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Authors

Roistacher, C. N.

Nauer, E. M.

Wagner, R. L.

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Transmissibility of Cachexia, Dweet Mottle, Psorosis, Tatterleaf and Infectious Variegation Viruses on Knife Blades and Its Prevention

C. N. Roistacher, E. M. Nauer, and R. L. Wagner

Mechanical transmission of citrus viruses and viruslike diseases has been reviewed by Price (1968), Weathers (1969), and Weathers *et al.*, (1974). Mechanical transmission from citrus to citrus has been recorded for the viruses causing infectious variegation (IVV), including crinkly leaf (CLV), satsuma dwarf (SDV), and citrus leaf rugose (CLRV), psorosis A (PV), citrus necrotic ringspot (CRSV), tatterleaf-citrange stunt complex (TL-CSV), tristeza, and seedling yellows tristeza, and citrus exocortis viroid (CEV) (Weathers, *et al.*, 1974; Garnsey and Timmer, 1980; Timmer and Garnsey, 1980; and Garnsey *et al.*, 1977).

Disinfection of clippers or knife blades has been successful for CEV using a 2 per cent formaldehyde plus 2 per cent sodium hydroxide solution (Garnsey, 1967), or a 1 per cent sodium hypochlorite solution (Roistacher *et al.*, 1969), and both are also effective for TL-CSV and CLRV (S. Garnsey, personal communication).

This paper presents evidence for the transmission on knife blades from citrus to citrus of two viruses not previously reported, and the chemical inactivation of five citrus viruses on knife blades.

MATERIALS AND METHODS

Exploratory tests were made to determine the transmissibility of certain citrus viruses by knife from virus-infected citrus to citron. Symptoms developing on citron were sufficient to determine directly transmissibility of PV and IVV. Since citron is a symptomless carrier of cachexia virus (CV), Dweet mottle virus (DMV), and TL-CSV, subpropagations were made by budgrafts from citron to specific indica-

tor plants. Indicators were: Parsons Special mandarin budded on rough lemon seedlings for CV (Roistacher *et al.*, 1973); Dweet tangor for DMV; and *Citrus excelsa* and Troyer citrange for TL-CSV.

All viruses were established in seedlings or clonal propagations of Arizona 861 citrons. Citrons were selected as donor and receptor plants because of their proven superiority in mechanical transmission of citrus viruses (Garnsey, 1974; Garnsey *et al.*, 1977). All plants were grown in a U.C. potting mix modified for citrus (Nauer *et al.*, 1968) in 4-liter plastic containers. Glasshouse temperatures averaged 26°C daytime maximum and 19°C nighttime minimum.

Inoculation by knife cut was as described for exocortis by Roistacher *et al.* (1969). After exploratory tests, each of the five viruses was tested for chemical inactivation on knife blades in two larger experiments. In the first experiment, two cuts were made into the infected donor citron, the blade dipped for 1 second in a 20 per cent solution of household bleach (1.05 per cent sodium hypochlorite), wiped with tissue paper, and then a slice cut into the receptor citron. This operation was repeated 10 times per plant for each of 15 citrons. Fifteen additional citrons were knife-cut without chemical dip as controls. After cutting, the sliced area was wrapped with polyethylene tape. In the second experiment, the procedures were the same except 10 plants were used for each of the following treatments: 1) sodium hypochlorite as in the first experiment; 2) a 2 per cent sodium hydroxide plus 5 per cent formaldehyde dip; and 3) a control consisting of knife-

cut inoculations without chemical dip.

The effect of a water-dip control on transmissibility was studied in separate tests for PV, TL-CSV, and IVV. Ten to 15 citrons were inoculated with each virus by 10 knife cuts as previously described; however, prior to each inoculation cut, the blade was dipped in water and wiped dry with tissue paper, simulating the technique used for chemical dip. Non-dip controls were also tested.

After inoculation, citrons were cut back to force new growth. After at least 8 weeks, the new growth was observed for symptoms of PV or IVV, or grafted to appropriate indicator plants for detection of CV, DMV, and TL-CSV.

PRELIMINARY TESTS AND RESULTS

Cachexia. The appearance of two unexplained CV infections, during 1976 and 1977, in Parsons Special mandarin indicators suggested possible mechanical transmission of CV. In an exploratory test, five CV-infected plants, a navel orange, two mandarins, a grapefruit, and a Lisbon lemon, served as donors. Twenty-five knife cuts were made into each of two citrons from each donor. After 3 months, buds from each of the CV-inoculated citrons were grafted onto Parsons Special mandarin/

rough lemon indicator plants and were observed for symptoms 12 months later. Only two citrons inoculated from the Lisbon lemon source were infected.

In a second exploratory test, with citron as the donor plant, knife blades were first cut into the CV-infected citron, then dipped for 1 second in a 20 per cent solution of commercial bleach (1.05 per cent hypochlorite), wiped dry, and used to cut citron receptors. Ten knife cuts were made into each of five citrons for dipped and non-dipped treatments. In addition, three citrons were bud-inoculated with donor tissue as a positive control. After 3 months, buds from the new growth of each of the 13 citron receptors were graft-inoculated to clonal Parsons Special mandarin propagated on rough lemon seedlings, and were observed for symptoms 13 months later. Results showed 0/5, 4/5, and 3/3 positive transmissions for dipped, non-dipped, and bud-inoculated controls, respectively.

The two larger transmission tests show that CV is readily transmissible by knife cuts from citron to citron, and that transmission by knife cuts was prevented by sodium hypochlorite treatment (table 1).

Dweet mottle virus. DMV was shown by Roistacher and Blue (1968) to be separate and distinct from psorosis-A

TABLE 1
TRANSMISSION OF FIVE CITRUS VIRUSES BY CONTAMINATED KNIFE BLADES
WITH AND WITHOUT TREATMENT IN TWO DISINFECTANTS

Virus	Number of transmissions/total plants inoculated					
	Test 1			Test 2		
	Sodium hypochlorite dip	Control no dip	Sodium hypochlorite dip	Sodium hydroxide + formaldehyde dip	Control no dip	
Cachexia (CV)	0/15	15/15	0/10	2/10	10/10	
Dweet mottle (DMV)	0/15	2/15	0/10	0/10	6/10	
Psorosis (PV)	0/30	13/30	0/20	0/20	6/20	
Tatterleaf (TL-CSV)	0/15	14/15	0/10	0/10	9/10	
Infectious (IVV) variegation	0/15	15/15	0/10	0/10	9/10	

and concave gum viruses, and is detected only in Dweet tanger indicator seedlings. In preliminary tests, knife cuts from an infected Dweet tanger donor to Dweet indicator seedlings failed to transmit DMV to six plants, whereas DMV was transmitted to one of four citrons knife-cut inoculated (10 cuts/plant) from infected Dweet tanger. This mechanically infected citron was used as the donor plant for further transmission experiments. Two larger transmission tests showed 8/25 (32 per cent) positive transmission from citron to citron in the non-dip controls and no transmission when knife blades were disinfected in either of the two chemicals tested (table 1).

Psorosis. In preliminary tests, six isolates of PV and one isolate of concave gum were graft-inoculated to citron, and knife-cut inoculations were made from citron to citron (10 cuts/plant). One PV isolate was mechanically transmitted to three of four citrons. This isolate, P-203 in a Kao Panne pummelo (CRC 2249), was introduced from Thailand to the citrus variety collection at the University of California, Riverside, in 1930. Indexing of this pummelo in 1963 showed no reaction in Mexican lime, a strong mottle in leaves of *Citrus excelsa* and King mandarin, a mild leaf curl and mottle in sour orange, and some leaf curl in sour lemon. The citrons inoculated by mechanical transmission showed a severe shock reaction with pronounced leaf flecking, mottle, curl, necrotic spotting, and leaf drop.

Results of the two larger tests are given in table 1. Nineteen of 50 mechanically inoculated citrons in control treatments were infected. There was no infection when knife blades were disinfected in either of the two chemicals. In a separate test, PV was not transmitted to 15 citrons when knife blades were dipped in water and wiped with tissue paper, but was transmitted to 7/15 plants of the dry-cut controls.

To ascertain whether P-203 was related to psorosis A, sweet orange seedlings were bud-inoculated with tissue from a citron infected with P-203 by

mechanical inoculation. Four months later seedlings were challenge-inoculated with psorosis-A lesion inoculum. Preinoculated seedlings were protected, whereas challenge-noninoculated controls developed severe psorosis-B lesions. This suggests that the mechanically transmitted P-203 is a psorosis-A virus.

Tatterleaf. In preliminary tests, knife-cut transmissions were attempted from a Meyer lemon donor plant containing TL-CSV to each of six *C. excelsa*, Troyer citrange, and citron receptor plants. The *C. excelsa* and Troyer plants remained symptomless, but all six citron receptors were found infected when subsequently indexed on *C. excelsa* and Troyer citrange indicator plants by graft inoculation.

In two larger transmission tests, TL-CSV was transmitted from citron to citron in 23/25 plants (92 per cent) of the nondipped controls (table 1). Buds from all 23 infected citrons induced symptoms in *C. excelsa* and Troyer citrange indicators, indicating no separation of tatterleaf and citrange stunt components by mechanical transmission. No transmission of TL-CSV from citron to citron occurred when knife blades were dipped in the two chemicals. In a separate test, 0/10 citrons were infected when inoculated with contaminated knife blades dipped in water and wiped dry prior to cutting, whereas 9/10 were infected in dry-cut controls.

Infectious variegation. In early transmission trials, IVV was transmitted from infected citron to citron in 3/14 plants using 10 knife cuts per plant. The virus source was IV 401, a sap-transmitted strain of IVV free of CEV, collected originally from Glendora, California, in 1939 by J. M. Wallace. No transmissions occurred when knife blades were dipped in 20 per cent bleach.

An IVV isolate collected by E. C. Calavan (IV 402) from a sour lemon in the Alta Loma area of Southern California in 1977 was used in the two larger

tests. The source plant was also infected with severe CEV, and Eureka lemon and citron bud-inoculated from this source showed strong leaf curl and mottle. Citrons mechanically inoculated with this isolate by knife cuts, which showed very mild CEV symptoms, were used as donor plants. In the two larger transmission tests, 24/25 (96 per cent) of the plants in the nondipped controls became infected (table 1). In contrast, 0/35 were infected when blades were disinfected in either chemical. In a separate test, there was no infection in 10 citrons inoculated with contaminated knife blades which had been dipped in water and wiped dry prior to cutting, whereas dry-cut controls showed 9/10 transmissions.

CONCLUSIONS

1. The CV and DMV were transmitted from citrus to citrus by knife-cut inoculation. This is the first report of knife-cut transmission of these viruses.

2. The sodium hypochlorite dip (1 per cent active) or a mixture of sodium hydroxide (2 per cent) plus formaldehyde (5 per cent) dip was completely effective in preventing transmission of four of five citrus viruses on contaminated knife blades. None of these four viruses was transmitted by 10 knife cuts

from citron to citron after a 1-second dip between cuts, whereas nondipped blades transmitted the viruses in 32 to 100 per cent of inoculated citrons. Only the sodium hypochlorite dip completely prevented transmission of the cachexia pathogen.

3. When blades were dipped in water and wiped dry prior to inoculation cutting, PV, TL-CSV, and IVV were not transmitted, whereas blades neither dipped nor wiped transmitted the viruses at 47, 90, and 90 per cent rates, respectively. The water-dip plus wiping was as effective in preventing transmissions as the two chemical treatments.

4. The high transmissibility of CV by knife-cut inoculation could explain the appearance of CV symptoms in unbudded seedlings or in nucellar selections by Reichert and Perlberger (1934), Childs (1952, 1956), Norman *et al.* (1959), Reichert (1959), and Carpenter and Furr (1960). Mechanical transmission could be partially responsible for the widespread distribution of CV in many of the citrus-growing areas of the world. Childs *et al.* (1965) found no evidence of seed transmission of CV.

5. TL-CSV and IVV were also found to be highly transmissible by knife cuts from citron to citron, whereas DMV and PV P-203 were comparatively less transmissible.

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