

The Status of *Citrus tristeza virus* in the Fars and Bushehr Provinces of Iran

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ABSTRACT. *Citrus tristeza virus* (CTV) was massively introduced into Mazandaran Province in the north of Iran through import of thousands of Satsuma mandarins from Japan in 1969. The southern citrus growing provinces, including Fars and Bushehr, were assumed to be tristeza-free, first because they were free of infected Satsumas and secondly because of prevailing high temperatures especially during the summer months. However, a survey of citrus varieties in Fars and Bushehr Provinces showed widespread occurrence of the virus. The virus was detected by ELISA in sweet orange, sour orange, “local” mandarin, Kinnow and other types of mandarin, sweet lime and Mexican lime. Most “local” mandarin plants on Mexican lime rootstocks in certain areas were infected. The virus was best detected in the plants from January to April. The extent of loss due to CTV was not determined but the major symptoms were general unthriftiness and slow decline in most citrus varieties, stem pitting in “local” mandarin and Mexican lime, occasional vein clearing in Mexican lime and yellowing and browning of veins in a navel sweet orange selection. *Aphis gossypii*, *A. craccivora* and *Myzus persicae* experimentally transmitted CTV at low efficiency. Aphid transmission explains occurrence of the virus in Mexican lime and sour orange seedling trees. Most southern isolates could be distinguished from a common northern isolate by their different symptoms on Mexican lime and their different reactivity with two antisera in ELISA. This indicated different origins for CTV in the north and south of the country. Wide distribution, aphid transmissibility and high rate of detection in certain varieties indicate that CTV was introduced in the south of Iran long before its massive introduction into the north.

The major citrus-growing regions of Iran are in the lowlands south of the Caspian Sea in the north and several provinces in the south. Mazandaran Province in the Caspian Sea region ranks first in citrus production followed by Fars Province in the south. *Citrus tristeza virus* (CTV) was massively introduced into Mazandaran Province through importation of over 40,000 Satsuma mandarin plants on trifoliolate orange rootstocks from Japan in 1969 (2, 5, 8). The virus remained confined to the area until its recent spread by *Aphis gossypii* (6). The southern citrus producing provinces were assumed to be tristeza-free because of prevailing high temperatures and absence of introduced Satsumas. The first report on the presence of CTV in southern Iran appeared in 1996 (7). The present paper reports widespread occurrence of the virus and its aphid transmission in the southern provinces of Fars and Bushehr.

Common citrus varieties were examined for diagnostic symptoms

of CTV over a period of 5 yr in all major citrus-growing regions of Fars and Bushehr Provinces. Direct double-antibody sandwich ELISA (1) was performed to detect the virus in symptomatic or randomly selected plants. A commercial (BioReba) and a locally produced antiserum were used. ELISA specimens consisted of bark samples from the trunk or green shoots. Occasionally, samples were subjected to polymerase chain reaction (PCR) for verification, using the CTV-specific primers CTVv: AAC GCC CTT CGA GTC TGG GGT AGG A/CTVc: TCA ACG TGT GTT GAA TTT CCC AAG C (designed by A. Rowhani, Univ. Calif., Davis, personal communication). Extracts of selected samples were adsorbed to formvar-coated electron microscope grids, stained with uranyl acetate and examined in a LEO 906 electron microscope.

Three aphid species, *Myzus persicae*, *A. gossypii*, and *A. craccivora*, were collected from infested citrus trees, reared on herbaceous plants in the greenhouse and used in trans-

mission studies, following the method of Hermoso de Mendoza et al. (3). A Mexican lime isolate of CTV from Darab, Fars Province, was used as the source of the virus (4). The aphids were given a 48 h acquisition feeding access on the source plant followed by 48 h of inoculation feeding access on young Mexican lime seedlings, after which the plants were sprayed with an insecticide. Approximately 200 aphids were used per test seedling (3) and 20 seedlings were used for each aphid species. The results were assessed by ELISA 6 mo after inoculation.

CTV was detected in Darab, Fassa, Jahrom, Khafr, Kazeroon and Shiraz in the Fars Province and in several locations in the Bushehr Province. General unthriftness, die-back and slow decline were observed in most citrus varieties, especially in older plantations in both provinces. A high proportion of Mexican lime seedling trees, mandarins and Mexican lime rootstocks under "local mandarin" showed stem pitting. Vein clearing was also detected in Mexican lime. All plants with stem pitting and/or vein clearing tested CTV-positive by ELISA. The highest ELISA readings were obtained with samples taken in winter and early spring. Filamentous particles were observed when crude bark extracts or partially purified preparations from these trees were studied in the electron microscope. CTV was also associated with a graft-transmissible decline of navel sweet orange in Darab characterized by vein yellowing, vein corking (Fig. 1), and die back. Infection of these plants with CTV was verified by ELISA, PCR and electron microscopy. Table 1 shows ELISA results of a preliminary survey of various citrus varieties for CTV.

Low but significant rates of CTV transmission were obtained with all three aphid species in greenhouse tests; the proportions of CTV-positive plants were 3/19, 2/20, and 1/20 with *A. gossypii*, *A. craccivora*, and *M. persicae*, respectively.



Fig. 1. Vein corking in a declining Navel sweet orange infected with *Citrus tristeza virus* in Darab, Fars Province.

Natural field transmission of the virus is implied from the following results: When 280 Mexican lime seedling trees were tested by ELISA using the BioReba and locally-produced antisera, the numbers of CTV-positive trees were 43 and 128, respectively; similar tests with 110 sour orange seedling trees gave 33 and 60 positives, respectively. Thus the positive results were clear, although the antisera performed differently.

The above studies show that CTV is widespread in Fars and Bushehr provinces. The virus may have been introduced recently through exotic varieties such as Satsuma and Kinnow mandarins, smuggled into the area. However, presence of the virus in remote and isolated locations suggests alternative routes for its introduction. The isolates in the south have as yet to be characterized but they seem to be different from those present in the north as they induce milder symptoms on Mexican lime seedlings when compared with a

TABLE 1
RESULTS OF A PRELIMINARY SURVEY FOR *CITRUS TRISTEZA VIRUS* IN THE FARS PROVINCE OF IRAN USING DAS-ELISA

Citrus species	No. positive/No. tested
Sweet orange	91/190
Palestine sweet lime	15/38
Mexican lime	8/40
Satsuma	30/51
Mandarins:	
Kinnow	35/87
Local	35/57

common northern isolate in parallel inoculation tests. In ELISA, most southern isolates reacted strongly with the locally-produced antiserum while weaker reactions were obtained with BioReba antiserum. The reverse was true with the northern isolate.

We conclude that CTV has had a long history in southern Iran and

was introduced into the region with citrus varieties which are now considered “native” or “local”.

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