A Psorosis-Like Virus Causing Symptoms Only on Dweet Tangor

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METHODS FOR detecting mild strains of citrus viruses were studied as part of the Variety Improvement Program of the Citrus Research Center, University of California, Riverside. Detection of very mild strains of virus has been enhanced by the use of a modified U.C. soil mix (3) to produce maximum plant growth free from patterns of nutrient deficiency or excess, and by supplemental lighting during the winter months (4).

Investigations on indicators for concave-gum virus revealed that the Dweet tangor [*Citrus reticulata* Blanco x *C. sinensis* (L.) Osb.] is a very efficient indicator for a mild strain of the virus and is also sensitive to psorosis-A and crinkly-leaf viruses (4). Despite a mild spotting that may appear on uninoculated seedlings, the superior sensitivity of Dweet tangor to psorosis-type viruses makes it a desirable indicator plant. After 1963, all new candidates entering the Variety Improvement Program were indexed on Dweet tangor, and over 150 candidates not previously indexed on Dweet were tested. One result of this re-indexing was detection of a psorosis-like mottle (Fig. 1,A) on leaves of the Dweet indicator inoculated from a Cleopatra mandarin (*C. reticulata* Blanco) (CRC 270 in 8A-13-50). The parent tree showed no visible evidence of damage or decline due to virus infection, although the fruits were small, the twigs

were dying back, and little new growth was apparent. The trunk appeared normal and no pitting or bark discoloration was evident. (This selection was introduced from Florida in 1914 by Dr. H. S. Fawcett.) Since the virus seemed to cause a reaction only in Dweet tangor it was named Dweet mottle virus (DMV), pending further investigation.

This paper reports experiments designed to investigate sweet orange [*C. sinensis* (L.) Osb.] as a symptomless carrier of the virus, protection of sweet orange by DMV when challenged by other psorosis-like viruses, leaf reactions of other citrus indicators to DMV, and detection of DMV when other viruses are present.

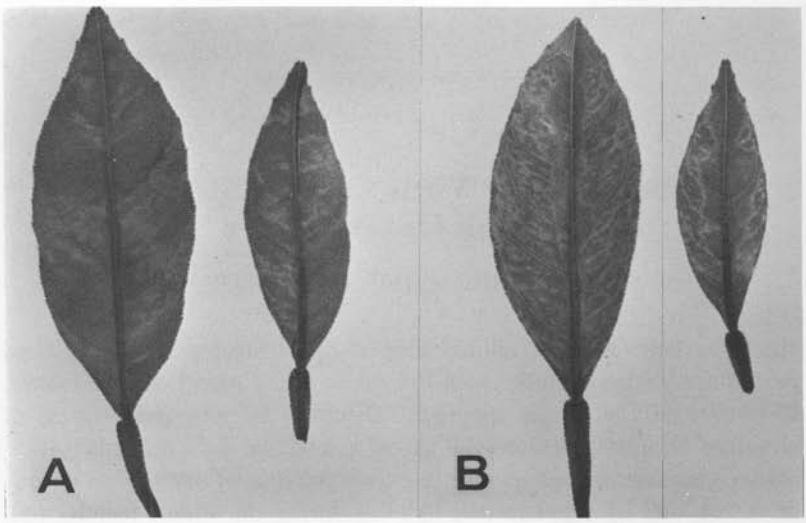

FIGURE 1. *Dweet tangor leaves showing symptoms of: A. Dweet mottle virus and B. Concave-gum virus.*

Methods and Results

SWEET ORANGE AS A SYMPTOMLESS CARRIER.—Indexing the Cleopatra tree (CRC 270) on seedlings of West Indian lime [*C. aurantifolia* (Christm.) Swing.], sweet orange, sour orange (*C. aurantium* L.), Kara mandarin, and *C. excelsa* Wester showed that it is free of psorosis-A, concave-gum, crinkly-leaf, tristeza, vein-enation, yellow-vein, and tatter-leaf viruses. The possibility that sweet orange may be a symptomless carrier of DMV was tested as follows: 4 seedlings of Madam Vinous sweet orange and 4 of Dweet tangor were inoculated with buds of CRC 270 and cut back immediately after inoculation to force new growth. Symp-

toms appeared on the 4 Dweet seedlings within 4 weeks, but no reaction appeared on leaves of the 4 sweet orange seedlings within three and one-half months. The DMV in the inoculated sweet orange seedlings was challenged with concave-gum virus by bud inoculation. To ascertain whether the sweet orange seedlings were infected with DMV and to test the possibility that sweet orange is a symptomless carrier, the top growth removed from the sweet orange was used to inoculate healthy Dweet tangor seedlings. Dweet mottle virus failed to protect the sweet orange seedlings when challenged with concave-gum virus, but the sweet orange grafts did transmit the DMV back to Dweet. This indicates that sweet orange can be a symptomless carrier of DMV. The symptoms of DMV and concave-gum virus on Dweet leaves are compared in Figure 1.

CROSS-PROTECTION AGAINST OTHER VIRUSES.—To investigate possible relationships between DMV and other psorosis-like viruses, the following experiment was performed: 2 of 4 Madam Vinous sweet orange seedlings, growing 4 per can, were inoculated with buds from CRC 270; the remaining seedlings were left as controls. The treatment was replicated 4 times and all plants were cut off about 20 cm above the soil level to force new growth. After 3 months, no symptoms had appeared on any of the sweet orange plants. Presumably, 2 seedlings of each can contained DMV. To test for cross-protection, the DMV in the 2 seedlings in each of the 5 cans was challenged by bud graft inoculation with the following viruses: concave gum, infectious variegation, crinkly leaf, and psorosis A (both lesion and non-lesion forms). The remaining 2 seedlings in each can were inoculated with the 5 viruses as positive controls. After 6 weeks, strong symptoms were produced by all 5 viruses on seedlings pre-inoculated with DMV, and on the control seedlings. Thus, no cross-protection occurred when orange seedlings containing DMV were challenged by concave-gum, infectious-variegation, crinkly-leaf, or psorosis-A viruses.

TESTING ADDITIONAL INDICATORS FOR DWEET MOTTLE VIRUS.—This experiment was designed to test other varieties of citrus for their reactions to DMV. Buds of CRC 270 were grafted into 2 seedlings each of Hinkley sweet orange, Eureka lemon [*C. limon* (L.) Burm. f.], King mandarin, Kara mandarin, Etrog citron [*C. medica* (L.) var. *ethrog* Engl.], Orlando and Sexton tangelos (*C. reticulata* x *C. paradisi*), Troyer citrange (*C. sinensis* x *Poncirus trifoliata*), Jochimsen grapefruit (*C. paradisi* Macf.), and Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.]. After 3 months, no visible leaf reaction appeared on any of these citrus varieties except the Etrog citron which showed a very mild exocortis reaction. Each inoculated plant was re-indexed on Dweet tangor, and

the results showed that DMV was transmitted back to Dweet. Under the conditions of this experiment, all of the varieties of citrus tested are apparently symptomless carriers. The tangelos and Palestine sweet limes inoculated with DMV will be observed for several years for xyloporosis virus symptoms.

The Cleopatra tree (CRC 270) was indexed for stubborn disease virus by the method of Calavan and Christiansen (2), i.e., side-grafting 2 shoots of CRC 270 into each of 5 Madam Vinous sweet orange seedlings and forcing a shoot of the latter. The results indicate that stubborn disease virus is not present in the tree CRC 270.

MIXING DMV WITH OTHER VIRUSES.—This experiment was performed to test possible synergistic effects on host reaction when DMV is mixed with other viruses. Buds from CRC 270 were inoculated into 2 seedlings of Dweet tangor and 2 of Valencia sweet orange. The same number and kind of plants were also inoculated with DMV in combination with yellow-vein, vein-enation, exocortis, tatter-leaf, cachexia, and tristeza viruses. The source plants containing the respective viruses had been indexed previously and apparently were free of other viruses. One set of plants was inoculated with DMV in combination with both yellow-vein and vein-enation viruses. As a positive control, Dweet tangor and sweet orange seedlings were inoculated with the previously mentioned viruses, but without DMV. After 8 weeks, the following results were observed: Dweet mottle virus produced symptoms on all Dweet tangor seedlings inoculated with CRC 270 budwood. No symptoms appeared on the inoculated Valencia sweet orange seedlings, with the exception of those trees inoculated with yellow-vein virus which developed typical symptoms of yellow-vein virus whether or not DMV was present. There was no evidence of a synergistic effect, or that other viruses enhanced the symptoms of DMV on Dweet, or produced symptoms on sweet orange.

Discussion and Conclusions

In 1960, Cleopatra mandarin, CRC 270, containing the Dweet mottle virus was rated virus-free when indexed on standard indicators. However, the use of new and more sensitive indicator plants showed certain viruses to be present. By these tests, the tree was found to contain a mild strain of exocortis virus, detected by the Etrog citron test (1), and a transmissible entity that causes a reaction only in Dweet tangor. Transmission studies indicate that many other citrus varieties are symptomless carriers of the Dweet mottle virus.

Weathers (9) demonstrated a synergistic effect between certain citrus

viruses. This occurs when two unrelated viruses are inoculated into the same host plant and the symptoms of one of the viruses are enhanced. No such effect was noted on Dweet tangor seedlings inoculated with DMV in combination with yellow-vein, vein-enation, yellow-vein and vein-enation, exocortis, tatter-leaf, cachexia, or tristeza viruses. Attempts to produce symptoms on otherwise symptomless sweet orange by combining DMV with these viruses failed.

There is no evidence that the symptoms produced by DMV on Dweet tangor are caused by exocortis, cachexia, or stubborn disease virsuses. These viruses produced no symptoms when tested on Dweet in these experiments or in the past. Dweet mottle virus appears to be unrelated to concave-gum virus because no clearly defined oak-leaf pattern is present in the leaf mottle produced by DMV on Dweet, whereas concave-gum virus usually produces such patterns (Fig. 1). Furthermore, DMV produces no symptoms on leaves of Kara or King mandarin, whereas these varieties are excellent indicators for concave gum (4). On the other hand, a mild strain of concave-gum virus that causes no symptoms on sweet orange protects against a challenge from a strain that can cause a more severe reaction (6), and the presence of a non-lesion strain of psorosis A in sweet orange usually protects against a challenge from the bark lesion form (6, 7). The failure of DMV to protect against a challenge from psorosis-A lesion or non-lesion strains indicates that psorosis A is unrelated to DMV. Wallace (8) recently demonstrated that crinkly-leaf virus will protect against a challenge from infectious variegation; he implied that they are related forms of the same virus, and that crinkly leaf is the milder strain. Because DMV failed to protect against concave-gum, psorosis-A, or infectious-variegation viruses, and because it affects only Dweet tangor, the authors consider it a distinct and previously unreported virus of citrus.

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