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Primocane Morphology and Leaf Surface Characteristics of Greenhouse-grown Red Raspberry Cultivars

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Abstract. **Abaxial and adaxial surfaces of the distal leaflet of the third leaf from the apex, and primocane morphology of the following internode, of six greenhouse-grown red raspberry (*Rubus idaeus* L.) cultivars ('Algonquin', 'Chilliwack', 'Comox', 'Haida', 'Meeker', and 'Tulameen') were examined. Scanning electron micrographs revealed tangled, woolly pubescence, irregularly shaped cells, and stomates on abaxial leaf surfaces. 'Algonquin' exhibited the densest pubescence, and 'Haida' the sparsest. Smooth-walled, uniserate trichomes were present on adaxial surfaces. 'Chilliwack' displayed greatest trichome abundance. Trichomes were less commonly seen on the adaxial leaf surface of 'Algonquin', 'Haida', and 'Meeker'. Relative abundance of spines on 3-month-old primocanes was dense for 'Tulameen', moderate-dense for 'Meeker' and 'Comox', moderate for 'Chilliwack', sparse-moderate for 'Algonquin', and sparse for 'Haida'. Spine length was greatest for 'Tulameen', and was greater in 'Meeker' than in 'Haida'.**

The major red raspberry producing region in North America is the Pacific Northwest, including areas of western Oregon and Washington, and southwestern British Columbia (Moore and Daubeny, 1993). While fruit characteristics such as size, flavor, color, ripening time, harvestability, storability, and disease and pest reactions of raspberries have received much attention (Daubeny, 1973, 1987; Daubeny and Anderson, 1991; Daubeny et al., 1991; Jennings et al., 1991; Moore and Daubeny, 1993), little information is available concerning leaf surface characteristics and primocane morphology.

Trichomes or pubescence on leaf surfaces have been linked to plant defense (Levin, 1973), temperature regulation (Ehleringer and Bjorkman, 1978), and water economy (Ehleringer and Mooney, 1978; Johnson, 1975). In general, pubescent leaves have a higher reflectivity and a lower absorptive capacity than nonpubescent leaves (Eller, 1977).

Genetic fingerprinting is a reliable technique for identification of raspberry cultivars held by germplasm repositories, propagators, and breeders (Cousineau and Donnelly, 1989a, 1989b, 1992). Payne and Morris (1976) found pubescence to be an effective aid in differentiating soybean (*Glycine max* L.) varieties. Kelsey (1984) reported differences in trichomes to be a useful taxonomic character for black sagebrush (*Artemisia nova* Nels.). Scanning electron microscopy has also been used to analyze drupelet morphology of red raspberry and related *Rubus* genotypes (Robbins et al., 1988). Leaf surface characteristics and primocane spine morphology could be used as additional aids for cultivar identification. This study was undertaken to characterize the adaxial and abaxial leaf surfaces and the primocane spine morphology of several red raspberry cultivars currently available in the Pacific Northwest.

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Materials and Methods

Root cuttings of the red raspberry cultivars Algonquin, Chilliwack, Comox, Haida, Meeker, and Tulameen were obtained from the research field of the Pacific Agricultural Research Centre (Abbotsford, B.C., substation) in Nov. 1993 and Nov. 1995. Root cuttings were placed in a sterilized and screened 20 loam : 1 peat moss mixture in 10-cm plastic pots in a greenhouse. Osmocote slow-release fertilizer (14N–6.1P–11.6K; Grace Sierra Co., Milpitas, Calif.) was added at the time of planting. Cuttings were placed beneath supplemental fluorescent lighting (≈330 μE $m^{-2} \cdot s^{-1}$; 16-h photoperiod) and watered as necessary. Additional fertilizer (20N–8.7P–12.8K; 3 $g \cdot L^{-1}$) was applied weekly starting 1 month after planting. Shoots arising from four cuttings of each cultivar were allowed to grow for 3 months before any observations were taken. Preliminary observations were recorded on two plants (replications) per cultivar in 1993–94. The experiment was repeated in a different year with four replications per cultivar in 1995–96. The results were subjected to analysis of variance using SYSTAT software (Wilkinson et al., 1992).

Two 0.5-cm^2 sections (one abaxial and one adaxial) per replicate were excised from the distal leaflet of the third leaf from the apex of greenhouse-reared plants. The leaf sections were fixed immediately in 5 mL of 2.5% glutaraldehyde (in 0.1 M sodium cacodylate buffer, pH 7.3) for 6 h, rinsed three times with sodium cacodylate buffer, and subsequently fixed in 2% osmium tetroxide for 1 h. The samples were dehydrated in a 50%, 70%, 85%, 95%, and 100% ethanol series, and embedded with liquid CO_2 (at 15 °C) in a CPD 020 critical point dryer (Balzers Union, Balzers, Liechtenstein). The chamber temperature was increased to 40 °C to evaporate the CO_2. Samples were mounted on aluminum stubs using double-sided adhesive tabs. Specimens were gold-coated (≈20- to 30-nm thickness) using a Semprep 2 sputtering device (Nanotech, Manchester, U.K.) and adaxial and abaxial leaf surfaces were examined using a Cambridge 250T scanning electron microscope (Cambridge Instruments, Cambridge, U.K.) at 20 kV. The number of trichomes in a 800 × 600 μm area of adaxial leaf surface was counted from the scanning electron micrographs of each replicate and expressed as number/mm^2. Trichomes were counted if greater than half the trichome base was included in this area.

A primocane section 4.5 to 5.0 cm in length was excised from the base of the third leaf from the apex of each specimen. Primocane sections were sliced in half longitudinally and the surfaces photographed. Relative spine density and spine length were measured directly from photographs. Average spine length was determined by measuring 10 spines from each replicate.

Results and Discussion

Scanning electron micrographs of abaxial surfaces of leaves revealed tangled, woolly pubescence (Fig. 1A–D). Stomates were observed in areas with less dense pubescence (Fig. 1 A and B). The epidermal surface appeared to be smooth with no evidence of crystalline epicuticular wax.

Notable differences in abundance of pubescence among red raspberry cultivars were detected. When grown under greenhouse conditions, abaxial leaf surfaces of 'Algonquin' appeared matted with dense pubescence (Fig. 1C). 'Haida' was sparsely pubescent with much of the abaxial leaf surface consisting of irregularly shaped cells (Fig. 1D). 'Chilliwack', 'Comox', 'Meeker', and 'Tulameen' exhibited intermediately abundant pubescence with no clearly discernible differences (micrographs not shown). Whether these differences can be attributed to number or to length of leaf hairs could not be determined.

Scanning electron micrographs of adaxial leaf surfaces revealed an absence of woolly pubescence (Fig. 1 E and F) and stomates. No crystalline epicuticular wax was observed. Smooth-walled, uniserate trichomes of varying lengths were present on adaxial surfaces. Cultivars differed in abundance of trichomes. In both experiments, the cultivar Chilliwack displayed greatest trichome abundance (Fig.

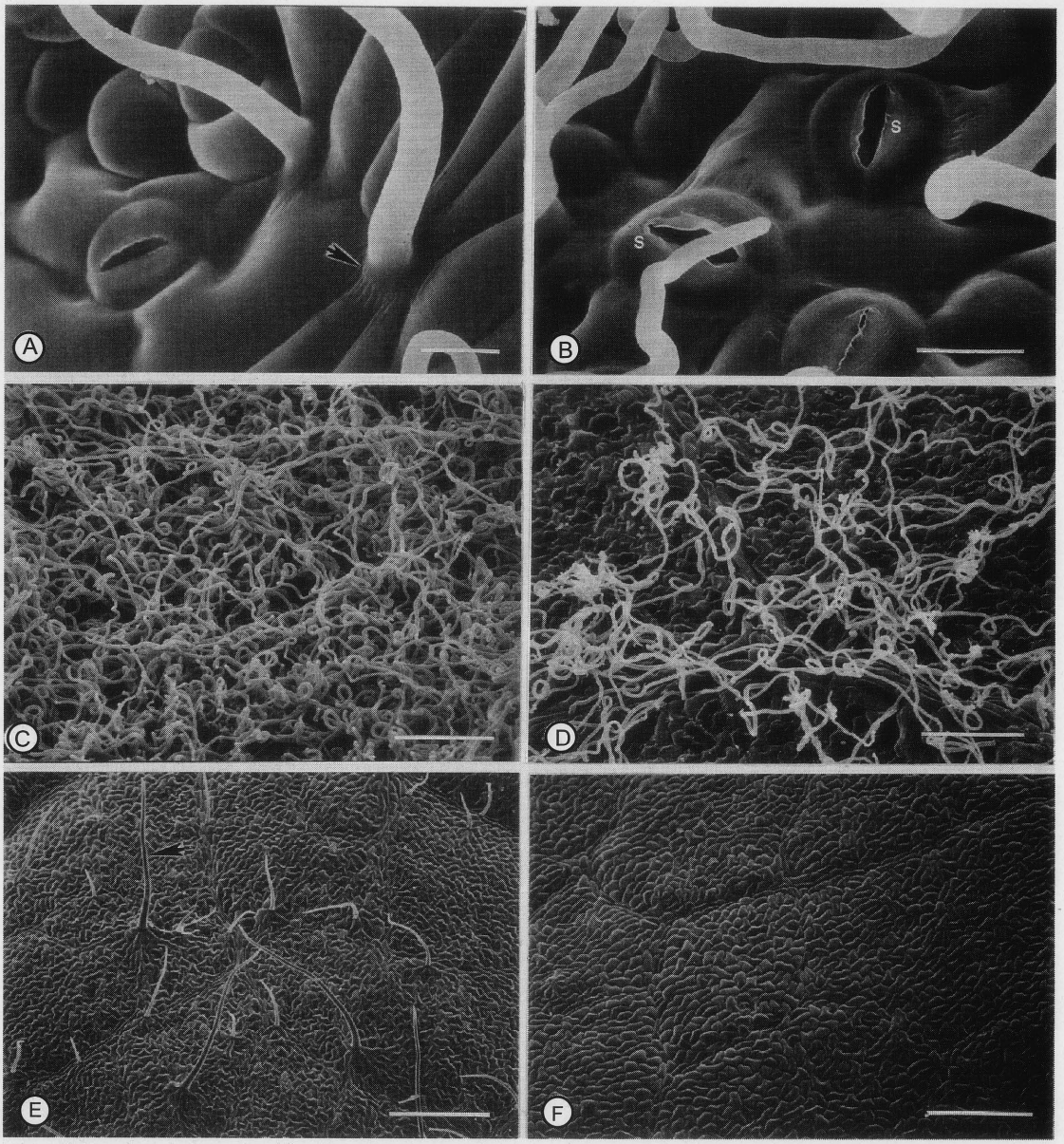

Fig. 1. Electron micrographs of red raspberry leaf surfaces. (**A**) and (**B**) Close-up of abaxial surface showing point of origin of woolly pubescence (**arrow**) and stomates (**s**) (bar = 10 µm); (**C**) abaxial surface of 'Algonquin' showing dense pubescence (bar = 100 µm); (**D**) sparsely pubescent abaxial surface of 'Haida' (bar = 100 µm); (**E**) smooth-walled, uniserate trichomes (**arrow**) on adaxial surface of 'Chilliwack' (bar = 200 µm); and (**F**) trichome-free adaxial surface of 'Meeker' (bar = 200 µm).

1E; Table 1). In the first experiment, 'Comox' had higher abundance of trichomes than 'Algonquin', 'Haida', and 'Meeker' (Table 1). The differences among 'Algonquin', 'Comox', 'Haida', 'Meeker', and 'Tulameen' were not statistically significant in the second experiment.

Primocanes of 3-month-old greenhouse-reared red raspberry plants differed in spine characteristics such as density, length, and thickness of spines on the third internode (Fig. 2; Table 1). Relative abundance of spines on the third internode was dense for 'Tulameen', moderate-dense for 'Meeker' and 'Comox', moderate for 'Chilliwack', sparse-moderate for 'Algonquin', and sparse for 'Haida'. Mean spine length was greatest for 'Tulameen' and greater in 'Meeker' than in 'Haida' in both experiments (Table 1). Other differences were not consistent in the two experiments. Spines arose from enlarged purplish bases in all cultivars except 'Meeker'. The spines in this cultivar lacked enlarged bases, were weak, and easily broken. Primocane morphology and leaf surface characteristics described in this study may provide additional means for distinguishing some of the greenhouse-grown red raspberry cultivars.

Trichome density is significantly affected by environmental factors including light inten-

Table 1. Abundance of trichomes on adaxial leaf surface and primocane spine characteristics of greenhouse-grown Pacific Northwest red raspberry cultivars.[z]

			Primocane spine characteristics		
	Trichome no. (mm^{-2})		Length (mm)		Relative density
Cultivar	Expt. I	Expt. II	Expt. I	Expt. II	
Algonquin	7.4	10.9	3.4	0.7	Sparse-moderate
Chilliwack	32.4	82.3	2.9	1.2	Moderate
Comox	15.0	37.7	5.4	1.2	Moderate-dense
Haida	6.3	1.8	2.5	0.7	Sparse
Meeker	0.0	10.0	5.2	1.5	Moderate-dense
Tulameen	9.3	30.0	7.2	2.8	Dense
$LSD_{0.05}$	7.2	48.9	2.0	0.6	

[z]Two replicates per cultivar in Expt. I and four replicates per cultivar in Expt. II. Relative primocane spine density assessment was based on results of both experiments.

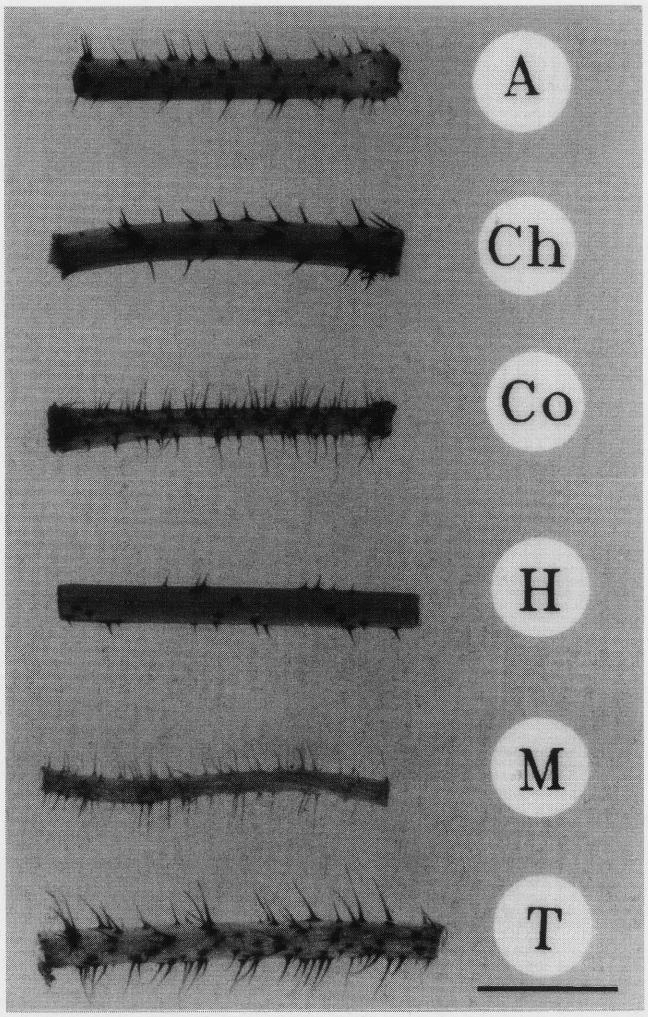

Fig. 2.Red raspberry primocane segments showing cultivar differences in density, length, and thickness of spines. A = 'Algonquin', Ch = 'Chilliwack', Co = 'Comox', H = 'Haida', M = 'Meeker', and T = 'Tulameen' (bar = 1 cm).

sity (Upadhyaya and Furness, 1994), soil texture (Beemster and Masle, 1996), and resource availability (Wilkins et al., 1996). In this study, trichome density in red raspberry cultivars was generally greater in the second experiment, whereas the primocane spine density was greater in the first experiment. Whether environment played any role in the differences between the two experiments remains to be determined. Relative trichome and primocane spine densities among the cultivars, however, were fairly similar in the two experiments.

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