**California Grape Rootstock Improvement Commission**
**California Grape Rootstock Research Foundation**
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**Project Title: Development of next generation rootstocks for California vineyards.**

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**Progress Summary:**
The **2012 crosses** focused on drought and salt resistance from newly screened highly resistant accessions of southwestern *Vitis* species; mapping of 101-14 x 110R; introgression of *M. rotundifolia* into 161-49C, and 5BB; GRN rootstock crosses with better rooting rootstocks and lower vigor; Ramsey x 1616C for mapping root architecture and salt tolerance; Freedom x St. George to allow study of virus tolerance; 101-14 x SG for virus and salt tolerance; and combining nematode and salt with GRNs and southwestern *Vitis* spp;

**Fanleaf** – We have made some progress on confirming the role of cytokinins and their precursors in O39-16's ability to induce tolerance to fanleaf infection. We have tested a subset of the 101-14 x *rotundifolia* 'Trayshed' population for their cytokinins and there is variation. We will begin applying these potential biomarkers for fanleaf tolerance to this population and field trials. Our *Xiphinema index* resistance gene (*XiR1*) gene candidates have been transformed into the susceptible St. George and we should have results on the effect of these *XiR1* candidates on preventing feeding by Summer. We have also launched a project to examine O39-16's effect as a bionematicide. We have identified a field site at Niebaum Coppola; developing potted collections of common cover crops and weeds for *X. index* feeding studies; and healthy and infected O39-16 root systems to determine whether *X. index* can reacquire GFLV from them.

**Salt and Drought Resistance** – we made good progress on a root architecture x drought resistance assay, and have almost finished examining 30 rootstocks with a rhizotron to examine rooting angles and architecture, and confirmed that rooting angles from herbaceous cuttings are an effective trait and that these angles segregate in several potential mapping populations. A second generation cross involving V. berlandieri c9031 and vinifera now established to test this apparently single gene source of chloride exclusion from c9031. A subset will soon be tested and if salt exclusion varies it will be used as a mapping population. Extensive tests documented that relative growth rate has a large impact on salt uptake and extra controls are now included on all tests to assess relative growth rate.

**Southwest Vitis and Salt Tolerance** – Three collection trips were made in 2012 – the Red River of northern Texas and southern Oklahoma; the east flank of the Rockies in Colorado and New Mexico; a transect from Las Vegas to St. George Utah – with the goals of acquiring more highly chloride resistant germplasm and to complete a wide ranging collection of *Vitis* germplasm from the southwestern US, which now consists of over 800 accessions. These collections will allow us to better understand this complex group of taxa which possess very strong tolerance to salt and drought in addition to *X. index* and PD resistance, and may also be useful for boron and sodium tolerance.

**Root Anatomy and Drought Tolerance** – Studies on the anatomical differences related to drought tolerance continue and correlations are being made between vessel size and distribution, and suberin deposition (which prevents water loss through the root surface.

**Leaf Longevity and Senescence** – Cabernet Sauvignon plots grafted on 101-14 and 110R at the Oakville Station are being studied to examine their impact on the longevity of leaf function and onset of ripening. If we can ripen fruit earlier less water will be required. Conversely, if we can control leaf longevity then quality of later harvests may be improved. This study found that leaf senescence varies and that it also segregates in the Ramsey x Riparia Gloire population, which we have already genetically mapped.

**Phylloxera Resistance** – Karl Lund's phylloxera studies are winding up. He documented clear genetic and phenotypic differences among 8 strains of California phylloxera collected from the roots of Chardonnay, AXR#1, 101-14 (leaves and roots), St. George (leaves) and Freedom. A three level examination of phylloxera genetic diversity (UCD campus; California; and Native range) is also nearing completion. Karl found that asexual/clonal reproduction occurs primarily in California including with the apparently introduced leaf gall forms, and that sexual recombination is more important in the native range. We have key populations that will allow mapping of resistance in the coming years.

**PROGRESS REPORT**

**2012 Crosses:** In addition to these crosses we tried to pollinate or use pollen from 25 of the 101-14 x Trayshed progeny, with little success. These progeny are expected to be sterile, but there may be a low percentage of fertile types (given Olmo's experience). Any fertile types would be tremendously valuable for breeding and genetic mapping. We will continue efforts to identify fertile members of this and other *rotundifolia*-based populations

Table 1. 2012 crosses.

| Cross # | Female | Male | Seeds | Purpose |
|---|---|---|---|---|
| 2012-080 | arizonica A44 | monticola T 03-02 S01 | 45 | Drought and salt |
| 2012-081 | arizonica A53 | monticola T 03-02 S01 | 23 | Drought and salt |
| 2012-084 | arizonica A44 | SC3 (girdiana) | 14 | Drought and salt |
| 2012-092 | SC1 (girdiana) | GRN-4 9365-85 | 1 | Drought, salt, nematodes |
| 2012-095 | treleasei ANU23 | candicans T56 | 10 | Drought and nematodes |
| 2012-102 | 101-14 Mgt | NM03-17 (treleasei) | 237 | Salt, nematodes |
| 2012-104 | 101-14 Mgt | 110R | 65 | Mapping |
| 2012-106 | 101-14 Mgt | 9024 (doaniana) | 89 | Salt |
| 2012-108 | 101-14 Mgt | 9028 (doaniana) | 99 | Salt |
| 2012-109 | 101-14 Mgt | berlandieri 9031 | 230 | Salt and mapping |
| 2012-110 | 101-14 Mgt | GRN-5 9407-14 | 572 | Nematodes |
| 2012-111 | 101-14 Mgt | St. George | 213 | Virus, salt, nematode mapping |
| 2012-112 | 101-14 Mgt | GRN-2 9363-16 | 230 | Nematodes |
| 2012-113 | 101-14 Mgt | GRN-4 9365-85 | 300 | Nematodes |
| 2012-115 | 161-49C | Trayshed | 224 | Nematodes, phylloxera, lime |
| 2012-116 | 161-49C | berlandieri 9043 | 250 | Lime and salt |
| 2012-117 | 161-49C | 110R | 379 | Mapping vigor |

| | | | | |
|---|---|---|---|---|
| 2012-118 | 161-49C | GRN-4 9365-85 | 132 | Nematodes and vigor control |
| 2012-125 | OKC-1 SO1 (acerifolia) | GRN-2 9363-16 | 106 | Nematodes, salt |
| 2012-126 | OKC-1 SO1 (acerifolia) | GRN-4 9365-85 | 240 | Nematodes, salt |
| 2012-129 | OKC-1 SO1 (acerifolia) | St. George | 420 | Salt |
| 2012-132 | champinii | arizonica A56 | 141 | Salt |
| 2012-133 | 5BB Kober | 1616C | 129 | Nematodes |
| 2012-136 | 5BB Kober | 9024 (doaniana) | 5 | Salt |
| 2012-138 | 5BB Kober | Trayshed | 66 | Pests, rotundifolia |
| 2012-142 | girdiana -11 | arizonica A56 | 1200 | Salt |
| 2012-143 | girdiana -22 | arizonica A56 | 322 | Salt |
| 2012-144 | girdiana Scotty's Castle | arizonica A56 | 205 | Salt |
| 2012-146 | Ramsey | Trayshed | 5 | Pests, salt, drought |
| 2012-148 | Ramsey | 1616C | 500 | Mapping vigor, leaf senescence |
| 2012-149 | Ramsey | ANU77(girdiana) | 97 | Salt |
| 2012-153 | Ramsey | 9028 (doaniana) | 171 | Salt |
| 2012-154 | Ramsey | St. George | 139 | Salt, nematodes, rooting |
| 2012-158 | 161-49C | St. George | 371 | Vigor, salt, drought |
| 2012-178 | Dog Ridge | Trayshed | 110 | Pests, rotundifolia |
| 2012-181 | GRN-3 9365-43 | ANU77(girdiana) | 138 | Nematodes, salt |
| 2012-185 | GRN-3 9365-43 | berlandieri 9031 | 118 | Nematodes, salt |
| 2012-186 | GRN-3 9365-43 | GRN-5 9407-14 | 230 | Broad nematode |
| 2012-186? | GRN-3 9365-43 | GRN-5 9407-14 | 15 | Broad nematode |
| 2012-187 | GRN-3 9365-43 | berlandieri 9043 | 100 | Nematodes, salt |
| 2012-188 | Dog Ridge | 110R | 59 | Salt, PD |
| 2012-189 | Dog Ridge | 140Ru | 70 | Salt, PD |
| 2012-190 | Dog Ridge | St. George | 111 | Salt and rooting |
| 2012-197 | Freedom | St. George | 300 | Virus tolerance |
| 2012-198 | Fry | munsoniana | 36 | Rooting in rotundifolia |

**Breeding Nematode Resistant Rootstocks:** We continue to work with Howard Ferris and Liang Zheng, Dept. Nematology, to test selections based on crosses designed to combine multiple forms of nematode resistance and improve horticultural characters. We first select within the populations for seedlings with strong growth, good internode length and limited lateral production (brushy appearance). Then we test these selections for their ability to root and the best ones are advanced to nematode testing against the aggressive root-knot nematode strains, HarmA and HarmC, followed by testing against *X. index*. Table 2 lists selections that are resistant to aggressive root-knot nematode strains HarmA and C and will now move to *X. index* testing. Nina Romero is becoming quite expert in this area and is helping with the testing.

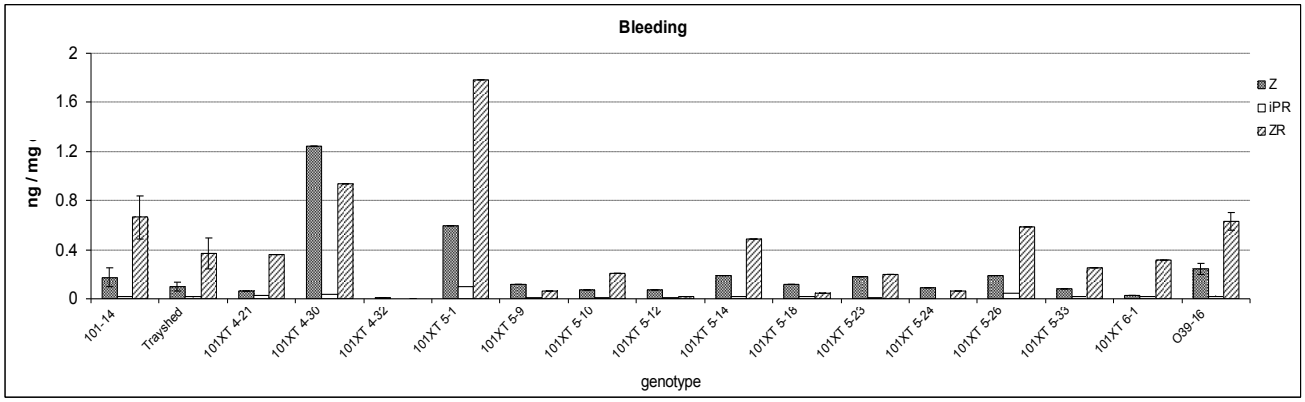
Table 2. Selections resistant to HarmA and HarmC root-knot nematodes, and with good horticultural characteristics (rootability, lack of laterals and good internode length) tested during 2012.

| Population | Cross | # Resistant |
|---|---|---|
| 2002-122 | 5BB x 9365-85 (GRN-4) | 1 |
| 2010-115 | 161-49C x Trayshed | 17 |
| 2011-133 | OKC-1 SO3 acerifolia x St. George | 6 |

| | | |
|---|---|---|
| 2011-137 | 161-49C x T9 doaniana | 3 |
| 2011-138 | 5BB Kober x Trayshed | 6 |
| 2011-143 | Ramsey x 08314-15 (PD resistance) | 9 |

**Rootstock-Induced Fanleaf Tolerance:** Cecilia Agüero is directing efforts with the UCD Metabolomics Center to determine which compounds are responsible for tolerance to fanleaf disease. The goal of this work is to find a metabolite associated with, or responsible for, this tolerance that we can use to rapidly screen populations such as the 101-14 x 'Trayshed'. Without these biomarkers we will have to field test for the ability to induce fanleaf tolerance, which will take many years.

Analysis of xylem sap collected from healthy and infected 'Chardonnay' grafted on O39-16, which induces fanleaf tolerance on the scion, and St. George at bleeding and fruit set resulted in the selection of cytokinins zeatin (Z), its precursor zeatin riboside (ZR), and isopentenyladenine riboside (iPR) as potential biomarkers. Xylem sap from 101-14, Trayshed and individuals of their *Vitis* x *Muscadinia* (VM) hybrid progeny, were collected in Spring 2012 and sent to the Metabolomic Facility to test these compounds using UPLC-QTRAP MS/MS analysis. Results showed a considerable variability in the contents of Z, ZR and iPR among the individuals tested, and encourage the testing of fanleaf tolerance on them (Fig. 1). Currently, we are conducting grafting experiments *in vitro* and under greenhouse conditions to assay the development of fanleaf degeneration in infected Chardonnay grafted on VM individuals. In addition, we will test treatments of VM plantlets with colchicine to duplicate their chromosome number in order improve the fertility of the cross. We also want to test the effect of inflorescence treatments with ZR on fruit set since, as in our previous experiments, ZR was the prevalent cytokinin present in bleeding and fruit set of xylem. Results also showed again that cytokinin levels were higher at bleeding and that iPR gained relevance at fruit set.



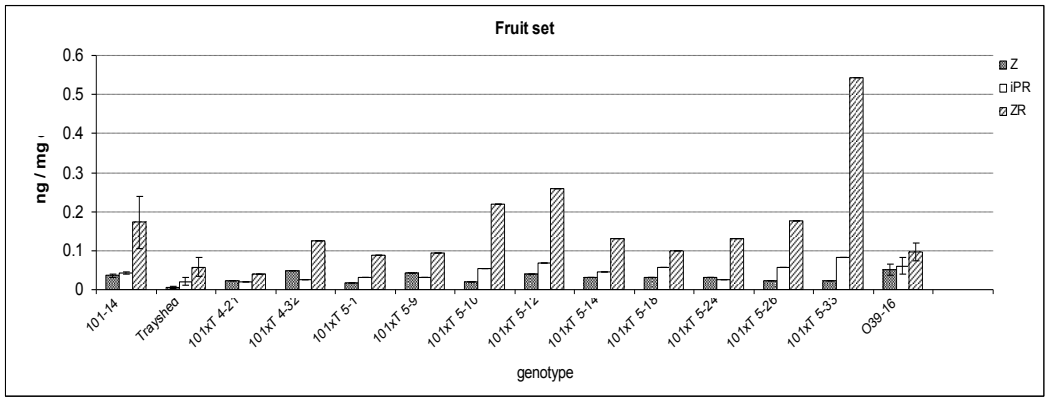



Figure 1. Peak areas of cytokinins in xylem sap collected in 2012.

**Genetic Mapping and the Physical Location of the *X. index* resistance gene, *XiR1***: The genetic and physical mapping of the *X. index* resistance gene, *XiR1* was completed and published (Hwang, C-F., K. Xu, R. Hu, R. Zhou, S. Riaz and M.A. Walker. 2010. Cloning and characterization of *XiR1*, a locus responsible for dagger nematode resistance in grape. Theoretical and Applied Genetics 121:780-799). Dr. Agüero is working on verifying the function of *XiR1* by genetically engineering two candidate genes from the *XiR1* region/locus (see last year's report for specifics) into *X. index* susceptible Thompson Seedless, St. George and tomato to verify the function of *XiR1*. We now know that the resistance gene candidates, *XiR1.1*, and *XiR1.2*, are members of the NB-LRR (nucleotide binding-leucine rich repeat) resistance gene family often involved in the signaling and promotion of resistance mechanisms.

Transformation experiments of tomato and grape with *XiR1.1* and *XiR1.2* are finished; with 8-10 independent lines that tested PCR positive for the genes in each combination. Plants were micropropagated to produce replicates, transferred to the greenhouse and tested with *X. index* this fall, however they were not able to survive transplantation to infected soil. As a consequence, multiplication of the plants is now being conducted through green cuttings under mist propagation, which produces considerably stronger roots, and they have survived transplanting in preliminary testing (Fig. 2). Plants will be evaluated for resistance to *X. index* based on the number of galls formed in 4–8 weeks after inoculation with 100 nematodes. Tomato will be tested afterwards because of greenhouse space constrains.

Table 3. Summary of accomplished goals

| | Cloned into binary plasmid | Grape transformation / plants | Tomato transformation / plants |
|---|---|---|---|
| *XiR1.1* | √ | √ / plants in GH | √ / plants *in vitro* |
| *XiR1.2* | √ | √ / plants in GH | √/ plants *in vitro* |

Fig. 2. Transgenic acclimated plantlets growing in the greenhouse and their multiplication under mist propagation.

***Xiphinema index* Resistance:** Sonet van Zyl finished with her PhD and took a faculty position in the Viticulture & Enology Department at CSU Fresno. She has not yet completed writing up her dissertation work, but we did publish a review of *Xiphinema index* resistance (VanZyl, S., M.A. Vivier and M.A. Walker. 2012. *Xiphinema index* and its relationship to grapevines: a review. South African Journal of Enology and Viticulture, 33: 21-32). Papers on the following topics are in preparation and will hopefully be submitted this year: a novel method of evaluating grape plants for *X. index* resistance; an inheritance study of *X. index* resistance in *V. arizonica*; and a potential genetic mapping paper.

**Long-term efficacy of O39-16 as a "bionematicide" against *Xiphinema index*:** Evan Goldman is evaluating the effectiveness of O39-16 in reducing (and possibly eliminating) *X. index* populations from infected fields, based on their presumed inability to feed on the highly resistant O39-16. A test plot in Napa was identified and work began to assess the level of fanleaf infection. Initial testing of the vines in July 2012 was unsuccessful, however RNA extraction methodology was refined in this process, leading to more rapid sample processing and reduced opportunities for contamination. Cuttings were collected from the test block in January 2013 and they are now being warmed and callused to provide fresh material GFLV testing. Once infected vines have been identified, soil samples will be collected from the drip zones, and nematodes will be extracted and identified.

**Grape fanleaf virus (GFLV) infection of O39-16:** Previous studies have shown that although O39-16 is resistant to *X. index* feeding, GFLV may be introduced to the plant and infect the scion. However, it is unclear whether O39-16 itself is capable of becoming infected, or whether the vascular system is merely serving as a conduit to the susceptible scion. In the coming year, we will test the host status of O39-16 for GFLV. GFLV-positive bud wood will be grafted to virus-free O39-16 and allowed to grow for at least nine months. Following this "inoculation" period, the vines will be cut back to a bud below the graft, and allowed to regrow; this growth will then be tested for GFLV.

**GFLV acquisition by *X. index* feeding on O39-16:** Although vines grafted on O39-16 are capable of being infected by GFLV, little is known about the ability of O39-16 to transmit the

virus. This experiment will determine if GFLV-positive vines grafted on O39-16 allow the roots to act as virus reservoirs that could re-infect *X. index* individuals. Virus-free *X. index* colonies are currently being established. In December 2012, six 12" pots filled with *X. index*-infested soil were planted with figs (a non-host for GFLV). The nematodes will be allowed to feed on the figs for at least nine months, at which point samples of nematodes will be tested for GFLV. Once the test confirms that the nematodes are virus-free, they can transferred to GFLV-positive vines rooted on O39-16.

**Weedy host evaluation:** Vineyards often maintain cover crops or resident weed populations between rows to help minimize soil compaction, reduce noxious weed populations, add organic matter to the soil, and maintain soil moisture. The reproductive potential of *X. index* on many of the weed and cover crop species is unknown. It's possible that *X. index* may be able to reproduce on some of these species, effectively mitigating any bionematicidal effects that O39-16 may be having on populations of *X. index* in vineyards. The possibility that *X. index* may reproduce on weeds and cover crops has serious implications for current weed management practices in California vineyards

A range of different vineyard weeds and cover crops will be evaluated for their ability to host *X. index* reproduction against positive controls (St. George rootstock) and negative controls (fallow). Cuttings from St. George were collected in January 2013 and are currently being rooted. Treatments will be carried out in small pots and inoculated with 100 *X. index* individuals (adults and $J_4$) when the plants have developed sufficient root systems (approximately two to four weeks following initiation). Extractions will be performed at six to eight weeks (perhaps longer depending on growing conditions) following inoculation, and final populations will be counted and compared to the positive and negative controls.

**Grape root longevity:** Grape roots are very durable in the soil; they may still be alive and capable of hosting populations of *X. index* and GFLV for more than nine years following removal of the vine. This is especially problematic when considering the use of O39-16 as a bionematicide – populations of virulent *X. index* may be sustained and increased on susceptible roots for long periods of time. We are searching for ex- St. George or AXR#1 fanleaf sites that have been without vines for over 10 years. We will excavate roots and assay their viability and any *X. index* populations that might be present.

**Salt and Drought Resistance –** Kevin Fort is continuing his work on salt and drought resistance. At the beginning of 2012, four primary objectives were outlined that encompassed salt tolerance, drought resistance, and the interaction of these two variables, and sought to advance the breeding program towards the goal of obtaining molecular markers for genes responsible for improvements in these traits. These objectives were all completed, and additional unplanned projects that furthered this end were also either completed or initiated. One academic paper on salt tolerance was accepted for publication, and three additional papers were completed and revised, and will soon to be submitted for publication.

**Completion of study on the interactions of salt and drought resistance –** A greenhouse study of salt and drought stress interactions was completed near the end of 2011, but involved an inordinately large number of samples (approximately 800) to be processed for root and shoot

biomass, leaf tissue chloride concentration, and root architecture. The first goal of 2012 was to complete this data set and to analyze the data. Initial findings of growth effects were presented in the 2012 proposal, and an analysis of chloride exclusion and root architecture effects were summarized in the mid-year report. A key finding has since emerged and will be discussed under "2012 Additional Work" below.

**Completion of root architecture assay for drought resistance –** A variety of container systems (11 total) were tested for an ideal geometry, volume, and growth regime that would allow the efficient harvesting of roots and differentiation of their architecture. In the end, all standard container sizes--and some very large, non-standard sizes--fell short of a desirable outcome. Small containers suffered from root restriction that tended to homogenize innate architectural differences, and roots grown in larger containers lost their important architectural characteristics once the media was washed away. A soilless system also suffered due to the lack of a supporting matrix. Dormant, lignified root systems excavated after a full season of growth appeared to maintain their architectural characteristics, but excavating these root systems was resource intensive and the root systems were large and complex, leading to problems evaluating and comparing them. Nevertheless, some useful data was obtained for a limited number of genotypes (e.g., Fig. 3). The most informative data were obtained from weekly monitoring of root growth along a plexiglass surface in rhizotron containers. Excellent data were also obtained from adventitious root angles of herbaceous cuttings grown in a coarse, shallow media, though perhaps a bit less informative than data obtained from the rhizotrons. However, the adventitious root assay was rapid and inexpensive, permitting a level of throughput that is not possible with the rhizotron containers. Examples of the two successful assay systems are shown in Fig. 4.

**Mapping development for deep rooting for drought resistance –** This objective was centered on the evaluation of 180 hybrid individuals from a Ramsey x Riparia cross, and for which an SSR-based genetic map is available. We evaluated a subset of this population for variation in their root architecture, but the values were all very similar, indicating that there was no segregation occurring in this F1 population, likely due to a dominant gene or genes present in all of the F1 individuals. Fortunately, this conclusion was reached prior to the pollination season in 2012, and F2 generations were created from three sibling crosses within the Ramsey x Riparia population. We expect these to segregate for deep and shallow rooting. Seed from these crosses were successfully produced and plans for their use in 2013 is described in "Projects Planned for 2013," below.

**Propagation of two Vitis populations for chloride exclusion mapping –** Population-level analysis of hybrids produced from strong and weak chloride-excluding parents indicates, at least in two important crosses, that strong chloride exclusion is dominant in the F1 generation and does not segregate, preventing genetic mapping of this trait. A second round of crosses to weak chloride-excluding parents were performed to produce the necessary segregating populations. In 2012 these populations were established and screening for salt tolerance is set to begin in the next several weeks. The most promising of these populations is derived from the parent *V. berlandier*i c9031, which in earlier screens exhibited a capacity for chloride exclusion equal to, and possibly better than, the exclusion capacity of currently available rootstocks. Although salt tolerance screening of F2 level crosses could have been accomplished in 2012 with an earlier-developed "primary screening" methodology, the phenotyping of ungrafted plants for salt

tolerance can be confounded by growth rate differences inherent in different genotypes. It was considered prudent to delay this screening somewhat until refinements were made to the assay, described in "2012 Additional Work," below.

**2012 Additional Work**

**Important modification to the salt tolerance assay –** The 14-day fritted clay-based assay developed in previous years minimizes the confounding that can occur in rates of chloride translocation to the shoot when relative growth rate differs between genotypes. Nevertheless, in some experiments also performed in previous years, chloride concentration in the leaves correlated to final dry weight of the shoot. This reflects the influence of differing growth rates among genotypes, and could impact the quality of the data. Because this effect could undermine the highly resource-intensive screening of a segregating population where precise phenotypes are critical to successfully linking markers to traits, two experiments were performed in 2012 to quantify the exact relationship of relative growth rate (RGR) and chloride uptake into the shoot. In one trial, precise RGR measurements were taken during successive two-week periods during the growth and development of herbaceous rootings exposed to high salinity conditions (Fig. 5). In a second trial, herbaceous rootings were propagated over successive weeks and then exposed to a single, common two-week period of high salinity (Fig. 6). Surprisingly, analyses of the data obtained indicated that the optimal approach to decoupling RGR from chloride uptake occurs when cuttings are propagated simultaneously, but grown across a range of RGRs induced by different watering regimes. This is the type of data produced in the salt and drought stress interactions study also completed in 2012. When genotype trends are intentionally generated across a range of RGRs, a chloride uptake phenotype emerges (Fig. 7) that is resistant to differing growth rates that occur in some experiments using a single watering regime. This understanding provides a new tool with which to control data quality in experiments where growth rate confounding cannot be risked.

**Initiation of root architecture characterizations of all California rootstocks -** Although grape root architecture has been examined by other researches, the difficulty of obtaining root data generally limits the number of genotypes being studied. After it was determined early in 2012 that rhizotron containers were well suited to the task, a series of root architecture characterizations were initiated on all of the generally available rootstocks in California. This work is now approximately 75% complete. The following 33 genotypes have been or soon will be examined in this study: O39-16, 5BB, 5C, 41B, 44-53, 99R, 101-14, 110R, 140Ru, 161-49C, 420A, 1103, 1616C, 3309, Colombard, Dog Ridge, Freedom, GRN-1, GRN-2, GRN-3, GRN-4, GRN-5, Harmony, Ramsey, Riparia, RR19, RR29 (siblings from the Ramsey x Riparia population), Schwarzmann, SO4, St. George, Thompson Seedless, and two southwest *Vitis* accessions GC5 and SC-1.

**Projects planned for 2013**

**Screen a *V. berlandieri*-derived population for salt tolerance using newly-developed, robust methodology –** As described, the *V. berlandieri* c9031 genotype is potentially as strong or stronger in chloride exclusion capacity as currently available rootstocks, and may possess a single dominant gene for chloride exclusion. A 2012 cross with Malaga Rosada (*V. vinifera* Malaga Rosada x [*V. vinifera* F2-35 x *V. berlandieri* c9031]) was performed to create salt tolerance segregation not observed in the uniformly strong chloride-excluding F1 hybrid

population of *V. vinifera* F2-35 x *V. berlandieri* c9031. A population of approximately 75 individuals is currently being multiplied for screening with our most robust non-grafted greenhouse screening method, using multiple watering regimes to better characterize the salt tolerance phenotype of each genotype. Further work with this population will depend on the nature of the data obtained. If the chloride exclusion pattern indicates a single dominant gene, genetic markers could be derived from this population. If more genes appear to be involved, the population would need to be expanded to at least 200 individuals. Seeds are available from the initial cross and are in cold storage for this purpose, if necessary. The screen of approximately 75 individuals is expected to be complete by summer 2013; the processing of tissue samples for chloride concentration will be complete by the end of 2013.

**Screen three additional mid-sized populations for salt tolerance -** In 2011, additional crosses were performed involving strong chloride excluding-parents, generating additional hybrid populations from significantly different taxonomic backgrounds that may possess additional genes conferring strong chloride exclusion. If so, such genes could be combined to create rootstocks with exceptional exclusion capacity. It is currently unknown whether screening these populations requires the additional effort and resources being applied to the most important of these populations, the *V. berlandieri*-9031-derived population described above. When objective number one is complete, an analysis of the data set should provide guidance for subsequent screens, and a more streamlined, resource-efficient screen may result. For work this year, the methodology employed will be decided depending on available greenhouse space and other resources following the completion of objective one. The populations available for additional salt tolerance screening are: 2A - *V. vinifera* Rosa Minna x (*V. vinifera*-F27 x *V. rupestris*-St. George); 2B - *V. vinifera* Rosa Minna x (Ramsey x Riparia); 2C - *V. riparia*-1411 x 140Ru. Because populations 2A and 2B have been crossed a second time to a weak chloride-excluding parent, they have an increased probability of exhibiting segregation for exclusion in the hybrid progeny.

**Assess the chloride exclusion capacity of the GRN 1 through 5 rootstocks -** The GRN rootstocks were developed for broad nematode resistance. Their parentage suggests that some or all of them may contain excellent chloride exclusion. However, the relative exclusion capacity of these rootstocks as compared with currently available rootstocks is unknown. Therefore, a greenhouse screen for salt tolerance will be performed with these rootstocks to compare them to well-characterized rootstock genotypes. This work will be completed by the end of 2013.

**Compliment rhizotron characterizations of root architecture characterizations with herbaceous rooting assay -** All genotypes already assayed or soon to be assayed in the rhizotron system are also currently in propagation for parallel characterization in the herbaceous (adventitious) rooting assay. This additional analysis will provide important insight into how much additional data is obtained when using the rhizotron system, and whether or not this additional information would be important in molecular marker development. As with the rhizotron data set, a full set of adventitious root data across the 33 genotypes above is expected by summer 2013.

**Root architecture characterizations of novel Ramsey x Riparia sibling hybrids -** Three crosses of Ramsey x Riparia siblings were performed in spring 2012 – all three crosses

individually generated a sufficient number of seeds to be used in molecular mapping. The unexpected lack of variation in rooting angle seen in the F1 generation of Ramsey x Riparia, which implies genes for deep rooting are dominant, is expected to re-appear in these pseudo-F2 generations in individuals lacking one or more dominant genes for repression of the shallow rooting phenotype. Because of the relatively large number of individuals in these populations, the high-throughput adventitious root assay will be used to phenotype the root architecture, although selected individuals may be assayed in the rhizotrons following the initial adventitious root characterization. All three populations are currently in propagation, and will be planted in the field this spring.

**Root architecture characterization of a 101-14 x 110R population, and possible correlation to phenological traits -** 101-14 is thought to induce relatively early ripening in the fruit of a grafted scion, in contrast to the relatively late ripening when grafted to 110R. To create a segregating hybrid population for use in molecular marker development these rootstocks were crossed and will be evaluated for phenological traits. Approximately 100 individuals were generated and planted to the field, and now have a full season of growth. Fortuitously, the root systems of 101-14 and 110R are shallow and deep, respectively, analogous to that observed in Riparia and Ramsey. However, unlike Riparia, 101-14 is itself an interspecies hybrid (*V. riparia x V. rupestris*), and therefore the 101-14 x 110R hybrids may segregate for major root architecture traits, unlike the non-segregation observed in Riparia x Ramsey F1 hybrids. All individuals in this hybrid population are currently being clonally amplified in a greenhouse, and will be assayed with the adventitious rooting assay. A completed data set for this population is expected by summer of 2013. If root architecture segregation is found, this will be useful not only for molecular marker generation for rooting, but correlations of root architecture to phenological traits could prove insightful in determining the basis for phenology differences in the scion.

**Root architecture characterization of a Riparia 1411 x 140Ru population – We have a small** 36-progeny population of Riparia 1411 x 140Ru. This population will be explored for segregation of salt tolerance in 2013 due to the excellent chloride exclusion capacity of 140Ru. Because this population is already being clonally amplified for this end, and because the parentage backgrounds of each parent are expected to differ for root architecture, these individuals will also be assessed for root architecture in the adventitious root assay. A completed data set for this relatively small population is expected to be complete by the summer of 2013.

Fig. 3. *Top:* Data obtained from field excavation of grapevine rootstocks. Replicate number indicated below each data point. Error bars are ± 1 standard deviation. *Bottom:* Photographs of representative excavated root systems.

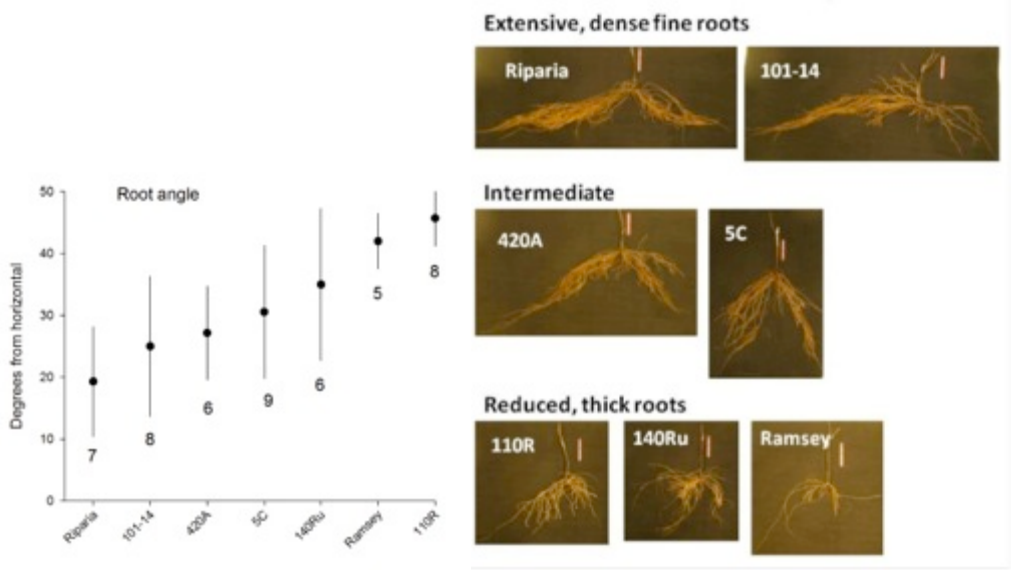



Fig. 4. Successful root architecture assay systems. *Top:* Rhizotron container and derived root tracings. *Bottom:* Root systems from adventitious roots assay. Two leftmost root systems: Riparia Gloire; two rightmost root systems: Ramsey.

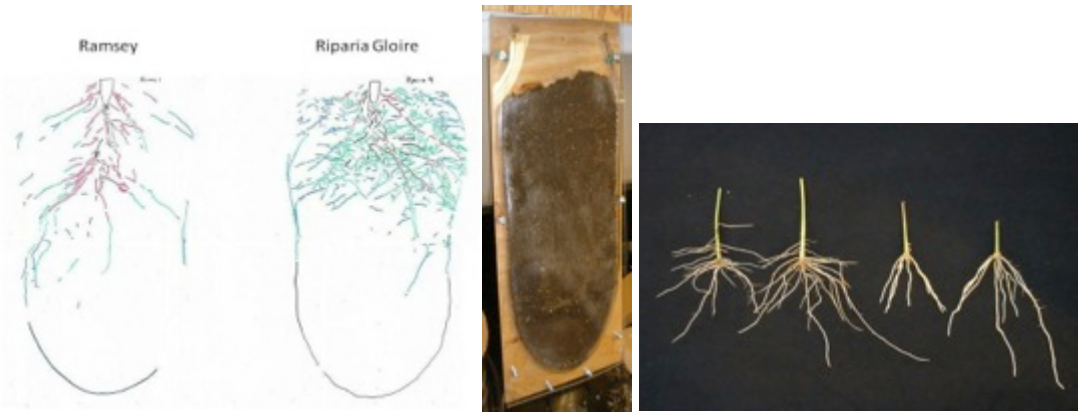


Fig. 5. Relative growth rate (RGR) trials, to determine the impact of different RGRs on the rate of chloride uptake. *Top:* Rootstocks growing in the last of four sampling periods. *Bottom:* Vines propagated over five successive weeks and exposed to high salinity in the same sampling period.

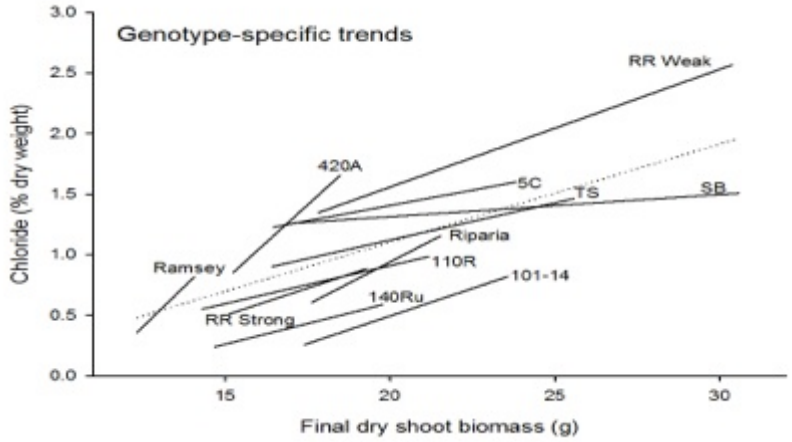



Fig. 6. Overall (dotted line) and genotype-specific (solid lines) trends in chloride uptake over a range of four increasingly frequent watering regimes.

**Germplasm Exploration for Chloride Exclusion –** Claire Heinitz has almost completed her MS research and has started on her PhD projects. Her progress and future plans are detailed below.

**Chloride exclusion in wild *Vitis* populations –** After confirming the efficacy and reliability of our greenhouse screen (see June 2012 report for details), we have continued to use it for the detection of wild genotypes that take up low levels of chloride into their shoots and/or roots. The screen involves greenhouse grown vines propagated from herbaceous cuttings, which are transferred to fritted clay media and exposed to 25mM NaCl irrigation for 2 weeks. These parameters have consistently shown the greatest separation between different genotypes, and the fritted clay allows us to recover fine roots without excessive washing and to avoid any added salinity effect due to salt building up in the media. Since the last report, we have finished analyzing the results from the 2011 screens, which included more of the genotypes collected by the Walker lab and some of Olmo's Mexican collections. See Figure 7 for a map that indicates the exclusion status of all accessions screened in 2010 and 2011. We are currently processing samples from the 2012 screens, which include our new collections from 2011 (Fig. 8) and targeted collections from the USDA germplasm repository. Our greenhouse screen continues to identify new sources of chloride exclusion, though excluders are often mixed geographically with non-excluders. This is likely due to the complex hybrid origins of the wild grapes in the southwest U.S.

**Investigating effects of growth rate on chloride uptake –** Following the work of research groups in Australia that also study chloride exclusion in grapevine (Schachtman and Thomas, 2003, Tregeagle et al., 2010 Functional Plant Biology), we evaluated a new method of generating plants for greenhouse screens which involves rooting the petioles of single leaves. We had hoped that this would allow us to propagate plants more quickly, take up less space in the greenhouse, and eliminate the potential effects of differential growth rate (because rooted leaves don't grow). However, we found that plants screened in this way performed very differently based on how they were propagated. We compared a small set of genotypes that had been previously characterized in our normal screen using various types of cuttings, including our

typical 2 node cuttings as well as the intact rooted leaves detailed in the references above. Each different cutting type either changed the ranking of the genotypes relative to our regular screen or eliminated any statistical difference between them. Therefore, we couldn't rely on the rooted leaf system to reliably rank the chloride uptake in the wide range of genotypes that we screen. Figure 9 illustrates the difference in chloride uptake for the same genotypes either grown from 2-node herbaceous cuttings or intact rooted leaves.

While the rooted leaf system proved ineffective for our purposes, it still showed that growth rate can have an impact on chloride uptake. To further investigate this effect, we included a second set of plants in all of the 2011 screens that had been "debudded" – at the start of salt treatment, all actively growing shoot tips, lateral buds, and expanding leaves were removed. We maintained this condition by continually removing buds every 1-2 days as they became active, effectively holding the growth rate of these plants at zero. Figure 10 presents the effects of debudding on chloride uptake in one screen. In genotypes where uptake was fairly high, debudding reduced the chloride concentration drastically; however in genotypes with already low uptake, debudding did not further reduce leaf chloride concentration. The effects in the roots were more pronounced and widespread, potentially indicating that growth rate is more tightly linked with root uptake than with transport to the leaves. This could also explain why root chloride concentration is more variable between screens than leaf concentration. In future screens, when it is more important to compare chloride exclusion in the root, we will include extra plants in order to measure the relative growth rate of each genotype.

**Mapping populations –** In 2011, Claire made crosses between several wild accessions that had performed well in the 2010 screens and the highly susceptible *V. rupestris* accession "Pumpstation," which continually displays dramatically high leaf chloride concentration. In 2012, several of these populations were moved to the field – one each with a *V. acerifolia, V. doaniana, V. arizonica,* and *V. girdiana* excluder parent. If these populations segregate for chloride exclusion, they can be used in the future in genetic mapping to find markers linked to chloride exclusion.

**New collections –** In late summer of 2012 we made three collection trips to Nevada, Utah, Colorado, New Mexico, Texas and Oklahoma to expand our collection of wild *Vitis*. These vines are currently in propagation for chloride screening, and will be moved to the field in the spring. We targeted locations where we had previously collected material that proved to be promising for chloride exclusion (Ash Meadows NV, Red River TX), where we hoped to find hybrid taxa in intermediate habitats (southern Colorado), and finally where we suspected a complicated mix of genotypes (southwest Utah). These accessions will be useful in rootstock breeding, but they will also help build a larger study set for genetic analysis. These collections also serve as a conservation library of wild materials that are threatened in their native habitat by changing land use and gene flow from the increasing numbers of cultivated *V. vinifera* vineyards.

**Genetic analysis –** This year, Claire will be performing the bulk of the genetic analyses to investigate the origins of chloride exclusion in wild *Vitis* and the hybrid relationships between different species in the southwest. Using a set of ~20 microsatellite markers and over 800 distinct grapevine accessions, we hope to address the following questions:

- How many distinct genetic "clusters" are present in the larger study set, and do any of them correlate with chloride exclusion capability?
- What is the extent of gene flow from *V. vinifera* to various wild vineyard neighbors?
- Can we find evidence of long-distance seed migration (possibly via birds)?

Our ultimate goal is to gain a greater understanding of the genetic structure of wild southwestern *Vitis*, which will guide breeding decisions. Previous attempts to characterize these species both by morphology and by genetics have failed, most likely due to small sample sizes and a failure to account for high levels of hybridization. Our study group is unprecedented in size, and because of our access to a high throughput genetic analyzer, we can rapidly screen our samples with a larger number of markers than any other study.

A preliminary analysis of accessions of three species from Texas and Oklahoma illustrates the potential power of population-level genetic analysis. Munson first suggested in 1909 that a unique species found only along the Red River (*V. doaniana*) may be a hybrid of *V. acerifolia*, found in southern Oklahoma, and *V. candicans*, found throughout Texas. We became interested in this population when we observed the excellent performance of *V. doaniana* accessions in our chloride exclusion screen. To test the theory of a hybrid origin, we extracted DNA from several of our collections and genotyped them using a small set of 6 microsatellite markers (Figure 10). Though this represents only a small amount of data, it was enough to confirm the possibility that *V. doaniana* is the result of continual hybridizations between *V. acerifolia* and *V. candicans* where their distributions meet. Using the program STRUCTURE, we determined that the 3 putative species represented only 2 genetic groups. *Vitis acerifolia* accessions belonged mostly to one group and *V. candicans* to the other, while *V. doaniana* accessions appeared as a mixture of the two (Figure 11). While these are only preliminary results, we are confident that this type of analysis will allow us to answer many important questions at large and small scales throughout our study set, and the results will help inform breeding decisions and conservation recommendations.

Figure 7. Map of all accessions screened for low chloride uptake in 2010 and 2011. Green circles indicate excluders (leaf chloride concentration similar to St. George), and red triangles indicate non-excluders (leaf chloride concentration statistically higher than St. George).

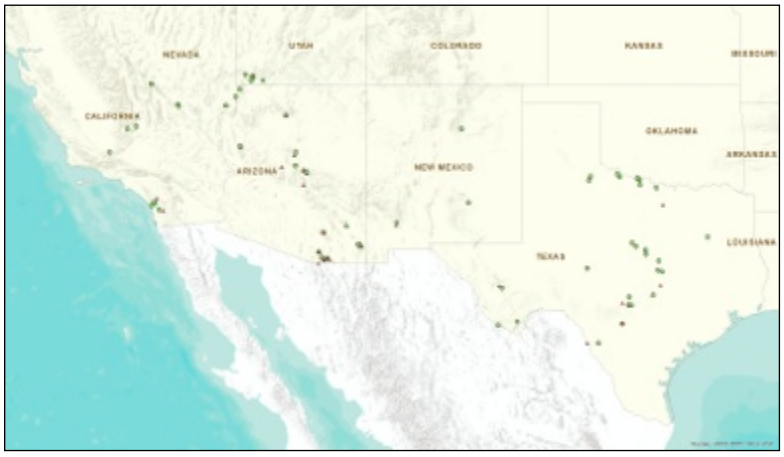
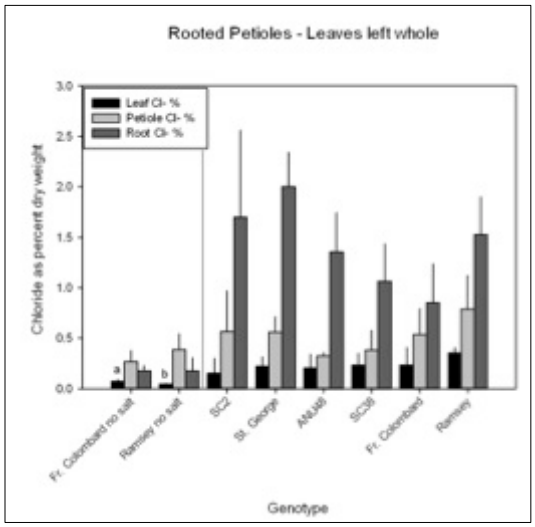


Figure 8. Map of chloride exclusion results for new collections from 2011. Green circles indicate excluders (leaf chloride concentration similar to St. George), and red triangles indicate non-excluders (leaf chloride concentration statistically higher than St. George).

Figure 9. A comparison of chloride uptake in the same genotypes using different propagation methods – either a 2-node herbaceous cutting or a rooted leaf. Lowercase letters indicate mean separation within each tissue type separately – leaves, petioles and roots.



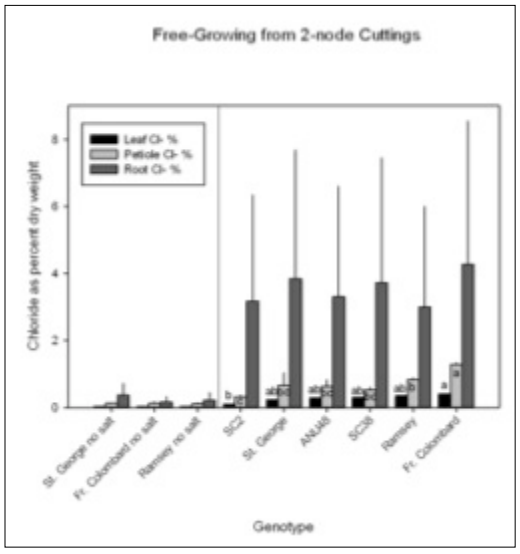




Figure 10. Comparison of root and leaf chloride concentration between free-growing and debudded plants. Red circles indicate a significant difference between debudded and control for that genotype and tissue type only.

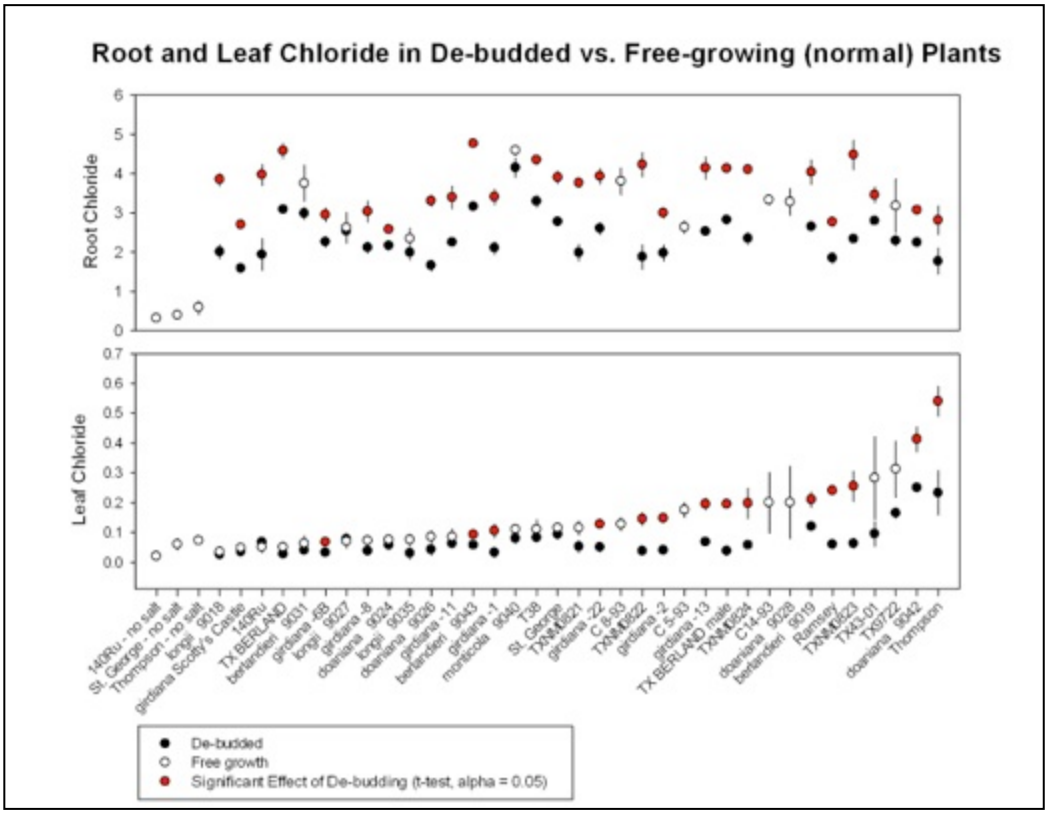
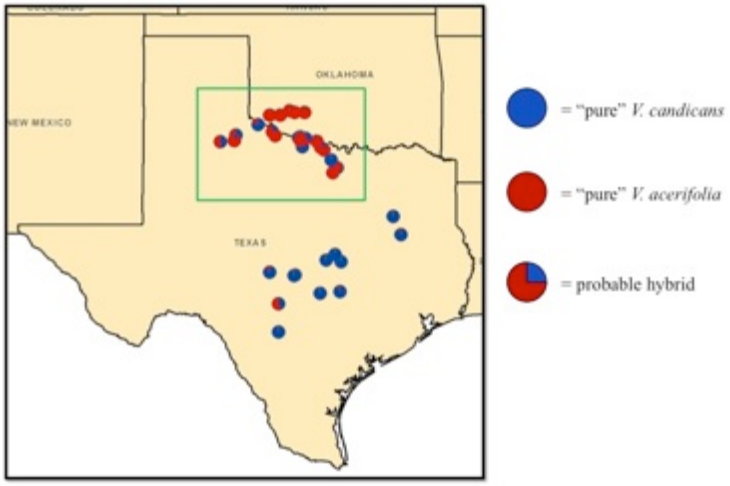



Figure 11. Accessions included in a preliminary genetic analysis to investigate the origins of *V. doaniana* (probable hybrid of *V. acerifolia* x *V. candicans*). The pie charts represent the percentage assignment to each genetic cluster based on STRUCTURE analysis.



**Root architecture and drought tolerance:**
Last summer we reported preliminary results on Cecilia Ozorio's anatomical differences between Ramsey, a drought resistant rootstock, and Riparia Gloire, a drought susceptible rootstock. At the time we had focused our efforts on the stem internodes of such cultivars, mainly for ease of access and sectioning. However, having discovered significant differences between the two, we began examining the roots of Ramsey and Riparia as well as those of *V. arizonica*, *V. girdiana*, St. George, 110R, 101-14Mgt, 420A and 5C. We focused on quantifying tissues related to water transport, water loss prevention and nutrient/water storage. The measurements included the total amount of tissue allotted for water transport (the xylem area), number and size of the vessels, cortex, rays and fibers accordingly.


Figure 12 - Left picture is the rooting node of Riparia. Notice Ramsey (right) at the same stage. Both came from dormant cuttings.

Preliminary observations show great variability in the tissue percent composition among the genotypes studied. The greatest variability seems to lie in the amount of tissue dedicated to the cortex in the woody roots, with St. George devoting over 50% of the total area to the cortex, compared to less than thirty percent in both Ramsey and Riparia (Fig. 13). Of all the rootstocks compared so far, 110R has the largest amount of vessel lumen per unit square area (Fig. 14) and its average vessel diameter is second in size only to that of St. George (Fig. 15), as a result of the reduced fiber and ray tissue areas found in St. George (Fig. 13 and 15). Although the average vessel diameter of 110R is similar to St. George, 110R's vessels vary in size but St. George's vessels are consistently large. We also noticed that Ramsey and Arizonica seem to have the smallest vessel diameters among all the varieties examined so far (Fig 14). This trend is important, since both individuals are drought resistant and because a similar trend was observed when vessel diameters were compared between Ramsey and Riparia internodes last year. Though at the time it was not found to be significantly different, we suspect that recently acquired microscopy equipment will allow for more accurate quantification of all vessels regardless of their size.

**The role of suberin in grape drought resistance**
Based on previously reported observations of anatomical differences of the cortex and its apparently variable biochemical composition among different rootstocks, a more detailed quantifiable analysis seems essential. Sections of samples are being made to determine if there may be a significant difference between the suberization and lignification of the cortex in woody roots. Suberization and lignification of the periderm and cortex are believed to prevent water loss and differences in the amount of each of these components may be a useful character for selecting drought resistance in the future. We are also interested in understanding the timing of when these water loss-insulating components are deposited in the cells of young roots. Last fall, green cuttings of each of the above rootstocks were rooted in the greenhouse. These are now

being processed for sequential sectioning. In addition, woody roots will be harvested and their cortex will be sent out for biochemical analysis.

**Correlation of rootstock architecture and drought resistance**

This experiment will determine the correlation between root angles and root length distribution of Ramsey, Riparia, 110R, 101-14, 5C, 420A, and Thompson Seedless. We hypothesize, that in drought resistant cultivars root length density will be linearly related to soil depth, but inversely related to root angle. During the summer of 2011 eight completely randomized field blocks were planted. Each block contained ten replicates for each of the species. The blocks were drip irrigated once per week throughout the first summer to allow the plants to establish. During their second summer, four of the blocks were drip irrigated and the other four were not irrigated. Harvest of this plot and measurement of root density and architecture is planned in the near future.

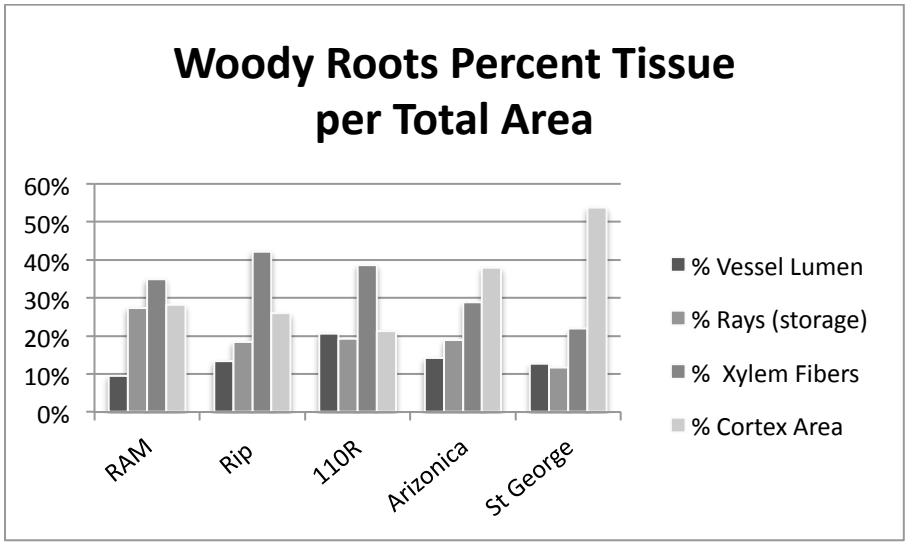



Figure 13. Woody root tissue ratios per unit square cross section.

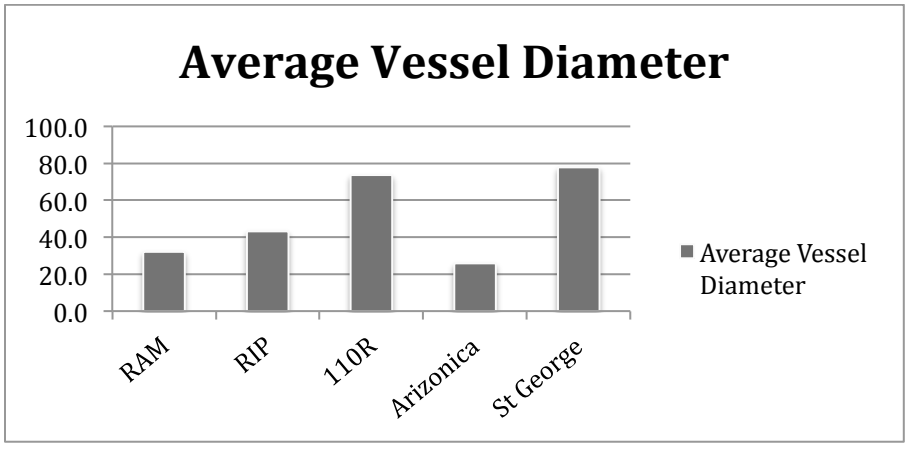



Figure 14. Woody root vessel diameters.

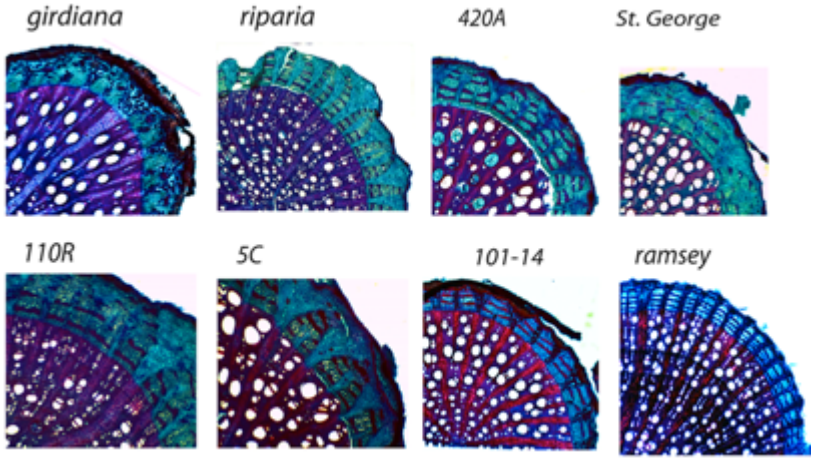



Figure 15. Woody root cross sections.

**Grape rootstock-scion interactions and influence on ripening, dormancy and leaf senescence –** Jean Dodson has finished her second year of field and shadehouse trials towards her doctorate exploring the influence rootstock selection has on the ripening of fruit and the longevity of leaf function. The purpose of this project is to clarify the role rootstock parentage has in rootstock-scion interactions, specifically on extended or shortened growth cycles and their influence on drought tolerance.

In 2011, field trials at the Oakville Research Center south vineyard began on Cabernet Sauvignon vines grafted to 101-14Mgt and 110R. The first year of data collection at this site was meant to be a broad survey tracking multiple physiological traits throughout the growing season. Data was collected on berry set percentages for primary and secondary clusters, total nodes per shoot, shoot-tip activity, internode length above the 2$^{nd}$ cluster and shoot caliber, leaf water potentials, berry sugar accumulation, leaf drop percentages and canopy density. Data from the first year was presented in the June 2012 Report.

The 2012 field trials were expanded to include a Cabernet Sauvignon vineyard in the Station's North vineyard grafted to 101-14Mgt and 110R. This year a more focused approach was used and data was taken on internode length and shoot caliper above the second cluster, light bar readings at the fruiting zone, shoot-tip activity, leaf water potentials, photosynthetic activity via Li-Cor analyzer, and vine senescence. We also have unanalyzed pruning weights, Brix, pH and TA data for 2012.

The Oakville South Station demonstrated significant differences between rootstocks on scion internode length, shoot caliber above the second cluster, leaf longevity over the course of the season and canopy density via light bar measures (Figures below). Results for pruning weight and berry analysis will not be analyzed until late Spring 2013. These results are consistent with the previous growing season, 2011, at this site. We will repeat this evaluation during the 2013 growing season.

The Oakville North Station had similar findings to the South Station, however, Red Blotch disease pressure likely influenced the findings. Significant differences were found between

rootstocks on leaf-holding ability over the course of the season, canopy density via light bar measures and leaf photosynthetic activity (Figures Below). Results for pruning weights and berry analysis will not be analyzed until late Spring 2013. The 2013 growing season will re-evaluate the previous season's work and add a protocol to score virus symptoms present on the leaves.

A shade house assay was developed during the 2012 season. Dormant cuttings of St. George, Riparia Gloire, 101-14MGT, 420A and 110R were taken and five were grafted to Cabernet Sauvignon and five were left un-grafted. A deficit irrigation treatment was imposed on a portion of both the grafted and un-grafted vines. The vines were then periodically evaluated for leaf water potential and the initiation of leaf senescence. The purpose of this assay is to find a means to quickly and effectively evaluate rootstock leaf holding capacity and the initiation of dormancy. These data are currently being evaluated.

This was also Jean's second year evaluating and scoring the Ramsey x Riparia Gloire mapping population for leaf senescent and canopy density. Selections from the extremes of this population will be grafted to Cabernet Sauvignon and screened using the shade-house assay to evaluate rootstock influence on scion dormancy initiation timing during the 2013 research season.

The 2011 crosses of 101-14MGT x 110R were planted in the field in spring of 2012. Field screening for leaf-holding capacity took place during fall of 2012. Selections from this population will be grafted to Cabernet Sauvignon and screened using the shade-house assay to evaluate rootstock influence on the timing of scion leaf senescence during the 2013 research season. This population may serve an important role in mapping genes associated with differences in fruit ripening and leaf function and senescence.

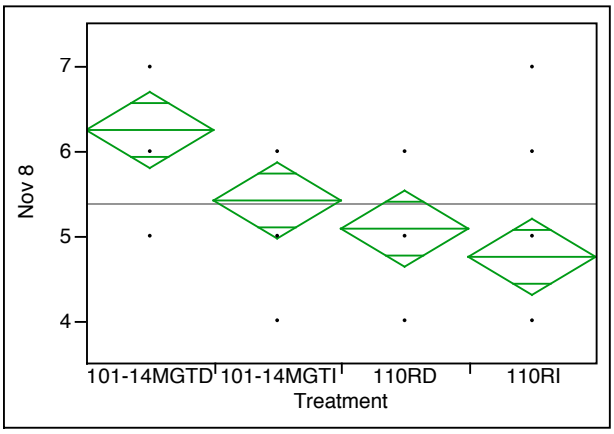



Figure 16. Vine senescence scores for November 8th 2012 for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry (higher numbers reflect earlier senescence). There was a clear – 101-14MGT has a shortened growing season compared to that of the 110R. The deficit irrigation treatment serves to accentuate the differences between the two rootstocks.

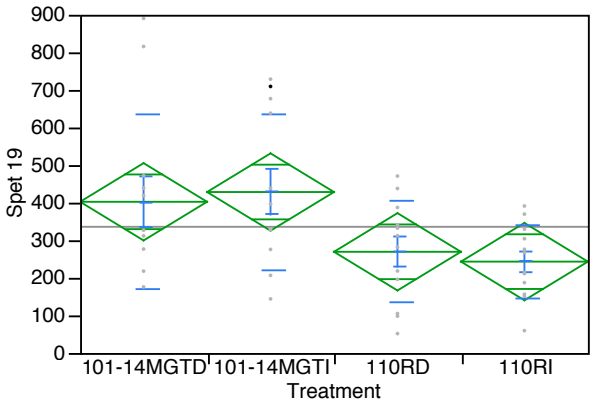



Figure 17. Light bar canopy density measurements (September 19th 2012) for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry. Although irrigation did not play a role in performance difference, there was a clear separation between rootstocks. 101-14MGT had lower canopy density and allowed greater light penetration into the fruiting zone and canopy when compared to 110R.

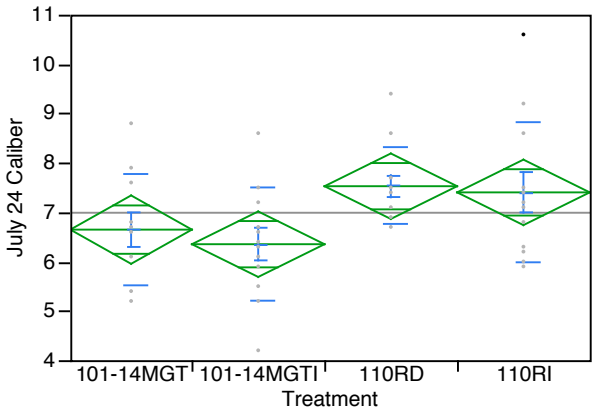



Figure 18. Internode length above the primary cluster (July 24th 2012) for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry. Although irrigation did not play a role in observed differences, there was a clear separation between rootstocks. The shoot caliber was significantly greater for 110R compared to the 101-14MGT.

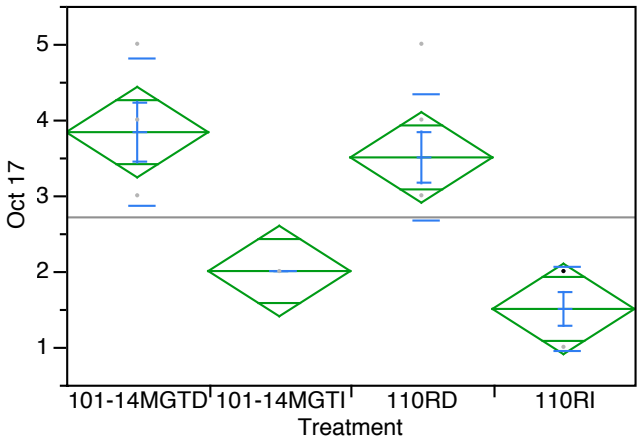



Figure 19. Vine senescence score (Oct 17th 2012) for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry. There was a clear separation between the irrigation treatments. The deficit irrigated treatments had earlier vine senescence than those rootstocks that were fully

irrigated. This trend might be reflective of the virus status in this vineyard, suggesting deficit irrigated vines were less impacted by Red Blotch and had a more normal senescence pattern.

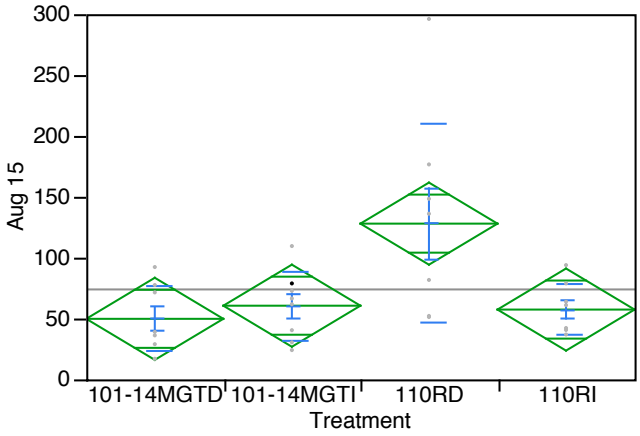



Figure 20. Light bar canopy density measurements (August 15th 2012) for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry. The greatest light penetration through the canopy was in the deficit-irrigated 110R. 110R deficit irrigated vines were less impacted by virus present in this block, as virus infested vines tend to hold onto leaves longer than normal, impacting the light penetration into the fruiting zone.

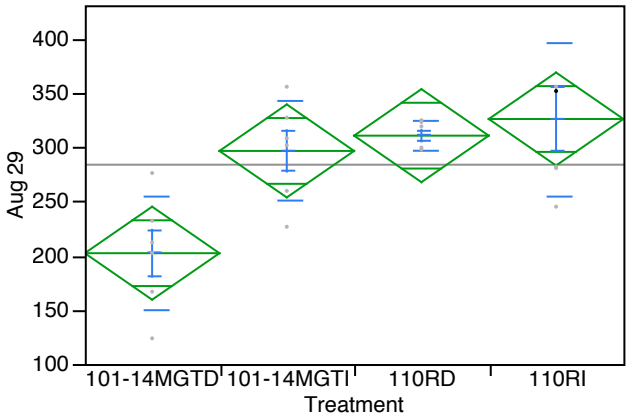



Figure 21. Photosynthetic activity via Li-Cor (August 29th 2012) for 101-14MGT Irrigated, 101-14MGT Dry, 110R Irrigated and 110R Dry. 110R had a higher activity than 101-14MGT and 101-14MGT under deficit irrigation had the lowest photosynthetic activity.

**Phylloxera resistance studies**: Last year we presented extensive phenotyping data on the feeding of six phylloxera strains on eight *Vitis* species roots (*V. vinifera*, AXR#1, *V. rupestris* 'St. George', *V. rupestris* 'Ganzin', *V. riparia* 'Riparia Gloire', *V. berlandieri* 9043, *V. berlandieri* 9031, and *M. rotundifolia* 'Trayshed'. This year we added more data across these root systems with two strains collected from the roots of Freedom rootstock in Napa Valley (Free R1 and Free R2 – Table 3). This feeding was scored from nodosities (young root tip feeding – common on most resistant species and rootstocks). Tuberosities (feeding on mature roots that leads to vine death) were only seen on *V. vinifera* 'Colombard'.

Table 3. Source and identification code for phylloxera strains used in root bioassays.

| Phylloxera Strain ID | Collected from – | Old name |
|---|---|---|
| VIN R1 | Chardonnay roots; UC Davis vineyard | Campus |
| AXR R1 | AXR #1 roots; Mendocino County | Mendo |
| 101 R1 | 101-14 roots; Healdsburg, CA | Jordan |
| 101 R2 | 101-14 roots; Geyserville, CA | Wasson |
| 101 L1 | 101-14 leaf galls; Dunnigan, CA | Boris |
| STG L1 | St. George leaf galls; Winters, CA | Wolf |
| Free R1 | Freedom roots; Oakville | Oak |
| Free R2 | Freedom roots; Spring Mtn, St. Helena | Spring |

The results of these feeding assays are summarized below (please see last year's report for data). The VIN R1 strain collected from own-rooted Chardonnay and shows the classic 'biotype A' phenotype. It has the ability to reproduce very efficiently on Colombard and on *V. rupestris* roots, although less effectively. The VIN R1 strain was unable to reproduce on AXR#1 or 101-14. The AXR R1 strain was collected off AXR#1 roots in Mendocino county and acts like a typical 'biotype B' strain. It has the ability to reproduce efficiently on both Colombard and AXR#1, but was unable to reproduce on 101-14. Strains collected off of 101-14 roots in Sonoma county (1-1 R1 and 101 R2), a strain collected from 101-14 leaves in Dunnigan CA (101 L1), and a strain collected from St. George leaves in Winters CA (STG L1) all had a similar phenotype – the ability to reproduce very effectively on 101-14. These 101-14 specialized strains retain the ability to reproduce on AXR#1, but at a lower level compared to the AXR R1 strain. Interestingly, the 101-14 specialized strains did not reproduce well on Colombard. The final two strains were both isolated from the rootstock Freedom. The first came a vineyard east of Oakville, and the second from a vineyard west of St. Helena. These strains have the ability to reproduce effectively on 101-14, AXR#1 and Colombard. While the Freedom strains are not as aggressive on any of the single root sources as other strains, their ability to reproduce effectively across many different roots makes them unique.

Six of the above strains (the Freedom strains were not included) were also tested against four rootstocks (101-14 Mgt, 1103P, Börner, and St. George, which was included to allow comparisons with the species screen). Strains collected from 101-14 (both root and foliar) reproduced well on 101-14, while other strains struggled to reproduce. Börner had the best resistance allowing only very limited reproduction from the 101 L1, 101 R2 and Free R1 strains, but the plants were hard to maintain and root quality was often poor. 1103P allowed all strains to reproduce within 21 days, but their egg laying ability dropped after that point. The VIN R1 and AXR R1 strains could only produce 3 and 7 eggs per adult on 1103P, respectively, while the other strains produced 32 eggs per adult or more. St. George allowed the six strains to reproduce within 20 days. Only the 101 R2 strain had a slightly lower fitness producing less than 10 eggs per adult, while the other strains tested produced between 13 and 17 eggs per adult. The rootstock Freedom allowed slow but consistent reproduction from the Free R1 strain, and poor reproduction from the VIN R1 and 101 L1 strains. Finally, the Ramsey results were interesting – the 101 L1, Free R1, 101 R2 and 101 R1 strains all reproduced quickly while feeding on Ramsey, however the VIN R1 and AXR R1 strains induced a necrotic hypersensitive response, which did not allow either strain to reproduce.

Ramsey was the first of a group of species that displayed a necrotic response to the feeding of a specific strain or set of strains. *Vitis berlandieri* 9043 also had a similar response, but was more specific than Ramsey, only responding with hypersensitivity to VIN R1. *Vitis arizonica/girdiana* b42-26 had the opposite result allowing the VIN R1 strain to reproduce while the AXR R1 strain induced a strong necrotic response. The 101 L1, Free R1 and 101 R2 phylloxera also induced a necrotic response from b42-26, but it was slower and less consistent (Table 4).

Table 4. Age to first reproduction (AFR) and eggs produced when comparing six phylloxera strains on b42-26.

| b42-26 | AFR | #Eggs@AFR + 2 Days | Eggs/Adult |
|---|---|---|---|
| VIN R1 | 10.50 | 65.25 | 18.78 |
| VIN R1 on Colombard | 11.33 | 33.17 | 19.86 |
| Free R1 | 27.00 | 0.75 | 0.75 |
| 101 R2 | 27.00 | 0.25 | 0.25 |
| 101 L1 | 27.50 | 5.75 | 3.00 |
| AXR R1 | 32.00 | 0.00 | 0.00 |

These results lead to the testing of a subset of 11 individuals from a b42-26 mapping population (05347 = *V. vinifera* F2-35 x b42-26) with the VIN R1 and AXR R1 strains. The VIN R1 strain was able to feed on all tested members of this population, and formed both nodosities and tuberosities. The AXR R1 strain only fed on half of the tested set and again produced both nodosities and tuberosities. These results highlight the need to understand phylloxera diversity. For unknown reasons the VIN R1 strain is better adapted to b42-26, and reproduced on all members of the 05347 population, while the AXR R1 strain only fed on half of the tested b42-26 progeny. The 05347 population segregates as though resistance is controlled by a single heterozygous dominant gene when challenged by the AXR R1 strain – half the progeny are resistant.

**Phylloxera genetic diversity studies**:

**California** – Several studies into the genetic diversity have been analyzed over the last year. The first of these was made up of 170 foliar phylloxera samples along with 4 previously phenotyped control strains. The foliar samples were collected between 2009 and 2011 from the Wolfskill Experimental Orchard (WEO), 2 fields sites and a greenhouse associated with UCD's Foundation Plant Services (FPS), and 6 commercial rootstock nurseries in Yolo and Solano. The four control strains, VinR1, AxRR1, 101R2 and FreeR2, have all been previously phenotyped for feeding behavior. These samples were analyzed using 10 Simple Sequence Repeat (SSR) markers, 5 of which we recently developed. The results demonstrate the clonal asexual aspect of the phylloxera life cycle. Of the 170 foliar samples analyzed, 147 samples fit into 9 genotypes. The only differences among eight of the nine groups were missing data points. The final group was also very similar; it was homozygous at one allele that was heterozygous in the rest of the groups. The groups were spread over all sampling locations, and sampling years. The remaining foliar samples showed little variation and grouped as a tight cluster. These were strong indicators that the foliar infestation started as a single introduction, and has maintained itself through asexual reproduction.

We also analyzed a large set of samples collected from the Oakville area. These samples were primarily from the UC Davis' Oakville Research Station. The collections were made in the summer of 2006, the winter of 2007, and the summer of 2011. A few additional samples from local vineyards were also included in this set, which included the source for the FreeR2 line. These samples were analyzed using a total of 12 SSR markers (6 newly developed in our lab). These results also indicated that phylloxera primarily has a clonal asexual life cycle. Of the 148 samples, 69 samples fall into 18 genotypic groups. These groups span all three collection times and across the different blocks at the Station. The samples from the Oakville area do show an overall larger range of diversity than did the foliar samples. Factorial analysis found that the samples split into three groups. These groups were supported by the genetic analysis software STRUCTURE.

The smallest of these groups was associated with the VinR1 and AxRR1 control samples. The Oakville samples found in this section come from the winter 2007 and summer 2011 collections. The winter 2007 sample are on two breeding selections (8909-05 and 9365-85), which are the only samples from these hosts. The final 5 members of this small group contain all samples collected from 110R. These hosts appear to be producing a selective pressure on the phylloxera to maintain this small group. The remaining two groups are represented in all three collection time, in all sampled blocks at the Oakville Station, and across the remaining hosts (101-14, 5C, 1103P, St. George and Freedom). These two groups appear to be closely related, but independent infestations. Both are equally adapted to the hosts available in the region. Therefore, their placement is dependent on which crawlers manage to survive the winters, and the random chance of their decedents being collected during sampling.

To follow up on diversity found in the UC Davis campus vineyard additional root-based samples were collected during the summer of 2012 by an undergraduate student, Bryan Ramirez-Corona. These samples were compared to the standard type A strain – VIN R1, the type B strain – AXR R1 and the newly discovered foliar strain. All three types were found in the campus vineyards and were scattered across the acreage but not associated with location or root host.

A final look at California genetic diversity came with a combined set that included samples from the Oakville area, Yolo county foliar samples, UC Davis campus samples, samples from Napa and Sonoma county commercial vineyards, and a few old foliar phylloxera samples collected in 1998 and 1999. This collection gave a broad overview of California phylloxera diversity. The results were consistent with the findings of each subset previously looked at. Again, the bulk of the Oakville area phylloxera split into two large groups. Most of the Napa and Sonoma county vineyard samples, along with the 101R2 and FreR2 samples correspond to these two groups. The same subgroup of Oakville samples still grouped with the AxRR1 and VinR1 controls. These were joined by the UC Davis campus samples described by Bryan. As a smaller subset was used the samples clustered in one group. A single sample from the Napa Sonoma set grouped very close to the VinR1. This sample was found on 1103P making it much different than the other rootstock samples found in this group. The foliar samples again formed a distinct group. A couple of the UC Davis campus samples joined this group, which supports Bryan's finding above. The only real surprise in this group was the groupings of the old foliar phylloxera samples from 1998 and 1999. Two of the old foliar samples grouped with the two large groups populated with the Oakville area samples. The final old foliar sample grouped with the UC

Davis samples in the AxRR1/VinR1 group. This gives the impression that the current foliar outbreak is of an entirely different origin than the older occurrences of foliar phylloxera.

**National Diversity –** We collected a large set of foliar phylloxera samples by stopping every 50 miles on a 3,000 mile wandering transect from 19 states across phylloxera's native range. Three or four adults from each plant were isolated and collected for DNA extraction. An initial analysis of one sample per plant was conducted using 32 SSR markers. Additional samples from California and 5 international commercial vineyards were included. The genetic analysis software STRUCTURE identified three large groups in this initial set. Figure 22 shows a factorial analysis with the groups highlighted. Each group is comprised of two smaller subsets of samples that are labeled. The results show the predominance of northeastern phylloxera on the wine growing regions represented in the study.

To test the diversity within a single site the additional DNA extractions from six diverse collection sites were tested. The resulting dendrogram is shown in Figure 23. The Arizona and Arkansas samples showed a low level of diversity with most samples being nearly clonal. Three of the remaining sites show a large level of diversity. In these sites only samples from the same plant were clonal. The final site in New York showed no clonal samples, implying that the sexual cycle predominates there. The excess of diversity in these sites showed that there is still a wealth of information to be gathered from the remaining unanalyzed samples. Currently all remaining national diversity samples are being run. It is hoped that this extra data can shed more light on phylloxera's native genetic diversity.

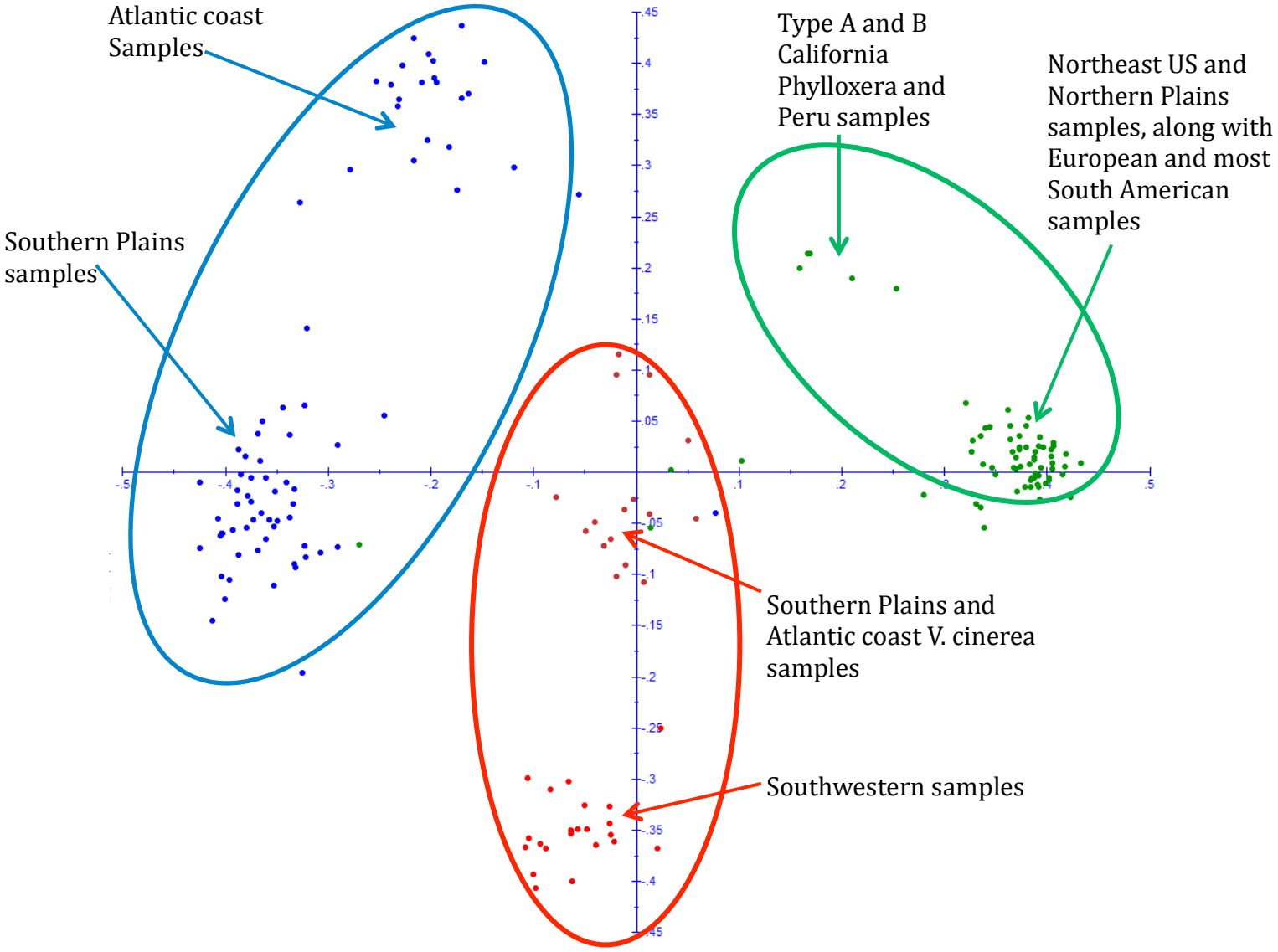



Figure 22. Factorial analysis of national phylloxera collections.

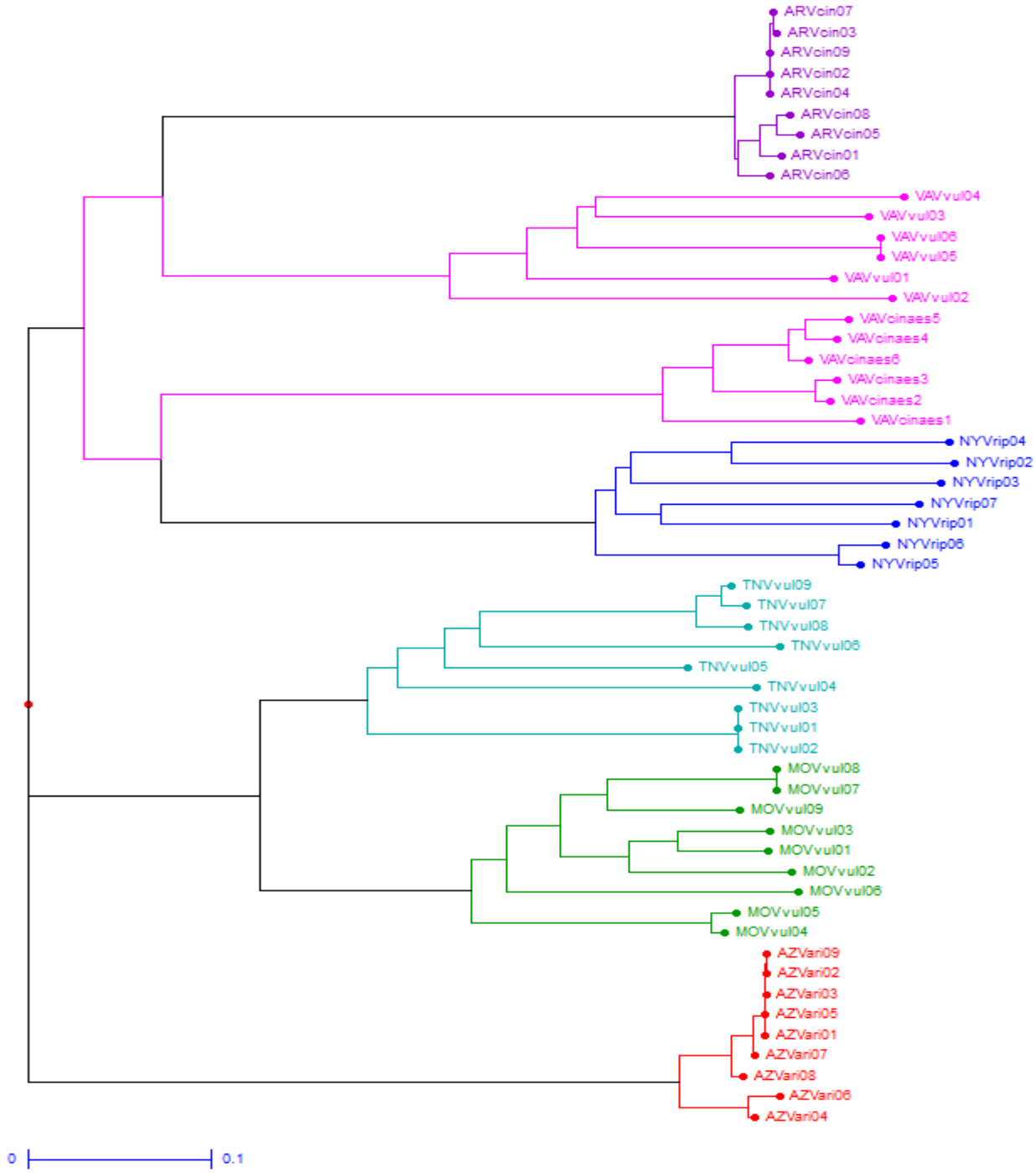



Figure 23. Dendrogram of a sub-sample of the National diversity samples. Arkansas samples are Purple, Virginia samples are pink, New York samples are dark blue, Tennessee samples are light blue, Missouri samples are green, Arizona samples are red.

**Rootstock Related Presentations December 2011 – January 2013**

Appropriate rootstock use. J. Lohr Winery Staff, Paso Robles, CA, December 19

UCD's grape breeding program. Wilbur Ellis Viticulture Team, Santa Rosa, CA, February 9, 2012.

Rootstock breeding and use issues. Napa Valley Vit Roundtable, Calistoga, CA February 27, 2012.

Breeding for pest and disease resistance. Ag Unlimited Annual Meeting, Napa, CA March 1, 2012.

Breeding resistant grapevines. Recent Advances in Viticulture and Enology, UC Davis, CA, March 15, 2012.

Current issues in grape diseases and pests. Wine Executive Program, UC Davis, CA, March 27, 2012.

Grape breeding for new rootstocks and winegrapes. University of Chihuahua, Mexico, March 29, 2012.

Breeding salt and drought tolerant rootstocks. Paso Robles Grower Association, J. Lohr Winery, April 11, 2012.

Issues to deal with when replanting vineyards. Paso Robles Grower Association, J. Lohr Winery, April 11, 2012.

Rootstocks 101. Texas Grape Growers Association, Lubbock, TX, April 27, 2012.

Establishing vineyards on rootstocks & Current breeding efforts. Texas Grape Growers Association, Lubbock, TX, April 27, 2012.

Understanding and using grape rootstocks. Lodi/Woodbridge Grape Day, Lodi, CA Mary 8, 2012.

Advances in scion and rootstock breeding. Sonoma Vit Tech Group, Santa Rosa, CA, May 16, 2012.

Overview of the Walker grape breeding program. Board of Visitors and Fellows, UC Davis, May 18, 2012.

Current research on fanleaf and new rootstock breeding. Napa Valley Sustainable Viticulture Seminar, Yountville, Mary 29, 2012.

What have we learned about rootstocks. Wooden Valley Grapegrowers Meeting, Suisun, CA, June 12, 2012.

Breeding programs from around the world: what is their likely contribution. ASEV Alternative Varieties Symposium, Portland, OR, June 19, 2012.

Nematode resistant rootstocks and crown gall. Roberts Integrated Viticulture Client Meeting, Santa Rosa, CA, July 9, 2012.

Advances in scion and rootstock breeding. Coppola Grower Meeting, Healdsburg, CA, July 17, 2012.

Vineyard issues. Calaveras Growers Meeting, Murphys, CA, July 27, 2012.

Advances in rootstock and scion breeding. Roll Global Meeting, UC Davis, August 16, 2012.

Breeding salt tolerant grape rootstocks. SCRI Meeting, Fresno, CA, August 23, 2012.

Nematode resistant rootstocks. International Fruit Genetics Meeting, Bakersfield, CA, August 24, 2012.

Breeding new rootstocks and fruiting varieties. VEN 290 Seminar, UC Davis, November 2, 2012.

101-14 and phylloxera. Roberts Integrated Viticulture Client Meeting, Santa Rosa, CA, November 29, 2012.

UC Davis grape breeding program. INIA Seminar, Santiago, Chile, December 13, 2012.

Development and management of rootstocks for table grapes. Hermosillo Table Grape Growers Association, Hermosillo, Mexico, January 25, 2012.

**Abstracts**

Osorio, C. G. and M.A. Walker. 2012. Anatomical variations related to drought tolerance in contrasting grape rootstocks. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts

Heinitz, C., and M. A. Walker. 2012. Continued screening for chloride exclusion in wild grapevines – new collections and genetic information. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts.

Agüero, C.B., S. Riaz, C-F. Hwang, R-R Hu, R. Hu, C. Bistue and M.A.Walker. 2012. Map-based cloning of Pierce's disease and *Xiphinema index* resistance genes from *Vitis arizonica*. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts.

Lund, K., S. Riaz and M.A. Walker. 2012. Genetic analysis of foliar phylloxera in northern California. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts.

Fort, K. and M.A. Walker. 2012. Developing a screen for assaying drought avoidance in *Vitis* rootstocks. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts.

Andrade, S., A. Nunez-Barrios, J. Martinez, D. Ojeda and A. Walker. 2012. Rootstocks and grape cultivars response to increasing soil water deficit in the northern part of Mexico. 63rd National Conference, American Society for Enology and Viticulture, Portland, OR. Technical Abstracts.

**Publications on Rootstock Related Topics**

Fort, K. and A. Walker. Breeding salt tolerant rootstocks. FPS Grape Program Newsletter Oct. 2011. Pp. 9-11

VanZyl, S., M.A. Vivier and M.A. Walker. 2012. *Xiphinema index* and its relationship to grapevines: a review. South African Journal of Enology and Viticulture 33: 21-32.

Riaz, S., R. Hu and M.A. Walker. 2012. A framework genetic map of *Muscadinia rotundifolia*. Theoretical and Applied Genetics 125:1195-1210.

Gambetta, G.A., C.M. Manuck, S.T. Drucker, T. Shanghasi, K. Fort, M.A. Matthews, M.A. Walker and A.J. McElrone. 2012. The relationship between root hydraulics and scion vigour across *Vitis* rootstocks: what role do root aquaporins play? Journal of Experimental Botany 63:6445-6455.

Aradhya, M., Y. Wang, M.A. Walker, B.H. Prins, A. M. Koehmstedt, D. Velasco, J.M. Gerrath, G.S. Dangl and J.E. Preece. 2012. Genetic diversity, structure, and patterns of differentiation in the genus *Vitis*. Plant Systematics and Evolution DOI 10.1007/s00606-012-0723-4

Ferris, H., L. Zheng and M.A.Walker. 2013. Resistance of grape rootstocks to plant-parasitic nematodes. Journal of Nematology (In Press).

Ferris, H., L. Zheng and M.A. Walker. 2013. Soil temperature effects on the interaction of grape rootstocks and plant-parasitic nematodes. Journal of Nematology (In press).

Fort, K.P., K.M. Lowe, W.A. Thomas and M.A. Walker. 2012. Cultural conditions and propagule type influence relative chloride exclusion in grapevine rootstocks. American Journal of Enology and Viticulture 64: (2) In press