Use of Electron Microscopy in Eradication of Tristeza Sources Recently Found in Israel

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Tristeza in Israel was first reported in 1956, in Meyer lemons (*5*). Subsequently, 17 citrus varieties growing in introduction plots and Nagami kumquat from various locations were found to be infected. All infections were traced to infected propagative stock, with no indication of natural spread (*3*).

Natural spread of tristeza in the Mediterranean area became evident in the early 1960s in Spain. This finding posed a threat to our tristeza-susceptible varieties and rootstocks grown in Israel, and prompted us to seek a more rapid method for identification of tristeza than the currently-used lime test (*4*). Earlier electron microscopy (EM) of bark extracts revealed threadlike particles (TLP) not observed in extracts from noninfected control trees (*2*). Results were obtained within two to three days, as compared with six to 12 weeks when indexing was done on lime seedlings. It was hoped that EM might be useful for identifying tristeza in suspect trees.

This paper reports spread of tristeza in one location in the Sharon, and the use of EM in identifying tristeza and leading to an eradication program.

MATERIALS, METHODS, AND RESULTS

Ten to 15 branches, 10 cm long and 10 to 15 mm in diameter, were collected from different parts of a tree. About 25 gm of bark were extracted as previously described (*2*). Partially purified extracts from three trees were combined, two thirds from each extract, and centrifuged for 90 minutes at 100,000 *g*. The resulting pellet was resuspended in 1.2 ml 0.01 M phosphate buffer, pH 8.2, centrifuged for 10 minutes at 4,000 *g*, and examined by EM. If TLP were observed, the remaining sample from each tree was then reexamined. Three technicians were able to prepare the material and diagnose 24 trees in one day.

Concurrently three buds from each of three to five trees were indexed on one lime plant. Buds were collected from various parts of each tree. If the indicator plant reacted positively, the sources were examined by EM.

In October, 1970, two declining 14-year-old Valencia trees were found to be infected by tristeza. To locate the source of infection, 100 to 200 trees from each surrounding orchard were randomly sampled. Altogether, 750 trees were examined by EM, and a cluster of infected trees was located. Electron microscopy examination of 3,000 trees within a radius of 150 meters from the center located 53 infected trees. They were found in plots differing in age, source of budwood, and stock-scion combination, the majority being latently infected mandarins. Their locations indicated natural spread of the disease in this area along a dispersal gradient (fig. 1).

Supplementary visual surveys of the major citrus-growing regions, carried out by advisors and orchard managers, revealed six declining tristeza-infected trees located at three other locations. Checking adjacent trees by EM revealed only 17 additional infected trees, however, with no indication of natural spread. Infection in several infected Calamondin trees in nurseries was traced to budwood sources by means of EM.

As a result of these surveys, an eradication program was initiated in the first location (Hibbat Ziyyon). From June,

1971, to May, 1972, 40,000 trees extending outward from the infection center were indexed by the conventional lime method. This was economically advantageous since a substantial saving of indicator plants was obtained by grafting budwood from three to five sources on each lime seedling and examining the sources by EM if any indicator reacted positively.

These tests revealed 180 additional infected trees, suggesting natural spread of the disease in this area. Natural spread in Hibbat Ziyyon may be the result of high transmission rates of this tristeza source by *Aphis gossypii* Glov., compared with very low rates of transmission of two other tristeza sources (unpublished).

All infected trees were destroyed immediately, and if more than 15 per cent of the trees in any one grove were found to be diseased, the entire orchard was destroyed. During 1972–1973, the previously indexed trees and an additional 20,000 in this area will be indexed.

During the surveys, EM was tested for its reliability in detecting TLP from various citrus cultivars. Marked differences in TLP among cultivars were observed (table 1), which should be considered when collecting tissue for EM. High numbers of TLP were regularly found in partially purified preparations from Shamouti orange, Clementine and Yousuf Effendi mandarins, and Calamondin. This made it possible to combine extracts from three trees for EM. Electron microscopy was unsuitable for screening Valencias, due to very low numbers of TLP. All the other cultivars examined contained sufficient TLP for rapid diagnosis by EM.

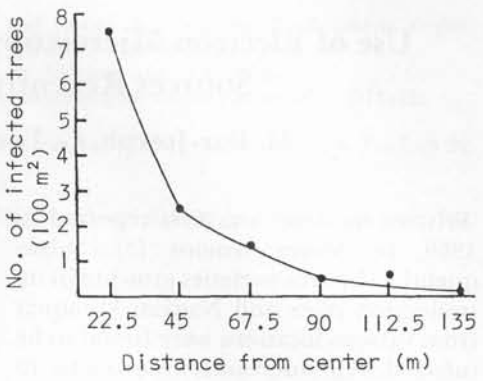

Fig. 1. Dispersal gradient of tristeza-diseased trees at Hibbat Ziyyon, Israel.

TABLE 1

THREADLIKE PARTICLES (TLP) IN PARTIALLY PURIFIED EXTRACTS FROM 25 gm OF BARK FROM VARIOUS CITRUS CULTIVARS

Cultivar	Probable means of infection	Number of trees examined	Number of TLP in one opening of a 200-mesh grid
Shamouti sweet orange	Vector	34	>100
Washington navel orange	Vector	2	>100
Valencia sweet orange	Vector	32	0–10
Early Nuris orange	Graft	4	>50
Marsh Seedless grapefruit	Vector	2	±50
Clementine mandarin	Vector	148	>100
Yousuf Effendi mandarin	Graft	10	>100
Calamondin	Graft	10	>100

CONCLUSIONS

Electron microscopy seems to be particularly useful in locating infection centers in areas where tristeza is not yet established. It is also useful in combination with the lime test in a large-scale eradication program. However, since TLP content varies from cultivar to cultivar, and is affected by temperature (*1*), it has to be evaluated and adapted for varying situations.

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