

## Studies on Recovery of Citrus Plants from Seedling Yellows and the Resulting Protection Against Reinfection

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IN AUSTRALIA, South Africa, Brazil, India, and the Philippines—where the efficient aphid vector of tristeza virus, *Toxoptera citricidus* (Kirkaldy), occurs—seedling-yellows (SY) virus is widely distributed in orchard trees of sweet orange and mandarin. In California—where *Aphis gossypii* Glover is the principal vector—SY virus has not been found in the commonly grown orchard varieties. Nevertheless, some citrus selections maintained in varietal collections of the University of California and many trees of the widely distributed Meyer lemon proved to be symptomless carriers of the virus complex. There is good evidence that the original sources of these trees were already infected when introduced into the United States.

On the basis of present knowl-

edge, the authors conclude that seedling yellows is caused by a mixture of two or more components, one of which is tristeza virus. It is referred to as seedling-yellows tristeza (SYT) virus in order to indicate its origin and distinguish it from naturally occurring tristeza (T) virus in California. SYT virus can be separated from the SY complex, but it has not been possible to isolate a virus free of SYT virus that will cause seedling yellows. There is at least the suggestion that symptoms of seedling yellows result from the combined action of SYT virus and another unidentified virus.

The seedling-yellows virus complex causes severe yellowing and dwarfing of lemon, sour orange, and grapefruit seedlings. California field tristeza (T) or SYT virus separated

from SY have little effect on these plants, except that some isolates cause slight stunting, vein clearing on some plants, and strong stem pitting, particularly on grapefruit.

In efforts to isolate and identify the virus that alone or in combination with SYT virus causes seedling yellows, the authors have separated SYT virus by 3 different methods. Separation was accomplished first in plants that recovered from symptoms of seedling yellows. Fully recovered plants contain virus that does not cause seedling yellows but that, like the naturally occurring tristeza virus, causes the lime reaction and, in some instances, decline of budded indicator trees.

When it was discovered that after recovery from seedling yellows, plants of lemon, sour orange, and grapefruit were protected against reinfection with the seedling-yellows source from which they had recovered, extensive studies were made of this protective reaction. The studies led to separation of SYT virus from the seedling yellows complex by aphid transmission and by passage through highly resistant citrus hosts. At the same time, isolates of SY virus were established in various ways. In order to identify the many different virus isolates under study, it was necessary to code them, using letters and numbers to show the origin of each and how it was derived. This coding system is explained as the various isolates are described and discussed.

This paper reports primarily on the transfer of protection from seedling-

yellows-recovered plants to other citrus and on differences in the degree of protection afforded lemon seedlings graft-inoculated with SYT virus separated by different methods from the seedling-yellows virus mixtures.

### *Materials and Methods*

STOCK SOURCES OF SEEDLING-YELLOWS VIRUS.—Fourteen different sources of SY virus were established and maintained by graft-inoculation to seedlings of West Indian lime or in propagations from the source trees. These stock sources were held in insect-proof glasshouses. Originally, because of plant host reactions, each source was considered to be a strain of seedling-yellows virus, but subsequent developments showed that the individual sources usually consisted of multiple SY and SYT components. The respective stock sources of seedling yellows were designated SY1, SY2, etc. All infections by graft-inoculations from the established stock sources carried the code letters and numbers of the stock sources. Data presented in this paper are based chiefly on studies of the SY sources listed in Table 1.

SEPARATION OF SYT VIRUS FROM SEEDLING YELLOWS BY PLANT RECOVERY.—Fraser (1, 2) reported that Eureka lemon seedlings that developed symptoms of seedling yellows after inoculation by *T. citricidus* later resumed normal growth and that inoculations from them failed to reproduce the disease in young lemon seedlings. McClean (3, 4) observed loss of symptoms in seedling-yel-

lows-affected grapefruit; subinoculations from these plants resulted in a normal tristeza lime reaction but gave either no seedling yellows or caused symptoms of reduced virulence.

In studies of 14 SY sources in California, plants of Eureka lemon, sour orange, or grapefruit recovered from symptoms induced by 8

plants. For example, isolates obtained from different plants that recovered from SY1 are identified as R<sub>1</sub>SY1, R<sub>2</sub>SY1, etc. This new designation became necessary for identification purposes when it was determined that SYT virus separated from the seedling-yellows virus complex by other means differed from that obtained by plant recovery, and the

TABLE 1. ORIGIN OF 10 SOURCES OF SEEDLING-YELLOWS VIRUS USED IN INVESTIGATIONS IN CALIFORNIA

Virus source no.	Location of source tree	Variety	Country of origin	Introduced to U.S.A.
SY 1	CRC <sup>a</sup>	African lemon	South Africa	1913
SY 2	Orland, Calif.	Meyer lemon	China	1908
SY 4	Riverside, Calif.	Meyer lemon	China	1908
SY 5	Riverside, Calif.	Meyer lemon	China	1908
SY 6	CRC	Kona sweet orange	Hawaii	1914
SY 7	CRC	Meyer lemon	China	1908
SY 8	UCLA <sup>b</sup>	Red Ling Mung	China	Prior to 1931 <sup>c</sup>
SY 9	UCLA	Beauty mandarin	Australia	Prior to 1942 <sup>c</sup>
SY 10	Riverside, Calif.	Meyer lemon	China	1908
SY 11	UCLA	Scarlet mandarin	Australia	Prior to 1931 <sup>c</sup>

a. CRC, Citrus Research Center, Riverside, Calif.

b. UCLA, University of Calif. at Los Angeles.

c. Propagative material received in California this date from U.S. Dept. of Agriculture, Plant Introduction Section, Glenn Dale, Maryland.

sources. The remaining 6 sources are of such virulence that growth is almost totally stopped, and there has been no recovery in plants held from 1 to 3 years. As reported by Wallace and Drake (6), plants that make complete recovery from symptoms contain SYT virus, but the yellows-inducing virus is no longer present. The virus remaining after recovery was described originally by Wallace, Martinez, and Drake (7) as seedling-yellows-tristeza (SYT) to distinguish it from naturally occurring tristeza (T) virus in California orchard trees. The symbol RSY is now used to identify SYT virus derived from recovered

symbol SYT was needed to identify these isolates.

SEPARATION OF SYT VIRUS FROM SEEDLING-YELLOWS VIRUS BY APHIDS. — Martinez and Wallace (5) reported that *Aphis gossypii* transmits the seedling-yellows virus complex (SY) and also can selectively transmit the SYT component after feeding on a plant with the yellows complex. The writers have substantiated this on many occasions and have established numerous aphid isolates of SYT virus from SY. Because these isolates are direct selections from SY by aphids and have not been associated with plant recovery, those re-

tained for study were assigned the code letters ASYT with other necessary numerical symbols to show their origin. For example, aphid isolates of SYT from the stock source of SY1 are coded  $A_1$ SYT1,  $A_2$ SYT1,  $A_3$ SYT1, etc., the subnumerals showing that each is a separate aphid transfer.

SEPARATION OF SYT VIRUS FROM SEEDLING-YELLOWS VIRUS IN PLANT HOSTS.—In some previous trials, seedlings of trifoliate orange and Troyer citrange developed no symptoms when graft-inoculated with California field sources of tristeza virus, and subinoculations from them were negative. Subsequently, when seedlings of these 2 kinds of citrus were graft-inoculated with seedling-yellows virus, some plants became infected. They were symptomless but retained virus for several months. Inoculations from them gave erratic infections, indicating that they contained a low concentration of unevenly distributed virus. Graft transfers sometimes resulted in seedling yellows, but frequently only the SYT virus was transferred. In order to identify these host-separated isolates of SYT virus from those separated by plant recovery (RSY) or by aphids (ASYT), isolates separated from SY in trifoliate orange and citrange were designated TSYT and CSYT, respectively. Similarly, isolates of seedling-yellows virus obtained by graft-inoculation from these host plants were identified by the symbols TSY and CSY.

APHID ISOLATES OF SEEDLING-YELLOWS VIRUS.—Some isolates of

SY virus were established by aphid transfer from stock sources. When it was learned that they often differed from the source from which they were derived and that 2 aphid isolates from the same stock source also differed, it became necessary to identify and study each as a distinct substrain or isolate. For example, different aphid isolates from stock source SY1, which caused seedling yellows, were coded  $A_1$ SY1,  $A_2$ SY1, etc.

Some of the ASY isolates were inoculated into lemon or sour orange seedlings, which developed characteristic symptoms followed by recovery and loss of the seedling-yellows virus component. The SYT virus components remaining in these recovered plants were maintained as individual isolates and identified as RASY. For example,  $R_1A_1$ SY1 indicates recovery number 1 from seedling-yellows symptoms caused by infection with aphid isolate number 1 ( $A_1$ SY1) derived from the original source of SY1 virus.

### *Experimental Data*

In the course of these investigations more than 60 different isolates of SY and SYT viruses were established from the 14 original stock sources of SY virus. They have been used primarily in cross-protection experiments. This paper discusses protection in plants recovered from symptoms of seedling yellows and presents some of the data obtained in experiments in which the various SYT isolates were graft-inoculated to healthy Eureka and Lisbon lemon

seedlings for subsequent challenge-inoculations with different SY isolates.

One or more plants recovered from all SY sources listed in Table 1 except SY9 and SY11. Thus, 1 or more recovery SYT virus isolates (RSY) were obtained from 8 SY sources. Aphid-selected SY (ASY) and SYT viruses (ASYT), virus from plants that recovered from aphid isolates of SY virus (RASY), other aphid transmissions of various isolates, and some host-selected SY (CSY) and SYT (CSYT) accounted for the large number of virus sources used in these studies. Examples of most of these are in Table 2.

RECOVERY FROM SYMPTOMS OF SEEDLING YELLOWS.—In these studies, recovery was for the most part in seedlings of Eureka lemon, but in some instances it occurred in plants of sour orange and grapefruit. Symptoms induced in lemon

seedlings from the original 14 stock sources of SY virus ranged from moderate to extreme severity. Frequency and rate of recovery varied widely among the different sources.

With the less virulent sources of SY, virus-infected plants developed strong symptoms but terminal growth continued or was renewed in a short time, gradually showing less severe symptoms. In other instances, infected plants showed strong symptoms, made very little growth for 3 months or longer, and then began to recover. With several sources of SY virus, growth was stopped completely and there was no recovery.

#### PROTECTION IN RECOVERED PLANTS.

—Results from further investigations of protection in SY-recovered plants support the statement by Wallace, Martinez, and Drake (7) that the degree of protection depends upon the "strain" (isolate) of SY virus from

TABLE 2. DESCRIPTION OF SOME ISOLATES AND SUBISOLATES OF SY AND SYT VIRUSES FROM STOCK SOURCES OF SY1 AND SY6

Isolate	Virus	Origin and how derived	Reaction of lemon <sup>a</sup>
SY1	SY	Stock source from African lemon by graft transfer	+
R <sub>1</sub> SY1	SYT	From SY1 recovered sour orange 99-30	—
A <sub>1</sub> R <sub>1</sub> SY <sub>1</sub>	SYT	Aphid transfer no. 1 from isolate R <sub>1</sub> SY1	—
A <sub>2</sub> R <sub>1</sub> SY1	SYT	Aphid transfer no. 2 from isolate R <sub>1</sub> SY1	—
A <sub>1</sub> SY1	SY	Aphid transfer no. 1 of SY from stock source SY1	+
A <sub>1</sub> SYT1	SYT	Aphid selection no. 1 of SYT from stock source SY1	—
SY6	SY	Stock source from Kona sweet orange by graft transfer	+
R <sub>1</sub> SY6	SYT	From SY6 recovered Eureka lemon 91-41	—
R <sub>2</sub> SY6	SYT	From SY6 recovered sour orange 149-58	—
A <sub>1</sub> R <sub>1</sub> SY6	SYT	Aphid transfer no. 1 from isolate R <sub>1</sub> SY6	—
A <sub>1</sub> SY6	SY	Aphid transfer no. 1 of SY from stock source SY6	+
R <sub>1</sub> A <sub>1</sub> SY6	SYT	From A <sub>1</sub> SY6 recovered sour orange 142-43	—
A <sub>1</sub> SYT6	SYT	Aphid selection no. 1 of SYT from stock source SY6	—
A <sub>1</sub> A <sub>1</sub> SY6	SY	Aphid transfer of SY from aphid isolate A <sub>1</sub> SY6	+
A <sub>1</sub> SYT6-A <sub>1</sub> SY6	SYT	Aphid selection of SYT from aphid isolate A <sub>1</sub> SY6	—
C <sub>1</sub> SY6	SY	From citrange graft-inoculated with stock source SY6	+
C <sub>1</sub> SYT6	SYT	From citrange graft-inoculated with stock source SY6	—

a. +, seedling yellows; —, no reaction. All sources induced symptoms in West Indian lime.

which a plant recovers and that with which it is challenge-inoculated. It is now established that plants recovered from infection with one of the field or parent sources of SY generally have a much broader protection than is found in plants recovered from an aphid isolate of SY virus from that same parent source. In fact, it has been found that plants recovered from some aphid isolates and containing RASY virus are not protected against the ASY isolate from which they recovered. This unexplained reaction is now known to have been responsible for some inconsistent results obtained in early studies of recovery and protection when aphid isolates (ASY) were selected purposely to provide "pure" sources of SY virus.

TRANSFER OF PROTECTION FROM RECOVERED PLANTS TO LEMON SEEDLINGS.—Wallace, Martinez, and Drake (7) reported that healthy lemon seedlings infected by graft-inoculation from recovered plants were protected only partially and temporarily against challenge inoculations with SY virus. Subsequent study has shown that, as in the case of recovered plants, the degree of protection conferred on lemon seedlings by SYT virus varies among SYT isolates.

After finding the explanations for some of the variable reactions and developing standard experimental tests, it was demonstrated that protection in healthy lemon seedlings graft-inoculated with certain SYT virus isolates from recovered plants equals that in plants propagated directly from the same recovered

plants. On the other hand, as shown in Table 3, other isolates of SYT virus provided very little or no protection.

From studies of SYT virus isolated from the SY complex in various ways, and T virus from orchard trees, the following cross-protection reactions were obtained.

1. Plants recovered from seedling yellows after graft-inoculation with the field sources of SY virus were fully protected against the SY source from which they recovered. They were fully protected against certain other SY sources but reacted slightly or fairly strongly to others.

2. The SYT virus remaining in the recovered plants described in 1, identified as RSY virus, conferred the same degree and range of protection when graft-inoculated to lemon seedlings as existed in the recovered plants (Fig. 1).

3. Recovery SYT virus (RSY), after transmission by aphids (ARSY), sometimes protected lemon seedlings completely, as shown in Figure 2, but different aphid transfers (isolates) from the same RSY source varied widely in the degree of protection they afforded lemon seedlings (Table 3).

4. SYT virus selectively transmitted by aphids (ASYT) from stock sources of SY conferred no significant protection to lemon seedlings against reinfection with SY virus. However, the presence of these isolates sometimes resulted in a delay in appearance of symptoms and slightly less stunting compared with check plants.

5. SYT virus screened from the SY









