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Exploration of Grapevine Rootstocks to Combat Chloride-Induced Toxicity

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Exploration of Grapevine Rootstocks to Combat Chloride-Induced Toxicity

By

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DISSERTATION

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Abstract

Chloride-induced grapevine toxicity is a leading contributor to abiotic stress in viticultural regions characterized by aridity and limited water availability. Although an essential element for plants, in large enough quantity chloride can reduce net photosynthesis, leaf area, and water uptake in grapevines. Furthermore, the scarcity of water for irrigation has promoted the use of water use efficient irrigation methods such as dripline application, which can limit leaching of nutrients and toxins, and a build-up of salts on the periphery of the wetting-zone. Deficit irrigation practices can also be utilized to improve fruit quality. This dissertation explores the range of tolerance to toxic soil-chloride concentrations in genus *Vitis*, limitations and thresholds for chloride tolerance in grapevines, and the potential for breeding chloride tolerant grapevine rootstocks. Each topic is expanded in chapters 1, 2, and 3 of this dissertation, respectively.

The historic threat of Grapevine Phylloxera in vineyards has resulted in the proliferation of grapevine rootstock cultivars to address this pest and other stressors. Relative to many other perennial crops, grapes have a wide selection of stock to choose from. However, there are innumerable, untested, and often undiscovered species and landraces undisturbed in their native habitats of the Americas that may hold further benefits to vineyards worldwide. To better define the range of chloride tolerance in *Vitis* germplasm, we examined the resistance to chloride toxicity in six species of wild grapevine collected from the south and southwestern United States alongside several cultivated and widely utilized rootstock cultivars. Presented in chapter 1, results from this survey suggest untapped potential for quasi-halophytic properties in some wild grapevine species such as *V. acerifolia* and *V. doaniana*. In chapter 2 common varieties were tested against a gradient of applied sodium chloride ranging from 0 – 100 mM NaCl to better define the chloride concentration threshold at which photosynthetic function begins to decline in commercial

rootstock cultivars and chloride becomes toxic. Chapter 2 establishes a range of NaCl exposure between 25-75 mM NaCl in which photosynthetic function begins to break down in many rootstock varieties. Finally, chapter 3 crosses a highly tolerant and highly susceptible parent to explore the breeding potential of chloride tolerance phenotypes in grapevine offspring. Throughout the 77 tested offspring we observed a wide range of continuous increments of foliar chloride accumulation, suggesting possible heritability of chloride-excluding phenotypes. These experiments suggest an untapped source of chloride tolerance in grapevines exists, the range at which these semi-tolerant varieties have the highest efficacy, and support the heritability of chloride-excluding phenotypes in subsequent generations. Taken together, this project increases the likelihood a breeding program can introduce chloride-tolerant grapevine rootstock phenotypes that could be successful.

Acknowledgments

The nature of this work required much time, knowledge, care, and commitment from a team of researchers at the Department of Viticulture and Enology at the University of California Davis. All plant material used in this study were collected and maintained by the Walker Grapevine Breeding lab at UC Davis; these were personally collected by Dr. M. Andrew Walker and a successive string of researchers and graduate students, some of which I did not have the pleasure of meeting personally. However, I'd like to offer my sincere gratitude to those who drove tirelessly across the United States of America and trekked into marshy swamps and arid deserts to provide me the opportunity to conduct these studies.

Propagation, growth, and plant care must be mostly attributed to Nina Romero, Gonzalo, and Joves, who are all astounding viticulturalists and aided in my work while providing me a unique and highly valued educational experience. Instrumental to the quantification of plant-assimilated chloride was student assistant, Jorge Rocha-Figueroa, who is the most tireless and reliable colleague I have ever had. Support from numerous individuals in the Walker lab came in various forms and I would like to thank Dr. Kevin Fort, Dr. Alan Tenscher, Dr. Summaira Riaz, Dr. Andy Nguyen, Dan Ng, and all members of the Walker lab who have made this work possible.

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Dedication

For all who wish to learn.
Knowledge should be shared.

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Chapter 1. Screening *Vitis spp.* for degrees of tolerance to high-input external sodium chloride concentrations and traditional breeding potential of candidate cultivars

1.1 Abstract

Unstable climatic conditions have begun to impact the health and productivity of grape vineyards throughout the world. These conditions have also stimulated the development of new viticultural practices to address decreases in precipitation, increases in diurnal temperature shifts, and greater water scarcity. Among the indirect effects of climate change is an increase in salinity concentrations of previously arable land. Although intended to increase water use efficiency and improve fruit quality, implementation of low-input drip irrigation followed by deficit irrigation practices in vineyards can exacerbate buildup of salts such as sodium chloride in vineyard soils. A demand for grapevine rootstocks tolerant to high sodium chloride concentrations has emerged to address vineyard productivity in the face of increasing soil salinity. However, the evaluation of a select pool of grapevine rootstocks resulted in a limited set of potential sources of salt tolerance. This study includes four separate trials and investigates the tolerance of 152 wild grapevine species, 20 established rootstock cultivars, and a breeding population to chloride toxicity under exposure to 75 mM NaCl concentrations over 21 days. Large variability was observed in the final chloride accumulation in leaf and petiole tissues among tested accessions. Established and cultivated rootstocks accumulated more chloride than many wild grapevines examined, suggesting the potential for a large pool of NaCl resistance in untested, wild germplasm. Results from the testing of a population resulting from susceptible GRN3 x tolerant *Vitis acerifolia* 9018 implied

continuous variation in chloride accumulation in above ground tissues in the offspring, but no variation in root chloride accumulation. Our findings suggest the mechanism of chloride tolerance in grapevines may be found in the ability of grapevine roots to limit chloride ion entry into xylem vessels and prevent long distance transport to photosynthetic tissues.

Key words: Cultivar, Salt sequestration, Salt exclusion, Salinity tolerance

1.2 Introduction

Vitis species, particularly *V. vinifera*, have long been useful in grapevine breeding. However, a relatively new development is the use of *Vitis* species outside of *V. vinifera* in rootstock breeding programs to combat pests, diseases, and other soil-based limitations (Carbonneau 1985, Ollat et al. 2016b). As such, developments have paved the way for rootstocks which are tolerant or resistant to phylloxera, nematodes, grapevine fanleaf virus, toxic nutrients in soils, and other critical concerns in vineyards (Christensen et al. 2003, Dandekar et al. 2019, Keller 2010).

Over 80% of rootstocks originally utilized for these purposes consisted of four species or hybrids: *V. rupestris*, *V. berlandieri*, *V. riparia*, and *V. vinifera* (Ollat et al. 2016a). Grapevine rootstocks have been utilized to control scion vigor, as a method of pest and disease tolerance, and to help tolerate environments adverse to productivity (Sabbatini and Howell 2013). However, the aforementioned four species provide limited sources of genetic material to combat biotic and abiotic stress. Wild species have the potential to address concerns the original four species could not, such as nematode resistance or drought tolerance (Ferris et al. 2012, Padgett-Johnson et al. 2003).

Global temperatures have increased 0.2ºC per decade since the pre-industrial era, and this has impacted multiple industries and environmental factors (Masson-Delmotte et al. 2018). Although climatic shifts are not to be ignored, it is also important to confront the more subtle responses of our changing climate; one of these is the concern that agricultural soils will become more saline as precipitation patterns shift and water becomes more limited in regions with intensive agriculture. Cultural practices such as drip-irrigation and deficit irrigation may contribute significantly to soil salinization under these conditions.

Much of the world's arable land is already heavily laden with salts which can limit the productivity of high-output agriculture. In Tunisia, approximately 25% of arable land is high in salinity levels (Askri et al. 2012). South Australia is also of concern, with predictions of increased irrigation demands, increased drought incidence, and increases in root zone salinity in grape vineyards as precipitation decreases in the approaching decades (Phogat et al. 2018). The aquifers in the San Joaquin Valley of California are also predicted to experience salinization (Schoups et al. 2005). Grapevines, like most agronomic crops are glycophytic and thus, do not tolerate high sodium chloride concentrations very well (Flowers 2004, Heinitz et al. 2015). Thresholds for grapevine salt tolerance begin at 2 $dS\ m^{-1}$, or about 5% the salinity of seawater, and most varieties cannot survive past 16 $dS\ m^{-1}$ (Zhang et al. 2002).

Because differences in chloride tolerance in members of *Vitis* spp. are widely observed, it is likely that rootstocks could be selected for the purposes of enhancing NaCl tolerance in highly salinized vineyards. Given that salt-tolerance can be selectively bred into a population, and the importance of novel candidates likely to pass down this trait, it is essential to expand the genetic pool of salt tolerant accessions for further use in breeding initiatives (Antcliff et al. 1983). Previous work from Heinitz et al. (2015) helped to identify many candidate grapevines for chloride-

tolerance testing but worked at a low concentration of 25mM NaCl. Testing accessions found in Heinitz et al. (2015) at higher NaCl concentrations contributed to more pronounced differences in foliar chloride accumulation. Additionally, expanding the tested accessions to many more individuals provided a clearer image of grapevines' potential for chloride tolerance and for heritability of chloride-tolerant phenotypic traits.

Our study seeks to assess the viability of a rapid and reliable method to identify accessions of wild and cultivated grapevines whose tolerance to chloride-induced toxicity is greater than average and may be used in future breeding efforts. More than 152 accessions from across the south and southwestern United States collected over 40 years were tested in three trials at 75 mM NaCl, or 7.5 $dS\ m^{-1}$, in fertigated water applied to an external media of fritted clay (Alizadeh et al. 2010). Leaf chloride concentrations were determined following each trial using destructive methods. The identification of an accession particularly tolerant to chloride-induced toxicity was then assessed for breeding potential using a single cross with the recently developed grape rootstock GRN3.

1.3 Materials and Methods

A series of four exploratory experiments were conducted to survey the potential for chloride tolerance in a wide range of grapevine species, both domestic and wild. Each experiment was designed and performed with a few shared species referred to as standards but separately explored chloride accumulation of multiple novel individuals. Methods for propagation, treatments, and sample processing were identical in all trials. The first experiment looked closely at leaf and root chloride sequestration in unique individuals of four wild species of grapevines found throughout the western and southwestern United States. The second experiment used many of the genotypes from the first study but added non-salted control replicates to observe any inherent differences in chloride uptake in each accession. Trial three was designed to test the response of unique *Vitis*

berlandieri individuals to high chloride exposure in rooting zones. The final study was designed to observe the ability of offspring to inherit leaf-chloride exclusion traits from a parent vine. This study exposed offspring of a highly salt tolerant parent and salt susceptible parent to high NaCl concentrations.

Plant Materials

Accessions of *Vitis* spp., originally collected in regions spanning from Arkansas to California, were tested for chloride-induced toxicity tolerance. These individuals have been collected over four decades and reside at the University of California, Davis experimental vineyards. Species included in this study include *V. acerifolia, V. arizonica, V. berlandieri, V. doaniana, V. girdiana, V. rupestris*, and cultivars of commercially used rootstocks. Trials ran year-round, however, all cuttings taken to propagate new plants were herbaceous, actively growing shoots collected between the months of March and July in 2018, 2019, and 2020.

Trials varied by tested accessions but contained the same set of standards with known NaCl tolerance: 44-53 M, Rupestris St. George, and Ramsey (Salt Creek). A total of 152 wild accessions and 20 established cultivars were tested across four trials. Results were relativized with respect to the standards used across trials. A cross of GRN3 (L514-10 x *V. champinii* cv c9038) x *V. acerifolia* 9018 was produced and 74 of the resulting offspring were grown from seed and assessed for tolerance to chloride applied to the external rooting media at 75mM NaCl, or approximately 7.5 $dS\ m^{-1}$ using a hand-mixed solution. The saline solution was applied via hand watering.

Propagation

Segments with two to three buds were separated and dipped in a 1:20 dilution of liquid rooting hormone of 1.03% Indol-3-butyric acid and 0.66% 1-naphthalene acetic acid (Wood's rooting compound ™; Earth Science Products Corp; Wilsonville, OR USA), consisting of 10,000 ppm

indole-3butyric acid and 5,000 ppm naphthalene acetic acid, then placed in a 1:1 (w/w) media mixture of vermiculite and perlite in a flat tray. Vegetative cuttings were allowed to leaf out in a fog room for 14 days following collection. To harden them and allow active root growth, they were then moved to a greenhouse set for a diurnal temperature range of 13°C and relative humidity shift of 16 percent from 0300 to 1500 hr daily for 14 days. Each rooted cutting was then transferred to a round 2548 cm^3 plastic pot and filled with a non-swelling, fritted illite clay compound (Turface Pro MVP ™, Profile Products LLC; Buffalo Grove, IL, USA) to prevent soil flocculation effects from applied NaCl. Plants were then allowed to acclimate to the new environment over the course of 21 days while being fertigated with a modified Hoagland's solution (Fort et al. 2015) of N, P, K, Ca, Mg, S, Fe, Cu, B, Mn, Mo, and Zn in appropriate proportions and applied to distilled water via an onsite water-powered chemical injector.

The media used was a large-particle, calcined, non-swelling illite clay with high porosity and relatively high cation exchange capacity of 30 $mEq\ 100g^{-1}$. This 2:1 layer silicate was used to ensure proper binding of potassium, which acts as an interlayer cation, to prevent sodium from over competing for binding sites within the media. Due to the large particle size of the clay, it was also selected to help guard against dispersion and flocculation of soils under NaCl concentrations high enough to promote changes in soil structure. Water holding capacity of this material was very low and required daily irrigation applications.

NaCl application

Following the 56-day growth and acclimation period, based on previous NaCl tolerance studies (Fort et al. 2013) plants were exposed daily to 75 mM, or 7.5 $mEq\ 100g^{-1}$, of NaCl dissolved in 500 mL aliquots of fertigated greenhouse water. Application of 75 mM NaCl concentrated mixture was chosen based on reported limitations for grapevine survival under saline irrigation regimes

(Alizadeh et al. 2010). This application rate continued for 21 days prior to harvest. In both treatments water-powered pressure mixers were used to homogenize control and salt solutions. Salt and control solutions both underwent a primary mixing with Hoagland's solution. Only salt solutions then underwent a secondary mixing to dilute a concentrated solution of 8.6 mol NaCl to 75mMol NaCl for this experiment. Prior to application, 500 mL aliquots of salinized or control water were measured by hand into large beakers and applied by hand to each vine directly. Applicators were cautious not to allow above ground tissue to contact the highly saline water.

Sample collection and preparation

At 21 days of NaCl exposure, all leaves and petioles from each plant were carefully removed, weighed for fresh mass, and individually stored and labelled in brown paper bags. In one trial, roots were washed clean of particles and weighed for fresh mass, and individually stored and labelled in brown paper bags. Weighed samples then were transferred to a drying room at 50°C for 14 days before dry mass was recorded. Shoots and original propagation tissue were excluded from all samples due to inconsistencies in size and age, respectively.

Once fully dried, samples of root and/or leaves were recorded for dry mass and then pulverized to a fine powder. An aliquot of 25 mg of dried and powdered tissue was taken from each sample and added to individual screw cap bottles with 25 mL de-ionized water. All samples were then, simultaneously loaded onto a modified orbital-shaker table and allowed to mix at 200 rpm for 1 h. Preliminary data [not shown] indicated that allowing this for 1 h resulted in minimal differences in chloride readings to those left for 18 h, overnight. Extracted samples were then filtered through 11µm filter paper and the resulting liquid was diluted by a factor of two in order to increase accuracy of the subsequent chloride readings. Chloride content readings from harvested tissues were realized using a silver-ion titration chloridometer (Model 926, Nelson-Jameson Inc.,

Marshfield, WI, USA), and repeated three times per sample, following manufacturer's guidelines for use.

Trial 1: Potential candidates and establishing biocontrol cultivars

A set of 60 accessions were tested for leaf and root chloride accumulation at an external applied concentration of 75mM NaCl dissolved in fertigated water for a 21-day period. Four of these were chosen as standards based on purported NaCl tolerance or susceptibility (Heinitz et al. 2015). Ramsey (Salt Creek), 44-53 M, 140 Ru, and *V. rupestris* St. George were selected for this role. The reference standards were chosen with the intent to span the range of likely Cl- accumulation in leaf and petiole tissues at harvest. Accessions were tested under 75 mM NaCl applied to deionized irrigation water containing a Hoagland's solution mixture. This solution was used for the four trials included in this study. Salinized fertigation solution was applied for 21 consecutive days in all cases. Individual accessions were initially replicated twelve times in this study.

This trial, tested at 75 mM NaCl, included promising candidates from previously tested species. These accessions were mostly collected in the wild and were originally collected from Texas to California across the southern United States. However, some replicates died prior to harvest and thus could not be used to quantify the chloride contents after 21 days; *ANU4* and *b42-34* particularly lost many individuals with only three replicates remaining of each at the time of harvest

Trial 2: Select species with purported NaCl tolerance

The second trial in this study was conducted with similar objectives of establishing baseline Cl^- accumulation ranges for a variety of wild accessions. Controls irrigated with 0 mM NaCl were included for each individual. The previously used standard accession, *V. rupestris* St. George was excluded in this study due to its poor survival in preliminary growth phases. It was replaced with

the previously tested *V. acerifolia* 9018, which performed well in initial trials, accumulating one of the lowest concentrations of Cl^- in leaf and petiole tissue with a low variance in the results.

Trial 3: Comparing accessions of V. berlandieri and previously explored cultivars

Previous work has focused on *V. berlandieri* and closely related rootstocks (Downton 1977, Gong et al. 2010, Sykes 1987, Upadhyay et al. 2013). The reported salt tolerance of *V. berlandieri* and its close relatives gave rise to trial 3 focusing on cultivated and wild accessions. As in previous trials, standards with consistent Cl^- accumulation in leaf and petiole tissue were chosen and represented the range of potential uptake rates in the study population. Standards in this study were limited to *V. acerifolia* 9018 and rootstocks 44-53 M, 110 R, and 140 Ru.

Trial 4: Exploring breeding potential of V. acerifolia 9018

In this trial a population of 74 offspring from GRN3 (L514-10 x *V. champinii* 9038) x *V. acerifolia* 9018 were tested under 75 mM NaCl dissolved in irrigation water for 21 days for Cl^- accumulation in petiole and root tissues.

1.4 Results

Trial 1: Potential candidates and establishing biocontrol cultivars

Results from this initial trial found a wide range of Cl^- accumulation in leaf and petiole tissues throughout the tested accessions (Table 1.1), and illustrated that the variance in Cl^- accumulation among accessions was high. In tested accessions with higher Cl^- contents at harvest, there tends to be higher variability in the recorded values, which may have arisen in relation to growth inhibition. Root Cl^- concentration at harvest displayed much greater variability in the majority of accessions tested than leaf Cl^- concentration and generally spanned a wider range of values (Table 1.1).

Table 1.1 illustrates three distinct relationships between petiole and root Cl^- concentrations in response to high external Cl^- in the tested accessions. For individuals such as *V. acerifolia* 9018, *V. doaniana* 9024, and *V. girdiana* 9024, Cl^- was most strongly accumulated in the root system; this is evidenced by Cl^- concentration values nearly ten-fold higher in roots than petiole tissues for *V. acerifolia* 9018. The opposite was observed for accessions like Rupestris Pump Station, which allowed Cl^- to accumulate in above ground tissues at much higher concentrations than in roots. There were also individuals with near equal concentrations of Cl^- in both tissue types. This final category includes most of the selected standards used in this study. These results indicate that there is little correlation between root and petiole Cl^- values following external NaCl application.

Trial 2: Select species with purported NaCl tolerance

The second trial in this study showed significant differences in the amount of Cl^- accumulated in above ground tissues in both treatments. However control treatments had fewer significant groupings and a more consistent range of values recorded (Figure 1.1). *Vitis acerifolia* 9018 and V. doaniana 9024 were the only accessions in this trial that did not have significantly different petiolar chloride concentrations between the control and the replicates exposed to 75 mM NaCl (Table 1.2).

Under control conditions (0 mM NaCl) the 27 tested accessions separated into 4 distinct groupings based on leaf and petiole Cl^- accumulation showing that significant differences in foliar chloride accumulation occur at low NaCl exposure. However, values recorded never exceeded 30 ppm and vines did not display any chloride toxicity symptoms.

The 27 tested accessions separated into 11 distinct groups based on leaf and petiole [Cl^-] following the 21-day application period. Under 75 mM NaCl applied salt accession choice is highly significant (Table 1.2). Compared with the four groups under control conditions, these divisions

under applied NaCl suggest that there is a strong influence by the rootstock cultivar on their ability to minimize Cl^- accumulation in above ground tissues in wild grapevines. However, latent [Cl^-] with 0 mM NaCl applied via fertigation still was, in some cases, significantly different by the cultivar with a *p value* < 0.0001. Although under control conditions, accession is still a significant variable in this trial, under 75 mM NaCl applied salt the accession variable is also significant with a *p value* = < 0.0001 (Table 1.2).

When quantifying leaf and petiole chloride accumulation in 75 mM NaCl treatments we observed a maximum 3473% increase in 44-53M when compared with a minimum 108% increase in *V. acerifolia* 9018 compared with control replicates (Table 1.2). While baseline chloride accumulation is significantly different in non-saline conditions, under 75mM NaCl the range of foliar chloride accumulation drastically increases and more greatly distinguishes successful and poor chloride excluders.

Trial 3: Comparing accessions of V. berlandieri and previously explored cultivars

Following treatment application and sample processing it was shown that variation in Cl^- accumulation within this closely related test group was still high when treated with 75 mM NaCl in irrigation solution (Figure 1.2). As in previous trials the variation in the control group was minimal. Dry masses of roots and foliar tissues were recorded. In general, the mass of either tissue type did not change in a significant way in response to applied NaCl concentrations in the irrigation water. The mass of roots at harvest tended to be more variable in control treatments than in salt-treated individuals with little distinction in overall root mass among the treatments (Table 1.4). Leaf weights were more variable overall, but still showed little difference between treatments. Leaf and petiole tissue outweighed root tissue in all cases however.

While there were many more significantly different groupings under high NaCl applications than under control conditions where no NaCl was applied (Table 1.2), there were hardly any more significant differences in above-ground tissue mass and no differences in root masses between the treatments. Although leaf and petiole dry weights did vary significantly by genotype under salt treatments, this did not correlate with any of the differences observed in Cl^- accumulation in above ground tissues. Likewise, when not treated with excess NaCl, there were no inherent differences in Cl^- accumulation, leaf and petiole mass, or root mass among the tested rootstocks in this study. However, although not directly correlated with Cl^- accumulation, dry-mass of leaf and petiole tissues did become significant to genotype tested in accessions treated with 75 mM NaCl where otherwise non-significant under control conditions.

Trial 4: Exploring breeding potential of V. acerifolia 9018

Offspring from GRN3 (L514-10 x *V. champinii* 9038) x *V. acerifolia* 9018 were tested for chloride accumulation in leaf and petiole tissues under 75 mM NaCl irrigation regimes. At harvest, ten distinct groupings of leaf and petiole tissue Cl^- concentrations emerged from the 76 tested selections, which included the parents (Table 1.3). However, there were no separations in root Cl^- accumulation between any of the offspring or parents in this study (Figure 1.4). Stem chloride accumulation was not recorded in our studies due to difficulties processing the more fibrous tissue on a large scale. Further studies are needed to investigate chloride sequestration in tissues prior to deposition in petiole and leaf tissues.

Root tissues had at least double the chloride concentrations at harvest than foliar tissues for each accession tested (Table 1.3). Additionally, offspring in this population had potential to accumulate more chloride in leaf and petiole tissues than their worst-performing parent, GRN3. However, the

offspring could not exclude chloride from petiole tissues more effectively than the other parent, *V. acerifolia* 9018.

While it is clear that this population resulting from a cross of a relatively poor Cl^- excluder and a relatively good Cl^- excluder varies significantly only in total leaf and petiole tissue Cl^- accumulation, a trend of continuous variation in both roots and shoot Cl^- content was observed (Figure 1.3). However, when focusing on leaf and petiole tissue, continuous variation in the offspring becomes more apparent.

1.5 Discussion

The purpose of these trials was to study the complex trait of salt-tolerance in grapevines and the variation present in a wide array of selections available for testing. Generally considered a glycophytic, perennial crop, grapevines are not well adapted to soils with high concentrations of NaCl. However, as climate change progresses and alters ecosystems and agricultural conditions indefinitely, acclimation to new conditions is an essential process that must be pursued. Beginning with a small trial of a few species, which were selected based on potential for exhibiting tolerance to NaCl in some form. We found a wide variation in chloride accumulation in tissues of grapevines exposed to high external NaCl applications. The primary responses we observed to excessive NaCl presence in rooting media across all tested accessions were separated into three response categories: 1) limiting Cl^- accumulation to roots, 2) equally distributing Cl^- between roots and petiole + leaf tissues, and 3) primary accumulation of Cl^- in leaf and petiole tissues. High variations in chloride accumulation in plant tissues only occurred when high concentrations of external NaCl were applied to rooting media.

While sodium concentrates to toxic levels first in most crops, chloride causes toxicity to grapevines before sodium under high NaCl concentrations in the rootzone (Henderson et al. 2014, Walker et al. 1997). Recent work has shown that grapevines' greater ability to tolerate high sodium than many perennial crops may be due to the *HKT*1;1 transporter, which regulates potassium entry and is selective against other ions (Henderson et al. 2014, 2018). The *HKT*1;1 in grapevine appears to have high affinity and selectivity for potassium and hence successfully excludes sodium when Na^+ is present in large amounts (Abbaspour et al. 2014, Storey et al. 2003). The tolerance of *Vitis* spp. to chloride, however, is less clear. For citrus, it has been suggested that chloride enters cells through non-selective anion channels and/or chloride-outward-rectifying channels (CLOR) (Moya et al. 2003). This has yet to be shown to be the case in grapevine. However, similar transporters likely moderate chloride uptake and transport within the vine (Henderson et al. 2014). Extrusion of excess NaCl via several types of ion transporters and/or channels has been suggested as a mechanism involved in removal of NaCl from the symplast, particularly efflux of Cl^- to the rhizosphere (De Boer and Volkov 2003, Henderson et al. 2014). Although it is apparent that some grapevine species and cultivars may tolerate and/or avoid chloride-induced toxicity better than others, we do not have a clear explanation for the mechanism that enables this variability in chloride-toxicity tolerance.

Results from this set of four experiments suggest a wide range of chloride tolerance in both established rootstock cultivars and wild *Vitis* species collected across the southern and southwestern United States. Among the most successful Cl^- excluders were individuals of *V. acerifolia*, *V. doaniana*, and *V. girdiana*, and the rootstocks 99 Richter and 140 Ruggeri. Each of the three highest performing wild species were collected from relatively arid geographical regions with the potential to have higher NaCl soil content. Applied NaCl concentrations were not high

enough to induce large differences in biomass production in leaves, petioles, or roots. However, statistically distinct groupings emerged for chloride accumulation in above ground tissues (i.e., leaves and petioles) and in roots of different species analyzed where these tissues were compared for Cl^- accumulation following a 21-day treatment. The relative lack of separation observed in root chloride accumulation may be partially affected by the high variation observed in root tissues when compared with the much lower variation in leaf and petiole tissue chloride accumulation within individual accessions. In all individuals root chloride accumulation was significantly greater than in leaf or petiole tissue.

Although the overall dry masses of roots, leaves, and petioles were not altered significantly by the NaCl treatment, leaf and petiole combined mass did differ at harvest based on genotype only under a salt-regime of 75 mM NaCl applied daily. In contrast, when no excess NaCl was applied, dry mass of leaf and petiole tissues did not vary among genotypes tested. As previously explored, the introduction of high NaCl concentrations may have altered the growth rate of leaf and petiole tissues, changed their morphology, or affected them in some other way. However, this degree of change induced in above ground tissues was influenced by the selection tested. From the initial trials *V. acerifolia* 9018 and various members of *V. doaniana* and *V. girdiana* were selected as having potential for use in the development of salt-tolerant rootstock cultivars.

Prior studies of the mechanisms of salt tolerance in grapevine (Gong et al. 2011, Munns and Tester 2008) also indicate Cl exclusion between root and shoot is important for rootstock tolerance. Focus can be concentrated on chloride accumulation in above ground tissues when searching for suitable candidates to contribute toward the development of a commercially viable, chloride-tolerant rootstock. Further, we have identified three wild *Vitis* species and at least two

commercially available rootstock cultivars, which have exhibited a high degree of chloride tolerance for immediate consideration.

Trial 1: Potential candidates and establishing biocontrol cultivars

This trial focused on exploring the potential ranges of NaCl tolerance in wild *Vitis*, candidate accessions. An exceptional range in Cl^- accumulation in both leaf and petiole combined material, and root tissue was observed (Table 1.1). Accessions could be separated into three distinct groups when analyzed using Tukey's honest significant differences test *V. acerifolia*, *V. girdiana*, and *V. doaniana* each had at least one accession, which greatly outperformed other individuals tested in this study, accumulating negligible concentrations of Cl^- in leaves and petioles. However, in the majority of these 'good-performers', root Cl^- accumulation was relatively higher when compared with more salt-susceptible accessions indicating that Cl^- transport from roots to shoots was restricted. While all roots were washed in clean, distilled water three times to minimize impact of surface contamination the possibility for surface contamination of roots cannot be ruled out due to their direct contact with salinized irrigation water.

Trial 2: Select species with purported NaCl tolerance

As with the standards in Trial 1, both treatments in Trial 2 were analyzed in physical separation from one another. The interspecific variation under the 0 mM NaCl (control) treatment was much lower than the variation under the 75 mM NaCl treatment (Table 1.2). Only *V. doaniana* 9024 showed a significant difference in Cl^- accumulation in leaf and petiole tissues when no additional NaCl was included in irrigation water. Under control conditions, the 27 tested grapevines separated into four distinct groupings. Table 1.2 also shows that the 27 tested accessions separated into 11 distinct groups based on leaf and petiole [Cl^-] following the 21-day application period. Compared with the four groups under control conditions, these divisions under applied NaCl suggest that

there is a strong influence by the cultivar on their ability to minimize Cl^- accumulation in above ground tissues in wild grapevines. While not technically different between treatments, there is a wide gap between the measurements and a notable difference in interquartile ranges when comparing the control values with those from 75 mM NaCl treated vines.

Trial 3: Comparing accessions of V. berlandieri and previously explored cultivars

This trial, although relatively limited in scope, did allow for the identification of two factors affected by high external NaCl concentrations. Both Cl^- accumulation and mass of above ground tissues were affected by the application of NaCl in irrigation water, dependent upon the accession tested. We also showed here that members and relatives of *V. berlandieri* are relatively susceptible to chloride accumulation, despite previous claims of tolerance. Changes in leaf dry mass suggest a change in leaf/petiole genesis, retention, growth rates, and/or morphology in response to the high external NaCl applications, but not a direct link to Cl^- accumulation in above ground tissues.

Trial 4: Exploring breeding potential of V. acerifolia 9018

Trial 4 was conducted to test the efficacy of using salt-tolerant grapevine selections as parents in breeding programs directed at chloride tolerant rootstock development. Throughout studies we have conducted prior to this, the accession *V. acerifolia* 9018 was consistently among the tested individuals with the lowest Cl^- accumulation in leaf and petiole tissues following exposure to 75 mM NaCl in irrigation water for 21 days. *V. acerifolia* 9018 also had some of the lowest variability in chloride measurements of all selections tested.

As salt accumulates in leaf tissues, leaf senescence rates increase while growth rates decrease in most plant species as a result of over accumulation and cell death (Flowers and Colmer 2008, Flowers 2004, Phogat et al. 2018). The results from this trial point toward a mechanism of Cl^-

tolerance, which avoids the loss of photosynthetic tissue as a primary strategy in acclimating to high NaCl concentrations in the surrounding rooting media.

Prior studies related to salt-tolerance in grapevine focused on potential mechanisms such as NaCl uptake, NaCl extrusion from tissues, NaCl sequestration, and NaCl exclusion from long-distance transport streams. From the results of this study, it appears that the latter two options are most likely to be present in selections highly resistant/avoidant to accumulation of Cl^- in tissues, which would cause the most damage to the productivity and growth capacity of the vine. It remains to be determined if the high levels of Cl^- accumulation seen in the roots of accessions tested in this study would greatly affect their potential for growth and development.

The observed lack of separation in root chloride-uptake points toward a difference in long-distance transport of chloride within *V. acerifolia* 9018 and those related to it. While we cannot rule out the potential for other physiological mechanisms of Cl^- exclusion from above ground tissues, *V. acerifolia* 9018 may achieve its relatively low chloride accumulation in leaves and petioles through exclusion of Cl^- anions from entering xylem vessels or by sequestering Cl^- in tissues upstream from those used in photosynthesis.

1.6 Conclusions

Prior work has shown that in grapevines under high NaCl exposure chloride acts as the primary agent in saline-toxicity and can damage the plant long before sodium (Henderson et al. 2014, Walker et al. 1997). Given this information, we chose to focus exclusively on chloride uptake in our trials. To minimize impacts of other nutrient flux we applied a constant application of other

essential nutrients using a standardized Hoagland's solution mixture. While the influence of other nutrients on vine growth and their potential for damage should not be ignored, these studies sought to identify the response of each accession to chloride exclusively.

From trials 1, 2, and 3 we observed that there is a significant difference in chloride uptake among grapevine species even at very low application rates, but a more pronounced difference in response to higher levels of salinity in the rootzone. This observation can be used to justify classifications of good, moderate, and poor chloride excluders among the accessions tested.

Through a population of offspring from GRN3 x *V. acerifolia* 9018 we observed continuous variation and significant differences in the accumulation of Cl^- in leaves and petioles following a treatment period of 21 days with 75 mM NaCl dissolved in irrigation water. This study points toward an avoidance strategy of salt-tolerant grapevines, which inhibits long-distance transport of chloride to leaves and petioles.

1.7 Tables

Table 1. 1. Accumulated leaf and root chloride content of select individuals with relatively low or high leaf chloride content at harvest and p-value for leaf chloride values as a function of accession and root chloride values; Trial 1.

Accession	Leaf + Petiole Chloride		Post hoc	Root Chloride		Post hoc
75mM NaCl applied	($mg \cdot L^{-1}$)	SE	HSD	($mg \cdot L^{-1}$)	SE	HSD
Pumpstation	335.05	38.8	a	182.17	65.3	ef
44-53 Mgt	308.89	46.05	a	266.83	45.4	de
V. arizonica AZ11-09	297.07	65.1	a	393.00	46.2	bc
Ramsey	279.92	20.49	a	282.60	35.3	de
St George	153.67	5.53	b	289.25	20.9	cde
140 Ruggeri	141.40	28.95	b	389.40	47.0	bc
V. girdiana 9024	113.08	14.3	bc	547.83	78.0	a
V. girdiana 8	66.08	5.0	bc	248.58	25.2	ef
V. doaniana 9042	64.67	6.5	bc	131.50	14.4	f
V. doaniana 9024	59.05	6.2	c	357.71	30.6	bcd
V. acerifolia 9018	44.83	5.5	c	440.83	44.5	ab
p value	< 0.001*			< 0.001*		
Leaf Cl^- *X*	*Accession*			Root *Cl*$^-$		
p value	< 0.001*			0.988		

Table 1. 2. Accumulated leaf + petiole combined tissue [Cl-]; 21-day application, 75 mM [NaCl].

Leaf and Petiole tissue [Cl^-]	75 mM NaCl			0 mM NaCl		
Accession	[Cl^-] ($mg \cdot L^{-1}$)	se	HSD	[Cl^-] ($mg \cdot L^{-1}$)	se	HSD
44-53 M	444.56	41.2	a	12.44	1.7	cd
Ramsey	311.67	34.8	b	20.11	2.6	abcd
Pumpstation	270.42	22.1	bc	15.00	2.1	bcd
V. arizonica AZ11-103	267.13	26.9	bc	17.00	2.2	bcd
V. doaniana 9042	213.22	9.7	de	23.67	4.8	abc
V. berlandieri 9043	190.89	16.7	def	20.00	3.9	abcd
b42-26	180.92	23.4	def	14.83	2.5	bcd
V. girdiana 22	179.13	15.7	def	13.50	2.5	cd
V. acerifolia OK12-019	176.17	28.5	defg	20.00	6.2	abcd
NV12-061	171.25	3.9	defg	19.44	3.7	abc
V. girdiana 1	155.67	25.5	efgh	13.33	1.5	bcd
T1	141.44	18.3	fghi	22.00	3.9	abcd
V. girdiana 13	139.33	16.3	fghi	17.33	4.1	bcd
V. girdiana 11	135.83	15.1	fghij	14.17	0.8	bcd
ANU 25	120.78	7.4	ghij	14.00	1.7	bcd
V. girdiana 6b	115.07	10.1	hij	15.42	2.7	bcd
NV12-050	106.58	6.3	hij	11.67	1.5	d
V. girdiana 2	102.44	19.6	hijk	14.67	1.3	bcd
140 Ruggeri	101.20	8.0	ijk	14.78	3.6	bcd
ANU 21	98.89	5.1	ijk	19.78	4.5	abcd
V. girdiana SC	88.75	10.9	ijk	17.73	2.8	bcd
V. acerifolia 9035	82.92	15.9	jk	15.78	3.7	bcd
V. girdiana 8	77.67	8.3	jk	18.08	4.2	abcd
V. doaniana 9026	64.67	13.8	jk	11.83	3.5	cd
V. doaniana 9024	53.50	8.3	k	29.17	2.2	a
V. acerifolia 9018	51.75	10.9	k	24.78	7.3	ab
p value	< 0.001 ***			< 0.001 ***		

Table 1. 3. Accumulated leaf + petiole combined tissue, and root tissue, Cl- concentration at harvest following 21-day application period for 75 mM NaCl applied treatment; representative accessions from each HSD posthoc group.

75 mM [NaCl]

Accession	Leaf and Petiole tissue [Cl^-]			Root tissue [Cl^-]		
	($mg \cdot L^{1}$)	se	Post hoc	($mg \cdot L^{1}$)	se	Post hoc
18113-077	98.00	28.2	a	327.67	82.36	a
18113-008	85.67	26.2	ab	257.50	91.30	a
18113-055	83.75	27.3	abc	286.42	70.60	a
18113-038	81.59	19.7	abcd	366.00	42.40	a
18113-018	78.67	20.3	abcde	294.75	34.50	a
18113-046	76.42	12.2	abcdef	298.92	26.20	a
18113-058	75.83	35.7	abcdefg	355.83	122.9	a
18113-076	73.67	21.1	abcdefgh	345.75	42.50	a
GRN3	67.67	26.4	abcdefghi	145.50	27.80	a
18113-048	66.50	22.6	abcdefghi	256.17	45.20	a
18113-043	47.83	15.6	bcdefghij	371.08	93.10	a
18113-026	36.09	8.80	cdefghij	234.92	58.90	a
18113-007	33.58	5.20	defghij	228.92	97.00	a
18113-027	32.34	7.50	efghij	404.92	127.9	a
18113-051	28.67	5.80	fghij	265.58	61.60	a
18113-034	26.92	4.40	ghij	340.67	74.60	a
18113-024	26.42	6.10	hij	288.67	37.30	a
18113-001	23.99	6.80	ij	322.67	96.90	a
V. acerifolia 9018	15.92	1.60	j	165.50	34.50	a
p value	< 0.001 ***			0.054		

Table 1. 4. Leaf and petiole chloride concentrations with leaf and root dry weights following 75mM NaCl treatment for 21 days; Trial 3.

	Leaf and Petiole tissue [Cl^-]			Dry Weight Leaves			Dry Weight Roots		
Accession	75 mM NaCl ($mg\ L^{-1}$)	se	HSD	Mass (g)	se	HSD	Mass (g)	se	HSD
44-53 M	366.61	32.0	a	7.92	1.0	abcde	2.48	0.3	abc
225 R	210.53	39.9	b	15.01	3.0	a	2.72	0.5	abc
TX16-032	206.00	81.7	bc	5.88	4.7	bcde	0.95	0.7	bc
TX16-022	198.89	45.5	bc	7.09	2.3	bcde	1.42	0.3	abc
Cosmos10	185.39	42.8	bc	9.99	1.5	abcd	2.54	0.5	abc
TX16-034	184.40	59.9	bc	5.17	2.0	de	3.46	4.3	abc
8B	182.50	21.1	bcd	13.78	0.6	ab	4.14	0.5	a
125 AA	176.39	45.4	bcd	12.80	1.9	ab	3.57	0.2	ab
1103 Paulson	176.07	24.6	bcd	10.03	1.8	abcd	1.8	0.2	abc
5C	170.93	33.8	bcd	10.78	2.3	abcd	2.58	0.6	abc
TX16-012	167.56	44.6	bcd	5.39	1.5	de	1.32	0.3	abc
TX15-073	166.39	51.2	bcd	2.38	1.4	e	0.63	0.3	bc
TX16-085	163.53	44.0	bcd	5.42	0.9	cde	1.38	0.2	abc
5BB	162.50	34.8	bcd	14.08	1.8	a	2.91	0.6	abc
110 Ruggeri	162.11	45.1	bcd	9.99	2.4	abcd	2.28	0.8	abc
TX16-015	150.94	68.2	bcd	6.38	3.2	bcde	1.39	0.8	abc
1045 Paulson	147.67	53.5	bcd	8.74	1.7	abcde	1.59	0.5	abc
5A	146.67	24.5	bcd	9.27	3.7	abcde	2.01	0.7	abc
TX16-009	143.08	54.7	bcd	5.52	2.3	bcde	1.15	0.6	abc
Cosmo2	142.50	30.9	bcd	12.60	3.5	abc	2.47	0.7	abc
EVEX13-5	137.72	34.2	bcd	10.59	2.7	abcd	2.14	0.4	abc
SO4	137.67	30.6	bcd	10.22	2.5	abcd	2.26	0.8	abc
Resseguier 2	135.67	36.5	bcd	7.92	2.2	abcde	1.09	0.3	abc
TX16-018	133.40	32.3	bcd	7.80	2.9	abcde	1.46	0.5	abc
TX16-026	124.50	71.8	bcd	3.58	2.3	de	1.04	0.8	abc
Riparia Gloire	119.33	44.2	bcd	6.67	2.0	bcde	1.57	0.5	abc
TX15-099	116.83	41.4	bcd	4.62	1.8	de	0.98	0.4	bc
TX16-065	116.78	20.5	bcd	4.67	1.2	de	1.49	0.8	abc
TX15-003	114.00	37.3	bcd	6.56	2.1	bcde	1.59	0.8	abc
TX15-091	107.53	23.6	bcd	3.50	1.0	de	1.18	0.4	abc
140 Ruggeri	103.00	30.5	bcd	8.16	1.9	abcde	1.59	0.4	abc
TX16-069	101.61	38.0	bcd	4.78	1.4	de	0.98	0.3	bc
TX15-059	89.72	27.9	bcd	4.93	2.3	de	0.93	0.5	bc
TX16-016	85.00	29.1	bcd	4.28	2.5	de	0.94	0.5	bc
TX16-068	82.89	24.8	bcd	6.54	2.5	bcde	1.06	0.4	abc
99R	71.22	24.9	cd	6.78	2.1	bcde	1.63	0.6	abc
V. acerifolia 9018	19.33	6.80	d	6.84	5.7	bcde	1.21	1.1	abc
p value	< 0.001 ***			< 0.001 ***			0.228		

1.8 Figures

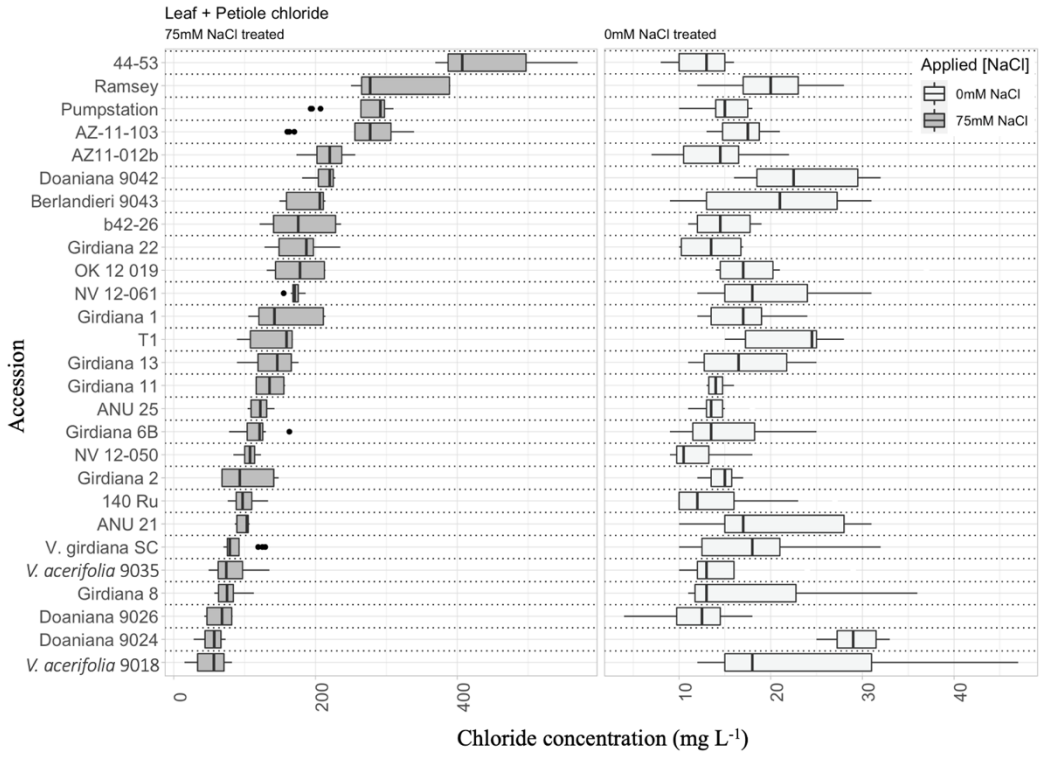

Figure 1. 1. Combined leaf and petiole chloride concentrations ([Cl-] mg · L^{-1}) at harvest; 75mM NaCl (left), 0mM NaCl (right) applied for 21 days prior to harvest.

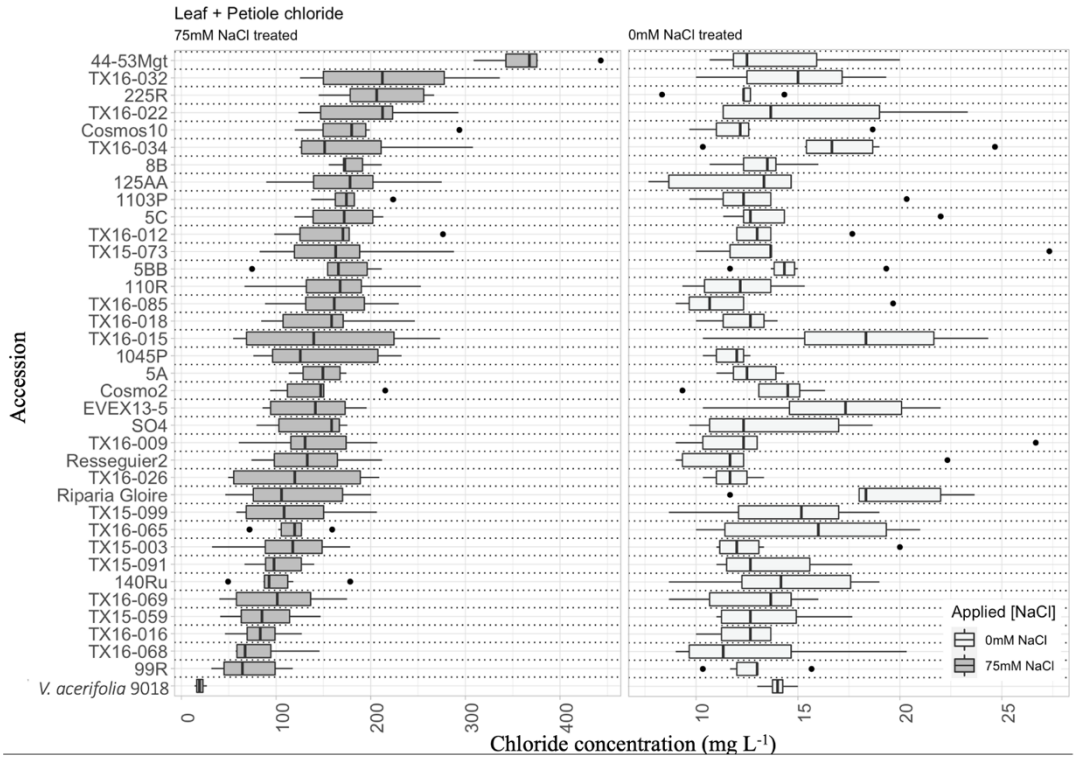

Figure 1. 2. Combined leaf and petiole chloride concentrations ([Cl-] mg · L^{-1}) of *V. berlandieri* and related accessions at harvest; 75mM NaCl (left), 0mM NaCl (right); applied 21-days.

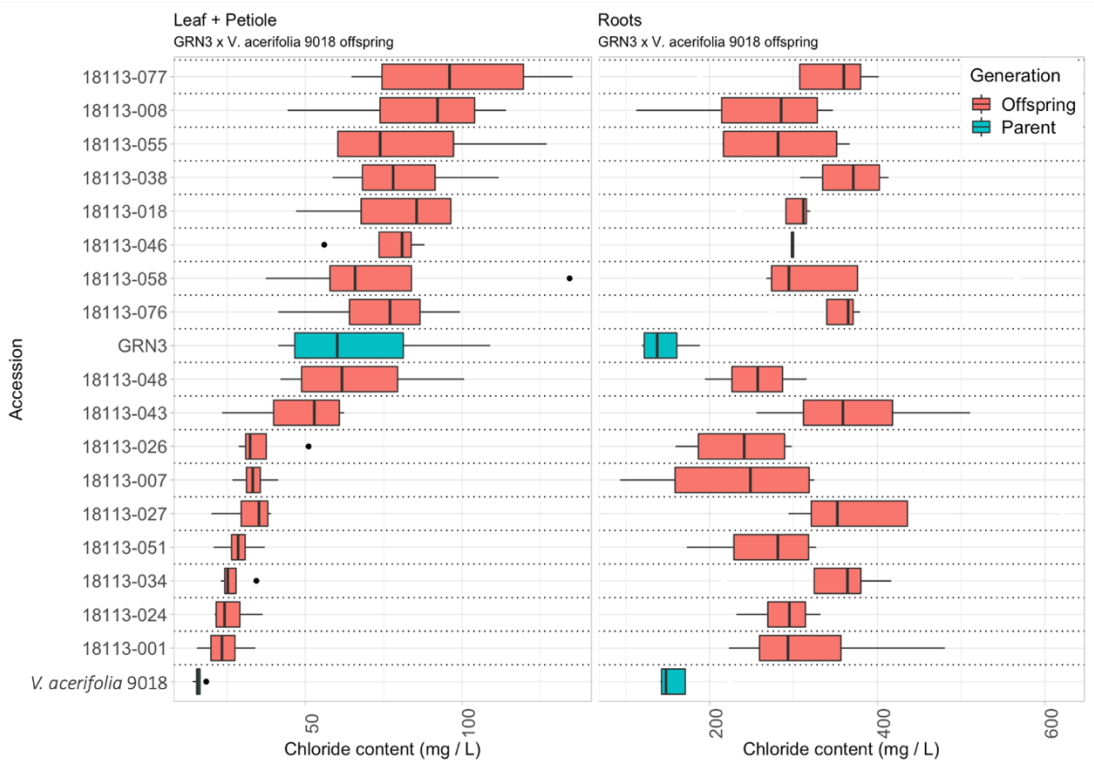

Figure 1. 3. Leaf and petiole (left) and root (right) chloride concentrations at harvest in a subset of offspring of GRN3 x V. *acerifolia* 9018 with parent accessions following 21-day exposure to 75mM NaCl treatments.

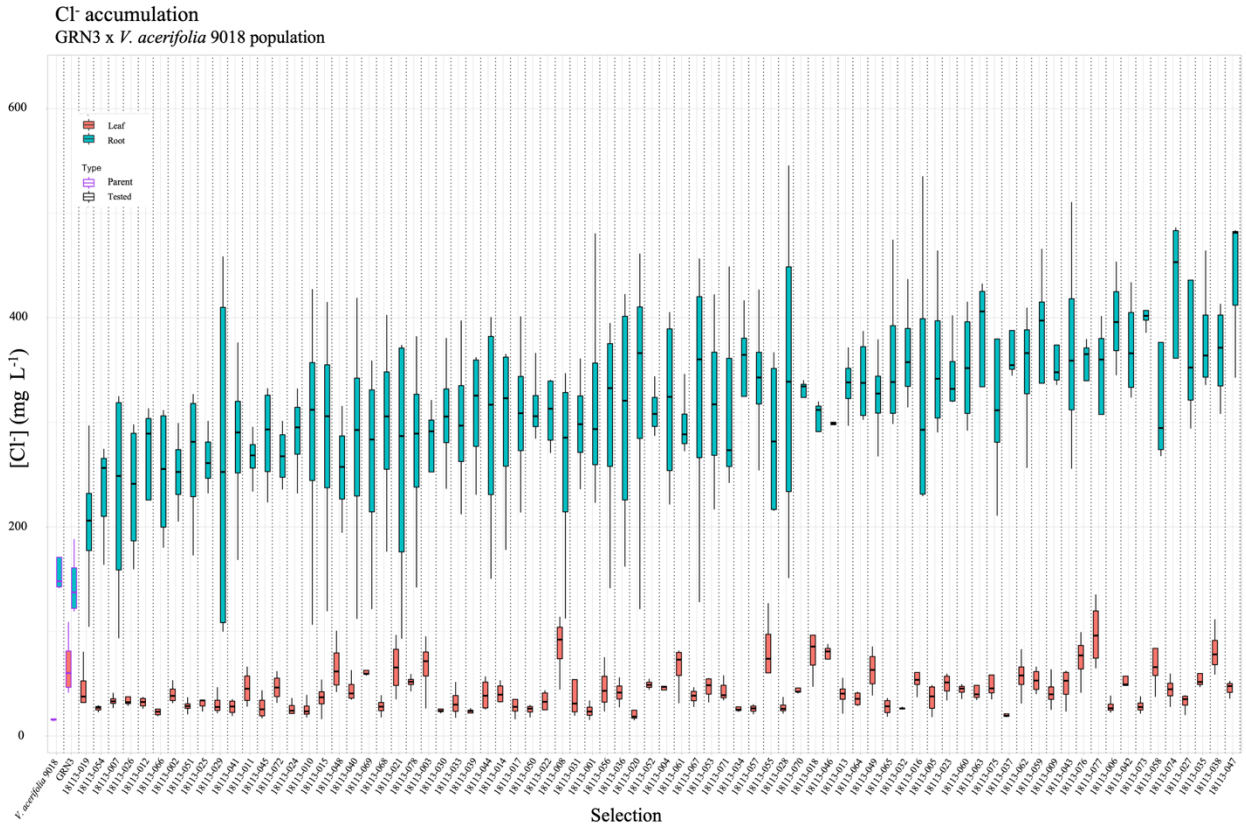

Figure 1. 4. Leaf and petiole (left) and root (right) chloride concentrations at harvest in all offspring of GRN3 x *V. acerifolia* 9018 with parent accessions following 21-day exposure to 75mM NaCl treatments.

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Chapter 2. Physiological response of Vitis spp. accessions to dissolved external sodium chloride applied at three distinct concentrations

2.1 Abstract

Accumulation of sodium chloride (NaCl) in vineyard soils is a pressing concern in arid viticultural regions around the world. Associated with decreases in productivity, salt-toxicity can impact vine nutrient status, photosynthetic capacity, and other aspects of importance to high-output agriculture such as soil structure (Henderson et al. 2014, Liang et al. 2021, Moya et al. 2003, Munns and Tester 2008). Soils with NaCl in concentrations of 40 mMol, or an electroconductivity (EC) of approximately 4 $dS\ m^{-1}$, are considered at the threshold for classifying a soil as saline, or sodic if sodium is the major contributor. However, this threshold is not crop specific and does not account for the diversity of NaCl tolerance observed in individual crops. In grapevines, chloride becomes toxic before sodium in a NaCl laden environment and can be introduced to vineyards in many ways, but a threshold to where NaCl in soils becomes toxic to grapevines has not yet been determined. This study applied three discrete NaCl concentrations and a non-salinized control to nine different accessions of grapevine rootstocks to observe the individual photosynthetic responses of transpiration rate (E), carbon assimilation (A_{net}), intracellular CO_2, and stomatal conductance (g_s) in each genotype exposed to each NaCl concentration. Total chloride accumulation was also measured in aboveground tissues. While each accession behaved distinctly at different NaCl exposures, measures of photosynthetic activity fell by 30-60% between lower (25mM) and higher (75mM) NaCl exposure in all cases. However, there were no significant differences in vine photosynthetic response at NaCl levels between 0-25mM NaCl or from 75-100mM NaCl. Results suggest that losses to vine productivity begin to occur in grapevines when

NaCl concentrations are between 25mM and 75mM NaCl with few differences below or above those concentrations, respectively.

Key words: Concentration, Cultivar, Exclusion, mMol, Tolerance, Toxicity

2.2 Introduction

With a rapidly increasing global population, higher water prices, and the desire to maximize the utility of scarce resources, there has been a move to use reclaimed water for irrigation in many vineyards worldwide (Laurenson et al. 2012, Stevens 2009). Over 90% of wineries in South Africa reuse wastewater for vine irrigation purposes (Van Schoor 2005). While this practice reduces the cost of irrigation and improves the economic viability of grape cultivation, it may also introduce elements in the water supply that are harmful to the well-being of a vineyard. Consequences of recycled irrigation water can be detrimental to vineyard health if the water reclamation process is ineffective at removing harmful or toxic compounds. Among the concerns is the accumulation of chloride (Cl^-) and sodium (Na^+) in reclaimed water (Kumar A. 2014). Commonly used in winery cleaning procedures, potassium chloride (KCl) often makes it into the vineyard water supply through ineffective waste-water remediation processes (Buelow et al. 2015b). While K^+ and Cl^- are both considered essential nutrients for grapevines, the amount of potassium needed for a healthy vineyard far exceeds the plants' needs for chloride (Nicholas 2004). Vineyards irrigated in this fashion may suffer from chloride-toxicity (Buelow et al. 2015a). Soil structure may also degrade due to high influx of monovalent cations such as Na^+ and excessive K^+ found in these reclaimed irrigation waters (Liang et al. 2021). This pathway for chlorine to enter a vineyard is by no means the only way it may be introduced, but it could become more commonplace as water prices increase and water itself becomes a scarce resource and a cause for concern.

Unlike many perennial crops, grapevines are relatively tolerant of high sodium levels in the rhizosphere, excluding Na^+ via cell-membrane transporters (*HKT* 1;1) with high-affinities for K^+ passage over Na^+, and by sequestering Na^+ cations in cell walls and vacuoles (Gong et al. 2011, Henderson et al. 2014). However, Cl^- in the root-zone can be excessively damaging and result in early leaf senescence, poor fruit set, lower transpiration rates, decreased carbon assimilation rates, and high levels of NaCl in ripe berries (Henderson et al. 2014, Moya et al. 2003, Munns and Tester 2008, Walker et al. 2010). These responses may directly contribute to poor photosynthetic capacity, lowered yields, less vigorous shoot growth, and the harvest of unmarketable fruit of poor quality. Due to the imbalanced response of grapevines to Cl^- when compared with Na^+, we chose to focus this project on the sole response of grapevines to toxic levels Cl^-. Chloride accumulation in vineyard soils can be an issue of great concern and is likely to become more prevalent as agricultural methods change to accommodate a growing global population.

The accepted threshold for what is considered a saline soil in vineyards is vague and not crop specific. Soils are generally considered to be saline at, or above 4 $dS\ m^{-1}$ (Qadir et al. 2000). Thresholds for *V. vinifera* cv. Sultana have been proposed around EC_e = 2.2 dS m^{-1}, or approximately 22 mM NaCl (Zhang et al. 2008). Tests have been conducted with grapevine NaCl tolerance using concentrations of 155 mM NaCl and above (Charbaji and Ayyoubi 2004, Troncoso et al. 1999). However, the majority of sodium chloride tolerance trials have been conducted at much lower concentrations, often between 25 and 50 mM NaCl (Abbaspour et al. 2013, Heinitz et al. 2015). These studies have recorded changes in growth rate, total biomass accumulation, photosynthetic response, and more parameters in response to varying applied NaCl concentrations. However, most have tested only a handful of *V. vinifera* cultivars and lack analysis of any wild

species accessions, instead choosing to test only existing rootstock cultivars (Abbaspour et al. 2013, Charbaji and Ayyoubi 2004, Troncoso et al. 1999).

Heinitz et al. 2015 tested 11 wild species under 25 mM NaCl over 14 days including *V. acerifolia*, *V. aestivalis*, *V. arizonica*, *V. berlandieri*, *V. champinii*, *V. cinerea*, *V. doaniana*, *V. girdiana*, *V. monticola*, *V. rupestris*, and *V. treleaseii*. The study conducted by Heinitz et al. 2015 included the common rootstock cultivars *V. rupestris* cv. St. George, 101-14 Mgt, and 44-53 Mal as well. NaCl tolerance in *Vitis* rootstocks is relatively uncommon, which may have directed attention toward obtaining and testing new germplasm for the development of NaCl tolerant rootstock cultivars. However, testing at a single NaCl concentration provides limited information for future studies. In particular, a relatively low applied NaCl concentration may not support findings of NaCl tolerance or susceptibility in wild or cultivated individuals. Thus, there is a need for a study examining a wide range of *Vitis* species using multiple applied NaCl concentrations.

Using nine rootstock-genotypes previously screened at 75mM NaCl for chloride tolerance (data not shown), this study sought to compare the responses of tolerant, moderately tolerant, and susceptible individuals to four concentrations of NaCl: 0, 25, 75, and 100mM. We sought to define a general range of NaCl exposure where an accession considered salt-tolerant becomes most useful in avoidance of chloride toxicity damages. Weekly measures of key physiological processes, such as stomatal conductance (g_s), net carbon assimilation (A_{net}), intracellular CO_2 levels, and transpiration rates (E), were recorded to track the performance of each genotype to exposure of variable sodium chloride concentrations ([NaCl]). Combined leaf and petiole chloride accumulation was recorded at harvest. These parameters were analyzed to better inform how to select an individual accession as a potential candidate for rootstock breeding efforts and to establish soil NaCl concentrations that become damaging to most grapevine rootstock cultivars.

2.3 Materials and Methods

Plant Material

Accessions selected for this experiment were based on preliminary data (not shown) from four prior exploratory trials in which they separated into distinct groupings using Tukey Honest Significant Difference posthoc analysis (Mendiburu 2019). Three accessions were selected from each of the groups considered highly tolerant, moderately tolerant, and susceptible. The highly tolerant individuals were 99 Richter (99R), 110 Richter (110R), and *V. acerifolia* 9018 (9018); moderately tolerant individuals were 140 Ruggeri, Dog Ridge, and Ramsey; susceptible individuals were 101-14 Mgt, *V. riparia* cv. Gloire (Riparia Gloire), and 44-53 Malegue (44-53 Mal).

Propagation

All herbaceous cuttings of each accession were taken from a single mother vine found within the University of California Davis, Department of Viticulture and Enology collection at Hopkins Vineyard in Davis, California. The use of vegetative propagules was chosen to remain consistent with previous findings of variability by propagule type (Fort et al. 2013). Each cutting was rooted using liquid rooting hormone of 1.03% Indol-3-butyric acid and 0.66% 1-naphthalene acetic acid (Woods Rooting Compound; Earth Science Products Corp; Wilsonville, OR, USA) diluted by a factor of 1:20, planted in a flat tray of 1:1 perlite to vermiculite media, and allowed to root in a high-humidity, fine-mist fog room for 14 days. Individual seedlings were than transplanted to their own 2548 cm^3 round pots using a non-swelling, fritted-illite clay compound as a growing medium (Profile Products LLC; Buffalo Grove, IL, USA) chosen to discourage soil flocculation and potassium leaching resulting from high sodium inputs. Once transplanted and moved to a

controlled-greenhouse setting, plants were grown for 56 days to develop sufficient root and photosynthetically active tissues before being exposed to NaCl fertigation.

NaCl application

Laboratory grade 99% NaCl was added to Hoagland's solution (Fort et al. 2015) greenhouse fertigation water at three distinct concentrations throughout the duration of the experiment. Mixed in large vats, 82.95g, 248.84g, and 331.79g NaCl were dissolved in of 56.8 liters of distilled, fertigated water to achieve 25mM, 75mM, and 100mM NaCl concentrations, respectively. Accuracy of the mixture was twice-checked for each new batch prior to application using an automated silver-ion titrator (Model 926, Nelson-Jameson Inc., Marshfield, WI, USA). Each treatment was applied by hand with 500mL of its respective NaCl solution using a graduated pitcher once daily at 10:00h PST with the intention to prevent damages related to water-stress. Sodium chloride treatments continued in this fashion for 21 days and were halted on the day of harvest. Irrigation water was carefully applied to prevent contact with all leaf and petiole tissues; contamination of a leaf led to its immediate excision from the plant to prevent artefacts of external NaCl from interfering with final sequestered-chlorine content evaluations. Few leaves were removed in this way. The sodium absorption ratio (SAR) of the irrigation solution was 28.95 in this experiment.

Photosynthetic measurements

Measurements of photosynthesis acquired using the LiCOR 6800 portable photosynthetic system were taken at three time points during the period of the experiment and consisted of single point measurements from four replicates of each rootstock ~ applied [NaCl] combination. Recording of g_s, A_{net}, intracellular CO_2, and E occurred once per seven days throughout the 28-day test period for each experimental unit and were measured using a portable photosynthetic system

(LiCOR model LI-6800; Lincoln, NE, USA). Leaves chosen from the same node position on each individual plant were measured each cycle to mitigate the influence of leaf age on recorded values. A new, undamaged leaf was chosen for each weekly measurement. Leaves selected for this process were left on the plants and allowed to continue transpiring and providing photosynthates to their associated vines. All leaf and petiole tissue remaining on each vine was collected during harvest including those previously used for photosynthetic measurements.

Sample collection and preparation

Following 21 days of NaCl exposure, the above and below-ground tissues were destructively harvested and separated into two categories: petioles and roots. The fresh mass of each tissue type and leaf areas for each plant were recorded using a portable leaf-area meter and belt conveyer (LiCOR models LI-3000a, LI3050A; Lincoln, NE, USA). Each category of harvested tissue was then separated into individual paper drying bags by individual plant then by tissue type and allowed to dry in a tissue drying room for 14 days. Once fully dried, the dry mass of each bag was recorded, and tissues were crushed to a fine powder in isolation from other samples. Crushed samples were then placed back into the drying room for another seven days to ensure any moisture potentially reabsorbed during the crushing process was removed. Leaf chloride content was evaluated for each harvested sample by adding 0.25g of powdered tissue to 25mL of deionized water and shaken at 2500 rpm for 60 minutes on a modified orbital shaker table. The resulting mixture was filtered through 11µm filter paper into a 50mL centrifuge tube and doubled in volume with deionized water for better sample homogeneity during chloride readings. Tissue chloride content was measured in mg L^{-1} using an automated silver-ion titrator (Model 926, Nelson-Jameson Inc., Marshfield, WI, USA) under the manufacturer's standard procedures for food grade plant tissues. Preparations for measurement and recording of chloride accumulation values in leaf and petiole tissues were

completed on a per-sample basis and in isolation of all other samples processed to avoid cross-contamination risks.

2.4 Results

Leaf and petiole chloride accumulation

As with previous trials (Chapter 1), it was clear that accumulation of Cl^- in leaf and petiole tissues is influenced by the rootstock tested. Although the range of average chloride readings in this experiment was 190 mg L^{-1} results from a Tukey Honest Significant Difference (Mendiburu 2019) test on results across all NaCl application levels distinguished one group of high chloride excluders and another of poor excluders. The accession 44-53 Malegue (44-53Mal) accumulated more than double the concentration of chloride in leaf and petiole tissues than any other tested rootstocks in this trial. Meanwhile *V. acerifolia* 9018 accumulated less than half the chloride than other tested accessions (Figure 1.4). Accessions did not vary significantly in leaf area, leaf mass, or root mass in this trial.

Root mass was weakly correlated with both leaf area and leaf mass. These variables were more strongly correlated when NaCl concentrations of 25 mMol or less were applied to the tested individuals. Leaf area and root mass obtained a maximum R^2 value of 0.66 while root mass and leaf mass obtained a R^2 of 0.56 under 25 mM applied NaCl. This correlation coefficient decreased with both greater and lesser concentrations of applied NaCl. However, when separated properly by NaCl treatment both accession and NaCl exposure were significant to Cl^- tissue accumulation and some photosynthetic responses. These results suggest there is not linear control of chloride tolerance in grapevines, but a ramped response when exposed to progressively greater concentrations of NaCl in external rooting media. Leaf and petiole chloride concentrations at harvest, as well as primary photosynthetic variables are further explored in the following sections.

When separated by rootstock and [NaCl] applied, eight of nine tested accessions had R^2 values of 0.76 or greater when comparing tissue Cl^- accumulation at harvest by applied [NaCl] treatment. However, *V. acerifolia* 9018, which has consistently tested as the most tolerant accession under high external NaCl exposure, only had a R^2 of 0.61 in this comparison with a significant p-value of 0.0481 (Table 2. 1). While all other tested individuals became significantly different from their respective, unsalted control replicates at, or below, 75 mMol applied NaCl, 9018 leaf and petiole Cl^- accumulation only differed from non-salted controls at 100 mMol applied NaCl.

Final leaf and petiole chloride concentrations at harvest separated into three distinct groups when comparing among accessions (Table 2. 2). Accession was a significant factor in Cl^- accumulation in any situation where excess NaCl was applied via the fertigation methods described above. While the cultivar 44-53Mal only varied within genotype between 25-75mM NaCl treatments, 44-53Mal accumulated significantly greater levels of Cl^- in leaf and petiole tissues than all other tested accessions in all treatments where NaCl was applied. Under 100mM NaCl only one of four 44-53Mal replicates survived through to harvest. The response of 44-53Mal is inversely mirrored in the extremely low Cl^- levels in leaf and petiole tissues of 9018. While 44-53Mal saw a 1779% increase in leaf and petiole Cl^- accumulation between unsalted vines and those exposed to 100mM NaCl, 9018 Cl^- levels only increased 93% within the same context.

Photosynthetic responses

Among accessions there was little observed difference in photosynthetic responses. Without separating by accession, stomatal conductance (g_s), transpiration rates (E), and net carbon assimilation rates (A_{net}) were significantly different in all tested accessions, delineating between the 25 mM and 75 mM NaCl treatments in all cases (Table 2. 3). At applied concentrations of 75 mM NaCl or higher, values for these variables dropped by at least half that of non-salted controls.

While intracellular CO_2 readings did decrease with increasing applied NaCl concentrations, these were not significantly different at any range of tested NaCl applications. Intracellular CO_2 concentrations were not significantly affected by increased exposure to external NaCl, but still decreased by 23% of their control values under exposure to 100 mM NaCl.

2.5 Discussion

Soils are widely considered 'saline' once the EC_e reaches 4 $dS * m^{-1}$ while some arguments have been made to lower this threshold to 2 $dS * m^{-1}$ (Sparks 2003). This trial has shown that, regarding grapevines, a threshold of 2 $dS * m^{-1}$, or approximately 20 mMol NaCl, is too low to be considered a threat to photosynthetic activity or vine productivity. Although exposure to high levels of soil salinity are problematic for many glycophytic species, excess salinity can also be detrimental to soil structure and chemistry. High concentrations of sodium can displace other cations resulting in dispersed or swelling clays, often decreasing the hydraulic conductivity of an afflicted soil (Halliwell et al. 2001). While we focused on chloride toxicity based on previous findings with grapevines, sodium can be important for other aspects of soil management in vineyards. Water infiltration rates at the soil surface can be further impacted by high NaCl content developing a surface seal through the drying of dispersed, sodium-impacted clay particles and greatly decreasing water infiltration at those sites (Agassi et al. 1981). These impacts have the potential to become detrimental to vine root growth as dispersed clay particles can directly alter the geometry of soil structures (Shainberg and Letey 1984) and indirectly alter nutrient availability. However, due to the nature of the rooting medium we used in this experiment these indirect impacts were greatly diminished.

Leaf and petiole chloride accumulation

As in previous trials, final Cl^- accumulation in leaf and petiole tissues are highly influenced by the variety tested and more pronounced in accessions which are more susceptible to accumulating chloride in these tissues at even minimal NaCl exposure (Table 2. 1). When separated by differing NaCl exposure, these differences by genotype become more divisible with unique groupings emerging between 0 mMol and 25 mM exposure, between 25- and 75-mM exposure, and in the most tolerant accession only occur from no NaCl controls at an applied NaCl concentration of 100mM.

The effect of NaCl concentrations on leaf and petiole Cl^- accumulation often differed within individual genotypes when exposed to between 25mM and 75mM applied NaCl in six of the nine accessions tested (Table 2. 1). At lower applied NaCl concentrations the accessions Dog Ridge, Ramsey, and Riparia Gloire were significantly distinct within their genotype between 0-25mM applied NaCl suggesting these varieties are more sensitive to low NaCl concentrations than the other six accessions included in this study. Furthermore, the rootstock 44-53 Mal, initially considered to be the least salt-tolerant variety included this study, did not differ with other 44-53 Mal individuals until an applied NaCl concentration of 75mM. These responses may suggest either a more rapid osmotic adjustment occurred in Dog Ridge, Ramsey, and Riparia Gloire or a more overall sensitive response to NaCl in these varieties. It should also be noted that Cl^- accumulation in leaf and petiole tissues separated into three distinct groups in each of these cultivars. While Cl^- accumulation in leaf and petiole tissues in Dog Ridge and Ramsey each separated from others of their respective cultivar between 0-25mM NaCl and then again between 25-75mM NaCl, Riparia Gloire separated among others of its variety between 0-25mM NaCl and then again between 75-100mM NaCl (Table 2. 1). This difference in Riparia Gloire may be indicative of a response differing from Dog Ridge and Ramsey.

The relatively high Cl^- accumulation of 44-53Mal under excess applied NaCl suggests a lack of ability to restrict or limit Cl^- ion movement from rhizosphere to photosynthetic tissues. Under exposure to high NaCl concentrations genotype greatly influences the amount of Cl^- which makes it to the leaf and petiole tissues. The vines tested never produced fruit throughout the duration of this study and so the response observed in Tables 1 and 2 cannot be attributed to Cl^- deposition into a fruit acting as a sink for chloride anions.

When considering the potential impact of damaging levels of soil NaCl accumulation, photosynthetic activity is of prime concern for production-based viticulture. Often in regions of high-volume grape productivity limited water availability for agriculture in conjunction with high temperatures and rapid evapotranspiration easily lead to accumulation of these salts in vineyard soils. While widely supported in states like California, drip irrigation can exacerbate the buildup of both sodium and chloride in vineyard soils due to insufficient available water to leach harmful ions below the rooting zone in fields under this irrigation management system. Limited precipitation can compound these impacts by preventing sufficient natural leaching needed to remove salts from agricultural soils. When paired with persistent drought, irrigation systems may be too costly to facilitate the necessary soil leaching. Facing the likely accumulation of damaging NaCl in the direct region around vine roots, photosynthetic responses of different rootstock accessions become an essential avenue of research to address high soil NaCl accumulation.

Photosynthetic responses

Our results show that applications of 25 mMol NaCl are not significantly different from 0 mMol applied NaCl in observed g_s, E, A_{net}, or intracellular CO_2 levels. However, at applied amounts of 75 mM NaCl or greater significant decreases in the measured photosynthetic parameters are extreme. The observed differences in E, g_s, and A_{net} only occurred at applied NaCl increments of

75 mMol or greater in this trial. A change in photosynthetic response to rhizosphere NaCl content appears to occur within the range of 25 mM and 75 mM soil NaCl, which agrees with the widely disseminated limit of 40 mMol NaCl, or roughly 4 dS, for a soil to be considered saline.

The competitive nature of K^+ and Na^+ for uptake, transport, and function in metabolism of most perennial crop species is often attributed to the similarity in size and charge of the two cations (Henderson et al. 2014, Sauer et al. 2013). Particularly the cases of uptake through high affinity potassium transport proteins in the root system and functionality of K^+ in gating stomatal guard cells suggest a strong influence of Na^+ on the resulting stomatal conductance and transpiration values presented here. E and g_s in this study had R^2 value of 0.99 and are clearly linked as well described in other fundamental works (Yu et al. 2009). However, stomatal conductance also affects the rate of carbon uptake from atmospheric CO_2, although at a much slower rate than water loss through transpiration (Rodrigues et al. 1993). When A_{net} was correlated to E and g_s the R^2 values were 0.65 and 0.62, respectively. Without separating by rootstock, the measurements taken of photosynthetic activity suggest that levels of NaCl of 75 mM or greater in soils can reduce grapevine vigor and growth rates by as much as half, regardless of accession exposed. A similar response may also occur at some NaCl concentrations between 25-75mM and is likely directly related to changes in stomatal guard cell activity responding to reductions in K^+ availability and accumulation of an 'imposter' cation in the form of Na^+. While intracellular CO_2 also decreased, likely due to changes in g_s, these values were not significant in this trial.

Contrarily, when we separate the results by rootstock and not applied NaCl concentration we see that intracellular CO_2 concentrations become significantly different between tested accessions (Table 2. 4). It is essential to note that this result does not reflect the change by treatment. Rather, it signifies a more general trend of differences in CO_2 accumulation among leaf mesophyll by

genotypes. This trend is further developed when plant CO_2 response to both the differing applied NaCl concentrations and the rootstocks is observed (data not shown). Further, we can see that stomatal conductance and transpiration rates differ among accessions only at the highest [NaCl] application rate of 100 mMol NaCl. Table 2. 4 suggests a similar photosynthetic response to presence of NaCl at any concentration in all grapevines. While in Table 2. 4 intracellular CO_2 is significantly different among genotypes tested, this observation may be attributed to an inherent difference in intracellular CO_2 when no excess NaCl is introduced into the environment.

These results lead us to two related conclusions. First is that CO_2 accumulation in leaf tissue varies by rootstock accession under ideal conditions. However, once NaCl is applied or accumulates near the roots this innate difference disappears. The response of rootstock CO_2 accumulation to excess, external NaCl is likely related to the much more responsive stomatal conductance (g_s). Observed in this trial, g_s only becomes significantly different among accessions when the plant is exposed to at least 100 mMol NaCl and is significantly different when separated by applied NaCl concentration regardless of rootstock (Table 2. 3). As they are highly correlated with one another, the same pattern emerges in E as observed in g_s. Because transpiration rates and CO_2 uptake are somewhat, if not heavily, reliant on the rate of g_s, it may be surmised that the responses in E and intracellular CO_2 recorded here are related to changes in stomatal conductance.

Regardless of these specific changes in the face of differing rootstocks and applied NaCl concentrations, introduction of NaCl at concentrations of 75 mM or greater appeared to greatly suppress photosynthetic activity in all varieties tested. Transpiration rates and stomatal conductance decreased by half or more in tested accessions exposed to this high NaCl concentration when compared with those exposed to 25 mMol NaCl or less. Net carbon assimilation also drops by 22 % when comparing results of those exposed to 25 mM and 75 mM

NaCl and these changes are also significant (Table 2. 2). Suppression of photosynthetic capacity is a consistent phenomenon in this trial and poses a serious challenge to regions threatened by high NaCl accumulation in vineyard soils.

From this study, it is clear that NaCl concentrations of 75mMol, or approximately 7.5 $dS * m^{1}$, have the potential to reduce photosynthetic activity in rootstocks by as much as half that of the corresponding, non-salt-stressed individual. Higher NaCl concentrations reduced transpiration rates, stomatal conductance, and net carbon assimilation in all varieties we tested here. However, it is unknown if this response is transmissible to a grafted scion, which would be assuming the responsibilities of photosynthesis in production settings.

2.6 Conclusion

While valuable information on photosynthetic responses of rootstocks to high external NaCl concentrations was gleaned from this study, it remains to be shown if the observed change in photosynthetic activity is transmissible to a grafted scion. Further studies are necessary to reveal the potential for highly salt tolerant rootstocks such as *V. acerifolia* 9018 to serve as a rootstock, or breeding stock, in highly saline viticultural regions.

Concerns about the potential of the tested rootstocks to confer the observed NaCl tolerance need to be addressed and answered before we proceed in pursuing these varieties as a solution to NaCl accumulation in vineyards. Ease of rooting and grafting are critical factors to consider, as well as root architecture and yield output on varieties grafted to such rootstocks. This knowledge is not trivial and will require multiple field trials to assess adequately. However, this study presented useful knowledge to contribute to the general problem of vineyard salinity. It serves as a start and not a solution to addressing this issue. Latter studies will focus on assessing the potential of

rootstocks which have displayed high, low, and moderate NaCl tolerance when grafted to a common scion.

2.7 Tables

Table 2. 1. Correlation of each NaCl concentration applied against each accession tested; significant groupings within each rootstock grouped by applied NaCl concentration.

Summary of fit Accession ~ [NaCl]		Tukey HSD [NaCl] applied			
Accession		0 mM NaCl	25 mM NaCl	75 mM NaCl	100 mM NaCl
101-14 Mgt		a	a	b	b
110 Richter		a	a	b	b
140 Ruggeri		a	a	b	b
44-53 Mal		a	a	b	b
99 Richter		a	a	b	b
Dog Ridge		a	b	c	c
V. acerifolia 9018		a	a	ab	b
Ramsey		a	b	c	c
V. riparia 'Gloire'		a	b	b	c

Table 2. 2. Final leaf and petiole Cl- concentrations at harvest by rootstock and applied NaCl treatment; "NA" = dead plants.

	[NaCl] applied											
Accession	0 mM NaCl			25 mM NaCl			75 mM NaCl			100 mM NaCl		
	mg L ^{1}	se		*mg L* ^{1}	se		*mg L* ^{1}	se		*mg L* ^{1}	se	
101-14 Mgt	17.50	4.9	a	44.56	8.1	bcd	120.3	19.6	b	161.6	28.29	ab
110 Richter	17.00	2.9	a	26.00	NA	cd	140.3	NA	ab	90.17	17.61	bc
140 Ruggeri	14.83	2.6	a	28.50	4.30	cd	105.0	NA	b	151.1	79.96	ab
44-53 Mal	17.45	0.6	a	120.3	10.3	a	328.2	68.0	a	266.6	NA	a
99 Richter	16.00	3.4	a	27.84	3.18	cd	85.6	43.0	b	113.6	24.83	bc
Dog Ridge	13.89	4.0	a	71.78	21.4	b	127.3	57.1	b	135.6	NA	abc
V. acerifolia 9018	14.67	2.4	a	17.66	2.52	d	25.20	2.89	b	28.44	8.15	c
Ramsey	16.55	6.6	a	65.89	15.0	bc	134.00	37.8	b	162.2	20.12	ab
Riparia Gloire	21.00	NA	a	46.67	NA	bcd	66.00	24.2	b	135.0	NA	abc
p-value	0.8007			< 0.001 **			< 0.001 **			0.0024 *		

Table 2. 3. Parameters of net photosynthesis based on LiCOR measurements of transpiration (E), net carbon assimilation (A_{net}), intracellular CO_2 (CO_2), and stomatal conductance (g_s) by applied NaCl concentration treatment.

Net photosynthesis by applied [NaCl]												
NaCl applied	*E*			*A_{net}*			*CO_2*			*g_s*		
	Mol m^{-2} s^{-1}	se		*Mol m^{-2} s^{-1}*	se		*Mol mol^{-1}*	se		*Mol m^{-2} s^{-1}*	se	
0 mM	0.002	0.0004	a	9.10	0.9	a	220.45	14.8	a	0.13	0.03	a
25 mM	0.002	0.0003	a	9.45	0.9	a	216.71	12.4	a	0.12	0.02	a
75 mM	0.001	0.0001	b	6.41	0.7	b	177.54	11.2	a	0.06	0.01	b
100 mM	0.0009	0.0001	b	6.35	0.6	b	169.37	11.3	a	0.05	0.01	b
p value	< 0.001*			< 0.001*			0.573			< 0.001*		

Table 2. 4. Parameters of net photosynthesis based on LiCOR measurements of transpiration (E), net carbon assimilation (A_{net}), intracellular CO_2 (CO_2), and stomatal conductance (g_s) by accession tested.

Net photosynthesis by genotype tested

Accession	*E*			A_{net}			CO_2			g_s		
	Mol m $^{-2}$ *s* $^{-1}$	se		*Mol m* $^{-2}$ *s* $^{-1}$	se		*Mol mol* $^{-1}$	se		*Mol m* $^{-2}$ *s* $^{-1}$	se	
101-14 Mgt	0.0018	0.0006	a	7.39	1.39	a	203.78	24.54	ab	0.1	0.04	a
110 Richter	0.0015	0.0005	a	7.2	1.22	a	195.49	26.04	ab	0.08	0.03	a
140 Ruggeri	0.0017	0.0003	a	8.61	1.1	a	202.96	12.91	ab	0.09	0.02	a
44-53 Mal	0.0015	0.0004	a	8.03	1.19	a	201.13	17.26	ab	0.09	0.02	a
99 Richter	0.0012	0.0003	a	7.13	1.16	a	178.64	14.91	ab	0.07	0.02	a
Dog Ridge	0.0017	0.0004	a	7.72	1.04	a	216.18	22.25	a	0.09	0.02	a
V. acerifolia 9018	0.002	0.0006	a	8.48	1.25	a	214.47	19.99	a	0.12	0.04	a
Ramsey	0.0013	0.0004	a	7.94	1.27	a	171.75	14.46	ab	0.08	0.03	a
p value	0.302			0.775			0.018**			0.409		

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Chapter 3. Ability of selected rootstock cultivars to confer chloride tolerance to grafted *Vitis vinifera* cv. Cabernet Sauvignon scion in greenhouse and near-field conditions

3.1 Abstract

Development of grapevine rootstocks with resistance to Cl^- toxicity damage has become more urgent as viticultural regions around the world begin to experience the damage imposed by insufficient water for irrigation and declining water quality. Through rapid depletion and pollution, groundwater throughout the southwestern United States has seen less abundance and an increase in brackish waters (Ferguson et al. 2018). Some regions engaged in viticulture rely heavily, if not solely, on the use of groundwater for irrigation and are at high risk for agricultural collapse due to low water availability and salt toxicity damage. Beginning with the Phylloxera crises of the late 19^{th} century, the use of grapevine rootstocks has long provided associated scion varieties direct protection from numerous pests, diseases, and abiotic stressors. However, new concerns of water and soil salinity have prompted development of new rootstocks with potential to directly limit sodium and chloride damage to photosynthetic tissues and harvestable fruit. Eight grapevine accessions, either already utilized as commercial rootstocks or with potential to be used in vineyards, were grafted to a 'Cabernet Sauvignon' Clone 8 scion and exposed to 50 mM NaCl via fertigation solution. Two complete sets of these individuals with replicates were separated into an indoor, greenhouse-based trial and a corresponding outdoor trial to test viability of results obtained in the highly controlled greenhouse setting with more realistic conditions outdoors. Photosynthetic responses were recorded in the associated scions at three time points over the 21-day experimental

period. Following the application time frame, accumulation of Cl^- was measured in scion leaf and petiole tissues as well as in the corresponding rootstocks' root systems. Indoor conditions resulted in definitive separation of scion Cl^- accumulation among the eight tested individuals. However, in outdoor conditions these differences were not as pronounced. Photosynthetic measurements suggest the inconsistency between indoor and outdoor trial results may be associated with shedding and replacement of Cl^- laden leaves by outdoor vines, but not vines grown in greenhouse conditions. Further work focused on salinity impact to fruit quality and yields is needed.

3.2 Introduction

Analysis of newly introduced grapevine rootstocks with the purposes of addressing novel concerns in vineyard settings requires many parameters be examined. Under conditions of biotic or abiotic stress, candidate rootstocks must also be assessed for rooting ability, grafting ability, impacts on vigor, effects on crop quality and yield, and many other traits related to growth and yield capacities (Barrios-Masias et al. 2015, Peterson et al. 2019, yıldırım et al. 2018). Likewise, when acquiring knowledge about salt tolerance in grapevine rootstocks the same features and factors should be quantified and recorded. Although specific responses of rootstocks to the conditions present in their immediate environment is of essential concern, addressing effects of those same rootstocks on the scion is as significant to the introduction of a newly developed grapevine rootstock. Again, many issues should be assessed during this screening phase. Among these is the scion-rootstock interaction and how a rootstock can alter phenotypic traits of a well-documented scion variety (Migicovsky et al. 2019, Walker et al. 2014). In grapevine, rootstock selection has been known to impact water uptake, berry skin composition, nutrient uptakes, and yields for their corresponding scion (Tramontini et al. 2013, Zhang et al. 2008, Zombardo et al. 2020). Alternatively, scion choice may also impact the biomass allocation and root architecture of

a paired rootstock (Tandonnet et al. 2010). Subsequently, interactions of specific rootstock and scion varieties are essential avenues of research to include in any grapevine development program. These concerns are also valid in the testing of Cl^- tolerance of varying accessions, both wild and cultivated.

Prior studies have identified potential candidates for the use in the UC Davis grapevine rootstock breeding program designed to address soils with high NaCl concentrations (Chapter 1). Chapter 2 examined the photosynthetic response of tolerant and susceptible accessions focusing heavily on the differences observed in rootstock stomatal conductance, transpiration rates, intracellular CO_2, and net carbon assimilation rates. Although understanding the response of rootstock photosynthetic tissue to high NaCl exposure is an important piece of the puzzle, observing the photosynthetic response of scions grafted to NaCl exposed rootstocks may be of greater importance. In production settings rootstocks serve the purpose of a buffer between the producing scion and the many detrimental pests, diseases, and toxic substances in the soil. Functionally, a Cl^- tolerant rootstock should be capable of conferring this trait to a wide selection of grafted scions.

Previous studies have measured aspects of photosynthesis and growth on scions grafted to well-known rootstock varieties (Verma et al. 2010, Zhang et al. 2016). However, many of these studies were conducted under the assumption that salinity tolerance traits were present in limited varieties with a lack of supporting data and few alternatives. There has been a limited pool of NaCl tolerant grapevine species to conduct high quality salinity tolerance trials with. Our previous work helped to target both individual rootstocks with the ability to exclude chloride from leaf and petiole tissues and those lacking Cl^- exclusion characteristics. With no significant differences observed in root Cl^- accumulation, this study separates salt-tolerant, mildly salt-tolerant, and salt-susceptible rootstocks by their previously observed accumulation of chloride in leaf and petiole tissues.

Choice of scion is also of concern when testing the effectiveness of rootstocks to impart their specific tolerances to the grafted *V. vinifera* scion. Founding studies on grapevine salt tolerance primarily used Sultana as a flagship scion for testing salt tolerances imparted by selected rootstocks (Downton and Crompton 1979, Walker et al. 2002). However, Sultana's large leaves and variable response to NaCl stress removed this variety as an option for the study presented here. Additionally, Sultana is no longer widely planted with only 119 acres planted in California, USA, since 2011 (California Department of Food and Agriculture and Agriculture 2019). Considering the declining interest in Sultana as the dominant grape cultivar in California, we selected Cabernet Sauvignon as our standard scion for this trial. Current acreage for Cabernet Sauvignon in California sits at 94,854 acres, both bearing and non-bearing (California Department of Food and Agriculture and Agriculture 2019). As a prominent scion and current industry standard, Cabernet Sauvignon is used here as a representative and functional cultivar for the potential of tested rootstocks to confer Cl^- tolerance to a grafted scion under sodic soil conditions.

All previous studies we have approached were conducted indoors and in greenhouse conditions. This trial was designed to represent the culmination of our research over three years into potential salt-tolerant rootstock varieties and we wanted to ensure that the results were comparable to our previous studies. However, we also believed it essential that we conducted a parallel trial outdoors and in a more realistic setting to display the efficacy of the proposed NaCl tolerance of different rootstock accessions.

3.3 Materials and Methods

Plant Material

Rootstocks tested here were chosen based on observations made previously in Chapters 1 and 2. Nine accessions were chosen in this trial to represent the three discrete groupings imposed upon the experiment. One of the chosen accessions did not survive the experiment and was removed from the final data set. Once grafted, two sets of experimental units were separated and placed both outdoors in 0.038 m^3 (38 Liter) plastic pots and indoors under controlled greenhouse settings in 0.002548 m^3 (2.548 L) pots. Each of the eight accessions was classified as either highly salt-tolerant, moderately salt-tolerant, or salt-susceptible, with three rootstocks chosen from the original nine in each classification. The salt-susceptible grouping ended the experiment with two distinct rootstocks due to the loss of one mentioned previously. Distinctions were based on evidence from previous results of leaf and petiole chloride accumulation following 21 days of exposure to 75 mM NaCl (data not shown). Those separated into the highly salt-tolerant category consistently displayed Cl^- concentration of 150 $mg * L^{-1}$ or less under the conditions described. Moderately salt-tolerant accessions recorded between 151 and 250 $mg * L^{-1}$ of Cl^- and salt-susceptible varieties regularly accumulated Cl^- concentrations of 251 $mg * L^{-1}$ or greater during preliminary testing.

Highly salt-tolerant rootstock varieties included *V. acerifolia* 9018, *V. girdiana* 8, and *V. doaniana* 9026. Moderately salt-tolerant rootstocks included the commonly utilized rootstocks 140 Ruggeri, *V. rupestris* 'St. George', and 1103 Paulsen. Salt-susceptible rootstocks included *V. rupestris* Pumpstation, 44-53 Malegue, and Ramsey.

Propagation

For each accession tested all propagated cuttings were sourced from a single mothervine, each of which provided multiple cuttings for replicates of three in this trial. Vegetative cuttings were

taken during active growing periods from March through July of 2019. Each cutting was taken from the terminal 0.5 meter of actively growing shoots and approximately 1 to 1.5 cm in diameter. Each cutting was limited to two buds to encourage root genesis over foliage growth in the early stages of propagation. Shoots or leaves with visible nutrient deficiencies, physical damage, and/or disease symptoms were avoided during collection. Once excised with clean pruning shears, cuttings were stored in plastic bags on ice until transported to the greenhouse for propagation preparations.

Two-bud segments were separated and dipped in a 1:20 dilution of liquid rooting hormone of 1.03% Indol-3-butyric acid and 0.66% 1-naphthalene acetic acid (Wood's rooting compound ™; Earth Science Products Corp; Wilsonville, OR, USA), consisting of 10,000 ppm indole-3butyric acid and 5,000 ppm naphthalene acetic acid, then individually placed in cellulose propagation plugs and distributed in a flat tray. Vegetative cuttings were allowed to leaf out in a fog room for 14 days following collection and preparation. Rooted cuttings were then moved to a greenhouse with consistent temperature fluctuations for 14 days to acclimate to a cooler and less humid environment. Each cutting was then transferred to either a round 2.548 L plastic pots, or a 38 L plastic pot, and filled with a non-swelling, fritted-illite clay growing media (Turface Pro MVP ™, Profile Products LLC; Buffalo Grove, IL, USA) to prevent soil flocculation effects from NaCl applied in excess. Plants were then allowed to acclimate under these conditions over the course of 21 days while being fertigated with N, P, K, Ca, Mg, S, Fe, Cu, B, Mn, Mo, and Zn in previously reported concentrations (Fort et al. 2015). All nutrients applied were first dissolved in distilled water via an onsite water-powered chemical injector.

Medium used for cuttings was a large-particle, calcined, non-swelling illite clay with high porosity and relatively high cation exchange capacity of 30 $mEq\ 100g^{-1}$. This 2:1 layer silicate was

used to ensure proper binding of potassium, which acts as an interlayer cation, to prevent sodium from over competing for binding sites within the media. This clay medium was also used to help guard against dispersion and flocculation of soils when sodium chloride concentrations, now referred to as [NaCl], were high enough to promote soil structure change as a result of high Na^+ levels. Water holding capacity of this material was very low relative to other potential rooting media and required daily irrigation applications.

Photosynthetic measurements

Photosynthetic measurements were taken using the LiCOR 6800 portable photosynthetic system (LiCOR model LI-6800; Lincoln, NE, USA) at three time points during the period of the experiment and consisted of single point measurements from three replicates of each rootstock ~ applied [NaCl] combination. Photosynthetic measurements were only taken in outdoor trials due to a lack of availability of equipment. Recording of g_s, A_{net}, intracellular CO_2, and E occurred once per seven days throughout the 21-day test period for each experimental unit. Leaves chosen from the same node position on each individual plant were measured each cycle to mitigate the influence of leaf age on recorded values. A new, undamaged leaf was chosen for each weekly measurement. Leaves selected for this process were left on the plants and allowed to continue transpiring and providing photosynthates to their associated vines.

NaCl application

Following the growth and acclimation period based on previous work (Fort et al. 2013) greenhouse tested plants were exposed daily to either fertigated greenhouse water with no NaCl added or 50 mM NaCl, or 5.0 $mEq\ 100g^{-1}$, of NaCl dissolved in fertigated, distilled water from the greenhouse irrigation system. This application rate continued for 21 days prior to harvest. Unlike

the nutrients in the fertigated water, which were mixed by a water-powered pressure injector directly, NaCl was incorporated and measured by hand in large, highly-concentrated stock solutions prior to being diluted to the required concentration by a secondary pressure injector and applied via overhead watering by hand. Stock solution was diluted by a factor of 65x prior to application on any tested rootstocks. Above ground tissue did not come into contact with the highly saline water to prevent accumulation of precipitated NaCl on the exterior of leaves or other tissues. Plants transplanted to 38 L pots and allowed to acclimate outdoors required more time for growth and development and were not treated with salt for 120 days. Imposition of a longer period designated for growth and development were meant to simulate conditions in real-world vineyards where vines do not produce yields for two to three years minimum. During the allotted growth phase outdoor vines were fertigated with greenhouse water used for the indoor trial with no NaCl added and a base level between 2-3 mM Cl^- in irrigation water. Following 120 days of growth, outdoor vines were exposed to either fertigated greenhouse water with no NaCl added or 50 mM NaCl water applied in the same manner as to the indoor vines.

Sample collection and preparation

Following 21 days of NaCl exposure, 10 leaves and petioles from each plant in the greenhouse trial were carefully removed, weighed for fresh mass, and individually stored and labelled in brown paper bags. Similarly, in our outdoor trial, 10 leaves and petioles from each plant were excised and treated in the same manner. Weighed samples then were transferred to a drying room at 50°C for 14 days before dry mass was recorded. Shoots and original propagation tissue were excluded from all samples due to initial inconsistencies in size and age.

Once fully dried, samples of leaf and petiole tissues were recorded for dry mass then pulverized to a fine powder while remaining isolated in their original harvest bags. An aliquot of

25 mg of dried and powdered tissue was taken from each sample and added to individual screw cap bottles with 25 mL de-ionized water. All samples were then, simultaneously loaded onto a modified orbital-shaker table and allowed to mix at 200 rpm for 1 hr. Preliminary data [not shown] indicated that allowing this for 1 hr resulted in minimal differences in chloride readings to those left for 18 h, overnight. Extracted samples were then filtered through 11µm filter paper and the resulting supernatant was diluted by a factor of 2 to increase accuracy of the subsequent chloride readings. Chloride content readings were determined using a silver-ion titration chloridometer (Model 926, Nelson-Jameson Inc., Marshfield, WI, USA), and repeated three times per sample, following manufacturer's guidelines for use.

3.4 Results

Outdoor-grafted trial

Photosynthetic responses of Cabernet Sauvignon scions to application of 50mM NaCl via fertigation were inconsistent over the 21-day application period. Among the eight surviving rootstocks tested in this study, there were no observed differences in transpiration (E), net carbon assimilation (A_{net}), intracellular CO_2, or stomatal conductance (g_s) when comparing the values for these parameters in associated scions (Table 3. 1). General increases were observed in E, A_{net}, and g_s, but not intracellular CO_2 from pre-application of salts to six days into the experiment. However, no notable trend could be established in these parameters between the 6- and 21-day measurements. With no significant differences in photosynthesis among the tested rootstock genotypes exposed to 50mM NaCl, comparing the NaCl treatments together did reveal some trends. Table 3. 2 shows transpiration rates generally increased throughout the experiment regardless of treatment. However, unsalted control vines had a more rapid increase in *E* overall. Net carbon assimilation initially increased notably between day 0 and day 6 but proceeded to decrease by day 21 in both

treatments. Stomatal conductance followed the same trend as A_{net}. Intracellular CO_2 reacted inversely and had an initial decrease followed by an increase with final values similar to readings taken before treatments began. Change in these measurements were variable and did not consistently increase or decrease any parameter with exception of *E*, which continued to increase throughout the project.

Accumulation of Cl^- was consistent throughout all vines of the same NaCl treatment. All Cl^- values of leaves and petioles of vines treated with 50mM NaCl were not significantly different with a single exception from Cabernet Sauvignon grafted to 44-53 Mal rootstock, which accumulated nearly three-fold greater Cl^- concentrations than the next highest accumulator (Figure 3. 1). Among the individuals that did not receive additional NaCl there were not significant differences in Cl^- accumulation in leaves and petioles between any individuals (Figure 3. 1). When Cl^- accumulation is compared by NaCl treatment within each rootstock genotype only three of the eight rootstocks did not show a significant Cl^- accumulation difference in scion leaves and petioles (Figure 3. 2). Both *Vitis acerifolia* 9018 and *Vitis doaniana* 9026 facilitated high salt exclusion from scion photosynthetic tissues were flagged as 'good chloride excluders' prior to the experiment. In the six other rootstock accessions Cl^- accumulation in leaf and petiole tissues was always greater in vines treated with 50mM NaCl than unsalted controls.

Indoor Grafted Trial

Under controlled greenhouse conditions results varied from the outdoor study. Accumulation of Cl^- in 50mM NaCl treated vines was generally much greater in scion leaves and petioles in all cases except when grafted to *V. acerifolia* 9018 or *V. doaniana* 9026 (Figure 3. 3). Furthermore, rootstock differentiation based on leaf and petiole Cl^- accumulation were grouped into three categories. Poor Cl^- excluders included scions grafted to 1103P and 44-53 M with Cl^-

concentrations near 300 $mg\ L^{-1}$. Moderate Cl^- excluders included *V. rupestris* 'St. George', 140Ru, and Ramsey. Good Cl^- excluders included *V. acerifolia* 9018, *V. doaniana* 9026, and *V. girdiana* 8. However, three rootstock genotypes, each representing a different category of excluder quality, were selected to have additional individuals included without a grafted scion. These three rootstocks were 140Ru, 44-53 M, and *V. acerifolia* 9018. Figure 3. 3 showed that there was no significant difference in leaf and petiole Cl^- accumulation with or without scions within the same genotype by the end of the experiment. While the results from this study conducted in a controlled greenhouse environment were partially expected, they do not align with the observations of the concomitant outdoor trial.

3.5 Discussion

Results from the indoor trial showed separation in leaf Cl^- accumulation among rootstock genotypes tested while Cl^- accumulation in the outdoor trial did not separate by rootstock genotype with only one exception when using 44-53 M. Discrepancies in Cl^- accumulation of scion leaf and petiole tissues of outdoor and indoor studies must be addressed. Separation of rootstock Cl^- effect on a shared scion was less pronounced when tested outdoors than in a greenhouse setting. Given this data, concerns may arise that rootstock choices do not actually impact the accumulation of Cl^- in scion tissues except in the most drastic cases, such as through the use of 44-53 M as a rootstock. However, this may not be true. Table 3. 2 shows that both A_{net} and g_s both initially increased drastically followed by a decrease at day 21 while E increased steadily in the outdoor study. Intracellular CO_2 had the opposite response with initial decreases followed by subsequent increases by the end of the project. These trends follow established behavior for these parameters in grapevines when analyzed by age of the leaves (Intrieri et al. 1992, Schubert et al. 1996).

These findings are particularly relevant due to the design and timing of each experiment. Indoor studies took place between the months of June and July of 2020 while the outdoor study occurred throughout the month of September, both in Davis, California, USA. The time gap between these instances is directly influenced by seasonal changes in day length, ambient temperatures, and available solar radiation. Additionally, outdoor vines were initially planted in April 2019 with the goal of establishing mature, fruiting vines to test impacts of NaCl application on fruit quality while indoor vines were planted in May of 2020. Although both studies occurred in 2020, the vines included in each experiment were transplanted to their final position nearly one year apart. Vines in indoor conditions were rigorously maintained with each individual loosing no more than two leaves each. Outdoor vines likely shed many more leaves than indoor vines due to both their age and the closer temporal proximity to dormancy these vines were tested at. It has been shown that leaf removal can elevate net photosynthetic rates of remaining leaves in grapevines (Petrie 2000). Table 3. 2 suggests that this may have occurred in outdoor-tested vines displaying trends in E, A_{net}, intracellular CO_2, and g_s similar to observations by Schubert et al. (1996) in younger leaves at day six and older leaves at day 21. It is possible that leaves tested for Cl^- accumulation in this study's outdoor trial were much younger when NaCl application began than those of their corresponding indoor treatments and were potentially shed and replaced during the experiment. However, when analyzing each study separately we can come to some meaningful conclusions.

Indoor Grafted Trial

Vines grown indoors showed that rootstock choice dramatically impacts Cl^- accumulation in scion leaves and petioles. Significant differences observed in Figure 3. 3 reflect the initial hypothetical groupings stated prior to the start of this project. Each rootstock had similar levels of

efficacy for Cl^- exclusion from leaf and petiole tissues with Cabernet Sauvignon scions grafted to them as they did in previous studies where they were not grafted to any scion. Additionally, those rootstocks within this study with both grafted and non-grafted individuals showed no difference in leaf and petiole Cl^- accumulation regardless of absence or presence of a Cabernet Sauvignon scion (Figure 3. 3). These findings suggest that the observed ability to limit Cl^- accumulation in above ground tissues in individual rootstocks can be conferred to a grafted scion and is not distinctly different from the response observed in rootstocks without a grafted scion. Of the observable tissue above ground in all individuals the vast majority belonged to the grafted scion here suggesting the location of chloride sequestration or restriction is likely located somewhere between the soil-root interface and the graft-union.

The conservation of observed rootstock Cl^- restriction qualities from ungrafted rootstock to a rootstock-scion combination suggests that the mechanism that limits Cl^- accumulation in leaf and petiole tissues is chiefly located at the root level and not likely to occur in the long-distance xylem transport of the trunk, but prior to ion entry into xylem vessels. Additionally, this does not seem to be impacted by leaf mass (Figure 3. 4) but may be affected by leaf area. These findings are supported by previous work, which credit passage through, and sequestration by, xylem parenchyma cells as the site of action for Cl^- tolerance in grapevines (Abbaspour et al. 2013, Gong et al. 2011, Henderson et al. 2014, Munns and Tester 2008).

Outdoor-grafted trial

It is likely that the discrepancies in Cl^- accumulation observed between the indoor and outdoor experiments were influenced by unaccounted for artefacts of the project. Particularly, the senescence of Cl^- laden leaves may have occurred in vines tested outside of a controlled environment at much higher rates than in those vines tested in a controlled greenhouse setting.

This type of response is not uncommon and has been shown to occur in rice leaves exposed to NaCl induced stress (Lutts et al. 1996). The greater age of outdoor vines, timing of treatment application, and lack of controlled environment may have contributed to possible increased rates of leaf senescence in the outdoor experiment in contrast to the indoor trial. Table 3. 2 shows surprisingly low photosynthetic values prior to NaCl application, and following exposure to 50mM NaCl each of the parameters increase or decrease in the same direction for both NaCl treatments. However, those treated with 50mM NaCl tended to have less overall change in transpiration, net carbon assimilation, and stomatal conductance than unsalted controls. It is possible that leaves chosen for photosynthetic measurements were relatively young at day zero of the treatment application, increasing rapidly in all measured parameters but less so in NaCl treated vines.

While in all cases scion-rootstock combinations grown outdoors accumulated less Cl^- in leaf and petiole tissues than their indoor counterparts, Cabernet Sauvignon grafted to 44-53 Mal still acquired significantly high levels of Cl^- in leaves and petioles. Additionally, scions grafted to another high Cl^- accumulator indoors, 1103P had 1500% less Cl^- accumulated outdoors than 1103P grafted scions in a greenhouse environment. The differences observed in some cases here suggest the removal of some Cl^- from the rootstock-scion system, possibly in the form of leaf senescence. If this is indeed the case then there are further problems to address in these studies. While removing accumulated Cl^- may be effective in the short-term, it likely depletes carbohydrate reserves in the vine and alters the rate of photosynthate production at crucial phenological timepoints during the growing season. Expansion of new leaves can also require a large amount of water to provide additional turgor for cell expansion, a problem particularly devastating in water-limited regions.

Concerns may arise with the development of berry clusters. Unlike leaf and petiole tissue, grapevines cannot drop fruit impacted by high Cl^- accumulation. Although not tested in this study, fruit act as a major sink for most crops and are likely to accumulate high levels of Cl^- in a salt-laden environment. Salt effected clusters often cannot be dropped manually either due to concerns of yield or profit losses.

3.6 Conclusion

Observations of leaf and petiole Cl^- accumulation of scions grown in a controlled climate showed that rootstock choice can impact the amount of Cl^- that accumulates in these tissues. Rootstocks selected to be compared with and without a grafted scion displayed no significant differences in leaf and petiole While this study provided insight into the response of a scion grafted to rootstocks of varying Cl^- tolerance, it was limited in scope. Further work is necessary to elucidate the movement of Cl^- from source to sink in grafted grapevines. Vines tested under 50mM NaCl in a controlled environment displayed an expected response dependent upon rootstock choice. However, vines tested under 50mM NaCl in a less controlled outdoor environment did not reflect findings observed in the former group. There may be many artefacts influencing this discrepancy. Some of these may be due environmental conditions such as excessive particulate matter in the atmosphere from wildfire smoke, which occurred at a large scale during this experiment. Leaf area may also be an important factor to include to help explain photosynthetic responses. Timing of the experiment is also of concern, as outdoor grafted vines were tested much later in the season than indoor grafted vines. Among possible related factors, the analysis of Cl^- accumulation in grapevine berry tissue should be examined to further understand the ability of selected rootstocks to confer Cl^- tolerance to a grafted scion. While leaf and petiole tissues can be easily shed by grapevines, fruit clusters cannot be removed so efficiently by the vine itself. To

further explore the data analyzed in this article it would be beneficial to collect senesced leaf and petiole tissue throughout the study and examine Cl^- accumulation of shed leaves per individual. Quantification of berry Cl^- accumulation in conjunction with analysis of shed leaves would serve to support or refute the findings of this study.

3.7 Tables

Table 3. 1. Transpiration rates (E), carbon assimilation (Anet), intracellular CO2 (Ci), and stomatal conductance (gs) based on LiCOR measurements by accession tested; Outdoor trial.

Photosynthetic responses to 50 mM NaCl												
0 days since treatment start												
Accession	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
1103 Paulson	0.001	0.0007	a	2.21	2.44	a	180.79	97.24	a	0.02	0.01	a
140 Rugerri	0.0012	0.0008	a	3.09	2.26	a	183.15	42.46	a	0.02	0.02	a
44-53 mal	0.0006	0.0006	a	0.39	0.24	a	258.73	105.47	a	0.01	0.01	a
Ramsey	0.0041	0.004	a	7.42	7.92	a	239.64	86.42	a	0.07	0.08	a
V. acerifolia 9018	0.0012	0.0008	a	2.08	2.48	a	241.73	71.3	a	0.02	0.02	a
V. doaniana 9026	0.0012	0.0008	a	1.68	2.3	a	267.15	98.3	a	0.02	0.01	a
V. girdiana 8	0.0007	0.0008	a	0.84	0.56	a	231.42	30.31	a	0.01	0.01	a
St. George	0.0019	0.0027	a	3.4	4.8	a	184.38	78.04	a	0.04	0.05	a
p value	0.781			0.435			0.759			0.441		
6 days since treatment start												
Accession	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
1103 Paulson	0.0029	0.0005	a	7.12	3.45	a	237.24	51.01	a	0.08	0.02	a
140 Rugerri	0.0043	0.0013	a	13.31	3.21	a	216.27	7.32	a	0.14	0.03	a
44-53 mal	0.0023	0.0014	a	8.13	4.07	a	196.32	12.74	a	0.08	0.05	a
Ramsey	0.0034	0.0015	a	10.87	4.63	a	215.1	24.53	a	0.12	0.06	a
V. acerifolia 9018	0.0025	0.0002	a	9.28	1.52	a	180.7	17.63	a	0.08	0.01	a
V. doaniana 9026	0.0019	0.0006	a	5.83	1.33	a	212.72	24.01	a	0.06	0.02	a
V. girdiana 8	0.003	0.0002	a	10.78	1.43	a	194.16	15.97	a	0.1	0.02	a
St. George	0.0022	0.0009	a	7.3	3.48	a	209.58	24.72	a	0.07	0.03	a
p value	0.131			0.144			0.282			0.096		
21 days since treatment start												
Accession	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
1103 Paulson	0.0045	0.0018	a	12.02	5.28	a	231.59	14.01	a	0.14	0.07	a

140 Rugerri	0.0034	0.0006	a	8.61	0.63	a	248.78	9.93	a	0.1	0.004	a
44-53 mal	0.0028	0.001	a	7.4	3.57	a	242.2	11.99	a	0.09	0.05	a
Ramsey	0.0046	0.0008	a	12.06	2.06	a	231.07	15.25	a	0.13	0.03	a
V. acerifolia 9018	0.0026	0.0004	a	6.99	0.7	a	212.73	18.16	a	0.07	0.01	a
V. doaniana 9026	0.0024	0.0009	a	6.44	3.27	a	245.67	13.07	a	0.08	0.03	a
V. girdiana 8	0.0029	0.0006	a	7.28	0.99	a	239.86	17.92	a	0.08	0.01	a
St. George	0.003	0.0003	a	8.4	1.5	a	241.27	4.47	a	0.1	0.02	a
p value	0.217			0.125			0.106			0.217		

Table 3. 2. Transpiration rates (E), carbon assimilation (Anet), intracellular CO2 (Ci), and stomatal conductance (gs) based on LiCOR measurements by NaCl treatment tested; Outdoor trial

Photosynthetic responses to 0 mM or 50 mM NaCl treatments												
0 days since treatment start												
[NaCl] treatment	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
0 mM NaCl	0.0012	0.0005	a	1.9	1.08	a	229.5	32.6	a	0.02	0.01	a
50 mM NaCl	0.0015	0.0007	a	2.64	1.31	a	223.37	26.54	a	0.03	0.01	a
p value	0.5347			0.4629			0.8013			0.5823		
6 days since treatment start												
[NaCl] treatment	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
0 mM NaCl	0.0035	0.0004	a	10.97	1.13	a	217.85	8.9	a	0.12	0.01	a
50 mM NaCl	0.0028	0.0004	b	9.08	1.24	b	207.76	9.49	a	0.09	0.01	b
p value	0.0178*			0.0266*			0.1894			0.0072**		
21 days since treatment start												
[NaCl] treatment	$E\ (mol * m^{-2} * s^{-1})$			$Anet\ (mol * m^{-2} * s^{-1})$			$CO_2\ (mol * mol^{-1})$			$g_s\ (mol * m^{-2} * s^{-1})$		
	mean	se		mean	se		mean	se		mean	se	
0 mM NaCl	0.0036	0.0004	a	8.88	1.16	a	248.1	7.16	a	0.11	0.01	a
50 mM NaCl	0.0033	0.0004	a	8.65	1.1	a	236.64	5.61	b	0.1	0.01	a
p value	0.3081			0.7871			0.0367*			0.2464		

3.8 Figures

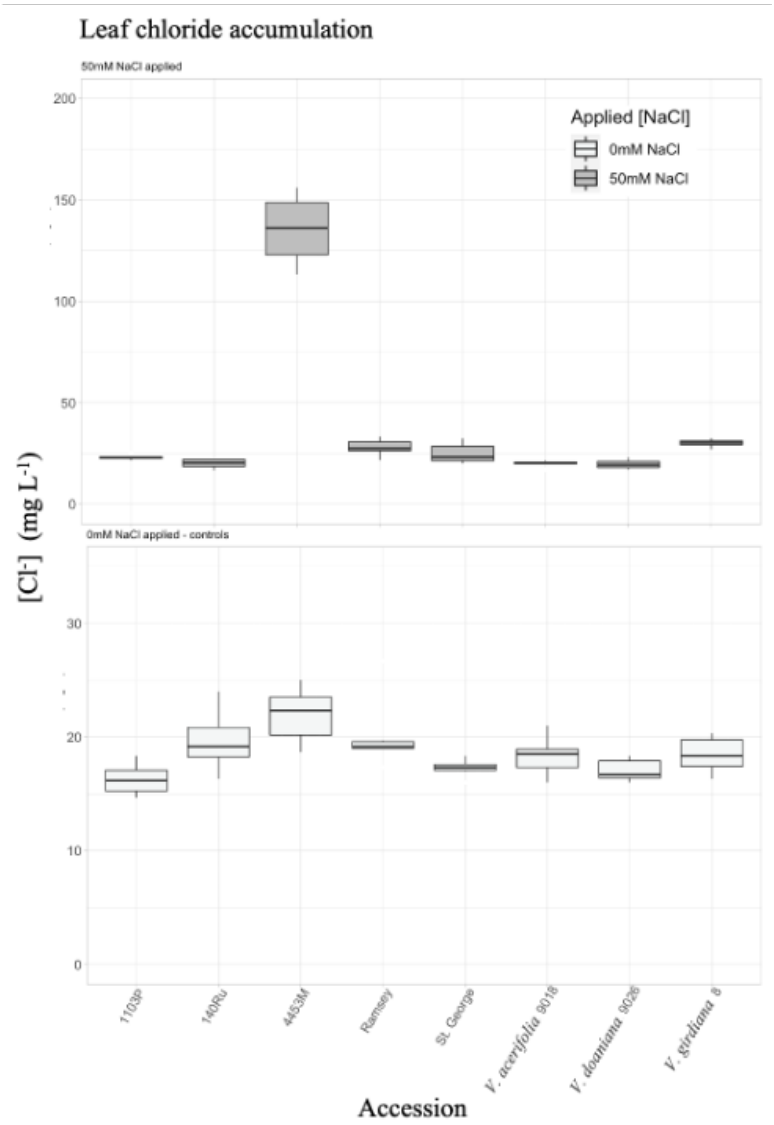

Figure 3. 1. Chloride accumulation in leaf and petiole tissues following 21-day exposure to 50mM or 0mM NaCl fertigation treatment; outdoor trial.

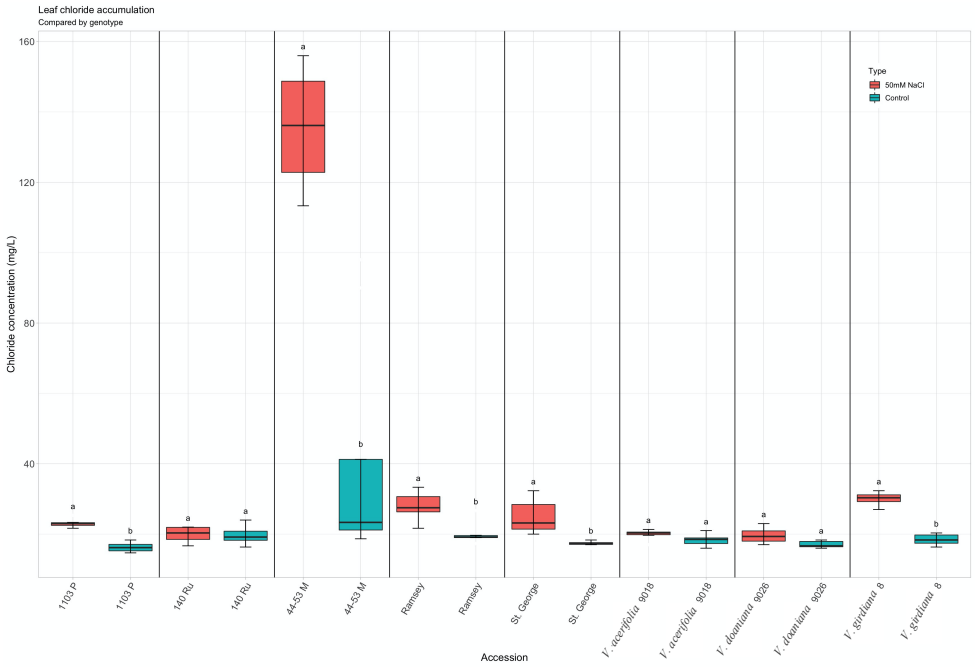

Figure 3. 2. Chloride accumulation in leaf and petiole tissues following 21-day exposure to 50mM or 0mM NaCl fertigation treatment; outdoor trial; compared by genotype, not NaCl treatment.

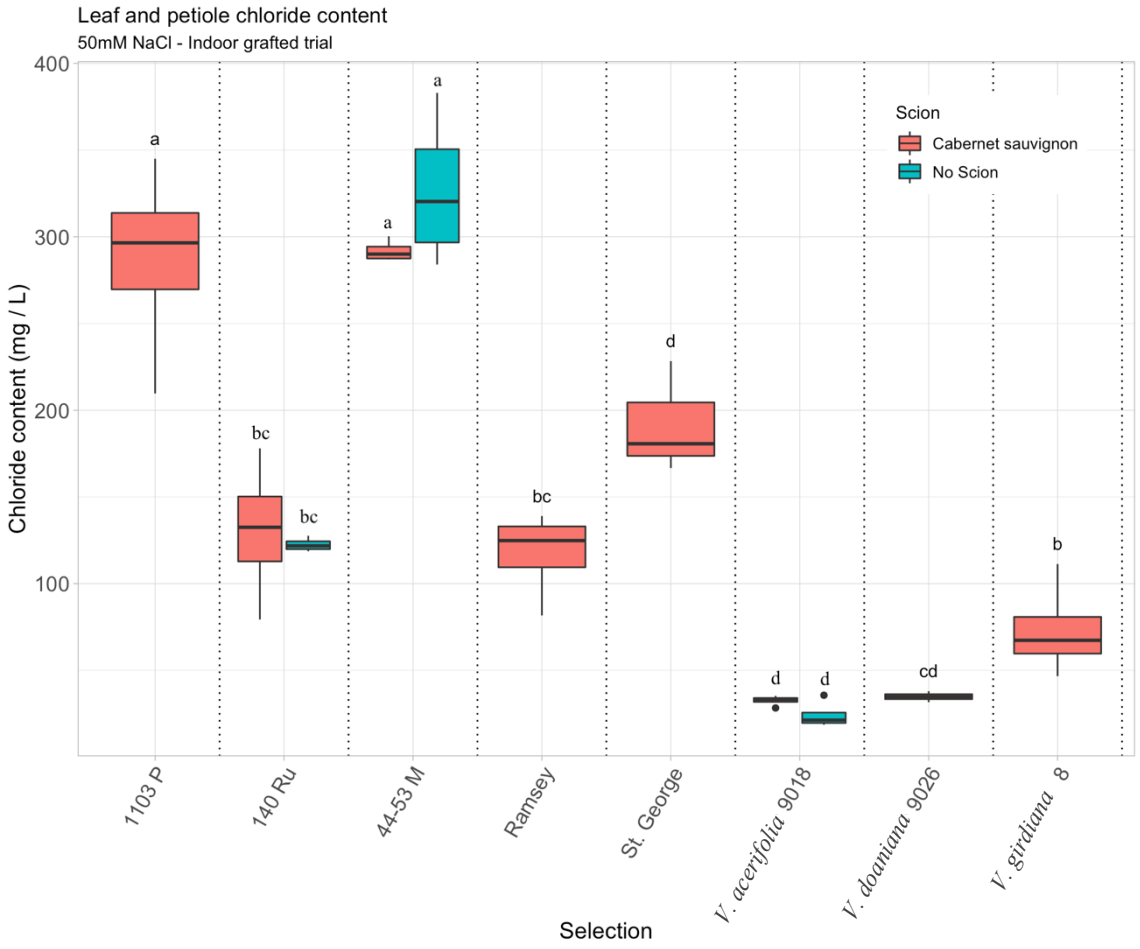

Figure 3. 3. Chloride accumulation in leaf and petiole tissues following 21-day exposure to 50mM or 0mM NaCl fertigation treatment; outdoor trial; compared by genotype, not NaCl treatment.

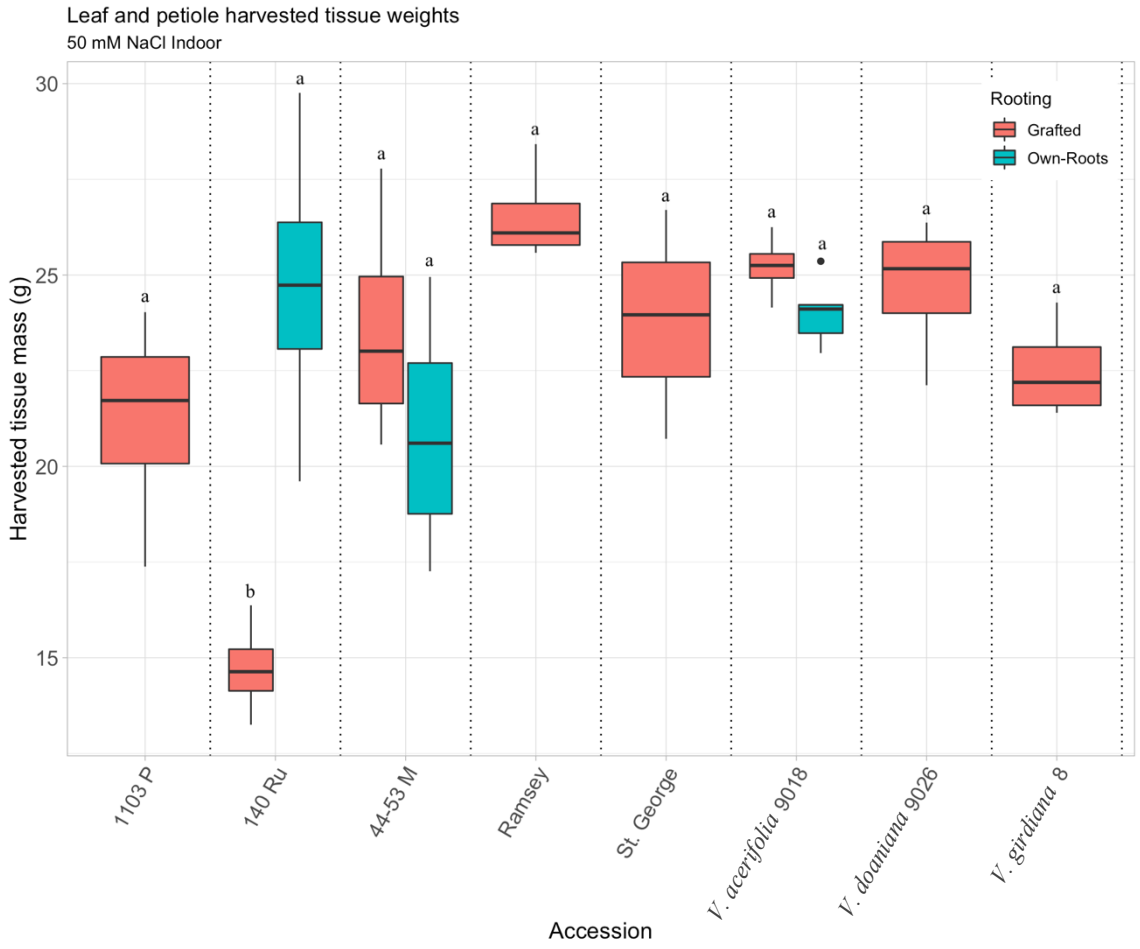

Figure 3. 4. Chloride accumulation in leaf and petiole tissues following 21-day exposure to 50mM or 0mM NaCl fertigation treatment; outdoor trial; compared by genotype, not NaCl treatment.

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