# THE MECHANISM OF THE TRANSMISSION OF POTATO LEAF ROLL VIRUS BY APHIDS

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[*Manuscript received May* 17, 1955]

*Summary*

The virus causing potato leaf roll can be recovered from the haemolymph of *Myzus persicae,* the aphid vector. Infective virus has also been separated from the bodies of infected vectors. The virus can be transmitted by an aphid after a moult, and infectivity is retained for at least 8 days; during this time the aphid is able to infect many plants. *M. persicae* is a much more efficient vector than *Macrosiphum euphorbiae.*

Experiments suggest that the virus multiplies to a limited extent in *M. persicae.* When this vector feeds on a plant containing a low concentration of virus there is generally a latent period (of approx. 20 hr at 25°C) between the acquisition feed and a successful inoculation feed. When the vector feeds on a source of high virus concentration occasional transmission is obtained with short (approx. 2 hr) acquisition and inoculation feeds. However, the percentage of transmissions under these conditions with the local strain of *M. persicae* is lower than that reported by American workers. No clonal differences in vector efficiency were found. Attempts to isolate strains of leaf roll virus with different vector relationships were unsuccessful.

The relevance of these results to the mechanism of transmission of viruses by insects is discussed. It is suggested that the occurrence of a latent period in a virus vector is indicative of the passage of the virus from the midgut into the salivary glands via the haemocoele, and the multiplication of the virus in that vector.

## I. Introduction

There are two opposing views on the mechanism of transmission by aphids of the virus causing leaf roll of the potato. Early work (Elze 1927; Smith 1931) demonstrated the existence of a period (the latent period) following a short acquisition feed during which the aphid was incapable of transmitting the virus. The duration of this latent period was estimated at 24-48 hr by Elze, 54 hr by Smith, and 30 hr by Webb, Larson, and Walker (1952). Kassanis (1952) showed that the latent period was variable, but in his experiments it exceeded 49 hr.

On the other hand, several groups of workers have reported transmission of potato leaf roll without a latent period. Loughnane (1943) in Ireland published preliminary results showing transmission following acquisition feeding periods of only 5 min when the inoculation feeding period was 5 days. Klostermeyer (1953) in the State of Washington reported transmission following combined acquisition and inoculation feeding periods of 20 min, and a more

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detailed study by Kirkpatrick and Ross (1952) in California demonstrated that potato leaf roll virus could be transmitted by aphids with a minimum latent period of 1½ hr. Larson (personal communication) has transmitted a severe and a mild strain of leaf roll after acquisition and inoculation feeding periods of 1 hr each, but no transmission was obtained when these periods were reduced to 5 min. Finally, MacCarthy (1954) has transmitted leaf roll following a 9½ hr latent period, but demonstrated that a somewhat longer period is usually necessary for transmission.

The discrepancy in the results reported in the previous paragraphs is basic, because it has been suggested (Day and Irzykiewicz 1954) that viruses transmitted following a latent period pass through the haemolymph into the saliva of their vectors, whereas those without a latent period are transmitted mechanically on the mouthparts. The resolution of this problem is therefore crucial to hypotheses concerning the mechanism of transmission of viruses by insects.

There are four possible explanations for the results of Elze, Smith and Kassanis on the one hand, and of Loughnane, Klostermeyer, Larson, and Kirkpatrick and Ross on the other. The early workers all used the potato as the host and as the test plant for work on leaf roll transmission. Later workers used species of *Datura* or *Physalis* in which the virus concentration appears to be greater. It seemed possible, therefore, that differences in virus content of the host plants could have accounted for the differences in the results. Secondly, leaf roll may be transmitted both "mechanically" (without a latent period) and "biologically" (with a latent period) by the same vector. The third possibility is that the two groups of workers were studying different viruses that produced similar symptoms. Finally, the conflicting reports may have been due to the use of strains of aphids differing in their ability to function as vectors.

The experiments reported in this paper were carried out to decide between these possibilities and to elucidate the mechanism of transmission of potato leaf roll virus by the aphid.

## II. Materials

Most of the work was done with a colony of *Myzus persicae* (Sulz.) maintained for over four years on *Datura stramonium* L. or *Solanum melongena* L. (egg plant). Some experiments were performed with clones of *M. persicae.* The method of obtaining these is described with the experimental results. A colony of *Macrosiphum euphorbiae* (Thomas), maintained on *D. stramonium,* was also used. All transmission experiments, unless the contrary is stated, were performed with one mature apterous insect per test plant.

The potato leaf roll virus used had been maintained by Dr. E. M. Hutton for several years in Katahdin potatoes in the glasshouse.

The test plants used were *Physalis floridana* Rydb. For some of the work the autotetraploid described by Hutton (1954) was employed. Results with either diploid or tetraploid could generally be assessed within 10 days, but the test plants were generally kept for 14 days after infection. During the winter symptoms developed more slowly and the indicator plants were kept for 3 or 4 weeks. These times are not long enough for root transmission (see Webb,

Larson, and Walker 1952) to have been detected. Very uniform test plants were obtained when the seed was germinated on wet filter paper in petri dishes and planted, 36 to a "flat," soon after the primary root had appeared. The seedlings were generally infected in the two-leaf stage, about 10 days after planting. Transplanting was practised in some early experiments, but the practice was discontinued because of occasional damage to the plants.

The test plants were kept in a conditioned insect-proof glasshouse provided with thermostatically controlled electric heating and evaporative cooling devices. Temperatures were generally maintained between 16 and 26°C during summer and winter, although local heating, due to insolation, often occurred. This glasshouse was regularly fumigated with nicotine and in only two instances were contaminative infections in control plants noted. The results of the experiment in which these occurred were disregarded. The aphid colony was kept, and the infections performed, in separate laboratories.

A total of about 15,000 indicator plants in over 50 different experiments was used in this work.

## III. Observations

### (*a*) *Infected* P. floridana *as a Source of Virus*

A preliminary experiment was performed to determine the optimum time to use infected plants as source plants, and to determine whether infected diploid or tetraploid *P. floridana* or infected potato was the preferable source plant. Eight diploid *P. floridana* and eight tetraploid *P. floridana* were infected with potato leaf roll. At weekly intervals each infected plant was colonized for 4 hr by *M. persicae* that had been starved for 1 hr. These aphids were then placed on test plants, one per plant, and left for 2 days. A young leaf roll infected potato from an infected tuber was similarly used as a source plant. Although it is appreciated that this technique cannot demonstrate small differences in virus concentration of the host plants, it is the only one at present available for potato leaf roll. (Some advantages of the method have been enumerated by Sylvester (1953).) The results (Table 1) demonstrate that the diploid *P. floridana* increased in efficiency as a source of virus until about the third week after infection. Autotetraploid *P. floridana* was a poor source although it was no less attractive to aphids, and epidermal hairs are, if anything, sparser on the leaves of the tetraploid than they are on the leaves of the diploid plant. Hutton (1954) has shown that the autotetraploid reacts more markedly than the diploid *P. floridana.* It is therefore apparent that the efficiency of the plant as a source of virus is not correlated with symptom expression, thus confirming with *Physalis* a conclusion reached by Kassanis (1952) for *Datura tatula.*

A second experiment was carried out to determine the optimum stage to infect diploid *P. floridana* for use as a source plant. The results (Table 2), though based on small numbers, suggest that the infection of plants at about 3 weeks of age is satisfactory for the production of good virus sources. In most subsequent experiments plants of diploid *P. floridana* approximately 3 weeks after infection were generally used as source plants. Subsequent tests showed that diploid *P. floridana* plants remain good sources for at least 7 months after infection.

### (*b*) *Details of Transmission of Potato Leaf Roll by Aphids*

On the hypothesis of insect transmission of viruses put forward by Day and Irzykiewicz (1954) a number of predictions are possible. If potato leaf

TABLE 1

EFFICIENCY OF VARIOUS INFECTED PLANTS AS SOURCES OF LEAF ROLL VIRUS

Numbers of *P. floridana* infected (out of 36) by single *M. persicae* following acquisition feed of 4 hr and inoculation feed of 48 hr

| Time after Infection | Virus Source | | |
|---|---|---|---|
| | Diploid *P. floridana* | Autotetraploid *P. floridana* | Potato |
| 2 days | 0 | 0 | 3 |
| 1 week | 2 | 1 | 1 |
| 2 weeks | 6 | 1 | 3 |
| 3 weeks | 13 | 0 | 7 |
| 4 weeks | 6 | 3 | 7 |

roll is caused by a vector-latent virus, it should be transmitted by the aphid after the latter moults; the virus should occur in the haemolymph of the infected

TABLE 2

EFFICIENCY OF DIPLOID *P. FLORIDANA* INFECTED AT VARIOUS AGES AS SOURCES OF POTATO LEAF ROLL VIRUS

Number of *P. floridana* infected (out of 36) by single *M. persicae* following acquistion feed of 4 hr and inoculation feed of 24 hr

| Age of Host at Infection (days) | Time after Infection (days) | | | | |
|---|---|---|---|---|---|
| | 19 | 26 | 34 | 40 | 54 |
| 13 | 1 | 3 | 1 | 3 | – |
| 16 | 5 | 2 | 0 | 1 | 0 |
| 22 | 5 | 5 | 2 | 1 | 1 |
| 24 | 2 | 5 | 0 | 0 | 0 |
| 30 | 2 | 4 | 0 | 1 | 0 |
| 35 | 1 | 0 | 3 | 2 | 0 |

aphid; the infective aphid should retain its infectivity for a long period; there should be some degree of vector specificity; and the aphid should be able to acquire the virus from solution. In addition, some of the vector-latent viruses

have been shown to multiply in their insect vectors. These points have been studied and the results are presented in the following paragraphs.

(i) *Transmission by Aphids Following a Moult.*—Elze (1931) and Smith (1931) both reported that the transmission by an aphid of potato leaf roll is not influenced by the intervention of a moult between successive feeds. Confirmation of these reports was sought with the local strain of leaf roll virus. A culture of *M. persicae* was maintained on a potato sprout from a leaf roll infected tuber. Periodically the plant was searched for aphids in the process of moulting. Each moulting aphid was carefully removed to a *P. floridana* indicator plant, and a feeding aphid, as a control, was placed on a similar indicator plant, each plant being covered by a celluloid and muslin cage. The inoculation feeding period was approximately 2 days. Thirty freshly moulted aphids produced 20 infec-

TABLE 3

PERSISTENCE OF POTATO LEAF ROLL VIRUS IN *M. PERSICAE* INFECTIONS (+) FROM SUCCESSIVE TRANSFERS TO *P. FLORIDANA* SEEDLINGS. ACQUISITION FEED 4 DAYS. INOCULATION FEEDS 1½ HR. APHIDS TRANSFERRED OVERNIGHT TO CHINESE CABBAGE

| Aphid | Day 1 | | | | | Day 2 | | | | | Day 3 | | | | | Day 4 | | | | | Total Infections out of 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | |
| 1 | − | − | + | + | − | − | − | − | + | + | + | − | − | + | − | + | + | − | − | − | 8 |
| 2 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 0 |
| 3 | − | − | + | − | − | − | − | + | + | + | − | − | − | + | − | − | + | + | − | + | 8 |
| 4 | − | + | + | − | − | + | + | + | + | + | + | − | + | + | + | + | − | − | + | − | 13 |
| 5 | − | + | + | − | − | − | − | + | + | − | − | − | + | − | + | − | − | − | + | − | 7 |
| 6 | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | − | − | + | − | + | 5 |

tions, and the 30 normal aphids produced 12 infections. It is apparent that moulting does not render the aphids non-infective and this is strong evidence that the virus is present in the body of the vector and is not carried mechanically on the stylets.

(ii) *Recovery of Virus from the Haemolymph.*—If the virus passes through the midgut of the vector into the haemolymph it should be possible to recover it from that tissue. Haemolymph from leaf roll infected aphids could be drawn into glass micro-injection needles and then injected into normal aphids. Mortality after 24 hr was less than 20 per cent. Satisfactory inoculations were performed on 146 aphids from donors that had fed for 2 weeks on infected potato sprouts. The recipients were immediately placed singly on *P. floridana* indicator plants and left for 2 days. Six infections resulted from these indicators demonstrating that virus could occasionally be recovered from the haemolymph, but that recovery was too infrequent to permit the technique to be used for passaging the virus.

(iii) *Retention of Infectivity.*—Experiments showed that single aphids were able to infect a series of indicator plants during a period of several days. In one experiment six aphids were transferred after an acquisition feed of 4 days on a young leaf roll infected potato sprout to *P. floridana* indicator plants at intervals of 1½ hr. Overnight they were placed separately on Chinese cabbage seedlings in which the virus does not multiply. Twenty plants of the indicator were infested by each aphid. One aphid transmitted the disease to 13 of these, two to 8, one to 7, one to 5, and one did not transmit at all (Table 3).

In a second experiment *M. persicae* that had been more than one week on infected potato were placed on a vigorous Chinese cabbage seedling. At intervals aphids were transferred to *P. floridana* indicator plants. Infections were obtained from aphids removed up to the 7th day, but thereafter aphids from the Chinese cabbage were non-infectious. The great majority of original apterae were by that time replaced by their offspring. The length of life of apterae under these conditions was about 10-15 days.

The retention of infectivity was also studied by a third method. In this experiment 36 mature apterous *M. persicae* from an infected potato were placed singly on indicator plants. Every 24 hr for 8 days they were transferred to new indicator seedlings so that the transmission behaviour of each aphid could be evaluated. The temperature approximated 25°C. It might be expected that this method would indicate longer virus retention times than the previous methods, because each insect could possibly reinfect itself when left for 24 hr on a sensitive plant and because the aphids were likely to live longer when they were handled only once a day, than when they were transferred every 1½ hr. Four aphids transmitted the disease the 8th day after they were first placed on the indicator plants.

These experiments demonstrate that *M. persicae* can retain the virus of potato leaf roll for at least a week. It is probable that the virus is retained for the life of the insect (MacCarthy 1954).

(iv) *Vector Specificity.*—Published data on specificity of vectors of potato leaf roll virus have been summarized by Day and Bennetts (1954), and by MacCarthy (1954). The latter considers that only four species of aphids are effective vectors of leaf roll in the field. These are *M. persicae*, *M. ornatus* Laing, *M. circumflexus* (Buckt.), and *M. ascalonicus* (Donc.).

It was planned to repeat with *Macrosiphum euphorbiae* several of the experiments that had been done with *M. persicae*. Under the name of *M. gei*, *M. euphorbiae* had been reported by Smith (1929) and Cottier (1931) to be incapable of transmitting the virus, and early attempts to demonstrate transmission by this species using *P. floridana* were uniformly negative. However, transmission was obtained in our experiments when *Datura tatula* L. was used as source and indicator plants. Even under these conditions transmission was too inefficient for detailed experiments to be completed. For example, after an acquisition feed of 3 days on a *D. tatula* infected for 3 weeks, 5 aphids were placed on each of 9 indicator seedlings. After 4 weeks only 2 of these showed symptoms. It was therefore concluded that, although *M. euphorbiae* could

transmit potato leaf roll, the difficulties of working with it were too great to warrant detailed tests. Similar results reported after the completion of our tests, were obtained by MacCarthy (1954) with *Macrosiphum solanifolii* (Ashm.) which is probably the same species of aphid.

Further information is required for generalizations concerning the vector specificity of potato leaf roll, but it is already apparent that this specificity is greater than would be expected in a vector-direct virus.

(v) *Acquisition from Solution of Virus by the Vector.*—It is known that aphids cannot acquire non-persistent viruses from solution and reasons for this have been suggested by Day and Irzykiewicz (1954). If a virus is ingested and passes from the midgut into the salivary glands via the haemocoele there is no reason why the aphid should not be able to acquire it from solution, although a number of workers have tried to accomplish this without success. The following experiments have been carried out: 44 highly infectious plants of *P. floridana* 3 weeks after infection were ground in a mortar with a little sand. The juice was squeezed through cheese cloth and yielded 21 ml of sap. This was centrifuged for 10 min at 10,000 *g* in a refrigerated centrifuge. The supernatant was then clarified at 100,000 *g* for 45 min in a Spinco ultracentrifuge at 0°C. The pellet was redissolved in M/15 phosphate buffer at pH 7·0 and the solution run again in the ultracentrifuge under the same conditions. The resulting pellet was resuspended in about 0·1 ml of buffer and was fed to previously starved *M. persicae* through a plastic membrane. None of these aphids became infectious.

An attempt was then made to isolate the virus from the insect vectors. Almost 3 *g* (estimated at 10,000) of *M. persicae* that had been feeding on infected potatoes for 2 weeks were washed from the plants. These aphids were ground with 5 ml of 0·05 per cent. gelatine in distilled water chilled in ice. The macerated insects were then centrifuged at 10,000 *g* for 10 min in a refrigerated centrifuge. The supernatant was centrifuged at 40,000 *g* for 30 min in a Spinco ultracentrifuge. The pellet was designated fraction 1. The supernatant was then centrifuged for 30 min at 100,000 *g* and the pellet designated fraction 2. The greater part of both pellets was easily resuspended in 0·05 per cent. gelatine. Solutions from fractions 1 and 2 were hand-inoculated on to *P. floridana* leaves, fed to aphids through plastic membranes, and inoculated into the haemocoele of normal aphids. Infections were obtained only from inoculation of fraction 2 into aphids and then in only 2 of 18 plants on which the inoculated aphids were permitted to feed.

This experiment was repeated using aphids from infected *P. floridana*. The supernatant from the preliminary low speed centrifugation at 10,000 *g* for 30 min was followed by ultracentrifugation at 100,000 *g* for 30 min and the pellet resuspended in 0·05 ml of 0·05 per cent. gelatine. This was injected into aphids of which 30 survived. When these were placed singly on *P. floridana* indicator seedlings for 3 days, three infections were obtained. The virus can therefore be recovered from infected aphids.

It is suggested that the apparent inability of aphids to acquire leaf roll from solution is due to the fact that too little active virus was present in the solution for it to infect the aphid vectors.

(vi) *Possibility of Multiplication of Potato Leaf Roll Virus in the Aphid.*— All of the experiments reported in the previous pages are explicable on the hypothesis that an aphid reinjects into a plant only a fraction of the virus it ingests and that no multiplication of virus need occur in the insect. Maramorosch (1953) has concluded that leafhopper-borne viruses that have a latent period undergo multiplication in their vectors. It is therefore important to determine whether multiplication of viruses can also occur in aphids. Unfor-

TABLE 4

TRANSMISSION OF POTATO LEAF ROLL VIRUS BY A CLONE OF *M. PERSICAE*. INOCULATION FEED ON A SINGLE INFECTED PLANT OF *D. TATULA*

| Duration of Acquisition Feed (hr) | Period on Chinese Cabbage (hr) | Duration of Inoculation Feed (hr) | Infections | |
|---|---|---|---|---|
| | | | Number | Percentage of Seedlings Inoculated |
| 1 | 24 | 4 | 0 | 0 |
| 1 | 48 | 4 | 0 | 0 |
| 1 | 72 | 4 | 0 | 0 |
| 1 | 96 | 4 | 1 | 3 |
| 3 | 0 | 4 | 1 | 3 |
| 3 | 24 | 4 | 3 | 5 |
| 3 | 48 | 4 | 6 | 9 |
| 3 | 72 | 4 | 15 | 21 |
| 3 | 96 | 4 | 12 | 17 |
| 3 | 144 | 4 | 14 | 20 |
| 6 | 24 | 4 | 3 | 10 |
| 6 | 48 | 4 | 10 | 28 |
| 6 | 72 | 4 | 10 | 28 |
| 6 | 96 | 4 | 15 | 42 |

tunately many of the techniques found satisfactory for demonstrating multiplication of viruses in leafhoppers are inapplicable to aphids. For example, leaf roll virus is not transmitted by a viviparous aphid to its offspring. This was reported by Elze (1927) and Smith (1929) and has been repeatedly confirmed with the local virus and vector. Inoculation of the aphid (see above), though occasionally successful, was too uncertain to permit the technique, so successfully applied by Maramorosch (1952), to be used with aphids. Heat treatment used by Kunkel (1937) to render infective leafhoppers free from virus could not be employed with *M. persicae* because of the proximity of the thermal death points

of the aphids (Broadbent and Hollings 1951) and that of the virus (Kassanis 1950). With so short a latent period it was not easy to devise an experiment to measure the relation of the dosage of the virus to the duration of the latent period; and techniques for measuring virus concentration were too inaccurate to permit measurement of an increase in virus concentrations in the insect during the latent period.

In view of the difficulties outlined in the previous paragraph, the following experiment was designed in an attempt to obtain data on the possibility of the multiplication of potato leaf roll virus in *M. persicae*. Large numbers of aphids were given short acquisition feeds (1, 3, and 6 hr) on leaf roll infected *Datura tatula*. They were then removed to Chinese cabbage which is immune to potato leaf roll. At 24 hr intervals groups of aphids were placed singly on *P. floridana* indicator seedlings in the two-leaf stage and the percentage of infective aphids determined (Table 4).

Precautions were taken to ensure that aphids selected at the later stages were not progeny of those placed on the Chinese cabbage. One transmission following a latent period of 7 hr was obtained. The percentage of transmission continued to increase for 3 days, in spite of the fact that the source of virus was removed after 3 hr. The most likely explanation is that the concentration of virus in the haemolymph continued to increase after the ingested virus was distributed through the tissues of the vector.

### (*c*) *The Occurrence of a Latent Period*

The data in the previous section confirm the view that the virus of potato leaf roll is transmitted by the "biological" mechanism, and suggest that limited multiplication of the virus may occur in the aphid. If multiplication does occur a latent period would be expected, and studies were therefore undertaken to attempt to resolve the conflicting reports on the existence of a latent period.

(i) An experiment duplicating in all published details that outlined in Table 7 of Kirkpatrick and Ross (1952) resulted in no transmission, although these authors reported transmission by 4 out of 5 aphid colonies.

A similar experiment (except that the 12 aphids were moved every 1½ hr during the day and were placed at night on individual Chinese cabbage seedlings) was carried on for 4 days, and it was only on the last day that infections were obtained. Many other results confirm the conclusion that "mechanical" transmission does not occur. Day and Irzykiewicz (1954) have demonstrated that mechanical transmission by aphids is most efficient when the acquisition and inoculation feeding periods approximate 2 min each. Hundreds of tests using 5 min feeding periods have not resulted in a single inoculation of potato leaf roll virus.

(ii) Larson (personal communication) found with both "mild" and "severe" strains of leaf roll about 20 per cent. transmission with acquisition feeds and inoculation feeds of 60 min each. But when an experiment using the same conditions was performed, no infections occurred with our strain of *M. persicae* in the short feeding periods (Table 5).

It appeared from the above results that under most conditions a latent period actually occurs in the transmission of potato leaf roll virus. Two experiments were performed to determine the duration of this latent period. The results are presented in Table 6. It would seem that the latent period under these conditions approximates 20 hr at 25°C. However, it was nearer 3 days in the experiment described in the preceding section and only a few hours in Table 4. The results confirm conclusions of Kassanis (1952) and of MacCarthy (1954) that the latent period is very variable. The explanation of this variability will be put forward in Section IV.

The above experiments dispose of two of the possible explanations advanced in Section I. The use of *P. floridana* and *D. tatula* as source plants did not generally result in transmissions except following a long latent period. Similarly, transmission in these experiments never occurred first by a mechanical

TABLE 5

TRANSMISSION OF POTATO LEAF ROLL BY *M. PERSICAE* FROM *D. TATULA* TO *P. FLORIDANA* (THREE-LEAF STAGE) AT 22°C. 10 APHIDS PER TEST PLANT

| Duration of | | Number of Infections |
|---|---|---|
| Acquisition Feed | Inoculation Feed | |
| 10 min | 10 min | 0/36* |
| 60 min | 60 min | 0/36 |
| 120 min | 120 min | 0/72 |
| 24 hr | 24 hr | 17/22 |

* Denominator indicates the number colonized, the numerator the number that became infected.

and later by a biological mechanism, and this suggestion certainly does not explain the conflicting results on the occurrence of the latent period. Two other possibilities were put forward, namely that the results were explicable by differences in the viruses used, or by differences in the vectors. These possibilities will now be considered.

(iii) *Vector Relationships of Strains of Leaf Roll Virus.*—The occurrence of "strains" of leaf roll has been clearly established by Webb, Larson, and Walker (1952). It is possible that certain strains may have vector relationships differing from the typical strain. Some evidence that Klostermeyer (1953) was dealing with a virus differing from typical leaf roll is suggested by his statement that "visitors from several eastern States and from foreign countries declare such pronounced symptom expression does not occur elsewhere."

In order to test the possibility that more than one virus causing leaf rolling symptoms was present in Australia, leaf roll infected potatoes were obtained from widely separated localities and each of these was tested in the manner

of the experiment set out in Table 5. Of 41 isolates examined none reacted differently from the typical strain. Details of the isolates examined are as follows:

2 isolates from A.C.T. from varieties Sebago and Katahdin,
6 isolates from four districts in New South Wales from varieties Exton, Factor, Katahdin, Saranac, and Sebago,
7 isolates from six districts in Victoria from varieties Sebago and Sequoia,
16 isolates from three localities in South Australia in varieties Adina, Delaware, Exton, Katahdin, Kennebec, Monak, and Sebago, and
10 isolates from five localities in Tasmania in varieties Woolnorth and Medium Brownell.

TABLE 6

LATENT PERIOD OF POTATO LEAF ROLL VIRUS IN *M. PERSICAE* AT 27-28°C. ACQUISITION FEED ON INFECTED *P. FLORIDANA*. INOCULATION FEEDS ON *P. FLORIDANA* IN TWO-LEAF STAGE

| Acquisition Feed (hr) | Inoculation Feed (hr) | Total Time (hr) | Infections (out of 36) |
|---|---|---|---|
| 15 | 4 | 19 | 0 |
| 22 | 4 | 26 | 3 |
| 28 | 4 | 32 | 1 |
| 39 | 4 | 43 | 3 |
| 51 | 4 | 55 | 7 |
| 15 | 3 | 18 | 0 |
| 15 | 5 | 20 | 2 |
| 15 | 7 | 22 | 8 |
| 15 | 9 | 24 | 0 |
| 15 | 11 | 26 | 2 |
| 15 | 13 | 28 | 6 |
| 15 | 24 | 39 | 10 |
| 15 | 29 | 44 | 10 |

It is, of course, virtually impossible to prove that only one virus causing the symptoms of potato leaf roll occurs, but the suggestion that two such viruses account for the divergent results published on the latent period of the virus in the vector is made less attractive by the fact that Larson (personal communication, see above) has found that transmission of two of his strains was practically the same when they were compared by the method set out in Table 5. Certainly no evidence has been obtained in the present work of the existence of two leaf rolling viruses with different vector relationships.

(iv) *Differences in Strains of Vectors.*—Although most of the results presented so far in this paper confirm the views of Elze, Smith, and Kassanis, a few transmissions were obtained following short acquisition and inoculation feeds. One such instance is shown in the 3-hr acquisition feeds of Table 4.

But such instances of latent periods of only a few hours were very infrequent by comparison with those reported by the American workers.

These and other results suggested that differences in strains of aphids might account for the reported differences in duration of the latent period. All work so far described in this paper was done with aphids from a colony of *M. persicae* that had been maintained in the laboratory for several years. A new colony was initiated from a number of field-caught alates, and clones were selected from this colony by the following method. Mature apterae were permitted an acquisition feeding period on a leaf roll infected *P. floridana* plant for 3 to 5 days. They were then placed singly for 24 hr on *P. floridana* indicator seedlings and moved daily to new seedlings for 4 days. The results of 205 aphids were as follows: 35 transmitted to all of the four indicators, 68 to three indicators, 58 to two indicators, 31 to one indicator, and 13 failed to transmit to any of the four indicator seedlings. Each surviving aphid was then placed

TABLE 7

TRANSMISSION OF POTATO LEAF ROLL VIRUS BY CLONES OF *M. PERSICAE* IN FOUR SEPARATE EXPERIMENTS

| Clone | Ability to Transmit | | | |
|---|---|---|---|---|
| | Number Infected (out of 4) in 24-Hr Test | Number Infected (out of 36) in: | | |
| | | 5-Day Test | 4-Day Test | 6-Day Test |
| 1 | 0 | 1 | 9 | 16 |
| 2 | 0 | 1 | 6 | 18 |
| 3 | 0 | 2 | | |
| 4 | 0 | 2 | | |
| 5 | 0 | 4 | | |
| 6 | 0 | 10 | | |
| 7 | 0 | 15 | | |
| 8 | 0 | 17 | | |
| 9 | 1 | 4 | | |
| 10 | 1 | 8 | | |
| 11 | 1 | 12 | | |
| 12 | 3 | 0 | 6 | 14 |
| 13 | 3 | 4 | 12 | 23 |
| 14 | 4 | 1 | | |
| 15 | 4 | 3 | | |
| 16 | 4 | 4 | | |
| 17 | 4 | 5 | | |
| 18 | 4 | 14 | | |
| 19 | 4 | 16 | 3 | 23 |
| 20 | 4 | 17 | 5 | 25 |

on a Chinese cabbage seedling to propagate the clone. Twenty of these clones were then studied further in the following way. Approximately 50 aphids from each clone were placed for 3 days on uniform, infected *P. floridana* plants; they were then placed singly on 36 *P. floridana* indicator seedlings for 2 days and the percentage of infectious aphids determined. The results (Table 7) demonstrate that selection based on one test did not influence the performance of the clones in a subsequent test. This suggests that the major component in apparent differences in vector efficiency is the operation of chance differences presumably in feeding, but the magnitude of the differences between the performance of, for example, clones 1 and 8 and between clones 14 and 20 suggested that real differences in vector efficiency may exist between clones. However, subsequent tests with these selected clones failed to substantiate this suggestion and it is apparent from columns 4 and 5 of Table 7 that the preliminary selection of clones did not result in strains of aphids that differed in vector ability. A latent period experiment similar to that set out in the lower part of Table 6 was carried out with clones 1 and 19. No differences were found.

It has thus not proved possible to isolate from the material available clones of *M. persicae* that differ in their ability to transmit potato leaf roll virus. However, the experiments demonstrate the marked variability in transmission which can occur within an experiment and that large numbers of vectors must be tested before conclusions are justified concerning the efficiency of clones to transmit the virus.

Simons (1954) reported considerable differences in the ability to transmit pea enation mosaic virus between young and mature apterae. Most workers have used mature apterae, but it seemed possible that differences in the stages used may have accounted for some of the differences in the results reported by various authors. However, a test with young and mature apterae transmitting potato leaf roll virus showed that both groups transmitted with equal efficiency. In a comparative experiment the mature apterae transmitted the disease to 18 of 72 indicators, and the young apterae to 16 of 72 indicators.

The suggestion that strains of aphids differing in their vector efficiency may explain the differences in the latent period reported by European and American workers cannot be studied by using aphids available to us. It remains, however, the most attractive hypothesis.

## IV. Discussion

Insects transmit viruses by several mechanisms (Day 1955); "direct mechanical transmission" is effected by mosquitoes transmitting rabbit myxomatosis; "modified mechanical transmission" is effected by aphids transmitting mosaic viruses; "delayed mechanical transmission" has been suggested as a designation for those viruses transmitted by ingestion and subsequent excretion by the vector; finally, "propagative transmission" is the type of transmission in which the virus is ingested, multiplies in the vector, and the "offspring" of the infecting virus are released. The first two types include the "vector-direct" viruses

as defined by Day and Irzykiewicz (1954). Potato leaf roll virus is clearly excluded from this category for the following reasons:

(i) It is transmitted by an aphid following a moult.
(ii) It can be isolated from the haemolymph.
(iii) The vector is infective for many days.
(iv) Vector specificity is well marked.
(v) There is often a latent period between an acquisition feed before a vector is capable of transmitting the virus.

Potato leaf roll is, thus, not a vector-direct virus, as defined by Day and Irzykiewicz (1954). Tables 4 and 6 provide data relevant to a decision between the delayed mechanical and the propagative types of transmission:

(1) The ability of the vector to transmit would be proportional to the duration of the acquisition feed in delayed mechanical transmission but would be unrelated in propagative transmission. Results with potato leaf roll virus (Table 6) favour the second alternative.
(2) The maximum efficiency of transmission should occur with shorter intervals between acquisition and transmission with delayed mechanical transmission but should increase with time in propagative transmission. The data of Tables 4 and 6 again confirm the second alternative.
(3) The frequency distribution of the number of infections plotted against the duration of the inoculation feeding period would follow a normal distribution curve if transmission was by the delayed mechanical mechanism, but would follow an exponential curve with propagative transmission. In this also the data of Tables 4 and 6 confirm the second alternative.

These considerations taken together indicate strongly that the potato leaf roll virus multiplies to a limited extent in the aphid vector. The route of the virus in the vector has been traced as far as the haemolymph, but not into the salivary glands or saliva. Blattny (1931) reported changes in the cytology of the aphid salivary glands after infection with leaf roll, but efforts to confirm this report have been unsuccessful.

The differences reported in the literature on the occurrence of a latent period are explained mainly by differences in the vectors used, in conjunction with the efficient source plants *P. floridana* and *D. tatula* used by recent investigators. The latent period is thus the time required for the virus to move from the midgut to the saliva of the vector. The data suggest the hypothesis that the virus decreases in activity as it moves along this path. When the amount of virus ingested is small, sufficient to reach the saliva is not present until multiplication has occurred. When the amount ingested is large some of it may occasionally reach the saliva before multiplication has occurred. But the barriers between midgut and saliva may differ in effectiveness between strains of aphids and, even with sources of relatively high virus concentration, sufficient to reach the saliva may not be ingested in those strains in which a latent period is demonstrable. Watson (1940) is correct in concluding that no fixed latent period occurs in several aphid-borne persistent viruses, and it

is now suggested that this may be due to the strains of the vector as well as to the characteristics of the virus.

It appears from published data that the mechanism of transmission of certain other viruses, e.g. beet yellows (Watson 1940), beet yellow-net (Sylvester 1949), and carrot motley dwarf (Stubbs 1948) may be similar to that of potato leaf roll. There may, in fact, be a series of aphid-borne viruses between these and such viruses as that of the lily symptomless disease described by Brierley and Smith (1944) and even that of strawberry virus 3 (Prentice and Woollcombe 1951), which has a very long latent period in its vector.

Leafhopper-borne viruses, with the exception of that causing beet curly-top, have longer latent periods in their vectors and multiplication of virus in the vector has been demonstrated in a number of them (Black 1953). On the basis of published work on the transmission of beet curly-top by *Circulifer tenellus* (Baker) it seems likely that the mechanism of transmission in this vector is similar to that described for potato leaf roll.

It will be apparent from the above that the occurrence of a very short latent period is not incompatible with the view that a virus is transmitted by the vector-latent mechanism, or even with multiplication of the virus in the insect vector. Black (1950) suggested the generalization that "most, if not all, plant viruses with *long* incubation (= latent) periods in their leafhopper vectors multiply in those vectors." Further work with beet yellows, beet yellow-net, and carrot motley dwarf virus may well permit the extension of this generalization to state that plant viruses with latent periods in their vectors multiply in those vectors.

## V. Acknowledgments

Thanks are due to Dr. E. M. Hutton for supplying the strain of potato leaf roll on which most of this work has been done, the *Physalis floridana* autotetraploid, and for much help in the early stages of the work. Infected tubers were kindly supplied by Mr. N. E. Grylls, Mr. E. Riek, and the officers of the Departments of Agriculture of N.S.W., Victoria, South Australia, and Tasmania: among these I would particularly thank Mr. R. J. Conroy, N.S.W.; Mr. R. Anderson, Victoria; Mr. H. K. Kemp, South Australia; and Dr. J. H. Wilson, Tasmania. Dr. R. H. Larson, University of Wisconsin, kindly permitted reference to his unpublished data. The author also wishes to acknowledge the careful technical assistance of Mrs. L. Abbot.

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