Title

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TRISTEZA AND RELATED DISEASES

Transmission of Tristeza and Seedling Yellows Tristeza Virus by *Aphis gossypii* from Sweet Orange, Grapefruit and Lemon to Mexican lime, Grapefruit and Lemon

C. N. Roistacher and M. Bar-Joseph

ABSTRACT. Over ten months, 645 transmission tests were made with *Aphis gossypii* Glover as vector for two isolates of seedling yellows tristeza virus (CTV-SY) and one of citrus tristeza virus (CTV). Acquisition hosts were seedlings of sweet orange, grapefruit, and lemon, and the receptor hosts were seedlings of Mexican lime, grapefruit and lemon. Twenty-seven combinations were tested with three virus isolates grafted onto three different acquisition hosts and vectored into three different indicator hosts. All three virus isolates in sweet orange were highly transmissible by aphids to Mexican lime (96-100%). The two CTV-SY isolates in sweet orange were transmitted to grapefruit at 93 and 79% whereas the CTV isolate was transmitted at only 14%. All three virus isolates in sweet orange were transmissible to lemon at 18 to 41%. CTV-SY in grapefruit was transmitted at 40 and 24% to Mexican lime; 31 and 14% to grapefruit, but was not transmitted to lemon, whereas CTV in grapefruit was transmitted to the three receptor hosts at 3, 0, and 4%, respectively. All 3 virus isolates were transmitted poorly from lemon. In general, transmission from grapefruit or lemon to grapefruit and lemon was quite low with only 14 transmissions in 226 tests (6.2%).

The epidemiological consequences of variable transmission from and to different cultivars are discussed.

Index words. Vector transmission, ELISA.

The rate of transmission of citrus tristeza virus (CTV) by *Aphis gossypii* has undergone dramatic changes over the last 30 years in several citrus areas of the world. In the early 1950's in California, Dickson *et al.* (11) reported a transmission rate of less than 5% by *A. gossypii* and in Florida, Norman and Grant (16) reported a 4% transmission rate. In the early 1970's, Bar-Joseph and Loebenstein (1) showed that most CTV isolates transmitted at less than 5% in Israel, but one seedling yellows tristeza (CTV-SY) isolate transmitted at 40% and caused widespread damage in the Hibbat-Zion area. Bar-Joseph (2) suggested that the shift in transmission rate was a function of a change in transmissibility of the virus and not the vector.

In recent years *A. gossypii* has also become a highly efficient vector for tristeza in California. Under controlled conditions at Riverside, California, Roistacher *et al.* (18) and Roistacher (20) reported that 46 field isolates of CTV and CTV-SY were transmitted at 100% efficiency by *A. gossypii* from sweet orange acquisition host plants to Mexican lime indicator-receptor seedlings. This increase in transmissibility in the virus was apparently responsible for the recent spread of CTV-SY isolates through the Citrus Research Center (CRC) field plots at Riverside (6, 17).

Transmission studies have been made with *Toxoptera citricida* (Kirk.) as vector of CTV from sweet orange to other cultivars. Costa *et al.* (9) in Brazil showed transmission rates of 70 and 50% from sweet orange to two cultivars of sweet orange, 47 and 31% from sweet orange to two cultivars of grapefruit and less than 15% from sweet orange to sour orange. Stubbs (23) in Australia transmitted CTV-SY from Mexican lime to grapefruit in 5 of 30 plants (14%) using 5 aphids per inoculation. Limited experimental transmission in Israel by *A. gossypii* from several acquisition hosts inoculated with the VT (CTV-SY) strain showed variability depending on the host. Transmission rates were 45.4 and 42.3% from sweet orange and mandarin, respectively, to Mexican lime, compared with only 9.0 and 5.2% from sweet lime and grapefruit respectively to Mexican lime (3).

This paper reports the results of experiments which tested in detail the rates of transmission of three tristeza isolates in three acquisition hosts (sweet orange, grapefruit, and lemon) vectored to three receptor hosts (Mexican lime, grapefruit, and lemon). The epidemiological consequences of variable transmission from and to different citrus cultivars are discussed.

MATERIALS AND METHODS

Plants used and conditions of growth. Acquisition host plants were carefully selected nucellar seedlings of Madam Vinous sweet orange, Duncan grapefruit and Eureka lemon. Indicator-receptor plants were selected nucellar seedlings of Mexican lime, Duncan grapefruit and Eureka lemon. Since Mexican lime is an excellent indicator for CTV, and our previous studies (3, unpublished) showed similar transmissibility of CTV by *A. gossypii* from sweet orange to Mexican lime, sweet orange or mandarin, Mexican lime was the preferred receptor host.

All seedlings were grown and fertilized by the U. C. system for plant growth modified for citrus (13). Seedlings were grown in 4-liter plastic containers in a glasshouse at temperatures of 26/19 C (daytime maximum/nighttime minimum). Five or more seedlings each of sweet orange, grapefruit and lemon were bud-grafted with inoculum tissue containing each of the three virus isolates and these reservoir plants were used as the acquisition feeding hosts. During the course of the experiment, emerging young leaves were periodically sampled and indexed for presence of CTV by ELISA (enzyme-linked immunosorbent assay) (4), or by leaf-graft inoculation to Mexican lime seedlings. This was done to test for the presence of CTV in conjunction with aphid feeding on these acquisition plants.

Aphid transmission. Young growth flushes of the acquisition host plants were periodically exposed to feeding by *A. gossypii* over a 10-month period. The aphid inoculation technique used is illustrated and described in detail by Roistacher (20). Aphids were first reared on leaves of muskmelon (*Cucumis melo* L., 'PMR 45'), which were cut up into small strips and transferred to cages enclosing the acquisition host plants. After a feeding period of 24 hours at 24 C in an illuminated temperature cabinet, young leaves of the acquisition host, covered with aphids in all stages of instar development, were removed and loosely tied to young leaves of the inoculation or receptor host. The inoculation feeding period was also 24 hours at 24 C, after which aphids remaining on the leaves were counted on each receptor plant, then destroyed by spraying with an insecticide. The aphid-inoculated receptor plants were held 3 to 5 months for observation of symptoms, or leaves were collected for indexing by ELISA after 4 to 6 weeks. A total of 645 individual transmission tests were done from January through October 1981, averaging 24 replicates for each of the 27 combinations tested. Growth flushes were coordinated between acquisition and receptor host plants for aphid transmission feeding by simultaneously cutting back acquisition and receptor seedlings to force new flushes of growth.

Virus sources used. All three virus isolates had previous histories of 100% transmission by *A. gossypii* from sweet orange to Mexican lime. All three isolates in reservoir plants of sweet orange were negative for exocortis and all induced typical severe leaf vein clearing and stem pitting symptoms when graft-inoculated to Mexican lime seedlings.

CTV-514 is a CTV isolate from a naturally infected Valencia orange, from Althouse-A ranch in central California. This typical tristeza isolate induced a mild yellows reaction in grapefruit, no seedling yellows reaction or stunting in sour orange or lemon and no stem pitting in grapefruit or sweet orange seedlings.

CTV-SY-563 is a CTV-SY isolate from a Brazil navel orange in the citrus variety collection at the CRC Riverside, CRC 597, field 7B, row 52, tree 12. Earlier indexing of certain suspect trees in the variety collection by Roistacher **et al.** (17) revealed that virus from this tree induced very severe yellows and stunting in seedlings of grapefruit, sour orange and lemon. Inoculated grapefruit seedlings were severely pitted whereas Madam Vinous sweet orange seedlings were only mildly pitted.

CTV-SY-568 is a CTV-SY isolate from a Minneola tangelo formerly in field 12B, row 49, tree 8 at the CRC shown by Calavan *et al.* (6) to have a very severe form of CTV-SY. This tree was stunted and pitted (21) and nearby grapefruit trees naturally infected by this isolate were also stunted and pitted. Budwood indexed from this tree induced a very severe seedling yellows reaction in grapefruit, sour orange and lemon, and also induced severe stunting, vein corking of leaves and extreme stem pitting in Madam Vinous seedlings. Navel and Valencia orange stems are also severely pitted by this isolate.

RESULTS

Presence of virus in the acquisition host plants. The results of 381 index tests by ELISA or leaf-graft inoculation to Mexican lime seedlings showed the presence of CTV in young leaves of all graft-inoculated acquisition of sweet orange. Tristeza isolates CTV-SY 568, CTV-SY 563 and CTV-514 were found respectively in 94, 94 and 88% of the young leaves of grapefruit acquisition plants and 98, 75 and 69% of the young leaves of lemon acquisition plants. The systemic spread of the virus from the inoculation site to the young lemon leaves was variable and apparently in proportion to severity of the virus; the more severe CTV-SY 568 infected 98% of the leaves whereas CTV-514 infected 69% of the lemon leaves.

In nearly all tests, plant condition and aphid transfer were good, and satisfactory aphid populations were recorded on the young leaves of the receptor plants. Table 1 gives the results of virus transmission, and the average number of aphids found for each of the 27 combinations tested. There were 253, 214, and 178 individual aphid transfer tests respectively from sweet orange, grapefruit and lemon acquisition hosts and the average number of aphids counted was 39.4, 41.3 and 45.9 on receptor plants inoculated from these respective

TABLE 1
TRANSMISSION BY *APHIS GOSSYPII* OF THREE ISOLATES OF CTV FROM THREE ACQUISITION HOSTS TO THREE RECEPTOR HOSTS

		Receptor Hosts					
		Mexican lime		Grapefruit		Lemon	
Virus isolates	Acquisition hosts*	Avg. no. aphids	Transmission rate	Avg. no. aphids	Transmission rate	Avg. no. aphids	Transmission rate
SY-563	SwO.	33	23/24†	35	26/33	28	5/28
	Gft.	38	7/29	21	3/22	53	0/18
	Lemon	54	2/28	29	0/15	23	0/20
SY-568	SwO.	46	31/31	37	24/26	61	14/50
	Gft.	33	10/25	32	8/26	38	0/15
	Lemon	72	5/29	37	2/15	52	0/16
T-514	SwO.	49	17/17	26	3/22	40	9/22
	Gft.	65	1/30	42	0/25	50	1/24
	Lemon	77	1/25	32	0/15	37	0/15

* SwO. = sweet orange; Gft. = grapefruit.
† No. plants infected/no. plants inoculated.

hosts. The average number of aphids found per plant for all 645 tests was 42.2.

Transmission from sweet orange. Vector transmission for all three isolates from sweet orange to Mexican lime was 99%. This was consistent with previous published results where 46 CTV and CTV-SY isolates in sweet orange or mandarin transmitted at 100% efficiency to seedlings of Mexican lime (18, 20). Transmission from sweet orange to grapefruit was 92 and 79% for the two CTV-SY isolates but only 14% for the CTV isolate. Transmission from sweet orange to lemon was 28 and 18% for the two CTV-SY isolates and 41% for the CTV isolate.

Transmission from grapefruit. Transmission from grapefruit to Mexican lime was 40 and 24% for the two CTV-SY isolates but only 3% for the CTV isolate. Transmission from grapefruit to lemon was negative for the CTV-SY isolate with only 1 positive in 25 tests for the CTV isolate.

Transmission from lemon. Transmissions from lemon to all receptor plants were generally poor. Transmission from lemon to Mexican lime was 12, 7 and 4% respectively for isolates CTV-SY 568, CTV-SY 563 and CTV-514. Transmission from lemon to grapefruit was negative for CTV-SY 563 and CTV-514 and 15% for the very severe CTV-SY isolate 568. None of the three isolates in lemon was transmitted to lemon.

There appears to be a correlation between the severity of CTV (as measured by reactions in indicator seedlings) and the efficiency of virus transmission by *A. gossypii.* This is illustrated in figure 2 which shows transmission by vector from sweet orange to grapefruit; from grapefruit to Mexican lime and grapefruit, and from lemon to Mexican lime for the three isolates tested. The one

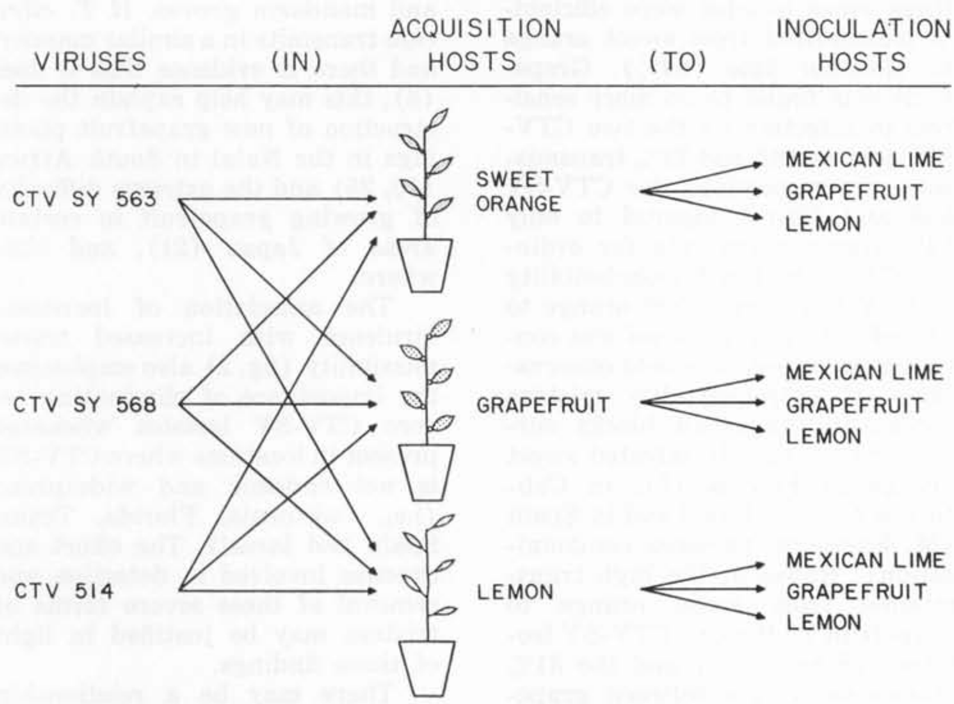

Fig. 1. A schematic of the procedure for transmission of three tristeza isolates in three acquisition hosts to three inoculation or receptor hosts.

exception was a reversal shown by isolate CTV-514 in sweet orange which transmitted at 41% to lemon, compared to 18 and 28% for CTV-SY isolates 563 and 568. A supplementary test was made with another CTV isolate (CTV-524) found in a lemon tree in the Central Valley of California (7). Distribution of CTV in a two-year-old bud propagation from this lemon tree was very uniform. Transmission tests by *A. gossypii* from a bud-inoculated sweet orange acquisition host were made to 25 lemon seedling receptor hosts (averaging 80 aphids per seedling). No transmission was obtained in marked contrast with the 41% transmission for CTV-514.

DISCUSSION

The present study was designed to compare the ability of *A. gossypii* to transmit CTV and CTV-SY from three important commercial citrus cultivars to similar receptor cultivars. All three virus isolates were efficiently transmitted from sweet orange to Mexican lime (99%). Grapefruit was found to be more sensitive to infection by the two CTV-SY isolates (92 and 79% transmission rate respectively for CTV-SY 568 and 563) compared to only 14% transmission rate for ordinary CTV. The low transmissibility of CTV-514 from sweet orange to grapefruit by *A. gossypii* was consistent with previous field observations of extremely low tristeza spread in grapefruit blocks surrounded by heavily infected sweet orange in Florida (5), in California (22), in Israel and in Spain (M. Fabreget, personal communication). However, the high transmission from sweet orange to grapefruit by the two CTV-SY isolates (79 and 93%) and the 31% transmission rate between grapefruit by CTV-SY 568 indicate that certain severe isolates of seedling yellows present in sweet orange, and grapefruit (or mandarin) might be hazardous to nearby plantings of grapefruit. Current studies (Roistacher, unpublished, and 24) show that stem pitting is readily transmissible by *A. gossypii,* and although stem pitting may be a separate part of the CTV-SY complex, it appears to be transmitted by *A. gossypii* as readily as seedling yellows.

There is an extensive program for the detection and eradication of severe isolates of CTV-SY in the experimental fields at CRC, Riverside, with complete replanting of the variety collection in 1983. This program includes the detection and elimination of CTV-SY from infected cultivars (6, 17, 19, 27). The findings in this paper support continued efforts for this CTV-SY eradication program, since widespread distribution of severe CTV-SY would be a hazard endangering future grapefruit plantings in the vicinity of infected sweet orange and mandarin groves. If *T. citricida* transmits in a similar manner, and there is evidence that it does (8), this may help explain the destruction of new grapefruit plantings in the Natal in South Africa (10, 25) and the extreme difficulty of growing grapefruit in certain areas of Japan (21), and elsewhere.

The association of increased virulence with increased transmissibility (fig. 2) also emphasizes the importance of eliminating severe CTV-SY isolates whenever present in locations where CTV-SY is not endemic and widespread (i.e., California, Florida, Texas, Spain and Israel). The effort and expense involved in detection and removal of these severe forms of tristeza may be justified in light of these findings.

There may be a relationship between virus severity (as measured by indicator reaction) and

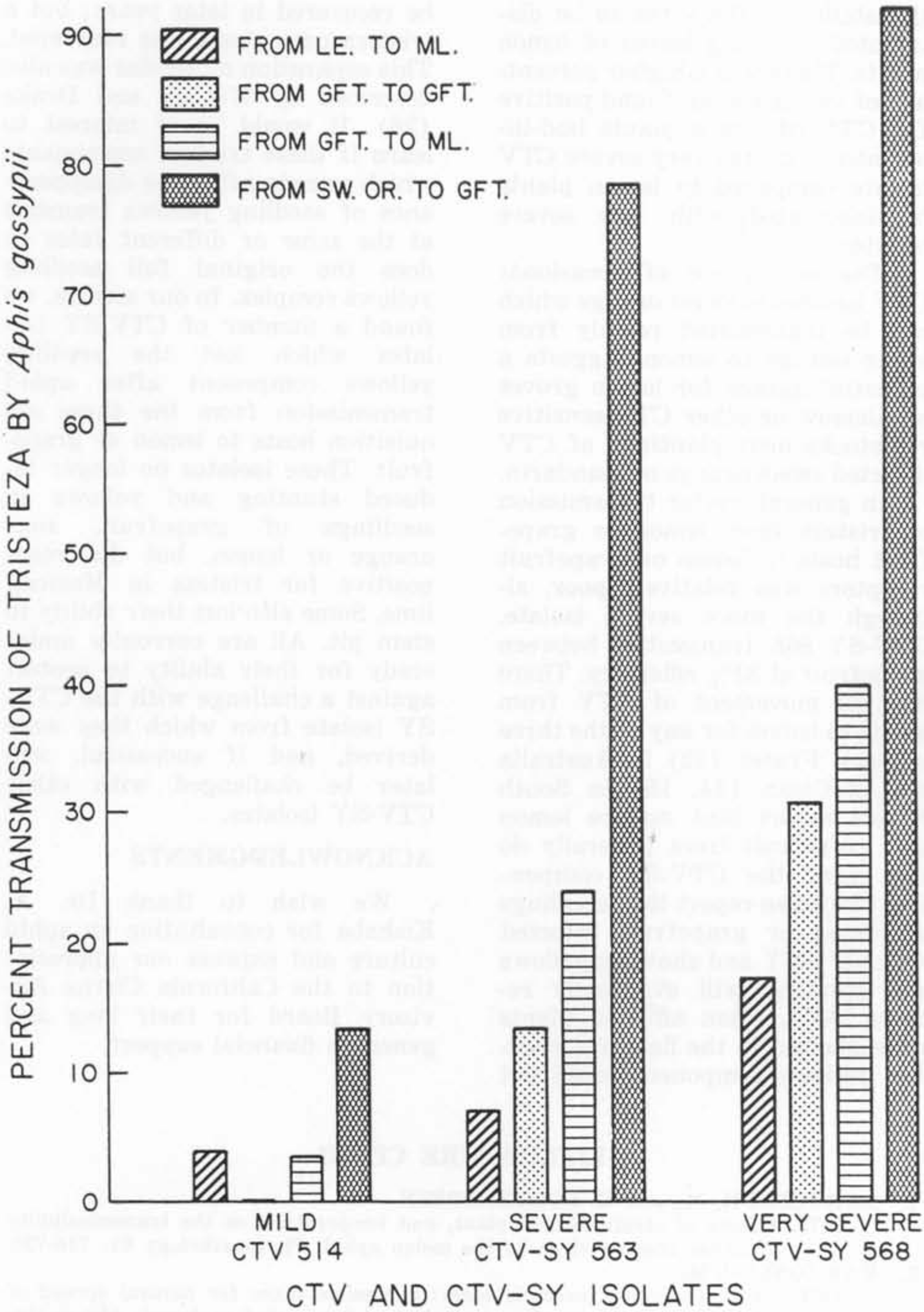

Fig. 2. Vector movement of two isolates of seedling yellows tristeza virus (CTV-SY) and one of tristeza virus (CTV) in sweet orange (SW.OR.), grapefruit (GFT.) and lemon (LE.) to Mexican lime (ML.) and grapefruit showing the relationship between severity of tristeza and efficiency of vector movement by *Aphis gossypii*.

the ability of the virus to be distributed in young leaves of lemon plants. There was a higher percentage of young leaves found positive for CTV of lemon plants bud-inoculated with the very severe CTV isolate compared to lemon plants bud-inoculated with less severe isolates.

The occurrence of occasional CTV isolates in sweet orange which can be transmitted readily from sweet orange to lemon suggests a potential hazard for lemon groves on alemow or other CTV-sensitive rootstocks near plantings of CTV infected sweet orange or mandarin.

In general, vector transmission of tristeza from lemon or grapefruit hosts to lemon or grapefruit receptors was relatively poor, although the more severe isolate, CTV-SY 568, transmitted between grapefruit at 31% efficiency. There was no movement of CTV from lemon to lemon for any of the three isolates. Fraser (12) in Australia and McClean (14, 15) in South Africa report that mature lemon and grapefruit trees generally do not carry the CTV-SY component. They also report that seedlings of lemon or grapefruit infected with CTV-SY and showing yellows and stunting will eventually recover. Also, when affected plants were planted in the field, the seedling yellows component could not be recovered in later years; but a tristeza component was recovered. This separation of strains was also described by Wallace and Drake (26). It would be of interest to learn if these tristeza components which remain after the disappearance of seedling yellows transmit at the same or different rates as does the original full seedling yellows complex. In our studies, we found a number of CTV-SY isolates which lost the seedling yellows component after aphid transmission from the three acquisition hosts to lemon or grapefruit. These isolates no longer induced stunting and yellows in seedlings of grapefruit, sour orange or lemon, but did react positive for tristeza in Mexican lime. Some also lost their ability to stem pit. All are currently under study for their ability to protect against a challenge with the CTV-SY isolate from which they were derived, and if successful, will later be challenged with other CTV-SY isolates.

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