Oxygen Radical Absorbance Capacity (ORAC) and Phenolic and Anthocyanin Concentrations in Fruit and Leaf Tissues of Highbush Blueberry

Mark K. Ehlenfeldt*,† and Ronald L. Prior‡

Marucci Center for Blueberry and Cranberry Research and Extension, Agricultural Research Service, U.S. Department of Agriculture, Chatsworth, New Jersey 08019, and Arkansas Children's Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Little Rock, Arkansas 72202

Antioxidant capacity, as measured by oxygen radical absorbance capacity (ORAC), and total phenolic and total anthocyanin contents were evaluated in fruit tissues of 87 highbush blueberry (*Vaccinium corymbosum* L.) and species-introgressed highbush blueberry cultivars. ORAC and phenolic levels were evaluated in leaf tissues of the same materials. Average values for ORAC, phenolics, and anthocyanins in fruit were 15.9 ORAC units, 1.79 mg/g (gallic acid equivalents), and 0.95 mg/g (cyanidin-3-glucoside equivalents), respectively. Cv. Rubel had the highest ORAC per gram of fresh weight values, at 31.1 units, and cv. Elliott had the highest values on the basis of ORAC per square centimeter of surface area. In leaf tissue, values for both ORAC and phenolics were significantly higher than in fruit tissue, with mean values of 490 ORAC units and 44.80 mg/g (gallic acid equivalents), respectively. Leaf ORAC had a low, but significant, correlation with fruit phenolics and anthocyanins, but not with fruit ORAC. An analysis of ORAC values versus calculated midparent values in 11 plants from the 87-cultivar group in which all parents were tested suggested that, across cultivars, ORAC inheritance is additive. An investigation of ORAC values in a family of 44 cv. Rubel × Duke seedlings showed negative epistasis for ORAC values, suggesting Rubel may have gene combinations contributing to ORAC that are broken up during hybridization.

Keywords: *Antioxidants; inheritance*

INTRODUCTION

Fruits and vegetables contain many different phytonutrients, many of which have antioxidant properties. Research has shown that fruits and vegetables contain other antioxidant nutrients, in addition to the well-known vitamins C and E, and carotenoids, which significantly contribute to their total antioxidant capacity (*1*, *2*). For example, flavonoids (including compounds such as flavones, isoflavones, flavonones, anthocyanins, and catechins) that are components of fruits and vegetables have strong antioxidant capacity (*3*, *4*). There is convincing evidence showing that fruits and vegetables are beneficial to health and contribute to the prevention of degenerative processes (*5*−*7*). Thus, it is important to characterize the beneficial phytonutrients present in these foods and the mechanisms responsible for these effects. The protection provided against diseases by fruits and vegetables has been attributed to the various antioxidants contained in these foods (*7*−*9*). At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radicals may lie at the heart of the etiology or natural history of a number of diseases, including cancer, heart, vascular, and neurodegenerative diseases (*10*, *11*). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these disease states.

In recent years there have been increasing numbers of studies that have quantified the total antioxidant capacity in foods. Studies by the USDA-ARS at Tufts University were among the first to measure the total antioxidant capacity of fruits and vegetables (*1*, *2*). The automated oxygen radical absorbance capacity (ORAC) procedure used in these studies lends itself well to identifying foods with high antioxidant capacity and to evaluating in vivo responses to dietary antioxidant manipulation (*1*, *2*, *12*, *13*). Results from these studies using a peroxyl radical generator, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), indicated that the antioxidant capacities of common fruits, vegetables, and teas had a considerable range. The edible portions of kale, strawberry, and spinach had relatively high antioxidant capacities of 17.7, 15.4, and 12.6 μmol of Trolox equivalents (TE)/g of fresh weight (fw), respectively (*1*, *2*). Interestingly, additional analyses indicated that the major source of antioxidant capacity of most of these fruits is not vitamin C (*2*).

Blueberries have become of special interest to those studying antioxidants because of their high antioxidant capacity (*14*) and their wide range of anthocyanin values (*15*). Our previous studies (*14*) had demonstrated ORAC values in highbush cultivars and other *Vaccinium* species ranging from 13.9 to 45.9 μmol of TE/g of fw. Means for the five highbush and five southern highbush cultivars were 24.0 and 28.5 μmol of TE/g of fw, respectively, with values ranging from 17 to 42 μmol of TE/g of fw. The wide range of values suggested that

† Research Geneticist, Blueberry and Cranberry Research Center.

‡ Research Chemist, Arkansas Children's Nutrition Research Center.

10.1021/jf0013656
Published on Web 04/27/2001

antioxidant levels in blueberries could be improved through further breeding. Prior et al. (*14*) also reported significant correlations of 0.72 and 0.92 between ORAC values and anthocyanins and between ORAC values and phenolics, respectively, suggesting that phenolics might present an especially useful means for selecting for higher ORAC values within a breeding program.

The purpose of this study was to compare total phenolics and anthocyanins concentrations and antioxidant capacity (ORAC) in berry samples from a large collection of highbush blueberry (*Vaccinium corymbosum* L.) and species-introgressed highbush blueberry cultivars. We also wanted to compare total phenolics and antioxidant capacity in leaf tissue from the same materials and to evaluate the utility of leaf tissue measurements as a means of hastening the selection of potential cultivars high in antioxidant activity. We also sought to make a preliminary evaluation of the inheritance of antioxidant capacity.

MATERIALS AND METHODS

Fruit and Leaf Sampling. Fruits from 87 cultivars were sampled from a field-grown collection that had been planted in 1995 at the Philip E. Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers University, Chatsworth, NJ. The bushes were grown in USDA Plant Hardiness Zone 6, on soils that are mostly Atsion sand containing 3−15% organic matter. Cultural practices in the plots included clean cultivation and the use of solid set irrigation for irrigation and frost protection. From mid-June through mid-July in 1998, daily high temperatures averaged 33 °C and cumulative rainfall totaled 4.1 cm; from mid-July through mid-August daily high temperatures averaged 35 °C and cumulative rainfall totaled 2.1 cm. Irrigation was applied, as needed, at a rate of ∼2.5 cm of water per week. Because of early flowering and subsequent cold damage, relatively light crops were present on some of the cultivars adapted to North Carolina and areas further south. Northern-adapted highbush cultivars were represented by five plants (these include Legacy, Sierra, and Ozarkblue, which have some *V. darrowi* ancestry). All other cultivars including North Carolina-adapted highbush, southern highbush (*V. darrowi* introgressed), half-highs, processing types, rabbiteyes, and rabbiteye hybrids were represented by two-plant plots (except as noted in Table 1). In 1998, fruits were collected across the available bushes for a total of 100 g of fruit from each cultivar. Fruit was collected at optimum ripeness, 15−25% ripe. Berry weights were determined on separately collected samples on groups of ∼50 berries. Fruits of a Rubel × Duke family were collected from single 4-year-old plants grown in a plot adjacent to the cultivars. No measurements of fruit weight were taken for these plants. Leaf samples were collected from the same group of plants in late July. Twenty-five leaves were collected per cultivar. Special attention was given to collect leaves that were fully expanded and cuticularized and of approximately equivalent physiological stage and condition. Fruit and leaves were frozen at −70 °C and shipped on dry ice to the USDA Human Nutrition Research Center on Aging (HNRCA) in Boston. Samples received at the USDA-HNRCA were stored at −70 °C (for ∼2 months) until analyzed. Extractions and analyses were performed on six duplicate samples per cultivar at a time.

Chemical Analyses. *Chemicals.* R-phycoerythrin (R-PE), ascorbic acid, gallic acid, and acetonitrile (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). AAPH was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Methanol (HPLC grade) was from Fisher Scientific (Boston, MA). HPLC grade water was obtained from J. T. Baker Inc. (Phillipsburg, NJ).

Sample Preparation for Determination of ORAC, Total Anthocyanins, and Total Phenolics. Blueberries were extracted essentially as described by Prior et al. (*14*) using acetonitrile/acetic acid for the analysis of ORAC, total anthocyanins, and tota1 phenolics. A sample of ∼10 g of each blueberry source was added to an equal volume (10 mL) of acetonitrile containing 4% acetic acid and homogenized in a blender for 2 min. After recovery of the homogenate, 5 mL of acetonitrile/acetic acid was used to wash the blender and pooled with the first homogenate. The pooled homogenate was left at room temperature with shaking every 3 min for at least 30 min and then centrifuged at 5000*g* for 15 min at 4 °C. Samples were diluted with buffer for ORAC analyses or with distilled deionized water (DDW) for the phenolic assay. The pellet following centrifugation was washed with 50 mL of acetonitrile containing 4% acetic acid and centrifuged, and the resulting supernatants were combined with the initial extract. Triplicate extractions were prepared from each blueberry source. Blueberry leaves were ground using a mortar and pestle with liquid nitrogen to keep the sample frozen. After grinding, 0.5 g of sample was added to 10 mL of 49.5% acetone/50% H_2O containing 0.5% glacial acetic acid. The samples were extracted with shaking for 1 h at room temperature and then centrifuged for 15 min at 5000*g*. Samples were diluted with buffer for ORAC analyses or with DDW for the phenolic assay.

Automated $ORAC_{ROO\cdot}$ Assay. The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ; emission filter = 565 nm). The procedure was based on that given in a previous paper by Cao and co-workers (*12*), as modified for the COBAS FARA II (*13*). Briefly, in the final assay mixture (0.4 mL total volume), R-PE (16.7 nM) was used as a target of free radical attack with AAPH (4 mM) as a peroxyl radical generator. Trolox (1.0 μM/L), a water-soluble analogue of vitamin E, was used as a control standard. The analyzer was programmed to record the fluorescence of R-PE every 2 mm after the addition of AAPH. All fluorescence measurements are expressed relative to the initial reading. Final results were calculated using the differences of areas under the R-PE decay curves between the blank and a sample and expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (fw).

Total Anthocyanin Assay. The total anthocyanin content was estimated by using a pH differential method (*16*). Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$, with a molar extinction coefficient of cyanidin-3-glucoside (c3g) of 29600. Results were expressed as milligrams of equivalent c3g per gram of fresh weight.

Total Phenolics Assay. Total soluble phenolics in the acetonitrile extracts were determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (*17*) using gallic acid as a standard. Results were expressed as gallic acid equivalents (GAE) per gram of fresh weight.

Calculations of Berry Weight and ORAC per Square Centimeter of Surface Area and Statistical Analyses. The average berry weight was determined by weighing ∼50 g of berries and counting the number of berries. For each cultivar, total ORAC per berry was calculated by multiplying ORAC TE/g of fw × g/berry. An estimated density value of 1.1 g/mL and average berry weight were used to calculate berry volume. Subsequent calculations to derive ORAC/cm^2 were based on the assumption that a berry was a perfect sphere. Sphere volume was used to derive a radius value based on the relationship $V_{sphere} = {}^4/_3\pi r^3$. The radius value was used to calculate a theoretical surface area based on the relationship $A_{sphere} = 4\pi r^2$. Total ORAC/berry and surface area/berry were used to derive an estimate of ORAC/cm^2 for each cultivar. Correlations and *t* tests were performed using MSTAT-C (Michigan State University).

RESULTS

Cultivar Assay. *Fruit.* A wide range of fruit ORAC values were observed among blueberries, ranging from 4.6 TE/g of fw (cv. Avonblue) to 31.1 TE/g of fw

Table 1. ORAC and Phenolic and Anthocyanin Concentrations in Fruit and Leaf Tissues of 87 Highbush (*V. corymbosum* L.) and Species-Introgressed Highbush Blueberry Cultivars

		fruit					leaf	
cultivar	type[a]	berry wt (g)	ORAC (μmol of TE/g of fw)	ORAC (μmol of TE/cm^2)	phenolics (mg of GAE/g of fw)	anthocyanins (mg of c3g/g of fw)	ORAC (μmol of TE/g of fw)	phenolics (mg of GAE/g of fw)
Ama[b]	HB	1.1	10.6	2.4	0.59	1.25		
Angola	HB	1.0	6.6	1.5	0.53	1.18	509.7	41.45
Atlantic	HB	2.7	9.6	2.9	0.90	1.50	308.4	25.53
Avonblue	SHB	1.5	4.6	1.2	0.48	1.36	454.6	42.87
Berkeley	HB	2.5	5.5	1.6	0.84	1.54	603.8	55.40
Bladen	HB	1.5	9.5	2.4	0.43	1.73	479.2	46.29
Bluechip	HB	2.2	10.9	3.1	0.65	1.29		
Bluecrop	HB	1.9	10.4	2.9	0.48	1.82		
Bluegold	HB	2.3	14.9	5.4	0.58	2.11	410.2	47.04
Bluehaven	HB	1.8	6.6	1.7	0.55	1.51	418.4	45.17
Bluejay	HB	1.7	7.2	1.9	0.72	1.37	418.3	51.34
Blueray	HB	2.0	11.1	3.1	1.01	1.60	474.2	45.08
Bluetta	HB	1.6	21.5	5.5	1.18	2.01	536.8	51.44
Bonus[b]	HB	2.1	18.6	5.2	0.95	1.66	411.7	41.24
Bounty	HB		19.5		1.29	1.97	549.7	32.42
Brigitta Blue[b]	HB	1.4	17.7	4.3	0.93	1.65	511.7	48.09
Burlington	HB	1.8	26.0	7.0	1.75	2.53	547.5	58.33
Cabot	HB		25.2		1.35	1.89	576.4	58.32
Cape Fear	SHB		16.7		1.10	2.55	547.6	49.49
Cara's Choice	HB		17.2		0.98	1.81		
Chandler	HB	2.5	17.8	5.3	0.76	1.98	543.1	46.13
Chanticleer	HB	1.3	17.7	4.2	0.83	2.09	513.8	53.85
Collins	HB	1.9	15.1	4.1	0.63	1.72	431.5	49.11
Concord	HB	1.1	18.0	4.1	1.34	2.22	684.9	66.29
Cooper	SHB	1.3	19.4	4.6	0.99	2.31	501.0	41.94
Coville	HB	2.4	15.4	4.5	0.93	2.07	378.5	38.17
Croatan	HB		13.5		0.86	1.91	433.6	38.40
Darrow	HB	2.9	14.8	4.6	1.07	2.07	714.2	60.34
Dixi	HB	2.7	11.3	3.5	0.90	1.64	351.4	31.65
Duke	HB	1.8	16.1	4.3	1.03	2.16	335.0	29.81
Duplin	SHB		12.8		0.74	1.86	425.5	40.43
Earliblue	HB	1.4	19.7	4.9	0.75	1.76	472.4	47.84
Elizabeth	HB	3.0	10.5	3.3	0.68	1.11	322.4	35.03
Elliott	HB	1.8	30.5	8.2	1.84	3.11	651.1	58.79
Friendship	HH	0.6	26.7	5.0	1.99	2.65		
Georgiagem	SHB	1.9	12.6	3.4	0.56	1.56	487.6	52.89
Gulfcoast	SHB	2.5	14.0	4.2	0.90	1.92	356.3	31.91
Harding[b]	HB	0.7	19.2	3.8	1.47	2.39	490.3	44.51
Hardyblue[b]	HB	1.3	17.4	4.1	0.68	1.74	485.1	47.78
Harrison	HB	1.9	18.1	4.9	1.26	2.11	489.5	40.38
Heerma[b]	HB	1.3	13.9	3.4	0.93	1.71		
Herbert	HB	2.6	19.7	6.0	0.95	2.12	480.0	42.55
Ivanhoe	HB	2.7	16.1	4.9	0.95	2.01	509.6	45.87
Jersey	HB	2.3	19.3	5.6	1.06	2.05	517.9	46.57
Jubilee	SHB	1.1	15.5	3.5	0.60	1.66	504.3	53.59
June	HB	1.2	12.0	2.8	0.63	1.56	568.3	45.58
Lateblue	HB	2.1	15.8	4.5	1.01	1.76	291.3	25.37
Legacy	HB	3.3	13.5	4.4	0.65	1.17	308.7	32.63
Little Giant[c]	PROC	0.6	20.8	3.8	1.21	2.38	971.3	77.43
Magnolia	SHB	1.9	9.4	2.5	0.55	1.16	691.6	61.97
Marimba	SHB	1.7	11.4	3.0	1.04	1.70	468.7	47.11
Meader	HB	2.0	11.8	3.3	1.01	1.72	622.3	57.85
Misty	SHB	2.1	13.9	3.9	0.81	1.58	678.3	49.02
Morrow	HB	1.1	20.2	4.6	1.21	2.22	469.2	45.72
Murphy	HB		11.7		1.33	1.87	412.2	38.93
Nelson	HB	3.3	17.4	5.7	0.70	1.47	245.0	25.58
Northblue	HH	1.4	18.4	4.6	1.10	1.92		
Northland	HB	1.7	17.2	4.5	1.37	1.29	577.5	56.65
Nui[b]	HB	2.1	13.7	3.8	1.63	1.22	447.6	46.69
O'Neal	HB	1.5	14.1	3.6	1.05	1.39	399.4	36.14
Olympia[b]	HB	1.5	14.6	3.7	1.62	1.54	564.2	54.05
Ornablue[d]	ORN	1.7	30.5	8.0	1.49	3.31	478.1	41.74
Ozarkblue	SHB	3.4	17.0	5.6	1.60	1.58	574.8	51.03
Patriot	HB	2.1	14.4	4.1	1.51	1.77	636.3	56.86
Pearl River	SHB/RE	1.5	9.3	2.3	0.25	0.89	679.6	74.24
Pemberton	HB	1.8	20.6	5.5	0.97	2.00	632.7	47.44
Pender	SHB	1.7	16.0	4.2	0.96	1.62	548.7	45.18
Pioneer	HB	1.1	15.6	3.5	0.59	1.29	591.2	46.38
Polaris	HH	1.1	19.0	4.3	1.37	2.19	437.7	37.15
Puru[b]	HB	1.9	22.1	6.0	0.20	1.57	373.4	30.88
Rancocas	HB	1.2	25.0	5.8	0.66	1.93	527.7	51.90
Reka[b]	HB	1.1	15.5	3.6	0.78	1.49	313.9	23.58
Reveille	SHB	1.4	15.4	3.8	0.47	1.44	463.3	34.86

Table 1 (Continued)

		fruit					leaf	
cultivar	type[a]	berry wt (g)	ORAC (μmol of TE/g of fw)	ORAC (μmol of TE/cm²)	phenolics (mg of GAE/g of fw)	anthocyanins (mg of c3g/g of fw)	ORAC (μmol of TE/g of fw)	phenolics (mg of GAE/g of fw)
Rubel	HB	0.8	31.1	6.2	1.65	3.25	624.1	50.88
Sampson	SHB	3.4	14.2	4.7	0.75	1.53		
Sharpblue	SHB	1.8	22.3	6.0	0.61	1.95		
Sierra	SHB	1.8	18.8	5.1	0.87	1.86	366.5	34.33
Snowflake	RE	1.0	13.6	3.0	0.48	1.13		
Spartan	HB	2.3	12.1	3.5	0.84	1.26	394.6	35.29
Stanley	HB	2.6	12.0	3.6	0.86	1.57	379.2	31.51
Star	SHB	2.0	8.5	2.4	0.50	1.17	511.3	42.86
Sunrise	HB	1.7	15.2	4.0	1.07	1.73	573.0	44.35
Sunshine Blue[d]	ORN		11.7		0.95	1.77	498.5	46.72
Toro	HB	3.6	19.8	6.7	1.09	2.28	378.6	31.76
Wareham	HB	1.2	16.2	3.8	1.11	1.72	276.9	26.23
Weymouth	HB	1.3	15.5	3.7	0.98	1.85	561.7	46.95
Wolcott	HB	1.3	18.0	4.4	0.91	1.47	389.7	33.78
av		1.9	15.9	4.2	0.95	1.79	490.4	44.80

[a] HB, highbush; SHB, southern highbush; HH, half-high; RE, rabbiteye; ORN, ornamental; PROC, processing. [b] Samples taken from two plants. [c] Samples taken from five plants. Cv. Little Giant is a *V. constablaei* × *V. ashei* hybrid. [d] Samples taken from one plant. Cv. Ornablue is a *V. corymbosum* × *V. pallidum* hybrid. Cv. Sunshine Blue is a *V. corymbosum* × *V. darrowi* hybrid.

Table 2. Correlation Coefficients between Fruit ORAC, Fruit Total Phenolic, Fruit Total Anthocyanin, Leaf ORAC, and Leaf Total Phenolic Concentrations in 87 Highbush Blueberry (*V. corymbosum* L.) and Species-Introgressed Blueberry (*V.* × *corymbosum* L.) Cultivars[a]

	fruit ORAC[b] (TE/g of fw)	fruit ORAC (TE/cm²)	fruit phenolics (GAE/g of fw)	fruit anthocyanin (c3g/g of fw)	leaf ORAC[c] (TE/g of fw)	leaf phenolics (GAE/g of fw)
fruit TE/g of fw		0.90***	0.76***	0.57***	0.18ns	0.10ns
fruit TE/cm²			0.70***	0.49***	0.00ns	−0.05ns
fruit phenolics				0.61***	0.23*	0.14ns
fruit anthocyanin					0.25*	0.18ns
leaf TE/g of fw						0.87***
leaf phenolics						

[a] *, **, ***, and ns designate significance at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and nonsignificance, respectively. [b] Correlations among fruit values are based on sample sizes of 87 except for correlations involving ORAC/cm², which are based on sample sizes of 79. [c] Correlations among leaf values are based on sample sizes of 77 except for correlations involving ORAC/cm², which are based on sample sizes of 70.

(cv. Rubel). This represents a >6-fold difference between highest and lowest values. The cultivars Rubel, Elliott, Ornablue, Friendship, and Burlington were highest in ORAC values. Across the 87 cultivars the mean value was 15.9 TE/g of fw (Table 1). For ORAC/cm², the mean value was 4.2 TE/cm². In contrast to the ORAC/g of fw values, cv. Elliott had the highest values (8.2 TE/cm²). The correlation of fruit ORAC/g of fw and fruit ORAC/cm² was $r = 0.90$ (79 samples) (Table 2). Most cultivars maintained similar rankings between TE/g of fw and TE/cm²; however, the rankings of cv. Rubel and Friendship changed from first and fourth to fifth and nineteenth, respectively. Both fruit phenolics and total anthocyanins exhibited a similarly wide range of values with a ~4-fold difference between the low and high values for both and respective means of 0.95 GAE/g of fw and 1.79 c3g/g of fw.

Highly significant correlations were observed among all fruit characteristics evaluated (Table 2). The correlations between fruit ORAC/g of fw and fruit phenolics ($r = 0.76$, 87 samples) and between fruit ORAC/g of fw and fruit anthocyanins ($r = 0.57$, 87 samples) were generally higher than the corresponding correlations between fruit ORAC/cm² and fruit phenolics ($r = 0.70$, 79 samples) and between fruit ORAC/cm² and fruit anthocyanins ($r = 0.49$, 79 samples). The correlation between fruit phenolics and fruit anthocyanins was $r = 0.61$ (87 samples). All of these are somewhat lower than the corresponding values observed by Prior et al. (*14*).

Leaves. Like the fruit assay, blueberry leaf assays exhibited a large range of ORAC and phenolic values. However, there were large differences between ORAC values in fruit and ORAC values in leaf tissue. ORAC values in leaf tissue ranged from 245 TE/g of fw (cv. Nelson) to 971 TE/g of fw (cv. Little Giant), with a mean value across 77 samples of 490 TE/g of fw. On a fresh weight basis this represented a ~25-fold difference. If fruit dry weight is considered, this still represents a ~5-fold difference based on the calculations of Prior et al. (*14*).

Leaf phenolic values ranged from 23.6 GAE/g of fw (cv. Reka) to 77.4 GAE/g of fw (cv. Little Giant), with a mean of 44.80 GAE/g of fw. Like the leaf ORAC values, leaf phenolics were ~30 times that observed in fruit on a fresh weight basis. Cv. Little Giant was considerably higher in leaf ORAC than most of the other cultivars surveyed and ranked 36% higher than the next highest cultivar, Darrow. Cv. Little Giant has *V. constablaei* Gray (50%) in its ancestry. Cv. Little Giant ranked tenth of 87 cultivars for fruit ORAC/g of fw. Among other cultivars at the high end of the distribution were Concord, Magnolia, Misty, and Pearl River. All of these except Concord have rabbiteye (*V. ashei* Reade) ancestry.

A highly significant correlation existed between leaf ORAC and leaf phenolic values ($r = 0.87$, 77 samples). Leaf ORAC had a low but significant correlation with fruit phenolics and anthocyanins, but not with fruit ORAC (Table 2).

Table 3 lists ORAC/g of fw values of 11 cultivars for which ORAC/g of fw values of both of the parents are available. Across these cultivars, the mean ORAC value (16.5 TE/g of fw) is not significantly different from the

Table 3. Highbush Blueberry Cultivars for Which Progeny and Parent ORAC Values Are Available

				ORAC (TE/g of fw)		ORAC (TE/cm^2)	
cultivar	pedigree			cultivar	midparent	cultivar	midparent
Atlantic	Jersey	×	Pioneer	9.6	12.2	5.6	3.5
Burlington	Rubel	×	Pioneer	26.0	23.3	6.2	3.5
Collins	Stanley	×	Weymouth	15.1	13.8	3.6	3.7
Earliblue	Stanley	×	Weymouth	19.8	13.8	3.6	3.7
Hardyblue	Pioneer	×	Rubel	17.4	23.3	3.5	6.2
Lateblue	Herbert	×	Coville	15.8	17.6	6.0	4.5
Meader	Earliblue	×	Bluecrop	11.8	15.1	4.9	2.9
Olympia	Pioneer	×	Harding	14.6	17.4	3.5	3.8
Toro	Earliblue	×	Ivanhoe	19.8	17.9	4.9	4.9
Wareham	Rubel	×	Harding	16.2	25.2	6.2	3.8
Weymouth	June	×	Cabot	15.5	18.6	–[a]	–[a]
av				16.5	18.0	4.5	4.4
t test				$t = 1.18$	$P = 0.26$	$t = 0.20$	$P = 0.84$

[a] No berry weights were available for cv. Cabot; therefore, ORAC/cm^2 and midparent value could not be determined.

calculated midparent value (18.0 TE/g of fw). If the same calculation is made for the cultivars on an ORAC/cm^2 basis (10 cultivars), values are again found to be not significantly different.

Family Data. For the Rubel × Duke family evaluated, highly significant ($P \leq 0.001$) correlations existed between fruit ORAC and fruit phenolics, between fruit ORAC and fruit anthocyanins, and between fruit phenolics and fruit anthocyanins, with *r* values of 0.78, 0.60, and 0.73, respectively (47 samples) (data not shown). Fruit ORAC values ranged from 1.3 TE/g of fw (clone 131) to 20.6 TE/g of fw (clone 137), with a mean value of 12.6 TE/g of fw (data not shown). These values are all lower than those found for Rubel (31.1 TE/g of fw), and 87% (41/47) were lower than those found for Duke (16.1 TE/g of fw). Fruit anthocyanins ranged from 0.39 c3g/g of fw (clone 131) to 1.65 c3g/g of fw (clone 139), with a mean of 0.96 c3g/g of fw (data not shown). All but one anthocyanin value was lower than that of Rubel (1.65 c3g/g of fw), and 60% (28/47) were lower than that of Duke (1.03 c3g/g of fw). Fruit phenolics ranged from 0.76 GAE/g of fw (clone 131) to 2.61 GAE/g of fw (clone 137), with a mean of 1.65 GAE/g of fw (data not shown). All phenolics were lower than the level found in Rubel (3.25 GAE/g of fw), and 89% (42/47) were lower than the level found in Duke (2.16 GAE/g of fw).

DISCUSSION

Cultivar Assay. *Fruit.* This study has expanded the list of cultivars assayed for ORAC and phenolic and anthocyanin contents and reports ORAC on both a fresh weight basis and a surface area basis. For commercial growers, ORAC/g of fw is of greater interest if they wish to market blueberries for their health benefits. Preliminary evaluations of ORAC activity in skins and seeds of blueberry suggest, however, that the majority of the antioxidants are concentrated in the skin (M. Mainland, personal communication), unlike fruits such as grapes, for which significantly higher antioxidant levels are found in the seeds due to tannins. Therefore, for breeding purposes, the standardized ORAC/cm^2 value is more meaningful because breeders would be interested in the highest concentration per unit area of skin. For fruit ORAC, cv. Rubel, Elliott, Ornablue, Friendship, Burlington, Cabot, and Rancocas have some of the highest levels. There is considerable common ancestry in these high ORAC clones. Cv. Burlington and Rancocas both have Rubel as a parent; cv. Elliott has Burlington as a parent (and hence Rubel as a grandparent). Cv. Cabot has Brooks (which was not tested) and Chatsworth (also not tested) as parents; interestingly, cv Ornablue has Brooks and Rubel as grandparents and the species *V. pallidum* Ait. as a parent. It is possible that cv. Brooks, which is no longer extant, might have had genes for high ORAC content. *V. pallidum* may also represent a source for genes for high ORAC content. Cv. Friendship is reportedly a highbush/lowbush introgressant (*18*). Lowbush clones have repeatedly been reported to be high in ORAC content (*19*, *20*).

Leaves. The wide range of leaves sampled show leaves possess high levels of antioxidants. In leaf ORAC, cv. Little Giant was shown to be considerably higher in antioxidants than most of the other cultivars. Little Giant has 50% *V. constablaei* and 50% *V. ashei* in its ancestry. This fact suggests that rabbiteye may be a source of foliar antioxidants. Our results suggest that *V. ashei* may be a source of elevated leaf ORAC values because levels were high in several clones with rabbiteye ancestry. *V. constablaei* may also be a significant contributor to leaf ORAC values. Luby and co-workers have suggested that *V. constablaei* might be a source of high fruit ORAC values (personal communication); however. its leaf values are unknown.

It was originally hoped that assaying seedling foliage early in the breeding process would hasten the selection of plants with high levels of fruit antioxidants. The low or nonsignificant correlations of leaf ORAC with fruit characteristics indicate that foliar selection would be difficult and progress, if any, would be slow. Assays in cranberry, similar to those done here, have shown that levels of antioxidants in foliar tissue can vary considerably with physiological age and harvest date (A. Howell, personal communication). Selection on foliar tissue awaits the development of a suitable molecular marker.

Inheritance. Evidence on inheritance is preliminary and limited. Analysis of the 11 highbush cultivar pedigrees demonstrates that, across cultivars, inheritance of ORAC levels is probably additive. In a tetraploid crop such as blueberry this is expected and is of some value because it means that parental selection can be utilized in a methodical way. This conclusion is tempered by the data from the Rubel × Duke family that suggest that ORAC and phenolics and anthocyanins contents (at least in this family) are controlled by epistatic gene action and surprisingly have large negative epistatic components. Nothing is known about the inheritance of ORAC using specific parents, but a good hypothesis for this case is that cv. Rubel, which has the

highest ORAC/g of fw values, has epistatic interactions which contribute to its high ORAC activity and that these interactions are broken up when it is used as a parent. Fruit sizes were not available in this family to correct for fruit size effects, but it seems unlikely from other results that size correction would have altered the results substantially. Further studies are underway with a broader range of families to clarify inheritance.

This study greatly expands the number of blueberry cultivars assayed for antioxidants in both fruit and leaf tissues and assays them under a common set of conditions. These data reveal general trends across cultivars, but specific values should be treated with caution. The data represent values from a single year and location and might be expected to vary with year and growing conditions. Nonetheless, this study demonstrates the wide range of antioxidants found in blueberry fruit and leaf tissues and should be of value to those studying antioxidants and to agricultural researchers seeking to enhance antioxidant levels.

LITERATURE CITED

(1) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem*. **1996**, *44*, 3426−3431.

(2) Wang, H.; Cao, G.; Prior, R. L. Tota1 antioxidant capacity of fruits. *J. Agric. Food Chem*. **1996**, *44*, 701−705.

(3) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med*. **1997**, *22*, 749−760.

(4) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem*. **1997**, *45*, 304−309.

(5) Joseph, J. A.; Shukitt-Hale, B.; Denisova, N. A.; Bielinski, D.; Martin, A.; McEwen, J. J.; Bickford, P. C. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci*. **1999**, *19*, 8114−8121.

(6) Bickford, P. C.; Chadman, K.; Taglialatea, G.; Shukitt-Hale B.; Prior, R. L.; Cao, G.; Joseph, J. A. Dietary strawberry supplementation protects against the age-accelerated CNS effects of oxidative stress. *FASEB J*. **1997**, *11*, A176.

(7) Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 7915−7922.

(8) Gey, K. F.; Puska, P.; Jordap, P.; Moser, U. K. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr*. **1991**, *53*, 326S−334S.

(9) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol: Modification of low-density lipoprotein that increases its atherogenicity. *New Engl. J. Med*. **1989**, *320*, 915−924.

(10) HalliwelI, B. Free radicals and antioxidants: A personal view. *Nutr. Rev*. **1994**, *52*, 253−265.

(11) Yu, B. P. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev*. **1994**, *76*, 139−162.

(12) Cao, G.; Alessio, H. M.; Culter, R G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radicals Biol. Med*. **1993**, *14*, 303−311.

(13) Cao, G.; Verdon, C. P.; Wu, A. H. B.; Wang, H.; Prior, R. L. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. *Clin. Chem*. **1995**, *41*, 1738−1744.

(14) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, M. Antioxidant capacity is influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem*. **1998**, *46*, 2686−2693.

(15) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, 1993; p 362.

(16) Cheng, G. W.; Breen, P. J. Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hortic. Sci*. **1991**, *116*, 865−869.

(17) Slinkard, K.; Singleton, V. L. Total phenol analysis: Automation and comparison with manual methods. *Am. J. Enol. Vitic*. **1997**, *28*, 49−55.

(18) Stang, E. J.; Dana, M. N.; Weis, G. G.; McCown, B. H. 'Friendship' blueberry. *HortScience* **1990**, *25*, 1667−1668.

(19) Kalt, W.; McDonald, J. E. Chemical composition of lowbush blueberry cultivars. *J. Am. Soc. Hortic. Sci*. **1996**, *121*, 142−146.

(20) Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem*. **1999**, *47*, 4638−4644.

Received for review November 15, 2000. Revised manuscript received March 14, 2001. Accepted March 19, 2001.

JF0013656