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Xyloporosis and Other Diseases

Studies on Two Mechanically Transmissible Citrus Viruses

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THE PURPOSE of this paper is to report the results of experiments on the identification, host range, and physical properties of 2 mechanically transmissible citrus viruses—latent Meyer lemon virus (LMLV) and citrus multiple sprouting virus (CMSV).

Materials and Methods

VIRUS ISOLATES.—Two isolates of LMLV were obtained from 2 different Meyer lemon trees (Nos. 547 and 186), growing in a rootstock trial in South Africa (3). Tree 547 was also infected with tristeza and greening viruses. The name LMLV does not necessarily imply that the virus is identical with that described by Yarwood (8). The CMSV came originally from a Joppa sweet orange tree with severe multiple sprouting symptoms (Fig. 1); this tree was growing in Transvaal, South Africa. There is presently insufficient evidence to decide whether CMSV is the cause of multiple sprouting or merely associated with the disease. The 2 isolates of this virus used in the present study came from leaves and flowers, respectively, of an artificially inoculated Valencia sweet orange tree

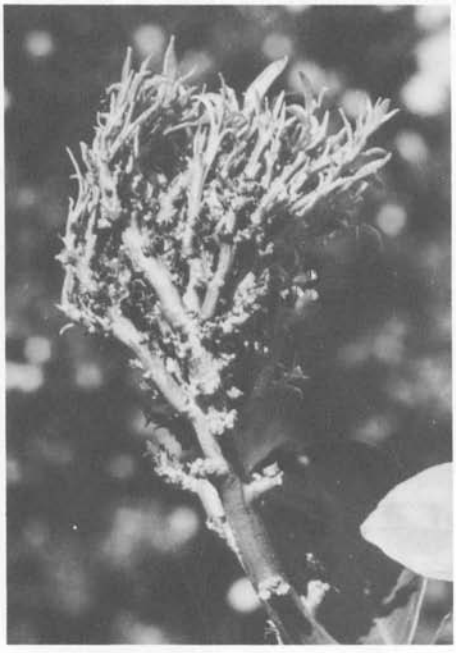

FIGURE 1. *Severe multiple sprouting symptoms in a shoot from Joppa sweet orange field tree from which CMSV was originally isolated.*

with multiple sprouting symptoms.

PROPAGATION AND MECHANICAL TRANSMISSION.—Test plants were maintained in a shaded greenhouse at 20–24° C.

Inoculum was prepared by triturating infected leaves or flowers in a mortar with twice their weight (w:v) of phosphate buffer 0.1M, pH 7.0. The sap thus obtained was rubbed over leaves previously dusted with celite. Initial transmissions were made from leaves of Meyer lemon (for LMLV) and from leaves and flowers of sweet orange (for CMSV) to seedlings of *Chenopodium quinoa* Willd. at the 6–8 leaf stage and to the primary leaves of Lady Finger Round cowpea. The viruses were subsequently maintained in *C. quinoa*.

Experimental Results

HOST RANGE.—Inoculum prepared from *C. quinoa* in the previously described manner was used to test a number of species of plants for susceptibility to LMLV and CMSV. Those species that became infected are listed in Tables 1 and 2. Symptoms appeared 6–15 days after inoculation. A higher percentage of transmission (80–100 per cent) was obtained when *C. quinoa* was the source of inoculum than when other species served as the source.

Symptoms produced by LMLV on *C. quinoa* consisted of diffuse yellow areas in inoculated leaves of young seedlings; yellow local lesions in inoculated leaves of plants at the 8–10 leaf stage, or older; and a faint chlorotic mottle in systemically infected leaves about 10 days after inoculation. In *C. amaranticolor* the local lesions were inconsistent, but inoculated leaves sometimes were distorted, and the systemic mottle was milder than in *C. quinoa*. Symptoms of CMSV in the 2 species were similar to those of LMLV but were more severe and appeared sooner (6 days).

The varieties of cowpea tested responded about the same to both viruses. The Iron Clay variety and the unidentified variety developed the most severe symptoms; they consisted of occasional necrotic spots in inoculated primary leaves; yellowing of veins and diffuse yellowing and necrosis of the blade of trifoliate leaves.

Three other species were infected with LMLV but developed no symptoms (Table 1), whereas the same 3 species, and 4 additional species, proved to be susceptible to latent infection with CMSV (Table 2).

Nicotiana glutinosa L. developed a diffuse yellowing of systemically infected leaves 10–15 days after inoculation with CMSV, but failed to become infected when inoculated with LMLV.

The following species failed to become infected with either virus: *Crotalaria spectabilis* Roth., *Cucumis sativus* L., *Nicotiana tabacum* L., *Ocimum basilicum* L., and *Phaseolus vulgaris* L. In addition *Capsicum anuum* L., *Cynara scolymus* L., *Helianthus annuus* L., *Cucumis melo* Naud., and *Zinnia elegans* Jacq. failed to become infected when inoculated with LMLV.

TABLE 1. HOST REACTION TO A LATENT VIRUS ISOLATED FROM MEYER LEMON

Host	Infection[a]		
	Local	Systemic	Latent
Amaranthaceae	—	—	+
Gomphrena globosa L.			
Chenopodiaceae			
Chenopodium amaranticolor Coste & Reyn.	±	Mo	
C. quinoa Willd.	ChS	Mo, Ma	
Leguminosae			
Vigna sinensis (L.) Endl.			
cv. unknown	—	Lr, N, Vy, Y	
cv. Black crowder	NS	Lr, Vy, St	
cv. Iron clay	NS	Lr, N, Y	
cv. Lady finger round	NS	Lr, Y	
cv. Phoenix	—	Lr, N, Y	
cv. Poona	±	Y	
Solanaceae			
Nicotiana clevelandii Gray	—	—	+
N. rustica L.	—	—	+

a. Infection determined by back-transmission to *C. quinoa*. Host reaction is denoted by: ChS, chlorotic spots; Lr, leaf roll; Ma, malformation; Mo, mottle; N, necrosis; NS, necrotic spots; St, stunting; Vy, vein yellowing; +, infection; ±, doubtful; —, no infection.

TABLE 2. PLANTS SUSCEPTIBLE TO THE VIRUS ASSOCIATED WITH CITRUS MULTIPLE SPROUTING

Host	Reaction[a]		
	Local	Systemic	Latent
Amaranthaceae			
Celosia argentea L.	—	—	+
Gomphrena globosa L.	—	—	+
Chenopodiaceae			
Chenopodium amaranticolor Coste & Reyn.	—	Mo, Ma	
C. quinoa Willd.	ChS	Mo, Ma	
Compositae			
Helianthus annuus L.	—	—	±
Leguminosae			
Vigna sinensis (L.) Endl.	—	Lr, N, Vy, St	
cv. Black crowder	—	Lr, Vy, Y	
cv. Iron clay	NS	N, Vy, Y	
cv. Lady finger round	NS	Lr, N, Vy, Y	
cv. Phoenix	NS	Lr, N	
cv. Poona	NS	N, Y	
Solanaceae			
Nicotiana clevelandii Gray	—	—	±
N. glutinosa L.	—	Y	
N. rustica L.	—	—	+
Petunia hybrida Vilm.	—	—	+
Physalis floridana L.	—	—	±

a. Infection determined by back-transmission to *C. quinoa*. Host reaction is denoted by: ChS, chlorotic spots; Lr, leaf roll; Ma, malformation; Mo, mottle; N, necrosis; NS, necrotic spots; St, stunting; Vy, vein yellowing; Y, yellowing; +, infection; ±, doubtful; —, no infection.

PHYSICAL PROPERTIES.—The physical properties of the 2 viruses—determined with standard procedures (2)—are very similar irrespective of the 4 isolates and 2 donor hosts, *C. quinoa* and *N. glutinosa*. Both had dilution end points between 10^{-4} and 10^{-5}, thermal inactivation points between 50 and 60°C, and withstood aging in vitro for 6–24 hours. Their infectivity was greatly reduced by freezing at −18°C for 2–4 weeks. Their infectivity was preserved for at least 6 months when infected leaves of *C. quinoa*—or infectious sap plus additives (1)—were freeze-dried.

PURIFICATION.—Attempts to purify both viruses by the methods described by Steere (6) and Semancik and Weathers (5) were only partly successful. Partially purified preparations obtained when infected plants of *C. quinoa* and cowpea were used as a source of virus were as infectious as crude sap but never more so.

Examination of the partially purified preparations, and of leaf dip preparations, under an electron microscope failed to reveal viruslike particles. No viruslike particles were seen in ultrathin sections when they were examined by electron microscopy.

Partially purified preparations of both viruses, as well as crude sap, failed to react with antiserum to viruses of tobacco necrosis, cucumber mosaic, alfalfa mosaic, and arabis mosaic in Oucterlony agar diffusion tests.

Discussion

Thus far, LMLV and CMSV can be differentiated only by minor differences in their host ranges. The latter seems to have a somewhat broader host range than the former. The differences may or may not be significant. The differential response of *N. glutinosa* offers a possible means of separating CMSV from a mixture of it and LMLV.

The 2 viruses discussed in this paper resemble, in their host range and physical properties, citrange stunt virus described by Wallace and Drake (7) and previously shown by Semancik and Weathers (4) to be mechanically transmissible. Lack of knowledge of the morphology of LMLV and CMSV prevents us from establishing whether or not either one of them is closely related to citrange stunt virus. There is presently no reason for believing that either virus is related to citrus-variegation virus or crinkly-leaf virus.

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