A SEPARATION OF VIRUS STRAINS FROM A TRISTEZA—SEEDLING-YELLOWS COMPLEX BY HEAT TREATMENT OF INFECTED LIME SEEDLINGS[1]

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INTRODUCTION

In recent years numerous investigators have used heat treatment of virus-infected plants or plant parts to obtain virus-free whole plants or virus-free tissue for propagation (*1, 7, 8, 9, 10, 11, 13, 14, 15*). Grant (*4, 5*) has reported success in obtaining citrus budwood free of tristeza and psorosis viruses by heat treatment of Key lime plants infected with the two viruses. Grant (reported elsewhere in this volume) has described a separation of strains of tristeza virus by heat treatment, and Grant and Higgins (*6*) have reported strain separation by a method of continuous selection of mild and severe strains from serial inoculations. In these studies they classified the strains as very mild, mild, or severe, depending on the mildness or severity of symptoms they produced on Key lime, lemon, and sour orange.

The purpose of the present paper is to describe some results obtained by propagation from heat-treated Mexican lime seedlings infected with the tristeza—seedling-yellows complex, which indicate a separation of virus strains. In addition we wish to report a confirmation of Grant's work on freeing citrus tissue of the tristeza virus.

MATERIALS AND METHODS

All lime seedlings that received heat treatment, as well as the control plants which received no heat treatment, were inoculated by grafting with scions obtained from a Meyer lemon (C.E.S. 1–54) known to be infected with a virus or virus complex which causes (*a*) tristeza (quick decline) symptoms on sweet orange on sour orange rootstock, (*b*) vein clearing and stem pitting on Mexican lime seedlings, and (*c*) seedling-yellows reaction on lemon seedlings. This source was one of many used by Wallace (*16*) in his work on seedling yellows. The seedling-yellows component of the complex is considered to be similar to that described by Fraser (*3*). Verification of infection of the lime seedlings was made prior to initiation of the heat treatment.

The method of heat treatment has been described (*2*). The seedlings involved in the work reported here were grown for four weeks at 38° to 40° C. Immediately upon termination of the heat treatment, shoots approximately 2 inches long that had developed during treatment were used for graft-inoculation of healthy lime seedlings. In some instances these shoots were cut into two pieces (tip half and lower half) and grafted separately to indicator seedlings. At the same time, scions were removed from some of the infected untreated check plants and grafted into other lime seedlings.

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EXPERIMENTAL RESULTS

About six weeks after inoculation of the lime seedlings directly from heat-treated plants, it was found that the infection reactions could be divided into three groups: (*a*) Some of the test seedlings developed no symptoms, thus indicating that the tissue used for inoculation was free of virus. (*b*) Some of the heat-treated tissue carried a virus strain or strain complex which induced mild symptoms on the lime seedlings. (*c*) Other pieces of tissue contained a virus strain or strain complex that induced severe symptoms on lime seedlings. The mild symptoms were characterized by slight vein clearing but no reduction in leaf size or stunting of the plant. The severe symptoms on lime included pronounced vein clearing, reduction in leaf size, and stunting of the plant.

Inoculations from control plants that had received no heat treatment produced uniformly severe symptoms on the lime plants with no indication of strain separation.

The two halves of a single shoot from each of two heat-treated plants, when used as scions to inoculate two lime plants, caused different reactions on the test plants. In the following discussion these separate shoots are referred to as shoot A and shoot B. In each case one of the test plants inoculated with one half of the original shoot exhibited mild symptoms, while the test plant inoculated with the other half exhibited

Fig. 1. Reaction of lime seedlings to graft-inoculation with different parts of a single shoot from a heat-treated infected lime plant: A) mild infection from the lower half of shoot B (see fig. 2); B) severe infection from the tip half of the same shoot.

a severe reaction. It was of interest, however, that infections from the tip half and lower half of shoot A were mild and severe, respectively, while the infections from the two halves of shoot B were in reverse order. The different reactions of lime seedlings infected from the two halves of shoot B are shown in figure 1.

After the reactions just described had been obtained, transfers were made to a second series of lime seedlings to determine if the reactions were consistent, and to two series of lemon seedlings to determine if the seedling-yellows virus strain had been affected by the heat treatment. The reactions obtained are illustrated diagrammatically in figure 2. As can be seen, the tip half of shoot A carried a strain of the virus that was mild in reaction on both lime and lemon, while the lower half carried a strain that was severe in reaction on both lime and lemon. The two virus strains obtained from shoot A are similar to those described by Grant (reported elsewhere in this volume) and by Grant and Higgins (*6*).

As illustrated in figure 2, different results were obtained with the two halves of shoot B. In this case the tip half of the shoot appeared to be carrying a strain that was severe on lime but mild on lemon, while the lower half carried a strain that was mild on lime but produced typical seedling yellows in lemon.

Figure 3 shows the reactions of lemon seedlings to the two virus strains obtained from shoot B, in comparison with a noninoculated control plant (*A*). Plants *B* and *C* in this figure were inoculated, respectively, from lime plants *A* and *B*, shown in figure 1. Plants *D* and *E* were inoculated from the same respective sources two months later. This demonstrates a consistent reaction of the strains separated in shoot B. The transfers from the lime test plants to other lime seedlings, made at the same time as the second transfers to lemon, also gave reactions consistent with those obtained in the original lime test plants and were similar to the reactions of the respective source plants shown in figure 1.

The subsequent transfers from the two lime test seedlings inoculated from the two halves of shoot A also continued to give the same reactions described for the two hosts in figure 2.

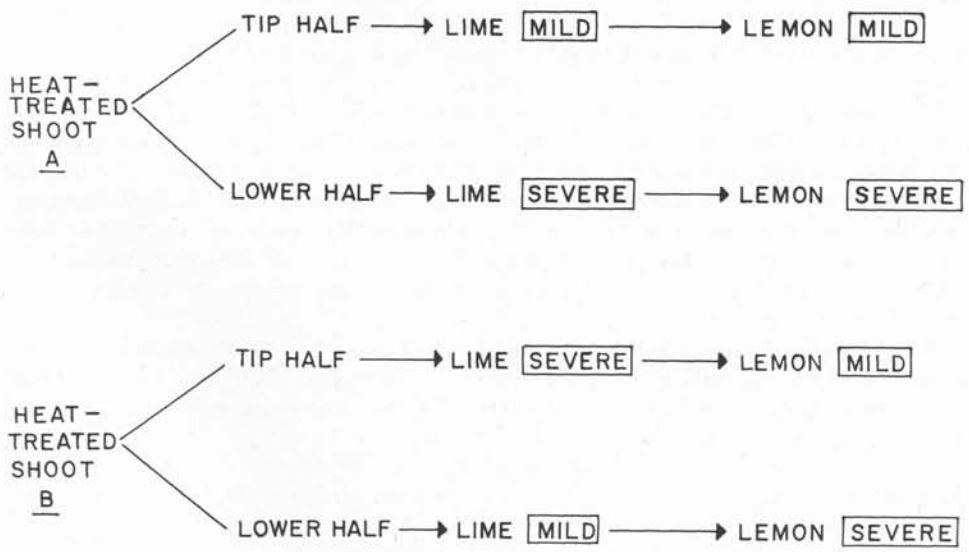

Fig. 2. Diagram of reactions of lime and lemon seedlings to virus strains obtained from the tip and lower halves of shoots A and B from two different heat-treated lime seedlings.

Fig. 3. Reaction of lemon seedlings to the two virus strains obtained from shoot B of infected, heat-treated lime seedling: A) self-grafted, noninoculated lemon seedling; B) and C) lemon plants inoculated, respectively, from plants A and B (shown in fig. 1); D) and E) lemon plants inoculated from the same respective sources two months later.

DISCUSSION

In other unreported studies of different field sources of the so-called seedling yellows in California, some strains of this virus induced mild symptoms on Mexican lime seedlings. The results of the heat-treatment studies reported in this paper confirm the existence of such strains. It cannot be stated with certainty that the virus obtained from the lower half of shoot B (fig. 2) was completely separated from all other strains of seedling-yellows virus originally present in the treated tree. Results indicated, however, that this part of the shoot contained no strain of virus capable of causing severe symptoms on lime. The writers agree with McClean and van der Plank (*12*) that the seedling-yellows virus is not distinct from that which causes tristeza. With this assumption in mind, it would seem that proof of complete separation of strains may have to await actual purification and mechanical transmission of the virus, because the likelihood of finding a host susceptible to one strain but immune to another closely related strain is rather remote.

With the exception of the two instances described in detail above, no other inoculations, where the two halves of a single shoot from a treated plant were involved, gave any indication of separation of virus strains. The two halves gave the same reaction, or one of the halves was found to be free of virus.

Although the separation of what appear to be different virus strains was obtained by heat treatment, it seems fortuitous that the separation in the treated shoot was such that the two strains could be isolated by an arbitrary cutting of the shoot into two pieces. The consistent reactions obtained indicate that this occurred. However, the possibility remains that different mixtures of virus strains, instead of individual or distinct strains, were separated by the heat treatment. In that case, interference between

strains may have influenced the reactions. With this in mind, additional work on heat treatment of the various strains described in figure 2 will be undertaken to see if further separation from these sources can be accomplished.

The results of these studies indicate that the virus strains that induced the severe seedling-yellows reaction in lemon moved more slowly in the heat-treated lime than the other strains. This conclusion is based on the fact that in both cases illustrated in figure 2 these severe strains were present only in the lower halves of the shoots taken from the heat-treated lime seedlings.

SUMMARY

By means of heat treatment, virus-free tissue has been obtained from lime seedlings infected with the tristeza–seedling-yellows virus complex. This confirms the results reported earlier by Grant (5).

An apparent separation of virus strains from the complex was accomplished by selection of tissue for propagation from heat-treated sources. Also, a strain of virus was obtained which does not induce severe symptoms in lime but causes severe symptoms of the seedling-yellows type in lemon.

There was some indication that the strains of the virus which induce the severe seedling-yellows reaction in lemon were slower than other strains of the complex in invading the new growth that developed on the lime plants during the heat-treatment period.

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