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## Use of Seedling-Yellows Recovery and Protection Phenomena in Producing Tristeza-Tolerant, Susceptible, Scion-Rootstock Combinations

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CITRUS seedling yellows is caused by a complex of (a) tristeza virus, which is easily separated, and (b) an unidentified virus, which has not been isolated free of tristeza virus (1, 2, 3, 4, 5, 6). Seedlings of sour orange, grapefruit, and lemon are severely yellowed and stunted by the seedling-yellows virus complex but normally show very little evidence of infection with tristeza virus. Seedlings of sweet orange and mandarin are hosts of both the seedling-yellows virus complex and tristeza virus but are not seriously affected by either.

The seedling-yellows virus complex has not been found in orchard trees in California but is present in numerous introduced citrus selections maintained in varietal collections and in most trees of Meyer lemon. Studies have been made on 14 different field sources maintained as SY1, SY2, SY3, etc. One or more plants of sour orange, lemon, or grapefruit infected with 8 sources of this virus complex recovered from severe stages of seedling yellows and thereafter resumed very normal growth. When recovery was complete, inoculations from these plants did not cause seedling yellows, but contained the tristeza virus that Wallace, Martinez, and Drake (6) designated SYT virus. Isolates of SYT

derived from plants that recover from seedling yellows are now designated by the letters RSY to distinguish them from SYT isolates separated from the SY complex in other ways.

Plants of lemon that had recovered from seedling yellows after graft-inoculation with the original sources of SY virus were not affected by inoculation with the SY source from which they recovered. They were protected also from some other SY sources, but the degree and extent of protection depended upon the SY virus isolate from which the plants recovered and the isolates used for challenge-inoculations (5).

This paper describes some preliminary results of efforts to transfer the protection in SY-recovered citrus to tristeza-susceptible budded trees. Experimental trees consisted of Valencia orange on rootstocks of sour orange or Eureka lemon. These susceptible combinations were prepared as described below, to provide trees carrying RSY virus—and virus-free control trees—for study of their performance under field conditions. Some of the trees were experimentally inoculated with naturally occurring tristeza virus before or after they were planted in the field. All were exposed to natural infection but were in a location where in-

fection by aphid vectors occurred somewhat slowly and irregularly.

### Results

EXPERIMENT 1A: HEALTHY VALENCIA BUDS PROPAGATED ON CUTTINGS FROM SY2 RECOVERED SOUR ORANGE.

—Ten trees were propagated from healthy Valencia buds on cuttings rooted from sour orange seedling No. 99-65, which had recovered from seedling yellows stock source SY2 derived originally from a Meyer lemon. These cuttings carried R<sub>1</sub>SY2 virus isolate. The rootstock cuttings were budded in a glasshouse in November 1965. In April 1966, 5 of these budlings were inoculated with tristeza virus, each receiving inoculum from 3 different isolates, T1, T2, T3. Controls consisted of 5 healthy Valencia on healthy sour orange cutting rootstocks inoculated with the 3 tristeza isolates and 5 not inoculated. These 20 trees were planted in the field in July 1966.

After 4 years in the field, with 1 exception, the 10 trees on R<sub>1</sub>SY2-infected sour orange rootstock are either normal or show only slight indications that they are infected. One tree that developed normally for 3 years began to show symptoms of tristeza thereafter and reached a severe stage of disease within 6 months. Because its reaction differs so strikingly from others in this group, tests are being made to determine whether—by error—its rootstock was a healthy sour orange cutting. If the virus from it does not protect lemon seedlings against SY2, this will establish that it does not

belong with the R<sub>1</sub>SY2-protected group. Among the 10 control trees, 5 have developed tristeza within 4 years, and some of these are in advanced stages of disease.

Some of the R<sub>1</sub>SY2-infected trees differ very little from the healthy control trees that remained free of symptoms, but others are slightly stunted and have smaller than normal leaves. Indications are that the R<sub>1</sub>SY2 virus in the sour orange rootstocks has had a very slight effect on the Valencia tops up to this time. It appears that the trees on the R<sub>1</sub>SY2-infected rootstocks have thus far been protected against natural infection as well as against the experimental inoculation they received with 3 isolates of tristeza virus.

EXPERIMENT 1B: HEALTHY VALENCIA BUDS PROPAGATED ON CUTTINGS GROWN FROM SOUR ORANGE NOT COMPLETELY RECOVERED.—Cuttings were made from sour orange plant 99-53, which appeared to have recovered from stock source SY1, a virulent isolate of seedling-yellows virus from a tree of African lemon introduced in 1913 from South Africa. Some of the rooted cuttings grew poorly and showed symptoms of seedling yellows, but others from the same source appeared normal. After budding these sour orange cuttings with Valencia orange buds, some budlings developed symptoms of tristeza but some showed no obvious symptoms while growing in the glasshouse. Twelve of the most normal trees were planted in the field in June 1967. During the first year under field conditions 5

of them developed tristeza. By the summer of 1969, 1 of the 5 was dead and the other 4 were severely affected and, upon indexing, yielded seedling-yellows virus.

Some of the remaining 7 trees of this group appear normal, but others have been affected slightly by the virus in them. They have not been indexed but apparently carry only recovery SYT virus ( $R_3SY1$ ). Had SY virus been present in the sour orange cuttings used for rootstocks, symptoms would certainly have developed by this time. Other experiments not discussed in this paper have shown that under field conditions, tristeza symptoms appear quickly in trees developed from healthy sweet orange buds propagated on sour orange rootstocks infected with SY virus.

EXPERIMENT 2: BUDS OF VALENCIA CARRYING RECOVERY SYT ( $RSY$ ) VIRUS PROPAGATED ON HEALTHY SOUR ORANGE AND EUREKA LEMON SEEDLINGS.—A 2-year-old tree developed in the glasshouse from a healthy Valencia bud on a cutting from SY4-recovered Eureka lemon seedling No. 101-28, carrying  $R_2SY4$  virus, provided buds for propagation to healthy seedlings of sour orange and Eureka lemon. Fifteen months after budding, 8 of the  $R_2SY4$ -infected trees on sour orange rootstock and 4 of the same infected Valencia on Eureka lemon rootstock were transferred to a field plot. Four controls of healthy Valencia on sour orange and 4 on Eureka lemon rootstocks were included.

After nearly 4 years in the field, the

$R_2SY4$ -infected trees on sour orange rootstock are continuing growth without showing strong symptoms of tristeza, but on some trees leaf size is reduced. Some early fruiting and a heavy set of fruit in 1969 indicate that the virus in these trees is having a mild effect. All 4 control trees have become infected by aphid inoculation and 3 of these are in an advanced stage of tristeza.

During the first 3 years in the field, the 4 trees developed from  $R_2SY4$  Valencia buds on healthy Eureka lemon rootstock grew more vigorously than trees of the same buds on sour orange rootstock. Only 1 of them showed symptoms of tristeza—dull, smaller leaves and slightly reduced vigor. In comparison, 2 of the 4 healthy controls had died from tristeza and another was in an early disease stage within 2 years (Fig. 1).

Considering the extreme tristeza susceptibility of trees of sweet orange on Eureka lemon rootstock, the vigorous growth of the  $R_2SY4$ -infected trees and the almost total absence of disease symptoms for more than 4 years from time of propagation indicated that they would continue to develop quite normally and not be affected by reinfection with tristeza virus. However, during the fourth year in the field, 3 of the 4  $R_2SY4$ -"protected" trees on Eureka lemon rootstock developed moderately severe tristeza symptoms. It is suspected that this resulted from a delayed or gradual reaction to the  $R_2SY4$  virus rather than from reinfection with strains of tristeza virus against which  $R_2SY4$  virus does not

protect. Regardless of the explanation, it is evident that this isolate of RSY virus does not provide permanent protection to such susceptible scion and rootstock combinations as sweet orange on Eureka lemon. Whether or not RSY isolates from other sources of SY virus will do so can be determined only by further experimentation.

EXPERIMENT 3: TREES GROWN FROM HEALTHY VALENCIA BUDS ON SOUR ORANGE SEEDLINGS PREVIOUSLY INOCULATED WITH RSY4 VIRUS.—In the glasshouse, healthy sour orange seedlings were graft-inoculated in September 1964 from lemon seedling No. 89-437, which had recovered from symptoms of seedling yellows after infection with SY4 virus. Numerous tests showed that only re-

covery seedling-yellows-tristeza virus—in this instance R<sub>1</sub>SY4—remained in lemon 89-437. Three months later, some of the inoculated seedlings (group 1), were challenge-inoculated with SY4, the seedling-yellows isolate used for the original inoculation of lemon 89-437. Others of the R<sub>1</sub>SY4-infected sour orange seedlings (group 2) were inoculated with A<sub>1</sub>SY6, an aphid isolate of seedling-yellows virus from stock source SY6. Group 3 consisted of R<sub>1</sub>SY4-infected seedlings that received no challenge-inoculation. The sour orange seedlings carrying R<sub>1</sub>SY4 virus (group 1) were unaffected when challenged with SY4, but those inoculated with A<sub>1</sub>SY6 virus (group 2) showed some leaf mottle and were slightly stunted.

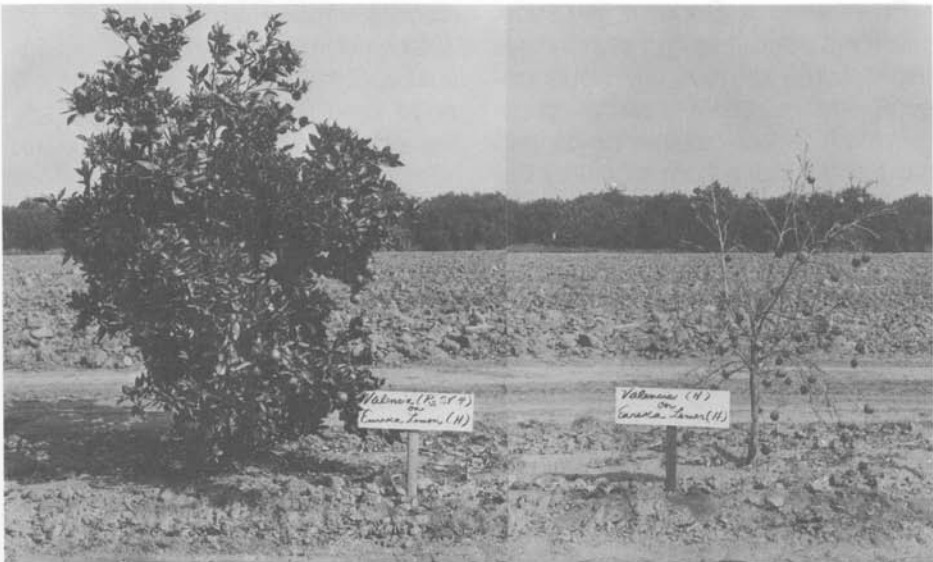


FIGURE 1. Trees of Valencia orange on Eureka lemon rootstock, a combination extremely sensitive to tristeza. Tree on left grown from Valencia bud carrying recovery SYT virus (isolate R<sub>2</sub>SY4). Tree on right grown from healthy Valencia bud. Both 4½ years from budding and exposed to natural infection in field 3½ years.

Group 3 sour orange seedlings developed normally.

In July 1965, the 3 groups of sour orange seedlings described above, and healthy sour orange seedlings, were budded with healthy Valencia orange. As the Valencia tops developed, all trees of group 1 on sour orange seedling rootstocks inoculated with R<sub>1</sub>SY4 and challenged with SY4 before budding, all of group 3 on unchallenged R<sub>1</sub>SY4 infected rootstocks, and the healthy controls developed normally. But even under glasshouse conditions, the budded trees in group 2 on the R<sub>1</sub>SY4-infected sour orange rootstocks, which prior to budding were challenge-inoculated with A<sub>1</sub>SY6, developed symptoms of tristeza. In July 1966, 4 trees from each of the 3 groups and 4 healthy control trees

were planted in the field. The trees of group 2 (challenge-inoculated with A<sub>1</sub>SY6) developed tristeza symptoms rapidly and became severely diseased. Index tests showed that they contained SY virus. The R<sub>1</sub>SY4-infected trees challenged with SY4 (group 1), and those not challenged (group 3), appeared normal in the summer of 1969 and did not differ from 3 of the 4 healthy control trees that had not yet developed symptoms from natural infection with tristeza virus. A "protected" tree and a control tree that developed tristeza symptoms by 1968 from natural infection and then reached an equilibrium stage are shown in Figure 2.

Inasmuch as the trees of sweet on sour orange rootstocks infected with R<sub>1</sub>SY4 virus have not developed tristeza symptoms up to their present



FIGURE 2. Trees of Valencia orange on sour orange rootstock. Left. Healthy Valencia bud propagated on a sour orange seedling inoculated 10 months previously with recovery SYT virus (isolate R<sub>1</sub>SY4). Tree is in normal condition after 3½ years in field. Right. Naturally infected control. This tree developed tristeza symptoms after 2 years in field, apparently from a mild strain of virus, which has permitted the tree to reach an equilibrium stage of tristeza.

age of 4 years, the chances seem good that they will not be affected by any of the naturally occurring strains of tristeza virus to which they may be exposed in the field. The fact that R<sub>1</sub>SY4 virus did not protect the trees against seedling-yellows isolate A<sub>1</sub>SY6 demonstrates, however, that field protection would not be assured if the seedling-yellows virus complex was being spread by aphid vectors in California, as is known to occur in some countries.

EXPERIMENT 4: TREES DEVELOPED FROM HEALTHY VALENCIA ON HEALTHY SOUR ORANGE ROOTSTOCKS WITH INTERMEDIATE STEM SCION FROM SEEDLING-YELLOWS RECOVERED SOUR ORANGE.—Trees were formed from healthy sour orange rootstocks and healthy Valencia tops with an intermediate stem section of 3–4 in. from seedling-yellows recovered sour orange 99-65 carrying R<sub>1</sub>SY2 virus. Ten of these trees and 5 control trees propagated similarly but from healthy parts were planted in the field in 1966. In late summer of 1969 4 of the 5 control trees had developed tristeza symptoms as a result of natural infection. The R<sub>1</sub>SY2-infected "sandwich" trees were either normal or showed very slight tristeza symptoms. Most of them are slightly smaller than the 1 unaffected control tree. The presence of R<sub>1</sub>SY2 virus is apparently causing some retardation of growth.

#### *Discussion and Conclusions*

On the basis of preliminary studies it appears that seedling-yellows-tristeza (RSY) virus from plants that

recover from some sources of the seedling-yellows virus complex provides protection against tristeza to trees of some stionic combinations that normally are very susceptible to tristeza virus. As is the case with lemon seedlings, however, a given isolate of RSY virus does not protect against all SY isolates. Also, from other studies, there is a suggestion that RSY virus from some SY sources is of such virulence that it causes tristeza symptoms on healthy budded indicator trees when inoculated to them. This is being investigated further to establish that such RSY isolates are entirely free of SY virus because it has been determined that small amounts of SY virus, even of reduced virulence, cause the tristeza reaction on budded indicator trees.

The results of these investigations may explain why McClean (2, 3) sometimes obtained virus from grapefruit trees in South Africa that caused the tristeza lime reaction but did not cause decline of trees of sweet orange on sour orange rootstock. Possibly some of McClean's isolates were similar to the experimentally selected isolates of RSY virus described in this paper. From studies in Australia, Fraser (1) reported that bud-inoculation from orchard trees of lemon, grapefruit, and sour orange to budded indicators caused no tristeza symptoms or only slightly reduced growth. These reactions also may have resulted from the recovery reactions and protection described herein.

Conferring this type of "artificial" protection against tristeza to citrus

trees of sweet orange on sour orange rootstocks is largely of academic interest at present. Despite the existence of several satisfactory tristeza-tolerant rootstock species, sour orange would be a useful and possibly a preferred rootstock in some regions if this type of protection can be developed to the point

where trees on sour orange rootstock will produce economic yields continuously under exposure to tristeza. Continued observation of the experimental trees described and other investigations now in progress will determine whether this type of protection is lasting and whether it has any practical application.

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