Progress Report – Due March, 2023

Fourth Quarterly Report

Mealybug species composition and management in Virginia vineyards

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Main areas of accomplishment in 4th Quarter:

This project represents the fourth year of a doctoral program of Ms Pragya Chalise. Ms Chalise made progress in her survey of mealybugs, and control trials for mealybugs, as well as ants tending those pests.

> **Objective 1:** Confirm the species composition of mealybugs in Virginia vineyards, comparing aerial populations with those inhabiting roots, emphasizing central and eastern Virginia
> **Objective 2:** Carry out two insecticidal efficacy trials
> **Objective 3:** Determine the most common ant species in association with root infestations
> **Objective 4:** Determine whether ants are moving bug from vine to vine
> **Objective 5:** Conduct a control trial targeting ant populations in vineyards

Progress to date:

Objective 1: Confirm the species composition of mealybugs in Virginia vineyards, comparing aerial populations with those inhabiting roots, emphasizing central and eastern Virginia

Mealybugs along with scales insects are grouped together under the superfamily Coccoidea and are closely related to aphids and whiteflies. They differ from scale insects in the fact that they retain their legs throughout their life, unlike scale insects. Mealybugs are adapted for plant parasitism and have developed different metamorphoses. Adults exhibit distinct sexual dimorphism. The female exhibits pedomorphism, where adult females retain the external morphology of the immature forms even though they are sexually mature. Males exhibit complete metamorphosis and adult males look like gnats and may be winged or wingless depending on the species. Adult males do not feed and their sole function is reproduction. Parthenogenesis is common in these insects as well.

Family Pseudococcidae is a species-rich family of phloem feeders, including serious agricultural pests. The subfamily Pseudococcinae includes the majority of economically important grape-infesting mealybugs. Grapevines accommodate a number of polyphagous species of mealybugs, that infest not only the grapes but also a number of deciduous fruit crops or some ornamental greenhouse plants. Some of the major vineyard-infesting mealybugs include species like grape mealybug (GMB), *Pseudococcus maritimus* (Ehrhorn), obscure mealybug (OMB), *Pseudococcus viburni* (Signoret), long-tailed mealybug (LtMB), *Pseudococcus longispinus* (Targioni- Tozzeti), vine mealybug (VMB), *Planococcus ficus* (Signoret), citrus mealybug (CMB), *Planococcus citri* (Risso), and Gill's mealybug (GiMB), *Ferrisia gilli* (Gullan). Mealybugs feed on all parts of the plants including the trunk, cordon, leaves, roots, and fruits.

The economic damage due to the presence of mealybugs in the grapevines is not only limited to feeding on phloem sap, but it also leads to the development of sooty mold growth due to the excretion of honeydew on different parts of the vines but also reduces plant vigor, health, and grape yield. Another damage is seen during harvest when different stages of mealybugs appear on grape clusters, causing cosmetic damage to the berries. Mealybugs are also vector grape leafroll disease, a viral plant disease caused by grape leafroll viruses (closteroviruses) in the genus *Ampelovirus*.

Grape mealybugs have been a predominant pest of the Virginia vineyard in the past. An earlier part of research on grapevine leafroll-associated viruses (GRLaV) in wine grape varieties and native grape species in Virginia has identified GMB, GiMB, and a minor number of obscure mealybugs (Jones, 2016). GMB and GiMB are considered native to the east coast. VMB has been found in all the grape-growing regions of California and recently discovered in Oregon; however, it has not been reported in Virginia and other states in the US in previous studies.

A monitoring program for a pest is an integral part of a pest management program. The objective of this study is to:
i. Determine the species composition of mealybugs in vineyards in Virginia, and
ii. The population distribution of mealybugs in Virginia vineyards.

2.2 Material and Methods

2.2.1 Sample Sites

We scouted five commercial vineyards with a previous record of mealybug infestation or grape leafroll virus (GLRaV) infection. The commercial sites were visited with permission from the vineyard owners. Commercial vineyards such as Horton (Orange County), Silver Creek (Nelson County), Virginia Mountain Vineyard (Botetourt), Pearmund Cellars (Fauquier County), Grace Estate Winery (Albemarle County), Barboursville Vineyard (Orange county-Albemarle county) and Barren Ridge Vineyard (Augusta county)] were visited for sampling.

2.2.2 Visual Sampling

Visual surveys are implemented to monitor the abundance and diversity of insect pests in the field, with the help of naked eyes or nets. These surveys typically document the total number of insects and the presence of particular species during the sampling duration. The research sites were monitored once a week from the end of April 2019 to October 2022. The traps were set up annually in each of the sites. Aerial samples (mealybugs on cordons, shoots, canes, and clusters) and the root samples were surveyed by visually examining different rows of vines per vineyard per day. During the early season, when insects were not spotted in the field by visual inspection, some leaf/shoot samples were taken back in 70% alcohol to check for the presence of mealybugs. An attempt was made to sample mealybugs in GLRaV-positive vines and those without known GLRaV. Mealybugs were photographed before being collected into 70% ethanol.

i. Sticky trap count: Males often use the pheromone cues left behind by the females to locate her for mating. Most mealybug pheromones consist of carboxyl esters of monoterpene alcohols and their racemic mixtures. Plastic delta traps from Alpha Scents, Inc were deployed in the field with sticky trap insert. We changed the plastic delta trap (from white to red one in 2020). The pheromone lures were ordered from Evergreen Growers Supply. Species-specific pheromone lures available on the market were deployed in the field. Lures specific to a GMB, VMB, CMB, OMB, and LtMB were used in the site to monitor the male mealybug population.

ii. 5-min count: Visual inspection of the vines for about five minutes per vine was carried out in the field sites. Each of the life stages was recorded separately and examined the aerial parts of the plants including spurs, leaves, and trunks (Geiger and Daane, 2001).

iii. Sticky Tape Band: We deployed sticky tape bands on ten vines, one each on the cordon as well as the trunk of each of the vines (hence a total of 20 tapes). Sticky tapes were cut out in a size of 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 tapes were placed by removing the outer bark of the vines. In each of the sampling methods, each of the life stages of mealybugs including egg masses was counted and recorded.

2.2.3 Genetic Analysis

The genetic analysis of mealybugs is based on a similar tool developed in California. DNA extraction was carried out using DNeasy Blood and Tissue kit. Due to the limitation in the reagents available, we pooled the sample. We carried out a genetic analysis of 24 samples from three different sites (7 samples from GEW, 4 from VMV, and 13 samples from Barboursville). Several genomic regions have been used for the identification of mealybugs and other insects. One of these regions that have been used is the mitochondrial cytochrome oxidase subunit I gene (COI). The species-specific primers designed for GMB, scarlet mealybug (*Pseudococcus calceolariae* Maskell), LtMB, VMB, CMB, OMB, and GiMB were used for the species identification. PCR was carried out in BIO-RAD C1000 thermal cycler using multiplex PCR plus kit. An initial denaturation step at 95 °C for 5 min was followed by 30 cycles of 30 sec at 94 °C, 90 sec at 53 °C and 90s at 72 °C, with a final extension of 10 minutes at 72 °C. All reactions used 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 sample. Our gel reading was divided into two replicates of each sample and two replicates of a notemplate control (no DNA). The positive control contains the DNA samples of GMB and GiMB from previous research by Taylor Jones in 2016 from AREC lab, Winchester. The first replicate was loaded with forward primer for scarlet mealybug (PCa), vine mealybug, citrus mealybug, Gill's mealybug, and the reverse primer. The second replica was loaded with forward primer for grape mealybug, long-tailed mealybug, obscure mealybug, and the reverse primer.

Results

Two species of mealybugs remain dominant throughout the vineyards sampled - GMB and GiMB. A few samples of obscure mealybug were also recorded. Male mealybugs captured on the trap was numerically higher in 2019 than in 2020, although trap capture data only indicated the presence of mealybug from the end of July up to the end of September. Male mealybugs were recorded from all the traps containing either of the lures used, although the number of trap captures was considerably higher in the grape mealybug lure

Objective 2: Carry out two insecticidal efficacy trials
The mealybug spray trials have been completed and analysis is being completed. Initial examination of the data revealed that not all assumptions of parametric statistics were met, so further use of non-parametric statistics was needed. Results will be reported later.

The ant related objectives will be discussed together below.
Objective 3: Determine the most common ant species in association with root infestations
We used a combination of pitfall traps, and trunk examination to determine ant activity. In pitfall traps, *Tetramorium,* the pavement ant remained the dominant ant in both of the vineyards, followed by the thief ant *Solenopsis molesta.*

Objective 3: Determine whether ants are moving bug from vine to vine
Field experiments are in progress, and analysis is pending.

Objective 5: Conduct a control trial targeting ant populations in vineyards
Field experiments are in progress, and analysis is pending. The active ingredient of the toxic ant bait is 1% disodium octaborate tetrahydrate.

Ant Portion Materials and Methods

The ant-mealybug experiment was carried out in two vineyard sites: Horton Vineyard, Orange County, and Pearmund Cellars, Fauquier County. 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 0.7 to 0.2 acres 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 four to 7 meters or 2-3 vine rows distance was maintained between the sugar bait and ant bait plots.

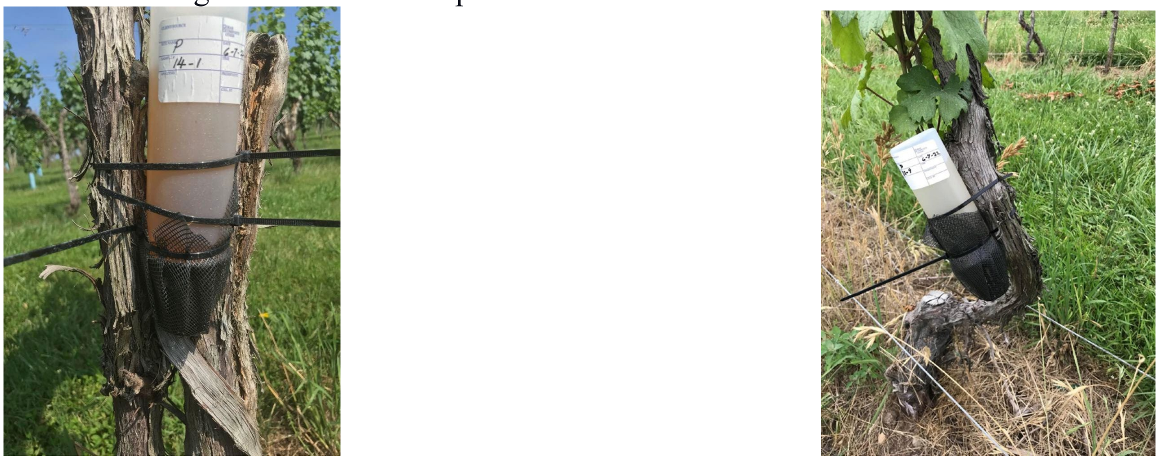


Figure 3. Ant dispensers containing a. 25% sugar solution (sugar bait) and b. Greenway liquid ant-killing bait (ant bait) deployed in the field

Sugar Dispensers

The liquid dispenser we used in the field is based on earlier research by Daane et al. (2008) and repeated by Parrilli et al. (2021). The research made use of falcon centrifuge tubes as sugar dispensers, or 250 ml HDPE narrow mouth bottles, assembled with white polypropylene closure from Lab Depot. 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 two-inch 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). Sugar dispensers, if improperly set up, could have a detrimental effect on bees or natural enemies in the vineyard.

Sugar dispensers (Figure 3. a) were deployed in the field in every 5-10th plant over an area of three rows of vines. They were deployed at the beginning of June and removed in the second week of September. We deployed 12-16 sugar dispensers in three rows of vines, evenly placing them through the experimental plot. 12 dispensers containing insecticides (Figure 3. b) were evenly deployed in three rows, maintaining a gap of two to 5 rows of grapevines 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. Ant activity was documented by 1. Pitfall trapping 2. One-minute visual count. Pitfall trapping is a standard method of sampling surface-dwelling invertebrates (Spence and Niemela, 1994; Ward et al., 2001). 50 ml falcon centrifuge tubes were used for pitfall trapping, with 75% alcohol and a few drops of ethylene glycol. 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 one foot below the vine canopy and above the dispensers on the trunk for one 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.

Ants were collected in 70% alcohol and taken back to the lab for identification. Identification was accomplished using the identification keys.

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 did not follow the normal distribution, the data 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. Data were analyzed based on count per sampling date and total count throughout the season.

Before commercial harvest, 25 fruit clusters per treatment in each replicate were evaluated using the scoring system. Injury was categorized as follows: 0= no mealybug or honeydew, 1=honeydew and 5 or fewer mealybugs, 2=honeydew and 5-9 mealybugs, 3=honeydew and more than 10 mealybugs, and 3=honeydew and egg masses). Fruit clusters with a score 2 and 3 were considered unmarketable or extremely infested. Fruit cluster infestation was analyzed using Wilcoxon paired test (Kruskal-Wallis test).

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.

Results

Ant activity in the vineyards

A total number of 1131 specimens of ants were collected in the two field sites (457 samples from Pearmund and 674 from Horton) over the whole field season, representing 12 genera of ants. According to the pitfall trap data (figure 3.1), *Tetramorium,* the pavement ant remained the dominant ant in both of the vineyards, followed by the thief ant *Solenopsis molesta.* According to the one-minute data count on the vine (figure 3.2), the top three leading foragers in the vineyards include *S. molesta*, *Pheidole* ants, and the pavement ant.

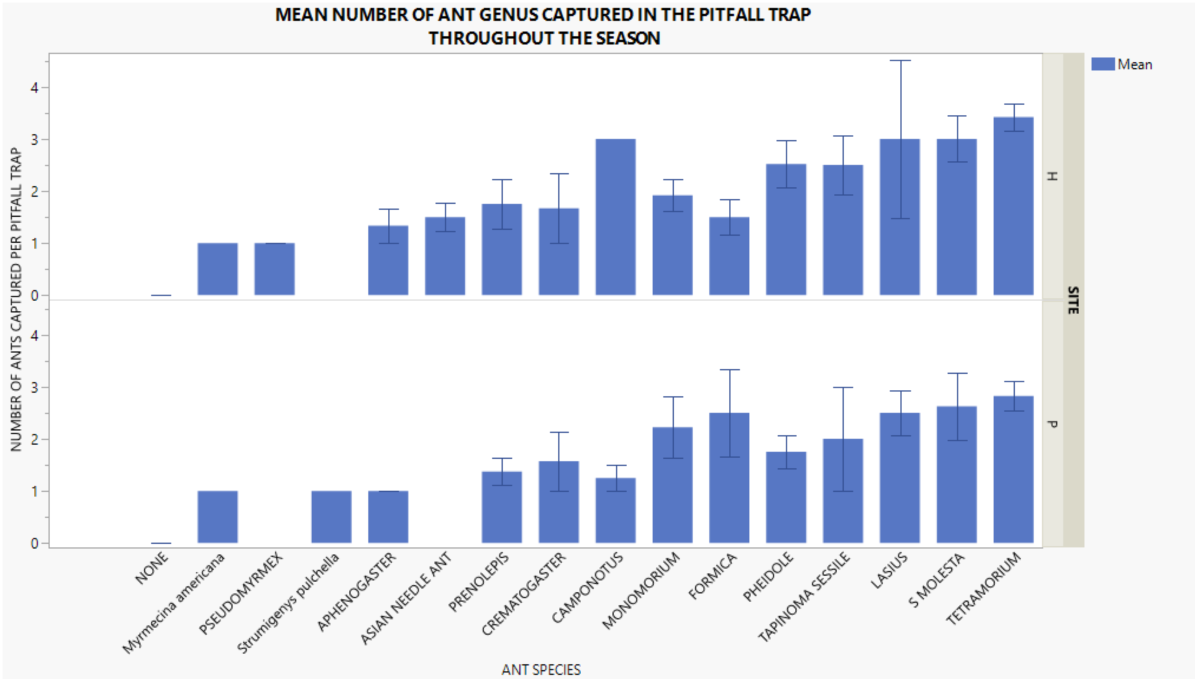


FIGURE 3.1. The mean number of ants captured in the pitfall trap throughout the season (June-September, 2022). Each error bar is constructed using 1 standard error from the mean.

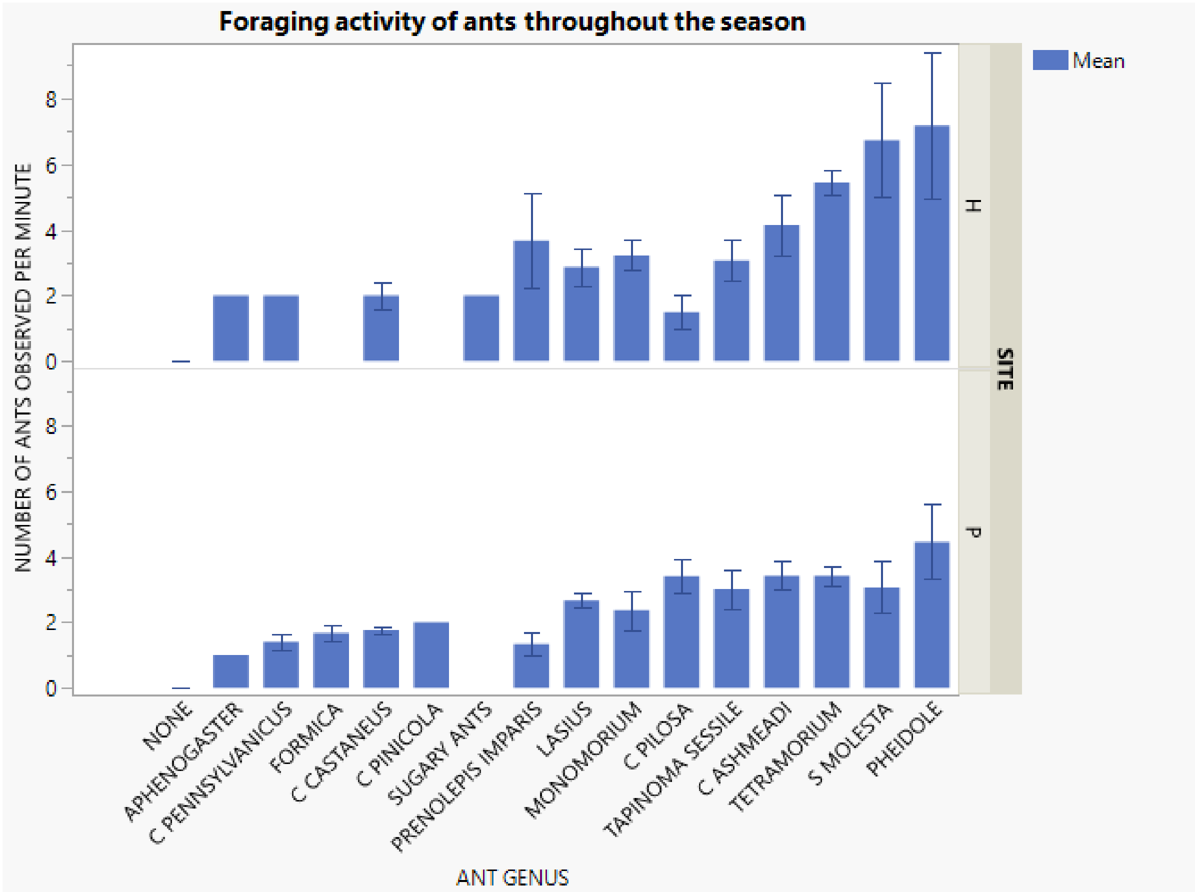


FIGURE 3.2. The mean number of ants observed in the trunk per minute throughout the season (June-September, 2022). Each error bar is constructed using 1 standard error from the mean.

3.3.2 Field studies with dispensers

Fewer numbers of ants were observed and captured in the control treatment throughout the season. The ant bait containing toxicants for ants 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 3.3 and 3.4).

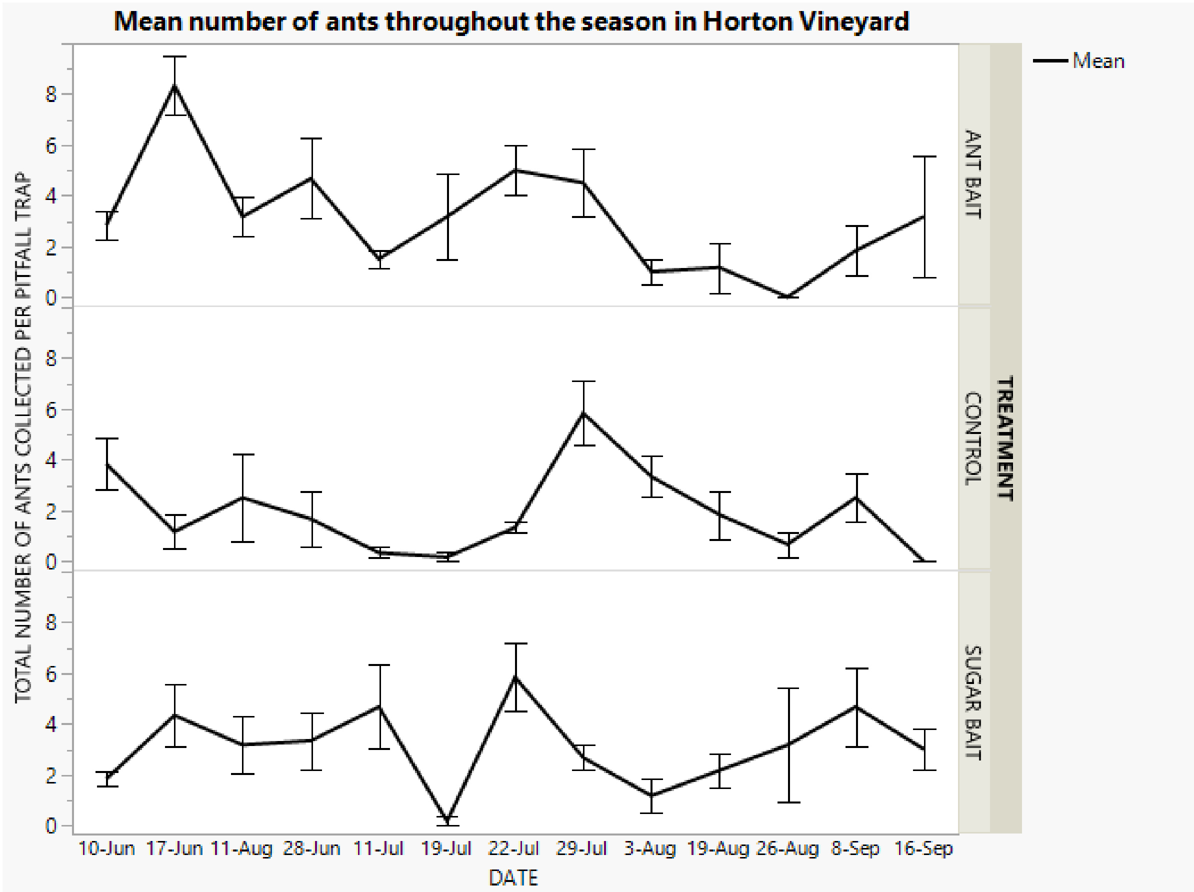


Figure 3.3 Mean number of ants throughout the season in Horton Vineyard. Each error bar is constructed using 1 standard error from the mean.

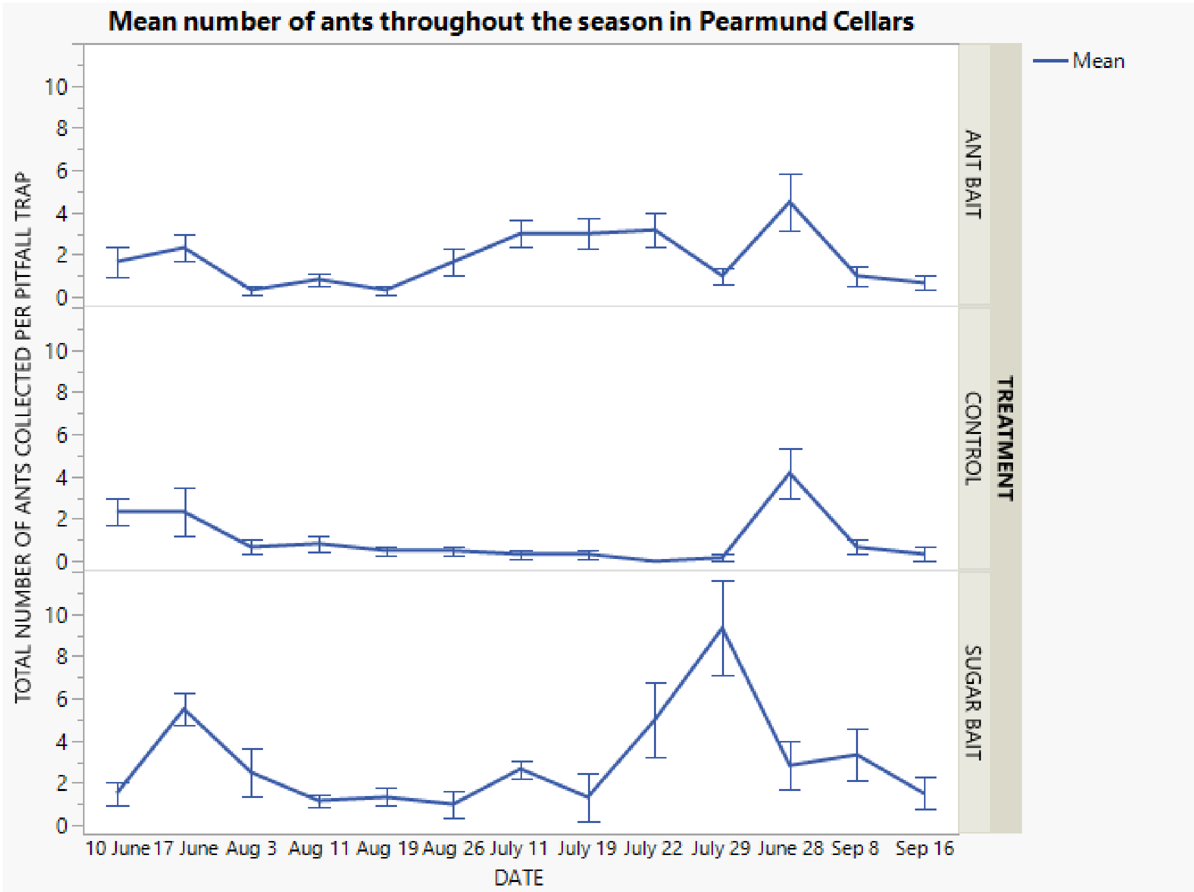
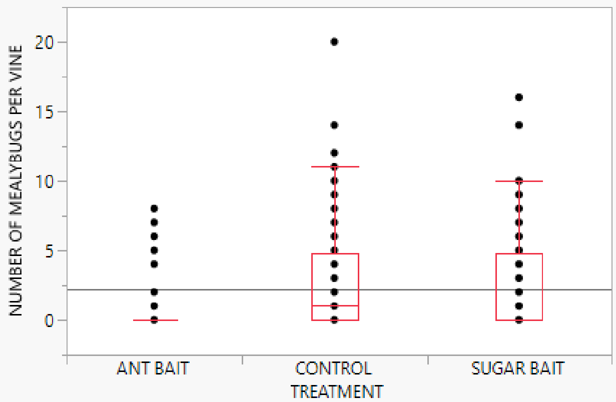


Figure 3.4 Mean number of ants throughout the season in Pearmund Cellars. Each error bar is constructed using 1 standard error from the mean.

3.3.3 One-minute count on the trunk

Field analysis of the mean number of ants throughout the season was calculated as well as per sampling dates. During the one-minute count data analyses, 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 3.1.a: Site P: Z- score: -0.2744, p=0.9464 for sugar bait: control; Z- score: -0.5084, p=0.8299 for ant 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 ant bait: control).

Oneway Analysis of NUMBER OF MEALYBUGS PER VINE By TREATMENT SITE=H



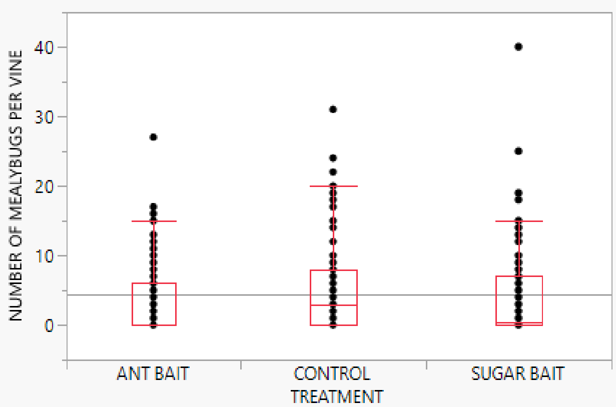
Nonparametric Comparisons With Control Using Steel Method

Control Group = CONTROL

q*	Alpha
2.21285	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	Difference Plot
SUGAR BAIT	CONTROL	-7.2125	6.827191	-1.05644	0.4665	0	-1.00000	0	
ANT BAIT	CONTROL	-23.1049	6.363245	-3.63100	0.0006*	0	-1.00000	0	

Oneway Analysis of NUMBER OF MEALYBUGS PER VINE By TREATMENT SITE=P



Nonparametric Comparisons With Control Using Steel Method

Control Group = CONTROL

q*	Alpha
2.21305	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	Difference Plot
SUGAR BAIT	CONTROL	-15.7091	8.277478	-1.89781	0.1044	0	-1.00000	0	
ANT BAIT	CONTROL	-19.5798	8.118400	-2.41178	0.0299*	0	-2.00000	0	

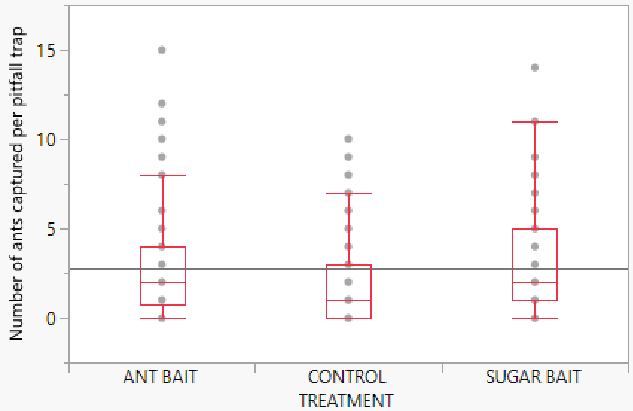
Figure 3.5.a. Nonparametric test (Steel test with control) for mean number of ants observed per vine for one-minute visual count data. Each error bar is constructed using 1 standard error from the mean.

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 data analysis results. There was no significant difference in the mean densities of ants present in ant bait treatment compared to the control in site P ((Table 3.1.b: Site P: Z- score: -0.1728, p=0.9785 for ant bait (Fig 3.5.a)). 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 (Fig 3.5.a)). The result was just the opposite for site H. There is a significant difference in the mean densities of ants present in ant bait treatment compared to the control in site H ((Table 3.1.d: Site H: Z- score: 2.409, p=0.0423 for ant 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).

3.3.4 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 ant bait: control). The result remains the same 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 ant bait: control).

Oneway Analysis of Number of ants captured per pitfall trap By TREATMENT SITE=H



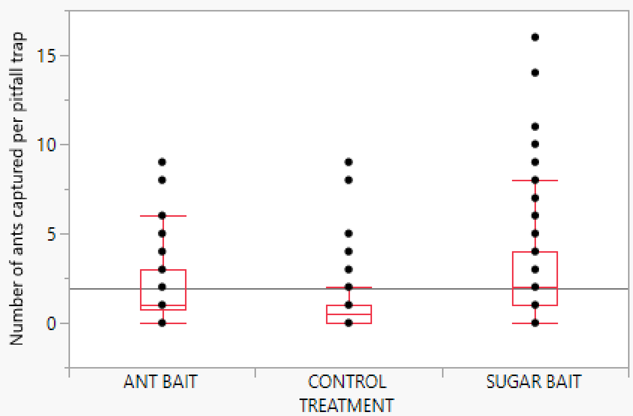
Nonparametric Comparisons With Control Using Steel Method

Control Group = CONTROL

q*	Alpha
2.21213	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	Difference Plot
SUGAR BAIT	CONTROL	21.48718	7.090185	3.030553	0.0047*	1.000000	0	2.000000	
ANT BAIT	CONTROL	17.80769	7.062758	2.521351	0.0221*	1.000000	0	2.000000	

Oneway Analysis of Number of ants captured per pitfall trap By TREATMENT SITE=P



Nonparametric Comparisons With Control Using Steel Method

Control Group = CONTROL

q*	Alpha
2.21213	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL	Difference Plot
SUGAR BAIT	CONTROL	35.20513	6.982153	5.042160	<.0001*	1.000000	1.000000	2.000000	
ANT BAIT	CONTROL	24.89744	6.906764	3.604790	0.0006*	1.000000	0.000000	1.000000	

Figure 3.5.b. Nonparametric test (Steel test with control) for mean number of ants observed per vine for pitfall trap data. Each error bar is constructed using 1 standard error from the mean.

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 ant 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 ant bait (Fig 3.5.b)). There was also a significant difference in the mean densities of ants present in both the ant 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 ant bait (Fig 3.5.b)).

Fruit Cluster Damage due to the presence of mealybugs

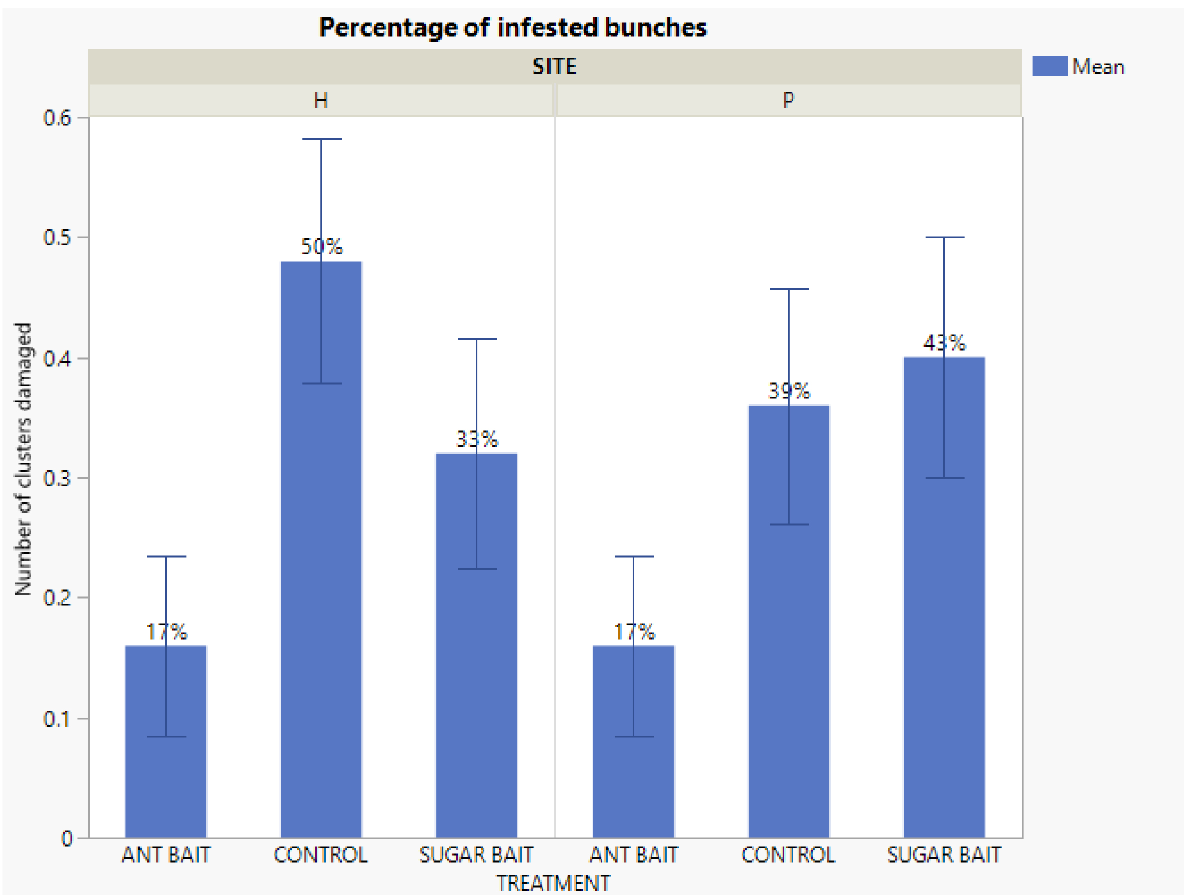
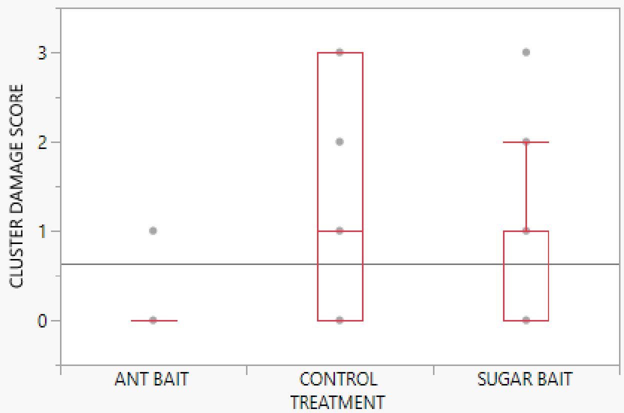


Figure 3.6. Percentage of infested bunches. Each error bar is constructed using 1 standard error from the mean.

Oneway Analysis of CLUSTER DAMAGE SCORE By TREATMENT SITE=H



Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
CONTROL	ANT BAIT	10.4000	3.460919	3.00498	0.0027*	0	0.00000	2.000000
SUGAR BAIT	ANT BAIT	3.7600	2.983629	1.26021	0.2076	0	0.00000	0.000000
SUGAR BAIT	CONTROL	-6.7600	3.627559	-1.86351	0.0624	0	-1.00000	0.000000

Difference Plot

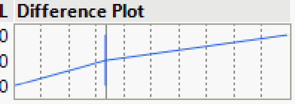
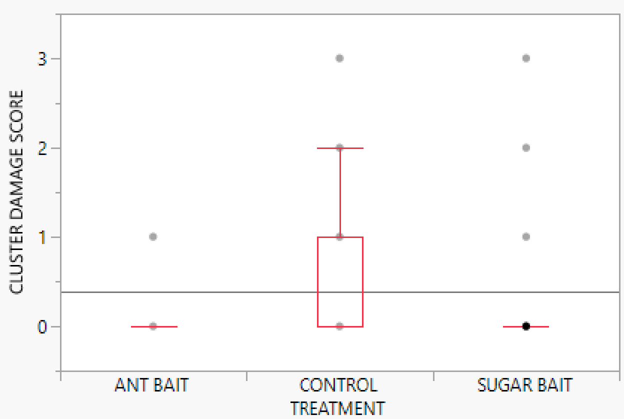


Figure 3.7.a. Fruit cluster damage analysis using the Wilcoxon test in Horton Vineyard

Oneway Analysis of CLUSTER DAMAGE SCORE By TREATMENT SITE=P



Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
CONTROL	ANT BAIT	6.60000	3.243581	2.03479	0.0419*	0	0.00000	1.000000
SUGAR BAIT	ANT BAIT	0.44000	2.628416	0.16740	0.8671	0	0.00000	0.000000
SUGAR BAIT	CONTROL	-5.32000	3.255764	-1.63403	0.1023	0	-1.00000	0.000000

Difference Plot

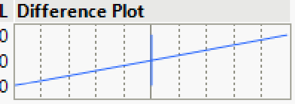


Figure 3.7.b. Fruit cluster damage analysis using the Wilcoxon test in Pearmund Cellars

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 ant-bait treatment. In another site, P, 39% of the infested clusters evaluated were from the control treatment, while 43% were from the sugar-bait treatment and 17% from the ant-bait treatment (figure 3.6). The nonparametric test for the fruit cluster infestation had similar results in both sites P and H. There is a significant difference in the cluster infestation between ant bait and control for both of the sites (Figure 3.7.b: Site P: Z- score: 2.034, p=0.0419; Figure 3.7.a: Site H: Z- score: 3.005, p=0.0027 for ant bait and control). On the other hand, there is no significant in the cluster infestation difference between sugar bait and control in both of the sites (Site P: Z- score= -1.634, p=0.1023; Site H: Z- score: -1.863, p=0.0624 for sugar bait and control).

3.3.6 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 (rs=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 (rs=0.0641; p=0.7608 for ants number; rs=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 ant 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 (rs=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 (rs=0.3266; p=0.1111). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (rs=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 (rs=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 (rs=0.1756; p=0.4010 for ant number; rs=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 (rs=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 (rs=0.1772; p=0.3968 for ant number; rs=0.2617; p=0.2063 for mealybugs number).

For the ant 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 highly significant

(rs=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 (rs=0.0773; p=0.7135). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (rs=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 (rs=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 (rs=0.1035; p=0.4746). The number of mealybugs per vine and cluster damage has a moderate correlation coefficient with a highly significant effect (rs=0.7797, p<0.0001).

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 species diversity of ants in vineyards in Virginia. Despite some of the drawbacks, this data best represents the specimens captured during the field trial.

One of the drawbacks of using of pitfall trap for is the underrepresentation of subterranean ants and the high representation of epigeic ants. Some of the arboreal ants included in the sample, are also active on the surface like *Crematogaster* and *Lasius*. 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 reaching 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.

Fifteen genera of ants were recorded foraging around commercial vineyards in the year 2022. Among those ants, the pavement ant remains the dominant ant in both of the 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 lik *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 in the field, they also pick mealybugs up and transfer them to safe place 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 dominant genera of ants in the vineyard, *Pheidole,* and *S. molesta* is a widespread generalist (Wilson 2003; Delabie and Fowler, 1995).

The use of two different types of dispensers for the ants 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 of 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).

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 ant baits compared to sugar bait and control. The distance between ant bait and sugar bait treatment was maintained at 14 to 21 feet 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 first week of July, most of the ant nests around the ant bait treatment region were gone, with only a few foraging/wandering ants or a few new ant nests seen on that section of the field.

Contrary to ant 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).
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. 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.

List of Tables

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 Pearmund Cellars 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 solution	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
		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
Ant 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 the sampling season A. Mean number of ants per vine per minute ±SE from Pearmund Cellars 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*
Ant 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 Horton Vineyard 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
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 Horton Vineyards (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
Ant 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 Pearmund Cellars 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
Ant 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. -0.685	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. 0.71	C. 0.65	C. 1	C. 0.012	C. 0.012	C. 0.005	C. 0.15	C. 0.74	C. 1	C. 0.85	C. 0.97	C.	C.

		62	45		5*	7*	2*	28	7		4	7	0.8737	0.5950

Table 3.2.b. Total ant activity throughout the season in Pearmund Cellars (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*
Ant 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 Horton Vineyards 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*

Ant 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 Horton Vineyards (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*
Ant Bait	1 % disodium octaborate tetrahydrate	A. 3.102±0.3865 B. 2.5213 C. 0.0221*

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