A Survey for Citrus Tristeza Virus in Italy by the Use of Enzyme-Linked Immunosorbent Assay

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ABSTRACT. A survey conducted by use of ELISA (enzyme-linked immunosorbent assay) has shown the presence of citrus tristeza virus (CTV) in a restricted citrus area of Calabria district. The affected trees, about 30-years-old, are 15 Golden Buckeye sweet oranges, 6 Satsuma Wase mandarins, 1 Marsh seedless grapefruit and 1 Ceylon lemon. All of them were introduced from abroad. Trees of local varieties, near affected ones or located in other areas always indexed virus-free. Other varieties of commercial interest introduced from Spain (Navelina sweet orange, Nules and Oroval Clementine) and or from other countries (Duncan, Ruby, Ruby Red, Marsh Seedless, Pink and Shambar grapefruit) were unaffected. Assays of aphids collected in the field by ELISA and by feeding on Mexican lime were also negative.
Index words. Aphids, vectors.

In Italy, citrus tristeza virus (CTV) infections have been noticed only in Meyer lemon and Satsuma mandarin trees (3, 4, 9, 10) introduced from abroad. Thousands of indexing tests on Mexican lime and examination of hundreds of thousands of trees in the field have shown that citrus cultivars of commercial interest are unaffected (3, 5). A recent study carried out using an ELISA test (1) revealed some infected trees introduced from abroad (6). This stressed the need for other investigations. A comprehensive review of all the results is reported in the present paper.

MATERIALS AND METHODS

Trees surveyed. An initial survey was undertaken to test some trees of the most important commercial citrus varieties and some others which were introduced for experimental purposes. A second survey was carried out from May 1982 to April 1983, mostly on samples taken in orchards where trees propagated from budsticks obtained abroad were noticed. At first, some Golden Buckeye sweet orange trees, which showed marked dwarfing were tested. Since these trees were infected, tests were extended to all the other imported trees and to some others cultivated locally. Other tests were carried out on samples taken in neighbouring orchards, where some budsticks of Golden Buckeye sweet orange and Satsuma Wase had been grown. Other samples were taken from trees in different localities of Basilicata, Calabria and Sicily, which are the most important citrus areas of Italy.

A total of 604 citrus trees were tested: 178 from local varieties (from orchards and experimental plots) and 426 belonging to the species and cultivars obtained from foreign introductions (table 1).

ELISA. For these assays an antiserum obtained from the T4 isolate of CTV (8) (kindly supplied by Dr. S. M. Garnsey, U.S. Horticultural Research Laboratory, USDA, Orlando, Florida) was used.

The citrus extracts were prepared from leaf vein samples and/or from cortex taken the day before or stored for a few days at —20°C. The standard procedure was used (1). Mexican lime seedlings inoculated with an isolate of CTV from Japan (TJ) (3) and sour orange seedlings inoculated with isolate T4 (7) were used as positive controls. Both isolates, free of other viruses, produce striking vein clearing symptoms on Mexican lime and suberization of the leaf veins.

Indexing. Affected trees were

TABLE 1
RESULTS OF ASSAYS FOR CITRUS TRISTEZA VIRUS (CTV) BY ELISA TEST IN DIFFERENT CITRUS SPECIES AND VARIETIES INTRODUCED IN ITALY FROM ABROAD

Citrus species and varieties	No. trees tested	No. trees affected	Countries of introduction*
Sweet orange			
Atwood Early Navel	1	0	Ca
Golden Buckeye	57	15	Ca, Sp
Hamlin	1	0	Fl
Navelate	27	0	Sp
Navelina	29	0	Sp
Newhall	1	0	Gr
Pineapple	7	0	Ca, Fl
Skaggs Bonanza	7	0	Ca
Thomson Navel	2	0	Ca
Tulegold	1	0	Ca
Valencia late (many selections)	22	0	Ca, Sp
Washington Navel (many selections)	19	0	Ca, Sp
Mandarin and mandarin-like			
Clementine Comune	12	0	Sp
Clementine Nules	18	0	Sp
Clementine Oroval	8	0	Sp
Clementine SRA 63	4	0	Co
Carvalhal	2	0	Sp
Fairchild	2	0	Ca
Ponkan	2	0	Sp
Wilking	6	0	Ca
Murcott	6	0	Fl
Satsuma Owari	4	0	J
Satsuma Wase	42	6	J, Sp
Satsuma	85	0	?
Grapefruit			
Duncan	5	0	Fl, ?
Marsh Seedless	4	1	Sp
Pink	5	0	Fl
Red Blush	1	0	Te
Ruby Red	2	0	Te
Ruby	8	0	Te
Shambar	5	0	Ca, Fl
Star Ruby	7	0	Te, ?
Lemons			
Eureka	2	0	Sp
Lemon of Ceylon	1	1	Cy
Verna	2	0	Sp
Unknown	2	0	?
Pummelo			
Reiking	1	0	Ca
Webber	1	0	Ca
Unknown	4	0	?
Lime			
Persian lime	1	0	?
Unknown	1	0	?

TABLE 1. (Continued)

Citrus species and varieties	No. trees tested	No. trees affected	Countries of introduction*
Kumquat			
Nagami	8	0	Sp, ?
Calamondin	1	0	?
Total	426	23	

*Ca = California; Co = Corsica; Cy = Ceylon; Fl = Florida; Gr = Greece; J = Japan; Sp = Spain; Te = Texas; ? = not identified.

also indexed on Mexican lime seedlings grown in 3-5 liter pots on a composite substrate of 2/3 peat and 1/3 volcanic soil, sterilized with steam, and kept in a glasshouse at 20-35C. During the test period all the seedlings were protected by nets to avoid contact with aphids or other insects.

Aphid assays. Aphids growing on infected field trees of Satsuma Wase mandarin and Golden Buckeye sweet orange, were assayed for the presence of the virus by ELISA (2) and were also fed on Mexican lime seedlings. The following aphid species were collected and assayed: *Myzus persicae* (Sulzer), *Aphis gossypii* Glover, *A. fabae* Scopoli, *A. craccivora* Koch, *Aulocarthum solani* (Kaltenbach) and *Macrosiphum euphorbiae* (Thomas). Colonies were collected with a brush to obtain not less than 100-120 apterae. These were weighed and homogenized in a phosphate buffered saline, pH 7.2, containing polyvinyl pyrrolidone (1%) and tested by ELISA. Other groups of the same aphid colonies were transferred to Mexican lime seedlings for 24 hours under a cage. Both assays were replicated at least three times.

RESULTS AND DISCUSSION

None of 129 trees of local varieties of lemon, sweet orange, and mandarin were positive for CTV. These consisted of 55 sweet oranges (3 Biondo comune, 29 Sanguinello and 19 Tarocco) and also 4 Avana mandarins. Since all were growing in the same orchard near the CTV-affected trees for 30 years, apparently there was no spread. These results are similar to those reported earlier (3).

Tests carried out on trees introduced from abroad revealed 15 trees of Golden Buckeye sweet orange, six Satsuma Wase mandarins, one Marsh seedless grapefruit and one Ceylon lemon were infected by CTV.

Golden Buckeye sweet orange trees showed die-back, loss of leaves and a noticeable reduction of growth. Trees about 30 years old reached a height of 1.5 m and showed reduced foliage. Many trees were devoid of new growth and had very little fruit. In some cases we noticed a scion overgrowth and on the cambial face of the cortex, there were very tiny pits in the bark with corresponding pegs on the wood. The young roots were severely damaged, while secondary roots were dead. Leaves were small and chlorotic.

Satsuma Wase mandarin trees, grafted on trifoliate orange, Marsh seedless grapefruit and Ceylon lemon trees did not show any symptoms.

Two months after inoculation, the young leaves of Mexican lime inoculated with bark patches of CTV-positive sweet orange trees showed the characteristic vein clearing. Later, some leaves showed vein corking with light pitting in

the wood. No positive reaction was detected for vein enation although some of the tested trees were introduced from countries where the disease is present.

None of the assays of aphids by ELISA or by feeding on Mexican lime were positive. This agrees with previous results (3).

Since 22 of the 23 trees positive for tristeza are concentrated in only one farm, this can be considered the first large concentration of diseased trees discovered in Italy. The results are of notable significance because the infected trees are in an important orchard area, which could be a serious threat. Fortunately the tests carried out on the trees near the infected ones did not show any sign of infection and the vectors appear unable to transmit the CTV isolate. Thus, there is hope for the effectiveness of the eradication program now under way. A broader survey is now in progress.

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