Incidence of Tomato Ringspot Virus and Tobacco Ringspot Virus in Grapevines in Pennsylvania

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ABSTRACT

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In southeastern Pennsylvania, tomato ringspot virus (TmRSV) and tobacco ringspot virus (TbRSV) were detected by ELISA both individually and together in declining Cascade grapevines and in symptomless vines adjacent to infected ones but not in other symptomless vines in one vineyard. In a survey of randomly selected grapevines (*Vitis* spp.) from 12 other vineyards in southeastern Pennsylvania, TmRSV and TbRSV were detected in one and 11 of 60 Seyval and one and two of 35 Chancellor vines, respectively. Forty Chelois grapevines were ELISA-negative for both viruses. In Erie County (the major grape-producing region of the state, located in northwestern Pennsylvania), grapevine varieties Niagara, Chelois, Aurora, and Concord exhibiting a variety of viruslike symptoms were also ELISA-negative for TmRSV and TbRSV. However, two declining Seyval and one declining Cascade grapevines were infected with TbRSV, and two declining Cascade grapevines were infected with TmRSV. Several declining vines of both cultivars were ELISA-negative.

Tomato ringspot virus (TmRSV) and tobacco ringspot virus (TbRSV) are widely distributed in orchards throughout the northeastern and central United States (15). Both viruses are efficiently vectored by endemic dagger nematodes (*Xiphinema* spp.) (4,11,20). TmRSV causes economically important disease in many woody deciduous fruit crops including peaches (1,16), grapevines (3,9,21), raspberries (18), sweet cherries (13), prunes (12), blueberries (10), and apples (19). TbRSV is economically important in grapevines (21) and possibly in cherries (23).

The role of TmRSV in diseases of peaches (1,17) and apples (19) has been studied extensively. Both TmRSV and TbRSV have been associated with decline of Cascade grapevines (21). These viruses have been reported to infect vines of European but not American origin (6,7). Infected vines exhibit stunting, shortened internodes, and irregularly shaped leaves (5,6). The viruses can cause significant loss of yield (21). Data on the susceptibility of grapevine varieties to TmRSV (8) and TbRSV (22) have been reported.

The current study was done to determine the incidence of TmRSV and TbRSV in declining Cascade grapevines and in other French hybrids grown in Pennsylvania, and to detect mechanically transmissible virus(es) in ELISA-negative grapevines exhibiting viruslike symptoms in northwestern Pennsylvania.

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MATERIALS AND METHODS

Assays for TmRSV and TbRSV in southeastern Pennsylvania vineyards. One vineyard planted exclusively with the cultivar Cascade was selected. Approximately 1% of the vines showed symptoms of virus-induced grapevine decline (6). Most of the diseased vines occurred in groups of five to 20 vines. Twenty symptomatic vines from three separate groups, 20 asymptomatic vines adjacent to each of the symptomatic vines, and 20 vines at least 30 m away from any symptomatic vine were sampled. A sample consisted of five leaves collected from five random positions on the grapevine. The samples were analyzed for TmRSV and TbRSV by ELISA (2). The coating conditions for both viruses consisted of incubating microtiter plates (Dynatech) at 4 C for 16 hr with 10 μg/ml of antivirus gamma globulins in 0.05 M sodium carbonate buffer, pH 9.6. Samples were triturated in 7.5 ml of 0.015 M sodium phosphate, 0.15 M sodium chloride, 0.05% Tween 20, and 20% polyvinylpyrrolidone (M_r = 40,000), pH 7.0, (PBS-Tween-PVP) with a tissuemizer (Tekmar) and incubated in the coated plates for 4 hr at 37 C. The antivirus alkaline phosphatase enzyme conjugates (1:5,000 dilution in PBS-Tween-PVP) were also incubated for 4 hr at 37 C. Twenty-six other vineyards sampled contained grapevines of the cultivars Seyval, Chelois, or Chancellor, which are commonly grown in this region of the state. Five samples per vineyard were collected; each sample consisted of five leaves from an individual vine and three soil subsamples from the base of that vine. The leaves were combined and analyzed for TmRSV and TbRSV by ELISA, and the soil subsamples were combined and analyzed for dagger nematodes. Individual vines were selected by randomly choosing a spot in the vineyard, walking in five rows toward the center, collecting a sample, walking in another five rows, collecting another sample, and continuing in this manner until five samples were collected.

Assays for TmRSV and TbRSV in northwestern Pennsylvania. Erie County is the major grape-growing region in Pennsylvania. Here, several different cultivars displaying a variety of viruslike symptoms were sampled. Leaf samples were collected from 10 vineyards. Tissue extracts were assayed by ELISA and by inoculation to *Chenopodium quinoa* Willd. In addition, soil samples were collected (three subsamples of approximately 200 g taken within 0.6 m from the base of the vine). The subsamples were mixed and analyzed for dagger nematodes (*Xiphinema* spp.) as previously described (16). All leaf analyses were conducted with spring season growth.

RESULTS

For a southeastern Pennsylvania vineyard of Cascade, 13 of 20 grapevines with classic symptoms of virus-induced decline (21) were infected with TmRSV alone, two of 20 were infected with TbRSV alone, and five of 20 were infected with both viruses (Table 1). The results confirm that virus-induced grapevine decline can be associated with either virus, and symptoms in vines containing one or both viruses were not visually different.

Several symptomless vines adjacent to declining vines were also infected. Of 20 vines, seven were infected with TmRSV, two with TbRSV, and three with both viruses. These results indicate that it is possible to detect TmRSV and TbRSV before symptom development. In a follow-up survey of this vineyard (during the fall season), approximately one-half of these symptomless infected vines exhibited poor growth. A year later, all infected vines were in a state of severe decline. None of the 20 Cascade grapevines located at least 30 m away from the disease pockets were infected with virus.

Other southeastern Pennsylvania vineyards of Seyval, Chelois, and Chancellor grapevines (which are the three most

popular grape cultivars in the region) were surveyed to test for the prevalence of TmRSV and TbRSV (Table 2). The grapevines were sampled randomly because no viruslike symptoms were evident in any of these vineyards. Of 35 Chancellor vines, we detected one with TmRSV and two with TbRSV. Among 60 Seyval vines, one was infected with TmRSV and 11 with TbRSV. A year later, none of the infected vines had developed symptoms, suggesting that these viruses do not cause overt disease in these cultivars under field conditions in southeastern Pennsylvania. All 40 samples of Chelois tested negative. Dagger nematode counts were highly variable, ranging from 0 to 167 per 100 cm^3 of soil and were not correlated with virus infection.

Six cultivars of grapevine (Niagara, Seyval, Chelois, Aurora, Cascade, and Concord) exhibited a variety of viruslike symptoms including mosaic, dieback, shortened internodes, and cupping of leaves. Ten vines each of Niagara and Chelois with dieback, 10 Aurora vines with shortened internodes, and 10 Concord vines with cupping of leaves, mosaic, or dieback were negative by ELISA and by *C. quinoa* bioassay. However, two of 10 Seyval with dieback were positive for TbRSV, two of 10 Cascade with dieback had TmRSV, and one had TbRSV (Table 3). These results indicate that most of the viruslike symptoms prevalent in vineyards in Erie County were not associated with TmRSV, TbRSV, or other mechanically transmissible viruses. Even in Cascade, which is highly susceptible to TmRSV and TbRSV and in which these viruses are readily detectable, most of the dieback symptoms were exhibited in vines apparently devoid of TmRSV and TbRSV. *Xiphinema* spp. counts were relatively low, ranging from 0 to 35 per 100 cm^3 of soil and were not correlated with any disease symptom or with virus infection.

Table 1. Incidence of tomato ringspot and tobacco ringspot viruses in a Cascade vineyard in Pennsylvania

Vine type	TmRSV alone[a]	TbRSV alone[a]	Both viruses[a]
Declining	13/20	2/20	5/20
Symptomless, adjacent to declining vine	7/20	2/20	3/20
Symptomless, at least 30 m from declining vines	0/20	0/20	0/20

[a] The number of vines positive for virus by ELISA over the number tested.

Table 2. Prevalence of tomato ringspot and tobacco ringspot viruses and nematode vectors in Chancellor, Seyval, and Chelois grapevines in southeastern Pennsylvania[a]

Variety	Vineyards tested (no.)	Vineyards positive (no.)		Plants positive[b]		*Xiphinema* spp.[c]
		TmRSV	TbRSV	TmRSV	TbRSV	
Chancellor	7	1	2	1/35	2/35	70
Seyval	11	1	5	1/60	11/60	34
Chelois	8	0	0	0/40	0/40	23

[a] Five individual plants from each vineyard were chosen at random. A sample consisted of five small leaves collected from different branches.
[b] Number of plants positive over the number tested.
[c] Average number of *Xiphinema* spp. per 100 cm^3 of soil.

DISCUSSION

TmRSV and TbRSV are serious threats to Cascade vineyards in Pennsylvania. Both viruses were associated with declining and symptomless vines, the latter of which developed disease symptoms in the second growing season. A high percentage of infected, symptomless vines adjacent to declining vines suggests onsite spread of these nepoviruses. The rate of spread of these viruses and their effects on the host will certainly vary with a variety of factors including climate, nematode population density, and cultural practices such as weed control. The particular vineyard studied is in an area of the United States renowned for development of severe TmRSV-induced symptoms in deciduous fruit trees. The nematode population density (25–35 *Xiphinema* spp. per 100 cm^3 of soil) was a little higher than the average for Pennsylvania. Weed control in this vineyard was much better than average; few dandelions or other broad leaf weeds were present in the grapevine rows. This perhaps explains why no infection occurred away from the disease pockets.

There was little TmRSV or TbRSV present in grapevines in Erie County. There are several possible explanations for this. First, the grapevines may be immune to infection by these viruses. Based on the results of Gonsalves (8), this explanation is likely to be correct for in the cultivars Seyval, Niagara, and Aurora. This information is not available for TbRSV. A second explanation may be that our detection methods lacked sufficient sensitivity or that the virus was not detected because of its uneven distribution (7). The second explanation is unlikely because our detection procedures readily detected virus in Cascade grapevines known to contain TmRSV and TbRSV, and exhaustive sampling (20–25 samples from different locations on symptomatic vines) still failed to detect any virus in several of the symptomatic vines. A third possible explanation is that *Xiphinema* spp. are inefficient vectors of TmRSV and TbRSV to grapevines. There are little data on this subject, but field transmission to Cascade has been demonstrated by Gonsalves (8) and by us. A fourth possible explanation is that one of the components necessary for disease (virus or vector) is not present. It is known that the dagger nematode is prevalent in Erie County, and bait tests have shown that some of them are carrying TmRSV. In addition, Prunus stem pitting is frequently found in Erie County. It is not known what the threshold of viruliferous nematodes must be for significant infection of grapevines to occur.

It is curious that the close association between virus infection and decline of Cascade grapevines in southeastern Pennsylvania did not hold true for Erie County. The symptoms in individual vines were similar but the field patterns were different. In Erie County, whole sections of fields were affected rather than spreading pockets as in southeastern Pennsylvania. Decline of Cascade grapevines probably has at least three causes—TmRSV, TbRSV, or at least one unknown cause. The unknown cause is usually designated as drought, winter injury, or Eutypa canker (14), but data to support these hypotheses are lacking.

Table 3. Analysis of grapevines in Erie County, Pennsylvania, for TmRSV and TbRSV

Variety	Symptom[a]	TmRSV[b]	TbRSV[b]
Niagara	M	0/10	0/10
Niagara	D	0/10	0/10
Niagara	S	0/10	0/10
Seyval	D	0/10	2/10
Seyval	S	0/10	0/10
Chelois	D	0/10	0/10
Chelois	S	0/10	0/10
Aurora	SI	0/10	0/10
Aurora	S	0/10	0/10
Cascade	D	2/10	1/10
Cascade	S	0/10	0/10
Concord	C	0/10	0/10
Concord	M	0/10	0/10
Concord	D	0/10	0/10
Concord	S	0/10	0/10

[a] C = cupping of leaves, D = dieback, M = mosaic, S = symptomless, SI = shortened internodes.
[b] The number of vines positive for virus over the number tested.

Our survey of grapevines in southeastern Pennsylvania yielded two surprises. First, one Seyval and one Chancellor grapevine were found to be infected with TmRSV. This indicates there are isolates of TmRSV that can infect these cultivars at least at low efficiency. Second, a high percentage of

Seyval were infected with TbRSV. TbRSV is present but not common in weeds in Pennsylvania (15). This suggests that TbRSV may be prevalent in some grape nursery stock.

We have confirmed that both TmRSV and TbRSV are associated with severe decline in Cascade vineyards and that the virus can spread under Pennsylvania conditions. We have shown that most viruslike symptoms common in grapevines in Erie County are not caused by TmRSV or TbRSV. We have shown that some grapevines cultivars can be symptomless carriers of TmRSV and TbRSV.

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