TRISTEZA VIRUS STRAINS

Theodore J. Grant[1]
U. S. Horticultural Field Station,
Orlando, Florida

INTRODUCTION

Tristeza virus strains and strain mixtures are identified at present largely on the basis of visible differences in growth and in degrees of symptom expression on infected test plants, and on the reproducibility of these visible differences by repeated transmissions to comparable test plants.

A tristeza virus strain that caused mild symptoms and another that caused severe symptoms were demonstrated by Grant and Costa (*9*) in Brazil. Both strains were transmitted by the same vector, *Toxoptera citricidus* (Kirk.). The differential behavior of various rootstock-scion combinations three years after inoculation with mild and severe strains of tristeza virus has been described by Costa, Grant, and Moreira (*4*). The freedom of these sources of tristeza virus from viruses causing psorosis, xyloporosis, and exocortis was indicated by the fact that no symptoms of these diseases were present on symptom-expressing species or rootstock combinations (*10*) inoculated with tristeza virus by means of aphids.

On the basis of differences in degree of symptoms on West Indian lime plants, Hughes and Lister (*12*) recognized two strains of the virus causing lime dieback in the Gold Coast, and McClean (*13*) recognized two strains of the causal agent of stem pitting in South Africa. Similarity of the causal agents of lime dieback in the Gold Coast and of stem pitting in South Africa and tristeza in Brazil (*2*) was indicated by transmissibility by the same aphid vector and by production of comparable symptoms on several citrus species and rootstock-scion combinations.

In Australia, Fraser (*6*) made bud collections from tristeza-infected field trees, and when she budded these on seedling test plants of Eureka lemon, sour orange, and grapefruit, obtained evidence of a qualitative difference in the virus content of different citrus species. The virus complex occurring naturally in orchard trees of several varieties of sweet orange and mandarin caused severe stunting and yellowing of the test plants. However, the virus in trees of Eureka lemon, sour orange, and grapefruit caused no leaf symptoms or only occasional vein flecking in the spring growth and apparently did not stunt the test plants. Fraser concluded that the virus causing seedling yellows is distinct from that responsible for what she designated the "tristeza—stem pitting complex," although she never found seedling-yellows virus occurring separately from the latter.

In South Africa, McClean and van der Plank (*14*) studied the seedling-yellows reaction in relation to tristeza and agreed with Fraser (*6*) in regard to symptomatology, host range, and the consistent presence of the "stem pitting component" in mixture with seedling-yellows component. McClean and van der Plank are of the opinion that tristeza is caused by a virus complex and that seedling-yellows virus is sometimes a part of that complex. Wallace (*17*) found that Meyer lemon, some satsuma varieties, and other miscellaneous noncommercial citrus introductions in California are carriers

[1] Plant Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Orlando, Florida.

of a strain or mixture of strains of tristeza virus that causes the seedling-yellows reaction described by Fraser (*6*). From his preliminary studies Wallace agreed with McClean and van der Plank that seedling yellows is not caused by a virus distinct from the tristeza virus.

Olson (*16*) studied the reactions to mild and severe strains of tristeza virus in Texas and reported that a mild strain from a Meyer lemon tree inhibited the development of severe symptoms in lime plants subsequently bud-inoculated with the virus from Sueoka satsuma.

Grant and Higgins (*11*) reported that tristeza virus from selected sources in Florida caused very mild, mild, or severe stunting, vein and veinlet clearing in the leaves, and pitting of the stems beneath the bark of Key lime test plants. Lineal branch growth of inoculated Key lime plants was proportional to the severity of other symptoms induced by the tristeza virus employed. Repeated selections and transmissions by leaf pieces from a mildly affected source plant indicated that the mild tristeza virus in Florida mutates readily or is a mixture of strains, some that consistently cause stem pitting and others that cause few or no stem pits on Key lime plants. Cross-protection tests with virus from selected source plants resulted in varying degrees of protective effects (*11*). The presence of virus strain mixtures in a given plant appeared to be related to the failure of any one strain to become thoroughly systemic, thus affording susceptible sites for the development of other strains.

It is the purpose of this report to describe further studies of tristeza virus strain mixtures and to discuss the results. The sources of tristeza virus T_0 (very mild), T_1 (mild), and T_3 (severe), the methods of transmission, and the care of test plants are the same as previously described (*11*). All inoculated and control plants illustrated were cut back to a single 6-inch main stem, leaving only the three uppermost leaves at the time of inoculation.

RANGE IN SYMPTOM EXPRESSION IN PLANTS INFECTED WITH TRISTEZA VIRUS

Typical differences in growth of Key lime plants following inoculation with leaf material from the T_3 and T_1 sources of tristeza virus are represented by the plants shown in figure 1. Key lime plants inoculated with T_3 virus showed vein clearing in the young leaves soon after inoculation. This was followed by yellowing and cupping of the leaves. In nine months these plants made about 26 per cent as much branch growth as the control plants, and they had many pits and striations in the stems under the bark. Comparable Key lime plants inoculated with leaf pieces from the T_1 virus source showed scattered vein clearing in young leaves. On subsequent growth under summer conditions in the greenhouse there were fewer leaf symptoms. In nine months these plants made about 70 per cent as much branch growth as the control plants and had relatively few stem pits.

Responses of sour orange and Eureka lemon plants to inoculation with the $T_3$and T_1 sources of tristeza virus are represented by the plants shown in figure 2. The plants inoculated with the T_3 virus were stunted, and their terminal leaves were yellow. The plants inoculated with the T_1 virus were shorter than the control plants but did not show the yellowing noted on the T_3 virus-inoculated plants.

It has been reported (*11*) that the aphid-transmitted T_1 tristeza virus was modified by leaf-piece selections and serial transmissions so that it produced both very mild and severe tristeza symptoms on Key lime plants. Comparative differences in the growth of five plants inoculated with leaf pieces from the third serial selection for mildest symptoms, and of five plants inoculated at the same time with leaf pieces from the fifth serial selection for severest symptoms, are shown in figure 3. Records on total branch length 178 days after inoculation showed that plants in group *A* with mildest

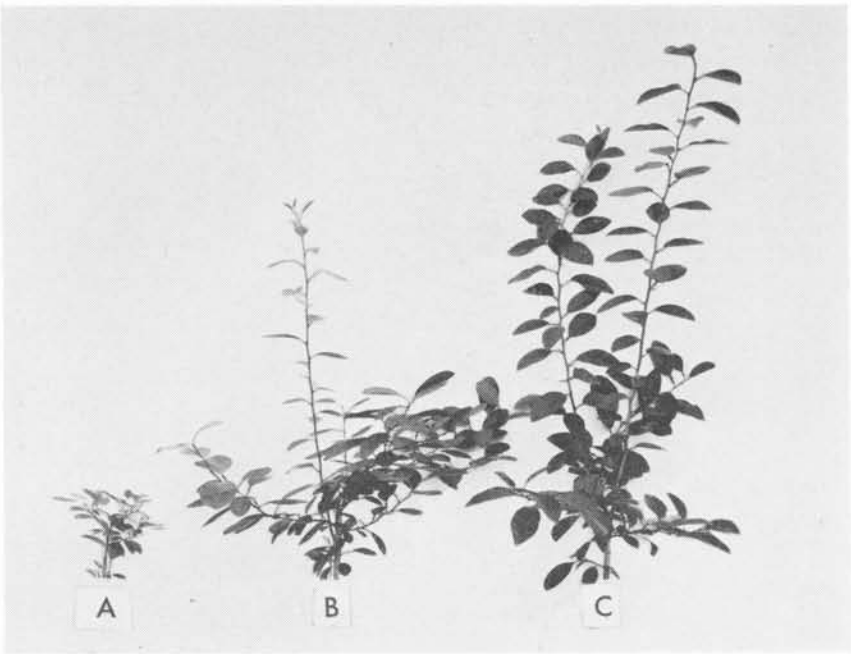

Fig. 1. Representative Key lime plants nine months after being cut to 6-inch single stems and inoculated with leaf pieces containing tristeza virus: A) T_3 (severe); B) T_1 (mild); C) comparable non-inoculated control plant.

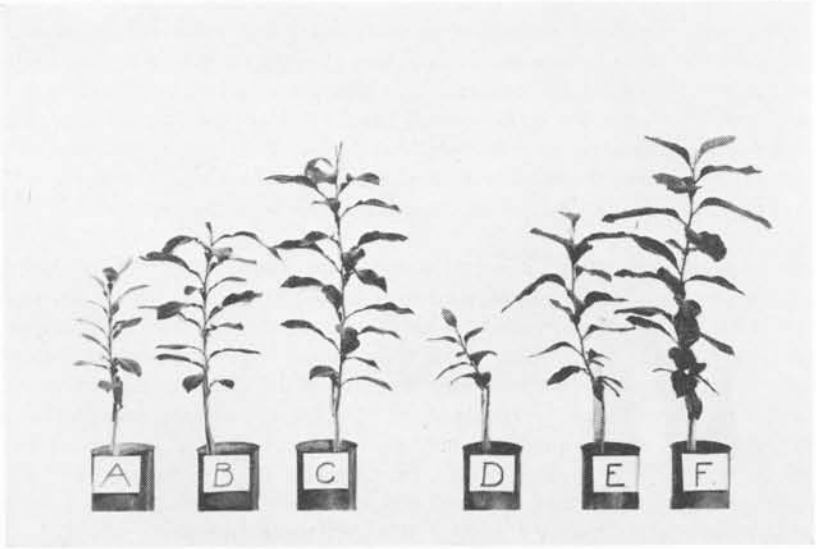

Fig. 2. Representative sour orange plants (A, B, C) and Eureka lemon plants (D, E, F) four months after inoculation with leaf pieces containing tristeza virus: A and D) T_3 (severe); B and E) T_1 (mild); C and F) comparable noninoculated control plants.

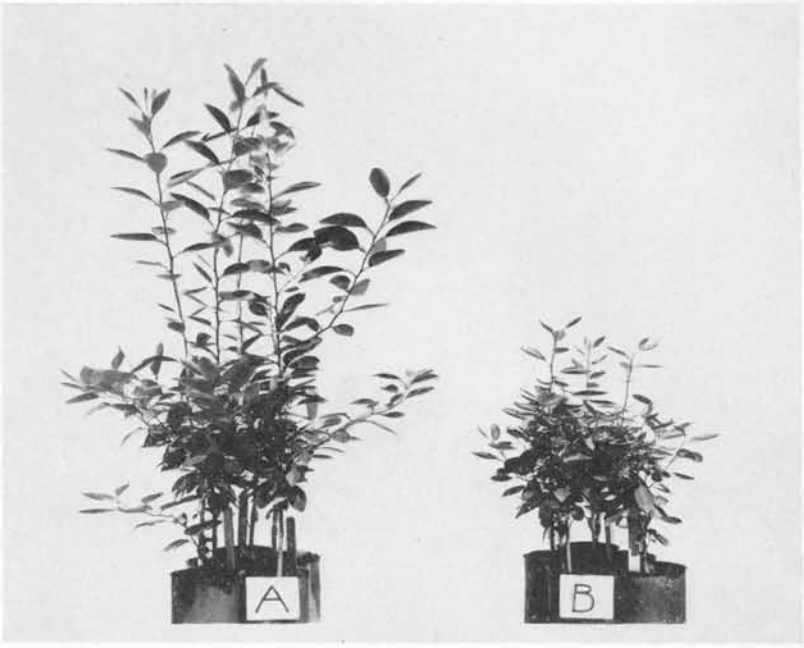

Fig. 3. Comparative differences in growth of Key lime plants 178 days after inoculation with leaf pieces from a single original source plant infected with aphid-transmitted T_1 tristeza virus. A) five plants inoculated with leaf pieces from the third serial selection for mildest symptoms; B) five plants inoculated with leaf pieces from the fifth serial selection for severest symptoms.

symptoms averaged 185 cm, and those in group *B* with severest symptoms averaged 94 cm. The total length of branch growth of the healthy control plants (not shown in fig. 3) averaged 213 cm. Since the serial T_1 leaf-piece inoculum originated from a single Key lime source plant infected as a result of virus transmission by *Aphis spiraecola* Patch, which acquired the virus from a single field tree (*15*), it must be presumed either that the aphids transmitted a mixture of strains or that the virus mutates readily.

By leaf-tissue transmissions to Key limes from the T_3 virus source that usually produces severe tristeza symptoms, it has also been possible to obtain an occasional plant that shows only mild symptoms. These results indicate also that tristeza virus exists as mixtures of strains which can be separated by leaf-piece transmission to Key lime plants.

In tests of cross protection, it was reported that where simultaneous inoculations were made with a mild and a severe source of tristeza virus, the plants showed severe symptoms (*11*). Plants inoculated first with virus from the T_0 (very mild) virus source and then, four months later, with virus from the T_3 (severe) source subsequently showed great variation in leaf-symptom intensity on the different branches. This suggested that these doubly inoculated plants had an erratic and unequal distribution of the virus from the T_3 source (*11*). To test this concept I selected five leaves from each of two of these doubly inoculated plants. Some leaves were young and succulent and others were old; some had vein clearing and others had no symptoms. A piece from each leaf was used to inoculate a Key lime plant. All ten plants were cut back to a single main stem and to uniform height at the time of inoculation. Their subsequent development in a ten-month period is shown in figure 4.

One plant (fig. 4, *A*) inoculated with a young leaf piece produced symptoms typical of infection with T_3 tristeza virus. Another plant (fig. 4, *E*), also inoculated with a

Fig. 4. Key lime plants ten months after inoculation with single leaf pieces from source plants doubly inoculated, first with T_0 (very mild) tristeza virus, and then, four months later, with T_3 (severe) tristeza virus (two months before leaf-piece transfer). A) plant showing stunted growth typical of infection with the T_3 tristeza virus. B, C, D) plants showing gradation in growth indicative of infection with varying proportions of T_3 and T_0 tristeza strains. E) plant with good growth typical of infection with T_0 tristeza strain. The average total branch length per plant was as follows: A, 76 cm; B, 171 cm; C, 232 cm; D, 348 cm; and E, 424 cm. The average numbers of stem pits per 10 cm of stem for the various plants were as follows: A, 100; B, 100; C, 22; D, 12; and E, 0.

young leaf piece, was typical of infection with T_0 virus. Eight plants (fig. 4, *B, C, D*), each inoculated with a single young or old leaf piece, some with and some without vein-clearing symptoms, produced a wide range in branch growth and leaf symptoms, thus indicating that these plants were infected with varying proportions of very mild and severe tristeza virus. The gradation in range from severe to very mild tristeza symptoms on the Key lime plants obtained from the doubly inoculated source plants provides evidence that in a two-month period, distribution of the challenging T_3 virus in the doubly inoculated source plants was unequal.

MODIFICATION OF TRISTEZA SYMPTOMS BY VARIOUS PROCEDURES

Passage of Virus Through Host Plants. The T_3 (severe) tristeza virus was used to inoculate five Duncan grapefruit seedlings. After two months, a piece of a mature terminal leaf from each grapefruit plant was used to inoculate a Key lime test plant. Subsequent growth of the five inoculated lime plants and of a representative healthy control plant in a two-month period is shown in figure 5. One plant (fig. 5, *A*) was stunted and had vein-cleared, cupped yellow terminal leaves characteristic of infection with T_3 virus. Four plants (group *B*) had milder symptoms but had not made as good growth as the healthy control plants. These results confirm the tendency previously noted in Australia (*6*) and South Africa (*14*), that transmissions from grapefruit, Eureka lemon, and sour orange trees produced milder symptoms than similar transmissions from sweet orange and mandarin trees.

That a particular plant may influence the development of a tristeza virus strain complex was also indicated by results in Florida (*11*), when serial selections were made to

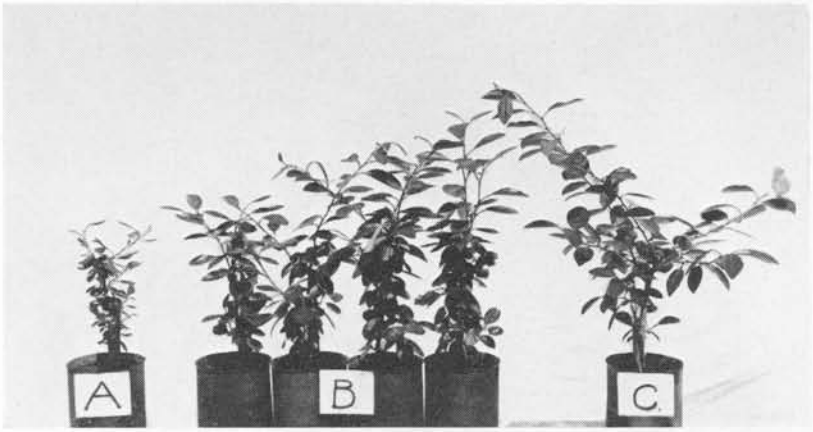

Fig. 5. Effect of passage of T_3 (severe) tristeza virus through grapefruit seedlings. A) Key lime plant showing characteristic symptoms of T_3 infection two months after inoculation from one T_3-infected grapefruit seedling. B) milder symptoms on four lime seedlings inoculated, respectively, from four T_3-infected grapefruit seedlings. C) comparable noninoculated control plant.

obtain strains that differed in their ability to cause stem pitting. It was noted that in a second selection for increasing stem pits an off-type Kalpi lime plant had 9 to 40 times as many pits as the other three typical Kalpi plants in the same series. These results suggested that dominance of the strain or strains inducing stem pitting may be influenced by physiological or genetical differences in the inoculated plants, since, once established, the high level of stem pitting was reproduced in other plants by leaf-tissue transfers (*11*).

Repeated Removal of Branches. Key lime plants showing very mild symptoms characteristic of infection from the T_0 (very mild) source are not weakened appreciably more than healthy plants when they are subjected to the complete removal of all branches three times in the course of a year. There was no evidence of any appreciable change in the very mild virus symptoms on either the leaves or the stems. With the T_1 (mild) tristeza virus source it was demonstrated that by serial leaf-piece selection from infected plants, one strain could be obtained that produced very mild symptoms and one that produced strong symptoms (fig. 3). Repeated cutting back of Kalpi and Key lime plants infected with the T_1 source that produced mildest symptoms did not appreciably change the symptoms, but, with one exception, repeated cutting back of the plants with strongest symptoms did reduce both leaf symptoms and stem pitting. This indicated that in the ordinary nucellar seedlings of Kalpi and Key lime the very mild virus tended to predominate when branches were repeatedly removed. The one exception was the off-type Kalpi lime previously mentioned as having 9 to 40 times as many stem pits as the other plants in the same series. In this plant the average number of stem pits per 10 cm of stem increased with the 3-times-repeated removal of branches in the course of a year.

Heat Treatment. It has been reported (*11*, *15*) that growth of Key lime plants infected with mild tristeza virus during hot summer weather in the greenhouse showed few vein-cleared leaves and less stem pitting than did growth made under cooler weather conditions. These observed reactions led to the building of a heat chamber for exposure of infected plants. The results (*7*) showed that some young branch growth that developed during continued exposure to high temperatures was free of tristeza virus even though old leaves and stem tissues of treated plants were not. The suppression of symptoms on tristeza-virus-infected Mexican lime seedlings by heat treatment was in-

Fig. 6. Selected Key lime plants showing range in tristeza symptoms in heat-treatment tests: A) typical nontreated plant infected with T_3 tristeza virus; B) comparable plant after 161 days in the heat chamber; C-F) indicator plants graft-inoculated at different intervals with tissues from heat-treated plants similar to that shown in pot B; C) plant with severe virus predominating; D) plant with severe and mild viruses in approximately equal mixture; E) plant with mild virus predominating; F) plant free of virus.

dependently demonstrated by Desjardins *et al.* (*5*). Further heat-treatment experiments by Grant (*8*) showed that the tristeza virus was present in practically all young branch growth that developed on infected plants during the early part of their exposure period in the heat chamber. With continued exposure of the infected plants, virus inactivation appeared to be faster in young tissues than in old, and virus distribution in the plants was gradually reduced. Exposure to temperatures of 98° to 104° F for 86 to 100 days was sufficient to inactivate the virus in some of the young and old tissues tested.

Inoculations made with tissues from tristeza-infected lime plants exposed to insufficient heat to rid all tissues of virus resulted in subculture plants that showed variable severity of symptom expression as illustrated in figure 6. On the basis of differences in growth and symptoms observed on the subculture plants, it was evident that the T_3 (severe) strain of tristeza virus had been modified by heat treatment. The results suggested that a wide range in the proportional amounts of mild and severe strains might be expected from different parts of some subculture plants. In order to test this, a T_3 subculture plant that appeared to contain a predominance of mild strain but had some vein clearing suggesting the presence of a severe strain was selected from the heat-treatment tests. Leaf-piece transfers were made to healthy Key lime plants. The results are illustrated in figure 7. The range in symptoms in these Key lime plants indicated that the source plant contained varying mixtures of tristeza virus strains.

The results of the heat-treatment tests indicated that the virus or strain mixtures causing yellowing and stunting and severe symptoms are more readily altered by heat treatment than are those responsible for milder symptom expressions.

DISCUSSION

It is generally recognized that the tristeza virus is distinct from those of psorosis, xyloporosis, and exocortis of citrus. The tristeza virus has been variously discussed as composed of mild and severe strains (*4, 9, 12, 13, 16*); as composed of two distinct viruses, tristeza and seedling yellows (*6*); as composed of a complex with a stem-pitting component and a seedling-yellows component (*14, 17*); and as composed of very mild, mild, and severe strains that may occur in mixtures (*11*). It is agreed that all of the component parts of the causal agent are transmitted by the insect vector