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Phenotypic diversity in antioxidant phytochemical composition among fruits from several genotypes of red raspberry (*Rubus idaeus* L.)

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Abstract. The Pacific Northwest in North America, Russia, and Eastern Europe are three major regions of commercial raspberry production worldwide. In British Columbia, Canada, most raspberries are produced for machine harvesting and processing, while some are selected for the fresh market. Due to increasing public awareness of the benefits of consuming antioxidants for improving human health, breeding of functional foods based on phytochemical composition pyramided with other economically important traits in raspberry is desirable. In this study, genotypes of raspberry destined for the fresh market or processing were each investigated for ascorbic acid and anthocyanin compositions. Variations in these compositional traits were assessed along three consecutive years as well as among three sites in the Fraser Valley of British Columbia. There was a wide range of ascorbic acid contents among fruits from different genotypes with a trend among sample years that appeared to be dependent on seasonal temperatures. For two cultivars, eight different anthocyanins were identified, where the rest of the cultivars contained from four to six. Growing conditions influenced anthocyanin levels, while the profiles stayed consistent. Results from this study can aid in selections by geneticists for crosses to improve antioxidant traits through breeding of new raspberry genotypes.

Keywords: Ascorbic acid, anthocyanins, antioxidants, LC-MS, soft fruit

1. Introduction

Nutritional qualities of fruits have become increasingly relevant for a healthy human diet. The antioxidant action of anthocyanins has been previously reported in the prevention of cancer proliferation in human cell lines [1], lowering of blood pressure in hypertensive stroke-prone rats [2], and the activation of endothelial nitric oxide synthase which induces endothelial-dependent relaxation [3]. Reduction of estrogen-induced mammary tumors in rats treated with ascorbic acid [4] and the promotion of bone health in older humans [5] are some examples of the potential benefits of increasing ascorbic acid in animal diets. Ascorbic acid is widely recognized as an oxygen radical scavenger and an essential nutrient for humans because eight different enzymes use ascorbic acid as electron donor [6]. These

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enzymes function in collagen formation [7], carnitine synthesis [8], norepinephrine biosynthesis [9] peptide hormone stabilization [10], and tyrosine metabolism modulation [11].

Molecular breeding approaches are now viable for enhancing the contents of specific healthy phytochemicals in fruits. Genetic background can play an essential role in determining the nutritional qualities of fruits. Until recently, reports of introgressions of nutritional phytochemical traits to breeding programs in fruit species have been limited [6]. Scalzo et al. [12] analyzed a number of phytochemical parameters in selected strawberry genotypes and found significant differences among genotypes for total antioxidant capacity; however, a poor correlation between antioxidant capacity and horticultural attributes such as yield and fruit size was also determined. When crosses were made between clones with desirable horticultural attributes and those with high antioxidant capacity, the authors [12] were able to select progeny that had both desirable commercial attributes and high antioxidant capacity. These results, albeit focused on strawberry, indicate that breeding of functional fruits with improved nutritional properties is feasible and can be coupled with selections for traditional agronomic traits of economic importance.

Raspberry (*Rubus idaeus* L.) bears red-pigmented, soft fruits containing several antioxidant compounds, including ascorbic acid (vitamin C) and anthocyanin flavonoids. Beekwilder et al. [13] determined that ascorbic acid accounted for 20% of the total antioxidant capacity in raspberry fruits. We considered that genetic potential may exist among *Rubus* spp. germplasm to further improve such healthy traits as ascorbic acid content in commercial genotypes through conventional breeding. The extent to which antioxidant capacity or nutritional phytochemical composition differ among raspberry genotypes by season or production site has not been previously reported from a single study. Tosun et al. [14] reported significant differences in antioxidant capacities as well as ascorbate and total phenolic contents among fruits from 11 raspberry genotypes. Our objective here was to extend the findings of Tosun et al. [14] by evaluating genotypic diversity in ascorbic acid and anthocyanin contents from 12 raspberry genotypes and determining whether significant differences for these traits among genotypes may be confounded by variability attributed to harvest season or site.

2. Materials and methods

2.1. Plant material

Fruits from the floricane raspberry (*R.idaeus)* cvs., Algonquin, Chilcotin, Cascade Delight, Chilliwack, Cowichan, Haida, Malahat, Meeker, Nootka, Sumner, Tulameen, Washington, Willamette, and two experimental genotypes, 36 and 62, were each harvested in each of three years at commercial maturity (22 July 2008, 17 July 2009, 14 July 2010). Collections were made from one experimental (PARC) and two commercial production sites (HK and AK) located in the Fraser Valley of British Columbia. Year-to-year variability analyses were done using samples from the Pacific Agri-Food Research Centre (PARC) substation in Abbotsford, BC. Site-to-site analyses were performed on the samples from PARC and two commercial production sites, South Alder Farms and Krause Berry Farms, owned by Harvey Kraus (HK) and Alfred Kraus (AK), respectively. Harvest date was determined using the following commercial maturity parameters: 9.3 to 13.0 ^{o}Brix, titratable acidity of 1.7 to 2.5%, pH of 2.6 to 3.9, and total soluble solids of 15 to 18%. Although agricultural practices were quite similar among three sites, the main soil differences were in the thickness of the veneer. PARC is characterized by orthic humo-ferric podzol soil, HK is 70% orthic humo-ferric podzol and 30% luvisolic humo-ferric podzol, while AK is 70% rego humic gleysol and 30% gleyed ortstein humo-ferric podzol. Samples were collected in plastic containers or sealed bags and kept on wet ice until they were snap-frozen with liquid nitrogen and stored at –80°C within five hours from harvest. Compositional chemistry analyses were performed on extracts from frozen samples; moisture content comparisons revealed no significant difference among years (data not shown). Mean temperature data for three years was retrieved from a government website, Weather Office, for the Abbotsford area [15]. Trendlines and their linear equations for each year were created using MS Excel software.

2.2. Determination of ascorbic acid content

The extraction procedure was modified from Frenich et al. [16] as follows. Two g of fruit drupelets were placed in a solution containing 5 mL methanol (Sigma-Aldrich Co., Oakville, Ontario, Canada), 25 mL 3% meta-phosphoric

acid (Sigma-Aldrich), and 8% acetic acid (Sigma-Aldrich). Each sample was homogenized using a Polytron mixer for one min and then centrifuged at 4°C for 30 minutes at 3600 g_n. 10 mL of the supernatant were then mixed with 15 mL of 0.1% acetic acid solution. An aliquot of the final mixture was transferred to a 1.5 mL screw-cap amber vial (Agilent Technologies Inc., Mississauga, Ontario, Canada).

An LC/MSD-Trap XCT Plus system equipped with electrospray ionization (ESI) was used to quantify L-ascorbate in each sample. Linear gradient separation was performed on a Zorbax SB-C18 rapid resolution HT 4.5 × 50 mm, 1.8 μm column (Agilent) starting with 2% solvent B (acetonitrile with 0.2% formic acid) in 98% solvent A (water with 0.2% formic acid) to reach 90% (acetonitrile with 0.2% formic acid) at 2.33 minutes along each 5 minute run. Flow rate was 1.0 mL·min and 2 μL of the sample were injected in triplicate. Quantification was performed by constructing a standard curve with commercially available ascorbic acid (Sigma-Aldrich) and hippuric acid (Sigma-Aldrich) as internal standards. During optimization of MS parameters, it was determined that for ascorbic acid, ESI negative mode showed the best response, whereas for ascorbic and hippuric acids, unprotonated molecular ions [M-H]- *m/z* 175 and 178, respectively, were monitored (data not shown).

2.3. Determination of anthocyanin content

Anthocyanins were extracted according to the method of Mullen et al. [17] with minor modifications. Two g of each fruit sample were extracted with a 30 mL solution containing 0.1% HCl in methanol using a Polytron homogenizer for one minute, followed by vortexing. The extract was centrifuged for 20 min at 3600 g_n. One mL supernatant was transferred to a screw cap amber vial and 1 μL was then injected into the LC-MS in triplicate.

An LC/MSD-Trap XCT Plus system equipped with electrospray ionization (ESI) was used to quantify anthocyanin composition in each sample. A linear gradient separation was performed on a Zorbax SB-C18 rapid resolution HT 4.5 × 50 mm, 1.8 μm column (Agilent) starting with 2% solvent A (acetonitrile with 2% (v/v) formic acid) in 98% solvent B (water with 2% (v/v) formic acid) and reaching 90% (acetonitrile with 2% (v/v) formic acid) (Sigma-Aldrich). Two μL of each sample were injected in triplicate and the flow rate was set at 1.0 mL.min. During optimization of MS parameters, it was determined that for anthocyanins, an ESI positive mode gave the best response (data not shown).

Table 1

Ascorbic acid contents (mg per 100 g fresh fruit) among three years for 12 genotypes. Overall mean and standard error of the mean (SEM) for two technical replicates are shown. *P*-values for 2-way ANOVAs for genotype, year, and genotype × year interaction are presented below

Genotype	2008		2009		2010		Overall genotype mean
	Mean	SEM	Mean	SEM	Mean	SEM	
Genotype 62	24.5	0.46	16.04	0.8	33.61	0.77	24.72
Algonquin	10.29	0.26	16.54	0.84	27.27	0.1	18.03
Chilcotin	13.55	0.41	27.22	0.14	29.7	3.59	23.49
Chilliwack	13.65	0.14	24.67	3.02	25.59	0.24	21.30
Cowichan	17.92	0.48	30.27	0.03	21.87	2.82	23.35
Haida	7.02	0.12	11.26	1.04	14.55	1.09	10.94
Meeker	14.1	0.02	34.41	0.59	34.07	0.1	27.53
Nootka	8.54	0.07	16.67	0.04	18.01	0.41	14.41
Sumner	8.06	0.04	13.16	0.72	26.93	1.88	16.05
Tulameen	21.41	0.07	40.61	2.39	24.44	1.34	28.82
Washington	10.81	0.09	22.83	0.08	25.58	0.9	19.74
Willamette	14.87	0.45	27.55	1.98	25.04	0.2	22.49
Overall annual mean	13.73		23.44		25.56		
Genotype	<0.001						
Year	<0.001						
Genotype × Year	<0.001						

Table 2
Ascorbic acid contents (mg per 100 g of fresh fruit) among three sites: PARC, HK, and AK. Overall mean and SEM of two technical replicates are shown. *P*-values for 2-way ANOVAs for plant, site, and plant × site interaction are presented below each genotype

	Sites					
	PARC		HK		AK	
	Mean	SEM	Mean	SEM	Mean	SEM
Genotype 36						
Plant						
1	25.12	0.76	33.28	0.00	30.60	0.71
2	30.15	3.46	29.99	0.19	43.96	1.87
3	25.99	2.75	42.67	3.72	26.53	3.19
Overall site mean	27.09		35.31		33.70	
Plant	0.071					
Site	0.004					
Plant × Site	0.002					
cv. C.Delight						
Plant						
1	17.88	2.01	18.55	0.91	20.94	1.05
2	23.14	0.24	24.22	1.06	19.49	0.7
3	23.86	1.14	18.69	0.51	21.57	2.5
Overall site mean	21.63		20.49		20.67	
Plant	0.042					
Site	0.541					
Plant × Site	0.035					
cv. Malahat						
Plant						
1	19.30	1.06	18.85	1.01	36.18	1.44
2	39.13	2.12	28.39	0.40	38.89	2.92
3	34.03	0.16	21.92	0.61	37.79	0.71
Overall site mean	30.82		23.05		37.62	
Plant	<0.001					
Site	<0.001					
Plant × Site	0.002					

Table 3
Anthocyanin peak identities corresponding to Fig. 2A

Peak no.	Ion mass	Time	Anthocyanin
1	611, 287	2.6	Cy-3-sophoroside
2	449, 287	3.1	Cy-3-glucoside
3	757, 611, 287	3.2	Cy-3-(2^G)-glucosylrutinoside
4	595, 271	3.3	Pg-3-sophoroside
5	595, 449, 287	3.6	Cy-3-rutinoside
6	433, 271	3.7	Pg-3-glucoside
7	741, 271	3.8	Pg-3-(2^G)-glucosylrutinoside
8	579, 271	4.2	Pg-3-rutinoside
9	491, 287	5.2	Cy-3-*O*-(6-acetyl)-glucoside

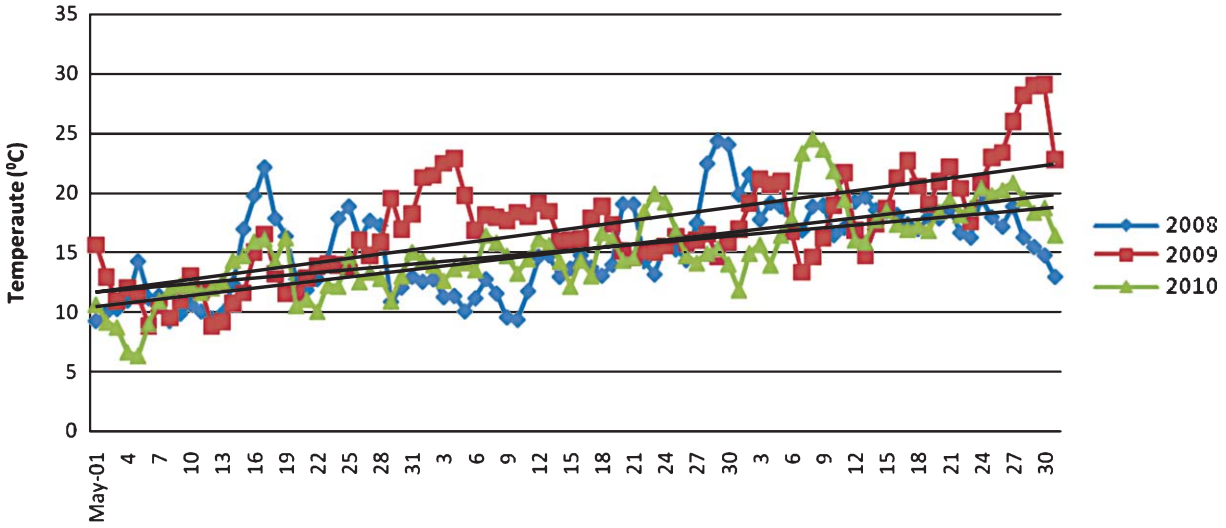

Fig. 1. Average temperature (°C) data along three months (May, June, July) for three years (2008, 2009, 2010) for the Abbotsford, BC, area as recorded by the government agency (Weather Office, http://www.weatheroffice.gc.ca [15]). Trendlines and equations for each line are shown.

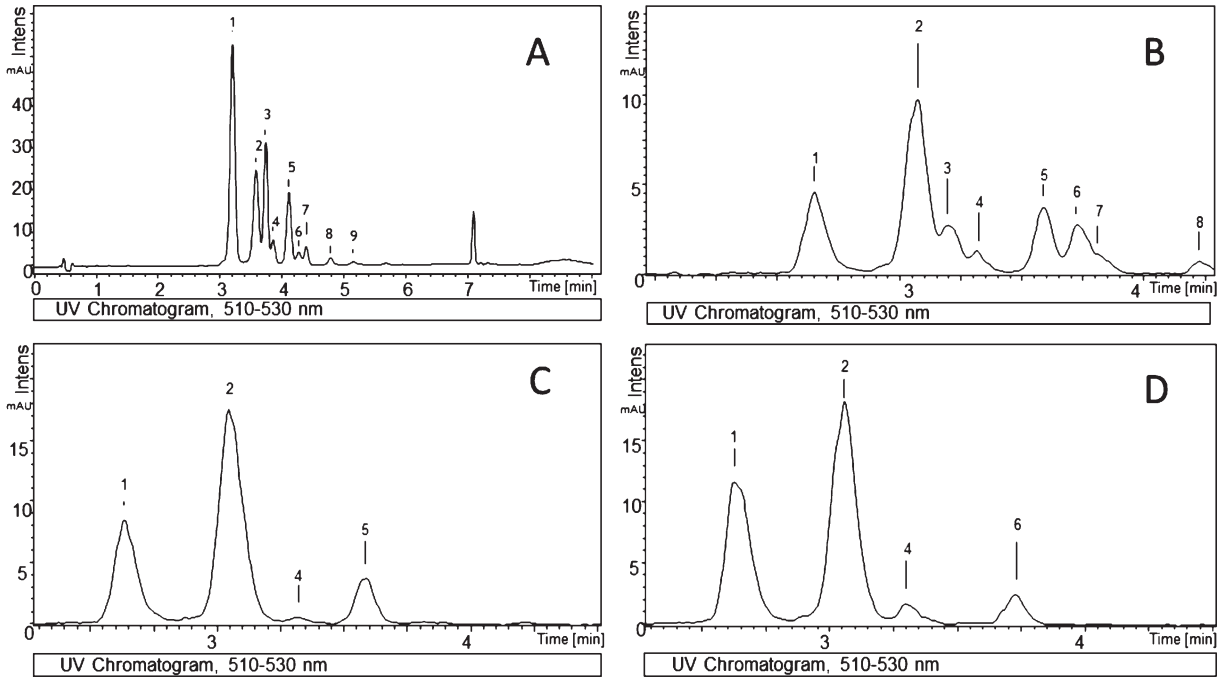

Fig. 2. HPLC chromatograms at 520 nm of raspberry extracts. For peak annotations, refer to Table 3. A: Pooled raspberry extract for method development and peak identification. B: cv. Cascade Delight. C: genotype 36. D: cv. Malahat.

Commercially available standards were purchased from Extrasynthese (Genay, France); these were cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside, pelargonidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside. Other peaks, for which no commercial standard was available, were inferred from matching to the MS spectra from Mullen et al. [17] and Beekwilder et al. [13].

Table 4

Anthocyanin profile comparison as absolute peak areas (per 2 g of fresh fruit) for 12 genotypes in 2010 at PARC. For anthocyanin peak (1–8) identifications, refer to Table 3. Overall mean and SEM for two technical replicates are shown. *P*-values for 2-way ANOVAs of each anthocyanin peak area for genotype significance are shown

	Anthocyanin peaks															
	1		2		3		4		5		6		7		8	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Genotype 62	2.00E+07	6.00E+05	6.00E+07	8.00E+05	4.00E+06	6.00E+05	ND	ND	7.00E+06	2.00E+05	9.00E+05	1.00E+05	ND	ND	ND	ND
Algonquin	7.00E+07	3.00E+06	2.00E+08	1.00E+06	7.00E+06	5.00E+05	6.00E+06	3.00E+05	2.00E+07	1.00E+06	2.00E+07	7.00E+05	ND	ND	ND	ND
Chilcotin	6.00E+07	5.00E+06	6.00E+07	5.00E+06	1.00E+07	5.00E+05	2.00E+06	73293	7.00E+06	4.00E+05	2.00E+06	27093	ND	ND	ND	ND
Chilliwack	8.00E+07	1.00E+06	2.00E+08	3.00E+06	3.00E+07	2.00E+05	5.00E+06	3.00E+05	4.00E+07	2.00E+05	2.00E+07	4.00E+06	3.00E+06	2.00E+05	4.00E+06	1.00E+06
Cowichan	4.00E+07	8.00E+05	9.00E+07	8.00E+06	9.00E+06	7.00E+05	ND	ND	1.00E+07	2.00E+06	ND	ND	ND	ND	ND	ND
Haida	5.00E+07	2.00E+05	8.00E+07	2.00E+06	8.00E+06	67506	3.00E+06	68719	8.00E+06	3.00E+05	8.00E+06	61114	ND	ND	ND	ND
Meeker	9.00E+07	4.00E+06	1.00E+08	7.00E+06	7.00E+06	4.00E+05	7.00E+06	2.00E+06	6.00E+06	4.00E+05	1.00E+07	3.00E+06	ND	ND	ND	ND
Nootka	1.00E+08	3.00E+06	2.00E+08	8.00E+05	ND	ND	5.00E+06	3.00E+05	ND	ND	1.00E+07	2.00E+06	ND	ND	ND	ND
Sumner	7.00E+07	2.00E+06	8.00E+07	1.00E+06	1.00E+07	5.00E+05	7.00E+06	7.00E+05	5.00E+06	3.00E+05	7.00E+06	47869	ND	ND	ND	ND
Tulameen	5.00E+07	3.00E+06	1.00E+08	9.00E+06	3.00E+06	2.00E+05	1.00E+06	2.00E+05	5.00E+06	7.00E+05	5.00E+06	6.00E+05	ND	ND	ND	ND
Washington	3.00E+07	2.00E+06	9.00E+07	1.00E+06	3.00E+06	3.00E+05	ND	ND	7.00E+06	5.00E+05	3.00E+06	3332	ND	ND	ND	ND
Willamette	1.00E+08	4.00E+06	1.00E+08	9.00E+05	ND	ND	6.00E+06	6.00E+05	ND	ND	8.00E+06	6.00E+05	ND	ND	ND	ND
Genotype	<0.0001		<0.0001		<0.0001		0.0015		<0.0001		<0.0001					

Table 5
Anthocyanin profile comparison (as absolute peak areas) for cv. Cascade Delight among three sites (PARC, HK, AK) for each of three biological replicates (per 2 g of fresh fruit). For anthocyanin peak (1–8) identifications, refer to Table 3. Overall mean and SEM for two technical replicates are shown. *P*-values for 2-way ANOVAs of each anthocyanin peak area for plant, site, and plant × site interaction are presented below

			Anthocyanin peaks							
Plant			1	2	3	4	5	6	7	8
PARC	1	Mean	2.58E+07	5.69E+07	1.05E+07	2.85E+06	1.00E+07	4.51E+06	1.47E+06	1.88E+06
		SEM	1.01E+06	3.39E+06	1.03E+06	3.43E+05	2.78E+06	4.70E+05	4.81E+05	6.11E+05
	2	Mean	3.71E+07	7.68E+07	1.96E+07	5.87E+06	2.20E+07	8.10E+06	3.96E+06	4.74E+06
		SEM	4.95E+05	4.13E+06	5.33E+05	1.05E+06	8.26E+05	5.16E+05	4.29E+05	1.93E+05
	3	Mean	3.00E+07	5.85E+07	1.53E+07	3.63E+06	1.59E+07	4.87E+06	2.66E+06	3.91E+06
		SEM	2.01E+06	5.26E+06	1.52E+06	1.02E+06	1.21E+06	8.06E+05	6.69E+05	9.27E+05
HK	1	Mean	2.46E+07	1.83E+07	1.94E+07	1.14E+07	2.37E+07	1.49E+07	2.43E+07	4.01E+07
		SEM	8.00E+06	3.02E+06	4.85E+06	1.75E+05	1.59E+07	1.19E+06	7.11E+06	2.34E+07
	2	Mean	2.45E+07	5.93E+07	1.23E+07	5.72E+06	1.46E+07	1.08E+07	3.20E+06	4.49E+06
		SEM	3.46E+04	2.54E+06	1.21E+06	2.89E+05	2.05E+06	3.52E+06	3.02E+05	4.39E+05
	3	Mean	3.59E+07	8.28E+07	2.53E+07	7.40E+06	3.05E+07	1.70E+07	7.17E+06	7.89E+06
		SEM	2.77E+05	3.17E+06	8.31E+05	1.43E+04	3.78E+05	1.46E+06	2.64E+05	7.58E+05
AK	1	Mean	3.37E+07	8.31E+07	2.38E+07	7.35E+06	3.44E+07	2.24E+07	7.02E+06	1.19E+07
		SEM	1.95E+05	3.51E+06	7.52E+05	1.62E+04	1.42E+06	1.86E+06	2.02E+04	6.96E+05
	2	Mean	3.48E+07	8.07E+07	3.36E+07	8.52E+06	4.01E+07	2.21E+07	1.08E+07	1.15E+07
		SEM	2.76E+06	3.75E+06	1.48E+06	1.79E+05	3.85E+06	8.07E+05	9.97E+04	1.26E+06
	3	Mean	2.54E+07	3.54E+07	1.02E+07	2.72E+06	6.99E+06	3.88E+06	1.39E+06	2.04E+06
		SEM	6.83E+05	3.43E+06	1.02E+06	3.64E+05	8.28E+05	2.66E+05	3.75E+05	5.10E+05
Plant			0.28	<0.001	0.03	<0.001	0.28	0.003	0.014	0.14
Site			0.43	0.004	0.004	<0.001	0.09	<0.001	0.004	0.14
Plant × Site			0.02	<0.001	<0.001	<0.001	0.014	<0.001	0.003	0.15

2.4. Statistical analysis

Overall means and standard errors of the means (SEMs) presented in the tables were calculated in MS Excel software using means of two replicates each analyzed in triplicate. Analyses of variance (ANOVAs) were done using SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Statistical significance of differences between parameters was evaluated using two-way ANOVA, where significance of interaction between plant and site (plant × site) or genotype and year (genotype × year) was determined.

3. Results and discussion

3.1. Ascorbic acid

Ascorbic acid content was determined for fruit extracts from each of 12 genotypes (Table 1). Ascorbic acid contents ranged from a low of 7 to 14 mg per 100 g of cv. Haida fruit to a high in cv. Tulameen which contained up to 40.6 mg per 100 g. Significant differences were obtained in ascorbic acid content for the 12 genotypes sampled from the same site in each of three successive years (Table 1). We detected a general trend that plants exhibiting high concentrations of ascorbic acid also maintained this relative ranking among genotypes irrespective of sampling year, with the exception of genotype 62 for which a decrease in ascorbic acid content occurred in 2009. The differences in ascorbic acid content among the harvest years within genotypes suggest that climate plays an important role in determining the total ascorbic acid content in raspberries at harvest (as can be seen by higher ascorbic acid amounts

Table 6
Anthocyanin profile comparison (as absolute peak areas) for cv. Malahat among three sites (PARC, HK, AK) for three biological replicates (per 2 g of fresh fruit). For anthocyanin peak (1, 2, 4, and 6) identification, refer to Table 3. Overall mean and SEM for two technical replicates are shown. *P*-values for 2-way ANOVAs of each anthocyanin peak area for plant, site, and plant × site interaction are presented below

			Anthocyanin peaks			
Plant			1	2	4	6
PARC	1	Mean	6.43E + 07	9.20E + 07	4.72E + 06	1.03E + 07
		SEM	1.51E + 05	3.76E + 06	4.95E + 05	1.85E + 06
	2	Mean	8.08E + 07	1.28E + 08	9.90E + 06	1.46E + 07
		SEM	8.18E + 05	5.32E + 06	8.64E + 05	7.68E + 05
	3	Mean	5.99E + 07	9.57E + 07	5.18E + 06	8.09E + 06
		SEM	5.67E + 06	6.39E + 06	1.50E + 06	1.08E + 05
HK	1	Mean	7.09E + 07	1.13E + 08	6.20E + 06	1.22E + 07
		SEM	3.42E + 05	4.27E + 04	1.27E + 05	8.72E + 04
	2	Mean	9.07E + 07	1.34E + 08	9.41E + 06	1.20E + 07
		SEM	2.19E + 06	8.89E + 05	5.60E + 04	6.40E + 05
	3	Mean	5.59E + 07	1.02E + 08	3.84E + 06	9.73E + 06
		SEM	1.94E + 06	6.10E + 06	1.54E + 05	1.56E + 06
AK	1	Mean	5.75E + 07	1.42E + 08	5.86E + 06	2.45E + 07
		SEM	2.37E + 05	3.03E + 06	1.17E + 05	2.93E + 05
	2	Mean	5.02E + 07	1.03E + 08	3.35E + 06	1.15E + 07
		SEM	3.62E + 06	1.10E + 07	1.42E + 05	9.73E + 05
	3	Mean	5.51E + 07	9.83E + 07	6.89E + 06	1.02E + 07
		SEM	1.00E + 06	1.20E + 06	2.16E + 06	3.02E + 05
Plant			<0.001	0.0013	<0.001	<0.001
Site			<0.001	0.062	<0.001	<0.001
Plant × Site			<0.001	<0.001	<0.001	<0.001

in 2009 where mean temperatures were generally higher towards July) but the reasonably consistent relative amounts among genotypes argue against a strong environmental component to heritability of this trait. When we examined site and plant variation in ascorbic acid (Table 2), we detected significant differences ($p < 0.05$) in plant to plant variation for cvs. Cascade Delight and Malahat and significant site differences for genotype 36 and cv. Malahat. There was a significant plant × site interaction, which suggests that growing conditions on each site affect the total ascorbic acid content on plants cultivated in different areas in the Fraser Valley area.

3.2. Anthocyanin identification and variation

It was previously determined that anthocyanins also contribute to the total antioxidant capacity of red raspberries [13]. The major anthocyanin determined for raspberry was cyanidin-derived glucosides, with a relatively minor presence of pelargonidin-derived glucosides (Table 3), which is consistent with thefindings of Mullen et al. [17], Scalzoet al. [18], and Wu et al. [19].

Once the anthocyanin profile was established, it was then used as a reference for further analyses requiring peak identification (Fig. 2A and Table 3). Profiles for Cascade Delight (Fig. 2B), genotype 36 (Fig. 2C), and Malahat (Fig. 2D) were determined and compared among three sites for the 2010 samples, as well as for 12 cultivars from PARC. Amounts of individual anthocyanins for each sample are represented in Tables 4, 5, 6, and 7 as absolute peak areas.

Table 7
Anthocyanin profile comparison (as absolute peak areas) for genotype 36 among three sites (PARC, HK, AK) for three biological replicates (per 2 g of fresh fruit). For anthocyanin peak (1, 2, 4, and 5) identification, refer to Table 3. Overall mean and SEM for two technical replicates are shown. *P*-values for 2-way ANOVAs of each anthocyanin peak area for plant, site, and plant × site interaction are presented below

			Anthocyanin peaks			
	Plant		1	2	4	6
PARC	1	Mean	6.43E + 07	9.20E + 07	4.72E + 06	1.03E + 07
		SEM	1.51E + 05	3.76E + 06	4.95E + 05	1.85E + 06
	2	Mean	8.08E + 07	1.28E + 08	9.90E + 06	1.46E + 07
		SEM	8.18E + 05	5.32E + 06	8.64E + 05	7.68E + 05
	3	Mean	5.99E + 07	9.57E + 07	5.18E + 06	8.09E + 06
		SEM	5.67E + 06	6.39E + 06	1.50E + 06	1.08E + 05
HK	1	Mean	7.09E + 07	1.13E + 08	6.20E + 06	1.22E + 07
		SEM	3.42E + 05	4.27E + 04	1.27E + 05	8.72E + 04
	2	Mean	9.07E + 07	1.34E + 08	9.41E + 06	1.20E + 07
		SEM	2.19E + 06	8.89E + 05	5.60E + 04	6.40E + 05
	3	Mean	5.59E + 07	1.02E + 08	3.84E + 06	9.73E + 06
		SEM	1.94E + 06	6.10E + 06	1.54E + 05	1.56E + 06
AK	1	Mean	5.75E + 07	1.42E + 08	5.86E + 06	2.45E + 07
		SEM	2.37E + 05	3.03E + 06	1.17E + 05	2.93E + 05
	2	Mean	5.02E + 07	1.03E + 08	3.35E + 06	1.15E + 07
		SEM	3.62E + 06	1.10E + 07	1.42E + 05	9.73E + 05
	3	Mean	5.51E + 07	9.83E + 07	6.89E + 06	1.02E + 07
		SEM	1.00E + 06	1.20E + 06	2.16E + 06	3.02E + 05
Plant			0.03	0.019	0.80	0.33
Site			<0.001	0.01	0.60	0.15
Plant × Site			0.12	0.69	0.14	0.12

Anthocyanin profiles for each of the 12 genotypes in PARC in 2010 were also determined (Table 4). Cv. Chilliwack had the most diverse profile for which eight peaks were found and identified, while the rest of the varieties ranged from four to six different anthocyanins. Peak area comparison also revealed that cv. Chilliwack not only had the most complex profile, but also one of the highest amounts of cyanidin-3-*O*-sophoroside (peak 1).

Among the three genotypes tested, cv. Cascade Delight was the one other cultivar that had the most complex profile with eight different anthocyanins (Table 5), whereas only four anthocyanins were resolved for cv. Malahat and genotype 36 (Tables 6 and 7). Figure 2 (B, C, D) shows the different anthocyanin compositions for cvs. Cascade Delight, genotype 36, and Malahat, respectively. Tables 5, 6, and 7 show specifically which anthocyanins significantly differed among plants, sites, and plant × site. With the exception of genotype 36, the site × plant interaction was significant, suggesting that there is an effect of the growing conditions of each site on the amount of a particular anthocyanin present.

4. Conclusion

Raspberries are a good natural source of ascorbic acid and anthocyanins, as well as other antioxidant compounds, including tannins such as ellagic acid. Compelling epidemiological evidence supports the consumption of pigmented soft fruits such as raspberries as being part of a healthy diet. Increasing consumer attention to antioxidant phenotypes in a healthy diet provides an economically sound basis for incorporating selections for these traits to existing raspberry breeding programs. The data from our study support the feasibility of this strategy given the significant differences

among genotypes in the antioxidant phytochemicals, ascorbic acid and several anthocyanins. It is unclear from *in vitro* and animal model studies as to which individual anthocyanins may be most important for antioxidant traits. Future studies, including breeding of raspberry genotypes with varied anthocyanin composition phenotypes and testing of progeny using similar experimental tools as above, will provide an applied means of assessing heritable effects of different anthocyanin compositions on total antioxidant capacity.

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