Anthocyanins, Phenolics, and Antioxidant Capacity in Diverse Small Fruits: *Vaccinium*, *Rubus*, and *Ribes*

RICHARD A. MOYER,† KIM E. HUMMER,‡ CHAD E. FINN,§ BALZ FREI,‖ and RONALD E. WROLSTAD*⊥⊥

King College, 1350 King College Road, Bristol, Tennessee 37620; National Clonal Germplasm Repository, Agricultural Research Service, U.S. Department of Agriculture, 33447 Peoria Road, Corvallis, Oregon 97330-2521; Horticultural Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 3420 NW Orchard Avenue, Corvallis, Oregon 97330; Linus Pauling Institute, Oregon State University, Corvallis, Oregon 97331; and Department of Food Science and Technology, 100 Wiegand Hall, Oregon State University, Corvallis, Oregon 97331-6602

Fruits from 107 genotypes of *Vaccinium* L., *Rubus* L., and *Ribes* L., were analyzed for total anthocyanins (ACY), total phenolics (TPH), and antioxidant capacities as determined by oxygen radical absorbing capacity (ORAC), and ferric reducing antioxidant power (FRAP). Fruit size was highly correlated ($r = 0.84$) with ACY within *Vaccinium corymbosum* L., but was not correlated to ACY across eight other *Vaccinium* species, or within 27 blackberry hybrids. Certain *Vaccinium* and *Ribes* fruits with pigmented flesh were lower in ACY, TPH, ORAC, and FRAP compared to those values in berries with nonpigmented flesh. ORAC values ranged from 19 to 131 µmol Trolox equivalents/g in *Vaccinium*, from 13 to 146 in *Rubus*, and from 17 to 116 in *Ribes*. Though ACY may indicate TPH, the range observed in ACY/TPH ratios precludes prediction of ACY from TPH and vice versa for a single genotype. In general, TPH was more highly correlated to antioxidant capacity than ACY was. This study demonstrates the wide diversity of phytochemical levels and antioxidant capacities within and across three genera of small fruit.

KEYWORDS: Anthocyanins; phenolics; ORAC; FRAP; blueberry; blackberry; black currant; rabbiteye; highbush; lowbush; marionberry; jostaberry; gooseberry

INTRODUCTION

Increasing epidemiological evidence associates diets rich in fruits and vegetables with reduced risk of heart disease, cancer, and other chronic diseases (1, 2). A major benefit from such a diet may be increased consumption of antioxidants (3), including carotenoids, ascorbate, tocopherols, and phenolics. One phenolic fraction, the flavonoids, are potent in vitro antioxidants (4–6) and include compounds such as flavones, isoflavones, flavonones, catechins, and the red, blue, and purple pigments known as anthocyanins (7). Wang et al. (8) observed that compounds other than vitamin C are major sources of antioxidant capacity in fruits. Blueberries (*Vaccinium* L. species), blackberries (*Rubus* L. hybrids), and black currants (*Ribes nigrum* L.) are rich sources of dietary anthocyanins and antioxidants (8–10). Many cultivars and native species of these berries exist, some with substantially higher antioxidant levels than others (11, 12). Plant anthocyanin levels vary according to season and growing location (11, 13, 14), confounding attempts to compare reported values within or across species and genera. Differing laboratory methods of extraction and analysis may also contribute to variance in reported levels of anthocyanins, phenolics, and antioxidants.

Prior et al. (11) compared total anthocyanins (ACY), total phenolics (TPH), and oxygen radical absorbing capacity (ORAC) of four *Vaccinium* species and 23 genotypes. Our objectives were similar, but our methodology and study group were broader. Our objectives were to (1) determine the ACY, TPH, and two measures of antioxidant capacity, ORAC and FRAP, in 107 genotypes of dark-colored, small fruits with representatives of nine *Vaccinium* L., seven *Rubus* L., and five *Ribes* L. species; (2) determine whether these measurements are correlated; (3) determine whether berry size is correlated with ACY, TPH, ORAC, or FRAP; and (4) compare the ACY, TPH, ORAC, and FRAP in first vs last ripe *Vaccinium corymbosum* cv. Summit.

MATERIALS AND METHODS

Safety. There is an explosion hazard as liquid nitrogen becomes gaseous. For proper venting procedures during liquid nitrogen milling of frozen materials, refer to Rodriguez-Saona and Wrolstad (15).

Sampling Procedures. Ripe fruit samples, as judged by flavor and color, were harvested during summer 2000 from two Willamette Valley

* Corresponding author: phone (541) 737-3591; fax (541) 737-1877; email ron.wrolstad@orst.edu.
† King College.
‡ National Clonal Germplasm Repository.
§ Horticultural Crops Research Laboratory.
‖ Linus Pauling Institute.
* Department of Food Science and Technology.
Molecular Devices, Foster City, CA). FRAP values, derived from
tables were filled with milled fruit powder and weighed, and then the
powder was extracted with acetonitrile, followed by two additional
extraction with 70:30 acetone/water. The pooled supernatants were
partitioned with two volumes of chloroform. The nonpolar phase was
partitioned with two volumes of chloroform. The nonpolar phase was
determined by ORAC and FRAP assays at the Linus Pauling Institute,

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{genotype} & \textbf{ACY} & \textbf{TPH} & \textbf{ORAC} & \textbf{FRAP} & \textbf{ACY/}
\hline
 Brunswick & 208 & 4.0 & 324 & 692 & 3.7 & 87.8 & 97.9 & 0.30 \\
 Bluegem & 242 & 6.0 & 161 & 417 & 2.6 & 31.0 & 140.1 & 0.34 \\
 CVAC 200.003 & 383 & 9.4 & 287 & 787 & 5.0 & 130.7 & 127.1 & 0.44 \\
 CVAC 1161.001 & 484 & 1.8 & 142 & 461 & 1.5 & 129.4 & 161.4 & 0.50 \\
 CVAC 1170.001 & 515 & 3.2 & 218 & 595 & 2.5 & 122.8 & 157.3 & 0.54 \\
 \textbf{ory} & \textbf{FRAP} & \textbf{TPH} & \textbf{berry} & \textbf{ACY/}
\hline
 V. angustifolium Alton & & & & & & & & \\
 V. ashel Readea (\textit{V. virgatum Alton}) & & & & & & & & \\
 CVAC 200.003 & 2 & 6 & 1 & 100 & 0 & 1 & 100 & 0 \\
 CVAC 1161.001 & 3 & 2 & 1 & 100 & 0 & 1 & 100 & 0 \\
 CVAC 1170.001 & 3 & 3 & 1 & 100 & 0 & 1 & 100 & 0 \\
 \hline
\end{tabular}
\caption{Table 1. Total Anthocyanin Content (ACY), Berry Size, Total Phenols (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 30 Vaccinium Genotypes}
\end{table}

\section*{RESULTS AND DISCUSSION}

\subsection*{Determination of Total Anthocyanins (ACY).}
Antioxidant capacity was
determined by ORAC and FRAP assays at the Linus Pauling Institute,

\subsection*{Antioxidant Capacity.}
Antioxidant capacity was
determined by ORAC and FRAP assays at the Linus Pauling Institute,

\subsection*{Statistical Analysis.}
Correlation and regression analyses were
performed using Microsoft Excel Data Analysis. Differences at \(p = 0.05\) were considered significant.

\section*{RESULTS AND DISCUSSION}

\subsection*{ACY, TPH, ORAC, and FRAP.}
Wide ranges of ACY, TPH, ORAC, and FRAP were observed within each genus (Tables 1, 2, and 3), consequently the genus means were not significantly different. Having chosen dark fruited, highly pigmented genotypes, we were not surprised to observe that many values of ACY, TPH, ORAC, and FRAP for genotypes in our tests were higher than that reported generally for fruits and vegetables (8). Species effects were apparent. Black raspberries, \textit{Rubus occidentalis} L. cvs. Munger, Jewel, and Earlysweet, (Table 2) had the highest ACY (627, 607, and 464 mg ACY/100 g, respectively), and FRAP (169, 184, and 206 mmol/L, respectively) levels of tested fruits, and high levels of TPH and ORAC (Tables 1, 2, and 3). Wild selections of rabbiteye blueberry, \textit{Vaccinium ashei}, from Florida and Georgia (Table 1) had the highest ORAC (131, 129, and 122 mmol TE/g) and higher levels of ACY and FRAP than did many other fruits. \textit{Ribes validi-\textit{anum}}, a black fruited cultivar from Chile, and \textit{Ribes nigrum} cvs. Consort and Willoughby (Table 3) had the highest TPH of all fruits tested (1790, 1342, 1122 mg TPH/100 g, respectively).

\subsection*{Vaccinium.}
The red huckleberry, \textit{V. parvilatrum}, had the lowest ACY (34 mg ACY/100 g), yet its ORAC and FRAP values were higher than those of many other Vaccinium
genotypes. \textit{Vaccinium} samples ranged from 34 to 515 mg ACY/100 g; ORAC values in \textit{Vaccinium} ranged from 19 to 131 mmol TE/g (Table 1).

Within highbush blueberry (\textit{V. corymbosum} L.) seedlings and cultivars, ACY varied from 73 to 430 mg/100 g, compared to reported values of 25–495 mg/100 g (7), 93–235 mg/100 g (11), or 39–331 mg/100 g (20). Bilberry, \textit{V. myrtillus}, was unavailable for our analyses.

Values of ACY, TPH, ORAC, and FRAP for the cultivated highbush blueberry, \textit{V. corymbosum} cvs. Bluecrop, Duke, and Rubel, and \textit{V. constablaei x ashei} hybrid cv. Little Giant, were consistent with those in other reports (11, 21). Reported ORAC for Bluecrop\(^2\) was 17.0 (11) or 60.1 mmol TE/g (21); we report a value of 50.0 mmol TE/g. ORAC values for the blueberry
Table 2. Total Anthocyanin Content (ACY), Berry Size, Total Phenolics (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 37 Rubus Species and Cultivars

<table>
<thead>
<tr>
<th>genotype</th>
<th>ACY mg/100 g¹</th>
<th>berries/100 g</th>
<th>TPH mg/100 g¹</th>
<th>ORAC µmol TE/g</th>
<th>FRAP µmol/g</th>
<th>ACY/TPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubus species blackberries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. cym Juz.</td>
<td>143 ± 3.8</td>
<td>42</td>
<td>545 ± 14</td>
<td>46.2</td>
<td>71.4</td>
<td>0.26</td>
</tr>
<tr>
<td>R. georgicus Focke</td>
<td>89 ± 2.9</td>
<td>57</td>
<td>561 ± 8.2</td>
<td>41.6</td>
<td>96.3</td>
<td>0.16</td>
</tr>
<tr>
<td>R. insularis F. Aresch.</td>
<td>170 ± 3.5</td>
<td>27</td>
<td>472 ± 14</td>
<td>51.4</td>
<td>97.1</td>
<td>0.36</td>
</tr>
<tr>
<td>R. ursinus (Cham. &amp; Schult.) G4-19</td>
<td>206 ± 1.0</td>
<td>87</td>
<td>678 ± 13</td>
<td>78.8</td>
<td>93.5</td>
<td>0.30</td>
</tr>
<tr>
<td>R. ursinus G4 bulk</td>
<td>211 ± 0.2</td>
<td>108</td>
<td>629 ± 7.2</td>
<td>60.4</td>
<td>78.8</td>
<td>0.34</td>
</tr>
<tr>
<td>means</td>
<td>164 ± 3.6</td>
<td>64.2</td>
<td>577 ± 5.6</td>
<td>55.7</td>
<td>87.6</td>
<td>0.29</td>
</tr>
<tr>
<td>Rubus hybrid blackberries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORUS 1112-2 (Siskiyou × OSC 1717)</td>
<td>94 ± 0.3</td>
<td>13</td>
<td>412 ± 5.5</td>
<td>36.4</td>
<td>86.7</td>
<td>0.23</td>
</tr>
<tr>
<td>ORUS 1122-1 (Oallaie × ORUS 728-3)</td>
<td>181 ± 3.7</td>
<td>10</td>
<td>620 ± 10</td>
<td>56.7</td>
<td>80.4</td>
<td>0.29</td>
</tr>
<tr>
<td>ORUS 1316-1 (ORUS 817R-6 × ORUS 1122-1)</td>
<td>106.5 ± 1.5</td>
<td>12</td>
<td>381 ± 16</td>
<td>40.6</td>
<td>75.5</td>
<td>0.28</td>
</tr>
<tr>
<td>ORUS 1234-1 (ORUS 834-5 × ORUS 1045-14)</td>
<td>124.0 ± 1.1</td>
<td>11</td>
<td>458 ± 19</td>
<td>26.7</td>
<td>46.5</td>
<td>0.27</td>
</tr>
<tr>
<td>ORUS 1369-3 (ORUS 828-42 × ORUS 1122-1)</td>
<td>161.7 ± 1.4</td>
<td>14</td>
<td>454 ± 7.8</td>
<td>38.6</td>
<td>63.3</td>
<td>0.35</td>
</tr>
<tr>
<td>ORUS 1382-2 (ORUS 1117-11 × ORUS 728-3)</td>
<td>217.3 ± 9.0*</td>
<td>15</td>
<td>650 ± 25*</td>
<td>70.5</td>
<td>91.2</td>
<td>0.33</td>
</tr>
<tr>
<td>ORUS 1431-1 (Black Douglass × Lumpy × Walt)</td>
<td>130.4 ± 2.7*</td>
<td>18</td>
<td>609 ± 28*</td>
<td>53.5</td>
<td>93.3</td>
<td>0.21</td>
</tr>
<tr>
<td>ORUS 1439-1 (Black Douglass × (Long Black × Mono))</td>
<td>106.0 ± 0.2</td>
<td>17</td>
<td>381 ± 5.6</td>
<td>36.1</td>
<td>53.8</td>
<td>0.28</td>
</tr>
<tr>
<td>ORUS 1452-1 (Black Douglass × Kataka)</td>
<td>128.0 ± 0.1</td>
<td>21</td>
<td>481 ± 2.3</td>
<td>52.4</td>
<td>69.4</td>
<td>0.27</td>
</tr>
<tr>
<td>ORUS 1714 (Chester × Cherokee)</td>
<td>168.3 ± 3.1</td>
<td>26</td>
<td>428 ± 6.3</td>
<td>47.8</td>
<td>60.5</td>
<td>0.39</td>
</tr>
<tr>
<td>ORUS 1719A (R. caucasicus × Chester)</td>
<td>148.6 ± 0.6</td>
<td>35</td>
<td>641 ± 30</td>
<td>60.5</td>
<td>87.2</td>
<td>0.23</td>
</tr>
<tr>
<td>ORUS 1719F (R. caucasicus × Chester)</td>
<td>156.0 ± 2.5</td>
<td>26</td>
<td>395 ± 5.6</td>
<td>39.0</td>
<td>40.6</td>
<td>0.29</td>
</tr>
<tr>
<td>ORUS 1719H (R. caucasicus × Chester)</td>
<td>134.1 ± 1.4</td>
<td>24</td>
<td>560 ± 3.3</td>
<td>45.2</td>
<td>73.7</td>
<td>0.24</td>
</tr>
<tr>
<td>ORUS 1719K (R. caucasicus × Chester)</td>
<td>109.1 ± 1.1</td>
<td>34</td>
<td>441 ± 0.6</td>
<td>40.4</td>
<td>63.2</td>
<td>0.25</td>
</tr>
<tr>
<td>ORUS 1722 (R. caucasicus × Cherokee)</td>
<td>80.0 ± 0.1</td>
<td>26</td>
<td>355 ± 15</td>
<td>33.3</td>
<td>56.8</td>
<td>0.23</td>
</tr>
<tr>
<td>ORUS 1723 (R. caucasicus × Cherokee)</td>
<td>152.0 ± 0.0</td>
<td>37</td>
<td>433 ± 12</td>
<td>45.0</td>
<td>48.9</td>
<td>0.35</td>
</tr>
<tr>
<td>ORUS 1726-1 (R. georgicus × Cherokee)</td>
<td>143.0 ± 2.9</td>
<td>29</td>
<td>509 ± 11</td>
<td>70.6</td>
<td>92.7</td>
<td>0.28</td>
</tr>
<tr>
<td>ORUS 1880 (R. georgicus × Cherokee)</td>
<td>97 ± 0.5</td>
<td>12</td>
<td>394 ± 10</td>
<td>43.8</td>
<td>48.8</td>
<td>0.25</td>
</tr>
<tr>
<td>NZ 9125R-1</td>
<td>113 ± 0.6*</td>
<td>13</td>
<td>378 ± 4.0</td>
<td>35.1</td>
<td>47.9</td>
<td>0.30</td>
</tr>
<tr>
<td>NZ 9351-4</td>
<td>169 ± 3.6</td>
<td>13</td>
<td>444 ± 1.4</td>
<td>51.2</td>
<td>64.7</td>
<td>0.38</td>
</tr>
<tr>
<td>NZ 9620R-1</td>
<td>215.0 ± 0.5</td>
<td>13</td>
<td>545 ± 5.8</td>
<td>52.7</td>
<td>78.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Cherokee [erect]</td>
<td>123.4 ± 4.5</td>
<td>13</td>
<td>407 ± 20</td>
<td>39.7</td>
<td>58.7</td>
<td>0.30</td>
</tr>
<tr>
<td>Chester [semierect]</td>
<td>164.1 ± 1.1</td>
<td>15</td>
<td>361 ± 8.1</td>
<td>47.5</td>
<td>56.9</td>
<td>0.45</td>
</tr>
<tr>
<td>Marion [trailing]</td>
<td>230.0 ± 2.1</td>
<td>21</td>
<td>560 ± 5.3</td>
<td>69.5</td>
<td>98.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Navaho [erect]</td>
<td>126 ± 2.5</td>
<td>17</td>
<td>304 ± 6.2</td>
<td>38.8</td>
<td>58.9</td>
<td>0.41</td>
</tr>
<tr>
<td>Siskiyou [trailing]</td>
<td>133.7 ± 3.7</td>
<td>11</td>
<td>543 ± 10</td>
<td>47.3</td>
<td>106.6</td>
<td>0.24</td>
</tr>
<tr>
<td>Triple Crown [semierect]</td>
<td>113 ± 3.9</td>
<td>10</td>
<td>275 ± 0.3</td>
<td>35.1</td>
<td>43.4</td>
<td>0.41</td>
</tr>
<tr>
<td>means</td>
<td>141 ± 27</td>
<td>18.7</td>
<td>460 ± 71.8</td>
<td>46.4</td>
<td>68.3</td>
<td>0.31</td>
</tr>
<tr>
<td>Rubus species raspberries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. innominatus S. Moore</td>
<td>52.6 ± 0.6</td>
<td>64</td>
<td>126 ± 0.3</td>
<td>13.1</td>
<td>19.9</td>
<td>0.41</td>
</tr>
<tr>
<td>R. niveus Thumb.</td>
<td>230.2 ± 2.2</td>
<td>56</td>
<td>402.0 ± 0.7</td>
<td>45.2</td>
<td>69.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Rubus occidentalis L. and hybrid black raspberries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earlysweet</td>
<td>464.7 ± 7.8</td>
<td>74</td>
<td>897 ± 32</td>
<td>100.3</td>
<td>205.6</td>
<td>0.52</td>
</tr>
<tr>
<td>Jewel</td>
<td>607 ± 2.5</td>
<td>52</td>
<td>1079 ± 34</td>
<td>146.0</td>
<td>184.1</td>
<td>0.56</td>
</tr>
<tr>
<td>Munger</td>
<td>627 ± 8.3*</td>
<td>71</td>
<td>800 ± 30</td>
<td>104.6</td>
<td>169.1</td>
<td>0.70</td>
</tr>
<tr>
<td>means</td>
<td>566 ± 63</td>
<td>66</td>
<td>955 ± 76</td>
<td>117.0</td>
<td>186.3</td>
<td>0.60</td>
</tr>
<tr>
<td>overall means</td>
<td>179 ± 8.9</td>
<td>31</td>
<td>505 ± 127</td>
<td>52.4</td>
<td>79.2</td>
<td>0.34</td>
</tr>
</tbody>
</table>

¹ Mean ± SEM (n = 2, unless: ² for n = 3, ³ for n = 4, or ⁴ for n = 5).


**Table 3. Total Anthocyanin Content (ACY), Berry Size, Total Phenolics (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 40 Ribes Genotypes**

<table>
<thead>
<tr>
<th>genotype</th>
<th>ACY mg/100 g</th>
<th>TPH mg/100 g</th>
<th>ORAC µmol TE/g</th>
<th>FRAP µmol/g</th>
<th>ACY/TPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captivator</td>
<td>14 ± 0.4</td>
<td>27</td>
<td>191 ± 17</td>
<td>17.0</td>
<td>25.2</td>
</tr>
</tbody>
</table>

**R. uva-crispa L. (** *R. grossularia* L.) **Gooseberry

<table>
<thead>
<tr>
<th>cultivars</th>
<th>ACY mg/100 g</th>
<th>TPH mg/100 g</th>
<th>ORAC µmol TE/g</th>
<th>FRAP µmol/g</th>
<th>ACY/TPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alagan</td>
<td>169 ± 6.0</td>
<td>121</td>
<td>694 ± 33</td>
<td>92.0</td>
<td>106.7</td>
</tr>
<tr>
<td>Baldwin</td>
<td>186 ± 2.0</td>
<td>99</td>
<td>807 ± 4.7</td>
<td>54.0</td>
<td>64.6</td>
</tr>
<tr>
<td>Beloruskaja sladkaja</td>
<td>157 ± 0.8</td>
<td>116</td>
<td>910 ± 7.9</td>
<td>51.3</td>
<td>101.2</td>
</tr>
<tr>
<td>Ben Conan</td>
<td>162 ± 4.2</td>
<td>66</td>
<td>498 ± 15</td>
<td>47.4</td>
<td>74.1</td>
</tr>
<tr>
<td>Ben Lomond</td>
<td>261 ± 5.2</td>
<td>91</td>
<td>933 ± 36</td>
<td>45.5</td>
<td>88.8</td>
</tr>
<tr>
<td>Ben Nevis</td>
<td>252 ± 6.2</td>
<td>60</td>
<td>815 ± 25</td>
<td>44.9</td>
<td>77.1</td>
</tr>
<tr>
<td>Blackdown</td>
<td>216 ± 1.0</td>
<td>128</td>
<td>812 ± 33</td>
<td>38.0</td>
<td>78.4</td>
</tr>
<tr>
<td>Boskoop</td>
<td>240 ± 2.7</td>
<td>125</td>
<td>796 ± 3.6</td>
<td>69.2</td>
<td>92.8</td>
</tr>
<tr>
<td>Consort</td>
<td>411 ± 12</td>
<td>176</td>
<td>1342 ± 28</td>
<td>93.1</td>
<td>114.0</td>
</tr>
<tr>
<td>Coronet</td>
<td>231 ± 0.6</td>
<td>178</td>
<td>704 ± 4.0</td>
<td>48.4</td>
<td>91.6</td>
</tr>
<tr>
<td>Crusader</td>
<td>319 ± 0.6</td>
<td>158</td>
<td>727 ± 10</td>
<td>50.5</td>
<td>89.4</td>
</tr>
<tr>
<td>Dosz Sibejeck</td>
<td>259 ± 2.0</td>
<td>201</td>
<td>928 ± 7.7</td>
<td>44.9</td>
<td>79.0</td>
</tr>
<tr>
<td>Hystawneznaja</td>
<td>156 ± 1.2</td>
<td>107</td>
<td>791 ± 11</td>
<td>49.2</td>
<td>89.4</td>
</tr>
<tr>
<td>Kantata</td>
<td>180 ± 4.8</td>
<td>87</td>
<td>552 ± 5.6</td>
<td>42.5</td>
<td>62.8</td>
</tr>
<tr>
<td>Kantata 50</td>
<td>207 ± 2.4</td>
<td>115</td>
<td>551 ± 30</td>
<td>36.9</td>
<td>67.2</td>
</tr>
<tr>
<td>Kirovchanka</td>
<td>263 ± 10</td>
<td>118</td>
<td>675 ± 31</td>
<td>65.5</td>
<td>118.2</td>
</tr>
<tr>
<td>Koamiczeskaja</td>
<td>221 ± 16</td>
<td>88</td>
<td>763 ± 27</td>
<td>62.2</td>
<td>108.7</td>
</tr>
<tr>
<td>Minaj Smylyrov</td>
<td>158 ± 1.2</td>
<td>126</td>
<td>808 ± 18</td>
<td>44.5</td>
<td>84.1</td>
</tr>
<tr>
<td>Neospjusjastaja</td>
<td>220 ± 7.1</td>
<td>165</td>
<td>554 ± 24</td>
<td>52.7</td>
<td>67.3</td>
</tr>
<tr>
<td>Nikkala Xi</td>
<td>257 ± 0.8</td>
<td>105</td>
<td>650 ± 14</td>
<td>62.7</td>
<td>99.4</td>
</tr>
<tr>
<td>Ojeyb</td>
<td>165 ± 3.5</td>
<td>102</td>
<td>830 ± 33</td>
<td>54.9</td>
<td>61.5</td>
</tr>
<tr>
<td>Pinot Deboir</td>
<td>208 ± 2.7</td>
<td>102</td>
<td>710 ± 13</td>
<td>47.7</td>
<td>88.8</td>
</tr>
<tr>
<td>Polar</td>
<td>213 ± 3.1</td>
<td>132</td>
<td>752 ± 19</td>
<td>59.4</td>
<td>95.5</td>
</tr>
<tr>
<td>Risager</td>
<td>181 ± 5.8</td>
<td>119</td>
<td>797 ± 24</td>
<td>54.4</td>
<td>108.7</td>
</tr>
<tr>
<td>Silvigerets Zwarte</td>
<td>346 ± 10</td>
<td>130</td>
<td>1059 ± 16</td>
<td>65.1</td>
<td>105.8</td>
</tr>
<tr>
<td>Siltsa</td>
<td>128 ± 1.7</td>
<td>109</td>
<td>632 ± 0.8</td>
<td>64.0</td>
<td>62.4</td>
</tr>
<tr>
<td>Strata</td>
<td>298 ± 0.6</td>
<td>134</td>
<td>883 ± 23</td>
<td>57.5</td>
<td>100.3</td>
</tr>
<tr>
<td>Titania</td>
<td>281 ± 3.9</td>
<td>92</td>
<td>890 ± 28</td>
<td>54.7</td>
<td>100.4</td>
</tr>
<tr>
<td>Tsma</td>
<td>180 ± 6.0</td>
<td>90</td>
<td>800 ± 6.7</td>
<td>52.1</td>
<td>102.4</td>
</tr>
<tr>
<td>Tunnja</td>
<td>275 ± 4.9</td>
<td>107</td>
<td>900 ± 33</td>
<td>77.7</td>
<td>99.0</td>
</tr>
<tr>
<td>Wasall</td>
<td>199 ± 6.9</td>
<td>94</td>
<td>742 ± 4.1</td>
<td>58.9</td>
<td>95.5</td>
</tr>
<tr>
<td>Willoughby</td>
<td>275 ± 3.9</td>
<td>131</td>
<td>1122 ± 26</td>
<td>73.4</td>
<td>145.9</td>
</tr>
<tr>
<td>means</td>
<td>229 ± 44</td>
<td>117</td>
<td>799 ± 121</td>
<td>57.1</td>
<td>92.0</td>
</tr>
</tbody>
</table>

**R. odoratum Wendl.**

| Crandall | 273 ± 1.0 | 74 | 958 ± 33 | 68.0 | 107.8 | 0.28 |

**R. valdivianum Phil.**

| overall means | 207 ± 61 | 126 | 748 ± 209 | 53.9 | 86.7 | 0.28 |

---

*yoxycantho"s L. cv. Jahn’s Prairie displayed anthocyanin—phenolic degradation, even when the acetone extract was heated to boiling as recommended for samples with high polyphenol oxidase activity (15). Thus, ACY, TPH, and antioxidant capacities were not measured for these gooseberries.

Ribes × nigroliria ORUS selections ACY values were higher than those for Captivator, but were lower than R. nigrum cultivars (Table 3). Our ACY values for R. nigrum cvs. Ojeyb, Ben Lomond, and Titania were lower than those reported from Poland (26). Our TPH samples ranged from 14 to 411 mg ACY/100 g; Banaszczyk and Pluta (26) observed R. nigrum cv. Ben Alder to have 467 mg ACY/100 g. Our ORAC values in Ribes ranged from 17 to 116 µmol TE/g (Table 3).

**Antioxidant Capacity: ORAC and FRAP.** The range of our ORAC values may be higher than those previously reported (20, 21, 24) because of the following:

1. The goal of our extraction method (15), is to determine the maximal amount of ACY, TPH, and antioxidant capacity that exist in a plant sample. The liquid nitrogen milling and acetone extraction procedures minimize enzyme activity and oxidation, especially after thawing or grinding has damaged the fruit. Some published extraction methods contain steps in which berries are allowed to thaw or incubate at room temperature before steps are taken to limit enzyme activity and oxidation. *Rubus* samples are especially susceptible to berry damage during picking, transportation, and thawing before extraction. A portion of the relatively large *Rubus* seeds were also ground during high-speed milling and thus contributed to TPH and antioxidant capacity.

2. Berries were picked in small lots and placed on ice in the field immediately, then frozen the same day, usually within 2 h.
Table 4. Correlation Coefficients ($r$) of Size, Total Anthocyanins (ACY), Total Phenolics (TPH), and Antioxidant Capacity (ORAC and FRAP)*  

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinium ($n = 31$)</td>
<td>0.55**</td>
<td>0.93**</td>
<td>0.73**</td>
<td>0.88**</td>
<td>0.79**</td>
<td>0.93**</td>
</tr>
<tr>
<td>V. corymbosum ($n = 15$)</td>
<td>0.84**</td>
<td>0.93**</td>
<td>0.78**</td>
<td>0.96**</td>
<td>0.81**</td>
<td>0.94**</td>
</tr>
<tr>
<td>Vaccinium, 8 species ($n = 16$)</td>
<td>0.29</td>
<td>0.93**</td>
<td>0.69**</td>
<td>0.85**</td>
<td>0.76**</td>
<td>0.92**</td>
</tr>
<tr>
<td>Rubus ($n = 37$)</td>
<td>0.45**</td>
<td>0.83**</td>
<td>0.90**</td>
<td>0.85**</td>
<td>0.92**</td>
<td>0.90**</td>
</tr>
<tr>
<td>Rubus hybrids (blackberries) ($n = 27$)</td>
<td>0.003</td>
<td>0.57**</td>
<td>0.70**</td>
<td>0.38**</td>
<td>0.73**</td>
<td>0.75**</td>
</tr>
<tr>
<td>Rubus ($n = 40$)</td>
<td>0.46**</td>
<td>0.82**</td>
<td>0.71**</td>
<td>0.74**</td>
<td>0.81***</td>
<td>0.88**</td>
</tr>
<tr>
<td>Rubus nigrum ($n = 32$)</td>
<td>0.41*</td>
<td>0.63**</td>
<td>0.58*</td>
<td>0.46**</td>
<td>0.44**</td>
<td>0.55**</td>
</tr>
<tr>
<td>all samples ($n = 108$)</td>
<td>0.43**</td>
<td>0.73**</td>
<td>0.79**</td>
<td>0.80**</td>
<td>0.84**</td>
<td>0.84**</td>
</tr>
</tbody>
</table>

* $* = p < 0.05; ** = p < 0.005.$

(3) The highest ORAC values observed in the Vaccinium population belonged to wild plants and seedlings, not the cultivars.

(4) Significant location and year-to-year effects on antioxidant activity have been reported in nine highbush blueberry cultivars (13). ACY levels of eight of the hybrid blackberries in this study ranged from 62 to 165% of the values reported by Fan-Chiang (23). The same extraction method was used in both studies; but the fruit samples of Fan-Chiang (23) were collected during the 1996, 1997, or 1998 growing seasons.

Correlations. ACY and TPH. Anthocyanins comprise a significant fraction of the TPH in Vaccinium and Rubus (ACY/TPH ratios, Tables 1, 2, and 3). The ACY/TPH ratio was lowest (0.28) in Ribes, not due to less ACY than the other two genera but to markedly higher TPH. Correlations of ACY to TPH were highest for Vaccinium, but were significant ($p = 0.005$) for all three genera (Table 4). Though ACY may indicate TPH, the range observed in ACY/TPH ratios (Tables 1–3) precludes prediction of ACY from TPH and vice versa, for a single genotype.

Influence of Anthocyanin and Phenolic Levels on Antioxidant Capacity. ACY averaged 34% of TPH in the 108 samples we studied, thereby contributing significantly to the overall antioxidant capacity (4, 6). Ascorbate levels contributed <1% to the total antioxidant capacity in highbush and lowbush blueberries (21) and <10% in a diversity of Rubus species (12), and are not reported for the samples in this study. Correlation coefficients between ACY or TPH and ORAC or FRAP (Table 4) are significant across the Vaccinium, Rubus, and Ribes sample sets and their subsets. ACY and TPH correlated highly with ORAC values, though in every case ($n = 7$) TPH:ORAC correlations were slightly higher than ACY:ORAC correlations. TPH:FRAP correlations were higher in six of seven cases compared to ACY:FRAP correlations (Table 4).

Ribes inominatus (Table 2) and Captivator cv. gooseberry (Table 3) are lowest in ACY and TPH within their respective genus, leading to the lowest ORAC and FRAP values as well. Despite the lowest ACY and low TPH, the red-fruited V. parvifolium (Table 1) has average ORAC and FRAP values for the 30 Vaccinium genotypes tested. Deighton et al. (12) reported an extreme case of decoupled ACY and FRAP when the yellow-fruited Rubus lambertianus Ser., devoid of ACY but high in TPH, displayed a FRAP value higher than several other red or black fruited Rubus species, many of which contained significant ACY. We primarily chose from among the most highly pigmented small fruits available, which may have influenced the significant correlations observed between ACY and TPH, as well as between ACY and total antioxidant capacities as measured by ORAC or FRAP.

The correlation of TPH:ORAC for Ribes nigrum ($r = 0.44$) is much lower than that for Vaccinium and Rubus species (Table 4). This low Ribes TPH:ORAC correlation may be due to the high ascorbic acid content of black currant fruit (26) that could contribute to ORAC but could not be measured by TPH. This could also account, in part, for the regression line slope differences in Figure 1.

The high correlation between ORAC and FRAP for all 108 samples ($r = 0.84$) suggests that either of these two measurements have validity for determining antioxidant activity with these fruits. The mechanisms of these two assays are distinct: the ability to trap a free radical with ORAC vs ferric ion reduction with FRAP. It should be pointed out, however, that in vitro measurement of antioxidant capacity may or may not reflect what happens in vivo. Very little is known about the absorption and metabolism of these compounds.

Regression Analysis. Scatterplots of TPH vs antioxidant capacity (as FRAP) are presented in Figure 1 for Vaccinium, Rubus, or Ribes genotypes. The spread of values within each genus can be observed, as well as two clusters of highest FRAP values: Vaccinium ashei Reade (rabbiteye blueberries) and Rubus occidentalis L. (black raspberries). See Table 1 (Vaccinium) and Table 2 (Rubus) for individual values. Ribes validianum is not included in Figure 1 Ribes, as the TPH for this species is 63% more than the next highest value, Ribes nigrum cv. Willoughby (Table 3).

Berry Size Correlation. The highest correlation ($r = 0.82$, $p = 0.005$) between berry size and ACY (Table 4) was observed for highbush blueberry genotypes ($n = 15$). In highbush blueberries, pigments reside exclusively in the skin. For a given volume of fruit, the amount of skin or surface area increases as berry size decreases; leading to the general observation that smaller highbush blueberries contain more anthocyanins per unit volume (11). Yet, ACY and berry size was not correlated ($r = 0.29$; $n = 16$) across eight other Vaccinium species and hybrids (Table 4). Perhaps if a range of clones differing in berry size within each species were examined, a significant correlation with total ACY could be observed. The relatively large berries of CVAC 1161 and CVAC 1170, rabbiteye blueberries (V. ashei Reade), contained the highest ACY within the 31 Vaccinium samples (Table 1). Therefore, larger berries of one Vaccinium species may still contain more ACY as compared to smaller berries of a different species. We made no attempts to compare blueberry skin thickness or berry shape, other potential sources of divergent ACY levels, though we do note that the large-fruited highbush cultivars had less spherical berries than the smaller-fruited highbush species material in our study.

The lack of a berry size-to-ACY correlation ($r = 0.003$, $n = 27$) for blackberry hybrids (Table 4) was anticipated, as druplet skin is quite thin and flesh is very dark. The current trend in Rubus breeding toward larger berry size appears to have had little effect on ACY, for the full range of ACY is encompassed both by cultivars and advanced breeding selections (Table 2).

ACY in black currant cultivars (Ribes nigrum, $n = 32$) was significantly affected by berry size ($r = 0.41$, $p = 0.05$), but to
a much lesser degree than in highbush blueberries. Black currants have undergone selection for hundreds of years (27), whereas cultivars of highbush blueberries were developed much more recently (28). Ribes valdivianum, a black currant native to Chile, is an extreme example of the relationship between fruit size and ACY within Ribes (Table 3). Even at 907 berries/100 g, ACY of R. valdivianum was comparable to that of R. nigrum cultivars 5 to 8 times as large. Only one gooseberry, cv. Captivator (R. uva-crispa L.) was evaluated (Table 3).

**Anthocyanin Levels and Flesh Color.** A few samples we examined contained dark skin and dark flesh: the ORUS 6-10 series of Ribes × nidigrolaria Bauer (Table 3), and the V. membranaceum Douglas ex Torrey selections (Table 1). Surprisingly, these were among the lowest in ACY, as compared to that of fruits with nonpigmented flesh. We note that the ACY extraction method used in this study removes virtually all the skin pigments, whereas after commercial pressing for blueberry juice, substantial ACY remains in the skin-rich press-cake (29). Thus for certain food processing applications, dark-fleshed Vaccinium or Ribes berries may still represent a valued source of ACY.

**Maturity Effects.** Summit, a Southern highbush blueberry cultivar, is represented twice (Table 1), as “Summit” and “Summit II”. Ripe fruit was picked from the same field very early (July 10) and very late (August 24) in the harvest season for this cultivar. For ACY, TPH, ORAC, and FRAP, increases of 163%, 175%, 182%, and 129% occurred during the growing season. Both maturity (11) and postharvest storage (21, 30) have been reported to increase Vaccinium ACY levels. The Summit ACY/TPH ratios remained nearly constant over time, 0.345 and 0.323, perhaps due to a concomitant rise in ACY and TPH. A significant 250% decrease in berry size was observed over time, which altered the skin to whole fruit ratio between the two samples. In Vaccinium, changes in berry size and thus skin area per berry should be considered in studies of maturity effects on ACY or antioxidant capacity.

**Implications for Fruit Selection and Breeding.** Vaccinium. The upper range of ACY (Table 1) for seedling highbush blueberries such as CVAC 5.001 and CVAC 23.001 indicates that cultivars with increased ACY, TPH, and antioxidant capacity may still be selected or developed from wild V. corymbosum L. material. Four rabbiteye selections had among the highest antioxidant capacities of all Vaccinium samples examined (n = 31) reflecting very high levels of both ACY and TPH (Figure 1 Vaccinium). Bluegem, the lone V. ashei cultivar tested, had an ORAC value more than twice that of any highbush (V. corymbosum L.) cultivar tested (n = 7). Magee (30) reported a similar 2-fold level of ACY in two rabbiteye cultivars compared to three southern highbush cultivars, whereas Prior et al. (11) observed equivalent ORAC means for several V. ashei vs V. corymbosum cultivars. The two Vaccinium ovatum (“evergreen huckleberry”) selections we tested were also quite high in ACY, TPH, and antioxidant capacity.

Ribes. Of the 32 blackberries tested, Marion cv. had the highest ACY levels and was among the highest in ORAC and FRAP values (Table 2). Yet, the full range of TPH and FRAP diversity is displayed among hybrid blackberry cultivars, recent crosses, and advanced selections (Table 2, Figure 1 Ribes). There is a significant gap reported (Table 2, Figure 1 Ribes) between R. ursinus hybrid blackberry and black raspberry (R. occidentalis) values; Earlysweet, Jewel and Munger black raspberries cultivars have phytochemical and antioxidant values well above those of all other Ribes in this study. Deighton et al. (12) found the blackberry R. caucasicus Focke with the highest antioxidant capacity of 18 selections from 12 Ribes species, and posited R. caucasicus as a donor to increase antioxidant capacity in blackberry hybrids. R. caucasicus fruit was unavailable for this study, but six R. caucasicus × Chester Thornless or Cherokee hybrids were examined (Table 2). One of these, ORUS1719A (R. caucasicus × Chester Thornless) was

**Figure 1.** Influence of total phenolic content (TPH) on antioxidant capacity (as ORAC) of Vaccinium, Rubus, and Ribes samples. The Ribes regression (n = 39) does not include R. valdivianum.
high in TPH and antioxidant capacity as compared to other blackberry hybrids, confirming the potential value of R. cassinus crosses, as predicted by Deighton et al. (12).

Ribes. Compared to highbush blueberries, blackcurrant (R. nigrum L.) cultivars displayed a lower correlation ($r = 0.41$) between size and ACY levels (Table 4). In addition, the correlation ($r = 0.30$) between size and TPH in blackcurrants ($n = 32$) was not significant. Larger fruited R. nigrum cultivars might be selected without concomitant lowering of ACY or TPH.

CONCLUSION

Our survey of small fruit germplasm for ACY, TPH, and antioxidant capacity shows distinctions in each of three genera examined, and confirms each as an excellent source of dietary phytochemicals.

ABBREVIATIONS USED

ACY, total anthocyanin content; TPH, total phenolic content; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant power; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

ACKNOWLEDGMENT

We thank Bob Durst for technical support, and Deborah J. Hobbs for performing the ORAC and FRAP analyses. We acknowledge Matthew J. Glaser for helping with sample extractions.

LITERATURE CITED


Received for review August 8, 2001. Revised manuscript received November 7, 2001. Accepted November 7, 2001. This research was partially supported by USDA ARS CRIS 53582-21000-026-00D, Oregon State University, and the King College sabbatical program. This is technical paper no. 11786 from the Oregon Agricultural Experiment Station.