Detection of Tomato Ringspot Virus in Peach Orchards

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ABSTRACT

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Peach orchards in northeastern West Virginia were surveyed for tomato ringspot virus (TmRSV) in peach trees and dandelions, using enzyme-linked immunosorbent assay (ELISA). In 10 orchards where peach trees with *Prunus* stem pitting symptoms and nearby dandelions were surveyed, TmRSV was detected in an average of 48% of the trees and 64.5% of the dandelions sampled. The low rate of detection of TmRSV in stem-pitted peach trees suggests that sampling in the trunk-root transition zone may be an inefficient methodology. Of 34 orchards in which dandelions were sampled, 80.6% were infected with TmRSV, indicating that TmRSV is widespread in peach orchards in the survey area.

Since the initial description of symptoms of *Prunus* stem pitting (PSP) in peach trees by Barrat et al (3), the causal agent has been identified as tomato ringspot virus (TmRSV) (14). The dagger nematodes *Xiphinema americanum* and *X. rivesi* are agents of transmission to fruit trees (4,6,7), and broadleaf weeds have been identified as virus reservoirs (13). PSP is widespread throughout many stone fruit growing areas of the United States (11,16). The distribution of PSP has been associated with infected planting stock (15,16), and control programs have been implemented in commercial nurseries to prevent dissemination of PSP through infected stock (2,9). Yet, after approximately 10 yr, the disease remains a persistent problem in Pennsylvania orchards (10). Trees free from disease before planting become infected after planting. Similar conditions prevail in northeastern West Virginia. Apparently, reservoir plants and nematode vectors have established the disease in Pennsylvania.

In the orchard, elimination of nematode vectors or weed hosts presents a formidable challenge. This study was undertaken to determine the current level of TmRSV infestation in the peach-growing region of northeastern West Virginia, an area of serious infestation in the early 1970s (3), and to test the efficiency of enzyme-linked immunosorbent assay (ELISA) in detecting TmRSV in peach trees.

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MATERIALS AND METHODS

Ten orchards with declining trees were selected for peach tree sampling. Ten trees with typical PSP symptoms were selected from each orchard (3). Field symptoms resembled general girdling symptoms—stunting, poor growth, and chlorotic foliage. The soil at the base of tree trunks was removed, the crown cut into, and a flap of bark pulled back to detect wood pitting. Only trees with wood pitting symptoms were sampled. In the Middle Atlantic states, these symptoms are associated with TmRSV infection (14,16), although in the Northwest, TmRSV has not been isolated from stem-pitted fruit trees (1). Two samples per tree were taken at least 4 in. apart from the pitted area of the trunk-root transition zone (approximately 4 in. below ground level). Samples of cambial tissue were collected by extracting a bark plug with a 1.2-cm diameter (No. 6) cork borer and scraping the cambium from the bark plug and from the wood exposed by bark plug removal. Samples were immediately placed in cold PBS-Tween PVP buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 2% polyvinylpyrrolidone MW 40,000) and stored on ice. The composition of PBS (g/L) was 8 NaCl, 0.2 KH_2PO_4, 1.15 Na_2HPO_4, 0.2 KCl, 0.2 NaN_3. Samples from each tree were combined, then assayed by ELISA for TmRSV.

One leaf from each of five dandelions closest to each sampled peach tree was assayed for TmRSV to determine possible correlation between peach and dandelion infection. A combined sample from five leaves was used for ELISA indexing.

Thirty-four randomly chosen orchards, including the 10 sampled in the peach tree and dandelion survey, were sampled for TmRSV infestation based on dandelion infection alone. Dandelions sampled for the peach tree-dandelion survey were not included in this survey. A block of approximately 2–5 acres per orchard was chosen randomly and sampled in five areas: north, south, east, and west corners and middle section. The samples from each of the five areas consisted of the composite of leaf samples from five dandelion plants.

All peach trees and dandelions were sampled between 8 July and 1 September 1982. Samples were stored on ice in PBS-Tween PVP buffer and processed within 4 hr of collection. General health of the 34 orchards was rated for each in the area where only dandelions were surveyed. Ratings were based on approximate percentages of replants and general tree vigor, taking tree age into consideration. A rating of 1 represented a healthy, vigorous orchard with 80–100% of the original trees surviving; 2, a healthy but less vigorous orchard with 60–79% of the original trees surviving; and 3, a declining orchard with 40–59% of the original trees surviving.

ELISA indexing was performed following the procedures of Clark and Adams (5). Antiserum to a grapevine isolate of TmRSV (8) was supplied by D. Gonsalves, New York State Agricultural Experiment Station, Geneva, NY. Wells of microtiter plates were coated with 200 µl of globulin at 5 µg/ml.

Tissue extracts were ground with a Tissuemizer (Tekmar Co., Cincinnati, OH 45222) in 1:20 ratio (w/v) with PBS-Tween PVP buffer. Enzyme-globulin conjugates were used at 1:400 v/v. Reaction intensity was measured photometrically at 410 nm with a Dynatech Microelisa Mini-Reader MR590 (Dynatech Laboratories, Inc., Alexandria, VA 22314). Controls included two healthy peach leaves, two healthy dandelion leaves, two infected dandelion leaf extracts, and four buffer wells per 60 sample wells. ELISA reactions giving absorbances equal to or greater than twice the average reading for healthy control samples were regarded as positive (10). The minimal optical density (OD) regarded as a positive indication of TmRSV was 0.38.

RESULTS

Peach trees and dandelions infected with TmRSV were detected in all 10 orchards surveyed (Table 1). Although all trees sampled had visual symptoms of

stem pitting (3), ELISA detection of TmRSV was inconsistent. Only 10–80%, or an average of 48%, of the stem-pitted trees per orchard gave positive reactions in the ELISA test. Composite dandelion samples collected in the vicinity of each sampled tree gave positive reactions in 30–95%, or an average of 64.5%, per orchard. Average OD readings for all positive reactions ranged from 0.90 to 1.96.

The general survey of 34 orchards for TmRSV-infected dandelion plants revealed that an average of 80.6% contained dandelions infected with TmRSV and that an average of 40.8% of the dandelion samples were infected (Table 2). The incidence of TmRSV infection in dandelions increased with orchard age. General orchard health declined in orchards over 5 yr of age (Table 2).

Table 1. Enzyme-linked immunosorbent assay (ELISA) detection of tomato ringspot virus (TmRSV) in apparently infected peach trees and adjacent dandelions

Orchard age (yr)	Trees TmRSV-positive[a] (%)	Composite dandelion samples TmRSV-positive[b] (%)
5	30	90
5	70	50
6	40	60
7	20	95
7	40	50
7	60	30
7	70	90
8	60	80
10	10	40
10	80	60
Avg.	48	64.5

[a]Ten trees per orchard sampled.
[b]Dandelions near indexed peach trees sampled.

DISCUSSION

Root tissue samples have provided the most reliable material for detection of TmRSV in peach by the ELISA technique (10). Root sampling from mature orchard trees is too difficult and time-consuming for routine survey purposes. We felt that sampling from the trunk-root transition zone would be less difficult and still result in a high percentage of detection. This was not the case. TmRSV was serologically detected from only 48% of obviously diseased trees. Serological detection of TmRSV may be inhibited by compounds present in peach tissue, but the higher rates of detection, up to 80%, in some of the sampled orchards make this unlikely. Failure to associate ELISA detection of TmRSV with recognized visual symptoms of peach stem pitting (3) suggests that further investigation of TmRSV distribution in peach trees is necessary. Lister et al (10) were able to detect TmRSV in leaves and shoots of inoculated peach seedlings. We tested young leaf tissue from one obviously infected 7-yr-old peach tree sampled on 8 July and obtained a positive ELISA reaction. Random sampling from leaves during the spring or early summer growth may be a more efficient method of ELISA detection of TmRSV in peach. Sampling date may have influenced the detection of TmRSV in both peach and dandelion (10), although within the time period covered by our sampling, 8 July to 1 September, no correlation was found between sample date and percent infection detected within peach or dandelion samples.

Linear regression analysis indicated no correlation between infection detected in peach trees and nearby dandelions ($r = 0.075$). The inability to correlate tree and adjacent weed infection is likely the result of low rates of virus detection in the peach tree. Multiple linear regressions involving general orchard health, percent composite dandelion samples infected with TmRSV, and orchard age suggest that orchard health is not strongly correlated with the percent infected dandelion samples (Table 3). The absence of correlation between dandelion infection and orchard health may be due in part to the general practice in local orchards of roguing-out declining trees after harvest and replanting and, in some orchards, of fumigating before replanting. Orchard health and percent infected dandelion samples are correlated with orchard age (Table 3). In general, as orchard age increases, so does the percentage of orchards combining infected dandelions, the percentage of dandelions infected with TmRSV in each orchard, and the number of replanted or declining trees (Table 2). The dispersal of TmRSV through infected dandelion seed (12) would allow for the spread and buildup of dandelion infection in older orchards.

Our study shows that TmRSV is widespread in the surveyed area, as indicated by infection in both peach trees and dandelions. The buildup of infected dandelions in older orchards suggests that TmRSV will continue to be a problem in the survey area despite introduction of TmRSV-free planting stock. Development of programs to control or eradicate the virus in the orchard or of TmRSV-resistant peach germ plasm is needed.

Table 2. Comparison of peach orchard age, tomato ringspot virus (TmRSV) infection of composite dandelion samples detected by enzyme-linked immunosorbent assay (ELISA), and general orchard health

Orchard age (yr)	No. of orchards	Percent orchards with infected dandelions[a]	Percent dandelion samples infected[b]	Orchard health rating[c] Avg.	Range
2–4	5	60	20	1.2	1–2
5	7	57	20	1.4	1–3
6–7	8	100	53	2.3	1–3
8–10	7	86	57	1.9	1–3
12–20	7	100	54	2.0	2
Avg.		80.6	40.8		

[a]Each orchard sample consisted of a composite of five dandelion leaf samples from each of five areas: north, south, east, west, and middle of a 2- to 5-acre block.
[b]Average optical density at 410 nm of positive samples.
[c]1 = Healthy and vigorous, with 80–100% of original trees surviving; 2 = healthy but less vigorous, with 60–79% of original trees surviving; 3 = declining, with 40–59% of original trees surviving.

Table 3. Multiple linear regressions of general orchard health rating, percent dandelion samples infected with tomato ringspot virus (TmRSV), and orchard age[a]

Y	X_1	X_2	R
General orchard health	Percent dandelion samples infected		0.18
	Orchard age		0.39*[b]
	Percent dandelion samples infected	Orchard age	0.39*
Percent dandelion samples infected	General orchard health		0.18
	Orchard age		0.44**
	General orchard health	Orchard age	0.44**

[a]Survey of 34 orchards.
[b]Probability of obtaining as large or larger R value by chance alone = 0.05. **Probability of obtaining as large or larger R value by chance alone = 0.01.

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