

June 2019 Progress Report
California Grape Rootstock Improvement Commission
California Grapevine Rootstock Research Foundation
American Vineyard Foundation
California Table Grape Commission
CDFR Improvement Advisory Board

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

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Overall Summary: Since my last report (Jan 2019) we have made good progress in upgrading and streamlining our nematode resistance screening. Nina Romero has made excellent improvements to our screening and is currently re-vamping our ring nematode resistance screening. There are 444 genotypes in testing either for nematodes, salt or both. Our 2019 crosses again focused on using fertile and tetraploid VR hybrids to get *rotundifolia* forms of resistance into better rooting backgrounds. The tetraploids were created by Cecilia Agüero. Seed from the 2018 crosses are mostly in storage, except for the 18113 (GRZN3 x *V. acerifolia* 9018. Chris Chen is working on his PhD with this population which brings excellent and broad nematode resistance to our best form of salt tolerance (which also has strong root-knot resistance). We have improved our phylloxera screening in the greenhouse and have verified a number of fertile VR hybrids also have strong phylloxera resistance. We hope to hire a post-doc to assist with examining phenolic compounds responsible for phylloxera and nematode resistance. They will also coordinate our efforts to determine how O39-16 induces fanleaf degeneration resistance. We are also making good progress on identifying the basis of red leaf virus tolerance. A new post-doc, will work with two visiting professors). We have rootstock examples of strong tolerance (St. George and AXR1) and very sensitive (Freedom and 101-14) and rapid tissue-culture and greenhouse-based screens that are rapid and mimic field tests.

2019 Pollinations: Table 1 presents the 2019 crosses which were designed to use tetraploid and diploid *Vitis Muscadinia* hybrids from crosses of 101-14 x *M. rotundifolia* Trayshed (the 07107 population. We are advancing these as rootstocks but have thus far not been able to find fertile forms that we could introgress the strong resistance from *rotundifolia* into other backgrounds that root well. The few seeds we have produced were not viable. We did produce a few mapping populations this year to complete our examinations of rupestris and riparia. The crosses with T6-42 (19-064, -065 and -066) are exciting as T6-42 (a fertile VR – *vinifera rotundifolia*) hybrid has repeatedly tested as resistant to phylloxera and ring nematodes.

2018 Pollinations and planting: None of the 2018 crosses were germinated except 18-113 (GRN-3 x longii 9018) – Table 2. The first 78 genotypes have been planted in the field. Copies are being readied for salt and HarmAC testing.

Nina ran a trial with this set to see if the seedling root architecture could predict the architecture of green and hardwood cuttings. She completed the comparison with greens and there was poor correlation both in respect to root angle ($R^2=0.49$) and root thickness ($R^2=0.15$). In addition, she tried different containers, both 4" pots and Styrofoam cups as well as two media, perlite and our standard seedling mix which is the UC agronomy mix cut about $\frac{1}{4}$ with perlite. The correlations were not strong, but observations indicated that perlite gave better results especially for root diameter.

Nematode resistance breeding: During this reporting period Becky Dykes and Daniel Pap both left the lab. They were leading the nematode resistance screening. I added supervision of the nematode screening to Nina Romero's duties and she worked very short-handed to get the screening completed. I hired two assistants for Nina to help with the nematode screening. We also have a visiting scholar from China who is assisting. I expect the results to be more consistent and that we will get much more completed.

Nematode screening: This year we completed 2 different screens for HarmAC (our combined Harmony and Freedom damaging root-knot nematode RKN strains) and tested 127 genotypes. Seventeen of these were being tested a second time and 16 a third time. Two separate screens for Ring resistance were completed and 129 genotypes were tested. Of these 64 were initial screens, 47 were second tests and 18 a third test. These results have been used to update Table 3 of our most promising nematode resistance candidates. Copies of 4 of the promising selections 2011-188-16, 2012-110-2, 2012-125-21, and 2012-185-8 are ready to go to the field.

Nematode testing across a wide range of species identified 6 accessions in the species *candicans*, *champinii*, *doaniana*, *monticola*, *palmata* and *rupestris* as highly resistant to HarmAC and possible new sources of RKN resistance. Five accessions showed high resistance to ring nematodes for the first time. Three were F1 *Vitis* x *Muscadinia* (VM) progeny while 2013-173-004 (T6-38 x 1103P) is a second generation VM and 2012-113-6 (101-14Mgt x GRN-4) derives its ring resistance from other *Vitis* sources.

To further evaluate our advanced nematode selections, in April we sent 6 copies each of 18 selections and 7 commercial rootstocks for evaluation by Andreas Westphal at Parlier. Table 3 details a grafted trial of 10 selections, 3 commercial stocks and one *Vitis* species which will be planted in Davis in the next few weeks so that we can evaluate their viticultural traits.

We have run most of our ring nematode resistance screens by inoculating with a given amount of nematodes and counting those that survive after 3 months, in addition to examining root damage and comparing nematode numbers to standard controls (O39-16, GRN-1, St. George and Colombard). Nina has modified the trial in several ways. She now runs a pot with soil and without plants to verify the survival of nematode under potted greenhouse conditions. She inoculates with 120 ring nematodes in each pot (with and without plants), and after 60 days she extracts the soil from the plantless pots and determine the number of survivors. This is then subtracted from each test plant score to indicate the degree of increase in ring population. Scores are assigned from 1-4 using O39-16 as standard: 1 Susceptible (statistically different from O39-16), 2 Moderately Susceptible (numerical break group below group 1), 3 Moderately Resistant (numerical break just below group 4), 4 Resistant (numerical break group that contains O39-16).

Our dagger nematode testing effort is currently focused on getting a larger pure culture for inoculation. At present there are 6 St. George plants inoculated with 300 nematodes per pot in Styrofoam cups. In 3 months these will go to 5 gal pots with 4-5 host plants/pot. We also have 2 – 5 gallon pots of pure culture on Colombard that have been building nematode numbers for the last 7 months. These will need another 3 months before they have sufficient numbers to serve as inoculum. Current projections suggest that by the end of the year there will be enough nematodes to inoculate a trial of about 25 of the most promising selections that have passed HarmAC and ring testing multiple times.

Phylloxera: Table 4 follows up and expands on results presented in the January 2019 report and documents that some VRs don't exhibit tuberosity formation yet have *vinifera* in their background. Table 4 reports on results from tests performed in 4" pots. Table 5 reports on the rootability of fertile VR hybrids. There was good agreement between the 4" pots and the same selections grown loose in tanks. It was found that the 2" pots were too small for good testing. Testing in pots is necessary to constrain the

roots and prevent root entanglement when selections are tested growing loose in the tanks of perlite. The tank is ready for the next round of testing.

Current testing and projections: There are 444 genotypes in testing either for nematodes, salt or both. Forty genotypes tested for ring have been sampled, extracted and await counting. Unfortunately, 2 significant ring trials, not counted as part of the above total, were lost late in testing when the irrigation failed. We have charged irrigation oversight to another staff. In various stages of preparation are 158 genotypes destined for nematode testing. These are mostly 2016 crosses with resistance from VRs and the 18-113 GRN-3 x *acerifolia* 9018 population seedlings. There are also 332 genotypes, mostly species with the exception of 49 F1 crosses with salt resistance from *arizonica*, *acerifolia*, or *girdiana* in various stages of preparation for salt trials.

Phenolic compounds in grapevine roots: We are also studying the association between phenolic compounds and phylloxera resistance. Phenolics do play a major role in the hypersensitive response (HR) against insect herbivores and microorganisms. We are also exploring an association between grape color and infestation level of own-rooted vines that suggests that white cultivars might exhibit a higher susceptibility (Arancibia et al. 2018). We have extracted phenolic compounds from red, pink and white varieties plus 2 rootstocks to compare their phenolic composition through LCMS-QTOF profiling. Based on PCA analysis of first round of results using the UC Davis database, we have selected anthocyanins and 10 additional compounds to continue towards target analysis.

Drought tolerance/avoidance: Kevin Fort left the lab to work for an environmental consulting agency in Sacramento. We are continuing his work on root fibrosity/depth and salt tolerance. *In vitro* evaluation of root growth using increasing concentrations of ‘agar’ (we are using Gelzan, Phytotechnology Labs) to develop a simple system to discriminate deep vs shallow root growth.

Apices from micro-plants of rootstocks 1103P, 110R, 101-14Mgt, Ramsey, and Riparia Gloire de Montpellier, were sub-cultured into clear Falcon tubes containing 30 ml of Nitsch and Nitsch medium supplemented with 20 g/l sucrose, 5 µg/l NAA and 5 µg/l biotin. Medium was solidified with 1, 6 or 12 g/l of Gelzan (PhytoTechnology Labs). Each treatment was replicated 5 times. The time required for roots to reach the base of the tube, root branching (fibrosity), and shoot - root fresh weight after 10 weeks were recorded. Results showed that this system allows discrimination among the different genotypes. As expected, higher root branching and fresh weights were found in 110R and 1103P. Interestingly, they also displayed higher root:shoot ratios, which increased with increasing agar concentrations. On the contrary, Ramsey, 101-14 and Riparia only modified their partitioning at 12 g/l. Ramsey roots reached the base of the tube as fast as 1103P, but branching was less pronounced.

We are also collaborating with MS student Idan Reingwartz, from the McElrone lab, who is studying root anatomical and morphological differences between 110R and 101-14. Together, we are developing a two-layered system where the lower half has been infused with PEG to modify the water potential of the medium.

Using CRISPR technology to study grape aquaporins: PIPs proteins (plasma membrane intrinsic proteins) are aquaporins, which facilitate the transport of water and small neutral molecules across cell membranes. We have designed gRNAs targeting the *V. vinifera* *PIP2-1* gene to knock it out. Plasmid construction with DNA harboring CRISPR-Cas9 and guides has been performed by Dr. M. Ron at Britt’s lab. Transformations of embryogenic callus of Thompson Seedless, Chardonnay, and St George via *Agrobacterium* have been initiated, and callus are currently growing in selection medium.

Chloride exclusion, germplasm and mapping population screening: We are using 75mM (12% sea water) salt concentrations to test germplasm previously identified as salt tolerant at 25-50 mM

concentrations. We hope this more severe test will identify the most useful parents for crosses. Table 6 lists the germplasm being tested at 75 mM NaCl, samples harvested May 29, 2018 and currently being processed for chloride tolerance.

Chris Chen recently successfully completed his PhD qualifying exam and he will be continuing our salt tolerance work. Recent progress in rootstock chloride tolerance has focused on establishing a mapping population for screening; following methods established throughout the past 11 months as standard protocol for greenhouse studies. This methodology requires use of special medium to prevent dispersion in response to high sodium concentrations in the soil, allow for sufficient aeration and drainage, and retain cations which compete for binding sites with sodium. Applied to the soil is a complete nutrient solution amended with $[\text{NaCl}] = 75\text{mM}$; a concentration which invokes chloride toxicity symptoms within a short time frame without causing plant death. To discover a source of natural tolerance to chloride toxicity we are testing wild-type grapevines from the arid regions of the southwestern United States. In total we have tested 60 individuals using our screening protocol for each of two propagation methods, herbaceous propagules and hardwood-dormant propagules, to compare effects of chloride uptake between the two methods of propagation. Both root and shoot chloride concentrations will be quantified. Samples are still in processing for these trials.

A novel individual of high chloride exclusion potential, *V. acerifolia* 9018 (same as *longii* 9018), was identified as having the lowest chloride accumulation in leaves following the NaCl application period (Fig.1); consistent with previous reporting. For purposes of establishing reliable source material for a salt-tolerance breeding program, a cross of GRN3 (mother) with *V. acerifolia* 9018 was made to determine heritability of salt-tolerant phenotypes in this salt-excluding species; this is with consideration that GRN3 has been reported as a poor chloride excluder in grapevine. Currently 78 individuals have been established and more than 200 seeds may be included in this mapping population; all have been DNA screened and are true to type. Chloride screening will commence on this population once the individuals reach maturity following procedure used to screen Ramsey x *acerifolia* 9018 mapping population.

Developing a consensus DNA fingerprint database of the Walker lab southwestern US germplasm for diversity and population genetic studies: I have amassed a very large collection of grape germplasm from the southern US – particularly the southwestern States (over 700 accessions). This collection is a very valuable resource for the rootstock breeding program. We are developing a consensus SSR fingerprint database to carry out population diversity studies that would help us to identify germplasm from different genetic groups. The collection also serves as the foundation for a NSF project to sequence many of these species and selections that is now underway. The sequencing and testing of these individuals for salt tolerance and PD resistance continues.

Transcriptomic analysis of grapevine infected by red leaf viruses: Plants have evolved RNA silencing as an efficient defensive mechanism to ward off virus infections (Dunoyer and Voinnet, 2005). This defensive pathway is triggered in response to virus invasion and generates small-interfering RNAs (siRNAs) to specifically target and cleave the viral genome into smaller nonfunctional fragments in a genome homology-dependent manner. Apart from siRNA-mediated gene silencing, microRNAs (miRNAs), another class of sRNAs, which play a regulatory role in many aspects of plant development and plant responses to biotic and abiotic stresses (Sunkar *et al.*, 2012), are also probably involved in the modulation of plant-virus interactions and the expression of disease symptoms.

Prof. Nihal Buzkan is on a 1.5 yr-long sabbatical with me and is working on this virus tolerance project. Experiments were carried out with Cabernet franc infected with red leaf viruses; leafroll (GLRaV-1) and rugose wood viruses (GVA) and two rootstocks Freedom (highly sensitive to red leaf viruses) and St. George (tolerant to red leaf virus disease) in field and *in vitro* conditions. Virus strains were LR131 for GLRaV-1 and LR132 for GVA.

Cabernet franc plants with LR131 and LR132 were bench grafted onto Freedom and St. George, then transplanted into field conditions in March 2017. Leaf-reddening symptoms were first observed on Cab franc plants with both virus strains grafted on Freedom by October, and became severe by November (Figure 2). St. George rootstocks with scions infected with both virus strains had mild symptom compared to those on Freedom.

The grafted plants in the vineyard were monitored for symptom progress in the canopy and the graft union from July to November in 2018. Leaf reddening started in few leaves by July and spread around the canopy by November (Figure 3). Swelling at graft union was first recorded in September 2018 in two year post-grafting. Three replicates from each graft combination according to symptom severity and healthy controls were selected for total nucleic acid extraction to prepare cDNA library for plant transcriptomic analysis by high throughput sequencing. Results from plant transcriptomic analysis will be correlated to symptom progress on plants.

The same experiment was also conducted in *in vitro* conditions. In February 2018, greenhouse-forced shoot-tips from virus infected Cabernet franc were collected and micrografted onto healthy rootstocks (Freedom and St. George). The first symptoms appeared on graft combinations 5 weeks after grafting (Figure 4).

Viral RNA was isolated from leaf petioles of Cabernet franc with LR131 and LR132 from grafted plants in field and *in vitro*. They were then subjected to two-step PCR test to confirm the presence of the viruses (Figure 5). PCR DNAs were sequenced in two directions with both primers in order to characterize the virus strains. An approx. 400-450 bp-nucleotide sequence from coat protein (CP) of LR131 strain was found to be closely related to the GLRaV-1 strain registered in GenBank from the US (JF811797) and grouped in the same cluster at phylogenetic tree (Fig. 6). The portion of CP (400-450 bp) of LR132 strain had high nucleotide homology with GVA strains from USA (KF013849) and Jordan (AY594176) (Figure 7). The plants *in vitro* were then acclimatized into greenhouse conditions for further testing.

A Western blot was used to confirm the expression of virus CP in infected *in vitro* plants. Leaf petioles were used to extract viral protein, run in SDS-PAGE to separate the protein fragments. It was then blotted onto a PVDF membrane with 0.45 µm pores and blocked with virus-specific polyclonal antibody (GLRaV-1 and GVA from BIOREBA). Goat anti-rabbit IgG (H+L) conjugated with Dy Light 550 (fluorescent dye) was used as secondary antibody. Visualization was carried out at 550 nm. St George rootstocks did not have detectable LR-1 CP while Freedom rootstocks had the virus CP at 21K in size (Figure 8). Further work will be carried out to have clean background with better visibility of protein bands on the membrane for both viruses.

Screening of rootstock population 08-180 (Freedom x St. George) for red leaf virus tolerance:

Dormant cuttings from the 08180 population and Cabernet franc with LR-1 and GVA were collected and stored at 36F for chilling requirement for about 6 weeks. These cuttings were bench grafted in mid-March 2018, then they were transferred into greenhouse conditions for virus replication and symptom observation (Fig. 9). Seventeen progenies with LR131 and thirteen progenies with LR132 were grafted. Six replicates for each virus/rootstocks combination were prepared as well as negative (healthy) and positive (infected) controls, then they were periodically checked for virus presence with an ELISA test starting from 3-month post grafting (mpg) up to one year.

The first symptoms of leaf roll in the 80-180 population grafted with both virus strains was observed 7 mpg (Figure 10). Leaf reddening was hard to see because the plants were overgrown in the greenhouse. The virus titer was found to be high in all 08-180 progenies with LR-1 until 6th and 7th mpg, when the titer

remarkably dropped. They were still infected, but 50% of the grafted progenies had low virus titer compared to the positive controls. The highest virus titer was measured at 6 and 7 mpg when the first symptom appeared on the plants. GVA titer was always found at low titers in all 08-180 progenies and this might be due to an avirulence property of the virus strain. We need to biologically characterize LR132 for its level of virulence.

Movement and localization of the viruses in positive (Cabernet franc with LR131 and LR132 grafted on Freedom and St George rootstocks) and negative controls (healthy Cabernet franc on both rootstocks) phloem tissue was investigated with immunofluorescent stained tissue sections in 10 µm thickness with fluorescent microscopy. Virus specific-polyclonal antiserum (BIOREBA) was used in 1:100 dilution and secondary antibody was anti-sheep IgG-FITC antibody produced in rabbit at 1:100 dilution. Microscopy observation was done at 500-550 nm wavelength

GVA particles in St George were erratically localized in phloem tissue up to 10-20 cm from the grafting point. Beyond that point, no virus particles were seen (Fig. 11). It seems virus particles are not moving to the upper level on tolerant rootstocks.

LR-1 particles were also visualized in phloem tissue of St George. The virus was localized starting up to 60 cm. Between 20-40 cm above the grafting point, LR-1 was not seen in phloem tissue. This might be due to irregular distribution and low virus titer in plant tissue (Figure 12). The same experiment for LR-1 and GVA in Freedom is under way.

Inheritance of GFLV Tolerance Trait in a 101-14 x Trayshed Population: Ph.D. student Andy Nguyen is making progress on the inheritance of rootstock-induced fanleaf degeneration tolerance that has been observed in O39-16. Field evaluation for rootstock-induced GFLV tolerance in genotypes from the 101-14 x Trayshed population and fertile VR hybrids is currently underway. The rootstock trial consists of vines with GFLV-infected Cabernet Sauvignon scions grafted on top of cuttings from these genotypes. Fruit set for each graft combination was quantified by counting the number of berries on a cluster and dividing that by the number of calyptras (a proxy for the number of flowers on the original inflorescence) to arrive at a percentage representing fruit set. Results from the 2018 season are shown in Figure 13. Promisingly, most of the genotypes performed better than the susceptible controls. We also looked at total berry weight (per cluster) and pruning weight for each rootstock genotype, shown in Figures 14 and 15. We are repeating the trial again for 2019.

GFLV resistance screening: Greenhouse evaluation of GFLV resistance in fertile VR hybrids and genotypes from the 101-14 x Trayshed population is nearly complete (Fig. 16). Most of the 101-14 x Trayshed progeny tested so far have lower levels of GFLV compared to 101-14, but not as low as O39-16. Notably, 07107-065 and 07107-133 show similar levels of resistance as O39-16. When comparing this data to some preliminary numbers from the fruit set field screening the rootstock genotypes with the lowest virus titers in our resistance screen show relatively high fruit set in our disease tolerance screen. However, the rootstock genotypes that harbored high levels of GFLV in our resistance screen can also confer some degree of disease tolerance, as shown by their elevated fruit set in comparison to the susceptible controls.

Mechanism of GFLV Tolerance: A new post-doc with experience in biochemical assays will be entering our lab to assist Ph.D. student Andy Viet Nguyen in studying the mechanism of rootstock-induced tolerance observed in O39-16. Before bud break, we collected xylem sap from GFLV-infected vines and healthy vines grafted on O39-16, GRN-1, and St. George. We are planning to analyze the sap to look for differences between the healthy and infected vines and between different rootstocks. Possibilities for analysis include phytohormones, amino acids, and proteins. We have also collected flowers during

different stages of flowering for RNA extraction to look for differences in expression of hormone biosynthesis genes.

Presentations to Industry Groups

- Walker, M.A. 2018. PD causes and cures. Lecture and tasting. D. Roberts Grower Meeting, Santa Rosa, Jan 12.
- Walker, M.A. 2018. Developing PD resistant wine grapes. Lecture and Tasting. Chateau Elan, Braselton, GA. Georgia Wine Producers Meeting, Jan 23
- Walker, M.A. 2018. Understanding plant material selection for vineyard redevelopment: including rootstock and plant material selection and soil pest and virus considerations, South State Gallo Growers Meeting, Fresno, CA Feb 15.
- Walker, M.A. 2018. Understanding plant material selection for vineyard redevelopment: Including rootstock and plant material selection and soil pest and virus considerations, North State Gallo Growers Meeting, Lodi, CA Feb 16.
- Walker, M.A. 2018. Grape breeding update. Current Issues in Viticulture, UC Davis, Feb 21.
- Walker, M.A. 2018. Rootstock breeding update. CDFA IAB Nursery Board meeting, UC Davis, Apr 11.
- Walker, M.A. 2018. Grape breeding update and PD wine tasting. UC Davis for the PD/GWSS Grower Advisory Board, April 23.
- Walker, M.A. 2018. UCD PD breeding program update and tasting. Temecula Winemakers Meeting, Wilson Creek winery, Temecula, June 8.
- Walker, M.A. 2018. Grape breeding at UC Davis. Lebanon Table Grape Growers Group, July 17.
- Walker, M.A. 2018. Grape breeding update. CGRIC Nursery Meeting, July 24.
- Walker, M.A. 2018. Fanleaf Field Day, discuss plot and breeding – Healdsburg, CA, Aug. 16 .
- Walker, M.A. 2018. Rootstock breeding program update. CDFA IAB meeting, UC Davis, Nov 14.
- Walker, M.A. 2018. New/replanted vineyard establishment concerns. UCD/On the Road Presentations, Escondido, CA, Nov 29.
- Walker, M.A. 2018. Current and future objectives of the grape breeding program at UCD. Recent Advances in Viticulture and Enology, UC Davis, Nov 30
- Walker, M.A. 2018. Current and future objectives of the UCD grape breeding program. Foundation Plant Services Annual Meeting, UC Davis, Dec. 4
- Walker, M.A. 2018. PD resistant winegrape breeding program update. CDFA PD/GWSS Board Symposium, San Diego, CA Dec. 12
- Walker, M.A. 2019. An update on the performance of the GRN rootstocks. Daniel Roberts Client Meeting, Jan 18
- Walker, M.A. 2019. How to select rootstocks. Viticulture Short Course, Napa, Feb 13.
- Walker, M.A. 2019. Grape vine pruning demo and instruction, UC Davis for Folsom Lake College, Feb 23.
- Walker, M.A. 2019. Stacking PD resistance genes for durable resistance. Current Advances in Wine and Grape Research, UC Davis, Feb. 27
- Walker, M.A. 2019. Current and future objectives of the grape breeding program at UC Davis, Salinas Farm Advisor Office, On the Road Presentation, March 8
- Walker, M.A. 2019. Grape rootstock breeding update. California Grape Rootstock Improvement Commission, Coalinga, CA March 11.
- Walker, M.A. 2019. The grape breeding program at UC Davis: where it's been and where it's going. CSU Fresno, March 20.
- Walker, M.A. 2019. An update on the performance of the GRN rootstocks, Lakeport, On the Road Presentation, March 28.
- Walker, M.A. 2019. Rootstock breeding update for the IAB. UC Davis, April 9, 2019.
- Walker, M.A. 2019. Potencial de las vides silvestres en la viticultura comercial. Toluca College, Toluca, MX, May 17, 2019.

- Walker, M.A. 2019. Rootstock breeding update for the CGRIC, UC Davis, June 13, 2019.
- Walker, M.A. 2019. Alternative rootstocks for use with wine and table grapes in Chile. Redagricola Viticulture Conference, Santiago, Chile. June 5, 2019.
- Walker, M.A. 2019. Management of traditional and alternative rootstocks and use with wine and table grapes in Chile. Redagricola Viticulture Conference, Santiago, Chile. June 6, 2019.
- Walker, M.A. 2019. Fundamentals of rootstock use with wine and table grapes in Chile. Redagricola Viticulture Conference, Santiago, Chile. June 6, 2019.
- Walker, M.A. 2019. Breeding rootstocks for use with table and wine grapes. Redagricola Viticulture Conference, Santiago, Chile. June 6, 2019.
- Walker, M.A. 2019. Performance of the GRN rootstocks in a fanleaf site. Lodi Winegrape Growers Association/ Gallo Winery, Lodi, CA, July 11, 2019.

Posters/Abstracts at Scientific Meetings

- Walker, M.A. 2018. 2017 AJEV Best Paper Award. Population diversity of grape phylloxera in California and evidence of sexual recombination. 69th ASEV National Meeting, Monterey, CA, June 20
- Weibel, J. and M.A. Walker. 2018. Wild *Vitis* species offer diverse sources of resistance and susceptibility to *Xiphinema index*. 69th ASEV National Meeting, Monterey, CA, June 20
- Riaz, S., A. Tenschler and M.A. Walker. 2018. Identification of the Pierce's disease resistance locus PDR2 from the Mexican grape species accession b42-26. 69th ASEV National Meeting, Monterey, CA, June 20
- Pap, D., S. Riaz, R. Wheeler-Dykes, N. Romero and M.A. Walker. 2018. Sources of resistance to root-knot nematode and phylloxera. 69th ASEV National Meeting, Monterey, CA, June 20
- Fayyaz, L., S. Riaz, R. Hu, M.A. Walker. 2018. Characterizing grapevine powdery genes from the Chinese species *Vitis piasezkii*. 69th ASEV National Meeting, Monterey, CA, June 20
- Cui, Z., C. Agüero and M. A. Walker. 2018. Greenhouse evaluation of grapevine leafroll associated virus on different rootstocks, 69th ASEV National Conference, Monterey, CA, 06-20-18.
- Nguyen, A., C. Agüero, H. Padre and M. A. Walker. 2018. Grapevine fanleaf virus resistance screening in a 101-14 x *rotundifolia* population, 69th ASEV National Conference, Monterey, CA, 06-20-18.
- Nguyen, A.V., C.B. Agüero, H. Padre, A. Phan, M.A. Walker. 2018. Characterizing grapevine fanleaf virus resistance and tolerance in a 101-14 Mgt. x *rotundifolia* population. Recent Advances in Viticulture & Enology, UCD, Nov. 30
- Huerta-Acosta, K., S. Riaz, O. Franco-Mora and M.A. Walker. 2018. Genetic diversity of wild grapevines in central and northern Mexico. Recent Advances in Viticulture & Enology, UCD, Nov. 30
- Walker, A., A. Tenschler and S. Riaz. 2018. Breeding Pierce's disease resistant winegrapes. CDFA PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12
- Riaz, S., R. Hu, C. Agüero, a. Tenschler and A. Walker. 2018. Molecular breeding support for the development of Pierce's disease resistant winegrapes: new sources of resistance and markers. CDFA PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12
- Agüero, C.B., S. Riaz, A. Tenschler and M.A. Walker. 2018. Molecular breeding support for the development of Pierce's disease resistant winegrapes – genetic transformation with *PdR1b* candidates. CDFA PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12
- Huerta-Acosta, K., S. Riaz, O. Franco-Mora and M.A. Walker. 2019. Search for new sources of resistance to Pierce's Disease: characterization of the PD resistant accession b46-43. ASEV National Conference, Napa, CA, June 19, 2019.
- Summaira Riaz, Alan Tenschler, Rong Hu and M Andrew Walker. 2019. Characterization of Pierce's disease resistance in b41-13, an accession collected from Tamaulipas, Mexico. ASEV National Conference, Napa, CA, June 19, 2019.
- Ines Hugalde, Summaira Riaz, Cecilia B. Agüero, Marcos Paolinelli, Nina Romero, Andy V. Nguyen, Hernán Vila, Sebastian Gomez Talquenca, M. Andrew Walker. 2019. Genetic determination of

vegetative vigor in a Ramsey x Riparia GM population. ASEV National Conference, Napa, CA, June 19, 2019.

Cecilia B. Agüero, Marco Rocha-Figueroa, and M. Andrew Walker. 2019. Effect of Agar on Growth of Roots of Six Grapevine Rootstocks. ASEV National Conference, Napa, CA, June 19, 2019.

Summaira Riaz, Cecilia Agüero, Rong Hu and M Andrew Walker. 2019. Comparative sequence analysis of the PD resistance locus *PdR1* in two resistant accessions –b43-17 and b40-14. ASEV National Conference, Napa, CA, June 19, 2019.

Christopher Cody Lee Chen, Nina Romero, and M A Walker. 2019. Rapid screening for salt-stress tolerance through chloride-ion accumulation in leaves of wild *Vitis* spp. rootstocks. ASEV National Conference, Napa, CA, June 19, 2019.

Laila Fayyaz, Alan Tenschler, Huma Qazi, M. Andrew Walker. 2019. Powdery mildew resistance varies in western US *Vitis* accessions. ASEV National Conference, Napa, CA, June 19, 2019.

Publications

Arancibia, C., S. Riaz, C. Agüero, B. Ramirez-Corona, R. Alonso, F. Buscema, L. Martinez and M.A. Walker. 2018. Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in Argentina: ecological associations to diversity, population structure and reproductive mode. Australian Journal of Grape and Wine Research 24:284-291.

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Table 1. 2019 Pollinations and purposes.

cross #	Female parent	Male parent	Purpose
2019-020	07107-062 FH 05-18 T=tetraploid	07107-079 FH 05-35 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-021	07107-062 FH 05-18 T=tetraploid	07107-079 FH 05-35 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-022	07107-062 FH 05-18 T=tetraploid	07107-050 FH 05-08 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-023	07107-062 FH 05-18 D=diploid	07107-050 FH 05-08 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-024	07107-062 FH 05-18 D=diploid	07107-044 FH 05-02 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-032	07107-062 FH 05-18 T=tetraploid	Schwarzman	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-033	07107-062 FH 05-18 D=diploid	Teleki 5C	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-035	07107-062 FH 05-18 D=diploid	Schwarzman	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-072	07107-062 FH 05-18 T=tetraploid	Teleki 5C	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-074	07107-062 FH 05-18 D=diploid	110R	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-037	Dog Ridge	<i>acerifolia</i> 9018	Deep roots and salt
2019-041	Riparia Barrett #50	GRN-2 9363-16	Improve GRN2
2019-042	Riparia Barrett #50	GRN-4 9365-85	Improve GRN4
2019-043	Vru 104	TX9725	Rupestris genetics
2019-047	Vru 110	TX9726	Rupestris genetics
2019-049	Rupestris A. de Serres	St. George	Rupestris genetics
2019-050	Rupestris A. de Serres	St. George	Rupestris genetics
2019-051	Rupestris A. de Serres	St. George	Rupestris genetics
2019-052	Rupestris A. de Serres	Rupestris Ganzin	Rupestris genetics
2019-059	R65-44	Riparia Gloire	Riparia genetics
2019-064	T6-42	Teleki 5C	Fertile VR crosses to standard stocks
2019-065	T6-42	1103 Paulsen	Fertile VR crosses to standard stocks

2019-066	T6-42	110R	Fertile VR crosses to standard stocks
2019-067	T6-42	Schwarzman	Fertile VR crosses to standard stocks
2019-068	T6-42	140Ru	Fertile VR crosses to standard stocks
2019-073	T6-38	1103 Paulsen	Fertile VR crosses to standard stocks
2019-075	T6-38	Schwarzman	Fertile VR crosses to standard stocks
2019-076	T6-38	140Ru	Fertile VR crosses to standard stocks
2019-077	T6-38	Teleki 5C	Fertile VR crosses to standard stocks
2019-078	T6-38	110R	Fertile VR crosses to standard stocks
2019-149	101-14 Mgt	07107-079 FH 05-35 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-150	101-14 Mgt	07107-079 FH 05-35 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-151	101-14 Mgt	07107-050 FH 05-08 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-152	101-14 Mgt	07107-050 FH 05-08 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-155	5BB Kober	07107-079 FH 05-35 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-156	5BB Kober	07107-079 FH 05-35 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-157	5BB Kober	07107-050 FH 05-08 T=tetraploid	101-14 x Trayshed crosses (fanleaf and nematodes)
2019-158	5BB Kober	07107-050 FH 05-08 D=diploid	101-14 x Trayshed crosses (fanleaf and nematodes)

Table 2. 2018 pollinations.

cross #	Female parent	Male parent	Purpose	# Seeds
2018-109	8909-05 GRN-1	Schwarzmann	Fertile Vitis/Muscadinia (VM) progeny	0
2018-110	8909-05 GRN-1	1103 Paulsen	Fertile VM progeny	0
2018-111	8909-05 GRN-1	3309 Couderc	Fertile VM progeny	0
2018-112	8909-05 GRN-1	Riparia Gloire	Fertile VM progeny	0
2018-113	GRN-3 9365-43	<i>acerifolia</i> 9018	Salt and broad nematode resistance	1080
2018-114	GRN-3 9365-43	<i>acerifolia</i> 9035	Salt and broad nematode resistance	444
2018-120	Dog Ridge	<i>acerifolia</i> 9018	Salt/RKN/deep roots	31
2018-121	Dog Ridge	<i>acerifolia</i> 9035	Salt/RKN/deep roots	268
2018-124	Ramsey	<i>doaniana</i> 9028	Salt/RKN/deep roots	225
2018-136	11-188-16	1103 Paulsen	Ring/RKN	3
2018-137	11-188-16	NM 03-17 S01 <i>treleasei</i>	Ring/RKN/salt	2
2018-138	11-188-16	ANU77 <i>girdiana</i>	Ring/RKN/salt	36
2018-139	11-188-16	ANU57 <i>treleasei</i>	Ring/RKN/salt	19
2018-141	11-188-16	GRN-4 9365-85	Dagger/Ring/RKN/salt	0
2018-142	11-188-16	GRN-2 9363-16	Dagger/Ring/RKN/salt	19
2018-143	11-188-16	<i>acerifolia</i> 9018	Ring/RKN/salt	10
2018-144	11-188-16	<i>acerifolia</i> 9035	Ring/RKN/salt	0
2018-145	11-188-16	3309 Couderc	Ring/RKN	22
2018-146	11-188-16	110R	Ring/RKN	0
2018-147	11-188-16	SO4	Ring/RKN	0

2018-149	101-14 Mgt	07107-079 FH 05-35 T=tetraploid	<i>rotundifolia</i> -based resistance and fertility	29
2018-150	101-14 Mgt	07107-079 FH 05-35 D=diploid	<i>rotundifolia</i> -based resistance and fertility	5
2018-151	07107-062 FH 05-18 T=tetraploid	GRN-4 9365-85	<i>rotundifolia</i> -based resistance and fertility	0
2018-152	07107-062 FH 05-18 T=tetraploid	GRN-2 9363-16	<i>rotundifolia</i> -based resistance and fertility	0
2018-153	07107-062 FH 05-18 D=diploid	GRN-4 9365-85	<i>rotundifolia</i> -based resistance and fertility	0
2018-154	07107-062 FH 05-18 D=diploid	GRN-2 9363-16	<i>rotundifolia</i> -based resistance and fertility	0
2018-155	101-14 Mgt	101-14 x T 48T	<i>rotundifolia</i> -based resistance and fertility	0
2018-156	101-14 Mgt	101-14 x T 48D	<i>rotundifolia</i> -based resistance and fertility	0
2018-157	101-14 Mgt	101-14 x T 42T	<i>rotundifolia</i> -based resistance and fertility	0
2018-158	101-14 Mgt	101-14 x T 42D	<i>rotundifolia</i> -based resistance and fertility	0
2018-170	2011-175-7	GRN-2 9363-16	Broad nematode resistance with PD	0

Table 3. Promising root-knot resistant selections grafted to Cabernet Sauvignon for a new rootstock trial at UCD. Nematode resistance is measured on a 1 to 4 scale with 1 highly susceptible and 4 resistant with virtually no nematode damage. Salt resistance employs a similar scale with a score of 1 accumulating high levels of leaf chloride while 4 represents very low levels of chloride. Rootability is reported from typical duration (6-7 weeks) with media and plant in 2" x 2" paper sleeves. Scale is 0 with no usable plants and 5 excellent shoots and roots.

Genotype	Parentage	Ave HarmAC Resist-ance	Times HarmAC tested	Ave Ring Resist-ance	Times Ring tested	Ave Salt Resist-ance	Times Salt Tested	Ave Sleeves - typical rootability
101-14Mgt		3.5	2	2.0	1	2.0	1	
1103P		1.0	1	2.0	1			
140Ru		2.0	3	1.0	1	2.9	14	
2010-115-22	161-49C x Trayshed	4.0	1	4.0	1			0.7
2011-148-42	Ramsey x NM 03-17 S01	3.7	3	2.7	3			3.8
2011-188-16	T6-42 x St. George	3.8	5	4.0	2	2.0	1	3.0
2012-108-32	101-14Mgt x doaniana 9028	3.5	2			3.5	2	4.0

2012-113-16	101-14Mgt x GRN-4	3.3	3	2.8	4			3.8
2012-113-28	101-14Mgt x GRN-4	3.0	3	3.0	3			3.8
2012-125-21	OKC-1 S01 x GRN-2	3.5	4	3.3	3	1.5	2	3.3
2012-153-24	Ramsey x doaniana 9028	3.5	2	2.5	2	3.0	1	4.0
2012-185-8	GRN-3 x berlandieri 9031	3.5	2	3.5	2			3.0
2012-190-26	Dog Ridge x St. George	4.0	1	1.0	1	3.0	2	3.0
	longii 9018	2.0	4	1.0	1	4.0	6	3.0

Table 4. Phylloxera (Type A) impacts on VR hybrids grown in the greenhouse either loose or in 2" pots in the tanks of perlite.

Genotype	Loose in tanks			2" pots		
	Nodosity %	Tuberosity %	# reps	Nodosity %	Tuberosity %	# reps
06725-01	3%	0%	4	13%	0%	4
b55-1				61%	14%	7
b59-45	15%	5%	13			
B59-47	15%	0%	2			
B59-50 v11	40%	40%	1	75%	0%	1
b59-50 v12	50%	54%	4	51%	0%	4
JB 81-107-11	18%	6%	9	9%	2%	4
Karadzhandal	85%	60%	2	100%	0%	5
N53-32	1%	0%	9	18%	47%	6
NC194-1				0%	0%	6
NC6-15 DVIT1741	0%	0%	3	0%	0%	6
NC74C049-10	7%	0%	12	0%	0%	5
T6-38	1%	0%	16	0%	0%	11
T6-42	0%	0%	18			
Zehnder 01-20-4	19%	0%	4	40%	4%	7
Zehnder 88-19-5	50%	68%	2	100%	0%	3
Zehnder 93-6-2	0%	0%	1	0%	0%	2

Table 5. Rootability of VR hybrids from dormant cuttings. We had 12 replicates for most of the genotypes.

Genotype	Percentage of Surviving Grafts	Rootability Score
NC194-1	8.3%	1
b55-1	83.3%	4
NC74C049-10	41.7%	2

JB81-107-11	88.9%	5
T6-42	16.7%	1
T6-38	Not assessed in this screen	
Zehnder 88-19-5	100%	5
Zehnder 01-20-4	100%	5
NC6-15	41.7%	3
Zehnder 93-6-2	41.7%	2
Zehnder 97-60-3	0%	0
06725-01	100%	5
N53-32	41.7%	3
b59-45	100%	5
b59-47	100%	5
b59-50	100%	5

Table 6. Grape germplasm that has tested well under previous tests at 25 to 50 mM NaCl concentrations and currently in testing at 75mM.

Genotype	
03300-048	101-14 x F8909-08
1103 P	<i>berlandieri</i> x <i>rupestris</i>
17:043	
2011-175-007	08314-31 x Schwarzmann
2011-175-015	08314-31 x Schwarzmann
ANU 21	<i>arizonica</i> / <i>girdiana</i>
ANU 71	<i>arizonica</i>
AZ 11-099	<i>arizonica</i> slight <i>riparia</i>
<i>doaniana</i> 9026	<i>doaniana</i>
F8909-08	<i>rupestris</i> x <i>arizonica</i>
<i>girdiana</i> Scotty's Castle	lobed <i>arizonica</i>
GRN-2	(<i>V. rufotomentosa</i> x (<i>V. champinii</i> 'Dog Ridge' x <i>V. riparia</i> 'Riparia Gloire')) x <i>V. riparia</i> 'Riparia Gloire'
KS14-032	<i>acerifolia</i> Kansas
Longii 9018	<i>acerifolia</i> TX
OK12-005	<i>doaniana</i>
OK14-002	<i>acerifolia</i>
R8916-22	<i>rupestris</i> x <i>arizonica</i>
R8916-32	<i>rupestris</i> x <i>arizonica</i>
St. George	<i>rupestris</i>
TXNM-088	<i>treleasei</i>
UT 12-092	<i>girdiana/treleasei</i> (<i>rip?</i>)
UT 12-099	<i>girdiana</i>
UT 12-100	<i>girdiana/treleasei</i> (<i>rip?</i>)

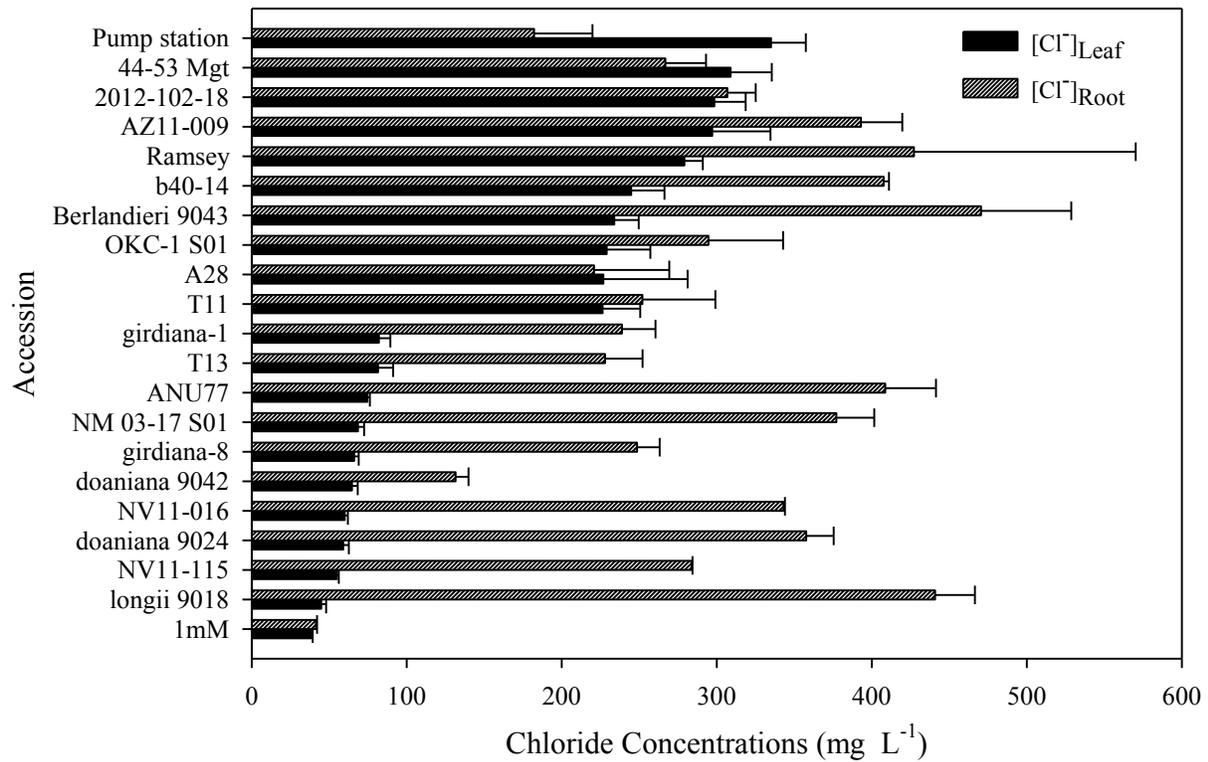


Figure 1. Chloride concentrations compared in leaves and roots of resistant breeding selections



Figure 2. Red leaf symptoms in Cabernet franc with both virus strains grafted on Freedom in the field by October and November 2017 (7 months after grafting). Healthy Cabernet franc grafted on Freedom and St George rootstocks as negative controls remained symptomless. Virus presence was confirmed in all graft combinations by RT-PCR.



Figure 3. Symptom progress on Cabernet franc with LR131 and LR132 virus strains grafted on Freedom rootstock by July (A), August (B), September (C), October (D), November (E) 2018.

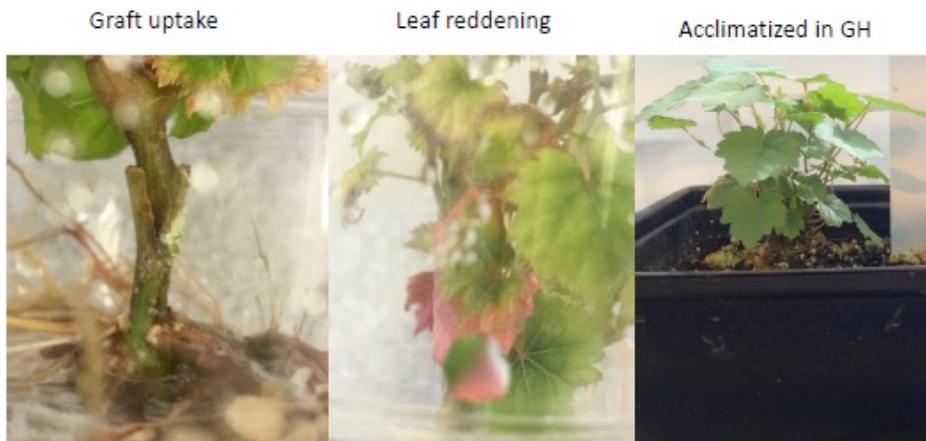


Figure 4. Establishment of micrografting and red leaf symptom caused by LR-1 in *C. franc* after 5 weeks of micrografting onto Freedom.

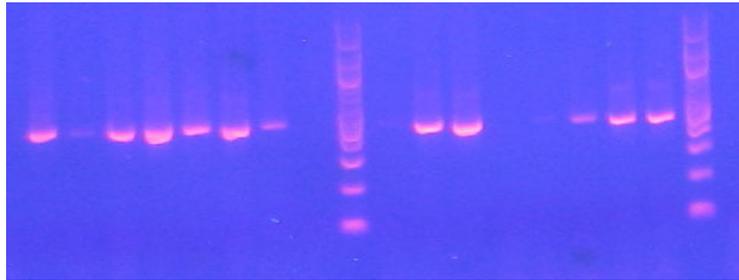


Figure 5. Electrophoretic analysis of PCR DNAs for LR-1 and GVA .

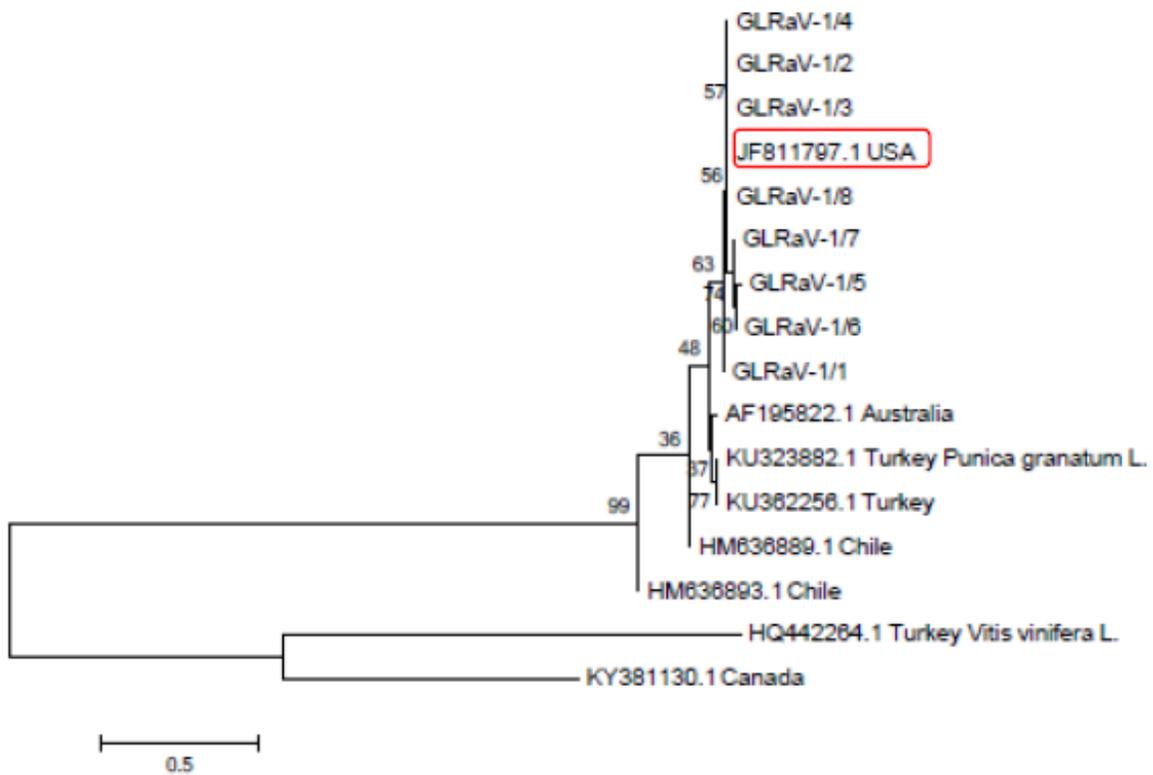


Figure 6. Phylogenetic tree of GLRaV-1 strain LR131 and other closely related LR-1 isolates.

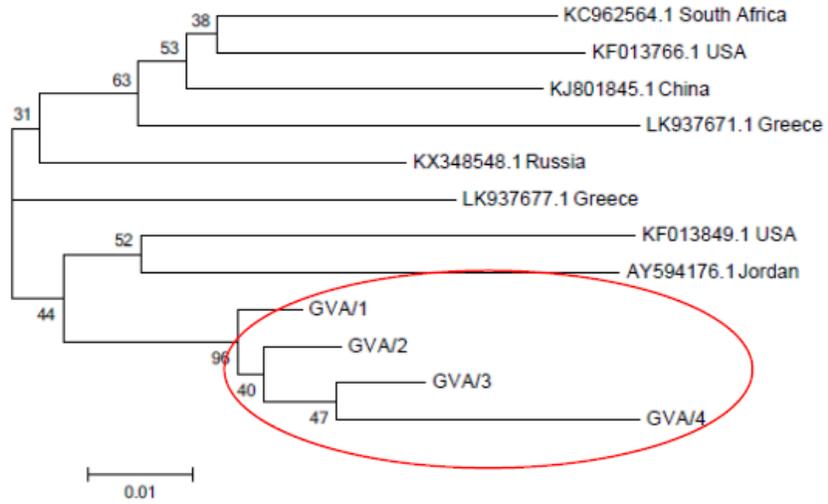


Figure 7. Phylogenetic tree of GVA strain LR132 and other closely related GVA isolates

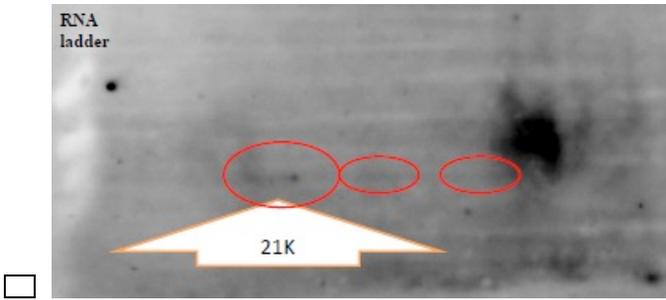


Figure 8. Western blot to visualize LR-1 CP in vitro grafted Freedom and St George rootstocks.

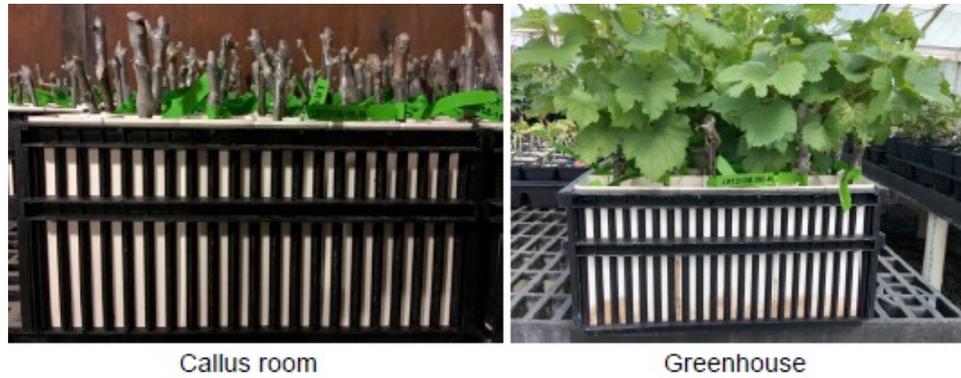


Figure 9. Cabernet franc with LR131 and LR132 grafted onto 08-180 (Freedom x St George) population in callus room and transferred into the greenhouse.

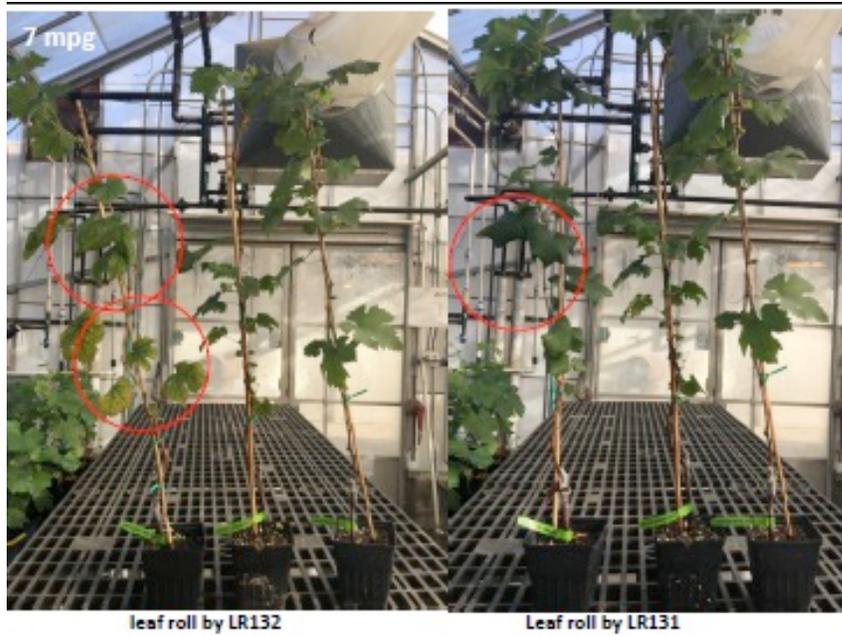


Figure 10. Leafroll symptom on 08-180 progeny induced by LR131 and LR132 in 6 mpg.

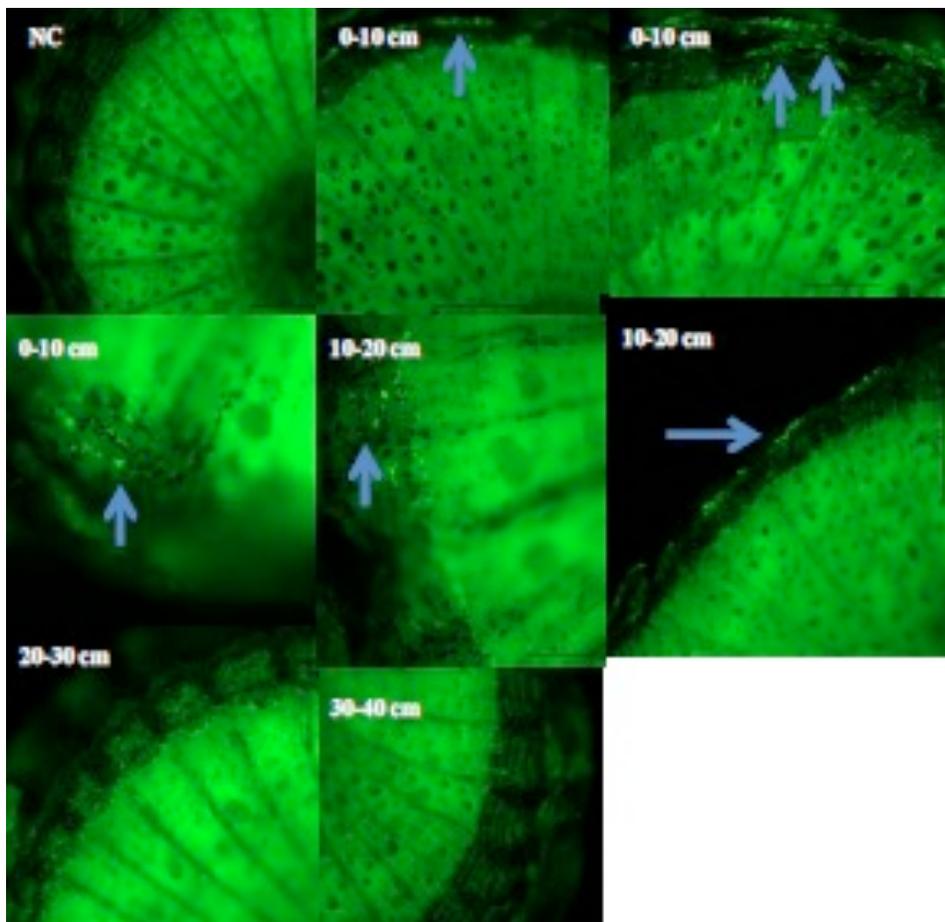


Figure 11. Fluorescence microscopy detection of immune-stained GVA in St George phloem tissue.

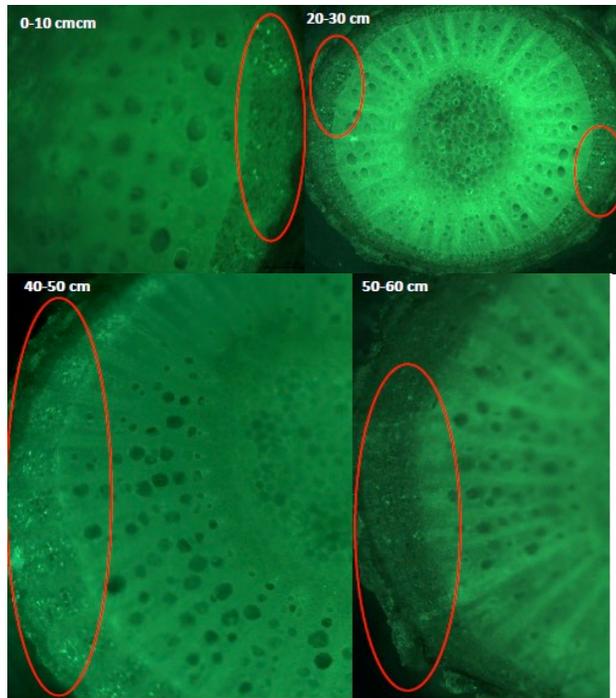


Figure 12. Fluorescence microscopy detection of immune-stained LR-1 in St George phloem tissue.

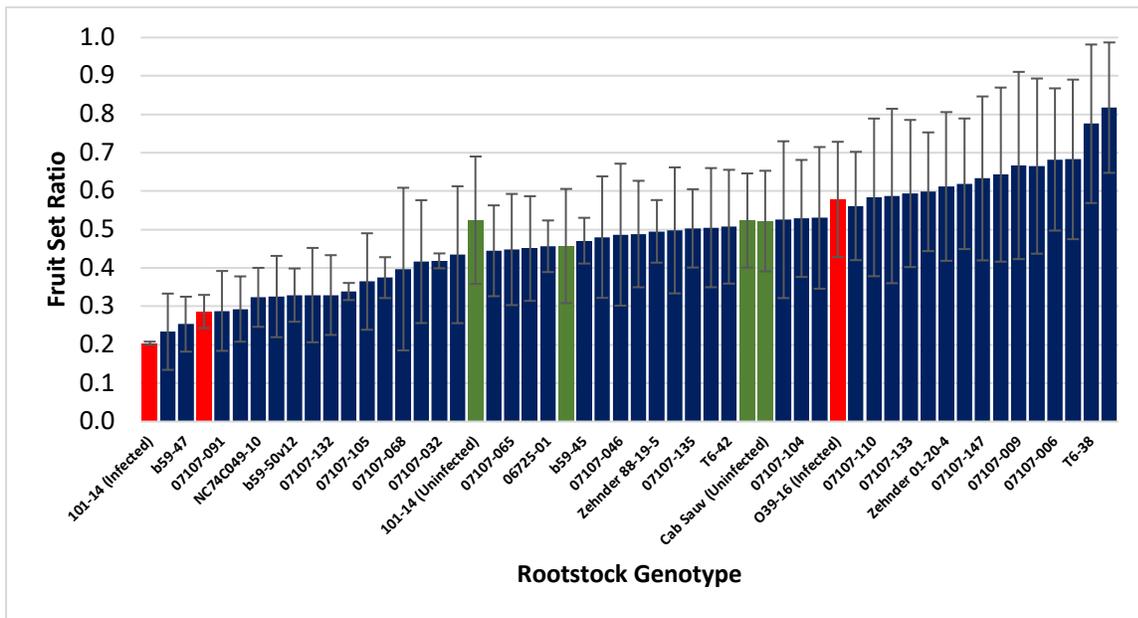


Figure 13. Fruit set ratio for GFLV tolerance screen studying rootstock-induced tolerance from the 101-14 x Trayshed population and fertile VR hybrids. Fruit set was quantified for Cabernet Sauvignon clusters.

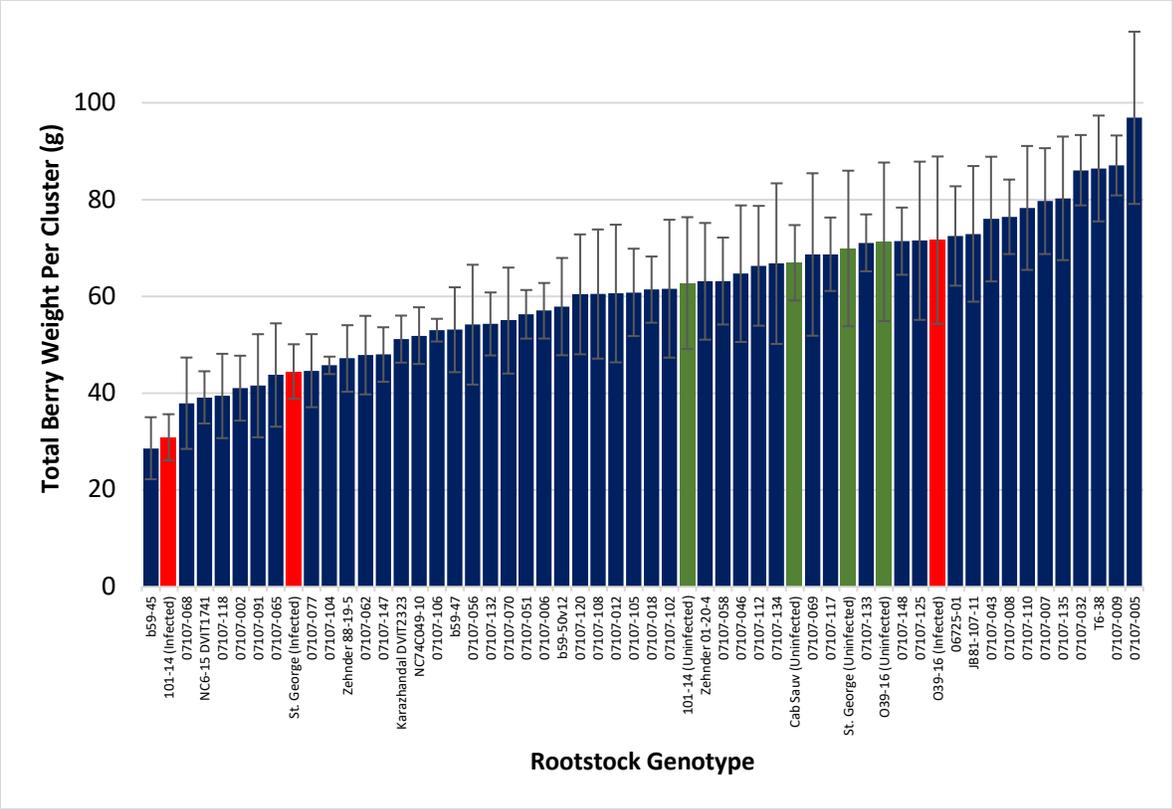


Figure 14. Total berry weight (per cluster) for the GFLV tolerance screen.

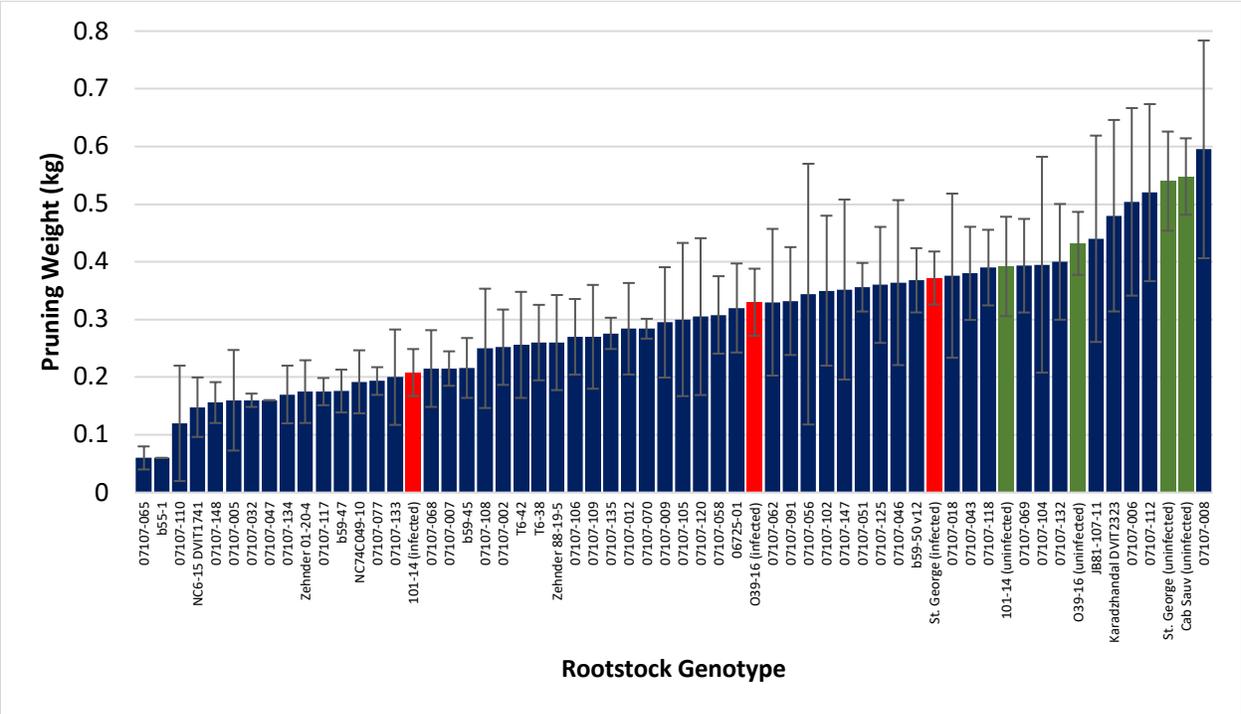


Figure 15. Pruning weight for the GFLV tolerance rootstock trial.

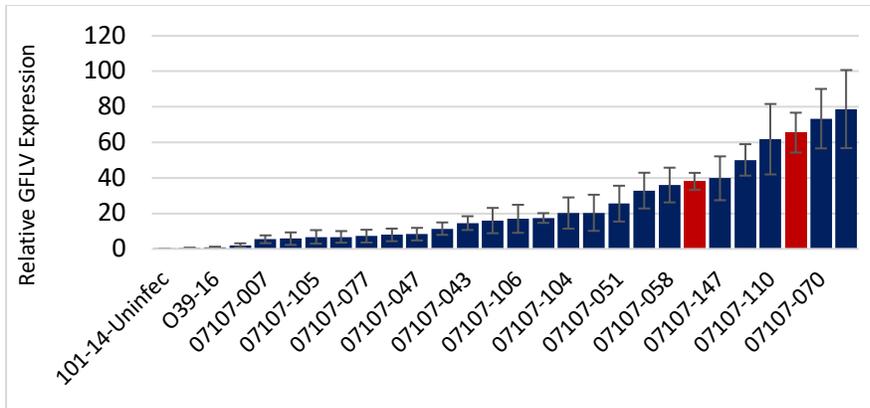


Figure 16. GFLV concentration in the rootstocks normalized to the 18SrRNA housekeeping gene and expressed relatively to O39-16, the tolerant control sample. Error bars represent standard error of the mean.