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Experiments on Heating Budwood to Eliminate Exocortis Virus

HEAT TREATMENT has been used successfully by several investigators (8-11, 13) to obtain virus-free plants or to eliminate virus strains from infected plants. Heat treatment has also been used by several authors (1-7, 14) in attempts to obtain sources of citrus budwood free from viruses. The present work, which was concerned with attempts to free citrus budwood from exocortis virus by hot-water treatment, was stimulated by the success attained by Grant (5, 6) and by Desjardins *et al.* (1) in eliminating tristeza virus and the seedling yellows virus complex from citrus plants. The method of hot-water treatment used, the results on survival of buds, and the negative results secured in attempts to obtain exocortis-virus-free tissue are described in this paper.

Materials and Methods

Budwood sticks about 25 cm long and 0.8 cm or less in diameter were collected from healthy and exocortis-infected trees of Rangpur lime and Baianinha sweet orange at the Limeira Citrus Experiment Station. Healthy Rangpur lime budwood was obtained from a 10-year-old seedling showing no symptoms of exocortis; exocortis-infected Rangpur lime budwood was obtained from a 3-year-old tree that had been inoculated with a severe strain of exocortis virus and was showing typical yellow patches and cracking of the bark on the branches. Healthy Baianinha orange budsticks were collected from a nucellar 8-year-old tree that had been grafted on Rangpur lime and was showing no symptoms of exocortis; exocortis-infected material of the same variety was from a 15-year-old tree grafted on Rangpur lime and showing typical scaling of the bark on the trunk of the stock.

After all leaves were removed from the budsticks, the pieces were assembled into bundles of about 20 sticks and immersed in a hot-water bath, a brass box 120 cm long, 80 cm wide, and 80 cm deep containing a volume of water of about 0.7 cubic meters. The bath was thermostatically controlled and a special device provided for homogeneous distribution of the heated water; the large volume of water minimized the variation in temperature during treatment.

Two experiments were carried out. In the first experiment, budwood sticks from all 4 parent trees were treated for 1, 5, and 10 hours at 30°-31°C and at 39.5°-40.5°C. At the same time, similar budsticks were maintained at room temperature to serve as controls. Ten hours after treatment was started, all the budwood sticks were taken to the Limeira Citrus Experiment Station and budded into 1-year-old Rangpur lime seedlings in the field. Ten seedlings were employed for each treatment and its control, each seedling receiving 2 buds.

A second experiment was carried out on February 7, 1963, in which budsticks of the same parent trees were treated for 1, 5 and 10 hours at 44.5°-45.5°C and for 1 hour at 50°-52°C. The treated buds from the second experiment were also budded into 1-year-old Rangpur lime seedlings at the Limeira Citrus Experiment Station.

When it was certain that the buds had survived, the Rangpur lime rootstock was cut back above the union and 3-4 new shoots of the rootstock were allowed to grow, above and below the point of inoculation, and were examined for visible symptoms of exocortis, such as yellow patches and cracking of the bark. Observations were made 6 and 12 months after grafting the budwood of the second experiment. The plants were given heavy nitrogenous fertilization in order to accelerate the production of symptoms (15).

Results and Discussion

Due to the severe drought that prevailed during the period of experimentation, exocortis symptoms developed much later than they normally do in Moreira's test for the disease (12).

Buds treated for 1 hour at 50°C did not survive, and there was no transmission of the virus. Of the buds treated for 10 hours at 45°C, only 20 per cent of the healthy Rangpur lime buds survived and none of them developed into branches; none of the other buds survived nor did the infected ones transmit the virus. These results are similar to those obtained by Fawcett and Cochran (3) when they attempted to eliminate

psorosis virus A by treating bud sticks in hot water. Of the buds treated for 5 hours, 54 per cent survived and none developed into branches; nevertheless all the infected buds that survived transmitted the virus, in both varieties. Of the buds treated for 1 hour at 45°C, about 86 per cent survived but only 25 per cent of them developed into branches. Two of the plants budded with infected buds of Baianinha orange have not shown symptoms of exocortis up to the present time.

Of the buds treated at 40°C for 10 hours, about 68 per cent survived and 69 per cent of them developed into branches. Eight plants budded with infected Rangpur lime buds that had been heated at 40°C for 10 hours developed symptoms of exocortis, despite the fact that in only 3 of them had the buds survived. Most probably, the buds on the remaining 5 plants survived long enough to transmit the virus and then died. The same thing probably occurred in the case of two plants that were budded with buds that had been treated at the same temperature for 5 hours. These results are similar to those of Price and Knorr (14). Almost all the buds of this treatment developed into branches.

Of the buds treated at 30°C for 1, 5, and 10 hours and at 40°C for 1 and 5 hours, almost 100 per cent survived; all the infected ones transmitted the virus, and the plants developed typical exocortis symptoms.

None of the plants grafted with buds from non-infected parent trees developed exocortis symptoms, irrespective of the hot-water treatment they received. Control plants grafted with non-treated buds from infected parent trees always showed exocortis symptoms, whereas the ones budded with healthy buds did not. In general, healthy buds, especially those from Rangpur lime, appeared more tolerant to the treatments than the infected ones.

The results indicate that the hot-water treatment of citrus budwood infected with exocortis virus, as used in these experiments, has not proved satisfactory for securing exocortis-free bud sources. Two plants that were grafted with infected Baianinha buds that were treated for 1 hour at 45°C have not developed symptoms up to the present time. These plants continue under observation.

Literature Cited

1. Desjardins, P. R., Wallace, J. M., Lange, C. T., and Drake, R. J. 1957. The suppression of tristeza virus symptoms in Mexican lime seedlings by heat treatment. Plant Disease Reptr. 41: 230-231.
2. Desjardins, P. R., Wallace, J. M., Wollman, E. S. H., and Drake, R. J. 1959. A separation of virus strains from a tristeza-seedling-yellows com-

plex by heat treatment of infected lime seedlings, p. 91-95. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.
3. Fawcett, H. S., and Cochran, L. C. 1941. Resistance of citrus tissue and psorosis virus A to heat. Phytopathology 31: 861.
4. Grant, T. J. 1957. Effect of heat treatments on tristeza and psorosis viruses in citrus. Plant Disease Reptr. 41: 232-234.
5. Grant, T. J. 1957. Heat treatments for obtaining sources of virus free budwood. Citrus Industry 38: 20-21.
6. Grant, T. J., and Higgins, R. P. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopathology 47: 272-276.
7. Grant, T. J., Jones, J. W., and Norman, G. G. 1959. Present status of heat treatment of citrus viruses. Florida State Hort. Soc. 72: 45-48.
8. Hughes, C. G., and Steindl, D. R. 1955. Ratoon stunting disease of sugar cane. Queensland Bur. Sugar Exp. Sta. Tech. Com. 2.
9. Kassanis, B. 1954. Heat-therapy of virus-infected plants. Ann. Appl. Biol. 41: 470-474.
10. Kunkel, L. O. 1936. Heat treatments for the cure of yellows and other virus diseases of peach. Phytopathology 26: 809-830.
11. Kunkel, L. O. 1952. Transmission of alfalfa witch's broom to non-leguminous plants by dodder, and cure in periwinkle by heat. Phytopathology 42: 27-31.
12. 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.
13. Posnette, A. F. 1953. Heat inactivation of strawberry viruses. Nature 171: 312.
14. Price, W. C., and Knorr, L. C. 1956. Kinetics of thermal destruction of citrus tissues in relation to the virus disease problem. Phytopathology 46: 657-661.
15. Weathers, L. G. 1960. The effect of host nutrition on the development of exocortis in Poncirus trifoliata. Phytopathology 50: 87.