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Author

Cohen, Mortimer

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A Leaf Insert Graft Used for Virus Transmission in Citrus

M. COHEN

THIS PAPER describes a new method of grafting leaf tissue from one citrus plant into a leaf of a second. Leaf tissue was first used for transmission of a citrus virus by Wallace (4), who placed a small portion of a leaf of the plant being indexed under a bark flap of an indicator plant. He observed long-time survival of the leaf piece and transmission of psorosis virus to 90–100 per cent of plants inoculated in this manner. Wallace (5) also used this method for tristeza virus inoculations. McWhorter and Allen (2) used a leaf piece of an infected plant to transmit lily curl stripe virus to the leaves of healthy plants. Although virus transmission was obtained, a true graft union did not develop.

Schwarz (3) introduced tristeza virus into inoculated plants by connecting the distal portion of the leaf of an infected West Indian lime seedling to a matching proximal part of a leaf of a healthy seedling. No transmission was obtained unless a callus connection was formed between the donor leaf piece and the receptor leaf. Callus was more likely to form and transmission more likely to occur when old, dark green leaves were connected than when young leaves were used.

The new technique involves the setting of a leaf piece from the donor

plant into a space of exactly the same size and shape in a leaf attached to a second plant (Fig. 1). The grafting is done by detaching a leaf or leaf piece from the plant providing the graft and laying it flat over a leaf on the second plant. A sharp-pointed scalpel or razor blade is then used to cut through both leaves at the same time. Usually the

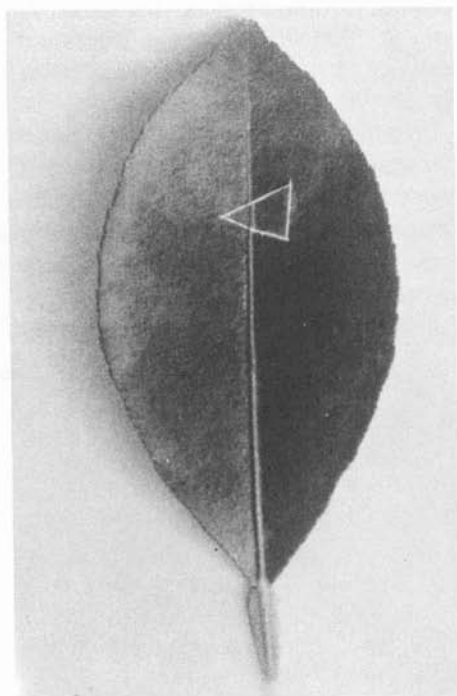


FIGURE 1. Leaf insert graft. "Midrib" graft in which midrib of leaf piece lines up with the midrib of the receptor leaf. A callus connection with the leaf has been made on all 3 sides of the leaf piece.

leaf piece is triangular with sides about 5 mm long. The diseased piece is then set into the hole in the healthy leaf. The leaf piece is kept in place between 2 squares of transparent or opaque, inert, flat, rigid, plastic sheeting. The plastic squares should have sides about 5 times as long as the leaf piece. They are fastened together with a wire paper clip. The plant is covered with a flexible plastic bag, supported by a stake that serves to keep the bag from clinging to the grafted leaf; then the plant is placed in a shady location for about 2 weeks.

The range of graftability of leaf pieces among the various species and varieties of citrus is about the same as with conventional budding or grafting. Leaf pieces of the following plants have been successfully grafted into West Indian lime: sweet orange, rough lemon, West Indian lime, grapefruit, sour orange, trifoliate orange, and Troyer citrange. Other leaf insert grafts made were: West Indian lime into sweet orange and into *Triphasia trifolia* (Burm. f.); sweet orange into sour orange; citron into sweet orange and sour orange; *Severinia buxifolia* (Poir.) Tenore and sweet orange into *S. buxifolia*.

Grafting success varied somewhat in different experiments and under different conditions. All trials with citrus were conducted in a greenhouse, but a lower percentage of successful grafts was obtained when trees in the field provided the graft material than with leaf pieces from greenhouse donors.

One trial, begun on March 5, 1968, involved grafting of single leaf pieces from plants of a number of varieties into 46 West Indian lime seedlings; all but 2 of the initial grafts lived. The 2 failures were successfully reinoculated.

Other experiments were less successful; but of 136 plants grafted in various experiments during a 2-year period, 85 per cent had at least 1 live leaf insert 2 months after grafting. It should be noted that often 2 leaf inserts were placed in 1 plant.

A comparison was made between 2 types of leaf insert grafts. One, the "midrib" type (Fig. 1), involved a leaf piece containing a section of the midrib; it was matched to a similar portion of the midrib in the receptor leaf. The second, the "blade" type, involved a leaf piece that did not contain any part of the midrib; it was inserted into a similar portion of the receptor leaf blade. A somewhat higher proportion of successful leaf inserts was obtained with midrib grafts than with blade grafts.

West Indian lime and sour orange seedlings that received leaf pieces by midrib graft developed tristeza symptoms more often than those that received leaf pieces by blade graft when the donor plant carried virus from a Meyer lemon (Table 1). When the donor plants carried Grant's T3 strain (1), transmission efficiency was almost 100 per cent for both types of graft. When the donor plants had been inoculated first with the Meyer lemon strain and 2 months later with T3, the

pattern of transmission resembled that obtained with the Meyer lemon strain only. The midrib leaf graft presumably provides a better vascular connection between leaf piece and leaf than the blade graft. The difference in transmissibility of the 2 virus strains with the 2 types of graft may be related to virus content

cotyledonous plants, including ginger lily (*Hedychium* spp.), *Dracaena* spp., corn (*Zea mays*), and 7 species of palms; but no true grafts were obtained although some leaf pieces remained green for a number of weeks. A few trials were also made with the dicotyledonous plants *Pittosporum* spp. and croton

TABLE 1. TRANSMISSION OF TRISTEZA VIRUS FROM SWEET ORANGE SEEDLINGS WITH BLADE AND MIDRIB TYPES OF LEAF-INSERT GRAFTS

Strain of virus carried by sweet orange seedlings	Indicator seedling	Blade grafts	Midrib grafts
Meyer lemon only	West Indian lime	1/7 ^a	7/7
	Sour orange	2/4	5/6
Meyer lemon plus T3 ^b	West Indian lime	0/5	3/4
	Sour orange		
T3 only	West Indian lime	7/8	7/7
	Sour orange	2/2	3/3

a. Numerator, number of seedlings infected; denominator, number of seedlings inoculated; only plants in which leaf-insert grafts lived for 1 month or more are counted.

b. Sweet orange seedlings inoculated with T3 strain about 2 months after inoculation with Meyer lemon strain.

or it may indicate a fundamental strain difference.

Exocortis virus also was transmitted by leaf insert graft in 2 of 3 attempts.

A number of trials were made to investigate the value of leaf insert grafts in plant groups other than citrus. Trials in the field were run with 10 different species of mono-

(*Codiaeum* spp.). A true graft was obtained only in croton.

The leaf graft technique for virus transmission may prove of value in inoculating very young citrus plants too small to inoculate by conventional methods. The technique may also reveal some aspects of virus transmissibility not shown by other methods.

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