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Transmission of Carrot Mosaic Virus by Aphids

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Abstract. The transmission of the carrot mosaic virus (CMV) by the aphids *Acyrthosiphon pisum* HARRIS, *Cavariela aegopodii* SCOP. and *Myzus persicae* SULZ was proved experimentally. It was observed simultaneously that CMV has a non-persistent character. CMV can be transmitted already 2 min after acquisition feeding by the aphids *Myzus persicae* SULZ and *Cavariella aegopodii* SCOP. When the time of acquisition feeding is prolonged to 4 min, CMV is transmitted also by aphid *Acyrthosiphon pisum* HARRIS. The host range of the investigated virus was also determined and its transmission to 8 plant species, belonging to 4 families, was achieved. On the basis of studies of the vector-virus relationship and of the host range, further proof was given for the different character of the Australian Carrot motley dwarf virus, the *Apium* virus 1 ROLAND and CMV. The experiments showed that preliminary starving of the aphids for 1 h increases their ability to transmit the virus by 3·3%.

In the studies concerning the carrot mosaic virus (CMV) it was necessary to clarify the problem of the virus vector to attempt at differentiating in this way the studied virus from the other viruses which occur in carrots and are by their symptoms close to the virus investigated.

By studies of the symptoms, the properties of the virus, its transfer by the sap to various host plants and by investigating the virus with the electron microscope we have been able in the preceding paper to differentiate this hitherto undescribed virus from the *Apium* virus 1 ROLAND, the *Cucumis* virus 1 DOOLITLE, SMITH, the carrot mosaic virus described in Belgium by ROLAND (1961) and the carrot dwarf disease virus (CONNERS, SAVILE 1947) (CHOD 1964, 1965).

Material and Methods

The CMV transmission experiments were preceded by analysis of the entomofauna in cultures of the seed generation and in the first year of carrot cultivations. Repeated trappings were carried out in the middle of June, at the end of July and in the second half of August in two localities — Litol near Lysá nad Labem and Ruzyně. We attempted to comprise at least 5% of the total area of investigated carrot cultures in the trappings.

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After evaluating the analysis of the entomofauna, the species of aphids which predominated in the carrot cultures were taken into the experiment. The following aphids were employed: *Acyrthosiphon pisum* HARRIS and *Cavariella aegopodii* SCOP. In order to exclude that the Australian carrot dwarf virus is concerned, the aphid *Myzus persicae* SULZ which does not transmit this virus was also included in the virus transmission experiment.

The species of aphids used for CMV transmission were taken from *Leguminosae* pea and spring ketch mized and in order to test the possibility of their natural infection they were allowed to feed on the plants *Vigna sinensis* L. ENDLICHER, which proved suitable as test plants in the virus transmission by the sap. No symptoms of mosaic or spots appeared on these plants after the assumed incubation time (14—18 days), the aphids were, therefore, considered non-infectious and were included in the further tests.

Results

1) Transmission of CMV by aphids

Material and Methods. From the mosaic infected carrot plants of the seed generation the aphids were shaken off onto filter paper and classified into two groups according to the species *Cavariella aegopodii* SCOP. and *Acyrthosiphon pisum* HARRIS. The aphids were then transferred with a hair brush into Petri dishes, 10 aphids into each dish. Then they were transferred to healthy carrot plants 3—5 cm high, cultivated from seeds in the isolator made of silon tissue. On each plant 10 female aphids without wings were placed. The control, so far not tested for their state of health, were aphids from carrot plants of the first year of carrot cultivations which did not have any habitual mosaic virus symptoms. In these informative experiments each species of aphids was transmitted to 20 plants and each experiment was repeated twice. The aphids were allowed to feed on the young carrot plants for 24 hr, then they were killed with phosphothion.

The experiments were carried out in silon tissue isolators. The plants with the aphids were covered with small isolators made of silon tissue, the experimental sites were protected from direct sunlight.

Results. A preliminary experiment showed that the aphids *Cavariella aegopodii* SCOP. and *Acyrthosiphon pisum* HARRIS are able to transmit the CMV. The first species of aphids transmitted the CMV in a higher percentage than the second. In the case of *Cavariella aegopodii* SCOP., positive CMV transmission was achieved with 75% of the plants, in the case of *Acyrthosiphon pisum* HARRIS only with 55% of the plants. The first mosaic symptoms appeared after an incubation time of 8—14 days. The control carrot plants, to which virus infected aphids had been transmitted remained free of symptoms.

2) Classification of CMV according to the vector-virus relationship

Material and Methods. The preliminary results obtained were the basis of detailed studies of the properties of virus, especially of the vector-virus relationship. For this purpose it was necessary to start a culture of aphids free of virus which were grown on plants of the family *Brassica* assumed not to be the host of the investigated virus. In the experiment various acquisition feeding times were tested. For this purpose 3 combinations of acquisition

feeding times were chosen. It was observed that the highest percentage of positive transmissions was attained with short acquisition feeding times. Detailed data is given in Tab. 1.

Table 1

Transmission ability for carrot mosaic virus of aphids (*Cavariella aegopodii* Scop. and *Acyrthosiphon pisum* Harris) after various feeding periods on the infected plant-without previous starving

Species of aphids tested	Time of feeding on the source of infection					
	15 min		30 min		60 min	
Number of plants in the experiment	u * / i	%	u / i	%	u / i	%
Acyrthosiphon pisum	20 / 10	50	20 / 8	40	20 / 5	25
Cavariella aegopodii	20 / 16	80	20 / 5	25	20 / 3	15

*) Note: u — numerator = used
i — denominator = infected

The leaves used as the source of infection were cut from mosaic infected carrot plants shortly before the start of the experiment in order to prevent any essential decrease of the turgor. When the acquisition feeding time was prolonged to 2 hr, only few positive virus transmissions were attained. In order to intensify feeding during virus acquisition by the aphid, a preliminary starvation period was inserted with the aphids tested.

With regard to the intensive movement of aphids in the period of acquisition feeding the insects were transferred to Petri dishes 30 min after the termination of the chosen starvation period, the dishes were covered by silon tissue and placed in the refrigerator at a temperature of about +4° C. The aphids exposed to this temperature were then transferred to the source of infection (CMV infected leaves) and were mostly feeding in one place and did not move.

Since the highest percentage of positive transmissions was attained at short periods of acquisition feeding below 5 min, the effect of the period of acquisition feeding on the transmission ability of the aphids was investigated in detail. The time of acquisition feeding was measured with a stop watch from the moment of the insertion of the aphid stylet into the plant tissue. Feeding of individual aphids was observed. For infectious feeding carrot plants in the stage of the first leaves were used in this experiment; 6 aphids were placed on each plant. In the experiment the time of acquisition feeding was also investigated with the aphid *Myzus persicae* Sulz. The results obtained are given in Tab. 2.

Table 2

Transmission ability for carrot mosaic virus of aphids (*Myzus persicae*, *Acyrthosiphon pisum* and *Cavariella aegopodii*) without previous starvation and after 1 hr previous starvation

Species of aphid	Time of previous starving	Time of feeding on the source of infection							
		1 min		2 min		4 min		5 min	
		Number of plants in the experiment							
		u/i*	%	u/i	%	u/i	%	u/i	%
Myzus persicae	0	10	—	10/4	40	10/6	60	10/6	60
Acyrthosiphon pisum	0	10	—	10	—	10/3	30	10/3	30
Cavariella aegopodii	0	10	—	10/3	30	10/5	50	10/5	50
Myzus persicae	1 hour	10	—	10/6	60	10/8	80	10/8	80
Acyrthosiphon pisum	1 hour	10	—	10	—	10/4	40	10/3	30
Cavariella aegopodii	1 hour	10	—	10/3	30	10/6	60	10/5	50

*) Note: u — numerator = used
i — denominator = infected

It follows from the results that CMV can be classified into the group of non-persistent viruses. The tests for the acquisition feeding times were carried out on 10 plants for each species of aphids.

Results.

a) CMV was found to be transmissible by the aphids *Myzus persicae* SULZ and *Acyrthosiphon pisum* HARRIS and *Cavariella aegopodii* SCOP.

b) The ability for virus transmission by aphids was proved to increase when aphids are used which were allowed to starve previously, and decreases with the increasing feeding period on the source of infection. Previous starvation of the aphids for 1 hr increases their ability to transmit the virus by 3·3%.

c) CMV was found to be transmissible already 2 min after acquisition feeding of the aphids *Myzus persicae* SULZ. and *Cavariella aegopodii* SCOP. When the time of acquisition feeding is prolonged to 4 min, CMV is transmitted also by the aphid *Acythosiphon pisum* HARRIS. When the time of acquisition feeding is prolonged to 15 min, the ability to transmit the virus by aphids increased, at 30 min, however, it shows a decreasing tendency.

d) The incubation time of the appearance of the CMV symptoms varies, the CMV symptoms appeared first after the transfer of virus by the aphid *Myzus persicae* SULZ.

For transmission of CMV to another host 20 plants of identical size were chosen from each tested plant species, the same number of plants was used in the controls. On each plant 6 female aphids without wings and on the control plants the same number of non-infectious aphids was placed. The time of acquisition feeding was 5 min, the time of preliminary starving of the aphids was 1 hr, the time of infectious feeding was 18—24 hr. After the termination of infectious feeding, the aphids on the plants were killed with phosphothion. The plants tested were observed for a period of 3—4 weeks in isolators made of silon tissue. From all plants to which the virus was transmitted successfully, back-transmissions were made to the initial plant of carrots.

3) Host plants for CMV Results. Positive transfer of CMV by all species of aphids tested was achieved to the following plants: *Daucus carota* L., *Petroselinum hortense* HOFFM., *Apium graveolens* L., *Chenopodium quinoa* WILLD, *Chenopodium capitatum* (L.) ASCH., *Chenopodium ambrosioides* L., *Vigna sinensis* L. ENDLICHER, *Medicago sativa* L. and *Datura stramonium* L.

It was not possible to transmit CMV by *aphids* to *Solanum lycopersicum* L., *Chenopodium murale* L., *Amaranthus retroflexus* L., *Cucumis sativus* L., *Anethum graveolens* L., *Nicotiana tabacum* L., *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L.

In the experiments the effect of the number of aphids per plant on the appearance of infection was also investigated. Two, 4, 6 and 10 aphids were placed on one plant. Optimum results of infection were attained with 6 aphids per plant, for infection with the aphid *Myzus persicae* SULZ two aphids were sufficient for one plant. Symptoms on the test plants caused by CMV after transfer by aphids:

Daucus carota L., variety Stupická. Seven to ten days after infection a fine mosaic appears on the central leaves. The external leaves remain without symptoms. Later, the internal leaves begin to distort slightly. At temperatures above 18° C the mosaic is often masked.

Petroselinum hortense HOFFM., variety Hanácká. Fine yellowish spots appear on the leaves after about 10 days, later the spots merge and the margins of the leaves turn yellow, the yellowing passes somewhat into the surrounding tissue. The mosaic infected plants show a growth depression.

Apium graveolens, var. *rapaceum* DC, variety Nerez. Within 10—12 days dispersed small spots appear, which gradually spread over the whole lamina; the spots merge occassionally. The spots have no sharp margin, their diameter at the start is about 1—2 mm. At temperatures above 18° C the symptoms are frequently masked.

Chenopodium quinoa WILLD. After 10—14 days the nervature becomes transparent and gradually turns yellow. The surrounding tissue does not change colour initially, then the yellow spreads systemically over the whole plant, the leaves begin to distort. The infected plants show growth depression (Fig. 1 and 2).

Chenopodium capitatum L. ASCH. Within 14—18 days all the leaves start turning yellow, the lamina becomes transparent, the nerves remain green,

the leaves begin to distort 18—20 days after infection. The leaves fall off too early and the plant dies.

Chenopodium abrosioides L. The leaves begin to turn yellow after 21 days, the colour change proceeds from the base of the lamina, the leaves begin to dry and to fall off. The plants die early due to infection.

Vigna sinensis L. ENDLICHER. Dispersed yellowish spots, 2—3 mm in diameter, without sharp margin appear on the leaves within 12—16 days. Later the spots merge, then the whole lamina turns yellow. The spots appear on all leaves.

Medicago sativa L., variety Kaštická. Fine yellowish spots, 1 mm in diameter, appear on the youngest leaves within 10—14 days. The external leaves are without symptoms. The spots are unequally distributed over the lamina, at higher temperatures the mosaic is often masked.

Datura stramonium L. After 10—12 days the lamina begins to turn yellow, the colour change spreads from the base of the leaf, the nervature remaining green. The leaves become fragile, within about 21 days all the leaves become completely yellow and fall off (Fig. 3).

Results of back-transmission. From all species of test plants to which CMV was transmitted, back transmission of CMV to carrots was carried out successfully. It was difficult to transmit the CMV from *Datura* to carrots (positive back transmission was attained only with 60% of the plants), only a fine mosaic was formed after back-infection, however, the carrot leaves did not distort. The presence of CMV in carrot leaves was, therefore, investigated with the electron microscope. The filamentous CMV particles were found.

Discussion

Transfer of CMV by the aphids *Acyrthosiphon pisum* HARRIS, *Cavariella aegopodii* SCOP. and *Myzus persicae* SULZ was achieved to 9 plant species from 4 families, i.e. *Daucus carota* L., *Petroselinum hortense* HOFFM., *Apium graveolens* L., *Chenopodium quinoa* WILLD., *Chenopodium capitatum* L. ASCH., *Chenopodium ambrosioides* L., *Vigna sinensis* L. ENDLICHER, *Medicago sativa* L. and *Datura stramonium* L.

Studies of the vector-virus relationship indicate that CMV belongs to the group of non-peristent viruses (stylet — borne type), differing thus from the Australian Carrot motley dwarf virus, which has a persistent character and is transmitted by the CMV transferring aphid *Cavariella aegopodii* SCOP. The Australian Carrot motley dwarf virus differs from CMV also by the impossibility of transmitting the former by the aphid *Myzus persicus* SULZ as against CMV.

It can be seen from the investigated plant host range that CMV is not highly specific for the family *Daucaceae* as in the case of *Apium* virus 1, thus differing from this virus.

It is of interest that on *Chenopodium quinoa* WILLD and *Datura stramonium* L. inoculated with infectious sap, lesions appeared, the virus infection localized in these and did not proceed further, whereas a systemic infection became manifest on both plants in the case of transfer by aphids. One possible explanation of this phenomenon is damage only to the parenchymatic cells during inoculation with the sap, the virus spreads only very slowly through

these cells, whereas the virus is introduced into the deeper phloem tissue by feeding the aphid and thus spreads throughout the plant. This explanation is in agreement with the observation of BENNET (1940) who placed the infectious sap of a cucumber mosaic virus strain on sugar beet leaves and observed that local lesions are formed whereas a systemic infection appeared on transfer by aphids.

With mosaic viruses the fact has to be taken into consideration that they can be present in the phloem as well as in the parenchymatic tissue. In the family Chenopodiaceae the temperature and illumination during infection has also a certain influence on the character of the symptoms (HOLLINGS 1956).

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J. CHOD, Ústřední výzkumný ústav rostlinné výroby — Ruzyně: **Přenos viru mozaiky mrkve mšicemi.** — Biol. Plant. **7** : 53—59, 1966.

Experimementálně byl prokázán přenos viru mozaiky mrkve (VMM) mšicemi, a to mšicí: *Acyrthosiphon pisum* HARRÍS, *Cavariella aegopodii* SCOP. a *Myzus persicae* SULZ. Zároveň bylo zjištěno, že VMM má neperzistentní povahu. VMM je přenosný již po 2 min. nabývacího sání mšicemi *Myzus persicae* SULZ a *Cavariella aegopodii* SCOP., při prodloužení doby nabývacího sání na 4 min. je VMM přenášen i mšicí *Acyrthosiphon pisum* HARRIS. U studovaného viru byl dále vyšetřován jeho hostitelský okruh a podařilo se ho přenést na 9 druhů rostlin, pocházejících ze 4 čeledí. Na základě studia vztahu vektor—virus a hostitelského okruhu byl podán další důkaz odlišení VMM od viru australské zakrslosti mrkve (Carrot motley dwarf virus) a od viru mozaiky celeru (*Apium* virus 1 ROLAND). Při pokusech bylo zjištěno, že předběžné hladovění mšic po dobu 1 hod. zvyšuje jejich schopnost přenést virus o 3,3 %.

И. Ход, Центральный научно-исследовательский институт растениеводства, Прага-Рузыне: **Перенос вируса мозаики моркови тлями.** — Biol. Plant. **7** : 53—59, 1966.

Экспериментальным путем доказан перенос вируса мозаики моркови (ВММ) тлями, а именно тлями: *Acyrthosiphon pisum* HARIS, *Cavariella aegopodii* SCOP., *Myzus persicae* Sulz. Установлено также, что ВММ неперсистентной природы. ВММ переносима уже после 2-минутного сосания тлями *Myzus persicae* SULZ и *Cavariella aegopodii* SCOP., в случае удлинения времени сосания на 4 мин. ВММ переносима и тлей *Acyrthosiphon pisum* HARIS. У изучаемого вируса исследован также округ его растений — хозяев и удалось его перенести на 9 растений из 4 семейств. На основании изучения отношений вектор — вирус и округа растений — хозяев принесено дальнейшее доказательство для отличения ВММ от вируса австралийской карликовости моркови (Carrot motley dwarf virus) и от вируса мозаики сельдерея (*Apium* вирус 1 ROLAND). В опытах установлено, что предварительное голодание тлей в течение 1 часа повышает их способность переносить вирус на 3,3%.