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THE EFFECT OF OIL ON THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS DURING SHORT INOCULATION PROBES

BY

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The transmission efficiency of pea enation mosaic virus by *Acyrthosiphon pisum* (Harris) in short inoculation periods to pea plants sprayed with oil prior to the test, was compared with that to plants which were not coated with an oil film. The transmission of virus to the former group was lower (approximately 11%). However, this difference was not significant. The probing behaviour measured by parameters such as test-probe frequency prior to phloem-seeking probes, duration of the probes and the cumulative duration of the short inoculation periods, did not differ for aphids probing on plants with and without oil film. The results suggest that the mechanism of pea enation mosaic virus transmission in short inoculation access periods differs from that of the non-persistent viruses.

Aphid transmission of stylet-borne viruses is counteracted by a film of oil on plants (Bradley, 1963; Hein, 1971). Reduction of transmission efficiency has been observed in the laboratory as well as in field experiments (Bradley *et al.*, 1966; Loebenstein *et al.*, 1964; Loebenstein *et al.*, 1970; Vanderveken & Semal, 1966). The efficiency could be reduced by 80—100%. The transmission of viruses that are circulative in aphids does not seem to be impaired by such a film on the leaves (Hein, 1971; Vanderveken, 1968). This may be ascribed to the mechanism of transmission and to the deeper location of the susceptible tissues in the plant.

Pea enation mosaic virus (PEMV), though one of the viruses circulative in aphids, can be transmitted in short probes (McEwen *et al.*, 1957; Nault & Gyrisco, 1966). This can be attributed to the susceptibility to the virus of such tissues as the epidermis and parenchyma. Nault & Gyrisco (1966) studied short-term inoculations and found that viruliferous pea aphids, *Acyrthosiphon pisum*, were able to transmit PEMV to pea plants during single test probes as short as 7 sec. An increase in transmission occurred with longer probes. Since the transmission of circulative viruses is not impaired by oil, investigations were carried out to examine whether oil affects the transmission of PEMV in short-term inoculations. To compare the behaviour pattern of aphids on plants either coated or free of oil, the probing activity was observed and measured by several parameters. Results of these investigations are reported here.

MATERIAL AND METHODS

The experiments were carried out with 7-day-old pea aphids (*Acyrthosiphon pisum*). Colonies were established by adults on pea plants, *Pisum sativum*,

"Koroza", which had been infected 10 days in advance. After 24 hr the adults were removed, so that the nymphs did not vary in age by more than 24 hr.

The plants were sprayed one day before the test inoculations were made, using an emulsion of 0.2% Bayol, produced and kindly supplied by Esso (the Netherlands), mixed with 0.2% of an emulsifier (Agral, produced by I.C.I. Holland). The plants were sprayed until the first droplets dripped from the leaves. Control plants were sprayed with water containing 0.2% emulsifier. The oil used reduced the transmission efficiency of potato virus by about 80%. This figure was the average of three experiments in which 200 aphids (*Myzus persicae* (Sulz.)) each were tested on control and oil-coated test plants (*Physalis floridana*). This reduction equals the effects of the oil samples used by Bradley (1963).

The pea aphids were starved 2 hrs before they were allowed to probe on the test plants, so that they would settle and probe readily. Each aphid was allowed to make four probes on a single test plant. The duration of the probes was measured. A probe began when the aphid placed its rostrum at the leaf surface and the antennae moved slowly backwards. The probe was considered to end when the rostrum was lifted or removed. All observations were made with a hand lens and timed with a stop watch. A probe which had not ended before 60 sec was terminated artificially by lifting the aphid gently with a brush. Such an aphid could then make new probes until it had finished its series of four. According to the terminology used by Nault & Gyrisco (1966), a probe which is terminated naturally will be called a test probe. These probes generally lasted 25 sec or less. Those probes which were ended artificially at 60 sec are considered to be phloem-seeking probes, as the aphid could have reached the phloem if not interrupted.

Plants were grown, aphids reared, and experiments conducted in an aphid-proof greenhouse at 20—22°C.

RESULTS

Transmission. In these experiments each of the 365 specimens of *A. pisum* used (187 on plants sprayed with oil and 178 on control plants) was allowed to make four probes on a single test plant. Of the 187 oil-sprayed plants 87, and of the 178 control plants 102 became infected (Table I). The percentage of plants infected was thus lower (11%) in the group sprayed with oil.

The duration of the probes made by each aphid on the test plant was accumulated and then divided into four categories. The transmission rate increased with the cumulative duration of the probes (Table I). This is an agreement with the results of Nault & Gyrisco (1966), who demonstrated that the rate of transmission was positively correlated with the duration of the probes.

Moreover, it can be derived from the distribution of the categories (Table I) that the cumulative probe duration did not differ for aphids on leaves either coated or free from oil. The averages of these durations appeared to be 31.1 and 31.7 sec, respectively. This indicates that the behaviour of the aphids on plants coated with oil did not differ from that of aphids which probed on plants without

TABLE I

Transmission of pea enation mosaic virus by pea aphids (A. pisum) *in short inoculation access periods to pea plants with or without an oil-coating*

Cumulative durations of successive test probes (sec)	Aphids probing on plants (%)		Plants infected with PEMV (%)	
	coated with oil	not coated with oil	coated with oil	not coated with oil
0— 60	10	8	15	20
61—120	42	40	42	59
121—180	32	36	51	56
181—240	16	16	66	75
Numbers of aphids tested and plants infected	187	178	87	57

an oil film. Bradley (1963) also failed to detect any effect of oil on the probing activity of aphids.

The cumulative durations were somewhat longer for the transmitting aphids than for those whose inoculations were negative. The average values were 33.8 and 29.1 sec, respectively.

Probing behaviour. Nault & Gyrisco (1966) studied the probing behaviour of the pea aphid. The behaviour of aphids on plants with or without oil film was compared by us in an identical manner. Our results show that the aphids (84%) had a strong tendency to make one or more test probes (Table II) before they initiated a phloem-seeking probe. The duration of the test probes ranged from 5 to about 22 sec, with an average duration of 16.2 sec. The duration of the first test probe was slightly shorter than that of the other probes. It can be seen in

TABLE II

Number of test probes made by pea aphids (A. pisum) *on pea plants with or without an oil-coating before they made a phloem-seeking probe*

No probes	No and % of aphids making zero, one or more test probes in a series on plants				Total No aphids	Average duration of test in sec
	coated with oil [2]	coated with oil [3]	not coated with oil [2]	not coated with oil [3]		
0	15 (17%)	14 (14%)	21 (20%)	7 (10%)	57 (16%)	
1	27 (31%)	13 (13%)	34 (33%)	30 (41%)	104 (29%)	15.9
2	20 (23%)	26 (26%)	27 (26%)	15 (21%)	88 (24%)	16.5
3	13 (15%)	14 (14%)	11 (11%)	8 (11%)	46 (13%)	16.5
4[1]	12 (14%)	33 (33%)	11 (11%)	14 (19%)	70 (19%)	16.5

[1]) Aphids were not allowed to make more than four probes.
[2]) Probings on plants which had been infected by the aphids.
[3]) Probings on plants which had not been infected by the aphids.

Table II that the tendency to make several test probes before a phloem-seeking probe is equally strong for both groups of aphids. The aphids on plants coated with oil-made test probes of 16.4 sec; the test probes on the control plants lasted 16.1 sec.

Among the 365 aphids, 57 made a phloem-seeking probe in the first probe, and of these 11 made these probes in all the subsequent probes. The other aphids of this group made both test probes and phloem-seeking probes in the subsequent tests. The average of the test probes was 16.4 sec, indicating that these probes do not differ in character or function from test probes made by aphids which initially probed one or more times.

DISCUSSION

It has been emphasized by several authors (Bradley, 1963; Vanderveken & Semal, 1966) that aphids do not change their probing pattern on plants sprayed with oil. We measured this activity by parameters such as the duration of the test probes, the test probe frequency prior to phloem-seeking probes and the distribution of cumulative durations of the short inoculation periods. The probing pattern did not differ for the aphids which probed on plants with or without an oil-coating.

In our experiments the transmission rate of PEMV by *A. pisum* obtained in short inoculation periods was not significantly affected by oil. The average duration of each short-term inoculation was about 30 sec and the aphids were not permitted to make phloem-seeking probes for longer than a minute. In a period of 30 sec approximately 30% of the aphid stylets passed through the epidermis and began to penetrate the parenchyma (Nault & Gyrisco, 1966). At 60 sec the stylets of all aphids were in the parenchyma. There is no reason to assume that the duration or the character of the probings made by vectors transmitting non-persistent viruses will differ from that of pea aphids. The probing pattern of *M. persicae*, *Macrosiphum euphorbiae* (Thomas), *Aphis nasturtii* (Kaltenbach) and *A. fabae* (Scopoli) seems to be identical to that described for the pea aphid (Nault & Gyrisco, 1966). This means that during probes, non-persistent viruses and PEMV will be transmitted to the same tissues. Therefore, the differences observed on the effects of oil film on the transmission rate of stylet-borne viruses and PEMV cannot be due to infection of different tissues during the probes. Thus, this difference has to be explained by the mechanism by which the non-persistent viruses and PEMV are transmitted. Stylet-borne viruses are carried at the tip of the stylet and infect the plant when the vector makes test or phloem-seeking probes. PEMV circulates through the vector and has to reach the salivary glands prior to transmission. Small amounts of saliva, containing virus, will be produced during the probes and will be the source by which the plant can be infected. It may be assumed that oil covering the cuticle may inactivate the virus. In this way non-persistent viruses may be inactivated at the tip of the stylets, whereas PEMV can be protected in the saliva against oil. However, Loebenstein *et al.* (1964)

showed that oil did not impair the infectivity of tobacco mosaic and cucumber mosaic virus. Besides Külps (1971) noted that oil not only penetrated the cuticle, but also the cell walls of the underlying tissues. This makes it likely that after transmission, oil somehow prevents the non-persistent viruses from starting or developing the infection after being introduced at the infectable sites by the vector. The occurrence of PEMV in the saliva may then be the reason why the infection cannot be disturbed by oil at these sites. These sites will probably be situated between the cell walls, since the penetration of the stylets occurs primarily intercellularly in the tissues. Occasionally, the stylets pierce through a cell wall (Lopez-Abella & Bradley, 1969), so that it is also plausible that the sites may be found within the wall.

ZUSAMMENFASSUNG

DIE WIRKUNG VON ÖL AUF DIE ÜBERTRAGUNG DES ERBSENENATIONEN-MOSAIK-VIRUS BEI KURZFRISTIGEN INFEKTIONSSTICHEN

Die Übertragung nicht-persistenter Viren wird durch eine Behandlung der Pflanzen mit Mineralöl eingeschränkt. Auf die Übertragung persistenter Viren haben Öle keinen Einfluß. Dies ist offensichtlich auf einen anderen Übertragungsmechanismus bei diesen Viren zurückzuführen. Erbsenenationen-Virus — ein persistentes Virus — kann aber auch in kurzfristigen Infektionszeiten auf Pflanzen übertragen werden.

In dieser Untersuchung wurde geprüft, ob die Übertragung dieses Virus bei solchen Infektionszeiten von Öl gehemmt werden kann. Es wurde jedoch keine entscheidende Einschränkung der Übertragungsraten gefunden, wenn die Pflanzen mit Öl behandelt worden waren.

Auch das Verhalten der Blattläuse auf diesen Pflanzen wurde durch Anwendung verschiedener Parameter analysiert. Verglichen wurden die Häufigkeit der Probestiche, bevor ein Saugstich gemacht wird, die Dauer der Probestiche und die Summe der Dauer kurzfristiger Infektionszeiten auf Pflanzen, die mit Öl behandelt wurden oder nicht. Die Saugstiche wurden nach einer Dauer von 1 min abgebrochen. Jede Blattlaus machte 4 Stiche an ein und derselben Pflanze.

Die genannten Parameter zeigen, daß im Verhalten der Blattläuse auf mit Öl bedeckten Planzen keine Änderung eintritt.

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