Neth. J. Pl. Path. 75 (1969) 360–368

The effect of potato leafroll virus on the biology of Myzus persicae

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Accepted 23 June 1969

Abstract

Since potato leafroll virus multiplies in the green peach aphid, *Myzus persicae*, the effect of the virus on the biology of its vector was investigated. Observations were made regarding the longevity and the reproduction rate of viruliferous and non-viruliferous aphids on leafroll-diseased and healthy plants of *Physalis floridana*. The same matters were investigated for both viruliferous and non-viruliferous aphids on seedlings of Chinese cabbage (*Brassica pekinensis*). It was shown that on leafroll-diseased plants of *P. floridana* the aphids produced more progeny than on healthy ones, although the average number of progeny produced per day in both cases was almost the same. On healthy Chinese cabbage seedlings there was no difference in average length of the larval and adult stages, number of progeny per aphid, and number of progeny per day, between viruliferous and non-viruliferous aphids. Evidence was obtained that the virus does not influence the development of its vector. Measurements of oxygen consumption of both viruliferous and non-viruliferous aphids point in the same direction.

Introduction

There has been some discussion as to whether or not potato leafroll virus (PLRV), which multiplies within its vector *Myzus persicae* (Sulz.) (Stegwee and Ponsen, 1958), affects the development of this aphid. Janssen (1929) and Arenz (1951) reported a more rapid increase in number of *M. persicae* on leafroll-diseased potato plants than on healthy ones. MacKinnon (1961) ascertained that adults had a longer life and consequently produced more offspring on leafroll-diseased *Physalis floridana* than on healthy controls. These results suggest that the virus is not pathogenic towards its vector. On the other hand, Ehrhardt (1960) reported that *M. persicae* infected with PLRV respired much less than non-viruliferous individuals of the same species. Although the experiments were carried out with adults of unknown age, he attributed the decrease in oxygen consumption of viruliferous aphids to the effect of virus multiplication.

The present study was designed to determine the effect of PLRV on the biology of the aphid by means of observations on longevity, reproduction and measurements of oxygen consumption.

Materials and methods

The experiments were carried out with the isolate of PLRV used by Stegwee and Ponsen (1958). The virus was maintained in *P. floridana* by repeated aphid transfers. To produce uniform leafroll-diseased plants, virus-free larval aphids from Chinese cabbage (*Brassica pekinensis* cv. 'Granaat') were allowed to feed for 24 h on a leafroll

source (*P. floridana* plants about 5 weeks old), after which they were placed singly for another 24 h on one-leaf-stage seedlings of *P. floridana*. Stock colonies of *M. persicae* were kept in aphid-proof cages on Chinese cabbage. In winter the aphids were exposed to additional light during 18 h a day. Throughout this study apterous viviparous aphids were used.

P. floridana was used as a test plant for PLRV. The plants were maintained in an insect-proof greenhouse provided with thermostatically controlled heating and cooling at a temperature varying between 18° and 22 °C and with a relative humidity of 60–80 %. During winter the plants received additional light to provide a day-length of 16 h.

Longevity and reproduction. New-born larvae were selected from a virus-free colony on Chinese cabbage and were individually allowed to feed on a leafroll-diseased and a healthy *P. floridana* plant of about 5 weeks. The caged plants were maintained in a cabinet at 20 °C. In other experiments new-born aphids were allowed to feed for 24 h only on leafroll-diseased and healthy *Physalis* plants 5 weeks old. After this acquisition period the aphids were placed individually on healthy one-leaf-stage *Physalis* seedlings, or on one-leaf-stage Chinese cabbage seedlings. The caged plants were maintained at room temperature. To eliminate as far as possible differences due to environmental conditions, the plants with infected larvae and those with non-viruliferous ones were arranged at random. The aphids were examined daily and the time of each moult, number of progeny produced, and mortality recorded, the offspring being removed and killed.

Oxygen consumption. New-born larvae were selected from a virus-free colony on Chinese cabbage and transferred either to Chinese cabbage seedlings in the one-leaf-stage or to leafroll-diseased *Physalis* plants of 5 weeks. After an acquisition period of 24 h on the leafroll source these aphids were allowed to feed on one-leaf-stage Chinese cabbage plants. About 5–10 larvae were kept on one Chinese cabbage seedling. This procedure was repeated in several parallels in order to measure the oxygen consumption of 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. The aphids spent their whole life on the same cabbage plants. To determine the oxygen consumption, aphids of a certain age were collected at random from several plants and placed in a respirometer vessel. Depending on their age, the number of aphids per vessel varied between 6 and 60.

The oxygen consumption was measured at 25 °C with a Barcroft respirometer according to the procedure described by Umbriet et al. (1957). The respirometer consisted of two reaction vessels (volume about 12 ml), each provided with a centre well and one side arm. The centre well of each vessel contained 1 ml of 10 % KOH, in which a piece of filter paper (10 × 25 mm) was dipped to increase the absorbing surface. The side arm of each vessel was fitted with a piece of filter paper (5 × 23 mm) which could serve in the experimental vessel as a substrate for the aphids. The side arms were plugged with cotton wool. Readings were taken at regular intervals during 2–3 h.

After oxygen measurement the aphids were killed, except those 10 days old. They

were transferred singly to *P. floridana* seedlings for 7 days to test them for the presence of PLRV. Subsequently, the seedlings were placed in an insect-proof greenhouse.

Cabinet. In the growth cabinet (Zephyr, Zoetermeer) only light and temperature were controllable. The temperature was 20 °C (± 2 °C), while the relative humidity was 70–90 %. Artificial light was given for 18 h per day be 12 Philips TLF 65 W/33 daylight fluorescent tubes. The tubes (6 on each side of the cabinet) were situated behind double glass in order to avoid any unwanted effect on the temperature inside the cabinet.

Results

Longevity and reproduction. The results presented in Table 1 show that on leafroll-diseased *P. floridana* plants highly significantly ($P < 0.01$) more aphids produced progeny than on healthy ones (2 × 2 table, exact test). Moreover, on diseased plants the aphids produced highly significantly ($P < 0.01$, Wilcoxon test) more progeny than on healthy ones, although the average number of progeny produced per day in both cases was almost the same. Also the average duration of the adult stage was longer on diseased (19 days) than on healthy plants (7 days). In both cases the average duration of the larval stage did not differ significantly (Wilcoxon test). On the other hand it appeared that on healthy *Physalis* seedlings (Table 2, exp. 1 and 3) the aphids developed faster than on healthy 5-week plants (Table 1). Moreover, on healthy seedlings there were no differences in average length of the larval and adult stages, number of progeny per aphid, and number of progeny per day between viruliferous aphids and non-viruliferous ones. Thus, healthy *Physalis* seedlings (Table 2) are more suitable for aphids than old healthy plants (Table 1). PLRV does not affect the development of its vector as was demonstrated by using immune Chinese cabbage (Table 2, exp. 2); no differences in longevity or average number of progeny per aphid between viruliferous and non-viruliferous aphids were observed. The aphids had a longer life on leafroll-

Table 1. Length of larval and adult stages, and number of progeny of *Myzus persicae* on leafroll-diseased and healthy *Physalis floridana* plants at 20 °C.

	Transfer of new-born larvae from Chinese cabbage to	
	leafroll-diseased *P. floridana*	healthy *P. floridana*
Total number of aphids	16	18
without progeny	1	11
with progeny	15	7
average number of progeny per aphid	36 ± 3.94	13 ± 2.14
average number of progeny per day	2.0 ± 0.23	1.9 ± 0.75
Number of aphids which have become adult	16	8
average length of larval stage (days)	10 ± 0.21	11 ± 1.03
average length of adult stage (days)	19 ± 2.82	7 ± 1.28

The sign ± is followed by the standard deviation of the mean ($s/\sqrt{n}$).

Tabel 1. Duur van larvale stadium, volwassen stadium en aantal nakomelingen van Myzus persicae op bladrolzieke en gezonde planten van Physalis floridana.

Table 2. Length of larval and adult stages, and number of progeny of viruliferous *Myzus persicae* at room temperature. New-born larvae from Chinese cabbage were allowed to feed for 24 h on leafroll-diseased (A) and healthy (B) *Physalis floridana* plants and thereafter placed singly on healthy *P. floridana* seedlings or on Chinese cabbage seedlings.

Exp.	Larvae	Adults	Total number of aphids n	Average length of larval stage (days) $\bar{x} \pm s/\sqrt{n}$	Average length of adult stage (days) $\bar{x} \pm s/\sqrt{n}$	Average number of progeny per aphid $\bar{x} \pm s/\sqrt{n}$	Average number of progeny per day $\bar{x} \pm s/\sqrt{n}$	Number of diseased plants/ number of test plants
1	A	*P. floridana* seedlings	25	9 ± 0.17	11 ± 1.27	27 ± 4.00	2.5 ± 0.19	23/25
	B		19	10 ± 0.24	11 ± 1.36	27 ± 3.52	2.6 ± 0.20	0/19
2	A 1*	Chinese cabbage seedlings	10	10 ± 0.23	12 ± 1.60	36 ± 5.46	3.0 ± 0.16	
	A 2*		21	9 ± 0.13	12 ± 0.78	43 ± 3.20	3.6 ± 0.19	
	A 3*		20	9 ± 0.20	14 ± 1.45	39 ± 5.26	2.7 ± 0.20	
	B 1*		20	10 ± 0.22	14 ± 1.37	43 ± 4.16	3.1 ± 0.14	
	B 2*		20	9 ± 0.15	13 ± 1.16	42 ± 3.25	3.5 ± 0.15	
	B 3*		21	10 ± 0.15	13 ± 1.22	38 ± 4.72	2.6 ± 0.16	
3	A Chinese cabbage seedlings**	*P. floridana* seedlings	20	10 ± 0.18	10 ± 0.92	31 ± 3.90	2.9 ± 0.21	19/20
	B		20	10 ± 0.19	11 ± 0.86	31 ± 3.13	2.9 ± 0.18	0/20

* Parallel experiments respectively.

** After the last ecdysis the aphids were transferred singly to one-leaf-stage healthy *P. floridana* seedlings.

Table 2. Duur van larvale stadium, volwassen stadium en aantal nakomelingen van virushoudende en virusvrije Myzus persicae. Pas geboren larven geselecteerd van Chinese kool, werden gedurende 24 uur gezet respectievelijk op een bladrolzieke (A) en gezonde (B) plant van Physalis floridana en daarna afzonderlijk geplaatst op gezonde zaailingen van P. floridana of zaailingen van Chinese kool.

diseased *Physalis* plants (Table 1) than on Chinese cabbage seedlings, but the average number of progeny per aphid was about the same (Table 2, exp. 2). In all experiments the number of moults was 4, but a few individuals in each experiment moulted only three times. The maximum number of progeny an aphid could produce within 24 h was 12.

After an acquisition-feeding period of 24 h on a diseased *P. floridana* plant 23 out of 25 aphids were able to transmit the PLRV (Table 2, exp. 1). The results in Table 2, exp. 3 show that 19 out of 20 aphids retained infectivity during their larval stage on the immune host plant. None of the non-viruliferous aphids became infective.

Oxygen consumption. Average weights of viruliferous and non-viruliferous aphids of the same age did not show significant differences. In both cases the average weight increased continuously up till the tenth day, and thenceforth remained almost constant. The average weight of an adult aphid is about 18-fold that of a new-born one (Table 3).

Viruliferous and non-viruliferous aphids of the same age did not differ significantly in oxygen consumption (Table 3). The average duration of the larval stage was 9 days and during that time the increase in respiration seemed to be irregular. During the adult stage the decrease in respiration intensity was very uniform.

Of the aphids fed for 24 h on a leafroll source, about 92 % retained infectivity during the larval stage on the immune host. None of the non-viruliferous aphids became infective (Table 3).

Discussion

Many investigators have reported that several species of insect vectors developed faster, lived longer, and gave more offspring on virus-diseased plants than on healthy hosts (Janssen, 1929; Carter, 1939; Severin, 1946; Kennedy, 1951; Arenz, 1951; Hijner and Cordon, 1953; Maramorosch, 1958; Baker, 1960; Miller and Coon, 1964; Saini and Peterson, 1965). However, the opposite has also been reported (Jensen, 1959, 1960; Shinkai, 1960; Okuyama, 1962; Lowe and Strong, 1963; Nasu, 1963).

For PLRV Janssen (1929) and Arenz (1951) showed that *M. persicae* multiplied substantially faster on virus-diseased than on healthy potato plants. MacKinnon (1961) established that adults of this species had a longer life and consequently more progeny on leafroll-diseased *P. floridana* plants than on comparable healthy plants. The results of the present study with *M. persicae* on *Physalis* agree with those of MacKinnon. In my experiments, however, the average duration of the larval stage and the average number of progeny per day were statistically not influenced by infection (Table 1). On the other hand, there was no difference between viruliferous and non-viruliferous aphids on healthy *Physalis* seedlings (Table 2, exp. 1 and 3). Kunkel (1926) working with young aster plants (*Callistephus chinensis*) also found no difference in longevity between *Macrosteles divisus* (= *Cicadula sexnotata*) infected with the causal agent of aster yellows and non-viruliferous leafhoppers. Aster yellows, however, may be due to a pathogen belonging to the group of *Pleuropneumonia*-like organisms (Doi et al., 1967).

By choosing Chinese cabbage, immune from PLRV, as host, nutritional differences between viruliferous and non-viruliferous aphids could be eliminated. On this host

the average lengths of the larval and adult stages, number of progeny per aphid, and number of progeny per day were nearly equal for both groups (Table 2, exp. 2). Thus, the propagative PLRV was not pathogenic to its aphid vector, *M. persicae*. These

Table 3. Weight and oxygen consumption of viruliferous and non-viruliferous *Myzus persicae* on successive days. New-born larvae selected from Chinese cabbage were allowed to feed for 24 h on a leafroll-diseased *Physalis floridana* plant and thereafter placed on Chinese cabbage seedlings (A). The controls were placed directly on Chinese cabbage seedlings (B). Afterwards the larval stage aphids were tested on *P. floridana* seedlings for 7 days. The oxygen consumption was measured at 25 °C.

Age of the aphids (days)	Total number of aphids	Average weight of 1 aphid (mg) $\bar{x} \pm s/\sqrt{n}$	Number of tests (n)	Number of aphids per test	ml O_2/g/h $\bar{x} \pm s/\sqrt{n}$	Number of diseased plants/number of test plants
Viruliferous aphids (A)						
Larva 4	125	0.08 ± 0.00	5	25	2.65 ± 0.37	
5	117	0.11 ± 0.01	6	17, 20	2.91 ± 0.17	
6	100	0.18 ± 0.02	5	20	2.74 ± 0.17	
7	100	0.26 ± 0.01	5	20	3.15 ± 0.20	
8	180	0.33 ± 0.01	10	15, 20	3.29 ± 0.05	
9	200	0.43 ± 0.01	11	15, 20	3.18 ± 0.07	
Adult 10	180	0.51 ± 0.02	12	15	3.21 ± 0.08	96/104
12	145	0.58 ± 0.03	10	10, 15, 20	3.05 ± 0.10	
14	145	0.54 ± 0.02	10	10, 15	2.94 ± 0.10	
16	150	0.60 ± 0.02	12	10, 15	2.87 ± 0.09	
18	113	0.58 ± 0.02	12	6, 7, 10	2.58 ± 0.12	
20	64	0.53 ± 0.01	7	6, 8, 10	2,23 ± 0.19	
22	87	0.53 ± 0.02	11	5, 7, 8, 9, 14	2.16 ± 0.14	
Non-viruliferous aphids (B)						
Larva 1	100	0.03 ± 0.01	2	50	1.73 ± 0.10	
2	120	0.05 ± 0.00	2	60	2.50 ± 0.04	
4	125	0.08 ± 0.00	5	25	2,75 ± 0.22	
5	130	0.12 ± 0.01	6	20, 25	3.01 ± 0.23	
6	100	0.17 ± 0.01	5	20	2.69 ± 0.14	
7	130	0.25 ± 0.00	7	15, 20	3.10 ± 0.07	
8	160	0.29 ± 0.01	10	15, 20	3.28 ± 0.20	
9	185	0.40 ± 0.01	11	15, 20	3.29 ± 0.10	
Adult 10	180	0.53 ± 0.02	12	15	3.19 ± 0.07	0/98
12	160	0.53 ± 0.02	10	15, 20	3.12 ± 0.07	
14	165	0.60 ± 0.02	12	10, 15, 20	2.89 ± 0.08	
16	165	0.57 ± 0.02	12	10, 15	2.73 ± 0.10	
18	118	0.52 ± 0.02	11	10, 11, 12, 15	2.58 ± 0.10	
20	70	0.55 ± 0.03	7	10	2.38 ± 0.10	
22	85	0.56 ± 0.03	11	5, 6, 7, 8, 10	2.16 ± 0.12	

Tabel 3. Het gewicht en de zuurstofconsumptie van virushoudende en virusvrije Myzus persicae op achtereenvolgende dagen. Pas geboren larven geselekteerd van Chinese kool werden gedurende 24 uur gezet op een bladrolzieke plant van Physalis floridana en daarna geplaatst op zaailingen van Chinese kool (A). De controle-luizen werden direct geplaatst op zaailingen van Chinese kool (B). Na het larvale stadium werden de luizen gedurende 7 dagen getoetst op zaailingen van P. floridana. De zuurstofconsumptie werd bij 25 °C gemeten.

results were similar to those of Severin (1947), who through the use of an immune host plant found the propagative aster yellows "virus" not to be pathogenic to its leafhopper vector, *Macrosteles divisus*. Likewise, the vertebrate viruses, yellow fever and Japanese B encephalitis multiplying in *Aëdes aegypti* and *Culex pipiens*, respectively, were not pathogenic to their respective mosquito vectors (Whitman, 1937; La Motte, 1960). The experiments surveyed in Table 2 and 3 show that after an acquisition feeding period of 24 h on a leafroll source, the percentages of viruliferous aphids varied between 90 and 95 %. The low percentages of aphids which did not transmit the virus, probably have not influenced the mean values.

The conclusion that PLRV is not pathogenic to the vector is further supported by the fact that no differences in weight and oxygen consumption could be detected between viruliferous aphids and non-viruliferous ones of the same age (Table 3). Ehrhardt (1960) found that the oxygen consumption of adults of *M. persicae*, fed for 8 h on a leafroll-diseased *P. floridana* plant and subsequently for 30 h on Chinese cabbage, was 30 % lower than that of adults taken from Chinese cabbage. Although his experiments were carried out with adults of unknown age, the reduction in oxygen consumption was attributed to the effects of virus multiplication. However, I have not found such a difference in oxygen consumption, but an increase during the larval stage and a decrease in the adult stage. Thus, the oxygen consumption is not influenced by the multiplication of PLRV in *M. persicae*. Ehrhardt's results might be attributed to the transfer of adult aphids to *P. floridana* and thence to Chinese cabbage plants.

Miller and Coon (1964) reported a reduction of 13.8 % in the oxygen consumption of *Macrosiphum granarium* reared on oat plants infected with barley yellow dwarf virus. Yoshii and Kiso (1957) demonstrated changes in oxygen consumption and a reduction in total amounts of phosphorus in orange trees with dwarf disease virus and in its planthopper vector, *Geisha distinctissima*. On the other hand Yoshii (1959) found that the leafhopper *Nephotettix bipunctatus cincticeps*, transovarially transmitting the rice dwarf virus, showed a higher oxygen consumption and higher total amounts of phosphorus on diseased than on healthy rice plants. These results do not warrant the conclusion that the virus is harmful to the insect vectors; the physiological condition of the virus-diseased plant might also have affected the oxygen consumption of the vector.

Acknowledgments

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

Samenvatting

Het effect van het aardappelbladrolvirus op de biologie van Myzus persicae

Met het oog op de vermeerdering van het aardappelbladrolvirus in de groene persikluis, *Myzus persicae*, werd het effect van het virus op de ontwikkeling van deze vector onderzocht. Daartoe werden de levensduur en het aantal nakomelingen van pasgeboren luizen op zowel bladrolzieke als gezonde planten van *Physalis floridana* nagegaan

(Tabel 1). Eveneens werd dit onderzocht voor zowel virushoudende als virusvrije luizen op zaailingen van *P. floridana* en Chinese kool (Tabel 2). Tevens werd de zuurstofconsumptie van zowel virushoudende als virusvrije luizen op Chinese koolzaailingen gemeten (Tabel 3). Uit dit onderzoek is gebleken dat het bladrolvirus geen schadelijk effect heeft op de levensduur, de omvang der nakomelingschap en de zuurstofconsumptie van de vector, hoewel het zich daarin wel vermeerdert.

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