trained on to upright stakes before being allowed to run along wires approximately 5 ft. from ground level. Side shoots are removed from time to time until the vines reach the wires, the vines are pruned, growth of adjacent vines eventually overlaps on the wires, wind may cause rubbing and abrasion of runners or shoots, and the vines are disturbed when fruits are picked. A good deal of wounding and abrasion of tissues may occur under normal conditions, and thus there are opportunities for ready transference of the woodiness virus by mechanical means.

Observations over a period of ten years indicate that, although a number of species of insects may feed and breed on passion vines in New South Wales, the populations of the various species observed have usually been so limited that serious injury to the vines has not resulted. In fact, generally in field examinations close search has

been necessary to find any insects at all. Only occasional brief infestations of jassids, thrips, and aphids have been observed.

Insects of the orders *Hemiptera* and *Thysanoptera* which have been found feeding on passion vines include the green vegetable bug, *Nezara viridula*, the passion vine leafhopper, *Scolypopa australis*, three species of jassids, including one belonging to the genus *Erythroneura*, the brown olive scale, *Saissetia oleæ*, the soft brown scale, *Coccus hesperidum*, the long-tailed mealy bug, *Pseudococcus adonidum*, the green peach aphid, *Myzus persicæ*, the potato aphid, *Macrosiphum solanifolii*, and five species of thrips, including *Hercinothrips bicinctus* (Bagnall). Of the aphid species mentioned, *Macrosiphum solanifolii* was collected even less frequently than *Myzus persicæ*.

Insects Used in Vector Transmission Experiments.

In a series of feeding experiments in 1936, 1937, and the early part of 1938, five species of thrips and three species of jassids were used, but under the conditions of these experiments negative results only were recorded.

In August, 1938, a further series of experiments were conducted with *Hercinothrips bicinctus*, a gregarious foliage-feeding species of thrips, and negative results were again obtained under conditions in which the aphid vectors, referred to later, transmitted the disease.

In a limited series of experiments later in 1937, there were indications that aphids were capable of transmitting the disease, but conditions at the time of the experiments were not ideal for the expression of symptoms.

Detailed experiments with aphids were commenced in 1938 and four species were used, viz. *Myzus persicæ*, *Macrosiphum solanifolii*, and two dark species belonging to the genus *Aphis*. The latter were obtained from velvet beans (*Stizolobium* sp.) and from cotyledons (*Cotyledon valida*), and resembling one another closely were thought to be one species until critical examination by Mr. E. H. Zeck, Assistant Entomologist of the N.S.W. Department of Agriculture, indicated that the populations on each host were distinct species. Both forms belong to the group in which are included *Aphis rumicis*, *Aphis medicaginis*, etc., in which there is considerable divergence of opinion as to the synonymy of the species concerned.

In Tables 2 and 3, Mr. E. H. Zeck has set out comparisons of certain morphological features of the two species.

TABLE 2.—*Essential Morphological Characters* of* Aphis *sp.* A† *from* Stizolobium sp.

Alate Forms.						Apterous Forms.					
Specimen No.	Antennal Segments.			Length of Cornicles.	No. of Hairs on Cauda.	Specimen No.	Antennal Segments.		Length of Cornicles.	No. of Hairs on Cauda.	
	III.		IV+V.				III.	IV+V.			
	Length.	No. of Sensoria.	Length.				Length.	Length.			
1	7	3–4	10	5	4	1	8	10	9	4	
2	8	3–4	11	5	4	2	6	8	6	4	
3	7	3–4	9	5	4	3	8	12	8	—	
4	7	6–3	9	5	4	4	8	10	6	3	
5	7	3–4	10	6	4	5	7	9	7	5	
6	8	4–5	11	5	4	6	6	8	5	—	
7	7	4–5	10	5	4	7	7	10	8	3	
8	7	3–3	11	5	—	8	7	8	6	4	
9	8	4–3	11	5	4	9	7	9	6	6	
10	7	3–4	10	5	4	10	7	8	6	4	

* Figures for length indicate micrometer divisions.

† Collected Sydney, N.S.W., Aug., 1938.

TABLE 3.—*Essential Morphological Characters* of* Aphis *sp.* B† *from* Cotyledon valida.

Alate Forms.						Apterous Forms.				
Specimen No.	Antennal Segments.			Length of Cornicles.	No. of Hairs on Cauda.	Specimen No.	Antennal Segments.		Length of Cornicles.	No. of Hairs on Cauda.
	III.		IV+V.				III.	IV+V.		
	Length.	No. of Sensoria.	Length.				Length.	Length.		
1	9	5–4	12	5	5	1	9	13	10	4
2	10	7–6	14	5	6	2	—	—	8	6
3	11	9–6	14	6	5	3	9	12	8	5
4	9	7–8	13	5	6	4	7	10	8	5
5	11	8–8	16	7	5	5	9	11	8	4
6	10	8–9	13	6	6	6	8	12	8	—
7	9	9–6	12	6	5	7	8	12	8	6
8	9	7–7	13	6	6	8	10	13	8	6
9	11	7–6	14	6	6	9	9	13	9	4
10	11	10–9	14	7	—	10	9	12	9	6

* Figures for length indicate micrometer divisions.
† Collected Sydney, N.S.W., Aug., 1938.

Critical differences between them are also illustrated by him in text-fig. 1. According to his summary also, the two species may be distinguished as follows :

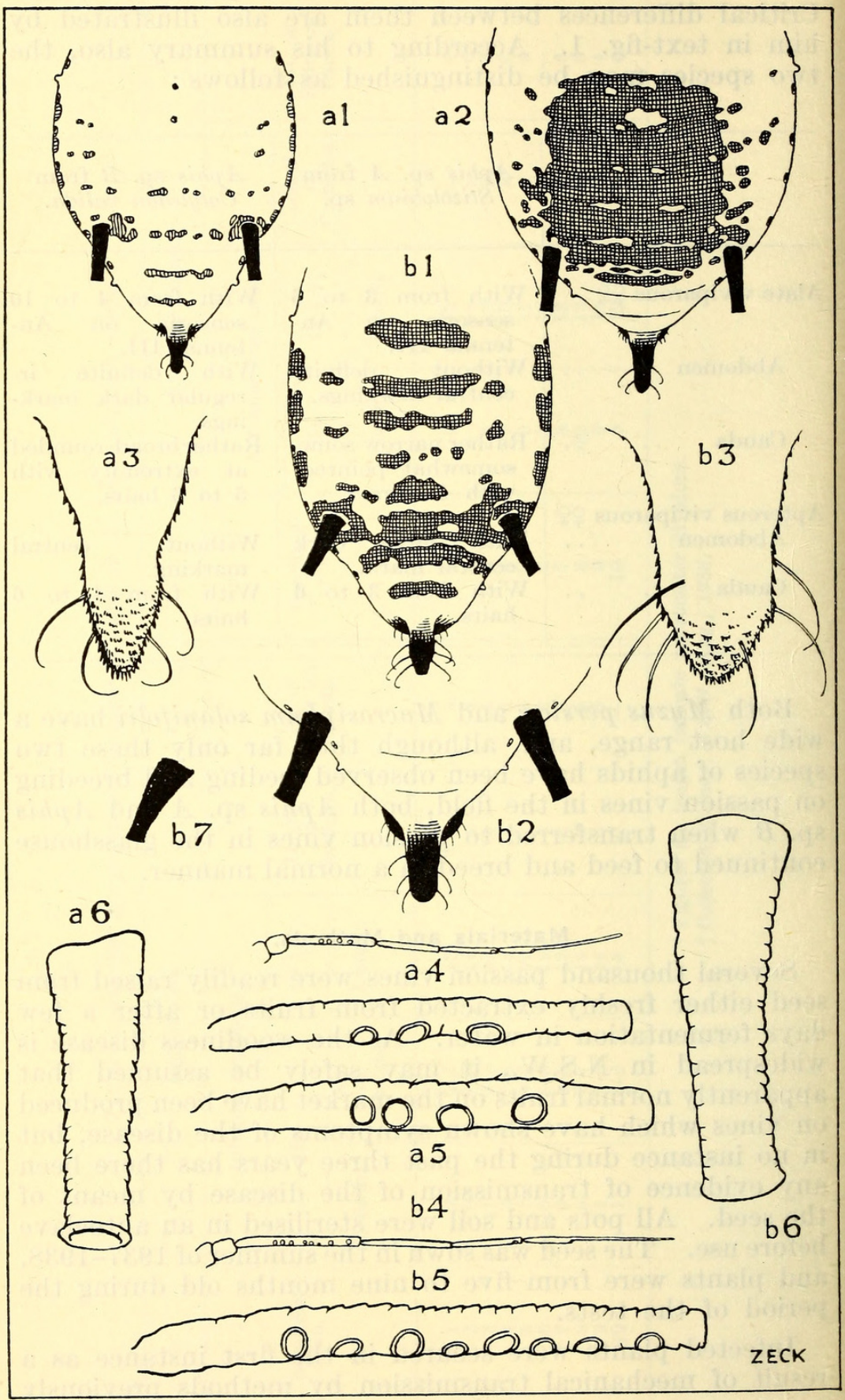
—	*Aphis* sp. *A* from *Stizolobium* sp.	*Aphis* sp. *B* from *Cotyledon valida*.
Alate viviparous ♀♀	With from 3 to 6 sensoria on Antennal III.	With from 4 to 10 sensoria on Antennal III.
Abdomen ..	Without definite central markings.	With definite irregular dark markings.
Cauda	Rather narrow somesomewhat pointed, with 4 hairs.	Rather broad, rounded at extremity with 5 to 6 hairs.
Apterous viviparous ♀♀		
Abdomen ..	With large dark central mark.	Without central marking.
Cauda	With from 3 to 6 hairs.	With from 4 to 6 hairs.

Both *Myzus persicæ* and *Macrosiphum solanifolii* have a wide host range, and, although thus far only these two species of aphids have been observed feeding and breeding on passion vines in the field, both *Aphis* sp. *A* and *Aphis* sp. *B* when transferred to passion vines in the glasshouse continued to feed and breed in a normal manner.

Materials and Methods.

Several thousand passion vines were readily raised from seed either freshly extracted from fruits or after a few days fermentation in water. As the woodiness disease is widespread in N.S.W., it may safely be assumed that apparently normal fruits on the market have been produced on vines which have shown symptoms of the disease, but in no instance during the past three years has there been any evidence of transmission of the disease by means of the seed. All pots and soil were sterilised in an autoclave before use. The seed was sown in the summer of 1937–1938, and plants were from five to nine months old during the period of the tests.

Infected plants were secured in the first instance as a result of mechanical transmission by methods previously


a1
a2
b1
a3
b3
b7
b2
a6
a4
a5
b4
b6
b5
ZECK

reported.[6] The aphids were collected from various sources as indicated later. During the feeding process the insects were kept in association with diseased or with healthy plants by means of cellophane sleeves four to five inches in diameter in which the upper portions of the plants were enclosed. The sleeves were prepared from sheets of cellophane, the edges of which were sealed with gold size, thus forming an open cylinder. The sleeves were placed over the plant, tied with raffia at the base round the lower part of the stem, and after insertion of insects were tied at the upper end round a piece of small glass tubing, plugged with cotton wool and supported by a stake. (Pl. VII, fig. 2.)

In tests with single vectors, the latter were enclosed in tubes held in position over portion of the leaf by means of a spring clip and pad in a slight modification of the method devised by Storey.[12]

After removal from original host plants, some insects were enclosed with healthy passion plants and others with virus infected plants. For convenience these were termed healthy "feeder" and diseased "feeder" plants. After varying periods, the insects from each type of plant were transferred to healthy passion plants known as test plants.

In some instances in order to facilitate expression of a virus, if any, carried by the vectors from the original non-passion fruit host plants, the insects were first placed in bulk on apparently healthy plants—known as "pre-feeder" plants—before subsequent transfer to healthy

---

Explanation to Text-figure 1.

a1. Abdomen of alate viviparous female. (× 45.)
a2. Abdomen of apterous viviparous female. (× 45.)
a3. Cauda of alate viviparous female. (× 225.)
a4. Antenna of alate viviparous female. (× 45.)
a5. Third segments of antenna of alate viviparous female. (× 225.)
a6. Cornicle of alate viviparous female. (× 225.)
b1. Abdomen of alate viviparous female. (× 45.)
b2. Cauda and cornicles of apterous viviparous female. (× 45.)
b3. Cauda of alate viviparous female. (× 225.)
b4. Antenna of alate viviparous female. (× 45.)
b5. Third segment of alate viviparous female. (× 225.)
b6. Cornicle of alate viviparous female. (× 225.)
b7. Cornicle of apterous viviparous female showing variation of form. (× 45.)

a. *Aphis* sp. *A* from *Stizolobium* sp.
b. *Aphis* sp. *B* from *Cotyledon valida*.

feeder and virus-infected feeder plants and thence to the healthy test plants.

On completion of feeding, insects were destroyed by spraying with nicotine sulphate or were removed by means of a brush or by hand. In the case of the latter method, which was utilised in the early tests only, hands were well washed in soapy water before and after treating each plant. Precautions were taken to avoid possible transfer of the disease mechanically during vector removal operations. The glasshouses also were fumigated periodically during the progress of the tests and no evidence was obtained of transfer of the disease to untreated or to check plants during this period.

Transmission Tests with Aphis sp. A.

Large numbers of these aphids were transferred from velvet bean plants to a mechanically infected passion vine which had shown first reaction symptoms on 12/7/38. The results of tests with this vector are summarised in Table 4. In one test (Table 4, Experiment 1), after five days' association, during which the aphids were observed to have fed on the foliage of the diseased plant, approximately forty wingless aphids were transferred to each of three healthy plants and were allowed to feed on these plants for four days. In a somewhat similar test (Experiment 1x) the aphids fed for six days on the same reactor and then were allowed to feed on the test plants for six days.

At the conclusion of the feeding period on these test plants, sleeves and aphids were removed. After six to nine days from the time of transfer of the aphids to the healthy test plants, terminal leaf curl reactions characteristic of the passion fruit woodiness disease developed in all six plants.

The reactions noted in these first experiments (1 and 1x) however, could have resulted from introduction of a virus derived from sources other than that of a diseased passion vine. This, however, would seem most improbable in view of the results obtained with further collections of this aphid from the same velvet bean plants and which consistently failed to demonstrate that the aphids on these plants were naturally infected with a virus capable of bringing about an apparent woodiness virus reaction when transferred to normal passion fruit plants.

TABLE 4.—*Transmission of Passion Fruit Woodiness Virus by Dark* Aphis *sp.* A.

Experiment.	History and Feeding Period. (Days.)	Date of Transfer to Test Plants and Feeding Period. (Days.)	Number and Types of Vector per Plant.	Number of Days after Transfer when Reaction Noted.	Result.*
1	On– M reactor of 12/7/38. (5)	18/7/38 (4)	Approx. 40 wingless.	7–9	3/3
1x	On M– reactor of 12/7/38. (6)	19/7/38 (6)	Approx. 40 wingless.	6–9	3/3
2	On healthy plants. (4)	2/8/38 (2)	Approx. 10 wingless.	Check.	0/6
2a	On V– or M– reactor of 26/7/38. (4)	2/8/38 (2)	10 to 20 wingless including few winged.	6	1/6
3	On " prefeeder " healthy plants 2 days thence to healthy plants. (1)	1/9/38 (4)	20 winged on each of four plants. 10 winged on each of two plants.	Check.	0/6
3a	On " prefeeder " healthy plants 2 days thence to V– reactors of 27/7/38, 8/8/38 or 15/8/38. (1)	1/9/38 (4)	20 to 80 wingless.	7–25	6/10
4	On healthy plants. (5)	28/9/38 (33)	Several hundred in bulk.	Check.	0/15
4a	On V– reactor of 25/7/38. (5)	28/9/38 (33)	Several hundred in bulk.	12†	8/15

M– reactor = mechanically infected passion plant.
V– reactor = diseased plant resulting from vector transmission.
(Date is that of record of first symptoms in reactors.)

* Numerator = number of infected plants.
* Denominator = total number of test plants used.
† First reaction observed.

In Experiment 2, populations of this *Aphis* sp. *A.* were placed on healthy passion plants prior to transference to the healthy test plants. Aphids from the same source were placed (Experiment 2a) also on diseased passion plants which had developed woodiness symptoms as a result of mechanical or insect transmission infections and on which first symptoms were recorded on 26/7/38. One of the six test plants in the infective vector series developed the leaf curl reaction.

In Experiments 3 and 3a the aphid populations were transferred from the velvet beans firstly to normal passion plants (prefeeder plants), portion of this population was then transferred to several healthy passion plants, and another portion to passion plants which had been infected as a result of previous vector transmissions, then in turn populations from the healthy feeder plants were transferred to healthy test plants and aphids from the diseased feeder plants were transferred to a corresponding series of healthy test plants. The prefeeding plants of both series, the feeding plants in Experiment 3 and check test plants all remained healthy, whereas six of the ten plants in the infective vector series developed symptoms of the woodiness virus. This experiment again indicated that the reactions noted in the test plants arose from a virus which had been obtained from diseased passion vines. Temperatures in the glasshouse in early September were rather higher than was considered desirable, and test plants of Experiments 3 and 3a were held in insect-proof cages at lower temperatures prevailing outdoors for six days prior to removal to the glasshouse for final observations. This procedure had the effect in some cases of checking growth of passion vines, and in some instances symptoms were not observed until 25 days after transfer of the aphids to the test plants, whereas, previously, symptoms were noted six to eleven days after transfer of infective vectors.

Experiments 4 and 4a were conducted in muslin-covered insect-proof cages placed outdoors owing to the existence of high temperature day conditions in the glasshouses. This test was of the same general character as the earlier experiments, except that a bulk aphid population was not transferred to healthy passion fruit plants in the first instance. It was conducted for general comparison with a test in which *Macrosiphum solanifolii* was tested as a vector and here again the dark *Aphis* sp. *A* populations which had previously fed on virus-infected passion vines

were demonstrated to be capable of transmitting the woodiness disease to other passion vines. As in this instance no steps were taken to eliminate the aphid population after a fixed feeding or association period with the test plants, infective aphids may have moved to certain of the test plants some time after the date of their introduction to the cage. The earliest infection was observed twelve days after commencement of the test and diseased plants were recorded at intervals up to the conclusion of the test on 31/10/38. Low night temperatures again temporarily slowed up plant growth and thus also possibly resulted in increasing time required for expression of first symptoms of the disease.

It was notable, also, that practically all of the reacting plants occurred in the northern section of the cage—nearest the source of strongest illumination. It would appear that the vectors, after liberation, were attracted to the plants in this portion of the cage.

It should be noted, also, that, although the passion plants proved to be congenial hosts for all aphid vectors tested, critical observations on actual feeding times were not made. For purposes of convenience, times of association of vector on the various plants are designated in the tabulated results as feeding times.

Transmission Tests with Aphis sp. B.

Aphids from *Cotyledon valida* were utilised in a series of transmission experiments the results of which are recorded in Table 5. The methods adopted were similar to those previously utilised except that a bulk population was not transferred in the first instance to a healthy passion plant for prefeeding. It was thought that if the aphids were carrying naturally a virus capable of causing an apparent woodiness reaction in test passion plants, random selection of aphids would most probably result in the development of symptoms in the feeder plants and the test plants of check plant series.

It will be observed from the tabulated results that reactions were obtained only when populations of this aphid had been rendered infective by feeding in the first place on virus-infected passion plants.

Reactions were secured six to eleven days after transfer of the infective aphids to healthy test plants after feeding in the first place on diseased passion plants during an

TABLE 5.—*Transmission of Passion Fruit Woodiness Virus by Dark* Aphis *sp.* B.

Experiment.	History and Feeding Period. (Days.)	Date of Transfer to Test Plants and Feeding Period. (Days.)	Number and Types of Vector per Plant.	Number of Days after Transfer when Reaction Noted.	Result.*
5	On healthy plants. (4)	8/8/38 (3)	10 winged or 10 wingless.	Check.	$\frac{0}{6}$
5a	On V– reactor of 25/7/38. (4)	8/8/38 (3)	10 winged or 10 wingless.	7–9	$\frac{4}{6}$
6	On healthy plants. (2)	19/8/38 (5)	20 wingless.	Check.	$\frac{0}{6}$
6a	On M– reactor of 19/4/38 or V– reactors of 25/7/38 or 8/8/38. (2)	19/8/38 (5)	Approx. 20 wingless on each of 9 plants. Approx. 100 wingless on each of 2 plants.	7–11	$\frac{9}{11}$
7	On M– reactor of 22/7/38. (4)	23/8/38 (2)	1 wingless.	13–21	$\frac{2}{24}$
7b	On M– reactor of 22/7/38. (4)	23/8/38 (3)	Approx. 100 wingless.	6–8	$\frac{5}{6}$

M– reactor = mechanically infected passion plant.
V– reactor = diseased passion plant resulting from vector transmission.
(Date is that of record of first symptoms in reactors.)

* Numerator = number of diseased plants.
* Denominator = total number of test plants used.

association period with these plants of from two to four days.

Tests with single aphids resulted in the development of woodiness symptoms in two plants of the 24 plants tested. In this small experiment no special significance is to be attributed to an apparent delay in the expression of a definite reaction. In tests with *Myzus persicæ* reported later, reactions were observed to occur within the same general time limits whether single aphids or small populations of aphids were utilised.

Transmission Tests with Myzus persicæ Sulz.

During the progress of tests with the dark aphid species, vector transmission experiments were conducted also with *Myzus persicæ*, the green peach aphid. Populations of this aphid were collected from sprouting potatoes. As in the preliminary experiments with the dark aphids, the first tests merely involved transfer of populations of *Myzus persicæ* after the latter had fed for from one to three days on passion plants mechanically infected with the woodiness virus. As indicated in Table 6 (Experiments 8 and 9) reactions were obtained seven to eleven days after transfer of the aphids to test plants.

The subsequent series of tests involved the use of check plants and of prefeeder as well as check plants as in tests with other vectors, and demonstrated that woodiness virus disease symptoms arose only as a result of the use of vectors which became infective after having fed previously on vines affected with woodiness.

Experiments 8 to 12a, 16, 16a, and 17a were conducted with individual plants enclosed in cellophane. Experiments 15 and 15a were bulk tests conducted in insect-proof cages, in which the check population comprised aphids which had fed on healthy passion plants and the reacting series comprised those which had fed on infected passion plants. The cages were held at low temperatures during the feeding period, growth was checked in some plants, and the reaction period ranged from 10 to 21 days.

The vectors freshly collected from potatoes were not carrying a virus which resulted in the development of reaction in healthy passion plants, as in all cases check plants and prefeeder plants remained healthy.

It is of interest to note, also, that in the tests with *Myzus persicæ* the reacting plants used as feeders were frequently those in which leaf curl symptoms had developed

TABLE 6.—*Transmission of Passion Fruit Woodiness Virus by* Myzus persicæ.

Experiment.	History and Feeding Period. (Days.)	Date of Transfer to Test Plants and Feeding Period. (Days.)	Number and Types of Vector per Plant.	Number of Days after Transfer when Reaction Noted.	Result.*
8	On M– reactor of 19/4/38. (1)	26/7/38 (3)	Approx. 30 winged and wingless.	7–9	$\frac{2}{3}$
9	On M– reactors of 19/4/38 and 30/5/38. (3)	28/7/38 (6)	Approx. 40 winged and wingless.	7–11	$\frac{6}{6}$
10	On healthy plants. (2)	28/7/38 (5)	Approx. 12 winged and 100 wingless.	Check.	$\frac{0}{6}$
10a	On M– reactor of 25/7/38. (2)	28/7/38 (5)	Approx. 10 winged and 100 wingless.	7–10	$\frac{2}{6}$
11	On M– reactor of 25/7/38. (4)	29/7/38 (5)	Approx. 50 winged and wingless.	9	$\frac{1}{2}$
12	On healthy plants. (1)	9/8/38 (3)	10 winged on one plant, 20 wingless on each of five others.	Check.	$\frac{0}{6}$
12a	On M– reactor of 8/8/38. (1)	9/8/38 (3)	10 to 20 winged or wingless.	6–9	$\frac{2}{6}$

M– reactor = mechanically infected passion fruit.
V– reactor = diseased plant resulting from vector transmission.
(Date is that of record of first symptoms in reactors.)

* Numerator = number of infected plants.
* Denominator = total number of test plants used.

TABLE 6.—*Transmission of Passion Fruit Woodiness Virus by* Myzus persicæ.—Continued.

Experiment.	History and Feeding Period. (Days.)	Date of Transfer to Test Plants and Feeding Period. (Days.)	Number and Types of Vector per Plant.	Number of Days after Transfer when Reaction Noted.	Result.*
15	On healthy plants. (4)	19/8/38 (5)	Approx. 60 winged and several hundred wingless. (Bulk.)	Check.	0/9
15a	On M– reactor of 12/7/38 and V– reactors of 27/7/38 and 8/8/38. (1)	19/8/38 (5)	Approx. 60 winged and several hundred wingless. (Bulk.)	10–21	6/9
16	On " prefeeder " healthy plants one day thence to healthy passions. (1)	26/8/38 (3)	7–10 winged.	Check.	0/6
16a	On " prefeeder " healthy plants one day, thence to M– reactors of 24/6/38 and 19/7/38 and V– reactor of 27/7/38. (1)	26/8/38 (3)	10 winged for each of 6 plants. 50 winged for each of 2 plants.	6–11	3/8
17	On " prefeeder " healthy plants one day, thence to V– reactor of 27/7/38. (1)	31/8/38 (1)	1 winged.	7–8	3/25
17a	On " prefeeder " healthy plants one day, thence to V– reactor of 27/7/38. (1)	31/8/38 (1)	7 winged.	8	1/1

M– reactor = mechanically infected passion fruit.

V– reactor = diseased plant resulting from vector transmission.
(Date is that of record of first symptoms in reactors.)

* Numerator = number of infected plants.

* Denominator = total number of test plants used.

just prior to their use as feeder plants. This was the case also in a number of the experiments in which *Aphis* sp. *A* was tested as a vector. Observations, however, were made in the *Myzus persicæ* tests 10a, 11, and 12a on the portions of the reactor plants from which the presumably infective aphids were obtained. It was noted that reactions were subsequently recorded in test plants only when the vectors were secured from the upper leaves of the diseased feeder plants. It would appear that the virus was not present in the symptomless leaves of the freshly diseased feeder plants during the feeding period of these tests.

Tests with single aphids as recorded in Experiment 17 demonstrated that the virus was transferred to three plants of the 25 plants tested. No attempt was made to determine minimum feeding or transmission times as has been demonstrated for this vector in the case of other virus diseases. The experiment indicated, however, that one aphid might secure the virus after feeding for 24 hours or less on a diseased passion plant and that it might transfer the disease to a healthy passion plant after feeding on it for 24 hours or less.

Transmission Tests with Macrosiphum solanifolii (Ashm.).

During August and September, 1938, populations of *Macrosiphum solanifolii* became available, and, although glasshouse conditions were becoming unfavourable on account of high day temperatures, it was decided to test this aphid as a possible vector of the passion fruit woodiness virus. Populations of the aphid were collected from sow thistles or milk thistles (*Sonchus oleraceus*), from tomatoes and later from gladioli.

In experiments 18 and 18a as indicated in Table 7, aphid populations were enclosed on healthy passion plants and on passion plants affected with the woodiness disease. After transfer of aphids from diseased to healthy test plants, leaf curl symptoms were subsequently recorded in two of the four test plants used.

In Experiments 19 and 19a bulk populations of aphids after identification were transferred to healthy prefeeder plants, and portions of these populations were then transferred to healthy feeder and to diseased feeder plants before subsequent transfer to the test plants. Three of the five test plants in the infective vector series developed the leaf curl reaction.

TABLE 7.—*Transmission of Passion Fruit Woodiness Virus by* Macrosiphum solanifolii.

Experiment.	History and Feeding Period. (Days.)	Date of Transfer to Test Plants and Feeding Period. (Days.)	Number and Types of Vector per Plant.	Number of Days after Transfer when Reaction Noted.	Result.*
18	On healthy plants. (2)	1/9/38 (4)	10 wingless.	Check.	$\frac{0}{3}$
18a	On M– reactors of 19/4/38 and V– reactor of 4/8/38. (1)	1/9/38 (4)	10 wingless.	7–11	$\frac{2}{4}$
19	On " prefeeder " healthy plants one day, then to healthy plants. (2)	2/9/38 (3)	10–20 wingless.	Check.	$\frac{0}{3}$
19a	On " prefeeder " healthy plants one day, then to M– reactors of 19/4/38 and 24/6/38 and V– reactor of 27/7/38. (2)	2/9/39 (3)	15.	8–10	$\frac{3}{5}$
20	On healthy plants. (4)	26/9/38 (35)	About 100. (Bulk.)	Check.	$\frac{0}{18}$
20a	On V– reactors of 27/7/38 and 2/9/38. (4)	26/9/38 (35)	About 100. (Bulk.)	10†	$\frac{7}{18}$

M– reactor = mechanically infected passion fruit.
V– reactor = diseased plant resulting from vector transmission.
(Date is that of record of first symptoms in reactors.)

* Numerator = number of infected plants.
* Denominator = total number of test plants used.
† First reaction observed.

In Experiments 20 and 20a, the aphids were enclosed in bulk with eighteen test plants in butter-muslin-covered insect-proof cages after having fed in one instance on healthy passion plants and in the other on plants affected with woodiness. In this test, the aphids were not destroyed at the close of a definite feeding or association period. First reactions were observed ten days after transfer of the presumably infective aphids to the test plants and further records of transmission were noted up to 31/10/38 when the experiment was terminated. In this outdoor test, it was noted as in the outdoor test with *Aphis* sp. *A*, that most of the reacting plants were situated in the northern portion of the cage in the region close to the source of strongest light intensity.

In all cases as before prefeeder and feeder healthy plants as well as the check or control plants remained healthy. Evidence was obtained as recorded in Table 7 to the effect that *Macrosiphum solanifolii* was capable of functioning as a vector of the virus of passion fruit woodiness.

Discussion.

Tests with two species of the genus *Aphis* and with *Myzus persicæ* and *Macrosiphum solanifolii* have demonstrated that these aphids are capable of transmitting the passion fruit woodiness virus from affected to healthy passion plants. Experiments with various species of thrips and of jassids during the past three years did not result in transmission, and it would appear that such insects are not capable of transmitting the disease. Further tests, however, are necessary before it can be stated conclusively that certain aphids only are capable of transmitting the disease.

Myzus persicæ and *Macrosiphum solanifolii* are recorded as vectors of other plant viruses. It is possible also that the *Aphis* sp. *A* and *B* are also known as plant virus vectors under specific designations among the dark coloured forms in the genus *Aphis*. Such designations, however, can be accepted only with reserve. According to Smith,[11] (p. 526), *Aphis rumicis* is recorded as a vector of five distinct plant viruses, *Myzus persicæ* (*ibid.*, p. 541) as a vector of at least 21 and possibly 23 plant viruses and *Macrosiphum solanifolii* (*ibid.*, p. 533) of ten plant viruses. Figures such as the foregoing are dependent on the characterisation of the viruses under consideration and on the acceptance of a specific determination for an aphid

such as *Aphis rumicis*. There is, however, an absence of specificity in the abovementioned aphids for transmission of a particular virus.

Furthermore, investigation has shown that in some cases at least, a large number of aphid species may be capable of transmitting a single plant virus. Drake *et al.*[4] have recorded that the Onion Yellow Dwarf virus may be transferred by 53 species of aphids as well as by several undetermined species. Zaumeyer and Kearns[14] record that at least eleven species of aphids are capable of transmitting the common bean mosaic virus. It is now generally conceded that in such instances the vectors are merely mechanical conveyors of the virus from one plant to another.

It would appear probable that aphid species other than the four recorded in this paper may be capable of transmitting the virus of passion fruit woodiness, but under field conditions in N.S.W. it is possible that *Myzus persicæ* is the most important of the insect vectors concerned.

Recommendations for the control of woodiness have stressed the value of sanitation measures, and such measures have been adopted with success by a number of growers. These measures include the planting only of healthy seedlings, removal of young plants showing woodiness symptoms, and finally the complete eradication of diseased plantations before replanting on the same area or in its vicinity.

Watson[13] has recorded that the adoption of spraying measures for destruction of *Myzus persicæ* during the first few weeks of growth of *Hyoscyamus niger* resulted in increased yields of the crop when cultivated as a biennial.

As passion vines in N.S.W. apparently are subject to visitation by aphids only at infrequent intervals, there is justification for the hope that adoption of aphicidal measures at appropriate periods may be economically practicable and may be of material value in increasing yields and in prolonging the productive life of commercial plantations.

Such measures, in any case, appear worthy of adoption in conjunction with sanitation measures for the development of passion fruit seedlings free from the virus of the woodiness disease.

Summary.

1. Statistical information on the production of passion fruit in N.S.W. indicates the existence of a low level of production as well as a decline in the average production per vine in recent years.

2. The woodiness disease is now regarded as the most serious of the diseases affecting passion fruit.

3. Further observations on the development of symptoms are reported.

4. There was no indication of transmission of the disease by means of the seed.

5. Observations particularly during the past three years, indicated that passion vines are not subject, as a rule, to serious infestation by insects. Occasional infestations by species of jassids, thrips, and aphids were recorded. The aphids *Myzus persicæ* and *Macrosiphum solanifolii* were recorded from time to time; the latter, however, appeared to occur even less frequently than the former.

6. It was demonstrated that two species of dark aphids characterised in this paper as *Aphis* sp. *A* and *Aphis* sp. *B*, *Myzus persicæ* and *Macrosiphum solanifolii* were capable of transmitting the virus of woodiness disease to passion plants. The disease developed as a rule in from six to eleven days after infective aphids had fed on healthy plants under the conditions of the experiments.

7. In a limited series of tests it was demonstrated, in the case of *Aphis* sp. *B* and *Myzus persicæ*, that one aphid could obtain the virus by feeding for 24 hours or less on a diseased passion plant and could transmit the disease after feeding on a healthy passion plant for 24 hours or less.

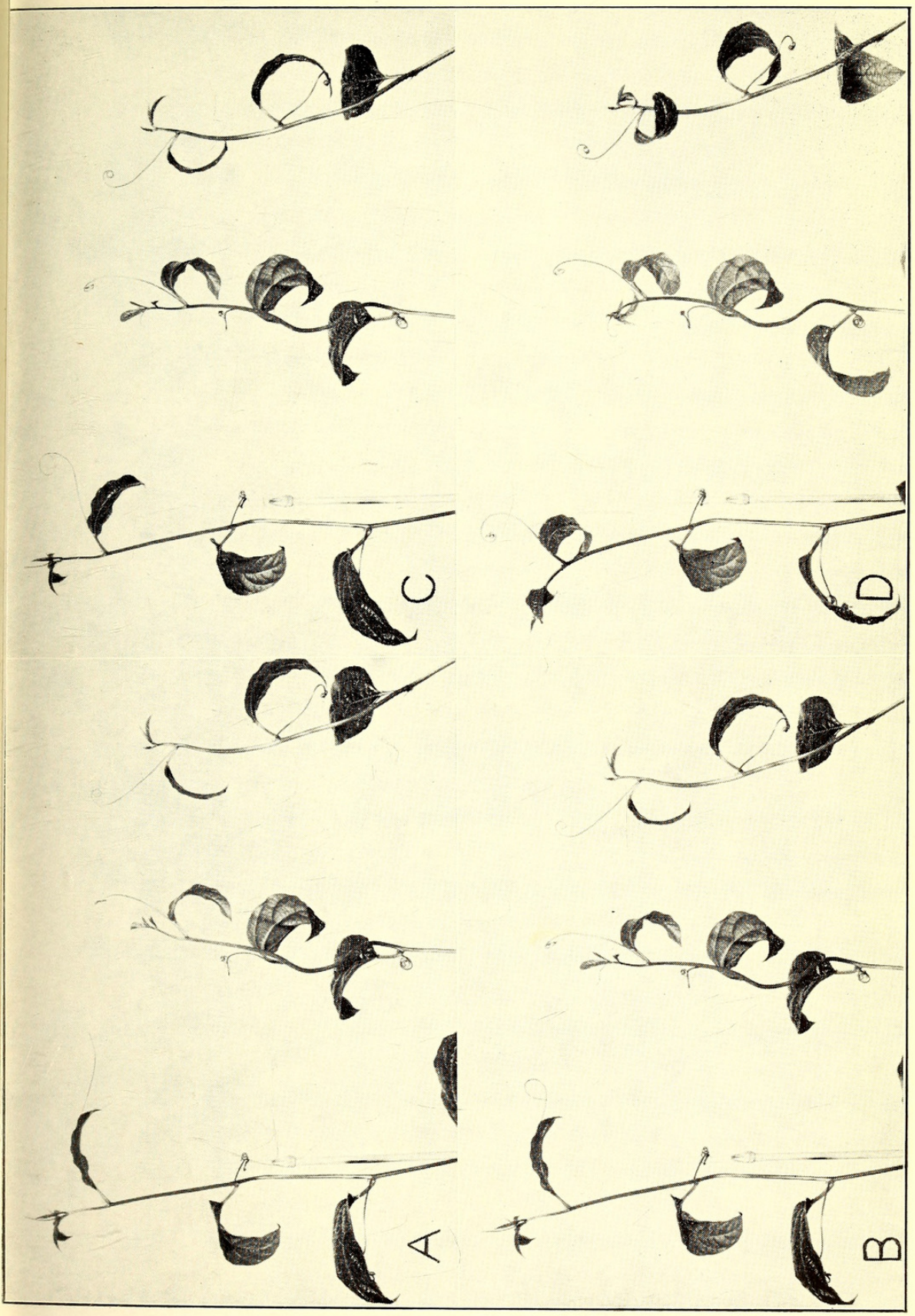
8. Tests failed to demonstrate that insects other than aphids were capable of functioning as vectors of the woodiness virus.

9. It is suggested that the adoption of aphicidal measures at appropriate periods may be economically practicable. Such measures appear to be worthy of adoption at least in beds of seedling plants in conjunction with other measures designed to minimise the incidence of the woodiness disease.

Literature Cited.

(1) Bewley, W. F.: Mycological Report. *Eighth Ann. Rept. Cheshunt Exp. and Res. Stn.*, Hertfordshire, 1922 (1923), pp. 34–45.* (Abstracted in *Rev. Appl. Mycol.*, 1923, *2*, 489.)

* Original papers not seen by authors.


C
D
A
B

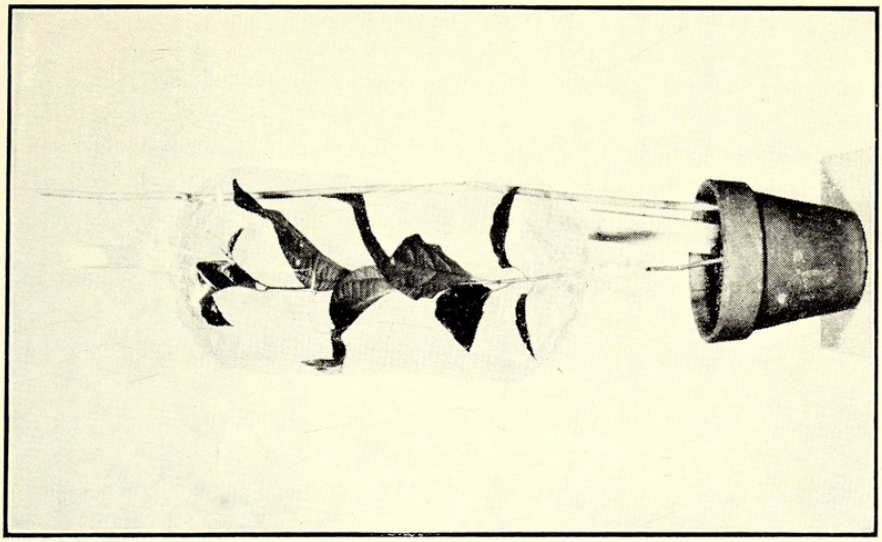


Fig. 2.

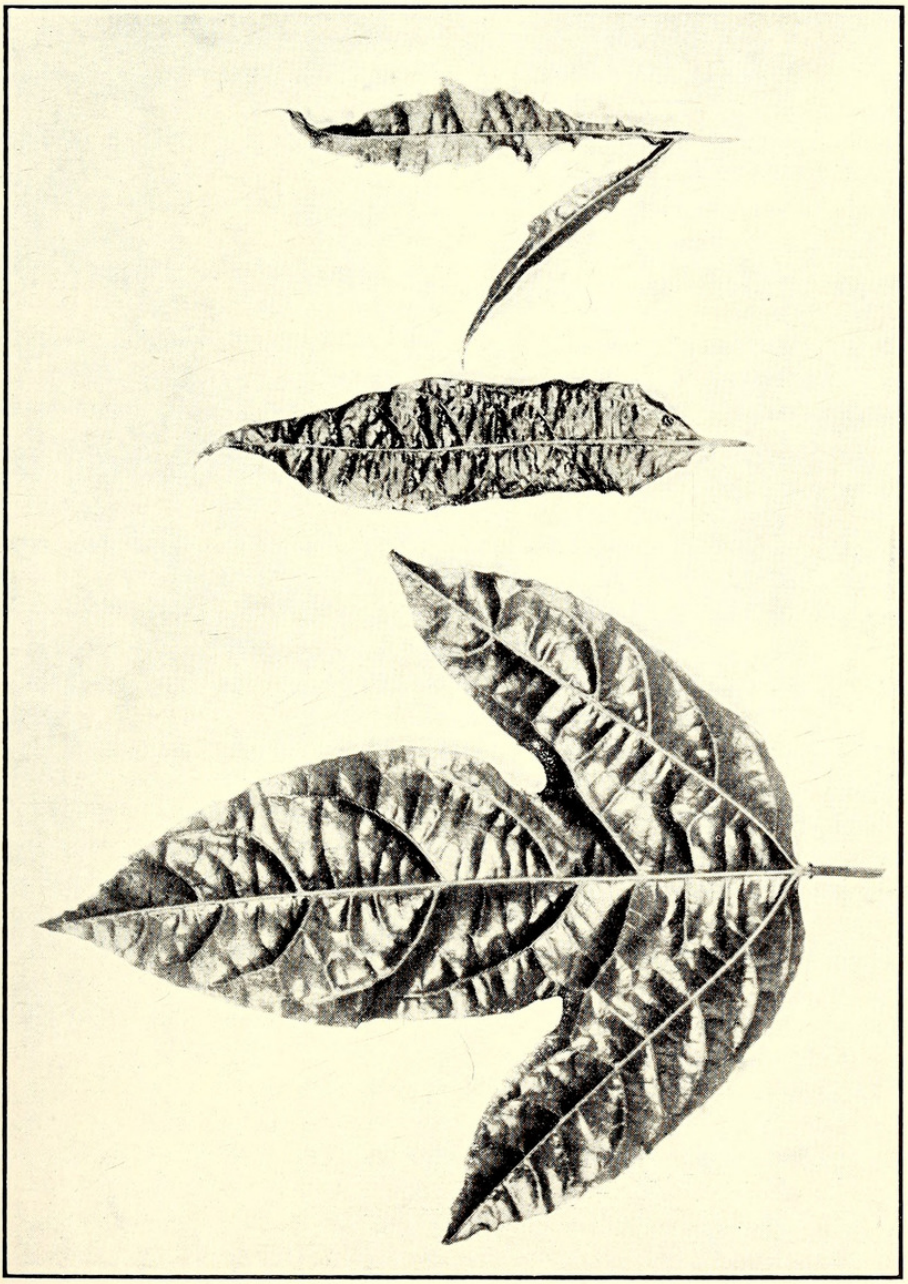


Fig. 1.

(2) Butler, E. J. : Some Aspects of the Morbid Anatomy of Plants, *Ann. Appl. Biol.*, 1930, *17*, 175–212.

(3) van der Byl, P. A. : Agriculture on the Winter Rainfall Area. The Work of an Important Institution. *Farming in South Africa*, 1931, *6*, 357.

(4) Drake, C. J., H. D. Tate and H. M. Harris : The Relationship of Aphids to the Transmission of Yellow Dwarf of Onions. *Jour. Econ. Entom.*, 1933, *26*, 841–846.

(5) McDonald, J. : Report of the Senior Plant Pathologist. *Rep. Dept. Agr. Kenya*, 1936 (1937), *2*, 1–12.

(6) Noble, R. J. : Some Observations on the Woodiness or Bullet Disease of Passion Fruit, *Jour. Roy. Soc. N.S.W.*, 1928, *62*, 79–98.

(7) ———— : Report of the Biologist, *Rept. Dept. Agr. N.S.W.*, for year ended 30th June, 1930 (1931), p. 20.

(8) Palm, B. T. : Verslag van het Deli Proefstation over 1 Juli 1920–30 Juni 1921,* *Meded. Deli. Proefstat. te Medan-Sumatra*, Series II, *21*, 72 pp., 4 figs., 1921. (Abstracted in *Rev. Appl. Mycol.*, 1923, *2*, 35.)

(9) Simmonds, J. H. : Powdery Spot and Fruit Scab of the Passion Vine, *Queensl. Agr. Jour.*, 1932, *38*, 143–152.

(10) ———— : Passion Vine Diseases, *Queensl. Agr. Jour.*, 1936, *45*, 322–330.

(11) Smith, K. M. : A Text Book of Plant Virus Diseases, 615 pp., illus., 1st Edn., London, J. and A. Churchill, 1936.

(12) Storey, H. H. : Transmission Studies of Maize Streak Disease, *Ann. Appl. Biol.*, 1928, *15*, 1–25.

(13) Watson (Hamilton), M. A. : Field Experiments on the Control of *Aphis*-transmitted Virus Diseases of *Hyoscyamus niger*, *Ann. Appl. Biol.*, 1937, *24*, 557–573.

(14) Zaumeyer, W. J., and C. W. Kearns : The Relation of Aphids to the Transmission of Bean Mosaic, *Phytopath.*, 1936, *26*, 614–629.

Explanation of Plates.

Plate VI.

Passion fruit plants affected with the woodiness virus and showing development of terminal leaf curl symptoms. ($\times \frac{1}{5}$.)

A	photographed	10.30 a.m.	26/8/38.
B	,,	12.30 a.m.	26/8/38.
C	,,	4.30 p.m.	26/8/38.
D	,,	10.00 a.m.	29/8/38.

Plate VII.

Fig. 1.—Leaves from virus-infected passion fruit plants showing abnormal lamina developments.
Left : Leaf from healthy plant.
Right : Leaves from virus-infected plants. ($\times \frac{4}{5}$.)

Fig. 2.—Passion fruit plant enclosed in cellophane sleeve for insect vector feeding test. ($\times \frac{1}{8}$.)

(Photos by Mr. E. G. Pont.)