Field Performance of Tristeza-susceptible Citrus Trees Carrying Virus Derived from Plants that Recovered from Seedling Yellows

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Earlier papers (*1, 3, 4, 5, 6*) described recovery of plants of lemon, sour orange, and grapefruit, the disappearance of yellows-inducing virus during recovery, and retention of a virus having all of the attributes of tristeza virus. It was also reported that the recovered plants were unaffected when reinoculated with the strain or source of seedling yellows virus from which they had recovered, and had degrees of protection ranging from slight to complete against other isolates of seedling yellows virus. Furthermore, seedlings of Eureka lemon and sour orange infected by tissue-graft inoculation from recovered plants were protected against seedling yellows to the same degree as recovered plants. In other words, citrus seedlings which normally develop severe symptoms when inoculated with seedling yellows virus were unaffected if previously inoculated with virus from a recovered plant (*5*).

In 1969, the authors presented results of attempts to transfer the protection found in seedling yellows-recovered plants to tristeza-susceptible budded trees of Valencia orange on rootstocks of sour orange and Eureka lemon (*6*). This paper constitutes a second report on the performance of "preimmunized" budded trees grown at Riverside, California, under field exposure to natural infection with tristeza virus.

SOURCES AND IDENTITY OF VIRUS ISOLATES

The virus that causes seedling yellows has not been found in orchard trees of commercially-grown citrus varieties in California, but has been obtained from many Meyer lemon trees as well as some trees of other introduced varieties maintained in varietal collections. Early in the studies of seedling yellows in California it was discovered that seedling yellows virus obtained from different carrier trees differed in virulence. It also was determined that subcultures obtained from the original field sources likewise differed in virulence and that a subculture did not consist of a single strain of virus. For that reason, the original sources of seedling yellows virus established for study by graft transmission from an infected tree are referred to as "stock sources" or "stock cultures," and the various subcultures obtained by different methods are described as virus "isolates." Stock cultures of seedling yellows virus from different carrier trees were maintained in the greenhouse, either in propagations from the source or by inoculation to seedlings of West Indian lime. The various subcultures were maintained in lime seedlings. Lime has not recovered from symptoms nor caused any detectable qualitative change in the seedling yellows virus complex.

Each stock source of seedling yellows established for study was identified by the letters SY, and assigned a number; for example, SY1, SY2, SY3. When seedlings of lemon, sour orange, or grapefruit recovered from symptoms caused by these SY sources, the tristeza virus isolates remaining in them, if retained for study, were identified as RSY, with each isolate carrying the number of the stock source of SY from which it was

derived. When two or more separate recovery isolates came from the same SY stock source, they were assigned subnumerals, R_1SY1, R_2SY1, R_3SY1, R_1SY2, and so forth.

Martinez and Wallace (*1, 2*) demonstrated that aphid vectors of tristeza virus, *Aphis gossypii* Glover and *Toxoptera citricidus* Kirk., can either transmit the entire SY complex or they can selectively transmit the tristeza component from a plant carrying that virus complex. Studies of such transfers showed that an aphid-transmitted isolate of SY usually differed from the SY stock source from which it was derived. Thus, when these isolates were maintained for study they were identified by the letters ASY, with the number of the stock culture from which they were transmitted, ASY1, ASY2, and so forth. When aphids selectively transmitted tristeza from an SY source, these virus isolates differed from the tristeza virus (RSY) separated from SY by plant recovery, in their ability to protect against SY virus. It was necessary to refer to these as aphid-separated seedling yellows-tristeza (ASYT). These isolates were maintained separately, and identified by appropriate numbers indicating their origin. It was also necessary to use additional code symbols for other virus isolates studied, particularly in experiments on the interaction or cross-protection between different isolates of the seedling yellows complex. For this paper, however, a complete list of the identifying symbols is not needed.

RESULTS

This paper refers largely to experiments 1A, 2, 3, and 4 previously described by Wallace and Drake (*6*), in which virus from seedlings of lemon and sour orange that had recovered from seedling yellows was graft-inoculated, by different methods, to trees of Valencia orange on rootstocks of either Eureka lemon or sour orange. These experimental trees were developed in greenhouses, and planted in the field in 1966, where they are exposed to naturally-occurring tristeza virus, but where reinfection takes place somewhat slowly and irregularly. This paper describes the condition of these experimental trees as of July, 1972.

Experiment 1A. In this experiment, healthy Valencia buds were propagated on rooted cuttings from SY2-recovered sour orange. Ten trees were developed from healthy Campbell Valencia orange buds on rooted cuttings from sour orange 99–65 (experiment and plant number) that had recovered from seedling yellows stock source SY2, originally from a Meyer lemon tree. These sour orange cuttings carried virus isolates R_1SY2. Five of these trees (group 1) received no further treatment. Three months before being planted in the field, five trees (group 2) were experimentally inoculated with three field isolates of tristeza virus, T1, T2, and T3. Controls consisted of five trees of healthy Valencia on healthy sour cuttings not inoculated experimentally (group 3) and five inoculated with tristeza isolates T1, T2, T3 (group 4).

After three years in the field, the 10 protected trees were either normal or only slightly reduced in vigor. Three of the noninoculated controls were in early stages of tristeza from natural infection, and two of the five experimentally inoculated controls were showing mild symptoms.

In July, 1972, six years after planting, the condition of these trees was as follows:

Group 1. Three trees were normal, one was 11 feet (fig. 1) and two were 8 feet in height, all with medium crops of ripe fruit. The two remaining trees of this group were 8 feet in height, but showed some symptoms of tristeza—chiefly dull leaves, slightly thin foliage, and a heavy crop of ripe fruit.

Fig. 1. Left, tree developed from healthy Valencia bud propagated on a rooted cutting from sour orange that recovered from SY2. The protecting virus is R_1SY2. Right, check tree of healthy Valencia on healthy sour orange cutting. This tree developed symptoms of tristeza in 1969 from natural infection. It showed typical symptoms throughout 1970, but made some recovery thereafter and reached an equilibrium stage. Trees planted in field July, 1966; photographed July, 1972.

Group 2. Three trees were rated good to excellent in appearance, and were 7 to 9 feet in height, with a good crop of ripe fruit. One tree that showed mild tristeza symptoms in October, 1969, collapsed in 1970 and subsequently died. The severe reaction of this protected tree suggested that, by mistake, the sour orange cutting rootstock had been virus-free when it was budded. Possibly it had been intended for use as one of the healthy control trees. Seedling limes inoculated from it gave the tristeza reaction, but lemon seedlings inoculated with this source of virus reacted severely when challenge-inoculated with SY2 virus. This established that the tree that collapsed from tristeza did not carry the protecting virus R_1SY2. The fifth tree of this group also collapsed in May of 1972. No tests were made as to the ability of the virus in that tree to protect lemons against SY2, and thus, no conclusions can be drawn.

Group 3. One tree was normal, 8 feet in height, with a medium crop of ripe fruit. One tree positive for tristeza since 1969 was now in equilibrium stage, stunted and without ripe fruit (fig. 1). One tree positive for mild tristeza was stunted, with thin foliage, and bore a medium crop of ripe fruit. Two trees have been dead since 1970.

Group 4. One tree showed slight tristeza symptoms, but in equilibrium stage. It had thick foliage of good color,

and a light crop of ripe fruit. The remaining four trees were in good condition, ranging in size from 7 to 9 feet. One tree had no crop of ripe fruit; three trees had medium crops. Four of the five trees in this group were indexed on lime seedlings in 1969 and all were positive for tristeza. Up to 1972 this group was in better condition than were the controls in group 3. Apparently the tristeza virus strains used in the early greenhouse inoculation have had very little effect on the group 4 budlings and have possibly provided them with some protection against field infection.

Experiment 2. In this experiemnt, buds of Valencia orange carrying RSY4 virus were propagated on healthy seedling rootstocks of sour orange and Eureka lemon. The experiment was described (*6*) as consisting of trees of Campbell Valencia orange on rootstocks of sour orange and Eureka lemon carrying a single isolate of RSY4 virus, mistakenly identified in the original report as R_2SY4. A review of the records showed that two SY4-recovered isolates were used in preparing the trees of experiment 2 that eventually were placed in field plantings. The trees now under study in this experiment were developed on sour orange and Eureka lemon, with buds from two 2-year-old Valencia trees propagated earlier, in the greenhouse, on rooted cuttings from two different SY4-recovered lemon seedlings, 101-28 and 89-439. Because another isolate identified originally as R_2SY4 is now under study in other field trees, it is necessary to identify the two isolates used in experiment 2 as R_4SY4 and R_5SY4. Four trees on sour orange and four on Eureka lemon carry R_4SY4, and four trees on sour orange have R_5SY4 as the protecting virus. Controls consist

Fig. 2. Left, tree grown from R_4SY4-infected Valencia bud on healthy sour orange seedling after six years in field. Center, healthy check tree that developed mild tristeza in 1969 and continued to make some growth. Right, naturally infected check tree that developed symptoms in 1969 and collapsed in spring of 1970. Right, photograph taken July, 1970; other trees photographed July, 1972.

of four trees on sour orange and four trees on Eureka lemon, started as healthy plants.

Three of the four controls on sour orange rootstocks had developed tristeza from natural infection within three years after planting. In 1972 two of these were dead, one was in a stunted, tristeza equilibrium condition, and the fourth appeared normal except for some stubborn-like foliage. In July, 1972, the four trees of Valencia on sour orange carrying R_4SY4 virus were 8 to 9 feet in height and in good condition, except that on some, leaves were smaller than normal, and one tree had dense, upright shoots somewhat like the growth of stubborn-affected trees. Figure 2 shows one of these trees in comparison with a check tree which developed mild tristeza in 1969 but reached an equilibrium stage of disease, and a check tree that collapsed in the spring of 1970.

The four trees of Valencia on sour orange carrying R_5SY4 virus were smaller than those with R_4SY4 virus, with heights of 6 to 7 feet. All had good color, but dense foliage and a slight appearance of stubborn-affected trees.

Two of the four check trees of Valencia on Eureka lemon became infected soon after planting in the field, and were dead by early 1969. Another developed early-stage symptoms in 1970, but continued to make some growth. In the summer of 1972, it was 5½ feet tall, with small leaves and general chlorosis. The fourth tree of this group was 9 feet in height, and normal except for a slight stubborn appearance.

Among the four trees of Valencia on Eureka lemon rootstocks carying R_4SY4 virus, two were 8 feet tall and almost normal in appearance. Another was 6½ feet in height and showed slight symptoms of tristeza. The fourth tree in this group was in an equilibrium stage, with thick foliage of large, slightly pale leaves. We believe that the disease effects shown by these trees are caused by the R_4SY4 virus carried by them. It is not surprising that trees of this extremely susceptible combination should suffer some injury from this virus. Apparently, however, the presence of this "protecting" virus has prevented severe tristeza reactions from natural infection.

At this stage of investigation we may conclude that (1) both R_4SY4 and R_5SY4 virus isolates have protected tristeza-susceptible citrus trees against field infection with tristeza virus; (2) both isolates cause some injury to the host trees; and (3) one isolate causes more injury than the other even though both were derived through plant recovery from the same stock source of SY4 virus.

Experiment 3. Trees were grown from healthy Valencia buds on sour orange seedlings previously inoculated with R_1SY4 virus. Healthy sour orange seedlings were graft-inoculated in September, 1964, from lemon 89-437 that had recovered from symptoms after inoculation with SY4 virus. The protecting virus, R_1SY4, was established in the rootstock seedlings for three months before some (group 1) were challenge-inoculated with SY4, the virus source used for the original inoculation of lemon 89-437, and others (group 2) were challenge-inoculated with an aphid-transmitted isolate of SY6. Seedlings inoculated only with R_1SY4 comprised group 3; group 4 was made up of healthy, noninoculated seedlings. Group 1 seedlings were not affected by inoculation with SY4, and this virus did not become established in them. The R_1SY4-infected sour orange seedlings in group 2 developed some symptoms of seedling yellows after inoculation with A_1SY6. All seedlings in groups 1, 3, and 4 developed normally in the greenhouse. Nine months after the original inoculation, all seedlings of groups 1, 3, and 4 and the most vigorous of those in group 2 were budded with healthy Chapman Valencia buds. For a period of one year, in the greenhouse, the budling trees of groups 1, 3, and 4 de-

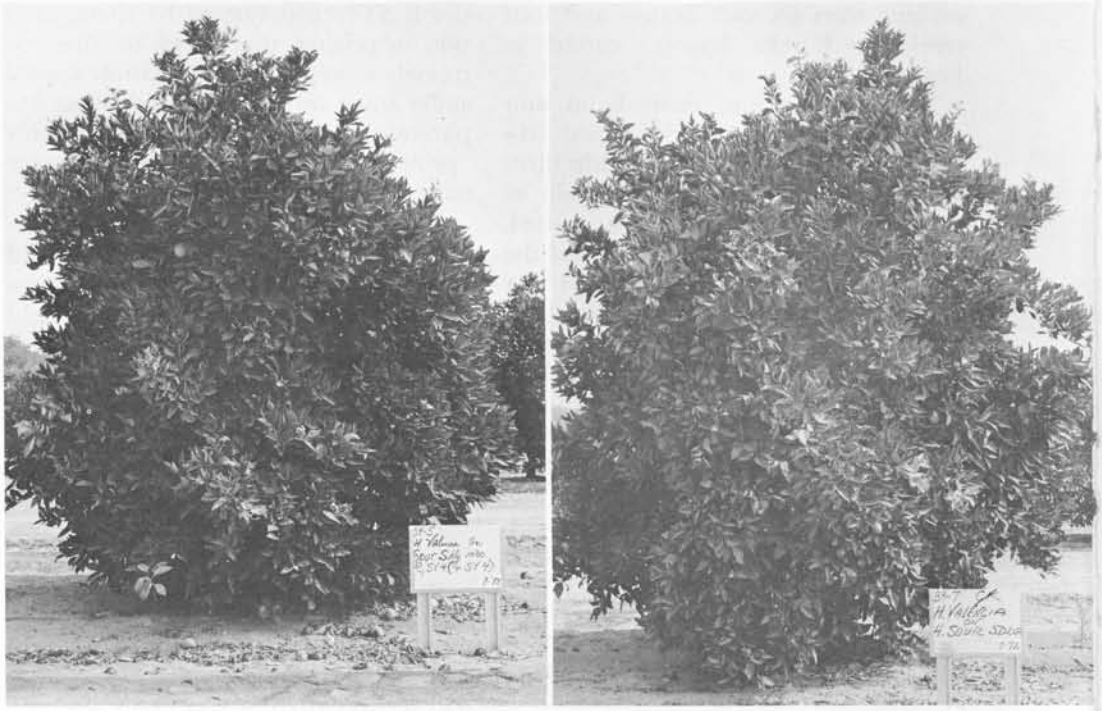

Fig. 3. Comparative growth of R_1SY4-infected and healthy Valencia on sour orange trees. Left, tree of group 1 (exp. 3) grown from healthy bud on sour seedling previously infected with R_1SY4 virus and challenged with SY4. Right, a check tree of healthy Valencia on healthy sour seedling that apparently had escaped natural infection for six years in field. It indexed negative in 1970.

veloped normally. Those in group 2, on R_1SY4-infected sour orange rootstocks challenge-inoculated with A_1SY6 before being budded, grew slowly, and developed symptoms of tristeza. When the four best trees of this group were planted in the field, they made very little growth. Indexing tests demonstrated that they contained SY virus. This proved that the presence of R_1SY4 virus did not keep A_1SY6 from becoming established in the sour orange seedlings and subsequently causing severe tristeza on trees developed from healthy Valencia buds propagated on them. These trees (group 2) are given no further consideration. After six years in the field, the conditions of the trees in groups 1, 3, and 4 were as follows:

Group 1. All four trees appeared normal in 1972, with medium to heavy crops of fruit, but three were stunted, with heights of 7 to 8 feet. The fourth tree was 10 feet tall, with no evidence of tristeza. All had indexed tristeza-positive in 1970. A tree of this group is shown in figure 3 in comparison with the check tree that escaped natural infection.

Group 2. All four trees were uniform in appearance, with heights of 10 feet. The 1972 crop of fruit was light to medium. Type of growth resembled that of trees with mild effects of stubborn disease.

Group 3. Two trees were normal in appearance, and 9 to 10 feet in height. One of these indexed negative for tristeza in 1970, but the other indexed positive. One of the four control trees that first showed tristeza symptoms in the summer of 1971 had reached an advanced stage in the spring of 1972. The fourth control tree, which had shown

mild tristeza by the summer of 1968, had developed to an equilibrium stage of the disease. It was 6 feet in height, with thick foliage of good color.

We may conclude from this experiment that: (1) R_1SY4 virus isolate has had little, if any, effect on trees of Valencia on sour orange for a period of seven years after propagation; and (2) R_1SY4 virus has apparently protected tristeza-susceptible trees against injury from the naturally-occurring strains of tristeza to which they have been exposed during the six years they have been in the field.

Experiment 4. Stem sections from SY2-recovered sour orange 99-65 were established on healthy sour orange seedlings. Later, healthy Campbell Valencia buds were placed in the grafted sections and forced into growth to provide "sandwich" trees containing R_1SY2 as the "protecting" virus isolate. Healthy control trees were grown in the same manner, with intermediate stem sections from healthy sour orange seedlings.

Three of five healthy controls of this experiment had collapsed by 1970, and soon died. A fourth control tree was in a severe stage of tristeza in 1972, with a very heavy crop of ripe fruit, suggesting an imminent collapse. The fifth control tree showed no tristeza symptoms. It was 9 feet tall, and in excellent condition. It produced a small crop of fruit in 1972.

All 10 trees with the R_1SY2-infected sour orange intermediate scion were in good condition, with thick, dark green foliage. In comparison with the one control that showed no tristeza symptoms, these protected trees were somewhat reduced in size, ranging in height from 6 to 8 feet. The leaves were smaller than those of a normal healthy tree, but this may have resulted partially from the density of the foliage. Apparently R_1SY2 virus in these trees has produced a slight stunting effect and probably caused some irregularity in fruit production. None of this group produced a crop in 1972, but all of them showed a very heavy new set of fruit. The striking difference between the naturally infected controls and the protected trees is strong evidence that the latter have not been affected, to this time, by reinfection with tristeza virus.

DISCUSSION AND CONCLUSIONS

It is evident that tristeza-susceptible stionic combinations tolerate infection with virus isolates derived from plants that recover from certain sources of the seedling yellows virus complex. It is also evident that some of these isolates of RSY virus have provided field protection against tristeza virus for a period of six years. But it is obvious that the experimentally protected trees in these limited trials are not so vigorous and productive as healthy trees of the same rootstock and scion varieties would be in the absence of tristeza infection. These limited studies have demonstrated that individual isolates of RSY virus vary widely in their direct effect on budded trees of tristeza-susceptible combinations. Possibly, from more extensive trials, isolates of RSY virus could be found that would have no significant effect on tristeza-susceptible budded trees and would at the same time prevent injury from naturally occurring tristeza strains. Even with that order of artifically-induced protection, however, any practical use would be limited to regions where the SY virus complex either is not present or is not being spread to orchard trees by vectors. This conclusion is supported by experimental study in California, where it has been demonstrated that individual isolates of RSY virus protect against some sources of SY virus but do not protect against other sources or isolates.

A few control trees that were healthy when planted have developed mild symptoms from natural infection, and two controls in group 4 of experiment

3 showed no symptoms in 1972 even though one was shown to be infected. No cross-protection studies were made of the virus in that tree. Possibly it and other mildly affected control trees were infected by aphids which had acquired virus from adjacent trees carrying the protecting RSY viruses. On the other hand, there are strains of tristeza virus in nature that cause slight or mild disease effects on stionic combinations generally characterized as susceptible to tristeza. In countries such as Australia, South Africa, and Brazil, for example, where the seedling yellows virus complex is being disseminated by the aphid vector to trees of grapefruit, lemon, and sour orange, these so-called mild, protecting strains of tristeza virus may, for the most part, be what we designate RSY virus, having originated through recovery of trees from seedling yellows. But in the United States, where the SY complex apparently has not been spread into orchard plantings, the avirulent strains of tristeza probably have not been derived from seedling yellows. Although some of these strains cause only slight injury to tristeza-susceptible budded trees, and may provide protection against some virulent strains, limited investigation by the authors suggests that, in California, it is easier to select good preimmunizing isolates through the seedling yellows plant-recovery reaction than by search for such isolates in mildly affected orchard trees. Certainly this is true when these two sources of virus are compared in preimmunization of plants of grapefruit lemon, and sour orange against reinfection with seedling yellows virus. Although studies were not conducted under field conditions, where the seedling yellows virus complex is being spread by the aphid vector, it is anticipated that in such places, lime trees preimmunized with selected RSY virus isolates will have a broader range of protection than those preimmunized with an ordinary mild strain of tristeza virus that has not originated from seedling yellows. This assumption is based on the knowledge that California field sources of tristeza virus give lemon, grapefruit, and sour orange only slight protection against many isolates of SY virus.

From the standpoint of establishing the nature of the protective reactions of the RSY virus that remains in citrus plants after they recover from symptoms of seedling yellows, to our knowledge nothing further can be added to our hypothetical explanation presented previously (*5*).

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