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Exocortis in Corsica

MOST OF THE CITRUS TREES in Corsica are grafted on sour orange (*Citrus aurantium* L.), as is usual in the Mediterranean area. In the course of a survey of citrus orchards for the presence of virus diseases (6), we found a Thomson Navel orange [*C. sinensis* (L.) Osbeck] grove where both sour orange and trifoliate orange [*Poncirus trifoliata* (L.) Raf.] had been used as rootstocks. In each row, the trees on trifoliate orange were generally in alternation with those on sour orange. The trees on trifoliate orange were severely stunted as compared with those on sour orange, but no exocortis-like scaling could be seen. In the same grove, we found several other varieties besides Thomson Navel.

The composition of the grove is as follows:

Category 1—60 Thomson Navel orange trees on sour orange (3.5 m high).

Category 2—30 Thomson Navel orange trees on trifoliate orange, very much stunted (1.5 m high) but without scaling.

Category 3—30 Thomson Navel orange trees on trifoliate orange, less stunted (2.5 m high) but showing a very pronounced bottleneck.

Category 4—1 Marsh grapefruit (*C. paradisi* Macf.) tree on trifoliate orange severely stunted with slight but typical scaling of exocortis.

Category 5—1 Clementine mandarin (*C. reticulata* Blanco) on trifoliate orange stunted, with severe scaling.

Category 6—4 trifoliate orange trees very severely stunted, showing typical scaling on the trunk and the main branches, and yellow blotches and cracks on the younger branches and shoots as described by Moreira (5).

Category 7—3 trifoliate orange trees very stunted without scaling, but with faint yellow blotches on the bark of the 1- or 2-year-old twigs.

In this grove, the trifoliate orange trees are scattered among the Thomson Navel orange trees. It is very likely that these trifoliate orange plants had been grafted with Thomson Navel orange buds about 20 years ago. As a matter of fact, on one of the trifoliate orange trees, one can still see a Thomson Navel orange side branch; this tree belongs to category 6 and has severe exocortis symptoms. It is thus likely that those trifoliate orange trees that are infected with exocortis virus received the virus from the Thomson Navel orange buds, which practically all died later on. In this grove, there are two types of infected trifoliate orange trees, one with severe symptoms (category 6), one with very mild symptoms (category 7), both severely stunted. This means either that two different sources of Thomson Navel orange buds have been used to build this grove, one carrying a severe strain of exocortis virus, the other a mild form; or, more likely, that only one Thomson Navel orange source was used but it carried two virus strains. Thus, knowing that the virus present in the Thomson Navel orange source used in this grove is able to induce exocortis symptoms on certain trifoliate orange trees, why do the 20-year-old Thomson Navel orange trees on trifoliate orange rootstock show only mild or severe stunting but no scaling?

The reason cannot be found in the local growing conditions since severe scaling does exist in the same grove not only on trifoliate orange trees but also on the 20-year-old Clementine mandarin tree on trifoliate orange. It is not impossible that this Clementine mandarin tree resulted from a top graft of a former Thomson Navel tree on trifoliate orange.

In another grove, typical exocortis symptoms were also found in the fall of 1962 on 4-year-old mandarin and orange trees on trifoliate orange introduced from Algeria; within 6 months the scaling, starting at the soil level, had moved up more than 20 cm.

Inoculation Experiments

Two-year-old Rangpur lime (*C. limonia* Osbeck) seedlings grown in the field were inoculated in August, 1960, with buds from trees of categories 2, 3, and 4. All the Rangpur lime seedlings inoculated with buds from the Marsh grapefruit tree (category 4) showed, eight months later, yellow blotches and small gum-producing cracks in the bark of certain shoots. In the following months, these symptoms grew more

severe. Now, the 1-year-old twigs still have yellow blotches and 0.5-1 cm long cracks with gumming, whereas the older twigs display only large necrotic zones with cracks. Certain shoots are entirely girdled by the necrotic zones; they dry out and die. Often the necrotic zones and cracks are most pronounced in the vicinity of a thorn.

The Rangpur lime seedlings inoculated with buds from either the Thomson Navel orange trees of category 2 or those of category 3 have shown the same symptoms, but in a much milder way and not so fast. Later, only a few lighter zones in the bark and a few very small cracks distinguished these inoculated seedlings from the non-inoculated checks grown in the same place and under the same conditions. Now, these symptoms are hardly visible.

The histochemical test of Childs, Norman, and Eichhorn (3) applied to bark from the grapefruit and Thomson Navel trees used as inoculum sources for the Rangpur lime seedlings was positive for the grapefruit tree and negative for the Thomson Navel tree.

Biochemical Analyses

To quantitate the yellowing of the bark caused by exocortis virus in Rangpur lime and trifoliate orange seedlings, we determined the chlorophyll content in the bark of the inoculated and non-inoculated Rangpur lime and trifoliate orange seedlings of categories 6 and 8. The results show a marked decrease in chlorophyll content of the bark of Rangpur lime seedlings as a result of virus inoculation; the decrease amounted to 54 per cent on a fresh weight basis or 62 per cent on a dry weight basis in young shoots (0.5 cm in diameter) and 77 per cent on a fresh weight basis or 83 per cent on a dry weight basis in older shoots (1 cm in diameter). The decrease in the case of trifoliate orange was less, 7 per cent on a fresh weight basis or 4 per cent on a dry weight basis in young shoots and 60 per cent on a fresh weight basis and 63 per cent on a dry weight basis in older shoots.

Discussion

It was hoped that Moreira's test (5) and Child's histochemical test (3) would give an answer to the following question: Do the 20-year-old Thomson Navel orange trees on trifoliate orange that are severely stunted but show no scaling carry exocortis virus? It was found that, for the stunted Thomson Navel orange trees, (a) Child's test was negative,

and (b) the Brazilian test for exocortis virus was only slightly positive under the conditions used. On the contrary, the Marsh grapefruit tree on trifoliate orange, also very stunted but showing scaling, reacted positively to the histochemical test as well as to the Rangpur lime test.

Fraser *et al.* (4) have demonstrated the existence of a transmissible stunting factor not associated with scaling. Calavan and Christiansen (1) have also shown the presence of a transmissible stunting factor in certain stubborn-affected trees, and some of their experiments have been confirmed by Cassin (2) in Morocco.

It is thus possible that in certain so-called exocortis-affected trees two factors are present: a stunting factor and a scaling factor. In certain trees, only one of these two factors was present. The stunted Thomson Navel trees contained only this stunting factor. If so, the histochemical test as well as the Rangpur lime test are much more sensitive for the scaling factor than for the stunting factor.

An alternative is to suppose that the scaling factor is a severe strain of exocortis virus, whereas the stunting factor is a mild strain of the same virus. If so, mild and severe can refer only to the reaction of the inoculated Rangpur lime test plants and not to the reaction of the orange tree on trifoliate orange; it is difficult to call a virus strain mild that has the ability to induce severe stunting in certain stock-scion combinations.

Conclusion

By using the Rangpur lime test and the histochemical test, we have not been able so far to correlate a transmissible factor with the stunting of 20-year-old Thomson Navel orange trees on trifoliate orange, nor could we find the reason why these trees show no symptoms of exocortis when in the same grove trifoliate orange trees, formerly grafted with Thomson Navel orange buds, do show mild or severe symptoms.

We feel that in testing for exocortis virus and a stunting factor related to exocortis virus or not, the histochemical test, the Rangpur lime test, or the traditional trifoliate orange test are insufficient. So we now use, in addition to these tests, the new Etrog citron test developed in California.

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Literature Cited

1. CALAVAN, E. C., and CHRISTIANSEN, D. W. 1961. Stunting and chlorosis induced in young-line citrus plants by inoculations from Navel orange trees having symptoms of stubborn disease, p. 69-76. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
2. CASSIN, J. 1965. Research on stubborn disease in Morocco, p. 204-206. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
3. CHILDS, J. F. L., NORMAN, G. G., and EICHHORN, J. L. 1958. A color test for exocortis infection in Poncirus trifoliata. Phytopathology 48: 426-432.
4. FRASER, L. R., LEVITT, E. C., and COX, J. 1961. Relationship between exocortis and stunting of citrus varieties on Poncirus trifoliata rootstock, p. 34-39. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
5. MOREIRA, S. 1961. A quick field test for exocortis, p. 40-42. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
6. VOGEL, R., and BOVÉ, J. M. 1963. Citrus diseases in Corsica. Brochure. I.F.A.C., Paris.