Transmission of Citrus Tristeza Virus by *Aphis gossypii* Reflecting Changes in Virus Transmissibility in California

C. N. Roistacher, E. M. Nauer, A. Kishaba, and E. C. Calavan

In the early 1950's, the transmission rate of citrus tristeza virus (CTV) by the melon aphid, *Aphis gossypii* Glover, was very low. Dickson *et al.* (1956) induced five transmissions in 207 tests (2.4 per cent) averaging 50 aphids per test, and 26 infections in 412 tests (6.3 per cent) averaging 221 aphids per test. Similar low rates of transmission were reported in the early 1960's from Florida by Norman *et al.* (1968) with *A. gossypii* and *A. spiraecola* Patch. A sudden outbreak of tristeza in Israel in 1970-73 prompted investigations by Bar-Joseph and Loebenstein (1973) on the transmission rates of three strains of CTV by four populations of *A. gossypii* collected from various parts of their country. Using 100 aphids per test, they found that one strain, from citrus in the Sharon Plains area where tristeza was spreading, was transmitted to over 40 per cent of the receptor plants by all four aphid populations. The other two strains were transmitted at less than 5 per cent.

These findings from Israel and the earlier report of Martinez and Wallace (1964), who found that seedling yellows tristeza virus (CTV-SY) from a few varieties in the collection at the University of California, Riverside, was transmissible by *A. gossypii* at relatively high rates, led to a survey for CTV-SY in some of the early citrus introductions in the variety collection at Riverside. This survey showed that 50 per cent of 38 early introductions had CTV-SY, and in a second test, 48 per cent of 27 declining trees nearby also had CTV-SY (Roistacher *et al.*, 1979). Since many of the infected trees in the second test were recent seedling introductions, CTV-SY apparently was spreading from infected to nearby trees. Therefore, transmissibility of CTV and CTV-SY isolates from many of these infected trees in the variety collection by the melon aphid, *A. gossypii*, was studied and is reported in this paper.

MATERIALS AND METHODS

Experimental procedures generally were those of Bar-Joseph and Loebenstein (1973) and Raccah *et al.* (1976). Budwood was collected from 20 CTV- and CTV-SY-infected trees in the variety collection at the Citrus Research Center (Roistacher *et al.*, 1979), and from three CTV-infected trees in central California. One to four Madam Vinous sweet orange seedlings in 4-liter containers were graft-inoculated with buds from each infected tree to provide a uniform acquisition host. Bar-Joseph and Loebenstein (1973) showed considerable differences in transmissibility of CTV from different hosts, with sweet orange and mandarin showing the highest transmission rates.

Madam Vinous sweet orange acquisition host plants were grown in a U.C. potting mix (Nauer *et al.*, 1968) in a glasshouse at temperatures averaging 26/19°C (daytime maximum/nighttime minimum). Two to 18 months after inoculation, they were cut back to force new growth for acquisition feeding. The Mexican lime seedlings used for inoculation feeding and indexing were grown in groups of three per 4-liter container in a U.C. mix at warmer temperatures (35/20°C). All plants were maintained in optimum growth flush to attract aphids for feeding.

Aphids for most of the transmission studies were progeny of R. C. Dickson's (A. G. 64) collection from watermelon

in 1960 in the Palo Verde Valley near Blythe, California. The aphids had been maintained on melon plants continuously for 18 years at Riverside. For comparative studies, aphids were collected in 1978 from melons growing near Brawley in the Imperial Valley, and from a melon vine near Tulare, in the central Valley of California.

Aphids were reared on muskmelon (*Cucumis melo* L., 'PMR 45') in rearing cages in a small glasshouse, with temperatures maintained at 21° ± 3°C. Melon leaves containing mixtures of immature and mature aphids were collected, cut into segments, and placed in cages attached to young shoots of the Madam Vinous acquisition plants. Norman and Sutton (1969) and Raccah *et al.* (1976) showed no differences in transmission rates between mature and immature aphids. Acquisition and inoculation feedings of 24 hours each were made at 24°C in a growth chamber. Light was provided for 16 hours daily. After the inoculation feeding, cages were removed, and the live aphids on leaves counted and killed with a 1 per cent nicotine sulfate spray. The inoculated Mexican lime plants were held in the glasshouse at 26.3°C/18.7°C (daytime maximum/nighttime minimum) at least 6 months and observed for tristeza symptoms. Most symptoms appeared within 4 to 8 weeks after inoculation feeding. Plants positive for tristeza were held for future subpropagations on seedling yellows indicator plants. Transmission tests for each isolate were repeated at least once.

RESULTS

Transmission rates of CTV and CTV-SY. The results of transmission of 23 CTV and CTV-SY isolates by *A. gossypii* (Blythe culture) are given in table 1. With the exception of two isolates, T-515 and *Citrus junos* (1487), the transmission rates for repeated tests were consistent. Thirteen isolates were transmitted at 100 per cent efficiency in at least one experiment, two above 90 per cent, and four isolates averaged 21-28 per cent transmission. The Meyer lemon isolate was transmitted to 3/42 Mexican limes (7 per cent). There was no transmission of two isolates (from Honey mandarin and *C. webberi*).

In 786 transmission tests made to Mexican limes with the Blythe culture, the average number of aphids per test was 44.3, and aphid counts ranged from zero to over 400 per plant. In some individual tests, transmission was achieved by few aphids, i.e., nine positive transmissions were obtained when no aphids survived and 14 transmissions were obtained with two to five surviving aphids per plant. Allowing for aphid mortality, the total number of aphids feeding during the 24-hour inoculation period was probably somewhat higher than the counts made after 24 hours.

The low transmission rates of CTV-SY isolates from Meyer lemon, Honey mandarin, and *C. webberi* suggest that these isolates were still being transmitted at rates similar to or below those reported by Dickson *et al.* (1956) for CTV in the early 1950's.

The three isolates of CTV from central California (T-505, T-514, and T-515) from D. Cordas of the Central Valley Tristeza Eradication Program were all natural field infections in Valencia or navel oranges. Isolate T-505, from a 1970 collection, was transmitted at 18 and 32 per cent in two tests. Isolate T-515, collected in 1977, was transmitted in six tests at rates of 0 to 91 per cent, whereas T-514, a severe tristeza isolate collected in 1977, was transmitted 100 per cent to 46 test plants.

Graft transmissions to other varieties. Graft inoculations from a number of vector-infected Mexican lime plants to grapefruit, sour orange, and Eureka lemon indicator seedlings were made to determine if the yellows-inducing potential of CTV-SY had been transmitted by *A. gossypii.* With few exceptions, the severe potential of CTV-SY was transmitted from the Madam Vinous acquisition host to Mexican limes by *A. gossypii* as indicated by the reactions on grapefruit, sour orange, and Eureka lemon indicator seedlings (table 2). The reactions in indicator seedlings were

comparable to those made by bud-inoculations from infected Madam Vinous host plants or field source trees (table 1). In some cases, strains were separated by vector, as from Brazil navel and Kona oranges. However, the large majority of vector-infected Mexican lime buds appeared to transmit unchanged the CTV-SY isolates shown in table 2, and many other isolates in unreported tests.

Comparison of different aphid cultures. Two additional cultures of *A. gossypii* were obtained, one from melons in the southern part of California and one from a melon in the Central Valley of California, to confirm that the current very high transmissibility of many CTV and CTV-SY isolates depends on the nature of the virus and not on differences in aphid cultures. Eleven virus isolates in Madam Vinous were selected: six showing high transmission, three moderate, and two no transmission with the Blythe aphid culture. The acquisition and inoculation procedures were the same as in previous tests. A total of 193 and 171 transmission tests were made to Mexican limes with the Brawley and Tulare aphid cultures, and the number of aphids per test averaged 55.9 and 49.5, respectively. Results conclusively show that virus transmissibility by all three aphid cultures was similar (table 3). This agrees with results of Bar-Joseph and Loebenstein (1973), who found little to no variability in the transmission rates of CTV among four aphid cultures collected from various localities in Israel.

DISCUSSION

The very high transmission rates found in these studies for 16 CTV and CTV-SY isolates suggest that *A. gossypii* may now be as efficient a vector for CTV or CTV-SY in California as *Toxoptera citricida* (Kirkaldy) is elsewhere in the world. However, *A. gossypii* populations in California, especially in the warmer areas of the state, are still relatively low compared to the abundance of *T. citricida* where it occurs.

Many of the citrus trees at Riverside which are now infected with highly transmissible CTV-SY are progeny of very early introductions. Wallace *et al.* (1956) reported the presence of CTV-SY in a few varieties in the CRC Riverside citrus variety collection and believed the virus was present when they were imported about 1930 or earlier. Martinez and Wallace (1964) showed that three CTV-SY isolates from the variety collection were transmitted by *A. gossypii* from sweet orange to Mexican lime in 4/16, 1/4, and 7/9 tests. Yearly surveys of trees in the UCR variety collection by W. P. Bitters (personal communication) revealed that few trees were removed because of decline or poor performance between 1940 and 1970 but, within the past 9 years, the increase of declining trees has been exponential and rather alarming. It is probable that the recent increase in declining trees in the UCR variety collection (Roistacher *et al.*, 1979) is due to the change in transmissibility of severe CTV and CTV-SY strains. Raccah *et al.* (1977) reported a segregation of CTV isolates from a single sweet orange source plant showing varying rates of transmission. Transmissibility of CTV by *A. gossypii* in 17 receptor sweet orange plants ranged from 0 to 38.5 per cent. Bar-Joseph *et al.* (1977) and Bar-Joseph (1978) postulated a theory to explain the increased transmissibility of CTV by *A. gossypii*. He suggested that "the tristeza isolates introduced from the Far East, Brazil, and South Africa were transmissible by *T. citricida*, but possibly not by *A. gossypii*. The presence of a non-transmissible strain should reduce, possibly by cross protection, the multiplication and spreading of transmissible mutants which arise from non-transmissible strains. However, under certain conditions, so far unidentified, cross protection fails or is incomplete and *A. gossypii* transmissible isolates might be acquired and transferred by this aphid to healthy trees. These newly infected trees will carry only the transmissible strain and will serve as sources for natural spread."

The varying rate of transmission ranging from 0 to 100 per cent for two isolates (*C. junos* 1487 and navel orange

TABLE 1
SOURCE TREES, REACTION RATINGS, AND TRANSMISSIBILITY* BY *APHIS GOSSYPII* FOR 23 CTV AND CTV-SY ISOLATES

CRC No.	Variety†	Reaction rating†				No. Aphids‡ per plant/ no. plants§	Per cent transmission§	
		M	G	S	L		Range	Overall
3369	De Ba Ahmed mand.	4	3+	0	3	60/12	—	100
266	Florida satsuma	5	2+	0	3	67/12	—	100
3558	Fremont mand.	3	4	1	0	46/22	—	100
3370	Spanish mand.	4	2+	4	0	60/9	—	100
602	Weshart mand.	4	3	0	0	44/19	—	100
——	Valencia or. T514	5	2	0	1+	45/46	—	100
560	*C. macroptera*	3	5	5	4	53/27	94-100	96
950	Lima or.	3	3	0	1	36/38	88-95	92
597	Brazil navel or.	4	5	3+	5	32/44	71-100	89
3144	*C. keraja*	4	3	1	0	42/8	67-100	88
562	Selecta or.	3+	3	0	4	38/47	78-100	87
561	*C. junos*	5	4	0	5	29/62	79-91	85
321	Imperial variegated or.	3	5	1	5	50/30	64-100	83
2446	*C. macrophylla*	4	0	1	0	55/15	56-100	73
1487	*C. junos*	4	5	1	1	62/27	0-100	41
——	Navel or. T515	3	3	1	0	34/68	0-91	40
——	Valencia or. T505	3	2+	2+	0	37/39	18-32	28
300	Parsons Special mand.	4	3	4+	5	57/89	17-38	26
451	Kona or.	5	4	4+	5	33/37	24-25	24
452	Kusaie lime	5	4	5	5+	44/57	11-60	21
662	Meyer L	3	4	4	4+	83/42	0-11	7
3177	Honey mand.	4	5	3	0	26/35	—	0
1455	*C. webberi*	5	4+	0	0	21/15	—	0

* From Madam Vinous sweet orange to Mexican lime.

† Results from Roistacher *et al.*, 1979. Ratings: 0 = negative, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe. M = Mexican lime, mand. = mandarin, G = grapefruit, or. = orange, S = sour orange, L = sour lemon; seedlings used for all ratings.
All isolates derived from trees in the Citrus Research Center (CRC) variety collection at Riverside, except T505, T514, and T515 from Tulare County in central California.

‡ Aphid culture was Dickson AG 64, collected in 1964 from Blythe, California. Numbers are averages.

§ From two or more experiments.

T-515) might be explained if we consider them to be in a dynamic state of change, moving from a less transmissible to a more transmissible form with protection varying from complete to incomplete. This was postulated by Raccah *et al.* (1977), who also found varying transmissibility from a single sweet orange source plant.

The results of these studies have important implications for California citrus: 1) the variety collection at the University of California will now be indexed and all severe CTV-SY-infected trees removed, and the important or desirable selections secured under screen until they can be freed of CTV-SV by thermotherapy or shoot-tip grafting; 2) trees surrounding the variety collection will be indexed for CTV-SY-infected trees and removed if infected (Calavan *et al.*, 1980); 3) continued vigilance needs to be maintained in the Central California Tristeza Eradication Program for any instances of tristeza infection, and especially any CTV-SY; 4) a statewide survey will be initiated and vigilance maintained for the detection of trees in decline, which will then be tested for CTV-SY.

In other states or countries where tristeza is present and *A. gossypii* is the prime vector, a search for severe forms of tristeza virus should be encouraged. In countries where indexing has been limited but tristeza may be present, indexing and eradication should command a very high priority.

These studies were confined to *A. gossypii* and transmissibility by other aphid species was not tested. *A. spiraecola* is known to transmit CTV, perhaps as efficiently as *A. gossypii* in Florida, and transmissibility studies for these and other aphids warrant investigation.

These studies suggest why it has been difficult to eradicate tristeza completely in some locations in central California. Two isolates, T-514 and T-515, were from specific locations in central California where tristeza persistently reappeared, even after eradication was presumed complete. Transmission tests showed both isolates to be highly transmissible (100 and 91 per cent in individual tests).

ACKNOWLEDGMENTS

The authors wish to acknowledge the invaluable technical advice extended by M. Bar-Joseph, the Volcani Institute, Israel, who, while on a visit to Riverside in May 1977, helped initiate the first transmission tests. Also, the invaluable technical assistance of R. Wagner is gratefully acknowledged. We wish to thank T. Kono of the California Department of Food and Agriculture Entomology Laboratory Services for identification of the Tulare and Brawley cultures of *A. gossypii*.

TABLE 2
THE REACTION OF CTV-SY ISOLATES SUBPROPAGATED FROM APHID-INFECTED MEXICAN LIME PLANTS BY GRAFTING TO SEEDLINGS OF GRAPEFRUIT, SOUR ORANGE, AND EUREKA LEMON, AND COMPARISON TO ORIGINAL ISOLATES

Virus sources for Mexican limes	Grapefruit		Sour orange		Eureka lemon	
	Original isolate	AT* isolate	Original isolate	AT isolate	Original isolate	AT isolate
Kusaie lime	4†	4.2†	5	4.9	5	5
Kona orange	4	4.1	4	4.4	5	4.2
Citrus macroptera	5	5.0	5	4.4	4	4.4
Brazil navel orange	5	5.0	3	4.5	5	4.5
Parsons Special mandarin	3	4.8	4	4.9	5	4.0
Meyer lemon	4	4.0	4	4.3	4	5.0

* AT = aphid-transmitted isolate of original.

† Average rating of infected plants: 0 = negative; 1 = very mild; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe.

TABLE 3
TRANSMISSION OF CTV AND CTV-SY ISOLATES BY 3 CULTURES OF *A. GOSSYPII* COLLECTED FROM DIFFERENT LOCATIONS IN CALIFORNIA

Sources of isolates in Madam Vinous	Blythe culture*		Brawley culture		Tulare culture	
	Avg. no. aphids per plant / No. Plants inoculated	Plants infected per cent	Avg. no. aphids per plant / No. Plants inoculated	Plants infected per cent	Avg. no. aphids per plant / No. plants inoculated	Plants infected per cent
De Ba Ahmed mandarin	60/12	100	30/11	100	58/6	67
Fremont mandarin	46/22	100	32/9	78	57/6	100
Citrus macroptera	53/27	96	64/30	100	70/29	100
Lima orange	49/38	92	65/24	100	66/11	100
Selecta orange	38/47	87	58/10	100	77/8	100
C. junos (561)	29/62	85	74/31	97	51/24	100
Navel orange T515	38/68	40	41/31	48	50/29	52
Parsons Special mandarin	59/89	26	66/12	50	62/17	47
Kona mandarin	33/37	24	61/9	89	18/9	33
Honey mandarin	26/35	0	43/14	0	34/20	0
C. webberi	21/15	0	53/12	0	62/7	14

* Average of results from table 1.

LITERATURE CITED

BAR-JOSEPH, M.
1978. Cross protection incompleteness: a possible cause for natural spread of citrus tristeza virus after a prolonged lag period. Phytopathology 68: 1110-11.

BAR-JOSEPH, M., and G. LOEBENSTEIN
1973. Effect of strain, source plant, and temperature on the transmissibility of citrus tristeza virus by the melon aphid. Phytopathology 63: 716-20.

BAR-JOSEPH, M., B. RACCAH, and G. LOEBENSTEIN
1977. Evaluation of the main variables that affect citrus tristeza virus transmission by aphids, p. 958-61. *In* 1977 Proc. Int. Soc. Citriculture Vol. III. Lake Alfred.

CALAVAN, E. C., M. K. HARJUNG, R. L. BLUE, C. N. ROISTACHER, D. J. GUMPF, and P. W. MOORE
1980. Natural spread of seedling yellows and sweet orange and grapefruit stem pitting tristeza viruses at the University of California, Riverside, p. 69-75 this volume.

DICKSON, R. C., M. M. JOHNSON, R. A. FLOCK, and E. F. LAIRD, JR.
1956. Flying aphid populations in southern California citrus groves and their relation to the transmission of the tristeza virus. Phytopathology 46: 204-10.

MARTINEZ, A. L., and J. M. WALLACE
1964. Studies on transmission of the virus components of citrus seedling yellows by *Aphis gossypii*. Plant Dis. Rep. 48: 131-33.

NAUER, E. M., C. N. ROISTACHER, and C. K. LABANAUSKAS
1968. Growing citrus in modified U.C. potting mixtures. Calif. Citrograph 53: 456, 458, 460-61.

NORMAN, P. A., and R. A. SUTTON
1969. Efficiency of mature and immature melon aphids in transmitting tristeza virus. J. Econ. Entom. 62: 1237-38.

NORMAN, P. A., R. A. SUTTON, and A. K. BURDITT, JR.
1968. Factors affecting transmission of tristeza virus by melon aphids. J. Econ. Entom. 61: 238-42.

RACCAH, B., M. BAR-JOSEPH, and G. LOEBENSTEIN
1977. The role of aphid vectors and variation in virus isolates in the epidemiology of tristeza disease. Contribution from the Volcani Center Agr. Res. Organization, 1977 series No. 273-E. p. 221-27.

RACCAH, B., G. LOEBENSTEIN, and M. BAR-JOSEPH
1976. Transmission of citrus tristeza virus by the melon aphid. Phytopathology 66: 1102-04.

ROISTACHER, C. N., E. C. CALAVAN, E. M. NAUER, and W. P. BITTERS
1979. Spread of seedling yellows tristeza at Research Center. Citrograph 64: 167-69.

WALLACE, J. M., P. C. J. OBERHOLZER, and J. D. J. HOFMEYER
1956. Distribution of viruses of tristeza and other diseases of citrus in propagative material. Plant Dis. Rep. 40: 3-10.