Attempts to Transmit the Causal Agent of a Cherry Stem Pitting Disorder in Washington Sweet Cherry Trees

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ABSTRACT

AL MUSA, A. M., G. I. MINK, and J. L. PARSONS. 1980. Attempts to transmit the causal agent of a cherry stem pitting disorder in Washington sweet cherry trees. Plant Disease 64:1081-1083.

Although dagger nematodes resembling *Xiphinema americanum* were present in most Washington sweet cherry orchards, we were unable to isolate any virus resembling tomato ringspot virus from stem-pitted sweet cherry trees, from bait plants grown in orchard soils, or from orchard weeds. No pitting developed on stems of cv. Lovell peach, *Prunus tomentosa* seedlings grown 28 mo in soils taken from under stem-pitted cherry trees, or *P. tomentosa* or *P. mahaleb* seedlings graft-inoculated with sweet cherry trees from three orchards. However, some pitted sweet cherry trees in one orchard induced interveinal chlorotic bands on lower leaves of graft-inoculated *P. tomentosa* seedlings. The causal agent and its relationship to the pitting disorder in sweet cherry trees were not determined. The disorder of sweet cherry trees in Washington appears to differ in etiology from similar diseases in the eastern United States and in California.

Additional key words: *Prunus avium,* Prunus stem pitting agents

Approximately one-fifth of the commercial sweet cherry (*Prunus avium*) trees in Washington have mild to severe stem pitting on the trunks of scion varieties with little or no pitting of rootstocks (4). Superficially, this pitting resembles that described on *Prunus* spp. elsewhere (1,6,8–11). However, unlike *Prunus* stem pitting disease in the eastern United States and California where pitting is accompanied by general tree decline (1,7), most of the stem pitted cherry trees in Washington exhibit nearly normal growth. This disorder appears to be of minor economic importance in Washington despite its wide occurrence. However, the general distribution of stem-pitted cherry trees throughout the cherry-growing regions of eastern Washington raised a significant question regarding the causal agent(s) of the disease.

In the East, stem pitting in different *Prunus* spp. appears to be caused by the same or related strains of the same causal agent, which can be transmitted readily through soil (6). A virus serologically related to tomato ringspot virus (Tom RSV) has been associated with stem-pitted peach and other stone fruit trees (6,9,10). The American dagger nematode, *Xiphinema americanum*, a vector of Tom RSV (12), has been found in soils under stone fruit trees affected by stem pitting disease (6). However, no consistent correlation was found between viruses resembling Tom RSV in soil and stem pitting in orchard trees (6). In California, Tom RSV has also been associated with stem pitting of sweet cherry trees (8), but its causal relationship remains unclear.

In the East, the *Prunus* stem pitting disease usually spreads from infected to adjacent trees with no random occurrence of newly infected trees (6). This pattern was not found in any orchard in Washington (4). Pitted trees occurred more or less at random in all orchards. Furthermore, general symptoms on the trees were quite unlike those in California sweet cherry orchards where trees were propagated on *P. cerasus* 'Stockton Morello' rootstocks. Symptoms did, however, resemble those reported in California orchards where *P. mahaleb* 'Mahaleb' and *P. avium* 'Mazzard' rootstocks were used (4,8).

If Tom RSV or a similar virus were involved in the stem pitting disorder of sweet cherry trees of Washington, it seemed reasonable to anticipate that this virus might be found in orchards where the disease occurred. To our knowledge, Tom RSV has not occurred naturally in central Washington. In our attempts to identify a causal agent for the Washington sweet cherry stem pitting disorder, we therefore attempted to detect Tom RSV or similar viruses in orchard soils and source trees.

MATERIALS AND METHODS

Graft transmission was attempted from field-grown, pitted sweet cherry trees to 3- to 5-mo-old *P. tomentosa* and *P. mahaleb* seedlings grown from seed in sterilized soil. The seedlings were graft-inoculated with two or three root chips collected from stem-pitted or healthy sweet cherry trees as described by Mircetich et al (7). The inoculated indicator plants were grown in a greenhouse or lathhouse and observed for symptoms. During winter the inoculated trees were kept in a cooler at 6 C for 3–4 mo. Because Prunus necrotic ringspot virus (NRSV) is a likely contaminant in most field-grown cherry trees (7), indicator plants with various leaf symptoms were tested for NRSV by enzyme-linked immunosorbent assay (3). All *P. mahaleb* and some *P. tomentosa* trees were also tested for NRSV and prune dwarf virus by bud inoculation on *Prunus serrulata* 'Shirofugen.'

Attempts were made to recover viruses from leaves of graft-inoculated *P. tomentosa* and *P. mahaleb* by rub-inoculation to *Chenopodium quinoa*, cowpea (*Vigna unguiculata* 'California Blackeye'), and cucumber (*Cucumis sativus* 'National Pickling'). Each indicator tree was examined for stem pitting 15–28 mo after inoculation.

Bark and cambial tissues from roots of each field source tree were indexed on *C. quinoa*. Other roots from each source tree were kept in moist plastic bags at 24 C for 1 mo. The etiolated sprouting buds and callus tissue that developed were indexed on *C. quinoa*, cowpea, and cucumber.

In all attempts to transmit viruses from

Fig. 1. Interveinal chlorotic bands on lower leaves of *P. tomentosa* graft-inoculated with root chips from a sweet cherry tree exhibiting severe trunk pitting.

Portion of a thesis submitted by the senior author in partial fulfillment of a Ph.D. degree. Present address of senior author: Faculty of Agriculture, University of Jordan, Amman, Jordan.

Washington State University College of Agriculture Research Center, Scientific Paper 5527, Project 1719.

This work was supported in part by funds provided by WSU-UOJ Contract AID-278-UNN-81-T.

0191-2917/80/12108103/$03.00/0

sweet cherry roots or from the leaves and bark of graft-inoculated *P. mahaleb* or *P. tomentosa*, the tissue was triturated in 0.01 M phosphate buffer, pH 8.0, or 0.01 M phosphate buffer containing 2% nicotine (final pH 8.5).

Soil samples were collected from various depths and positions under sweet cherry trees at different dates during 1975, 1976, and 1977. To determine if a *Xiphinema* sp. was consistently associated with the stem pitting disease, the presence of dagger nematodes was determined in 250-cc aliquots of each sample. In two cases, soil extracts containing *Xiphenima* spp. were poured onto roots of cucumber and cowpea seedlings growing in sterilized sand.

Attempts were made to transmit a virus from the soil samples to possible indicator plants. Bait plants including cucumber, bean (*Phaseolus vulgaris* 'Bountiful'), *Gomphrena globosa, C. quinoa,* tobacco (*Nicotiana tabacum* 'Connecticut Havana 423'), and cowpea were planted and grown 2–3 mo in growth chambers at 24 C. The herbaceous plants were observed for symptoms, and after various time intervals, root and leaf samples were indexed by rub-inoculation on *C. quinoa,* cowpea, and cucumber. In another series of experiments, detached leaves from *C. quinoa,* cowpea, and *G. globosa* were placed at 22 C in moist soil samples taken from under sweet cherry trees. After 2 days, the leaves were removed from the soil, incubated 2 days in illuminated (about 7,500 lux for 16 hr) moist chambers, observed for symptoms, and then indexed by rub-inoculation on *C. quinoa*, cucumber, and cowpea.

The possibility of transmitting the stem pitting agent through the soil to various woody plants was tested by growing peach (*P. persica* 'Lovell') and *P. tomentosa* seedlings in soil samples collected from under sweet cherry trees. These seedlings were observed for leaf symptoms and examined for stem pitting symptoms 28 mo after planting. Leaf and bark tissues from these seedlings were indexed by rub-inoculation on herbaceous indicator plants.

Seedlings of *P. tomentosa* and *P. mahaleb* were inoculated with viruses isolated during this study to determine if they induced stem pitting symptoms. Leaves and roots or stem cambium of newly germinating and 3–5 mo old *P. tomentosa* and *P. mahaleb* seedlings were graft- or rub-inoculated with infected tissue or purified viruses isolated from under stem-pitted trees. The inoculated indicator trees were examined for stem pitting 12 and 24 mo after inoculation.

RESULTS

Graft-inoculation. In 1976, 28 trees with no pitting or mild to severe pitting (4) were selected from two orchards and indexed on *P. tomentosa* seedlings by using root chips. Trees in orchard A were planted in 1962 and consisted of scion and rootstocks free from all cherry viruses recognized at that time. Each of the 16 trees tested in orchard A was indexed on Shirofugen flowering cherry in 1976 and found to be free of NRSV and prune dwarf virus. None of 70 inoculated *P. tomentosa* seedlings developed symptoms on leaves or stems during 20 mo of observation. No virus was transmitted by rub-inoculation from roots or leaves of the source trees or from roots or leaves of the inoculated indicator.

Orchard B was also established in 1962 but with trees of unknown virus content. Of the 37 *P. tomentosa* seedlings graft-inoculated with root chips from 12 trees, several developed symptoms of NRSV. However, only one of the 37 indicator plants developed a symptom on the stem that might be interpreted as stem pitting. This symptom consisted of five tiny depressions in the woody cylinder near the point of inoculation with inoculum from tree 14-9. No virus other than NRSV was transmitted by rub-inoculation to herbaceous plants from either leaves or roots of the source trees or the graft-inoculated indicator plants.

In August 1977, 10 additional *P. tomentosa* seedlings were graft-inoculated with root chips from tree 14-9 (orchard B). None of the 10 exhibited pitting on the stem when examined 15 mo after inoculation. During this period, one inoculated *P. tomentosa* seedling developed unusual leaf symptoms consisting of a diffuse chlorotic band between the larger veins on leaves near the base of each shoot (Fig. 1). No symptoms developed on young expanding leaves even when the plant was defoliated and new growth forced. No virus was transmitted from this plant by rub-inoculation.

In a second graft-transmission experiment, 36 trees from a third orchard (orchard C—planted with noncertified trees) were indexed individually on two *P. tomentosa* and five *P. mahaleb* seedlings (Table 1). As expected, NRSV was graft-transmitted from each of several trees with and without trunk pitting. In addition, the interveinal chlorotic banding symptom developed on one or both *P. tomentosa* seedlings inoculated with five of six severely pitted orchard trees. In three of these cases, NRSV was not detected in the source tree or in the inoculated *P. tomentosa*. One of the severely pitted source trees induced very severe NRSV symptoms in both *P. tomentosa* seedlings. Consequently, we could not be certain whether the interveinal chlorotic banding symptom was transmitted from this tree. The interveinal chlorotic banding symptom was transmitted from two of six trees with intermediate pitting symptoms but from none of 24 trees with mild or no pitting (Table 1).

In this experiment, only one of 72 inoculated *P. tomentosa* seedlings had a symptom that might be interpreted as trunk pitting—a few small depressions near the point of inoculation. None of 180 inoculated *P. mahaleb* seedlings showed any sign of pitting 15 mo after inoculation.

No virus resembling Tom RSV was transmitted to herbaceous plants by rub-inoculation from leaves or roots of the 36 source trees listed in Table 1 or from any of the 252 inoculated woody indicators, including the *P. tomentosa* seedlings exhibiting interveinal chlorotic bands.

Association of nematodes with stem pitting. Dagger nematodes resembling *X. americanum* were common in most Washington cherry orchards. The populations varied widely from tree to tree, ranging from none to more than 400 per 250 cc of orchard soil. There was no apparent correlation between dagger nematode populations and the degree of trunk pitting in any orchard.

Soil and nematode transmission. Herbaceous plants including bean, *C. amaranticolor, C. quinoa, G. globosa*, tobacco, and petunia were planted (two to four each) in 1-kg soil samples collected from under each tree in each orchard and grown 2–3 mo in growth chambers. No virus was recovered from leaves and roots of most of these plants by rub-inoculation to *C. quinoa*, cowpea, and cucumber. A virus identified as beet necrotic yellow vein virus was isolated from roots of one *G. globosa* plant grown in soil from under two trees (one pitted tree in orchard B and one nonpitted tree in orchard C). Back transmission attempts strongly suggested that beet necrotic yellow vein virus was not associated with the trunk pitting disorder of sweet cherries (A.M. Al Musa and G. I. Mink, *unpublished*).

Table 1. Symptoms on *Prunus tomentosa* and *P. mahaleb* seedlings graft-inoculated with root chips from trees exhibiting stem pitting symptoms in orchard C[a]

Pitting	No. of trees indexed	No. of trees producing leaf symptoms on *P. tomentosa*	No. of trees producing trunk pitting on *P. tomentosa*	No. of trees producing trunk pitting on *P. mahaleb*
Severe	6	5(1)[b]	(1)[b]	0
Intermediate	6	2	0	0
Mild	6	0	0	0
None	18	0	0	0

[a]Two *P. tomentosa* and five *P. mahaleb* seedlings were inoculated with each source tree.
[b]Results from one source tree uncertain.

Tree 14-9 in orchard B induced interveinal chlorotic banding on leaves of one of 10 inoculated *P. tomentosa* seedlings. Soil samples indicated the dagger nematode population around this tree was somewhat higher than that under surrounding trees. Herbaceous species planted in soil taken from under this tree and grown in growth chambers for 2–3 mo were: bean, *C. amaranticolor, C. capitatum, C. quinoa,* cowpea, cucumber, *G. globosa, N. tabacum* H 423, *N. glutinosa,* and petunia. Roots and leaves of each plant were then indexed on cucumber, cowpea, *C. quinoa, C. amaranticolor,* bean, and *N. clevelandii.* A virus identified as tobacco necrosis virus was isolated from roots of one *G. globosa* plant. All other bait plants indexed negative on the herbaceous indicators. Each pot was examined for dagger nematodes as the roots were removed for indexing. Each contained from 1 to more than 100 live nematodes when the root samples were tested.

In a second experiment, 400+ dagger nematodes extracted from soil under tree 14-9 were poured on the roots of bean, cowpea, cucumber, *C. amaranticolor,* and *C. quinoa,* and the plants were grown in greenhouse soil for 3 mo. Although live nematodes were found in each pot when root samples were indexed, no virus was recovered from the roots or leaves of any plant.

Two *P. tomentosa* and two Lovell peach seedlings (5–6 mo old) were grown 28 mo in soil taken from under each of the 28 trees in orchards A and B (including tree 14-9) and from under eight severely pitted trees in orchard C. Although all soil samples contained dagger nematodes when the experiment began and some containers contained them when the experiment ended, no leaf or stem symptoms developed on any plant.

Detached leaves of *C. quinoa, G. globosa,* and cowpea were buried 2 days in moist soil samples collected from under each tree listed in Table 1. The "bait" leaves were then incubated for 2 days in moist chambers and indexed for virus on *C. quinoa*, cucumber, and cowpea. No virus was recovered.

Finally, representatives of the following weed species growing near pitted cherry trees were indexed on cucumber, *C. quinoa,* and cowpea: *Asparagus officinalis, Amaranthus retroflexus, Brassica nigra, Chenopodium album, Cirsium arvense, Salsola kali, Solanum nigrum,* and *Taraxacum officinale.* Other than asparagus viruses I and II (5), which were found in two different asparagus plants, no viruses were detected in orchard weeds.

DISCUSSION

Despite intensive efforts, no virus resembling Tom RSV was isolated from Washington sweet cherry trees, from bait plants grown in orchard soil samples, or from orchard weeds. This and the fact that Lovell peach seedlings remained healthy in orchard soils for more than 2 yr strongly suggest that the stem pitting disorder in Washington cherry orchards differs from the peach stem pitting disease so prevalent in the eastern United States. Although an agent was transmitted by graft-inoculation to *P. tomentosa* from some pitted trees, the symptoms on this indicator differed from those described for Prunus stem pitting (6,8). In our study, a diffuse chlorotic mottle between major veins appeared on lower leaves 50–60 days after inoculation and remained confined to the lower leaves of each shoot. Furthermore, except for two highly questionable cases, no pitting developed on either *P. tomentosa* or *P. mahaleb* seedlings observed for 15–28 mo. The interveinal chlorotic banding symptoms were observed only on *P. tomentosa* inoculated with root chips from trees with intermediate or severe trunk pitting. Furthermore, this agent was detected in only one of three orchards where stem pitting was severe. The nature of this causal agent and its relationship to the stem pitting disorder in Washington have not yet been determined. If this agent is associated with stem pitting of sweet cherry trees in orchard C, it seems likely that pitting symptoms observed in orchards A and B may have a different etiology.

We detected two viruses vectored by fungi in Washington soils; neither appears to be associated with the trunk pitting disorder. Our inability to isolate Tom RSV despite an abundance of dagger nematodes resembling *X. americanum* raises the question of whether the nematode is the same as *X. americanum* found in the east and whether it may be a vector of either Tom RSV or tobacco ringspot virus. According to Lamberti and Bleve-Zacheo (2), *X. americanum* is a complex of different species that may vary in their ability to transmit plant viruses.

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