Translocation of Tristeza and Psorosis Viruses

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THIS PAPER presents the results of studies on the translocation of tristeza and psorosis viruses. The suggestion has been made (2) that tristeza virus is phloem-limited, since it apparently is unable to move across the ringed portion of a stem during a period of six months. Results of anatomical studies on citrus plants infected with tristeza virus led to the conclusion that the virus probably moves through the sieve tubes and infects adjacent parenchyma-like cells (11). Nour-Eldin and El-Banna (7) obtained no evidence that tristeza virus can enter the xylem of baladi lime [*Citrus aurantifolia* (Christm.) Swing.] or Parson Brown sweet orange [*C. sinensis* (L.) Osb.] plants. They reported, however, that psorosis virus occasionally entered the xylem of sweet orange seedlings and moved downward, less frequently upward, across an area from which a ring of bark had been removed.

The size and shape of tristeza virus is still somewhat problematical, although the work of Kitajima *et al.* (4) provides much circumstantial evidence that the virus is a thread-like particle of approximately 11 x 2,000 mμ. Kitajima *et al.* (4, 5) and Silva *et al.* (12) correlated the presence of tristeza virus in plants of various varieties of citrus with the presence of such thread-like particles as revealed by electron microscopy of sap of the same plants or of partially purified preparations made from the sap. Similar thread-like particles have been found by electron microscopy of ultrathin sections through phloem cells of West Indian lime (*C. aurantifolia*) plants infected with tristeza virus (9). At present the morphology of psorosis virus is not known.

Materials, Methods, and Results

Both viruses were obtained from diseased trees in the variety block of the Citrus Experiment Station at Lake Alfred, Florida. West Indian lime was used as a host plant for tristeza virus and Pineapple sweet orange was used as a host for psorosis virus. Inoculations were made by grafting a bud from a diseased seedling into the test plant; hereafter this bud will be referred to as the inoculation bud.

Experiments were carried out to determine whether or not tristeza virus will move up or down the stem of West Indian lime seedlings across a girdle from which a ring of bark has been removed. In each

experiment, a ring of bark about 1 in. wide was removed from the stem about 6 in. above ground level, and the woody cylinder was scraped. The seedlings were then inoculated with tristeza virus, the inoculation bud being placed above the girdle in 6 seedlings, and below it in the other 6. Non-girdled seedlings were inoculated in the same way to serve as controls. The seedlings were examined periodically for symptoms. The most obvious result was that the tops of the girdled plants became so yellowed that it was not possible to tell whether their leaves had vein clearing. Consequently, 70 days after inoculation, the yellowed tops were removed and rooted in a mist propagator. When they rooted, their leaves became green, and vein clearing was apparent where infection had occurred. Vein clearing developed above the girdle when the site of inoculation was below. There was no evidence that tristeza virus passed the girdle, confirming the observations of Costa *et al.* (2), Price and Knorr (8), and Nour-Eldin and El-Banna (7), and thus suggesting that the virus is not ordinarily translocated in the xylem vessels. Vein clearing developed both above and below the site of inoculation in all the control plants.

The following experiment was designed to determine how soon after inoculation the viruses of tristeza and psorosis would pass from the scion to the stock. The procedure was to inoculate a number of healthy seedlings and then remove the inoculation buds at intervals; the grafted plants were observed from time to time to detect symptoms.

Tristeza virus passed the graft union in a minimum of 8 days, but sometimes failed to pass within 17 days (Table 1). Psorosis virus passed the union in a minimum of 9 days, but often apparently failed to pass within 17 days (Table 1). There was considerable variation among test plants in the time required for virus to pass the union. This variation is undoubtedly related to the growth of the test plants and thus to the time required for the tissues of the stock and scion to unite. In some instances, both viruses infected the test plant even though the inoculation bud died; apparently, sufficient portions of tissues united to allow passage of the virus to the stock.

Experiments were performed to determine the time required for tristeza and psorosis viruses to move from the inoculation bud to the top of the test plant, and to the base of the test plant. The test seedlings were 12-15 in. tall. A bud from an infected tree was grafted into the seedlings about halfway between the top and the base. At various intervals thereafter, cuttings were taken from the top of the seedlings and rooted in a mist propagator; the decapitated seedlings were also retained and allowed to sprout. Three cuttings were taken from above the point of

inoculation and 3 from below. The cuttings were examined for vein clearing and were discarded when symptoms appeared. Cuttings that failed to develop symptoms were planted in soil in pots and kept under observation for 6 months. Many cuttings did not contain virus even though taken as long as 21 days after inoculation of the seedling (Fig. 1). This lack probably represents a failure of the virus to pass from the site of inoculation to the seedling; the time required for movement to oc-

TABLE 1. INCIDENCE OF SYMPTOMS OF TRISTEZA IN KEY LIME SEEDLINGS AND OF PSOROSIS IN SWEET ORANGE SEEDLINGS WHEN BUDS USED FOR INOCULATION WERE REMOVED AT VARIOUS INTERVALS

	Tristeza[a]		Psorosis[b]	
Days[c]	Number positive	Number negative	Number positive	Number negative
3			0	2
4	0	2		
7			0	2
8	3			
9	3		2	2
10	2	3 (1)[d]	5	6 (1)
11	2	1	3	6
12	5	1	8	1 (1)
13	9 (1)	5 (2)	6 (1)	3
14	7 (1)	2 (2)	7	4
15	8 (3)	1	6 (1)	0
16	6	3 (2)		
17	7	4 (3)	2	0
18	1	2 (2)		
20	1	1		
Check	11 (4)	3 (2)	5	3

a. Summary of 5 experiments.
b. Summary of 4 experiments.
c. Days after inoculation when buds were removed.
d. Numbers in parentheses represent the number of inoculation buds that failed to live.

cur apparently was a variable in these tests, as in the preceding experiments. In other seedlings, the virus moved out of the inoculation bud, infecting some sections, but not all. In a few instances, both tristeza and psorosis viruses passed through a section of a stem without infecting it. These results are reminiscent of the discovery by Samuel (10), subsequently confirmed by Kunkel (6), that tobacco mosaic virus can pass through long sections of tomato stems without infecting them. Both viruses moved to the top of the plant about as rapidly as to the base.

The results on movement of tristeza virus in seedlings may be com-

pared with those obtained by Burnett (1) in a study of the movement of tristeza virus in a single citrus tree infected naturally by aphids in the field. When buds from this tree were indexed on West Indian lime test plants, thirteen and one-half months elapsed between the first indication that the tree was infected and the time when all the buds taken from various parts of the tree indexed positive.

The observations reported here, together with the results of others, suggest that the translocation of tristeza and psorosis viruses is mainly through the phloem, probably through the sieve tubes. Psorosis virus

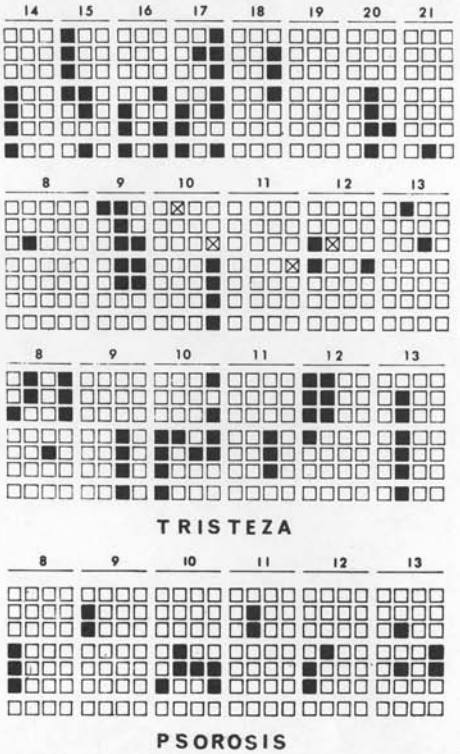

FIGURE 1. *Diagrams illustrating the translocation of tristeza virus in West Indian lime seedlings and of psorosis virus in sweet orange seedlings. Each column in a block represents a seedling from which six cuttings were made, top to bottom, at the indicated number of days after inoculation; the inoculation bud had been placed at a point between the third and fourth cutting from the top; the bottom square represents the decapitated plant with its attached roots. Blackened squares indicate cuttings that developed symptoms; those with an X indicate cuttings that failed to live.*

evidently can occasionally enter xylem cells and move through them (7), but tristeza virus appears to be restricted to phloem cells (9).

Figure 2 is an electron micrograph of an ultrathin section through a

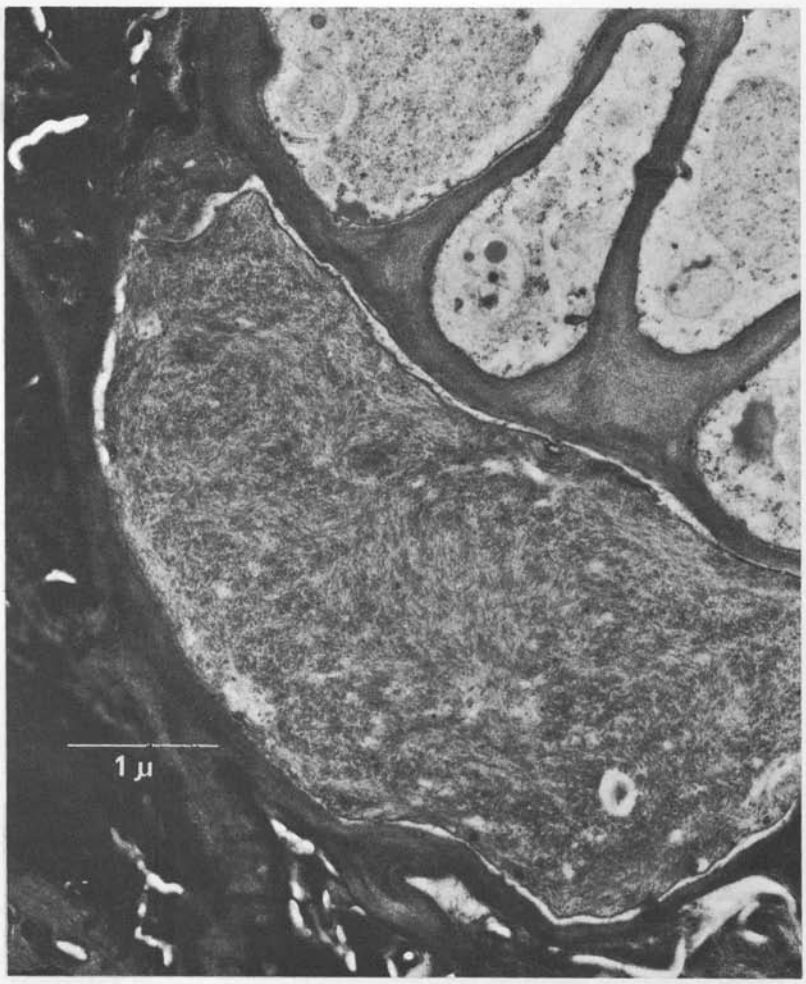

FIGURE 2. *Portion of an electron micrograph of an ultrathin section through a West Indian lime leaf infected with tristeza virus. The micrograph shows a single phloem cell that contains a large number of thread-like particles. Other phloem cells at the bottom left became necrotic as a result of infection. Phloem cells at the upper right appear to be normal. Thread-like particles sectioned transversely appear as dots in the micrograph; the arrangement of the dots suggests that the particles occur in bundles.*

fibrovascular bundle in a leaf of a West Indian lime plant infected with the T_3 strain (3) of tristeza virus. One cell in the micrograph was filled with thread-like particles similar to those described by Kitajima *et al.* (4, 5). The cells at the bottom left of the figure were so necrotic that thread-like particles could not be detected. The cells at the upper right appeared to be normal, without thread-like particles. In all ultrathin sections examined by electron microscopy, the thread-like particles were limited to phloem cells; they were never found outside vascular bundles nor in tissue from citrus seedlings not inoculated with tristeza virus. Only one or a few phloem cells per bundle contained threads, but nearly every bundle examined did contain them.

The evidence from electron microscopy of ultrathin sections confirms the hypothesis of Costa *et al.* (2) that tristeza virus is a phloem-limited virus. Regardless of whether the thread-like particles be considered as the virus itself or as by-products of virus activity, it seems reasonable to assume that virus activity is largely within phloem tissues and that the phloem is the main channel of translocation.

Electron micrographs of ultrathin sections of sweet orange leaf tissue showing young-leaf symptoms of psorosis have thus far failed to reveal the presence of virus-like particles in the cells. Thus, there is no evidence as yet that psorosis virus accumulates in phloem cells.

Discussion

The rate of movement of psorosis and tristeza viruses is difficult to determine because the method of inoculation does not supply the exact time for virus contact between healthy and diseased tissue. Evidently, there is considerable variation in the time required for tissues of the stock and scion to complete a union and, therefore, in the time required for virus to move from scion to stock. Even in the case of tobacco mosaic virus, which can be transmitted by mechanical means, the virus does not move from inoculated leaves into the stem of all tomato plants at the same rate (6).

Evidently, both tristeza and psorosis viruses are phloem viruses in the sense that their main channel of translocation is the phloem. After the virus reaches the phloem, translocation both upward and downward is relatively rapid (Fig. 1). Thus, there seems to be little relation between the speed at which the virus is translocated and the time required for it to kill a citrus tree.

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