Progress Report of Studies in California on Preimmunization Against Tristeza in Budded Citrus Trees

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The writers have reported previously (Wallace and Drake, 1972*b*, 1974) on the field performance of trees of Valencia orange on sour orange and Eureka lemon rootstocks preimmunized by virus isolates which remain in seedlings of sour orange and Eureka lemon after the plants recover from severe symptoms of seedling yellows. From other studies (Wallace and Drake, 1972*a*) it was concluded that seedling-yellows symptoms are caused by the action of an unidentified virus component in combination with tristeza virus. That conclusion is based primarily on the following evidence:

(1) After infection with a source of seedling-yellows (SY) virus and development of strong symptoms, seedlings of sour orange, lemon and grapefruit usually recover and thereafter show no symptoms or, at most, only a slightly reduced growth. Fully recovered plants retain only a tristeza-like virus, which we identify as RSY, that causes the tristeza lime reaction but does not cause seedling-yellows symptoms when inoculated into healthy seedlings of lemon and other seedling-yellows indicators. RSY virus also causes tristeza on budded citrus trees but, depending on the stock sources of SY virus from which the recovery isolates are derived, reaction of tristeza-susceptible budded trees ranges from extremely slight to typically severe tristeza symptoms.

(2) Aphid vectors can transfer the seedling-yellows virus complex but often after the aphids have fed on SY infected sweet orange they transmit only the tristeza component.

(3) Seedlings of trifoliate orange and Troyer citrange can be infected with SY virus but show no symptoms. When tissue-graft inoculations are made from these infected plants some of the resulting infections prove to be seedling yellows while others are tristeza.

Separation of tristeza virus from the tristeza-seedling yellows complex by the three methods described, namely, through plant recovery, by means of aphid vectors, and by passage through certain citrus hosts proves that tristeza virus is part of the SY complex. Further evidence of this is that all sources of inocula which caused seedling yellows have been found to contain tristeza virus.

The relationship between the unidentified component of the complex and tristeza virus has not been established. The inactivation or disappearance of that component during plant recovery indicates that it is not closely related to tristeza virus. On the other hand, the protection afforded by the RSY virus against reinfection with many sources of SY virus suggests relationship.

Details of the origins of SY virus sources used in our studies, their virulence, rate of plant recovery and degree of protection, and the description of many virus isolates or substrains separated from the stock sources by various procedures have been presented earlier (Wallace and Drake, 1972*a*). Our present paper constitutes a third report on field performance of trees of Valencia orange on rootstocks of sour orange and Eureka lemon preimmunized by isolates of RSY virus. It refers primarily to the condition of trees previously described (Wallace and Drake, 1972*b*, 1974) in experiments 1A, 2, 3, 4, which involve trees grown in field plantings at Riverside, California under natural exposure to tristeza infection.

EXPERIMENTS AND RESULTS

Experiment 1A. Healthy Valencia buds propagated on rooted cuttings from a SY2 recovered sour orange seedling. Cuttings from a sour orange seedling which had recovered from symptoms after inoculation with SY2 were rooted and budded with healthy Valencia orange. Five of these (group 1) were planted in the field without further treatment. Five similar trees (group 2) were experimentally inoculated with three field strains of tristeza virus before planting. Controls consisted of five trees (group 3) which were not experimentally inoculated and five trees (group 4) which were inoculated with the three sources of tristeza. Field plantings were made in July 1966. Trees in groups 1 and 2 carried R_1SY2 as the protecting virus.

Except for increases in size, the condition of the trees in this experiment was much as reported earlier (Wallace and Drake, 1974). In 1975, five preimmunized trees in group 1 ranged from 8 to 14 feet in height. All of these had dense, dark green foliage and heavy crops of ripe fruit. Two of the five trees had died in preimmunized group 2, which was challenge-inoculated with tristeza virus prior to planting in the field (Wallace and Drake, 1974). It has been demonstrated experimentally that one of these trees did not contain the R_1SY2 protecting virus. That suggested that this tree and possibly the other severely affected tree were healthy trees mistakenly placed in this group or else their sour orange rootstock cuttings, by chance, did not carry R_1SY2 virus. The three surviving trees of the preimmunized challenge-inoculated groups were 9, 10, and 12 feet in height, respectively. The two smallest showed slight indications of tristeza, chiefly small leaves. The largest tree was graded normal in appearance.

In group 3, consisting of five healthy controls not experimentally inoculated with tristeza virus, there were three surviving trees. One of these was normal, 13 feet in height. It must be assumed that this tree had escaped natural infection. The other two trees were not more than 7 feet high, definitely stunted from tristeza infection, but now in somewhat of an equilibrium stage of the disease. One of these and a preimmunized tree of group 1 are shown in fig. 1.

Fig. 1. Ten-year-old trees in experiment 1A. Left, grown from healthy Valencia bud on sour orange cutting carrying R_1SY2 virus. Right, a naturally-infected control tree developed from a healthy Valencia bud on a healthy sour orange cutting.

As reported previously (Wallace and Drake, 1974), it appeared that the experimental inoculation of the group 4 control trees with tristeza virus before they were planted in the field had little effect on them and that the tristeza virus strains used actually gave them some field protection. This still appeared to be so with one exception in 1975 when one tree developed general yellowing, presumably resulting from tristeza infection. It is of interest that this affected tree proved to be the one tree of this group that indexed negatively for tristeza in a test made in 1969. As was found occasionally by indexing, the experimental inoculation had failed. Thus with no protection from the mild tristeza virus used for the inoculation, this tree was more apt to react to subsequent natural infection. The remaining four trees of this group ranged in height from 8 to 13 feet with only slight foliage symptoms of tristeza on two of the smaller trees.

Experiment 2. Buds of Valencia orange carrying R_4SY4 or R_5SY4 propagated on healthy seedling rootstocks of sour orange and Eureka lemon. Buds were derived from 4-year-old greenhouse grown Valencia trees on rootstocks of rooted cuttings from two lemon seedlings which recovered from SY4. The sweet orange buds carried either R_4SY4 or R_5SY4 virus and were propagated on healthy sour orange and Eureka lemon seedlings. In July 1966, eight of these trees on sour orange rootstock and four on Eureka lemon stock were planted in the field. Four trees of healthy Valencia on sour orange and four on Eureka lemon were planted as controls.

In July 1975, only three of the eight control trees survived. Two of these were normal or nearly normal in appearance, apparently having escaped natural infection. The third tree was stunted and chlorotic, having shown evidence of being infected since 1968.

After 10 years in the field, seven of the eight immunized trees on sour orange rootstock were free of tristeza symptoms except for being stunted in varying degrees. The eighth showed some foliage symptoms of tristeza. Two of the four protected trees on Eureka lemon rootstock were small and bushy and showed some aspects of tristeza-affected trees.

The early, severe reaction of six of the eight control trees in this group and the performance of the immunized trees make it obvious that the presence of the RSY4 virus in these trees has provided good protection against naturally occurring strains of tristeza virus although the RSY4 virus itself has reduced the growth rate of most of the trees. Three preimmunized Valencia on sour orange trees of this experiment are shown in fig. 2.

Fig. 2. Three 10-year-old trees of experiment 2 grown from Valencia buds carrying R_4SY4 virus when propagated on healthy sour orange seedlings. The two control trees adjacent to this group died early from natural infection.

Experiment 3. Trees grown from healthy Valencia buds on sour orange seedlings inoculated with R_1SY4 virus before budding. This experiment originally consisted of four groups of trees but records on group 2 are no longer maintained because they involved a source of virus, R_1SY6, which caused severe tristeza damage to budded trees. The trees of this experiment still under study involve isolate R_1SY4 which has caused slight, if any, tristeza effects, and healthy control trees.

Group 1 was composed of four

healthy Valencia trees on rootstocks of sour orange inoculated with R_1SY4 and challenge-inoculated with SY4 prior to being budded. The challenge inoculation with SY4 had no effect on the sour seedlings and tests showed that the SY virus did not become established in the pre-immunized seedlings. After 9 years in the field all trees of this group are normal in appearance but are slightly smaller than the one control tree which has apparently escaped infection.

Group 3 trees were healthy Valencia propagated on sour orange seedlings pre-immunized with R_1SY4 virus but receiving no challenge inoculations. All trees graded good to excellent condition with no evidence of tristeza symptoms.

Group 4 trees were controls of healthy Valencia on healthy sour orange rootstock. One tree died from tristeza after reaching a height of 7 feet. Two trees, respectively 6 and 8 feet in height, have strong tristeza symptoms. The fourth control tree is 14 feet in height and normal in appearance. Apparently this tree has escaped natural infection. It has been useful for judging the stunting effect of the protecting viruses carried by the pre-immunized trees.

Experiment 4. Healthy Valencia on healthy sour orange rootstock with intermediate stem section of sour orange carrying R_1SY2 virus. Three-inch stem sections from a sour orange seedling which had recovered from SY2 were grafted to healthy sour orange seedlings. Later, a healthy Valencia bud was placed in these stem sections and forced into growth. The intermediate sections presumably carried R_1SY2 virus but no tests were made to determine if all were infected. Ten trees of this type were planted in the field in 1966. Five control trees propagated similarly but having healthy intermediate stem sections were included.

Three of the five healthy controls in this experiment showed tristeza symptoms by 1969, three years after planting, and all of these collapsed in 1970 and eventually died. Another control tree had developed severe tristeza symptoms by 1970 but remained alive and developed into a stunted tree with an equilibrium type of tristeza symptom. The fifth control tree, shown in fig. 3, was 10 feet high in 1975 and normal except for slightly dull foliage, indicating recent infection.

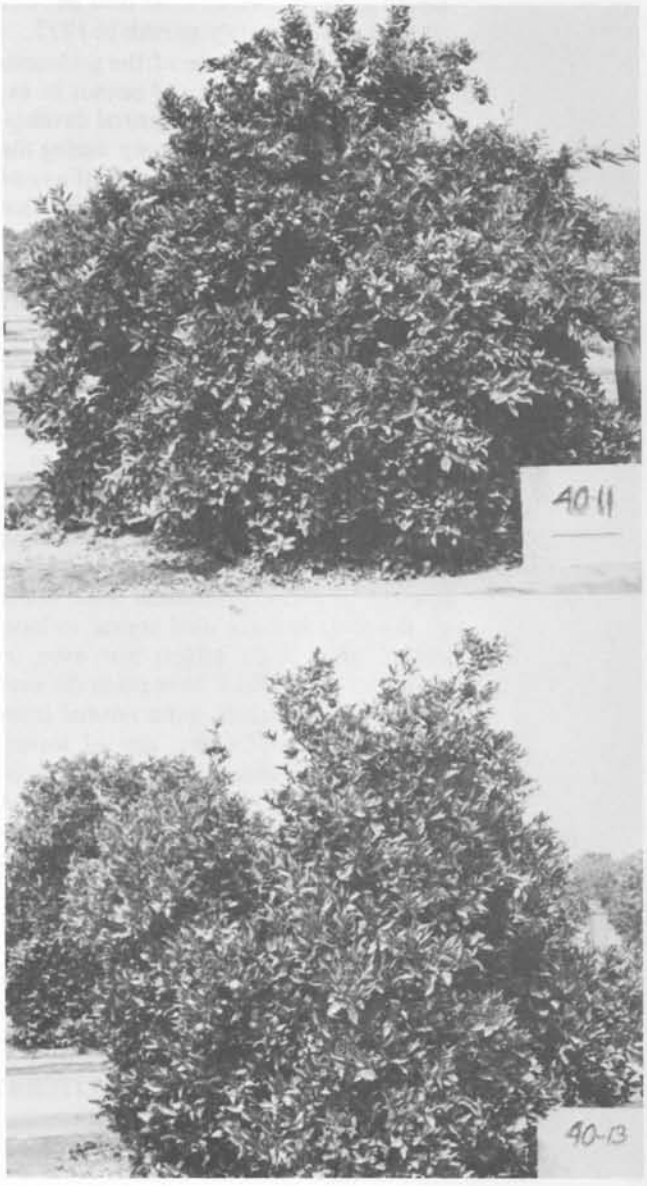

Fig. 3. Upper, a normal appearing "sandwich" tree of experiment 4, Valencia on sour orange with intermediate sour orange graft carrying R_1SY2 virus. Lower, a healthy control tree which was normal until 1975 when it began to show a slightly dull leaf color.

In the preimmunized group, 4 of 10 trees were graded normal in appearance in 1975, ranging in height from 9 to 11 feet (see fig. 3) and three were normal except for stunting and reduced size of leaves. The other three trees of this group collapsed in 1974. Two of these died but one showed slight recovery growth in 1975.

The collapse of three of the preimmunized trees in experiment 4 cannot be explained. The growth and general development of all trees in this group during the first 5 years was as good as that of any of the groups of protected trees. The fact that tristeza symptoms appeared 3 to 4 years later on the three severely-reacting trees of this protected group than on the four control trees that collapsed indicated that, initially, the preimmunized trees had some protection against reinfection in the field. This suggests that all 10 of the trees in the preimmunized group carried the R_1SY2 virus isolate when they were established. If that is the case, it will have to be concluded either that R_1SY4 does not provide protection against all of the tristeza virus strains to which these trees were exposed under field conditions, or that under certain conditions the R_1SY2 virus itself is capable of causing severe tristeza effects on trees of sweet orange on sour orange rootstock. In experiment 1A, some of the trees carrying this same R_1SY2 virus isolate have not held up as well as has been the case of trees protected by certain other RSY isolates. Some later planted trees carrying R_1SY2 virus are now under study and these may provide more information on the degree and duration of protection afforded by this particular virus isolate.

CONCLUSIONS

It is evident that the preimmunizing viruses have had some effect on the development of the experimental trees. Some of the virus isolates used appear to have caused only slight effects but even in groups of trees which have made the best growth and remained quite normal from the standpoint of color, size of leaves, and density of foliage, there is some variation in tree size. Certainly some of the virus isolates have provided tristeza-sensitive trees with a fairly high degree of protection against the naturally occurring field strains of tristeza virus to which they have been exposed for a period of nine years. On the other hand, a few preimmunized trees have developed symptoms as severe as those of healthy control trees except that, on the preimmunized trees, symptoms have been later in developing. The failure of some of the RSY virus isolates to provide uniform, permanent protection under field conditions is being investigated further.

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