Interspecific Variation in Anthocyanins, Phenolics, and Antioxidant Capacity among Genotypes of Highbush and Lowbush Blueberries (*Vaccinium* Section *cyanococcus* spp.)

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Recent interest in the possible protective effects of dietary antioxidant compounds against human degenerative disease has prompted investigation of foods such as blueberries (*Vaccinium* sp.), which have a high antioxidant capacity. Fruit obtained from genotypes of highbush blueberries (*Vaccinium corymbosum* L.) and lowbush blueberries (*Vaccinium angustifolium* Aiton) were analyzed for their antioxidant capacity, their content of anthocyanins, and total phenolic compounds, to evaluate the intraspecific and interspecific variation in these parameters. The method of extraction influenced the composition of fruit extracts; the highest anthocyanin and total phenolic contents and antioxidant capacity were found in extracts obtained using a solvent of acidified aqueous methanol. Regardless of the method, lowbush blueberries were consistently higher in anthocyanins, total phenolics, and antioxidant capacity, compared with highbush blueberries. There was no relationship between fruit size and anthocyanin content in either species.

Keywords: *V. angustifolium; V. corymbosum; V. darrowi; southern highbush; ORAC*

INTRODUCTION

Lowbush "wild" blueberries (*Vaccinium angustifolium* Aiton) and highbush "cultivated" blueberries (*Vaccinium corymbosum* L.) are both native to North America and have been commercially produced for many years. Lowbush blueberry production is localized in eastern Canada and the northeastern United States, whereas highbush blueberries are produced in temperate regions throughout North America, particularly the Pacific northwest, the Great Lakes region, and the Atlantic states of the United States. Southern highbush blueberry varieties have been developed from *V. corymbosum* L. introgressed with *Vaccinium darrowi* Camp. Compared to *V. corymbosum*, southern highbush varieties require less chilling to stimulate reproductive development and are therefore commonly grown in the southern United States. Southern highbush and highbush blueberries are referred to collectively as highbush, or cultivated, blueberries.

Although they share the common "blueberry" name, the production systems for highbush and lowbush blueberries are distinctly different. Lowbush blueberries grow wild, so that commercial blueberry fields are composed of many genetically and phenotypically different clones (*1*). Phenotypic variation in fruit and leaf color as well as plant height is readily apparent among wild clones in a lowbush blueberry field. In contrast, commercial highbush blueberries are grown on plantations, using varieties that have been bred for their production and food characteristics. In North America, production levels of lowbush and highbush fruit are similar, with the total North American annual production of blueberries (highbush and lowbush) between 170 and 193 million kg (John Sauvé, personal communication). The market for fresh blueberry fruit is met almost entirely by highbush blueberries, whereas lowbush blueberries are first frozen and then used mainly as an an ingredient in processed foods. Some highbush blueberries are also frozen and processed.

In a survey of 22 different fruits and vegetables, blueberries had the highest antioxidant capacity when measured with the oxygen radical absorbing capacity (ORAC) assay (*2*−*4*). Dietary antioxidant compounds may aid in mitigating oxygen free radical damage in the body, and their consumption is purported to promote good health and decrease the risk of degenerative diseases such as cardiovascular disease and various cancers (*5*, *6*). Phenolic compounds, which include anthocyanins, possess antioxidant properties (*3*) and are highly concentrated in blueberries. Significant positive relationships were reported between ORAC antioxidant capacity and the content of anthocyanins and total phenolics in ripe blueberries (*4*). Because blueberries

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Table 1. Summary of Blueberry Surveys Conducted Using Extraction Method A, Method B, or Methods A, B, and C (Methods Are Described in Table 3)

survey	*n*	genotype	extraction method
1	80	highbush blueberry varieties, including 17 southern highbush	A
2	135	lowbush blueberry clones	B
3	40	lowbush clones and highbush varieties	A, B, C

Table 2. Highbush and Southern Highbush Varieties Included in Survey 1

Ama	Chanticleer	Ivanhoe	Pearl River
Angola	Collins	Jersey[a]	Pemberton
Atlantic	Concord	Jubilee[b]	Pender[b]
Avonblue[b]	Cooper[b]	June	Pioneer
Berkeley[a]	Coville[a]	Lateblue[a]	Puru[a]
Bladen	Croatan	Legacy[a]	Rancocas
Bluechip	Darrow	Little Giant	Reka[ab]
Bluecrop[a]	Dixi	Mangolia[b]	Reveille
Bluegold[a]	Duke[a]	Marimba[b]	Rubel[a]
Bluehaven	Duplin[b]	Meader	Sampson[b]
Bluejay[a]	Earliblue[a]	Misty[b]	Sharpblue[b]
Blueray	Elizabeth	Morrow	Sierra[a,b]
Bluetta	Elliott[a]	Murphy	Spartan
Bonus	Georgiagem[b]	Nelson[a]	Star
Bounty	Gulfcoast[b]	Northland	Stanley[b]
Brigitta Blue[a]	Harding	Nui[a]	Sunrise
Burlington[a]	Hardyblue	Olympia	Toro[a]
Cabot	Harrison	O'Neal	Wareham
Cape Fear[b]	Heerma	Ozarkblue[b]	Weymouth
Chandler	Herbert	Patriot[a]	Wolcott

[a] These varieties were also included in survey 3. [b] Southern highbush.

are an important source of dietary antioxidants, the present study examined phenotypic diversity within and between blueberry species with respect to anthocyanin and phenolic contents and ORAC.

MATERIALS AND METHODS

Plant Collection. Three separate blueberry surveys were conducted (Table 1). For survey 1, northern and southern highbush varieties were harvested from a demonstration plot at the Rutgers Blueberry and Cranberry Research Center in Chatsworth, NJ, during 1998. Ripe fruit from five plants of 63 northern highbush varieties and two plants of 17 southern highbush varieties (Table 2) were collected when all fruits on the plant were between 25 and 30% ripe. Fruits collected for extraction were frozen at −40 °C. Fruit weight (grams per fruit) was determined on separate samples. In survey 2 (Table 1), fruits from 135 lowbush blueberry clones were collected from three regions (Queens, Hants, and Cumberland Counties) of Nova Scotia, Canada, in 1997. Within each region, three commercial blueberry fields (<10 km apart) were selected; within each field 15 clones were tagged, and mature fruit were hand-harvested. The 15 clones from each field included 5 clones each of the "hairy", "blue", and "black" morphological types (*7*). Prior to freezing at −40 °C, a subsample of 20 fruits of each clone was weighed. Fruit for survey 3 (Table 1), collected during 2000, included ripe fruit of 20 varieties of highbush blueberries from a commercial site near Kentville, NS, and fruit from 20 lowbush blueberries clones from Hants County, NS, ~60 km away. All fruit was frozen at −40 °C prior to extraction and chemical analysis. No attempt was made in any of the surveys, to select commercially acceptable fruit on the basis of size.

Sample Preparation and Extraction. The following procedures were common to the extraction methods A−C (Table 3). Before extraction, a large quantity (>100 g) of frozen fruit was shredded in a food processor, and frozen material was repackaged and returned to storage at −40 °C. A weighed amount (10−15 g) of frozen shredded fruit was ground in the appropriate solvent for 2 min at room temperature, using a Virtis homogenizer (The Virtis Co., Gardner, NY). For all samples, an aliquot of extract was brought to dryness under nitrogen using a Zymark concentrator (Zymark Corp., Hicksville, NY) and resolubilized in water for measurement of ORAC antioxidant capacity. All extracts were stored at −70 °C prior to analysis. Extracts of the 20 highbush and 20 lowbush blueberry (survey 3) were prepared once using methods A (four) and B and twice using method C (eight).

In methods A and B, samples were clarified by centrifugation using a DuPont Sorvall RC 5B (Newtown, CT) at 17000*g* for 5 min. In method C, samples were filtered using glass 9 cm fiber filters (Fisher Scientific, Nepean, ON).

Chemical Measurements. Anthocyanin content was measured using the pH differential method of Wrolstad (*9*), which was adapted for a 96-well microplate reader (Molecular Devices, Menlo Park, CA). Content of monomeric anthocyanins was calculated using the extinction coefficient for cyanidin 3-glucoside (29600) and expressed as cyanidin 3-glucoside equivalents per gram of fresh weight (C3-glu equiv/g of FW). Total dissolved phenolics were measured using the Folin−Ciocalteu assay (*10*) and a 96-well microplate reader. Absorbance was read at 700 nm, using gallic acid as a phenolic standard, with results expressed as gallic acid equivalents per gram of fresh weight (GA equiv/g of FW).

Antioxidant capacity was measured as ORAC in appropriately diluted aqueous extracts using the automated ORAC assay of Cao et al. (*2*, *11*) and a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Nutley, NJ). In the final assay mixture (0.4 mL total volume), β-phycoerythrin (β-PE; Sigma, St. Louis, MO) was used (16.7 nM) as the target of free radical attack. 2,2′-Azobis(2-amidinopropane dihydrochloride (AAPH) (Wako Chemicals, Richmond, VA) was used (4 mM) as the peroxyl radical generator. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma), a water soluble analogue of vitamin E, was used (1.0 mM) as a standard for antioxidant activity. Reagents were dissolved in 75 mM phosphate buffer, pH 7.0. Fluorescence of β-PE was measured every 2 min after the addition of AAPH. The total running time for the ORAC assay was 70 min. Two blank samples (phosphate buffer) and two Trolox standards were included in each run of 12 duplicate blueberry samples. Final results were calculated using the ratio between areas under the decay curves of β-PE for the Trolox standard and a sample and were expressed as micromoles of Trolox equivalents per gram of fresh weight (μmol of TE/g of FW).

Statistical Design and Analysis. Survey 3 was conducted in two parts. In the first part, 20 highbush and 20 lowbush

Table 3. Summary of Extraction Methods and Variation in the Extractability of Blueberry Anthocyanins, Phenolics, and Oxygen Radical Absorbing Capacity (ORAC), among Methods

method	extraction solvent	ratio (mL/g of FW)	incubation (h)	clarification	coefficient of variation (%) anthocyanins	phenolics	ORAC
A	96% acetonitrile, 4% acetic acid	1	0.5[a]	centrifugation	33	18	13
B	88% methanol, 12% water, 0.1% formic acid	3	18[b]	centrifugation	15	35	11
C	40% methanol, 20% water, 40% acetone, 0.1% formic acid	2	0	filtration	19	14	15

$P = 0.0003$[c] $P < 0.0001$ $P = 0.2168$

[a] Stirring every 3 min. [b] In darkness. [c] Significance probability to test homogeneity of variance among methods A−C.

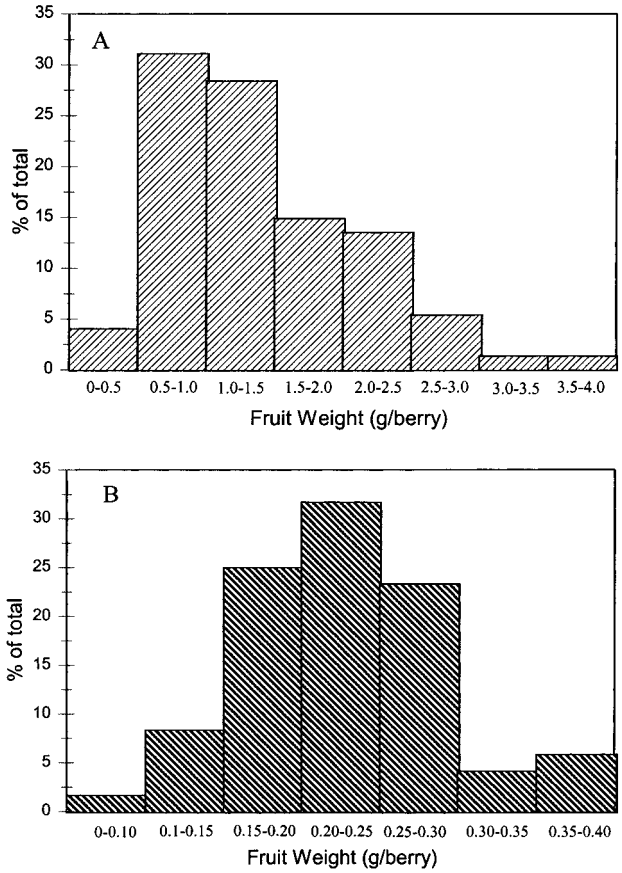

Figure 1. Distribution in the weight of blueberry fruit (grams per berry) in surveys 1 and 2: (A) highbush and southern highbush blueberries; (B) lowbush blueberries.

blueberry genotypes were extracted using methods A and C, and in the second part the same samples were extracted using methods B and C. In each part, the material was randomly divided into groups of five fruit samples (two highbush, two lowbush, plus a repeat of one of four selected genotypes) and were extracted with both methods in a single session. All samples were processed by using the two methods, in a total of 10−12 groups of five samples per group. The additional repeats of the selected genotypes were included to assess the reproducibility of the methods.

The combined data from survey 3 were analyzed using Restricted Maximum Likelihood as described by the SAS procedure MIXED (*13*). The full model for the results of survey 3 included group, method, species, and interaction between method and species, as fixed effects. The random components included session nested within group, subsession nested within the interaction of session and group, genotype nested within the species × method interaction, and finally the genotype × method interaction. In the full model, the residual error was assumed to be different for each method. The likelihood ratio test was used to test the equality of the residual errors and to develop a reduced model.

Using the reduced model from the survey 3, the data from surveys 1 and 2 were analyzed in combination with the data from survey 3. Predicted means were obtained by combining the estimated means for the species × method interaction and the best linear unbiased predictors of the genotype effects (*13*).

RESULTS

Fruit Size. The weight of fruit from the highbush and southern highbush cultivars varied over a relatively wide range, from <1 to 4 g/fruit (Figure 1A), although most highbush fruits were between about 1 and 3 g/fruit. In contrast, fruits of the lowbush clones were smaller and had a much narrower range in fruit weight; all clones had fruit no greater than 0.5 g (Figure 1B). Whereas the fruit size distribution profile was asymmetrical for the fruit of the highbush varieties, it was symmetrical for the fruit of the lowbush genotypes.

Table 4. Significance Probability of Species-Dependent and Extraction Method-Dependent Differences in Anthocyanins, Phenolics, and Oxygen Radical Absorbing Capacity (ORAC) of 20 Highbush Blueberry Varieties and 20 Clones of Lowbush Blueberries, Extracted Using Methods A−C

	significance probability		
	species	method	species × method
anthocyanins[a]	0.0004	<0.0001	NS[b]
phenolics[c]	<0.0001	0.009	NS
ORAC[d]	<0.0001	<0.0001	0.021

[a] Milligrams of cyanidin 3-glucoside equivalents per gram of fresh weight. [b] NS, not significant. [c] Milligrams of gallic acid equivalents per gram of fresh weight. [d] Micromoles of Trolox equivalents per gram of fresh weight.

Extraction Method. Methods A−C differed in their coefficient of variation (percent) in the extraction of identical samples (Table 3). Method B yielded the most consistent results (i.e., lowest coefficient of variation) for anthocyanins and ORAC, whereas method C yielded the most consistent values for phenolics. There was insufficient evidence to suggest that the coefficients of variation for ORAC were different among the three methods ($P = 0.216$).

Differences were found in the composition of extracts of 20 highbush varieties and 20 lowbush clones (survey 3), depending on the method of extraction. Extraction by method B resulted in a higher level of anthocyanins ($P = 0.0004$), phenolics ($P < 0.0001$), and ORAC ($P < 0.0001$) compared to method A or C (Tables 4 and 5). There was no interaction between the extraction method and the blueberry species for either anthocyanins or total phenolics; however, there was an interaction between extraction method and species for ORAC ($P = 0.021$) (Table 4).

Because the highest levels of the components were found using method B (Table 5), these data were combined with data obtained in surveys 1 and 2, after survey 1 and 2 values were adjusted to account for the constant differences in extraction method.

Variation within Species. Box plots (Figure 2) summarize the variation in the highbush and southern highbush varieties (surveys 1 and 3) and lowbush blueberry clones (surveys 2 and 3), and mean values are given in Table 6. For the highbush and southern highbush varieties, the 10th and 90th percentiles of anthocyanin content extended over a 1.6-fold range between 0.927 and 1.48 mg of C3-glu equiv/g of FW (Figure 2). The median anthocyanin content of 1.19 mg of C3-glu equiv/g of FW (Figure 2) was essentially the same as the mean anthocyanin content of 1.18 mg of C3-glu equiv/g of FW (Table 6). For phenolic content, the 10th and 90th percentiles extended over a 1.3-fold range, between 1.65 and 2.16 mg of GA equiv/g of FW (Figure 2). The median and mean total phenolic contents of the highbush and southern highbush varieties were both 1.91 mg of GA equiv/g of FW (Table 6; Figure 2). The 10th and 90th percentiles in ORAC values extended over a 1.8-fold range, between 33.2 and 58.2 μmol of TE/g of FW (Figure 2). The median ORAC value was 46.3 μmol of TE/g of FW (Figure 2), which was very close to the mean ORAC of 45.2 μmol of TE/g of FW for the highbush and southern highbush blueberries (Table 6).

Table 5. Mean Anthocyanin and Total Phenolic Content and ORAC Antioxidant Capacity of 20 Genotypes Each of Highbush and Lowbush Blueberries

	species		method A	method B	method C
anthocyanins[a]	highbush	mean $\log_{10}$	−0.161 (0.101)[b]	0.067 (0.099)	−0.050 (0.098)
		mean[b]	0.690	1.17	0.893
	lowbush	mean $\log_{10}$	0.001 (0.101)	0.241 (0.099)	0.095 (0.098)
		mean	1.00	1.74	1.24
phenolics[d]	highbush	mean $\log_{10}$	0.211 (0.025)	0.292 (0.035)	0.239 (0.020)
		mean	1.63	1.96	1.73
	lowbush	mean $\log_{10}$	0.473 (0.025)	0.573 (0.035)	0.510 (0.020)
		mean	2.97	3.74	3.24
ORAC[e]	highbush	mean $\log_{10}$	1.49 (0.096)	1.69 (0.096)	1.55 (0.095)
		mean	31.0	49.0	35.3
	lowbush	mean $\log_{10}$	1.74 (0.096)	1.88 (0.096)	1.80 (0.095)
		mean	55.0	75.9	63.1

[a] Milligrams of cyanidin 3-glucoside equivalents per gram of fresh weight. [b] Standard error of the $\log_{10}$ means are given in parentheses. [c] Back-transformed from $\log_{10}$. [d] Milligrams of gallic acid equivalents per gram of fresh weight. [e] Micromoles of Trolox equivalents per gram of fresh weight.

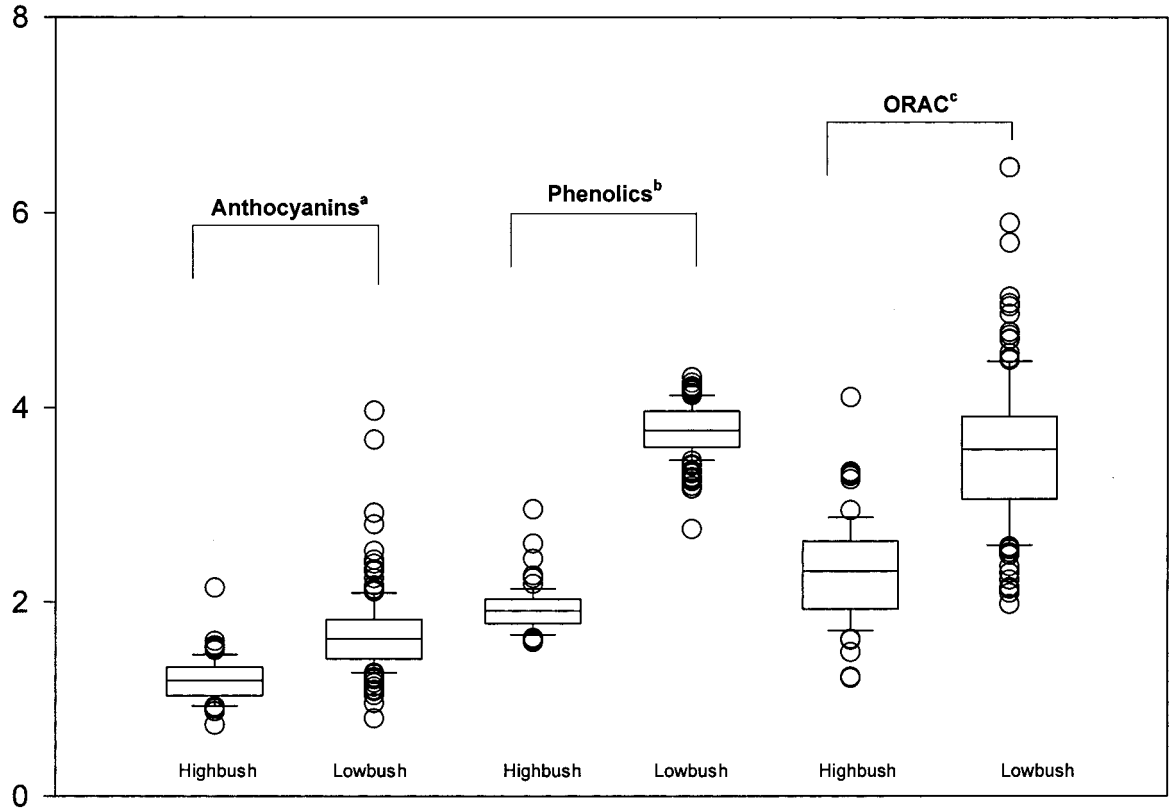

Figure 2. Box plots illustrating intra- and interspecific variation in adjusted values of 155 lowbush blueberry clones and 80 northern (including 17 southern) highbush blueberry varieties for the content of anthocyanins (milligrams of C3-glu equivalents per gram of FW)[a], total phenolic content (milligrams of gallic acid equivalents per gram of FW)[b], and ORAC (micromoles of Trolox equivalents ×20 per gram of FW)[c]. The median is indicated by the midline of the shaded box, whereas the 75th and 25th percentiles are the upper and lower boundaries, respectively, of the box. Error bars indicate the 90th and 10th percentiles, and all points that lie outside these percentiles are indicated by dots.

Among the lowbush clones, the 10th and 90th percentiles of anthocyanin content spanned a 1.6-fold range, between 1.27 and 2.1 mg of C3-glu equiv/g of FW, respectively (Figure 2). The median and mean lowbush anthocyanin contents were both 1.63 mg of C3-glu equiv/g of FW (Table 6). The 10th and 90th percentiles in phenolic content among the lowbush clones extended over a 1.2-fold range between 3.46 and 4.12 mg of GA equiv/g of FW, respectively (Figure 2). The median and mean total phenolic content was 3.76 mg of GA equiv/g of FW (Figure 2; Table 6). The 10th and 90th percentiles in ORAC for the lowbush clones extended over a 1.7-fold range between 51.5 and 90.1 μmol of TE/g of FW, respectively. The median ORAC value for the lowbush clones was 71.4 μmol of TE/g of FW (Figure 2), which was slightly higher than the mean of 69.8 μmol of TE/g of FW for these fruits (Table 6).

Difference between Species. In survey 3 the 20 lowbush blueberry clones had higher levels of anthocyanins ($P = 0.0004$), total phenolics ($P < 0.0001$), and ORAC ($P < 0.0001$) compared with the 20 highbush varieties, irrespective of the extraction method (Tables 4 and 5). The highest values for anthocyanins, phenolics, and ORAC were obtained with method B (Table 5). When fruits of survey 3 (i.e., 20 genotypes per species) were extracted using method B, the anthocyanin content of lowbush fruit exceeded that of highbush fruit by 49%, whereas phenolic content was 91% greater and ORAC

Table 6. Content (Back-transformed from log_{10}) of Anthocyanins, Phenolics, and Oxygen Radical Absorbing Capacity (ORAC) for Highbush and Southern Highbush Blueberry Varieties (Surveys 1 and 3) and Lowbush Clones (Surveys 2 and 3)

mean	species	predicted[a]	coefficient of variation (%)	significance probability, species
anthocyanins[b]	highbush	1.18[c]	8.7	0.0001
	lowbush	1.63[d]	8.5	
phenolics[e]	highbush	1.91[c]	2.0	<0.0001
	lowbush	3.76[d]	5.1	
ORAC[f]	highbush	45.2[c]	9.1	<0.0001
	lowbush	69.8[d]	9.3	

[a] Predicted mean for method B. [b] Milligrams of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] Surveys 1 and 3. [d] Surveys 2 and 3. [e] Milligrams of gallic acid equivalents per gram of fresh weight. [f] Micromoles of Trolox equivalents per gram of fresh weight.

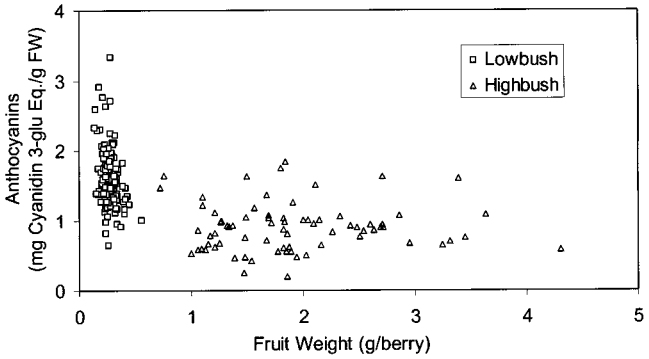

Figure 3. Scatter plot of blueberry fruit weight and anthocyanin content for 135 lowbush clones and 80 highbush (including 17 southern highbush) blueberry varieties.

55% greater (Table 5). The same was true in surveys 1 and 2 after values were adjusted to method B and combined with results of survey 3 (Table 6). The anthocyanin content of the lowbush blueberry clones was 38% greater ($P = 0.0001$) than the level found in the highbush samples (Figure 2; Table 6), whereas the lowbush phenolic content exceeded ($P < 0.0001$) the highbush by 97% (Figure 2; Table 6). Similarly, the mean ORAC of the lowbush clones was 54% greater ($P < 0.0001$) than those of the highbush and southern highbush varieties (Figure 2; Table 6) in the combined results of all surveys.

Fruit Size and Pigment Content. There was no relationship between fruit weight and anthocyanin content among either the 135 lowbush clones or the 80 highbush and southern highbush clones (data not shown). When both species were plotted together (Figure 3), it was evident that the lowbush anthocyanin content varied over a wide range relative to fruit weight, whereas the opposite was true for highbush varieties, for which a narrower range in anthocyanin content and a wider range in fruit weight were found.

DISCUSSION

Fruit Size. In a discussion of factors that affect blueberry fruit size, the terms "fruit size" and "fruit weight" are used synonymously (*14*). In the present study, the narrower distribution in the size of the lowbush fruit, compared to the highbush fruit, may be of interest to those who use blueberries in various food products. For example, the bakery trade may seek fruit of uniform size because they may perform more consistently in baked goods. The smaller lowbush fruit may also be appealing in bakery products because, compared to highbush fruit, a smaller cavity remains after the fruit softens during baking. The sizes of highbush and lowbush fruit are both affected by genetic and environmental factors (e.g., pollination) (*14*).

Extraction Method. Differences in the extractability of anthocyanins, total phenolics, and resulting ORAC, depending on the method employed, illustrate the differential solubility of anthocyanins, other phenolics, and other ORAC-active substances in blueberry fruit. Method C was included because acetone was considered a good solvent for the extraction of proanthocyanidins (*15*). However, the solvent used in method B, which did not contain acetone, resulted in extracts that had the highest levels of anthocyanins, total phenolics, and ORAC and, in general, the most consistent results.

Variation within Species. Similar ranges in anthocyanin and phenolic contents and ORAC were found in the lowbush and highbush fruits. Values for the 10th and 90th percentiles were <2-fold different, for all variates and both species. This may not be expected because commercial highbush and southern highbush varieties have been developed through artificial selection from a limited germplasm, compared to lowbush blueberries, which comprise a multitude of wild genotypes.

The synthesis of anthocyanins and other phenolic compounds can be influenced by various abiotic and biotic factors, including temperature, irradiation, herbivory, and pathogenic infection (*16*). The anthocyanin content of the highbush cultivar Bluecrop was 1.02 mg of C3-g equiv/g of FW (survey 3, method B), whereas earlier studies report 0.718 mg of C3-g equiv/g of FW (*17*) and 0.832 mg of C3-g equiv/g of FW (*18*), using similar methods. Bluecrop was extracted using method A in the study by Prior et al. (*4*) and had an anthocyanin content of 0.931 mg of C3-g equiv/g of FW. In the present study, using method A, the pigment content of Bluecrop was 0.577 mg of C3-g equiv/g of FW (survey 3, method A). Similarly, Bluecrop extracted using method C (*8*) was 1.12 mg of C3-g equiv/g of FW, compared to 0.759 mg of C3-g equiv/g of FW in the present study (survey 3, method C). The anthocyanin contents of select genotypes of lowbush blueberries were different by up to 30% between two seasons of study (*19*).

Differences between Species. The 155 clones of lowbush blueberries had on average higher levels of anthocyanins, total phenolics, and ORAC antioxidant capacity than the 80 varieties of highbush blueberries; this is consistent with earlier reports for these species (*4*, *8*, *17*, *18*). Also consistent with earlier studies (*4*) was the positive relationship between anthocyanin content and total phenolic content, as well as the positive relationship between these contents and ORAC antioxidant capacity. Although environmental factors can influence the synthesis of the compounds responsible for the ORAC of blueberry fruit (*16*), the differences between the species were great enough so that lowbush fruit was consistently higher than highbush fruit in their major antioxidants, that is, anthocyanins and total phenolics. The differences between highbush and lowbush fruits were greatest with respect to their total phenolic content, which was almost 2-times greater in lowbush than in highbush genotypes. Total anthocyanin content was only 38% greater and ORAC 54% greater in the lowbush clones compared to the highbush variet-

ies (Table 6). This result suggests that anthocyanins may make a greater contribution to ORAC than other phenolics. Other phenolics, such as proanthocyanidins, flavonols, and hydroxycinnamates, which contribute to ORAC (*20*, *21*), may vary between lowbush and highbush blueberries (*22*, *23*).

Fruit Size and Pigment Content. Highbush and lowbush blueberries both belong to the *Vaccinium* section *cyanococcus* and have pigment only in the peel of the fruit. Some members of the *Vaccinium* section *myrtillus* have anthocyanin pigment in both the peel and the flesh of the fruit. The lack of any relationship between fruit size and anthocyanin content in the present study (Figure 3) was surprising in light of the difference in size between highbush and wild blueberries and may reflect differences in the evolutionary histories of these two species. Environmental factors such as water availability may also influence the relationship between fruit size and pigment content. A greater proportion of large fruit was found in the lowbush clones Brunswick and Fundy in plants that were irrigated, as compared to those sheltered from rainfall (*24*). (Several lowbush blueberry clones, including Brunswick and Fundy, were selected from wild stands, propagated, and named several decades ago; they are not produced commercially.) Another factor may be the distribution of anthocyanin pigment in the epidermal and subepidermal layers of the peel. These layers were recently compared in the highbush varieties Burlington, Coville, and Elliott (*25*). The epidermis of Burlington fruit had one exterior layer of small pigment-containing cells and one interior layer of larger cells that contained small amounts of pigment. Coville epidermis consisted of three layers of medium-sized pigment-containing cells, whereas Elliott epidermis had one exterior layer of medium-sized pigment-containing cells that were loosely associated with larger thin-walled cells and contained pigment deposits (*25*). Such differences could influence fruit pigment content and obscure any relationship that may exist between fruit size and pigment content.

Although different in their relative amounts, highbush and lowbush blueberries both have a high antioxidant capacity compared to other fruits (*2*−*4*). For this reason their consumption is encouraged as part of a diet rich in fruits and vegetables. Phenolic antioxidants, which make the greatest contribution to the ORAC of blueberry fruit, are negatively affected by processing treatments such as heat and exposure to air (*26*). Thus, although blueberries are often consumed in processed forms, they should be consumed raw to obtain the highest ORAC from these fruit.

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