

Aphid-Transmissibility Variants of Citrus Tristeza Virus in Infected Citrus Trees

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ABSTRACT

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Each of four orange trees infected with citrus tristeza virus (CTV), contained several variants of the virus that differed in aphid transmissibility. In order to ascertain the presence of these variants, small pieces of budwood of two trees, from which an overall low rate of transmission (7.5%) had been obtained, were grafted on 2-yr-old orange plants. When those plants were used in aphid-transmission experiments, highly (above 30%), intermediate (5-20%), and poorly (less than 5%) aphid-transmissibility variants were obtained. One of these trees came from an orchard in which no natural spread had been observed for two decades, although recently natural spreading had become apparent, and the second tree

was grafted with budwood originating from this orchard. Using the same procedure with two trees from a region where natural transmission was evident during the last decade and overall transmission rates reached 25%, poorly transmissible variants also were obtained. Aphids apparently transmit simultaneously more than one isolate, as a spectrum of variants was observed from aphid-inoculated seedlings. It is suggested that tristeza-infected trees may harbor more than one variant; and that trees from a location where only limited natural spread is observed could contain CTV variants that are highly transmissible, but which are quantitatively suppressed by a dominating poorly transmissible variant.

Citrus tristeza disease was found in Israel as early as 1956, mainly in introduction plots, but with no indication of natural spread (10). Natural spread in Israel was first observed in 1970 in one location in the Sharon plain (3). Subsequently it was shown that citrus tristeza virus (CTV) from this area is efficiently transmitted by the melon aphid *Aphis gossypii* Glov. (2). CTV transmission rates with 10 aphids per test plant reached 36%, compared with less than 5%

transmission from plants infected with virus from one of the introduction plots where no natural spread had been observed (2,9). The sudden appearance of this highly transmissible CTV strain raised the possibility that the host plant might harbor more than one strain of the virus. Thus, trees from the location where no natural transmission was observed also could contain a strain(s) that is highly transmissible by aphids, but which is, however, quantitatively suppressed by a dominating nontransmissible strain.

It is well known that often more than one strain of virus systemically infects the same host plant and that one generally predominates. One example is the occurrence of yellow strains of

tobacco mosaic virus (TMV) in tobacco plants systemically infected with an ordinary strain; another is the presence of a TMV substrain virulent to resistant tomatoes within the original parent nonvirulent inoculum (12). Furthermore, even a single cell can be simultaneously infected by two virus strains, as noted in a review by Loebenstein (6); and recently Otsuki and Takebe (8) reported that parallel replication of two TMV strains occurs within one tobacco mesophyll protoplast.

The citrus tristeza disease may be considered to be a complex of strains and components, as discussed by McClean (7), who distinguished between stem-pitting, seedling yellows, and tristeza (decline) reactions. A tree may harbor more than one component, as in seedling yellows and stem-pitting; the two may be related—stem-pitting strains protect against reinfection with seedling yellows (11). Aphid transmissions from cross-protected donors have been attempted (1).

Therefore, we were interested in determining whether tristeza-infected citrus trees harbor more than one isolate which differ in aphid transmissibility and, if so, whether they can be separated by graft transmission from the original plant.

MATERIALS AND METHODS

A clone of melon aphids, *Aphis gossypii* Glov., was reared on *Cucumis sativus* L. "Elem" cucumber plants in growth chambers at 25 ± 1 C, under continuous illumination.

For virus source plants 2-yr-old seedlings of orange cultivar Mme. Vinous and 2-yr-old plants of Shamouti orange (grafted on sour orange rootstock) were grafted with budwood from four trees infected with CTV (designated A-donors in Fig. 1). Two of the infected source trees were from the Hibbat Ziyon area, where natural spread of the disease has been known since 1970 (3). A third (~35 yr old) tree was grafted in 1972 with budwood that derived

originally from Miqwe Yisra'el (ST), where for two decades no natural spread had been found (2). The fourth tree was taken in 1977 from Miqwe Yisra'el, when natural spread had become apparent (4). In 1978, a marked increase in natural spreading was observed in Miqwe Yisra'el. CTV from Hibbat Ziyon (VT) was highly transmissible by melon aphids in laboratory tests (transmission rates averaging 40%), whereas that from Miqwe Yisra'el had a low transmission rate, averaging 5% (2,9). Virus source plants were cut back 1–2 mo after graft inoculation. When new growth appeared, plants were transferred to 20–22 C for 2 wk, before being used as donors. Aphid transmission tests from these donors were done over a period of 1 mo following the flush of new growth (Fig. 1).

Infectivity was tested on 3- to 5-wk-old Egyptian sour lime (*Citrus aurantifolia* (Christm.) Swing) seedlings. For transmission tests, batches of 60 1-wk-old aphids were caged 24 hr on the young growth of a single virus source plant. Subsequently, leaves with about 10 adults and their larvae were caged on one Egyptian sour lime seedling for an inoculation period of 24 hr. Indicator plants were kept for 3 mo of observation at 25 C, although in most cases symptoms appeared within 10–28 days after inoculation. The transmissibility of CTV variants obtained by aphid transmission was studied by grafting several orange plants with small pieces of budwood from indicator seedlings that reacted positively to CTV. The B-donors were designated b-1, b-2..., or d-1, d-2... depending upon the indicator from which they were taken. Transmission rates from B-donors were studied similarly to compare them with those from A-donors. An outline of the scheme is presented in Fig. 1.

Transmission rates from donors deriving from the same infected tree were statistically analyzed by analysis of variance and least-significant differences. Most experiments were carried out within one or two flushes of growth. No significant variation was observed within each of the donors, during tests carried out on the same flush

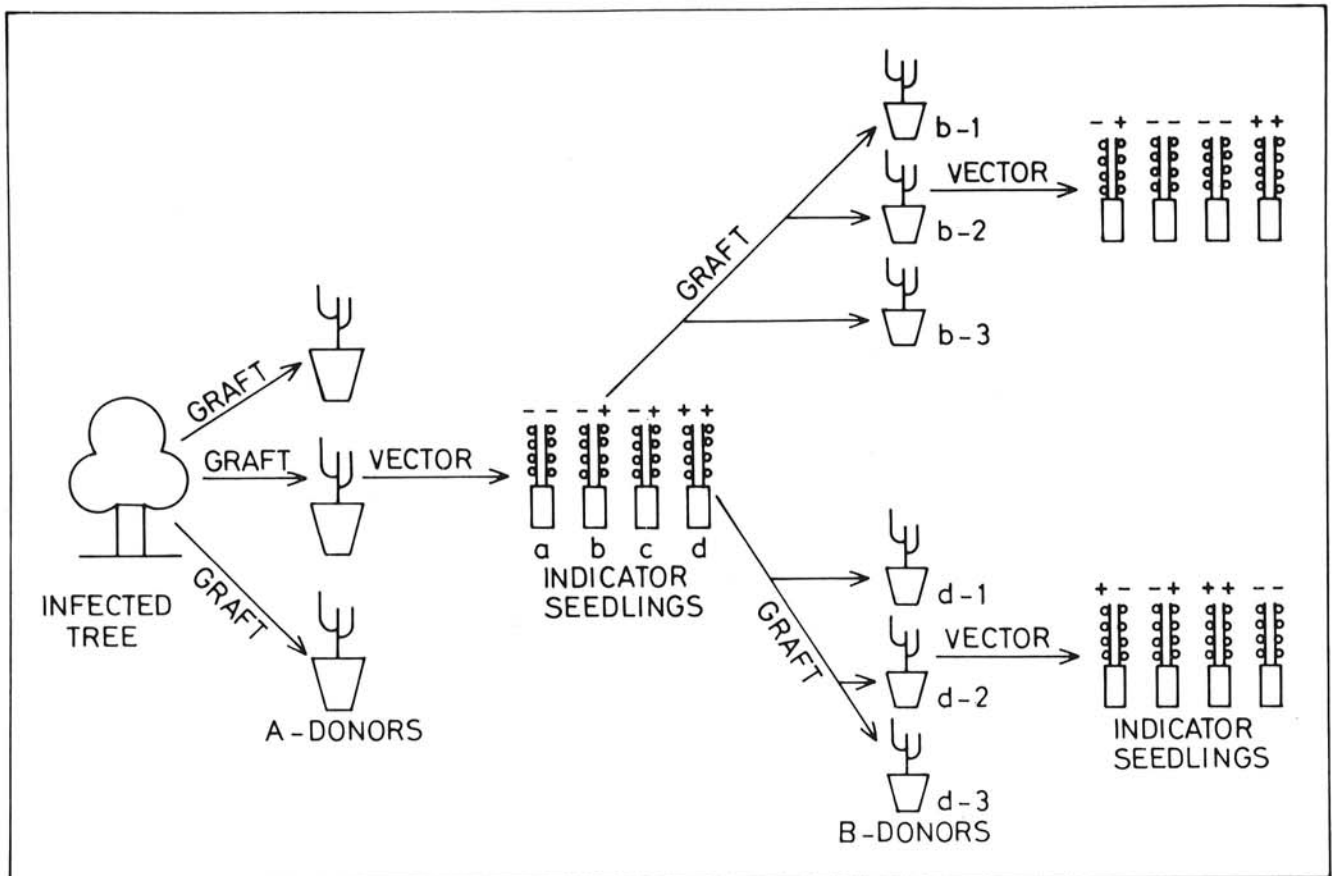


Fig. 1. Scheme for separation of citrus tristeza virus variants by graft inoculation and subsequent testing for aphid transmissibility. Donors (A and B) were 2-yr-old Mme. Vinous sweet orange seedlings or 2-yr-old plants of Shamouti sweet orange grafted on sour orange. Indicator plants were Egyptian sour lime seedlings, inoculated by *Aphis gossypii*. + indicates positive reaction and - indicates negative reaction.

of growth. Differences found between flushes of growth were not significant as summarized in Table 1.

RESULTS

The overall transmission rate from virus source plants grafted with budwood from the Hibbat Ziyon area (natural transmission) was 317/1,269 (25%). The overall transmission rate from donors grafted with budwood from Miqwe Yisra'el (limited natural spread) was 146/1,934 (7.5%).

Aphid transmission rates from each individual donor plant grafted with budwood from the two infected trees from Hibbat Ziyon are summarized in Fig. 2. From four donors grafted with budwood from tree H-A, low transmission rates (less than 5%) were obtained, from four other donors intermediate rates (5–20%), and from six donors high rates (above 30%). Similar results were observed with donors grafted with budwood from tree H-B. Intermediate transmission rates were obtained from the donors, while there was a high rate of aphid transmission from two donors.

Aphid transmission rates from each donor grafted with budwood from two trees in Miqwe Yisra'el (limited natural transmission) are summarized in Fig. 3. Among donors grafted with budwood from tree M-A, three had low transmission rates (<5%), four others had intermediate rates (5–20%), and one had a high transmission rate (38%). Among those grafted with budwood from tree M-B, two donors with low and three with intermediate rates of aphid transmission were obtained.

To exclude the possibility that the differences in transmission rates from the various donors originating from one tree were due to seasonal events or to incomplete invasion of the plant by the virus, aphid-transmission rates from several donors were retested after 2–3 mo. As summarized in Table 1, transmission rates from most donors—both from those grafted with budwood from Hibbat Ziyon or from Miqwe Yisra'el—were similar at the two times of assay; i.e., donors that gave low, intermediate, or high transmission rates, respectively, at the first date of assay, gave similar readings at the second assay. With some donors, such as H-A-8, H-A-11, M-B-3, and M-B-4, although transmission rates varied somewhat between the two assays, differences were not statistically significant.

In order to determine whether the CTV transmitted by aphids from the donors (A-donors) to indicator seedlings is homogenous or still contains several variants, budwood from each of four positive-reacting indicators (Fig. 1) was grafted to three or four new sweet orange plants, which then served as donors (B-donors) for determining aphid transmission rates. All A-donors originated from tree M-A from Miqwe Yisra'el. From one A-donor (M-A-1) low transmission rates (1/54, 1.8%) were obtained, whereas the other two (M-A-7 and M-A-9) gave intermediate (5/27, 18.5%) and high (10/26, 38.5%) rates, respectively. From four indicator

TABLE 1. Aphid transmission rates from several citrus tristeza virus-infected donors determined from two subsequent flushes of growth at a 2- to 3-mo interval

Donor ^a	Date	Transmission		Date	Transmission		Statistical significance ^c
		Rate ^b	%		Rate	%	
H-A-1	11/77	1/49	2.0	3/78	0/11	0	n.s.
H-A-2	11/77	1/40	2.5	2/78	1/14	7.1	n.s.
H-A-8	11/77	4/33	12.1	5/78	4/22	18.2	n.s.
H-A-11	9/77	15/40	37.5	11/77	6/25	24.0	n.s.
H-B-3	4/78	12/52	23.1	7/78	5/32	15.6	n.s.
M-A-1	3/77	1/54	1.9	5/77	0/32	0	n.s.
M-A-2	3/77	1/47	2.1	5/77	0/48	0	n.s.
M-B-2	2/78	1/52	1.9	5/78	2/36	5.6	n.s.
M-B-3	3/78	1/16	6.3	6/78	5/36	13.9	n.s.
M-B-4	3/78	4/28	14.3	5/78	6/20	30.0	n.s.
M-B-5	2/78	10/49	20.4	5/78	7/40	17.5	n.s.

^aDonors were the same as those in Fig. 2 and 3. H-donors were grafted with budwood from Hibbat-Ziyon and M-donors with budwood from Miqwe Yisra'el, both in Israel. A and B designate two different budwood source trees from each area.

^bDenominator, number of plants used; numerator, number of plants infected with batches of 10 1-wk-old aphids. Acquisition access period = 24 hr, inoculation access period = 24 hr.

^cTransmission rates between the two dates were statistically analyzed by Student's *t*-test; ($P < 0.05$). n.s. = not significant.

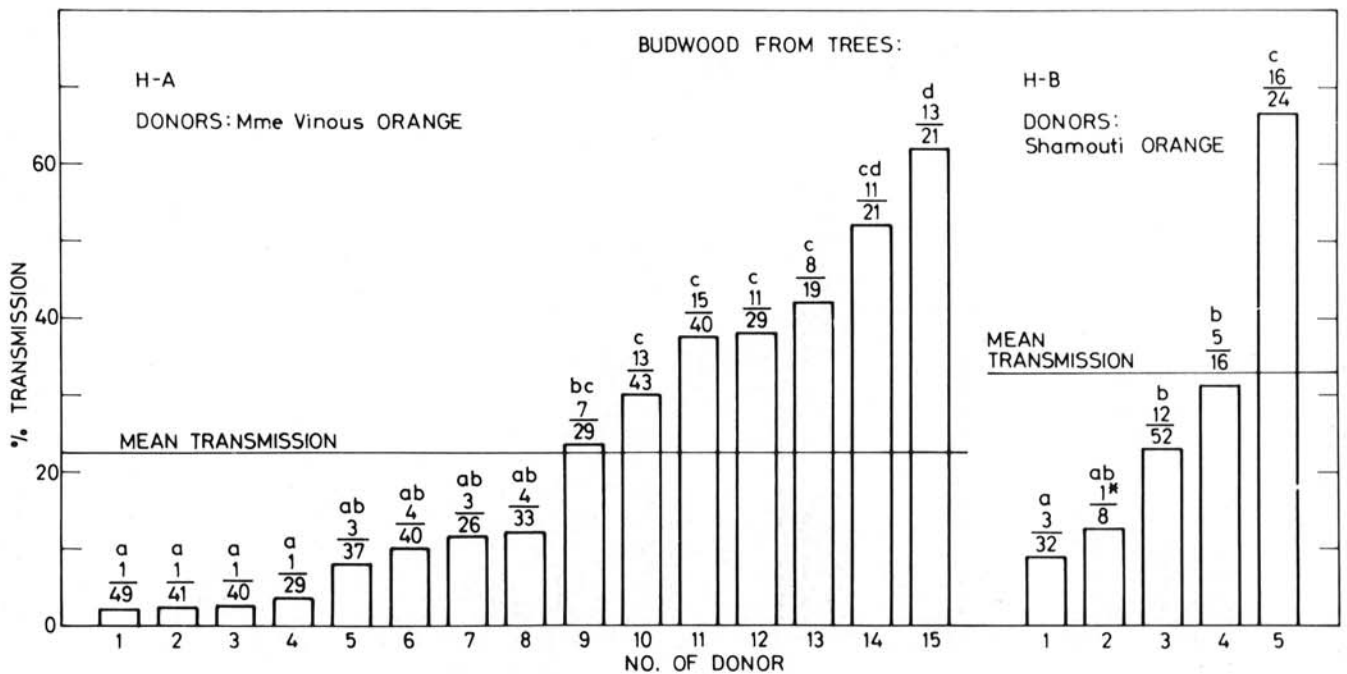


Fig. 2. Distribution of aphid transmission rates from donors (A-donors) grafted with budwood from two trees from Hibbat Ziyon, Israel. Budwood from tree H-A was grafted on 2-yr-old Mme. Vinous sweet orange seedlings, and that from tree H-B on 2-yr-old plants of Shamouti sweet orange grafted on sour orange. Ratios above bar: denominator, number of plants used; numerator, number of plants infected, with batches of 10 1-wk-old aphids. Acquisition access period = 24 hr, inoculation access period = 24 hr. Ratios below the same letter are not significantly different (analysis of variance, mean LSD; $P < 0.05$). *Donor died during the experiment.

seedlings grafted to B-donors, two gave high and intermediate rates of transmission, one gave low and intermediate rates, while from the fourth no significant differences between the B-donors was observed, all were in the intermediate range (Fig. 4). This segregation was independent of the transmission rate obtained from the original A-donor. Thus, while the transmission rate from the A-donor (M-A-1) was only 1.8% (1/54), the resulting B-donors gave both high (25.8 and 31.6%) and intermediate (7.7 and 9.1%) rates. Vice versa, from the A-donor M-A-7 (which gave intermediate rates of transmission [5/27, 18.5%]), the resulting B-donors gave both low (2.4%) and intermediate (6.5 and 18.2%) rates. Apparently, aphid-inoculated indicator seedlings contained more than one tristeza variant.

DISCUSSION

Each of the four infected trees harbored several variants of CTV which differed in transmissibility by aphids. Thus, a tree from Miqwe Yisra'el, where the overall transmission rate was low, contained intermediate and highly transmissible variants. The average transmission rate from these trees was low; it is suggested that a poorly transmitted variant(s) of CTV dominates—and is present at a comparatively high titer, thereby suppressing quantitatively (but not excluding) other intermediate or highly transmissible variants. Conversely, in trees from Hibbat Ziyon where a marked natural spread was evident and overall transmission rates were high, poorly transmitted variants also

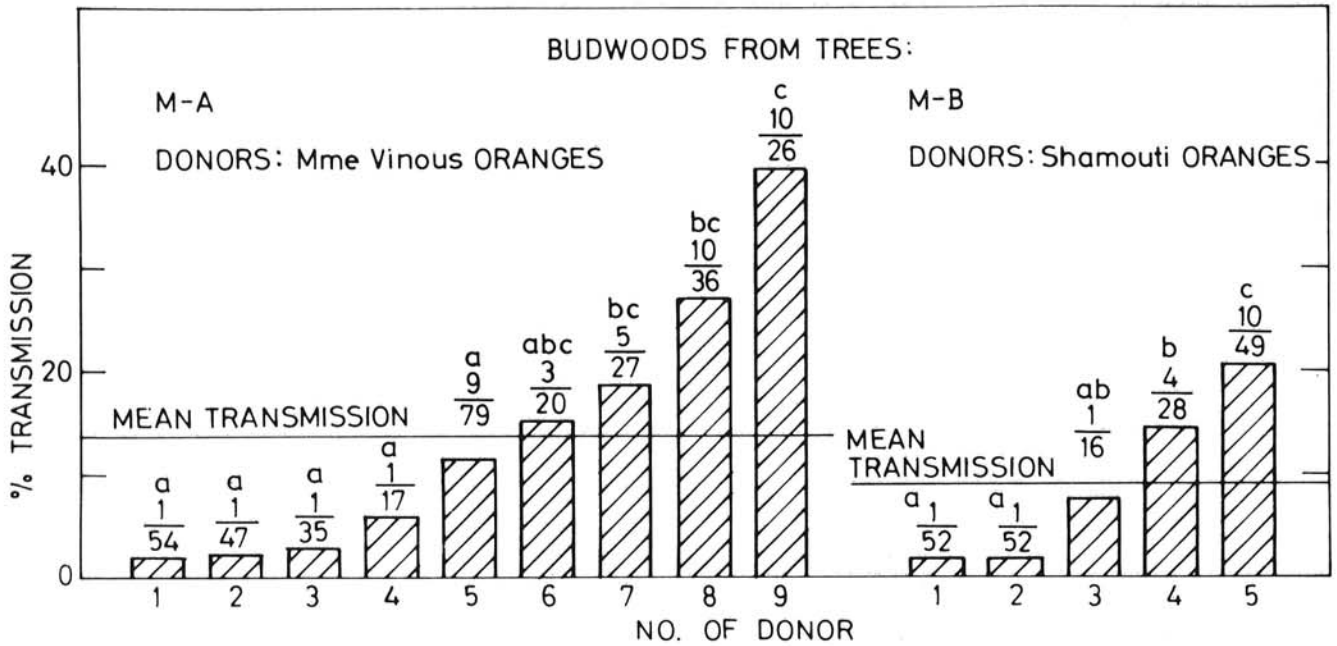


Fig. 3. Distribution of aphid transmission rates from donors (A-donors) grafted with budwood deriving from Miqwe Yisra'el. Budwood from tree M-A (a tree grafted with budwood taken earlier from Miqwe Yisra'el) was grafted on 2-yr-old Mme. Vinous sweet orange seedlings, and from tree M-B on 2-yr-old plants of Shamouti sweet orange grafted on sour orange. Ratios above bar: denominator, number of plants used; numerator, number of plants infected, with batches of 10 1-wk-old aphids. Acquisition access period = 24 hr, inoculation access period 24 hr. Ratios below the same letter are not significantly different (analysis of variance, mean LSD; $P < 0.05$).

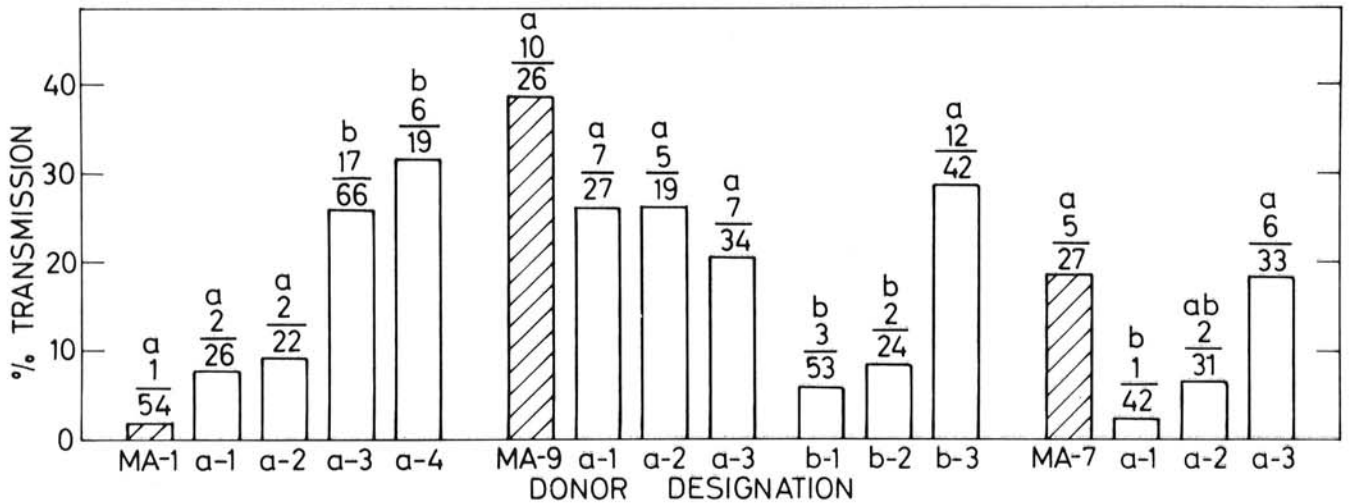


Fig. 4. Distribution of aphid transmission rates from B-donors for evaluating homogeneity of aphid-transmitted tristeza. To obtain B-donors sweet orange plants were grafted with budwood from positive-reacting Egyptian sour lime seedlings inoculated by aphids from several A-donors originating from tree M-A. \square - transmission rate from A-donor preceding those from the respective B-donors \square . Ratios above bar: denominator, number of plants used; numerator, number of plants infected, with batches of 10 1-wk-old aphids. Acquisition access period = 24 hr, inoculation access period = 24 hr. Ratios below the same letter are not significantly different (analysis of variance, mean LSD; $P < 0.05$).

could be recovered. It seems that these were quantitatively suppressed by a dominant, highly transmissible variant(s) that is present in the tree at a high titer. Another possibility could be a variation in the mobility of variants, or in their site of multiplication in the citrus tree. This could allow one variant to be more accessible to the aphid during probing; though with another virus aphid transmissibility seems to be an intrinsic property of the virus, associated with the protein coat (5).

Two possible explanations could be suggested for the segregation of tristeza variants by grafting. One is based on the assumption that budwood taken from different parts of the tree contains different variants, or the same variants but in different ratios, or a combination of the two. The other assumes that some variations exist among seedlings or plants of the same variety serving as donors, allowing differential multiplication of the variants.

A group of aphids confined to one donor apparently transmit more than one isolate, as a spectrum of variants was observed from aphid-inoculated seedlings. The spectrum of variants in one donor does not seem to change markedly during short periods, as retests from the same donor after 2-3 mo did not reveal marked differences in transmission rates. However, under orchard conditions it may well be that stress conditions or severe pruning and subsequent intense new flushes of growth may cause quantitative changes between variants. Thus, a highly transmissible variant, previously present at low concentration, may establish itself in the new growth and become dominant. This could provide an explanation for the natural spread of CTV associated with highly transmissible strains, in areas where previously no indication of natural spread had been observed.

For two strains of cucumber mosaic virus evidence has been presented that transmission by aphids is associated with their protein coat (5). It might therefore be of interest to see, once pure isolates of tristeza virus have been obtained, if transmissibility of tristeza variants is also associated with their coating proteins.

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