Progress Report
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Strong progress continues to be made. The return of Becky Wheeler from maternity leave, her now well-trained assistant (Alyssa) and the addition of a post-doc (Daniel Pap who is studying the genetics of nematode and phylloxera resistance) have greatly accelerated our nematode resistance efforts. We also made strong progress on salt tolerance, root architecture and better understanding the key rootstock species *Vitis berlandieri*.

2017 Pollinations / 2016 Seedlings
The 2017 crosses are presented in Table 1. This year's crosses focused on combining strong sources of chloride exclusion with deep rooting and broadly based nematode resistance, with salt and boron tolerance and fanleaf tolerance. The 2016 seedlings will be planted in June and their parentages and purposes are detailed in Table 2.

Nematode screening since January 2017
Screening of seedling populations for resistance to nematodes continues. Since January, we have tested 133 genotypes in either initial or confirmational screens for resistance to a combined inoculum of HarmA and HarmC root knot nematodes (RKN). Of the 24 genotypes tested in secondary assays, 13 were confirmed resistant. The remaining 11 genotypes scored moderately resistant, and will be tested once more to confirm degree of resistance. The remaining 109 genotypes tested against RKN were initial screens. Fifty-four of those scored resistant and they will be moved forward to confirmational testing for RKN and initial screens for resistance to ring nematode. Nine genotypes already found to be resistant to RKN were tested for resistance to ring nematode. Two of these genotypes were resistant to ring, and will be moved into secondary testing to confirm ring resistance as well as testing against dagger nematode. Table 3 details the results of the root-knot nematode (RKN) screening and the movement of selections through the nematode screening pipeline. If strong mothervines exist of the advancing selections, they will be further evaluated for horticultural traits, and the best moved on to be bench-grafted prior to field testing.

We currently have 60 genotypes in initial testing for resistance to RKN. We also have 50 genotypes in initial testing for resistance to ring nematode. These are genotypes that have been resistant to RKN in initial screens. Those with good ring resistance will be moved into dagger testing, as our dagger nematode population is now large enough to use for bioassays. At the scheduled rate of testing, we will complete all initial screens for 2012 crosses by year's end.

Root-knot nematode (RKN) testing and mapping – Daniel Pap
Efficient and quick root-knot nematode (RKN) screening is essential to develop new resistant rootstock varieties. Daniel Pap (new post-doc) has been improving our RKN screen to develop a quick and robust phenotyping system to screen new germplasm and mapping populations at a larger scale. Recent results successfully prove that altering our inoculum source from juveniles to eggs, makes the screening process much faster – shortening the incubation time from 4 month to 6 weeks. Subsurface irrigation in the greenhouse, with a capillary mat instead of drip emitters, has also greatly improved the screening process by providing more uniform irrigation and allowing more pots per bench.

The major screening bottleneck continues to be scoring the plants in the trial, where within a limited time many roots need to be examined under the microscope. We are developing a semi-automated system to calculate the number of eggs from each plant in the screen. Previous data shows that egg counts correlate well with the number of gelatinous egg matrixes (Cousins et al., 2001). In the new system, eggs are extracted from infested roots with the 5% bleach solution and separated with stacked sieves. The resulting eggs are in a ~50 ml suspension in a conical tube. For the ease of visualization acid fuchsine is added to stain all root-knot nematode eggs a bright magenta color. The stained eggs are filtered onto a 1 cm diameter Whatman filter paper using a vacuum system. Images were taken with microscope with standardized settings. ImageJ software is used to generate the script with multiple image processing steps, which allows a count of "particles" automatically in batches of pictures. Figure 1 shows the correlation of automated egg counts vs. stain concentrations.

Screening germplasm and using existing mapping populations – We possess an extensive grape germplasm collection. We are using this collection to examine a wide range of species for nematode resistance. The first germplasm screening trial resulted in two new accessions that appear to be resistant to both HarmA and HarmC strains, and others that might possess moderate resistance (Figure 2). These and other known resistant genotypes are being used in crosses this year with susceptible *V. vinifera* with the aim of unraveling the genetics of resistance from multiple backgrounds (Table 4).

An existing population was screened for segregation of RKN resistance (*V. vinifera* F2-35 X *V. berlandieri* 9031). *Vitis berlandieri* 9031 is thought to have a moderate level of resistance to RKN, however in our screens it appeared as moderately susceptible, moreover the segregation pattern for ~50 genotypes did not predict the presence of a major resistance QTL (Figure 3). We are testing this population with phylloxera in an attempt to map a resistance locus.

Data from existing breeding populations allows us to explore RKN resistance further. For instance, populations of 101-14Mgt crossed with GRN2, GRN4 and GRN5. Both parents have resistance to RKN, hence the expected segregation ratios in the progeny is 3:1 if the resistance is controlled by one locus. Accumulated data shows this pattern presented in Table 5. Collection of more accurate phenotypic data and generated genotypic data allowed us to confidently identify a resistance locus on chromosome 18. In the following months more phenotypic and genetic data will be collected from existing breeding lines to confirm our finding on this and other chromosomes.

We successfully germinated decade-old archived seed lots made by a previous MS student who made crosses with the GRN rootstocks. The Colombard x GNR4 and Colombard x GRN5 population, 163 and 133 seedlings, respectively, were promising and were screened with SSR markers to verify their parentage. Unfortunately, only 21 seedlings were from that cross and the others were off-types, perhaps selfed Colombard. These crosses will be made again this year. We may be able to use the selfed Colombard seedlings as female flowered parents for mapping and marker development.

Molecular verification of purity in the 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 have proven that HarmA is more virulent than HarmA in our first screen (Figure 4). We inoculated with a single egg mass of these strains separately along with the I3 strain on Harmony, Freedom, GRN1 and Colombard to monitor their virulence, and more importantly to purify a single line. We also tested DNA extraction methods from eggs that yield good quality and quantity of DNA. 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 resistance – Kevin Fort

Drought resistance in Ramsey x Riparia Gloire hybrids – In July 2015 rooted cuttings of Ramsey, Riparia Gloire, and subsets of Ramsey x Riparia Gloire F1 and F2 hybrids were planted to the field and grown for the duration of the season. In February 2016 the dormant vines were excavated, washed and stored at ~4 ºC until they could be scanned and digitally analyzed. As was described in our January 2016 report using 12 *Vitis* genotypes, the average root thickness can serve as an effective index for drought stress resistance when vines are grown in

relatively heavy field soil. Under these conditions, drought susceptible root systems are relatively fibrous and drought resistant root systems are relatively thick-rooted. The intention of the hybrid planting was to use the root morphology index (i.e., relatively fibrous-rooted versus thick-rooted) to gain insight into the genetic basis of drought resistance. As can be observed in Figure 5, the small set of F1 progeny and the larger set of F2 progeny were generally intermediate and evenly spread between the values of Ramsey and Riparia Gloire, an indicator of multigenic inheritance. This field trial also provided validation for the method itself, as such a field trial had only been completed one previous time in the trial using the 12 *Vitis* genotypes. Lastly, two individuals in the F2 generation were more fibrous than the F1 parent that exhibited greater fibrosity, a phenomenon known as transgressive segregation. One explanation for this phenomenon is a dominance effect of genes responsible for thick rooting originating in Ramsey over genes responsible for fibrous rooting originating in Riparia Gloire. A release from this genetic dominance is then observed for a subset of individuals in the F2. A similar increase in the variability of the F2 population relative to the F1 population was observed in the mean rooting angle of herbaceous cuttings reported in January 2014, and we have observed from several previous experiments that shallow-rooted rootstocks such as Riparia Gloire, 5C, 101-14 and 1616C are also relatively fibrous, and that deeply-rooted rootstocks such as 110R, 1103P and Ramsey are also relatively thick-rooted. Confirmation of this transgressive segregation and its correlation to rooting angle will require the analysis of a much larger population and is currently underway, described below.

Completed analysis of root morphology from four experiments – In addition to the two root morphology data sets described in the previous section, root morphology was also investigated in a shadehouse population of *Vitis* genotypes grafted with Cabernet Sauvignon and a population of ungrafted herbaceous cuttings. Preliminary data from these two latter experiments were also reported in our January 2016 report. A full analysis of all four data sets is now complete and a draft publication has been written. One important finding was that the absolute quantity of the finest root fraction of fibrous, drought-susceptible root systems grown in the field was much larger than that seen in thick-rooted, drought-resistant root systems (Fig. 6A). Although the absolute quantity of thick roots of drought-resistant genotypes was greater than that seen in drought-susceptible genotypes (Fig. 6B), the degree of difference was small relative to the inverse relationship in the fine root fraction. This finding implies that the fine roots are the most readily distinguishing variable to separate drought resistant and susceptible genotypes. When this principle was applied to herbaceous cuttings, which after four weeks had insufficient time to develop any thick roots (relative to that observed in the field), the fine root fraction could alone easily distinguish drought resistant and susceptible rootstocks (Fig. 6C). *Vitis vinifera* genotypes tested in these two environments were found to have qualitatively different root systems that produced not only high root biomass (data not presented), but both high root length of fine and thick roots (Fig. 6A-C). Root morphology data derived from the shadehouse, which used a coarse potting media and a full season of growth, produced very high total root length of fibrous roots regardless of genotype, and had no reliable predictive value for drought resistance (data not presented). The root systems of herbaceous cuttings which can be generated over only four weeks should therefore be sufficient for the genetic analyses of crosses involving standard rootstocks derived from *V. riparia*, *V. rupestris*, *V. berlandieri* and *V. champinii*. This principle can be seen in the strong correlation of the fine root fraction of rooted herbaceous cuttings and field-grown rootstocks (Fig. 7), a surprising result given the large disparity of soil media, environments and growth periods.

Preliminary analysis of rootlet populations from fabric containers in the field – In January 2017 we reported preliminary results of a combined greenhouse and field experiment of 20 *Vitis* genotypes for drought resistance. At that time, the field experiment had not yet been harvested. This field component involved the use of large fabric pots that were filled with field soil. Ordinarily, field soil cannot be used in containers because it results in drainage problems and anoxic soil conditions. However, the fabric containers promised to alleviate this issue by providing a hydraulic continuity between the field soil in the fabric container and the soil surface on which the containers were placed. These vines were harvested in early March 2017. In contrast to earlier root system analyses, which scanned entire root systems at once, in this analysis individual rootlets were scanned and the entire root system of an individual was treated as a population of adventitious rootlets. A much more detailed and informative set of data was obtained which helped to resolve earlier questions. Riparia Gloire was found to have only fibrous rootlets, whereas Ramsey contained a diverse population of rootlets that ranged from very fibrous to very thick (Fig. 8A). Approximately 20% of the Ramsey rootlets were markedly thicker than the remainder of the Ramsey rootlet population (Fig. 8A). Using Riparia Gloire and Ramsey as standards for drought susceptibility

and resistance, respectively, other rootstocks were assessed. In Fig. 8B it can be observed that the rootstock St. George has an intermediate phenotype, with the bulk of the population lying between Riparia Gloire and Ramsey. However, as with Ramsey, a small percentage of roots were very thick. This result explains the similarity of deep plunging roots seen in both Ramsey and St. George in rhizotron containers analyzed in 2014, yet published data indicates that St. George is more drought susceptible than Ramsey. Our previous speculation included the possibility that this susceptibility might be an artifact of the nematode sensitivity of St. George that could stunt its root system, but these current root system data indicate that St. George is merely a more fibrous root system than Ramsey. Using this system as a predictor of drought resistance, it appears that the untested GRN-2 will prove to be as drought sensitive as Riparia Gloire (Fig. 8C). *Vitis vinifera* 'Colombard' might be expected to have even greater drought resistance than Ramsey if not for its phylloxera susceptibility (Fig. 8D). This data set is in progress, and upon completion a more thorough, replicated analysis will be produced and will also compare drought-stressed and well-watered root systems.

Drought resistance screen for a large population of Ramsey x Riparia Gloire hybrids – By combining our conclusions derived from the field population of Ramsey x Riparia Gloire F1 and F2 hybrids and the fine root fraction component of rooted herbaceous cuttings, we are currently investigating an expanded population of 109 genotypes of herbaceous F2 hybrids. A large bottom-heated "tray" of 50:50 perlite and vermiculite was created in half of the greenhouse used for the mist propagation of cuttings (Fig. 9). Twenty replicates were planted for each genotype together with the F1 parents and Ramsey and Riparia Gloire. After four weeks of growth, these plantlets will be harvested, the roots washed and scanned, and root fibrosity will be assessed as earlier described. Because all of these F2 individuals were previously DNA fingerprinted with SSR markers, a QTL analysis can rapidly be performed. We plan to also analyze a similar-sized population of F1 individuals following this experiment.

Refining the genetic, geographic, and environmental characterization of *Vitis berlandieri* for germplasm conservation and rootstock breeding – Jake Uretsky

As previously reported, we are describing the wild grape species *Vitis berlandieri* (*V. cinerea* var. *helleri*) genetically, geographically, and environmentally while comparing it with closely related taxa, especially *V. cinerea*. The lime tolerant *V. berlandieri* was instrumental in developing many of the important rootstocks currently used in grape production, and rootstocks derived from this species, particularly *V. berlandieri* x *V. rupestris* hybrids (e.g., '110R', '140Ru', and '1103P'), have increasingly important traits like drought and/or salinity resistance. Better characterization of *V. berlandieri* will help focus our germplasm collection efforts to minimize redundancy and maximize value and diversity for breeding purposes. Presented here are the refined results from a population structure analysis, as well as principle environmental data that indicate differences in adaptation between *V. berlandieri* and *V. cinerea* populations. The results of initial phenotypic screens of *V. berlandieri* accessions are also reported.

Analysis of population structure – The analysis of population structure included *V. berlandieri* and *V. cinerea* accessions collected in 2015-2016, previously collected accessions from Texas and northeastern Mexico, and accessions from the Wolfskill and Montpellier germplasm repositories. Accessions of *V. candicans* were included in addition to those of *V. berlandieri* and *V. cinerea* to reduce sampling bias. Our results using the population genetics software STRUCTURE showed evidence for two, three, or four subpopulations within the Texas accessions (Figure 10). The strongest evidence was for either two or four subpopulations, with the two population grouping consisting of *V. candicans* versus all other taxa and with *V. berlandieri*, *V. cinerea*, the Mexican b-series seedlings, and *V. candicans* all grouped independently in the four population grouping. Morphological differences among groups provide additional evidence for four subpopulations within the analyzed accessions. Principle coordinates analysis (PCoA) and pairwise F_{st} tests also supported the STRUCTURE results (Figure 11). PCoA visualizes the genetic relationships among accessions without any prior assumptions concerning population structure and divergence, while pairwise F_{st} tests indicate the relationships among individuals within subpopulations compared to relationships within pooled subpopulations. The most appropriate interpretation of these data is that *V. berlandieri* and *V. cinerea* populations are closely related but that significant genetic differences exist between them.

Relationships between genetic and environmental data – We investigated a range of temperature, precipitation, and soil variables for evidence of relationships between environmental and genetic differences among populations. Such relationships can indicate the fitness of accessions for specific environments and, in turn, appropriateness for breeding objectives. Of twenty-three variables tested, mean annual precipitation and soil pH were among the most important features distinguishing between *V. berlandieri* and *V. cinerea* collection locations (Figure 12). Mean annual precipitation was 79.3 cm for *V. berlandieri* accessions and 1070.7 cm for *V. cinerea* accessions, and mean soil pH was estimated at 7.2 for *V. berlandieri* accessions and 6.0 for *V. cinerea* accessions. A Mann-Whitney-Wilcoxon non-parametric test showed that these differences were highly significant ($p << 0.0001$). The relatively small variance in values at *V. berlandieri* collection locations reflect the restricted range of the species compared to *V. cinerea*.

We performed Mantel tests to examine the relationship between genetic and environmental differences among accessions, and found that there was a moderate but highly significant ($r = 0.22$; $p < 0.001$) correlation between genetic and environmental variance. This is important in justifying the concentration of our collection activities to *V. berlandieri* accessions in the Texas Hill Country, as opposed to all *cinerea*-like specimens throughout Texas and even beyond into more eastern and northern states. The genetic-environmental relationship is confounded, however, by a strong correlation ($r = 0.85$; $p < 0.001$) between environment and geography due to the east-west gradient of environmental values (Figure 12). In fact, the genetic-environmental correlation was lost in a partial Mantel test, which tests the genetic-environmental relationship while controlling for geographic distance. In other words, we cannot disassociate environment from geography and, thus, cannot make strong conclusions about the adaptation of our accessions based on our current data. We will return to Texas in mid-June to sample grapevines from the region between the Hill Country and east Texas with the aim of determining the relationship between genetic and environmental differences we have clearly observed between the *V. berlandieri* and *V. cinerea* populations.

Screening for nematode resistance – Ten new *V. berlandieri* accessions were recently tested for resistance to the HarmC of root-knot nematode (RKN). The plants were grown from herbaceous cuttings in pure sand, inoculated with RKN egg masses, and evaluated after six weeks. Although none of the new accessions showed total resistance to nematode infection, most of the accessions possessed significantly fewer egg masses per root biomass than the 'Colombard' control plants (Table 6). This partial resistance could prove useful for stacking resistance genes for more durable resistance in future rootstock cultivars. We are currently propagating an expanded set of *V. berlandieri* accessions to screen for RKN resistance and better assess the diversity for this trait within the species.

Powdery mildew resistance – We screened twenty new *V. berlandieri* accessions for their resistance to powdery mildew. Four young, fully expanded leaves were collected from plants of each accession grown in the greenhouse. The leaves were disinfected in a 1:1 bleach to water solution and rinsed in four changes of autoclaved water. Leaves were inoculated in a settling tower and allowed to incubate for two weeks. Infection was scored on a scale of 1 to 5, with 1 representing no spore germination and 5 representing complete infection and uniform reproduction of the pathogen. A score of 2 or lower indicated that the pathogen did not form reproductive structures. All accessions showed some form of infection; however, two accessions, TX15-073 and TX16-017 showed very limited or no development of reproductive structures, indicating these accessions might possess partial or field-level resistance to the pathogen (Table 7). We will be replicating this screen and including the broader collection of *V. berlandieri* accessions.

Additional phenotypic screens – In addition to the screens previously mentioned, we are awaiting results of a screen of Pierce's disease resistance in a set of *V. berlandieri* accessions that will be taken down in early July. Also, plants are currently being propagated to evaluate salinity tolerance and for an assay using iron reductase synthesis as a proxy for lime tolerance.

Evaluation of Root Traits Associated with Chloride Exclusion-Cassandra Bullock-Bent (with help from K. Fort and C. Agüero)
In January 2017, results were presented from a greenhouse assay that was designed to investigate the effect of salinity stress on root growth of four grape rootstocks in an effort to understand of how root traits relate to salt

tolerance. Rootstocks 140Ru, O39-16, Ramsey and Riparia Gloire (Riparia), represented vines from varying genetic backgrounds and offered a range of chloride exclusion capability based on previous studies. The results highlighted a strong correlation between the percent of fine roots produced and the amount of chloride accumulated in the shoots after three weeks of applied salt (R=0.7124; R=0.9779 using mean values).

Root traits are likely the key to understanding salt tolerance, since roots adapt to environmental signals while acquiring nutrients and water. Chloride enters the grapevine via the roots across the symplastic pathway and accumulates at the highest concentrations in the cortex and pericycle cells compared to the hypodermis and endodermis. The pericycle sequesters Na+ and Cl- and is responsible for initiating lateral root primordia, which may play a vital role in chloride exclusion in grapevine roots. In addition to ion sequestration, auxin development and distribution changes with the onset of salt stress and also affects the growth and production of lateral and primary roots. This suggests that root trait phenotyping, particularly fine and lateral root production, may be used to screen for salt resistance in addition to our current screening methods.

The next step is to expand the greenhouse screen to include more rootstocks in order to determine if the results are repeatable and to evaluate root phenotype across diverse species backgrounds. It would be beneficial to assess root growth development under salt stress via tissue culture, which allows us to scan roots in clear growth media on a weekly basis without destructively harvesting plants. This would allow us to compare an individual plants' growth pattern to itself, which may reduce some of the variability caused by the differences in individual plant growth phase or environmental effects.

14. March through May 2017, we expanded the greenhouse screen to 16 genotypes including the original 140Ru, O39-16, Ramsey, and Riparia, adding: 101-14 Mgt, 110R, 44-53, GRN1, Longii 9018, Longii 9035, NM 03-17, Pumpstation, SC-12, SC-2, Schwarzmann, and St. George. Herbaceous cuttings were collected from established plants maintained in the greenhouse over winter. Cuttings were dipped in 1:20 auxin dilution and established in the mist room in perlite flats for 3 weeks. Cuttings were then transplanted to 1 gallon pots with fritted clay, repeating the process of the previous trial. Pots were arranged in a split plot design according to the treatment application (0mM or 75mM NaCl), with 8 replicates of each genotype for the given treatment for a total of 256 plants. Plants were given 3 weeks of an establishment period, then received 3 weeks of the designated treatment to then be destructively harvested and analyzed as described in the previous report.

Data collection is underway and we hope to complete harvest in June. Roots that were scanned and analyzed using WinRHIZO^{TM} software are yielding promising results, as shown in Figure 13 and 14. The original rootstock varieties used in the first trial are distributed in a slightly different order with O39-16 having the highest percentage of fine roots, followed by Riparia, 140Ru, and Ramsey. These results are not alarming since previous assays have shown that rootstocks rankings that are based on chloride accumulation can change from screen to screen. Rootstocks that have been associated with high chloride accumulation from past studies, including 44-53, Pumpstation, and O39-16 had some of the highest percentages of fine roots, while rootstocks that tend to accumulate lower concentrations of chloride had a lower percentage of fine roots. Interestingly, the two accessions of *Vitis acerifolia* (Longii 9018 and Longii 9035), which are characterized as salt tolerant, have a high percentage of fine roots. This may suggest that *V. acerifolia* has a unique trait that contributes to chloride exclusion, unless the chloride accumulation data show otherwise. It is difficult to draw conclusions without the complete data analysis that includes the leaf chloride percent as dry weight.

Root Trait Analysis in Tissue Culture – January through May 2017, we screened 5 genotypes in vitro including 140Ru, O39-16, Ramsey, 110R and Thompson Seedless with different salt concentrations: 0, 25, 50, and 75mM NaCl. Cuttings were repeatedly micro propagated and grown in agar medium starting in January, to ensure plantlet uniformity and the development of root initials at the start of the trial. Observations from pilot studies showed that rootstocks would yield highly variable growth rates or not grow at all when cuttings were different sizes and directly placed in the treatment media. Each genotype was replicated 10 times for each treatment, with the exception of Thompson Seedless, which had only 4 replicates. The growth medium was composed of Murashige and Skoog (MS) medium, sucrose, NAA, biotin, agarose, and the concentration of NaCl corresponding to the treatment type. All tubes were scanned once a week for 5 weeks and analyzed using WinRHIZO^{TM}. Plants were extracted from the tubes and destructively harvested at the end of the treatment duration. Roots from

harvested plants were scanned again. Fresh weight was recorded for roots and shoots, then tissue was stored in coin envelopes and dried for ≥ two weeks at 37ºC. Dry weight has been recorded, but we still need to measure chloride accumulation in the root and shoot tissue (Figure 15).

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 Viet Nguyen
We continue our work studying the inheritance of grapevine fanleaf virus (GFLV) tolerance that has been observed in O39-16. As previously planned, we have successfully bench grafted cuttings of GFLV-infected Cabernet Sauvignon to hardwood cuttings from individuals of the 101-14 Mgt. x *Muscadinia rotundifolia* 'Trayshed' population. From the 50 genotypes we selected for testing, 41 genotypes grafted and rooted to a degree adequate for field trials. These grafted plants were planted at UCD in early May and we plan to train the plants aggressively this summer in order to see fruiting next year. The plants will later be observed for differences in fruiting characteristics (mainly the presence of the characteristic disrupted fruit set symptoms of GLFV). In order to observe any differences in GFLV multiplication among the different members of the population, at least three replicates of each bench graft combination were also grown in the greenhouse for a future GFLV assay. In June (four months after the initial bench grafting), the roots of the surviving greenhouse plants will be assayed for GFLV using RT-qPCR. We hope to find a correlation of relative GFLV concentration with severity of GFLV fruit set symptoms in the field.

Screening of Fertile VR Hybrids for GFLV Tolerance – As previously planned, we also included several fertile VR (*vinifera* x *rotundifolia*) hybrid genotypes in our GFLV tolerance and multiplication screen. From our original 15 VR selections, only 13 genotypes grafted and rooted successfully. These plants were grafted and planted simultaneously with the 101-14 x Trayshed progeny, so we expect a similar timeline for the progression of this project.

GFLV Multiplication in GRN-1 – We are waiting for our field vines grafted on GRN-1 (*V. rupestris* x *M. rotundifolia*) to be inoculated with GFLV in order to study the potential rootstock-induced GFLV tolerance from this rootstock. As this will take time, we have started study on the degree of GFLV multiplication in GRN-1 under greenhouse conditions. We approach grafted GFLV-infected Chardonnay plants to GRN-1 plants for inoculation purposes. After four months, leaf samples from the GRN-1 scions were assayed for GFLV using RT-qPCR. Our results show that GRN-1 does not resist GFLV multiplication under these conditions (Figure 16). This is in contrast to O39-16, which does resist GFLV multiplication (Figure 17). However, it is possible that the mechanism for the induction of fanleaf tolerance may not interact directly with the virus, so we should not eliminate the possibility that GRN-1 may also be able to induce fanleaf tolerance.

Describing the Impact of O39-16 on Fanleaf Sites - We have started work on conclusively proving that O39-16 suppresses the typical fruit set symptoms associated with GFLV. Although this is generally an accepted fact, there are no recent publications describing the impact of O39-16 in fanleaf sites. In an established vineyard in Lodi, CA, we have bagged four flower clusters pre-bloom on 10 infected vines grafted on O39-16, 10 uninfected vines grafted on O39-16, 10 infected vines grafted on St. George, and 10 uninfected vines grafted on St. George.

Infection status will be verified by PCR. We will obtain the flower to berry ratio on each bagged flower cluster to quantify percent fruit set and subsequently be able to compare fruit set between healthy and infected vines grafted on O39-16 and also fruit set between infected vines grafted on either O39-16 or St. George.

Rootstock tolerance to red leaf viruses – Zhenhua Cui

We have been utilizing in vitro micrografting to examine tolerance to red leaf virus in grape rootstocks. When grafting on both Freedom and St. George (St.G), Cabernet franc (Franc) had significantly greater growth than LR131 (Fig.19A). Although Franc had much greater growth on Freedom than on St.G, when the Franc was infected by leaf roll (LR131) growth was similar on those two rootstocks (Fig.19A). When grafted with LR131 infected scions, both Freedom and St.G had less root growth than when grafted by clean Franc (Fig.19B). It was apparent that LR131 had a greater inhibition on the root growth of Freedom than St.G (Fig.19B). ANOVA showed that both rootstock genotype and virus had significant effect on scion growth and root growth after grating (Table 8), but there was no interaction between them. LR132 is a very aggressive leafroll strain, which greatly inhibits the growth of *in vitro* plants. When LR132 was used for micrografting, none of the grafted plants survived (Table 9).

Herbaceous grafting – Besides Freedom and St.G, 101-14 and AXR were also used for green herbaceous grafting with LR131 + and - Franc. Overall, when grafted by either LR131+ or - Franc, AXR resulted a lower survival rate than the other three rootstocks (Table 9). But among all the combinations, there was no significant difference in survival rate. However, scion biomass showed dramatic differences. When grafting on Freedom and 101-14, LR131 caused significant reduction in biomass of 19.6% and 13.4%, respectively, compared with clean Franc (Fig. 20). Yet there was no significant change in biomass between LR131 infected and clean Franc when grafting on St.G and AXR (Fig.2).

The degree of grape leafroll disease (GLD) symptoms on LR131 was assessed 6 months after grafting. A scale of GLD symptoms was made using two criteria, degree of leaf-roll of the leaf blade, and reddish coloration (Fig. 21). The leaves were scored from 1 to 5 (most severe). The assessment of GLD symptom degree on LR131 is shown in Fig. 22. LR131 showed a higher degree of GLD symptoms when grafted on 101-14 and Freedom with a total score of 6.7 and 5.5, respectively, than when grafted on St.G and AXR with a total score of 3.1 and 2.8, respectively (Fig. 22).

Bench grating – Since LR131 woody cuttings were not available last winter, only LR132 was used for dormant cane bench grafting. Although LR132 performed better in bench grafting than micrografting, it still showed a low survival rate (18%) when grafting on Freedom compared with other rootstocks (Table 9). 101-14 also had a low survival rate below 50% when grafted with LR132 infected wood (Table 2). Even though LR132 had a highest survival rate of 58% when grafting on St.G, it was still much lower than when grafted with clean Franc (Table 9).

Plans for the next 6 months:

Expand the micrografting, herbaceous and dormant cane bench grafting with Franc, LR131 and LR132 scions on Freedom, 101-14, St.G and AXR.

Complete the assessment of the degree of GLD symptom expression caused by LR132 by herbaceous grafting on the four rootstocks in greenhouse.

Quantify the amount LR131 and LR132 viurses after grafting on the 4 rootstocks.

Finalize the impact of LR131 and LR132 on biomass development by quantifying both virus levels and GLD expression on different rootstocks.

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

Talks at Grower Meetings (Extension/Outreach) – July 2016 – June 2107

Grape rootstocks – what's known, what's assumed and what's coming. 11th Annual Enology and Viticulture Conference, Penticton, BC, Canada, July 18,2016

Grape flowering. Daniel Roberts Client Group Seminar, Martinelli Winery, Santa Rosa, CA July 22, 2017.

UCD vineyard tour. Lake County Winegrape Growers, UCD, August 17, 2016

Growing winegrapes in California, Chinese Agricultural Delegation, 9, 2016

Improving grape rootstocks for table grape use. Chilean Table Grape Growers, UCD Oct 3, 2017

Grape breeding above and below ground. Cal Poly SLO Seminar, Oct 6, 2016
Grape breeding update. CDFA NT, FT, Grapevine Improvement Advisory Board, UCD/FPS, Nov 1, 2016
PD resistant winegrapes and rootstocks. Texas A&M Grape Grower meeting, Driftwood, TX, Nov 18, 2016
The next steps for the PD resistant grape breeding program. UCD Conference Center, Nov 29, 2016
UCD grape breeding program update. FPS Annual Meeting, Dec 1, 2016
Progress in the grape breeding program. Vine Health Seminar, UCD ARC, Dec 9, 2016
Update on the breeding of slat and drought resistant grape rootstocks. San Joaquin Valley Grape Symposium, C.P.D.E.S Hall, Easton, CA, Jan 11, 2017
Breeding grapes to adapt to climate change. 3rd International Symposium on Grapes, Hermosillo, Sonora, Mexico, Jan 27, 2017
The origin of winegrapes. Daniel Roberts Client Group Seminar, Martinelli Winery, Santa Rosa, CA, Jan 30, 2017
Rootstock breeding update. Current Wine and Grape Research, UC Davis Conference Center, Feb 13, 2017
Gape roots a primer. Napa Valley Grape Grower Meeting, Napa, CA, Mar 1, 2017
Establishing and managing grape vines with less water. Santa Carolina Growers meeting, Chile, Mar 23, 2017
Vineyard challenges, Wine Executive Program, UCD Business School, Mar 28, 2017
Development of grape rootstocks for control of pests and diseases, 63rd Conference on Soil-borne Plant Pathogens, UCD, Mar 30, 2017
Grape breeding update, CDFA IAB meeting, Apr 19, 2017

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

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 142:36-46.

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).

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 12 (1): e0170678. doi:10.1371/journal.pone.0170678.

Wolkovich, E.M., D.O. Burge, M.A. Walker and K. Nicholas. 2017. Phenological diversity provides opportunities for climate change adaptation in winegrapes. Journal of Ecology. **DOI:**10.1111/1365-2745.12786.

Zhen-Hua, C., BWen-Lu, H. Xin-Yi Hao, L. Peng-Min, D. Ying, M. A. Walker, X. Yan and W. Qiao Chun. 2017. Drought stress enhances up-regulation of anthocyanin biosynthesis in Grapevine leafroll-associated virus 3 infected *in vitro* grapevine (*Vitis vinifera*) leaves. Plant Disease (In Press).

Table 1. 2017 pollinations, completed and planned.

Cross #	Female	Male	Purpose
2017-027	101-14 Mgt	07107-050 FH 05-08 T=tetraploid	Fanleaf tolerance
2017-028	101-14 Mgt	acerifolia 9018	Salt and nematode, improved rooting
2017-029	101-14 Mgt	07107-050 FH 05-08 D=diploid	Fanleaf tolerance
2017-030	101-14 Mgt	07107-044 FH 05-02 T=tetraploid	Fanleaf tolerance
2017-031	101-14 Mgt	07107-044 FH 05-02 D=diploid	Fanleaf tolerance
2017-032	101-14 Mgt	acerifolia 9035 K4	Salt and nematode, improved rooting
2017-033	101-14 Mgt	treleasei NM 03-17 S01 K1	Salt and nematode, improved rooting
2017-034	101-14 Mgt	girdiana SC11	Salt and improved rooting
2017-035	101-14 Mgt	2012-142-25	Salt and nematode, improved rooting
2017-036	101-14 Mgt	2012-144-24	Salt and nematode, improved rooting
2017-037	101-14 Mgt	2012-144-39	Salt and nematode, improved rooting
2017-038	101-14 Mgt	07107-079 FH 05-35 T=tetraploid	Fanleaf tolerance
2017-039	101-14 Mgt	07107-079 FH 05-35 D=diploid	Fanleaf tolerance
2017-040	101-14 Mgt	11188-003	Fanleaf tolerance
2017-044	12108-032	GRN-2 9363-16	Salt and broad nema
2017-045	12108-032	GRN-4 9365-85	Salt and broad nema
2017-046	12108-032	GRN-5 9407-14	Salt and broad nema
2017-047	06104-002	GRN-2 9363-16	Salt and broad nema
2017-048	06104-002	GRN-4 9365-85	Salt and broad nema
2017-049	06104-002	GRN-5 9407-14	Salt and broad nema
2017-056	2012-144-41	Schwarzmann	Salt and broad nema
2017-057	2012-144-41	Teleki 5C	Salt and broad nema
2017-058	2012-144-41	1616C	Salt and broad nema
2017-059	2012-144-41	GRN-2 9363-16	Salt and broad nema
2017-060	2012-144-41	GRN-4 9365-85	Salt and broad nema
2017-061	2012-144-41	110R	Salt and broad nema
2017-062	2012-144-41	1103 Paulsen	Salt and broad nema
2017-065	5BB Kober	NM 03-17 S01 K1	Salt and broad nema
2017-069	5BB Kober	acerifolia 9018	Salt and broad nema
2017-070	5BB Kober	acerifolia 9035 K4	Salt and broad nema
2017-072	5BB Kober	2012-142-25	Salt and broad nema
2017-073	5BB Kober	2012-144-24	Salt and broad nema
2017-074	5BB Kober	2012-144-39	Salt and broad nema
2017-075	5BB Kober	07107-079 FH 05-35 T=tetraploid	Fanleaf tolerance
2017-076	5BB Kober	07107-079 FH 05-35 D=diploid	Fanleaf tolerance
2017-077	5BB Kober	07107-050 FH 05-08 D=diploid	Fanleaf tolerance
2017-078	5BB Kober	11188-003	Fanleaf tolerance
2017-079	5BB Kober	07107-044 FH 05-02 D=diploid	Fanleaf tolerance
2017-093	GRN-3 9365-43	girdiana SC11	Salt, boron, nematodes
2017-095	GRN-3 9365-43	acerifolia 035 K4	
2017-096	GRN-3 9365-43	11188-003	Broad nema resistance, B tolerance
2017-098	GRN-3 9365-43	2012-144-39	Broad nema resistance, B tolerance
2017-099	GRN-3 9365-43	12142-021	Broad nema resistance, B tolerance
2017-101	GRN-3 9365-43	12108-028	Broad nema resistance, B tolerance
2017-102	GRN-3 9365-43	12149-021	Salt and nema resistance
2017-103	GRN-3 9365-43	12149-030	Salt and nema resistance

2017-104	GRN-3 9365-43	2012-142-25	Broad nema resistance, B tolerance
2017-105	GRN-3 9365-43	10115-022	Ring and RKN
2017-106	12142-021	GRN-2 9363-16	Broad nema resistance, B tolerance
2017-107	12142-024	GRN-4 9365-85	Broad nema resistance, B tolerance
2017-113	GRN-3 9365-43	acerifolia 9018	Salt and nema resistance
2017-173	SC1	GRN-2 9363-16	Salt, boron, nematodes
2017-174	SC1	GRN-4 9365-85	Salt, boron, nematodes
2017-175	SC1	GRN-5 9407-14	Salt, boron, nematodes
2017-176	SC1	110R	Salt, boron
2017-177	SC1	1103 Paulsen	Salt, boron
2017-178	SC1	140Ru	Salt, boron
2017-182	SC12	NM 03-17 S01 K1	Salt, boron
2017-183	SC12	SC11	Salt, boron
2017-184	SC12	GRN-2 9363-16	Salt, boron
2017-185	SC12	GRN-4 9365-85	Salt, boron
2017-186	SC12	GRN-5 9407-14	Salt, boron
2017-187	SC12	1103 Paulsen	Salt, boron
2017-188	SC12	110R	Salt, boron
2017-189	SC12	140Ru	Salt, boron
2017-193	2012-108-28	GRN-2 9363-16	Salt and nema resistance
2017-194	2012-108-28	GRN-4 9365-85	Salt and nema resistance
2017-195	2012-108-28	GRN-5 9407-14	Salt and nema resistance
	F2-7	GRN-2 9363-16	Mapping
	F2-7	GRN-4 9365-85	Mapping
	F2-7	GRN-5 9407-14	Mapping
	F2-35	420A Mgt	Mapping
	F2-35	GRN-2 9363-16	Mapping
	F2-35	GRN-4 9365-85	Mapping

Table 2. 2016 seedlings ready for field planting, expected late June 2017.

Cross ID	Female	Male	# To Field	Cross Purpose
2016-029	101-14 Mgt	arizonica GC5 K1	48	Salt resistance and better rooting, moderate vigor
2016-036	101-14 Mgt	2012-144-24 (161-49C x arizonica)	50	Salt resistance and better rooting, moderate vigor
2016-046	161-49C	arizonica GC5 K1	50	Lime, salt, nematodes
2016-050	161-49C	b55-1 fertile VR	1	VR hybrid, lime, rootability
2016-051	161-49C	2012-142-25 (161-49C x arizonica)	10	Salt resistance
2016-052	161-49C	2012-144-24 (161-49C x arizonica)	50	Salt resistance
2016-053	161-49C	2012-144-39 (161-49C x arizonica)	50	Salt resistance
2016-063	5BB Kober	b55-1 fertile VR	50	Add VR resistance to berl x riparia rootstock
2016-064	5BB Kober	2011-188-06 (T6-42 x St. Geo)	10	Add VR resistance to berl x riparia rootstock
2016-069	5BB Kober	berlandieri 9031 K3	50	Add better drought and salt to 5BB
2016-072	5BB Kober	2012-142-25	50	Salt resistance and better

				rooting, moderate vigor
2016-073	5BB Kober	2012-144-24	50	Salt resistance and better rooting, moderate vigor
2016-090	GRN-3 9365-43	NM 03-17 S01 K1	41	Add salt and drought resistance to GRN3
2016-095	GRN-3 9365-43	acerifolia 9035 K4	8	Add salt and drought resistance to GRN3
2016-096	GRN-3 9365-43	2012-142-25	40	Add salt and drought resistance to GRN3
2016-097	GRN-3 9365-43	2012-144-24	50	Add salt and drought resistance to GRN3
2016-110	doaniana 83 K3/4	GRN-4 9365-85	23	Deep roots and very high nema resistance as well as TX root rot
2016-113	GRN-3 9365-43	acerifolia 9018	5	
2016-131	Dog Ridge	girdiana SC11	50	Better salt resistance to Dog Ridge and TX root rot
2016-134	Dog Ridge	arizonica GC5 K1	38	Drought and salt with very deep roots
2016-135	Dog Ridge	acerifolia 9035 K4	50	Drought and salt to Dog Ridge
2016-136	Dog Ridge	2011-175-15	56	Drought and salt with very deep roots
2016-141	9026 (doaniana)	GRN-4 9365-85	4	Deep roots high vigor to GRN4
2016-143	Ramsey	arizonica TX12-003	41	Better roots and salt resistance
2016-158	Ramsey	arizonica GC5 K1	50	Better roots and salt resistance
2016-162	Ramsey	acerifolia 9035 K4	50	Better roots and salt resistance, lime tolerance, Drought and salt in low vigor background
2016-165	riparia 1411	arizonica GC5 K1	37	Drought and salt in low vigor background
2016-168	riparia 1411	b55-1 fertile VR	5	VR in a weak good rooting background
2016-169	riparia 1411	2012-142-25	48	Better rooting, salt and nematodes
2016-170	riparia 1411	2012-144-24	4	Better rooting, salt and nematodes
2016-171	riparia 1411	2012-144-39	30	Better rooting, salt and nematodes
2016-190	SC2 K2	GRN-2 9363-16	22	Salt and boron to GRN nema
2016-191	SC2 K2	GRN-4 9365-85	29	Salt and boron to GRN nema
2016-196	SC2 K2	2012-144-24	23	Salt, boron, nematodes
2016-197	SC2 K2	2012-144-39	47	Salt, boron, nematodes

2016-198	berl 9019 K3	Schwarzman	40	Salt, nema, good rooting
2016-203	berl 9019 K3	110R	5	Salt, nema, lime

Table 3. Seedlings and populations tested for resistance to a combined inoculum of RKN strains HarmA&C, with a few ring nematode results as noted. These are evaluations conducted since January 2017. The results are for listed as R = resistant (<2 egg masses) or S = susceptible (>1 egg mass). There were many examples of moderate resistance but these were discarded. If more than one individual in a seedling population was tested the results are listed as numbers R/S.

Population or Seedling	Parentage	Seedlings tested R/S	Decision	Purpose
06104-028	101-14 Mgt x 9363-16	1S	Remove From Pipeline	Low vigor, broad nema, good rootability
06105	101-14 Mgt x 9407-14	2R	Move Forward To Ring Nema Assay	low vigor, good rootability, broad nema
06109	101-14 Mgt x 9365-85	2/1	Move Forward To Ring Nema Assay	low vigor, broad nema
0707-027	5BB x b40-14	R	Move Forward To Ring Nema Assay	*X. index* resistance
0708-022	5BB x R8916-22	S	Remove From Pipeline	*X. index* resistance
07170	9365-43 x 8916-20	3/1	Move Forward To Ring Nema Assay	*X. index* resistance, RKN
07171	9365-43 x 8916-22	2S	Remove From Pipeline	*X. index* resistance, RKN
08140-007	Cosmos 2 x 8908-19	1S	Remove From Pipeline	*X. index* resistance, RKN
08143	Cosmos 2 x b57-39	3S	Remove From Pipeline	*X. index* resistance, RKN
08171-002	9365-43 x 8916-22	S	Remove From Pipeline	*X. index* resistance, RKN
08314-031	03300-048 x 06301-93	R	Move Forward To Ring Nema Assay	PD, RKN
10115-029	161-49C x Trayshed	1/1	Move Forward To Ring Nema Assay	*X. index*, ring, RKN
11143-005	Ramsey X 08314-15	1/3	Move Forward To Ring Nema Assay	PD, RKN
11144-015	Ramsey X 08314-46	S	Remove From Pipeline (AT keep for PD use)	PD, RKN
11188	T6-42 X St. George	1/1	Move Forward To Ring Nema Assay	Fertile VR, ring, RKN, *X. index*
12110	101-14 Mgt x GRN-5 9407-14	12/11	Move Forward To Ring Nema Assay	broad nema, improved rooting
12112	101-14 Mgt x GRN-2 9363-16	6/4	Move Forward To Ring Nema Assay	low vigor, broad nema
12113	101-14 Mgt x	11/9	Move Forward To Ring	decent vigor, broad nema

	GRN-4 9365-85		Nema Assay	
12115	161-49C x Trayshed	2Ring	Move Into Confirmation Ring Nema Assay And Xi Assay	phylloxera, broad nema
12118	161-49C x GRN-4 9365-85	5/13	Remove From Pipeline	vigor, broad nema
12125	OKC-1 SO1 (acerifolia) x GRN-2 9363-16	5/4	Remove From Pipeline	Salt, broad nematode
12126	OKC-1 SO1 (acerifolia) x GRN-4 9365-85	10/2	Move Forward To Ring Nema Assay	Salt, broad nematode
12129-007	OKC-1 SO1 (acerifolia) x St. George	1R	Move Forward To Ring Nema Assay	Salt, broad nematode
12129	OKC-1 SO1 (acerifolia) x St. George	0/4	Remove From Pipeline	Salt, broad nematode
12178-002	Dog Ridge x Trayshed	1R	Move Forward To Ring Nema Assay	soil pests
12185	GRN-3 9365-43 x berlandieri 9031	3/2	Remove From Pipeline	Salt, lime, broad nematode
12187	GRN-3 9365-43 x berlandieri 9043	1/2	Move Forward To Ring Nema Assay	Salt, broad nematode
13180-001	T6-42 x 1616C	RingS	Remove From Pipeline	Fertile VR, ring, RKN, *X. index*
13183-001	T6-42 x GRN-2 9363-16	S	Remove From Pipeline	Fertile VR, ring, RKN, *X. index*
Ramsey	Breeding Collection	S	Characterization for Ring	
Girdiana SC12	Collection	S	Remove From Pipeline	

Table 4. Resistant parents of proposed crosses.

Species	**Rootstocks**
V. doaniana T9	101-14Mgt
V. acerifolia 9027	1616C
V. acerifolia 9035	420A
V. champinii 9021	GRN2
V. champinii 9037	GRN3
V. cinerea b45-26	GRN4
V. cinerea-arizonica b41-23	GRN5
V. doaniana 9024	
V. doaniana 9026	
V. doaniana 9028	
V. girdiana SC12	

V. candicans T56
V. candicans T64
V. vulpina 9006

Table 5. Segregation pattern of Resistant X Resistant crosses. Desired segregation ration for two single loci is 3:1.

Cross	Resistant	Susceptible
101-14Mgt x GRN-2	16	9
101-14Mgt x GRN-4	16	6
101-14Mgt x GRN-5	12	7

Table 6. Mean root-knot nematode egg masses, dry root biomass, and egg masses per root biomass of ten *V. berlandieri* accessions, and 'GRN-1' and 'Colombard' controls.

Genotype	Egg Masses	Dry Root Biomass (g)	Egg Masses / Biomass
Colombard	64.8	2.18	29.7
GRN-1	0.0	1.32	0.0**
TX15-003	3.0	1.77	1.7*
TX15-091	0.8	1.25	0.6**
TX16-015	7.0	2.10	3.3*
TX16-018	3.8	2.02	1.9**
TX16-022	1.0	1.78	0.6**
TX16-026	10.0	1.70	5.9*
TX16-032	4.5	3.24	1.4**
TX16-034	2.5	1.57	1.6**
TX16-065	28.3	2.54	11.1
TX16-068	1.0	1.53	0.7*

Asterisks represent significantly fewer (*$p < 0.05$; **$p < 0.01$) egg masses per root biomass than 'French Colombard' as determined by Dunnett's test ($n = 4$).

Table 7. Powdery mildew Infection scores for twenty *V. berlandieri* accessions and the control 'Carignan'.

Genotype	Infection Score
Carignan	5.0
TX15-003	4.1
TX15-063	3.3
TX15-073	1.8**
TX15-091	4.3
TX16-012	5.0
TX16-015	3.4
TX16-016	4.3
TX16-017	2.1*
TX16-018	3.1
TX16-022	4.2
TX16-025	4.7
TX16-026	4.0
TX16-030	4.8
TX16-032	3.8
TX16-034	3.8
TX16-035	4.8
TX16-063	3.9
TX16-064	3.3

TX16-065	5.0
TX16-068	4.4

Asterisks represent significantly lower (*p < 0.005; **p < 0.001) infection scores than 'Carignan' as determined by Dunnett's test (n = 4).

Table 8. ANOVA of effects of virus, rootstock and their interactions on the growth of micrograft of *in vitro Vitis.*

Investigated indexes	Virus	Rootstock	Virus x Rootstock
Shoot growth	**	*	ns
Root growth	**	**	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.

Table 9. Grafting trials with Cabernet franc infected with LR131 or LR132 or clean (Franc) in different combinations.

Combination	Survival/# attempted	Survival rate (%)
Micrografting		
Franc/Freedom	27/30	90
LR131/Freedom	0/30	0
Franc/St. Geo	27/30	90
LR131/St.Geo	0/30	0
Green Herbaceous Grafting (3 reps)		
Franc/Freedom		80±12
LR131/Freedom		77±11
Franc/St.Geo		82± 8
L131/St.Geo		76± 10
Franc/101-14		82± 8
LR131/101-14		79± 9
Franc/AXR		65 ±15
LR131/AXR		67± 10
Dormant Cane Bench-grafting		
Franc/Freedom	33/40	83
LR132/Freedom	11/60	18
Franc/St.Geo	40/40	100
LR132/St.Geo	35/60	58
Franc/101-14	37/40	93
LR132/101-14	28/60	47
Franc/AXR	39/40	98
LR132/AXR	30/60	90

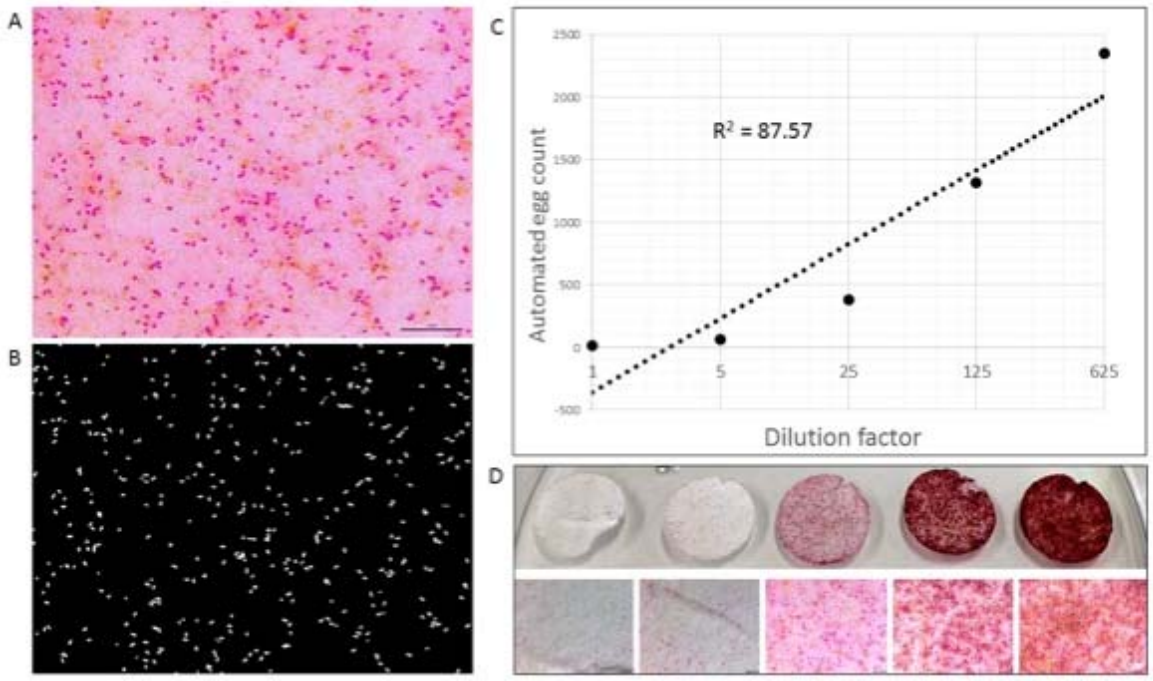


Figure 1 A, Acid Fuchsine stained RKN eggs on the filter paper. **B**, Processed image from ImageJ software, the count masks of RKN eggs from the pictures above. **C**, Logarithmic dilution series and correlation with the automated egg counts from ImageJ software. **D**, The respective Whatmann filter paper discs and the processed micro images.

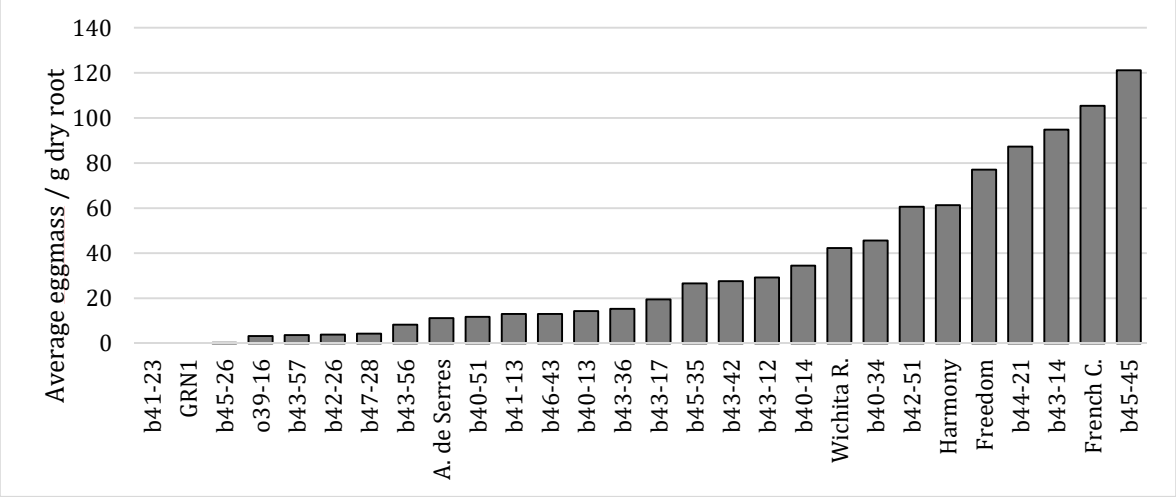


Figure 2 Average eggmass counts (both for HarmA and HarmC) of selected accessions from "b-series" of Mexican grape species. The identified resistant accession b41-23 showed no eggmasses, while b45-26 showed very limited nematode reproduction with only HarmA strain.

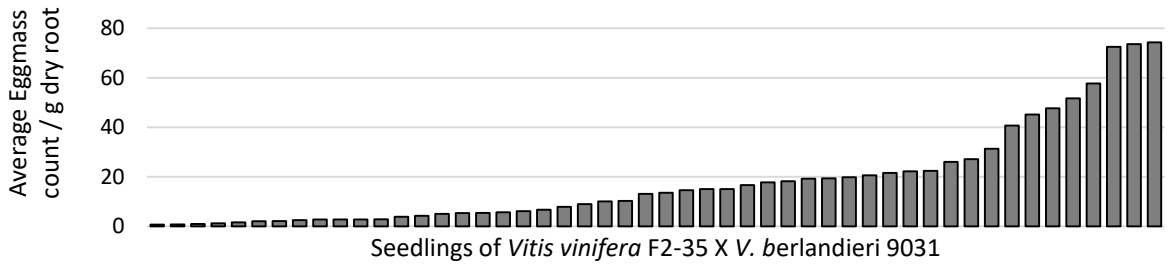


Figure 3. RKN evaluation of the population derived from *V. berlandieri* 9031.

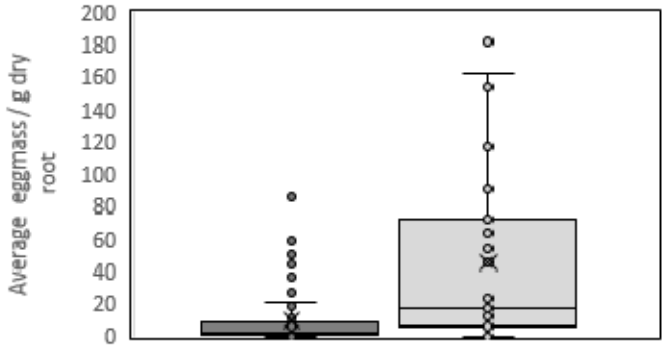
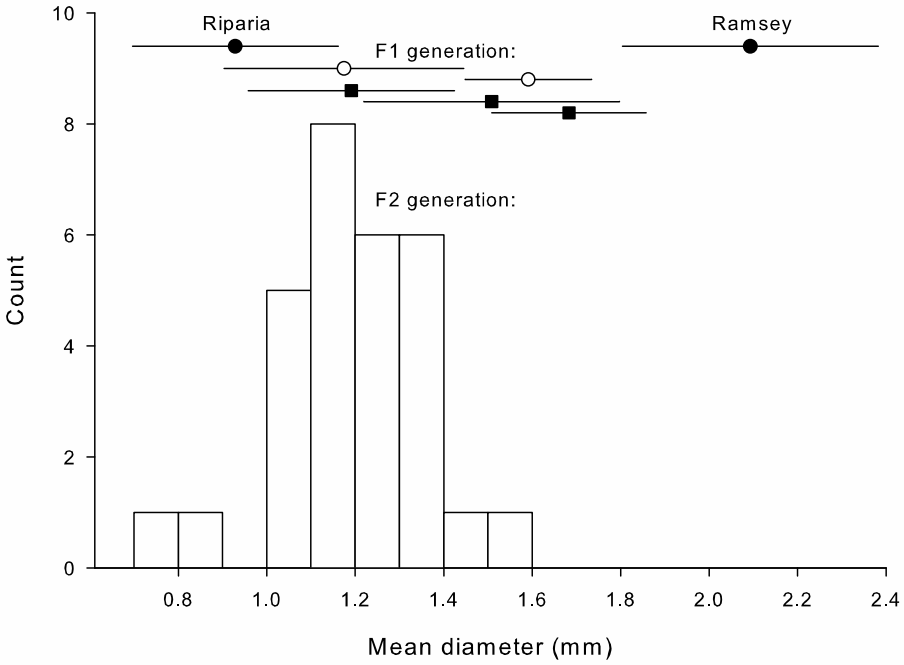
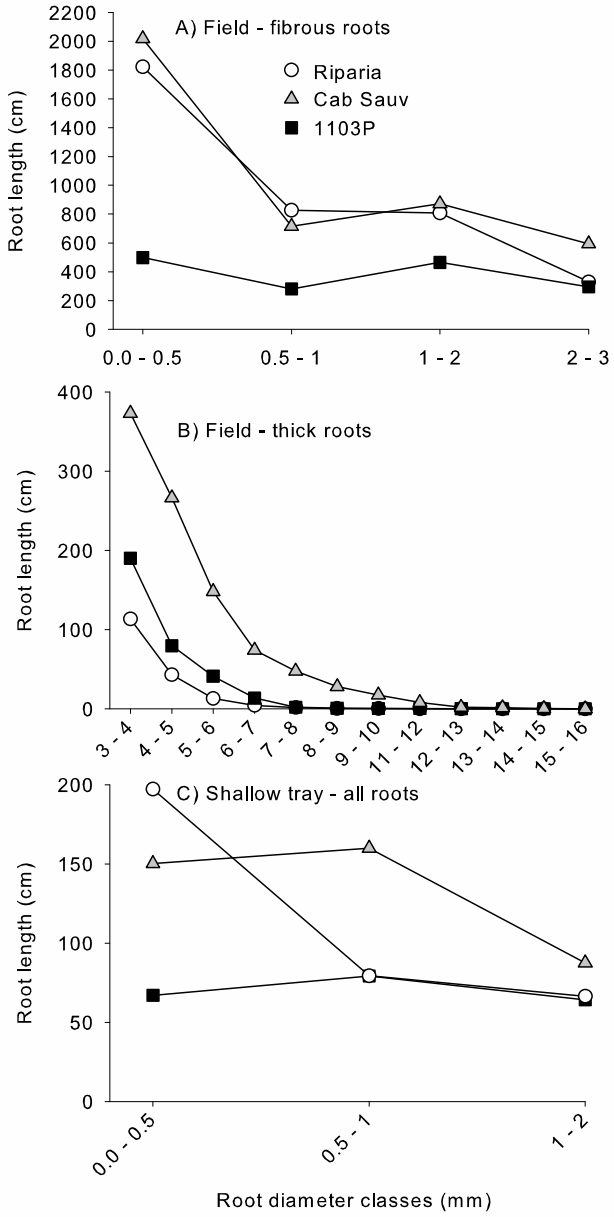


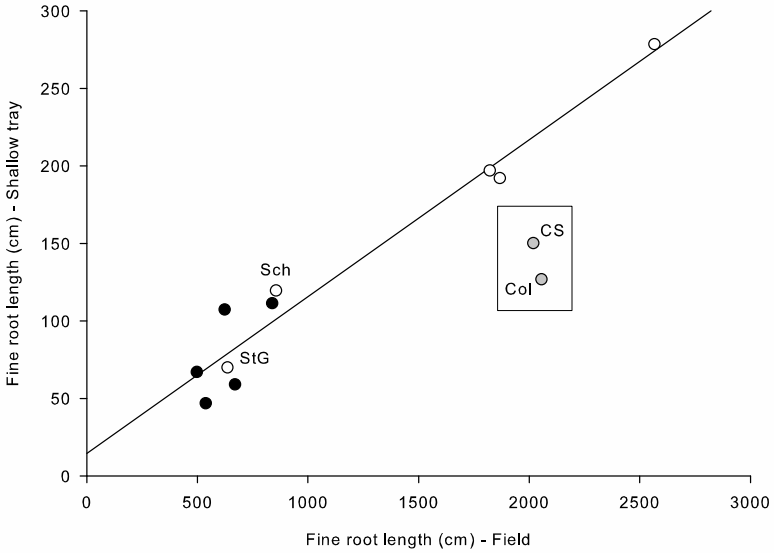
Figure 4. Average eggmass counts with HarmC (dark grey) and with HarmA (light grey) across the genotypes of the first screen of germplasm.



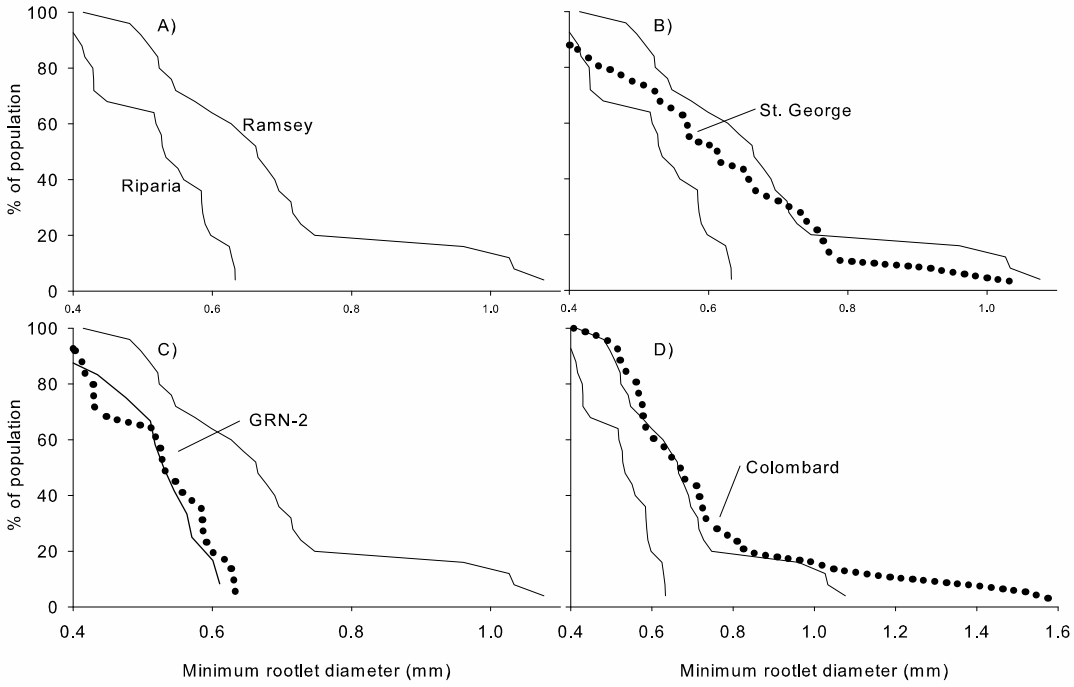
ve selections
ıre the parents



s: *Vitis berlandieri* X
) and *Vitis vinifera*
lants, B) thick root
house-grown



ɔaceous cuttings of ten *Vitis*
plants. Gray symbols (CS =
by a rectangle to indicate
hwarzmann'
ɾived



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ght
paria. Note

Figure 9. Full population screen of Ramsey x Riparia F2 hybrids for root characteristics related to drought tolerance.

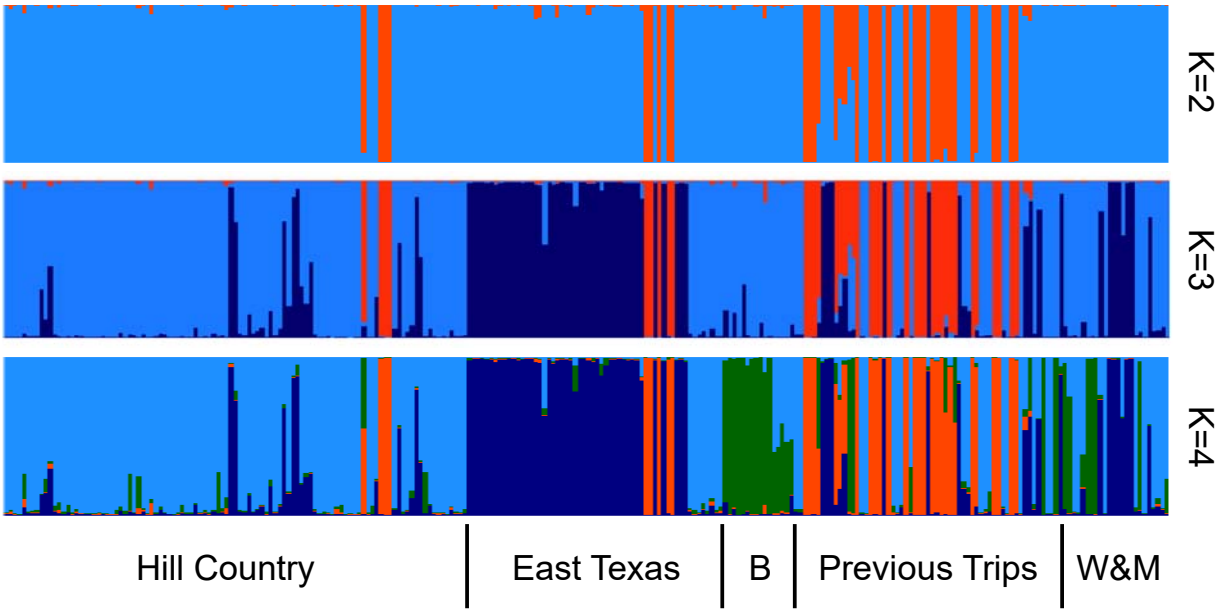

Figure 10. Population structure of *V. berlandieri* and related taxa in Texas and northeastern Mexico. 'Hill Country' and 'East Texas' accessions were collected in 2015-2016; 'B' refers to b-series, *V. cinerea*-like seedlings procured by H. Olmo from northeastern Mexico and southwestern Texas; 'Previous Trips' are previously collected accessions; 'W&M' are accessions from the Wolfskill and Montpellier germplasm repositories. For K = 2, LIGHT BLUE = *berlandieri, cinerea,* and b-series; ORANGE = *candicans*. For K = 3, LIGHT BLUE = *berlandieri* and b-series; DARK BLUE = *cinerea*; ORANGE = *candicans*. FOR K = 4, LIGHT BLUE = *berlandieri*; DARK BLUE = *cinerea*; GREEN = b-series; ORANGE = *candicans*.

Figure 11. PCoA and table of pairwise F_{st} values of *berlandieri*, *cinerea*, b-series, and *candicans* accessions. These results support the close but independent grouping of *berlandieri* and *cinerea*.

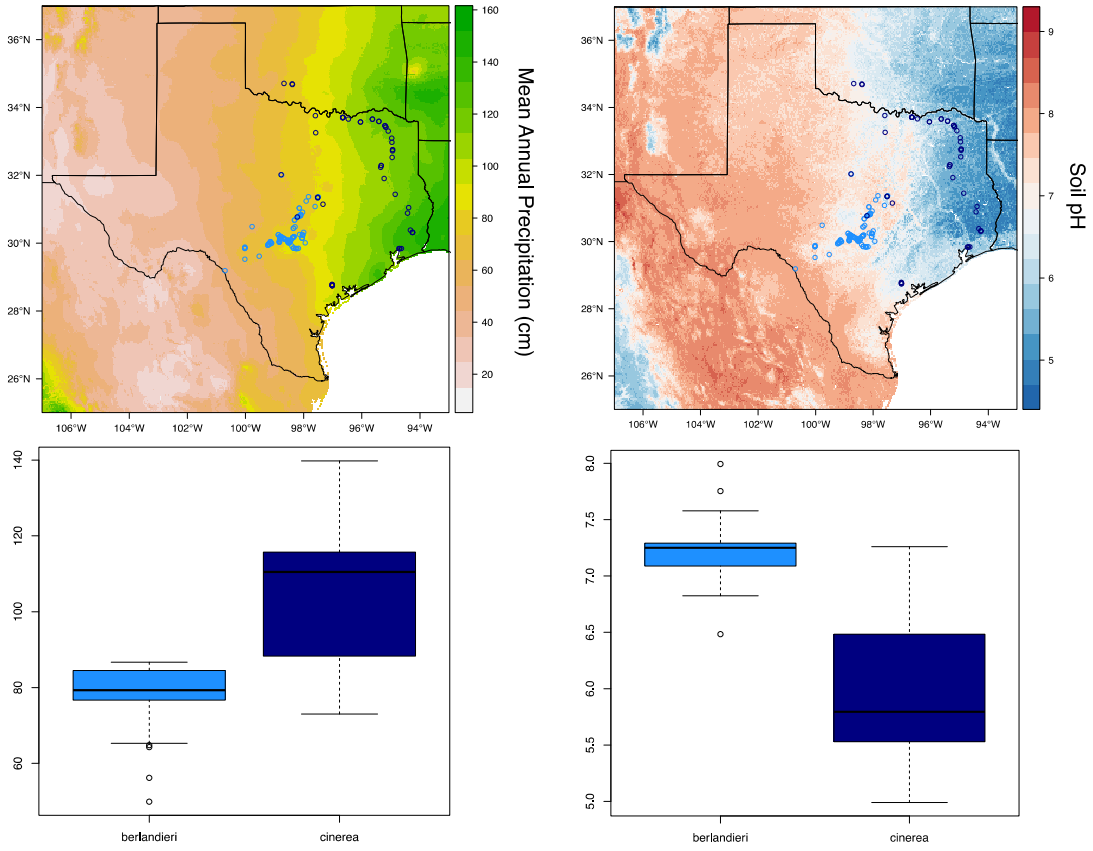

Figure 12. Mean annual precipitation (left) and soil pH (right) at collection locations for *V. berlandieri* and *V. cinerea* accessions and DNA samples. The small environmental variance for *V. berlandieri* collection locations indicates the restricted range of the species. Differences in mean annual precipitation and soil pH were highly significant ($p << 0.0001$) between *V. berlandieri* and *V. cinerea* collection locations according to a Mann-Whitney-Wilcoxon non-parametric test.