Nutraceutical quality analysis of several genotypes of *Sambucus* spp. grown in Florida

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Objective. *Sambucus* (elderberry and elderflower) is a new alternative crop with potential for commercial production in Florida. There is limited knowledge on the effects of local growing conditions on the chemical properties of flowers and fruits of genotypes which can be successfully grown in the Southeast. This study evaluated the nutritional composition of several *Sambucus* genotypes grown in Levy County, Florida.

Materials and Methods. An initial group of thirteen genotypes cloned from selected native stock and commercial varieties were planted in replicated blocks with a minimum of four plants in each block in 2018. An additional fifty-four genotypes were planted in four expansion plots totaling two acres of cultivation area between 2019 and 2021. Not all genotypes displayed productivity over the course of the study, with less than half of genotypes producing sufficient materials for testing. Elderberry fruit was harvested during the 2019 through 2021 summer season (July-August). Certain genotypes were evaluated on berries collected in winter months as available. Elderflower samples were collected in summer of 2020. All material analyzed in the study was grown and collected by Hyldemœr + Co., then vacuum-sealed and frozen at -20 °C (Fig. 1) until being transported to the University of Florida Postharvest Lab and kept frozen (-30 °C) for later analysis. Berries were analyzed for soluble solids content (SSC), total titratable acidity (TTA), pH, juice content, total anthocyanin content and total antioxidant capacity (FRAP); while flowers were measured for phenolic content.

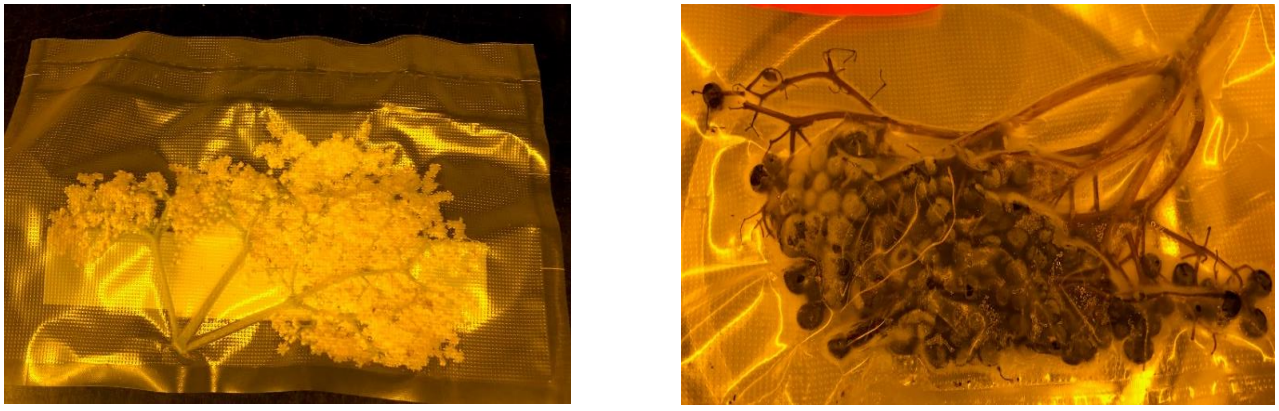

Figure 1. Flowers (left) and berries (right) vacuum sealed and frozen. Image taken under yellow light to avoid degradation of antioxidants.

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Quality analyses. All analyses were performed under yellow light (UV filtering) to prevent degradation of nutritional compounds. Fruit samples (Fig. 2) were ground by hand using a small metal spatula then centrifuged at 12,000 rpm for 20 minutes at 4 °C. The blended berry samples (10 to 20 g) resulted in 5 to 10 ml of juice. The juice was filtered through four layers of cheesecloth and was used to assess SSC, TTA, and FRAP.

A digital Refractometer (model r2i300, Reichert Analytical Instruments) was used to determine soluble solids content (SSC, °Brix); pH and total titratable acidity (TTA, % citric acid basis) were measured using an automatic potentiometer (model 719 S Titrino, Metrohm). TTA was determined by diluting 6 ml of juice with 50 ml deionized water, and then titrating with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2 and expressed as percent citric acid (Fig. 2).

Figure 2. Stems were removed from the berries before grinding (left). Refractometer (middle) and automatic titrimeter (right).

Antioxidant capacity was measured using the FRAP (ferric reducing antioxidant power) assay (Benzine and Strain, 1996; Pulido et al., 2000). Clarified juice was evaluated against a Trolox standard, mixed with a working solution of 1:1:10 FeII TPTZ to FeIII TPTZ to a sodium acetate buffer and read at an absorbance of 593 nm. Results were expressed as Trolox equivalents (TE µmol/g f.w.) (Fig. 3).

Total anthocyanin content was extracted using acidified methanol outlined in Lee et al. (2005). Elderberry tissue (0.3 g) was homogenized with 10 mL of 0.5% formic acid in methanol (v/v) then centrifuged at 12,000 rpm at 4 °C for 20min. The supernatant was retained, and the sample was re-extracted with 5 ml acidified methanol, vortexed 1 min then centrifuged again. The supernatant was combined with the previously retained supernatant and completed to 15 ml with acidified methanol. The extract (0.6 ml) was mixed with 2.4 ml potassium chloride pH 1 buffer and pH 4.5 buffer and measured using a spectrophotometer (Powerwave XS2, BioTek, Winooski, VT, USA) at 510 and 700 nm. Pigment concentration was calculated using the following formula: Abs520 × dilution factor × (molecular weight (MW) of cyanidin-3-glucoside equivalents (CGE)/molar extinction coefficient) where MW of CGE = 449.2 and the molar extinction coefficient = 26,900. Results were expressed as CGE (mg/g f.w.) (Fig. 3).

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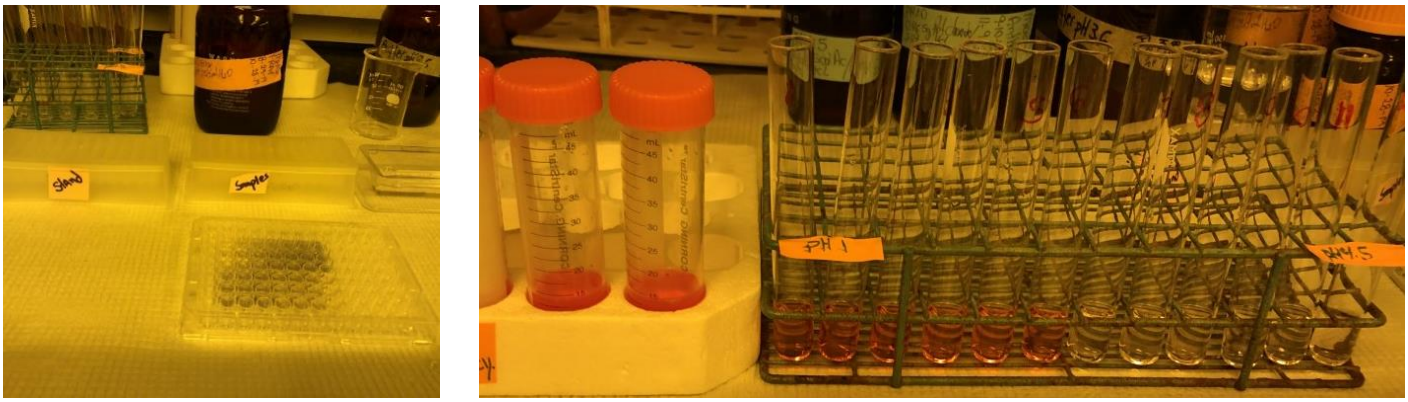

Figure 3. Antioxidant capacity FRAP (left) anthocyanin (right).

Total phenolic content for flowers were analyzed with the Folin-Ciocalteu method reported by Singleton and Rossi (1965) using gallic acid as the standard. Once the flowers were removed from the vacuum sealed bag, they immediately turned brown (Fig. 4). Elderflower (2 g) was homogenized with 2 mL of calcium chloride then centrifuge at 4,500 rpm at 4 °C for 20 minutes. The sample (0.1 mL) was diluted with 5.9ml water then added to 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2.0 mL of 7.5 % sodium carbonate then incubated in a water bath at 45 °C for 15 min. Aliquots of 250 µL were pipetted into a 96-well plate and absorbance read at 765 nm in a microplate reader (PowerWave XS2, Biotek). The results were expressed as gallic acid equivalents (GAE mg/100 g) of flower fresh weight.

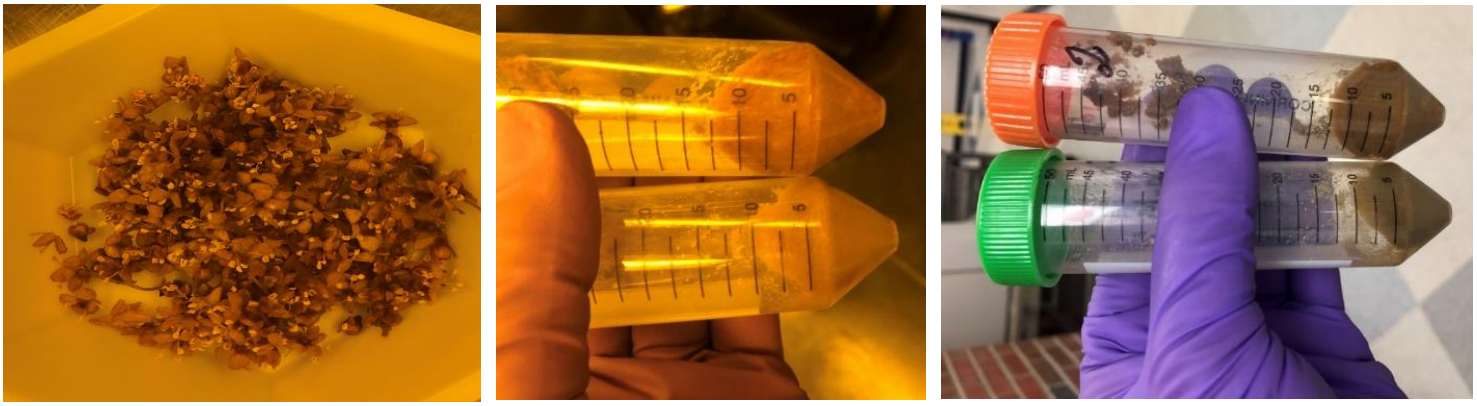

Figure 4. After opening the flower bag (left), after homogenizing the flowers on ice (middle) and at room temperature (right).

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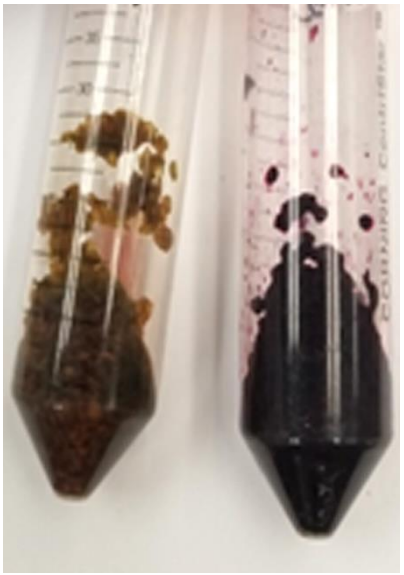
Results – 2019 season. For the elderberry genotypes tested, the SSC ranged from 6.95 to 13.65 °Brix, TTA from 0.27% to 0.59% and the SSC/TTA ratio from 17.12 to 33.93. The pH was similar for all genotypes and averaged 5.2. The antioxidant activity (FRAP) ranged from 1.25 to 18.73 (µmol/g). Genotypes 34 and 38 showed promise in the field so they were analyzed for total anthocyanins. Genotype 34 (8.67 CGE mg/g) had much higher average anthocyanin content than the average of five samples of genotype 38 taken in the main harvest season during July (1.12 CGE mg/g) (Table 1). The phenolic content for elderflower ranged from 4.7 to 8.85 (GAE mg/g) (Table 2).

Results – 2020 season. The values for most parameters measured were higher for the 2020 harvest season. It was also observed that the fruit were larger and contained more seeds. For the genotypes tested in 2020, the SSC ranged from 7.37 to 15.93 °Brix, TTA from 0.38% to 0.58%, and SSC/TTA ratio from 20.85 to 37.32. The pH was similar for all genotypes and averaged 5.0. The antioxidant activity (FRAP) ranged from 6.7 to 37.9 (µmol/g) and total anthocyanins from 1.49 to 12.92 (CGE mg/g) (Table 3).

Results – 2021 season. Genotype 38, Sample 49 (38-00N-Z1.5) had the lowest FRAP (1.17 µmol/g) and anthocyanin (0.65 mg/g) compared to other samples. Genotype JLAV-1, Sample 54 (JLAV 1-03-Z0.5) had both high FRAP (32.59 µmol/g) and anthocyanin (13.24 mg/g) results compared to other samples. The image shows the color difference between blended samples 49 (38-00N-Z1.5) on the left and 54 (JLAV 1-03-Z0.5) on the right. According to the field notes, sample 49 was picked underripe. Sample 54 was taken from an establishment year plant at only 10 months in-ground in the same plot and on the same harvest date as sample 49. Table 4 (page 7) shows the average analysis per genotype. Full data for all individual samples in the study is available from Hyldemœr + Co. upon request. For the genotypes tested in 2021, the average SSC ranged from 8.02 to 13.83 °Brix, TTA from 0.30% to 0.67%, and SSC/TTA ratio from 13.39 to 27.28. The pH was similar for all genotypes and averaged 4.8. The antioxidant activity (FRAP) ranged from 6.27 to 32.57 (µmol/g) and total anthocyanins from 3.6 to 26.57 (CGE mg/g) (Table 4).



Summary. The cumulative results from three harvest seasons (2019, 2020 and 2021) were similar or slightly higher than the values found in the literature, though several samples tested at significantly higher ranges than found in the literature in terms of antioxidant capacity and anthocyanin content. In 2020 when the flower sample bags were opened they immediately oxidized (turned brown); however, the data was similar to other reported results. The reported values for elderberry (*Sambucus canadensis* L.) were 8 to 12 °Brix, 0.75 to 1.4% TTA, 4.6 pH (Kaack et al., 2015; Ozgen et al., 2010; Perkins-Veazie et al., 2015), 2 to 6 Cy-3GE mg/g Anthocyanin content, and 20 to 24 TE µmol/g Antioxidant capacity (Ozgen et al., 2010; Perkins-Veazie et al., 2015). Elderflower phenolic content was reported as 10 GAE mg/g (Mikulic-Petkovsek et al., 2015).

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Table 1. Season 2019: Elderberry fruit antioxidant activity, total anthocyanins, SSC, TTA, SSC/TTA ratio, pH and juice content - postharvest.

Sample from Genotype Specimen	Harvest date	Antioxidant activity - FRAP		Anthocyanin		SSC		TTA		SSC/TTA Ratio		pH		Juice content (ml/g)
		TE (µmol/g)	± *s.d.*	Cy-3GE (mg/g)	± *s.d.*	°Brix	± *s.d.*	Citric acid %	± *s.d.*	SSC/TTA	± *s.d.*	pH	± *s.d.*	
27-01-400	7/25/2019	9.77	*1.58*			10.17	*0.45*	0.59	*0.05*	17.12	*0.02*	4.58	*0.02*	0.45
27-02-400	7/6/2019	5.38	*0.41*			9.07	*0.15*	0.32	*0.03*	28.66	*0.02*	5.39	*0.02*	0.44
29-01-100	7/11/2019	13.13	*0.80*			10.87	*0.45*	0.45	*0.05*	24.09	*0.06*	4.98	*0.03*	0.52
29-01-100	7/25/2019	16.26	*3.93*			10.73	*0.21*	0.39	*0.01*	27.47	*0.03*	5.31	*0.06*	0.53
29-03-100	7/11/2019	15.92	*0.50*			10.60	*0.00*	0.50	*0.02*	21.22	*0.01*	4.92	*0.01*	0.62
30-02-200	8/30/2019	8.39	*0.31*			12.80	*0.42*	0.38	*0.00*	33.68	*0.01*	5.37	*0.01*	0.38
31-01-100	7/25/2019	5.54	*0.57*			8.60	*0.36*	0.37	*0.02*	22.99	*0.06*	5.21	*0.06*	0.41
31-03-400	7/25/2019	4.16	*0.21*			9.13	*0.47*	0.38	*0.01*	23.81	*0.03*	5.45	*0.03*	0.37
34-01-200	7/11/2019	9.15	*1.01*	7.20	*0.76*	10.47	*0.47*	0.52	*0.05*	20.13	*0.01*	4.75	*0.01*	0.31
34-02-200	7/15/2019	6.15	*1.54*	10.72	*2.23*	12.73	*0.15*	0.49	*0.03*	26.04	*0.04*	4.93	*0.04*	0.22
34-15-400	7/11/2019	5.76	*0.39*	8.09	*0.11*	11.70	*0.87*	0.45	*0.01*	25.92	*0.05*	5.26	*0.05*	0.30
38-02-200	7/9/2019	2.92	*0.13*	2.24	*0.03*	8.55	*0.07*	0.33	*0.03*	25.79	*0.04*	5.00	*0.04*	0.21
38-02-400	11/6/2019	15.88	*0.19*	4.30	*1.00*	10.15	*0.07*	0.45	*0.00*	22.66	*0.04*	4.87	*0.04*	0.43
38-04-400	11/6/2019	18.73	*2.55*	5.73	*0.04*	9.70	*0.42*	0.47	*0.01*	20.70	*0.01*	4.70	*0.01*	0.43
38-09-400	7/18/2019	2.67	*0.47*	1.39	*0.16*	9.45	*0.35*	0.28	*0.02*	33.93	*0.02*	5.71	*0.02*	0.22
38-14-400	7/18/2019	1.49	*0.20*	0.33	*0.03*	7.80	*0.00*	0.32	*0.00*	24.15	*0.06*	5.38	*0.06*	0.20
38-15-400	7/18/2019	2.32	*0.07*	0.98	*0.18*	9.15	*0.07*	0.30	*0.01*	30.55	*0.02*	5.75	*0.02*	0.22
38-19-400	7/11/2019	1.25	*0.20*	0.66	*0.07*	6.95	*0.35*	0.27	*0.00*	25.60	*0.01*	5.36	*0.01*	0.22
50-01-400	7/25/2019	8.88	*0.76*			12.80	*0.42*	0.49	*0.01*	26.10	*0.01*	5.08	*0.01*	0.31
51-01-200	8/30/2019	13.56	*0.13*			13.65	*0.64*	0.48	*0.06*	28.59	*0.00*	5.36	*0.00*	0.28
53-01-100	7/11/2019	8.95	*0.25*			9.60	*0.14*	0.36	*0.00*	26.34	*0.01*	5.29	*0.01*	0.30

TE= trolox equivalents; Cy-3GE= cyanidin 3-glucoside equivalents. Data based on fresh weight and represents the mean (n=3).

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Table 2. Season 2019: Elderflower total phenolics - postharvest.

Sample from genotype specimen	Harvest date	Total phenolics (mg/g GAE)	± *s.d.*
27-01-400	6/6/2019	7.53	*0.06*
27-03-100	5/30/2019	6.73	*0.02*
29-03-100	5/30/2019	6.59	*0.04*
29-03-100	6/6/2019	6.16	*0.02*
30-02-200	7/19/2019	4.66	*0.04*
31-04-400	6/4/2019	6.22	*0.06*
34-19-400	6/14/2019	5.57	*0.01*
38-02-200	5/30/2019	8.85	*1.26*
50-F1-400	5/30/2019	6.63	*0.02*
51-01-200	8/30/2019	7.76	*0.02*

GAE = gallic acid equivalents. Data based on fresh weight and represents the mean (n=3).

Table 3. Season 2020: Elderberry fruit antioxidant activity, total anthocyanins, SSC, TTA, SSC/TTA ratio, pH and juice content - postharvest.

Sample from genotype specimen	Harvest date	Antioxidant activity - FRAP		Anthocyanin		SSC		TTA		SSC/TTA Ratio		pH		Juice content (ml/g)
		TE (µmol/g)	± *s.d.*	Cy-3GE (mg/g)	± *s.d.*	°Brix	± *s.d.*	Citric acid %	± *s.d.*	SSC/ TTA	± *s.d.*	pH	± *s.d.*	
27-08-400	8/1/2020	37.88	*0.18*	12.92	*0.80*	15.93	*0.59*	0.58	*0.06*	27.93	*3.97*	4.63	*0.06*	0.6
28-01-200	7/23/2020	16.72	*0.18*	8.97	*1.72*	13.90	*0.20*	0.39	*0.10*	37.32	*10.13*	5.20	*0.01*	0.6
29-03-800	8/1/2020	21.61	*0.15*	10.78	*0.97*	10.37	*0.31*	0.47	*0.13*	23.18	*7.14*	5.11	*0.01*	0.5
31-08-400	8/1/2020	21.24	*0.02*	7.52	*0.09*	11.33	*0.25*	0.54	*0.07*	20.85	*2.54*	4.88	*0.04*	0.6
32-03-400	5/25/2020	18.07	*0.11*	2.63	*0.23*	11.83	*0.12*	0.49	*0.11*	24.58	*5.30*	4.85	*0.03*	0.6
33-08-400	7/23/2020	17.05	*0.07*	6.19	*1.05*	11.60	*0.44*	0.38	*0.09*	31.40	*6.13*	5.29	*0.05*	0.6
34-19-400	8/1/2020	21.76	*0.04*	10.89	*0.56*	10.83	*0.12*	0.53	*0.12*	21.12	*4.77*	4.88	*0.01*	0.6
38-03-400	8/1/2020	6.68	*0.01*	1.49	*1.49*	7.37	*0.23*	0.38	*0.19*	22.24	*10.98*	4.88	*0.03*	0.6

TE= trolox equivalents; Cy-3GE= cyanidin 3-glucoside equivalents. Data based on fresh weight and represents the mean (n=3).

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Table 4. Season 2021: Elderberry fruit antioxidant activity, total anthocyanins, SSC, TTA, SSC/TTA ratio, pH and juice content - postharvest.

Sample average of genotype	Antioxidant activity - FRAP		Anthocyanin		SSC		TTA		SSC/TTA Ratio		pH		Juice content (ml/g)
	TE (µmol/g)	± *s.d.*	Cy-3GE (mg/g)	± *s.d.*	°Brix	± *s.d.*	Citric acid %	± *s.d.*	SSC/TTA	± *s.d.*	pH	± *s.d.*	
27	9.36	*na*	14.34	*na*	11.45	*na*	0.58	*na*	19.83	*na*	4.65	*na*	0.45
28	22.41	*4.03*	14.51	*4.41*	11.23	*2.93*	0.51	*0.03*	22.09	*4.51*	4.79	*0.21*	0.59
29	14.21	*1.28*	17.91	*5.29*	10.82	*1.96*	0.51	*0.09*	21.57	*5.78*	4.83	*0.16*	0.51
31	15.55	*4.34*	12.04	*4.11*	12.01	*1.58*	0.57	*0.07*	21.19	*2.63*	4.81	*0.18*	0.53
32	9.79	*na*	13.61	*na*	10.70	*na*	0.48	*na*	22.18	*na*	4.87	*na*	0.65
33	12.97	*0.34*	10.35	*1.78*	11.95	*0.67*	0.54	*0.01*	21.99	*1.47*	4.81	*0.13*	0.58
34	12.00	*0.40*	7.21	*2.51*	10.06	*0.23*	0.38	*0.06*	26.72	*4.08*	5.04	*0.27*	0.51
36	15.28	*1.47*	23.30	*6.33*	13.83	*4.00*	1.03	*0.03*	13.39	*3.50*	4.26	*0.14*	0.44
38	6.27	*1.01*	3.60	*2.50*	8.21	*1.15*	0.30	*0.03*	27.28	*2.90*	4.93	*0.10*	0.58
DNS-01	18.51	*na*	26.57	*na*	10.15	*na*	0.61	*na*	16.69	*na*	4.41	*na*	0.54
DNS-07	18.19	*na*	17.21	*na*	8.05	*na*	0.43	*na*	18.94	*na*	4.74	*na*	0.49
DNS-10	18.11	*na*	13.89	*na*	10.50	*na*	0.51	*na*	20.58	*na*	4.68	*na*	0.48
DNS-11	28.63	*na*	22.91	*na*	10.15	*na*	0.66	*na*	15.47	*na*	4.50	*na*	0.60
DNS-15	26.39	*na*	17.04	*na*	11.85	*na*	0.52	*na*	22.77	*na*	4.68	*na*	0.51
DNS-16	23.31	*3.24*	13.63	*0.34*	10.05	*0.78*	0.39	*0.05*	25.81	*5.32*	5.00	*0.23*	0.53
DNS-19	24.51	*na*	23.79	*na*	12.20	*na*	0.57	*na*	21.25	*na*	4.86	*na*	0.53
DNS-23	26.65	*na*	22.56	*na*	11.40	*na*	0.67	*na*	17.09	*na*	4.66	*na*	0.48
DNS-26	32.57	*na*	25.39	*na*	11.60	*na*	0.48	*na*	23.95	*na*	4.99	*na*	0.51
DNS-30	9.11	*2.30*	6.79	*4.92*	8.07	*0.88*	0.44	*0.05*	18.52	*3.68*	4.84	*0.19*	0.44
FGWR	20.68	*1.91*	22.85	*6.98*	12.30	*2.12*	0.63	*0.01*	19.71	*3.68*	4.49	*0.26*	0.56
JLAV1	20.71	*7.70*	12.21	*4.01*	8.02	*0.75*	0.35	*0.02*	23.12	*3.01*	5.13	*0.14*	0.54
JLAV5	30.15	*3.38*	17.95	*1.50*	9.81	*0.88*	0.37	*0.01*	26.88	*2.67*	5.15	*0.20*	0.59

TE= trolox equivalents; Cy-3GE= cyanidin 3-glucoside equivalents. Data based on fresh weight and represents the mean (n=3).

Discussion. Though approximately 40% of genotypes present in the trial plots proved sufficiently productive to analyze for compositional quality, relatively few genotypes displayed meaningful, consistent productivity. Of the most consistently productive genotypes, a relatively large group were demonstrated to be capable of producing fruit with high concentrations of antioxidants and anthocyanins by previously reported standards. Some potentially productive genotypes were eliminated from further trialing expansion. For example, genotype 38 reliably produced harvest volumes in excess of nearly any other genotype. Because of its high productivity and large cymes, this genotype needs to be trellised to keep the fruit off the ground effectively. This additional management, labor and material cost was not demonstrated to be worthwhile long-term given the relatively poor compositional quality analysis for genotype 38 over

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the 3-year study period. Conversely, the JLAV and DNS genotypes series grown in the same trial plots, but with only one year of analysis performed, demonstrated early precocity and very high levels of antioxidant capacity and anthocyanins. Ongoing trialing of genotypes selected through field and compositional quality analysis will focus on a limited number of genotypes, only 4 of which were present in the original 13 genotypes at the beginning of the study period: genotypes 29, 31, 33, and 34. Additional genotypes added over the 2019-2021 establishment period which warrant further study for cultivation in the Southeast include: the FGW, DNS and JLAV series of genotypes.

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