Transmission of Tomato Ringspot Virus to Apple Rootstock Cuttings and to Cherry and Peach Seedlings by *Xiphinema rivesi*

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ABSTRACT

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Apple and geranium strains of tomato ringspot virus (TmRSV) were transmitted by *Xiphinema rivesi* to seedlings of Wisconsin SMR-18 cucumber, Samsun tobacco, Halford Peach, and mazzard cherry and to rooted cuttings of apple rootstocks M9, M7A and MM106. The virus was detected by enzyme-linked immunosorbent assay (ELISA), primarily in the roots of the woody test plants after 8 wk of exposure to viruliferous nematodes. Woody plants were then stored under refrigeration (0–2 C) for 14 wk to induce dormancy. The virus was not detected in the roots of any more test plants after dormancy but was recovered from leaves and roots of infected apple and peach plants by both infectivity assay and ELISA. All TmRSV-infected apple plants remained symptomless; the infected peach seedlings displayed foliar symptoms of peach yellow bud mosaic regardless of the TmRSV strain.

Tomato ringspot virus (TmRSV) has become increasingly important in deciduous fruit crops grown in Pennsylvania and much of the eastern United States. This virus is known to cause Prunus stem pitting disease (PSP) in peach (14). The characteristic pitting symptoms of the lower trunk have been observed in many other commercial stone fruit species under both field (10) and greenhouse experimental conditions (9). In addition, TmRSV has been associated with several other diseases, including apple union necrosis and decline (AUND) (17,19), virus-induced grapevine decline (6,21), ringspot disease of red raspberry (16), and a blueberry decline (3). All of these diseases have been observed in Pennsylvania.

Peach yellow bud mosaic (PYBM) also is caused by TmRSV; however, the foliar symptoms typical of this predominantly California disease (13) have not been associated with PSP in the East. *Xiphinema americanum* sensu lato has been demonstrated to transmit PYBM isolates of TmRSV to apricot, peach, and plum (20). Recently, *X. californicum*, a species formerly considered *X. americanum* sensu lato was demonstrated to differentially transmit *Prunus* strains of TmRSV to cucumber and certain *Prunus* spp. (7).

Experimentally, TmRSV has not been transmitted by nematodes to clonal apple rootstocks or mazzard cherry. Our investigations were conducted to determine whether *X. rivesi* Dalmasso would transmit TmRSV strains to apple rootstock cuttings and cherry and peach seedlings. *X. rivesi*, a nematode vector of TmRSV (5) recently identified from Pennsylvania (22), was used in this study because it is more frequently associated with TmRSV-incited diseases in Pennsylvania than *X. americanum* (4).

MATERIALS AND METHODS

Soil containing a population of *Xiphinema* sp. was collected from the root zone of a symptomless black cherry (*Prunus serotina* J. F. Ehrh.) tree, placed in four 66-L tubs planted to either chewings fescue (*Festuca rubra* L.) or sudangrass (*Sorghum bicolor* (L.) Moench var. *bicolor*), and maintained in a greenhouse. The species was identified by examining 200 adult females. Shape of the head and tail and length of the odontostyle and odontophore (22) were used to identify the population as containing only *X. rivesi*. Specimens have been deposited with the USDA nematode collection, Nematology Laboratory, Beltsville, MD. Four 400-cc samples from each tub were planted with two Wisconsin SMR-18 cucumber (*Cucumis sativus* L.) seedlings; after 4 wk, each seedling was assayed for TmRSV and tobacco ringspot virus by enzyme-linked immunosorbent assay (ELISA) (2) to ensure that nematodes used in subsequent transmission experiments were not already carrying either of the two viruses.

TmRSV isolates. The TmRSV-A isolate was originally obtained from the MM106 rootstock portion of an AUND-affected apple tree and had been shown to cause PSP in peach (18). The TmRSV-G was isolated from a geranium (*Pelargonium* × *hortorum* L. H. Bailey) with ring spot symptoms. The TmRSV-A isolate has antigenic determinants both in common with and distinct from those of TmRSV-G (12).

Acquisition phase. *X. rivesi* were removed from naturally infested field soil by a modified wet-sieving technique. Two hundred cubic centimeters of soil was placed in a 5-L plastic bucket containing about 1 L of water and allowed to soak for about 1 hr. The bucket was subsequently filled with water and the soil thoroughly mixed and allowed to settle for 30 sec, then poured through two 100-mesh (149-μm) sieves. The nematodes retained on the sieves were washed into a container. About 3,000 *X. rivesi* were added to each of twelve 400-cc Styrofoam pots containing two cucumber seedlings in a sand/vermiculite (1:1, v/v) mix. The seedlings in each of the pots were previously rubbed with one of the following: 1) 0.01 M potassium phosphate buffer, pH 7.0 (control), 2) TmRSV-A, or 3) TmRSV-G. The nematodes were given a 3-wk acquisition period.

Transmission phase. Nematodes were removed by the wet-sieving technique from the soil contained in each of the 12 acquisition pots. In the transmission phase, each seedling or rootstock cutting was placed in a 400-cc Styrofoam pot containing the sand/vermiculite mix. Ten-cubic-centimeter aliquots of water containing 34 ± 12 *X. rivesi* previously exposed to TmRSV-A were added to the roots of each of the following plants: 13 cucumber, 10 tobacco (*Nicotiana tabacum* L. 'Samsun'), 21 MM106 apple (*Malus pumila* Mill.), 7 M7A apple, 7 M9 apple, 4 M26 apple, 5 mazzard cherry (*Prunus avium* L.), and 12 Halford peach (*P. persica* (L.) Batsch.). Apple rootstocks used in this study were rooted cuttings from virus-tested rootstocks maintained in the greenhouse. The peach and cherry

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seedlings originated from seed that was harvested from virus-tested trees and grown in steam-treated potting medium under greenhouse conditions. Ten-cubic-centimeter aliquots containing 42 ± 18 *X. rivesi* previously exposed to TmRSV-G were added to the roots of 17 cucumber, 18 tobacco, 10 MM106 apple, 7 M7A apple, 7 M9 apple, 1 M26 apple, and 5 Halford peach plants. Ten-cubic-centimeter aliquots containing 92 ± 24 *X. rivesi* previously exposed to uninfected cucumber were added to the roots of five cucumber, five tobacco, five MM106 apple, five M9 apple, three M7A apple, five mazzard cherry, and five Halford peach plants. The roots and leaves from each plant were indexed for TmRSV by ELISA about 4 wk after adding the nematodes. All herbaceous plants were discarded after the assay. The woody plants were maintained in the greenhouse for another 4 wk, assayed again for TmRSV, placed in cold storage at 0–2 C for a 14-wk dormancy period, and returned to the greenhouse. This artificially induced dormany was used because the study was initiated in January and the woody plants discontinued growth after 3 mo. Peach seedlings and the apple rootstock cuttings were indexed after dormancy by ELISA and by triturating leaf or root tissue in 0.01 M potassium phosphate buffer, pH 7.0, and inoculating onto *Chenopodium quinoa* Willd. indicator plants.

ELISA procedure. ELISA was performed as described by Clark and Adams (2). Antibody to TmRSV was prepared and fractionated as described previously (12). The microtiter plates (Dynatech) were coated at 4 C for 16 hr with 10 mg of anti-TmRSV gamma globulins per milliliter in 0.05 M sodium carbonate, pH 9.6. Samples were prepared by trituration of leaf or root tissue (about 0.3 g) with a mortar and pestle in 5 ml of 0.02 M potassium phosphate, 0.15 M sodium chloride, 0.05% Tween 20, 20% polyvinyl-pyrrolidone, mol wt 40,000, pH 7.4 (PBS-Tween-PVP), placed in duplicate wells of precoated plates, and incubated at 4 C for 16 hr. The anti-TmRSV alkaline phosphatase-enzyme conjugate (1/5,000 dilution) was incubated at 37 C for 4 hr. Samples were scored as positive or negative by visual observation of yellow color. Spectraphotometric analysis was not necessary because there were no questionable (faint color) reactions.

RESULTS

The results of TmRSV transmission tests to woody and herbaceous hosts by *X. rivesi* are shown in Table 1. The virus (TmRSV) was not detected in any of the herbaceous or woody control plants that had received *X. rivesi* previously exposed to healthy cucumber "donor" plants. Because there was no significant difference in transmission efficiency or in symptom development between the two TmRSV strains, the results were combined. Of the apple rootstocks tested, only M26 did not become infected under our test conditions.

After 4 wk of exposure to the viruliferous nematodes, TmRSV was not detected in the roots or leaves of any of the woody plants, whereas the virus was detected in the roots of 25/30 cucumber and 19/28 tobacco plants. After 8 wk, TmRSV was detected in the roots of 37 of

Table 1. Transmission of tomato ringspot virus (TmRSV) to woody and herbaceous hosts by *Xiphinema rivesi*[a]

	TmRSV detection[b]					
	4 Wk		8 Wk		After dormancy[c]	
Host	Root	Leaves	Root	Leaves	Root	Leaves
Apple (MM106)	0/31	0/31	13/31	1/31	13/31	13/31
Apple (M7A)	0/14	0/14	7/14	0/14	7/14	7/14
Apple (M9)	0/14	0/14	4/14	0/14	4/14	4/14
Apple (M26)	0/5	0/5	0/5	0/5	0/5	0/5
Cherry (mazzard)	0/5	0/5	4/5	0/5	*[d]	*
Peach (Halford)	0/17	0/17	9/17	1/17	9/17	9/17
Cucumber (Wis.SMR-18)	25/30	6/30	*	*	*	*
Tobacco (Samsun)	19/28	18/28	*	*	*	*
Controls[e]	0/33	0/33	0/23	0/23	0/18	0/18

[a] *X. rivesi* (34 ± 12 or 42 ± 18) having access to either an apple or geranium strain of TmRSV were added to each of the experimental plants except the controls.

[b] No. of TmRSV-positive plants/total no. of plants tested; enzyme-linked immunosorbent assay (ELISA) technique was used at the fourth week, eighth week, and after dormancy; bioassay on *Chenopodium quinoa* was also conducted after dormancy.

[c] After the 8-wk assay, all apple and peach plants were placed under refrigeration (0–2 C) for 14 wk to induce dormancy, then returned to greenhouse conditions. Roots and new leaf growth were assayed for virus. Bioassay and ELISA results were identical.

[d] * = Plants not assayed.

[e] *X. rivesi* (92 ± 24) having access to healthy cucumber were added to each of the following: five MM106 apple, five M9 apple, three M7A apple, five mazzard cherry, five Halford peach, five cucumber, and five tobacco plants.

Fig. 1. Halford peach seedlings. (Left) Seedling infected with a strain of tomato ringspot virus originally recovered from apple is showing symptoms similar to peach yellow bud mosaic. (Right) Control.

86 woody plants but was detected in the leaves of only 1/9 infected peach seedlings and 1/13 infected MM106 apple rootstocks. The induced dormancy treatment did not result in detection of TmRSV in the roots of any more woody plants after the dormancy treatment. After dormancy, however, TmRSV was detected from the leaves of all the apple rootstocks and peach seedlings, the roots of which had previously tested positive. Additionally, all TmRSV-infected peach seedlings developed symptoms typical of PYBM regardless of the virus isolate (Fig. 1). TmRSV was readily recovered from leaves showing PYBM symptoms by mechanical inoculation to *C. quinoa*. The virus was also mechanically transmitted to *C. quinoa* from TmRSV-infected MM106 apple leaves, although these plants showed no obvious symptoms.

DISCUSSION

The TmRSV strains were transmitted to herbaceous and woody plants by *X. rivesi*. That more herbaceous plants than woody plants become infected is not surprising; herbaceous plants generally developed a more extensive root system than the woody plants under our greenhouse conditions, and as a result, may have had more favorable feeding sites for the nematodes. No difference in transmission efficiency was noted between the two TmRSV strains (TmRSV-A and TmRSV-G), although the experimental design would not detect small differences. To our knowledge, this is the first report of *Xiphinema* transmission of TmRSV to mazzard cherry and the apple rootstocks MM106, M7A, and M9. The transmission of TmRSV to M7A and M9 raises an interesting point because these rootstocks have not previously been associated with the AUND disease in the field. This disorder has been reported mainly in association with certain MM106/scion combinations such as Delicious, Tydeman's Red, and Quinte. Because of AUND and the greater susceptibility of MM106 to the *Phytophthora*-incited collar rot disease, this rootstock is being replaced by M7A in Pennsylvania. The increased planting of M7A rootstock makes it important to determine if trees grafted on this rootstock develop AUND under field conditions.

Previous reports of TmRSV transmission to peach (1,20) were with *X. americanum* sensu lato. A recent report by Hoy et al (7) indicates that the vector employed by Teliz et al (20) was probably *X. californicum*. Because both *X. americanum* sensu stricto and *X. rivesi* occur in Pennsylvania, the *Xiphinema* sp. used by Bloom et al (1) is uncertain. Perhaps of greater interest than the TmRSV transmission to peach is the development of PYBM-like symptoms in peach seedlings infected with either an eastern apple strain or a geranium strain of TmRSV. Mircetich et al (11) have suggested that PYBM isolates of TmRSV do not cause stem pitting in peach and vice versa; however, stem pitting symptoms were reported from yellow bud mosaic virus-infected peach in California (15). Because the apple strain of TmRSV has caused stem pitting under field conditions (18) and yellow bud mosaic symptoms in peach under greenhouse conditions in our study, perhaps factors other than the strain of TmRSV are important in symptom development. Other factors that may be involved include juvenility of the peach tree and environmental conditions. Lister et al (8) reported an apparent difference among TmRSV strains in their ability to move systemically in 1-yr-old Elberta peach seedlings; PYBM isolates were recovered from leaves more readily than other isolates. The peaches used in our studies were 6-mo-old Halford seedlings. Because our test plants were young and vigorous, the movement of TmRSV into peach leaves and the subsequent development of PYBM symptoms may have been favored. Additionally, the induced dormancy may have improved the movement of TmRSV from peach roots to leaves. The presence or absence of PYBM symptoms on TmRSV-infected peach trees may be determined by environmental conditions. The generally colder winter temperatures in the northeastern United States compared with California could be responsible for death of the weakened buds of TmRSV-infected peach trees and the lack of PYBM symptoms. Recently, in Pennsylvania, shortly after leaf initiation in the spring, a single nectarine tree in an orchard with high incidence of stem pitting was observed to have PYBM-like symptoms. The TmRSV was readily recovered from the leaves of the tree; however, the leaves on this tree died within 3 wk. The tree also showed typical symptoms of PSP.

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