# June Amendment to the January 2018 Progress Report California Grape Rootstock Improvement Commission California Grape Rootstock Research Foundation

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

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# Reporting Period: June 2017 to January 2018

*Overall Summary:* We are making strong progress in streamlining the assays for nematode, salt and phylloxera screening to test new germplasm, existing breeding populations to single out best rootstock selections, and test breeding and mapping populations through the efforts of Daniel Pap. We have built up inoculum to carry out germplasm screening for the dagger nematode. Salt screening of germplasm that was promising in earlier screens was initiated at higher concentrations (75mM) to select optimum accessions that we could use in crosses. At the same time, we are initiating salt screening of breeding populations with different accessions of *V. acerifolia* and 140Ru to look for segregation in order to genetically map this trait. We are making good progress towards better understanding of root architecture in multiple rootstock species including *V. berlandieri*. Specific root length and root diameter are key features that could be used to test rootstock selections. Trials of selected accessions that pass the screening for horticultural features, nematode, phylloxera and salt tolerance are in pipeline.

# 2018 Pollinations / 2017 Seedling Planting

Table 1 presents the crosses made in 2018. This has been a very peculiar year with an early start to a long and delayed bloom. GRN-1 seemed to produce fertile flowers this year and it was pollinated with several standard rootstocks that were blooming at the same time. These efforts are to gain a fertile bridge to allow rotundifolia traits to be combined with salt tolerance, better rooting/grafting, and broadened nematode resistance. We also made crosses with *Vitis/Muscadinia* hybrids, but that had been treated with colchicine n an effort to produce tetraploids capable of hybridizing with other *Vitis* species. These efforts will allow *rotundifolia* traits to be widely utilized without taking the risk of phylloxera susceptibility from using available fertile *vinifera/rotundifolia* hybrids in rootstock crosses.

We have completed planting this year's rootstock seedlings (Table 2)

# Nematode resistance breeding

Since January, we have generated nematode resistance data for 172 seedlings in either initial or confirmation screens. Of these, 72 tested resistant to RKN and 5 resistant to ring nematode. We have identified 2 genotypes (2012-188-16 and 2012-110-02) that have tested resistant to both ring and RKN twice; both have been propagated to develop multiple mothervines and will be planted in the vineyard this summer. 2012-188-16 derives resistance from *rotundifolia* (via the fertile VR hybrid T6-42) and 2012-110-02 derives its resistance from GRN-5 and 101-14. Fifteen other seedlings tested resistant to RKN and ring nematode at least once each; these have been propagated and will tested a second time. Overall, 104 seedlings have tested resistant to RKN and will move into ring testing, and 24 seedlings have tested resistant to ring nematode and will move into RKN testing. Ninety-eight seedlings have been removed from the nematode pipeline for poor horticultural characteristics, poor rootability or nematode susceptibility since January (Table 3). Table 4 presents the best of the nematode resistant selections from the last year of testing.

#### Assay developments

We have recently started using smaller pots for nematode bioassays, enabling us to increase the throughput without investing in more greenhouse space. This allows us to simultaneously test horticulturally promising seedlings for resistance to both RKN and ring nematodes. This process was used to identify 2012-110-2, which has been moved to a mothervine block a year earlier than projected.

#### Current testing and projections

One hundred and thirty seedlings are currently in testing for resistance to ring nematode, and 62 seedlings are in testing for resistance to RKN. Ninety-nine genotypes are in propagation to begin initial rootability and nematode resistance testing. More materials are expected to be tested by January 2019.

Becky Wheeler-Dykes, who has been doing our nematode screens resigned on May 31, 2018. We will hire a replacement over the next couple of months and Nina Romero will take over the nematode work in the interim.

#### Dagger nematode resistance

Student has been preparing for qualifying exam since January.

#### Root-knot nematode and phylloxera resistance optimization and genetic mapping- Daniel Pap

**Optimizing RKN testing and mapping:** Efficient and quick root-knot nematode (RKN) screening is needed to rapidly develop new resistant rootstock varieties and we continue to optimize the screen. We have reduced the time for the screen to 6 weeks, and we are now using a more uniform subsurface irrigation system. For the hot summer months, we are testing the use of red shade cloth to reduce the temperature of the media since overheated sand media could negatively impact the nematodes.

We have further tested the feasibility of using 2" pots on capillary mats, but these small pots were not adapted to the sub-surface irrigation mats. Results with 4" pots are presented in Figure 1.



**Figure 1.** Number of RKN egg masses on susceptible St. George and Colombard hosts after variable number of weeks post inoculation and with sub-surface irrigation in 4" pots.

*Screening germplasm for RKN resistance:* The previously reported new resistant accessions were propagated for retesting. Each was inoculated with both HarmA and HarmC, and with a combined HarmA/C inoculum. The results confirm previously reported resistance levels.

*Crosses under investigation for RKN mapping:* Populations were screened to identify segregating populations that are suitable for mapping the genetic region responsible for RKN resistance. For this purpose, crosses that include susceptible *V. vinifera* are best, and we developed mapping populations with the susceptible *V. vinifera* female F2-35 or F2-7 last year. Table 5 lists the germinated populations and the number of seedlings produced. Subsets of these seedlings will be tested in the greenhouse for RKN resistance. The first populations based on GRN4, b41-23 and b45-26 are under investigation with subsets of fifty seedlings. GRN4 was selected because of previous preliminary results that placed resistance loci on chromosome 18. Populations with b45-26 and b41-23 were selected to study a potentially new resistance source. b41-23 is also being tested for dagger nematode resistance. Populations will be only established in the field if greenhouse tests suggest they are useful for mapping.

**Phylloxera resistance traits** We are also evaluating phylloxera resistance and attempting to map it. Strain specific resistances and broadly-based resistances need to be explored and combined into the ideal rootstock. Phylloxera feeding can result in large swollen galls on lignified roots called tuberosities. Tuberosities only form on susceptible species (*V. vinifera* and Asiatic *Vitis*) and they kill infested vines. Evaluating for tuberosity resistance is cumbersome due to the relatively long periods of time they take to form and the inability to maintain significant amounts of 1/4" inch thick roots. Nodosities, hooked gals on young feeder roots are much easier to assess in the greenhouse or lab environment. We are looking for quick and obvious responses, for example the HR (hypersensitive response) resistance that can be seen *in vitro* and allows quick and quantifiable nodosity level feeding resistance.

**Phylloxera isolates maintained** Besides the widespread A and B types of phylloxera, there are also other emerging types including types that feed and form leaf galls on rootstock foliage. To study these types and strains we have isolated and are maintain eight different phylloxera lines that are maintained *in vitro* on detached roots that are assayed every two weeks. These isolates are molecularly unique and continue to be studied. The strains are:

Campus – isolated from *V. vinifera* roots from UC Davis vineyard (biotype A); phy1103 – isolated from 1103P leaf/roots from UC Davis field; phyWR – isolated from *V. rupestris* Wichita Refuge from Wolfskill USDA-Repository; phy1616 – isolated from Napa, Flora Springs Vineyard on the roots of 1616C; #1, #2, #3 – isolated from three different locations in Napa; and Sonoma – isolated from Sonoma Valley

# Phylloxera phenotyping systems in progress

1. An extensive quick test: In the greenhouse, four bins filled with perlite and soil mix are planted with V. *vinifera* plants to maintain biotype A (Campus-strain) and biotype B (#3 isolate) and to provide an environment for a quick test. Rooted cuttings are placed in this media. Roots are examined after about two months under microscope and nodosites are counted.

2. *In vivo* – greenhouse method: Plants are planted in four-inch pots in perlite and inoculated depending on inoculum availability with leaf galls (phy1103 isolate) or infected root pieces. This test system is limited by the quantity and quality of the phylloxera inoculum available at the time of testing.

3. *In vitro* detached roots (Granett assay): 2-4 mm diameter roots are excised and cultured in Petri dishes. The root pieces are inoculated egg-by-egg with a small paint brush under a microscope and then incubated in the dark at 24C. All phylloxera isolates are maintained on *V. vinifera* roots with this method. Infested plants are examined after 30 days for hypersensitive reactions, possible nodosities, pseudotuberosities and specific phylloxera feeding habits, and reproduction. The main limitation of this method is the time for preparation of roots and maintaining the inoculum.

**Phylloxera mapping efforts;** Phylloxera mapping efforts are aimed at identifying resistance loci in mapping populations, in which highly susceptible *V. vinifera* are crossed with resistant accessions. Phylloxera mapping populations are listed in Table 6.

Earlier results found that accession b42-26 develops a HR reaction upon infestation with biotype B. A genetic map was developed with this background for identifying the PdR2 locus, so we can use the genetic map and apply a new trait to this existing map. Genetic data applied to a small subset of phylloxera from population 05-347 clearly indicate one genetic region responsible for this HR reaction. An *in vitro* screen of 50 seedlings from this population will be completed by mid-June of 2018. We are also conducting another test on the same plants with B biotype. To better characterize the location of the responsible region on chromosome 18 more molecular markers are being added.

We have tested a subset of 45 plants of  $F2-35 \times V$ . *berlandieri* 9031 cross (07-135) in the bins with biotype A and have seen evidence of segregation. We have also tested the parents of this cross with the phyl103 isolate under *in vitro* assay by placing leaf galls on excised root pieces. phyl103 grows easily on leaves and roots of 1103P, and are maintained on the leaves in isolation chambers (mesh-tents) in the greenhouse. It is capable of providing large quantities of inoculum at one time.

We observed extensive feeding on the entire root surface of the susceptible F2-35 and very limited feeding on the *berlandieri* 9031 roots that were localized on wounds and callus tissue with no reproduction after light feeding on these tissues. Our hypothesis was that this behavior could segregate, and this was confirmed by initial tests with the biotype A. *In vitro* tests provide evidence that resistance to tuberosity formation could segregate in this populations. In vitro excised roots infested with phy1103 were examined by two or three independent scorers at 7, 14, 21 and 28 days after inoculation. Initial observation included nodosity-like feeding, pseudo-tuberosity formation, number of adults and their location (wounds, cortex, root tips, tuberosities) and reproduction rates. After two weeks it was clear, that the initial hypothesis was true that tuberosity and associated feeding behaviors segregated (Figure 2). We repeating and expanding this assay.



**Figure 2**. Total number of tuberosities per genotype on the 07135 population (resistance source *V*. *berlandieri* 9031). The in vitro excised roots were tested after 4 weeks with the phy1103 isolate.

In addition, the 15-134 cross (susceptible *V. riparia* DVIT1411  $\times$  *V. berlandieri* 9031) could serve to confirm our finding in a non-*vinifera* background and help refine the putative resistance loci.

The RKN screen of the 07-135 (*V. vinifera* x *V. berlandieri* 9031) population suggests continuous segregation for resistance. We will develop a framework genetic map for this population after completing the phenotyping with phylloxera. Thus, we potentially could explore resistance in *V. berlandieri* 9031 for both RKN and phylloxera.

An additional three populations are being examined for the segregation of their resistance to biotype A using greenhouse bins with the resistant backgrounds of Riparia Gloire and *V. cinerea* B9. Furthermore, the resistance derived from 101-14 is being examined in a cross with St. George, which allows biotype A to feed and reproduce *in vitro*.

# Phenolic compounds in grapevine roots

We are also studying the association between phenolics 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 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.

# Drought tolerance/avoidance understanding

Kevin Fort has left the lab to work for an environmental consulting agency in Sacramento. We are continuing his work on root fibrosity/depth and salt tolerance. I am taking on a new PhD student in July and he is deciding whether to work on root architecture or salt tolerance.

**Understanding of the genetics of root architecture:** Multiple replicates of an F1 population of Ramsey (deep rooted) x Riparia Gloire (shallow rooted) were planted in the field in Spring 2017. We are growing these plants for two years to allow root structure to fully develop. These plants will be undercut and dug in Fall 2018 in an effort to identify genetic factors that control root architecture for marker development.

**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 7 lists the germplasm being tested at 75 mM NaCl, samples harvested May 29, 2018 and currently being processed.

Last year, two crosses with Dog Ridge were made with salt excluding accessions *V. acerifolia* 9018 and 9035. We observed 1:1 segregation in 15 tested seedlings of cross 14-138 (Dog ridge x *longii* 9018) and one-way analysis of variance indicated a highly significant genotypic effect. In Spring 2018, more crosses were made to expand the size of this population for genetic mapping. Plants are being propagated to repeat the salt screen in Summer 2018.

**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. 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. It will also serve as the foundation for a NSF project to sequence many of these species and selections that is just now getting underway.

# Tolerance to redleaf virus disease.

# Transcriptomic analysis of grapevine infected by red leaf viruses -Nihal Buzkan (Visiting Professor)

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 year-long sabbatical with me and is working on this virus tolerance project. Experiments were carried out with grapevine cv. Cabernet franc infected with redleaf 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 on Freedom and St. George, then transplanted into field conditions in march 2017. Symptom expression was observed by October 2017 (Fig.3).



Fig 3 and 4

The same experiment was also conducted in *in vitro* conditions (Fig. 5). In February 2018, greenhouse-forced shoot-tips from virus infected Cabernet franc were collected and micrografted onto diseased and healthy rootstocks (Freedom and St. George). The first symptoms appeared on graft combinations 5 weeks after grafting (Fig. 6 and 7).



Fig.5. Establishment of micrografting

**Fig.6.** Red leaf symptom caused by LR-1 in C. franc after 5 weeks of micrografting onto Freedom.

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 and absence of the viruses (Fig. 7). PCR DNAs were sequenced in two directions with both primers in order to characterize the virus strains. Moreover, virus quantification was done with SyberGreen real time PCR before microRNAs were isolated. They will be then subjected to high throughput sequence analysis in order to understand the effect of virus infection on plant gene regulations for symptom expression.



Fig. 7. Electrophoretic analysis of PCR DNAs for LR-1 and GVA.

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

Dormant cuttings from the 08180 population (Freedom x St. George) 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. 8 and 9). They were periodically checked for virus presence with an ELISA test. Real time PCR will be carried out to quantify LR-1 and GVA in the 08180 population to correlate virus titer with symptom severity.





Fig. 8 and 9. Grafted plants in greenhouse.

# Inheritance of GFLV Tolerance Trait in a 101-14 x Trayshed Population -

Ph.D. student Andy Nguyen continues to make progress on the inheritance of rootstock-induced fanleaf degeneration tolerance that has been observed in O39-16.

**Update:** As expected, many of the 101-14Mgt x *M. rotundifolia* Trayshed vines flowered this season. We bagged two inflorescences per vine before bloom. In the early summer, we will harvest the bags in order to count the collected calyptras and berries to calculate fruit set and determine the impact of grapevine fanleaf virus for each graft combination.

*Field Screening of Fertile VR Hybrids for GFLV Tolerance* – Screening 13 selections of fertile VR (*vinifera x rotundifolia*) hybrids. Eighty vines in the field grafted with these VR rootstocks, and the impact of fanleaf for each graft combination will also be assessed this upcoming spring with the method described above.

**Update:** Similar to the above study, these vines also flowered this season and we bagged two inflorescences per vine in order to conduct the same assessment.

#### GFLV Resistance in 101-14 x Trayshed Progeny and Fertile VR Hybrids -

**Update:** Greenhouse evaluation of GFLV resistance in genotypes from the 101-14 x Trayshed population is still underway. Results obtained so far are shown in Figure 1. Two genotypes (07107-065 and 07107-120) are promising and had similar levels of resistance as O39-16. We plan to study these genotypes and any other potentially interesting genotypes further by taking root samples from the same graft combinations currently grafted in our field plot and then quantifying GFLV levels in those samples (these field vines are the same vines we are using for our tolerance screen). In doing so, we can be more confident that our greenhouse resistance screen can accurately predict GFLV resistance in rootstocks on field vines. We also finished bench-grafting a second set of plants to repeat this resistance screen again this summer.

*Mechanism of Rootstock-Induced GFLV Tolerance* – We are also evaluating the cause behind the observed fanleaf tolerance induced by O39-16.

**Update:** Since a year has passed since chip-bud inoculation, we will verify infection in the inoculated vines with RT-qPCR in the coming month.

*Determining GFLV Infection Status in Rootstock Field Trials* – In May, we collected shoot tip samples from two separate rootstock trials grown on fanleaf sites (Lodi / Gallo and Healdsburg/Vino Farms) sites. These rootstock trials include the GRN series. We will determine the infection status of these vines through ELISA. Upon determination of infected vines, we can begin to observe the impact of the different rootstocks on GFLV symptoms. The trials have GRN-1 thru GRN-5, O39-16, RS-3 and RS-9, and 1103P in common. The Lodi site also includes the susceptible St. George, 3309C, 101-14, and Harmony, and between each of the 5 five vine reps a St. George vine so that the overall infection level of the plot can be assessed. The Healdsburg site also includes 1616C, Schwarzmann. Fanleaf is now expressing at these sites – both were planted in 2011.

#### **Presentations/Abstracts at Scientific Meetings**

- Walker, M.A. 2017. Development of grape rootstocks for control of pests and diseases. 63<sup>rd</sup> Conference on Soil-borne Plant Pathogens, UCD, Mar 30
- Huerta, K., S. Riaz, O. Franco-Mora, A. Walker. 2017. Evaluation of genetic diversity in wild *Vitis* material from northern and central Mexico. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
- Ellis, D., B. Robertson, C. Gillespie, M. Anderson, M.A. WA. Walker, J.D. Peterson. Effect of pruning on grapevine shoot and cluster development as a function of arm position along the cordon. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
- Bullock-Bent, C., K. Fort, M.A. Walker. 2017. Salt tolerance of four grape rootstocks is related to root architecture traits. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
- Uretsky, J., M.A. Walker. 2017. A preliminary examination of taxonomic and geographic relationships among accessions of *Vitis berlandieri* and associated taxa. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
- Walker, M.A. 2017. The southwestern *Vitis*: a grape breeding mother lode. ASEV 2017 Merit Award. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
- Walker, M.A. 2017. *Vinifera* hybrids and resistance to Pierce's disease. ASEV Eastern Section Meeting, Charlottesville, VA, July 12
- Walker, M.A. 2017. Development of next generation grape rootstocks. International Conference on Grape Phylloxera and Nematodes, UCD, Aug. 21
- Walker, M.A. 2017. Walker lab grape breeding progress, North American Grape Breeder's Conference,

UCD, Aug. 24

- Walker, M.A. 2017. Breeding winegrapes to resist Pierce's Disease. Mallorca, Spain, Nov 11
- Walker, M.A. 2017 What are the next steps for the PD resistant wine grape program? Current Issues in Wine Health, UC Davis, Dec 5
- Walker, M.A. 2017. Current breeding efforts in drought- and salt-tolerant rootstocks. Winegrpae Short Course, UC Davis, Dec 12
- 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, 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, SouthState 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.
- M.A. Walker 2018. Grape breeding update. Current Issues in Viticulture, UC Davis, Feb 21.
- M.A. Walker 2018. Developing PD resistant wine grapes. Lecture and tasting, UC Davis, May 4.
- M.A. Walker 2018. Developing PD resistant wine grapes. Lecture and tasting, UC Davis, May 11.

#### **Publications**

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

- Pap, D., S. Riaz, I.B. Dry, A. Jermakow, A.C. Tenscher, D. Cantu, R. Olah and M.A. Walker. 2016.
- 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.
- 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.
- Fresnedo-Rameriez, J., Q. Sun, C-F. Hwang, C. A. Ledbetter, D. W. Ramming, A. Y. Fennell, M. A. Walker, J. J. Luby, M. D. Clark, J. P. Londo, L. Cadle-Davidson, G-Y. Zhong, and B. I. Reisch. 2016. Toward the elucidation of cytoplasmic diversity in North America grape breeding programs. Molecular Breeding 36:116
- 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.
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- Forneck, A., V. Dockner, R. Mammerler, K.S. Powell, L. Kocsis, D. Papura, J. Fahrentrapp, S. Riaz and M.A. Walker. 2017. PHYLLI – an international database for grape phylloxera. International Organization for Biological and Integrated Control (IOBC) West Palaerartic Regional Section (WPRS) 128:45-51.

- Cui, Z-H., W-L. Bi, X-Y. Hao, P-M Li, Y. Duan, M.A. Walker, Y. Xu, Q-C. Wang. 2017. Drought stress enhances up-regulation of anthocyanin biosynthesis in grapevine leafroll-associated virus 3 infected *in vitro* grapevine (*Vitis vinifera*) leaves. Plant Disease 101:1606-1615.
- Arancibia, C., S. Riaz, C. Agüero, B. Ramirez, 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 (In Press)
- Fort, K. and M. A. Walker. 2018. Root system morphology predicts drought tolerance capacity in ten grape rootstocks. American Journal of Botany (submitted)

cross #	Female parent	Male parent	Purpose
		Schworzmonn	Fertile Vitis/Muscadinia
2018-109	8909-05 UCD GRN-1	Schwarzhann	(VM) progeny
2018-110	8909-05 UCD GRN-1	1103 Paulsen	Fertile VM progeny
2018-111	8909-05 UCD GRN-1	3309 Couderc	Fertile VM progeny
2018-112	8909-05 UCD GRN-1	Riparia Gloire	Fertile VM progeny
			Salt and broad nematode
2018-113	GRN-3 9365-43	acerifolia 9018	resistance
			Salt and broad nematode
2018-114	GRN-3 9365-43	acerifolia 9035	resistance
2018-120	Dog Ridge	acerifolia 9018	Salt/RKN/deep roots
2018-121	Dog Ridge	acerifolia 9035	Salt/RKN/deep roots
2018-124	Ramsey	doaniana 9028	Salt/RKN/deep roots
2018-136	11-188-16	1103 Paulsen	Ring/RKN
2018-137	11-188-16	NM 03-17 S01 treleasei	Ring/RKN/salt
2018-138	11-188-16	ANU77 girdiana	Ring/RKN/salt
2018-139	11-188-16	ANU57 treleasei	Ring/RKN/salt
2018-141	11-188-16	GRN-4 9365-85	Dagger/Ring/RKN/salt
2018-142	11-188-16	GRN-2 9363-16	Dagger/Ring/RKN/salt
2018-143	11-188-16	acerifolia 9018	Ring/RKN/salt
2018-144	11-188-16	acerifolia 9035	Ring/RKN/salt
2018-145	11-188-16	3309 Couderc	Ring/RKN
2018-146	11-188-16	110R	Ring/RKN
2018-147	11-188-16	SO4	Ring/RKN
		07107-079 FH 05-35	rotundifolia-based
2018-149	101-14 Mgt	T=tetraploid	resistance and fertility
		07107-079 FH 05-35	rotundifolia-based
2018-150	101-14 Mgt	D=diploid	resistance and fertility
	07107-062 FH 05-18		rotundifolia-based
2018-151	T=tetraploid	GRN-4 9365-85	resistance and fertility
	07107-062 FH 05-18		rotundifolia-based
2018-152	T=tetraploid	GRN-2 9363-16	resistance and fertility
	07107-062 FH 05-18		rotundifolia-based
2018-153	D=diploid	GRN-4 9365-85	resistance and fertility
	07107-062 FH 05-18		rotundifolia-based
2018-154	D=diploid	GRN-2 9363-16	resistance and fertility
			rotundifolia-based
2018-155	101-14 Mgt	101-14 x T 48T	resistance and fertility

Table 1. 2018 pollinations.

			rotundifolia-based
2018-156	101-14 Mgt	101-14 x T 48D	resistance and fertility
			rotundifolia-based
2018-157	101-14 Mgt	101-14 x T 42T	resistance and fertility
			rotundifolia-based
2018-158	101-14 Mgt	101-14 x T 42D	resistance and fertility
			Broad nematode
2018-170	2011-175-7	GRN-2 9363-16	resistance with PD

Table 2.	Rootstock crosses	planted in the	UCD vineya	ard 2018
			2	

				# Planted
Cross	Female Parent	Male Parent	Purpose	In Field
2017-028	101-14 Mgt	acerifolia 9018	Salt, nema	55
2017-032	101-14 Mgt	acerifolia 9035	Salt, nema	55
2017-046	12108-032	GRN-5	Nema	9
2017-074	5BB Kober	2012-144-39	Salt, nema	55
2017-078	5BB Kober	11188-003	VR hybrid, Nema	18
2017-115	doaniana 83	2012-144-24	Salt	55
2017-172	SC1	NM 03-17 S01	Salt	13
2017-173	SC1	GRN-2	Salt, nema	55
2017-174	SC1	GRN-4	Salt, nema	55
2017-175	SC1	GRN-5	Salt, nema	17
	2012-113-46 (101-14 X GRN-			
2017-601	4)	GRN-2	Nema	55

Table 3. Current testing and materials in the pipeline to be tested by January.

# Genotypes Tested For Nema Resistance Since January	172
# Genotypes Tested for RKN Resistance (Initial and Confirmation)	157
# Genotypes Moved Forward for Initial RKN Resistance	72
# Genotypes Tested for Ring Resistance (Initial and Confirmation)	15
# Genotypes Move Forward for Initial Ring Resistance	5
# Genotypes Removed From Pipeline Since January (Poor nema resistance, poor rootability, etc.)	98
# Genotypes Currently In Testing	131

Table 4. Best nematode resistant rootstocks candidates.

	Completed Nematode			
Selection	Testing	Rooting	Female parent	Male parent
	Resistant to RKN (2X),			
2011-188-16	<b>Resistant to Ring (2X)</b>	Good	T6-42	St. George
	Resistant to RKN (2X),			
2012-110-2	<b>Resistant to Ring (2X)</b>	Good	101-14 Mgt	GRN-5 9407-14
	Resistant to RKN (2X),			
2012-113-8	Resistant to Ring (1X)	Good	101-14 Mgt	GRN-4 9365-85

	Resistant to RKN (2X),			
2012-118-17	Resistant to Ring (1X)	Very good	161-49C	GRN-4 9365-85
	Resistant to RKN (2X),		OKC-1 SO1	
2012-125-21	Resistant to Ring (1X)	Good	(acerifolia)	GRN-2 9363-16
	Resistant to RKN (2X),			
2012-154-2	Resistant to Ring (1X)	Good	Ramsey	St. George
	Resistant to RKN (1X),			treleasei NM 03-
2011-148-42	Resistant to Ring (2X)	Good	Ramsey	17 S01
	Resistant to RKN (1X),			
2012-113-16	Resistant to Ring (2X)	Good	101-14 Mgt	GRN-4 9365-85
	Resistant to RKN (1X),		GRN-3 9365-	
2012-185-8	Resistant to Ring (2X)	Good	43	berlandieri 9031
	Resistant to RKN (1X),			
06301-138	Resistant to Ring (1X)	Very good	03300-018	9365-85
	Resistant to RKN (1X),			
2011-175-7	Resistant to Ring (1X)	Very good	08314-31	Schwarzmann
	Resistant to RKN (1X),			
2012-110-14	Resistant to Ring (1X)	Very good	101-14 Mgt	GRN-5 9407-14
	Resistant to RKN (1X),			
2012-110-33	Resistant to Ring (1X)	Good	101-14 Mgt	GRN-5 9407-14
	Resistant to RKN (1X),			
2012-112-17	Resistant to Ring (1X)	Good	101-14 Mgt	GRN-2 9363-16
	Resistant to RKN (1X),			
2012-112-33	Resistant to Ring (1X)	Good	101-14 Mgt	GRN-2 9363-16
	Resistant to RKN (1X),			
2012-113-11	Resistant to Ring (1X)	No Data	101-14 Mgt	GRN-4 9365-85
	Resistant to RKN (1X),		OKC-1 SO1	
2012-125-34	Resistant to Ring (1X)	Good	(acerifolia)	GRN-2 9363-16

Table 5. Seedling populations developed for RKN mapping. Seedlings have been DNA tested and they are true-to-type.

<b>Cross ID</b>	Female	Male	Mapping Purpose	#Seeds planted	#Seeds germinated
17-513	F2-35	GRN2	Phylloxera and RKN	100	68
17-514	F2-35	GRN4	Phylloxera and RKN	197	129
17-517	F2-07	b41-23	RKN and PD	274	146
17-518	F2-07	b45-26	RKN and Dagger	327	308
17-521	F2-35	GRN5	Phylloxera and RKN	320	139
17-506	F2-35	T64	RKN	16	12
17-507	F2-07	longii 9027	RKN	139	94
17-510	F2-07	champinii 9021	RKN	145	99
17-515	F2-35	T56	RKN	81	13

Table 6. Populations under investigation for mapping phylloxera resistance.

Cross ID	Female		Male	#Seedlings
05-347	F2-35	×	V. arizonica b42-26	370
07-135	F2-35	×	V. berlandieri 9031	110
15-134	<i>V. riparia</i> DVIT 1411	×	V. berlandieri 9031	200
09-140	Almeria	×	Riparia Gloire	114
09-390	Malaga Rosada	×	V. cinerea B9	225
12-111	101-14	×	St. George	100
05-803	Colombard	×	GRN4	19

Table 7. Grape germplasm that has tested well under previous tests at 25 to 50 mM NaCl concentrations and currently in testing.

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