Studies on Citrus Tristeza Virus Disease

II. Distribution and Movement of the Casual Virus in Citrus Plants

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Tristeza is the most important virus disease affecting citrus and causes great losses (Costa, 1956). This disease was first observed in Egypt in 1957 in a varietal collection near Cairo (Nour-Eldin and Bishay, 1958). However, no subsequent spread was noticed, indicating the lack of an effective vector under local conditions. Apparently the virus was introduced with imported budwood. Most citrus varieties on their own roots can be symptomless carriers. However, Key lime shows characteristic vein clearings (Hughes and Lister, 1949).

In a previous paper (Tolba *et al.*, 1968), several aspects of epidemiology were discussed. In the present work, distribution and movement of the virus in diseased plants were studied.

MATERIALS AND METHODS

Balady lime seedlings previously inoculated by grafting from a tristeza-infected Valencia sweet orange tree in the Barrage Experiment Station varietal collection were used as the source of tristeza virus.

Virus movement from the inoculation site. To determine the least time required for the virus to move from infected inoculum into healthy tissues of the host plant, leaf patches from tristeza-infected Balady lime were grafted onto healthy Balady lime seedlings in 25-cm clay pots by the method of Wallace (1947). Each seedling received two patches. The inoculated seedlings were divided into six groups, each consisting of six seedlings. Beginning on the fifth day after inoculation inoculum patches were removed daily from one of the groups so that by the tenth day all inocula had been removed. The leaf patches were pulled out by the tip of a budding knife. To ensure that all parts of infected patches were removed, some of the lime tissues surrounding the patches were also removed. All inoculated seedlings were kept in a greenhouse for one month, then were examined.

Virus movement through the woody cylinders of infected plants. To determine if movement through the xylem occurs in infected plants, 2-year-old healthy seedlings of the varieties Balady lime, Brazilian sour orange, sweet lime, Orlando tangelo, Duncan and Marsh seedless grapefruit, citron, Poorman grapefruit, Troyer citrange, Rangpur lime, rough lemon and Calamondin mandarin, grown in 25-cm clay pots in an insect-free greenhouse, were ringed by removing a bark bracelet about 3 cm long from each stem. The cambial surface of the wood under the rings was left exposed for two weeks to dry out. Each seedling was then bud inoculated above the ring by stem patches of infected Balady lime. Seedlings were kept in the greenhouse and pruned periodically below the ring in the hope that partial carbohydrate starvation in the roots, induced by the pruning, might result in subsequent movement of stored carbohydrates and the virus downward across the ring. A year later the growth below the ring on each seedling was indexed on two Balady lime plants for the presence of tristeza virus.

Virus distribution in infected plants. To study the distribution of the virus in infected plants, 1-year-old seedlings of Balady lime, Brazilian sour orange, sweet

lime, Orlando tangelo, Duncan grapefruit, citron, Poorman grapefruit, Cleopatra mandarin, Troyer citrange, and trifoliate orange were bud inoculated with tristeza virus. A year later, different parts of the inoculated seedlings were grafted to healthy Balady lime seedlings to detect tristeza virus.

RESULTS

Movement of tristeza virus from the site of inoculation. Eight days seems to be the minimum time required for the virus to move from infected leaf patches to the surrounding healthy tissues of the test plants (table 1).

Movement of tristeza virus through the woody cylinder. It is obvious from the results (table 2) that the virus did not move downward through the woody cylinders of the 12 varieties tested even when drastic pruning was done below the rings.

Virus distribution in infected plants. In all varieties tested (table 3) the virus was recovered from leaves, stem bark, and roots but all attempts to recover it from the woody cylinder, even with the cambium attached, failed.

TABLE 1
TIME REQUIRED FOR TRISTEZA VIRUS TO MOVE FROM THE SITE OF INOCULATION

Period before removal of the tristeza-virus-infected leaf graft (days)	Lime seedlings inoculated	Lime seedlings infected
5	6	0
6	6	0
7	6	0
8	6	3
9	6	6
10	6	6

TABLE 2
LACK OF VIRUS MOVEMENT THROUGH THE WOODY CYLINDER

Citrus species and varieties	Seedlings ringed and inoculated	Surviving seedlings tested	Seedlings with tristeza virus below the ring
Balady lime	10	2	0
Brazilian sour orange	10	8	0
Sweet lime	7	3	0
Orlando tangelo	10	3	0
Duncan grapefruit	10	2	0
Marsh seedless grapefruit	10	6	0
Citron	10	7	0
Poorman grapefruit	10	7	0
Troyer citrange	10	2	0
Trifoliate orange	10	3	0
Rangpur lime	10	10	0
Rough lemon	10	7	0
Calamondin mandarin	10	2	0

TABLE 3
VIRUS DISTRIBUTION IN INFECTED PLANTS

Plants tested	Tissues tested			
	Root*	Stem bark	Leaf patch	Woody cylinder with cambium
Balady lime	4 / 4†	4 / 4	4 / 4	4 / 0
Brazilian sour orange	4 / 4	4 / 4	4 / 4	4 / 0
Sweet lime	4 / 4	4 / 4	4 / 4	4 / 0
Orlando tangelo	4 / 4	4 / 4	4 / 4	4 / 0
Duncan grapefruit	4 / 4	4 / 4	4 / 4	4 / 0
Poorman grapefruit	4 / 4	4 / 4	4 / 4	4 / 0
Citron	4 / 4	4 / 4	4 / 4	4 / 0
Cleopatra mandarin	4 / 4	4 / 4	4 / 4	4 / 0
Troyer citrange	4 / 4	4 / 4	4 / 4	4 / 0
Trifoliate orange	4 / 3	4 / 4	4 / 4	4 / 0

*Root grafts consisted of a piece of bark attached to a piece of wood.
†Number of lime seedlings grafted/number of virus transmissions.

DISCUSSION

Transmission of viruses by grafting is an inevitable sequel to their ability to survive in and to spread between vegetative cells inside plants. Several horticultural grafting techniques have been applied in experimental virus transmission. Other forms of grafting or pseudografting not used in horticulture have also been effective for transmitting viruses. The insertion of infected leaf pieces under the bark of a host plant can lead to virus transmission. In this case, transmission apparently depends on tissue formed between the cut edges of the leaf pieces and the stocks. This method was used by Wallace (1947) to transmit citrus psorosis virus.

Our results show that a minimum of 8 days is required for the virus to move into healthy tissue of Balady lime seedlings. Obviously the time required for union depends on the growth condition of both scion and stock as well as the conditions, especially temperature, under which the combination is grown. Under standard conditions, different viruses are transmitted in different minimal times. Fridlund (1967) classified 12 Prunus viruses into three classes on the basis of minimal time requirements for transmission. Minimal time was correlated with the kind of tissue that supports virus replication. Thus, he found that viruses of class 3 required longer than those of class 2 or class 1 for transmission, apparently because the former are nearly or wholly confined to phloem cells. Phloem differentiation and connections necessary for graft transmission are believed to require more time than connections of meristemic or parenchymatous tissues. In histological studies of the graft union of Turkish tobacco, meristematic unions between scion and stock occurred with 48 hours of contact, and phloem differentiation in new tissue at the union occurred at least by the seventh day (Bennett, 1943). This agrees with results obtained in the present study indicating that tristeza virus is most probably confined to phloem tissues. The facts that the virus is not transmitted by sap (Tolba, *et al.*, 1968) but by phloem-feeding aphids (Meneghini, 1946), that its symptoms on lime are restricted mainly to the conductive tissues, plus the results of histological studies of tristeza-infected sweet orange on different rootstocks (Tolba, *et al.*, 1968) all clearly indicate that the main tissue for virus replication is the phloem. More direct evidence was obtained by electron microscopy of tristeza-infected lime plants showing phloem cells packed with viruslike rods. These

rods were lacking in parenchymatous tissues (Price, 1966). Obviously, the tristeza virus can be replicated only in phloem tissue and seems to be confined there.

Two types of movement are recognized for systemic viruses, a slow cell-to-cell spread and a relatively rapid movement for greater distances through the vascular tissues. In cell-to-cell movement it was estimated that a virus moves at a rate of 8 μ per hour (Uppal, 1934). Soon after a virus enters a susceptible host, it increases for a time, reaches a certain concentration and then moves to neighboring cell via plasmodesmata. Direct evidence for this type of movement was obtained by Esau *et al.* (1967), who obtained micrographs of virus particles in plasmodesmata. This process may go on for sometime until the virus reaches a vein after which the second type of movement may occur. The virus moves from the minor vein to a main vein, then down the midrib into the stem to initiate systemic infection. It has been suggested that this movement occurs in the phloem and that its direction is determined by the flow of food coming from the leaves (Samuel, 1934).

In our work, tristeza virus remained in the top portion of ring-girdled plants where it originally was introduced while the low portion remained free of virus even when treatments were applied to encourage food flow to the roots. In a similar experiment, Bennett (1940) reported that TMV moved through a ring girdle in tobacco but took longer than normal. He attributed this result to slow cell-to-cell movement. Still, beet curly top virus does not move across pieces of tobacco stem in which continuity of the phloem is broken by ringing, which indicates that this virus may be largely confined to the phloem (Bennett, 1934, 1956). It is often assumed that viruses which move systemically become evenly distributed throughout the plant, but this rarely happens (Bawden, 1964).

In our work tristeza virus was recovered from leaves, stem bark, and roots of infected plants, but all efforts to recover it from xylem, even with the cambium attached, failed. These results and those from ringing indicate that the virus is not replicated in wood parenchyma and that xylem is not the normal route for the rapid spread of the virus in infected plants. Our results also show that the virus does not move in the cambium between phloem and wood, thus confirming earlier results (Nour-Eldin and Elbanna, 1963). However, other possibilities exist: 1) the virus might enter the xylem and not be replicated there so that by the time union between wood pieces occurs the small amount of virus present is inactivated and nothing is transmitted from the wood; and 2) the virus may enter xylem vessels but fail to move to surrounding susceptible tissues. We believe that the second possibility is more probable for tristeza virus, especially in the light of results obtained by ringing. In agreement with this hypothesis are results obtained by Caldwell (1931, 1934), who concluded that when TMV was experimentally introduced into xylem vessels of tobacco it remained there without causing systemic infection unless the vessles were mechanically damaged. All these results indicate sieve tubes to be the usual pathway for rapid movement of virus.

It should be obvious that pathogens that are more or less restricted to xylem, e.g., Pierce's disease of grape, can only multiply or be replicated and spread in xylem tissue (Houston, *et al.*, 1947).

We concluded from our results that tristeza virus is mainly restricted to phloem tissue where it is replicated and spread and that the xylem is not a normal route for the rapid translocation of the virus.

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