Comparison of Time Requirement for Graft Transmission of Citrus Tristeza Virus With Other Fruit Tree Viruses

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Many fruit tree viruses, although transmissible by sap inoculation to herbaceous plants, cannot be transmitted to trees by sap inoculation, but can be transmitted easily by grafting. We tried to ascertain the minimum inoculum-receptor contact period for successful transmission to determine the mechanism of graft transmission in woody plants.

METHODS AND RESULTS

A section of bark approximately the size of the inoculum shield was cut from the stem of a Mexican lime seedling. A 'lip' was formed at the lower end of the cut to hold the inoculum shield in place. An inoculum shield was cut from a Eureka lemon infected with citrus tristeza virus (CTV, HS-46, courtesy of Dr. Sasaki) and the lower end was tapered sharply by cutting off the front surface, then inserted under the 'lip' of the stock and wrapped with polyethylene tape (fig. 1). The inoculum shields were removed from five Mexican lime plants at 1, 2, 4, 8 and 16 days after inoculation; the rest were left as controls with continuous contact. Careful observation of symptoms was made on newly developed leaves each month after cutback. Eight months after inoculation, stems were examined for pitting. Mexican lime seedlings which had 4 days of contact with the inoculum shield showed no vein clearing or stem pitting, but two plants of the 8-day contact seedlings showed leaf symptoms and one had stem pitting. All control plants showed leaf symptoms and stem pitting. Apparently, at least 8 days of contact are necessary for successful graft transmission of CTV (table 1). This coincides with data presented by Tolba *et al.* (1976).

Similar experiments were done with prunus necrotic ringspot virus (NRSV) using Shirofugen as an indicator. The method of inoculation was the same as above. Establishment of infection was indicated by local bark necrosis and gummosis at the inoculation site. At least 3 days were required to transmit NRSV to Shirofugen (table 1).

Apple seedlings were inoculated with inoculum shields of apple stem infected with apple chlorotic leafspot virus (CLSV, B-81, courtesy of Dr. Mink). The inoculation method was as above. Infection was determined by grafting an indicator variety, Maruba-kaido, onto the inoculated apple seedlings. Apple seedlings infected with CLSV cause leaf necrosis and/or distortion symptoms in the Maruba-kaido scion. The minimum contact period for transmission of CLSV was only 2 days (table 1), but 4 days may be needed to obtain a stable infection.

DISCUSSION

In contrast to the 3-4 days required for transmission to apple of CLSV and to Shirofugen of NRSV, 8 days were required for graft transmission of CTV to Mexican lime. Fridlund (1967) observed three distinct groups according to the speed of graft transmission using 12 prunus viruses. Bennett (1943) postulated that phloem-limited viruses would take longer to transmit since phloem differentiation in the newly developed tissue of the graft union is slower than for other tissues. This is apparently why CTV, a phloem-limited virus, takes longer to transmit than CLSV and

NRSV.

According to Mendel (1936, 1937), initial callus formation may occur as early as 24-48 hours after budding of Shamouti orange to Palestine sweet lime and sour orange. However, tissue differentiation, such as tracheid formation in the callus, occurs in 15 days and cambium regeneration in 25 days. Therefore, a complete tissue union between inoculum donor and receptor tissue is not formed at 8 days, and virus might not be transmitted through the phloem tissue at this time. Wallace (1947) and Cohen (1972) succeeded in transmitting citrus viruses using leaf inserts. We obtained the necrotic reaction of Shirofugen by inserting cucumber leaf tissue infected by NRSV under the bark of Shirofugen stems. This suggests that only a union of the callus between donor and receptor tissues and a supply of the virus from the donor for a considerable time may be sufficient for virus transmission in grafting.

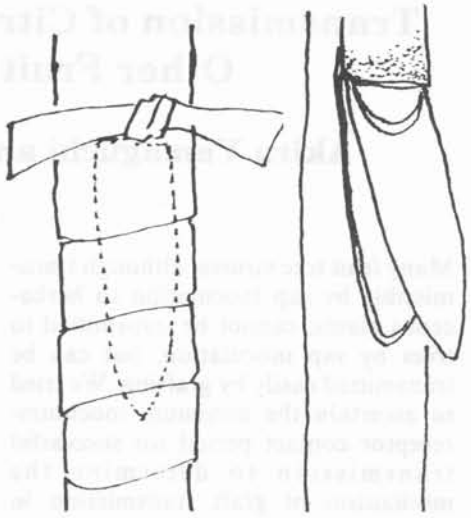

Fig. 1. Method of graft inoculation. In treatments where inoculum shield was removed after a specified time; inoculation site was rewrapped.

TABLE 1
INOCULUM-RECEPTOR CONTACT PERIOD FOR GRAFT TRANSMISSION OF APPLE CHLOROTIC LEAFSPOT VIRUS (CLSV), CITRUS TRISTEZA VIRUS (CTV), AND PRUNUS NECROTIC RINGSPOT VIRUS (NRSV)

	Contact period (days)					
Virus	1	2	4	8	16	Cont.*
CTV†	0/5	0/5	0/5	2/5	3/5	5/5
CLSV‡	0/5	1/5	4/5	5/5	5/5	5/5
NRSV§	0/20	0/20	19/20	10/10	—	20/20

* Inoculum left in place.
† Based on vein clearing symptoms in Mexican lime; no. infected/no. inoculated.
‡ Based on leaf symptoms in *Malus prunifolia* var. *ringo*; no. infected/no. inoculated.
§ Based on bark symptoms in Shirofugen cherry; no. infected/no. inoculated.

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