Distribution of Tomato Ringspot Virus in Peach Trees: Implications for Viral Detection

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ABSTRACT

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The distribution of tomato ringspot virus (TmRSV) in peach was determined by indexing different parts of naturally infected, orchard-grown trees and graft-inoculated Halford seedlings by enzyme-linked immunosorbent assay (ELISA) using antisera prepared to TmRSV particles. TmRSV and symptoms of peach stem pitting (PSP) associated with TmRSV infection were restricted in their distribution within trees. Bark from the belowground portion of the stems was the most reliable source of viral antigen. Both antigen incidence and titer gradually declined toward the upper parts of the stems and toward the roots. The distribution pattern in graft-inoculated Halford peach seedlings was apparently influenced by the isolate of TmRSV. Of five isolates tested on a very limited scale, only peach yellow bud mosaic (PYBM) was readily detected in all parts of the tree. The implications for detecting TmRSV in peach and for the control of PSP through cross-protection are discussed.

Additional key words: probability of virus detection, Prunus stem pitting, tomato ringspot virus isolates

The peach stem pitting (PSP) disease, described in 1968 (2,14), is widespread throughout the Middle Atlantic area of the United States (3,21), where it is a limiting factor for peach production (11,13,20,22). To a lesser extent, the disease also occurs on the West Coast (18). Tomato ringspot virus (TmRSV), a nepovirus (19), has been identified as the causal agent of PSP (17).

Although the etiology of PSP has been established, the reliable detection of TmRSV in peach trees has remained a problem. Published reports have suggested that TmRSV might be unevenly distributed in peach. Mircetich et al (12) found that the PSP-causal agent(s) was transmitted to peach seedlings readily by root chips but erratically by buds from naturally infected orchard-grown peach trees. In a survey of mature orchard trees in West Virginia, TmRSV was detected by enzyme-linked immunosorbent assay (ELISA) in only 48% of the trees with PSP symptoms, when bark from the trunk-root transition zone was sampled (3). After a dormancy period, TmRSV was detected in nine of 17 samples by ELISA in roots and leaves of Halford peach seedlings that had been inoculated via the dagger nematode *Xiphinema rivesi* Dalmasso and were maintained in the greenhouse (7,15). In a study in which graft-inoculated peach seedlings (grown in the greenhouse) were indexed by ELISA and bioassay, TmRSV was consistently recovered from roots in all samples tested but inconsistently from leaves and lateral shoot stems (10).

Accurate and reliable detection of TmRSV in peach (and other fruit crops) is essential for disease assessment as well as for the development of control strategies. The distribution of TmRSV in peach trees might be a crucial factor in the control of PSP through cross-protection, which is currently being investigated in our laboratory. The objectives of this study were to develop an efficient technique for indexing TmRSV infections in peach trees, to determine the distribution of TmRSV and PSP symptoms in peach, and to determine if there is a correlation between TmRSV isolates and distribution in peach trees.

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MATERIALS AND METHODS

Trees. Six peach trees (three Redskin and three Redhaven on Halford or Lovell rootstocks) in two commercial orchards in Berkeley County, West Virginia, were selected for sampling in July 1984 when bark was readily slipping. The selected trees were 7 yr old and showed typical symptoms of PSP, such as poor growth, chlorotic and scarce foliage, thick spongy bark and wood pitting at the base of the trunk, and general decline (2). Because these trees were usually standing in clusters with other affected trees, it was presumed that they had been inoculated by nematodes in the orchards, although an infection in the nursery could not be ruled out.

An attempt was made to assess the influence of virus isolates on the distribution in peach plants, even though only a limited number of test plants per isolate were available. Virus distribution was assayed in 12 Halford seedlings that had been inoculated separately with five TmRSV isolates: Amberg, Mazzard, Staff, Chickadee, and Peach Yellow Bud Mosaic (PYBM) These had been inoculated with bark chips into the stems (about 20 cm above soil level) either 3 yr (one seedling each with Amberg, Staff, and PYBM) or 4 yr before this study. The Amberg and Mazzard isolates originated from a Malling-Merton 106 (MM106)

apple rootstock and a cherry tree in New York, respectively; Staff was from grapevine in Ontario, Canada; Chickadee was from an MM106 apple rootstock in Oregon; and PYBM was from peach in California. The Halford seedlings had been growing in a nursery at Geneva, NY, and generally displayed no typical PSP symptoms, except for the PYBM-infected tree, which had symptoms (16) on a few leaves in spring.

Tissue sampling. The trees were dug out to facilitate the collection of the samples. As far as possible, the same sampling pattern was used for each tree. From the PSP-affected orchard trees in West Virginia, 44 samples per tree were collected: 12 from leaves (taken from four branches), eight from scaffold branches (two samples per branch), eight from the trunk above and eight from the trunk below the soil line from four points equidistant around the circumference, and eight from main roots usually within 60 cm from the tree trunk (Fig. 1, Table 1). The graft union between scion cultivar and rootstock was not unequivocally discernible in all trees but coincided nearly with the soil line in the recognizable cases. Therefore, trunk sampling positions are presented in relation to the soil line rather than the graft union. About half the number of samples (between 12 and 26 per tree) were taken from the Halford nursery trees at Geneva.

Leaf samples were composed of three or four subsamples taken from adjacent leaves and weighing about 0.3 g. Leaves were immediately stored in envelopes on ice. Bark and root samples were obtained by cutting out with a knife an oval-shaped slice about 5 cm long and 2 cm wide and scraping cambial tissue from the removed slice as well as from the exposed wood of the tree. The knife was immersed in 95% ethanol and blotted dry between samplings. The samples were placed in cold ELISA extraction buffer in the field.

ELISA procedure. All tissue samples were ground with a Tissumizer (Tekmar, Cincinnati, OH) the same day they were collected and processed in ELISA plates (Dynatech, Chantilly, VA). The double-antibody sandwich ELISA procedure as described by Clark and Adams (5) was followed. Rabbit antiserum to the grape yellow vein (GYV) strain of TmRSV (9), supplied by G. V. Gooding, Jr. (North Carolina State University, Raleigh), was used for detection of the Chickadee isolate of TmRSV. For the detection of the other isolates in the nursery and the orchard trees, rabbit antiserum to the PYBM strain, produced in our laboratory, was applied. ELISA plates were coated with γ-globulin at 5 μg/ml for GYV and 1 μg/ml for PYBM. Tissue samples were diluted about 1:20–50 (w/v). Alkaline phosphatase-labeled γ-globulin was used at 1:800 and 1:1,000 (v/v) dilutions for GYV and PYBM, respectively. The hydrolysis reaction was stopped after about 1 hr with 3 M NaOH. Plates were read visually, and the absorbance was measured at 410 nm (A_{410nm}) with a Microelisa Mini Reader MR 590 (Dynatech) for the orchard-grown trees in West Virginia and at 405 nm with a Microelisa Auto Reader MR 580 (Dynatech) for the Halford seedlings at Geneva. Substrate buffer was used as a reference.

Extraction buffer and bark from rootstocks and leaves from two TmRSV-free peach trees (at the Appalachian Fruit Research Station, Kearneysville, WV) were used as negative controls, and greenhouse-grown peach seedlings that had been infected by mechanical inoculation or by graft inoculation with the Amberg or PYBM isolates were used as TmRSV-positive controls. Leaves of *Gomphrena globosa* L. or *Nicotiana benthamiana* Domin infected with the Amberg or PYBM isolates of TmRSV were also used as controls in each plate. All samples were tested in two replicate wells. The absorbance readings from replicate wells were averaged. ELISA reactions with an absorbance reading equal to or greater than three times that of the healthy samples (separately for leaves and bark) and with a visually detectable yellow color were rated positive.

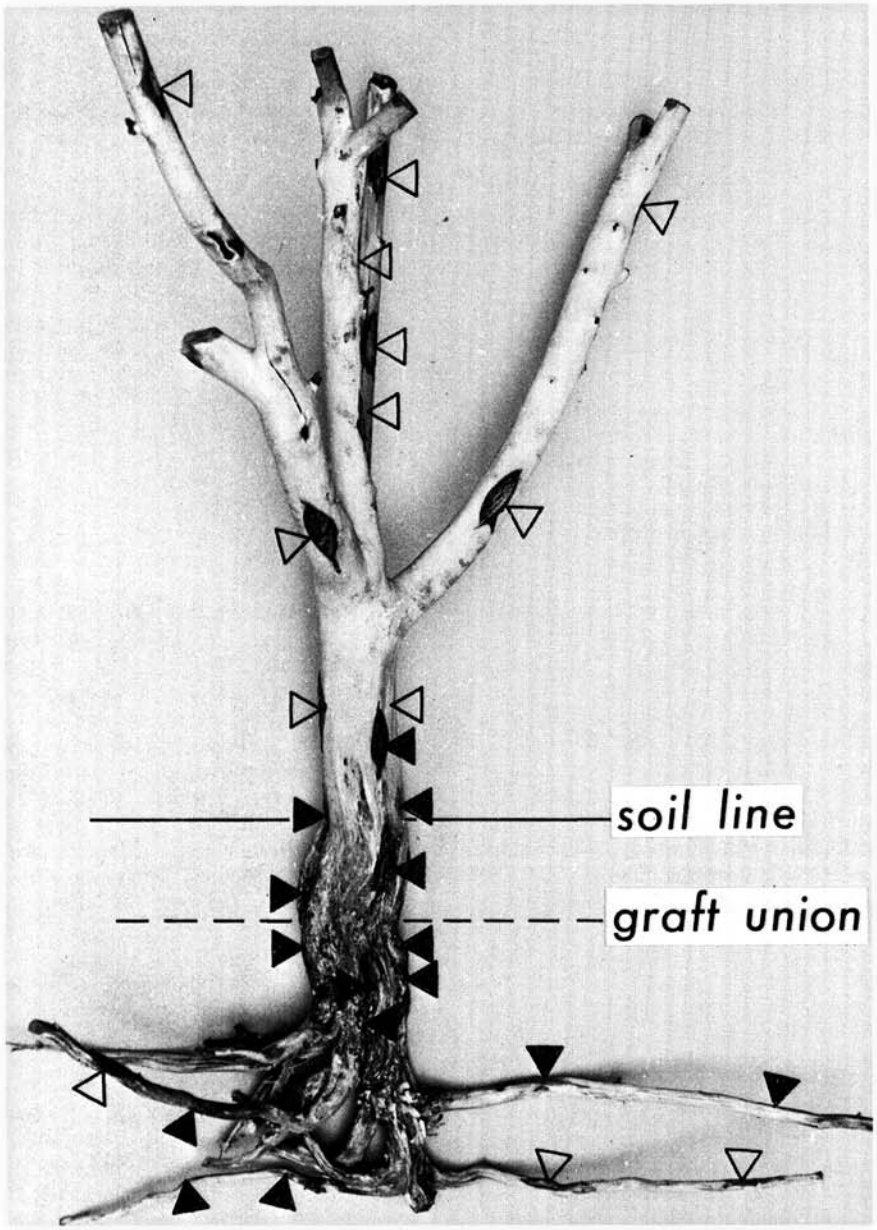

Fig. 1. Distribution of tomato ringspot virus (TmRSV) and stem pitting in Redskin/Halford peach (tree 1, Table 1). The tree was 7 yr old, grown in a commercial orchard in West Virginia, and severely declining. The assay was done in July 1984 by taking 44 samples from different parts of the tree. Open triangles = ELISA-negative for TmRSV; closed triangles = ELISA-positive for TmRSV.

RESULTS

Detection of TmRSV and PSP symptoms in orchard-grown peach trees. Absorbance values (A_{410nm}) for healthy peach bark used for negative control averaged 0.01 (range −0.03 to 0.03) when the readings were zeroed on buffer. The healthy peach leaves (negative control), however, resulted in an A_{410nm} of 0.145 (0.06–0.08 from one TmRSV-free tree and 0.21–0.24 from a second tree). Therefore, different thresholds for positive reactions were set: 0.10 for bark and 0.44 for leaf samples.

TmRSV was not evenly distributed within trees, but the distribution pattern was somewhat similar in all six trees. ELISA detected TmRSV mostly in bark samples taken from the lower trunk (Fig. 1, Table 1). The highest percentage of ELISA-positive samples was obtained from stems within the first 10 cm below the soil line (Table 1) followed by those collected 13–23 cm below the soil line and from roots. Virus detection was substantially lower in distal portions of the roots (28–60 cm) than in proximal portions (3–27 cm). ELISA also detected TmRSV in bark of trunks up to 25 cm above the soil line but mostly within 10 cm of it. Sometimes, ELISA-positive and ELISA-negative bark samples were located only 1 or 2 cm apart. No TmRSV was detected in bark of branches or in leaves.

The graft union did not seem to be a barrier for virus movement, because TmRSV was detected in the scion portions as well as in the rootstocks of at least some trees. For example, five ELISA-positive samples in the tree in Figure 1 were taken from the Redskin scion tissue. In other trees, the graft union was not clearly discernible.

The highest A_{410nm} values were obtained in bark extracts of the trunk from slightly above the soil line to the trunk-root transition zone (Table 1). Root samples and stem samples from higher locations gave low readings. Leaf samples, all considered negative for TmRSV, produced variable A_{410nm} values. The samples from the Redhaven cultivar gave an average absorbance of 0.05 (range 0.03–0.12), whereas the values for the Redskin cultivar were consistently higher (average 0.21, range 0.13–0.34). However, TmRSV was readily detected in positive control leaves of peach seedlings, *G. globosa*, or *N. benthamiana* infected with the Amberg isolate of TMRSV.

Only pitting and grooving were rated as positive PSP symptoms, because thick bark was not unequivocally definable in all cases. Symptoms such as pitting and grooving were distributed in a pattern similar to the ELISA-positive samples (Table 1), but there was not complete agreement between symptoms and TmRSV by ELISA; 47 samples were both ELISA-positive and symptomatic (either pitting or grooving), 21 were ELISA-positive but symptomless, and four were ELISA-negative but symptomatic. In the last case (symptoms but ELISA-negative), the tissue was already dead in three of the four samples. Eleven of the 21 TmRSV-infected but symptomless bark samples were derived from roots.

The probability of detecting TmRSV infections by ELISA and symptoms (pitting or grooving) in different tree locations was calculated from pooled data of all six trees (Table 1). The probability of successful detection (P) was determined with the equation: $P = 1 - (1 - p)^n$, where n = number of samples used and p = fraction (number of ELISA-positive samples/total number of samples indexed), and $(1 - p)^n$ = probability of failing detection (Table 2). For example, to estimate the probability of virus detection by ELISA for sampling stems 0–23 cm below soil (Table 2), p was obtained by averaging the values 0.95 and 0.82 from Table 1 from stems 0–10 cm below the soil line and stems 13–23 cm below the soil line, respectively. Only two samples per tree were required to obtain at least a 95% probability of successful detection in each case by ELISA or by symptomatology (Table 2). To achieve the same probability of detection with root sampling (roots 3–60 cm from

Table 1. Detection of tomato ringspot virus by enzyme-linked immunosorbent assay (ELISA) and stem pitting in six orchard-grown peach trees in West Virginia

	ELISA reactions vs. stem-pitting symptoms									
	Cultivar (tree no.)						Total			
	Redskin[a]			Redhaven[a]					A_{410nm}	
Part of tree	1	2	3	1	2	3	All trees	Percent	Mean	Range
Leaf	0/12[b]	0/12	0/12	0/12	0/12	0/12	0/72	0	...	...
Bark from:										
Branches	0/8	0/8	0/8	0/8	0/8	0/8	0/48	0	...	...
	[0][c]	[0]	[0]	[0]	[0]	[0]	[0]	[0]		
Stem, 15–45 cm above soil	1/4	0/4	1/4	0/4	0/4	0/4	2/24	8	0.13	0.11–0.15
	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]		
Stem, 0–10 cm above soil	4/4	0/4	4/4	0/4	1/4	0/4	9/24	38	0.51	0.12–1.46
	[4]	[0]	[3]	[0]	[0]	[1]	[8]	[33]		
Stem, 0–10 cm below soil	4/4	4/4	3/4	4/4	2/2	4/4	21/22	95	0.78	0.24–1.61
	[4]	[4]	[3]	[1]	[2]	[4]	[18]	[82]		
Stem, 13–23 cm below soil	4/4	3/4	3/4	3/4	2/2	3/4	18/22	82	0.85	0.30–1.84
	[3]	[2]	[3]	[3]	[2]	[4]	[17]	[77]		
Root, 3–27 cm from trunk	3/4	1/4	3/4	3/4	0/4	3/4	13/24	54	0.20	0.10–0.66
	[0]	[0]	[2]	[3]	[0]	[1]	[6]	[25]		
Root, 28–60 cm from trunk	2/4	0/4	1/4	1/4	0/4	1/4	5/24	21	0.22	0.10–0.36
	[1]	[0]	[0]	[1]	[0]	[0]	[2]	[8]		
Tree total	18/44	8/44	15/44	11/44	5/40	11/44	...	...	...	...
	[12]	[6]	[11]	[8]	[4]	[10]				

[a] Cultivars Redskin and Redhaven are on either Halford or Lovell rootstocks.
[b] Numerator is number of ELISA-positive samples, denominator is total number of samples.
[c] In brackets are numbers of samples with stem-pitting symptoms.

trunk), however, seven and 17 samples had to be taken for ELISA and symptomatology, respectively.

Distribution of TmRSV isolates in peach trees. These nursery trees at Geneva did not generally display obvious PSP symptoms, although some trunks had developed a few very small pits or grooves and slightly sunken and flattened areas above the points of inoculation, which are indicative for PSP (12). Reading pitting symptoms was somewhat difficult because the trees were in a poor state of health, presumably resulting from Cytospora canker and cold damage. No leaf symptoms were observed, except on the tree infected with PYBM, which developed irregularly shaped chlorotic blotches along the veins ("oak-leaf pattern"), which is one of the symptoms of yellow bud mosaic (16).

The distribution pattern of the Amberg, Mazzard, Staff, and Chickadee isolates (Table 3) was similar to that found in the naturally infected, orchard-grown peach trees in West Virginia (Table 1), but the proportion of ELISA-positive samples was generally lower in Table 3 than in Table 1. However, in the tree infected with the PYBM isolate, TmRSV was also detected by ELISA in bark obtained from branches and in leaves (Table 3). In previous years, we recovered the PYBM isolate from symptomatic leaves of several other Halford trees (now dead, data not shown), also indicating systemic infection of peach by this isolate.

There was little dissimilarity of absorbance values between samples taken from different tree parts with the Amberg, Mazzard, Staff, and Chickadee isolates; the average A_{405nm} values were 0.39, 0.34, and 0.29 for root, stem below soil, and stem above soil, respectively. With the PYBM, however, much higher A_{405nm} values were obtained in leaves (average 1.04) and bark from the above-soil portion of the stem (average 1.09) than in the lower trunk zone, roots, and side branches (av. 0.47, 0.37, and 0.21, respectively).

DISCUSSION

It should be noted that our ELISA detected TmRSV coat protein. Thus, our data do not distinguish between the detection of TmRSV coat protein, which is not assembled into virions and the detection of infectious TmRSV particles. In this communication, we refer to ELISA detection of virus or virus titer but fully recognize that this means serological detection of TmRSV coat protein.

Although TmRSV was unevenly distributed in peach trees, our data indicate some clear trends on the detection of the virus. Bark samples from the lower trunk part were the most reliable tissue source in the naturally infected, orchard-grown trees in West Virginia and in most of the Halford seedlings (infected with several different isolates) grown at Geneva, NY. This tissue source for ELISA tests was most reliable with regard to highest percentage of ELISA-positive samples and highest absorbance values, which presumably reflected highest virus titer in this portion of the trees. Both virus incidence and titer gradually declined toward the upper part of the stems and toward roots. This fact is

Table 2. Probability of detecting tomato ringspot virus infections in known-infected, orchard-grown peach trees by enzyme-linked immunosorbent assay (ELISA) and by symptomatology

Sample location	No. samples per tree	Detection[a] By ELISA	Detection[a] By symptomatology
Stem, 0–23 cm below soil	1	0.89	0.80
	2	0.99	0.96
Root, 3–27 cm from trunk	1	0.54	0.25
	2	0.79	0.44
	3	0.90	0.58
	4	0.96	0.68
	8	...	0.90
	9	...	0.92
	10	...	0.94
	11	...	0.96
Root, 3–60 cm from trunk	1	0.38	0.17
	2	0.61	0.31
	3	0.76	0.42
	4	0.85	0.52
	5	0.90	0.60
	6	0.94	0.67
	7	0.96	0.72
	13	...	0.91
	17	...	0.96

[a] Based on pooled data of six trees (Redskin or Redhaven cultivars on Halford or Lovell rootstocks) in West Virginia.

Table 3. Distribution of five tomato ringspot virus isolates detected by enzyme-linked immunosorbent assay (ELISA) in Halford peach seedlings[a]

Part of tree	Virus isolate: Amberg 4[b]	Mazzard 3	Staff 3	Chickadee 1	PYBM 1	Total: Without PYBM 11	Without PYBM Percent	All trees 12	All trees Percent
Leaf	0/21[c]	0/18	0/18	0/6	4/8	0/63	0	4/71	6
Bark from:									
Branches	0/6	0/6	0/7	0/2	2/2	0/21	0	2/23	9
Stem above soil	4/8	3/12	1/15	2/4	2/2	10/39	26	12/41	29
Stem below soil	8/17	3/18	7/15	1/6	3/3	19/56	34	22/59	37
Root	4/19	1/12	6/14	0/4	7/11	11/49	22	18/60	30
Total pos./indexed	16/71	7/66	14/69	3/22	18/22				
Percent	23	11	20	14	69				
Total pos./indexed[d]		40/228							
Percent[d]		18							

[a] Seedlings had been inoculated 3 or 4 yr before this study and were field-grown in Geneva, NY.
[b] Number of trees per virus isolate.
[c] Number of (ELISA)-positive samples/number of samples indexed.
[d] For Amberg, Mazzard, Staff, and Chickadee.

also recognizable from the calculations of probability for successfully detecting TmRSV (Table 2), which indicated that two to four times as many root samples must be analyzed by ELISA to reach the same probability level as sampling the lower stem. Obviously, bark from the stem portion below soil is the preferred choice for indexing peach by ELISA.

Our data agree with those of Mircetich et al (12) who demonstrated that root chips of orchard-grown peach trees are a better source than buds for transmitting the causal agent of PSP. In their study, stem tissue in naturally infected, orchard-grown trees was not assayed. In their experimentally inoculated peach seedlings grown in the greenhouse, the distribution of TmRSV was limited to stems and roots (12). Similarly, TmRSV is irregularly distributed in apple trees (4), grapevine (8), and plum rootstocks (6).

Our findings contradict the conclusions of a previous study (10), in which TmRSV was most reliably detected in root tissue of peach seedlings. However, Lister et al (10) did their study on young trees growing in the greenhouse and 1–2 yr after they had been bud-inoculated with TmRSV. The orchard-grown trees in our study had presumably been inoculated by nematodes and were 7 yr old, which might have allowed a more even distribution of TmRSV in the lower stem. It can be expected that the extent of systemic infection of TmRSV in peach is dependent on the severity of virus infection, as was shown in grapevines (8). Probably, factors other than different time periods since inoculation and/or different modes of inoculation might also have contributed to the much higher detection rate in the orchard-grown peach trees in West Virginia than in the Halford seedlings at Geneva. Environmental conditions such as temperature and/or length of growing season might be more conducive to virus replication and movement in peach trees in West Virginia. Furthermore, the trees in West Virginia and Geneva were infected with different isolates of TmRSV.

The low detection efficiency of TmRSV from bark of the trunk-root transition zone of stem-pitted trees reported by Barrat et al (3) might be explainable by different reasons. First, they might have used less severely infected trees in their study and, therefore, the virus might have been less evenly distributed in the lower stem. Second, because they used smaller and fewer bark samples (1.1 cm^2, two per tree) than we used in our study (about 5 cm^2, four to eight per tree), their chance of missing the presumed "virus pockets" on the trunk was increased.

The uneven distribution of TmRSV is a problem for virus indexing, particularly in the early stage of infection, when the distribution of TmRSV is quite localized. Thus, some caution should be exercised in interpreting our calculations on the probability of detecting TmRSV in orchard trees, because these calculations are based on the results obtained from severely PSP-affected and known-infected trees. We would have preferred to include also some PSP-affected orchard trees with milder symptom expression in our study in West Virginia. However, understandably, growers are reluctant to sacrifice trees that still bear a significant crop.

The knowledge of TmRSV distribution in peach trees is also essential for the development of cross-protection as a strategy to control the peach stem-pitting disease. It can be expected that systemically infecting virus isolates would cross-protect trees more effectively than "less systemic" isolates. Uneven infection of protecting isolates of citrus tristeza virus was indeed suggested to be a possible cause of incomplete cross-protection in sweet orange trees (1). Because of the low number of trees tested, we could not determine the relative capability of TmRSV isolates to systemically infect belowground and aboveground parts of Halford seedlings under field conditions (Table 3). Our data do show that PYBM isolate was readily detected in upper parts of the stem, branches, and in leaves as well as in the lower trunk and in roots of one tree. Lister et al (10) also showed that the PYBM isolate was more consistently detected, compared with the TmRSV-G and TmRSV-SP isolates, in leaves of graft-inoculated Elberta peach seedlings grown under greenhouse conditions. Based on these observations, PYBM may serve as a good parent strain for obtaining chemically induced mild mutants for cross-protection studies in peach and other fruit trees.

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