SURVEY OF DIFFERENT MEALYBUG SPECIES AND ATTENDING ANTS AND THEIR MANAGEMENT IN VIRGINIA VINEYARDS

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Survey of different mealybug species and attending ants and their management in Virginia vineyards

Pragya Chalise

Abstract (Academic)

Mealybugs (Order Hemiptera: Superfamily Coccoidea) represent a persistent problem in grape-growing regions globally, with a notable increase in infestations in recent times. The current study investigates the species composition and seasonal dynamics of mealybugs in vineyards. *Pseudococcus maritimus* and *Ferissia gilli* remain the dominant species, while *Pseudococcus viburni* was also spotted during two separate instances and *Planococcus ficus* remained absent. Commercially available pheromone delta traps and adhesive bands were deployed to capture the male mealybugs, while numerically higher efficacy was observed in the adhesive band trap. Notably, the placement of adhesive band traps on the cordon and trunk revealed differential male mealybug captures, probably indicating their movement within the vines. The research also divulges into the life cycle of mealybugs and their population densities during the sampling season. The main highlight has been the lower population density of earlier nymphal stages of the first generation of mealybugs followed by the colonization of grapevines by the second-generation crawlers. Adults of second generations give rise to the overwintering stages of mealybugs. Traditionally the research also explores the species and seasonal population dynamics of ants along with mealybugs and their management in Virginia vineyards. The activity of fifteen genera of ants in the vineyard was recorded, with some of the dominant ant genera like *Tetramorium*, *Crematogaster*, and *Lasius* were recorded in the vineyard actively tending and defending the mealybugs and moving them around. Fruit cluster infestation was also higher in the control treatment in comparison to sugar dispenser and ant bait dispenser treatments. This comprehensive assessment of ant diversity further deepens our understanding of the intricate ecosystem within vineyards. A spray trial using three different insecticides with distinct modes of action (Buprofezin, Bifenthrin, and Spirotetramat) in two of the commercial vineyards. Preliminary results indicate that on some days after treatment, insecticides effectively controlled mealybug populations, offering a glimmer of hope to vineyard owners grappling with infestations. Mealybugs pose a significant threat to grape cultivation, and this research provides valuable insights that can help vineyard owners and grape growers develop more targeted and effective control strategies. Species identification, understanding their behavior, and exploring potential allies in the fight against mealybugs are all crucial steps toward maintaining the health and productivity of vineyards in Virginia. While challenges remain in the battle against mealybugs, this research marks a significant step forward in safeguarding grape cultivation in Virginia and potentially reshaping strategies for controlling these persistent pests in vineyards. Growers and researchers alike eagerly await further developments and the practical application of these findings to protect the flourishing vineyards of the region.

Survey of different mealybug species and attending ants and their management in Virginia vineyards

Pragya Chalise

Abstract (General Audience)

Mealybugs are common grapevine pests, recently causing a nuisance in vineyards across the United States and Canada. Some of the previously documented species of mealybugs common in the eastern part of the States include grape mealybug, obscure mealybug, Gill’s mealybug, and long-tailed mealybugs. The research has mainly addressed the mealybugs and their tending ant species and their management in vineyards in Virginia. The study identifies grape mealybug and Gill’s mealybug as the dominant species with occasional sightings of obscure mealybug and a lack of vine mealybug throughout the sampled vineyard sites. The male mealybugs were monitored using commercially available pheromone delta traps and adhesive band traps. Placement of adhesive band traps in the cordon and trunk of grapevines revealed different male mealybug capture data suggesting their movement within the grapevines. Two generations of mealybugs including the first generation with lower population density emerging out from the overwintering life-stage and the subsequent second generation responsible for colonizing different parts of the grapevines and producing overwintering life-stages were observed. Fifteen genera of ants were sampled from the vineyard via pitfall trap with some ants like pavement ants, acrobat ants, and medium garden ants actively tending and protecting the mealybugs. The use of sugar dispensers in the vineyards also led to lower fruit cluster infestations with mealybugs in comparison to the area without dispensers. The insecticidal spray trial using three different insecticides was also effective in controlling populations of mealybugs on some of the dates after treatment. The research offers valuable insights for grape growers, aiding in the development of effective control strategies for mealybug control. It emphasizes the importance of identifying the species present, understanding their behavior, and exploring potential allies and insecticide options in pest management.

Dedication

Those mountains that you are carrying, you were only supposed to climb.

Najwa Zebian

Dedicated to my dad and mum for being the backbone to everything I stand for and my son for being the hope I cling to.

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Table of Contents

Chapter 1: Introduction and Literature Review

Chapter 2: A survey of mealybug species and their distribution in Piedmont grape-growing region in Virginia

Chapter 3: Association between ants and mealybugs in Virginia vineyards

Chapter 4: Determine the efficacy of different insecticides against mealybugs in vineyards

Chapter 5: Summary, Implications, and Suggestions for Future Research

Chapter 1

Introduction and Literature Review

1.1 Background

Grapes are a high-valued fruit crop around the globe, consumed in a fresh form and a variety of processed forms. Over twenty-six million metric tons of grapes were produced in the world in 2021, out of which about 6 million metric tons were produced in the US alone. Grapevines were estimated to cover 7.3 million hectares globally in 2021. United States continues to maintain its top position among the leading vine-growing countries, covering 5.5% of the total vineyard surface area globally (OIV, 2022). The US ranks fourth in the wine-producing countries in 2021 with its 24.1 million hectoliters of wine production. The US also leads the world in wine consumption (OIV, 2022). California is the wine capital of the states, with over 6000 wineries and Virginia occupies the top ten wine-producing states (OIV, 2022).

The area occupied by grapevines in Virginia has increased by 14% since 2015 to 1740 ha in 2021. Cabernet Franc is the most widely planted variety in the state, surpassing Chardonnay, the long-time leading cultivar. Some of the top-planted varieties in Virginia include *Vitis vinifera* grapes (82%) followed by hybrid varieties (14%) and then American (*V. labrusca*) varieties (4%) (Virginia Wine, 2021). About two dozen grape varieties are grown in Virginia, which includes popular white varieties such as Petit Manseng, Viognier, Chardonnay, Vidal Blanc, etc., and red varieties such as the Cabernet Franc, Norton, Petit Verdot, etc. Some of the lesser-known varieties to make it to the list include Albarino, Barbera, Nebbiolo, Tannat, and Touriga Nacional (Gans, 2019).

Every year, grapevines receive tremendous pressure from pests and the environment. Among the common cryptic pests commonly encountered in grapevines are mealybugs. Mealybugs are grouped with scale insects in the order Hemiptera, the suborder Sternorrhyncha, and the superfamily Coccoidea. Coccoidea is represented by 13 families of 214 species in the US (McGavin & Zimmerman, n.d.). Scales differ from mealybugs in that they are permanently attached to the host plant surface and covered in a waxy covering. Mealybugs differ from scale insects because they retain legs throughout their life. This superfamily contains insects that are phloem feeders, where females are soft-bodied, motile, or non-motile insects and males are small fly-like, with or without wings. According to the mealybug catalog maintained by Ben-Dov (1995), 7800 species from this superfamily have been reported worldwide. Mealybugs infest 250 families of host plants. The most common host families infested by mealybugs include Poaceae, Asteraceae, Fabaceae, Rosaceae, Rubiaceae, and Euphorbiaceae. Mealybugs tend to occur more in herbaceous plants.

The family Pseudococcidae is a species-rich family of mealybugs, including serious agricultural pests. The subfamily Pseudococcinae includes many economically important grape-infesting mealybugs (Hardy et al. 2008). Grapevines accommodate several polyphagous species of mealybugs that infest not only the grapes but also several deciduous fruit crops or some ornamental greenhouse plants (Daane et al. 2012). Some of the economically important vineyard-infesting mealybugs include grape mealybug, *Pseudococcus maritimus* Ehrhorn, obscure mealybug, *Pseudococcus viburni* Signoret, longtailed mealybug, *Pseudococcus longispinus* Targioni- Tozzeti, vine mealybug, *Planococcus ficus* Signoret, citrus mealybug, *Planococcus citri* Risso, and Gill’s mealybug, *Ferrisia gilli* (Gullan) (Daane et al. 2012).

1.2 Distribution in the United States

Grape mealybug has been the predominant mealybug in Virginia in the past (Pfeiffer 2008). In an earlier survey of mealybugs in Virginia (part of a larger study on grapevine viruses), Jones (2016) identified 100 mealybugs, composed of 67 grape mealybug, 31 Gill’s mealybug, and 2 obscure mealybugs. Gill’s mealybug is a recently described species of mealybug found infesting pistachios, almonds, grapes, persimmons, and stone fruits as well as mulberry (Gullan et al. 2003). It is believed to have originated from the southeastern US and was reported in the Sierra foothills and Lake County, California for the first time in 2003. It has been reported in Alabama, Georgia, Louisiana, Oregon, Virginia, and Florida. Although a primary pest in almonds, they also infest wine grapes (Haviland et al. 2006; Wunderlich et al. 2013).

Longtailed mealybug appears cosmopolitan in tropical and subtropical environments, while they are present in greenhouses and homes in temperate regions (Tenbrink and Hara, 2007). Citrus mealybug is present throughout the citrus growing regions in Mexico and the US and in greenhouses. Obscure mealybug, longtailed mealybug, and grape mealybug are the predominant mealybugs in vineyards on the West Coast (Flint, 2015). Another invasive pest vine mealybug was reported in California in 1994 (Gullan et al. 2003) and has spread throughout the state since then. It was sighted in Oregon in the summer of 2021 (Oregon Department of Agriculture, 2022). Vine mealybug has been found in all grape-growing regions of California and recently documented in Oregon; however, it has not been reported in Virginia in previous studies.

1.3 Life Cycle

A slight variation in the life cycle is evident between different species of mealybugs. One common characteristic feature between different species is their adaptation to plant parasitism and modified metamorphosis. Adults exhibit distinct sexual dimorphism. The female exhibits paedomorphism, where adult females retain the external morphology of the immature forms even though they are sexually mature. Males undergo nymphal, prepupal, and pupal stages before molting into winged/ wingless adults. Parthenogenesis is common in these insects as well.

Mature females start growing, as their reproductive organs start developing. Females release species-specific pheromones to attract the males. Mated females oviposit in waxy/cottony ovisacs that contain 100-500 eggs. In some species such as Longtailed mealybug, nymphs hatch out immediately from the eggs and hence, the egg stage remains within the female until they are hatched. Adult females in this group of insects often protect the newly hatched crawlers by covering them with their abdomen (McKenzie 1967).

Generally, mealybug females go through three larval instars, and males through four instars. Newly hatched first instars are quite active and mobile and are known as crawlers. Crawlers hatch out from the eggs in about 3-16 days after oviposition. The crawlers have longer legs and antennae with fewer segments than the other stages. The crawler stage is usually responsible for covering diverse areas of the host. The male and female first instars often appear nearly identical. Second-instar nymphs still have proportionally larger legs and antennae in comparison to the body and fewer antennal segments than in the adults. The second and later instars are often covered in a waxy covering over their body. Male mealybugs only feed during the first two instars. By the end of the second instar, the male instars secrete a thin filamentous cocoon over their body. It has also been noted that males often lose their rostrum after the first molt (McKenzie 1967).

The third instar females often resemble an adult with smaller antennae and legs in comparison to their body size. Males start looking different from the third instar stage. The third instar stage is known as a male prepupa. The male prepupa is much smaller and elongated than the female third instar. It also has wing pads, more antennal segments, and lacks a rostellum (McKenzie 1967).

Adult female is large and characterized from the nymphal stages by the presence of a vulva. She also has numerous pores in the body that help in the identification of species. Adult females of some species also have two pairs of dorsal cavities or ostioles. The posterior pair is present on the seventh abdominal segment, while the anterior pair is present on the head. These ostioles secrete a defensive fluid when disturbed. Antennal segments in adult females vary from 2 to 9. They also have anal rings and lobes (McKenzie 1967).

The fourth instar male resides in a cocoon and is called a pupa. This stage differs from the third instar in having longer wing pads and more antennal segments. The fourth instar molts to form an adult male. An adult male is winged and gnat-like. They are feeble fliers having 9-10 segmented antennae. The mouthparts are vestigial and limited to a small circular opening found in the posterior-ventral part of the head. A pair of wings developed from the mesothorax. Different species of mealybugs may have males with fully developed wings (macropterous), some may have reduced and nonfunctional wings (brachypterous), and some may be wingless (apterous). Adult males have much longer legs than females. Adult males are easily identified under the hand lens due to the presence of beaded antennae, a thorax broader than the abdomen, two pairs of caudal setae (tail filaments), and a posterior end of the abdomen broadly triangular (McKenzie 1967).

Morphologically, grape mealybug and obscure mealybug look similar to each other, except when disturbed, grape mealybug releases bright red/orange liquid through ostioles toward the rear and front-top of the body, while obscure mealybug releases transparent liquid. Gill's mealybug does not secrete such a liquid. Grape mealybug has four slender caudal whitetails and short lateral projections on sides, while Gill's mealybug has two thick broad tails and lacks any lateral projections on sides. It has also been observed that female grape mealybug lays eggs in egg sacs and the immature stage crawls away from their mother, while the immatures of Gill's mealybug remain aggregated around the mother, especially on the underside of the body.

1.4 Pest Biology and Movement

Seasonal population dynamics of grape mealybug, citrus mealybug, and vine mealybug have been documented in California vineyards (Geiger and Daane 2001, Becerra et al. 2006, Grasswitz and James 2008, Cid et al. 2010). A slight variation is observed within and among species of mealybugs regarding seasonal feeding location and movement on the vine and depends on factors such as regional temperature and vineyard management practices (Geiger and Daane 2001, Grasswitz and James 2008). Mealybugs often overwinter as eggs or first instars or sometimes even second instars under the bark in the trunk or cordon. Throughout the season, the overwintering stage migrates from the overwintering site to the site having plant resources i.e., root to shoot to fruits/leaves. Some species have also been reported to overwinter in the roots. With the onset of warm weather during spring, overwintering instars often migrate to new growth or spurs to feed. When fully grown, adult females often migrate to the bark to oviposit, and young nymphs oviposit in two weeks (Valera 2005). The newly hatched nymphs then disperse to infest leaves or fruits (Geiger and Daane 2001). During late adulthood, females often migrate to the underside of the bark of the trunk or cordon to lay their eggs towards the end of the season (Geiger and Daane 2001, Grasswitz and James 2008). Grape mealybug has two generations per year in California vineyards, obscure mealybug has 2-3 overlapping generations, vine mealybug has up to 6 overlapping generations, and Gill's mealybug has 2-3 generations per year in California (Valera and Smith 2009).

1.5 Economic Importance of Mealybugs

One of the primary effects of the presence of mealybug is the production of honeydew, which supports the growth of sooty mold and attracts ant populations toward the grapevines. These insects use their piercing and sucking mouthparts to feed directly on the phloem sap. Phloem sap contains sugar in a relatively higher proportion than other essential nutrients needed by these tiny insects. Hence, a large amount of sticky, sugary fluid is excreted by these insects, which is known as honeydew. Honeydew produced by these insects is often deposited on the surface of grapevines, which supports the growth of sooty mold and attracts ant populations toward the grapevines. Healthy plants can tolerate low populations without significant damage while high populations reduce the plant vigor, yield, fruit quality, and wine quality (Kovacs et al. 2001, Martinson et al. 2008). Sometimes the mealybug infestation will not be evident in the vineyard until harvest when the appearance of mealybugs on the clusters forces the grape growers to drop the clusters. Crop loss in severely infested vines and loss of vigor in infested vines making them more vulnerable to cold injury and other environmental stress are other drawbacks associated with this disease (Martinson et al. 2008).

The greatest economic impact resulting from mealybugs is their potential role as vectors of important vineyard viral diseases, notably grapevine leafroll-associated viruses (GLRaV).

1.6 Grapevine Leafroll Disease

The grapevine leafroll disease triad has three components: 1. Virus complex 2. Grapevine (host), and 3. insect vector. The grapevine leafroll disease is caused by the virus complex named Grapevine leafroll-associated viruses (GLRaV). GLRaVs consist of virus species in different genera and belong to the family Closteroviridae, containing several closteroviruses in the genus *Ampelovirus*. About ten species of GLRaV are associated with GLD. Among these species, GLRaV-3 is the most prominent, causing GLD worldwide. It is introduced into a new vineyard by infected plant materials (Lo et al. 2006). An insect vector facilitates the movement of grapevine leafroll disease depending on the virus present. Scales and mealybugs transmit GLRaV (Wilcox and Wolf 2008, Jones 2012, Miles et al. 2020).

Symptoms of the disease differ depending on the varieties of grapes affected. In red-fruited varieties, the leaf tissues between the veins turn red, along with the downward curling of the leaves. In white varieties, leaf tissues between the margin turn yellow with downward curling of the leaves. It causes stunted growth of the vines, delaying ripening and reducing the sugar content and coloration of the fruit (Wilcox and Wolf 2008, Jones 2012, Miles et al. 2020). Visual symptoms associated with GLD first appear on the leaves and fruit during the growing season and become more visible as the season progresses (Martinson et al. 2008). GLD symptoms are not always distinct on the host, as explained earlier. Some varieties can show distinct symptoms while others, although infected, may be asymptomatic. Furthermore, the typical symptoms associated with GLD can easily be confused with nutrient deficiency, herbicide damage, and other plant diseases (Rayapati et al. 2008).

Management of viral diseases can be a daunting task. The only two ways to manage viral disease are either to control the host plant or control the vector responsible for disease transmission. Elimination of the host plants infested with GLD and replacement with new plants after a duration can be economically challenging both for large-scale and small-scale vineyards. In smaller vineyards, replacing vines as soon as the symptoms appear can be reasonable. As explained earlier, as visual assessment is not the best tool, testing the vines for the presence of GLRaV would be a better option. The second option would be the control of insect vectors responsible for the transmission of GLD, which does not eliminate the virus but prevents spreading to new and healthy vines (Jones 2016).

The most common mealybug in Virginia, grape mealybug, is a known vector of GLRaV-3; this is the most severe of the eight types of grapevines leafroll reported so far. Golino et al. (2002) reported that they could confirm that GLRaV-3 isolates are transmitted by four species in California – obscure mealybug, longtailed mealybug, citrus mealybug, and grape mealybug have the potential to transmit. This was the first experimental evidence of grapevine leafroll virus transmission by mealybugs. Management of mealybugs will be critical to the management of GLRaV (Cooper et al. 2018). The mitigation of damage due to arboviruses is largely dependent on the control of its vector. It would be interesting to study the species composition and their management in Virginia vineyards. Recent research by Taylor and Nita (2019) has documented GLRaV-1, GLRaV-4, grapevine virus A, grapevine virus B, GRSPaV, and obscure mealybug for the first time in Virginia.

1.7 Association with Ants

Ants have been observed in proximity to honeydew-producing insects, including mealybugs. The interactions between ants and honeydew-producing hemipterans have been studied extensively in multiple ecosystems, and this association has been found to be beneficial to both insects (Renault et al. 2005, Styrsky and Eubanks 2006, Brightwell and Silverman 2010, Wilder et al. 2011). The mutualism of mealybugs with ants is based on the consumption of honeydew provided by mealybugs in exchange for protecting honeydew producers against their natural enemies (Delabie 2001, Mansour et al. 2012, Feng et al. 2015).

Ant-mealybug association has also been reported to have a mediating effect on the niche they occupy. The oviposition preference of the leafroller *Sylepta derogata* Fabricius (Order Lepidoptera: Family Pyralidae), was higher on plants with ant-mealybug mutualism. The larval parasitoid of *S. derogata*, *Apanteles derogatae* Watanabe (Hymenoptera: Braconidae), also had higher parasitism on mutualist absent plants (Xu et al. 2019). In a recent study of three ant species native to the Mediterranean, foraging on grapevine canopies was found to induce a population increase in vine mealybugs. However, in the same study, only 16% of the total mealybug population on the site were tended by ants (Beltrà et al. 2017).

Most mealybugs can form root colonies on grapes, although the tendency varies among species. Ants play an important role in the transfer of mealybugs to the roots, and movement in the vineyard (Daane et al. 2007). Grasswitz and James (2008) studied the movement of grape mealybug between vines, including self-directed movement by walking, or movement aided by the wind. Although crawlers are the most mobile stage, they show a little tendency to move away from the original point of infestation. Any mealybug life-stages had limited movement from the original point of infestation. However, the study did not include an ant-assisted movement of mealybugs (Grasswitz and James 2008). The ability of mealybugs to disperse is limited due to their slow movement and is often facilitated by the movement of infested plant materials or debris, wind, or contaminated machinery around the field (Lo et al. 2006). In a study of mealybugs and grapevine leafroll associated viruses, Jones and Nita (2016) found that the movement of the grapevine leafroll disease was not affected by wind– this would be consistent with the ant-assisted movement of the vector mealybugs.

Crawlers of four species of armored scale insects (Hemiptera: Diaspididae) have been recorded to attach themselves to three different insect species common housefly (*Musca domestica* L.), mealybug destroyer (*Cryptolaemus montrouzieri* Mulsant), and Argentine ants (*Linepithema humile* Mayr). This process is called phoresis. They utilize hairs at the end of each of their legs ending up in suction cups to attach themselves to their host (Magsig-Castillo et al. 2010). Another instance of ant-mealybug association has been observed in the mealybug *Paraputo anomala* Newstead (Hemiptera: Pseudococcidae), where juvenile females disperse by attaching themselves to the founding queen ant *Aphomomyrmex afer* Emery (Hymenoptera: Formicidae) via phoresis (Gaume et al. 2000). While sampling vineyard-infesting mealybugs from roots, Pfeiffer (2018) collected at least three species of ants, the most common being the smaller yellow ant (*Lasius claviger* Roger), pavement ant (*Tetramorium caespitum*), and thief ant (*Solenopsis molesta*) (Hymenoptera: Formicidae) in Albemarle County (pers. comm.). When smaller yellow ants were collected into a container that contained a root sample with mealybugs attached, a worker ant picked up a mealybug and ran around the container in an agitated fashion. An understanding of the role of ants may provide a clearer view of the epidemiology of grapevine leafroll disease (Pfeiffer, pers comm).

In California where most of the research on mealybugs has been conducted, Argentine ants have a mutualistic relationship with honey-excreting homopterans and affect their natural enemies as well (Tillberg 2007). An association with ants not only increases the density of obscure mealybugs but also lowers the density of parasitoids *Pseudaphycus flavidulus* Brèthes and *Leptomastix epona* Walker (Hymenoptera: Encyrtidae). Densities of the mealybug destroyer have also been reported to be higher in the ant-tended vines having a higher density of mealybugs. They avoid being detected due to their striking resemblance to mealybug adults (Daane et al. 2007). The effect of ants on mealybug parasitism depends on the species of ants and the latter also affects the magnitude of ant attacks. The Argentine ant largely influences the oviposition attempts and offspring of parasitoid *Anagyrus vladimiri* Triapitsyn (Hymenoptera: Encyrtidae). Argentine ants have been observed to reduce the foraging time, oviposition attempts, and the number of offspring produced in a parasitoid wasp (*Anagyrus pseudococci* Girault) but not in *Coccidoxenoides perminutus* Girault (Sime and Daane 2014). Mealybug parasitism rates were also higher in ant-excluded vines (Daane et al. 2003, Daane et al. 2007, Mansour et al. 2012, Beltrà et al. 2017).

1.8 Pest Management

The concept of Integrated Pest Management (IPM) introduced as early as 1967 has never been more relevant before than in the current time when overdependence on chemical control has resulted in the ever-emerging pest resistance against insecticides, the toxic effect of insecticides against natural enemies and the pollinator and the continual need of research for the new mode of action to cope up with it. IPM focuses on controlling pests rather than completely eradicating them (Smith and Bosch 1967). This method focuses not only on the grapevines but also on pest population dynamics, behavior, natural enemies, and host preferences. Cryptic pests such as mealybugs require the development of effective monitoring and management tools. Integrated pest management is a long-term approach to maintaining a healthy vineyard with low risk to workers and the environment.

Cultural Control

The first step in pest control can be the use of cultivars with genetic resistance against the mealybugs. A recent study investigating potted plants in vine mealybugs in California vineyards has documented Cabernet Sauvignon and Chardonnay as the most suitable varieties for vine mealybug population growth (Naegele et al. 2020). Rootstock varieties IAC 572, 10-17A, and RS-3 confer some degree of resistance against vine mealybug. The *Vitis vinifera* varieties appear susceptible to vine mealybug, however, resistance can vary with scion variability. However, table grape varieties Valley Pearl and Flame Seedless appear to confer more resistance to vine mealybug than wine grape cultivars Chardonnay and Cabernet Sauvignon (Naegele et al. 2020).

Insecticides are the most common option to control mealybugs in commercial vineyards. However, their waxy covering protects them from the chemicals such that the growers must explore alternative options (Copeland et al. 1985, Walker 2000). An increased use of nitrogen fertilizer over the season resulted in an increase in the survival of immatures and the size of adult pink sugarcane mealybug females in laboratory and semifield settings. However, the study failed to produce similar results in field settings probably due to natural enemies and environmental conditions (Rae and Jones 1992). In a more recent study with vine mealybugs, the application of nitrogen fertilizers had a positive effect on fecundity, survival, and shorter developmental times (Hogendorp et al. 2006, Cocco et al. 2021). Nitrogen fertilizer also increases the pruning weight and cluster weight of grapes. However, it does not influence anthocyanin content, titratable acidity, and soluble solids during harvest (Cocco et al. 2021).

Cover cropping adopted in vineyards helps in improving soil structure, organic matter, and carrying capacity, minimizing soil erosion, and improving plant vigor. Inter- and intra-row cover cropping not only affects grapevine growth and fruit yield and quality, but also vine mealybug development and reproduction (Muscas et al. 2017). The use of legume cover crops and soil tillage in vineyards promotes mealybug development, particularly survival, fecundity, and fertility, and reduces developmental time, more likely due to incorporation of nitrogen into the soil. Hence, cover cropping and tillage favors establishing ant colonies in vineyards and reduces parasitoid activity; which in turn promotes mealybug infestations (Serra et al. 2006; Mgocheki and Addison 2010, Cocco et al. 2020). Hence, no-tillage practice in the east coast seems to favor management of mealybugs.

Mealybugs have a broad host range that commonly includes ornamental plants making the sampling of pest-infested sample tasks daunting. Weed management can be an option for checking the mealybug population on the field, as they serve as reservoir hosts that provide safe refuse to the pest and can promote breeding grounds (Daane et al. 2004). Mealybug density and cluster infestation are also higher in spur-pruned varieties than in the cane-pruned varieties, as spur-pruned varieties provide more bark for residing than the cane-pruned vines (Geiger and Daane 2001). Summer pruning, directed in the vineyard to remove excess shoots and leaves in grapevines and promotes aeration and sunshine in the canopy, might negatively affect the mealybug population in the vines by promoting natural enemies (Walton and Pringle 2004b).

Another step in cultural control can be studying the history of the site and avoiding planting new vines in areas having a history of mealybug/scale insect infestation. One labor-intensive practice can be cluster thinning and bark stripping (Mansour et al. 2018). Bark stripping incorporated with mineral oil applications also reduces vine mealybug density (Pavlović et al. 2019). Multiple factors stimulate grape yield and the mealybug development on the vine such that vineyard management practice cannot be generalized; and the mealybug management practice depends on the climate, soil condition, grape varieties, rootstock, etc.

Biological Control

Biological control has been categorized into three categories: 1. Classical biological control 2. Conservation 3. Augmentation (Cate 1990). Classical biological control is the management of a pest that has been accidentally introduced from another continent (invasive pest) by importing and releasing biological control agents from the same continent as the invasive pest. Parasites that attack the native mealybug species (*Pseudococcus* species), do not attack vine mealybug which is widely spread and devastating in vineyards in California and Oregon. Two species of parasitoid wasps (*A. pseudococci* and *A. vladimiri*) are potential candidates for biological control of invasive vine mealybug (Triapitsyn et al. 2007, Daane et al. 2012 Cocco et al. 2020). These are the tropical and subtropical species, *A. pseudococci* from Sicily (Italy), while *A. vladimiri* from Italy, Israel, Russia, Spain, Turkmenistan, Tunisia, and California (Triapitsyn et al. 2007, Mansour et al. 2017a, Cocco et al. 2020). Despite having a broad host range, *A. vladimiri* exhibits differential host preference among the Pseudococcidae host family, with a clear preference for vine mealybug (Bugila et al. 2014a, Bugila et al. 2014b). Both sexes of *A. vladimiri* are attracted to the vine mealybug sex pheromone, a kairomonal response (Franco et al. 2008, Franco et al. 2011).

Biological control via conservation focuses on habitat modification and pesticide selection to enhance the survival of existing biological control agents (native or introduced). Selection of pesticides having low effect on pollinators and natural enemies might be one of the primary steps in enhancing biological control. The natural enemies of mealybugs commonly encountered in the vineyard include mealybug predators and parasitoids such as lacewings (*Chrysoperla* spp.) (Neuroptera: Chrysopidae), parasitoid wasps, mealybug destroyers, cecidomyiid flies (*Diadiplosis koebelei* Koebele), etc. The presence of wildflower spots or strips in the vineyards also supports parasitoid fecundity and population growth (Benelli et al. 2017). Conservation of the wildflowers and wooded area along the edge of the vineyard promotes the survival of natural enemies.

Augmentation is a biological control procedure which is the release of an overwhelming number of biological control agents to reduce a pest problem. Some studies have researched the efficacy of inoculative or inundative releases of parasitoids in the population density of mealybugs in the field. The mass-reared parasitoid *A. pseudococci,* when released to control the vine mealybug population in California vineyards lowered the vine mealybug density and cluster damage in parasitoid-infested plots relative to the control (Daane et al. 2006). However, the results were inconclusive that the three inundative releases of parasitoids were solely responsible for controlling mealybug density; as mealybug population density was already decreasing before the release of parasitoids and the season-long parasitism was not significantly different between the treatments (Daane et al. 2006). A similar biological control program had been launched with the release of 10,000 or 50,000 *A. pseudococci* per hectare to control citrus mealybug in citrus orchard in Israel. This augmentative release increased the parasitoid density; however, did not decrease the mealybug density and fruit cluster infestations (Mendel et al. 1999). Another inoculative release of *A. vladimiri* released in vineyards in Tuscany, Italy heavily infested with vine mealybug documented some promising results. *A. vladimiri* at a density of 1000 insects per ha in May and *C. montrouzieri* at densities of 500 insects per hectare in June and July were released with effective results in a significant reduction in the mealybug population (Lucchi and Benelli 2018).

Chemical Control

Chemical control is one of the primary methods implemented by vineyard managers against grape pests in Virginia (Franco et al. 2009). The intensive and repetitive use of chemicals has not only reduced natural populations of mealybugs' natural enemies but also the development of insecticide resistance in the pest itself leading to continuous and increasing use of the chemicals (Franco 2004, Franco 2009, Venkatesan 2016). Newer products with better coverage against mealybugs have been introduced with mixed results in recent times.

Delayed dormant spray of insecticidal soaps and dormant oils to control pests provides some control in mealybugs and scale insects if the populations are not too high (Varela et al. 2009). Some of the commercially available insecticides against mealybugs available in the market include insect growth regulators such as buprofezin (Applaud), neonicotinoids like acetamiprid (Assail), dinotefuran (Venom), imidacloprid (Admire), and spirotetramat (Movento), organophosphates like phosmet (Imidan) and malathion, and even pyrethroids like cyfluthrin (Baythroid).

Some research has documented the population reduction of natural enemies due to the use of pesticides against mealybugs. Organophosphates (chlorpyrifos, malathion), fipronil, pyrethroids (cypermethrin), imidacloprid, and buprofezin have been documented to have an adverse effect on natural enemies and pollinators (Mgocheki and Addison 2009, Mansour et al. 2011, Whitehorn et al. 2012, Vanaclocha et al. 2013, Tirado et al. 2013, Byrne et al. 2014, Mgocheki and Addison 2015, Mansour et al. 2018). On the other hand, spirotetramat does not exhibit adverse effects against natural enemies and pollinators and has the capability to move up and down through the vascular system of plants (Maus 2008, Mansour et al. 2018).

The mating disruption technique is another option that can be a useful tool against vine mealybugs. Mating disruption is when pheromones, released by females and used by males to locate females, are released in large amounts in the vineyard such that any kind of communication between males and females is disrupted. Recent research on mating disruption in California vineyards has promising results in limiting the spread of vine mealybugs over large vineyard areas. The tool seems effective when the pest population is low and when used complementary to the chemical treatment (Hogg et al. 2021).

As ants tend to defend mealybugs against their natural enemies, biological control agents released or already present in the field have often been used in addition to sugar dispensers to enhance pest control efficiency (Daane et al. 2006, Rahmouni et al. 2013, Beltrà et al. 2017, Parrilli et al. 2021, Ricciardi et al. 2021, Pérez-Rodríguez et al. 2021). Some of the commonly used ant toxicants in bait include boric acid, hydramethylnon, imidacloprid, or thiamethoxam (Rust et al. 2004, Tollerup et al. 2004, Daane et al. 2006, Cooper et al. 2008, Nondillo et al. 2016, Parrilli et al. 2021).

Liquid ant baits containing arsenical insecticides mixed with honey were used to control ants in the past (Barber 1916). When tested in the laboratory, these killed workers quickly but failed to kill queens (Knight and Rust 1991). Ant toxins having delayed toxicities have been tested against Argentine ant populations. Important attributes of these toxicants are prolonged delayed toxicity over a wide dosage range, readily transferable from one ant to another, and not repelled or avoided by ants when used in baits. Markin (1970) used 32P-labeled baits and determined that a single worker ant typically feeds 4-12 other workers. The toxin-receiving ants then passed the food to the other workers in the nest such that within forty-eight hours, 156 workers had received food from the first ant. Hence, immediate killing of ants removes foraging from the equation such that the delayed toxicity would have more effect on the larger part of the population. The bait was diluted in each exchange (Rust et al. 2004). The use of trail pheromones by the ants when they detect the ant baits, in turn, increases the recruitment and consumption of toxins by 29% in 3 hours (Greenberg and Klotz 2000).

Liquid ant baits have been evaluated for their efficiency in the control of ants and associated pests in commercial vineyards (Klotz et al. 1998, Rust et al. 2004, Daane et al. 2006, Nelson and Daane 2007, Beltra et al. 2017, Parrilli et al. 2021). Either 25% sucrose solution or toxins incorporated with 25% sucrose solutions have been tested in the field. In addition to sugar baits, biological control agents have also been used with promising effects against ants and mealybugs (Beltra et al. 2017, Parrilli et al. 2021). Honeydew is the main reason why ants tend after mealybugs. The use of sugar baits or toxic sugar baits allocated in small doses over the vineyard as an alternative food source is more effective in targeting not only the foragers but the entire nest that derives food from it (Cooper et al. 2008, Daane et al. 2008). The forager ants when fed on toxicants present on sugar dispensers baited with insecticides are not killed immediately, but the toxicants are diluted when passed on from one ant to another during feeding. This process of transferring food or other fluids among members of the community through mouth-to-mouth (stomodeal) or through anus-to-mouth (proctodeal) feeding, called trophallaxis, helps baits with a low concentration of toxicants to reach from vines to ant nests and recruit more ants to the bait (Rust et al. 2004).

Different methods can be deployed for ant management in the vineyards which may include barrier sprays or selective baits. The tactics deployed depend on the budget and size of the commercial vineyard. One of the primary methods includes the use of exclusion barriers such as a sticky trap around the trunk to prevent access of ants to the cordon (Schwartz 1988; Addison and Samways, 2012). Chemical barriers include the use of a sticky band trap impregnated with insecticides for use as an ant barrier. Among the thirteen chemical barriers tested against Argentine ants and common pugnacious ants (*Anoplolepis custodiens* Smith), chlorpyrifos and terbufos-impregnated slow-release bands were highly effective (Addison 2002). Barrier treatment works effectively against foragers, however, does not affect the ants in the nest or field.

Surveillance of the ant species, the foraging distance of ants, bait size, distribution, and density are the key to controlling ants in the field using low-toxicity baits. Furthermore, the success of the program depends on the attractiveness of the carrier and null/minimal damage to the non-target species. The foraging activity of Argentine ants was higher, thus more baits are necessary compared to the ant *A. custodiens* F. Smith and acrobat ant *Crematogaster peringueyi* Emery (Nyamukondiwa and Addison 2014). Argentine ants are also known to forage at distances less than 36 meters in California vineyards such that it would be effective to deploy bait stations at intervals of 36m or less (Hogg et al. 2018).

The effectiveness of the bait can also be increased by changing the design and deployment patterns. The higher the number of bait stations, the more effective the control (Daane et al.

2006). Earlier season deployment of dispensers is also recommended to target spring foragers. 250 ml dispensers are found to work best in controlling ants in their relative advantage to being efficient in deployment on the field, and easy to clean and refill. Smaller-sized dispensers require frequent maintenance to refill and deploy and hence need more manpower (Daane et al. 2006).

Justification of Research

The incorporation of a multidisciplinary approach in pest management can be an effective tool for controlling mealybugs. Decisions regarding the use of different methods of pest control should be based on the physiological and bio-ecological characteristics of pests (Mansour et al. 2018). For insect pests such as mealybugs, it is important to time the spray schedule with vulnerable life-stages (crawler stage) in comparison to adults with a thick layering of wax such that the chemical can penetrate the cuticle layer. Furthermore, in comparison to the older pesticides, the use of newer chemicals provides more penetration to the protected areas inside the vines.

Mealybugs are cryptic pests, which once appeared on vineyard sites are very hard to control. It is therefore a necessity to develop pest monitoring and management tools for addressing certain research questions. Most of the work on mealybugs and ants is carried out on the west coast. This dissertation describes and documents the distribution of species of mealybugs in Virginia via various sampling methods. The species of ants and their relation to the mealybugs have been established on the West Coast. To develop an effective pest management tool for mealybugs, another important tool is understanding the species composition of ants in Virginia in close association with the mealybugs. We also aimed to quantify the species composition of ants in Virginia in close association with the mealybugs. We also study the effect of control of ant populations on the research sites on the population of mealybugs. Finally, we examine the efficacy of some insecticides against the mealybugs in the vineyard.

Research Objectives

Objective 1: A survey of mealybug species and their seasonal activity in vineyards of the Piedmont region, Virginia

Objective 2: Association between ants and mealybugs in Virginia vineyards.

Objective 3: Determine the efficacy of different insecticides against mealybugs in the vineyard.

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Chapter 2

A survey of mealybug species and their seasonal activity in vineyards of the Piedmont region, Virginia

Abstract

Mealybugs (Superfamily Coccoidea: Family Pseudococcidae) are a cryptic and nuisance pest that is common in vineyards and deciduous fruit crops across the US. With the introduction of invasive species of mealybug i.e., vine mealybug (*Planococcus ficus* Signoret) in the West, growers often encounter challenges related to the issue of declining vine health and potential for economic losses. While the research has been extensive in the west, the current research in Virginia focuses on species-level identification and population distribution of these species in different commercial vineyards around Virginia. Pheromone-baited sticky traps were deployed with commercially available species-specific lures to monitor male mealybugs around the vineyards. Adult females and males and nymphal stages in the grapevines were monitored by a 5-minute count on grapevines randomly. The species present in the vineyard, the life-stages of each species of mealybugs were recorded weekly from April to the end of September. Two species of mealybugs- grape mealybug, *Pseudococcus maritimus* (Ehrhorn), and Gill's mealybug, *Ferrisia gilli* (Gullan) were prevalent throughout the sampled areas in Virginia. The nymphal stages were active as early as the first week of May, often found migrating to new growth spurs. The first generation emerging from overwintering life-stage although low in number, exhibited an increase in numbers when the season started warming up.

2.1 Introduction

A notable increase in mealybug infestations has been observed in the North American vineyards during recent periods (Daane et al. 2006, 2008, 2012). Mealybugs are ubiquitous pests within grape cultivation regions in the United States and Canada, feeding on virtually all sections of grapevines, encompassing even the subterranean roots. The mealybug species that inhabit vineyards are classified within the subfamily Pseudococcinae, family Pseudococcidae, and superfamily Coccoidea (McKenzie 1967). Among the multitude of mealybug species that encroach upon grapevines, this discourse will center on certain species that have attained a primary pest status. Noteworthy among the species of prime concern during our investigation is the grape mealybug, *Pseudococcus maritimus* (Ehrhorn); the obscure mealybug, *Pseudococcus viburni* (Signoret); the longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzeti); the vine mealybug, *Planococcus ficus* (Signoret); the citrus mealybug, *Planococcus citri* (Risso); and Gill's mealybug, *Ferrisia gilli* (Gullan) (Daane et al. 2012).

Grape mealybug has been the predominant mealybug pest of grapes in Virginia (Pfeiffer 2008). An earlier research project on grapevine leafroll-associated viruses in wine grape varieties and native grape species in Virginia has identified grape mealybug, Gill's mealybug, and obscure mealybug (Jones 2016). Grape mealybug, although primarily reported from grapes, is reported to infest deciduous fruit crops as well (Burts and Dunley 1993). Vine mealybug has been found in all the grape-growing regions of California; however, it has not been reported in Virginia in previous studies. Longtailed mealybug appears to be cosmopolitan in tropical and subtropical environments, while they are present in greenhouses and homes in temperate regions (Tenbrink and Hara 2007). Citrus mealybug is an important pest in vineyards in Spain and Brazil (Cid et al. 2010). It is a polyphagous pest, that prefers citrus plants. It is a common pest of citrus and ornamental plants primarily in greenhouses and nurseries. Gill’s mealybug is a newly described species of mealybug found in pistachio-growing regions of California on almonds, grapes, persimmons, stone fruits and mulberry (Gullan et al. 2003).

The introduction of mealybugs to vineyards has the potential to cause significant damage (Millar et al. 2002, Daane et al. 2006, 2008, 2012, CDFA 2013, Wunderlich et al. 2013). The presence of mealybugs in the vineyard causes the production of honeydew, which remains as the residue in different parts of grapevines where they are located. This causes the growth of sooty mold in the vines, attracting ants to the grapevines. The greatest economic impact resulting from mealybugs is their potential role as vectors of important vineyard viral diseases, notably grapevine leafroll-associated viruses (GLRaV). The most common mealybug in Virginia, grape mealybug, is a known vector of GLRaV-3. It is the most severe of the eight types of grapevines leafroll reported so far (Jones and Nita 2016). Golino et al. (2002) reported that they were able to confirm that four species found in California – obscure mealybug, longtailed mealybug, citrus mealybug, and grape mealybug have the potential to transmit GLRaV-3 isolates. This was the first experimental evidence of grapevine leafroll virus transmission by obscure mealybug and grape mealybug. In addition, it was also reported for the first time that GLRaV-5 could be transmitted by a longtailed mealybug.

Management of mealybugs will be critical to managing grapevine leafroll viruses in the vineyard (Cooper et al. 2018). The mitigation of damage due to arboviruses or due to the presence of mealybugs is dependent primarily on the control of its mealybug vectors. Investigating the mealybug species composition and their management in Virginia vineyards would provide meaningful insights.

2.2 Materials and Methods

Sampling Sites

We scouted several commercial vineyards with a previous record of mealybug infestation or grape leafroll disease for the presence of mealybugs (Orange County (site H), Nelson County (site S), Botetourt County (site VMV), Fauquier County (site P), Albemarle County (site GEW), Orange-Albemarle County (site B), and Augusta County (Site BR)). Among these sites, five vineyards (H, GEW, P, S, and VMV) were monitored once a week from the end of April 2019 to October 2021. The above-ground parts of the grapevines (mealybugs on cordons, shoots, canes, and clusters) as well as the root samples were surveyed by visual examination of at least one row of vines per vineyard per day. When spotted in the field, mealybugs were photographed before being placed into 70% ethanol. During the early season, when insects were not spotted in the field, some leaf/shoot samples were taken back in 70% alcohol to check for the presence of mealybug nymphs.

Mealybug Species Identification

Morphological Identification

Most of the mealybugs were identified and photographed in the field based on morphological tools. Some samples were taken back to the lab for identification, while others were sent to us for identification.

Genetic Analysis

The genetic analysis of mealybugs is based on a tool developed by Daane et al. (2011). DNA extraction was carried out using a DNeasy Blood and Tissue kit. Due to the limitation in the reagents available, we pooled the sample and then carried out a genetic analysis of 24 samples from three different sites. Several genomic regions have been used for the identification of mealybugs and other insects. One of these regions that has been used is the mitochondrial cytochrome oxidase subunit I gene (COI). The species-specific primers designed for grape mealybug, scarlet mealybug, longtailed mealybug, vine mealybug, citrus mealybug, obscure mealybug, and Gill's mealybug were used for the species identification (Table 2.1). PCR was carried out in a BIO-RAD C1000 thermal cycler using a multiplex PCR plus kit. An initial denaturation step at 95 ℃ for 5 min was followed by 30 cycles of 30s at 94 ℃, 90s at 53 ℃, and 90s at 72 ℃, with a final extension of 10 minutes at 72 ℃. All reactions used a QIAGEN multiplex PCR master mix that includes $MgCl_2$ (3mM), buffer, dNTPs, and *Taq* polymerase.

After amplification, 4µl of each PCR product was visualized by electrophoresis on a 2% agarose gel using GelRed. Each reading consists of a single mealybug. Our gel reading was divided into two replicates of each sample and two replicates of a no-template control (no DNA). The positive control contains the DNA samples of grape mealybug and Gill's mealybug from previous research by Taylor Jones in 2012 from AREC lab, Winchester. The first replicate was loaded with forward primer for scarlet mealybug (PCa), vine mealybug (PF), citrus mealybug (PC), and Gill's mealybug (FG), and the reverse primer. The second replicate was loaded with forward primer for grape mealybug (PM), Longtailed mealybug (PL), obscure mealybug (PV), and reverse primer.

Table 2.1. Size and Name of species-specific primers used for mealybugs.

Amplicon lengths	Size	Primers used
Scarlet mealybug	650 bp	PCa / MB-R
Long-tailed mealybug	600 bp	PL / MB-R
Vine mealybug	450 bp	PF / MB-R
Grape mealybug	400 bp	PM / MB-R
Citrus mealybug	350 bp	PC / MB-R
Obscure mealybug	250 bp	PV/ MB-R
Gill’s mealybug	150 bp	FG/ MB-R

Primer sequences:

FG	5’-GAA TCA TTA ATT TCT AAA CGT TTA CTA A-3’
MB-R	5’-CAA TGC ATA TTA TTC TGC CAT ATT A-3’
PC	5’-TAA TCT ATT TTT ATC TAT CAA TTT AAC C-3’
PCa	5’-TGC AAC AAT AAT TAT TGC CAT C-3’
PF	5’-CTT TGT TGT AGC TCA CTT TCA C-3’
PL	5’-CCA TTT ATC TTT GAT CCA CAG-3’
PM	5’-CTG ATT TCC TTT ATT AAT TAA TTC AAC-3’
PV	5’-ATA TTT CTT CTA TTG GTT CAT TC-3’

Male mealybug activity

Grape mealybug has been reported to have two generations per year in California vineyards (Geiger and Daane 2001, Varela et al. 2006), while Gill’s mealybug has two to three generations per year (Gullan et al. 2003, Varela et al. 2006). Vine mealybugs in the same region can have up to seven generations per year (Varela et al. 2006, Gutierrez et al. 2008), while obscure mealybugs have 3-4 generations per year (Walton and Pringle 2004, Varela et al. 2006). Most of these mealybugs overwinter as eggs or in the first instar except vine mealybugs which do not overwinter (Varela et al. 2006).

Pheromone Delta Trap

Male mealybug activity in the field was recorded using pheromone-baited delta traps from Alpha Scents, Inc., OR. This method uses the species-specific sex pheromones, (pheromones released by the female to attract males of the same species to locate her for mating). The seasonal flight activity of male mealybugs was monitored in the vineyard by deploying commercially available species-specific pheromone lures. We used a white pheromone delta trap and sticky liner in 2018, however, it attracted more bees to the trap. Hence in 2019, we changed the plastic delta trap (from white to red) to monitor the male mealybug population. The pheromone lures were ordered from Evergreen Growers Supply, OR. Lures specific to grape mealybug, vine mealybug, citrus mealybug, obscure mealybug, and longtailed mealybug were used in the site. The pheromone delta traps were placed randomly in the mealybug-infested regions in all the sampling sites.

Figure 2.1.a. Pheromone Delta Trap; b. Adhesive Band Trap; and c. 5-minute visual count on the grapevine.

Adhesive band traps

The adhesive band traps were deployed in the vineyards as a part of the ant-mealybug experiment. We deployed adhesive band traps on ten vines, one each on the cordon and the trunk of each of the vines (hence a total of 20 tapes). The tapes were cut out 6 cm long, placed after removing the bark layer, and replaced a week after the trap had been placed. We deployed these tapes weekly to monitor ant and mealybug movement up and down the vines.

The captured male mealybugs on each of the traps were counted and averaged each week over the five traps per site and standardized to calculate the average number of mealybugs per day throughout the growing season. All data were analyzed using SAS JMP software. For trap counts, a chi-square test was performed to calculate if there is an effect of placement of sticky band trap on the trap capture data among vineyard sites and on each sample date.

Mealybug Life-stage Activity

The activity of mealybugs was monitored in the vineyard weekly or biweekly during the sampling period (April to September). Insects like mealybugs have an uneven clumped distribution in the vineyards (Geiger and Daane 2001). During sampling, the mealybug population was checked under the bark and in fruit clusters and leaves next to the wooded regions of the vines (Geiger and Daane 2001). The relative population of mealybugs was recorded by visual inspection of the vines for about five minutes per vine. Crawlers were counted separately from other life-stages, while the second and third instars were counted together as nymphal stages and adult stages were recorded. The male mealybugs, either second instar, pupal stage, or adult stage present on the trunk were counted together as the number of male mealybugs. The total number of each of the life-stages was summed and analyzed using the Generalized Linear Model (GLM) with Poisson distribution and log link function. Analysis was carried out separately for 2020, 2021, and 2022. The model used the total mealybugs per 5-minute search per vine as a function of life-stages, date, year, and date*year interaction.

2.3. Results

Morphological Identification

Grape mealybug and Gill's mealybugs remain the dominant species of mealybugs throughout the sampled vineyard sites in Virginia. All the vineyards we visited throughout our study, or the samples sent to us for species identification of mealybugs in vineyards comprised the same two species of mealybugs. Obscure mealybug was observed on two separate instances, while vine mealybug was absent from our sampled data.

Figure 2.2.a. Grape mealybugs; b. Grape mealybug egg mass; c. Gill's mealybugs; and d. Adult Gill's mealybugs with the crawler stage.

Genetic Identification

The species of mealybugs collected from the root samples as well as the shoot samples when analyzed PCR revealed the same two species of mealybugs (grape mealybug and Gill's mealybug). In Figure 2.3.a, our 22 samples are compared against the positive grape mealybug sample from Jones 2016 and has positively identified them as grape mealybug having 400bp amplicon length. In Figure 2.3.b, our 2 samples are compared against the positive Gill's mealybug sample from Jones 2016 and has positively identified them as Gill's mealybug having 150bp amplicon length.

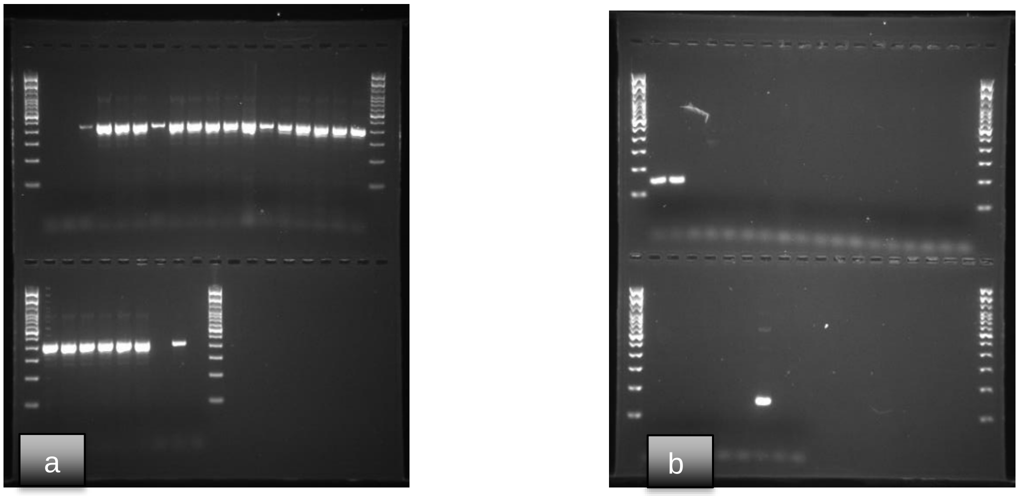


Figure 2.3. Mealybug samples from three different locations on two separate gel experiments. 2% agarose gel with DNA revealing the presence of 22 Grape mealybugs (a); and 2 Gill's mealybugs (b).

Male Mealybug Activity

Pheromone Delta Trap Capture

The number of male mealybugs captured in the pheromone delta traps showed some significant variation across some of the sampling dates and across the sampling sites (Table 2.2.a). Meanwhile, male mealybugs' mean trap capture data exhibited no significant differences across most of the vineyard sites, however there were some significant differences in mealybug densities in some of the annual average data (Figure 2.2.b).

Table 2.2.a. Male mealybug densities on different field sites from 2019 to 2021. P values less than 0.05 signifies significant difference.

Field Site	**Year**	**May**	**June**	**July**	**August**
GEW	2019	χ^2=7.45e-6 p=0.9978	χ^2=7.45e-6 p=0.9978	χ^2=7.588 p=0.0059	χ^2=102.98 p<0.0001
GEW	2020	χ^2=7.091 p=0.0077	χ^2=0.6198 p=0.4311	χ^2=18.615 p<0.0001	χ^2=9.546 p=0.0020
H	2019	χ^2=2.68e-6 p=0.9987	χ^2=2.68e-6 p=0.9987	χ^2=2.68e-6 p=0.9987	χ^2=2.174 p=0.1403
H	2020	χ^2=5.136e-6 p=0.9982	χ^2=6.766 p=0.0093	χ^2=0.631 p=0.4269	χ^2=4.328 p=0.0375
H	2021	χ^2=5.689e-6 p=0.9981	χ^2=6.415 p=0.0113	χ^2=6.415 p=0.0113	χ^2=0.575 p=0.4479
P	2019	χ^2=1.145e-6 p=0.9991	χ^2=1.145e-6 p=0.9991	χ^2=1.145e-6 p=0.9991	χ^2=64.377 p<0.0001
P	2020	χ^2=4.115 p=0.0425	χ^2=12.829 P=0.0003	χ^2=6.355 p=0.4253	χ^2=6.355 p=0.4253
P	2021	χ^2=6.546 p=0.0105	χ^2=9.206 p=0.024	χ^2=1.98 p=0.1592	χ^2=0.218 p=0.6400
S	2019	χ^2=4.62e-6 p=0.9983	χ^2=4.62e-6 p=0.9983	χ^2=4.62e-6 p=0.9983	χ^2=19.293 p<0.0001
S	2020	χ^2=4.78 p=0.0287	χ^2=6.79 p=0.0091	χ^2=4.74 p=0.0294	χ^2=0.456 p=0.4995
S	2021	χ^2=5.385e-6 p=0.9981	χ^2=9.074 p=0.0026	χ^2=5.385e-6 p=0.9981	χ^2=6.24 p=0.0125

Table 2.2.b. Annual average male mealybug densities captured in pheromone delta traps on different field sites from 2019 to 2021. P values less than 0.05 signifies significant difference.

Field Site	**Year**	**May**
GEW	2019	χ^2=79.356 p<0.0001
GEW	2020	χ^2=12.23 p=0.0005
H	2019	χ^2=15.616 p<0.0001
H	2020	χ^2=1.6223 p=0.2028

H	2021	$\chi^2=0.8705$ $p=0.3508$
P	2019	$\chi^2=4.54$ $p=0.0331$
P	2020	$\chi^2=0.0893$ $p=7650$
P	2021	$\chi^2=1.916$ $p=0.1663$
S	2019	$\chi^2=1.622$ $p=0.2028$
S	2020	$\chi^2=0.338$ $p=0.5608$
S	2021	$\chi^2=30.9126$ $p<0.0001$

Adhesive Band Trap Capture

The strategic positioning of sticky band traps on both the main stem (trunk) and lateral branches (cordon) exhibited a substantial influence on the number of male mealybugs captured within these specific areas. In site H, the application of adhesive bands was observed to have a remarkably substantial consequence on the capture of mealybugs (Figure 2.4.a; $\chi^2=74.028$; $p<0.0001$). Similarly, in site P, the arrangement of adhesive bands yielded a significant effect on the quantity of captured mealybugs (Figure 2.4.b; $\chi^2=164.558$; $p<0.0001$). Moreover, in site S, the implementation of adhesive bands also significantly impacts on the number of mealybugs captured (Figure 2.4.c; $\chi^2=34.673$; $p<0.0001$). These data underscore the potency of adhesive band traps in attracting and capturing male mealybugs, and the consistent outcomes across distinct sites underscore the efficacy of this approach. Numerically, the adhesive band trap had a higher trap male mealybug capture than the pheromone trap capture.

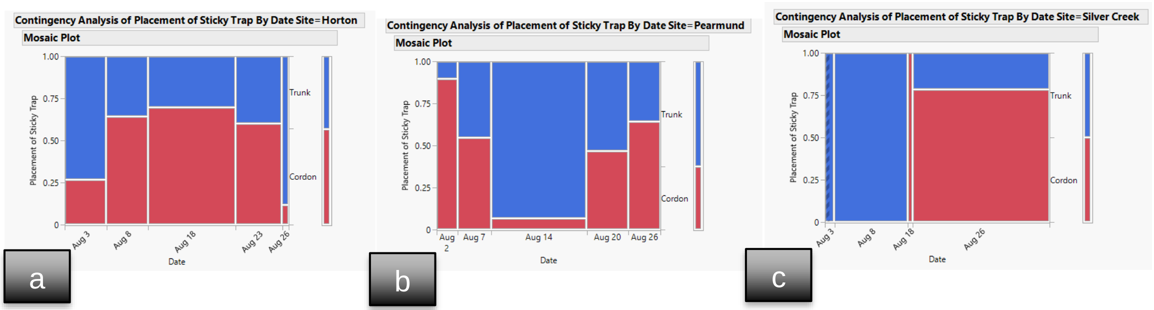
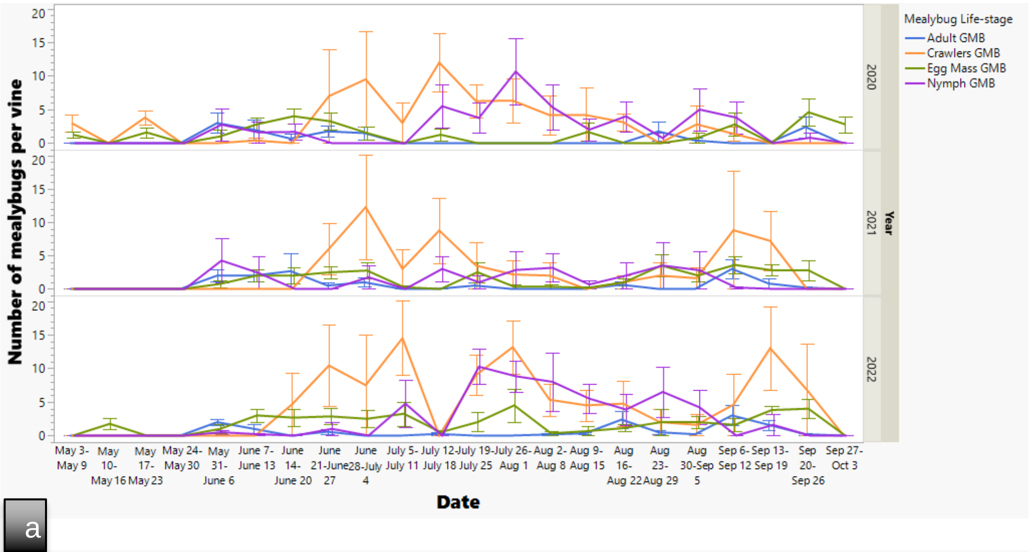


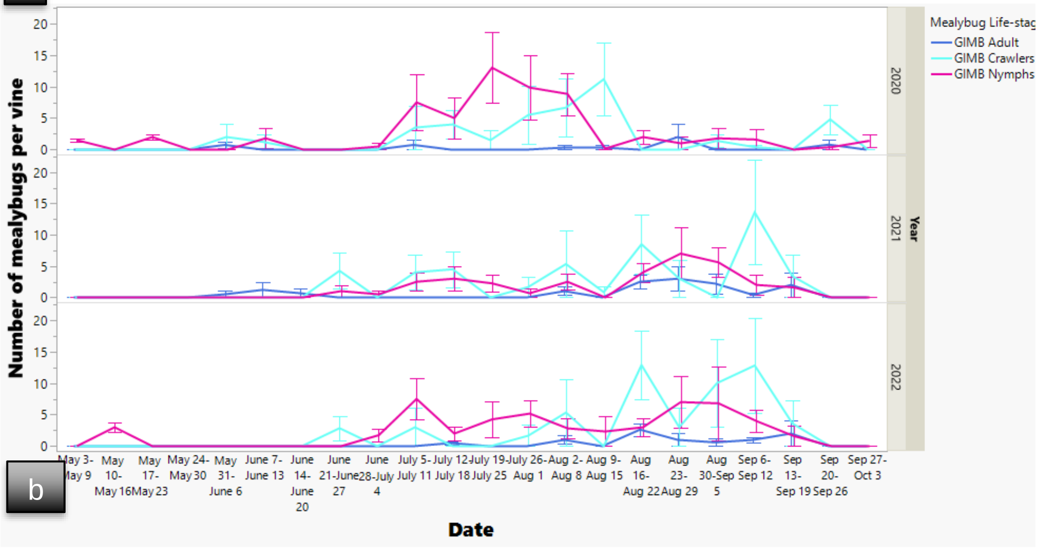
Figure 2.4.a.b.c Effect of placement of adhesive band tape on number of mealybugs captured in sites H, P and S respectively.

Mealybug Life-stages Activity

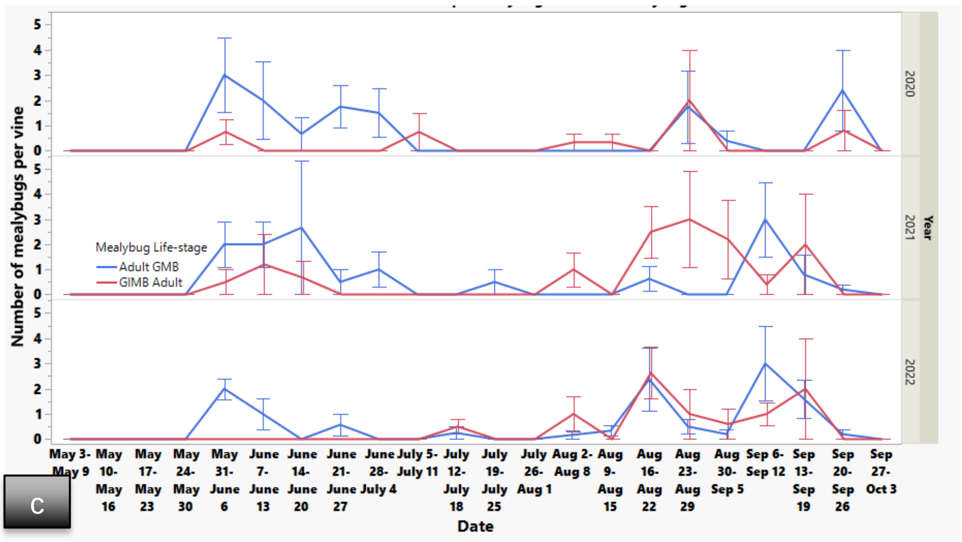
The densities of different mealybug life-stages remained significantly different throughout the season. The effect of date, each of the mealybug life-stage, year, and interaction between date*mealybug life-stage*year and mealybug life-stage*year on a 5-minute mealybug density count over the season were analyzed. Densities of mealybugs remained significantly different throughout the season on different sampling dates (Figure 2.5, and 2.6). The mealybug densities were not significantly different in different sampling years and in the interaction mealybug life-stage*year (Figure 2.5, and 2.6). All the interactions analyzed date*mealybug life-stage*year, date*year and mealybug life-stage*year had a significant effect on mealybug densities per vine (Figure 2.5, and 2.6). Figure 2.5 also demonstrated that although the population densities of the first generation emerging from overwintering life-stage remains low, the population tends to build up during the summer in the succeeding generation.



Number of mealybugs per vine
Date
Year
2020
2021
2022
Mealybug Life-stage
Adult GMB
Crawlers GMB
Egg Mass GMB
Nymph GMB
0
5
10
15
20
May 3-May 9
May 10-May 16
May 17-May 23
May 24-May 30
May 31-June 6
June 7-June 13
June 14-June 20
June 21-June 27
June 28-July 4
July 5-July 11
July 12-July 18
July 19-July 25
July 26-Aug 1
Aug 2-Aug 8
Aug 9-Aug 15
Aug 16-Aug 22
Aug 23-Aug 29
Aug 30-Sep 5
Sep 6-Sep 12
Sep 13-Sep 19
Sep 20-Sep 26
Sep 27-Oct 3
a



Number of mealybugs per vine
Date
Year
2020
2021
2022
Mealybug Life-stag
GIMB Adult
GIMB Crawlers
GIMB Nymphs
0
5
10
15
20
May 3-May 9
May 10-May 16
May 17-May 23
May 24-May 30
May 31-June 6
June 7-June 13
June 14-June 20
June 21-June 27
June 28-July 4
July 5-July 11
July 12-July 18
July 19-July 25
July 26-Aug 1
Aug 2-Aug 8
Aug 9-Aug 15
Aug 16-Aug 22
Aug 23-Aug 29
Aug 30-Sep 5
Sep 6-Sep 12
Sep 13-Sep 19
Sep 20-Sep 26
Sep 27-Oct 3
b



Number of mealybugs per vine
Date
Year
2020
2021
2022
Mealybug Life-stage
Adult GMB
GIMB Adult
0
1
2
3
4
5
May 3-May 9
May 10-May 16
May 17-May 23
May 24-May 30
May 31-June 6
June 7-June 13
June 14-June 20
June 21-June 27
June 28-July 4
July 5-July 11
July 12-July 18
July 19-July 25
July 26-Aug 1
Aug 2-Aug 8
Aug 9-Aug 15
Aug 16-Aug 22
Aug 23-Aug 29
Aug 30-Sep 5
Sep 6-Sep 12
Sep 13-Sep 19
Sep 20-Sep 26
Sep 27-Oct 3
c

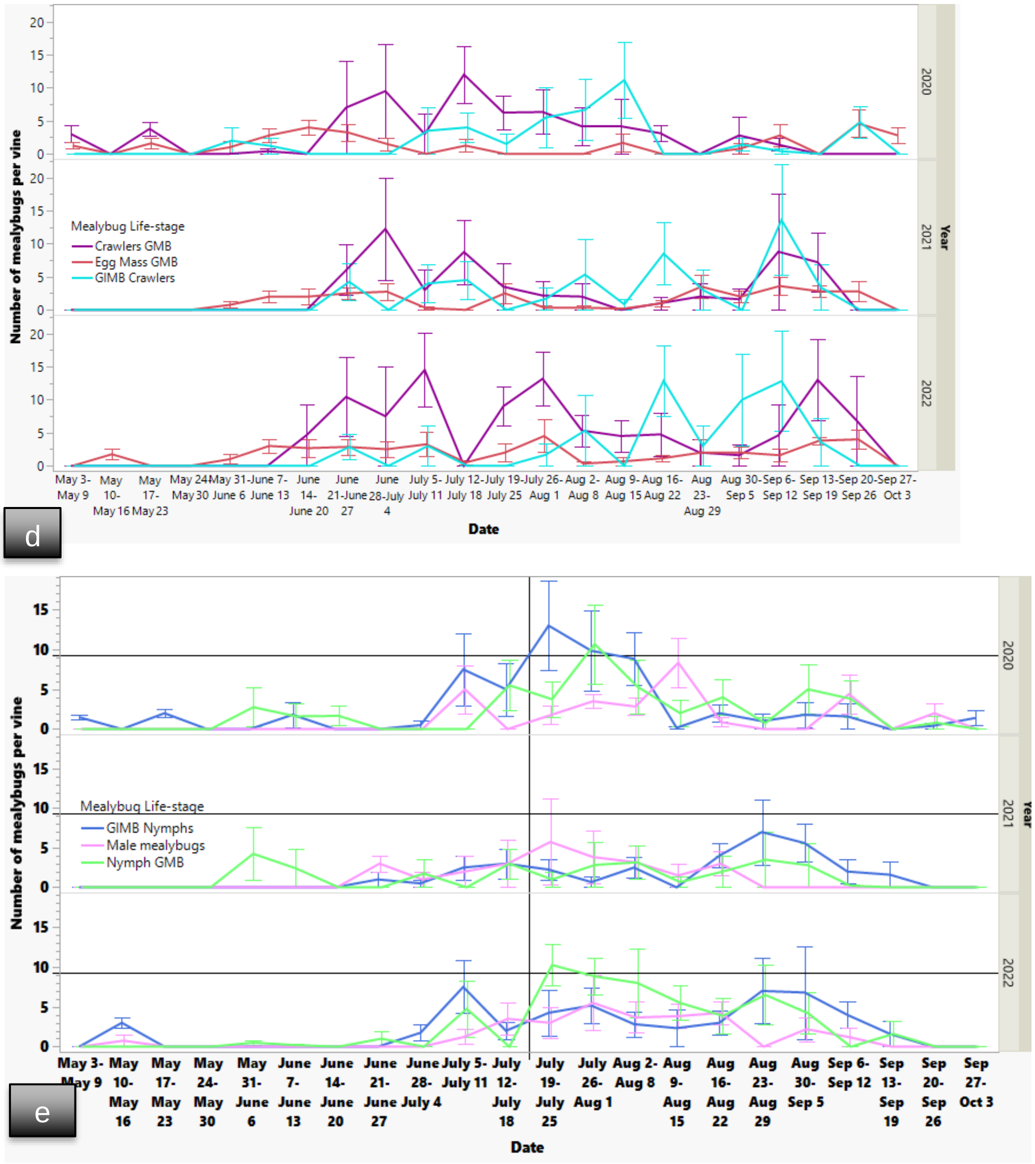


Figure 2.5. a,b,c,d,e. Seasonal distribution of different life-stages of grape mealybug and Gill's mealybug from 2020 to 2022.

Effect Tests

Source	DF	L-R ChiSquare	Prob>ChiSq
Date	21	1208.6974	<.0001*
Date*Mealybug Life-stage*Year	168	1045.4923	<.0001*
Mealybug Life-stage	4	476.13004	<.0001*
Year	2	0.0006999	0.9997
Date*Year	42	423.56955	<.0001*
Mealybug Life-stage*Year	8	0.0022608	1.0000

Figure 2.6. Effect of date, each of the mealybug life-stage, year, and interaction between date*mealybug life-stage*year and mealybug life-stage*year on five-minute mealybug density count

Figure 2.7. Gill’s mealybug and grape mealybug nymphs on new growth spur of grape.

Grape mealybug overwinters as egg mass/ crawler stage while Gill’s mealybug overwinters as nymphs. The first generation is often seen during early spring (May) dispersing to feed on new shoot growth/ new growth spurs, while some of them remain underneath the bark feeding on the trunk. Later nymphal stages of Gill’s mealybug were observed and collected from the new growth spurs, while a minority of them were present inside the trunk. Most of the crawlers and second instar nymphs of grape mealybug were observed and collected from the trunk, while a minority of them were present along with Gill’s mealybug in the new growth spurs (Figure 2.7).

Adult Gill’s mealybug was recorded from the vineyard as early as the last week of May, while adult grape mealybug was recorded as early as the first week of June (Figure 2.5). The overwintering stage starts ovipositing or producing crawler stage from mid-week of June until the second week of July, while the first generation starts infesting vineyards from late July.

2.4 Discussion and Conclusion

Previous research by Jones (2016) has underlined the widespread presence of grapevine virus infections and the presence of its vectors-the mealybugs. The research has also recorded the presence of grape, Gill’s, striped and obscure mealybugs in Virginia vineyards. This research and the subsequent detections in a few of the vineyards in Virginia (D.G. Pfeiffer, pers comm) has highlighted the necessity to develop effective pest management strategies for the control of this cryptic pest. Previous studies and reports have signified the presence of some of the mealybug species and some of the ant species in mutualistic relation with mealybugs as reported from the West Coast. Some of the main questions my research had attempted to address are the species composition of mealybugs and their attending ants and their management in Virginia vineyards. Using a 5-minute visual count, my results revealed the consistent and dominant presence of two main species: grape mealybug and Gill’s mealybug. The analysis of specimens collected from root and shoot samples using PCR consistently identified these two species across the vineyard sites. I also spotted obscure mealybug during two separate instances, which positively confirms the previous study by Jones et al., 2016. The absence of vine mealybug from our data is a big relief for the grape growers, as this mealybug species have up to seven generations per year, produce more honeydew and more resistant to chemical control (Daane et al. 2012, Nilima et al. 2012).

The study further examined male mealybug capture using pheromone-baited delta traps, indicating generally stable and low capture counts across most sampling dates and sites. However, a notable exception occurred in August 2019 at the GEW field site (χ^2=4.668; p=0.0307). This observation highlights the potential for variations in trap capture data. One of the reasons for the higher trap capture data might be due to higher population density of mealybugs in the field site. The impact of sampling dates on the average daily count of mealybugs was statistically significant, indicating temporal variations in mealybug populations. Notably, there were no significant differences in male mealybug trap capture data across different vineyard sites, suggesting a consistent distribution of these insects. One of the shortcomings of our experiment has been inability to identify the male mealybugs into species level, due to which our confirmation for mealybug generations per year is based on crawler stage emergence entirely.

The utilization of adhesive band traps strategically placed on main stems and lateral branches significantly influenced male mealybug capture rates. This effect was observed consistently across different zones within the vineyards. Adhesive bands exhibited higher efficacy in capturing male mealybugs compared to pheromone traps. The adhesive traps are sticky and a little bit difficult to handle. However, it would be interesting to study further if the use of adhesive band trap might provide some kind of relief against male mealybugs under small scale settings.

Regarding mealybug life-stages, distribution and densities remained significantly distinct throughout the season. Factors such as date, mealybug life-stage, year, and their interactions all contributed to the density of mealybugs per vine. Overwintering behavior was observed in both grape and Gill’s mealybug, with different patterns of infestation and presence on new shoot growth and trunks. My study revealed that first generation grape mealybugs began ovipositing or producing crawler stages from mid-June to mid-July, with the first-generation infesting vineyards in late July. Adult Gill’s mealybug was recorded earlier in the season compared to adult grape mealybug.

Overall, the study provides valuable insights into the dominant mealybug species in Virginia vineyards, their distribution, life-stage dynamics, and the efficacy of different trapping methods. The lack of vine mealybugs during our survey provides relief, as this nuisance invasive pest would be a complicated factor in mealybug management. Our findings contribute to a better understanding of mealybug behavior and can inform pest management strategies in vineyard environments.

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Chapter 3

Association between ants and mealybugs in Virginia Vineyards

Abstract

The mutualism between ants and honeydew-producing hemipterans like aphids, whiteflies, scales, mealybugs, treehoppers, and leafhoppers changes the behavior of ants such that they become more aggressive even towards other non-honeydew-producing herbivores as well as other predators, which they would ignore otherwise. While the ant-mealybug relation has been researched more on the West Coast, this research focuses on the identification of ant genus around vineyards, the identification of ants in close association with mealybugs, deployment of sugar dispensers to study its effect on ants and mealybug population distribution, and monitoring fruit cluster infestations. Ant populations were monitored using a pitfall trap and a 1-minute count on the trunk, while mealybug populations were monitored using a 5-minute count on the trunk. Fruit cluster infestation was monitored by recording the number of mealybugs and the presence of honeydew. Pavement ant (Genus *Tetramorium* Mayr) remains the dominant ant present in the vineyard, while ants like pavement ants, acrobat ants (Genus *Crematogaster* Lund), medium garden ants (Genus *Lasius* Fabricius) and thief ants (*Solenopsis molesta* Buren) were found tending mealybugs closely. Ant number remains low in the control treatment area, lacking any dispensers. The dispensers baited with ant toxicants, although had initially a higher number of ants, the number started decreasing to a minimum after the second week of July. The ant densities were similar in all the treatments before the deployment of

dispensers, it was different after deploying sugar dispensers and sugar-toxicant dispersers. The fruit cluster infestation was also higher in the control treatment lacking any ant dispensers.

3.1 Introduction

Ants (Hymenoptera: Formicidae) are among the diverse insects and play a prominent role in the ecosystem they inhabit. Thus, it is essential to monitor population biodiversity and other interactions happening at the niche level (Gibb et al. 2017, 2023). Ants are eusocial insects, where members are divided into both reproductive and nonreproductive members of overlapping generations and hence form superorganisms. Superorganisms are individuals belonging to the same social unit and same species, which synergistically interact with each other such that there are selective pressures on individuals and the colony (Gibb et al. 2023).

Ants have often been found in close association with honeydew-producing hemipterans and have been studied extensively in multiple ecosystems (Renault et al. 2005, Styrsky and Eubanks 2006, Brightwell and Silverman 2010, Wilder et al. 2011). In the association, ants tend and protect the honeydew-producing hemipterans from predators and parasitoids as well as maintain hygiene in the mealybug colony, while hemipterans provide them with an important food supply i.e., honeydew. This association has often been observed in aphids, whiteflies, scales, mealybugs, treehoppers, and leafhoppers. This mutualistic interaction due changes the behavior of ants such that they become more aggressive even towards other non-honeydew-producing herbivores as well as other predators, which they would ignore otherwise.

Ants receive honeydew from mealybugs and in return maintain hygiene in the mealybug colony, transport them to new feeding sites, and provide protection against natural enemies

(Daane et al. 2007, Cheng et al. 2015, Xu et al. 2019). Honeydew is the main reason why ants tend and protect mealybugs. The use of sugar baits or toxic sugar baits allocated in small doses over the vineyard as an alternative food source to mealybugs is more effective in targeting not only the foragers, but the entire nest is fed by them (Cooper et al. 2008, Daane et al. 2008). The forager ants when fed on toxicants present in insecticide dispensers baited with sugar are not killed immediately, but the toxicants are diluted when passed on from one ant to another during feeding. The process is trophallaxis, which is a nutritive fluid-exchange observed in social insects and some nonsocial insects. It helps baits with a low concentration of toxicants to reach from vines to ant nests and recruit more ants to the bait (Rust et al. 2004, Wcislo, 2016). Hence rather than killing a single ant, this process can effectively control ants on a larger scale.

Granular insecticides, liquid baits and insecticide-laced sugar provisioning have been tested in the field to control ant activity (Daane et al. 2006, Daane et al. 2008, Nondillo et al. 2016, Beltrà et al. 2017, Parrilli et al. 2021) and have provided effective control of ants and mealybugs. Artificial sugar dispensers have been deployed in the field with or without insecticides (Daane et al. 2008, Beltrà et al. 2017). These methods could be effective in controlling ant populations, as instead of providing immediate control, ants take these materials back to the nest and hence divert attention from the mealybugs.

3.2 Materials and Methods

Field Sites and Experimental Design

The ant-mealybug experiment was carried out in two vineyard sites: Orange County (Site H), and Fauquier County (Site P). Vineyards were selected based on the availability and pest pressure recorded by the researcher in the previous years. These are conventional vineyards, relying on synthetic insecticides for pest management. Each vineyard block was more than 10 years old and had a previous history of mealybug infestations. The trial was carried out in an area of 0.283 to 0.081 hectare inside each vineyard. Each experimental area was divided into three plots: control plots, sugar bait plots, and ant bait plots. The control plots were separated from other treatment plots by a distance of 10 to 20 meters. A distance of 4 to 7 meters or 2-3 vine rows distance was maintained between the sugar bait and ant bait plots.

Figure 3.1 a. Ant dispensers containing a. 25% sugar solution (sugar bait) and b. 1% disodium octaborate tetrahydrate bait (sugar-toxicant bait) deployed in the field.

Sugar Dispensers

The liquid dispenser I used in the field is based on earlier research by Daane et al. (2008) and repeated by Parrilli et al. (2021). 250 ml HDPE narrow mouth bottles, assembled with white polypropylene closure was modified into dispenser (The Lab Depot, Dawsonville, GA). A 1 cm circular hole was drilled in the cap of the tube and a permeable mesh was placed between the tube and the cap. A 5.08 cm garden slotted mesh net cup was placed outside the cap with a plastic mesh outside to allow the entry of ants, but not bees (Figure 3.1). Sugar dispensers, if improperly set up, could have a detrimental effect on bees in the vineyard.

We deployed 12-16 dispensers (Figure 3.1) in four rows of vines, evenly placing them after every 5-10 plants through the experimental plots. They were deployed at the beginning of June and removed in the second week of September. A gap of 2-5 rows of grapevines was maintained in between each of the two treatments. The insecticide used for ant control was Greenway liquid ant-killing bait with the active ingredient 1% disodium octaborate tetrahydrate. Each of the dispensers was refilled and cleaned every one to two weeks.

Ant Activity

Ant populations in the vineyard were monitored and sampled weekly in all three treatment plots by both 1. Pitfall trapping 2. 1-minute visual count. 50 ml falcon centrifuge tubes were used for pitfall trapping, with 75% alcohol and a few drops of ethylene glycol as the preservative (Bestelmeyer and Wiens 1996, Calixto et al. 2007, Wike et al. 2010, Johnson et al. 2014, Sheikh et al. 2018). The advantage of using ethanol is that it does not attract ant species differentially. A total of 5-8 pitfall traps were placed randomly per experimental plot per site. Ant activity was also monitored by counting the number of ants crossing an imaginary line of 20 cm in length and in between the vine canopy and above the dispensers on the trunk for 1-minute. The vines for the visual count and pitfall trap placement were selected randomly to represent the whole plot. The mealybug numbers were also counted in the vineyard by using a 5-minute visual count. The ants were collected in 70% ethanol and taken back to the lab for identification using the identification keys (Fisher et al. 2007).

Data Analysis

The foraging activity of the different ant species was calculated by the mean number of each ant species collected throughout the season. The data were first transformed using log transformation to check for homogeneity. As the data were not normally distributed, they were analyzed using Steel's method for nonparametric multiple comparisons with control. The Steel criterion uses the Wilcoxon test statistic in the pairwise comparisons of the standard control sample with each of the treatment samples (Critchlow and Fligner 1991). Data were analyzed based on count per sampling date and total count throughout the season.

Before the commercial harvest, 25 fruit clusters per treatment in each replicate were evaluated using following scoring system: 0= no mealybug or honeydew, 1=honeydew and five or fewer mealybugs, 2=honeydew and six to nine mealybugs, 3=honeydew and more than ten mealybugs, and 3=honeydew and egg mass). Fruit clusters with a score of 2 and 3 were considered unmarketable or extremely infested. Fruit cluster infestation was analyzed using Wilcoxon paired test (Beatty, 2018).

Multivariate analysis was used to analyze the relation between the number of ants per minute, the number of mealybugs per vine, and the percentage of cluster damage for each of the sites. In the results, correlation coefficients, covariance matrix, correlation probability, and scatterplot matrix were shown for each pair. For the statistical analysis, we used the JMP Pro software package.

3.3 Results

Ant activity in the vineyards

A total number of 1131 specimens of ants were collected in two of the field sites (457 samples from site P and 674 from site H) over the whole field season, representing 12 genera of ants. Ants were identified up to genus level due to time constraint. According to the pitfall trap data (Figure 3.2), *Tetramorium*, the pavement ant remained the dominant ant in both vineyards, followed by the thief ant *Solenopsis molesta.* According to the one-minute data count on the vine (Figure 3.3), the top three leading foragers in the vineyards include *S. molesta*, *Pheidole* ants, and the pavement ant.

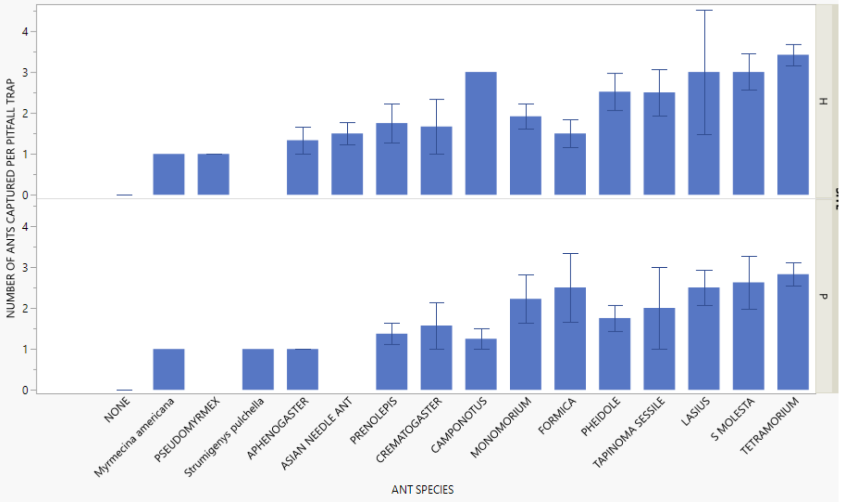

FIGURE 3.2. The mean number of ants captured in the pitfall trap throughout the season (June-September 2022) in sites H (Orange County, VA) and P (Fauquier County, VA). Each error bar is constructed using 1 standard error from the mean.

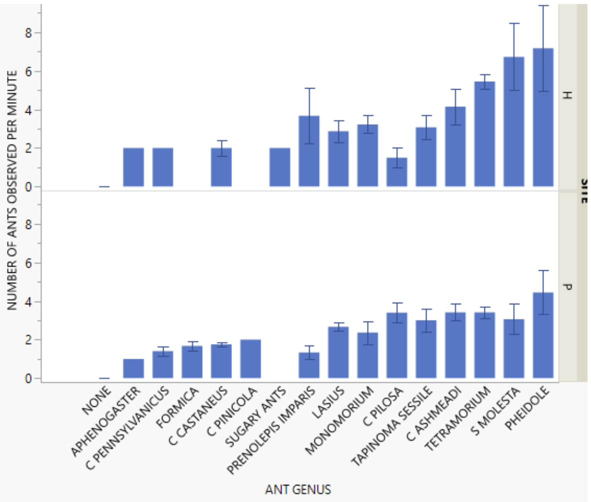

FIGURE 3.3. The mean number of ants observed in the trunk per minute throughout the season (June-September 2022) in sites H (Orange County, VA) and P (Fauquier County, VA). Each error bar is constructed using 1 standard error from the mean.

Field studies with dispensers

Fewer numbers of ants were observed and captured in the control treatment throughout the season. The sugar-toxicant bait initially attracted a higher number of ants during the initial few weeks of deployment and started decreasing throughout the season. In comparison to the other treatments, sugar baits attracted a higher number of ants throughout the season (Figures 3.4).

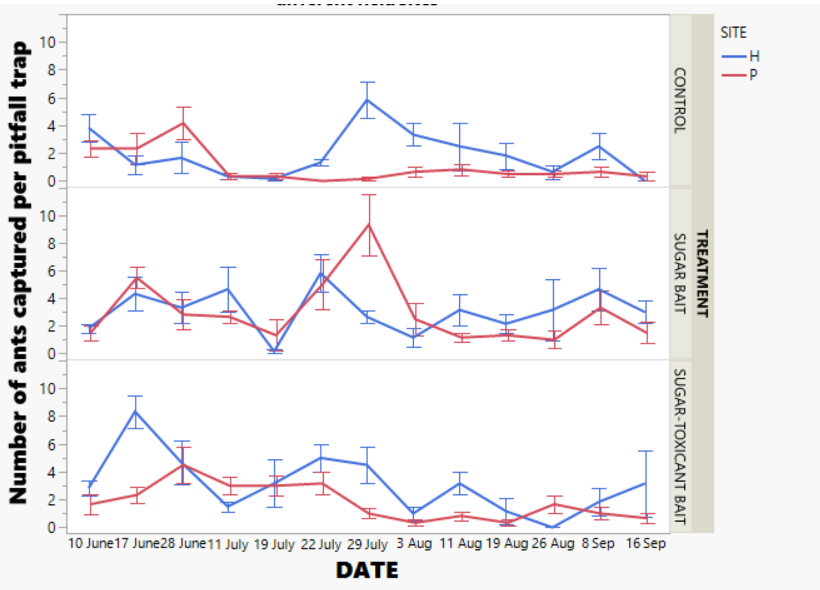

FIGURE 3.4. Mean(±SEM) number of ants captured per pitfall trap in different treatments throughout the season in sites H (Orange County, VA) and P (Fauquier County, VA).

Table 3.1.a. Variation in the mean number of ants per minute throughout the sampling season A. Mean number of ants per vine per minute ±SE from site P (Fauquier County, VA) B. Z-score and C. p-value on each of the dates. (1-minute count data)

Treatment	Content of treatment	June 7	June 16	June 28	July 11	July 19	July 28	Aug 3	Aug 11	Aug 20	Aug 26	Aug 31
Control	Empty	A. 2.4±0.7746	A. 3.9±1.12	A. 2.5±0.9457	A.2.3±0.8439	A.0.6±0.3399	A.1±0.4216	A.0.9±0.5044	A.1.2±0.5333	A.1.6±0.7024	A. 2.8±1.073	A. 0.9±0.6046
Sugar bait	25% sucrose	A. 2.1±0.862	A.3±1.505	A.0.7±0.2603	A.3.2±0.5538	A.4.9±0.8465	A.5.6±0.636	A.2.5±0.6540	A.4.2±0.8666	A.3.3±0.8825	A.3.9±1.1	A.1.1±0.5044

	solution	B. -0.2744	B. 0	B. -0.599874	B. 0.9958	B. 3.4948	B. 3.5266	B. 1.71	B. 2.46	B. 1.45	B. 1.002	B. 0.9772
		C. 0.9464	C. 1	C. 0.7728	C. 0.5065	C. 0.0009*	C. 0.0008*	C. 0.1549	C. 0.0260*	C. 0.2528	C. 0.5025	C. 0.5170
Sugar-toxicant bait	1 % disodium octaborate tetrahydrate	A. 1.6+0.4761	A. 3.7+1.2653	A.1.6+0.8589	A.1.4+0.3399	A.1.1+0.4819	A.2.7+0.5783	A.0.8+0.4163	A.0.6+0.2666	A.0.8+0.3266	A. 1.1+0.4069	A. 0.5+0.5
		B. -0.508463	B. -0.6871	B. -0.9994	B. -0.2108	B. 0.86866	B. 1.8213	B. 0.0914	B. -0.32021	B. -0.5012	B. -0.82286	B. -0.4868
		C. 0.8299	C. 0.7149	C. 0.5024	C. 0.9682	C. 0.5921	C. 0.1232	C. 0.9939	C. 0.9285	C. 0.8353	C. 0.6236	C. 0.8426

Table 3.1.b Total variation in the mean number of ants per minute throughout sampling season A. Mean number of ants per vine per minute +SE from site P (Fauquier County, VA) B. Z-score and C. p-value on each of the dates. (1-minute count data)

Treatment	Content of treatment	Overall mean value of ants per minute
Control	Empty	A. 1.82727+0.31045
Sugar bait	25% sucrose solution	A. 3.13636+0.45232 B. 4.023 C. 0.0001*
Sugar-toxicant bait	1 % disodium octaborate tetrahydrate	A. 1.44545+0.29213 B. -0.17279 C. 0.9785

Table 3.1.c. A. Mean number of ants per vine per minute +SE from site H (Orange, County, VA) B. Z-score and C. p-value on each of the dates. (1-minute count data)

Treatment	Content of treatment	June 10	June 17	June 29	July 12	July 21	July 29	Aug 5	Aug 10	Aug 22
Control	Empty	A.2.8+0.9285	A.2.6+0.8969	A.2.4+0.7774	A.3.4+2.0231	A.3.2+1.0729	A.0.9+0.7951	A.5.8+2.225	A.5.9+0.1.852	A.5.1+1.649

Sugar bait	25% sucrose solution	A. 1.9±0.8226	A.7.3 ±1.317	A.2± 0.6667	A.2.3 ±0.6333	A.2.3±0.6506	A.4±0.715	A.2.4± 0.5259	A.2.9±0.5259	A.5.3±1.2914
		B. -1.168	B. 2.48	B. -0.2745	B. 0.9473	B. 3.4948	B. 3.14	B. -0.9152	B. -0.533	B. 0.3814
		C. 0.3972	C. 0.0248*	C. 0.9464	C. 0.5419	C. 0.0009*	C. 0.0033*	C. 0.6307	C. 0.8163	C. 0.9004
Sugar-toxic ant bait	1 % disodium octaborate tetrahydrate	A. 1.3±0.5587	A. 5.2± 0.8138	A.0.5 ±0.5	A.2.9 ±0.9363	A.1.6±0.4268	A.2±1.5275	A.1.444±0.7	A.2.5±1.376	A.0.666±0333
		B. -1.509	B. 1.9491	B. -2.087	B. 0.758	B. 0.86866	B. -0.449	B. 1.616	B. -1.777	B. -2.17
		C. 0.2265	C. 0.0930	C. 0.0676	C. 0.6712	C. 0.5921	C. 0.8647	C. 0.2386	C. 0.1352	C. 0.0557

Table 3.1.d. Total ant activity throughout the season in site H (Orange County, VA) (1-minute count)

Treatment	**Content of treatment**	**Overall mean value of ants per minute**
Control	Empty	A. 3.567±0.4949
Sugar bait	25% sucrose solution	A. 3.378±0.3354 B. 1.418 C. 0.3315
Sugar-toxicant bait	1 % disodium octaborate tetrahydrate	A. 1.7647±0.2712 B. 2.409 C. 0.0423*

Table 3.2.a. Variation in the mean number of ants per minute throughout the sampling season A. Mean number of ants per vine per minute ±SE from site P (Fauquier country, VA) B. Z-score and C. p-value on each of the dates. (Pitfall-trap data)

Treatment	**Content of treatment**	**June 10**	**June 17**	**June 28**	**July 11**	**July 19**	**July 22**	**July 29**	**Aug 3**	**Aug 11**	**Aug 19**	**Aug 26**	**Sep 8**	**Sep 16**

Control	Empty	A.2.334±0.6146	A. 2.334±1.145	A. 4.16±1.167	A. 0.33±0.2108	A. 0.333±0.2108	A0±0	A.0.1667±0.1667	A.0.667±0.333	A. 0.8±0.4014	A.0.5±0.2236	A. 0.5±0.2236	A.0.667±0.333	A. 0.33±0.33
Sugar bait	25% sucrose solution	A. 1.5±0.5627	A.5.5±0.7637	A.2.83±1.08	A2.667±0.4216	A.0.166±0.1667	A.5±1.807	A.9.33±2.231	A.2.5±1.176	A.1.166±0.3073	A.1.33±0.4216	A.1±0.6324	A.3.334±1.229	A.1.5±0.7637
		B. -0.8615	B. 1.96	B. -0.0813	B. 2.916	B. 0.096	B. 3.015	B. 2.911	B. 1.414	B. 0.5924	B. 1.482	B. 1.482	B. 2.138	B. 1.434
		C. 0.5951	C. 0.908	C. 0.6282	C. 0.0068*	C. 0.9932	C. 0.0050*	C. 0.0070*	C. 0.2679	C. 0.776	C. 0.2375	C. 0.237	C. 0.0599	C.0.2585
Sugar toxicant bait	1 % disodium octaborate tetrahydrate	A. 1.667±0.7149	A. 2.334±0.6146	A.4.5±1.335	A.3±0.6324	A.3.16±1.701	A.3.166±0.793	A.1±0.365	A.0.333±0.2108	A.0.833±0.3073	A.0.33±0.2108	A. 1.667±0.6146	A. 1±0.4472	A. 0.66±0.333
		B. -dd0.685	B. 0.7754	B. 0	B. 2.719	B. 2.714	B. 3.0032	B. 1.715	B. -0.64	B. 0	B. -0.467	B. 0.178	B. 0.4316	B. 0.8616
		C. 0.7162	C. 0.6545	C. 1	C. 0.0125*	C. 0.0127*	C. 0.0052*	C. 0.1528	C. 0.747	C. 1	C. 0.854	C. 0.977	C. 0.8737	C. 0.5950

Table 3.2.b. Total ant activity throughout the season in site P (Fauquier County, VA) (pitfall trap data)

Treatment	Content of treatment	Overall mean value of ants per minute	
Control	Empty	A.	1.013±0.1900
Sugar bait	25% sucrose solution	A.	3±0.3849
		B.	5.042
		C.	<0.0001*
Sugar toxicant bait	1 % disodium octaborate tetrahydrate	A.	1.8077±0.2152
		B.	3.604
		C.	0.0006*

Table 3.2.c. Variation in the mean number of ants per minute throughout the sampling season A. Mean number of ants per vine per minute ±SE from site H (Orange County, VA); B. Z-score; and C. p-value on each of the dates. (Pitfall-trap data)

Treatment	**Content of treatment**	**June 7**	**June 17**	**June 29**	**July 11**	**July 14**	**July 19**	**July 29**	**Aug 5**	**Aug 10**	**Aug 30**	**Sep 5**	**Sep 9**	**Sep 15**
Control	Empty	A. 3.83±1.0138	A. 1.16±0.654	A. 1.16±1.115	A. 0.33±0.2108	A. 0.1667±0.1667	A1.33±0.211	A.5.83±1.276	A.3.33±0.8027	A2.5±1.708	A.1.83±0.945	A. 0.667±0.4944	A. 2.5±0.957	A. 0±0
Sugar bait	25 % sucrose solution	A. 1.83±0.307	A.4.33±1.202	A.3.33±1.115	A4.667±1.646	A.0.166±0.1667	A. 5.83±1.327	A.2.66±0.494	A.1.16±0.654	A.3.16±1.108	A.2.16±0.654	A.3.16±2.227	A.4.66±1.542	A.3±0.816
		B. -0.575	B. 2.75	B. 1.702	B. 2.573	B. 1.789	B. 2.884	B. -0.72439	B. -1.89	B. 1.063	B. 0.667	B. 0.716	B. 1.067	B. 3.003
		C. 0.788	C. 0.0114*	C. 0.1567	C. 0.0191*	C. 0.1312	C. 0.0076*	C. 0.6895	C. 0.105	C. 0.461	C. 0.728	C. 0.6954	C. 0.4587	C. 0.0052*
Sugar toxicant bait	1 % disodium octaborate tetrahydrate	A. 2.83±0.542	A. 8.33±1.174	A.4.667±1.585	A.1.5±0.3415	A.3.16±1.701	A. 5±1	A.4.5±1.335	A.1±0.0.516	A.3.16±0.7923	A.1.16±0.9803	A. 0±0	A. 1.83±0.9803	A. 3.166±2.37
		B. -1.562	B. 1.952	B. 1.464	B. 2.355	B. 0.000	B. 2.25	B. -1.534	B. -2.056	B. 0.9201	B. -0.8632	B. -1.354	B. -0.501	B. 2.647
		C. 0.2052	C. 0.0923	C. 0.2454	C. 0.0347*	C. 1	C. 0.0455*	C. 0.2161	C. 0.0728	C. 0.555	C. 0.594	C. 0.2966	C. 0.8344	C. 0.0155*

Table 3.2.d. Total ant activity throughout the season in site H (Orange County, VA) (pitfall trap data)

Treatment	**Content of treatment**	**Overall mean value of ants per minute**
Control	Empty	A. 1.936±0.2893

Sugar bait	25% sucrose solution	A. 3.089±0.3419 B. 3.030 C. 0.0047*
Sugar toxicant bait	1 % disodium octaborate tetrahydrate	A. 3.102±0.3865 B. 2.5213 C. 0.0221*

1-minute count on the trunk

Field analysis of the mean number of ants was calculated as average value for the entire season as well as per sampling dates. During the 1-minute count data analyses, the mean densities of ants in the sugar bait and the sugar-toxicant bait treatments were not significantly different from the density in the control before the placement of ant dispensers (Table 3.1.a: Site P: Z-score: -0.2744, p=0.9464 for sugar bait: control; Z- score: -0.5084, p=0.8299 for sugar-toxicant bait: control). The result remains the same for site H (Table 3.1.a: Site P: Z- score: -0.168, p=0.3972 for sugar bait: control; Z- score: -1.509, p=0.2265 for sugar-toxicant bait: control).

When data were compared per sampling dates, the densities were not statistically significant for most of the sampling dates (Table 3.1.a and 3.1.c) in both site H and site P during the deployment of ant dispensers. The mean densities of ants for the entire sampling period have different data analysis results. There was no significant difference in the mean densities of ants present in sugar-toxicant bait treatment compared to the control in site P (Table 3.1.b: Site P: Z- score: -0.1728, p=0.9785 for sugar-toxicant bait). However, there was a significant difference between the mean densities of ants present in the sugar bait dispenses in comparison to the control in site P (Table 3.1.b: Site P: Z- score: 4.023, p=0.0001 for sugar bait). The result was just the opposite for site H. There is a significant difference in the mean densities of ants present in sugar-toxicant bait treatment compared to the control in site H (Table 3.1.d: Site H:

Z- score: 2.409, p=0.0423 for sugar-toxicant bait). However, there was no significant difference between the mean densities of ants present in the sugar bait dispenses in comparison to the control in site H (Table 3.1.d: Site H: Z- score: 1.418, p=0.3315 for sugar bait).

Pitfall trap data

The mean densities of ants in the sugar bait and the ant bait treatments were not significantly different from the density in the control before the placement of ant dispensers (Table 3.2.a: Site P: Z-score: -0.8615, p=0.5951 for sugar bait: control; Z-score: -0.685, p=0.6545 for sugar-toxicant bait: control). Similar results occurred for site H (Table 3.2.a: Site P: Z-score: -0.575, p=0.788 for sugar bait: control; Z-score: -1.562, p=0.2052 for sugar-toxicant bait: control).

Like the one-minute data count, the pitfall trap data were analyzed for each of the sampling dates as well as the total sampling duration. When data were compared per sampling dates, the densities were not statistically significant for most of the dates (Table 3.1.a and 3.1.c) in both site H and site P during the deployment of ant dispensers. The mean densities of ants for the entire sampling period have different results compared to the one-minute count. There was a significant difference in the mean densities of ants present in both the sugar-toxicant bait and sugar bait treatment compared to the control in site P ((Table 3.2.b: Site P: Z- score: 5.042, p=<0.001 for sugar bait, and Site P: Z- score: 3.604, p=0.0006 for sugar-toxicant bait). There was also a significant difference in the mean densities of ants present in both the sugar-toxicant bait and sugar bait treatment compared to the control in site H ((Table 3.2.d: Site H: Z- score: 3.03, p=0.0047 for sugar bait, and Site P: Z- score: 2.521, p=0.0221 for sugar-toxicant bait).

Fruit cluster injury due to the presence of mealybugs

In one of the sites H, 50% of the infested clusters evaluated were from the control treatment, while 33% were from the sugar bait treatment and 17% from the sugar-toxicant bait treatment. In another site, P, 42% of the infested clusters evaluated were from the control treatment, while 37% were from the sugar bait treatment and 21% from the sugar-toxicant bait treatment (Figure 3.5). The nonparametric test for the fruit cluster infestation had similar results in both sites P and H. The cluster infestation level was significantly different between sugar-toxicant bait and control for both sites (Figure 3.6: Site P: Z- score: 2.034, p=0.0419; Figure 3.6: Site H: Z-score: 3.005, p=0.0027 for sugar-toxicant bait and control). On the other hand, the cluster infestation level between sugar bait and control in both sites was not significantly different (Site P: Z- score= -1.634, p=0.1023; Site H: Z- score: -1.863, p=0.0624 for sugar bait and control).

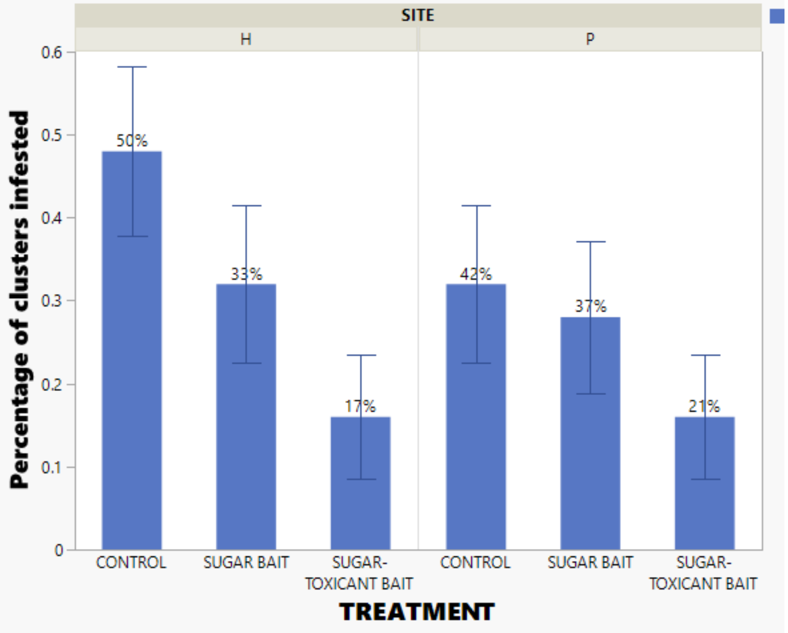

Figure 3.5. Percentage of infested bunches (site H (Orange County, VA) and P (Fauquier County, VA)). Each error bar is constructed using 1 standard error from the mean.

Site H

Sugar bait: Control= Score mean difference: -6.76, p=0.0624

Control: Sugar-toxicant bait= Score mean difference: -6.76, p=0.0027

Sugar bait: Sugar-toxicant bait: Score mean difference: 3.76, p=0.2076

Site P

Sugar bait: Control= Score mean difference: -5.32, p=0.1023

Control: Sugar-toxicant bait= Score mean difference: 6.6, p=0.0419

Sugar bait: Sugar-toxicant bait= Score mean difference: 0.44, p=0.8671

Figure 3.6. Score means difference and p-value for fruit cluster damage analysis using the Wilcoxon test in site H (Orange County, VA) and P (Fauquier County, VA).

Relation between ants, mealybugs, and cluster infestation

For the multivariate data analysis, we compared the data of ant densities, mealybug densities, and cluster injury data for the whole sampling season. For site P, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is low, positive, and not significant (r=0.1587; p=0.0977) in the control treatment. In the same treatment, the correlation coefficient between both the number of ants per minute as well as the number of mealybugs per vine and cluster damage is low, positive, and not significant (r=0.0641; p=0.7608 for ant number; r=0.0622; p=0.7679 for mealybugs number). These weak-to-low correlation coefficient values imply that changes in one domain are not correlated strongly with changes in the related domain.

For the sugar-toxicant bait treatment in site P, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is low, positive, and significant (r=0.1882; p=0.0489). In the same treatment, the correlation coefficient between both the number of ants per minute and the cluster damage is weak, positive, and not significant (r=0.3266; p=0.1111). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (r=0.7686, p<0.0001).

For the sugar bait treatment in site P, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is low, positive, and not significant (r=0.0374; p=0.6984). In the same treatment, the correlation coefficient between both the number of ants per minute as well as the number of mealybugs per vine and cluster damage is low, positive, and not significant (r=0.1756; p=0.4010 for ant number; r=0.0776; p=0.7122 for mealybugs number).

For site H, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is weak, positive, and significant (r=0.2524; p=0.0239) in the control treatment. In the same treatment, the correlation coefficient between both the number of ants per minute as well as the number of mealybugs per vine and cluster damage is weak, positive, and not significant (r=0.1772; p=0.3968 for ant number; r=0.2617; p=0.2063 for mealybugs number).

For the sugar-toxicant bait treatment in site H, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is weak, positive, and significant (r=0.3374; p=0.0022). In the same treatment, the correlation coefficient between the number of ants per minute and the cluster damage is weak, positive, and not significant (r=0.0773; p=0.7135). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (r=0.5956, p=0.0017).

For the sugar bait treatment in site H, the correlation coefficient between the number of ants per minute and the number of mealybugs per vine is weak, positive, and not significant (r=0.1195; p=0.2912). In the same treatment, the correlation coefficient between the number of ants per minute and the cluster damage is weak, positive, and not significant (r=0.1035;

p=0.4746). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (r=0.7797, p<0.0001).

3.4 Discussion and Conclusions

This work represents the ant species collected by pitfall trap and one-minute count in two different vineyards in Virginia. This collection does not account for all the species of ants encountered in vineyards in Virginia. More intensive data collection is needed to reveal the species diversity of ants in vineyards in Virginia. Despite some of the drawbacks, these data best represent the specimens captured during the field trial.

Fifteen genera of ants were recorded foraging around commercial vineyards in Virginia in 2022. Among those ants, the pavement ant remains the dominant ant in both vineyard sites followed by the thief ant, the *Lasius* genus (garden ant), the odorous house ant (*Tapinoma sessile*), and the *Pheidole* genus (big-headed ants). During the field research, some of the ant species seen in close association to and tending after the mealybugs include genus like *Crematogaster*- the acrobat ant, especially species *C. ashmeadi* Emery and *C. pilosa* Emery, *Tetramorium*- the pavement ant, *Lasius*- the garden ant, and *Solenopsis molesta*- the thief ant.

Crematogaster ashmeadi and *C. pilosa* are arboreal ants native to the southeastern united states (Tschinkel 2002, Saarinem 2021, MacGown 2022). They are commonly known as acrobat ants because of the way the workers hold up their abdomen over the rest of the body when disturbed. They mostly nest in trees, logs, fallen branches, and in hollow stems of plants. These ants were seen in the grapevine under the bark, actively tending mealybugs and raising their abdomen up when alarmed or disturbed. As seen on the field, they also pick mealybugs up and transfer them to safe places when disturbed.

Tetramorium and *Lasius* have been two of the dominant genera seen in association with mealybugs, the former is subterranean, and the latter is arboreal (Stock and Gauge 2022). Like Crematogaster, they were seen actively defending and moving mealybugs around to safer sections of the grapevines. Among the common genera of ants in the vineyard, *Pheidole*, and *S. molesta* are widespread generalists (Delabie and Fowler 1995, Wilson 2003).

One of the drawbacks of using of pitfall trap is the underrepresentation of subterranean ants and the high representation of epigeic ants. Some of the arboreal ants (e.g. *Crematogaster* and *Lasius*) included in the sample are also active on the surface. Some of the epigeic and subterranean ants are underrepresented in the one-minute visual count for ants in the trunk. The pitfall traps were installed as soon as they reached the field early in the morning and taken back when leaving the field, the same day. In addition, the vineyards are mostly surrounded by wooded areas and hence may have contained ant species present in the forests as well.

The use of two different types of ant bait dispensers had a varying effect on the pest densities in the vineyards. The result can be traced back to the varying effects of these dispensers on the activities of ants. The ant densities were similar in all the treatments before the deployment of dispensers. Although the ant densities were not significantly different across different treatments for each of the sampling dates for pitfall trap data, the data evaluation for the entire sampling period revealed significant differences in ant densities across different treatments when compared to the control. This result is similar to previous research on the use of dispensers (Parrilli et al. 2021). The data evaluation for the entire sampling period for one-minute visual count data revealed a significant difference in ant densities in sugar bait compared to the control in one of the sites and in ant bait compared to the control in another site. The result is comparable to the previous research by Beltrá et al. (2017) and Pérez-

Rodríguez et al. (2021). Thus, insecticide-laced sugar dispensers may reduce ant populations in vineyards, which could help increase biological control of mealybugs in vineyards.

One of the important aspects of the experiment was the ant distribution in the presence of dispensers. During the initial days of dispenser deployment, ant densities were numerically higher in the baits containing ant toxicants compared to sugar bait and control. The distance between sugar-toxicant bait and sugar bait treatment was maintained at 4.3 to 6.4 meters distance. One of the promising aspects of using ant baits was seen two weeks later when numerous groups of two ants were seen carrying around many dead/sick ants. By the second week of July, most of the ant nests around the treatment region having ant toxicants were gone. I only saw a few foraging/wandering ants or a few new ant nests seen on that section of the field.

Contrary to sugar-toxicant bait, sugar bait treatment had lower densities of ants during the initial days of dispenser deployment and slowly the ant number starts increasing in a few weeks. By the third week of August, even in the sugar bait treatment, more ants were seen around mealybugs than on the dispensers. The number of ant foragers at ant baits increasing over time can be explained by pheromone recruitment and the establishment of foraging trails (Greenberg and Klotz 2000).

The use of sugar/ant dispensers has often been combined with other methods of biological control like the use of predators or parasitoids (Beltrá et al. 2017, Parrilli et al. 2021). The parasitization rate and predation rates on different treatments were not included in our study due to time constraints. Previous studies have recorded a significant increase in the predation pressure and parasitization rates in mealybugs when sugar dispensers were deployed (Beltrá et al. 2017, Parrilli et al. 2021, Pérez-Rodríguez et al. 2021).

A weak to low correlation exists between ant densities and mealybug densities in the control ant bait and sugar bait treatments, which suggests the changes in ant densities have a very little or weak effect on mealybug densities. A moderate correlation exists between mealybug densities and cluster injury due to mealybugs, which suggests changes in mealybug densities have a moderate effect on cluster injury. Although some of the results were significant, a strong correlation between the number of ants per minute, and the number of mealybugs per vine with the cluster infestation was lacking in our data. There is a weak correlation, however the use of dispensers in our study has led to numerically lesser ant activities, mealybugs numbers and cluster injury in the treatment area having ant baits with ant toxicants. A further study might be needed to study the effect of multiple years of placement of dispensers on ant activity, mealybug levels and cluster infestation rate on the vineyard. The dispensers should be continuously deployed for more than two consecutive years for increasing their efficacy against vineyard-dwelling ant populations (Daane et al. 2007).

One of the time-consuming aspects of the dispenser used in the field started from the assemblage of all the tiny pieces of the sugar dispenser and its delivery. The current dispenser is more suitable for small- to medium-sized vineyards, which require a limited number of dispensers. More research should be carried out to optimize and improve the installation and maintenance to make it more friendly for vineyards of varying sizes.

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Chapter 4

Determine the efficacy of different insecticides against mealybugs in vineyards

Abstract

Mealybugs (Superfamily Coccoidea and Family Pseudococcidae) are cryptic nuisance pests, prevalent in vineyards and deciduous fruit crops across the United States. Although insecticides are the first option for the control of agricultural pests, it is often complicated for cryptic pests like mealybugs, which often hide in concealed locations like under the bark of trunk or cordon and sometimes in the roots. The present study aims to investigate the effect of three different insecticides (buprofezin, bifenthrin, and spirotetramat) on the survival of mealybugs. The efficacy of these insecticides was tested on individual grapevines by using single-vine treatment, we conducted spot spray applications with twelve single vine replicates of each treatment. The mealybug densities were recorded in the year 2021 and 2022, before conducting the spray trial and every week after the spray trial. Mealybug densities were similar on each of the treatments before the chemical spray, while the densities were different in the chemical spray trial are in comparison to the control. Further details and insights regarding these results are elaborated more in detail in the subsequent chapter.

4.1 Introduction

The family Pseudococcidae is the second largest family of superfamily Coccoidea represented by 53 genera and 320 species in the United States (Ben-Dov et al. 2016). Mealybugs are common pests of perennial crops, grapevines, deciduous fruit crops, greenhouses, and ornamental plants in the United States. They are named after the powdery white wax covering their body (Millar 2002, Franco et al. 2009). They are highly polyphagous pests, found infesting 250 families of host plants. Some of the important vineyard-infesting mealybugs include grape mealybugs (*Pseudococcus maritimus* Ehrhorn), Gill's mealybugs (*Ferrisia gilli* Gullan), longtailed mealybug (*Pseudococcus longispinus* Targioni-Tozzetti), citrus mealybug, *Planococcus citri* (Risso), citrophilus mealybug (*Pseudococcus calceolariae* Maskell), vine mealybug (*Planococcus ficus* Signoret), striped mealybugs (*Ferrisia virgata* Cockerell), and obscure mealybugs (*Pseudococcus viburni* Signoret) (Daane et al. 2012). Grape mealybug, Gill's mealybug, striped mealybug, and obscure mealybug have been reported in grapes in the past (Pfeiffer 2008, Jones and Nita 2016).

Mealybugs are sexually dimorphic. The females look more like the larger nymphal stage and feed on the phloem sap throughout the life-stages. The males look like gnats with a pair of wings and undergo complete metamorphosis. Males stop feeding after the second instar and have the sole purpose of reproduction (McKenzie 1967).

Synthetic chemicals may be used on mealybugs either for direct control or as semiochemicals for their surveillance, mass trapping, or mating disruption. Chemical control is one of the primary control methods vineyard managers use against grape pests in Virginia (Franco 2004, 2009). The intensive and repetitive use of chemicals has not only reduced natural populations of mealybugs' natural enemies but also the development of insecticide resistance in the pest itself leading to continuous and increasing use of the chemicals (Franco 2004, 2009, Venkatesan 2016). Newer products with better efficacy against mealybugs have been introduced with mixed results in recent times (Haviland et al. 2023, 2015, Lopez et al. 2022).

Although insecticides are the first option vineyard managers settle for during the crisis, it is often complicated for cryptic pests like mealybugs, which often hide in concealed locations like under the bark of trunk or cordon and sometimes in the roots (Daane et al. 2003). It is further complicated by the association of mealybugs with ants. In California, where most of the research on mealybugs has been conducted, Argentine ants *Linepithema humile* Mayr have a mutualistic relationship with honeydew-excreting Sternorrhyncha and affect their natural enemies as well (Tillberg 2007). An association with ants increases the density of obscure mealybug and lowers the density of parasitoids *Pseudaphycus flavidulus* and *Leptomastix epona* (Hymenoptera: Encyrtidae). Densities of mealybug destroyer, *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) have also been reported to be higher in the ant-tended vines having a higher density of mealybugs. They avoid detection due to their striking resemblance to mealybug adults (Daane et al. 2007).

The present study aims to investigate the effect of three different insecticides (buprofezin, bifenthrin and spirotetramat) on the survival of mealybugs. Field experiments were conducted to test the efficacy of three different insecticides having different modes of action groups (Table 4.1). Buprofezin (Applaud) is an insect growth regulator (IRAC Group 16) that has been recommended for the control of red scale, white louse scale, cicadellids (leafhoppers), and mealybugs in grapes and pears (De Cock and Degheele 1998). It inhibits chitin biosynthesis, suppresses the oviposition of adults, and reduces the viability of eggs. Bifenthrin

(Brigade) is a broad-spectrum pyrethroid (IRAC Group 3A) that is effective against chewing, flying, and sucking insects like stink bugs, bollworms, mites, etc., and either ingested or works on contact. Spirotetramat (Movento) has a unique mode of action impacting lipid biosynthesis in certain insects (IRAC Group 23) and is considered a revolutionary tool in the control of aphids, psyllids, whiteflies, mealybugs, etc. Spirotetramat exhibits systemic movement within the host plant, effectively traversing both xylem and phloem tissues, allowing it to be translocated to new shoots and down to the roots (Brück et al. 2009). Translocated through the vascular system of the host plant, it is particularly effective against immature stages of sucking pests such as mealybugs, scales, aphids, psyllids, and whiteflies (Nauen et al. 2008).

The experiment was designed to quantify the effect of insecticides to control mealybugs in the vineyard and its effect on cluster infestation. These chemicals are all registered for use on grapes. To determine the efficacy of these insecticides on individual grapevines used as single-vine treatment, I conducted spot spray applications with twelve single vine replicates of each treatment.

Table 4.1. IRAC Mode of Action classification for the insecticides (Buprofezin, Bifenthrin and Spirotetramat) that were field-tested against mealybugs (IRAC, 2016).

Brand Name (Common Name)	Active Ingredient	IRAC Group/ subgroup	Primary Site of Action/Mode of Action (Based on IRAC MoA Classification)
Applaud®	70% Buprofezin	16 (Inhibitors of Chitin Biosynthesis, Type I)	Insect Growth Regulator/ Chitin Synthesis Inhibitor
Brigade®	25.1% Bifenthrin	3A(Pyrethroids)	Sodium Channel Modulator
Movento®	22.4% Spirotetramat	23	Lipid Biosynthesis Inhibitor

4.2 Materials and Methods

Field Sites and Experimental Design:

The insecticide field trial experiment was carried out in two vineyard sites: Orange County (Site H), and Nelson County (Site S), VA. Vineyards were selected based on the availability and pest pressure recorded by the researcher in the previous years. These are conventional vineyards, relying on synthetic insecticides for pest management. Each vineyard block had more than 10 years old Cabernet Franc grapes and had a previous history of mealybug infestations. The spray trial was carried out randomly in twelve single vine replicates of each treatment. There were four treatments: 1. Untreated Control; 2. Buprofezin; 3. Bifenthrin; and 4. Spirotetramat insecticide. The treatment plots were separated from each other by 1-2 vines. Prior to the spray trial, between the years 2019 and 2021, the vineyard sites were thoroughly scanned for the presence of mealybugs on the vines. These vines were flagged for the presence of mealybugs and allocated for the insecticidal trial.

The concentration of products used in the experiments is listed in Tables 4.2. The label and the safety data sheet of the insecticide were followed thoroughly. A SOLO 3-gallon backpack sprayer was used for the experiment. The spray date was selected on a date with no rainfall up to 2 days after the insecticide application. Each of the chemical concentrations of the three insecticides was calculated and diluted in the field site. The insecticide application was carried out two times per growing season halving the recommended application rate per growing season.

The pretreatment mealybug number was recorded using a five-minute sampling of the vine, where we recorded the number of mealybugs encountered under the bark. I have the five-minute sampling data on mealybugs from May 10, 2022, for the year 2022. The first spray trial for the year 2022 was conducted on May 31, 2022, and the second spray trial was conducted after a month on July 11, 2022. We have the 5-minute presampling data on mealybugs for the year 2021. The spray trial for the year 2021 was conducted on July 11, 2021.

Table 4.2. List of materials applied and spray rates for the insecticides that were field-tested against mealybugs.

Brand Name (Common Name)	Active Ingredient	Company	rate/100 L water
Applaud®	Buprofezin	NICHINO America, Inc Wilmington, DE	0.53 lb ai
Brigade® 2EC	Bifenthrin	FMC Corporation Philadelphia, PA	0.1 lb ai
Movento®	Spirotetramat	BAYER CropScience St. Louis, MO	0.13 lb ai

Data Analysis

The five-minute counting of mealybugs per vine was recorded in each of the treatments. The five-minute sampling was carried out before the spray treatment as well as on different dates after the treatment. The mealybug densities were analyzed 20 days before treatment (DBT), 1, 10, 17, and 29 days after the first treatment (DAT), and 1, 10, 18, and 31 days after the second treatment (DA2T) for the year 2022. For the year 2021, because only one spray trial was carried out, mealybug densities were analyzed 1 and 10 days after the treatment (‘DAT). Each of the field locations and the field dates was analyzed separately. Analysis of the mean number of mealybugs on different treatments was carried out using nonparametric comparisons for each pair using Wilcoxon Test (Beatty, 2018). For the statistical analysis, I used the JMP Pro software package.

4.3 Result

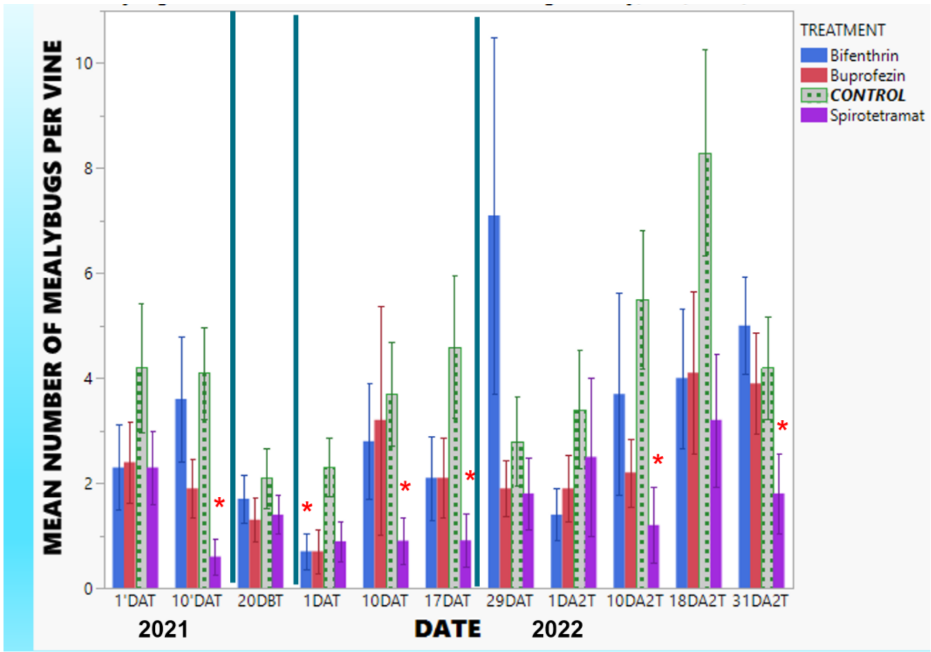

Figure 4.1. The mean number of mealybugs per vine according to a 5-minute visual count on the vine in different treatments (Orange County, Site H). Each error bar is constructed using 1 standard error from the mean. 1’ and 10’ DAT indicate 1 and 10 days after treatment in 2021. 20DBT indicates 20 days before treatment before the first spraying (May 2022), and the 1DAT, 10DAT, 17DAT, and 29DAT indicate 1, 10, 17, and 29 days after the first spraying in the year 2022. 1DA2T, 10DA2T, 17DA2T, and 29DA2T indicate 1, 10, 17, and 29 days after the second spraying in the year 2022. * sign indicates significance difference in comparison to control or untreated check.

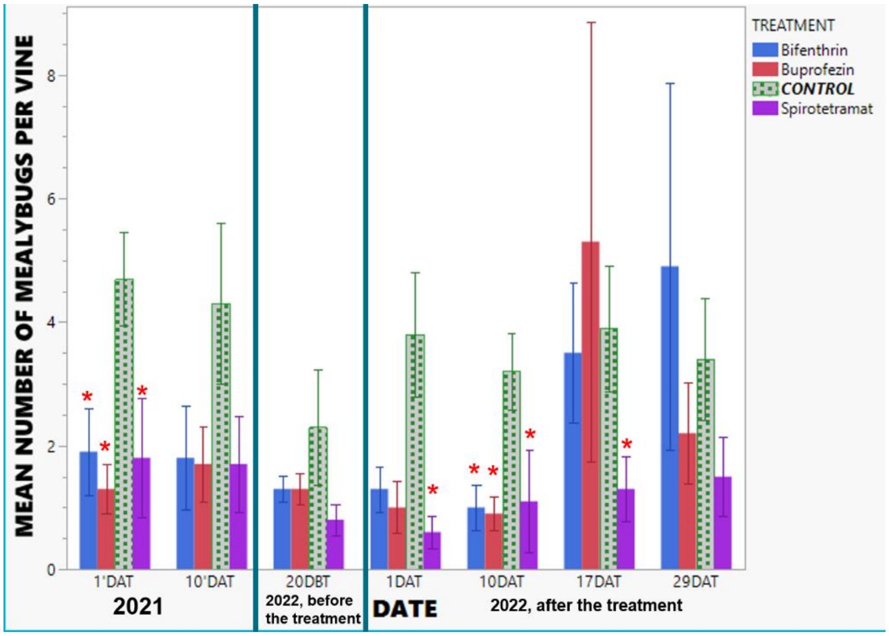

Figure 4.2. The mean number of mealybugs per vine according to a 5-minute visual count on the vine in different treatments (Nelson County, Site S). * sign signifies significant difference compared to the control. Each error bar is constructed using 1 standard error from the mean. 1’ and 10’ DAT indicate 1 and 10 days after treatment in 2021. 20DBT indicates 20 days before treatment before the first spraying (May 2022), and the 1DAT, 10DAT, 17DAT, and 29DAT indicate 1, 10, 17, and 29 days after the first spraying in the year 2022.

Spray trial data at Orange County, VA (Site H)

The spray trial data in the year 2021 revealed a significant difference in the mealybug densities only in spirotetramat treatment in comparison to the control at 10 days after the treatment (‘DAT) (Table 4.3). Mealybug densities were numerically lower in all of the treatments in comparison to the control at 1DAT and 10 DAT. There was no significant difference in the mean mealybug densities between treatments and control before the spray trial in year 2022 (Table 4.3). Although the mealybug densities were numerically lower in all of the treatment in comparisons to the control in 1DAT, 10DAT and 17DAT after first spray trial in 2022, only spirotetramat treatment led to significantly lower number of mealybugs in comparison to the control (Table 4.3). Mealybug density in bifenthrin treatment was significantly lower in comparison to the untreated control one day after the first spray trial in 2022. The data exhibited no significant difference (for most of the data) in mealybug densities during the second spray trial in the year 2022, except for spirotetramat which was effective up to 31 days after the second spray trial (Table 4.3).

Spray trial data at Nelson County, VA (Site S)

The mealybug density in all of the treatments had significantly lower mealybug density in comparison to the control (Table 4.4) in comparison to the untreated control in the year 2021. The data exhibited no significant differences in mealybug densities 10' DAT during the first spray in 2021 and 20DBT in the year 2022 (Site S). The mealybug densities were significantly different and lower in comparison to the control one day after treatment (1DAT) after the first spray only in spirotetramat treatment (Figure 4.2) and was effective to control mealybugs upto 17 days after the treatment. The mealybug densities were significantly different and lower in comparison to the control ten days after treatment (1DAT) after the first spray in 2022 (Table 4.4)

The mean densities of mealybugs remained numerically higher in the control treatment than in the rest. Rest of the dates, a higher number of mealybug densities in some of the treatments is consistent with the emergence of the crawler stage (Figure 4.1 and Figure 4.2).

Table 4.3. Z-score and p-values calculated by comparing the mean number of mealybugs in the spray trial (buprofezin, bifenthrin and spirotetramat) against the mean number of mealybugs in control (Site H).

DATE→	**1’DAT**	**10’DAT**	**20DBT**	**1DAT**	**10DAT**	**17DAT**	**29DAT**	**1DA2T**	**10DA2T**	**18DA2T**	**31DA2T**
CONTROL-Buprofezin	Z=0.97 p=3318	Z=1.92 p=0.0548	Z=1.0 p=0.317	Z=1.95 p=0.501	Z=1.49 p=0.134	Z=1.2 p=0.229	Z=0.626 p=0.5311	Z=1.2 p=0.2283	Z=1.77 p=0.0755	Z=1.69 p=0.09	Z=0.269 p=0.7874
CONTROL-Bifenthrin	Z=1.05 p=0.2898	Z=0.422 p=0.6723	Z=0.48 p=0.6312	Z=1.99 p=0.0461*	Z=0.85 p=0.393	Z=1.24 p=0.2125	Z=0.50 p=0.615	Z=1.49 p=0.1355	Z=1.29 p=0.194	Z=1.47 P=0.1415	Z=-0.58 p=0.5615
CONTROL-Spirotetramat	Z=.97 p=0.3316	Z=3.09 p=0.0020*	Z=0.78 p=0.433	t=1.75 p=0.079	Z=2.24 p=0.046*	Z=2.2 p=0.0273*	Z=0.667 p=0.5046	Z=1.44 p=0.1492	Z=2.45 p=0.0139*	Z=1.82 p=0.0684	Z=2.4 p=0.0160*

Table 4.4. Z-score and p-values calculated by comparing the mean number of mealybugs in the spray trial (buprofezin, bifenthrin and spirotetramat) against the mean number of mealybugs in control (Nelson County, Site S).

DATE→	**1’DAT**	**10’DAT**	**20DBT**	**1DAT**	**10DAT**	**17DAT**	**29DAT**
CONTROL-Buprofezin	Z=3.08 p=0.0019*	Z=1.28 p=0.20	Z=0.672 P=0.5014	Z=1.88 p=0.0597	Z=2.77 p=0.0055*	Z=1.002 p=0.3159	Z=0.842 p=0.3994
CONTROL-Bifenthrin	t=2.29 p=0.0216*	Z=1.16 p=0.244	Z=0.668 p=0.5037	Z=1.5 p=0.132	Z=2.54 p=0.0120*	Z=0.346 p=0.729	t=0.311 p=0.755
CONTROL-Spirotetramat	t=2.53 p=0.0400*	t=1.32 p=0.1836	Z=1.45 p=0.1464	Z=2.18 p=0.0288*	Z=2.43 p=0.0150*	Z=2.05 p=0.0399*	t=1.02 p=0.306

4.4 Discussion and Conclusions

The mealybug management in the vineyards is usually focused on the repeated use of chemical control throughout the season (Franco et al. 2009; Daane et al. 2012). The management of mealybugs in vineyards poses significant challenges due to their cryptic nature and the potential non-target effects of conventional chemical control methods on beneficial arthropods. To address these issues, this discussion explores the use of targeted insecticides, with a particular focus on the effectiveness of buprofezin, bifenthrin and spirotetramat.

The use of three different insecticides in our field trial had different effects on mealybug densities on different dates. A single spray trial was conducted in the second week of 2021 and double spray trial during year 2022 (last day of May and second week of July). Compared to the populations of overwintering generations emerging out during spring, mealybug densities of subsequent first generation were numerically higher during and after July. The spray trial conducted during July (the single spray trial of 2021 and second spray trial of 2022) revealed significant difference in mealybug densities in either of the insecticides used for treatment. This observation is similar to the study by Daane et al. (2019), where the delayed dormant applications of buprofezin at two different concentrations exhibit no significant treatment differences in mealybug densities until the week when mealybug numbers in large numbers begin the migration from trunk and cordon to leaves. We also observed a similar trend where no significant difference in densities were observed before the treatment and 1 day and 10 days after the treatment in one of the sites having larger mealybug density. Although in the field site having lower mealybug density, there was significant difference in mealybug densities in all the treatment sites compared to the control a day after the treatment. Daane et al. (2006) has also mentioned the higher effectiveness of buprofezin if applied earlier in the season when the population is low and does not have overlapping generations unlike the late-season application. However, only in spirotetramat treatment, significant difference in mealybug densities were observed even 17 and 29 days after treatment in comparison to the control after the first spray of the season.

Efficacy variation of different insecticides and the lack of significance in the data might be due to clumped and uneven distribution of mealybugs on the sites. One of the problems of using conventional chemicals for mealybug control is their inability to reach this cryptic pest which is often found hiding in concealed locations inside the bark and root up to 30 cm deep (Walton and Pringle 2004a, Walton et al. 2004b, Daane et al. 2012). Another effect is their non-target effect on beneficial arthropods (Desneux et al. 2007, Biondi et al. 2012, Pisa et al. 2015, Mansour et al. 2018). Another important step in incorporating integrated pest management in the commercial setting has been checking the compatibility of insecticides with biocontrol agents (Stark et al. 2007, Biondi et al. 2012). One of the future directions of the current study might be checking the predators and parasitoid populations in the spray trial area in addition to the population of mealybugs present.

In a recent study by Steenwyk et al. (2016), nine treatments were replicated four times in the vine mealybug control program with no significant difference observed among the treatments after the buprofezin-delayed dormant application. However, significantly reduced cordon infestation was observed with Applaud at 24 oz/acre, Applaud (12.0 oz/acre) combined with Assail (2.5 oz/acre), and Applaud (16.0 oz/acre) combined with Assail (2.5 oz/acre) compared to untreated control. In addition to this, a lower fruit infestation rate was observed in all the treatments compared to the untreated control. My observation recorded fruit infestations in control, sprayed zone, and the area with ant dispensers and did not record fruit cluster infestation in each of the insecticide treatments. A future study recording fruit infestation rates in each of the treatment sites would be important to underline the efficacy of these insecticides.

Our study focuses on commercial vineyard sites and spray trial was dated based on availability of the site and restricted-entry interval of other pesticides used in the vineyard. In one of the sites having lower number of mealybugs, the analysis has recorded lower number of mealybug densities in comparison to the control. Although this study was not replicated across multiple vineyards, a further study may provide a different direction especially by targeting crawler stage before the populations are relatively higher in the later season.

My field trial was divided into two applications of insecticides per season to target these pests effectively. However, a single application of spirotetramat at a rate of 72-88 g of active ingredient per hectare is effective to control mealybugs in grapevine with an efficiency exceeding 90 % according to study by Brück et al. (2009) and Mansour et al. (2010). It exhibits long-lasting protection and residual activity to control mealybug nymphs up to 40 days after the treatment (Mansour et al. 2010). A future study evaluating the effectiveness of a single application per season in comparison to double application with lower spray volume would be interesting. Spirotetramat has also been incorporated into the spray program in California vineyards against vine mealybugs during late spring to early summer or post-harvest spray (Daane et al. 2012). The April, May, or June foliar applications were highly effective against vine mealybugs, especially the April application when damages were reduced from 50% to 97% (Haviland et al. 2010). 100% mortality of citrus mealybugs was observed in citrus orchards even after seven days of treatment (Satar et al. 2013, Mansour et al. 2017). Similar to this study, my study has also demonstrated effectiveness of spirotetramat 1 day and 10 days after the application. Recent application of spirotetramat in pistachio orchards in California also resulted in significant reductions of Gill’s mealybugs 42, 56, 69, and 96 days after treatment (Haviland and Rill 2021). One of my field observations on some of the sites over the years has been lower number of Gill’s mealybug numbers on the field compared to year 1 when the vineyard managers just started using spirotetramat for mealybug control. A future study focusing on the effect of different insecticides on species level would be interesting.

Applications of these insecticides resulted in significant reductions in mealybug densities compared to the untreated check (control) on most of the evaluation dates where data were statistically significant. Some of the dates after treatment, when mealybug densities were significantly higher in the treatments, indicate many crawlers on the grapevines. As the data have been based on a 5-minute visual count on vines for mealybugs, sometimes it took a long time to find the hiding spot of mealybugs which likely affected the outcome.

Ideally, it would have been more satisfactory to have more replication plots than I assigned and work on more varieties of grapes affected in the greenhouse as well as field settings. However, as most of my field sites have been commercial vineyards, 48 vines with 12 single-vine treatments of each treatment were ambitious enough to utilize in commercial sites. Due to the time constraints and limited personnel involved, I was unable to incorporate the number of predators and parasitoids after the spray trial, cluster infestation rate during harvest, and number of ants per treatment. These variables might have given a different picture of each of the insecticidal treatments.

In summary, the research presented demonstrates the efficacy of buprofezin, bifenthrin and spirotetramat in reducing mealybug densities in Virginia vineyards. However, the study also acknowledges some limitations, such as the difficulty in locating well-hidden mealybugs during visual counts and the need for further investigation into the impact on predators, parasitoids, cluster infestation rates, and ant populations. In conclusion, the discussion highlights the importance of integrated pest management practices and targeted insecticides in enhancing mealybug management in vineyards. By adopting a holistic approach that considers the interactions between pests, natural enemies, and host plants, sustainable and effective pest control strategies can be developed for the benefit of vineyard ecosystems and agricultural sustainability. Our research was only able to cover the chemical aspect on the management of mealybugs on the field and fails to incorporate the role of these insecticides in the ant populations, natural enemies, and pollinators in the field. Further research and collaboration between researchers, vineyard managers, and growers are essential to fine-tune and optimize these strategies for specific vineyard environments and pest pressures.

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Chapter 5

Summary, Implications, and Suggestions for Future Research

5.1 Results Summary and Implications

The research conducted in this dissertation has undermined species composition, population density, and management of mealybugs and their attending ants in vineyards in Virginia. The arrival of invasive *Planococcus ficus* in the West Coast of the United States in the early 1990s and its invasion throughout the grape-growing regions in California and its recent appearance in Oregon (2021) has caused a havoc among the grape-growers. In addition to the invasive species *P. ficus,* there are other species of mealybugs prevalent in the US. The presence of these cryptic species, which often lies hiding in concealed locations, affects the grapevine health and yield. Mealybugs are often attended by ants, which feed on their honeydew excrement. A thorough knowledge of species composition, and the management of mealybugs and their attending ants in vineyards is necessary to implement management decisions. Based on the current knowledge on research conducted in the West Coast, this dissertation has attempted to address some of the gap in information in mealybugs in Virginia.

My research has demonstrated that *Pseudococcus maritimus* and *Ferrisia gilli* are common throughout vineyards in Virginia (Botetourt County, Albemarle County, Nelson County, Orange County, Culpepper County, and Augusta County). *Pseudococcus viburni* was also spotted on two instances while *P. ficus* was missing in our study. We present evidence suggesting that nymphal stages of *P. maritimus* and *F. gilli* were active as early as May in Virginia vineyards and this first generation of mealybugs emerging from overwintering stages are lower in populations. Adults are seen during the month of June and produce second generation that are responsible for maximum damage on the grapevines in summer. These second generations are ultimately responsible for producing the overwintering life-stages.

My research also revealed fifteen genera of ants active in vineyards via pitfall tap study. *Tetramorium, Lasius, Solenopsis molesta, Tapinoma sessile* and *Pheidole* remain some of the dominant ant genera in the sampled vineyards. Among the ant genera, *Tetramorium, Lasius,* and *Crematogaster* were some of the ants seen actively tending after and defending the mealybug species. The deployment of dispensers in the vineyards also had some interesting results. Fewer ants were recorded from untreated control treatment in comparison to areas having ant dispensers. Although ant number in the pitfall trap and ant nests around the treatment area were higher in treatment with sugar dispensers laced with insecticide in comparison to the treatment area with dispensers with only sugar solution. Two weeks after the placement of ant insecticides in dispensers, the treatment area was seen with dead and sickly ants. The area had a minimum number of ant nests after the second week of July, almost a month after the placement of dispensers (having ant insecticide). The ants caught on the pitfall traps were largely comprised of ants wandering around that treatment area or new nests established. The sugar dispensers had consistently higher number of ants feeding on it throught the sampling season. Although after the month of August, more ants were seen tending after the mealybugs than on the dispensers placed just on the next vine. The fruit cluster infestation rate was also significantly lower in the areas having dispensers in comparison to the control area lacking dispensers. Similar results were recorded in the recent research by Parrilli et al (2021), and Beltrà et al. (2017).

In chapter 4, I evaluated to address the efficacy of buprofezin, bifenthrin and spirotetramat against mealybug densities in grapevines. Buprofezin and bifenthrin were effective up to 10 days after the treatment, while spirotetramat remains effective up to 31 days after the treatment. We therefore recommend spirotetramat to be incorporated as one of the management tool for the chemical control of mealybugs.

5.2 Future Direction

Our research has highlighted the species composition, their seasonal fluctuations in densities of mealybugs and attending ants in vineyards and have also addressed some of the management techniques for their control. Based on the data recorded, adhesive tape band were more effective to trap male mealybugs in comparison to the control. However, both traps have higher chances of trapping unwanted animals including small birds. Hence there is a need to improve the selectivity of these traps to reduce the capture of non-target animals. A field or lab study observing the efficiency of traps of different colors and sizes on the number of male mealybugs captured in the trap. Another interesting approach to reduce the capture of nontarget animals is by adding netting around the trap to avoid other non-target animals to stay away from the sticky trap (Sétamou et al. 2019). The male mealybugs were captured in all lures in varying numbers in all species-specific lures deployed on the field i.e., *P. maritimus, P. viburni, P. longispinus, P. ficus,* and *P. citri.* As evident by Bahdur et al. (2013), and Waterworth et al. (2011), the males are caught most likely caught in field trials with multiple species-specific pheromone lures might just be a random act. The selectivity of male mealybugs *P. maritimus* and *F. gilli* to different trap colors and sex-pheromone lures remains to be explored, as we failed to identify them.

The data presented here summarizes the ant genera prevalent in the vineyard and some of the dominant ant genera as evident by pitfall trap capture and 1-minute visual count. We addressed the ant and density fluctuations in vineyard after the placement of sugar dispensers and its effect on fruit cluster infestation. The presence of sugar dispensers on the field has lowered the fruit cluster infestation in comparison to the area without sugar dispensers. The data presented here did not show the effect of deployment of sugar dispensers on the natural enemies' populations (predator and parasitoid) of mealybugs in the vineyard or even the use of biological control agents in control of mealybugs in addition to the use of dispensers. Parrilli et al (2021) has demonstrated a great potential of biological control agents in the control of mealybugs in the vineyard.

Another area in need of study is the effect of buprofezin, bifenthrin and spirotetramat on ants and the natural enemies' population in the vineyard. Recent studies have demonstrated that the presence of buprofezin in the vines also has a significant impact on the parasitization activity and development of immature stages of the generalist coccinellid predators of the mealybug host (James, 2004; Suma et al., 2009). Another study using five different treatment methods were applied to control fire ants and hibiscus mealybugs in citrus have demonstrated all four treatments (Hot water treatment, chlorpyrifos/bifenthrin treatments, Clinch, and Extinguish treatments) had significantly fewer ant colonies compared to the untreated check. However, chlorpyrifos/bifenthrin treatments had more ant colonies compared to the other chemical treatments. However, there was no effect of overall treatments on ant-tending hibiscus mealybug colonies as well as on the abundance of predators or parasitoids (Middleton et al., 2023). It is possible that the use of these chemicals would influence the ants and natural enemies’ population in the vineyard. Measuring the population densities of ants and natural enemies after the insecticide treatment may help address this question.

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