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NonTransmission of Tristeza Virus by Aphids in Northern Iran During the Last Eighteen Years

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ABSTRACT. Tristeza virus was reported by Ebrahimi in Iran in 1971 on 50,000 satsuma trees which were imported from Japan in 1968. Efforts at eradication did not work. The infected trees which are on trifoliate orange rootstock are 18-yr-old now, interplanted with blood orange Thompson navel oranges, and old-line satsuma and Clementine mandarins on sour orange rootstock of the same age. For 6 yr, shoots of interplanted trees on sour orange rootstock which are fed upon by *Aphis citricola* Van Der Goot and *Toxoptera aurantii* (Boyer de Fonsicolombe) were collected and five buds from five shoots of each tree were budded on a single Mexican lime seedling. This was repeated twice a year in June and October. No symptom of vein clearing was observed on Mexican lime seedlings, and no decline of interplanted trees on sour orange occurred.

Tristeza had not been reported in Iran until 1969. During 1969 and early 1970, one of the members of last Royal family imported 50,000 two-yr-old satsuma mandarin trees from Japan budded on trifoliate orange under the names Ishykawa and Sugyama. Upon their arrival in Teheran, trees were shipped directly to the prepared land, the main grove of Mahdashte near Sari, the Capital of Mazandaran province, without conducting quarantine operations.

The two varieties of high quality and fancy appearance attracted Y. Ebrahimi's attention and involved him in an investigation of the health of the plants with emphasis on virus and viruslike disease. Budwood was collected and inoculated on Etrog citron Arizona 861, Orlando tangelo, local sweet orange seedling and Mexican lime for detection of exocortis, cachexia, psorosis, tristeza and vein enation, respectively (5).

This investigation resulted in a report to the Dept. of Agriculture of Iran in 1975, announcing that all 50,000 imported Satsuma mandarin trees were carrying tristeza virus (CTV) (4). Immunosorbent electron microscopy by Ebrahime-Nesbat and Nienhaus (6) confirmed the occurrence of CTV in Iran. Specialists' knowledge about the extent of damage of such viral diseases on citrus industries throughout the world indicated a serious study of CTV in the

Northern citrus belt of the country was needed. The vectors were determined and the following aphids were reported in the Caspian citrus growing areas: *Aphis gossypii* Glover, *Aphis citricola*, *Aphis craccivora* Koch, *Myzus persicae* Sulzer and *Toxoptera aurantii*. Ghorbani (7) and Minassian (8) sampled many trees in different citrus growing areas of the North and also collected aphids which had fed on tristeza-infected trees in Mahdasht grove and suggested some natural spread may have occurred.

However, natural spread of tristeza in Northern Iran was doubtful, since no decline of interplanted varieties on sour orange among the infected satsuma trees was observed. Therefore, a study on the possibility of transmission of CTV by aphids fed from infected trees was conducted at the Ramsar Citrus Experiment Station (1, 2, 3, 9, 10).

MATERIALS AND METHODS

Budwood for this study was collected from the Mahdasht grove where 50,000 contaminated satsuma trees were planted, twice a year (in June and October) from 1979 to 1985. The trees selected for sampling were Clementine tangerine, old-line satsuma tangerine and blood orange on sour orange rootstock and were all surrounded by tristeza-infected trees imported from Japan. The total number of trees sampled was 100.

Care was taken to choose aphid damaged shoots only. Such shoots were then collected from trees which had coexisted next to tristeza-infected trees from Japan for 18 yr. Five shoots were collected from each tree and five buds (one from each shoot) were inserted into a 6-month-old Mexican lime seedling. Three Mexican lime seedlings were used for each pre-selected tree. Seedlings were planted in black plastic bags of 15x30 cm filled with a methyl-bromide treated mixture of river sand, leaf compost and aged cow manure. Three Mexican lime seedlings, inoculated with tristeza-infected buds, were used as controls.

Parallel to the above experiment, the possibility of transmission of CTV by aphids was studied. Fifty green peach aphids were collected from a nucellar satsuma tree and were transferred to the tender young shoots of a 2-yr-old tristeza-infected Ponkan mandarin in a screen cage. Three days later, when most of young shoots had been fed on and the young leaves were curled, three Mexican lime seedlings, two months old, with tender leaves, were exposed to the colony of aphids which had fed on the tristeza-infected Ponkan. Two days later, all the young leaves of the Mex-

ican lime seedlings were curled. These young seedlings were exposed to aphids for 74 h and then sprayed with malathion and moved to the greenhouse where the temperature ranged from 15 to 27 C.

A 2000 ppm Greenzit solution was administered as a supplement to keep the lime seedlings in vigorous condition. Seedlings were inspected regularly for symptoms of tristeza.

RESULTS AND DISCUSSION

The positive control seedlings showed signs of vein clearing in 23 days, but no symptoms appeared on the others. Seedlings were repruned and 25 days later, new leaves of control seedlings showed vein clearing again and none was observed on the others. Finally, no tristeza symptoms were noted on aphid-inoculated Mexican lime seedlings for the next 5 yr. These results were expected since all of the varieties budded on sour orange which had coexisted for 18 yr with the CTV-infected trees in the Mahdasht grove had remained free of decline.

It is concluded that none of the known aphids present in Caspian region are capable of transmitting CTV from infected trees to others so far.

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