Interim Progress report for CDFA Agreement Number #17-0331-000-SA-2

Project Title: Identification of grape cultivars and rootstocks with resistance to vine mealybug

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Reporting Period. The results reported here are from work conducted 8/01/2017 to 6/30/2018.

Introduction.

Mealybugs are soft-bodied, sap-sucking insect pests of grapevines and other plants. Besides the direct losses attributed to damaged leaves and fruit in grape, mealybugs can transmit the economically important Grapevine Leaf Roll Associated Virus (GLRaV). It is estimated that GLR disease control costs growers $12,106 to $91,623 per acre annually in California (Ricketts et al., 2015). Of that expenditure, mealybug control costs are estimated at $50 per acre, in vineyards with small mealybug populations and many natural predators, up to $500 per acre for vineyards with moderate populations and few parasitoids (Ricketts et al., 2015). Vine mealybug (*Planococcus ficus*) is one of six mealybug species that threaten the California grape industry. This introduced (ca. 1994) pest can rapidly reproduce and spread, outcompeting other mealybug species and making it the most important mealybug pest of grape in California (Daane et al., 2012).

Vine mealybug development is temperature, not seasonal, dependent, and the insect can complete its life-cycle during winter months if days are warm. This season-independent development leads to high population numbers, which has contributed to the difficulty of controlling this insect. For vine mealybug, up to seven generations per year have been observed in California vineyards compared to the two observed in grape mealybug (Geiger and Daane, 2001; Gutierrez et al., 2008). Females reach maturity as soon as 30 days from egg, and once mature, can produce 50 to 800 viable offspring depending on nutrient availability (Waterworth et al., 2011, Berning et al., 2014). Even using a low estimate of 50 viable offspring, a single mealybug could produce millions of individuals over the course of a growing season.

Insecticides are the main form of control. Mating disruption and parasitoids have been implemented with success in vineyards, however these forms of control are more expensive (Daane et al., 2007; Mansour et al., 2011; UC IPM Pest Management Guidelines: Grape). Optimization of insecticide control strategies (application timing and efficacy) have garnered much attention. However, the vine mealybug spends much of its life and development on the roots and under the bark protecting it from chemical sprays (Daane et al., 2012). This makes contact insecticides often ineffective, and systemic insecticides difficult to time. An effective complement to insecticides is the use of resistant grapes. Resistant grapes, and specifically resistant rootstocks, could directly reduce mealybug populations developing or overwintering under the bark and on roots in the vineyard.

Few sources of natural resistance to mealybug have been identified in grape. In Brazil, one study identified a single rootstock with lab-based resistance to mealybug (Filho et al, 2008, Figure 2). This resistance was described as a reduction in the number of viable offspring produced per female compared to susceptible cultivars, Cabernet Sauvignon and Isabel (Filho et al, 2008). This was later confirmed in a similar lab experiment performed by a different lab group (Bertin et al, 2013). These results, while promising, were based on mealybug species (*Dysmicoccus brevipes* and *Planococcus citri*) of minor importance to California. The only other report of mealybug resistance in grape comes from observations by Michael McKenry and David Ramming (*unpublished*), suggesting that rootstock RS-3 has resistance to an unknown species of mealybug in addition to nematode resistance. While early work has shown that these two sources are likely to be resistant to mealybugs, further work is needed to confirm their use against vine mealybug outside of lab conditions, in addition to identifying new sources of resistance.

Objectives.

This project seeks to develop novel control strategies for vine mealybug using host resistance as part of an integrated management program. Identifying grape material with resistance to vine mealybug could serve in the short term as rootstocks and over the long term as source of resistance for traditional cultivar breeding.

Objective 1: Develop a method to evaluate mealybug host resistance and identify grape material with leaf resistance to vine mealybug.

Objective 2: Evaluate grape materials with identified resistance to vine mealybug.

Objective 3: Determine multi season sustainability of resistance to vine mealy bug in identified grape rootstocks and cultivars.

Description of Activties and Summary of Accomplishments

Objective 1

Potted grapevines of four accessions (Table 1) were planted into pots and grown in a growth chamber for detached and attached leaf assays for mealybug resistance. Three crawlers (stage 1 or 2) were transferred to a single grape leaf and enclosed in a clip cage (Figure 1.) Three leaves were evaluated per plant for 3 plants per grape accession. High crawler mortality was observed and no significant differences were detected among accessions.

Figure 1. Clip cage with mealybugs on a grape leaf in the greenhouse.

Table 1. Grape accessions evaluated in Objective 1.

Line	Type	Species	Special notes
Cabernet Sauvignon	Wine Grape	*V. vinifera*	Susceptible
Chardonnay	Wine Grape	*V. vinifera*	Susceptible
Flame Seedless	Table Grape	*V. vinifera*	Susceptible
17-01	Wild species	*V. champinii*	Potential resistant
IAC572	Rootstock	*V. caribeae*	Potential resistant

Objective 2 and 3
In summer of 2017, potted grapevines of seven accessions (Table 2) were placed into outdoor screen cages and evaluated for mealybug severity. Plants were evaluated for the number of mealybugs and ovisacs visible in a 1-minute period, number of mealybugs and ovisacs detected on 3 randomly selected leaves, the presence of predators such as lacewings, spiders, and ladybird beetles, and the number of ants on each cultivar. Southern Fire ants were detected among cultivars, and were visibly maintaining mealybug colonies. Differences in mealybug severity and ant presence were detected among cultivars with Cabernet Sauvignon showing the highest number of mealybugs and ants (Figure 2). Visible mealybugs were removed from grape plants at the end of the season (December), and no ants were detected. Few mealybugs, <1 per plant on average, were detected on any of the grape cultivars evaluated in spring 2018 (April – May). Not all plants survived the winter, and death was observed in rootstocks IAC 572 and 10-17A (1 plant each), and *V. australis* (4 plants). Grape plants were re-evaluated biweekly beginning in June 2018 for mealybugs from overwintering (bark and roots) or wind-dispersed crawlers (Figure 3). Plants are being evaluated for number of mealybugs and ovisacs visible in a 1-minute period, number of mealybugs and ovisacs detected on 3 randomly selected leaves, the presence of predators such as lacewings, spiders, and ladybird beetles, and the number of ants on each cultivar. The project is ongoing and data will be collected colonization throughout the summer.

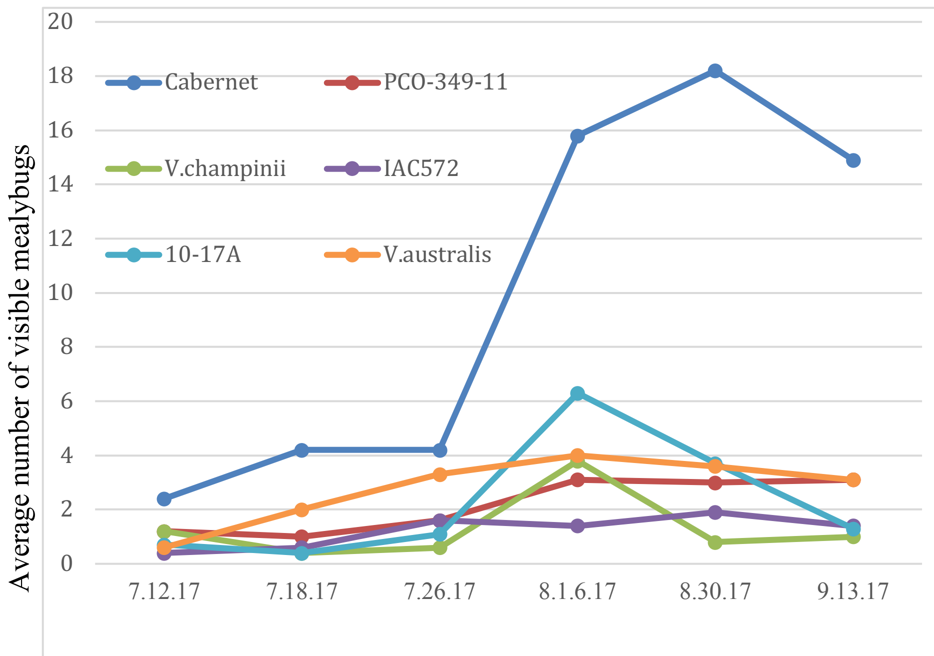

Figure 2. Average number of visible mealybugs on grape in outdoor screen cage studies from July 12 (7.12.17) to September 13 (9.13.17).

Figure 3. Ants "farming" mealybugs on Cabernet Sauvignon after recolonization (June 2018)

Table 2. Grape accessions evaluated for mealybug resistance in Objectives 2 and 3.

Line	Type	Species	Special notes[A]
10-17A	Rootstock		Nematode resistance
IAC 572	Rootstock	*V. caribeae*	Mealybug resistance
Cabernet Sauvignon	Wine grape		Known susceptible
17-01	Wild species	*V. champinii*	
17-02	Wild species	*V. candicans*	
PCO-349-11	Rootstock		Nematode resistance
17-03	Wild species	*V. australis*	

Publications pending and presentations
None to date

Research relevance statement
This research will contribute to novel management strategies for vine mealybug including breeding for disease resistance (through identifying novel sources of resistance) and the use of resistant rootstocks.

Layperson summary of project accomplishments
As part of a two-year study, mealybug cultivars were evaluated for resistance to vine mealybug. Potential resistance to vine mealybug was identified in grape rootstock IAC572.

Status of funds
To date, $14,293.07 has been spent on technical assistance on propagating, planting, maintaining and evaluating the mealybug studies. An estimated $18,825.93 remains for project support. An additional $1,139.55 has been spent on squash (maintaining mealybug colonies in the lab), field irrigation supplies (timers, hoses, and nozzles), potting materials, clip cages, and filter paper (mealybug transfers).

Intellectual Property
No intellectual property rights are associated with this project, and rootstock IAC572 is available through Foundation Plant Services.

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