J. Amer. Soc. Hort. Sci. 111(6):945–950. 1986.

Anthocyanin Patterns in Ripening Thornless Blackberries

Gerald M. Sapers, Kevin B. Hicks, Anita M. Burgher, Donna L. Hargrave, Scott M. Sondey, and Alexander Bilyk

Agricultural Research Service, U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Additional index words. *Rubus*, cultivars, composition

Abstract. **The pigments of thornless blackberry fruit were investigated by analytical and preparative high performance liquid chromatography (HPLC) to characterize anthocyanin patterns in representative cultivars and to determine the effects of ripening on these patterns. Cyanidin-3-glucoside and cyanidin-3-rutinoside were positively identified. In addition, a cyanidin derivative containing xylose and 2 dicarboxylic acid-acylated derivatives of cyanidin-3-glucoside were tentatively identified. Five different anthocyanin patterns were seen among the 33 cultivars and selections compared. During ripening, cyanidin-3-glucoside increased to a greater extent than the other pigments. The accumulation of individual anthocyanins was linearly related to the total anthocyanin content and to the soluble solids : acidity ratio. Cultivar differences in anthocyanin patterns and changes in individual anthocyanins in fruit approaching full ripeness are insufficient to influence fruit color.**

The characteristic color of blackberries and other fruits of *Rubus* species is due to the presence of various glycosides of cyanidin and pelargonidin (2). These anthocyanins have been shown to fall into characteristic patterns for different species and cultivars (3, 17) that are under genetic control (4, 15). In a study of 19 blackberry species (17), cyanidin-3-glucoside (Cy-3-Glc) and cyanidin-3-rutinoside (Cy-3-Rut) were reported as the major pigments with pelargonidin-3-glucoside present in only one species. Traces of 5 other unidentified pigments were reported. The anthocyanins of thornless blackberry cultivars have not been well-characterized; 'Black Satin' and 'Dirksen Thornless' fruit were reported to contain only Cy-3-Glc and small amounts of 2 unidentified pigments (17). Jennings and Carmichael (8) have demonstrated that the color of blackberries tends to change from black to red when they are frozen—the extent of this change reflecting incomplete ripeness.

Previously, we reported differences in the titratable acidity, soluble solids, total anthocyanin, and pectin content in fruit of 40 thornless blackberry cultivars and selections (13). It was of interest to us in the present study to isolate and characterize the anthocyanins in fruit of thornless blackberries, to determine the extent of quantitative differences in the proportions of individual anthocyanins in representative cultivars, and to determine the effects of ripening on the accumulation of individual anthocyanins in thornless blackberry fruit.

Materials and Methods

Source of blackberries. Ripe fruit of various thornless blackberry cultivars and selections, obtained from plantings at the Beltsville Agricultural Research Center in 1982 and 1983, were cleaned, packaged in polyethylene freezer containers, and stored at −13°C, as described previously (13). After several months' storage, the frozen blackberries were sorted into 2 subsamples: fruit that had retained their original black color, and fruit that had turned red during frozen storage. Additional samples of 3 cultivars ('Black Satin', 'Hull Thornless', and 64-21-3), obtained from Beltsville in 1984, included under-ripe (red and reddish purple) as well as ripe fruit. After freezing, the blackberries were sorted into 8 subsamples, varying in surface color from light red to black.

Fruit analysis and anthocyanin extraction. Anthocyanins were extracted from frozen subsamples by blending about 50 g of fruit with an equal weight of solvent I [95% ethanol : 1.5 M HCl (85:15, v/v)] for 2 min at high speed in a semimicro blending container. The homogenate, mixed with 4 g Celite Analytical Filter Aid, was transferred to a Whatman No. 5 filter disk in a Buchner funnel, and clarified extract was collected under vacuum. The clarified extracts were filtered through a 0.2 μm Millipore membrane filter prior to analysis by HPLC.

To permit the comparison of blackberry composition and HPLC data (1984 ripeness study only), the sample preparation procedure was modified as follows: about 50 g of frozen fruit were homogenized for 2 min at high speed in the semimicro blending container. About 10 ml of homogenate was removed for the determination of soluble solids and titratable acidity, as described previously (13). The remaining homogenate was blended with an equal weight of solvent I for 1 min, and the extract was analyzed spectrophotometrically for total anthocyanin (13). A portion of the extract was analyzed by HPLC.

Analytical HPLC of blackberry anthocyanins. Individual anthocyanins in blackberry extracts were determined by HPLC with a Waters Associates chromatographic system similar to that used previously for blueberry anthocyanins (14). Sample extracts (3–10 μl) were injected onto a Waters 3.9 mm × 15 cm Resolve 5-μ C_{18} steel column. The mobile phase comprised 0.1 M pH 2.0 (pH 1.5 in 1983 and 1984) phosphate buffer (solvent A) and acetonitrile (solvent B), with a gradient of 12% B to 20% B in 25 min, following program 7 of the Waters solvent programmer. The mobile phase flow rate was 2.0 $ml \cdot min^{-1}$. Peaks absorbing at 546 nm were integrated, and the area percentages were calculated.

Pelargonidin was used as an internal standard in the quantitative study of anthocyanin patterns in fruits of different ripeness. One milliliter of a 1% solution of pelargonidin Cl in solution I was added to clarified fruit extracts, which were then made

Received for publication 7 Feb. 1985. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned. We acknowledge Gene J. Galletta and Lester W. Greeley of the USDA's Beltsville Agricultural Research Center in Beltsville, Md., who provided us with samples of thornless blackberries, and Samuel Asen and Phyllis S. Budin, from the same Center, who provided anthocyanin standards and assisted us in the anthocyanin identifications. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

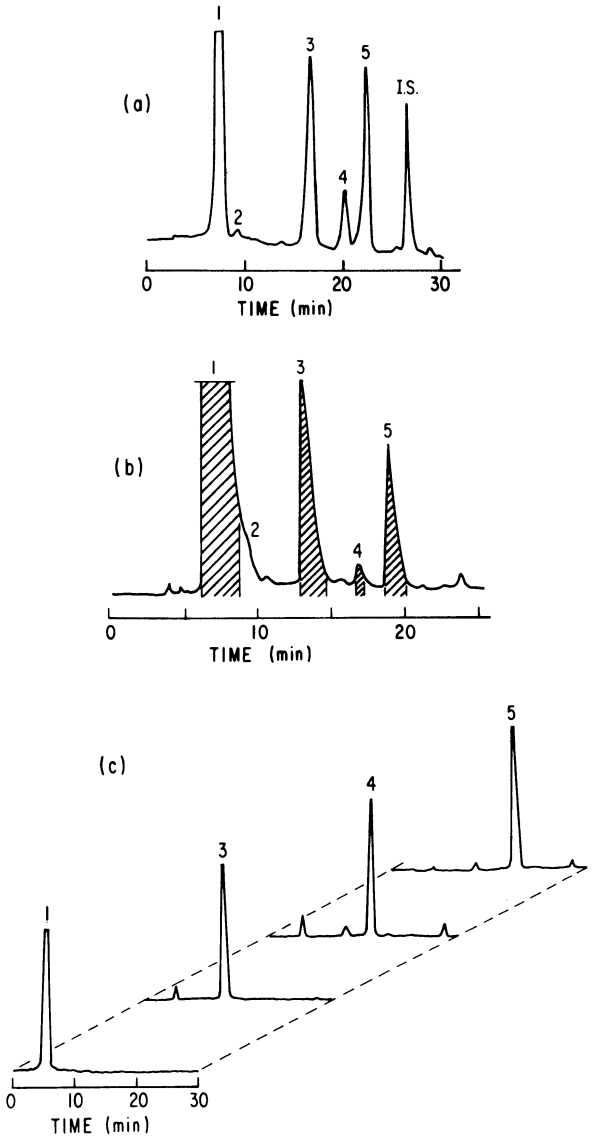

Fig. 1. Analytical and preparative HPLC of blackberry anthocyanins. (**a**) Analytical HPLC of 'Hull Thornless' blackberry. A 5-μl injection of extract analyzed as described. Anthocyanins are labeled 1–5. Pelargonidin was added as an internal standard (I.S.). Detection at 546 nm, 0.1 absorbance units full scale. (**b**) Single injection of blackberry extract (250 μl) on Gilson Gradient Auto Prep. HPLC system with Rainin Dynamax C-18 preparative column as described in the text and elsewhere (7). Peaks 1–5 (cross-hatched areas) were collected, treated as described, and lyophilized. (**c**) Analytical HPLC of fractions collected in b; conditions as in a.

up to 50 ml with solution I. HPLC peak areas were normalized and multiplied by appropriate dilution factors so that the amounts of individual anthocyanins in fruit samples could be estimated. Anthocyanin contents were expressed as area units per 100 g of fruit, since extinction coefficients of the pure pigments in the HPLC mobile phase at 546 nm were not available.

Preparative HPLC of blackberry pigments and their subsequent identification. Blackberry extract (300 ml, prepared as described above from a mixture of 6 different cultivars) was evaporated in vacuo (25°C) in the presence of water (100 ml) to remove alcohol, and then the syrupy concentrate (about 50 ml) was loaded onto a column (9.2 cm × 30 cm) of Amberlite CG-50 (Rohm and Haas, Philadelphia). After eluting soluble sugars and nonbound components with distilled water (2 liters), a crude anthocyanin fraction was eluted with 0.025% (v/v) HCl in methanol. This solution was evaporated in vacuo (25°) in the presence of water to yield a thick syrup (2 ml), which was diluted with 6 ml of 50% (aqueous) methanol and then filtered (nylon 66, 0.2 μm). Samples of this solution (250 μl) containing 15 mg of solids were injected on a Rainin (Rainin Instrument, Woburn, Mass.) Dynamax C-18 preparative (2.2 cm × 25 cm) column in a Gilson Automatic Preparative HPLC System (Gilson Medical Electronics, Middleton, Wis.). The HPLC system and conditions for the separation and isolation of blackberry anthocyanins have already been described (7). Purified anthocyanins from preparative HPLC were characterized by the following procedures. The ultraviolet/visible absorption spectra of pigments (freshly dissolved in 0.1% HCl in methanol) were obtained with a Perkin-Elmer (Oak Brook, Ill.) Model 559 spectrophotometer. Controlled acid hydrolyses were performed by refluxing pigments in EtOH : 2 N HCl (1:1, v/v) for timed intervals as long as 90 min (1). Anthocyanin acyl groups were removed by reacting the pigment in 1% (w/v) KOH (under N_2) for 15 min at room temperature, followed by acidification with 1% HCl. Anthocyanins and the products of acid or base hydrolyses were analyzed by analytical HPLC, or by chromatography on cellulose plates vs. authentic standard compounds or well-characterized natural products. Solvents for TLC analysis of anthocyanins were BAW [4 butanol : 1 acetic acid : 5 H_2O (by volume), upper layer]; 4 phenol : 1 H_2O (v/v); and 2 ethyl acetate : 1 pyridine : 2 H_2O (by volume), upper layer. Sugars were identified by cellulose TLC using the following solvents: 15 acetic acid : 3 HCl : 82 H_2O (by volume); 1% HCl (w/v); and BAW. Visualization was with aniline-hydrogen phthalate reagent.

Results and Discussion

Preliminary characterization of blackberry anthocyanins. HPLC of blackberry extracts revealed 1 major (peak 1), 4 minor (peaks 2–5), and several trace components that could be detected at 546 nm (Fig. 1a). Preparative chromatography (Fig. 1b) of an extract of ripe blackberries (a mixture of 'Chester', 'Hull Thornless', and selections C-48, C-56, C-57, and C-67) yielded purified fractions of components 1, 3, 4, and 5, which were treated with Amberlite CG-50 to remove phosphate buffer (7), lyophilized, and then subjected to analytical HPLC (Fig. 1c) and additional qualitative analyses. Peak 2 was similarly isolated from 'Comanche', a cultivar that contained higher levels of this pigment.

Controlled acid hydrolysis of a sample of the combined extracts (before purification), followed by HPLC, revealed the presence of cyanidin but not pelargonidin or other aglycones. The spectral properties of the purified compounds (Table 1) suggest that all components were 3 substituted glycosides of cyanidin. The wavelengths of maximum absorption in the ultraviolet (UV) (about 280 nm) and in the visible region (about 527 nm) and their absorbance ratios (56–58) are consistent with those previously reported for cyanidin but not for pelargonidin glycosides (6). The ratio of absorbance at 440 nm to the absorbance at the visible maximum was about 22, which is evidence for 3-substituted cyanidin derivatives. The lack of an absorption maximum in the 310–320 nm region suggests that none of the pigments are acylated with aromatic compounds.

Peak 1 was positively identified as Cy-3-Glc by its coelution with that standard compound on analytical HPLC and cellulose thin-layer plates. In addition, peak 1 had appropriate spectral

Table 1. Spectral properties of anthocyanins isolated from blackberries

Peak	Amount isolated (mg)[z]	Purity (%)[y]	λmax Visible	λmax UV	A_{UVmax}/A_{vismax}	A_{440}/A_{vismax}
1	90.8	98	526	280	56 (60)[x]	23 (24)[x]
2	<<1mg	87	528	282	---	---
3	12.4	95	527	280	58	22
4	1.0	78	527	280	68	23
5	9.8	92	528	280	64	22

[z]Amount of lyophilized pigment obtained from 20 repetitive injections as in Fig. 1b.
[y]Based solely on peak area percentage from analytical chromatograms.
[x]Values reported for Cy-3-Glc (6).

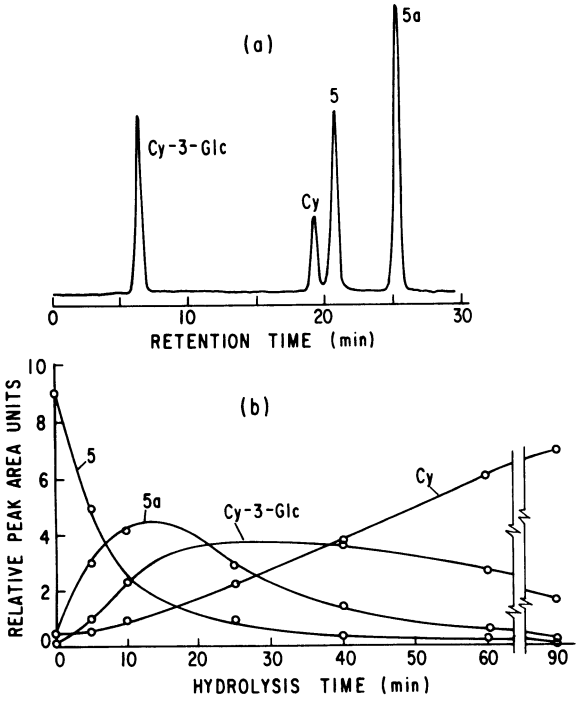

Fig. 2. Controlled acid hydrolysis of peak 5, isolated from blackberry extract. (**a**) Analytical HPLC of partial acid hydrolysis products (10 min of heating) from reaction of pure compound 5. (**b**) Appearance and disappearance of reactant and products over time during the hydrolysis of purified compound 5. See text for explanation.

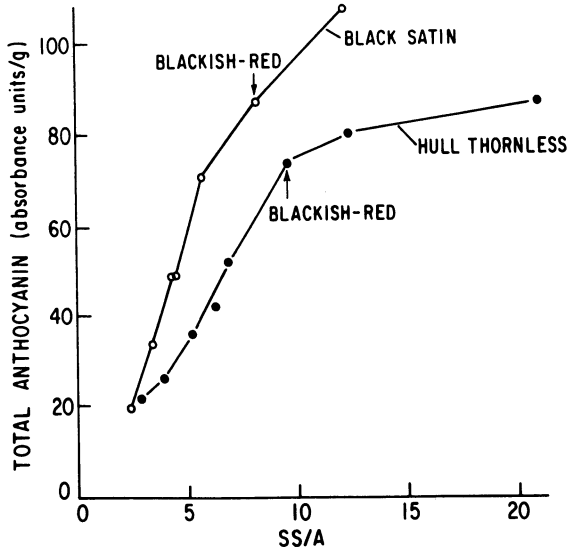

Fig. 3. Total anthocyanin content vs. soluble solids : acidity ratio (SS:A) for 'Black Satin' and 'Hull Thornless' fruit samples.

properties (Table 1, see ref. 6); its acid hydrolysis yielded cyanidin and glucose. This compound is known (17) to be a major pigment of *Rubus* species.

Peak 2 was identified as Cy-3-Rut, another previously identified (17) pigment in *Rubus*. Cy-3-Glc and Cy-3-Rut extracted from rhubarb (18) coeluted with peaks 1 and 2, respectively, on the analytical HPLC system. Upon acid hydrolysis, peak 2 produced Cy-3-Glc and cyanidin as expected. Because of the extremely small amounts of this pigment that were isolated, complete UV/visible spectra could not be obtained.

Peak 3 also is a cyanidin derivative. Controlled base hydrolysis caused no change in R_f values precluding an acylated compound. Acid hydrolysis produced cyanidin and xylose. The presence of xylose is especially noteworthy, since it has been reported (16) that blackberries are incapable of synthesizing xylose-containing pigments. No authentic pigment was available for comparison with compound 3, but reversed phase HPLC separation principles established for anthocyanins (5) would predict that since the putative cyanidin-3-xyloside would be more hydrophobic than Cy-3-Glc or Cy-3-rut, its retention time should be longer than those of the latter pigments, as was observed.

Compounds 4 and 5 were both extremely unstable, based on their rapid fading under ambient conditions. Compound 4, available in very small quantities, gave a complicated acid hydrolysis pattern, producing glucose, Cy-3-Glc, cyanidin, and an unidentified pigment. Compound 5, present in larger quantities, gave similar results and was investigated further. Upon controlled acid hydrolysis, compound 5 gave 3 additional peaks by HPLC (Fig. 2a). During hydrolysis, compound 5 initially produced a 2nd peak, 5a, which in turn declined in concentration as the level of Cy-3-Glc increased (Fig. 2b). Ultimately the Cy-3-Glc peak declined, as expected, to form cyanidin (peak Cy). Controlled base hydrolysis (not shown) yielded only Cy-3-Glc. We suggest that at least peak 5 (and perhaps peak 4) are dicarboxylic acid-acylated derivatives of Cy-3-Glc. Anthocyanins whose sugars are acylated with simple dicarboxylic acids such as malonic or succinic acid recently (1) have been shown to be extremely unstable and to display complex acid hydrolysis behavior. When such pigments are hydrolyzed in acidic aqueous ethanol as in Fig. 2a, the free carboxyl group of the dicarboxylic acid becomes esterified, resulting in a considerably less polar and later-eluting compound on reversed-phase HPLC (see ref. 1; Fig. 2, peak 5a). These unstable compounds rapidly decompose under acidic conditions to produce the deacylated anthocyanin. When compound 5 was hydrolyzed in aqueous acid (no ethanol), only Cy-3-Glc and cyanidin were detected by HPLC. These prelim-

Table 2. Anthocyanin distribution patterns in ripe thornless blackberries

Pattern	Representative clone	Year	Mean HPLC peak area (%) Peak no.[z] 1	2	3	4	5
A	Black Satin	1982	81.3	0.9	8.5	2.4	5.6
		1983	87.5	0.2	5.8	1.7	4.0
B	SIUS 50	1982	94.2	0.2	0.1	1.0	3.8
C	Hull Thornless	1982	72.2	1.0	12.3	3.1	9.4
		1983	72.1	1.0	16.9	2.8	6.4
D	C-57	1982	84.8	0.5	4.2	0.9	8.7
		1983	86.1	0.3	3.3	0.9	7.0
E	Comanche[y]	1983	88.9	7.6	0.2	1.7	---

[z]Pigments detected at 546 nm and designated as peaks 1–5 on basis of HPLC retention time.
[y]Not classified as thornless cultivar.

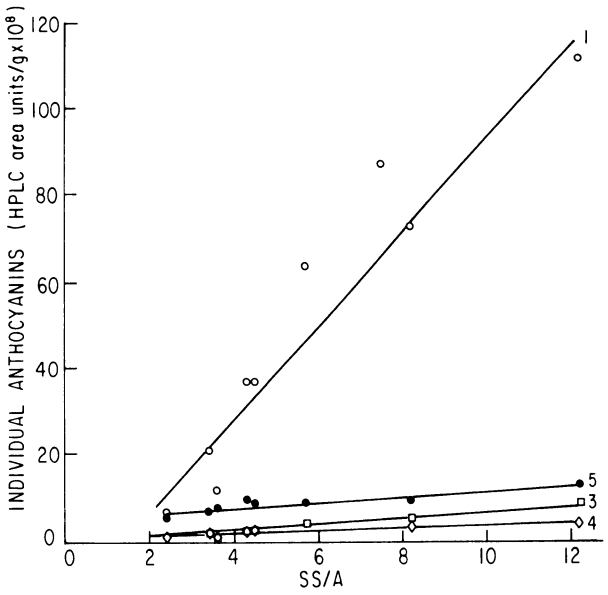

Fig. 4. Individual anthocyanins vs. soluble solids : acidity ratio (SS:A) for 'Black Satin' fruit samples.

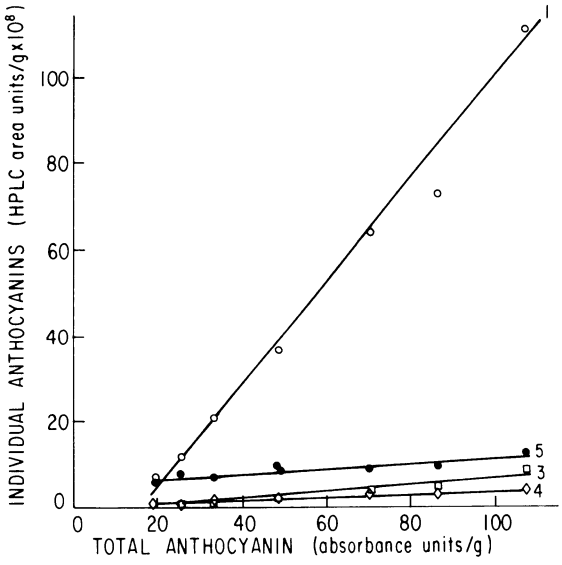

Fig. 5. Individual anthocyanins vs. total anthocyanin content for 'Black Satin' fruit samples.

inary investigations reveal that blackberry contains 2 hitherto unknown types of anthocyanins—those containing xylose, and those that may be acylated with dicarboxylic acids.

Anthocyanin patterns in blackberry clones. The proportions of individual anthocyanins in different cultivars and selections, as determined by HPLC (Fig. 1a), appear to fall into 5 distinct patterns (Table 2). The most common pattern, (A), represented by 'Black Satin' and also seen with 'Chester', 'Smoothstem', 'Thornfree', C-33, C-52, C-62, C-65, SIUS 64-9-3, SIUS 64-21-3, SIUS 64-21-4, SIUS 64-21-10, SIUS 64-36-1, SIUS 64-39-2, SIUS 64-40-2, and US 1520, has a large peak 1, a trace peak 2, and small peaks 3, 4, and 5. A 2nd pattern, (B), represented by SIUS 50 and also seen with C-47 and C-51, is similar to pattern A but with trace amounts of peak 3. A third pattern, (C), represented by 'Hull Thornless' and also seen with 'Dirksen Thornless', C-48, and C-67, is characterized by a smaller proportion of peak 1 and a larger proportion of peak 3 than is obtained with the other patterns. The fourth pattern, (D), represented by C-57 and also seen with C-55, C-56, C-58, C-59, C-60, and C-61, resembles pattern A but with a larger proportion of peak 5, especially in slightly under-ripe samples. These patterns all were obtained with thornless cultivars and selections. A distinctly different pattern, (E), was obtained with samples of the thorny blackberry cultivars 'Comanche', 'Cheyenne', and 'Cherokee', all of which were derived from crosses of 'Darrow' and 'Brazos' at the Univ. of Arkansas (9–11). This pattern is characterized by the elevation of peak 2 and disappearance of peaks 3 and 5. Data collected for 25 clones over 2 seasons indicate that anthocyanin patterns for thornless blackberries were reproducible. The anthocyanin patterns described above did not correspond to any differences among cultivars in spectral properties or tristimulus parameters that would be indicative of an observable color difference in juice or fruit. This probably is a consequence of the fact that the anthocyanins of all blackberry cultivars compared in this study were dominated by one component, Cy-3-Glc, which represented more than 70% of the total pigments.

Anthocyanin patterns in ripening berries. Preliminary comparisons of anthocyanin patterns for red- and black-colored fruits in frozen blackberry samples revealed several consistent differences, the black subsamples containing a greater proportion of component 1 and smaller proportions of components 3 and 5 than the red subsamples. Presumably, this difference represents

Table 3. Anthocyanin pattern and content vs. ripeness.

Cultivar	Subsample	Color[z]	SS:A[y]	Total anthocyanin[x]	Anthocyanin distribution (%)[w] Peak no. 1	3	4	5	Anthocyanin content[v] Peak no. 1	3	4	5
Black Satin	1	Light red	2.4	19.4	43.4	2.6	5.6	41.1	6.8	---	0.9	6.1
	3	Med. red	3.4	33.2	63.9	3.9	4.9	21.3	20.5	1.2	1.6	6.8
	5	Dark red	4.5	48.9	71.4	4.3	3.8	15.8	36.7	2.2	2.0	8.1
	7	Blackish red	8.2	86.6	79.5	5.4	3.3	10.1	72.7	5.0	3.0	9.2
	8	Black	12.2	107.3	80.5	6.2	2.7	8.9	111.8	8.6	3.8	12.4
Hull Thornless	1	Light red	2.9	21.6	31.6	---	7.7	50.5	5.8	0.6	1.4	9.2
	3	Med. red	5.2	31.2	47.5	7.4	6.2	34.3	16.1	2.5	2.1	11.6
	5	Dark red	6.8	46.8	61.5	11.0	5.6	17.9	36.6	6.6	3.3	10.7
	6	Blackish red	9.6	73.3	65.2	12.5	4.4	13.9	55.7	10.7	3.7	11.9
	7	Black	12.4	81.0	66.3	14.5	4.4	12.9	60.8	13.3	4.0	11.9
	8	Black	21.0	86.2	67.0	14.1	3.9	12.0	64.8	13.6	3.7	11.6

[z]In frozen state.
[y]Soluble solids (percentage at 20°C)/titratable acidity (percentage of citric acid)
[x]Absorbance units per gram.
[w]Mean HPLC peak area percentage.
[v]Area units per gram ($\times 10^8$).

an effect of ripening, since the black subsamples also had larger values of the soluble solids : acidity ratio (SS:A) and greater total anthocyanin contents than the red subsamples (13).

To shed more light on changes in blackberry anthocyanins during ripening, we compared a series of fruit samples of several cultivars (1984 season) that differed greatly in degree of ripeness, as judged by surface color and SS:A values (Table 3). These samples showed a progressive increase in total anthocyanin content in fruits of greater SS:A—the rate of increase decreasing as fruits attained a black color (Fig. 3). The proportions of anthocyanins in these samples varied greatly, with components 1 and 3 increasing and components 4 and 5 decreasing with increasing ripeness. However, changes in anthocyanin proportions were small in fruit approaching full ripeness (blackish red to black in the frozen state, corresponding to shiny black to black in fresh state). Therefore, it is unlikely that the color differences in these fruits were due to differences in the proportions of individual anthocyanins. In a study of ripening effects on raspberry pigments, Barritt and Torre (3) reported a small quantitative change in the anthocyanin composition in fruits of increasing ripeness, with 2 different cultivars ('Willamette' and 'Meeker') giving similar results. Sági et al. (12) found similar anthocyanin patterns in unripe, ripe, and overripe fruit of 3 raspberry cultivars.

Changes in the proportions of individual anthocyanins during ripening reflect differing rates of anthocyanin accumulation in blackberry fruit. Our data show a 10- to 16-fold increase in component 1 and much smaller increases in components 3, 4, and 5 in fruit of increasing ripeness. The relationship between individual anthocyanin content and SS:A (Fig. 4) or total anthocyanin content (Fig. 5) appeared to be linear. 'Black Satin' and 'Hull Thornless' fruit, which have slightly different anthocyanin patterns (A and C), respectively, showed similar anthocyanin accumulation behavior.

Since all of the blackberry anthocyanins appear to contain the same aglycone (cyanidin), the differing rates of anthocyanin accumulation may reflect differences in the rate of glycosylation and in the case of components 4 and 5, acylation. The linear relationship between the individual and total anthocyanin contents suggests that the rates of glycosylation are constant during ripening. The basis for the linear relationship between anthocyanin accumulation and SS:A is not clear, but indicates a temporal linkage between these presumably independent manifestations of ripening.

Literature Cited

1. Asen, S. 1984. High pressure liquid chromatographic analysis of flavonoid chemical markers in petals from Gerbera flowers as an adjunct for cultivar and germplasm identification. Phytochemistry 23:2523–2526.
2. Barritt, B.H. and L.C. Torre. 1973. Cellulose thin-layer chromatographic separation of *Rubus* fruit anthocyanins. J. Chromatogr. 75:151–155.
3. Barritt, B.H. and L.C. Torre. 1975. Fruit anthocyanin pigments of red raspberry cultivars. J. Amer. Soc. Hort. Sci. 100:98–100.
4. Barritt, B.H. and L.C. Torre. 1975. Inheritance of fruit anthocyanin pigments in red raspberry. HortScience 10:526–8.
5. Castelle, K.V., H. Geiger, R. DeLoose, and C.F. Van Samere. 1983. Separations of some anthocyanidins, anthocyanins, proanthocyanidins, and related substances by reversed-phase high performance liquid chromatography. J. Chromatogr. 259:291–301.
6. Francis, F.J. 1982. Analysis of anthocyanins, p. 181–207. In: P. Markakis (ed.). Academic, New York.
7. Hicks, K.B., S.M. Sondey, D. Hargrave, G.M. Sapers, and A. Bilyk. 1985. Automated preparative HPLC of anthocyanins. LC Mag., Liquid Chromatography and HPLC. 3(11):981–984.
8. Jennings, D.L. and E. Carmichael. 1979. Colour changes in frozen blackberries. Hort Res. 19:15–24.
9. Moore, J.N., E. Brown, and W.A. Sistrunk. 1974. 'Cherokee' blackberry. HortScience 9:246.
10. Moore, J.N., E. Brown, and W.A. Sistrunk. 1976. 'Cheyenne'—a new blackberry for Arkansas. Ark. Farm Res. 25(6):3.
11. Moore, J.N., E. Brown, and W.A. Sistrunk. 1974. 'Cherokee' blackberry. HortScience 9:246.
12. Sági, F., L. Kollányi, and I. Simon. 1974. Changes in the colour and anthocyanin content of raspberry fruit during ripening. Acta Alimentaria 3:397–405.
13. Sapers, G.M., A.M. Burgher, J.G. Phillips, and G.J. Galletta. 1985. Composition and color of fruits and juice of thornless blackberry cultivars. J. Amer. Soc. Hort. Sci. 110:243–248.
14. Sapers, G.M., A.M. Burgher, J.G. Phillips, S.B. Jones, and E.G. Stone. 1984. Color and composition of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 109:105–111.

15. Tesovic, Z. 1978. Study of anthocyanins in the fruit of inbred seedlings obtained by selfing some red raspberry (*Rubus idaeus* L.) cultivars. Jugosl. Vocarstvo 12 (44–45):39–42. [Chem. Abstr. 92:143440d; 1980).
16. Timberlake, C.F. and P. Bridle. 1982. Distribution of anthocyanins in food plants, 125–162. In: P. Markakis (ed). Anthocyanins as food colors. Academic, New York.
17. Torre, L.C. and B.H. Barritt. 1977. Quantitative evaluation of *Rubus* fruit anthocyanin pigments. J. Food Sci. 42:488–490.
18. Wrolstad, R.E. and D.A. Heatherbell. 1968. Anthoxyanin pigments of rhubarb, Can. Red. J. Food Sci. 33:592–594.

J. Amer. Soc. Hort. Sci. 111(6):950–955. 1986.

Flowering and Fruiting Characteristics of *Vaccinium ashei* and *Vaccinium ashei–Vaccinium constablaei* Derivative Blueberry Progenies

J.R. Ballington[1] and Y.M. Isenberg[2]
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7601
A.D. Draper[3]
Agricultural Research Science, U.S. Department of Agriculture, PGGI, Beltsville, MD 20705

Additional index words. blueberry breeding, species hybridization, *Vaccinium corymbosum*, breeding methods, disease resistance, *Monilinia vaccinii–corymbosi*

Abstract. **Hexaploid *Vaccinium* hybrid progenies, including F_1, F_1 intercross, F_1 x F_2, BC_1, BC_1 intercross, and BC_1 x F_2 crosses between *V. ashei* Reade and *V. constablaei* Gray, and an intercross between late-blooming *V. ashei* genotypes, established in the commercial blueberry production area in eastern North Carolina, were compared among themselves and with 2 highbush blueberry (*V. corymbosum* L.) cultivars for flowering, ripening, primary mummy berry infection, crop, and fruit characteristics. There were significant differences among progenies for all traits, with sufficient variability for selection within most progenies. Differences reflected specific parent combinations rather than type of cross with the *V. ashei–V. constablaei* derivative progenies. The experiment included both *V. ashei* and *V. ashei–V. constablaei* derivative progenies that produced a high percentage of seedlings flowering with or later than highbush blueberries. Two percent of the *V. ashei–V. constablaei* derivative seedlings bloomed and ripened with the early ripening highbush cultivar 'Croatan'. Crop ratings were variable in all progenies, and high SDs for the cultivars indicated that a high percentage of the variation was environmental. Primary mummy berry infection significantly reduced the crop in several progenies but was not responsible for the poor overall crop performance of most. Mean fruit size of the *V. ashei* intercross was large enough for hand harvest, while all but the 2 smallest-fruited *V. ashei–V. constablaei* derivative progenies were large enough for mechanical harvesting. Fruit of most progenies were commercially acceptable for color, picking scar, firmness, and flavor.**

Commercial blueberry culture in southeastern North Carolina is based on the tetraploid (2n = 4× = 48) highbush blueberry (*Vaccinium corymbosum* L.), which is noted for early ripening, high-quality fruit of moderate to large size (5). Current highbush cultivars are limited in ecological amplitude, being adapted only to sandy soils containing >2% organic matter with a high water table providing constant, but not excessive, moisture (1). Since soils suitable for highbush production (without extensive soil modification) occur only sporadically, expansion of blueberry culture in eastern North Carolina is seriously limited (6).

A 2nd species, the hexaploid (2n = 6× = 72) rabbiteye blueberry (*V. ashei* Reade), also has a number of desirable horticultural characteristics, including productivity, light-blue fruit color, excellent fruit firmness, small picking scar, superior shelf life, adaptation to mechanical harvesting, and tolerance to upland soils (2). In comparison to highbush, rabbiteye is later-ripening, and, being native to the south climatically, has a low chilling requirement. Low chilling requirement is considered a contributing factor to early flowering (4), resulting in susceptibility to frost or freeze injury (2). Attempts have been made to overcome the deficiencies of the rabbiteye blueberry through interspecific hybridization with the compatible hexaploid species *V. constablaei* Gray, which is native to high elevations in the southern Appalachians (3, 4). Hybrids from this combination appear to combine late flowering with a relatively short interval between flowering and ripe fruit, but are often deficient in fruit size and quality. A recent report comparing *V. ashei* and *V. constablaei* advanced generation and backcross progenies in a severe frost and freeze year found significant differences among progenies for vigor and cropping ability (2). However, vigor was acceptable for all progenies. Specific parental combination appeared more important than type of cross. Additionally, there appeared to be sufficient variability for selection for cropping ability when large progenies were grown. The objective of this study was to evaluate these same advanced generation and backcross progenies between *V. ashei* and *V. constablaei* for relative flowering date and period, seasoning of ripening, fruit characteristics, and productivity to ascertain their adaptation and potential for commercial blueberry production.

Received for publication 24 Feb. 1986. Paper no. 10344 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. Appreciation is expressed to Cavell Brownie, Dept. of Experimental Statistics, N.C. State Univ., for assistance with statistical analysis; C.M. Mainland, Horticultural Science Dept., for supplying climatological data; J.J. Luby, Dept. of Horticulture and Landscape Architecture, Univ. of Minnesota, St. Paul, for assistance in data collection; and Joseph Fedrowitz for performing the statistical analysis. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Associate Professor.
[2]Graduate Assistant.
[3]Research Geneticist.