Distribution of Tomato Ringspot Virus in Dandelion in Pennsylvania

C. A. POWELL and W. L. MOUNTAIN, Pennsylvania Department of Agriculture, Harrisburg 17110; T. DICK, Department of Plant Pathology, Pennsylvania State University, Fruit Research Laboratory, Biglerville 17307-0309; L. B. FORER, M. A. DERR, and L. D. LATHROP, Pennsylvania Department of Agriculture, Harrisburg 17110; and R. F. STOUFFER, Department of Plant Pathology, Pennsylvania State University, Fruit Research Laboratory, Biglerville 17307-0309

ABSTRACT

Powell, C. A., Mountain, W. L., Dick, T., Forer, L. B., Derr, M. A., Lathrop, L. D., and Stouffer, R. F. 1984. Distribution of tomato ringspot virus in dandelion in Pennsylvania. Plant Disease 68: 796-798.

Population densities of *Xiphinema* spp. (*X. americanum* and *X. rivesi*) and common dandelion (*Taraxicum officinale*) and relative infection of dandelion with tomato ringspot virus (TmRSV) were determined in peach orchards with TmRSV-induced disease (Prunus stem pitting [PSP]), peach orchards without PSP, and nonorchards in eight geographic regions of Pennsylvania representing both intensive and nonintensive peach production areas. There were no significant differences ($P = 0.05$) in *Xiphinema* or dandelion density among types of sites or geographic regions. The percentage of dandelion infection with TmRSV was significantly higher ($P = 0.05$) in orchards with PSP (29%) than in either orchards without PSP (7%) or nonorchards (5%). Prevalence of TmRSV in dandelion was also greater ($P = 0.05$) in intensive peach production areas (21%) than in nonintensive peach production areas (7%). There were two geographic regions in which no TmRSV-infected dandelion was detected. These data indicate that in Pennsylvania, TmRSV is not yet endemic in dandelion and that the presence of TmRSV, not the nematode vector or dandelion, is the limiting component of the PSP disease. Results support the hypothesis that TmRSV is initially introduced in an orchard via either infected nursery stock or dandelion seed and subsequently becomes established in dandelion and other weeds over a period of years.

Prunus stem-pitting disease (PSP), which is caused by tomato ringspot virus (TmRSV) (8), is a lethal disease of peach that occurs throughout many peach-growing regions of the eastern United States. Initial attempts to control the disease focused on the presumed source of diseased trees, TmRSV-infected nursery stock. Pennsylvania and some other states have implemented regulatory programs requiring that peach nursery stock be produced from TmRSV-free scion and rootstock sources and that the nursery stock be grown on fumigated soil to protect against infection via dagger nematode vectors (*Xiphinema* spp.). Although these practices have eliminated TmRSV from Pennsylvania peach nursery stock for almost a decade, PSP in Pennsylvania orchards remains a problem.

Recent surveys in Indiana, New York, and Pennsylvania have shown that many common orchard weeds are hosts for TmRSV and could serve as reservoirs of the virus (7). The most frequently infected ubiquitous weed is common dandelion. In addition, it is known that TmRSV is transmitted through dandelion seed and that the dagger nematode can acquire TmRSV from infected dandelion (6).

Although dandelion in orchards with PSP is frequently infected with TmRSV (6,7), it was not known whether dandelion in other locations was also infected. We therefore compared the dagger nematode density, dandelion density, and relative TmRSV infection of dandelion in orchards with PSP, orchards without PSP, and nonorchards in both intensive and nonintensive peach production areas in Pennsylvania.

Accepted for publication 16 March 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Experimental design and sampling procedures. Eight geographic regions in Pennsylvania were selected for sampling. Four regions, in northern and southern Adams, York, and Franklin counties, represented intensive peach production areas (much of the agricultural land in tree fruit production), and four regions, in Columbia, Lehigh, Lycoming, and Snyder counties, represented nonintensive peach production areas (less than 10% of the agricultural land in tree fruit production). In each geographic region, three sites were randomly selected. These sites consisted of an orchard containing peach trees with visual symptoms of the PSP disease (at least 5% of the trees with visual symptoms), a peach orchard with no PSP symptoms, and a fallow field (nonorchard). Sites within a region were located within 1–5 mi. of each other. Each site was sampled at four randomly selected places so that there were four replicates per site.

The sampling procedure for each replicate was as follows: A single peach tree, or spot in the field for nonorchards, was selected as the reference point and assigned the number 1. The corresponding tree in the adjacent row was given the number 2, etc., until five trees in successive rows were numbered. A number (1–5) was then randomly selected and the corresponding tree served as the starting point (reference tree) for the sampling. A sampling area within the orchard drive row was defined by two 8-m strings attached to two 20-cm wooden cross-bar stakes placed diagonally from the drip line of the reference tree to the drip line of the adjacent orchard row. The dandelions within this 1.6-m^2 area were counted starting from the stake near the reference tree. If there were fewer than 100 dandelions in the first sampling area, a second number from the remaining four was selected and the dandelions in a second sampling area were counted as described before. This procedure was repeated until either 100 dandelions were counted or all the dandelions in five sampling areas were counted. Dandelions number 20, 40, 60, 80, and 100 were collected for virus analysis. About 100 cm^3 of soil was collected with each of these five dandelions for nematode analysis. On rare occasions, when fewer than 100 dandelions were present in all five sampling areas of a replicate, additional dandelion and soil samples were randomly collected from the same general area so that a total of five dandelion and five soil samples were collected for each replicate.

Nematode analysis. Nematodes were extracted by elutriation combined with the Baermann funnel technique (1,9). For each replicate, a 100-cm^3 subsample from the thoroughly mixed 500-cm^3 (approximate) sample of soil was suspended in 300–400 ml of tap water. The suspension remained undisturbed for 0.75–1.5 hr and then was washed into the receiving funnel of the elutriator. Larger debris was collected on a 45-mesh sieve, and the overflow was collected on a 400-mesh sieve. Nematodes were then extracted from the residue on the 400-mesh sieve with a Baermann funnel (1). Nematodes were drawn off 20–24 hr after the residue was put in the funnel.

Virus analysis. A subsample consisting of about 0.5 g of root and 0.5 g of leaf tissue from each of the 480 dandelion samples was triturated in 10 ml of 0.05 M

Table 1. Dagger nematode density[w]

Orchard category[x]	Region[y] Intensive	Non-intensive	Means[z]
With PSP	8.9	12.1	10.5 a
Without PSP	12.2	24.8	18.5 a
Non-orchards	23.2	5.9	14.5 a
Means[z]	14.8 a	14.2 a	

[w] Data represent average *Xiphinema* spp. per 100 cm^3 of soil.
[x] PSP = Prunus stem pitting.
[y] Intensive represents four geographic regions of Pennsylvania in which most agricultural land was in tree fruit production; nonintensive represents four regions in which less than 10% of the agricultural land was in tree fruit production.
[z] Means in columns or rows followed by the same letter are not significantly ($P = 0.05$) different from other means in the same column or row according to Fisher's LSD.

Table 2. Dandelion density[w]

Orchard category[x]	Region[y] Intensive	Non-intensive	Means[z]
With PSP	28.5	44.1	36.3 a
Without PSP	46.8	40.0	43.4 a
Non-orchards	23.9	21.6	22.8 a
Means[z]	33.1 a	35.2 a	

[w] Data represent average number of dandelions per square meter.
[x] PSP = Prunus stem pitting.
[y] Intensive represents four geographic regions of Pennsylvania in which most agricultural land was in tree fruit production; nonintensive represents four regions in which less than 10% of the agricultural land was in tree fruit production.
[z] Means in columns or rows followed by the same letter are not significantly ($P = 0.05$) different from other means in the same column or row according to Fisher's LSD.

Table 3. Percentage of dandelions infected with tomato ringspot virus (TmRSV)[w]

Orchard category[x]	Region[y] Intensive	Non-intensive	Means[z]
With PSP	45	14	29 a
Without PSP	8	6	7 b
Non-orchards	10	0	5 b
Means[z]	21 a	7 b	

[w] Data represent average percentage of dandelions infected with TmRSV.
[x] PSP = Prunus stem pitting.
[y] Intensive represents four geographic regions of Pennsylvania in which most agricultural land was in tree fruit production; nonintensive represents four regions in which less than 10% of the agricultural land was in tree fruit production.
[z] Means in columns or rows followed by the same letter are not significantly ($P = 0.05$) different from other means in the same column or row according to Fisher's LSD.

sodium carbonate buffer, pH 9.0. The resulting sap was mechanically inoculated to Carborundum-dusted *Chenopodium quinoa* leaves and placed in microtiter plates (Dynatech) for indirect enzyme-linked immunosorbent assay (ELISA) (5) using antiserum to an apple isolate of TmRSV prepared in a rabbit and commercially available antiserum to rabbit immunoglobulin (IgG) prepared in a goat (Miles). Controls consisted of 17 positive and 17 negative dandelion samples (one of each per ELISA plate). If positive mechanical transmission and negative ELISA results were obtained, the infected *C. quinoa* was tested by ELISA to determine if it contained TmRSV. If positive ELISA and negative mechanical transmission were obtained, the analysis was repeated with a fresh portion of the sample.

RESULTS

Xiphinema population densities were not significantly different ($P = 0.05$) among nonorchards, orchards without PSP, and orchards with PSP or between intensive and nonintensive peach production regions (Table 1), confirming previous survey conclusions that the dagger nematode is widely distributed throughout Pennsylvania on a variety of hosts (4). It should be noted that the data in Table 1 represent averages, and there are frequently large local differences in dagger nematode densities within a distance of even a few feet. Virtually all samples contained numbers of *Xiphinema* sufficient to vector TmRSV. Dandelion population densities were also similar among nonorchards and orchards with and without PSP and between intensive and nonintensive peach-growing regions (Table 2). The percentage of TmRSV-infected dandelion was higher ($P = 0.05$) in orchards with PSP than in orchards without PSP or in nonorchards. The percentage of TmRSV-infected dandelion also was higher in intensive than in nonintensive peach production regions (Table 3).

DISCUSSION

Peach trees with PSP-like symptoms have been observed for many years (2,3); however, the disease has only become epidemic within the last two decades. Perhaps not coincidentally, this dramatic increase in PSP followed a shift from the cultural practice of disking the entire orchard floor, which eliminated most weeds, to the minimum-tillage practice of establishing sod in the drive rows and treating the tree rows with herbicide. The minimum-tillage practice increases traction for heavy machinery and reduces soil erosion.

One possible explanation for the dramatic increase in PSP during the last 20 yr is that TmRSV has been prevalent for many years in native weed hosts, and the shift to minimum tillage has allowed these weeds to grow within the root zones of peach trees, providing a source of virus for the dagger nematode vector. This explanation, however, is not compatible with our data from Pennsylvania. There were no differences in dandelion or dagger nematode densities among orchards with PSP, orchards without PSP, and nonorchards or between intensive and nonintensive peach production regions, indicating that neither dandelion density nor dagger nematode density was a limiting factor in the PSP disease. However, a significantly higher percentage of dandelion was infected with TmRSV in intensive peach production regions versus nonintensive peach production regions and in orchards with PSP versus orchards without PSP or nonorchards. This indicates that the presence of TmRSV in dandelions may be a limiting factor in the PSP disease and that TmRSV is not prevalent in all regions in Pennsylvania.

A second explanation for the dramatic increase in PSP, which is compatible with our data, is that TmRSV was initially introduced in the intensive fruit-growing regions via infected nursery stock. Minimum-tillage practices facilitated movement of the virus via dagger nematodes into dandelion and other weeds. Over a period of time, the percentage of infected dandelion increased through nematode and seed transmission. As the number of TmRSV-infected dandelions within an orchard or region increased, the probability of virus transmission to healthy trees also increased.

Although TmRSV is not yet established throughout Pennsylvania, this may not be true for other states or regions. In fact, TmRSV is becoming prevalent throughout intensive peach production areas of Pennsylvania. Practices such as vigorous broadleaf weed control, nematode control, and planting only TmRSV-free nursery stock must become a routine component of orchard management not only to protect an individual orchard but also to prevent or delay establishment of TmRSV in other regions.

ACKNOWLEDGMENTS

We wish to thank Caroline Herzog and Rodney Shaffer for technical assistance.

LITERATURE CITED

1. Baermann, G. 1917. Eine einfache Methode zur Affindung von Ankylostonum (Nematoden) Larven in Erdproden. Geneeskd. Tijdschr. Ned. Indie. 57:131-133.
2. Barrat, J. G. 1964. Problems of young peach trees. Hortic. News N.J. Hortic. Soc. 45:15-18.
3. Christ, E. G. 1960. New peach problem. Hortic. News N.J. Hortic. Soc. 41:4006.
4. Forer, L. B., and Longenecker, J. L. 1978. Distribution of *Xiphinema americanum* in cereal and corn fields in Pennsylvania. (Abstr.) Phytopathol. News 12:228.
5. Lommel, S. A., McCain, A. H., and Morris, T. J. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant

viruses. Phytopathology 72:1018-1022.
6. Mountain, W. L., Powell, C. A., Forer, L. B., and Stouffer, R. F. 1983. Transmission of tomato ringspot virus from dandelion via seed and dagger nematodes. Plant Dis. 67:867-868.
7. Powell, C. A., Forer, L. B., Stouffer, R. F., Cummins, J. N., Gonsalves, D., Rosenberger, D. A., Hoffman, J., and Lister, R. M. 1984. Orchard weeds as hosts of tomato ringspot and tobacco ringspot viruses. Plant Dis. 68:242-244.
8. Smith, S. H., Stouffer, R. F., and Soulen, D. M. 1973. Induction of stem pitting in peaches by mechanical inoculation with tomato ringspot virus. Phytopathology 63:1404-1406.
9. Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. Minist. Agric. Fisheries Food Technol. Bull. 2. London, Her Majesty's Stationary Office.