# Distribution of Tobacco Streak, Tomato Ringspot, and Raspberry Bushy Dwarf Viruses in *Rubus ursinus* and *R. leucodermis* Collected from the Pacific Northwest

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#### ABSTRACT

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Clonal and seed propagules of Rubus ursinus, the trailing blackberry, and seed of R. leucodermis, the western black raspberry, were collected from throughout the Pacific Northwest (Oregon, Washington, and British Columbia). The collections were made in four general environmental regions: at sea level along the Pacific coast, in the coastal mountains adjacent to the coast, in the Willamette Valley, and in the Cascade Mountains. After the resulting propagules were established, leaves were tested by enzyme-linked immunosorbent assay according to standard procedures. None of the R. leucodermis samples tested positive for raspberry bushy dwarf idaeovirus (RBDV), tomato ringspot nepovirus (TomRV), or tobacco streak ilarvirus (TSV). Rubus leucodermis is either resistant to these viruses or their vectors, the viruses are not seed transmitted in this species, or the viruses were not detected due to inadequate sampling. No samples of R. ursinus tested positive for RBDV or TomRV. Samples from 77% of the sites where cuttings were taken and 37% of the seedling populations tested positive for TSV. Along the Pacific coast, only 20% of the sites sampled by cuttings tested positive for TSV. However, the percentage of infected sites where cuttings were taken increased to 88 and 100% in the coastal mountains and the Cascade Mountains, respectively. Along the Pacific coast, 14% of seedling sites tested positive for TSV. The number of TSV positive seedling sites increased to 45 and 36% in the Cascade Mountains and the coastal mountains, respectively. The Willamette Valley site tested negative for TSV. With one exception, all of the low elevation sites tested negative for TSV. Prevailing winds or earlier flowering may prevent some of these Pacific Coast populations from becoming infected. Many of the TSV positive sites had some samples that tested negative, indicating that TSV is unevenly distributed within these populations.

Additional keywords: breeding, germ plasm

Rubus ursinus Cham. & Schlechtend. and R. leucodermis Douglas ex Torr. & A. Gray were collected from throughout the Pacific Northwest to examine variability within these species and potential value as germ plasm for the USDA-ARS Rubus breeding program. The distribution of viruses in native Rubus spp. is of interest because of the large commercial industry located in the same region and because the collection will be utilized in breeding programs designed to develop improved blackberry and raspberry germ plasm. This provided an opportunity to examine the distribution of viruses in native species. Because genotypes used in crosses must also be free of viruses, plant material was

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tested for the presence of tomato ringspot nepovirus (TomRV), raspberry bushy dwarf idaeovirus (RBDV), and tobacco streak ilarvirus (TSV), all of which can be seedborne in *Rubus*.

TSV was first reported by Johnson (11) and Rosaceae was identified as a host family by Fulton (9). TSV is vectored by both pollen and thrips (13,15). In Rubus, TSV has been found in eastern black raspberry (Rubus occidentalis L.) (4,12,16). TSV is generally considered symptomless in Rubus (2,16). However, Frazier (8) reported transmitting the causal agent of strawberry necrotic shock, TSV (4), from "degenerate-appearing" plants of cv. Boysen (an R. ursinus derivative). Converse (5) also found that cv. Santiam, a selection that is presumed to be largely derived from R. ursinus, plants infected with TSV produced 32% fewer primocanes than did a Santiam clone that was free of TSV.

TSV was isolated from 34.5% of *R. ursinus* plants sampled from scattered populations in southwest British Columbia (2,17). The authors did not report on the distribution of these sites nor on the percentage of sites that had infected plants.

Converse and Bartlett (6) reported on the occurrence of TSV, RBDV, and TomRV in *R. ursinus* collected from 26 sites that were located primarily within the Willamette Valley of Oregon. Seventy-nine percent of their sites and 33% of their plants from these sites tested positive for TSV.

RBDV is pollenborne and can be a serious problem in susceptible cultivars, causing interveinal chlorosis, crumbly fruit, and decreased yields (7,14). TomRV, which is vectored by nematodes, can cause chlorotic ringspots, crumbly fruit, a decline in plant growth, and a decrease in productivity in susceptible red raspberry cultivars (18). *Rubus ursinus* is either immune or only mildly affected by TomRV (18). None of the 63 samples of *R. ursinus* nor the one sample of *R. leucodermis* tested positive for RBDV or TomRV in studies by Converse and Bartlett (6).

Our objective was to determine the presence and distribution of RBDV, TomRV, and TSV in *R. ursinus* and *R. leucodermis* collected from throughout their native growing range in the Pacific Northwest.

## MATERIALS AND METHODS

Collection sites. In 1993, sites were chosen from the eastern edge of the Cascade Mountains to the Pacific Ocean in Oregon, Washington, and British Columbia (Fig. 1). The major types of sites were (i) the Cascade Mountains, from Crater Lake (OR) to Hope (BC), (ii) coastal mountains, from the Siskiyou Mountains (OR), through the Coastal Range to the Olympic Mountains (WA) as well as from mountains on Vancouver Island (BC), and (iii) Pacific coast sites, which were within a mile of the Pacific Ocean or adjacent bays, from Florence (OR) to Vancouver and Gabriola Islands (BC). We also collected from an additional site in the Willamette Valley (OR).

Clonal collections of *R. ursinus*. Clonal collections of *R. ursinus* consisted of cane sections that were collected from 20 to 30 genotypes no closer than 30 m from each other at each site (Table 1; Fig. 1). The canes were stored in a chest cooler and transported back to Corvallis, OR. The cane sections were cut into two to four node cuttings and placed under mist in media-filled flats. Rooted cuttings were then transferred to pots in a greenhouse.

Seedling material of *R. ursinus* and *R. leucodermis*. Seed was collected from 22 sites (Fig. 1; Table 2; specific locations of

R. leucodermis collection sites are available from authors). Eleven of these sites were the same as sites where canes of R. ursinus had been collected. Seeds from an additional 14 R. ursinus and 11 R. leucodermis sites, which had been collected in 1983 or 1985, were obtained from the USDA-ARS National Clonal Germplasm Repository. The seeds were stratified for 2 months at 2°C under moist conditions prior to germination. Thirty-two seedlings representing each site were transplanted to pots and maintained in the greenhouse.

Sampling for ELISA. Leaf samples were taken from seedlings and asexually propagated plants. For the cuttings, 6 to 20 genotypes were sampled from each of 21 sites (Table 1). For the seedling collection, 32 genotypes from each site were sampled (Table 2). Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect RBDV and triple antibody sandwich (TAS)-ELISA was used to detect TSV and TomRV in raspberry plants. All reagents were used at 100 µl per well in Linbro flat-bottom microtiter

**British** Columbia Δ Washington )regon 37

- R. ursinus seed □ TSV-free TSV-infected
- R. ursinus cuttings o TSV-free TSV-infected
- R. leucodermis collection sites riangle TSV-free

Fig. 1. Locations of sites where cuttings of Rubus ursinus (circles), seed of R. ursinus (squares), or seed of R. leucodermis (triangles) were collected. Open symbols are tobacco streak ilarvirus (TSV) negative; solid symbols are TSV positive. Locations associated with numbers for R. ursinus are given in Tables 1 and 2, and specific site descriptions for R. leucodermis are available from the authors.

plates (Flow Laboratories, Mclean, VA) except for the blocking step, which was 200 µl per well. Plates were washed extensively after each step except for blocking. Coating antibody, purified polyclonal IgG, was diluted in coating buffer (carbonate, pH 9.6). Plates were coated for 2 to 4 h at room temperature. Plates were then blocked with phosphate-buffered saline Tween containing 0.1% nonfat dried milk powder and 0.05% Tween 20 (blocking buffer) for 1 h at room temperature.

Three to five leaflets from each population were homogenized (1:40, wt/vol in blocking buffer containing 2% polyvinylpyrrolidone (mol wt 44,000) and 100-µl samples loaded onto the duplicate plates for each virus (a total of six) and incubated overnight at 4°C. RBDV-specific monoclonal antibody conjugated to alkaline phosphatase 0.1µg/ml was loaded into the plates and incubated at 30°C for 2 h. For TSV and TomRV the plates were washed again and crude ascitic fluid diluted in blocking buffer was added at dilutions of 1:10,000 and 1:5,000, respectively, and incubated for 2 h at 30°C. After an additional washing, rabbit anti-mouse (IgG + IgM) alkaline phosphatase conjugate was added to the plates for the TSV and TomRV test and incubated an additional 2 h at 30°C. Substrate (p-nitrophenyl phosphate) at 0.5 mg/ml in 10% diethanolamine, pH 9.8, was incubated for 1 h at room temperature, then overnight at 4°C.

Absorbance of each well at 405 nm  $(A_{405})$  was read in an ELISA plate reader (MR5000 Dynatech, VA) after the 1 h incubation and after the overnight incubation at 4°C. Reactions were considered positive if the  $A_{405}$  values were greater than 5 times the values obtained for healthy controls. All samples were tested in duplicate.  $A_{405}$  values of healthy samples ranged from 0.0 to 0.05; any values greater than 0.25 were considered positive.

#### RESULTS

Rubus ursinus. Individual populations were not uniformly infected with TSV (Tables 1 and 2). Samples from 12 of 21 clonal sites and 14 of 41 seedling sites gave inconsistent results; some tested positive and others tested negative for TSV. Three of the 11 R. ursinus sites that had clonal and seed samples collected in 1993 tested positive for TSV in the clonal samples but negative in the seed samples. This is either due to insufficient sampling or may indicate a lower transmission rate through seed.

Sites sampled by cuttings. Forty-four percent of the samples tested positive for TSV, representing 71% of the sites. None of the samples tested positive for RBDV or TomRV. Only 20% of the sites on the Pacific coast tested positive for TSV, whereas 100% of the Cascade Mountain sites and 88% of the sites in the coastal mountains tested positive. Samples from the Willamette

Table 1. Enzyme-linked immunosorbent assay for tobacco streak ilarvirus of clonally collected populations of Rubus ursinus from sites throughout the Pacific Northwest

No. infected samples/ total no. samples tested	Location [Fig. 1 reference number]	State/ province	Elevation (m)	Year collected
0/5	Pacific coast, Florence, Siuslaw NF (GP 04)*[1]	OR	1 to 15	1993
0/2	Pacific coast, Crescent Bay (LIG 06) [2]	WA	1 to 5	1993
0/5	Pacific coast, Pacific City (GP 08) [3]	OR	1 to 15	1993
0/3	Pacific coast, Aberdeen (GP 06) [4]	WA	15 to 30	1993
0/5	Willamette Valley, Corvallis (GP 14) [5]	OR	91	1993
0/5	Coastal mountains, east of Agness, Siskiyou NF (GP 03) [6]	OR	275 to 305	1993
1/5	Cascade Mountains, Mt. Baker, Mt. Baker-Snoqualmie NF (LIG 17) [7]	WA	460 to 472	1993
1/5	Cascade Mountains, Chilliwack Lake (GP 18) [8]	BC	640	1993
2/5	Coastal mountains, Horne Lake, Vancouver Island (GP 19) [9]	BC	137 to 152	1993
2/5	Coastal mountains, Triangle Lake, Siuslaw NF (GP 11) [10]	OR	244	1993
2/5	Coastal mountains, NW Quilcene, Olympic NF (LIG 04) [11]	WA	700	1993
2/5	Cascade Mountains, Fraser Valley, NE Hope (GP 20) [12]	BC	183 to 213	1993
3/5	Coastal mountains, Saddle Mountain St. Park (GP 07) [13]	OR	381 to 427	1993
3/5	Cascade Mountains, Mt. St. Helens/Mt. Adams, Gifford Pinchot NF (LIG 38) [14]	WA	460	1993
3/5	Cascade Mountains, Iron Mountain, Willamette NF (GP-10) [15]	OR	1,250	1993
4/5	Coastal mountains, Mary's Peak, Siuslaw NF (GP 05) [16]	OR	762 to 823	1993
4/5	Cascade Mountains, Lake Wenatchee, Wenatchee NF (LIG 30) [17]	WA	780	1993
4/5	Coastal mountains, SW Quilcene, Olympic NF (LIG 02) [18]	WA	792	1993
2/2	Pacific coast, Dungeness (LIG 05) [19]	WA	15	1993
4/4	Coastal mountains, Mt. Hebo, Siuslaw NF (GP 09) [20]	OR	914 to 945	1993
5/5	Cascade Mountains, Huckleberry Mtn., Rogue River NF (GP 02) [21]	OR	1,645 to 1,740	1993

<sup>&</sup>lt;sup>a</sup> LIG refers to populations collected by Luby et al. (unpublished, 1993); GP refers to USDA-ARS Hortic. Crops Lab. accessions.

Table 2. Enzyme-linked immunosorbent assay for tobacco streak ilarvirus of seedling populations of Rubus ursinus from sites throughout the Pacific Northwest

No. infected samples/ total no. samples tested	Location [Fig. 1 reference number]	State/ province	Elevation (m)	Year collected
0/3	Pacific coast, Deception Pass St. Park (LIG 11)*[1]	WA	1.5	1993
0/3	Pacific coast, Crescent Bay (LIG 06) [2]	WA	1 to 5	1993
0/3	Pacific coast, Dungeness (LIG 05) [3]	WA	15	1993
0/3	Pacific coast, Aberdeen (GP 06) [4]	WA	15 to 30	1993
0/2	Pacific coast, Gabriola Island (GP 16) [5]	BC	15 to 30	1993
0/3	Pacific coast, Port Orchard (RUB 605) [6]	WA	70	1984
0/3	Coastal mountains, SW Crescent Bay, Olympic NF (LIG 07) [7]	WA	191	1993
0/3	Coastal mountains, (BLJ-9), Florence/Mapleton, Siuslaw NF (RUB 703) [8]	OR	200	1985
0/3	Coastal mountains, east of Agness, Siskiyou NF (GP 12) [9]	OR	305	1993
0/1	Coastal mountains, Saddle Mountain St. Park (GP 07) [10]	OR	381 to 427	1993
0/3	Coastal mountains, SW Quilcene, Olympic NF (LIG 01) [11]	WA	457	1993
0/3	Coastal mountains, (BLJ-14) near Agness, Siskiyou NF (RUB 649) [12]	OR	510	1985
0/3	Coastal mountains, SW Crescent Bay, Olympic NF (LIG 09) [13]	WA	610	1993
0/3	Coastal mountains, (BL-75), near Shelton, Olympic Mtns. NF (RUB 679) [14]	WA	760	1985
0/3	Coastal mountains, Mary's Peak, Siuslaw NF (GP 05) [15]	OR	762 to 823	1993
0/3	Cascade Mountains, Mt. Baker, Mt. Baker-Snoqualmie NF (LIG 15) [16]	WA	305	1993
0/3	Cascade Mountains, Mt. St. Helens/Mt. Adams, Giff. Pinch. NF (LIG 38) [17]	WA	460	1993
0/3	Cascade Mountains, Mt. Baker, Mt. Baker-Snoqualmie NF (LIG 17) [18]	WA	460 to 472	1993
0/3	Cascade Mountains, Baker Lake, Mt. Baker-Snoqualmie NF (LIG 12) [19]	WA	460 to 610	1993
0/3	Cascade Mountains, N of Trout Lake, Gifford Pinchot NF (LIG 42) [20]	WA	640	1993
0/1	Cascade Mountains, Chilliwack Lake (GP 15) [21]	BC	640	1993
0/3	Cascade Mountains, N. of Packwood, Gifford Pinchot NF (LIG 32) [22]	WA	790	1993
0/3	Cascade Mountains, Lake Wenatchee, Wenatchee NF (LIG 31) [23]	WA	975 to 1035	1993
0/3	Cascade Mountains, McKenzie, Willamette NF (RUB 395) [24]	OR	1,042	1983
0/3	Cascade Mountains, Cougar Reservoir, Willamette NF (RUB 396) [25]	OR	1,050	1983
0/3	Cascade Mountains, (BL-58), Wind River, Gifford Pinchot NF (RUB 620) [26]	WA	1,300	1985
1/3	Pacific coast, Westport (GP 13) [27]	WA	31	1993
1/3	Coastal mountains, (BLJ-4), Deadwood, Siuslaw NF (RUB 708) [28]	OR	150	1985
1/3	Coastal mountains, SW Crescent Bay, Olympic NF (LIG 08) [29]	WA	380	1993
1/3	Coastal mountains, NW Quilcene, Olympic NF (LIG 04) [30]	WA	700	1993
1/3	Cascade Mountains, Lake Wenatchee, Wenatchee NF (LIG 29) [31]	WA	760 to 900	1993
1/3	Cascade Mountains, Lake Wenatchee, Wenatchee NF (LIG 30) [32]	WA	780	1993
1/3	Cascade Mountains, N. of Packwood, Gifford Pinchot NF (LIG 33) [33]	WA WA	930	1993
1/3	Cascade Mountains, (BL-45), Opal Lake, Willamette NF (RUB 662) [34]	OR		1995
1/3		OR	1,000	
	Cascade Mountains, Iron Mountain, Willamette NF (GP 10) [35]		1,250 740	1993 1993
1/2	Cascade Mountains, Stephens Pass, Mt. Baker-Snoqualmie NF (LIG 24) [36]	WA		
2/3 2/2	Coastal mountains, Mt. Hebo, Siuslaw NF (GP 09) [37]	OR	914 to 945	1993
2/3 2/2	Cascade Mountains, (BL-70), Gifford Pinchot NF (RUB 677) [38]	WA	1,100	1985
2/3	Cascade Mountains, (BL-40) Link Lake area, Deschutes NF (RUB 660) [39]	OR	1,130	1985
2/3	Cascade Mountains, Huckleberry Mtn., Rogue River NF (GP 02) [40]	OR	1,645 to 1,740	1993
3/3	Coastal mountains, (BL-81), near Quinalt, Olympic NF (RUB 686) [41]	WA	677	1985

<sup>&</sup>lt;sup>a</sup> BL and BLJ refer to original citation in Ballington et al. (1); RUB are USDA-ARS National Clonal Germplasm Repository accessions; LIG refers to populations collected by Luby et al. (unpublished, 1993); GP refers to USDA-ARS Hortic. Crops Lab. accessions.

Table 3. Summary of incidence of tobacco streak ilarvirus (TSV) in R. ursinus samples and sites collected in the Pacific Northwest

Type of site	Type of propagule	Samples TSV+/totala	Sites TSV+/totala
Pacific coast	Clonal	2/17	1/5
	Seed	1/20	1/7
Coastal mountains	Clonal	21/39	7/8
	Seed	8/40	5/14
Cascade Mountains	Clonal	19/35	7/7
	Seed	12/57	9/20
Willamette Valley	Seed	0/5	0/1

<sup>&</sup>lt;sup>a</sup> Number of samples or sites that tested positive for TSV/total number of samples or sites.

Valley site tested negative for TSV (Table 3). With one exception, all of the low elevation sites (0 to 90 m) tested negative for TSV (Table 1).

Seedlings. Thirty-six percent of the samples tested positive for TSV (Table 2). None of the samples tested positive for RBDV or TomRV. Only 12.5% of the Pacific coast sites tested positive for TSV, whereas 38.5% of the coastal mountain sites, and 45.5% of the Cascade Mountain sites tested positive (Table 3). Incidence of TSV was higher in populations from higher elevation than from those collected at lower elevation.

R. leucodermis. None of the samples of R. leucodermis seedlings tested positive for TSV, TomRV, or RBDV.

#### DISCUSSION

While TSV commonly occurs in native R. ursinus, RBDV and TomRV do not. This finding agrees with earlier observations (2,6) based on testing of R. ursinus collected from a narrow geographic range.

The lack of uniformity of infection within a population could result from several factors. Converse (5) found an uneven distribution of TSV in a single blackberry clone. While our seed collections came from many canes of many plants within a population, if fruit were harvested from a TSV-free cane, the seedlings from that cane would be less likely to carry TSV. If seed infection occurred via pollenborne virus, then the mother plant might not have been infected. A similar phenomenon might exist with our clonal material; within a composite sample some of the plants might be free of TSV or the individual cane(s) taken from an infected plant could be free of TSV.

Explaining the distribution pattern of infected sites is difficult. The low incidence of TSV in coastal populations might be due to prevailing winds or earlier, nonsynchronous flowering that may prevent the infected pollen from inland locations from moving to the coastal populations, or it may be due to insufficient sampling.

The TSV distribution may be related to species diversity in R. ursinus. Taxonomists described the range of the octoploid R. ursinus as being primarily coastal, whereas the dodecaploid R. macropetalus, which is now more commonly included under the R. ursinus species designation, was found at higher elevation (3,10). Further taxonomic analysis and chromosome counting may help determine whether there are true botanical differences between these types. Subsequently, botanical ranges can be compared with the range of infected sites.

The presence of TSV in Rubus plants generated from seed has not been previously reported. Plant breeders in the Pacific Northwest, who routinely test seedlings for virus as part of their cultivar development program, report that RBDV is transmitted to 0 to 8% of the progeny (H. Daubeny and P. Moore, personal communication). It was interesting that TSV remained viable in dry, cold-stored, R. ursinus seed for 8 years.

None of the three viruses examined were found in R. leucodermis. This is interesting especially given the widespread locations of the collection sites and the report that TSV is native to the region (6). All of the samples from sites that overlapped between the two Rubus species tested negative for the three viruses. Several possibilities for the low incidence of TSV exist. Rubus leucodermis could be resistant to these viruses; this would be surprising since the closely related R. occidentalis (eastern black raspberry) is susceptible. These viruses may not be seed transmitted in R. leucodermis. Finally, these populations may not have had the opportunity to become infected with these viruses. The fact that R. leucodermis might be resistant to these viruses deserves further study because it might be a valuable germ plasm resource in red and black raspberry breeding programs.

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