Comparison of honeydew production to explain vine mealybug dominance in California

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Abstract The recent introduction of vine mealybug (VMB) has elevated the mealybug's pest status and is causing significant damages throughout California vineyards. There have been field observations that noted higher honeydew accumulations on the vines infested with VMB, and these observations have led many researchers to assume VMB's higher individual honeydew production as one of the reasons for its huge success. However, this assumption had not been investigated. Mealybug gets a powerful protection from the ants that obtain honeydew and in return reduce the abundance and efficacy of mealybug's natural enemies. This mutualistic association significantly contributes to the success of mealybug survival, and it has been established that the honeydew quantity provided is positively correlated with ant tending intensity. This research aims to explain the wide prevalence and dominance of VMB. I hypothesized that VMB does not produce more honeydew on an individual basis, given its comparable body size with naturalized mealybug species and other biological attributes like higher reproduction and development rate. I collected honeydew droplets produced by the second instars of VMB and the two naturalized species, obscure and longtailed mealybugs (OMB and LTMB). Then, I compared the average honeydew productions among the three species. The ANOVA determined that there is no significant difference among the honeydew productions. This result suggests that ant tending is not a main reason for VMB's success, and that biological control programs should focus more on controlling mealybug population itself to successfully manage infestations in California vineyards.

Introduction

Mealybugs (Hemiptera: Psuedococcidae) are widely known as a pest in California vineyards. These oval shaped unarmored scale insects with a white wax layer covering their bodies can be found on barks, stems, and leaves of the vine. Mealybug infestations result in reduced crop value through their physical presence in grape clusters and the accumulation of honeydew that serves as a substrate for fungal pathogens and closterol viruses (Godfrey *et al.* 2002, Daane *et al.* 2006a, Daane *et al*. 2007, Nelson and Daane 2007). Severe mealybug outbreaks can cause defoliation or even death of the plant (Godfrey *et al.* 2002). Until recently, mealybug infestations in California were considered mild because natural enemies—mainly the species of parasitoids that lay eggs inside the mealybugs—maintained mealybug populations at a relatively constant and manageable level (Daane *et al.* 2006b, Daane *et al*. 2007, Nelson and Daane 2007). However, with the introduction of vine mealybug (VMB), *Planococcus ficus*, their pest status has elevated over the past decade, calling for more attention and research on the effective control of mealybug population densities and prevalence (Daane *et al*. 2006b, Daane *et al.* 2007, Nelson and Daane 2007).

There are several species of mealybug in California. The obscure mealybug (OMB), *Pseudococcus viburni*, and the longtailed mealybugs (LTMB), *Pseudococcus longispinus*, have persisted in California from at least the early 1900s (Daane *et al.* 2008a). (Throughout this paper, the acronyms VMB, OMB, LTMB are used to indicate the vine, obscure and longtailed mealybugs to avoid confusion with using P to denote the mealybug genera *Planococcus* and *Psuedococcus*). Consequently, enough time and effort were put into research and management strategies to establish their natural enemies, which successfully maintained the mealybug populations (Ben-Dov 1994, Daane *et al*. 2007, Daane *et al*. 2008b). For this reason, infestation damages were generally below economically damaging levels and mealybugs were not considered as a primary vineyard pest (Ben-Dov 1994, Daane *et al.* 2007). However, in the few years since the identification of VMB in the Coachella Valley of southern California in 1994, significant damages in the state's grape-growing regions were observed (Millar *et al.* 2002, Daane *et al*. 2004, Daane *et al*. 2006a) and mealybugs were recently ranked as the most important pest problem for California table grapes (Geiger *et al.* 2001). Moreover, while the OMB and LTMB distributions are restricted to coastal vineyards, VMB is found in almost all grape-growing regions in the state, indicating its overwhelming prevalence and dominance compared to naturalized mealybug species (Daane *et al.* 2004, Daane *et al.* 2006b, Gutierrez *et al.* 2008).

VMB has several advantageous characteristics that may contribute to its dominant status. Since VMB has been recently introduced, efforts to establish natural enemies have not yet resulted in reliable and consistent biological control (Millar *et al*. 2002). VMB has an unusually high reproductive rate with the female depositing more than 500 eggs, compared to the other species' average of 300 eggs (Daane *et al*. 2008a). There are four to seven generations per year for VMB, in contrast with two to three generations per year for the other *Pseudococcus* species (Millar *et al*. 2002, Daane *et al*. 2006b, Gutierrez *et al*. 2008); this results in multiple life stages at any given time over the season, leading to more difficulty in controlling the populations. With these characteristics, VMB can outnumber and outcompete other mealybug species in the resources- and space-limited vineyard habitats.

Interestingly, it has been observed on field visits that there is more honeydew buildup on the vines infested with VMB than the vines with OMB (Cooper 2008, pers. comm., Gutierrez *et al*. 2008). These observations have led many researchers to conclude that VMB produces more honeydew on an individual basis than other species, because it would explain VMB's dominance as honeydew encourages ant tending—a powerful protection from their natural enemies.

The Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae) was first reported in 1905 and since then has spread throughout California (Cooper *et al*. 2008). Although commonly recognized as a household pest, the ants cause severe problems by displacing native ant species and disrupting natural interactions in ecosystem (Cooper *et al*. 2008). In agricultural fields, *L. humile* is often associated with outbreaks of phloem-feeding insects like aphids and mealybugs (Cooper *et al.* 2008). In the strong mutualistic association called ant tending that *L. humile* and mealybugs form, ants collect the sugar-rich honeydew droplets and in return repel or kill adult parasitoids that seek mealybugs to lay eggs in them (Bartlett 1961, Way 1963, Way *et al*. 1997, Daane *et al*. 2006a, Daane *et al*. 2007, Cooper *et al*. 2008, Silverman and Brightwell 2008). The aggressive behaviors of *L. humile* towards mealybug natural enemies significantly reduce the abundance and efficacy of biological control (Nelson and Daane 2007). For example, when the natural enemies of VMB were imported from Chile and implemented in California vineyards, it was found that foraging ants diminished the success of natural enemies establishment and resulted in higher mealybug densities (Daane *et al*. 2008a).

Since honeydew greatly enhances the survival and establishment of mealybugs, it is crucial to investigate and compare honeydew productions of mealybug species to confirm the reason for VMB dominance. As previously mentioned before, the field observations indicated that the vines infested with VMB have a higher honeydew buildup. However, the cause for this phenomenon has not been researched. It is possible that VMB excretes more honeydew on an individual basis, or there might have simply been a higher population density of VMB per vine and resulted in a greater honeydew accumulation. My specific research question is: “does a single VMB produce more honeydew than individuals of other naturalized mealybug species?” If so, it would establish VMB as a more destructive pest than others since honeydew is direct evidence of plant nutrient loss. Moreover, since the past findings confirmed that the intensity of ant and scale insect mutualism is positively correlated with the honeydew quantity provided (Holldobler and Wilson 1990, Fischer *et al.* 2001), my study would confirm again the significance and implication of ant tending. The outcome of research can also be applied to mealybug control programs; insecticide treatment to directly control mealybug population is limited in the effectiveness and often incompatible with sustainable farming practices (Daane *et al.* 2005, Walton *et al*. 2006). If VMB is found to get significant benefits from ant tending, it may be much more effective to enforce the ant bait program that would result in the increased efficacy of mealybug natural enemies.

Nonetheless, I hypothesized that VMB does not produce more honeydew on an individual basis, and that ant tending does not contribute to its success. The size of VMB does not particularly differ from that of the other species, and the high fecundity and developmental rate seem significant enough to explain its prevalence. In order to test my hypothesis, I collected honeydew droplets from the individuals of VMB, OMB and LTMB over a 24-hour period. By quantifying and comparing the honeydew produced on an individual basis, I was able to deduce the reasons for the dominance and prevalence of VMB in California vineyards.

Methods

Methods and Objectives Several trials were run to collect honeydew droplets produced by the second instars of each mealybug species over a 24 hour period. I counted and calculated the droplets to determine the difference among the mean individual honeydew productions of the study species: VMB, OMB, and LTMB.

Study System I conducted an experimental greenhouse study at the Natural Resources Laboratory, located at the corner of Hearst and Oxford, Berkeley. The data collection spanned from late August to early December of 2008. My subjects are the invasive vine mealybug VMB and the two naturalized mealybugs, the obscure mealybug OMB and the longtailed mealybug LTMB. The vine species chosen is Chardonnay *Vitis vinifera*.

Data Collection, Technique of Analysis and Rationale I chose to conduct my study at the greenhouse to eliminate possible outdoor confounding factors like weather conditions and other interacting organisms that may obscure the data. The controlled experiment would also ensure the same growth condition for mealybugs and vine plants, strengthening the assumptions required for ANOVA. The naturalized mealybugs OMB and LTMB were chosen because of their long history in California and availability to the lab. The vine *V. vinifera* is chosen also because of its availability in the greenhouse.

Each mealybug population was grown in several containers that contain one or two butternut squash (*Cucurbita moschata*), which ensured portability and easy detection of mealybugs. The containers were put in separate rooms of the insectary to avoid contamination. To inoculate mealybugs to the vines in the greenhouse, about 20 to 30 pieces of filter paper (approximately 30 mm x 40 mm) in total were placed on the mealybug-infested squashes for about ten minutes. This was a sufficient time for the newly hatched first instars to cover the papers. These mobile first instars of mealybug are also known as the "crawlers" because it is the only life stage that the mealybugs are actively mobile and crawl around. Once they find a favorable spot, mealybugs become almost immobile as they stick their mouthpart into the plant surface and begin to feed. Unless they feel threatened or disturbed, they are not likely to move much.

At the favorable spots, the crawlers become the settled first instar, and molt into second and third instars that are morphologically identical except for the increased body size. Attempting to transfer the latter stages of mealybug to vines would be very difficult because of their tiny size (adult being about 3 mm) and extremely vulnerable mouthpart.

The mealybug-covered papers were collected with tweezers in a large Petri dish and carried to the species-specific rooms of the greenhouse, where 12 to 15 potted *V. vinifera* were grown at constant temperatures of 23 ± 2°C. Each piece of paper was pinned on a leaf, allowing the crawlers to move over, settle, and grow into latter stages. This inoculating procedure was repeated until approximately 30 to 50 mealybugs per vine were observed.

As the amount of honeydew produced differs across the life stages (Cooper 2008, pers. comm.), individuals from the same life stage were chosen for an objective comparison. The honeydew production of second instars of each species was investigated because they are the easiest and fastest to obtain; the crawlers are too mobile and too small with the body size of about 1 mm, and the mealybugs from the latter stages not only take a relatively long time to grow, but also tend to retreat to hidden places like under the bark for safety, making it difficult to capture their honeydew.

A single second instar mealybug per vine was investigated to avoid confounding variables resulting from possible varying plant conditions; since honeydew is mealybug’s direct byproduct of plant phloem intake (Cooper 2008, pers. comm.), plant conditions greatly influence the honeydew production. To properly catch the honeydew droplets, I searched for the leaf with a single second instar mealybug underneath by gently flipping the leaves; other individuals on the same leaf, if found, were removed. Once the leaf was ready for each vine plant, a thick foam tape was wrapped around the petiole to prevent the mealybug from moving off the leaf.

With wooden stands, water-sensitive paper (Water Sensitive Paper, Quantifoil, Gena, Germany) (50mm x 70mm) was placed 3 to 5 cm underneath each leaf where the identified mealybug was located. If the paper were placed too close from the leaf, the leaf transpiration would turn the paper from yellow to blue before the trial was completed. The paper for each specimen was replaced every four hours to prevent over-accumulation of honeydew droplets. All trials started and ended at 2 p.m. The trial was repeated until around 12 honeydew production data for each species were collected. This number of data was sufficient to estimate and compare the mean amount of honeydew produced (Cooper 2009, pers. comm.).

After each trial, the honeydew droplets on water-sensitive papers were measured and counted under the microscope with micrometer and recorded on a spreadsheet. From a previous research, it was discovered that mealybugs produce honeydew droplets of certain diameters, which could be categorized by 0.01mm, 0.2mm, 0.3mm, 0.5mm, and 0.7mm (Cooper 2008, pers. comm.). The number of droplets was multiplied by the respective diameter to get the overall area of honeydew produced by each specimen of one species. Then, the mean amount of production by species was estimated and compared. The single-factor ANOVA was used to test the significance of the resulting difference in mean honeydew productions of the three mealybug species.

Results

The mean honeydew production of VMB (28.3 mm^2) was higher than the production of LTMB (17.3 mm^2), but very similar to that of OMB (27.9 mm^2) (Fig. 1). In addition, the range of error bars is large for VMB (±9.2 mm^2) and OMB (±10.4 mm^2), in contrast with the small error bar of LTMB (±4.0 mm^2) (Fig. 1).

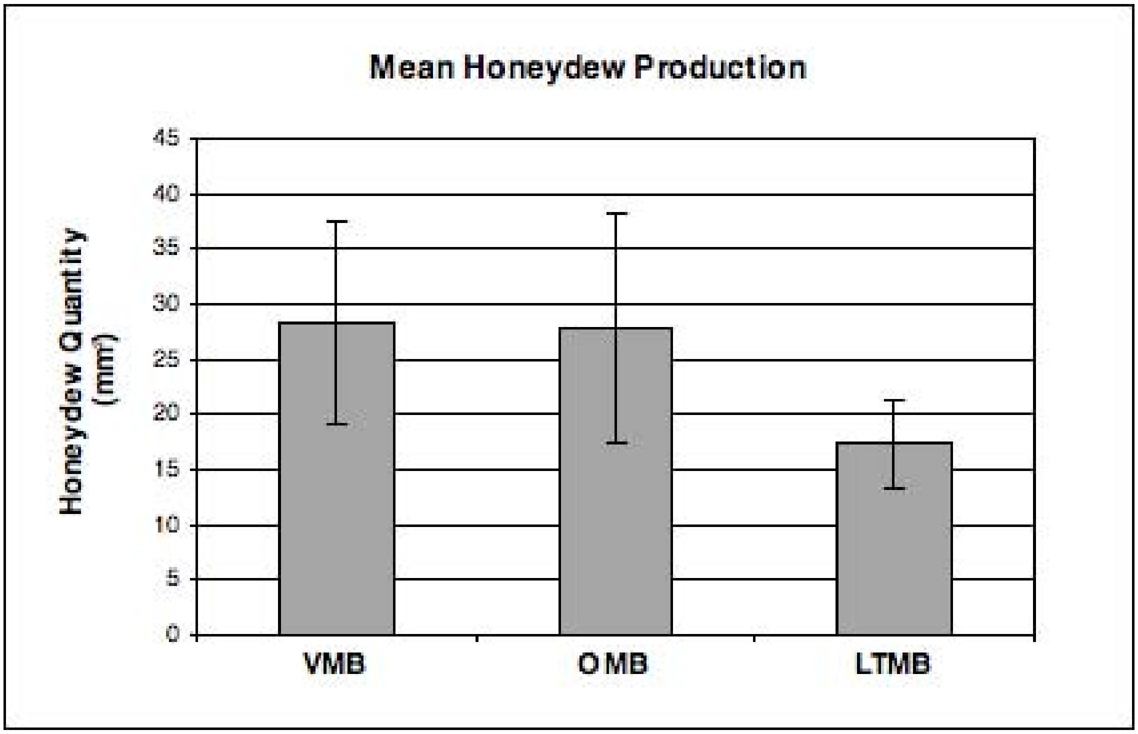

Figure 1. Average honeydew quantity produced (mm^2) over 24 hours by the three mealybug species (VMB n=12, OMB n=11, LTMB n=14) between September and December 2008 at the Daane Laboratory Insectary, Berkeley, CA. Bars indicate ±1 S.E. Significant difference (single-factor ANOVA) indicates the p-value=0.54, F=0.63, df=2.

All three standard error bars overlap with one another and the ANOVA indicates the p-value 0.54 and F statistic 0.63, which are more than 0.05 and less than the F critical value 3.27, respectively. Therefore, the mean honeydew production of VMB (28.3 mm^2 ±9.2, n=12) was not significantly higher than that of OMB (27.9 mm^2±10.4, n=11) and LTMB (17.3 mm^2±4.0, n=14).

Discussion

The comparison of average area of honeydew produced by VMB, OMB, and LTMB over 24 hours indicates that VMB does not produce more honeydew on an individual basis than the other two naturalized species (Fig. 1). This result supports my hypothesis and implies that the stronger ant tending is not a contributing factor to the VMB dominance in California vineyards.

This conclusion contradicts the past findings that listed VMB's higher honeydew production as one of the advantageous biological characteristics over other mealybug species (Millar *et al*. 2002, Bentley *et al.* 2002, Daane *et al.* 2006b, Gutierrez *et al.* 2008). Nonetheless, the field observations that might have misled researchers indicate that VMB tend to aggregate in a higher population density. This phenomenon results from VMB's high reproductive rate and fast development time, which enables a rapid population buildup (Daane *et al.* 2004, Daane *et al.* 2008a). As the phenomenological definition of competition holds (Tilman 1987), this characteristic may enable VMB to prevail in vineyards simply by outnumbering others in the space- and resource-limited environment.

The range of the error bars (Fig. 1) also supports my hypothesis that ant tending does not play a significant role in VMB dominance. The narrow error bar of LTMB in contrast with the wide error bars of VMB and OMB indicates that the individual honeydew production of LTMB is much more consistent; therefore, it seems reasonable to deduce that the ants would be more attracted to the reliable honeydew production of LTMB and show a stronger ant tending intensity. Nevertheless, VMB is evidently doing much better than LTMB in vineyards (Daane *et al*. 2004, Daane *et al*. 2006b, Daane *et al.* 2008b, Gutierrez *et al*. 2008) despite the comparable quantities of individual honeydew productions of the three species and the more consistent honeydew production of LTMB. This indicates a negligible impact of ant tending on the VMB dominance.

Mealybug control programs may consider utilizing this finding to maximize their effectiveness. Although insecticide use can provide adequate mealybug control, it can be prohibitively expensive for some grape producers and the repeated use often lead to adaptive response from mealybugs as well as reduced numbers of natural enemies (Walton and Pringle 1999, Daane *et al.* 2004, Daane *et al.* 2006b). Moreover, VMB feeds and is located primarily in protected sites like leaf axils and bark crevices, reducing the effectiveness of chemical treatments (Millar *et al.* 2002, Daane *et al.* 2008a, Gutierrez *et al*. 2008). Overlapping generations of VMB that result in the coexistence of all life stages at any given time also complicates the timing of chemical applications, as chemicals are timed to attack the pest when it is at its most vulnerable life stage (Daane *et al.* 2008a). In addition, VMB's wide host range allows extensive distribution and residual populations within and outside the vineyard even after chemical application is implemented (Millar *et al*. 2002, Daane *et al.* 2006b, Walton *et al*. 2006, Daane *et al.* 2008a, Gutierrez *et al.* 2008).

Since the ant tending was attributed for VMB's dominance based on field observations, ant baits have been employed in some mealybug control programs (Cooper *et al*. 2008). Ant bait program is a system that capitalizes on the ants' sugar-feeding requirements and social structure to deliver small doses of toxicant throughout the colony (Cooper *et al.* 2008). But the result from my research suggests that to manage VMB populations, rather than using chemical applications or ant baits, it may be more effective to focus on controlling the reproductive and development rate of VMB since its biological attributes result in rapid population increases. For example, by utilizing the mealybug's sex pheromone that females emit to attract males, the vineyard growers can disrupt mealybug mating and eliminate problems inherent in pesticide use (Millar *et al.* 2002, Daane *et al.* 2005, Walton *et al.* 2006, Daane *et al.* 2006a, Daane *et al.* 2008a). However, because the mating disruption alone provides an adequate control only when there is a low mealybug population density (Walton *et al.* 2006), future research to further investigate the effective control methods for VMB should focus on other means to control reproductive and development rate of VMB using its biological attributes. The limitations of my study would be a small sample size and controlled environment. A bigger sample size in future research could reduce the overlapping of error bars, and field study would reflect a more realistic aspect.

The result of this research suggests that the VMB's higher individual honeydew production, commonly assumed as one of the major causes for its dominance, may be inaccurate. VMB's unique attributes like high reproduction and development rate, lack of firmly established natural enemies, behavioral advantages, and overlapping generations explain its prevalence. I recommend future mealybug control programs to integrate biological disruption methods to effectively manage this formidable vineyard pest.

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References

Bartlett, B. R. 1961. The influence of ants upon parasites, predators, and scale insects. Annual Entomological Society of America 54:543-551.

Ben-Dov, Y. 1994. A systematic catalogue of the mealybugs of the world (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with data and geographical distribution, host plants, biology and economic importance. A systematic catalogue of the mealybugs of the world (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with data and geographical distribution, host plants, biology and economic importance: 1-686.

Bentley, W. J., W. W. Cone, K. M. Daane, and N. M. Korfanta. Mealybugs in North American vineyards. L. T. Wilson T. J. Dennehy Vineyard Pest Management 2002. Pergammon Oxford, UK.

Cooper, M. L. Staff Research Associate, Environmental Science, Policy and Management, University of California, Berkeley. 2009, personal communication.

Cooper, M. L. Staff Research Associate, Environmental Science, Policy and Management, University of California, Berkeley. 2008, personal communication.

Cooper, M. L., K. M. Daane, E. H. Nelson, L. G. Varela, M. C. Battany, N. D. Tsutsui, and M. K. Rust. 2008. Liquid baits control Argentine ants sustainably in coastal vineyards. California Agriculture 62:177-183.

Daane, K. M., W. J. Bentley, V. M. Walton, R. Malakar-Kuenen, J. G. Millar, C. A. Ingels, E. A. Weber, and C. Gisper. 2006a. New controls investigated for vine mealybug. California agriculture 60: 203-231.

Daane, K. M., M. L. Cooper, S. V. Triapitsyn, V. M. Walton, G. Y. Yokota, D. R. Haviland, W. J. Bentley, K. E. Godfrey, and L. R. Wunderlich. 2008a. Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. California Agriculture 62:167.

Daane, K. M., M. L. Cooper, S. V. Triapitsyn, J. W. Andrews, and R. Ripa. 2008b. Parasitoids of obscure mealybug, Pseudococcus viburni (Hemiptera: Pseudococcidae) in California: establishment of Pseudaphycus flavidulus (Hymenoptera: Encyrtidae) and discussion of related parasitoid species. Biocontrol Science and Technology 18:43-57.

Daane, K. M., R. D. Malakar-Kuenen, and V. M. Walton. 2004. Temperature-dependent development of Anagyrus pseudococci (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, Planococcus ficus (Homoptera: Pseudococcidae). Biological Control 31:123-132.

Daane, K. M., K. R. Sime, B. N. Hogg, M. L. Bianchi, M. L. Cooper, M. K. Rust, and J. H. Klotz. 2006b. Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards. Crop Protection 25:592-603.

Daane, K. M., K. R. Sime, J. Fallon, and M. L. Cooper. 2007. Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. Ecological Entomology 32:583-596.

Daane, K. M., R. J. Smith, K. M. Klonsky, and W. J. Bentley. 2005. Organic vineyard management in California. Organic-Research.com 5: 37-55.

Fischer, M. K., K. H. Hoffmann, and W. Volkl. 2001. Competition for mutualists in an ant-homopteran interaction mediated by hierarchies of ant attendance. Oikos 92:531-541.

Fischer, M. K. and A. W. Shingleton. 2001. Host plant and ants influence the honeydew sugar composition of aphids. Functional Ecology 15:544-550.

Geiger, C. A., K. M. Daane, W. J. Bentley, G. Y. Yokota, and L. A. Martin. 2001. Sampling program for grape mealybugs improves pest management. California Agriculture 55: 19-27.

Godfrey, K. E., K. M. Daane, W. J. Bentley, R. J. Gill, and R. Malakar-Keunen. 2002. Mealybugs in California vineyards. UC ANR Publication 21612.

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. Journal of Applied Ecology 45:524-536.

Holldobler, B. and E. O. Wilson. 1990. Host tree selection by the neotropical ant Paraponera-Clavata (Hymenoptera, Formicidae). Biotropica 22:213-214.

Millar, J. G., K. M. Daane, J. S. McElfresh, J. A. Moreira, R. Malakar-Kuenen, M. Guillen, and W. J. Bentley. 2002. Development and optimization of methods for using sex pheromone for monitoring the mealybug Planococcus ficus (Homoptera: Pseudococcidae) in California vineyards. Journal of Economic Entomology 95:706-714.

Nelson, E. H. and K. M. Daane. 2007. Improving liquid bait programs for argentine ant control: Bait station density. Environmental Entomology 36:1475-1484.

Silverman, J. and R. J. Brightwell. 2008. The Argentine ant: Challenges in managing an invasive unicolonial pest. Annual Review of Entomology 53:231-252.

Tilman, D. 1987. The American Naturalist 129 (5):769-774.

Walton, V. M., K. M. Daane, W. J. Bentley, J. G. Millar, T. E. Larsen, and R. Malakar-Kuenen. 2006. Pheromone-based mating disruption of Planococcus ficus (Hemiptera: Pseudococcidae) in California vineyards. Journal of Economic Entomology 99:1280-1290.

Walton, V. M. and K. L. Pringle. 1999. Effects of pesticides used on table grapes on the mealybug parasitoid Coccidoxenoides peregrinus (Timberlake) (Hymenoptera: Encyrtidae). South African Journal for Enology and Viniculture 20:31-34.

Way, M. J., M. E. Cammell, M. R. Paiva, and C. A. Collingwood. 1997. Distribution and dynamics of the Argentine ant Linepithema (Iridomyrmex) humile (Mayr) in relation to vegetation, soil conditions, topography and native competitor ants in Portugal. Insectes Sociaux 44:415-433.