January 2017 Report
California Grape Rootstock Improvement Commission
California Grape Rootstock Research Foundation
American Vineyard Foundation
CDFA Improvement Advisory Board
California Table Grape Commission

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

Reporting Period: July 2016 to January 2017

2016 Pollinations – The 2016 crosses are presented in Table 1. The 47 crosses addressed combining strong sources of chloride exclusion with deep rooting and broadly based nematode resistance. Some of these crosses also incorporated boron resistance and rotundifolia based resistance from fertile VR hybrids.

Screening of seedling populations for resistance to nematodes – We first examine populations for horticultural characters and screen out brushy, short internode seedlings. The remaining seedlings are tested for ease of rooting and then go into testing against our combination of two strains of root-knot nematode we call HarmA&C. One of these strains is *Meloidogyne incognita* and the other *M. arenaria* (although testing is underway to better characterize these strains). These nematodes are reared on tomato but tested on Harmony every 2-3 generations to ensure that their aggressive feeding behavior is not lost. We also use Harmony (occasionally Freedom) and St. George as our susceptible controls in these tests. Selections with successful results in these bioassays will be moved forward to ring nematode testing. Dagger nematode testing has been slowed by a lack of inoculum due to very low numbers in the field as a result of the drought.

Screening of the backlog of genotypes as well as new populations for nematode resistance (root knot nematode RKN, ring and dagger) continues. 91 nematode tests were conducted in the second half of 2016; many were to further characterize the rootstock selections used in field trials in L block in the UC Davis vineyards. The remaining tests were on rootstocks with high priority parentages, good horticultural characteristics and good propagation. Of these, 22 were found resistant in their respective bioassays, and will be moved forward and tested on other nematodes this year (Table 2). 115 genotypes were propagated and are in the greenhouse and will go into testing starting in 3 weeks as bioassay bench space becomes available. In the second half of 2016, we secured another greenhouse to be used exclusively for bioassays, and will be using a shorter bioassay length. With these adjustments, we hope to test about 175 genotypes by June 2017.

Approximately 700 vines in the vineyard have recently been marked for removal due to poor horticultural characteristics, poor rootability, or nematode susceptibility as seen in bioassays. We have taken cuttings of 299 genotypes this winter; these are now in cold storage and the callus stage, ready to be used in bioassays. The genotypes include the field trial selections that didn't make it into last year's bioassay pipeline, as well as those genotypes with previous good results that are moving forward into further testing. Work continues on building a large enough population of dagger nematodes to include the species in all RKN testing, cutting out another step on the road to a rootstock resistant to all three nematodes.

Root-knot nematode testing and mapping – Efficient and quick root-knot nematode (RKN) screening is essential to develop new resistant rootstock varieties. We are improving our RKN screen to develop quick and robust phenotyping system to screen new germplasm and mapping populations at a larger scale. We are maintaining nematodes on tomato plants; the roots of which are loaded with gelatinous nematode egg masses. Previously a modified Baermann funnel technique under mist has been used to extract juveniles from egg masses to provide inoculum for screening. However, extracting sufficient numbers of nematodes takes up to week, even when many tomato plants are used. Nematode viability might be impacted due to extended extraction times. We have found that extracting the eggs directly from the egg masses produces a good quality and quantity of

inoculum in one hour. Moreover, a highly infected single tomato plant can provide over ~600 000 eggs compare to ~12 000 juveniles using the previous technique. Preliminary inoculation trials show that inoculation with eggs is more effective and results in a higher infection rate (Figure 1). We have also effectively reduced the screening time from four months to six weeks. Our trials with the eggs/juvenile inoculations show that this reduced time is enough for the nematodes to infect the plants and generate egg masses. The screening bottleneck continues to be scoring the plants in the trial, where within a limited time a large number of roots need to be examined under microscope. Reduced screening time facilitate higher number of plants to be screened. When root systems are smaller, it is easier to stain and count egg masses. Besides that, smaller plants also mean more efficient use of greenhouse space.

Screening germplasm and using existing mapping populations – We possess an extensive germplasm collection as a result of 25yrs of field work. We are beginning to use this collection to examine a wide range of species for nematode resistance. Based on genetic diversity analysis we selected 79 genotypes. Figure 2 represents the geographical distribution of the selected accessions and Table 3 lists the accession that are propagated and will be tested in the Spring 2017. Existing populations are being screened for segregation of RKN resistance (for eg: *Vitis vinifera* F2-35 X *V. berlandieri* 9031). New crosses are proposed for Spring 2017 with known resistant germplasm to develop new mapping populations. All these populations will be screened to determine inheritance of resistance. Developing a rootstock with resistance from multiple backgrounds of RKN resistance is desirable and is more likely to provide a long lasting and durable field resistance.

Molecular identification of purity of existing RKN isolates – Currently we are maintaining three isolates from two RKN species. Existing molecular markers show limited to no levels of diversity below species level. We are characterizing the isolates and monitoring the purity of the strains. We will be running a limited coverage genome sequencing to gain more information on our strains. The generated data with the published genome and EST sequences could allow us to develop molecular markers capable of characterizing the isolates.

Drought, salt and boron resistance – Kevin Fort

Nutrient testing of leaf tissue from shadehouse chloride screen – In January 2016, results of a two-year common-scion study were presented that validated the superior chloride exclusion capacity of four wild accessions of *Vitis*: two accessions of *Vitis acerifolia* ('longii 9018' and 'longii 9035'), one accession of *Vitis treleasei* ('NM03-17') and one accession of *Vitis girdiana* ('SC12'). During this study, leaves of salt-treated plants developed a classic chloride toxicity symptom: necrosis of the leaf margin that spreads inward until the entire leaf dies. However, leaves of some genotypes also developed an interveinal necrosis that we had not observed in previous salt screens (Fig. 3). This symptom was consistent with that observed in studies of other crop plants exposed to high levels salinity and generally indicates a deficiency of one or more cations, typically potassium, calcium and magnesium. It was possible that we had not observed this symptom in the past given that we usually screen at toxic, but relatively moderate, salinity concentrations corresponding to those frequently seen in salt-affected vineyard soils (25-50 mM NaCl). On occasion we had used high salt levels (≥ 75 mM NaCl), but these screens employed relatively short exposure periods (2-4 weeks). Because of the value of the results in this experiment, it was important to ensure that this leaf symptom was in fact a cation deficiency and not an unexplained result that was in some way relevant to the superior performance of the aforementioned *Vitis* genotypes. In this study, St. George exhibited the highest frequency of this leaf phenotype, and nutrient data of leaf tissue for St. George are shown in Fig. 3. For salt-treated vines, values of K, Ca and Mg were significantly reduced. Also assayed were the leaves of salt-treated Ramsey and Dog Ridge, and these also showed reduced K uptake. Ca and Mg did not differ for Ramsey and Dog Ridge in salt-treated plants versus controls, but this is likely due to both a higher uptake of these nutrients and to the use of sampled basal leaves that may have acquired their Ca and Mg stores prior to the onset of the salt treatment. The wild accessions did not have unsalinized controls, and so could not be compared as with St. George, Ramsey and Dog Ridge; however, the leaves of the wild accessions were completely symptom-free, indicating that these genotypes can acquire adequate levels of essential nutrients even in the presence of very high salinity.

Drought resistance screen of 20 *Vitis* genotypes– In June 2016 we reported the final analyses of a rhizotron project wherein four grafted rootstocks (Merlot on 101-14, Riparia Gloire, 110R and Ramsey) were subjected to a

period of sustained drought stress, followed by a daily-irrigated recovery period. In that study, stomatal conductance during the recovery period was found to be an excellent correlate of drought resistance capacity in these genotypes. Unfortunately, a significantly expanded screen for drought resistance would inevitably face the time constraints imposed by the porometer (~2.5 minutes per measured leaf sample) and the need to make measurements within a two-hour window of time at midday. Therefore, it was also reported in June 2016 that we had tested the use of a thermographic camera and an infrared thermometer, both of which can theoretically be used as a substitute for stomatal conductance and both of which provide nearly instantaneous measurements. These methods could potentially be used in much larger screens of drought resistance. The promising results we obtained initiated a greenhouse-based drought resistance study of potted vines and with a corresponding field component. The goal of this study was to determine if principles obtained from the rhizotron work could be applied to a relatively large screen of plants under drought stress that would result in a reasonable rank order of drought resistance capacity using rapid phenotyping methods, especially leaf temperature. Other variables measured included specific leaf area, leaf area at harvest, root and shoot dry biomass, root suberization data derived from stained root cross sections and root fibrosity derived from digitized root systems. Also included in this study were the same 20 genotypes planted in the field in fabric pots that permitted the use of heavy field soil rather than standard potting media. Our report in January 2016 indicated that growth in heavy field soil is critical for obtaining high quality phenotypic data. In the greenhouse, two harvests were conducted, allowing for an analysis of growth rate. Seventeen rootstocks and three *Vitis vinifera* controls were used, as follows: 101-14, Riparia Gloire, 1616C, 5C, Schwarzmann, Freedom, 110R, 1103P, St. George, 140Ru, Ramsey, Dog Ridge, GRN-1-5; for *V. vinifera* controls: Colombard, Cabernet Sauvignon and Chardonnany.

Leaf temperature using an infrared thermometer performed to expectation, allowing the rapid collection of data. For 101-14 and Ramsey, leaf temperature data was compared to stomatal conductance readings also taken on these rootstocks (Fig. 4). In this figure stomatal conductance was high for daily-irrigated plants, as expected, and the cooling effect of this higher transpiration rate was reflected in corresponding lower leaf temperatures. The leaf temperature data generated over the course of the three drought and recovery periods in this experiment is currently being analyzed.

Leaf area and biomass data sets are now complete in both their collection and analysis. As was demonstrated in the rhizotron study, rootstocks that are devigorating to the scion and are known to be drought sensitive from field trials also produce relatively rapid growth in greenhouse container culture, likely due to the rapid proliferation of roots that are also drought sensitive. Because of this sensitivity, these genotypes show a greater reduction in leaf area production in plant cohorts concurrently exposed to drought conditions. By multiplying leaf area at harvest by the reduction of leaf area in drought-exposed plants, an index for drought sensitivity was produced from plants in this study that correspond with previously-established characterizations of drought sensitivity (Fig. 4). Five genotypes that grew poorly in this study (all three *V. vinifera* genotypes, 1616C and GRN-1) were not comparable and are not presented in Fig. 5. Interestingly, three of the four remaining GRN rootstocks ranked on par with two drought-resistant rootstocks, Ramsey and Dog Ridge. Because the GRN rootstocks have not been formally evaluated for drought resistance in previous work, it is a promising result that these rootstocks that have high nematode resistance might also already have excellent drought tolerance. It is also a promising result that drought resistance can be obtained from a rapidly-obtained measure from the shoot system, given the generally labor- and time-intensive nature of obtaining data from root systems. Lastly, and as expected, shoot biomass correlated very strongly with leaf area, indicating that the same results can be obtained by merely weighing the shoot system—an even more rapid method of obtaining these data.

The final phase of the drought resistance experiment involves the characterization of suberin within the root of all genotypes both for drought-treated and daily-watered controls (Fig. 6). This data, currently being generated in the collaborating laboratory of Dr. Andrew McElrone, should be complete in early 2017. It is possible that drought-resistant genotypes produce thicker suberin layers within the roots that aid in water conservation and therefore maintained hydration during drought periods, and this data set will address this possibility. Lastly, field vines (Fig. 6) will be harvested in January 2017 and analyzed both for root fibrosity and thickness, previously established as a high-quality indicator of drought resistance, and for specific root length, which could be a more rapid indicator of drought resistance in the roots than digitizing entire root systems.

Boron exclusion screen of 20 *Vitis* genotypes– In June 2016 we reported on the successful development of an in-house boron concentration assay that could be used in future screens of wild *Vitis* germplasm to discover novel sources of boron exclusion. As was true in our work on chloride exclusion, an in-house assay is critical for the affordable analysis of large numbers of plants, given the exorbitant cost of outsourcing leaf tissue samples. Unlike chloride analysis, the analysis of boron from leaf tissue is more technically challenging, and a number of methodological hurdles needed to be overcome to make this a working assay. With this stage complete, we applied this assay to a study of wild *Vitis* species screened in 2013, and the resulting data is presented in Fig. 7. In this study, potted vines of ungrafted wild species were irrigated with a 3 ppm B solution or a 0.5 ppm B control solution. Five accessions of *Vitis girdiana* were found to have relatively strong boron exclusion, and one accession (SC11) had a boron concentration similar to 0.5 ppm B controls. A separate study of commercial rootstocks was performed at the same time as the wild species screen, and boron concentration in the leaf tissue is currently being measured.

Publication

A manuscript detailing the rhizotron-based drought tolerance study was completed, submitted to the Journal of the American Society for Horticulture Science, and was accepted. The title and abstract for this paper, to be published in 2017, follows:

Early Measures of Drought Tolerance in Four Grape Rootstocks
Kevin Fort, Joaquin Fraga, Daniele Grossi and M. Andrew Walker

Abstract. Recent and severe droughts in major grape-growing regions of the United States and Australia underscore the importance of more efficient agricultural use of water. Grape rootstock breeding for increased drought tolerance could contribute to continued sustainable yields as fresh water supplies decline. Rhizotron containers were used in a greenhouse to investigate predictive measures of drought tolerance in young grapevine rootstocks. Deeper rooting distributions were found for the drought-tolerant rootstocks '110R' (*Vitis berlandieri* x *Vitis rupestris*) and 'Ramsey' (*Vitis champinii*, a natural hybrid of *Vitis candicans* x *V. rupestris*) as opposed to shallower distributions observed in the more drought-sensitive rootstocks '101-14Mgt' (*Vitis riparia* x *V. rupestris*) and 'Riparia Gloire' (*V. riparia*). Production of new roots during a 6-d non-irrigated period declined 45% to 53% for 'Riparia Gloire' and '101-14Mgt', respectively, but showed no change in '110R' and 'Ramsey'. Slow growth, a hallmark of abiotic stress tolerance, was evident in the drought-tolerant rootstocks in their relatively slow shoot growth prior to drought stress and their relatively slow new root growth during recovery, especially

***Vitis berlandieri* and root system architecture – Jake Uretsky –**
The lime-tolerant grape species *Vitis berlandieri* (*V. cinerea* var. *helleri*) was initially incorporated into rootstock breeding programs after iron-chlorosis was observed in vines grafted to early rootstock cultivars planted in limestone-based soils. In addition to lime-tolerance, rootstocks with *Vitis berlandieri* parentage, particularly *V. berlandieri* x *V. rupestris* hybrids, generally perform well in dry conditions, and adaptation to the shallow soils and acute moisture fluctuations characteristic of the Texas hill country where *V. berlandieri* naturally resides indicates the value of the species for developing rootstocks for sub-optimal growing conditions. Despite the historical and future breeding potential of *V. berlandieri*, very few accessions exist in germplasm repositories. We previously reported the acquisition of new *Vitis berlandieri* accessions in the Texas hill country during the summer of 2015 and winter of 2016.

Our objectives with these accessions include: 1) Expand *V. berlandieri* germplasm available for breeding; 2) Define the species genetically, geographically, and phenotypically; 3) Assess the genetic and phenotypic diversity in the species, especially for characteristics relevant to rootstock breeding; and, 4) Incorporate the new material into our rootstock breeding.

Genetic and geographic delineation. We used molecular markers to characterize the genetic and geographic extent of *V. berlandieri* as a distinct entity from *V. cinerea*, a species with limited breeding value. DNA samples

for *V. cinerea* utilized in this study were collected in eastern Texas in the summer of 2016. To reduce sampling bias, our analysis included accessions of *V. candicans*, which is morphologically, phenologically, and genetically distinct from *V. cinerea* and *V. berlandieri* but is found across the range of both species within the collection areas. DNA from *V. berlandieri* accessions in the Wolfskill and Montpellier repositories was also included to compare classic and current *berlandieri* accessions.

Our results show a clear grouping of *V. berlandieri* collected in the Texas hill country apart from *V. cinerea* collected in East Texas (Figures 8 and 9). Also, recent *V. berlandieri* collections group with the classic *V. berlandieri* maintained in germplasm repositories (Figure 1). Accessions that strongly group as *V. berlandieri* are concentrated in a fairly narrow region, and *berlandieri* x *cinerea* hybrids are most prevalent along the eastern boundary of that region (Figures 9 and 10). Lastly, some accessions formally grouped with *V. berlandieri* as *V. cinerea* var. *helleri* form a distinct grouping, based on our analysis (Figures 8 and 9). This group has been labeled 'b-series' after the accession IDs.

Environmental adaptability. We are also utilizing environmental data to help predict the phenotypic variation and adaptability that might exist in newly collected material. Figure 3 shows the geographic range of any accessions currently in our vineyard collection that are either *V. berlandieri* or hybrids that possess a large proportion of molecular markers associated with *V. berlandieri*. Our germplasm represents a range of soil environments, particularly regarding depth to a restrictive layer and limestone content, and mean annual precipitation diminishes along an east to west gradient of which are germplasm is representative (Figure 10; Table 4). The ability for root systems to penetrate deeply into soil, even through compacted soil, is likely a vital trait for maintaining water uptake in dry conditions and throughout the growing season. Accessions with combined adaptation to low rainfall and shallow soils are particularly interesting from a breeding perspective. Also, some lime-tolerant *V. berlandieri* rootstocks have been shown to exclude chloride in our trials, suggesting an association between these traits that could be exploited for breeding rootstocks with improved salinity tolerance.

It should be noted that certain environmental variables, especially soil characteristics, are difficult to ascertain on a fine scale and must be interpolated from other known data. In other cases, land use, including agricultural, industrial, and residential, may have drastically changed the environmental characteristics since accessions were collected. Nevertheless, these data can be useful guides to maximize phenotypic diversity in the germplasm and to inform germplasm utilization in the breeding program. We will continue to refine our genetic and environmental data to better grasp the variation in our material and, possibly, to identify molecular markers associated with particular environmental conditions that could be useful in breeding.

Phenotypic variation. Little is known about phenotypic variation in *V. berlandieri*. We are currently assessing the germplasm for various traits, including rootability, salt tolerance, nematode resistance, Pierce's disease resistance. Also, we are attempting to optimize techniques for propagating this germplasm, since the species is initially slow growing and has thus far required hybridization with other *Vitis* spp. for commercially viable rootstocks. Our initial observations indicate that there is significant variation in our accessions for ease of propagation. Improving the ability to propagate *V. berlandieri* accessions and selections would allow better utilization of traits that make this species unique.

2016 *In Vitro* Chloride Toxicity Evaluation in Grapevines – Cassandra Bullock — Grapevine salt tolerance has primarily been assessed in our lab through a verified greenhouse assay in which we determine chloride exclusion capability (Fort et al. 2013). This method involves growing plants in the greenhouse in fritted clay, applying a measured concentration of NaCl for the duration of usually around two weeks, destructively harvesting all tissue and determining the chloride that has accumulated in the tissue. This method gives clear and repeatable rankings as far as how much a given genotype accumulates or excludes chloride compared to other genotypes in the same assay.

July through September 2016, we screened 4 genotypes in the greenhouse including 140Ru, O39-16, Ramsey and Riparia with different salt concentrations: 0, 25, 50, and 75mM NaCl. 300 green cuttings of each genotype were taken from FPS, dipped in 1:20 auxin dilution, and were left to establish in the mist room in perlite flats for 2

weeks. The cuttings were then transplanted from the perlite flats into 1 gallon pots with fritted clay. Over the course of 4 days, plants were checked for survival and replaced if they prematurely died.

Pots were then arranged into a split-split plot design, so that salt level (0, 25, 50, and 75mM NaCl) was the main plot, the harvest date (3 weeks with 3 harvests) was subplot, and the genotype was the sub-subplot. 8 replicates were planted for each genotype, salt treatment level, and harvest date, for a total of 384 pots.

After a week of establishment in the greenhouse, the salt treatments were applied. Plants were harvested in 3 time periods over the course of 3 weeks, with the first harvest occurring after 1 week of salt treatment. Roots from the harvested plants were scanned and analyzed with WinRHIZO^{TM}, which is an image analysis system that can measure different root attributes including: root length, area, volume, and can separate varying root sizes into classes. Leaves and roots were then dried in individual bags for two weeks, ground, and then analyzed for chloride content with the Nelson-Jameson M926 Chloride Analyzer System. We are still analyzing week 2 data, grinding roots for all of the weeks, and we will then need to analyze the root tissue for chloride content.

All genotypes experienced a general decline in biomass and root elongation, with an increase in leaf chloride percentage as the concentration of salt applied increased (Table 5). Genotypes that have been characterized for salt tolerance in previous screens show the expected segregation of genotypes, with O39-16 accumulating the most chloride, followed by Ramsey, Riparia, and 140Ru, respectively (Figure 11)($p<0$, $R^2 = 0.7488$). Standard deviations are low, which may be the result of a shorter study time frame and/or earlier application of salt treatment from the time of cuttings. Under salinity stress, especially in the higher concentrations (50mM and 75mM), the analysis of variance determined that the lateral to structural root ratio was significantly affected by the percent of chloride accumulated in leaf tissue ($p <0$). Figures 11 and 12 show a correlation between the lateral to structural root ratio and chloride accumulation in the leaf tissue at higher concentrations of applied salt (At 75mM, $R^2 = 0.7309$). The genotypes segregate according to leaf chloride accumulation in leaf tissue. With salt application, all genotypes showed a slight increase in the lateral to structural root ratio, but genotypes 140Ru and Riparia, which accumulated less chloride, did not have as significant of an increase as O39-16. This phenomenon may be attributed nutrient uptake inhibition as the result of salt stress. It is also interesting to note that all genotypes, except Ramsey, showed a decline in the total length of lateral roots. When only the lateral roots are compared to percent chloride accumulation without the ratio, R^2 drops to 0.3206. This may suggest that the use of ratios, or comparing an individual plants' growth pattern to itself, may reduce some of the variability caused by the differences in individual plant growth phase or environmental effects.

We also examined specific root length (SRL), which measures the total length of the root with the root dry weight (cm/g). SRL increases for all genotypes with increased application of salt, meaning less biomass was produced as roots were lengthening (Figure 13) (At 75mM, $p<0$). O39-16 had the highest values for SRL at all concentrations and was the only genotype statistically different than the rest ($p<0$). However, genotypes with lower SRLs were not statistically different from each other at a given concentration (Fig. 14; At 75mM, Riparia, Ramsey, and 140Ru $p<0.1$).

Boron tolerance in different rootstock varieties – Spencer Falor-Ward – Due to drought and the increased use of poor quality groundwater, soil concentrations of boron (B) are reaching damaging levels in some of California's grape growing regions. Grapevines are considered to be a B sensitive crop with a threshold value of B in soil solution of 0.5 to 0.75 mg L^{-1} (0.05 to 0.074 mM). At concentrations of 0.80 mg L^{-1}, toxicity symptoms, such as chlorosis, necrosis of older tissues and reduced growth of young tissues, begin and result in decreased vine vigor, yield and longevity. It is often not possible to leach B from the soil with high-quality water, nor use organic compounds to immobilize or inactivate it. The use of B tolerant rootstock cultivars is one means by which B could be managed. The identification of B tolerant wild species or commercial rootstock cultivars is needed to breed new tolerant rootstocks capable of growing in high B soils. This study examined 15 grape rootstocks and *Vitis* species using *in vitro* growing conditions and four concentrations of B ranging from 1ppm to 20ppm in an effort to identify B tolerance. The results indicated that there were different degrees of growth and B uptake given the B concentration in the tissue culture media. Order ranking based on index scoring, dry wt. and B % in dry wt. indicated that the *Vitis* species accessions NM 03-17-S01, T 03-15 and Longii 9018 were B tolerant. These accessions will be retested under *in vitro* and field conditions.

Inheritance of GFLV Tolerance Trait in a 101-14 x Trayshed Population (Andy Nguyen) – We have started work on a project to study the inheritance of grapevine fanleaf virus tolerance that has been observed in O39-16. Crosses of 101-14 Mgt x *Muscadinia rotundifolia* 'Trayshed' were made several years ago and the resulting progeny have been growing in our vineyard. We have recently taken hardwood cuttings from 50 of these vines to use for bench grafting later this season. These cuttings will be grafted to cuttings of GFLV-infected Cabernet Sauvignon obtained from a vineyard in Rutherford, CA. We will be using the infected Cabernet Sauvignon as both a scion and a rootstock for our grafts. The grafted plants will be planted at UCD and observed for differences in fruiting characteristics (mainly the presence of the characteristic fruit set symptoms of GFLV). For the reciprocal graft (infected Cabernet Sauvignon as the rootstock with the 101-14 x Trayshed progeny as the scion), we will be taking leaf samples from the grafts three to four months after grafting for RNA extraction and use qPCR to quantify relative GFLV concentrations in the scion. The purpose of this portion of the project is to observe any differences in GFLV multiplication among the different members of the population. Additionally, we hope to find a correlation of relative GFLV concentration with severity of GFLV fruit set symptoms when the respective 101-14 x Trayshed progeny used in these experiments. We expect initial results as soon as May 2017 (mainly the differences in the population regarding relative GFLV concentration), but any observations regarding fruiting characteristics will take longer as these vines need time to grow in the field.

Screening of Fertile VR Hybrids for GFLV Tolerance – Simultaneously, we will also test 15 VR (*vinifera/rotundifolia*) hybrid genotypes with *Muscadinia rotundifolia* in their backgrounds using the same method. These VR hybrids have some degree of fertility, which makes them more attractive for study as they can potentially be used for future crosses.

Induction of Fanleaf Tolerance by O39-16 – For the purposes of starting a study regarding the mechanics of the induction of GFLV tolerance by O39-16, we have young vines of healthy Chardonnay grafted on O39-16, GRN-1, and St. George (20 of each combination) in our fields. We plan to inoculate half these vines with GFLV by bud grafting this upcoming spring. Through high throughput sequencing done at FPS, we verified that our infected plant (our inoculum source) is infected with GFLV, but also with both known yellow speckle viroids and the hop stunt viroid. These viroids should not greatly affect GFLV expression, so this inoculum should be adequate for our purposes. After successful inoculation, we will be ready to start future GFLV-related projects, such as metabolomic comparison studies of healthy and infected plants on both GFLV-tolerant and susceptible rootstocks or using RNA-Seq for a transcriptomic approach.

***Xiphinema americanum*, a potential vector of GFLV (Cecilia Agüero) –** Cecilia Agüero and Andy Nguyen in collaboration with Xuyun Yang, Liang Zheng and Howard Ferris, have been examining the potential GFLV vectoring ability of *X. americanum*. In order to test the hypothesis that *X. americanum* can vector GFLV, *in vitro* roots of GFLV infected Chardonnay were inoculated with 10 *X. index* or *X. americanum* nematodes. After 96 h nematodes were transferred to *in vitro* roots of healthy St George plantlets for another 96 h and then removed. Two weeks later, roots were collected and frozen in liquid nitrogen. RNA extraction from roots was performed using a CTAB method and the RNA pellet was further purified using the RNeasy Plant Mini Kit (Qiagen®) including a DNAse treatment (1). cDNA was synthesized from the prepared RNA, concentrated to 8 µL with a vacuum centrifuge, using Superscript III (Invitrogen ®) and subjected to qRT-PCR on a StepOnePlus PCR System using Fast SYBR Green Master Mix (Applied Biosystems). All qRT-PCR reactions were performed with the following cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 3s and 60°C for 30s and primer sequences: GFLV-F: GTTGTGTGTTAGGGGAGGTACTATTA; GFLV-R: TTCCACATACACCCCGGGATA; 18SrRNA-F: GTGACGGAGAATTAGGGTTCGA; 18SrRNA-R: CTGCCTTCCTTGGATGTGGTA.

A second experiment was performed following the same procedure except that feeding on GFLV infected Chardonnay and healthy St. George was prolonged to 1 week and root samples were collected 25 days after nematodes were removed. A third experiment was conducted under greenhouse conditions. GFLV infected Chardonnay plants, previously multiplied in vitro, were grown in 1-gallon pots, lined with fiberglass cloth and filled with autoclaved sand. Each pot was inoculated with 1 ml of inoculum containing 50 nematodes. Five pots were inoculated with *X. americanum*, 5 with *X. index* and 3 with water. Three weeks later, healthy St. George

plants were transplanted to each of the 13 pots. At this point, every pot contained 2 plants: one infected Chardonnay and one healthy St. George. Four months later**,** qPCR for GFLV testing in leaves was run on all St George plants as described previously. Only plants inoculated with *X. index* were clear positives for GFLV. The efficiency was low in *in vitro* experiment #1 and in the greenhouse experiment, with 1 plant infected out of 5 plants inoculated. The efficiency was improved *in vitro* experiment #2, in which the time of root exposure to the nematodes was increased to 1 week and the time until root collection was increased to 25 days. The lower efficiency found in the greenhouse could be due to a low number of nematodes used as inoculum, plus small size of plants in proportion to pot size. The fact that no clear positives were found among the *X. americanum* treatments indicate that either *X. americanum* does not vector GFLV or it is not as efficient as *X. index* in vectoring it.

Rootstock tolerance to red leaf viruses – Zhenhua Cui – Zhenhua is doing a 2-yr post-doctoral study in my lab. He has been working on leaf roll viruses in China and is very well suited for efforts to determine how the tolerance and hypersensitivity we have seen functions and whether we can detect graft incompatibility or red leaf sensitivity at an early stage with greenhouse and *in vitro* grafting. The first stage of the project is underway and we have collected plant material from Adib Rowhani at FPS. He has been examining field-grown vines with various virus rootstock combinations and was able to suggest the best (most responsive and most aggressive) rootstock and virus combinations. We will be using two highly sensitive rootstocks (Freedom and 101-14 Mgt) and two tolerant rootstocks (St. George and AXR#1). The scion for grafting experiments will be the red leaf indicator Cabernet Franc and we will use three virus treatments: LR1; LR1 + GVA; and virus-free.

Micrografting – In the past 6 months, most of the micrografing experiments have been done. Vegetative growth of different combinations were investigated (Figure 17). It takes a longer time for bud elongation when grafting on Freedom than grafting on St.George. But it makes no difference between LR131 (infected with GRLaV-1) and Cab Franc (virus-free) (Figure 17A). ANOVA shows that the rootstock plays a significant effect on bud elongation of the scions (Table 6). St.George takes less time to produce roots whether grafted by Cab Franc or LR131 when compared with Freedom (Figure 17B). However, the root initiation time was prolonged in Freedom when grafted by LR131 than when grafted by Cab Franc (Figure 17B). Table 6 shows that rootstock plays a significant effect on root initiation and has a significant interaction with the sanitary status of the scion.

The survival rate of the micrografting goes down from 2 weeks to 8 weeks after grafting (Figure 18). The reserves of the scions allow them grow under *in vitro* system in the first two weeks even when the scions and rootstocks are not contacted well. However, But the bad connection killed the graftings along with the duration. Virus effect on the grafting should appear after connection forms and the virus moves, thus only the graftings surviving after 8 weeks would be used for further test. The survival rate of the graftings on St.George is significantly higher than those on Freedom. But the survival rate of Cab Franc grafting on Freedom is lower than LR131 on Freedom since 4 weeks after grafting (Figure 18), probably because of grafting difficulties. Data from the dry weight of scion and roots of rootstock are being analyzed.

Greengrafting – In the past 6 months, plants became ready for grafting and two more rootstocks (101-14 and AXR#1) were used for the experiment. To date, 8 different combinations of green graftings have been tried and the data are being collected.

Campus rootstock trials – We are now planting advanced selections in trials on campus with three 3-vine replicates. 101-14 Mgt and 1103P are included as controls/standards so that growth and yield can be compared these low and high vigor rootstocks. Nathan Kane is a second year MS monitored this rootstock plot. Advanced selections are planted in three 3-vine replicates. During the dormant season of 2016 the vines were pruned and trained into a bi-lateral cordon system. Pruning weights were recorded during pruning. After bud break shoots were removed to establish arm positions. During the 2016 growing season bud break, bloom, veraison, total cluster count, average berry weight, average cluster weight, harvest weight, brix, pH, and TA were evaluated and recorded, data are being analyzed. Data will be presented in the June 2017 report.

Presentations/Abstracts/Scientific Meetings/Publications Related to Rootstock Breeding
Talks at Grower Meetings (Extension/Outreach) – 2016

"Walker grape breeding program update and tasting". Silverado SIMCO Growers Management Seminar, Napa, CA Jan 13
"Rootstock and scion breeding overview: current and future options". Lodi Grape Day, Feb 2.
"Rootstock breeding update". Unified Grant Management Seminar, UC Davis Feb 9.
"Rootstock possibilities for the Sacramento Valley". Clarksburg Grape Day, Mar 2.
"Vineyard challenges from the ground up". UCD Wine Executive Program, Mar 22.
"Grape rootstock breeding progress". CDFA IAB Reports, UC Davis, Apr 13.
"Grape breeding update". John Dyson and William Salem staff, UC Davis, Apr 13.
"California viticulture and its place in the World". Haas Business School Lecture, Mondavi Winery, Apr 16.
PD breeding update and tasting. Oak Knoll Growers Group, Napa, CA Jan 7, 2016
Walker grape breeding program update and tasting. Silverado SIMCO Growers Management Seminar, Napa, CA Jan 13, 2016
PD resistant winegrapes – update and tasting Napa/Sonoma growers meeting, Napa, CA Jan 21, 2016
Rootstock and scion breeding overview. Lodi Grape Day, Lodi, CA Feb. 2 2016
PD resistant winegrape breeding and tasting, Silverado Vineyards meeting, Napa, CA April 4
PD resistant winegrape breeding. Talk and discussion with John Dyson and Williams Salem staff, UC Davis, April 13
PD resistant winegrape breeding and tasting for California Association of Winegrape Growers, Sacramento, CA Apr 18
Breeding PD resistant winegrapes. Temecula Grape Day, Temecula, CA Apr 21
Breeding PD resistant winegrapes. Alan Tenscher presenting to the AVF Board in Livermore, Apr 29
Breeding PD resistant winegrapes. Talk and tasting for Napa winemakers and viticulturists, UC Davis, May 4
Winegrape breeding at UC Davis. Vintage Nursery Open House, Wasco, CA May 18
Winegrape breeding at UC Davis. International Cabernet Sauvignon Conference, Pine Ridge Winery, Napa, CA June 22 2016
Grape breeding Daniel Roberts Growers group, Santa Rosa, CA July 22
Grape breeding at UCD. Chilean table grape growers association, UCD Oct 3
Grape breeding above and below ground. Cal Poly San Luis Obispo, CA Oct 6
Grape breeding update. CDFA Industry Advisory Board, UC Davis, Nov 1.
PD resistant wines – lecture and tasting. Sacramento Private School Auction with Darrel Corti. UCD, Nov. 13
Breeding PD Resistant Winegrapes. Texas A&M, Driftwood, TX, Nov, 18
What are the next steps for the PD resistant wine grape breeding program? Vineyard Health Seminar, UCD, Nov. 29
PD Breeding program update. FPS Annual Meeting, UCD, Dec. 1
Progress in the Grape Breeding Program, Recent Advances in Viticulture and Enology, UCD, Dec. 9
Classical and molecular breeding to combat PD. CDFA PD Board Annual Meeting, San Diego, CA, Dec. 13

Presentations/Abstracts at Scientific Meetings

Xiaoqing Xie, Cecilia B. Agüero, Yuejin Wang, M. Andrew Walker. 2016. Optimizing the Genetic Transformation of Grape Fruiting and Rootstock Cultivars. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Hugalde, Inez, Cecilia B. Agüero, Nina Romero, Felipe Barrios-Masias, Andy V. Nguyen, Summaira Riaz, Andrew Walker, Andrew McElrone, and Hernán Vila. 2016. A Mechanistic Model for Vegetative Vigor in Grapevine. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Hugalde, Inez, Summaira Riaz, Cecilia B. Agüero, Nina Romero, Felipe Barrios-Masias, Andy V. Nguyen, Hernán Vila, Andrew McElrone and M. Andrew Walker. 2016. Physiological and Genetic Control of Vigor in a Ramsey x Riparia Gloire de Montpellier Population. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Robertson, Brooke, Courtney Gillespie, M.A. Anderson, M. Andrew Walker, and J.C. Dodson Peterson. 2016. Grapevine Shoot and Cluster Development as a Function of Arm Positioning along the Cordon. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Fort, Kevin, Claire Heinitz and M. Andrew Walker. 2016. Superior Salt Tolerance in Grafted Accessions of Wild *Vitis* Species. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Uretsky, Jake and M. Andrew Walker. 2016. Evaluating Grape Root Architecture in a 101-14Mgt x 110R

Genetic Mapping Population. 67th ASEV National Meeting, Monterey, CA June 29, 2016.

Publications

Fort, K. and A. Walker. 2016. Breeding for drought tolerant vines. *Wines & Vines*, January.

Viana, A.P., M.D.V. de Resende, S. Riaz and M.A. Walker. 2016. Genome selection in fruit breeding: application to table grapes. Scientia Agricola 73:142-149.

Pap, D., S. Riaz, I.B. Dry, A. Jermakow, A.C. Tenscher, D. Cantu, R. Olah and M.A. Walker. 2016. Identification of two novel powdery mildew resistance loci, *Ren6* and *Ren7*, from the wild Chinese grape species *Vitis piasezkii*. BMC Plant Biology 16(1):170

Forneck, A., K. Powell and M.A. Walker. 2016. Scientific opinion: Improving the definition of grape phylloxera biotypes and standardizing biotype screening protocols. American Journal of Enology and Viticulture 47: 64:371-376..

Xie, X., C.B. Agüero, Y. Wang and M.A. Walker. 2016. Genetic transformation of grape varieties and rootstocks via organogenesis. Plant, Cell, Tissue and Organ Culture 126:541-552.

He, Rr; Jiao Wu; Yali Zhang; Shaoli Liu; Chaoxia Wang; Andrew M. Walker; Jiang Lu. 2016 Overexpression of a thaumatin-like protein gene from *Vitis amurensis* improves downy mildew resistance in *Vitis vinifera* grapevine. Protoplasma DOI: 10.1007/s00709-016-1047-y (In press)

Fort, K.P., J. Fraga, D. Grossi and M.A. Walker. 2016. Early measures of drought tolerance in four grape rootstocks. Journal of the American Society for Horticultural Science. (In press)

Riaz, S., K.T. Lund, J. Granett and M.A. Walker. 2017. Population diversity of Grape Phylloxera in California and evidence for sexual reproduction. American Journal of Enology and Viticulture 68: In Press.

Lund, K.T., S. Riaz and M.A. Walker. 2017. Population structure, diversity and reproductive mode of the Grape Phylloxera (*Daktulosphaira vitifoliae*) across its native range. PLOS One (In press).

Table 1. 2016 crosses and their purpose

Cross #	Female	Male	# berries	# sinkers	Purpose
2016-029	101-14 Mgt	GC5 arizonica	309	592	Salt resistance and better rooting, moderate vigor
2016-031	101-14 Mgt	berlandieri 9031	97	249	Salt resistance and better rooting, moderate vigor, lime tolerance
2016-032	101-14 Mgt	berlandieri 9035	269	578	Salt resistance and better rooting, moderate vigor, lime tolerance
2016-036	101-14 Mgt	2012-144-24	41	81	Salt resistance and better rooting, moderate vigor
2016-046	161-49C	GC5	96	118	Lime, salt, nematodes
2016-050	161-49C	b55-1	16	3	VR hybrid, lime, rootability
2016-051	161-49C	2012-142-25	315	633	
2016-052	161-49C	2012-144-24	190	410	
2016-053	161-49C	2012-144-39	81	171	
2016-061	2012-144-41	110R	0		110R berlandieri drought and salt/nema
2016-062	2012-144-41	1103 Paulsen	0		1103P root architecture and salt/nema
2016-063	5BB Kober	b55-1	136	111	Add VR resistance to berl x riparia rootstock
2016-064	5BB Kober	2011-188-06 (T6-42 x St. Geo)	25	11	Add VR resistance to berl x riparia rootstock
2016-069	5BB Kober	berlandieri 9031	43	59	Add better drought and

					salt to 5BB
2016-071	5BB Kober	St. George	220	322	Mapping population for lime tolerance and root architecture
2016-072	5BB Kober	2012-142-25	64	85	Salt resistance and better rooting, moderate vigor
2016-073	5BB Kober	2012-144-24	107	137	Salt resistance and better rooting, moderate vigor
2016-090	GRN-3 9365-43	NM 03-17 S01	26	47	Add salt and drought resistance to GRN3
2016-096	GRN-3 9365-43	2012-142-25	472	1003	Add salt and drought resistance to GRN3
2016-097	GRN-3 9365-43	2012-144-24	156	368	Add salt and drought resistance to GRN3
2016-110	doaniana 83	GRN-4 9365-85	33	52	Deep roots and very high nema resistance as well as TX root rot
2016-121	Dog Ridge	140Ru	73	181	Better salt resistance to Dog Ridge
2016-131	Dog Ridge	SC11	51	122	Better salt resistance to Dog Ridge and TX root rot
2016-134	Dog Ridge	GC5	93	277	Drought and salt with very deep roots
2016-135	Dog Ridge	9035	96	126	Drought and salt to Dog Ridge
2016-136	Dog Ridge	2011-175-15	28	68	Drought and salt with very deep roots
2016-137	Dog Ridge	berlandieri 9031	41	111	Drought and salt to Dog Ridge
2016-141	9026 (doaniana)	GRN-4 9365-85	0	6	Deep roots high vigor to GRN4
2016-143	Ramsey	TX12-003	57	82	Better roots and salt resistance
2016-157	Ramsey	ANU77	163	482	Better roots and salt resistance
2016-158	Ramsey	GC5	96	222	Better roots and salt resistance
2016-162	Ramsey	9035	338	909	Better roots and salt resistance, lime tolerance
2016-165	riparia 1411	GC5	171	130	Drought and salt in low vigor background
2016-167	riparia 1411	berlandieri 9031	46	75	Drought, salt and lime tolerance
2016-168	riparia 1411	b55-1	54	23	VR in a weak good rooting background
2016-169	riparia 1411	2012-142-25	222	251	Better rooting, salt and nematodes
2016-170	riparia 1411	2012-144-24	179	188	Better rooting, salt and nematodes
2016-171	riparia 1411	2012-144-39	136	117	Better rooting, salt and nematodes
2016-172	riparia 1411	selfed/OP	157	186	Partial hermaphrodite?

2016-176	NM12-114	selfed	0		Partial hermaphrodite?
2016-177	101-14 Mgt	selfed	13	13	Partial hermaphrodite?
2016-190	SC2	GRN-2 9363-16	16	23	Salt and boron to GRN nema
2016-191	SC2	GRN-4 9365-85	23	37	Salt and boron to GRN nema
2016-196	SC2	2012-144-24	19	25	Salt, boron, nematodes
2016-197	SC2	2012-144-39	45	55	Salt, boron, nematodes
2016-198	berl 9019	Schwarzmann	34	44	Salt, nema, good rooting
2016-203	berl 9019	110R	307	329	Salt, nema, lime

Table 2. Root knot resistant seedlings selected during 2016. Selections with 2 or fewer egg masses are considered resistant

Genotype	Mean Egg Masses	Std. Dev.	Female	Male	Purpose
GRN1	0.0	0.0	Rupestris A. de Serres	rotundifolia Tray	Resists all nematodes
GRN1	0.0	0.0	Rupestris A. de Serres	rotundifolia Tray	Resists all nematodes
GRN1	0.0	0.0	Rupestris A. de Serres	rotundifolia Tray	Resists all nematodes
12125-049	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12126-048	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12126-047	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12125-042	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12125-040	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12125-039	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12125-034	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12126-035	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12126-034	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12125-028	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12126-023	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12125-021	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12126-003	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12126-001	0.0	0.0	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Salt, broad nematode
12185-006	0.0	0.0	GRN-3 9365-43	berlandieri 9031	Salt, lime, broad nematode
11188-003	0.3	0.5	T6-42	St. Geo	Fertile VR
12125-023	0.3	0.6	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12185-009	0.3	0.5	GRN-3 9365-43	berlandieri 9031	Salt, lime, broad nematode
12126-013	0.8	1.5	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
12185-007	1.0	2.0	GRN-3 9365-43	berlandieri 9031	Salt, lime, broad nematode
12125-009	2.0	1.6	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12125-002	2.3	2.6	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12129-015	4.3	3.1	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
07143-001	5.3	3.5	girdiana -22	arizonica A56	Salt, drought, boron
11115-020	8.5	14.5	161-49C	Trayshed	Fertile VR
12129-050	9.8	14.2	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
12129-021	45.4	70.0	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
St. George	52.0	82.3			Susceptible controls
12129-044	62.5	93.2	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
12129-011	81.3	145.9	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting

12129-046	86.3	84.4	OKC-1 SO1 (acerifolia)	St. George	Salt, improved rooting
12125-029	95.8	140.2	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Salt, broad nematode
12129-026	127.5	115.2	OKC-1 SO1 (acerifolia)	St. George	Salt, broad nematode
St. George	134.0	124.4			Susceptible controls
12129-022	150.0	70.7	OKC-1 SO1 (acerifolia)	St. George	Salt, broad nematode
St. George	225.0	86.6			Susceptible controls
Harmony	240.0	103.9			Susceptible controls
Harmony	386.7	361.5			Susceptible controls
Harmony	412.5	725.0			Susceptible controls
Colombard	31.6	74.6			Susceptible controls
Colombard	420.8	722.3			Susceptible controls
Colombard	712.5	566.2			Susceptible controls

Table 3. Number of accession by species propagated to screen in the Spring 2017.

Species	Number of accessions
V. acerifolia	10
V. arizonica	9
V. berlandieri	14
V. champinii	4
V. cinerea	11
V. doaniana	3
V. girdiana	8
V. monticola	1
V. mustangensis	4
V. riparia	5
V. rupestris	5
V. treleasei	2
M. rotundifolia	3

Table 4. Depth to restrictive layer, pH, and percent limestone ($CaCO_3$) of soils and mean annual precipitation at the collection sites of *V. berlandieri* accessions currently in our collection. The dotted line separates new versus previously collected accessions.

	Soil Characteristics			Mean Annual
Accession ID	Depth (cm)	pH	% $CaCO_3$	Precipitation (cm)
TX15-003	27	7.3	2	68.6
TX15-005	200+	7.9	71	68.6
TX15-017	200+	8.2	30	81.3
TX15-026	200+	8.2	64	83.8
TX15-059	43	8.2	50	78.7
TX15-060	46	7.4	0	78.7
TX15-063	30	7.3	2	76.2
TX15-073	61	8.2	58	81.3
TX15-075	46	8.2	51	81.3
TX15-078	200+	8.2	11 - 51	86.4
TX15-079	38	7.9	9	88.9
TX15-082	200+	7.9	25	88.9
TX15-084	39 - 74	8.2	65	88.9
TX15-091	36 - 86	7.3 - 8.2	7 - 36	78.7

TX15-093	33	7.6	12 - 36	81.3
TX15-096	36 - 200+	8.2	26 - 63	83.8
TX15-099	43	7.9	50 - 63	88.9
TX15-103	200+	8.2	28 - 64	86.4
TX16-015	43 - 200+	8.2	30 - 55	86.4
TX16-016	43 - 200+	8.2	30 - 55	86.4
TX16-018	43	7.9	60	78.7
TX16-022	71	8.0	47	81.3
TX16-024	43	8.2	50	81.3
TX16-025	43	8.2	50	78.7
TX16-026	43	8.2	50	78.7
TX16-030	41	8.2	26	76.2
TX16-032	43	8.2	50	78.7
TX16-034	200+	8.2	64	83.8
TX16-046	48	7.0	2	83.8
TX16-047	46	8.2	55	83.8
TX16-053	46	8.2	55	88.9
TX16-064	43	7.9	63	86.4
TX16-065	43	7.9	50 - 63	88.9
TX9724	200+	7.9	22	53.3
T17	200+	8.1	42	63.5
T23	200+		0	78.7
T25	38	8.2	60	78.7
T38	38	8.2	60	83.8
TX9717	43	8.2	26	78.7
TX9722	137	8.2	17	53.3
TX43-01	46	7.9	63	83.8
T03-05 S02	200+	6.5	0	76.2
T03-05 S03	200+	6.5	0	76.2
TX12-134	38	8.2	7 - 55	78.7
TX12-136	48	8.2	51	78.7
TX PALMATA 2	200+	6.7	2	109.2
berl 9019	200+	7.4	6	76.2
berl 9031	200+	8.2	28	86.4
berl 9043	200+	7.4	6	76.2

Table 5. Mean values with standard deviation for different genotype attributes

	140Ru		**Ramsey**		**Riparia**		**O39-16**	
	0mM	**75mM**	**0mM**	**75mM**	**0mM**	**75mM**	**0mM**	**75mM**
Leaf Biomass (g)	1.11± 0.34	0.64± 0.43	0.84± 0.42	0.45± 0.2	1.12± 0.27	0.75± 0.55	0.97± 0.46	0.4± 0.14

Leaf% Cl	0.05± 0.04	1.74± 0.44	0.02± 0.02	2.55± 0.45	0.05± 0.02	2.36± 0.4	0.06± 0.02	3.7± 0.41
Root Dry Weight (g)	0.24± 0.08	0.16± 0.07	0.23± 0.12	0.16± 0.08	0.36± 0.11	0.24± 0.13	0.3± 0.12	0.15± 0.05
Specific Root Length (cm/g)	0.47± 0.12	0.63± 0.14	0.47± 0.10	0.58± 0.11	0.47± 0.06	0.59± 0.16	0.55± 2.39	0.86± 0.12
Total Length (cm)	1097.17± 345.64	981.31± 311.48	990.28± 321.57	911.5± 332.94	1665.25± 404.54	1267.4± 443.79	1605.81± 442.7	1213.88± 313.19
L:S Ratio	0.91± 0.13	1.15± 0.2	1.04± 0.16	1.55± 0.39	1.12± 0.26	1.25± 0.19	1.91± 0.36	2.53± 0.52
Lateral Root Length (cm)	527.56± 202.95	519.34± 159.62	502.07± 176.95	553.77± 219.7	881.64± 291.41	693.2± 217.98	1041.01± 293.65	862.67± 229.8
Structural Root Length (cm)	569.59± 150.85	461.95± 162.83	488.2± 151.75	357.71± 128.91	783.58± 142.73	574.1± 232.55	564.8± 164.29	351.18± 106.5

Table 6 ANOVA of effects of virus, rootstock and their interactions on the time required for bud break and root initiation after grafting of *in vitro*.

Investigated indexes	Virus	Rootstock	Virus X Rootstock
Bud breaking	ns	*	ns
Root initiation	ns	**	*

**=significant difference at $P \leq 0.01$ by LSD test; *=significant difference at $P \leq 0.05$ by LSD test; ns=no significant difference.

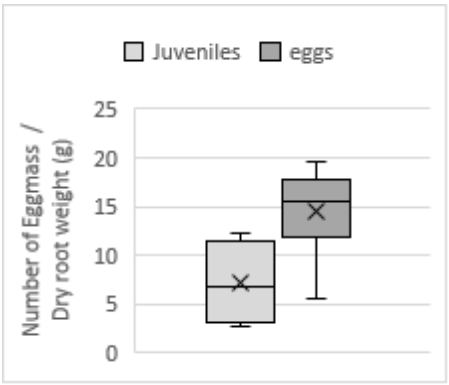


Figure 1. Inoculation rate of eight replicates of *V. vinifera* 'Carignane' plants inoculated with 3,000 juveniles or eggs. Roots are examined after six week of inoculation.

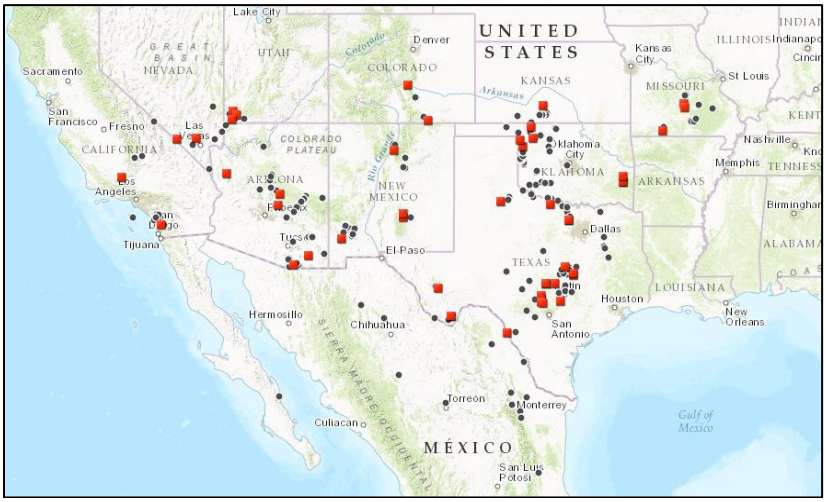
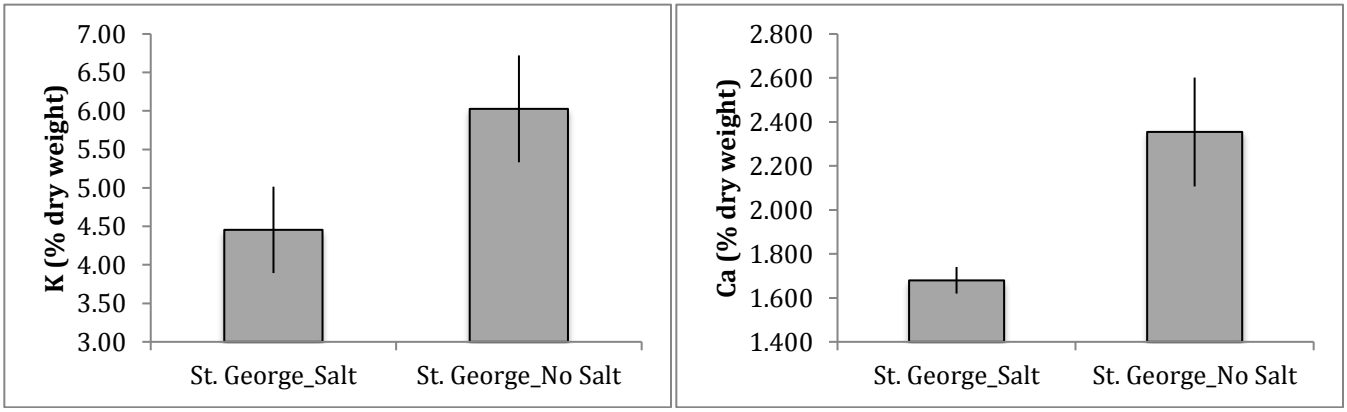


Figure 2. Accession of plants from the southwestern *Vitis* diversity study. Red square dots represent selected accession from the most diverse genetic groups.



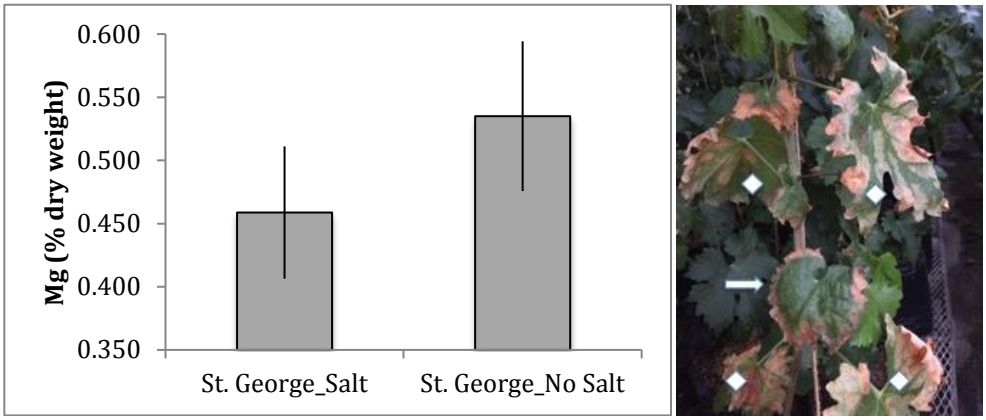


Figure 3. Selected leaf nutrient concentrations from Cabernet Sauvignon grafted onto St. George in salinity screen performed in 2015. Salinized vines were irrigated for two months with 75 mM NaCl, and following a one-month ramping period that began with 15 mM NaCl. Interveinal chlorosis, a common symptom of cation deficiency (see photograph), was noted at the end of the ramping period. In photograph, diamonds depict cation deficiency symptom; arrow depicts leaf margin necrosis, a symptom resulting from chloride toxicity.

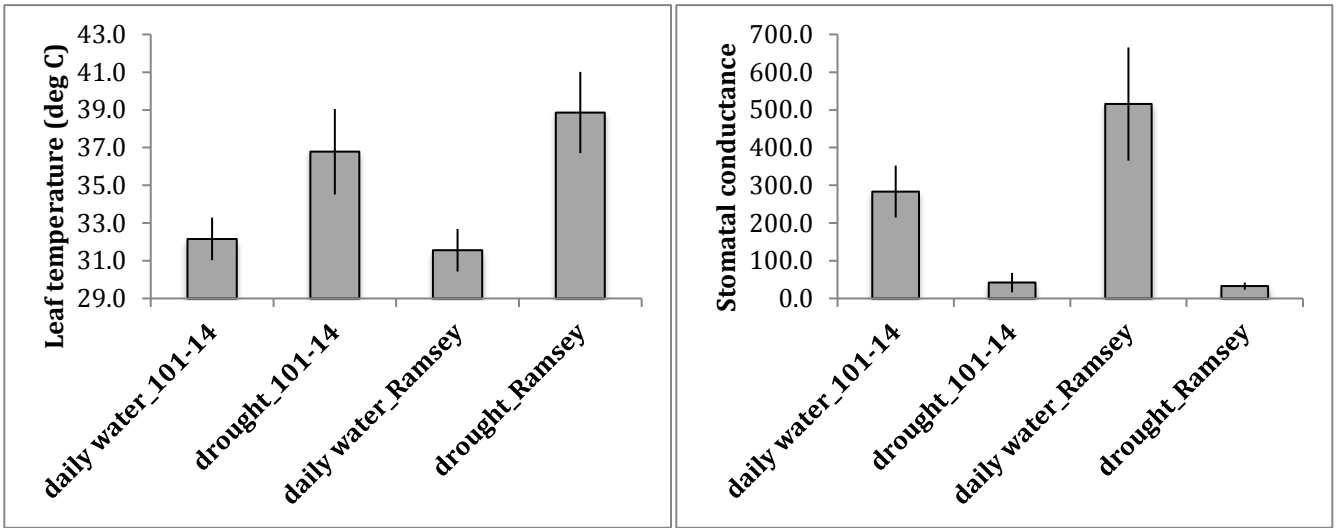


Figure 4. Leaf temperature (*left*) and stomatal conductance (*right*) for potted rootstocks 101-14 and Ramsey following two days of daily irrigation or drought in a greenhouse.

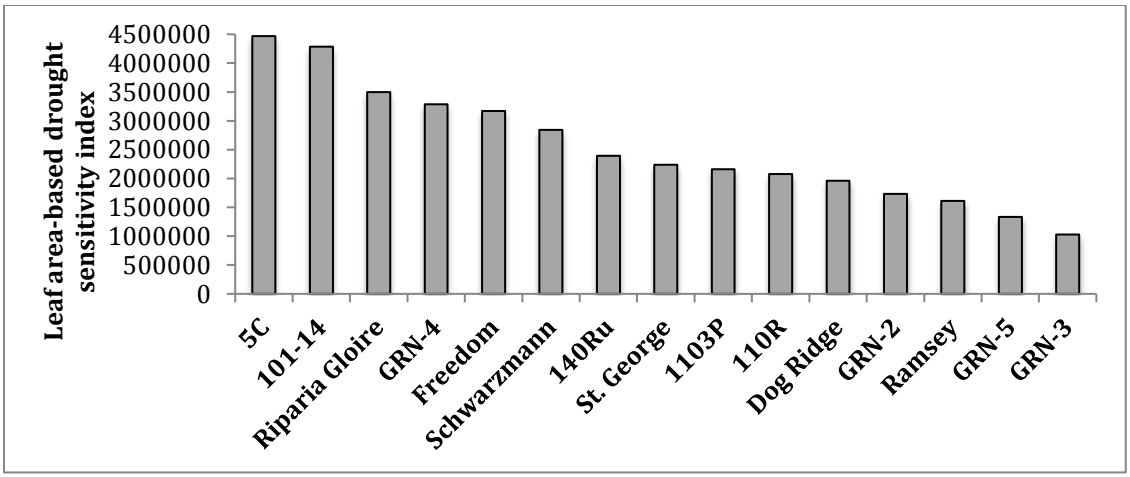


Figure 5. Drought sensitivity index based exclusively on leaf area of potted vines. Rootstocks 1616C, GRN-1, and three varieties of *Vitis vinifera* were excluded due to excessively slow growth rates that made their results incomparable.

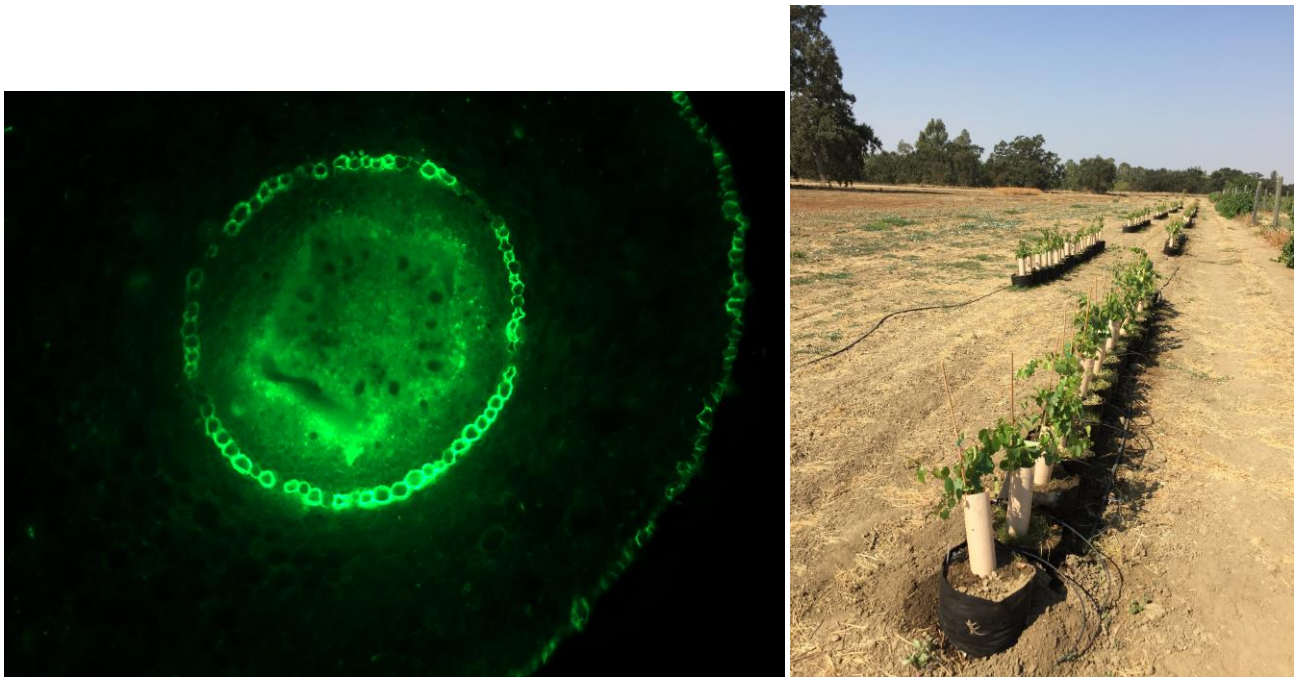


Figure 6. (*left*) Suberin presence in root cross section of a sample rootstock, from potted vine drought study. (*right*) Early season growth of 20 *Vitis* genotypes planted in horticultural fabric containers, to be harvested for root analysis following dormancy in winter 2016-2017.

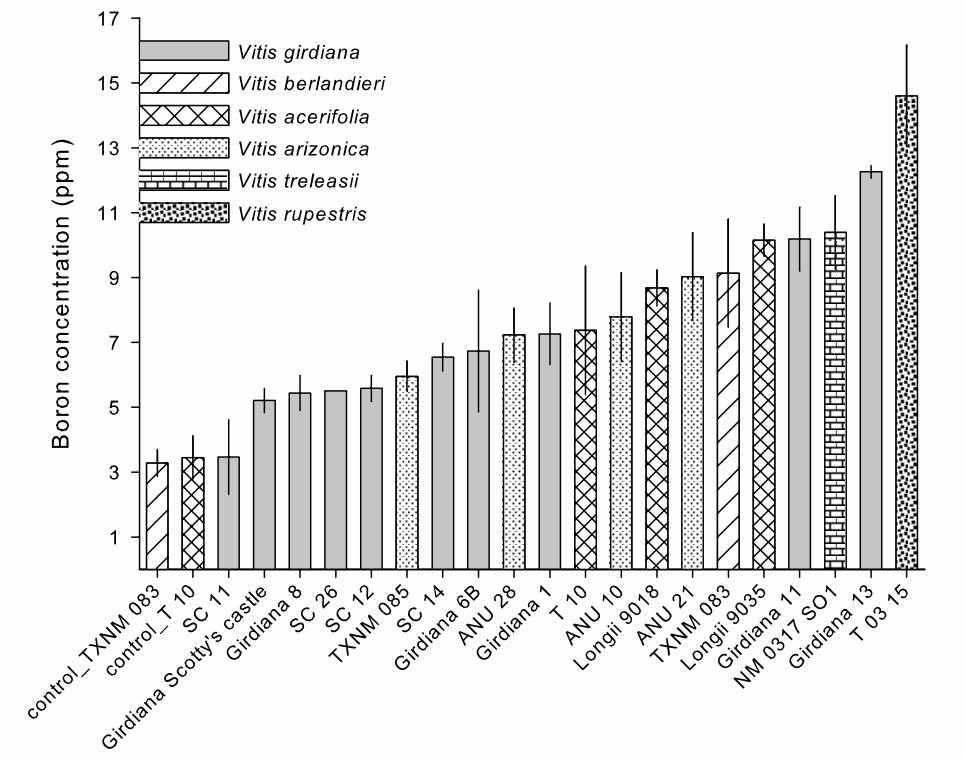


Figure 7. Boron concentration in leaf extracts for 20 wild *Vitis* species grown in containers in a greenhouse and irrigated with fertilized water containing 3 ppm boron. Two controls (leftmost columns) were grown with no added boron. Common species are indicated with a common fill pattern.

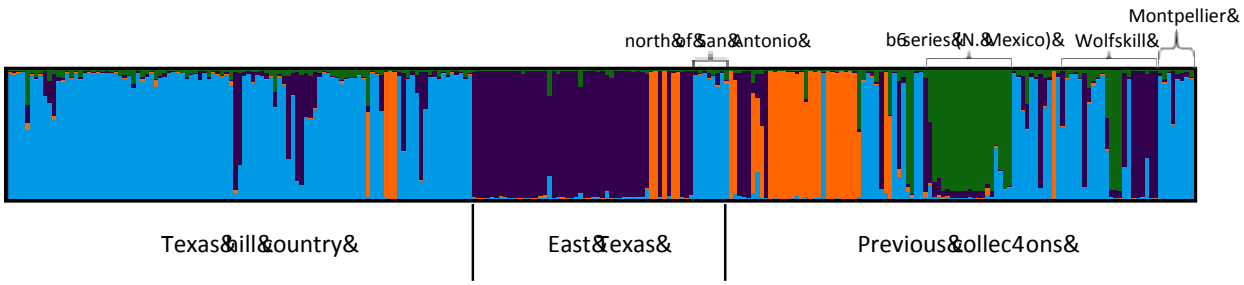


Figure 8. Groupings of *V. berlandieri* accessions and related accessions from recent and past collection trips, based on genetic analysis using SSR markers. Each vertical bar represents one accession. LIGHT BLUE = *berlandieri*; PURPLE = *cinerea*; ORANGE = *candicans*; GREEN = b-series collections from N. Mexico.

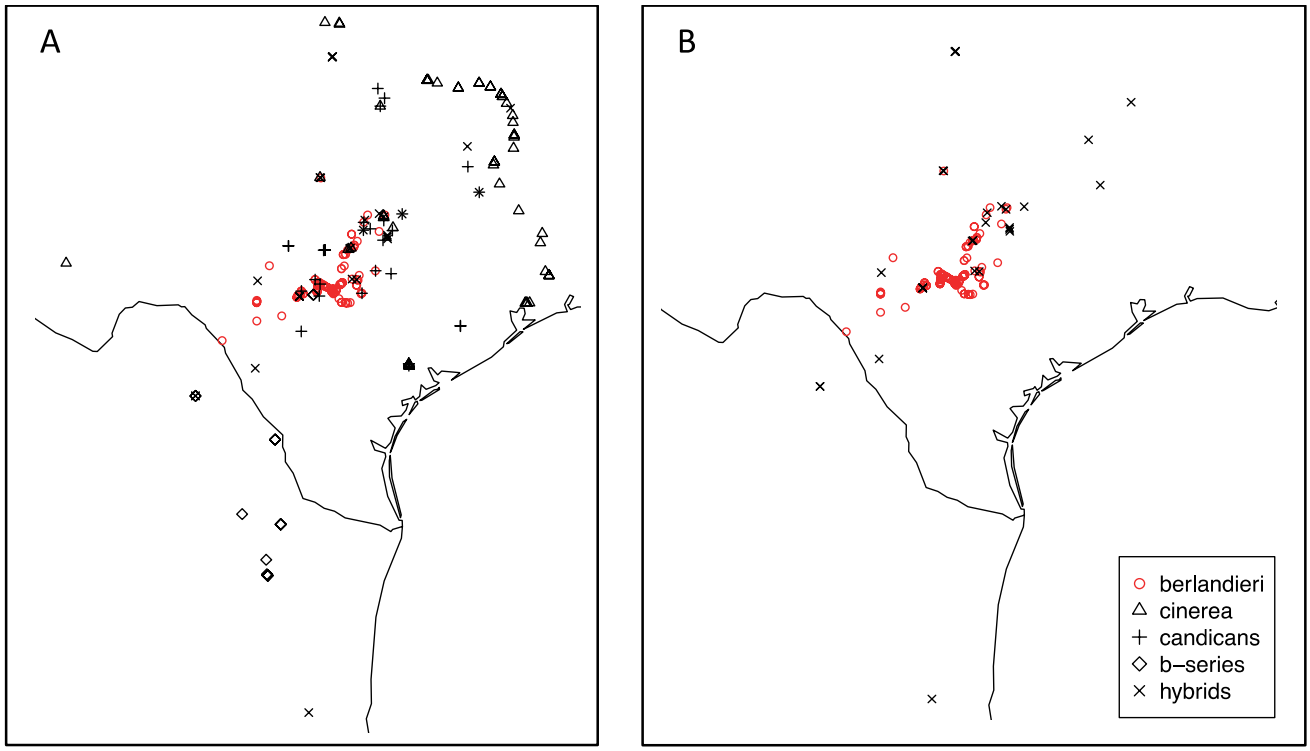


Figure 9. The map to the left (**2A**) depicts the collection location and species grouping of *V. berlandieri* accessions and related accessions, based on genetic analysis. Map **2B** only includes accessions identified as *V.*

berlandieri or as hybrids among species groups.

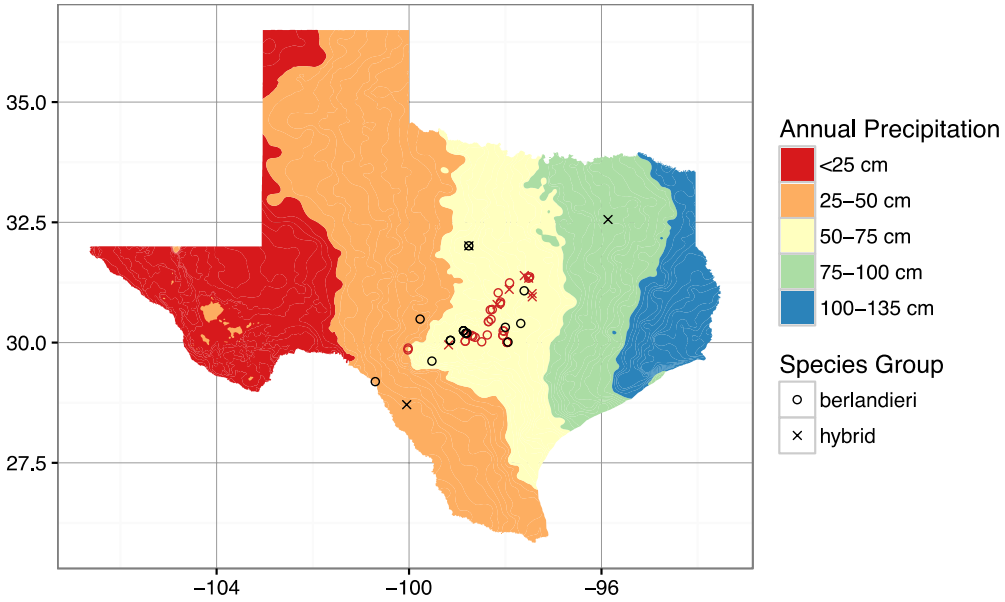


Figure 10. Average annual precipitation and geographic range of *V. berlandieri* (O) and *berlandieri* hybrids (×) currently in our collection. RED = new collections; BLACK = previous collections. The precise precipitation values for each location are estimated, due to scale.

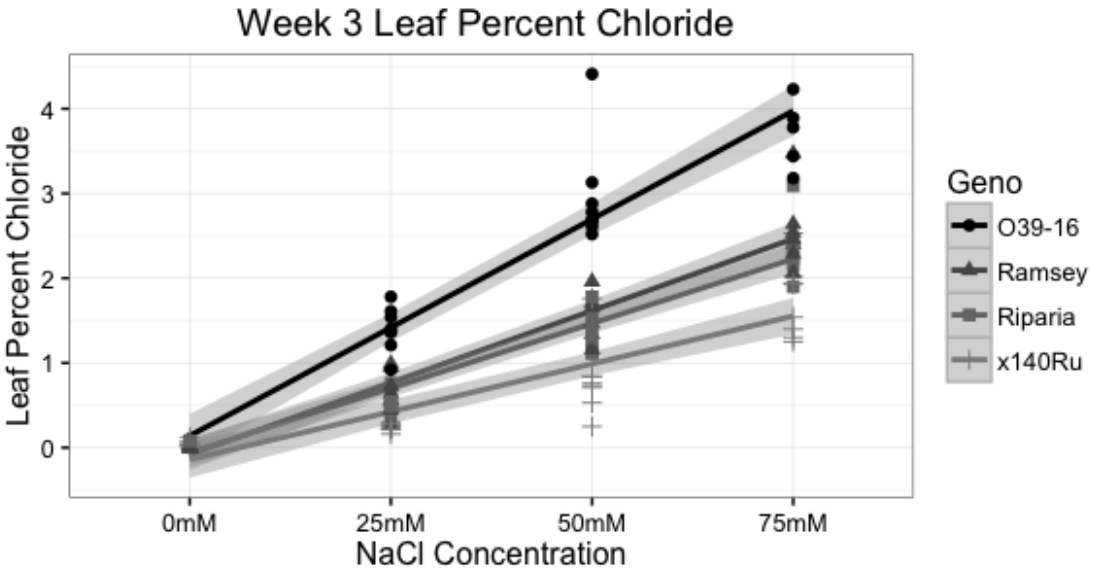


Figure 11. Leaf percent chloride for all plants after 3 weeks of given salt treatment (0, 25, 50, and 75mM NaCl) concentrations.

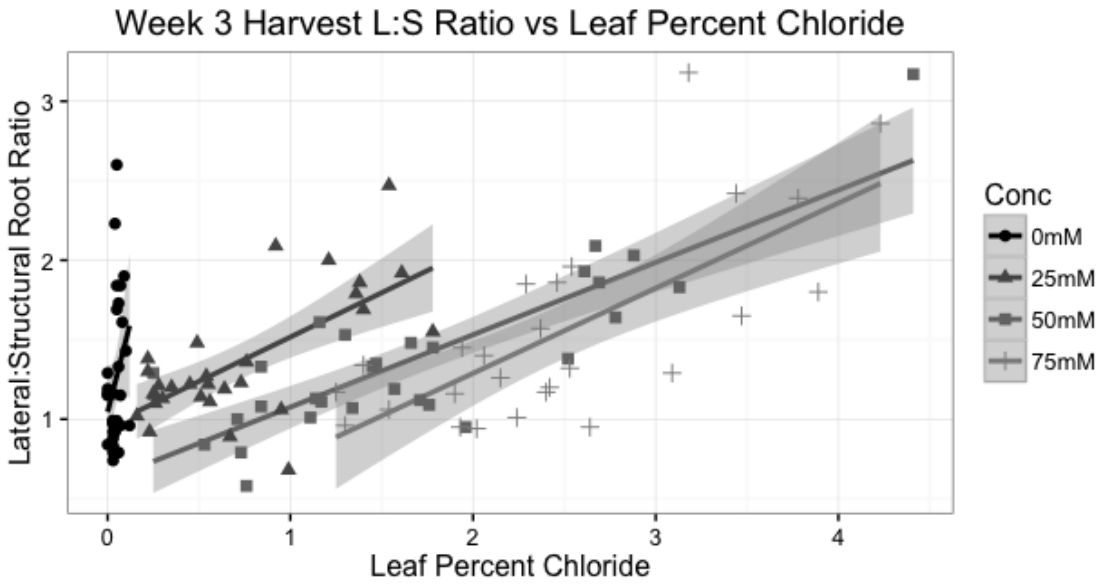


Figure 12. Correlations between lateral to structural root ratio and leaf percent chloride for all plants after 3 weeks of given salt treatment (0, 25, 50, and 75mM NaCl)

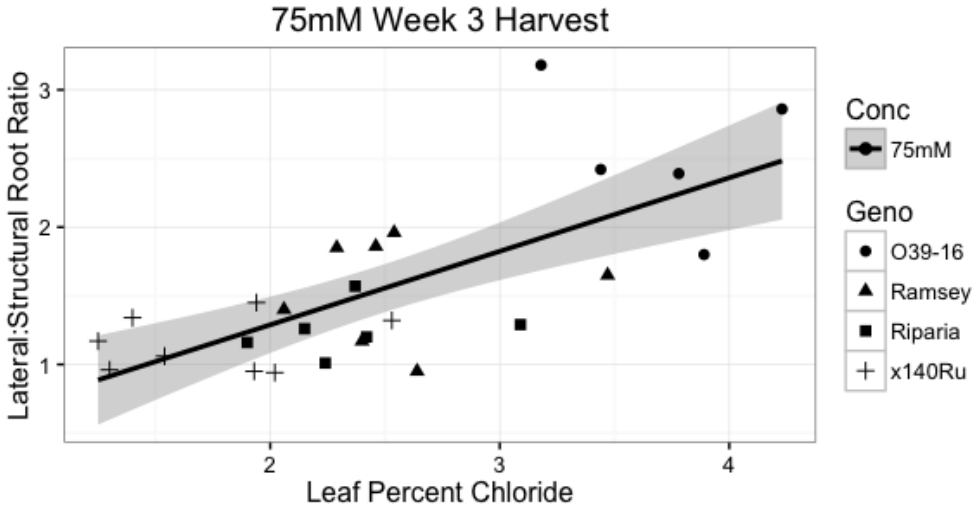


Figure 13. Correlation using all genotypes between lateral to structural root ratio and leaf percent chloride for all plants after 3 weeks of 75mM NaCl

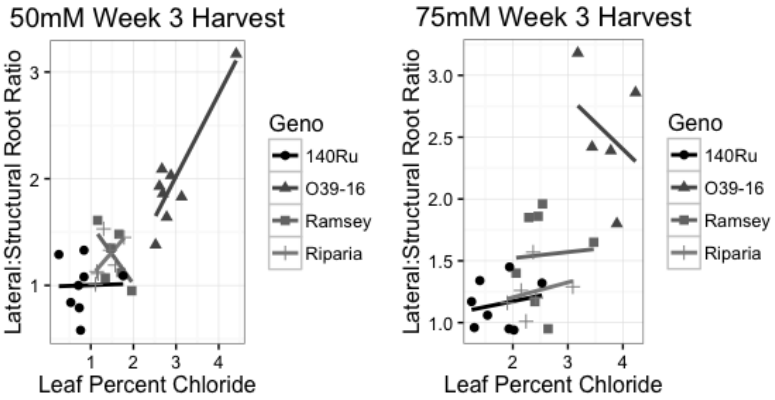
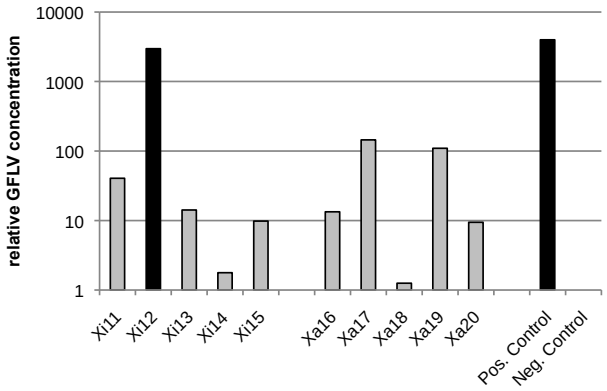


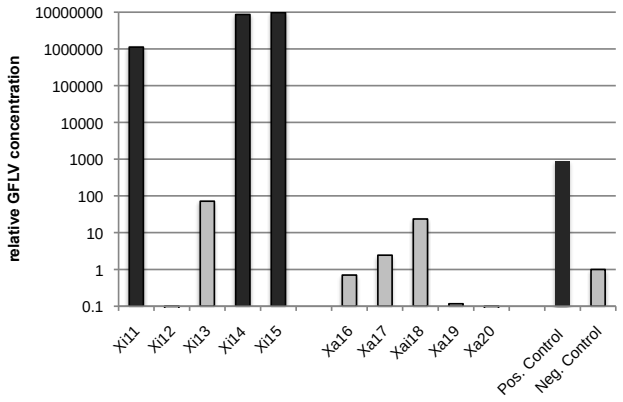
Figure 14. Genotypes separating based on lateral to structural root ratio and leaf percent chloride after 3 weeks 50mM NaCl (left) and 75mM (right).

Figure 15. Roots at the time of collection and RNA extraction (left) and soil inoculation in the greenhouse

a)



b)



c)

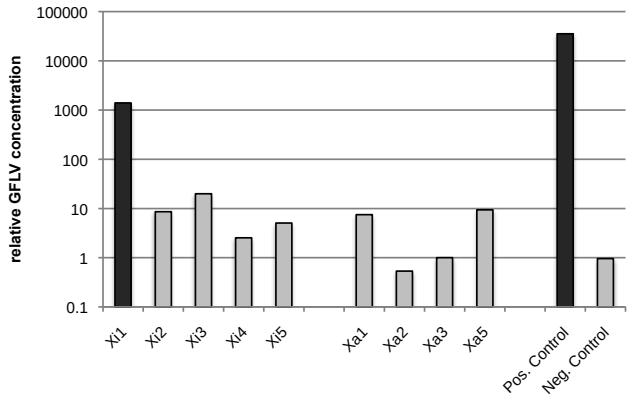


Figure 16. GFLV concentration relative to housekeeping gene 18SrRNA and negative control ($\triangle\triangle$Ct). Negative control was healthy St George. a) in vitro experiment #1, b) in vitro experiment #2 and c) greenhouse experiment. Note log scale in y axis of graphs

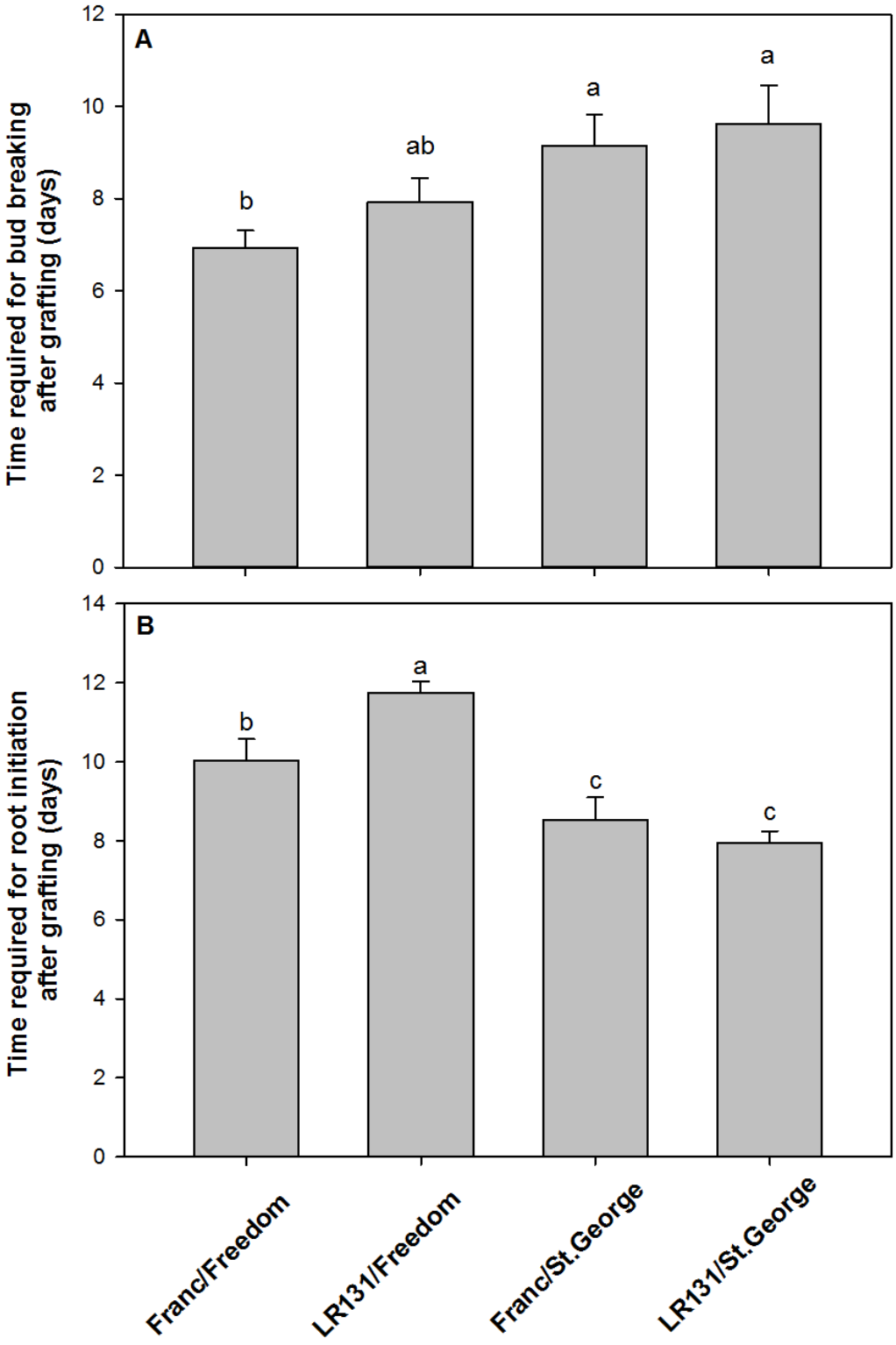


Figure 17. Vegetative growth of different combinations of micrografting of *in vitro Vitis.* Data were presented as means ± SE. Different letters within the same parameter are significantly different at $P \leq 0.05$ by LSD test.

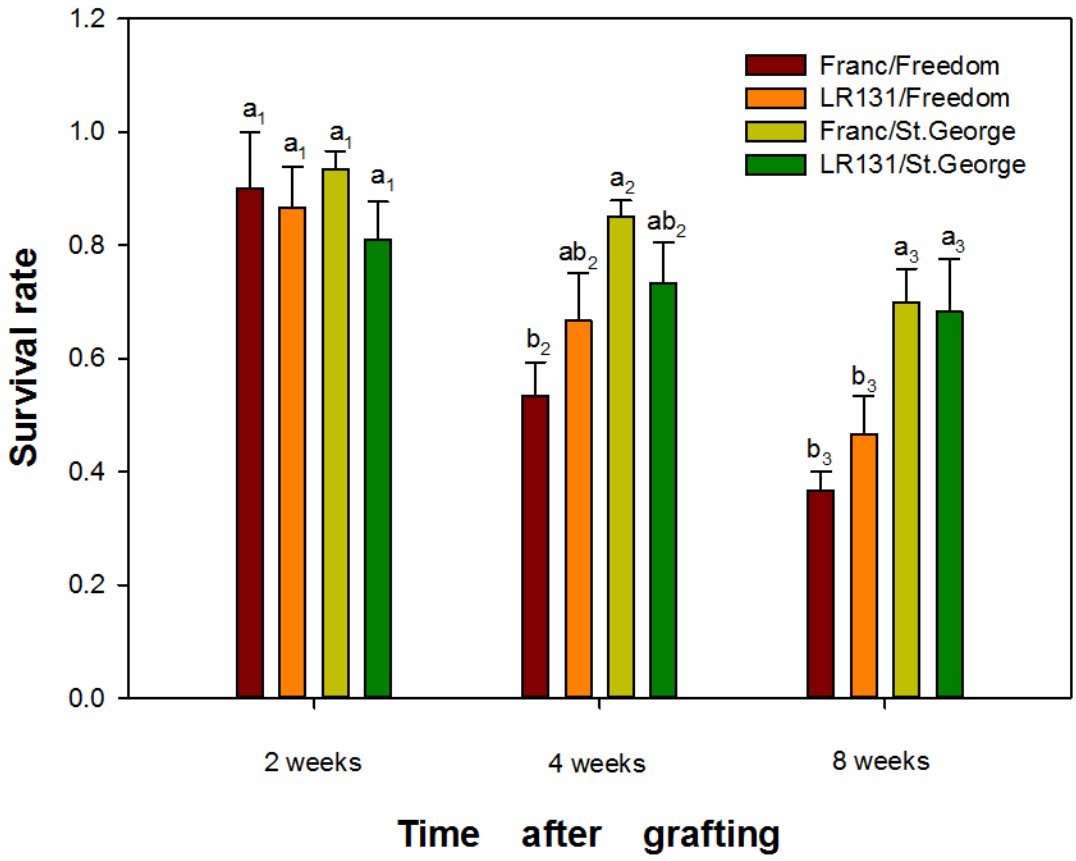


Figure 18. Survival rate of different combinations of micrografting of *in vitro Vitis*. Data were presented as means ± SE. Different letters within the same time of analysis are significantly different at $P \leq 0.05$ by LSD test.