PROGRESS REPORT FOR CDFA AGREEMENTS 15-0427-SA AND 16-0556-SAFP

IMPROVING VINE MEALYBUG WINTER AND SPRING CONTROLS: I. BIOASSAYS, II. USING HPLC TO FOLLOW INSECTICIDE MOVEMENT IN THE VINE

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REPORTING PERIOD: The results reported here are from work conducted April 2016-February 2017.

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 this multi-billion-dollar commodity. 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. 2013). Researchers, PCAs and farmers 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. The vine mealybug population is primarily on the trunk and upper root zone near the soil line 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 the most difficult to control even with systemic insecticide applications (Daane, pers. obsrv.). Moreover, mealybugs can remain on even the remnant pieces of vine roots after vineyard removal, hosting pathogens and infesting new vines after replanting the vineyard (Bell et al. 2009).

Insecticides with systemic action are the best materials to control this protected population – but their proper use can vary among vineyards and regions. Moreover, vineyards with mealybug damage typically 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.

A delayed dormant (typically in February) application of chlorpyrifos (Lorsban®) was the standard post- harvest or pre-season control that targeted mealybugs on the trunk and cordon (Daane et al. 2006). The best in-season insecticide for vine mealybug that move from the trunk and cordon to the leaves, canes and fruit has been an application of Movento® (Bayer Crop Science), with the active ingredient Spirotetramat, which may also help control root feeding

nematodes (Mike McKenry, pers. comm.). Still, the effectiveness of any systemic material will depend on application timing, soil moisture, vine condition and age and commodity (for example, post-harvest application timing). Our objectives are to improve controls that target the winter-spring vine mealybug population and to better determine the spring emergence of vine mealybug crawlers to better time foliar applications. Specifically, we are conducting field bioassays to determine the effect of application timing, soil moisture, vine condition and age and commodity (for example, post-harvest application timing, wine *vs*. raisin management practices) on systemic insecticide effectiveness. We plan to work with all vineyard-registered insecticide materials, but this past year's work has focused on the field application bioassays and movement of Movento® in the vine. To follow the movement of Movento® we are collecting vine samples and using an "HPLC" to determine amounts of different metabolites associated with Movento® in different parts of the vine. For example, two of the questions we plan to address is whether Spirotetramat converts to the metabolite Enol-Spirotetramat (which is the primary toxicant) similarly under different vines condition, such as nutrient status or cultivar and where on the vine the metabolites move to and in what concentration are the metabolites found on different vine sections – such as the leaves versus the roots? We will also use our protocols to help confirm the presence of Spirotetramat metabolites in the root system, in support of Dr. Andreas Westphal's proposal.

LIST OF 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.

I: Bioassay

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.

II: Using HPLC to follow the movement of Movento® in the vine

1) Improve the protocols to determine levels of Spirotetramat and its first metabolite, the enol form, in vine tissue samples.
2) Investigate the dissipation and transformation mechanisms of the active ingredient of the pesticide Movento® after application.

RESULTS AND DISCUSSION

I: Bioassay: Insecticide controls for vine mealybug

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. 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 1).

Fig. 1: Sampling different vine sections (leaves and petioles, low and high trunk sections, and roots) using both (A) timed (1 minute) visual counts for the bioassay and (B) taking leaf or bark chip samples for HPLC analyses.

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. In 2016, the visual count of mealybugs took place from April and October. 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 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 will continue this in the coming spring (Fig. 2 - right).

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.

We also have 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. The economic damage of clusters took place from June 2016 through harvest. We evaluated the clusters when the grape clusters were not ready to be harvested, and when they were ready to be harvested.

Taking into consideration all the sample areas, approximately 600 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.

Much of the data remains to be analyzed, especially the late season (just before harvest) and

post-harvest sprays that will need additional sampling in spring and summer 2017 to determine treatment impact. Moreover, in our commercial fields the overall density of the mealybug was very low, making treatment comparisons difficult. As detailed in the previous report, one clear result was that vines sprayed with Movento® in May (the recommended standard treatment would be 8 oz in April or May) had less fruit damage compared to untreated and to the mid July spray treatments (Chi Square $P < 0.001$). Combining these same treatments across the different vineyards sampled in the Central Valley, even though mealybugs were found in low numbers throughout all the sampling areas, spray treatment had a statistically significant effect on the numbers of individuals found in each developmental stage ($F_{2,2} = 5.3586$, $P = 0.004$).

We have yet to analyze the post-harvest treatments, but by the end of the season vines treated from mid- to late-May had fewer vine mealybug compared to untreated vines; however, there was no significant difference between May and mid-July treatments (Fig. 4). These results indicate that the metabolites of Movento® are moving through the vine and killing mealybugs even in the pre-harvest application treatment, but the earlier treatments are killing the mealybugs before they get into the fruit.

In our bioassay studies, 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 were not sufficient tools to evaluate details of the vine mealybug population's response to pesticide applications. One problem is their clumped distribution in the host plant, which requires a great number of samples to get an accurate estimate of population response. There was also a repeated issue of grower overspray on the control plots, reducing our number of control replicates.

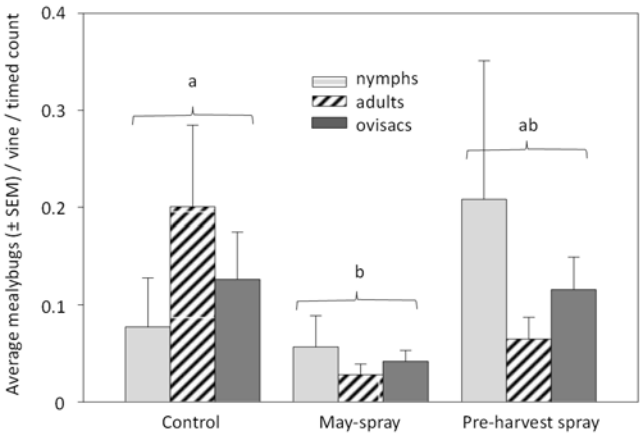

Fig. 3: In the San Joaquin Valley, there was more fruit damage in the pre-harvest treatment even though there was a reduction in the number of adult mealybugs as compared with the control. Figure 3 (right) shows the average number of nymphs, adults and ovisacs on vines treated in mid- late-May (farmer standard treatment), pre- harvest and a no-spray control. There was significant difference between the mid-May and pre-harvest (mid-July) treatments in total mealybugs.

Data from the Napa Valley and Lodi Woodbridge vineyards has not yet been analyzed. In most of these sites, we found it difficult to make comparisons among bioassay treatments because the levels of mealybugs were too low, including the control treatments.

II. HPLC to follow the movement of systemic insecticides

To study how the pesticide Movento® moves through the vines, the pesticide uptake and movement of key metabolites in the plant was followed by means of high pressure liquid chromatograph methodology (HPLC). To better understand our purpose, a description of how Movento® works to kill mealybugs is needed. Spirotetramat is sprayed onto the leaves where it has translaminar activity and gets absorbed. It is not the Spirotetramat that primarily kills the

mealybug but the first breakdown product or metabolite called "Enol". The Enol can change to other metabolites such as Enol-Glycoside and Ketohydroxy as some of the primary metabolites found, but it is the Enol metabolite that is most important for killing the mealybugs. The change from Spirotetramat to Enol appeared to be most effective in the leaf tissue, as described in Bayer-sponsored studies in apple, cotton and other crops. Whereas some translaminar pesticides remain in the leaves, Spirotetramat and its metabolites can be transported by the phloem (and to some extent the xylem) to other plant parts – and this is key in moving the product to where the mealybugs are.

We used the HPLC to obtain the concentration of the active ingredient of Movento® (Spirotetramat) and two of its primary metabolites, Spirotetramat -enol and Ketohydroxy (the latest metabolite is not active against mealybugs as far as we know). To analyze the quantity of Spirotetramat, Enol and other metabolites in leaves, the extraction method "QuEChERS" (Quick Easy Cheap Effective Rugged Safe) was followed. This methodology allows the preparation and analyses of several samples at one time, and provides extracts of several structurally different substances with good efficiencies.

Adapting this method includes trying different solvents and mobile phases to clean and extract the desired compounds and testing various elution times. Afterwards the obtained results are compared to a standard curve for the desired compound. In this process, the most appropriate and reproducible cleaning and extraction process was determined for leaves, canes and roots. We also modified the process for smaller bark samples (<10 g) that can be completed without the addition of a "Mass Spectrophotometer" (MS).

Our analyzed samples are collecting in association with our field bioassays. After counting mealybugs (see bioassay above), 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 2016 resulted in approximately 6000 samples, which are being analyzed using the HPLC technology

Results from leaf tissue analyses show that Spirotetramat is quickly converted into Enol (remember that Enol is the metabolite responsible for killing the mealybugs) (Fig. 4).

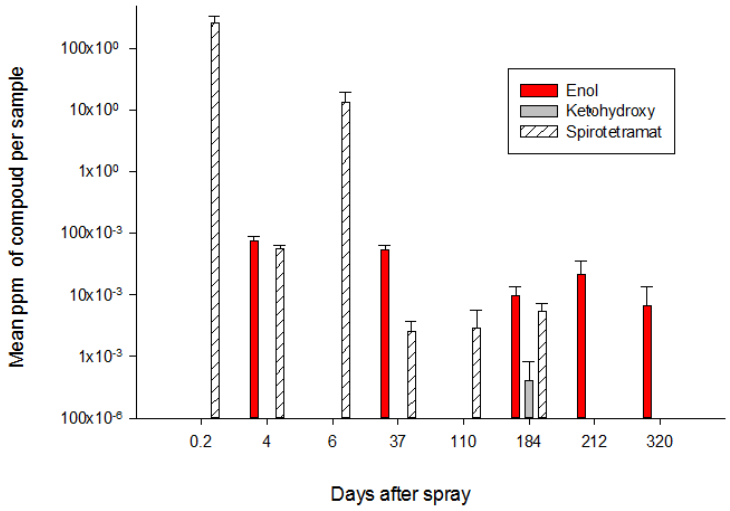

Fig. 4. Mean concentration (parts per million) of Spirotetramat and two of its metabolite in leaf samples from 5 hours after spray to 10 months after spray.

Note that the Y-axis is using a log scale so there are great differences in the amounts of metabolites. Most important was that some Spirotetramat and Enol was found in the leaf tissue up to 320 days after treatment. It is still unclear (from our studies) if the Spirotetramat found long after the application will eventually convert to Enol, or if this conversion process slows as it is surprising that the initial conversion to Enol that we still find Spirotetramat unconverted 5 months after spray treatments. These tested vines will continue to be sampled until leaf drop, and other vine tissue (e.g., bark) will be sampled up to a year after the spray application. At his point, we assume that Enol found after 3-5 months is from either relative stable Enol remaining in the leaves, or Spirotetramat that in the leaves that is much later (in time) converted to Enol. Note also that we found the Ketohydroxy metabolite only on the last sample date and at a very low amount (Fig. 4).

When looking closer at the amount of Spirotetramat and Enol in leaf tissue over the sampling period, it's clear that the amount Spirotetramat is reduced quickly, from about 261 ppm (parts per million) 5 hrs after spray to about 13 ppm after 6 days, and <0.03 ppb after 1 month (Fig. 4). There is not a corresponding increase in Enol, which is lower than Spirotetramat initially but shows a more stable presence during the ten-month sampling period, around 0.002 ppm (20 ppb) (Fig. 4). Note that on three sample dates (5 hours later treatment,6 and 110 days) we did not detect any Enol and this analysis will be repeated with stored samples to determine if this unusual finding was a data entry error. What is needed now is a field bioassay on the amount of Enol in the plant that is toxic to mealybugs and for how long the mealybug must feed to acquire this lethal dose.

During May 2016, we applied Movento® in Thompson grape vines. Before being treated, 6 leaves per vine were covered to prevent the material to reach them. As soon as the neighboring leaves stopped dripping, the "bagged leaves" were uncovered. Four days later, the leaves were sampled as usual.

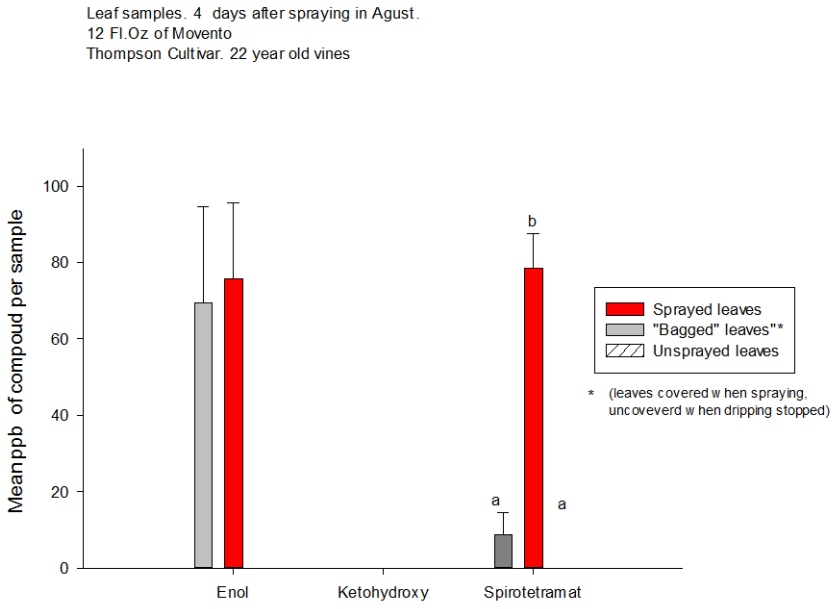

Figure 5. Spirotetramat and Enol content in covered and later uncovered leaves (in parts per billion) treated with a label rate (12 fl. oz per acre) of Movento® in May. Samples were collected four days after treated.

The analyses show that the amount of Spirotetramat is significantly different from the

"bagged" and control treatments ($F_{(2,67)}$=22.14, p<0.001). However, the amount of Enol and Ketohydroxy is no significantly different among treatments (Fig. 5). This result shows that the metabolites are moving with the phloem from the treated leaves to leaves rapidly and transforming rapidly as well.

Our initial results from the trunk tissue analyses show that only Spirotetramat-Enol is found in the bark tissue, 212 and 320 after spraying in Crimson cultivars Crimson cultivar, 14 years old (Fig. 6). This result shows that the metabolites are moving with the phloem from the leaves to other vine sections. One question this does raise is whether the Spirotetramat found in the trunk is easily converted to Enol. We assume that the metabolites flow passively in the phloem and so it is possible that, depending on vine needs, the metabolites could be carried back to the leaves. There was no significant difference in the amount of Enol present 212 and 320 days after treatment. There was no difference between samples collected in upper and lower portions of the leaves.

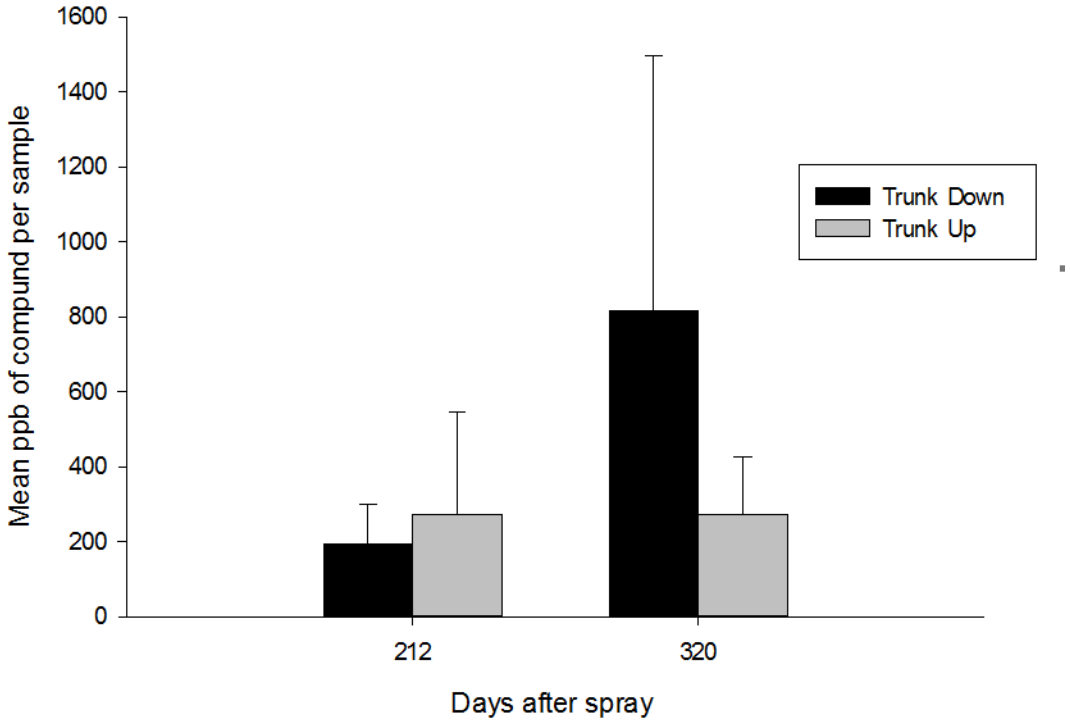

Fig. 6. Mean concentration (parts per billion) of Spirotetramat-Enol at the bark from samples collected 212 and 320 days after Movento® was applied to the leaves at label rate (8 oz per acre) in May 2015. Vines are Crimson cultivar, 14 years old. No control treatment is available as the entire field got sprayed.

We also analyzed the effect of application rate in the bark. Two label rates were applied (8 fl. oz and 12 fl. oz) and one off label (24 fl. oz). Our results show that there is no effect of the application rate on the amount of compound that reaches the bark 6 and 30 days after treatment (Fig. 7). The next step is to analyze the amounts of compounds that reaches the bark tissue in a longer period.

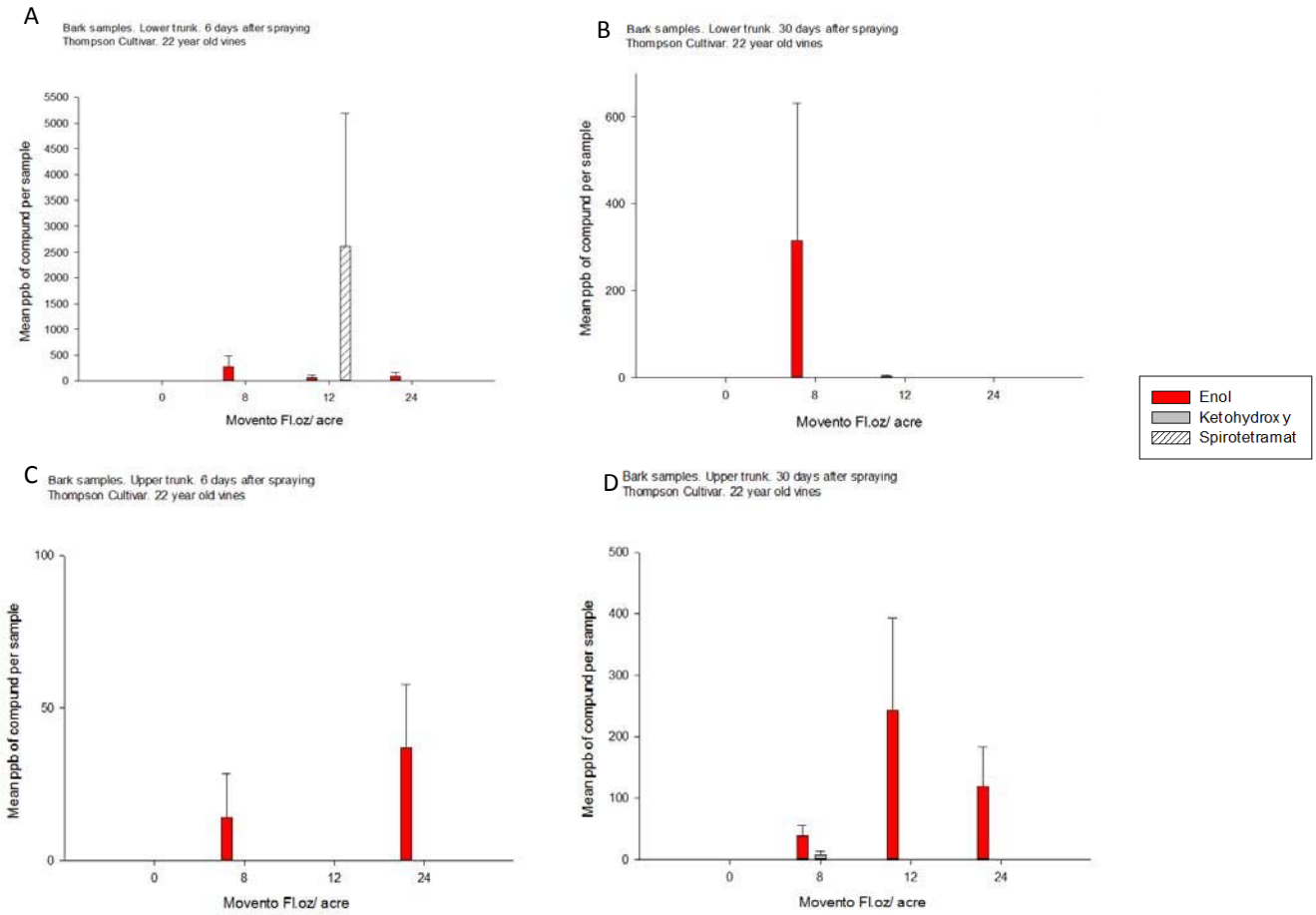

Fig. 7. Mean concentration (parts per billion) of Spirotetramat and its two metabolites, Enol and Ketohydroxy at the bark from samples collected 6 and 30 days after Movento® was applied to the leaves at label rate (8 and 12 fl. oz per acre) and off the label application (24 fl. oz per acre). Vines are Thompson, 22 years old.

III. Temperature development of vine mealybug.

These data have not yet been analyzed.

PUBLICATIONS AND PRESENTATIONS

Publications:
No peer-reviewed publications to report.

Proceedings articles (2016-present).

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., 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 (2016-present):

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.

K.M. Daane: Update on mealybug controls – what works and what can be improved. Central Coast Wine Grape Seminar. Salinas. CA. Mar. 2016.

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

K.M. Daane: Improving insecticide controls for mealybugs – following the movement of translaminar insecticides in the vine mall and large bug pests UCCE Seminar: Vineyard Pests and Disease Management. San Luis Obispo, CA, Nov. 2016.

K.M. Daane: Improving vine mealybug winter and spring controls. 2016 Pierce's Disease Research Symposium. San Diego, CA, Dec. 2016.

K.M. Daane: Mealybug pests in California vineyards – their role in the transmission of plant pathogens and their controls. Unified Wine and Fruit Outreach Day. Walla Walla, WA, Jan. 2017.

K.M. Daane: Using HPLC to follow the movement of a Movento through the vine to improve controls of vineyard mealybug pests. Bayer CropScience Tree Fruit and Vineyard Growers Meeting. Monterey, CA, Jan. 2017.

K.M. Daane: Mealybug controls as an example of the development of an IPM program (1 hr lecture). Integrated Pest Management class at West Hills College. Coalinga, CA. Feb. 2017.

K.M. Daane: Using HPLC to follow the movement of an insecticide through the vine. 65th Annual Lodi- Woodbridge Grape Day. Lodi, CA, Feb. 2017.

K.M. Daane: Insect pest management - grapes. Bayer Crop Science, 2017 Grape and Citrus Symposium. Monterey, CA, Mar. 2017.

K.M. Daane: Control tools for mealybugs and their impact on grape leafroll associated viruses. 2017 E&J Gallo Winery Mealybug, Leafroll and Insecticide Update Meeting. Fresno, CA, Mar. 2017.

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 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 we selected vineyards in three regions and have taken spring through fall samples. 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. We collected approximately 6000 tissue

samples at vineyards being used for the field bioassays, as well as from vineyards with unusual vine mealybug densities, or where we can manipulate spray application to test movement of key metabolites of Movento®. For analyses, we have developed protocols for tissue analysis using an HPLC, and verified that the procedure is accurate. Currently, we have processed <10% of the collected samples, but this fall and winter, now that the field work has been largely completed, we are focusing on tissue analyses with the goal of having 2015 and 2016 samples processed before we collect 2017 material.

LAY PERSON SUMMARY

The vine mealybug has become one of the most important insect pests of California vineyards. Researchers, PCAs and farmers 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. 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 to better time foliar applications. In 2016, research focused on bioassays (e.g., the number of live or dead mealybugs) and movement of Movento® - or more correctly its metabolites - in the vine, using high pressure liquid chromatograph methodology (HPLC). Preparing samples and running the HPLC can be time consuming and we have processed around 20% of the 6000 samples collected. We have confirmed that Spirotetramat is rapidly converted in the leaves to the metabolite called Enol-Spirotetramat, and this metabolite can remain in the leaves for most of the season. The Enol-Spirotetramat can change to other metabolites such as Enol-Glycoside and Ketohydroxy, but it is the Enol-Spirotetramat that is most important for killing the mealybugs. There is a gradual decline in the amount of Enol-Spirotetramat, but we found Spirotetramat in leaves 184 days after

application, suggesting that this material might still yet be converted to Enol-Spirotetramat. As we process more of the samples we will be better able to determine the metabolic pathways of Spirotetramat and what influence vineyard conditions and application methodology has on the effectiveness of Movento®.

STATUS OF FUNDS

Funds are being spent appropriately and are on schedule – as of October 2016, approximately $83,600 has been spent on employee wages and supplies from the 2015-2017 “field bioassay grant”. No funds have been spent from the 2016-2018 “HPLC to follow insecticide movement grant” as UC Berkeley has not yet provide a fund number. This second grant was processed slowly as UC Berkeley and CDFA determined if changes to the “IDC” were needed.

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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