

Resistance to Cucumber Mosaic Virus Transmission by Aphids in *Cucumis melo*

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ABSTRACT

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Cucumber mosaic virus (CMV)-resistant *Cucumis melo*, PI 161375 (SC) when infected by CMV strain 14 (CMV-14) is a less efficient source of virus for *Myzus persicae* and *Aphis gossypii* than is the CMV-susceptible cultivar Cantaloup Charentais (CH). SC also is less susceptible than CH to CMV-14 transmission by *M. persicae* and virtually completely resistant

to CMV-14 transmission by *A. gossypii*, although observed rates of transmission to CH by this aphid are very high. This resistance to CMV transmission by the melon aphid is not virus strain-specific and represents an additional form of resistance of SC to CMV.

RESUME

La lignée de melon PI 161375 (SC), résistante au virus de la mosaïque du concombre (CMV) est, lorsqu'elle est infectée par la souche 14 de CMV (CMV-14), une moins bonne source pour l'acquisition de virus par *Myzus persicae* et *Aphis gossypii* que le cultivar sensible Cantaloup Charentais (CH). SC est également moins sensible que CH à la transmission du CMV-14 par *M. persicae*, et pratiquement complètement résistante à la

transmission du CMV-14 par *A. gossypii*, bien que les taux de transmission à CH, observés avec cette espèce, soient très élevés. Cette résistance à la transmission du CMV par le puceron du Melon n'est pas spécifique de la souche virale utilisée et représente pour SC une forme supplémentaire de résistance à ce virus.

Cucumber mosaic is one of the major diseases affecting muskmelon (*Cucumis melo* L.) crops in southeastern France. Every year, nearly 100% of the plants show cucumber mosaic virus (CMV) symptoms by the middle of June and the yields of late crops are drastically reduced (5). Risser et al (6) have started a breeding program to introduce resistance to CMV into the Charentais type muskmelons. Line PI 161375, also called Songwan Charmi (SC), was used as a source of resistance.

A recent survey (3) has shown that 65% of 1,124 CMV isolates collected among naturally infected vegetables and weeds in southeastern France were unable to infect SC systemically; they will be referred to hereafter as CMV "common strains". Those strains that can infect SC tentatively have been grouped in a new "Song" pathotype. The symptoms induced in SC following mechanical inoculation range from light mottle to severe mosaic and stunting, and virus concentration is lower than in the susceptible cultivar Cantaloup Charentais (CH) (H. Lecoq and M. Pitrat, unpublished). The presence of "Song" strains in the major muskmelon production areas prompted the study of the efficiency with which they are acquired and transmitted by the common vectors *Myzus persicae* Sulz. and *Aphis gossypii* Glov. between CH and SC. Leclant and Messiaen (2) observed the green peach aphid to be associated with the primary spread of CMV in muskmelon fields in southeastern France, and the melon aphid to be mainly involved in the secondary development of the disease.

MATERIALS AND METHODS

Plants. Muskmelon plants were sown in flats and transplanted 1 wk later into 10-cm square pots filled with a potting soil. Plants were maintained at temperatures ranging 18–25 C in an insect-protected greenhouse that regularly was sprayed or fumigated with

aphicides. Unless otherwise stated, plants were inoculated 15–20 days after sowing, when the first true leaf had begun to expand.

Virus strain. CMV strain 14 (CMV-14), a "Song" pathotype, was used throughout this study. This strain was isolated from a CMV resistant melon showing mosaic symptoms in the field, and was passed through serial single local lesions from and to *Vigna sinensis* 'Black' and *Chenopodium quinoa*, before being multiplied on CH and kept in tissue dried over CaCl₂. Mechanical inoculations were done by the method currently used in our laboratory (4). Under these conditions, CMV-14 induces severe mosaic symptoms on SC and CH within 5–7 days.

Unless otherwise stated, symptomatic terminal leaves of plants that had been mechanically inoculated 7–9 days prior to the transmission experiments, were used as virus sources for the aphids. Different source plants were used for each experiment. Virus infectivity in source leaves was determined by mechanically inoculating opposite leaves of *V. sinensis* 'Black' with diluted sap and counting the local lesions that formed.

Aphid colonies. Colonies of nonviruliferous *M. persicae* and *A. gossypii*, originated from single aphids, were maintained on pepper cultivar Yolo Wonder and muskmelon cultivar Cantaloup Charentais, respectively. The colonies were kept in different growth chambers with 16 hr of continuous light at 21 C and 8 hr of darkness at 16 C. To get homogeneous aphid populations for the experiments, 10–15 adults were placed on healthy plants and allowed to produce larvae for 2–3 days. The adults were then transferred to new healthy plants and the remaining larvae were used 6–8 days later, when they had reached either the late instar or adult stage.

Transmission technique. Following a 2- to 4-hr starvation period, groups of five to 10 aphids were placed in a small leaf cage which was affixed for 3.5 min to young infected leaves. The leaf cage was removed and aphids found to be in probing position were carefully transferred to healthy plants with a camel hair paint brush. To randomize the experimental conditions the aphids were placed alternatively on the different sources and test plants. Unless

TABLE 1. Efficiency of Cantaloup Charentais (CH) and Songwhan Charmi (SC) as sources of cucumber mosaic virus strain CMV-14 for aphids

Aphid ^a	Source plant	Time after inoculation of source plant (days)	Local lesions per leaf of <i>V. sinensis</i> ^b (no.)	Test plant	Number of experiments	Transmission rate ^c	Probability of equality ^d
<i>Myzus persicae</i>	CH	7	224	CH	10	50/100	$P < 0.005$
	SC	7	30	CH	10	22/100	
	CH	7	224	SC	10	38/100	$P = 0.005$
	SC	7	30	SC	10	14/100	
<i>Aphis gossypii</i>	CH	7-8	108	CH	10	64/100	$P < 0.005$
	SC	7-8	17	CH	10	31/100	
	CH	14	75	CH	8	13/80	$P = 0.025$
	SC	14	4	CH	8	4/80	
	CH	21	38	CH	9	13/90	$P = 0.025$
	SC	21	2	CH	9	5/90	

^aOne viruliferous aphid was deposited on each test plant.

^bMean number of local lesions produced by rubbing diluted (1/1000) sap, from SC or CH source leaves used in transmission experiments, on 10 opposite leaves of *Vigna sinensis* 'Black'.

^cResults are expressed as number of plants infected divided by the number of plants inoculated.

^dResults analyzed by the Wilcoxon test.

TABLE 2. Comparison between the susceptibility of Cantaloup Charentais (CH) and Songwhan Charmi (SC) to transmission of cucumber mosaic virus strain CMV-14 by *Myzus persicae*.

Source plant ^a	Aphids per test plant (no.)	Test plant	Number of experiments	Transmission rate ^b	Probability of equality ^c
CH	1	CH	15	85/150	$P < 0.005$
CH	1	SC	15	59/150	
SC	1	CH	15	29/150	$P < 0.05$
SC	1	SC	15	16/150	
CH	3	CH	15 ^d	50/75	$P < 0.025$
CH	3	SC	15 ^d	34/75	
CH	5	CH ^e	6	47/60	$P = 0.025$
CH	5	SC ^e	6	26/60	

^aSource plants were used 7-8 days after inoculation.

^bResults are expressed as number of plants infected divided by the number of plants inoculated.

^cResults were analyzed by the Wilcoxon test.

^dEach experiment contained five plants of SC and CH.

^eTest plants were at the four- to five-leaf stage.

TABLE 3. Comparison between the susceptibility of Cantaloup Charentais (CH) and Songwhan Charmi (SC) to the transmission of cucumber mosaic virus strain CMV-14 by *Aphis gossypii*

Source plant ^a	Aphids per test plant (no.)	Test plant	Number of experiments	Transmission rate ^b	Probability of equality ^c
CH	1	CH	10	64/100	$P < 0.005$
CH	1	SC	10	0/100	
SC	1	CH	10	31/100	$P < 0.005$
SC	1	SC	10	0/100	
CH	3	CH	10 ^d	48/50	$P < 0.005$
CH	3	SC	10 ^d	0/50	
CH	5	CH ^e	6	56/60	$P = 0.025$
CH	5	SC ^e	6	0/60	

^aSource plants were used 7-8 days after inoculation.

^bResults are expressed as number of plants infected divided by the number of plants inoculated.

^cResults analyzed by the Wilcoxon test.

^dEach experiment contained five plants of SC and CH.

^eTest plants were at the four- to five-leaf stage.

otherwise stated, 10 test plants were used per treatment and each was exposed to one viruliferous aphid. At least 3 hr after exposure to viruliferous aphids, plants were either sprayed or fumigated with an insecticide. Usually the first mosaic symptoms appeared 5 days after inoculations. However, the plants were kept an additional 3 wk for further observation before being discarded. During the incubation period plants were regularly sprayed or fumigated with aphicides. The results were analyzed by the Wilcoxon test (7).

RESULTS

Efficiency of Cantaloup Charentais and Songwhan Charmi as CMV source for aphids. As previously mentioned, the concentration of CMV strains belonging to the "Song" pathotype is higher in CH than in SC. It was necessary to learn whether these cultivars differed when used as virus sources for aphids. The results summarized in Table 1 show that significantly fewer aphids (*M. persicae* and *A. gossypii*) transmitted CMV-14 from SC than from CH. SC also was a less efficient source of virus than CH for the melon aphid, when plants inoculated 14 and 21 days prior to the experiments were used as virus sources.

When virus infectivity in the source leaves was estimated by inoculating diluted sap from these leaves on *V. sinensis*, larger numbers of local lesions were produced by CH sap than by SC sap, confirming a lower concentration of virus in SC than in CH (Table 1).

Relative susceptibility of Cantaloup Charentais and Songwhan Charmi to CMV transmission by aphids, *Myzus persicae*. Results of the transmission efficiency experiments proved that CH was a better source of CMV-14 for *M. persicae* than was SC. In the following experiment, the susceptibility of both cultivars to the transmission of CMV-14 by this vector was examined. In all the cases tested (Table 2) significantly more CH than SC plants became infected indicating a lower susceptibility of SC to CMV-14 transmission by the green peach aphid.

***Aphis gossypii*.** Differences in susceptibility to virus transmission were much greater between SC and CH when the melon aphid was used as vector. In conditions under which almost all the CH plants developed mosaic symptoms none of the SC became infected (Table 3). However, in an experiment intended to check whether a mass transfer of viruliferous aphids (10 per plant) to young SC, would achieve transmission, three plants of 30 became infected.

Trials were done with two other CMV isolates (isolates TEZ and MG-18 obtained from field samples in 1978) which induce severe mosaic symptoms and stunting when mechanically inoculated to

CH and SC. Infected CH plants (7 days after inoculation) served as virus sources and three *A. gossypii* were used per test plant. Fifteen of 15 CH developed mosaic symptoms and none of 15 SC were infected by each of these isolates.

To determine whether the resistance of SC to virus transmission by *A. gossypii* is an aspect of the resistance of this line to CMV "common strains," experiments were carried out comparing SC, CH, and *C. melo* line La Jolla 90436 (LJ). The latter, which was supplied by the Plant Science Research Division, USDA, La Jolla, California, was observed (6) to have the same oligogenic recessive resistance to CMV "common strains" as SC. CMV-14-infected CH, 7 days after inoculation, were used as virus sources and three aphids deposited per test plant. Of 20 test plants of each line, 20 CH, 18 LJ, and no SC plants became infected in these experiments.

Finally, in order to check that the lower susceptibility of SC to virus transmission by *A. gossypii* was not due to an atypical behavior of that aphid on SC, single aphids were placed on SC and CH after a 3-hr starvation period. Probing usually started quickly on both SC and CH. Four hours later the percentage of the remaining aphids was 100% (140 of 140) on CH and 99% (139 of 140) on SC. In another experiment, however, aphid counts after 16 hr of feeding were 99% (99 of 100) on CH and only 59% (59 of 100) on SC.

DISCUSSION

Differences in rates of virus transmission by aphids according to the virus source plant and the test host used have been reported previously (8,9,12). Availability of virus to aphid vectors (13) and susceptibility of plants to infection (10,11) also have been implicated as important resistance factors which limit the amount of virus spread in the field. In the present study, plants of two melon cultivars differed in virus source efficiency and in relative susceptibility to virus infection.

The CMV-susceptible muskmelon cultivar CH is a better source for CMV-14 than SC when *M. persicae* or *A. gossypii* are used as vectors (Table 1). This difference may be related to the lower virus concentration in SC than in CH (Lecoq and Pitrat, unpublished). However, differences in leaf structure or in virus location in cells, as well as different probing behavior on SC and CH also may modify the ability of these aphids to acquire CMV-14 from CH and SC.

SC resistance to CMV-14 transmission by *A. gossypii* apparently cannot be accounted for by a wandering behavior of the aphids on this cultivar; they begin probing quickly after being deposited on SC and 4 hr later as many remain on SC as on CH. Neither can the lack of transmission be attributed to the possibility that *A. gossypii* does not inject sufficient inoculum to infect SC. Indeed, although no simultaneous comparisons were made between *A. gossypii* and *M. persicae* as CMV-14 vectors, data in Tables 2 and 3 suggest that *A. gossypii* is a more efficient vector when CH is used as a test plant indicating a better uptake and/or transmission of virus for *A. gossypii* than for *M. persicae*. Theoretically, when SC is used as a test plant, *A. gossypii* should be at least so efficient as *M. persicae*. Also, when infected SC plants are used as a virus source and CH as a test plant, *A. gossypii* acquired CMV-14 so efficiently as *M. persicae* (Table 1), which shows that this aphid is able to probe on SC.

SC resistance to CMV transmission by *A. gossypii* apparently is not strain specific, because two other CMV isolates of the "Song" pathotype also were not transmitted to SC by this aphid. The resistance observed in young plantlets at the first-leaf stage, also

was expressed in test plants at the fourth- to fifth-leaf stage. However, mass transfer of viruliferous aphids to young plantlets may break the resistance partially.

Resistance to CMV transmission by *A. gossypii* was not observed in LJ, a line which shares with SC the same oligogenic resistance to CMV "common strains" (6). Therefore, resistance to CMV transmission involves another mechanism. However it is not yet known whether this mechanism may be efficient in the absence of resistance to CMV "common strains" and whether this mechanism also includes the partial resistance observed for CMV-14 transmission by *M. persicae*. SC has been observed to be resistant to the curling caused by the Western biotype of *A. gossypii* and also to express some antibiosis against that aphid (1). Whether these properties are related to the resistance to CMV transmission is not known, but they may be responsible for our repeated failure to establish *A. gossypii* cultures on SC, and for the aphid's tendency to leave SC plants after 16 hr.

The resistance of SC to "common strains" of CMV and its resistance to transmission of "Song" strains by *A. gossypii* makes this line important material for breeding purposes. The association of these two mechanisms is likely responsible for the satisfactory behavior of SC in our field conditions, a behavior better than that of lines such as LJ which are resistant only to "common strains" of CMV (6).

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