SALT EXCLUSION AND ALKALINE SOIL TOLERANCE OF COMMON MUSCADINE AND BUNCH GRAPE ROOTSTOCK CULTIVARS

A Thesis

by

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ABSTRACT

Bunch grapes (*Vitis* spp.) are classified as moderately salt tolerant. However, little is known about the salt tolerance of muscadine (*Vitis rotundifolia*) grapes. The objective of this research was to evaluate the salt exclusion capacity of muscadine grapes relative to common bunch grape rootstocks and own-rooted hybrids. In two separate experiments, 31 muscadines, 6 bunch grape rootstocks, and 5 own-rooted hybrid cultivars were irrigated daily with a 25mM NaCl salt solution for a period of 14 days and destructively harvested to determine Na^+ and Cl^- concentrations in roots and shoots. At harvest, leaf necrosis was rated on a scale of 0 to 4. In greenhouse test one and two, Blanc Du Bois accumulated higher concentrations of both Na^+ and Cl^-, but with lower leaf necrosis ratings than all six rootstock cultivars. Own-rooted hybrid and muscadine cultivars exhibited a greater range of accumulation of Na^+ and Cl^- than the rootstocks, and generally had higher ratings of leaf necrosis. The muscadine cultivar Janebell displayed generally lower concentrations of both Na^+ and Cl^- than most other muscadine cultivars, and overall there was no clear separation between the exclusion capacity of the muscadines and bunch grape rootstocks.

To evaluate the relative alkaline soil tolerance of muscadines, 31 muscadine cultivars, 6 bunch grape rootstock cultivars, and 5 own-rooted hybrids were evaluated under field conditions in an alkaline (pH 8.2) Weswood silt loam soil. At the end of the growing season, tissue samples were collected from each cultivar for nutrient analysis. Significant differences in Na^+ and Cl^- exclusion capabilities between some muscadine and rootstock cultivars were observed, although the salinity of the soil and irrigation water were within recommended ranges for commercial grape production. All six rootstock cultivars exhibited generally higher rates of vigor than the muscadines under field conditions. The hybrid cultivars Blanc Du Bois and Dunstan's Dream accumulated higher concentrations of Cl^- than the rootstocks but did not have high marginal necrosis ratings. This research suggests that Blanc Du Bois may benefit from grafting on sites where salinity is limiting, and that muscadines are not less salt tolerant than bunch grapes. Furthermore, the range in salt exclusion capacity observed in the muscadines under study suggest that grafting may be a viable option for muscadine growers when salinity is thought to pose a risk.

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NOMENCLATURE

CEC	Cation Exchange Capacity
EC	Electrical Conductivity
IBA	Indole-3-butyric acid
ICP	Inductively Coupled Plasma
ISE	Ion Selective Electrode
μg/g	Microgram per Gram = mg/kg
mg/kg	Milligram per Kilogram
mmho/cm	Millimhos per Centimeter
NASS	National Agricultural Statistics Service
NRCS	Natural Resources Conservation Service
RO	Reverse Osmosis
rpm	Revolutions per Minute
SAR	Sodium Adsorption Ratio
TABC	Texas Alcoholic Beverage Commission
TDS	Total Dissolved Salts
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The state of Texas is home to thirteen different native grape species, all with unique attributes that allow them to adapt to the various climates and soil regions that exist within the state (USDA-NRCS, 2017). The vast majority of cultivated grapes used for wine production are *Vitis vinfiera* or bunch grape cultivars. Only a small amount of *Vitis rotundifolia* or muscadine cultivars are produced for local fresh markets and wine production. Over the past decade, the Texas wine industry has expanded significantly despite challenges associated with producing grapes and wine in Texas (TABC, 2018). One major challenge is the buildup of salts in the soil profile in irrigated vineyards. Irrigated vineyards are at a greater risk from salinization than non-irrigated vineyards due to the relatively higher concentration of dissolved salts in the ground water (Keller, 2015). The naturally high sodium ion (Na^+) concentration of ground water from five major underground aquifers used for irrigation in Texas further compounds this issue (George et al., 2011). Another challenge is the amount of alkaline soil present in some parts of the state. These can lower the productivity of grapevines in these soils, which lead to the use of alkaline soil tolerant rootstocks. A major limiting factor for muscadine grape production is the inability to tolerate alkaline soil conditions, which has historically restricted production to the acidic soils of the eastern portion of the state. Expanding grape production in Texas to potential vineyard sites where salinity is a restricting factor requires a greater understanding of salinity tolerance of both muscadine and bunch grapes.

1.2 Salinity

One of the greatest challenges in the agriculture industry throughout the world today is the issue of salinity. Salinity is defined as the concentration of dissolved mineral salts present in the soils, soil solution, and water (Tanji, 2002). A saline soil is one that is characterized by excessive levels of soluble salts in the soil solution with greater than or equal to 4 dS/m ECe, approximately equal to 40mM NaCl (Brown, 2008). In saline soils, the salt NaCl is generally the most soluble and abundant salt present in the soil solution (Munns and Tester, 2008). Soil salinization results from the buildup of dissolved solids in the soil and water profile over time and is estimated to have affected over 76 million ha of irrigated land throughout the world (Oldeman et al., 1991). Nelson and Mareida (2001), estimated that some 12 million ha of irrigated farm land may have already gone out of production due to soil salinization.

Throughout the world, dryland farming areas comprise around 85% of the food production. These crops are generally not affected by soil salinization. Although the amount of salt-affected land (about 900×10^6 ha) is imprecisely known, its extent is sufficient to pose a threat to agriculture since most crop plants, will not grow in high concentrations of salt (Flowers and Yeo, 1995; Munns, 2002). Munns and Tester (2008) have estimated that up to 20% of the world's irrigated farmland is affected by salinity, while others claim that value to be closer to 50% (Tanji, 2002). Nevertheless, increasing production pressure on the world's current irrigated farmland will only increase issues with soil salinity in the future. This reality of agriculture in our world today is the major force behind the steadily increasing research push for developing more salt tolerant crops (Lauchili, 2002).

1.3 Salinity – Grapes

Texas is home to many wild grape species that produce fruit on a yearly basis without any form of irrigation, however this is not representative of commercial grape production. The state has over 2,800 hectares of irrigated grape land supplying grapes to over 490 licensed wineries in 2018 (USDA-NASS, 2012; TABC, 2018). The Texas wine and grape industry is currently valued at over $13.1 billion as of 2017 (Rimerman, 2015), making it the fifth largest wine industry in the United States and the seventh largest grape producing state (Wines and Vines, 2017; USDA-NASS, 2015). Groundwater, the most common source of irrigation in Texas vineyards, is provided by nine major underground aquifers, five of which are classified as having slightly saline total dissolved-solids concentrations (1,000 – 3,000 mg/l) (George et al., 2011). These soil and water salinity factors coupled with the increasing demand of water for cities and municipalities create unique problems for grape growers in Texas (Townsend 2016).

Further compounding this problem is that the methods of soil remediation on saline soils are not often economical nor practical. Grapevines are considered to be a moderately salt tolerant crop (Downton, 1977a), but saline soil remediation with regard to grapevines presents a unique situation. As a woody perennial crop, rotation for soil fallowing is impractical (Adcock et al., 2007). Second, using an additional fallow field adjacent to the vineyard to act as a salt sink requires up to ten times the amount of area planted in order to be successful which is almost as impractical as moving grapevines (Konukcu, 2006). These specific issues have led to the development of rootstock breeding programs with the objective of developing grape rootstocks with high levels of tolerance to salinity (Fort and Walker, 2011).

1.4 Salinity – Physiology

Many physiological responses of grapevines to salinity have been reported, these include: reductions in stomatal conductance and photosynthesis, systemic disturbances that lead to reductions in both growth and vegetative biomass, as well as reductions in yield (Downton, 1977a; Prior et al. 1992; Walker et al., 2002). Reductions in growth in response to salinity are usually attributed to either ion toxicity or low external osmotic potential (Munns and Termaat, 1986). The stresses that are imposed by salinity relate to ion composition and ion concentration within the plants. When dissolved salt ion concentrations in the soil solution increase, water energy gradients decrease, making it more difficult for water and nutrients to move through root membranes and into the plant (Volkmar et al., 1998). The osmotic effects of increasing ionic concentrations within the aqueous transport streams affects all of the internal plant membranes, not just the root membrane. Increased internal concentrations of particular salt ions can cause membrane damage, interfere with solute balance, and cause shifts in nutrient concentrations (Volkmar et al., 1998). In cases of prolonged exposure, salt-stressed grapevine symptoms develop as necrotic areas on leaves; initially at leaf margins and progressing inwards (Walker et al. 2008). As the level of salt stress increases, the rate of leaf necrosis and defoliation will increase to a point where the plant itself cannot maintain new growth, ultimately leading to decreased productivity and eventually plant death (Thomas, 2011).

As glycophytes, grapevines react to high concentrations of salinity in the soil in two ways. First is the uptake and sequestration of NaCl from the soil solution into cell vacuoles followed by osmotic adjustment. Second is by diminishing the NaCl entrance into the cells effectively excluding it from the plant altogether (Harborne 1993). With regard to viticulture, chloride ions (Cl^-) were identified as early as 1933 to be the most problematic ion for grapevines in salt affected soil (Hickinbotham 1933). In contrast with other plants such as citrus, Cl^- are significantly more toxic to leaf tissue than Na^+ in grapes (Storey and Walker, 1999). However, Na^+ are more effectively sequestered in the root tissue of grapevines than Cl^- (Munns and Tester, 2008; Prior et al., 1992). Also, Cl^- transport to the shoots from the roots has been demonstrated to controlled by Cl^- concentration in root tissue (Storey et al., 2003). Cl^- are passively loaded into xylem tissue and circulated almost exclusively throughout the xylem and excluded from the phloem tissue (Gillingham and Tester, 2007). Once Cl^- concentrations in the plant become excessive, the plant will begin to segregate additional Cl^- into the vacuole of leaf cells until critical concentrations are reached (Munns, 2005). Once critical concentrations of Cl^- are reached in the vacuoles of leaves, membrane degradation occurs and Cl^- begins to increase in concentration in the cytoplasm, disrupting multiple cellular functions and enzyme activity (Munns, 2005). This leads to marginal leaf necrosis or leaf burn symptoms in the lower leaves progressing upward causing leaf drop and defoliation prior to vine death.

The first study that established a correlation between high concentrations of Cl^- in leaves and excessive salt uptake symptoms such as marginal leaf necrosis was published by C. F. Ehlig in 1960. Sample leaves with marginal leaf burn symptoms contained significantly higher Cl^- concentrations when compared to Na^+ and had significantly higher concentrations of Cl^- compared to Na^+ at each stage of expression. Further studies have demonstrated relatively low levels of Na^+ in grape leaves exhibiting salt stress symptoms (Downton, 1977a; Downton, 1977b; Sykes 1987). However, variability in Cl^- accumulation in grape leaves by different genotypes has also been demonstrated, as a result of differential exclusion of Cl^- in the root tissue (Tregeagle et al., 2010).

1.5 Salinity – Soils

The four primary cations that compose soluble salts are Na^+, K^+, Ca^{2+}, and Mg^{2+}, along with the major anions consisting of Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-, and in strongly alkaline soil, CO_3^{2-} (Tanji, 1990). Most salts occur naturally in the soil and are also found in irrigation water and fertilizers (Thompson and Walworth, 2006). The relative concentrations of different ions vary between soil types and water sources, but the ions most often associated with the effects of salinity on grapevines are Na^+ and Cl^-. The most common cause of salt stress is a high concentration of Na^+ and Cl^- in the soil solution. Both of which are essential plant nutrients involved in osmotic regulation at the cellular level. These dissolved ions in the soil solution increase the electrical conductivity (EC) of the water fraction and therefore the salinity of irrigation water or water extracts of the soil and are expressed in units of electrical conductivity (Keller, 2010).

Salt-affected soils are a result of a salt accumulation and result in the three types of soil classifications, each with its own management requirements: saline, saline-sodic, and sodic. Saline soils contain salt concentrations that disrupt the growth cycle of most plants, common salts include $NaCl$, $CaCl_2$, gypsum ($CaSO_4$), and $MgSO_4$. Saline soils are classified as having an EC that is 4 mmho/cm^{-1} or greater, and with sodium adsorption ratio (SAR) of 13 or less. The SAR is a useful index to predict the tendency of a solution to produce excess exchangeable Na^+ (Bresler et al., 2012).

Generally alkaline soils in Texas naturally range from a pH of 7.5-8.3. Leaching salts from this soil type will not increase the pH of a saline soil (Provin and Pitt, 2004). Any sodic soil is one that has at least 15% exchangeable Na^+ percentage (ESP), and they can be identified by a lack of plants due to the tough salt crusts that can develop on the soil surface. Sodic soils are also low in soil permeability, they display hard and dry surfaces, and have very dispersed soil particles (Tanji, 1990). Saline-sodic soils are similar to saline soils, only with significantly higher concentrations of Na^+ relative to Ca^{2+} and Mg^{2+} salts. Saline-sodic soils are classified as having an EC that is 4 mmho/cm^{-1} or lower, and the pH is generally less than 8.5. The exchangeable Na^+ percentage is greater than 15% of the cation exchange capacity (CEC). CEC is a measure of a soil's capacity to hold soil cations, specifically: Ca^{2+}, Mg^{2+}, Na^+, K^+, H^+, and Al^{3+}. Water will move through saline-sodic soils much like saline soils, however management techniques for saline-sodic soils are different. Any attempt to simply leach the salt from this soil type like a saline soil will transform a saline-sodic soil to a sodic soil (Provin and Pitt, 2004). Sodic soils are low in soluble salts, but relatively high in exchangeable Na^+. Sodic soils are notoriously bad for plant life due to their high

Na^+ concentration, causing rooting problems for most plant species. They have a relatively high pH range of 8.5 to 12.0, which also allows their high Na^+ levels to disrupt the physical and chemical composition of soil clay particles. This causes the soil surface to have extremely low permeability to air and water, which in turn causes extensive surface crusting and clodding in areas with significant water application.

1.6 Alkalinity – Soils

Soil alkalinity is a soil condition that results from the accumulation of soluble salts in the soil profile. Most alkaline soils are found in dry desert environments, humid regions affected by sea water, and in low lying areas used for agriculture where salts from irrigation ground water and surface water have been concentrated in the soil due to minimal leaching and high evaporation rates (Day and Ludeke, 1993). Soil pH indicates the hydrogen ion activity of a soil solution, and defines ranges of soil acidity, alkalinity, and neutrality in terms of a 14-level logarithmic scale centered on a pH of 7 which is considered neutral. Therefore, a soil solution with a pH 8.0 is ten times more alkaline than that of a soil solution with a pH of 7.0. Alkaline soils are characterized by the presence of the cations: Ca^{2+}, Na^+, Mg^{2+}, and K^+. Along with the accompanying anions: Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-, HPO_4^{2-}, and H_3BO_3. Alkaline soils exhibit the ionic forms of Na^+, K^+, Cl^-, and NO_3^- in increasing amounts in the presence bicarbonate and other complex forms of sulfates and bicarbonates (Merry, 2009).

The macronutrient phosphorus (P) is commonly deficient in alkaline soils because it is tied up in insoluble calcium and magnesium phosphate mineral forms including $Ca_3(PO_4)_2$ and $Mg(HPO_4)_2$ (Brady and Weil, 2002). Potassium (K) absorption may also be limited in soils with high amounts of exchangeable Ca^{2+} and Mg^{2+} (Wolf and Bates, 2008) as well as Na^+. High amounts of exchangeable Na^+ in the soil allow the ion to compete with K^+ in the process of transport across the cell membrane during uptake (Brady and Weil, 2002). Magnesium deficiency is quite common in very acidic (pH $<$4.5) sandy soils, however high Ca^{2+} and/or K^+ levels in very alkaline (pH > 8.5) soils can also curb Mg^{2+} uptake and induce deficiencies due to competition among these cations for root uptake (Delas and Pouget, 1984). The same is true with regard to competition with Na^+ in saline soils (Shaul, 2002). The solubility of iron (Fe) and zinc (Zn) is strongly dependent on soil pH. In alkaline (pH > 7.0) soils, ion availability for uptake is low, particularly in calcareous soils. High pH in alkaline sodic-soils also affects the population of soil bacterial micro flora and their ability to access nutrients found in organic matter (Keller, 2010).

1.7 Grape Rootstocks

Grape rootstocks have been bred over the years to compensate for many different growing conditions as well as insect and nematode problems affecting grapevines. However, the choice of rootstock for a particular location depends on the complex interactions between soil type, depth, physical and chemical properties, pests, diseases, water availability and environmental factors (Sivritepe, 2011). Reynolds and Wardle (2001) outlined seven major criteria for choosing rootstocks in the order of importance. They include phylloxera resistance, nematode resistance, adaptability to high pH soils, adaptability to saline soils, adaptability to low pH soils, adaptability to wet or poorly drained soils and adaptability to drought.

The ability of the grapevine to uptake and transport Cl^- is highly correlated to the characteristics of a particular cultivars root system (Bernstein et al., 1969). The most salt tolerant rootstocks are those that can maintain ion concentrations of Cl^- in either their own foliage or that of the scion (Alexander and Groot-Obbink, 1971; Downton, 1977a; Downton, 1977b). The majority of rootstocks in use today are hybrids of three species: *V. riparia, V. rupestris,* and *V. berlandieri* However, new cultivars are being bred from *V. mustangensis,* a wild grape native to Texas, that is fairly resistant to phylloxera, drought, and downy mildew (Galet, 1998). Rootstock characteristics can often be generally well described in terms of species and hybrids, but each rootstock has its own unique set of horticultural characteristics that allows us to differentiate them (Cousins, 2005).

V. riparia x V. rupestris rootstocks consist of dense, relatively shallow root systems which are reported to be most suitable for planting in loam to clay loam soils (Dry, 2007; Pongrácz, 1983). Common selections include 3309C, 101-14 Millardet et de Grasset (Mgt), and Schwarzmann. These rootstocks tend to root and graft easily and they also provide excellent resistance to phylloxera. Neither the parents, nor these hybrids, are known for tolerance to calcareous soils (Cousins, 2005).

V. berlandieri x V. rupestris rootstocks consist of dense, relatively deep root systems which perform well in all soil types. These hybrids are adapted to deep, well-drained soils, such as hillsides, and are much more tolerant of drought as well as calcareous soil conditions (Dry, 2007;

Pongrácz, 1983). Due to their deep root systems and ability to acclimate to a wide range of soil types, these hybrids require less water than own rooted vine and other hybrid rootstocks (Dry, 2007). Common selections are 110R, 140Ru, and 1103P. Many are noted for their high vigor as well as excellent protection against phylloxera (Cousins, 2005).

V. berlandieri x V. riparia rootstocks consist of shallow root systems that perform well in clay soils and can tolerate calcareous soil but cannot tolerate high levels of salinity. These hybrids do require less water than *V. riparia x V. rupestris* hybrids but are not well suited for dealing with prolonged drought conditions. Their root systems can become extensive over time in deep soils under ample irrigation (Dry, 2007; Pongrácz, 1983). Common selections include SO_4, Teleki 5C, Kober 5BB and 420A Mgt. These rootstocks tend to be of lower to moderate vigor and are adapted to vineyard site, with ample moisture (Cousins, 2005).

New hybrid rootstock cultivars *(V. riparia x V. rupestris) x (V. mustangensis x V. rupestris)* were developed and released in 2011. Matador and Minotaur which are full sibling rootstocks with the same seed parent 101-14 Mgt. and pollen parent 3-1A resulted from the controlled hybridization of selected seedlings. These rootstocks were identified as seedlings due to their complete suppression of root-knot nematode reproduction in their root tissue in greenhouse evaluations. Both are easily rooted and propagated from dormant cuttings. Reliable salinity and soil type tolerance information has been difficult to locate (Cousins, 2011).

1.8 Muscadine grapes

One of the oldest and first grape species to be cultivated in North America is the muscadine grape, *Vitis rotundifolia* Michx. synonym *Muscadinia rotudifolia*, Michx., which is native to the southeastern United States (Andersen et al., 2010). The vines' natural range extends from Delaware to central Florida and back west along the Gulf of Mexico through the southern states into eastern Texas (L.H Bailey Hortorium, 1976; Dearing, 1948; Munson, 1909). Also, extending north along the Mississippi river into Missouri and along the Appalachian Mountains from both the eastern and western ranges (Olien, 1990). These vines grow best on fertile sandy loams and alluvial soils and perform quite poorly on wet heavy soils with little to no drainage. Natural populations are found in shady, well- drained bottom lands along rivers that are not subject to either constant drought or excessive water logging (Hedrick, 1908; Munson, 1909; McEachern and Baker 1997). Wild muscadine vines are functionally dioecious due to incomplete stamen formation in female vines and incomplete pistil formation in male vines (Dearing, 1947; Hedrick, 1908). Female vines growing in the wild can produce fruit in numerous clusters of anywhere from one to 40, but most commonly produce four to 10 thick-skinned berries containing two to six large seeds (Husmann and Dearing, 1916; Young, 1920). The vines are generally late in breaking bud in the spring and also require a longer growing season which normally consists of over 100 days in order to fully mature the fruit (Hedrick, 1908).

Members of *V. rotundifolia* have 40 somatic chromosomes ($2n = 2x = 40$), along with fruit that is borne in many clusters with four to 10 berries per cluster depending on cultivar. Other characteristics include: the formation of an abscission zone between fruit and rachis and smooth, thin, tight, non-shedding bark that contains warty shoots on young wood. The bark of *V. rotundifolia* will separate in scales from older wood, vines have a continuous pith throughout the entire length of the vine, along with unbranched tendrils (L.H Bailey Hortorium, 1976; Einset et al., 1975; Munson, 1909, Hedrick, 1908; Williams, 1923). *Euvitis* grapes, in contrast only have 38 somatic chromosomes (2n = 2x = 38), many berries per cluster, no abscission zone between fruit and rachis, striated bark on young wood, and thicker, rough bark that peels in strips on older wood. Bunch grapes also produce branched tendrils but have a pith that is interrupted by diaphragms at each node (Olien, 1990). Muscadine grapes propagated from woody cuttings generally root poorly when compared to *Euvitis* cultivars, and are therefore commercially propagated via layering (Woodruff, 1936) and softwood cuttings utilizing a mist system (Goode and Lane, 1983). Muscadine grapes have a high degree of tolerance to pests and diseases that commonly plague grapes in North America. It is this degree of tolerance that would make muscadine grapes a natural choice as a rootstock for *Euvitis*, however the two genera are graft-incompatible (Winkler et al., 1974). This graft incompatibility has not diminished the desire or efforts of breeders to genetically incorporate pest and disease tolerances from muscadine grapes into bunch grape cultivars and rootstocks. Simultaneously, breeders seek to incorporate *Euvitis* traits into muscadine cultivars to increase juice yield per tonne of fruit, to modify juice chemistry for improved juice and wine stability, and to incorporate seedlessness from *Euvitis* into muscadine-like hybrids. (Carroll, 1985; Goldy et al., 1989; Lane, 1978).

Muscadine grapes are still in the early stages of improvement whether through active breeding programs or wild selections. In contrast, *vinifera* grapes, have been cultivated for close to 6000 years, with passive selection likely occurring long before any attempted breeding (Goldy and Onokpise, 2001). Over 100 years of breeding muscadine grapes have resulted in the release of many improved cultivars (Olien, 1990). One of the early breeding goals was to develop perfect-flowered, self-fertile cultivars, the first of which was released in 1948 (Dearing, 1948). Since then there have been over 100 improved muscadine cultivars released (Mortensen, 2001). Controlled improvements of *V. rotundifolia* have almost exclusively been through intraspecific crosses with its two closest related species *V. munsoniana* and *V. popenoei*. Traits that have been improved include: fruit retention, yield, fruit size, and flower type (Goldy and Onokpise, 2001). The second long-standing goal among both *V. vinfera* and *V. rotundifolia* breeders has been to produce hybrids, which is difficult given the difference in chromosome number, and success has only been achieved when *Euvitis* is used as the female parent (Einset and Pratt, 1975).

Within southern and central Texas, the two most common hybrid wine grape cultivars grown that are tolerant to Pierce's Disease (PD) and can be grown on their own roots are Black Spanish (Lenoir) and Blanc Du Bois. Pierce's Disease is caused by a xylem limited bacterium *Xyllela fastidiosa* that clogs the vascular tissue of susceptible cultivars (Kamas et al., 2010). All muscadine grapes grown in Texas are also grown without using a rootstock. Most grapes grown in Texas are planted on a rootstock, specifically to increase their production potential or mitigate stresses found in their environment. Salinity and alkaline soil tolerance are two specific challenges that have traditionally been overcome by using rootstocks in the Texas wine and grape growing industry. The objectives of this research were to evaluate the salt exclusion and alkaline soil tolerance properties of un-grafted hybrid wine grape cultivars, commercially relevant muscadine grape cultivars, and common bunch grape rootstocks.

Determining the ability of select, un-grafted hybrid wine grapes, muscadine grapes, and bunch grape rootstocks to exclude salts and tolerate alkaline soils will lead to the potential expansion of grape production in Texas. Allowing grape production to expand into areas previously determined to be unsuitable for grape production can be accomplished using selected cultivars with high tolerances to salinity and soil alkalinity. The ability of grapevines to exclude Na^+ and Cl^- will be an essential selection tool in future breeding programs developing vines for growth in Texas and other regions that experience high levels of salinity stress coupled with of soil alkalinity. Increasing muscadine grape production in Texas will enhance the potential for westward expansion, beyond their native range. Cultivars that can handle higher levels of soil pH and salinity will have more success. As grape production increases, the need to mitigate salinity stresses from irrigation water and soil pH will only increase.

CHAPTER II

MATERIALS AND METHODS

2.1 Plant material

All plant material was propagated via softwood cuttings. Herbaceous cuttings of all cultivars were taken from the basal region of grapevine shoots in late June to early July Goode and Lane (1983). All cuttings consisted of one-bud, green shoots with one attached leaf approximately 7.6cm – 10.1cm in length. Cuttings were trimmed and treated with 1000µg/g Indole-3-butyric acid and 500µg/g 1-Napthaleneacetic acid liquid rooting concentrate (IBA; Dip'N Grow, Clackama, Oregon) as a 1000 mg/L IBA concentration. Cuttings were placed into round 1.89L green thin walled polypropylene pots containing 100% coarse, premium grade Perlite (Sungro Horticulture, Agawam, Massachusetts) and placed on a shaded mist bench in the greenhouse under intermittent RO-water misting. Cuttings remained on the mist bench for 28 days in perlite prior to transfer to testing media.

Rooted plantlets for greenhouse tests were then removed from the mist bench and placed into square 0.62L black thin walled polypropylene pots containing 100% fritted clay media (Turface MVP, Turface Athletics, Buffalo Grove, Illinois) with a pH of 6.0. Plantlets remained in fritted clay media for an additional 28 days prior to treatment application to allow for bud break and additional vine growth. Once adequate growth occurred vines were vertically staked to prevent excess contact of leaves with saline irrigation solution.

Rooted plantlets for field test were removed from the mist bench and placed into round 1.89L black polypropylene pots containing 100% commercial potting mix (Metro-Mix 900, Sungro Horticulture, Agawam, Massachusetts). Plantlets remained in the mix for an additional 28 days to allow for bud break and additional vine growth. Once adequate vine growth occurred, vines were vertically staked to encourage vertical growth. Vines were placed under 30% shade cloth for 14 additional days to allow acclimatization, prior to planting into a Weswood silt loam field plot with a pH of 8.0 at the Texas A&M University (TAMU) Research Farm.

2.2 Greenhouse tests

Two rounds of greenhouse testing were conducted to evaluate the Na^+ and Cl^- exclusion capability of six rootstock, five own-rooted hybrid, and 31 muscadine grape cultivars (Table 1). Greenhouse vines were watered with RO-water at 48-hour intervals during growth in fritted clay media prior to saline irrigation solution application. Vines were fertilized every 7 days with a 100mg/L concentration 21-7-7 liquid fertilizer (Peters, J.R. Peters Inc., Allentown, Pennsylvania) by fertilizer injector (Dosatron, Dosatron USA, Clearwater, Florida). To equalize the quantity of leaf tissue and minimize direct irrigation solution contact to leaves, any lateral shoot growth was removed 7 days prior to the first NaCl irrigation application.

A 25mM NaCl irrigation solution was applied by hand across all replications once per day for a period of 14 consecutive days. The irrigation solution was prepared by adding 110.64g NaCl, anhydrous, free-flowing, ACS reagent, ≥ 99% Halite (Redi-Dri™, Sigma-Aldrich, St. Louis,

Table 1 : Parentage, origin, and primary market use of rootstock, own-rooted interspecific hybrid, and muscadine cultivars.

Cultivar	Parentage[z]	Origin[y]	Primary use
101-14 Mgt.	*V. riparia* x *V. rupestris*	France	Rootstock
1103P	*V. rupestris* x *V. berlandieri*	Sicily	Rootstock
140Ru	*V. rupestris* x *V. berlandieri*	Sicily	Rootstock
420A	*V. berlandieri* x *V. riparia*	France	Rootstock
Matador	101-14 Mgt. x 3-1A (*V. mustangensis* x *V. rupestris*	USDA	Rootstock
Schwarzmann	*V. riparia* x *V. rupestris*	Czechoslovakia	Rootstock
Black Spanish	*V. aestivalis* x *V. cinerea* x *V.vinifera*	Southern U.S.	Wine
Blanc du Bois	Fla. D6-148 x Cardinal	UF	Wine
Dunstans Dream	Fla. W1521 x DRX 69-99	Florida	Wine
Southern Home	Summit x Fla. P9-15	UF	Home use
Victoria red	Ark. 1123 x Exotic	UA & TAMU	Fresh market
Alachua	Southland x Fry	UF	Fresh market
Albemarle	Burgaw x Topsail	NCSU	Home use
Black Beauty	Fry x 12-12-1	Ison	Fresh market
Black Fry	Fry x Cowart	Ison	Fresh market
Bountiful	Creek x seedling of Topsail	USDA MS	Fresh market
Carlos	Howard x NC11-173 (Topsail x Tarheel)	NCSU	Wine
Creek	Open pollinated seedling of San Monta	UG	Wine
Darlene	5-11-3 x Carlos	Ison	Fresh market
Delicious	AA10-40 X CD8-81	UF	Fresh market
Dixie	Topsail x NC 28-193 (Lucida x Wallace)	NCSU & USDA	Wine
Dixiered	Seedling 44-6 x S. 44-7	Ison	Fresh market
Doreen	Higgins x Dixie	NCSU & USDA	Wine
Eudora	Fry x Southland	UF & USDA	Fresh market
Fry	Ga. 19-13 x USDA 19-11	UG	Fresh market
Granny Val	Fry x Carlos	Ison	Fresh market
Hall	Fry x Tara	UG	Fresh market
Higgins	Yuga x a white male pollinator	UG	Fresh market
Hunt	Flowers x a white male muscadine	UG	Juice & Jelly
Janebell	Fry x Senoia	Ison	Fresh market
Late Fry	Fry x Granny Val	Ison	Fresh market
Loomis	Creek x US 15	USDA Mississippi	Fresh market
Magnolia	[(Hope x Thomas) x Scuppernong)] x (Topsail x Tarheel)	NCSU & USDA	Home use
Magoon	Thomas x Burgaw	USDA Mississippi	Fresh market
Pam	5-11-3 x Senoia	Ison	Fresh market
Scuppernong	*V. rotundifolia*	Roanoke Island, NC	Juice & Jelly
Southern Jewel	Granny Val x DB-63	UF	Fresh market
Southland	Thomas x seedling of Topsail	USDA Mississippi	Home use
Sterling	NC 50-55 x Magnolia	NCSU & USDA	Juice & Jelly
Supreme	Black Fry x Dixieland	Ison	Fresh market
Triumph	Fry x GA29-49	UG & UF	Fresh market
Welder	Dearing x unknown	Florida	Wine

[z] Information obtained from Clark, 1997; and Riaz et al., 2008.

[y] Ison = Isons Nursery and Vineyard, Brooks, Ga; NCSU = North Carolina State University, Raleigh; UG = University of Georgia, Griffin; UF = University of Florida, Leesburg; MS = Mississippi State University, Poplarville; USDA = United States Department of Agriculture.

Missouri) to 75.7 liters of RO-water. Concentration of 25mM NaCl solution was measured with a handheld total dissolved solids meter (Pocket Size Tester, HM Digital Inc., Redondo Beach, California).

Experimental design in the greenhouse study consisted of a randomized block design. Each experiment contained four replications and each replication contained three vines representing each cultivar tested. Vines were placed in flats on 10 cm spacing to allow for application of NaCl irrigation application and to increase air flow between vines.

After saline irrigation solution treatment, grapevines were destructively harvested. Roots were rinsed with RO-water to remove any excess fritted clay media remaining. Root and shoot materials were separated at the base, then oven dried at 80° C for 48 hours (Jones, 2001). Root and shoot samples were ground using a blade coffee grinder (Kitchen Aid, Benton Harbor, Michigan) until completely pulverized and then passed through a No. 10 U.S.A. Standard Testing Sieve (The Murdock Co., Mundelein, Illinois) to ensure sample particle size uniformity across all root and shoot samples.

2.3 Field Test

A one-acre field plot was planted at the TAMU research farm to evaluate vine vigor, mineral uptake, and the Na^{+} and Cl^{-} exclusion capability of six rootstock, five own-rooted hybrid, and 31 muscadine grape cultivars grown under alkaline soil conditions. Vines were drip irrigated at 7-day intervals with local groundwater containing 1000+ ppm total dissolved salts (TDS).

Field plot experiment consisted of a randomized block design, containing four replications of three vines per cultivar in each. Vines were planted at 3.04m between row and at 0.6m in row spacing to allow for ease of access, observation, positioning and sample collection.

Leaf and petiole samples were taken after the typical harvest period in late July early August, specifically Julian date 260, prior to senescence at complete cane maturation for this region of Texas. All samples were rinsed with RO-water by hand for 30 seconds prior to testing by the Texas A&M AgriLife Extension Soil, Water and Forage Testing Lab (College Station, Texas) for mineral nutrient concentrations. Recommended muscadine nutrient leaf concentrations reported by Mills and Bryson (2015) have been reprinted with permission and are listed in Table 2.

Table 2 : Diagnostic levels based on whole-leaf nutrient concentrations for muscadine grapevines sampled in mid- or later summer (Mills and Bryson, 2015)

Element	Units	Deficient	Sufficient	Excessive
N	%	1.65	1.65 - 2.15	> 2.15
P	%	0.12	0.12 - 0.18	> 0.18
K	%	0.80	0.8 - 1.20	> 1.20
Ca	%	0.70	0.7 - 1.10	> 1.10
Mg	%	0.15	0.15 - 0.25	> 0.25
B	ppm	< 15	15 - 25	> 25
Cu	ppm	< 15	15 - 25	> 25
Fe	ppm	< 60	60 - 120	> 120
Mn	ppm	< 60	60 - 150	> 150
Mo	ppm	< 0.14	0.15 - 0.35	> 0.35
Zn	ppm	< 18	18 - 35	> 35

[z] Critical level is the point which no additonal recommended.

2.4 Sodium and chloride analysis

Root and shoot tissue samples were prepared and extracted separately, following a rapid quantification method developed by Iseki et al., (2017). Sample Na^+ and Cl^- were extracted from 1.0g of tissue using RO-water, samples were then agitated with a vortex mixer (Thermo Fisher Scientific, Beverly, MA) at 2500rpm for five minutes, then centrifuged at 4000rpm for five minutes for extraction. Then 20mL of supernatant was extracted and tested directly via ion probe. Ion concentrations in root and shoot material were determined using a ROSS sodium ion selective electrode (Thermo Fisher Scientific, Beverly, MA) and a ROSS chloride ion selective electrode (Thermo Fisher Scientific, Beverly, MA). To ensure accuracy and precision, ten percent of root and shoot samples were sent to the Texas A&M Soil, Water and Forage Testing Lab for Na^+ and Cl^- analysis via ICP analysis of a nitric acid digest for Na^+, and a RO-water extraction for Cl^- to compare to results obtained from ion selective probe analysis.

2.5 Quantification of leaf necrosis

Visual ratings of marginal leaf necrosis were recorded on irrigation day 12, two days prior to conclusion and destructive harvest of vines in the green house tests. Visual ratings provided a basis of the degree of cultivar sensitivity to excess salt uptake. The total percentage of leaves on the vine displaying marginal leaf burn or salt burn was recorded.

Field plot visual leaf necrosis was recorded two days prior to leaf and petiole sampling of grapevines. A second round of visual leaf ratings were recorded the following year during E-L stage 23; Full Bloom 50% caps off period to monitor any salt damage progression.

The percentage of marginal leaf necrosis was defined using a five-tiered index, as follows: 0 = asymptomatic, 1=1-25% of all leaves displaying any amount of necrosis symptoms, 2=26-50%, 3=51-75%, and 4=76-100 (Fort et al., 2013).

2.6 Bud break

Budbreak data was recorded over a one-month period at the beginning of the second year between Julian day 70 to Julian day 100. To evaluate differences in transitioning from dormancy to shoot development across all cultivars. Budbreak was identified using the E-L number scale developed by (Lorenz et al., 1995), which consists of 47 growth stages that describe grapevine phenological growth across shoot and inflorescence development, flowering, berry development, ripening, and senescence. Specifically, E-L number 4, Green tip: first leaf tissue visible or budburst was the stage used to identify bud break. All vines were visually assessed once daily during budswell stage and woolly bud stage to budbreak.

2.7 Vigor

Vine vigor or shoot growth over time was evaluated during the post bloom period on Julian day 170 by measuring shoot length in centimeters. During the dormancy period preceding the second year of growth, all vines were trimmed back to two buds per vine prior to budbreak to ensure uniformity across all replications. Shoot length measurements consisted of measuring only the longest of the two shoots.

2.8 Statistical analysis

All data collected was analyzed using JMP Pro 10 software (SAS Institute Inc. Cary, NC), and subjected to one-way ANOVA, any significant results were subjected to Tukey's Honest Significant Difference Test (HSD) for mean comparison. All visual marginal leaf necrosis ratings recorded across both greenhouse and field tests were subjected to Kruskal-Wallace non-parametric analysis to determine if there was any significant difference in marginal leaf necrosis symptoms.

CHAPTER III

RESULTS

3.1 Greenhouse Test 1

Dry root material for all cultivars tested ranged in mass from 1.24g - 6.00g (Figure 1). The rootstock cultivar140Ru had the least amount of root dry mass of 1.24g, while the muscadine cultivar Eudora had the greatest root dry mass of 6.00g. All six rootstock cultivars ranged from a dry mass of 1.24g – 2.23g, and none were significantly different from any other. Significant differences in the dry weight of root material were observed across the own-rooted hybrid, and muscadine grape cultivars under study. The five own-rooted hybrid cultivar dry root mass ranged from 1.24g – 5.11g, Southern Home was significantly greater in mass than all other hybrid cultivars except Black Spanish, which were not statistically different from one another. Muscadine dry roots mass ranged from 2.21g – 6.00g, only two cultivars, Eudora and Carlos were significantly higher in mass than 17 other muscadine cultivars, the remaining 12 muscadine cultivars were not statistically greater in dry root mass.

Dry shoot material for all cultivars tested ranged in mass from 1.74g – 9.42g (Figure 2). The rootstock cultivar 1103P had the least amount of shoot dry mass of 1.74g, and the own-rooted hybrid cultivar Southern Home had the greatest shoot dry mass of 9.42g. All six rootstock cultivars ranged from a dry mass of 1.72g – 2.49g, with no significant differences between them. Significant differences in the dry mass of shoot material were observed across the own-rooted hybrid and muscadine cultivars under study. The five own-rooted hybrid cultivar dry shoot mass

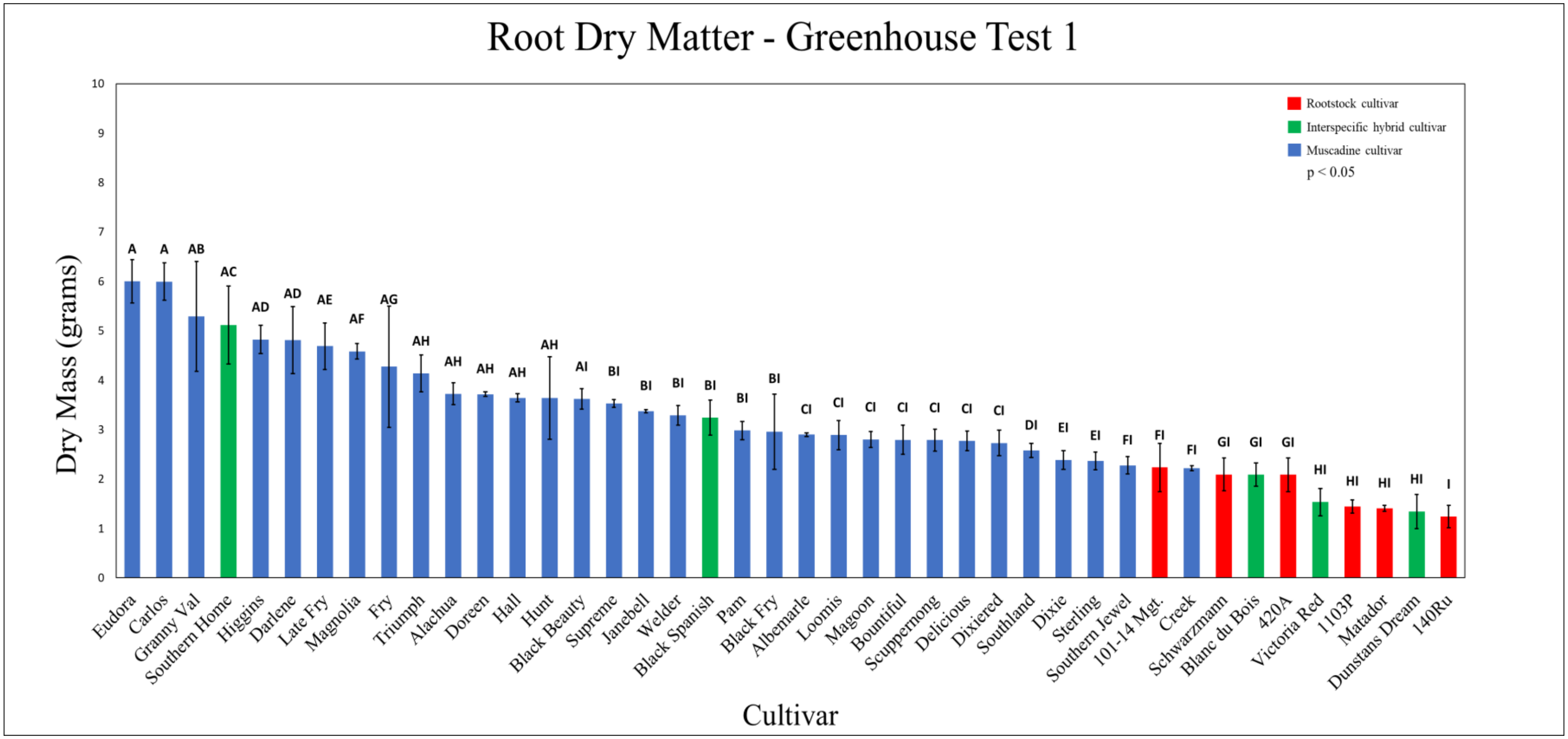

Figure 1: Dry mass of root plant material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

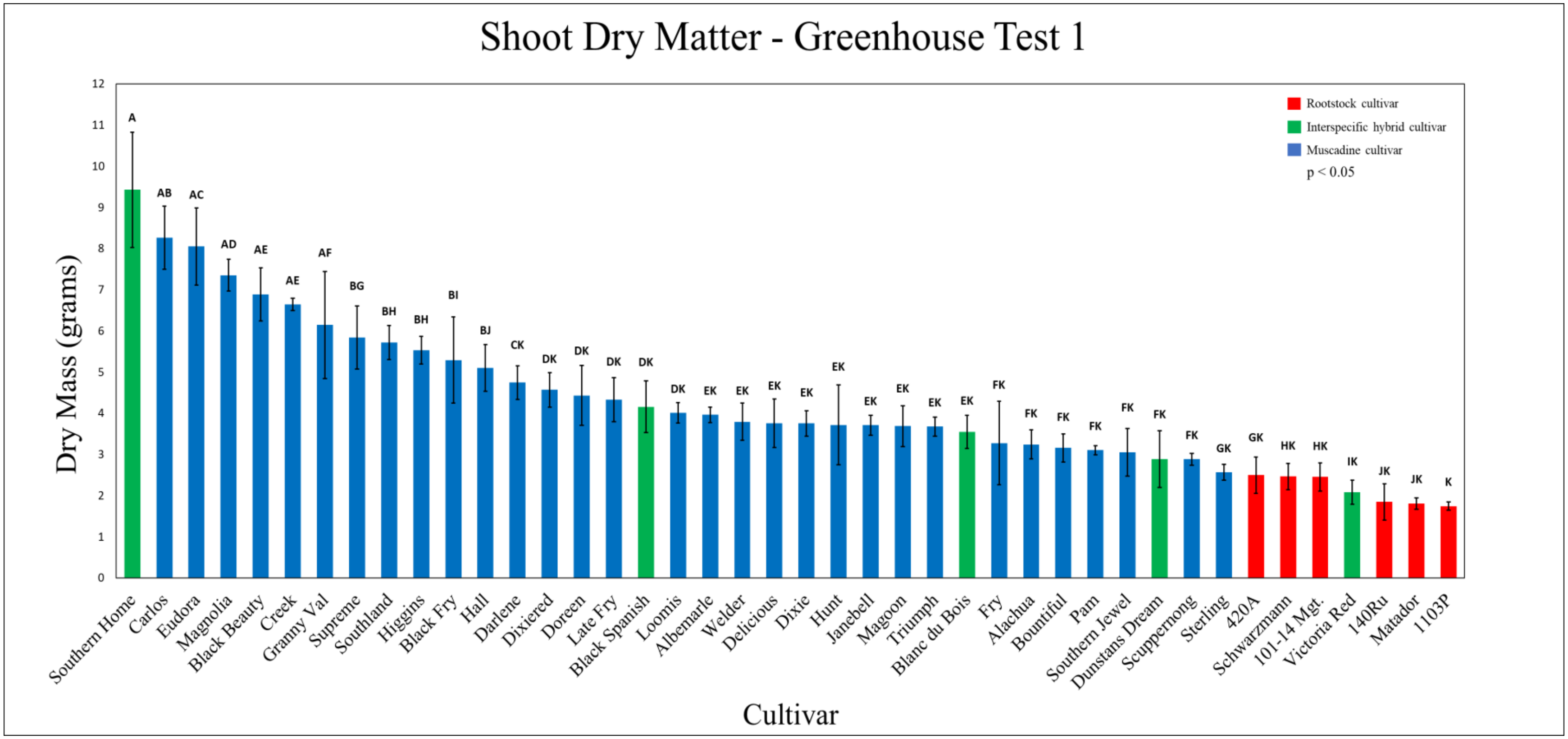

Figure 2: Dry mass of shoot plant material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

ranged from 2.07g – 9.42g, Southern Home was significantly greater in mass than the other four cultivars. Significant differences were observed across the 31 muscadine cultivars. Shoot dry mass ranged from 2.56g - 8.26g, Carlos and Eudora were significantly greater in shoot mass than 19 other muscadine cultivars.

Root Na^+ concentration for all cultivars tested ranged from 50µg/g - 189µg/g, a greater than three-fold difference in root Na^+ concentration (Figure 3). The rootstock cultivar 101-14 Mgt. displayed the least concentration of root Na^+ at 50µg/g but was not statistically different than 23 other cultivars. The own rooted hybrid cultivar Blanc Du Bois exhibited the greatest root Na^+ concentration of 189µg/g but was not statistically different than six other cultivars. Significant differences in root Na^+ concentration were observed across the rootstock, own-rooted hybrid, and muscadine cultivars. The six rootstock cultivars ranged from 50µg/g- 125µg/g, Schwarzmann was significantly higher in root Na^+ concentration than 140Ru and 101-14 Mgt. The five own-rooted hybrid cultivar root Na^+ concentrations ranged from 83µg/g - 126 µg/g. Two hybrid cultivars, Blanc Du Bois and Dunstan's Dream were statistically higher in Na^+ concentration than Southern Home, but not Victoria Red or Black Spanish. Muscadine cultivar root Na^+ concentrations ranged from 58µg/g - 189µg/g across all 31 cultivars tested. The cultivar Hall displayed a higher concentration of root Na^+ than 27 muscadine cultivars.

Root Cl^- concentrations for all cultivars tested ranged from 83µg/g - 611µg/g demonstrating a greater than seven-fold increase in root Cl^- concentration (Figure 4). The rootstock cultivar 101-14 Mgt. displayed the least amount of root Cl^- concentration of 83µg/g but was not statistically different than 23 other cultivars. Dunstan's Dream and Blanc Du Bois presented with the greatest root Cl^- concentration of 611µg/g and 587µg/g respectively. Significant differences in

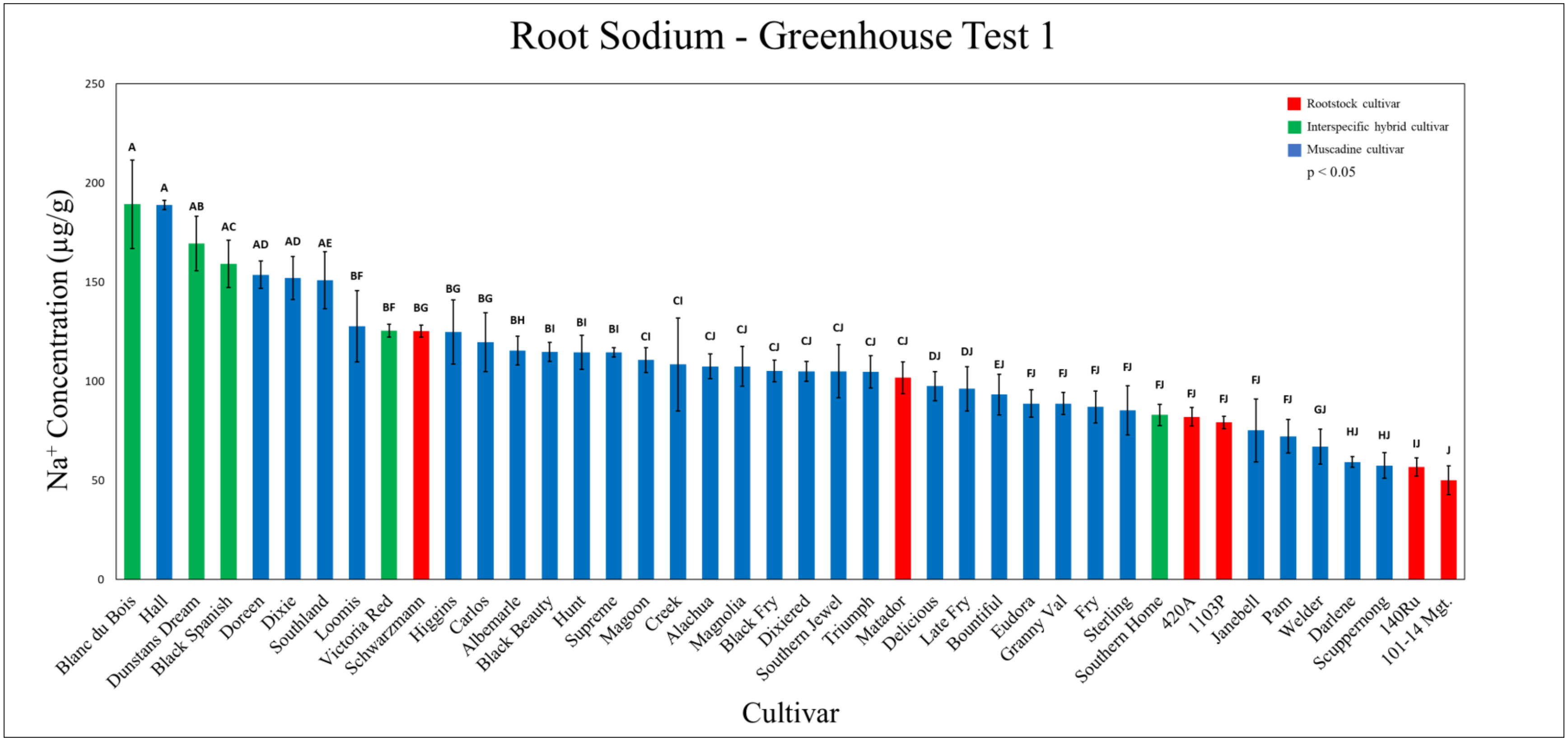

Figure 3: Sodium ion concentration of root material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

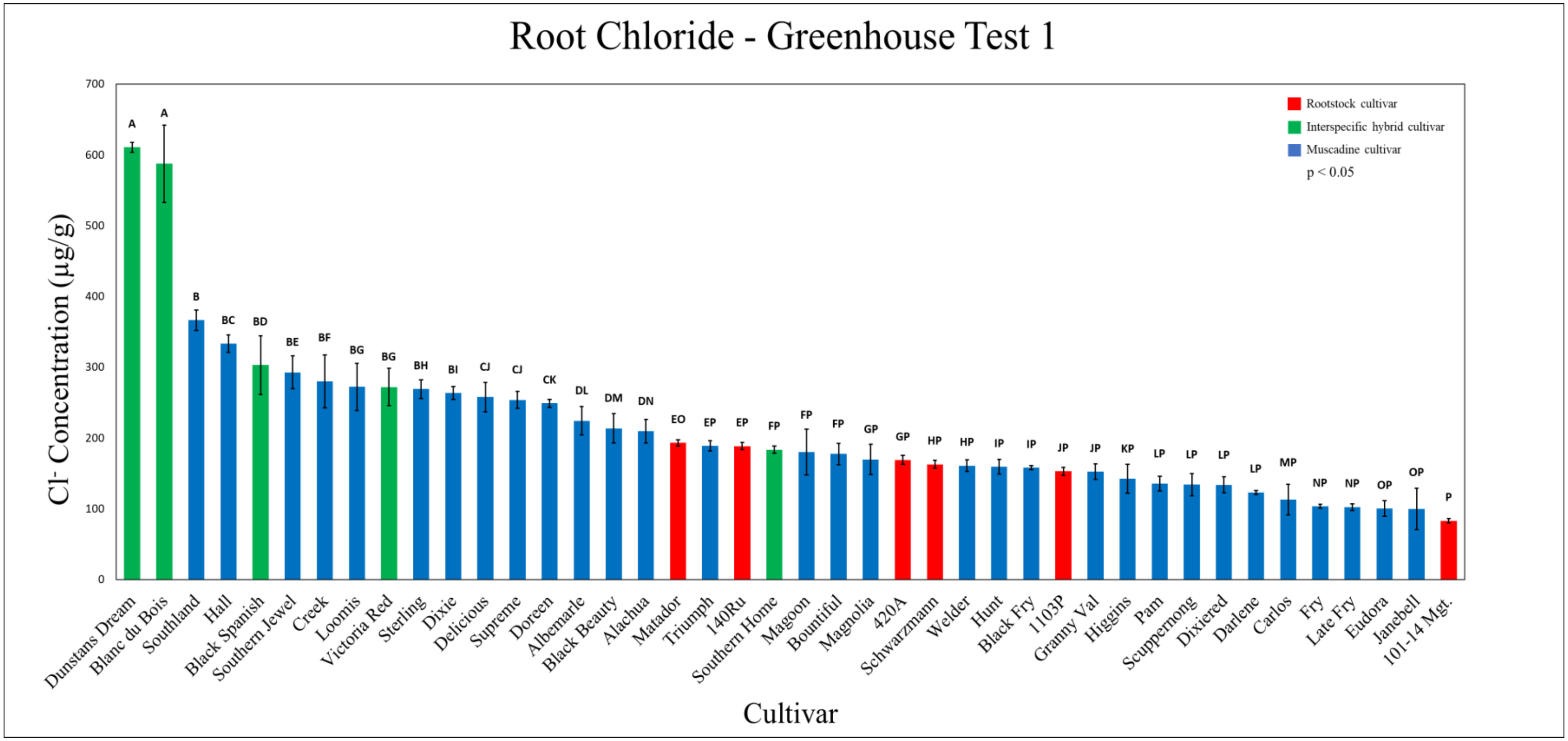

Figure 4: Chloride ion concentration of root material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

root Cl^- concentration were observed across the rootstock and own-rooted hybrid, and muscadine cultivars. Rootstock cultivars ranged from a Cl^- concentration of 83µg/g -193µg/g, 101-14 Mgt was significantly lower in Cl^- concentration than only Matador which was the greatest in concentration. The own-rooted hybrid cultivars root Cl^- concentrations ranged from 183µg/g - 611µg/g, Dunstan's Dream and Blanc Du Bois had significantly higher Cl^- concentrations than all other cultivars tested. The 31 muscadine cultivars root Cl^- concentrations ranged from 100µg/g - 366µg/g, the cultivars Southland and Hall were significantly higher in Cl^- concentration than 20 other muscadine cultivars.

Shoot Na^+ concentrations for all cultivars tested ranged from 69µg/g - 204µg/g (Figure 5). The rootstock cultivar 101-14 Mgt displayed the least amount of shoot Na^+ concentration of 69µg/g, though not statistically different than 29 other cultivars. The own-rooted hybrid cultivar Blanc Du Bois had the greatest shoot Na^+ concentration of 204µg/g but was also not statistically different than three other cultivars. Significant differences in shoot Na^+ concentration were observed across the own-rooted hybrid, and muscadine cultivars. Rootstock shoot Na^+ concentrations ranged from 69µg/g - 126µg/g, with no significant difference between any cultivars. The own-rooted hybrid cultivars Na^+ levels ranged from 98µg/g - 204µg/g. Blanc Du Bois was significantly higher in Na^+ concentration than Southern Home, Dunstan's Dream, and Black Spanish. All 31 muscadine cultivars ranged from 69µg/g - 180µg/g, Doreen was significantly higher in shoot Na^+ concentration than 20 other muscadine cultivars.

Shoot Cl^- concentration for all cultivars tested ranged from 62µg/g - 330µg/g, exhibiting a five-fold difference in shoot Cl^- concentrations across all cultivars (Figure 6). The muscadine variety Scuppernong displayed the least shoot Cl^- concentration of 62µg/g, though not significantly

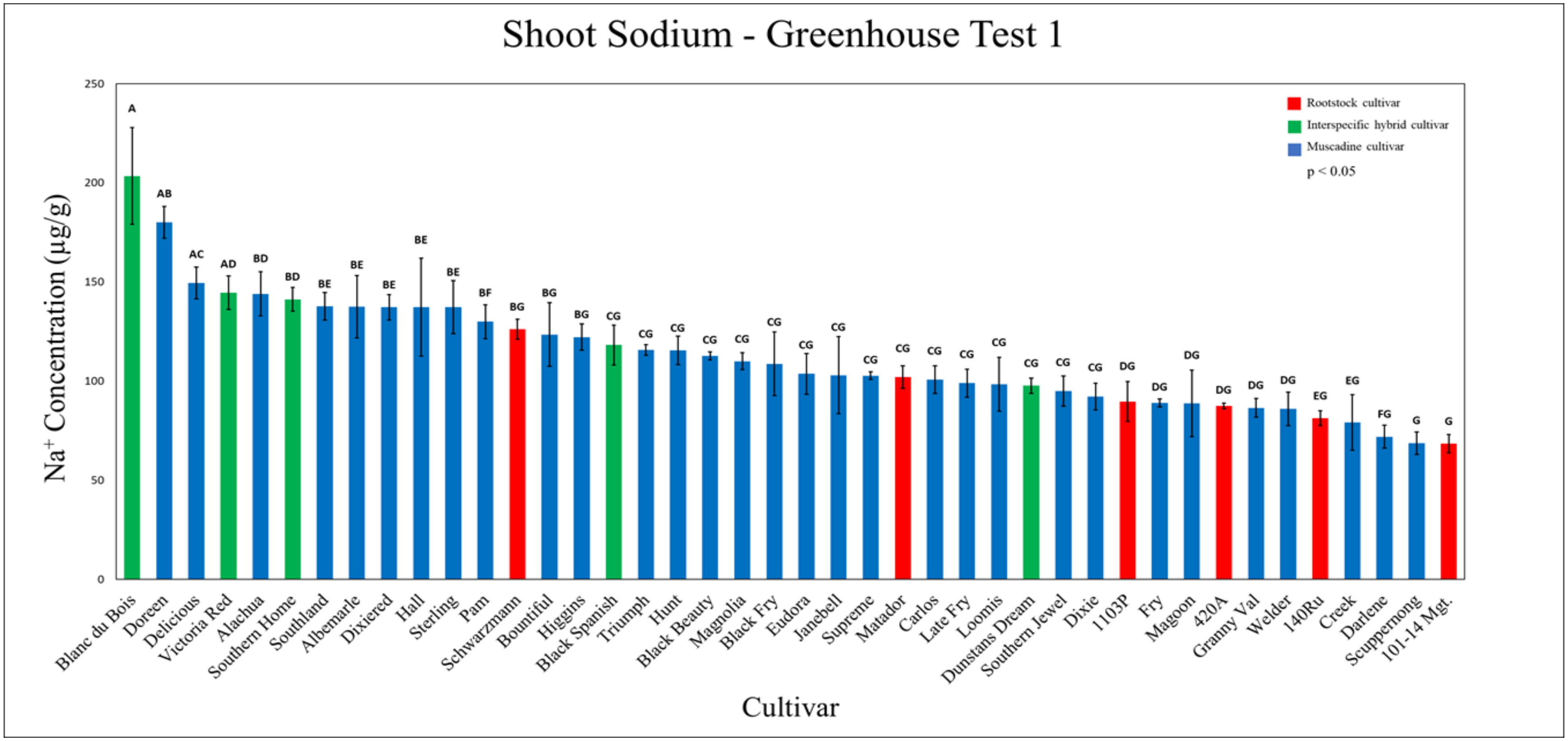

Figure 5: Sodium ion concentration of shoot material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

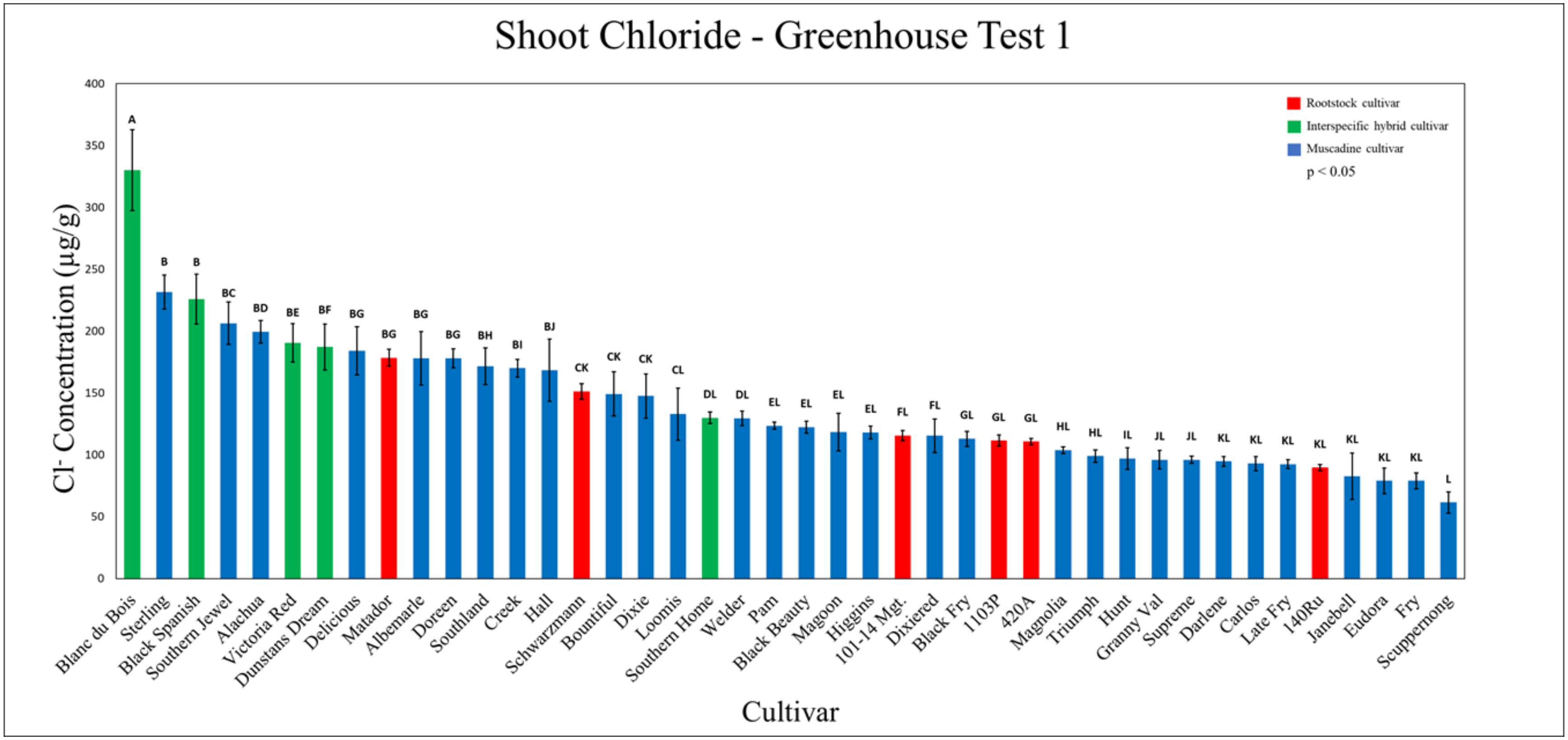

Figure 6: Chloride ion concentration of shoot material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

different than 24 other cultivars. The own-rooted hybrid cultivar Blanc Du Bois displayed a significantly higher concentration of 330µg/g, than all other cultivars. Significant differences in shoot Cl^- concentration were observed across rootstock, own-rooted hybrid, and muscadine cultivars. All six rootstock cultivars ranged from 90µg/g - 179µg/g Cl^- concentration, 140Ru was significantly lower in concentration than only Matador. The own-rooted hybrids shoot Cl^- concentrations ranged from130µg/g - 330µg/g, Blanc Du Bois had a significantly higher concentration of Cl^- than any other cultivar tested. All 31 muscadine cultivars ranged from a concentration of 62µg/g - 232µg/g, Sterling was significantly higher in shoot Cl^- concentration than 22 other muscadine cultivars.

Marginal leaf necrosis ratings for all cultivars tested are displayed in Table 3. Leaf necrosis ratings ranged from 0.1 – 2.0 for all cultivars. Muscadine cultivars overall had significantly lower ratings of marginal leaf necrosis than the rootstock cultivars but were not statistically different as a group from the hybrid cultivars. The rootstock cultivars were also not statistically different from the hybrid cultivars. The own-rooted hybrid Dunstan's Dream displayed the least amount of marginal leaf necrosis rating, and the rootstock cultivar 1103P exhibiting the greatest rating of marginal leaf necrosis. Significant differences in marginal leaf necrosis ratings were observed across rootstock, own-rooted hybrid, and muscadine cultivars. Six rootstock cultivars ratings ranged from 1.0 - 2.0; 1103P, 420A, 101-14 Mgt and 140Ru all displayed significantly higher ratings of leaf necrosis than Matador and Schwarzmann. The five own-rooted hybrid cultivars ratings ranged from 0.1 – 1.83, Dunstan's Dream and Southern Home displayed significantly lower ratings of leaf necrosis than all other hybrids. All 31 muscadine cultivars displayed some level of

Table 3 : Greenhouse Test 1, Kruskal-Wallis inclusive and pairwise comparisons of visual marginal necrosis ratings[a] for rootstock, own-rooted interspecific hybrid, and muscadine cultivars.

GH1	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis	0.99	2	12.3272	0.0021***
MUS vs. RS	1.02	1	12.6029	0.0004***
MUS vs. HY	0.89	1	0.6181	0.4318
RS vs. HY	1.23	1	3.7087	0.0541

[a] Leaf marginal necrosis ratings range from 0 (asymptomatic) to 4 (100%) - described in text.
*** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level

marginal leaf necrosis ranging from 0.5 – 1.25, the cultivar Triumph displayed significantly lower ratings of leaf necrosis than only 20 cultivars.

3.2 Greenhouse Test 2

Dry root material for all cultivars tested ranged in mass from 1.32g – 4.71g (Figure 7). The rootstock cultivar 140Ru had the least amount root dry mass of 1.32g while the own-rooted hybrid cultivar Black Spanish had the greatest root dry mass of 4.71g. All six rootstock cultivars ranged from a dry mass of 1.32g – 2.03g, and none were significantly different from any other.

Significant differences in dry mass root material were observed across the own-rooted

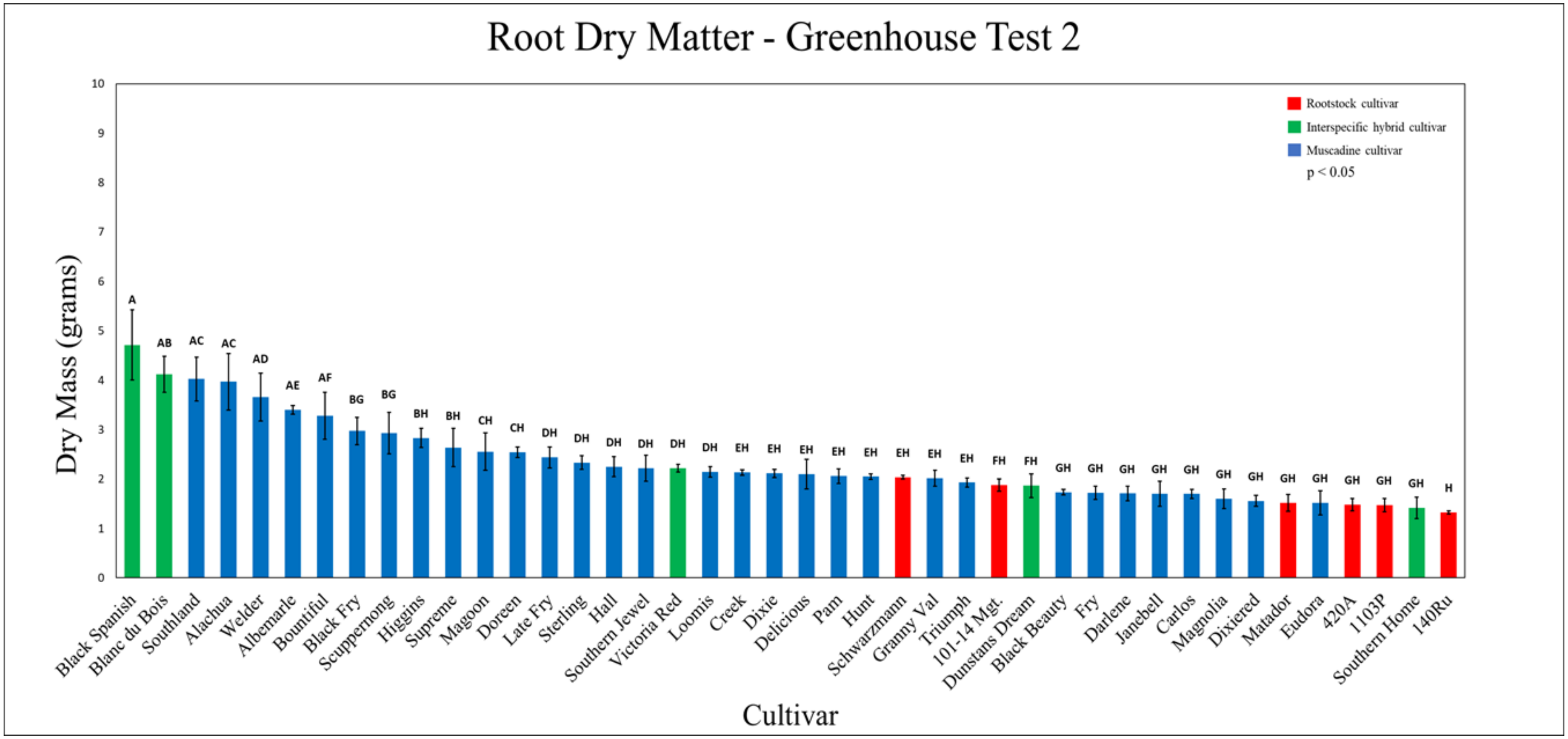

Figure 7: Dry mass of root plant material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

hybrid, and muscadine grape cultivars tested. The five own-rooted hybrid cultivar dry root mass ranged from 1.41g – 4.71g, Black Spanish and Blanc Du Bois were significantly higher in mass than the other three hybrid cultivars which were not statistically different from one another. Thirty-one muscadine cultivars dry root mass ranged from 1.51g – 4.02g only seven cultivars were significantly greater in mass than the remaining 24 muscadine cultivars.

Dry shoot material for all cultivars tested ranged in mass from 1.63g – 6.25g (Figure 8). The muscadine cultivar Triumph had the least shoot dry mass with 1.63g, and the muscadine cultivar Southland had the greatest shoot dry mass of 6.25g. All six rootstock cultivars ranged from a dry mass of 1.81g – 2.35g, none were significantly different from any other, and all were less in mass than the all hybrid cultivars. Significant differences in dry mass of shoot material were observed across the own-rooted hybrid, and muscadine grape cultivars tested. The five own-rooted hybrid cultivar dry shoot mass ranged from 2.46g – 5.45g, Blanc Du Bois and Black Spanish were significantly higher in dry shoot mass than Victoria Red. Muscadine cultivars shoot dry mass ranged from 1.63g – 6.25g, the cultivar Southland was among the greatest in dry shoot mass, and the cultivar Triumph was among the least in shoot dry mass.

Root Na^+ concentration for all cultivars tested ranged from 59µg/g - 353µg/g, more than a five-fold difference in root Na^+ concentration (Figure 9). The muscadine cultivar Janebell displayed the least root Na^+ concentration of 59µg/g but was not statistically different than 26 other cultivars tested. The own rooted hybrid cultivars Dunstan's Dream and Blanc Du Bois exhibited the greatest root Na^+ concentrations of 353µg/g and 306µg/g respectively. The root Na^+ concentrations of the six rootstock cultivars ranged from 74µg/g- 149µg/g, with no significant

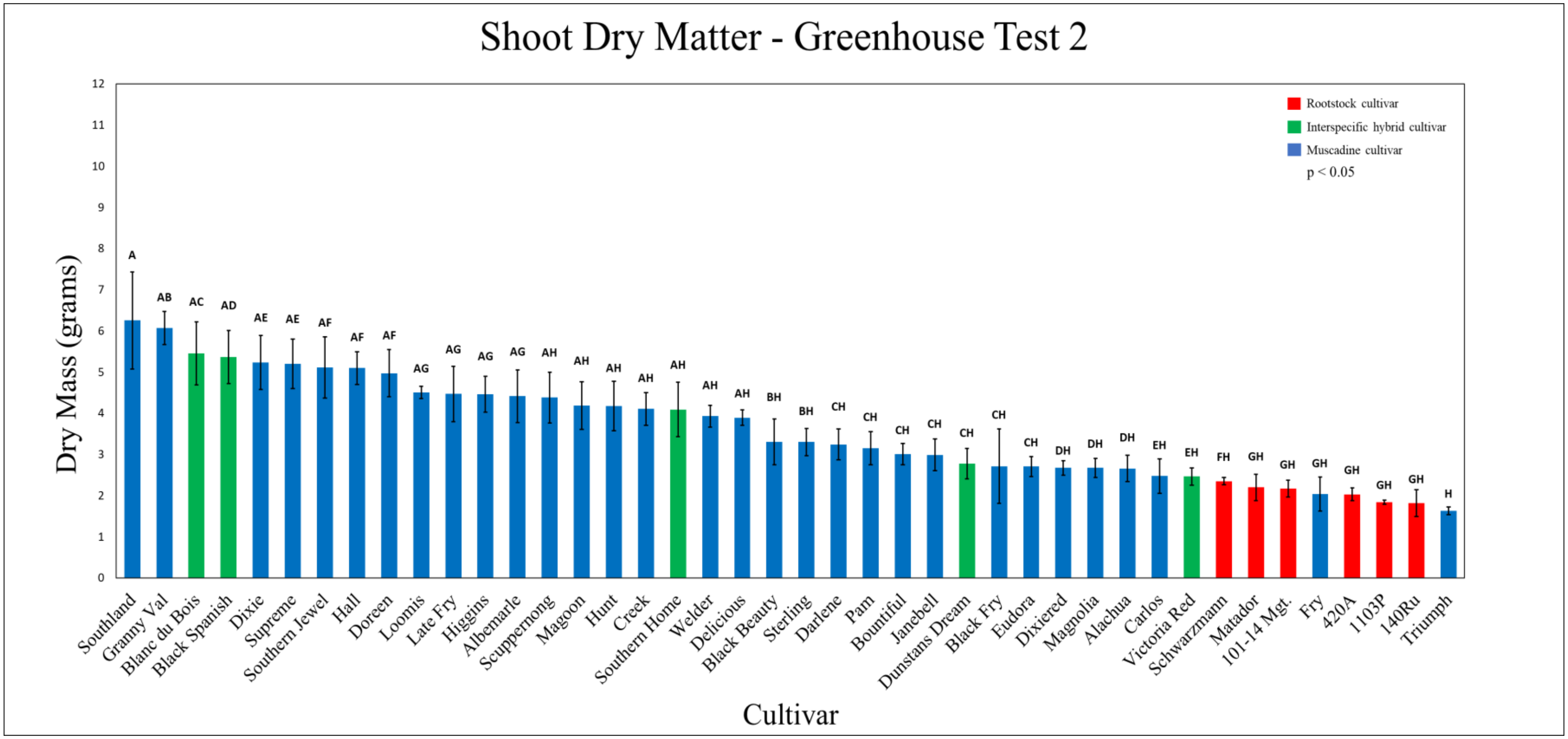

Figure 8: Dry mass of shoot plant material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey’s HSD.

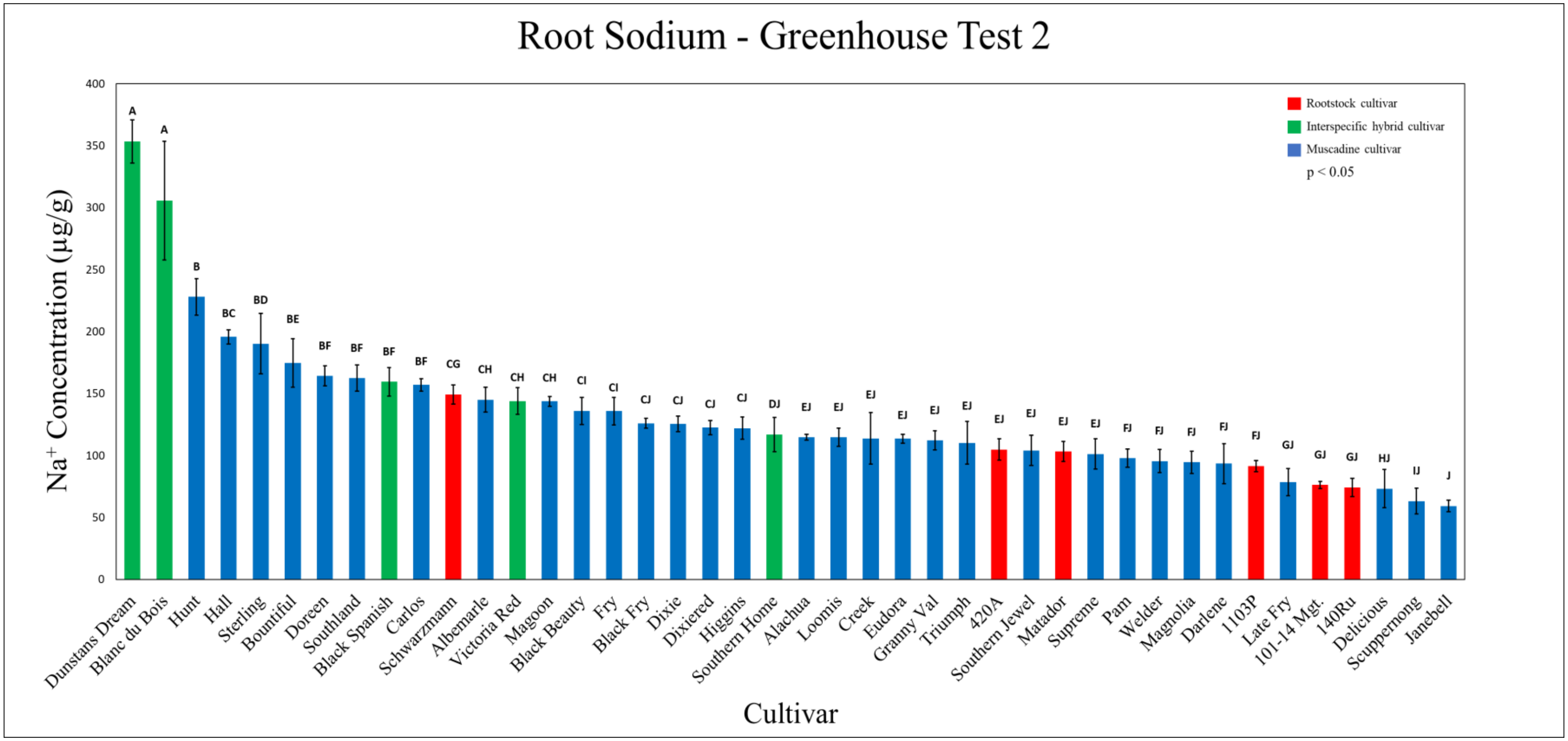

Figure 9: Sodium ion concentration of root material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

differences observed across rootstocks. Significant differences in root Na^+ concentration were observed across the own-rooted hybrid, and muscadine grape cultivars. All five own-rooted hybrid cultivar Na^+ levels ranged from 117µg/g - 353µg/g, Dunstan's Dream and Blanc Du Bois displayed significantly higher concentrations of root Na^+ than all other cultivars tested. Muscadine cultivar root Na^+ levels ranged from 59µg/g - 228µg/g, Hunt displayed the greatest root Na^+ concentration, and was significantly higher in concentration than 24 other muscadine cultivars.

Root Cl^- concentration for all cultivars tested ranged from 70µg/g - 1209µg/g, demonstrating more than a 14-fold difference in root Cl^- concentration across all 42 cultivars tested (Figure 10). The muscadine cultivar Janebell displayed the least root Cl^- concentration of 70µg/g, and Dunstan's Dream presented with the greatest root Cl^- concentration of 1209µg/g. Significant differences in root Cl^- concentration were observed across the rootstock, own-rooted hybrid, and muscadine grape cultivars All six rootstock cultivars ranged from a concentration of 115µg/g - 265µg/g, 101-14 Mgt. had a significantly lower concentration than Matador. root Cl^- concentrations of the own-rooted hybrid cultivars ranged from 254µg/g - 1209µg/g. Dunstan's Dream had a significantly higher Cl^- concentration than all other cultivars tested, while Blanc Du Bois was significantly higher than all other cultivars tested apart from Dunstan's Dream. The 31 muscadine cultivars root Cl^- concentrations ranged from 70µg/g - 569µg/g and, the cultivar Hunt was significantly higher in Cl^- concentration than all other muscadine cultivars tested.

Shoot Na^+ concentrations for all cultivars tested ranged from 69µg/g - 205µg/g (Figure 11). The muscadine cultivar Southern Jewel displayed the least shoot Na^+ concentration of 69µg/g and the muscadine cultivar Bountiful exhibited the greatest shoot Na^+ concentration of 205µg/g. The

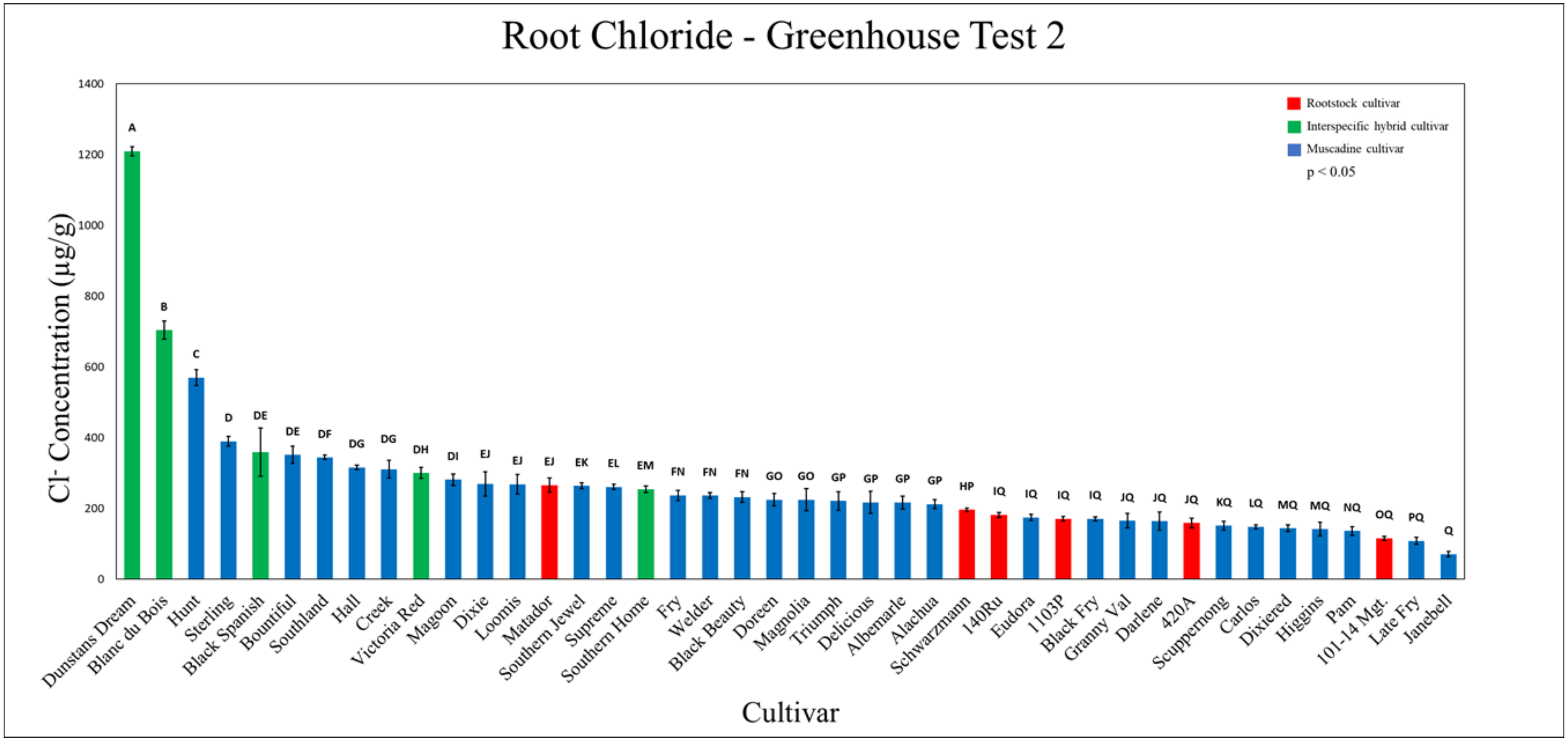

Figure 10: Chloride ion concentration of root material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

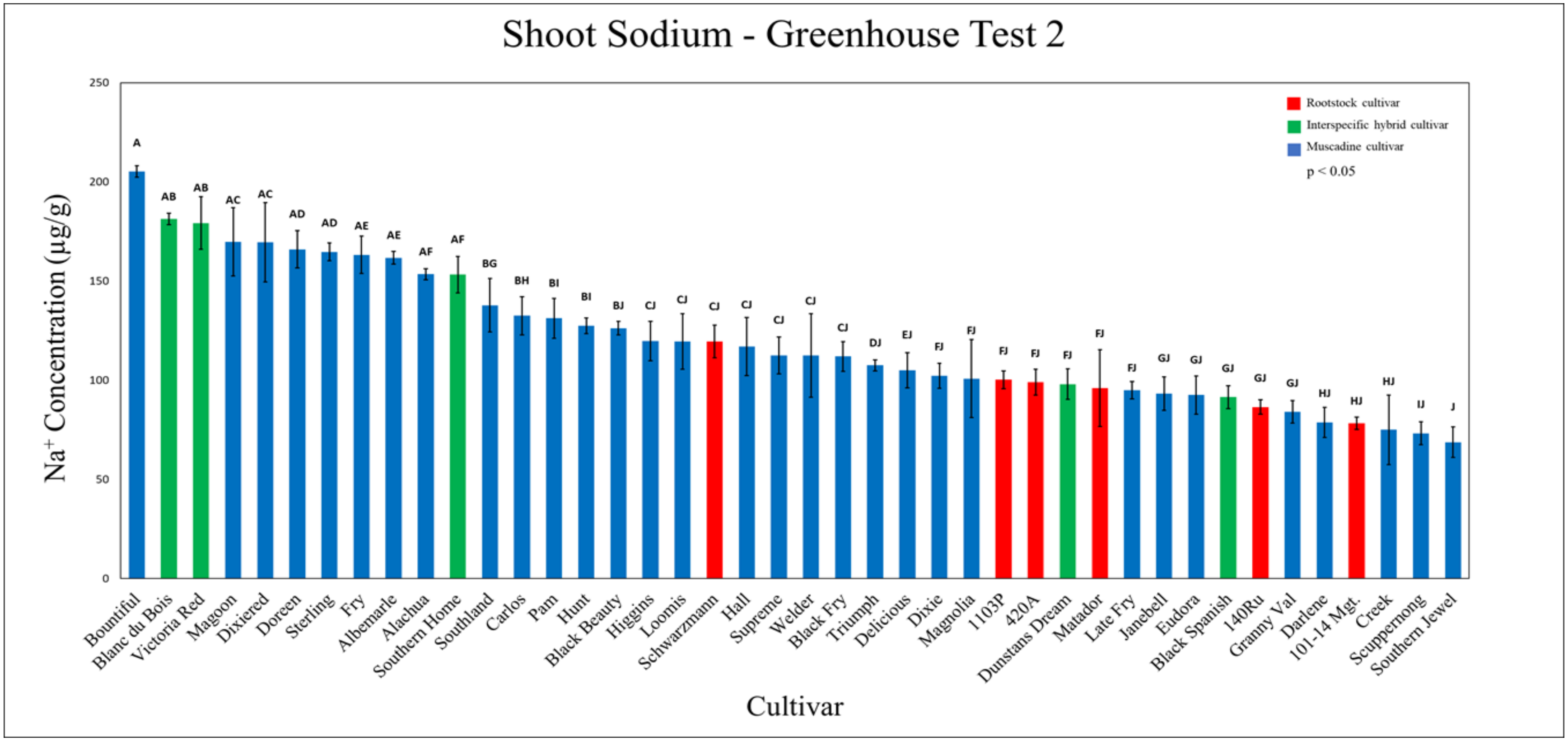

Figure 11: Sodium ion concentration of shoot material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

rootstock cultivars ranged from a concentration of 78µg/g - 119µg/g, no significant differences were observed across the rootstock cultivars. Significant differences in shoot Na^+ concentrations were observed across the own-rooted hybrids, and muscadine cultivars. The own-rooted hybrid cultivars Na^+ concentration ranged from 91µg/g - 181µg/g, Blanc Du Bois and Victoria Red were significantly greater in shoot Na^+ concentration than Dunstan's Dream and Black Spanish. All 31 muscadine cultivars ranged from 69µg/g - 205µg/g, and Bountiful was significantly greater in shoot Na^+ concentration than 23 other muscadine cultivars.

Shoot Cl^- concentration for all cultivars tested ranged from 69µg/g - 347µg/g, exhibiting a more than five-fold difference in shoot Cl^- concentration across all cultivars tested (Figure 12). The muscadine variety Scuppernong displayed the least shoot Cl^- concentration of 69µg/g and the muscadine cultivar Sterling the greatest concentration of 347µg/g. Significant differences in shoot Cl^- concentration were observed across rootstock, own-rooted hybrids, and muscadine cultivars. All six rootstock cultivars ranged from 98µg/g - 178µg/g Cl^- concentration, and Schwarzmann was significantly higher in concentration than 140 Ru. Shoot Cl^- concentrations of the own-rooted hybrids ranged from 148µg/g - 319µg/g, Southern Home had a significantly lower shoot Cl^- concentration than all other hybrids. All 31 muscadine cultivars ranged from a concentration of 69µg/g - 347µg/g, and Sterling was significantly higher in shoot Cl^- concentration than all other muscadine cultivars.

Marginal leaf necrosis pairwise comparison for all cultivars tested are displayed in Table 4. Leaf necrosis ratings ranged from 0.08 – 2.08 for all cultivars. Muscadine cultivars overall had significantly lower ratings of marginal leaf necrosis than the rootstock cultivars but were not

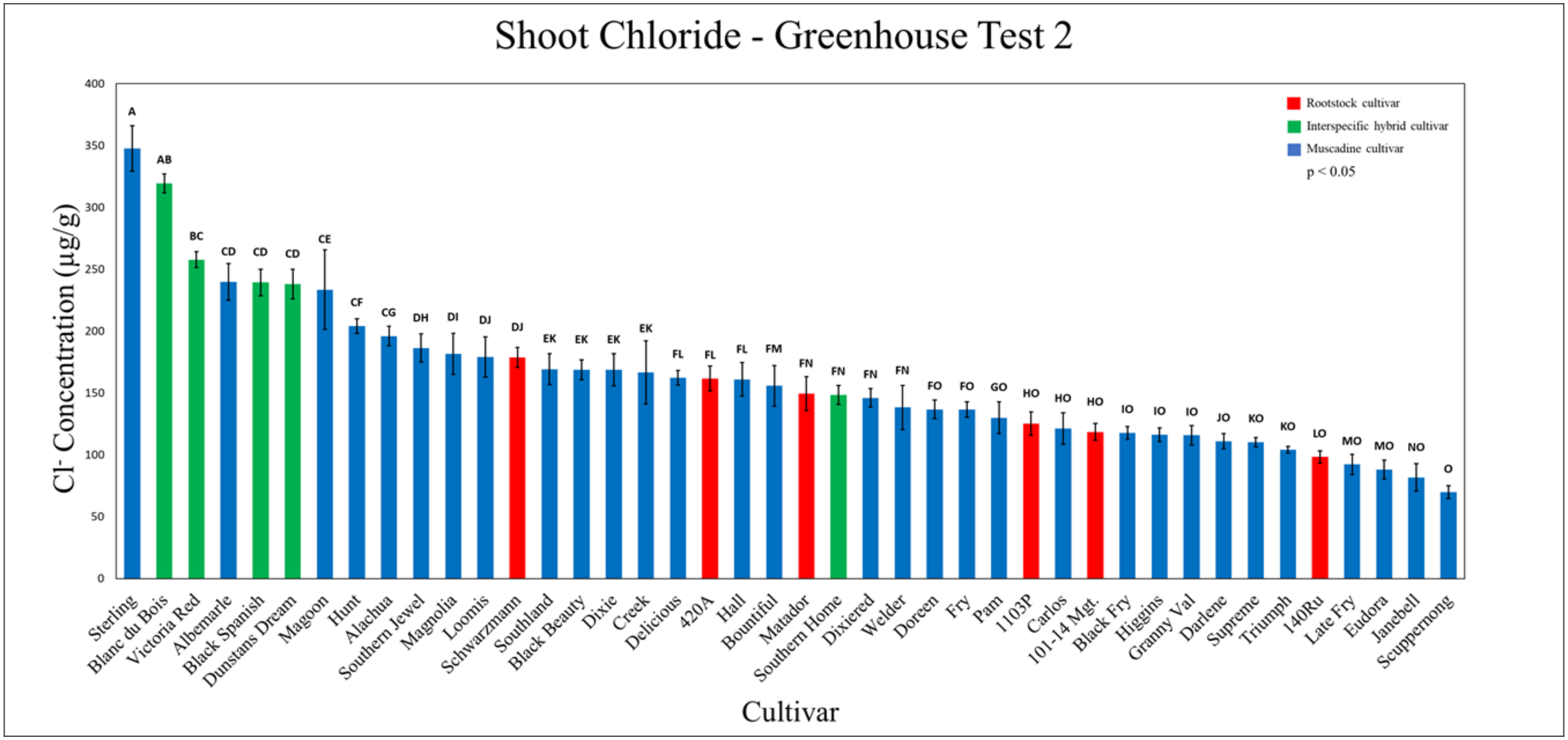

Figure 12: Chloride ion concentration of shoot material from rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

Table 4 : Greenhouse Test 2, Kruskal-Wallis inclusive and pairwise comparisons of visual marginal necrosis ratings[a] for rootstock, own-rooted interspecific hybrid, and muscadine cultivars.

GH2	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis	0.98	2	10.3148	0.0058***
MUS vs. RS	1.00	1	10.1403	0.0015***
MUS vs. HY	0.89	1	1.3683	0.2421
RS vs. HY	1.20	1	2.7123	0.0996

[a] Leaf marginal necrosis ratings range from 0 (asymptomatic) to 4 (100%) - described in text.
*** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level

statistically different as a group from the hybrid cultivars. The rootstock cultivars were also not statistically different from the hybrid cultivars. The own-rooted hybrid Dunstan's Dream displayed the least amount of marginal leaf necrosis rating, and the rootstock cultivar 1103P displayed the greatest rating of marginal leaf necrosis. Significant differences in marginal leaf necrosis ratings were observed across rootstock, own-rooted hybrids, and muscadine cultivars. Six rootstock cultivars ratings ranged from 1.0 - 2.08, 1103P, 420A, 101-14 Mgt., and 140Ru all displayed significantly higher ratings of leaf necrosis than Matador and Schwarzmann. The five own-rooted hybrid cultivars ratings ranged from 0.08 – 2.08, Dunstan's Dream and Black Spanish displayed significantly lower ratings of marginal leaf necrosis than all other hybrid cultivars. All 31 muscadine cultivars displayed some level of marginal leaf necrosis within the range of 0.41 – 1.41, the cultivar Triumph displayed significantly lower ratings of leaf necrosis than only 19 cultivars.

3.3 Field Test

Mineral nutrient concentration means for all grape cultivar leaf and petiole samples taken are listed in Table 5. Leaf and petiole Na^+ concentrations for all cultivars tested ranged from 129µg/g - 883µg/g (Figure 13). The rootstock cultivar 101-14 Mgt. displayed the least leaf and petiole Na^+ concentration of 129µg/g and the own-rooted hybrid cultivar Southern Home exhibited the greatest concentration of 883µg/g. The rootstock cultivars ranged from a Na^+ concentration of 129µg/g - 286µg/g, with no significant differences between any of the cultivars. Significant differences in leaf and petiole Na^+ concentration were observed across the own-rooted hybrid, and muscadine cultivars. Sodium concentrations of the own-rooted hybrid cultivars ranged from 278µg/g - 883µg/g, and the Na^+ concentration of Dunstan's Dream was significantly lower than all but two hybrid cultivars; Victoria Red and Black Spanish. Muscadine Na^+ concentrations ranged from 435µg/g - 810µg/g, and Carlos was significantly higher in leaf and petiole Na^+ concentration than eight other muscadine cultivars.

Leaf and petiole Cl^- concentration for all cultivars tested ranged from 238µg/g - 1310µg/g (Figure 14). The rootstock cultivar 140Ru displayed the least leaf and petiole Cl^- concentration of 238µg/g, while the own-rooted hybrid cultivar Dunstan's Dream exhibited the greatest concentration of 1310µg/g. All six rootstock cultivars ranged from 238µg/g - 530µg/g leaf

Table 5 : Mineral nutrient concentration of leaf and petiole sampling of rootstock, own-rooted interspecific hybrid, and muscadine cultivars.

	%					µg/g							
Cultivar	N	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S	B	Cl
101-14 Mgt.	1.89 ad	0.24 bf	2.02 ad	1.36 i	0.17 mn	129 j	25 b	330 ab	11 b	142 f	1799 cd	56 be	318 g
1103P	1.66 bh	0.27 af	1.80 bf	1.67 ei	0.32 dm	169 ij	33 ab	807 ab	11 b	113 f	1716 de	60 bc	308 g
140Ru	1.73 bg	0.36 a	1.57 dh	1.43 hi	0.37 bl	166 ij	27 ab	401 ab	11 b	284 ef	1901 cd	69 b	239 g
420A	1.87 ae	0.23 bf	1.54 ei	1.94 ci	0.40 aj	171 hj	43 ab	695 ab	12 b	475 cf	2050bc	89 a	279 g
Matador	1.76 bg	0.30 ac	2.46 a	1.81 di	0.26 in	239 gj	39 ab	1285 ab	18 ab	127 f	2340 ab	51 bf	531 eg
Schwarzmann	1.98 ab	0.29 ad	2.15 ac	1.95 ci	0.21 kn	287 ej	46 ab	271 b	12 ab	164 f	2096 bc	67 b	346 fg
Black Spanish	1.62 ci	0.21 bf	1.87 be	2.15 bg	0.28 gn	572 af	33 ab	573 ab	10 b	147 f	1472 ef	68 b	970 ad
Blanc du Bois	1.90 ac	0.23 bf	1.67 dg	2.42 ad	0.20 ln	616 ad	39 ab	345 ab	12 ab	101 f	1783 ce	59 bd	1090 ac
Dunstans Dream	1.83 af	0.32 ab	1.72 cg	2.19 bg	0.30 fm	278 fj	53 a	1228 ab	16 ab	159 f	2009 cd	71 ab	1311 a
Southern Home	1.44 gm	0.25 af	1.38 fm	2.34 ae	0.43 ah	883 a	34 ab	1774 ab	17 ab	484 cf	1257 fi	43 cg	536 eg
Victoria red	2.11 a	0.26 af	2.24 ab	2.09 bh	0.13 n	528 bg	34 ab	314 ab	9 b	96 f	2418 a	67 b	598 dg
Alachua	1.23 km	0.20 bf	1.00 ko	2.04 ch	0.35 bl	580 af	34 ab	863 ab	10 b	271 ef	1060 gi	33 fg	562 dg
Albemarle	1.22 lm	0.19 cf	0.94 lo	2.15 bg	0.46 af	464 ci	27 ab	1475 ab	10 b	316 ef	1039 hi	31 fg	507 eg
Black Beauty	1.45 gm	0.18 ef	1.27 go	1.66 fi	0.24 jn	507 bg	42 ab	917 ab	11 b	117 f	1097 gi	27 g	480 eg
Black Fry	1.19 m	0.17 ef	0.91 mo	2.06 bh	0.38 bk	579 af	31 ab	993 ab	9 b	253 ef	987 i	30 g	519 eg
Bountiful	1.33 hm	0.16 f	0.81 o	2.05 ch	0.33 dm	601 ad	35 ab	816 ab	9 b	266 ef	1081 gi	29 g	488 eg
Carlos	1.41 gm	0.19 cf	1.07 io	1.92 ci	0.32 dm	810 ab	24 b	1185 ab	10 b	306 ef	1099 gi	34 fg	601 dg
Creek	1.31 im	0.17 ef	0.86 no	1.88 ci	0.51 ab	669 ad	30 ab	1202 ab	13 ab	148 f	1107 gi	35 fg	1184 ab
Darlene	1.47 gm	0.20 bf	1.19 ho	1.92 ci	0.27 hn	509 bg	27 ab	580 ab	10 b	245 ef	1219 fi	35 fg	754 cf
Delicious	1.33 hm	0.25 af	0.86 no	1.88 ci	0.54 a	692 ad	28 ab	1373 ab	10 b	201 ef	1134 gi	33 fg	492 eg
	***	***	***	***	***	***	**	*	***	***	***	***	***

Prob > F *** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level
Mean values labeled with the same letter were not statistically different at p ≤ 0.05 Tukey’s HSD.

Table 5 : (continued)

	%					µg/g							
Cultivar	N	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S	B	Cl
Dixie	1.35 hm	0.20 cf	1.07 io	1.83 di	0.41 ai	488 cg	41 ab	799 ab	9 b	148 f	1189 fi	42 cg	584 dg
Dixiered	1.48 fm	0.18 df	0.99 no	2.53 ac	0.30 fm	652 ad	37 ab	1612 ab	9 b	313 ef	1256 fi	31 fg	768 bf
Doreen	1.28 im	0.22 bf	1.01 ko	2.73 ab	0.46 af	579 af	39 ab	1845 ab	14 ab	231 ef	1229 fi	40 cg	477 eg
Eudora	1.60 ci	0.21 bf	1.35 fm	2.95 a	0.33 dm	598 ae	48 ab	1897 ab	16 ab	191ef	1473 ef	38 eg	419 eg
Fry	1.58 cj	0.23 bf	1.44 ek	2.07 bh	0.28 gn	473 ci	43 ab	1144 ab	12 ab	245 ef	1334 fh	34 fg	478 eg
Granny Val	1.57 ck	0.24 bf	1.50 ei	2.29 af	0.41 ai	664 ad	36 ab	1476 ab	18 ab	147 f	1288 fi	44 cg	528 eg
Hall	1.42 gm	0.22 bf	1.33 gn	1.90 ci	0.45 ag	436 dj	35 ab	1740 ab	19 ab	147 f	1236 fi	37 eg	391 eg
Higgins	1.63 ci	0.18 df	1.48 ej	1.83 di	0.30 fm	665 ad	33 ab	1355 ab	15 ab	321 ef	1191 fi	33 fg	540 eg
Hunt	1.54 em	0.21 bf	1.41 el	1.93 ci	0.31 em	558 bf	39 ab	1036 ab	11 b	222 ef	1326 fh	43 cg	399 eg
Janebell	1.44 gm	0.19 cf	1.14 ho	1.60 gi	0.30 fm	546 bg	26 b	809 ab	9 b	203 ef	1152 fi	29 g	366 fg
Late Fry	1.35 hm	0.22 bf	1.01 jo	1.62 gi	0.44 ag	627 ad	35 ab	1018 ab	10 b	592 be	1110 gi	41 cg	505 eg
Loomis	1.36 hm	0.20 bf	0.99 ko	1.69 ei	0.40 aj	753 ac	28 ab	906 ab	9 b	145 f	1299 fi	35 fg	494 eg
Magnolia	1.63 ci	0.21 bf	1.28 go	1.70 ei	0.30 fm	575 af	33 ab	1518 ab	15 ab	103 f	1326 fh	39 dg	464 eg
Magoon	1.46 gm	0.28 ae	1.34 fm	1.89 ci	0.47 ae	646 ad	33 ab	1278 ab	11 b	741 ad	1355 fh	45 cg	549 dg
Pam	1.37 hm	0.18 cf	1.15 ho	2.18 bg	0.36 bl	497 cg	41 ab	2684 a	21 ab	270 ef	1150 gi	29 g	425 eg
Scuppernong	1.32 hm	0.23 bf	1.26 go	1.75 di	0.48 ad	600 ad	29 ab	1309 ab	12 ab	923 ab	1242 fi	28 eg	489 eg
Southern Jewel	1.56 dl	0.19 cf	1.15 ho	1.94 ci	0.37 bl	482 ch	28 ab	685 ab	10 b	373 df	1176 fi	35 fg	804 be
Southland	1.43 gm	0.20 cf	1.53 ei	1.69 ei	0.35 cl	436 dj	34 ab	900 ab	11 b	795 ac	1215 fi	38 eg	465 eg
Sterling	1.33 hm	0.23 bf	1.16 ho	1.64 fi	0.50 ac	579 af	34 ab	1912 ab	16 b	1017 a	1239 fi	36 eg	600 dg
Supreme	1.42 gm	0.24 bf	1.19 ho	1.56 gi	0.33 dm	584 af	34 ab	965 ab	11 b	748 ad	1232 fi	38 eg	565 dg
Triumph	1.25 jm	0.20 bf	1.26 go	1.89 ci	0.37 bk	587 af	43 ab	1666 ab	13 b	172 f	1178 fi	30 g	467 eg
Welder	1.29 im	0.20 bf	1.15 ho	1.58 gi	0.44 ag	494 cg	32 ab	2276 ab	24 a	1025 a	1097 gi	37 eg	497 eg
	***	***	***	***	***	***	**	*	***	***	***	***	***

Prob > F *** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level
Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

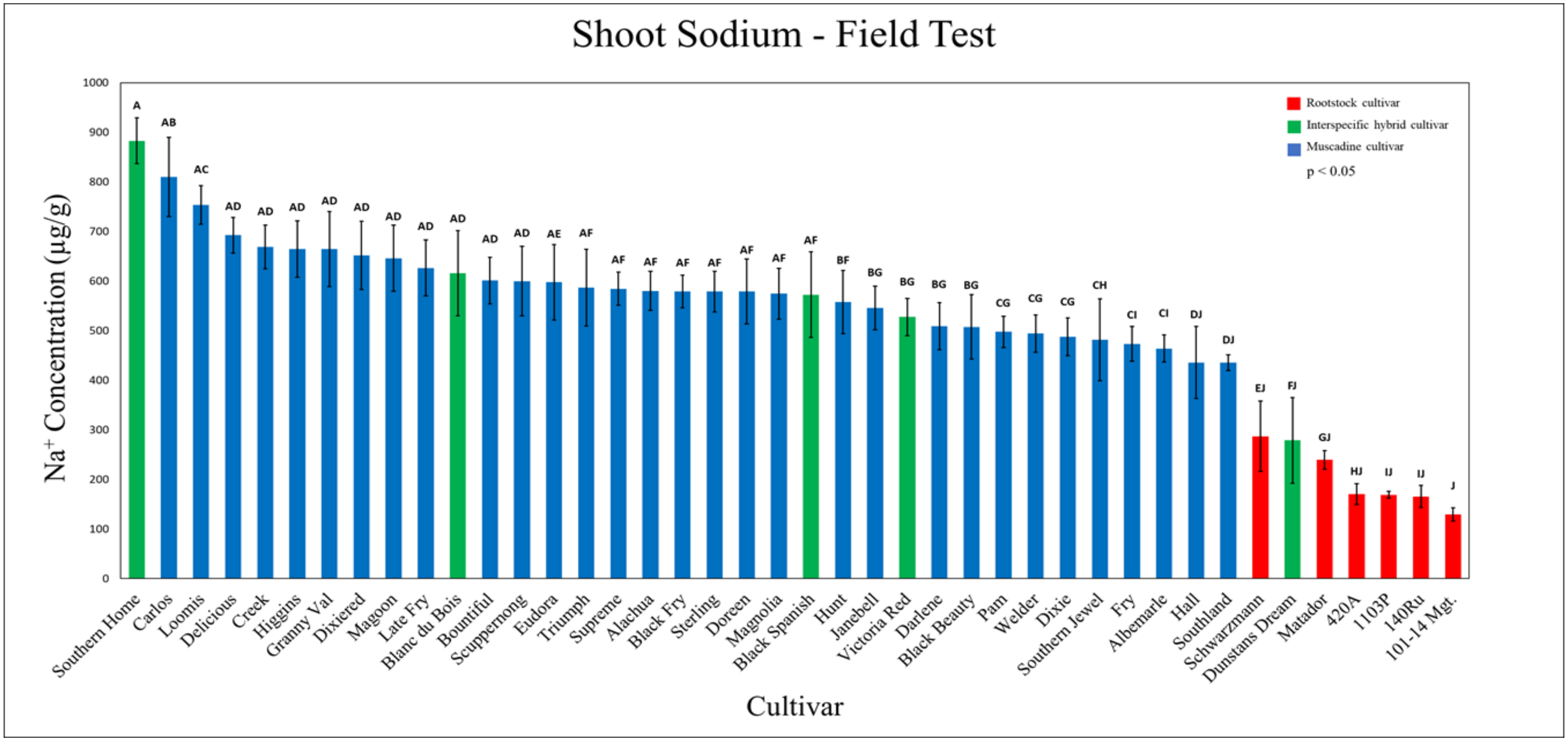

Figure 13: Sodium concentration of total leaf and petiole samples of rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

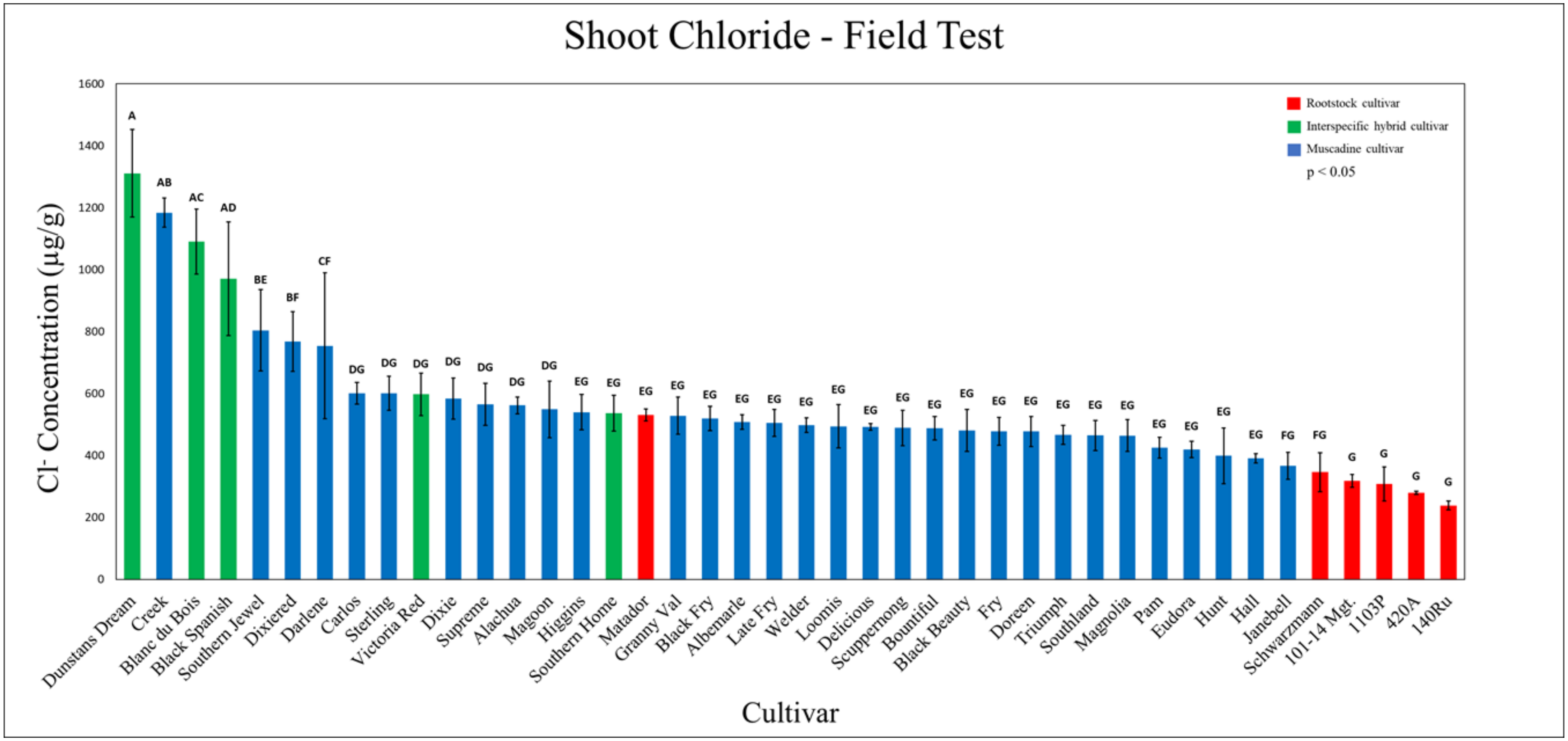

Figure 14: Chloride concentration of total leaf and petiole samples of rootstock, own rooted interspecific hybrid, and muscadine cultivars. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

and petiole Cl^- concentration, with no significant differences observed across all rootstock cultivars. Significant differences in leaf and petiole Cl^- concentration were observed across the own-rooted hybrid, and muscadine cultivars. Shoot Cl^- concentration of the own-rooted hybrids ranged from 536µg/g - 1310µg/g, Dunstan's Dream and Blanc Du Bois were significantly greater in Cl^- than all other hybrids. Muscadine cultivars ranged from a concentration of 366µg/g - 1183µg/g, and Creek was significantly higher in leaf and petiole Cl^- concentration than 28 other muscadine cultivars.

Post-harvest marginal leaf necrosis pairwise comparisons for all cultivars tested are displayed in Table 6. Muscadine cultivars overall had significantly lower ratings of marginal leaf necrosis than the rootstock cultivars at $p \leq 0.05$ but were not statistically different as a group from the hybrid cultivars. Rootstock cultivars were also not statistically different from the hybrid cultivars. Leaf necrosis ratings ranged from 1.00 – 1.66 for all cultivars, the rootstock cultivar 101-14 Mgt. displayed the least amount of marginal leaf necrosis ratings, and the own-rooted hybrid cultivar Victoria Red exhibiting the greatest rating of marginal leaf necrosis. Six rootstock cultivars ratings ranged from 1.0 - 1.08, with no significant differences among any of the rootstock cultivars. The five own-rooted hybrid cultivars ratings ranged from 1.0 – 1.66, Victoria Red only had a significantly higher rating of marginal leaf necrosis than Blanc Du Bois. All 31 muscadine cultivars displayed some level of marginal leaf necrosis within the range of 1.00 – 1.41, however there were no significant differences among any of the 31 cultivars tested.

Pairwise comparisons of marginal leaf necrosis recorded at full bloom for all cultivars tested in the second year of the field study are displayed in Table 7. Significant differences were observed across all groups of cultivars. Muscadine cultivars were significantly higher in marginal

Table 6 : Field Test-post harvest, Kruskal-Wallis inclusive and pairwise comparisons of visual marginal necrosis ratings[a] for rootstock, own-rooted interspecific hybrid, and muscadine cultivars taken at E-L stage 41 After harvest; cane maturation complete, 2017.

2017	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis	1.16	2	4.7200	0.0944
MUS vs. RS	1.15	1	4.6973	0.0302**
MUS vs. HY	1.17	1	0.0267	0.8701
RS vs. HY	1.12	1	2.0833	0.1489

[a] Leaf marginal necrosis ratings range from 0 (asymptomatic) to 4 (100%) - described in text.
*** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level

Table 7 : Field Test-flower, Kruskal-Wallis inclusive and pairwise comparisons of visual marginal necrosis ratings[a] for rootstock, own-rooted interspecific hybrid, and muscadine cultivars taken at E-L stage 23 Full Bloom 50% caps off period, 2018.

2018	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis	1.58	2	18.3789	0.0001***
MUS vs. RS	1.69	1	14.7753	0.0001***
MUS vs. HY	1.84	1	5.3506	0.0207**
RS vs. HY	0.32	1	4.3676	0.0366**

[a] Leaf marginal necrosis ratings range from 0 (asymptomatic) to 4 (100%) - described in text.
*** --- significant at .001 level, ** --- significant at .05 level, * --- significant at .01 level

leaf necrosis than both the muscadine and hybrid cultivars. Rootstock cultivars displayed significantly lower ratings of marginal leaf necrosis than the hybrid cultivars. Leaf necrosis ratings ranged from 0.0 – 3.33 for all cultivars, all six rootstock cultivars displayed no visual leaf necrosis symptoms, and thus were not significantly different from one another. The five own-rooted hybrids leaf necrosis ratings ranged from 0.0 – 2.83, Southern Home had a significantly higher rating than all others. All 31 muscadine cultivars displayed some level of marginal leaf necrosis ranging from 0.58 – 3.33, the cultivar Hall displayed significantly lower ratings of marginal leaf necrosis ratings than four other muscadine cultivars.

Bud break data is displayed in (Figure 15). All cultivars ranged from 78.5 days – 96.5 days, the own-rooted hybrid cultivar Dunstan's Dream was the cultivar with least amount of days until budbreak at 78.5 days, while the muscadine cultivar Delicious was the cultivar with greatest amount of days until budbreak at 96.5 days. All six rootstocks ranged from a period of 80.5 days – 85.2 days, no significant differences were observed across all rootstock cultivars. Significant differences in bud break date were observed across own-rooted hybrid cultivars, and muscadine cultivars. The own-rooted hybrids all ranged from a period of 78.5 days – 92.2 days, and all hybrids reached bud break significantly earlier in the year than Southern Home at 92.2 days. The muscadine cultivars ranged from a period of 85.5 days – 96.5 days, Delicious was significantly later in reaching budbreak than 20 muscadine cultivars.

Vine vigor as determined by differences in shoot length are displayed in (Figure 16). Vine shoot lengths ranged from 91cm – 276cm in length, the rootstock cultivar Matador

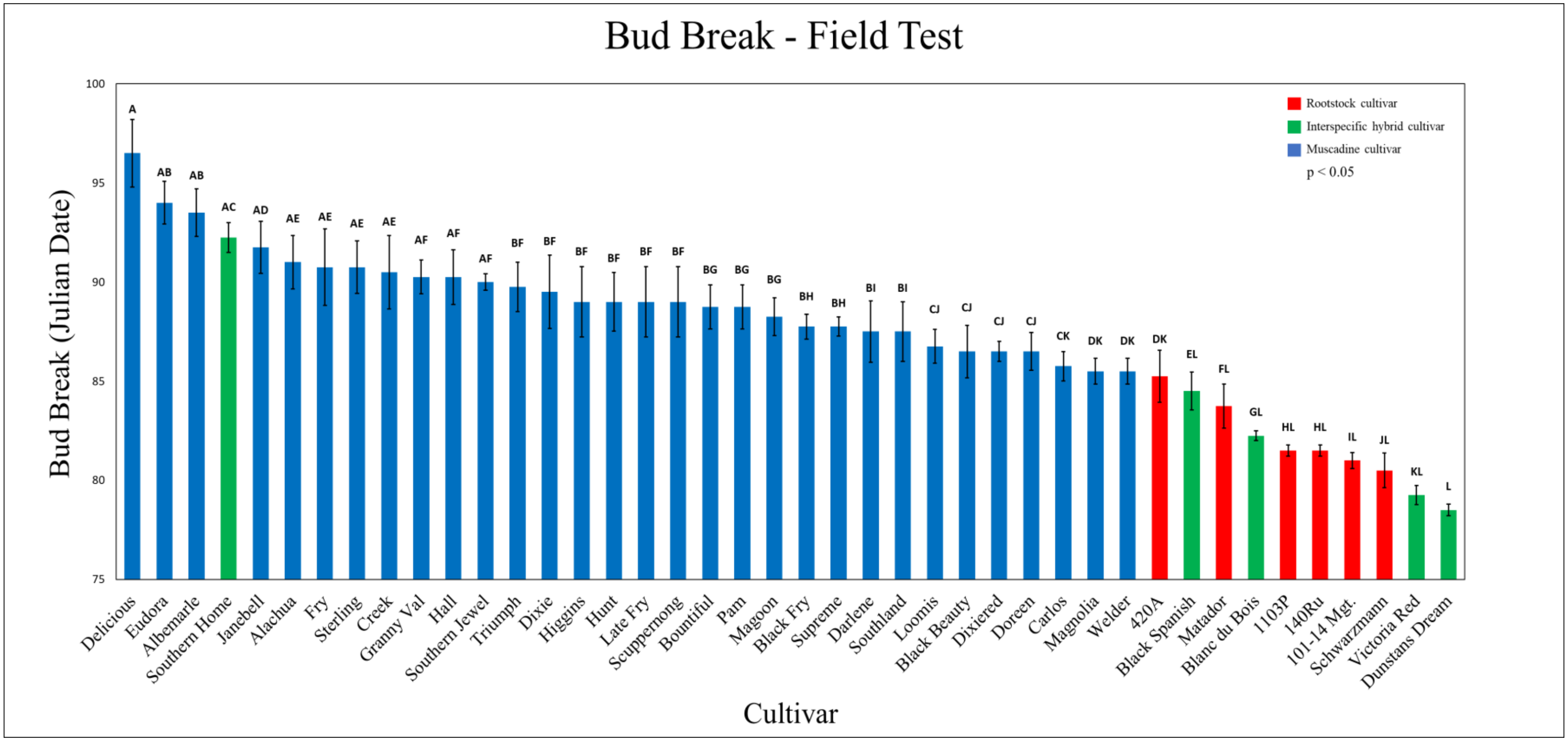

Figure 15: Budbreak of rootstock, own rooted interspecific hybrid, and muscadine cultivars. Budbreak defined as E-L stage 4 Budburst, reported in Julian days. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

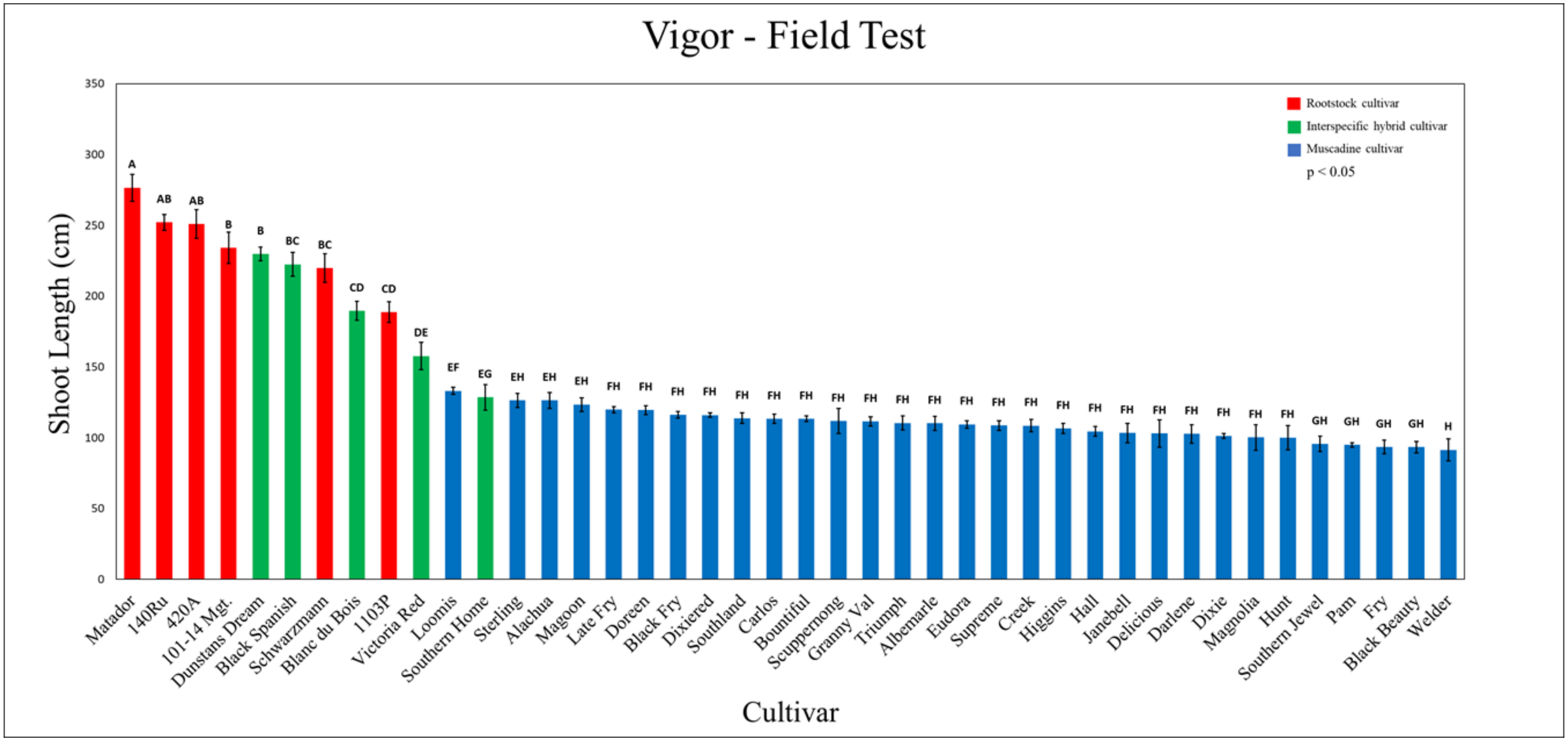

Figure 16: Vine vigor as determined by shoot length of rootstock, own rooted interspecific hybrid, and muscadine cultivars recorded on Julian day 170. Each measurement represents an average of four replicates. Mean values labeled with the same letter were not statistically different at $p \leq 0.05$ Tukey's HSD.

displayed the greatest degree of vigor, while the muscadine cultivar Welder exhibited the least degree of vigor. Significant differences in vine vigor were observed across rootstock, own-rooted hybrid cultivars, and muscadine cultivars All six rootstock cultivars ranged in length from 188cm – 277cm, and Matador was significantly greater in shoot length than 101-14 Mgt. and 1103P. The five own-rooted hybrid cultivars ranged from a shoot length of 129cm – 229cm, Dunstan's Dream was significantly greater in shoot length than Blanc Du Bois, Victoria Red, and Southern Home. Finally, all 31 muscadine cultivars ranged from 91cm – 133cm in length, Loomis was significantly greater in shoot length than five other muscadine cultivars.

CHAPTER IV

DISCUSSION

Significant advances in the effort to understand and mitigate salt stress in grapes have been made in recent years. Applied research has focused on developing new rootstocks derived from *V. champini, V. berlandieri, and V. vinifera*. Comparing new hybrid cultivars to the current industry standards to find naturally superior salt excluders, will allow for greater vigor, and higher yields (Walker et al., 2008). Efforts to understand the mechanisms of Cl^- exclusion in rootstocks are underway, evaluating differences in transpiration rates between multiple cultivars known for their varied Cl^- exclusion capabilities. Understanding simply how Cl^- is absorbed and moved throughout the vine at different times of the year could provide insight into what aspect of the plant controls movement (Tregeagle et al. 2010). In 2013, Fort et al (2013) observed that differences occurred between strong excluders of Cl^- of the same genotype leading to the conclusion that Cl^- exclusion appeared to be a quantitatively inherited trait. Genetic mechanisms for controlling Na^+ exclusion in grapevines were unknown until researchers in Australia mapped a dominate quantitative trait loci (QTL) associated with leaf Na^+ exclusion under salinity stress. This revealed that the dominant high-affinity potassium transporter (HKT) variants exhibited greater Na^+ conductance with less rectification than other recessive variants (Henderson et al., 2017). However, the focus of current research is on bunch grapes and the author is unaware of any efforts toward the development of muscadine rootstocks.

4.1 Greenhouse Tests

Root and shoot dry weights of the rootstock cultivars over the two rounds of greenhouse testing were not significantly different but were generally lower than the own-rooted hybrids and muscadine cultivars. However, Na^{+} concentrations across both root and shoot material did not exhibit any significant differences across six rootstocks cultivars. Significant differences between root and shoot Na^{+} concentrations could indicate that compartmentalization had occurred in the root tissue. For most species, Na^{+} reaches toxic concentrations before Cl^{-}, however in species such as grapevines, soybeans, and citrus Cl^{-} is considered to be the more toxic ion. The association is between genetic differences in the rate of Cl^{-} accumulation in the leaves and the plants salinity tolerance. These differences may arise due to Na^{+} being withheld so effectively in woody roots and stems that lower concentrations will reach the leaf tissue, allowing for K^{+} to become the major ion associated with osmotic adjustment. This also allows Cl^{-} pass directly to the leaf tissue, where it becomes the more significantly toxic component present in leaf tissue (Munns and Tester, 2008). However, the correlation between Na^{+} shoot and roots concentrations for the rootstocks in greenhouse test 1 and 2 were 0.95 and 0.88, respectively (Figure 17, Figure 18). This is not indicative of exclusion at the shoot level. Furthermore, no correlation was observed between shoot and root Cl- in either test.

The concentration of Cl^{-} across all the rootstock cultivars except Matador was not significantly different. In one greenhouse test Matador did accumulate a significantly higher concentration of Cl^{-} in its root and shoot tissues, but this was not observed in the second test. Fort

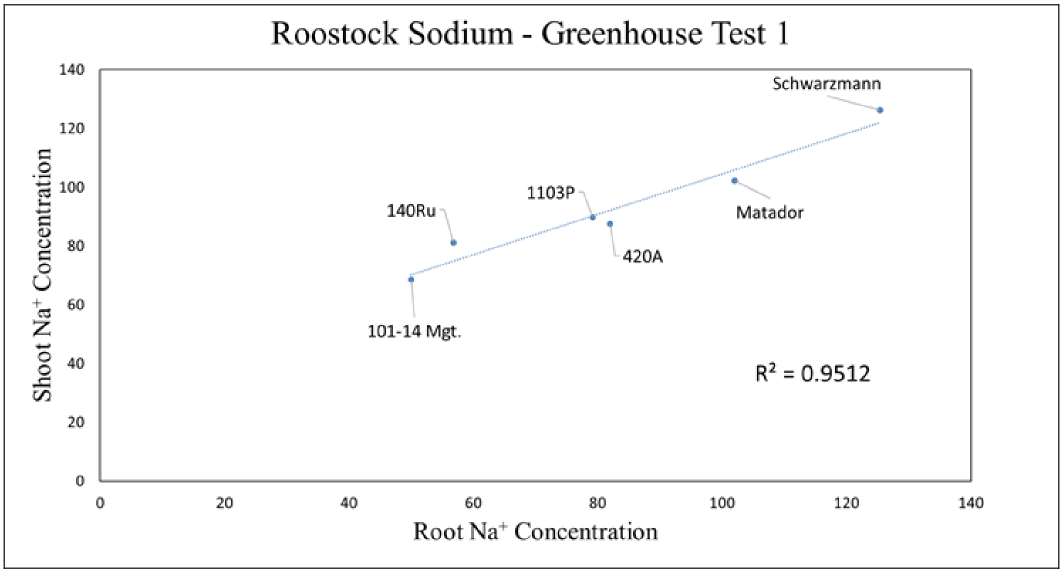

Figure 17: Correlation of sodium ion concentration of root and shoot material from six rootstock cultivars in greenhouse test 1.

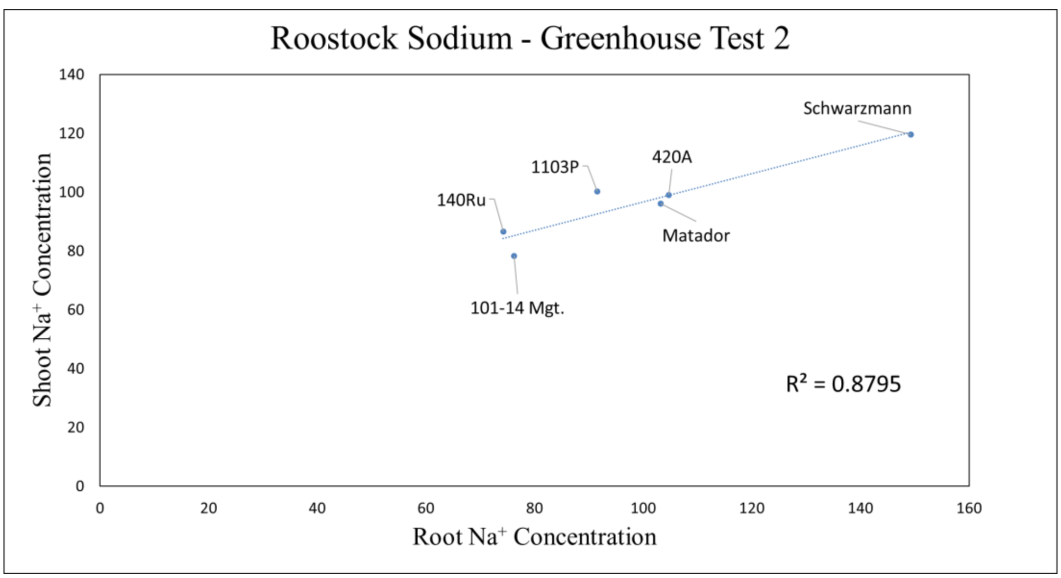

Figure 18: Correlation of sodium ion concentration of root and shoot material from six rootstock cultivars in greenhouse test 2.

et al., (2013) evaluated four rootstock cultivars Schwarzmann, 140Ru, 101-14 Mgt, and 1103P using a similar protocol to this study and found no significant differences in Cl^- exclusion across those four rootstock cultivars. However, this is in contrast to Downton (1977) who reported specific differences in the Cl^- accumulation capabilities of different *Vitis* species: *V. rupestris* < *V. berlandieri*, *V. riparia* < *V. candicans*, *V. champini*, *V. longii* < *V. cineria*, < *V. cordifolia* < *V. vinifera*. In comparison to the muscadine cultivars under study, the six of the rootstock cultivars would not be considered superior in their capability to exclude either Na^+ or Cl^- from their root systems. The most effective Cl^- excluding muscadine cultivar in this test, Janebell, contained only an average of 85ug/g across both tests in its shoot tissue, and the most effective salt excluding rootstock 101-14 Mgt. had 100 ug/g Cl^-. This is the first published report on salt exclusion in muscadine grapes.

The wide range of ion exclusion capabilities, dry weight plant matter, and marginal leaf necrosis observed across the muscadine cultivars in the greenhouse studies is significant, particularly as it relates to the potential of using one of these cultivars as a rootstock. However, it should be noted that there are currently no leaf tissue nutrient concentration recommendations for Na^+ or Cl^- for muscadine grapes. The muscadine cultivars that where statistically superior in their ability to exclude both root and shoot Na^+ were: Janebell, Granny Val, Eudora, Fry, Black Fry, Late Fry, Triumph, Southern Jewel, Scuppernong, Magnolia, and Darlene. However, Janebell was the only cultivar that was statistically superior in its ability to exclude excessive concentrations of Na^+ and Cl^- from its root and shoot tissues. It is interesting to note that the common ancestor that the first eight share is the cultivar Fry. This suggests the possibility of a genetic link explaining their performance. The Scuppernong variety is the oldest muscadine variety still in production and is also part of the parentage of Magnolia. Finally, the Ison seedling 5-11-3 is a parent of the cultivar Darlene, and the parent of other cultivars that have demonstrated superior exclusion of Cl^-. Fry and Scuppernong share some similar characteristics including; both are pistillate flower types requiring a pollinizer. Scuppernong is 100% *V. rotundifolia,* while the parents of Fry are Ga 19-13 x USDA 19-11, both having combinations of *V. rotundifolia* and *V. munsoniana* in their parentages. Fry was developed and released by R. Lane in 1970 at the University of Georgia, and Scuppernong was first reported to have been discovered by Sir Walter Raleigh in 1584. The Scuppernong vine, known as the Mother Vine, on Roanoke Island is reported to be over 200 years old.

All six of the rootstock's cultivars had significantly lower concentrations of both Na^+ and Cl^- in both root and shoot tissue than the own-rooted hybrid Blanc Du Bois. This could be significant because most Blanc Du Bois grown in Texas is grown on its own roots. On average, the concentration of shoot and root Cl^-, the most acutely toxic ion associated with salinity, was two to three times higher in Blanc Du Bois compared to the rootstock cultivars. These findings suggest that a rootstock could be beneficial if Na^+ or Cl^- are present in the soil or irrigation water at limiting concentrations.

High concentrations of Cl^- in grape leaf tissue have been demonstrated to cause marginal leaf necrosis (Ehlig, 1960), and grapes are reported to be more sensitive to Cl^- than Na^+. One method grapevines use to tolerate these high concentrations is compartmentalization of both Na^+ and Cl^- at the cellular and intracellular levels. This allows for the avoidance of toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. If unfavorable conditions continue, ion concentrations ultimately become too high causing membrane degradation, and enzyme inhibition leading to the breakdown of cellular function. This sequestration of excessive Na^+ and Cl^- and occurs first in the older leaves, eventually causing senescence. However, despite having relatively low tissue concentrations of Cl^-, the rootstock cultivars demonstrated higher marginal necrosis ratings compared to the muscadines. The cultivars:1103P, 420A, 101-14 Mgt. and 140Ru, all have *V. rupestris*, *V. berlandieri*, or *V. riparia* parentage, and these three *Vitis* species have been shown to accumulate lower concentrations of Cl^- in scion leaf tissue than *V. vinifera* (Downton, 1977). Nevertheless, they consistently demonstrated the greatest marginal leaf necrosis ratings across two rounds of greenhouse tests. This suggests that either the muscadines were more tolerant of higher tissue concentrations or the necrosis observed was caused by another factor. The correlation between tissue Na^+ and Cl^-, and the combination of tissue Na^+ and Cl^-, and leaf necrosis ratings was not significant.

Based on the root and shoot concentrations of Na^+ and Cl^-, the own-rooted hybrid cultivars demonstrated a greater range of ion exclusion capability when compared to the rootstock cultivars. This is not surprising considering the diverse genetic background that the own-rooted hybrids represent. The hybrid cultivars were also able to accumulate much higher concentrations of Na^+ and Cl^- without demonstrating high levels of marginal leaf necrosis. As previously stated, Blanc Du Bois demonstrated the greatest concentration both Na^+ and Cl^- in root and shoot tissue. Blanc Du Bois accumulated 587 μg/g Cl^- in its root tissue, more than double than the topmost accumulating rootstock Matador's root Cl^- concentration, while simultaneously demonstrating a significantly lower overall rating of marginal leaf necrosis than the four rootstocks mentioned above. In the second round of greenhouse testing, Dunstan's Dream accumulated 1209 μg/g Cl^- in its root tissue compared to the next closest cultivar Blanc Du Bois with 704 ug/g Cl^-. Southern

Home a hybrid with *V. rotundifolia* in its parentage accumulated significantly less Cl^- in its root tissue (183ug/g).

Dunstan's Dream exhibited a shoot Cl^- concentration of only 238 ug/g Cl^-, while displaying the least amount of marginal leaf necrosis rating of any cultivar tested. The ability of Dunstan's Dream to accumulate, compartmentalize and tolerate more than a ten-told higher Cl^- concentration is surprising. It should also be mentioned that the cultivar Black Spanish also performed extremely well under these conditions, it accumulated 239 ug/g Cl^- in its shoot tissue and along with Dunstan's Dream displayed the least amount marginal necrosis ratings across two rounds of testing.

4.2 Field Test

Leaf and petiole tissue analysis is a direct measure of the vine's mineral nutrient status. Mineral concentrations of select tissues can account for a vine's nutrient uptake, movement, accumulation and compartmentalization. Nutrient concentrations vary with tissue type, growth stage, shoot and canopy position, cultivar and growing season (Christensen, 2005). This affects recommendations for tissue selection for analysis. Some viticulturists sample only petioles, while others recommend both leaf and petiole (Christensen, 2005) (Davenport et al., 2017). Therefore, specific sampling techniques and nutrient standards have been established according to the needs of the vines at different times of the year. Nitrogen, for example, is needed earlier in the year during shoot growth and flowering in greater concentration than later during veraison or harvest, and this nutrient is mobile. In this study, whole leaves were sampled on the third fully expanded leaf from the shoot tip. This tissue location is consistent with commercial recommendation for nutrient analysis post-veraison.

Muscadine grapes are native to the southeastern United States, an area characterized by high rainfall and acidic soil. Muscadines geographic isolation to this area has resulted in a lack of information to be available regarding any potential to tolerate alkaline soil conditions, and this study suggest an inability to tolerate alkaline soils. All 31 muscadine cultivars were below the recommended ranges for NO_3 and would be considered deficient. However, this may be attributed to excessive rainfall prior to sampling and or the mobility of nitrogen in the vine during the latter part of the season when sampling took place. Phosphorus leaf and petiole concentrations were within the sufficient range for three muscadine cultivars and excessive concentrations were observed in the remaining 28 cultivars. This was not expected due to the decreasing availability of phosphorous at soil pHs of 8.0 or greater. These high concentrations were also observed in the rootstocks and hybrids. Calcium and magnesium concentrations in all 31 muscadine cultivars exceeded recommended values, but initial soil tests indicated high concentrations of calcium and magnesium in the soil profile prior to planting. Zinc concentrations were in the sufficient range for 20 muscadine cultivars and the remaining 11 excessive. This was also surprising because zinc availability diminishes under alkaline soil conditions. Iron concentration of leaf and petiole samples was extremely high, ten to twenty times the recommend range, however even with this excessive concentration, iron chlorosis symptoms were observed on a number of muscadine cultivars. Iron can be abundant in calcareous soils, but it is often precipitated as insoluble Fe^{3+} oxides and hydroxides making them unavailable for uptake by the roots. Species that have evolved in calcareous soil conditions such as *V. vinifera* and *V. berlandieri* can pump out protons (H^+) and organic acids (malate and citrate) which acidify the soil solution and improves Fe solubilization and uptake. Iron-inefficient species such as *V. labrusca*, *V. riparia*, and *V. rotundifolia* are unable or less efficient in releasing H^+ (Keller, 2015). Although the release of H^+ may enhance the uptake and transport of iron, bicarbonate (HCO_3^-) from calcareous soils leads to changes in the apoplast that inhibit conversion of the inactive Fe^{3+} form to the active form Fe^{2+}. Consequently, Fe^{3+} becomes bound in the apoplast, unable to enter the mesophyll cells which leads to yellowing between veins on young leaves or chlorosis (Mengel et al., 1984). This is the most probable explanation of the high concentrations of iron observed in the muscadines that expressed interveinal chlorosis in the apical regions of their shoots.

There was no statistical difference between the six rootstock cultivars in for Na^+ accumulation in leaves and petioles. All six were significantly lower than all but two muscadine cultivars; Southland and Hall, with a range of 129µg/g to 287µg/g.

The concentration of Cl^- in the leaves varied greatly among the hybrids and muscadines, but no significant differences were observed in the rootstocks. Dunstan's Dream had a higher Cl^- concentration that is higher than the rootstocks by a factor of five. This capability of the own-rooted hybrid cultivars to accumulate higher Cl^- concentrations in their shoot tissue without displaying higher rates of marginal leaf necrosis is consistent with their performance under greenhouse conditions.

The significant differences in vigor may be explained by nutrient availability under alkaline soil conditions. The ability of all six rootstock cultivars to outgrow the longest muscadine by 60cm may be directly related to their capacity to access nutrients in the soil profile. When grown commercially, muscadines are often given twice as much trellis space than bunch grapes due to their inherent vigor. All 31 muscadine cultivars grew relatively evenly only differing by a maximum of 42cm which suggests that the effect of the alkaline soil pH, and low nitrogen availability in the soil profile restricted growth for cultivars that did not possess a vigorous root system to actively seek out nutrients, or do not have mechanisms to sequester soil nutrients that function at the same capacity as bunch grapes.

CHAPTER V

CONCLUSIONS

Salinity is a challenge for agriculture around the world. Grapes are considered to be moderately salt tolerant, and the use of salt tolerant rootstocks is often recommended for bunch grapes when salinity in the soil or water is present at levels that are thought to be limiting. Grapes are more sensitive to Cl^- than Na^+, but both can produce toxic effects at high concentrations. Rootstocks that are considered to be salt tolerant are thought to more effectively exclude these ions at the root soil interface. The sensitivity to salinity and exclusion capacity of muscadine grapes and the interspecific hybrid grapes Blanc Du Bois and Black Spanish, which are most commonly grown un-grafted, has not been previously reported. This research aimed to compare the salt and alkaline soil tolerance of muscadine grapes, interspecific hybrid grapes, and common bunch grape rootstock cultivars using greenhouse and field studies.

In two greenhouse tests, there was no clear difference in salt exclusion between the muscadines and bunch grape root stocks. However, a wide range of exclusion properties was observed across the muscadine cultivars under study suggesting the potential of using muscadine rootstocks on vineyard sites where salinity poses a risk. This is the first report on salt tolerance in muscadines.

In the field study, a wide range of tissue Na^+ and Cl^- was observed across the forty-two cultivars studied, although salinity in the soil and irrigation water used was within a commercially acceptable range. As a whole, there was not a clear difference in shoot Cl^- concentrations between the bunch grape rootstocks and most of the muscadines, but the bunch grape rootstocks generally contained lower concentrations of shoot Na^+. In the greenhouse studies, the muscadine cultivars generally exhibited less leaf necrosis and greater biomass than the bunch grape rootstocks, but the opposite was observed in the field likely as a result of their nutritional status and poor alkaline soil tolerance.

In both field and greenhouse studies, the hybrid white wine grape cultivar Blanc Du Bois contained among the greatest concentrations of tissue Na^+, and Cl^- suggesting a poor capacity to exclude salts. This was also observed to a lesser extent in the red wine hybrid grape cultivar, Black Spanish, which is also grown commercially as ungrafted. This research suggests that grafting may be beneficial for these wine grapes on sites where salinity is a problem. Further studies should be conducted to evaluate the potential of salt excluding muscadines as rootstocks. Bunch grapes are routinely grafted, but muscadines are not due to their incompatibly with bunch grape species.

LITERATURE CITED

Adcock, D., McNeill, A.M., McDonald, G.K. and Armstrong, R.D., 2007. Subsoil constraints to crop production on neutral and alkaline soils in south-eastern Australia: a review of current knowledge and management strategies. Aust. J. Exp. Agric., 47(11), pp.1245-1261.

Alexander, D. McE. and J. Groot-Obbink. 1971. Effect of chloride in solution culture on growth and chloride uptake of Sultana and Salt Creek grape vines. Aust. J. Exp. Agric. 11:357-361.

Andersen, P.C., T.E. Crocker, and J. Breman. 2010. The muscadine grape. University of Florida, IFAS Extension :1-18.

Brady, N.C. and Weil, R.R., 2002. Soil of dry regions: alkalinity, salinity, and sodicity. p. 412-448. In: The nature and properties of soils. Thirteenth ed. Pearson.

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.

Bresler, E., B.L. McNeal and D.L. Carter. 2012. Saline and sodic soils: principles-dynamics-modeling (Vol. 10). Springer Science & Business Media.

Carroll, D.E. 1985. Muscadine grapes: Factors influencing product quality, p.177-197. In: H.E. Pattee (ed.). Evaluations of quality of fruits and vegetables. Springer, Boston, MA.

Christensen, L.P. and Smart, D.R., 2005. Proceedings of the soil environment and vine mineral nutrition symposium, San Diego, California, USA, 29-30 June, 2004. In Proceedings of the soil environment and vine mineral nutrition symposium, San Diego, California, USA, 29-30 June, 2004. Amer. Soc. Enol. Vit..

Clark, J.R. 1997. Grapes. In: The Brooks and Olmo register of fruit and nut varities. Third ed. ASHS Press, Alexandria, Virginia, pp. 248-299.

Cousins, P. 2005. Evolution, genetics, and breeding: viticultural applications of the origins of our rootstocks. In Grapevine Rootstocks: Current Use, Research and Application. Peter Cousins and R. Keith Striegler (eds.), pp. 1-7, Osage Beach, Missouri.

Cousins, P. 2011. Three root-knot nematode resistant rootstocks released by USDA Agricultural Research Service. FPS grape program newsletter.

Davenport, J.R., J.D. Lunden, and T. Winkler. 2012. Wine grape tissue nutrient concentrations in the inland Pacific Northwest. Comm. Soil Sci. Pl. Anal. 43(1-2), pp.21-27.

Day, A.D. and K.L. Ludeke. 1993. Soil alkalinity, p.35-37. In: A.D. Day and K.L. Ludeke (eds.). Plant nutrients in desert environments. Springer-Verlag, Berlin.

Dearing, C. 1947. Muscadine grapes. US Dept. of Agriculture, Farmers' Bul. 1785.

Dearing, C. 1948. New muscadine grapes. U.S. Dept.of Agr. Circ. 769.

Delas, J. and Pouget, R., 1984. Action de la concentration de la solution nutritive sur quelques caractéristiques physiologiques et technologiques chez Vitis vinifera L. cv." Cabernet-Sauvignon". II.-Composition minérale des organs végétatifs, du moût et du vin. Agronomie, 4(5), pp.443-450.

Downton, W.J.S. 1977a. Influence of rootstocks on the accumulation of chloride, sodium and potassium in grapevines. Aust. J. Agric. Res. 28:879-889.

Downton, W.J.S. 1977b. Chloride accumulation in different species of grapevine. Sci. Hort. 7: 249-253.

Dry, N. 2007. Grapevine Rootstocks: Selection and Management for South Australian Vineyards. Lythrum Press, Adelaide.

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

Einset, J. and C. Pratt. 1975. Grapes, p. 130-153. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Flowers, T.J. and A.R. Yeo. 1995. Breeding for salinity resistance in crop plants—where next? Aus. J. of Plnt. Phys. 22: 875–884.

Fort, K. and A. Walker. 2011. Breeding salt tolerant rootstocks. FPS Grape Program Newsletter 10: 9-11.

Fort, K.P., Lowe, K.M., Thomas, W.A. and Walker, M.A., 2013. Cultural conditions and propagule type influence relative chloride exclusion in grapevine rootstocks. Amer. J. Eno. & Vit., pp. ajev-2013.

Galet, P. 1998. Grape Varieties and Rootstock Varieties. Oenoplurimédia, Chaintré, France.

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

Gillingham, M, and M.Tester. 2005. The regulation of anion loading to the maize root xylem. Plant Physiol. 137:819-828.

Goldy, R.G., E.P. Maness, H.D. Stiles, J.R. Clark, and M.A. Wilson. 1989. Pigment quantity and quality characteristics of some native Vitis rotundifolia Michx. Amer. J. Eno. & Vit. 40: 4: 253-258.

Goldy, R.G. and O.U. Onokpise. 2001. Genetics and breeding, p.51-90. In: F.M. Basiouny, and D.G. Himelrick (eds.). Muscadine grapes. ASHS Press, Alexandria, VA.

Goode, D.Z. and R.P. Lane. 1983. Rooting leafy muscadine grape cuttings. Hort. Sci. 18:944-946.

Harbone, J. B. 1993. The plant and its biochemical adaptation to the environment. In: Introduction to ecological biochemistry. 5-54. Acad. Press, Harcourt Brace & Co. Publ., London.

Hedrick, U.P. 1908. The Grapes of New York. State of New York Dept. Agr. 15th Annu. Rpt. vol. 3, Pt. 2.

Hickinbotham, A.R. 1933. Soluble Salts in Non-Irrigated Vineyards. Bulletin 279, pp. 217-223. Department of Agriculture of South Australia, Adelaide.

Husmann, G.C. and C. Dearing. 1916. Muscadine grapes.U.S. Dept. of Agr. Farmers' Bul. 709.

Iseki, K., Marubodee, R., Ehara, H. and Tomooka, N., 2017. A rapid quantification method for tissue Na+ and K+ concentrations in salt-tolerant and susceptible accessions in Vigna vexillata (L.) A. Rich. Plant Production Science, 20(1), pp.144-148

Jones, J.B. 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, Boca Raton, FL.

Kamas, J., L. Stein, and M. Nesbitt. 2010. Pierce's Disease Tolerant Grapes. 15 December 2017. <http://aggie-horticulture.tamu.edu/fruit-nut/files/2010/10/pd-grapes.pdf>

Keller, M. 2010. The science of grapevines: anatomy and physiology. Academic Press.

Konukcu, F. 2006. Potential of dry drainage as a sustainable solution to waterlogging and salinization. In – Biosaline Agriculture and Salinity Tolerance pp 129-135. M. Ozturk, Y. Waisel, M.A. Kahn, G. Gork (Eds). Birkhauser, Basel, Switzerland.

Lane, R.P. 1978. Bunch grape research in Georgia. Vin. Wine Gro. J. 5. 63-65.

Lauchli A. 2002. In Salinity: Environment—Plants—Molecules. A. Lauchli and U. Luttge (Eds.), pp. ix-x Kluwer Academic Publishers, Boston, U.S.A.

Lorenz, D.H., K.W. Eichhorn, H. Bleiholder, R. Klose, U. Meier and E. Weber. 1995. Growth Stages of the Grapevine: Phenological growth stages of the grapevine (Vitis vinifera L. ssp. vinifera)—Codes and descriptions according to the extended BBCH scale. Aus. J. Gra. & Wine Res. 1(2) pp.100-103.

Henderson, S.W., J.D. Dunlevy, Y. Wu, D.H. Blackmore, R.R. Walker, E.J. Edwards, M. Gilliham, and A.R. Walker. 2018. Functional differences in transport properties of natural HKT1; 1 variants influence shoot Na+ exclusion in grapevine rootstocks. New Phyt. 217(3), pp.1113-1127.

Hortorium, L.H.B. 1976. Hortus third: A Concise Dictionary of Plants Cultivated in the United States and Canada. 3rd ed. Macmillan, New York.

McEachern, G.R. and M.L. Baker. 1997. Muscadine fact sheet. 10 November 2017. <https://aggie-hoticulture.tamu.edu/fruit-nut/fact-sheets/muscadine/.

Mengel, K., M.T. Breininger and W. Bübl, W. 1984. Bicarbonate, the most important factor inducing iron chlorosis in vine grapes on calcareous soil. Plant and soil. 81(3), pp.333-344.

Merry, R.H. 2009. Acidity and alkalinity of soils, p. 115-119. In: A. Sabljic (ed.). Environmental and ecological chemistry. Eolss Publishers, Oxford. U.K.

Mills, H.A. and G. M. Bryson. 2015. Plant analysis handbook IV: A guide to sampling, preparation, analysis, and interpretation for agronomical and horticultural crops. Micro Macro Publishing, Athens, GA.

Mortensen, J.A., 2001. Cultivars, p.91-106. In: F.M. Basiouny, and D.G. Himelrick (eds.). Muscadine grapes. ASHS Press, Alexandria, VA.

Munns R. 2002. Comparative physiology of salt and water stress. Plant, Cell and Environment 25,239–250.

Munns, R. 2005. Genes and salt tolerance: Bringing them together. New Phytologist 167:645-663.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59:651-681.

Munns, R. and Termaat, A., 1986. Whole-plant responses to salinity. Functional Plant Biology, 13(1), pp.143-160.

Munson, T.V. 1909. Foundations of American grape culture. T.V. Munson & Son, Denison, Texas.

Nelson, M. & Mareida, M. 2001. Environmental impacts of the CGIAR: an assessment, in Doc. No. SDR/TAC:IAR/01/11 presented to the Mid-Term Meeting, 21-25 May, Durban, South Africa.

Oldeman, L., Hakkeling, R. & Sombroek, W. 1991. World map of the status of human-induced soil degradation. Wageningen, ISRIC and Nairobi, UNEP.

Olien, W.C. 1990. The muscadine grape: botany, viticulture, history, and current industry. HortScience 25 7:732-739.

Pongrácz, D.P. 1983. Rootstocks for Grape-vines. David Philip Publisher (Pty), Capetown, South Africa.

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.

Provin, T. and J. L. Pitt, 2004. Managing soil salinity. Texas Agric. Ext. Serv. Bull. E- 60. Texas A&M Univ., College Station.

Reynolds, A.G. and D.A. Wardle. 2001. Rootstocks impact vine performance and fruit composition of grapes in British Columbia. Hort. Tec. 11:419-427.

Riaz, S., A.C. Tenscher, 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. Am. Soc. Hort. Sci. 133(4), pp.559-568.

Rimmerman, F. 2015. The economic impact of wine and wine grapes on the state of Texas. Helena Ca.

Shaul, 2002. Magnesium transport and function in plants: the tip of the iceberg. Biometals 15:307-321.

Sivritepe, N., H.O. Sivritepe, H. Celik, and A.V. Katkat. 2010. Salinity responses of grafted grapevines: Effects of scion and rootstock genotypes. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(3), p.193.

Storey, R. and R.R Walker. 1999. Citrus and salinity. Sci. Hort. 78:39-81.

Storey, R., D.P. Schachtman, and M.R. Thomas. 2003. Root structure and cellular chloride, sodium and potassium distribution in salinized grapevines. Plant Cell Environ. 26:789-800.

Sykes, S.R. 1987. Variation in chloride accumulation in hybrids and backcrosses of Vitis 573 berlandieri and Vitis vinifera under glasshouse conditions. Am. J. Enol. Vitic. 38: 313-320.

Tanji, K.K. (ed.) 1990. Agricultural salinity assessment and management. American Society of Civil Engineers Manuals and Reports on Engineering Practice No. 71. American Society of Civil Engineers, New York, NY.

Tanji, K.K. 2002. Salinity in the soil environment. In Salinity: Environment – Plants – Molecules. A. Lauchli and U. Luttge (Eds.), pp. 21-51. Kluwer Academic Publishers, Boston, U.S.A.

TABC. Texas Alcoholic Beverage Commission. 2018. License/Permit Information by Class. G. Winery Permit. https://www.tabc.state.tx.us/public_information/listing_by_class_location.asp.

Thomas, W.A. 2011. Development of a Repeatable, Low-Cost, High-Throughput and Precise Salt Tolerance Assay for Grapevines. University of California, Davis.

Thompson, T.L. and J.L. Walworth. 2006. Salinity management and soil amendments for southwestern pecan orchards. Rep. Dep. of Soil, Water, and Environmental Science, Univ. of Arizona, Tucson.

Townsend, C.G., D.R. Butler, and R.W. Dixon. 2016. The Challenge of Growing Wine Grapes in the Hill Country: An Evaluation of Changing Grower Perceptions of Natural Hazards in Texas Vineyards. Papers in Applied Geography, 2(1), pp.105-112.

Tregeagle, J.M., J.M. Tisdall, M. Tester, and R.R. Walker. 2010. Cl- uptake, transport and accumulation in grapevine rootstocks of differing capacity for Cl- exclusion. Funct. Plant Biol. 37: 665-673

U.S. Department of Agriculture. n.d. Grape production in the United States in 2015, by state (in 1,000 tons). Statista. Accessed 27 August 2017. Available from

<https://www.statista.com.ezproxy.library.tamu.edu/statistics/193913/top-10-grape-producing-us-states/>

U.S. Department of Agriculture, Natural Resources Conservation Service. 2017. State search for genus. 20 September 2017. <https://plants.usda.gov/java/stateSearch>..

Volkmar, K.M., Y. Hu, and H. Steppuhn. 1998. Physiological responses of plants to salinity: a review. Can. J. Plt. Sci. 78(1), pp.19-27.

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) 1. Yield and vigour inter-relationships. Aust. J. of Gra. Win. Res. 8; 3–14.

Williams, C.F. 1923. Hybridization of Vitis rotundifolia. Inheritance of anatomical stem characters. North Carolina Agr. Sta. Tech. Bul. 23.

Wines & Vines. n.d. Number of wineries in the United States in 2017, by state*. Statista. Accessed 27 August, 2017. Available from <https://www.statista.com.ezproxy.library.tamu.edu/statistics/259365/number-of-wineries-in-the-us-by-state/.>

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. 1974. General viticulture. Univ. of Calif. Press, Berkeley.

Wolf, T.K. and T.R. Bates. 2008. Nutrient management. In: Wine grape production guide for eastern North America. T. K. Wolf (ed.). pp. 141-169. Plant and Life Sciences Publishing, Ithaca, New York.

Woodroof, J.G. 1936. Developments in growing muscadine grapes in the south. Proc. Amer. Soc. Hort. Sci. 33:447-449.

Young, W.J. 1920. Muscadine grapes: Culture and varieties. South Carolina Agr. Expt. Sta. Bul. 205.

APPENDIX A

A - 1 : Greenhouse test one data means.

	g		µg/g				0-4
Cultivar	Root	Shoot	Root Na	Root Cl	Shoot Na	Shoot Cl	Rating
101-14 Mgt.	2.23	2.45	50	83	69	116	1.67
1103P	1.44	1.74	79	153	90	112	2.08
140Ru	1.24	1.85	57	188	81	90	1.67
420A	2.09	2.50	82	169	88	111	2.00
Matador	1.41	1.81	102	193	102	179	1.08
Schwarzmann	2.09	2.46	125	163	126	151	1.00
Black Spanish	3.24	4.16	159	303	118	226	0.25
Blanc du Bois	2.09	3.55	189	587	204	330	1.00
Dunstans Dream	1.34	2.89	170	611	98	187	0.17
Southern Home	5.12	9.43	83	183	141	130	0.83
Victoria red	1.53	2.08	126	272	145	191	1.83
Alachua	3.72	3.24	108	209	144	200	1.25
Albemarle	2.90	3.96	116	224	138	178	1.08
Black Beauty	3.62	6.89	115	214	113	122	0.75
Black Fry	2.96	5.29	105	158	109	113	0.92
Bountiful	2.79	3.16	93	177	124	149	1.00
Carlos	6.00	8.26	120	113	101	93	1.00
Creek	2.22	6.64	109	280	79	170	1.00
Darlene	4.82	4.75	59	123	72	95	0.75
Delicious	2.77	3.75	98	258	150	184	0.92
Dixie	2.39	3.75	152	264	92	148	1.00
Dixiered	2.73	4.57	105	134	137	116	0.58
Doreen	3.72	4.43	154	249	180	178	1.00
Eudora	6.00	8.05	89	100	104	79	1.00
Fry	4.27	3.28	87	103	89	79	0.92
Granny Val	5.29	6.14	89	152	87	96	0.92
Hall	3.64	5.10	189	333	137	168	1.00
Higgins	4.83	5.53	125	142	122	118	0.75
Hunt	3.64	3.72	115	159	116	97	0.75
Janebell	3.38	3.71	75	100	103	83	0.83
Late Fry	4.69	4.33	96	102	99	93	1.00
Loomis	2.89	4.01	128	272	99	133	0.67
Magnolia	4.59	7.35	108	170	110	104	0.92
Magoon	2.80	3.69	111	180	89	119	1.00
Pam	2.98	3.10	72	135	130	124	1.00
Scuppernong	2.79	2.88	58	134	69	62	1.00
Southern Jewel	2.28	3.05	105	293	95	206	1.00
Southland	2.58	5.72	151	366	138	172	0.83
Sterling	2.37	2.56	85	269	137	232	0.92
Supreme	3.53	5.84	115	254	103	96	1.00
Triumph	4.14	3.67	105	189	116	99	0.50
Welder	3.29	3.79	67	161	86	130	1.00

A - 2 : Greenhouse test two data means.

	g		µg/g				0-4
Cultivar	Root	Shoot	Root Na	Root Cl	Shoot Na	Shoot Cl	Rating
101-14 Mgt.	1.88	2.17	76	116	78	119	1.58
1103P	1.47	1.84	92	171	100	125	2.08
140Ru	1.32	1.82	74	182	87	98	1.59
420A	1.48	2.03	105	159	99	162	1.92
Matador	1.52	2.20	103	266	96	150	1.00
Schwarzmann	2.04	2.35	149	196	120	179	1.00
Black Spanish	4.71	5.37	160	359	92	239	0.17
Blanc du Bois	4.12	5.46	306	704	181	319	0.92
Dunstans Dream	1.87	2.77	354	1209	98	238	0.08
Southern Home	1.42	4.09	117	255	153	149	0.83
Victoria red	2.22	2.46	144	300	179	258	2.08
Alachua	3.97	2.66	115	212	154	196	1.42
Albemarle	3.40	4.41	145	217	162	240	1.17
Black Beauty	1.73	3.31	136	232	126	169	0.67
Black Fry	2.97	2.71	126	171	112	118	0.92
Bountiful	3.28	3.01	175	351	205	156	1.00
Carlos	1.70	2.47	157	148	133	121	0.92
Creek	2.13	4.10	114	311	75	167	0.92
Darlene	1.71	3.24	94	164	79	111	0.75
Delicious	2.10	3.89	73	217	105	162	1.00
Dixie	2.12	5.23	126	269	102	169	1.08
Dixiered	1.56	2.67	123	144	170	146	0.50
Doreen	2.55	4.97	164	225	166	137	1.00
Eudora	1.52	2.71	114	175	93	88	1.00
Fry	1.72	2.04	136	237	163	137	0.92
Granny Val	2.02	6.07	112	165	84	116	0.67
Hall	2.25	5.10	196	316	117	161	0.92
Higgins	2.83	4.46	122	141	120	116	0.83
Hunt	2.05	4.18	228	570	128	204	0.75
Janebell	1.70	2.99	59	71	93	82	0.92
Late Fry	2.44	4.47	79	108	95	92	1.00
Loomis	2.15	4.51	115	268	120	179	0.67
Magnolia	1.60	2.67	95	225	101	182	0.83
Magoon	2.55	4.19	144	281	170	234	1.00
Pam	2.06	3.15	98	136	131	130	1.00
Scuppernong	2.93	4.38	63	151	73	70	1.00
Southern Jewel	2.22	5.11	104	264	69	186	1.00
Southland	4.03	6.26	163	344	138	169	0.75
Sterling	2.33	3.30	190	389	165	348	1.00
Supreme	2.64	5.20	101	261	113	110	1.00
Triumph	1.93	1.63	110	221	108	104	0.42
Welder	3.66	3.93	96	237	113	138	1.00

A - 3 : Field test data means.

	Julian days	0-4		cm
Cultivar	Bud Break	Rating 1	Rating 2	Vigor
101-14 Mgt.	81.00	1.00	0.00	234.08
1103P	81.50	1.25	0.00	188.67
140Ru	81.50	1.08	0.00	252.08
420A	85.25	1.00	0.00	251.00
Matador	83.75	1.00	0.00	276.58
Schwarzmann	80.50	1.00	0.00	219.83
Black Spanish	84.50	1.08	0.00	222.42
Blanc du Bois	82.25	1.00	0.67	189.67
Dunstans Dream	78.50	1.17	0.00	229.83
Southern Home	92.25	1.17	2.83	128.58
Victoria red	79.25	1.67	0.08	157.67
Alachua	91.00	1.08	1.25	126.33
Albemarle	93.50	1.42	0.83	110.17
Black Beauty	86.50	1.00	2.08	93.33
Black Fry	87.75	1.00	1.42	116.25
Bountiful	88.75	1.25	1.50	113.33
Carlos	85.75	1.33	2.25	113.42
Creek	90.50	1.25	2.00	108.50
Darlene	87.50	1.25	1.83	102.67
Delicious	96.50	1.08	1.50	103.08
Dixie	89.50	1.08	3.33	101.33
Dixiered	86.50	1.00	2.08	116.00
Doreen	86.50	1.33	2.83	119.50
Eudora	94.00	1.17	1.33	109.42
Fry	90.75	1.17	2.17	93.50
Granny Val	90.25	1.08	2.83	111.50
Hall	90.25	1.17	0.58	104.50
Higgins	89.00	1.08	2.08	106.50
Hunt	89.00	1.25	3.25	100.08
Janebell	91.75	1.25	2.00	103.33
Late Fry	89.00	1.17	2.67	119.83
Loomis	86.75	1.17	2.67	133.08
Magnolia	85.50	1.00	1.58	100.17
Magoon	88.25	1.42	2.08	123.42
Pam	88.75	1.17	1.00	94.92
Scuppernong	89.00	1.42	2.17	111.75
Southern Jewel	90.00	1.17	1.42	95.50
Southland	87.50	1.08	2.42	113.75
Sterling	90.75	1.00	2.92	126.33
Supreme	87.75	1.17	3.00	108.58
Triumph	89.75	1.25	1.92	110.42
Welder	85.50	1.08	1.83	91.42

APPENDIX B

B - 1 : Soil sample results from field plot.

Analysis	Result	Critical Level[z]	Units
pH	8.00	5.8	-
Conductivty	316	-	µmho/cm
Nitrate -N	14	-	µg/g
Phosphorus	31	0	µg/g
Potassium	369	0	µg/g
Calcium	6127	180	µg/g
Magnesium	239	50	µg/g
Sulfur	18	13	µg/g
Sodium	55	-	µg/g
Iron	5.52	4.25	µg/g
Zinc	0.35	0.27	µg/g
Manganese	2.13	1.00	µg/g
Copper	0.53	0.16	µg/g
Boron	0.69	0.60	µg/g
Chloride	10.4	-	µg/g

[z] Critical level is the point which no additonal nutrient is recommended.

B - 2 : Well water sample results

Parameter		Results	Units	Method
Calcium (Ca)		124	µg/g	ICP
Magnesium (Mg)		38	µg/g	ICP
Sodium (Na)		116	µg/g	ICP
Potassium (K)		4	µg/g	ICP
Boron (B)		0.89	µg/g	ICP
Carbonate (CO_3)		0	µg/g	Titr.
Bicarbonate (HCO_3)		719	µg/g	Titr.
Sulfate (SO_4)		35	µg/g	ICP
Chloride (Cl^-)		36	µg/g	Titr.
Nitrate -N (NO_3-N)		0.68	µg/g	Cd-red.
Phosphorus (P)		0.08	µg/g	ICP
pH		6.97		ISE
Conductivty		1304	µmhos/cm	Cond.
Hardness		27	grains CaCO3/gallon	Calc.
Hardness		467	µg/g CaCO3	Calc.
Alkalinity		589	µg/g CaCO3	Calc.
Total Dissolved Salts (TDS)		1074	µg/g	Calc.
SAR		2		Calc.
Iron (Fe)		86	µg/g	ICP
Zinc (Zn)	<	0.01	µg/g	ICP
Copper (Cu)	<	0.01	µg/g	ICP
Manganese (Mn)		0.09	µg/g	ICP

ICP - Inductively coupled plasma; Titr. - Titration; ISE - Ion selective electrode; Cd-red. - Cadmiun reduction; Cond. - Conductivity; Calc. - Calculated