Interim Progress Report for CDFA Agreement Number 17-0331-000-SA

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

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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). Mealybug control costs are estimated at $50 per acre, in vineyards with small mealybug populations and many natural predators, 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).

Insecticides are the main form of mealybug control. Mating disruption and parasitoids have been implemented with success in vineyards, however these forms of control are more expensive or can be impeded by Argentine ant populations which "tend" the mealybugs (Daane et al., 2007; Mansour et al., 2011; UC IPM Pest Management Guidelines: Grape). Resistant grapes, and specifically resistant rootstocks, could directly reduce mealybug populations developing or overwintering under the bark and on roots in the vineyard.

In Brazil, one study identified a single rootstock with lab-based resistance to citrus mealybug (Filho et al, 2008). 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, are 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only other report of mealybug resistance in grape comes from field observations by Michael McKenry and David Ramming (unpublished), suggesting that rootstock RS-3 may have resistance to an unknown species of mealybug in addition to nematode resistance.

This project will have long-term impact by facilitating the breeding of mealybug resistance into commercial wine, table and raisin grape cultivars. In the short term, these materials may be used as rootstocks to reduce mealybug populations on susceptible commercial cultivar scions. The information from these studies will be distributed to growers, nurseries, breeders, PCAs and extension personnel to accelerate the implementation of these materials into breeding programs, rootstock evaluations, and nurseries.

Objectives of Proposed Research:

This proposal seeks to develop a novel control strategy for vine mealybug using host resistance as part of an integrated management program. This will be accomplished by identifying grape material with resistance to vine mealybug that can be used as rootstocks and a 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: Multi season sustainability of resistance to vine mealy bug in identified grape rootstocks and cultivars.

Description of Activities Conducted, and Summary of Accomplishments:

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

Figure 1. Insect clip cages on grapes.

A vine mealybug colony was established in the lab on butternut squash, as per Dr. Daane's recommendations and clip cages were constructed to complete Objective 1. Grape plants were propagated for 'Flame Seedless', 'Autumn King', 'IAC 572', 'Tampa' and 'Cabernet Sauvignon' in the greenhouse. Three 1st or 2nd stage mealybug crawlers were placed into a clip cage (Figure 1) on a single leaf from each cultivar. Three leaves per cultivar were evaluated. Surviving mealybugs and life stage were evaluated after 3 and 6 weeks. High crawler mortality was observed for each cultivar, making statistical comparisons impractical. Currently clip cage testing is continuing using 2nd stage crawlers to minimize mortality due to molting issues.

Detached leaves from each of the listed cultivars were placed into petri dishes in the lab and ten 1st or 2nd stage mealybug crawlers were placed on each leaf. Five leaves were evaluated for each cultivar. Similar to clip cages, high mortality rates among crawlers were observed. **Summary:** Mealybug colonies were established, and clip cages were compared to detached leaf assays for mealybug screenings. Clip cages provided better information.

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

Cuttings were collected for each of the proposed grape cultivars to be evaluated for potential resistance to vine mealybug (Table 1). Cuttings are currently being rooted in a growth chamber and will be transplanted to outdoor pots for mealybug infestation this spring. Plants will be enclosed in mesh bags to prevent ants from moving mealybugs between cultivars. **Summary:** Plants are being rooted and

experiments will begin at the end of April.

Table 1: Cultivars and species being evaluated for mealybug resistance being propagated.

Cultivar	Species	Features
Flame Seedless	*V. vinifera*	Susceptible control
Cabernet Sauvignon	*V. vinifera*	Susceptible control
IAC 572	*V. caribbea*	Citrus mealybug resistance
RS-3	*Interspecific hybrid*	Mealybug resistance (anecdotal)
USDA 1-1	*V. champinii*	Pierce's Disease resistance
10-17A	*Interspecific hybrid*	Nematode resistance
USDA 1-2	*V. australis*	Pierce's Disease resistance
PCO-349-11	*Interspecific hybrid*	Nematode resistance

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

Of the initial 10 *Vitis* genotypes proposed for evaluations, six were successfully obtained and propagated (Table 2). In an outdoor screenhouse, rooted cuttings were planted into pots and allowed to grow for 3-4 weeks prior to mealybug application. Plants were fertilized biweekly to promote plant health and sprayed with wettable sulfur weekly to control powdery mildew, with the exception of those genotypes that demonstrated a sensitivity to sulfur. Two mealybug ovisacs (average of 10-20 crawlers per ovisac) were placed onto each plant to promote colonization by the insect. Visible mealybugs, ovisacs, predators, and ants were counted every 2 weeks (July - Sept.) on each plant. In November, plants were pruned and visible mealybugs removed from above ground tissues. The experiment was duplicated and mealybug evaluations will resume this spring.

Figure 2. Adult vine mealybugs Cabernet Sauvignon grape.

Figure 3. Ants on Cabernet Sauvignon grape.

Highest numbers of mealybugs were observed in mid-August, with visible mealybug numbers decreasing into September. Initial results suggest that mealybug colonization was higher on 'Cabernet Sauvignon' than the other species evaluated (Figure 2). This is thought to be, in part, due to the preference of 'Cabernet Sauvignon' by the ant species present (Figure 3). High variability was observed among replicate plants, with most plants having few to no visible mealybugs. 'Cabernet Sauvignon', was the exception with moderate to high levels (10-50) of mealybugs visible on most replicates. Cultivars IAC 572, USDA 1-1, and 10-17A had low numbers of mealybugs detected throughout the season. Evaluations are expected to resume in early April. **Summary:** Potential differences were detected among cultivars in ant and mealybug preferences for feeding and colonizing grape.

Table 2: Cultivars and species currently being evaluated for mealybug colonization and

overwintering.

Cultivar	Species
USDA 1-1	*V. champinii*
PCO-349-11	*Interspecific hybrid*
IAC 572	*V. caribbea*
10-17A	*Interspecific hybrid*
USDA 1-2	*V. australis*
USDA 1-3	*V. candicans*
Cabernet Sauvignon	*V. vinifera*

Publications Produced or Pending, and Presentations: No publications or presentations are pending or have been produced. Presentations and publications are anticipated to occur after results have been confirmed in year 2 (Objective 3) or completed (Objective 1 and 2).

Research Relevance Statement: Vine mealybug is quickly becoming a major pest to the California grape industry. Growers spend an estimated $123 to $500 per acre each year to manage mealybugs, with losses still being observed. Insecticide sprays often provide inconsistent control due to problems associated with spray timing and poor contact with the insect. As concerns about the development of insecticide resistance increase, alternate systems for controlling mealybug are essential. This research has provided the grape industry with the first evaluation for vine mealybug resistance.

Layperson Summary of Project Accomplishments: Clip cages were determined to be a better method for evaluating mealybug survival and growth than detached leaf assays for grape. A mealybug colony for research purposes was successfully established and used to infest grape plants in an outdoor screenhouse. Six grape cultivars were evaluated biweekly for susceptibility to vine mealybug including rootstocks 10-17A and IAC 572. Plants were evaluated for the total number of visible mealybugs, egg sacs, and ants. High variability in the number of mealybugs was observed between plants, but differences among cultivars was evident. Greater numbers of mealybugs, ants, and mealybug egg sacs were observed on the grape cultivar Cabernet Sauvignon compared to each of the other species evaluated. Potential sources of resistance, IAC 572 and 10-17A, had low or no mealybugs present on most, but not all, of the plants evaluated. Mealybugs were allowed to overwinter on grapes, and plants will be evaluated biweekly after bud break has occurred. Dormant cuttings of grape species evaluated in 2017 are being rooted for continued evaluations in 2018, as well as additional cultivars (e.g. rootstock RS-3).

Status of funds: The PI received the funds as in August 2017. To date, $197.33 has been spent. For Objectives 2 and 3, $98.40 was spent on filter paper to transfer mealybugs from squash to grapes for whole plant assays, $89.02 on timers and irrigation for screen cage potted plant studies. An additional $9.91 has been spent on organic squash for maintaining mealybug colonies. The PI is in the process of hiring a technician (GS-5) to manage, maintain and assist with mealybug evaluations. A candidate has been selected and accepted, we are presently completing a background check.

Summary and Status of Intellectual Property Associated with the Project: No intellectual property will be generated through this project, as all materials evaluated are publically available.

Literature Cited:

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