Transmission of Citrus Tristeza Virus by the Melon Aphid

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ABSTRACT

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The VT isolate of citrus tristeza virus was transmitted efficiently by ten aphids (36%), and even with five aphids per test plant substantial transmissions (11%) were obtained. No transmission was obtained from acquisition or infection feeding periods of 5 minutes, and about 6 hours for each period was required to obtain near-maximal transmissions. Melon aphids retained inoculativity after 3.5 hours of post-acquisition fasting and when feeding for 2 or 6 hours on an intermediate cucumber plant or lime seedling, respectively. These features are characteristic of a semipersistent mode of transmission.

Citrus tristeza virus (CTV) is spread efficiently in the southern hemisphere by *Toxoptera citricidus* (Kirk), whereas transmission rates by *Aphis gossypii* (Glov.) and *A. spiraecola* Patch. – prevalent in California and Florida – are significantly lower (3, 4, 7).

Natural spread of CTV in Israel first was observed in 1970 in one area in the Sharon plain (2). However, the disease had been found since 1956, mainly in introduction plots, but without any indication of natural spread of the virus (13). Subsequently, it was shown that the tristeza strain-VT, from the area where natural spread was observed, is efficiently transmitted by the melon aphid, *A. gossypii* (1). Transmission rates averaging 40% were obtained, compared with less than 5% with two other isolates.

This paper is a report on transmission features of the VT strain by *A. gossypii*.

MATERIALS AND METHODS

In general, experimental procedures followed those described previously (1).

The VT isolate originated from a naturally infected sweet orange cultivar Valencia. Seedlings of sweet orange cultivar Mme. Vinous were graft-inoculated and used for aphid (acquisition) feeding after 2 to 12 months. Infectivity was tested on 1- to 2-month-old and 5- to 10-cm-tall Egyptian sour lime seedlings.

Melon aphids were reared on *Cucumis sativus* L. 'Bet Alpha' cucumber plants in screened cages. Density of aphid populations on source plants was adjusted to maintain a high proportion of apterae. Alatae were collected from time to time to verify their identity.

Transmission tests were done in an air-conditioned plastic chamber at 25 ± 2 C. Cucumber leaves infested with aphids were transferred into a plastic cage (1) and the cage was placed on the tristeza-infected source. After various periods of access to the virus source plant (acquisition), apterous aphids were transferred, with a camel's hair brush, to test plants. During infection feeding, test plants were kept isolated under cages made from 30-ml plastic tubes. The cages were subsequently removed and plants were sprayed with an aphicide. Observations for symptoms started after 20 days and continued for a period of 3 months.

RESULTS

Preliminary experiments showed that both mature and immature alatae aphids transmitted the VT strain to a degree similar to mature apterous aphids.

Effect of numbers of aphids on transmission rates.—Mature apterous melon aphids were placed for 24 hours on virus source plants. Subsequently, various numbers of feeding aphids were collected and placed on test plants for 24 hours. Transmission rates of 1/200 (0.5%), 8/69 (11.6%), 35/96 (36.5%), and 69/118 (58.5%) were obtained with 1, 5, 10, and 100 aphids per test plant, respectively.

Acquisition and infection periods.—Groups of 10 aphids were used for each test. When various acquisition periods were evaluated, aphids were left for 24 hours of infection feeding on test plants, whereas when infection periods varied, acquisition periods were 24 hours. No transmission was obtained in 20 trials with 5 minutes of acquisition feeding, compared to transmission rates of 1/24 (4.2%), 2/30 (6.7%), 6/30 (20%), 31/85 (36.5%), and 6/20 (30%) following acquisition periods 0.5, 2, 6, 24, and 120 hours, respectively.

No transmission was obtained in 26 and 30 trials following infection periods of 5 and 30 minutes, respectively. Transmission rates reached 1/33 (3%), 6/45 (13.3%), 4/37 (10.8%), 10/31 (32.2%), and 35/96 (36.5%) following infection periods of 1, 2, 4, 6, and 24 hours, respectively.

Postacquisition fasting.—Following 24-hour

acquisition feedings on infected plants, aphids were placed in a cloth-sealed glass tube. After various periods they were transferred in groups of 10 for a 24-hour infection feeding period on virus-free test plants. Transmission rates of 18/43 (42%), 1/14 (7.1%), and 1/44 (2.3%) were obtained after 3.5, 14, and 24 hours of fasting, respectively. No transmission was obtained in 27 trials following 48 hours of fasting.

Successive transmissions through citrus or cucumber.—Aphids after a 24-hour acquistion period were transferred for various periods of time onto healthy lime seedlings. Then, aphids observed feeding were placed in groups of 25 onto test plants for a 24-hour infection period. Aphids retained their inoculativity while feeding for 1 and 2.5 hours on an intermediate lime seedling, with transmission rates of 8/23 (34.8%) and 4/11 (36.4%), respectively. After 24 hours on that host, inoculativity was lost. With colonies of 10 aphids 2/10 (20%) transmissions were obtained following 6 hours and none after 24 hours of intermediate feeding on lime.

In further experiments, aphids exposed to a 24-hour acquisition period immediately were transferred serially to a succession of healthy lime seedlings. As seen in Table 1, more than 20% transmissions were obtained after 1-hour feeding on one intermediate (Exp. 1), and even after two and three intermediate feedings, aphids still were inoculative (Exp. 2, 3). However, no transmissions were obtained after two passages with infection feeding periods of 3 hours on each intermediate host (Exp. 4).

When groups of 10 viruliferous aphids were placed for 2- or 24-hour feedings on an intermediate cucumber plant and then for a 24-hour infection feeding period on lime seedlings, 2/14 and 0/20 transmissions, respectively, were obtained.

DISCUSSION

The high transmission rates of the VT strain obtained with small numbers of melon aphids were considerably higher than those reported for transmissions with similar or larger numbers of *A. gossypii* in California (5) and Florida (8). The transmission rates of VT by *A. gossypii* approached those reported for *Toxoptera citricidus*, although single aphids of *T. citricidus* transmitted tristeza virus much more efficiently (3). The high transmission rate seems to be an intrinsic property of the VT strain; transmission was poor with other tristeza virus strains (1). This increases the potential of natural spread of tristeza in Israel, although preliminary surveys indicated that melon aphids comprise only 10-20% of the aphid population in our citrus groves and that *A. spiraecola, Myzus persicae,* and *Toxoptera aurantii* did not transmit the VT isolate (12).

Short acquisition and infection periods were not sufficient for transmission of the VT strain by groups of 10 melon aphids, and about 6 hours for each were required for near-maximal transmissions. However, Dickson et al. (5) were able to obtain 3% transmission after only a 5-minute acquisition when larger numbers of aphids were used. The relatively long transmission times required are in accordance with the feeding behavior of the melon aphid and the site of tristeza virus in the plant. Pollard (10) reported that after 42 seconds of probing, the stylets of the melon aphid remained in the epidermis, and 20 minutes were required for penetration into the vascular system. As tristeza virus is present mainly in the phloem (11), it will not be regularly transmitted by *A. gossypii* during short probes. Similarly, with *T. citricidus* Costa and Grant (3) found that a 4-hour feeding period was requisite for maximum transmissions.

Melon aphids remained inoculative after 3.5 hours of postacquisition fasting and after feeding for 2 and 6 hours on an intermediate cucumber or lime seedling, respectively. Some inoculativity was retained even after three 1-hour passages on intermediate lime seedlings. They lost inoculativity almost completely when fasting, and completely when feeding on an intermediate host for 24 hours. Similar results were obtained by Norman et al. (9) with large numbers of melon aphids, inoculativity was lost almost completely when aphids fed for 24 hours on an intermediate lime seedling. Retention of tristeza virus by *T. citricidus* differs markedly, as no noticeable decrease in inoculativity was noted after 24 hours of fasting (6); but even so, in nature, viruliferous aphids probably are able to infect more than one tree.

The relatively long acquisition and infection periods necessary for transmissions of the VT strain by the melon aphid, and the retention of inoculativity during fasting and feeding on an intermediate host, are typical for a semipersistent mode of transmission.

TABLE 1. Transmission of isolate VT of citrus tristeza virus by melon aphids (*Aphis gossypii*) after serial transfers on lime seedlings[a]

Experiment	Transfer	Feeding period (hours)	Transmission[b]
1	1st	1	1/38
	2nd	24	8/38
2	1st	1	0/30
	2nd	1	0/21
	3rd	24	1/20
3	1st	1	0/20
	2nd	1	0/20
	3rd	1	0/20
	4th	24	3/20
4	1st	3	2/25
	2nd	3	1/25
	3rd	14	0/25

[a]Transmissions were via 5-15 aphids per plant following an acquisition period of 24 hours.

[b]Numerator = number of plants infected. Denominator = number of plants inoculated.

LITERATURE CITED

1. BAR-JOSEPH, M., and G. LOEBENSTEIN. 1973. Effects of strain, 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* Proc. 6th Conf. Int. Org. Citrus Virol., University of California, Richmond. 232 p.
3. COSTA, A. S., and T. J. GRANT. 1951. Studies on

transmission of the tristeza virus by the vector, Aphis citricidus. Phytopathology 41:105-113.
4. DICKSON, R. C., R. A. FLOCK, and M. M. JOHNSON. 1951. Insect transmission of citrus quick-decline virus. J. Econ. Entomol. 44:172-176.
5. DICKSON, R. C., M. M. JOHNSON, R. A. FLOCK, and E. F. LAIRD. 1956. Flying aphid populations in southern California citrus groves and their relation to the transmission of the tristeza virus. Phytopathology 46:204-210.
6. MENEGHINI, M. 1948. Experiencias de transmissao doc doenea "tristeza" dos citrus pelo pulgo da laranjera. Biologico 14:115-118.
7. NORMAN, P. A., and T. J. GRANT. 1956. Transmission of tristeza virus by aphids in Florida. Proc. Fla. State Hortic. Soc. 69:38-42.
8. NORMAN, P. A., R. A. SUTTON, and E. A. K. BURDITT. 1968. Factors affecting transmission of tristeza virus by melon aphids. J. Econ. Entomol. 61:238-242.
9. NORMAN, P. A., R. A. SUTTON, and A. G. SELHIME. 1972. Further evidence that tristeza virus is transmitted semipersistently by the melon aphid. J. Econ. Entomol. 65:593-594.
10. POLLARD, D. G. 1958. Feeding of the cotton aphid, Aphis gossypii Glover. Emp. Cotton Grow. Rev. 35:244-253.
11. PRICE, W. C. 1966. Flexuous rods in phloem cells of lime plants infected with citrus tristeza virus. Virology 29:285-294.
12. RACCAH, B., M. BAR-JOSEPH, and G. LOEBENSTEIN. 1975. Attempts to transmit citrus tristeza by aphids prevalent on citrus trees in Israel. Hassadeh 55:1275-1278. (in Hebrew).
13. REICHERT, I., and A. BENTAL. 1960. Citrus varieties in Israel infected with tristeza. Ktavim 10:53-58.