

NYWGF RESEARCH - FINAL REPORT

Funding for fiscal year: 2025-2026

SECTION 1:

Project title: Identification of gut-associated fungi in grape berry moth larvae

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New Research **Continued Research**

Amount Funded \$10,058

SECTION 2:

Project Summary Impact Statement:

This proposal aimed to identify gut-associated fungi in grape berry moth larvae. The grape berry moth (GBM) is a prevalent pest of grapes in the northeastern U.S. The larvae of this insect feed on grape berries, causing crop loss and promoting the development of pathogens, compromising juice and wine quality. Immature grapes either heal from GBM damage or fall off from the vine, but mature grapes decompose, affecting juice and wine quality when harvested and mixed with healthy berries. Despite this known phenomenon, very little is known about the contribution of GBM to the development of fungal diseases in grape clusters. We found significant differences in fungal communities between larvae grown on grapes at different ripening stages. Ascomycota was the dominant phylum (99%) present in the guts of larvae fed on mature grapes, while larvae fed on immature grapes mainly contained fungi within the Cryptomycota (51%) phylum. Furthermore, larvae fed on ripe grapes had a higher abundance of yeasts (Saccharomycetales) compared with those fed on immature grapes. This information demonstrates the influence of grape chemistry on the GBM gut bacterial community. Furthermore, many of the fungal taxa found in the insect gut have been reported growing in the carposphere of grapes, suggesting that GBM takes up these microorganisms from its diet and can potentially move the pathogens within grape clusters, facilitating their spread.

Objectives:

- 1) To identify gut-associated fungi in grape berry moth larvae.

Materials & Methods:

Insect samples. GBM-infested grapes were collected from infested vineyards in Erie County, PA, in late July and early September 2025. The grapes were taken to the laboratory at Penn State Behrend and were dissected under a stereomicroscope to extract the larvae feeding inside. We used last-instar larvae to facilitate the dissection of their guts. The larvae were washed with distilled water, surface sterilized with 70% ethanol for 30 s, and rinsed with sterile distilled water. The larvae were placed in plastic tubes, chilled in ice, and dissected to extract their guts. Ten guts were placed in a sterile 1.7 ml plastic tube representing one biological replicate. We collect a total of 10 replicates at each sampling time.

DNA extraction and quantification. Individual samples, comprised of a pool of 10 guts each, were disrupted by hand in 1 mL of 1X phosphate-buffered saline (PBS) using a sterile plastic pestle. The total microbial DNA was purified using the EZNA Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany) following the manufacturer's protocol. The DNA was quantified using a NanoDrop™ OnceC spectrophotometer and stored at -20°C for further use.

PCR amplification. The genomic DNA extracted from the samples was used for amplifying the fungal internal transcribed spacer (ITS1) region using the following primer pairs: ITS7/ITS4 (5'-GTGARTCATCGARTCTTTG-3' and 5'-TCCTCCGCTTATTGATATGC-3'). A polymerase chain reaction (PCR) mixture (20 µl final volume) was prepared with 0.6 U of Phusion™ High-Fidelity DNA Taq Polymerase (New England BioLabs, Ipswich, USA), 0.5 µM of each forward and reverse primers, 200 µM of dNTPs, 1X Phusion HR reaction buffer, and 50 ng of DNA template. The PCR cycle had an initial denaturation step of 95 °C for 3 min, followed by 34 cycles at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 68°C for 1 min with a final extension step at 68°C for 7 min. The PCR products were visualized in 2% TAE agarose gel electrophoresis and stained with Gel Red®. The PCR products were purified using ExoSAP-IT following the manufacturer's protocols. Purified products were sent to the Penn State Genomics Facility for library preparation and DNA sequencing using the Illumina platform.

Bioinformatics analyses. The raw paired-end ITS sequences were processed and analyzed using the QIIME 2 software package (*Quantitative Insights Into Microbial Ecology*, <https://qiime2.org>). The Amplicon Sequence Variants (ASV) were used to identify the fungal species using the Greengenes database. The alpha and beta diversity metrics of fungal communities from each sampling time were calculated with QIIME. Statistical differences in gut-associated microbial abundance from GBM larvae sampled at different time points were analyzed using General Linear Models (GLM) with a negative binomial distribution.

Data collection:

The following data were collected in this study:

- Sequencing data that allowed the identification of GBM gut-associated fungi
- Fungal taxa abundance

Our Results are presented in the Attachments section below.

Results/Outcomes/Next Steps:

We obtained 1,074,066 effective tags for our samples. From these sequences, 800 ASVs were identified at 70% identity. A total of 156 unique amplicon sequence variants (ASVs) were conserved and used in comparative analyses between the two larval groups feeding on unripe and ripe grapes. However, due to marked differences between the two larval groups, the number of sequences per sample could not be normalized to carry out alpha and beta diversity analyses of the microbiota. All samples from larvae fed on immature grapes presented very low ASV frequencies compared to those fed on mature grapes. Therefore, diversity analyses of fungi in GBM gut-associated communities were not carried out, and data were analyzed descriptively.

Detected fungi and yeasts were classified into 11 phyla, 19 classes, 36 orders, 49 families, 66 genera, and 36 species. Those sequences that were not assigned to known microbial taxa were designated as "Unclassified." Ascomycota was the most dominant phylum across the larval group fed on mature grapes, representing 99% of relative abundance, whereas the larvae fed on immature grapes contained mainly taxa in the Cryptomycota (51%) and Ascomycota (34.3%) phyla (Figure 1). Taxa in the Basidiomycota were also present across all samples with a mean of 1.7% relative abundance. At the class level, Saccharomycetes was ubiquitous in all samples, being the dominant class in the mature grape group (87.5%). Leotiomycetes (11.9%) and Dothideomycetes (5.3%) were also found in the gut contents of both larval groups. The classes Aphelidea, Chytridiomycetes, Peronosporomycetes, the clade LKM11, and an incertae sedis class belonging to the Cryptomycota were absent in larvae fed on immature grapes but present in those fed on mature grapes.

At the genus level, the classification rate was 79.5%. Eight genera were absent in the immature grape-fed larval samples: Clavispora-Candida clade, *Hanseniaspora*, *Issatchenkia*, *Martiniozyma*, Ogataea-Candida clade, Starmerella-Candida clade, *Tilletiopsis*, and one unclassified genus from the order Hypocreales. Four genera were absent in the mature grape-fed larval samples: *Haliphthoros*, *Paramicrosporidium*, and two unclassified genera from the Pleosporaceae family and the Rhizophydiales order. Additionally, the genera LKM11 and *Malassezia* were significantly more abundant in larvae fed on immature grapes compared to those fed on mature grapes (GLM, $p < 0.001$ and $p < 0.05$, respectively). The most abundant genus in the mature and immature grape-fed larval samples was *Hanseniaspora* (45.7%) and the clade LKM11 (43.5%), respectively (Figure 1). The top three genera with the highest abundance in the mature grape-fed samples were *Hanseniaspora*, *Pichia*, and *Issatchenkia*.

The majority of the classified features found in GBM larval guts fed on mature grapes belonged to the yeasts *Hanseniaspora* spp and *Pichia* spp (phylum Ascomycota, class Saccharomycetes). Yeasts grow naturally in sugar-rich environments like fruits and fermented products, and have been isolated from grape berries and grape must. Therefore, GBM larvae likely acquired these microorganisms from their diet. *Hanseniaspora* species are known to mediate the early stages of spontaneous fruit fermentation and add desirable volatile compounds to wine aroma, but they can also affect the fermentation quality by producing acetic acid. *Pichia* spp. is considered a low- or non-fermentative yeast and is used

in enology to increase the quality of specific varietal wines. Yeasts are common gut residents of many insect species that obtain them from their food sources. Previous studies have demonstrated that some yeast strains improve *D. suzukii* larval development and survival, likely by nutrient provisioning. However, when feeding on overripe fruits, the fermentation mediated by yeasts produces alcohol, which is detrimental to living tissues. This may be alleviated in insects by acetic acid bacteria, like *Gluconobacter* and *Acetobacter* that metabolize alcohol into acetic acid. This mechanism may be present in GBM larvae that harbor both groups of microorganisms, yeasts and acetic acid bacteria. *Acetobacter* helps metabolize the alcohol derived from *Saccharomyces* yeasts in *Drosophila melanogaster*.

Gluconobacter was the most abundant genus of the family Acetobacteraceae in our samples. This genus belongs to the group of acetic acid bacteria commonly found in fruits and fermented products due to their ability to grow in sugar-rich environments with low pH. *Gluconobacter* was more prevalent in GBM larvae fed on ripe grapes. *Gluconobacter* has also been found in the frugivores *G. molesta*, *Carposina sasakii*, *Drosophila suzukii*, and *Drosophila melanogaster*. GBM larvae may be taking these bacteria from their diet, as previous studies (in a different grape cultivar) have demonstrated a higher abundance of *Gluconobacter* in the carposphere of ripe grapes compared to unripe ones.

Technology Transfer Plan:

Results from this project will be published in a peer-reviewed scientific journal. We will also prepare an extension article to disseminate these results. Additionally, these results will be presented in growers' meetings in both NY and PA.

Attachments

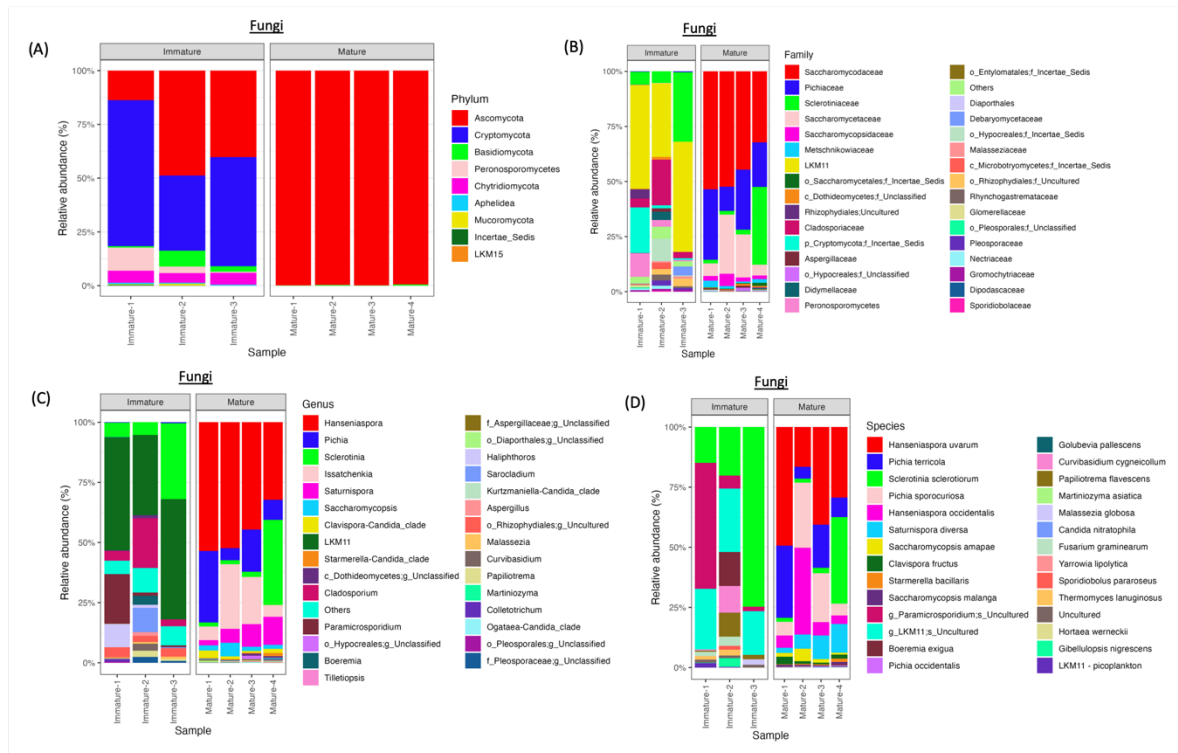


Figure 1. Relative abundance (%) of fungal communities from GBM larvae guts across samples fed on mature and immature Concord grapes at the (A) phylum, (B) family, (C) genus, and (D) species levels.

SECTION 3:

Project summary and objectives:

This proposal identified gut-associated fungi in grape berry moth (GBM) larvae. The larvae of this insect feed on grape berries, causing crop loss and promoting the development of pathogens. Grapes infested with GBM late in the season decompose, affecting juice and wine quality when harvested and mixed with healthy berries. Despite this known phenomenon, very little is known about the contribution of GBM to the development of fungal diseases in grape clusters. In addition to injuring the grapes, GBM larvae may be able to move fungal spores from infested to healthy clusters. This information is helpful to better understand the contribution of GBM to the development of cluster diseases.

Importance of research to the NY wine industry:

The GBM larvae damage grape clusters by direct feeding and by increasing susceptibility to fungal and bacterial pathogens. GBM infestations are particularly damaging late in the season when grape injury leads to cluster rot and decay. The results of this proposal suggest that GBM larvae harbor fungal gut symbionts that can contribute to cluster diseases. It may also be possible for the insect to transmit fungal pathogens from diseased to healthy clusters, but further research will be needed to confirm this.

Project Results/next steps:

We found significant differences in fungal communities between larvae grown on grapes at different ripening stages. Ascomycota was the dominant phylum (99%) present in the guts of larvae fed on mature grapes, while larvae fed on immature grapes mainly contained fungi within the Cryptomycota (51%) phylum. Furthermore, larvae fed on ripe grapes had a higher abundance of yeasts (Saccharomycetales) compared with those fed on immature grapes. Changes in grape composition throughout the ripening process, such as the increase in Brix degrees, provide sugar-rich environments that are very favorable for the growth and development of these fungi within ripe grapes. Since GBM larvae grow inside decaying fruit late in the season, most gut-associated yeasts might be either environmental or pathogenic opportunistic microorganisms of rotten grapes. Many of the fungal taxa found in the insect gut have been reported growing in the carposphere of grapes, suggesting that GBM takes up these microorganisms from its diet and can potentially move the pathogens within grape clusters, facilitating their spread, but the ability of GBM to transmit these pathogens has not been experimentally confirmed.

Supporting attachments:

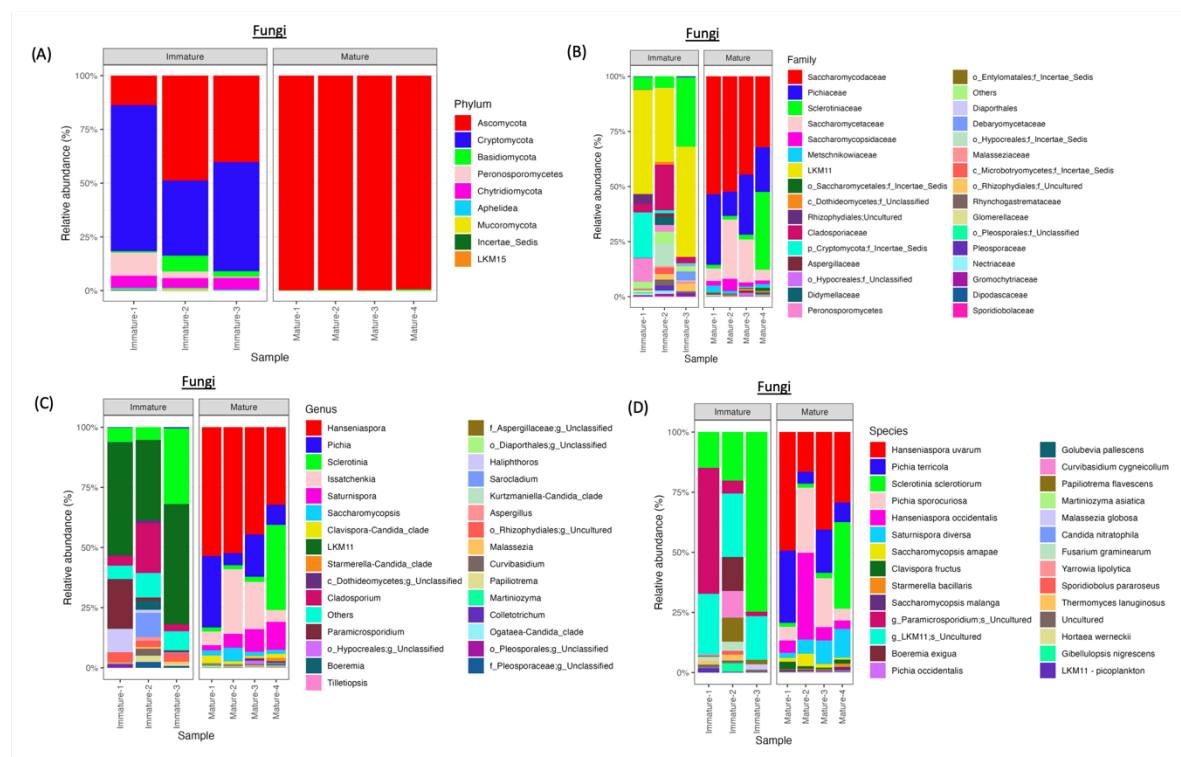


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