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Ring Callus as a Path for Non-Graft-Transmitted Aeglopsis Chevalieri Vein-Clearing Virus

THE FACT that the virus of tristeza can be transmitted from plants of lime [*Citrus aurantifolia* (Christm.) Swing.] to plants of Aeglopsis (*Aeglopsis chevalieri* Swing.) by means of the aphid vector, [*Toxoptera citricidus* (Kirk.)], but not by grafting (1, 2) was interpreted to result from the absence of sieve tube connections in the graft union (2, 3). The present study was undertaken to determine what types of grafts, if any, would transmit the virus between these two species.

Methods and Materials

METHOD OF GRAFTING.—Seedlings of beladi lime and Aeglopsis about two years old in 20-cm clay pots were grafted in one or another of four ways. (a) A ring of bark about 10 cm long was removed from each seedling, exposing the cambial surfaces of the woody cylinder. The woody cylinders of the two seedlings were then bound together with strips of plastic (Fig. 1, A). (b) The second method was the same as the first except that a one-cm length of the woody cylinder at each end was scraped to remove the cambium (Fig. 1, B). (c) The seedling of Aeglopsis was prepared as in method (a) but the beladi lime seedling was prepared by slicing through the bark and wood (Fig. 1, C). (d) Both seedlings were sliced through the bark and wood (Fig. 1, D). In the first three methods, a strip of plastic was placed at each end of the ringed area to prevent physical contact at this point between the two seedlings. To serve as controls (e) healthy seedlings of lime were grafted as in (a) to Aeglopsis.

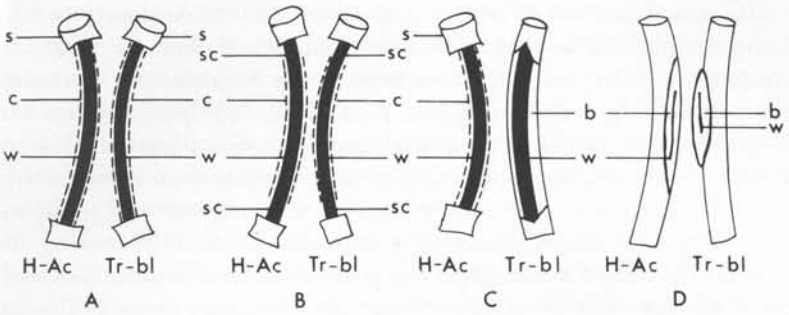

FIGURE 1. *Diagram illustrating four different methods of approach-grafting healthy seedlings of Aeglopsis chevalieri (H-Ac) to tristeza-infected beladi lime seedlings (Tr-bl); s, stem; w, woody cylinder; c, cambium; sc, scraped cambium; b, bark.*

Experimental Results

TRANSMISSION THROUGH RING CALLUS.—Seedlings of Aeglopsis were approach-grafted in each of the four ways mentioned above to beladi lime seedlings previously inoculated with tristeza virus by budding; each of five different sources of tristeza virus was used. Results obtained by each of the four methods of grafting were as follows:

(a) Ten of 15 Aeglopsis seedlings, those in which ring callus had formed, developed yellow pin points and very short yellow dashes along the lateral veins and veinlets. No pitting developed in the wood of the seedlings within 16 months.

(b) Twelve grafts of this type were made. In seven of the Aeglopsis seedlings, the ring callus extended so as to unite with the bark at the end of the ring. In only two of the beladi lime seedlings attached to these seven Aeglopsis seedlings did the ring callus extend far enough to unite with the bark. Nevertheless, all seven Aeglopsis seedlings developed pin points and short dashes as in (a), indicating not only that the causal agent was transmitted from the lime seedlings to the Aeglopsis seedlings but also that it was present in the isolated cambial tissue that developed into ring callus on the lime seedlings.

(c) Twelve of 15 Aeglopsis seedlings developed symptoms as in (a) within two to eight months after grafting.

(d) None of 15 Aeglopsis seedlings developed symptoms. Failure of the virus to move from lime to Aeglopsis in these tests suggests that the tissues differentiating from ordinary wound callus are structurally, or developmentally, different from those differentiating from ring callus.

(e) None of the seven healthy controls developed symptoms.

RETRANSMISSION OF THE CAUSAL AGENT.—Every Aeglopsis seedling approach-grafted to tristeza-virus-infected beladi lime by means of methods (a), (b), and (c) was indexed to one Aeglopsis and two beladi lime seedlings by leaf-patch grafts. Each of the Aeglopsis seedlings subinoculated from seedlings that developed pin points along the veins became infected and developed continuous vein clearing on all new leaves within two months. The infected plants were stunted and produced leaves that were cupped. Six months after inoculation of the seedling, the woody cylinder of the main stem was pitted. Pitting subsequently extended to mature twigs. None of the beladi lime seedlings developed symptoms nor were symptoms induced when these seedlings were indexed back to Aeglopsis. No symptoms developed in any of the seedlings grafted with leaf patches from Aeglopsis seedlings that failed to show symptoms after being approach-grafted to infected beladi lime seedlings.

LACK OF INTERFERENCE.—The 15 Aeglopsis seedlings that failed to develop symptoms after being approach-grafted to infected beladi lime seedlings were infected 10 months later by grafting them with leaf patches from Aeglopsis seedlings showing yellow pin-point symptoms. All beladi lime seedlings that had been grafted with leaf patches from Aeglopsis seedlings were inoculated 72 days later with one of the sources of tristeza virus; all developed the characteristic vein clearing.

TRANSMISSION FROM AEGLOPSIS TO VARIOUS SPECIES OF CITRUS.—Inoculations were made by grafting with leaf patches from (a) an Aeglopsis seedling with yellow pin-point symptoms, (b) two Aeglopsis seedlings with continuous vein clearing, (c) a previously inoculated Aeglopsis seedling that did not develop symptoms, and (d) a healthy Aeglopsis seedling to 12 different species and varieties of citrus. Only the Orlando tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) plants developed any symptoms. They produced tumor-like growths along the edges of the bark where the Aeglopsis leaf patch was inserted, whether inoculated with a leaf having yellow pin points or with one having continuous vein clearing. Tangelo seedlings grafted with leaf patches from healthy Aeglopsis did not develop this abnormal growth. When indexed to beladi lime seedlings, plants of the 12 species gave no indication of being infected with a virus.

DISTRIBUTION OF TRISTEZA VIRUS IN BELADI LIME TISSUES.—Decorticated woody cylinders, which were ensheathed with cambial tissues, were removed from tristeza infected beladi lime seedlings and were side grafted into 12 beladi lime seedlings about one year old. None became infected,

suggesting that tristeza virus was not present in these cambial tissues.

ANATOMICAL STUDIES.—Cross sections through the graft-union between the woody cylinders of Aeglopsis and lime revealed a bridge of tracheary elements and fibers connecting the cylinders (Fig. 2). This

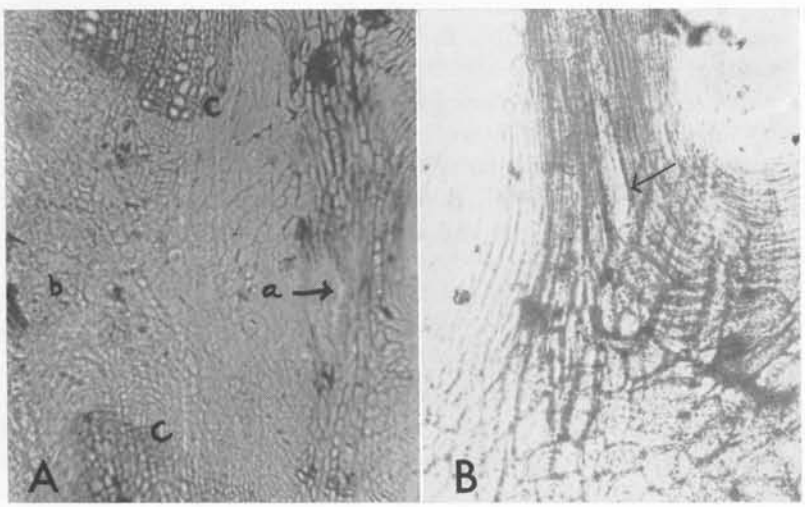

FIGURE 2. *A. Cross section through a union between Aeglopsis and beladi lime seedlings approach-grafted after removing a bracelet of bark from the stem of each seedling; (a) bridge connecting the two cylinders; (b) ring callus filling the cavity; (c) differentiated xylem cells from each of the two cylinders. B. Bridge extending from the woody cylinder of Aeglopsis, with a tracheary element indicated by the arrow.*

bridge is formed across the narrowest distance between the two woody cylinders. At first, the callus cells presumably fill the cavity between both woody cylinders. Later, a vascular cambium differentiates in the vicinity of each woody cylinder. The xylem formed from the vascular cambium in the vicinity of the lime woody cylinder is continuous and in line with the primary wood rays and vessels. On the other hand, it seems that the vascular cambium differentiating from the callus cells of Aeglopsis is not regularly formed. This is indicated by the presence of tracheary elements scattered within the callus formed around the primary xylem of Aeglopsis, without being in line with or even in the direction of the primary vessels. It has not been possible to detect sieve tube formation in the vicinity of both woody cylinders.

Discussion

The fact that yellow pin points were induced in Aeglopsis seedlings when the ring-callus method of grafting was used but not when ordinary methods of grafting were used suggests that tissues differentiating from the ring callus are structurally or developmentally different and that they thus provide for a sufficient union between the two species to allow movement of the virus from the tristeza-infected lime seedling to the Aeglopsis seedling. Schneider (3) attributed failure of transmission to lack of sieve-tube connections between Aeglopsis and tristeza-infected grafts of citrus. We are inclined to agree with Schneider's hypothesis, although we could detect no sieve-tube connections between Aeglopsis and lime grafted by the ring-callus method. Phloem connections might form so scantily in the ring callus as to be overlooked. Removal of the entire bark might give the developing callus the chance to differentiate in a certain arrangement under the influence of the adjacent lime tissue instead of being influenced by the presence of lateral tissues of Aeglopsis as they would be when the ordinary approach-grafting method is used.

The yellow pin points exhibited by Aeglopsis are thought to be induced by a causal agent other than tristeza virus. Tristeza virus could not be detected in the cambial tissue ensheathing the woody cylinders of tristeza-infected citrus plants. Still, Aeglopsis seedlings developed pin-point symptoms when their exposed woody cylinders were approach-grafted to exposed woody cylinders of tristeza-infected lime seedlings after the ends of the lime woody cylinders had been scraped to remove any possible connection between the cambium and the bark.

Vein clearing and wood-pitting symptoms, similar to those reported by Knorr (1) and McClean (2), were induced when healthy Aeglopsis seedlings were grafted with leaf patches from seedlings of Aeglopsis that had exhibited only the yellow pin-point symptoms after having been infected with an agent that had moved through ring callus. This result suggests that the causal agent that induced yellow pin points is a virus complex rather than a single virus.

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