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Physiochemical Changes in Floricane and Primocane Blackberries Harvested from Primocane Genotypes

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***Abstract*. Fresh-market blackberries (*Rubus* subgenus *Rubus* Watson) have a growing global market, with continued release of cultivars to meet demand for consumer-quality fruit. The release of primocane-fruiting blackberry plants that produce crops on both floricanes and primocanes has expanded blackberry production. This study investigated the physiochemical attributes of fresh-market blackberries harvested from two cane types (floricane and primocane) from four primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') grown at the University of Arkansas Fruit Research Station, Clarksville in 2015 and 2016. Year-to-year differences were evident as blackberries harvested in 2016 were smaller (6 g) and less acidic (0.7% titratable acidity) than berries harvested in 2015 (8 g berries with 0.9% titratable acidity); however, soluble solids in each year were similar (≈10.2%). Differences in genotypes were also a factor. 'Prime-Ark® Traveler' (2015) and APF-268 (2016) had the highest berry firmness (7.8–8.3 N). In both years, APF-238 had the lowest firmness (5.7–6.0 N), highest isocitric acid (0.8–1.1 g/100 g), and highest total anthocyanins (239–353 mg/100 g). Floricane fruit harvested from 'Prime-Ark® Traveler' had the highest berry weights (8.3–10.4 g) in both years. Blackberries harvested from primocanes were wider (21.3–22.9 mm), had higher soluble solids (11.6% to 12.6%), and had lower titratable acidity (0.6%) when compared with floricane fruit in both years. Major year-to-year differences were found for several variables in this study, indicating that environmental effects can be substantial and growers should be aware of this influence on berries harvested from the different cane types. Evaluation of quality properties of floricane and primocane fruit of primocane plants in other locations would be valuable, particularly from areas where commercial blackberry production is established.**

Fresh-market blackberries (*Rubus* subgenus *Rubus* Watson) have a growing market worldwide, with the continued release of new blackberry cultivars to meet demand. Like other dark-pigmented fruits, blackberries are a rich source of bioactive components that can significantly affect human health (Clark et al., 2002; Conner et al., 2005; Seeram, 2008; Siriwoharn et al., 2004). Many factors affect the production and quality of fresh-market blackberries including the genetic background, soil type, weather conditions, and plant management.

Fresh-market blackberries need to have attributes that appeal to consumers, but also withstand the market chain from harvest to commercialization. Key attributes for quality fresh-market blackberries include firm berries with a good balance between acidity and sugar content. Achieving sugar and acid balance and maintaining fruit quality during storage are major goals of fresh-market blackberry breeders. Based on consumer sensory studies, a desired fresh-market blackberry should have a berry weight of 8–10 g, soluble solids of 9% to 10%, and titratable acidity between 0.9% and 1.0% (Threlfall et al., 2016a). In addition to sugars and acids, phytochemicals play an important role in consumer perception of flavors. Soares et al. (2013) reported that phenolic compounds, such as anthocyanins and hydrolysable tannins, affect bitterness in fruits and derived products. Other important fresh-market blackberry quality attributes include firmness, seediness, red drupelet incidence, and decay resistance.

More recently, primocane fruiting has become a major focus in blackberry breeding (Clark and Finn, 2008; Strik et al., 2007). In the last years, the University of Arkansas has released primocane-fruiting cultivars such as 'Prime-Jim®', 'Prime-Jan®', 'Prime-Ark 45®', and 'Prime-Ark® Traveler' (Clark and Perkins-Veazie, 2011; Clark and Salgado, 2016; Clark et al., 2005). Blackberries typically produce fruit on the second-year canes (floricanes), requiring canes to be overwintered to produce a crop. However, primocane-fruiting blackberry plants can bear fruit on current-season canes (primocanes). Thus, primocane-fruiting blackberry genotypes (selections and cultivars) can produce two cropping seasons, one during the summer on floricanes and the other during midsummer to fall on primocanes. Primocane fruiting has contributed to the expansion of blackberry production similar to the expansion achieved with primocane-fruiting red raspberries (*Rubus idaeus* L.) (Clark and Perkins-Veazie, 2011). Primocane-fruiting blackberries show great promise for improving the availability of fresh-market blackberries worldwide using off-season production systems (Strik et al., 2007).

In 2004, the University of Arkansas released 'Prime-Jim®' and 'Prime-Jan®', the first commercial primocane-fruiting cultivars (Clark et al., 2005). The first shipping-quality, primocane-fruiting blackberry was released, as 'Prime-Ark® 45' (Clark and Perkins-Veazie, 2011) in 2009. 'Prime-Ark® Traveler' is the world's first commercially released, thornless primocane-fruiting blackberry with shipping-quality fruit (Clark and Salgado, 2016).

In Arkansas, primocane-fruiting blackberry genotypes typically flower and fruit during mid to late summer, when average daily high temperatures range from 32 to 40 °C. Primocane-fruiting cultivars such as 'Prime-Jim®' and 'Prime-Jan®' exhibited poor summer heat tolerance in field trials (Clark et al., 2005) in Arkansas, resulting in low berry weight, crumbly berries, and poor flavor. Double and otherwise misshapen fruit have been seen on primocane-fruiting genotypes and are associated with high heat during bud, flower, and fruit development (Clark, 2008).

Primocane cultivars have better fruit production in areas with daily high temperatures of 25 to 30 °C during flowering and fruit development. According to Thompson et al. (2009), the yield of primocanes from 'Prime-Jan®' grown in Oregon was 0.3–3.6 kg/plant with berry weights 4.0–8.1 g. Although 'Prime-Jan®' does not produce primocane fruit well in Arkansas, subsequent releases

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such as 'Prime-Ark® Traveler' produced 4306–10,287 kg·ha^{-1} (0.95–2.3 kg/plant) with berry weights from 4.7 to 7.3 g, and 'Prime-Ark® 45' produced 4978–10,540 kg·ha^{-1} (1.1–2.3 kg/plant) with berry weights from 5.1 to 7.3 g (Clark and Salgado, 2016).

In recent sensory studies on fresh-market blackberries grown in Arkansas, floricane berries from primocane cultivars have had positive consumer attributes. 'Prime-Ark® Traveler' had the highest liking values for overall impression and overall flavor; consumers liked the shape and firmness of this cultivar. 'Prime-Ark® Traveler' and 'Prime-Ark® 45' had the highest liking values for appearance and size (Threlfall et al., 2016b). Consumer and physiochemical studies on fresh-market blackberries can provide positive findings to guide the growth of the blackberry industry.

The physiochemical attributes that affect quality of fresh-market blackberries from floricanes have been researched, but there is little information on these attributes of blackberries from primocanes of the same genotypes to describe any differences in berries harvested from the different cane types. The objective of this study was to investigate physiochemical attributes of fresh-market blackberries harvested from two cane types (floricane and primocane) of four primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') grown at the University of Arkansas Fruit Research Station, Clarksville in 2015 and 2016.

Materials and Methods

Blackberry plants and culture

Four University of Arkansas blackberry breeding program primocane-fruiting blackberry genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') were used for this study. The blackberry plants were grown at the Fruit Research Station in Clarksville, AR (West Central Arkansas, lat. 35°31′58″N and long. 93°24′12″W). Plants were trained to a T-trellis with two lower wires ≈0.5 m from the soil surface spaced 0.5 m apart and two upper wires ≈1.0 m high spaced 0.8 m apart. The plants were established in 2011 and 2012 in three plots with five plants per plot. Standard cultural practices for erect blackberry production were used including annual spring nitrogen fertilization (56 kg·ha^{-1} N) using ammonium nitrate with an additional application of (23 kg·ha^{-1} N) after the floricane crop was completed. The plants were irrigated as needed using trickle irrigation. Primocanes were tipped at 1.1 m in mid-June and again in late July to early August. Dormant pruning consisted of removing dead floricanes and also removing primocane tissue to a point below the flowering area on the primocanes. The plants received a single application of liquid lime sulfur (94 L·ha^{-1}) at budbreak for control of anthracnose (*Elsinoë veneta* [Burkholder] Jenk.). Raspberry crown borer (*Pennisetia marginata* [Harris]) was controlled by a single application of a labeled insecticide with bifenthrin as the active ingredient in October of each year. Insecticides labeled for commercial use in Arkansas were used for spotted wing drosophila (*Drosophila suzukii* Matsumura) control.

Harvest

Blackberries were harvested from the primocane blackberry genotypes in June (floricane fruiting) and in July/August (primocane fruiting) in 2015 and 2016 (Table 1). Blackberries were harvested before 11:00 AM at the shiny-black stage of ripeness. Approximately 2 kg of berries were harvested from each genotype and cane type. Berries were harvested into 240-g vented clamshells in triplicate for each genotype, placed in chilled coolers, and transported to the University of Arkansas Department of Food Science, Fayetteville, AR for physiochemical analysis after harvest.

Physiochemical analysis

At harvest, firmness and the incidence of red drupelets were analyzed on fresh berries. For berry and pyrene attributes, basic composition, and phytochemical analysis, three replications of ≈150 g of berries from each genotype were stored at –20 °C until analysis. All analyses were performed on berries or juice from the berries at room temperature (24 °C).

Berry and pyrene attributes. Three randomly selected berries per genotype and replication were evaluated for berry and pyrene attributes including berry weight, berry length, berry width, and pyrenes/berry. The three-berry samples were weighed on a digital scale (Explorer; Ohaus Corporation, Parsippany, NJ), and the width and height (at the widest or longest sections of the berry) of each blackberry was measured using a digital caliper. To determine pyrene attributes, the pyrenes were separated from the skin and pulp using 0.1 mL of Pec5L enzyme (Scott Laboratories, Petaluma, CA) added to each bag containing the three-berry frozen sample. Once the berries thawed, they were hand mashed in the bags. After 2 h at 21 °C, distilled water was added to each bag. To obtain pyrenes, samples were washed using a strainer under running water. The pyrenes were placed onto paper towels and dried at ambient temperature (21 °C) for 2 h. Pyrenes from each three-berry sample were counted and then dried in a laboratory oven (Isotemp®, Model 655F; Fisher Scientific, Pittsburg, PA) at 55 °C for ≈24 h, and weighed.

Firmness. The firmness of the fresh blackberries was measured by compression using a TA.XTPlus Texture Analyzer (Texture Technologies Corporation, Hamilton, MA) with a 5 kg load cell. Compression was done by placing an individual blackberry horizontally on a flat surface, and a 7.6-cm diameter cylindrical and plane probe compressed each blackberry 5 mm. From each genotype and replication, five blackberries were evaluated. The results of firmness were expressed in newtons (N).

Red drupelet incidence. The red drupelet incidence (%) of the fresh blackberries was calculated by counting the total number of drupelets and the number of red drupelets per berry. From each genotype and replication, five blackberries were evaluated.

Basic composition analysis. Titratable acidity, pH, and soluble solids were performed using juice extracted from the thawed blackberries. Blackberry samples were removed from the freezer and thawed; three-berry samples of each genotype and replication were strained through cheese cloth to extract the juice. Titratable acidity and pH were measured by an 877 Titrino Plus (Metrohm AG, Herisau, Switzerland) pH meter standardized with pH 2.0, 4.0, 7.0, and 10.0 buffers. Titratable acidity was determined using 6 g of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide to an endpoint of pH 8.2; results were expressed as percent citric acid. Total soluble solids (expressed as percent) was measured with a Bausch & Lomb Abbe Mark II refractometer with automatic temperature compensation (Scientific Instrument, Keene, NH).

Phytochemical analysis. To evaluate phytochemical compounds, three-berry samples (≈25 g of frozen blackberries) for each genotype and replication were used for extractions. The frozen berries were homogenized with a Euro Turrax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, OH) using 20 mL of methanol/water/formic acid (60:37:3 v/v/v) and then centrifuged for 5 min at 10,000 rpm. After filtering the samples through Miracloth (Calbiochem, La Jolla, CA), the filter cakes were isolated, and the extraction was repeated. This extraction process was repeated with acetone/water/acetic acid (70:29.5:0.5 v/v/v) to assure complete extraction of the phytochemical compounds. The sample extracts were adjusted to a final volume of 200 mL using a mix of the previous extraction solvents (50:50 v/v) and stored at –80 °C until analysis.

Table 1. Harvest date for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane), Clarksville, AR, 2015 and 2016.

Yr	Genotypes	Floricane harvest date	Primocane harvest date
2015	APF-238	22 June	23 July
	APF-268	22 June	24 Aug.
	Prime-Ark® 45	16 June	10 Aug.
	Prime-Ark® Traveler	16 June	10 Aug.
2016	APF-238	10 June	18 July
	APF-268	13 June	16 Aug.
	Prime-Ark® 45	13 June	1 Aug.
	Prime-Ark® Traveler	13 June	1 Aug.

Ellagitannins and flavonols. Sample extracts (3 mL) were dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and resuspended in 1.0 mL of 50% methanol. The reconstituted samples were passed through 0.45-μm polytetrafluoroethylene (PTFE) syringe filters (Varian, Inc., Palo Alto, CA) before high pressure liquid chromatography (HPLC) analysis. The ellagitannins and flavonols were analyzed according to previous methods (Hager et al., 2008a, 2010). The ellagitannin peaks were quantified at 255 nm with results expressed as milligrams of ellagic acid equivalents per 100 g of fresh berry weight. The flavonols were quantified at 360 nm with results expressed as milligrams of rutin equivalents per 100 g of fresh berry weight.

Anthocyanins. Sample extracts (3 mL) were dried using a Speed Vac concentrator (ThermoSavant) and resuspended in 1 mL of 3% formic acid. The reconstituted samples were passed through 0.45-μm PTFE syringe filters (Varian, Inc.) before HPLC analysis. The anthocyanin analysis by HPLC was performed based on previous methods (Cho et al., 2004; Hager et al., 2008b). All anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, cyanidin 3-malonylglucoside, and cyanidin 3-dioxalylglucoside) were quantified as cyanidin 3-glucoside equivalents (C3GEs) with total monomeric anthocyanins results expressed as milligrams C3GEs per 100 g fresh berry weight.

Total phenolics. Total phenolics were determined using the Folin–Ciocalteu assay (Slinkard and Singleton, 1977) with a gallic acid standard and a consistent standard curve based on serial dilutions. Absorbencies were measured at 760 nm, and results were expressed as milligrams of gallic acid equivalents per 100 g of fresh berry weight.

Organic acids and sugars. Individual acids and sugars were analyzed using juice extracted from the thawed blackberries. Glucose, fructose, and isocitric, isocitric lactone, and malic acids of blackberries were measured using HPLC procedures described in Walker et al. (2003). The HPLC was equipped with a Bio-Rad HPLC Organic Acid Analysis Aminex HPX-87H ion exclusion column (300 × 7.8 mm) and a Bio-Rad HPLC column for fermentation monitoring (150 × 7.8 mm) in series. A Bio-Rad Micro-Guard Cation-H refill cartridge (30 × 4.5 mm) was used for a guard column. Columns were maintained at 65 °C by a temperature control unit. Mobile phase consisted of a pH 2.28 solution of sulfuric acid and water with a resistivity of 18 M obtained from a Millipore Milli-Q reagent water system, with 0.65 mL·min^{-1} flow rate. The solvent delivery system was a Waters 515 HPLC pump equipped with a Waters 717 plus autosampler. Samples were diluted using 1 mL of blackberry juice in 5 mL of distilled water and then mixed. Samples were filtered through a 0.45-μm PTFE, transferred into a vial, and 20 μL of the sample was used for the analysis. A Waters 410 differential refractometer detector to measure refractive index connected in series with a Waters 996 photodiode array detector (PDAD) monitored the eluting compounds. Acids were detected by PDAD at 210 nm, and sugars were detected by a differential refractometer. The peaks were quantified using external standard calibration based on peak height estimation with baseline integration.

Statistical design and analyses

Four primocane genotypes were evaluated in this study, with blackberries harvested from both floricanes and primocanes. After each harvest, the blackberries were randomly placed into three replications for each physicochemical analysis with three to five berries per replication. Genotypes were evaluated in triplicate, and data were analyzed using JMP® (version 12.0; SAS Institute, Cary, NC). The physiochemical data were analyzed by analysis of variance. Tukey's honestly significant difference was used for mean separation (P = 0.05), and Slice test was used to detect significant differences for interactions between cane type and genotypes (P < 0.05).

Results and Discussion

As with most field studies, weather conditions varied between the 2 years (2015 and 2016) of the blackberry growing and harvest seasons in Clarksville, AR. Temperatures were similar in both years, with average minimum temperatures of 11 °C in April and average maximum temperatures of 32 °C in July (2015) and 31 °C in June (2016) (Figs. 1 and 2). In both years, rainfall was 94–101 mm in April and 39–47 mm in September. However, the major year differences were when the maximum rainfall occurred. In 2015, rainfall was highest in May (456 mm) and June (189 mm) as compared to 2016 when rainfall was less and more widely distributed throughout the summer with the most rain in July (174 mm). Fruit set of floricane berries occurs during spring when

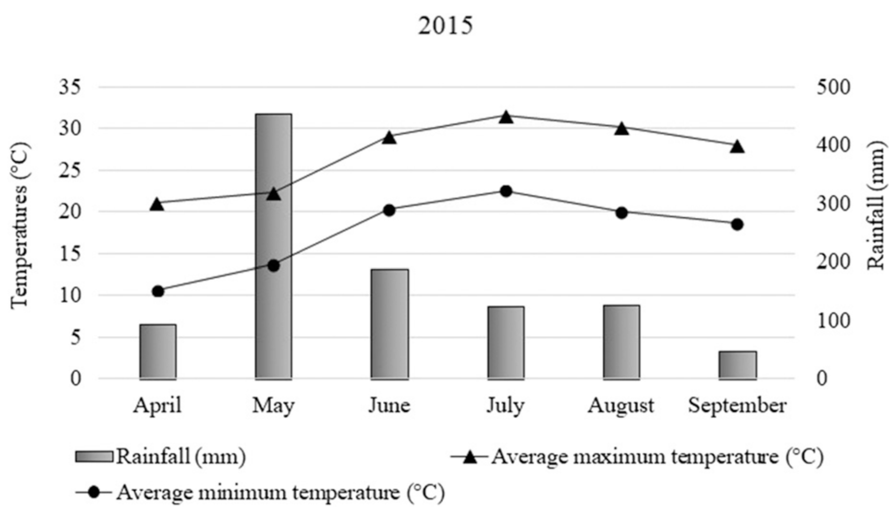

Fig. 1. Temperature and rain conditions at the University of Arkansas Fruit Research Station, Clarksville, 2015.

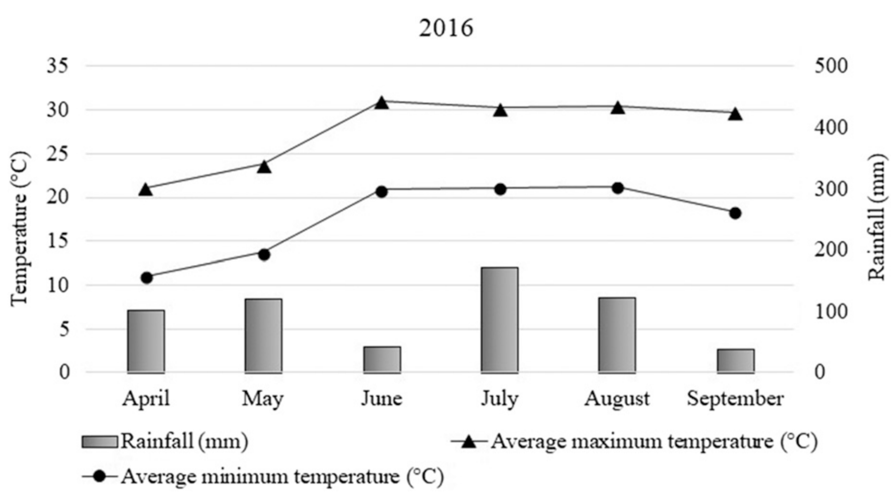

Fig. 2. Temperature and rain conditions at the University of Arkansas Fruit Research Station, Clarksville, 2016.

normally temperatures are mild and availability of water is higher, supporting the development of drupelets and pyrenes. However, fruit set of primocane berries occurs during the summer when temperatures are higher and water uptake is more dependent on irrigation. These weather and management conditions affect quality and physiochemical composition of blackberries.

This study evaluated blackberries from four primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') on the two cane types (floricane and primocane). Regardless of genotype and cane type, the blackberries harvested in 2016 were smaller (6 g) and less acidic (0.7% titratable acidity) as compared to berries harvested in 2015 that there were larger (8 g) and more acidic (0.9% titratable acidity) (data not shown). Interestingly, the soluble solids of the blackberries harvested in each year were similar with 10.2% soluble solids in 2015 and 10.3% in 2016 (data not shown). Since rainfall varied greatly, the data for this study were analyzed by year. The main effects and interactions of the fruit for the primocane genotypes and cane type (floricane and primocane) were evaluated (Tables 2 and 3). There were substantially more dependent variables with interactions between genotype and cane type in 2016 than in 2015.

For 2015 main effects, primocane fruit was wider and firmer with higher soluble solids and pH, and lower titratable acidity than floricane fruit (Table 4). Floricane fruit had higher isocitric lactone acid levels, but lower malic acid, glucose, total anthocyanins, and total phenolics than primocane fruit (Table 5). The higher levels of glucose, total anthocyanins, and total phenolics in primocane fruit might be due to the lower availability of water from rainfall during fruit development and harvest (June–July) compared with the period of development of floricane fruit (May–June) (Fig. 1). In 'Cabernet Sauvignon' grapes, water deficits accelerated anthocyanin accumulation and increased the expressions of genes responsible for the biosynthesis of anthocyanins and increased the initial rate of sugar accumulation (Castellarin et al., 2007). There was no effect for genotype for berry width, red drupelet incidence, or soluble solids. APF-268 had the highest pyrenes/berry (79.1) and the highest titratable acidity (1.02%). 'Prime-Ark® Traveler' had the highest firmness (8.29 N) and APF-238 the lowest (6.01 N). APF-238 had the highest isocitric acid (1.06 g/100 g), glucose (4.05 g/100 g), total anthocyanins (353 mg/100 g), and total phenolics (473 mg/100 g). These results agree with those of Segantini et al. (2017) that reported firmness for primocane genotypes ranging from 6.0 to 7.2 N, total anthocyanins ranging from 124 to 213 mg/100 g, and total phenolics ranging from 336 to 472 mg/100 g. Thompson et al. (2009) reported anthocyanins from 192 to 267 mg/100 g and total phenolics from 677 to 733 mg/100 g for primocane berries from 'Prime-Jan®'.

When data were analyzed using the Slice test, there were interactions between genotype and cane type for berry weight, berry length, total flavonols, and total ellagitannins in 2015 (Table 6). Variability of berry weight for the different genotypes and canes as the floricane fruit from 'Prime-Ark® Traveler' had the highest berry weight (10.37 g) and

Table 2. Analysis of variance main and interaction effects by year for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) on berry and pyrene attributes, firmness, red drupelet incidence, and composition, Clarksville, AR, 2015 and 2016. Highest order interactions are bolded.

Yr	Source	Berry wt (g)	Berry length (mm)	Berry width (mm)	Pyrenes/berry	Firmness (N)	Red drupelets (%)	Soluble solids (%)	pH	Titratable acidity (% citric)
2015	Cane type	0.6248	0.0481	0.0001	0.5656	0.0060	0.1210	<0.0001	0.0003	<0.0001
	Genotype	0.0003	0.0008	0.0783	0.0001	0.0164	0.0587	0.0720	0.0003	0.0115
	Cane type × genotype	**0.0244**	**0.0058**	0.1479	0.3038	0.7646	0.0580	0.2443	0.5290	0.8955
2016	Cane type	0.5247	<0.0001	0.0002	<0.0001	0.0088	0.0033	<0.0001	0.1518	0.0014
	Genotype	0.0040	<0.0001	0.0290	0.4508	0.0083	0.0176	0.0012	0.0066	0.0641
	Cane type × genotype	**0.0350**	**0.0027**	0.4548	**0.0003**	0.0600	0.7866	**0.0001**	**0.0060**	0.0698

Table 3. Analysis of variance main and interaction effects by year for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) on organic acids, sugars, and phytochemicals, Clarksville, AR, 2015 and 2016. Highest order interactions are bolded.

Yr	Source of variation	Isocitric acid (g/100 g)	Isocitric lactone acid (g/100 g)	Malic acid (g/100 g)	Glucose (g/100 g)	Fructose (g/100 g)	Total anthocyanins[z] (mg/100 g)	Total flavonols[z] (mg/100 g)	Total ellagitannins[z] (mg/100 g)	Total phenolics[z] (mg/100 g)
2015	Cane type	0.0571	<0.0001	0.0001	<0.0001	<0.0001	0.0015	<0.0001	0.9828	0.0038
	Genotype	0.0168	0.2152	0.1978	0.0117	0.0035	0.0001	0.0024	0.1533	<0.0374
	Cane type × genotype	0.2084	0.5775	0.6340	0.9519	0.3604	0.1370	**0.0007**	**0.0123**	0.4396
2016	Cane type	0.3778	0.096	0.0016	0.4458	0.1065	0.0007	0.8140	0.1746	0.0673
	Genotype	<0.0001	0.0638	0.0007	<0.0001	<0.0001	0.0002	0.0121	0.3698	0.0110
	Cane type × genotype	0.0760	**0.0049**	**0.0016**	0.2546	0.2889	0.1303	**0.0105**	**0.0016**	**0.0191**

[z]Phytochemicals of blackberries calculated as fresh weight for total ellagitannins, total flavonols, total anthocyanins [mg cyanidin 3-glucoside (acy)/100 g], and total phenolics.

Table 4. Main effect means for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) for berry and pyrenes attributes, firmness, red drupelets incidence, and composition, Clarksville, AR, 2015.

Main effect[z]	Berry width (mm)	Pyrenes/berry	Firmness (N)	Red drupelets (%)	Soluble solids (%)	pH	Titratable acidity (% citric)
Cane type							
Floricane	20.92 b	73.02 a	6.33 b	2.23 a	8.05 b	3.16 b	1.08 a
Primocane	22.93 a	75.41 a	8.05 a	2.14 a	12.63 a	3.73 a	0.61 b
P value	0.0001	0.5656	0.0060	0.121	<0.0001	0.0003	<0.0001
Genotype							
APF-238	21.58 a	52.33 b	6.01 c	3.74 a	11.15 a	3.28 b	0.97 ab
APF-268	21.61 a	79.11 a	8.09 ab	3.52 a	10.08 a	3.24 b	1.02 a
Prime-Ark® 45	21.36 a	84.72 a	6.36 ab	1.16 a	10.38 a	3.68 a	0.67 b
Prime-Ark® Traveler	22.87 a	80.72 a	8.29 a	2.32 a	9.75 a	3.57 a	0.73 ab
P value	0.0783	0.0001	0.0164	0.0587	0.0720	0.0003	0.0115

[z]Cane types and genotypes were evaluated in triplicate. Mean values with different letter(s) for each attribute within main effect are significantly different ($P < 0.05$) using Tukey's honestly significant difference.

APF-238 had the lowest (5.52 g) (Fig. 3). These results are similar to those found by other authors. Clark and Salgado (2016) reported berry weight from 5.2 to 7.2 g for primocane fruit and from 6.7 to 7.6 g for floricane fruit from 'Prime-Ark® Traveler'. Thompson et al. (2009) found berry weight of primocane fruit from 'Prime-Jan®' to range from 4.0 to 8.1 g during two years of study in Oregon. Fernandez and Ballington (2010) reported berry weight of primocane genotypes cultivated in the Southern Appalachian Mountains to range from 3.1 to 7.9 g. Differences in berry weight could also be due to cultivars, management techniques, weather, and soil conditions, often referred to as the genotype × environmental interaction. Floricane fruit from APF-268 had the longest berry (33.6 mm), and AFP-238 the shortest (25.3 mm). The primocane fruit from APF-238 had the highest total flavonols (33.76 mg/100 g), but the floricane fruit had the lowest (6.40 mg/100 g) (Table 6). The floricane fruit from APF-238 developed during May and was harvested toward the end of June during a period with higher availability of water (645 mm) compared with June–July (313 mm), the period of development and harvest for primocane fruit from APF-238 (Fig. 1). The lower amount of water available during the development of primocane berries probably increased the levels of anthocyanins vs. floricane berries.

For 2016 main effects, primocane fruit had wider berries and higher incidence of red drupelets as compared to floricane fruit (Table 7). Floricane fruit had firmer berries, higher titratable acidity, and higher total anthocyanins compared with primocane fruit. The development and harvest of floricane fruit occurred during May–June, when the rainfall was lower (165 mm) compared with July–August (297 mm), the period of development and harvest of primocane fruit (Figs. 1 and 2). As stated prior, the amount of water during the development of floricane berries can impact levels of anthocyanins in berries.

There was not an effect for genotype for titratable acidity. APF-268 had the highest berry width (21.3 mm) and firmness (7.7 N). APF-238 had the highest incidence of red drupelets (3.90%), highest isocitric acid content (0.76 g/100 g), and total anthocyanins levels (239 mg/100 g). 'Prime-Ark® 45' had the highest glucose level (4.58 g/100 g), whereas 'Prime-Ark® Traveler®' had the highest fructose level (3.74 g/100 g), although these levels were similar among these two cultivars (Table 7). In this study, isocitric acid was the major organic acid present in blackberries, followed by malic acid and isocitric lactone acid; fructose and glucose were the main sugars detected. These results are similar to those found in the literature. Fructose and glucose have been reported as the main sugars in blackberries (Du et al., 2010; Kafkas et al., 2006), with fructose ranging from 3.53 to 6.53 g/100 g and glucose ranging from 2.22 to 4.74 g/100 g (Wrolstad et al., 1980). According to Mikulic-Petkovsek et al. (2012), the levels of citric acid ranged from 0.41 to 0.56 g/100 g, and the levels of malic acid ranged from 0.11 to 0.22 g/100 g in wild and cultivated blackberries.

There were interactions between genotype and cane type (Table 8). The Slice test showed that floricane fruit from 'Prime-Ark® Traveler' had the highest berry weight (8.36 g) and 'Prime-Ark® 45' had the lowest (5.45 g). Floricane fruit from APF-268 had the longest berries (35.0 mm) and 'Prime-Ark® 45' the shortest (22.4 mm).

Floricane fruit from 'Prime-Ark® 45' had 60% more pyrenes/berry (81.7) and 63% lower soluble solids than the primocane fruit. Primocane fruit from 'Prime-Ark® 45' had the highest pH (4.05) of all the other fruit. Primocane fruit from APF-238 had the highest isocitric lactone acid and malic acid levels. Floricane fruit had the highest total flavanols ('Prime-Ark® 45') and total ellagitannins (APF-238), whereas primocane fruit had the highest total phenolics (APF-238).

Conclusions

Our study compared a substantial number of sources of variation and dependent variables for primocane-fruiting blackberries.

Table 5. Main effect means for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) for organic acids, sugars, and phytochemicals,[z] Clarksville, AR, 2015.

Main effect[z]	Isocitric acid (g/100 g)	Isocitric lactone acid (g/100 g)	Malic acid (g/100 g)	Glucose (g/100 g)	Fructose (g/100 g)	Total anthocyanins[y] (mg/100 g)	Total phenolics[y] (mg/100 g)
Cane type							
Floricane	0.96 a	0.29 a	0.26 b	2.67 b	2.34 b	202.54 b	360.82 b
Primocane	0.83 a	0.16 b	0.38 a	4.43 a	3.98 a	297.81 a	466.93 a
P value	0.0571	<0.0001	0.0001	<0.0001	<0.0001	0.0015	0.0038
Genotype							
APF-238	1.06 a	0.28 a	0.34 a	4.05 a	3.61 a	353.12 a	472.55 a
APF-268	0.94 ab	0.25 a	0.27 a	2.90 b	2.55 b	278.17 ab	391.14 ab
Prime-Ark® 45	0.79 b	0.19 a	0.34 a	3.69 ab	3.50 a	236.23 b	450.41 ab
Prime-Ark® Traveler	0.77 b	0.22 a	0.33 a	3.56 ab	2.98 ab	133.18 c	341.40 b
P value	0.0168	0.2152	0.1978	0.0117	0.0035	0.0001	<0.0374

[z]Cane types and genotypes were evaluated in triplicate. Means with different letter(s) for each attribute within main effect are significantly different ($P < 0.05$) using Tukey's honestly significant difference.
[y]Phytochemicals of blackberries were calculated as fresh weight for total anthocyanins [mg cyanidin 3-glucoside (acy)/100 g] and total phenolics.

Table 6. Interaction means for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) for berry attributes and phytochemicals, Clarksville, AR, 2015.

Cane type[z]	Genotype	Berry wt (g)	Berry length (mm)	Total flavonols[y] (mg/100 g)	Total ellagitannins[y] (mg/100 g)
Floricane	APF-238	5.52 c	25.29 c	6.40 c*	24.96 a
	APF-268	10.28 a	33.59 a*	7.36 c*	35.32 a
	Prime-Ark® 45	7.09 abc	28.44 bc	10.13 c	40.62 a
	Prime-Ark® Traveler	10.37 a*	30.31 ab	6.27 c	24.73 a
Primocane	APF-238	6.19 bc	26.63 bc	33.76 a*	37.01 a
	APF-268	8.94 ab	27.77 bc*	22.39 ab*	27.40 a
	Prime-Ark® 45	9.16 ab	28.98 bc	15.61 bc	30.93 a
	Prime-Ark® Traveler	7.96 abc*	28.68 bc	12.38 bc	30.50 a
	P value	0.0244	0.0058	0.0007	0.0123

[z]Genotypes were evaluated in triplicate. Mean values with different letter(s) for each attribute are significantly different ($P < 0.05$) using Tukey's honestly significant difference.
[y]Phytochemicals of blackberries were calculated as fresh weight for total flavonols and total ellagitannins.
Mean values followed by an asterisk (*) within a genotype were significantly different for cane type using the Slice test ($P < 0.05$).

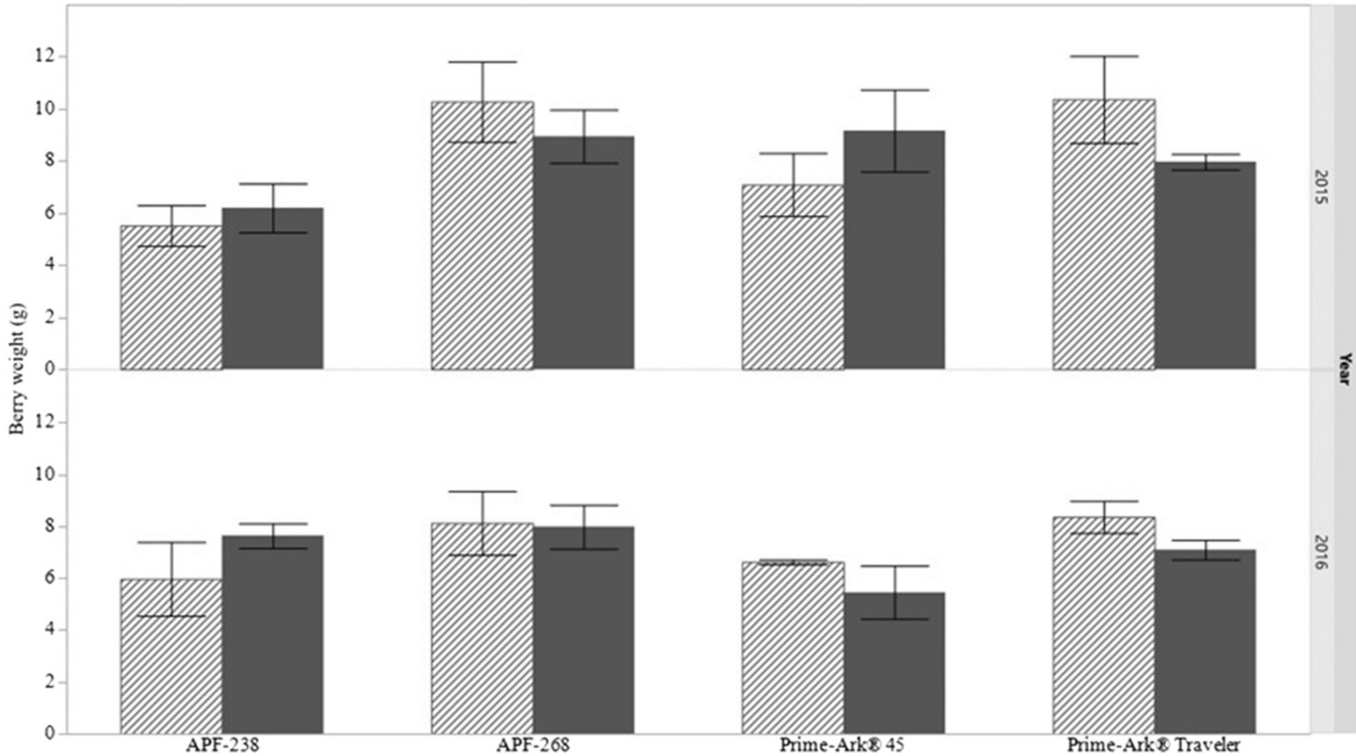

Fig. 3. Berry weight average and standard deviation by year for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler'), Clarksville, AR, 2015 and 2016. Floricane cane type in hatch and primocane cane types in dark gray.

Table 7. Main effect means for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) for berry attributes, firmness, red drupelets incidence, composition, and phytochemicals,[z] Clarksville, AR, 2016.

Main effect[z]	Berry width (mm)	Firmness (N)	Red drupelets (%)	Titratable acidity (% citric)	Isocitric acid (g/100 g)	Glucose (g/100 g)	Fructose (g/100 g)	Total anthocyanins[y] (mg/100 g)
Cane type								
Floricane	19.52 b	7.24 a	2.48 b	0.85 a	0.56 a	4.00 a	3.23 a	207.05 a
Primocane	21.35 a	5.98 b	2.63 a	0.60 b	0.59 a	4.11 a	3.42 a	151.84 b
P value	0.0002	0.0088	0.0033	0.0014	0.3778	0.4458	0.1065	0.0007
Genotype								
APF-238	20.29 ab	5.66 b	3.90 a	0.72 a	0.76 a	4.21 a	3.43 a	238.55 a
APF-268	21.28 a	7.71 a	3.18 ab	0.83 a	0.48 c	2.91 b	2.45 b	145.10 b
Prime-Ark® 45	19.49 b	5.90 b	1.56 b	0.78 a	0.62 b	4.58 a	3.70 a	191.70 ab
Prime-Ark® Traveler	20.69 ab	7.18 ab	1.59 b	0.56 a	0.46 c	4.52 a	3.74 a	142.33 b
P value	0.0290	0.0083	0.0176	0.0641	<0.0001	<0.0001	<0.0001	0.0002

[z]Cane types and genotypes were evaluated in triplicate. Mean values with different letter(s) for each attribute within main effect are significantly different ($P < 0.05$) using Tukey's honestly significant difference.
[y]Phytochemicals of blackberries were calculated as fresh weight for total anthocyanins [mg cyanidin 3-glucoside (acy)/100 g].

Table 8. Interaction means for fresh-market blackberries from primocane genotypes (APF-238, APF-268, 'Prime-Ark® 45', and 'Prime-Ark® Traveler') and cane types (floricane and primocane) for berry and pyrenes attributes, composition, and phytochemicals, Clarksville, AR, 2016.

Cane type[z]	Genotype	Berry wt (g)	Berry length (mm)	Pyrenes/berry	Soluble solids (%)	pH	Isocitric lactone acid (g/100 g)	Malic acid (g/100 g)	Total flavonols (mg/100 g)[y]	Total ellagitannins (mg/100 g)[y]	Total phenolics (mg/100 g)[y]
Floricane	APF-238	5.97 ab*	25.29 cd	56.55 c	11.00 ab	3.51 b	0.13 b*	0.26 b*	11.59 ab*	30.08 a	457.67 ab*
	APF-268	8.13 a	34.96 a*	77.22 ab*	9.70 bc	3.34 b	0.22 ab	0.19 b	14.01 ab*	46.14 ab*	481.32 ab
	Prime-Ark® 45	6.62 ab	28.35 bc*	81.66 a*	7.96 c*	3.41 b	0.21 b	0.34 b	15.66 a	38.00 ab	489.18 ab
	Prime-Ark® Traveler	8.36 a	30.00 b*	78.11 ab*	8.76 bc*	3.43 b*	0.16 b	0.29 b	11.13 ab	29.21 ab	418.35 b
Primocane	APF-238	7.64 ab*	25.16 cd	67.66 abc	12.36 a	3.28 b	0.49 a*	0.58 a*	15.47 a*	31.17 ab	527.57 a*
	APF-268	7.98 a	26.74 bcd*	51.22 c*	9.00 bc	3.34 b	0.14 b	0.27 b	8.42 b*	22.73 b*	425.39 b
	Prime-Ark® 45	5.45 b	22.41 d*	49.44 c*	12.66 a*	3.48 b	0.22 ab	0.35 b	12.92 ab	36.07 ab	527.17 a
	Prime-Ark® Traveler	7.10 ab	24.02 d*	59.00 bc*	12.36 a*	4.05 a*	0.16 b	0.29 b	9.10 b	39.33 ab	471.63 ab
	P value	0.0350	0.0027	0.0053	0.0001	0.0060	0.0049	0.0096	0.0105	0.0016	0.0191

[z]Genotypes were evaluated in triplicate. Mean values with different letter(s) for each attribute are significantly different ($P < 0.05$) using Tukey's honestly significant difference.
[y]Phytochemicals of blackberries were calculated as fresh weight for total ellagitannins, total flavonols, and total phenolics.
Mean values followed by an asterisk (*) within a genotype were significantly different for cane type using the Slice test ($P < 0.05$).

We found major year-to-year differences for several variables, and this indicates that environmental effects can be substantial and growers should be aware of this influence on berries harvested from the different cane types. Likewise, for some variables, cane type and genotype had significant effects. One of the more noteworthy findings that can be important to growers and consumers is berry weight. The significant interaction effects reflect substantial influence from several factors, indicating that berry weight cannot be expected to be the same among genotypes, canes, or years. Evaluating cultivars in specific locations will be needed to determine performance capabilities. From a fruit quality standpoint, growers and consumers are interested in berry firmness, the tendency for red drupelet development, and influences on berry sweetness. Firmness from floricane vs. primocane fruit differed in both years and is interesting as it was anticipated that there would be differences among genotypes, but that cane types would have more consistency. This also suggests an environmental effect at different locations. Red drupelet development showed significance in some comparisons, but overall the impact of cane and year were much less than the genotype. Cane type and genotype had substantial influence on soluble solids, which may have been influenced by crop load, although fruit yield was not evaluated in this study. It will be important to continue the evaluations of these unique plants in other regions for influences of year, cane type, and genotype. This is particularly critical because yield affects fruit quality. Our findings provide the first data for these influences on primocane blackberries, and further research will expand knowledge for this new type of blackberry.

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