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 herein include work conducted January 2016-July 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 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. Moreover, mealybugs can remain on 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 and spring 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 mealybug as they 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. Still, the effectiveness of any systemic material will depend on application timing, soil moisture, vine condition, 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 using systemic insecticide 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). We are also working with other insecticides registered in vineyards, but this report will focus on the field application bioassays and the movement of metabolites of Movento® in the vine by 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 are addressing is whether Spirotetramat converts to the metabolite Spirotetramat-Enol (which is the primary toxicant) similarly under different vineyard conditions, 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 counts were then used to block treatments against the initial 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 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).

Fig. 2: Applying Movento®

A standardized application method was used across all vineyards so that surfactant and application rate would not be an influence. At each site, there were 15 replicates (individual vines) per treatment per vineyard, with treatments placed in a complete randomized design.

Taking into consideration all the sample areas, approximately 600 vines were sampled for mealybugs as well as cluster evaluation. Together, the treated vineyards ranged across several factors that could affect pesticide efficiency, such as the age of vineyards, irrigation type, commodity (table, raisin and wine grapes), the presence of a girdle, and geographical area.

The post-harvest sprays needed additional sampling in summer 2017 to determine treatment impact, and these data are still being processed. One problem that we encountered is that in our commercial fields the overall mealybug density was low, making treatment comparisons difficult among the sampled areas; nevertheless, spray treatment had a statistically significant effect on the numbers of individuals found in each developmental stage ($F_{(2,2)} = 5.3586$, $P = 0.004$).

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 were 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, active ingredient of Movento®, 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-Spirotetramat". 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 the most important for killing the mealybugs. The change from Spirotetramat to Enol is assumed to occur 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 Spirotetramat and its first metabolites, Enol-Spirotetramat. To analyze the quantity of Spirotetramat and Enol in leaves and other vine tissues 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, producing extracts of several structurally different substances with good efficiency.

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).

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. 3).

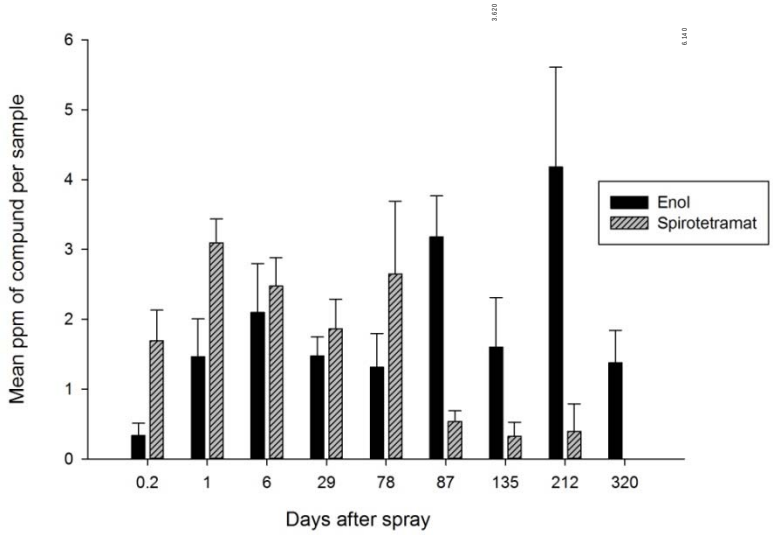

Fig. 3. Mean concentration (parts per million) of Spirotetramat and its first metabolite, Spirotetramat-Enol in leaf samples from 5 hours after spray to 10 months after treatment.

Note that most important was that some Spirotetramat was found in the leaf tissue up to 212 days after treatment and Enol up to 320 days after spray. It is still unclear (from our studies) the

conversion rate to Enol and how this process slows as we found Spirotetramat long after the application. We still find Spirotetramat unconverted 6 months after spray treatments.
When looking closer at the amount of Spirotetramat and Enol in leaf tissue over the many different sampling periods, the amount Spirotetramat shows a consistent presence but a reduction in concentration over time – although in this example vineyard the level of Enol remained relatively stable even up to 320 days. (Fig. 3). We intend now to determine 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.

In our previous report, we showed 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, 14 years old (Fig. 4.A). This result shows that the metabolites are moving with the phloem from the leaves to other vine sections. One question this raises 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.

When looking at the leaf samples from the same vines (Fig.4.B), results show that the Spirotetramat-Enol concentration at 28 days after spray is significantly higher than the concentration after 212 and 320 days after treatment, when it is no longer present a ($F_{2.97}$ =18.73, P< 0.0001). Spirotetramat concentration at 28 days after spray is significantly higher than the concentration after 212 and 320 days after treatment, when it is no longer present a ($F_{2.97}$ =11.090, P< 0.0001).

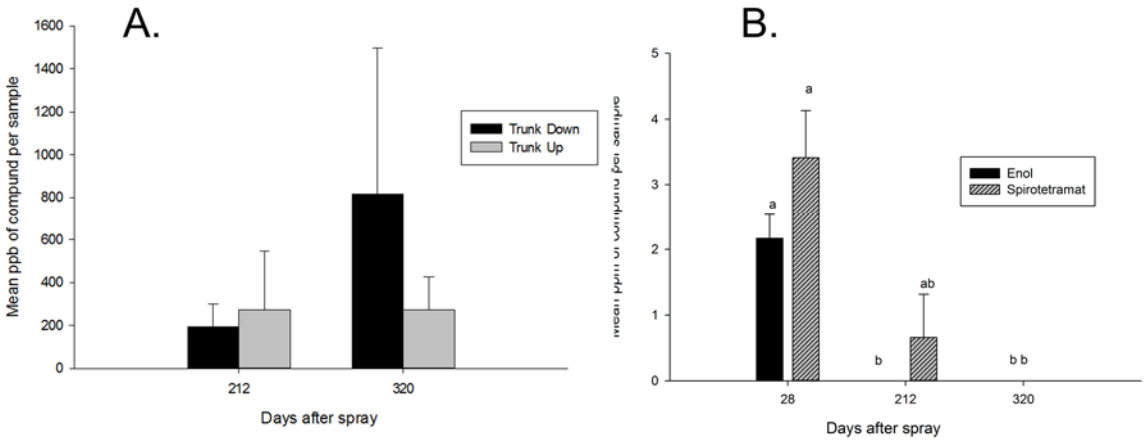

Fig. 4. A. Mean concentration (parts per billion) of Spirotetramat-Enol at the bark from samples collected 212 and 320 days after Movento® was applied to the leaves at label rate (8 oz per acre) in May 2015. Vines are Crimson cultivar, 14 years old. B. Mean concentration (parts per million) of Spirotetramat and its first metabolite, Spirotetramat-Enol in leaf samples from the same vines. No control treatment is available as the entire field got sprayed. Notice that the y-axis on Fig. A is in parts per billion (ppb) and in Fig .B is in parts per million (ppm). The letter above the columns shows significant difference within each compound.

We also analyzed the effect of leaf exposure to the pesticide during application. Mature (22 years old) Thompson seedless vines were sprayed with Movento at a rate of 12 fl. oz. Leaf samples were collected 5 hours and 1, 6 and 30 days after treatment. Our results show that the interaction of days after spray and leaf exposure has an effect of the amount of compound that is found on the leaves for both analyzed, compounds Spirotetramat and Spirotetramat -Enol ($F_{(7,57)}$= 8.0687, P<0.0001; $F_{(7,57)}$= 4.6006, P=0.0004, respectively) (Fig 5. A and Fig 5.B respectively).

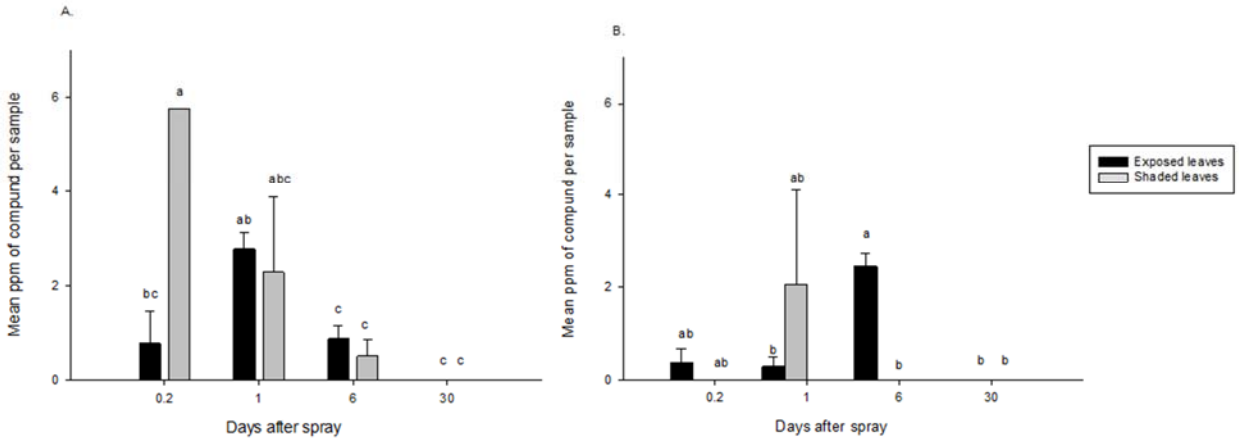

Fig. 5. Mean concentration (parts per million) of Spirotetramat (A) and its first metabolite, Enol (B) at leaves from samples collected 5 hours, 1, 6 and 30 days after Movento® was applied at label rate 12 fl. oz per acre. Vines are Thompson, 22 years old.

III. Temperature development of vine mealybug.

These data have not yet been analyzed.

PUBLICATIONS AND PRESENTATIONS

Publications:

No peer-reviewed publications to report.

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: 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.

P. Yang et al.: Vine mealybug controls; using HPLC to follow the movement of a systemic insecticide through vine to optimize application. *101th Annual Meeting, Pacific Branch of the Entomological Society of America.* Portland, OR. Apr. 2017. (Poster)

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.* Lodi, CA, April. 2017 (same talk as above, different location).

K.M. Daane: Using HPLC to follow the movement of an insecticide through the vine. *Spring 2017 Viticulture Program Team Meeting.* Parlier, CA, Apr. 2017.

V. Hochman Adler: Improving vine mealybug winter and spring controls: following insecticide movement in the vine. Viticulture Research Roadshow. San Joaquin Valley Winegrowers Association, Fresno, CA. June. 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 <30% of the collected samples.

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 212 days after application but no longer found after320 days. 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 June 2017, we have closed out the "field bioassay grant," and we have spent (or encumbered) approximately $95,000 from ongoing grant "HPLC to follow insecticide movement grant."

SUMMARY AND STATUS OF INTELLECTUAL PROPERTY ASSOCIATED WITH THE PROJECT

There is no intellectual property associated with this project.

LITERATURE CITED

Bell, V. A., R. G. E. Bonfiglioli, J. T. S. Walker, P. L. Lo, J. F. Mackay, and S. E. McGregor. 2009. Grapevine leafroll associated virus 3 persistence in *Vitis vinifera* remnant roots. J Plant Pathol 91: 527-533.

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Gutierrez, A. P., K. M. Daane, L. Ponti, V. M. Walton, and C. K. Ellis. 2008. Prospective evaluation of the biological control of vine mealybug: refuge effects and climate. J Appl Ecol 45: 524-536.

Prabhaker, N., C. Gispert, and S. J. Castle. 2012. Baseline susceptibility of *Planococcus ficus* (Hemiptera: Pseudococcidae) from California to select insecticides. J Econ Entomol 105: 1392-1400.