Sap Transmission of Chlorotic Leaf Spot and Stem-Grooving Viruses to Apple

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ABSTRACT

Six of nine apple stem-grooving virus isolates were transmitted mechanically from infectious Zucchini squash sap to 8-day-old McIntosh apple seedlings. An apple chlorotic leaf spot virus isolate was similarly transmitted from infectious *Chenopodium* sap. More transmissions were achieved in apple seedlings predarkened for 72 hr before inoculation than in those grown at a 16-hr photoperiod. Neither virus incited symptoms in infected apple seedlings, but each was recovered 22-82 days after inoculation.

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The major obstacle in relating certain apple viruses to the diseases they incite is the difficulty in reinfecting apple after the viruses are isolated in herbaceous plants. Cropley (1) failed to transmit chlorotic leaf spot virus (CLSV) to any of 40 apple seedlings inoculated with infectious sap of *Chenopodium amaranticolor* Coste & Reyn. or to any of 11 Spy 227 apple trees in which he implanted infected *C. amaranticolor* leaf tissues. He did, however, transmit CLSV to 4 of 7 Spy 227 trees by inarching with infected *Chenopodium*. Lister et al. (3) similarly infected 1 of 14 apple seedlings by inarching with CLSV-infected *C. quinoa* Willd., but 100 attempts to infect apple seedlings with partly purified preparations of CLSV from *C. quinoa* were not successful. Saksena & Mink (5), in 80 attempts, were not able to transmit CLSV to apple from herbaceous hosts with sap inoculations, tissue implants, or inarches. Stouffer (6) infected 4 of 8 apple seedlings with a CLSV isolate by *C. quinoa* tissue implants, but did not transmit a second CLSV isolate by this means. Stem-grooving virus (SGV or "Type 2" virus) was transmitted to apple seedlings by inarching with infected herbaceous hosts (4) or, with difficulty, by sap inoculation (R. M. Lister, *personal communication*).

Many attempts in our laboratory to transmit CLSV or SGV by sap inoculation of large apple seedlings, by tissue implants of infected *C. quinoa* leaves in apple, or by dodder (*Cuscuta subinclusa* Dur. & Hilg.) did not succeed. A single isolate of each virus was transmitted to 1 of 10 and 1 of 10 Spy 227 trees, respectively, by inarching them with infected *C. quinoa*, but the procedure was very laborious and many other attempts failed (2). A more efficient method of infecting apple from herbaceous plants was urgently needed.

In May 1971, we again attempted to transmit SGV from herbaceous plants to apple. Inoculum of eight SGV isolates was prepared by concentrating and partly purifying infected Zucchini squash (*Cucurbita maxima* Duchesne) sap with chloroform-bentonite treatment and ultracentrifugation (7). This host was relatively free of virus inhibitors, and a high concentration of SGV was attained in plants 14-21 days after inoculation. Eight-day-old McIntosh apple seedlings were dusted with 400-mesh corundum and the young leaves, cotyledons, and succulent stems were gently rubbed with purified virus inoculum. Each virus isolate was inoculated to 10 apple seedlings, of which five were subjected to complete darkness for 72 hr before inoculation and the remaining five were grown at a 16-hr daylength. The various SGV inocula induced 41-97 lesions/leaf in inoculated cowpea controls [*Vigna sinensis* (Torn.) Savi].

TABLE 1. Sap transmissions of stem-grooving virus (SGV) to apple seedlings from purified squash inocula

SGV isolate[c]	Plants infected/plants inoculated[a]	
	Predarkened[d]	16-hr day
Mutsu	3/5	1/5
Duke of Clarence	3/5	0/5
Queen Cox	0/5	2/5
Austin Gravenstein	1/5	0/5
Red Gravenstein	1/5	1/5
Totals	8/25	4/25

[a] McIntosh apple seedlings inoculated 8 days after emergence.

[b] Complete darkness for 72 hr before inoculation.

[c] Apple cultivars from which SGV isolates were initially obtained.

The inoculated McIntosh apple seedlings grew vigorously without symptoms. When they were initially indexed 22 days after inoculation on *C. quinoa* indicators, SGV was recovered from 4 of 80 seedlings. In later indexings, 64 and 82 days after inoculation, 12 of 50 seedlings in five inoculum groups were infected; and 0 of 30 seedlings, in the remaining three inoculum groups. SGV was not isolated from any of 10 noninoculated seedlings in any of the three indexings. Twice as many infections occurred in predarkened seedlings as in those grown at 16-hr daylength (Table 1).

In August, the procedure was repeated with yet another SGV isolate and with a CLSV isolate. The CLSV inoculum was purified and concentrated from *C. quinoa* sap. Each of the viruses was transmitted to young predarkened apple seedlings. Extensive trials with these same inocula on 5-month-old budlings of Idared and R-12740-7A apple are not concluded, but neither virus was recovered from inoculated budlings in the initial indexing 34 days after inoculation.

This technique afforded an easy and relatively efficient means of transmitting SGV or CLSV from herbaceous hosts to apple. Although a preinoculation dark period was not essential, its use improved rates of transmission. The data suggested that very young

apple seedlings were much more susceptible to infection by sap inoculation than older plants.

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