Received: 22 April 2020 | Accepted: 7 April 2021 | Published: 10 May 2021
DOI:10.20870/oeno-one.2021.55.2.3387

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

Tolerance of Muscadine grapes (*Muscadinia rotundifolia*) to alkaline soil

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Associate editor: Nathalie Ollat

ABSTRACT

Muscadine (*Muscadinia rotundifolia*) grapes have been used in grape variety and rootstock development due to their inherent pest and disease resistance, but little is known about their alkaline soil tolerance. In this study, Muscadine varieties, commercial rootstock and interspecific hybrid grape (*Vitis* spp.) cultivars were evaluated for alkaline soil tolerance under field conditions to determine the potential suitability of muscadines for rootstock development. Thirty-one muscadine and eleven interspecific hybrid grape cultivars were grown in a moderately alkaline soil (pH = 8.1) over a three-year period. Alkaline soil tolerance was determined by relative vine vigour (shoot length), vine nutrient status (whole leaf tissue testing) and visual chlorosis. Additional data were collected on the timing of budbreak. Overall, the muscadines studied expressed low vigour and had greater chlorosis symptoms than the interspecific hybrid rootstocks (Paulsen 1103, Millardet et de Grasset 101-14, Millardet et de Grasset 420A, Ruggeri 140, Schwarzmann, and Matador). These parameters were not correlated with the concentration in any specific nutrient, although nutrient deficiencies (nitrogen, copper) and excesses (calcium, boron) were observed in the muscadine varieties.
Overall, the muscadine grapes expressed poor alkaline soil tolerance compared to interspecific hybrid grape rootstocks (1103P, 101-14 MGt., 140Ru, Schwarzmann, 420A, and Matador), even the ones having poor alkaline soil tolerance (101-14 MGt., Schwarzmann) and own-rooted cultivars (Black Spanish, Blanc Du Bois, Dunstan's Dream and Victoria Red). Nevertheless, some variability in chlorosis symptoms and nutrition was observed across the muscadine group, suggesting some interests to select Muscadine hybrid rootstocks less sensitive to iron chlorosis.

KEYWORDS

Muscadine, rootstock, hybrid, alkaline

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/3387

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INTRODUCTION

Muscadines grapes (*Muscadinia rotundifolia*) are native to the southeastern United States where they are grown commercially for fresh market consumption, wine and nutraceutical products (Olien, 1990). As a native from warm, humid climates, muscadines have excellent fungal disease resistance as well as resistance to Pierce's Disease (*Xylella fastidiosa*), phylloxera (*Daktulosphaira vitifoliae*) and multiple species of nematodes including the dagger nematode (*Xiphinema index*) (Olmo, 1986; Walker *et al*., 1989; Walker *et al*., 1994; Kellow *et al.,* 2002; Ferris *et al*., 2012; Rubio *et al.*, 2020). While the muscadine grape industry in the U.S. is very limited in scale, there is increasing interest in potential health benefits of muscadines due to high levels of polyphenolics, particularly, stilbenes (Yi *et al.*, 2005), and breeders have used muscadines to develop nematode-resistant rootstocks such as VR 039-16 and VR 043-43 (Lider *et al*., 1988; Walker *et al*., 1991; Bavaresco *et al*., 2005a; Ferris *et al.*, 2012). The recently released *V. rupestris* x *M. rotundifolia* rootstock, UCD GRN-1, is reported to have moderate to strong resistance to at least seven species of nematodes. However, Scheiner *et al.* (2020) and Kamas *et al.* (2020) reported that it performed poorly on alkaline soils.

Lime-induced chlorosis, associated with iron (Fe) deficiency, has been extensively reported in a variety of crops grown on alkaline soils. Low soil Fe may cause chlorosis, but Mengel (1994) reported that Fe uptake from the root apoplast into the cytosol of root cells is a more important process leading to chlorosis than soil Fe availability. Bicarbonates (HCO_3^-) play an important role in lime-induced chlorosis through the HCO_3^- buffer effect (Nikolic and Kastori, 2000). The term "Fe chlorosis paradox" has been used to describe observations of higher Fe concentrations in chlorotic leaves than healthy, green leaves of plants grown on alkaline sites (Bavaresco *et al.*, 1999; Römheld, 2000). Bavaresco *et al.* (1994) ranked the grape species *V. berlandieri*, *V. champini*, and *V. cinerea* as highly tolerant of alkaline soils, *V. arizonica*, *V. californica*, *V. longii*, *V. monticola* as tolerant, and *V. aestivalis*, *V. amurensis*, *V. andersonii* and *V. riparia* as susceptible. Genotypic variability in susceptibility to lime-induced chlorosis has also been reported within individual species of *Vitis* (Ksouri *et al.*, 2007). Muscadine grapes have not been characterized for alkaline soil tolerance, but Bavaresco *et al.* (2005a) reported that the *V. vinifera* x *M. rotundifolia* rootstock VR 043-43 was susceptible to lime induced chlorosis and Ollat *et al.* (2016) reported similar problems with Muscadine hybrid rootstocks. While the genetic basis of alkaline soil tolerance in grapes has not been fully elucidated, Bert *et al*. (2013) identified two quantitative trait loci (QTLs) in an F1 population of *V. vinifera* x *V. riparia* that may be involved in polygenic control.

The native range of muscadines is restricted to acidic soils in the Southeastern U.S. from Texas to Delaware and poor alkaline soil tolerance could limit muscadine's potential for use in developing rootstocks as well as expanding the range of muscadine fruit production (Olmo, 1986). Our project screened thirty-one muscadine cultivars for potential alkaline soil tolerance by comparing their nutrient status, growth and iron deficiency symptoms under field conditions to common alkaline soil tolerant and alkaline soil susceptible commercial rootstocks, and other interspecific hybrid grapes commonly grown on their own roots.

MATERIALS AND METHODS

1. Experimental design

The study was conducted from 2017 to 2019 at Texas A&M University research vineyard in Brazos County, Texas USA (30°53'N, 96°'W; 103 m asl). The soil was, as classified by the United States Department of Agriculture Natural Resource Conservation Service, a Weswood series clay loam with moderate alkalinity (Table 1).

2. Plant material

Thirty-one muscadine varieties, six commercial interspecific hybrid rootstocks, and five interspecific hybrid fruiting cultivars were planted in a vineyard in early spring 2017 (Table 2). Vines were propagated from softwood cuttings and first grown in 0.87 L (9.3 × 9.3 × 10.1 cm) pots to get uniformed vine size and age before transplanting into the vineyard. The experimental design consisted of a randomized block, with four three-vine replications. Spacing was 3.0 m between rows and 0.6 m between vines. Vines were trained vertically on stakes to a height of 1.8 m and a single shoot was retained each season. Lateral shoots were removed as they developed. During the dormant season, all vines were pruned back to two buds to ensure uniformity, and to prevent trellis overgrowth. No shoot topping was performed. Drip irrigation was applied

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TABLE 1. Soil chemical characteristics at the research site and soil target values.

Nutrient	Soil concentration (mg kg^{-1})	
	Research vineyard	Target range[c]
N[a]	2.50 ± 0.70[b]	-
P	11.50 ± 2.47	20–50
K	163.0 ± 26.1	75–100
Ca	6,230 ± 843	500–2000
Mg	141.5 ± 3.89	100–250
S	16.00 ± 6.01	-
Na	20.50 ± 14.6	-
B	0.20 ± 0.14	0.3–2.0
Fe	6.33 ± 0.42	20
Mn	2.71 ± 0.33	20
Cu	0.57 ± 0.04	0.5
Zn	0.27 ± 0.08	2
Cl	21.60 ± 2.21	-
pH	8.10 ± 0.07	-
Organic matter (%)	1.02 ± 0.23%	-
Active lime	10.1%	-

[a]Nitrate.
[b]Mean value ± SE of four composite soil samples taken at a depth of 0–25 cm taken across the test plot.
[c]Target ranges of soil nutrients based on Wolf (2008).

throughout the growing season as required, and vines were fertirrigated with urea ammonium nitrate every 28 days for a total application rate of 16.8 kg ha^{-1} of nitrogen per year (Table 3). All inflorescences were removed before bloom to maintain uniformity across the study.

3. Data Collection

3.1 Phenology

Budbreak was recorded at the beginning of the second growing season to evaluate differences in the timing of shoot emergence across cultivars. All vines were visually assessed daily beginning at the bud swell stage and budbreak was determined when 50 % of vines reached E-L number 4 (Lorenz *et al.*, 1995).

3.2. Vine nutrition

Whole leaf (blade and petiole) tissue samples, consisting of two most recently matured (approximately 1/3 of final size) leaves per vine, were taken in late summer (Julian day 232–242) in the second and third growing season at a timing that coincided with the commercial harvest of many winegrape cultivars in the region. Whole leaves were selected to ensure adequate sample quantity for testing and to compare with existing reference for Muscadine vines (Mills and Jones Jr, 1996). All samples were washed with RO–H_2O, dried at 80 °C, and sent to the Texas A&M AgriLife Extension Soil, Water and Forage Testing Laboratory for mineral nutrient analysis via ICP-MS.

3.3. Canopy chlorosis

Visual leaf chlorosis was recorded two days prior to tissue sampling in 2018 and 2019. The percentage of the grapevine canopy expressing leaf chlorosis (Pouget index ≥ 1) was recorded according to a five-tiered index, as follows: 0 = asymptomatic, 1 = 1–25 % of all leaves

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TABLE 2. Cultivar, abbreviation, and parentage of grapes used in the field STUDY.

Cultivar Roostocks	Abbreviation	Parentage
Millardet et de Grasset 101-14	101-14 MGt.	*V. riparia* x *V. rupestris*
Paulsen 1103	1103P	*V. rupestris* x *V. berlandieri*
Ruggeri 140	140Ru	*V. rupestris* x *V. berlandieri*
Millardet et de Grasset 420A	420A	*V. berlandieri* x *V. riparia*
Matador	Matador	101-14 MGt. x 3-1A (*V. mustangensis* x *V. rupestris)*
Schwarzmann	Schwarzmann	*V. riparia* x *V. rupestris*
Hybrids		
Black Spanish	Black Spanish	*V.* spp.
Blanc du Bois	Blanc Du Bois	Fla. D6-148 x Cardinal
Dunstans Dream	Dunstans Dream	Fla. W1521 x DRX 69-99
Southern Home	Southern Home	Summit x Fla. P9-15
Victoria red	Victoria Red	Ark. 1123 x Exotic
Muscadines		
M. rotundifolia cv. Alachua	Alachua	Southland x Fry
M. rotundifolia cv. Albemarle	Albemarle	Burgaw x Topsail
M. rotundifolia cv. Black Beauty	Black Beauty	Fry x 12-12-1
M. rotundifolia cv. Black Fry	Black Fry	Fry x Cowart
M. rotundifolia cv. Bountiful	Bountiful	Creek x seedling of Topsail
M. rotundifolia cv. Carlos	Carlos	Howard x NC11-173 (Topsail x Tarheel)
M. rotundifolia cv. Creek	Creek	Open pollinated seedling of San Monta
M. rotundifolia cv. Darlene	Darlene	5-11-3 x Carlos
M. rotundifolia cv. Delicious	Delicious	AA10-40 X CD8-81
M. rotundifolia cv. Dixie	Dixie	Topsail x NC 28-193 (Lucida x Wallace)
M. rotundifolia cv. Dixiered	Dixiered	Seedling 44-6 x S. 44-7
M. rotundifolia cv. Doreen	Doreen	Higgins x Dixie
M. rotundifolia cv. Eudora	Eudora	Fry x Southland
M. rotundifolia cv. Fry	Fry	Ga. 19-13 x USDA 19-11
M. rotundifolia cv. Granny Val	Granny Val	Fry x Carlos
M. rotundifolia cv. Hall	Hall	Fry x Tara
M. rotundifolia cv. Higgins	Higgins	Yuga x a white male pollinator
M. rotundifolia cv. Hunt	Hunt	Flowers x a white male muscadine
M. rotundifolia cv. Janebell	Janebell	Fry x Senoia
M. rotundifolia cv. Late Fry	Late Fry	Fry x Granny Val
M. rotundifolia cv. Loomis	Loomis	Creek x US 15
M. rotundifolia cv. Magnolia	Magnolia	[(Hope x Thomas) x Scuppernong)] x (Topsail x Tarheel)
M rotundifolia cv. Magoon	Magoon	Thomas x Burgaw
M. rotundifolia cv. Pam	Pam	5-11-3 x Senoia
M. rotundifolia var. Scuppernong	Scuppernong	*V. rotundifolia*
M. rotundifolia cv. Southern Jewel	Southern Jewel	Granny Val x DB-63
M. rotundifolia cv. Southland	Southland	Thomas x seedling of Topsail
M. rotundifolia cv. Sterling	Sterling	NC 50-55 x Magnolia
M. rotundifolia cv. Supreme	Supreme	Black Fry x Dixieland
M. rotundifolia cv. Triumph	Triumph	Fry x GA29-49
M. rotundifolia cv. Welder	Welder	Dearing x unknown

[z]Based on Clark and Rom, 1997 and Riaz *et al.*, 2008.

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TABLE 3. Chemical characteristics of irrigation water at the research site.

Parameter	Water concentration (mg L^{-1})	
	Mean value[a]	Range[b]
Calcium (Ca)	133.5	124–143
Magnesium (Mg)	36.5	35–38
Sodium (Na)	112.0	108–116
Potassium (K)	4.0	4–4
Boron (B)	0.78	0.67–0.89
Carbonate (CO_3)	0	0
Bicarbonate (HCO_3)	714.5	710–719
Sulfate (SO4)	36.0	35–37
Chloride (Cl^-)	48.5	36–61
Nitrate (NO_3^-)	0.35	0.01–0.68
Phosphorus (P)	0.08	0.07–0.08
Iron (Fe)	3.28	3.28–3.28
Alkalinity ($CaCO_3$)	585.5	582–589
pH	6.82	6.67–6.97
Conductivity	1287.5 umhos/cm	1271–1304
Sodium Absorption Ratio (SAR)	2.2	2.1–2.3
Soil Composition	Weswood Series silt loam[c]	

[a]Mean of two separate samples.
[b]Range of two separate samples.
[c]United States Department of Agriculture Natural Resources Conservation Service.

displaying chlorosis symptoms, 2 = 26–50 %, 3 = 51–75 %, and 4 = 76–100 % (adapted from Pouget and Ottenwaelter, 1978).

3.4. Vine vigour

Vine vigour was assessed in year two at a timing that coincided with post-veraison for many winegrape cultivars (Julian day 176). The length of the main shoot was measured on individual vines for a relative comparison of growth.

4. Statistical Analysis

All parametric data were analysed using JMP Pro 14 software (SAS Institute Inc. Cary, NC), and subjected to one-way ANOVA followed by Tukey's Honest Significant Difference Test (HSD) for means separation. Data pooled by variety type were analysed with Welch's t-test to account for unequal sample size and means separated by Games-Howell. Visual chlorosis data were subjected to Kruskal-Wallis non-parametric analysis.

RESULTS

Across the forty-two grape cultivars studied, budbreak varied by 18 days, with the muscadines averaging a later budbreak (range = 85.5 to 96.5 Julian days) than the interspecific hybrid grapes (range = 80.5 to 85.2 Julian days) (Figure 1). Indeed, in 2018, the muscadines broke bud an average of one week (7.7 days) later than the ten interspecific hybrid grapes. Differences in timing of budbreak were observed across the muscadine cultivars, but not significantly among the six rootstocks.

Vine vigour assessed by shoot length is shown in Figure 2. Shoot lengths ranged from 91 to 276 cm. The six rootstock cultivars ranged in length from

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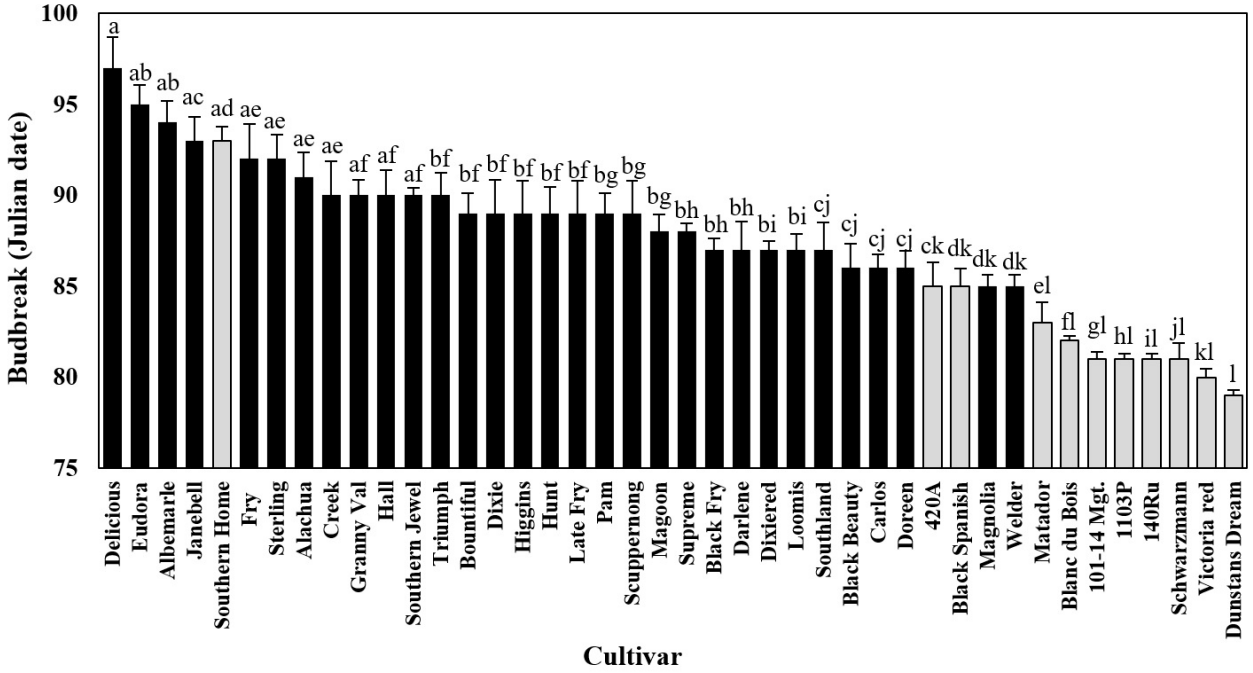


FIGURE 1. Mean budbreak for all cultivars tested in 2018.

Values are mean ± SE. Means indicated by different letters are significantly different at $p \leq 0.001$, Tukey's HSD. Solid bars indicate muscadine grapes (*M. rotundifolia*) and grey bars interspecific hybrids (*Vitis* spp.).

188 to 277 cm and were all significantly longer than the muscadines (range = 91 to 133 cm). The other five fruiting interspecific hybrid grapes were generally less vigorous than the rootstocks but had greater shoot length than the muscadines.

Leaf mineral composition for each studied cultivar in years 2 (2018) and 3 (2019) is presented in Table 4. Differences between cultivars were observed for all nutrients in both years with the exception of Fe in 2019. Due to significant cultivar x year interaction for most nutrients, data were not pooled over years. As a group, the muscadines contained lower N (–22.6 %), P (–27.6 %), K (–39.4 %) and B (–45.8 %) than the rootstock group, and higher Ca (12.9 %), Mg (23.7 %), Na (76.6 %), Fe (51.4 %), Mn (40.0 %) and Cl (42.0 %) in 2018. In 2019, very similar results were obtained, except for iron which did not differ by group. Among the muscadines, the cultivars Eudora, Granny Val, Delicious, Fry, Magoon, Darlene, Higgins, and Late Fry maintained the highest macronutrient status (see supplemental Table 1 for data by cultivar). The highest micronutrient status was most consistently observed in Granny Val, Eudora, Fry, and Welder and the lowest in Albemarle, Black Beauty, Janebell, and Loomis. Sodium and chloride concentrations varied greatly by cultivar and by type of grape. The muscadines consistently accumulated more sodium in leaves than the rootstocks and more chloride in 2018. Sodium ranged from 129.2 (101-14 MGt.) to 810.0 mg/kg (Carlos) in 2018 and 30.6 (Scharzmann, Dunstan's Dream) to 610.9 mg/kg in 2019 (Carlos). Chloride concentration in leaves for all cultivars tested ranged from 238.7 (140Ru) to 1310 g/kg (Dunstan's Dream), and the muscadines displayed a 3.2-fold range across cultivars (366.2, Janebell to 1183.7 mg/kg, Creek). Chlorosis was visible in the first season of the study and canopy chlorosis incidence was recorded the following two years (Figure 3). The muscadine group ranged from 0.87 to 2.2 while the rootstock group ranged from 0.50 to 0.65. The other interspecific hybrids ranged from 0.50 to 2.08. The muscadine group was more chlorotic than the interspecific hybrid grapes, but chlorosis ratings varied across muscadine cultivars by a factor of 2.5.

DISCUSSION

Muscadine grapes are native to the southeastern United States, an area characterized by high rainfall and acidic (pH < 7.0) soils and are characterized as one of the latest grapes to commence growth in the spring (Olmo, 1986). Due to their limited production, little is known about potential alkaline soil tolerance. Among *Vitis* species, Bavaresco *et al.* (1994) suggested two possible mechanisms of response to lime induced chlorosis: an adaptive mechanism characterized by a high rate of growth

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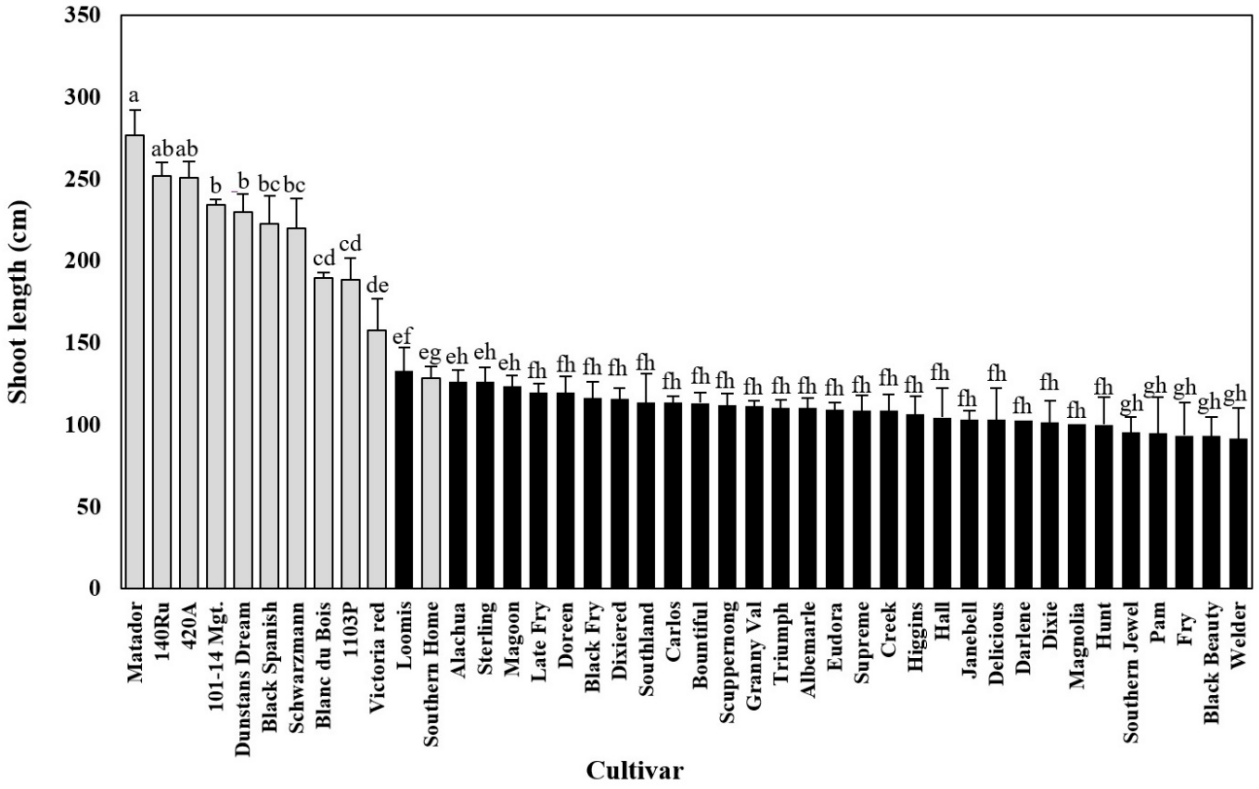


FIGURE 2. Mean shoot length for all cultivars tested in 2018.

Values are mean ± SE. Means indicated by different letters are significantly different at $p \leq 0.001$, Tukey's HSD**.** Solid bars indicate muscadine grapes (*M. rotundifolia*) and grey bars interspecific hybrids (*Vitis* spp.).

and high iron uptake and a protective mechanism with a slow growth rate and low iron uptake.

In our field study, shoot length and visual symptoms of nutrient deficiency symptoms (chlorosis) were recorded to account for the appearance of nutrient deficiency. Tissue testing was conducted in years 2 and 3 to assess leaf nutrient status. Symptoms of deficiencies became apparent by mid-summer in the first growing season. Muscadine varieties were all characterized by reduced shoot growth and more severe chlorotic symptoms. Visual chlorosis ratings showed a strong negative correlation (R^2 = 0.69) with shoot length. Shoot growth measured only in one year of our study did not correlate with a specific mineral nutrient concentration in the leaves. We attribute the stunted growth of the muscadines to their inability to access and use soil nutrients, particularly Fe although it could partly be linked to a later budbreak and development.

The chlorosis symptoms observed were primarily associated with Fe deficiency as interveinal chlorosis beginning in the youngest leaves. Chlorosis ratings in the muscadines were on average greater in 2018 than in 2019. Interestingly, leaf Fe concentrations in 2018 were ten to twenty times the recommended range for muscadine grapes. As a group, the muscadines had also a 2-fold higher concentration of Fe in 2018 than the rootstock group but were much more chlorotic. In 2019, leaf Fe concentration were much lower for all the genotypes and not significantly different between the muscadines and the hybrid group.

It was previously reported that Fe may not be accurately represented by tissue testing on alkaline soils (Mengel and Geurtzen, 1988, Bavaresco *et al.*, 1999). Iron can be present in leaf chlorotic tissue of deficient plants in relatively high levels due to the concentration effect from restricted leaf expansion and shoot growth (Bavaresco *et al*., 1994; Gruber and Kosegarten, 2002), but unavailable for use by the plant as a result of HCO_3^- (Mengel, 1994). Bavaresco *et al*. (1994) observed a wide range in Fe and leaf K/Ca ratios across eleven *Vitis* spp. that were not related to the occurrence of chlorosis. Thus, the authors suggested that it is not suitable to discriminate genotypes by their nutritional status alone.

Most research studies have evaluated relative alkaline soil tolerance of grapevines under

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TABLE 4. Whole leaf nutrition in late summer by type in 2018 and 2019.

	N	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S	B	Cl
2018													
Cultivar	% dry weight					mg/kg dry weight							
Muscadine	1.41b	0.21c	1.17b	1.95b	0.38a	578.4a	34.14	1297.9a	12.56	361.2a	1198.7b	35.45b	580.2b
Rootstock	1.82a	0.29a	1.93a	1.70c	0.29b	193.4b	35.69	631.5c	12.61	217.6b	1983.8a	65.38a	336.7c
Hybrid	1.79a	0.26b	1.78a	2.23a	0.27b	562.9a	38.45	853.5b	12.93	199.7b	1805.0a	60.82a	872.5a
Significance	***	**	***	*	*	***	ns	**	ns	***	***	***	**
2019													
Muscadine	1.94b	0.17b	0.86b	1.93ab	0.21b	299.32a	32.61b	82.97b	6.21c	136.94a	1490.09b	44.05b	-
Rootstock	2.51a	0.23a	1.08ab	1.79b	0.26a	38.35c	33.75b	94.35a	8.50b	107.00b	1927.63a	56.15a	-
Hybrid	2.47a	0.24a	1.13a	2.44a	0.23b	202.19b	37.67a	82.76b	10.63a	144.65a	1937.93a	54.73ab	-
Significance	***	***	***	***	**	***	*	**	***	***	***	***	-
Interactions													
Year	**	***	***	ns	***	***	ns	**	***	***	***	***	-
Cultivar x year	ns	***	**	***	***	***	ns	*	***	***	***	***	-

[a]ns, and *, **, *** indicate not significant and statistically significant at the 0.05, 0.01, 0.001 level of probability, respectively.
[b]Data not available.
[c]Means followed by different letters are significantly different at the 95 % level (Tukey's HSD).

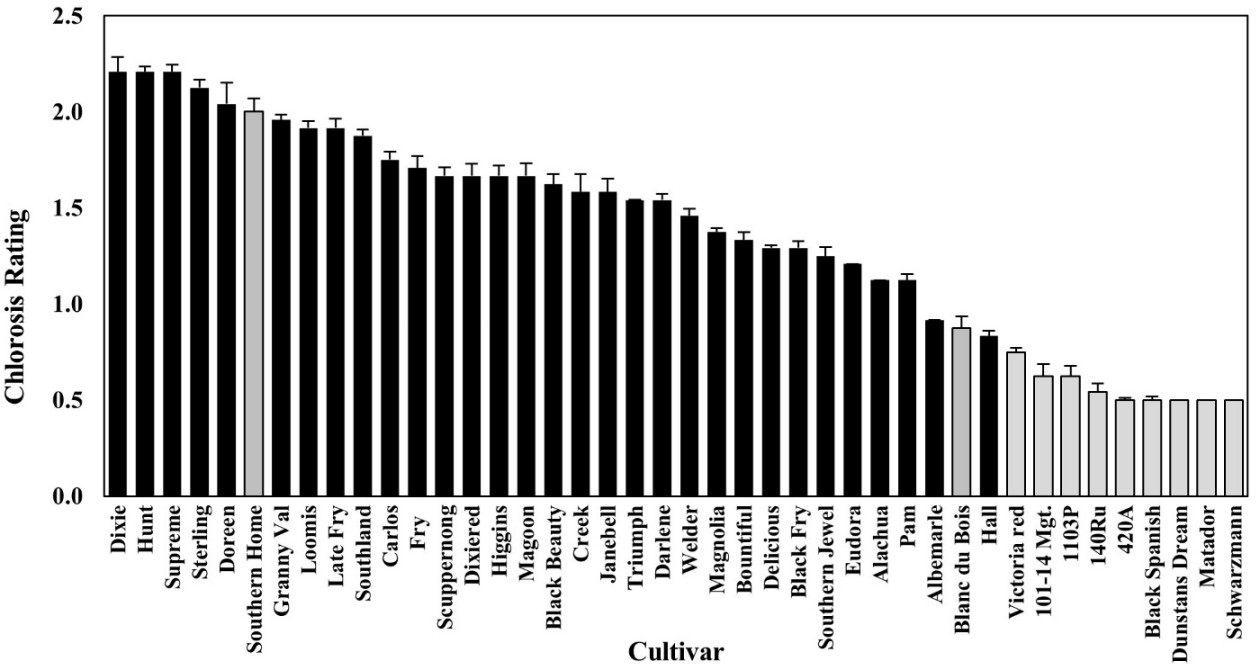


FIGURE 3. Mean canopy chlorosis rating by grape cultivar in 2018 - 2019.
0 = asymptomatic, 1 = 1–25 % of all leaves displaying any amount of chlorosis symptoms, 2 = 26–50 %, 3 = 51–75 % and 4 = 76–100 %. Values are mean ± SE. Solid bars indicate muscadine grapes (*M. rotundifolia*) and grey bars interspecific hybrids (*Vitis* spp.).

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controlled conditions and high soil HCO_3^- (Spiegel-Roy *et al.*, 1971; Mengel *et al.*, 1984; Bavaresco *et al.*, 1994; Bavaresco and Lovisolo, 2000, Bavaresco *et al.*, 2005b), but the present study was carried out under field conditions representative of Texas terroir to evaluate vine response from year to year. Bavaresco *et al.* (1994) were able to separate eleven species of *Vitis* into three distinct groups based on alkaline soil tolerance using a more basic soil medium (pH = 8.4) with higher active lime (19 %) than for the current study. However, the present conditions were sufficient to induce clear differences between the performance of the muscadines and interspecific hybrid grapes.

Although the rootstocks 101-14 MGt. and Schwarzmann are considered to have poor alkaline soil tolerance, relative to 1103P and 140Ru, symptoms of lime-induced chlorosis did not vary greatly among the six rootstocks tested. This may be attributed to the soil at the research site which was only moderately alkaline, and greater differences may have been observed on a site with more active lime.

However, it is also important to consider that ungrafted vines were used in this study. Grafting may have produced different results due to the interaction between scion and rootstock as reported by other authors (Pouget and Ottenwalter, 1973, Bavaresco and Lovisolo, 2000). Pouget and Pily (1975) utilized reciprocal grafting with the lime-induced chlorosis resistant rootstock 41 B as a control to screen new rootstocks for tolerance, however, a similar method could not be used in the current study due to the graft incompatibility of muscadines and *Euvitis* (Goldy, 1992).

In addition to Fe, other macro- and micronutrients varied widely by cultivar type, which may have also contributed to the differences in chlorosis and low vigour observed in the muscadines. Based on recommendations for muscadines (Mills and Jones Jr, 1996), all cultivars were deficient in N in 2018 and Cu in both years. Nitrogen was applied via fertigation at a rate of 16.8 kg ha^{-1} per year, which is consistent with recommended maintenance applications for *V. vinifera* grapes, but possibly not sufficient for muscadines. In contrast, P concentrations were excessive for the 28 muscadine cultivars in 2018. This was not expected due to relatively low soil P. However, Mengel *et al.* (1984) observed significantly higher P in chlorotic leaves of grapevines grown on a calcareous soil versus green leaves of vines grown on non-calcareous soil. It was concluded that the high P observed was not a cause of Fe chlorosis, but rather a result possibly from an increased availability of P due to higher solubility of Ca phosphates or increased H_2PO4^- from the excretion of H^+ from roots. As for Fe, high P concentrations observed in the muscadine leaves may also result from restricted growth. Although soil Zn was well below the target range in our experiment and the availability of Zn is reduced under alkaline conditions (Marschner, 1993), leaf Zn content was within recommended ranges for all cultivars. Tissue Ca also exceeded recommended values in both years and Mg in 2018, but high concentrations of both nutrients were present in the soil. As reported by Bavaresco *et al.* (1994), the different species of grapes used in this study likely have their own distinct nutritional requirements and thus different sufficiency ranges.

The year-to-year variability in vine nutrition, particularly higher Fe, Na and Mn in 2018, may be attributed to low precipitation and a greater dependence on irrigation, or possibly to the variation in vine growth and vigour. Indeed irrigation was applied on a weekly basis over a 4-month period (May–August) in 2018, and only on 3 occasions in 2019. Precipitation over that time frame was 149.9 mm and 385.1 mm, respectively. The irrigation water used in this study had a relatively high level of alkalinity (582 mg L^{-1} $CaCO_3$), Fe (3.28 mg L^{-1}), and bicarbonates (710 mg L^{-1} HCO_3^-) (Table 2). The concentration of HCO_3^- in irrigation water has been used as a means to study alkaline soil tolerance and nutritional responses in grapevines (Mengel and Geurtzen, 1986; Nikolic and Kastori, 2000; Ksouri *et al.*, 2007) and apples (Shahabi *et al.*, 2005). For example, Ksouri *et al.* (2007) observed a decrease in chlorophyll content and tissue Fe in grapevines when irrigated with varying concentrations of bicarbonates (0, 4, 8, 12 and 16 mM). This HCO_3^- buffering effect could explain the greater chlorosis observed in 2018, but not the higher Fe tissue concentrations. While we did not measure the specific chemical forms of Fe present in the irrigation water, it is possible that Fe uptake and subsequent inactivation via HCO_3^- may have occurred in-plant resulting in elevated tissue concentrations.

Salinity was not the focus of this study, but the wide range in tissue Na and Cl observed among the muscadines as well as the interspecific hybrid winegrape cultivars tested was notable. Differential Cl exclusion has been widely observed among Vitis species as well as across different cultivars (McEAlexander and Obbink, 1971; Downton, 1977; Antcliff *et al.*, 1983), but

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no information is available on the salt exclusion capacity of muscadines. On average, the muscadines contained 72.3 % higher tissue Cl than the rootstock group, suggesting a low ability to exclude salt for muscadine. However, a 5.5-fold range was observed across individual muscadine cultivars, indicating some intraspecific variability for this trait. Under field conditions, salt tolerance is defined by a vine's ability to continue to grow and produce fruit under high salinity (May, 1994). Thus, additional work is necessary to characterize the relative salt tolerance of muscadines.

When grown commercially for fruit production, muscadines are commonly spaced at 6.1 m between vines due to their rampant vigour (Olien, 1990). However, the relatively poor growth under the current studied conditions would have prohibited sustainable fruit production. Although supplementing nutrients through fertilizer additions may have improved nutrient status, the most cost-effective, durable solution would be to graft onto a tolerant rootstock. However muscadines are graft incompatible with commercial cultivars of *Euvitis* as a result of a different chromosome number and associated structural differences (muscadines 2n = 40, *Euvitis* 2n = 38) (Goldy, 1992), and commercial muscadine production is restricted by this limitation. *Euvitis* x *M. rotundifolia* rootstocks such as VR 039-16, VR 043-43 and GRN-1 have shown to be graft compatible with *Euvitis,* but their compatibility with *M. rotundifoia* is unknown. The differential response in chlorosis and nutrition status observed among the thirty-one muscadine cultivars evaluated suggests there is potential to select some of them for greater alkaline soil tolerance.

CONCLUSIONS

While the size of the muscadine industry in the U.S. is small in scale, breeding programs are developing improved fruiting cultivars and *M. rotundifolia* x *Euvitis* rootstocks based on increasing interest in the health benefits of muscadines and inherent pest and disease resistance. Our study suggests that muscadine production and rootstock development may be restricted by their relatively poor alkaline tolerance, but differences in performance across cultivars suggest a potential for improvement through selection and breeding. Further experiments using grafted material should be performed.

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