**Renewal Progress Report for CDFA Agreement Number 15-0427-SA**

**Project Title**: Improving vine mealybug winter and spring controls.


| **Principal Investigators** | **Co-Principal Investigator:** |
|---|---|
| Kent Daane | Monica Cooper |
| Dept. ESPM | UC Cooperative Extension |
| University of California | 1710 Soscol Ave, Suite 4 |
| Berkeley, CA 94720-3114 | Napa, CA 94559 |
| kdaane@ucanr.edu | mlycooper@ucanr.edu |


**Reporting Period:** The results reported here are from work conducted Jan 2016 to Jun 2016.

## INTRODUCTION

The vine mealybug, *Planococcus ficus*, has become one of the most important insect pests of California vineyards, threatening economic production and sustainable practices in these multi-billion dollar state commodities (raisin, table, wine and juice grapes). Insecticides are the primary control tool for vine mealybug (Daane et al. 2006, Prabhaker et al. 2012, Daane et al. 2013, Bentley et al. 2014), especially when leafroll diseases (GLDs) are a concern (Daane et al. 2012). Because the vine mealybug population is primarily on the trunk and upper root zone during the winter and early spring (Daane et al. 2013). This population has a refuge from natural enemies (Gutierrez et al. 2008) and can be difficult to control with insecticide applications (Daane, pers. obsrv.). Moreover, mealybugs can remain on even the remnant pieces of vine roots after vineyard removal, hosting both pathogens and the mealybug (Bell et al. 2009).

A delayed dormant (typically in February) application of chlorpyrifos (Lorsban®) was the standard post-harvest or pre-season control (Daane et al. 2006), but more recent work suggests that a post-harvest application of spirotetramat (Movento®) provides equal or better control in some regions of the state (Haviland, pers. comm). Still, effectiveness will depend on application timing, soil moisture, vine condition and age and commodity (for example, post-harvest application timing). Our objectives are to improve pre or post-harvest controls that target the winter-spring vine mealybug population and to better determine the spring emergence of vine mealybug crawlers in order to better time foliar applications.

Researchers have developed relatively good controls that target exposed vine mealybugs – those on the leaves or canes. However, controlling the more protected mealybug population found under the bark of the trunk or on the roots has been more difficult, both for biological controls and insecticides. The application of insecticides with systemic action has helped control this protected population – but their proper use appears to vary among vineyards and regions. Work in Kern County has helped provide guidelines for insecticide use in table grapes in this region (Castle, Haviland, and Prabhaker, unpubl. data). However, similar studies in the central San Joaquin Valley and north coast wine grapes should also be conducted. Typically, vineyards with mealybug damage have large overwintering populations that are never fully regulated, and annually are the source for new generations throughout the summer that infest leaves and fruit of that vineyard and can disperse to other vineyards. Therefore it is critical to develop better control programs for this overwintering population.

Our proposed work will improve pre- and post-harvest systemic insecticide treatments and produce vine mealybug temperature development models that will better predict emergence of crawlers from these protected locations in order to time foliar insecticide treatments. This information will be disseminated to farmers, PCAs and extension personnel, thereby having a practical, direct and immediate impact on insecticide application methods. Because these are straightforward and applied objectives, we foresee the insecticide trials conducted in the two-year timeframe and the temperature model developed in the first year and field validated in the second year of the proposed project. If these results suggest other variables significantly impact insecticide effectiveness than these will be tested in a future proposal.

**OBJECTIVES**
The proposal seeks to develop better controls for the overwintering vine mealybug population found primarily under the bark of the trunk or on the roots at the soil line.

1. Investigate population dynamics and controls for overwintering vine mealybug.

2. Determine the temperature relationship of vine mealybug and grape mealybug to better predict spring emergence and spray timing.

**RESULTS AND DISCUSSION**

**1. Insecticide controls for vine mealybug**
Typically, vineyards with mealybug damage have large overwintering populations that are never fully regulated, and annually are the source for new generations throughout the summer that infest leaves and fruit of that vineyard and can disperse to other vineyards. Therefore it is critical to develop better control programs for this overwintering population (Fig. 1).

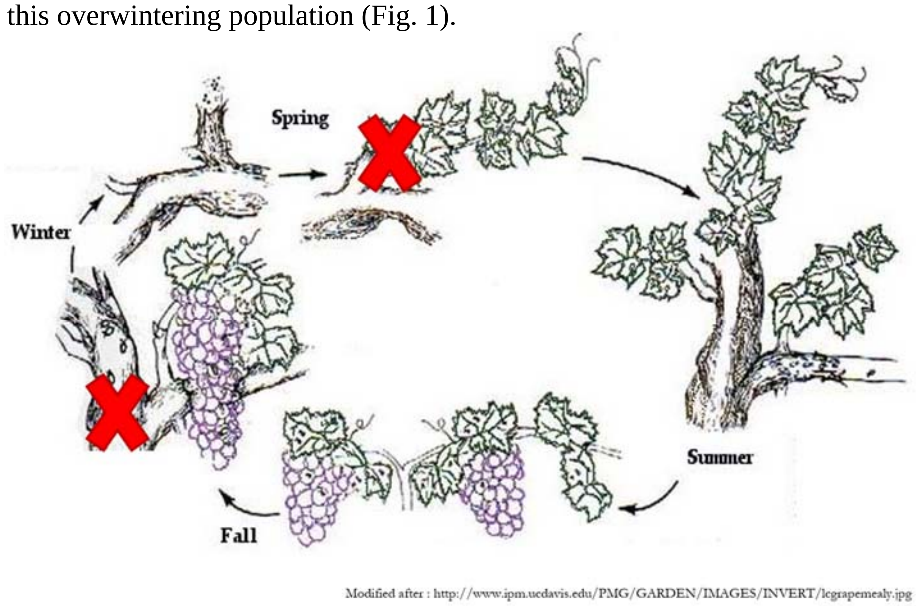


Fig.1: Scheme showing seasonal mealybug movement in the grape vine, with the red x's representing the population location and seasons where we hope to improve control. Our goal is to develop better controls for the trunk population – especially in the spring and early summer before they move out onto the leaves, or in the fall to kill the population before they move under the bark.

Our overall objective is to improve pre or post-harvest controls that target the winter-spring vine mealybug population and to better determine the spring emergence of vine mealybug crawlers in order to optimize timing of foliar applications. Understanding the systemic uptake of the pesticide by the vine is vital in order to make management decisions.

During 2015 and 2016 we used bioassays (visual counts of mealybugs) to look at control effectiveness across vineyards in different regions and with different management practices or vine structures (this work was funded via the CDFA PD GWSS project). Commercial vineyards were selected in the central San Joaquin Valley (Fresno County) with four vineyard blocks near Fresno (1 Thompson seedless raisin grapes, 1 Crimson seedless table grapes and 2 Thompson seedless table grapes); the Lodi-Woodbridge wine grape region (Stockton county) with three vineyards near Lodi (1 Cabernet Sauvignon, 1 Pinot Noir, 1 Chardonnay); and North Coast wine grape region (Napa County) with two vineyards at a site in the Carneros region of Napa (1 Pinot Noir, 1 Chardonnay). We are also sampling numerous 'experimental' vineyard blocks at the Kearney Agricultural Research and Extension Center that represent wine and table grape blocks undergoing studies for nitrogen, irrigation, and wine grape cultivars. At each site, we have counted mealybug densities on the vine, measured cluster damage and taken vine fresh tissue samples before and after Movento® applications (sections from the leaf, cane and trunk) (Fig 2).

Fig. 2: Sampling trunk live tissue, leaves and petioles, canes, cordons, trunk (above and below girdle when present) and roots.

We applied the insecticide Movento® at different application timings – as measured by calendar date as well as by weeks before or after harvest (Movento® has a 7 day Pre-Harvest Interval). We applied Movento® at the label rate and determined the percentage kill of mealybugs on different sections of the vine during the summer, fall (completed), and the coming spring (Fig. 3). A standardized application method was used across all vineyards so that surfactant and application rate would not be an influence. At each site, there are 15 replicates (individual vines) per treatment per vineyard, with treatments placed in a complete randomized design.

Fig 3: Spraying Movento® in grape vines

Pre-treatment mealybug counts were taken using a timed count. In brief, on each sampled vine, an experienced sampler searched for mealybugs for a 1-minute period. The areas of the vine searched change with the seasonal movement of the mealybug population (i.e., during the winter the roots and lower trunk sections are the most likely regions to find vine mealybug). The pre-treatment mealybug density was then used to block treatments against density because vineyard mealybug populations can be clumped. The visual count of mealybugs took place from June 2015 through November 2015 and April 2016 to the present (June 2016 – these count will continue through November 2016). This allows us to monitor mealybug populations at different phenological stages of the crop. We monitored when the grape clusters were not ready to be harvested, when they were ready to be harvested and after they were harvested.

We completed a measurement of economic damage on five clusters on each vine using a 0–3 scale: 0 means no mealybug damage, 1 means honeydew present but the bunch is salvageable, 2 means honeydew and mealybugs present but at least part of the bunch is salvageable, and 3 means a total loss (Fig 4). The economic damage of clusters place from August 2015 through the 2015 harvest, and this process will be repeated in the 2016 harvest period.

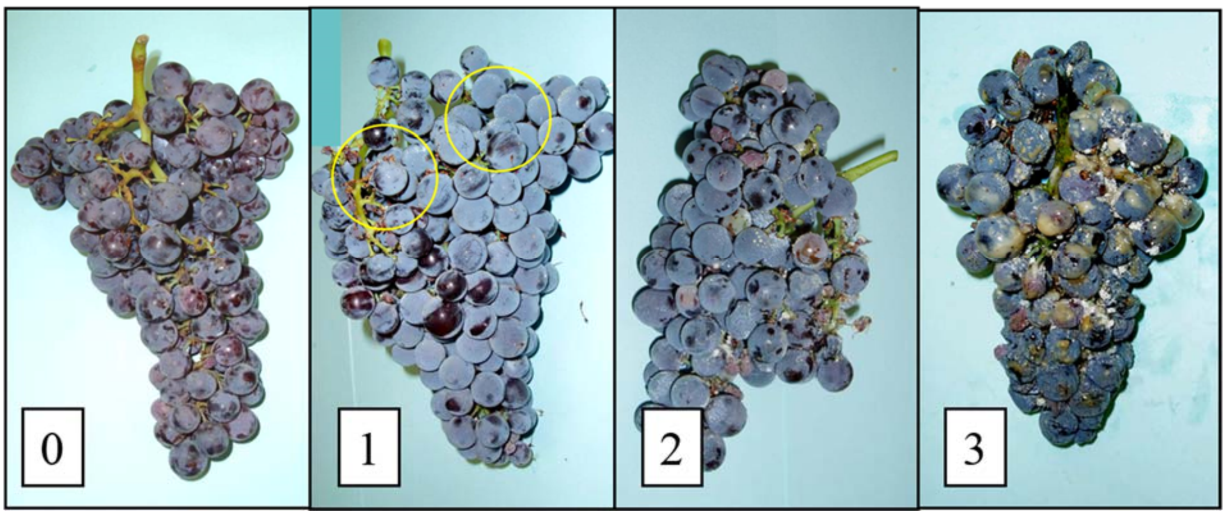


Fig 4. A visual rating of 0 to 3 cluster economic damage for mealybug infestation in the fruit clusters.

In 2015, taking into consideration all the sample areas, approximately 530 vines were sampled for mealybug counts and for cluster evaluation. Together, the treated vineyards include several factors that could be affecting the pesticide efficiency, such as the age of vineyards, irrigation type, commodity (table, raisin and wine grapes), the presence of a girdle, and geographical area. Similar counts have been made in 2016, although this work is in progress and these data have not been only partially analyzed, as described below.

Result highlights show that vines treated with Movento in May (grower standard treatment) and before harvest (preharvest treatment) had significantly fewer mealybugs (Fig.5) and less fruit damage (Fig 6) compared with untreated vines. Even though mealybugs were found in low numbers, the spray treatment had a significant effect on the numbers of individuals found in each developmental stage ($F_{6,6}$= 13.88, P <0.001). This was relatively consistent in all vineyards studied and in all regions.

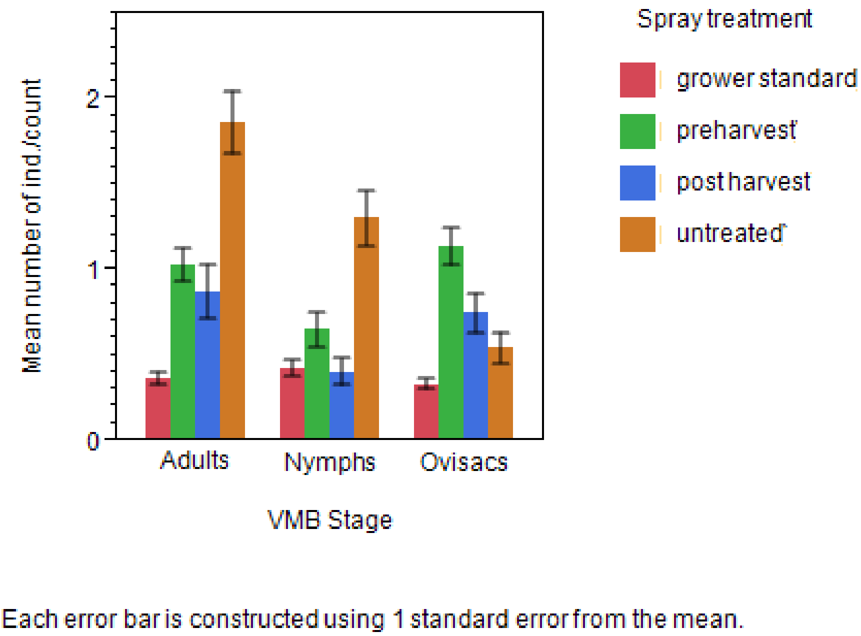


Fig. 5. In the central San Joaquin Valley, treated vines show significantly less VMB individuals compared to untreated vines.

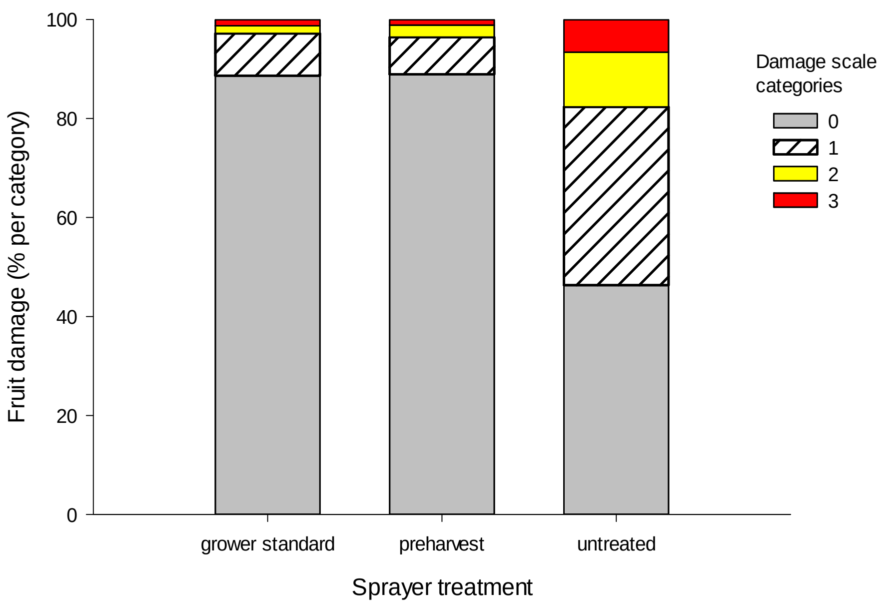


Fig. 6. In tested San Joaquin Valley vineyards, for example, treated vines show significantly less fruit damage compared to untreated vines.

That Movento applications reduced mealybug densities is not surprising and has been shown in numerous studies. What we will complete this year is a comparison of mealybug densities on different parts of the vine in different regions and different grape commodities. It also important to note that the grower standard treatment (typically between May and June depending on the region) was still the best application timing.

The low number of mealybugs found in all the monitoring sites and the low constant damage recorded suggest that visual counts and cluster damage evaluation alone are not sufficient tools to evaluate vine mealybug population's response to pesticide applications. This could be as a result of their clumped distribution in the host plant. This and as several factors might affect the rate of the pesticide uptake on the vine, and therefore, its efficiency to kill the pest we added another evaluation tool to better study and improve the vine mealybug winter and spring population dynamic and their control. We are currently on the initial steps of studying the movement through the vines of the pesticide Movento®, the most common pesticide used nowadays against vine mealybugs in California.

In order to study how the pesticide Movento® moves through the vines, the pesticide uptake by the plant was followed by means of high pressure liquid chromatograph methodology (HPLC). After counting mealybugs, five portions of the vine were sampled for living tissue: leaves and petiole, trunk above and below the girdle, cane, and arm. If girdle is not applicable, a bottom and middle part of the trunk were taken. If arm is not applicable, an upper part of the trunk was sampled. This fresh tissue sampling effort in 2015 resulted in approximately 6000 samples, from which a subsample was gathered for HPLC analysis, based on bioassays. A smaller number of samples are being collected in 2016 (approximately 2000) because of the time and expense in processing the samples. However, we are using results from 2015 to concentrate our efforts on more important aspects of Movento application and timing.

The HPLC methodology allows the preparation of several samples in a relatively short period of time and extracts several structurally different substances with good efficiencies. We applied this methodology in order to obtain the concentration of the active ingredient of the Movento® pesticide and its first metabolite, spirotetramat and spirotetramat -enol, respectively. In order to analyze the quantity of spirotetramat and spirotetramat-enol in leaves, the extraction QuEChERS (Quick Easy Cheap Effective Rugged Safe) method is followed. We adapted this QuEChERS extraction methodology to our samples to achieve the most trustful results. Adapting this method includes trying different solvents and mobile phases to clean and extract the desired compounds and testing various elution times.

Initially, we study only the concentration of spirotetramat and spirotetramat -enol, respectively. Later, we included the analyses of two more spirotetramat metabolites in plants: Enol-Gic (glucoside) and Ketohydroxy.

Our initials results in leaf tissue analyses show that spirotetramat is fast converted into spirotetramat-enol. These two compounds seem to be transformed into other metabolite or fade through time while another metabolite, glucoside, increases its presence in an oscillating manner through time. The metabolite ketohydroxy seems to be fast transformed by the plant into another metabolite or fade as well (Fig.7).

In addition, tissue sampling for continuing this analysis is being carried out since April 2016 through November 2016. We are currently adapting the QuEChERS extraction methodology to our bark tissue samples.

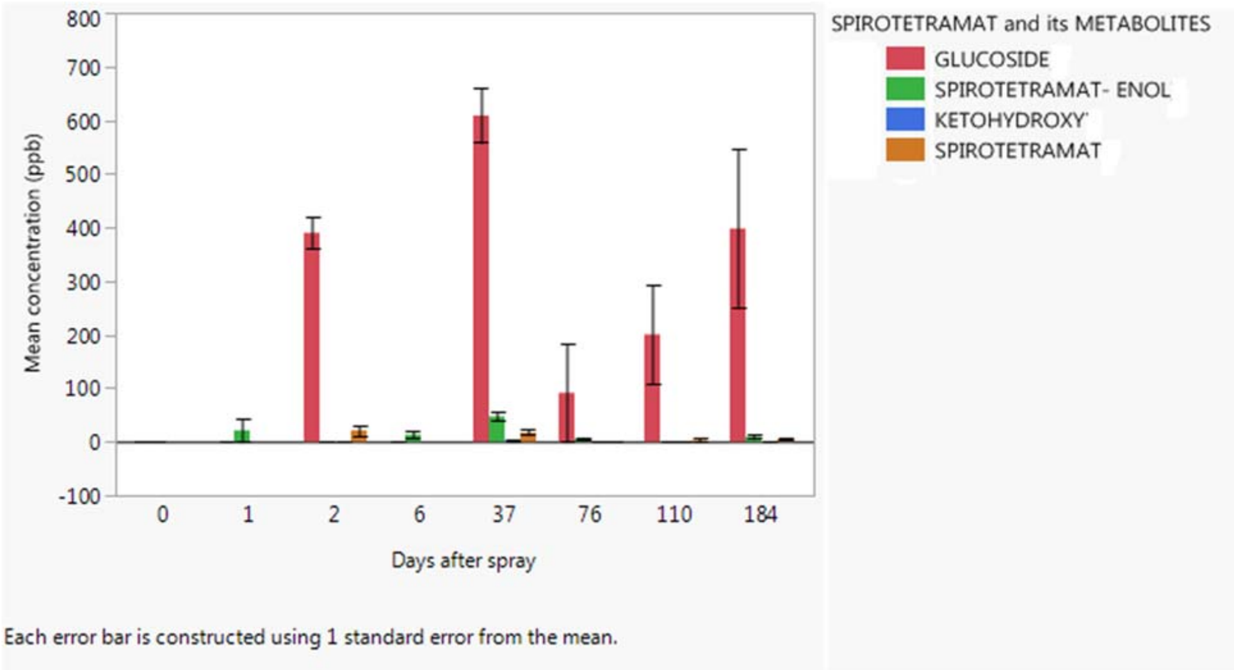


Fig. 7. Mean concentration (ppb) of spirotetramat and its metabolite through time.

2. Temperature development of vine mealybug.

Temperature models have not yet been developed.

**PUBLICATIONS PRODUCED AND PENDING, AND PRESENTATIONS MADE THAT RELATE TO THE FUNDED PROJECT.**

**Publications (January-June 2016):**

No peer-reviewed publications to report.

Proceedings articles.


Hochman Adler, V., Lutz, T. M., Hutchins, J. Cooper, M. L., and Daane, K. M. 2016. Identification and control of vine mealybug, pp. 6-11. In: *Proceedings, San Joaquin Valley Grape Seminar, January, 2016*. University of California Cooperative Extension and Allied Grape Growers. Easton, CA.

Daane, K. M., Hochman Adler, V., Lutz, T. M., Wilson, H., Hutchins, J., Cooper, M. L., Hogg, B. N., Blaisdell, K., Dervishian, G., Van Zyl, S., Kurtural, K., Chen, J., Oh, H., Fonseca-Espinoza, N., Oneto, R., Golino, D., and Almeida, R. 2016. Vine mealybug controls – investigating improvement to current control programs, pp 23-29. In: *Proceedings, Sonoma County Grape Day Seminar*, February 10, 2016. California Table Grape Commission. Fresno, CA.

Daane, K. M., Lutz, T. M., Yang, M. N., Truong, L., Islas, B., Badalyan, S. M., Tomajan, S., Molinar, A. J., and Yokota, G. Y. 2016. Black widow spider biology and control in vineyards, pp 7-17. In: *Proceedings, San Joaquin Valley Table Grape Seminar*, February 17, 2016. California Table Grape Commission. Fresno, CA.

Daane, K. M., Hochman Adler, V., Lutz, T. M., Wilson, H., Hutchins, J., Cooper, M. L., Hogg, B. N., Blaisdell, K., Dervishian, G., Van Zyl, S., Kurtural, K., Chen, J., Oh, H., Fonseca-Espinoza, N., Oneto, R., Golino, D., and Almeida, R. 2016. Vine mealybug controls – investigating improvement to current control programs, pp 23-29. In: *Proceedings, San Joaquin Valley Table Grape Seminar*, February 17, 2016. California Table Grape Commission. Fresno, CA.

**Presentations (January-June 2016):**

K.M. Daane: Identification and control of vine mealybug. *2016 San Joaquin Valley Grape Symposium*. Easton CA. Jan. 2016.

K.M. Daane: Mealybug research – from pesticide movement in the vine to their role as vectors of plant viruses. *Sonoma County Grape Day*. Santa Rosa. CA. Feb. 2016.

K.M. Daane: Vine mealybug controls – investigating improvement to current control programs. *San Joaquin Valley Table Grape Day*. Visalia. CA. Feb. 2016.

## RESEARCH RELEVANCE STATEMENT

The vine mealybug has become one of the more important insect pests of California vineyards, threatening economic production and sustainable practices in this multi-billion dollar state industry. This work has just begun to better understand and optimize registered insecticides used to control the vine mealybug in the winter and spring periods, when the mealybug bug population is located primarily under the bark on the trunk and cordons. In the initial four month period we selected vineyards in three regions. We both applied treatments of Movento and we monitored commercial spray applications in vineyards for different commodities (e.g., wine vs table grape) and with various management practices (e.g., trellis systems). We monitored mealybug densities but found little difference among the plots, in part because of the low mealybug populations. The work suggests that using HPLC to follow the movement of the pesticide and its enol may provide better resolution of differences in application timing and the impact of vineyard management practices.

## LAYPERSON SUMMARY

The vine mealybug has become one of the more important insect pests of California vineyards, threatening economic production and sustainable practices in this multi-billion dollar state industry. Researchers have improved biological and chemical controls, but this pest remains in vineyards and can quickly build in numbers during the summer and damage the crop near harvest-time. One reason that insecticides do not provide complete control is that a portion of the vine mealybug population remains under the bark of the trunk or on the roots and emerges from this refuge in the spring and summer. Our first objective is to improve pre and post-harvest insecticide application to control this overwintering population. Our second objective is to develop temperature-based models to better predict the spring emergence of the mealybug 'crawlers' in order to better time spring foliar insecticide treatments. In the first four months of this project we have made considerable progress towards our first objective, taking samples from vineyards in Napa and Lodi-Woodbridge wine grapes and San Joaquin Valley table grapes, wine grapes and raisin grapes. At each site we have counted mealybug densities on the vine and taken vine samples (sections from the leaf, cane and trunk) to be analyzed for the presence of the insecticide (spirotetramat) and its enols. These samples are being stored at -20 °C and the proper protocol to process them by HPLC is being improved.

## STATUS OF FUNDS

Funds are being spent appropriately and are on schedule – as of January 2016, approximately $32,000 has been spent on employee wages and supplies. UC Berkeley is often slow to invoice the granting agency.

**SUMMARY AND STATUS OF INTELLECTUAL PROPERTY ASSOCIATED WITH THE PROJECT**

There is no intellectual property associated with this project.

**LITERATURE CITED**


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Prabhaker, N., Gispert, C., and Castle, S. J. 2012 Baseline susceptibility of Planococcus ficus (Hemiptera: Pseudococcidae) from California to select insecticides. J. Econ. Entomol. 105: 1107-1476.