Apparent Elimination of Exocortis and Yellowing Viruses in Lemon by Heat Therapy and Shoot-Tip Propagation

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ETROG CITRON (*Citrus medica* L.) seedling 60-13 has been used by the author as an indicator for exocortis virus since January, 1964. When graft-inoculated from citrus sources infected with exocortis, it reacted under glasshouse conditions as described by Calavan *et al.* (2, 3). Conversely, it failed to react when grafted with material not infected with this virus. This indicator also developed marked chlorosis and transient vein clearing of terminal leaves when grafted with old-line lemon [*C. limon* (L.) Burm. f.] varieties, including several not infected with exocortis virus. When the indicator was propagated on sour orange (*C. aurantium* L.) seedling rootstocks, the symptoms were accentuated and accompanied by severe stunting.

In Victoria, Australia, old-line lemon varieties decline on trifoliate orange [*Poncirus trifoliata* (L.) Raf.] and on Carrizo and Troyer citrange (*C. sinensis* x *P. trifoliata*) rootstocks. The Eureka and Villafranca selections are infected with mild and severe strains, respectively, of exocortis and induce typical exocortis symptoms when propagated on trifoliate orange. Two lines of Lisbon lemon, however, are not so affected, but they lack vigor on these rootstocks and produce sparse chlorotic foli-

age. The virus or virus complex responsible for the chlorosis syndrome in citron 60-13 may be responsible for this disorder, and the purpose of this study was to test that hypothesis.

Methods and Results

Eureka (Rodwell), Lisbon (Doncaster and Rix), and Villafranca lemons propagated on trifoliate orange, and grown in a U.C. soil mixture composed of equal quantities of fine sand and peat with added nutrients (4), were subjected to heat therapy in a naturally lighted phytotron cabinet operating at 38°C (5).

Shoot-tips, usually 1 cm or less in length, were taken at regular intervals from succulent, vigorously growing shoots of plants in the cabinet. They were cut in wedge form and grafted to stems of similar size and succulence of either trifoliate orange or of citron 60-13 propagated from stem cuttings. The graft was made in a leaf axil from which a similar wedge of tissue containing the axillary bud was first removed. The operation was usually performed with the aid of a low power head-mounted binocular magnifier (Berger Loupe, Gowllands Ltd., Croydon, England).

The virus causing the chlorosis and stunting of citron 60-13 was inactivated throughout the Villafranca lemon plant when it was grown in the heat-therapy cabinet for 184 days. The exocortis virus, however, was not inactivated by this treatment. This plant was then given additional heat treatment, and shoot-tip propagations were made as follows: 5 at 230 days, 2 at 342, 1 at 378, and 1 at 412. These plants all indexed negative for exocortis on citron 60-13.

Eureka lemon tips were propagated as follows: 3 at 398 days, 3 at 583, 1 at 600, and 1 at 652 days. All indexed negative for exocortis and the yellowing virus on citron 60-13. One Rix Lisbon plant was subjected to 1,022 days of continuous heat treatment and was then extremely vigorous and too large to be retained in the cabinet. Two tip propagations were made from this plant and both indexed negative for both viruses.

After a brief delay, treated plants of the three varieties made fairly vigorous growth during heat therapy. However, their leaves were shaped abnormally and shoot-growth was rosetted due to shortened internodes. The plants made normal growth after removal from the cabinet, or when propagated from shoot-tips. It would appear, therefore, that the altered habit of growth in the cabinet was not a heat-induced mutation.

In January, 1966, bud-sticks of the Arizona 861 citron were received from L. G. Weathers. This selection is more sensitive to the exocortis virus than is the citron 60-13 indicator (1) and might be expected to

detect mild, possibly heat-resistant strains not revealed by citron 60-13.

The amount of indicator budwood available initially was insufficient to re-index all the plants derived from the heat-treated lemons. It was decided, therefore, to index the plant of each variety that had received the shortest period of treatment. If these plants failed to induce exocortis symptoms on the more sensitive indicator, then the plants propagated after longer periods of treatment could scarcely be infected. The lemons selected for testing were Doncaster Lisbon (155 days), Villafranca (230 days), Rodwell Eureka (398 days), and Rix Lisbon (602 days). These selections were propagated on Rough lemon [*C. limon* (L.) Burm. f.] seedling stocks and made vigorous growth. The indicator buds were inserted in the lemon tops which were pruned after an interval to stimulate growth of the indicator. Eight months after budding, the Arizona 861 indicator had made vigorous symptomless growth on the four lemon varieties.

All heat-treated material, whether propagated on the citron 60-13 clone or on Rough lemon seedlings, is growing vigorously in an insect-screened glasshouse. This material may be free of all known citrus viruses, but only long-term testing will determine whether this is the case.

The lemon selections exposed to heat treatment were obtained from varieties in a lemon rootstock trial in progress at Mildura since 1961. It is planned to replant this trial in 2 or 3 years with trees propagated from the healthy material and on the same series of rootstocks. The behavior of the virus-infected combinations will have been adequately assessed by then.

Discussion

The identity of the virus in lemons that is responsible for chlorosis and stunting of the citron 60-13 indicator has not been determined. All lemon varieties were infected with mild strains of tristeza virus. However, such mild strains can scarcely be responsible for the severe stunting that occurred when citron 60-13, propagated on sour orange, was bud-inoculated with lemon. Tristeza transmitted by aphids from lemon, grapefruit, and mandarin failed to induce detectable symptoms on citron 60-13 propagated on Rough lemon stocks.

West Indian lime plants inoculated with tristeza from lemon by aphids (*Toxoptera citricida* Kirk.) exhibited less severe symptoms than when graft-inoculated from lemon. However, limited attempts to transmit the yellowing syndrome from lemon by aphids were unsuccessful. The more severe symptoms on the graft-inoculated plants cannot be attributed to

synergism between exocortis and tristeza because several of the lemon (inoculum source) trees are free of exocortis virus.

Thus, an unknown entity may be responsible for the symptoms observed on West Indian lime and citron 60-13, and it may be responsible for the early decline of lemons on trifoliate orange and Carrizo and Troyer citrange rootstocks in Victoria.

These results are believed to be the first record of inactivation of exocortis virus by a combination of heat-therapy and shoot-tip propagation. Their validity depends entirely upon the reliability of the citron indicators for detecting the virus because the heat-treated clones have not yet been grown under field conditions on trifoliate orange rootstocks.

If the field performance of the heat-treated lemons supports the indicator evidence, then production of other exocortis-free citrus bud lines should be relatively easy. Shoot-tip propagation in conjunction with heat-therapy appears to be essential for success, since the virus, believed to be exocortis, causing epinasty of citron indicators was not inactivated by heat alone in 652 days, but was eliminated by combining heat treatment with shoot-tip propagation after 230 days.

Literature Cited

1. Allen, R. M., and Oden, W. R. 1964. Variability of seedling clones of citron as indicators of exocortis virus of citrus. (Abstr.) Phytopathology 54: 1431.
2. Calavan, E. C., Frolich, E. F., Carpenter, J. B., Roistacher, C. N., and Christiansen, D. W. 1963. Rapid-index methods for exocortis of citrus. (Abstr.) Phytopathology 53: 1138.
3. Calavan, E. C., Frolich, E. F., Carpenter, J. B., Roistacher, C. N., and Christiansen, D. W. 1964. Rapid indexing for exocortis of citrus. Phytopathology 54: 1359-1362.
4. Matkin, O. A., and Chandler, P. A. 1957. The U.C.-type soil mixes, p. 68-85. *In* K. F. Baker [ed.], The U.C. System for Producing Healthy Container-Grown Plants. Manual 23. Univ. Calif. Div. Agr. Sci., Berkeley.
5. Stubbs, L. L. 1963. A phytotron cabinet for heat-therapy studies with virus-infected plants. Comm. Phytopath. News 9: 49-52.