Dispersal Behavior of Vine Mealybug (*Planococcus ficus*) in California Grapevines

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ABSTRACT

The vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae), has been a pest of the California grape vineyards since the early 1990s. Thus, many studies have contributed to understanding its life cycle, damage, chemical and biological controls, and its distribution within the vineyards. However, the dispersal rates and movement have not been studied. *Pl. ficus* can be detrimental to the vineyard because of many reasons including transmitting viruses within vineyards, lowering crop quality by releasing honeydew on parts of the vine, and decreasing the grape's aesthetic value. There are many control and monitoring methods to prevent the mealybug from damaging the vineyards, but insecticides and pheromone traps appear to be more efficient than counting and mating disruption methods. However, due to the complexity of *Pl.ficus*, effective control of the infestations has yet to be completely successful. Many studies have discussed the negative effects of various methods in the vineyard such as time- consuming labor for a counting method, killing natural enemies with insecticide sprays, and lack of adequate food resources for natural enemies in biological control methods. Although there are numerous amounts of management methods, this literature review shows that there is room for improvement. In particular, *Pl. ficus* dispersal behavior within the vineyard would increase efficiency by providing a specified target range for the grower.

KEYWORDS

literature review, insecticides, pheromone traps, mating disruption, biological control

INTRODUCTION

Since the concept of integrated pest management was accepted in the late 1960s and 1970s, growers aimed to control pests rather than completely eradicate them (Smith and Bosch 1967). Rather than spraying the whole field with pesticides, this management method requires knowledge of not only the vineyard plants but about the pests as well. This is because understanding the pest's life cycle, predators, behavior, and plant preferences can provide an efficient management method and reduce the amount of pesticide used on the vineyard. The vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae), has grown to be a pest of wine and table grapes in California since its introduction to the Coachella Valley in the early 1990s (Gill 1994). Although there is currently an abundant amount of information on the distribution, damage, and chemical/biological controls of the mealybug in vineyards (Daane et al. 2004), the dispersal rates and movement has not been studied in detail. This literature review will serve to analyze the various studies done on the vine mealybug and its relatives to determine what type of behavior study would be appropriate for the pest and predict possible findings.

The vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae), is a phloem feeder that uses its mouthpart to suck out plant fluids (McKenzie 1967). *Pl. ficus*, along with other vineyard mealybug species, can also lower crop quality by releasing carbohydrate-rich honeydew on the parts of the vine which becomes a substrate for sooty mold (Flaherty et al. 1992). Although most of the vineyard mealybug species have the power to damage the grapes, *Pl. ficus* is unique because of its high reproductive rate of >250 eggs per female (Walton 2003) and a faster developmental time than the other species. This means that the *Pl. ficus* infestations are greater in number and damage to the vineyard. For table grape growers, the presence of the mealybugs, honeydew, and molds decrease cosmetics of the grape cluster and therefore reduce its market value (Daane et al. 2011). In addition, the vine mealybug has a wider host range than the other mealybug species including avocado trees (*Persea Americana* Mill.), mango trees (*Mangifera indica* Blume), walnut trees (*Juglans spp.),* and common weeds (Cox 1989; Ezzat and McConnell 1956; Ben-Dov 1994). This polyphagous nature makes it even more difficult to control because there are a variety of hosts it can thrive in.

The control of *Pl. ficus* became more crucial with the discovery that they can transmit viruses within vineyards (Engelbrecht and Kasdorf 1984; Cabeleiro and Segura 1997).

Specifically, *Pl. ficus* can vector viral diseases of grapevines like grapevine leafroll disease (GLD) which impacts the berry by delaying bud-break, flowering, and berry maturation (Martelli et al. 2002). Research has focused on the grapevine leafroll associated virus-3 (GLRaV-3) because it is the predominant virus species in relation to the GLD spread (Tsai et al. 2010). *Pl. ficus* is capable of transmitting GLRaV-3 in all of its life stages, but the crawler and first instar stages have found to be highest in efficiency (Petersen and Charles 1997; Tsai et al. 2008). The diseased vine plant shows changes in color, reduced sugar content, increased acidity in fruit juice (Charles et al. 2006) and thus *Pl. ficus* can be detrimental to the vine grower even in small populations.

In addition to the grapevine leafroll disease, a newly discovered disease called grapevine red blotch disease has raised concerns not only about the grapevines but about the possible insect vectors. This new disease is caused by grapevine red blotch associated virus (GRBaV) which is a DNA virus similar to the grapevine leafroll associated virus (Rwahnih et al. 2013). Grapevine red blotch disease causes reduction of sugar accumulation in grape harvest (Poojari et al. 2013) which affects the quality of the wine and again economically damaging to the grower. Since *Pl. ficus* is known to be a vector of the grapevine leafroll associated virus (Golino et al. 2002), there is a possibility it could also be a vector of the grapevine red blotch associated virus because it is found in the grapevines. Researchers have not studied mealybugs as a vector for the grapevine red blotch disease because they are still trying to understand the disease. Thus, it is important to understand *Pl. ficus* dispersal rate and behavior because of their current impact on the vineyard and possible future damages as vectors of viruses.

Currently, there are various methods to control and monitor *Pl. ficus* including pheromone traps, aerial and walking studies, insecticides, mating disruption, and biological control. It has been found that there is a variation in the *Pl. ficus* seasonal feeding location and movement on the vine that depend on the regional temperatures and management practices (Daane et al. 2012). The most effective type of monitoring method so far for *Pl. ficus* appears to be the pheromone based monitoring system because the vine mealybugs are hard to find by searching for them on the vines (Millar et al. 2002). In regards to control, mating disruption and insecticides are common for *Pl. ficus* (Walton et al. 2006; Daane et al. 2006) but insecticide sprays have shown higher success rates. However, due to the complexity of the insect, effective control of the infestations has yet to be completely successful. In addition, studies on the *Pl. ficus* dispersal rate and behavior have yet to be conducted and thus can only be inferred from behavior studies of related species. There have

only been visual counting studies on *Pl. ficus* (Daane et al. 2006; Walton et al. 2004; Franco et al. 2009). However, aerial and walking studies have been conducted on its relatives, *Planococcus citri* and *Pseduococcus maritimus* (Cid et al. 2010; Grasswitz and James 2008). Their studies results are helpful in predicting *Pl. ficus* movement because *Pl. citri* is in the same genus *Planococcus* and *P. maritimus* is in the same order and family, Homoptera: Pseudococcidae. Both *Pl. citri* and *P. maritimus* are also vectors of GLRaV-3 and have the same life cycle as *Pl. ficus* (Daane et al. 2012). As a result, the studies on *Pl. citri* and *P. maritimus* were used in my review to help understand the possible dispersal behavior of *Pl. ficus.*

This literature review will thus analyze the different types of methods in monitoring and controlling the vine mealybug species. If a monitoring and controlling method study was not done on *Pl. ficus*, I used studies based on similar species relatives of *Pl. ficus*. This review aims to find effective methods of monitoring and controlling the vineyard mealybug. The results will serve as a foundation for future studies on *Pl. ficus* dispersal rates and behavior. In addition, a small dataset on a greenhouse study of *Pl. ficus* dispersal rates and behavior will be included in the end to provide preliminary results for a long-term study.

Life Cycle and Reproduction

The vine mealybug is sexually dimorphic, meaning that it has physical differences between the male and female (Holm 2008). The developmental stages of egg, first instar, second instar, and third instar are the same in both sexes (Walton and Pringle 2004). These instar stages are molts that resemble the previous stage with an increased size and amount of wax secretion (Daane et al. 2012). Once the third instar stage is reached the male vine mealybug has a prepupa stage followed by the pupa, which is where the winged male emerges (Kriegler 1954). The males are about 1 mm in body length and do not have any mouth parts. The female mealybug reaches the adult stage after the third instar stage and does not have wings; it looks the same as the third instar stage but is clearly segmented with more wax secretion and size of approximately 4 mm long, 1.5 mm thick, and 2 mm long (Holm 2008). Once mature, the female *Pl. ficus* releases pheromones to attract adult males for reproduction (Hinkens et al. 2001). When the male and female copulation is complete, the female lays up to 360 eggs in an egg sac (ovisac) that has filamentous waxy hairs (Franco et al. 2009). Lower and upper temperature threshold for their development has been

observed at 16.59ºC and 35.61ºC, respectively (Walton 2003). Due to their small size and temperature dependent development, it is difficult to visually monitor their population and oftentimes the vine grower discovers the infestation only after *Pl. ficus* is mature.

Monitoring *Planococcus ficus*

Counting

Visual monitoring and counting vineyard mealybugs is a time-consuming process that requires a large number of samples. However, small-scale samplings have been conducted to help growers predict damages on their vineyards and measure disease.

In 2001, Kent Daane and Chris Geiger sampled six commercial table grape vineyards; three in central San Joaquin Valley and three in Kern County. Each vineyard block was >10 years in age and had grape mealybug, *Pseudococcus maritimus*, infestations. The experimental design and analysis included cane-pruning and spur pruning systems because the two were the predominant pruning systems used in table-grapes. They did absolute sampling and within-vine mealybug distribution, where they sampled six vines (one from each site) each month from March to October 1998 and bimonthly until February 1999. Mealybugs were first counted in position on each sample date then the bark, spurs, canes, leaves, and bunches were taken into the laboratory to be examined for mealybugs, old mealybug ovisacs, and natural enemies. In addition, they performed five relative sampling techniques to test concurrently with absolute samples on the same vines. These sampling techniques were: 5 minute counts, excised spur counts, nondestructive spur/cane counts, sticky tape counts, and counts on standard-sized samples of bark from the trunk (Geiger and Daane 2001).

Sampling methods revealed that there is a seasonal movement of mealybugs in the spring vertically up the vines (Daane et al. 2003). Toward the summer, the mealybugs are found in more exposed locations because of the new canes and leaves (Geiger et al. 2001; Walton 2003; Malakar-Kuenen et al. 2001). Thus, temperature appears to be the biggest influence on mealybug development and distribution (Daane et al. 2012). In the Coachella Valley and San Joaquin Valley sampling study in 2001, *Pl. ficus* density in the Coachella Valley vineyards rapidly increased to an early peak in April with a rapid decline in late April to June (Figures 1 and 2). In the San Joaquin

Valley vineyards, density continued to increase until July and August and reduced in August and September (Figures 3 and 4). Instead of looking at just the seasonal density, they also looked at the generations per year in both locations. It was then they concluded that temperature had an impact because they believed the vine mealybug could have upper temperature thresholds that slow development and/or increase mortality in Coachella Valley populations. There were five to six generations per year in the San Joaquin Valley while the Coachella Valley saw four to five generations per year (Daane et al. 2003). This shows that the temperature plays a role in *Pl. ficus* development and can affect the visual sampling methods because it will vary on the weather.

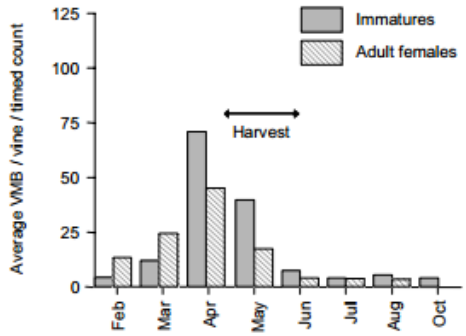


Figure 1. Seasonal abundance of immature (excluding crawlers) and adult vine mealybug in Coachella Valley. Data combined from 3 vineyard blocks sampled in Coachella and Thermal, California, 2001 (Daane et al. 2003).

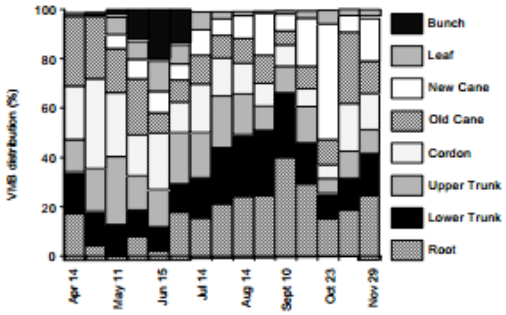


Figure 2. Vine mealybug population distribution (%) on the vine in Coachella Valley. Data combined from three vineyard blocks sampled in Coachella and Thermal, California, 2001 (Daane et al. 2003).

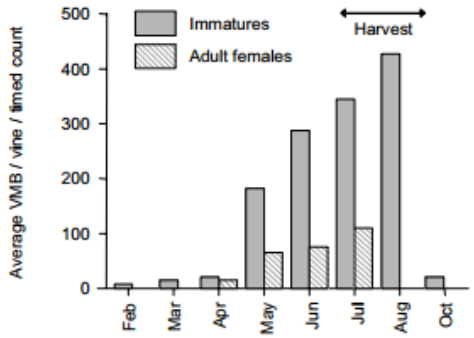


Figure 3. Seasonal abundance of immature (excluding crawlers) and adult vine mealybugs in the San Joaquin Valley. Data from raisin vineyard near Del Rey, California, 2001 (Daane et al. 2003).

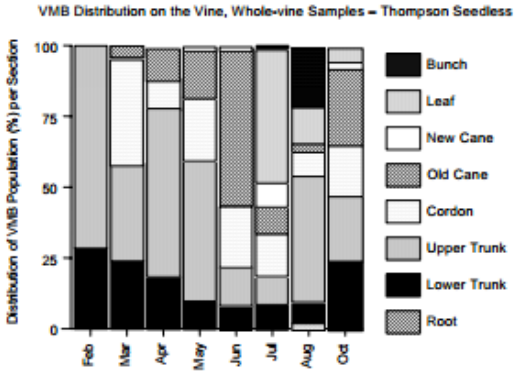


Figure 4. Vine mealybug population distribution (%) on the vine in San Joaquin Valley. Data from raisin vineyard near Del Rey, California, 2001 (Daane et al. 2003).

Aerial and Walking with Traps

Vineyard mealybug aerial and walking studies have been crucial in better understanding their movement because of two reasons: the use of traps and the fact that male mealybugs have wings. Although each vineyard species has different biological characteristics, host plant preferences, and economically damage the vineyard, the all of the vineyard mealybug males have wings and the females do not (Daane et al. 2012). It is reported that adult males and crawlers, which are newly emerged first-instar nymphs, in the majority of vineyard mealybugs are more active in dispersal than other stages and adult females (Franco et al. 2009). Thus, traps are helpful in capturing the male vineyard mealybugs and help in assessing their movement.

Aerial and walking studies can be done in both the field and/or in the laboratory/shade house to document the movements. In the field studies, the movements are observed for multiple

seasons (Grasswitz and James 2008; Cid et al. 2010) to obtain counts on all stages. Cid et al. performed a field study in Spain to determine the movement of the citrus mealybug, *Planococcus citri*, in a vineyard confirmed with GLRaV-3 and mealybug infestation. They sampled over three seasons with adhesive traps placed on three height levels of five plants. The mealybugs that were trapped on the tapes were counted in their lab and the movement at each level was calculated as average per plant of diary net movement (number of mealybugs trapped in lower trap minus number of mealybugs in upper trap divided by time expressed in days).

Similarly, traps were used in a dispersal study of grape mealybugs performed in the field and the shade house (Grasswitz and James 2008). They used cardboard sticky traps stapled to wooden stakes in the field to trap the airborne mealybugs. To observe any dispersal by walking, they performed both field and shade-house studies where pairs of vines shoots were connected and designated as 'donor' and 'recipient' of the grape mealybug in the field. At first they used egg mealybug masses to perform the walking study but soon chose to use newly hatched first-instar mealybugs when the egg masses failed to hatch. In the shade-house, circular arrays were set up to allow the vines to grow next to each other and newly hatched first-instar mealybugs were transferred to the leaves at the base of the plants. The number and location of the mealybugs were recorded for a month.

Despite female mealybugs' limited power of dispersal, they can still be found in most of the wine producing regions around the world. The vine mealybug distribution has doubled over the past 13 years (Ben-Dov 1994). Long distance dispersal has been the result of unsanitary pruning and harvesting practices, but shorter distances can be covered when strong winds carry the young instars (Holm 2008). It was found in the field and shade house studies (Figure 5) that the grape mealybugs showed little tendency to disperse away from the original point of infestations (Grasswitz and James 2008). They also observed that in the first generation, the mealybugs did not show any differences in the distance moved or time taken to settle on new versus old shoots (Figure 6). Lastly, the aerial dispersal results showed that there was a highly significant difference between numbers caught on the traps close to the vine as opposed to those on traps farther away. Although this study was conducted on grape mealybugs, they are similar to the vine mealybug in that the males have wings and the females stay remote after laying eggs (Daane et al. 2012). They also have the same life cycle, with 3-4 stages of instars after emerging out of the ovisacs. This study suggests that *Pl. ficus* dispersal could be short distances as well.

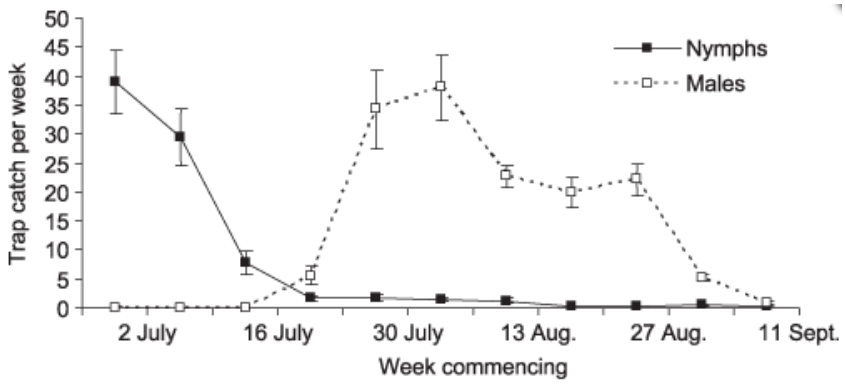


Figure 5. Aerial dispersal (mean number ± SE) of grape mealybug nymphs and adult males at site 2, second generation, 2007.

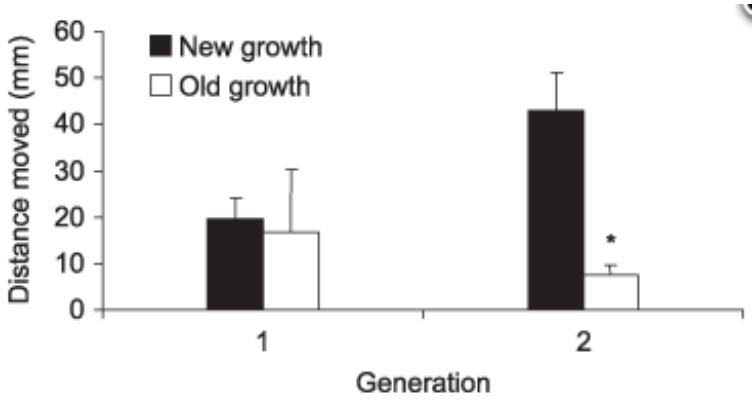


Figure 6. Distance (mean ± SE) moved by first-instar grape mealybugs on old vs. new grape shoots. *indicates significant difference within a generation (Mann-Whitney test: P<0.05).

Pheromone Traps

Due to the inefficiency of counting methods, pheromone traps are now popular in monitoring vineyard mealybugs (Millar et al. 2005). Lavandulyl senecioate has been identified as the sex pheromone of vine mealybug *Planococcus ficus* (Millar et al. 2002) and can be used in rubber septum lures. This pheromone can be used to monitor male mealybug flight activity in vineyards which helps in determining the infestation density. A pheromone-baited trap study was conducted in nine South African vineyards to compare the density from the traps with density that was recorded by visual monitoring methods (Walton et al. 2004). They loaded rubber setpa with

100 ug dose of racemic lavandulyl senecioate and placed them in the vineyards at distances ranging from 0 to 200m.

In California, field trials with the same sex pheromone, lavandulyl senecioate, were conducted in vineyards that had vine mealybug infestations (Millar et al. 2005). They also used rubber septa but loaded them with hexane solutions of pheromone components as well as butylated hydroxytoluene stabilizer in 1% of the pheromone dose. The experiment tested effects of the different blends of racemic lavandulol with racemic lavandulyl senecioate, different doses of only lavandulyl sencioate, field longevity of the lures, and compared the racemic pheromone with the chiral pheromone. Lastly, they compared the pheromone trap catches with mealybug densities in three vineyards near Del Rey, which is in Fresno County, California.

In contrast to physical monitoring methods, pheromone traps are less time consuming and more efficient because they are more sensitive than physical sampling (Millar et al., 2002; Walton et al., 2003). The pheromone-baited traps were attractive to male *Pl. ficus* for about 12 weeks and had a range of 50m in a 2001 study by Hinkens et al. 2001 in California. In addition, the number of *Pl.ficus* males in the traps was positively correlated to the female mealybug infestation which was determined by using the physical sampling methods (Walton et al. 2004). Thus, population estimates can be made by the number of male *Pl. ficus* captured and since it is more convenient, it is used more often than physical monitoring methods. Figure 7 shows that stem infestation was a significantly positive function of trap counts when comparing seasonal averages of the regions (Walton et al. 2004).

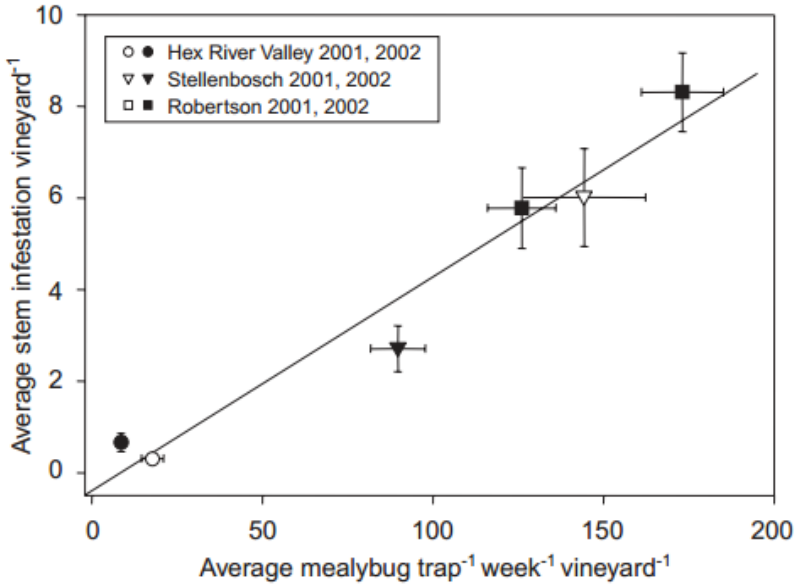


Figure 7. Positive and significant relationship of seasonal average (±SEM) percent stem infestation to seasonal average (±SEM) *P. ficus* adult males caught in pheromone-baited traps, for each region and growing seasons ($y = -0.391 + 0.047x$, $df = 1, 5$, $F = 90.97$, $P < 0.001$, $r^2 = 0.947$)

Controlling *Planococcus ficus*

Mating Disruption

Once the sex pheromone was identified for *Pl. ficus*, mating disruption was possible but this method is not popular because other mealybug pheromones are not readily synthesized. A study was done to test the mating disruption program in California vineyards for *Pl. ficus* with a sprayable, microencapsulated formulation of racemic sex pheromone lavandulyl snecioate (Walton et al. 2006). The formulation was mixed with water and applied to the vines with an airblast spray two times in May, once on June, and between August 2nd and 4th of 2003. In 2004 they sprayed four times, once each month in April, May, June, and July. They then determined mealybug densities by visual count and also looked at the pheromone's effect on mealybug egg productions.

Mating disruption is useful because the vine mealybug females do not migrate from one area to another as easily as their male counterparts (Franco et al. 2009). However, many studies have not been conducted on the vine mealybug with the sex pheromone because the complex structure of the pheromones restricts large scale synthesis. The Walton et al. study in 2006 with

pheromone application for mating disruption experiment showed that grapevine crop damage was reduced from 9-11% in control plots to 3-4% in treated plots. However, efficiency of the pheromone formulation in the field was reduced after 3 weeks and thus they had to apply more than four applications per season. Figure 8 shows their results of the 2003 and 2004 sprays, where there were more first instars but fewer ovisacs in the mating disruption treatment than control in 2003, and fewer first stars and ovisacs in the mating disruption treatment in 2004.

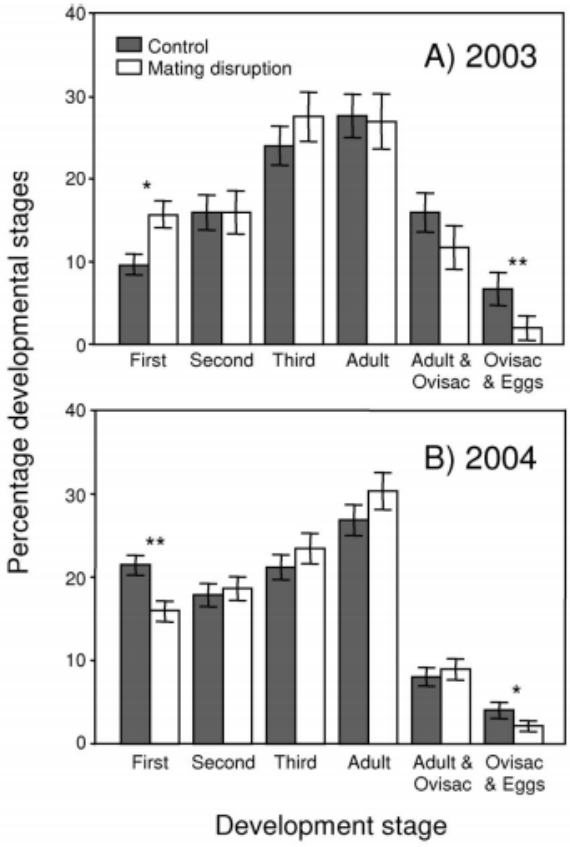


Figure 8. Proportion of *P. ficus* development stages from timed counts of field populations taken from May through August collections in (A) 2003 and (B) 2004 showing non-significant treatment for percentages of first instars in 2003 (t=1.81, df=174, P =0.071) but significant differences in 2004 (t=2.01, df= 468, P=0.045), and significant differences in ovisacs produced in 2003 (t=2.37, df=174, P=0.018) but not 2004 (t=1.67, df=468, P=0.095).

Biological Control

Biological control of vineyard mealybugs is a common practice but it has been found that lack of adequate food resources for natural enemies within the agro-ecosystems could restrict the

control agents (Franco et al. 2009). Although there are many natural enemies of vineyard mealybugs, *Anagyrus pseudococci* is the most commonly used parasitoid from vine mealybug in California (Malakar-Kuenen et al. 2001). It should also be noted that ants have a mutualistic association with vineyard mealybugs, including *Pl. ficus*, and thus have been seen to disrupt biological control (Daane et al. 2007; Mgocheki and Addison 2009). This disruption is mainly due to the ants' attraction to the honeydew produced by the mealybug, and thus interrupting the parasitoid activity of *Anagyrus* species on vine mealybugs (Mgocheki and Addision 2010). In addition, it was found that exclusion of ants from the vine canopy allowed the parasitoids better access to the vine mealybugs that were feeding on exposed areas of the vine, like leaves (Daane et al. 2007). A study was done to observe any influence of temperature on *A. pseudococci* development because it would improve biological control of *Pl. ficus* in California (Daane et al. 2004). In this study, they experimented with egg, larval, and pupal development of *A. pseudococci* in different temperatures and compared them to *A. pseudococci* populations in the field. They found that late spring *A. pseudococci* emergence was correlated to the vine mealybug movement from the bark region of the vines, to the exposed locations like the leaves (Malakar-Kuenen et al., 2001). In addition, there was a sharp increase in parasitism soon after in early July, of about 25% in 2001 and 60% in 2002.

To obtain the highest success in biological control, the introduction of the parasitoid population in the spring has been suggested (Mendel et al. 1999) because that is when the mealybugs leave their refuges and colonize on new areas of the host plant. It has also been found that the parasitoids look for mealybugs in the range of the different pheromone release points (Franco et al. 2009) so the intensity of the parasitization in the treated plots could be greater. It was also found that the season-long vine mealybug density was significantly lower in the *Anagyrus* species release than in the control treatment (Figure 9) in a 2006 study (Daane et. al 2006). However, they could not conclude that the released *Anagyrus* were the only factors in reducing the population because there was no season-long difference in percentage parasitism and there were inconsistent density counts on different times of the season regardless of the *Anagyrus* presence. With that in mind, it still shows enough evidence to use the *Anagyrus* for biological control of the vine mealybug in grape vineyards (Daane et. al 2006).

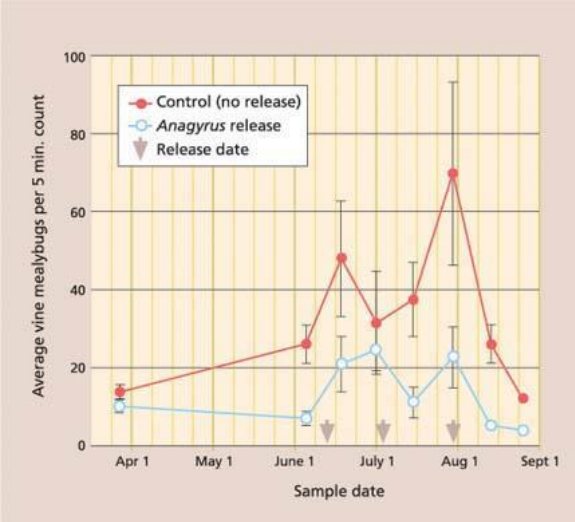


Figure 9. Season-long average (+- SEM) of settled (second instar to adult) vine mealybugs was significantly lower in treatments with *Anagyrus pseudococci* release, as compared to no-insecticide control plots (repeated measures ANOVA: F-13.27; df=1, 76; P=< 0.001).

In addition, parasitoids are only able to attack the vine mealybugs when they are in exposed locations and thus their effect can be varied depending on the mealybug movements (Le Vieux and Malan 2013). This fact, with the potential biological control disturbance of ants, can significantly affect the success of biological control attempts.

Insecticide

Three main modes of insecticide application are used: foliage cover spraying, application of insecticide solution to the soil, and chemigation by application of systemic compounds through irrigation system (Franco et al. 2009). Insecticides are the primary control tool for *Pl. ficus* and the most commonly used insecticides are: imidacloprid, buprofezin, acetamiprid, clothianidin, and chlorpyrifos (Daane et al. 2012). Buprofezin is an insect growth regulator that is effective against nymph stages of mealybug and suppresses the oviposition of adults as well as reducing egg viability (Izawa et al. 1985). Imidacloprid is a chloro-nicotinyl which affects the nervous system by blocking the post synaptic acetyl cholinesterase receptors (Stenersen 2004). Chlorpyrifos belongs to the organophosphates class and is a broad-spectrum nerve insecticide (Franco et al. 2009). Acetamiprid and clothianidin are neonicotinoids that act on the central nervous system and

while they are highly successful against mealybugs, they have been found to affect the immune responses of other insects such as bees (Prisco et al. 2013).

The efficiency of insecticides is constantly tested because sometimes their repeated use could kill natural enemies of the mealybug (Walton and Pringle 1999). For example, imidacloprid can kill beneficials that feed on nectar (Mgocheki and Addison 2009). The effectiveness of imidacloprid and buprofezin on *Pl. ficus* were tested in 2006 because they were considered less disruptive than the organophosphates (Daane et al. 2006). The imidacloprid insecticide was applied systemically in two vineyards in California, one via drip irrigation and the other by furrow irrigation. The mealybug densities were monitored before treatment application and after. In the next year, imidacloprid and buprofezin were applied in the existing plots in different dosages.

Effective control of the vine mealybugs is achieved when the population is in the crawler stage or young nymphal instars (Franco et al. 2009). The host plant should also not provide shelter from the sprays. It was found that imidacloprid provided the greatest reduction in cluster damage when it was applied in April or May via drip-irrigation system on the grapevines (Daane et. al 2006). It was less effective when it was used through the furrow-irrigation system and even when it was timed correctly, the application did not locally kill the vine mealybugs (Figure 10). They found that the vine mealybug population recovered in all of the treatment plots between the summer of 2002 and spring 2003 which was most likely due to the fact that the imidacloprid did not reach all parts of the vine.

It was also found that the *Pl. ficus* populations hide in the bark and in the crevices of stems so it is difficult to target them with insecticide sprays (Berlinger 1977). Oils have also been used for control of scale insects but they have not been successful with mealybugs (Franco et. al 2009). However, integration of narrow refined oils with other insecticides has been found to improve insecticide efficacy (Cranshaw et al. 2000).

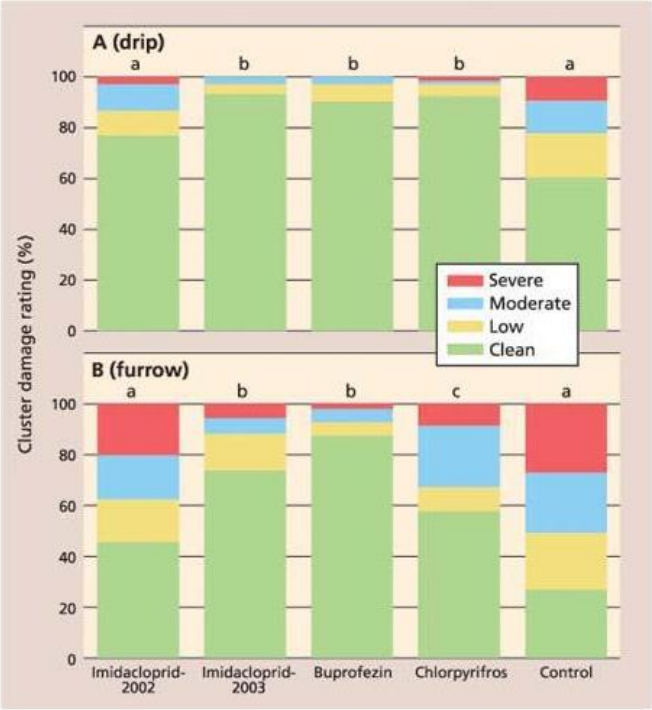


Figure 10. Percentage cluster damage rating for insecticide and control treatments (A) drip-irrigated and (B) furrow-irrigated vineyard. Clean = no mealybug damage; low= honeydew, indicating presence of mealybugs; moderate = honeydew and mealybugs present; severe= unmarketable. Different letters indicate significant difference among treatments ($P \leq 0.05$).

METHODS

Study system

To perform my dispersal trial study, I used the grapevine (*Vitis vinifera*) plants provided by the Daane Lab in the Oxford Tract in Berkeley, California. These grapevine plants were in pots and were grown in the greenhouse in a separate room from the other lab plants to avoid any type of contamination or infestation. I was not told of their exact age but they were taken from the Napa Valley vineyard in August 2013 and constantly trimmed. They were watered daily and the greenhouse had a temperature of about 22ºC. Light came in through the roof of the greenhouse and reached the plants. I inspected for vine mealybugs on the plants before I began the trial to make sure they were clean. Six vine plants were chosen for the first trial based on the foliage and overall health of the vine; the vine leaves did not have any red blotches or brown marks.

For the study system, I used vine mealybugs reared in the Oxford tract. The vine mealybugs were cultured on squash and were kept in a large glass box in a room away from the plants. These mealybugs were originally taken from a Napa Valley vineyard during the summer field days and brought back to the Daane lab to culture. This room was in a separate building from the greenhouse and had room temperature with artificial lights. I carefully placed 6 female mealybugs in a petri dish lined with filter paper and put the petri dish in an incubator that was set at 22ºC because I wanted the female mealybugs to begin laying ovisacs in room temperature. After 4 days, the ovisacs were ready and I transferred three ovisacs to the source plant in the greenhouse.

Data Collection

The six plants were lined up in a row in a greenhouse room that did not have any other plants inside. Five plants were connected via one vine cane and I used a twist-tie to connect the ends of the canes together (Figure 11). If the plants had more than one cane, I trimmed them so that there would only be two canes per plant, one opposite of the other. The five plants were numbered consecutively, with the source plant as number 1 and the last plant as number 5 (Figure 12). The sixth plant was placed behind the source plant, opposite of plant number 2. This was to check if there were other means of dispersal besides walking. The greenhouse was room temperature (about 22ºC) and light reached the plants. In addition, I watered them every day to make sure they did not dry up.

The three ovisacs were placed on the bark of the source plant. I checked daily to see if the crawlers emerged from the ovisacs, and four days after the initial transfer, the ovisacs appeared to have broken. This day was considered Day 1 and I began counting. To count for the mealybugs, the twist-ties were taken off and the plants were taken to the Daane lab room. To make sure the crawlers would not transfer through direct contact, the plants were taken separately to the room. I checked each leaf and the bark of the plants to count the number of mealybugs and their life stages. I did not use a microscope because the crawlers were visible and placing the plant under the microscope could have damaged the leaves and the mealybugs on them. Once the mealybug counts were done, the plants were taken back to the greenhouse and set up once again. This was repeated on the fourth day and the seventh day.

I used mealybug crawlers that had already emerged from the ovisacs in the Emerged Crawlers Trial because I wanted to have a higher count. These crawlers were taken from the same squash room and about 300 were collected in a petri dish. This petri dish was then balanced on top of the bark of the source plant (Figure 13). This was to ensure that the crawlers would have access to the bark and the leaves of the source plant and could then disperse to the connected plants. I did not want to scatter the crawlers on the leaves of the source plant because I wanted to make sure they had the same starting point on the plant. I came back the next day to count the mealybug crawlers and that day was considered Day 1. I did the same count procedure as the Ovisac Trial and counted again in Days 4 and 7.

Figure 11. Vine canes connected together with white twist-ties (circled in red).

Figure 12. Plants lined up from Source plant (#1) to Plant 5 from right to left

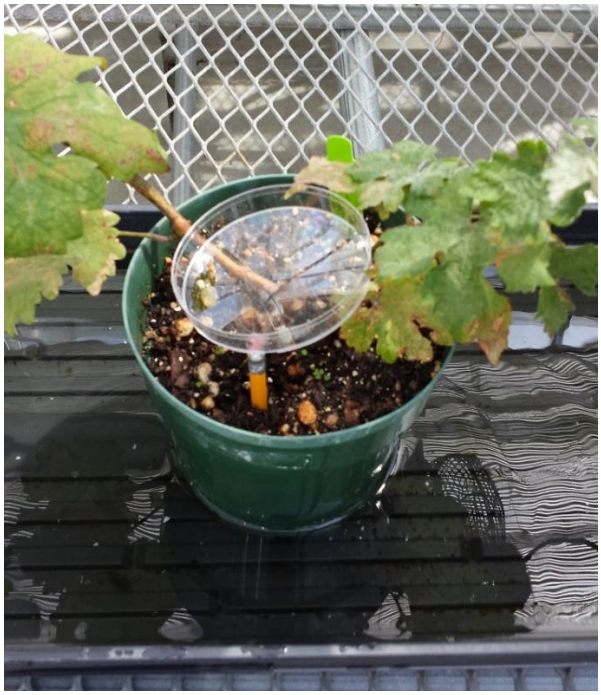


Figure 13. Crawlers in petri dish balanced on source plant (#1) bark.

RESULTS

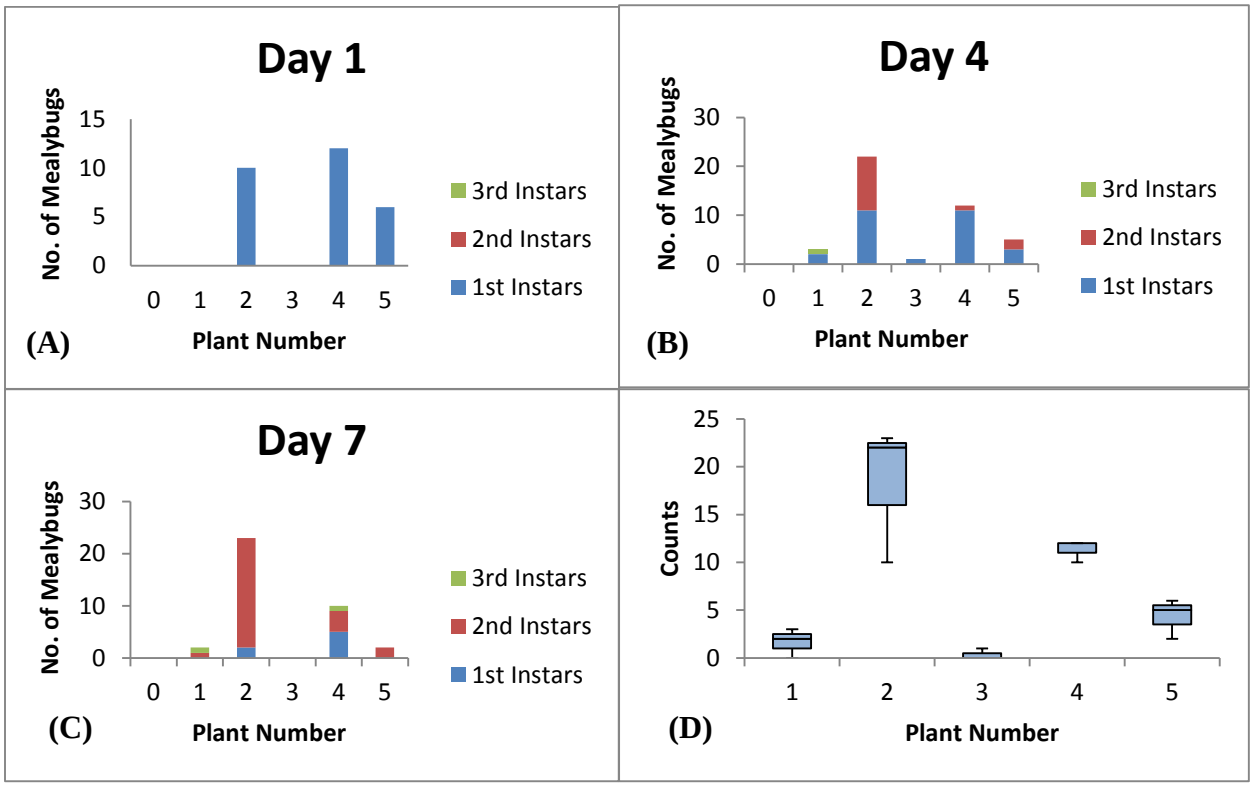
Ovisac Trial

The vine mealybug crawlers were able to reach Plant 5 (Table 1). Plant 0, the plant behind the source plant, did not have any mealybugs on any of the days of the count which means that the mealybugs did not have any other means of dispersal besides walking. Plant 2 had the most mealybugs in total over the counting days (Figure 14). Kruskal-Wallis rank sum test of the total counts by day showed a P value of 0.7803, df=2, and chi-squared value of 0.4961. Thus, there was no significant difference among the counts in the days. In addition, the Paired Wilcoxon test of the plants and counts showed V=27.5 with a P value of 0.1228, which also meant that there was not a significant difference. However, there were various instar stages in the plants throughout the week which meant that the crawlers could have been hidden under the bark before the first counting day (Day 1). It could have also been warmer in the greenhouse than room temperature and sped up the development. Since Plant 2 appeared to have significantly more total vine mealybugs than the

other plants, I performed a 2 tailed distribution t-test with two-sample unequal variance between the total number of mealybugs on Plant 2 with the total number of mealybugs on Plant 3. This gave me a P value of 0.049, which was just below 0.05 and was the closest I could get to find a significant difference among the highest count Plant (Plant 2) versus the lowest count Plant (Plant 3). It should be noted that the t-test assumes the data is normalized, but my data was not because I did not have enough data points.

Table 1. Vine mealybug counts on each plant over 7 days.

	Plant 0	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Day 1	0	0	10 first instar	0	12 first instar	6 first instar
Day 4	0	2 first instar, 1 third instar	11 first instar, 11 second instar, 6 third instar	1 first instar	11 first instar, 1 second instar	3 first instar, 2 second instar
Day 7	0	2 second instar, 1 third instar	2 first instar, 21 second instar, 3 third instar	0	5 first instar, 4 second, 1 third instar	2 second instar



Emerged Crawlers Trial

This trial did not see any development into the second or third instars (Table 2). Out of the 300 crawlers placed in the petri dish in the middle of the source plant (Plant 1), the highest number of first instar mealybugs was 137. In addition, the majority of the mealybugs remained on Plant 1 and did not move to the other plants (Figure 15).

Table 2. Vine mealybug counts on each plant over 7 days.

	Plant 0	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Day 1	0	95 first instar	1 first instar	0	0	0
Day 4	0	132 first instar	2 first instar	1 first instar	0	0
Day 7	0	137 first instar	1 first instar	1 first instar	4 first instar	0

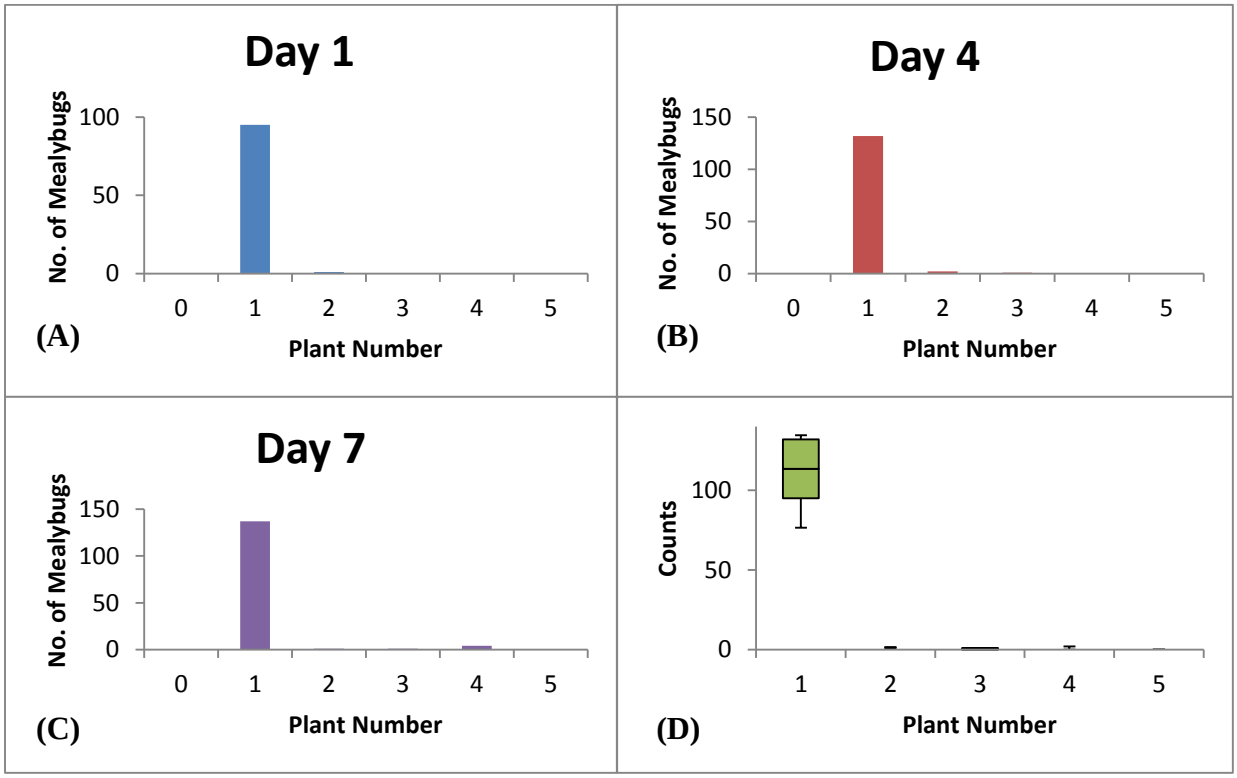


Figure 15. Mealybug count of crawlers (A) Mealybug counts on Day 1. (B) Mealybug counts on Day 4. (C) Mealybug counts on Day 7. (D) Boxplot of total mealybug counts of all 3 counting days (Day 1, 4, and 7).

DISCUSSION

Based on my literature review, monitoring vine mealybugs using a combination of pheromone traps and visual sampling is the most efficient method. This is because the labor of visual sampling is limited to one or two surveys a season while the pheromone traps can be used the anytime of the year. In regards to monitoring, the insecticide use is still the most popular method to rid of *Pl. ficus* infestations. While insecticides may be less labor intensive than biological control and mating disruption, the sprays are not able to kill *Pl. ficus* when they hide under the bark (Berlinger 1977). The sprays can also harm natural enemies of the vine mealybug which creates more problems for the grower (Walton and Pringle 1999). Thus, more studies should be done on biological control and mating disruption to encourage growers to use them and decrease their dependence on insecticides. However, given the amount of sampling and pheromone trap studies, *Pl. ficus* dispersal has not been observed.

The closest study to the vine mealybug dispersal behavior was the behavior study done by Grasswitz and James in 2008 of the grape mealybug, *Pseudococcus maritimus*, between host plants. *P. maritimus* is not under the same genus as *Pl. ficus* but it is under the same order and family, Homoptera: Pseudococcidae (Hardy et al. 2008). In addition, both *Pl. ficus* and *P. maritimus* are vectors of GLRaV-3 and have the same life cycle. They found that in regards to walking dispersal, *P. maritimus* did not move far from the source plant (47cm). In addition, the aerial dispersal traps showed that the male grape mealybugs did not get to far distances. They only caught 4 first instar nymphs in 8m distance and the majority of the instars were found in the traps placed less than 3m from the source plant. With this information, they concluded that there was poor dispersal ability and removing the infected plants should reduce the damage. However, while the grape mealybug and vine mealybug are similar in their life cycle and feeding, the vine mealybug has shown to have more generations than the grape mealybug and does not diapause through winter (Le Vieux and Malan 2013).Thus, the dispersal behavior of vine mealybug is crucial in attempts to improve current management methods.

Similar to the Grasswitz and James study, my two trial studies have shown that the vine mealybug crawlers do not disperse very far from their point of release. In the Ovisac Trial, the vine mealybug crawlers reached Plant 5. However, their jump from Plant 2 to Plant 4 appeared out of the ordinary. This means that the crawlers either walked through Plant 3 or chose not to settle on

any of the plants, the crawlers in Plant 3 were hidden in the bark, or that the crawlers in Plant 4 and 5 were from another source (perhaps from a previous infestation that remained dormant in the bark). Nonetheless, the Ovisac Trial showed more dispersal than the Emerged Crawlers Trial, where the latter used already emerged crawlers. Emerged Crawlers Trial results can be explained in a variety of ways. However, the most probable explanation is that the crawlers were not as motivated to move and find a source of food because they have been emerged from the ovisac for a few days. Although the exact time lapse between the instar stages has not been determined, the newly emerged crawlers of most mealybug species disperse more actively than the other life stages (Kosztarab and Kozar 1988).

The results suggest that when the crawlers are freshly emerged from the ovisacs, they will move outward in a faster rate and higher density. However, once they find a food source, they will settle and no longer disperse, creating an aggregative distribution (Gullan and Kosztarab 1997; Nestel et al. 1995). Once more studies have been done to narrow down the time frame of this emergence and dispersal, spray times and/or trap methods can be improved to target the immediate stages of dispersal.

Limitations

Since time only allotted two successful trials, further studies should take into consideration several factors. One main factor to consider is the fact that the greenhouse room temperature does not reflect the field environment. As the season changes, the temperature will change and it can affect the generations of the vine mealybugs (Daane et. al 2012). In addition, the vineyards have more foliage than the plants I used in my study, which means that the vine mealybug crawlers will have more ways to disperse among the vines. Yet another factor to consider is the wind. Dispersal of first instars by wind currents has been shown in various species of mealybugs that infest trees (Barrass et al. 1994; Cornwell 1960). This has not been studied specifically for the vine mealybug, but since the first instar size is similar in all of the mealybug species, dispersal via wind currents is also a possibility.

Conclusion

From my literature review, it is clear that there is still much to study about *Pl. ficus*. To improve current management methods we must try to understand as much as we can about *Pl. ficus* movement throughout the vineyard as well as its natural enemies. Although pheromone studies and samplings have progressed, monitoring *Pl. ficus* is difficult because of their small size. Even without the natural dispersal factor the vine mealybugs can also be transferred by direct contact of the vine plants whenever the grower moves the vine plants around (Franco et al. 2009). However, knowing their natural movement between vine plants in the vineyard can narrow down the insecticide spray locations. Since the vine mealybugs are difficult to see when they have just emerged, the grower can predict the range of dispersal when he finds emerged ovisacs and/or infestations. Then the grower can spray the insecticide or use biological control in the specified plot and avoid harming other parts of the vineyard. My trial studies have shown that the newly emerged crawlers moved farther distances, to Plant 5, than the already emerged crawlers that had been out of the ovisacs for a few days. This suggests that their dispersal range can be estimated and utilized in the current integrated pest management methods. Future studies should also look at the effect of temperature on their dispersal rates to make sure a variety of vineyards throughout California can use the range.

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