

Evaluation of *Rubus leucodermis* Populations from the Pacific Northwest

Chad Finn and Kirsten Wennstrom¹

USDA–ARS, HCRL, Northwest Center for Small Fruit Research, 3420 NW Orchard Avenue, Corvallis, OR 97330

Janessa Link²

Waldport High School, Waldport, OR 97394

Jill Ridout³

Department of Horticulture, Oregon State University, Corvallis, OR 97331

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Abstract. Sixteen western black raspberry (*Rubus leucodermis* Douglas ex Torrey & A. Gray) populations, collected from throughout the Pacific Northwest, and ‘Munger’, the most widely grown black raspberry (*R. occidentalis* L.) cultivar, were established in 1994 in a replicated trial in Corvallis, Ore. In 1996 and 1997 the seedlings were evaluated for date of budbreak, flowering date, ripening date, fruit size, and disease tolerance. Fruit within a replication were pooled for evaluation of pH, soluble solids, titratable acidity, and anthocyanin content. The plants were vigorous and had produced solid hedges by the time evaluation commenced. The populations were significantly different within each year for all traits except for anthocyanin content in 1996. *Rubus leucodermis* populations were identified that broke bud and ripened fruit earlier or later than ‘Munger’; however, all flowered with or sooner than ‘Munger’. Despite the fact that *R. occidentalis* is native to eastern North America and *R. leucodermis* to the West, ‘Munger’ was much less affected by foliar and cane diseases than the *R. leucodermis* populations. Several populations were as vigorous as ‘Munger’. ‘Munger’ had fruit that were 30% larger than the mean for any *R. leucodermis* population. Generally, *R. leucodermis* had higher pH and lower titratable acidity than ‘Munger’, but many populations had similar soluble solids; lower acidity may partly explain the blandness of *R. leucodermis* fruit compared with ‘Munger’. Despite the lighter appearance of *R. leucodermis*, the anthocyanin levels of some populations were higher than ‘Munger’. *Rubus leucodermis* may be a source of earlier fruiting, later budbreak, and vigor when used in breeding but careful selection for fruit size (for the fresh market), acidity (for the processing market), and disease resistance must be done. *Rubus leucodermis* may also be an excellent source of raspberry bushy dwarf virus (RBDV) resistance in black and red raspberry breeding programs.

The native North American black raspberries are represented by *Rubus leucodermis* Douglas ex Torrey & A. Gray in the West and *R. occidentalis* L. in the East. *Rubus leucodermis* is found from British Columbia to southern California and from the Pacific Coast inland to Montana, Utah, and Nevada (Hitchcock and Cronquist, 1973). Typically, they are found in disturbed sites, such as recent clear-cuts or along forest roads, in fields and canyons, and from sea level to ≈1300 m (C. Finn, personal observation; Hitchcock and Cronquist, 1973). The plants can grow to 2 m and have a typical black raspberry semi-erect, arching growth habit. However, *R. leucodermis* foliage tends to be lighter green and more coarsely toothed, the canes more heavily glaucous, the

spines shorter and more hooked, and the fruit softer and dull purple-colored rather than the shiny-black of *R. occidentalis* (Jennings, 1988, personal observation).

The commercial black raspberry processing industry is concentrated in the Willamette Valley (Oregon), where there are 450 to 500 ha in production (NASS, 2002). ‘Munger’, which accounts for nearly all of the production in Oregon, is reputed to have been selected from open-pollinated ‘Shaffer’ seed (*R. occidentalis*) in western Ohio about 1890 (Hedrick, 1925). Production of black raspberries for fresh market sales is scattered in small plantings throughout North America, but is most common in the eastern United States, where ‘Jewel’, ‘Bristol’, and ‘Haut’ are the primary cultivars grown but others, such as ‘Black Hawk’, ‘Cumberland’, and ‘MacBlack’, are also important. These cultivars are all derived primarily from *R. occidentalis*, although one recently released cultivar, ‘Earlysweet’, has some *R. leucodermis* in its background (Galletta et al., 1998).

The processing industry is facing increasing problems with yield loss due to fungal diseases

and viruses, and it has long been recognized by growers that ‘Munger’ is subject to pollination problems in wet, cool springtime conditions. Traditionally, the processing industry has dug tip-layers from production fields to use as planting stock for new fields. Due to an increased awareness of virus problems, nursery stock is now either purchased as virus-negative or is self-propagated in nursery fields that are not allowed to flower, thereby avoiding potential raspberry bushy dwarf virus (RBDV) transmission. While these practices and better fungal control programs will help, the industry would benefit from cultivars better adapted to the region’s climate and pests.

Since *R. leucodermis* is native to the West and is closely related to *R. occidentalis* (Jennings, 1988), it may be a valuable source of adaptive traits to incorporate into *R. occidentalis* cultivars. The red (*R. idaeus* L.) and black raspberry industries are struggling with how to manage the pollen-borne RBDV (Murant, 1987). *Rubus leucodermis* has never tested positive for RBDV when wild stands or plants adjacent to commercial raspberry fields have been surveyed (Converse and Bartlett, 1979; Finn and Martin, 1996; Martin, 1998). Whether *R. leucodermis* is immune to RBDV or somehow escapes infection is unknown, although graft testing would suggest escape (R. Martin, personal communication). Finn and Martin (1996) also found no tobacco streak virus (TSV) or tomato ringspot virus (ToRSV) in seedling populations of *R. leucodermis* collected from throughout the Pacific Northwest. Stace-Smith and Martin (1988) did find double-stranded RNA (dsRNA) in native *R. leucodermis* seedlings but none of the plants with dsRNA showed any symptoms indicative of virus infection, nor were any virus-like particles observed with electron microscopy.

In our program, *Rubus leucodermis* and *R. occidentalis* can be easily crossed and the resulting progenies are fertile and productive. Ourecky and Slate (1966) used *R. leucodermis* to try to increase variability in their black raspberry (*R. occidentalis*) program (Jennings, 1988). Seedlings from these crosses were very vigorous and productive but tended to have many of the inferior traits of *R. leucodermis*, including dull-colored, soft, and smaller fruit.

The objectives of this research were to collect *R. leucodermis* populations from throughout the Pacific Northwest and then characterize them to determine whether they would be useful in improving the adaptation of commercially grown black raspberries.

Materials and Methods

Seeds representing 10 populations from the eastern edge of the Cascade Mountains to the Pacific Ocean in Oregon and Washington were collected by us in 1993 (Table 1). Seeds from an additional six sites were also obtained from the USDA–ARS National Clonal Germplasm Repository, which were collected by Ballington et al. (1985). The seed from each sample were generally extracted from large quantities of randomly collected fruit. The major types of sites were: 1) the Cascade Mountains, from Crater

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¹Retired.

²High School and Saturday Academy Student.

³Undergraduate Student; in partial fulfillment of an undergraduate thesis requirement.

Lake (Ore.) to the North Cascades (Wash.); 2) coastal mountains, from the Siskiyou Mountains (Ore.), through the Coastal Range to the Olympic Mountains (Wash.); 3) Pacific coast sites, which were directly along the coast of the Pacific Ocean or the Puget Sound.

Seeds were scarified following standard procedures (Ourecky, 1975) and stratified for 2 months at 2 °C under moist conditions prior to germination. Thirty-two seedlings representing each population were transplanted to pots, tested for TSV, RBDV, and ToRSV by ELISA (Finn and Martin, 1996), and maintained in the greenhouse.

In Spring 1994, the plants were established in a field at the USDA-ARS National Clonal Germplasm Repository (Corvallis, Ore.) in a randomized complete-block design with four replications and eight plants per replication. In vitro-propagated, plug-size 'Munger' (*R. occidentalis*) was included in each block as a standard for comparison. Plants were left untrained during the 1994 growing season and cut to the ground in the dormant season. From 1995 until the experiment's conclusion, the plants were trained similar to commercial black raspberries, including summer tipping of primocanes at 0.75 m and dormant season cane hedging (Crandall, 1995).

In 1996 and 1997, the plants were evaluated weekly for the date of budbreak, flowering, and 5% ripe fruit. In 1997, each plant was rated for vigor and crop load (1–5 scale; 1 = poor, 5 = outstanding). In 1996 and 1997, the plants were given an overall disease rating on a 1–5 scale (1 = most disease symptoms; 5 = fewest disease symptoms). The primary cane and foliar diseases were anthracnose [*Elsinoe veneta* (Burkholder) Jenk.] and leaf spot [*Septoria darrowii* Zeller].

In 1996 and 1997, 25 ripe fruit were randomly harvested from each plant and weighed. Fruit samples from each replication for a population were pooled and frozen for later laboratory evaluations. For analysis, the samples were thawed to room temperature and pureed in a blender for 20 s. The pH and soluble solids (°Brix) were measured directly from this puree. A 5-g subsample of puree was diluted with 50 mL distilled water, placed on a stir plate, and titrated with 0.1 N NaOH to pH 8.2 to determine the titratable acidity of the sample. A separate 1-g sample of the puree was placed in a flask with 50 mL solvent (1.5 N HCl: 95% EtOH, 15:85). The sample was stirred for 30 min and then vacuum-filtered (Whatman's #1 filter). Enough solvent was added to bring the volume to 100 mL. About 15 mL were subjected to spectrophotometry (DU-70 Spectrophotometer, Beckman, Fullerton, Calif.) at $A = 535_{OD}$. The anthocyanin content (mg:100 g⁻¹) was calculated as: $C = [(A \times MW \times 10^3 \times 0.10 \text{ L solvent}) / (E \times \text{g puree})] \times 100$; where $A = 535_{OD}$, $MW = 445.2$ (molecular weight of cyanidin-3-glucoside [chrysanthemine or asterin] a major black and red raspberry anthocyanin) and $E = 29,600$, the molar absorbance of chrysanthemine or asterin.

Plants were sampled and tested by ELISA for RBDV, ToRSV, and TSV in Spring 1999.

'Munger' is the only black raspberry

Table 1. Locations where *Rubus leucodermis* was collected in the Pacific Northwest.^z

Population	Location	State	Elevation (m)
Pacific coast			
LIG 11	Deception Pass State Park	WA	2
LIG 5	Dungeness	WA	15
Coastal mountains			
RUB 646	Elk Creek, Siskiyou NF (BLJ-12-1)	OR	180
RUB 645	Florence/Mapleton, Siuslaw NF (BLJ-9-1)	OR	200
GP 3	East of Agness, Siskiyou NF	OR	275–305
LIG 01	Southwest of Quilcene, Olympic NF	WA	457
RUB 651	Near Agness, Siskiyou NF (BLJ-14-4)	OR	510
LIG 9	Southwest of Crescent Bay, Olympic NF	WA	610
RUB 705	Raspberry Mountain, Siskiyou NF (BLJ 15-2)	OR	730
Cascade Mountains			
LIG 38	Mt. St. Helens/Mt. Adams, Gifford Pinchot NF	WA	460
LIG 12	Baker Lake, Mt. Baker-Snoqualmie NF	WA	460–610
LIG 37	Mt. St. Helens/Mt. Adams, Gifford Pinchot NF	WA	535
LIG 19	Klipchuck Campground, Okanogan NF	WA	900
LIG 21	Mazama, Okanogan NF	WA	1020
RUB 678	Northeast of Packwood, Gifford Pinchot NF (BL-70-2)	WA	1100
RUB 658	Benham Falls, Deschutes NF (BL-32-3)	OR	1280

^zAbbreviations: BL and BLJ refer to original citations in Ballington et al. (1985); GP refers to USDA-ARS Hort. Crops Lab accessions; LIG refers to populations collected by Luby et al. (unpublished, 1993); NF = National Forest; RUB are USDA-ARS National Clonal Germplasm Repository accession numbers.

cultivar grown in the Northwest. Any of our efforts to utilize *R. leucodermis* in our breeding program will involve 'Munger' as a prominent parent and the breeding effort and it will be the genotype to which all selections will be compared. For this reason, we included 'Munger' in the PROC GLM (SAS Institute, 1991) analysis. In an attempt to better characterize our collection of *R. leucodermis*, we used multivariate analysis of variance [PROC GLM-MANOVA, (SAS Institute, 1991)] to compare populations by the provenances where they were collected. This analysis did not elucidate any clear patterns and is not presented here.

Results and Discussion

General. After two growing seasons, the plants were vigorous and had produced solid hedges. Due to our long term interest in tolerance of biotic and abiotic stress, we left the planting in for two additional seasons. By 1997, disease pressure was beginning to take its toll on the vigor of many plants. By 1998, about 50% of the plants were dead or had been removed due to susceptibility to disease and by 2000, about 80%.

Populations were significantly different between years. There was a significant population × year interaction for all traits evaluated in the field except there was no effect due to year for flowering date (Table 2). For pH, soluble solids, and titratable acidity there were significant differences due to population and year, and there was a significant population × year interaction (Table 2). However, for anthocyanin content, there were no population differences in 1996 and no year or population × year effects.

Plant characteristics. Budbreak and ripening dates were earlier in 1997 than 1996 (Table 2). While average first flowering date was spread over 2 weeks in 1997, in 1996 all of the *R. leucodermis* populations began flowering within a 3-d period. These differences probably reflect the differences in the weather/temperature; there was a relatively rapid change between cold, winter weather

and warm, sunny days in 1996, whereas in 1997, there was a gradual warming that began earlier in the season but it did not warm as quickly.

Populations were identified that broke bud either significantly earlier or later than 'Munger' (Table 2). In the Pacific Northwest, later budbreak to avoid spring frost and decrease the length of time the foliage is exposed to disease pressure would be a desirable characteristic. All *R. leucodermis* populations flowered with or sooner than 'Munger' (Table 2). This would not be a desirable trait in developing black raspberry cultivars for the Pacific Northwest, as even 'Munger' with its relatively late flowering is often exposed to cool, wet weather that inhibits pollination and reduces fruit set. Populations were also identified that ripened fruit either significantly earlier or later than 'Munger' (Table 2). For the commercial black raspberry processing industry, ripening date is not generally a concern as having most of the fruit ripen within a short time frame is ideal for the processing plants. However, the significantly earlier and later ripening of some *R. leucodermis* populations could be a source of altered ripening date for the fresh market industry.

Scatter plots showed no obvious correlation between elevation of the collection site and plant characteristics with the exception of budbreak, which was later in the populations gathered from high elevations (data not shown).

One of the biggest early disappointments with this planting was the lower vigor and higher disease susceptibility of the *R. leucodermis* populations compared to 'Munger' (Table 2). In some ways this is not surprising, as 'Munger' has stood up in commercial plantings for decades despite the fact that it was selected from germplasm that evolved in eastern North America. We expected to see several *R. leucodermis* genotypes and a few populations that would be at least as well adapted as 'Munger', but this was not the case. The plants showed more foliar and cane

Table 2. Year and population means for six plant and five fruit characteristics in *Rubus leucodermis* populations collected in the Northwest and 'Munger'.

Source	Plant characteristics						Fruit characteristics				
	Budbreak ^y	Flowering date ^y	Ripening date ^y	Crop ^z	Disease rating ^z	Vigor ^z	Fruit size (g)	pH	Soluble solids (°Brix)	Titrateable acidity (% as citric acid)	Anthocyanins (g)
Year											
1996	75.4	122.8	176.1	---	2.4	---	1.23	4.10	10.58	0.63	3.19
1997	69.6	126.1	166.0	3.3	3.3	2.4	0.98	3.78	9.50	0.79	2.71
Population/cultivar											
GP 3	71.5	125.4	176.9	3.3	2.5	2.0	1.20	3.83	9.51	0.77	2.24
LIG 1	74.6	126.9	171.9	3.7	2.5	2.8	1.17	4.03	9.43	0.61	2.75
LIG 5	70.6	124.2	175.0	3.8	2.7	3.2	0.84	3.89	10.94	0.76	2.98
LIG 9	75.4	125.7	171.7	3.4	2.4	2.2	1.13	4.03	10.04	0.64	3.34
LIG 11	72.2	124.5	171.9	3.4	2.7	1.9	1.04	4.05	10.35	0.65	3.56
LIG 12	73.0	121.7	169.1	3.3	2.5	2.2	1.09	3.96	9.31	0.68	2.69
LIG 19	73.8	121.8	170.5	2.8	2.2	1.6	1.05	3.90	9.16	0.77	3.31
LIG 21	76.4	124.9	172.3	3.4	2.2	1.9	1.14	3.94	8.90	0.71	3.00
LIG 37	72.3	123.7	169.7	3.6	2.5	2.9	1.09	3.94	10.15	0.75	3.01
LIG 38	71.7	120.5	167.7	3.6	2.3	2.5	1.20	3.86	9.95	0.77	3.12
RUB 645	68.2	122.5	170.6	3.4	2.9	2.4	1.08	3.86	10.99	0.74	3.14
RUB 646	66.5	121.2	169.9	3.8	2.7	2.9	0.93	4.03	11.13	0.63	2.36
RUB 651	68.9	126.0	172.9	3.6	2.4	2.3	1.02	4.16	10.18	0.55	2.32
RUB 658	74.8	122.4	170.7	2.5	2.1	1.2	1.08	3.95	8.85	0.72	3.28
RUB 678	72.6	121.6	167.9	3.0	2.6	2.7	1.17	3.83	10.16	0.77	3.44
RUB 705	73.5	128.7	176.3	3.2	2.3	2.0	1.08	4.14	9.73	0.56	2.47
Munger	75.1	130.6	171.0	3.3	4.2	2.9	1.53	3.59	11.98	0.97	3.05
Significance (P)											
Replication	0.001	0.001	0.141	0.370	0.001	0.004	0.111	0.310	0.850	0.407	0.001
Year (Y)	0.001	0.072	0.001	---	0.001	---	0.001	0.001	0.001	0.001	0.001
Population (P)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
P × Y	0.001	0.001	0.001	---	0.001	---	0.001	0.033	0.001	0.002	0.332

^yDays from 1 Jan.

^zCrop, disease rating, and vigor, all scored on a subjective scale, where 1 = smallest crop, most disease, and poorest vigor; and 5 = largest crop, least disease, and greatest vigor.

disease symptoms in 1997 than 1996 based on the disease rating score. This is probably due to the cumulative effects of no disease control in the planting and to a warmer and wetter than typical late spring in 1997. While most of the *R. leucodermis* populations were less vigorous than 'Munger', five populations (LIG 1, LIG 5, LIG 37, RUB 646, and RUB 678) had equivalent average vigor ratings.

Fruit characteristics. Fruit size, pH, soluble solids, and anthocyanin content decreased and titrateable acidity increased from 1996 to 1997 (Table 2). The possible causes for a decrease in fruit size include a change in crop load or environmental conditions, or the cumulative effect of disease and other stress. The last reason seems the most likely, as the plants appeared to be carrying a full crop in each year, the plot was irrigated, and fruit set, which can be affected by cool, wet weather, seemed to be excellent.

There were very few differences in crop load ratings suggesting that genotypes within the *R. leucodermis* populations can be selected that should not negatively impact yield when used in a breeding program (Table 2). However, crop load ratings were difficult to make consistently and accurately, as the quantity of ripe fruit on the evaluation dates can bias the estimate.

'Munger' fruit size was 30% greater than the largest fruited *R. leucodermis* populations (Table 2). Large size is important for hand-picked fruit for the fresh market; however, it is not important for machine-harvested fruit for the processing industry as long as the overall yield is still acceptable. If *R. leucodermis* is crossed with *R. occidentalis* cultivars, it will be

critical to select strongly for increased fruit size for markets where fruit size is important.

In the field, *R. leucodermis* drupelets appear dull and purple rather than shiny and black like those of *R. occidentalis*. In addition, the fruit is generally bland flavored when compared with 'Munger'. Analyses of the fruit purees showed that 'Munger' generally had a lower pH and higher titrateable acidity than the *R. leucodermis* samples (Table 2). Three populations (LIG 5, RUB 645, and RUB 646) had soluble solids equivalent to 'Munger'. This combination of traits may partly explain *R. leucodermis*'s bland flavor as they do not have the same "tartness" as 'Munger'. 'Munger's low pH and high titrateable acidity are valuable for stabilizing anthocyanins after processing. The anthocyanin content of the *R. leucodermis* populations were surprisingly high with very few differences among populations or between 'Munger' and *R. leucodermis* (Table 2). The difference between the perceived color and glossiness in the field and the anthocyanin levels could be due to fruit morphology, anthocyanin composition, pH, and/or compositional and physical factors. *Rubus leucodermis* fruit is covered with fine epidermal hairs that may cause a part of the perceived difference in color. Cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sambubioside, and cyanidin 3-xylosylrutinoside are the primary fruit anthocyanins in *R. occidentalis* (Nybom, 1968), including 'Munger' (Torre and Barritt, 1977). The relative proportion of each of these, as opposed to total anthocyanin content, affected the perceived color of each (Torre and Barritt, 1977). *Rubus leucodermis* may have similar anthocyanins

but their relative proportion may be different, thereby affecting the perceived color.

Conclusions

The primary hope in the initial collecting of this species was that *R. leucodermis* would be a source of increased tolerance to the abiotic and biotic stresses typical of the Pacific Northwest. This species might then be crossed with 'Munger' or other *R. occidentalis* cultivars to develop cultivars that were better adapted to the Pacific Northwest. Based on our short-term results this does not seem likely as, in general, *R. leucodermis* was less vigorous and more disease susceptible than 'Munger'. However, the fact that *R. leucodermis* was not initially infected with RBDV, TSV, and ToRSV and has not become infected after three flowering seasons suggests that it may be a potential source of resistance to these viruses. Resistance to RBDV would be an extremely valuable trait for red and black raspberry breeding.

Twenty-two superior selections of *R. leucodermis* from these 16 populations have been made. Fifteen of these selections came from three sites (LIG 9, LIG 11, and RUB 646). These selections are available from C.E.F. to interested parties and, after these selections are directly compared, a subset will be placed in the USDA-ARS National Clonal Germplasm Repository.

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