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Survey of Seasonal Variation of Leaf Tissue Nutrient Concentration of Southeastern Blackberry

Amanda Lay-Walters
Department of Horticulture, University of Arkansas, 495 N. Campus Drive, Fayetteville, AR 72701, USA

Jayesh Samtani
School of Plant and Environmental Science, Virginia Polytechnic and State University, 1444 Diamond Springs Road, Virginia Beach, VA 23455, USA

Gina Fernandez
Department of Horticultural Science, North Carolina State University, 270 Kilgore Hall, Raleigh, NC 27695, USA

John Havlin
Department of Crop and Soil Sciences, 101 Derieux Place, North Carolina State University, Raleigh, NC 27695, USA

Elina Coneva
Department of Horticulture, Auburn University, 101 Funchess Hall, Auburn, AL 36849, USA

Eric Stafne
Coastal Research and Extension Center, South Mississippi Branch Experiment Station, Mississippi State University, 810 Highway 26 West, Poplarville, MS 39470, USA

Natalie Bumgarner and David Lockwood
Department of Plant Sciences, University of Tennessee, 301 Ag & Natural Resources, Knoxville, TN 37996, USA

Zilfina Rubio Ames
Department of Horticulture, University of Georgia, 2360 Rainwater Road, Tifton, GA 31793, USA

Karen Blaedow
North Carolina Cooperative Extension, 100 Jackson Park Road, Hendersonville, NC 28792, USA

Amanda McWhirt
Department of Horticulture, University of Arkansas System Division of Agriculture, Cooperative Extension Service, 2301 S. University Avenue, Little Rock, AR 72204, USA

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Abstract. **The southeastern blackberry (*Rubus* subgenus *Rubus*) industry has expanded rapidly in the past two decades. However, fertilizer rate recommendations have been adopted primarily from other regions without verification of their suitability for the cultivars and soils of the southeastern United States. Blackberry leaf tissue nutrient sampling is a practice growers use to monitor plant nutrient status and adjust their fertility programs. Current blackberry leaf tissue nutrient sufficiency ranges used for nutrient monitoring for the region are not based on a regionwide study and have, instead, been adapted from ranges used in other regions. These ranges are developed exclusively for samples collected postharvest, limiting growers' ability to assess blackberry leaf tissue nutrient status during the growing season. A regionwide survey of the nutrient status of southeastern blackberry was undertaken to verify existing sufficiency ranges and determine whether sufficiency ranges for earlier sampling timings could be developed. In 2022 and 2023, leaf tissue nutrient samples were collected across nine locations in seven southeastern US states (Alabama, Arkansas, Georgia, Mississippi, North Carolina, Tennessee, and Virgina) and analyzed for N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B. Not all locations had the same cultivars; but, in total, 12 cultivars were sampled and are representative of the cultivars grown in the region. The most recent mature leaves were collected individually from blackberry primocanes and floricanes at four and five phenological stages, respectively. Phenological stage had a significant effect on all nutrients in primocanes and on all nutrients in floricanes except Mn. Notably, average primocane leaf tissue N, S, Fe, and Mn concentrations from sampling in this study did not fall within currently published leaf tissue nutrient sufficiency ranges for the region. Blackberry primocane leaf N and S concentrations in this study fell within ranges recommended for other regions. Location of sampling (state) and cultivar were found to have some impacts on the leaf tissue nutrient concentration of most nutrients; however, these differences were generally small and no practical differences were observed that would necessitate the development of leaf tissue nutrient ranges specific to subregions or specific cultivars. Instead, phenological stage was the primary influence driving observed seasonal changes in leaf tissue nutrient concentration. Uniform variation in nutrient concentration of primocane leaf tissues across most phenological stages was observed, which indicates there is the potential to develop new sufficiency ranges for phenological stages earlier in the season. Updated sufficiency ranges are recommended for the southeastern blackberry primocane leaf macronutrients N (2.0%–3.0%) and S (0.10%–0.20%) postharvest, whereas the micronutrients Fe and Mn require further investigation.**

The southeastern United States has greatly expanded its fresh-market blackberry industry during the past 15 years (Fernandez 2021), and has over 2400 ha in production (US Department of Agriculture 2022). To keep up with industry growth and the growing popularity of new

Table 1. The recommended blackberry leaf tissue nutrient sufficiency ranges for various regions across the United States and Canada.[i]

Nutrient	Southeast[ii]	Oregon[iii]	Washington and Oregon[iv]	California[v]	Eastern, midwestern, and northeastern Canada[vi]
N (%)	2.50–3.50	2.3–3.0	2.0–3.0	2.0–3.0	2.0–3.0
P (%)	0.15–0.25	0.19–0.45	0.15–0.40	0.25–0.40	0.25–0.40
K (%)	0.90–1.50	1.3–2.0	0.9–1.8	1.5–2.5	1.5–2.5
Ca (%)	0.48–1.00	0.60–2.0	0.5–1.5	0.6–2.5	0.6–2.0
Mg (%)	0.30–0.45	0.30–0.60	0.25–0.60	0.3–0.9	0.6–0.9
S (%)	0.17–0.21	0.10–0.20	0.10–0.20	ND	0.4–0.6
Fe ($mg \cdot kg^{-1}$)	60–100	60–250	70–500	50–200	60–250
Mn ($mg \cdot kg^{-1}$)	50–250	50–300	50–300	50–200	50–200
Zn ($mg \cdot kg^{-1}$)	20–70	15–50	20–50	20–50	20–50
Cu ($mg \cdot kg^{-1}$)	8–15	6–20	5–15	7–50	6–20
B ($mg \cdot kg^{-1}$)	25–85	30–70	30–70	30–50	30–70

[i] All recommendations are for the phenological stage of floricane postharvest, which is generally late July or early August; however, this can vary by region.

[ii] Source: Southeast Regional Caneberry Guide (Fernandez et al. 2023).

[iii] Source: Caneberries: Nutrient Management Guide (Hart et al. 2006).

[iv] Source: A review of N, P, K, Ca, and Mg nutrition in red raspberry and blackberry (Strik et al. 2024).

[v] Source: Fresh Market Caneberry Production Manual (Bolda et al. 2012).

[vi] Source: Raspberry and Blackberry Production Guide for the Northeast, Midwest, and Eastern Canada (Bushway et al. 2008).

ND = no data.

cultivars in the southeastern United States, there is a need to update regionally specific recommendations on blackberry nutrient management. Commercial blackberry growers are encouraged to develop fertilization programs based on recommend N fertilizer rates of 56 to 90 $kg \cdot ha^{-1}$ (Fernandez et al. 2023; Strik 2017). In addition, using leaf tissue and soil nutrient analyses from the previous year, growers can then adjust regional recommendations to their farm. Recommended leaf tissue nutrient sufficiency ranges for blackberry vary by region (Table 1). However, only the ranges for Washington and Oregon proposed by Strik et al. (2024) are based on peer-reviewed research. To our knowledge there have been only limited regionwide surveys of blackberry leaf tissue nutrient content, and no surveys that have assessed seasonal variation has been for blackberry in the southeastern United States.

Leaf tissue nutrient surveys have been used in multiple horticultural crops to gain insight on plant nutrient status and to refine established nutrient sufficiency ranges (Lukas et al. 2022; Pond et al. 2006; Rana et al. 2021; Thompson et al. 1997; Veazie et al. 2024; Wells 2009). In blackberry, a regionwide leaf tissue nutrient survey for the southeastern region had never been conducted until 2021 (McWhirt et al. 2024). In the Pacific Northwest, a considerable amount of work has been done to understand blackberry nutrient needs and to establish nutrient sufficiency ranges for primarily trailing-type blackberries (Fernandez-Salvador et al. 2015; Harkins et al. 2014; Strik 2015; Strik and Vance 2016, 2017, 2018; Strik et al. 2024). In contrast, the southeastern United States grows predominately erect and semierect cultivars.

In the southeastern region, current nutrient sufficiency ranges published in Fernandez et al. (2023) were adapted from ranges developed for other regions or are based on data from grower-submitted leaf tissue nutrient samples from blackberries in North Carolina (Hicks K, personal communication).

Blackberry leaf tissue nutrient concentration for most nutrients is known to vary throughout the season (Clark et al. 1988; Strik 2015; Strik and Vance 2017, 2018). For crop nutrient monitoring in blackberry, growers are recommended to sample the most recently mature primocane leaves during late July or early August, which is the postharvest period for floricane-fruiting cultivars (Clark et al. 1988; Fernandez et al. 2023; Hart et al. 2006; Strik and Vance 2017). However, primocane-fruiting cultivars are recommended to be sampled at the green-fruit stage (Strik 2015). Despite these different recommendations for sampling time, the recommended nutrient sufficiency ranges for these cultivars are the same (Bolda et al. 2012; Bushway et al. 2008; Fernandez et al. 2023; Hart et al. 2006; Strik et al. 2024). Regardless of fruiting type, blackberry leaf tissue nutrient sufficiency ranges have only been established for primocane leaves for this one phenological stage, which coincides with when nutrient content has been previously reported to be most stable (Strik 2015; Strik and Vance 2017, 2018). This sampling window does not allow commercial growers to adjust their fertilizer programs based on plant nutrient status during the growing season. The limitation adds an additional challenge because previous research has determined that the majority of nutrients applied in-season are allocated to the primocanes whereas floricanes rely more on stored nutrients (Strik 2017). Thus, identification of a nutrient imbalance via leaf tissue nutrient sampling on primocanes late in the season may be difficult to correct completely before the subsequent floricane harvest season. There is also a lack of published data on floricane leaf tissue nutrient status, which could be useful to help diagnose floricane nutrient disorders. The ability to sample earlier in the season and to have accurate leaf tissue nutrient sufficiency ranges would enable growers to make informed decisions about fertility management while plants are still actively growing.

Variation in leaf tissue nutrient concentration by cultivar has been observed in blackberry (Fernandez-Salvador et al. 2015; Harkins et al. 2014; Strik 2015; Strik and Vance 2017, 2018), raspberry (*Rubus ideaus*) (Horuz et al. 2013; John et al. 1976), blueberry (*Vaccinium corymbosum*) (Lukas et al. 2022; Strik and Vance 2015), and muscadine grape (*Vitis rotundifolia*) (Rana et al. 2021). In blackberry, nutrient content variation by cultivar is often small, and leaf tissue nutrient ranges account for this level of variability. In addition, no cultivar-specific nutrient sufficiency ranges for blackberry exist to our knowledge. However, it is still currently recommended to sample blackberry leaf tissue for nutrient status separately by cultivar (Fernandez et al. 2023; Hart et al. 2006; Strik and Vance 2017).

The expansion of the southeastern blackberry industry requires up-to-date nutrient sufficiency ranges and the ability for growers to assess plant nutrient status during the growing season. Therefore, the objectives of this study were 1) to verify leaf nutrient sufficiency ranges for southeastern blackberries through a regionwide survey and 2) to investigate nutrient

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A.M. is the corresponding author. E-mail: amcwhirt@uada.edu.

Table 2. States, locations, cultivars, and selected soil analyses (depth, 0–20 cm) of sites where blackberry leaf tissue nutrient samples were collected in 2022 and 2023.[i]

			Soil characteristics				
State	Location	Cultivar	Soil type	pH	EC (μmhos·cm^{-1})	P (mg·kg^{-1})	K (mg·kg^{-1})
Alabama	Chilton Research and Extension Center, Clanton, AL, USA (lat. 32.9200124°N, long. –86.6704287°W; elevation, 209 m)	Natchez, Osage, Ouachita, Prime-Ark® Freedom, Prime-Ark® 45	Ruston series (fine-loamy, siliceous, semiactive, thermic Typic Paleudults)	6.4	69	148	124
Arkansas	Fruit Research Station, Clarksville, AR, USA (lat. 35.530371°N, long. –93.402952°W; elevation, 277 m)	Ouachita	Linker series (fine-loamy, siliceous, semiactive, thermic Typic Hapludults)	6.3	73	81	108
Georgia	Lanier County, GA, USA; private farm	Osage, Ouachita	Fuquay series (loamy, kaolinitic, thermic Arenic Plinthic Kandiudults)	6.2	78	110	79
	Tift County, GA, USA; private farm	Ouachita, Von	Tifton series (fine-loamy, kaolinitic, thermic Plinthic Kandiudults)	6.8	63	94	48
Mississippi	University of South Mississippi Branch Experiment Station, Poplarville, MS, USA (lat. 30.839062°N, long. –89.545924°W; elevation, 102 m)	Chickasaw, Kiowa, Sweetie Pie	Ruston series (fine-loamy, siliceous, semiactive, thermic Typic Paleudults)	5.7	95	44	112
North Carolina	Henderson County, NC, USA; private farm	Ouachita	Hayesville series (fine, kaolinitic, mesic Typic Kanhapludults)	6.5	125	34	124
Tennessee	Middle Tennessee Ag Research and Education Center, Spring Hill, TN, USA (lat. 35.7512°N, long. –86.9300°W; elevation, 233 m)	Caddo, Kiowa, Natchez, Osage, Ouachita, Ponca, Prime-Ark® Freedom, Prime-Ark® 45, Prime-Ark® Traveler, Von	Maury series (Fine, mixed, active, mesic Typic Paleudalfs)	6.3	116	80	203
Virginia	Hampton Roads Agricultural Research and Extension Center, VA Beach, VA, USA (lat. 36.89286°N, long. –76.177050°W; elevation, 7 m)	Ouachita, Prime-Ark® 45, Prime-Ark® Traveler	Tetotum series (fine-loamy, mixed, semiactive, thermic Aquic Hapludults)	6.2	100	297	67
	Hampton County, VA, USA; private farm	Natchez, Ponca, Von	Pamunkey (fine-loamy, mixed, semiactive, thermic Ultic Hapludalfs)	6.7	83	23	115

[i] For the privacy of growers, global positioning satellite information of private farms is not included.
EC = electrical conductivity.

stability of leaf tissue across phenological stages to determine whether developing earlier season leaf tissue nutrient sufficiency recommendations are viable.

Materials and Methods

Study sites. Our observational study was conducted in 2022 and 2023, with leaf tissue nutrient sampling at nine mature blackberry plantings across seven southeastern US states (Table 2). Locations included public university research stations and private commercial farms (Table 2). Locations' US Department of Agriculture plant hardiness zones ranged from 7b to 9a (US Department of Agriculture, Agricultural Research Service 2023).

Cultivars. Twelve blackberry (*Rubus* subgenus *Rubus*) cultivars—Caddo, Chickasaw, Kiowa, Natchez, Osage, Ouachita, Ponca, Prime-Ark® 45, Prime-Ark® Freedom, Prime-Ark® Traveler, Sweetie Pie, and Von—were included in the dataset. All cultivars were not present at every location (Table 2); however, there were at least four replicate leaf tissue samples for each cultivar collected at each sampling date, with the exception of 'Caddo', which had two replicate leaf tissue samples.

Soil sampling. A minimum of one soil sample was collected in a randomized pattern from each site every year (28 Mar–22 Apr 2022 and 14 Mar–12 Apr 2023) at a depth of 20.3 cm using open-sided, chrome-plated steel soil probes. Soil cores were split in 0- to 10.2-cm and 10.2- to 20.3-cm depths and were analyzed separately. Samples consisted of three homogenized cores taken from the row middles of the cultivars present. The soil test data presented in Table 2 were averaged over the two depths. All soil samples were submitted to the University of Arkansas Fayetteville Agricultural Diagnostic Laboratory (Fayetteville, AR, USA). Soils were analyzed for all key nutrients, but only results for P, K, pH, and electrical conductivity (EC) are presented (Table 2). Mineral nutrients were analyzed using Mehlich-3 via inductively coupled plasma mass spectrometry (Zhang et al. 2014). Soil pH was determined using a 1:2 soil-to-water ratio (Sikora and Kissel 2014); EC was determined using a 1:2 soil-to-water ratio by electrode (Wang et al. 2014).

Production systems. Plantings included in this study ranged in age but were established between 2012 and 2021, with a 0.8-m spacing between plants in 0.9- to 1.5-m wide rows. Mowed mixed-species groundcover grew between rows at all locations. Plants were irrigated via drip irrigation and were trained on T- or V-trellis systems, except in Mississippi, where plants were not trellised. Locations followed the regionally recommended methods and timing suggested by Fernandez et al. (2023) for summer primocane tipping, and winter pruning and training for plants.

Plantings were fertilized annually from primocane emergence until the end of harvest, ~15 weeks. Fertilization rate ranged between 67 and 101 $kg \cdot ha^{-1}$ N, based on standard recommendations (Fernandez et al. 2023) and results from soil analysis at each location. Fertilization was applied via drip irrigation on a weekly basis or across two to three equally portioned hand applications during the growing season. Although fertilizer source, application method, and timing within the season all varied, each were representative of the diverse cultural practices of the region. In-row weed barriers varied across states. In Alabama and North Carolina, USA, plants were grown on bare ground. Plants in Arkansas and Tennessee, USA, were grown under black landscape fabric. In Georgia and Virginia, USA, either white or black plastic mulch was used for in-row weed control. In Mississippi, USA, plants were grown under pine bark mulch.

Leaf sampling. Leaf tissue nutrient samples were collected from 28 Mar to 29 Aug 2022 and 14 Mar to 23 Aug 2023. Because of regional differences in crop development, a 2- to 3-week difference occurred between when all locations started sampling each year. Samples began being collected first in Mississippi each year; the location latest to start sample collection was North Carolina. Floricane leaf tissue nutrient samples were collected in a randomized pattern at five phenological stages: floricane bloom; primocanes at 15 cm tall; small, green fruit on the floricane; peak harvest on the floricane; and postharvest. Primocane leaf tissue nutrient samples were collected at four phenological stages: primocanes at 15 cm tall; small, green fruit on the floricane; peak harvest on the floricane; and postharvest. Primocanes were not sampled at floricane bloom because of the concern that canes were too small. Small, green fruit were characterized as fruit beginning to develop after petal drop up until fruits developed color. Peak harvest was determined by visual assessment of when crop load was greatest. In addition, postharvest was characterized as late July or early August, after floricane harvest ended. The rate of growth and development of primocanes and reproductive organs in plantings did not appear to differ between years. Yield data were not recorded. Plantings were observed to have typical commercial yield for these cultivars in the southeastern region, except in Mississippi, USA, where ‘Chickasaw’ and ‘Kiowa’ were observed to have below-average yields as a result of disease pressure.

Approximately 25 to 50 whole, most recently mature, fully expanded floricane or primocane leaves, not including petioles, were collected per sample. Leaves were sampled uniformly from both sides of the planting rows. Plot sizes varied across locations; however, all consisted of at least five plants. Leaves were unwashed per standard recommendation (Hart et al. 2006) and were stored in paper bags before shipping for laboratory analysis. Floricane and primocane leaves were sampled, stored, and analyzed separately. Leaf samples from all locations were submitted to the University of Arkansas Fayetteville Agricultural Diagnostic Laboratory. Leaves were analyzed for N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B. Leaf tissue N concentration was analyzed via combustion (Campbell 1992). Leaf tissue concentration of other mineral nutrients was analyzed via acid digestion (Jones and Case 1990).

Data analysis. Data were analyzed separately by cane type (primocane or floricane), because the goal of our study was to determine the changes in leaf tissue nutrient concentration for each tissue type across phenological stage and to verify the recommended leaf tissue nutrient sufficiency ranges, which are currently based on primocane leaf samples. In total, 873 leaf tissue nutrient samples were collected and analyzed. One data point was removed as an outlier from the 2022 primocane Fe concentration data. Three data points were removed as outliers from 2022 data for floricane Cu concentration. One data point was removed as an outlier from the 2023 primocane B concentration data. The ability to sample the same cultivars at all locations in each state was not possible for this survey, thus “cultivar” was evaluated initially as a nested factor within the main effect of “state” for its impact on blackberry plant tissue nutrient status.

Exploratory data analysis was used to assess normality of distribution of leaf tissue nutrient content. Leaf tissue nutrient data were analyzed for the effect of phenological stage and year using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC, USA). In a separate model, leaf tissue nutrient data were analyzed for the effect of state, cultivar, and year using PROC GLIMMIX SAS version 9.4 (SAS Institute, Cary, NC, USA). Main effect interactions between phenological stage, cultivar, and state were not investigated, to focus instead on the study objective of determining the effect of these separate factors on leaf tissue nutrient concentration. However, because of replication limitations at some locations, cultivar was nested within an interaction of state by year. Post hoc analyses were conducted on least-squared means determined to be significant ($P < 0.05$) using Tukey’s honestly significant difference test. All tables and figures display the true means of our data.

Results and Discussion

Influence of cane type. Nutrient concentrations of floricane and primocane leaf tissues were statistically different for all nutrients except Cu ($P = 0.2902$) (Tables 3 and 4). Leaf tissue N, P, K, Mg, S, and Zn concentrations were generally greater in primocane than in floricane tissues, whereas floricane leaf Ca, Fe, Mn, and B concentrations were generally greater than in primocane tissues. Inherent differences in nutrient uptake and nutrient concentration between cane types have been established previously in blackberry (Bryla and Strik 2008; Mohadjer et al. 2001; Naraguma et al. 1999). For this reason, floricane and primocane leaf tissue nutrient data were separated for all analyses of the impact of phenological stage, state, and cultivar on leaf nutrient content.

Current leaf tissue nutrient ranges are developed for primocane leaf tissues due to the generally greater variability in floricane leaf tissue nutrients (Strik and Vance 2016, 2017), which is reflected in our data. We include

Table 3. Blackberry primocane leaf tissue nutrient concentration in the southeastern United States across four phenological stages, year, and phenological stage-by-year interaction for samples collected in 2022 and 2023.

		Leaf										
Variable	*n*	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	B ($mg \cdot kg^{-1}$)
Phenological stage												
Primocane 15 cm	76	3.54 a[i]	0.34 a	1.49 a	0.47 b	0.33 c	0.21 a	72 a	277 ab	40 ab	10.6 a	17 c
Small, green fruit	86	3.25 b	0.30 b	1.52 a	0.52 b	0.37 b	0.20 b	54 ab	250 b	43 a	9.3 b	21 bc
Harvest	128	2.40 c	0.22 c	1.33 b	0.68 a	0.38 b	0.16 c	50 b	253 b	32 b	8.4 b	29 ab
Postharvest	113	2.18 d	0.21 c	1.23 c	0.77 a	0.41 a	0.16 c	46 b	343 a	34 b	9.1 b	32 a
P value	—	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0061	0.0012	<0.0001	<0.0001
Year												
2002	216	2.66 b	0.25 b	1.36	0.62	0.39 a	0.18	59 a	233 b	36	9.5 a	28 a
2023	187	2.82 a	0.26 a	1.38	0.64	0.36 b	0.18	48 b	339 a	38	8.8 b	23 b
P value	—	0.0009	0.0479	0.1229	0.6535	<0.0001	0.4422	0.0008	<0.0001	0.2176	0.0014	<0.0001
Phenological stage × year												
P value	403	0.2588	0.0061	0.0012	0.1718	0.8298	0.0250	0.0003	0.8900	0.0893	0.0185	0.9342

[i] Values in the same column followed by the same letter indicate they are not significantly different by Tukey’s honestly significant difference test at $P \leq 0.05$.

Table 4. Blackberry floricane leaf tissue nutrient concentration in the southeastern United States across five phenological stages, year, and phenological stage-by-year interaction for samples collected in 2022 and 2023.

		Leaf										
Variable	*n*	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	B ($mg \cdot kg^{-1}$)
Phenological stage												
Bloom	104	3.01 a[i]	0.29 a	1.21 bc	0.69 c	0.32 a	0.19 a	65 ab	477	37 a	18.8 a	26 b
Primocane 15 cm	74	2.95 a	0.24 b	1.29 ab	0.72 c	0.31 ab	0.17 b	52 c	607	35 ab	10.1 ab	28 b
Small, green fruit	102	2.73 b	0.21 bc	1.31 a	0.88 b	0.32 a	0.16 c	65 ab	518	31 bc	809 ab	35 ab
Harvest	112	2.16 c	0.20 c	1.18 c	1.25 a	0.32 a	0.14 d	73 a	493	32 abc	7.7 b	41 a
Postharvest	78	1.76 d	0.18 d	1.14 c	1.27 a	0.29 b	0.12 e	59 bc	556	27 c	6.0 b	44 a
P value	—	<0.0001	<0.0001	<0.0001	<0.0001	0.0034	<0.0001	<0.0001	0.0836	<0.0001	<0.0001	<0.0001
Year												
2022	251	2.52	0.22 b	1.20 b	1.00 a	0.34 a	0.16 a	69 a	474 b	33	13.0 a	39 a
2023	219	2.53	0.23 a	1.25 a	0.93 b	0.29 b	0.15 b	58 b	579 a	32	7.7 b	30 b
P value	—	0.5072	0.0152	0.0084	0.0363	<0.0001	0.0070	<0.0001	0.0006	0.6456	0.0001	<0.0001
Phenological stage × year												
P value	470	0.0080	0.2692	0.0015	0.1918	0.0004	0.0087	0.0194	0.6662	0.7922	0.0413	0.8301

[i] Values in the same column followed by the same letter indicate they are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

floricane leaf tissue data as a reference point, but focus our analysis on the impact of state/location and cultivar on only primocane leaf tissue nutrients relative to the development of updated leaf tissue ranges for the southeastern United States.

Influence of phenological stage. Phenological stage at the time of leaf sampling had a significant effect on all primocane leaf tissue nutrients ($P < 0.01$) (Table 3). In general, primocane leaf N, P, K, S, Fe, Zn, and Cu concentrations decreased as the season progressed, whereas Ca, Mg, Mn, and B concentrations increased (Figs. 1 and 2). Despite these differences across phenological stage, standard deviations were reasonably similar at each stage, which indicates that early-season stages have similar nutrient stability as postharvest. Year of leaf sampling had a significant effect on primocane leaf tissue nutrient concentration for N, P, Mg, Fe, Mn, Cu, and B. In 2022, primocane leaf Mg, Fe, Cu, and B concentrations were greater than in 2023, whereas leaf N, P, and Mn concentrations were greater in 2023 than 2022 ($P < 0.05$). Significant ($P < 0.05$) phenological stage-by-year interactions for a few nutrients were observed, but they did not reveal a different trend than the one related to the effect of phenological stage (Figs. 1 and 2).

Phenological stage also had a significant effect on all floricane leaf tissue nutrients (Table 4), with the exception of Mn ($P = 0.0086$). In general, N, P, K, Mg, S, Fe, Zn, and Cu floricane leaf tissue nutrient concentrations decreased as the season progressed, whereas Ca and B concentrations increased (Figs. 1 and 2). Year of leaf sampling had a significant effect on floricane leaf tissue nutrient concentration for P, K, Ca, Mg, S, Fe, Mn, Cu, and B. In 2022, Ca, Mg, S, Fe, Cu, and B concentrations were greater than in 2023 ($P < 0.05$), whereas floricane leaf P, K, and Mn concentrations were greater in 2023 than in 2022 ($P < 0.05$). The concentrations of N, K, Mg, S, Fe, and Cu in floricane leaf tissues were significantly affected by an interaction between year and phenological stage ($P < 0.05$). Similar to primocane leaf tissue nutrients, these statistically significant interactions did not reveal a different trend than the one related to the effect of phenological stage (Figs. 1 and 2).

The mean value of primocane leaf N concentration at postharvest sampling (Table 3) did not fall within the current range recommended (2.5%–3.5%) for the southeastern region of the United States (Fernandez et al. 2023). Primocane leaf N showed similar variability (determined by standard deviation) across all phenological stages (Fig. 1), with less than a 0.2% difference in mean standard deviation. In general, mean leaf tissue N concentration in floricanes followed a similar decreasing pattern as that of primocanes throughout the season. Previous work has found similar results for season-long decreases in primocane leaf tissue N concentration (Clark et al. 1988; Strik 2015; Strik and Vance 2017, 2018) and floricane leaf tissue N concentration (Strik and Vance 2017, 2018).

The mean primocane leaf P and K concentrations at postharvest sampling (0.21% P and 1.23% K) fell within the current range recommended for the southeastern region of the United States (Fernandez et al. 2023). Primocane leaf P and K stability (determined by standard deviation) was similar across all phenological stages (Fig. 1B and 1C) within the same nutrient, differing less than 0.04% and 0.08%, respectively. In general, leaf P concentration of both cane types followed a similar decreasing trend throughout the season. Despite leaf K concentration generally decreasing in both cane types, floricane leaf K peaked at the stage of small, green fruit (1.28% K in 2022 and 1.34% K in 2023) before decreasing. Trends of decreasing blackberry primocane and floricane leaf P and K concentrations for erect and semierect blackberry cultivars have been reported previously (Clark et al. 1988; Strik and Vance 2017, 2018); however Strik and Vance (2017) did not observe this pattern in trailing cultivars.

The mean primocane leaf Ca and Mg concentrations at postharvest sampling (0.77% Ca and 0.41% Mg) fell within the current ranges recommended for the southeastern region of the United States (Fernandez et al. 2023) (Table 1). Variation in primocane leaf Ca and Mg standard deviation across phenological stages was minimal (Fig. 1D and 1E), differing less than 0.20% for leaf Ca, with the greatest variability at postharvest, and 0.04% for leaf Mg. In general, leaf Ca concentration in floricanes was greater than in primocanes; however, the opposite was true for leaf Mg concentration. Both cane types generally followed a similar trend throughout the season for leaf Ca concentration. However, mean leaf Mg concentration differed between canes. Primocane leaf Mg concentration increased throughout the season, whereas it decreased in floricanes. Previous research in Oregon, USA, reported an increase in primocane and floricane leaf Ca and Mg concentration during the season in semierect and erect blackberry cultivars (Strik and Vance 2017). Strik and Vance (2018) reported a peak in Ca and Mg concentration in blackberry primocane leaves during harvest, and then a decline postharvest to similar concentrations as Spring, whereas blackberry floricane leaf Ca and Mg concentrations generally increased. By contrast, Clark et al. (1988) observed in Arkansas, USA, that blackberry primocane leaf Ca generally increased, but was stable from June to August, which matches our results more closely.

At postharvest sampling, the mean leaf S concentration (0.16%) fell below the current range recommended for the southeastern region of the United States (Fernandez et al. 2023). Primocane leaf S was similar in stability across all phenological stages (Fig. 1F), with less than a 0.01% difference. In general, primocane leaf S was greater than floricane leaf S; however, both followed a similar downward trend throughout the season, which has been observed previously (Clark et al. 1988; Strik and Vance 2017, 2018).

Mean primocane leaf Fe and Mn concentrations at postharvest (45.57 and 342.88 $mg \cdot kg^{-1}$, respectively) did not fall within the current

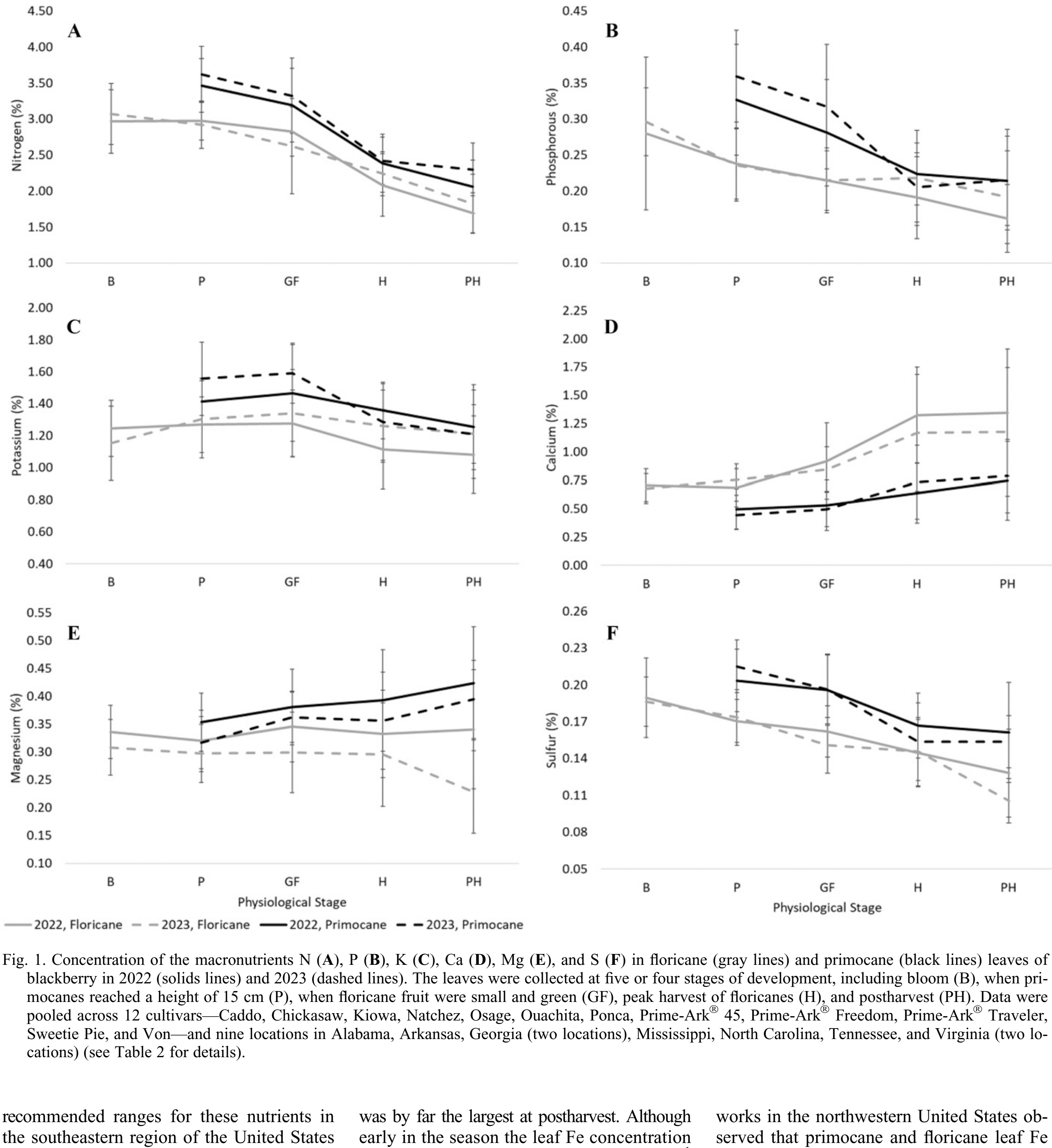

Fig. 1. Concentration of the macronutrients N (**A**), P (**B**), K (**C**), Ca (**D**), Mg (**E**), and S (**F**) in floricane (gray lines) and primocane (black lines) leaves of blackberry in 2022 (solids lines) and 2023 (dashed lines). The leaves were collected at five or four stages of development, including bloom (B), when primocanes reached a height of 15 cm (P), when floricane fruit were small and green (GF), peak harvest of floricanes (H), and postharvest (PH). Data were pooled across 12 cultivars—Caddo, Chickasaw, Kiowa, Natchez, Osage, Ouachita, Ponca, Prime-Ark® 45, Prime-Ark® Freedom, Prime-Ark® Traveler, Sweetie Pie, and Von—and nine locations in Alabama, Arkansas, Georgia (two locations), Mississippi, North Carolina, Tennessee, and Virginia (two locations) (see Table 2 for details).

recommended ranges for these nutrients in the southeastern region of the United States (Fernandez et al. 2023) (Table 1). Primocane Fe fell below the current range, and leaf Mn was above the current range. Water sources can be contaminated with Mn by soils, which can cause increased Mn application depending on irrigation source. High Mn in groundwater has been documented in North Carolina and Georgia, USA, (Gillispie et al. 2016). Primocane leaf Fe was similar in stability across phenological stage (Fig. 2A), with a range of 11.25 to 27.34 $mg \cdot kg^{-1}$, with the smallest value being at the small, green fruit stage. Leaf Mn stability varied widely across phenological stage relative to mean nutrient concentrations (Fig. 2B); however, the standard deviation was by far the largest at postharvest. Although early in the season the leaf Fe concentration was greater in primocanes (71.78 $mg \cdot kg^{-1}$ at primocane at 15 cm) than in floricanes (52.24 $mg \cdot kg^{-1}$ at primocane at 15 cm), by the end of the season the primocane leaf Fe concentration (45.57 $mg \cdot kg^{-1}$ postharvest) was less than in floricanes (59.09 $mg \cdot kg^{-1}$ postharvest). Throughout the season, floricane leaf Mn concentration (473.82 $mg \cdot kg^{-1}$ at bloom and 556.31 $mg \cdot kg^{-1}$ postharvest) was greater than in the primocanes (277.04 $mg \cdot kg^{-1}$ at primocane 15 cm and 342.88 $mg \cdot kg^{-1}$ postharvest). Previous work in the southeastern United States by Clark et al. (1988) observed a similar trend of decreasing primocane leaf Fe concentration throughout the season. Conversely, works in the northwestern United States observed that primocane and floricane leaf Fe and Mn concentrations increased throughout the season (Strik and Vance 2017, 2018).

Mean primocane leaf Zn, Cu, and B concentrations at most phenological stages were within the current range recommended for the southeastern region of the United States (Fernandez et al. 2023) (Table 1), except for leaf B concentration at the small, green fruit stage and primocanes at 15 cm tall. Although statistical differences among phenological stages were identified, these differences lacked practical implications for real-world production, and as such a discussion of these micronutrients is limited herein. Previous work in the southeastern United States also observed blackberry

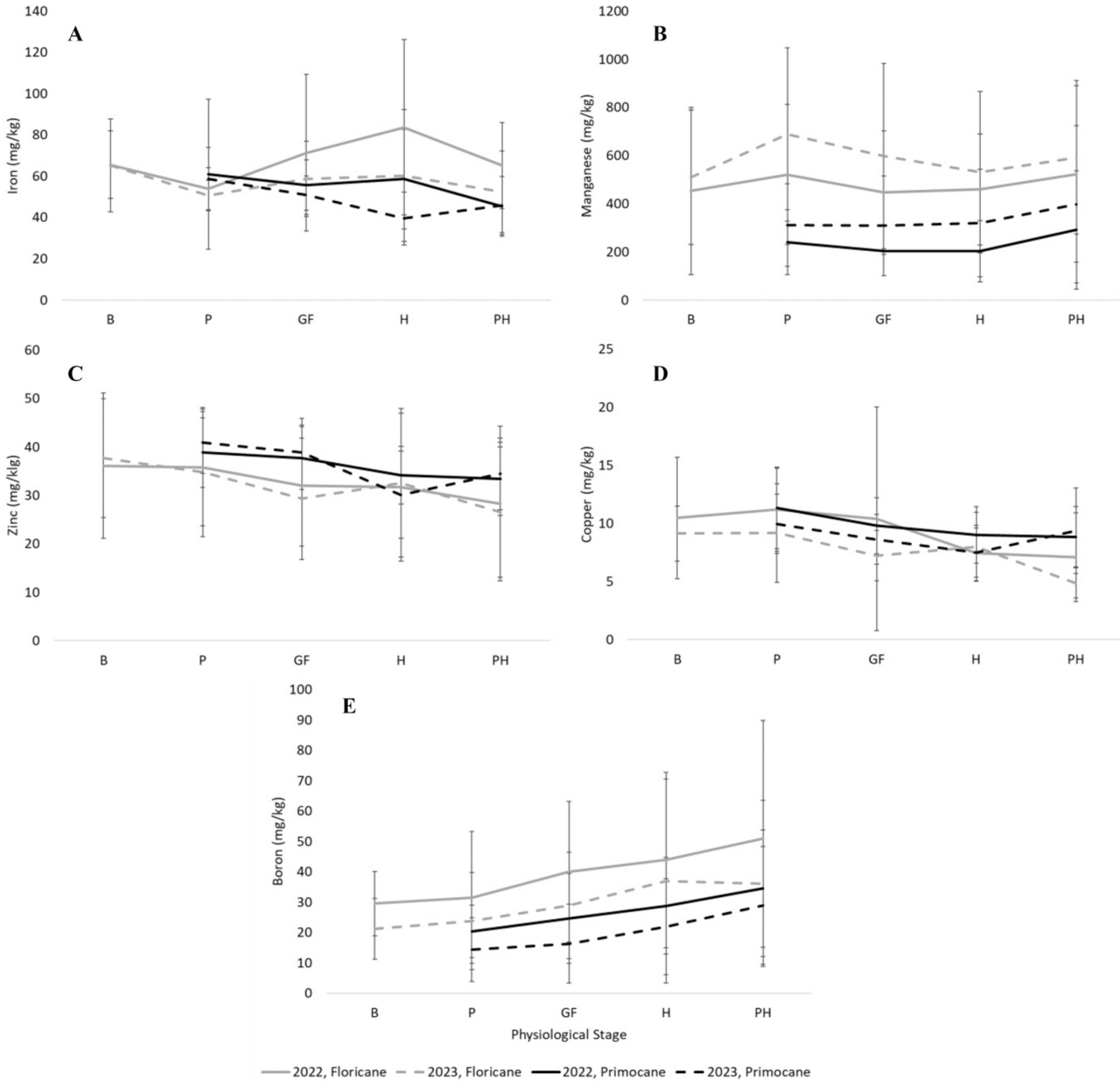

Fig. 2. Concentration of the micronutrients Fe (**A**), Mn (**B**), Zn (**C**), Cu (**D**), and B (**E**) in floricane (gray lines) and primocane (black lines) leaves of blackberry in 2022 (solids lines) and 2023 (dashed lines). The leaves were collected at five or four stages of development, including bloom (B), when primocanes reached a height of 15 cm (P), when floricane fruit were small and green (GF), peak harvest floricanes (H), and postharvest (PH). Data were pooled across 12 cultivars—Caddo, Chickasaw, Kiowa, Natchez, Osage, Ouachita, Ponca, Prime-Ark® 45, Prime-Ark® Freedom, Prime-Ark® Traveler, Sweetie Pie, and Von—and nine locations in Alabama, Arkansas, Georgia (two locations), Mississippi, North Carolina, Tennessee, and Virginia (two locations) (see Table 2 for details).

primocane leaf Zn and Cu concentrations decreasing throughout the season (Clark et al. 1988). In the northwestern United States, blackberries of various growth habits generally increased in primocane leaf Zn and Cu (Strik and Vance 2017, 2018). Strik and Vance (2017) observed floricane Zn and Cu concentrations decreased throughout the season before increasing, having greater concentrations postharvest than in Spring. Strik and Vance (2018) observed a similar trend with blackberry floricane leaf Zn, whereas floricane leaf Cu remained stable throughout. Previous works observed a similar trend of increasing blackberry primocane and floricane leaf B concentration (Strik and Vance 2017, 2018).

Influence of cultivar and state on primocanes. As a part of our regionwide survey of blackberry leaf tissue nutrient content, we evaluated the effects of state/location and cultivar on plant tissue nutrient status, as these factors could potentially affect the use of regionwide leaf tissue sampling guidelines. In our analysis, some variability in nutrient status of blackberries for certain nutrients was observed among the states where we collected samples, whereas cultivar was observed to have very little effect on mean primocane leaf tissue nutrient concentration. Ultimately, for simplicity, cultivar was dropped from our presented data because state was the driving factor in observed differences in primocane leaf tissue nutrient concentration. Previous literature and production guides recommend that cultivars should be sampled separately to assess leaf tissue nutrient concentration in blackberry (Fernandez et al. 2023; Hart et al. 2006; Strik 2017; Strik and Vance 2017, 2018; Strik et al. 2024) and raspberry (Horuz et al. 2013; John et al. 1976; Strik et al. 2024). However, nutrient sufficiency ranges for specific cultivars have never been developed or recommended for blackberries or raspberries (Table 1). Our results indicate cultivar-specific leaf tissue nutrient ranges were not likely needed for the cultivars evaluated in our trial, but small regional differences in blackberry leaf tissue nutrient content do occur, which is likely a result of differences in soil type, climate, and management practices.

Differences in primocane leaf tissue nutrient content for all nutrients, except for Mn and Zn, were observed among the seven states included in our trial (Table 5); however, in some cases, significant state-by-year interactions were also observed. In general, observed differences in nutrient concentration among states or between years within the same state are differences of less than 0.2% or less than 20 mg·kg^{-1} and do not reveal trends that would require states to have separate recommended blackberry leaf tissue nutrient sufficiency ranges. In addition, some state-by-year interactions were present, but these specific data are not shown in a figure because observed differences were very small and do not provide additional insight into regional variability beyond state-by-state differences.

Primocane leaf N concentration was less in Tennessee, USA (2.29%), than all other states when nutrient status of blackberries by state was averaged across year and stage (Table 5). These differences were attributed to inefficient fertilizer application at planting and during plant establishment (Bumgarner N, personal communication). Primocane leaf P was less in Mississippi, USA, than in all other states except North Carolina, USA (Table 5). Mississippi's low P concentration may be related to the low soil pH in Mississippi, USA (5.7 pH) (Table 2), limiting plant-available P (Brady and Weil 2002). Primocane leaf K varied by state and year (Table 5); however, differences among states were only observed in 2023, when mean leaf K in Alabama, USA (1.54%), was greater than in Arkansas, USA (1.25% K), and Tennessee, USA (1.25%). Furthermore, leaf K in Mississippi, USA (1.31%), was also greater than in Arkansas, USA, during the same year. Variation in fertilizer source, fertilizer application history, or soil types likely contribute to these small differences in leaf K. Primocane leaf Ca varied by state within each year of sampling (Table 5). Leaf Ca in 2022 in Mississippi, USA (0.31%), was less than in all other states except Mississippi, USA, in 2023 and both years in North Carolina, USA. In 2023, mean leaf Ca in Mississippi, USA (0.37% Ca), was less than all other states, except North Carolina, USA (0.60%), and Virginia, USA (0.61%). Primocane leaf Mg concentration varied by state and year (Table 5). When analyzed within year, leaf Mg was not significantly different across states in 2023, but was greater in Georgia, USA (0.51%), than Mississippi, USA, in 2022 (0.32%). Liming sources and practices can affect both Ca and Mg levels in soils and plant tissues, which could account for some of the observed regional variability. Similarly, with the lowest soil pH in Mississippi, USA (Table 2), plant-available Ca and Mg may have been limited. Primocane leaf S varied by state and year; however, when states were compared in the same year, no significant differences were observed across states in 2023. In Alabama and Georgia, USA, soil S levels can generally be very low (Smith et al. 2017b) and can be affected by fertilizer and liming practices. Sulfur concentration in blackberry primocane leaf tissues has also been observed to vary depending on weed management strategies (Harkins et al. 2014).

Primocane leaf Fe varied by state and year (Table 5). However, when states were compared within year, no significant differences were observed in leaf Fe concentration in 2023. But, in 2022, primocane leaf Fe in Tennessee, USA (71.90 mg·kg^{-1}), was greater than in Georgia, Alabama, and Mississippi, USA (52.21, 48.95, and 40.84 mg·kg^{-1}, respectively). Primocane leaf Fe in Arkansas, USA (64.57 mg·kg^{-1}), was also greater than in Alabama and Mississippi, USA, in 2022, and leaf Fe in Virginia, USA (54.56 mg·kg^{-1}), was higher than in Mississippi, USA. Southeastern US soils can be lower in Fe than the average US soils, especially compared with the Pacific Northwest, which could contribute to lower primocane leaf Fe concentrations in our survey (Smith et al. 2017a). Primocane leaf Cu varied by state and year (Table 5); however, all differences among states within the same year were less than 4 mg·kg^{-1} Cu. Primocane leaf Mn and B varied by state and year (Table 5); however, when states were compared in the same year, no significant differences were observed among states in either year.

The practical application of these results indicates that although small statistical differences were found, relative uniformity in blackberry nutrient content across the southeastern region of the United States was evident. The small differences identified were likely a result of differences in soil type and cultural practices in the region, including application timing and sources of inputs such as lime, fertilizer, and irrigation water (McWhirt et al. 2024). Some nutrients have been documented to vary in blackberry primocane leaf tissue depending on weed management strategy (Harkins et al. 2014). Although these differences are important to take into consideration when interpreting individual sample results, these small regionwide differences do not indicate that state-by-state blackberry leaf tissue nutrient sufficiency ranges should be developed. Instead, the current regional recommendation presented by Fernandez et al. (2023) should be updated to account for the standards in our region.

All phenological stages were included in the analysis of primocane leaf tissue nutrient concentrations reported herein. Because of the small sample size, comments on the statistical differences across states exclusively for leaf tissue nutrient concentration postharvest were not pursued. However, through exploratory analysis, states' mean primocane leaf tissue nutrient concentrations postharvest were assessed. Most fit within the ranges recommended by Fernandez et al. (2023) (Table 1). However, primocane leaf tissue nutrient concentrations of all states fell below those

Table 5. Southeastern blackberry primocane leaf tissue nutrient concentration across state, year, and state-by-year interaction for samples in 2022 and 2023.

		Leaf										
Variable	*n*	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (mg·kg^{-1})	Mn (mg·kg^{-1})	Zn (mg·kg^{-1})	Cu (mg·kg^{-1})	B (mg·kg^{-1})
State												
Alabama	65	2.86 a[i]	0.22 b	1.45 a	0.68 bc	0.39 ab	0.18 ab	48 bc	461	38	7.9 c	29 ab
Arkansas	89	2.80 a	0.22 b	1.33 b	0.81 a	0.37 ab	0.18 ab	54 ab	213	34	9.0 bc	18 ab
Georgia	30	3.02 a	0.23 b	1.30 b	0.79 ab	0.50 a	0.18 ab	54 ab	326	37	8.2 bc	53 a
Mississippi	120	2.73 a	0.31 a	1.38 ab	0.34 d	0.32 b	0.16 b	42 c	308	35	9.8 ab	10 b
North Carolina	9	3.10 a	0.30 ab	1.49 ab	0.57 c	0.42 ab	0.21 a	63 ab	82	44	12.1 a	38 ab
Tennessee	60	2.29 b	0.25 b	1.32 b	0.76 ab	0.40 ab	0.17 ab	67 a	205	33	9.3 bc	39 ab
Virginia	30	2.77 a	0.24 b	1.47 ab	0.70 abc	0.42 ab	0.20 ab	51 bc	172	34	9.9 abc	39 ab
P value	—	<0.0001	<0.0001	0.0006	<0.0001	0.0212	0.0131	<0.0001	0.9447	1.0000	<0.0001	0.0314
Year												
2002	216	2.66	0.25	1.36	0.62	0.39 a	0.18	59 a	233 b	36	9.5 a	28 a
2023	187	2.82	0.26	1.38	0.64	0.36 b	0.18	48 a	339 a	38	8.8 b	23 a
P value	—	0.7248	0.7711	0.6536	0.1248	0.0004	0.1022	0.0295	0.0055	0.9072	0.0048	0.0319
State × year												
P value	403	0.1124	0.1364	<0.0001	0.0035	0.0117	0.0029	0.0002	0.0367	0.7596	0.0005	0.0131

[i] Values in the same column followed by the same letter indicate they are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

recommended ranges for N (2.5%–3.5%), S (0.17%–0.21%), and Fe (60–100 ppm) postharvest. Previous literature (Strik and Vance 2017, 2018) found that across eight blackberry cultivars of various growth habits, all fell within the postharvest primocane leaf tissue N range of 2.0% to 3.0%. The trailing cultivars Obsidian (Strik and Vance 2018) and Black Diamond (Strik and Vance 2017) were the only exceptions, falling less than 0.05% outside the recommended ranges. Both of these cultivars are not grown in the southeastern United States. Clark et al. (1988) did not observe significant differences in blackberry primocane leaf N concentrations across three cultivars in Arkansas, USA, and theorized this could be a result of the cultivars studied having shared parentage. Through exploratory analysis of our data, we observed that primocane leaf tissue nutrient concentration of primocane-fruiting cultivars in general had similar nutrient concentrations to most floricane-fruiting cultivars at the same phenological stages for all nutrients. Our survey of blackberry nutrient status in the southeastern United States indicates that primocane leaf N concentration postharvest fell well within the range of 2.0% to 3.0%, which is recommended for blackberry production in the Pacific Northwest, California, and the northern United States, as well as in parts of Canada (Table 1).

Conclusion

Across the United States, small differences in certain blackberry leaf tissue nutrients (such as Fe and Mn) have been observed, indicating regional differences can have some influence on plant nutrient uptake and accumulation for the crop. However, in our study, leaf tissue nutrient variation across state/location and cultivar within the southeastern region of the United States was small and could more often be attributed to cultural management practices or differences in soil type. Thus, region-specific nutrient sufficiency ranges for blackberry are adequate for nutrient management across cultivars in the southeastern United States. Based on our results, we suggest lowering the recommended blackberry primocane leaf tissue nutrient sufficiency ranges for postharvest sampling for N to 2.0% to 3.0% and for S to 0.10% to 0.20% for the Southeast. Primocane leaf Fe concentrations are, on average, less in the Southeast, whereas Mn concentrations are greater and more variable, which requires further investigation to refine leaf tissue nutrient ranges for the region. In our study, variability (assessed through standard deviation) in primocane leaf tissue nutrient concentration was similar across different phenological stages for many nutrients. Therefore, we recommend the development of new nutrient sufficiency ranges for primocane leaf tissues at the earlier phenological stages of primocanes at 15 cm tall; small, green fruit on the floricane; and floricanes at peak harvest for blackberry in the southeastern United States.

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