California Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB)

2017 Research Progress Reports

Nursery Services Program

California Department of Food and Agriculture

Sacramento, CA 95814

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REPORTS

Progress report on a research grant proposal to: Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB)

January 30, 2018

Project Title: Study of the Effects of Little cherry virus-1 and Little cherry virus-2 on Different Cherry Rootstocks 170636000SA

Fiscal Year and Project Duration: Second year of a 4-year project

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Objectives:

1. To test a collection of plants by qRT-PCR to locate infected source material needed for the experiment.

2. To evaluate the effects of LChV-1 and LChV-2 on 16 different popular *Prunus* rootstocks. All rootstocks will be grafted with the same cherry scion cultivar, 'Bing'.

3. To test the inoculated plants in year 2 for the selected viruses and monitor the virus movement and record the symptom observation.

Accomplishments:

Objective 1:

In 2017 laboratory staff screened 35 FPS positive controls selections as well as accessions from the USDA ARS NCGR Wolfskill collection and UCD Plant Pathology Department Armstrong collection. All trees were tested by a RT-qPCR panel for 16 different viruses, including Apple chlorotic leafspot virus (ACLSV), American plum line pattern virus (APLPV), Apple mosaic virus (ApMV), Cherry green ring mottle virus (CGRMV), Cherry leaf roll virus (CL RV), Cherry necrotic rusty mottle virus (CNRMV), Cherry raspleaf virus (CRLV), Cherry virus A (CVA), Hop stunt viroid (HSVd), Little cherry virus-1 and -2 (LCV-1) and (LCV-2), Plum bark necrosis stem pitting associated virus (PBNSPaV), Prune dwarf virus (PDV), Peach latent

mosaic viroid (PLMVd), Prunus necrotic ringspot virus (PNRSV), and Peach Rosette Mosaic Virus (PRMV). Two selections were chosen to serve as the inoculation source for LChV-1 and LChV-2. We identified a single infection source of LChV-1 but were unable to do so for LCHV-2. The inoculation source that we selected is co-infected with CVA.

	Group ID	Disease Profile
Virus Positive Sample	11454	LChV1
Virus Positive Sample	13157	CVA, LChV2

Objective 2:

Material from both inoculation sources was independently T-budded onto four potted *P. avium* cv. Bing trees. The Bing trees were propagated using FPS rootstock seed and scion material and confirmed to be negative for all 16 viruses and viroids prior to inoculation. Six buds of virus positive inoculation material were T-budded onto each potted tree to ensure virus transmission.

Foundation Mahaleb and Mazzard seed was collected, stratified, and germinated for establishing rootstock seedlings to include in the trial. All other rootstock cultivars were received as a donation from Sierra Gold Nurseries. All rootstocks have been advanced to PropTek Deep Pots, staked pruned, and treated with dormant sprays.

The site for the field trial has been cultivated and drip irrigation lines have been installed.

Summary:

Little cherry disease (LCD), associated with Little cherry virus-1 (LChV-1) or -2 (LChV-2), is a common problem of cherries (*Prunus avium*) which occurs worldwide, causes unmarketable fruit and often results in tree or orchard removal (Jelkmann and Eastwell, 2011). Most of the new cherry rootstocks used in cherry production are interspecific *Prunus* hybrids which introduces an increased risk of an adverse reaction (hypersensitivity) to some viruses (Lang and Howell, 2001). Hypersensitive reactions exhibit graft union gum exudation, premature abscission, and tree death within one or two growing seasons and have been shown to occur in *Prunus* when infected with Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) (Howell and Lang, 2001, Lang and Howell, 2001, Lang et al., 1998). We propose to evaluate the effects of LChV-1 and LChV-2 on 16 different popular *Prunus* rootstocks. All rootstocks will be grafted with a scion variety from the same accession. Observations of budtake and tree performance will be recorded and evaluated for two years. Rootstocks will be rated for sensitivity to LChV-1 and LChV-2 and this information will be shared with growers and nurseries to assist in making rootstock selection decisions.

Project's Benefit to Nursery Industry:

In the US, sweet cherry fresh market production totaled 254,906 tons and was valued at $703 million in 2015 (NASS, 2017). Washington, California and Oregon account for more than 90% of sweet cherry industry in the US, with 34,786, 34,742, and 13,416 acres planted to sweet cherries in 2012, respectively (NASS, 2017). Interest in sweet cherry production has increased in recent years due to the high value of fresh market cherries and the increasing availability premium quality varieties and new rootstocks with exciting horticultural traits (Lang and Howell, 2001).

Little cherry disease is a concern to growers wherever cherries are grown. LCD is associated with LChV-1 or LChV-2, which can be found in single and mixed infections. Trees with LCD produce cherries of small size and poor color making fruit unmarketable. The problem results in unpicked limbs or trees, tree removal and even orchard removal. The disease is readily transmitted by grafting and LChV-2 is vectored by mealybugs (Jelkmann and Eastwell, 2011). To date, no breeding programs have been successful in finding resistance to the disease.

In orchards worldwide, cherries (*P. avium*) are either budded or grafted onto rootstocks. Rootstocks provide protection from soil-borne pests and improved tolerance to abiotic stresses, such as heavy soils, drought conditions, salinity, and cold winter temperatures, thus, increasing the survival of the scion material. Traditionally, cherries in the US were grown on Mazzard or Mahaleb rootstocks or clonally-propagated 'Colt' which are generally tolerant of infection by pollen-borne viruses, PDV and PNRSV (Lang et al. 1998). It has been increasingly well-documented that new *Prunus* rootstock selections can show hypersensitive reactions to viruses that have been typically well tolerated by traditional rootstocks (Lang et al. 1997, Lang et al. 1998, Lang and Howell 2001, Howell and Lang 2001). These new rootstock selections are derived from species other than or are hybrids with *P. avium* which offers genetic diversity and novel horticultural traits, but with an increased risk of hypersensitivity. Hypersensitive (rapid and lethal) reactions exhibit graft union gum exudation, premature abscission, and tree death within one or two growing seasons. Viruses with documented hypersensitivity include PNRSV and PDV (Howell and Lang, 2001). It is not currently known if LChV-1 and LChV-2 can cause similar hypersensitive reactions in the common *Prunus* rootstocks.

We plan to conduct a field trial to investigate hypersensitivity reactions to LChV-1 and LChV-2 in the top *Prunus* rootstocks. Currently, we anticipate using GiSelA®3, GiSelA®5, GiSelA®6, GiSelA®12, Krymsk®5, Krymsk®6, Krymsk®7, EMLA Colt,' MaxMa®14, Cass, Clare, Clinton, Crawford, Lake and seedlings of Mazzard and Mahaleb in the trial. We will assess the sensitivity of these rootstocks to LChV-1 and LChV-2 and share the results of our research.

This research has a great benefit to the cherry growing industry as the results of our research will assist growers and nurseries in rootstock selection for new plantings. Informed rootstock selection will result in healthier, more productive cherry trees.

Progress Report - Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB)
(Note: This is a joint report for two related and collaborative projects)

Project Titles:
1) Integrated management of Fusarium canker in bareroot nursery stock and container-propagated stone fruit trees (Bostock, Lead PI)
2) Managing the water relations of bareroot nursery stock to improve establishment, performance, and disease resistance (Shackel, Lead PI)

Fiscal year: 2017-18 (Year 2 of 2)

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Personnel: Junior Specialist, **Randy Kuffel**, is supported by the IAB funds allocated to the two projects, and is primarily responsible for conducting the experiments.

Introduction

The overall focus of these related projects concerns the health of bareroot nursery trees during cold-storage and after planting. Our research seeks to clarify the relationship between tree water status and development of a fungal canker disease that primarily occurs in bareroot stone fruit trees. Related research within these projects seeks to identify management options that can be implemented by the California fruit and nut tree nursery industry. Nurseries have experienced sporadic losses to a canker disease caused by opportunistic fungi that attack young trees weakened by stress. The disease can occur in dormant bareroot trees maintained in cold storage in refrigerated warehouses, with disease signs and symptoms developing during storage or soon after planting. Diseased trees display molds growing on the bark and roots, and necroses of the inner bark, cambium and sapwood, which girdle and kill the trees. Weak establishment in new plantings also may be associated with the presence of these pathogenic fungi on roots, and infected but non-symptomatic dormant trees can develop symptoms later and collapse in the field. Our previous research demonstrated the involvement of five opportunistic fungal pathogens: *Fusarium avenaceum*, *Fusarium acuminatum*, *Fusarium brachygibbosum*, *Cylindrocarpon obtusiusculum*, and *Ilyonectria robusta* (**Fig. 1**)[1]. Loss of bark turgidity in almond stem segments due to desiccation stress correlates with

[1]Marek, S. M., Yaghmour, M. A., and Bostock, R. M. 2013. *Fusarium spp.*, *Cylindrocarpon spp.*, and environmental stress in the etiology of a canker disease of cold-stored fruit and nut tree seedlings in California. *Plant Dis*. 97:259-270

significantly increased susceptibility. In addition, pathogenic *Fusarium spp.* can be isolated from every aspect of the production system including symptomatic and non-symptomatic almond bareroot trees, budwood, wheat rotation cover crops and residues in nursery fields, cold storage facility air and surfaces, and nursery equipment. In two nurseries, genetic testing confirmed that isolates from the various sources were highly genetically similar, suggesting these materials are potential inoculum sources (manuscript in preparation). The overall objective of this research is to implement an integrated disease management plan based on our findings. Our collaboration also seeks to determine more precisely water status of bareroot trees during processing and storage, and the relationship to tree establishment, performance and disease.

With these considerations, the **objectives** of this research are as follows:

Project 1.

1. Evaluate alternative cover crops to wheat for use in rotation with trees in nursery production fields.
2. Conduct a systematic survey of the incidence of pathogenic *Fusarium* species in almond budwood, bareroot and container trees at several nurseries and in newly planted and mature orchards.
3. Document the role of stem water potential (SWP) in tissue susceptibility to *Fusarium* spp.
4. Evaluate fungicide treatments for efficacy in managing the canker disease.

Project 2.

1. Compare the sensitivity and reproducibility of the stem water potential method (SWP) to the only other previously published method for measuring the water status of dormant tissue (relative bark water content, RWC).
2. Conduct an initial survey with cooperating nurseries, documenting the effects on SWP at each step of current nursery handling practices, from digging through storage and delivery.
3. Document the effects of contrasting levels of SWP on tree establishment in the orchard.
4. Document the role of SWP in tissue susceptibility to *Fusarium spp*. (same as Objective 3, Project 1)
5. Assess practical steps or treatments designed to avoid the occurrence of desiccation that leads to low SWP during handling of bareroot nursery material

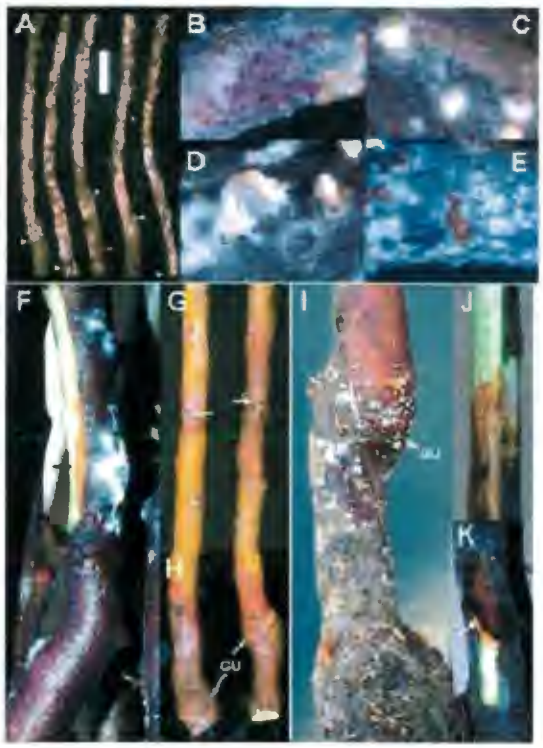

Fig. 1. Various symptoms of the cold-storage canker disease in almond and apple tree. (from *S. M. Marek et al. (2013) Plant Dis. 97:259-70.*)

PROGRESS ON PROJECT OBJECTIVES AND PLANS

Project 1.

1. Evaluate alternative cover crops to wheat for use in rotation with trees in nursery production fields.

We initiated small-scale experiments under greenhouse conditions to assess survival and persistence of soilborne inoculum of *F. avenaceum* as this species is commonly isolated and highly virulent in disease assays with almond branches. After conferring with nursery staff and cover crop experts, we selected two

mustard species (*Sinapis alba* and *Brassica juncea*) to compare with wheat (*Triticum aestivum* cv. Patwin 515). The latter is a known host for the *Fusarium* species of interest and used in nursery production fields, and highly genetically similar isolates of the pathogen occur in both the wheat material and nursery trees. We are conducting small-format experiments under highly controlled conditions to see if there is benefit of the mustards in terms of depressing, or at least not further enriching, soilborne populations of the pathogenic *F. acuminatum* relative to the wheat. We are in the process of analyzing the number of CFUs (Colony Forming Units) in each sample's soil. This will provide suggestive evidence on the efficacy of each cover crop treatment in decreasing *F. acuminatum* pathogen load in the soil. We have also begun additional replicate experiments with larger sample sizes and higher densities of plant cover in each pot. We expect to end these experiments in summer 2018.

2. Conduct a systematic survey of the incidence of pathogenic *Fusarium* species in almond budwood, bareroot and container trees, and clonal rootstock at several nurseries and in newly planted and mature orchards.

These studies are ongoing and will continue during the course of the current and new fiscal year. During 2016, we sampled healthy-appearing cuttings of clonal rootstock material used for propagation of Myrobalan 29C, a plum rootstock commonly used with various stone fruits. These fresh cuttings were subjected to the ONFIT (overnight freezing induction test) to allow for out-growth of potentially latent infections. In a sample of over 100 cuttings, no *Fusarium* species were detected. Only apparently saprophytic species were cultured: *Phoma* spp., *Alternaria alternata*, *Alternaria* sp., and *Epicoccum nigrum*. These determinations were based on morphology in culture and ITS sequences. During 2017, we sampled healthy-appearing cuttings of almond budwood material from cvs. Aldrich, Monterey, and Nonpareil mother trees. These fresh cuttings were subjected to the ONFIT. In a sample containing 28 cuttings from Aldrich, 30 cuttings from Monterey, and 28 from Nonpareil, no *Fusarium* species were detected. In addition, we collected extensive soil and dried cover crop stubble from two nurseries in 2017. Here, we subcultured onto *Fusarium* selective media. We are still working to complete the molecular analyses to confirm *Fusarium* species.

3. Document the role of stem water potential (SWP) in tissue susceptibility to *Fusarium* spp.

This objective seeks to establish more precisely the relationship between host susceptibility and branch water status by measuring SWP with a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA). Our previous work showed that relative water content (RWC) of the bark (or bark turgidity), a traditional measure used in disease predisposition research in trees, was an important factor in almond susceptibility to *Fusarium* spp. SWP is a more contemporary and precise measure of tree water status. It will be important to establish how RWC and SWP values compare, as well as the relationship of SWP to host susceptibility. SWP measurement with a pressure bomb also could afford nursery technical staff a means to monitor tree water status during cold storage or during other stages of operation. The outcome of recent and ongoing experiments are discussed below in more detail within Project 2, objective 1.

4. Evaluate fungicide treatments for efficacy in managing the canker disease.

Our previous research found several fungicides currently registered for use on almond to be particularly effective *in vitro* and *in vivo* against the *Fusarium* pathogens of concern. These are fludioxonil (Scholar), fluopyram/trifloxystrobin (Luna Sensation), and fluxapyroxad/ pyraclostrobin (Merivon). We conducted an experiment at a commercial nursery on how certain almond varieties respond to fungicide treatments. The varieties were Nonpareil on Lovell root, Monterey on K86 root, Fritz on Lovell root, and NePlus on Lovell root. The fungicide application on bare-root trees included Merivon (6 oz/100 gal) and Aliette (3.2 oz/100 gal). After conducting ONFIT on the fungicide-treated and untreated control bareroot trees, we found relatively equal amounts of *Fusarium* species between treatments, including *Fus. proliferatum*, *Fus.*

avenaceum, *Fus. acuminatum*, *Fus. oxysporum*, and *Fus. equiseti*. In addition, we found equal amounts of *Neonectria* species as well as *Cylindrocarpon* between treatments. Between all varietals and treatments, we found *Fusarium proliferatum* the most frequently. We plan to continue experiments of directly applied fungicides on infested branches in spring, 2018.

Project 2.

1. Compare the sensitivity and reproducibility of the SWP method to the only other previously published method for measuring the water status of dormant tissue (RWC, relative bark water content).

Stem water potential (SWP) is a quick and reliable method to determine the water status of dormant xylem tissue. However, after a series of experiments, we have evidence that relative bark water content (RWC) is not easily translated into a measure for xylem water status. Although our initial data suggested that SWP could be an indicator of RWC, subsequent analyses indicate that this relationship is more complicated. Our research shows that the relationship between SWP and RWC varies throughout the course of the year (**Fig. 2**). In the winter months, we see branches at low RWC despite high SWP, suggesting that the hydration capacity of almond bark changes, perhaps due to anatomical differences.

Our initial method for determining RWC, called the bark disc method, may have exaggerated our findings of low RWCs despite high SWPs. This is due to two main problems: 1) The cork borer used in the bark disc method partially crushes bark tissue and squeezes water out of fresh samples, resulting in low fresh weights, and 2) lengthy and non-standardized hydration times impart high imbibed weights. Both of these factors potentially can yield low RWCs (**see equation below**). However, this method fails to account for the significant differences in slope and intercept that we see between the regressions in **Figure 2**.

$$\frac{Fresh\ Weight - Dry\ Weight}{Imbibed\ Weight - Dry\ Weight} * 100 = Relative\ Water\ Content\ \%$$

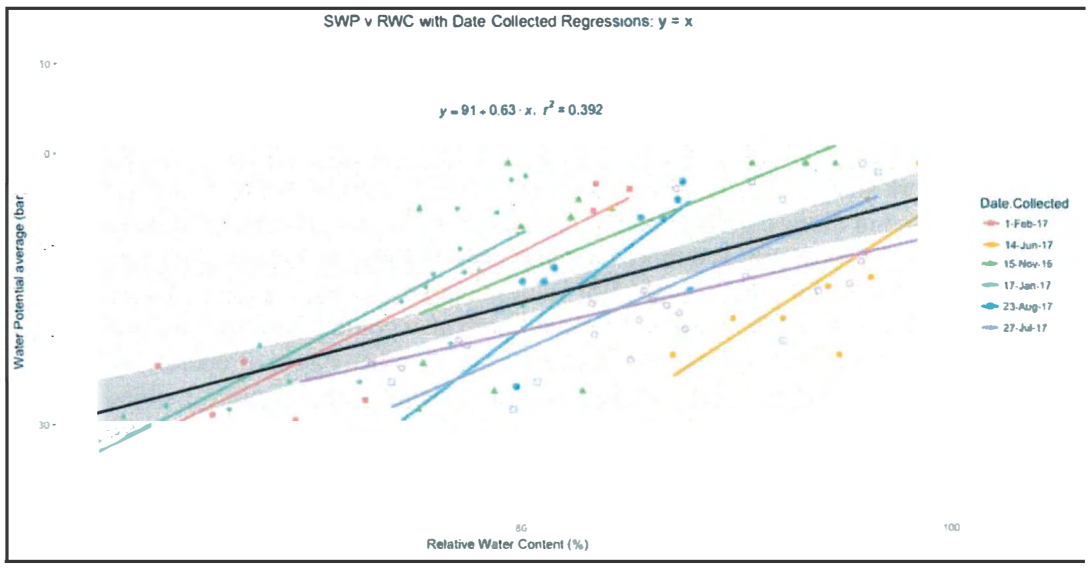

Fig. 2. Relationship of RWC and SWP during January to August, 2017. Monthly stem water potentials (SWP) and bark relative water content (RWC) in almond branches (cv. Nonpareil) allowed to dry for various periods at room temperature. The solid black line is a linear regression of the composite data from all sampling dates, described by the equation RWC = 91 + 0.63(SWP), r^2 = 0.392. SWP determined with a pressure bomb and RWC determined as described in Marek et al (2013) Plant Dis. 97:259-70

We have developed an alternative method for determining relative water content. This method uses razor blades to gently excise bark tissue without losing fresh weight, and has a standardized hydration time of two hours. However, we still see low RWCs despite high SWPs (**Fig. 3**). This suggests that the anatomy of almond bark is more complex than we initially suspected. We have begun monthly sectioning of bark (**Fig. 4**) to assess by microscopy if there are seasonal differences in the cellular architecture that may help explain the variation we see in SWP and RWC. This work began in January 2018, and will continue through August 2018.

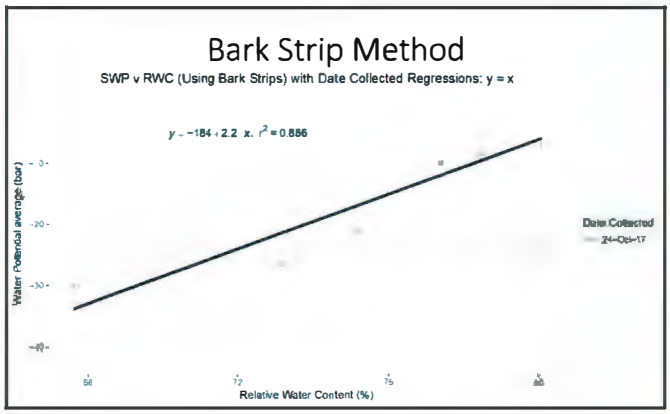

Fig. 3. Relationship of RWC and SWP using the new bark strip excision method.
SWP = -184 + 2.2(RWC), r^2 = 0.886

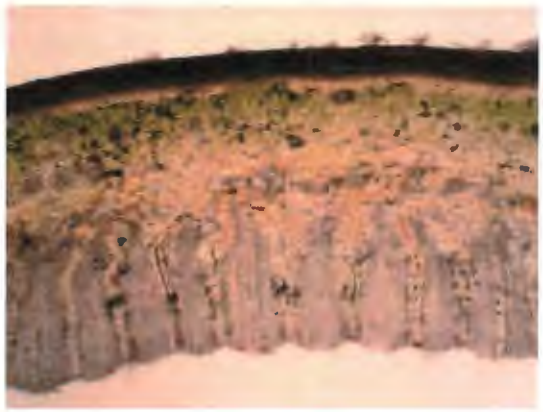

Fig. 4. Cross-section of Nonpareil almond bark obtained using a vibrating microtome and sampled on March 5, 2018.

2. Conduct an initial survey with cooperating nurseries, documenting the effects on SWP at each step of current nursery handling practices, from digging through storage and delivery.

Prior work conducted by Dr. Shackel established that there is variation in SWP in bareroot trees within the nursery production chain and within cold-storage. There are logistical and resource challenges in conducting a more thorough study of this nature; however, we think the tree establishment experiment (objective 3 below) addresses this issue adequately.

3. Document the effects of contrasting levels of SWP on tree establishment in the orchard.

In April 2017, we received 30 bareroot trees of Aldrich on Krymsk 86 directly from cold-storage from a commercial nursery. The trees were protected from dehydration by bagging them in plastic during transit (approximately 1 hour). They were brought to the lab and allowed to hydrate overnight in the plastic bags. To create contrasting levels of SWP, we stressed 15 trees by opening the bag and allowing them to dry at ambient temperature and humidity indoors for two days, while the remaining 15 trees remained bagged and hydrated for two days at ambient temperature. After these treatments, we close-planted all trees in five randomized blocks of six trees each (three stressed and three hydrated) at the UCD Armstrong Field Research facility. Before planting, all stressed trees sampled had SWPs below -28 bars, while all hydrated trees sampled had an average SWP of -5 bars. To evaluate time to leaf out and mortality rates, we allowed the trees to grow for four months. All trees were placed on drip irrigation and received the same watering regime during the course of the experiment. On average, all control trees leafed out within 27 days, while the living, stressed trees leafed out later but within 50 days. Out of 15 control trees, 13 were alive after four months, while only 7 of the 15 stressed trees survived.

4. Document the role of SWP in tissue susceptibility to *Fusarium* spp. (same as Objective 3, Project 1, see above).

5. Assess practical steps or treatments designed to avoid the occurrence of desiccation that leads to low SWP during handling of bareroot nursery material.

We will assess this during spring and summer of 2018.

Publications. In earlier reports, we noted the publication of a peer-reviewed paper on this disease in the highly respected journal *Plant Disease* (*S. M. Marek et al. (2013) Plant Dis. 97:259-70*). Mrs. Abigail Stack (neé Seidle) completed her M.S. degree and thesis in Plant Pathology (2016)[2], presented a poster of her research findings at the 2015 Annual Meeting of the American Phytopathological Society[3], published a disease note in *Plant Disease*[4], and two manuscripts are in preparation for journal submission.

Acknowledgments

We thank Prof. Judy Jernstedt, UC Davis Plant Sciences Department, for advice and assistance in the anatomical studies of bark.

[2] Seidle, A.J. 2016. Etiology, Epidemiology, and Management of *Fusarium* spp. Causing Cryptic Cankers in Cold-Stored, Bare-Root Propagated Almond Trees in California Nurseries. M.S. thesis. University of California, Davis. 81 pp.

[3] Seidle, A., Gordon, T.R., and Bostock, R.M. 2015. Etiology and management of *Fusarium* spp. causing cryptic cankers in cold-stored, bare-root propagated almond seedlings. Phytopathology 105 (Suppl.4):S4.125 (abstr).

[4] Stack, A.J., Yaghmour, M.A., Kirkpatrick, S.C., Gordon, T.R., and Bostock, R.M. 2017. First report of *Fusarium brachygibbosum* causing cankers in cold-stored, bare-root propagated almond trees in California. Plant Dis. 101: 390.

Final progress report on a research grant proposal to: Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB)

August 2018

Project Title: Optimization of meristem-tip culture methodologies for virus elimination for Prunus cultivars 170651000SA

Fiscal Year and Project Duration: Second year of a two year project

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Executive Summary for Project;

The framework of the tree virus eradication program is basically set for the selected/researched varieties of cherries, almonds, and peaches. However, high genetic diversity in the FPS tree material substantiates further media optimization for specific cultivars and potentially different developmental phases (initiation, maintenance, etc.).

- **Cherry and almond** projects are at the last phase of research with repeated data sets. To complete the manuscripts, final results for PCR tests from rooted plants are needed. Some explants are large enough for preliminary PCR.
- Meristem culture of *Prunus* tree varieties seem to go through distinct developmental phases that may require different medium recipes (in most cases: establishment, elongation, and rooting). Within the same group of *Prunus* (e.g. cherries), medium preferences can be can vary. Media customization is needed depending on cultivars and phases.
- Explants are stressed during the in vitro establishment phase and also when heat treated. This combination of stresses may affect overall thermotherapy efficiency (e.g. acclimatization period was too short). There is evidence that acclimatized cuttings may tolerate high temperatures for a longer periods. However, it may take several months for the explants to be fully acclimatized before heat treating.
- Multiple virus eradication procedures (direct meristemming from field-grown trees, indirect meristemming from in vitro shoots, thermotherapy in combination with grafting) were tested on infected almonds (3 cultivars) and cherries (5 cultivars). Explants from these experiments are under further evalution.
- **Peach** meristems from field-grown trees were successfully established on a series of media.

Objectives:

1. Develop meristem establishment media and culture methods for *Prunus* cultivars from the FPS fruit tree collection

2. Develop shoot elongation media for established shoots from micro-shoot tips

3. Develop rooting procedures for meristem-derived shoots

4. Evaluate effectiveness of different virus elimination procedures using RT-qPCR and HTS

Accomplishments:

Accomplishments are described according to cultivar type since each taxonomic group of *Prunus* cultivar has unique needs for media, environment, and horticultural strategies.

PROJECT 1: CHERRY MERISTEM-TIP CULTURE AND CVA ERADICATION:

Disease status of five non-symptomatic cherry cultivars (*Prunus* spp.) were tested using a panel including eight biological indicators and 21 assays (ELISA and PCR). Cherry virus A (CVA) was detected in four out of five cultivars. Lateral shoots from the CVA-negative cherry trees (*Prunus lannesiana* cv. 'Krymsk ®7, or P2G9) were used as an explant source for media optimization and meristem culture experiments. A virus eradication via meristem culture was tested on the infected cherry cultivars. In vitro-derived meristems exhibited higher survival rates on different media.

Current status: Meristems from P2G9 cherry, a non-infected model cultivar, were established on severeal growth media by two methods (1) directly from the field or (2) from in vitro cuttings. In this cultivar, meristem survival was higher when were established from in vitro shoots (100% survived, 240 meristems tested in 6 medium recipes). Initiation, regeneration, and rooting were all performed using one medium for this cultivar – additional optimization does not appear necessary.

For four CVA+ cultivars, the efficiency is varied. Meristems of two cultivars can be excised directly from field-grown trees. 23 of the 36 English Morello meristems grew plantlets with shoots and leaves. (64%). For Montmorency, 13 of 36 meristems grew shoots and leaves (36%). For these two cultivars, the shoots are ready for rooting inducement. Both meristem and shoot explants from the other three cultivars (Lapins, Black Republican, and Lambert) did not respond to MSB for some unknown reasons – explants declined without any signs of growth.

Some of the tested cultivars had bacterial endophytes, resulting in high contamination percentages for of those explants. This contamination issue poses a potential complication APHIS method due to the cutting establishment requirement.

1. **Zeatin does not enhance meristem initiation in P2G9, (neither in vitro nor field-derived meristem).**

This experiment indicated that zeatin does not enhance meristem initiation success, while our standard media (MSB) performed best. The first run of this experiment have been completed.

Table 1: *Effect of zeatin on cherry in vitro meristem initiation*

Media	Number of meristem	%Survive	% shoot	Average shoot size (mm)
MS + 1.1 mg/L BA	40	100	50	10.2
MS + 0 mg/L Zeatin	40	45	25	5.2
MS + 0.1 mg/L Zeatin	40	70	35	8.0
MS + 0.2 mg/L Zeatin	40	50	15	5.0
MS + 0.5 mg/L Zeatin	40	85	35	5.6
MS + 1 mg/L Zeatin	40	45	15	6.7

Fig*. Effects of zeatin on in vitro meristem survival*

2. **P2G9 meristems perform best when established from in vitro cuttings (100% survive + 2 months).** Adding this step (+1-3 months) can completely reverse the results. As a part of the experiment, cherry meristems from field-grown trees were excised on the same set of media and none survived. In addition to the finding that that P2G9 can be established on other media (MSmTFe or WPMmTFe), this experiment indicated that meristem survival rate can be improved significantly when meristem is excised from in vitro plants.

3. **Agar concentration significantly affects in vitro cherry shoot growth. Reduction of agar concentration may potentially improve meristem survival rates of other crops.**
Shoots elongated on standard MSB exhibited chlorosis, shoot tip necrosis, stunting, and hyperhydricity. Supplementmention of MS with meta-topolin and FeEDDHA significantly reduced this problem. Increased ventilation of the culture vessel showed potential to increase leaf

size, but resulted in unacceptable rates of moisture loss in the culture medium. The reduction of agar concentration appears to mitigate this issue of media desiccation.
Media solidified with 6g/L agar reductions in cherry shoot growth, fresh weight, dried weight, and shoot height. **Decreased concentrations of agar to 4g/L significantly improved explant growth.** This experiment was repeated.

***Table 2**: Effect of agar concentration on in vitro cherry after 5 weeks*

Medium	Height (mm)	Fresh weight (mg)	Dried weight (mg)	Leaf number	Shoot number
MsmTFe 4g/L agar	35.7	528	67.8	30.4	4.0
MsmTFe 5g/L agar	25.9	297	39.7	21.0	3.4
MsmTFe 6g/L agar	25.9	270	48.6	17.9	2.5

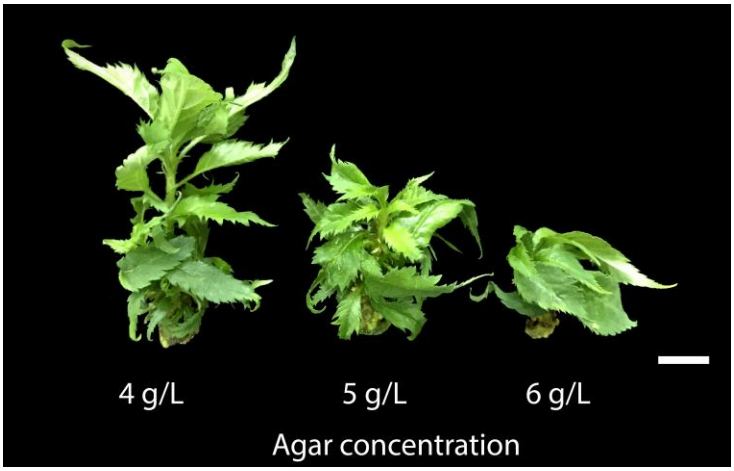


***Fig**. Standard agar concentration 6 g/L inhibited in vitro shoot growth in Cherry P2G 9-01*

4. **Cherry in vitro thermotherapy is complicated due to endophytes.** Montmorency trees are infected with endophytic bacteria (30-40% contaminated). Multiple trials were conducted to ramp up the number and the endophyte contamination level were different from tree to tree.
5. **Cherry in vitro thermotherapy may require long acclimatization period.** Most cultivars decline precipitously if subjected to thermotherapy immediately following establishment in media. A buffer period of approximately 2 weeks at 23° C may potentially improve survival during heat treatment.

***Table**: Establishment of cherry cuttings under high temperature was affected by endophytic contamination and different heat tolerance thresholds*

Cultivars	Location	Total # of Cuttings	# of Contamination	% Survival (under 30°C)
English Morello	GOH B12 9-12	41	2	40%
Lapins	GOH B12 19-17	41	6	0%
Montmorency	GOH B13 1-4	36	13	25%
Lambert	GOH B13 33-36	41	0	9%
Black Republican	GOH B14 1-4	41	1	11%

6. **To evaluate the protocol, five CVA-positive cherries were established with varying success (0-64% survival).** However, due to conformation to a rigid set of methodology for publication, no modification was made to improve survival rate of Lapins, Lambert and Black Republican. These cultivars did not respond to MSB at either 26°C or 30°C. Media optimization needs to be investigated, but initial survival rates could be improved with MSmTFe or WPMmTFe.
Table. *Meristem establishment efficiency of five cherry cultivars, and how cuttings respond to the media. Meristems excised in Summer 2017*

Cultivars	Location	Total # of Meristem	# of Living Plants	In vitro Cuttings' Status
English Morello	GOH B12 9-12	36	23	(Did not need to try)
Lapins	GOH B12 19-17	36	0	Cuttings did not respond to MSB
Montmorency	GOH B13 1-4	36	16	(Did not need to try)
Lambert	GOH B13 33-36	36	0	Cuttings did not respond to MSB
Black Republican	GOH B14 1-4	36	0	Cuttings did not respond to MSB

Among the infected cherry cultivars, Lapins, Lambert, and Black Republican did not grow well on MSB. Their cutting were established as another source of meristems. However, these cutting were either contaminated with endophytes or did not respond to MSB.

Fig. *English Morello(left) and Montmorency (right) (CVA positive) meristem-derived plants. The meristems were excised in the summer 2017 as a part of the experiment. Survival rates is 64% and 45%, respectively*

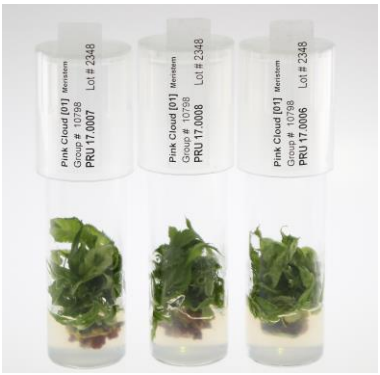


***Fig**. Pink Cloud (CVA positive) meristem-derived plants. The meristems were excised in the Summer 2017 by Waclawa Pudlo using the cherry procedure. Survival rate is 55% (20 plants/36 meristem).*

Current cherry meristem culture protocol:

1. Collect only apical shoots as meristem source from field or potted plants
2. Excise meristems onto three media WPMmTFe, MSmTFE, or MSB. Culture at 23°C, 16/8hrs photoperiod, 35-50 $\mu molm^{-2}s^{-1}$ for 2 week, transfer once.*
3. After a month, transfer to maintenance media (MSmTFE or to-be-developed) under normal light intensity 80-100 $\mu molm^{-2}s^{-1}$. Some cultivars can be very sensitive to ethylene accumulation; tubes with larger plants should be vented to prevent hyperhydricity.*
4. Repeatedly transfer on maintenance media until large enough for rooting (variety-dependent)
5. If not rooted by itself, transfer to rooting media (to be developed) for 1 week in isolation frm light
6. Transfer from rooting media to MS or Rose 16.9 without BA

* If all the established meristems do not survive. It should fall into our two case studies (P2G 9-1 or Lapins)

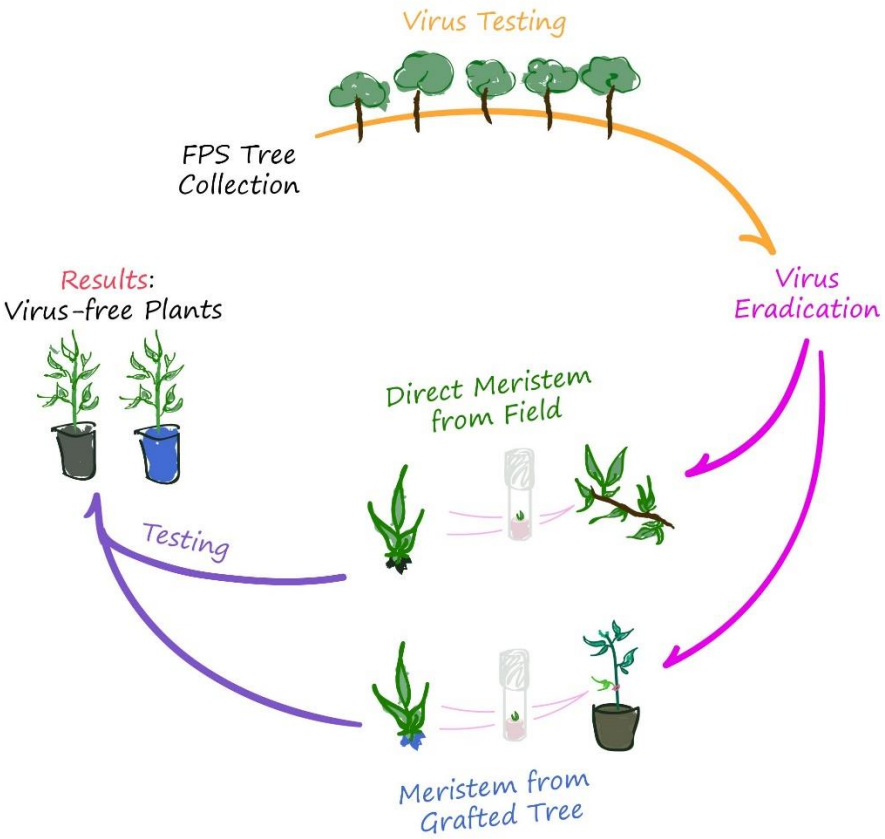
- Sterilize cuttings with FPS standard protocol (P001) then excise meristems from in vitro shoots.
- If endophyte is a problem, excise large meristems to get around endophytes, then excise meristem from that in vitro shoot explants.
- If both the meristem and cutting explants are not responding, then media optimization should be conducted with focus only on basal salt (MS, DKW, QL, WPM) and sugar (sucrose, fructose, glucose)

PROJECT 2: MEDIA OPTIMIZATION FOR MERISTEM CULTURE OF ALMOND

Abstract:

Virus detection and elimination procedures using meristem culture were successfully developed and evaluated on almond (*Prunus dulcis*) cultivars. *Prunus dulcis* 'Nonpareil' was used as a model for meristem culture development. In vitro shoots and meristems were evaluated on different media, including Quoirin and Lepoivre (QL), Murashige and Skoog (MS), Driver & Kuniyuki (DKW), McCown's woody plant medium (WPM), or Chu-N6 medium. WPM supplemented with meta-Topolin, Fe-EDDHA, and 4g agar proved to be the most effective for meristem culture while MS was most suitable for cutting establishment and shoot culture. In vitro shoot establishment was affected by position on the branch and tree, although this effect was not seen in meristem culture. The meristem culture procedure was evaluated on *P. dulcis* 'Bennet Hickman', 'Fowler', and 'P2G#9-11'. Meristems from different sources of material (field grown trees, grafted and heat-treated plants) were tested for virus infection statuses using qPCR.

Personnel involve: Hoang Nguyen, Maher Al Rwahnih, Erin Hsu, Ninh Khuu, James Shoulders, Sue Sim, Josh Pucket, John Preece, Deborah Golino


Virus Testing
FPS Tree Collection
Virus Eradication
Direct Meristem from Field
Meristem from Grafted Tree
Testing
Results:
Virus-free Plants

Current status: Although cutting establishment was difficult and complicated, Nonpareil almond meristems can be established consistently using both lateral nodes and apical nodes on WPMmTFe. A meristem establishment procedure (not including rooting) was developed and tested on three FPS cultivars: P2G #11-01 (Peach/Almond Hybrid), Bennett-Hickman (Almond), and Fowler (Almond).

All three tested cultivars have live plants. P2G11 already has 1 plant to soil that is awaiting testing. This plant was excised directly from the tree into WPMmTFe media during Spring 2017. There are more meristem-derived plants that were excised from heat treated scions. Those plants are expressing mild to severe signs of decline but can hopefully be maintained to the end. This number of explants is not large enough to test other media.

Future plan:

- Optimize maintenance media, focusing on media salts (MS, QL, WPM, DKW, or mixed) and sugar (sucrose, glucose, fructose, or mixed)
- Test rooting procedures with dark treatments

1. **Nonpareil almond can be established with a 57% success rate on WPM supplemented with meta-Toplin, FeEDDHA and solidified by 4g/L agar. This result could potentially be improved if we change our material preparation practice (only select apical shoots).**
Among five tested media, WPM was the best one for meristem initiation. However, this media promote branching. This is true for many other species. Media with lower salt levels are better for meristematic tissue regeneration, but shoot growth usually involves a medium with higher salt concentrations.

***Table**. Effects of media on Nonpareil meristem growth and survival rates after a month (combined data from both Spring and Summer trial)*

Media	Number of meristem	Number of shoots	% Success	Shoot size	Shoot number
QL	30	16	53	-	+
MS	30	11	37	+	+
1/2xMS	29	13	45	+	+
WPM	30	17	57	++	++
DKW	30	15	50	+	+
Chu N6	30	0	0	-	-

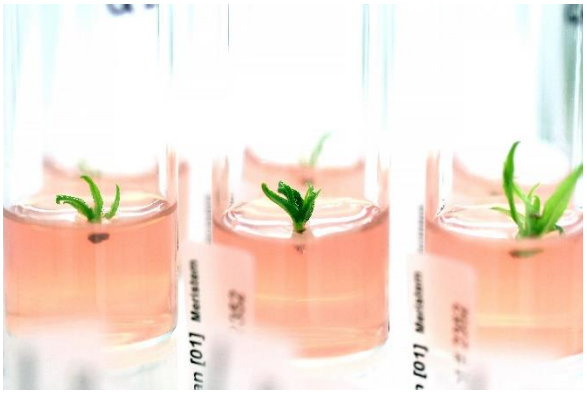


***Fig**. Nonpareil almond meristem on WPM supplemented with 0.5 mg/L mT 0.1 mM FeEDDHA and solidified by 4 g/L agar*

Because WPM is a very low salt media, we suspected that ½ x MS would provide the same effects. This hypothesis were tested in order to simplify the protocol. However, ½ x MS did not seem to be as good as WPM, at least for Nonpareil.

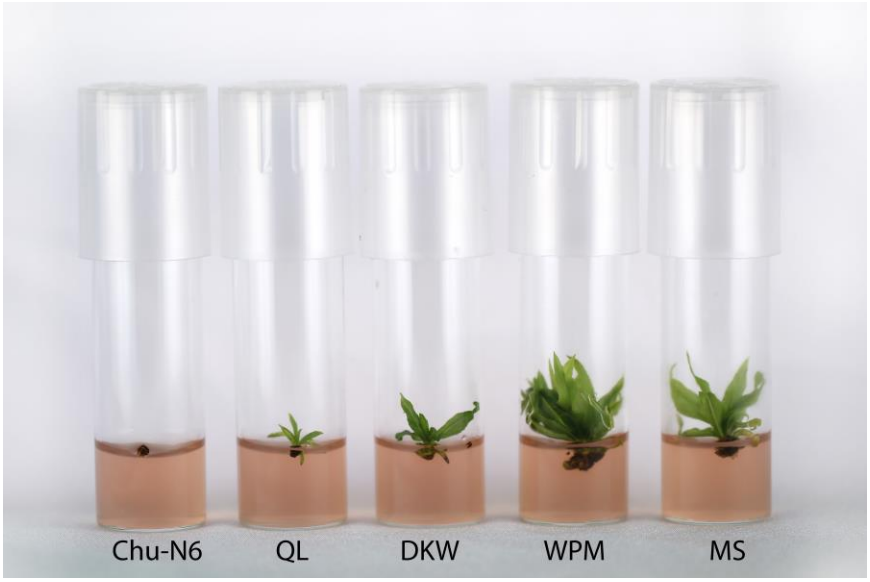


***Fig**.Almond meristem on different basal salt recipes supplemented with 0.5 mg/L mT 0.1 mM FeEDDHA and solidified by 4 g/L agar*

2. **Nonpareil cuttings were best established on MS based media. However, multiple trials on FPS material slowly indicated that this media is still not optimal for long-term performance.** Lateral nodes were used as an explant source for elongation stage media optimization. The results are very interesting: if meristems from previous experiment can be excised onto most media, especially WPM, the low performance of WPM in this experiment clearly indicated that there are inhibitors surrounding the meristem tip.

- Shoots collected from the top and the bottom perform differently. Explants should be collected from above-shoulder height (>5ft), from branches that are growing upward.

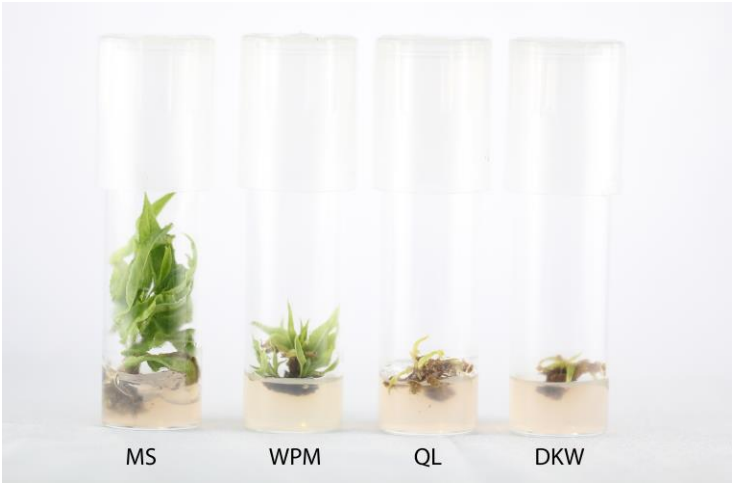


***Fig**. Almond shoot cultures on different media*

3. **Season and cutting location, in combination, may have tremendous impact on cutting establishment due to endogenous inhibitors. Therefore, in vitro cutting establishment should only be conducted in the summer, not early Spring or late Fall. Cuttings should be selected from upper parts of the tree (above 1.5 meters) and only vertical shoots should be selected.** Knowing that MSmTFe was best for shoot culture, a different set of experiments were conducted to investigate if the position of shoot can affect in vitro shoot culture.

Early Spring Late Spring

Fig. *Effect of lateral node position on almond shoots (30 day culture)*

During late Spring or Summer, almond lateral nodes are best taken from the middle part of the branches (No. 4-8).

Almond vertical shoots and horizontal shoot behave very differently.

Table. *Effects of position on in vitro almond cutting establishment. Results were combined from two repeated experiment in Summer 2017 (n=80)*

	Vertical Cuttings	**Horizontal Cuttings**
Shoot size (mm)	26.68	18.68
Leaf number	7.58	3.37
Fresh weight (mg)	295	157

Fig. *Horizontal cutting-derived shoots (left) and vertical cutting-derived shoots (1 month old)*

As endogenous auxin act as an inhibitor, anti-auxin compounds were hypothesized to have a significant effect on almond tissue culture. This hypothesis was tested with TIBA and PCIB as two most effective anti-auxins. However, this experiment was compromised by the lights used at the Germplasm. A light source with strange wavelengths bleached the media from pink to white. Aside from this, no significant improvement was observed from TIBA and PCIB, so we terminated this experiment.

The Nonpareil almond meristem procedure was evaluated on FPS almonds (Bennett-Hickman, Fowler, and P2G #11-01**). Meristem-establishment media work consistently for these cultivars. However, media for plant maintenance after initial establishment still requires fine tuning.**

This work will be conducted on Bennett-Hickman, Fowler, and P2G #11-01 (Peach/Almond Hybrid). Permissions were granted by the nurseries.

Fig*. Meristem-derived plant of Bennett-Hickman, Fowler, and P2G #11-01 (directly excised from trees during Summer 2017)*

If meristems excised directly from potted trees do not result in virus free plants, thermotherapy of grafted plants may provide a cleaner source of almond meristems. 20 meristems of three cultivars (*Bennett-Hickman, Fowler, and P2G #11-01*) were excised from each heat treated scion and their survival rates were the same as untreated plants.

Fig. *Grafted almond and peaches under heat treatment*

Fig. *A rooted P2G11 plant, meristem-derived (excised in May2017), in acclimatization chamber.*

<u>Current almond meristem culture protocol</u>:

1. Collect only apical shoots as meristem source
2. Excise meristems onto WPMmTFe. Culture at 23°C, 16/8hrs photoperiod, 35-50 $\mu molm^{-2}s^{-1}$ for 2 week, transfer once.*
3. After a month, transfer to maintenance media (MSmTFE or to-be-developed) under normal light intensity 80-100 $\mu molm^{-2}s^{-1}$. Some cultivars can be very sensitive to ethylene accumulation, when those plants get larger, tubes should be vented to prevent hyperhydricity.*
4. Repeatedly transfer on maintenance media until large enough
5. If not rooted by itself, transfer to rooting media (to-be-developed) for 1 week in dark treatment
6. Transfer from rooting media to rooting media without BA

   * If all the established meristems do not survive, attempt excision of larger meristems. If meristems at larger sizes are not responding, media optimization should be conducted with a focus on basal salt (MS, DKW, QL, WPM) and sugar (sucrose, fructose, glucose)

PROJECT 3: PEACH VIRUS ELIMINATION USING MERISTEM TIP CULTURE

Abstract:

Many woody plant species, especially peaches, are recalcitrant and difficult to propagate *in vitro* due to their poor responses to different culture media. In this study, meristems (0.5-1mm) and cuttings of three peach varieties (*Prunus persica* cv. Stanislaus, Lovell, and Red Haven) are tested across various media to identify the optimal recipes for meristem or shoot culture. Field and in vitro meristems of the clean variety Stanislaus are excised and tested on media varying in salt, sugar, and vitamins to determine the best combination for both cultures. The optimal media resulting from this experiment will be used for excised meristems of other peach varieties to test its overall efficacy. The entirety of this project will determine the optimal procedures and media to cultivating recalcitrant peach varieties from meristems to potted plants.

Personnel involved: Hoang Nguyen, Maher Al Rwahnih, Erin Hsu, Ninh Khuu, James Shoulders, Sue Sim, Josh Pucket, John Preece, Deborah Golino

Current status:

Peach meristems were among the most recalcitrant as they always turned black and died within a week. Many medium optimization experiments were carried out on Stanislaus field material, including meristem excision from *in vitro* shoots and micro-grafting, but none of these resulted in positive results.

A series of treatments that were recently developed based on QL salts and fructose allowed meristem establishment of two peach cultivars (Lovell and Red Haven). Repeated experiment confirmed the stability of these recipes (PR17.10-19). Both runs are still awaiting data collection, but their survival on optimal media after 5 weeks is about 70-80%. Now we know that apical meristems of these two peach cultivars prefer QL, fructose, and a fairly low temperature of 23°C.

Future plan:

- Test PR17.10-19 on different peaches
- Develop maintenance media using both SierraGold and FPS plant materials, focusing first on sugar (sucrose, glucose, fructose, or mixed), and then salts (MS, QL, WPM, DKW, or mixed)

Stanislaus peach meristems from potted trees or from in vitro shoots should not be established onto these media because they will not last for more than a week.

Table. *Stanislaus peach meristem establishment result on different media (July 2017)*

Media Recipes	Total # of Meristems	# Living Meristems from Potted Trees	# Living Meristems from in vitro Shoots
0.5x MSmTFe	20	0	
MSmTFe	20	0	
1.5x MSmTFe	20	0	
3x MSmTFe	20	0	
QLmTFe	20	0	
WPMmTFe	20	0	
PR 17.1	20	-	0
PR 17.2	20	-	0
PR 17.3	20	-	0
PR 17.4	20	-	0
PR 17.5	20	-	0

Stanislaus peach cuttings showed no difference when tested on WPM media supplemented with 20 g/L sucrose, 0-15 g/L glucose, 0-10g/L sorbitol, and mixed. They did not perform well on any media specifically, and also declined after a month or two. WPM and MS may not be a good basal salt for peaches, therefore we focused on QL in our later experiments.

When grafted on GF305 and heat treated at 35°C, peach cultivars (Bonita 3 PID 61897 and Harvester PID 61905) demonstrated very poor heat tolerance capacity, in comparison to three other almond cultivars. We did not harvest any peach meristem from this treatment. We are very curious what temperature can peaches tolerate.

An experiment, performed on UCD Seed biotech center's thermogradient table suggested that Red Haven peach cuttings should not be established under temperature higher than 27.5°C. Temperature ranges that are lower than 25°C should be tested using a different culture system, not petri dishes, to identify optimal temperature for meristem establishment and shoot maintenance.

Fig*. Thermogradient table set up and cutting establishment after a month. Note that media dried out very quickly on petri dishes (June 2017).*

Table. *Effect of temperature (*25-35°C*) on Red Haven peach cutting growth and survival (July 2017).*

Temperature	Total # of explants	# Living Explants	# Shoots
25°C	39	14	4
27.5°C	39	8	3
30°C	39	2	1
32.5°C	39	0	0
35°C	39	0	0

Establishing peach meristems via micrografting on in vitro stem cuttings was a failed attempt. Stanislaus peach meristems lived for a month after being micrografted. Only meristems placed on the ring (vascular cambium) responded. A weak connection between living meristems and cuttings was present only through callus and not vascular vessels; therefore, meristems developed only for a short period and then declined. 30 meristems were established with this method in June 2017.

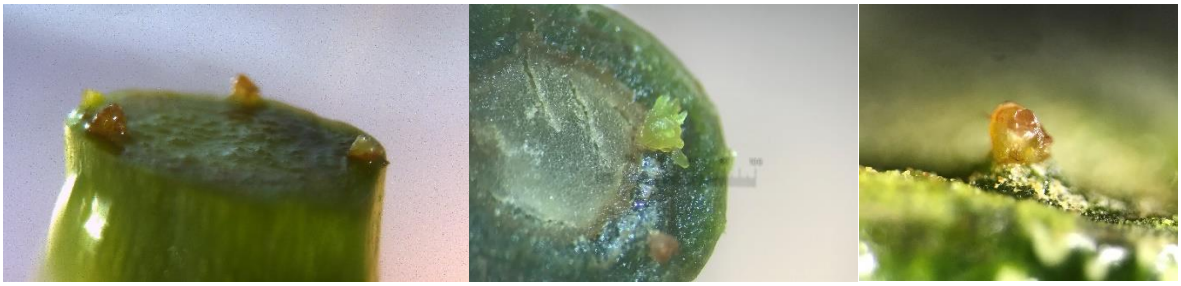


Fig. *Stanislaus meristem set up on fresh-cut internodes. Callus formation underneath meristems was observed but growth cessation happened after a month or two.*

Table*. Growth and performance of Stanislaus and Red Haven peach cuttings on 7 media recipes. Data were collected in September 2017 and combined from two repeated experiments (n=60)*

Cultivars	Media	Height (mm)	Fresh Weight (g)	Avg. Shoot Number	Avg. Leaf number
Stanislaus	MSmTFe	16.9	0.120	0.48	5.1
	PR 17.1	17.4	0.136	0.57	5.6
	PR 17.2	16.8	0.126	0.52	5.5
	PR 17.3	11.3	0.100	0.52	3.7
	PR 17.4	15.0	0.145	0.48	5.3
	PR 17.5	13.0	0.105	0.40	4.7
	WPMmTFe	10.8	0.066	0.42	3.8
Redh Hven	MSmTFe	7.4*	0.043*	0.23*	2.6*
	PR 17.1	18.2*	0.110*	0.58*	7.1*
	PR 17.2	13.1	0.083	0.45	5.7
	PR 17.3	16.0	0.099	0.45	4.9
	PR 17.4	10.8	0.057*	0.38	3.2*
	PR 17.5	21.6*	0.119*	0.65*	7.0*
	WPMmTFe	12.3	0.043*	0.42	3.1*

Stanislaus and Red Haven Peach cuttings should not be established on MSmTFe, WPMmTFe and PR 17.1-5. Peach cuttings establishment media should be developed using QL salts supplemented with 15g fructose (PR17.10-19).

Meristems from peach cultivar Red Haven and Lovell were recently excised directly from the field successfully on PR 17.10-17.19. This experiment is undergoing with very high survival rates (70-80%) after 5 weeks. This clearly indicates that the carbohydrate source is of highest importance in media optimization for these cultivars.

Fig. *5-week old Lovell meristems on PR17.12-14. 800 meristems of two cultivars (Lovell and Red Haven)*

<u>Current peach meristem culture protocol</u>:

1. Collect only apical shoots as meristem source
2. Excise meristems onto QL media supplemented with 15g/L fructose. Culture at 23°C, 16/8hrs photoperiod, 35-50 $\mu molm^{-2}s^{-1}$ for 2 week, transfer to the same media.*
3. After a month, transfer to maintenance media (same media or to-be-developed) under normal light intensity 80-100 $\mu molm^{-2}s^{-1}$. Some cultivars can be very sensitive to ethylene accumulation, when those plants get larger, tubes should be vented to prevent hyperhydricity.*
4. Repeatedly transfer on maintenance media until large enough
5. If not rooted by itself, transfer to rooting media (to-be-developed) for 1 weeks in dark treatment
6. Transfer from rooting media without BA

   * If all the established meristems do not survive, larger meristems should be excised. If meristem at larger sizes are not responding, media optimization should be conducted with focus only on basal salt (MS, DKW, QL, WPM) and sugar (sucrose, fructose, glucose)

IAB-ABC Joint Project : **Rootstock Breeding** 170726000SA

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Objectives:

A. Separate out general hybrid-vigor effects from specific major gene control of desired rootstock traits to allow more predictable progress by public and private breeding programs targeting rootstock improvement.

B. Compile a more comprehensive knowledge of breeding value and deficiencies for this genetically diverse germplasm.

C. Improve methods to generate and clonally propagated large interspecies-hybrid populations to capture targeted traits within a commercially viable background. Concurrently, developed methods to generate large segregating progeny populations from species and hybrids in order to sort out major gene effects of *Objective A.*

D. Generate new and diverse species-hybrids with promising rootstock potential for testing and selection. Also, develop and test methods for generating binary or chimeric rootstocks, that is, rootstocks combining 2 or more segments from different species.

Executive Summary

Changes in orchard land and water quality and subsequent management and cultural practices have led to the need for a new generation of rootstocks with improved disease and environmental stress resistance. Responding to this need, a number of public and private efforts have been initiated to develop and test new rootstocks for California tree crops. Germplasm derived from interspecies hybrids is often pursued to attain the greatest range of vigor and desirable

Item	Genetic background
Atlas	Almond, Peach, P. davidiana, Plu8m
Bright Hybrid	Almond, Peach, P. davidiana
Cadaman	Peach x P. davidiana
Citation	Almond, Plum
Compass	P. besseyi x P. americana
Controller 5	P. salicina x Peach
Cornerstone	Peach x Almond
Empyrean#1	Peach x P. davidiana
Flordaguard	Peach x P. davidiana
Hansen 536	Almond, Peach, P. davidiana
Hiawatha	P. besseyi x P. salicina
Ishtara	P. cerasifera, P. saliciana, Peach
Krymsk#86	Peach x P. cerasifera
Marianna 2624	P.munsoniana x P. cerasifera x P. hortulana
Nemaguard	Peach x P. davidiana
Nemared	Peach x P. davidiana
Nickels	Almond, Peach, P. davidiana
Paramount	Peach x Almond
Viking	Almond, P. blireiana, P. cerasifera, P. Mume

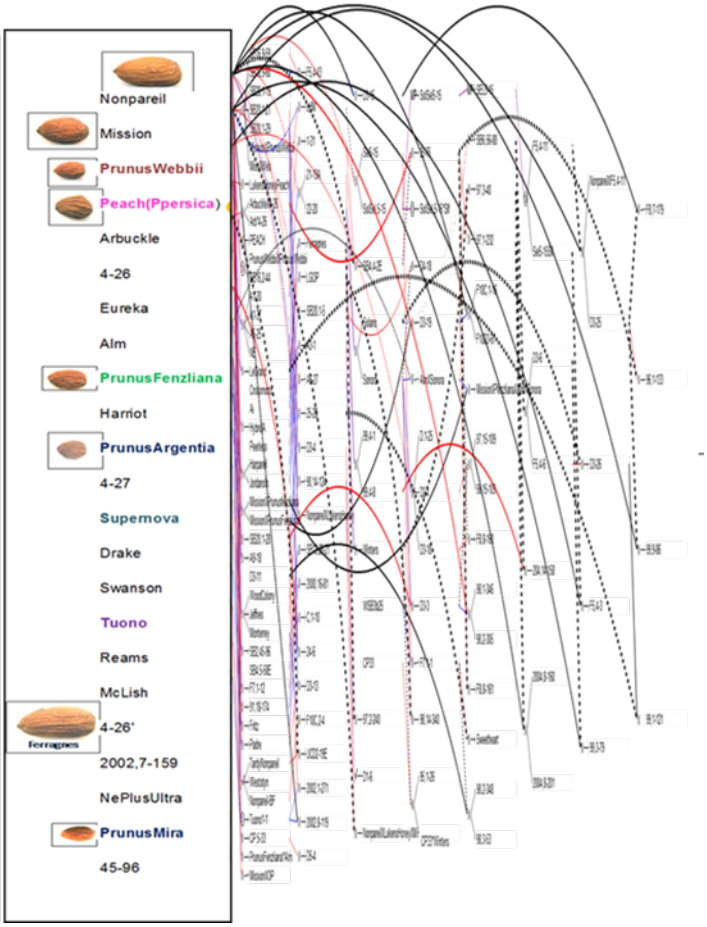


Figure 1. A list of currently important Prunus rootstocks resulting from interspecific hybridization (left). Examples of a diverse range of species parents and breeding crosses from early generations of the UCD almond/peach breeding and germplasm improvement programs programs (right); {solid lines denote seed parent while dotted lines denote pollen parent}.

horticultural traits (Figure 1) [Citation 1]. However, the development of such exotic germplasm is often difficult and time-consuming and, as has been recently shown with the UCB1 pistachio hybrid rootstock, the genetic, genomic and cultural interactions can be complex and unpredictable. As part of our long-term UCD almond and peach variety development programs, breeding lines have been and continue to be developed combining almond, peach and plum as well as with an extensive diversity within related Prunus species (Figure 1 and 2) [Citations1,3,5].

Early selections within this germplasm have demonstrated traits which appear desirable for rootstocks, including possible drought, nutrient, insect and disease tolerance, and modified tree size/structure. This germplasm is being made available to interested public and private rootstock development programs as clonal as well as segregating seedling populations to facilitate and accelerate comprehensive testing. Over 2,000 genetically diverse genotypes derived from this diverse UCD germplasm including peach (P. persica), almond (P. dulcis), P. mira, P. davidiana, P. scoparia, P. tangutica, P. webbii, P argentea, P. orthosepala, and P. bucharica (see Figure 2) have been transferred in 2017 for evaluation in several public and private programs for resistance to drought, salinity, boron toxicity, as well as diseases and pests (Table 1). The development of effective molecular markers for nematode and disease resistance allows improved selection and so breeding efficiency.

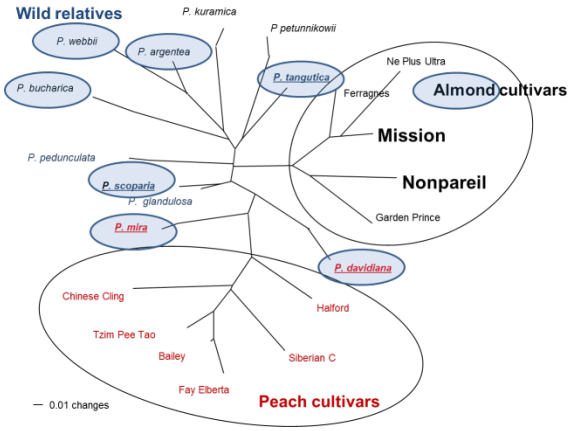
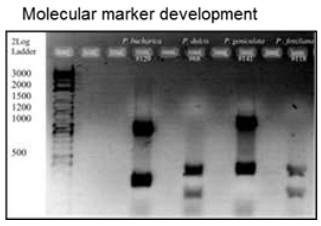
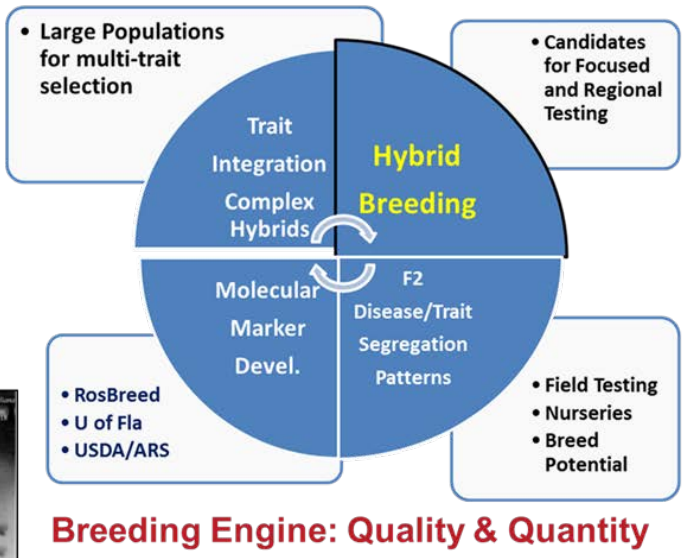


Figure 2. . Diagram showing genetic relationships among diverse species currently included in the UCD breeding programs. (Length of connecting lines indicates increasing genetic diversity.)

2017 Interspecific hybrids at UCD
Large populations required for:
-accurate molecular mapping
-recombine the numerous traits required for commercial success



Molecular marker development



Nematode screening at KAC

Figure 3. UCD Prunus Breeding cycle. Interspecies hybrid rootstock candidates from Hybrid Breeding are evaluated simultaneously for specific disease resistance and overall field performance, as well as trait inheritance patterns in progeny and subsequently, molecular marker development. The driving engine of the breeding program remains the ability to produce/propagate large numbers of diverse interspecies hybrids.

However, the number and diversity of rootstock characteristics needed for commercial success, require the development of breeding populations well beyond those manageable through traditional molecular marker assisted breeding alone{1,2] (see Fig. 7). Consequently, an aggressive breeding strategy has been developed which allows efficient concurrent assessment of both hybrid rootstock candidates as well as progeny derived from these hybrids for subsequent inheritance and molecular marker studies. The most promising species, as well as individual genotypes within species are then selected (based on disease resistance, molecular marker assessment as well as overall field performance) for a subsequent round of hybridization and testing (Fig. 3). By Dec., 2017, over 300 additional species-hybrids and over 1,400 segregating F2 seed were recovered from controlled pollinations.

Current methods for generating chimeric rootstocks have now been summarized and published [19] including potential appication for applied breeding.

A updated draft compiliolation of currentknowledge/opinion of breeding value and deficiencies for Prunus intra- and interspecific germplasm is included in the Appendix. Updates for individual collaborations listed in Table 1 are provided in the following text.

Workplans:

Workplans and methods are generally be the same for different years but with differing levels of detail.

Year 1 has completed the 1st stage of cooperator test-plantings including plot mapping, and the collection of initial information on species hybrid growth-vigor, potential disease resistance and plant architecture. Hybrid , F2 and/or BC2 seed has been generated for concurrent in-house and cooperator studies. Segregating F2and BC populations from targeted interspecies hybrids have been generated from controlled crosses for heritability, including molecular-marker studies.

Year 2 and 3 will involve the collection of data from different cooperators and the compilation of results for different species donors. New interspecies hybrid as well as F2/BC populations will also be developed based on cooperator feedback. Crossing goals for 2018 target the generation of over 500 new inter-species hybrids between almond, peach, plum as well as their related species. Preliminary molecular data available from RosBreed [8] and other UF, Clemson University and USDA cooperators may allow initial characterization of interspecies potential. If year 2&3 results continue to look promising, future funding will be solicited primarily from the Almond Board of California.

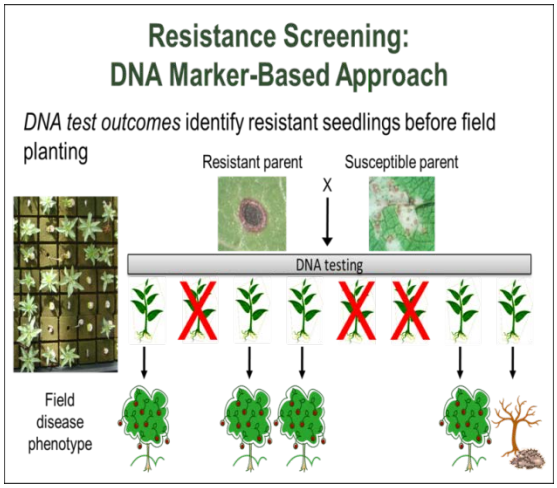


Figure 4. In breeding populations segregating for a gene with a major resistance affect, hundreds to thousands of established molecular markers can be evaluated to find a marker that co-segregates with resistance because it is located close enough to the actual disease gene.

Methods:

Traits are controlled by genes in which the required information is coded by specific sequences of DNA. Rootstock breeding, like variety breeding, essentially involves selecting for desirable genes while selecting against undesirable genes. Some traits, such as rootknot nematode resistance, are controlled by single genes with major affects, while others such as vigor are controlled by a large number of genes, each having only small individual affects. While we cannot yet identify most important genes by their DNA sequence, by statistically comparing large populations segregating for targeted traits, we can use the association of known molecular markers with the trait of interest as markers or indicators for the presence of that trait (Figure 4). This is because the marker is located on the DNA close enough to the trait of interest that, on average, they are inherited together. Because we know the DNA sequence of the marker, we now have a powerful tool to select for that trait even at the seedling stage. Molecular markers tend to be filler-DNA so that the DNA sequence does not have to be precise and so often mutates or changes over evolutionary time. Because of this, markers developed for one species, such as peach, may not be useful for even closely related species, such as almond, because of the large amount of time separating their evolution from a common ancestor [2]. Normally this is not a problem because most crop breeding involves only the single ancestral species. In rootstock breeding, however, diverse interspecies hybrids are common because they tend to be exceptionally vigorous and so inherently tolerant of wider environmental differences [1,2]. Because this growth vigor will often mask and so delay disease expression, is very difficult to determine whether the hybrid shows disease tolerance because of the possession of useful resistance genes or whether the vigorous growth can initially compensate for the lost diseased tissue and so delay final disease expression. To overcome this difficulty, we are developing both a series of inter-species hybrids as well as segregating seedling progeny populations which allow the identification of major resistance genes through their inheritance patterns (Figure 5). For example, in figure 5, the inherent vigor of almond by peach hybrids will confer tolerance to nematode damage, thus requiring many additional years before resistance/susceptibility can be truely determined. By generating segregating progeny populations, the hybrid vigor is removed while specific inheritance patterns denote the

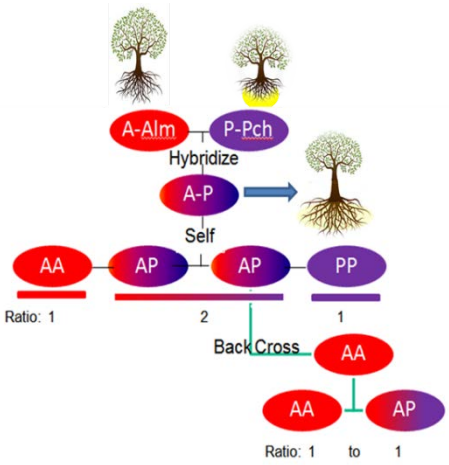


Figure 5. Segregation of root knot nematode resistance in Almond by Nemaguard peach lineages.

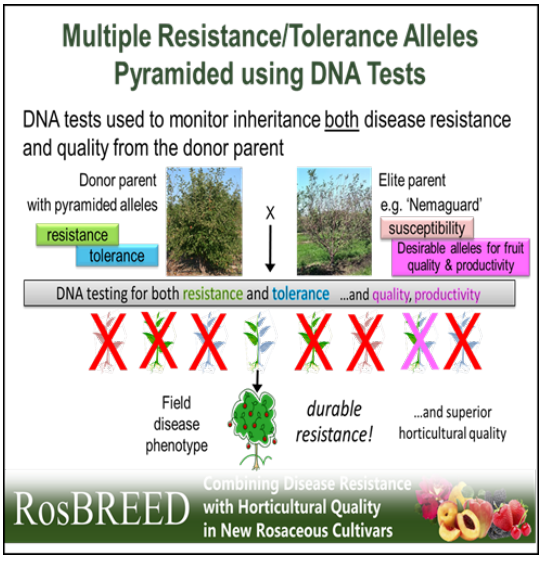


Figure 6. By simultaneously selecting for multiple molecular markers targeting separate resistance/quality genes, seedlings can be recovered in which the desired genes have been consolidated or pyramided.

segregation of major resistance genes (1:2:1 for F2 populations, and 1:1 segregation for backcross (BC) backcross populations). Species hybrids identified by progeny inheritance patterns as having desired major genes can then be advanced to further field testing as well as the generation of more advanced species hybrids. Similarly, individual F2 or BC progeny possessing the desired trait as well as good general rootstock potential can be selected for additional targeted inter-species crosses.

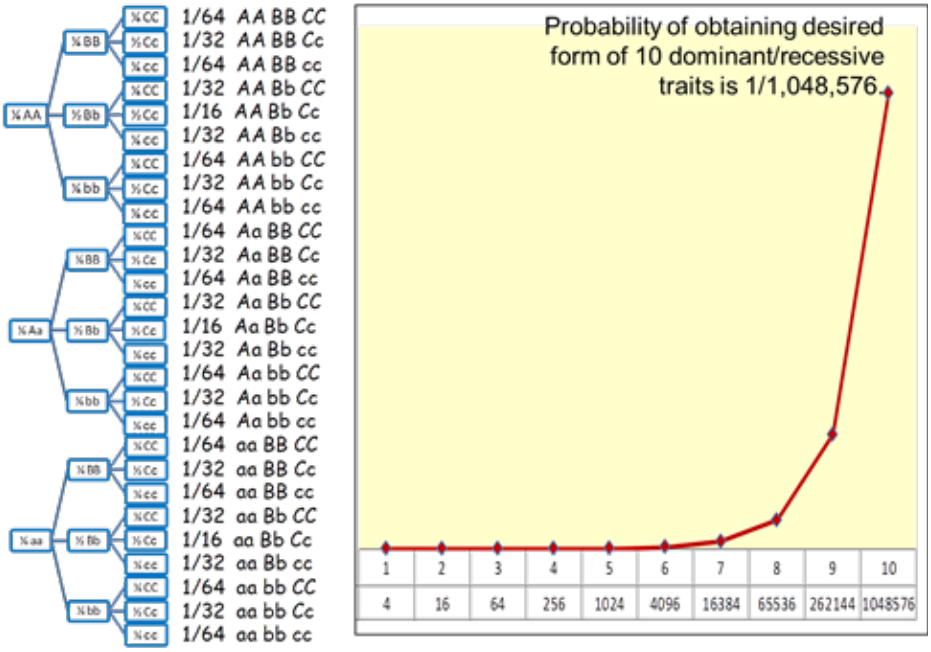


Figure 7. Segregation ratios following selfing for 3 separate genes (left). Minimum population sizes required to recover at least one of each possible genotype for increasing number of genes (right).

When molecular markers are available for several separate traits, the opportunity exists of identifying plants with a high probability of having each desired traits even at the seedling stage (Figure 6) [4,6,7]. However, because the traits are typically inherited independently, the minimum probability of obtaining multiple desired traits is the product of the probability of each individual trait. For example, to obtain the desired genotypes AABBCC in Figure 7, the minimum population size would have to be at least 64 and the number increases logarithmically for each additional gene/trait added quickly reaching over a million trees required for just 10 genes. Because commercially successful rootstocks will need more than just rootknot and/or ring nematode resistance (for example, graft compatibility, lack of high susceptibility to other diseases or pests, the ability to propagate in high numbers and true-to-type, desirable root and scion architectures, etc.), molecular marker assisted breeding capacity is limited and needs to be combined with quantitative breeding methods requiring large (though not as large) breeding populations. Consequently the UCD Prunus Breeding cycle (Figure 3) incorporates four complementary components: hybridization, progeny inheritance assessment, molecular marker development, and the development of breeding methods generating large numbers of diverse inter-species hybrids, including successful recovery of viable plantlets and subsequent successful clonal propagation for replicated testing.

Progress update: 2017

Breeding. Approximately 80 clones (~50 species hybrids and 30 species parents) and over 2,000 seed/seedlings from segregating progeny populoations were moved to cooperator evaluation in 2017. Subsequently, over 300 additional species-hybrids and over 1,400 segregating F2 seed were recovered from controlled pollinations made in spring 2017. Goals for 2018 include over 500 species hybrids and approximately 3,000 segregating seedling progeny from select parents.

Propagation. Over 50 species hybrids were propagated for replicated trials. Using methods optimized for individual species combinations, propagation 'takes' using harwood cuttings usually exceeded 60%. (Figure 8). {Lower propagation successes were frequently attributed to poor initial plant quality}. Similar or improved takes are indicated by current propaqgation trials using softwood cuttings. [Propagation taqkes of >50% are considered satisfactory for this stage of replicated testing since typically only 5 to 20 plants are required for each replicated test].

Progress for individual projects is summarized in Table 1 with details, including goals for 2018 provided in the following updates.

ID	Origin	Prop.	Rooted	Take (%)
NSW6-50	Almond by P.argentea	20	12	60.00
NSW6-18	Almond by P.argentea	20	14	70.00
FSC2-29	Almond by P.bucherica	20	7	35.00
STU2-29	Almond by P.bucherica	20	7	35.00
NSW6-24	Almond by P.scoparia	20	5	25.00
06,1-107	Almond by P.tangutica	20	15	75.00
08,7-310	Almond by P.webbii	20	2	10.00
PG13-6	Almond interspecies	20	2	10.00
PG13-3	Almond interspecies	20	15	75.00
07,13-250	Peach by Almond by P.scoparia	20	3	15.00
05,20-139	Peach by Almond by P.scoparia	20	8	40.00
2008,18-63	Almond by P.mira	20	2	10.00
NSW6-16	Almond by P.mira	40	28	70.00
08,6-210	Almond by P.mira	50	36	72.00
05,20-192	Peach by Almond	30	19	63.33
2009,19-32	Peach by Almond complex	20	4	20.00
2009,19-85	Peach by Almond complex	20	4	20.00
2009,20-89	Peach by Almond complex	20	6	30.00
2009,17-336	Peach by Almond complex	20	7	35.00
2009,33-175	Peach by Almond complex	20	7	35.00
2009,19-150	Peach by Almond complex	20	10	50.00
2009,21-168	Peach by Almond complex	20	11	55.00
2009,18-87	Peach by Almond complex	20	12	60.00
07,12-209	Peach by Almond complex	40	25	62.50
2009,22-1	Peach by Almond complex	20	13	65.00
2008,53-47	Peach by Almond complex	20	13	65.00
2008,61-38	Peach by Almond complex	30	21	70.00
2009,28-152	Peach by Almond complex	30	21	70.00
2009,32-214	Peach by Almond complex	40	29	72.50
2009,19-112	Peach by Almond complex	40	29	72.50
08,58-18	Peach by Almond complex	30	22	73.33
2009,23-28	Peach by Almond complex	20	15	75.00
2009,23-109	Peach by Almond complex	20	16	80.00
2009,29-15	Peach by Almond complex	20	16	80.00
2008,25-101	Peach by Almond complex	20	16	80.00
2009,19-18	Peach by Almond complex	20	16	80.00
10,10-420	Peach by Almond complex	30	24	80.00
2009,29-107	Peach by Almond complex	40	33	82.50
2009,19-252	Peach by Almond complex	20	17	85.00
2008,25-113	Peach by Almond complex	50	37	74.00
05,17-186	Peach by P.davidiana	40	32	80.00
2008,44-28	Peach by P.mira	40	12	30.00
STU2-32	Almond by P.orthosepala	40	22	55.00
NSW7-32	Peach by Plum	20	2	10.00
NSW7-34	Plum interspecies	40	14	35.00
PG3-29	Plum interspecies	20	14	70.00
PG14-2	Plum interspecies	30	19	63.33

Figure 8. Propagation success in 2017 for selected species hybrids using hardwood cuttings.

Table 1. Status of current cooperator and in-house resistance evaluation projects.

Trait	Cooperator	Material under evaluation	*Speciesevaluated*	Status
Heat Tolerance	M. Gilbert	15 clones	***a, f, m, p, w***	Under analysis
Botryophaeria resistance	J. Chaparro (U. Fla)	40 cl., 100 sdlings	***a, b, f, m, pd, p, plsp, t, tr, w***	Field plots established with preliminary results
Root lesion Ring, and Root-knot nematode	A. Westphal	25 clones	***a, dv, m, p, t, w***	Field plots established for 7 cl. with 19 clones propagated.
Phytophthora	Greg Browne	3 clones	***pl***	Plants established
Crown gall	D. Kluepfel	~200 seedlings	***p, t,***	>100 sdlings in field, ~100 sdlings greenhouse,
Salinity tolerance	P. Brown	12 clones	***d, a, , f, m, p, t, w***	Greenhouse testing
Botryophaeria, Oxyporus and other wood rot diseases	Rizzo/Johnson	15 clones	***d, a, , f, m, p, t, w***	10 clones under test with 10 to 20 additional clones to be added
Effect on scion architecture	Fowler/Wonderful	7 clones	***a, dv,***	Field plots in commercial production
Nonpareil Compat. & Replant decline	Burchell Nursery	50 clones	***a, b, dv, m, p, plsp, s, t, w***	Field testing
Replant decline	Sierra Gold Nursery	20 clones & ~1000 seed	***a, dv, m, p, s, t, w***	Field testing
Dryland culture	A. Langford	Almond seedlings	***d***	Field testing
Armillaria	In-house	~200 seedlings	***d, p***	Seed being prepared for planting
Asphyxia	In-house	~100 seed	***d, p***	Seed being prepared for planting
Verticillium & Phytophthora	In-house	6 cl. & ~240 sdlings	***d, p***	Seed being prepared for planting
Architecture & disease	In-house	90 cl., ~40, 000 sdlings	***a, b, dv, m, p, s, t, w***	Field testing
High density plantings.	G. Thorp, Australia	20 cl., ~400 seedlings	***d, , f, m, p, w***	12 clones propagated, >1000 crosses (hybrids and F2's)
Tissue culture, plant-regeneration, transformation	Abhaya Dandekar	~200 developing seed; 6 clones	***d, p, dv***	Ease of in-vitro regeneration underway
Almond {P.dulcis} (d), Peach {P.persica} (p), P.argentea (ar), P.fenzliana (f), P.mira (m), P.webbii (w),				
P.bucharica (b), P.pedunculata (pd), Plum spp. (pl), P.tangutica (t), P.triloba (tr), P.davidiana (dv), P.scoparia (s)				

1. Heat Tolerance. Cooperator: Mathew Gilbert. Material under evaluation: 15 clones. Species evaluated: P. *argentea*, *P. fenzliana, P. mira, P. persica*, *P. webbii*. Status: Germplasm differences based on a preliminary screening of critical leaf temperature completed and presented in annual reports[5]. Recent models have suggested that vascular structure in addition to leaf architecture may be the principal components leading to heat tolerance. Whether such vascular structural differences among species has important rootstock implications has yet to be determined though vascular structure has been shown by Ted

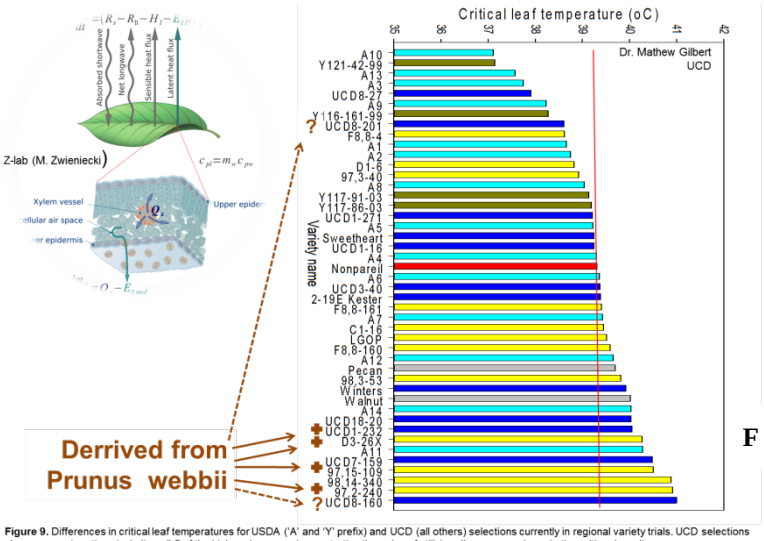


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Figure 9. Differences in critical leaf temperatures for USDA ('A' and 'Y' prefix) and UCD (all others) selections currently in regional variety trials. UCD selections show a range in ratings including all 5 of the high-end scores, demonstrating the value of utilizing diverse germplasm in the cultivar breeding program.

Dejong to affect both rootsctock-to-scion vigor and final scion size. Notably, most of the UCD clones showing exceptional levels of heat tolerance are almond introgression lines derived from Prunus webbii. Future goals: identify improved predictors of rootstock-conferred heat/drought tolerance for future studies. Evaluate own-rooted accessions of almond-P webbii introgression lines, including accessions UCD04, 8-160 and UCD04, 8-210 under dryland production conditions [see project 10].

2. Botryophaeria resistance. Cooperator: Jose Chaparro (UF). Material under evaluation: 40 clones, 200 seedlings. Species evaluated: *P. argentea, P. bucharica, P. fenzliana, P. mira, P. pedunculata, P. persica, Plum spp., P. tangutica, P. triloba, P. webbii*. Status: disease plots established and the 1st round of disease and molecular marker evaluations completed. A major molecular marker for botryophaeria resistance in several Prunus dulcis (almond) accessions has been identified by UF cooperators with a summary of results in preparation for submission for publication in mid-2018. Future goals: an additional 60 almond by P davidiana and almond by P mira hybrids were generated in 2017 at UCD for UF testing beginning in 2018. In addition, based on present results 80 additional hybrids as well as a complex intra-species cross (Jeffries by Nonpareil) will be made in 2018 with a target of 100 segregating seedling progeny.
3. Root lesion, Ring, and Root-knot nematode. Cooperator: Andreas Westphal and Burchell nursery. Material under evaluation: 40 clones. Species evaluated: *P. argentea, P. davidiana, P. mira, P. persica, P. tangutica, P. webbii.* Status: Field plots established. Within the propagated group is a tandemly-grafted rootstock composed of a Nemaguard upper rootstock cleft-grafted to a Hansen basal-rootstock as a test-of-concept of binary-rootstocks engineered to rapidly combine desirable traits from different rootstocks or to selectively target soil strata differences in pathogen, nutrient, drought, etc. conditions. Selection showing promise in 2017 for both root knot and ring nematode include UCD 05, 17-186 ((P persica x P davidiana) x P persica) and STU 2-32 [4]. (P dulcis x P. x orthosepala). Future goals: continue multiyear evaluation of resistance. Add 40 clones (primarily P mira and P davidiana) currently being propagated for spring, 2018 planting. Generate F2 of UCD05, 17-186 as well as an 100 additional ((P persica x P davidiana) x P persica) hybrids.

Figure 10. Almond by P orthosepala (left) in ring nematode infested evaluation block

4. Phytophthora. Cooperator: Greg Browne. Material under evaluation: 3 clones. Species evaluated: Plum interspecies. Status: Plants established. Future plans: four additional P. dulcis accessions identified as having potential resistance based on long-term survival in

Phytophthora infested soils are being clonally propagated for control testing. Concurrently, these items are also being used as parents in 2018 inter-and intra--species hybridizations.

5. Crown gall. Cooperator: Dan Kluepfel and M. Aradhya. Material under evaluation: ~200 seedlings, ~400 seed. Species evaluated: *P. persica x P. tangutica* (F_2, F_3). Status: The Kluepfel research lab had previously identified potential crown gall resistance in the interspecies hybrid *P. persica x P. tangutica* [4]. To test for progeny segregation patterns indicating a control by major genes (i.e. heritable in any future

Figure 11. tree segregating for bacterial canker susceptibility in an F2 of peach by P tangutica.

Figure 12. Lethal crown gall on a P tangutica accession in the USDA germplasm collection.

controlled hybridizations), we have generated F_2 and F_3 peach by *P. tangutica* progeny populations from the USDA *P. tangutica* source used. Over 200 seed have been field planted for test inoculations, with an additional 400 seed reserved to allow inoculations/testing under laboratory conditions. While controlled inoculations have not yet occurred, field plantings have shown evidence for a high heritability for susceptibility to bacterial canker in F2 progeny (Figure 11) while a sibling of 1 of the P tangutica USDA parents was found to had been killed by crown gall (Figure 12) though whether the infection was on P tangutica or an as yet unknown rootstock species remains unknown. Future goals: provide hybrid clones and F2 seed/seedlings for 2018 controlled inoculations.

6. Salinity tolerance. Cooperator: Patrick Brown et al.. Material under evaluation: 6 clones. Species evaluated: Almond, *P. argentea, , P. fenzliana, P. mira, P. persica, P. tangutica, P. webbii*. Status: 12 different interspecies clones were initially targeted for evaluation. However we were unable to supply the 8-10 trees per clone required for 2017 trials. Future goals: propagate 8-12 trees of an additional 6-8 clones to be included in future evaluations.

7. Oxyporus, Botryophaeria, and other wood rot diseases. Cooperator: Rizzo/Johnson. Material under evaluation: 16 clones (9 from UCD breeding and 7 standard rootstocks). Species evaluated: Almond, P. argentea, , P. fenzliana, P. mira, P. persica, P. tangutica, P. webbii. Status: preliminary studies have identified potential resistance and several species sources. Future goals: provide additional accessions (species hybrids and species parents) for 2018 evaluations as requested.
8. Effect on scion architecture. Cooperator: Fowler Nursery/Paramount. Material under evaluation: 7 clones; in-house-5 clones. Species evaluated: *P. argentea, P. davidiana, P. dulcis, P. persica, P. scoparia.* Status: Field plots established and in commercial production with multi-year evaluations completed. Future goals: evaluate relative production performance as well as orchard longevity for different species sources. (see also project 14).
9. Replant decline. Cooperators: Burchell Nursery, Sierra Gold Nursery. Material under evaluation: 50 clones. Species evaluated*: P. argentea, P. bucharica, P. davidiana, P. mira, P. persica, Plum spp., P. scoparia, P. tangutica, P. webbii*. Status: Field plots established (see ring nematode evaluations for project 3). Future goals: continue multiyear field evaluations combined with 2018 nematode intensity ratings by Andreas Westphal.
10. Dryland culture. Cooperator: Andrew Langford and in-house. Material under evaluation: Almond spp., seedlings & seed. Status: Potted almond seedlings have been planted are currently being prepared for field propagations in 2018 in dryland Capay

Figure 13. Own-rooted accessions of almond-P webbii I introgression lines including accessions UCD04, 8-160, UCD04, 8-210 and siblings (identified in project 1 as having potential heat tolerance), under dryland production conditions (trees in 2017 fowwowing 4 years without supplementary water; inset: selfed crop of drland UCD04, 8-160 in 2017) .

orchards. In addition, we continue to evaluate own-rooted accessions of almond-P webbii introgression lines including accessions UCD04, 8-160 and UCD04, 8-210, identified in project 1 as having potential heat tolerance, under dryland production

conditions [see figure 13 and project 1]. We also are monitoring a 60-year-old almond-rooted dryland Nonpareil/Mission orchard for growth habits and production consistency.

11. Armillaria. In-house. Material under evaluation: ~200 seedlings. Species evaluated: almond and peach spp., Seedlings & seed. Status: seed was collected from parent trees from almond by peach introgression lines showing continued good growth in known *Oak root*

Figure 15. Five year survival of almond introgression lines derived from peach by P. webbii in a test plot highly infested with oak root fungus.

fungus hotspots. Open-pollinated seed was collected and will be tested in plots previously shown to have high *Armillaria* damage. New plantings are scheduled for spring 2018. Heritability will be estimated from progeny inheritance patterns. At a very basic level, if no evidence of any heritable resistance/tolerance is evident in the progeny, the value of that parent for continued breeding will have been greatly diminished. Over 300 plum F2 seed have been generated for future inheritance and molecular studies. Future goals: Replant UCD Oak-root fungus plot with next generation of accessions for screening. Generate 200 almond by resistant/tolerant plum species hybrids in 2018.

12. Asphyxia. In-house. Material under evaluation: ~100 seed. Species evaluated: Almond and Peach spp. seedlings & seed. Status: Seed was collected from almond species lines showing continued good growth in areas having highly saturated soils. Open-pollinated seed was collected and is being prepared for testing under greenhouse conditions were soil saturation can be more accurately maintained. Almond by almond and almond by peach crosses were made in 2017 for resistance and heritability trials. Heritability of tolerance/resistance will be estimated from progeny inheritance patterns as described above. Future goals: generate an additional 20 almond by peach and almond by plum hybrids from parents showing promise of resistance.

Figure 14. Drought tolerance associated with a strong vertical tap root identified in some almond introgression lines derived from peach by P webbii hybrids.

13. Hybrid architecture. UCD Almond and Peach variety breeding

programs material under evaluation: 90 clones & ~70,000 seedlings. Species evaluated:

Figure 16. Vigor differences in peach by P. mira hybrids after 4 mo. field growth in 2017.

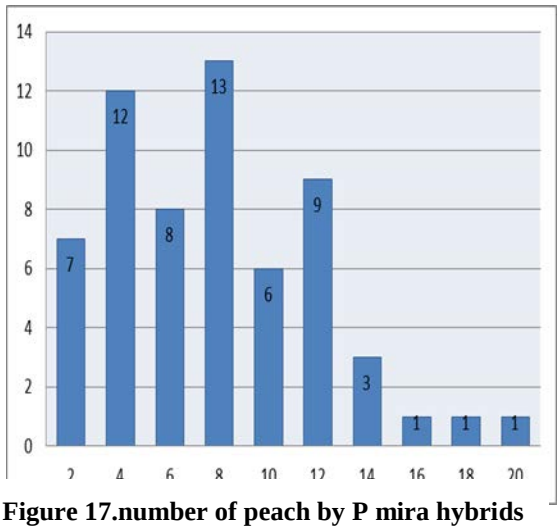

Figure 17.number of peach by P mira hybrids different height categories (inches) after 6 month of field growth.

P. argentea, P. bucharica, P. davidiana, P. mira, P. persica, P. scoparia, P. tangutica, P. webbii. Status: Over 90 species hybrids or species introgression parents, as well as ~70,000 seedling progeny are currently being evaluated as part of the UCD Almond and Peach breeding programs. Genetic opportunities for rootstock improvement are now being considered concurrently with those for scion (variety) improvement in the ongoing evaluation of this germplasm. For example, interspecies progeny breeding blocks scheduled for removal are now retained for an additional season but without supplemental irrigation to evaluate differences in survival. Drought tolerance associated with a strong vertical tap root was identified in some almond introgression lines derived from peach by P webbii hybrids (Figure 14). Similarly, Figure 15 shows progeny from a peach by P mira hybridization after 6 months of field growth demonstrating variability in the levels of hybrid vigor and plant architecture in these species hybrids. Height/vigor distribution profile of 60 hybrid progeny from a peach by P davidiana by p mira cross are shown in Fig. 17. Future goals: continue drought and tree/root architecture studies. Identify predictors of tolerance/resistance.

14. High density plantings. Work with Bruce Lampinen and Australian collaborators, Grant Thorp at *Plant and Food Research*, and Michelle Wirthensohn, at the *University of Adelaide* has found that the greatly reduced basal-tree productivity is a major limitation in the development of very high density orchards. Reduced spur-bearing from harvest damage as well as lower-wood shading has been identified as a major contributor to this loss. High-density, compact and lateral-bearing selections are being developed from *Prunus webbii* and *Prunus mira* lineages which contribute to 2nd and 3rd year fruit-bearing wood having higher bearing-densities as well as greater fruit-wood renewal (Fig. 18 at left). (Much of the production is on short dard-type lateral shoots similar to those seen on the *Winters* variety). Approximately 12 advanced UCD breeding selections showing this trait are being propagated for testing with cooperating nurseries under very

high density systems. In addition, a large (> 200 seedlings) F2 population segregating for this bearing habit, self-compatibility, kernel quality and tree architecture has been generated in 2017 for studying desirable/undesirable trait associations and the possible development of molecular markers for targeted traits. In 2017, novel pillar-type almond breeding lines derived from P mira introgression was also identified which show promise for productive, high density plantings (Fig. 18 at right). In addition, peach by P mira hybrids and BC1 progeny also show a pillar trait with evidence for control by a single major gene. Future goals: continue to evaluate the inheritance of the compact and pillar-type growth habits and determined what affect compact and pillar-trees confer when used as rootstocks. Establish a high density trial at Wolfskill Experiment Station (with Lampinen and Thorp) to assess tree architecture differences.

Figure 18. Compact (left) and pillar (right) almond tree architectures (inset: crop in 2017).

15. Tissue culture, plant-regeneration, transformation. Cooperator: Abhaya Dandekar. Material under evaluation: ~200 developing seed. Species evaluated: *P. dulcis, P. persica, P. davidiana*. Status: The Dandekar project aims to re-synthesize commercially successful almond by peach hybrids to use as foundation for plantlet-regenerable tissue-culture callus for subsequent genetic engineering of additional desirable rootstock characteristics. Over 50 seed approximating the initial parentage of the Nickels almond by peach by P. davidiana rootstock have been generated in 2017 and provided to the Dandekar lab for culture and evaluation. Future goals: Since a major barrier to genetically engineering almond rootstocks is the ability to regenerate from tissue-culture callus, we plan to generate approximately 150 additional almond by peach/P davidiana / P mira hybrid seed in the spring of 2018 but harvest the developing seed at very early stages of embryo development (globular to heart stage) to test whether early embryonic tissue is more amenable to the adventitious callus/plantlet regeneration required for successful genetic engineering. We also plan to assess methods for direct meristem transformation using recent modifications of the Biolistic gene gun [see 9, 10].

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Appendix. Draft summary of Rootstock characteristics.

Group	Item	Genetic background	Wet Soil	Anchorage	Vigor	Phytophthora	Bacterial Canker	Crown Gall	Root Knot Nematode	RingN	Lesion	Oak Root Fungus	Non-Pareil Compatible?	Suckers?
Pe	11 1 1	(Flordaguard x Alnem) x Y105 208 94												
Pe	11 3 1	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 3 2	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 7 1	Y115 175 97 *x P. davidiana* 'Potanni'												
Pl	12 hybrids of	P. dulcis x P. webbii												
Al	198 13	*P. webbii x P. dulcis*												
Al	198 17	*P. tangutica x P. dulcis*												
Pe	198 18	Nemared x *P. kensuensis*												
Pe	198 3	*P. argentia x P. dulcis*												
Al x Pl	AC 941(Mirobac)	P. dulcis x P. cerasifera												
Pe	AC 9000	P. dulcis x P. persica												
Pe	AC 9502	P. dulcis x P. persica												
Pl	AC 952	P. insititia												
Pl	AC 959	P. insititia x P. domestica												
Pl	AC 960	P. insititia x (dulcis x persica)												
Pe	Adarcias	'Garnem' almond x 'Nemared peach'												
Pe	Adefuel	P. dulcis x P. persica	1		2	1					1	1		
	Adesoto				2									
Al	Alnem 1	bitter almond												
Al	Alnem 201	?												
Al	Alnem 88	bitter almond												
ex hyb	Atlas	Nemaguard * (Jordanolo * Prunus blireiana.	2?	3	3	2	2	2	3	2	2	1	Yes	ne
Pe	Bright Hybrid #2	P. dulcis x Nemaguard												
Pe	Bright Hybrid #3	P. dulcis x Nemaguard												
Pe	Bright Hybrid #6	P. dulcis x Nemaguard												
Pe	Bright Hybrid 106	P. persica x P.dulcis	2						3		2			
Pe	Bright Hybrid"	P. persica x P. davidiana												
Pe	Bright Hybrid 1	P. persica x P.dulcis	1	4	4	1	1	2	3	2		1	Yes	ne
Pe	Bright Hybrid 4	P. persica x P.dulcis												
Pe	Bright Hybrid 5	P. dulcis (Titan) x Nemaguard				1	1	1	1	1	1	1		
Pe	Cadaman	P. persica x P. davidiana	1		2	3	3	1	1	2	1			
Pl	Citation	OP Red Beaut Plum OR Siberian C x (plum x almond)				2	1	1	3	2	2			
unk	Compass	Prunus besseyi x Prunus americana					2		2	2	2	1		
Pe	Contoller 9.5 (HBOK	P. persica (cv Harrow Blood) x (cv Okinawa)							3					
Pl	Controller 5 (=K146 4	P. salicina x P. persica				2	1		2	1	1			
unk	Controller 7 (HBOK 32)					1			3					
Pe	Controller 8 (HBOK 1	P. persica (cv Harrow Blood) x (cv Okinawa)												
unk	Controller 9					2	1		1	1	2		no	
Pe	Cornerstone	P. persica x P.dulcis	1			2	1	2	2	2	2	1		
Pl	Empyrean 101 (Aedes	P. isititia	3	2		2	1		3	1	1	2		
unk	Empyrean 3 (Tetra)	(Prunus domestica)	2	2	1	2	2	3	2		2	1		
Pe	Empyrean#1 (Barrier	P. persica x P. davidiana				1	1	1	3	2	1			
Pl	Empyrean#2 (Penta)	O.P. P. domestica	2	2	2	2	2		3	1	1	2		
Pe	Flordaguard	{('Shau Thai OP' x Prunus davidiana) x (3 OP	1	2			3		3	2	2			
unk	Flordaguard	P. persica x P. davidiana							4	2.4	2.4			
Pe	Flordaguard x Alnem	P. persica x Israeli bitter almond												
Pe	Flordaguard x Israeli I	(P. persica x P. davidiana) x P. dulcis												
Pe	Flordaguard x weepin	P. persica x P. davidiana												
Pe	Floridaguard x almon	(P. persica x davidiana) x P. dulcis	1											
	Guardian	P. persica sdling							3	3	4			
	Guardian	P. persica sdling					2			2	2	1		
Pe	Guardian (SC 17)	Nemaguard							3	2	2			
Pe	GxN 15(Garnem)	P. dulcis x P. persica (Nemared)												
Pe	GxN 22 (Felinem)	P. dulcis x P. persica (Nemared)												
Pe	GxN 9(Monegro)	P. dulcis x P. persica (Nemared)												
Pe	H184	Titan almond x Nemaguard												
Pe	Hansen 536	almond MVM	1	4	4	1	2	2	3	1	2	1	Yes	ne
Comple	Hansen 536 x P. blieriana									RingN				
Comple	Hansen 536 x P. cerasifera					3								
Comple	Hansen 536 x P. domestica					3		3						
Pe	Harrow Blood x Okina	P. persica x P. davidiana												
Pe	Harrow Blood x Okina	P. persica x P. davidiana												
Pl	Havens 2B	P. insititia												
unk	HBOK 15							3	2	2				
unk	HBOK 28													
unk	HBOK1							4	3	3				
unk	HBOK10								3					
	HBOK15								4	3	1			
unk	HBOK17													
Pl	Hiawatha	P. besseyi x P. salicina							2	1	2			
Pe	IS 5/19	P. dulcis x P. persica												
Pe	IS 5/8	P. dulcis x P. persica												
Pe	IS 29 5	P. dulcis x P. persica												

Appendix. Draft summary of Rootstock characteristics. (Cont.)

Group	Item	Genetic background	Wet Soil	Anchorage	Vigor	Phytophthora	Bacterial Canker	Crown Gall	Root Knot Nematode	RingN	Lesion	Oak Root Fungus	Non-Pareil Compatible?	Suckers?
Pe	11 1 1	(Flordaguard x Alnem) x Y105 208 94												
Pe	11 3 1	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 3 2	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 7 1	Y115 175 97 *x P. davidiana* 'Potanni'												
Pl	12 hybrids of	P. dulcis x P. webbii												
Al	198 13	*P. webbii x P. dulcis*												
Al	198 17	*P. tanqutica x P. dulcis*												
Pe	198 18	Nemared x *P. kensuensis*												
Pe	198 3	*P. argentia x P. dulcis*												
Al x Pl	AC 941(Mirobac)	P. dulcis x P. cerasifera												
Pe	AC 9000	P. dulcis x P. persica												
Pe	AC 9502	P. dulcis x P. persica												
Pl	AC 952	P. insititia												
Pl	AC 959	P. insititia x P. domestica												
Pl	AC 960	P. insititia x (dulcis x persica)												
Pe	Adarcias	'Garnem' almond x 'Nemared peach'												
Pe	Adefuel	P. dulcis x P. persica	1		2	1					1	1		
	Adesoto				2									
Al	Alnem 1	bitter almond												
Al	Alnem 201	?												
Al	Alnem 88	bitter almond												
exhyb	Atlas	Nemaguard * (Jordanolo * Prunus blireiana.	2?	3	3	2	2	2	3	2	2	1	Yes	ne
Pe	Bright Hybrid #2	P. dulcis x Nemaguard												
Pe	Bright Hybrid #3	P. dulcis x Nemaguard												
Pe	Bright Hybrid #6	P. dulcis x Nemaguard												
Pe	Bright Hybrid 106	P. persica x P.dulcis	2						3		2			
Pe	Bright Hybrid"	P. persica x P. davidiana												
Pe	Bright Hybrid 1	P. persica x P.dulcis	1	4	4	1	1	2	3	2		1	Yes	ne
Pe	Bright Hybrid 4	P. persica x P.dulcis												
Pe	Bright Hybrid 5	P. dulcis (Titan) x Nemaguard				1	1	1	1	1	1	1		
Pe	Cadaman	P. persica x P. davidiana	1		2	3	3	1	1	2	1			
Pl	Citation	OP Red Beaut Plum OR Siberian C x (plum x almond)				2	1	1	3	2	2			
unk	Compass	Prunus besseyi x Prunus americana					2		2	2	2	1		
Pe	Contoller 9.5 (HBOK	P. persica (cv Harrow Blood) x (cv Okinawa)							3					
Pl	Controller 5 (=K146 4	P. salicina x P. persica				2	1		2	1	1			
unk	Controller 7 (HBOK 32)					1			3					
Pe	Controller 8 (HBOK 1	P. persica (cv Harrow Blood) x (cv Okinawa)												
unk	Controller 9					2	1		1	1	2		no	
Pe	Cornerstone	P. persica x P.dulcis	1			2	1	2	2	2	2	1		
Pl	Empyrean 101 (Aedes	P. isititia	3	2		2	1		3	1	1	2		
unk	Empyrean 3 (Tetra)	(Prunus domestica)	2	2	1	2	2	3	2		2	1		
Pe	Empyrean#1 (Barrier	P. persica x P. davidiana				1	1	1	3	2	1			
Pl	Empyrean#2 (Penta)	O.P. P. domestica	2	2	2	2	2		3	1	1	2		
Pe	Flordaguard	{('Shau Thai OP' x Prunus davidiana) x (3 OP	1	2			3		3	2	2			
unk	Flordaguard	P. persica x P. davidiana							4	2.4	2.4			
Pe	Flordaguard x Alnem	P. persica x Israeli bitter almond												
Pe	Flordaguard x Israeli I	(P. persica x P. davidiana) x P. dulcis												
Pe	Flordaguard x weepin	P. persica x P. davidiana												
Pe	Floridaguard x almon	(P. persica x davidiana) x P. dulcis	1											
	Guardian	P. persica sdling							3	3	4			
	Guardian	P. persica sdling					2			2	2	1		
Pe	Guardian (SC 17)	Nemaguard							3	2	2			
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Pe	H184	Titan almond x Nemaguard												
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Comple	Hansen 536 x P. domestica					3		3						
Pe	Harrow Blood x Okina	P. persica x P. davidiana												
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Pl	Havens 2B	P. insititia												
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unk	HBOK1							4	3	3				
unk	HBOK10								3					
	HBOK15								4	3	1			
unk	HBOK17													
Pl	Hiawatha	P. besseyi x P. salicina							2	1	2			
Pe	IS 5/19	P. dulcis x P. persica												
Pe	IS 5/8	P. dulcis x P. persica												
Pe	IS 29 5	P. dulcis x P. persica												

Progress report for CDFA project: 170278000SA

Title of Proposed Research: Development of an ELISA assay for the rapid screening of cherry for Little cherry virus 2.

Proposed Duration: 1 Year **Start Date:** 7/1/17

Total Amount Requested: $9,163 **Department:** Plant Pathology

Principal Investigator: Dr. Scott Harper, Director CPCNW

The objective of this project was to develop polyclonal antisera for the development of an Enzyme-Linked Immunosorbent Assay (ELISA) protocol for the detection of *Little cherry virus 2* (LChV-2) in cherry selections in clean plant, breeding and nursery programs, as well as for orchard screening, in the US. An ELISA was proposed as, although it is an older technology, is extremely robust and tolerant of crude sample preparation, in the field if required, and can readily scale to sample large numbers of samples cost-effectively.

Three sequential milestones were proposed for this project, which necessarily must be accomplished in order.

Task 1: *Isolate and clone the coat protein (capsid) gene of the three major LChV2 strains present in the US into a binary expression vector.*

The commencement of this objective, and thus the rest of the project, was delayed by staff changes at the CPCNW. The postdoctoral research associate who was assigned to work on this project departed in April 2017, and a new researcher arrived in mid-July, with a delay while they familiarized themselves with the project.

To ensure that the putative antisera would capture the range of LChV-2 isolates present in the U.S, and from countries that send propagative material into the U.S., it was first necessary to examine the genetic diversity of LChV-2. Using a total of 48 isolates sourced from grower and nursery samples in Washington and Oregon, as well as samples from international locations, including Europe and Asia, we performed Single-Stranded Conformation Polymorphism (SSCP) analysis to examine genetic diversity. We found that despite their being nine distinct haplotypes based on polymerase sequence, there was only one haplotype for the coat protein (CP). This suggests that this gene is extremely conserved, as expected for a structural protein, and that antisera raised against any particular variant should, theoretically, detect all known isolates.

With this information we have begun constructing the expression cassette. Primers were designed to amplify the duplicated 35s promoter and NOS terminator sequences, with a linker sequence containing a polyhistidine tag for protein affinity-purification, and unique restriction enzyme site to allow later modification or replacement of the LChV-2 CP gene. Complimentary primers were designed for the LChV-2 CP gene, and were used to amplify this gene from Washington isolate LC-5; the 35s and NOS terminator were amplified from plasmid pCAM-CTV-p65, described in Killiny et al. (2016). Overlap PCR was used, using both pairwise and complete approaches, to

assemble the three fragments. The assembled cassette was then ligated into the digested pCAMBIA 1380 vector. We found at this point that coat protein insert may be toxic to *E. coli*, suggested by low colony number and poor growth rates. We have therefore begun rebuilding the construct on a stepwise basis, inserting each of three components (35s promoter, CP, terminator) separately. This has necessitated redesign of the cloning/primer strategy with new, unique restriction enzyme sites. We are also exploring whether use of a different cloning vector, pRI-201-AN would be more effective and reliable than the current pCAMBIA vector. It is to be hoped that we will solve the cloning problems by May 2018 for subsequent protein expression.

Task 2: *Raise polyclonal antisera to these proteins, and test the efficacy of the received antisera.*

The raising of antisera against the expressed LChV-2 coat protein is contingent on completion of the first task, and submission of the expressed and purified proteins to a third-party antisera production company. This task will be begin once the proteins have been successfully expressed.

Task 3: *Develop and validate an ELISA protocol.*

As with the previous task, development of an ELISA protocol is contingent on the production of viable antisera against LChV-2. This task will be begin once the antibodies have been raised and received.

Timeframe for remaining activities:

May 2018 – Complete cloning and expression of LChV-2 coat protein.
Jun 2018 – Submit expressed protein for antisera production
Aug 2018 – Receive antisera, test efficacy.
Oct 2018 – Complete optimization and validation of the ELISA protocol.

Progress report for CDFA project: 170279000SA

Title of Proposed Research: Heat therapy and indexing of stone fruit and pome fruit cultivars

Proposed Duration: 2 Years **Start Date:** 7/1/17

Total Amount Requested: $30,000 **Department:** Plant Pathology

Principal Investigator: Dr. Scott Harper, Director CPCNW

This project was intended to support the development and distribution of virus-tested 'clean' plants for nurseries within the state of California for use in the state certification program. Each year, the CDFA Improvement Advisory Board (IAB) financially supported nominations for California industry members for submission to the Clean Plant Center Northwest at Washington State University's Irrigated Agriculture Research and Extension Center, at Prosser, WA.

Objective 1: *Perform assays to detect viruses and virus-like organisms in CDFA-IAB submitted stone and pome fruit selections*

In 2017 a total of nine selections were sponsored by the CDFA-IAB from three California-based nurseries, representing three stone fruits and six pome fruit cultivars. Buds from each were propagated on appropriate rootstocks after arrival and maintained under greenhouse conditions until the plants were of suitable size to begin the diagnostic process. Each submitted selection then underwent a series of ELISA and PCR analyses, high-throughput sequencing for known and unknown pathogens, as well as traditional herbaceous and 'woody' greenhouse indexing; pome fruit cultivars also entered field indexing for diseases of unknown etiology.

A total of two selections were found to be infected using molecular analyses, one stonefruit selection had *Cherry virus A* (CVA), whilst one pome fruit selection had *Apple stem grooving virus* (ASGV). No novel viruses were identified during testing, although the pome fruit field indexing will be only complete in September 2019 as it requires observation of fruit development on indicators.

Based on this data, a total of two selections, each from a different nursery, will move forward to heat therapy. The remainder will be retested by PCR in spring-summer of 2018 to ensure that no pathogens were missed, through low titer or long latency, during the initial screening. However, with the recent discovery of two new pome fruit infecting virus-like organisms in late 2017, all six selections will be screened for these new agents during early spring, and the number of plants requiring therapy may increase.

Objective 2: *Perform heat therapy on virus-infected selections to produce 'clean' plants.*

Heat therapy of the CVA infected stone fruit and ASGV infected pome fruit selection is scheduled to begin once the plants emerge from winter dormancy and begin growing. Given the small size of the mother plants and paucity of available tissue we anticipate that this will be performed beginning March 2018. The time a plant needs for heat treatment is variable, between 50-90 days

with some experimentation needed for each virus-cultivar combination, therefore we estimate June for completion. Pending the success of heat treatment, which will be confirmed by follow up testing, we anticipate that the stone fruit cultivars would be available for release in January of 2019, with the pome fruit being available for full release after September 2019.

Timeframe for remaining activities:

Mar 2018 – Begin heat therapy of infected selections, screen all pome selections for newly reported virus-like organisms.
Jun 2018 – Complete heat therapy, produce micro-grafts from treated trees. Secondary screening of uninfected selections for confirmation of virus-free status.
Aug 2018 – Screen micrografts for virus presence, repeat heat therapy if needed.
Mar 2019 – Secondary screening of heat-treated selections for confirmation of virus-free status.
Sept 2019 – Complete field indexing of pome fruit cultivars and release material to owners.

Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board

Impact of carbon-source selection on effectiveness of Anaerobic Soil Disinfestation in controlling *Agrobacterium tumefaciens*

Progress Report

August 2017

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Title: Impact of carbon-source selection on effectiveness of Anaerobic Soil Disinfestation in controlling *Agrobacterium tumefaciens*

1. Introduction

Preplant crown gall management strategies in California have been based on soil fumigation with methyl bromide (MeBr), 1, 3-dichloropropene (1, 3-D), chloropicrin and others (Epstein et al., 2008, Yakabe et al., 2010, Yakabe et al., 2012a, Strauss et al., 2015). However, international regulations have phased out MeBr production and subsequent measures have placed increasingly strict limitations its usage, allowing exemptions only for select crops. In addition, newly-implemented restrictions governing usage of 1, 3-D in California have also taken effect in 2017.

These increasingly stringent regulations have driven the development of alternative methods to manage soilborne pests. Practices such as soil steaming (Melander & Kristensen, 2011, Samtani et al., 2012), solarization (Stapleton, 2000, Simmons et al., 2013), soil flooding (Strandberg, 1987) and planting catch crops (Yamagishi et al., 1986, Evenhuis et al., 2004) are or have been used, but they have not been widely adopted due to high energy costs and practical limitations.

One approach that has gained popularity due to its relatively simple mode of implementation, adaptability to a variety of cropping systems and reduced environmental impact is anaerobic soil disinfestation (ASD). Independently developed as an alternative to soil fumigation in the Netherlands (Blok et al., 2000) and Japan (Momma et al., 2006), ASD incorporates elements of solarization and biofumigation to suppress soilborne phytopathogens. Since 2003, ASD has been used to control soil pathogens by berry growers in California (Shennan et al., 2014, Muramoto et al., 2016), who have approximately 1,000 acres of strawberries and cane berries now being produced annully

using ASD. Recent studies have also shown inhibition of fungal and nematode pathogens causing apple replant disease (Hewavitharana & Mazzola, 2016) and significant reductions in *A. tumefaciens* populations under walnut nursery conditions (Strauss et al., 2017).

ASD relies on incorporating a labile carbon source into infested soil which is then irrigated to field capacity and sealed under a gas-impermeable plastic tarp for several weeks to prevent gas exchange. This promotes the formation of anoxic soil conditions and favor the proliferation of anaerobic microbes (Strauss & Kluepfel, 2015). The synergistic effect of these processes is thought to be the driving force behind pathogen suppression, though the precise mechanism by which ASD functions is not entirely understood. As microbial respiration depletes soil oxygen, the treated area quickly becomes anaerobic, promoting proliferation of anaerobic organisms that consume the carbon amendments in the soil and generate metabolic waste products including short chain fatty acids (e.g. acetic, butyric and propionic) and other volatile organic compounds toxic to soilborne phytopathogens (Blok et al., 2000, Momma et al., 2006, Hewavitharana et al., 2014).

Much attention has been focused on identifying a single, broad spectrum C-source that is effective against many pathogens in various cropping systems. Rice bran is commonly chosen as a research model in California ASD studies because of its proven ability to reduce pathogen populations (Muramoto et al., 2014, Strauss & Kluepfel, 2015). However, rice bran and the associated irrigation and tarping supplies required for ASD drive the cost per acre to rates similar to, or higher than C35 applications. Consequently, it is necessary to identify other abundant, effective and cheap C sources.

The objectives of this study were to evaluate the efficacy of a variety of locally produced C-sources in the form of agricultural waste products, soil amendments and other materials as determined by their potential to 1) generate and sustain anaerobic soil conditions in a greenhouse environment and 2) reduce soil populations of *A. tumefaciens* in greenhouse ASD experiments.

2. Materials and Methods

Trial location and experiment design: Greenhouse experiments were conducted in an unlit, ventilated, climate controlled (80-85° F) chamber within a facility on the University of California Davis campus in Davis, CA, USA. Bioreactors were constructed from polyvinyl chloride (PVC) tubing and measured 12" in length with a diameter of six inches. A square piece of polyester mesh fabric was glued to the bottom of each unit and a small hole was cut in the mesh fabric to facilitate the insertion of soil sensors. Approximately 10 kg of Hanford sandy loam soil from a USDA-certified organic field at the Kearney Agricultural Center in Parlier, CA, USA was adjusted to 20% moisture content and packed tightly into each bioreactor. Subsequently, the upper 6" of soil was removed from the bioreactor and manually mixed with a carbon (C) source at a rate of 9 tons $acre^{-1}$.

Carbon sources: The C sources evaluated in this study were obtained from local industries and included: acid whey residue from a whey tank (AWT) and from a cheese vat (AWV), almond hulls (AH), almond shells (AS), a commercial biochar (BC) formulation, spent brewer's barley (BAR), used cooking oil (CO), 5% ethanol (EtOH), almond orchard wood (AOW), molasses (MOL), mustard seed meal (MSM), red grape pomace (RGP), rice bran (RB), tomato pomace (TP), trub slurry (TSL) containing inactivated pilsner yeast, walnut hulls (WH) and walnut shells (WS). Unamended

treatments where soil was not covered with plastic and was not irrigated were used as non-treated controls (NTC). RB-amended soil treatments that were either "not irrigated" (RB_{NW}), not covered with a plastic tarp (RB_{NT}) or were not irrigated or tarped (RB_{NTNW}) were also included to evaluate the efficacy of individual components of ASD.

Measurement of soil oxidation reduction potential: Oxidative reduction potential (ORP) was recorded by placing sensors (Sensorex, Garden Grove, CA, USA) at the boundary between non-amended and C source-amended soil (6"). Measurements were recorded hourly using CR1000 and CR10X dataloggers (Campbell Scientific, Logan, UT, USA) for 21 d. Five C sources were tested in each experiment with four replications per treatment. Each treatment was included in at least two independent experiments to provide a minimum of eight replications per treatment. No C source was added to "no treatment" control (NTC) pots, which were included in all experiments. Cumulative mV hr was quantified for each treatment by summing the difference between average mV hr measured hourly and the critical reduction potential (200 mV) indicating anaerobicity (Strauss et al., 2017)

Inoculum Preparation: Each C-source was assessed for its efficacy in reducing populations of *A. tumefaciens*. Inoculum was prepared as described in Strauss et al., (2017). Prior to inoculating soil with *A. tumefaciens,* soil from the UC Kearney Agricultural Center (Parlier, CA, USA) was adjusted to 20% moisture content with sterile water and baked twice at 85° C for 18 h. A previously constructed rifampicin-resistant mutant of *A. tumefaciens* 186r (Yakabe et al., 2014) was grown in 5 ml tryptic soy broth overnight in a rotary shaking incubator at 29° C. Cells were pelleted, washed three times and adjusted to 10^9 CFU ml^{-1} using a spectrophotometer. Inoculum was subsequently pipetted into a sealable plastic bag containing oven-sterilized soil to achieve a final inoculum density of 10^6 CFU g^{-1}. Soil was thoroughly mixed to ensure even distribution of bacterial cells. Inoculum pouches were prepared by adding 10 g of inoculated soil to 5" x 5" mesh squares then securing each pouch with nylon twine.

Pouches were placed at a depth of 6" and C source-amended soil was packed tightly, preserving approximately 1" headspace in each bioreactor. 200 ml of water was added to the surface and allowed to infiltrate the soil after which a 6" x 6" section of totally impermeable film (TIF, VAPORSAFE, Raven Engineered Films, Sioux Falls, SD, USA) was used to seal the opening of each bioreactor. Bioreactors were arranged in a completely randomized design within plastic bins (24" x 18" x 6") that were periodically flooded to maintain a water level of 1-2" above the base of each bioreactor for the duration of each experiment. Treatments where water was withheld following the initial surface irrigation (i.e. RB_{NTNW}, RB_{NW} and NTC) were placed on platforms that always remained above water level.

Quantification of *A. tumefaciens* and total bacterial populations post ASD: To assess the effect of ASD on populations of *A. tumefaciens* 186r and other soilborne bacteria, inoculum pouches were removed after 21 d and 1 g of soil was suspended in sterile water and vortexed for 5 m. One ml of this suspension was serially diluted and plated on tryptic soy agar (TSA) containing 100 mg L^{-1} cyclohexamide and 100 mg L^{-1} rifampicin using a spiral plater. To assess the effect of ASD treatments on total soil

bacterial populations, 1 ml of the soil suspension was also plated on 10% TSA containing cyclohexamide 100 mg L^{-1}. All culture plates were incubated at 28° C and bacterial colonies on 10% TSA and *A. tumefaciens* colonies on TSA were counted with an automated colony counter (Flash and Go, IUL, Barcelona, Spain) after 24 h and 48 h, respectively. Colony PCR was performed on 2-3 colonies from each ASD treatment to confirm of *A. tumefaciens* 186r identity. Additional confirmations were made with Benedict's reagent (Bernaerts & De Ley, 1963) and by streaking colonies on tellurite-amended (60 µg ml^{-1}) semi-selective medium 1A (Mougel et al., 2001).

3. Results

Of the solid C sources tested, all but AH, AS, AOW and BC had C/N ratios of less than 30:1. As a general trend, the C sources which were most effective (as determined by microbial enumeration and soil Eh measurements) had higher levels of total NPKS than ones which were least effective. For the solid C sources, the percentage of total P, K and S was highest in RB, RGP and MSM, respectively. WS had the lowest percentage of total P and S and total K was lowest in B. All of the liquid substrates tested (AWT, AWV and M) had C/N ratios of less than 25:1. Total P, K and S was uniformly highest in MOL.

Cumlative mV hrs Results: Soil from ASD treatments amended with RB, MOL, TP, TSL, MSM, AH, AS, BAR, RGP, AS, ETOH and CO generated significantly more mean cumulative Eh mV hrs (CEmVh) than non-amended and non-tarped NTC soils, but means among individual treatments did not differ significantly (Fig 2). No differences in CEmVh were observed among the C sources that did not differ significantly from NTC (AOW, AWT, AWV, BC, WH, WS, RB_{NT}, RB_{NW} and RB_{NTNW}). In general, effective carbon-sources induced a sharp increase in anaerobic conditions during the first three days of ASD followed by consistent anaerobic levels for the duration of the experiment. All treatments except RB_{NTNW} and NTC generated mean CEmVh greater than zero. The strongest anaerobic soil conditions occurred in RB and RGP, with 201,000 and 197,000 mean CEmVh, respectively. NTC and RBNTNW did not generate mean CEmVh greater than zero (-130,000 and -15,800, respectively) and did not become anaerobic during the course of the experiments. RB, TP, TSL, MSM, AH, BAR and RGP were the only treatments where all eight replications generated mean CEmVh greater than zero and exhibited consistent microbial kill. All replications remained aerobic in the NTC treatment. Variability among one or multiple replications within a treatment was regularly observed for the remaining C sources. For example, MOL, ETOH, CO and RB_{NT} each had one replication which remained aerobic and AOW, AS, AWT, AWV, BC, RB_{NW}, RB_{NTNW}, WH and WS each had two or more replications which remained aerobic.

***A. tumefaciens* and total bacterial populations post ASD:** ASD treatment had a significant effect on the survival of *A. tumefaciens* and total bacterial colonies recovered from inoculum pouches. Fourteen treatments (AH, AS, AWT, BAR, CO, ETOH, MOL, MSM, RB, RB_{NT}, RGP, TP, TSL, and WS) all significantly reduced populations of *A. tumefaciens* compared to NTC. However, 8 of the 14 c-sources had exhibited superior performance in terms of pathogen kill and cumulative Eh hours. Those 8 carbon sources were RB, MOL, TP, TSL, MSM, AH, BAR and RGP.

ASD treatment also impacted the survival of total soil bacteria, though markedly less than on *A. tumefaciens*. MSM, RB, MOL, TP and RGP were not statistically different from one another and significantly reduced total soil bacterial populations as compared to the other carbon sources and the non-treated control. MSM and RB had the strongest suppressive effect, reducing populations by 32-33% compared to NTC, whereas while the remaining C sources only reduced populations by 15% or less. Additionally, MSM and RB both significantly reduced total bacterial colonies compared to AWT, AWV, RBNTNW, WH and WS by 31% or less.

Discussion/Key points

The type of carbon source used in the process of Anaerobic Soil Disinfestation has a dramatic influence on a number of key parameters. These include, cumulative Eh mV hours and microbial kill. In general, the greater the N content of the carbon-source, the greater the effectiveness during the ASD process. Of the 17 carbon-sources examined, 8 carbon sources (RB, MOL, TP, TSL, MSM, AH, BAR and RGP) were found to be statistically superior in terms of their ability to kill the target pathogen *Agrobacterium tumefaciens* and establish and maintain effective anaerobic conditions. Interestingly, the other less effective nine carbon sources, while effective in a few replications, were much more variable and inconsistent in their effectiveness. Given the consistency of the top 8 carbon sources in microbial kill we are confident in the quality of the data coming out of the in vitro microcosms used in these trials. Consequently, the inconsistent effectiveness of the nine carbon sources appears to be a function of the given carbon source and not a characteristic of the experimental system. However, even though a given carbon source performed poorly/inconsistently, it may not be an inherently poor C-source, it may just indicate we need to more clearly understand the mechanisms of ASD in order to modify the system to enhance effectiveness of these "less-effective" c-sources. For example, if the C/N ratio is a key driver in the system, it may be possible to add or delete a key factor by making simple inexpensive amendments to the c-source prior to soil incorporation. This would greatly expand the available c-source options that will drive down costs and facilitate year-round availability.

The this relative ranking of c-source effectiveness, as revealed by our in vitro tests, has laid the ground work for us to begin larger scale field trials to confirm these rankings in a variety of conditions with a variety of crop plants. In addition, given the fact that the cost of many of these top 8 agricultural waste carbon-sources are less than rice bran, the current gold standard for ASD, growers and nursery operators now have other cost effective options from which to choose. In addition, given the apparent similar level of effectiveness of these top 8 carbon sources, growers will now have options should a given carbon source be of limited supply at a given time of year.

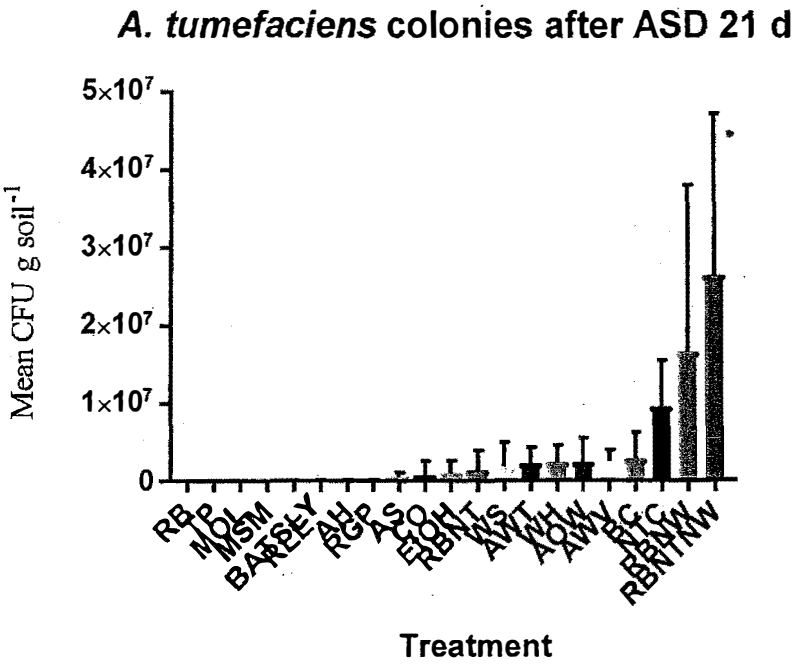

Figure 1.

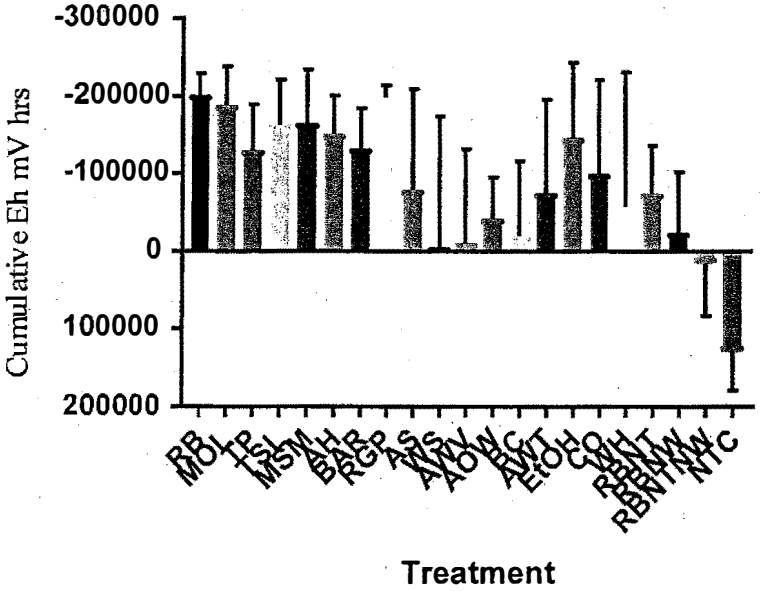

Figure 2.

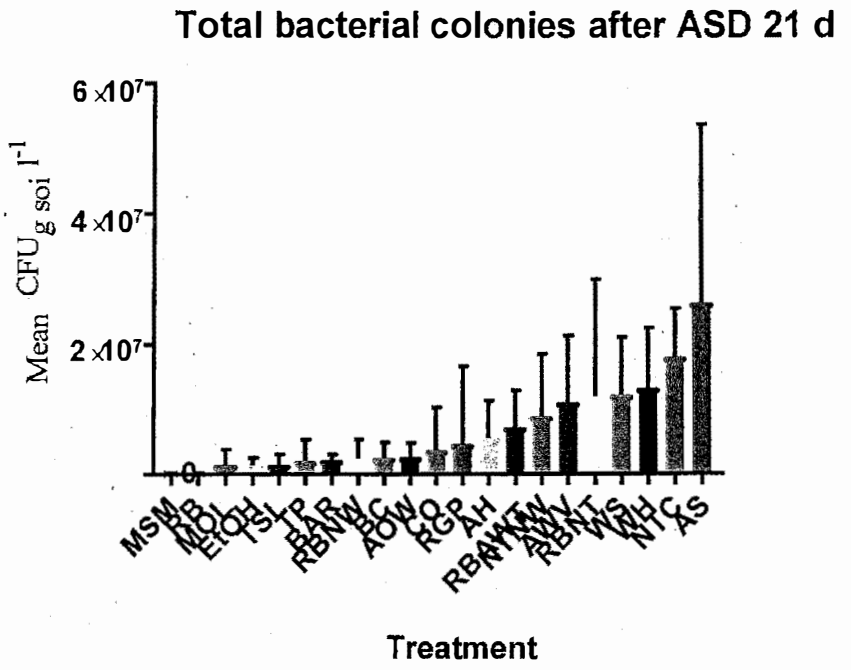
Total bacterial colonies after ASD 21 d
6 x10⁷
4 x10⁷
2 x10⁷
Mean CFU g soi l⁻¹
Treatment

Figure 3.

Legends:

Figure 1. The impact of carbon-source on *Agrobacterium tumefaciens* populations post ASD. See materials and methods in text for carbon source codes indicated across the x-axis. Y-axis represents the total colony forming units (CFU)/ g soil. Error bars represent ±1 SD

Figure 2. Cumulative Eh mV hrs measured over the 21 day ASD period for each carbon source. See materials and methods in text for carbon source codes indicated across the x-axis. Values above the "midline" (0) represent anaerobic conditions. Values below the mid line represent aerobic conditions. Error bars represent ±1 SD

Figure 3. The impact of carbon-source on total aerobic bacterial populations post ASD. See materials and methods in text for carbon source codes indicated across the x-axis. Y-axis represents the total colony forming units (CFU)/ g soil. Error bars represent ±1 SD

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Progress report on a research grant proposal to: Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB)

April 2, 2018

Project Title: Study of the Effects of Red Blotch Disease on Different Grapevine Rootstocks and Different *Vitis vinifera* Plants

Fiscal Year and Project Duration: Fifth year of a 6 year project 170423000SA

Project Leader: Deborah Golino, Department of Plant Pathology/Foundation Plant Services, University of California, One Shields Avenue, Davis, CA 95616
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Objectives:

1. To evaluate the effects of "Red Blotch" disease on 10 different popular grapevine rootstocks. All rootstocks will be grafted with a scion variety from the same accession of Cabernet Sauvignon.
2. To evaluate the effects of "Red Blotch" disease on 12 different popular grapevine scion varieties (*V. vinifera*), scions will be propagated on two different rootstocks of 101-14 and St. George 18.
3. To evaluate the effects of 'Red Blotch" disease on 7 different rootstocks only, no scion grafted. This experiment was added to the project in 2016.

Accomplishments:

Objective 1 and 2:

Samples were collected from the 10 rootstocks listed for this project including: Couderc 3309, Freedom, 101-14 MGT, 420A MGT, 039-16, 1103P, RS-3, 140Ru, Salt Creek and St. George 18 and the 12 scion varieties of: Primitivo, Cabernet franc, Cabernet Sauvignon, Merlot, Syrah, Pinot Noir, Chardonnay, Durif, Petite Verdot, Zinfandel, Thompson Seedless and Flame Seedless and tested for the full panel of grapevine viruses by RT-qPCR to check their freedom from these viruses. Canes were collected from these source vines and proceeded with bench grafting. Bench grafting was completed in the spring of 2014 and rooted plants were delivered in the month of July (bench grafting services was generously provided by Martinez Orchards, Inc.). Total number of bench grafted plants were 1052 plants. In August 2014, Half of the Cabernet Sauvignon plants on 10 different rootstocks were chip bud inoculated into the rootstock from sources either infected with Grapevine red blotch virus (GRBV) or a healthy source (negative control) (Table 1). The plants were kept in the greenhouse for approximately a month

for the chip bud to heel and after acclimatization for a short period, the buds take were inspected and recorded and planted in the field. At the same time, half of the 12 grape cultivars propagated on two different rootstocks of 101-14 and St. George 18 were also chip bud inoculated and planted in the field as described above (Table 2).

All non-inoculated plants (the second halves of Cabernet Sauvignon on 10 different rootstocks and the 12 grape cultivars propagated on two different rootstocks of 101-14 and St. George 18) were also planted in the field. We let these plants grow for a year in the field and in July-August 2015 they were inoculated by chip budding from GRBV and healthy sources into the scion.

The field was prepared before planting using the California Sprawl trellis system with a cordon wire at 46 inches and a single foliage catch-wire at 64 inches and a drip irrigation system.

Table 1: Shows number of Cabernet Sauvignon plants produced on each of the 10 rootstocks. Half of the plants were chip bud inoculated on the rootstock (RS inoc.) from sources infected with *Grapevine red blotch virus* (RB) or healthy, virus-free source (H) and planted in the field. The other half (Scion inoc.) were planted in the field and let them to grow through the growing season and in July-August of 2015 the scion part of the plants was inoculated with the RB and H sources.

Rootstock	Inoculum. source	RS inoc.	Scion inoc.
3309C	RB	10	10
	H	8	8
Freedom	RB	10	10
	H	6	5
101-14	RB	8	8
	H	4	4
420A	RB	6	6
	H	6	0
03916	RB	10	10
	H	8	8
1103P	RB	10	10
	H	8	8
RS-3	RB	9	9
	H	5	5
140R	RB	10	10
	H	6	6
Salt Creek	RB	8	8
	H	4	4
St. George 18	RB	10	10
	H	8	8

Table 2: Shows the list of the number of plants produced from grapevine scion cultivars propagated on two different rootstocks of 101-14 and St. George 18. Half of the plants were chip bud inoculated on the rootstock (RS inoc.) from sources infected with *Grapevine red blotch virus* (RB) or healthy, virus-free source (H) and planted in the field. The other half (Scion inoc.) were planted in the field and let them to grow through the growing season and in July-August of 2015 the scion part of the plants was inoculated with the RB and H sources.

Scion cultivars	101-14 Mgt			St. George 18	
	Inoc. source	RS inoc.	Scion inoc.	RS inoc.	Scion inoc.
Cab franc 01	RB	10	10	10	10
	H	7	8	8	8
Chardonnay 04	RB	8	8	9	9
	H	4	4	5	5
Cab Sauv 04	RB	8	8	10	10
	H	4	4	8	8
Flame Sdless 01	RB	10	10	10	10
	H	8	7	8	8
Merlot 03	RB	10	10	10	10
	H	8	8	8	8
Pinot Noir 2A	RB	10	10	10	10
	H	7	8	8	8
Durif 03	RB	7	8	10	10
	H	4	3	6	8
Petite Verdot 02	RB	9	9	9	10
	H	4	4	8	8
Syrah 08	RB	10	10	10	10
	H	7	8	8	8
Thomson Sdless 02A	RB	10	10	10	10
	H	8	8	8	8
Zinfandel 01A	RB	8	10	10	10
	H	5	4	7	8
Primitivo 03	RB	5	5	10	10
	H	0	4	8	8

In October-November 2016 leaf samples were collected from each vine in the experiment (total of 1063 plants) and tested by real time PCR (qPCR) for the presence and movement of the virus (Tables 3 rootstock trial and Table 4 scion trial). To mention, we used two different sources of GRBV, Chardonnay 41 (Ch. 41) and Orange Muscat 02 (OM 02), because enough wood material was not available from a single source for inoculation. Both sources were repeatedly tested by qPCR for the presence of the virus. The healthy source for inoculation was Pinot Noir 90 (PN 90) from the foundation collection. However, the efficiency of virus movement from these two sources were different. The 2016 qPCR data showed 94% of the plants inoculated with the virus from OM 02 source were tested positive by qPCR while only 62% of the plants inoculated from Ch 41 source were positive. In the scion trial (Table 4), Cab. Sauv. on two different rootstocks of 101-14 and St. George 18 were inoculated with the OM 02

virus source and the transmission success rate was 94% (34 from 36 inoculated plants tested positive), but in the rootstock trial (Table 3) the transmission success of Ch. 41 virus source to Cab. Sauv. propagated on the same rootstocks of 101-14 and St. George 18 was 64% (22 from34 plants tested positive). The virus load from the two inoculum sources and the sequence identity between the two GRBV isolates (Ch. 41 and OM 02) were investigated by high throughput sequencing (HTS). The sequence data showed that the nucleotide identity between the two isolates were very similar and had 98% identity. However, the data showed that the OM 02 source in addition to GRBV was also infected with *Grapevine syrah virus 1* (GSyV-1) and two viroids (*Hop stunt viroid* and *Grapevine yellow speckle viroid*). Ch. 41 source was infected with GRBV and *Grapevine yellow speckle viroid*. It is not clear yet if GSyV-1 has any interaction with GRBV in OM 02 inoculum source and helping in the movement of red blotch associated virus. In 2017 446 vines were selected and retested by qPCR for the virus. This test included all Cab. Sauv. plants on 10 different rootstocks (279 plants, Table 1), and the remaining were mostly the plants in the neg. or H group which produced inconsistent test results in 2016, all plant in the neg. or H group which tested positive in 2016 and more randomly selected vines in the control group to check for the spread of the virus in the vineyard by biological vector. The combination test results from 2016 and 2017 are presented in Tables 3 and 4 (No qPCR++ column).

In 2016 RT-qPCR testing of the collection, we found total of 8 plants inoculated with healthy bud wood (PN90/neg) or not inoculated (NI) were tested positive by PCR. This number in 2017 testing increased to 11 (Tables 3 and 4 presented as red fonts). The possible explanation for these results: 1) error in planting and labeling the vines in the vineyard, 2) laboratory error in sampling and testing the plants, 3) possibility of spread in the vineyard by the vector. However, the increased number from 8 to 11 plants in 2017 will indicate the high possibility of the spread of the virus in the field by the biological vector.

Symptoms expression on Cab. Sauv. propagated on different rootstocks (Table 3) and on different scion varieties propagated on two different rootstocks of 101-14 and St. George 18 were rated and recorded. The symptoms severity on different vines were rated on the scale of 1 to 4 (Fig. 1). Scale 1 when less than ¼ of the vine canopy showing symptoms, scale 2 when leaves on more than a quarter and less than ½ of the vine canopy on both cordons were symptomatic, scale 3 when leaves on more than half and less than ¾ of the vine canopy were symptomatic and scale 4 when the leaves on more than 3/4 of the vine canopy were symptomatic (Tables 3 and 4, Sym. Rating). Among all scion cultivars tested, Cab. franc was the one showing the most severe leaf symptoms, then in order of less severity: Merlot and Zinfandel; Primitivo; Pinot Noir and Syrah; Cabernet Sauvignon, Durif and Petit Verdot (Table 4 and Fig. 2). Flame seedless and Thompson Seedless were asymptomatic (Table 4). Symptoms on white varieties were rated based on leaf scorching. Severe leaf scorching symptoms were observed on Chardonnay cultivar and less sever leaf scorching on Pinot Noir and Zinfandel (Table 4 and Fig. 2).

In January 2018 the pruning weight was measured and recorded and its analysis is in progress.

Table 3: qPCR and symptom evaluation of Cabernet Sauvignon 04 (Cab Sauv 04) bench grafted on 10 different rootstocks and chip bud inoculated on the rootstock portion (RS) or scion (SC) of the plants with GRBV-infected Chardonnay 41 (Ch. 41), from a healthy source Pinot Noir 90 (PN 90) as control or not inoculated (NI). The Table shows number of plants inoculated per treatment (No. inoc), number of plants tested positive by qPCR (No. qPCR^{++}) for GRBV, number of plants which are showing characteristic red blotch symptoms (No. Sym.$^{++}$) and symptom rating (Sym. Rating).

Scion	Rootstock	Inoc. Site	No. inoc.	Inoc. Source	No. qPCR^{++}	No. Sym.$^{++}$	Sym. Rating
Cab Sauv 04	3309C 05	RS	10	Ch. 41/pos	9	6	1
Cab Sauv 04	3309C 05	SC	10	Ch. 41/pos	10	9	1
Cab Sauv 04	3309C 05	Rs	8	PN90/neg	0	0	0
Cab Sauv 04	3309C 05	NI	8	-	0	0	0
Cab Sauv 04	Freedom 01	RS	10	Ch. 41/pos	9	8	1-2
Cab Sauv 04	Freedom 01	SC	9	Ch. 41/pos	9	9	1
Cab Sauv 04	Freedom 01	RS	6	PN90/neg	0	0	0
Cab Sauv 04	Freedom 01	NI	3	-	0	0	0
Cab Sauv 04	101-14 01	RS	8	Ch. 41/pos	8	7	1
Cab Sauv 04	101-14 01	SC	8	Ch. 41/pos	6	6	1
Cab Sauv 04	101-14 01	RS	4	PN90/neg	1	0	0
Cab Sauv 04	101-14 01	NI	4	-	0	0	0
Cab Sauv 04	420A 04	RS	5	Ch. 41/pos	5	5	1
Cab Sauv 04	420A 04	SC	6	Ch. 41/pos	5	5	1
Cab Sauv 04	420A 04	RS	6	PN90/neg	1	0	0
Cab Sauv 04	039-16 01	RS	10	Ch. 41/pos	10	10	1
Cab Sauv 04	039-16 01	SC	9	Ch. 41/pos	9	9	1
Cab Sauv 04	039-16 01	RS	8	PN90/neg	3	1	2?
Cab Sauv 04	039-16 01	NI	8	-	0	0	0
Cab Sauv 04	1103P 01	RS	9	Ch. 41/pos	9	8	1
Cab Sauv 04	1103P 01	SC	10	Ch. 41/pos	10	10	1
Cab Sauv 04	1103P 01	RS	8	PN90/neg	3	2	1-2?
Cab Sauv 04	1103P 01	NI	7	-	0	0	0

Cab Sauv 04	RS-3 01.1	RS	8	Ch. 41/pos	7	6	1
Cab Sauv 04	RS-3 01.1	SC	8	Ch. 41/pos	8	8	1
Cab Sauv 04	RS-3 01.1	RS	5	PN90/neg	1	1	1?
Cab Sauv 04	RS-3 01.1	NI	3	-	0	0	0
Cab Sauv 04	140R 01	RS	9	Ch. 41/pos	9	7	1
Cab Sauv 04	140R 01	SC	10	Ch. 41/pos	10	9	1
Cab Sauv 04	140R 01	RS	6	PN90/neg	0	0	0
Cab Sauv 04	140R 01	NI	6	-	0	0	0
Cab Sauv 04	Salt Crk 08	RS	8	Ch. 41/pos	8	6	1
Cab Sauv 04	Salt Crk 08	SC	8	Ch. 41/pos	8	7	1
Cab Sauv 04	Salt Crk 08	RS	4	PN90/neg	0	0	0
Cab Sauv 04	Salt Crk 08	NI	4	-	0	0	0
Cab Sauv 04	St. G. 18	RS	10	Ch. 41/pos	10	10	2
Cab Sauv 04	St. G. 18	SC	9	Ch. 41/pos	9	9	1
Cab Sauv 04	St. G. 18	RS	7	PN90/neg	0	0	0
Cab Sauv 04	St. G. 18	NI	8	-	0	0	0

Table 4: qPCR and symptom evaluation of 12 scion varieties each bench grafted on two different rootstocks of 101-14 01 and St. George 18. The plants were chip bud inoculated on the rootstock portion of the plants (RS) or scion (SC) with GRBV-infected Orange Muscat 02 (OM 02). Controls were chip buds from a healthy source Pinot Noir 90 (PN 90) or not inoculated (NI) plants. Only Durif 03 plants were inoculated with the Chardonnay 41 (Ch 41) virus source. The Table shows number of plants inoculated per treatment (No. inoc), the sources used for inoculation (Inoc. Source), number of plants tested positive by qPCR (No. qPCR^{++}) for GRBV, number of plants which are showing characteristic red blotch symptoms (No. Sym.$^{++}$) and symptom rating (Sym. Rating). Leaf scorching symptom (LS) was also recorded.

Scion	**Rootstock**	**Inoc. Site**	**No. Inoc.**	**Inoc. Source**	**No. qPCR^{++}**	**No. Sym.$^{++}$**	**Sym. Rating**
Cab. franc 01	101-14 01	RS	10	OM 02	9	9	3
Cab. franc 01	101-14 01	SC	10	OM 02	10	10	4
Cab. franc 01	101-14 01	RS	7	PN 90	0	0	0
Cab. franc 01	101-14 01	NI	8	-	0	0	0
Cab. franc 01	St. G. 18	RS	10	OM 02	10	10	4
Cab. franc 01	St. G. 18	SC	10	OM 02	10	10	3-4
Cab. franc 01	St. G. 18	RS	7	PN 90	0	0	0

Cab. franc 01	St. G. 18	NI	8	-	0	0	0
Chard. 04	101-14 01	RS	8	OM 02	8	8	3-4 LS
Chard. 04	101-14 01	SC	8	OM 02	8	8	3-4 LS
Chard. 04	101-14 01	RS	4	PN 90	2	2	1-2 LS
Chard. 04	101-14 01	NI	4	-	0	0	0
Chard. 04	St. G. 18	RS	9	OM 02	9	6	1 LS
Chard. 04	St. G. 18	SC	9	OM 02	9	9	2-3 LS
Chard. 04	St. G. 18	RS	5	PN 90	0	0	0
Chard. 04	St. G. 18	NI	4	-	0	0	0
Cab. Sauv. 04	101-14 01	RS	8	OM 02	8	7	1
Cab. Sauv. 04	101-14 01	SC	8	OM 02	8	7	1
Cab. Sauv. 04	101-14 01	RS	4	PN 90	0	0	0
Cab. Sauv. 04	101-14 01	NI	4	-	0	0	0
Cab. Sauv. 04	St. G. 18	RS	10	OM 02	10	9	1
Cab. Sauv. 04	St. G. 18	SC	10	OM02	10	10	1
Cab. Sauv. 04	St. G. 18	RS	8	PN 90	0	0	0
Cab. Sauv. 04	St. G. 18	NI	7	-	0	0	0
Flame Sdl 01	101-14 01	RS	10	OM 02	10	2	1
Flame Sdl 01	101-14 01	SC	10	OM 02	10	0	0
Flame Sdl 01	101-14 01	RS	8	PN 90	0	0	0
Flame Sdl 01	101-14 01	NI	7	-	0	0	0
Flame Sdl 01	St. G. 18	RS	10	OM 02	10	0	0
Flame Sdl 01	St. G. 18	SC	10	OM 02	10	0	0
Flame Sdl 01	St. G. 18	RS	8	PN 90	0	0	0
Flame Sdl 01	St. G. 18	NI	8	-	0	0	0
Merlot 03	101-14 01	RS	10	OM 02	9	9	3
Merlot 03	101-14 01	SC	10	OM 02	10	10	3-4
Merlot 03	101-14 01	RS	8	PN 90	0	0	0
Merlot 03	101-14 01	NI	8	-	0	0	0
Merlot 03	St. G. 18	RS	9	OM 02	9	9	3.4
Merlot 03	St. G. 18	SC	10	OM 02	10	10	2
Merlot 03	St. G. 18	RS	8	PN 90	0	0	0
Merlot 03	St. G. 18	NI	7	-	0	0	0

Pinot Noir 2A	101-14 01	RS	10	OM 02	9	9	2-3 also LS
Pinot Noir 2A	101-14 01	SC	10	OM 02	10	10	3-4
Pinot Noir 2A	101-14 01	RS	5	PN 90	0	0	0
Pinot Noir 2A	101-14 01	NI	8	-	0	0	0
Pinot Noir 2A	St. G. 18	RS	10	OM 02	10	10	1-2
Pinot Noir 2A	St. G. 18	SC	9	OM 02	9	9	2-3
Pinot Noir 2A	St. G. 18	RS	7	PN 90	0	0	0
Pinot Noir 2A	St. G. 18	NI	8	-	0	0	0
Durif 03	101-14 01	RS	7	Ch. 41	7	7	1
Durif 03	101-14 01	SC	7	Ch 41	7	7	1
Durif 03	101-14 01	RS	4	PN 90	0	0	0
Durif 03	101-14 01	NI	3	-	0	0	0
Durif 03	St. G. 18	RS	10	Ch. 41	10	10	1
Durif 03	St. G. 18	SC	10	Ch 41	10	9	1-2
Durif 03	St. G. 18	RS	6	PN 90	0	0	0
Durif 03	St. G. 18	NI	8	-	0	0	0
Pet. Verdot 02	101-14 01	RS	9	OM 02	5	7	1
Pet. Verdot 02	101-14 01	SC	9	OM 02	9	9	1
Pet. Verdot 02	101-14 01	RS	4	PN 90	0	0	0
Pet. Verdot 02	101-14 01	NI	4	-	0	0	0
Pet. Verdot 02	St. G. 18	RS	8	OM 02	8	9	1
Pet. Verdot 02	St. G. 18	SC	9	OM 02	9	9	1-2
Pet. Verdot 02	St. G. 18	RS	8	PN 90	0	0	0
Pet. Verdot 02	St. G. 18	NI	8	-	0	0	0
Syrah 08	101-14 01	RS	10	OM 02	10	10	2
Syrah 08	101-14 01	SC	10	OM 02	10	10	2
Syrah 08	101-14 01	RS	7	PN 90	0	0	0
Syrah 08	101-14 01	NI	7	-	0	0	0
Syrah 08	St. G. 18	RS	10	OM 02	10	10	2-3
Syrah 08	St. G. 18	SC	10	OM 02	9	9	2-3
Syrah 08	St. G. 18	RS	8	PN 90	0	0	0
Syrah 08	St. G. 18	NI	8	-	0	0	0
Thom. Sdl 2A	101-14 01	RS	10	OM 02	9	0	0

Thom. Sdl 2A	101-14 01	SC	10	OM 02	10	0	0
Thom. Sdl 2A	101-14 01	RS	8	PN 90	0	0	0
Thom. Sdl 2A	101-14 01	NI	8	-	0	0	0
Thom. Sdl 2A	St. G. 18	RS	9	OM 02	9	0	0
Thom. Sdl 2A	St. G. 18	SC	10	OM 02	10	0	0
Thom. Sdl 2A	St. G. 18	RS	8	PN 90	0	0	0
Thom. Sdl 2A	St. G. 18	NI	8	-	0	0	0
Zin. 01A	101-14 01	RS	8	OM 02	8	8	3-4 also LS
Zin. 01A	101-14 01	SC	10	OM 02	10	10	3-4
Zin. 01A	101-14 01	RS	5	PN 90	0	0	0
Zin. 01A	101-14 01	NI	3	-	0	0	0
Zin. 01A	St. G. 18	RS	10	OM 02	10	10	3-4
Zin. 01A	St. G. 18	SC	8	OM 02	7	8	3-4
Zin. 01A	St. G. 18	RS	7	PN 90	0	0	0
Zin. 01A	St. G. 18	NI	8	-	0	0	0
Primitivo 03	101-14 01	RS	5	OM 02	4	4	3
Primitivo 03	101-14 01	SC	5	OM 02	4	4	3
Primitivo 03	101-14 01	RS	4	PN 90	0	0	0
Primitivo 03	St. G. 18	RS	10	OM 02	10	10	4
Primitivo 03	St. G. 18	SC	10	OM 02	9	9	2-3
Primitivo 03	St. G. 18	RS	8	PN 90	0	0	0
Primitivo 03	St. G. 18	NI	7	-	0	0	0

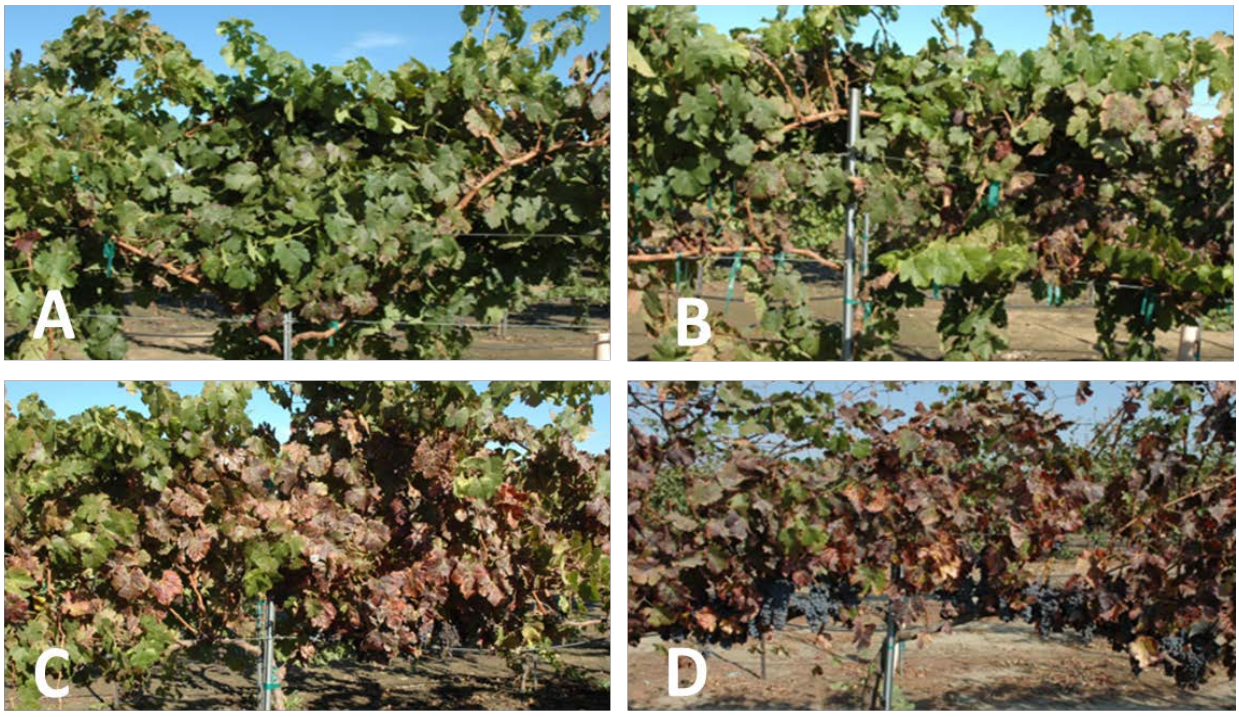

Fig. 1: Symptom expression was rated from scale 1 to 4. A) Scale 1 when ¼ or less of the vine canopy was showing symptos, B) Scale 2 between ½-1/4, C) Scale 3 between 2/3-1/2 and D) more than 2/3 of the canopy was showing symptoms.

Cab franc

Cab Sauv

Chardonnay

Merlot

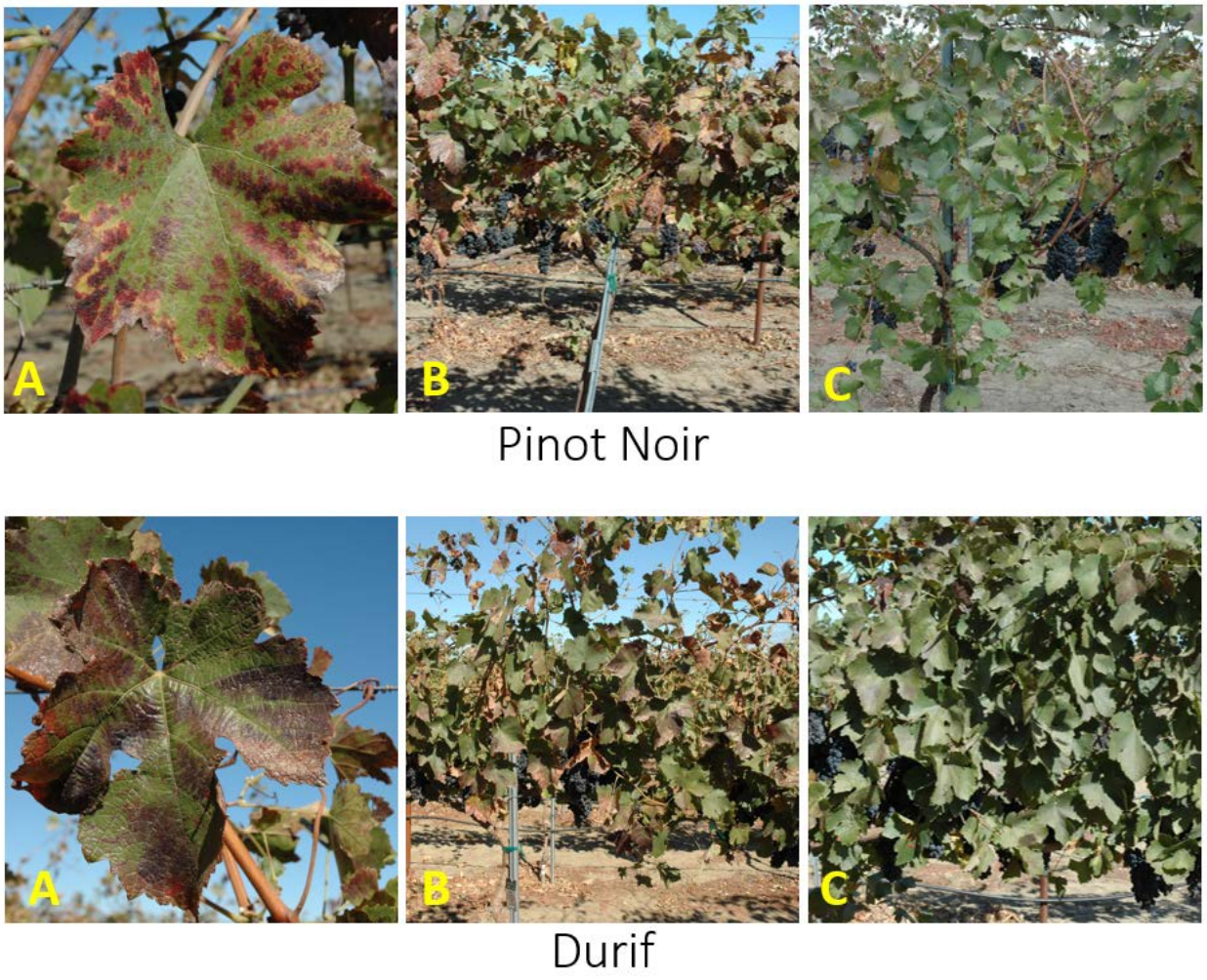

Fig. 2: Leaf symptom expression of GRBV on different grape varieties of Cab franc, Cab Sauvignon, Chardonnay, Merlot, Pinot Noir and Durif. A and B are leaf symptoms from virus-inoculated plants and C is healthy plant.

Objective 3:

In 2016 we added 7 different popular rootstocks to the project in order to evaluate the effects of the GRBV on each one of these rootstocks. We designated and prepared land for planting the rootstocks for the experiment. In our recent qPCR testing data from Russell Ranch where FPS's protocol 2010 materials are maintained, we found in our 2017 testing of the vineyard that GRBV has spread by biological vector from neighboring backyard grown grapevines infected with the virus to the foundation vineyard. Based on this finding we decided to withhold on the experiment due to the proximity of the land from FPS greenhouse and shade house facilities. The designated land is close to the FPS facilities (approximately 200-300 yds) that the experimental plantings may put the materials kept in the facility in danger of becoming infected

with GRBV. The fund we received for this part of the project will be extended to 2018-2019 to cover the partial cost of the maintenance and the evaluation of the vine performances described in objectives 1 and 2.

In addition, in 2018-2019 funding cycle we decided not to apply for additional funds for the project. We will apply for an extension without charge to the CDFA to use the remaining funds from 2017-2018 to cover part of the cost. The remaining cost for the project for this period will be covered by FPS. In 2018 we will continue evaluating vine performance, yield, and juice composition for different scions and rootstock varieties used in this experiment.

Project Title: Study of the Effects of Red Blotch Disease on Different Grapevine Rootstocks and Different *Vitis vinifera* Plants

Project Leader: Deborah Golino

Summary:

To date more than 75 different graft-transmissible agents including viruses have been reported in grapevine. More recently a new virus, named *Grapevine red blotch virus* (GRBV) was found in grapevine and as its name indicates this virus was found to be associated with red blotch type symptoms in red grape varieties. The virus also has been reported in white grape varieties with undefined leaf symptoms. The virus likely can be found in all types of grape cultivars and hybrids including: rootstocks, wine grapes, table- and raisin grapes. However, the associated virus has been sequenced and its genome has been characterized. How and to what extent the red blotch disease affects the performance of vines propagated on different rootstocks and on different grapevine scion varieties is not clear yet and much needed information is missing. This project is planned to study the effects of GRBV on plants propagated on different rootstocks and also on different scion varieties (*Vitis vinifera*). In this project we have inoculated the GRBV onto Cabernet Sauvignon plants on 9 different rootstocks and onto 12 different scion varieties each on two different rootstocks. Due to the lack of enough virus-infected material from a single source plant for inoculation, we used the inoculum from two different sources of Orange Muscat 02 (OM 02) and Chardonnay 41 (Ch. 41) to inoculate the plants. All the Cabernet Sauvignon plants on 9 different rootstocks and the 12 scion varieties (total of 1052 plants) were planted in the field in 2015. All these plants were tested by real time PCR (qPCR) for GRBV to check the movement of the virus to the plants. The test results showed that 94% of the Cabernet Sauvignon plants on 9 different rootstocks and 97% of the 12 scion varieties on two different rootstocks of 101-14 and St. George 18 were positive for the virus. The qPCR test results also showed that 11 healthy control plants were also tested positive in 2017. Eight healthy control plants were tested positive in 2016 and this increment by 3 in 2017 is an indication of the presence of active biological vector that has spread the virus in the vineyard. Symptom expression on different scion varieties were rated from 1 to 4 and found that different varieties express different symptom severity. The symptom severity on different scion varieties in order were: Cabernet franc; Merlot and Zinfandel; Primitivo; Pinot Noir and Syrah; Cabernet Sauvignon, Durif and Petit Verdot. Severe leaf scorching symptoms were observed on Chardonnay cultivar and less sever leaf scorching on Pinot Noir and Zinfandel. In January 2018 the pruning weight was measured, recorded and the analysis is in progress.

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Synergy between grapevine vitiviruses and grapevine leafroll viruses

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Abstract An interactive relationship between vitiviruses and grapevine leafroll viruses was characterized in grapevine. *Grapevine viruses A* and *B* (GVA and GVB) were found more frequently in the presence of co-infecting *Grapevine leafroll associated viruses* (GLRaV-1, −2 or −3) than in their absence. The titers of the vitiviruses in co-infection with leafroll viruses were found to be higher than were their titers in the absence of leafroll virus infection. The occurrence of vitivirus-associated stem-pitting symptoms was correlated with leafroll virus co-infection. Specific pairing associations on the species level were found between different viti- and leafroll virus species: GVB was associated preferentially with GLRaV-2; GVA was associated preferentially with GLRaV-1 and GLRaV-3. In contrast to the increase in vitivirus titer seen with leafroll virus co-infection, the incidence and titer of grapevine leafroll virus appeared to be unaltered by vitivirus co-infection. The potential for a synergistic enhancement of grapevine disease in co-infected vines is discussed.

Keywords Grapevine leafroll virus · Grapevine vitivirus · *Grapevine virus A* · Viral synergy

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Introduction

Synergistic interactions between co-infecting pairs of plant viruses from different families can result in increased viral multiplication and disease symptom severity, compared to single virus infections (Syller 2012). The classic example is the interaction between *Potato virus Y* (PVY) and *Potato virus X* (PVX) in *Nicotiana tabacum* (Rochow and Ross 1955). In the co-infection, the PVX titer may increase ten-fold, while the PVY titer remains unchanged. This synergy involves an increase in disease severity compared to infections in which the viruses occur singly. In this report, we describe a similar synergy between grapevine leafroll viruses and grapevine vitiviruses.

The viruses associated with leafroll disease constitute a significant problem for vineyards worldwide (Maree et al. 2013). Interactions that potentiate leafroll virus pathogenesis could contribute to that problem. Interactions between *Grapevine leafroll associated viruses* (GLRaV) and vitiviruses have been reported (Fortusimi et al. 1997; Credi and Babini 1997; Golino et al. 2000; Mannini et al. 2003; Hommay et al. 2008; Komar et al. 2007, 2010; Santini et al. 2011). Their co-occurrence has been commonly observed (Namba et al. 1991; Goszczynski and Jooste 2003; Saldarelli et al. 2005; Le Maguet et al. 2012), and the possibility that the co-infection causes severe disease in *Vitis vinifera* has been raised. Credi and Babini (1997) noted that in mixed infections in grapevine, the most severe combinations involved *Grapevine virus A* (GVA) co-infecting with GLRaV spp. Monis and Bestwick (1997) associated graft

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incompatibility with the coinfection of *Grapevine virus B* (GVB) with GLRaV-2. Golino et al. (2000) considered the severity of GLRaV-2 coinfecting with GVB to be a possible synergy.

Proposed bases for such interactions have ranged from vector mealybug co-transmission of leafroll viruses with vitiviruses (Zorlini et al. 2006; Hommay et al. 2009; Bertin et al. 2016a, b) to interactions among viral gene products. The impact of these co-infections has been found to be dependent on the host varietal backgrounds, with infection severities influenced by rootstock choices (Golino et al. 2003; Komar et al. 2010).

We have tabulated the incidences of co-occurrence of vitivirus and leafroll virus species. In the subset of naturally occurring infections that contain pairings of either GVA or GVB together with either GLRaV-2 or (GLRaV-1 and or GLRaV-3) the distribution of species was found to be non-random. The asymmetric affiliation frequencies demonstrated specific associations between the two vitiviruses and their respective co-occurring leafroll virus partners.

Materials and methods

Plant material The grapevine accessions in the 435 plant sample (from which the analysis in Fig. 1 was derived) were from a wide variety of accessions screened by Foundation Plant Services (U. C. Davis, California) between 2007 and 2015. These plants were tested by PCR for grapevine leafroll viruses and grapevine vitiviruses. The vines in the 1048 plant sample (from which the analyses in Figs. 2 and 3 were derived) were members of a survey from a selection of producing commercial vineyards in California (Arnold et al. 2015). A 204 plant subset of the 435 plant sample was screened for the presence of GVB by graft inoculation to grapevine indicator host LN33 ((solonis x Othello) x *V. berlandieri*)

	GVA	GVB	none
GLRaV-2	**3**	**26**	31
GLRaV-3 and/or GLRaV-1	**89**	**4**	118
no leafroll virus	20	14	

Fig. 1 Analysis of the co-incidence of vitivirus and leafroll virus infection. Top left quadrant: number of vines (**in bold**) infected with either GVA or GVB, plus either GLRaV-2, or [GLRaV-1 and/or GLRaV-3]. Also shown: the number of vines infected singly with GLRaV-1 and/or GLRaV-3, GLRaV-2, GVA or GVB. None = no GVA or GVB

	GVA	GVB	none
GLRaV-2	**5**	**46**	46
GLRaV-3	**34**	**3**	121
no leafroll virus	2	2	

Fig. 2 Co-incidence analysis of infections in vineyard plants. Top left quadrant: number of vines (**in bold**) infected with either GLRaV-2 or GLRaV-3, plus either GVA or GVB. Also shown: the number of vines infected singly with GLRaV-2, GLRaV-3, GVA or GVB. None = no GVA or GVB

(minimum of 3 indicator plants each per test) to test for the induction of stem pitting symptoms.

Tabulation of co-infections Analysis of synergies between specific viti- and leafroll viruses entailed the characterization of plants carrying only one viti- and one leafroll virus (we found that the incidences of GLRaV-1 and GLRaV-3 could be combined for analysis, with their pooled numbers treated as a single leafroll virus). Such co-infected vines were identified as a subset of all of the infected plants in a sample. The incidence of specific associations between viti- and leafroll viruses was assessed by first scoring all plants in each sample for GVA, GVB, GLRaV-1, −2, and −3. Plants infected with GLRaV-2, or with GLRaV-1 and/or GLRaV-3 were identified in subsets of all tested plants that were singly-infected with either GVA or GVB. The identified double-infections fell into four categories: 1) those containing only GVA plus GLRaV-2; 2) those containing only GVB plus GLRaV-2; 3) those containing GVA plus GLRaV-1 and/or GLRaV-3; and 4) those containing GVB plus GLRaV-1 and/or GLRaV-3. The numbers of vines that were found to be singly infected by any of these viruses were also recorded in four categories: 5) GVA alone, 6) GVB alone, 7) GLRaV-2 alone, and 8) GLRaV-1 alone, GLRaV-3 alone, and GLRaV-1 plus GLRaV-3 co-infected. Preferential associations between specific viti- and leafroll virus species pairs were revealed from the numbers of plants in each of these four categories (see Figs. 1 and 2).

PCR analysis of viruses GVA, GVB, GLRaV-1, −2 and −3 were specifically detected by RT-PCR (Osman et al. 2008) in the analysis of the 435 plant sample characterized in Fig. 1. RT-qPCR (Al Rwahnih et al. 2012) was used to detect and quantitate these same virus species in the analyses of the 1048 plant sample characterized in Figs. 2 and 3 (GLRaV-1 was not detected in that sample).

	GVA	GVB
GLRaV-2	26.9	23.7
GLRaV-3	19.3	25.8
no leafroll	31.5	27.5

Fig. 3 Average Ct values for GVA or GVB, co-infected by GLRaV-2, or GLRaV-3, or by no leafroll virus

Relative fold increases (RI) in virus levels derived from the RT-qPCR analysis in Fig. 3, comparing one Ct value (Ct_1) to a second Ct value (Ct_2) were calculated as:

$$RI_{(1\ vs\ 2)} = 2^{(Ct_1 - Ct_2)}.$$

Results

Co-occurrence of vitiviruses and leafroll viruses Field-infected grapevines were analyzed by specific RT-PCR tests for the presence of grapevine leafroll viruses (GLRaV-1, −2, and −3) and grapevine vitiviruses (GVA and GVB). To assess whether or not infection by a specific leafroll virus species influences the possibility of infection by a specific vitivirus species, a subset of the multiply-infected plants was generated. This subset contained those plants that were infected by only two viruses, one member each of the vitiviruses and leafroll viruses (GLRaV-1 and -3 were pooled and treated statistically as a single virus in this analysis.)

Preferential pair-wise associations between specific viti- and leafroll virus species were revealed from the relative numbers of plants infected in each of the four possible cross-species pairings (see Figs. 1 and 2). The numbers of infected plants in each of the four categories in this subset are seen in bold in the upper left quadrants in Figs. 1 and 2. In Fig. 1 the top left quadrant tabulates the categories of 122 co-infections that make up the subset of plants singly infected with just one vitivirus and one leafroll virus species, abstracted from an initially 435 plant sample.

Analysis of the frequencies of the four possible pair-wise associations between vitiviruses and leafroll viruses (top left quadrant in Fig. 1) revealed that GVB associated with GLRaV-2. Similarly, GVA was seen to associate specifically with GLRaV-1 and GLRaV-3. GVA occurred with GLRaV-1 or −3 significantly more frequently than it occurred with GLRaV-2. GVB occurred nine times more frequently in the presence of GLRaV-2 than did GVA. The probabilities of these asymmetric association frequencies occurring by chance was very low, $p < 0.0001$ in chi^2 analysis.

Also shown in Fig. 1 is the numbers of plants in the total sample that were singly infected, either with grapevine leafroll or vitiviruses. These incidences show that vitiviruses occurred infrequently in the absence of leafroll viruses. Such single vitivirus infections were detected in only 34 plants in this survey. In comparison, single vitiviruses in coinfection with single leafroll viruses occurred in 122 plants (Fig. 1); infections containing a single leafroll virus and two vitiviruses occurred in another 51 plants in the total data set (data not shown).

A similar presentation of the data from another survey of 1048 vineyard plants is given in Fig. 2. This revealed an interaction between leafroll viruses and vitiviruses similar to that seen in Fig. 1. As in Fig. 1, the incidence of infection by vitivirus in the absence of leafroll virus was low ($n = 4$). The presence of GVA was elevated in the presence of GLRaV-3, occurring ~7 times more frequently ($n = 34$) than it occurred in the presence of GLRaV-2 ($n = 5$). GVB was found to occur in the presence of GLRaV-2 some 15 times more frequently ($n = 46$) than it occurred in the presence of GLRaV-3 (n = 3). (There were no GLRaV-1 infected plants identified in this field survey.)

In contrast to the vitiviruses, the incidence of infection with leafroll virus did not appear to be contingent upon the presence of vitivirus co-infection. In the survey represented in Fig. 2, there were 167 cases of leafroll virus infection in the absence of vitivirus co-infection, compared with 200 cases of leafroll virus infection in the presence of one or more vitiviruses (in the entire dataset).

Effect of co-occurrence with leafroll virus on the vitivirus titer In those few instances where vitiviruses occurred in the absence of leafroll viruses, the vitivirus titers were low. In Fig. 3, an average of the Ct values for all the GVA and GVB infections in the 1048 plant data set is tabulated. In that comparison, the relative vitivirus titer was found to be significantly higher in the presence of leafroll virus co-infection. The relative average GVA Ct value in plants co-infected with GLRaV-3 was 4700 fold lower than the average Ct value of GVA in plants in which leafroll virus was not detected. The average GVB Ct value in plants co-infected with GLRaV-2 was 14 fold lower than the value from GVB-infected plants in which

the leafroll virus was absent. And though GVB associated preferentially with GLRaV-2 and GVA associated preferentially GLRaV-3, a slight enhancement of the average vitivirus Ct value was also noted in the non-preferential associations, e.g., of GVB with GLRaV-3 and GVA with GLRaV-2.

In contrast, the titers of leafroll virus appeared unaffected by vitivirus co-infection. A tabulation of the relative GLRaV-3 titers in the 1048 plant sample found an average leafroll virus Ct value of 20.4 ($n = 128$) in the presence of vitivirus co-infection, and of 20.7 ($n = 129$) in the absence of co-infection.

Vitivirus infection at very low levels was occasionally detected by RT-PCR in the absence of leafroll virus infection (Fig. 3). However, we did not detect foliar disease symptoms associated with vitivirus infection at any level, unless those plants were also infected with leafroll virus. Those foliar disease symptoms in the co-infection were characteristic of leafroll virus infection symptoms.

Co-infection involved with rugose wood symptoms Rugose wood disease symptoms include pitting and cracking of the bark of hybrid grapevine indicator host LN33 infected by GVB (Martelli 1993). A 204 vine subset of the 435 plant sample was assessed for its capacity to induce stem pitting after graft inoculations from each vine to that indicator host. In this subset, five vines had tested positive for GVB by RT-PCR; grafts from all five of these vines induced stem-pitting symptoms on LN33. Four of those vines had been found by RT-PCR analysis to be co-infected with GLRaV-2. This showed that the induction of stem pitting symptoms by GVB on LN33 correlated with co-infection with GLRaV-2. One GVB RT-PCR positive vine was scored as positive for stem-pitting though that vine had tested negative for GLRaV-2 infection. That exception might be ascribable to non-specificity in the LN33 bioassay for stem pitting (see next section).

Note on the specificity of the stem pitting bioassay for GVB on grapevine hybrid LN33

From the 204 vine subset of plants that were inoculated to host LN33, most of the positive stem symptoms tests were from source plants that tested negative for GVB. 17 of the source plants that tested positive for GVA but negative for GVB induced stem pitting symptoms on the indicator host. Though the stem pitting response of LN33 is considered diagnostic for GVB infection, the induction of the response by GVA shows a lack of vitivirus specificity for this bioassay. 15 of those 17 GVA infected source plants also tested positive for GLRaV-1 and /or GLRaV-3. That showed that the induction of stem pitting symptoms by GVA on LN33 correlated with co-infection with GLRaV-1 and GLRaV-3. None of the co-infected LN33 plants that showed stem symptoms were found to have been inoculated with the reciprocal pairings, e.g., GVA with GLRaV-2, or GVB with GLRaV-1 or GLRaV-3.

In the bioassay on indicator host LN33, a subset of source vines ($n = 53$) that were found by specific PCR analysis to be uninfected by either GVA or GVB were scored as positive in the stem-pitting assay. This apparent false positive rate of 26% is discussed below.

Discussion

In the results described here, co-infection with both vitivirus and leafroll virus correlated with increased incidence of vitivirus occurrence and increased relative vitivirus titer. Vitivirus occurrence in the absence of leafroll virus co-infection was found to be rare: e.g. vitivirus occurred in the absence of grapevine leafroll virus in only 38 examples out of 1483 *V. vinifera* plants in the surveys represented in Figs. 1 and 2. In such cases of vitivirus infecting singly in the absence of leafroll virus co-infection, the vitivirus showed higher relative Ct values (Fig. 3).

The induction of rugose wood stem disease was also found to be associated with the co-infected state. Plants scored as GVB-infected by their induction of stem pitting symptoms on indicator host LN33 were found to be co-infected with GLRaV-2 in four out of five cases. We also saw that inoculation from GVA-infected source plants induced stem pitting symptoms on that indicator host in the absence of detectable GVB infection. This response to GVA demonstrated that stem pitting symptom induction on indicator host LN33 is not specific for GVB. The source plants for the GVA inoculation that induced stem symptoms

were co-infected with GLRaV-1 and/or GLRaV-3 in 88% of the cases, further correlating the co-infected state with induction of rugose wood stem disease.

We occasionally saw rugose wood symptoms on LN33 in the absence of any vitivirus infection ($n = 53$), or in the presence of GVB without GLRaV-2 co-infection ($n = 1$) or in the presence of GVA without GLRaV-1 or GLRaV-3 co-infection ($n = 2$). These apparently false positive reactions may have been due to infection, or co-infection, with viruses other than the species for which we were screening. This would include infection by undiscovered viral species, or unknown strains of known viral species.

Specificity of the vitivirus / leafroll virus interaction The specific association between viti- and leafroll viruses is illustrated in Figs. 1 and 2. Two different species-specific co-infections were found to predominate: GVB was found to associate principally with GLRaV-2; GVA was predominantly associated with GLRaV-1 and -3. (The similarity of GLRaV-1 and GLRaV-3 in their interactions with GVA is consonant with their previously noted genetic similarities (Fazeli and Rezaian 2000)).

The rise in vitivirus incidence and decline in vitivirus Ct values with leafroll virus co-infection was not paralleled by similar changes in leafroll virus incidence or Ct values. In Figs. 1 and 2 the numbers of leafroll-infected plants in which no vitivirus co-infection was present were comparable to their numbers in the presence of single vitivirus co-infection. Leafroll virus Ct values were similar in the presence or absence of vitivirus infection. This asymmetric effect on the titers of the two co-infecting viruses is similar to that seen in the classic synergy between PVX and PVY (Vance 1991) in which PVX is raised in titer by the co-infection, while PVY levels are not affected.

In the synergy between PVX and PVY (Vance 1991), the increased titer of PVX correlated with increasing severity of the co-infection. The uncontrolled background of infections by other viruses in the vines we have characterized precludes our definitive demonstration of a correlation between the synergistic increase in vitiviral titer and an increase in severity of the co-infection. However, the following observations of infection severities are consistent with such an increase in the severity of co-infections with these viruses. A) The enhancement of the severity of grapevine leafroll disease by vitivirus coinfection has been described in specific cases (Rowhani et al. 2016). Severe disease symptoms (including vine death) have been reported in grapevine leafroll virus plus vitivirus co-infection (Golino et al. 2015). Credi and Babini (1997) noted that in mixed infections, the most severe combination was GVA plus GLRaV-3, followed in severity by GVA plus GLRaV-1. The severities of the co-infections have been seen to be dependent on the choice of the grapevine rootstock accessions. B) Leaf symptoms of vitivirus infections in the absence of leafroll virus co-infection have been found to be mild or asymptomatic (Goheen 1989; Martelli and Boudon-Padieu 2004). GVB is latent in hybrid rootstock Kober 5BB; both GVB and GVA are latent in *V. rupestris* (Gambino et al. 2010). Vitiviruses are generally latent in non-grafted grapevines, and latency can also occur in grafted vines (Martelli and Boudon-Padieu 2004). Vitiviruses are not associated with specific foliar symptoms (Martelli and Boudon-Padieu 2004). In the present study, we were unable to find *V. vinifera* plants, or published reports of such plants, showing vitivirus-associated disease symptoms in the absence of a leafroll virus co-infection. These observations suggest that vitivirus infections that are not co-infected with leafroll virus may be low in titer and asymptomatic.

The above observations do not definitively demonstrate increased infection severity by the leafroll virus plus vitivirus co-infection, due to the possible effects of other uncharacterized superinfections, such as *Grapevine rupestris stem pitting associated virus* (GRSPaV), *Grapevine fleck virus* (GFkV) or grapevine nepoviruses. Also, comparisons of infections among different cultivars are not controlled for variables introduced by different accessional backgrounds and their different rootstocks, both of which may affect the response to disease. E.g., the response to GVA plus GLRaV-1 co-infection resulting from inoculation from a Maduar accession source included severities that varied significantly with the rootstock vs. scion combination (Golino et al. 2015). Conclusions about the disease severity of synergistic co-infection with grapevine leafroll viruses and vitiviruses will await the development of purifiable infectious clones of both members of the co-infection, which can be inoculated in pure form into standardized, pathogen-free grapevine host stocks.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest. No human subjects or animals were involved with this study.

Human and animal rights No human subjects or animals were involved with this study.

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Progress Report
California Grape Rootstock Improvement Commission
California Grape Rootstock Research Foundation
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California Table Grape Commission
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Project Title: Development of next generation rootstocks for California vineyards. 170634000SA

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Reporting Period: June 2017 to January 2018

Over all Summary: We are making strong progress in streamlining the assay's 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. We have dynamic duo of Becky Wheeler and post-doc Daniel Pap to greatly accelerate nematode screening efforts. 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. longii* and 140Ru to look for segregation in order to carry out marker development process. We are making good progress in better understanding of root architecture in multiple rootstock species including *Vitis berlandieri*. The key point from different drought screens is that specific root length and root diameter is 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 for 2018-2019 funding cycle.

2017 Pollinations / 2016 Seedlings
A total of 74 crosses were made in 2017 with objectives of improving and combining strong sources of chloride exclusion with deep rooting and broadly based nematode resistance; combining salt and boron tolerance; and developing fanleaf tolerance (Table 1). A total of 1,265 seedlings from 36 crosses were planted in the field in spring of 2017. Table 2 present details of parentages, number of seedlings for each cross and purposes.

Nematode resistance breeding
Key objectives: improve the screening assay, test and identify new germplasm for crosses, test mapping populations to identify genomic regions for marker development, repeat resistance screens for multiple nematodes.
We made strong progress in all aspects of nematode resistance breeding. Since June, we have tested 252 genotypes to a combined inoculum of HarmA and HarmC root knot nematodes (RKN). Some of these genotypes were tested for first time and others were to confirm the results of a previous screen. Of these, 40 tested resistant to RKN and 30 resistant to ring nematode. We have identified 1 genotype that has tested resistant to both ring and RKN twice; it has been propagated and will be tested for resistance to dagger nematode and phylloxera in the 2018. We have 5 genotypes that have shown resistance to RKN and ring nematode in one screen; these have been propagated and will again tested to confirm the results of the initial screen. Twenty-eight genotypes were resistant to RKN and will move to screening for ring nematode. Eleven genotypes show resistance to ring nematode and will move to RKN testing. After reviewing data on germplasm resistance to RKN generated by Daniel Pap, a populations from 2012 were added to the nematode-testing pipeline; these should be through initial screening by mid-year. We began testing breeding populations generated from the 2014 crosses. From the 2012 to 2015 crosses, we planted 4,653 seedlings for nematodes, salt, deep rooting, boron, and fanleaf). They were evaluated for field characteristics and 1,694 genotypes did not meet the criteria for horticultural traits and are scheduled for removal in Spring 2018 (Table 3). Table 4 outlines summary of progress since May 2017.

Optimizing RKN testing and mapping

Efficient and quick root-knot nematode (RKN) screening is essential to develop new resistant rootstock varieties. Daniel Pap has improved our RKN screen to evaluate new germplasm and mapping populations. Recent results verify optimizing efforts by changing our inoculum from juveniles to eggs and shortening the incubation time from 16 weeks to 6 weeks. Subsurface irrigation in the greenhouse, with a capillary mat instead of drip emitters, has also greatly improved the screening process by providing more uniform irrigation and allowing more pots per bench.

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

Screening germplasm for RKN resistance

Selections from our extensive germplasm collection were made based on SSR fingerprint data to examine a wide range of accession representing most species and geographic regions. A total of 122 accessions were examined including a diverse set of *Vitis arizonica* and more extensive set from a recent collection in Texas (*V. berlandieri*). We have also included parents from existing mapping populations that were developed to explore Pierce's Disease resistance. All accessions were tested separately with HarmA and HarmC, with the optimized screen. The results are summarized in Table 5 and 6. We identified 19 accessions with resistance to RKN.

Preliminary analyses show no pattern of distribution of the resistance across species or genetic data, moreover, there was no indication that resistance originated from one specific geographic region. The new resistant accessions were propagated for retesting. Further work, examining the resistant accessions in crosses with susceptible parents are necessary understand the genetic differences of these resistances, if they evolved separately or if one resistance locus is distributed randomly across these species. Mapping resistances from different genetic sources and different loci is necessary to establish durable resistance in the field.

Molecular verification of purity in the existing RKN isolates

We maintain three isolates from two RKN species. Existing molecular markers show limited to no levels of diversity below the species level. We have proven that HarmA is more virulent than HarmC in our first screen (Figure 2). We inoculated a single egg mass of these strains separately along with the I3 strain on Harmony, Freedom, GRN1 and Colombard to monitor their virulence, and more importantly to purify a single line. We also tested DNA extraction methods from eggs that yield good quality and quantity of DNA. We are in the process of comparing our strains to previous work on the sequencing of RKN strains. More details regarding molecular markers capable of characterizing the isolates will be available Summer 2018.

Crosses under investigation for RKN mapping

Populations were screened to identify segregating populations that are suitable to map the genetic region responsible for the RKN resistance. Molecular markers flanking this genetic region could then be utilized in marker-assisted selection, not only to speed up the rootstock development, but also to make it possible to combine multiple different resistance loci into the same rootstock. For this purpose, the crosses that include the most susceptible *V. vinifera* are most suitable, and we developed mapping populations with the susceptible *V. vinifera* female F2-35 or F2-7.

The crosses that are already established in the field, and are of interest for RKN resistant mapping are summarized in Table 7. The RKN screen of the 07-135 (*V. vinifera* x *V. berlandieri* 9031) population suggests continuous

segregation for resistance. This population is also investigated for phylloxera resistance by two different screening assays (in vitro and in the greenhouse). We will develop a framework genetic map for this population after completing the phenotyping with phylloxera. Thus, we could explore resistance in *V. berlandieri* 9031 for both RKN and phylloxera.

Three crosses with 101-14 × GRN2/GRN4/GRN5 were investigated for RKN segregation. Results indicate a clear evidence of two separate loci possibly from two different resistant parents (Table 7). New results from a *V. vinifera* × GRN4 (05803) cross indicates that resistance from GNR4 segregates in a Mendelian manner, however further confirmation is needed with the expanded population (Figure 3). New crosses are made with all three fertile male GRN rootstocks (Table 8A.) with susceptible vinifera and after germination a subset of each population will be tested for segregation.

The populations with *V. arizonica* males ANU67, b47-32 and *V. cinerea* DVIT2236.2 are developed primarily for Pierce's Disease resistance mapping. Recent results show that these accessions are resistant to RKN. For the population with ANU67 the development of framework genetic is in the pipeline, and we could utilize this map for exploring RKN resistance as well. Two smaller populations with b47-32 and DVIT2236.2 will be tested for segregation, and if needed seedling numbers will be increased in the Spring 2018. Three more crosses developed in 2017 involved two *V. cinerea*, b41-23 and b45-26 and *V. longii* 9035. We are in process of seed germination and seedlings will be passed through testing in the greenhouses (Table 8A). The accession b45-26 shows strong resistance to dagger nematode, and a subset of population will be tested for resistance segregation. There is also evidence that *V. longii* 9035 is a good candidate for salt tolerance, this population could supplement other salt mapping efforts.

More crosses are available from last year and these are listed Table 8B. Crosses with resistant parents of *V. mustangensis* T64, *V. longii* 9027, *V. champinii* 9021, *V. doaniana* 9024 and *V. mustangensis* T56 are in pipeline for germination and will be planted in the field in the Summer 2018. These populations will serve to examine resistance from other species. 1The remaining three crosses indicated with bold and underscored cross ID in Table 8B could serve to refine the mapping efforts in the aforementioned population with same resistant background and will be germinated upon successful identification of genetic region(s) that are responsible for the RKN resistance.

Dagger nematode resistance

Graduate student Jordan Weibel is working on exploring the dagger nematode resistance in a wide range of germplasm collected in last two decades. Both hardwood and green cuttings are used to test the germplasm in greenhouse assay that consisted of use of soil infested with dagger nematodes. Last year, we expanded the inoculum to carry out the screens by growing susceptible host plants in large bins with infested soil. To speed up the process, we are examining whether rooted plants can be directly planted into infested soil. If successful, this method will save the 4-6 weeks of time that was needed to establish plants before inoculating. Table 9 lists 47 accessions that are currently in the greenhouse screen. In Spring 2018, crosses will be made with most resistant germplasm emerging from the above-mentioned trial. Efforts are also underway to refine the genetic map of the *XiR2* locus from b40-14 and get it ready for publication. We repeated the cross of the 0705 population in 2014 to search more seedlings to detect recombinants closer to the previously mapped *XiR2* locus. We tested 350 seedling plants with markers and selected 42 recombinants of interest that will be screened for dagger nematode in Fall 2018.

Drought tolerance/avoidance understanding

Key objective: understand root architecture genetics and develop molecular tools; develop assay that reliably mimic root structure; test commercial rootstocks to monitor drought response in relation to root architecture; develop assays that could predict drought response in high throughput manner.

Roots are dynamic and have the ability to adapt to changing soil environments. Grape rootstocks vary for root architecture and response to the soil moisture availability. It is a known fact that deep thick rooted rootstocks perform better in low water/drought conditions when compared to the shallow rooted rootstocks. Root

architecture is a difficult trait and is influenced by various environmental factors. Development of an assay that is fast, reliable, less resource and labor intensive and mimic field results is our overall objective. We have carried out multiple screens with commercial rootstocks with different root architecture to determine what feature of root structure is most reliable to study in both field and greenhouse conditions. Preliminary results with different assays indicated that the average root thickness could serve as an effective index that may be potentially linked to drought stress tolerance when vines are grown in relatively heavy field soil.

In January and June of 2017 we reported on a drought resistance screen using 20 cultivars (17 rootstocks and 3 wine grape cultivars). This screen had a greenhouse component that measured growth, stomatal conductance and root suberization of drought-treated and well-watered plants. The significant result was an inverse correlation between root system fibrosity and drought tolerance capacity in a relatively large subset of cultivars indicating that this trait could be a robust indicator of drought resistance. Drought susceptible cultivars produced adventitious roots that were all fibrous in comparison to drought-resistant rootstocks such as Ramsey (Fig. 4, *left*). Rootstocks such as Freedom that are known to be intermediate in drought tolerance from field trials and grower experience had more fibrous roots than drought tolerant rootstocks, but also more thick roots than drought susceptible rootstocks (Fig. 4, *left*). Drought conditions had no detectable effects on the fibrosity of drought susceptible root systems, but drought resistant rootstocks had more variable responses to drought (Fig. 4, *right*). In most drought resistant rootstocks, fewer thick roots were produced in drought-treated plants, possibly from a reduction in growth rate.

The conclusion of another study on a set of rootstocks was that fibrous and thick-rooted rootstocks could be accurately—and possibly optimally—characterized from young, four week-old roots generated from herbaceous cuttings. In this study, the production of fine roots was 4.2-fold higher in Riparia Gloire relative to Ramsey, and the phenotypic separation was more distinct than any other root system characteristic (paper submitted for publication to the journal Annals of Botany). To test out these results, we used Ramsey x Riparia Gloire F1 and F2 hybrid populations that were propagated in a fog room. However, results were very variable and no inferences could be drawn. So far, the clearest results have been obtained by comparing specific root length of plants grown in the field conditions; none of the greenhouse assays provided the results that mimic field conditions or provide reproducible results.

We are working in collaboration with Dr. Andrew McElrone to develop a reliable assay to test drought tolerance or drought avoidance. His group is focused on the study of physiological mechanisms linked to the differential responses of drought sensitive and tolerant rootstocks. They have tested 110R, drought resistant and 101-14 drought sensitive. The results of their work also support our conclusions that specific root length and root diameter are two key parameters that could be used to screen for drought tolerant rootstocks. We also obtained similar results from an experiment carried out in the fabric pots described above that drought resistant rootstocks develop fewer thick roots. The pliability of the rootstock to re-establish its growth after a bout of low water conditions also plays a huge role and drought resistant rootstocks possess that elasticity (See report of McElrone for more details)

The second aspect of this work is to understand the genetic factors that control root architecture. If we could identify genomic regions that potentially control root structure then markers could be developed. To understand the genetics of root architecture, we have planted multiple replicates of the F1 population of Ramsey (deep rooted) x Riparia Gloire (shallow rooted) in the field in Spring 2017. They will be excavated from ground by the end of 2018, and X-ray examined in collaboration with Dr. Daniel Chitwood. We hope to identify a reliable feature that could be used to detect genomic regions associated with root architecture for use in rootstock breeding.

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

Key objective: Collect new V. berlandieri *germplasm; understand population structure in relation to other species, test for traits to identify accessions that are resistant to nematodes, and have salinity and lime tolerance for rootstock breeding.*

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

Analysis of population structure – The analysis of population structure included *V. berlandieri* and *V. cinerea* accessions collected in 2015-2016, previously collected accessions from Texas and northern Mexico, and accessions from the Wolfskill and Montpellier germplasm repositories. Accessions of *V. candicans* were included in addition to those of *V. berlandieri* and *V. cinerea* to reduce sampling bias. Our results using the population genetics software STRUCTURE showed evidence for two, three, or four subpopulations within the Texas accessions (Figure 5). The strongest evidence was for either two or four subpopulations, with the two population grouping consisting of *V. candicans* versus all other taxa and with *V. berlandieri*, *V. cinerea*, the Mexican b-series seedlings, and *V. candicans* all grouped independently in the four population grouping. Morphological differences among groups provide additional evidence for four subpopulations within the analyzed accessions. Principle coordinates analysis (PCoA) and pairwise F_{st} tests also supported the STRUCTURE results (Figure 6). PCoA visualizes the genetic relationships among accessions without any prior assumptions concerning population structure and divergence, while pairwise F_{st} tests indicate the relationships among individuals within subpopulations compared to relationships within pooled subpopulations. The most appropriate interpretation of these data is that *V. berlandieri* and *V. cinerea* populations are closely related but that significant genetic differences exist between them.

Relationships between genetic and environmental data – We investigated a range of temperature, precipitation, and soil variables for evidence of relationships between environmental and genetic differences among populations. Such relationships can indicate the fitness of accessions for specific environments and, in turn, appropriateness for breeding objectives. Of 23 variables tested, mean annual precipitation and soil pH were among the most important features distinguishing between *V. berlandieri* and *V. cinerea* collection locations (Figure 7). Mean annual precipitation was 79.3 cm for *V. berlandieri* accessions and 1070.7 cm for *V. cinerea* accessions, and mean soil pH was estimated at 7.2 for *V. berlandieri* accessions and 6.0 for *V. cinerea* accessions. A Mann-Whitney-Wilcoxon non-parametric test showed that these differences were highly significant ($p << 0.0001$). The relatively small variance in values at *V. berlandieri* collection locations reflects the restricted range of the species compared to *V. cinerea*.

We performed Mantel tests to examine the relationship between genetic and environmental differences among accessions, and found that there was a moderate but highly significant ($r = 0.22$; $p < 0.001$) correlation between genetic and environmental variance. This is important in justifying the concentration of our collection activities to *V. berlandieri* accessions in the Texas Hill Country, as opposed to all *cinerea*-like specimens throughout Texas and even beyond into more eastern and northern states. The genetic-environmental relationship is confounded, however, by a strong correlation ($r = 0.85$; $p < 0.001$) between environment and geography due to the east-west gradient of environmental values (Figure 7). In fact, the genetic-environmental correlation was lost in a partial Mantel test, which tests the genetic-environmental relationship while controlling for geographic distance. In other words, we cannot disassociate environment from geography and, thus, cannot make strong conclusions about the adaptation of our accessions based on our current data. New grapevine sample data from the region between the Hill Country and east Texas may help us determine the relationship between genetic and environmental differences we have clearly observed between the *V. berlandieri* and *V. cinerea* populations.

Nematode resistance screening – Ten new *V. berlandieri* accessions were recently tested for resistance to HarmC RKN). The plants were grown from herbaceous cuttings in pure sand, inoculated with RKN egg masses, and evaluated after six weeks. Although none of the new accessions showed total resistance to nematode infection,

most of the accessions possessed significantly fewer egg masses per root biomass than the 'Colombard' control plants (Table 10). This partial resistance could prove useful for stacking resistance genes for more durable resistance in future rootstock cultivars. We are currently propagating an expanded set of *V. berlandieri* accessions to screen for RKN resistance and better assess the diversity for this trait within the species.

Chloride Exclusion, germplasm and mapping population screening
Key objective: Screen germplasm identified in earlier salt screens and new berlandieri *accessions at higher salt concentrations; screen small breeding populations with different backgrounds to identify segregation; understand how high salt effects root growth.*

Germplasm screening - In previous reports, we described the salt screen protocol that was developed by Kevin Fort and then Ph.D. student Claire Heinitz used it to screen a vast range of germplasm to identify salt excluders. In earlier stages, screening was carried out at 25mM of salt concentration and in later stages 50mM of salt concentrations were used. Currently, we are using 75mM (12% sea water) of salt concentrations and all germplasm that passed previous screens will be tested again. These screens are in the pipeline for the Summer 2018. We tested a subset of *berlandieri* accessions collected from Texas to find other unique germplasm that is salt and lime tolerant to use in the breeding program. Table 11 shows results of screen that was carried out at 75 mM sodium chloride concentration. We identified two very unique salt tolerant accessions that had better visual ratings and lower salt uptake in comparison to the standard highly resistant 140Ru, and the previously identified *berlandieri* 9031. We are planning to repeat the screen on most promising eight accessions that had visual scores of 4.5 or higher and lower salt accumulation. In parallel, we are planning to make crosses in spring with most promising selections from this trial to have seeds for breeding populations available for testing in 2019.

Screening of small breeding populations to look for segregation – Each year we make crosses to develop small breeding populations with good salt excluders. Once established in the field, they go through rigorous screening for horticultural traits, and testing for different pests and diseases. Testing of these breeding populations allow us to choose the best selections for use as rootstocks as well as allow us to search for the segregation of trait to understand its genetics. In 2014, we made crosses with two accessions of *V. longii*, 9018 and 9035 with the low to moderate salt excluder Ramsey. Both *longii* accessions are good salt excluders at 75 mM of sodium chloride in multiple salt screens carried out from 2012-2017. A total of 84 seedling plants were established in the field with these two crosses. In 2016, both *longii* accessions were crossed with the poor salt excluder rootstock Dog Ridge and 73 seedling plants were established in the field. In summer 2017, we marker tested these four populations to keep only true-to-type progeny. DNA was extracted for all seedling plants and 7 SSR markers were used to genotype them. We identified 23 off-types. All verified seedlings were propagated for salt screening in the greenhouse. We have completed salt screen of two crosses where Dog Ridge was crossed with *longii* 9018 and 9035 and are currently in screen. We observed 1:1 segregation in 15 tested seedlings of cross 14-138 (Dog ridge x *longii* 9018) and one-way analysis of variation indicated highly significant genotypic effect. These are very promising results to move forward for marker discovery by mapping. We intend to repeat this cross in spring 2018 to increase the population size for mapping. The second cross with *longii* 9035 also showed strong genotypic effect and more plants showed lower salt accumulation, but with continuous segregation ratios. Both populations are going to be screened a second time to verify the results of first screen. Results of the other two breeding populations with Ramsey will be available in Summer 2018.

Understand how high salt effect the root growth - Cassandra Bullock-Bent recently finished her MS study on the effect of salinity on the growth of four different rootstocks (140Ru, O39-16, Ramsey and Riparia Gloire). She observed a strong correlation between the percent of fine roots produced and the amount of chloride accumulated in the shoots after three weeks of applied salt. Further work was needed on a wider range of rootstocks to see if these findings hold up and if rooting structure could be used to access the ability of the plant to avoid salt. Last year, we expanded the greenhouse screen to 16 genotypes including the original 140Ru, O39-16, Ramsey, and Riparia, adding: 101-14 Mgt, 110R, 44-53, GRN1, *longii* 9018, *longii* 9035, *arizonica* NM 03-17, *rupestris* Pumpstation, *girdiana*s SC-12 and SC-2, Schwarzmann, and St. George. Plants were screened in the greenhouse based on our established assay for salt. Roots were scanned and analyzed using WinRHIZO^{TM} software. However no correlation was observed for the root structure to high salt accumulators. Figure 8 shows the salt trial results. Both *longii* accessions 9018 and 9035 are proven to be consistently good excluders in multiple screens.

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. As mentioned in our previous research update, we have 240 newly planted field vines as part of our project to study the degree of fanleaf tolerance that can be induced by 101-14 Mgt. x *Muscadinia rotundifolia* 'Trayshed' progeny. There are 41 different genotypes from this population that are being screened in this study. During the summer, these vines were trained by laying down a single strong shoot on the trellis wire in order to promote flowering and fruiting as early as next season. We predict that these plants will flower in Spring 2018. We will count the calyptras and berries to calculate fruit set, and determine the impact of grapevine fanleaf virus for each graft combination.

Screening of Fertile VR Hybrids for GFLV Tolerance – Simultaneously with the 101-14 x Trayshed fanleaf tolerance study, we will also be screening 13 selections of fertile VR (*vinifera* x *rotundifolia*) hybrids. We have 80 newly-planted 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.

GFLV Resistance in 101-14 x Trayshed Progeny and Fertile VR Hybrids – The rootstock genotypes chosen for our fanleaf degeneration tolerance study will also be assayed for GFLV resistance. There is a differentiation between resistance and tolerance because both terms describe different virus-host interactions that impact disease management in distinct ways. Resistance is the plant's ability to suppress virus multiplication to a degree (either completely or partially), and tolerance is the ability to lessen the damage caused by virus infection. In previous research updates, we showed that O39-16, as well as cultivars of pure *M. rotundifolia*, exhibit GFLV resistance. We are now also evaluating resistance in genotypes from the 101-14 x Trayshed population, as well as the selections of fertile VR hybrids. Similar to the tolerance study, plants for this resistance study were also bench-grafted with GFLV-infected Cabernet Sauvignon scions (the genotypes of interest were treated as rootstocks). We verified GFLV infection with RT-qPCR (Figure 9). We are currently using RT-qPCR to quantify GFLV levels for each of the rootstocks. Preliminary data with five genotypes from the 101-14 x Trayshed population show that these individuals show some degree of resistance when compared to the susceptible 101-14 control (Figure 10). We hope to finish testing all the plants by March. We plan to repeat this resistance screen once again this year with the same genotypes. We will be starting the bench-grafting for these new plants during this winter.

Since we are testing the same genotypes in both the tolerance and resistance screen, we hope to be able to determine any correlation between the two traits. Preliminary data shown in previous research updates show that when O39-16 was graft-inoculated with GFLV, virus concentrations in the rootstock were much lower when compared to the highly-susceptible St. George, indicating that there may be a correlation between a rootstock's ability to induce fanleaf tolerance and its GFLV resistance when graft-inoculated.

Mechanism of Rootstock-Induced GFLV Tolerance – We are also evaluating the cause behind the observed fanleaf tolerance induced by O39-16. We are examining this induced tolerance with four-year-old vines of Chardonnay grafted on either O39-16 or St. George (21 plants of each scion/rootstock combination). Last spring, we successfully inoculated ten vines of each graft combination with GFLV-infected buds. During Spring 2019 (thus giving the virus adequate time to spread and multiply in the inoculated vines), we plan to use high-performance liquid chromatography (HPLC) to extract and quantify major cytokinins and gibberellins known to be key during fruit set. We expect to find significant differences between scions grafted on O39-16 and scions grafted on St. George, and will be able to compare between infected and uninfected vines.

Rootstock tolerance to red leaf viruses – Zhenhua Cui
Leafroll Associated Viruses - GLRaV - produce great damage on fruit yield and quality. Specific strains also affect the graft-union severely, killing plants 3-4 years after grafting. Field observations and trials have shown that some rootstocks (Freedom, 1103P, 101-14, 3309C) react strongly, while others (St. George and AXR1) do not express severe symptoms and rarely show graft incompatibility (Golino et al. 2015). In order to better understand this response we grafted rootstocks Freedom, 101-14, St. George, and AXR1 with Cabernet franc infected with isolates LR131 (GLRaV1) or LR132 (GLRaV co-infected with grapevine virus A – GVA). Experiments were performed under greenhouse and in vitro conditions.

Greenhouse - Green grafts of Cabernet franc infected with LR131 or LR132 showed minor leafroll symptoms when grafted on St. George and AXR1, but symptoms were more severe when grafted on Freedom and 101-14, especially with the LR132 isolate (Figures 11 and 12). Similar differences were observed with scion dry weights, showing significant differences between Cabernet franc infected with LR132 grafted on Freedom or 101-14 and healthy controls (Figure 13). GLRaV-1 concentration was similar in all LR131 green grafts but was affected by in LR132: St. George had the highest level of both GLRaV-1 and GVA, while 101-14 had the lowest level of both GLRaV-1 and GVA (Figure 14).

Infection with LR132 also markedly reduced graft survival rate on Freedom and 101-14, with no significant differences on St. George and AXR1. This response was more pronounced with bench grafts that were transplanted to the field (Table 12). Overall, grafting methods, virus status, rootstock genotypes and their interactions, all affected the survival rate of grafts. Despite higher virus concentrations found at the union graft, St. George had the highest tolerance, followed by AXR1. Both Freedom and 101-14 were very sensitive to leafroll virus. MicroRNA (miRNA) seq libraries have been produced at the UCDavis DNA Technologies Core from the different green graft combinations to study their miRNA profile and generated data is being analyzed at present. MiRNAs have been reported to regulate plant growth, development, metabolism and disease resistance.

In vitro - The same scion/rootstock combinations were performed in vitro. The objective was to compare this system with greenhouse conditions and to identify early signs/symptoms of graft incompatibility derived from virus infections. Micrografting requires tissue culture skills but has the advantage of requiring less space and the potential for faster development of symptoms (Figure 15). In fact, under in vitro conditions LR132 was so severe that inhibited all micrograft growth (Figure 16). Healthy Cabernet franc and Cabernet franc infected with LR131 showed similar survival rates, however LR131 infection delayed bud break and root initiation when grafted on Freedom and 101-14. The vegetative growth of LR131 micrografts was also lower on Freedom and 101-14 (Figure 17). Differences in GLRaV-1 concentration in LR131 micrografts were not significant among the different rootstocks (Figure 18). Interestingly, histological observations of the unions showed that LR132 infection delayed callus formation between the scion and rootstock interfaces and the absence of vascular connection between them, which probably caused the failure of all LR132 micrografts. On the contrary, obvious callus was observed between scion and rootstock interfaces in both healthy and LR131 infected micrografts regardless of the rootstocks, and a strong vascular bundle connection between scion and rootstock was observed 2 months after grafting (Figure 19). Considering all the performances of the different micrografts, St. George and AXR1 showed a high level of GLRaV-1 tolerance in micrografted plants, while Freedom and 101-14 were sensitive when grafted with GLRaV-1-infected scions. LR132 killed all grafts, although we are not sure whether synergistic effects between GVA and GLRaV-1 contribute. Results here are accordant with our green grafting results and also with the results of Golino et al. (2015) in field. Micrografting seems to be a more sensitive system for virus infection with a shorter period and lower cost, which makes it a potential tool to select virus tolerant rootstocks.

Presentations/Abstracts/Scientific Meetings/Publications Related to Rootstock Breeding
Talks at Grower Meetings (Extension/Outreach) – Jan 2107 to Jan 2018

Progress in the grape breeding program. Vine Health Seminar, UCD ARC, Dec 9, 2016
Update on the breeding of slat and drought resistant grape rootstocks. San Joaquin Valley Grape Symposium, C.P.D.E.S Hall, Easton, CA, Jan 11, 2017
Breeding grapes to adapt to climate change. 3rd International Symposium on Grapes, Hermosillo, Sonora, Mexico, Jan 27, 2017
The origin of winegrapes. Daniel Roberts Client Group Seminar, Martinelli Winery, Santa Rosa, CA, Jan 30, 2017
Rootstock breeding update. Current Wine and Grape Research, UC Davis Conference Center, Feb 13, 2017
Grape roots a primer. Napa Valley Grape Grower Meeting, Napa, CA, Mar 1, 2017
Establishing and managing grape vines with less water. Santa Carolina Growers meeting, Chile, Mar 23, 2017
Vineyard challenges, Wine Executive Program, UCD Business School, Mar 28, 2017
Grape breeding update, CDFA IAB meeting, June 2, 2017
Grape breeding in CA, Vina San Pedro growers, UCD, June 5, 2017

Grape breeding and UCD tour, Lake County Growers tour (Paul Zellman), UCD, July 19, 2017
Rootstocks: development, use and needs. Sonoma County Vineyard Tech Group, Santa Rosa, CA July 20, 2017
GRN rootstocks. Talk and tour to Chilean growers, UCD, July 24, 2017.
Current breeding efforts in droughty and salt –tolerant rootstocks. Wine Grape Short Course, UCD Conference Center, Dec 12, 2017.

Presentations/Abstracts at Scientific Meetings

Walker, M.A. 2017. Development of grape rootstocks for control of pests and diseases. 63rd 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. 68th 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. 68th 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. 68th 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. 68th ASEV National Meeting, Bellevue, WA, June 29

Walker, M.A. 2017. The southwestern *Vitis*: a grape breeding mother lode. ASEV 2017 Merit Award. 68th 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

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.

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Dodson Peterson, J.C. and M.A. Walker. 2017. Influence of grapevine rootstock on scion development and initiation of senescence. Catalyst: Discovery into Practice 2:48-54.

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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. Annals of Botany (submitted)

Table 1. 2017 pollinations.

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

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

Table 2. 2016 seedlings to the field.

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

				rooting, moderate vigor
2016-073	5BB Kober	2012-144-24	50	Salt resistance and better rooting, moderate vigor
2016-090	GRN-3 9365-43	NM 03-17 S01 K1	41	Add salt and drought resistance to GRN3
2016-095	GRN-3 9365-43	acerifolia 9035 K4	8	Add salt and drought resistance to GRN3
2016-096	GRN-3 9365-43	2012-142-25	40	Add salt and drought resistance to GRN3
2016-097	GRN-3 9365-43	2012-144-24	50	Add salt and drought resistance to GRN3
2016-110	doaniana 83 K3/4	GRN-4 9365-85	23	Deep roots and very high nema resistance as well as TX root rot
2016-113	GRN-3 9365-43	acerifolia 9018	5	
2016-131	Dog Ridge	girdiana SC11	50	Better salt resistance to Dog Ridge and TX root rot
2016-134	Dog Ridge	arizonica GC5 K1	38	Drought and salt with very deep roots
2016-135	Dog Ridge	acerifolia 9035 K4	50	Drought and salt to Dog Ridge
2016-136	Dog Ridge	2011-175-15	56	Drought and salt with very deep roots
2016-141	9026 (doaniana)	GRN-4 9365-85	4	Deep roots high vigor to GRN4
2016-143	Ramsey	arizonica TX12-003	41	Better roots and salt resistance
2016-158	Ramsey	arizonica GC5 K1	50	Better roots and salt resistance
2016-162	Ramsey	acerifolia 9035 K4	50	Better roots and salt resistance, lime tolerance, Drought and salt in low vigor background
2016-165	riparia 1411	arizonica GC5 K1	37	Drought and salt in low vigor background
2016-168	riparia 1411	b55-1 fertile VR	5	VR in a weak good rooting background
2016-169	riparia 1411	2012-142-25	48	Better rooting, salt and nematodes
2016-170	riparia 1411	2012-144-24	4	Better rooting, salt and nematodes
2016-171	riparia 1411	2012-144-39	30	Better rooting, salt and nematodes
2016-190	SC2 K2	GRN-2 9363-16	22	Salt and boron to GRN nema
2016-191	SC2 K2	GRN-4 9365-85	29	Salt and boron to GRN nema
2016-196	SC2 K2	2012-144-24	23	Salt, boron, nematodes
2016-197	SC2 K2	2012-144-39	47	Salt, boron, nematodes
2016-198	berl 9019 K3	Schwarzman	40	Salt, nema, good rooting
2016-203	berl 9019 K3	110R	5	Salt, nema, lime

Table 3. Details of plants from different crosses that are scheduled for removal in Spring 2018. We routinely make field evaluation and compare results from different pest testing to make decision on what need to move forward in the breeding program.

Cross ID	Female	Male	Purpose	Planted	Kept	Remove
2012-080	arizonica A44	monticola T 03-02 S01	Mapping salt	25	5	20
2012-081	arizonica A53	monticola T 03-02 S01	Mapping salt	15	1	14
2012-084	arizonica A44	SC3 (girdiana)	Salt rootstock	10	4	6
2012-102	101-14 Mgt	NM03-17 (treleaseii)	Salt rootstock	50	8	42
2012-106	101-14 Mgt	9024 (doaniana)	Salt rootstock	50	7	43
2012-108	101-14 Mgt	9028 (doaniana)	Salt rootstock	50	8	42
2012-109	101-14 Mgt	berlandieri 9031	Salt mapping	90	0	90
2012-110	101-14 Mgt	GRN-5 9407-14	Nematodes	55	55	0
2012-111	101-14 Mgt	St. George	Virus and salt	50	0	50
2012-112	101-14 Mgt	GRN-2 9363-16	Nematodes	50	50	0
2012-113	101-14 Mgt	GRN-4 9365-85	Nematodes	50	50	0
2012-115	161-49C	Trayshed	Nematodes phylloxera, lime	50	1	49
2012-116	161-49C	berlandieri 9043	Lime and salt	50	0	50
2012-117	161-49C	110R	Mapping vigor	50	0	50
2012-118	161-49C	GRN-4 9365-85	Nematodes and vigor	50	2	48
2012-125	OKC-1 SO1 (acerifolia)	GRN-2 9363-16	Nematodes salt	50	7	43
2012-126	OKC-1 SO1 (acerifolia)	GRN-4 9365-85	Nematodes salt	50	50	0
2012-129	OKC-1 SO1 (acerifolia)	St. George	Salt and nematodes	50	0	50
2012-133	5BB Kober	1616C	Nematodes	50	50	0
2012-138	5BB Kober	Trayshed	Nematodes and rooting	5	0	5
2012-142	girdiana-11	arizonia A56	Salt	45	2	43
2012-143	girdiana-22	arizonia A56	Salt	50	4	46
2012-144	girdiana Scotty's Castle	arizonia A56	Salt	50	1	49
2012-148	Ramsey	1616C	Mapping vigor salt, leaf senescence	50	0	50
2012-149	Ramsey	ANU77 (girdiana)	Salt	50	19	31
2012-153	Ramsey	9028 (doaniana)	Salt	50	3	47
2012-154	Ramsey	St. George	Salt, nematodes, rooting	50	1	49
2012-158	161-49C	St. George	Rooting, salt, vigor	25	0	25
2012-178	Dog Ridge	Trayshed	Pests, rooting	10	1	9
2012-185	GRN-3 9365-43	berlandieri 9031	Nematodes, salt	9	0	9
2012-187	GRN-3 9365-43	berlandieri 9043	Nematodes, salt	5	0	5
2012-188	Dog Ridge	110R	Salt, PD, deep roots	40	5	35
2012-189	Dog Ridge	140Ru	Salt, PD, rooting depth	25	7	0
2012-190	Dog Ridge	St. George	Salt and rooting	50	0	50
2012-197	Freedom	St. George	Mapping virus tolerance	50	50	0
2012-198	Fry	munsoniana	Rooting test	16	16	0

2013-121	9715-17	Riparia Gloire		200	0	200
2013-133	riparia 1411	140Ru	Mapping population	170	94	76
2013-145	101-14 Mgt	1103 Paulsen	Root architecture	4	0	4
2013-146	Ramsey	Trayshed	Rotundifolia crosses for pest resistance and GFLV tolerance	10	0	10
2013-148	2009-133-11	2009-133-10	“”	50	0	50
2013-149	2009-133-11	2009-133-23	“”	19	0	19
2013-150	2009-133-11	2009-133-07	“”	17	0	17
2013-161	T6-38	420A Mgt	“”	1	1	0
2013-164	T6-38	110R	“”	3	3	0
2013-165	T6-42	110R	“”	2	2	0
2013-173	T6-38	1103 Paulsen	“”	4	4	0
2013-174	T6-42	1103 Paulsen	“”	1	0	1
2013-179	T6-38	1616C	“”	3	1	2
2013-180	T6-42	1616C	“”	2	2	
2013-182	T6-38	GRN-2 9363-16	“”	3	1	2
2013-183	T6-42	GRN-2 9363-16	“”	4	1	3
2014-015	Ramsey	Riparia Gloire	Expand mapping population	150	0	150
2014-016	Ramsey	Ramsey		2		
2014-084	OKC-1 SO3 acerifolia	GRN-2 9363-16	Cl exclusion and nema resistance	50	0	50
2014-085	OKC-1 SO3 acerifolia	GRN-4 9365-85	Cl exclusion and nema resistance	50	0	50
2014-088	SC2 girdiana	GRN-2 9363-16	Double Cl exclusion and nema resistance	50		
2014-089	SC2 girdiana	GRN-4 9365-85	Double Cl exclusion and nema resistance	15		
2014-091	SC2 girdiana	b40-14	Double Cl exclusion and X. index resistance and no Cl exclusion Mapping	50		
2014-094	GRN-3 9365-43	140Ru	Cl exclusion and X. index resistance	50		
2014-105	GRN-3 9365-43	UT12-099	Double Cl exclusion, deep roots, nema resistance	50		
2014-106	GRN-3 9365-43	UT12-100	Cl exclusion and nema resistance	9		
2014-108	GRN-3 9365-43	ANU21	Cl exclusion and nema resistance	7		
2014-112	GRN-3 9365-43	berlandieri 9031	Cl exclusion, drought and nema resistance	32		
2014-114	GRN-3 9365-43	UT12-092	Double Cl exclusion and nema resistance	32		
2014-117	Dog Ridge	140Ru	Cl exclusion, deep roots, nema resistance	50		
2014-118	Dog Ridge	TX12-003	Double Cl exclusion, deep roots, nema resistance	6		
2014-119	Dog Ridge	NM11-068	Double Cl exclusion,	50		

			deep roots, nema resistance			
2014-120	Dog Ridge	NV11-116	Cl exclusion, deep roots, nema resistance	50		
2014-121	Dog Ridge	T9	Cl exclusion, deep roots, nema resistance	40		
2014-124	Dog Ridge	NV12-051	Double Cl exclusion, deep roots, nema resistance	11		
2014-125	Dog Ridge	UT12-078	Double Cl exclusion, deep roots, nema resistance	50		
2014-130	Dog Ridge	ANU21	Double Cl exclusion, deep roots, nema resistance	50		
2014-132	Riparia 1411	110R	Mapping population	128		
2014-133	Riparia 1411	140Ru	Mapping population	152		
2014-135	Dog Ridge	ANU77	Double Cl exclusion, deep roots, nema resistance	50		
2014-136	Dog Ridge	2011-175-15	PD resistance, nema and rooting	17		
2014-137	Dog Ridge	berlandieri 9031	Salt, deep roots, PD	40		
2014-138	Dog Ridge	longii 9018	Cl exclusion, deep roots, nema resistance	17		
2014-139	Dog Ridge	NV12-049	Double Cl exclusion, deep roots, nema resistance	11		
2014-143	Ramsey	TX12-003	Double Cl exclusion, deep roots, nema resistance	5		
2014-144	Ramsey	NM11-068	Double Cl exclusion, deep roots, nema resistance	50		
2014-145	Ramsey	NV11-116	Double Cl exclusion, deep roots, nema resistance	50		
2014-146	Ramsey	T9	Cl exclusion, deep roots, nema resistance	5		
2014-150	Ramsey	UT12-078	Double Cl exclusion, deep roots, nema resistance	15		
2014-152	Ramsey	UT12-099	Double Cl exclusion, deep roots, nema resistance	2		
2014-153	Ramsey	UT12-100	Double Cl exclusion, deep roots, nema resistance	30		
2014-158	Ramsey	2011-175-15	PD resistance, nema and rooting	50		
2014-159	Ramsey	berlandieri 9031	Cl exclusion, deep roots, nema resistance	50		
2014-160	Ramsey	longii 9018	Cl exclusion, deep	50		

			roots, nema resistance			
2014-164	T6-38	110R	VR resistance 110R roots	10		
2014-173	T6-38	1103 Paulsen	VR resistance 1103P roots	1		
2014-187	T6-38	GRN-4 9365-85	VR resistance / nema resistance	2		
2014-199	101-14 Mgt	berlandieri 9031	Cl exclusion and rooting depth mapping and rootstock potential	50		
2015-083	OKC-1 SO1 acerifolia	b40-14	Nematodes and salt	20		
2015-087	OKC-1 SO3 acerfolia	b40-14	Nematodes and salt	7		
2015-096	GRN-3 9365-43	NM11-068	Nematodes and salt	50		
2015-101	GRN-3 9365-43	NV12-049	Nematodes and salt	7		
2015-103	GRN-3 9365-43	UT12-078	Nematodes and salt	3		
2015-106	GRN-3 9365-43	UT12-100	Nematodes and salt	1		
2015-107	GRN-3 9365-43	AZ12-138	Nematodes and salt	11		
2015-108	GRN-3 9365-43	ANU21	Nematodes and salt	1		
2015-110	GRN-3 9365-43	ANU77	Nematodes and salt	10		
2015-137	Dog Ridge	berlandieri 9031	Nematodes and salt	1		
2015-152	Ramsey	UT12-099	Nematodes and salt	3		
2015-156	Ramsey	SC11	Nematodes and salt	2		
2015-157	Ramsey	ANU77	Nematodes and salt	50		
2015-170	NM12-114	GRN-2 9363-16	Nematodes and salt	50		
2015-181	2011-188-16	b40-14	Nematodes and salt	3		
2015-185	2011-175-07	GRN-4 9365-85	Nematodes and salt	20		
2015-186	2011-175-06	longii 9018	Nematodes and salt	20		
2015-187	T6-38	GRN-4 9365-85	Nematodes and salt	6		
2015-188	2011-175-06	GRN-2 9363-16	Nematodes and salt	20		
2015-154	Ramsey	AZ12-138	Nematodes and salt	1		
Tetraploid plants (chromosome doubling)				14	14	0

Table 4. Progress since May 2017 to test selections for different nematode assays.

# Genotypes Tested For Nema Resistance Since May	252
# Genotypes Tested for RKN Resistance (Initial and Confirmation)	167
# Genotypes Moved Forward for Initial RKN Resistance	40
# Genotypes In Testing	51
# Genotypes Tested for Ring Resistance (Initial and Confirmation)	85
# Genotypes Move Forward for Initial Ring Resistance	30
# Genotypes Removed From Pipeline Since May (Poor nema resistance, poor rootability, etc.)	301

Table 5. Total number of plants screened to explore the diverse resistance sources for RKN resistance and the number of resistant accession found by species.

Species	Number	Resistant
M. rotundifolia	1	1
V. acerifolia	6	1
V. aestivalis	1	0
V. arizonica	31	2
V. berlandieri	28	2
V. candicans	5	1
V. caribaea	5	0
V. champinii	5	2
V. cinerea	11	4
V. doaniana	6	1
V. girdiana	6	0
V. longii	2	1
V. monticola	1	0
V. riparia	5	1
V. rupestris	6	1
V. treleasei	2	2
V. vulpina	1	0
Total:	**122**	**19**

Table 6. List of new wild accessions that show resistance or high level of tolerance to RKN infections. Accessions IDs indicated with bold letters and underscores are parents of existing crosses.

Accession	Species	Egg mass / g for dry root	
		Harmony A	Harmony C
ANU67	*V. arizonica*	0.5	-
b41-23	*V. cinerea*	0	0
b45-26	*V. cinerea*	0	1.0
b47-32	*V. arizonica*	0.8	0
9021	*V. champinii*	0.3	0
CO12-103	*V. riparia*	0	0
9026	*V. doaniana*	0	0
DVIT2236.2	*V. cinerea*	0	0
9035	*V. longii*	0	0
NM11-072	*V. treleasei*	1.0	0
OK14-023	*V. acerifolia*	0.8	0
T 03-06 S01	*V. champinii*	0	0
T54	*V. cinerea*	0	0
T56	*V. candicans*	0	0
T6-42	*M. rotudnifolia*	0	0
TX15-059	*V. berlandieri*	0	-
TX15-105	*V. berlandieri*	0	-
Vru42	*V. rupestris*	0.5	0
NM11-072	*V. treleasei*	1.0	0

Table 7. The following crosses are being used to study the genetics of RKN resistance.

Cross ID	Female	Male	#seedlings
07-135	F2-35	*V. berlandieri* 9031	110
12-112	101-14	GRN2	56
12-110	101-14	GRN5	57
12-113	101-14	GNR4	57
05-803	French Colombard	GRN4	19
14-362 / 16-361 / 14-344	F2-35	*V. arizonica* ANU67	276
13-344 / 12-307	F2-35	*V. arizonica* b47-32	38
14-360	F2-35	*V. cinerea* DVIT2236.2	30

Table 8A. Seeds available from crosses made in 2017 that will be germinated in February 2018 and subset will be screened with RKN in the greenhouse in the 2018 Spring.

Cross ID	Female		Male	#Seeds
17-513	F2-35	×	GRN2	100
17-514	F2-35	×	GRN4	197
17-521	F2-35	×	GRN5	320
17-517	F2-07	×	*V. cinerea* b41-23	274
17-518	F2-07	×	*V. cinerea* b45-26	327
17-502	F2-35	×	*V. longii* 9035	93

Table 8B. Further crosses from 2017 that are will be germinated and planted in the field this season, or will be germinated (bold and underscored Cross IDs) after successful mapping efforts from pervious populations from the same resistant background**.**

Cross ID	Female		Male	#Seeds
17-506	F2-35	×	*V. mustangensis* T64	16
17-507	F2-07	×	*V. longii* 9027	291
17-510	F2-07	×	*V. champinii* 9021	145
17-515	F2-35	×	*V. mustangensis* T56	81
17-505	F2-35	×	*V. doaniana* 9024	124
17-501	F2-35	×	*V. longii* 9027	215
17-519	F2-35	×	*V. cinerea* b41-23	209
17-520	F2-35	×	*V. cinerea* b45-26	39

Table 9. Species currently being screened for X. index resistance in the greenhouse .

Genotype	Species
longii 9027	*V. acerifolia*
TX12-018	*V. acerifolia*
OK14:002	*V. acerifolia*
OK14:019	*V. acerifolia*
OK14:059	*V. acerifolia*
OK14:072	*V. acerifolia*
OK14:031	*V. acerifolia*
OK14:053	*V. acerifolia*
KS14:032	*V. acerifolia*
b43-17	*V. arizonica*

b43-12	*V. arizonica*
b42-24	*V. arizonica*
b44-11 M50	*V. arizonica*
b47-27 M79	*V. arizonica*
b40-14 M4	*V. arizonica*
TXNM081	*V. arizonica*
A14 female	*V. arizonica*
SAZ7	*V. arizonica*
AZ14:087	*V. arizonica*
AZ11-001	*V. arizonica*
A55 female	*V. arizonica*
NM11-021	*V. arizonica*
TXNM0816	*V. arizonica*
NM11-043	*V. arizonica*
ANU43	*V. arizonica*
AZ11-099	*V. arizonica*
AZ12-138	*V. arizonica*
ANU71	*V. arizonica*
GC6	*V. arizonica*
UT12-064	*V. arizonica*
DVIT2211.7	*V. arizonica*
DVIT1269	*V. arizonica*
TX9722	*V. berlandieri*
TXNM088	*V. berlandieri*
T 03-01 S01	*V. berlandieri*
TX PALMATA 2	*V. berlandieri*
TX43-01	*V. berlandieri*
T17	*V. berlandieri*
TXNM083	*V. berlandieri*
berlandieri 9019	*V. berlandieri*
doaniana 9042	*V. doaniana*
OK12-005	*V. doaniana*
TX12-035	*V. doaniana*
OK12-007	*V. doaniana*
OK14:030	*V. doaniana*
doaniana 9024	*V. doaniana*
OK12-015	*V. doaniana*

Table 10. Mean root-knot nematode egg masses, dry root biomass, and egg masses per root biomass of ten *V. berlandieri* accessions, and ‘GRN-1’ and ‘Colombard’ controls.

Genotype	Egg Masses	Dry Root Biomass (g)	Egg Masses / Biomass
Colombard	64.8	2.18	29.7
GRN-1	0.0	1.32	0.0**
TX15-003	3.0	1.77	1.7*
TX15-091	0.8	1.25	0.6**

TX16-015	7.0	2.10	3.3*
TX16-018	3.8	2.02	1.9**
TX16-022	1.0	1.78	0.6**
TX16-026	10.0	1.70	5.9*
TX16-032	4.5	3.24	1.4**
TX16-034	2.5	1.57	1.6**
TX16-065	28.3	2.54	11.1
TX16-068	1.0	1.53	0.7*

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

Table 11. Chloride concentration (ppm) for *V. berlandieri* accessions treated 75 mM sodium chloride. Asterisks denote control genotypes. Higher visual ratings indicate more vigorous plants and reduced symptoms.

	Visual Rating	Mean Cl ppm ± SD
140Ru*	4.7	184 ± 62
St. George*	2.7	275 ± 160
Malegue 44-53*	2.0	417 ± 11
berlandieri 9031	4.5	174 ± 51
TX BERL	4.5	176 ± 47
TX BERL MALE	4.8	208 ± 8
TX15-003	**5.0**	**106 ± 33**
TX15-073	4.8	238 ± 11
TX15-091	2.8	156 ± 86
TX16-068	**5.0**	**73 ± 41**
TX16-016	4.2	162 ± 18
TX16-015	4.0	211 ± 52
TX16-032	4.8	218 ± 16
TX16-012	4.3	225 ± 27
TX16-065	1.8	264 ± 12
TX16-018	3.3	266 ± 13
TX16-022	2.3	273 ± 43
TX16-034	4.8	104 ± 20
TX16-035	3.6	146 ± 39
TX16-026	4.7	162 ± 29

Table 12. Survival rate (%) of green grafts in the greenhouse and bench grafts in the field

	Green grafting	Bench grafting
Franc/Freedom	80	100
Franc/St.George	82	95
Franc/101-14	82	100
Franc/AXR1	65	100

LR131/Freedom	77	95
LR131/St.George	76	100
LR131/101-14	79	95
LR131/AXR1	66	100
LR132/Freedom	51	0
LR132/St.George	70	93
LR132/101-14	63	27
LR132/AXR1	60	87

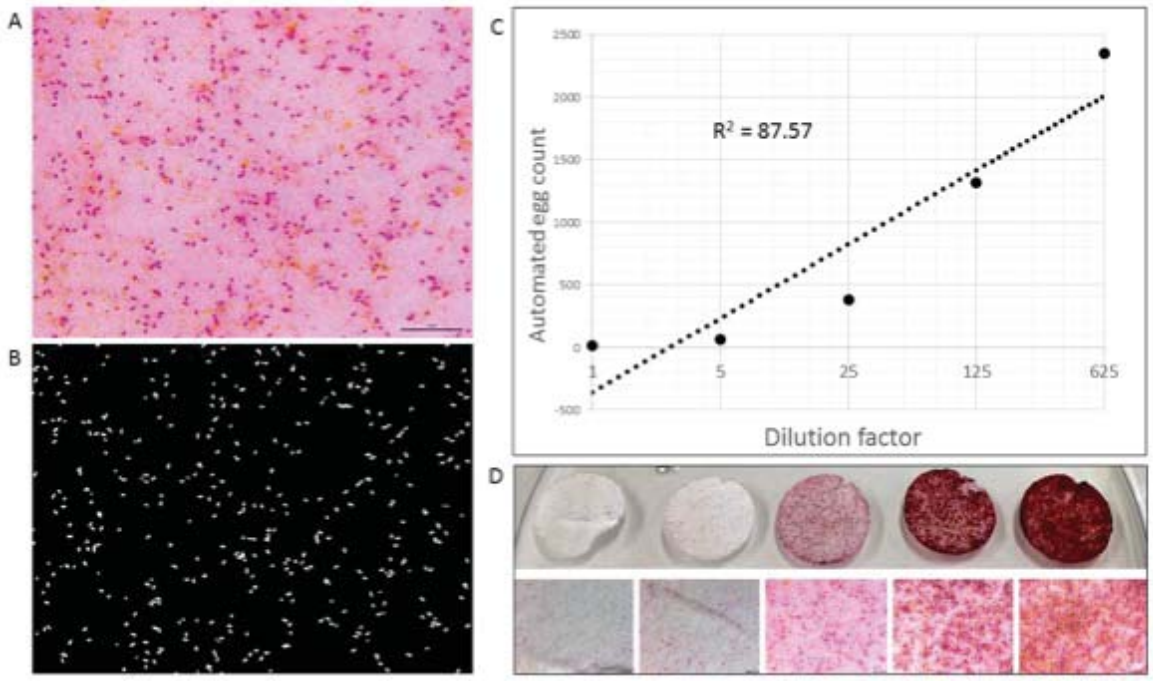


Figure 1 A, Acid Fuchsine stained RKN eggs on the filter paper. **B**, Processed image from ImageJ software, the count masks of RKN eggs from the pictures above. **C**, Logarithmic dilution series and correlation with the automated egg counts from ImageJ software. **D**, The respective Whatmann filter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

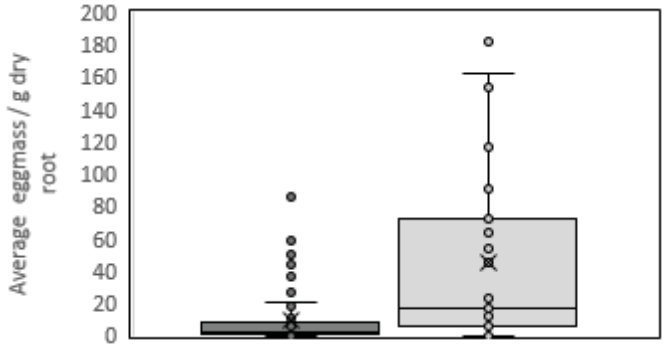


Figure 2. Average eggmass counts with HarmC (dark grey) and with HarmA (light grey) across the genotypes of the first screen of germplasm.

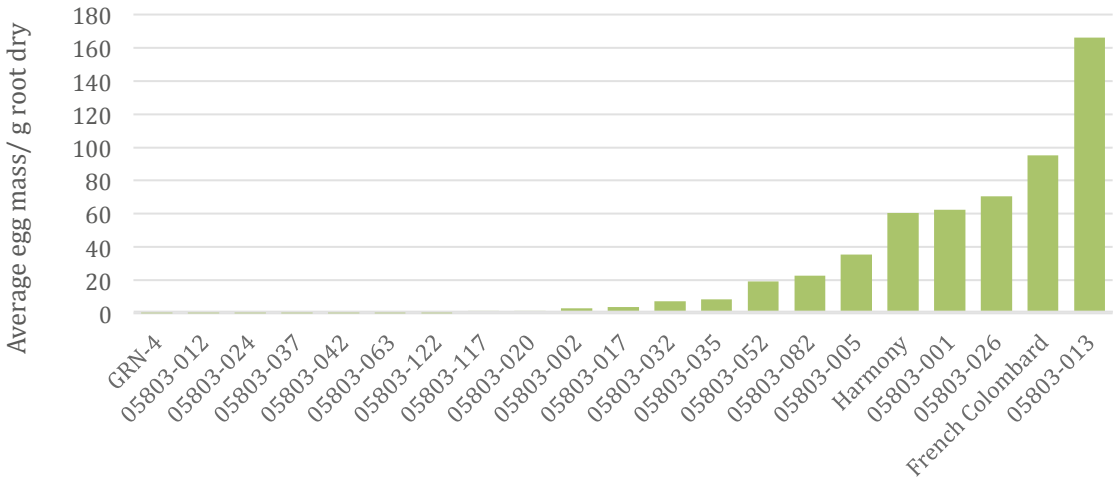


Figure 3. Results of RKN screen of the 05-803 population resulting from a cross of Colombard x GRN4. Eight out of the 18 tested seedlings showed sufficient resistance.

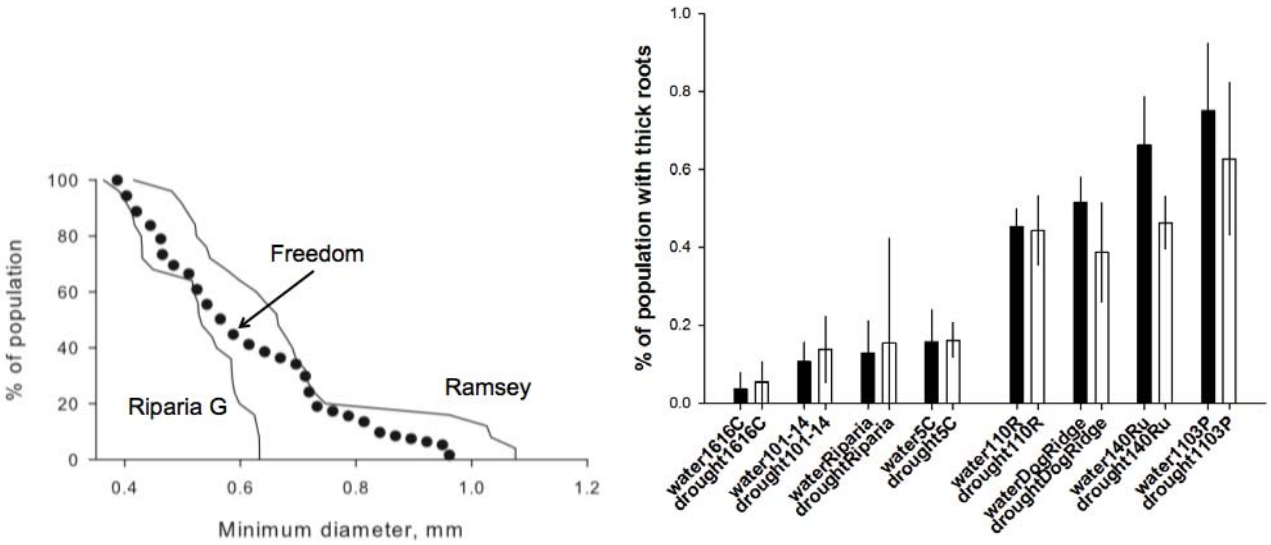


Figure 4. Patterns of root fibrosity from fabric containers using field soil and two irrigation regimes. (*left*) Percent of adventitious roots from three representative rootstock individuals under well-watered conditions, with minimum diameters as indicated. Drought resistant, moderately drought resistant, and drought susceptible genotypes are Ramsey, Freedom and Riparia G (Riparia Gloire), respectively. (*right*) Percent of adventitious roots exhibiting thick roots from four drought susceptible rootstocks (1616C, 101-14, Riparia Gloire and 5C) and four drought resistant rootstocks (110R, Dog Ridge, 140Ru and 1103P) under well-watered (filled columns) and drought (open columns) conditions. Error bars are ±1 standard deviation.

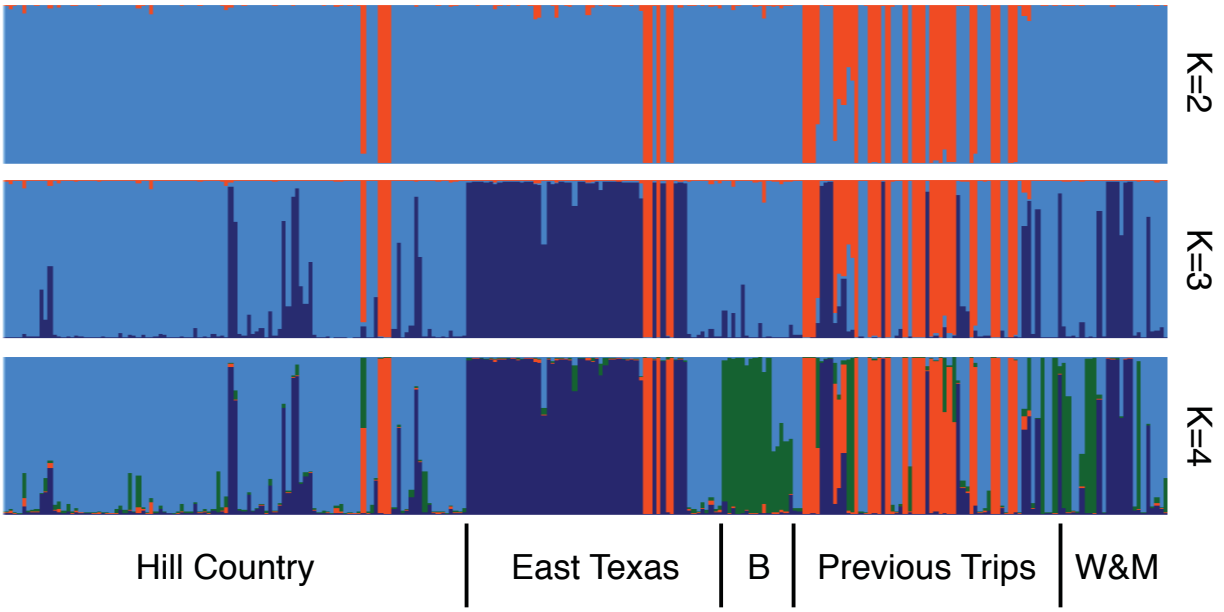
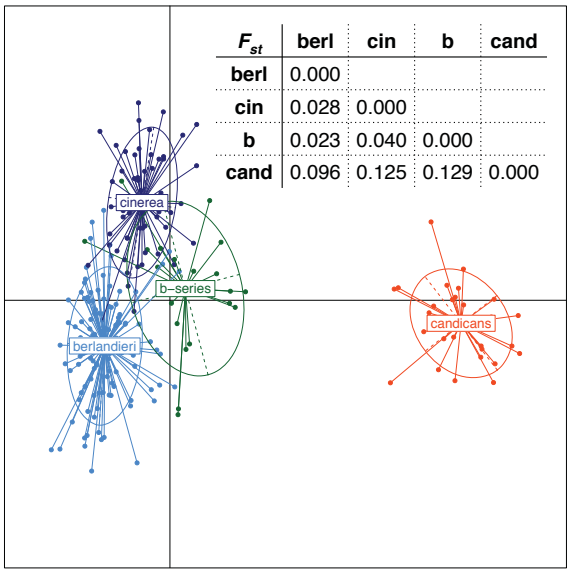


Figure 5. Population structure of *V. berlandieri* and related taxa in Texas and northeastern Mexico. 'Hill Country' and 'East Texas' accessions were collected in 2015-2016; 'B' refers to b-series, *V. cinerea*-like seedlings procured by H. Olmo from northeastern Mexico and southwestern Texas; 'Previous Trips' are previously collected accessions; 'W&M' are accessions from the Wolfskill and Montpellier germplasm repositories. For K = 2, LIGHT BLUE = *berlandieri*, *cinerea*, and b-series; ORANGE = *candicans*. For K = 3, LIGHT BLUE = *berlandieri* and b-series; DARK BLUE = *cinerea*; ORANGE = *candicans*. FOR K = 4, LIGHT BLUE = *berlandieri*; DARK BLUE = *cinerea*; GREEN = b-series; ORANGE = *candicans*.



F_{st}	berl	cin	b	cand
berl	0.000			
cin	0.028	0.000		
b	0.023	0.040	0.000	
cand	0.096	0.125	0.129	0.000

Figure 6. PCoA and table of pairwise F_{st} values of *berlandieri*, *cinerea*, b-series, and *candicans* accessions. These results support the close but independent grouping of *berlandieri* and *cinerea*.

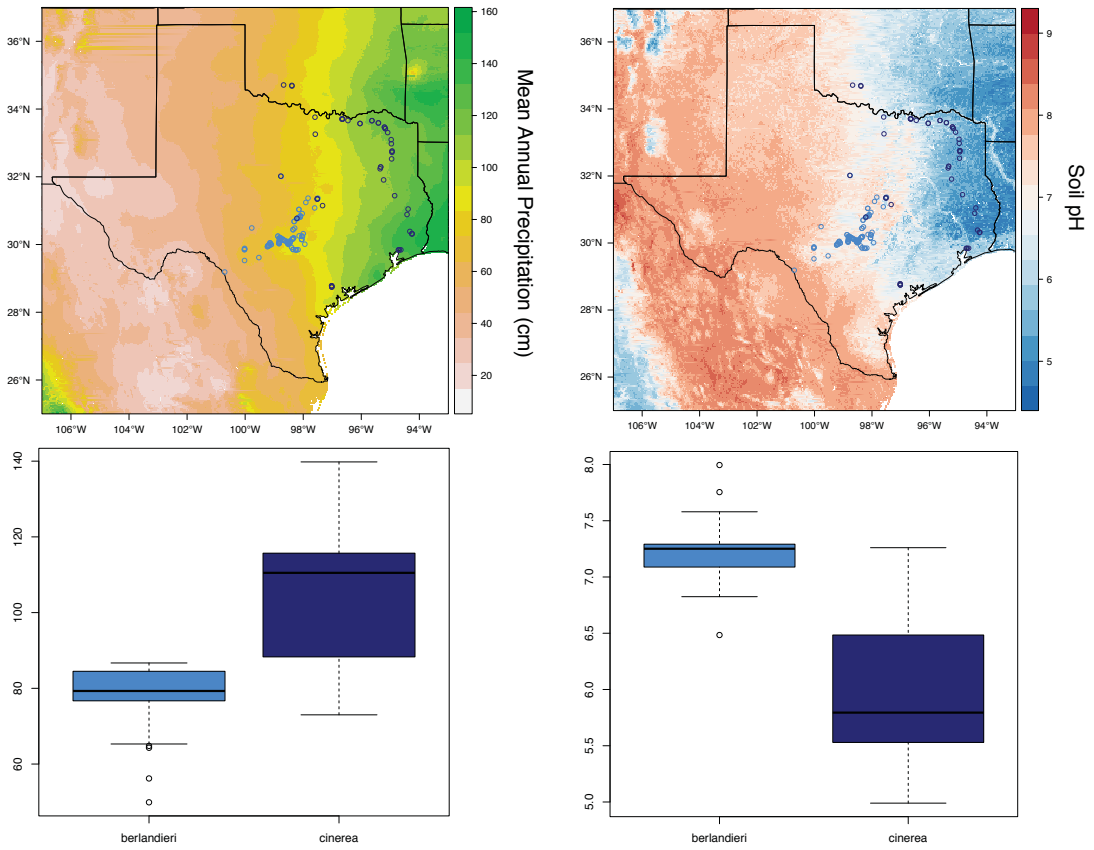
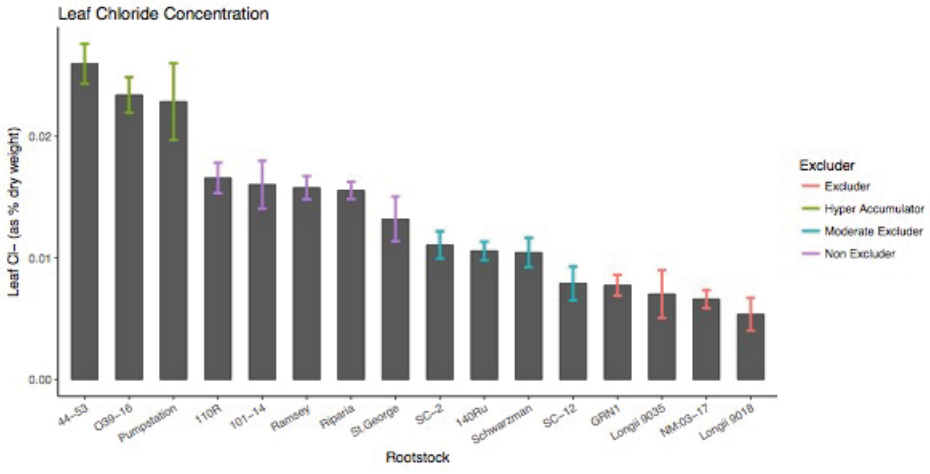


Figure 7. Mean annual precipitation (left) and soil pH (right) at collection locations for *V. berlandieri* and *V. cinerea* accessions and DNA samples. The small environmental variance for *V. berlandieri* collection locations indicates the restricted range of the species. Differences in mean annual precipitation and soil pH were highly significant ($p << 0.0001$) between *V. berlandieri* and *V. cinerea* collection locations according to a Mann-Whitney-Wilcoxon non-parametric test.

Figure 8. Salt screen data for the 16 rootstocks at 75 mM of salt concentrations. Both *longii* accessions 9018 and 9035 are extremely good salt excluders.



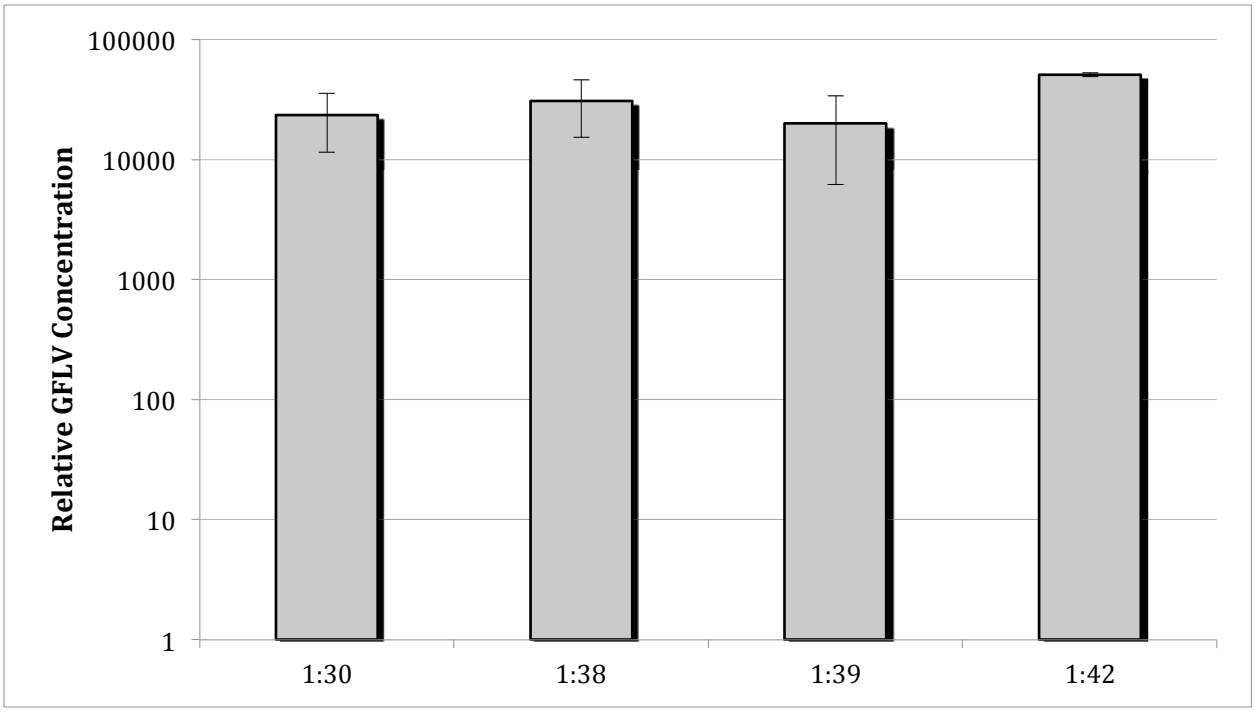


Figure 9. GFLV concentration in 4 vines randomly selected from the vines we used as our inoculum source for our GFLV tolerance and resistance screens. Concentrations are normalized to the 18SrRNA housekeeping gene and expressed relatively to a negative control sample. Error bars represent standard error of the mean (n=3).

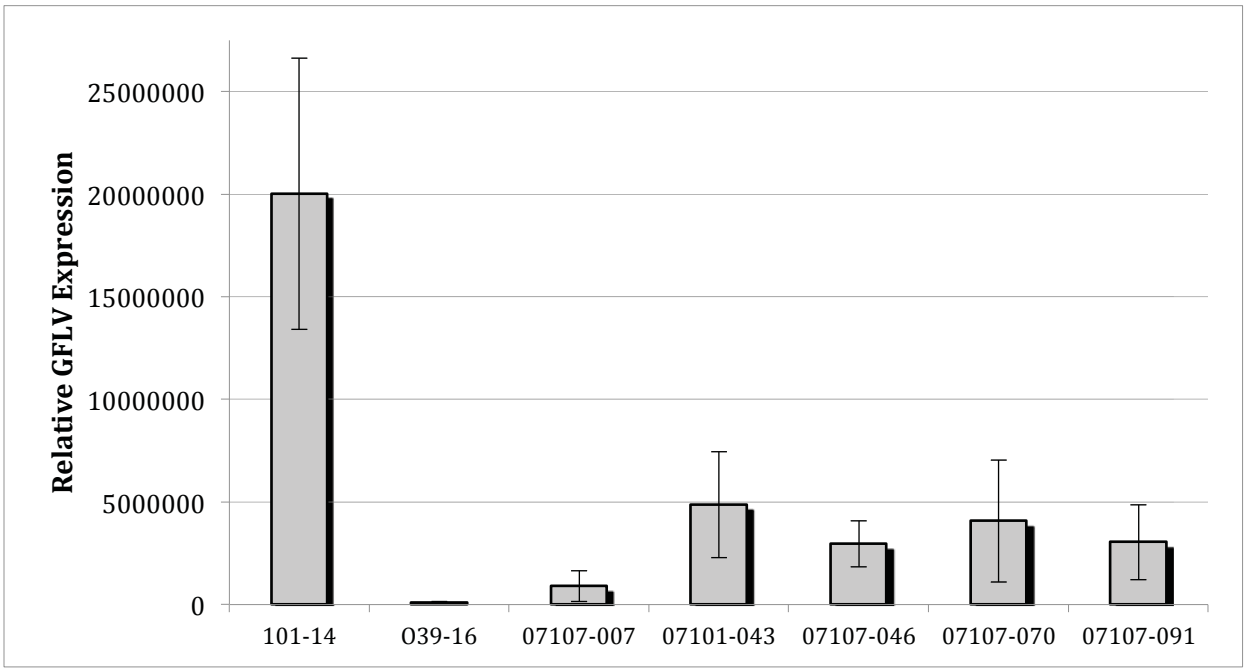


Figure 10. GFLV concentration in the rootstocks normalized to the 18SrRNA housekeeping gene and expressed relatively to a negative control sample. Error bars represent standard error of the mean (n=3).

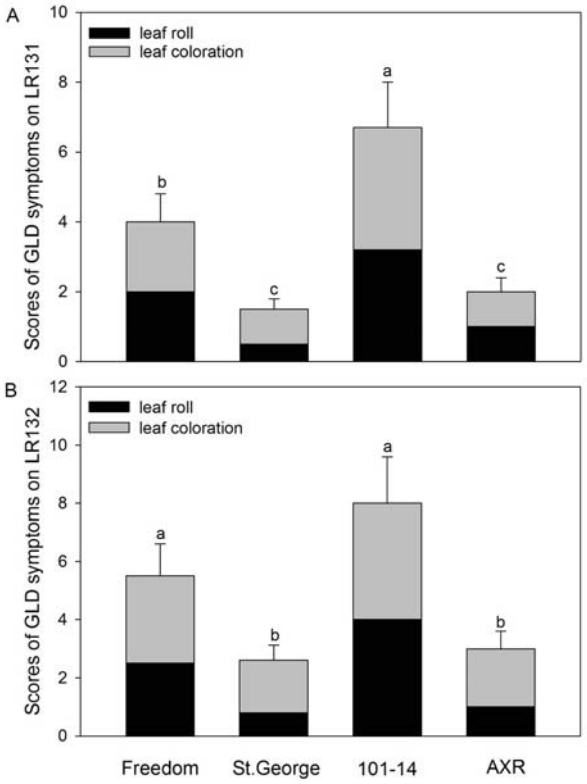


Figure 11. Evaluation of grapevine leafroll disease (GLD) on LR131 and LR132 green grafts 3 months after grafted on Freedom, St. George, 101-14 or AXR1. Symptoms were scored based on a 0-5 scale. Bars are SE of the total scores, and different letters mean significant difference at $P \leq 0.05$

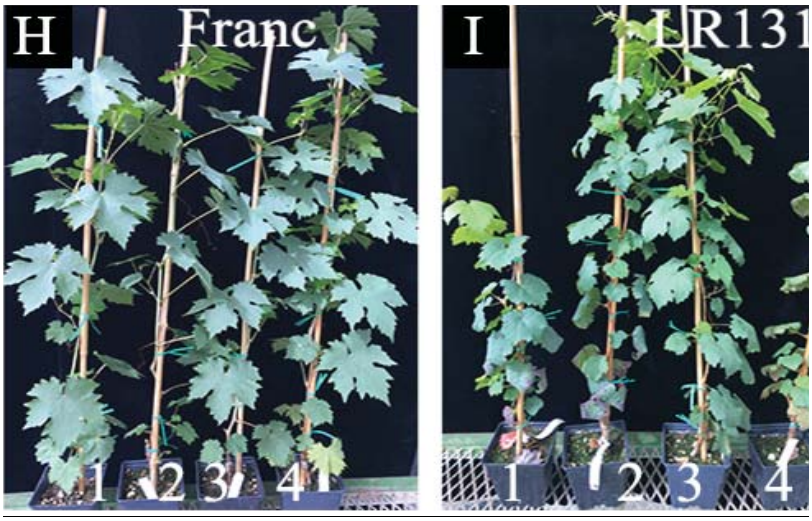


Figure 12. Grapevine leafroll disease symptoms on green grafts; H, I and J are healthy (H), infected LR131 (I) and infected LR132 (J) C. franc grafted onto Freedom (1), 101-14 (2), St.George (3) and AXR1 (4)

Figure 13. Effect of graft combination on the growth of green grafts measured as scion dry weight. Ns means no significant difference, * means significant difference at *P≤0.05* level by t-student test

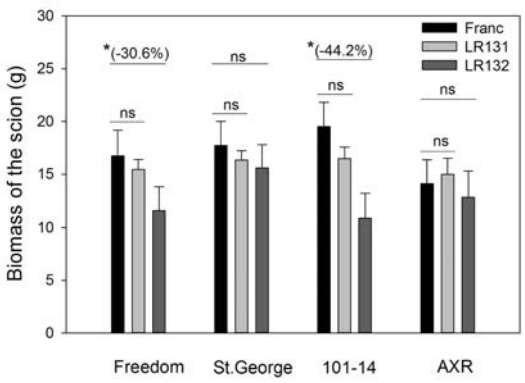
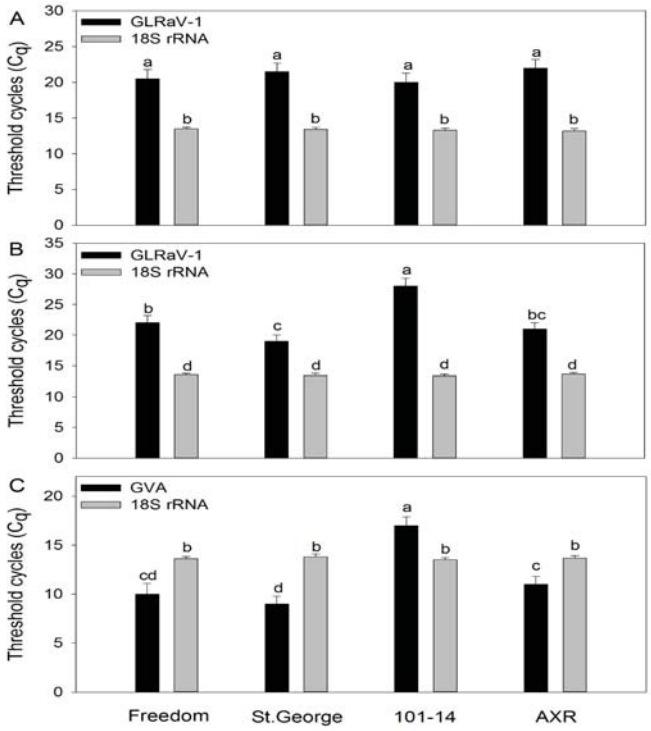


Figure 14. Virus concentration at union grafts of A) GRLaV-1 in LR131, B) GLRaV-1 in LR132 and C) GVA in LR132. Virus concentration is inversely proportional to Threshold cycles (Cq).



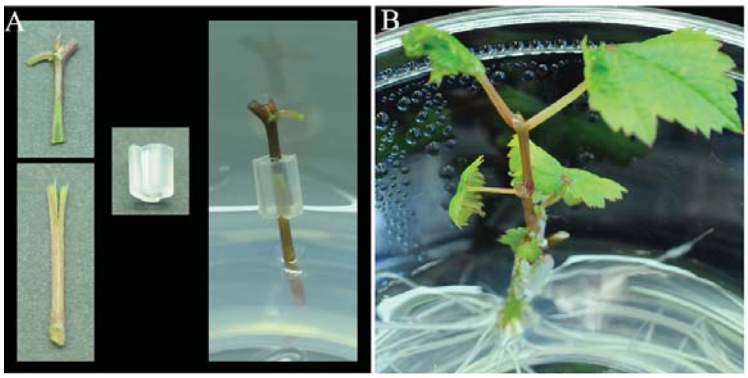
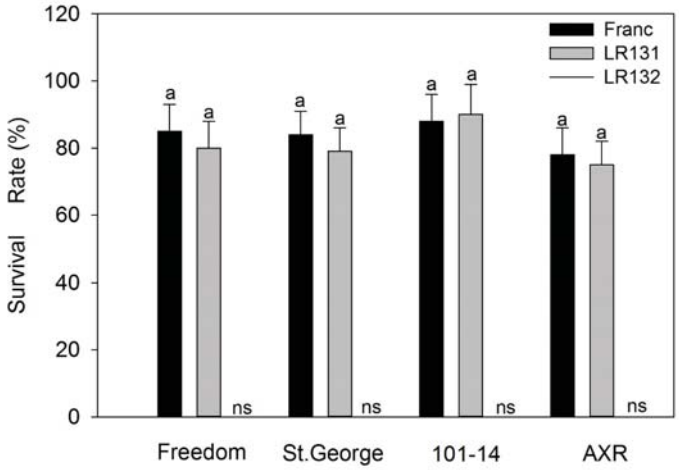


Figure 15. In vitro grafting

Figure 16. Survival rate of micrografts of micrografts of healthy and LR131 infected C. franc onto Freedom, St. George, 101-14 and AXR1after 2 months; ns means no survival



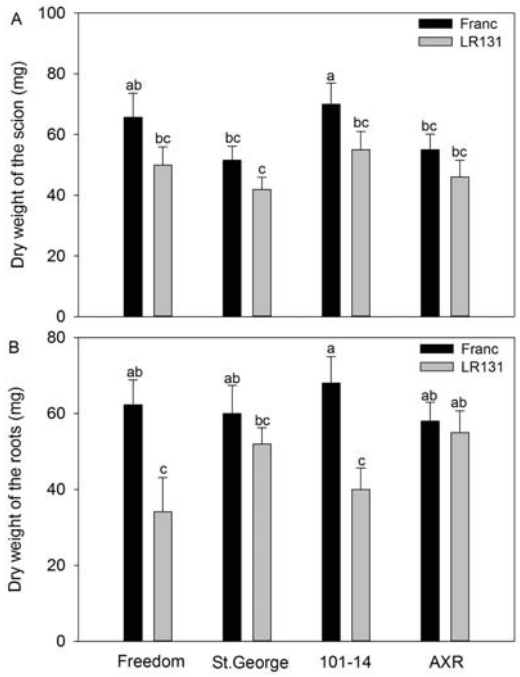


Figure 17. Effects of virus and rootstock on the vegetative growth of the scion (A) and the roots (B) of micrografts of healthy and LR131 infected C. franc onto Freedom, St. George, 101-14 and AXR1

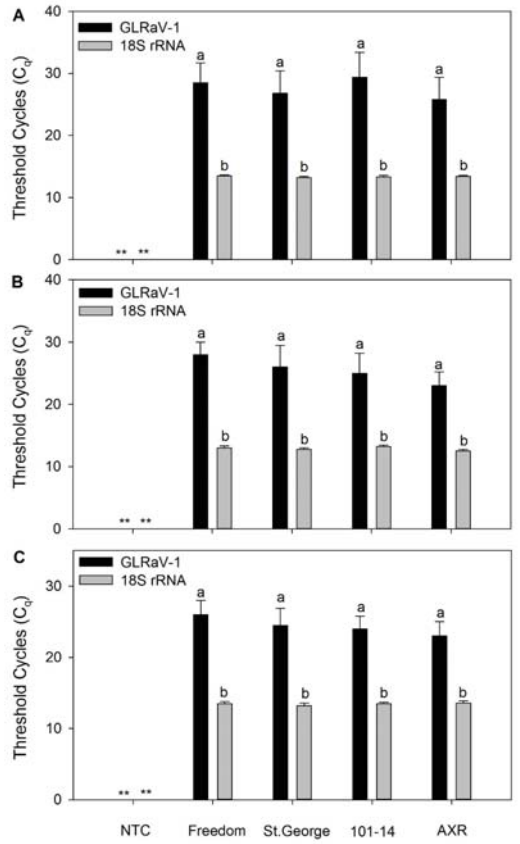


Figure 18. GLRaV-1 concentration after 7 days (A), 1 month (B) and 2 months (C), respectively, in LR131 infected C. franc grafted onto Freedom, St. George, 101-14 and AXR1. NTC means no template control; ** means undetectable.

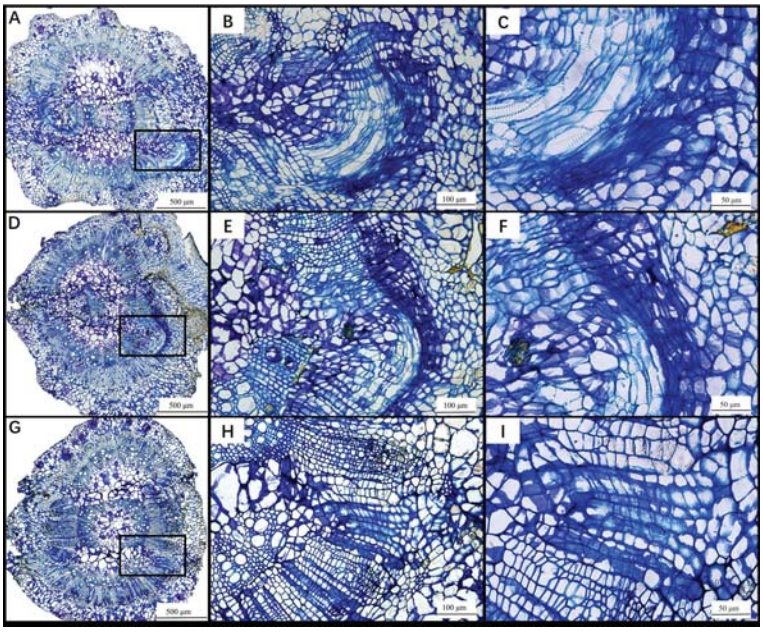


Figure 19. Histological observations of micrograft unions 2 months after grafting. A) Healthy micrografting union; B and C are closer views of the square in A, 10 x and 20 x respectively; D) LR131 infected micrograft union; E and F are closer views of the square in D 10 x and 20 x respectively; G) LR132 infected micrograft union; H and I are closer views of the square 10 x and 20 x respectively

170425000SA

Report: Evaluating novel nematicidal chemistry for usefulness in the nursery industry

PI: Andreas Westphal, Department of Nematology, University of California Riverside

This third year of the project started on July 1, 2017 after original initiation in 2015. The overall objectives are (1) test new nematicides for their efficacy against plant-parasitic nematodes important in perennial stock production; (2) test application methods for these non-fumigant nematicides; and (3) determine possible growth responses to nematode reduction.

Screening for effectiveness against perennial crop-typical nematodes in microplots

In spring 2016, two microplot experiments, one in sandy loam and a second in sandy soil at the Kearney Agricultural Research and Extension Center (KARE) infested with *Pratylenchus vulnus* had been initiated. Sixty microplots either filled with sandy soil and another sixty filled with sandy loam soil. Natural populations of the nematode had been established by cultivating two “liners” of the almond rootstock ‘Nemaguard’ during summer 2015, and inoculating with *Pratylenchus vulnus* at 1,995 vermiform lesion nematodes by adding excised highly infested walnut roots in pouches to each plot. Nematode populations built up on the Nemaguard root systems. In February 2016, these plants were removed, and the now nematode-infested soil mixed within each plot to simulate soil conditions at treatment in a nursery situation. Soil samples were collected, and nematode population densities determined. Material and water amounts were chosen based on the first microplot experiment (reported earlier) and long-year experience, to allow for delivery of materials >4 ft deep. After treatment, soil samples were taken to 5-ft depth and examined in 1-ft increments for live nematodes. Microplots were planted to clonal plugs of ‘Nemaguard’ (April 21, 2016). Four plants were established per plot. During the season, plants were excavated, and examined for nematode infection. In both soil types, the nematode numbers were lowest after Telone fumigation, and Vapam drench, three experimental biocides also had very low numbers (Fig. 1). Salibro (Q8U80) and treatments 4 and 5 had numerically higher numbers. This numerical reduction compared to the water control is noteworthy because these three non-fumigant materials represent minimal chemical amounts compared to the biocide treatments. Because the time from planting to end of the season was shorter than nursery stock is typically grown before sale, the decision was made to administer the test materials as post-plant applications to evaluate if this would continue to protect the roots from infection until the fall of 2017. At the beginning of the 2017 growing season, soil drenches in a post-plant use were repeated with beneficial selected nematicidal compounds because noticeable soil populations of *P. vulnus* had been detected (Fig. 2). The single plant will be used for evaluating the potential for keeping the root systems free of infection by *P. vulnus* for 18 months. This sampling is scheduled for within this month, and data will be presented at the research meeting.

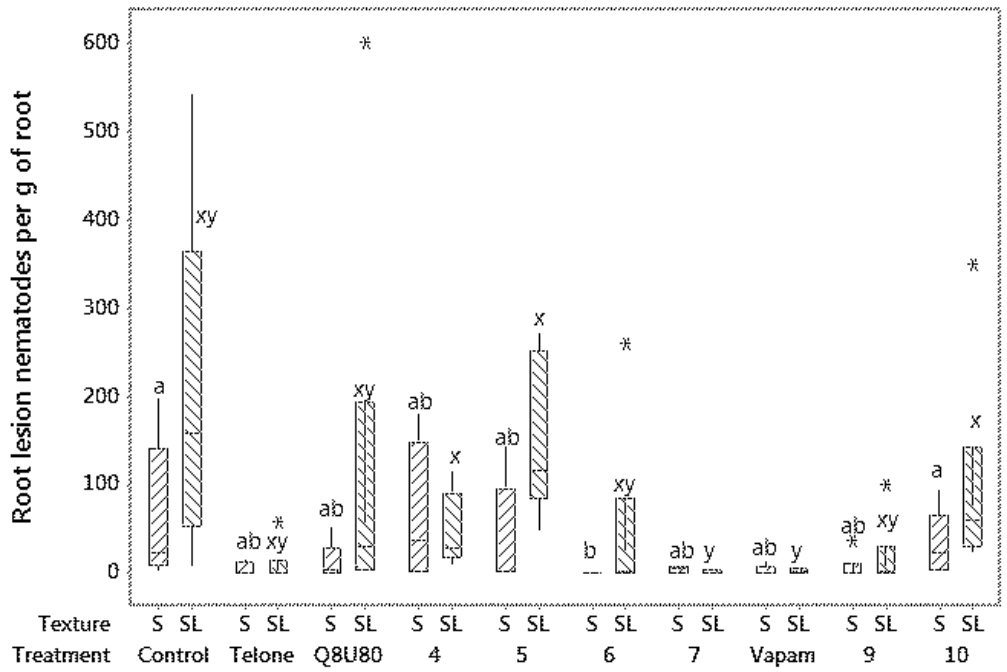


Fig. 1 *Pratylenchus vulnus* extracted from roots of Nemaguard grown in infested soil six Months after planting into pre-plant treated sand or sandy loam soil started in 2016 spring.

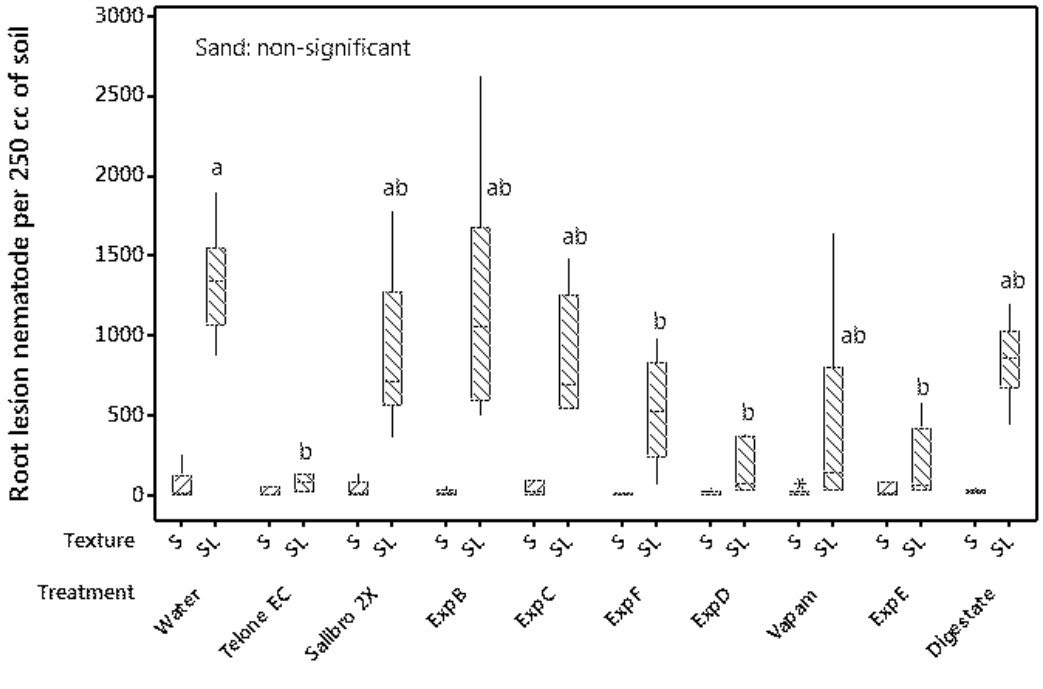


Fig. 2 Soil populations of *Pratylenchus vulnus* in sand (S) and sandy loam (SL) infested with *Pratylenchus vulnus* and cropped to 'Nemaguard' clonal plants at the beginning of the 2017 growing season.

In summer 2017, seedlings were harvested from the plots, and root extractions conducted. At that time, no statistical differences among treatments were detected, but there seemed to be numerical differences (Fig. 3).

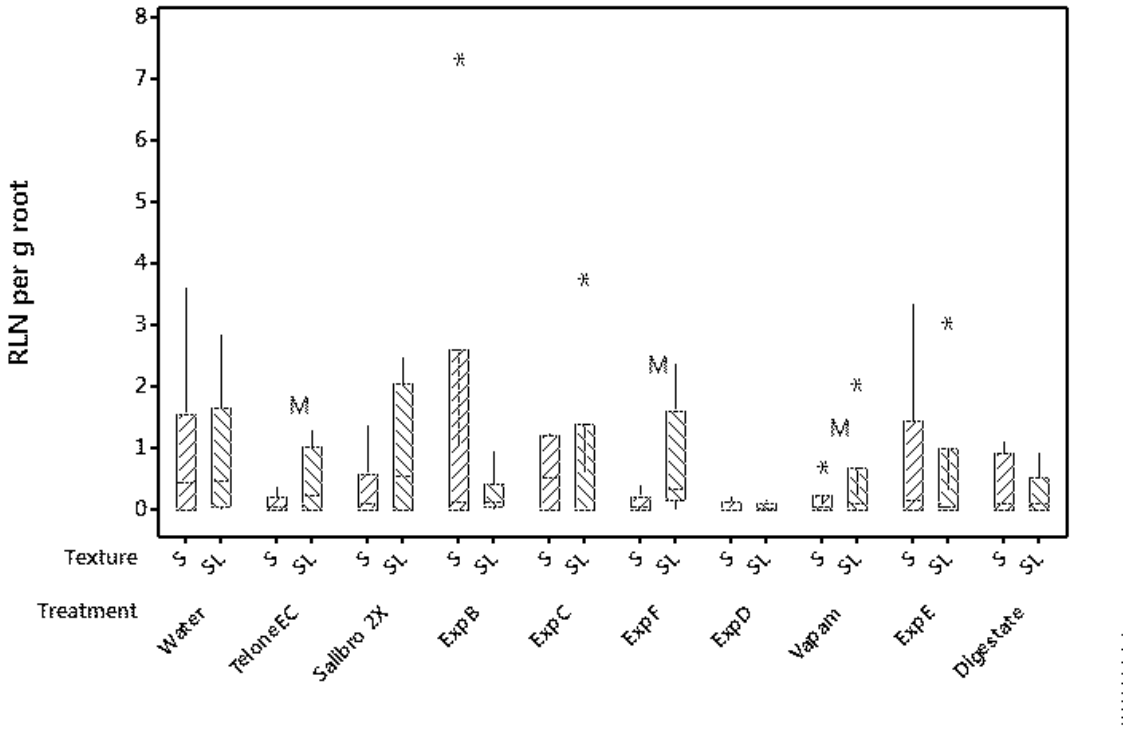


Fig. 3 Numbers of *Pratylenchus vulnus* extracted from roots grown in sand (S) and sandy loam (SL), and treated with potentially nematicidal compounds.

Application to small plots in a nursery-type setting at KAC

Two experiments were conducted in this objective. Both experiments had a water drench control, and a Telone EC fumigation treatment.

Emphasis on biocide applications

In August 2016, four replicate plots of 10 × 35 ft dimensions were prepared to allow for drench treatments in 6 acre inch of water a non-fumigant treatment combination and two biocidal materials. At planting of the nursery seeds of 'Nemaguard', soil samples were taken to a depth of 5 ft in 1-ft increments. On treatment averages, the two experimental biocides had similarly low numbers as the Telone EC treatment, the other experimental treatment had slightly higher numbers (Fig. 4).

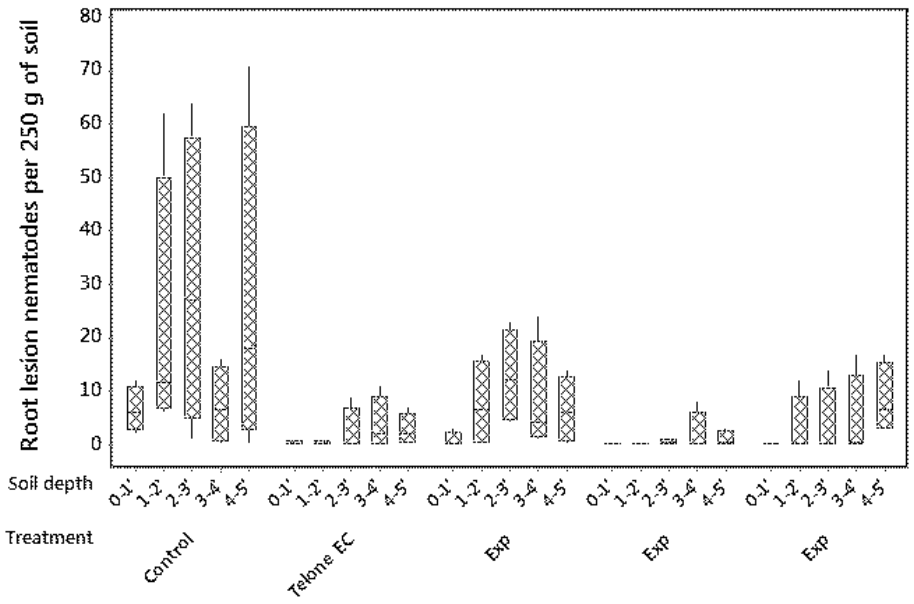


Fig. 4. Vermiform *Pratylenchus vulnus* in soil after various drench treatments in sandy loam soil started in mid-August 2016.

Emphasis in application techniques and schedules for non-fumigant nematicides

At beginning of October 2016, each of the drench treatments were applied to five replicate plots of 10 × 35 ft dimension in 6 acre inch of water. Treatment suspensions were either applied on the soil surface or through drip lines buried 22 inches deep in soil (Table 1). Soil samples were taken before treatment and 6 weeks later at planting of nursery seeds 'Nemaguard'. Preliminary examination showed treatment effects. The water control had mid-level population densities, whereas the Telone EC plots, and one experimental biocide had a reduction of nematode numbers (Table 1; Fig. 5). The other treatments were all non-fumigant materials. These seemed to have elevated numbers in the shallow soil layers, and observation that is routinely made in these drench treatment experiments. Experience from the microplot trials suggested that these detected nematodes are not necessarily infective. Information that we anticipate in the seedling examinations scheduled for the emerging plants in 2016 had shown.

The extreme weather conditions of winter 2017 made it difficult to conduct this trial. Despite all efforts to remove the flooding waters that repeatedly encumbered the plots, water logging could not be averted. As a result, the plots needed to be replanted repeatedly. A first examination of seedling growing in these plots revealed overall low infection rates (data not shown). A presumably more telling, end of the season evaluation is scheduled for the month of February.

Table 1. Numbers of *Pratylenchus vulnus* post treatment in a nursery experiment in small field plots at Kearney Agricultural Research and Extension Center in 2016

Treatment number	Soil profile shallow	deep	*Pratylenchus vulnus* per 250 ml of soil	Statistical grouping
1	Water control - S		3.5 ± 0.7	ab
2	Telone EC - S		1.0 ± 0.5	c
3	Q8U80 - S	Q8U80 - B	9.5 ± 2.6	a
4	ExpB - S	Q8U80 – B	2.5 ± 0.5	bc
5	ExpC - S	Q8U80 – B	3.6 ± 0.9	ab
6	ExpB - S	Q8U80 – P	6.3 ± 2.4	a
7	ExpC - S	Q8U80 – P	6.3 ± 1.1	a
8	Water control - S	Water control - B	9.0 ± 1.9	a
9	ExpD - S		2.9 ± 1.7	bc
10	Q8U80 - S		3.2 ± 0.7	ab
P-values				
Treatment (T)				<0.01
Depth (D)				<0.01
T × D				<0.01

S Surface applied, allowed to percolate as a surface drench
B Applied through drip lines buried at 22 inches depth
P Surface applied ("pulsed") the day prior to application to the shallow soil layer

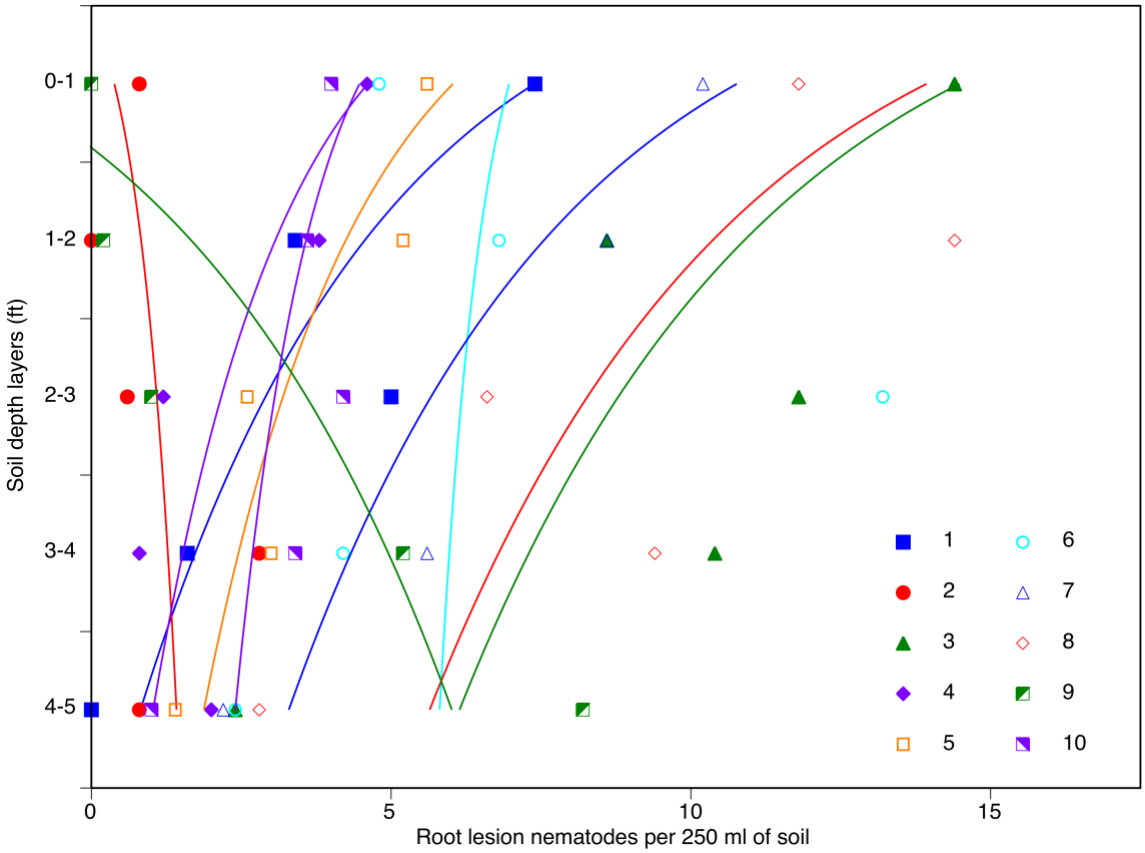


Fig. 5 *Pratylenchus vulnus* extracted from soil samples collected at 0-5 ft depth post treatment in 2016. Treatment 1 through 10 are summarized in Table 1. Trend lines were arbitrarily entered in the graph. Statistical analysis is provided for the treatment means in Table 1.

2017/2018: Refined application methods to small plots in a nursery-type setting at KAC

A second round of application method examinations was necessary before studies are extended to commercial scales. In this project, most effective treatments were repeated to determine the reliability of nematode suppression (Table 2). This was done to test if additional treatment patterns need to be designed. Treatments were applied in fall of 2017, and clonal plugs will be planted at the end of winter 2018. The same evaluation measures as in previous tests will be taken. Soil (and root) samples for nematode extraction will be taken at planting and at ½-year increments. Two rows spaced 2 ft apart will be planted in each plot. Plants of one of these rows will be excavated after at the necessary intervals for growth and root evaluations, and the second one will be maintained until harvest maturity of the planting stock after approximately 15 months. No comprehensive data are available on this trial yet.

Table 2. Treatment assignments and schedules of various chemicals in a nematode-infested field. Materials were applied in a total of 6 acre inch of water: Either as one solution ("surface continuous"), first for the deep layer and then the shallow layer on top ("pulsed"), or sprayed on the soil surface and then watered in ("surface spray + water")

Treatment no.	Shallow (0-2.5-ft)	Deep (2.5-5-ft)	Method
1	Water	Water	Surface continuous
2	Telone EC	N/A	Surface continuous
3	Salibro 2X	N/A	Surface continuous
4	ASD	N/A	Surface continuous
5	Salibro 2X	ExpB	Pulsed
6	ExpC 2X	ExpB	Pulsed
7	Salibro – total three times		Surface continuous
8	ExpD 2X	N/A	Surface continuous
9	ExpE	N/A	Surface continuous
10	Salibro 2X	N/A	Surface spray + water

Summary and conclusions

Summarizing the overall project, three materials seem to offer promise. Salibro and ExpB offered some benefit in reducing nematode numbers. The treatment schedules applied in Fall 2017 fully considered their behavior in soil. ExpD, a high-volume material, repeatedly suppressed nematode numbers, and thus should be moved forward in the program. Further efforts need to be made to confirm alternatives to Telone fumigation. It appears that the three candidate materials are fit to be moved to larger scale testing as outlined in the proposal.