

IMPROVING VINE MEALYBUG WINTER AND SPRING CONTROLS (15-0427-SA)
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ABSTRACT

Field trials were conducted to test winter and spring controls for the vine mealybug, *Planococcus ficus*, which is the most important insect pest of California vineyards. There are many insecticides that can be used to kill mealybugs on the exposed leaves or reduce mealybugs infestation in the fruit clusters; however, it has been more difficult to kill mealybugs under the bark or on the roots, where they have some protection from both insecticides and natural enemies. A delayed dormant (typically in February) application of chlorpyrifos (Lorsban®) was the standard control to kill mealybugs in the spring, and applications near bloom-time of either a systemic neonicotinoid or the lipid biosynthesis inhibitor Spirotetramat (Movento®) provided control of mealybugs moving to the leaves or fruit. In 2015 and 2016, we followed the effectiveness of a May application of Movento in vineyards in Napa, San Joaquin and Fresno Counties to determine if geographic region or grape cultural practices (e.g., wine vs. table grape) impacted effectiveness, but could not separate effectiveness because all applications resulted in relatively good control. Small plot tests showed that May and post-harvest (the previous year) application provided better control than a late season (July) application. The insect growth regulator buprofezin (Applaud®) applied as a delayed dormant provided mixed results, but did lower mealybugs compared with the control. A broad-spectrum material, calcium polysulfides (Sulforix®) was tested as a delayed dormant and provided good control under laboratory settings but was not as effective as Lorsban. Our second objective was to develop temperature-based models to better predict the spring emergence of the mealybug ‘crawlers’ to better time spring foliar insecticide treatments. We summarized temperature cabinet data for vine mealybug development, reproductive potential and life table parameters. A temperature model for spring emergence has not yet been developed.

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 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. We compared the May application (8 oz per acre) of the systemic insecticide Movento (Bayer Crop Science) in vineyards in Napa, Lodi-Woodbridge and Fresno regions, and across wine, raisin and table grapes and found good control regardless of the vineyard monitored. We also tested pre- and post-harvest Movento application to control the overwintering population and found that the May and post-harvest applications provided good control. We also tested other materials to be used in the spring, but the level of control achieved was not as good as the application of Movento. In the laboratory, we followed the development time, mortality and reproductive potential of the vine mealybug at seven different temperatures. Our goal was to develop a model to help predict when the mealybug will start to move from protected areas under the bark of the trunk and out onto the leaves in order to better time insecticide applications; however, other ongoing work suggests that Movento remains in the vine for a considerable amount of time reducing the importance of an exact application time.

INTRODUCTION

Mealybugs are pests to wine, raisin and table grapes merely because of their presence in the fruit clusters. We previously investigated mealybug biological controls (Daane et al. 2004, 2008b, 2008c, Fallahzadeh et al. 2011; Gutierrez et al. 2008; Sime and Daane 2014), pheromones (Bahder et al. 2013; Figadère et al. 2007, Miller et al. 2002, 2005; Walton et al. 2004, 2006, 2013; Zou et al. 2010), and ants effects on biological controls and ant

controls (Daane et al. 2006, 2007, 2008a, Nelson and Daane 2007; Hogg et al. 2018). Results from these studies show that while mealybugs can be suppressed they cannot be eradicated from the vineyard. Moreover, low mealybug densities are needed for table grapes and for wine grapes when considering mealybugs as vectors of plant pathogens (Daane et al. 2012, Almeida et al. 2013). Of the vineyard mealybug species in California, *Planococcus ficus* (the vine mealybug), has become the most important insect pest, threatening economic production and sustainable practices (Wilson and Daane 2017, Daane et al 2018) in this multi-billion-dollar state 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).

Our project sought to improve vine mealybug control during the winter-spring period. 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 (Daane et al. 2008, Gutierrez et al. 2008) and can be the most 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 control (Daane et al. 2006), but each year infested vineyards are typically treated with a bloom-time application of a neonicotinoid and/or a May-June application of spirotetramat (Movento®) to control mealybugs moving from the roots and trunk to the leaves and fruit during the season. Still, effectiveness will depend on application timing, soil moisture, vine condition and age and commodity (for example, post-harvest application timing). Our objectives were to improve controls that target the winter-spring mealybug population and to better determine the spring emergence of vine mealybug crawlers to better time foliar applications.

OBJECTIVES

1. Investigate controls for overwintering and spring vine mealybug populations.
2. Determine the temperature relationship of vine mealybug and grape mealybug to better predict spring emergence and spray timing.

RESULTS AND DISCUSSION

Application of Movento in different regions and vineyard practices. In 2015 and 2016, we monitored the effectiveness of a May application of Movento (8 oz per acre) in vineyards located in different regions and using different management practices. Selected sites were Pinot Noir and Chardonnay wine grapes in Napa County; Cabernet Sauvignon, Pinot Noir, and Chardonnay wine grapes in San Joaquin County; and a Thompson seedless raisin, and Crimson seedless table grape and two Thompson seedless table grapes in Fresno County. The goal of this trial was to compare the efficacy of Movento applied at 8 oz per acre in May in various geographic regions and in vineyards using different cultural practices (e.g., irrigation amounts and types, different cultivars and vine ages, etc.). Additionally, we tested the impact of pre-harvest and post-harvest applications of Movento in small plots (commonly using three replicates of five vine sub-plots). The pre-harvest applications varied from late July to late August, the post-harvest applications varied from late August through September.

Results found overall mealybug density to be low and highly variable among sites, especially in Napa and Lodi-Woodbridge regions, making treatment comparisons difficult (3 of the 5 Napa and Lodi sites had no or little cluster damage at harvest, after the May application of Movento). To account for the low numbers of mealybugs, data were pooled across all sites sampled in San Joaquin and Fresno County (where there was measurable mealybug densities) and show that the May and post-harvest (the previous year) application of Movento lowered mealybug numbers more than the control or pre-harvest application ($F = 3.816$, $df = 3,4280$, $P = 0.009$; Fig. 1). A late April to early June application of Movento has been the grower standard application timing, typically with the label 8 oz per acre rate. The post-harvest application (2015) gave similar results; however, many some of the vineyard replicates were lost when cooperating growers used a May application (2016) the following year. In the 2016 April or June counts (before the 2016 application period), our 2015 pre-harvest spray treatment (the sub-plot trial) did not show any impact the following year.

Results of cluster damage were similar to those of mealybug density. Data from wine grapes in Napa Valley and Lodi Woodbridge showed no difference among treatments using mid-May, or pre-harvest Movento applications. However, mealybug densities were too low to make any strong statements. There were higher mealybug densities at some sites in the Fresno area, where we found the May application of Movento had less fruit damage compared to

untreated, mid-July (pre-harvest) and post-harvest (the previous season) spray treatments (Chi Square = 65,659, $P < 0.001$; Fig. 2). In other words, the May-June application worked well at all monitored sites. We were pleased that the post-harvest application provided control, but this was conducted in relatively small plots and all but two of the replicates were lost as some grower-collaborators applied Movento the following year. One conclusion from these trials was that we needed to use other methods to follow Movento movement in the vine, and for this reason because work with the HPLC, in the second, coordinated, study.

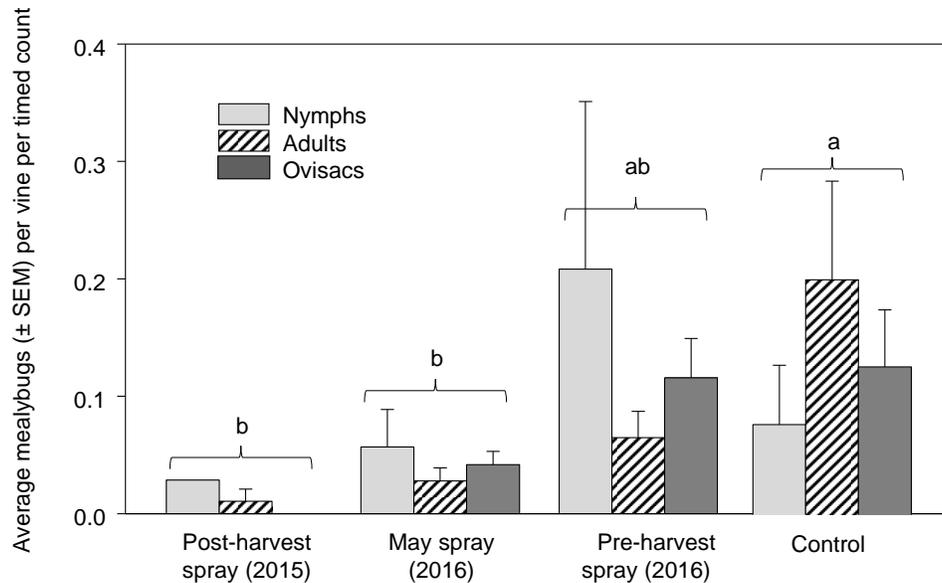


Fig. 1: Average number of nymphs, adults and ovisacs on vines treated in mid- late-May (farmer standard treatment), pre- harvest and post-harvest, and a no-spray control.

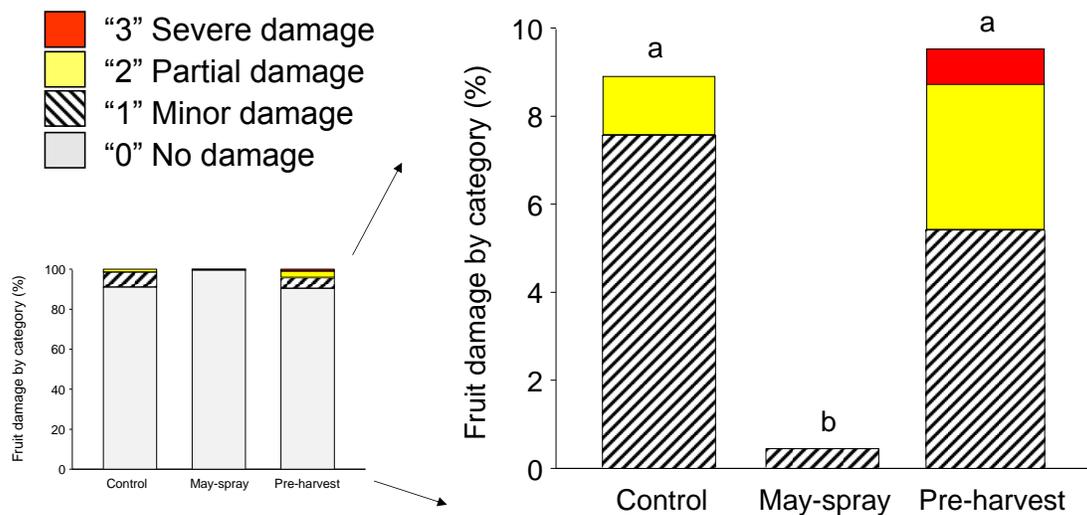


Figure 2. There was less cluster damage in vineyards receiving a May application of Movento than a July (pre-harvest) application, based on visual ratings (0 to 3) mealybug infestation in the fruit clusters.

Delayed dormant comparison. To test other insecticide materials for better winter-spring control of the vine mealybug, we used a 25-year-old raisin field (*cv.* Thompson Seedless) in Fresno County, where we had no among-vineyard variation impacting the treatments. Different delayed dormant and spring applications were applied with and without Movento (Table 1). Applaud® (buprofezin, Nichino) is an insect growth regulator that is typically applied in season against early stage mealybugs. We tested Applaud as an alternative delayed-dormant spray to Lorsban-4E (chlorpyrifos, Dow Chemical).

A standardized application method was used for each material so that surfactant and application rate would not be an influence. At each site, there were 15 replicates (individual vines) per treatment per vineyard, arranged in a complete randomized design. Note that the insecticides have different modes of action, such that we expected combinations to provide additive control (Movento is classified in the group 23, Applaud is Group 16, and Lorsban is group 1B, by the Insecticide Resistance Action Committee, IRAC).



Photo 1. Applying insecticides

Table 1. Schedule of spray treatments investigating novel insecticide combinations for a delayed dormant to spring application to control overwintering mealybugs. In all trials, Movento was applied at the full label rate (for a single application) of 8 oz per acre. The nine treatments (material, rate and application date) were:

Spray treatment	Insecticide, Application rate, and Application timing
1	
2	Applaud, 24 fl oz, 1 March 2017
3	Applaud, 12 fl oz, 22 March 2017
4	Applaud, 24 fl oz, 22 March 2017
5	Applaud, 12 fl oz, 22 March 2017 AND Movento, 4 May 2017
6	Applaud, 24 fl oz, 22 March 2017 AND Movento, 4 May 2017
7	Movento 8 fl oz, 4 May 2017
8	Lorsban 4E, 4 pts, 1 March 2017
9	Untreated control

Results from the delayed dormant spray trial comparing Applaud applied at different times (and with or without a Movento spray in May) with the standard Lorsban delayed dormant treatment significant effect on the numbers of individuals found per vine sample ($F = 6.258$; $df = 8,531$; $P < 0.001$; Fig. 3). There was no difference between Applaud applied at 12 oz as a late dormant (22 March and the control (treatments 3 vs. 8); however, Applaud applied 1 March (treatments 1 and 2) was similar to the Lorsban treatment (8). As described above, Applaud applied just 3 weeks later (22 March) was similar to the control at the 12 oz per acre rate, but lower at the off-label 24 oz rate. The three Movento treatments had the lowest counts, and the Movento treatments that included Applaud at 24 oz rate as a delayed dormant had the lowest counts (Fig. 3).

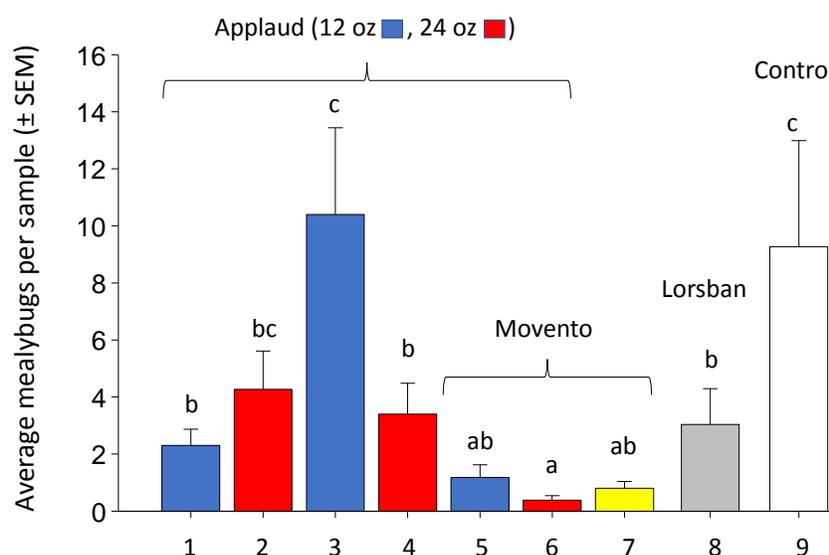


Figure 3: Average number of mealybugs per vine per 1-minute count on vines treated different with different pesticides (Table 1) at different rates and at timings (samples were taken during a timed count).

Delayed dormant comparison. We conducted a contact bioassay of Sulforix (Miller Chemical Inc.) against the vine mealybug as a possible dormant application. This material is registered for use on grapes, although the label carries a ‘danger’ signal word. The goal in this initial phase was simple to determine contact mortality of this product at 1% and 2% concentrations, with no surfactant, against the vine mealybug. To complete these laboratory bioassays, we have maintained a large colony of vine mealybug using a source population from the San Joaquin Valley (source populations from infested vineyards near Del Rey, CA, Fresno County). All mealybugs are reared on butternut squash, held at 75-82 °F.

For foliar materials, we used small ornamental pumpkins (e.g., Halloween pumpkins), which allowed us to better manipulate the mealybug numbers. The smaller pumpkins were placed inside insectary cages where the mealybugs would transfer (crawl) from the heavily infested butternut squash to the smaller pumpkins. The mealybugs were allowed 7 – 10 days to establish, and then the excess mealybugs were removed to create a squash with ~200 mealybugs of different age categories from first to third instars (no adults with ovisacs transferred). For each replicate, infested squash were randomly assigned an insecticide treatment, and at each application all insecticide treatments were tested.

Sulforix was mixed in 100 ml of water at 1% and 2% formulations. The insecticide was compared with a water spray control, and for another trial we also included Abacus (Abamectin 2%). Insecticides were applied using a hand sprayer, set at a fine mist. The pumpkins were sprayed outside, in the morning, and until wet. While drying, the squash were propped to their sides to drain off any insecticide so that it did not puddle or accumulate in cracks or depressions on the squash. After the squash were dry, they were placed on wooden platforms (to reduce rolling) inside a glasshouse. Each insecticide had 10 replicates, with each pumpkin serving as an individual replicate. Mealybug mortality was checked at 0, 1, 3, 7, 14, and 21 days after spray application until all mealybugs were either dead or had produced an ovisac. After 28 days, all ovisacs produced were recorded.

Mealybugs were randomly assigned to each treatment, and selected pumpkins had an estimated 200 mealybugs each. We always count the mealybugs again after the spray because some of the unsettled smaller stages often are washed off just by the liquid spray itself, regardless of insecticide. On the spray day (0), 3-4 hours after spray treatments, the average mealybug count was 154 ± 13 mealybugs per squash, so we met our desired initial population of 100-200 mealybugs per squash, although the range (50-450) was larger than desired. However, the 2% Sulforix treatment had 99 ± 19 mealybugs, whereas the 1% Sulforix, Abacus and Water Control had 160 ± 34 , 190 ± 21 , 167 ± 29 , respectively (Fig. 4). We suspect that there was some immediate kill in the Sulforix 2% treatment, and the normally sessile mealybugs moved off the pumpkins and died. On DAT 3, there were significantly fewer mealybugs on the Sulforix 2% treatment ($F=10.083$, $df = 3,36$, $P < 0.001$), and on DAT 7 there were significantly fewer mealybugs on both the Sulforix 1% and 2% treatments ($F=11.760$, $df = 3,36$, $P < 0.001$) (Fig. 4). This pattern held stable through DAT 21, although there was eventually a drop in the Abacus treatment as well, although no separation from the control. We note that Abacus was not really an appropriate test material, it was being used for another trial.

On DAT 28 and 35, we made counts of ovisacs because some insecticides will not kill the mealybug but will reduce their fecundity (they will not produce an ovisac). Results show a great reduction in Sulforix 2% as compared with all other treatments ($F=5.049$, $df = 3,36$, $P = 0.005$) (Fig. 5).

Sulforix at 1% and 2% significantly reduced mealybug density. Mortality was concentrated in the first seven days after treatment. Additionally, Sulforix 2% most likely had an immediate kill (within the first 3 hours) that were not captured by our data collection methods. There was a reduction in ovisacs in the Sulforix 2% treatment, but surprisingly there was not a significant reduction in Sulforix 1%, although the counts of ovisacs may have been earlier than development had allowed all the treated mealybugs to develop to this last stage. What is needed now is to test Sulforix in the field with vine mealybug naturally underneath the bark. In that field test Sulforix should be compared with the grower standard – chlorpyrifos – as a delayed dormant spray.



Photo 2. Insectary production and inoculation of small pumpkins with vine mealybug.



Photo 3. Spray application the day of treatment, pumpkins were later transferred to a glasshouse for long term counts.

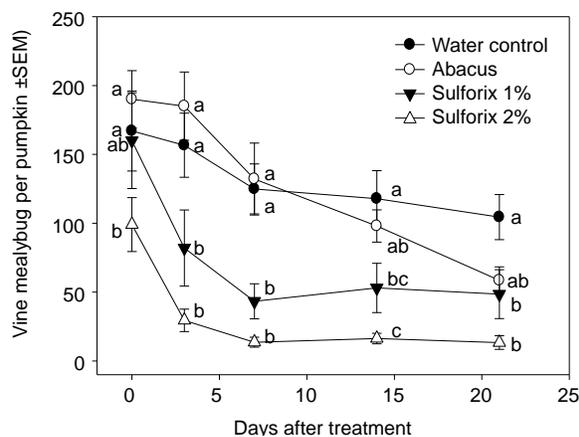


Figure 4. Mealybugs per pumpkin at different days after treatment application. For each sample date, means with different letters are significantly different.

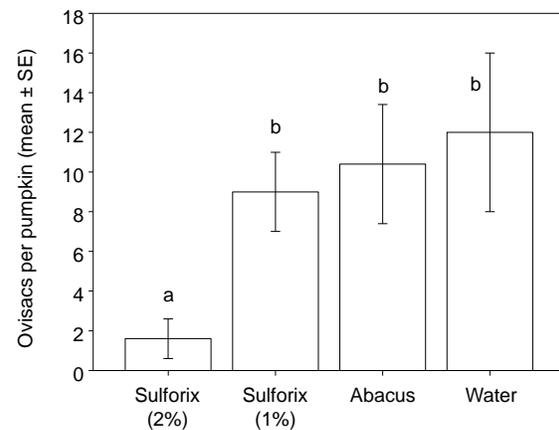


Figure 5. Mealybug ovisacs (egg sacs) per pumpkin averaged at 28 and 35 days after treatment application, means with different letters are significantly different.

Temperature development of vine mealybug. The effect of constant rearing temperatures on *P. ficus* development time was determined at 12, 16.5, 19, 23, 26, 30 and 34°C. Temperatures were at $\pm 1.5^\circ\text{C}$, as recorded by HOBO data recorders (Onset, Bourne, MA) placed in each cabinet. To begin each trial, 7–10 adult *P. ficus* females, which were beginning to produce ovisacs, were placed on each vine, which was then held at 25°C for a 24 h inoculation period. After which, the vines were checked for freshly deposited eggs, still in the ovisac, and the adults and excess eggs were removed. Inoculated plants were then randomly assigned to temperature treatments. Thereafter, plants were checked every 1–2 d for mealybug development and survival. After 2 wk., this period was extended to 3–6 d, depending on the development rate at each temperature. Mealybug density was recorded by the following developmental stages: egg, first instar, second instar, third instar female (pre-oviposition), third instar male (prepupa), adult female (producing an ovisac), male pupae, ovisac with eggs, and adult male (male pupa with an emergence hole).

Towards the end of each generation, adult females were individually numbered for future identification (after the ovisac deposition begins, there is very little movement of adult females) and to record eggs per individual females. For each ovisac, deposited eggs were collected on each observation date and placed in a gelatin capsule, which was then returned to the respective temperature treatment for 30 d or until egg hatch was complete. After this period, egg production and the proportion of hatched eggs were recorded for each female.

Temperature development. Vine mealybug, *Planococcus ficus*, completed developed from egg to adult (with ovisac) at temperatures from 16.5–30.0°C, but failed to complete development at the lowest (12°C) or highest (34°C) temperatures tested (Fig. 6). The estimated development times from egg to adult (based on the production

of adults with ovisacs) were fit to the Brière et al. (1999) temperature development rate model, which provided lower, optimal and upper temperature thresholds and is described as:

$$r(T) = aT(T - T_o)(T_L - T)^{1/b}$$

where T is the rearing temperature ($^{\circ}\text{C}$), T_o is the lower temperature threshold, T_L is the lethal (upper) temperature threshold, and a and b are empirical constants. The optimum temperature (T_{opt}) is calculated as:

$$T_{opt} = \frac{2bT_L + (b+1)T_o + \sqrt{4b^2T_L^2 + (b+1)^2T_o^2 - 4b^2T_oT_L}}{4b+2}$$

where T_L , T_o , a , and b , obtained from equation (1).

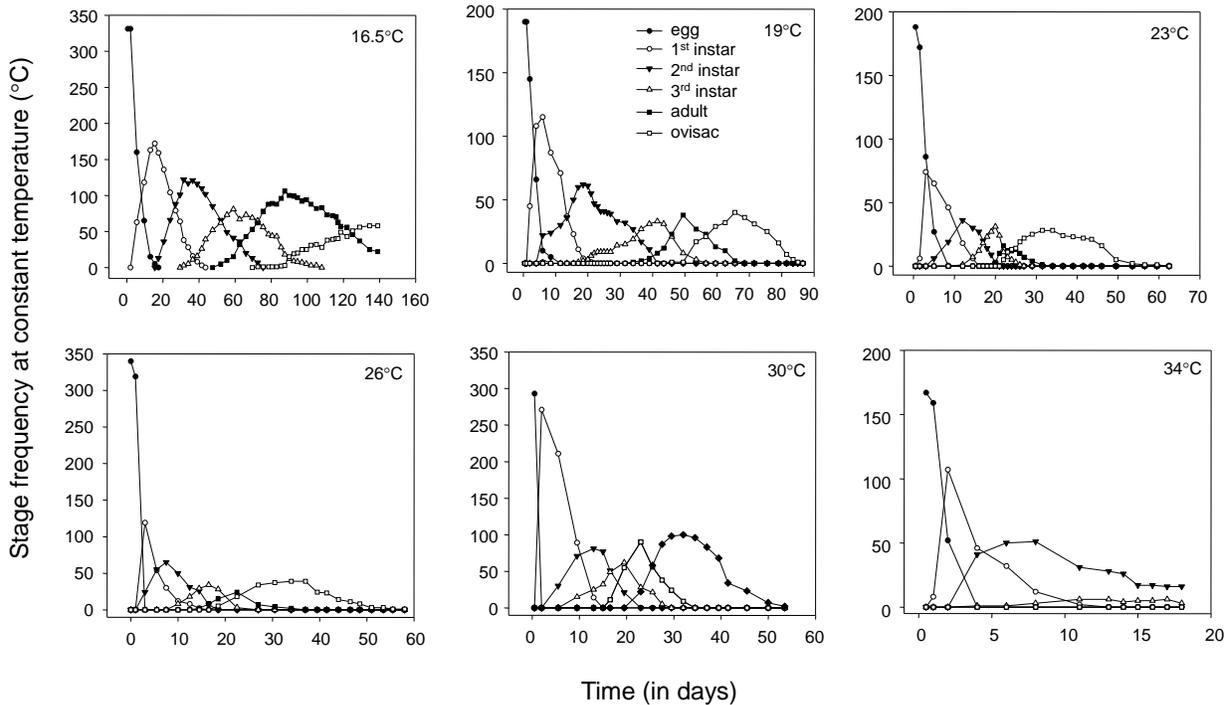


Figure 6. Development for each life stage of *Planococcus ficus* at six constant temperatures.

The low threshold temperature was also determined using simple linear regression ($r(T) = \alpha T + \beta$) with data from temperature treatments 16.5 – 23 $^{\circ}\text{C}$, which most closely resembles a straight line. The development rate is a linear function of temperature, and α and β are regression parameters fitted to the data. The low development threshold is calculated as $T_L = -\alpha/\beta$, and the thermal constant (k) from birth to adult, in required degree-days (DD), is calculated as $k = 1/\beta$ (Liu and Meng 1999).

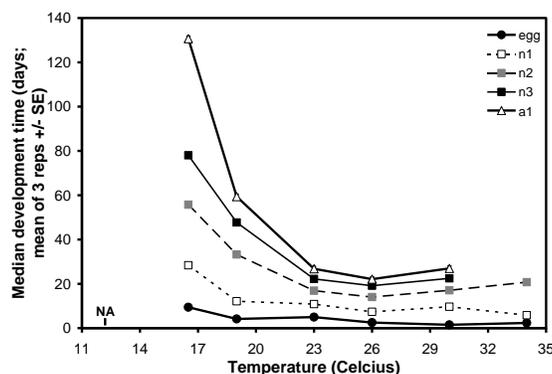


Figure 7. *Planococcus ficus* stage development times at different temperatures. Development time defined as the number of days required for 50% of the population to move beyond a given stage). N1, N2, N3, and A1 refer to first, second, third instar nymphs and pre-reproductive adults, respectively. Most error bars are obscured by symbols. Estimates were not possible for some stages at some temperatures (12, 30, 34 $^{\circ}\text{C}$).

Results show development times decreased as temperatures increased (Fig. 7), ranging from about 140 days (at 16.5°C) to about 25 days (at 30°C). The estimated lower and upper temperature thresholds for were 14.55 and 35.41°C, respectively, while the optimum developmental temperature was 26.93°C. Using linear regression with mid-range temperatures (19–30°C) a lower temperature threshold of 14.6°C was estimated ($y=0.00362x - 0.053$; $F_{1,2} = 156.84$; $P < 0.0507$; $R^2 = 0.987$). The thermal constant is 276.31 degree-days.

Reproductive parameters. The net reproduction rate (R_o) was greater than zero at all temperatures that permitted complete development, indicating positive population growth (Table 2). The maximum R_o (433.34) for was obtained from data collected at 26°C. The lowest estimated R_o (82.61) occurred at 16.5°C. The female: male ratio of offspring, which impacts R_o also varied among temperatures, ranging from 10.25: 1 at 19°C to 5.10: 1 at 16.5°C.

Mean generation times (T) values estimated for each of the trialed temperatures decreased with increasing temperatures with a gradual decrease in mean generation time as temperatures increased between 16 and 30°C (Table 2). The shortest generation time (T) was also recorded at this temperature. This decrease was more pronounced between 16, 19 and 23°C and reached a plateau between 23 and 30°C. The largest T -value was recorded at 16.5°C. These values decreased to 32.19 at 26°C after which there was a slight increase.

Intrinsic rate of natural increase (r_m) values were positive at temperatures ranging from 16.5 to 30°C, indicating positive population growth. The lowest estimated r_m value was 0.037 at 16.5°C; the highest was 0.26 at 26°C. At 30°C the r_m values dropped to 0.195. The fitted model was $y = (0.000000161) \times x(x-(34.04632)) \times ((15.8684)-x) \times \exp(1/0.151912)$ ($F_{1,4}=41.76$; $P = 0.11$; $R^2 = 0.9864$). Using these r_m values, the lower, upper, and optimal temperatures for population increase are estimated at 15.87, 34.05 and 26.47°C, respectively.

Table 2. Female fecundity, life table parameters and gender ratio of *Planococcus ficus* on Thompson Seedless grapevines at five temperatures (± 0.5 °C).

Temp (°C)	Eggs / female	Egg viability (%)	R_o	T	r_m	Female/Male ratio
12	-	-	-	-	-	-
16.5	155.25 \pm 0.01	98.33 \pm 0.37	82.61	130.62	0.037	5.1
19	176.19 \pm 0.04	87.78 \pm 1.67	200.81	66.88	0.082	10.25
23	210.44 \pm 0.10	96.81 \pm 0.93	316.86	32.33	0.21	9
26	362.41 \pm 0.08	88.94 \pm 1.00	433.34	32.19	0.26	8
30	201.89 \pm 0.02	52.61 \pm 1.34	274.41	33.18	0.195	6.545
34	-	-	-	-	-	-

Fecundity and egg viability. Across all temperatures at which ovisacs were produced (16.5–30°C), average life time egg production was 220.8 \pm 15.5 eggs per female. Temperature influenced egg production, which ranged from a maximum of 364.4 \pm 0.8 eggs per female at 26°C to a minimum of 155.25 \pm 0.1 eggs per female at 16.5°C. There was a decrease in egg production at lower and higher temperatures, indicated by a good fit ($R^2 = 0.94$) to the Briere et al. (1999) model modified for fecundity. The lower, upper, and optimal temperatures for egg laying were determined at 11.59, 34.08, and 25.22°C, respectively. Egg viability was highest at 16.5°C, similar between 19–26°C, and significantly lower at 30°C ($F_{4,2185} = 383.49$, $P < 0.0001$).

We have worked with two entomologists that are very qualified to model data (Dr. Mark Sisterson and Dr. Mathew Daugherty). One aspect of this study that failed was our inability to tract the development time of individual mealybugs. With our design, we expected more uniform development times for each life stage at each of the tested temperatures. We suspect that feeding on different parts of the vine may have added to mixed development times. The end result is that we used the “average” development based on peak population densities. This produced an informative figure showing life stage development and mortality; however, without being able to produce standard errors around each mean, we cannot complete a statistically accurate development model. For this reason, we have begun a simpler temperature development trial, counting only development from egg to ovisac. This will be combined with the presented data to develop a predictive model for spring emergence.

CONCLUSIONS

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. The most common control tool is an application of Movento around May, which provides control of mealybugs on the leaves. We investigated regional (Napa, Lodi-Woodbridge, and Fresno) and commodity (wine, raisin and table grapes) difference in this application but found in excellent in season control and no difference among regions. We compared pre-harvest and post-harvest applications of Movento to determine if we could concentrate this systemic material in the spring and found that the May application was the best period, although post-harvest (the previous year) application provided control as well. We tested other materials (buprofezin and calcium polysulfides) and found these materials provide some control. We also looked at vine mealybug temperature development and life stage parameters and sought to incorporate these data into a model to predict spring emergence. This model is still in development.

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