Title: Seasonal evaluation of muscadine leaf tissue across growing regions to identify nutrient levels for the development of phenology-based recommendations

Progress Report

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Research Proposal

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Objective: To identify nutrient levels in leaf tissue based on phenology of commercially grown bronze and dark-colored muscadine cultivars grown across three distinctive growing regions.

Justification and Description:
Muscadine (*Vitis rotundifolia* Michx., syn. *Muscadinia rotundifolia* [Michx.] Small) is a native grape to the southeastern U.S that has been selectively bred for commercial production. The southeastern U.S. is a diverse region with varying soil types, climates, and topography. Muscadine is cultivated in areas where low temperature extremes rarely fall below -12 °C. This limits muscadine production to hardiness zone 8a (-12.2 °C to -9.4 °C) or higher, which lies in the Atlantic U.S. Coastal Plain of Georgia, South Carolina, and North Carolina. Through this region, there are variations in chill hour accumulation and heat unit accumulation, which affects bud break, flowering, and harvest timing. Growers may follow phenological stages to apply cultural practices such as pruning, weed management, and insect/disease control (Cline, 2017). However, fertilization is often calendar based. Bud break is a key indicator to begin fertilizer

applications on a four to six week basis until nitrogen requirements are met (Krewer et al. 2002). Two methods are used to identify the efficacy of the nutritional program: soil and leaf tissue sampling. Soil sampling identifies the available nutrients. Tissue sampling provides insight into nutrient uptake. Testing of muscadine leaf tissue is suggested to be done June/early July using both leaf blade and petiole (Clark and Spiers, 2001). The sufficiency/deficiency range for macro and micro nutrients is well established for mid to late summer sampling (Bryson et al., 2014). However, information is lacking for nutrient sufficiency/deficiency ranges outside of this sample timing. Further, mid to late summer sufficiency/deficiency ranges could vary across regions as it relates to phenological progression between bud break, harvest, and postharvest sampling. Late, "mid to late summer" is subjective and poorly adaptable across phenologically distinct regions.

As a consequence of the above mention limitations, muscadine growers are likely to experience nutrient management issues with calendar based sampling of tissues, which potentially reduces the ability to make corrections. In contrast, bunch grape producers are provided with standardized tissue sampling at the phenological stages of bloom and veraison (fruit color change or ripening).While bunch grape growers widely adopt scientific advancements in agricultural production, muscadine growers have not had the same level of research to advance their production. For example, there is no mention of nutrient management in the cultural practice section of the integrated muscadine guide (Cline, 2017). Fertilization recommendations for muscadine in Georgia are generalized across cultivars and regions, which are based on soil testing only (Krewer et.al., 2002). Thus, information on regional nutrient concentrations for muscadine is lacking, though there is research that identifies temporal variability in muscadine (Cumming, 1977). The objective was to identify seasonal tissue nutrient changes in the most important commercially grown bronze and dark-colored muscadine grapes ('Carlos' and 'Noble', respectively) from sites in south Georgia, middle Georgia, and North Carolina. This work could potentially give growers, researchers, and extension personnel insight into temporal muscadine nutritional status and will perhaps highlight local and/or regional trends of nutrient demand. Though several other cultivars exist, this data will provide a sound baseline for muscadine vineyard nutritional management.

Methods

Materials

Leaf tissue was collected at bloom, veraison, and immediately postharvest from commercially grown 'Carlos' and Noble' vineyards in south Georgia, middle Georgia, and North Carolina. Soil samples were collected prior to fertilization within each block. All vineyards were at least 4 years old and considered commercially mature. The leaf tissue and soil sample collection took place at three farms: 1) Cauble Creek Vineyard, Salisbury, NC (35° 39' 48.28" N 80° 34' 3.01" W, elev. 799 ft); 2) Chateau Elan Vineyard, Braselton, GA (34° 05' 55.98" N 83 49' 04.50" W, elev. 927 ft), and 3) Still Pond Vineyard, Arlington, GA (31° 26' 21.09" N 84° 37' 20.88 W, elev. 213 ft). Cauble Creek Vineyard has soils of Lloyd clay loam with 2 to 8% slope, whose parent material is saprolite derived from diorite, gabbro, diabase, and gneiss. Chateau Elan Vineyard soil are Cecil sandy clay loam, 6 to 10% slope, whose parent material is residuum derived from granite, gneiss, and schist. Still Pond Vineyard soil is Greenville sandy loam, 2 to 5% slope, derived from marine deposits.

Treatments
Leaf tissue samples were collected in a complete block design with three blocks for each cultivar in each vineyard making a total of 18 blocks. From each block, tissue samples were collected randomly from at least 4 plants to make one tissue sample. Each sampling had 3 replications from each block making 18 samples on each sampling date at each vineyard. At each sampling, general plant health was assessed and phenological stage recorded.

Leaves were collected as leaf blade with attached petiole. Clark and Spiers (2001) suggested a double fist full of mature leaves located opposite fruit clusters on fruiting shoots. We considered a double fist full as 60 leaves per sample bag. Leaf samples were rinsed with distilled water and dried to a constant weight at 80 °C prior to sending the samples to an analytical laboratory. The samples were analyzed for nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), boron (B), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) (Waters Agricultural Laboratories, Inc., Camilla, GA), where the dried leaves were ground to pass through a 20-mesh screen. The samples were reduced to ash in a muffle furnace, acid digested, and measured by inductive coupled plasma spectrophotometer (ICP) coupled to a Digiblock 3000 (SCP Science, Baie D'Urfé, Quebec, Canada). Nitrogen was determined through combustion of plant tissue using a LECO FP-428 N analyzer (LECO, ST. Joseph, MI, U.S.).

A composite soil sample was taken in March 2018 before fertilization at each site. From under the drip line of the same plants used in this study, each sample was taken at 0 to 15 cm depth with the surface 2.5 cm removed. Soil nutrients were extracted (Waters Agricultural Laboratories, Inc., Camilla, GA) using a Mehlich I procedure and pH was measured using a 0.01molar calcium chloride solution in a 1:1 soil to $CaCl_2$ mixture and reported as an adjusted pH value of + 0.6 units (Kissel and Sonon, 2008).

Statistics
The data was analyzed using SAS's 9.4 Proc GLM (SAS Institute Inc., Cary, NC, U.S.). Means were separated at $P<0.05$ level using Fisher's least significant difference (LSD) test.

Results

Soil samples were collected in mid-March 2018 before fertilization (Table 1). Cauble Creek soils were significantly lower in phosphorous (P) and had significantly more magnesium (Mg), cation exchange capacity (CEC), and organic matter (OM) than the other sites. There was approximately 79% less P, 48% more Mg, 48% higher CEC, and 60% more OM at Cauble Creek than the average of Chateau Elan and Still Pond's soils. Interestingly, Mg was positively correlated between leaf tissue and soil across cultivars and sites, whereas, P was negatively correlated (Figure 1). Neither, Ca or K demonstrated strong influence in leaf tissue levels when regressed against available soil Ca and K (Figure 1).

'Carlos' and 'Noble' leaf tissue samples were collected at bloom, which occurred 5/29 at Still Pond, 6/7 at Chateau Elan, and 6/19 at Cauble Creek. At this sampling, N, P, K, Mg, S, B, Mn, and Cu nutrient levels that were sufficient to excess (Bryson et al., 2014). Zinc was deficient at

Cauble Creek and Still Pond and Fe was deficient in 'Noble' at Chateau Elan (Table 2). Iron was deficient in each treatment at various intervals without being chlorotic, suggesting the lower range of 60 ppm may need an adjustment downward (possibly 50 ppm).

The second tissue sampling occurred at veraison, which happened on 8/7 at Still Pond, 8/15 at Chateau Elan, and 8/30 at Cauble Creek. This sampling occurred at a point where peak N demand has passed for bunch grapes (Keller, 1997). Thus, sampling at veraison would reveal if reserves were depleted. Nitrogen, P, K, Ca, S, and B were all sufficient or in excess at all three sites (Table 2). Magnesium was deficient in both 'Carlos' and 'Noble at Chateau Elan and was in excessive amounts at Cauble Creek. Zinc levels at Cauble Creek and Still Pond's were <18 ppm; however, classic Zn deficiency of leaf chlorosis and/or excessive shoot development were not noted (Clark and Spiers, 2001), suggesting sufficient Zn was available to the plants. Copper levels were generally lower than the 5 ppm (the lower threshold for sufficiency) but shortened internode growth symptoms (Clark and Spiers, 2001) were not observed.

The final sampling was postharvest. Leaves were collected on 10/1 at Chateau Elan. 10/5 at Still Pond, and 10/26 at Cauble Creek. Sufficiency ranges were met or exceeded for N, P, Ca, B, and Mn at all the sites (Table 2). Deficiencies were observed in K, S, Zn, Fe, and Cu. Deficiencies at this sample timing would be expected due to nutrient removal associated with fruit harvest, recycling of translocatable nutrients, and the end of the seasonal cycle with slowing metabolism as plants prepare for dormancy (Jackson, 2000). Interestingly, N was never deficient in either cultivar at any site despite modest fertilizer regimes: no fertilizer was applied at Cauble Creek, a total of 80 lbs of 14-2-8 in split applications (30 lb at bud break and 50 lb at bloom), which amounted to 9.6 lb of N applied to both 'Carlos' and 'Noble', was applied at Chateau Elan, and 60 lb of N was applied at bud break, 600 lb of 10-10-10 per acre at Still Pond.

Nitrogen levels decreased with each sample timing with average N across cultivar and site at 2.9% (1st), 2.5% (2nd), and 2.1% (3rd). Sufficiency range for N mid-summer sampling is 1.65% to 2.15% (Bryson et al., 2014). Phosphorous showed a similar trend as N: P = 0.21% (1st), 0.17% (2nd), and 0.14% (3rd). Potassium is highly mobile in plants and tends to concentrate in actively growing tissue. This is partially why sampling leaf tissue adjacent to fruiting clusters is recommended (Clark and Spiers, 2001). These leaves should be fully expanded and separated distally from apical growing point, where K concentrations will be sensitive to sink demand of both actively growing tissue and developing fruit. Considering sites, the K concentrations in the minimally fertilized sites trended higher than Still Pond. However, deficiencies were not noted until the postharvest sampling and was likely a function of the removal of large amounts of K with fruit harvest. Potassium soil levels were similar at all sites, with the lowest K level at Chateau Elan (Table 1). Our results cover only a single season's data; further research should be conducted to better understand our observations.

Leaf tissue Mg dropped precipitously after the first sampling at Chateau Elan. However, Chateau Elan's leaf tissue were not expressing any symptoms of Mg deficiency. Leaf Mg levels can be affected by monovalent and divalent cations (e.g. K and Ca) competition in the soil matrix. However, similar levels of K and Ca were observed at Still Pond, where Mg levels were sufficient throughout the season. Which suggests the soil may have a significant influence on nutrient availability, especially with higher pH values as noted by the significant difference

between Chateau Elan and Still Pond soil pH (Table 1). Calcium leaf tissue levels trended to excessive through the season. Calcium is xylem mobile and accumulates in the leaves; our observations show that Ca increased in leaf tissue to levels of 2% at Chateau Elan in 'Carlos'. Sulfur was at sufficient levels in the 1st sampling and was deficient in 'Noble' at Cable Creek and Chateau Elan in the 2nd sampling (Table 2). All the cultivars were deficient for S at the 3rd sampling. Sulfur is translocatable being both xylem and phloem mobile; therefore, as the season progressed levels declined across the cultivars and sites.

Though B levels decreased throughout the season, deficiency levels were never observed. Zinc levels in leaf tissue were slightly deficient throughout the season at Still Pond and Cauble Creek. Zinc levels at Chateau Elan were significantly greater than the other vineyards. This may be attributed to Zn based fungicides, which can adhere to tissue surfaces or in the stomata. Manganese was in excessive amounts throughout the season. Manganese uptake is dependent on soil pH and is typically taken up in greater amounts in lower relative to higher pH levels (Clark and Spiers, 2001). However, the sites had soil pH from 5.4 to 6.8 and Cauble Creek had the highest levels of Mn with soil pH of 6.4. This suggests that pH limits at which differential Mn uptake occurs was not observed between the sites. Nonetheless, there were no visual Mn leaf tissue symptoms and there were no effects upon yield. 'Carlos' averaged 7 ton/A and 10 ton/A for 'Noble' across the three vineyards.

Conclusions

Seasonal testing of muscadine leaf demonstrated that N, P, K, S, decrease through the sampling dates and Ca and Mn increase in concentration. Nutrients Mg, B, Zn, Fe, Cu remain in relatively stable concentrations throughout the season. Sampling based on phenology shows there are significant differences between sample timing and when planning fertilization. Veraison appears to be the phenological point at which nutrient demand can be best estimated relative to bloom and postharvest. This work demonstrates there is variability in tissue nutrient concentration between sampling dates, sites, and cultivars; however, minor adjustments in the sufficiency ranges of Fe, Zn, and S should be considered.

Impact

Establishing a specific phenological leaf tissue timing will allow muscadine growers throughout the growing region of the Southeastern U.S. a point at which nutrient ranges can be used to determine fertilization program and estimate demand. By sampling at fruit set, veraison, and postharvest, the information provides a guide to determine plant health at critical stages of production. This work provides extension and growers with information to estimate nutrient needs and project budgets.

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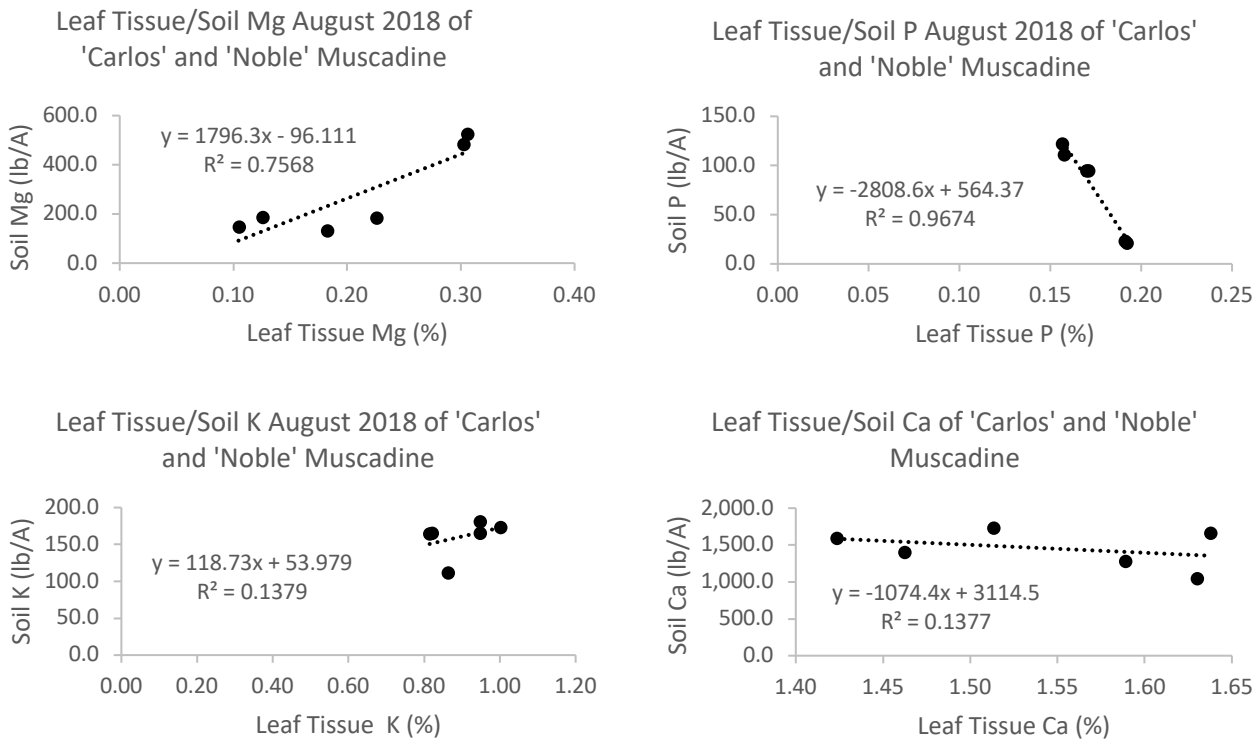

Figure 1 are the regressions for magnesium (Mg), phosphorous (P), potassium (K), and calcium (Ca) for August leaf sampling regressed against the soils collected from Cauble Creek, Chateau Elan, and Still Pond vineyards in 2018.

Table 1. 2018 soil analyses of 'Carlos' and 'Noble' muscadine grape grown commercially in South Georgia (Still Pond), North Georgia (Chateau Elan), and North Carolina (Cauble Creek) vineyards. Samples were collected in March prior to fertilization.

	Soil Analyses for 2018 for 'Carlos' and 'Noble' Muscadine Grape													
	P		K		Mg		Ca		pH		CEC		OM	
Soils	lb/A		lb/A		lb/A		lb/A							
Cauble Cr **Carlos**	21.3	c[z]	181.7	a	524.3	a	1,736.7	a	6.5	ab	10.1	a	4.6	a
Chateau Elan **Carlos**	95.0	b	112.0	a	148.7	b	1,670.3	a	6.9	a	6.9	b	1.8	b
Still Pond **Carlos**	122.3	a	164.7	a	184.7	b	1,282.7	ab	5.3	c	7.4	b	1.9	b
Cauble Cr **Noble**	23.3	c	173.3	a	482.7	a	1,596.0	ab	6.3	b	10.3	a	4.5	a
Chateau Elan **Noble**	111.3	ab	166.3	a	187.7	b	1,403.0	ab	6.6	ab	6.4	b	2.0	b
Still Pond **Noble**	95.0	b	166.3	a	132.7	b	1,054.3	b	5.5	c	6.5	b	1.8	b

[z]Means followed by a different letter within a column and within a cultivar are significantly different at $P \leq 0.05$ according to Fisher's least significant difference (lsd) test.

Table 2. 2018 Leaf tissue analyses of ‘Carlos’ and ‘Noble’ muscadine grape grown commercially in South Georgia (Still Pond), North Georgia (Chateau Elan), and North Carolina (Cauble Creek) vineyards. The phenological stages were used to determine sample timing fruit set (petal fall), veraison (highlighted), and postharvest. Means followed by a different letter within a column and within a cultivar are significantly different at $P \leq 0.05$ according to Fisher’s least significant difference (lsd) test. Sufficiency ranges are from Bryson et al. (2014).

	Leaf Tissue Analyses for 2018 for 'Carlos' and 'Noble' Muscadine Grape										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm
Sufficiency Ranges	1.65-2.15	0.12-0.18	0.80-1.20	0.15-0.25	0.70-1.10	0.19-0.27	15-24	18-35	60-150	60-120	5.0-10
Cauble Cr **Carlos** 6/19	2.99 b	0.23 a	1.32 b	0.28 bc	1.01 k	0.19 bc	26.0 de	13.8 f	355.6 fgh	52.1 gh	9.9 a
Chateau Elan **Carlos** 6/7	2.88 b	0.22 ab	1.63 a	0.26 de	1.44 gh	0.20 b	33.1 b	20.9 e	286.5 gh	59.6 cde	7.7 b
Still Pond **Carlos** 5/29	2.71 cd	0.17 de	1.01 cd	0.24 ef	1.24 j	0.17 ef	22.0 ghij	14.4 f	274.2 h	57.3 cdefg	5.6 cd
Cauble Cr **Noble** 6/19	2.91 b	0.22 a	1.41 b	0.28 bc	1.02 k	0.18 de	27.2 cd	14.3 f	301.0 gh	51.7 gh	9.6 a
Chateau Elan **Noble** 6/7	3.34 a	0.21 b	1.35 b	0.24 ef	1.35 hi	0.24 a	52.0 a	26.2 d	706.4 b	72.6 ab	7.4 b
Still Pond **Noble** 5/29	2.59 cde	0.18 d	1.05 cd	0.22 f	1.31 ij	0.17 def	24.1 efg	15.8 f	377.0 efg	56.0 efgh	5.2 de
Cauble Cr **Carlos** 8/30	2.49 efg	0.19 c	0.95 cdef	0.31 a	1.51 fg	0.18 cde	24.9 ef	13.8 f	547.2 cd	71.8 b	5.3 cde
Chateau Elan **Carlos** 8/15	2.71 c	0.17 de	0.86 fg	0.10 hi	1.64 de	0.19 bcd	23.2 fghi	52.5 b	269.6 h	57.6 cdefg	5.2 de
Still Pond **Carlos** 8/7	2.51 def	0.16 f	0.81 gh	0.23 f	1.59 ef	0.18 de	23.2 fgh	15.6 f	361.6 fgh	54.0 efgh	4.0 fgh
Cauble Cr **Noble** 8/30	2.29 hi	0.19 c	1.00 cde	0.30 ab	1.42 gh	0.16 fg	24.1 efg	14.8 f	701.7 b	63.2 cd	4.6 ef
Chateau Elan **Noble** 8/15	2.69 fgh	0.16 f	0.95 cdef	0.13 h	1.46 g	0.16 fg	28.9 c	69.0 a	473.0 de	55.9 efgh	4.3 fg
Still Pond **Noble** 8/7	2.34 gh	0.17 de	0.82 gh	0.18 g	1.63 de	0.18 bcd	21.5 hij	15.7 f	543.2 cd	53.5 fgh	3.5 h
Cauble Cr **Carlos** 10/26	2.02 j	0.14 g	0.61 ij	0.29 abc	1.64 de	0.14 ij	22.8 fghi	14.3 f	547.1 cd	53.6 efgh	4.0 fgh
Chateau Elan **Carlos** 10/1	2.39 fgh	0.16 ef	0.90 efg	0.07 j	2.08 a	0.17 def	17.0 k	48.1 c	412.7 ef	78.1 a	6.0 c
Still Pond **Carlos** 10/5	2.23 hi	0.13 g	0.73 hi	0.27 cd	1.96 b	0.15 hi	21.5 hij	15.8 f	465.3 de	53.7 efgh	3.5 h
Cauble Cr **Noble** 10/26	1.76 k	0.13 g	0.64 ij	0.31 a	1.58 ef	0.13 j	23.2 fghi	15.6 f	831.2 a	50.6 h	3.6 gh
Chateau Elan **Noble** 10/1	2.13 ij	0.14 g	0.91 defg	0.10 i	1.76 c	0.16 gh	20.0 j	66.3 a	585.7 c	63.6 c	5.6 cd
Still Pond **Noble** 10/5	2.05 j	0.13 g	0.72 hi	0.18 g	1.70 cd	0.15 hi	21.0 ij	13.8 f	547.6 cd	58.6 cdefg	3.9 gh