California Grape Rootstock Improvement Commission
California Grape Rootstock Research Foundation
American Vineyard Foundation
CDFA Improvement Advisory Board
California Table Grape Commission
January 2014

Project Title: Development of next generation rootstocks for California vineyards.

Principal Investigator: Andrew Walker, Dept. Viticulture and Enology, UC Davis.
530-752-0902 awalker@ucdavis.edu

Progress Summary:
The **2013 crosses** focused on developing rootstocks with deeper root systems, the genetics of root architecture traits, and introgressing the excellent soil pest resistance from *rotundifolia* into rootstocks using semi-fertile *vinifera* x *rotundifolia* (VR) hybrids (see Table 1). This may also be a way to incorporate fanleaf tolerance and allow improvement of O39-16. VR hybrids are normally sterile but a few were selected by Olmo to have some fertility. Unfortunately they are also crosses with *vinifera* so we must be assured of their phylloxera resistance (studies underway).

GRN Field Trials – This was the first year data was gathered from GRN rootstock trials; most of which are being overseen by farm advisers and Constellation. We took crop yields at a trial in Dunnigan with Franzia and another in Lodi with Gallo. This data will be combined with pruning weights (not yet taken) and presented with the next report and as a bulletin to nurseries and cooperators.

Nematode testing – We work closely with Howard Ferris and his technician to evaluate the nematode resistance of rootstock breeding populations. Nina Romero (my chief greenhouse and field technician) propagated and assisted with the nematode resistance screening of hundreds of seedlings this year. Nina and I first examined the populations and evaluated them for brushy growth, internode length, and vigor. Most were also evaluated for their ability to root from dormant cuttings. They were tested for resistance to the Harmony/Freedom aggressive root-knot strains (HarmA and HarmC) and *Xiphinema index*, and many were also screened for ring nematode resistance. The best 21 are shown in Table 2 and will be advanced to field testing on the UC Davis campus with 101-14 and 1103P comparison controls.

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 they reduced susceptibility to *X. index* resistance, but the transformed plants were still susceptible. There are more lines to test 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 Chardonnay plants 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. Xiaoqing has also produced a number of tetraploid VR hybrid 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. Olmo was able to produce some fertile VR hybrids but these are *vinifera* x *rotundifolia* and may be 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 to provide breeding material if they have good phylloxera resistance.

Evan Goldman is completing his MS on how long *X. index* persists in a O39-16 vineyard. He has very good data from a plot at BV in Oakville where he has compared large 22 year old blocks of O39-16 to the susceptible 3309C and 110R. He has more samples to analyze, but the results are promising and indicate O39-16 may be able to eliminate *X. index* from a vineyard.

Salt and Drought Resistance – Kevin Fort continues to make strong progress on salt and drought resistance. We have a new three-step assay system that results in material ready for field evaluation. He has looked at a wide range of rootstocks, which determined 140Ru has very strong chloride resistance. We have also identified many new sources of salt resistance in southwestern *Vitis* species. This work is led by Claire Heinitz (4^{th} yr PhD student). She is now examining where this resistance originates within this very complicated and highly mixed group of southwestern species. If we know the source of resistance we can breed with it more precisely.

Kevin is also working on drought resistance and focusing on the genetic basis of root architecture – particularly deep rooting and its ability to provide more growth in drier soils. We are working closely with Andrew McElrone's group on this project and are exploring many avenues including differences in root periderm, root regeneration rates, ability to take up water through storage roots, and hydraulic redistribution. He developed a very effective rhizotron system to look at roots and coordinated the efforts of Joaquin Fraga (MS student) to examine root angles and distribution of over 30 rootstocks. This study confirmed the close link between rooting depth particularly structural roots and known drought resistance.

Cecilia Osorio (finishing MS student) completed an examination of the root angles of 7 grape rootstocks under normal and drought conditions in field nursery bed. In general drought tolerant rootstocks allocate more photosynthate to the roots. She also examined the anatomical differences of the roots under normal and dry conditions and found large anatomical differences under drought stress. Xylem vessel diameters were different and can be altered with drought stress. She is completing the quantification of xylem characters and looking at changes in suberin and lignin.

Southwest Vitis and Salt Resistance – Claire Heinitz is leading the effort to examine southwestern *Vitis* species for chloride resistance. She is analyzing hundreds of accession we have collected from across the southwest. Her most recent efforts have focused on species from

north central Texas and southern Oklahoma, which are a complicated blend of *V. riparia*, *V. candicans*, *V. arizonica* and *V. rupestris* and all of their hybrid combinations – *V. doaniana*, *V. champinii* and others. Her work will help us sort out and utilize these species more effectively in breeding and mapping efforts.

Leaf Longevity and Senescence – Jean Dodson is completing her PhD (expected Summer 2014) on the impact rootstock have on leaf longevity and senescence with potted vine and field trials. She found clear differences and is analyzing abscisic acid levels and correlating them to a wide range of vine growth and leaf function measures. These studies will direct the evaluation of genetic mapping populations we have including Ramsey x Riparia Gloire and 101-14 x 110R.

Phylloxera Resistance – Karl Lund finished his PhD on the biodiversity of phylloxera in December 2013 and we are getting manuscripts submitted. The dissertation abstract is included in this report. He is currently developing our F2 generation Ramsey x Riparia Gloire genetic map and working with Kevin Fort to place root characters on this map (reported under the drought resistance section).

REPORT
2013 Crosses – See Table 1.

Nematode testing – See Table 2

Fanleaf Degeneration
Genetic Mapping and the Physical Location of the *X. index* resistance gene, *XiR1* – The genetic and physical mapping of the *X. index* resistance gene, *XiR1* is completed and published (Hwang et al. 2010. Theoretical and Applied Genetics 121:780-799). Dr. Agüero is working on verifying the function of *XiR1* by genetically engineering two candidate genes from the *XiR1* region/locus (see last year's report for specifics) into *X. index* susceptible Thompson Seedless, St. George and tomato to verify the function of *XiR1*. We now know that the resistance gene candidates, *XiR1.1*, and *XiR1.2*, are members of the NB-LRR (nucleotide binding-leucine rich repeat) resistance gene family often involved in the signaling and promotion of resistance mechanisms.

Transformation experiments with *XiR1.1* and *XiR1.2* are finished (Table 3) and 10 independent lines of each combination have been acclimated to greenhouse conditions. Five lines of St. George and 5 lines of Thompson Seedless were multiplied through green cuttings and evaluated for resistance to *X. index* based on the number of galls formed 10 weeks after transplanting into infested soil. Results obtained with St. George showed four lines, one transformed with *XiR1.1* and three with *XiR1.2*, had fewer galls than the untransformed control (Figure 1). Although the feeding intensity is greater with this inoculation system, these lines were susceptible to *X. index*. All five Thompson Seedless lines tested also were susceptible when compared to the untransformed control. In 2014, we plan to continue genotype evaluation in the greenhouse using new re-established *X. index* populations. We will also evaluate the expression of the transgenes through qPCR in order to determine the correlation between the observed phenotype and the level of transgene expression.

Rootstock-Induced Fanleaf Tolerance – Cecilia Agüero continues efforts to determine if a biochemical marker can be found for fanleaf tolerance. The goal of this work is to find a metabolite associated with, or responsible for, this tolerance that we can use to rapidly screen populations such as the 101-14 x 'Trayshed', which may contain tolerance to fanleaf due to their *rotundifolia* (Trayshed) parent. Without these biomarkers we will have to field test for the ability to induce fanleaf tolerance, which could take 10+ years.

Analysis of xylem sap collected from healthy and infected Chardonnay grafted on O39-16 (which induces fanleaf tolerance on the scion), and susceptible St George at bleeding and fruit set resulted in the selection of cytokinins zeatin (Z), its precursor zeatin riboside (ZR), and isopentenyladenine riboside (iPR) as potential biomarkers. Xylem sap from 101-14, Trayshed and individuals of their *Vitis* x *Muscadinia* (VM) hybrid progeny, were collected in spring 2012 and sent to the Metabolomic Facility to test these compounds using UPLC-QTRAP MS/MS analysis. Results showed a considerable variability in the contents of Z, ZR and iPR among the individuals tested, and encouraged the testing of induced fanleaf tolerance on them. Ph.D. student Xiaoqing Xie is conducting grafting experiments under greenhouse conditions to assay GFLV susceptibility on VM individuals and different varieties of *M. rotundifolia* grafted on infected Chardonnay. A preliminary experiment comparing *in vitro* V-shaped grafting with greenhouse V-shaped and approach grafting resulted in the selection of greenhouse approach grafting for subsequent experiments.

VR tetraploids – In addition, Xiaoqing has treated VM (*Vitis* x *Muscadina*) genotypes with antimitotic agents to duplicate their chromosome number in an effort to improve the fertility of the crosses between *Vitis* and *Muscadinia* (*Vitis* has 2n=38 and *Muscadinia* is 2n=40). She has treated in vitro shoot tips, anthers, preembryogenic callus and zygotic embryos with colchicine and oryzaline to select the best explant. Shoot tips have already produced plants that have been analyzed by flow cytometry, the majority of which were tetraploid (Table 4). Positive tetraploid plants will be acclimated to greenhouse conditions for further evaluation and future use as parents in *Vitis* x *Muscadinia* crosses.

Cytokinin treatments – We also tested the effect of inflorescence treatments with ZR on fruit set, based on the observation that ZR was the prevalent cytokinin present in xylem sap at bleeding and fruit set. Treatments were conducted in an infected commercial vineyard of Cabernet Sauvignon grafted on St. George or O39-16. The second cluster in the middle position of each arm was sprayed with water or 10 μM ZR at bloom or 14 days after bloom. Although ZR sprayed at bloom decreased cluster weight, two-way ANOVA analysis only found statistical differences between rootstocks for cluster weight and fruit set (Figure 2). A block of Chardonnay grafted on St. George or O39-16 has been established in our experimental vineyard. Plants are 2 year's old and will be chip inoculated with GFLV this spring. This experimental block will allow testing a larger array of growth regulators, concentrations and times of application.

Resistance to Salt, Drought, and Boron – Kevin Fort, Post-doc supported by E&J Gallo
Salt Resistance
Three levels of screening for chloride exclusion have been developed from previous work, and differ in the throughput capacity and robustness of the assay. Untested materials begin the

screening process at Stage I, a high throughput, 14-day assay that is highly effective at eliminating salt hyperaccumulators such as 44-53, and also identifies a potential subset of genotypes that are highly resistant to chloride accumulation. In some cases, apparent salt exclusion can result from an unusually slow growth rate, resulting in false positives generated from the rapid Stage I assay. A second, more resource-intensive assay was therefore developed to measure growth rate and chloride accumulation simultaneously, and is effective at identifying and eliminating these false positives, and is referred to as Stage II. The final Stage III assay, intended to closely mimic field conditions, tests plant material found promising from both prior assay stages. In this final assay, the rootstock is bench grafted to a common scion and sampled repeatedly for a full growing season under high chloride irrigation while growing in a large, 3.6-gallon container. In all three stages, several reliable biocontrols of known chloride exclusion capacity are used for comparison to the novel plant material.

Assess the chloride exclusion capacity of the rootstocks GRN 1-5, and experimental rootstocks SC-1 and GC-5 – We previously tested some widely available commercial rootstocks at the Stage II level, some of which had established performance documented in the scientific literature. We confirmed the reliability of this assay by showing excellent chloride exclusion capacity in 140Ru, perhaps the most consistently strong chloride-excluding rootstock currently available, and simultaneously demonstrating poor chloride exclusion in two separate *Vitis vinifera* genotypes. In 2013, we used this same Stage II assay to characterize the GRN rootstock series, and also co-tested two promising new genotypes, SC-1 and GC-5: *Vitis girdiana* and *Vitis arizonica*, respectively. This test also used two reduced container sizes, 4" and 2", to test the feasibility of reducing the greenhouse footprint for the assay. This screen, completed in 2013, awaits biomass and leaf tissue analysis of the harvested plants, planned for early 2014.

Stage II screen of a *Vitis berlandieri*-derived hybrid population for salt tolerance, for molecular marker development – In earlier work, recently submitted for publication, a hybrid population derived from *V. berlandieri* was found to have among the strongest chloride exclusion observed to date at the Stage I level. This source of chloride exclusion may be additionally important because only in *V. berlandieri* has simple segregation for chloride exclusion ever been documented in the scientific literature. This population may therefore be ideally suited for the development of molecular markers. In the F1 population, all individuals showed dominant inheritance of strong chloride exclusion. Therefore, in 2012, a cross was performed to produce an F2-like population of hybrids that may segregate for chloride exclusion and therefore form the basis for the genetic mapping of this trait. In early 2013, a population of approximately 70 individuals was produced, and approximately half of this population was later tested in a Stage II screen. As with the GRN screen, this completed test awaits biomass and leaf tissue analysis of the harvested plants, planned for early 2014.

Propagation and maintenance of a St. George-derived hybrid population, for molecular marker development – In parallel to the development of the *V. berlandieri*-derived hybrid population, a St. George-derived F2-like hybrid population was simultaneously developed. The purpose of this population was to produce molecular markers for strong chloride exclusion using an alternative source that differed taxonomically from *V. berlandieri*, and therefore might be combined using marker-assisted selection with the *V. berlandieri*-based trait to produce

exceptionally strong chloride exclusion. This population is currently slated for screening in 2014.

Drought Resistance

Drought resistance is widely regarded as a complex trait, controlled by numerous genes and multiple physiological traits. Because the trait of deep rooting has been successfully exploited in other crops to enhance drought resistance, our initial work focused on methods to efficiently assay this character. In earlier work, we found that growing herbaceous cuttings for 2-3 weeks on a mist bed in a mixture of perlite and vermiculite produced adventitious roots that were strong enough to maintain their inherent rooting angle upon removal from the media and root washing. Plants grown longer than this or in dense media were unreliable for root system architecture characterizations. This assay also has a throughput that was high enough to allow several categories of characterizations in 2013, as follows.

Adventitious root angle characterizations: commercial rootstocks – In most studies that examine the morphology and architecture of commercially available rootstocks, only a small subset of genotypes are studied due to the labor-intensive nature of characterizing root systems. The high throughput of the adventitious root angle assay permitted the simultaneous comparison of all widely-available rootstocks used in California. As shown in Figure 3, the mean root angle of reputably drought-resistant rootstocks such as 1103P, Dog Ridge, Ramsey, and St. George were deep relative to that seen in reputably drought-susceptible rootstocks such as Riparia Gloire, 1616C, and 420A. These data support the use of this assay in characterizing the mean rooting angle of novel rootstock genotypes, and for developing molecular markers in segregating populations.

Adventitious root angle characterizations: phenology and salt tolerance populations – The adventitious root angle assay was also employed at the population level. First, it was considered that a 101-14 x 110R hybrid population that was segregating for phenological characteristics might be doing so partly due to differences in the root architecture. As shown in Figure 4, all F1 individuals in this population were deeply rooted, with very little variability. Therefore, the deep-rooting phenotype appears to be dominant, as the distinctly shallow rooting phenotype always observed in the 101-14 parent was completely absent in the F1. Similarly, an F1 hybrid population of Ramsey x Riparia Gloire was earlier shown to be all deep rooted (Figure 4), again supporting the conclusion that shallow rooting is a recessive phenotype. Three subpopulations of hybrids were also examined that were developed for the sake of salt tolerance marker development, and were derived from 140Ru, *V. girdiana*, and *V. arizonica* (Figure 5). Some degree of segregation was observed in the 140Ru and *V. arizonica* populations, indicating heterozygosity in the parents for this trait, and therefore the possibility of using these populations to map root architecture. Surprisingly, the *V. girdiana* hybrid population exhibited a root architecture that was more shallow than even Riparia Gloire, previously thought to be at or near the biological extreme for shallow roots. It is possible, therefore, that the development of even more devigorating rootstocks than Riparia Gloire can be produced.

Adventitious root angle characterizations: Ramsey x Riparia Gloire F2 population – In an attempt to generate a hybrid population segregating for root architecture that could be used to develop molecular markers for this trait, F1 individuals were crossed in the Ramsey x Riparia

Gloire F1 population in 2012 and planted in early 2013. Unlike the dominant deep phenotype observed in the F1, 159 individuals assayed in the F2 generation had an excellent range of phenotypes, and the shallow rooting phenotype that characterizes Riparia Gloire was recovered (Figure 6). Forty F1 individuals were also screened to confirm the earlier deep-rooting observation (Figure 5). For reference, several commercial rootstocks were screened as well (Figure 6). This F2 population was also the first set of genotypes wherein roots were scored as vectors rather than root angles, increasing the accuracy of the measurements. From this data set, it has also been observed that what may be a more important measure of gross level root architecture is not necessarily the mean rooting angle, but instead the presence or absence of a significant fraction of the total roots in the upper soil profile. The data analyzed from this perspective is presented in Figure 7. Using both methods of analysis, data generated in this screen is currently being used to develop SSR molecular markers for the trait.

Root angle measurements on second season, field-grown commercial rootstocks – Although physical and chemical edaphic factors play a major role in the distribution of roots in the field, the influence of genetically-controlled shallow or deep rooting has nevertheless been well documented. As additional confirmation that rootstock plays an important role in the distribution of roots in the field, hardwood cuttings were planted in 2011 and excavated in each of the subsequent two dormant periods. To test whether drought significantly altered rooting patterns, both well-watered and sparsely-watered irrigation treatments were employed. Because quantifying large root systems using a rapid index is considerably less precise than measuring individual adventitious roots, subtle differences between genotypes were not distinguished. Nevertheless, the broad patterns of shallow versus deep rooting were confirmed and, surprisingly, were completely unaffected by irrigation treatment (Figure 7). This latter observation underscores the importance of breeding for rootstock architecture.

Genetic mapping of rooting angle in the Ramsey x Riparia F2 population – Karl Lund

Genetic mapping and QTL analysis is underway on a F2 population created from a cross of siblings from the Ramsey x Riparia Gloire population. We have a genetic map of the F1Ramsey x Riparia population but the progeny do not seem to segregate for many traits of interest – the progeny are the same as one of the parents, thus we created an F2 population by crossing two of the F1 progeny. A total of 320 simple sequence repeat (SSR) markers have been tested for utility on the parents of the F2 population; 171 were polymorphic with clear results. Of these SSR markers, 92 have been run on the full population of 160 progeny (more will be made in 2014), with an additional 12 markers being run each week. An initial genetic map placed 76 of the 92 SSR markers run on the full population into 15 linkage groups covering 14 chromosomes (Figure 8). Rooting angle data collected by Kevin Fort was used to conduct a QTL analysis for this character. On this very preliminary genetic map, QTL analysis shows a possible mapable locus on Chromosome 6 (Table 5). The consistency of both LOD and percent of variation explained across the lower segment of chromosome 6 give strong assurances that this is a real QTL involved in rooting angle. Examination of the remaining chromosomes shows only one more marker on chromosome 8 with a significant LOD score (Table 6). As only one marker in this region of chromosome 8 is above the threshold of significance it may not hold up to further testing. However, the relatively low percentage of the variation explained (7.4%) may be a result of the relatively low number of offspring in this population. We hope the addition of more

offspring into the F2 population, and the continued addition of SSR markers, will improve and refine the placement of rooting angle.

Analysis of Wolpert field data – The pruning weight and yield for approximately ten years of field data generated by Dr. Jim Wolpert at eight vineyard sites has been analyzed using principle components analysis (Figure 9). This analysis is an important first step needed for the preparation of a manuscript detailing the results of this extensive data set.

Rhizotron dry-down and recovery study – From published evidence in the scientific literature, drought resistance can involve more than the distribution of roots during the occurrence of drought, a plant strategy that exploits different volumes of soil to prevent dehydration. But even deeply-rooted plants do undergo tissue drying when soil water scarcity persists. Therefore, the rate at which a plant recovers from drought once soil water is replenished can also be important, and genetically-controlled differences in recovery may exist among rootstocks. Because large rhizotron containers have been used to successfully track the root architecture development of herbaceous grape cuttings, this same system was used to document whole shoot and root system responses to a soil dry-down and re-watering. Four rootstocks were used in this trial: two deep-rooted stocks, 110R and Ramsey, and two shallow-rooted stocks, 101-14 and Riparia Gloire. All individuals were bench grafted with a common Merlot scion. Working with Joaquin Fraga and Daniele Grossi from the Walker lab, measures of shoot and root growth and physiology were taken following establishment and during a soil dry-down period followed by re-watering. One important finding was the rapid recovery from drought seen in 110R and Ramsey (Figure 7). In 24 hours, these rootstocks re-established stomatal conductance values equal to or greater than that measured in well-watered controls, whereas the rootstocks 101-14 and Riparia Gloire were not observed to fully recovery even after multiple days of daily re-watering. This finding indicates that it might be possible to breed for recovery from drought with a simple shoot system measure, stomatal conductance, thereby avoiding the need to collect data from much more resource-intensive root system response variables. This possibility will be explored further in 2014.

Boron tolerance study – A pilot screen was initiated to test the boron tolerance of commercial rootstocks and select *Vitis* species from desert regions. The intensity of symptoms in these selections varied in response to irrigation water with 0, 1, 3 and 5 ppm of B under potted vine culture. Joaquin Fraga and Daniele Grossi also worked with Kevin Fort on these screens; the trials were completed and await symptom, biomass, and leaf tissue analysis of the harvested plants, are a top priority for early 2014. A considerable number of wildland *Vitis* accessions are in need of evaluation, and the 2013 screen marks the first step toward this end. In queue for screening are *V. arizonica* from boron deposits in southeastern Arizona, *V. girdiana* from gypsum flats in southern Nevada, and highly salt tolerant selections of *V. acerifolia* and *V. doaniana* from the Red River between Texas and Oklahoma. If boron tolerance can be identified, it will be bred into commercial rootstocks and genetic markers for this trait will be produced.

Cumulative root fractions of California commercial rootstocks and drought trials – Joaquin Fraga

We finalized the analysis of last year's root architecture characterizations by performing a cumulative root fraction analysis of the data. This type of analysis has been used before to describe the vertical rooting patterns of perennial plants (Smart et al. 2006. Am. J. Enol. Vitic. 57, 89–104; Gale et al. 1987. Can. J. For. Res. 17, 829–834). The cumulative root fraction graph (Fig. 10). These data show a relationship between the lack of superficial roots with higher drought resistance – examples include Ramsey, Ramsey hybrids RR19 and RR29, St. George, GRN1, GRN5 and 1103P. On the other side of the spectrum, genotypes of greater proportion of shallow roots are related to drought susceptibility – Riparia, 1616C and 420A. Differentiating behaviors will be fundamental for the search of QTLs responsible for deep rooting.

The rhizotron method was employed in a dry down experiment performed with four rootstocks (110R, 101-14, Riparia and Ramsey) grafted onto *V. vinifera* cv. Merlot. The same protocol for plant establishment was used as in the California rootstock characterization. Plants were well-watered for five weeks, followed by cessation of all irrigation, and compared to a daily-watered control. Root growth was quantified on a weekly basis. Some interesting conclusions were drawn. In the deficit treatment (Fig. 11), Ramsey and 110R produced a greater total number of roots and a greater number of roots at deeper layers relative to Riparia and 101-14. This summer, we will perform deficit experiments that explore other root parameters such as root rebirth, lateralization, and survivorship.

Germplasm exploration for chloride exclusion and genetic diversity of southwest U.S. *Vitis* – Claire Heinitz

Results of intensified chloride screen with increased concentration and duration – Using our rapid 2-week greenhouse screen, we have identified many chloride-excluding accessions that perform better than the current industry standards. Chloride concentration in the roots and leaves of these plants indicate that while most genotypes sequester a similar amount of chloride in the roots, transport to the shoot varies widely. In addition, some unique genotypes were identified which had reduced chloride in the roots as well as the shoots, and one accession displayed the reverse pattern with a higher concentration of chloride in the leaves than in the roots. These accessions appear to display differential chloride exclusion mechanisms; in addition, the contradictory results of genetic inheritance of the trait further indicate that chloride exclusion is a complex trait that may have multiple mechanisms. Our two week, 25mM greenhouse screen was developed to rapidly screen a large number of genotypes, but it only provides a snapshot of the exclusion potential of a particular genotype. This experiment was designed to test the effect of increasing treatment pressure on chloride uptake patterns, and addresses the following questions:

- What are the relative effects of increasing chloride concentration and longer treatment duration on accessions with potentially different chloride exclusion mechanisms? (Table 7)
- Does the "double exclusion" pattern of *V. girdiana* break down under higher treatment pressure?
- Is leaf chloride concentration related to the growth rate of the plant?

The 7 different accessions were subjected to a factorial treatment structure of 4 chloride concentrations (0, 25, 50, and 75 mM NaCl) and 2 treatment durations (3 and 6 weeks) in a randomized complete block design with 4 replicates. The plants were grown in a greenhouse and the excess chloride delivered in the irrigation water. Additional plants of each accession were grown and harvested at the beginning of the experiment to determine the relative growth rate of each accession*treatment combination. After 3 or 6 weeks, plants were destructively harvested and leaf, stem and root tissue separated, dried, and analyzed for chloride concentration.

Genotype rankings based on leaf chloride concentration change with increasing treatment pressure (Figure 12) – Leaf Cl- concentration in both Ramsey and St. George (both considered good excluders) increases dramatically with increasing treatment intensity, but SC1, SC2 and 9031 remain relatively low. The hyper-accumulator "pumpstation rupestris" remains the highest in chloride uptake across all treatments. After 6 weeks, relative genotype rankings are no longer consistent.

The "double exclusion" pattern in V. girdiana is consistent across all treatments (Figure 13) – Leaf Cl- concentration increased for all accessions with increasing treatment intensity, but the effects were different for each of the genotypes. In all treatments, SC1 and SC2 maintained the lowest Cl- concentration in both the leaves and the roots, while Thompson, Ramsey, and St. George all accumulated much more chloride in the leaves both with increasing time and treatment intensity. For most accessions, root Cl- concentration did not change with increased treatment time, which supports the model that the roots can sequester a defined amount of chloride before moving to the shoot.

Chloride uptake does not correspond to growth rate (Figure 14) – Accessions with lower chloride uptake are not necessarily growing more slowly – all of the genotypes show a general reduction in growth rate as the salt concentration increases, but especially over 6 weeks overall growth rate is fairly steady. The *V. girdiana* genotype SC2 stands out as an excellent chloride excluder that maintains a high growth rate. Relative growth rate over the shorter time span is more volatile, and may not be a useful measurement tool.

In conclusion, the different chloride uptake patterns that suggest multiple exclusion mechanisms are consistent over increasing external chloride concentration and treatment time. The *V. girdiana* accessions appear to have additional exclusion capabilities that allow concentrations to remain relatively low in the roots and shoots, without sacrificing growth rate. Industry standard excluders like Ramsey and St. George fail under high treatment pressure, but uptake remains relatively low in our new accessions of *V. girdiana* and *V. berlandieri*. In future screens of our most promising excluders, we will use the 75 mM / 3 week combination, as this level generated the best genotype separation in the shortest time frame.

Advancing primary germplasm and crosses to general rootstock breeding program – Throughout the continued greenhouse screening for chloride exclusion, we have also evaluated the best performers for dormant rooting capability. In addition, we have made several crosses between good excluders and commercial rootstocks in an attempt to improve general horticultural characteristics (Table 8). This year, we will begin to evaluate the progeny of these crosses and several promising wild accessions for grafting performance and longer-term chloride

exclusion (see above under Kevin Fort progress). We will be adding to the list this year as we gather more data on our new collections.

Update on preliminary study of the hybrid origin of *V. doaniana* – A preliminary analysis of accessions of three species from Texas and Oklahoma illustrates the potential power of population-level genetic analysis. Munson first suggested in 1909 that a unique species found only along the Red River (*V. doaniana*) may be a hybrid of *V. acerifolia*, found in southern Oklahoma, and *V. candicans*, found throughout Texas. We became interested in this population when we observed the excellent performance of *V. doaniana* accessions in our chloride exclusion screen. To test the theory of a hybrid origin, we extracted DNA from 41 of our collections from the Red River area which we categorized based on morphology as either *V. acerifolia, V. doaniana, V. candicans,* or as "hybrid/uncertain", meaning close to one of these species but not fitting completely. We then genotyped them using a set of 20 microsatellite markers and analyzed the data with the program STRUCTURE, which determines the most likely number of genetic groups and assigns each individual to a group (or groups). Surprisingly, what we thought would be 3 or even 2 genetic groups (with *V. doaniana* as a mix of *acerifolia* and *candicans*) appears most likely to be 5 groups. The percentage assignment of each individual in the study to these 5 groups is represented with different colors in Figure 15. The samples are grouped based on our original species determination, and you can see that the *V. acerifolia* and *V. candicans* samples largely belong to unique genetic groups (red and yellow, respectively). Some of the *V. doaniana* samples are a mix of red and yellow which could support the hybrid origin, but others appear to belong to completely distinct genetic groups. Most interestingly, all of the samples that were assigned to the pink, green, or blue groups were collected in along a single crossing point of the river, while the rest of the samples from the region all belonged to the red, yellow, or a mix of the two (Figure 16). From these results, it is clear that the origin of this rare and potentially valuable wild species is more complex than originally hypothesized, and hopefully by continuing to expand our sample size and include more examples of different species we will begin to get a clearer picture of the relationships between populations of wild *Vitis.* By combining the geographical origins, genetic profiles, and field phenotypic performance of all of our collections we are building a strong resource for rootstock breeding and conservation.

Correlation of rootstock architecture to drought resistance – Cecilia Osorio

Experimental Background - This experiment was designed to understand the effects of root angle and anatomical differences on drought resistance of seven commercially available rootstocks. Ramsey, Riparia, 110R, 101-14, 5C, 420A, and 140Ru*, were planted on a split block arrangement of eight field blocks during the summer of 2011. Each block contained ten replicates of each rootstock. All blocks were drip irrigation throughout the first summer. In the second summer half of the blocks were not irrigated. The vines were not pruned or trained.

Biomass Allocation –Strong root systems are important in the cultivation of grapevines under droughty conditions because they contain more surface area for water absorption, can reach and better compete for deep pockets of moisture and may have higher storage capacities. Our results show that drought tolerant cultivars allocated more biomass to their roots than drought susceptible cultivars under well watered conditions. Total root biomass did not change significantly in drought tolerant cultivars when exposed to drought vs. well watered conditions.

However, drought susceptible cultivars increased the amount of total root biomass significantly when exposed to drought (Figure 17).

Anatomical Tissue Quantification: Cortex area ratio –Along with the ability to reach and absorb water and nutrients, roots are also equipped with a thick lignified cortex to prevent not just infection and predators, but to limit water loss. Thus, we compared the amount of cortex area per total root area to determine if this was a relevant phenotype among drought tolerant rootstocks. We found that there was no significant difference among cultivars under well-watered conditions but under drought conditions 420A has a significantly greater cortex area ratio than Ramsey and Riparia Gloire (alpha 0.05) (Figure 18). Thus, cortex thickness may not be a reliable phenotype to examine water conservation within drought tolerant rootstocks, but cortex thickness is not entirely responsible for the prevention of water loss. Suberin and lignin are two major hydrophobic chemical components that engulf the endodermal cells on the outer layers of the cortex. Consequently, steps have been taken to explore a feasible method to quantify these two chemicals in conjunction with the McElrone lab.

Vessel Diameters – Vessel diameters are also an important drought tolerance phenotype that has been studied in the past. The sizes of vessels in a plant determine the amount of water and the pressure at which water is transported up to the shoot. For this project, roots were cut into 70um thick sections, stained and mounted for microscopic anatomical examination. Because of the variability in the thickness of the roots of some cultivars, statistical analysis was conducted using the rates of vessel diameters to root diameter. We first compared the cultivars to each other for each of the treatments separately. Under well-watered conditions Riparia Gloire had significantly smaller vessel diameters than the rest of the cultivars, but under drought treatment, both Riparia Gloire and 420A had significantly smaller vessel diameters than the rest of the cultivars. When both treatments were considered only 5C had significantly smaller vessels under drought than under well watered conditions.

Although these quantitative results seem to indicate that there is no correlation between vessel diameters and drought tolerance, we have observed peculiar patterns that may indicate vessel diameters are indeed an important phenotype related to drought tolerance in grape rootstocks. Although the method is still being refined, quantification of the frequency of different vessel diameter classes (e.g. <40um or >40um) seems to describe our initial observations; that Ramsey and 140Ru reduce their vessel diameters towards the end of the season under drought treatment only. This change is a natural occurrence in many species, but vessel sizes of these two cultivars appeared to have been reduced much earlier in the season when exposed to drought (Figure 19 and 20). In addition, none of the drought susceptible rootstocks (Riparia Gloire, 5C, 420A, 101-14) displayed this pattern (Figure 21). Additional and improved quantification methods are being developed to accurately define other anatomical characters such as lumen and storage cell capacity area.

Phylloxera Biodiversity, Karl Lund PhD Summary –
The failure of the rootstock AXR#1 in the 1980s revealed that grape phylloxera exhibit selective feeding behavior and that understanding phylloxera's phenotypic and genetic diversity as well as their population biology is key to combating future epidemics. Recently, two new phylloxera feeding behaviors have been identified in California. The first type feeds primarily on rootstock

leaves and has been found in rootstock nursery plantings and collections in Yolo and Solano Counties. The second type consists of phylloxera strains capable of aggressive feeding on immature unlignified root tips of rootstocks thought to have strong resistance. A series of studies was implemented to understand the phenotypic and genetic differences within and among these new phylloxera groups, as well as their relationship to older California phylloxera strains.

The first study examined the current phenotypic diversity of phylloxera across northern California. Eight single adult linage lines were created in the laboratory and maintained under growth chamber conditions. The ability of each strain to reproduce was assayed in a series of 30-day feeding trials on a set of hosts with different resistance levels. Statistical analysis found significant differences in each single adult linage's ability to reproduce on the different hosts. It was also shown that phylloxera collected from the same host had similar phenotypes. More importantly, this study identified multiple sources of general and line specific hypersensitive-like responses to phylloxera feeding attempts. These hypersensitive responses provide new sources of strong phylloxera resistance for use in future breeding work.

The second study examined the genetic diversity and reproductive mode of phylloxera strains in northern California. The sample set consisted of 403 phylloxera isolates collected from 20 sites across northern California grape-growing areas, which were analyzed using simple sequence repeat markers (SSR). These collections specifically focused on phylloxera infesting rootstocks thought to have strong phylloxera resistance, and on leaf feeding phylloxera, which until recently were uncommon in California. Results from the clustering software STRUCTURE were verified with neighbor-joining and principle coordinate analysis to construct a set of consistent genetic populations across all of these three analyses. Four populations were distinguished: leaf feeding phylloxera; samples collected from immature unlignified and structural suberized roots of *V. vinifera* and *V. vinifera* hybrids; and two closely related but separable populations found on immature unlignified roots of rootstocks with no *V. vinifera* in their parentage. The data also showed that samples collected from leaf galls were exclusively produced through asexual reproduction, but that there was evidence of rare sexual reproduction in the other groups of samples, especially at sites where the multiple populations overlapped. These results suggest that phylloxera could use sexual reproduction to expedite their adaptation to rootstock varieties.

The third study examined the genetic diversity of phylloxera across their native range in the eastern and southwestern United States to create a database for comparing phylloxera from outside the native range. Over 500 samples collected from 19 states were analyzed with SSR markers and analyzed with the same three analytical approaches as noted above. These samples clustered into five populations based primarily on the host species from which they were collected, with some impact of geographic separation. The data also showed that sexual reproduction was common across the native range. Comparisons to California samples showed that all California samples were members of, or hybrids of, the population of isolates collected on *V. riparia* in the northeastern United States. These data suggests that as new *Vitis* species are used to breed rootstocks with resistance to drought, salinity and nematodes, their ability to select new genetic types of phylloxera should be considered and evaluated.

Talks at Grower Meetings (Extension/Outreach)

Vineyard replanting/redevelopment decisions: pests and diseases. Unified Symposium, Sacramento, January 30, 2013.

Walker grape breeding program. Napa Valley Grape Growers, UC Davis, February 8, 2013.

Why are phylloxera still bugging us? Sonoma Grape Day, UCCE meeting, February 20, 2013.

Grape rootstock breeding progress. Current Issues in Wine and Grape Health, UC Davis, February 21, 2013.

Grape breeding. Napa Valley Vintners. UC Davis, Feb. 27, 2013.

Are phylloxera still important? Recent Advances in Viticulture and Enology, UC Davis, March 14, 2013.

Intro to rootstocks. WiVi Annual Central Coast Meeting, Paso Robles, March 20, 2013.

Vineyard challenges: wine growing from the ground up. Wine Executive Program, UC Davis, March 26, 2013.

The vineyard of the future. Wine Executive Program, UC Davis, March 28, 2013.

Walker lab rootstock breeding / Salt and drought resistance. E&J Gallo Winery Seminar, Modesto, March 28, 2013.

Breeding PD resistant wine grapes (including new PD rootstocks). Santa Rosa Winegrape Association Meeting, Santa Rosa, CA, April 5, 2013.

Grape rootstock breeding progress. Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board, UC Davis, April 9, 2013.

Sustainable Viticulture. Haas Business School, DNV Top Tech Program, Mondavi Winery, Oakville, CA, April 20, 2013

Walker lab rootstock breeding. AVF Meeting, Livermore, April 26, 2013.

Pest and disease threats: Decisions and the future of farming. Napa Valley 2030 – Ahead of the Curve, Napa Valley Grape Growers, Napa, May 7, 2013

Walker grape breeding program. Chilean Winegrowers Meeting, UC Davis, May 7, 2013

Using grape rootstocks to avoid drought. Roll Global / UCD meeting, UC Davis, May 8, 2013.

How to choose rootstocks for foothill vineyards and why phylloxera still matter. Foothill Grape Day, Placerville, June 6, 2013.

Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Grape Symposium, Ica, Peru June 19, 2013.

Nematode resistant grape rootstocks. International Table Grape Symposium, Ica, Peru June 20, 2013.

Breeding resistant grapes. Diageo Central Coast Growers Meeting, Asilomar, CA, July 24.

Breeding for salt and drought resistance. Australian Raisin Producers, UC Davis, September 6, 2013

Site evaluation and rootstock decision making. Foothills Grape Growers, Boeger Winery, October 11, 2013.

Grape breeding at UC Davis. Viticulture and Enology Seminar, UC Davis, Oct. 4, 2013.

Red blotch symptoms and impact. California Grape Rootstock Improvement Commission tour, Oakville, CA, October 18, 2013.

Grape rootstock decision making. Wente Vineyards, November 1, 2013.

Can we breed better drought and salt resistance into grape rootstocks. Paso Robles Grape Growers Water Use Seminar, Templeton, CA, November 8, 2013.

Grape roots: shallow or deep; young or old; healthy or dying. Daniel Roberts Client Group Meeting, Santa Rosa, CA, December 6, 2013.

Presentations at Scientific Meetings

Walker, M.A. 21013. Development and management of rootstocks for table grapes. First International Symposium on the Vine. Hermosillo, Sonora, Mexico, January 25, 2013.

Walker, M.A. 2013. Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Grape Symposium, Ica, Peru June 19, 2013.

Walker, M.A. 2013. Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Nematode resistant grape rootstocks. International Table Grape Symposium, Ica, Peru June 20, 2013.

Walker, M.A. 2013. Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Rootstock breeding for future needs. Rootstock Symposium, 64th ASEV National Meeting, Monterey, CA June 25, 2013

Goldman, E. and Walker, M.A. 2013. Reducing *Xiphinema index* populations using the resistant rootstock 'O39-16': the status of *X. index* after 20 years. 64th ASEV National Meeting, Monterey, CA, June 26, 2013.

Osorio, C., McElrone, A. and Walker, M.A. 2013. Identification of drought resistance morpho-anatomical characters among seven grape rootstocks. 64th ASEV National Meeting, Monterey, CA, June 26, 2013.

Lund, K., Riaz, S. and Walker, M.A. 2013. Genetic analysis of phylloxera across the eastern southeastern United States. 64th ASEV National Meeting, Monterey, CA, June 26, 2013.

Dodson, J.C. and M.A. Walker. 2013. Grapevine rootstock-scion interactions and their influence on ripening periods and the initiation of senescence. 64th ASEV National Meeting, Monterey, CA, June 26, 2013.

Riaz, S. and Walker, M.A. 2013. Using marker-assisted selection to optimize grape breeding. Grape Research Coordination Network Meeting, UC Davis, Davis, CA, July 11, 2013.

Fort, K., Heintiz, C., Fraga, J., Osorio, C., McElrone, A. and Walker, A. 2013. Breeding grape rootstocks for chloride exclusion and drought resistance. Specialty Crop Research Initiative Research and Coordination Meeting, Prosser, WA, August 6, 2013.

Walker, A. Walker lab grape breeding. 2013. North American Grape Breeders Meeting, Fayetteville, AR, August 15, 2013.

Walker, M.A., Lund, K., Riaz, S. and Romero, N. 2013. Breeding grape rootstocks for resistance to phylloxera and nematodes – it's not always easy. Keynote Address, 6th International Phylloxera Symposium, Bordeaux, France, August 29, 2013.

Walker, M.A. 2013. Grape breeding at UC Davis, Seminar at Missouri State University, Springfield, MO, August 10, 2103.

Walker, M.A. 2013. Optimizing grape improvement with molecular tools. University of Missouri, Colombia, MO, August 11, 2013.

Table 1. 2013 crosses

Cross #	Maternal	Paternal Parent	Seeds	Purpose
2013-121	9715-17	Riparia Gloire	329	(Ramsey x Riparia) x Riparia mapping salt and drought
2013-122	9715-46	Riparia Gloire	~800	(Ramsey x Riparia) x Riparia mapping salt and drought
2013-123	9715-63	Riparia Gloire	~700	(Ramsey x Riparia) x Riparia mapping salt and drought
2013-133	Riparia 1411	140Ru	>500	Salt and drought mapping and rootstock production
2013-145	101-14 Mgt	1103 Paulsen	14	Root architecture mapping
2013-146	Ramsey	Trayshed	60	Soil borne pest resistance
2013-147	09133-21	09133-10	8	Rip 1411 x 140Ru sib matings salt/drought
2013-148	09133-11	09133-10	138	Rip 1411 x 140Ru sib matings salt/drought
2013-149	09133-11	09133-23	28	Rip 1411 x 140Ru sib matings salt/drought
2013-150	09133-11	09133-07	31	Rip 1411 x 140Ru sib matings salt/drought
2013-154	09133-11	09133-35	5	Rip 1411 x 140Ru sib matings salt/drought
2013-155	09133-21	09133-06	19	Rip 1411 x 140Ru sib matings salt/drought
2013-160	NC 6-15	420A Mgt	0	Rootable and fertile VR x rootstock – nematodes and fanleaf
2013-161	T6-38	420A Mgt	13	“ “
2013-162	T6-42	420A Mgt	0	“ “
2013-163	NC 6-15	110R	0	“ “
2013-164	T6-38	110R	38	“ “
2013-165	T6-42	110R	6	“ “
2013-166	NC 6-15	SO4	0	“ “
2013-167	T6-38	SO4	2	“ “
2013-168	T6-42	SO4	0	“ “
2013-169	NC 6-15	St. George	0	“ “
2013-170	T6-38	St. George	10	“ “
2013-171	T6-42	St. George	5	“ “
2013-172	NC 6-15	1103 Paulsen	0	“ “
2013-173	T6-38	1103 Paulsen	14	“ “
2013-174	T6-42	1103 Paulsen	4	“ “
2013-175	NC 6-15	140Ru	0	“ “
2013-176	T6-38	140Ru	0	“ “
2013-177	T6-42	140Ru	0	“ “
2013-178	NC 6-15	1616C	0	“ “
2013-179	T6-38	1616C	16	“ “
2013-180	T6-42	1616C	16	“ “
2013-181	NC 6-15	GRN-2 9363-16	0	“ “
2013-182	T6-38	GRN-2 9363-16	42	“ “
2013-183	T6-42	GRN-2 9363-16	37	“ “
2013-186	NC 6-15	GRN-4 9365-85	0	“ “
2013-187	T6-38	GRN-4 9365-85	12	“ “
2013-188	T6-42	GRN-4 9365-85	0	“ “
2013-189	NC 6-15	GRN-5 9407-14	0	“ “
2013-190	T6-38	GRN-5 9407-14	3	“ “
2013-191	T6-42	GRN-5 9407-14	0	“ “

Table 2. Nematode resistant selections from 2013 screening, These selections root well and have strong resistance to the aggressive root-knot nematode strains Harm A & C, and X. index. Some also resist ring nematode, although none did so as well as the GRN1 control. They were designed to broaden nematode resistance, improve rooting ability, or incorporate nematode resistance with resistance to Pierce's disease.

Resistant to Harm A&C and Xi, designed to improve rooting of GRN5

06105-50	101-14 Mgt x 9407-14 (GRN5)
06105-16	101-14 Mgt x 9407-14 (GRN5)

Resistant to Harm A&C and Xi, designed to Broaden GRN resistance and reduce vigor

06104-02	101-14 Mgt x 9363-16 (GRN2)
06104-08	101-14 Mgt x 9363-16 (GRN2)
06104-12	101-14 Mgt x 9363-16 (GRN2)
06109-01	101-14 Mgt x 9365-85 (GRN4)
06109-17	101-14 Mgt x 9365-85 (GRN4)
06109-28	101-14 Mgt x 9365-85 (GRN4)

Resistant to Harm A&C and Xi, designed to incorporate strong *V. arizonica*-based nematode resistance and PD resistance

0707-39	5BB x b40-14
0707-45	5BB x b40-14
0708-21	5BB x R8916-22
11175-07	08314-31 X Schwarzman

Resistant to Harm A&C and Xi, designed to incorporate *M. rotundifolia*-based nematode resistance

11188-03	T6-42 X St. George

Resistant to ring nematode

10115-26	161-49C x Trayshed
11115-13	161-49C X Trayshed
11138-1	5BB Kober X Trayshed
11138-2	5BB Kober X Trayshed
11143-6	Ramsey X 08314-15

Resistant to HarmA&C; Xi; and ring

11175-6	08314-31 X Schwarzmann
11175-15	08314-31 X Schwarzmann
11188-6	T6-42 X St. George

Table 3. 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 4. Antimitotic treatments (Col = colchicine; Ory = oryzaline) conducted on shoot tips and regeneration of tetraploid plants. These tetraploids will be used as parents to determine if they improve the fertility of *Vitis* x *Muscadinia* hybrids.

Antimitotic agent	Duration time (hours)	No. of individuals treated	No. surviving	No. tested (flow cytometry)	No. tetraploid
Control	-	60	60	1	0
Col 0.01%	24	60	57	1	1
	48	60	54	1	0
	72	60	53	1	1
Col 0.025%	24	60	51	1	1
	48	60	50	1	1
	72	60	47	1	1
Col 0.05%	24	60	46	1	1
	48	60	45	1	1
	72	60	42	1	1
Ory 5µM	24	30	28	1	1
	48	60	55	1	1
	72	30	26	1	1
Ory 15µM	24	30	27	1	1
	48	30	25	1	1
	72	30	21	1	1
Ory 30µM	24	30	19	1	1
	48	30	17	1	1
	72	30	13	1	1

Table 5. LOD and percent variation explained (% Expl.) for chromosome 6. Map positions were calculated by JoinMap4 and are in centiMorgans. LOD and percent of variation explained were calculated by MapQTL6. Highlighted LOD scores indicate those that are above the 95% confidence threshold

Chromosome	Position	Locus	LOD	% Expl.
6	0	UDV90	0.18	0.5
6	15.664	UDV85	0.77	2.3
6	16.969	VMC2g2	0.87	2.6
6	17.069	VMC5c5	0.87	2.6
6	29.448	VVIc50	3.37	9.6
6	36.827	VVIp28	4.34	12.2
6	38.022	VMC5g1.1	5.11	14.2
6	38.023	VMC3a8	5.11	14.2
6	38.024	VMC3f12	5.11	14.2
6	39.85	VVMD21	5.17	14.3
6	40.18	VMC4h5	5.3	14.7
6	49.047	VMCNg4b9	6.69	18.

Table 6. LOD and percent variation explained (% Expl.) for remaining identified chromosomes. Map positions were calculated by JoinMap4 and are in centiMorgans. LOD and percent of variation explained were calculated by MapQTL6. Highlighted LOD score is above the 95% confidence threshold.

Chr	Position	Locus	LOD	% Expl	Chr	Position	Locus	LOD	% Expl
1	0	ctg8034	0.72	2.1	8	0	VMC2h10	1.76	5.1
1	2.44	ctg5664	0.71	2.1	8	16.935	VMC1e8	2.56	7.4
1	6.047	VMC7g5	0.38	1.1	8	27.311	VMC5h2	1.12	3.3
1	23.325	VMC2b3	1.48	4.3	8	32.306	VMC1b11	1.55	4.5
1	31.485	VMC9f2	1.78	5.2	8	56.901	VMC7h2	1.49	4.4
1	35.946	ctg6392	1	2.9	8	61.872	UDV125	1.16	3.4
1	43.154	VMC9d3	0.61	1.8	8	63.181	UDV75	0.93	2.7
3	0	UDV61	1.06	3.1	8	63.815	VMC6g8	1.1	3.2
3	32.073	VVMD28	0.11	0.3	8	66.568	VMC2f12	0.29	0.9
4	0	ctg6426	1.71	5	14	0	VVIN94	0.09	0.3
4	15.762	VMCNg1f1.1	0.51	1.5	14	0.332	VVIi51	0.09	0.3
4	0	ctg0624	0.69	2.1	14	4.95	VVIp26	0.08	0.2
4	1.975	VVIp77	0.4	1.2	14	5.282	VVIs70	0.08	0.2
5	0	VVC6	0.87	2.6	14	6.301	ctg5882	0.07	0.2
5	18.547	UDV53	1.28	3.8	14	9.186	UDV95	0.2	0.6
5	30.699	VCM5e11	0.44	1.3	14	44.342	VVC34	0.15	0.4
5	34.956	VMC6e10	0.25	0.8	14	51.956	VMC1e12	0.5	1.5
5	67.194	VMC4c6	1.28	3.8	17	0	UDV103	0.35	1
7	0	VMC7a4	2.01	5.8	17	26.001	VMC3a9	0.08	0.2
7	2.283	VMC6f5	1.73	5	17	43.508	VMC7c3	0.69	2.1
7	11.28	VMC5h5	1.72	5	18	0	VMC7f2	0.68	2
7	33.794	VrZAG62	0.46	1.4	18	10.452	VMCNg1e3	1.06	3.1
7	37.031	VVMD7	0.67	2	18	13.017	VMC2d2	1.29	3.8
7	41.271	VMC1h5	1.2	3.5	18	49.312	VVIv16	1.01	3
					18	50.234	VMC8b5	1.01	3
					18	52.354	VMC2b1.1	0.9	2.7
					19	0	VMC5e9	1.56	4.6
					19	9.957	VMC5h11	0.85	2.5

Table 7. Accessions included in high intensity / duration greenhouse chloride screen

Accession	Species	Exclusion pattern	Tentative exclusion category
St. George	*V. rupestris*	Leaves low Roots high	Typical Exclusion
Thompson Seedless	*V. vinifera*	Leaves high Roots high	Typical Non-exclusion
"Pumpstation"	*V. rupestris*	Leaves *very* high Roots low	Hyper-accumulation
SC 1	*V. girdiana*	Leaves low Roots low	Double Exclusion
SC 2	*V. girdiana*	Leaves low Roots low	Double Exclusion
9031	*V. berlandieri*	Leaves low Roots high	Typical Exclusion with potential single-gene inheritance
Ramsey	*V. champinii*	Leaves moderate Roots high	Typical Exclusion with potential multi-gene inheritance

Table 8. Primary germplasm and crosses advancing in the rootstock breeding program

Accession / Population ID	**Species / Parentage**	**Status**
T 03-15	*V. rupestris*	Primary germplasm, selected from 2-week greenhouse chloride exclusion screen, tested for dormant rootability
NM 03-17 S01	*V. treleasei*	
OKC-1 S01	*V. acerifolia*	
SC12	*V. girdiana*	
longii 9018	*V. acerifolia*	
longii 9035	*V. acerifolia*	
SC2	*V. girdiana*	
2011-133 pop	OKC-1 SO3 x St. George	Progeny from promising crosses; need to be screened visually for horticultural characteristics, then tested for chloride exclusion and rootability
2011-137 pop	161-49C x T9(*V. doaniana*)	
2011-148 pop	Ramsey x NM 03-17 S01	
2011-155 pop	OKC-1 S03 x Rip Gloire	
2011-156 pop	OKC-1 S03 x 1616C	
2011-157 pop	161-49C x ANU77(*V. girdiana*)	
2011-162 pop	161-49C x ANU21(*V. girdiana*)	

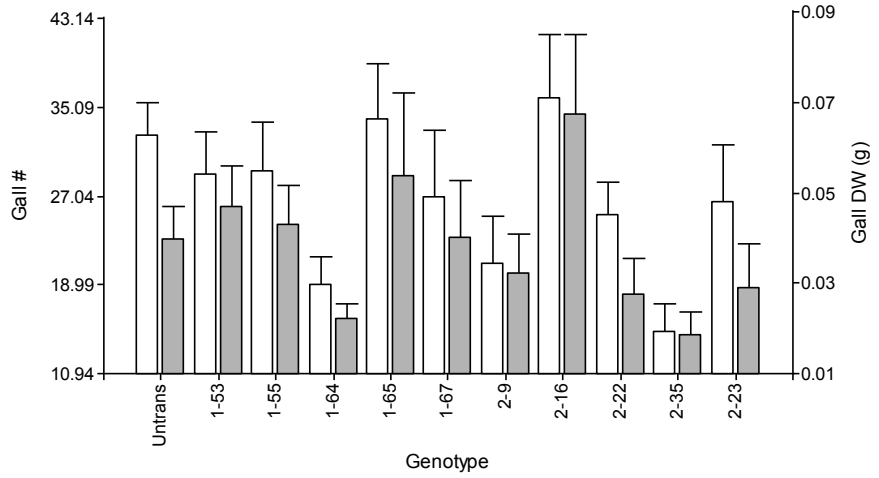


Figure 1. Gall number (white) and gall dry weight (DW) (gray) in 10 transgenic lines of St. George transformed with *XiR1.1* (1-) or *XiR1.2* (2-) after 10 weeks of inoculation. Error bars represent SE.

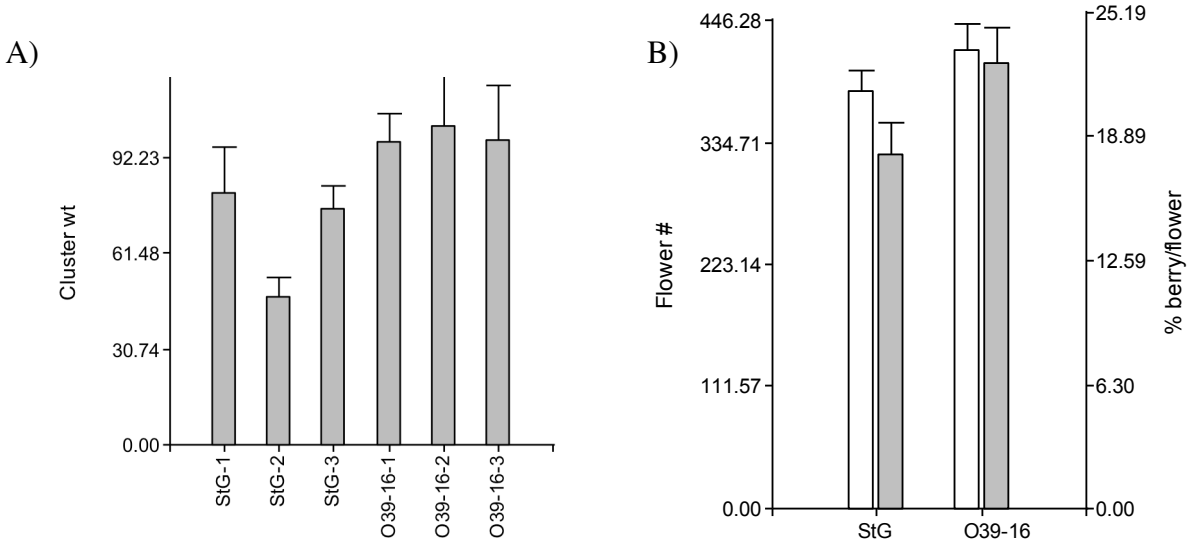


Figure 2. Cluster weight (A) and flower number and fruit % berry/flower (B) of clusters of Cabernet Sauvignon grafted on St George or O39-16. Clusters were sprayed with water: 1; 10µM ZR at bloom: 2, and 10 µM ZR 14 days after bloom: 3. Error bars represent SE.

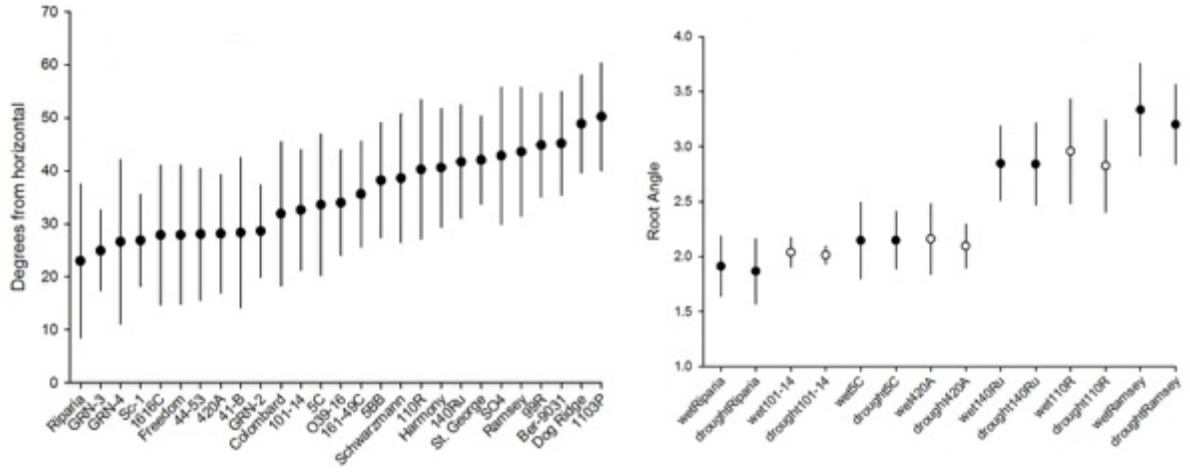


Figure 3. *Left:* Root angles of adventitious roots, derived from rooted herbaceous cuttings of all widely-available commercial rootstocks in California. *Right:* Root angle index of selected commercial rootstocks, grown for two seasons in the field from hardwood cuttings, and following excavation. Error bars represent 1 standard deviation.

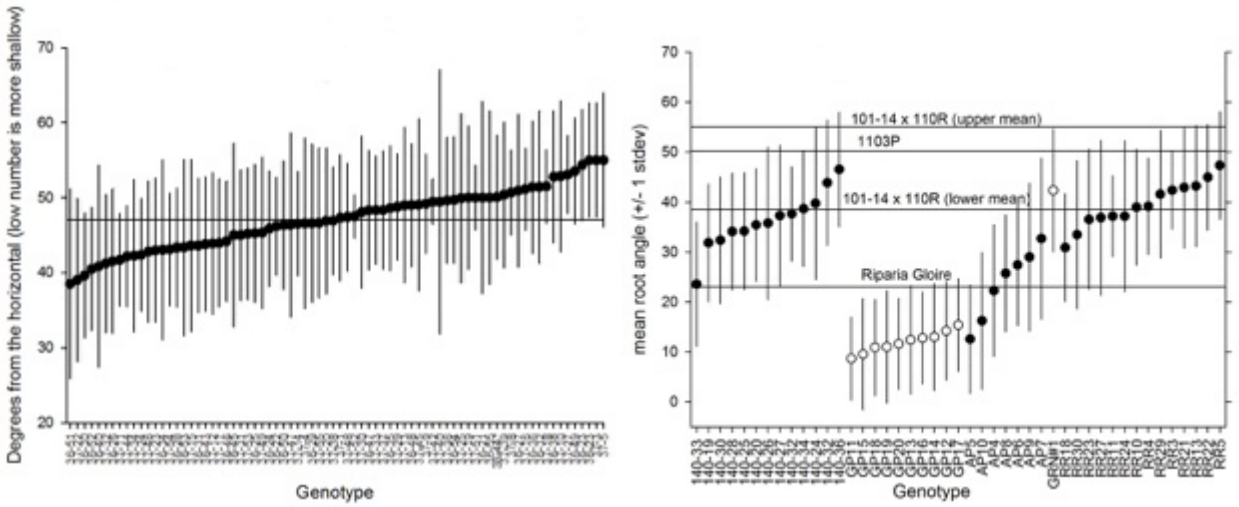


Figure 4. Root angles of adventitious roots. *Left:* 76 hybrids of 101-14 x 110R. Horizontal line at 48 degrees is the mean of all measurements. *Right:* Hybrid populations derived from140Ru, *V. girdiana*, *V. arizonica*, and Ramsey x Riparia. For comparison, horizontal lines refer to means from other screens. Error bars represent 1 standard deviation.

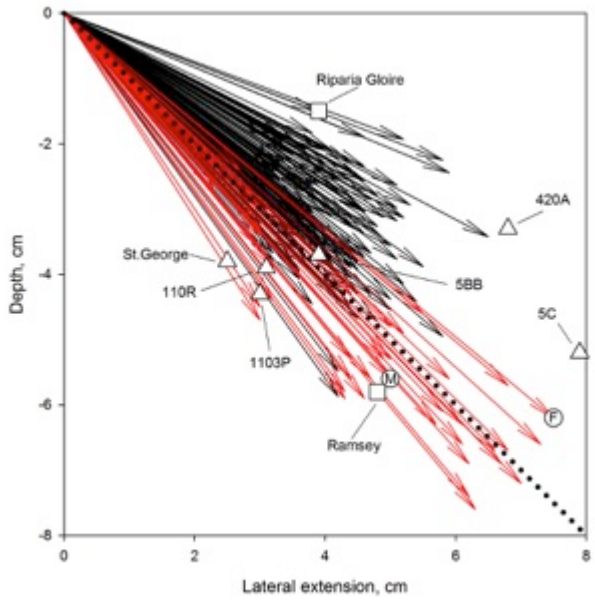


Figure 5. Mean root angles for Ramsey x Riparia Gloire F1 (red) and F2 (black) generations; roots quantified as vectors. Co-screened commercial rootstocks are shown as symbols; "M" and "F" are F2 parents. Dotted line is 45 degree reference line from the horizontal.

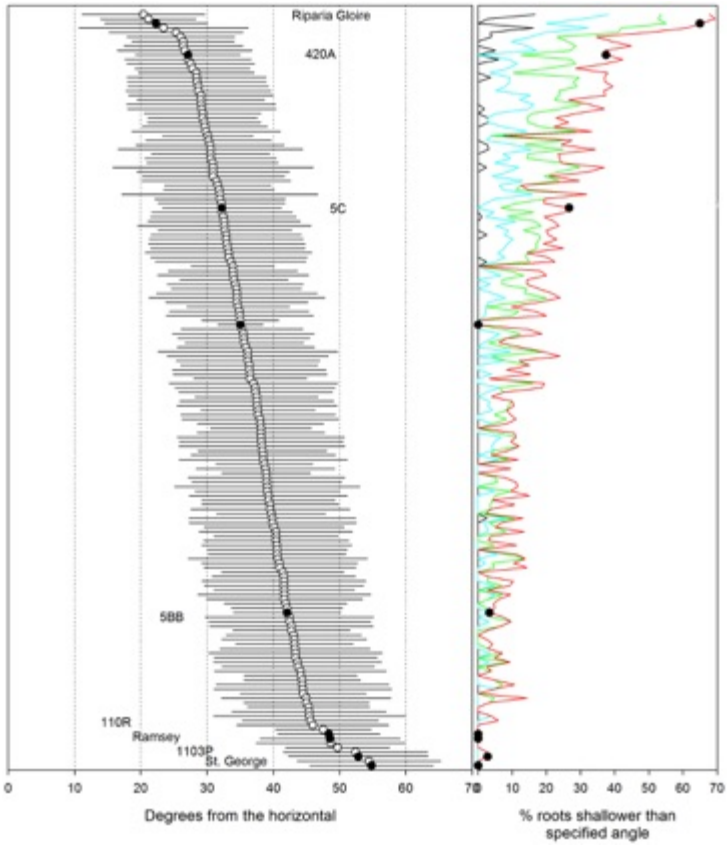


Figure 6. *Left:* Variation of root angles for Ramsey x Riparia Gloire F2 root angles, with commercial rootstock references. Error bars are 1 standard deviation. *Right:* Data analyzed as

root percentages that are below relaxed (red) and stringent (black) threshold values, and two intermediate thresholds (blue and green).

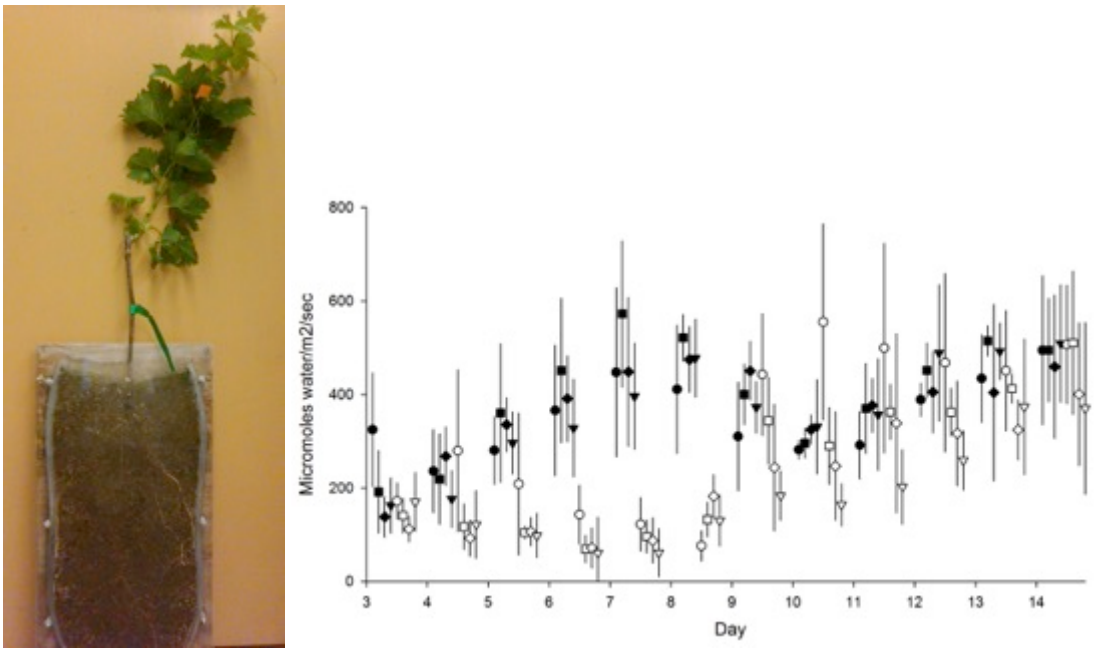
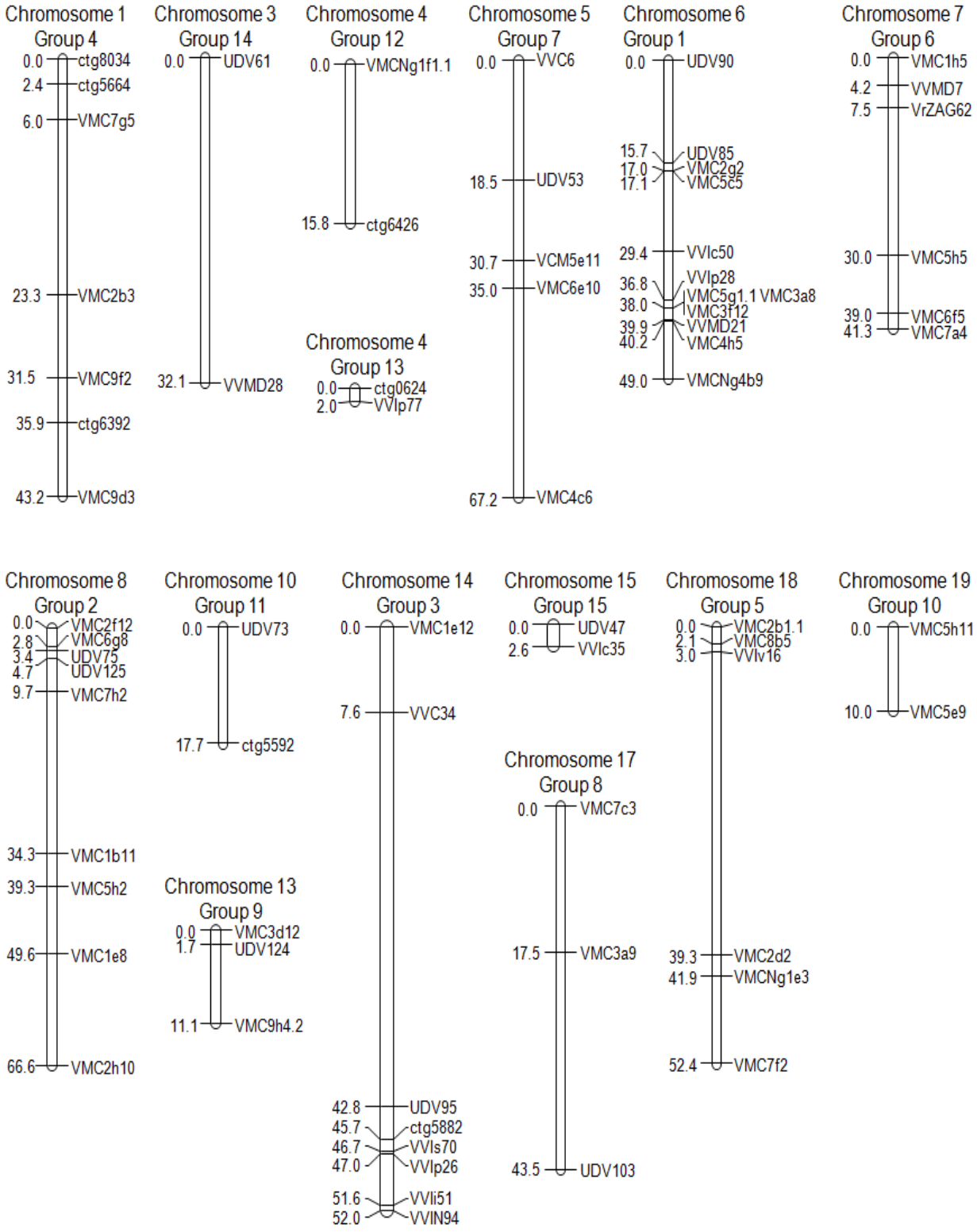


Figure 7. Rhizotron dry-down and recovery experiment, using a common scion (Merlot) on four rootstocks: 101-14, Riparia Gloire, Ramsey, and 110R. *Left:* rhizotron container. *Right:* midday stomatal conductance. Closed symbols, well-watered controls; open symbols, dry-down and recovery treatment.

Figure 8 Map of 15 Initial linkage groups. Linkage groups were calculated by JoinMap4 and map distances are displayed in centimorgans. Linkage groups were sorted into chromosomes through use of SSR markers with known chromosomal assignments.



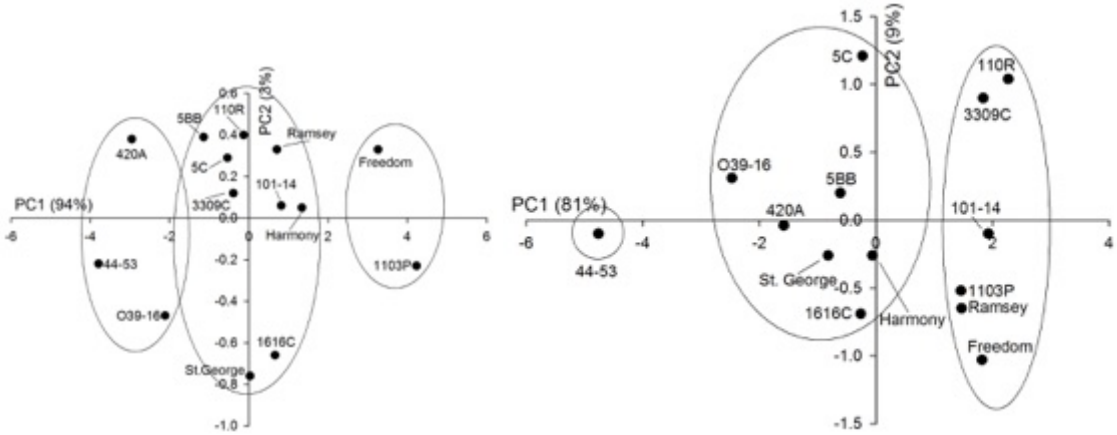


Figure 9. Principle components analysis of pruning weight (left) and yield (right) of Chardonnay grafted onto 13 rootstocks. Data from Dr. Jim Wolpert.

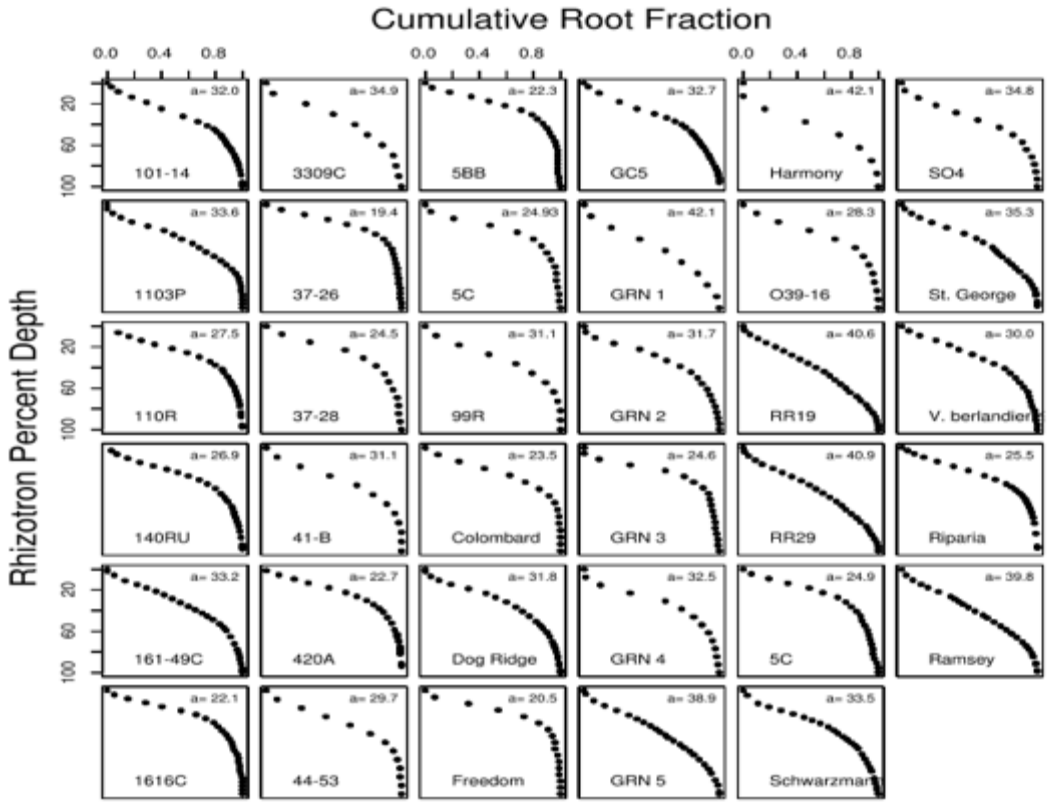


Figure 10. Cumulative Root Fraction of 35 commercially used rootstocks over percent maximum depth per genotype after 5 weeks of growth. Number associated with "a" refers to the area above the curve.

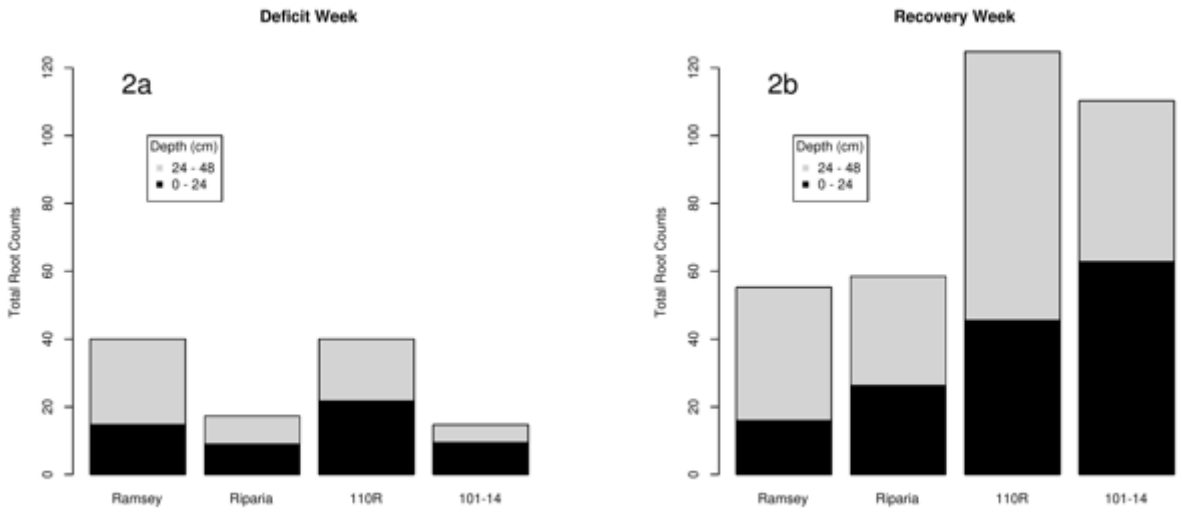


Fig. 11a shows new root counts after a week of no watering and Fig. 11b shows the new root counts after a week of recovery.

Figure 12. Comparison of genotypes over increasing chloride concentration

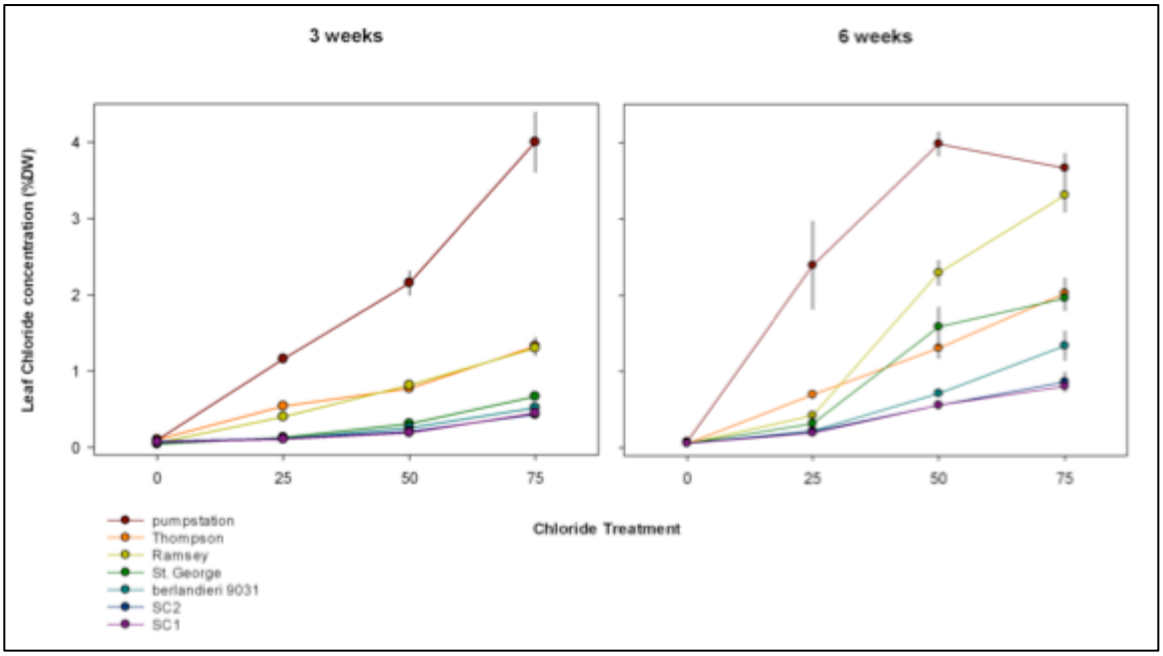


Figure 13. Leaf and Root chloride concentration at all treatment levels

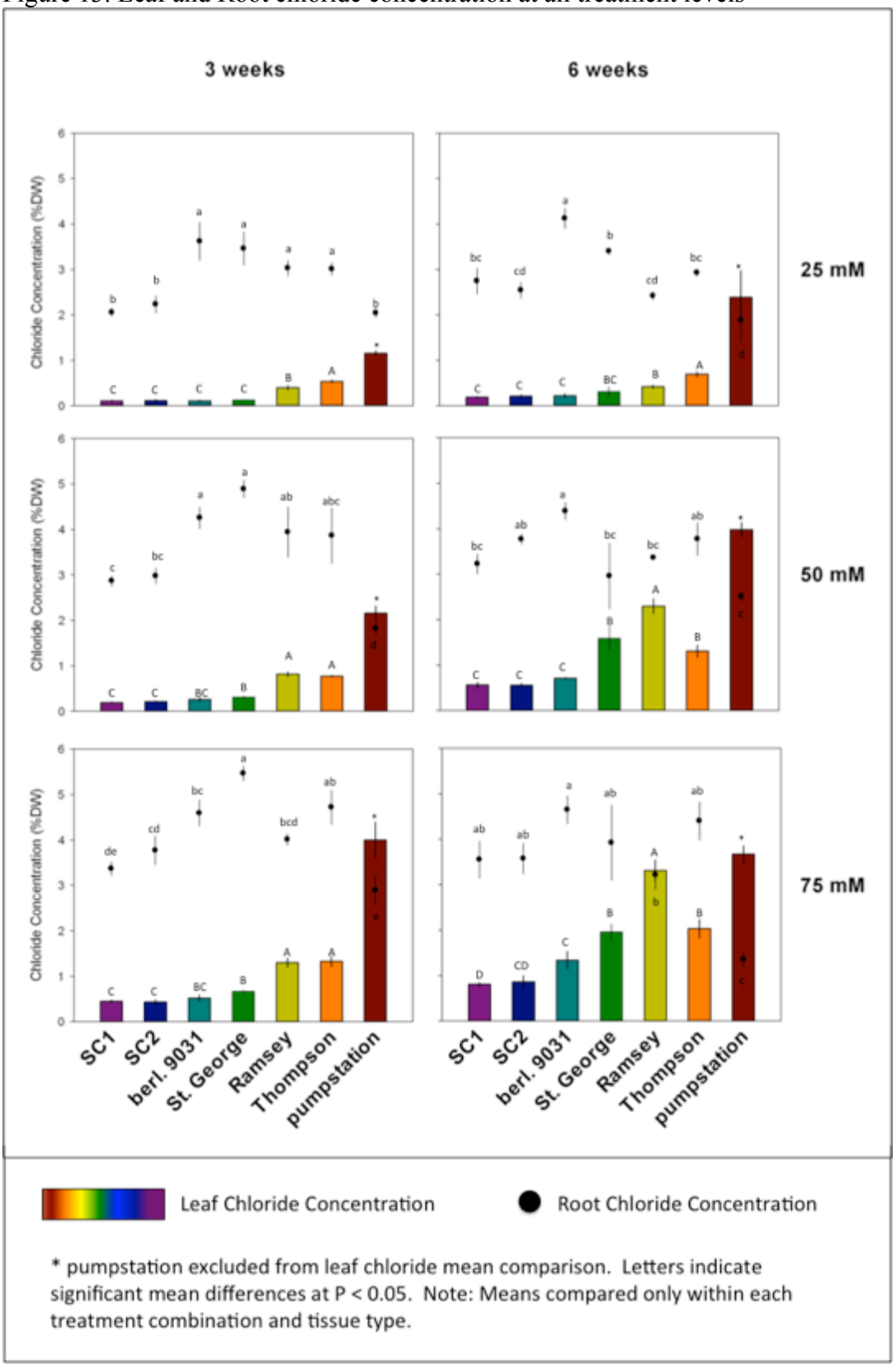


Figure 14. Comparison of the relative growth rate over increasing chloride

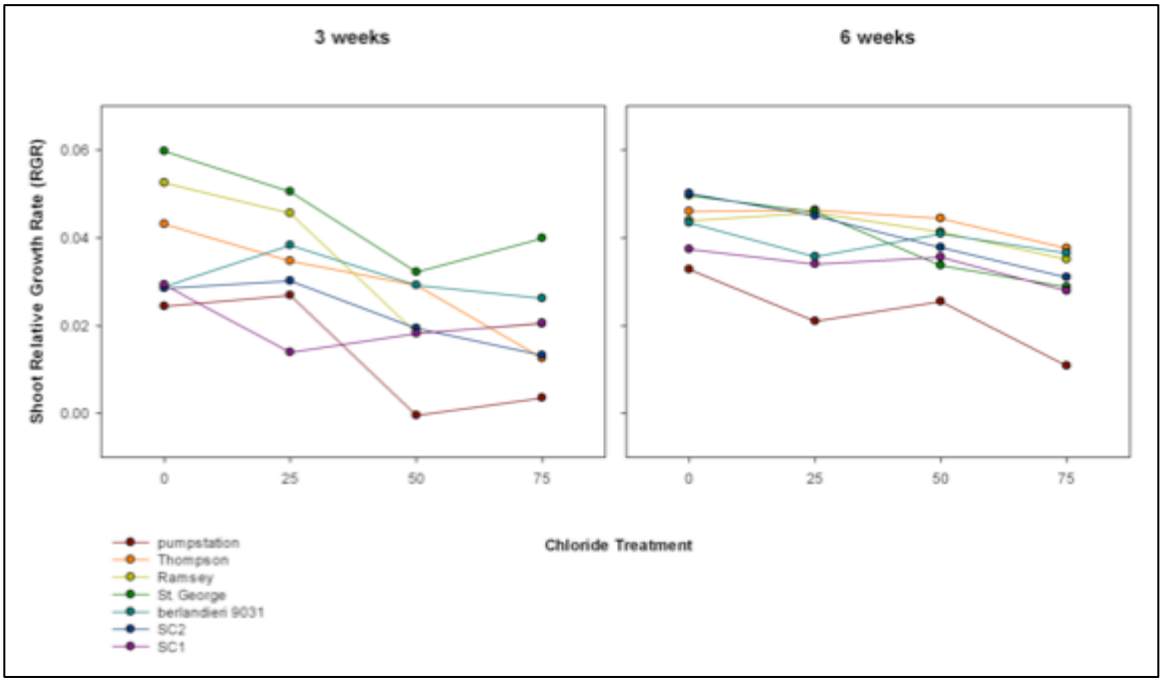


Figure 15. STRUCTURE diagram. Each vertical bar represents a single individual, and the colors represent the percentage assignment of that individual to one of 5 genetic groups. Each section (1-4) groups individuals based on our initial morphological species determination: 1 = *V. acerifolia*, 2 = unknown / hybrid, 3 = *V. doaniana*, 4 = *V. candicans*

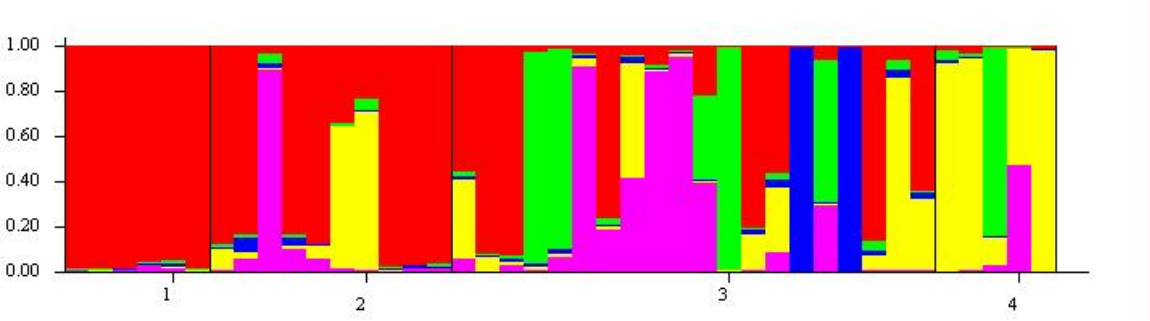


Figure 16. Map of individuals from the *V. doaniana* hybrid origin study. Each symbol on the map is an individual from the bar graph in Figure 15. The highlighted area shows the small region that contains all of the more admixed samples.

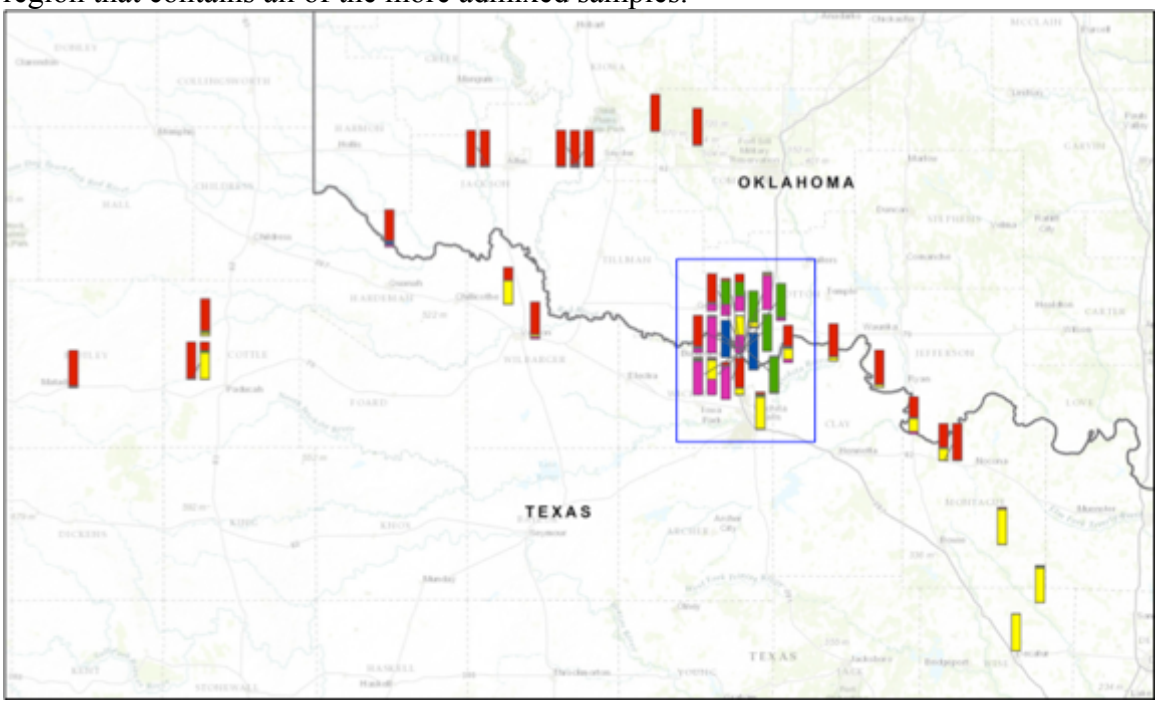
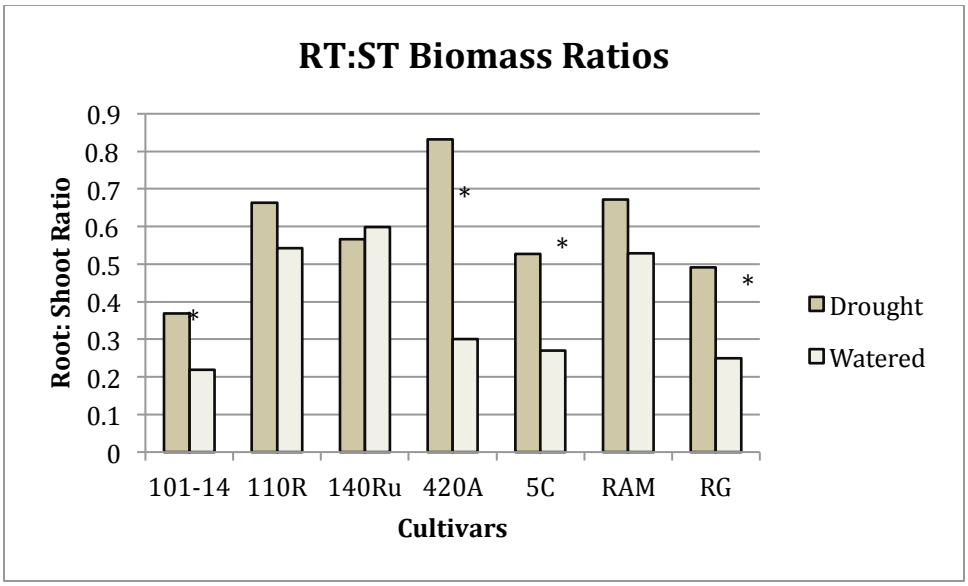


Figure 17. Root to shoot biomass ratios by treatment for each cultivar. Probability values table from analysis of variance statistical analysis between treatments per cultivar



Cultivar	101-14	110R	140Ru	420A	5C	RAM	RG
p-value	0.0003*	0.0402*	0.6300	0.0001*	0.0007*	0.1587	0.0004*

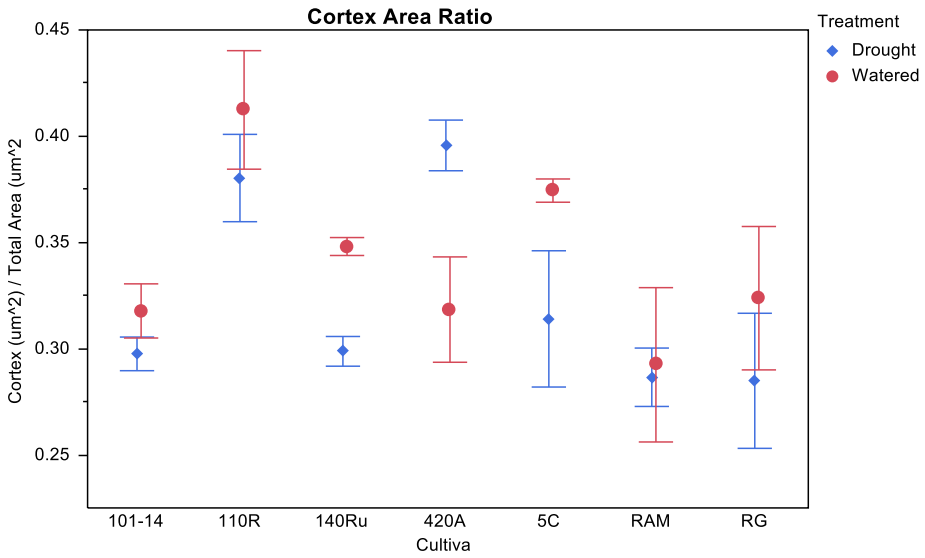


Figure 18. Root cortex area: root total area ratio per rootstock by treatment.

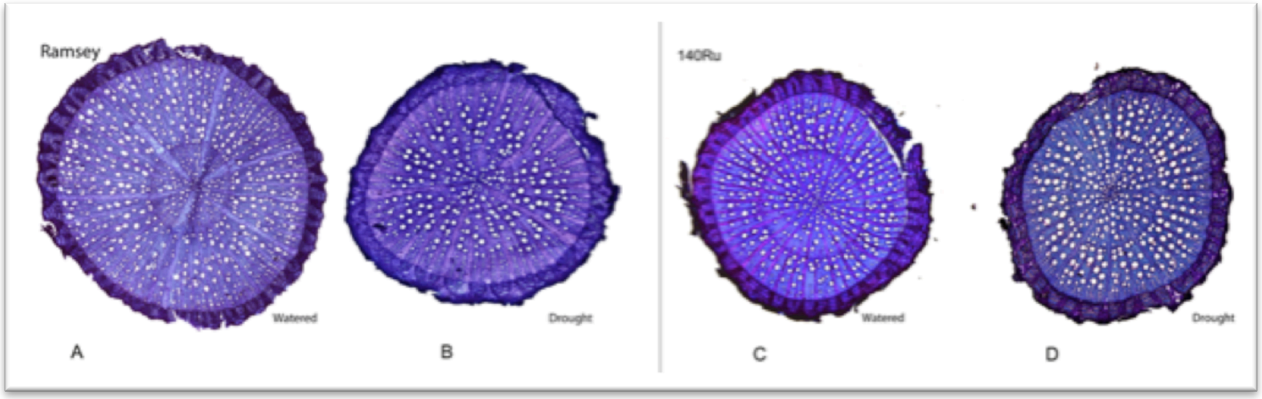


Figure 19. Root cross sections, drought and watered treatments of each cultivar. **A** Ramsey watered **B** Ramsey drought **C** 140Ru watered and **D** 140Ru drought. Smaller vessels started to appear earlier in the season when exposed to drought treatment.

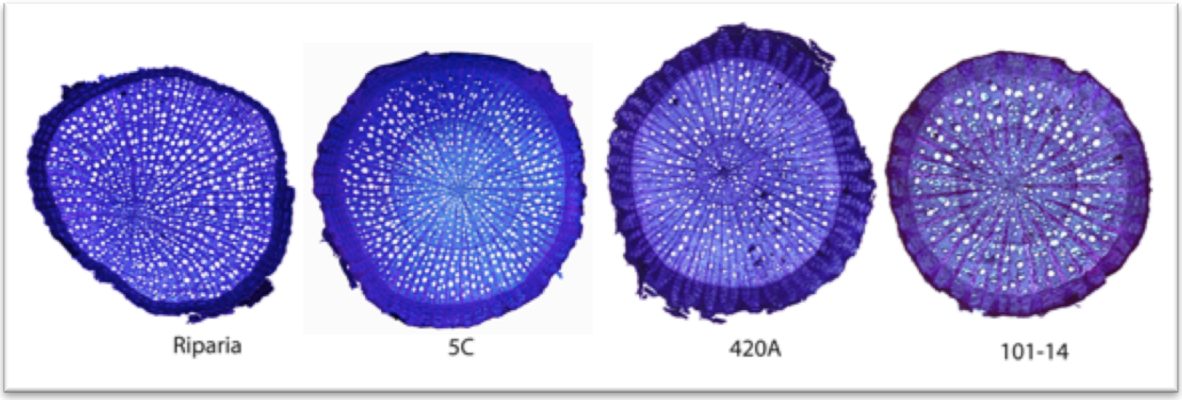


Figure 20. Drought susceptible root cross sections from plants exposed to drought treatment for one year.

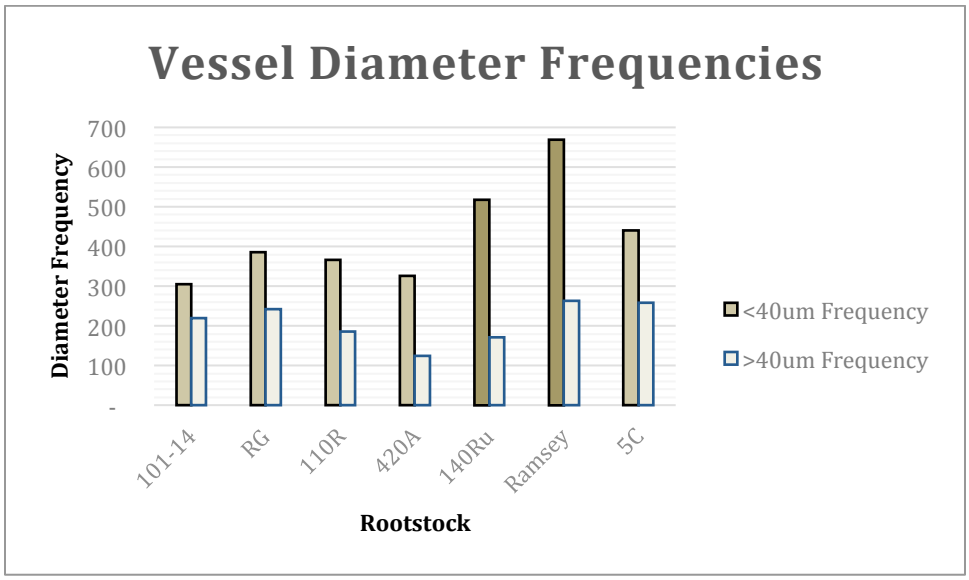


Figure 21. Frequency of vessel diameters <40um and >40um developed under drought for each rootstock.