Comparison of Salt Exclusion in Muscadine and Interspecific Hybrid Grapes Using a Greenhouse Screening Procedure

Danny Hillin¹, Pierre Helwi², and Justin J. Scheiner¹

ADDITIONAL INDEX WORDS. chloride, own-rooted, rootstock, sodium, *Vitis rotundifolia*

SUMMARY. Bunch grapes (*Euvitis*) are classified as moderately salt-tolerant. However, little is known about the salt tolerance of muscadine grapes (*Vitis rotundifolia*). The objective of this research was to evaluate the salt exclusion capacity of muscadine grapes relative to common bunch grape rootstocks and hybrid winegrapes using a greenhouse screening assay. In two separate experiments, 31 muscadine, six bunch grape rootstocks, and five hybrid winegrape cultivars were irrigated daily with a 25-mM sodium chloride salt solution for a period of 14 d, followed by a destructive harvest to determine sodium (Na) and chloride (Cl) concentrations in root and shoot tissues. Generally, the muscadines studied exhibited a greater range of salt concentration relative to bunch grape rootstocks. Total tissue (shoot and root) salt varied by 250% and 430% across muscadines and by 180% and 190% across bunch grape rootstocks for Na and Cl, respectively. Despite the wider range, muscadine grapes expressed significantly less leaf necrosis than the bunch grape rootstocks. The most effective salt-excluding muscadines, ‘Janebell’, ‘Scuppernong’, ‘Late Fry’, and ‘Eudora’, were not distinguishable from the bunch grape rootstocks [‘Paulsen 1103’ (1103P), ‘Ruggeri 140’ (140Ru), ‘Schwarzmann’, ‘Millardet et de Grasset 101-14’ (101-14 Mgt.), ‘Millardet et de Grasset 420A’ (420A), and ‘Matador’]. Overall, there was no discernable difference between the salt exclusion capacity of muscadine and bunch grapes. The hybrid winegrape ‘Blanc Du Bois’ displayed poor Na and Cl exclusion properties but showed only moderate leaf necrosis symptoms. In both experiments, ‘Blanc Du Bois’ accumulated more than two-fold higher root and shoot concentrations of Na and Cl compared with the best-performing rootstocks (1103P, 140Ru, 101-14 Mgt.), suggesting that ‘Blanc Du Bois’ could benefit from grafting if salinity is a limiting factor.

High soil salinity is an increasing challenge for global agriculture that affects a wide range of crops (Qadir et al., 2014). Grapes (*Vitis* sp.) are classified as moderately salt-tolerant, but high salinity can cause osmotic stress as well as specific ion toxicity (Downton, 1977a). Osmotic stress can result in decreased stomatal conductance and photosynthesis, leading

to declining yield and overall vine health (Downton, 1977a; Prior et al., 1992; Walker et al., 2008). High cellular concentrations of specific salt ions, salt toxicity, can cause membrane damage, interfere with solute balance, and cause shifts in nutrient concentrations (Volkmar et al., 1998). Prolonged exposure to high internal concentrations of salts leads to leaf necrosis that begins at leaf margins and progresses inward (Walker et al., 2008), ultimately causing defoliation and vine death (Thomas, 2011). Salinity is particularly challenging for arid production regions that rely heavily on irrigation

as well as areas with saline irrigation water sources (Ayars, 2013).

Bunch grapes (*Euvitis*) are more sensitive to Cl toxicity rather than Na (Downton, 1977a; Ehlig, 1960; Hickinbotham, 1933; Sykes, 1987); therefore, Cl exclusion, the ability of the plant to restrict uptake of Cl⁻ from the soil and subsequent transport in the xylem to the shoot, has been widely studied in European winegrapes (*Vitis vinifera*) and bunch grape rootstocks as an indicator of salt tolerance (McE Alexander and Obbink, 1971; Antcliff et al., 1983; Downton, 1977a, 1977b; Sauer, 1968; Sykes, 1985, 1987; Teakle and Tyerman, 2010). European winegrapes generally are considered to be poor Cl excluders compared with rootstocks derived from winter grape (*Vitis berlandieri*) and sand grape (*Vitis rupestris*) (Downton, 1977a; Sauer, 1968). Previous studies have reported tissue Cl concentrations of more than 10-fold across grape species and interspecific hybrids (Bernstein et al., 1969; Downton, 1977a, 1977b). Therefore, salt exclusion is an important criterion for rootstock selection when salinity is perceived to be a limiting factor for production, and developing salt-tolerant rootstocks has been a priority for grape breeders in the United States (Fort and Walker, 2008).

Muscadines (*Vitis rotundifolia*) represent a species of grape native to the southern United States from Texas to Delaware. Unlike bunch grapes, which have 38 somatic chromosomes, muscadines have 40 chromosomes, and graft incompatibility has been reported (Winkler et al., 1974). Commercially, muscadines are grown on their own roots; however, little is known about their salt tolerance. The objective of this study was to compare the salt exclusion capacity of muscadines relative to common bunch grape rootstocks and interspecific winegrapes that are usually grown on their own roots using a rapid greenhouse assay.

Received for publication 12 Apr. 2021. Accepted for publication 1 Sept. 2021.

Published online 1 November 2021.

¹Department of Horticultural Sciences, Texas A&M AgriLife Extension Service, HSBF, 2134 TAMU, College Station, TX 77843

²Texas A&M AgriLife Extension Service, 1102 E. Drew Street, Lubbock, TX 79403

This study represents a portion of the thesis submitted by Danny Hillin for completion of a Master's degree.

J.J.S. is the corresponding author. E-mail: jscheiner@tamu.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.21273/HORTTECH04627-20>

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
1	ppm	mg·L ⁻¹	1
1	ppm	µg·g ⁻¹	1
0.9464	qt	L	1.0567
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Salt tolerance in crop plants is a very active area of research (Munns and Gilliham, 2015; Munns et al., 2020; Zelm et al., 2020), and efforts to develop salt-tolerant grapevine rootstocks are ongoing (Cousins, 2005; Heinitz et al., 2014). Although advances in identifying species of grapes with superior salt exclusion properties (Heinitz et al., 2014) and mechanisms of salt tolerance have been made (Ahmad and Anjum, 2020; Das and Majumder, 2019; Gilliham and Tester, 2005; Henderson et al., 2014; Zelm et al., 2020), viticulture research has focused on bunch grapes, which are considered to be graft-incompatible with muscadines (Winkler et al., 1974). The goal of this study was to determine the relative salt tolerance of muscadines as well as several interspecific hybrid winegrapes that are frequently grown on their own roots by comparing them to rootstocks that have been previously characterized. A muscadine with superior salt tolerance would be better suited to grow at sites with high salinity or could potentially serve as a rootstock for other muscadines. The hybrid winegrapes presented here are most commonly grown on their own roots, although their salt tolerance is undocumented. The naturally high salt concentrations of groundwater from five major underground aquifers used for irrigation in Texas can lead to the buildup of high Na and Cl ion concentrations in the soil profile (George et al., 2011). Salt exclusion was evaluated at both the root (root-soil interface) and shoot levels (compartmentalization in roots) because both are recognized as mechanisms of salt tolerance (Munns and Tester, 2008). Leaf necrosis was measured as a relative indicator of tissue sensitivity to Na and Cl.

Chloride has been the focus of most viticulture research due to its greater toxicity to grapes; however, in many other crops, Na has been identified as more important (Munns and Tester, 2008; Teakle and Tyerman, 2010). Because of the lack of research available, we chose to study both ions because neither has been reported for muscadines.

Materials and methods

PLANT MATERIAL AND EXPERIMENTAL DESIGN. Two greenhouse experiments were conducted using a procedure described by Fort et al. (2013)

to evaluate the relative Na and Cl exclusion capacity of muscadines and interspecific hybrid bunch grapes (Table 1). This procedure involves applying a known molar concentration of both Na and Cl to plant material in a neutral medium and then analyzing relative Na

and Cl concentrations to establish a rank order of exclusion capability. The experiments were conducted during subsequent years from June to August in 2018 and 2019, under greenhouse conditions in College Station, TX. The experimental design was a randomized

Table 1. Cultivar and parentage of grapes used for greenhouse salinity tests 1 and 2 to compare the salt exclusion capacity of muscadine and interspecific hybrid grapes. Parentage based on Clark (1997) and Riaz et al. (2008).

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

block with four replications. Each replication consisted of three potted vines. Plant material was acquired from the U.S. National Plant Germplasm Repository (Davis, CA), and vines were propagated by softwood cuttings under intermittent mist. After sufficient rooting (28 d), the plantlets were removed from mist and placed in square 0.65-qt polypropylene pots containing 100% fritted clay media (Turface MVP; Turface Athletics, Buffalo Grove, IL) with a pH of 6.0. Pots were placed in flats with 4-inch spacing to increase air flow between plants. Vines were watered with reverse osmosis water (Series R18; Watts, North Andover, MA) at 48-h intervals and fertilized every 7 d with a 100-mg·L⁻¹ concentration of 21N–3.0P–5.8K fertilizer (JR Peters, Allentown, PA) for a period of 28 d. To equalize the quantity of leaf tissue across cultivars and replications, and to minimize direct contact between irrigation solution and leaves, vines were vertically staked and lateral shoot growth was removed 7 d before the initiation of a salt treatment. Starting on day 57, a 25-mm sodium chloride (NaCl) irrigation solution was applied daily by hand at a volume of 150 mL per pot for 14 consecutive days. A zero NaCl control was not included in this study because of cost and labor limitations.

Immediately after the completion of the salt treatment, vines were destructively harvested. Plants were rinsed with reverse osmosis water to remove any fritted clay media and surface contamination. Root and shoot materials were then separated at the base and oven-dried at 80 °C for 48 h. Root and shoot dry weight were subsequently determined with a balance (ME 204; Mettler Toledo, Columbus, OH).

SODIUM AND CHLORIDE ANALYSIS. Dried root and shoot tissue samples of each replication were prepared and extracted separately for the Na and Cl analysis according to a rapid quantification method described by Iseki et al. (2017). Root and shoot samples were ground using a blade coffee grinder (Kitchen Aid, Benton Harbor, MI) until completely pulverized. Samples were then passed through a number 10 U.S.A. Standard Testing Sieve (The Murdock Co., Mundelein, IL) to ensure particle size uniformity. The Na and Cl were extracted from a 1.0-g subsample of ground tissue using 50 mL of reverse osmosis water.

Samples were agitated with a vortex mixer (Thermo Fisher Scientific, Waltham, MA) at 2500 rpm for 5 min and then centrifuged at 2900 *g*_n for 5 min for extraction. Then, 20 mL of supernatant was removed and Na and Cl were tested directly using ion-selective electrodes (ROSS; Thermo Fisher Scientific). To ensure accuracy and precision, separate subsamples were collected from 10% of the root and shoot tissue samples and sent to the Texas A&M Soil, Water, and Forage Testing Laboratory (College Station, TX) for comparison. The correlation coefficients of the two methods used to determine Na and Cl in root and shoot materials were 0.99 and 0.99 and 0.98 and 0.99, respectively (data not shown).

LEAF NECROSIS RATINGS. Visual ratings of marginal leaf necrosis were recorded 3 d before destructive harvest. The percentage of marginal leaf necrosis was defined using the following five-tiered index described by Fort et al. (2013): 0 = asymptomatic; 1 = 1% to 25% of all leaves displaying any amount of necrosis symptoms; 2 = 26% to 50%; 3 = 51% to 75%; and 4 = 76% to 100%.

STATISTICAL ANALYSIS. All data were analyzed using statistical software (JMP Pro version 10; SAS Institute, Cary, NC). Na and Cl tissue concentration data were subjected to a one-way analysis of variance (ANOVA), and means were separated using the Tukey's honestly significant difference (HSD) test. Data from each test were not combined for analysis because of significant treatment × test interactions. The Na:Cl ratio data were subjected to the Proc GLM procedure, whereby data from each test were combined for analysis and means were separated using Tukey's HSD test. Leaf necrosis ratings were subjected to a Kruskal-Wallis nonparametric analysis, and rootstock tissue Na and Cl data were subjected to ordinary least squares regression.

Results and discussion

During the first greenhouse assay, a 330% difference in total (root and shoot) Na [119 µg·g⁻¹ for 'Millardet et de Grasset 101-14' (101-14 Mgt.) to 393 µg·g⁻¹ for 'Blanc Du Bois'] and a 470% difference in total plant Cl (196 µg·g⁻¹ for 'Scuppernong' to 919 µg·g⁻¹ for 'Blanc Du Bois') were observed across the studied cultivars (Figs. 1–4). During the second experiment, the total

Na changed by 350% (137 µg·g⁻¹ for 'Scuppernong' to 487 µg·g⁻¹ for 'Blanc Du Bois') and Cl changed by 950% (152 µg·g⁻¹ for 'Janebell' to 1447 µg·g⁻¹ for 'Dunstan's Dream'). Although we were unable to attribute the wider range of total Cl observed during the second experiment to a specific factor or testing conditions, the ranges observed during both experiments were consistent with those of other studies of salt exclusion in grapevines (McE Alexander and Obbink, 1971; Antcliff et al., 1983; Bernstein et al., 1969; Downton, 1977a; Fort et al., 2013) (Figs. 2 and 4). Fort et al. (2013) reported no differences in leaf Cl concentrations among the strong Cl-excluding rootstocks that we evaluated ['Schwarzmann', 101-14 Mgt., 'Paulsen 1103' (1103P), and 'Ruggeri 140' (140Ru)] using the same greenhouse procedure; however, they did observe a 200% to 500% difference between cultivars characterized as strong or weak excluders. More importantly, the rank order observed by Fort et al. (2013) was consistent with those of field studies of mature grapevines under conventional management.

The lowest total tissue Na concentrations were consistently observed in the rootstocks of 101-14 Mgt., 140Ru, and 1103P, and in the muscadines 'Scuppernong', 'Janebell', 'Darlene', 'Granny Val', 'Creek', 'Late Fry', and 'Eudora'. In contrast, the hybrid winegrapes 'Blanc Du Bois', 'Dunstan's Dream', and 'Victoria Red' and muscadines 'Doreen' and 'Hall' consistently accumulated the highest total Na concentrations. Several of these cultivars also showed similar responses to Cl. The rootstock 101-14 Mgt. and muscadines 'Eudora', 'Janebell', 'Scuppernong', 'Late Fry', 'Darlene', and 'Granny Val' displayed the lowest Cl concentrations, whereas the hybrid grapes 'Blanc Du Bois' and 'Dunstan's Dream' consistently accumulated the highest Cl concentrations.

Because exclusion from shoots by compartmentalization in roots is a known mechanism of salt tolerance, the root-to-shoot concentration ratios were determined for both Na and Cl (Table 2). The hybrid winegrape 'Dunstan's Dream' had the highest ratios for Na (2.25) and Cl (3.70), whereas the those of other grapes ranged between 0.67 ('Southern Home') and 1.61 ('Black Spanish') and between 0.92 (101-14 Mgt.) and

Fig. 1. Sodium concentrations in grape cultivar roots (shaded region) and shoots (unshaded region) during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. Top error bars indicate shoot values. Means indicated by different letters are significantly different at $P \leq 0.001$ via Tukey's honestly significant difference test; $1 \mu\text{g} \cdot \text{g}^{-1} = 1 \text{ ppm}$.

2.09 ('Scuppernon'), respectively. Although 'Dunstan's Dream' appeared to display a greater degree of compartmentalization in the roots as compared with the other cultivars, it had one of the highest shoot concentrations of both ions, suggesting poor exclusion

at the root level. 'Dunstan's Dream' is an interspecific hybrid with muscadine parentage; however, the graft compatibility between bunch grape $\times$ muscadine hybrids in this study ('Dunstan's Dream' and 'Southern Home') and muscadines is unknown.

The mean shoot concentration of Na in the six grape rootstocks studied was highly correlated [$R^2 = 0.98$ and 0.88 , respectively (Supplemental Figs. 1 and 2)] with the root concentration observed during both tests, suggesting that exclusion at the shoot level did not

Fig. 2. Chloride concentrations in grape cultivar roots (dark region) and shoots (light region) during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. Top error bars indicate shoot values. Means indicated by different letters are significantly different at $P \leq 0.001$ via Tukey's honestly significant difference test; $1 \mu\text{g} \cdot \text{g}^{-1} = 1 \text{ ppm}$.

Fig. 3. Sodium concentrations in grape cultivar roots (shaded region) and shoots (unshaded region) during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. Top error bars indicate shoot values. Means indicated by different letters are significantly different at $P \leq 0.001$ via Tukey's honestly significant difference test; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

occur. However, root age and root type could be a factor that we were unable to consider. Softwood cuttings were used during this study based on the work conducted by Fort et al. (2013), who determined that grape softwood cuttings provided the most reliable data and results consistent with published

field studies compared with hardwood cuttings and dormant bare root grapevines. Although similar, a much weaker relationship [$R^2 = 0.17$ and 0.30 , respectively (Supplemental Figs. 3 and 4)] between root and shoot Na concentrations was observed in the muscadines, and no relationship was observed

in the winegrape group (Supplemental Figs. 5 and 6). In contrast, stronger correlations were observed for root and shoot Cl in the muscadine group [$R^2 = 0.58$ and 0.39 , respectively (Supplemental Figs. 7 and 8)], whereas very weak correlations were observed for the rootstocks [$R^2 = 0.11, 0.06, 0.39$, and

Fig. 4. Chloride concentrations in grape cultivar roots (shaded region) and shoots (unshaded region) during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. Top error bars indicate shoot values. Means indicated by different letters are significantly different at $P \leq 0.001$ via Tukey's honestly significant difference test; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Table 2. Mean ratios of root-to-shoot sodium (Na) and chloride (Cl) concentrations of grape cultivars during greenhouse tests 1 and 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d.

Cultivar	Na	Cl
	(root:shoot ratio)	
Dunstan's Dream	2.25 a ^z	3.70 a
Black Spanish	1.61 ab	1.47 cd
Hall	1.47 bc	1.90 bd
Creek	1.46 bd	1.86 bd
Dixie	1.39 be	1.61 bd
Granny Val	1.30 be	1.58 bd
Welder	1.30 be	1.93 bc
Southern Jewel	1.25 be	1.59 bd
Carlos	1.24 be	1.29 cd
Hunt	1.20 be	1.95 bc
Schwarzmann	1.18 be	1.22 cd
Matador	1.18 be	1.40 cd
Magoon	1.17 be	1.39 cd
Higgins	1.15 be	1.50 cd
Loomis	1.15 be	1.84 bd
Southland	1.12 be	2.00 bc
Blanc du Bois	1.11 be	1.87 cd
Black Fry	1.07 be	1.60 bd
Supreme	1.05 be	2.50 b
Black Beauty	1.05 be	1.59 bd
Eudora	1.04 be	1.68 bd
Darlene	0.98 be	1.41 cd
Triumph	0.95 be	2.08 bc
Doreen	0.95 be	1.53 bd
Fry	0.95 be	1.53 bd
Late Fry	0.94 be	1.21 cd
Scuppernong	0.94 be	2.09 bc
Magnolia	0.93 be	1.35 cd
Millardet et de Grasset 420A	0.92 be	1.30 cd
Paulsen 1103	0.92 be	1.36 cd
Sterling	0.91 be	1.25 cd
Albemarle	0.90 be	1.14 cd
Victoria Red	0.87 be	1.26 cd
Millardet et de Grasset 101-14	0.86 ce	0.92 d
Bountiful	0.85 ce	1.57 bd
Ruggeri 140	0.82 ce	1.95 bc
Dixiered	0.81 ce	1.18 bd
Alachua	0.78 ce	1.11 cd
Janebell	0.74 ce	1.13 cd
Delicious	0.72 de	1.39 cd
Pam	0.70 e	1.23 cd
Southern Home	0.67 e	1.50 cd
Significance	***	***
Cultivar × test	NS	NS

^zMeans followed by different letters are significantly different at the 0.001% level (Tukey's honestly significant difference test).

*** indicates statistically significant at the 0.001 level of probability and NS indicates not statistically significant.

0.11, respectively (Supplemental Figs. 9–12)], suggesting that compartmentalization may have occurred differentially by subgenera. However, as previously discussed, it is unknown how root age and type influence compartmentalization, and it is unknown whether the durations of the salt treatments were sufficient to properly test

this response. Most research of the salt tolerance of grapes has quantified Cl in shoot tissue only, because this most directly correlates with toxicity symptoms (Ehlig, 1960).

Although some of the muscadine cultivars accumulated up to 300% higher shoot Cl concentrations compared with the rootstocks studied

during our experiments, they generally displayed less leaf necrosis (Figs. 5 and 6). When data were pooled into three groups based on type (muscadines, rootstocks, hybrid winegrapes), the muscadine group had less overall leaf necrosis than the rootstocks during both experiments (Table 3). This could be explained as a lower sensitivity to Cl in leaf tissue, which has been reported for salt-tolerant plants (Munns and Gilliam, 2015); however, more work is necessary to confirm this. Although the level of necrosis expressed by each group was consistent across both greenhouse tests, shoot Na and Cl did not correlate with necrosis ratings, possibly because of the low resolution of the 5-point scale used to assess necrosis. However, the results observed by type were consistent with those of previous research (Baneh et al., 2014; Fort et al., 2013) indicating individual differences in Cl toxicity among grape species and other accessions as determined by leaf necrosis. For rootstocks, this characteristic may be of little importance because exclusion from shoots would confer salt tolerance.

As a group, there were few differences in overall salt exclusion among the six rootstocks studied, in agreement with the findings of Fort et al. (2013) for 'Schwarzmann', 101-14 Mgt., 1103P, and 140Ru. When compared with many of the muscadine cultivars, these rootstocks did not display superior exclusion of Na or Cl at the root level or shoot level, although 1103P, 140Ru, and 'Schwarzmann' are regarded as superior salt excluders among bunch grape rootstocks (Fisarakis et al., 2001; Southey, 1992; Walker et al., 2010). Several of the most effective Cl-excluding muscadines ('Scuppernong', 'Janebell', and 'Eudora') contained less (mean = 80.9 $\mu\text{g}\cdot\text{g}^{-1}$) shoot tissue Cl during both experiments compared with the most effective Cl-excluding rootstocks 101-14 Mgt. and 140Ru (mean = 105.5 $\mu\text{g}\cdot\text{g}^{-1}$). In contrast, the muscadine cultivar Sterling contained an average shoot Cl concentration that was approximately three-times (290 $\mu\text{g}\cdot\text{g}^{-1}$) higher during both experiments. This overall wide range of exclusion among muscadine cultivars could be significant because it relates to potential use as rootstocks or for future breeding efforts. It may be noteworthy that the majority of the most effective salt-excluding muscadine cultivars share

Fig. 5. Leaf necrosis ratings of grape cultivars after daily irrigation with a 25-mm sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. 0 = asymptomatic; 1 = 1% to 25% of all leaves displaying any amount of necrosis symptoms; 2 = 26% to 50%; 3b = 51% to 75%; and 4b = 76% to 100%.

common parentage (Scuppernong). ‘Scuppernong’ is a wild selection found in Tyrell County, NC, before 1760 (Dearing, 1938). Although unconfirmed, its coastal origin may have resulted in some degree of salt tolerance.

‘Blanc Du Bois’, the most widely grown white winegrape in Texas (U.S. Department of Agriculture, 2019), maintained higher root and shoot Na

and Cl concentrations than the six rootstocks in our study. This is consistent with the findings of Scheiner et al. (2020), who reported more than 400% higher tissue Na in own-rooted ‘Blanc Du Bois’ compared with ‘Blanc Du Bois’ grafted on 1103P, and of Hillin et al. (2021), who observed a more than 300% difference between leaf Cl of own-rooted ‘Blanc Du Bois’ and

1103P. The average concentration shoot Cl in ‘Blanc Du Bois’ was more than double that of the six rootstocks. This may be significant because ‘Blanc Du Bois’ is most commonly grown on its own roots, and these data suggest that grafting onto a rootstock would be beneficial if Na or Cl is present in the soil or irrigation water at concentrations that would inhibit normal growth.

Fig. 6. Leaf necrosis ratings of grape cultivars after daily irrigation with a 25-mm sodium chloride salt solution for 14 d. Values are mean $\pm$ SE. 0 = asymptomatic; 1 = 1% to 25% of all leaves displaying any amount of necrosis symptoms; 2 = 26% to 50%; 3 = 51% to 75%; and 4 = 76% to 100%.

Table 3. Kruskal-Wallis inclusive and pairwise comparisons of mean necrosis ratings for muscadines, rootstocks, and hybrid winegrapes after daily irrigation with a 25-mm sodium chloride salt solution for 14 d.

	Mean	df	Chi-square	$P > \chi^2$
Test 1				
Kruskal-Wallis	0.99	2	12.32	0.0021***
Muscadines vs. rootstocks	1.02	1	12.60	0.0004***
Muscadines vs. winegrapes	0.89	1	0.62	0.4318
Rootstocks vs winegrapes	1.23	1	3.71	0.0541
Test 2				
Kruskal-Wallis	0.98	2	10.31	0.0058***
Muscadines vs. rootstocks	1.00	1	10.14	0.0015***
Muscadines vs. winegrapes	0.89	1	1.37	0.2421
Rootstocks vs. winegrapes	1.20	1	2.71	0.0996

*** indicates statistically significant at the 0.001 level of probability.

Conclusions

Grapes are characterized as moderately salt-tolerant, and salt-tolerant rootstocks are often recommended for bunch grapes when salinity is a limiting factor. Rootstocks that are characterized as salt-tolerant more effectively exclude salt ions from shoot tissue. This study used an established greenhouse screening procedure to compare the salt exclusion capacity of 31 muscadine grape and five interspecific winegrape cultivars to that of six bunch grape rootstocks. During two experiments, there was no clear difference among the salt-exclusion potential of muscadine and bunch grape rootstocks. However, the wide range in exclusion across muscadines suggests the potential for using specific muscadine cultivars as rootstocks when salinity is a limiting factor. The hybrid white winegrape ‘Blanc Du Bois’ consistently accumulated Na and Cl concentrations more than double those of the bunch grape rootstocks, indicating that it has a relatively poor capacity to exclude salts but only displayed moderate leaf necrosis.

Literature cited

Ahmad, R. and M.A. Anjum. 2020. Physiological and molecular basis of salinity tolerance in fruit crops, p. 445–464. In: A.K. Srivastava and C. Hu (eds.). Fruit crops diagnosis and management of nutrient constraints. Elsevier, Amsterdam, Netherlands. <https://doi.org/10.1016/B978-0-12-818732-6.00032-0>.

Antcliff, A.J., H.P. Newman, and H.C. Barrett. 1983. Variation in chloride accumulation in some American species of grapevine. *Vitis* 22:357–362.

Ayars, J.E. 2013. Adapting irrigated agriculture to drought in the San Joaquin Valley of California, p. 25–39. In: K. Schwabe, J. Albiac, J. Connor, R. Hassan, and L. Meza González (eds.). Drought in arid and semi-arid regions. Springer, Dordrecht, Netherlands. https://doi.org/10.1007/978-94-007-6636-5_2.

Baneh, H.D., A. Hassani, and F.G. Shaieste. 2014. Effects of salinity on leaf mineral composition and salt injury symptoms of some Iranian wild grapevine (*Vitis vinifera* L. ssp. *sylvestris*) genotypes. *OENO One* 48:231–235. <https://doi.org/10.20870/oeno-one.2014.48.4.1692>.

Bernstein, L., C.F. Ehlig, and R.A. Clark. 1969. Effect of grape rootstocks on chloride accumulation in leaves. *J. Amer. Soc. Hort. Sci.* 94:584–590.

Clark, J.R. 1997. Grapes, p. 248–299. In: R.M. Brooks and H.P. Olmo (eds.). Brooks and Olmo register of fruit & nut varieties. 3rd ed. ASHS Press, Alexandria, VA.

Cousins, P. 2005. Evolution, genetics, and breeding: Viticultural applications of the origins of our rootstocks, p. 1–7. In: P. Cousins, and R.K. Striegler (eds.). Grapevine rootstocks: Current use, research and application. U.S. Dept. Agr., Agr. Res. Serv., Plant Genet. Resour. Unit, Osage Beach, MO.

Das, P. and A.L. Majumder. 2019. Transcriptome analysis of grapevine under salinity and identification of key genes responsible for salt tolerance. *Funct. Integr. Genomics* 19:61–73. <https://doi.org/10.1007/s10142-018-0628-6>.

Dearing, C. 1938. Muscadine grapes. U.S. Dept. Agr. Farmers’ Bul. 1785.

Downton, W.J.S. 1977a. Influence of rootstocks on the accumulation of chloride, sodium and potassium in grapevines. *Aust. J. Agr. Res.* 28:879–889. <https://doi.org/10.1071/AR9770879>.

Downton, W.J.S. 1977b. Chloride accumulation in different species of grapevine. *Scientia Hort.* 7:249–253. [https://doi.org/10.1016/0304-4238\(77\)90021-8](https://doi.org/10.1016/0304-4238(77)90021-8).

Ehlig, C.F. 1960. Effects of salinity on four varieties of table grapes grown in sand culture. *Proc. Amer. Soc. Hort. Sci.* 76: 323–331.

Fisarakis, I., K. Chartzoulakis, and D. Stavarakas. 2001. Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agr. Water Manage.* 51:13–27. [https://doi.org/10.1016/S0378-3774\(01\)00115-9](https://doi.org/10.1016/S0378-3774(01)00115-9).

Fort, K.P., K.M. Lowe, W.A. Thomas, and M.A. Walker. 2013. Cultural conditions and propagule type influence relative chloride exclusion in grapevine rootstocks. *Amer. J. Enol. Viticult.* 64:241–250. <https://doi.org/10.5344/ajev.2013.12073>.

Fort, K.P. and M.A. Walker. 2008. Breeding salinity tolerant grape rootstocks. In: *Proc. 2nd Annu. Vitic. Res. Conf., Univ. California, Davis*. p. 11.

George, P.G., R.E. Mace, and R. Petrosian. 2011. Aquifers of Texas. Texas Water Dev. Board Rpt. 380.

Gilliam, M. and M. Tester. 2005. The regulation of anion loading to the maize root xylem. *Plant Physiol.* 137:819–828. <https://doi.org/10.1104/pp.104.054056>.

Heinitz, C.C., K. Fort, and M.A. Walker. 2014. Developing drought and salt resistant grape rootstocks. *Acta Hort.* 1082:305–312. <https://doi.org/10.17660/ActaHortic.2015.1082.42>.

Henderson, S.W., U. Baumann, D.H. Blackmore, A.R. Walker, R.R. Walker, and M. Gilliam. 2014. Shoot chloride exclusion and salt tolerance in grapevine is associated with differential ion transporter expression in roots. *BMC Plant Biol.* 14: 1–18. <https://doi.org/10.1186/s12870-014-0273-8>.

Hickinbotham, A.R. 1933. Soluble salts in non-irrigated vineyards. Dept. Agr. South Aust. Bul. 279.

Hillin, D., P. Helwi, and J. Scheiner. 2021. Tolerance of muscadine grapes (*Muscadinia rotundifolia*) to alkaline soil. *OENO One* 55:227–238. <https://doi.org/10.20870/oeno-one.2021.55.2.3387>.

Iseki, K., R. Marubodee, H. Ehara, and N. Tomooka. 2017. A rapid quantification method for tissue Na⁺ and K⁺ concentrations in salt-tolerant and susceptible accessions in *Vigna vexillata* (L.) A. Rich. *Plant Prod. Sci.* 20:144–148. <https://doi.org/10.1080/1343943X.2016.1251826>.

McE Alexander, D. and J.G. Obbink. 1971. Effect of chloride in solution culture on growth and chloride uptake of Sultana

- and Salt Creek grape vines. *Aust. J. Exp. Agr.* 11:357–361.
- Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59:651–681, <https://doi.org/10.1071/EA9710357>.
- Munns, R. and M. Gilliham. 2015. Salt tolerance of crops – What’s the cost? *New Phytol.* 208:668–673, <https://doi.org/10.1111/nph.13519>.
- Munns, R., D.A. Day, W. Fricke, M. Watt, B. Arsova, B.J. Barkla, J. Bose, C.S. Byrt, Z.H. Chen, K.J. Foster, and M. Gilliham. 2020. Energy costs of salt tolerance in crop plants. *New Phytol.* 225:1072–1090.
- Prior, L.D., A.M. Grieve, and B.R. Cullis. 1992. Sodium chloride and soil texture interactions in irrigated field grown Sultana grapevines I. Yield and fruit quality. *Aust. J. Agr. Res.* 43:1051–1066, <https://doi.org/10.1071/AR9921051>.
- Qadir, M., E. Quillrou, V. Nangia, G. Murtaza, M. Singh, R.J. Thomas, P. Drechsel, and A.D. Noble. 2014. Economics of salt-induced land degradation and restoration. *Nat. Resour. Forum* 38:282–295, <https://doi.org/10.1111/1477-8947.12054>.
- Riaz, S., A.C. Tenschler, B.P. Smith, D.A. Ng, and M.A. Walker. 2008. Use of SSR markers to assess identity, pedigree, and diversity of cultivated muscadine grapes. *J. Amer. Soc. Hort. Sci.* 133:559–568, <https://doi.org/10.21273/JASHS.133.4.559>.
- Scheiner, J.J., A. Labay, and J. Kamas. 2020. Rootstocks improve Blanc Du Bois vine performance and fruit quality on alkaline soil. *Catalyst Discovery Into Practice* 4:63–73, <https://doi.org/10.5344/catalyst.2020.19007>.
- Sauer, M.R. 1968. Effects of vine rootstocks on chloride concentration in Sultana scions. *Vitis* 7:223–226.
- Southey, J.M. 1992. Grapevine rootstock performance under diverse conditions in South Africa, p. 27–51. In: J.A. Wolpert, M.A. Walker, and E. Weber (eds.). *Proc. Rootstock Seminar: A worldwide perspective*. Amer. Soc. Enol. Vitic., Reno, NV.
- Sykes, S.R. 1985. Variation in chloride accumulation by hybrid vines from crosses involving the cultivars Ramsey, Villard Blanc, and Sultana. *Amer. J. Enol. Viticult.* 36:30–37.
- Sykes, S.R. 1987. Variation in chloride accumulation in hybrids and backcrosses of *Vitis berlandieri* and *Vitis vinifera* under glasshouse conditions. *Amer. J. Enol. Viticult.* 38:313–320.
- Teakle, N.L. and S.D. Tyerman. 2010. Mechanisms of Cl⁻ transport contributing to salt tolerance. *Plant Cell Environ.* 33:566–589, <https://doi.org/10.1111/j.1365-3040.2009.02060.x>.
- Thomas, W.A. 2011. Development of a repeatable, low-cost, high-throughput and precise salt tolerance assay for grapevines. Univ. California Press, Davis, CA.
- U.S. Department of Agriculture. 2019. Texas wine grape varieties. U.S. Dept. Agr., Washington, DC.
- Volkmar, K.M., Y. Hu, and H. Steppuhn. 1998. Physiological responses of plants to salinity: A review. *Can. J. Pl. Sci.* 78:19–27, <https://doi.org/10.4141/P97-020>.
- Walker, R.R., D.H. Blackmore, P.R. Clingeleffer, and R.L. Correll. 2008. Rootstock effects of salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana) I. Yield and vigour inter-relationships. *Aust. J. Grape Wine Res.* 8:3–14, <https://doi.org/10.1111/j.1755-0238.2002.tb00206.x>.
- Walker, R.R., D.H. Blackmore, and P.R. Clingeleffer. 2010. Impact of rootstock on yield and ion concentrations in petioles, juice and wine of Shiraz and Chardonnay in different viticultural environments with different irrigation water salinity. *Aust. J. Grape Wine Res.* 16:243–257, <https://doi.org/10.1111/j.1755-0238.2009.00081.x>.
- Winkler, A.J., J.A. Cook, W.M. Kliever, and L.A. Lider. 1974. General viticulture. Univ. California Press, Berkeley, CA. <https://doi.org/10.1097/00010694-197512000-00012>.
- Zelm, E., Y. Zhang, and C. Testerink. 2020. Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* 71:403–433, <https://doi.org/10.1146/annurev-arplant-050718-100005>.

Supplemental Fig. 1. Correlations among sodium concentrations in root and shoot tissues of six rootstock grape cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 2. Correlations among sodium concentrations in root and shoot tissues of six rootstock grape cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 3. Correlations among sodium concentrations in root and shoot tissues of five winegrape cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 4. Correlations among sodium concentrations in root and shoot tissues of five winegrape cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 5. Correlations among sodium concentrations in root and shoot tissues of 31 muscadine cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 6. Correlations among sodium concentrations in root and shoot tissues of 31 muscadine cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 7. Correlations among chloride concentrations in root and shoot tissues of six rootstock grape cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 8. Correlations among chloride concentrations in root and shoot tissue of six rootstock grape cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 9. Correlations among chloride concentrations in root and shoot tissues of five winegrape cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 10. Correlations among chloride concentrations in root and shoot tissues of five winegrape cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 11. Correlations among chloride concentrations in root and shoot tissues of 31 muscadine cultivars during greenhouse test 1 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

Supplemental Fig. 12. Correlations among chloride concentrations in root and shoot tissues of 31 muscadine cultivars during greenhouse test 2 after daily irrigation with a 25-mM sodium chloride salt solution for 14 d; $1 \mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.