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

2018 Research Progress Report

Nursery Services Program

California Department of Food and Agriculture

Sacramento, CA 95814

TABLE OF CONTENTS

Reports

I. Effects of Little cherry virus-1 and Little cherry virus-2 on Different Cherry

Pages 1-4

II. Development and validation of real-time quantitative PCR assays for the detection of fruit tree viruses.

Pages 5-11

III. Development of Next Generation Rootstocks for California Vineyards

Pages 12-31

IV. Development of an Armillaria resistance screen for clonal walnut rootstocks

Pages 32-34

V. Integrated management of Fusarium canker in bare root and container-propagated stone fruit trees
. . . In progress

VI. Support to Foundation Plant Services 2018/2019
. . . In progress

VII. Evaluating novel nematicidal chemistry for usefulness in the nursery industry
. . . In progress

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

January 30, 2019

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

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

Project Leader: Maher Al Rwahnih, Academic Administrator, Department of Plant Pathology, Foundation Plant Services, University of California

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

We also performed High Throughput Sequencing (HTS) analysis on both selected positive controls to confirm the previous RT-qPCR results and to exclude any possible infection with known or unknown virus/es.

Objective 2:

RT-qPCR was used to verify that all the selected rootstocks were negative for LChV1 and LChV2. In addition trees were also tested for the two common pollen vectored viruses PNRSV, PDV which are already known to cause hypersensitivity reaction in some of the selected rootstocks. Plants were also tested for CVA as its known to be seed transmitted.

For the first iteration of the trial, previously funded by IAB (funding cycle July 1, 2017 to June 30, 2018), green growing negative control material was sourced from Foundation Orchard Bing trees and positive material from container grown LChV-1 and LChV-2 Bing trees. Material was collected in late May of 2018 and T-bud grafted to container grown rootstocks with 2 buds per rootstock. Bud take success was evaluated post-grafting and additional buds were grafted where success was poor. The virus inoculated, T-budded and non-grafted control trees were planted in a randomized complete block early October 2018. In spring of 2019, rootstock vegetation above the scion buds will be tested to confirm successful transmission; after which, it will be removed to promote scion growth. Observations of bud take and tree performance will be recorded and evaluated. Continuation of this project is contingent upon Program approval and funding.

For the second iteration of the trial, during the 2018-19 funding cycle, material was collected from negative and positive source trees and T-budded to container grown rootstocks in October of 2018. Dormant grafted and non-grafted control trees are scheduled for winter 2019 planting. In the spring of 2019, following bud-break, rootstock vegetation above the scion buds will be tested to confirm successful transmission; after which, it will be removed to promote scion growth. Observations of bud take and tree performance will be recorded and evaluated. Continuation of this project is contingent upon Program approval and funding. The site for the field trial has been cultivated and drip irrigation lines have been installed. Weed and pest control maintenance will continue through the funding of the project.

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

January 30, 2019

Project Title: Development and validation of real time quantitative PCR assays for the detection of fruit tree Viruses

Project Duration: 07/01/2018 to 06/30/2018

Project Leader: Maher Al Rwahnih, Academic Administrator, Department of Plant Pathology, Foundation Plant Services, University of California

Objectives:

1. Screen select pome and Prunus tree populations for targeted pathogens to compile a representative set of genome sequences (as complete as possible), and evaluate current published primers.
2. Incorporate new genetic data into a more complete characterization of genetic variation across the targeted pathogens to inform assay design.
3. Construct improved assays utilizing multiple primers sets for detecting all existing targeted pathogen variants.
4. Empirically test and validate proposed assay designs using positive controls.
5. Disseminate research progress and results.

Accomplishments:

Objective 1:
From July to September 2018, plant material allegedly infected by the targeted viruses and other pathogens (Table 1) was obtained from the National Clonal Germplasm Repository (NCGR) in Davis, CA and Corvallis, OR, which includes pome and fruit trees collected from around the world. Material was also obtained from the Clean Plant Center Northwest (CPCNW), Washington State University in Prosser, WA and plants that are part of the Foundation Plant Services (FPS) pipeline of foreign and domestic introductions. In total, 60 pome or Prunus samples were obtained and included in the screening. In the cases of tomato ringspot virus (ToRSV), Arabis mosaic virus (ArMV) and tobacco ringspot virus (TRSV) were unable to obtain isolates infecting pome or Prunus trees, but isolates infecting grapevine were included in the study.

Table 1. Targeted pathogens of fruit trees.

Disease	Disease Agent	Host
Apple chlorotic leaf spot	Apple chlorotic leafspot virus (ACLSV)	Pome, Prunus
Apple mosaic	Apple mosaic virus (ApMV)	Pome, Prunus

Apple stem grooving	Apple stem grooving virus (ASGV)	Pome
Apple stem pitting, Pear stem pitting, Pear stony pit disease	Apple stem pitting virus (ASPV)	Pome
Flat apple disease	Cherry rasp leaf virus (CRLV)	Pome, Prunus
Tobacco ringspot	Tobacco ringspot virus (TRSV)	Pome
Apple union necrosis	Tomato ringspot virus (ToRSV)	Pome, Prunus
Pear decline	Pear decline Phytoplasma	Pome
Apple scar skin/Dapple apple disease	Apple scar skin viroid (ASSVd)	Pome
Pear blister canker	Pear blister canker viroid (PBCVd)	Pome
Cherry green ring mottle	Cherry green ring mottle virus (CGRMV)	Prunus
Cherry leafroll	Cherry leafroll virus (CLRV)	Prunus
Little cherry	Little cherry virus 1 (LChV-1); Little cherry virus 2 (LChV-2)	Prunus
Prune dwarf	Prune dwarf virus (PDV)	Prunus
Prunus necrotic ringspot	Prunus necrotic ringspot virus (PNRSV)	Prunus
Arabis mosaic	Arabis mosaic virus (ArMV)	Prunus

In order to confirm the pathogen-infection status of collected samples, total nucleic acid (TNA) extracts were prepared from plant material and later analyzed by high throughput sequencing (HTS). Additionally, plants free of any pathogen were included in the HTS analysis as negative controls. As a result, plants infected with targeted pathogens were identified (Table 2).

Table 2. Plant material infected with targeted pathogens of fruit trees.

Sample	Plant Material	Pathogen Infection	HTS
S1	Grapevine	ToRSV	Yes
S2	Prunus	PNRSV	Yes
S3	Prunus	PNRSV, ACLSV	Yes
S4	Grapevine	ArMV	Yes
S5	Grapevine	ArMV	Yes
S6	Grapevine	TRSV	Yes
S7	Prunus	CGRMV	Yes
S8	Prunus	PDV	Yes
S9	Pome	ASGV	Yes
S10	Pome	ASGV	Yes
S11	Pome	ASGV	Yes
S12	Pome	ASPV	Yes
S13	Pome	ASPV	Yes
S14	Pome	ASPV	Yes
S15	Pome	ASPV	Yes
S16	Pome	ASPV	Yes
S17	Pome	ASGV	Yes
S18	Pome	ASGV	Yes
S19	Pome	ASGV	Yes
S20	Pome	ASPV	Yes

S21	Pome	ASPV, ApMV, ASGV, ACLSV	Yes
S22	Pome	ASPV	Yes
S23	Pome	ASPV	Yes
S24	Pome	ASPV, PBCVd	Yes
S25	Pome	ACLSV, ASGV, ASPV	Yes
S26	Pome	ApMV, ASPV	Yes
S27	Pome	ASPV	Yes
S28	Pome	ASGV, ACLSV, ASSVd	Yes
S29	Pome	PBCVd	Yes
S30	Pome	ASPV	Yes
S31	Pome	ASPV, PBCVd	Yes
S32	Pome	ASPV, ASGV, ACLSV	Yes
S33	Pome	ASPV, ACLSV	Yes
S34	Pome	ASPV, ACLSV	Yes
S35	Pome	ASPV, ASGV, ACLSV	Yes
S36	Prunus	PDV, PNRSV	Yes
S37	Pome	ASGV	Yes
S38	Pome	ASPV, PBCVd	Yes
S39	Prunus	LChV1	Yes
S40	Prunus	LChV2	Yes
S41	Prunus	LChV2	Yes
S42	Prunus	CLRV	Yes
S43	Prunus	CLRV	Yes
S44	Prunus	CRLV	Yes
S45	Pome	ASGV, Pear decline	Yes
S46	Pome	ASGV, Pear decline	Yes

Published PCR detection systems (Table 3) for targeted pathogens were evaluated *in silico* to determine their detection capacity (i.e. number of virus, viroids and phytoplasma isolates that they can detect) using the sequence data deposited in GenBank and the HTS data generated at FPS. Based on this evaluation, several detection assays failed in their detection capacity, evidencing the need of an improved assay (with additional primers/probes) or the design of a completely new assay.

Table 3. Published PCR-based assays for targeted pathogens of fruit trees.

Disease Agent	Assay	Reference	Target Region
ACLSV	Multiplex RT-PCR	Menzel et al. 2002	CP gene
	RT-LAMP	Peng et al. 2017	CP gene
	Real-time PCR	Osman et al. 2017	CP gene
ApMV	Multiplex RT-PCR	Menzel et al. 2002	RNA 3
	One-step RT-PCR	Sanchéz-Navarro et al. 2005	RNA 3
	Real-time PCR	Gadiou and Kundu 2012	RNA 3

ASGV	Multiplex RT-PCR	Menzel et al. 2002	CP gene
	Multiplex RT-PCR	Ito et al. 2002	CP gene
	Real-time PCR	Gadiou and Kundu 2012	CP gene
ASPV	Multiplex RT-PCR	Menzel et al. 2002	CP gene
	RT-PCR	Kundu 2002	CP gene
	RT-PCR	Komorowska et al. 2010	CP gene
CRLV	RT-PCR	James et al. 2001	RNA 2
	RT-PCR	Villamor et al. 2016	RNA 2
	RT-PCR, Real-time PCR	Osman et al. 2017	RNA 2
TRSV	RT-PCR	Fuchs et al. 2010	RNA 1
	RT-PCR	Fisher 2013	RNA 1 & 2
ToRSV	RT-PCR	Griesbach 1995	RNA 1
	Immunocapture RT-PCR	Fuchs et al. 2009	RNA 1
	RT-PCR	Yao et al. 2018	RNA 1
Pear decline (*Phytoplasma pyri*)	PCR	Lorenz et al. 1995	16s rDNA gene
	Nested PCR	Chapa et al. 2003	16s rDNA gene
	PCR	Seemuller et al. 2004	16s rDNA gene
ASSVd	RT-PCR	Di Serio et al. 2002	Genome
	Real-time PCR	Kim et al. 2010	Genome
	RT-PCR	Kumar et al. 2014	Genome
PBCVd	RT-PCR	Joyce et al. 2003	Genome
	RT-PCR	Hassen et al. 2004	Genome
	RT-PCR	Hassen et al. 2006	Genome
CGRMV	RT-PCR	Hassan et al. 2006	CP gene
	RT-PCR	Fiore et al. 2013	CP gene
	Real-time PCR	Osman et al. 2017	CP gene
CLRV	Immunocapture RT-PCR	Werner et al. 1997	RNA 2
	Multiplex RT-PCR	Bertolini et al. 2001	RNA 2
LChV-1	RT-PCR	Bajet et al. 2008	RdRp region
	RT-PCR	Glasa et al. 2015	CP gene
	Real-time PCR	Katsiani et al. 2018	CP gene
	RT-LAMP	Tahsima et al. 2018	RdRp region
LChV-2	RT-PCR	Eastwell and Bernardy 2001	RdRp region
	RT-PCR	Rott and Jelkmann 2001	ORF1a
	RT recombinase polymerase amplification assay	Mekuria et al. 2014	CP gene
PDV	RT-PCR	Mekuria et al. 2003	CP gene (RNA 3 or sgRNA 4)
	Real-time RT-PCR	Jarosova and Kundu 2010	CP gene (RNA 3 or sgRNA 4)
	Nested PCR	Lee et al. 2014	CP gene (RNA 3 or sgRNA 4)
PNRSV	One-step RT-PCR	Sanchéz-Navarro et al. 2005	CP gene (RNA 3 or sgRNA 4)
	Real-time RT-PCR	Marbot et al. 2003	CP gene (RNA 3 or sgRNA 4)
	Real-time fluorescent PCR	Huo et al. 2017	CP gene (RNA 3 or sgRNA 4)

ArMV	Multiplex RT-PCR	Bertolini et al. 2001	CP gene (RNA 2)
	RT-PCR	Kominek et al. 2003	CP gene (RNA 2)
	RT-PCR	Fisher 2013	RNA 1 & RNA 2

Objective 2:
As a result of the HTS analysis conducted at FPS, 9 near-complete genomes of viruses or viroids were obtained from pome and Prunus tree samples. These sequences included apple chlorotic leafspot virus (ACLSV), apple mosaic virus (ApMV), apple scar skin viroid (ASSVd), apple stem grooving virus (ASGV), apple stem pitting virus (ASPV), pear blister canker viroid (PBCVd), Prunus necrotic ringspot virus (PNRSV). We are in the process of submitting this new sequence data to GenBank.

Objective 3:
To construct improved detection assays, sequence data available at GenBank and generated at FPS was aligned using a custom script developed in-house. This script identified potential candidates for primers/probes, later, candidate primers/probes were adjusted according to the parameter for TaqMan real-time PCR (MGB probes). Finally, adjusted primers/probes were aligned to determine identity and unique primers/probes were used in the assays; thus, multiple primers/probes were included in single quantitative PCR (qPCR) reactions. If the script identified a region similar to a previously published assay, the assay was updated with extra primers or probes for highly divergent variants of the virus or viroid. Currently, ten different detection assays (Table 4) for targeted pathogens have been developed (new assays) or updated via the multistep process described-above.

Table 4. Designed or updated assays for detection of fruit tree pathogens.

Pathogen	Type of Primer	# of Primers	5' Fluorophore	Target Region	Reference
ToRSV	Forward	2		CP/Polyprotein	Osman et al. 2014 (Updated)
	Reverse	2			
	Probe	1	FAM		
ArMV	Forward	2		RNA2/P2/CP	This study
	Reverse	1			
	Probe	3	FAM		
ApMV	Forward	4		CP	Osman et al. 2014 (Updated)
	Reverse	2			
	Probe	2	FAM		
ACLSV	Forward	1		CP	Osman et al. 2017 (Updated)
	Reverse	3			
	Probe	1	FAM		
PDV	Forward	4		CP	Osman et al. 2014 (Updated)
	Reverse	2			
	Probe	2	FAM		
PNRSV	Forward	4		CP	This study
	Reverse	2			
	Probe	2	FAM		
ASSVd	Forward	5		Viroid genome	This study
	Reverse	4			
	Probe	2	FAM		
ASPV	Forward	5		CP	This study

	Reverse	5			
	Probe	2	FAM		
ASGV	Forward	2		MP/Polyprotein	This study
	Reverse	1			
	Probe	1	FAM		
LChV2	Forward	2		RDRP	This study
	Reverse	1			
	Probe	2	FAM		

Objective 4:
As initial validation, all the new assays or updated assays were empirically tested using the virus-infected material (Table 2) and healthy plants (negative controls). As a result of this small-scale survey, the ten improved assays efficiently and specifically detected the different targeted pathogens. Plants determined positive for fruit tree viruses or viroids during the HTS analysis, tested positive during the screening using the improved qPCR assays. The next-step is challenge these assays against a large number of samples, including samples from commercial orchards.

Objective 5:
Preliminary results have been presented during growers' meetings organized by the UC Cooperative Extension, and scientific meetings organized by the California Department of Food and Agriculture (CDFA). Additionally, the novel detection tools will be shared with diagnostic labs involved in the fruit tree industry in the US, including the National Clean Plant Network.

Summary:

This project will evaluate the broad-range detection capacity of currently available pome fruit and Prunus fruit tree virus, viroid and phytoplasma assays and design new qPCR assays if current assays are inadequate. HTS will be used to screen select pome and Prunus tree populations for viruses and other pathogens. The CDFA is currently working to update the Pome Fruit Tree Registration and Certification regulations in order to create regulations that are harmonized with other state's pome industries. Current detection methods for viruses identified as targeted viruses by the pome fruit working group, in addition to primary Prunus viruses, will be investigated. Our objectives are to screen select pome fruit and Prunus tree populations for targeted viruses, viroids and phytoplasma to compile a representative set of genome sequences (as complete as possible) and evaluate current published primers (and probes), incorporate new genetic data into a more complete characterization of genetic variation across the targeted pathogens to inform assay design, construct improved assays utilizing multiple primers sets for detecting all existing targeted pathogen variant, empirically test and validate proposed assay designs using positive controls, and disseminate research progress and results. The overarching goal of this work is to design the most robust assays for virus, viroid and phytoplasma of pome fruit and Prunus fruit trees which will contribute to maintaining the highest quality nursery stock.

Project's Benefit to Nursery Industry:

New advances in qPCR have significantly improved the detection of pathogens, allowing quick, more sensitive and precise quantification compared to conventional PCR. This work will evaluate the broad-range detection capacity of currently available fruit tree virus, viroid and

phytoplasma assays and design new qPCR assays if current assays are inadequate. This work is especially timely as the development of these more robust pome fruit pathogen detection assays concurs with recent changes to the CDFA Pome Registration and Certification Program in anticipation of creating a program at FPS that harmonizes with other state's pome industries. In that sense, FPS is working closely with the CPCNW to standardize the testing process of new domestic and foreign fruit tree introductions.

HTS is a very useful new research tool for detecting viruses present in a variety of crops. In this project we will prescreen select pome and Prunus tree populations for viruses, viroids and phytoplasma and analyze select trees using HTS to develop the most robust qPCR assays. The CDFA has recently adopted qPCR-based methods for detecting some grapevine viruses. We anticipate the continued shift to qPCR-based methods given their potentially higher sensitivity. Any assays we develop as a result of this project will be made available to CDFA and private commercial diagnostic labs and will augment the production of certified propagation material and the effective control of fruit tree viruses and other pathogens in California orchards. The development of a robust, sensitive and reliable detection method with a broad-range detection capacity is very much desirable and needed for large scale virus, viroid and phytoplasma testing. This method will tremendously help the clean stock programs and the fruit tree industry for early detection of the viruses and other pathogens in their material with lower cost and in a shorter time.

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

Project Title: Development of next generation rootstocks for California Vineyards

Reporting Period: January 2018 to January 2019

Principal Investigator: Andrew Walker, Dept. Viticulture and Enology

Overall Summary: Although we experienced significant staffing changes this year (Kevin Fort, Daniel Pap, Becky Wheeler-Dykes, and a PhD Student), we continue to make strong progress on screening for nematode, salt and phylloxera resistance and evaluate new germplasm, existing breeding populations to single out the best rootstock selections, and test breeding and mapping populations. Salt screening of germplasm that was promising in earlier screens was initiated at higher concentrations (75mM) to select optimum accessions that we could use in crosses. At the same time, we are initiating salt screening of breeding populations with different accessions of *V. acerifolia* and 140Ru to look for segregation in order to genetically map this trait. We are making good progress towards better understanding of root architecture in multiple rootstock species including *V. berlandieri*. Fibrosity, specific root length and root diameter are key features that could be used to test rootstock selections. Trials of selected accessions that pass the screening for horticultural features, nematode, phylloxera and salt tolerance are in the pipeline. Four fertile VR (*vinifera* x *rotundifolia*) hybrids (T6-38, Zehnder 93-6-2, NC194-1 and T6-42) had very strong phylloxera resistance and will be used in crosses with *Vitis* rootstocks this season

2018 Pollinations / 2017 Seedling Plantings: Table 1 presents the crosses made in 2018. 2018 was a peculiar year with an early start before a long and delayed bloom. GRN-1 seemed to produce fertile flowers this year and it was pollinated with several standard rootstocks that were blooming at the same time (2018-109 to 2018-112). These efforts are to gain a fertile bridge to allow *rotundifolia* traits to be combined with salt tolerance, better rooting/grafting, and broadened nematode resistance. There are only a few seeds from these efforts and they failed to produce viable seeds. We will try again this year and try to obtain viable plants with embryo rescue. We also made crosses with *Vitis/Muscadinia* hybrids that had been treated with colchicine in an effort to produce tetraploids capable of hybridizing with other *Vitis* species. These efforts will allow *rotundifolia* traits to be widely utilized without taking the risk of phylloxera susceptibility from using available fertile *vinifera/rotundifolia* hybrids in rootstock crosses. We were successful at producing 34 viable seeds from two crosses (2018-149 and 2018-150). The progeny from these crosses will be DNA tested and cytogenetically examined to verify their chromosome number and that they are true-to-type. Crosses 2018-113 and 2018-114 have great potential to combine high salt resistance with excellent nematode resistance. Crosses 2018-120, -121 and -124 were made to produce progeny with root-knot and salt resistance and deep roots. Crosses 2018-136 through -145 were made to combine 2011-118-16 (the VR hybrid T6-42 x St. George, which has tested as very resistant to nematodes) with salt resistance. Crosses 2018-149 to 2018-170 were made with colchicine treated parents in an effort to produce viable seed from 2007-107 (101-14 x *rotundifolia*
'Trayshed') parents. These failed last year but we will try to embryo rescue berries before they abort this year. Table 2 presents the 2017 seedling populations that were planted in the vineyard in 2018.

Nematode resistance breeding: During this reporting period Becky Dykes and Daniel Pap both found other employment. They were leading the nematode resistance screening. I added supervision of the

nematode screening to Nina Romero's duties and she worked very short-handed to get the screening completed. I have just hired two assistants for Nina to help with the nematode screening. I expect the results to be more consistent and that we will get much more completed.

In 2018, we completed 12 different screens for HarmAC (our combined Harmony and Freedom damaging strains) and tested 437 genotypes. Forty-three of these were tested a second time and five a third time. Three separate screens for Ring resistance were completed and 145 genotypes were tested. Of these 128 were first tests, 11 were second tests and one was a third test. Through these efforts we have identified 2 genotypes (2011-188-16 and 2012-110-2) that are highly resistant to RKN and ring. 2011-188-16 derives resistance from *rotundifolia* (via the fertile VR hybrid T6-42) and 2012-110-2 derives its resistance from GRN-5 and 101-14. Table 3 presents the best of the nematode resistant selections from the 2018 testing. There are two other potential additions, 2010-115-22 is a cross of 161-49C x *rotundifolia* 'Trayshed' and 2012-110-8 is a cross of 101-14 x GRN-5. Both have excellent nematode resistance and need to be tested again to verify their rooting and grafting ability

We have run most of our ring nematode resistance screens by inoculating with a given amount of nematodes and counting those that survive after 3 months, in addition to examining root damage and comparing nematode numbers to standard controls (St. George and Colombard). Nina has modified the trial in several ways. She now runs a pot with soil and without plants to verify the survival of nematode under potted greenhouse conditions. She inoculates with 120 ring nematodes in each pot (with and without plants), and after 30 days she extracts the soil from the plantless pots and determine the number of survivors. This is then subtracted from each test plant score to indicate the degree of increase in ring population. Scores are assigned from 1-4: 1 Susceptible (>20 ring nematodes above the plantless standard), 2 Moderately Suscept (>5 and <=20 ring nematodes), 3 Mod Resistant (>2 and <=5 ring nematodes), 4 Resistant (<=2 ring nematodes).

Dagger nematode resistance: The student studying dagger nematode resistance did not pass his qualifying exam and will not be completing his PhD. Nina began the process of sorting through his germplasm and verifying some of his results, most of which were susceptible. While testing these Nina noticed that the typical red sandy soil created root deformation similar to what Xi feeding elicits. She ran a uninoculated trial using the red (crushed lava rock) soil and the white sandy soil she uses for HarmAC and Ring. The latter didn't induce the same root damage which mimicked nematode feeding damage and may have led to false positives we detected. We switched soil / sand mixes and the testing is back on track.

Crosses under investigation for RKN mapping: I am in the process of hiring a new post-doctoral scholar to work on resistance to soil-borne pests. I am searching for someone to study the molecular and biochemical basis of resistance to nematodes and phylloxera. To further the work we are keeping a wide range of mapping populations so that the new post-doc can "hit the ground running".

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

Phylloxera isolates maintained: We have characterized eight strains of phylloxera including types A and B that are currently being used for greenhouse testing. We also have the leaf gall form which is an excellent inoculum source. With the departure of Daniel Pap, we put the other strains on hold, but will start these colonies again depending on the new post-docs interest. The strains are:
Campus – isolated from *V. vinifera* roots from UC Davis vineyard (biotype A); phy1103 – isolated from 1103P leaf/roots from UC Davis field; phyWR – isolated from *V. rupestris* Wichita Refuge from Wolfskill USDA-Repository; phy1616 – isolated from Napa, Flora Springs Vineyard on the roots of 1616C; #1, #2, #3 – isolated from three different locations in Napa; and Sonoma – isolated from Sonoma Valley

Phylloxera phenotyping systems:

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

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

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

Phylloxera mapping efforts: Phylloxera mapping efforts are aimed at identifying resistance loci in mapping populations, in which highly susceptible *V. vinifera* are crossed with resistant accessions. Phylloxera mapping populations are listed in Table 4.

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

We have tested a subset of 45 plants of F2-35 × *V. berlandieri* 9031 cross (07-135) in the bins with biotype A and have seen evidence of segregation. We have also tested the parents of this cross with the phy1103 isolate under *in vitro* assay by placing leaf galls on excised root pieces. phy1103 grows easily on leaves and roots of 1103P, and can be maintained on the leaves in an isolation chambers (mesh-tents) in the greenhouse. It is capable of providing large quantities of inoculum at one time.

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

tuberosities) and reproduction rates. After two weeks it was clear, that the initial hypothesis was true that tuberosity and associated feeding behaviors segregated (Figure 1). This project is ready for the next post-doc to pursue.

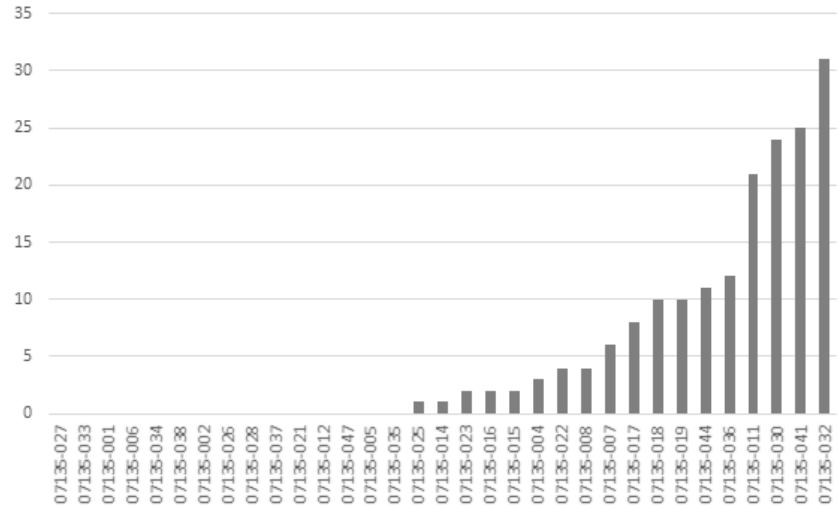

Figure 1. Total number of tuberosities per genotype on the 07135 population (*vinifera* x resistance source *berlandieri* 9031). The in vitro excised roots were tested after 4 weeks with the phy1103 isolate.

In addition, the 15-134 cross (susceptible *V. riparia* DVIT1411 × *V. berlandieri* 9031) could serve to confirm our finding in a non-*vinifera* background and help refine the putative resistance loci. The RKN screen of the 07-135 (*V. vinifera* x *V. berlandieri* 9031) population suggests continuous segregation for resistance. We will develop a framework genetic map for this population after completing the phenotyping with phylloxera. Thus, we potentially could explore resistance in *V. berlandieri* 9031 for both RKN and phylloxera.

We also screened many of our fertile VR hybrids for resistance to phylloxera as a first step in choosing parents with the greatest level of resistance to compare with past results and confirm the utility of our current screening system (Table 5).

Phenolic compounds in grapevine roots: We are also studying the association between phenolics and phylloxera resistance. Phenolics do play a major role in the hypersensitive response (HR) against insect herbivores and microorganisms. We are also exploring an association between grape color and infestation level of own-rooted vines suggests that white cultivars might exhibit a higher susceptibility (Arancibia et al. 2018). We have extracted phenolic compounds from red, pink and white varieties plus 2 rootstocks to compare their phenolic composition through LCMS-QTOF profiling.

Drought tolerance/avoidance: Kevin Fort has left the lab to work for an environmental consulting agency in Sacramento. We are continuing his work on root fibrosity/depth and salt tolerance. *In vitro* evaluation of root growth using increasing concentrations of 'agar' (we are using Gelzan, Phytotechnology Labs) to develop a simple system to discriminate deep vs shallow root growth. Apices from micro-plants of rootstocks 1103P, 101-14Mgt, Ramsey and *Vitis riparia* cv. Gloire de Montpellier, were sub-cultured into clear Falcon tubes containing 30 ml of Nitsch and Nitsch medium supplemented with 20 g/l sucrose, 5 µg/l NAA and 5 µg/l biotin. Medium was solidified with 0.5, 2.5 or 5 g/l of Gelzan (PhytoTechnology Labs). Each treatment was replicated 5 times. The time required for roots to reach the base of the tube was recorded. At the end of the experiment, root fresh weight was also measured.

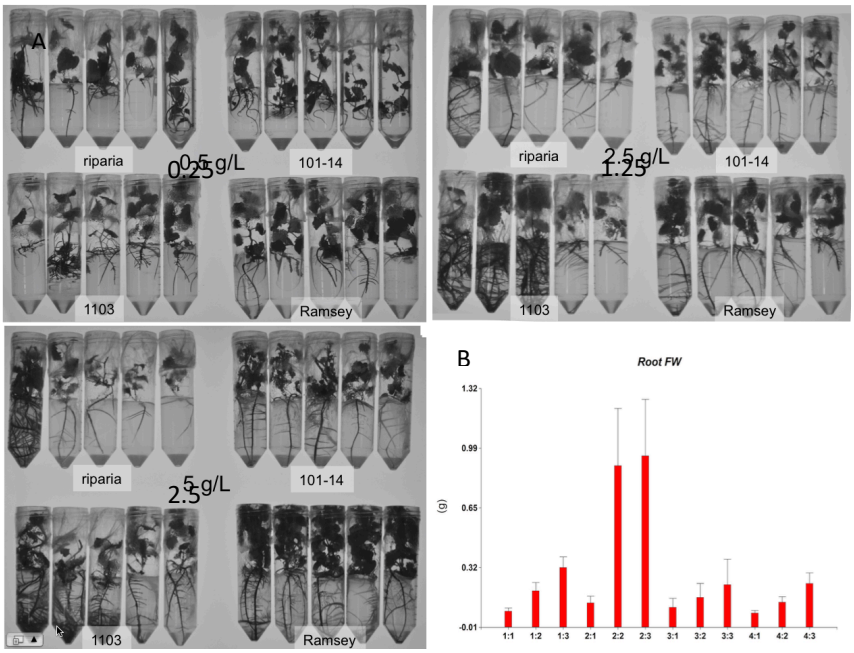

Figure 2. (A) Root development and (B) fresh root weight (FW) at the end of the experiment, which lasted 80 days. First number is cultivar; 1=Ramsey, 2=1103, 3=riparia and 4=101-14. Second number is Gelzan concentration; 1=0.25, 2=1.25 and 3=2.5 g/L. ANOVA analysis and Fisher Test of Root FW showed statistical differences at cultivar (1103 different from the rest) and agar concentration (0.5 g/L different from the rest).

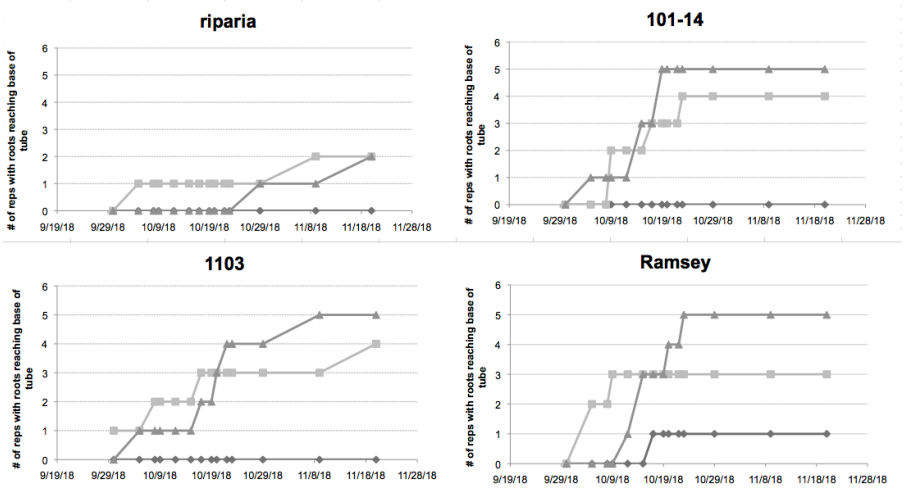

Figure 3. Root growth of riparia, 101-14, 1103 and Ramsey in time (0.25 ♦ , 1.25 ■ and 2.5 ▲ g/l).

Experiment will be repeated using 0.5, 1.5 and 6 g/L to attempt more discrimination in combination with root weights and shoot/root partitioning (although 101-14 show similar time to reach the base of the tube, its root biomass was much lower).

Chloride exclusion, germplasm and mapping population screening: We are using 75mM (12% sea water) salt concentrations to test germplasm previously identified as salt tolerant at 25-50 mM concentrations. We hope this more severe test will identify the most useful parents for crosses. Tables 6 and 7 list the germplasm being tested at 75 mM NaCl, samples harvested May 29, 2018 and currently being processed.

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

I have taken on a PhD student (Chris Chen) who will be working on salt tolerance and following up on these two crosses Ramsey x *acerifolia* 9018 and Ramsey x *acerifolia* 9035. The distribution of these populations is shown in Figure 4 and 5. The Ramsey x 9018 population is skewed towards resistance and the 9035 population is more evenly distributed. Both figures are based on leaf chloride levels after exposure to 75mM NaCl. The progeny from crosses with Dog Ridge instead of Ramsey were all determined to be off-type.

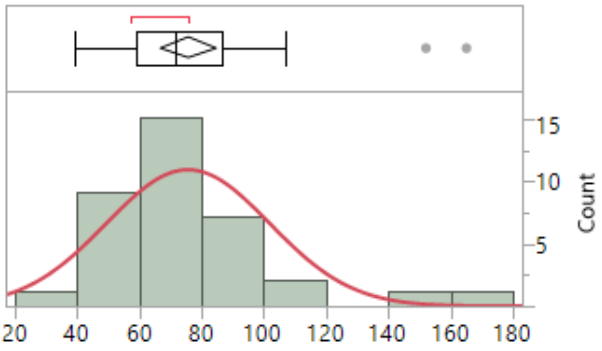

Figure 4. Distribution of progeny genotype leaf chloride (ppm) in the cross Ramsey x *longii* 9018. n=32 plus both parents. Leaf chloride of 9018 was 39 ppm and Ramsey 165 ppm. Distribution isn't normal and skewed to resistant.

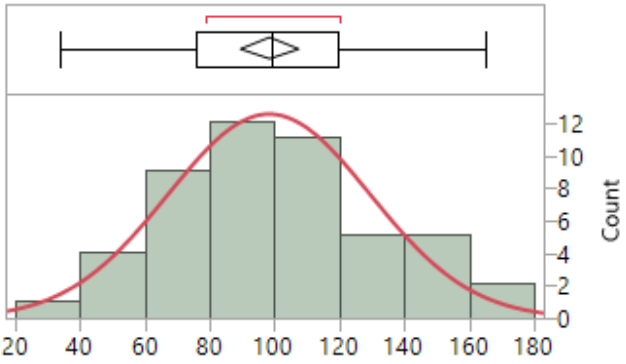

Figure 5. Distribution of progeny genotype leaf chloride (ppm) in the cross Ramsey x *longii* 9035, n=47 plus Ramsey at 165 ppm. Genotype means are normally distributed.

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

Tolerance to redleaf virus disease.

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

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

Cabernet franc plants with LR131 and LR132 were bench grafted on Freedom and St. George, then transplanted into field conditions in March 2017. Symptom expression was observed by October 2017 (Figure 6).

Figure 6 and 7

The same experiment was also conducted in *in vitro* conditions. In February 2018, greenhouse-forced shoot-tips from virus infected Cabernet franc were collected and micrografted onto diseased and healthy rootstocks (Freedom and St. George). The first symptoms appeared on graft combinations 5 weeks after grafting (Figure 8 and 9).

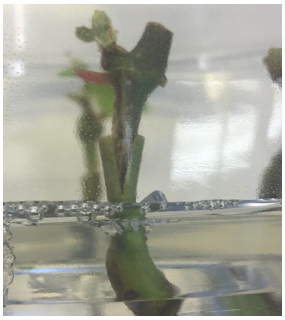

Figure 8. Establishment of micrografting

Figure 9. Red leaf symptom caused by LR-1 in C. franc after 5 weeks of micrografting onto Freedom.

Viral RNA was isolated from leaf petioles of Cabernet franc with LR131 and LR132 from grafted plants in field and in vitro. They were then subjected to two-step PCR test to confirm the presence and absence of the viruses (Figure 10). PCR DNAs were sequenced in two directions with both primers in order to characterize the virus strains. Moreover, virus quantification was done with SyberGreen real time PCR before microRNAs were isolated. They will be then subjected to high throughput sequence analysis in order to understand the effect of virus infection on plant gene regulations for symptom expression.

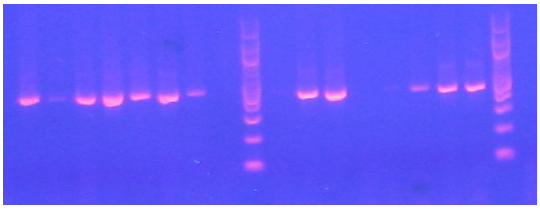

Figure 10. Electrophoretic analysis of PCR DNAs for LR-1 and GVA .

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

Dormant cuttings from the 08180 population (Freedom x St. George) and Cabernet franc with LR-1 and GVA were collected and stored at 36F for chilling requirement for about 6 weeks. These cuttings were bench grafted in mid-march 2018, then they were transferred into greenhouse conditions for virus replication and symptom observation (Fig. 11 and 12). They were periodically checked for virus presence with an ELISA test. Real time PCR will be carried out to quantify LR-1 and GVA in the 08180 population to correlate virus titer with symptom severity.

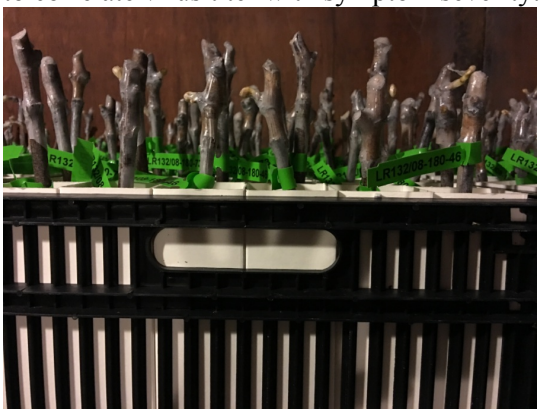

Figure 11 and 12. Grafted plants in greenhouse.

Figure 13. GLRaV-1 (LR131) and GVA (LR 132) virus isolates grafted onto Freedom and St. George rootstocks. (S2: LR132 on St George, F2: LR132 on Freedom; S1: LR131 on St George, F1: LR131 on Freedom).

Figure 14. Red leaf symptoms on Freedom and St. George rootstocks grafted with LR-1 (LR131) and GVA (LR132) isolates in 5 months post inoculation.

Tissue sectioning and immunofluorescent staining for virus localization in phloem tissue of virus infected susceptible and tolerant rootstocks: Stem pieces at 10 cm in size at and above the graft union of Freedom and St George rootstocks grafted with LR-1 and GVA isolates were prepared for tissue section. They were then cut into 2-cm sizes to fit into parafin embedding cassettes. Stem pieces were placed in 1XPBS buffer supplemented with 4% gluteraldehyde for fixation at room temperature for 2 hours. After fixation, they were then washed with 1X PBS for 10 min and dehydration process was carried out by two successive 1-h incubations in each of 70, 80, 95, and 100% ethanol. They were embedded in paraffin. Tissue sections of 10-15 µm in size were done by using a sliding microtome and placed into 1X PBS to prevent from dehydration.

Initial tissue sectioning and staining work has recently started to find the best antiserum dilution for virus capture and FITC staining. Tissue sections were incubated in various dilutions (1:20, 1:50, 1:80, 1:100) of virus specific polyclonal antiserum (Bioreba) in 1XPBS buffer and 1% Bovine serum albumin (BSA) at 37^{o}C for 2 hours. They were then washed three times with the same buffer combination used for antiserum dilution. FITC-conjugated rabbit antiserum [anti sheep IgG (whole molecule)-FITC antibody produced in rabbit] in 1:40 and 1:80 dilutions in 1XPBS and 1% BSA was used to stain viruses in the tissue. Visualization of the FITC stained tissues was done fluorescence microscope (Figure 15). The best result for virus antiserum and FITC dilution was obtained from the dilutions of 1:100 and 1:80, subsequently. Further study has been underway to understand virus localization in susceptible and resistant rootstocks.

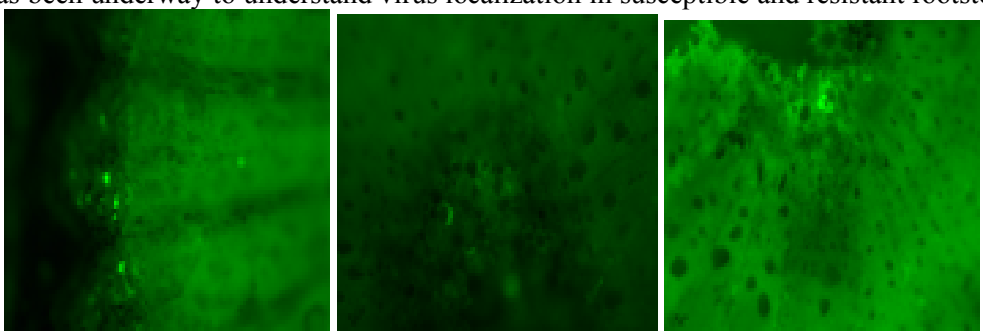

Figure 15. Preliminary results from tissue sectioning and immunofluorecent staining of GVA infected stem tissue.

Inheritance of GFLV Tolerance Trait in a 101-14 x Trayshed Population: Ph.D. student Andy Nguyen is making progress on the inheritance of rootstock-induced fanleaf degeneration tolerance that has been observed in O39-16.

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

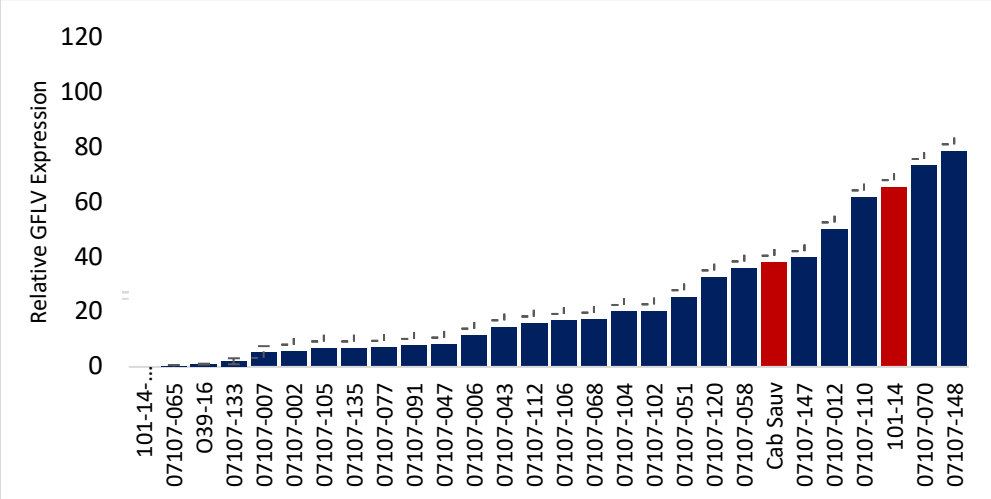

Figure 16. GFLV concentration in the rootstocks normalized to the 18SrRNA housekeeping gene and expressed relatively to O39-16, the tolerant control sample. Error bars represent standard error of the mean.

Fruit set field screening: As expected, many of the 101-14Mgt x *M. rotundifolia* Trayshed vines flowered this season. We bagged two inflorescences per vine before bloom. Bags were harvested in early summer in order to count the collected calyptras and berries to calculate fruit set and determine the impact of grapevine fanleaf virus for each graft combination. We are in the process of quantifying and analyzing the fruit set data we obtained from our fanleaf rootstock trial. This trial consists of fanleaf-infected Cabernet Sauvignon scions grafted on fertile VR hybrids or individuals from the 101-14 x Trayshed population. During the summer, all calyptras and berries from 369 sampled clusters were scanned using a digital scanner in order to later quantify flower count, berry count, and average berry size for each sampled cluster. We used ImageJ (an open platform scientific image analysis software) to process these images and obtain our data. There was an initial delay in our analysis since the calyptra mixture for each cluster also contained other dried flower parts, complicating the counting algorithm. However, after adjusting various settings, we have determined a method that can obtain an accuracy of about 95% in calyptra counting (Figure 17). At the moment, we are still in the process of perfecting the automation of the image analysis so we can process all images more rapidly and accurately. We have obtained preliminary results from a small subset of our control vines and some selected rootstock genotypes.

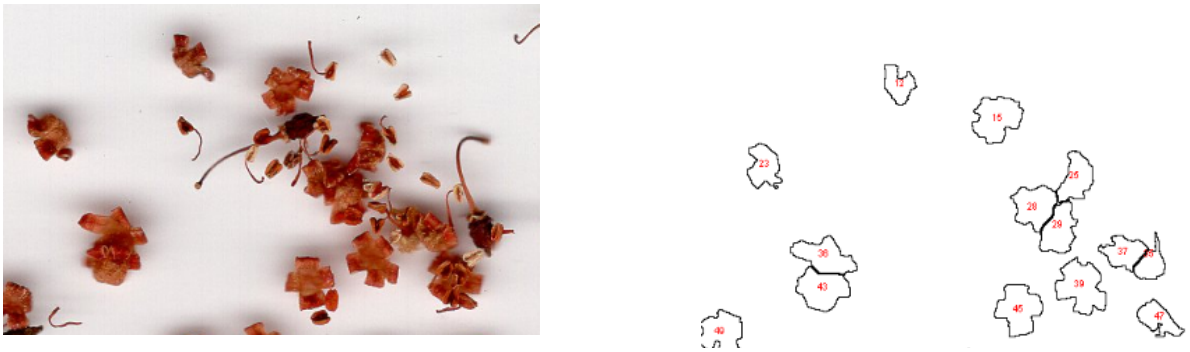

Figure 17. Using ImageJ to separate the calyptras from the other dried flower parts in the mixture to then accurately quantify the number of flowers originally present on the cluster.

We have also recorded the berry weight of each cluster to obtain another comparison point between the rootstock genotypes. Berries were removed from the rachis and the total berry weight for each cluster was recorded. Results are shown in Figure 18.

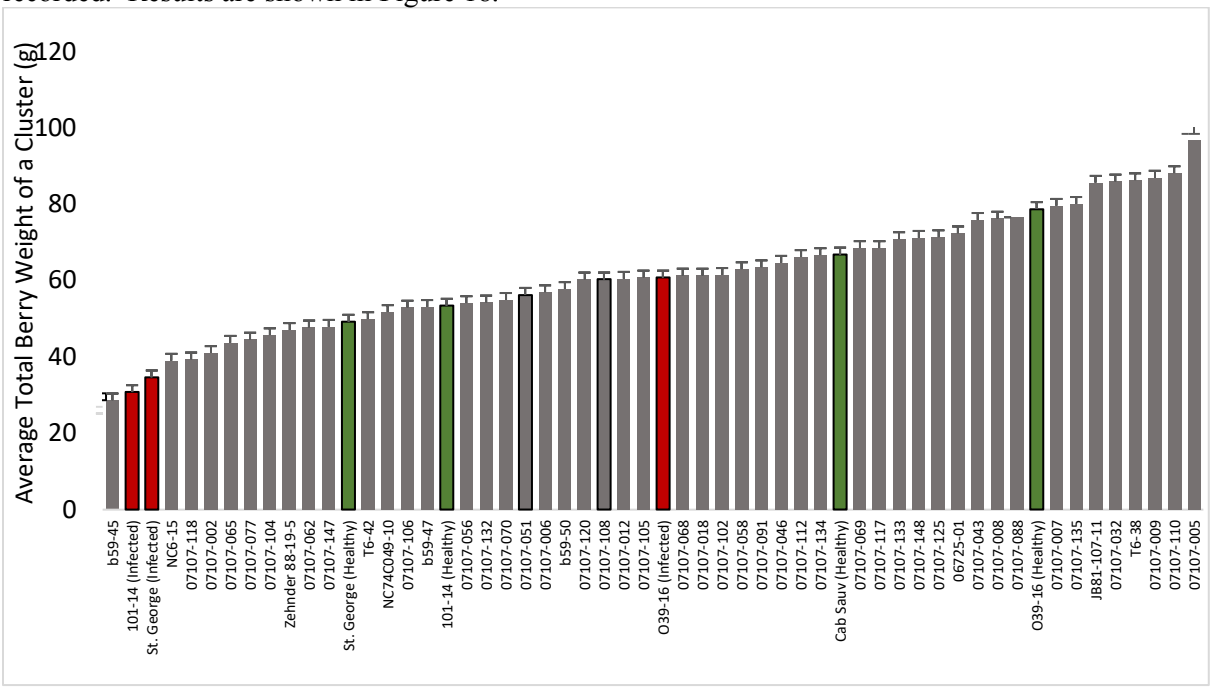

Figure 18. Total berry weight of clusters harvested from the fanleaf fruit set screen. Clusters were harvested in July. Error bars represent standard error of the mean.

Rootability of Fertile VR Hybrids: Rootability from green cuttings of the fertile VR hybrids was evaluated during the phylloxera screen. The root system for each plant was examined and scored on a five-point scale, where a score of 1 represents very thin and minimal rooting, while a score of 5 indicates a very vigorous and extensive root system. During the fanleaf resistance screen (discussed later in this report), rootability from dormant cuttings was also assessed under a similar scoring system. Results from this assessment are listed in Table 8.

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

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

Mechanism of Rootstock-Induced GFLV Tolerance: We are evaluating the cause behind the observed fanleaf tolerance induced by O39-16. A year has passed since chip-bud inoculation of a campus planting of O39-16, we will verify infection in the inoculated vines with RT-qPCR in the coming month. We are also sampling in two field trials (Lodi / Gallo and Healdsburg/Vino Farms). The trials have GRN-1 thru GRN-5, O39-16, RS-3 and RS-9, and 1103P in common. The Lodi site also includes the susceptible St. George, 3309C, 101-14, and Harmony, and between each of the 5 five vine reps a St. George vine so that the uniformity of the infection level can be assessed. The Healdsburg site also includes 1616C, Schwarzmann. Fanleaf is now expressing at these sites – both were planted in 2011.

Determining GFLV Infection Status in Rootstock Field Trials: ELISA testing of the vines from two separate rootstock trials grown on fanleaf sites (Lodi/Gallo and Healdsburg/Vino Farms) is complete. Both trials include the GRN series, as well as O39-16, RS-3, RS-9, and 1103P. The Lodi site additionally includes St. George, 3309C, 101-14, and Harmony. The Healdsburg site also includes 1616C and Schwarzmann. Due to time constraints and an issue with the initial shoot tip sampling, only data from the GRN series, O39-16, 101-14, and St. George was collected. A summary of the results is shown in Table 9. Soil from the Healdsburg site was analyzed for nematodes by an outside service and we plan to relate this data back to our ELISA results. We are also planning to sample all vines again this year (including those that were missed in 2018) to track the spread of the disease. We will also begin to observe the impact of the different rootstocks on GFLV symptoms.

Posters/Abstracts at Scientific Meetings

Weibel, J. and M.A. Walker. 2018. Wild *Vitis* species offer diverse sources of resistance and susceptibility to *Xiphinema index*. 69th ASEV National Meeting, Monterey, CA, June 20

Riaz, S., A. Tenscher and M.A. Walker. 2018. Identification of the Pierce's disease resistance locus PdR2 from the Mexican grape species accession b42-26. 69th ASEV National Meeting, Monterey, CA, June 20

Pap, D., S. Riaz, R. Wheler-Dykes, N. Romero and M.A. Walker. 2018. Sources of resistance to root-knot nematode and phylloxera. 69th ASEV National Meeting, Monterey, CA, June 20

Fayyaz, L., S. Riaz, R. Hu, M.A. Walker. 2018. Characterizing grapevine powdery genes from the Chinese species *Vitis piasezkii*. 69th ASEV National Meeting, Monterey, CA, June 20

Cui, Z., C. Agüero and M. A. Walker. 2018. Greenhouse evaluation of grapevine leafroll associated virus on different rootstocks, 69th ASEV National Conference, Monterey, CA, 06-20-18.

Nguyen, A., C. Agüero, H. Padre and M. A. Walker. 2018. Grapevine fanleaf virus resistance screening in a 101-14 x *rotundifolia* population, 69th ASEV National Conference, Monterey, CA, 06-20-18.

Nguyen, A.V., C.B. Agüero, H. Padre, A. Phan, M.A. Walker. 2018. Characterizing grapevine fanleaf virus resistance and tolerance in a 101-14 Mgt. x *rotundifolia* population. Recent Advances in Viticulture & Enology, UCD, Nov. 30

Huerta-Acosta, K., S. Riaz, O. Franco-Mora and M.A. Walker. 2018. Genetic diversity of wild grapevines in central and northern Mexico. Recent Advances in Viticulture & Enology, UCD, Nov. 30

Walker, A., A. Tenscher and S. Riaz. 2018. Breeding Pierce's disease resistant winegrapes. CDFA

PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12

Riaz, S., R. Hu, C. Agüero, a. Tenscher and A. Walker. 2018. Molecular breeding support for the development of Pierce's disease resistant winegrapes: new sources of resistance and markers. CDFA PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12

Agüero, C.B., S. Riaz, A. Tenscher and M.A. Walker. 2018. Molecular breeding support for the development of Pierce's disease resistant winegrapes – genetic transformation with *PdR1b* candidates. CDFA PD/GWSS Board Symposium Poster, San Diego, CA Dec. 12

Presentations at Scientific Meetings

Walker, M.A. 2017. Breeding winegrapes to resist Pierce's Disease. European conference on *Xylella fastidiosa*: finding answers to a global problem: Palma de Mallorca, 13-15 November 2017 Mallorca, Spain

Walker, M.A. 2018. 2017 AJEV Best Paper Award. Population diversity of grape phylloxera in California and evidence of sexual recombination. 69th ASEV National Meeting, Monterey, CA, June 20

Presentations to Industry Groups

Walker, M.A. 2017. What are the next steps for the PD resistant wine grape program? Current Issues in Wine Health, UC Davis, Dec 5

Walker, M.A. 2017. Current breeding efforts in drought- and salt-tolerant rootstocks. Winegrape Short Course, UC Davis, Dec 12

Walker, M.A. 2018. PD causes and cures. Lecture and tasting. D. Roberts Grower Meeting, Santa Rosa, Jan 12.

Walker, M.A. 2018. Developing PD resistant wine grapes. Lecture and Tasting. Chateau Elan, Braselton, GA. Georgia Wine Producers Meeting, Jan 23

Walker, M.A. 2018. Understanding plant material selection for vineyard redevelopment: Including rootstock and plant material selection and soil pest and virus considerations, South State Gallo Growers Meeting, Fresno, CA Feb 15.

Walker, M.A. 2018. Understanding plant material selection for vineyard redevelopment: Including rootstock and plant material selection and soil pest and virus considerations, North State Gallo Growers Meeting, Lodi, CA Feb 16.

Walker, M.A. 2018. Grape breeding update. Current Issues in Viticulture, UC Davis, Feb 21.

Walker, M.A. 2018. Rootstock breeding update. CDFA IAB Nursery Board meeting, UC Davis, Apr 11.

Walker, M.A. 2018. Grape breeding update and PD wine tasting. UC Davis for the PD/GWSS Grower Advisory Board, April 23.

Walker, M.A. 2018. UCD PD breeding program update and tasting. Temecula Winemakers Meeting, Wilson Creek winery, Temecula, June 8.

Walker, M.A. 2018. Grape breeding at UC Davis. Lebanon Table Grape Growers Group, July 17.

Walker, M.A. 2018. Grape breeding update. CGRIC Nursery Meeting, July 24.

Walker, M.A. 2018. Fanleaf Field Day, discuss plot and breeding – Healdsburg, CA, Aug. 16 .

Walker, M.A. 2018. Rootstock breeding program update. CDFA IAB meeting, UC Davis, Nov 14.

Walker, M.A. 2018. New/replanted vineyard establishment concerns. UCD/On the Road Presentations, Escondido, CA, Nov 29.

Walker, M.A. 2018. Current and future objectives of the grape breeding program at UCD. Recent Advances in Viticulture and Enology, UC Davis, Nov 30

Walker, M.A. 2018. Current and future objectives of the UCD grape breeding program. Foundation Plant Services Annual Meeting, UC Davis, Dec. 4

Walker, M.A. 2018. PD resistant winegrape breeding program update. CDFA PD/GWSS Board Symposium, San Diego, CA Dec. 12

Walker, M.A. 2019. An update on the performance of the GRN rootstocks. Daniel Roberts Client

Meeting, Jan 18

Publications

Riaz, S., K.T. Lund, J. Granett and M.A. Walker. 2017. Population diversity of Grape Phylloxera in California and evidence for sexual reproduction. American Journal of Enology and Viticulture 68: 218-227.

Lund, K.T., S. Riaz and M.A. Walker. 2017. Population structure, diversity and reproductive mode of the Grape Phylloxera (*Daktulosphaira vitifoliae*) across its native range. PLOS One 12 (1): e0170678. doi:10.1371/journal.pone.0170678.

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

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.

Forneck, A., V. Dockner, R. Mammerler, K.S. Powell, L. Kocsis, D. Papura, J. Fahrentrapp, S. Riaz and M.A. Walker. 2017. PHYLLI – an international database for grape phylloxera. International Organization for Biological and Integrated Control (IOBC) West Palaerartic Regional Section (WPRS) 128:45-51.

Cui, Z-H.,W-L. Bi, X-Y. Hao, P-M Li, Y. Duan, M.A. Walker, Y. Xu, Q-C. Wang. 2017. Drought stress enhances up-regulation of anthocyanin biosynthesis in grapevine leafroll-associated virus 3 infected *in vitro* grapevine (*Vitis vinifera*) leaves. Plant Disease 101:1606-1615.

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

Cui, Z.-H., C.B. Agüero, Q.C. Wang and M.A. Walker. 2019. Validation of micrografting to identify incompatible interactions of rootstocks with virus-infected scions of Cabernet Franc. Australian Journal of Grape and Wine Research doi: 10.1111/ajgw.12385

Fort, K. and M. A. Walker. 2019. Root system morphology predicts drought tolerance capacity in ten grape rootstocks. American Journal of Enology and Viticulture (submitted)

Table 1. 2018 pollinations. **cross #**	**Female parent**	**Male parent**	**Purpose**	**# Seeds**
2018-109	8909-05 GRN-1	Schwarzmann	Fertile Vitis/Muscadinia (VM) progeny	0
2018-110	8909-05 GRN-1	1103 Paulsen	Fertile VM progeny	0
2018-111	8909-05 GRN-1	3309 Couderc	Fertile VM progeny	0
2018-112	8909-05 GRN-1	Riparia Gloire	Fertile VM progeny	0
2018-113	GRN-3 9365-43	*acerifolia 9018*	Salt and broad nematode resistance	1080
2018-114	GRN-3 9365-43	*acerifolia* 9035	Salt and broad nematode resistance	444
2018-120	Dog Ridge	*acerifolia 9018*	Salt/RKN/deep roots	31
2018-121	Dog Ridge	*acerifolia* 9035	Salt/RKN/deep roots	268
2018-124	Ramsey	*doaniana 9028*	Salt/RKN/deep roots	225
2018-136	11-188-16	1103 Paulsen	Ring/RKN	3
2018-137	11-188-16	NM 03-17 S01 *treleasei*	Ring/RKN/salt	2
2018-138	11-188-16	ANU77 *girdiana*	Ring/RKN/salt	36
2018-139	11-188-16	ANU57 *treleasei*	Ring/RKN/salt	19
2018-141	11-188-16	GRN-4 9365-85	Dagger/Ring/RKN/salt	0
2018-142	11-188-16	GRN-2 9363-16	Dagger/Ring/RKN/salt	19
2018-143	11-188-16	*acerifolia 9018*	Ring/RKN/salt	10
2018-144	11-188-16	*acerifolia* 9035	Ring/RKN/salt	0
2018-145	11-188-16	3309 Couderc	Ring/RKN	22
2018-146	11-188-16	110R	Ring/RKN	0
2018-147	11-188-16	SO4	Ring/RKN	0
2018-149	101-14 Mgt	07107-079 FH 05-35 T=tetraploid	*rotundifolia*-based resistance and fertility	29
2018-150	101-14 Mgt	07107-079 FH 05-35 D=diploid	*rotundifolia*-based resistance and fertility	5
2018-151	07107-062 FH 05-18 T=tetraploid	GRN-4 9365-85	*rotundifolia*-based resistance and fertility	0
2018-152	07107-062 FH 05-18 T=tetraploid	GRN-2 9363-16	*rotundifolia*-based resistance and fertility	0
2018-153	07107-062 FH 05-18 D=diploid	GRN-4 9365-85	*rotundifolia*-based resistance and fertility	0
2018-154	07107-062 FH 05-18 D=diploid	GRN-2 9363-16	*rotundifolia*-based resistance and fertility	0
2018-155	101-14 Mgt	101-14 x T 48T	*rotundifolia*-based resistance and fertility	0
2018-156	101-14 Mgt	101-14 x T 48D	*rotundifolia*-based resistance and fertility	0
2018-157	101-14 Mgt	101-14 x T 42T	*rotundifolia*-based resistance and fertility	0
2018-158	101-14 Mgt	101-14 x T 42D	*rotundifolia*-based resistance and fertility	0
2018-170	2011-175-7	GRN-2 9363-16	Broad nematode resistance with PD	0

Table 2. Rootstock crosses planted in the UCD vineyard 2018

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

Table 3. Most promising nematode resistance candidates. Nematode resistance is measured on a 1 to 4 scale with 1 highly susceptible and 4 resistant with virtually no nematode damage. Propagation is reported from typical duration (6-7 weeks) with media and plant in 2" x 2" paper sleeves. Scale is 0 with no usable plants and 5 excellent shoots and roots.

Genotype	Parentage	Avg HarmAC Resistance	Times HarmAC tested	Avg Ring Resistance	Times Ring tested	Ease of Propagation
2011-188-16	T6-42 x St. George	4.0	4	4.0	2	3.0
2012-110-02	101-14Mgt x GRN-5	3.3	4	3.5	2	3.5
2012-113-8	101-14Mgt x GRN-4	4.0	2	3.0	1	3.3
2012-118-17	161-49C x GRN-4	3.5	4	3.0	1	4.0
2012-125-21	OKC-1 S01 x GRN-2	3.5	4	3.5	2	3.3
2012-154-2	Ramsey x St. George	3.0	3	3.0	1	3.8
2011-148-42	Ramsey x NM 03-17 S01	3.5	2	3.0	2	3.8
2012-113-16	101-14Mgt x GRN-4	3.3	3	3.0	3	3.8
2012-185-8	GRN-3 x berlandieri 9031	3.5	2	3.5	2	3.0
06301-138	03300-018 x GRN-4	3.0	2	3.0	1	4.0
2011-175-7	08314-31 x Schwarzmann	3.0	2	4.0	1	4.5
2012-110-14	101-14Mgt x GRN-5	3.7	3	3.5	1	4.5
2012-110-33	101-14Mgt x GRN-5	4.0	3	3.0	1	3.0
2012-112-17	101-14Mgt x GRN-2	3.0	2	4.0	1	3.0
2012-112-33	101-14Mgt x GRN-2	4.0	2	3.0	1	3.8
2012-113-11	101-14Mgt x GRN-4	4.0	1	3.0	1	
2012-125-34	OKC-1 S01 x GRN-2	4.0	1	4.0	1	3.0

Table 4. Populations under investigation for mapping phylloxera resistance.

Cross ID	Female		Male	#Seedlings
05-347	F2-35	×	*V. arizonica* b42-26	370
07-135	F2-35	×	*V. berlandieri* 9031	110

15-134	*V. riparia* DVIT 1411	×	*V. berlandieri* 9031	200
09-140	Almeria	×	Riparia Gloire	114
09-390	Malaga Rosada	×	*V. cinerea* B9	225
12-111	101-14	×	St. George	100
05-803	Colombard	×	GRN4	19

Table 5. Phylloxera resistance (Type A) in a selected group of VR hybrids tested using a greenhouse assay. Statistical resistance was determined using O39-16 as Dunnett's reference. Index is ranges from 1 (highly susceptible) to 4 (no apparent feeding).

Genotype	Phylloxera Resistance (rel to O39-16)	Mean Phylloxera Rating	Std Dev	Times Tested
O39-16	R	4.0	0.0	4
T6-38	R	4.0	0.0	4
Zehnder 93-6-2	R	4.0	0.0	4
NC194-1	R	3.8	0.5	4
T6-42	R	3.7	0.5	12
NC74C049-10	R	3.6	0.5	7
NC6-15	R	3.6	0.5	9
N53-32	R	3.0	1.1	6
B59-45	S	2.8	0.6	10
06725-01	S	2.7	1.2	3
Zehnder 01-20-4	S	2.3	1.3	4
B59-50	S	2.0	1.4	4
JB81-107-11	S	1.3	0.5	6
Zehnder 88-19-5	S	1.3	0.5	4
Karadzhandal	S	1.1	0.4	8
B59-47	S	1.0	0.0	3
Colombard	S	1.0	0.0	2

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

Genotype	
03300-048	101-14 x F8909-08
1103 P	*berlandieri* x *rupestris*
17:043	
2011-175-007	08314-31 x Schwarzmann
2011-175-015	08314-31 x Schwarzmann
ANU 21	*arizonica / girdiana*
ANU 71	*arizonica*
AZ 11-099	*arizonica* slight *riparia*
doaniana 9026	*doaniana*
F8909-08	*rupestris* x *arizonica*
girdiana Scotty's Castle	lobed *arizonica*

GRN-2	(*V. rufotomentosa* x (*V. champinii* 'Dog Ridge' x *V. riparia* 'Riparia Gloire')) x *V. riparia* 'Riparia Gloire'
KS14-032	*acerifolia* Kansas
Longii 9018	*acerifolia* TX
OK12-005	*doaniana*
OK14-002	*acerifolia*
R8916-22	*rupestris x arizonica*
R8916-32	*rupestris x arizonica*
St. George	*rupestris*
TXNM-088	*treleasei*
UT 12-092	*girdiana/treleasei (rip?)*
UT 12-099	*girdiana*
UT 12-100	*girdiana/treleasei (rip?)*

Table 7. Salt (chloride) resistance rating at 75mM NaCl using grape germplasm that tested Resistant at 25 or 50 mM NaCl. Cl exclusion ratings based on mean leaf Cl concentration: 1 susceptible – 4 resistant.

Genotype	Species or parentage	Chloride_ Exclusion _Rating	Mean_Leaf _Chloride_ ppm	Times Tested	Number_ Reps
ANU21	*arizonica / girdiana*	2	88	1	3
ANU71	*arizonica*	2	84	1	3
AZ11-099	*arizonica* slight *riparia*	3	36	1	4
doaniana 9026	*doaniana*	4	19	1	5
GRN-2	(*V. rufotomentosa* x (*V. champinii* 'Dog Ridge' x *V. riparia* 'Riparia Gloire')) x *V. riparia* 'Riparia Gloire'	1	111		3
acerifolia 9018	*acerifolia* TX	4	35	3	7
OK14-002	*acerifolia*	3	36	1	3
R8916-22	*rupestris x arizonica*	3	34	1	3
R8916-32	*rupestris x arizonica*	3	32	1	3
St. George	*rupestris*	2	91	2	3
TXNM088	*treleasei*	3	53	1	3
UT12-099	*Girdiana*	2	93	1	3

Table 8. Rootability of VR hybrids from dormant cuttings. We had 12 replicates for most of the genotypes.

Genotype	Percentage of Surviving Grafts	Rootability Score
NC194-1	8.3%	1
b55-1	83.3%	4
NC74C049-10	41.7%	2
JB81-107-11	88.9%	5
T6-42	16.7%	1
T6-38	Not assessed in this screen	

Zehnder 88-19-5	100%	5
Zehnder 01-20-4	100%	5
NC6-15	41.7%	3
Zehnder 93-6-2	41.7%	2
Zehnder 97-60-3	0%	0
06725-01	100%	5
N53-32	41.7%	3
b59-45	100%	5
b59-47	100%	5
b59-50	100%	5

Table 9. Preliminary fruit set data for selected genotypes. 101-14 and St. George are susceptible controls and O39-16 is the tolerant control. 2 replicates of 07107-133 and 07107-148 are shown here.

Graft Combination	No. of Berries	No. of Calyptras	Fruit Set %
Healthy on 101-14	169	328	51.5%
Infected on 101-14	110	651	16.9%
Healthy on St. George	157	294	53.4%
Infected on St. George	15	370	4.1%
Healthy on O39-16	220	438	50.2%
Infected on O39-16	187	457	40.9%
Infected on 07107-065	133	236	56.4%
Infected on 07107-133	155	255	60.8%
Infected on 07107-133	174	255	68.2%
Infected on 07107-110	134	362	37.0%
Infected on 07107-148	123	289	42.6%
Infected on 07107-148	144	385	37.4%

Table 10. Vine infection status for two separate rootstock trials grown on fanleaf sites.

Lodi (Gallo)

Rootstock	Number of Vines Sampled	Number of Vines Testing Positive for GFLV
GRN-1	25	2
GRN-2	25	2
GRN-3	25	3
GRN-4	25	2
GRN-5	20	3
O39-16	25	0
101-14	25	7
St. George	92	15

Healdsburg (Vino Farms)

Rootstock	Number of Vines Sampled	Number of Vines Testing Positive for GFLV
GRN-1	40	4
GRN-2	39	8

GRN-3	39	7
GRN-4	40	6
GRN-5	38	2
O39-16	38	0

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

Project Title: Development of an Armillaria resistance screen for clonal walnut rootstocks

Project duration: 2018-2019 Progress Report

Project Leader: Pat J. Brown, Chuck L. Leslie, and Wes Hackett

Objective 1: Several matrices and supplemental additives have been tested for their ability to support growth of Armillaria mellea in vitro which can then be used to inform trials to infect ex vitro rooted micro-shoots and bare root liner sized plants. These tests were carried out in the dark at 25 C in Magenta GA7 containers, petri dishes and shake cultures. Observations of mycelial and rhizomorph growth are summarized in Tables 1 and 2. These observations indicate that Armillaria mellea grows best in cultures supplemented with easily metabolized, soluble carbohydrate such as dextrose (glucose) and very little or not at all on insoluble carbohydrates such as cellulose(cotton balls). The medium to vigorous growth on walnut stems and shavings with only water added suggests that these materials after autoclaving have substantial concentrations of soluble carbohydrates and/or other compounds that Armillaria mellea can metabolize. The lack of growth on autoclaved pine shavings with only water added suggests that little or no soluble carbohydrates are released by autoclaving or that growth inhibitory substances are released by autoclaving or both. The latter is suggested by the observation that mycelial growth is not optimal on pine shavings even with Potato Dextrose Broth added. Moderate growth on water agar medium suggests that autoclaved agar contains some carbohydrates and other substances that Armillaria mellea can metabolize. These observations provide evidence that the rapid growth of Armillaria mellia mycelium and rapid infection of rooted microshoots on Driver and Kuniyuki Walnut medium may be due to high concentration of soluble carbohydrate (3% sucrose) in the medium. This hypothesis will be tested directly in an experiment that is currently being set up.

Objective 2: We've developed a growth chamber, controlled environment (high pressure sodium vapor lighting, refrigeration controlled temperature and humidity control with foggers and a humidistat) system for Armillaria infection of in vitro and ex vitro rooted microshoots which involves maintaining a high humidity of 82-92% RH at a constant temperature of 25C and a 16 hour daylength at an intensity of 350-450 micromoles/ meter squared/sec using sodium vapor lamps. Newly rooted microshoots are transplanted to 1 3/8 x 2 1/2 inch stabilized peat Q plugs that are partial split longitudinally and held in place with a twist tie. They are then placed in Magenta GA7 containers having one cm DI water in the bottom which maintains a saturated water root environment. Based on anecdotal evidence this should be conducive to Armillaria infection. Microshoot roots and shoots grow well in this environment. The saturated root environment can easily be modified by changing the level of the drain hole in the side of the Magenta. We will soon initiate an experiment using this system and one of the optimal inoculum substrates discussed under Objective 1.

Objective 3: We've also tested the growth chamber, controlled environment Armillaria infection assay system described under Objective 2 above using bare rooted liner-sized plants.

This involves washing the soil from the root system using a fine spray of water to expose both the new, white roots and the larger brown, woody roots. The bare rooted system is then covered with two Q plugs that have been split longitudinally. The Q plugs are held in place with twist ties. The Q plug covered root system is then placed in a Magenta GA7 container with one cm of water in the bottom maintained with a drain hole 1 cm up from the bottom. Roots grow well in this environment even when the shoots are quiescent. We've used this system for one experiment to test for Armillaria infection using a slurry containing homogenized mycelium that had been culture on Potato Dextrose Broth and carboxymethyl cellulose (a soluble form of cellulose). Bare root systems of liner sized plants were dipped in the slurry and covered with Q plugs and incubated as described above. Root systems grew well. After two months of growth none of the plants had symptoms of infection and when the Q plugs were removed no evidence of mycelial or rhizomorph growth could be detected.

Table 1. Growth of Armillaria Mellea walnut strain on SOLID matrices and supplemental additives. Stems were 3/8 to 1/2 inch diameter and cotton balls were 3/4 inch diameter. PDB is potato dextrose broth. Cultures were grown in the dark at 25° C.

Matrix Material	Supplemental Additive	Mycelial Growth	Rhizomorph Growth
Chandler Stems	PDB	++++	+++
	H20	+++	++
Burbank Paradox Stems	PDB	+++	++
	H20	++	+
Burbank Paradox Shavings	PDB	+++	++
	H20	++	+
Pine Shavings	PDB	++	+
	H20	-	-
Cotton Balls	PDB	+++	+
	H20	-	-
Agar	PDB	++++	++++
	H20	++	-

Table 2. Growth of Armillaria Mellea walnut strain as LIQUID and supplemental additives. PDB is potato dextrose broth. Cultures were grown on platform shaker in the dark at 25°C

Material	Supplemental Additive	Mycelial Growth	Rhizomorph Growth
Potato Dextrose Broth	N/A	++++	+
Sodium Carboxymethyl Cellulose	PDB	+++	-
	H20	+	-