Title of Project: Physiochemical and Post-Harvest Attributes of Blackberries from University of Arkansas Primocane Genotypes

Progress Report

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Research Proposal

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Objectives

1. Evaluate post-harvest attributes of primocane and floricane fruit from the primocane genotypes from the University of Arkansas Blackberry Breeding Program
2. Determine physiochemical attributes of the primocane and floricane fruit from the primocane genotypes from the University of Arkansas Blackberry Breeding Program

Justification

Blackberry (*Rubus subgenus Rubus*) is one of the best examples of a wild-harvested specialty crop that moved to commercial use through breeding efforts. This nutraceutical-rich, fresh-market fruit has the potential for an increased role in commercial markets due to consumers' increasing demand for food products with high functional/health properties. The introduction of plants and fruit from blackberry genotypes (cultivars and advanced non-released selections) with shipping-quality fruit and primocane-fruiting capability (plants capable of fruiting on both the first-year primocanes and the second-year floricanes), can expand the role of blackberries in increasing fresh-market fruit production in the United States. Because of the introduction of new fresh-market blackberry genotypes, there is a critical need to determine the marketing potential for this fresh fruit so southern U.S. growers can compete in fresh-market blackberry production and better market their blackberry products. Blackberries are grown throughout the South, with a number of large producers selling in the shipping market, but also with many growers that sell on-farm and in a variety of local markets.

Public and private blackberry breeding programs play a critical role in the future of the blackberry industry. From 1985 to the present, over 60 blackberry cultivars were released from breeding programs, including the first commercially viable fresh-market genotypes (Clark 1999, Clark 2008, Clark and Finn 2008, Moore 1984). In the South the largest blackberry breeding effort is conducted at the University of Arkansas,

Fayetteville (directed by John R. Clark). The Arkansas program contributes the majority of the varieties for the South and is the primary program to help southern blackberry industry development. Breeding traits of interest in fresh-market blackberries include fruit quality, flavor, shipping quality, thornlessness, plant adaptation, plant habit and disease/pest resistance. Primocane fruiting has become a major focus in blackberry breeding (Clark and Finn 2008).

Primocane-fruiting trait expression was first selected for in 1997 at University of Arkansas. Prime-Jim® and Prime-Jan®, released by the University of Arkansas in 2004, were the first commercial primocane-fruiting cultivars (Clark et al. 2005, Stanton et al. 2007). In 2009, the first shipping-quality, primocane-fruiting blackberry was released, Prime-Ark® 45 (Clark and Perkins-Veazie 2011), and commercial plantings were established in 2010. This primocane-fruiting blackberry has the potential to be a major player in commercial production since it can allow a diversification of time of ripening, particularly in fresh-market, local fruit production.

Fresh-market blackberries must be firm to survive the marketing chain. Generally, blackberry fruit must have a balance of astringency, acidity, and sweetness (about 10% soluble solids) (Hall 1990), and achieving this balance and maintaining it after storage is a major goal of blackberry breeders. Other important fresh-market blackberry quality attributes include harvestability, seediness, black color retention and decay resistance. Like other dark-pigmented fruits, blackberries are a rich source of bioactive components that can have significant impacts on human health (Clark et al. 2002, Conner 2005, Seeram 2008, Siriwoharn et al. 2004). Breeding for enhanced nutraceutical composition of blackberries has been hampered by lack of published information on nutraceutical composition of blackberries.

Methodologies

Harvest

Blackberries were harvested at shiny-black stage of maturity in the Fruit Research Station of University of Arkansas, in Clarksville, AR. Approximately 1,000 g of fruit of each genotype were harvested during the peak floricane fruiting in June and then during the peak of primocane fruiting in July-August. The was harvested prior to 10 am, and then transported in coolers to the Food Science Department, Fayetteville, AR where the blackberries were randomly selected and placed into 240g-vented clamshells. The fruit was stored for 7 days at 2°C and 90 ± 5% HR. The genotypes evaluated in this study were APF-238, APF-268, 'Prime-Ark 45®' and 'Prime-Ark Traveler®', all of these genotypes are primocane plants.

Physiochemical analysis

Three replications of approximately 150 g of berries were collected for each genotype, placed in plastic storage bags, and stored at -20°C until analyses.

Berry and pyrene attributes

From the frozen berries, three berries per genotype and replication were used to determine berry attributes (individual berry weight, berry length, and berry width) and pyrene attributes (number/berry and weight/berry). The three-berry samples were weighed on a digital scale and the width and height of each blackberry were measured with digital calipers. To determine pyrene attributes, a pectolytic enzyme was added to each bag containing the three-berry frozen sample to break down the skin and pulp. Once the berries thawed, they were hand-mashed in the bags. After 1.5 hours at 21°C, 100 mL of distilled water was added to each bag. The samples were poured into a strainer. Under

running water, the pulp was mashed against the strainer until only pyrenes remained. The pyrenes were placed onto paper towels and dried at ambient temperature (21°C) for 1.5 hours. The pyrenes for each three-berry sample were counted and weighed. The pyrenes were dried in a laboratory oven at 55°C for approximately 24 hours and weighed.

Soluble solids, pH, and titratable acidity

Three replicate three-berry samples of each cultivar and genotype were used to determine soluble solids, pH, and titratable acidity. Samples were thawed and placed in cheesecloth to extract the juice from the berries. Titratable acidity and pH were measured with an automated titrimeter and electrode standardized to pH 2.0, 4.0, 7.0, and 10.0 buffers. Titratable acidity was determined using 6 mL of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; results were expressed as g/100 g citric acid. Total soluble solids (expressed as %) was measured with a Bausch & Lomb Abbe Mark II refractometer (Scientific Instrument, Keene, NH).

Sugar and organic acid analysis

Glucose, fructose, isocitric, isocitric lactone and malic acids of the blackberries were identified and measured using HPLC procedures described in Walker et al. 2003. HPLC was equipped with a Bio-Rad HPLC Organic Acid Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) and a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm) in series. A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) was used as a guard column. Columns were maintained at 65 0.1 °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. The sulfuric acid solution was used as the solvent with 0.65 mL/min flow rate. The solvent delivery system was a Waters 515 HPLC pump equipped with a Waters 717 plus autosampler. One milliliter of blackberry juice was homogenized in 5 mL of distilled and 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 to measure refractive index connected in series with a Waters 996 photodiode array detector monitored the eluting compounds. Organic acids were detected by photodiode array 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.

Nutraceutical analysis.

Nutraceutical analysis was done on each genotype in triplicate. To obtain sample extracts, samples (25g) were homogenized with 20 mL of methanol/water/formic acid (60:37:3 v/v/v) with by a Euro Turrax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, OH). The samples were filtered through Miracloth (Calbiochem, La Jolla, CA), the filter cakes was isolated, and the extractions were repeated. This extraction process will be repeated with acetone/water/acetic acid (70:29:0.5 v/v/v) to assure complete extraction of the nutraceutical compounds. The filtrates were adjusted to a final volume of 200 mL with extraction solvent.

Ellagitannins and flavonols. Sample extracts (3 mL) were dried using a Speed Vac concentrator and re-suspended in 1.0 mL of extraction solvent. The reconstituted samples were passed through 0.45 μm PTFE syringe filters prior to High Pressure Liquid Chromatography (HPLC) analysis. The ellagitannins were analyzed on a Waters Alliance HPLC system equipped with a Waters model 996 photodiode array detector and Millenium version 3.2 software. Separation was performed using a

Phenomenex Aqua 5 μm C18 (250 x 4.6 mm) column with a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1 v/v) for mobile phase B at a flow rate of 1.0 mL/min. A linear gradient was run from 10 to 55% B (0-50 min), from 55 to 100% B (50-60 min), and from 100 to 10% B (60-65 min). The ellagitannins and flavonols were identified on the basis of comparison of HPLC retention times to our previous HPLC results obtained using the identical HPLC conditions and LC-MS analysis. The ellagitannin peaks were quantified at 255 nm as ellagic acid equivalents using external calibration curves of ellagic acid, with results expressed as milligram ellagic acid equivalents per 100 g of fresh berry weight. The flavonols were quantified at 360 nm as rutin with results expressed as equivalents per 100 g of fresh berry weight.

Anthocyanins. Sample extracts (3 mL) were dried using a Speed Vac concentrator and re-suspended in 1.0 mL of 3% formic acid. The anthocyanin analysis by HPLC were performed based on previous methods with a 250 × 4.6 mm Symmetry C_{18} column. The mobile phase will consist of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate was 1.0 mL/min with a linear gradient from 2 to 60% B over 60 min. The anthocyanin peaks were quantified at 510 nm using a photodiode array detector. 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 with total monomeric anthocyanins results expressed as milligrams per 100 g of berry fresh weight.

Total phenolics. Total phenolics were measured 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 will be expressed as mg of gallic acid equivalents (GAE) per 100 g fresh berry weight.

Postharvest quality

Blackberries for postharvest analyses were randomly selected in the laboratory. Only sound fruit (100% commercially acceptable) were used for analyses and placed in the clamshells in triplicate. Weight loss, percent unmarketable, firmness and color reversion were evaluated after 7 days of storage.

Percent unmarketable

After 7 days of storage, fruit was evaluated by removing all the fruit from each clamshell and counting the number of unmarketable (softness, mold, rot, and leakage) fruit.

Weight loss

Weight loss was calculated as percent weight decrease during storage of the total fruit in the clamshells from the initial prior to storage**.**

Firmness

Firmness was measured by compression using a TA.XTPlus Texture Analyzer (Texture Technologies Corporation, Hamilton, MA) with a 5 kg load cell. Fruit compression was measured by placing an individual blackberry horizontally on a flat surface using a 7.62 cm (3 inches) diameter cylindrical and plane probe. From each clamshell, five blackberries were evaluated randomly at day 0 and day 7.

Percent of color reversion

The percent of color reversion of individual blackberries was calculated by counting the total number of drupelets and the number of red drupelets per berry. Blackberries from each genotype were evaluated at harvest and after storage using five randomly selected berries from each clamshell/replication.

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Results

Table 1. Characterization of physical attributes for blackberry genotypes at harvest (day 0), Clarksville, AR, 2016.

Cane	Genotype	Berry length (mm)	Berry width (mm)	Berry weight (g)	Pyrenes/ Berry
Primocane (Fall)	APF -238	25.16	21.09	7.23	67.67
	APF -268	26.75	22.74	7.92	51.22
	Prime-Ark 45®	22.41	20.22	5.67	49.44
	Prime-Ark Traveler®	24.02	21.38	7.25	59.00
Floricane (Summer)	APF -238	25.29	19.50	6.04	56.56
	APF -268	34.96	19.82	8.01	77.22
	Prime-Ark 45®	28.36	18.77	6.06	81.67
	Prime-Ark Traveler®	30.00	20.02	7.64	78.11

Genotypes were evaluated in triplicate (n=3).

Table 2. Basic composition for blackberry genotypes at harvest (day 0) and after storage (day 7), Clarksville, AR, 2016.

		pH		SS		TA (%)		SS/TA ratio	
Cane	Genotype	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Primocane (Fall)	APF -238	3.28	3.33	12.37	12.80	0.75	0.79	16.46	16.26
	APF -268	3.35	3.34	9.00	8.07	0.67	0.71	13.47	12.39
	Prime-Ark 45®	3.48	3.43	12.67	12.07	0.66	0.67	13.27	18.00
	Prime-Ark Traveler®	4.05	3.68	12.37	11.27	0.33	0.46	40.03	25.87
Floricane (Summer)	APF -238	3.52	3.43	11.00	10.37	0.70	0.90	15.69	12.06
	APF -268	3.35	3.44	9.70	9.33	1.00	0.83	10.07	11.49
	Prime-Ark 45®	3.42	3.46	7.97	8.60	0.92	0.74	9.02	12.45
	Prime-Ark Traveler®	3.44	3.44	8.77	9.73	0.81	0.78	12.37	13.11

Genotypes were evaluated in triplicate (n=3). Fruit were stored in 240-g, low-profile, vented clamshells, at 2°C and 90% RH during 7 days.
SS means soluble solids; TA means titratable acidity as % of citric acid and SS/TA ratio means soluble solids/titratable acidity ratio.

Table 3. Phytochemical composition of blackberry genotypes at harvest (day 0) and after storage (day 7), Clarksville, AR, 2016.

		Ellagitannins (mg/100g)		Flavonols (mg/100g)		Anthocyanins (mg/100g)		Total Phenolics (mg/100g)	
Cane	**Genotype**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**
Primocane (Fall)	**APF -238**	31.17	44.02	15.47	15.29	182.63	226.33	527.57	531.40
	APF -268	22.73	38.98	8.42	9.91	130.68	222.93	425.39	428.65
	Prime-Ark 45®	36.07	50.63	12.93	14.09	167.41	175.93	527.17	572.77
	Prime-Ark Traveler®	39.33	58.53	9.10	14.35	126.65	132.54	471.63	497.93
Floricane (Summer)	**APF -238**	30.08	35.51	11.59	12.13	294.48	338.94	457.67	592.53
	APF -268	46.14	43.53	14.02	14.98	159.53	215.23	481.33	546.97
	Prime-Ark 45®	38.00	44.45	15.66	17.70	215.99	254.86	489.18	580.92
	Prime-Ark Traveler®	29.21	38.10	11.14	9.97	158.23	138.00	418.36	433.26

Genotypes were evaluated in triplicate (n=3). Fruit were stored in 240-g, low-profile, vented clamshells, at 2°C and 90% RH during 7 days.
Fresh weight of blackberries for total anthocyanins (mg cyanidin 3-glucoside/100 g); total flavonols (mg rutin equivalents/100 g); ellagitannins (mg ellagic acid equivalents/100 g); total phenolics (mg gallic acid equivalents/100 g).

Table 4. Organic acids and sugars of blackberry genotypes at harvest (day 0) and after storage (day 7), Clarksville, AR, 2016.

		Isocitric (mg/100g)		Isocitric lactone (mg/100g)		Malic acid (mg/100g)		Glucose (g/100g)		Fructose (g/100g)	
Cane	**Genotype**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**
Primocane (Fall)	**APF -238**	810.45	704.13	498.27	130.85	589.16	266.65	4.08	4.35	3.37	3.49
	APF -268	542.84	425.07	142.08	223.38	271.61	190.81	3.10	2.73	2.72	2.19
	Prime-Ark 45®	620.64	612.74	227.88	217.17	350.14	340.29	4.54	4.64	3.79	3.61
	Prime-Ark Traveler®	403.68	518.03	150.48	164.43	290.21	299.73	4.76	4.30	3.84	3.65
Floricane (Summer)	**APF -238**	809.34	855.40	186.52	253.99	297.23	213.51	3.93	3.23	3.33	3.08
	APF -268	1090.54	948.84	209.52	154.07	255.38	255.57	2.88	2.86	2.52	2.57
	Prime-Ark 45®	793.44	805.14	213.42	231.91	233.64	242.03	2.47	2.67	2.15	2.43
	Prime-Ark Traveler®	854.60	879.60	225.92	225.41	232.32	270.98	2.87	3.18	2.41	2.76

Genotypes were evaluated in triplicate (n=3).
Fruit were stored in 240-g, low-profile, vented clamshells, at 2°C and 90% RH during 7 days.

Table 5. Postharvest quality for blackberry genotypes at harvest (day 0) and after storage (day 7), Clarksville, AR, 2016.

		Unmarketable fruit (%)		Weight loss (%)		Firmness (N)		Color reversion (%)	
Cane	**Genotype**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**	**Day 0**	**Day 7**
Primocane (Fall)	**APF -238**	---	22.65	---	2.22	5.19	4.55	6.04	7.15
	APF -268	---	20.93	---	3.95	6.05	5.46	2.94	2.64
	Prime-Ark 45®	---	19.44	---	10.35	5.48	4.24	1.27	3.53
	Prime-Ark Traveler®	---	18.30	---	2.78	7.24	5.46	0.30	0.51
Floricane (Summer)	**APF -238**	---	7.01	---	7.41	6.14	5.30	1.77	3.28
	APF -268	---	16.75	---	2.59	9.38	9.13	3.43	3.47
	Prime-Ark 45®	---	17.34	---	5.97	6.33	7.16	1.86	2.40
	Prime-Ark Traveler®	---	44.27	---	6.19	7.12	5.97	2.88	5.70

Genotypes were evaluated in triplicate (n=3). Unmarketable fruit (%) and weight loss (%) after 7 days of storage at 2°C for blackberry genotypes in 240-g, low-profile, vented clamshells. Firmness and color reversion evaluated at harvest (day 0) and after storage (day 7).

Conclusions
The floricane and primocane fruit from the primocane genotypes in this study provided a substantial range in physiochemical and postharvest storage attributes (7 days at 2°C) that were measured. The fruit from the floricanes were harvested in June and the fruit from the primocanes were harvested in July and August. Fruit from the different canes of these genotypes performed differently in the terms of storage.

In terms of physiochemical data of the fruit, the basic composition (soluble solids, pH, and titratable acidy), the nutraceutical (ellagitannins, flavonols, anthocyanins and total phenolics) and the organic acid (isocitric, isocitric lactone and malic acid) and sugar (glucose and fructose) content were measured.

At harvest the fruit had 9.0-12.7% and 8.8-11.0% soluble solids, 0.33-0.75% and 0.70-1.0% titratable acidity, and 5.7-7.9 and 6.1-8.0 g berry size for primocane and floricane fruit, respectively. In general, the nutraceutical levels increased after storage. The predominant acid was isocitric, but isocitric lactone and malic acid also have a dominant role in acid content of these blackberry genotypes.

In terms of postharvest quality, initial data indicates that most of the fruit from these genotypes decreased firmness in storage for 7 days at 2°C. The weight loss during storage of the blackberries in the clamshells ranged from 2.2-10.45% and 2.6-7.4% for primocane and floricane fruit, respectively. While the unmarketable blackberries in the clamshells during storage ranged from 18.3-22.7% and 7.0-44.3%% for primocane and floricane fruit, respectively. The color reversion (drupelet color shifts from black to red) of the blackberries ranged from 0.5-7.2% and 2.4-3.5% for primocane and floricane fruit, respectively, during storage.

The data will be statistically analyzed for main effects and interactions of the factors for the attributes evaluated. Correlations will also be done to determine the relationships of the attributes.

Impact Statement
The data collected from this study will help characterize blackberry fruit quality and performance in storage of primocane genotypes for future U.S. blackberry breeding objectives. More specifically, the results will identify attributes and storage potential for both primocane and floricane fruit from primocane blackberries from the University of Arkansas blackberry breeding program.

Citation(s) for any publications arising from the project
The data is being analyzed and a journal publication is “in writing”. Data from this project will be used to present oral and poster presentations at regional and national meetings.

Journal Article: Segantini, D. M, R.T. Threlfall, O.S. Hines, J.R. Clark, L.R. Howard, C.R. Brownmiller, and L.J.R. Lawless. 2017 Postharvest and changes in physiochemical attributes of primocane blackberry genotypes. In progress. HortScience, In writing.

Presentation: Segantini, D.M., R.T. Threlfall, and J.R. Clark. 2017. Physiochemical Attributes Impacted by Post-harvest Storage and Harvest Season of Primocane Blackberry Genotypes. Sothern Region American Society for Horticultural Science Annual Meeting February 3-5. Mobile, Alabama.

Poster: Segantini, D.M., R.T. Threlfall, J.R. Clark, L.R. Howard, C.R. Brownmiller. 2017. Phytochemical Contents Impacted by Post-harvest Storage and Harvest Season of Primocane Blackberry Genotypes. Sothern Region American Society for Horticultural Science Annual Meeting February 3-5. Mobile, Alabama.