THE TRANSMISSION OF WITCHES' BROOM VIRUS DISEASE OF LUCERNE BY THE COMMON BROWN LEAFHOPPER, OROSIOUS ARGENTATUS (EVANS)

By G. A. H. HELSON*

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Summary

The disease was transmitted by Orosius argentatus (Evans) collected in infected lucerne crops, and also by groups of virus-free individuals reared under experimental conditions, after having been fed on plants infected with witches' broom virus.

The following plants became infected with the virus under experimental conditions: Beta vulgaris L.; Datura stramonium L., Erodium cicutarium (L.) L.Hér., Hypochaeris radicata L., Lycopersicon esculentum Mill., Medicago sativum L., Vinca rosea L. Lucerne was difficult to infect and the first symptoms took about seven months to appear (but see note on infection of lucerne).

In all plants the symptoms caused were severe stunting, proliferation of axillary shoots, and the production of green flowers. In tomato and other hosts these symptoms closely resembled those of tomato big bud virus. The same vector transmits them and it is suggested that the two diseases may be caused by the same or closely related strains of virus.

I. INTRODUCTION

Witches' broom, a virus disease of lucerne (Medicago sativum L.), is prevalent throughout the inland areas of Queensland, New South Wales, Victoria, and South Australia and has been reported from north-western Australia and the west coast of the United States of America (Menzies 1946). The disease is characterized by severe stunting and proliferation of shoots produced from the crown (Plate 1, Fig. 1). Usually, plants fail to flower, but occasionally very small blooms or green flowers are produced (Plate 1, Fig. 2). The disease has been in Australia for at least 40-45 years and was first recognized to be caused by a virus by Edwards (1935a, 1935b, and 1936) who was able to transmit it to healthy lucerne plants by grafting. Field surveys made by Edwards demonstrated that lucerne fields commonly showed 20-25 per cent. infection with the disease and occasionally old stands were observed in which 60-70 per cent. infection occurred. Diseased plants yielded 37 per cent. less weight of green fodder than was obtained from unaffected plants (Edwards 1935). In addition, the disease considerably shortened the life of lucerne stands in regions receiving an annual rainfall of 17-21 in. and, where grown for seed production, the diseased plants were a total loss (Edwards 1936).

* Formerly Division of Entomology, C.S.I.R.O. Present address: Department of Agriculture, Wellington, N.Z.
considered it highly probable that the disease was spread, under natural conditions, by an insect vector but his attempts to transmit the disease with insects were unsuccessful.

The present paper describes the results of the first year of an ecological survey of leafhoppers occurring in infected lucerne fields along the Lachlan River, N.S.W., and in the Australian Capital Territory, and the results of transmission experiments with one of these, *Orosius argentatus* (Evans).

**II. Field Survey**

Before experiments for the transmission of the disease could begin it was necessary to know what possible insect vectors frequented fields of lucerne where the disease was prevalent. As the disease was suspected of belonging to that group of virus diseases, most of which are transmitted by leafhoppers, it was decided to concentrate on these insects and to make collections from eight stations, along the River Lachlan between Cowra and Jemalong, N.S.W., at three-monthly intervals. In addition, weekly sweeps were made at Dickson Experiment Station, A.C.T., on a lucerne field infected with witches’ broom.

Where possible, on the field survey, sweeps were made with two 14-inch nets trawled over the lucerne from the sides of a truck moving at 10 miles per hour over a distance of one-fifth of a mile. Four such sweeps were made at each station. Where it was not possible to sweep from the truck, two hundred hand sweeps were made. At Dickson Experiment Station, all sweeps were made with two 14-inch nets from the sides of a jeep travelling at 10 miles per hour. Two traverses of 0.1 mile each were made across each diagonal of the field and the total number of insects caught in the four traverses was recorded.

Possible vectors included in the large numbers of insects caught were aphids, thrips, and leafhoppers and of these leafhoppers were the most abundant. During the 1947-48 survey the following jassids commonly occurred in lucerne fields at all eight stations on the Lachlan River and at Dickson Experiment Station: *Orosius argentatus* (Evans), *Erythroneura ix* Myers, *Empoasca viridigrisea* Paoli, *Balclutha* sp., *Eurinoscopus punctatus* Ev., *Nehela torrida* Ev., *Exitianus (Euscelis) indicus* (Dist.) (= *Euscelis norrisi* Ev.), *Nesoclutha obscura* Ev., *Euscelis* sp., and *Thamnotettix* sp. The first three were the most abundant at all places.

At Dickson, A.C.T. (Fig. 1), the weekly sweeps began on November 6, 1947. *O. argentatus* reached its maximum numbers by mid November, and except for a short period in December when heavy rains and cold weather prevailed, the number remained at a high level until the third week in December. During this period, *E. ix*, *E. viridigrisea*, *E. punctatus*, and *Balclutha* sp. were present in small numbers only. After the end of December, the number of *O. argentatus* decreased, whereas the other four leafhoppers all increased in numbers, but at no stage did they approach the maximum recorded for *O. argentatus* during the early summer. This species had a small autumn maximum in late February and March. By the end of May all species (except *E. ix*, small numbers of which occurred throughout the winter) ceased to be taken
in the sweeps and did not reappear until spring. *O. argentatus* began to appear in the sweeps again in the second week in September and reached a spring peak by early November. This peak, however, was only one-third as great as that of 1947. *O.argentatus* was therefore most abundant during spring and early summer whereas the other four species were most abundant during summer and autumn.

Of these species, *O. argentatus* was the only leafhopper known to be a vector of virus diseases. It transmits tobacco yellow leaf dwarf and tomato big bud virus diseases and was therefore chosen for the initial witches' broom transmission experiments in the glass-house. Also, it belongs to the sub-family Euscelinae, which contains a large number of vectors of virus diseases, including *Circulifer tenellus* (Baker), which transmits curly-top of sugar beet in California (Shaw 1910), and *Eutettix phycitis*, which transmits "little-leaf" of egg-plant in India (Thomas 1939).

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**III. GEOGRAPHICAL DISTRIBUTION OF THE VECTOR**

*Orosius argentatus* is widely distributed throughout Australia and extends from the moist tropics in the north to the dry temperate regions in the south and west. It feeds on a large number of host plants and is able to breed on many of these (Helson 1942). It also appears to be widely distributed throughout the Australian region, for Oman (personal communication) records having seen specimens from Eniwetok, Ulithi, Guam, Larat, Amboina, Fiji (collected 1908), and Canton I. (Oman 1943) in the Australian region and specimens that are probably *argentatus* from Sunday I. in the Kermadec Group, New Zealand region, and from the Philippines in the oriental region. To date this leafhopper has not been recorded from the Hawaiian Is. or from New Zealand.
IV. Bionomics of Vector

The habits and life cycle of *O. argentatus* have been recorded elsewhere (Helson 1942). In southern inland districts, there are three complete but overlapping generations in a year. The length of the life cycle varies from 32 to 50 days, depending on the time of the year. Breeding plants in addition to those already listed (Helson 1942) are: Brassica adpressa Boiss. (hairy brassica), Silybum marianum (L.) Gaertn. (variegated thistle), Sonchus oleraceus L. (common sow thistle), Plantago lanceolatum L. (ribwort), Callistephus chinensis (L.) Nees (aster), and Atriplex semibaccata R.Br. (creeping saltbush).

Aster is a favoured host plant, both for feeding and breeding, whereas tomato, tobacco, and lucerne are not preferred. In the laboratory, the leafhopper not only fails to breed on these but rapidly dies if fed exclusively on them. However, the insects thrive in the lucerne fields where other feeding plants are generally present.

V. Transmission Experiments

(a) *By Naturally Infected Leafhoppers*

In November 1947, numbers of wild *O. argentatus* were collected from a lucerne field at Dickson Experiment Station, by the method already described, and were placed in a cage in the glass-house with a plant of *Datura stramonium* and a small-flowered malva (*Malva parviflora*). Thirty-five days later the datura* leaves showed vein banding and interveinal chlorosis, and 62 days later green flowers were produced, the leaves were reduced in length and width, and axillary growth was stimulated. The malva did not develop any virus symptoms.

In December 1947, a second field collection of leafhoppers was made in the same way and these also were placed in a cage in the glass-house together with four datura plants and three malva plants. After a period of 44 days, one of the daturas produced green flowers and had vein banding on the young leaves, one had definite vein banding on the young leaves, and the other two plants showed interveinal chlorosis of the young leaves. Seventy-four days after being placed in the cage with leafhoppers, all datura plants were producing green flowers. The malva plants did not exhibit any symptoms of virus disease. Control plants used as food plants for the virus-free insect colonies were all flowering normally at this time.

A scion, taken from one of the daturas with the green flowers, was then grafted to a young tobacco seedling (variety Hickory Pryor) 156 days after the datura had been exposed to the insects. The graft was successful and within 36 days the veins on the young tobacco leaves began to whiten and the leaf blades took on a glazed appearance. One hundred and fifty-six days after grafting, the plant had produced green flowers and was producing small bunched terminal leaves, both pronounced symptoms of virus disease (Plate 2, Fig. 1).

As these first tests were made with wild insects collected from a field of lucerne, the virus disease transmitted to daturas in the two experiments just

*Unless otherwise stated, “datura” = *Datura stramonium.*
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described could have been acquired from weeds or other crop plants growing in the vicinity at that time.

(b) By Laboratory-Infected Leafhoppers

A series of laboratory experiments using virus-free insects from colonies established in the glass-house were conducted to show that the disease obtained from lucerne in the laboratory was the same as that carried by the leafhoppers collected in the field. Virus-free insects were reared from parents that had been picked off their breeding plants as they hatched and transferred to virus-free breeding plants before they had commenced feeding. No symptoms developed in these plants nor in other healthy daturas added to the colonies from time to time to test the virus-free insects.

A lucerne plant infected with witches' broom virus disease taken from the lucerne field in 1946 was used as the sole source of virus disease in all the later experiments (Plate 1, Fig. 1). The experimental results appear in Table 1.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Time in Days</th>
<th>Fed on infected lucerne</th>
<th>Fed on healthy host</th>
<th>To appearance of green flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago sativa</em></td>
<td>8</td>
<td>7</td>
<td>14</td>
<td>195</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>44</td>
<td>10</td>
<td>96</td>
<td>81</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>44</td>
<td>10</td>
<td>83</td>
<td>—</td>
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<tr>
<td><em>Datura stramonium</em></td>
<td>44</td>
<td>10</td>
<td>83</td>
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<tr>
<td><em>Datura stramonium</em></td>
<td>44</td>
<td>10</td>
<td>15</td>
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<tr>
<td><em>Lycopersicon esculentum</em> (var. Rouge de Marmonde)</td>
<td>44</td>
<td>10</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em> (var. Rouge de Marmonde)</td>
<td>44</td>
<td>10</td>
<td>35</td>
<td>—</td>
</tr>
<tr>
<td><em>Hypochaeris radicata</em></td>
<td>44</td>
<td>10</td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td><em>Erodium cicutarium</em></td>
<td>44</td>
<td>10</td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td><em>Vinca rosea</em></td>
<td>92</td>
<td>14</td>
<td>76</td>
<td>68</td>
</tr>
<tr>
<td><em>Beta vulgaris</em></td>
<td>100</td>
<td>20</td>
<td>20</td>
<td>123</td>
</tr>
<tr>
<td><em>Beta vulgaris</em></td>
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<td>3</td>
<td>14</td>
<td>129</td>
</tr>
</tbody>
</table>

Experiment 1.—Forty-four laboratory-infected *O. argentatus* were placed in a cage with one datura, one malva, and one lucerne plant. Eighty-one days after first exposure to the insects, the datura began to show vein banding and interveinal chlorosis of the leaves, and 96 days from the beginning of the experiment was producing green flowers. The datura was still showing pronounced virus symptoms and was producing green flowers (Plate 2, Fig. 2) 146 days after exposure. The malva died without showing any symptoms, and the lucerne plant remained healthy.
Experiment 2.—Two datura plants added to the same cage produced green flowers in 83 days and showed pronounced symptoms of virus disease after 112 days. Another datura was then added to the cage for 15 days, after which it was taken out and pruned. Forty-four days after pruning it too produced green flowers, and 62 days later was showing pronounced virus symptoms. Three other daturas and one D. tatula used as feeding and breeding plants in the cage likewise produced green flowers and other pronounced virus symptoms.

Experiment 3.—Two tomato seedlings were placed for 35 days in the cage containing the infected plants on which the leafhoppers were then breeding. At the end of this time, the veins of the leaves were beginning to appear translucent so the plants were removed and kept for observation. At the end of 44 days, the main veins of the young leaves and the growing tips were showing a pronounced purpling, the marginal leaflets were showing epinasty, and the leaves were reduced in size. The veins were translucent and prominent, giving a netted appearance. One plant had already produced green flowers and the second plant did so three days later. Fifty-six days from the beginning of the experiment, the plants were showing very definite signs of virus disease. These became more and more pronounced as time went on and the axillary buds of both plants grew and produced dwarf leaves with purple veins with a curling and twisting of the main vein. The leaves were rugose and at 89 days the plant showed a rosetted appearance at the top caused by the stunting of growth of the main stem and the proliferation of numerous axillary shoots with dwarf leaves and green flowers. One month later the plants were still dwarfed and remained so until death (Plate 3, Fig. 1).

Experiment 4.—One common crowfoot plant (Erodium cicutarium) was added to the same cage one month after the two tomato seedlings and after 15 days was removed and kept for observation. Forty-one days later, the older leaves of the plant were curled and rolled downwards and the new leaves were a light green, erect, and spindly. A week later the young leaves were light green, very much reduced in size, with vein banding, and the plant produced small, green flowers on thin, spindly stems. Similar symptoms were observed in the field locally (Plate 3, Fig. 2); 89 days after the experiment began, the plant was dying.

Experiment 5.—One month after the two tomato seedlings, a flatweed plant (Hypochoeris radicata) was also added to the same cage and allowed to remain for 15 days. Odd spindly leaves began to appear 41 days later and the new leaves were chlorotic. This was the same as the time taken for virus symptoms to appear in the crowfoot. One week later the plant had produced a large number of new leaves, much reduced in size, spindly, and with narrow, chlorotic leaf blades. The plant collapsed and died from a crown necrosis 54 days after the experiment began and before flowers were produced, so it is not known whether green flowers would have been formed. Plants with green flowers but no leaf symptoms were subsequently found in the field.
Experiment 6.—One hundred virus-free *O. argentatus* were allowed to feed for 20 days on two daturs, three sugar beet (*Beta vulgaris*), one malva, and the diseased lucerne in a cage. The plants were then removed and kept for observation. One of the three sugar beet plants began to throw spindly leaves with very narrow leaf blades 123 days later, and within 183 days was showing pronounced virus symptoms. The plant wilted and collapsed 219 days after the beginning of the experiment, and showed a crown necrosis very similar to that of the flatweed. Again no flowers were formed before death. All the other plants remained healthy. In a second experiment with a sugar beet on which 10 infective insects fed for three days, the plant became similarly affected in 129 days (Plate 4).

Experiment 7.—A *Vinca rosea* seedling was placed in the transmission cage for 76 days with 92 leafhoppers that had fed on the witches’ broom-infected lucerne. Forty-eight days after exposure commenced, leafhoppers were still feeding on the plant, which was flowering normally (white flowers) and resembled its control plant in every respect. Sixty-eight days from the beginning of the experiment, however, green flowers were produced and thereafter a few green and white flowers were formed, together with some entirely green flowers in which the corolla tube was shortened. In some instances the gynoecium became swollen and burst through the walls of the corolla. Thereafter the plant continued to produce green flowers.

Experiment 8.—As the disease had not so far been transmitted from lucerne to lucerne, 80 virus-free jassids were confined on the diseased lucerne plant for seven days. However, lucerne is not a preferred host plant and the leafhopper cannot breed upon it (Helson 1942). A high mortality always results where these insects are placed on lucerne for more than one or two days without an alternate host plant. Only eight leafhoppers were recovered at the end of the feeding time and these were placed in a cage with two healthy lucerne seedlings for 14 days. All the leafhoppers died by the end of this period, and the plants were retained for observation. One plant subsequently died, but at the end of 195 days, the other began producing small, light green rugose leaves more rounded than usual. This rugosity and roundness, apparently symptoms of witches’ broom, became more pronounced at 215 and 250 days.

VI. DISCUSSION

All the plants infected with witches’ broom virus developed similar symptoms, namely stunting of growth, proliferation of axillary buds and, most characteristic of all, production of green flowers, frequently after normal flowering. Green flowers have also been observed on lucerne in the field. In the autumn of 1948 these were fairly common on plants showing an advanced stage of the disease in an old stand at Canowindra and at Dickson, A.C.T. (Plate 1, Fig. 2).

The production of green flowers and the stunting of growth, on hosts other than lucerne, is so consistent and characteristic that it bears a striking
similarity to the effect of tomato big bud on its various hosts (Samuel, Bald, and Eardley 1933; Simmonds 1936; Hill 1943). Big bud is also transmitted by *O. argentatus* (Hill 1943) and on tomato, under green-house conditions, may produce very similar symptoms to witches' broom (Plate 5; cf. Plate 3, Fig. 1). Witches' broom of lucerne, when transmitted to tomato, causes a proliferation of the internal phloem as observed by Samuel, Bald, and Eardley (1933) in big bud. The symptoms of the two diseases on *D. stramonium*, tobacco, sugar beet, and crowfoot are also similar. For these reasons it is considered possible that the two diseases may be caused by the same virus or by strains of it. Further evidence is required before this can be said with certainty but the information already obtained justifies further investigation.

If it is true that the two diseases are caused by the same, or by strains of the same, virus, then the relationship between the incidence of big bud on tomato and the seasonal abundance of the vector at Dickson, A.C.T., in 1947-48 is of interest. As has already been pointed out, the leafhopper reached its greatest numbers early in November and the population remained at a high level until the end of December. The percentage of tomatoes in this area that became infected with big bud increased about the middle of February, following swarming of the leafhoppers in the last week of January 1948 (Fig. 2).

![Graph showing seasonal abundance of Orosius argentatus and incidence of big bud on tomato](image)

**Fig. 2.**—Seasonal abundance of *Orosius argentatus* (Evans) on lucerne, and incidence of big bud on tomato (broken line) at Dickson Experiment Station, A.C.T., 1947-48. The arrow indicates a flight.

The geographical distribution of diseases characterized by greening and proliferation is also very interesting. Such a disease has been reported on tobacco, egg plant, black nightshade, and thorn apple in Rumania (Ghipiu 1931). Fruit woodiness or stolbur on tomato has been reported by Richkow, Karatschevsky, and Michailova (1934), in the U.S.S.R., and Ryjkoff (1935) considered that the same disease infected tobacco, tomato, chilli, *Convolvulus arvensis*, *Atropa belladonna*, *Datura* spp., and other members of the Solanaceae. The changes in the flowers of tomatoes observed by Kostoff (1933) in Russia
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Fig. 1

Fig. 2

WITCHES' BROOM DISEASE OF LUCERNE

Fig. 1

Fig. 2

Witches' Broom Disease of Lucerne

Fig. 1

Fig. 2

agreed very closely with those described by Samuel, Bald, and Eardley (1933) for big bud on tomato in Australia. Ryjkoff therefore concluded the two diseases were caused by the same virus. The disease in Russia is transmitted by *Hyalesthes obsoletus* (Cixiidae, Sukhov 1948). In India, a closely related disease, "little-leaf" of egg plant, is transmitted by the leafhopper, *Eutettix phycitis* (Thomas and Krisnaswami 1939). Greening of *Crotalaria usaramoensis* has been observed in Java but its method of transmission is not known (J. van der Vecht, personal communication). In the Australian region, a similar disease has been reported on tomatoes, antirrhinum, zinnia, petunia, and false cape gooseberry from Suva (Parham, personal communication). Big bud of tomato has been reported from the Pacific north-west of the U.S.A. (Dana 1940), transmission in this case being effected by graft only. A similar disease on lucerne in the Pacific north-west is considered by Edwards (1935a) to be the same disease as witches' broom of lucerne in Australia. It is transmitted by another leafhopper, *Platymoides acutus* (Menzies 1946). To date no similar disease has been recorded from New Zealand or the Hawaiian Islands.

Thus it will be seen that a group of very similar virus diseases occur from Europe across Asia through the Netherlands East Indies to Australia and extend across the Pacific to the West Coast of the U.S.A. There is no evidence that the diseases are, in fact, related but further investigations should prove interesting and profitable.

VII. Acknowledgments

Many of the results of this investigation would not have been possible without the ready cooperation of lucerne growers in the Lachlan Valley, New South Wales. Grateful acknowledgment is made for the assistance rendered by Messrs. Weir Brothers, Mr. A. Sergeant, and Mr. W. C. Goodacre, on whose properties the ecological surveys were made; by Dr. J. W. Evans and Dr. P. W. Oman in the identification of leafhoppers; by Mr. N. Grylls, who assisted with field surveys; and by Mr. R. Brock, who supplied the figures on the incidence of big bud.

VIII. References


**EXPLANATION OF PLATES 1-5**

**PLATE 1**

Fig. 1.—Lucerne plant affected with witches’ broom in an advanced stage of the disease. Healthy plant on right.

Fig. 2.—Lucerne shoots from field affected with witches’ broom showing green flowers (arrows).

**PLATE 2**

Fig. 1.—*Nicotiana tabacum* showing witches’ broom transmitted by graft 156 days after grafting scion *Datura stramonium* to which the disease was transmitted by *Orosius argentatus*. Healthy plant on left.

Fig. 2.—*D. stramonium* plant infected with witches’ broom 146 days after exposure to infective *O. argentatus*. Healthy plant on left.

**PLATE 3**

Fig. 1.—Tomato plants (var. Rouge de Marmonde) with symptoms of witches’ broom 124 days after exposure to *O. argentatus*, showing rosette appearance. The diseased plant did not grow any further and began to die 31 days later. Healthy plant on left.

Fig. 2.—*E. cicutarium* collected in field, Canberra, A.C.T., naturally infected with virescence. Healthy shoot on left, shoot showing dwarfing and green flower in centre, and shoot with green flowers and incipient seed pods on right.

**PLATE 4**

*Beta vulgaris* showing plant in foreground affected by witches’ broom 183 days after exposure to infective *O. argentatus*. Plants in background remained healthy.

**PLATE 5**

Tomato plant showing big bud symptoms.