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Quantification of Total Phenolic, Anthocyanin, and Flavonoid Content in a Diverse Panel of Blueberry Cultivars and Ecotypes

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Abstract. **Blueberries are an important fruit crop in the Ericaceae represented by multiple *Vaccinium* species and ecotypes. In addition to their economic value, blueberry fruit is known for an abundance of specialized metabolites with known human health benefits. Phenolic compounds, which include flavonoids and anthocyanins, are an important class of compounds found in blueberry that are known to contribute to fruit flavor and quality and for having health-promoting properties. Previous surveys of phenolic compounds in blueberry have demonstrated considerable variability in concentration of these metabolites, which is associated with differences in environmental factors and cultivars surveyed. This study expands this knowledge by surveying total phenolic, flavonoid, and anthocyanin content in ripe fruits of 71 blueberry cultivars from one growing season in Michigan. Included in this diversity panel are three ecotypes of blueberry (northern highbush, southern highbush, and half highbush). Rubel, Legacy, and Friendship were among the seven cultivars with the highest content of each compound. Total phenolic content showed a 5.03-fold difference among the lowest and highest cultivars, and total flavonoid content and total anthocyanin content demonstrated a 2.66-fold and 6.37-fold difference between the lowest and highest content across cultivars, respectively. There was no significant impact of ecotype on phytochemical composition of ripe fruits. This study also represents the first large-scale analysis of total phenolic content using the Fast Blue BB (FBBB) reagent. Data from this study have the potential to aid in future breeding efforts to enhance the human health benefits of this economically important fruit crop.**

Blueberries (*Vaccinium* spp.) are an economically important fruit crop with many important cultivated species native to North America (Hancock et al., 2008). The United States is a global leader in blueberry production, with fresh market production amounting to $712 million in sales in 2020 (U.S. Department of Agriculture National Agricultural Statistics Service, 2020). Blueberries are represented by several species and ecotypes. A member of the Ericaceae, commercial *Vaccinium* species are represented by northern highbush blueberries (*V. corymbosum* L.), lowbush or "wild blueberries" (*V. angustifolium* Aiton), and rabbiteye blueberries (*V. virgatum* Aiton; syn. *V. ashei* Reade). Southern highbush is an additional ecotype of blueberry, which is characterized by low chilling requirements and generally has incorporation of genes from *V. darrowii* into the highbush background (Hancock et al., 2008). Northern and southern highbush blueberry ecotypes are the focus of most of the current blueberry breeding in the United States (Hancock et al., 2008) with breeding targets related to low chilling tolerance, disease resistance, drought tolerance, fruit flavor, and color improvement (Brevis et al., 2008).

Blueberry fruits are prized for their overall sweet flavor and known health-promoting compounds. The most well-known health-promoting compounds in blueberry include vitamin C, folate, phenolic, and flavonoid compounds (Wang et al., 2017). Blueberries are also well known for their high antioxidant capacity (Bunea et al., 2013; Guerra et al., 2005; Manganaris et al., 2014), attributable mainly to chlorogenic acid, flavonoid, anthocyanin, and procyanidin compounds (Moyer et al., 2002). Additionally, blueberries contain iridoid compounds, a large group of specialized metabolites with known human health benefits (Leisner et al., 2017).

In addition to their human health benefits, fruit quality has become associated with the content of phenolics, flavonoids, and antioxidant capacity in blueberry (Gündüz et al., 2015). Phenolic compounds are a family of secondary metabolites that includes polyphenols, flavonoids, and other polymers (Cheynier, 2012). Flavonoids themselves are also a large family, including more than 8,000 molecules (Andersen and Markham, 2006). Flavonoids can be further divided into subgroups, including anthocyanins, flavones, and flavonols (Tsao, 2010). Phenolic compounds are widespread across the plant kingdom and play a role in plant secondary metabolism; they are a source of color, flavor, and health-promoting properties in plant-based foods (Cheynier, 2012). Previous research has surveyed the content of phenolic compounds in blueberry fruits, which has demonstrated considerable variability attributable mainly to assay technique, tissues assayed, genotypes analyzed, and other environmental factors (Connor et al., 2002; Dragović-Uzelac et al., 2010; Ehlenfeldt and Prior, 2001; Gündüz et al., 2015; Kim et al., 2013; Okan et al., 2018). Less work has been reported however, investigating the significance of ecotype on the content of phenolic compounds. The current study presents data on the range of total phenolic, flavonoid, and anthocyanin content across ripe fruits of 71 blueberry cultivars and three ecotypes, previously screened for total antioxidant capacity (Colle et al., 2019) and iridoid content (Leisner et al., 2017). Results from this work not only increases our knowledge of specialized metabolites in blueberry but provides information to aid future breeding efforts to enhance the human health benefits of this economically important fruit crop.

Material and Methods

Blueberry fruit sampling

Ripe fruits from 71 commercial blueberry cultivars were collected from the Michigan Blueberry Growers Association (MBGA) in Grand Junction, MI (42°24′09.4″N, 86°04′20.9″W), in collaboration with Ed Wheeler, the blueberry breeder at MBGA. Samples were harvested on site over several weeks in July 2015 to account for differences in ripening times among cultivars. After harvest, fruits were placed on ice and transported to East Lansing, MI, where they were flash-frozen with liquid nitrogen and stored at –80 °C. Samples were shipped overnight on dry ice to Auburn, AL, in 2019 and stored at –80 °C.

Extraction of compounds

The evaluation of all phenolic compounds was conducted at Auburn University. Three to five fruits were ground in liquid nitrogen, transferred to a 25-mL tube, and placed back at

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–80 °C for storage. Extraction was performed according to Lester et al. (2012), with changes in the ratio solvent-to-sample, number of extractions, and in the separation of supernatant from sample residuals. In this study, 500 ± 2.5 mg of frozen ground tissue were transferred to a 2-mL microcentrifuge tube, followed by the addition of 1.3 mL solvent (MeOH 70%, CAS 67-56-1, VWR, product no. BDH1135). Tubes were vortexed for 1 min and centrifuged for 10 min (6700 g_n, at 20 °C) and the supernatant was transferred to another tube. The pellet was resuspended using 1.5 mL solvent, followed by shaking for 1 min and centrifugation for 10 min (7200 g_n, at 20 °C). To meet the proper solvent-to-sample ratio, the extractions were performed twice. The supernatants from the first and second extractions were combined and centrifuged again for 10 min (7200 g_n, at 20 °C). This final supernatant was transferred to a 10-mL vessel and stored at –80 °C for further determination of phenolic compounds.

Total phenolic content

Total phenolic content was assayed according to a modified version of Lester et al. (2012), with changes in the sample dilution, FBBB salt, and standard curve. Fast Blue BB Salt hemi (zinc chloride) salt (FBBB) ($C_{17}H_{18}N_3O_3Cl{\cdot}1/2\ ZnCl_2$, CAS 5486-84-0, Sigma-Aldrich, St. Louis, MO; product no. F3378) was used for total phenolic detection. One hundred microliters of FBBB solution (1000 mg/L) was added to 2-mL centrifuge tubes containing 1.0 mL of the diluted sample extract (1:30), deionized (DI) H_2O (blank), or a standard solution (gallic acid, $(HO)_3C_6H_2CO_2H$, CAS 149-91-7, Sigma-Aldrich, product no. G7384), followed by the addition of 100 µL NaOH (50 g/L, CAS 1310-73-2, VWR, product no. 0583). Tubes were vortexed after the addition of FBBB and NaOH. After incubation for 75 min at room temperature (20 °C), tubes were vortexed and 240 µL was transferred to microplate wells (Corning Falcon 96-well flat bottom, product no. 3370, Tewksbury, MA). All assays were performed using BioTek Cytation 3 microplate reader (Winooski, VT). Absorbance was read at 420 nm starting 90 min after the addition of NaOH. Total phenolic content (milligrams per liter) was determined based on standard solutions of gallic acid at concentrations of 0, 25, 50, 75, and 100 mg/L and expressed as gallic acid equivalents (GA eq).

Total flavonoid content

A modified protocol for determination of total flavonoid content was performed according to Zhishen et al. (1999). Because of the high number of samples and to obtain the right time interval between the addition of reagents, the final volume of the solution comprised by sample and reagents was reduced from 10 mL to 240 µL. In addition, aluminum chloride ($AlCl_3$) was substituted by $AlCl_3{\cdot}6H_2O$ due to laboratory safety protocols. DI H_2O (70.4 µL) was added into microplate wells (Corning Falcon 96-well flat bottom) containing 24 µL of diluted sample extraction (1:5), DI H_2O (blank), or a standard solution (catechin, $C_{15}H_{14}O_6{\cdot}xH_2O$, CAS 225937-10-0, Sigma-Aldrich, product no. C1251), followed by the addition of 20 µL of sodium nitrate ($NaNO_2$) (18 g/L, CAS 7632-00-0, Sigma-Aldrich, product no. S2252), 20 µL of $AlCl_3{\cdot}6H_2O$ (65 g/L, CAS 7784-13-6, Sigma-Aldrich, product no. 237078), 48 µL of sodium hydroxide (NaOH) (1 M, CAS 1310-73-2), and 57.6 µL of DI water. The microplate was manually shaken for 1 min after the addition of each reagent. $AlCl_3{\cdot}6H_2O$ was added 5 min after $NaNO_2$ and NaOH 6 min after $AlCl_3{\cdot}6H_2O$. Absorbance was read at 510 nm, 10 min after the addition of NaOH using the microplate reader. The concentration of total flavonoid content in each sample (mg/L) was determined based on standard solutions of catechin (0, 15, 30, 45, and 60 mg/L) and expressed as catechin equivalents (Cat. eq).

Total anthocyanin content

Total anthocyanin content was determined based on the pH differential method (Wrolstad, 1976) and expressed as malvidin-3-glucoside (Mvd-3-glu, $C_{23}H_{25}O_{12}$). In this method, two pH values are used to determine total anthocyanin content due to changes in the form of this phenolic compound, caused by each pH (a highly colored oxonium or flavylium form in pH 1.0 and the colorless carbinol form in pH 4.5). Initially, 1500 µL of pH 1.0 buffer (1.86 g/L KCl, CAS 7447-40-7, VWR, product no. BDH9258) was transferred into 2-mL microcentrifuge tubes containing 50 µL DI H_2O (blank) or sample extracts. Tubes were then vortexed after the addition of buffer and before transferring 240 µL to microplate wells (Falcon 96-well flat bottom, Tewskbury, MA). Absorbance was read at 510 and 700 nm, starting 50 min after the addition of buffer. All steps were repeated, using pH 4.5 buffer (32.81 g/L CH_3CO_2Na, CAS 127-09-3, Sigma-Aldrich, product no. S2889). pH values were adjusted using hydrogen chloride (HCl) (CAS 7647-01-0, VWR, product no. 0369). The sample-to-buffer ratio (1:31) was determined by a previous dilution test according to Wrolstad (1976).

Formulas

Total phenolic and flavonoid content. The final concentration of total phenolic and flavonoid content (mg/100 g fresh weight) per sample was determined by the formulas:

$$C = (A - b) \div a,$$

where C: GA eq or Cat. eq (mg/L); A: absorbance; b: intercept value; a: slope.

$$C_{mg/100gFW} = \{[(((C \times a) \div 1000) \times b) \div c] \times 100\} \div d,$$

where $C_{mg/100g\ FW}$: GA eq or Cat. eq (mg/100 g FW); d: tissue used for extraction (g); b: total solvent used for extraction (mL); c: sample used (mL); a: sample + buffer (mL); C: GA or Cat. eq (mg/L).

Total anthocyanin content. The final concentration of total anthocyanin content expressed as Mvd-3-glu (mg/L and mg/100 g fresh weight) per sample was determined as follows:

$$A = (510\ \text{nm} - 700\ \text{nm})_{\text{pH1}} - (510\ \text{nm} - 700\ \text{nm})_{\text{pH4.5}},$$

where A: absorbance.

$$C = \{[A \div (e \times L)] \times 10^3\} \times MW,$$

where C: concentration of Mvd-3-glu (mg/L); A: absorbance; e: molar absorbance (28,000); L: pathlength; 10^3: change of concentration unit (g to mg); MW: molecular weight of Mvd-3-glu (493.5 g/mol).

$$C_{mg/100gFW} = \{[(((C \times a) \div 1000) \times b) \div c] \times 100\} \div d,$$

where $C_{mg/100g\ FW}$: Mvd-3-glu (mg/100 g FW); d: tissue used for extraction (g); b: total solvent used for extraction (mL); c: sample used (mL); a: sample + buffer (mL); C: Mvd-3-glu (mg/L).

Experimental design and statistical analysis

Assays were performed using between one and three biological replicates per cultivar and four technical replicates per sample. Analysis of variance and Tukey's multiple comparison test ($P < 0.05$) were performed using R (version 4.0.2; R Core Team, 2020) to determine significant differences between cultivars and ecotypes. Assumptions of the analysis of variance (normality, homogeneity of variance, independence) were tested before analysis. Graphics and tables were developed using the ggplot2 package (version 3.3.5; Wickham, 2016).

Results

Total phenolic, flavonoid, and anthocyanin content of ripe blueberry fruits was determined from a panel of 71 cultivars using biochemical assays (Table 1). The content of compounds changed significatively among cultivars but not between ecotypes (Table 2). Total phenolic content ranged from 392.78 ± 34.70 to 1974.82 ± 84.73 mg/100 g FW, a 5.03-fold (Fig. 1; Table 3). The cultivar Rubel [Northern Highbush (NH)] had the highest concentration, followed by Legacy [Southern Highbush (SH)], Friendship (NH), Coville (NH), Cape Fear (SH), Duke (NH), and Pamlico (SH). In contrast, the lowest concentration of total phenolic content was found for the cultivar Puru (NH), followed by Draper (NH), Sampson (SH), Chippewa [Half Highbush (HH)], Chanticleer (NH), Summit (SH), and Duplin (SH).

Total flavonoid content ranged from 52.19 ± 5.93 to 138.59 ± 7.35 (Fig. 2; Table 3). Overall, a 2.66-fold difference between the lowest and highest flavonoid content was found among the 71 blueberry cultivars (Fig. 2). Friendship (NH) had the highest concentration, followed by Coville (NH), Rubel (NH), Legacy (SH), Blueray (NH), Northland (HH), and Bluegold (NH).

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Table 1. Blueberry cultivars and ecotypes evaluated in this study.

Cultivar	Ecotype	Acronym	n[z]
Aurora	Northern Highbush	NH	3
Beaufort	Southern Highbush	SH	2
Berkeley	Northern Highbush	NH	3
Bluechip	Northern Highbush	NH	2
Bluecrop	Northern Highbush	NH	1
Bluegold	Northern Highbush	NH	3
Bluehaven	Northern Highbush	NH	3
Bluejay	Northern Highbush	NH	3
Blueray	Northern Highbush	NH	3
Blueridge	Southern Highbush	SH	2
Bluetta	Northern Highbush	NH	3
Bounty	Northern Highbush	NH	1
Brigitta	Northern Highbush	NH	3
Cape Fear	Southern Highbush	SH	2
Cara's Choice	Southern Highbush	SH	3
Caroline	Northern Highbush	NH	3
Carteret	Southern Highbush	SH	2
Chandler	Northern Highbush	NH	3
Chanticleer	Northern Highbush	NH	3
Chippewa	Half Highbush	HH	3
Collins	Northern Highbush	NH	3
Concord	Northern Highbush	NH	2
Coville	Northern Highbush	NH	3
Craven	Southern Highbush	SH	2
Croatan	Northern Highbush	NH	2
Darrow	Northern Highbush	NH	3
Denise	Northern Highbush	NH	3
Draper	Northern Highbush	NH	3
Duke	Northern Highbush	NH	2
Duplin	Southern Highbush	SH	2
Earliblue	Northern Highbush	NH	3
Elizabeth	Northern Highbush	NH	3
Elliott	Northern Highbush	NH	3
Friendship	Northern Highbush	NH	2
Hannah's Choice	Northern Highbush	NH	3
Hardyblue	Northern Highbush	NH	3
Herbert	Northern Highbush	NH	3
Huron	Northern Highbush	NH	2
Jersey	Northern Highbush	NH	3
Lateblue	Northern Highbush	NH	3
Legacy	Southern Highbush	SH	3
Lenoir	Southern Highbush	SH	2
Liberty	Northern Highbush	NH	3
Meader	Northern Highbush	NH	3
Nelson	Northern Highbush	NH	3
North Blue	Half Highbush	HH	3
North Country	Half Highbush	HH	3
Northland	Half Highbush	HH	3
Northsky	Half Highbush	HH	3
Nui	Northern Highbush	NH	3
Olympia	Northern Highbush	NH	3
Ornablue	Half Highbush	HH	2
Osorno	Northern Highbush	NH	2
Ozarkblue	Southern Highbush	SH	3
Pamlico	Southern Highbush	SH	1
Patriot	Northern Highbush	NH	3
Pender	Northern Highbush	NH	2
Polaris	Half Highbush	HH	3
Puru	Northern Highbush	NH	3
Reka	Northern Highbush	NH	3
Rose	Northern Highbush	NH	3
Rubel	Northern Highbush	NH	3
Sampson	Southern Highbush	SH	2
Sierra	Southern Highbush	SH	3
Spartan	Northern Highbush	NH	3
St. Cloud	Half Highbush	HH	3
Stanley	Northern Highbush	NH	2
Summit	Southern Highbush	SH	2
Superior	Half Highbush	HH	3
Sweetheart	Northern Highbush	NH	2
Toro	Northern Highbush	NH	3

n[z] = total number of biological replicates per cultivar.

Table 2. Analyses of variance for metabolites

Parameters	df	Sum sq	Mean sq	*F* value	Pr(>F)
		Total phenolic content			
Cultivar	70	13,432,477	191,893.0	6.129	<2e-16***
Residuals	116	3,631,639	31,307.0		
Ecotype	2	66,110	33,055.0	0.358	0.700
Residuals	184	16,998,005	92,380.0		
		Total flavonoid content			
Cultivar	70	49,687	709.8	5.109	5.72e-15***
Residuals	116	16,117	138.9		
Ecotype	2	447	223.5	0.629	0.534
Residuals	184	65,357	355.2		
		Total anthocyanin content			
Cultivar	70	447,297	6,390.0	6.984	<2e-16***
Residuals	115	105,220	915.0		
Ecotype	2	3,377	1,689.0	0.563	0.571
Residuals	183	549,140	3,001.0		

***Significant at $P \leq 0.001$.

Chanticleer (NH) had the lowest flavonoid content, followed by Sampson, Elizabeth (NH), Cara's Choice (SH), Concord (NH), Bounty (NH), and Bluecrop (NH).

The concentration of total anthocyanin content ranged from 50.60 ± 11.77 to 322.54 ± 13.71 mg/100 g FW (Fig. 3; Table 3), representing a 6.37-fold difference between the lowest and highest anthocyanin content across the 71 blueberry cultivars. The highest concentration was observed for Rubel (NH), followed by Friendship (NH), Legacy (SH), Duke (NH), Cape Fear (SH), Earliblue (NH), and Berkeley (NH). Puru (NH) was the cultivar with the lowest anthocyanin content, followed by Draper (NH), Summit (SH), Duplin (SH), North Sky (HH), Chippewa (HH), and Sweetheart (NH).

Across all three analyses (total phenolic content, total flavonoid content, and total anthocyanin content), Rubel (NH), Legacy (SH), and Friendship (NH) were among the seven cultivars with the highest content of each compound. Differences between the lowest and highest concentrations of phenolic compounds between ecotypes was low compared with across cultivars (Table 4). Total phenolic content only differed by 6.43% across ecotypes, whereas total flavonoid and total anthocyanin content differed by 5.17% and 7.20%, respectively. The mean total values between ecotypes were 883.56, 86.99, and 163.68 mg/100 g FW for total phenolic, flavonoid, and anthocyanin content, respectively (Table 4).

Discussion

Total phenolic content. In this study, we surveyed a diverse panel of blueberry germplasm to gain insight into the content of phenolic compounds in blueberry fruits (Figs. 1–3; Table 3). The concentration of total phenolic, anthocyanin, and flavonoid compounds demonstrated significant variability among cultivars, as previously observed (Castrejón et al., 2008; Connor et al., 2002; Dragović-Uzelac et al., 2010; Ehlenfeldt and Prior, 2001; Fredes et al., 2014; Gündüz et al., 2015; Kim et al., 2013; Mengist et al., 2020; Okan et al., 2018; Shibata et al., 2021). In addition to expanding the list of cultivars surveyed for total phenolic, anthocyanin, and flavonoid contents, a diverse set of ecotypes was also included in this study to give insights into ecotype differences in these specialized metabolites from one growing environment.

This is the first large-scale study to quantify total phenolic content in blueberry fruits using FBBB as the reagent, as most previous studies used the Folin–Ciocalteu (F-C) method (Medina, 2011a). In this study, 43 cultivars were analyzed using FBBB that had previously been analyzed for total phenolic content using the F-C method (Dragović-Uzelac et al., 2010; Ehlenfeldt and Prior, 2001; Fredes et al., 2014; Gündüz et al., 2015; Kim et al., 2013; Li et al., 2017; Okan et al., 2018; Shibata et al., 2021). On average, the total phenolic content was 5.2 times higher than the F-C value (1.7–10.3), which is in accordance with previous work done to compare the FBBB and F-C methods (Lester et al., 2012; Medina, 2011a, 2011b).

Several factors may contribute to the higher values observed for total phenolic content in blueberry fruits when measured with FBBB compared with F-C (Granato et al., 2016; Lester et al., 2012; Medina, 2011a, 2011b). Unlike F-C, FBBB detects phenolics directly in a specific reaction between hydroxyl groups from the phenolic compounds and the diazonium group in the reagent (Medina, 2011a). Work previously cited in strawberry has also demonstrated that total phenolic content measured using FBBB has a greater concentration on average than total phenolics measured with F-C (Lester et al., 2012). This work also demonstrated that total phenolics measured with FBBB had a significant correlation with total phenolics measured via HPLC and that the FBBB assay does not interact with sugars or ascorbic acid, all limitations of the F-C reagent (Lester et al., 2012). Additionally, differences in the location, growing season, and fruit stage may contribute to differences in overall values of total phenolic content in this study compared with others (Connor et al., 2002; Dragović-Uzelac et al., 2010; Gündüz et al., 2015). For

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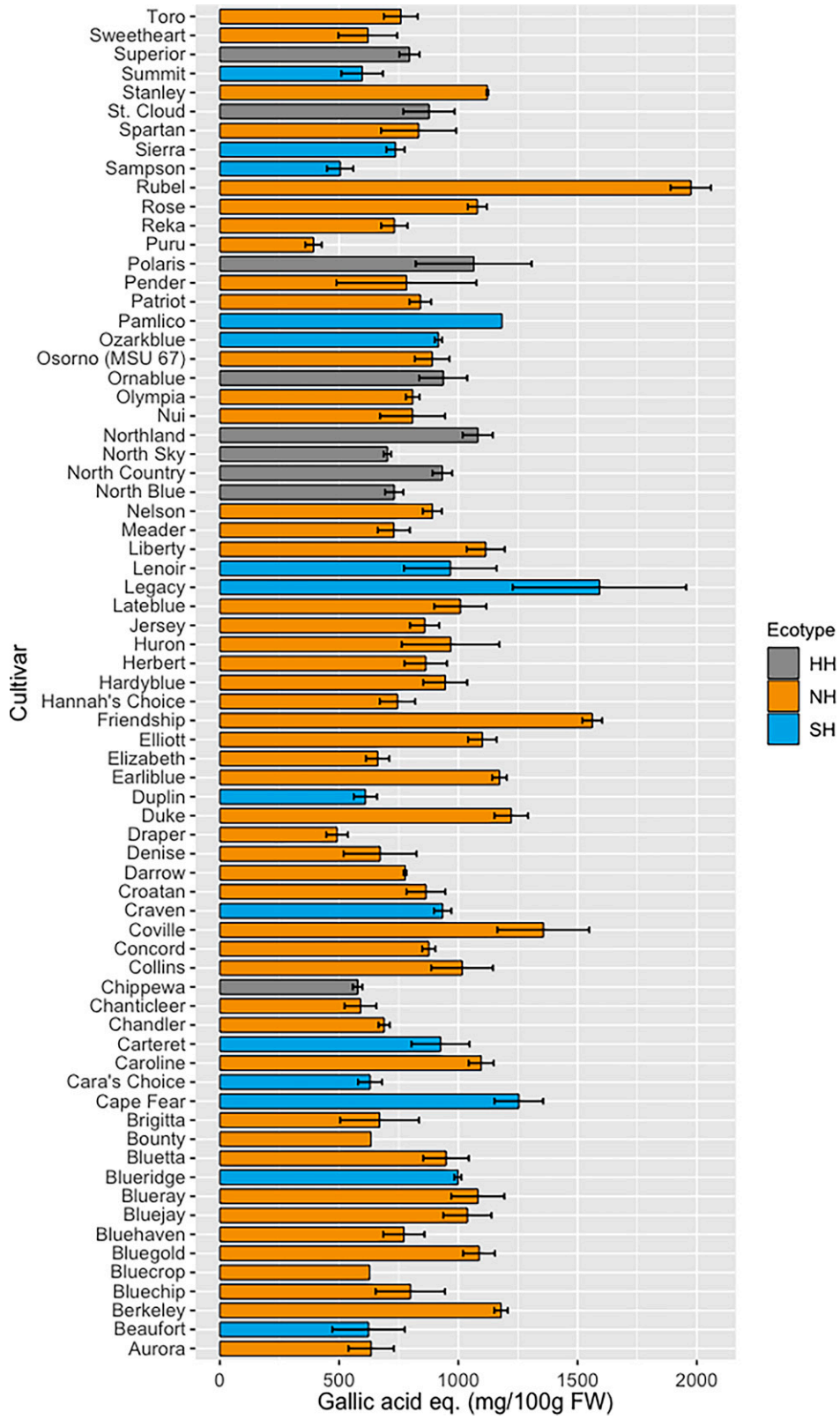

Fig. 1. Concentration of total phenolic content in fruit tissue for 71 blueberry cultivars. Values presented as gallic acid equivalents (mg/100 g FW) and represent mean ± SE.

example, in strawberries, an interval of 3 days between harvest of ripe fruit promoted differences up to 2.6-fold in total phenolic content (Lester et al., 2012), and work reported in blueberry found total phenolic content changed during maturation and with different cultivation and climate conditions (Castrejón et al., 2008). Blueberry fruit used in this study were collected at full maturity from one location, one harvest period, and in one growing season, which may limit the scope of the results. However, results from this work provide a resource for breeders and growers to understand general trends in total phenolic content when comparing across blueberry germplasm using a new assay technique.

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Table 3. Quantification of total phenolic, flavonoid, and anthocyanin content in fruit tissue for 71 blueberry cultivars

Cultivar	Total phenolic content	Total flavonoid content	Total anthocyanin content
	mg/100 g FW		
Aurora	634.40 ± 95.23	82.00 ± 4.25	124.61 ± 23.53
Beaufort	623.50 ± 152.11	80.92 ± 10.09	110.27 ± 37.53
Berkeley	1,178.92 ± 28.42	93.45 ± 5.99	224.29 ± 7.10
Bluechip	798.74 ± 145.43	77.21 ± 0.34	162.01 ± 32.95
Bluecrop	628.16 [z]	70.53 [z]	117.85 [z]
Bluegold	1,086.83 ± 66.76	106.87 ± 6.86	175.19 ± 17.91
Bluehaven	771.72 ± 86.39	72.28 ± 7.58	148.65 ± 21.70
Bluejay	1,038.00 ± 101.00	73.89 ± 4.36	213.59 ± 9.56
Blueray	1,081.55 ± 111.56	111.28 ± 12.10	175.56 ± 10.27
Blueridge	997.91 ± 14.64	103.34 ± 3.57	160.75 ± 2.98
Bluetta	948.47 ± 95.67	70.81 ± 2.27	194.19 ± 19.57
Bounty	633.41 [z]	69.93 [z]	128.15 [z]
Brigitta	669.82 ± 165.50	77.73 ± 10.92	122.65 ± 20.47
Cape Fear	1,253.63 ± 102.15	88.68 ± 0.75	250.37 ± 12.10
Cara's Choice	629.85 ± 50.48	67.19 ± 8.45	120.32 ± 6.08
Caroline	1,095.91 ± 52.38	83.61 ± 3.95	216.19 ± 9.73
Carteret	925.16 ± 121.79	75.88 ± 0.53	186.59 ± 34.16
Chandler	688.99 ± 23.65	77.32 ± 4.71	124.29 ± 6.77
Chanticleer	589.66 ± 66.69	52.19 ± 5.93	124.41 ± 2.09
Chippewa	577.71 ± 20.05	81.87 ± 7.71	107.09 ± 5.62
Collins	1,015.66 ± 129.58	97.37 ± 10.25	188.14 ± 23.23
Concord	876.10 ± 27.73	67.74 ± 0.03	170.71 ± 0.03
Coville	1,356.37 ± 192.85	129.29 ± 16.87	219.01 ± 24.39
Craven	934.22 ± 36.50	93.00 ± 4.53	183.10 ± 8.18
Croatan	864.06 ± 81.38	84.56 ± 5.67	185.17 ± 9.93
Darrow	776.54 ± 6.37	80.20 ± 2.96	148.79 ± 4.71
Denise	671.87 ± 153.04	76.86 ± 12.48	120.91 ± 18.58
Draper	491.58 ± 45.24	96.70 ± 5.35	65.50 ± 5.31
Duke	1,221.86 ± 70.59	90.05 ± 0.16	257.79 ± 11.06
Duplin	610.36 ± 48.76	89.92 ± 5.61	99.05 ± 3.24
Earliblue	1,172.58 ± 30.60	106.68 ± 9.55	239.45 ± 6.74
Elizabeth	661.60 ± 48.99	56.32 ± 2.56	149.22 ± 12.64
Elliott	1,100.72 ± 59.96	104.64 ± 4.21	217.74 ± 10.73
Friendship	1,561.46 ± 41.61	138.59 ± 7.35	281.56 ± 25.95
Hannah's Choice	744.74 ± 74.45	79.51 ± 1.70	143.85 ± 15.37
Hardyblue	945.12 ± 92.16	91.14 ± 3.11	175.24 ± 9.55
Herbert	863.45 ± 89.25	78.09 ± 3.00	164.23 ± 7.90
Huron	967.43 ± 204.84	84.73 ± 3.01	171.06 ± 25.61
Jersey	858.79 ± 61.41	74.24 ± 3.86	166.71 ± 4.33
Lateblue	1,008.30 ± 109.47	73.91 ± 7.98	205.91 ± 20.88
Legacy	1,591.72 ± 363.86	120.68 ± 18.25	267.84 ± 43.50
Lenoir	966.33 ± 194.31	81.15 ± 6.54	191.06 ± 20.21
Liberty	1,114.89 ± 79.92	83.19 ± 0.91	221.29 ± 6.39
Meader	729.56 ± 67.40	77.16 ± 2.95	130.52 ± 13.58
Nelson	890.99 ± 40.15	89.47 ± 3.30	141.33 ± 2.63
North Blue	731.24 ± 38.47	74.93 ± 11.38	148.89 ± 1.38
North Country	932.96 ± 41.50	87.41 ± 7.16	172.18 ± 10.30
North Sky	702.54 ± 15.76	84.54 ± 4.90	105.83 ± 8.95
Northland	1,081.27 ± 63.29	109.66 ± 2.92	188.75 ± 10.59
Nui	808.16 ± 136.35	77.24 ± 4.17	148.79 ± 29.82
Olympia	808.60 ± 28.78	75.37 ± 1.86	156.25 ± 9.72
Ornablue	936.70 ± 100.67	96.76 ± 9.97	187.47 ± 17.94
Osorno	890.29 ± 72.64	77.72 ± 8.92	193.59 ± 9.30
Ozarkblue	916.79 ± 15.55	104.39 ± 7.23	156.41 ± 1.15
Pamlico	1,182.33 [z]	97.10 [z]	222.82 [z]
Patriot	840.54 ± 45.54	74.19 ± 6.01	153.93 ± 5.98
Pender	782.45 ± 293.76	85.16 ± 1.30	135.83 ± 77.96
Polaris	1,064.49 ± 243.10	85.78 ± 10.91	193.11 ± 49.80
Puru	392.78 ± 34.70	80.35 ± 5.58	50.60 ± 11.77
Reka	731.76 ± 55.30	74.95 ± 3.64	139.06 ± 5.95
Rose	1,079.71 ± 40.13	77.75 ± 4.86	202.01 ± 12.22
Rubel	1,974.82 ± 84.73	128.69 ± 3.24	322.54 ± 13.71
Sampson	504.41 ± 55.23	53.96 ± 3.20	114.04 ± 18.46
Sierra	736.62 ± 38.41	78.43 ± 1.33	139.56 ± 14.44
Spartan	833.57 ± 157.67	72.17 ± 4.88	166.90 ± 21.78
St. Cloud	876.93 ± 107.77	97.79 ± 9.92	165.90 ± 17.17
Stanley	1,121.80 ± 3.93	99.76 ± 10.52	209.98 ± 7.75
Summit	596.58 ± 87.29	72.91 ± 5.34	94.88 ± 16.63
Superior	794.96 ± 42.42	87.53 ± 2.40	165.09 ± 9.35
Sweetheart	620.39 ± 123.49	77.01 ± 2.30	109.67 ± 28.21
Toro	758.77 ± 71.22	87.91 ± 4.33	122.56 ± 11.40
Mean	895.07	85.66	167.03
P value	<0.0001	<0.0001	<0.0001

[z] n = 1.

Data expressed as mean ± SE (n = 1–3).

Total flavonoid content. In addition to measuring total phenolic content, total flavonoid and total anthocyanin content were also measured (Figs. 2–3; Table 3). Across all 71 cultivars, the average total flavonoid content in this study was 85.66 mg Cat. eq/100 g FW (ranging from 52.19 to 138.59 mg Cat. eq/100 g FW). On average, results from this study are 1.77-fold higher than those found by Okan et al. (2018) (1.21–2.78, 20 cultivars) but 43% and 47% lower than values reported by Wang et al. (2017) and Li et al. (2017). The use of other flavonoid compounds as the standard (e.g., quercetin in Okan et al., 2018) can affect the specific value of total flavonoid content, as can differences in assay methodology (e.g., high-performance liquid chromatography), location fruit was collected, genotypes and cultivars surveyed, cultivation practices, and seasonal variation (Cezarotto et al., 2017; Harnly et al., 2006; Li et al., 2013; Wang et al., 2008; Wang et al., 2012). Much of the previous work on analysis of flavonoid compounds in blueberry are in relation to total antioxidant capacity, with a focus on determining which flavonoid compounds contribute most to antioxidant capacity (Bunea et al., 2011; Cezarotto et al., 2017; Huang et al., 2012; Li et al., 2013). This previous work demonstrates that flavonoid compounds are important phytochemicals in blueberry with positive human health benefits (Wang et al., 2017). Additional work is needed to survey individual flavonoid compounds in the current diversity panel of ripe blueberry fruits; however, general trends of which cultivars contain the highest total flavonoid content is presented.

Total anthocyanin content. In addition to total phenolic and total flavonoid content, total anthocyanin content was measured in ripe fruits across the 71 blueberry cultivars (Fig. 3; Table 3). The total anthocyanin content ranged from 50.60 to 322.54 mg malvidin-3-glucoside/100g FW, with an average of 167.03. Comparing results for 40 cultivars previously analyzed for total anthocyanin content this study reports values ≈8.3% lower on average (Connor et al., 2002; Ehlenfeldt and Prior, 2001; Fredes et al., 2014; Kim et al., 2013; Okan et al., 2018). Cultivars Legacy and Puru showed the largest differences in total anthocyanin content; Legacy had a 2.20-fold higher content that previously reported, while Puru had a 0.38-fold lower content compared with previously reported total anthocyanin content values. All data were generated based on the pH differential method (first published by Wrolstad, 1976). Although delphinidin-3-glucoside is the major anthocyanin pigment in blueberries, the previous studies used cyanidin-3-glucoside as reference for the quantification of this metabolite, while malvidin-3-glucoside was used in this work, as recommended by Wrolstad (1976). This, along with differences in molar absorbance and molecular weight, may slightly affect the comparison between results from this and previous studies.

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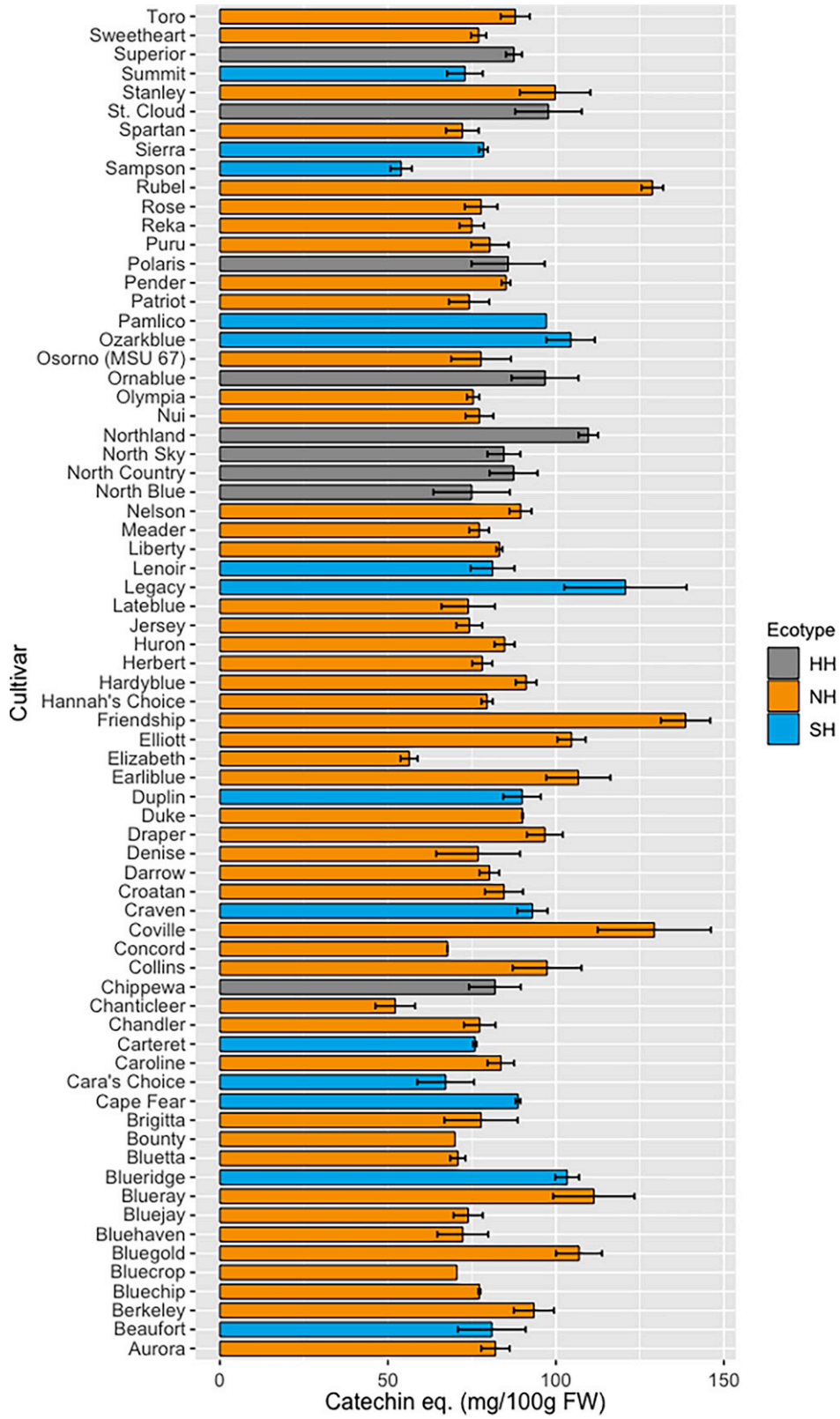

Fig. 2. Concentration of total flavonoid content in fruit tissue for 71 blueberry cultivars. Values presented as catechin equivalents (mg/100 g FW) and represent mean ± SE.

Previous studies have also demonstrated that growing season, harvest period and location all significantly affect both the composition of anthocyanin compounds and total anthocyanin content in blueberries (Chai et al., 2021; Connor et al., 2002). It has also been demonstrated that there is a significant correlation between total antioxidant capacity and anthocyanin content in fruit tissue (Ehlenfeldt and Prior, 2001). This indicates total anthocyanin content is a robust indicator of the nutrient quality of the fruit and its potential human health benefits (Kim et al., 2013).

Ecotype analysis. In addition to including many cultivars in the diversity panel, three

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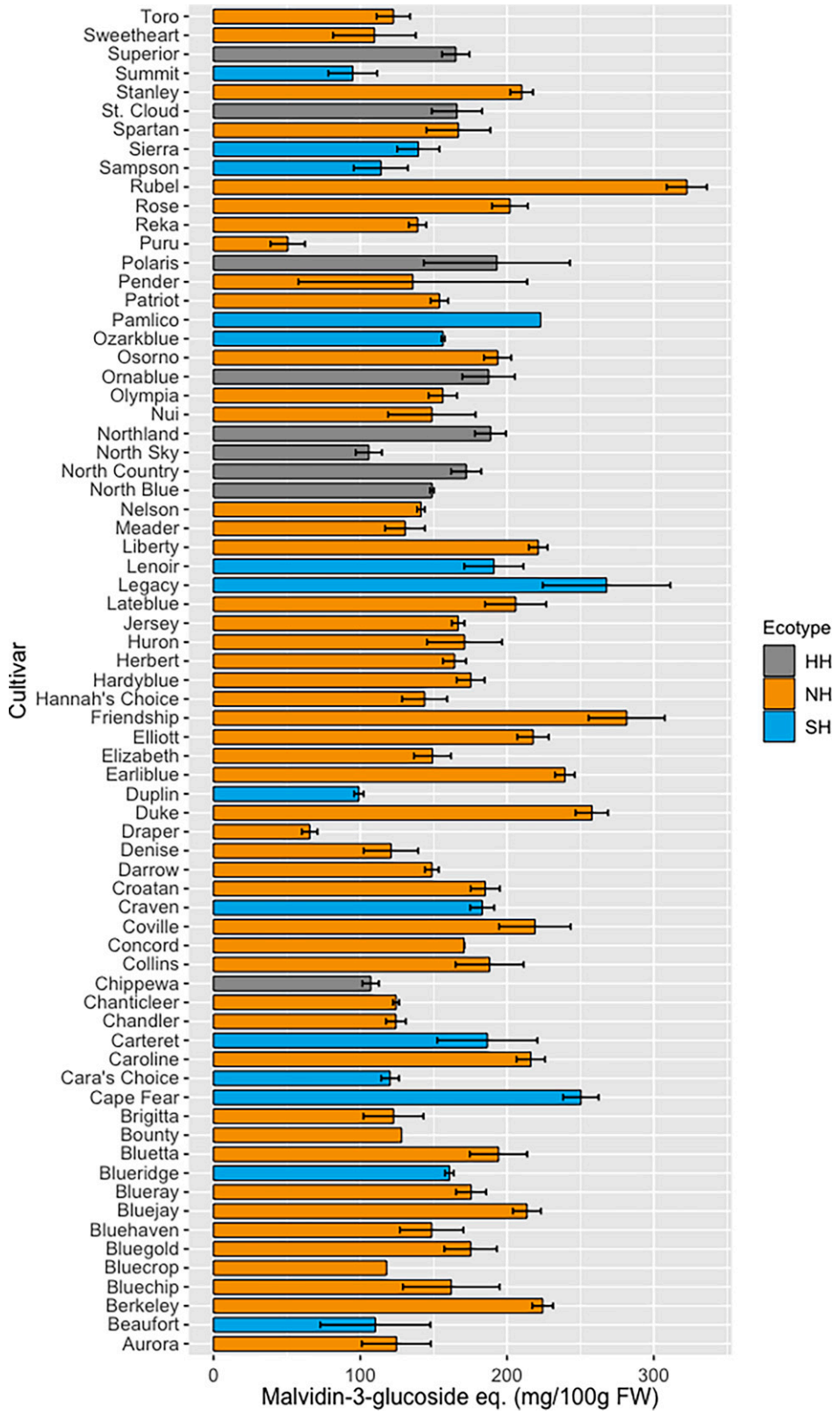

Fig. 3. Concentration of total anthocyanin content in fruit tissue for 71 blueberry cultivars. Values presented as malvidin-3-glucoside equivalents (mg/100 g FW) and represent mean ± SE.

ecotypes of blueberry were also represented in this study (Table 1). Although there was variability among ecotypes for phenolic compound measured, ecotype was not a significant variable contributing to changes in these metabolites (Tables 2 and 4). The panel was comprised mostly of the NH ecotypes, due to the origination of the diversity panel from Michigan. This may limit the ability to detect significant differences among the other ecotypes because only 14 SH cultivars and 9 HH cultivars were included in this analysis (Table 1). Additionally, it is possible that other factors involved in cultivar selection and blueberry breeding may be driving changes in phenolic content more so than ecotype, as SH cultivars were developed from NH ecotypes, and rabbiteye is a close relative of both

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Table 4. Analysis of total phenolic, total flavonoid, and total anthocyanin content in blueberry fruit tissue across three ecotypes

	Total phenolic content	Total flavonoid content	Total anthocyanin content
Ecotype		mg/100 g FW	
Northern Highbush	907.06 ± 26.62	84.92 ± 1.67	169.68 ± 4.88
Southern Highbush	891.34 ± 65.04	86.73 ± 3.69	163.08 ± 10.92
Half Highbush	852.30 ± 42.57	89.31 ± 2.95	158.29 ± 8.34
Mean	883.56	86.99	163.68
P value	>0.05	>0.05	>0.05

Data expressed as mean ± SE.

ecotypes (Gündüz et al., 2015; Mengist et al., 2020).

Previous surveys of phytochemical properties in blueberry across ecotypes have found varying results of ecotype impact on fruit metabolite composition (Chai et al., 2021; Gündüz et al., 2015). A survey of total anthocyanin content across 74 blueberry cultivars and five ecotypes from China (NH, SH, HH, rabbiteye, and lowbush) found that anthocyanin profiles were similar across cultivars but varied in the amount of each individual anthocyanin compound (Chai et al., 2021). This is in contrast with Li et al. (2017), who found that HH cultivars of blueberry had higher concentrations of anthocyanidins than NH cultivars. The authors hypothesize that this is due to the characteristics of each cultivar and genetic origin of the fruit quality traits in each cultivar's pedigree (Li et al., 2017). Gündüz et al. (2015) found the phytochemical properties in blueberry fruits were similar across ecotypes (NH, SH, and rabbiteye) and concluded that breeding new varieties of blueberry with different fruit quality parameters and phytochemical properties would not be limited by genetic barriers. Furthermore, recent work has identified genotypic effects that explain phytochemical and fruit quality trait diversity within and between different ploidy groups in blueberry (Mengist et al., 2020). Overall, results from this study and previous work demonstrate considerable variability among phenolic compounds in blueberry that is likely a product of the genotype and environment interactions, but further work is needed to survey phenolic compounds across diverse ecotypes and environments.

Conclusions

In this study, we surveyed a diverse panel of blueberry cultivars and ecotypes to gain insight into the range of concentrations of total phenolic, total anthocyanin, and total flavonoid compounds in ripe fruits. Results from this work demonstrate significant variability among cultivars, with no significant impact of ecotype on the phytochemical content of ripe fruits. This study also represents the first large-scale analysis of total phenolic content using the FBBB reagent. Although these data represent findings from a single year, harvest period, and location, trends in values of phenolic compounds are of value to those studying specialized metabolites in blueberry and breeders seeking to increase the content of phenolic compounds in blueberry germplasm. Further studies are needed to disentangle the genotype by environment, growing season, and cultivation interactions present in the concentration and composition of phenolic compounds in blueberry fruits, which will aid future breeding efforts to enhance the human health benefits of this economically important fruit crop.

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