Identification of grape cultivars and rootstocks with resistance to vine mealybug

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Abstract
Vine mealybug, *Planococcus ficus*, is the primary mealybug pest on grapes in California. This pest costs the industry an estimated $126 - $500/acre in pesticides and replant costs annually. In addition, this insect can transmit the economically important Grapevine Leaf Roll Associated Virus (GLRaV), which costs growers $12,106 to $91,623 per acre annually in California. Mealybug resistance cultivars are not available but will be integral for long-term management of this pest. Ten grape cultivars, breeding lines, and species were evaluated for mealybug resistance in two potted plant field trials. Grape species and rootstocks had lower mealybug incidence than the susceptible control, Cabernet Sauvignon, when evaluated in 2017 and 2018. In the second study, fewer mealybugs were observed on inter-specific hybrid rootstocks RS-3 and IAC 572 than on the *Vitis vinifera* cultivars evaluated. Overall, grape rootstocks IAC 572, RS-3 and 10-17A had fewer mealybugs on average than any of the grape cultivars evaluated and may serve as potential sources of resistance or tolerance.

Layperson Summary
Vine mealybug is a major pest to the California grape industry. Insecticide sprays provide inconsistent control due to problems associated with timing and poor contact with the insect. As concerns about the development of insecticide resistance increase, alternate systems for controlling mealybug are essential. Resistant grape cultivars are not currently available and could take more than a decade to breed. In the interim, resistant rootstocks could provide sufficient control either alone or in combination with insecticides. Ten grape lines were evaluated bi-weekly for susceptibility to vine mealybug including potentially resistant rootstocks 10-17A and IAC 572. Plants were evaluated for the total number of visible mealybugs and egg sacs. Greater numbers of mealybugs and 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 few mealybugs present on most, but not all, of the plants evaluated in 2017 and 2018 compared to Cabernet Sauvignon. In a separate outdoor cage study, rootstocks IAC 572 and RS-3 had few to no mealybugs compared to the four scion cultivars evaluated. From our results, RS-3, IAC 572, and 10-17A are all good potential candidates for breeding mealybug-tolerant cultivars. These materials are currently available to nurseries, researchers, and grape breeders through Foundation Plant Services.

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, 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).

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.

Objectives

The objective of this project is to develop a novel control strategy for vine mealybug using host resistance as part of an integrated management program. Identified grape material with resistance to vine mealybug will be further evaluated for use as rootstocks and 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.

Results and Discussion

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

Fig 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 (Fig 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.

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. Because of the low success rates of leaf assays, we transitioned to whole plant resistance studies in Objectives 2 and 3.

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

Dormant cuttings were collected from the San Joaquin Valley Agricultural Sciences Center located in Parlier, CA and rooted in a growth chamber for mealybug host resistance evaluations. Rooted cuttings of grape cultivars were transplanted to deepots and moved to the field (Table 1). One hundred crawlers (stage 1st and 2nd) were placed onto each plant, with a second set of 100 crawlers inoculated onto each plant one week later. Crawlers were gently transferred to filter paper from a colony growing on winter squash in the lab using a paintbrush. The filter paper with mealybugs was stapled to each plant to allow crawlers to move from the filter paper to the plant. After inoculation, each plant was covered with a mesh bag to minimize predators and ants and attached at the base using an industrial rubber band (Fig 2). Five replicate plants were used for each cultivar. Plants were evaluated bi-weekly for mealybug colony growth measured as the total number of visible mealybugs (crawler (1st and 2nd stage), 3rd stage instars, adults, and ovisac scored independently) on each plant. Plant health was scored on a scale of 1-5 with 1 being 90% dead and 5 being perfectly health. Plants were evaluated from 1st of August to 27th of September of 2018, with initial inoculations July 7th.

Fig 2. Mesh bag covering grape plant

Table 1. Cultivars and species evaluated for mealybug resistance.

Cultivar	Species	Features
Flame Seedless	*V. vinifera*	Table grape control
Cabernet Sauvignon	*V. vinifera*	Wine grape control
IAC 572	*Interspecific hybrid*	Citrus mealybug resistance
RS-3	*Interspecific hybrid*	Mealybug resistance (anecdotal)
Autumn King	*V. vinifera*	Table grape
Chardonnay	*V. vinifera*	Wine grape
Valley Pearl	*V. vinifera*	Table grape

For each plant, an area under the insect growth curve (AIGC) was calculated modified from the Area Under the Disease Progress Curve described by Shaner and Finney (1977) and the average AIGC was calculated per line using SAS statistical analysis software. Data was normalized using a log transformation prior to ANOVA and statistical differences were determined based on Tukey's Honest Significant Difference.

Greatest mealybug numbers and AIGC were observed on control cultivars Chardonnay, Autumn King and Cabernet Sauvignon (Table 2, Fig 3). Rootstocks IAC 572 and RS-3 had the lowest number of mealybugs, with RS-3 having no detectable mealybugs after the first two weeks of inoculations. The experiment was performed once. High variability in mealybug numbers were observed for highly susceptible genotypes, reducing the statistical separation among cultivars.

Table 2. Mealybug Area under the Insect Growth Curve for juvenile (Stage 1 and 2 crawlers) and adult mealybugs on grape genotypes

Cultivar	Juvenile	Adults
Autumn King	889	295.75
Cabernet	1022	485.8
Chardonnay	1758.4	736.4
Flame Seedless	95.2	133
IAC572	63	14
RS-3	7	9.8

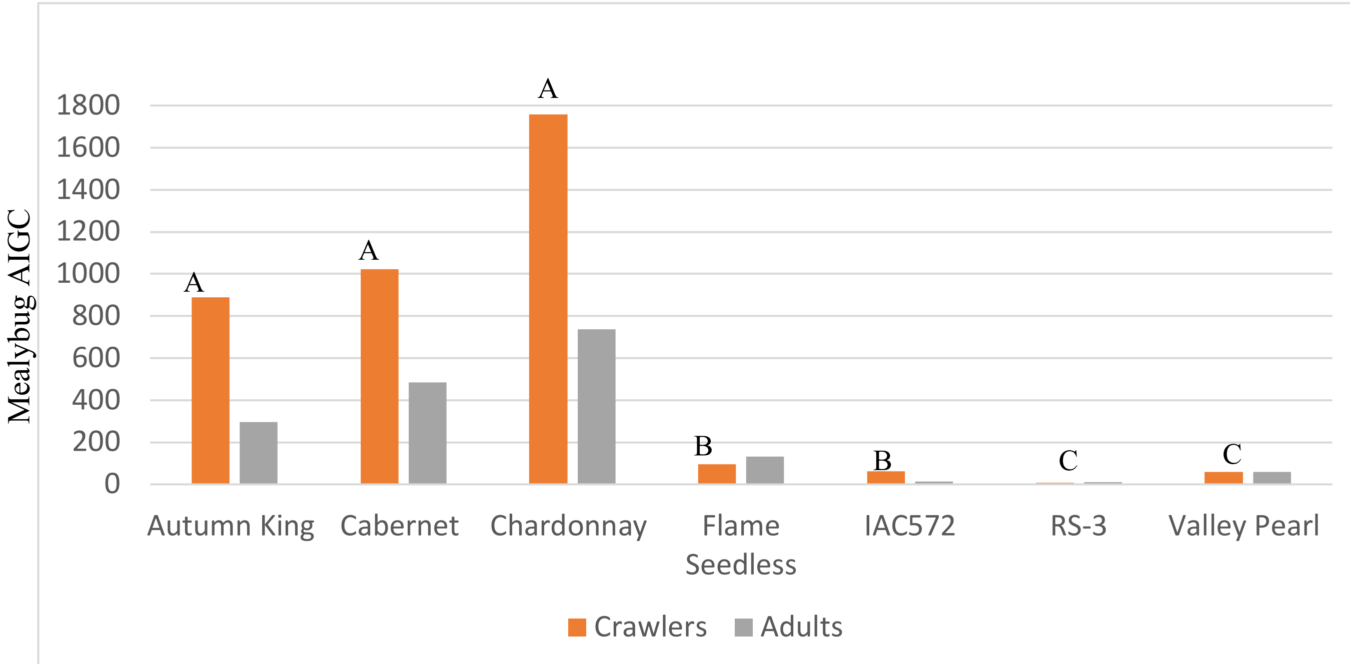

Fig 3. Insect under the growth curve for grape genotypes evaluated in outdoor cage studies. Bars with the same letter are not statistically different.

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

Six *Vitis* genotypes were evaluated for susceptibility to vine mealybug (Table 3). Two mealybug ovisacs (average of 10-20 crawlers per ovisac) were placed onto each plant to promote colonization by the insect. Ten replicate plants were used for each line, and the experiment was repeated. Visible mealybugs, ovisacs, predators, and ants were counted every 2 weeks (July - Sept.) on each plant. During the winter, plants were pruned, and visible mealybugs removed from above ground tissues. In year 2, mealybug evaluations began in June and continued through September. At the end of the experiment (October 2018), plants were returned to the lab, and soil was gently removed from the roots. Roots were evaluated for mealybug overwintering by visual inspection. No mealybugs were observed on the roots of any of the genotypes evaluated (*data not shown*).

Fig 4. Adult vine mealybugs Cabernet

For each cultivar or line, an AIGC was calculated based on the total number of insects detected at each rating. AIGCs were determined for 2017 and 2018. In addition, at the end

Table 3. Cultivars and species 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*
Cabernet Sauvignon	*V. vinifera*

of 2017 and 2018 a final mealybug count was taken to determine the total number of mealybugs present on each individual plant. In year one, 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 (Fig 4). This may be, in part, due to the ants, which preferentially colonized Cabernet Sauvignon (Fig 5). 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. In year two, mealybug numbers steadily increased across all cultivars compared to year one (Fig 6, Table 4). Numbers peaked in August, and steadily decreased throughout September. Experiment was performed over two growing seasons, 2017 and 2018. Mealybug juveniles and adults were not separated in mealybug evaluations and large fluctuations in number of mealybugs detected within a cultivar was evident from week to week.

Table 4. Mealybug population growth (AIGC) per cultivar for the 2018 field season

	2017		**2018**	
Cultivar	**Average Final[1]**	**Average AIGC[2]**	**Average Final**	**Average AIGC**
Cabernet Sauvignon	8.1	879.6	14.9	733.1
PCO-349-11	1.55	102.375	3.1	151.75
USDA 1-*1*	0.5	53.9	1	99.8
IAC572	0.7	49.15	1.4	89.5
10-17A	0.7	159.875	1.3	192
USDA 1-2	1.55	141.875	3.1	205.75

[1] Indicates the number of mealybugs detected at the final count (September 13, 2017 and September 18, 2018)
[2] Area under the insect growth curve

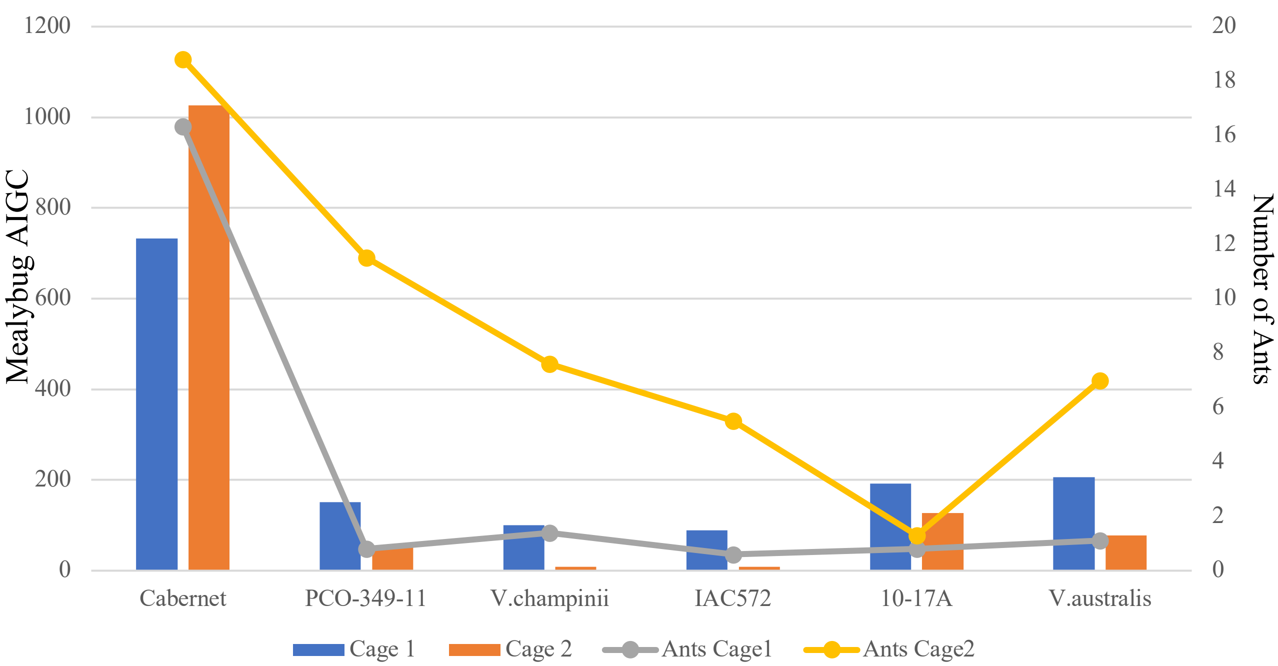

Fig 5. Mealybug Area under the insect growth curve (AIGC) for run 1 (Cage 1) and run 2 (Cage 2) of the mealybug overwinter project (2017). Orange and gray lines correspond to the average number of ants detected on each line in cage 1 and cage 2, respectively.

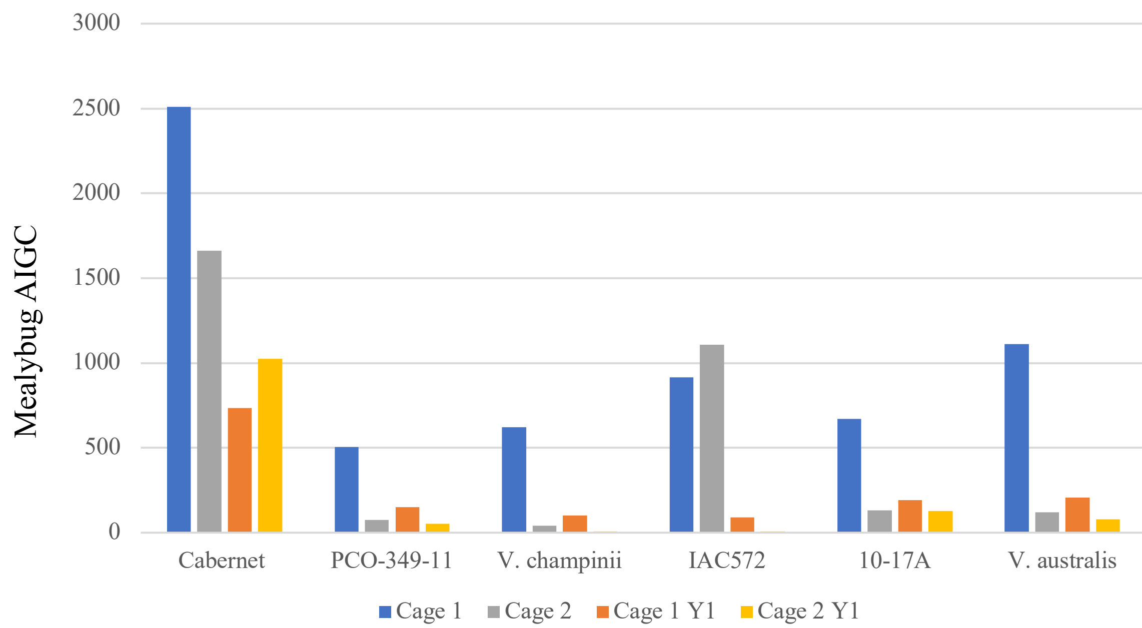

Fig 6. Mealybug area under the insect growth curve (AIGC) for run 1 (Cage 1) and run 2 (Cage 2) of the mealybug overwinter project in year 1 (orange and yellow) and year 2 (blue and gray). Numbers include juveniles and adults.

Findings to Date

Clip cages were a better method for evaluating mealybug survival and growth than detached leaf assays for grape, however whole plant assay was the best method for evaluating host resistance. Six grape cultivars were evaluated biweekly for susceptibility to vine mealybug including rootstocks 10-17A and IAC 572 in 2017 and 2018. 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 in Obj 2. This was also consistent between the first and second years of evaluation. In a separate outdoor cage study (Obj 1) evaluating 7 grape lines, rootstocks IAC 572 and RS-3 had few to no mealybugs compared to grape cultivars Chardonnay, Cabernet Sauvignon, Flame Seedless, Valley Pearl, and Autumn King. Based on these data, rootstocks RS-3, IAC 572, and 10-17A have greater tolerance to vine mealybug than scion cultivars and may be useful within a breeding program to incorporate insect tolerance or as rootstocks used in vineyards with susceptible scions.

Current Activities and Plans

Our previous CDFA-funded work has identified three rootstocks with possible vine mealybug resistance. Objective 2 was completed, and Objective 1 we've opted not to repeat because sample size was insufficient for meaningful separation. Because of the high variability observed across Objectives 1 and 2, we wish to repeat Obj 1 in a more controlled environment. We are currently finishing a pilot experiment to determine if the greenhouse would be more appropriate for mealybug resistance studies. Based on the data from the pilot experiment, it seems that the greenhouse will have less variability with fewer external insect pressures. Our new CDFA PD/GWSS proposed project is to repeat our evaluation of resistant rootstocks in a controlled (e.g. greenhouse) environment with larger replicate plant numbers building off of the previous proposals. This will reduce the effect on mealybug populations from predators and ants, and provide us with better data to confirm host resistance.

We anticipate that once this additional year of screening is complete, and sources of resistance have been confirmed, the project would transition to breeding resistant cultivars, identifying the different mechanisms of resistance, and vineyard rootstock trials. In support of those future activities, crosses were made in 2017 between susceptible and resistant table and wine grape cultivars for developing mapping populations. Seedlings were planted in spring of 2018 and are currently being grown for future cutting and evaluations. Drs. Monica Cooper and Rachel Naegele are in planning stages for a small on-site rootstock trial with a grower to determine if these materials can reduce mealybug populations in field-grown vines.

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