**Progress Report – Due March, 2023**

**Second Quarterly Report**

# Mealybug species composition and management in Virginia vineyards

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## Main areas of accomplishment in 2nd 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:** Determine the most common ant species in association with root infestations
**Objective 3:** Determine whether ants are moving bug from vine to vine
**Objective 4:** Carry out two insecticidal efficacy trials
**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 ℃ for 5 min was followed by 30 cycles of 30 sec at 94 ℃, 90 sec at 53 ℃ and 90s at 72 ℃, with a final extension of 10 minutes at 72 ℃. All reactions used QIAGEN multiplex PCR master mix that includes MgCl2 (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 no-

template 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:** 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 4:** Carry out two insecticidal efficacy trials
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.

**References**

Jones, T. (2016). "Documentation of Grapevine Leafroll-Associated Viruses in Wine Grape Varieties and Native Grape Species in Virginia, and Examination of the Movement of Grapevine Leafroll Disease to Develop Management Strategies." Edited by Mizuho Nita, Anton Baudoin, Sue Tolin, and Elizabeth Bush. Master of Science, Virginia Polytechnic Institute and State University.

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