Evaluation of Recently Developed Indexing Methods in Israel

A. BENTAL

PALESTINE SWEET LIME [*Citrus aurantifolia* (Christm.) Swing.] and sour orange (*C. aurantium* L.) are the principal rootstocks used for the commercial citrus production in Israel. The growers prefer sweet lime rooted trees because of their characteristics of early fruit, small crown, and easier picking. Unfortunately, its high susceptibility to xyloporosis disease renders sweet lime impractical as a rootstock unless the trees are inarched with xyloporosis-tolerant sour orange. The sour orange, on the other hand, is susceptible to the much-feared tristeza, and may have to be replaced by tristeza-tolerant rootstocks, most of which are susceptible to exocortis. Thus, Israeli growers are confronted with several problems and an urgent need for quick answers.

The new quick methods of indexing exocortis and xyloporosis-cachexia, developed in Brazil (5, 7, 8, 9) and California (2, 3), have aroused hope of obtaining a quick answer to the problem of finding virus-free propagation material. Investigations of these methods were carried out at The Volcani Institute of Agricultural Research to verify their reliability under Israeli conditions, in comparison with other indexing methods.

Materials and Methods

The two methods generally used were direct inoculation of indicator seedlings by inserting blind buds from the indexed trees, and double-budding, by simultaneous insertion of inoculum and buds of different indicator varieties, into Rough lemon or sweet lime seedlings. In one instance, a test for exocortis was made by budding the indicator variety directly on branches of a tree to be indexed.

Trifoliate orange [*Poncirus trifoliata* (L.) Raf.], Rangpur lime (*C. reticulata* var. *austera* hyb.), Kusaie lime [*C. aurantifolia* (Christm.) Swing.], and Etrog citron (*C. medica* L. var. *ethrog* Engl.), were used as indicator plants for exocortis virus; and Orlando tangelo (*C. reticulata* Blanco x *C. paradisi* Macf.) and Palestine sweet lime were used for indexing xyloporosis-cachexia virus.

The inoculum came from the following sources: 1) Exocortis-affected, 30-year-old Shamouti sweet orange [*C. sinensis* (L.) Osb.] on trifoliate orange, showing characteristic bark scaling on the rootstock; 2) xyloporosis-affected, 30-year-old Shamouti sweet orange on sweet lime, show-

ing characteristic pits and pegs on the rootstock; 3) cachexia-affected, 25-year-old Nacotee tangelo on sour orange, showing characteristic gum pockets and pegs in the bark of the scion; and 4) cachexia-affected, 25-year-old Clementine mandarin (*C. reticulata* Blanco) on sour orange, showing gum pockets and pegs in the bark of the scion. Check plants were either non-inoculated or budded with nucellar buds.

Results

EXOCORTIS.—The first positive results were observed in sprouts of 8 Etrog buds inserted into branches of Shamouti sweet orange on trifoliate with bark scaling of the rootstock. After 5 months, the young sprouts showed characteristic chlorotic blotches and cracks, which indicates that the local Etrog is a reliable indicator plant for exocortis virus. As checks, 8 potted sweet lime seedlings were budded with the same Etrog. Sprouts from the Etrog buds remained without any symptoms, which eliminated the possibility that the Etrog itself may have carried the exocortis virus. The Shamouti tree indexed in this way served as a source of exocortis inoculum for further trials.

Additional tests with direct inoculation and with the double-budding methods showed the following results within 7 months: 1) Both sources of inoculum, an exocortis-infected Shamouti tree on trifoliate rootstock and a xyloporosis-affected Shamouti of unknown exocortis status, induced characteristic blotches and cracks in *all* plants of the 4 mentioned indicator varieties within 4 to 7 months. These symptoms appeared regardless of which indexing method was used. 2) The first source of inoculum (Shamouti on trifoliate) induced stunted growth and leaf epinasty in 57 per cent of the Etrog seedlings, as early as 3 to 4 months after direct inoculation. Neither stunting nor epinasty appeared when the double-budding method on Rough lemon seedlings was used. The second source of inoculum (Shamouti on sweet lime) failed to cause any epinasty, but in *all* cases pronounced cracks were produced. 3) The check plants budded with nucellar Shamouti remained free of any exocortis symptoms.

To investigate the question of why stunting and epinasty appeared in only 57 per cent of the Etrog seedlings inoculated with the same source of exocortis, 9 sweet lime seedlings were inoculated with buds from an Etrog seedling showing epinasty, and 9 sweet lime seedlings were inoculated with buds from an Etrog seedling showing only cracks. All 18 seedlings were budded simultaneously with nucellar Etrog. The results show that both sources of inoculum induced almost equally high percent-

ages (78 and 71.5 per cent, respectively) of epinasty in the sprouts of the nucellar buds.

Growth of the inoculum buds was suppressed to allow quicker growth from the indicator bud. In a few cases, however, the inoculum bud was also allowed to sprout. Epinasty was usually perpetuated in the growth of the inoculum taken from the epinasty source, and the symptoms appeared also in growth from the nucellar bud. However, inoculum from the cracked bark source perpetuated the cracks when its buds grew and induced epinasty in the nucellar bud.

Some exceptions were noticed. For example, in one case the growth of inoculum from a source which had shown epinasty perpetuated it, but induced only cracks in the nucellar bud. In another case, the inoculum bud from a source showing only cracks produced epinasty, whereas the sprout of the nucellar bud produced cracks but no epinasty.

XYLOPOROSIS.—The xyloporosis experiments were started in November, 1964. No symptoms appeared by September, 1966, so details of these experiments are omitted from this report. However, it should be noted that 16 one-year-old budlings of Orlando tangelo on sweet lime inoculated either in stock or in scion with xyloporosis-affected Shamouti sweet orange or cachexia-affected Nacotee tangelo and 8 uninoculated check plants, showed a slight constriction and a yellow line at the bud-union on all budlings, including the non-inoculated checks.

Discussion and Conclusions

The results of quick indexing of exocortis in Israel generally confirm the results reported from Brazil (5, 7, 9) and California (2, 3), and show that under Israeli conditions, trifoliate orange, Rangpur lime, Kusaie lime, and Etrog citron can be used successfully as quick and reliable indicators for exocortis. However, these results differ slightly from those reported from Florida (4) and South Africa (11), in which the Rangpur lime responded slowly and gave inconsistent results.

Leaf epinasty in the inoculated Etrog seedlings appeared in much less time than the blotching and cracking. However, not *all* the inoculated Etrog plants showed epinasty, whereas cracks developed in 100 per cent of the plants. The inconsistency of the epinasty symptoms can perhaps be attributed to the variability of the Etrog seedling (1, 2, 3, 4). The fact that the first source of inoculum induced epinasty, while the second source failed to do so in any of the Etrog seedlings suggests that qualitative differences occur in different sources of exocortis virus, as noted by Calavan *et al.* (2). It is also noteworthy that both methods of indexing

gave good blotching and cracking symptoms, whereas epinasty was produced only by the direct inoculation method, and was not induced even then on Rough lemon seedlings double-budded with the same source of inoculum. In another test, however, when sweet lime seedlings were double-budded, a very high percentage (71.5 and 78 per cent) of epinasty was obtained. This suggests that variability of Etrog seedlings is not the sole reason for the appearance or absence of epinasty in Etrog, because all indicator buds were taken from one seedling.

The high percentage of epinasty obtained when the double-budding method was used on sweet lime stocks, and the absence of epinasty with the same method on Rough lemon stock, does not agree with the report of Calavan *et al.* (2). They stated that shoots of citron buds grown on exocortis-inoculated Rough lemon seedlings showed epinasty, sometimes even within 1 month. On the other hand, Garnsey and Cohen (4) reported that the length of the incubation period necessary for appearance of symptoms on the citron indicator was different on different rootstocks.

Our quick indexing tests for xyloporosis-cachexia were disappointing. In Brazil (8), 70 per cent of Rangpur lime seedlings inoculated with a known source of xyloporosis produced symptoms in the sprouts of Orlando tangelo buds within 1 year, and failure to show symptoms was attributed to an uneven distribution of xyloporosis virus in the source of inoculum. In our trials, however, not a single case of xyloporosis symptoms was recorded, even after 2 years.

Salibe and Roessing (10) reported that the presence of exocortis virus apparently delays the appearance of xyloporosis symptoms in the indicator. This may explain our results because, as was proven, the xyloporosis source also contained the exocortis virus. In view of this effect of exocortis on the appearance of xyloporosis symptoms, it is questionable to what extent this indexing method can be considered valid and reliable, particularly since exocortis virus is distributed widely in all citrus varieties.

Bud-union constriction in the budlings of Orlando tangelo on sweet lime rootstock was also observed in Brazil by Moreira (6). According to him, these symptoms developed following inoculations with xyloporosis virus. In our case, however, all the non-inoculated check plants also showed the same symptoms, thus challenging the opinion that the bud-union constriction in Orlando on sweet lime was induced by the xyloporosis virus.

It may be concluded that the new methods contributed a valuable and reliable indicator for detecting exocortis.

Further investigations are necessary to explain the failure of xyloporosis-cachexia indexing in Israel.

This paper is a contribution from the National and University Institute of Agriculture, Rehovot, Israel, 1967 Series No. 1109-E.

Literature Cited

1. ALLEN, R. M., and ODEN, W. R. 1954. Variability of seedling clones of citron as indicators of exocortis virus of citrus. Phytopathology 54: 1431.
2. 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.
3. FROLICH, E. F., CALAVAN, E. C., CARPENTER, J. B., CHRISTIANSEN, D. W., and ROISTACHER, C. N. 1965. Differences in response of citron selections to exocortis virus infection, p. 113-118. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
4. GARNSEY, S. M., and COHEN, M. 1966. Response of various citron selections to exocortis infection in Florida. Proc. Florida State Hort. Soc. 78: 41-48.
5. MOREIRA, S. 1961. A quick test for exocortis, p. 40-42. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
6. MOREIRA, S. 1965. Cachexia and xyloporosis, are they caused by the same virus? p. 56-60. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
7. ROSSETTI, V. 1961. Testing for exocortis, p. 43-49. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
8. SALIBE, A. A. 1965. A quick field test for xyloporosis virus, p. 95-98. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
9. SALIBE, A. A., and MOREIRA, S. 1965. New test varieties for exocortis virus, p. 119-123. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
10. SALIBE, A. A., and ROESSING, C. 1965. Testing citrus trees for viruses, p. 232-234. *In* W. C. Price [ed.], 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
11. SCHWARZ, R. E. 1965. Evaluation of the methods for indexing exocortis virus of citrus. S. African J. Agr. Sci. 8: 593-598.