Letter to the Editor

Cross Protection Incompleteness: A Possible Cause for Natural Spread of Citrus Tristeza Virus After a Prolonged Lag Period in Israel

M. Bar-Joseph

Virus Laboratory, Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.
The author thanks J. E. van der Plank for an encouraging evaluation of part of this paper and C. N. Roistacher for a critical review.

Citrus tristeza virus (CTV) is transmitted by several aphids (3, 8) of which the most efficient is the oriental aphid *Toxoptera citricidus* (Kirk). The combination of CTV-infected plants and the presence of *T. citricidus* has resulted in the nearly complete spread of CTV into all citrus-growing areas where this aphid is present. As a result of the CTV epidemic in South America millions of trees were killed and the citrus industry was severely affected. On the other hand, the melon aphid, *Aphis gossypii* (Glov.), appears to range from being a nonvector to being a fairly efficient vector at different times and places. In Texas, Dean and Olson (4) reported that natural spread did not occur and that no transmissions were obtained with *A. gossypii.* Similar conditions appeared to exist in 1959 in the San Joaquin Valley of California (5). In Israel, indexing tests made during 1954-1959 in several introduction plots in which imported infected trees had been growing for over two decades indicated that there had been no natural spread from the imported trees to other trees, for which virus-free budwood had been used (10). Subsequent laboratory virus transmission studies (6) established that several aphids prevalent in these groves, including *A. gossypii*, failed to transmit the virus. Beginning in 1970 this situation changed (2) and a limited natural spread was revealed in four separate areas. The newly infected trees in three of these areas were located within 100 m of groves in which CTV had been introduced over 35 yr ago. Transmission tests established a correlation between the field spread and the appearance of CTV isolates highly transmissible by *A. gossypii* (1).

Table 1 summarizes experiments with nine CTV isolates. Six of these (CT, NT, MT, BT, ST, PT) apparently were introduced to Israel during the 1928-1937 period and were preserved either in the original carriers or in local citrus cultivars that were top-grafted over 35 yr ago on infected carriers. In the last 7 yr three other isolates (B2T, HT, VT) were found in three separate groves (5 and 40 km apart) where natural spread is taking place. The results indicate that, compared to the highly transmissible isolates in the recently infected trees, the introduced isolates were transmissible at low rates.

Although it is possible that recent introductions of *A. gossypii*-transmissible isolates have occurred by the introduction of CTV-infected budwood, the distribution pattern for new infections, the results of indexing programs, and the data presented herein provide strong arguments against this possibility.

A hypothesis was suggested (3) to explain the lag period between the introduction of CTV to Israel and other citrus areas in which the oriental aphid is absent and its natural transmission by aphids prevalent in these areas. Indications of the validity of this hypothesis are given in this letter.

The CTV isolates introduced to Israel and other citrus-growing areas in the Mediterranean area and North America from the Far East and South Africa, probably were transmissible by *T. citricidus* but not by *A. gossypii* or the other aphids prevalent in these groves. The presence of an isolate not transmissible by *A. gossypii* should suppress, by cross protection, the multiplication and spread of *A. gossypii*-transmissible mutants which arise from nontransmissible strains. Evidence for cross protection between strains of CTV was obtained (Table 2)

TABLE 1. Transmission rates by *Aphis gossypii* of different citrus tristeza virus (CTV) isolates found in Israel

Source of isolate	Transmission rate		Ref.[b]
	Ratio[a]	(%)	
Isolates preserved in the original carrier trees			
CT	2/57[a]	3.5	(1)
NT	3/55	5.5	...[b]
MT	0/42	0	(3)
Isolates found in local cultivars probably infected by top-grafting on infected carrier trees			
BT	0/50	0	(9)
ST	2/68	2.9	(1)
PT	1/45	2.2	...[b]
Isolates found in groves where CTV is epidemic			
B2T	14/42	33.3	(3)
HT	14/35	40.0	(9)
VT	69/118	58.5	(1)

[a]Numerator = number of plants infected; denominator = number of plants inoculated.
[b]Data sources:
(1) Bar-Joseph and Loebenstein, Phytopathology 63:716-720.
(3) Bar-Joseph, Raccah, and Loebenstein. Proc. Int. Soc. Citriculture 3:(In press).
(9) Raccah, Loebenstein, Bar-Joseph, and Oren. 1976. Pages 47-49 *in* E. C. Calavan, ed. Proc. 7th Conf. Int. Org. Citrus Virologists, Univ. Calif. Riverside.
... Bar-Joseph, 1972, *unpublished.*

00032-949X/78/000 196$03.00/0

TABLE 2. Transmission rates of a transmissible citrus tristeza virus (CTV) isolate (VT) following cross protection with a low-transmissible CTV isolate (ST)

Primary or protective strain	Challenge strain[a]	Days after inoculation with: Primary or protective strain	Challenge strain[a]	Infection rate[b] Ratio	(%)
ST	VT	210	39	0/9	0
ST	VT	120	39	2/39	5.1
ST	VT	120	64	3/64	4.7
ST	...	90	...	2/27	7.4
ST	...	159	...	2/27	7.4
VT	...	55	...	7/19	36.8
VT	...	105	...	15/40	37.5

[a]Three-dot leader (...) indicates no challenge strain inoculum was introduced.

[b]Numerator = number of plants infected; denominator = number of plants inoculated. Seedlings of Mme. Vinous sweet orange were graft-inoculated with primary or protective strains, the inoculated seedlings were pruned and graft-inoculated with the challenge strain on the new branches. Aphid transmission was performed according to Bar-Joseph and Loebenstein (1973).

in experimental cross-protection tests; *Citrus sinensis* Osb. 'Mme. Vinous' sweet orange seedlings protected with the ST isolate, which has a low transmission rate by *A. gossypii*, were challenge-inoculated with the highly transmissible VT isolate of CTV. The presence of the low-transmissible isolate suppressed the transmission of the VT isolate. Tests for seedling yellows (SY) reaction indicated that only the ST ordinary strain and not VT (the SY isolate) was transmitted from the ST/VT cross protected combination. Based on these considerations, it is suggested that under certain conditions cross protection fails or is incomplete and transmissible mutants are acquired by *A. gossypii* and transferred to healthy trees. These newly infected trees will carry the readily transmissible isolates and will serve as sources for natural spread.

The factors responsible for cross protection failure or incompleteness among CTV isolates (12) are not known. In most citrus-growing areas in which *T. citricidus* was not present (e.g. California, Florida, Spain, and Israel) an average interval of over 30 yr existed between the introduction of CTV-infected budwood and the appearance of natural spread (7, 11). This suggested a possible relation between cross protection stability and the aging of the infected trees. The physiological conditions that affect cross-protection stability have not been examined. Probably CTV, a phloem-restricted virus (8), is transported in large amounts to new tissues only in young trees, whereas in older trees less virus is transported. Transportation of smaller amounts of virus might increase the likelihood of some cells and certain plant tissues containing only the transmissible mutant. These cells and tissues probably served as the original source from which *A. gossypii* acquired CTV and effected its natural spread.

Epidemiology is still more readily applied to discerning the patterns of diseases as they have occurred in the past, rather than to predicting future trends. Analysis of the change in CTV behavior from the nonspreading phase to the epidemic phase in Israel implies that for certain other viruses which have been transferred without their efficient vector into new areas, mutants may arise that are transmissible by insects prevalent in those areas and that infection caused by those mutants might develop to epidemic proportions after a prolonged lag period.

LITERATURE CITED

1. BAR-JOSEPH, M., and G. LOEBENSTEIN. 1973. Effects of strains, source plant, and temperature on the transmissibility of citrus tristeza virus by the melon aphid. Phytopathology 63:716-720.
2. BAR-JOSEPH, M., G. LOEBENSTEIN, and Y. OREN. 1974. Use of electron microscopy in an eradication program of new tristeza sources, recently found in Israel. Pages 83-85 *in* L. G. Weathers, and M. Cohen, eds. Proc. 6th Conf. Int. Org. Citrus Virol., Univ. California, Div. Agric. Sci., Berkeley. 232 p.
3. BAR-JOSEPH, M., B. RACCAH, and G. LOEBENSTEIN. 1978. Evaluation of the main variables that affect citrus tristeza transmission by aphids. Proc. Int. Soc. Citriculture 3:(In press).
4. DEAN, H. A., and E. O. OLSON. 1956. Preliminary studies to determine possibility of insect transmission of tristeza virus in Texas. J. Rio Grande Valley Hortic. Soc. 10:25-30.
5. DICKSON, C. R., and R. A. FLOCK. 1959. Insect vectors of tristeza virus. Pages 97-99 *in*: J. M. Wallace, ed. Citrus virus diseases. Univ. Calif., Div. Agric. Sci., Berkeley. 243 p.
6. HARPAZ, I. 1964. Inconsistency in the vector relations of the citrus tristeza virus. Riv. Patol. Veg. 4:549-558.
7. MARTI-FABRÉQAT, F. 1975. The tristeza disease of citrus. Pages 51-54 *in* Ciba-Geigy, Citrus Tech. Monogr. 4. Basle, Switzerland. 88 p.
8. PRICE, W. C. 1970. Citrus tristeza virus. Commonw. Mycol. Inst., Assoc. Appl. Biologists. Descriptions of Plant Viruses No. 33. (Unpaged).
9. RACCAH, B., G. LOEBENSTEIN, M. BAR-JOSEPH, and Y. OREN. 1976. Transmission of citrus tristeza virus by aphids prevalent on citrus, and operation of the tristeza suppression programme in Israel. Pages 47-49 *in* E. C. Calavan, ed. Proc. 7th Conf. Int. Org. Citrus Virol., Univ. California, Riverside. 227 p.
10. REICHERT, I., and A. BENTAL. 1960 . Citrus varieties in Israel infected with tristeza. Ktavim 10:53-58.
11. ROISTACHER, C. N. 1976 . Tristeza in the Central Valley: a warning. Calif. Citrog. 62:16-23.
12. WALLACE, J. M., and R. J. DRAKE. 1972. Use of seedlings-yellows recovery and protection phenomena in producing tristeza-tolerant, susceptible, scion-rootstock combinations. Pages 137-143 *in* W. C. Price, ed. Proc. 5th Conf. Int. Org. Citrus Virol. Univ. Florida, Gainesville. Univ. Fla. Press, Gainesville. 301 p.