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Cross-Protection Studies with Strains of Concave Gum and Psorosis Viruses

CROSS-PROTECTION phenomena in sweet orange seedlings infected with various California strains of psorosis virus, including concave gum, were first reported by Wallace (16, 17), who pointed out that in some instances protection was only partial. The cross-protection or virus-strain-interference reaction has sometimes been useful in diagnosing psorosis in cases where leaves of sweet orange seedlings failed to show definitive symptoms.

In 1960 an indexing test in the quarantine greenhouse used for the University of California Citrus Variety Improvement Program (9, 10, 18) revealed a faint oak-leaf pattern on leaves of King mandarin (*Citrus nobilis* Loureiro) inoculated with budwood of PI (Plant Introduction, U. S. Department of Agriculture) 209532, a sweet orange [*C. sinensis* (L.) Osbeck] variety, Sanguina Murteras from Spain (10). Sweet orange, Eureka lemon [*C. limon* (L.) Burm. f.], *Citrus excelsa* Wester, and West Indian lime [*C. aurantifolia* (Christm.) Swing.] seedlings developed no symptoms. In further studies, inoculations with buds of PI 209532 in ten varieties of sweet orange seedlings did not yield diagnostic symptoms, whereas King mandarin produced recognizable oak-leaf patterns indicative of concave gum virus infection (12). Additional studies showed four varieties of mandarin and mandarin hybrids to be superior indicators for this mild strain of virus (11).

After failure in a preliminary test to provide protection in sweet orange seedlings against the psorosis A lesion-bark reaction, experiments were begun in the quarantine house to obtain evidence on the possible relationship of this mild strain to a California strain of concave gum virus which causes strong oak-leaf patterns on sweet orange leaves and to two

California sources of psorosis A virus, one from a non-lesioned propagation of a sweet orange having "A" type symptoms, the other from a lesioned propagation of a sweet orange with "B" type symptoms. The results of these experiments and similar cross-protection studies employing mostly concave gum and psorosis virus strains from Sicily and Spain are reported here. For purposes of this paper, the severe strain of concave gum virus from California is called concave gum S, and the mild strain from PI 209532, concave gum M.

Experiments and Results

EXPERIMENT 1. *Concave gum M virus challenged by psorosis A virus from non-lesion and lesion inocula.* Seedlings of West Indian lime, Koethen sweet orange, and King mandarin, five cans of each variety with four plants per can, were used. Plants 1 and 2 in each can were inoculated with two buds infected with virus of concave gum M and all plants were cut back to single stems eight inches high. New growth was observed during nine readings over a three-month period. All inoculated mandarin plants developed mild oak-leaf patterns; all sweet orange and lime plants remained symptomless.

After three months, the stems of all plants were again cut back to a height of about nine inches, and plant 2, previously infected with virus of concave gum M, was budded with psorosis A non-lesion inoculum from Fawcett No. 604 sweet orange. An identical inoculation was made in plant 3. Plant 1 was left as the concave gum M infected control, and plant 4 as the non-inoculated control. All varieties and replicates were identically treated and new growth was examined 14 times over a nine-month period. Shock symptoms were found on young leaves and shoots of all sweet orange and lime seedlings inoculated with virus of psorosis A, indicating that the virus of concave gum M failed to protect against shock symptoms induced by virus of psorosis A. No differences in severity of shock reaction or leaf symptoms were observed between plants 2 and 3 in any can. Mandarin seedlings developed no shock reaction.

One year after the concave gum M inoculations (nine months after the psorosis non-lesion inoculations), all sweet orange plants were grafted with psorosis A lesion-bark inoculum from Wallace No. 158-8. Lesion-bark symptoms first appeared within eight weeks and soon developed in all five plants preinoculated only with virus of concave gum M and in the five previously non-inoculated controls. No bark lesions appeared within one year on plants previously infected with virus from psorosis A

non-lesion inocula. These results suggest no relationship between concave gum and psorosis A viruses.

EXPERIMENT 2. *Concave gum M virus challenged by concave gum S virus.* Seedlings of Bessie, Bidwells Bar, Indian River, Koethen, Madam Vinous, Olivelands, Parson Brown, and Pineapple varieties of sweet orange (one can of each variety with four plants per can) were grown for nine months. Three plants in each can were then bud-inoculated with virus of concave gum M, the fourth being left as a control. All plants were then cut back to a height of eight inches and allowed to grow for eight months, after which they were cut back again. Concave gum S virus from Fawcett No. 571 sweet orange was then graft-inoculated into the control and into two of the three previously inoculated plants of each variety. Leaves were examined in three subsequent flushes of growth during a seven-month period. The eight plants inoculated only with concave gum S virus developed oak-leaf symptoms in at least one flush of growth. No leaf symptoms were found in the other 24 plants.

This experiment was repeated in part, with 18 Koethen sweet orange seedlings in nine cans. One plant in each can was inoculated with virus of concave gum M and both plants were cut back. Nine months later all 18 plants were cut back again, graft-inoculated with virus of concave gum S, and observed for five months. The numbers of leaves showing oak-leaf symptoms on each of the nine plants inoculated only with virus of concave gum S were: 38, 20, 20, 33, 19, 16, 17, 29, and 21. Plants previously inoculated with virus of concave gum M remained symptomless when challenge inoculated with virus of concave gum S from Fawcett No. 516 sweet orange, except that seven leaves on one plant showed very mild vein-clearing patterns. Results from both parts of experiment 2 showed that virus of concave gum M is able to protect sweet orange seedlings against the oak-leaf reaction caused by virus of concave gum S, thus indicating close relationship between these viruses.

EXPERIMENT 3. *Concave gum or psorosis virus from various sources challenged by psorosis A virus from lesion bark.* Following the failure of concave gum M virus to protect against psorosis A virus from non-lesion or lesion inocula, a study was made to determine whether infection with concave gum S virus or with strains of psorosis or concave gum viruses from several foreign citrus importations would protect against a challenge inoculation by psorosis A lesion bark. Two seedlings each of sweet orange, West Indian lime, Eureka lemon, and King mandarin were inoculated singly with buds from each of 14 foreign imports previously

found to be infected with concave gum or psorosis viruses (9, 10). Other seedlings of the same varieties were inoculated with virus of non-lesion psorosis A or with concave gum S virus from Fawcett No. 516. Symptoms were observed during a nine-month period, then the sweet orange seedlings were challenge inoculated with psorosis A lesion-bark inocula from Wallace 158-8. The imports used were: *Citrus sinensis* varieties Blood Navel P.I. 209527, Cadenera P.I. 218258, Maceteras P.I. 209864, Ovale P.I. 218015, Salustiana P.I. 209530, Sanguina Doblefina P.I. 209531, Sanguina Murteras P.I. 209532, and Valencia P.I. 218261; *C. limon* varieties Cocuzzaro P.I. 218007, Femminello P.I. 209626, Interdonato P.I. 209627, Lunario P.I. 209628, and Monachello P.I. 209629; *C. reticulata* variety Clementine P.I. 247751.

Inoculations from all of the foreign imports in this experiment resulted in oak-leaf symptoms or typical concave gum vein-clearing in the leaves of one or more indicator varieties. Considerable variation in degree and type of symptoms caused by different inocula suggests differences in virus strain complexes infecting the different imports, with one or more strains of concave gum virus usually present in the complex. The reactions of the various indicator plants to virus of psorosis A non-lesion inoculum were severe shock, severe leaf curl, and strong spotting without oak-leaf patterns. This syndrome differed markedly from that caused by any virus from the 14 imported citrus selections. The typical lesion-bark reaction that occurred in sweet orange seedlings preinfected with concave gum virus and challenged by lesion-bark inocula is shown in Figure 1.

The results of the challenge inoculations suggest close relationship between a virus from Valencia orange PI 218261, from Spain, and psorosis A virus, despite some differences in symptoms. Infection by virus from no other import provided protection against psorosis A lesion-bark virus, thus indicating that the concave gum or other viruses in these imports probably are not closely related to psorosis A virus.

Discussion

It is widely agreed that a plant infected with one virus usually retains its susceptibility to unrelated viruses, but is protected to some degree against different strains of the same virus (1, 7, 13). Numerous exceptions to this generalization have been noted (1, 13). The current results, while not constituting proof, do indicate a probable lack of close relationship between concave gum and psorosis A viruses. Additional indications that concave gum and psorosis A viruses may be unrelated are: (a) psorosis A virus causes an eruptive scaly bark, whereas concave gum

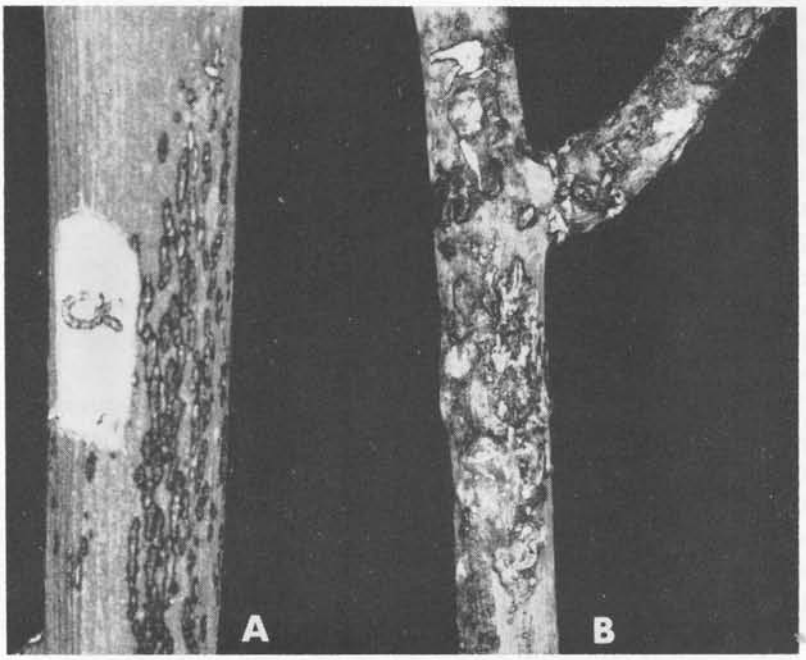

FIGURE 1. *Stems of sweet orange seedlings. A. Non-inoculated control showing normal vertical marks with normal cork development. B. Irregular circular patchy lesions produced by psorosis A lesion-bark inoculation made nine months after original infection by concave gum virus.*

virus causes concavities in sweet orange trunks and branches; (b) psorosis A virus, or a mixture of concave gum and psorosis A virus, usually causes a shock reaction of young leaves and shoots of West Indian lime and sweet orange seedlings, whereas concave gum virus alone apparently does not; (c) psorosis A virus rarely causes oak-leaf patterns but mixtures of psorosis A and concave gum viruses generally cause oak-leaf patterns on mandarin and sweet orange; (d) concave gum S virus does not cause small round spotting of leaves like that produced by psorosis A virus on sweet orange and West Indian lime seedlings following their recovery from shock.

The failure of concave gum virus to protect sweet orange seedlings against the lesion-bark reaction when challenged by psorosis A virus from lesion bark differs from results obtained with similar methods by Wallace (16, 17). Recent work by Fraser (5), indicating that preinfection with a crinkly leaf virus provided no cross protection against psorosis A virus, employed neither shock nor bark lesion reactions. The reasons for these

differing results are not known but may be due, wholly or in part, to different virus complexes or to different experimental methods and conditions. The current work used only four virus sources from California. Tristeza, yellow vein, vein enation, and tatter-leaf viruses were absent from these sources; but other viruses may have been present. The possible influence of exocortis virus, found in some of these sources, and other viruses on cross-protection phenomena with respect to concave gum and psorosis A has not been thoroughly studied. Lesion and non-lesion inocula from other sources might have caused different reactions in the preinfected plants used by the authors.

The protection afforded by psorosis virus from non-lesion bark of the California source and from Valencia PI 218261 against a challenge by lesion-bark psorosis virus substantiates earlier work by Wallace (16, 17), using similar materials. Although protection against lesion development was complete for the term of our experiments, it is assumed that lesions would eventually have developed near the bark-lesion grafts (16). The non-lesion inoculum of psorosis A was derived from a sweet orange seedling tree that required years, after its inoculation by H. S. Fawcett in 1939, to develop bark symptoms, as is customary in trees infected from non-lesion inocula. The fact that psorosis virus from non-lesioned tissues is able to provide complete but temporary protection against development of bark lesions, although by itself it eventually causes bark lesions, indicates, as suggested by Wallace (16), that two strains of virus, only one of which causes lesions, are present in all psorosis A infected trees. The lesion-forming strain, he reasoned, when circumstances permit, increases sufficiently in concentration to overcome the protection afforded by the non-lesion forming strain.

Recently Wu and Rappaport (19), in a study of interference between lesion and non-lesion strains of TMV, found the number of lesions to be a function of the total concentration of the mixed inoculum and the ratio of the strains. If interference between non-lesion and lesion-forming strains of the psorosis A complex functions similarly, inocula of lesion or non-lesion types would be expected to cause their respective symptoms only in tissues containing little or none of the interfering strain, except at the inoculation site where high concentrations of the dominant lesion-forming strain might cause a slowly developing bark lesion of the type mentioned above. Dominance of the lesion-forming strain of psorosis A virus, once achieved, usually is maintained in lesion bark and in unprotected sweet orange plants.

It appears that the concave gum M and S virus complexes used in

these experiments contained neither strain of the psorosis A complex and, therefore, were unable to protect against reaction to either type of psorosis A inoculum.

Concave gum is present in several countries of the Mediterranean region (2, 4, 14, 15) including Italy, where bark lesions are rare in some areas, and Spain, where bark lesions of psorosis A are common on Valencia and Washington Navel trees (6, 8). Apparently collectors of citrus budwood for the United States usually were able to detect and avoid psorosis A, but found no symptoms of concave gum in many infected trees from which they collected. The possibility that few trees of some varieties used in experiment 3 are infected by psorosis A virus (8) may have limited further the number of psorosis A infected imports.

It is apparent from these studies that mild strains of concave gum virus will not usually be detected when challenged by psorosis A virus, but may be detected in certain indicator plants directly or by challenge with a severe strain of concave gum virus.

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