Transmission of Tristeza and Seedling Yellows Tristeza Virus by Small Populations of *Aphis gossypii*

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ABSTRACT

Roistacher, C. N., Bar-Joseph, M., and Gumpf, D. J. 1984. Transmission of tristeza and seedling yellows tristeza virus by small populations of *Aphis gossypii*. Plant Disease 68:494-496.

Transmission studies with three isolates of citrus tristeza virus, using 1, 3, 9, and 27 individuals of *Aphis gossypii*, showed a clear dosage response to infection. Infection was 1.1, 6.7, 23.3, and 56.7% for 1, 3, 9, and 27 aphids applied, respectively. These results are strikingly similar to earlier studies in South America using *Toxoptera citricida*. Extrapolation of the infection curve indicates that 100% infection would be expected if about 52 aphids were applied and a minimum of 33 living aphids were found after a 24-hr feeding period at 24 C. These results show a very high level of efficiency for *A. gossypii* in transmitting certain strains of tristeza virus in California.

A change in transmissibility of citrus tristeza virus (CTV) vectored by *Aphis gossypii* Glover has been observed in recent years in several citrus-growing areas of the world (1,3,16). In the early 1950s, Dickson et al (7) estimated that in California, 5,600 aphids would be required to cause a single infection of a citrus tree under field conditions. Under controlled conditions in 761 transmission tests to individual plants, they reported low rates of transmission of 2.1, 2.4, and 6.3%, respectively, when an average of 21, 50, and 221 aphids were used per test. Similar low transmissions were obtained in Florida (9). In 1970, a rapid and destructive spread of tristeza was observed in the Sharon Plains region of Israel. Bar-Joseph and Loebenstein (1), using air-conditioned chambers held at 25 ± 2 C, reported that the Sharon Plains isolate transmitted to 40% of the indicator test plants using 100 aphids per test, whereas two other isolates from different regions transmitted to fewer than 5% of the test plants. Recently, Roistacher et al (16) and Roistacher (12) showed that many isolates of CTV and seedling yellows tristeza virus (CTV-SY) collected from field trees at the University of California Citrus Research Station at Riverside would transmit to 100% of the indicator test plants by *A. gossypii* using 50–200 aphids per test.

Previous transmission tests using small populations of *Toxoptera citricida* Kirkaldy were contradictory relative to the ability of individual aphids to transmit CTV. Thus, although Costa and Grant (5) reported a relatively high transmission (9/55 or 17% of the test plants infected) using single *T. citricida*, later studies by Costa et al (6) showed no infection (0/30) by single *T. citricida*. Many recently discovered California CTV isolates have been shown to transmit to 100% of the test plants using 200 or fewer *A. gossypii* per test. Aphid transmission studies with three of these isolates present, respectively, in three acquisition hosts, ie, sweet orange (SwO), (*Citrus sinensis* (L.) Osbeck), grapefruit (Gft), (*C. paradisi* Macf.), and lemon (*C. limon* (L.) Burm.) showed that transmission varied not only with the host but also with the severity of the isolate (14).

This paper examines the relationship between small aphid populations and the infection of Mexican lime indicator plants with the same three CTV isolates used previously (14). Results show a clear dosage effect between the numbers of aphids fed on test plants and CTV transmission.

MATERIALS AND METHODS

Three virus isolates with previous histories of infecting 100% of Mexican lime test plants by *A. gossypii* transmission (15,16) and which all induced typical severe stem pitting and leaf symptoms of tristeza in Mexican lime seedlings were used in this study. The isolates are designated CTV-514, CTV-SY-563, and CTV-SY-568. CTV-514 was isolated from a naturally infected Valencia orange tree in central California and produced a mild yellows reaction in Gft seedlings and no seedling yellows reaction in sour orange or lemon seedlings. It was classified as a typical tristeza isolate. Isolate CTV-SY-563 was obtained from a Brazil navel orange tree found in the citrus variety collection at the University of California at Riverside, CRC 597. Previous indexing (15) revealed that budwood from this tree induced very severe yellows and stunting in seedlings of Gft, sour orange, and lemon. Grapefruit seedlings were also severely pitted, whereas Madam Vinous SwO seedlings were only mildly pitted. CTV-SY-568 was isolated from a badly stunted and pitted Minneola tangelo tree and was found to have a very severe form of CTV-SY (4,13). Budwood indexed from this tree induced a very severe seedling yellows reaction in Gft, sour orange, and lemon, as well as severe stunting and vein corking of leaves and extreme stem pitting in Madam Vinous SwO seedlings. Navel and Valencia orange stems are also severely pitted by this isolate. The latter two isolates are classified as seedling yellows, with CTV-568 being an exceptionally severe isolate (13).

The three isolates were all maintained in plants of Madam Vinous SwO, which when indexed were found negative for other citrus viruses and viruslike pathogens.

In these experiments, 360 transmission tests were made using all permutations of four aphid populations and three tristeza isolates with 30 replicates. Aphids were reared on muskmelon (*Cucumis melo* L. 'PMR45') and transferred to the young growing leaves of virus-infected SwO seedlings for an acquisition feeding period of 24 hr at 24 C. Aphids were selected regardless of instar development. Our own observations, based on hundreds of tests, and the results of Norman and Sutton (10) and Raccah et al (11) showed no differences in the transmission of CTV by mature or immature *A. gossypii*. The technique used for transmission was similar to that illustrated by Roistacher (12). Young leaves of the acquisition host containing 1, 3, 9, or 27 apterous feeding aphids were cut into small segments holding the correct number of aphids. These were pinned to a young leaf of Mexican lime using a thorn as shown in Figure 1. Aphids were observed to move readily from the cut leaf segment to the leaf of Mexican lime. Plants were caged and placed in a controlled-temperature cabinet for an inoculation feeding period of 24 hr at 24 C, then the living aphids were counted with the aid of a 10-power Opti-visor binocular head lens. The aphids were then sprayed with insecticide and the Mexican lime indicator plants were held in the glasshouse at temperatures

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of 26/19 C (daytime maximum/nighttime minimum) for a minimum period of 3 mo for development of tristeza symptoms.

RESULTS AND DISCUSSION

Table 1 shows the results of aphid survival and transmission rates for the three tristeza isolates and the four aphid populations tested. Statistical analysis showed no significant difference in transmission to Mexican lime among the three isolates; all behaved similarly when aphid populations and infectivities were compared. The percentages of aphids found living after feeding periods of 24 hr at 24 C were 27, 46, 44, and 68 when 1, 3, 9, and 27 aphids were applied, respectively. These percentages were calculated from the combined results for the three virus isolates.

When results for the three isolates were combined (90 plants per aphid population), the percent transmission using 1, 3, 9, and 27 aphids was 1.1, 6.7, 23.3, and 56.7, respectively. These results compare rather closely with the results of Costa et al (6), where 1, 3, 9, and 27 aphids of *T. citricida* in 30 tests each resulted in 0, 10, 30, and 50% transmission, respectively.

Figure 2 shows the relationship among the number of aphids applied, the number of aphids found after 24 hr, and the percentage of test plants infected. A clear dose response of CTV-infected plants to the number of aphids feeding on test plants was found. Extrapolation of the infection curve indicates that 100% infection of test plants would be expected if about 52 aphids were applied and a minimum of 33 living aphids were found on the test plants after 24 hr of feeding. This extrapolation fits well with actual findings of 95.3, 100, and 100% infection of test plants with average aphid counts of 33, 46, and 49, respectively (14), using these same three isolates. In another report (16) representing 120 individual tests using six other isolates of CTV and CTV-SY, 100% infection was obtained where an average of 52.1 aphids were found per test.

There was little or no difference in transmissibility among virus isolates using low populations of *A. gossypii* in these studies. Previous studies using higher aphid populations showed 99–100% transmission for these same three isolates vectored from SwO acquisition hosts to Mexican lime indicator hosts (14). In that same study, however, these three isolates were found to be variably transmitted by *A. gossypii*, depending on the acquisition host (SwO, Gft, or lemon) and the receptor host (Mexican lime, Gft, or lemon). For example, transmission of the severest isolate CTV-SY-568 from SwO to Gft was 92% but only 14% for the milder isolate CTV-514. Transmissibility to Gft increased markedly with increased virulence of the virus. Transmission from Gft and lemon to Gft and lemon was generally poor.

Our results indicate that low populations of *A. gossypii* can readily transmit certain isolates of tristeza found in California, and in one case, a single aphid was capable of transmitting the virus (Table 1, isolate CTV-SY-568). This is in marked contrast to the poor transmission reported for *A. gossypii* in previous years in California (7), in Florida (9), and for certain isolates in Israel (1). This study demonstrates that *A. gossypii* is a relatively efficient vector for certain CTV isolates presently found in California and that these isolates appear to have been transmitted as efficiently by *A. gossypii* as the CTV or CTV-SY isolates were transmitted by *T. citricida* in Brazil. Despite the similarity in transmission efficiency, *T. citricida*, wherever present, is a very dominant citrus aphid species; it builds up large populations, is capable of rapid movement (8), and is adaptable to a wide range of temperatures. *A. gossypii* represents only 4% of the aphid population on citrus in California (7) but is the primary vector for tristeza in that state and is present in very low

Table 1. Survival rates and transmission efficiency of small populations of *A. gossypii* in tests with three isolates of tristeza from California

Total aphids applied per plant	Virus source	Total aphids found (30 plants)	No. of infected plants (30 plants)	Percent infection for three isolates combined (90 plants)
1	T-514	5	0	
	SY-567	13	0	
	SY-568	6	1	1.1
3	T-514	33	4	
	SY-567	50	1	
	SY-568	41	1	6.7
9	T-514	83	6	
	SY-567	147	7	
	SY-568	126	8	23.3
27	T-514	570	19	
	SY-567	580	19	
	SY-568	506	13	56.7

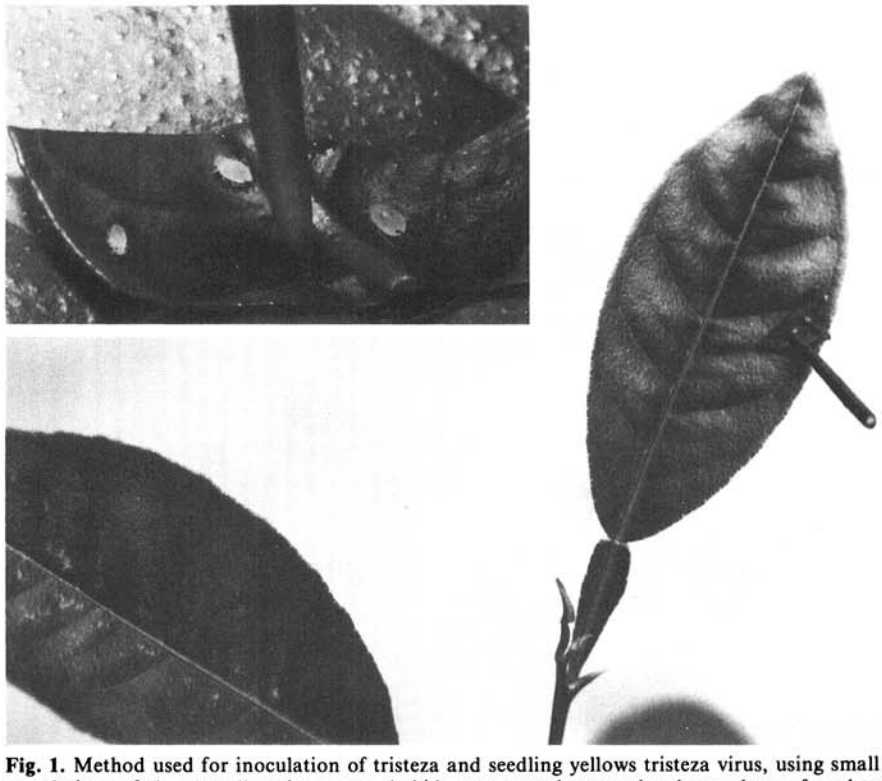

Fig. 1. Method used for inoculation of tristeza and seedling yellows tristeza virus, using small populations of *A. gossypii* as the vector. Aphids were reared on muskmelon and transferred to virus-infected young sweet orange leaves. A small leaf segment of sweet orange containing 1, 3, 9, or 27 aphids was cut out and pinned to the indicator host (Mexican lime) as illustrated. Aphids were observed to move rapidly from the leaf segment to the indicator plant.

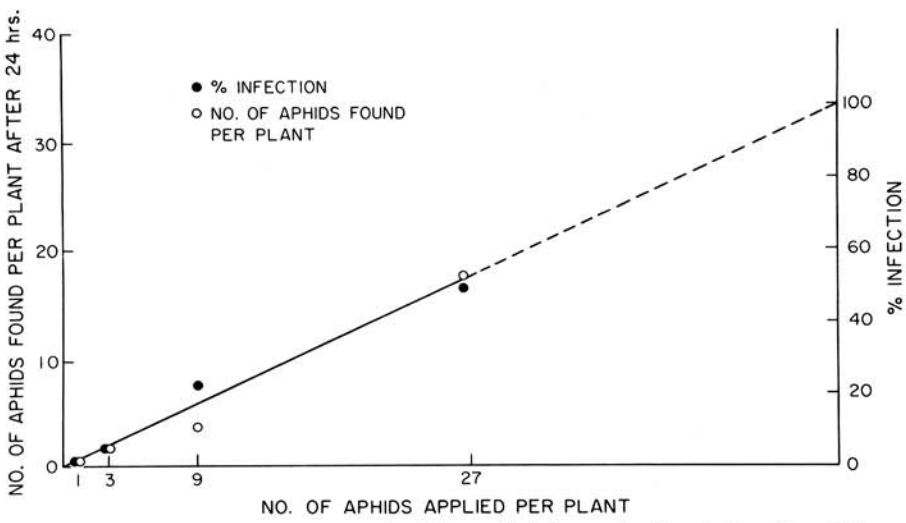

Fig. 2. Relationship among the number of aphids applied, the number found alive after a 24-hr feeding period on young leaves of Mexican lime at 24 C, and the percentage of lime plants found infected by tristeza.

populations in the desert and during the warmer summer months in central California.

These studies indicate that very small populations of *A. gossypii* could pose a significant threat to citrus production in areas where highly transmissible isolates of CTV occur. Also, the recent development of increasing transmissibility of severe CTV-SY isolates by *A. gossypii* (2,16) indicates a serious threat to areas of the world where *A. gossypii* is the primary vector of CTV and where destructive tristeza isolates remain undetected as potential reservoir sources.

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