

## Prune Brownline Disease: Susceptibility of Prune Rootstocks and Tomato Ringspot Virus Detection

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### ABSTRACT

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Tomato ringspot virus (TmRSV) was readily graft-transmitted to prune (*Prunus domestica*) trees on peach (*P. persica*) and Myrobalan plum (*P. cerasifera*) rootstocks and a brownline symptom developed at the scion/rootstock union when TmRSV-infected root chip inoculum from prune brownline (PBL)-affected orchard trees was grafted to rootstocks of trees. However, TmRSV was not transmitted and PBL did not develop in trees on Marianna 2624 plum (*P. cerasifera* × *P. munsoniana*) clonal rootstock similarly graft-inoculated with root chip inoculum from the same source. In addition, TmRSV isolates associated with the PBL, peach yellow bud mosaic, Prunus stem pitting, cherry leaf mottle, and California peach stem pitting diseases all infected and induced brownline formation at the scion/rootstock union of graft-inoculated prune trees on peach and

Myrobalan, but not those on Marianna 2624, rootstocks. TmRSV was consistently detected by direct and indirect enzyme-linked immunosorbent assay (D-ELISA and I-ELISA, respectively) and by radioimmunosorbent assay (RISA) in peach and Myrobalan plum rootstocks of trees naturally affected by PBL; however, I-ELISA and RISA were more reliable than D-ELISA for detecting TmRSV in diseased trees. *Xiphinema californicum* transmitted TmRSV-PBL to cucumber (*Cucumis sativus*) and to seedlings of the standard prune rootstocks, peach, and Myrobalan plum. Apparently, Marianna 2624 plum is resistant or immune to TmRSV infection, so control measures for PBL should include the use of trees propagated on Marianna 2624 plum instead of Myrobalan plum or peach rootstocks at sites threatened or affected by diseases caused by TmRSV.

*Additional key words:* European plum, NEPO virus.

Prune brownline (PBL) is a widespread, serious disease affecting prune (*Prunus domestica*) trees on Myrobalan plum (*P. cerasifera*) and peach (*P. persica*) rootstocks in California (14). PBL is associated with the infection of susceptible rootstocks by a strain of tomato ringspot virus (TmRSV), and it results in the development of a narrow strip of dark brown necrotic tissue, brownline

symptom, at the graft union with the resistant-hypersensitive prune scion (14). The brownline spreads around the union causing girdling, decline, and eventual death of the affected tree.

It is difficult to mechanically transmit TmRSV from host *Prunus* spp. (7,13), but the virus can be detected in *Prunus* spp. by direct enzyme-linked immunosorbent assay (D-ELISA) (7,11,14). Radioimmunosorbent assay (RISA) (6) is more reliable than D-ELISA for the detection of TmRSV in rootstocks of PBL-affected prune trees (7), but other variations of ELISA have not been compared.

TmRSV is endemic in California (5), and several strains cause different disease symptoms in *Prunus* species (13). The ability of

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different TmRSV strains to induce PBL has not been determined. PBL commonly spreads within an orchard from diseased to adjacent healthy trees, indicating that the disease is soilborne (14), but the vector of the pathogen has not been determined. *Xiphinema americanum* was shown to transmit a strain of TmRSV to peach (18). However, more recently it was reported that *X. americanum* is a species limited to eastern North America (10), and that the nematode resembling it in California is a new species, *Xiphinema californicum* Lamberti and Bleve-Zacheo. Transmission of TmRSV-PBL by *X. californicum* to Myrobalan plum and peach has not been reported.

In limited field observations, PBL has not been observed affecting any prune tree on Marianna 2624 plum (*P. cerasifera* × *P. munsoniana*) clonal rootstock, and a number of trees on Marianna 2624 have been observed growing directly adjacent to PBL-affected trees on peach or Myrobalan rootstocks for 5 yr without developing PBL (14). This suggests the possibility that Marianna 2624 is resistant to the agent that causes PBL.

The purpose of the research reported in this paper was to investigate the differential susceptibility of selected prune rootstocks to TmRSV infection and PBL development, the role of different virus strains in PBL, to compare the detection of TmRSV in PBL-affected trees by three different immunosorbent assay procedures, and to test transmission of TmRSV by *X. californicum* to cucumber (*Cucumis sativus*), peach, and Myrobalan plum.

## MATERIALS AND METHODS

**Graft transmission.** In a field experiment, we attempted to reproduce PBL by graft-transmitting TmRSV from a naturally PBL-affected prune (*Prunus domestica* L. 'French') tree on Myrobalan plum (*P. cerasifera* Ehrh.) rootstock to healthy French prunes on peach [*P. persica* (L.) Batsch 'Nemaguard'], Myrobalan plum, or Marianna 2624 plum (*P. cerasifera* × *P. munsoniana* [?] Wight and Hedr.) rootstocks. Inoculum consisting of small to medium-sized roots from the Myrobalan rootstock or branches from the prune scion of the PBL-affected tree was collected and assayed by an indirect enzyme-linked immunosorbent assay (I-ELISA) for TmRSV. Only roots testing positive for TmRSV were employed as inoculum. No TmRSV was detected in any scion branch inoculum. Indicator trees on each rootstock (1.5–2.0 cm in diameter) were inoculated in the scion or rootstock with three scion bark patches or root chips that were inserted into T-cuts on the trunk. Five trees of each indicator type received bark patch inoculum in the scion or rootstock, five received root chip inoculum in the scion, 14 received root chip inoculum in the rootstock, and six served as uninoculated controls. The indicators were observed during three growing seasons for the development of visible decline symptoms, and the scion and rootstock of each tree were assayed for TmRSV by I-ELISA during the second and third growing seasons. At the termination of the experiment, the bark was removed from all indicators, and the graft unions were observed for the presence of a brownline.

In a field and lathhouse experiment, we attempted to graft transmit five isolates of TmRSV from *Prunus* and induce brownline formation in healthy French prunes on Lovell peach, Myrobalan plum, or Marianna 2624 plum rootstocks. Inoculum was collected from naturally TmRSV-infected orchard trees that exhibited symptoms characteristic of the PBL (14), peach yellow bud mosaic (PYB) (17), Prunus stem pitting (PSP) (13), cherry leaf mottle (CLM) (8), and California stem pitting (CaSP) (15) diseases. Five trees of each indicator type were inoculated in the same manner as described above in the scion or rootstock with root chip or bark patch inoculum shown by I-ELISA to be infected with each virus isolate, and five controls were similarly inoculated in the scion and rootstock with tissue from healthy, symptomless orchard trees. Indicators that received inoculum infected with TmRSV-PSP were maintained in 18.9-L (five-gallon) pots in the lathhouse. Indicators that received inoculum infected with PBL, PYB, CLM, or CaSP TmRSV isolates or TmRSV-free inoculum were grown in the field. The indicators were observed for scion decline symptoms, assayed in the scion and rootstock for TmRSV with I-ELISA, and checked

for a brownline at the graft union after three growing seasons.

**Purification and antiserum production.** A TmRSV isolate obtained from a Myrobalan plum rootstock of a PBL-affected prune tree (14) was purified from young systemically infected cucumber (*Cucumis sativus* L. 'National Pickling') leaves that were harvested from plants growing in flats in the greenhouse, 10–14 days following inoculation of the cotyledons with the virus. The leaves were extracted in one and one-half volumes of 0.5 M borate buffer, pH 6.7, containing 0.06 M sodium sulfite, 0.02 M ethylenediaminetetraacetic acid (EDTA), and 2% mercapto-ethanol, and the extract was filtered through four layers of cheesecloth. Triton X-100 was added to a final concentration of 2.5%, and the extract was stirred for 30 min at 4 C. Following 15 min of low-speed centrifugation in a GSA rotor at 10,000 rpm, the supernatant was centrifuged for 2.5 hr at 27,000 rpm in a Beckman type 30 rotor in tubes containing 8-ml, 20% sucrose cushions. Pellets were resuspended in 0.05 M borate buffer, pH 6.7, and centrifuged in an SS-34 rotor at 10,000 rpm for 10 min. Virus bands were collected following cesium chloride isopycnic centrifugation (3) in a Beckman SW 50.1 rotor at 40,000 rpm for 35–40 hr.

An antiserum with a specific titer of 1:512 and a nonspecific titer of 1:4 (titers determined in agar gel double diffusion tests against sap extracted from virus-infected or healthy cucumber plants) was produced against TmRSV-PBL by immunizing a rabbit with a series of three weekly intramuscular injections of 1 mg purified virus in 1 ml of 0.05 M borate buffer, pH 6.7, emulsified with 1 ml of Freund's incomplete adjuvant (1 ml in each rear leg). Serum was collected from weekly bleedings from the marginal ear vein begun 1 wk after the last injection. Chicken egg yolk immunoglobulins against TmRSV-PBL were obtained from immunized chickens (2).

**Assays for TmRSV.** Naturally and experimentally PBL-affected prune trees propagated on peach and Myrobalan plum seedling rootstocks and on clonal Marianna 2624 plum rootstocks were assayed for TmRSV by D-ELISA (4) employing rabbit antiviral immunoglobulins conjugated with alkaline phosphatase, by I-ELISA employing chicken antiviral immunoglobulins (2) and horseradish peroxidase conjugated rabbit antichicken immunoglobulins (9), and by RISA (6). Cambial and inner bark tissues (0.5–1.0 g) of scion or rootstock portions of prune trees were triturated in 3 ml of ELISA grinding buffer (4) with nicotine added to a final concentration of 2%. The samples were then homogenized with a Polytron homogenizer and the extracts were placed in polystyrene ELISA plates (two plate wells per sample). Reactions were determined by scanning plates in a Titertek Multiscan colorimeter for absorbance at 405 or 450 nm for D-ELISA and I-ELISA, respectively, 30 min after the addition of enzyme substrates, or for RISA by radioactivity in a liquid scintillation counter. Samples with reaction values at least three times the value of healthy control samples were accepted as positive for TmRSV.

**Nematode transmission of TmRSV.** Field soil naturally infested with nematodes morphologically similar to *X. californicum* Lamberti and Bleve-Zacheo (10) was collected in an apple orchard in Sonoma County, CA, transferred to two wooden boxes (1 × 0.75 × 0.6 m) in the greenhouse, and the nematode population was maintained on sudan grass (*Sorghum vulgare* var. *sudanense* Hitchc. 'Piper'). Nematodes were washed free from soil samples and collected on a 149- $\mu$ m (100-mesh) screen. Large numbers of *X. californicum* (500–1,000) were hand-picked into distilled water within no more than 3 hr and then poured over exposed root systems of cucumber seedlings mechanically inoculated 1–2 wk previously with purified TmRSV-PBL or healthy cucumber seedlings in 100-ml plastic pots partially filled with a 1:1 mixture of sterilized sandy loam field soil and UC-mix soil (1) (1:1, v/v). The plant root systems were then covered with the same soil mix, and the pots were then placed in a growth chamber at 24 C with a 14-hr daylight period.

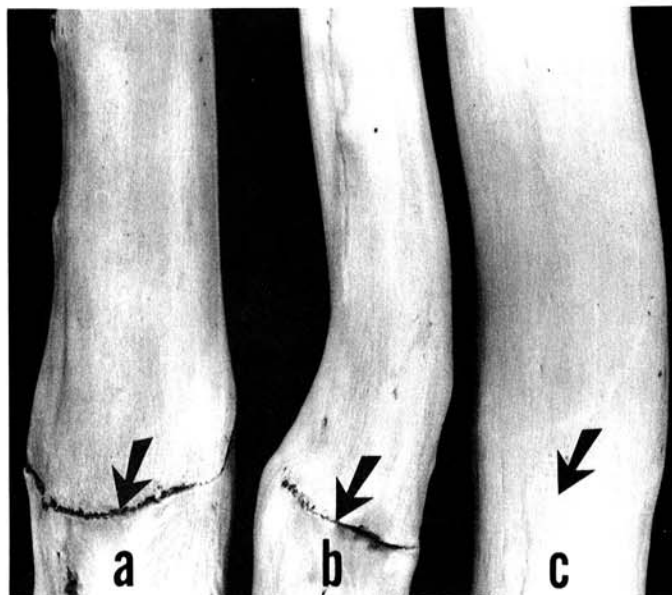
The nematodes were given a 14-day virus acquisition feeding then washed free, collected on a 149- $\mu$ m (100-mesh) screen, hand-picked into distilled water, and poured directly onto exposed root systems of healthy young cucumber (25 nematodes per plant), Lovell peach, or Myrobalan plum (100 nematodes per plant)

seedlings in partially filled 100-ml plastic pots or 150-ml clay pots for the cucumber and host *Prunus* spp., respectively. The pots were filled with the same soil mixture and placed in the growth chamber. Root samples were collected from all virus acquisition hosts and assayed for TmRSV with I-ELISA to be sure that TmRSV had infected the root systems.

The root systems of 25 cucumber bait plants were thoroughly washed, sampled, and assayed by I-ELISA for TmRSV 14 days after the addition of viruliferous nematodes. Young roots of 15 peach and 15 Myrobalan seedlings were collected, thoroughly washed, and assayed for TmRSV 60 days after the addition of viruliferous nematodes, and bark samples were collected from the main trunks and assayed for TmRSV after 1.5 yr. Controls consisting of five cucumber, five peach, and five Myrobalan seedlings exposed to nematodes given a previous feeding on healthy cucumber seedlings and healthy plants not exposed to nematodes were tested in the same way.

## RESULTS

**Differential susceptibility of prune rootstocks.** TmRSV-PBL was readily transmitted to healthy French prune trees on



**Fig. 1.** Differential susceptibility of French prune trees on different rootstocks to prune brownline (PBL) disease. Graft unions (arrows) of prune/peach (a), prune/Myrobalan (b), and prune/Marianna 2624 (c) trees graft-inoculated in the rootstock with root chips from a PBL-affected orchard prune tree. Note the presence of a brownline at the prune/peach and prune/Myrobalan graft unions and absence of a brownline at the prune/Marianna 2624 graft union.

Nemaguard peach or Myrobalan plum rootstocks when root chips from the Myrobalan rootstock of a tree naturally affected by PBL were grafted to the peach or Myrobalan rootstock of indicator trees, and all virus-infected trees developed a brownline at the graft union. No transmission of TmRSV-PBL or brownline development occurred when the same inoculum was grafted to Marianna 2624 plum rootstocks of indicator trees. Likewise, no TmRSV was transmitted and no disease developed when root chip inoculum was applied to French prune scions of indicators on all three rootstocks, or when bark patches from the French prune scion of a tree naturally affected by PBL were grafted to scions or rootstocks of indicators on each rootstock. All control indicator trees remained free of TmRSV-PBL and PBL.

Fourteen of 14 indicators on peach and Myrobalan rootstocks inoculated in the rootstock with virus-infected root chips became infected with TmRSV-PBL and developed a brownline at the graft union within three growing seasons, whereas none of 14 indicators on Marianna 2624 rootstock inoculated in the same fashion became infected with TmRSV-PBL or developed PBL. The brownline at the union of the French prune scion and peach or Myrobalan rootstock of indicator trees (Fig. 1a,b) was identical to those observed in orchard trees naturally affected by PBL, while the unions of indicators on Marianna 2624 rootstock (Fig. 1c) were identical to those of healthy trees.

Indicator trees that were completely girdled by a brownline at the graft union showed typical PBL scion decline symptoms (14). Indicators experimentally affected by PBL but not completely girdled by the brownline at the termination of the experiment showed reduced growth in tree height and trunk diameter compared to trees without PBL.

Five isolates of TmRSV associated with different diseases of *Prunus* spp. (TmRSV-PBL, TmRSV-PYB, TmRSV-CLM, TmRSV-CaSP, and TmRSV-PSP) were readily transmitted to healthy French prune trees on Lovell peach or Myrobalan plum rootstock when inoculum from virus-infected orchard trees was grafted to the rootstock portion of indicator trees, and a brownline developed at the graft union of TmRSV-infected indicators (Table 1). None of the five TmRSV isolates was transmitted to prune trees on Marianna 2624 plum rootstock when the same inoculum was applied to the rootstock of indicator trees (Table 1). Likewise, no TmRSV isolate was transmitted when the same inoculum was applied to the French prune scion of indicator trees on any of the three rootstocks. No TmRSV transmission or brownline development occurred in any control indicator tree that had received inoculum from healthy orchard trees (Table 1).

Brownlines induced at unions of indicator trees on peach and Myrobalan rootstocks as a result of infection by TmRSV-PBL, TmRSV-PYB, TmRSV-PSP, TmRSV-CLM, or TmRSV-CaSP (Fig. 2) were all identical to brownlines observed at unions of trees naturally affected by PBL. In contrast, the unions of indicators on Marianna 2624 rootstocks looked identical to those of healthy orchard trees. Indicators with PBL caused by different TmRSV

**TABLE 1.** Ability of the prune brownline (PBL), peach yellow bud mosaic (PYB), cherry leaf mottle (CLM), California stem pitting (CaSP), and Prunus stem pitting (PSP) isolates of tomato ringspot virus (TmRSV) to infect and induce PBL disease in graft-inoculated French prunes on Lovell peach, Myrobalan plum, or Marianna 2624 plum rootstocks

Indicator (scion/rootstock)	Part of indicator inoculated	Fraction of indicators with brownline induced at the graft union by five TmRSV isolates <sup>a</sup>					Detection of TmRSV in indicators <sup>b</sup>		Fraction of control indicators with brownline at the union <sup>c</sup>
		PBL	PYB	CLM	CaSP	PSP	Scion	Rootstock	
French prune/Lovell peach	Scion	0/5	0/5	0/5	0/5	0/5	—	—	0/5
	Rootstock	5/5	5/5	4/5	5/5	4/5	—	+	0/5
French prune/Myrobalan plum	Scion	0/5	0/5	0/5	0/5	0/5	—	—	0/5
	Rootstock	4/5	5/5	3/5	5/5	5/5	—	+	0/5
French prune/Marianna 2624 plum	Scion	0/5	0/5	0/5	0/5	0/5	—	—	0/5
	Rootstock	0/5	0/5	0/5	0/5	0/5	—	—	0/5

<sup>a</sup>Inoculum from naturally TmRSV-infected orchard trees showing symptoms characteristic for each disease. TmRSV infection confirmed with indirect enzyme-linked immunosorbent assay (I-ELISA).

<sup>b</sup>Indicators assayed for TmRSV by I-ELISA.

<sup>c</sup>Inoculum from healthy, symptomless orchard trees.



isolates showed visible scion decline symptoms typical of trees naturally affected by PBL (14).

**Serological detection of TmRSV in PBL-affected trees.** D-ELISA, I-ELISA, and RISA each consistently detected TmRSV in peach and Myrobalan plum rootstocks of naturally PBL-affected trees; however, I-ELISA and RISA detected TmRSV more reliably than D-ELISA (Table 2). In tests employing unmatched samples collected from rootstocks of PBL-affected trees in different orchards at different times, TmRSV was detected in 94% of the trees assayed by I-ELISA and in 96% of the trees assayed by RISA, whereas TmRSV was detected in 63% of the trees assayed by D-ELISA. TmRSV was never detected in the prune scion of any PBL-affected tree, and TmRSV was never detected in the rootstock or scion of any symptomless prune tree by any of the three immunosorbent assays.

The TmRSV detection abilities of RISA and D-ELISA were directly compared by using the same rootstock samples in both assays (7). In split sample experiments, RISA was more reliable than D-ELISA, detecting TmRSV in 34 of 35 (97%) trees compared to 18 of 35 (51%) for D-ELISA. The means of RISA TmRSV-positive and healthy control sample readings were  $834.2 \pm 552.1$  and  $42 \pm 22.8$  cpm, respectively, whereas D-ELISA TmRSV-positive and control sample  $A_{405 \text{ nm}}$  means were  $0.51 \pm 0.24$  and  $0.053 \pm 0.022$ , respectively. Additionally, the sensitivities of D-ELISA and RISA were compared using purified homologous TmRSV, and RISA detected TmRSV at a lower concentration than D-ELISA. RISA cpm readings were at least three times the buffer control readings for purified TmRSV at a virus concentration of 2 ng/ml, whereas D-ELISA could detect purified virus at a concentration of 7 ng/ml.

Results from I-ELISA tests on PBL-affected trees in which the brownline symptom was only found on one side of the tree further demonstrated the close association between rootstock infection with TmRSV and the presence of a brownline at the graft union. TmRSV was detected in 18 of 18 trees when samples were taken directly below the brownline, whereas TmRSV was detected in 5 of 18 (28%) of the same trees when rootstock samples were taken up to 15 cm around the circumference of a tree from the end of the brownline. No TmRSV was detected in 10 rootstock samples taken >15 cm from the end of a brownline.

**Nematode transmission of TmRSV-PBL.** *X. californicum* readily acquired TmRSV-PBL from virus-infected roots of mechanically infected cucumber plants and then transmitted virus to healthy cucumber, Lovell peach, and Myrobalan plum seedlings. TmRSV-PBL was detected by I-ELISA in washed roots of 24 of 25 (96%) cucumber plants exposed to viruliferous nematodes for 14 days, in 7 of 15 (47%) of the washed root samples from both peach and Myrobalan seedlings that were exposed to viruliferous nematodes for 60 days, and in 10 of 15 (67%) Myrobalan and 8 of 15 (53%) peach bark samples collected from the seedlings after 1.5 yr. TmRSV-PBL was not detected in any sample from cucumber, peach, or Myrobalan seedlings exposed to nematodes given an acquisition feeding on healthy plants. The  $A_{450 \text{ nm}}$  means for cucumber TmRSV-positive, control, and healthy root sample readings were  $0.27 \pm 0.09$ ,  $0.023 \pm 0.005$ , and  $0.028 \pm 0.02$ , respectively. The  $A_{450 \text{ nm}}$  means for Myrobalan TmRSV-positive, control, and healthy control root sample readings were  $0.32 \pm 0.12$ ,  $0.032 \pm 0.014$ , and  $0.027 \pm 0.023$ , respectively, and the means for peach TmRSV-positive, control, and healthy root samples were  $0.23 \pm 0.06$ ,  $0.019 \pm 0.006$ , and  $0.014 \pm 0.01$ , respectively. The  $A_{450 \text{ nm}}$  means for Myrobalan TmRSV-positive, control, and healthy bark sample readings were  $0.2 \pm 0.06$ ,  $0.02 \pm 0.01$ , and  $0.013$ , respectively, and the means for peach TmRSV-positive, control, and healthy bark samples were  $0.18 \pm 0.02$ ,  $0.02 \pm 0.01$ , and  $0.017$ , respectively.

## DISCUSSION

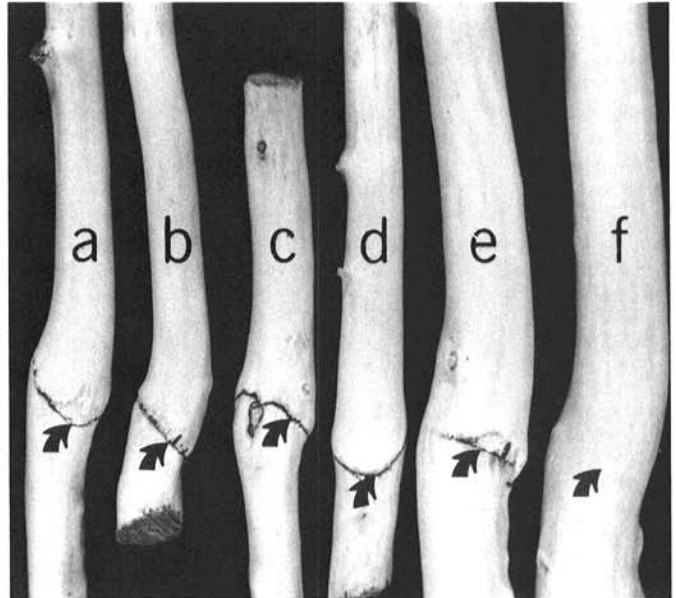
Detection of TmRSV in peach and Myrobalan plum rootstocks but not in scions of orchard trees naturally affected by PBL with immunosorbent assays and the consistent detection of TmRSV only in tree rootstock portions below the graft union brownline

symptom supports previous evidence (14) that PBL results from the hypersensitive reaction of prune scions to TmRSV infections in peach and Myrobalan rootstocks.

The widespread occurrence of different TmRSV strains in California (5,14,15), and the graft transmission of PBL, PYB, PSP, CLM, and CaSP isolates of TmRSV to peach and Myrobalan rootstocks and resultant PBL induction in prune trees suggests that PBL could be caused by different strains of TmRSV.

The common occurrence of dagger nematodes in prune orchards in California (12) and the transmission of TmRSV-PBL to peach and Myrobalan seedlings by *X. californicum* suggest that this nematode is a PBL-agent vector.

PBL-affected prune trees on Marianna 2624 plum rootstock were not observed in the field, and the natural spread of disease in one orchard from PBL-affected French prunes on Myrobalan



**Fig. 2.** Prune brownline (PBL) disease induction in French prune trees on Myrobalan rootstock graft-inoculated with five tomato ringspot virus (TmRSV) isolates associated with different diseases of *Prunus* spp. Graft unions (arrows) of trees inoculated in the rootstock with inoculum naturally infected with PBL (a), peach yellow bud mosaic (b), Prunus stem pitting (c), California stem pitting (d), or cherry leaf mottle (e) TmRSV isolates or with inoculum from a healthy, symptomless tree (f). Note the presence of a brownline at the graft union of trees that received inoculum infected with each TmRSV isolate.

**TABLE 2.** Detection of tomato ringspot virus (TmRSV) by direct enzyme-linked immunosorbent assay (D-ELISA), indirect ELISA (I-ELISA), and radioimmunosorbent assay (RISA) in unmatched samples collected from Myrobalan plum and peach rootstocks of prune brownline (PBL)-affected prune trees

Assay	Fraction of trees in which TmRSV was detected <sup>a</sup>	Mean assay readings for:	
		TmRSV-positive trees <sup>b</sup>	Healthy trees <sup>c</sup>
D-ELISA	33/52	$0.56 \pm 0.21$	$0.082 \pm 0.031$
I-ELISA	58/62	$0.27 \pm 0.12$	$0.025 \pm 0.013$
RISA	44/46	$784.3 \pm 491.8$	$58.5 \pm 17.6$

<sup>a</sup>Cambium and inner bark samples were collected independently for each assay from rootstocks of naturally PBL-affected trees, and the fraction represents the number of trees tested positive for TmRSV per number tested with each assay.

<sup>b</sup>Assay sample readings at least three times the value of healthy sample readings were accepted as positive for TmRSV. D-ELISA readings are expressed in sample light absorbance at 405 nm, I-ELISA readings in absorbance at 450 nm, and RISA in counts per minute of radioactivity.

<sup>c</sup>Cambium and inner bark samples were collected from PBL-free orchard trees and served as healthy controls for each assay.

rootstock to adjacent French prunes on Marianna 2624 rootstock did not occur over a period of nine years (14). These observations, combined with the failure to transmit TmRSV and induce PBL in prune trees on Marianna 2624 rootstock, and successful virus transmission and PBL induction in similarly inoculated trees on peach and Myrobalan plum rootstocks, strongly suggest that Marianna plum is resistant or immune to infection by TmRSV.

The apparent resistance of Marianna 2624 to infection by TmRSV may be utilized in combination with previous control measures (14) to effectively control PBL in prune orchards. PBL-affected trees should be rogued along with adjacent symptomless trees which may already have TmRSV infections started in their root systems, and trees should be transported out of orchards in a way that does not move soil from areas with diseased trees to areas with healthy trees. Likewise, any orchard management practice that results in the regular movement of soil or water from areas with diseased trees to areas with healthy trees should be avoided. Weeds commonly found in fruit tree orchards are now known to be reservoirs of TmRSV (16), so to prevent the natural spread of PBL to remaining healthy trees, diseased sites should be fumigated with a nematicide before replanting, then kept free from weeds for two growing seasons. Prune trees on Marianna 2624 clonal rootstock should be used to replant PBL-affected sites within orchards or to plant new sites with a history of TmRSV diseases. In addition, Marianna 2624 plum might also be used to control diseases caused by certain strains of TmRSV in other *Prunus* species, such as yellow bud mosaic in almond (17) and stem pitting in apricot (13), since certain cultivars of almond and apricot can be propagated on Marianna 2624 plum.

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