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# The Spread and Distribution of Citrus Tristeza Virus Isolates in Sour Orange Seedlings

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**ABSTRACT.** A rapidly spreading decline of several citrus varieties on sour orange rootstock in the Morasha area, in the coastal plain of Israel, was found to be caused by a severe "seedling yellows" isolate of the citrus tristeza virus (CTV). Repeated ELISA tests revealed great variation in distribution of the Morasha CTV isolate throughout the canopies, even in declining trees. The CTV spread and distribution in graft-inoculated sour orange seedlings was followed by ELISA tests. A preferential basipetal transport and accumulation of virus at the early stages of CTV infection was found. A working hypothesis discussing the consequences leading to a rapid decline on sour orange rootstock is presented.

Citrus tristeza virus (CTV), an aphid-transmitted closterovirus has caused considerable damage to citrus production worldwide (1). Natural spread of CTV has been observed in Israel since 1970 and continuous efforts have been made to contain the disease by regular surveys using enzyme linked immunosorbent assay (ELISA) for diagnosis and by elimination of all infected trees found.

In 1985 several trees of different citrus varieties, all grafted on CTV-sensitive sour orange rootstock, were found to display quick decline symptoms in a 100-ha commercial plantation near the Morasha Junction in the Coastal Plain of Israel. A large proportion of these declining trees were not CTV positive by ELISA tests before or immediately after quick decline was observed.

This report reviews information on the spreading quick decline observed in the Morasha area and analyses the systemic distribution of CTV in graft inoculated sour orange seedlings.

## MATERIALS

**Sample collection and assays.** Ten or twenty twigs were assayed per tree (3) and tested by the heterologous anti-globulin double antibody sandwich (Hadas) - ELISA using chicken egg yolk and anti-CTV antibodies (2). Standard CTV indicators including acid lime, alemow, sour orange, and a modified sweet orange/sour orange (SWO/SO) decline test (4) were used for diagnosis.

**Systemic distribution of CTV in graft inoculated sour orange seedlings.** Eight-month-old SO seedlings approximately 0.8 m high were, chip graft inoculated, 0.2 m above ground with Washington navel or Clementine mandarin buds infected by the Morasha seedling yellows (SY) isolate (3) and a CTV isolate from Aчитuv. Previous field observation did not indicate collapse problems in the Aчитuv area. At different intervals after inoculation groups of 2-4 plants from each treatment were removed and 1-g tissue samples from seven parts of each plant were tested by Hadas-ELISA.

## RESULTS

**Distribution of CTV in quick decline trees at Morasha, Israel.** The occurrence of CTV at two sampling dates in different twigs of nine Minneola trees in two blocks at Morasha are presented in Table 1. These trees had been assayed and found CTV-free in 1984. However, decline was observed since Oct-Nov 1985. In May 1986, CTV was detected in eight of the nine Minneola trees, but on two of the eight trees fewer than half of the twigs sampled contained detectable virus concentrations. CTV could not be detected in any of the sampled twigs of one of the trees in May which was six months following the start of decline symptoms in that tree.

Indexing assays were made to determine whether the limited detection of virus could be a result of low virus

TABLE 1.  
DETECTION BY ELISA OF CITRUS TRISTEZA VIRUS (CTV) IN THE CANOPY OF MINNEOLA TREES ON SOUR ORANGE AT MORASHA

No.	Twig positive (%) <sup>z</sup>	
	May 86 <sup>y</sup>	Sep. 86
1	100	100
2	100	100
3	0	50
4	90	100
5	60	90
6	70	90
7	20	30
8	40	90
9	70	100

<sup>z</sup>Ten twigs assayed per tree, results given as percentage of twigs infected.

<sup>y</sup>The decline was first observed in this block in the autumn of 1985 (3). A 1984 assay did not detect CTV in these trees.

titers in collapsing trees. Only seven of the 14 twigs sampled (1 twig/tree) from 14 declining Minneola trees induced vein clearing and stem pitting symptoms on lime and alemon seedlings and SY reaction on sour orange plants. ELISA assays of the non-reacting indicator plants were negative. The severity of the Morasha CTV isolates was confirmed by the SWO/SO decline test. The indicators developed severe stunting and yellowing with some wilt within 4 months of inoculation. The disease spread rapidly in the Morasha groves and 80% of the block had declined after 3 yr (3).

**Systemic distribution of CTV in graft inoculated SO seedlings.** Fig. 1A and M illustrate the kinetics of CTV movement in different parts of SO seedlings which had been graft inoculated 0.2 m above ground with two CTV isolates. Thirty seven days postinoculation (PI) CTV was detected by ELISA only in parts containing the inoculum graft. At the forty-fourth day PI, CTV was detected only in the basal parts of the plants regardless of the virus isolate. The virus continued to remain in only the basal parts of the plants inoculated by the Morasha isolate 51 days PI, and only 7 days later was CTV detected by ELISA both in the basal section and in the upper parts of plants infected by both isolates. The virus titer differed considerably between the

CTV isolates (Fig. 1A and M). The readings of the Achituv isolate were = > 1.000 units at 405 nm. The titres in the SY Morasha isolate, however, with the exception of the inoculated part, were much lower especially in the upper parts. Only low readings, < 0.3 OD unit, were obtained in the rootlets of plants inoculated with the SY isolate. Rootlets of plants infected by the ordinary Achituv isolate also gave low ELISA readings (< 0.5).

## DISCUSSION

The combination of uneven virus distribution within the canopy (Table 1), together with high virulence, could explain the failures to detect CTV infection in the Morasha groves using twigs from the scion. The pathological consequences leading to collapse on SO rootstock have not been characterized. The CTV distribution assays in SO seedlings following graft inoculation by the Morasha isolate indicate that the virus first spread basipetally from the inoculation site. Only 58 days after inoculation was the virus detected in the upper part. A similar distribution also was observed in plants inoculated with two other CTV isolates, a SY and an ordinary isolate, from Hedera and Mikveh Yisrael respectively (Bar-Joseph & Nitzan, unpublished). The basipetal movement of CTV might be accounted

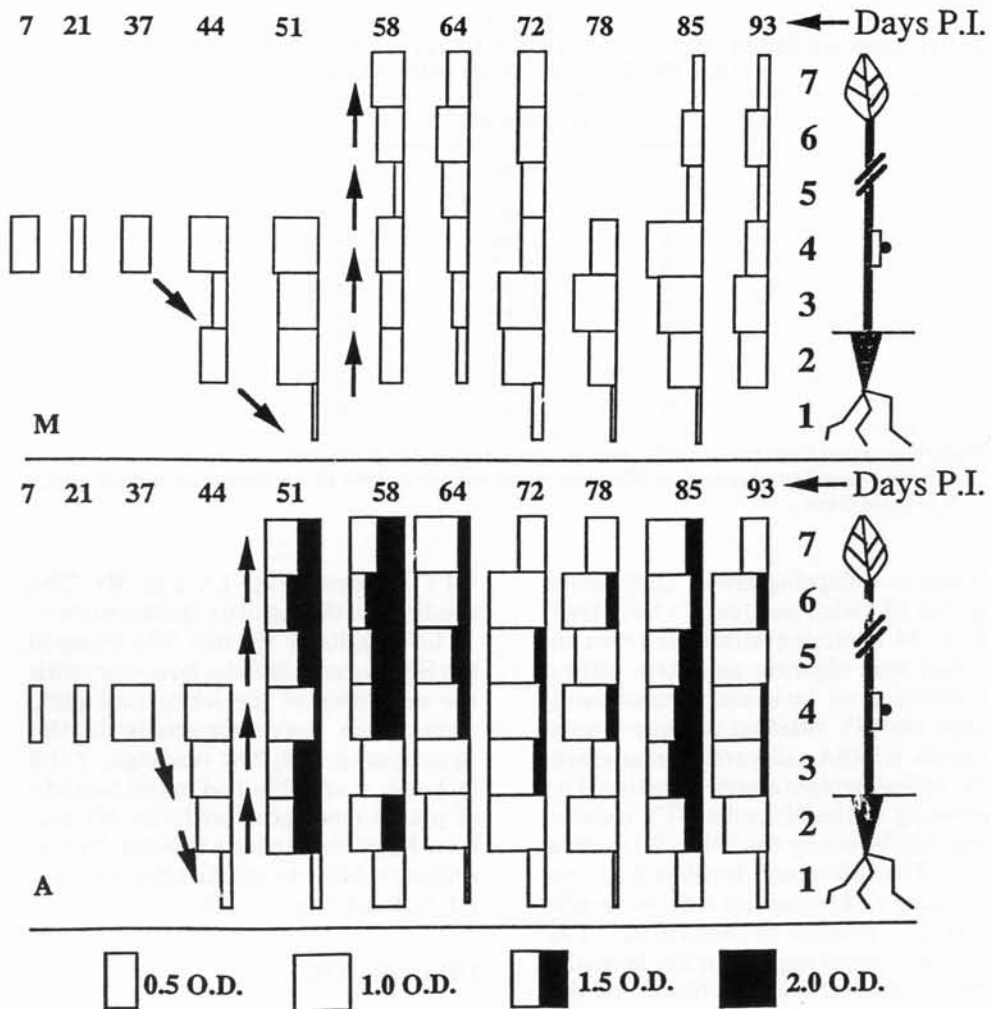


Fig. 1. The distribution of citrus tristeza virus (CTV) in different parts of young sour orange seedlings at different times post inoculation (PI) with the Achituv (A) and Morasha (M) strains of CTV. Plant parts (1-7) are illustrated on the right side of the figure. The graphic scale of the ELISA reading (OD 405nm) is indicated at the bottom line of the figure.

for by the association of this virus with the phloem tissue, although similar patterns of spreading were noticed previously with viruses not restricted to phloem (5). Preliminary results indicate that a similar pattern of virus spread also occurs in mature grafted trees on SO rootstock (Bar-Joseph, unpublished).

The following working hypothesis is suggested to account the rapid decline at Morasha: i) infection of young leaves by aphids, ii) limited virus increase at the infection site and virus transfer through the phloem to the SO

roots, iii) infection of the SO root system results in hypersensitive-like reaction (HIR) leading to phloem and rootlet degeneration, iv) rootlet degeneration causes a severe water deficit which eventually results in canopy collapse.

At this stage the virus is only sparsely distributed in the canopy parts. A drastic leaf drop enables water to be monopolized by a new flush of growth. The new flush at the peripheral parts of the scion serves as a sink, causing virus distribution throughout the canopy. Then reinfection from the canopy down towards the

depleted root system lead to tree death. A different theory suggests that a certain virus expressed product(s) diffuses from the scion part toward the sensitive SO and causes the bud union decline which leads to the starvation of the root system. Several aspects of these theories are now under investigation.

#### ACKNOWLEDGEMENTS

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