Neth. J. Pl. Path. 76 (1970) 234–239

The biological transmission of potato leafroll virus by Myzus persicae

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Accepted 4 December 1969

Abstract

The ability of the green peach aphid, *Myzus persicae*, to transmit potato leafroll virus after a six-hours' acquisition feeding period on leafroll-diseased plants of *Physalis floridana*, followed by a feeding for different lengths of time on Chinese cabbage (*Brassica pekinensis*) was determined. Virus transmission was optimally efficient at about the ninth day after aphid birth. If after a one-hour acquisition feeding period on a virus source the aphids were starved for different numbers of days at 4 °C, the efficiency increased with increasing starvation periods. Starvation at 4 °C prior to the one-hour acquisition feeding period had no effect on the ability to transmit the virus. Starvation of larvae at 4 °C did not prevent them completing their larval stage in about 9–10 days when transferred to plants at 20 °C.

Introduction

Elze (1927) found that viruliferous *Myzus persicae* (Sulz.) could transmit potato leafroll virus (PLRV) (Cryptogram: */*: */*: S/S: S,I/Ap)[1] to healthy potato plants after 7 or 10 days on the virus-immune host *Spinacia oleracea*. From this he concluded that an aphid, once it is infected, retains infectivity for a long time. Similar results were obtained by Smith (1929), MacCarthy (1954), and Day (1955). Moreover, Day (1955) found that the percentage of PLRV-diseased test plants increased with the time the aphids had stayed on the immune host *Brassica chinensis*. On the other hand, Stegwee and Ponsen (1958) demonstrated big variations in the infection ability of viruliferous aphids during their life.

The present study was undertaken to determine the ability of *M. persicae* to transmit PLRV during the course of their life.

Materials and methods

The same PLRV isolate was used as in earlier experiments (Ponsen, 1969). It was maintained in *P. floridana*, which was used as both source and test plant in all experiments. Stock colonies of *M. persicae* were kept in aphid-proof cages on Chinese cabbage (*Brassica pekinensis* Granaat). Throughout this study apterous viviparous aphids were used.

New-born larvae were selected from a virus-free colony on Chinese cabbage and were allowed to feed for 6 hours on a leafroll-diseased *P. floridana* plant about 5 weeks old. After this acquisition period the aphids were placed on one-leaf-stage Chinese

[1] See editorial note on cryptograms on page 227.

cabbage plants. About 5–10 larvae were kept on one Chinese cabbage seedling. This procedure was repeated in several parallels to get a large number of aphids of the same age. After 10 days the plants were examined daily for the presence of progeny and all young aphids were removed. The individually caged plants were kept in a cabinet at 20 °C (Ponsen, 1969). The aphids spent their whole life on the same cabbage plants. To test them for the presence of PLRV, aphids of a certain age were collected at random from several plants and transferred singly to one-leaf-stage *P. floridana* seedlings. After 24 hours the aphids were removed and the seedlings were placed in an insect-proof greenhouse at a controlled temperature varying between 18 ° and 22 °C and with a relative humidity of 60–80 %. In another experiment new-born aphids were allowed to feed for 1 hour on leafroll-diseased *P. floridana* plants 5 weeks old. After this acquisition period the larvae were stored at 4 °C for several days without food. Subsequently, they were transferred singly to *P. floridana* seedlings for 7 days to test them for the presence of PLRV.

Results

The results of the first experiment, presented in Fig. 1, show an optimal efficiency of transmission of PLRV by *M. persicae* at about the ninth day after their birth. The variation for each individual point of this curve is very large. It appears that of the aphids that spent a six-hours' acquisition feeding period on the leafroll source and thereafter 6 days on Chinese cabbage, 52 % could transmit PLRV with an infection feeding period of 24 hours on *P. floridana* seedlings (Fig. 1). However, if new-born aphids were allowed to feed for one hour on a leafroll source and thereafter for 7 days on test plants, the percentage virus transmission was 19 (Fig. 2). If, on the other hand, the latter were kept after their acquisition feeding period at 4 °C without food, it was found that the longer the aphids were kept at this temperature, the greater the increase in efficiency of transmission (Fig. 2). As in Fig. 1, the variation for each individual point of the curve in Fig. 2 is again very large. Starvation for 5 days at 4 °C prior to the acquisition feeding period of one hour, followed by an infection feeding period of 7 days on *P. floridana* seedlings, had no effect on the efficiency of virus transmission (Fig. 2, see*), for the percentage of transmission was not significantly different (14 % and 19 %, respectively), (χ^2 test, 2×2 table) from that obtained when an acquisition feeding period of one hour was given without storage at 4 °C (Fig. 2).

The results in Table 1 show that a longer acquisition feeding period on the virus source gives an increase of the number of leafroll-diseased plants. Similar results were obtained when the aphids were starved at 4 °C before testing. The results also show that at an acquisition feeding period of 4 hours or longer, starvation at 4 °C has no further effect on virus transmission (χ^2 test, 2×2 table).

The mortality of the new-born aphids, after starvation for 1–5 days at 4°C, varied between 0 and 10 %; after starvation for 6 days at the same temperature the mortality was about 50 %. During starvation at 4 °C the larvae produced no honeydew and did not moult; notwithstanding the storage of the larvae at 4 °C without food, they completed the larval stage in about 9–10 days when transferred to plants at 20 °C. Normally at this temperature the aphid completed the larval stage in 9–10 days (Ponsen, 1969).

Fig. 1. Efficiency of transmission of potato leafroll virus by *Myzus persicae* at 20 °C. New-born larvae selected from Chinese cabbage were allowed to feed for 6 hours on a leafroll-diseased *Physalis floridana* plant and were then placed on Chinese cabbage seedlings. At regular intervals samples of aphids were taken and tested singly on healthy *P. floridana* seedlings for 24 hours. The points represent the mean values of the several plots; each plot represents one experiment in which about 13 aphids were used.

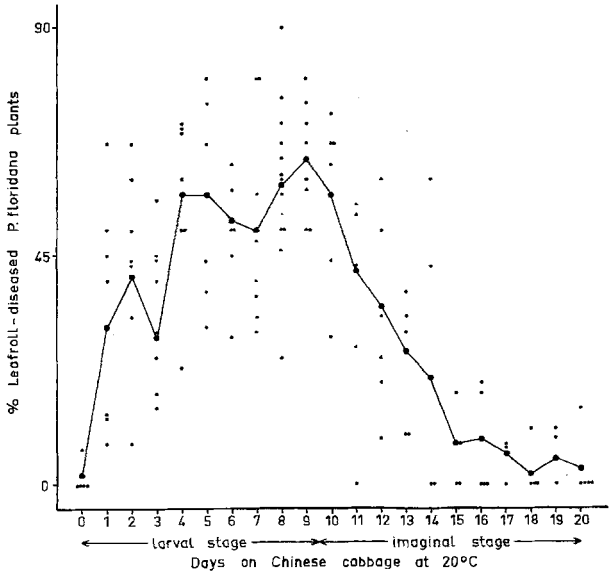

Fig. 1. De efficiëntie van de overdracht van het aardappelbladrolvirus door Myzus persicae bij 20 °C. Pasgeboren larven geselecteerd van Chinese kool werden gedurende 6 uur gezet op een bladrolzieke plant van Physalis floridana en daarna geplaatst op zaailingen van Chinese kool. Op geregelde tijden werd een aantal luizen genomen en afzonderlijk getoetst op gezonde zaailingen van P. floridana gedurende 24 uur. De punten vertegenwoordigen de gemiddelde waarden van de verschillende stippen; iedere stip vertegenwoordigt één proef waarbij ongeveer 13 luizen werden gebruikt.

Table 1. The ability of *Myzus persicae* to transmit potato leafroll virus after several hours' feeding on leafroll-diseased *Physalis floridana* followed by starvation at 4 °C. Infection feeding period 7 days (1 aphid per test plant).

Acquisition feeding period (hours)	Number of days at 4 °C without food before testing								
	0			4			5		
	a	b	c	a	b	c	a	b	c
4	4	43/78	55	2	18/29	62			
6	2	30/39	77	2	36/47	77			
24	8	83/92	90	2	32/34	94	2	33/34	97

a = Number of experiments
b = Number of diseased plants/number of test plants used
c = Percentage of plants infected

Tabel 1. Het vermogen van Myzus persicae om het aardappelbladrolvirus over te brengen na een voeding van verscheidene uren op een bladrolzieke plant van Physalis floridana gevolgd door een verblijf bij 4 °C zonder voedsel. Infectieperiode 7 dagen (1 luis per toetsplant).

Fig. 2. The ability of *Myzus persicae* to transmit potato leafroll virus after a storage at 4 °C without food. New-born larvae selected from Chinese cabbage were allowed to feed for 1 hour on a leafroll-diseased *Physalis floridana* plant and were then placed at 4 °C without food. At regular intervals samples of aphids were taken and tested singly on healthy *P. floridana* seedlings for 7 days. The points represent the mean values of the several plots; each plot represents one experiment in which about 17 aphids were used.

* The data in this column were obtained from new-born aphids stored at 4 °C without food followed by an acquisition feeding period of 1 hour on a leafroll source and thereafter tested singly on healthy test plants for 7 days.

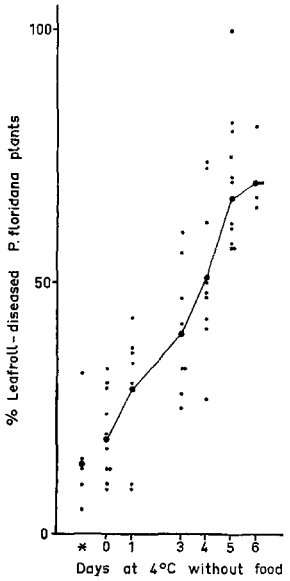

Fig. 2. Het vermogen van Myzus persicae om het aardappelbladrolvirus over te brengen na een verblijf bij 4 °C zonder voedsel. Pasgeboren larven geselecteerd van Chinese kool werden gedurende 1 uur gezet op een bladrolzieke plant van Physalis floridana en daarna weggezet bij 4 °C zonder voedsel. Op geregelde tijden werd een aantal luizen genomen en afzonderlijk getoetst op gezonde zaailingen van P. floridana gedurende 7 dagen. De punten vertegenwoordigen de gemiddelde waarden van de verschillende stippen; iedere stip vertegenwoordigt één proef waarbij ongeveer 17 luizen werden gebruikt.

** De gegevens in deze kolom zijn verkregen van pasgeboren luizen geplaatst bij 4 °C zonder voedsel, gevolgd door een opneemperiode van 1 uur op een bladrolbron en daarna afzonderlijk getoetst op gezonde toetsplanten gedurende 7 dagen.*

Discussion

Kassanis (1952) found that young or recently infected *Datura tatula* and potato plants were much better PLRV sources for *M. persicae* than older plants. He supposed that 'the PLRV content of the infected plants reaches an early maximum and then falls'. The results of Beemster (1960) pointed in the same direction. When leaves instead of whole plants were used, the same phenomenon was shown with PLRV (MacKinnon, 1962) and also with other virus-hostplant combinations (Stimmann and Swenson, 1967; Gill, 1969). Similar results have now been obtained with PLRV in *M. persicae*. After a six-hours' acquisition feeding period on a leafroll source the efficiency of virus

transmission by *M. persicae* increased to about the ninth day after birth, then decreased gradually in the imaginal stage during the time the aphids remained on Chinese cabbage (Fig. 1). Since PLRV multiplies in its vector (Stegwee and Ponsen, 1958) this increase during the larval stage of *M. persicae* must be due to virus multiplication in the aphid. In the imaginal stage of the aphid no virus multiplication probably takes place, although the virus could be recovered from the haemolymph (Stegwee and Ponsen, 1958). According to Harrewijn and Noordink (in prep.) the imaginal aphids (*M. persicae*) have a lower feeding ratio than the larvae; these authors also suggest that presumably the excretion of saliva is proportionally related to the uptake of food. The decrease in efficiency of PLRV transmission by imaginal aphids might well be explained in a similar way. Analogous results were obtained by Sylvester (1967). He found that the decline in the transmission rate of pea enation mosaic virus by *Acyrthosiphon pisum*, occurred with a similar decline in the rates of reproduction and excretion and presumably reflected a general lessening in the feeding activity of ageing aphid vectors.

The presence of PLRV in the honeydew from aphids feeding on a leafroll source (Ponsen, unpublished data) could indicate that part of the virus ingested during the acquisition period is lost with the honeydew. Moreover, Broadbent (1951) observed that aphids excreted only while feeding. During storage at 4°C without food the larvae did not produce any honeydew, so that during that time the ingested virus would presumably have the opportunity to penetrate into the haemolymph. The longer the larvae remained at 4 °C without food, the more virus might penetrate into the haemolymph and thus the more virus be in a position to multiply later at 20 °C. This would correlate with the increase in efficiency of transmission of PLRV by viruliferous aphids (Fig. 2). The aphids which were transmitted directly to *P. floridana* seedlings lost part of the ingested virus with the honeydew, which could account for the small percentage of leafroll-diseased plants in this group (Fig. 2). From this it may be concluded that the ingested virus was partly excreted with the honeydew, and partly penetrated through the gutwall into the haemolymph, and that the gutwall is no appreciable barrier for PLRV.

The large variation for each point on the curves is due to the occasional failure of viruliferous aphids to infect plants, as already demonstrated by Day (1955).

Acknowledgments

The author expresses his sincere thanks to Professor Dr J. P. H. van der Want and Dr D. Noordam for their stimulating advice, and to Mr C. A. van den Anker for the statistical analyses.

Samenvatting

De biologische overdracht van het aardappelbladrolvirus door Myzus persicae

Het vermogen van de groene perzikluis, *Myzus persicae*, om het aardappelbladrolvirus over te brengen werd gedurende haar leven nagegaan. Daartoe werden pasgeboren luizen zes uur op een bladrolzieke plant van *Physalis floridana* gezet en daarna op Chinese kool. Vervolgens werden de luizen op verschillende tijden gedurende 24 uur ge-

toetst op zaailingen van *P. floridana*. Het bleek dat maximale virusoverdracht door *M. persicae* plaatsvond op de negende dag na infectie (Fig. 1). Tevens werd gevonden dat het vermogen van virusoverdracht door de luis werd verhoogd wanneer ze na een opneemperiode van 1 uur op een virusbron werden weggezet bij 4 °C zonder voedsel. Werden de luizen vóór de opneemperiode van 1 uur op de virusbron weggezet bij 4 °C zonder voedsel, dan bleek deze behandeling geen invloed te hebben op de virusoverdracht (Fig. 2, zie*). Een opneemperiode op de virusbron van 4 uur of langer en een verblijf bij 4 °C zonder voedsel, had eveneens géén invloed op de virusoverdracht (Tabel 1).

References

Beemster, A. B. R., 1960. Translocation of leafroll and virus Y in the potato. Proc. 4th Conf. Potato Virus Dis. (Braunschweig): 60–67

Broadbent, L., 1951. Aphid excretion. Proc. R. ent. Soc. Lond. 26: 97–103.

Day, M. F., 1955. The mechanism of the transmission of potato leafroll virus by aphids. Aust. J. biol. Sci. 8: 498–513.

Elze, D. L., 1927. De verspreiding van virusziekten van den aardappel (Solanum tuberosum L.) door insecten. Meded. LandbHogesch. Wageningen 31:1–90.

Gill, C. C., 1969. Cyclical transmissibility of barley yellow dwarf virus from oats with increasing age of infection. Phytopathology 59: 23–28.

Harrewijn, P. & Noordink, J. P. W., in prep. Food uptake of Myzus persicae from artificial diets. Neth. J. Pl. Path.

Kassanis, B., 1952 Some factors affecting the transmission of leafroll virus by aphids. Ann. appl. Biol. 39: 157–165.

MacCarthy, H. R., 1954. Aphid transmission of potato leafroll virus. Phytopathology 44: 167–174.

MacKinnon, J. P., 1962. Comparative studies between the aphid transmission of potato leafroll and turnip latent viruses. Can. J. Bot. 40: 525–531.

Ponsen, M. B., 1969. The effect of potato leafroll virus on the biology of Myzus persicae. Neth. J. Pl. Path. 75: 360–368.

Smith, K. M., 1929. Studies on potato virus diseases V; Insect transmission of potato leafroll. Ann. appl. Biol. 16: 209–230.

Stegwee, D. & Ponsen, M.B., 1958. Multiplication of potato leafroll virus in the aphid Myzus persicae (Sulz.). Entomologia exp. appl. 1: 291–300.

Stimmann, M. W. & Swenson, K. G., 1967. Aphid transmission of cucumber mosaic virus affected by temperature and age of infection in diseased plants. Phytopathology 57: 1074–1076.

Sylvester, E. S., 1967. Retention of inoculativity in the transmission of pea enation mosaic virus by pea aphids as associated with virus isolates, aphid reproduction and excretion. Virology 32: 524–531.