Distribution of Citrus Variegation Virus within Citrus Hosts

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ABSTRACT. The distribution of citrus variegation virus (CVV) in Femminello lemon trees grafted on Volkamer lemon was investigated by enzyme-linked immunosorbent assay (ELISA) tests to determine the optimum period, tissues, and procedures for accurately detecting CVV from field samples. Thirty-four types of tissues from trees infected with a severe strain of CVV were tested at different seasons. The virus was recovered from collar bark in winter and spring and from young twigs, leaves, flowers, endocarp and seeds in spring and summer. The results show that the ELISA test for CVV in young tissues of field trees gives suitable results during spring and summer, but is inconsistent in autumn. In winter the test can be applied only to collar bark. A low percentage of seed tested were infected with CVV.
Index words. Seed infection, ELISA.

Citrus variegation virus (CVV) has been found in several citrus-growing areas in different countries. The virus is sometimes widespread in lemon and it can be a serious disease problem for lemons and mandarins (20). In an earlier paper (9), we reported preliminary results of enzyme-linked immunosorbent assay (ELISA) tests from field-collected material and suggested that ELISA could be a reliable indexing method for CVV.

It is often assumed that viruses which systemically infect plant hosts eventually become evenly distributed throughout the plant, however, this rarely happens (3), and many examples of erratic distribution of viruses within plant hosts have been reported. Cucumber mosaic virus (CMV) is unevenly distributed within symptom-expressing leaves; dark-green areas generally contain less virus than light green or yellow areas (13). Tobacco mosaic virus (TMV) is unequally distributed in different organs of some pepper cultivars, being present in symptomatic leaves, but rarely in roots or stems, and never in symptomless leaves of the same plants (18). Sharka virus (PPV) is distributed erratically in stone fruit, and this causes problems in indexing of potential parent trees for Sharka (14). Tobacco ringspot virus (TobRV) is also distributed unequally in cherry trees.

Examples of irregular virus distribution in citrus include tatterleaf citrange stunt virus (TL-CSV) which is restricted to leaf areas with visible symptoms in citrange hosts (10) and some isolates of citrus ringspot virus (CRSV) which are also irregularly distributed in citrus hosts (17). Limited systemic spread has also been observed in trees inoculated with the impietratura agent (1).

Seasonal and tissue age effects on serological indexing for citrus viruses have also been noted (2).

The purpose of the present study was to investigate the distribution of CVV by the ELISA test and to find the optimum period, tissues, and procedures for accurately detecting CVV in field trees.

MATERIALS AND METHODS

Virus. The severe isolate of CVV (FI-CVV) used in this study was discovered in Fior d'arancio lemon trees (6). It produces severe vein flecking in young leaves, crinkly-leaf in older leaves and occasional fruit symptoms (fig. 1) in field trees. It causes local lesions on mechanically inoculated cowpea plants.

Leaves of Volkamer lemon experimentally inoculated with two different strains of CVV, coded CVV-1 and CVV-2 (11), were used as positive controls in ELISA assays.

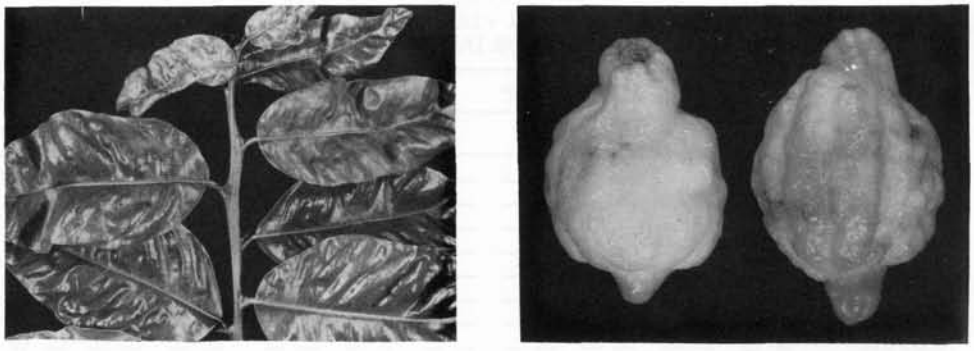

Fig. 1. Symptoms of citrus variegation virus infection in flush of Femminello Fior d'arancio lemon collected in June and in mature fruit (right).

Plant materials. Test trees were commercial Fior d'arancio lemon trees about 10 yr old in an experimental planting near Catania. Two of the trees were grafted on sour orange and two on Volkamer lemon rootstock. Fior d'arancio flowers many times a year and this allowed us to collect various kinds of tissue at every sampling interval. Tissue was collected from two plants at each sampling period.

Experimental procedure. Different parts of the trees were tested every 15 days from May 1982 until April 1983, except during December and January. We tested feeder roots, bark of old roots, bark from the collar trunk and main branches, bark from 1- and 2-yr-old twigs, bark from young twigs, young shoots, leaves (old, middle-age and young), veins from middle-age and young leaves, petioles and lamina from middle-age leaves, open and closed flowers, albedo collected from fruit 1, 2, 4, 6, and 7 cm in diameter, and seeds collected from fruit of different ages. Pedicel, button areas, epicarp and mesocarp of the equatorial area, columella and stylar end tissues each collected from an individual 7-cm fruit, were tested. Further details are shown in tables 1 and 2.

In other trials, we tested two lots of seeds (1,000 and 800, respectively) collected from mature fruit in summer. We also collected 500 seeds from mature summer fruit and tested half of them after washing to remove pectins and the remaining half after peeling away the seed coat.

In another experiment, we tested composite samples of three or four young leaves taken from single shoots collected from different parts of the tree to ascertain the distribution of the virus in the tree canopy.

All samples were ground on the same day or occasionally the day after collection. One gram of tissue was ground by mortar and pestle in the presence of liquid nitrogen, and extracted in 20 ml of buffer (PBS-T + 2% PVP (8) or 0.05 M Tris buffer, pH 7.8).

ELISA tests. A conventional double sandwich ELISA procedure was used in all tests (2, 8, 9). An antiserum to CVV-2 (9) was used. Plates were coated with a γ globulin at a 1 μg/ml concentration and the conjugate was diluted 1/1000. Dynatech M 129A, Nunclon Delta 163320 and 262162 Microelisa plates were used in different tests. Samples were tested at a dilution of 1/20 (w:v) in the extraction buffer.

The results were determined by visual observations, and in some cases, by measurement of absorbance at 405 nm with a Beckman Model 25 spectrophotometer. We considered all the samples positive which had an absorbance value 2.5 times that of extracts from the uninoculated control.

TABLE 1
DETECTION OF CITRUS VARIEGATION VIRUS FROM DIFFERENT PARTS OF BARK AND ROOTS OF FEMMINELLO FIOR D'ARANCIO LEMON TREES BY ELISA

Tissue samples	1982[z]							1983		
	M	J	J	A	S	O	N	F	M	A
Feeder roots	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Old roots	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Collar bark	M M	M W	W W	— —	— —	/ —	— —	W W	W W	M M
Trunk bark	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Branch bark	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
New flush	W W	W S	M M	— —	— —	/ —	— —	— —	— —	— M
Twigs 2 yr	— —	— —	— —	— —	— —	— —	/ —	— —	— —	— —
Twigs 1 yr	W M	— —	— —	— —	— —	/ —	— —	— —	— —	— W
Young twigs	M M	S S	M —	— —	— —	/ —	— —	/ /	— —	— W
Leaves										
Old	— —	— —	— —	— —	— —	—	— —	— —	— —	— —
Mid. age	W W	W M	S M	— —	— —	—	— —	— —	— —	— M
Young	M S	S S	S M	M —	— —	—	— /	/ /	/ —	W M
Vein, mid. age	M M	S S	S —	— —	— —	—	— —	— —	— —	W M
Vein, young	S S	S S	S M	M —	— —	—	— /	/ /	/ —	W M
Petiole, mid. age	S S	S S	S S	M —	— —	—	— —	— —	— —	W M
Lamina, young	S S	S S	S S	M —	— —	—	— /	/ /	/ —	M M

[z]Samples collected at 15-day intervals during months indicated. / = not tested; — = negative; W = weak reaction; M = moderate; S = strong. Each sample was collected from a single lemon tree. Samples were tested at a 1:20 dilution. Each value is the mean of two samples.

TABLE 2
DETECTION OF CITRUS VARIEGATION VIRUS FROM DIFFERENT PARTS OF FRUIT AND FLOWERS TO FEMMINELLO FIOR D'ARANCIO LEMON TREES BY ELISA

Samples site	1982[z]							1983		
	M	J	J	A	S	O	N	F	M	A
Open flowers	— W	W M	— —	/ /	— —	/ —	— —	— —	— —	W W
Closed flowers	— —	W —	— —	/ /	— —	/ —	— —	— —	— —	— —
Small fruit	— —	— —	— —	— —	— —	/ /	/ /	/ —	— —	— —
Fruit, 2 cm[y]	— —	— —	— —	— —	— —	/ /	/ /	/ /	/ /	— —
Fruit, 4 cm[y]	— W	M W	— —	— —	— —	/ —	— —	/ /	/ /	W W
Fruit, 6 cm[y]	— W	M M	— —	— —	— —	/ —	— —	/ /	/ /	— W
Fruit, 7 cm[y]	— —	M M	— —	— —	— —	/ —	— —	/ /	— —	— W
Seed, 2 mo fruit	— M	— —	— —	— —	— —	/ /	/ /	/ —	— —	— —
Seed, 4 mo fruit	— M	W M	M W	— W	— —	/ —	— —	/ /	/ /	/ —
Seed, 7 mo fruit	— M	W —	— —	— —	— —	/ —	— —	/ /	/ W	— —
Seed, mature fruit	— M	— M	— —	— —	— —	/ —	— —	/ /	/ —	— M
Pedicel[y]	— —	M S	— M	— —	— —	/ —	— —	— —	— W	— —
Button area[y]	— —	M S	— —	— —	— —	/ —	— —	— —	— —	— —
Epicarp[y]	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Mesocarp[y]	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Endocarp[y]	M M	M S	— —	— —	— —	/ —	— —	— —	— —	W W
Columella[y]	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —
Stylar-end[y]	— —	— —	— —	— —	— —	/ —	— —	— —	— —	— —

[z]Samples collected at 15-day intervals during months indicated. / = not tested; — = negative; W = weak reaction; M = moderate; S = strong. Each sample was collected from a single lemon tree. Samples were tested at a 1:20 dilution. Each value is the mean of two samples.
[y]Single or composite sample tested.

RESULTS

Results of seasonal sampling of different tissues are summarized in tables 1 and 2. The virus was detected in the collar bark of both rootstocks, but only in samples collected from February to July.

The virus was detected only three times in one-yr-old twigs (April and May), but more frequently in young twigs (table 1). It was consistently detected in petiole and leaf lamina of middle age and young leaves in April, May, June, and July samples and in some cases in August. Most leaves collected during the summer months were symptomless. The virus was detected five times in open flowers, but only one time in closed flowers and sporadically in fruits 4 to 7 cm in diameter (table 2). It was found in some seeds collected from 4 months postbloom to maturity. Pedicel, button areas and endocarp of fruit were positive in some samples. Other sites tested showed no virus (tables 1 and 2).

In tests of two large lots of seeds, 0.7% of a 1,000 seed lot and 2.1% of an 800 seed lot were infected. Citrus variegation virus was detected in a low percentage of washed seeds and peeled seeds (without hull).

In the experiment carried out to ascertain the distribution of the virus in the foliage, young leaves collected from all parts of the tree gave positive results.

DISCUSSION

Our results show that ELISA can be applied to detect CVV from young tissues of field trees in spring, summer, and in some cases, in the autumn when young flushes occur and unhardened leaves are available for testing. In winter, the test can be applied only to collar bark.

Roots, limbs and mature twigs were not good sampling sites. Open flowers and fruit yielded some positive samples, but were less consistent sources than young leaves.

Our results agree with those of Grasso and Catara (12), who transmitted CVV to different herbaceous species using leaves, albedo, petals and anthers as inoculum sources.

Our results did not show erratic distribution of CVV within the foliage of the host. Negative results at some periods must be related to a low concentration of CVV particles rather than to their complete absence.

This is the first report that CVV can be detected within seed. However, Wallace (21) reported two cases of transmission of crinkly leaf virus (CLV) through lemon seeds, and CLV and CVV are apparently closely related. Our results show that CVV is restricted to the inner portion of the seeds rather than to the outer tegument. Although seed transmission of plant viruses is quite common, only a few cases of seed transmission of citrus virus diseases have been reported. Bridges *et al.* (4) and Childs and Johnson (7) reported transmission of psorosis virus through the seed of Carrizo citrange at rates of 15 to 31% and Pujol (15) reported transmission through seeds of Troyer citrange. Campiglia *et al.* (5) reported transmission of psorosis through seeds of trifoliate orange at levels ranging from 1 to 10%. These researchers also reported that psorosis virus passes through the seeds of Florida rough lemon.

Presence of CVV in fruit was not surprising since citrus tristeza virus (CTV) particles are consistently found in the albedo of infected Hassaku fruits (16) and fruit pedicel (2). Tsuchizaki *et al.* (19) even suggested the pericarp as a tissue source for purification of CTV.

We did not detect CVV in roots, but tests carried out in a replanted grove site near Dundee, Florida, revealed CVV infection in sprouts originating from roots of trees removed several years earlier (11).

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