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ABSTRACT. Aphid transmission and natural spread of citrus tristeza virus (CTV) in different citrus receptor hosts were studied under field and laboratory conditions. After 10 years of field exposure, natural infection of 12 citrus cultivars segregated into three groups: 1) highly susceptible varieties (89-100%) which included Alemow, Volkamer lemon, Ridge Pineapple, Orlando tangelo, and rough lemon; 2) varieties with low susceptibility (27-53%) which included Rangpur lime, Cleopatra mandarin, sour orange, and Palestine sweet lime; and 3) resistant varieties (0%) which included Swingle citrumelo, trifoliate orange, and Carrizo citrange. In greenhouse tests, Alemow and Mexican lime seedlings were the best receptor plants for transmission of the T-67 isolate of CTV by *Aphis gossypii* Glover with infection rates of 38 and 31%, respectively, whereas infection rates in Pineapple and Hamlin sweet oranges and Duncan grapefruit were 19, 17, and 11%, respectively. No transmission was obtained to sour orange or Carrizo citrange. *Citrus hystrix* DC, sweet orange varieties, Temple, and grapefruit were good hosts for nymph survival and production of *A. gossypii* and *Aphis citricola* van der Goot in laboratory tests with detached leaves. In comparison, Mexican lime was a poor host while Alemow was a nonhost. Carrizo citrange and Swingle citrumelo were also nonhosts of the aphids. These observations indicated that many varieties susceptible to CTV were good hosts for citrus aphids under test conditions, but vector transmission to different receptor plants was not necessarily correlated to aphid host suitability.

Index words. Citrus rootstocks, aphid vectors, resistance, epidemiology, transmission, host range.

Citrus varieties differ in susceptibility to citrus tristeza virus (CTV). The mechanism of these differences is complex and not well understood. Garnsey et al. (4) have described differences in virus titer of different CTV isolates in different cultivars. Some varieties such as trifoliate orange, Swingle citrumelo, and Carrizo citrange are highly resistant to CTV infection, even by graft transmission, whereas sweet orange varieties support high titers of virus.

Natural spread of CTV is by aphid vectors. The variety of the acquisition host and the variety of the receptor affected CTV transmission under experimental conditions (2, 7, 9). Hence, natural spread of CTV depends on a complex of factors which includes susceptibility to inoculation, virus replication, vector feeding behavior, vector reproduction, and vector movement. The purpose of this study was to gain further insight into these interactions by comparing field spread of CTV into different cultivars with some laboratory tests of vector transmission, and to measure aphid reproduction on different citrus varieties.

METHODS AND MATERIALS

Trees and plot. A 13-row citrus plot was planted in a commercial citrus grove in 1976 in Indiantown, Florida. Ten rows were planted with Valencia orange trees infected with T-26, a mild CTV isolate, on different rootstocks. Greenhouse-grown, 1-year-old seedlings or rooted cuttings of 12 cultivars (Table 1) were planted as tristeza-free trees in rows 1, 8, and 13 in this plot. These trees were rootstock varieties and were planted in a randomized complete block design with six replications and three trees per replication. The entire plot area covered 1.4 ha with 80 trees per row and a tree spacing of 5.2 x 7.6 m. The primary purpose of this plot test was to monitor citrus blight incidence in grafted and nongrafted trees. Since other commercial plantings near this plot were infected with CTV, a survey was conducted of all rootstocks trees in the plot after 10 years of field exposure. These trees provided an opportunity to measure CTV spread in the field under replicated conditions.

CTV assay. Fully expanded young leaves or twigs were harvested from two sides of each field tree in the spring of 1985 and 1986. Bark and/or leaf midribs were excised from these samples and assayed for CTV infection by double antibody sandwich enzyme-linked immunosorbent assay (1) using antisera to whole, unfixed virus particles of CTV isolate T-4 (3).

Vector transmission. Seedlings of nine citrus varieties were grown in the greenhouse. These plants were used as receptor plants when they were ca. 10-20 cm tall. highly transmissible CTV isolate, T-67, was propagated in Madam Vinous sweet orange as the inoculum source for virus acquisition by *Aphis gossypii* Glover (9). Twenty aphids (late instars and adults) were transferred to each receptor plant. The virus acquisition access and the inoculation periods were each 24 h. Donor and receptor plants were maintained in an air-cooled, partly shaded glasshouse. Daily diurnal temperatures fluctuated between 20-32 C.

Aphid host range. Laboratory colonies of the melon aphid were reared on okra, *Hibiscus esculentum* L. var. Clemson spineless, while the spirea aphid, *Aphis citricola* van der Goot, was reared on *C. hystrix* DC. Both aphids were reared in environmental chambers at 25 C with a relative humidity of $80 \pm 10\%$ and a 16 h photoperiod at 5000 foot candles. Two reproductively mature apterous aphids were transferred from the colony to detached leaves of each cultivar on 1% water agar, as described by Yokomi and Gottwald (10). There were 10 replications and the test was repeated twice. Host suitability was evaluated by counting the number of live nymphs on each detached leaf at day 7. This value was called the reproductive rate. Because host preconditioning was not included prior to the test, the early reproductive rate was often influenced by the colony host. After 7 days, however, the number of live nymphs was indicative of the host suitability of the aphid. Statistical analysis was conducted by SAS (Raleigh, NC).

RESULTS

Field spread. After 10 years of field exposure to endemic and field sources of CTV and natural populations of aphid vectors, sufficient field spread had occurred to segregate the citrus varieties into three categories based on number or percentage of trees infected (Table 1). The first group was highly susceptible to CTV with infection percentages ranging from 89 to 100%. This group included Ridge Pineapple (100%), Alemow (100%), Volkamer lemon (100%), Orlando tangelo (94%), and rough lemon (89%). The second group was rather poorly susceptible to CTV with infection ranging from 27 to 53%. This group included Rangpur lime (53%), Cleopatra mandarin (50%), sour orange (52%), and Palestine sweet lime (27%). The last group, which included Carrizo citrange, remained free of CTV.

TABLE 1
NATURAL INFECTION BY CITRUS TRISTEZA VIRUS OF CITRUS CULTIVARS ATER 10 YEARS IN A COMMERCIAL GROVE IN INDIANTOWN, FLORIDA[z]

Cultivar	No. infected[y] no. tested	% Infected
Alemow	18/18	100
Volkamer lemon	17/17	100
Ridge Pineapple	2/2	100
Orlando tangelo	17/18	94
Rough lemon	16/18	89
Rangpur lime	8/15	53
Cleopatra mandarin	9/18	50
Sour orange	9/17	52
Palestine sweet lime	4/15	27
Swingle citrumelo	0/18	0
Trifoliate orange	0/18	0
Carrizo citrange	0/17	0

[z]Trees were planted in November 1976, with a row spacing of 5.2 x 7.6 m.

[y]CTV infection was determined by double antibody sandwich enzyme-linked immunosorbent assay. Total trees/variety was 18. Tree deaths due to freezes or *Phytophthora* foot rot accounted for tree counts less than 18/variety.

Vector transmission tests. Influence of host receptor variety on *A. gossypii* transmission of T-67 was determined under laboratory conditions. Infection rates in Mexican lime and Alemow were the highest at 31% and 38%, respectively (Table 2). Limited tests with pummelo and rough lemon receptors resulted in transmission rates of 30 and 20%, respectively. Rates for Pineapple and Hamlin sweet oranges were 19 and 17%, respectively. Infection in grapefruit was 11%, whereas no transmission to sour orange or Carrizo citrange was detected. Four hosts were common to the field test described above.

Aphid reproduction. Nymph production of *A. gossypii* under test conditions was generally lower than that of *A. citricola* on comparable hosts. *C. hystrix* was the best host for the melon aphid with 37.3 nymphs per adult ($P < .05$), whereas Valencia and Temple were the next best hosts at 15.9 and 15.7 nymphs per adult, respectively (Table 3). Some reproduction (4-5 nymphs) occurred on Pineapple and navel oranges, and on Mexican lime, but this group was not statistically different from nonhosts (0 to 1.5 nymphs). For spirea aphid, the highest reproduction was 25.6 nymphs on Valencia followed by *C. hystrix*, navel, and Temple with 20.9, 18.5, and 16.9 nymphs, respectively. Pineapple orange and Orlando tangelo were fair hosts along with Marsh grapefruit and Mexican lime (5-8 nymphs). However, the latter two hosts and the nonhosts (1.8 or less) were in the same statistical group. The nonhosts for both aphids in this test included trifoliate orange, Volkamer lemon, Cleopatra mandarin, rough lemon, Palestine sweet lime, Carrizo citrange, sour orange, alemow, and Swingle citrumelo.

TABLE 2
APHID TRANSMISSION OF CTV TO DIFFERENT RECEPTOR CULTIVARS OF CITRUS IN THE GREENHOUSE[z]

Receptor variety	No. positive[y]/ no. tested	% transmission
Alemow	37/97	38
Mexican lime	48/153	31
Pummelo	3/10	30
Rough lemon	2/10	20
Pineapple	15/81	19
Hamlin	6/35	17
Duncan grapefruit	8/70	11
Sour orange	0/25	0
Carrizo	0/25	0

[z]Vector = 20 *Aphis gossyii*/receptor plant; the CTV isolate was T-67. Data from several experiments were combined and account for the different numbers of replications/variety.
[y]Infection identified by symptom expression or by enzyme-linked immunosorbent assay.

TABLE 3
REPRODUCTION OF APHIDS ON DETACHED LEAVES OF DIFFERENT CITRUS CULTIVARS[z]

Host	Avg. no. nymphs/aphid[y] *Aphis gossypii*	*Aphis citricola*
Hystrix	37.3a[x]	20.9ab
Valencia	15.9 b	25.6a
Temple	15.7 b	16.9 b
Marsh	9.1 c	5.6 cd
Pineapple	5.1 cd	8.8 c
Navel	4.8 cd	18.5 b
Mexican lime	4.6 cd	5.6 cd
Orlando tangelo	1.5 d	8.5 c
Trifoliate orange	0.6 d	0.3 d
Volkamer lemon	0.6 d	0.1 d
Rangpur lime	0.4 d	1.8 d
Cleopatra mandarin	0.4 d	0.2 d
Rough lemon	0.3 d	0.2 d
Palestine sweet lime	0.1 d	0.2 d
Carrizo citrange	0.1 d	0.1 d
Sour orange	0.1 d	0.1 d
Alemow	0.1 d	0.1 d
Swingle citrumelo	0.0 d	0.9 d

[z]Leaves incubated on 1% water agar for 7 days in environmental chamber set for 25 C and 16 h photoperiod at 5000 foot candles (10).
[y]Two reproductively mature adult aphids were placed on each detached leaf on day 0. The data represent the averages of two separate tests per aphid host after 7 days. Each test had 10 replications.
[x]Means associated with the same letter are not statistically different ($P < .05$) by Duncan's multiple range test.

DISCUSSION

The CTV-infected Valencia orange trees adjacent to the seedling trees provided a good source of virus for natural spread of tristeza (2, 7, 9). Periodic field observations indicated both melon and spirea aphids were present in the Indiantown grove. Earlier vector tests had indicated that the T-26 isolate of CTV was not as readily transmissible as some other Florida isolates tested (9). In fact, some of the field-infected Alemow had stem pitting symptoms which are indicative of infection by a more severe tristeza isolate. Because T-26 does not induce stem pitting in Alemow (4) and CTV infection is so prevalent in Florida sweet oranges (5), it is believed that much of the CTV infection in the plot was from endemic sources rather than from the T-26-infected trees in the plot.

The susceptibility to natural CTV infection in the cultivars compared favorably with CTV susceptibility by graft inoculation. As expected, known CTV-resistant cultivars such as Carrizo, Swingle, and trifoliate orange remained free of CTV infection, while cultivars that were good replicative hosts of CTV, such as sweet orange varieties, became naturally infected with tristeza. The results of CTV transmission in the laboratory to different citrus receptor hosts generally corroborated infection observed in the field. Alemow and Mexican lime were the best receptor hosts. Sweet oranges and grapefruit were also receptors, but showed a lower infection rate. The lack of laboratory transmission to sour orange receptors correlated to the low rate of field infections observed in this cultivar after 10 years of field exposure. CTV problems on sour-rooted citrus are due to CTV infection to the sweet orange or grapefruit scion. Risk of CTV infection to nursery liners (seedling rootstocks before budding) in the field should be low for all CTV-resistant hosts and all hosts which were in the low infection group in our study.

The detached-leaf host range tests indicated that the good CTV receptor hosts Mexican lime and Alemow were poor or nonhosts for reproduction of the melon and the spirea aphids. Limited tests with rough lemon also showed lack of aphid host suitability coupled with good CTV transmission. Sweet oranges and grapefruit were good reproductive hosts for aphids, but were not as good receptors as the previous three. Roistacher et al. (8) reported preferential feeding by the melon aphid for several citrus varieties, which was associated with CTV and CTV-seedling yellows vector transmission to these hosts (7). The reproductive efficiency of an aphid on a citrus cultivar is not a measure of infection susceptibility, but may be important when citrus is the primary source for generating vector populations.

The high degree of aphid host suitability of Temple orange observed in our test was noteworthy. Outbreaks of tristeza decline in Florida have sometimes been discovered in proximity to Temple groves. Pelosi et al. (6) have suggested that this may be due to the attraction of aphids to the frequent flushes of growth in Temple orange groves. Our data support their suggestion that large aphid populations could build up in these groves which are also excellent hosts for CTV with subsequent spread of CTV if migration to other groves occurs. In contrast, the epidemiological importance of Mexican lime, an excellent CTV host, depends on the abundance of transient aphid vectors because the reproductive rate of the citrus aphids tested was low in relation to the other citrus varieties. However, the high rate of CTV vector transmission to Mexican lime would further complicate the epidemiology.

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