Report
California Grape Rootstock Improvement Commission
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Project Title: Development of next generation rootstocks for California vineyards.

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2014 Pollinations – The 2014 crosses are presented in Table 1. They focused on combining PD resistant rootstocks with nematode resistance from arizonica forms with *XiR1 X. index* resistance and the GRN rootstocks; using excellent forms of chloride exclusion from Claire Heinitz' work in crosses with GRN nematode resistance; using double chloride exclusion (shoot and root exclusion – most forms sequester chloride in the roots but prevent it from moving to the shoots, these prevent chloride from building up in the roots); combining drought resistance with chloride exclusion and GRN nematode resistance; combining deep rooting (Dog Ridge and 14uRu) with GRN nematode resistance; and combine *vinifera* x *rotundifolia* (VR) ring nematode resistance and potential for virus tolerance with GRN nematode resistance.

2014 Screening of Crosses for Nematode Resistance – Nina Romero and I walked about 1,100 of the 2010-2012 progeny and scored them for horticultural characteristics (cane length and brushiness and internode lengths. About 20% of these progeny were advanced to rooting tests with ten 2-3 node cuttings. Those that rooted well and scored highly for horticultural characters were advanced to nematode testing against a combined inoculum of HarmA and HarmC (Harmony and Freedom aggressive root-knot nematode strains) and then against ring nematode. Unfortunately, not all selections were tested for both nematodes, but we have selections that will be tested to confirm either resistance. We also tested these selections for salt tolerance in a quick screen to select those with strong resistance and potential for breeding and selection as rootstocks

Table 2 presents the best of the ring nematode resistant selections in comparison to nematode numbers and nemas/g of root obtained for O39-16, our highly resistant control. Plants were propagated by Nina and grown in 4 inch pots for testing. They were inoculated with 1,500 ring or 500 root-knot nematodes and evaluated for population development (ring) or egg masses (root-knot) after 3 months of growth. None were as highly resistant as either of our two standards the rotundifolia-based rootstocks O39-16 and GRN-1, but we will select the best in terms of rooting and root-knot and ring resistance to advance to further nematode testing against citrus and dagger nematodes.

Table 3 and 4 present the results of testing with the combined HarmA/HarmC root-knot nematode inoculum. The breeding objective for Table 3 progeny was to improve the rooting of the GRN series (particularly GRN-5) and moderate vigor by crossing with 101-14Mgt. Thirty of these with egg mass / g of root data below 2 will be advanced to further testing. The selections tested in Table 4 were hoped to combine salt tolerance, deeper rooting and broad nematode resistance. Thirty-three of these will be advanced to salt and additional nematode testing. They include a broad range of resistance backgrounds and have good promise.

Table 5 presents the parentage and number of selections that survived a severe salt screen that Nina devised. About 300 of the 1,100 we scored for horticultural characters and that rooted at 50% and above were tested for salt resistance by submerging them in 150 mM NaCl (about 30% of seawater) for 2 weeks to eliminate as many selections as possible prior to additional testing – 61 did not develop any salt burn symptoms, although they had reduced root and shoot growth; 2 of 30 *V. rupestris* selections from Missouri also passed this test. All of these

selections will be rested with our established screen to compare the effectiveness of this rapid screen. Selections that include the GRN rootstocks as parents will be advanced to screening against all the nematode strains.

Fanleaf – We continue to make progress on identifying and verifying the function of the *Xiphinema index* resistance gene from *V. arizonica* b42-26, and it resistance locus *XiR1*. Two gene candidates are members of the NB-LRR (nucleotide binding-leucine rich repeat) resistance gene family that control recognition of pests and diseases and the triggering of a defense reaction. These two candidates were transformed into St. George and Thompson Seedless and some lines exhibited reduced susceptibility to *X. index* (Figure 1), but the transformed plants were still susceptible. There are more lines to test (Table 6) and we are examining gene expression with qPCR and will pursue native promoters to determine if they can increase resistance.

Xiaoqing Xie and Cecilia Agüero have been producing green-grafted *M. rotundifolia* and GFLV infected Chardonnay plants to test resistance to the virus in different cultivars of *M. rotundifolia*. After initial success with Lucida and Trayshed (Figure 2), following experiments include five additional varieties and O39-16. Xiaoqing has also produced a number of tetraploid VR hybrids that we hope will be better able to hybridize with other rootstocks and allow us to introgress *rotundifolia*'s remarkable resistance, which is very difficult due to the differences in chromosome number (Table 7). The diploid and tetraploid forms of four VR genotypes have been established in the field for further analysis. Olmo was able to produce some fertile VR hybrids but because these are *vinifera* x *rotundifolia* some will susceptible to phylloxera. A new MS student Tarana Shaghazi is testing these to determine which have the best phylloxera and ring nematode resistance. Many of these were used in crosses in 2013, and a few were used in 2014, to provide breeding material if they have good phylloxera resistance. Cecilia Agüero is also conducting pre-bloom hormone treatments on clusters in the field to test the effect of candidate cytokinins on reducing fanleaf expression. These candidates were identified by our earlier studies of xylem constituents from O39-16 and associated with its ability to induce tolerance to fanleaf disease (Figure 3).

O9-16's potential to act as natural nematicide to *X. index* – Evan Goldman is finished his MS thesis on the ability of O39-16 to eliminate *X. index* from a vineyard. He sampled *X. index* numbers in a 22-year-old Oakville vineyard that as planted was a large replicated rootstock trial with 4 row x 50 vine blocks. He sampled over the season to compare *X. index* populations on O39-16, 110R and 3309C, the later two are susceptible to *X. index*. His results are summarized in the abstract below from his MS. I am including Figures 4 and 5 from the June 2014 report.

Potential to Eradicate *Xiphinema index* Using the Bioantagonistic Rootstock 'O39-16'
Evan Goldman MS Abstract

Abstract: Previous reproduction studies of *Xiphinema index* (the dagger nematode) on the grape rootstock 'O39-16' showed that populations decreased over time. In addition, the alternative host range of *X. index* is limited and does not seem to include many common vineyard weeds. This study was conducted to determine the most effective sampling method to recover *X. index* and to evaluate the possibility that the nematode can be eradicated over time from vineyards that have been planted with 'O39-16' rootstock. Two sampling methods (shovel vs. Oakfield tube) were used, and the nematodes were extracted and identified. Pearson's test determined that there was a poor correlation between the two methods and subsequent sampling used the shovel method. The populations of *X. index* and *X. americanum* on 'O39-16' were compared with adjacent populations on '3309C' and '110R' rootstocks, both susceptible to *X. index* feeding. Samples were collected from beneath drip emitters on three dates, and on each date the same drip zones were sampled. Nematodes were extracted and identified. Very few *X. index* were recovered from 'O39-16'; most samples were devoid of *X. index*. Significantly fewer *X. index* were recovered from 'O39-16' than from either '3309C' or '110R'. There was a tendency for 'O39-16' to have more *X. americanum* than either '3309C' or '110R', although the differences were usually not significant. To verify the absence of *X. index* on 'O39-16', soil pits were dug alongside previously sampled vines. Samples were collected at 25 cm, 50 cm, and 100 cm and nematodes were extracted and identified. Although the differences were not significant, there was a trend for fewer

nematodes at increasing depths. In conclusion, the likelihood that *X. index* can be eradicated through the use of 'O39-16' is high. However, these results need to be verified in other vineyards, especially those planted solely on 'O39-16'.

Drought and salt resistance – Kevin Fort

Completion of screen to assess the chloride exclusion capacity of the rootstocks GRN-1–5, and experimental rootstocks SC-1 and GC-5 – It was reported in January 2013 that a salt tolerance screen had been completed, and that included the rootstocks GRN-1-5 and two experimental rootstocks GC-5 (*V. arizonica*) and SC-1 (*V. girdiana*). This 2013 assay used a protocol we developed that mimics the field results of mature grafted vines. The leaf tissue chloride analysis is now complete from this screen and results are shown in Figure 6. From this data, a rank order for chloride exclusion capacity from strongest to weakest excluders is as follows: (1) GRN-1, (2) GC-5, SC-1, (3) St. George, (4) GRN-2, GRN-3, GRN-5, 101-14, and (5) GRN-4, 44-53. These results confirmed in part previous screens showing the strong exclusion capacity of GC-5 and SC-1, the strong but reduced exclusion capacity of St. George, and the very weak exclusion capacity of 44-53. The chloride uptake by Colombard illustrates the effectiveness of the assay, in that a conventional analysis (Figure 7, *left*) would lead to the false conclusion that Colombard is a strong chloride excluder. The measurement of growth rate (Figure 6) permits the more reasonable interpretation that the apparent exclusion by Colombard in this study is more likely an artifact of an unusually slow growth rate, which may be due leaf roll infection in this plant material. The surprising result of very strong chloride exclusion by GRN-1 is currently being re-tested. The salt tolerance screen presented here was also performed in much smaller, 2 inch containers, but resulted in aberrant patterns (Figure 7, *right*) that are likely the result of root constriction and an insufficient soil volume, and so the 4 inch containers may represent the smallest container size from which robust data can be reliably derived. These insights will help in maximizing the efficiency of future screening.

Expanded screen to assess the chloride exclusion capacity of commercial rootstocks and experimental rootstocks – The salt tolerance screen of GRN-1–5/GC-5/SC-1 represents our first application of multiple refinements to the screening protocol that have been in development since 2005. This assay has now been applied to an expanded set of 42 genotypes representing nearly all of the widely available commercial rootstocks in California, in addition to a set of experimental rootstocks that may have an even stronger capacity for chloride exclusion (Table 8, Figure 8). Some genotypes were included in this screen ("Research genotypes" in Table 8) that may lead to better insight into the heritability of chloride exclusion and hence could assist in future breeding efforts. This salt tolerance screen is now complete, and leaf tissue samples are being processed to quantify chloride accumulation.

Establishment of a common scion population of rootstocks and experimental rootstocks for full season evaluation of chloride exclusion – Following the early screening by Claire Heinitz of hundreds of wild *Vitis* genotypes for superior salt exclusion and rootability of dormant cuttings, six genotypes were selected for advancement. Five of these genotypes were included in the herbaceous screen described above to assess relative chloride exclusion in the context of nearly all commercially available rootstocks; the sixth genotype was excluded due to insufficient propagation material. However, dormant cuttings were obtained in January 2014 for all six genotypes and, together with ten rootstocks, were grafted to Cabernet Sauvignon (Table 9). In April 2014, nine replicates of each rootstock and each of the six experimental genotypes were planted into large 5 gallon containers and placed in a shadehouse (Figure 9). The goal of this salt tolerance screen is to test the advanced selections in a manner more closely aligned with field conditions, namely (1) plant material with a common scion, (2) reduced environmental buffering within a shadehouse, and (3) a full-season of exposure to high chloride in the soil solution, with leaf samples analyzed at monthly intervals. This screen represents the most stringent and final stage of testing, and is reserved for the most promising plant material. Because growth rate variation can confound the interpretation of chloride uptake data, an unanticipated high variability in the rate of establishment within and among genotypes in 2014 led us to delay salt treatments until 2015, at which time all sampled vines will have both a full season of growth and graft union healing, and a fully quantified history of pruning weights. This delay will help to ensure that the highest quality data is produced for properly characterizing these advanced selections for chloride exclusion.

Development of a two-dimensional soilless assay for root architecture characterizations of complex root systems – We are optimizing methods to analyze root system to optimize throughput and the predictive ability of the assay. A likely possibility is that more than one assay will be employed to overcome analytical tradeoffs present in each method. Our very high throughput screen of measuring root angles of adventitious roots from herbaceous cuttings is now complete and the data sets are presently being analyzed. Simultaneously, we have in 2014 developed a novel two-dimensional soilless method of analyzing more developed, complex root systems. This system is analogous to the soil media-based rhizotron characterizations completed in 2013 by Joaquin Fraga (reported earlier), and will complement that rhizotron data set by providing greater insight into the rooting angles of the primary roots and branching patterns of associated lateral roots. Whole root systems can be scanned (Figure 10), and will be analyzed in great detail using WinRhizo software, recently made available to the lab.

Development of a three-dimensional media-based assay for improved rooting angle characterizations – Many two-dimensional root architecture methods can yield important insights into root system development, have a moderate throughput, and because they are non-destructive are useful in characterizing structural changes over time. However, growing a grape root system within a thin layer can result in inaccuracies as root growth is directed by the container boundaries. To address this limitation measured root angles from plants grown for approximately one month in a large container that holds an inner basket (Figure 11). Plants in this system are inverted at harvest, the container carefully removed, and soil teased away from the roots that have grown through the inner basket openings, thereby retaining root position at the basket boundary. In some cases, a translucent plastic pot was used to allow root angle scoring without harvesting the plant. In the pilot study, this system showed that by one month of growth, what began as a cluster of adventitious roots developed into a system of approximately six dominant roots with extensive lateral root development. The rooting angle of the dominant roots maintained the root angle range measured in very young adventitious roots in many, but not all cases. This more resource-intensive assay may be used in the future to better characterize root systems when limited comparisons are needed with very high quality data.

Establishment of a common scion population of commercial and experimental rootstocks for a two-season container study of rooting traits – In contrast to our attempts to produce a rapid, high-throughput screen that can be used to characterize root systems, we are concurrently characterizing root architecture in plants grown for one or two full seasons. These studies will ensure that data produced in rapid characterizations is a reliable representation of more mature grape roots, and may show the strengths and weaknesses of rapid characterizations. The first of these longer-term studies is intermixed within the same shadehouse assay earlier described for the season-long analysis of chloride exclusion (shown in Figure 8; genotypes listed in Table 10). In winter 2014-2015, a subset of these plants will be harvested and the root systems digitally analyzed with WinRhizo. The data obtained will be compared both with rapid characterizations and with data obtained from harvested roots at the end of the 2015 growing season. Because deep-rooting rootstock genotypes such as 110R have thicker roots that may overwinter more effectively than other genotypes, all plants with harvested roots will be re-planted in 2015 as controls to determine if the establishment of root thickness differences can produce genotype-driven shoot growth differences following a single season of establishment.

Establishment of a common scion population of rootstocks for a full season field study of rooting traits and response to drought – In early 2013, a set of commercial rootstocks were excavated from a field plot at UC Davis following two seasons of growth, with a drought treatment imposed in the second season. The data from this trial was insightful in that it revealed important patterns of root:shoot biomass ratios in deep- and shallow-rooted rootstock genotypes. Additionally, it was found that genetically-driven patterns of root architecture were unaffected by the drought treatment. As a follow-up to this important study, we have established an expanded set of rootstock genotypes at the same study site (Table 10; Figure 12), having first grafted all individuals with a common scion (Cabernet Sauvignon). The study design and plot layout have both been improved, and the set of measurement variables is to be expanded. A treatment subset of these plants will be drought stressed in 2015, and the entire study harvested following the 2015 growing season.

Update on the Genetic mapping of rooting angle in the Ramsey x Riparia F2 population – Summaira Riaz, Karl Lund, Kevin Fort – Data analysis of this project continues (Please see June 2014 report).

Root architecture and drought resistance – Jake Uretsky

Update on genetic mapping of root angle in Ramsey x Riparia populations – We previously reported results of QTL analysis of rooting angle phenotype in a Ramsey x Riparia F2 population. Briefly, 160 F2 progeny derived from a sibling cross in the F1 were evaluated for rooting angle from green cuttings grown in a perlite/vermiculite mixture. A major QTL explaining 24.8% of phenotypic variation was identified on chromosome 6, and additional QTLs explaining a combined 15% of variation were identified on chromosomes 4 and 19. These results helped confirm a genetic basis for rooting angle and prompted an additional experiment evaluating rooting angle in the original F1 population.

Performing a similar analysis on the F1 population allows us to exploit the full genetic variability contained within the parent genotypes, as well as determine the contribution to rooting angle from each parent. We evaluated 140 F1 genotypes from green cuttings grown in perlite/vermiculite medium. Replicates were arranged in a randomized complete block design with three blocks to account for potential temperature and moisture variation in the propagation house. After two weeks of growth, data for root angle, length, depth, and count were recorded. Mean rooting angles for F1 progeny showed continuous segregation between the parental genotypes. In addition, rooting angle of the three longest roots per plantlet was highly correlated with overall rooting angle (r^2 = 0.955). Restricting measurements to these roots streamlines evaluation and data analysis and may reduce measurement error due to an overabundance of roots that would likely not persist into maturity.

QTL analysis supported our findings of QTLs correlated with rooting angle on chromosomes 6 and 19 in the F2 population, but the QTL initially identified on chromosome 4 was not significant in the F1. Additional QTLs of interest were identified on chromosomes 8, 13, and 16. We have expanded the Ramsey x Riparia F1 population and are currently refining our framework SSR map with 15 publicly available markers on each chromosome possessing a significant QTL. The improved map, combined with the expanded F1, will aid in confirming or rejecting the utility of these various QTLs for use in developing deep-rooted material.

Root system architecture, root growth and development, and root persistence and regeneration – Root architectural and morphological traits that optimize and maintain water uptake in plants are prime candidates for developing rootstock cultivars adapted to dry conditions. We have evaluated young roots in plants grown in a variety of substrates (perlite, sand, fritted clay, peat-based potting mix, etc.) for traits believed to influence drought tolerance. Our screens have included mapping populations and wild accessions, with well-defined commercial rootstocks as controls. We have given particular consideration to a 101-14 Mgt (*V. riparia* x *V. rupestris*, drought sensitive) x 110R (*V. berlandieri* x *V. rupestris*, drought tolerant) F1 mapping population that has been evaluated for root system architectural traits, including rooting angle, primary and secondary root length, and root diameter, ecological traits like specific root length (length x biomass^{-1}), and physiological traits, particularly root and shoot biomass and root dry-to-fresh weight ratio. Ultimately, we aim to rapidly and accurately predict the characteristics of mature root systems based on the young, developing root systems.

The dynamism of roots makes categorizing root systems and predicting mature plant phenotypes exceedingly difficult. The root system of a single plant resides in soil with variable moisture, fertility, temperature, texture, aeration, and biology, and many measurable traits are simultaneously altered by responses to the environment. For example, we previously reported that 101-14 Mgt possessed long, narrow, highly branched roots, while110R produced shorter, thicker roots with few laterals. In fritted clay, however, 101-14 roots can be thick and unbranched, and 110R roots develop long, uniform lateral branches, resulting in a root architecture resembling a fish spine. Confounding effects of shoot responses to the environment can also obscure root specific responses. Traits that are primarily influenced by feedback between the roots and shoot might be of limited value in a grafted plant. Finally, most roots are ephemeral and most of the adventitious roots emerging from a cutting will not persist as the vine matures. The relative importance of genetic versus environmental factors in root persistence is unknown, which presents a very difficult question – which roots do we measure?

We sought an experimental procedure for examining root systems in heterogeneous environments and have begun using a split-pot system for root analysis (Figure 13). Our fundamental objectives are to determine how microenvironments within root system spaces affect root development and maturation, root persistence and regeneration, and morphology and architecture. The anatomical and structural changes that occur early in plant growth are, in many cases, irreversible and so will affect the root system and the scion throughout the life of the plant.

The split-pot procedure is as follows; green cuttings are started in a 3:1 perlite/vermiculite mix in the propagation house using standard protocol. After about two weeks, when adventitious roots have developed to several centimeters in length, plantlets are removed from the medium for transplant. The root systems are pruned to two roots of similar developmental stage and length, measured, and placed in a 90° irrigation elbow that rests in notches cut in nursery pots. The pots contain a thin layer of perlite at the base and are filled with sand so that the surface of the sand is slightly beneath the top of the irrigation elbow (Figure 13). The potted plants are established in the propagation house for approximately one week before being placed in the greenhouse. For control plants, both pots of the split-pot are well-watered. Only one pot is watered regularly in the treated plants. The dual pot systems are weighed daily to determine gravimetric soil moisture and maintain consistent watering. After two dry-down cycles, the plants are re-watered to saturation, removed from the system, and evaluated (Figure 14).

The split-pot procedure provides several advantages for evaluating root growth and development. First, individual roots are measured at the start and conclusion of the experiment, providing a window into developmental changes occurring during this time. The system also limits confounding effects of environmental stress on the plant. For instance, imposing water stress on entire root systems necessarily stresses the shoot and results in reduced shoot growth, photosynthesis and transpiration. Any effects measured in the root system cannot be separated from effects in the shoot. With a split-pot, the shoot is not drought stressed because half of the root system can supply adequate water. In such a system, developmental differences measured in the roots are fundamentally driven by the root response. Finally, there are many the treatment combinations that include variable moisture, fertility, growing media, and combinations thereof. Alternating treatment regimes within one split-pot unit during the course of an experiment might be especially important for predicting responses to non-uniform or periodic changes in soil moisture found in vineyards.

We continue to focus on the commercial rootstocks 101-14 Mgt and 110R and their F1 progeny. Several observations from our initial results involving 101-14 Mgt and 110R are of note. Roots receiving regular moisture possessed the bulk of the overall root fresh weight and biomass in both rootstock cultivars (Figures 15a, 15b, and C). Similar observations were made for other genotypes but with fewer replicates. This pronounced difference in growth occurred regardless of initial root size, suggesting that root persistence might initially be driven by resource acquisition and photosynthetic demand, as opposed to being genetically predetermined.

Calculating root dry-to-fresh weight ratios (DW:FW) revealed that the response to drying soil was much more pronounced in 101-14 Mgt than in 110R. The DW:FW ratio is easy, quick, and cheap to measure and indicates the relative substance of a given plant tissue (structural and non-structural carbohydrates, waxes, suberin, proteins, etc.) in terms of water content. In 101-14 Mgt, the dry treatment resulted in a near doubling of root DW:FW compared with the wet treatment; the magnitude of this effect was much smaller in 110R (Figure Ac). Surprisingly, 110R DW:FW was similar in roots subjected to drying soil compared with well-watered 101-14 Mgt roots. These data suggest that differences in drought tolerance between these cultivars might result from an exaggerated environmental response in 101-14 Mgt and a muted response in 110R. The development of hydrophobic barriers in roots does limit water loss but also restricts water uptake to relatively small regions in the root system, usually near the root tip. Increased DW:FW might result from the production and deposition of substances for diminishing water loss to the external environment, such as lignin or suberin, and/or from a reduced capacity for water uptake after periods of

low moisture. Perhaps the relative performance of 110R and 101-14 Mgt rootstocks in deficit irrigation is linked to this response to drying soil and the subsequent ability to acquire water upon rewetting.

The use of the split-pot procedure is promising for isolating and understanding specific mechanisms under genetic control that improve the response to water stress in grape roots and that can be exploited in breeding improved cultivars. We will continue to evaluate an assortment of plant materials using this system with principle focus on 101-14 Mgt x 110R F1 progeny.

Decoupling drought tolerance from rootability – In addition to identifying genetic components for improved drought tolerance, the 101-14 Mgt x 110R F1 population is currently being evaluated for rootability. Dormant cuttings from several canes of each genotype of a population subsample, as well as parental genotypes, were collected from the vineyard. Relative cane position, length, diameter, and weight were recorded for each cutting. The cuttings were soaked and placed in the callus room following standard protocol. These cuttings will be removed after two weeks and evaluated for callus and root formation, number of roots, root length, and root diameter.

The aim of this experiment is two-fold: 1) determine if ease of propagation in terms of rootability is segregating in the 101-14 Mgt x 110R population; and 2) evaluate the effect of genotype, cutting position, and cutting size on rootability. The strength of the genetic link between drought tolerance and ease of propagation is not well understood. Evaluating root system responses to drought in conjunction with rootability in 101-14 Mgt x 110R F1 genotypes provides an opportunity to untangle these traits and develop easily propagated drought adapted rootstock cultivars.

Genetic analysis of southwest *Vitis* accessions – Claire Heinitz – We previously identified several potential sources of chloride exclusion among wild *Vitis* accessions that appear to be from different species based on morphology and geography. In order to determine whether these accessions arise from a common genetic background (and therefore have similar mechanisms of resistance), or if they are in fact from distinct genetic groups and represent multiple mechanisms of chloride exclusion, we need to understand the population dynamics of wild *Vitis* in the region. These vines are challenging from this perspective because they readily form interspecific hybrids and can move large distances through seed dispersal via birds and small mammals. Our previous work using nuclear microsatellite markers to investigate a possible hybrid origin of *V. doaniana* (a strong chloride excluder from the border region of Texas and Oklahoma) demonstrates the power of this technology to tease apart complex genetic relationships. However the nuclear microsatellites also showed us that, even in the case of a well-documented hybrid of two neighboring species, the story is not so simple (see January 2014 report). As a result, when we began to look at our entire collection of southwest *Vitis* as a whole, we decided to begin by using chloroplast microsatellite markers.

The chloroplast genome in plants evolves and is passed down independently from the nuclear genome, and only from the maternal (seed) line. There is no recombination; so maternal lineages remain identical until random mutation causes a change in the genome. This is why traditional plant taxonomy is usually performed with chloroplast gene sequences – it is possible to observe larger- scale evolutionary events that happened in the more distant past. However, chloroplast gene sequence analysis of the southwest *Vitis* germplasm failed to resolve relationships between the major species, indicating that we do need a tool that will reveal more recent divergence and hybridization events. Chloroplast microsatellite markers are an excellent compromise between conserved chloroplast gene sequences and nuclear markers – like the gene sequences, they are only inherited maternally so they ignore recent hybridization events, but like the nuclear microsatellites they mutate much more rapidly.

An analysis of 420 wild *Vitis* accessions using 13 chloroplast microsatellite markers revealed 89 unique haplotypes (genetic groupings), which clustered into 3 groups (A, B, and C; Figure 16). These major groups roughly correspond to morphological and geographical groups (Figure 16). The A group is more distinct from the other two, and contains mostly accessions with morphology consistent with *V. riparia* and *V. rupestris*. The B group is split between *V. girdiana* (in southern California, Nevada, and Utah), *V. treleasei* (in central New Mexico), and *V. berlandieri* (central Texas). The C group is made up almost exclusively of *V. arizonica*

accessions. While all three groups contain accessions previously designated as chloride excluders, 5 haplotypes in the A and B groups contain predominantly strong excluders (A1, A15, A19, B12, B28; highlighted in Fig. 17).

This analysis indicates that the two populations with the strongest chloride excluders in our collection – *V. girdiana* from western Nevada and *V. doaniana* from the Red River – may in fact derive from distinct genetic backgrounds, and therefore possess unique mechanisms for chloride exclusion. Another interesting result is the diversity in the B haplotype group. It will be very interesting to see if *V. girdiana* is actually more closely associated with *V. berlandieri* from Texas than with the neighboring *V. arizonica*. Additionally, all of the *V. berlandieri* accessions belong to a relatively small number of closely related haplotypes, compared to other species and regions with more haplotype diversity. This could be a signature of a "founder effect", an indication that the populations of *V. girdiana* that now extend from southern California to southern Utah originated from a small group of migrant plants.

The next steps for answering these questions and continuing the work involve sequencing some of the chloroplast microsatellite alleles to rule out homoplasy (a character shared by a set of species but not in a common ancestor), and following up with similar work using nuclear markers. Comparing results from chloroplast and nuclear markers will reinforce our findings and allow us to differentiate patterns of gene flow. Finally, the goal is to identify groups of accessions in our collection with genetically unique sources of traits (salt, drought, and nematode resistance) for rootstock breeding (these species also possess strong PD resistance.

Correlation of rootstock architecture to drought resistance – Cecilia Osorio
This work is currently being written up in thesis form and publication being organized. Please see the June 2014 report for its current status.

Grapevine rootstock-scion interactions and influence on ripening periods and the initiation of senescence – Jean Dodson – Jean completed her PhD in August 2014, and is now an Assistant Professor of Viticulture at Cal Poly San Luis Obispo. We are working at getting papers from her dissertation published. The summary follows.

Summary – Currently, the primary role rootstocks have in winegrape production is to address pest pressure and, to some extent, environmental factors such as drought and soil chemistry. Rootstock research continues to look for better resistance to a range of pests and disease issues that impact vineyard health, but studies examining how rootstocks influence ripening periods, the initiation of senescence and the timing of dormancy lack in comparison. Although nursery rootstock guides discuss the influence that rootstocks have on scion growth, the casual basis of these effects remains unclear.

Environmental conditions, irrigation practices, rootstock parentage and interactions between these factors all play a role in the physiological development of the scion (Keller et al. 2012, Harbertson and Keller 2012). It is thought that the influence rootstocks have on scion growth and phenology is associated with differences in rooting patterns (Swanepoel and Southey 1989), the availability of water and nutrients (Keller et al. 2001) and the nature of the soil at a given vineyard site (Morano and Kliewer 1994). However, it is unclear whether rootstock rooting patterns influence scion phenology, are primarily genetically determined or more directly a result of environmental influences such as soil depth, structure and water availability. It is likely a combination of genetic and environmental factors that determine the impact rootstocks have on scion phenology.

The primary objective of this research was to determine the role grapevine rootstocks have extending or hastening the timing of leaf senescence, and to examine the role drought and rootstock parentage have on senescence. By creating a more complete understanding of how rootstocks influence leaf senescence, this study would assist breeders in developing rootstocks that are better adapted to shorter or longer growing seasons as influenced by temperature, latitude and rainfall. Such rootstocks could extend or shorten fruit ripening and the time grapevines have to store assimilates before dormancy. Growers could potentially conserve water by selecting a rootstock that induces a shortened growing season resulting in earlier scion leaf senescence. Conversely, in winegrape regions a rootstock that extends the photosynthetically active period of scion leaves could increase the time available to accumulate phenolics in berries and impact sensory characteristics.

Before studies detailing rootstock impact on specific scion phenological traits could be performed, it was important conduct a comprehensive rootstock survey to establish if broad differences between rootstocks exists on major factors that address major industry concerns, such as yield and pruning weights. This initial rootstock field evaluation survey was done using field data from Dr. Jim Wolpert collected over the course of eleven years at eight vineyard sites in California, each of which had a distinct soil profile. This foundation work established that differences in pruning weights and yields (vine balance) existed for different rootstocks across sites of varying soil composition. Eighteen rootstocks were evaluated for pruning weight and yield, although not all rootstocks were present in each of the eight field trial locations. This general survey of rootstock-scion interaction for pruning weight and yield was conducted from 1989 – 2000, although not each site had data collected each year.

Based on the data gathered from the broad rootstock field evaluation study, rootstocks 101-14MGT (*Vitis riparia x V. rupestris*) and 110R (*V. berlandieri x V. rupestris*) were chosen as the initial material to be evaluated more closely in the Oakville Station field evaluations. 101-14MGT and 110R are commonly used rootstocks with different parentage that are thought to represent opposite ends of the spectrum with regard to scion yield output, perceived vigor induction and ability to tolerate drought conditions.

The Oakville Station field evaluations included three vineyard blocks, all of which were on deep gravelly clay loam soils (25% clay, 30-50% silt and the rest sand). Each of the three vineyard blocks had a normally irrigated and drought treatment imposed. During the initial year of study, a wide-range of phenological traits were tracked to determine which if any could be consistently linked with rootstock selection, and if the differences were exaggerated under drought conditions. The second and third years of field evaluations focused on the traits that initially indicated differences based on rootstock and that did not interfere with commercial vineyard operation procedures.

The rootstock 101-14MGT has been considered to be a lower vigor inducing rootstock with a shallow root system and 110R a higher vigor inducing rootstock with a deeper root system (Christensen et al. 2003). It was from this and the supportive data collected from the Wolpert rootstock field evaluations that it could be hypothesized that rootstock based and genetically determined differences existed for differential fruit ripening and leaf senescence timing, and that dry farmed conditions would magnify differences between these two rootstocks.

The second objective of this study was to develop an accurate and rapid method to screen for various scion phenological traits that are influenced by rootstocks. The creation of such an assay would be a valuable tool in grape rootstock breeding programs to screen for the impact new rootstock selections have on scion growing periods and the initiation of dormancy as tracked by leaf senescence. An assay was developed to determine whether shoot growth and leaf function could be evaluated in potted plants, how such evaluations relate to mature vines, how changes in irrigation regime influences these pot based evaluations, and whether these evaluations apply to grafted and un-grafted rootstock material.

Four pot-based assays were developed; three of which utilized 4 L pots in a shade-house; the fourth used 20 L pots outdoors in full sun. All pot assay trials were evaluated for leaf function over the growing season by assessing leaf senescence and leaf water potentials. The initial pot assay studied five commonly used rootstocks: Riparia Gloire (*V. riparia*), St. George (*V. rupestris*), 101-14MGT (*V. riparia x V. rupestris*), 420A (*V. berlandieri x V. riparia*) and 110R (*V. berlandieri x V. rupestris*) as grafted (to Cabernet Sauvignon) and un-grafted plants. These rootstocks range widely in their ability to induce scion vigor (Jones et al. 2009, Reynolds and Wardle 2001).

The second shade-house pot assay selected plant material from an existing Ramsey (*V. champinii*) x Riparia Gloire (*V. riparia*) population that had already been genetically mapped and had wide variation in shoot growth and the duration of leaf function. Selections chosen for the container assay demonstrated significantly extended or shortened ripening periods compared to the rest of the population under field conditions.

The third shade-house based assay included progeny chosen from a 101-14MGT x 110R population created earlier in this project. The purpose of this cross was to develop a genetic mapping population capable of examining the genetic basis of hastening or extending leaf function and vine dormancy.

The fourth container assay was conducted in full sun in 20 L pots. This trial mimicked the plant material being evaluated in the Oakville Station field evaluations and included fully irrigated and deficit irrigation treatments. The purpose was to show how a pot-based assay correlated with the field data.

The Oakville Station field evaluations were successful in documenting differences in rootstock-scion interactions and the impact rootstocks have on phenological development. 101-14MGT induced a shorter growing season than 110R and once senescence was initiated, Cabernet Sauvignon on 101-14MGT reached full dormancy faster than on 110R. 101-14MGT also was found to devigorate the scion canopy in comparison to 110R, allowing a greater degree of light to penetrate into the canopy and fruiting zone. The structure of the canopy of the two rootstocks also varied when it came to shoot development. The diameter of the shoots, the internode length, the cluster weight and pruning weights for 110R were all greater than 101-14MGT regardless of trellising system.

Tracking leaf water potential, stomatal conductance and abscisic acid (ABA) levels in leaf tissue was difficult and improvements need to be made in data collection protocols. Sampling for all three factors should limit the timing of collection to 1-hour at mid-day to reduce variation. Finally, the timing of ABA sampling during the growing season is important and starting earlier in the growing season is imperative to catching the hormone fluxes that trigger the initiation of senescence and the timing of dormancy.

The development and initial testing of the potted vine assay found that imposing irrigation treatments on such a small scale is difficult to control and maintain because the small volume of soil dries out quickly. It is best to select one irrigation protocol and evaluate all plant material under that condition. Unfortunately some of the plant material included in the Oakville pot assay exposed to full sun, although certified, was infected with Red Blotch virus, which negatively impacted the vine vigor and leaf senescence results. Additional assays were developed from both green and dormant cuttings; the assay developed from green cutting was easier to evaluate throughout the season. Leaving rootstock material un-grafted rather than grafting to scion wood is more efficient for initial testing. Additional work is needed to improve this high throughput, inexpensive assay.

GRN rootstock trials – We took yield data from the Gallo Lodi trials for the second year. This plot is well-established with 13 rootstocks including GRN1, -2, -3, -4, and -5, RS3 and RS9, Harmony, 1103P, St. George, 3309C, 101-14 Mgt, and O39-16. The scion is Malbec and it was planted of a modified Wye trellis in 2011. The site has fanleaf degeneration (although the *X. index* counts were low), low levels of *X. americanum*, high ring nematode counts, high to moderate root-knot nematodes, and very low levels of lesion nematode. No impact of nematode feeding on vine vigor is apparent yet. Yields were good (Table 12, Figure 18) and shoot lengths all seemed normal. We will take pruning weights for the first time in February (the plot was pruned before we could gather pruning weights last year.

Presentations/Abstracts/Scientific Meetings/Publications Related to Rootstock Breeding

Talks at Grower Meetings (Extension/Outreach)

Breeding grape rootstocks for drought and salinity resistance. San Joaquin Valley Viticultural Tech Group, Fresno, CA, June 11, 2014.

Walker rootstock breeding program. Presented to the American Vineyard Foundation Oversight Committee, UC Davis, June 17, 2014

Rootstock breeding progress report. Presented to the Calif. Grape Rootstock Improvement Commission, June 18, 2014

Can we breed better drought and salt resistance into grape rootstocks? Presented to the International Cabernet Sauvignon Symposium, UC Davis, July 19, 2014

Grape breeding in the Walker lab. VEN 290 Seminar, UC Davis, Oct. 3, 2014

Grape breeding at UC Davis. E&J Gallo Lab Techs, UC Davis, Dec. 5, 2014

Grape breeding at UC Davis. UC Chile LINC, UC Davis, Jan. 15, 2015

New rootstocks, old problems. A. Walker. Robert's Integrated Viticulture Annual Meeting, Santa Rosa, CA Jan. 23, 2015

Presentations/Abstracts at Scientific Meetings

Walker, M.A. 2014. Drought and salinity tolerance in grape rootstocks. Water Use Symposium. 65th ASEV National Meeting, Austin, TX June 25, 2014.

Fort, K., J. Fraga, D. Grossi, and M. A. Walker. 2014. Rapid recovery from drought stress in deeply-rooted *Vitis* rootstocks. 65th ASEV National Meeting, Austin, TX June 25, 2014 Xiaoqing, X.,[2], C. B. Aguero[2] and M A. Walker. 2014. In vitro induction of tetraploids in *Muscadinia* hybrid rootstocks. 65th ASEV National Meeting, Austin, TX June 25, 2014

Osorio, C. and M. A. Walker. 2014. Differences in the drought tolerance characteristics of seven grape rootstocks. 65th ASEV National Meeting, Austin, TX June 25, 2014.

Goldman, E. and M. A. Walker. 2014. Potential to eradicate *Xiphinema index* using the bioantagonistic rootstock 'O39-16'. 65th ASEV National Meeting, Austin, TX June 25, 2014.

Dodson, J.C. and M. A. Walker. 2014. Grape rootstock-scion interactions and their influence on ripening periods and the initiation of senescence. 65th ASEV National Meeting, Austin, TX, June 25, 2014.

Heinitz, C. and M. A. Walker. 2014. Population dynamics, gene flow and sources of chloride exclusion in wild *Vitis* from the southwest United States. 65th ASEV National Meeting, Austin, TX June 25, 2014.

Fraga, J., Fort, K. and Walker, M.A. 2014. Using a rhizotron to evaluate deep and shallow rooting in grape rootstocks. 65th ASEV National Meeting, Austin, TX June 25, 2014

Arancibia, C., L. Martínez, R. Alonso, F. Buscema, S. Riaz, C.B. Agüero, K. Lund, and M.A. Walker. 2014. Molecular analysis of phylloxera present in Argentinean vineyards. 65th ASEV National Meeting, Austin, TX June 25, 2014

Walker, A. Development of drought and salt resistant rootstocks Presented at the International Grape Genetics and Breeding Conference, Beijing, China July 31, 2014

Walker, A. Disease resistance in perennial crops: classical and molecular approaches using grape as an example. 2nd International Plant Breeding Symposium, Universidada Estadual do Norte Fluminense Darcy Ribeiro, Campos do Goytacazes, Oct. 4, 2014

Publications

Schuck, M.R., L.L. Biasi, F.M. Moreira, A.L. de Silva, S. Riaz and M.A. Walker. 2014. Use of microsatellite markers to assess the identity and genetic diversity of *Vitis labrusca* and *Vitis rotundifolia* cultivars. Acta Scientiarum Agronomy 36:301-308.

Hwang, C-F., K. Xu, R. Hu, S. Riaz and M.A. Walker. 2014. Cloning and characterization of the dagger nematode resistance gene *XiR1*. Acta Horticulturae 1046:391-394.

Van Zyl, S., M.A. Vivier, S. Riaz and M.A. Walker. 2014. The genetic mapping of *Xiphinema index* resistance derived from *Vitis arizonica.* Acta Horticulturae 1046:165-168.

Walker, M.A., K. K. Lund, C. Agüero, S. Riaz, K. Fort, C. Heinitz and N. Romero. 2014. Breeding grape rootstocks for resistance to phylloxera and nematodes – it's not always easy. Acta Horticulturae 1045:89-97

Fort, K.P., C.C. Heinitz and M.A. Walker. 2014. Chloride exclusion patterns in six grapevine populations. Australian Journal of Grape and Wine Research. In Press

Bao, L.V., I.B. Scatoni, C. Gaggero, L.Guitierrez, J. Monza and M.A. Walker. 2015. Genetic diversity of grape phylloxera leaf galling populations on *Vitis* species in Uruguay. American Journal of Enology and Viticulture 66: In press.

Walker, M.A. 2014. The *Vitis* species and rootstocks. IN: Compendium of Grape Diseases, 2nd Edition, Ed. W.F. Wilcox et al. American Phytopathology Press. In Press

Table 1. 2014 rootstock crosses with number of clusters pollinated, seeds produced and the purpose of the cross.

Cross #	Female	Male	Purpose	Seeds
2014-05	161-49C	R8916-22	PD Resistance, low vigor, *X. index*	1476
2014-81	OKC-1 SO1 *acerifolia*	GRN-4 9365-85	Double Cl exclusion and nema resistance	0
2014-84	OKC-1 SO3 *acerifolia*	GRN-2 9363-16	Cl exclusion and nema resistance	1096
2014-85	OKC-1 SO3 *acerifolia*	GRN-4 9365-85	Cl exclusion and nema resistance	1325
2014-86	OKC-1 SO3 *acerifolia*	*rupestris* Pump Station	Cl exclusion mapping population	573
2014-87	OKC-1 SO3 *acerifolia*	b40-14 *arizonica*	Cl exclusion and *X. index* resistance	23
2014-88	SC2 girdiana	GRN-2 9363-16	Double Cl exclusion and nema resistance	99
2014-89	SC2 *girdiana*	GRN-4 9365-85	Double Cl exclusion and nema resistance	21
2014-90	SC2 *girdiana*	*rupestris* Pump Station	Double Cl exclusion mapping population	37
2014-91	SC2 *girdiana*	b40-14 *arizonica*	Double Cl exclusion and *X. index* resistance and no Cl exclusion mapping	247
2014-94	GRN-3 9365-43	140Ru	Cl exclusion and *X. index* resistance	248
2014-99	GRN-3 9365-43	2011-188-06 (T6-42 x St. Geo)	GRN and VR resistance, deep roots	0
2014-102	GRN-3 9365-43	NV12-051 *girdiana*	Double Cl exclusion and nema resistance	0
2014-105	GRN-3 9365-43	UT12-099 *girdiana/riparia*	Double Cl exclusion and nema resistance	252
2014-106	GRN-3 9365-43	UT12-100 *girdiana/riparia*	Cl exclusion and nema resistance	41
2014-108	GRN-3 9365-43	ANU21	Cl exclusion and nema resistance	36
2014-109	GRN-3 9365-43	SC11 *girdiana*	Double Cl exclusion and nema resistance	130
2014-110	GRN-3 9365-43	ANU77 *girdiana*	Double Cl exclusion and nema resistance	0
2014-112	GRN-3 9365-43	*berlandieri* 9031	Cl exclusion, drought and nema resistance	140
2014-114	GRN-3 9365-43	UT12-092 *girdiana/riparia*	Double Cl exclusion and nema resistance	45
2014-117	Dog Ridge	140Ru	Cl exclusion, deep roots, nema resistance	118
2014-118	Dog Ridge	TX12-003 *candicans*	Double Cl exclusion, deep roots, nema resistance	20
2014-119	Dog Ridge	NM11-068 *arizonica/riparia*	Double Cl exclusion, deep roots, nema resistance	173
2014-120	Dog Ridge	NV11-116 *girdiana*	Cl exclusion, deep roots, nema resistance	186
2014-121	Dog Ridge	T9 *doaniana*	Cl exclusion, deep roots, nema resistance	57
2014-139	Dog Ridge	NV12-049 *girdiana*	Double Cl exclusion, deep roots, nema resistance	13
2014-124	Dog Ridge	NV12-051 *girdiana*	Double Cl exclusion, deep roots, nema resistance	14
2014-125	Dog Ridge	UT12-078 *girdiana/riparia*	Double Cl exclusion, deep roots, nema resistance	174
2014-128	Dog Ridge	2011-188-06 (T6-42 x St. Geo)	Nematodes, deep roots, VR resistance	0
2014-130	Dog Ridge	ANU21 *girdiana/riparia*	Double Cl exclusion, deep roots, nema resistance	160
2014-131	Dog Ridge	SC11 *girdiana*	Double Cl exclusion, deep roots, nema resistance	0
2014-135	Dog Ridge	ANU77 *girdiana*	Double Cl exclusion, deep roots, nema resistance	67
2014-136	Dog Ridge	2011-175-15 (083314-31 x Schwarzmann)	PD resistance, nema and deep rooting	21
2014-137	Dog Ridge	*berlandieri* 9031	Cl exclusion, deep roots, nema resistance	0
2014-138	Dog Ridge	*acerifolia* 9018	Cl exclusion, deep roots, nema resistance	58
2014-15	Ramsey	Riparia Gloire	Expand mapping population	959
2014-143	Ramsey	TX12-003 *candicans*	Double Cl exclusion, deep roots, nema resistance	38
2014-144	Ramsey	NM11-068 *arizonica/riparia*	Double Cl exclusion, deep roots, nema resistance	177

2014-145	Ramsey	NV11-116 *girdiana*	Double Cl exclusion, deep roots, nema resistance	91
2014-146	Ramsey	T9 *doaniana*	Cl exclusion, deep roots, nema resistance	11
2014-147	Ramsey	T12 *doaniana*	Cl exclusion, deep roots, nema resistance	0
2014-150	Ramsey	UT12-078 *girdiana/riparia*	Double Cl exclusion, deep roots, nema resistance	27
2014-152	Ramsey	UT12-099 *girdiana/riparia*	Double Cl exclusion, deep roots, nema resistance	9
2014-153	Ramsey	UT12-100 *girdiana/riparia*	Double Cl exclusion, deep roots, nema resistance	94
2014-157	Ramsey	ANU77 *girdiana*	Double Cl exclusion, deep roots, nema resistance	0
2014-158	Ramsey	2011-175-15 (083314-31 x Schwarzmann)	PD resistance, nema and rooting	109
2014-159	Ramsey	*berlandieri* 9031	Cl exclusion, deep roots, nema resistance	366
2014-160	Ramsey	*acerifolia* 9018	Cl exclusion, deep roots, nema resistance	464
2014-189	NM12-114	*rupestris* Pump Station	Cl exclusion mapping population	29
2014-179	2011-188-16 (T6-42 x St. Geo.)	b40-14 *arizonica*	VR and X. index resistance, no Cl exclusion Mapping	0
2014-183	2011-188-16 (T6-42 x St. Geo)	GRN-2 9363-16	VR resistance and GRN, no Cl exclusion Mapping	0
2014-184	2011-188-16 (T6-42 x St. Geo)	GRN-4 9365-85	VR resistance and GRN	4
2014-185	2011-175-07 (08314-31 x Schwarzmann)	GRN-4 9365-85	PD, nematodes	0
2014-188	2011-175-06 (08314-31 x Schwarzmann)	GRN-2 9363-16	PD, nematodes	0
2014-199	101-14 Mgt	*berlandieri* 9031	Cl exclusion and rooting depth mapping and rootstock potential	73
2014-132	*riparia* 1411	110R	Mapping population	329
2014-133	*riparia* 1411	140Ru	Mapping population	650
2014-134	*riparia* 1411	*berlandieri* 9031	Mapping population / rootstock potential	0
2014-164	T6-38 (VR)	110R	VR resistance 110R roots	68
2014-182	T6-38 (VR)	GRN-2 9363-16	VR resistance / nema resistance	17
2014-187	T6-38 (VR)	GRN-4 9365-85	VR resistance / nema resistance	45

Table 2. Ring nematode resistant selections from a tested group of 85 genotypes (first selected for their horticultural traits). Inoculated with 1,500 nematodes and evaluated after 3 months of growth in 4inch pots.

Selection	Parentage	Ring Nematodes/ pot	Ring Nematodes/g root
O39-16	*vinifera* x *rotundifolia*	10	3.9
2011-175-12	08314-31 X Schwarzmann	133	57.8
2010-115-22	161-49C x *rotundifolia* Trayshed	150	59.4
0708-21	5BB x R8916-22	120	59.4
2012-113-13	101-14 Mgt x GRN-4 9365-85	143	62.3
2011-175-1	08314-31 X Schwarzmann	165	70.7
2011-174-3	08314-31 X 420A	168	84.2
2012-185-5	GRN-3 9365-43 x *berlandieri* 9031	150	95.0
2011-133-44	OKC-1 SO3 *acerifolia* X St. George	170	103.2
2011-188-6	T6-42 X St. George	153	223.3
Harmony	1613C sdlg x Dog Ridge sdlg	2,700	1,262.7
Colombard	*vinifera*	2,870	1,296.4
St. George	*rupestris*	2,128	1,309.4

Table 3. Resistance to root-knot nematode HarmA&C in UW block sections designed to improve the rooting and cane production of the GRN rootstocks. This block was first selected for horticultural appearance and rooting.

Selection	Parentage	Mean Gall/g Root
Harmony	1613C sdlg x Dog Ridge sdlg	95.5 a
Colombard	*vinifera*	84.8 a
2012-110-43	101-14 Mgt x GRN-5 9407-14	21.3 b
2012-113-4	101-14 Mgt x GRN-4 9365-85	11.3 bc
2012-112-15	101-14 Mgt x GRN-2 9363-16	9.9 bc
2012-112-39	101-14 Mgt x GRN-2 9363-16	7.3 bc
2012-110-5	101-14 Mgt x GRN-5 9407-14	6.1 bc
2012-112-26	101-14 Mgt x GRN-2 9363-16	5.9 c
2012-113-7	101-14 Mgt x GRN-4 9365-85	4.3 c
2012-112-1	101-14 Mgt x GRN-2 9363-16	2.1 c
2012-110-37	101-14 Mgt x GRN-5 9407-14	2.1 c
2012-112-22	101-14 Mgt x GRN-2 9363-16	0.5 c
2012-110-41	101-14 Mgt x GRN-5 9407-14	0.4 c
2012-110-6	101-14 Mgt x GRN-5 9407-14	0.2 c
2012-110-23	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-110-7	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-110-45	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-110-48	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-110-2	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-110-33	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-112-9	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-11	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-12	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-3	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-16	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-18	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-110-28	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-112-18	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-110-35	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-112-28	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-36	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-110-13	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-112-36	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-44	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-45	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-48	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-112-50	101-14 Mgt x GRN-2 9363-16	0.0 c
2012-113-2	101-14 Mgt x GRN-4 9365-85	0.0 c
2012-110-8	101-14 Mgt x GRN-5 9407-14	0.0 c
2012-113-5	101-14 Mgt x GRN-4 9365-85	0.0 c
2012-110-14	101-14 Mgt x GRN-5 9407-14	0.0 c

Table 4. Resistance to root-knot nematode HarmA&C in D&E blocks designed to incorporate salt tolerance, deeper rooting, better rooting, and broader nematode resistance. This block was first selected for horticultural appearance and rooting.

Selection	Parentage	Mean Egg/G Root
St. George	rupestris	89.1 a
2011-137-5	161-49C X T9 doaniana	79.0 b
Colombard	vinifera	76.8 b
Harmony	1613C sdlg x Dog Ridge sdlg	68.7 c
08-143	Cosmos 2 x b57-39	64.9 c
2011-133-17	OKC-1 SO3 acerifolia X St. George	30.2 d
2011-156-1	OKC-1 SO3 acerifolia X 1616C	24.1 d
2011-133-32	OKC-1 SO3 acerifolia X St. George	13.8 e
2011-188-9	T6-42 X St. George	13.7 e
2011-148-1	Ramsey X NM 03-17 S01 treleasei	11.6 ef
2011-137-3	161-49C X T9 doaniana	9.7 efg
2011-137-17	161-49C X T9 doaniana	9.6 efg
2011-148-40	Ramsey X NM 03-17 S01 treleasei	6.8 efg
2011-148-32	Ramsey X NM 03-17 S01 treleasei	6.7 efg
06-104-06	101-14 Mgt x 9363-16	6.3 efg
2011-148-9	Ramsey X NM 03-17 S01 treleasei	6.1 efg
2011-133-38	OKC-1 SO3 acerifolia X St. George	5.7 efg
2011-148-36	Ramsey X NM 03-17 S01 treleasei	4.0 fg
08-143	Cosmos 2 x b57-39	2.3 fg
2011-137-9	161-49C X T9 doaniana	1.7 g
2011-133-50	OKC-1 SO3 acerifolia X St. George	1.5 g
2011-175-17	08314-31 X Schwarzmann	1.5 g
0707	5BB x b40-14	1.5 g
08-171	9365-43 x 8916-22	1.4 g
2011-143-17	Ramsey X 08314-15	1.4 g
2011-148-46	Ramsey X NM 03-17 S01 treleasei	1.0 g
2011-148-41	Ramsey X NM 03-17 S01 treleasei	0.7 g
2011-176-18	08314-31 X St. George	0.6 g
08-171	9365-43 x 8916-22	0.5 g
2010-115-18	161-49C x Trayshed	0.5 g
2011-137-1	161-49C X T9 doaniana	0.4 g
2011-143-10	Ramsey X 08314-15	0.3 g
07-170	9365-43 x 8916-20	0.3 g
2011-137-16	161-49C X T9 doaniana	0.2 g
06-105	101-14 Mgt x 9407-14	0.1 g
07-170	9365-43 x 8916-20	0.0 g
2011-144-19	Ramsey X 08314-46	0.0 g
07-07-10	5BB x b40-14	0.0 g
06-118	5BB x 9365-43	0.0 g
06-109	101-14 Mgt x 9365-85	0.0 g
2011-148-42	Ramsey X NM 03-17 S01 treleasei	0.0 g
06-104	101-14 Mgt x 9363-16	0.0 g
06-104	101-14 Mgt x 9363-16	0.0 g

2011-156-14	OKC-1 SO3 acerifolia X 1616C	0.0 g
07-07	5BB x b40-14	0.0 g
2011-174-1	08314-31 X 420A	0.0 g
2011-175-7	08314-31 X Schwarzmann	0.0 g
2011-175-11	08314-31 X Schwarzmann	0.0 g
06-109	101-14 Mgt x 9365-85	0.0 g
08-171	9365-43 x 8916-22	0.0 g
2010-115-33	161-49C x Trayshed	0.0 g
2011-156-18	OKC-1 SO3 acerifolia X 1616C	0.0 g
2011-148-33	Ramsey X NM 03-17 S01 treleasei	0.0 g

Table 5. Number of selections surviving severe (150mM NaCl for 2 weeks). Now destined for refined salt testing, propagation, root-knot nematode testing. All rooted and grew without salt damage symptoms; shoot growth was reduced by about 50%.

Selection	Parentage	#Salt tolerant
12-102-03	101-14 x NM03-17 (*treleasei*)	13
12-108-28	101-14 x 9028 (*doaniana*)	3
12-125-03	OKC-1 SO1 (*acerifolia*) x GRN-2 9363-16	4
12-126-02	OKC-1 SO1 (*acerifolia*) x GRN-4 9365-85	1
12-126-08	OKC-1 SO1 (*acerifolia*) x GRN-4 9365-85	2
12-129-22	OKC-1 SO1 (*acerifolia*) x St. George	4
12-142-04	*girdiana*-11 x *arizonica* A56	7
12-143-09	*girdiana*-22 x *arizonica* A56	2
12-144-01	*girdiana* Scotty's Castle x *arizonica* A56	9
12-149-21	Ramsey x ANU77 (*girdiana*)	3
12-153-18	Ramsey x 9028 (*doaniana*)	1
12-154-13	Ramsey x St. George	1
12-154-28	Ramsey x St. George	4
12-158-17	161-49C x St. George	1
12-185-03	GRN-3 9365-43 x *berlandieri* 9031	2
12-189-17	Dog Ridge x 140 RU	1
12-190-14	Dog Ridge x St. George	3
Vru 2	*rupestris* from Missouri	
Vru 85	*rupestris* from Missouri	

Table 6. Number of transgenic lines produced; lines in greenhouse are shown in parentheses

	T. Seedless	St George
XiR1.1	29 (10)	16 (10)
XiR1.2	12 (10)	13 (10)

Table 7. Percentage of survival rate and tetraploids obtained from treated shoot tips, anthers and pre-embryogenic calli of 101-14 Mgt × *M. rotundifolia* cv. Trayshed #75 as affected by treatments with different times and concentrations of colchicine and oryzalin. Shoot tips were obtained from hybrid plants grown in vitro, anthers from hybrid plants in the field and pre-embryogenic calli developed from in vitro cultured anthers.

Treatment		Shoot tip		Anther		Pre-embryogenic calli	
Antimitotic agent	Duration (hours)	Survival rate (%)	Tetraploids (%)	Survival rate (%)	Tetraploids (%)	Survival rate (%)	Tetraploids (%)
Control	-	100	-	20±1.1	-	86.67±0.7	-
Colchicine 0.01%	24	87.69±3	9.23±1.24	17.33±4.81	2.67±1.33	73.33±3.85	15.55±2.22
	48	82.95±5.15	12.01±2.6	9.33±4.81	2.67±1.33	62.22±8.01	24.44±5.88
	72	75.22±5.95	18.51±2.38	5.33±3.53	2.67±2.67	48.89±8.01	22.22±5.88
Colchicine 0.025%	24	81.79±4.31	30.34±2.34	11.11±4.84	3.33±1.93	57.78±5.88	28.89±4.45
	48	62.99±10.17	31.41±2.64	2.67±2.67	1.33±1.33	48.89±5.88	**37.78±2.22**
	72	50±10	**35.08±1.04**	2.67±2.67	2.67±2.67	40±3.85	28.89±2.22
Colchicine 0.05%	24	52.01±5.72	28.12±0.21	3.33±1.93	2.22±1.11	51.11±2.22	11.11±2.22
	48	40.3±8.35	27.65±2.1	2.22±1.11	2.22±1.11	42.22±5.88	24.44±4.45
	72	29.32±7.13	17.26±1.13	6.67±1.93	4.44±1.11	22.22±2.22	13.34±6.67
Oryzaline 5µM	24	97.78±1.47	2.69±0.52	15.55±2.22	2.22±2.22	73.33±3.85	11.11±2.22
	48	95.08±2.57	3.8±0.6	13.33±3.85	4.44±4.45	55.55±11.76	15.56±4.45
	72	86.71±4.37	5.43±0.63	4.45±2.22	2.22±2.22	55.56±5.88	17.78±4.45
Oryzaline 15µM	24	92.86±3.93	6.54±1.07	8.89±8.89	4.44±4.45	60±3.85	24.45±2.22
	48	79.01±2.32	11.28±1.61	6.67±3.85	4.44±4.45	28.89±5.88	13.33±3.85
	72	67.31±6.19	11.71±3.51	4.45±2.22	2.22±2.22	28.89±9.69	17.78±5.88
Oryzaline 30µM	24	60.77±7.96	22.09±1.92	8.89±2.22	6.67±3.85	37.78±8.01	17.78±2.22
	48	51.45±3.45	26.11±3.09	6.67±6.67	6.67±6.67	24.44±5.88	13.33±3.85
	72	38.08±7.66	14.1±2.12	2.22±2.22	2.22±2.22	20±7.7	17.78±5.88

Table 8. Categorized list of 42 genotypes tested in the 2014 salt tolerance screen.

Rootstocks	New accessions	*V. rotundifolia*	*V. vinifera* controls	Research genotypes
039-16	TO3-15 (*V. rupestris*)	NC184-4	Cabernet Sauvignon	A. de Serres
101-14	NMO3-17 (*V. treleasei*)	Supreme	Colombard	RR4
1103P	longii 9018 (*V. acerifolia*)	Southland		RR19
110R	longii 9035 (*V. acerifolia*)			RR23
140Ru	OKC-1 (*V. acerifolia*)			RR29
161-49C	GC-5 (*V. arizonica*)			*V. berlandieri*-9031
1616C	SC-1 (*V. girdiana*)			
420A				
44-53				
5BB				
5C				
99R				
Dog Ridge				
Freedom				
Harmony				

GRN-1
GRN-2
GRN-4
GRN-5
Ramsey
Riparia Gloire
Schwarzmann
SO4
St. George

Table 9. Categorized list of genotypes established for the containerized study using a common scion and a full season analysis of chloride exclusion and rooting traits.

Grafted rootstocks	Ungrafted rootstocks	New accessions	*V. vinifera* controls	Research genotypes
			Cabernet	
101-14	101-14	TO3-15 (*V. rupestris*)	Sauvignon	RR19
1103P	1103P	NMO3-17 (*V. treleasei*)	Colombard	RR29
110R	110R	longii 9035 (*V. acerifolia*)		RRF2♀
140Ru	140Ru	longii 9018 (*V. acerifolia*)		RRF2♂
1616C	1616C	OKC-1 (*V. acerifolia*)		
Ramsey	Ramsey	SC12 (*V. girdiana*)		
Riparia Gloire	Riparia Gloire			
Dog Ridge				
Schwarzmann				
St. George				

Table 10. Categorized list of genotypes established for the field study using a common scion and a full season of growth for the analysis of rooting traits and response to drought.

Rootstocks	*V. vinifera* controls
101-14	Cabernet Sauvignon
1103P	Colombard
110R	
140Ru	
1616C	
Dog Ridge	
Ramsey	
Riparia Gloire	
Schwarzmann	
St. George	

Table 11. 2013 / 2014 data from the GRN Dunnigan/Franzia GRN rootstock trial.

Rootstock	Cls #	Yield kg	Pruning Weights kg
RS3	88 a	10.64 a	0.63 a
44-53	93 a	11.13 abc	0.70 a
420A	125 ab	14.17 abcd	0.72 ab
RS9	134 ab	16.08 abcd	0.79 ab
5C	120 ab	15.69 abcd	0.82 ab
1616C	132 ab	15.88 abcd	0.84 ab

3309C	114 ab	14.68 abcd	0.86 abc
101-14	148 b	16.80 d	0.89 abc
1103P	117 ab	14.21 abcd	0.92 abc
GRN3	145 b	17.46 d	1.04 bcd
Freedom	114 ab	10.81 ab	1.19 cde
St.Geo	124 ab	14.87 abcd	1.28 de
GRN2	144 b	16.15 bcd	1.31 de
GRN4	125 ab	14.78 abcd	1.40 e

Table 12. 2014 yield and cluster numbers for rootstocks in the Gallo/Lodi trial grafted to Malbec and second year of crop.

Rootstock	Yield (kg), 29Sep14	Cluster number, 29Sep14
RS 9	11.2 A	88.2
RS 3	14.5 AB	99.5
GRN 5	14.7 AB	95.5
GRN 4	14.7 AB	87.7
St. Geo	15.5 AB	98.3
GRN 1	16.0 B	86.5
101-14	16.1 B	99.3
O39-16	16.3 B	100.5
Harmony	16.4 B	95.9
GRN 2	16.4 B	102.1
1103P	16.6 B	96.9
3309C	17.2 B	97.9
GRN 3	18.0 B	104.5

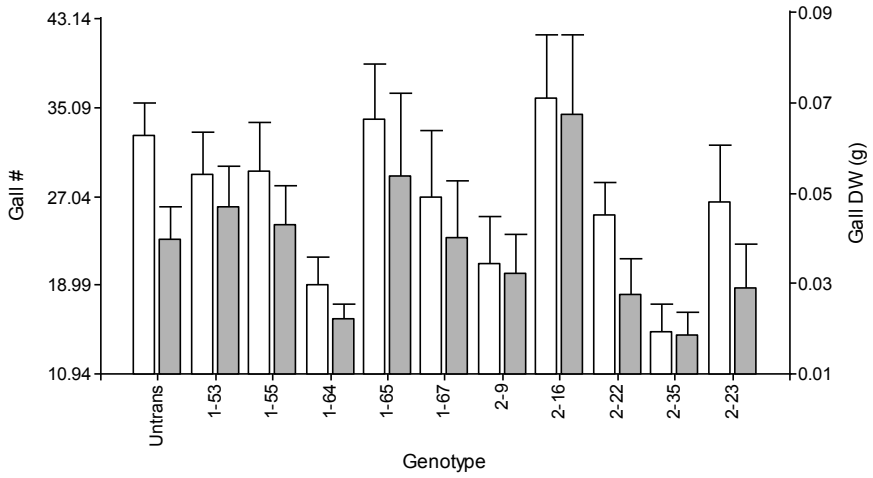

Figure 1. Gall number (white) and gall dry weight (DW) (gray) in 10 transgenic lines of St. George transformed with *XiR1.1* (1-) or *XiR1.2* (2-) after 10 weeks of inoculation. Error bars represent SE.

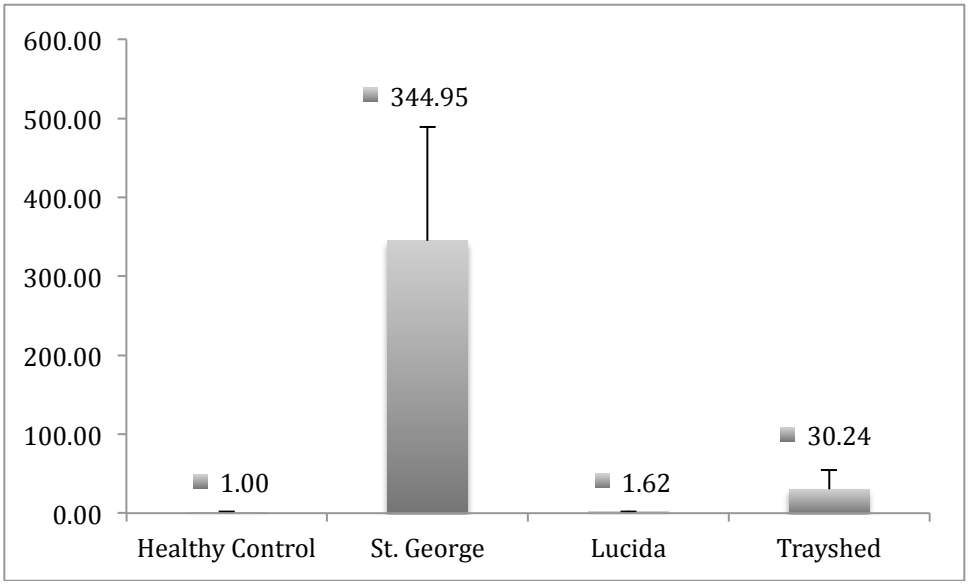

Figure 2. Fold change in GFLV concentration found in St. George, Lucida and Trayshed grafted on infected Chardonnay when compared with Healthy Control. Means represent an average of 3-6 plants. 18S rRNA was used as internal reference gene

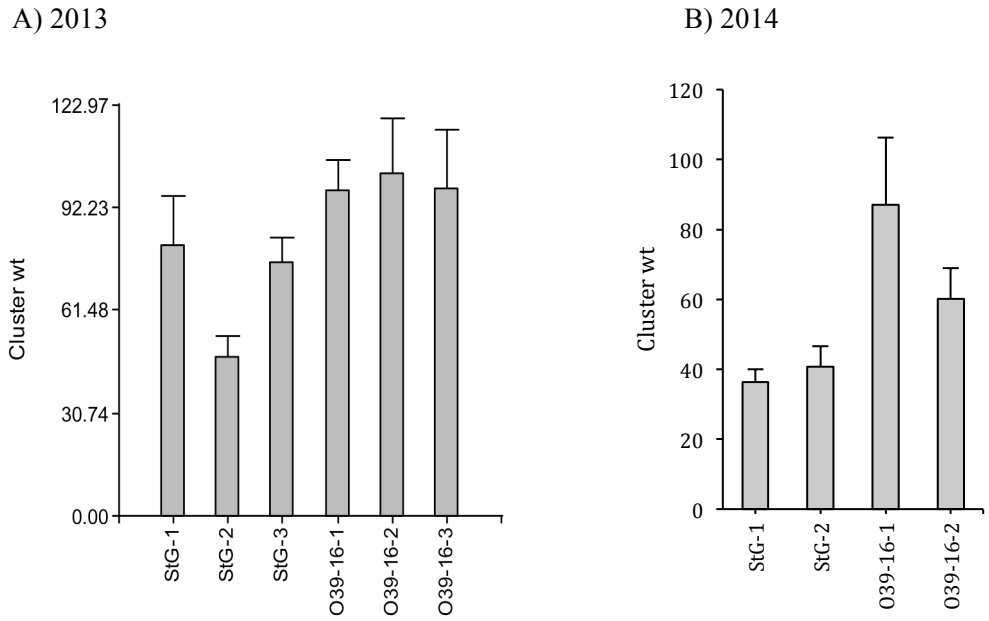

Figure 3. Weight of clusters of GFLV infected Cabernet Sauvignon grafted on St George or O39-16. A) 2013- Clusters were sprayed with water: 1; 10µM ZR at bloom: 2, and 10 µM ZR 14 days after bloom: 3 and B) 2014- water: 1 and 10 µM ZR at bud break: 2 in 2014. Error bars represent SE.

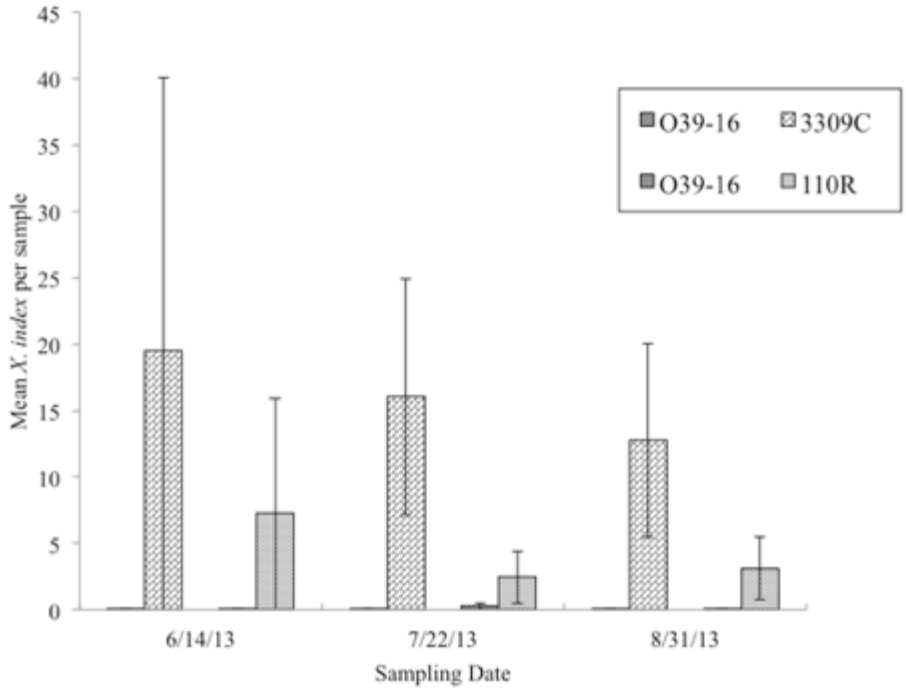

Figure 4. Mean number of *X. index* per sample. Error bars represent 95% confidence interval. Adjacent columns represent paired comparisons, hence 'O39-16' is present twice at each sampling date. *X. index* populations were significantly less on 'O39-16' than either rootstock on all sampling dates except '110R' on June 14 (Student's paired t test, one-tailed, $p < 0.05$).

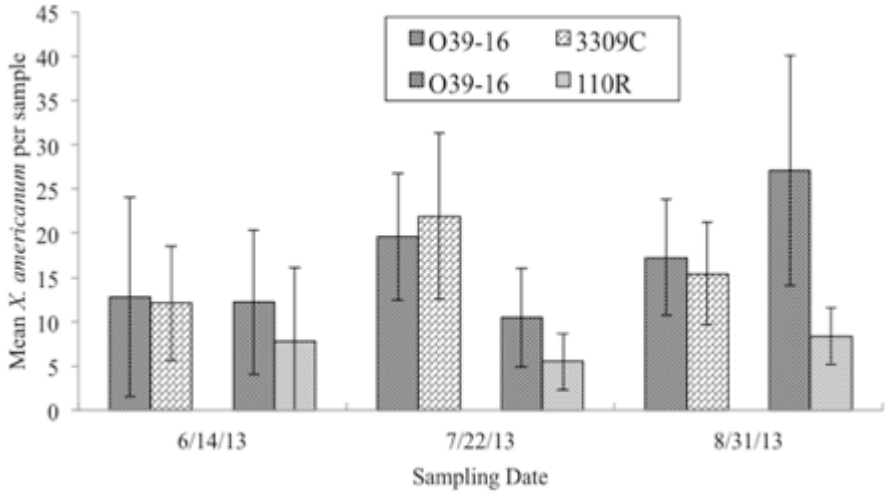

Figure 5. Mean number of *X. americanum* per sample. Error bars represent 95% confidence interval. Adjacent columns represent paired comparisons, hence 'O39-16' is present twice at each sampling date. No significant differences were seen among *X. americanum* populations except between 'O39-16' and '110R' on August 31 (Student's paired t test, two-tailed, $p < 0.05$).

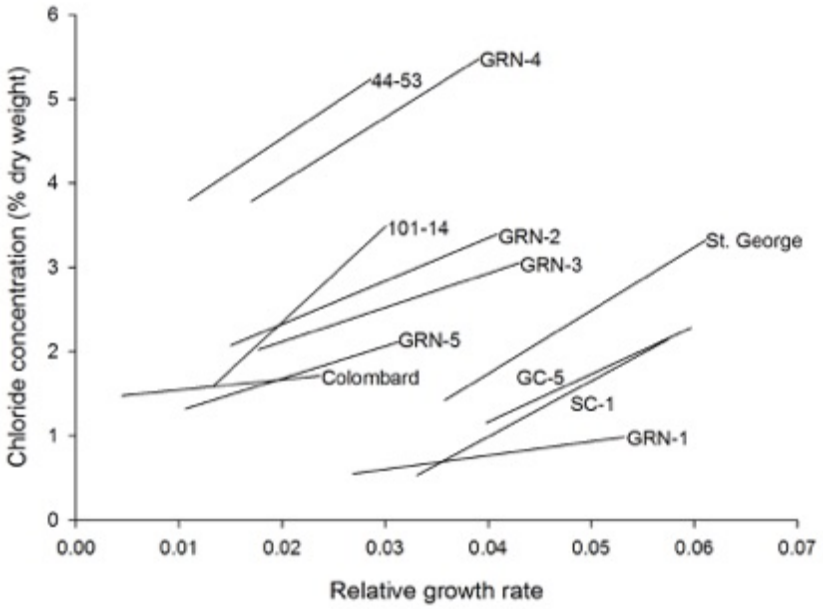

Figure 6. Chloride concentration in the pooled leaves of rootstocks GRN 1–5, experimental rootstocks SC-1 and GC-5, and four biocontrol genotypes. Lines are regressions of chloride concentration on the relative growth rate of each genotype subjected to three irrigation regimes: daily irrigation and two levels of less frequent irrigation. All containers used in this experiment were four inch.

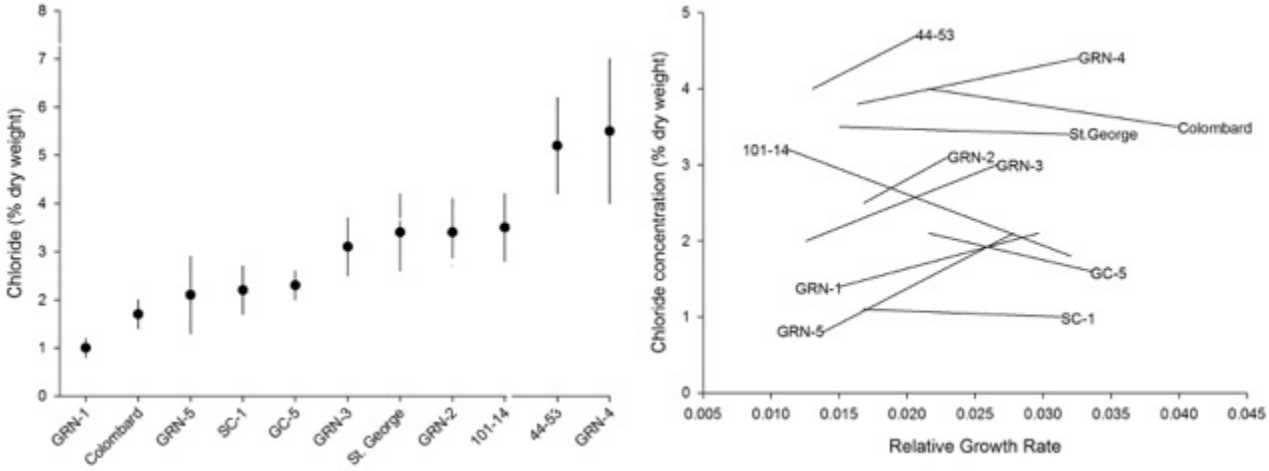

Figure 7. (*left*) Alternative presentation of chloride concentration in leaves from only the daily-irrigated treatment shown in Figure A. (*right*) Chloride concentration in the pooled leaves as presented in Figure A, but from plants grown in two-inch containers.

Figure 8. High throughput salt tolerance screen to assess the chloride exclusion capacity of 42 commercial and experimental rootstocks, plus biocontrols. Specific genotypes listed in Table 8.

Figure 9. Containerized common scion and own-rooted population of rootstocks and experimental genotypes for full season evaluation of chloride exclusion and a two-season study of rooting traits. Specific genotypes listed in Table 8.

Figure 10. Two-dimensional root systems of (*left*) Ramsey and (*right*) 110R obtained from a novel soilless assay for root architecture characterizations of complex root systems.

Figure 11. Three-dimensional media-based assay for improved rooting angle characterizations.

Figure 12. Common scion population of rootstocks for a full season field study of rooting traits and response to drought. Specific genotypes listed in Table 8.

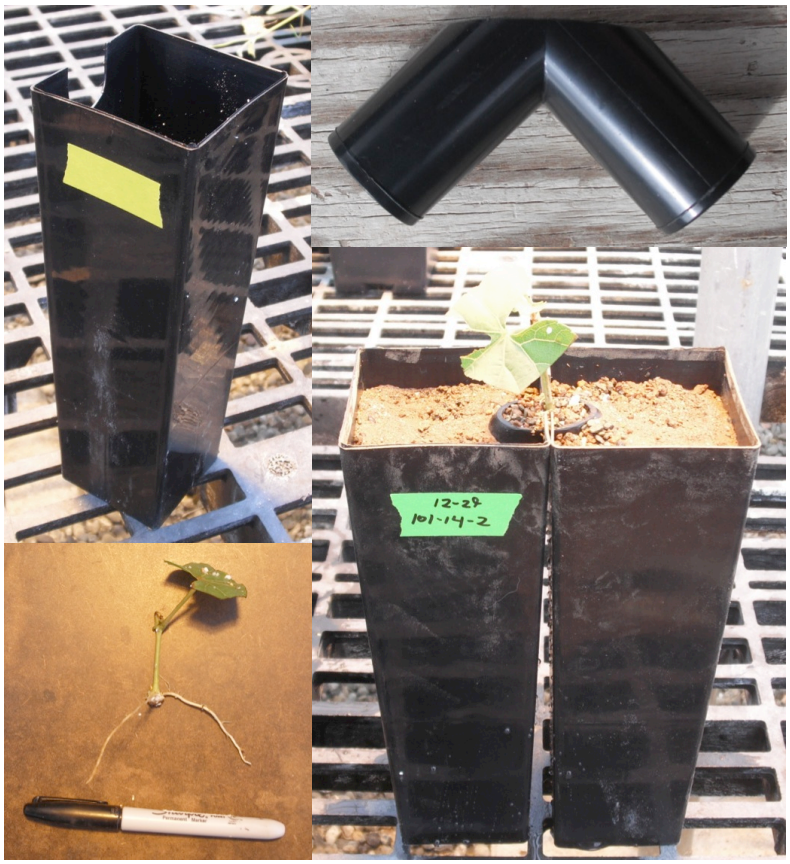

Figure 13. The split-pot planting system.

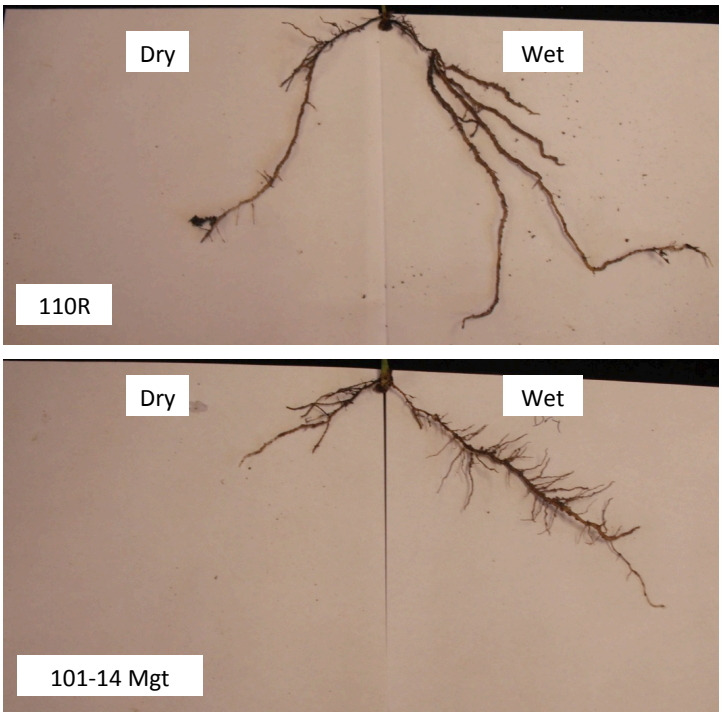

Figure 14. Root systems for 110R and 101-14 Mgt after removal from the split-pot planting system.

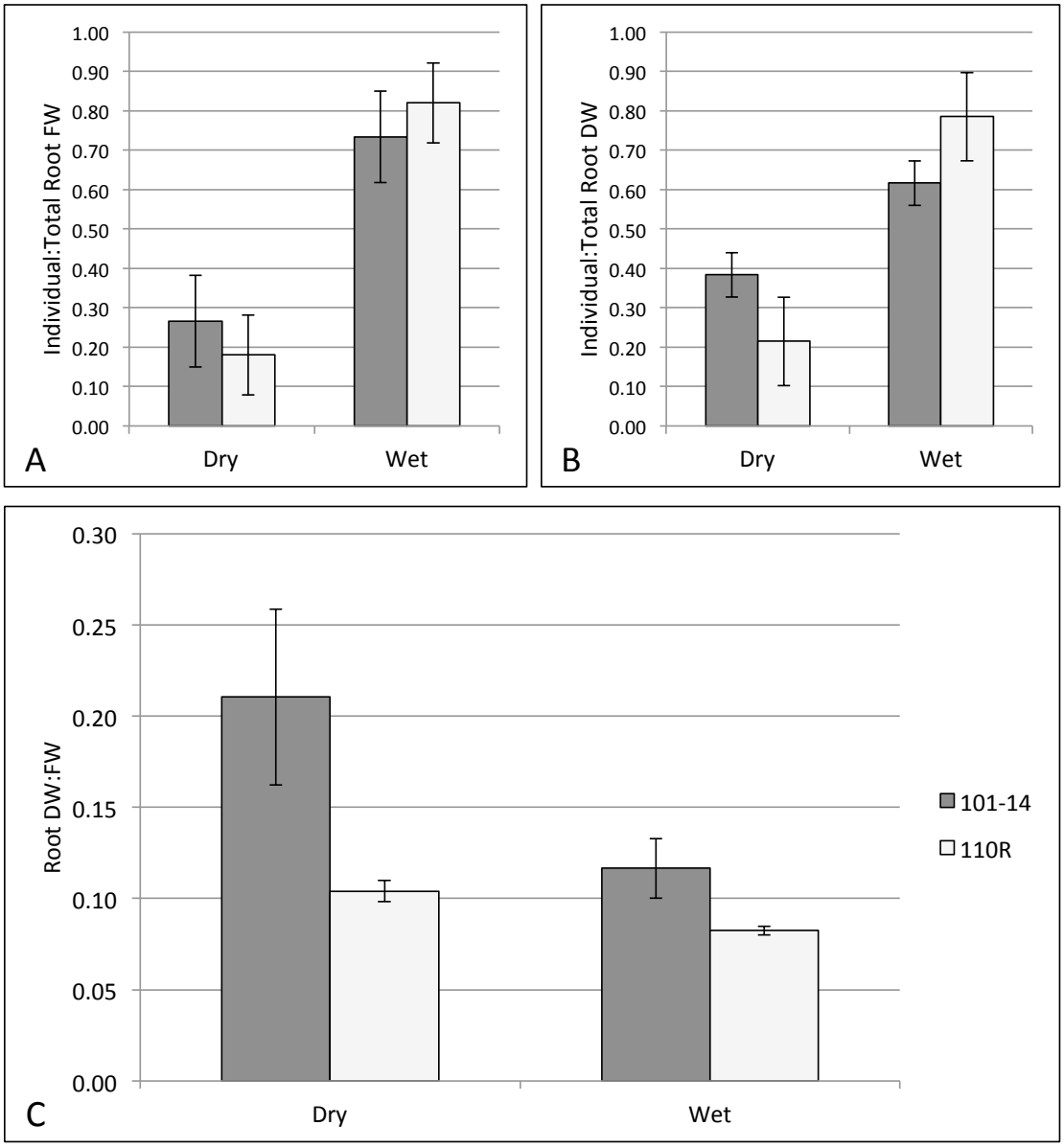

Figure 15. Root biomass in 101-14 Mgt and 110R plants grown in a split-pot system with wet and dry treatments. A) Ratio of individual root fresh weight (FW) to total root system fresh weight; B) Ratio of individual root dry weight (DW) to total root system dry weight; C) Individual root dry weight to fresh weight ratio (DW:FW). Error bars represent ±SD.

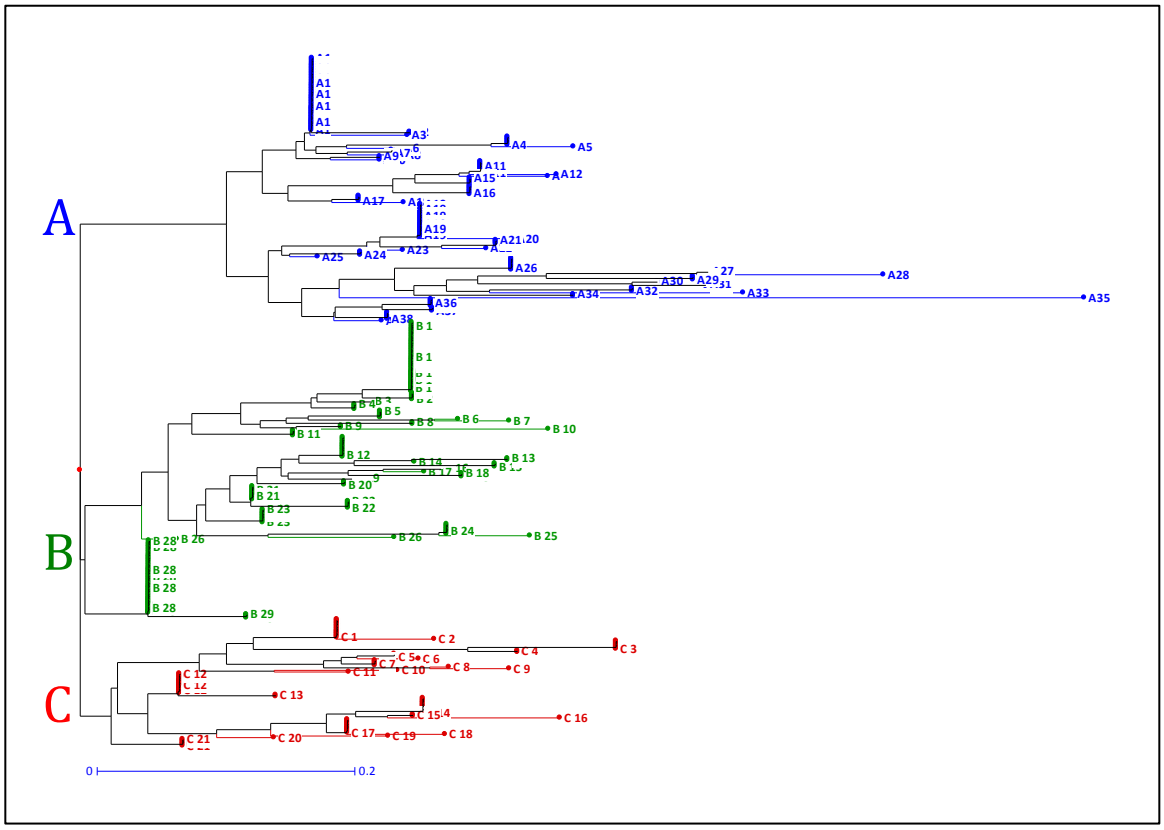

Figure 16. Neighbor-Joining Tree of chloroplast haplotypes from 420 accessions genotyped at 13 loci.

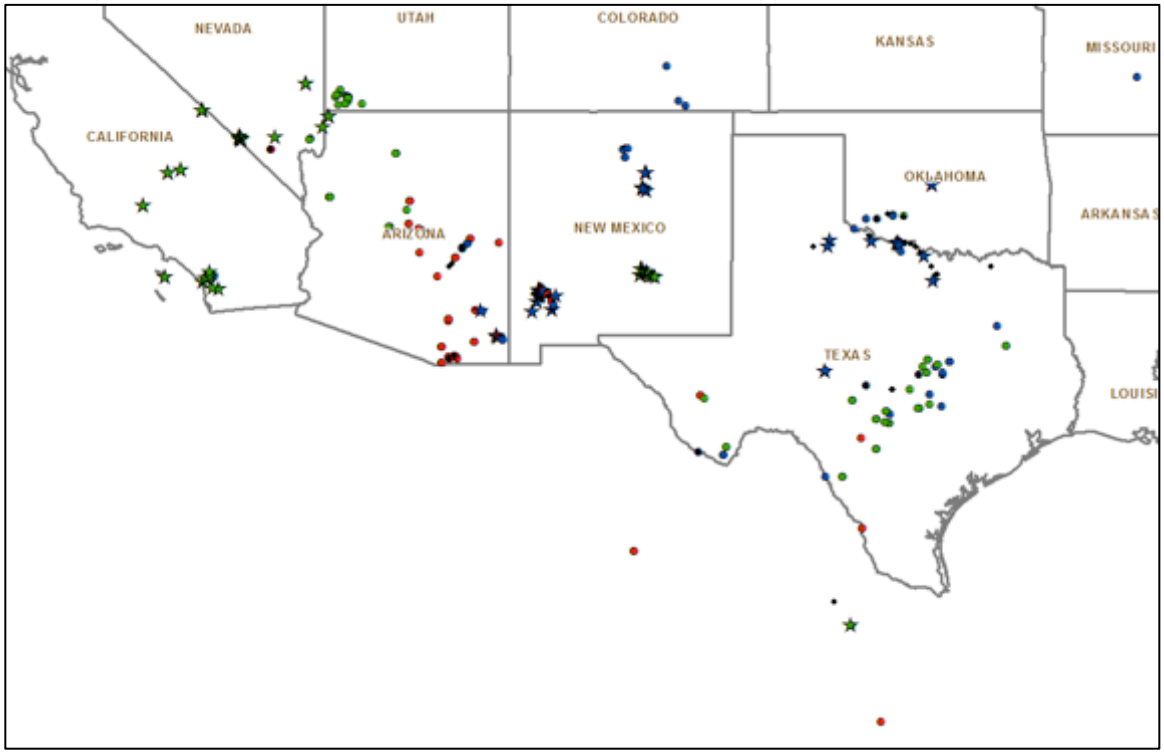

Figure 17. Map of accessions in this study, color-coded by haplotype group with "chloride-excluding haplotypes" highlighted. Blue – "A" haplotype group, Green – "B" haplotype group, Red – "C" haplotype group, Stars – haplotypes which contain predominantly strong chloride excluders [A1, A15, A19, B12, B28]

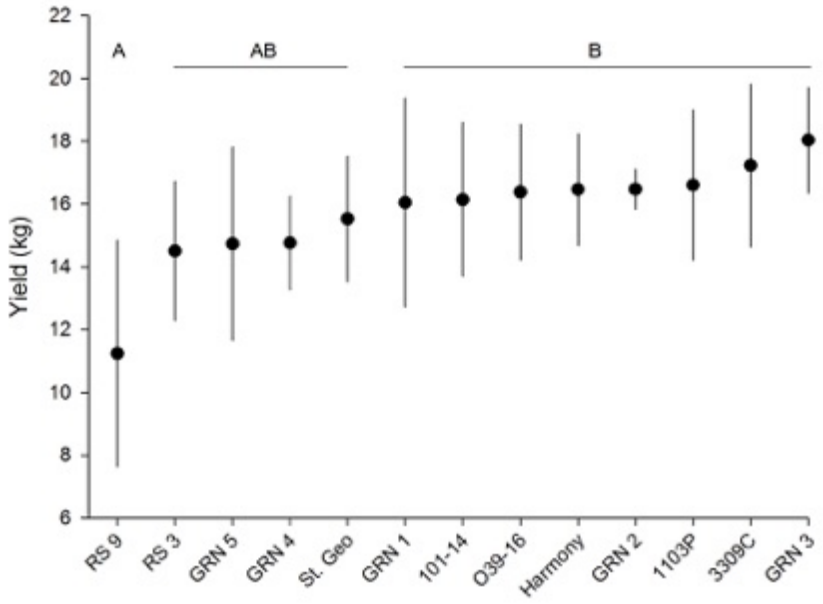

Figure 18. Fruit yields for the Gallo/Lodi trial grafted to Malbec and second year of crop.