Differential Transmission of Prunus Tomato Ringspot Virus Strains by *Xiphinema californicum*

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Accepted for publication 4 October 1983.

ABSTRACT

Hoy, J. W., Mircetich, S. M., and Lownsbery, B. F. 1984. Differential transmission of Prunus tomato ringspot virus strains by *Xiphinema californicum*. Phytopathology 74:332-335.

Three tomato ringspot virus (TmRSV) strains associated with the prune brownline (PBL), Prunus stem pitting (PSP), and cherry leaf mottle (CLM) diseases of various hosts in the genus *Prunus* were found to be serologically related, but not identical. TmRSV-PBL, TmRSV-PSP, and TmRSV-CLM were each transmitted by *Xiphinema californicum* from mechanically inoculated cucumber (*Cucumis sativus*) to cucumber and certain *Prunus* species. TmRSV-PBL was transmitted to peach (*Prunus persica*) and Myrobalan plum (*P. cerasifera*), TmRSV-PSP was transmitted to peach, and TmRSV-CLM was transmitted to Mahaleb cherry (*P. mahaleb*), but not to peach or sweet cherry (*P. avium*). *X. californicum* transmitted TmRSV-PBL from cucumber to 24 of 25 cucumber and eight of 15 peach. TmRSV-PSP was transmitted to 10 of 10 cucumber and eight of 10 peach, and TmRSV-CLM was transmitted to three of 22 cucumber and none of 10 peach. Apparently, *X. californicum* is an efficient vector of TmRSV-PBL and TmRSV-PSP, but an inefficient vector of TmRSV-CLM.

The dagger nematode, *Xiphinema americanum* Cobb, is known to be a vector of tomato ringspot virus (TmRSV) (10,17,18). Transmission of TmRSV to experimental hosts, such as cucumber (10,17), and to economic hosts, such as peach, apricot, and plum (18) has been demonstrated. Strains of TmRSV cause several important diseases of stone fruit trees, including stem pitting in numerous *Prunus* spp. (12), yellow bud mosaic in peach and almond (16), and prune brownline in European plum on both peach and Myrobalan plum rootstocks (13). The transmission of nepoviruses by nematodes may or may not be highly specific (6). Serologically related strains of some viruses, such as raspberry ringspot virus and tomato black ring virus, have different nematode vectors (5). With viruses transmitted by *X. americanum*, such as TmRSV and tobacco ringspot virus, no differences in transmission levels of virus strains have been detected (15,17), but transmission levels of strains of TmRSV associated with different diseases of *Prunus* spp. have not been compared.

Recently, it was reported (11) that *X. americanum* Cobb is limited to eastern North America. Results of limited field observations suggest that the *Xiphinema* species most commonly associated with fruit trees in California is *X. californicum* Lamberti & Bleve-Zacheo. *Xiphinema rivesi* Dalmasso, a species previously distinguished from *X. americanum* (3), transmits TmRSV from cucumber to cucumber (4), but the ability of the proposed new *Xiphinema* species to transmit TmRSV is uncertain.

This paper reports the results of experiments designed to test for transmission of three strains of TmRSV (originally isolated from *Prunus* spp.) to cucumber and *Prunus* spp. by *X. californicum*.

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MATERIALS AND METHODS

Nematodes. Nematodes morphologically similar to *X. californicum* were located and identified from an apple (*Malus domestica* Borch.) orchard in Sonoma County, California. Soil containing a high population of *X. californicum* was collected, transported to Davis, and placed in two wooden boxes (1 × 0.75 × 0.6 m) in the greenhouse. Nematodes were then maintained on Sudan grass (*Sorghum vulgare* var. *sudanense* Hitch. 'Piper'). Nematode specimens are on deposit in the University of California at Davis Nematode Collection (UCDNC, slides 40 a–f).

TmRSV isolates. Single TmRSV isolates were obtained from trees naturally affected by the prune brownline (PBL) (13), Prunus stem pitting (PSP) (12), or cherry leaf mottle (CLM) (7) diseases. A TmRSV-PBL isolate was obtained from the Myrobalan plum (*Prunus cerasifera* Ehrh.) rootstock of a PBL-affected prune (*P. domestica* L. 'French') tree. Cambial and inner bark tissues (0.5–1.0 g) were collected below the brownline symptom at the graft union and triturated with a mortar and pestle in chilled 0.1 M potassium phosphate buffer, pH 7.0, mixed with 5% aqueous nicotine (1:1.5, v/v), and the resulting extract was rubbed on Carborundum-dusted leaves of cucumber (*Cucumis sativus* L. 'National Pickling'), cowpea (*Vigna unguiculata* [L.] Walp. 'Ramshorn'), bean (*Phaseolus vulgaris* L. 'Bountiful'), and tobacco (*Nicotiana tabacum* L. 'Havana 425') in the field. Inoculated plants were then transferred to the greenhouse and observed for the development of virus symptoms. A TmRSV-PSP isolate was obtained from the stem-pitted lower trunk of a PSP-affected peach (*P. persica* (L.) Batsch 'Lovell') tree as described above, and a TmRSV-CLM isolate was obtained from a CLM-affected sweet cherry (*P. avium* L. 'Bing') tree on Mahaleb cherry (*P. mahaleb* L.) rootstock as described above except that the inoculum was prepared from leaves showing mottling symptoms. Virus isolates were maintained in the same four herbaceous host plants in the greenhouse.

Serology of TmRSV strains. Partial serological relationships among TmRSV-PBL, TmRSV-PSP, and TmRSV-CLM isolates were shown in gel double immunodiffusion tests employing an antiserum prepared against TmRSV-PBL (7) and a similarly prepared antiserum against TmRSV-PSP which had a specific titer of 1:512 and a nonspecific titer of 1:4. Tests were conducted in petri plates containing a 5-mm layer of agar (0.8% Difco Ionagar, 0.85% sodium chloride, and 0.1% sodium azide). Antisera against TmRSV-PBL and TmRSV-PSP (diluted 1:30 and 1:20, respectively, in phosphate-buffered saline [PBS], pH 7.0) and antigen and healthy control samples, consisting of sap expressed from systemically virus-infected or healthy cucumber leaves, were placed in wells cut in the agar layer, and then plates were observed after 48 hr for the development of precipitin reaction bands at the intersection of antigen and antiserum diffusion zones.

Assay for TmRSV. All cucumber and experimental hosts in *Prunus* spp. were assayed for TmRSV by an indirect enzyme-linked immunosorbent assay (I-ELISA) (2) employing chicken antiTmRSV-PBL immunoglobulins and horseradish peroxidase-conjugated rabbit antichicken immunoglobulins (9). Root or bark samples (1–3 g) were collected from individual plants, triturated in 3 ml of grinding buffer (PBS, pH 7.4, containing 2% polyvinylpyrolidone [M.W. 40,000], 0.2% bovine serum albumin, and 0.05% polyoxyethylene sorbitan monolaurate [Tween-20]) with a mortar and pestle, homogenized with a Polytron homogenizer (Brinkmann Instruments), and incubated in polystyrene ELISA plates (two plate wells per sample) overnight at 4 C. Sample reactions were determined by scanning plates in a Titertek Multiskan colorimeter (Flow Laboratories, Inc., Inglewood, CA 90301) for absorbance at 450 nm 15 min after the addition of peroxidase substrate (9). Samples with reaction values at least three times those of the healthy plant control samples were accepted as positive for TmRSV.

Nematode virus transmission experiments. Nematodes were washed from soil samples and collected on a 0.15-mm (100 mesh) sieve. Large numbers (500–1,000) of *X. californicum* were hand picked within no more than 3 hr and placed directly on exposed root systems of virus-infected cucumber seedlings (plants mechanically inoculated in cotyledon stage with TmRSV-PBL, TmRSV-PSP, or TmRSV-CLM 6–10 days prior to use), or healthy cucumber seedlings in 100-cc plastic pots partially filled with a 1:1 mixture of sterilized sandy loam field soil and UC mix (1). The pots were then filled with the same soil mix and placed in a growth chamber at 24 C with a 14-hr light period.

The nematodes were given a 14-day virus acquisition feeding on roots of virus-infected cucumber plants. Then they were extracted from the soil by sieving as before, counted, and separated into groups of 25 or 100. The nematodes were placed directly on exposed root systems of healthy 10- to 14-day-old seedlings of cucumber (25 nematodes per plant) or older seedlings of *Prunus* spp. (100 nematodes per plant) (3–8 mm in diameter) in partially filled 100-cc plastic pots (cucumber) or 150-cc clay pots (*Prunus* spp.). The pots were then filled with the same soil mixture and placed in the growth chamber. Twenty-five cucumber, 15 peach, and 15 Myrobalan plum seedlings were exposed to nematodes transferred from TmRSV-PBL infected cucumbers; 10 cucumber and 10 peach seedlings were exposed to nematodes transferred from TmRSV-PSP infected cucumbers; and 22 cucumber, 10 peach, 10 Mazzard cherry, and 10 Mahaleb seedlings were exposed to nematodes transferred from TmRSV-CLM infected cucumbers. Controls consisted of five plants each of cucumber, peach, Myrobalan plum, Mazzard cherry, and Mahaleb cherry seedlings exposed to nematodes transferred from healthy cucumber seedlings. Root samples were collected from all cucumber virus acquisition hosts and assayed for TmRSV with I-ELISA to confirm that TmRSV had infected the root systems.

To determine whether the three TmRSV isolates were acquired and transmitted by *X. californicum*, root samples were collected from cucumber, and root and bark samples were collected from test hosts of the *Prunus* spp. exposed to nematodes given previous acquisition feedings on TmRSV-PBL-, TmRSV-PSP-, or TmRSV-CLM-infected or healthy cucumber plants and assayed for TmRSV with I-ELISA. Root samples from cucumber plants were collected, thoroughly washed, and assayed for TmRSV after a 14-day exposure to nematodes. Sixty days after the addition of nematodes, young roots were collected from seedlings of the *Prunus* spp., thoroughly washed, and assayed for TmRSV. After 1.5 yr, bark samples were collected and assayed for TmRSV.

Twenty samples of 25, 50, 75, or 100 *X. californicum* were collected after virus acquisition feedings in 1 ml of I-ELISA grinding buffer, sliced with a scalpel in the esophageal and intestinal regions, stirred with a vortex mixer, and assayed for TmRSV with I-ELISA to determine if TmRSV could be detected in viruliferous nematode extracts. Four samples of 25 nematodes from the same acquisition feedings were placed on the exposed root systems of healthy cucumber seedlings which were handled and assayed for TmRSV as described above. All nematode samples from an acquisition feeding were considered viruliferous if TmRSV was transmitted to cucumber seedlings by the sample of 25 nematodes. Controls consisted of samples of nematodes that were collected after a feeding on healthy cucumber seedlings and were handled and tested in the same way.

RESULTS

Serology of TmRSV strains. Results of gel double immunodiffusion tests showed that the TmRSV-PBL, TmRSV-PSP, and TmRSV-CLM isolates were serologically related but not identical. In tests employing antiserum against TmRSV-PBL, TmRSV-PBL formed a heterologous precipitin spur with TmRSV-CLM, TmRSV-PBL was homologous with TmRSV-PSP, and TmRSV-PSP was homologous with TmRSV-CLM (Fig. 1A). In tests employing antiserum against TmRSV-PSP, TmRSV-PSP formed heterologous precipitin spurs with TmRSV-PBL and TmRSV-CLM, and TmRSV-PBL formed a precipitin spur with TmRSV-CLM (Fig. 1B).

Nematode transmission of TmRSV strains. *X. californicum* transmitted TmRSV-PBL, TmRSV-PSP, and TmRSV-CLM from virus-infected cucumber to cucumber and certain *Prunus*

species; however, the three strains were not transmitted with equal efficiency. *X. californicum* efficiently transmitted TmRSV-PBL and TmRSV-PSP to cucumber and to peach (Table 1), whereas TmRSV-CLM was transmitted very inefficiently to cucumber and not at all to peach (Table 1). In experiments to detect transmission of the three virus strains from cucumber to cucumber plants with I-ELISA, the $A_{450\,nm}$ means for root samples in which TmRSV was detected were 0.27 ± 0.09 for TmRSV-PBL, 0.37 ± 0.12 for TmRSV-PSP, and 0.11 ± 0.26 for TmRSV-CLM. The means for controls exposed to nematodes given a previous acquisition feeding on healthy cucumbers and healthy root samples were 0.018 ± 0.014 and 0.023 ± 0.005, respectively. In I-ELISA tests to detect transmission of the virus strains to peach, the $A_{450\,nm}$ means for TmRSV-positive root samples were 0.23 ± 0.06 for TmRSV-PBL and 0.25 ± 0.16 for TmRSV-PSP, and the means for TmRSV-positive bark samples were 0.18 ± 0.02 for TmRSV-PBL and 0.19 ± 0.04 for TmRSV-PSP. TmRSV-CLM was not detected in any peach samples. Control and healthy root sample means were 0.019 ± 0.006 and 0.014 ± 0.01, respectively, and control and healthy bark sample means were 0.02 ± 0.01 and 0.021 ± 0.006, respectively.

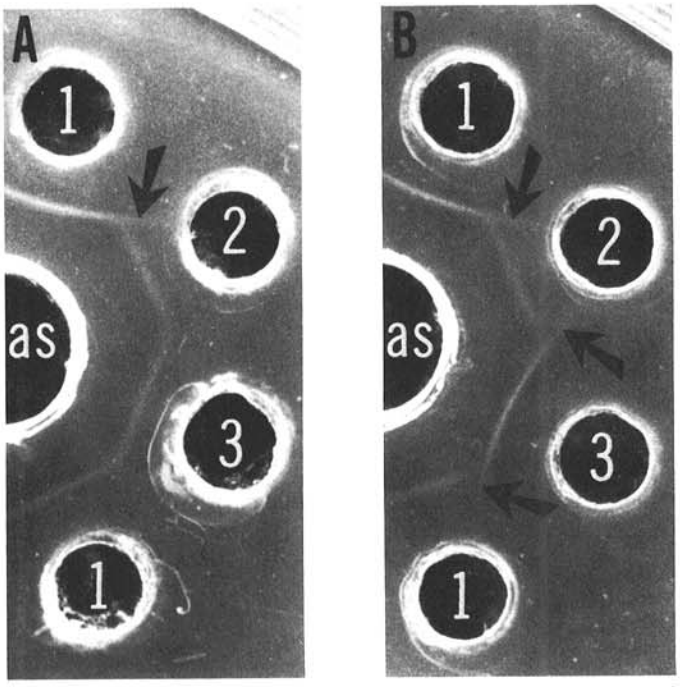

Fig. 1. Comparison of the prune brownline (PBL) (well 1), cherry leaf mottle (CLM) (well 2), and Prunus stem pitting (PSP) (well 3) strains of tomato ringspot virus (TmRSV) in gel double immunodiffusion tests employing antiserum (as) produced against TmRSV-PBL or TmRSV-PSP. **A,** Test employing TmRSV-PBL as. Note the precipitin spur (arrow) on the TmRSV-PBL (well 1) reaction line extending behind the reaction line of TmRSV-CLM (well 2). **B,** Test employing TmRSV-PSP as. Note presence of spurs (arrows) on reaction line of TmRSV-PSP (well 3) behind the TmRSV-PBL (well 1) and TmRSV-CLM (well 2) reaction lines and on the TmRSV-PBL (well 1) reaction line behind the TmRSV-CLM (well 2) reaction line.

TABLE 1. Transmission of the prune brownline (PBL), Prunus stem pitting (PSP), or cherry leaf mottle (CLM) strains of tomato ringspot virus (TmRSV) to cucumber and peach seedlings by *Xiphinema californicum*

	Fraction of indicators in which three TmRSV strains were detected[b]			Fraction of control indicators in which TmRSV was detected[c]
Indicator[a]	PBL	PSP	CLM	
Cucumber	24/25	10/10	3/22	0/5
Lovell peach	8/15	8/10	0/10	0/5

[a] Indicators exposed to nematodes given previous 14-day acquisition access feeding on cucumber seedlings mechanically inoculated with one TmRSV strain or sap from healthy cucumber seedlings. Cucumber indicators were exposed to 25 *X. californicum* for 14 days, and peach indicators were exposed to 100 *X. californicum* for 60 days.

[b] Root samples from indicators exposed to *X. californicum* given previous TmRSV-PBL, TmRSV-PSP, or TmRSV-CLM acquisition access feedings were collected, washed, and assayed for TmRSV with indirect enzyme-linked immunosorbent assay (I-ELISA).

[c] Root samples from indicators exposed to *X. californicum* given previous acquisition access feeding on healthy cucumber seedlings were collected, washed, and assayed for TmRSV with I-ELISA.

In additional experiments to transmit TmRSV-PBL and TmRSV-CLM from virus-infected cucumber to other *Prunus* seedlings, TmRSV-PBL was transmitted to 10 of 15 Myrobalan plum seedlings (8) exposed to nematodes from a TmRSV-PBL acquisition feeding, and TmRSV-CLM was transmitted to two of 10 Mahaleb cherry and 0 of 10 Mazzard cherry seedlings exposed to nematodes from a TmRSV-CLM acquisition feeding. No virus was detected in any of five each of control Myrobalan plum, Mazzard cherry, or Mahaleb cherry seedlings exposed to nematodes given a previous acquisition feeding on healthy cucumber plants or in healthy control plants.

TmRSV-PBL was not detected in 20 samples of extracts from 25, 50, 75, or 100 *X. californicum* collected, sliced with a scalpel, and assayed for TmRSV by I-ELISA immediately following virus acquisition feeding on TmRSV-PBL infected cucumber seedlings, while four samples of 25 *X. californicum* from the same acquisition feedings each transmitted TmRSV-PBL to cucumber seedlings. The $A_{450\,nm}$ mean readings for extracts from nematodes fed on virus-infected or healthy plants were 0.016 ± 0.006 and 0.013, whereas the $A_{450\,nm}$ means for cucumber root samples from bait plants exposed to viruliferous nematodes and healthy controls were 0.32 ± 0.025 and 0.028.

DISCUSSION

Apparently, *X. californicum* is a vector of TmRSV. It is the second species, in addition to *X. rivesi* (4), that has been separated from *X. americanum* and found to transmit TmRSV. Newly erected *Xiphinema* species should be tested to determine if they are vectors of TmRSV.

Strains of TmRSV cause several serious diseases of *Prunus* spp. (12,13,16). The peach yellow bud mosaic strain of TmRSV (TmRSV-PYB) was shown to be efficiently transmitted by California nematodes identified as *X. americanum* (18) to peach, apricot (*P. armeniaca* L.), and Damson plum (*P. insititia* L.). The nematodes employed in those experiments were probably *X. californicum*. However, no specimens remain from that work to confirm the relationship. In this study, *X. californicum* transmitted isolates of three other strains of TmRSV from *Prunus* spp. associated with the PBL, PSP, and CLM diseases to *Prunus* spp. which are affected by those diseases. The isolates were shown to be serologically related but not identical, and in additional tests (7), they also were found to be related but not identical to an isolate of TmRSV-PYB.

The failure to detect TmRSV with I-ELISA in extracts from up to 100 nematodes collected after virus acquisition feedings, when samples of 25 nematodes which were transferred to bait plants from the same feedings induced a TmRSV-positive root sample reading, indicates that the TmRSV detected in washed roots from cucumber and *Prunus* spp. was within the roots and not in the nematodes remaining on the root surface. In addition, the $A_{450\,nm}$ means for TmRSV-positive root samples from cucumber and peach bait plants were 14–17 times those for extracts of viruliferous nematodes. Standard slash tests, in which nematode extracts are mechanically inoculated to herbaceous plants, detect nepoviruses in vector *Xiphinema* very inefficiently (10,14), and have failed to detect TmRSV in *X. americanum* (10). Immunosorbent electron microscopy efficiently detects some nepoviruses in nematode vectors (14), but viruliferous vectors of TmRSV have not been assayed. In our tests, I-ELISA was not sensitive enough to detect TmRSV in *X. californicum*.

TmRSV-CLM appears to be transmitted by *X. californicum* much less efficiently than TmRSV-PBL or TmRSV-PSP.

Variability in the efficiency of nematode transmission of different strains of TmRSV has not been reported previously. The low rate of nematode transmission of TmRSV-CLM suggests that in some cases differences in the virus protein coat might affect TmRSV acquisition and transmission by nematodes. However, since three other serologically distinguishable Prunus TmRSV strains are efficiently transmitted, there is no clear correlation between differences detected serologically among TmRSV strains and nematode transmission levels. If *X. californicum* is the primary vector of TmRSV-CLM, then the low rate of TmRSV-CLM transmission would be expected to limit the occurrence of CLM-affected cherry trees. Limited field observations indicate that the incidence of CLM-affected trees is restricted in California commercial orchards. The widespread, serious diseases of *Prunus* species, such as PSP, yellow bud mosaic, and PBL, are caused by TmRSV strains for which high levels of virus transmission by nematodes have been demonstrated.

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