TRANSMISSION OF TRISTEZA VIRUS BY APHIDS IN FLORIDA

PAUL A. NORMAN
Entomology Research Branch
Theodore J. Grant
Horticultural Crops Research Branch
Agricultural Research Service
U. S. Department of Agriculture
Orlando

The mild tristeza virus was transmitted from Temple orange trees to Key lime test plants by two species of aphids in preliminary experimental work (16). The green citrus aphid (Aphis spiraeaeola Patch) gave positive transmissions to 9 of 128 test plants and the melon aphid (A. gossypii Glover) to 1 of 26 plants. Higher ratios of infection have been obtained in recent tests with the combined use of controlled sources of inoculum in several varieties of citrus seedlings, multiple-branched Key lime test plants, larger numbers of aphids per plant, and timely observations to detect initial symptoms. This report presents results obtained with this improved technique. It incriminates the black citrus aphid (Toxoptera aurantii (Fonsc.) ) as a vector, and describes tests with other insects and mites, so far negative, as vectors. Studies of Meyer lemon trees as sources of inoculum are also discussed.

METHODS

In order to establish tristeza virus in plants of different citrus varieties, two Key lime plants (T₁ and T₂) were selected as standard sources of the virus inoculum. These plants had been infected in March 1953 as a result of aphid transmissions from a stunted Temple orange tree on a red lime (Rangpur type) rootstock (16). Green citrus aphids were transferred to these plants after they had fed on the Temple orange for 116 hours, 75 to the T₁ plant for a 1-hour transmission feeding period and 30 to the T₂ plant for 23 hours’ feeding. Both these Key lime plants have been used in other pathological investigations (8) and the reactions on the Key lime are considered typical of the mild tristeza virus in Florida.

Leaf pieces from the T₁ and T₂ sources were used to inoculate greenhouse-grown citrus seedlings. The Valencia and Florida sweet seedlings were considered to be nucellar, and the Temple oranges were sexual seedlings selected for characteristics of the parent variety. Presence of the tristeza virus in these plants was confirmed by retransmission with leaf-piece transfers to Key lime plants. The infected Valencia and Florida sweet seedlings were transplanted to a field and the infected Temple orange plants were kept in pots in a screen-house. Individual plants were rechecked by leaf-piece inoculations into Key lime plants for proof of continued presence of the virus in the young growth at the time of each acquisition feeding by aphids.

In the previous tests (16), in the present tests with the black citrus aphid, and in studies of Meyer lemon as a source of virus, small Key lime plants 8 to 12 inches high with single stems and 25 to 150 aphids were employed. In the other tests healthy Key limes 18 to 20 inches high were cut back or the tops bent over to stimulate rebranching, and colonies of 300 to 700 aphids were used.

Pathological investigations had indicated that the optimum time to observe initial symptoms of vein clearing associated with the mild tristeza virus was 20 to 40 days following tissue inoculation. In insect-inoculated Key lime plants 30 to 60 days following infestation was found to be the optimum period. Thereafter the symptoms might diminish, especially under summer conditions in the greenhouse. Initial symptoms did not always occur on all branches. The branches showing symptoms were tagged so that they could be observed frequently and used for testing retransmission by means of leaf inoculations into Key lime plants.

Isolated aphid colonies of single species were placed on young, succulent growth of healthy citrus seedlings and allowed to feed for 24 hours, since previous tests with other species (8, 5) had indicated that such feeding would free them of tristeza virus. The young shoots with the aphids were then trans-
ferred to the infected seedlings that had been previously tissue-inoculated and tested, and the aphids were allowed to move over voluntarily. After a 25-hour period to acquire the virus, aphids on shoots from the infected seedlings were placed on the multiple-branched Key lime test plants in separate cages at the laboratory. Again the aphids were allowed to move over voluntarily. At the end of 24 hours counts per unit of leaf area were used as a basis for estimating the total number of aphids present on each test plant. Representative aphid specimens were collected for positive identification. The test plants were sprayed twice with 0.04 percent nicotine sulfate before they were transferred to the greenhouse.

Tests With Green Citrus and Melon Aphids

The results given in table 1, from tests carried out in March and April 1956, show that the green citrus aphid transmitted the virus from three varieties of infected citrus seedlings to Key lime plants. All test plants infested with melon aphids became infected. The proportion of successful transmissions by both species was much higher than in the previous tests (16).

<table>
<thead>
<tr>
<th>Source of Inoculum</th>
<th>Number of Aphids per Test Plant</th>
<th>Number of Test Plants Infected</th>
<th>Infected</th>
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</thead>
<tbody>
<tr>
<td>Green citrus aphid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valencia</td>
<td>300</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Florida sweet</td>
<td>400</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Temple</td>
<td>300</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Temple</td>
<td>400</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Melon aphid</td>
<td>700</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

In these tests initial symptoms of tristeza were detected on one or more branches of the test plants 5 to 6 and, in one case, 8 weeks after inoculation. New young leaves of infected branches showed distinct vein clearing and a veinlet pattern that frequently faded as the growth matured. After the initial vein-clearing symptoms disappeared, some leaf cupping and deficiency signs remained. Presence of the virus in all aphid-infected plants was confirmed by tissue transmissions to additional Key lime plants.

Tests With the Black Citrus Aphid

One positive transmission of tristeza virus was obtained in five tests with the black citrus aphid. In this test 25 alate adults and nymphs, all reared from one adult, were given an acquisition feeding period of 48 hours on an infected Valencia orange scion grown on a potted Key lime rootstock in the greenhouse. The transmission feeding period was 4 hours. Presence of the virus in the aphid-infected Key lime test plant was confirmed by leaf-tissue transfers. The identity of the aphid species was confirmed by Louise M. Russell, of the Entomology Research Branch. This is the first record of positive transmission of tristeza virus by this species.

Meyer Lemon As a Source of Tristeza Virus

Meyer lemon trees are present in dooryards or small plantings in most citrus areas. Some Meyer lemon trees have been found to carry tristeza virus (11, 17, 20), but investigations in Texas (4, 18) indicate that its spread from this host is not common. Because of the wide interest in Meyer lemon as a host, tests were made to transmit the virus from it. Three aphid species were used as vectors. Colonies of 5 to 50 apterous adults were employed. In 16 tests with the black citrus aphid and 21 tests with the melon aphid no transmissions were obtained. In 107 tests where the green citrus aphid was used, 2 transmissions were secured.

In the first positive transmission a Meyer lemon tree at Minneola, Fla., was the source of inoculum. Thirty apterous adult green citrus aphids fed for 42½ hours on this tree and 42 hours on the test plant. Scattered but distinct clearing of veins occurred on the young leaves of the Key lime test plant 5 months later. These symptoms became less evident as the leaves matured, and subsequent new growth showed no further symptoms. While these transmission tests were being carried out, budwood from the Meyer lemon branch that the aphids had fed on was brought to the greenhouse and side-grafted into 5 Key lime plants. All these plants showed strong vein- and veinlet-clearing symptoms, which were evident for a longer period and were more distinct than those observed on the Key lime plant infected as a result of aphid inoculation.
The Meyer lemon scion on one of the graft-inoculated Key lime plants was allowed to develop, and subsequently green citrus aphids were fed on it for 24 hours and then transferred to two Key lime plants for another 24 hours. One plant, on which 75 aphids fed, showed no symptoms, but the other, on which 50 aphids fed, developed transitory leaf symptoms 4 months later. This limited symptom expression of tristeza suggested that either the source of inoculum contained only a very mild tristeza-virus strain or the aphids had sorted out and transmitted only a portion of the virus strain mixture.

In order to obtain further information, leaf-piece transfers and scion grafts were made. The Key limes inoculated with tissue from the Meyer lemon at the end of 2 months showed striking vein- and veinlet-clearing symptoms. The Key limes inoculated with tissues from the aphid-transmitted source showed only slight deficiency symptoms and a tendency for slight cupping of some leaves. Three months after the inoculations observations were made for the presence of stem pits. Two plants tissue-inoculated from the Meyer lemon source had averages of 28 and 100 pits per 10 centimeters of stem; two of three plants tissue-inoculated from the aphid-infected Key lime source had no pits, and one plant had 1 pit per 10 centimeters of stem. These results show that a milder form of tristeza virus was transmitted from the Meyer lemon by the aphids than was transmitted by tissue grafts from the same source.

**Tests With Other Insects and Mites**

Tests were also made with other insects and mites found on citrus in Florida. The sources of inoculum were tristeza-infected Key lime seedlings. Thus far there have been no positive transmissions. The species tested as vectors, with the number of Key lime plants infested, were as follows: green peach aphid (*Myzus persicae* (Sulz.)) 4, citrus mealybug (*Pseudococcus citri* (Risso)) 49, leafhopper *Homalodisca triquetra* (F.) 35, blue sharpshooter leafhopper (*Oncocetopia undata* (F.)) 7, big-footed plant bug (*Acanthocepha-la femorata* (F.)) 14, southern green stink bug (*Nezara viridula* (L.)) 29, stink bug *Euschistus obscurus* (P. de B.) 7, citrus red mite (*Metatetranychus citri* (McG.)) 8.

**Test Plants As a Measure of Virus Transmission**

Tristeza of citrus was first recognized as a disease of sweet orange on sour orange rootstock. This scion-rootstock combination was used in initial studies, which showed that the disease is caused by a virus and can be transmitted by tissue grafts (1, 6) and by *Aphis citricidus* (Kirk) (1, 3, 13, 15). As information advanced, West Indian, Mexican, and Key lime plants were employed as means of detecting this virus (9, 10, 14, 19).

The primary symptoms of vein and veinlet clearing and stem pitting on the Key lime plants are useful. Improvements in the production and detection of symptoms on the test plants have been sought as means of obtaining further information on virus transmission. In the present investigations the use of standardized sources of inoculum, multi-branched Key lime plants, large aphid populations, and observations at critical periods have given high ratios of virus transmission under early-spring conditions. The recovery from initial symptom expression in the summer suggests that the Key lime plants are not as good indicators of tristeza virus under high-temperature conditions. Temperatures appear to affect not only the occurrence of vein clearing on the leaves, but also stem-pitting symptoms, as noted by Grant and Higgins (8).

The intensity of symptoms on the test plants also varies with the virus strain. Recent pathological investigations indicate that the mild tristeza virus in Florida may be a mixture of strains (8). By use of the aphid-transmitted mild-virus source plants T and T, and with leaf-piece transmissions to Key lime plants and successive selections of leaf pieces and transmissions to other Key lime plants, evidence was obtained of virus strains that cause many stem pits and some that cause few to no pits. Apparently the tristeza virus strains could exist in varying mixture levels in infected plants. Work in South Africa (12) and Brazil (7) has shown that aphids have transmitted a mild form of the virus from trees known to be carrying the severe form. In the present study of Meyer lemon as a virus source, the two transmissions obtained by means of aphids produced notably milder symptom expression on Key lime plants than those obtained by tissue transmission.
Transmission of Tristeza Virus in Citrus Groves

The green citrus aphid is the most abundant aphid on Florida citrus. It usually limits its feeding to seasonal growth flushes of succulent terminals which vary with the citrus variety and rainfall conditions, and its feeding curls the tender foliage. The black citrus aphid, appearing later in the season, feeds on more mature leaves. The melon aphid, although less prevalent on citrus than the green citrus aphid, is also found on young growth.

Recent studies in California (5) indicate that in four districts where measurements were taken the yearly average number of aphids of all species flying to a single orange tree ranged from 185,725 in the coastal area to 956,238 in the area around Covina and Azusa called the intermediate district. The respective figures for the melon aphid alone were 3,200 and 35,600. Since the melon aphid is the demonstrated vector of tristeza virus in southern California, it is not surprising that the disease spread most rapidly in the intermediate district. Green citrus aphids made up more than 85 percent of the aphids caught flying to the orange trees, but neither this species nor the black citrus aphid has been shown to carry the tristeza virus in California.

We do not have comparable data for aphid populations in Florida. However, our studies show that all three species are potential vectors of the tristeza virus.

Each tristeza-infected tree serves as a reservoir from which the aphids can obtain the virus. There are two types of reservoirs—(A) an infected tree on a nontolerant rootstock, as sour orange, which shows decline symptoms and produces delayed, weak flushes of new growth; and (B) a tree on a tolerant rootstock which has apparently healthy growth but carries the tristeza virus. The latter is a more dangerous source of the virus, because the succulent flushes of new growth are suitable for aphid feeding and transmission of the virus at the time other normal, healthy trees are flushing. The visibly diseased trees (type A) seem to be less dangerous sources of inoculum because of their 10-day to 2-week delay in producing new flushes of growth that are less vigorous.

In California Dickson et al (5) reported that the rate of spread of tristeza in the groves seldom exceeded two new infections each year from each diseased tree. They noted, however, that the most rapid spread was generally in the intermediate area where most orchards were ruined commercially about five years after the disease was first reported in them. This area had the largest number of flying aphids.

In Florida the visible spread of the disease has been greatest in a Temple orange grove where all trees were reported as being on sour orange rootstock. Actually some were growing on tristeza-tolerant rootstocks and it is believed that these trees have served as more favorable reservoirs of virus for aphid transmission than the visibly diseased trees on sour orange rootstock.

The more infected trees available, the greater is the chance for aphids to acquire the virus and transmit it to other trees. In Florida the number of visibly diseased trees is not always a reliable measure of the number of infected trees, for frequently there are mixtures of rootstocks.

Surveys made by the State Plant Board of Florida (2) show a widely scattered distribution of tristeza-infected trees. These trees serve as sources of virus, and as aphid infestations are not usually controlled by present spraying practices, the number of infected trees in the State may be expected to increase.

Summary

The green citrus aphid was found to transmit the tristeza virus from infected Valencia and Florida sweet seedlings as well as from the Temple orange variety previously reported. The black citrus aphid was shown for the first time to be a vector of the virus. Seven other insects and one mite species did not transmit the virus.

Improved techniques have given high ratios of transmission by the melon and green citrus aphids. The techniques utilize controlled sources of inoculum in several varieties of citrus seedlings, multiple-branched Key lime test plants, 500 to 700 aphids per test, and timely observations to detect initial symptoms.

Transmissions of virus by the green citrus aphid from Meyer lemon produced notably
The burrowing nematode, *Radopholus similis* (Cobb) Thorne, is now known to parasitize more than 125 different species of plants. Some species show no evident effects of the parasite, whereas other species such as rough lemon suffer severely. Many of the susceptible species are grown as ornamentals around homes and, if parasitized by burrowing nematodes, may be a source of infection for citrus growing close by. If all the burrowing nematodes that parasitize these plants are alike, then any infected plant could spread the infection to nearby citrus. On the other hand, should some colonies of burrowing nematodes be so specialized that they do not feed on citrus, then their presence on host plants would not be a threat to adjacent groves. Because of the extensive host range of the burrowing nematode, it is important to know whether physiologic races of burrowing nematodes occur in nature and whether there are races that do not parasitize citrus. A physiologic race is generally understood to be identical with the species in morphological respects but to differ from it in some aspect of its physiology, such as parasitism.

In Florida, clumps of banana, *Musa nana* and *M. sapientum*, are often planted in and about citrus groves bordering lakes, marshes, irrigation ponds and drainage ditches. The first evidence indicating the existence of a physiologic race of burrowing nematodes that differs from the burrowing nematodes causing spreading decline came from such a clump of banana plants. The banana roots were heavily parasitized with burrowing nematodes whereas the citrus roots were not. Roots from this location were examined four times during the following year. Each time the citrus roots were free of burrowing nematodes although the citrus roots were intermingled with the parasitized.