Effects of Strain, Source Plant, and Temperature on the Transmissibility of Citrus Tristeza Virus by the Melon Aphid

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ABSTRACT

Three citrus tristeza virus isolates, which were considered to be related strains in cross protection tests, differed markedly in their transmissibility by *Aphis gossypii*. High transmission rates, averaging 40%, were obtained with the VT isolate, compared with less than 5% with the CT and ST isolates; all three were acquired from 'M^{me} Vinous' sweet orange. The source of the aphid population had no significant effect on transmission rates. M^{me} Vinous was a much better host for virus acquisition than 'Palestinian' sweet lime, although no differences in the concentration of threadlike particles (TLP) were observed. The differences in transmissibility of the tristeza isolates were not correlated with TLP content. Significantly higher transmission rates were obtained when source plants were kept at 22 C, compared with those from plants held at 31 C. This was correlated with a decrease in TLP content.

The high transmissibility of the VT strain by *A. gossypii*, compared with the low rates of the two other strains, may explain the natural spread observed recently in Israel, as well as the lack of spread from previously located tristeza sources.

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Additional key words: Aphis gossypii, threadlike particles.

Transmission of citrus tristeza virus (CTV) by the melon aphid *Aphis gossypii* Glov. has been demonstrated in California (6), Florida (12), India (18) and the Philippines (11). Although the melon aphid is prevalent in Israel, there had been no indication of natural spread from tristeza-infected trees located mainly in introduction plots during 1956-1960 (15). Furthermore, attempts to transmit from these sources by *A. gossypii, Toxoptera aurantii* (B.de F.), *Myzus persicae* (Sulz.), and *A. pomi* de Geer either were not successful (9, 16), or occurred at a very low rate (2).

Recently, more than 230 tristeza-infected trees were found in one area in the Sharon plain. The infected trees were found in plots differing in age, source of budwood and stock-scion combination. This, as well as their location according to a dispersal gradient (5), indicated natural spread of the disease in this area. As *Toxoptera citricidus* Kirk. has not yet been found in Israel (17), a study of the vector capability of *A. gossypii* in Israel, as well as the factors affecting tristeza transmission, seemed valid. Factors affecting transmission of tristeza by melon aphids have also been studied by Norman and co-workers (13, 14).

MATERIALS AND METHODS.—The isolates of tristeza originated from a graft-infected *Citrus sinensis* (L.) Osbeck var. 'Shamouti' (ST), a naturally infected 'Valencia' (VT), and a graft-infected *C. mitis* Blanco var. 'Calamondin' (CT). All three induce vein-clearing, yellowing, cupping, and stunting on 'Egyptian' sour lime [*C. aurantifolia* (Christm.) Swing.]. However, ST induces no symptoms on sour orange (*C. aurantium* L.) or 'Eureka' lemon [*C. limon* (L.) Burm.], whereas VT causes partial stunting and somewhat smaller leaves on sour orange, and stunting and small leaves on Eureka lemon. CT causes partial

Fig. 1. Plastic cage used for testing aphid transmission of citrus tristeza virus.

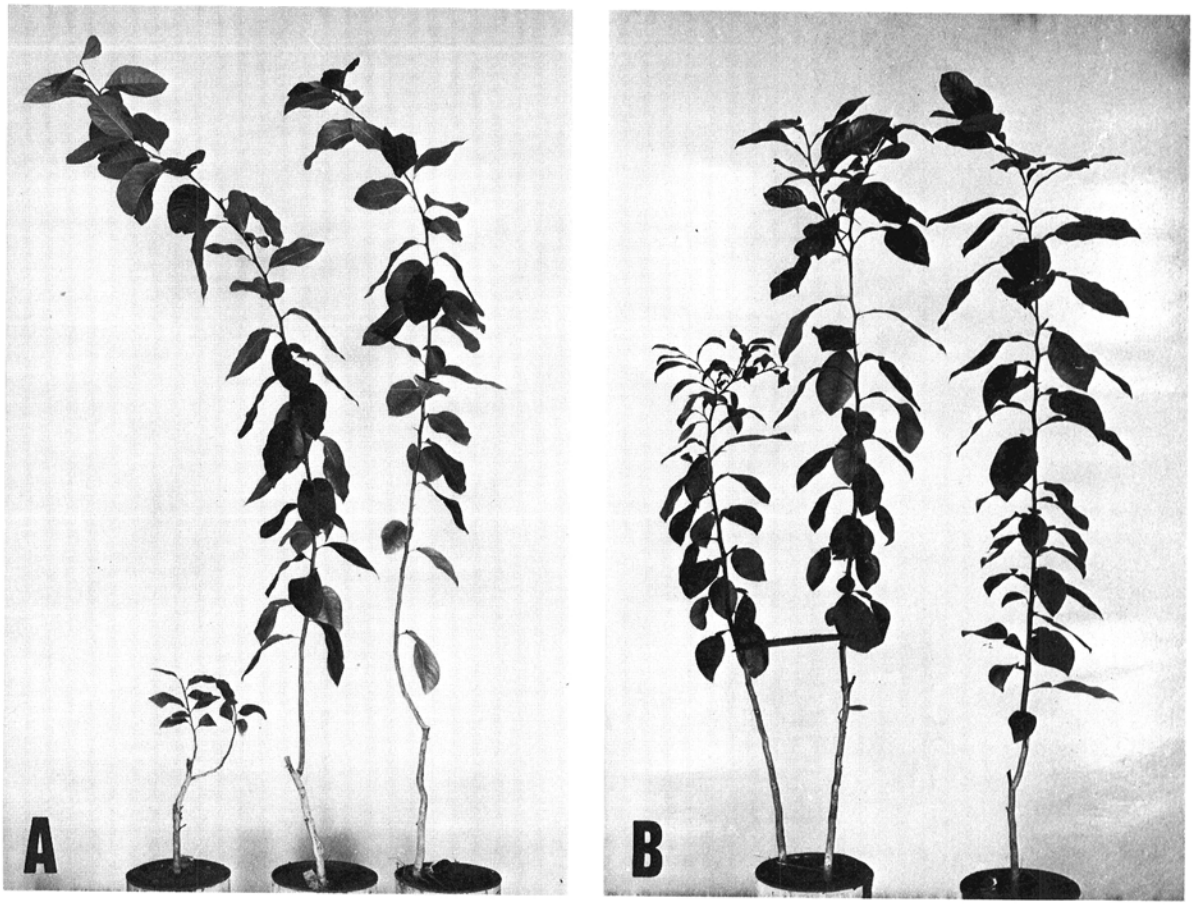

Fig. 2. (A-B). Cross protection. **A)** 'Eureka' lemon seedlings: left – graft inoculated with VT isolate; middle – challenged with VT after ST inoculation; and right – inoculated with ST. **B)** Sour orange seedlings: left – graft inoculated with CT isolate; middle – challenged with CT after ST inoculation; and right – inoculated with ST.

vein-clearing, small leaves and stunting on sour orange and some stunting on Eureka lemon. The CT and VT isolates apparently contain a seedling yellows component (11).

Seedlings of 'M^{me} Vinous' sweet orange and 'Palestinian' sweet lime [*C. limettioides* Tanaka) were grown for 14-18 months in 5-kg cans, containing soil, sand, and peat (2:1:1). The plants were then inoculated by chip grafting and used for aphid (acquisition) feeding after 2.5 to 5 months.

Infectivity was tested on Egyptian sour lime seedlings, grown in groups of three to five plants, in a 5-kg can. When the plants were 4-5 months old and 15-25 cm high, they were used for aphid inoculations.

Melon aphids were collected either from citrus at Ramat Gan, Ra'anana and Caesarea, 20 or 40 km apart, or reared on *Cucumis sativus* L. var. 'Bet Alpha' cucumber plants, in cages within a glasshouse.

Transmission tests were done in an air-conditioned plastic chamber, at 25 ± 2 C. Cucumber or citrus leaves, carrying about 100 apterous aphids, were transferred into a plastic cage (Fig. 1). The cage was then placed on the tristeza-infected source, so that 5-10 cm of the distal branch was included in the cage. After 24 hr the cage, together with the aphid-infested tip of the source plant, was transferred to the upper part of the test plant. The aphids were allowed access to the test plant for 24 hr. The cages were subsequently removed and the plants transferred to a greenhouse, after careful spraying with an aphicide mixture. Screening for symptoms of infection started after 20 days and continued for a period of 5 months.

The concentration of threadlike particles (TLP) was estimated after partial purification (4), using different concentrations of tobacco mosaic virus as reference (3).

RESULTS.–*Cross protection.*–The ST source protected sour orange and Eureka lemon seedlings against both the CT and VT isolates. Sour orange and Eureka lemon seedlings were grafted with healthy or ST-infected Shamouti budwood. After two weeks the plants were topped above the grafts, and one branch was allowed to develop. Four months later the plants were challenge-inoculated on the new shoot with CT or VT isolates. The plants were topped after three

TABLE 1. Transmission rates of tristeza isolates from 'M^me Vinous' sweet orange seedlings by *Aphis gossypii* from different sources

Tristeza isolate	Aphid source	Transmission[a]	% Transmission
VT	Ramat Gan	23/53	43.4
VT	Ra'anana	22/50	44.0
VT	Caesarea	36/104	34.6
VT	Cucumber colony	38/88	43.2
ST	Ra'anana	1/60	1.7
ST	Caesarea	1/21	4.8
ST	Cucumber colony	1/25	4.0
CT	Ra'anana	1/22	4.5
CT	Caesarea	2/57	3.5
CT	Cucumber colony	1/24	4.2

[a]Numerator = number of plants infected; denominator = number of plants inoculated.

weeks and kept for one year under observation in the greenhouse. Four healthy sour orange seedlings grafted with CT and four healthy Eureka lemon seedlings grafted with VT, developed typical symptoms within 3-4 months (Fig. 2). However, no symptoms developed on any of six sour orange or Eureka lemon seedlings, previously protected with ST, when inoculated with CT or VT, respectively. These isolates may therefore be considered as strains.

Aphid transmission of tristeza isolates.—The VT isolate was transmitted by four populations of *A. gossypii* at significantly higher rates than the ST or CT isolates (Table 1). Transmission rates of the VT strain averaged 40%, whereas with the other two isolates, transmission rates of less than 5% were obtained. The source of the aphid population had no significant effect on transmission rates.

Attempts to recover VT by aphids from VT-grafted M^me Vinous seedlings, protected by the ST strain, proved negative. Also, no increase in the transmission rate of ST was obtained from these plants.

Effect of source plant and temperatures on transmission rates.—Transmission rates of both the highly transmissible isolate (VT) and the weakly transmissible one (ST) were affected by the source plant serving for acquisition feeding. With VT acquired from M^me Vinous sweet orange seedlings, transmissions reached 45.4% (24/53); when acquired from Palestinian sweet lime only 9% transmission (3/33) was obtained. With ST, 3.4% transmission (2/59) was obtained when acquired from M^me Vinous and none (0/60) from Palestinian sweet lime.

Keeping VT-infected source plants at 31 C before acquisition feeding significantly reduced transmission rates, compared with those obtained from plants kept at 22 C (Table 2).

When M^me Vinous seedlings which had been infected for 80 days with VT and kept at 22 C were transferred to 31 C, transmission rates were not affected for the first 12 days (Fig. 3). However, after 20 days or more at 31 C, transmission rates were low, comparable to those obtained from plants kept constantly at 31 C. Transferring VT-infected M^me Vinous seedlings from 31 to 22 C, increased their suitability as source plants within six days. Nevertheless, even after 65 days, transmission rates from these plants were lower than those obtained from plants kept constantly at 22 C.

Concentration of threadlike particles (TLP) in source plants.—No significant differences in TLP content were observed when M^me Vinous seedlings

TABLE 2. Transmission rates of VT isolate by *Aphis gossypii* from source plants kept at two temperatures

	Temperature			
	22 C		31 C	
Source plant	Transmission[a]	% Transmission	Transmission[a]	% Transmission
Palestinian sweet lime	13/63	20.6	4/64	6.3
M^me Vinous sweet orange	31/51	60.8	6/49	12.2

[a]Numerator = number of plants infected; denominator = number of plants inoculated.

were infected with the ST, CT, or VT isolates; the content ranged between 1 and 5 μg/25 g of leaves. Furthermore, TLP content in Mme Vinous leaves was similar to that in Palestinian sweet lime. Both were infected with the VT isolate. TLP content averaged 10 μg/25 g leaves in both varieties, grown for three months at 22 C compared with 0.2-1.0 μg/25 g leaves in plants kept at 31 C.

DISCUSSION.—Marked differences in the transmissibility of CTV isolates by *A. gossypii* were observed; based on cross protection tests they may be regarded as related strains. High transmission rates, averaging 40%, were obtained from Mme Vinous with the VT isolate, compared with less than 5% with two other isolates. The source of the aphid population had no significant effect on transmission rates. Significantly more transmissions were obtained from Mme Vinous than from Palestinian sweet lime, although no differences in TLP content were observed. Mme Vinous is probably a better host for virus acquisition, or, alternately, differences in vector preference for the two source plants could account for the observed results. The differences in transmissibility of tristeza isolates were not correlated with TLP content, even though these are considered to represent the causal agent of tristeza (3, 10). The only effect correlated with TLP content was that of temperature.

A decrease in transmissibility during summer, compared to that in winter, was observed by Norman and co-workers (14). It has also been observed that some citrus varieties are better sources for acquisition than others (12). However, compared with data from California (7) and Florida (14), transmission rates with the VT isolate were significantly higher.

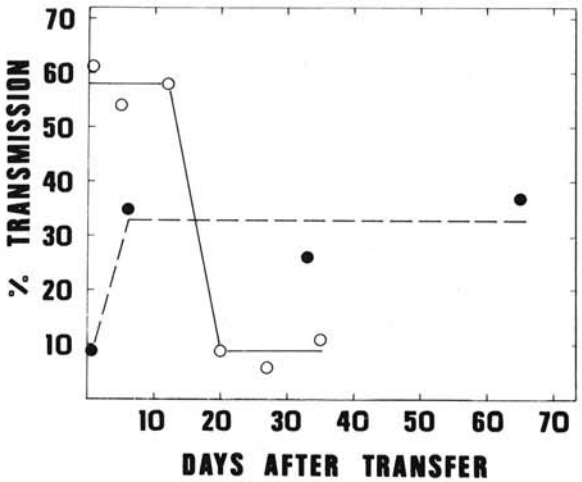

Fig. 3. Effect of temperature change on the suitability of infected Mme Vinous plants as a source for VT acquisition.
o——————o plants transferred from 22 to 31 C.
•- - - - - - - -• plants transferred from 31 to 22 C.

The high transmissibility of the VT isolate seems to be associated with an intrinsic property of this strain. Variations in transmission rates between strains of other stylet-borne viruses have been reported (1, 8, 19).

The high experimental transmission rates of the VT strain by *A. gossypii*, compared with the very low rates of the two other strains, may explain the natural spread observed recently in one area in Israel (5), as well as the lack of spread from previously identified tristeza sources (16). It might be assumed that strains which are experimentally transmitted from Mme Vinous at less than 5% have little epidemiological potential, whereas those transmissible by more than 20% are of major importance. However, these suggestions require confirmation from other areas where tristeza is being spread by the melon aphid.

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