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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 knowledge, 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-yellows-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 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 plants. For example, isolates obtained from different plants that recovered from SY1 are identified as R_1SY1, R_2SY1, 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 symbol ṠYT was needed to identify these isolates.

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[a]	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[b]	Red Ling Mung	China	Prior to 1931[c]
SY 9	UCLA	Beauty mandarin	Australia	Prior to 1942[c]
SY 10	Riverside, Calif.	Meyer lemon	China	1908
SY 11	UCLA	Scarlet mandarin	Australia	Prior to 1931[c]

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.

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 retained 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_1SYT1, A_2SYT1, A_3SYT1, 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_1SY1, A_2SY1, 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_1SY1 indicates recovery number 1 from seedling-yellows symptoms caused by infection with aphid isolate number 1 (A_1SY1) 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[a]
SY1	SY	Stock source from African lemon by graft transfer	+
R_1SY1	SYT	From SY1 recovered sour orange 99-30	—
$A_1R_1SY_1$	SYT	Aphid transfer no. 1 from isolate R_1SY1	—
A_2R_1SY1	SYT	Aphid transfer no. 2 from isolate R_1SY1	—
A_1SY1	SY	Aphid transfer no. 1 of SY from stock source SY1	+
A_1SYT1	SYT	Aphid selection no. 1 of SYT from stock source SY1	—
SY6	SY	Stock source from Kona sweet orange by graft transfer	+
R_1SY6	SYT	From SY6 recovered Eureka lemon 91-41	—
R_2SY6	SYT	From SY6 recovered sour orange 149-58	—
A_1R_1SY6	SYT	Aphid transfer no. 1 from isolate R_1SY6	—
A_1SY6	SY	Aphid transfer no. 1 of SY from stock source SY6	+
R_1A_1SY6	SYT	From A_1SY6 recovered sour orange 142-43	—
A_1SYT6	SYT	Aphid selection no. 1 of SYT from stock source SY6	—
A_1A_1SY6	SY	Aphid transfer of SY from aphid isolate A_1SY6	+
A_1SYT6–A_1SY6	SYT	Aphid selection of SYT from aphid isolate A_1SY6	—
C_1SY6	SY	From citrange graft-inoculated with stock source SY6	+
C_1SYT6	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

complex by resistant hosts, trifoliate orange (TSYT) and Troyer citrange (CSYT), did not protect lemon seedlings against the SY source they were derived from or against any other SY isolate (Fig. 1).

6. Aphid isolates of SY virus (ASY) differed from the SY stock source from which they were obtained. Individual ASY isolates from a single SY stock source also differed from each other. Apparently, stock sources of SY virus are comprised of multiple substrains, and aphids pick up and transfer only a portion of these, possibly single substrains at times.

7. Aphid-selected isolates of SY virus from a given SY stock source often caused symptoms as severe on lime and lemon as the SY source from which they were derived. Lemon seedlings inoculated with these ASY isolates sometimes recovered after developing characteristic symptoms of yellows, and these recovered plants contained only SYT virus, identified by the symbol RASY. When RASY virus isolates were inoculated to lemon seedlings, protection was of a low order, sometimes none at all, against the ASY from which the particular RASY virus was derived. (See R_1A_1SY6 and R_1A_2SY6, Table 3.) The lack of protection or only partial protection in lemon seedlings reported earlier by Wallace, Martinez, and Drake (7) resulted for the most part from the use of virus from plants that had recovered from aphid isolates of SY virus.

8. In tests with 5 sources of field tristeza (T) virus maintained as "strains" because of differences in virulence in Mexican lime and budling indicators of tristeza, none gave protection against certain SY iso-

TABLE 3. PROTECTION IN LEMON SEEDLINGS BY DIFFERENT ISOLATES OF SYT VIRUS

Seedlings inoculated with SYT isolate	Degree of protection when challenge-inoculated with SY isolates							
	SY1	A_1SY1	SY6	A_1SY6	A_2SY6	A_3SY6	SY11	C_1SY6
R_1SY1	Complete	Complete	Very good[a]	Very slight[c]			None	
A_1R_1SY1	Complete	Complete	Slight [b]	Very slight				
A_2R_1SY1	Complete	Complete	Slight	Very slight				
R_2SY1	Complete	Complete					None	
A_1SYT1	None	None	None	None				
R_1SY6	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
A_1R_1SY6	Complete	Complete	Complete	Very good				
A_2R_1SY6	None	None	None	None				
A_3R_1SY6	Complete	Complete	Complete	Very good				
A_4R_1SY6	None	None	None	None				
R_1A_1SY6	None	None	None	None	None	None	None	None
R_1A_2SY6	None	None	Very slight	Very slight	Complete	Very slight	None	Slight
$A_1R_1A_2$SY6			Slight	Slight	Very good			
A_1SYT6	None	None	None	None	Very slight	None	None	None
C_1SYT6			None	None	Very slight	None		None

a. Transitory symptoms of terminal leaves; mature leaves reduced in size; sometimes only slight stunting.
b. Delay in appearance of symptoms and slightly less stunting of plants.
c. Primarily a delay in appearance of symptoms.

lates. In other instances there was a delay in appearance of symptoms and sometimes a partial protection against some of the milder isolates of SY virus.

Discussion

The nature of the protection in plants recovered from seedling yellows is not understood. Certainly the SYT virus remaining in these plants plays an important role in providing protection. In experiments not described in this paper, elimination of SYT virus from recovered lemon plants by heat treatment also eliminated protection against reinfection with SY virus. On the other hand, SYT virus selected from stock sources of SY by aphids provided lemon seed-

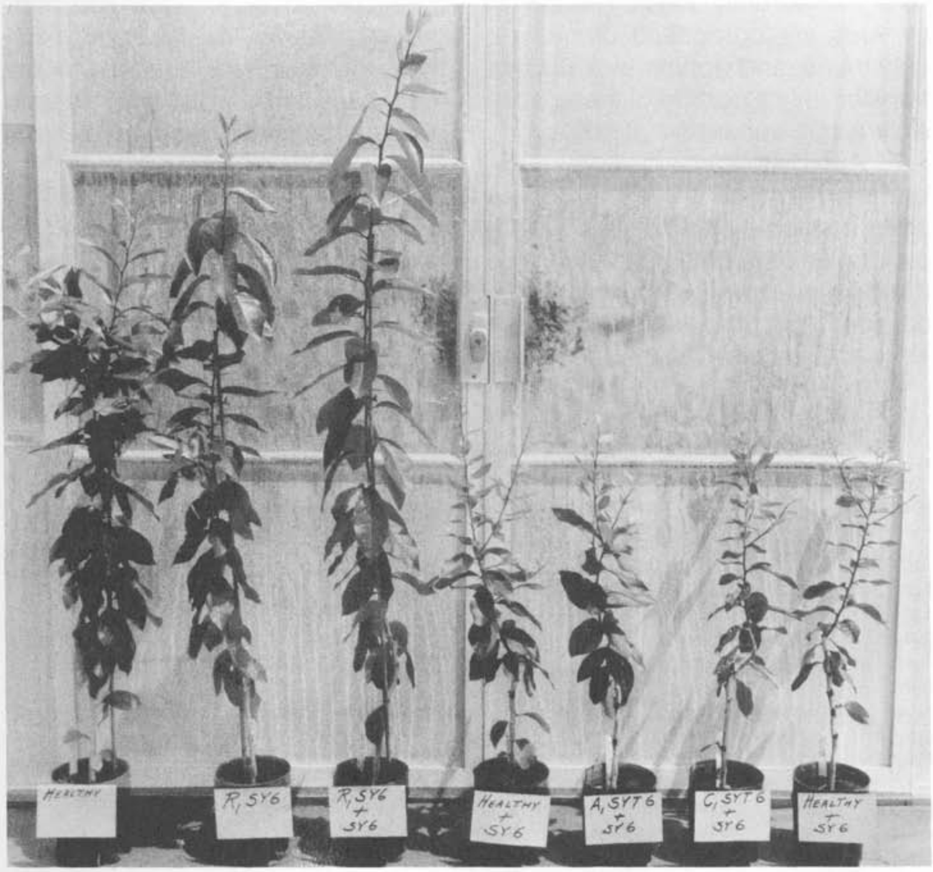

FIGURE 1. *Protection in lemon seedlings by SYT virus from a seedling-yellows-recovered plant and lack of protection by SYT virus separated from seedling-yellows complex by aphids or through a highly resistant plant host. Left to right: Healthy control; inoculated only with recovery SYT virus isolate R_1SY6; inoculated with isolate R_1 SY6 and challenge-inoculated with SY6; healthy inoculated with SY6; aphid-selected SYT6 from stock source of SY6 and challenge-inoculated with SY6; isolate of SYT6 screened from SY6 in Troyer citrange and challenge-inoculated with SY6; another healthy seedling inoculated with SY6.*

lings with no significant protection against challenge inoculations with SY virus. Similarly, SYT virus screened from the SY complex by passage through highly resistant plant hosts provided no protection.

Perhaps the most puzzling reaction encountered in these studies is that in plants that recovered from some aphid isolates of SY virus, the SYT virus remaining after recovery (see R_1A_1SY6 in Table 3) gave no protection against any of the SY isolates tested, including the aphid isolate of SY (A_1SY6) from which it was derived through recovery. It will be noted that after recovery from another aphid isolate (A_2SY6), the SYT virus remaining (R_1A_2SY6) gave complete protection against A_2SY6 from which it was derived but provided lemon seedlings very slight or no protection against other SY isolates. As is shown in Table 3, recovery SYT virus (R_1SY6) from stock source SY6 from which aphid isolates A_1SY6 and A_2SY6 were derived, gave complete protection against all 8 SY isolates used in the cross-inoculation tests.

The stock sources of SY virus used in these investigations have existed in the source trees for many years. There is good evidence that each contains multiple substrains of both the unidentified component and the SYT component. The plant that recovered from stock source SY6 provided isolate R_1SY6, which very probably consisted of many substrains of SYT virus. This isolate protected against numerous isolates of SY virus. On the other hand, aphid isolate A_1SY6, selected from SY6 by the

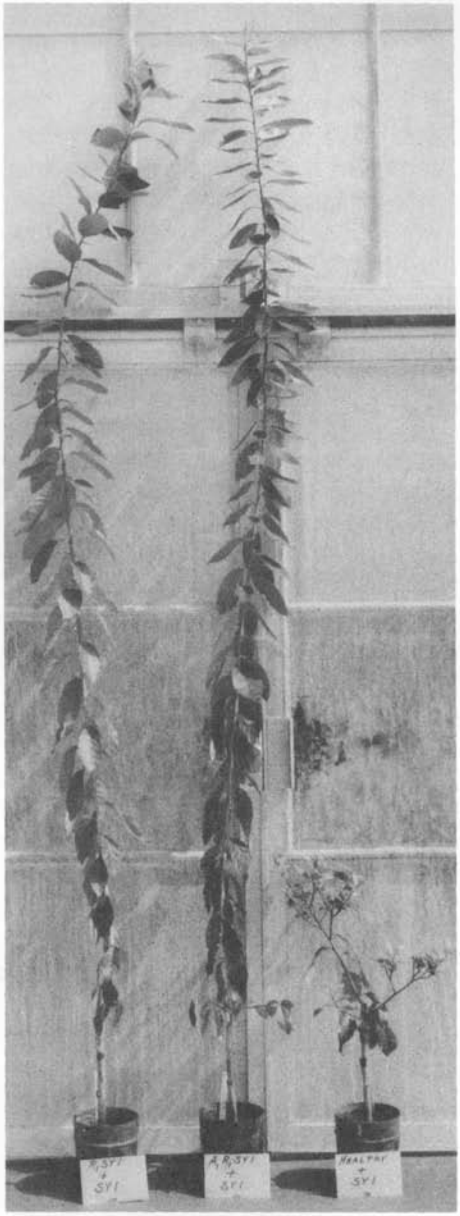

FIGURE 2. *Protection of lemon seedlings by SYT virus isolate R_1SY1 before and after transmission by aphids. Left. Seedling inoculated with R_1SY1 and challenge-inoculated with SY1. Center. Plant inoculated with aphid-transmitted R_1SY1 (isolate A_1R_1SY1) and challenge-inoculated with SY1. Right. Healthy inoculated with SY1.*

inefficient vector *Aphis gossypii,* probably contains very few or perhaps single substrains of the 2 components. In combination, the substrains of the 2 virus components present in some aphid isolates cause symptoms of seedling yellows, but possibly after plant recovery the SYT virus (R_1A_1SY6) remaining provides no protection against A_1SY6 because it does not contain a sufficient number of substrains or the necessary specific substrains of SYT6 virus. Hypothetically, this supposition would explain the failure of isolate R_1A_1SY6 to protect against A_1SY6 from which it was derived by plant recovery, but until the unidentified virus component of the seedling-yellows complex can be isolated there can be no experimental proof that this is the correct explanation.

Protection of lemon seedlings by SYT virus that remains in plants that recover after being infected with stock sources of seedling-yellows virus but not by SYT virus selected from SY sources by aphids—or screened through resistant hosts from the same stock sources—remains to be explained experimentally. There is a suggestion, at least, that protection in recovered plants does not result entirely from the mere presence of SYT virus.

The investigations have given no additional information on the nature and identity of the component of the seedling-yellows virus complex that disappears during plant recovery. The protection reactions described in this paper indicate that the unidentified component of the SY complex is related to the SYT virus always present in virus sources that cause seedling yellows. On the other hand, the disappearance of the yellows-inducing component in plants that recover suggests that it is not closely related to tristeza virus because Eureka lemon, for example, has proved to be a permanent host of all SYT isolates as well as numerous field sources of tristeza virus from California orchard trees.

Literature Cited

1. FRASER, L. 1952. Seedlings yellows, an unreported disease of citrus. Agr. Gaz. N. S. Wales 63: 125-32.
2. FRASER, L. 1959. The relation of seedling yellows to tristeza, p. 57-62. *In* J. M. Wallace (ed.), Citrus Virus Diseases. Univ. of Calif. Div. Agr. Sci., Berkeley.
3. MCCLEAN, A. P. D. 1960. Seedling yellows in South African citrus trees. S. African J. Agr. Sci. 3: 259-79.
4. MCCLEAN, A. P. D. 1963. The tristeza virus complex: Its variability in field-grown citrus in South Africa. S. African J. Agr. Sci. 6: 303-32.
5. MARTINEZ, A. L., and WALLACE, J. M. 1964. Studies on transmission of the virus components of citrus seedling yellows by Aphis gossypii. Plant Disease Reptr. 48: 131-33.
6. WALLACE, J. M., and DRAKE, R. J. 1961. Seedling yellows in California, p. 141-49. *In* W. C. Price (ed.), Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
7. WALLACE, J. M., MARTINEZ, A. L., and DRAKE, R. J. 1965. Further studies on citrus seedling yellows. p. 36-39. *In* W. C. Price (ed.), Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.