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Volatile Composition of Four Southern Highbush Blueberry Cultivars and Effect of Growing Location and Harvest Date

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ABSTRACT: The volatile composition of four southern highbush blueberry cultivars ('Primadonna', 'Jewel', 'Snowchaser', and 'FL02-40') grown in two locations (Gainesville and Haines City, FL) and harvested multiple times was investigated. A total of 42 volatiles were identified, including 8 esters, 12 terpenoids, 11 aldehydes, 7 alcohols, and 4 ketones. Twelve of these volatiles are reported for the first time in highbush blueberries, with 10 being positively identified: (*Z*)-3-hexenal, (*E*,*E*)-2,4-hexadienal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonedienal, methyl 2-methylbutanoate, butyl acetate, 2-methylbutyl acetate, and geranyl acetate. The dominant volatiles were aldehydes followed by terpenoids and esters, with distinct varietal profiles. 'Primadonna' was characterized by a large amount of esters and C-6 aldehydes. In contrast, fewer than 4 esters were found in 'FL02-40' and 'Snowchaser', respectively, but they produced more terpenoids than 'Primadonna' and 'Jewel'. Location and/or harvest date affected the production of volatiles in 'Primadonna', but not so much in the other cultivars.

KEYWORDS: blueberry volatiles, *Vaccinium* spp., southern highbush, Primadonna, Jewel, Snowchaser, 'FL02-40'

INTRODUCTION

Blueberries are popular fruits because of their nutritional value and eating quality. Because blueberries have such high levels of antioxidants with beneficial health attributes, there has been a plethora of research on the composition of these antioxidants, with a focus on genetic makeup and environmental factors.[1−5] In contrast, there are fewer studies on the volatile composition, which determines aroma and flavor. A recent consumer study demonstrated the importance of flavor (blueberry flavor and sweetness) on overall eating quality over textural (juiciness and texture characters) and visual (color and size) attributes.[6] However, this study did not correlate volatiles with flavor attributes.

Several types of blueberries are cultivated and marketed as fresh fruit in North America, depending on the region: The highbush blueberry (*Vaccinium corymbosum*) is grown in Michigan, New Jersey, Oregon, and North Carolina. The rabbiteye blueberry (*Vaccinium virgatum* (syn. *Vaccinium ashei*) and the southern highbush blueberry (interspecific hybrids of *Vaccinium virgatum*, *V. corymbosum*, and *Vaccinium darrowii*) are both mostly grown in the southeastern areas of the United States.[7] The wild blueberry (lowbush blueberry, *Vaccinium angustifolium*) naturally occurs in Maine and Canada and is harvested for the processing market. In addition to cultivated types, there are many wild native *Vaccinium* species that are gathered only locally (for example, bilberries including *Vaccinium myrtillus*, *Vaccinium deliciosum*, and *Vaccinium membranaceum*). Each blueberry type produces a different volatile profile. Fruits of the lowbush blueberry, *V. myrtillus*, and other wild *Vaccinium* species produce mostly esters: methyl acetate, ethyl acetate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and methyl butanoate, as well as acetaldehyde, (*E*)-2-hexenal, and linalool.[8−12] On the other hand, volatiles isolated from highbush blueberry fruit include "green compounds" such as (*E*)-2-hexenal, (*E*)-2-hexenol, hexanal, and (*Z*)-3-hexenol and terpene alcohols such as linalool, citronellol, nerol, α-terpineol, and geraniol.[13−15] The major volatiles in the rabbiteye blueberry were found to be ethyl acetate, *p*-cymene, hexanol, (*Z*)-2-hexenol, heptanol, cineralone, β-ionone, terpinen-4-ol, 2-undecanone, α-terpineol, carveol, nerol, and eugenol.[16−18] Although volatile constituents are different among different species of blueberries, (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, linalool, and geraniol have been found in common in most types and are considered to be typical aroma compounds for blueberry.[13,18]

Due to the low chilling requirements selected for southern highbush blueberry cultivars, these berries typically ripen earlier than all other cultivated blueberries (highbush, lowbush, and rabbiteye). This feature provides for a high-value market window from mid-April to late May, which has led to the expansion of Florida's blueberry acreage.[7] Various blueberry cultivars, which were released from the blueberry breeding program at the University of Florida and bred specifically for Florida's mild winter and subtropical growing environment, have been planted commercially. 'Primadonna' is an early-ripening southern highbush blueberry cultivar.[19] The fruit of 'Primadonna' are relatively large and have excellent firmness and sweetness. 'Jewel' is another early-ripening southern highbush blueberry cultivar.[7] The berry quality is also excellent but tends to be tart (high acid) until fully ripe. 'Snowchaser' is an extremely early season southern highbush

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blueberry.[19] The berry is medium to large and has good firmness and flavor. 'FL02-40' is a new highbush blueberry cultivar released in 2009 and commercialized under the name of Kestrel; it has been anecdotally described as possessing "fresh green", "fruity", and "floral" flavor.

To our knowledge, there has been no study reporting the volatile content of southern highbush blueberries. Therefore, the objective of this study was to investigate the volatile composition of four southern highbush blueberry cultivars, 'Primadonna', 'Jewel', 'FL02-40', and 'Snowchaser', and the impact of growing location and harvest date on these volatiles.

MATERIALS AND METHODS

Chemicals. Pure standards of methyl 2-methylbutanoate, ethyl 2-methylbutanoate, butyl acetate, (*Z*)-3-hexenyl acetate, 1-hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 1-nonanal, (*E*,*E*)-2,4-hexadienal, (*Z*,*E*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 1-pentene-3-ol, 1-pentanol, 1-octanol, 2-heptanone, 2-nonanone, 6-methyl-5-hepten-2-one, 2-undecanone, linalool (3,7-dimethylocta-1,6-dien-3-ol), 3-heptanone (internal standard), and ethyl undecanoate (internal standard) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Hexyl acetate, limonene (1-methyl-4-(1-methylethenyl)cyclohexene), and (*Z*)-linalool oxide ((2*S*,3*S*)-2-ethenyl-2,6,6-trimethyloxan-3-ol) were obtained from Fluka (Buchs, Switzerland). 1-Pentanal, 1-hexanol, and geraniol (3,7-dimethylocta-2,6-dien-1-ol) were obtained from Acros Organics (Fair Lawn, NJ). 1-Octanal, 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), α-terpineol (2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol), and geranyl acetate (3,7-dimethyl-2,6-octadiene acetate) were obtained from SunPure (Avon Park, FL). Internal standards 3-heptanone and ethyl undecanoate were prepared in methanol at 31.5 and 8.69 mg/kg in a single mixture. Methanol (HPLC grade, 99.9%) and sodium chloride were obtained from Fisher Scientific (Fair Lawn, NJ), whereas sodium fluoride (ACS graded) was obtained from Acros Organics.

Blueberry Samples. Highbush blueberries 'Jewel' and 'Primadonna' were hand-harvested from a local blueberry farm in Haines City, FL (28° 03′ 29″ N, 81° 33′ 55″ W). Harvest dates were April 27, May 5, and May 12, 2010. 'Jewel' and 'Primadonna' were also harvested from a University of Florida grower-cooperator farm near Gainesville, FL (29° 47′ 32″ N, 82° 07′ 22″ W). Harvest dates were May 19 and May 24, 2010. Two other highbush blueberry cultivars, 'Snowchaser' and 'FL02-40', were harvested from the same field on May 10, May14, and May 17, 2010. All fruits were harvested at commercial maturity, as determined by complete blue skin color. Both field locations used a typical harvest rotation of 3—5 days between picking. For 'Jewel', 'Primadonna', and 'FL02-40', the harvest dates were midseason with respect to the overall yield of the plants. Therefore, the fruits from each cultivar and location used in the study were considered to be similar in maturity. The majority of the total yield for 'Snowchaser' plants was harvested prior to the harvest dates of this study. However, 'Snowchaser' has a longer harvest window than the other cultivars utilized in this study, and fruits of similar maturity continued to be available during the month of May. Regardless, 'Snowchaser' plants were subject to the same commercial harvest schedule, so although the total numbers of fruits were lower, the maturity stage of individual fruits was similar to the other cultivars.

After harvest, blueberry fruits were washed with distilled water and dried with tissue paper. Fruits were sorted for the absence of surface defects and uniform blue coloration. Two hundred grams of blueberries was blended in a glass container (Waring Products Div., Dynamics Corp. of America, New Hartford, CO), with equal weight of distilled water, 20% sodium chloride, and 1% sodium fluoride. Sodium chloride was employed to reduce possible enzyme activity, and sodium fluoride was used to reduce microbial growth. The sample was pureed using a high-speed pulse mode and blended for 20 s. Ten grams of blueberry puree was put in a 40 mL vial loaded with a 3 mm Teflon-coated stir bar and previouly flushed with ultrahigh-purity (0.99999%) grade nitrogen for 30 s. Fifteen milliliters of internal standards 3-heptanone and ethyl undecanoate was added to the vials with the puree, for final concentrations of 47.25 and 13.04 μg/kg, respectively. Three vials were collected per sample and immediately frozen at −20 °C until volatile analysis within 24 h. Furthermore, soluble solids content (SSC) and titratable acidity (TA, expressed as percent citric acid) were measured in duplicate on a subset of fruit (not salted) using a PAL-1 pocket refractometer (Atago USA, Inc., Bellevue, WA) for SSC and a Metrohm 808 Titrando (Metrohm USA, Riverview, FL) for TA.

Blueberry Volatile Extraction with SPME. Sample vials with puree were equilibrated at 40 °C in a water bath for 20 min. After equilibration, headspace volatiles were collected on a 2 cm SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm film thickness, Supelco, Bellefonte, PA) for 40 min at 40 °C. After extraction, volatile desorption was performed by introducing the SPME fiber into a GC injection port for 3 min; injection was splitless.

GC-MS Identification. GC-MS analyses were performed using a Perkin-Elmer Clarus 500 GC-MS (Perkin-Elmer, Waltham, MA). Compound separation was achieved with a DB-Wax column (60 m × 0.25 mm i.d., cross-linked polyethylene glycol, 0.50 μm film thickness, J&W Scientific, Agilent Technique, Foster City, CA). The column flow rate was 2.0 mL/min. Initial oven temperature was 35 °C and held for a 1 min, then increased to 190 °C at a rate of 4 °C/min, and finally to 240 °C at a rate of 8 °C/min, with a 5 min hold at the final temperature. Injection port, MS transfer line, and ion source temperatures were 230, 240, and 180 °C, respectively. Electron ionization mass spectrometric data from m/z 25 to 300 were collected, with an ionization voltage of 70 eV. Compound identifications were made by comparing mass spectral data samples with the Wiley 275.L (G1035) database and confirmed by authentic pure standards (Table 1). Standard retention indices (RIs) were calculated using a series of standard linear alkanes C_5—C_{25} for GC-FID identification.

GC-FID Quantification. GC-FID quantification was conducted using an Agilent 6890N gas chromatograph system equipped with a flame ionization detector (FID) (Palo Alto, CA). Volatiles were analyzed on a DB-Wax column (30 m × 0.32 mm i.d., cross-linked poly(ethylene glycol), 0.50 μm film thickness, J&W Scientific, Folsom, CA). The column flow rate was 2.0 mL/min, with injection in the splitless mode. Injector and detector temperatures were 220 and 250 °C, respectively. Initial oven temperature was 35 °C and held for 1 min, then increased to 190 °C at a rate of 4 °C/min, and finally to 240 °C at a rate of 8 °C/min, with a 5 min hold at the final temperature. Compound identification was based on matching retention times with authentic pure standards, RIs, which were calculated using a series of standard linear alkanes C_5—C_{25}, and GC-MS. It should be noted that the same numbers of peaks and compounds were identified on the 60 m column (GC-MS) as on the 30 m column (GC-FID), indicating the resolution was similar on both columns.

Seven-point calibration plots were constructed using peak areas obtained by adding known amounts of standards to low volatile blueberry puree made with unripe fruit (green and turning-purple). Standards were added as mixtures so that final puree concentrations ranged as indicated in Table 1. Fifteen microliters of internal standard was also added to each calibration mixture at the same final concentrations as in the sample puree. After mixing and equilibration, the volatiles were extracted with SPME and analyzed with GC-FID under the same conditions as for sample analysis. Calibration plots for each volatile were constructed and were used to calculate the concentrations of volatiles in the samples. Peak areas corrected for internal standard in the calibration matrix were subtracted from the peak area of the calibration

Table 1. Chemical Standards and Calibration Curves Used for Quantification of Volatiles in Blueberry Samples

RI[a] (Wax)	compound	regression eq[b]	R^2	range (μg/kg)
1133	3-heptanone (IS)[c]			
959	1-pentanal	$y = 10.891x$	0.995	20−900
997	methyl 2-methylbutanoate	$y = 3.9588x$	0.999	8−900
1036	ethyl 2-methylbutanoate	$y = 1.5501x$	0.999	4−800
1053	butyl acetate	$y = 2.4741x$	0.998	5−600
1061	1-hexanal	$y = 3.1891x - 0.3202$	0.990	7−2000
1117	(*Z*)-3-hexenal	$y = 301.69x^2 + 9.1554x + 1.1066$	0.985	20−1400
1146	1-penten-3-ol	$y = 2.97x$	0.995	4−300
1166	2-heptanone	$y = 1.201x$	0.996	5−700
1182	1-methyl-4-(1-methylethenyl)cyclohexene (limonene)	$y = 2.4038x$	0.984	5−400
1188	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (1, 8-cineole)	$y = 0.5705x$	0.992	5−800
1202	(*E*)-2-hexenal	$y = 0.0238x^2 + 1.1467x + 5.5438$	0.999	600−6000
1239	1-pentanol	$y = 13.832x + 0.3779$	0.999	10−1100
1256	hexyl acetate	$y = 0.5226x$	0.993	5−200
1270	1-octanal	$y = 0.5286x$	0.997	10−1000
1302	(*Z*)-3-hexenyl acetate	$y = 0.5664x$	0.993	9−900
1331	6-methyl-5-hepten-2-one	$y = 0.3893x$	0.992	5−700
1341	1-hexanol	$y = 2.9284x$	0.984	7−700
1370	(*Z*)-3-hexenol	$y = 0.6232x^2 + 5.3607x + 0.2154$	0.999	10−1000
1373	2-nonanone	$y = 0.3848x + 0.7398$	0.990	6−1200
1378	1-nonanal	$y = 0.4943x + 0.3638$	0.982	7−700
1392	(*E*)-2-hexenol	$y = 0.5852x^2 + 4.7138x + 0.2917$	0.997	7−1400
1413	(*E*,*E*)-2,4-hexadienal	$y = 14.032x$	0.994	8−800
1426	(2*S*,3*S*)-2-ethenyl-2,6,6-trimethyloxan-3-ol ((*Z*)-linalool oxide)	$y = 7.9173x$	0.993	6−500
1534	3, 7-dimethylocta-1,6-dien-3-ol (linalool)	$y = 0.5111x$	0.990	12−1400
1724	ethyl undecanoate (IS)[c]			
1545	1-octanol	$y = 2.1387x$	0.977	10−1100
1604	(*E*,*Z*)-2,6-nonadienal	$y = 2.5293x$	0.996	10−1100
1611	2-undecanone	$y = 1.454x$	0.995	5−800
1677	2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol (α-terpineol)	$y = 8.8742x$	0.916	15−3000
1684	(*E*,*E*)-2,4-nonadienal	$y = 3.5672x + 1.1693$	0.998	7−1100
1706	3,7-dimethyl-2,6-octadiene acetate (geranyl acetate)	$y = 1.4314x$	0.984	4−900
1796	(*E*,*E*)-2,4-decadienal	$y = 7.2663x$	0.972	6−500
1825	3,7-dimethylocta-2,6-dien-1-ol (geraniol)	$y = 5.3268x$	0.952	7−700

[a] RI, retention index. [b] $x = A_{st}/A_{is} - A_{matrix}/A_{is\ in\ matrix}$ and $y = C_{st}/C_{is}$ with A_{st} = area of standard, A_{is} = areas of internal standard, A_{matrix} = area peak in matrix, $A_{is\ in\ matrix}$ = areas of internal standard in matrix, C_{st} = concentration of standard, and C_{is} = concentration of internal standard. 3-Heptanone was used as internal standard for compounds with RI from 959 to 1534 and ethyl undecanoate for compounds with RI from 1545 to 1825. [c] IS, internal standard.

standard, as indicated in the footnote of Table 1. Triplicate analyses were performed for each sample and standards.

Statistical Analysis. The effect of cultivar, harvest date, and interactions was examined for each location using two-way analysis of variance (ANOVA), using SAS statistical software (SAS System Software version 9.1, SAS Institute, Cary, NC). Because interactions were significant for most volatiles, one-way ANOVA was performed for each cultivar within location. Mean volatile contents were separated using Tukey's Honest Significant Difference (HSD) test for multiple comparison, with $\alpha = 0.05$. A principal components analysis (PCA) was performed on the mean ($n = 3$) data using Pearson's correlation method to account for large variation in scaling between volatiles[20] using XLSTAT software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Volatile Composition in Four Southern Highbush Blueberry Hybrids. A total of 42 volatiles were identified and quantified via GC-FID using authentic internal and external standards. The quantified volatiles were grouped into five chemical groups, including 8 esters, 12 terpenoids, 11 aldehydes, 7 alcohols, and 4 ketones (Table 2). In general, the amount and presence/absence of esters and terpenoids were the differentiating volatile factors between cultivars. Except for 1-penten-3-ol, 2-nonanone, and 2-undecanone, alcohols and ketones were present in all cultivars. The presence and amount of some aldehydes also varied among cultivars. Table 2 lists odor thresholds of each compound compiled from the literature[21] as an indication of possible contribution of the compound to the blueberry aroma. However, odor thresholds provide only a general indication and do not reflect the actual contribution of the compound to the aroma, because it does not take into account interactions among volatiles and between volatiles and fruit matrix.[22] Furthermore, an effort was made to list thresholds obtained from the same laboratory whenever possible, because

Table 2. Concentration Ranges (Micrograms per Kilogram) of Volatile Compounds Found in Four Florida Southern Highbush Blueberry Cultivars Harvested Over 1 Month in Two Locations (Published Odor Threshold in Water for Each Volatile)[21]

RI	compound	Primadonna	Jewel	Snowchaser	FL02-40	threshold[i]
997	methyl 2-methylbutanoate	10−40	10−16	13−15	7−21	0.25
1005	methyl 3-methylbutanoate[a]	269−3527	34−247	39−128	14−21	4.4
1036	ethyl 2-methylbutanoate	1.5−8.7	—[j]	—	—	0.1
1050	ethyl 3-methylbutanoate[b]	10−111	5−111	0−8	—	0.01
1053	butyl acetate	0−26	0.6−1.7	—	—	66
1104	2-methylbutyl acetate[c]	0−3	0−6	—	—	5
1256	hexyl acetate	0.7−4.3	0−3	—	—	2
1302	(*Z*)-3-hexenyl acetate	2.4−3.2	3−7	21−48	10−29	8
	total esters	*307−3638*	*52−379*	*74−192*	*45−59*	
1082	*β*-pinene[d]	8−22	13−18	11−14	9−15	140
1182	limonene	29−126	33−43	39−49	70−85	10
1188	1,8-cineole	3−4	—	40−46	6−10	1.3
1192	(*Z*)-dihydrolinalool oxide[e]	0−13	0.9−6.1	41−74	19−66	—
1224	(*E*)-dihydrolinalool oxide[e]	1.5−24	1−9	71−175	34−134	—
1426	(*Z*)-linalool oxide	—	—	2−3	9−13	320
1534	linalool	20−94	16−46	84−98	75−240	6
1677	α-terpineol	70−353	53−118	521−744	491−1343	330
1783	nerol[f]	16−100	—	—	49−130	30
1825	geraniol	—	—	—	53−102	40
1706	geranyl acetate	—	—	11−16	3−23	9
1832	geranylacetone[f]	75−503	35−205	18−28	308−510	60
	total terpenoids	*237−1203*	*171−416*	*970−1073*	*1416−2387*	
700	acetaldehyde[g]	0−5	5−21	6−11	9−16	15
959	pentanal	21−49	38−79	24−55	23−26	12
1061	hexanal	179−1590	44−277	66−198	156−205	4.5
1117	(*Z*)-3-hexenal	280−1125	317−413	256−340	464−612	0.25
1202	(*E*)-2-hexenal	1686−4777	1816−2039	1548−1967	2674−3286	17
1270	octanal	tr[k]	tr	tr	tr	0.7
1378	nonanal	60−61	59−62	59−60	60	1
1413	(*E*,*E*)-2,4-hexadienal	0−36	9−26	29−30	25−42	60
1604	(*E*,*Z*)-2,6-nonadienal	5−36	—	12−25	9−27	0.01
1684	(*E*,*E*)-2,4-nonadienal	109−143	99−176	91−299	113−153	0.09
1796	(*E*,*E*)-2,4-decadienal	—	—	54−73	tr	0.07
	total aldehydes	*2414−7640*	*2437−2926*	*2415−2845*	*3606−4313*	
1146	1-pentene-3-ol	0−13	—	—	9−13	400
1239	pentanol	60−117	83−132	60−90	73−82	4000
1341	hexanol	14−55	5−17	15−19	26−93	500
1370	(*Z*)-3-hexenol	56−86	67−78	52−59	105−111	70
1392	(*E*)-2-hexenol	79−144	79−131	84−106	155−165	1000
1513	2-heptanol[h]	105−232	101−151	80−200	122−150	70
1545	octanol	24−47	13−26	11−18	60−89	110
	total alcohols	*378−560*	*364−493*	*304−490*	*559−683*	
1166	2-heptanone	2−8	5−10	3−4	4−7	140
1331	6-methyl-5-hepten-2-one	7−10	5−10	3−4	6−8	50
1373	2-nonanone	tr	tr	tr	tr	5
1611	2-undecanone	0−7	—	12−24	8−31	7
	total ketones	*12−21*	*10−21*	*18−31*	*21−42*	

[a] Concentration estimated by methyl 2-methylbutanoate. [b] Concentration estimated by ethyl 2-methylbutanoate. [c] Concentration estimated by butyl acetate. [d] Concentration estimated by limonene. [e] Concentration estimated by 1,8-cineole. [f] Concentration estimated by geraniol. [g] Concentration estimated by pentanal. [h] Concentration estimated by (*E*)-2-hexenol. [i] Threshold values in water from ref 21 are presented as an indication of possible odor contribution. [j] —, no peak detected. [k] tr, trace.

threshold values can vary considerably between laboratories using different methods.

Esters were most predominant in 'Primadonna', followed by 'Jewel', 'Snowchaser', and 'FL02-40' (Table 2). The four methyl butyric acid esters, when present, were at concentrations well above their reported thresholds, indicating their likely overall fruity aroma contribution to these blueberries. In contrast, butyl acetate, 2-methylbutyl acetate, and hexyl acetate were present in 'Primadonna' and 'Jewel' at or below their reported thresholds, suggesting that they provide little to no aroma in these cultivars; they were absent from 'Snowchaser' and 'FL02-40'. (*Z*)-3-Hexenyl acetate was higher in 'Snowchaser' and 'FL02-40' than in 'Primadonna' and 'Jewel' and above its odor threshold in the former two cultivars. Esters constitute one of the largest groups of volatiles in many fruits,[23,24] as well as wild lowbush blueberries and *V. myrtillus*.[8−10,12] The overall lower ester amounts in 'Snowchaser' and 'FL02-40' might be explained by a common ancestor, *Vaccinium elliottii*, which was reported to have low volatile content including esters.[11] Few esters have been identified in highbush blueberries, and three of the eight esters are reported here for the first time in southern highbush blueberries: methyl 2-methylbutanoate, butyl acetate, and 2-methylbutyl acetate. Other identified esters such as ethyl 2-methylbutanoate and hexyl acetate have been identified in northern highbush blueberries (*V. corymbosum*),[13,14] whereas methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and (*Z*)-3-hexenyl acetate have been reported in *V. myrtillus*.[9,10,12] Concentrations of total esters ranged from 52 to 3638 μg/kg, which accounted for 0.7−28.4% of total volatiles.

Terpenoids were most abundant in 'FL02-40', followed by 'Snowchaser', 'Primadonna', and 'Jewel' (Table 2). β-Pinene, limonene, (*Z*)- and (*E*)-dihydrolinalool oxide, linalool, α-terpineol, and geranyl acetone were present in all of the studied cultivars. 1,8-Cineole ("minty like"[25]) was present at 30 times above its odor threshold in 'Snowchaser', but at much lower concentration in 'Primadonna' and 'FL02-40' and absent from 'Jewel' (Table 2). Two new terpenoids, (*Z*)-dihydrolinalool oxide and (*E*)-dihydrolinalool oxide, were only tentatively identified. Because standards for these two compounds are not commercially available, the tentative identification was based on fragmentation pattern matches with these compounds in the MS library. The role of dihydrolinalool oxide in southern highbush blueberries was unknown, for it is seldom reported in fruits. It is noted that, together with (*Z*)-linalool oxide, they were present in large amounts in 'Snowchaser' and 'FL02-40'.

Linalool and geraniol are considered to be major aroma-active terpenoids in rabbiteye blueberry *V. ashei*.[18] However, in this study, whereas linalool was well above its threshold in all cultivars, geraniol was present only in 'FL02-40' and was found at concentrations above its threshold (Table 2). Both linalool and geraniol were previously reported to contribute to floral flavor.[25] α-Terpineol and geranylacetone were present in all cultivars at levels above thresholds for most harvests, except α-terpineol in 'Jewel' and geranylacetone in 'Snowchaser' (Table 2). With such a range of concentrations and diverse odor characteristics,[25] terpenoids might be the group of compounds that help determine the characteristic aroma of each cultivar. For instance, in addition to esters, limonene and linalool might contribute to the "fruity" and "floral" aroma of 'Primadonna', respectively, whereas almost all terpenoids identified in 'FL02-40' and 'Snowchaser' would contribute to their unique flavor. In general, *Vaccinium* species have appreciable terpenoid content.[9,14,17]

Aldehydes represent the largest volatile group in the blueberries examined in this study. Total aldehyde content ranged from 2414 to 7640 μg/kg (Table 2), which accounted for 54−77% of total volatiles. Seven of the 11 aldehydes identified in this study have been previously reported in blueberries. However, four unsaturated aldehydes, (*Z*)-3-hexenal, (*E*,*E*)-2,4-hexadienal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-2, 4-nonadienal, are reported in cultivated highbush blueberries for the first time. Although these unsaturated aldehydes are generally considered to be products of fatty acid oxidation, the salt added during sample preparation should have inhibited enzyme activity that might have produced these aldehydes during fruit maceration. Furthermore, most aldehydes in this study except acetaldehyde, octanal, and (*E*,*E*)-2,4-hexadienal were found in concentrations well above their reported thresholds, suggesting major contributions to blueberry flavor. Hexanal, (*Z*)-3-hexenal, and (*E*)-2-hexenal have characteristic green/grassy odors, octanal and nonanal have citrus-like odor, (*E*,*Z*)-2,6-nonadienal has a characteristic cucumber odor, and (*E*,*E*)-2,4-nonadienal and (*E*,*E*)-2,4-decadienal are fatty/waxy.[25] Most aldehydes were present in all cultivars except for (*E*,*Z*)-2,6-nonadienal, which was absent from 'Jewel', and (*E*,*E*)-2,4-decadienal, which was found in significant amounts only in 'Snowchaser' (Table 2). Overall, one may consider that aldehydes contribute to the "fresh/green" flavor in blueberries. Hexanal and (*E*)-2-hexenal have been reported to be the major "green compounds" in rabbiteye blueberry.[18]

Like aldehydes, alcohols are common fruit volatiles. Total alcohol concentrations ranged from 304 to 683 μg/kg, but all of the alcohols were below their reported odor thresholds (Table 2), only possibly contributing to flavor by interaction with other volatiles. The seven identified alcohols were previously reported in blueberries,[14] with (*Z*)-3-hexenol and (*E*)-2-hexenol thought to be contributors to aroma.[18]

The four ketones identified in this study were present at relatively small concentrations, 130−163 μg/kg, contributing to <5% of total volatiles (Table 2). The only ketone above its odor threshold, with a reported descriptor of "fruity",[25] was 2-undecanone; it was present only in 'Snowchaser' and 'FL02-40'. Therefore, this class of compounds was a minor volatile component in the southern highbush blueberries in this study.

In summary, all cultivars were dominated by aldehydes, with 'Primadonna' and 'FL02-40' containing the largest concentrations. High concentrations of esters distinguished 'Primadonna', whereas 'FL02-40' contained larger concentrations of terpenoids and alcohols in comparison to the remaining cultivars. It is common that varietal flavor differences are due to quantitative differences, not qualitative differences.[26−30] However, the four southern highbush blueberries studied herein had qualitative differences, possibly due to the complexity of their genotype. All southern highbush blueberries have resulted from one or more interspecific hybridization events and, therefore, may have a greater diversity in volatile profiles. For example, the pedigree for 'FL02-40' includes crosses with all of the following *Vaccinium* species: *V. corymbosum*, *V. tenellum*, *V. darrowii*, *V. virgatum*, and *V. elliottii*. This type of complex pedigree is not uncommon among southern highbush blueberry cultivars. Significant differences in the quality of volatile contents have also been reported in other *Vaccinium* species.[11]

Growing Location. Growing location may affect fruit quality due to variations in factors such as soil, nutrition, water availability, climate/microclimate, and sunlight.[31] In recent years, blueberry production has expanded in Florida to include more

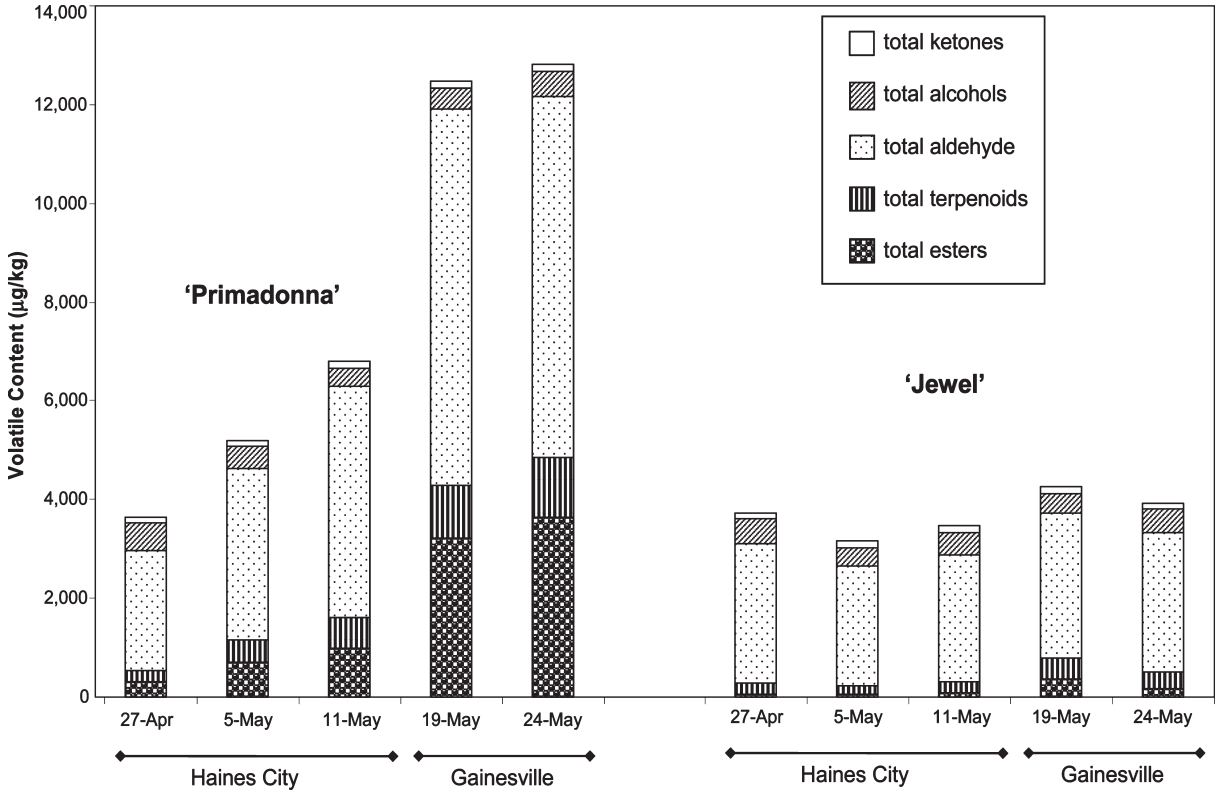

Figure 1. Volatile content of 'Primadonna' and 'Jewel' blueberries grown in Haines City and Gainesville (Florida) and harvested on different dates.

southerly locations. For example, Haines City is located at a more southern latitude than Gainesville, and blueberry fruit maturity typically occurs 2−3 weeks prior to that in Gainesville. Soils in the central ridge area of Florida where Haines City is located are sandier than flatwood soil types near Gainesville.[32] In this study, the volatile compositions of 'Primadonna' and 'Jewel' grown in Gainesville and Haines City in Florida were compared across harvest dates for each cultivar (Figure 1).

'Primadonna' from Gainesville had much higher total volatile content than fruit harvested from Haines City (Figure 1). The total volatile content in 'Primadonna' from Haines City was only 28−54% of that from Gainesville, with aldehydes, esters, and terpenoids being 32−64, 8−31, and 20−59%, respectively, of those from Gainesville. Furthermore, total aldehydes in 'Primadonna' from Haines City accounted for 68−70% of total volatiles, slightly higher than that from Gainesville (58−62%), whereas total esters accounted for only 9−15% of total volatiles, much lower compared to Gainesville (26−29%). Such differences in volatile concentrations would very likely result in different flavor, with fruit harvested in Gainesville being described as "fruitier". The total terpenoids in 'Primadonna' from Haines City was similar to that from Gainesville in percentage. The volatile composition of 'Primadonna' from two locations was not only different in quantity but also different in quality. Aldehydes including acetaldehyde and (*E*,*E*)-2,4-hexadienal were found in 'Primadonna' from Haines City, but could not be detected in 'Primadonna' from Gainesville (data not shown).

In contrast to 'Primadonna', the total volatiles and total aldehydes in 'Jewel' from Haines City and Gainesville appeared to be similar (Figure 1). However, total esters and total terpenoids in 'Jewel' from Haines City were lower than those from Gainesville, although it was not statistically tested (harvest dates and time between harvests being different). Total esters in 'Jewel' from Haines City were 52−78 μg/kg, only 14−43% of that from Gainesville. Moreover, total esters in 'Jewel' from Haines City accounted for only around 2% of total volatiles, much lower compared to 5−9% for those from Gainesville. Total terpenoids in 'Jewel' from Haines City was only 41−70% of that from Gainesville, and their percentage of total volatiles was also smaller than that from Gainesville. The overall volatile compositions of 'Jewel' from the two locations were very similar, slightly different in quantity but not in quality.

Even though an attempt was made to harvest fruit at the same commercial maturity stage, there may be a maturity gradient with later harvests. 'Primadonna' had increasing SSC (from 11.1 to 15.1%) and decreasing TA (from 0.89 to 0.13% citric acid) with harvest date (Haines City and Gainesville all together); 'Jewel' fruit harvested from Haines City had SSC from 9.5 to 10.8% and TA from 1.22 to 0.90%, whereas 'Jewel' from Gainesville had SSC of 12.0% and TA of 0.34% for both harvests. These data confirm earlier work[33] and may explain the increase in esters and overall volatiles observed in both cultivars and both locations. Similar increases in esters and other volatiles are well documented in other fruit.[34−36] Furthermore, nutrition differences between the two locations can also affect quality parameters such as sugars and acids[37] and also volatile content. The data in the present study show how the two cultivars responded differently to these external factors (soil, nutrition, and climate) and indicate the need for more thorough exploration of the potential genotype × environment interactions for aroma and flavor of blueberries.

Harvest Dates. The effect of cultivar, harvest date, and interactions was examined for each location, because it is less practical to compare fruit harvested at different dates in both locations (Tables 3 and 4). For 'Primadonna' and 'Jewel', there

Table 3. Effect of Cultivar and Harvest Date on Blueberry Volatiles for 'Primadonna' and 'Jewel' Harvested in Haines City, FL

		Primadonna[i]			Jewel[i]			*F* value (significance)[k]		
RI	compound	April 27	May 5	May 11	April 27	May 5	May 11	cultivar (C)	harvest (H)	C × H
997	methyl 2-methylbutanoate	20.6a	11.0b	9.7b	13.6a	9.5a	15.8a	0.19	4.97*	4.44*
1005	methyl 3-methylbutanoate[a]	268.7c	577.6b	884.6a	29.6b	34.1b	48.7a	593.74***	68.55***	60.55***
1036	ethyl 2-methylbutanoate	2.2a	2.2a	1.5b	—[j]	—	—			
1050	ethyl 3-methylbutanoate[b]	9.7c	110.9a	88.5b	10.5a	4.6c	7.0b	919.66***	199.61***	248.32***
1053	butyl acetate	0.0c	2.6a	2.6a	1.7a	0.96b	0.81b	26.25***	27.97***	102.18***
1104	2-methylbutyl acetate[c]	2.3a	0.0b	2.0a	3.9a	0.0b	0.0b	1.84	644.80***	202.98***
1256	hexyl acetate	0.9a	0.7a	0.8a	0.7b	0.4c	2.9a	343.00***	754.43***	731.29***
1302	(*Z*)-3-hexenyl acetate	2.4a	2.6a	2.6a	6.5a	2.7b	2.8b	36.66***	24.64***	30.99***
1082	*β*-pinene[d]	21.3a	21.9a	19.8a	13.0b	17.0a	17.9a	30.09***	2.23	4.21*
1182	limonene	29.2b	58.6a	75.7a	36.5b	32.5b	48.5a	27.80***	33.82***	15.15***
1188	1,8-cineole	3.9a	2.7a	3.1a	—	—	—			
1192	(*Z*)-dihydrolinalool oxide[e]	0.0c	5.4b	7.2a	6.1a	0.86b	1.0b	41.94***	8.33**	266.81***
1224	(*E*)-dihydrolinalool oxide[e]	1.5b	10.5a	13.4a	8.7a	1.3b	1.5b	61.43***	5.42*	102.17***
1534	linalool	19.9c	56.2a	43.9b	15.5c	31.4b	38.2a	82.14***	165.40***	26.10***
1677	α-terpineol	69.9b	89.0b	159.2a	96.7a	52.8b	76.7ab	16.58**	13.99***	17.68***
1783	nerol[f]	16.4b	17.8b	30.5a	—	—	—			
1832	geranylacetone[f]	74.8c	183.4b	272.4a	50.0a	34.7b	50.0a	151.63***	28.37***	28.94***
700	acetaldehyde[g]	4.7a	4.0a	1.4a	20.7a	18.9a	17.1a	13.23**	0.22	0.01
959	pentanal	48.5a	37.1a	25.3b	78.8a	44.6b	46.0b	84.37***	65.65***	9.67**
1061	hexanal	179.1c	345.2b	521.2a	67.8a	61.2a	43.8a	708.66***	70.62***	93.60***
1117	(*Z*)-3-hexenal	279.7b	476.7b	733.0a	356.4a	328.0a	316.6a	27.53***	14.96***	21.10***
1202	(*E*)-2-hexenal	1685.7c	2406.3b	3153.1a	2038.6a	1816.1b	1922.4ab	42.88***	27.87***	37.88***
1378	nonanal	59.8a	59.9a	59.8a	60.1a	59.4a	61.5a	1.66	2.14	2.49
1413	(*E*,*E*)-2,4-hexadienal	21.2b	36.1a	23.6b	20.9a	9.3a	15.7a	24.80***	0.57	11.38*
1604	(*E*,*Z*)-2,6-nonadienal	4.8b	6.9b	21.4a	—	—	—			
1684	(*E*,*E*)-2,4-nonadienal	129.1a	109.0a	134.6a	176.1a	98.4b	150.9ab	1.79	5.13*	1.59
1146	1-penten-3-ol	0.0b	1.9ab	3.4a	—	—	—			
1239	pentanol	117.1a	82.6b	71.2b	131.6a	89.8b	90.1b	20.65***	85.42***	1.30
1341	hexanol	55.0a	23.8b	15.9c	11.1b	5.4c	27.7a	713.97***	290.03***	653.00***
1370	(*Z*)-3-hexenol	86.2a	57.0b	55.9b	78.3a	67.2b	77.7a	62.59***	143.29***	72.24***
1392	(*E*)-2-hexenol	143.9a	125.3b	99.6c	94.7b	79.1c	131.1a	96.42***	22.79***	148.28***
1513	2-heptanol[h]	129.3a	117.5a	105.4a	150.7a	106.9b	100.5b	0.08	10.91**	2.13
1545	octanol	29.0a	24.2a	26.1a	26.3a	15.8b	20.6ab	4.15	2.63	0.37
1166	2-heptanone	1.9a	1.7a	2.2a	5.4b	7.3ab	9.8a	168.33***	10.01**	7.64**
1331	6-methyl-5-hepten-2-one	9.9a	10.3a	7.0b	4.5b	4.6b	10.3a	149.47***	18.62***	197.55***
1611	2-undecanone	0.0c	2.9b	4.5a	—	—	—			

[a] Concentration estimated by methyl 2-methylbutanoate. [b] Concentration estimated by ethyl 2-methylbutanoate. [c] Concentration estimated by butyl acetate. [d] Concentration estimated by hexanal. [e] Concentration estimated by 1,8-cineole. [f] Concentration estimated by geraniol. [g] Concentration estimated by pentanal. [h] Concentration estimated by octanal. [i] Data are the average of triplicate runs. Numbers followed by the same letter in a row within each cultivar are not significantly different by Tukey's Honest Significant Difference (HSD) test (α = 0.05). [j] —, no peak detected. [k] *, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001 respectively

was a cultivar effect for most volatiles, except for methyl 2-methylbutnoate, 2-methylbutyl acetate, nonanal, (*E*,*E*)-2,4-nonadienal, 2-heptanol, and octanol (Table 3). There was no harvest date effect for *β*-pinene, acetaldehyde, nonanal, (*E*,*E*)-2,4-hexadienal, and octanol. Interactions were significant for most compounds (Table 3). For 'Snowchaser' and 'FL02-40' the effect of cultivar was not seen for methyl 2-methylbutanoate, *β*-pinene, all of the unsaturated dienals, pentanol, 2-heptanol, and 2-undecanone (Table 4). Harvest date effect and interactions varied with compounds.

Within 'Primadonna', most compounds significantly increased with later harvest date (Table 3). Some compounds such as ethyl 3-methylbutanoate, butyl acetate, limonene, and (*Z*)- and (*E*)-dihydrolinalool oxide were lowest mostly at the first harvest,

Table 4. Effect of Cultivar and Harvest Date on Blueberry Volatiles for 'Snowchaser' and 'FL02-40' Harvested in Gainesville, FL

		Snowchaser[h]			FL02-40[h]			*F* value (significance)[k]		
RI	compound	May 10	May 14	May 17	May 10	May 14	May 17	cultivar (C)	harvest (H)	C × H
997	methyl 2-methylbutanoate	15.3a	14.0a	12.6a	7.1b	12.5b	20.8a	0.20	8.00**	17.53***
1005	methyl 3-methylbutanoate[a]	38.6c	128.3a	107.8b	13.7c	18.0b	20.8a	1129.61***	171.12***	133.80***
1050	ethyl 3-methylbutanoate[b]	0.0c	1.6b	8.0a	—[i]	—	—			
1302	(*Z*)-3-hexenyl acetate	20.5b	48.2a	47.0a	23.8b	28.6a	10.4c	621.00***	176.56***	265.89***
1082	*β*-pinene [c]	11.8a	11.3a	13.8a	15.3a	14.2a	9.2a	0.21	0.94	4.36*
1182	limonene	38.7a	48.6a	43.6a	70.1a	85.3a	77.5a	112.09***	5.04*	0.23
1188	1,8-cineole	39.5a	42.2a	45.5a	7.6b	9.7a	5.6c	1732.55***	3.18	9.50**
1192	(*Z*)-dihydrolinalool oxide[d]	41.2c	60.9b	73.9a	47.9b	65.7a	18.6c	66.62***	44.02***	127.37***
1224	(*E*)-dihydrolinalool oxide[d]	71.1c	134.9b	175.0a	93.4b	134.2a	34.4c	167.19***	97.46***	275.47***
1426	(*Z*)-linalool oxide	3.4a	2.3a	3.4a	11.4a	12.8a	9.4b	60.39***	0.48	1.53
1534	linalool	83.8a	94.7a	98.3a	207.9b	240.3a	74.5c	359.10***	125.64***	151.44***
1677	α-terpineol	743.7a	540.6ab	520.7b	1037.2a	1343.2a	491.0b	37.22***	22.30***	17.28***
1783	nerol[e]	—	—	—	110.9ab	48.6b	130.0a			
1825	geraniol	—	—	—	80.6ab	101.8a	52.9b			
1706	geranyl acetate	12.1ab	15.7a	10.6b	5.1b	23.4a	2.6b	5.84*	63.93***	25.41***
1832	geranylacetone[e]	27.6a	19.3b	17.6b	450.1a	308.2a	510.4a	119.31***	2.69	2.65
700	acetaldehyde[f]	5.6a	10.3a	10.5a	8.9b	13.3ab	16.4a	9.40**	7.79**	0.47
959	pentanal	54.6a	27.7b	23.5b	23.2b	22.6b	26.4a	99.81***	67.60***	85.04***
1061	hexanal	66.0c	254.7a	198.2b	194.8a	155.6b	205.2a	6.56*	103.56***	188.98***
1117	(*Z*)-3-hexenal	255.8b	339.7a	302.4ab	464.1b	612.4a	490.3b	168.79***	15.95***	2.21
1202	(*E*)-2-hexenal	1547.8c	1966.6a	1799.7b	2691.6b	3286.1a	2674.2b	590.91***	44.77***	7.99**
1378	nonanal	59.8a	59.1a	59.2a	59.9a	59.8a	59.8a	6.57*	2.18	1.22
1413	(*E*,*E*)-2,4-hexadienal	28.7a	30.1a	29.0a	25.3a	41.6a	27.1a	0.72	5.24*	3.76
1604	(*E*,*Z*)-2,6-nonadienal	25.1a	11.9b	12.1b	9.3ab	8.6b	26.8a	0.26	3.70	9.46**
1684	(*E*,*E*)-2,4-nonadienal	298.7a	90.5a	134.0a	128.4a	113.0a	153.3a	1.12	2.60	2.49
1796	(*E*,*E*)-2, 4-decadienal	73.1a	54.0a	71.1a	—	tr[j]	—			
1146	1-penten-3-ol	—	—	—	9.5a	12.7a	9.1a			
1239	pentanol	90.0a	60.2c	79.8b	81.5a	73.0a	78.1a	0.09	15.03***	4.76*
1341	hexanol	16.7b	14.8c	18.5a	93.1a	25.5a	29.1a	8.35*	3.83	3.79
1370	(*Z*)-3-hexenol	59.5a	52.1b	49.9b	104.9a	110.6a	107.1a	487.80***	0.83	2.94
1392	(*E*)-2-hexenol	106.4a	84.8b	83.9b	165.1a	155.2a	157.3a	250.50***	5.82*	1.10
1513	2-heptanol[g]	199.8a	79.9a	126.1a	150.2a	122.1a	127.2a	0.01	4.16*	1.56
1545	octanol	17.8a	11.8b	10.6b	78.4a	60.4a	88.9a	80.97***	1.53	1.55
1166	2-heptanone	3.7a	3.3a	3.0a	5.6a	3.5b	7.0a	50.40***	11.66**	14.80***
1331	6-methyl-5-hepten-2-one	3.0b	3.7a	3.9a	8.2a	7.4a	6.1b	489.28***	4.83*	27.24***
1611	2-undecanone	11.6b	18.4ab	24.2a	19.4b	30.8a	8.3c	0.80	13.29***	30.52***

[a] Concentration estimated by methyl 2-methylbutanoate. [b] Concentration estimated by ethyl 2-methylbutanoate. [c] Concentration estimated by hexanal. [d] Concentration estimated by 1,8-cineole. [e] Concentration estimated by geraniol. [f] Concentration estimated by pentanal. [g] Concentration estimated by octanal. [h] Data are the average of triplicate runs. Numbers followed by the same letter in a row within each cultivar are not significantly different by Tukey's Honest Significant Difference (HSD) test (α = 0.05). [i] —, no peak detected. [j] tr, trace. [k] *, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001 respectively.

in April, whereas other compounds (α-terpineol, nerol, (*Z*)-3-hexenal, (*E*,*Z*)-2,4-nonadienal, and 1-penten-3-ol) were highest at the last harvest. Most alcohols, except 1-penten-3-ol, 2-heptanol, and octanol, decreased with harvest (Table 3). For 'Jewel', in contrast to 'Primadonna', some esters decreased with harvest, and two esters (methyl 3-methylbutanoate and hexyl acetate) increased (Table 3). Likewise, terpenoid concentrations increased (*β*-pinene, limonene, and linalool) or decreased with harvest. As for aldehydes and ketones, they either decreased, decreased (from April 27 to May 5) and increased (from May 5 to May 11), or remained constant over time.

The few esters in cultivars harvested from Gainesville increased, except methyl 2-methylbutanoate ('Snowchaser') and (*Z*)-3-hexenyl acetate ('FL02-40') (Table 4). (*Z*)- and (*E*)-dihydrolinalool oxide increased with harvest in 'Snowchaser', and α-terpineol and geranylacetone decreased (Table 4). As with

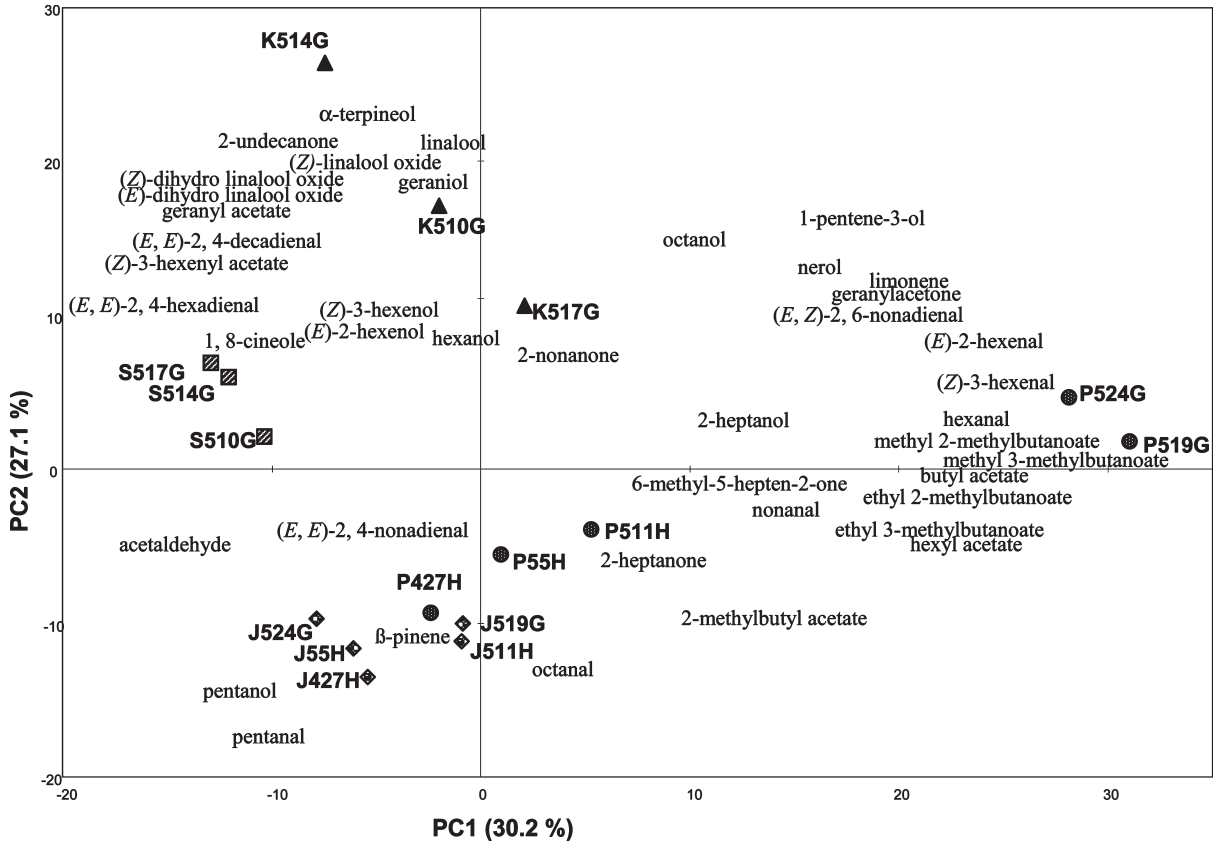

Figure 2. PCA plot of cultivars, growing locations, and harvest dates differentiated by volatile content (P = Primadonna; J = Jewel; S = Snowchaser; K = FL02-40; G = Gainesville, FL; H = Haines City, FL; 427 = April 27; 55 = May 5; 510 = May 10; 511 = May 11; 519 = May 19; 524 = May 24). Data are the average of three replications per cultivar/location/harvest.

'Primadonna', most alcohols decreased with harvest, except pentanol. Two ketones, 6-methylhepten-2-one and 2-undecanone, increased with harvest (Table 4). For 'FL02-40', the sample from the second harvest had levels of volatiles higher than in the first or third harvest.

The highest differences in volatile content between harvests were expressed by 'Primadonna'. Fruits for this study were harvested weekly, but the commercial farms we sampled from used a 3−5 day harvest schedule. Therefore, there may have been slight maturity differences among fruits from different harvest dates that could lead to changes in volatile content. However, the rapidly changing environmental conditions in Florida during late-April and May are also a likely contributor to changes in volatile content within a cultivar. For example, the nearest environmental weather station to the Haines City farm (14 km) recorded 6.6 cm of precipitation over the 2 days prior to the first harvest on April 27. The degree to which the precipitation may or may not have influenced volatile content is beyond the scope of the present study, but it is worth noting that 'Jewel' harvested from the same location and subject to the same precipitation did not show the same degree of variation between harvest dates.

Comparing All Cultivars and Location/Harvest Date Using PCA. The distribution of sample volatiles as affected by cultivar, growing location, and harvest date can be visualized in a PCA plot. PCA was performed on volatile concentration data (mean of the three replications) from all samples. The first three principal components (PCs) explained 72.3% of the variation, with PC1, PC2, and PC3 contributing 30.2, 27.1, and 15.0%, respectively. Figure 2 shows the biplot of PC1 versus PC2.

All aliphatic esters (straight and branched chain) had high positive loadings on PC1, as well as the C6 aldehydes hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenal (Figure 2). 'Primadonna' harvested in Gainesville had high scores on PC1, indicating high amounts in these specific esters and aldehydes. In contrast, the C6 alcohols hexanol, (*E*)-2-hexenol, and (*Z*)-3-hexenol were uncorrelated to their aldehyde counterparts (vectors at 90° of each other), with positive loadings on PC2 and PC3 (not shown), where 'FL02-40' had high scores. Interestingly, nerol had high positive loading on PC1, whereas its isomer geraniol was uncorrelated (vectors for nerol and geraniol at 90° of each other) and had high positive loadings on PC2 and PC3. Geranylacetone (PC1) and geranyl acetate (PC2) followed the same patterns. Pentanol and pentanal were correlated with each other with negative loadings on PC1 and PC2.

Whereas 'Primadonna' from Gainesville had high positive scores on PC1, 'Primadonna' from Haines City was on the negative side (April harvest) or had low scores on PC1. Scores for 'Jewel' were all on the lower left quadrant of the PCA, mostly characterized by pentanol, pentanal, acetaldehyde, and β-pinene. 'Snowchaser' was on the negative side of PC1, characterized by (*E,E*)-2,4-hexadienal, 1,8-cineole, and (*Z*)-3-hexenyl acetate and little variation due to harvest date. Finally, 'FL02-40' had high positive scores on PC2 and PC3, characterized by most terpenes (linalool, geraniol, α-terpineol, geranyl acetate, and (*Z*)- and (*E*)-dihydrolinalool oxide).

This one-year study showed that the volatile composition of 'Primadonna' was influenced by environmental factors, whereas 'Jewel' was unaffected. The higher volatile content in 'Primadonna' from Gainesville might be due to riper fruit, as indicated by higher SSC and lower TA. However, the variation of volatile composition in the four southern highbush blueberry cultivars planted in the same field indicated that plant genotype also influences fruit flavor. Genetics determines precursors, enzyme systems, and their activity in flavor formation, which causes differences in flavor metabolism pathways.

To our knowledge, this is the first report of the volatile composition of southern highbush blueberries. Some volatiles were reported in blueberries for the first time. The volatile composition and content were influenced by cultivar and to a lesser extent affected by environmental conditions such as growing location and harvest date.

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REFERENCES

(1) Ehlenfeldt, M. K.; Prior, R. L. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *J. Agric. Food Chem.* **2001**, *49* (5), 2222–2227.

(2) Connor, A. M.; Luby, J. J.; Tong, C. B. S.; Finn, C. E.; Hancock, J. F. Genotypic and environmental variation in antioxidant activity among blueberry cultivars. *Acta Hortic.* **2002**, *574*, 209–213.

(3) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric.* **2005**, *85* (13), 2149–2158.

(4) Howard, L. R.; Clark, J. R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83* (12), 1238–1247.

(5) McIntyre, K. L.; Harris, C. S.; Saleem, A.; Beaulieu, L.-P.; Ta, C. A.; Haddad, P. S.; Arnason, J. T. Seasonal phytochemical variation of anti-glycation principles in lowbush blueberry (*Vaccinium angustifolium*). *Planta Med.* **2009**, *75* (3), 286–292.

(6) Saftner, R.; Polashock, J.; Ehlenfeldt, M.; Vinyard, B. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biol. Technol.* **2008**, *49* (1), 19–26.

(7) Williamson, J. G.; Lyrene, P. M. Blueberry varieties for Florida. *University of Florida IFAS Extension* **2004**, *No. HS967*, 1–9.

(8) Hall, I. V.; Forsyth, F. R.; Lightfoot, H. J. Volatiles from developing fruit of *Vaccinium angustifolium*. *Can. Inst. Food Sci. Technol. J.* **1970**, *3* (1), 1–3.

(9) von Sydow, E.; Anjou, K. The aroma of bilberries (*Vaccinium myrtillus* L.). I. Identification of volatile compounds. *Food Sci. Technol. (London)* **1969**, *2*, 78–81.

(10) von Sydow, E.; Andersson, J.; Anjou, K.; Karlsson, G. The aroma of bilberries (*Vaccinium myrtillus* L.). II. Evaluation of the press juice by sensory methods and by gas chromatography and mass spectrometry. *Food Sci. Technol. (London)* **1970**, *3* (2), 11–17.

(11) Baloga, D. W.; Vorsa, N.; Lawter, L. Dynamic headspace gas chromatography-mass spectrometry analysis of volatile flavor compounds from wild diploid blueberry species. In *Fruit Flavors*; Rouseff, R. L., Leahy, M. M., Eds.; ACS Symposium Series 596; American Chemical Society: 1995; pp 235−247.

(12) Rohloff, J.; Nestby, R.; Nes, A.; Martinussen, I. Volatile profiles of European blueberry: few major players, but complex aroma patterns. *Latv. J. Agron.* **2009**, *12*, 98–103.

(13) Parliment, T. H.; Kolor, M. G. Identification of the major volatile components of blueberry. *J. Food Sci.* **1975**, *40* (4), 762–763.

(14) Hirvi, T.; Honkanen, E. The aroma of blueberries. *J. Sci. Food Agric.* **1983**, *34* (9), 992–996.

(15) Hirvi, T.; Honkanen, E. The aroma of some hybrids between high-bush blueberry (*Vaccinium corymbosum*, L.) and bog blueberry (*Vaccinium uliginosum*, L.). *Z. Lebensm. Unters. Forsch.* **1983**, *176* (5), 346–349.

(16) Horvat, R. J.; Schlotzhauer, W. S.; Chortyk, O. T.; Nottingham, S. F.; Payne, J. A. Comparison of volatile compounds from rabbiteye blueberry (*Vaccinium ashei*) and deerberry (*V. stamineum*) during maturation. *J. Essent. Oil Res.* **1996**, *8* (6), 645–648.

(17) Horvat, R. J.; Senter, S. D.; Dekazos, E. D. GLC-MS analysis of volatile constituents in Rabbiteye blueberries. *J. Food Sci.* **1983**, *48* (1), 278–279.

(18) Horvat, R. J.; Senter, S. D. Comparison of the volatile constituents from rabbiteye blueberries (*Vaccinium ashei*) during ripening. *J. Food Sci.* **1985**, *50* (2), 429–431.

(19) Clark, J. R.; Finn, C. E. Register of new fruit and nut cultivars list 45. *HortScience* **2010**, *45* (5), 716–756.

(20) Johnson, R. A.; Wichern, D. W. *Applied Multivariate Statistical Analysis*, 3rd ed.; Prentice Hall: Englewood Cliffs, NJ, 1992.

(21) Van Gemert, L. J. *Compilations of Odour Threshold Values in Air, Water and Other Media*; Oliemans Punter and Partners: Huizen, The Netherlands, 2003.

(22) Plotto, A.; Margaria, C. A.; Goodner, K. L.; Goodrich, R.; Baldwin, E. A. Odour and flavour thresholds for key aroma components in an orange juice matrix: terpenes and aldehydes. *Flavour Fragrance J.* **2004**, *19* (6), 491–498.

(23) Latrasse, A. Fruits III. In *Volatile Composition in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 329−387.

(24) Berger, R. G. Fruits I. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 283−304.

(25) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität Munchen: Garching, Germany, 1998; p 63.

(26) Dirinck, P. J.; De Pooter, H. L.; Willaert, G. A.; Schamp, N. M. Flavor quality of cultivated strawberries: the role of the sulfur compounds. *J. Agric. Food Chem.* **1981**, *29*, 316–321.

(27) Du, X. F.; Kurnianta, A.; McDaniel, M.; Finn, C. E.; Qian, M. C. Flavour profiling of 'Marion' and thornless blackberries by instrumental and sensory analysis. *Food Chem.* **2010**, *121* (4), 1080–1088.

(28) Baldwin, E. A.; Nisperos-Carriedo, M. O.; Baker, R.; Scott, J. W. Quantitative analysis of flavor parameters in six Florida tomato cultivars (*Lycopersicon esculentum* Mill). *J. Agric. Food Chem.* **1991**, *39* (6), 1135–1140.

(29) Paillard, N. Analysis of volatiles from some varieties of apples. *Fruits* **1967**, *22* (3), 141–151.

(30) Mehinagic, E.; Royer, G.; Symoneaux, R.; Jourjon, F.; Prost, C. Characterization of odor-active volatiles in apples: influence of cultivars and maturity stage. *J. Agric. Food Chem.* **2006**, *54* (7), 2678–2687.

(31) Reineccius, G. Flavor formation in fruits and vegetables. In *Flavor Chemistry and Technology*; Reineccius, G., Ed.; CRC Press Taylor and Francis Group: Boca Raton, FL, 2006; pp 73−101.

(32) Williamson, J. G.; Lyrene, P. M. State of the Florida blueberry industry. *Proc. Fla. State Hortic. Soc.* **1995**, *108*, 378–381.

(33) Kushman, L. J.; Ballinger, W. E. Acid and sugar changes during ripening in Wolcott blueberries. *Proc. Am. Soc. Hortic. Sci.* **1968**, *92*, 290–295.

(34) Pérez, A. G.; Sanz, C.; Olías, R.; Ríos, J. J.; Olías, J. M. Evolution of strawberry alcohol acyltransferase activity during fruit development and storage. *J. Agric. Food Chem.* **1996**, *44* (10), 3286–3290.

(35) Harb, J.; Streif, J.; Bangerth, K. F. Aroma volatiles of apples as influenced by ripening and storage procedures. *Acta Hortic.* **2008**, *796*, 93–103.

(36) Zhu, H.; Li, X. P.; Yuan, R. C.; Chen, Y. F.; Chen, W. X. Changes in volatile compounds and associated relationships with other ripening events in banana fruit. *J. Hortic. Sci. Biotechnol.* **2010**, *85* (4), 283–288.

(37) Ballinger, W. E.; Kushman, L. J. Relationship of nutrition and fruit quality of Wolcott blueberries grown in sand culture. *J. Am. Soc. Hortic. Sci.* **1969**, *94*, 329–335.