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Breeding Salt Tolerant Grapevine Rootstocks

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Breeding Salt Tolerant Grapevine Rootstocks

By

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Dedication

To

Jack Keegan and Bill, Heidi & Charlie

Thank you for inspiring me to pursue a lifetime in grape growing and winemaking and for your time and support toward achieving this dream.

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Abstract

The accumulation of salts in plant rootzones, known as “salinization,” is often a gradual process that can degrade soil structure and cause permanent plant damage. Salinization caused by ever increasing water demands and an increasingly dry climate is becoming problematic for agriculture in semi-arid to arid regions, including California’s Central Valley, which produces about one-quarter of the United States' food supply and almost half of its nuts and fruits, including grapes, according to the USGS California website (https://ca.water.usgs.gov). While grapevines are considered moderately salt tolerant (Walker *et al.* 2002), the current rate of salinization in many of California's vineyards is contributing to reduced crop yields and lower fruit quality (Keller 2020). Once symptoms of salt toxicity appear on the leaves, vine growth and crop yield may already be in serious decline (Fort and Walker 2011). To prevent salt toxicity from occurring in grapevines, the Walker Lab at UC Davis is applying traditional plant breeding techniques to some of the native population of wild North American grapevine species to improve the salt tolerance of commercial rootstocks (Fort *et al.* 2013). In recent years, the Walker Lab discovered a wild grapevine species called *Vitis acerifolia* 9018, which has consistently proven to be more salt tolerant than the most salt tolerant commercial rootstocks (Chen 2021). These physiological traits could be bred into existing popular commercial rootstocks to improve their salt tolerance. The research for this thesis was performed to observe the stress response and compare the salt tolerance of the recently discovered salt tolerant wild rootstock *V. acerifolia* 9018, against commercial rootstocks widely planted in California and other salinization-affected places, including Ramsey, 1103 Paulson and 140 Ruggeri.

Chapter 1: Literature Review

1.1.a. Salinization

Salinization has affected the rise and fall of human civilizations for millennia (Tanji and Wallender 2011). The earliest civilizations emerged with the rise of agriculture around 4000 BC in a region of the Middle East known as the Fertile Crescent, where the climate and soils were particularly suitable for farming. This is where the Sumerians, the world's first civilization, occupied the lower semi-arid region of Mesopotamia near the Tigris and Euphrates Rivers, in present-day Iraq. The Sumerians originally flourished as wheat farmers, but the waters which they used to irrigate their fields contained a high concentration of salts which accumulated in the rootzone over thousands of years, causing wheat yields to steadily decline from salt toxicity. Fortunately, the Sumerians discovered that they could replant wheat with barley, a more salt tolerant crop (Pitman and Lauchli 2002), but over time salts continued to accumulate in the soil, and eventually barley yields began to decline from salt toxicity as well (Fort and Walker 2011). Lacking a more salt tolerant crop alternative, the Sumerian civilization declined. This same dilemma ultimately led to the decline of other ancient civilizations in Mesopotamia and the rise of new civilizations in more distant, fertile lands (Pitman and Lauchli 2002).

In semi-arid to arid agricultural regions, such as the Fertile Crescent in ancient times and California's Central Valley today, surface evaporation and plant transpiration frequently exceed precipitation (Scudiero *et al.* 2016). In such areas, adequate seasonal rainfall or flood irrigation is necessary to leach salts below the rootzone. However, the Central Valley and other arid agricultural regions around the world are now experiencing enhanced water scarcity due to longer growing seasons, shorter off-seasons and reduced or irregular precipitation; most of the

Western United States is enduring a long-term drought (Buelow *et al*. 2015). Consequently, salinization is expected to become increasingly common and problematic.

Over a quarter of cultivated lands in the contiguous U.S., primarily in the western region, have a moderate to severe risk of developing saline, sodic or saline-sodic soils (Tanji and Wallender 2011). Saline soils contain a high concentration of the major soil salts including sodium (Na^+), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), sulfate (SO_4^{2-}), bicarbonate (HCO^3), carbonate (CO_3^{2-}) and nitrate (NO_3^-), while sodic soils contain a high concentration of Na^+ relative to Ca^{2+} and Mg^{2+} (Tanji and Wallender 2011). The relative composition and abundance of salts in the rootzone is related to soil type, water resources and land management (Keller 2020). Thus, certain regions are more prone to salinization or sodification based on natural and anthropogenic factors. In general, salinization leads to salt toxicity while sodificaiton leads to soil structure degradation. Waterlogging can also lead to salinization (Munns *et al.* 2006).

Worldwide, about 23% of cultivated lands are saline and 37% are sodic (Tanji 2002). Major wine producing countries with salt affected soils include Australia, Spain, and the U.S. (Wickey *et al*. 2011). Cultivated lands suffering the most from salt toxicity in the U.S. include areas of California's Central Valley and southern California (Pitman and Lauchli 2002). In the western San Joaquin Valley, which is a part of the Central Valley, over two-million acres, or 60% of cultivated lands, are considered saline, sodic, or saline-sodic (Scudiero *et al*. 2015). While the Central Valley only represents 1% of the U.S.'s cultivated land area, the state provides about 25% of the nation's food supply, including 40% of fruits and nuts, according to the USGS California website (https://ca.water.usgs.gov). To continue producing high quality produce in the

Central Valley, it is important to prevent salinization before it causes plant death or irreparable soil damage.

While flood irrigation was once used to prevent salinization in the Central Valley, this tactic is no longer sustainable in California and other semi-arid to arid regions around the world due to water scarcity caused by increasing water demands and reduced or irregular water supply (Fort and Walker 2011). Consequently, 17% of the world's cultivated lands conserve water by using drip irrigation (Pitman and Lauchli 2002).

Drip irrigation systems allow users to maximize water use efficiency by adjusting the time-of-day and application rate of irrigation to match the specific water requirements of crops (Pitman and Lauchli 2002). However, there is a strong correlation between drip irrigation and salinization (Flowers 2004). Drip irrigation systems are not designed to leach salts from the rootzone. Therefore, excess, or low-quality irrigation water and irrigation system inefficiencies can contribute to unintentional or *secondary* salinization (Flowers 2004).

Secondary salinization affects 20-50% of the world's irrigated lands, which account for over 30% of world's total food supply (Pitman and Lauchli 2002). Worldwide economic losses due to secondary salinization are estimated in the billions of dollars (USD) annually (Pitman and Lauchli 2002). Visible symptoms of secondary salinization include *salt rings* which form a pale discolored circular area in the soil beneath the drip emitter (Raine *et al*. 2007).

To prevent secondary salinization, it is important to monitor irrigation water quality, especially in semi-arid to arid regions, as high-quality irrigation water has a relatively low soluble salt concentration (White 2009). It is also important to monitor plant water status, so that the timing and application of irrigation water aligns with the plants' need and ability to absorb water and nutrients from the soil solution. Excess irrigation water can lead to the accumulation of

additional salts in the rootzone and contribute to upward intrusion, which can be detrimental, especially if the groundwater has a relatively high salt concentration (Pitman and Lauchli 2002). For example, the Western San Joaquin Valley suffers from secondary salinization, in part, because the water table is oftentimes less than 1.5m below the land surface (Tanji and Wallender 2011) and the soils derive from salt-laden parent material (Scudiero *et al*. 2015). It is also important to monitor the efficiency of the drip irrigation system since the application rate uniformity can vary by over 40% (Raine *et al*. 2007), and thereby thwart other calculated efforts to manage salinization. However, when irrigation water quality, plant water status and drip irrigation system efficiency are well monitored and managed, modern drip irrigation systems can help prevent or at least reduce secondary salinization while conserving water.

1.1.b. Grapevine physiological responses to salinity

Unlike most crops, which struggle to survive when exposed to salinity (Lauchli and Grattan 2011), grapevines are considered somewhat tolerant to salinity (Walker *et al*. 2002). Grapevine salt toxicity is most often related to excess Na^+ or Cl^- (Keller 2020), which are also the most common salt ions found in irrigation water (Walker *et al*. 2010). However, Cl^- is especially toxic to grapevines (Abbaspour *et al.* 2014) and is also relatively mobile in the soil profile (White 2009).

Salinization from salt ions leads to a decrease in soil water potential. If the water potential of the soil becomes lower than the water potential of the root cells, the roots will not be able to absorb water and nutrients, leading to osmotic shock (Munns and Tester 2008). Fortunately, grapevine roots can quickly respond to changes in soil salinity and use hormonal

signaling to alert the canopy, which is more sensitive to salinity than the roots, to reduce its growth and transpiration, and thereby reduce excess salt uptake (Munns 2002b).

The first visible symptoms to occur from salt toxicity are necrotic leaf margins or leaf burn beginning with the oldest leaves (Munns 2002b). One can differentiate Cl^- and Na^+ toxicity by the nature of leaf burn. Chloride toxicity is identified as marginal leaf burn beginning to one side of the leaf while Na^+ toxicity is identified as having marginal leaf burn beginning around the entire perimeter of the leaf. Unfortunately, these symptoms can easily be confused for drought stress or nutrient deficiencies (Munns 2002a), which may encourage incremental irrigation and exacerbate salinization and its mal effects.

If soil salinization occurs, grapevines can produce organic solutes and store them in root cells to decrease their water potential. However, producing organic solutes is energy intensive and reduces plant growth (Munns and Tester 2008). A natural stress response for the grapevine is to absorb solutes (salt ions) from the soil solution to lower the water potential of the root cells, which can lead to excessive Cl^- uptake.

Chloride toxicity reduces photosynthesis and water uptake before any visible symptoms occur (Keller 2020), leading to reduced root and shoot growth (Shani and Ben-Gal 2005). Chloride toxicity has been found to hasten veraison, leading to smaller berries (Walker *et al.* 2000) with concentrated sugars and lower acidity (Keller 2020), and decrease malic acid and raise ion concentrations in the resultant wine (Gong *et al*. 2010). Some wine producing countries including Australia have legal limits for salt ion concentrations in wine (Walker *et al*. 2010). Researchers in Australia have been particularly interested in studying salt toxicity in grapevines. The Murray-Darling drainage system, which provides water to over 60% of Australia's wine grape production, is increasing in salinity at a rate of approximately 1%

annually (Gibberd *et al*. 2003). As salinization becomes an increasing issue worldwide, grapevine geneticists and traditional grapevine breeding programs are seeking solutions to develop salt tolerant rootstocks to maintain quality grape and wine production in the future (Henderson *et al*. 2018).

Research on grapevine physiological responses to salinity has evaluated different rootstocks across a range of salt treatments as compared to a pure water standard over varying periods of time. The results demonstrate that some rootstocks are more salt tolerant than others (Munns and Tester 2008) and that salt tolerant rootstocks prevent Cl^- uptake (Abbaspour *et al*. 2013) by sequestering and storing it in root cell vacuoles (Munns 2002b). However, the specific mechanisms and heritable genes contributing to grapevine Cl^- tolerance remain uncertain (Henderson *et al*. 2014).

Gong *et al*. 2011 compared the salt tolerance of K51-40, a commercial rootstock with poor salt tolerance, to 140 Ruggeri ("140 R"), a commercial rootstock with relatively greater salt tolerance. They found that 140 R and K51-40 acquired about the same amount of Cl^-, but that the amount of Cl^- measured in the petiole and leaf samples of K51-40 was significantly higher than that in the 140 R (Gong *et al.* 2011). Gong *et al.* 2011 concluded that Cl^- acquired by K51-40 was readily transported by the xylem to the canopy, while Cl^- acquired by 140 R was more likely to be captured by proteins and sequestered into vacuoles inside xylem parenchyma cells. Further research suggests that the more salt tolerant 140 R may have a mechanism in its roots that reduces Cl^- transport to the xylem (Tregeagle *et al.* 2010). Since these mechanisms are not stress-inducible, there must be inherent genetic differences between K51-40 and 140 R that impact phenotypic Cl^- tolerance (Henderson *et al.* 2014). In an attempt to determine the gene(s) responsible for lending salt tolerance in grapevines, K51-40 and 140 R were crossed to make 60

hybrids that were subjected to a common salt treatment. Gong *et al.* 2011 found that the Cl^- concentrations in the petioles and leaves were continuous and normally distributed and provided evidence for Cl^- tolerance in grapevines as a multi-genetic trait (Flowers 2004).

1.1.c. Breeding salt tolerant rootstocks

Grapevines are among the most resilient plants on earth. They exist almost everywhere in the world as cultivated or wild species and thrive even in the harshest of environments. In North America, wild grapevine species are found in a broad spectrum of geographies and climates, ranging from the hot and arid deserts of Mexico and the southwestern U.S. to the lush flood plains and thick forests of the midwestern U.S. and Canada, including locations with freezing winter temperatures. These North American grapevine species have endured pests, disease and climate change for millennia, and the natural selection involved in surviving these numerous pressures is at least partially responsible for the extensive species diversity among grapevines in North America. Migratory birds further the hybridization of these numerous native species by broadly distributing the grapevine seeds they eat. Altogether, wild grapevines species in North America represent a trove of genetic diversity, which is sufficiently vast that many species and hybrids are difficult to identify and are poorly mapped.

Beginning with the phylloxera crisis, the use of wild American grapevine species as 'rootstocks' became existentially critical to the wine industry. However, in more than a century of study and cultivation, only a few North American grapevines species have been used in rootstock breeding programs around the world, including *Vitis riparia* (the river grape), *Vitis rupestris* (the rocky grape) and *Vitis berlandiari* (the desert grape) (White 2009). Over time, grape growers and winemakers noticed that certain rootstocks performed better than others in

response to different soil types, climates and grapevine pests and diseases. Nowadays, as climate change and grapevine pests and diseases appear to be getting worse, there is renewed interest in exploring the commercial viability of incremental North American grapevine species. Currently, these species are used not only as rootstocks, but as breeding material hybridized with *V. vinifera* to overcome some of the most challenging grapevine pests and diseases facing the wine industry (Riaz *et al.* 2009). The challenges grape growers now face and the recent successes in developing new rootstocks and scions with North American grapevines will lead to ever greater curiosity in the diverse North American population of wild grapevine species.

The increasing scientific and commercial interest in lesser-known wild grapevine species inspired grapevine geneticists at UC Davis to collect thousands of wild Native North American grapevine specie accessions over the past several decades with the intention of screening them for tolerance to different abiotic and biotic stresses facing the grape growing industry (Heinitz *et al.* 2019). Most of the accessions in the UC Davis collection were collected in the southwestern U.S., where there is a great diversity of wild grapevine species.

For nearly a decade, the M. Andrew Walker Lab in UC Davis' Department of Viticulture & Enology has been screening its extensive collection of wild grapevine species for Cl^- tolerance. Their intention was to use traditional breeding and gene marker selection to discover Cl^- tolerant rootstocks and eventually commercialize new salt tolerant rootstocks (Fort *et al.* 2015). Traditional breeding is thought to be the key to discovering genes responsible for salt tolerance and commercializing new salt tolerant rootstocks (Munns *et al.* 2006). Over time, the Walker Lab developed a reliable assay to efficiently analyze different wild grapevines (Fort *et al.* 2013), using vegetative cuttings planted in fitted clay media. Unlike a typical soil media, fritted clay retains its porosity and permeability when irrigated with salt water. This helps prevent soil

structure degradation and allows adequate leaching and drying between salt treatments (Halliwell *et al.* 2001). Furthermore, frequent leaching limits nutrient uptake from the fritted clay media (Adams et al 2014). While the assay technique has remained relatively constant over the past decade, the salt treatment concentration and period applied have been intentionally varied in comparisons of the most salt tolerant commercial rootstocks with the most salt tolerant wild North American grapevine species.

In early trials, grapevines were examined for chloride tolerant traits through irrigation with a fertigation solution mixed with 25 mM NaCl over a two-week period, while controls received the same fertigation solution without NaCl (Fort *et al.* 2015). The applied concentration of 25mM NaCl is relatively low compared with the traditionally accepted threshold for soil salinity at 40 mM equivalents of NaCl (Richards 1954) (Qadir et al. 2000). Results from these early experiments were comparable to long-term field studies in confirming the validity of the assay (Fort *et al.* 2013).

In later trials, grapevines were irrigated with a fertigation solution mixed with 25, 50, 75 and 100 mM NaCl over a four-week period, while controls received the same fertigation solution without NaCl. Increasing the salt treatment and trial period helped to create separation between the most salt tolerant rootstocks. Over the course of many such trials, the Walker Lab identified the most salt tolerant rootstocks in their possession. It was determined that, at some NaCl concentration level between 25 and 75 mM, there is meaningful separation between the best commercial rootstock Cl^- excluders and the best wild species Cl^- excluders in the UC Davis collection (Chen 2021). Many of the recent trials have focused on 50 mM NaCl, since it approximates the definition of a salt affected soil.

The most chloride tolerant wild North American grapevine species studied to date in the UC-Davis collection is called *Vitis acerifolia* 9018 ("9018"). The accession for this grapevine was found in southwestern Oklahoma. A species richness map of this area indicates that it is one of the most diverse wild grapevine species in North America (Heinitz *et al.* 2019). *V. acerifolia* 9018 has been the primary focus of the Walker Lab's salt tolerance project for several years. The most recent greenhouse salt trials were performed to ensure the reproducibility of previous salt tolerance test results among wild grapevine species in comparison to the top performing salt tolerant commercial rootstocks (Chen 2021). The most recent salt trials have also included photosynthetic analysis, achieved using a LI-COR to analyze photosynthetic activity between the different genotypes (Chen 2021). Future experiments will test the salt tolerance of 9018 in the vineyard.

Chapter 2: Breeding Salt Tolerant Rootstocks

2.1. Experiment 1

2.1.a. Introduction

Major wine producing regions around the world including California's Central Valley are increasingly threatened by salinization (Tanji and Wallender 2011). For the past decade the Walker Lab at UC Davis has been researching salt tolerance in grapevines (Fort 2012) (Fort *et al.* 2013) (Fort *et al.* 2015) (Heinitz 2016). Over the years, the Walker Lab has screened hundreds of wild grapevine accessions using an assay developed by the lab to quickly analyze the salt tolerance of grapevines (Fort *et al.* 2013), which has been demonstrated to mimic field results (Heinitz 2016). Using those methods, the Walker Lab recently identified the 9018 wild

grapevine species that has demonstrated superior salt tolerance compared to the most salt tolerant commercial rootstocks (Chen 2021). For several years the Walker Lab's research on 9018 has included a series of slightly modified experiments to determine the salt tolerance of 9018. The experimental variations included salt treatments ranging from 25 mM NaCl to 100 mM NaCl (Chen 2021), a range which straddles the lower and upper NaCl thresholds for a saline soil in semi-arid to arid regions (Scudiero *et al*. 2016). Those experiments determined that the maximum separation in Cl^- tolerance between the top performing wild grapevine species and the top performing commercially available rootstocks is somewhere between 25-75 mM NaCl (Chen 2021). Experiment 1 was designed to quantify over a 28-day period the photosynthetic and Cl^- storage progression of different genotypes using a salt treatment of 50 mM NaCl to compare the salt tolerance of 9018 against several commercial rootstocks including 140 R, 1103 Paulson ("1103 P") and Ramsey, which are thought to have moderate salt tolerance, and 44-53 Malègue ("44-53 M") which is considered to have low salt tolerance (Walker *et al.* 2010) (Fort 2012).

2.1.b. Materials & Methods

Experiment 1 began in the spring of calendar year 2020 when vegetative grapevine cuttings, each containing two buds, were acquired from the UC Davis vineyards. One bud from each cutting was then partially submerged for several seconds in a 1:20 dilution of liquid rooting hormone (Wood's Rooting Compound, Earth Science Products Corporation, Wilsonville, Oregon, USA), containing 1.03% Indol-3-butyric acid and 0.66% 1-naphthalene acetic acid. The buds exposed to rooting hormone were then inserted into propagation plugs evenly distributed on trays and transferred to a fog room where they were held at constant temperature and humidity for 14 days (Figure 1). Afterwards, the cuttings were transferred to a greenhouse for another 14 days and exposed to normal ambient daily temperature fluctuations and a regular irrigation cycle.

The irrigation water was a solution of essential plant nutrients including N, K, Ca, S, Mg, B, Mn, Zn, Cu, and Mo. Following the initial growth period in propagation plugs, all successfully rooted vegetative cuttings were transferred to 2548 cm^3 round pots filled with a permeable fritted clay media (Turface Pro MVP, Profile Products LLC in Buffalo Grove, Illinois, USA) to prevent flocculation upon exposure to NaCl. After 14 days in pots, 48 grapevines per genotype (240 pots total) were carefully selected based on their health and relative growth rates for the trial experiment. Each pot received a unique identification number. The numbered pots were randomized into six blocks which were evenly separated and grouped on greenhouse tables, each with an equal number of salt treatment and control pots per genotype for six scheduled harvests over a 28-day period, each harvest spaced approximately every four to five days apart (Figure 2). In total, there were 240 pots, or 40 pots per harvest period consisting of 20 salt treatment pots and 20 control pots with an even number of pots per genotype.

Prior to the first salt treatment, exactly 28 days after replanting the vegetative cuttings in pots, a portable photosynthetic system called a LI-6800 (LI-COR Biosciences in Lincoln, Nebraska, USA) was used to obtain initial photosynthetic measurements of leaves from each grapevine scheduled for the first two harvest periods (Figure 3). The net photosynthetic measurements included the transpiration rate (*E*), net carbon assimilation rate (*A*), intracellular CO_2 (*Ci*) and stomatal conductance (*gsw*). The salt-treated pots then received water mixed with the fertigation solution and 50 mM NaCl, while the control pots received water mixed with the fertigation solution and 0 mM NaCl (Table 1). All pots were completely saturated with the appropriate irrigation treatment twice daily about 12 hours apart; allowing ample time to allow the fritted clay media to become completely dry between irrigations.

At the beginning of each harvest period, the assigned grapevines were subjected to the same photosynthetic testing. Then the leaves and roots of each plant were separated, the roots were rinsed with distilled water, and weighed separately (Figure 4). The leaves and roots were then transferred to a heat storage room held at 50ºC to dehydrate the leaves. After this period the dry weights of the leaves and roots were recorded and the samples were crushed into a fine powder which was then tested for chloride concentration using silver-titration and a cold-water extraction process with a silver ion titration meter (M926 Chloride Analyzer System, Nelson Jameson Inc., Marshfield, Wisconsin, USA). Chloride concentrations were measured from the leaf and petiole, and root samples from each of the genotypes using 0 mM NaCl and 50 mM NaCl.

Statistical analysis was carried out using the R project (ver. 4.0.5; The R Foundation for Statistical Computing, Vienna, Austria). Both the photosynthetic data recorded from the LI-6800 and the chloride data recorded from the M926 Chloride Analyzer System were analyzed using the Tukey Honest Significant Difference test.

2.1.c. Results & Discussion

The results of Experiment 1 indicate a significant reduction in *E*, *A* and *Ci*, and a decrease in *gsw* in the salt treated vines over time. However, certain genotypes appear to have different photosynthetic responses to salinity.

Individual photosynthetic measurements were recorded from each genotype using 0 mM NaCl and 50 mM NaCl. Using 0 mM NaCl there were no significant differences in *E*, *A*, *Ci* or *gsw* among the genotypes (Table 2). However, when the photosynthetic activity of each genotype was measured individually using 50 mM NaCl there were significant differences in *Ci*,

and considerable differences in *E* and *gsw* among the genotypes (Table 3). At 50 mM NaCl, 9018 exhibited the lowest *Ci*, *E* and *gsw*. If 9018 is the most salt tolerant rootstock as Experiment 1 indicates, reducing photosynthesis could be a mechanism for increasing salt tolerance. However, 44-53 M, which has been understood to have the lowest salt tolerance, was not the most photosynthetically active genotype using 50 mM NaCl. Thus, photosynthetic activity is not likely to be the sole mechanism grapevines utilize to tolerate high soil salinity.

Under control conditions of 0 mM NaCl, 44-53 M exhibited significantly higher Cl^- concentrations in leaf and petiole samples than 1103 P and 9018 (Table 4). However, the differences in Cl^- uptake in the leaf and petiole samples at 0 mM NaCl between the wild rootstock 9018 and Ramsey, 1103 P, 140 R, 44-53 M, (Figure 5) were relatively minor in comparison to the differences in Cl^- uptake between the genotypes when 50 mM NaCl was applied (Figure 6). Under the 50 mM NaCl treatment, the Cl^- concentrations for leaf and petiole samples were significantly lower in 9018 than 44-53 M with two degrees of separation, and significantly lower than Ramsey with one degree of separation (Table 4). However, under the 50 mM NaCl treatment, the Cl^- concentrations for leaf and petiole samples showed no statistically significant differences between 9018, 140 R and 1103 P, and no statistically significant differences between 140 R and 1103 P (Table 4). The genotypes listed in order from least to greatest observed leaf and petiole Cl^- concentration under the 50 mM NaCl treatment were 9018, 1103 P, 140 R, Ramsey and 44-53 M with most genotypes steadily increasing in leaf Cl^- concentration over time, and only 9018 maintaining a relatively constant leaf Cl^- concentration throughout the 28-day test period (Figure 6).

In contrast to leaf and petiole analysis, the root Cl^- analysis showed roughly the opposite trend between genotype and Cl^- uptake. Root Cl^- accumulations over time with salt treatment

(Figure 8) showed more variability and less linear patterns than did the leaf Cl^- accumulations over time with salt treatment (Figure 6). For the root samples treated with 0 mM NaCl, 9018 acquired significantly more Cl^- than 44-53 M by two degrees of separation (Table 5). Further comparison of the root samples treated with 0 mM NaCl separates the genotypes into two groups, one consisting of 9018 and 140 R and the other consisting of 1103 P, Ramsey and 44-53 M, listed in order of greatest to least root Cl^- concentration. Similar to the petiole and leaf analysis, differences in root Cl^- uptake among genotypes was relatively minor for the 0 mM NaCl treatment (Figure 7) in comparison to the 50 mM NaCl treatment (Figure 8). Root Cl^- analysis for the 50 mM treatment determined that 9018 recorded the highest Cl^- concentrations, comparable to 140 R (Table 5), while 44-54 M recorded the lowest root Cl^- concentrations – significantly lower than *44-53 M* and 9018. The observed root concentration data for 9018 and 140 R, which are among the most salt tolerant of rootstocks, suggest that retention of Cl^- in the roots to prevent or lessen Cl^- transport to the shoots might be another mechanism for salt tolerance which could be explained by the xylem loading theory (Storey 2003).

According to the xylem loading theory, NaCl is sequestered into vacuoles in the xylem parenchyma cells before it has a chance to enter the xylem stream. Any NaCl that enters the xylem stream may be captured and sequestered by other xylem parenchyma cells before the salts reach the scion. Once the vacuoles fill with salt, the grapevine can abandon these root cells by abandoning the NaCl-loaded root. This salt exclusion strategy is far less taxing of plant vigor than maintaining the root cells by transporting the salts into the canopy, which will eventually lead to salt toxicity in the scion, and vine death. This root loading and abandonment strategy may give a salt tolerant rootstock such as 9018 an advantage in saline soils by better capturing, sequestering and preventing salts from reaching the scion, even if they have to occasionally

abandon salt-laden root cells and grow new roots. Furthermore, if salt tolerant rootstocks did shed more roots, that would likely reduce water and nutrient uptake which could explain why the salt tolerant rootstocks demonstrated less photosynthetic activity under salt stress.

Figure 1. Vegetative cuttings after 14 days in a fog room

Figure 2. Grapevines after 14 days in a greenhouse, organized according to a randomization scheme

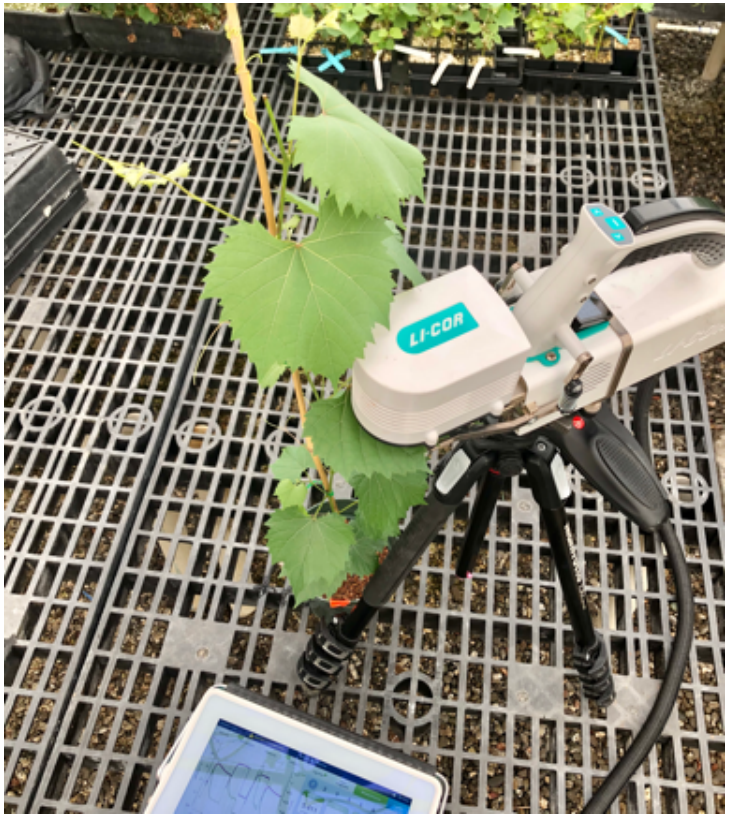

Figure 3. A portable photosynthetic system taking measurements prior to the beginning of the 28-day trial period

Figure 4. Roots separated from the grapevines were washed in distilled water before being transferred to a heat storage room prior to chloride analysis

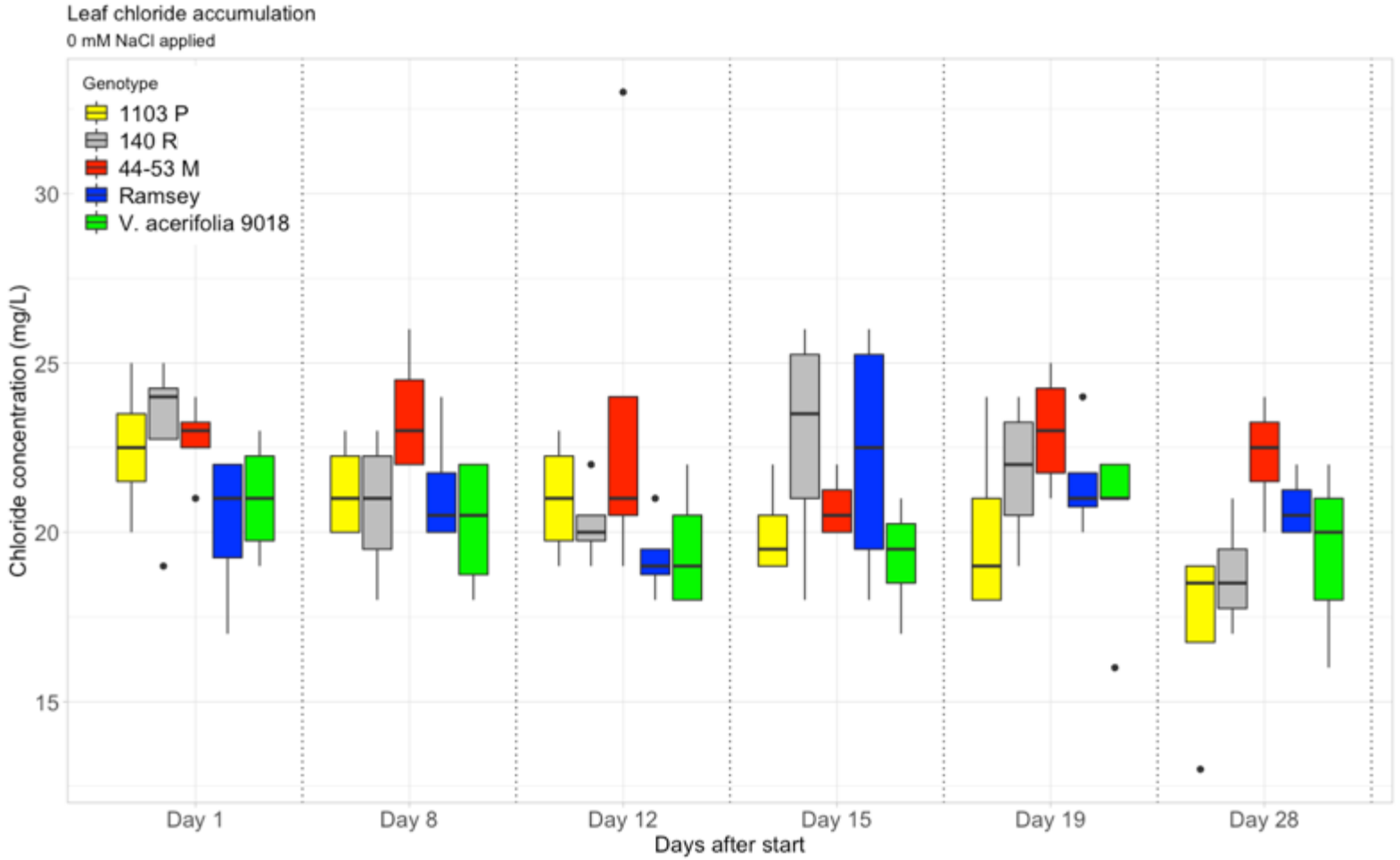

Figure 5. Leaf chloride accumulation without salt treatment; 0 mM NaCl applied

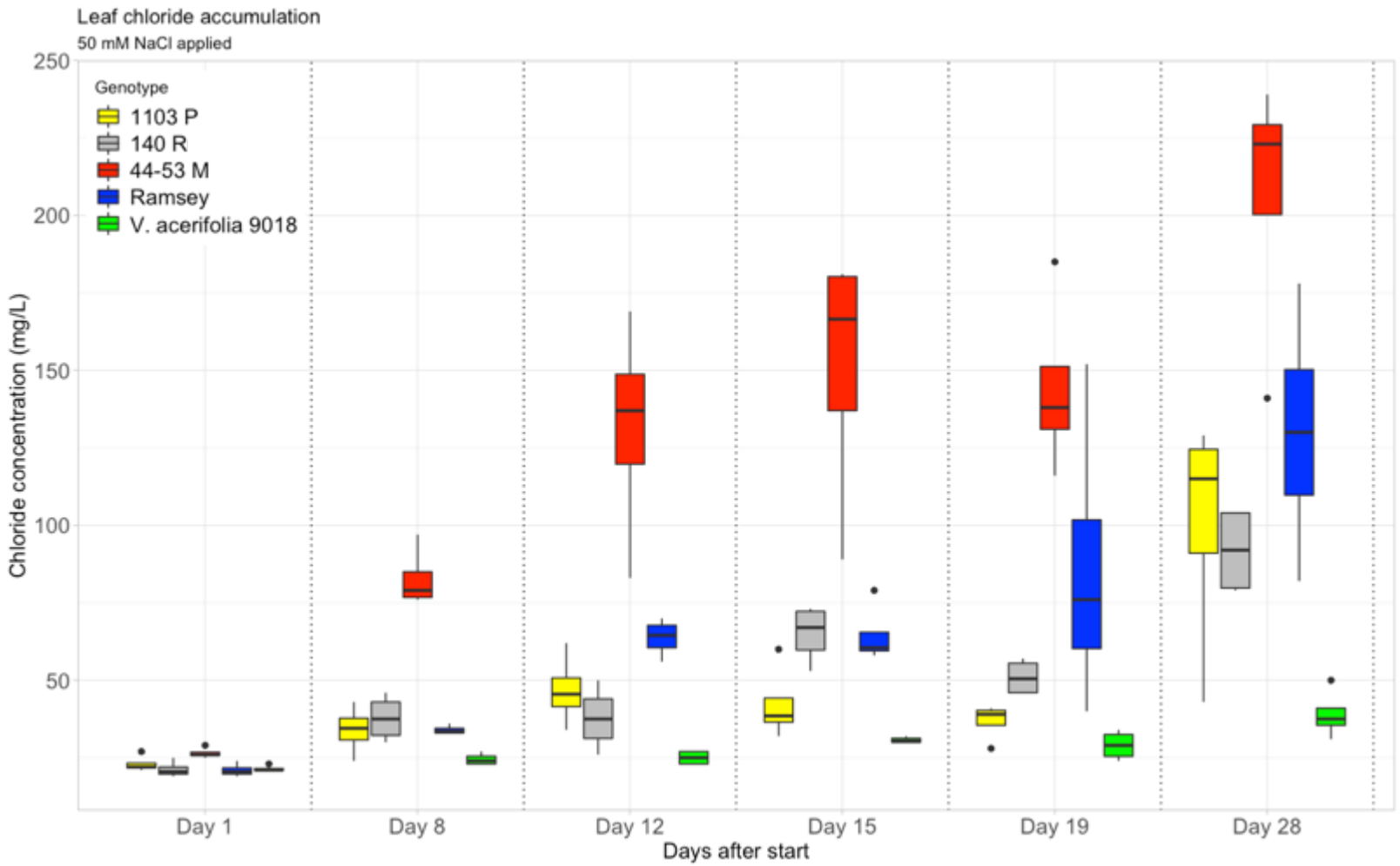

Figure 6. Leaf chloride accumulation with salt treatment; 50 mM NaCl applied

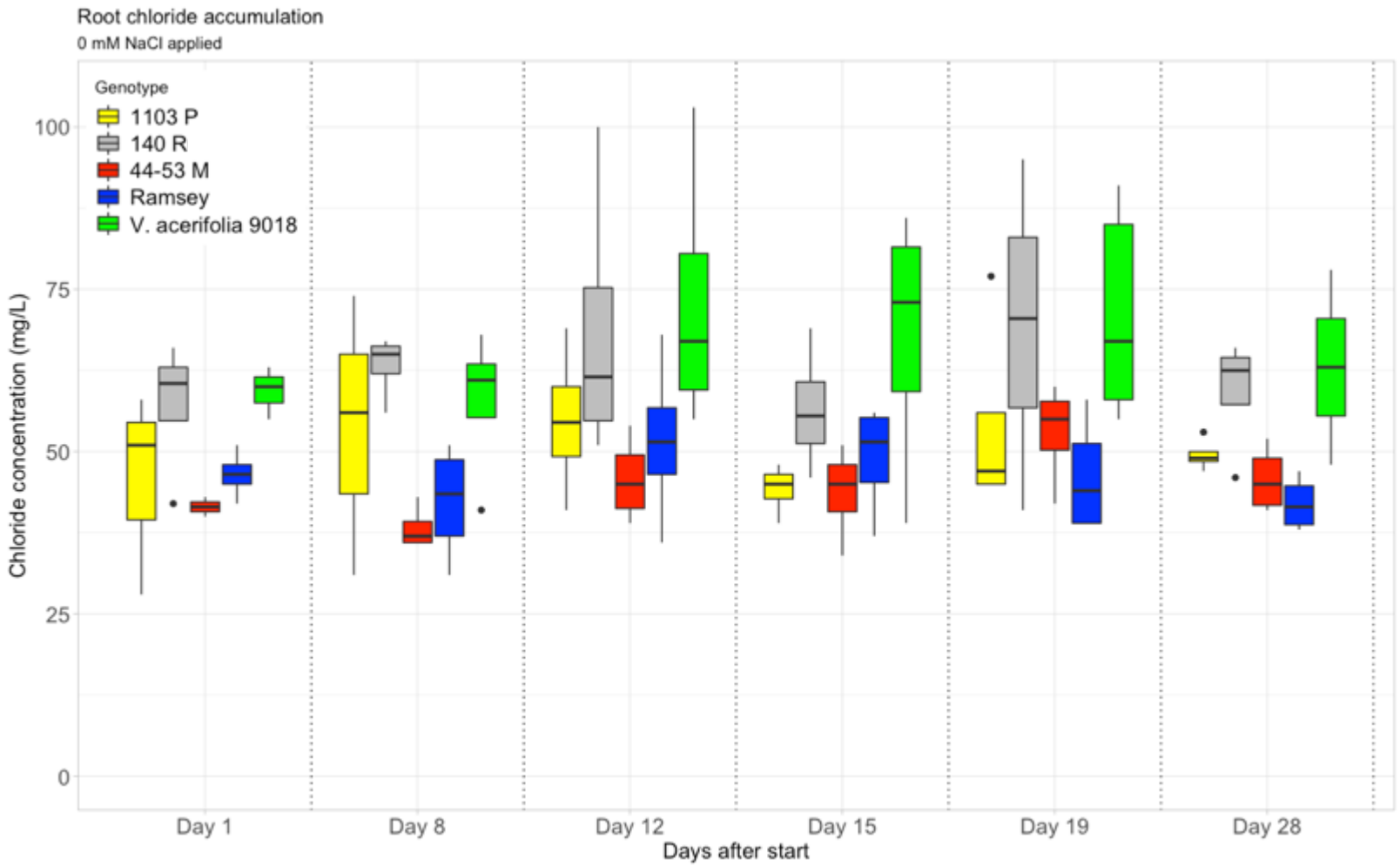

Figure 7. Root chloride accumulation without salt treatment; 0 mM NaCl applied

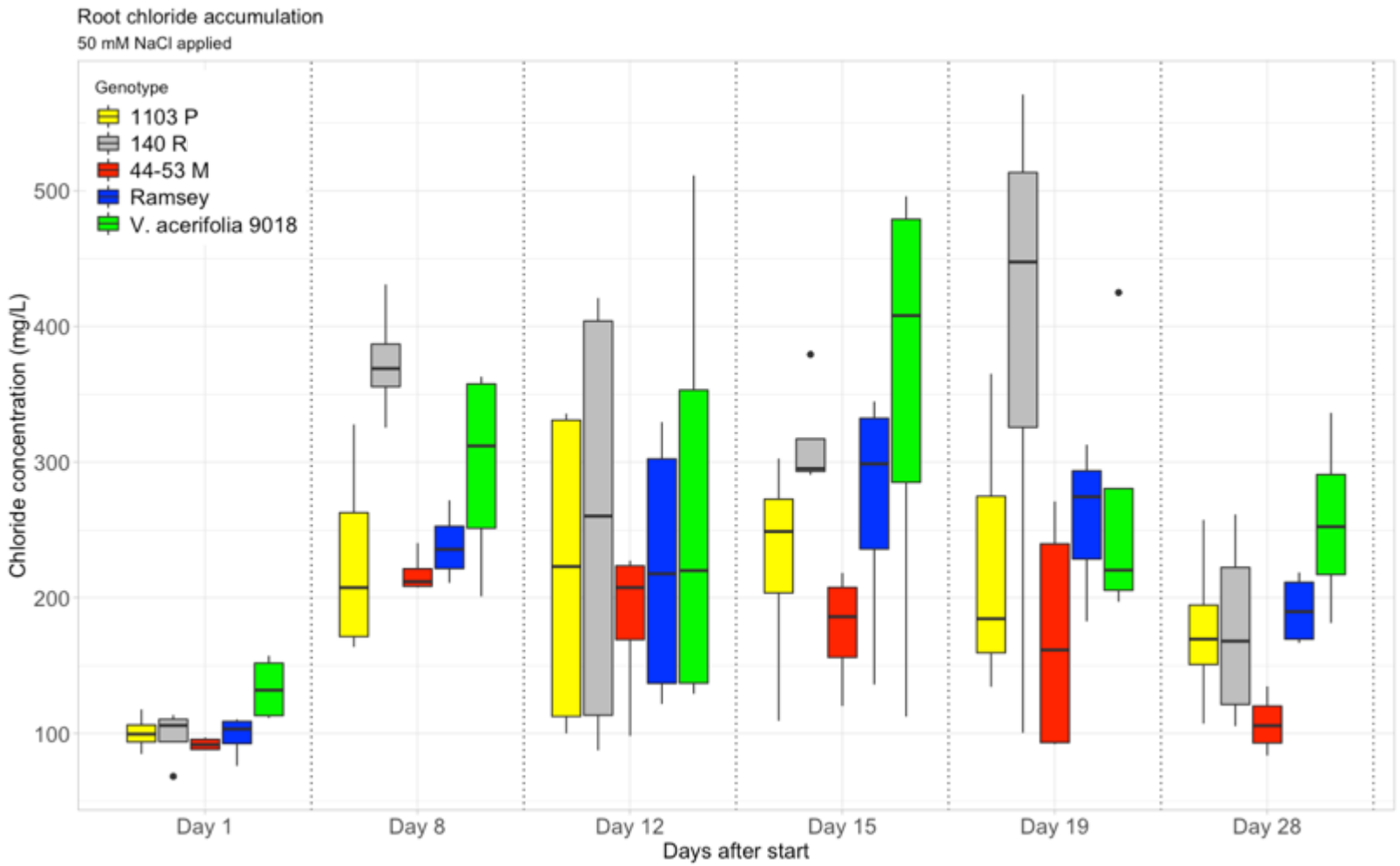

Figure 8. Root chloride accumulation with salt treatment; 50 mM [NaCl] applied

Table 1. Net photosynthesis of genotypes (1103 Paulson, 140 Ruggeri, 44-53 Malègue, Ramsey and V. acerifolia 9018) without salt treatment (0 mM NaCl applied*) and with salt treatment (50 mM NaCl applied)*

Net photosynthesis by applied [NaCl]												
NaCl	*E* (mol × m^{-2} × s^{-1})			*A* (mol × m^{-2} × s^{-1})			*Ci* (mol × mol^{-1})			*gsw* (mol × m^{-2} × s^{-1})		
	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group
0 mM	0.0032	0.0001	a	7.69	0.18	a	316.16	2.12	a	0.231	0.009	a
50 mM	0.0024	0.0001	b	6.02	0.23	b	312.77	2.77	a	0.161	0.008	b
p-value	< 0.001*			< 0.001*			0.413			< 0.001*		

Note: E = transpiration rate, *A* = net carbon assimilation, *Ci* = intracellular CO_2, *gsw* = stomatal conductance. *$p < 0.05$.

Table 2. Photosynthesis by genotype without salt treatment; 0 mM NaCl applied

Photosynthesis by genotype at 0 mM [NaCl]

Genotype	*E* (mol × m^{-2} × s^{-1})			*A* (mol × m^{-2} × s^{-1})			*Ci* (mol × mol^{-1})			*gsw* (mol × m^{-2} × s^{-1})		
	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group
44-53 M	0.0039	0.0001	a	9.75	0.11	a	309.75	1.93	ab	0.283	0.008	a
Ramsey	0.0042	0.0003	a	8.55	0.20	a	314.16	2.15	ab	0.305	0.011	a
140 R	0.0042	0.0001	a	8.75	0.13	a	322.85	1.44	a	0.301	0.008	a
1103 P	0.0027	0.0001	a	7.04	0.17	a	302.19	1.88	ab	0.184	0.008	a
V. acerifolia 9018	0.0027	0.0001	a	8.65	0.04	a	278.63	2.11	b	0.185	0.010	a
p-value		0.100			0.312			0.057			0.211	

Note: E = transpiration rate, *A* = net carbon assimilation, *Ci* = intracellular CO_2, *gsw* = stomatal conductance. **p* < 0.05.

Table 3. Photosynthesis by genotype with salt treatment; 50 mM NaCl applied

Photosynthesis by genotype at 50 mM [NaCl]

Genotype	*E* (mol × m^{-2} × s^{-1})			*A* (mol × m^{-2} × s^{-1})			*Ci* (mol × mol^{-1})			*gsw* (mol × m^{-2} × s^{-1})		
	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group	Mean	Standard Error	Group
44-53 M	0.0024	0.0001	a	6.46	0.23	a	310.82	2.15	ab	0.157	0.008	ab
Ramsey	0.0029	0.0001	ab	6.61	0.20	a	312.08	3.30	ab	0.208	0.011	a
140 R	0.0025	0.0001	ab	5.92	0.19	a	320.39	2.25	ab	0.168	0.007	ab
1103 P	0.0023	0.0001	ab	5.44	0.29	a	327.42	2.62	a	0.158	0.009	ab
V. acerifolia 9018	0.0018	0.0001	b	5.97	0.23	a	293.14	2.99	b	0.111	0.006	b
p-value		0.057			0.648			0.019*			0.056	

Note: E = transpiration rate, *A* = net carbon assimilation, *Ci* = intracellular CO_2, *gsw* = stomatal conductance. **p* < 0.05.

Table 4. Leaf chloride accumulation without salt treatment (0 mM NaCl applied) and with salt treatment (50 mM NaCl applied)

	[NaCl] applied					
Genotype	0 mM NaCl			50 mM NaCl		
	mg/L	Standard error	Group	mg/L	Standard error	Group
44-53 M	22.63	0.18	a	123.71	4.15	a
Ramsey	21.21	0.14	ab	66.54	2.77	b
140 R	20.88	0.17	ab	50.75	1.58	bc
1103 P	20.33	0.16	b	47.21	1.94	bc
V. acerifolia 9018	20.00	0.13	b	28.29	0.44	c
p-value	< 0.001*			< 0.001*		

*Note: * = $p < 0.05$*

Table 5. Root chloride accumulation without salt treatment (0 mM NaCl applied) and with salt treatment (50 mM NaCl applied)

	[NaCl] applied					
Genotype	0 mM NaCl			50 mM NaCl		
	mg/L	Standard error	Group	mg/L	Standard error	Group
44-53 M	44.67	0.44	c	158.61	4.07	b
Ramsey	46.29	0.54	bc	210.70	5.33	ab
140 R	62.33	0.92	ab	268.60	9.65	a
1103 P	60.96	2.59	abc	195.07	5.86	ab
V. acerifolia 9018	68.13	1.20	a	262.92	8.11	a
p-value	< 0.001*			0.002*		

*Note: *$p < 0.05$*

2.2. Experiment 2

2.2.a. Introduction

A second experiment was performed using methods and materials that were similar to Experiment 1, but Experiment 2 consisted of fewer genotypes, used more replicates of each genotype and used only a single harvest that did not allow measuring changes in salt concentration over time. The purpose of Experiment 2 was to demonstrate the reproducibility of results from Experiment 1. The genotypes selected for Experiment 2 were 44-53 M, 1103 P and 9018. These genotypes were selected to allow additional comparison between 9018 and 1103 P, because 1103 P is one of the most popular and widely planted rootstocks in California.

2.2.b. Materials & Methods

The materials and methods for Experiment 2 were the same as for Experiment 1 unless explicitly mentioned below. Prior to the first salt treatment, exactly 14 days after replanting the vegetative cuttings in pots, 18 grapevines per genotype (56 pots total) were carefully selected based on their health and observed growth rates for the trial experiment. Unlike Experiment 1, Experiment 2 utilized only a single harvest which was performed after a 28-day period. Also unlike Experiment 1, photosynthetic measurements were not recorded.

2.2.c. Results & Discussion

The results from the leaf and petiole analysis for the 0 mM NaCl and 50 mM NaCl treatments were consistent with Experiment 1. Grapevine genotypes grown without salt treatment showed similar leaf and petiole Cl^- concentrations (Figure 9), while grapevine genotypes grown with the 50mM NaCl salt treatment showed distinctly different leaf and petiole

Cl^- concentrations (Figure 10). There were no significant differences in leaf and petiole Cl^- concentrations between 44-53 M, 1103 P and 9018 (Table 6) without the salt treatment. However, with the salt treatment, 9018 had significantly lower leaf and petiole Cl^- concentrations than 44-53 M (Table 6) and exhibited roughly half the leaf and petiole Cl^- concentrations as 1103 P (Table 6).

The results from the root analysis for the 0 mM NaCl and 50 mM NaCl treatments were also consistent with Experiment 1. Root chloride concentrations without salt treatment showed that 9018 acquired significantly more Cl^- than 44-53 M. Although 9018 acquired more root Cl^- than 1103 P, the root chloride concentrations for 1103 P were not statistically different than 44-53 M or 9018. With the salt treatment 9018 acquired more root Cl^- than 44-53 M and 1103 P but there were no statistically significant differences in root Cl^- among all three genotypes. As with the results for leaf and petiole Cl^-accumulation, the root Cl^- accumulation results are also consistent with Experiment 1, again indicating that there might be a mechanism for salt tolerance that allows the more salt tolerant rootstocks to exclude Cl^- from the xylem thus preventing it from the canopy. That would explain why 9018 and 1103 P have significantly lower petiole and leaf Cl^- concentrations than 44-35 M with the 50 mM NaCl salt treatment in Experiment 1 and Experiment 2 while simultaneously having much higher root Cl^- concentrations when treated with 50 mM NaCl.

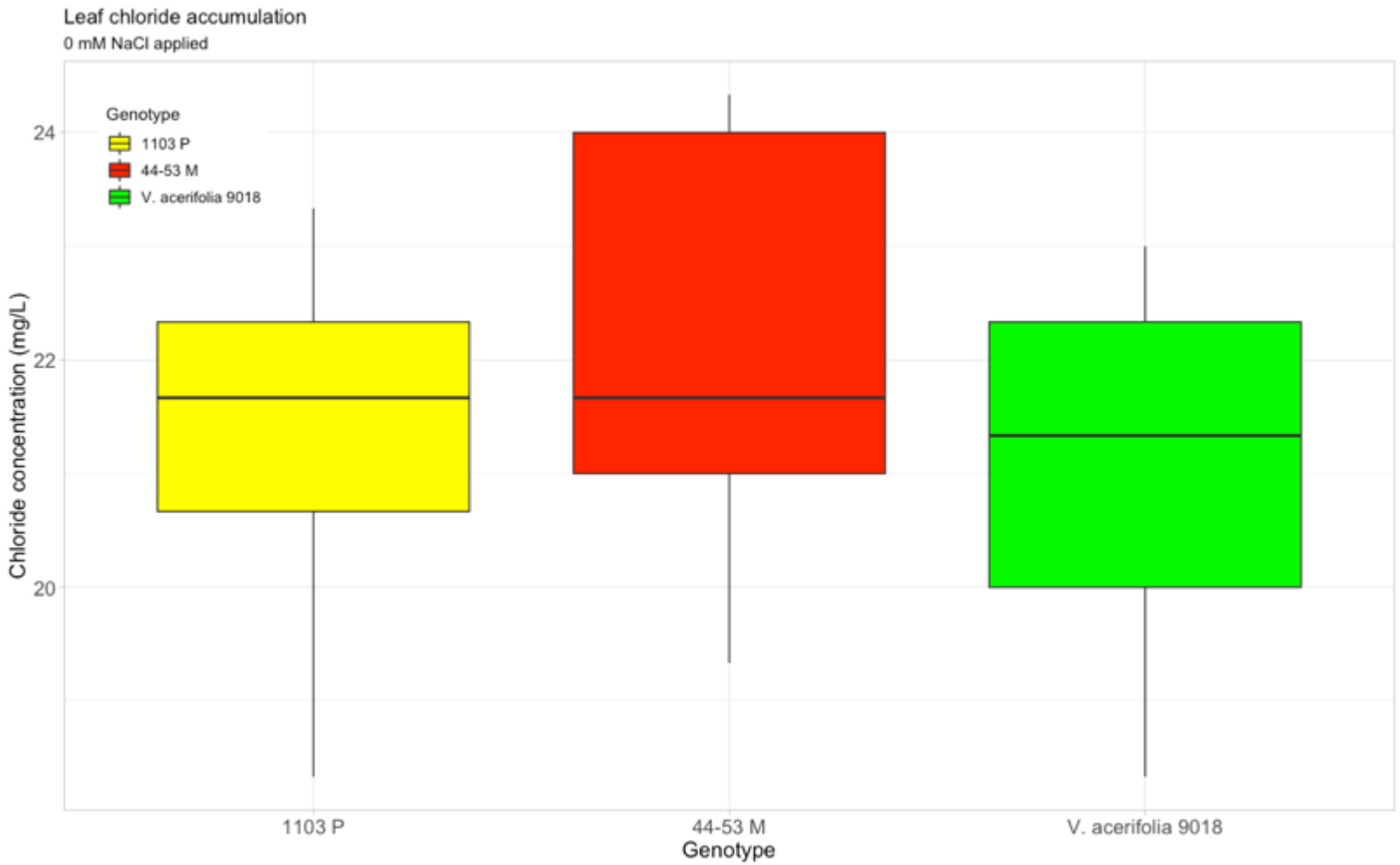

Figure 9. Leaf chloride accumulation without salt treatment; 0 mM NaCl applied

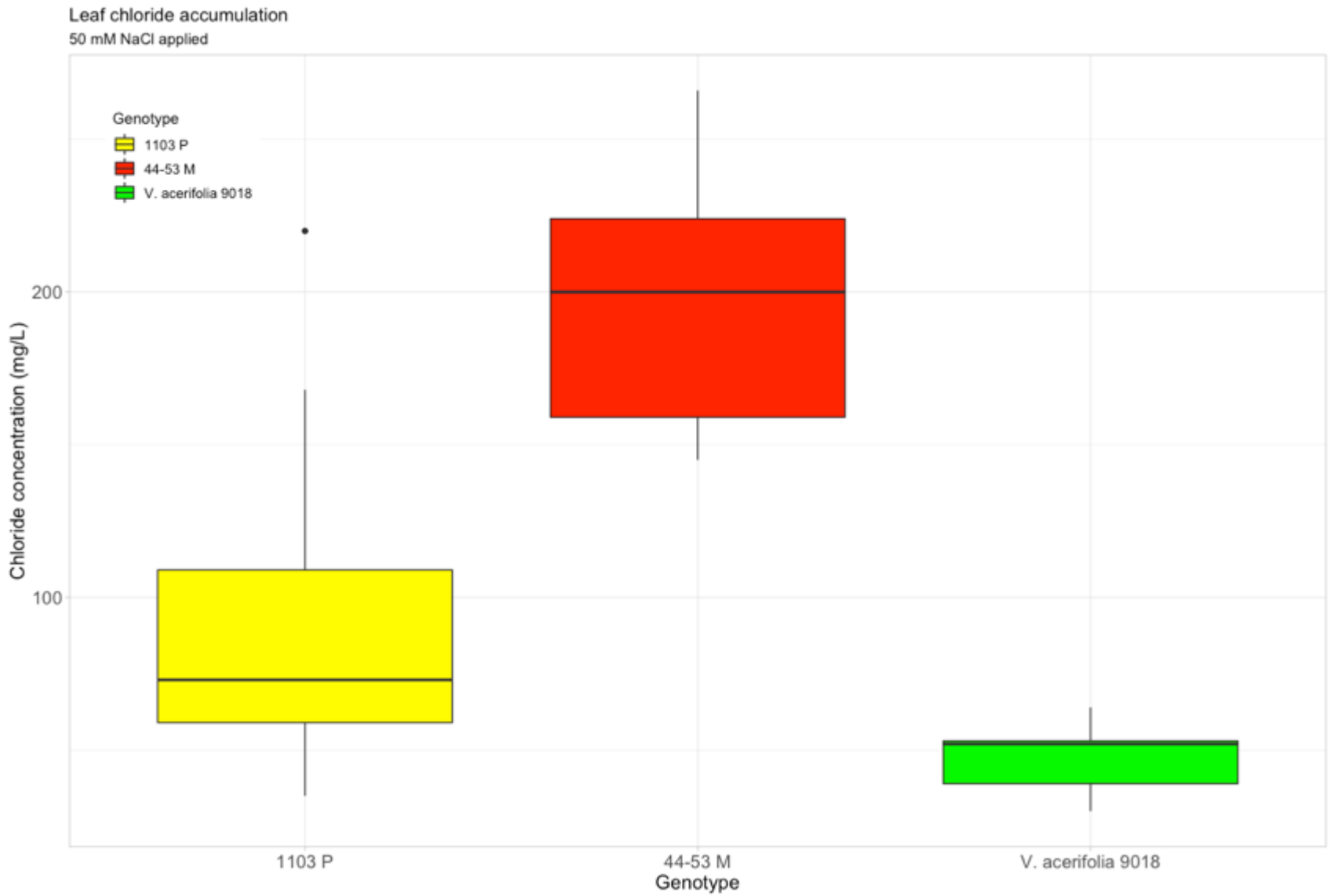

Figure 10. Leaf chloride accumulation with salt treatment; 50 mM NaCl applied

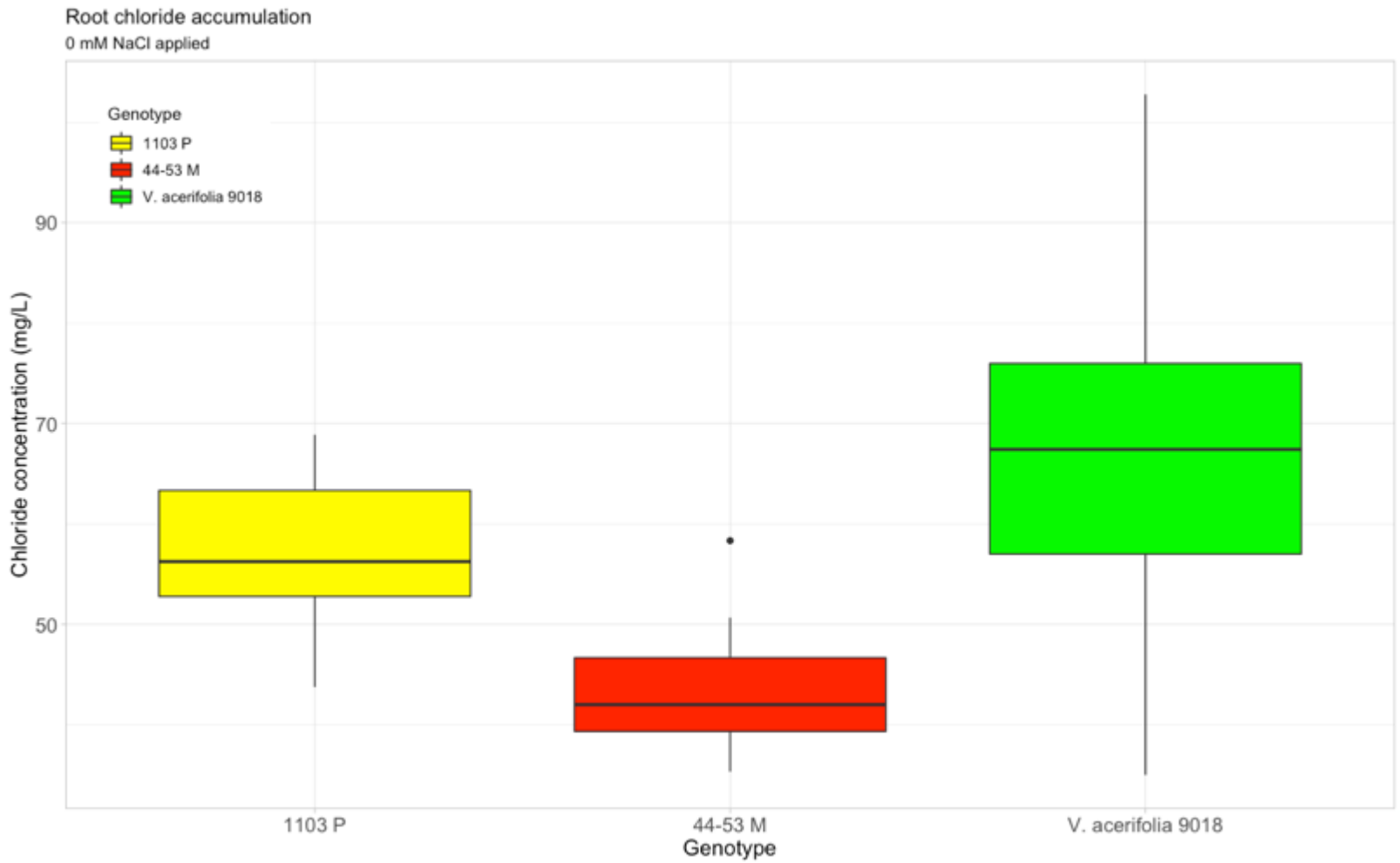

Figure 11. Root chloride accumulation without salt treatment; 0 mM NaCl applied

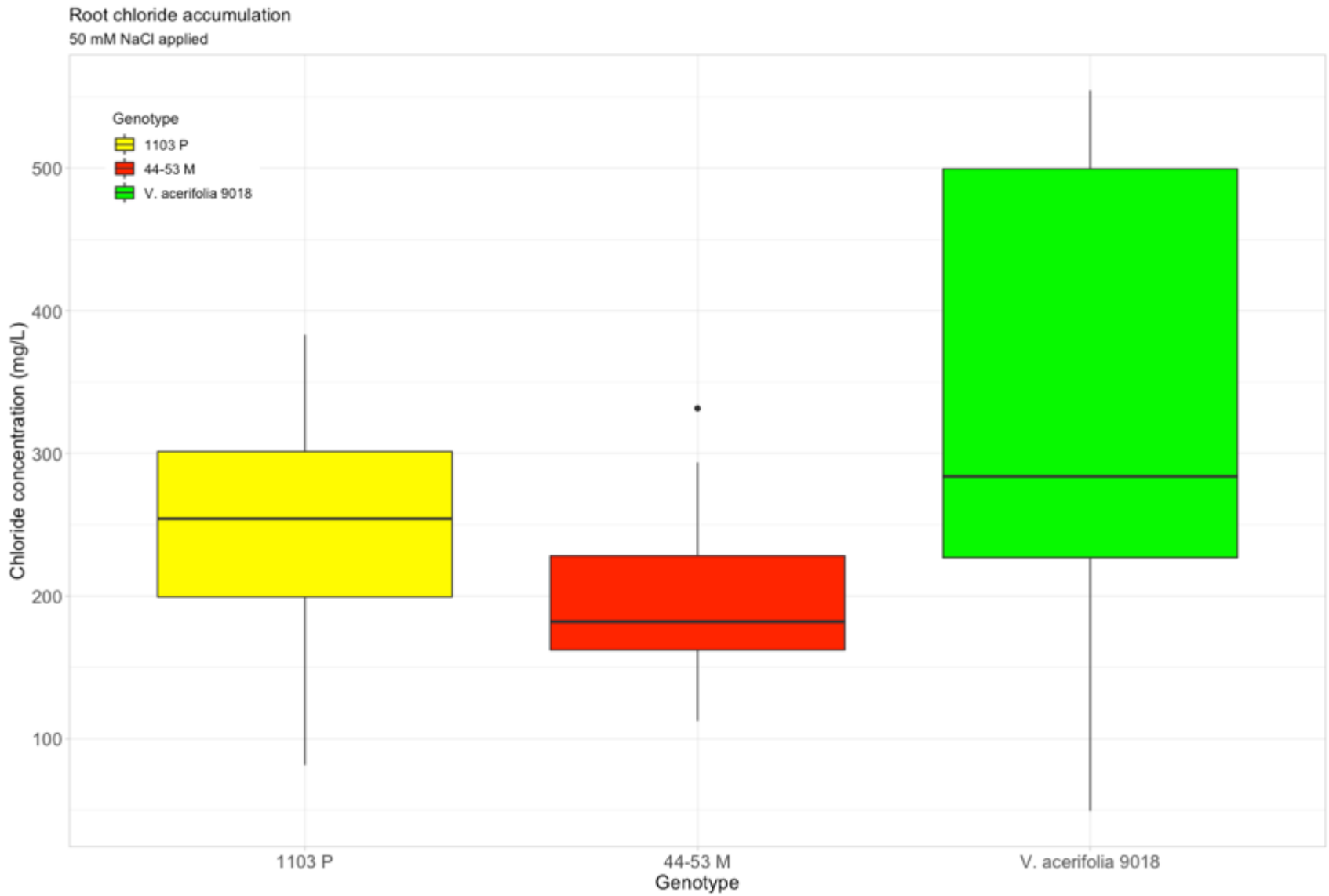

Figure 12. Root chloride accumulation with salt treatment applied; 50 mM NaCl

Table 6. Leaf chloride accumulation with salt treatment (50 mM NaCl applied) and without salt treatment (0 mM NaCl applied)

[NaCl] applied						
Genotype	0 mM NaCl			50 mM NaCl		
	mg/L	Standard error	Group	mg/L	Standard error	Group
44-53 M	22.26	0.26	a	198.33	5.66	a
1103 P	21.26	0.23	a	96.22	8.27	b
V. acerifolia 9018	21.19	0.23	a	48.11	1.51	b
p-value	0.364			< 0.001*		

*Note: *p < 0.05*

Table 7. Root chloride accumulation with salt treatment (50 mM NaCl applied) and without salt treatment (0 mM NaCl applied)

[NaCl] applied						
Genotype	0 mM NaCl			50 mM NaCl		
	mg/L	Standard error	Group	mg/L	Standard error	Group
44-53 M	43.59	0.98	b	202.7443	9.51	a
1103 P	56.59	1.18	ab	247.04	14.03	a
V. acerifolia 9018	66.18	9.01	a	319.07	23.15	a
p-value	< 0.001*			0.145		

*Note: *p < 0.05*

Chapter 3: Conclusions

Results from Experiment 1 are consistent with recent findings of the Walker Lab. Although there are no significant differences in photosynthesis between the commercial rootstocks tested and 9018 when no salt treatment is applied, there are significant differences between the commercially available rootstocks and 9018 when a salt treatment of 50 mM NaCl is applied. Under these conditions, 9018 demonstrates the lowest transpiration rates and stomatal conductance, suggesting that one possible mechanism used by 9108 to avoid salt uptake is to slow down canopy growth. During periodic or prolonged droughts, the decrease in growth may be sufficient to prevent salt toxicity until more favorable growth conditions arise.

Exposing grapevine roots to increasing salt concentrations progressively increases the risk of grapevine salt toxicity. Experiments 1 and 2 compared the Cl^- concentrations in leaves, petioles and roots of 9018 with several other commercial rootstocks ranging from low to high salt tolerance. While salt concentrations in the leaves and petioles across the genotypes did not materially differ without the salt treatment, significant differences were observed between the genotypes with the 50 mM NaCl treatment. *V. acerifolia* 9018 demonstrated the lowest leaf and petiole salt concentrations of all the tested genotypes: significantly less than 44-53 M and Ramsey, with half as much Cl^- accumulation than the 140 R and 1103 P, which were not significantly different from 44-53 M or Ramsey. Similar results were recorded in Experiment 2, with 9018 having significantly lower Cl^- concentration in leaf and petiole samples than 44-53 M, and half the Cl^- concentration of 1103 P. Another significant difference between the tested genotypes is apparent when salt concentration over time is measured, as shown by Experiment 1 where the low and stable leaf Cl^- concentration of 9018 over the entire 28-day salt tolerance

testing performed for this research stands in sharp contrast to the steadily increasing leaf Cl^- concentrations of the other tested genotypes (Figure 5).

The purpose of the Walker Lab's most recent greenhouse salt trials, including the Experiments 1 and 2, was to repeatedly demonstrate the high salt tolerance of 9018 compared to some of the most popular salt tolerant commercial rootstocks, including 140 R and 1103 P. Part of demonstrating the salt tolerance of 9018 requires finding the optimal experimental trial duration and salt concentration to demonstrate the rootstock's full potential. Experiments 1 and 2 used a 28-day trial period and 50 mM NaCl, consistent with some of the most recent trials performed in the Walker Lab, but also included the addition of 1103 P. To further demonstrate the extent of 9018's salt tolerance relative to the most salt tolerant commercial rootstocks, salt treatments may need to be raised from 50 mM NaCl to somewhere between 50 – 75 mM NaCl. 50 mM NaCl was chosen for these experiments because previous studies determined that significant differences between the salt tolerance of genotypes become obvious between 25 and 75 mM NaCl, and because the NaCl threshold for a soil to be considered saline is 40 mM equivalents of NaCl.

Although the results from Experiments 1 and 2 showed significant differences in leaf and petiole salt concentration among the tested grapevines, 9018 invariably demonstrated the lowest average mean Cl^- concentrations in these tissues, while the opposite was found for Cl^- concentration in the roots of all of the tested genotypes. In both Experiments 1 and 2, the root chloride concentrations were always highest in 9018, and in Experiment 1, they were significantly higher in 9018 than 44-53 M with the salt treatment. This discrepancy appears to suggest another mechanism that uses to exclude NaCl, which is possibly explained by the xylem loading theory (Storey 2003).

While the specific genes and mechanisms for grapevine salt tolerance remain elusive, the traditional breeding programs and assays like the one employed in Experiments 1 and 2 can be used to identify salt tolerant rootstocks, which can then be used to breed salt tolerant genes into existing commercial rootstocks. To accomplish that objective, the salt tolerance potential of wild North American grapevine species in genotypes like *V. acerifolia* 9108 must first be established through greenhouse testing and then vineyard testing. Ongoing trials in the Walker Lab use an experimental set up similar to that employed in Experiments 1 and 2, except that the grapevines are planted in 25-gallon buckets filled with fritted clay, which have been left outdoors for several years with cabernet sauvignon as the scion. The purpose of this series of experiments is to determine if the more salt tolerant rootstocks are effective in preventing salt accumulation in the fruit clusters. Further work is in progress to conduct a long-term study with grapevines planted in the vineyard. If these experiments demonstrate that 9018 is more salt tolerant than the most tolerant commercial rootstocks in the vineyard, and permits less salt accumulation in the fruit clusters than the commercial rootstocks, 9018 can then be crossed with existing commercial rootstocks to improve the salt tolerance of commercial rootstocks, and ease the identification of salt tolerant genes through further comparative genetic analysis.

Continued efforts to identify salt tolerant genotypes may lead to further exploration for wild grapevine species in the southwestern U.S., where 9018 and other relatively salt tolerant wild grapevine species were discovered, and the identification of other accessions with even better salt tolerance than 9018 through the greenhouse assay developed by the Walker Lab and genetic testing. Even if 9018 and other wild grapevines species are used commercially to improve the salt tolerance of commercial rootstocks, it is important to simultaneously develop and apply improved irrigation and farming practices to reduce salinization. These needed

improvements should become more easily achievable as irrigation systems and vineyard monitoring equipment become more advanced. By combining improved rootstocks with enhanced farming practices and irrigation equipment, vineyards will be able to survive more saline conditions and longer drought periods while maintaining production quality and yields.

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