NYWGF RESEARCH - FINAL REPORT

Funding for fiscal year: 2022-2023

SECTION 1:

Project title: Effect of plant biochemical defenses on grape berry moth survival

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New Research ☒ **Continued Research** ☐

Amount Funded **$** 9,274

SECTION 2:

Project Summary Impact Statement:

The aim of this proposal was to quantify plant defensive compounds in different grape cultivars with contrasting GBM development and survival. We measure differences in pH, degrees Brix, total phenolics, polyphenol oxidase, and peroxidase activity in pre-veraison grape berries of Concord, Niagara, Chambourcin, Riesling and Vidal; as well as in pre and post veraison Concord berries. We will also carried out bioassays to determine the correlation between plant defensive compounds and grape berry moth survival. This information improves our understanding of the influence of the host plant on grape berry moth growth which leads to direct management applications.

Objective:

To quantify plant defensive compounds in different grape cultivars with contrasting GBM development and survival.

Materials & Methods:

Grape berries. Clusters from Concord, Niagara, Chambourcin, Riesling, and Vidal were used for the quantification of plant defensive compounds and for survival bioassays of grape berry moth. All of these grape cultivars are currently grown in the Lake Erie Region.

a) Quantification of plant defensive compounds in grape berries.

We quantified primary and secondary metabolites in grapes from five different cultivars: Concord, Niagara, Chambourcin, Riesling, and Vidal at pre-veraison stage, and in Concord grapes at pre and post version. We measured degrees Brix and pH in fresh juice. Additionally, we quantified total phenolics, polyphenol oxidase and peroxidase activity in frozen grape tissue from the cultivars above. Each of these measurements was done in at least 10 grape clusters per cultivar. Each cluster was randomly selected from different grapevines.

- **Degrees Brix:** were measured in fresh berry juice using a hand-held ATAGO N1 refractometer that quantifies degrees Brix from 0-32%.

- **pH:** was measured in fresh berry juice using a calibrated Mettler Toledo pH meter.

- **Total phenolics:** The concentration of total phenolics in grape berries was quantified using spectrophotometric assays following the Folin-Ciocalteu protocol (Ainsworth and Gillespie, 2007). The content of phenolics was expressed as mM of gallic acid equivalents per gram of fresh tissue.

- **Polyphenol oxidase:** The activity of polyphenol oxidase was measured using spectrophotometric assays. Grape berries were grounded in liquid nitrogen, extracted with 1.25 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 5% insoluble polyvinylpolypyrrolidone, and centrifuged at 11,000 x g for 10 min at 4°C. Five microliters of supernatant were mixed with 200 µl of 3 mM caffeic acid and absorbance at 450 nm were monitored for 5 min. Enzymatic activity was expressed as change in absorbance/min/mg tissue.

- **Peroxidase:** The activity of peroxidase was assayed using spectrophotometric assays. Grape berries were grounded in liquid nitrogen, homogenized in 1.25 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 5% of cross-linked polyvinylpyrrolidone and centrifuged at 11,000 × g for 10 min at 4°C . The supernatant (5 µl) was mixed with 10 µl of 3% H_2O_2 and 190 µl of 3 mM guaiacol. The change in absorbance was measured at 450 nm for 5 min.

- **Grape berry moth survival bioassays.**

We carried out bioassays to determine the correlation between plant defensive compounds and grape berry moth survival. Immature grape clusters from Concord and Niagara (*Vitis labrusca*), Chambourcin and Vidal Blanc (*Vitis vinifera*, French-American hybrids), and Riesling (*Vitis vinifera*) cultivars were obtained from the Lake Erie Regional Grape Research and Extension Center in North East, Pennsylvania. Clusters were disinfected by immersing them in a solution of 20% ethanol for 15 min and rinsed three times with distilled water to remove any pesticide residue. Three grape clusters of each variety were placed in adult mating containers as oviposition substrate for 24 hours. Each container had approximately 30 adults. A total of 50-65 berries of each grape cultivar with eggs laid on the surface were placed individually in plastic cups and kept at 25 ± 1°C, 70 ± 2% RH, and a photoperiod of 16:8 h (L:D) in a Conviron Environmental Chamber (GEN200SH). Only one egg was retained per berry to guarantee the presence of one individual insect per replicate. The eggs were observed daily until they hatched. The newly emerged larva was also observed every day until it drilled into the berry. After drilling, the individuals were maintained in the chamber for a week since the entire larval development period occurs inside a berry. During this period, a new berry of the same grape cultivar was provided every four days to guarantee food availability. After one week, a piece of paper towel was placed inside each cup as a substrate for pupation. Subsequently, the berries were observed daily until the larvae came out to search for a place to pupate. Both the date on which the larva emerged from the grape and the pupation date were recorded for each larva. After obtaining the pupa, they were observed daily to record moth emergence.

- **GBM survival on immature (pre-veraison) and mature (post-veraison) Concord grapes**

A total of 81 mature and 55 immature concord grape berries, each containing an egg of Grape Berry Moth were collected in individual cups and kept under controlled conditions of temperature (25 $\pm$ 1ºC), relative humidity (70 $\pm$ 2%) and photoperiod (16:8 h L:D) in a B.O.D. incubator (Conviron, USA). The eggs were observed daily for their hatching and after that the larvae were monitored regularly to determine the different parameters which include egg hatching, larval period, pupal period, pupal weight, adult longevity, number of eggs laid by female adults, and egg hatching rate. During larval development a new grape was placed in each cup after every two days to ensure proper supply of food. After egg hatch, a piece of paper was added into each cup as a pupation substrate. The pupae were collected, weighed, and sexed using a stereomicroscope (Leica, USA) and placed back in the individual cups. Once the adults emerged, then a couple of adults (one male and one female) emerged at the same day were released in individual cylindrical cages specially designed for their rearing. The wire mesh cages of height 30cm and diameter of 10cm were made of plastic. The cage had a circular (4-5cm in diameter) front opening; usually large enough for grape berry moth adult transferring and this opening was kept tied with cloth to prevent the adults from fleeing the cage. About 20-30 of these cages were used to rear the adult moths and the cages were kept in an incubator. The adults were provided with 40% honey solution for nutrition and water-soaked sponges paced in cups for the humidity. About 2-3 grapes were placed in each cage for female oviposition. The grapes with eggs were collected in plastic cups and their hatching was recorded

after six days. The fecundity of the females was recorded daily by placing new grapes in the cages. The survival of the adults was also noticed daily, and the dead male moth were replaced until the female moth's death.

The variables of developmental time in days per biological stage and fecundity as the number of eggs laid per female were quantified for GBM fed on each of the two ripening stages, and values are reported as means ± standard error (SE). All statistics and figures for the GBM life cycle and fecundity analysis were performed in R (version 4.2.0). The original data were tested for normality using the Shapiro-Wilk normality test at a coefficient of > 0.05 (Shapiro & Wilk, 1965). For normally distributed data (e.g., adult longevity and pupal weight), we used two Sample t-test at $p < 0.05$ to assess the effect of treatment on each parameter. For non-normally distributed data (e.g., immature developmental times, fecundity and hatching rates), Wilcoxon test ($p < 0.05$) was used instead.

Data analysis. Differences in pH, degrees Brix, total phenolics, polyphenol oxidase, and peroxidase activity in grape berries was determined with Analysis of Variance (ANOVA). When data was not non-normally distributed, we used various transformations methods or non-parametric analyses. We determined the proportion of grape berry moth larvae that survived when feeding on Concord, Niagara, Chambourcin, Riesling, and Vidal grapes as percentages.

Results/Outcomes/Next Steps:

1) Quantification of plant defensive compounds in grape berries

pH. Grape berries from different cultivars (Chambourcin, Riesling, Concord, Niagara, and Vidal) were harvested on Aug 5, 2022. The grapes were macerated by hand using a ceramic mortar and pestle, the juice was used to measure pH with a pH meter (Mettler Toledo SevenCompact™ S220, Northbrook, Illinois). The average pH of the juice was 2.37 across treatments, with Chambourcin having the lowest pH (Average = 2.33) and Riesling having the highest (Average= 2.43) (ANOVA = $F_{4,46}$= 4.02, P=0.007). There were no significant differences in pH among the other cultivars (Tukey test alpha=0.05). The results are presented in Figure 1.

Degrees Brix: Fresh juice from macerated grapes (as indicated above for pH) was used to measure degrees Brix with a digital refractometer (Atago PAL-1). There were statistical differences in degrees Brix among different cultivars from grapes collected on Aug 5, 2022 (pre-veraison) as depicted by ANOVA and Tukey tests ($F_{4,50}$= 22.24, P=0.000). Riesling and Niagara had higher degrees Brix, and Chambourcin had the lowest when compared with Vidal and Concord (Figure 1)

pH and degrees Brix in mature and immature Concord grapes. There were significant differences in both pH (t= -16.72, df=10, P = 0.000) and degrees brix (t= -8.52, df = 9, P = 0.000) in Concord grapes at the stages of pre-veraison and post-veraison, both values being lower in pre-veraison grapes (Figure 2).

Total phenolics. Grape berries have a high concentration of phenolic compounds even when they are immature. These compounds bring protection against herbivores. From the cultivars tested, Chambourcin grapes in pre-veraison had the lowest amounts followed by Riesling, whereas Concord, Niagara and Vidal had similar concentration of phenolics (Figure 3).

Polyphenol oxidase (PPO) and peroxidase (POX) activity. We found no activity of PPO or POX in pre-veraison grapes of any cultivar. We did find PPO activity in matured Concord grapes. These results are most likely due to inhibition of the enzymatic assay by other components present in the fruit. There will be a need to chemically removed the enzymatic inhibitors to test the activity of these enzymes.

2) Bioassays

Grape berry moth survival on different grape cultivars (Concord, Niagara, Chambourcin, Riesling). GBM had both high egg viability (>87%) and adult emergence rate (>70%) (Table 1). Means comparisons show that the egg incubation time of GBM on Vidal (4.27 days) was significatively longer than on other cultivars (Kruskal-Wallis test: H = 26.62, $p < 0.001$). In addition, larvae developed faster when reared on Niagara (17.71 days) and Riesling (17.49 days) compared with those reared on the Chambourcin (19.02), Concord (19.67), and Vidal

(18.81) cultivars (Kruskal-Wallis test: H = 32.07, $p < 0.001$). No significant differences were found in the time that larvae remained outside the grape before pupating nor in the development time of the pupal stage among different host cultivars (Kruskal-Wallis test: H = 3.95, $p = 0.4134$ and H = 1.01, $p = 0.9082$, respectively).

GBM Development on immature (pre-veraison) and mature (post-veraison) Concord grapes. GBM egg viability was superior when oviposited on immature (60.49%) than on mature concord grapes (86.27%) (Table 2). Similarly, percentage of successful development from egg to adult on immature grapes (74.51%) was also higher than when reared on mature grapes (22.22%) (Table 2). Developmental times of life stages are presented in Figure 4. Mean comparisons showed that the egg incubation time of GBM on mature grapes (5.06 days) was significatively longer than on immature grapes (4.02 days) (Wilcoxon test: W = 0.27, $p < 0.001$) (Figure 4A). In addition, larvae developed faster when reared on mature (17 days) compared with those reared on immature grapes (19.67 days) (Wilcoxon test: W = 622.5, $p < 0.028$) (Figure 4B). In contrast, GBM pupa developed faster when fed on immature (9.61 days) than when reared on mature grapes (12.00 days) (Wilcoxon test: W = 146, $p = 0.004$) (Figure 4C).

The number of emerged males and females had a sex ratio close to 1:1 in both grape ripening stages tested as food source. Overall, GBM adult longevity was significatively longer when reared on immature (48.87 days) compared to those reared on mature berries (20.06 days) (two Sample t-test: T= -14.70, df = 33.21, $p < 0.001$) (Figure 4D). Consequently, female and male longevity were significatively longer when reared on immature (49.05 and 48.67 days, respectively) than when reared on mature grapes (19.80 and 20.43 days, respectively) (Two Sample t-test: T= -10.39, df = 16.68, $p < 0.001$; and T= -10.34, df = 15.27, $p < 0.001$, respectively) (Figure 4E-F). GBM pupae body mass didn't differ statistically between grape ripening stages (two sample t-test: T= 1.44, df = 28.77, $p = 0.16$) (Figure 5A).

No significant differences were found in the total fecundity per female between both grape ripening stages (Wilcoxon test: W = 16, $p = 0.34$) (Figure 5B). The mean of the total eggs laid per female on mature grapes seems to be slightly higher than on immature grapes; however, this might be influenced by the low number of females emerged from mature grapes (n= 17 and n=3 females on immature and mature grapes, respectively). The greatest fecundity occurred on immature grapes achieving a maximum of 127 eggs laid per female, while the maximum on mature grapes was 105 eggs (Figure 5B). Egg hatch from females reared on both grape ripening stages didn't differ between both food sources (Wilcoxon test: W = 29, $p = 0.749$) (Figure 2C).

Technology Transfer Plan:

Relevant results from this project will be published in two peer-reviewed scientific journals along with data from other experiments. We will also prepare an extension article to disseminate some of these results. Additionally, these results will be presented in growers' meetings in both NY and PA.

Attachments:

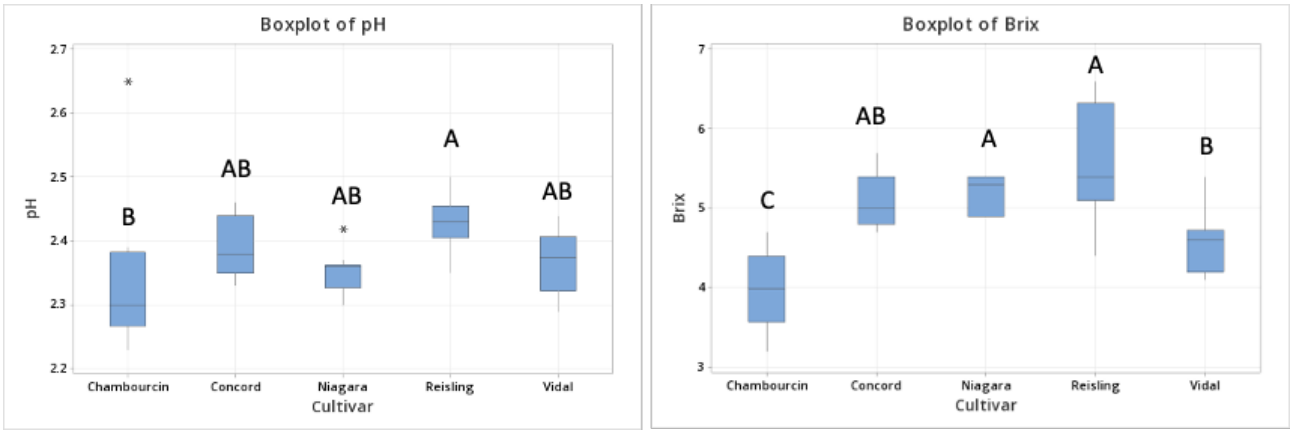

Figure 1. pH and degrees Brix of fresh juice from different grape cultivars at pre-veraison stage. Different letters indicate significant differences among treatment means obtained with Tukey at alpha=0.05.

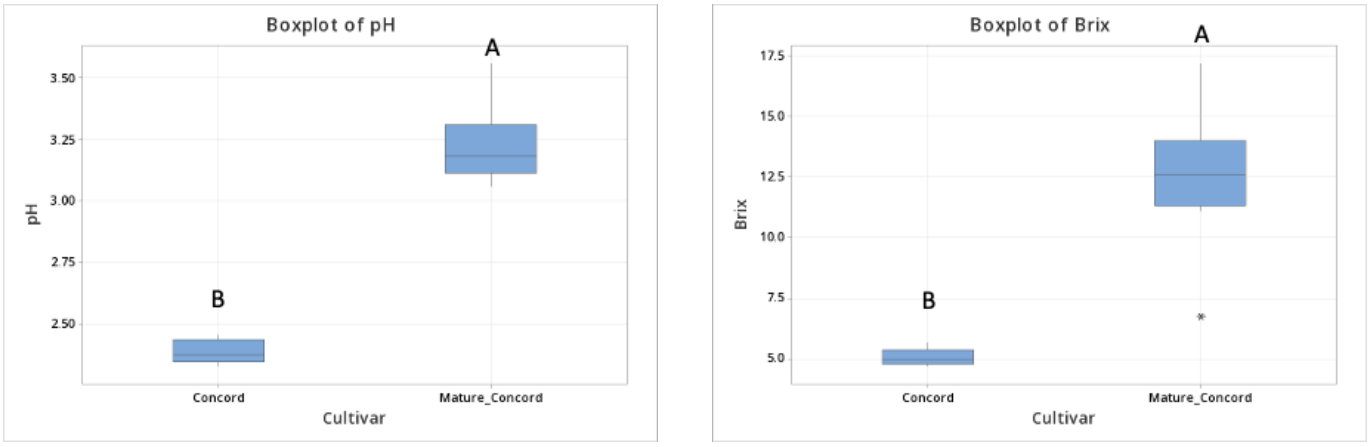

Figure 2. pH and degrees Brix of fresh juice from Concord grapes at pre-veraison and post-veraison stages. Different letters indicate significant differences among treatment means obtained with Tukey at alpha=0.05.

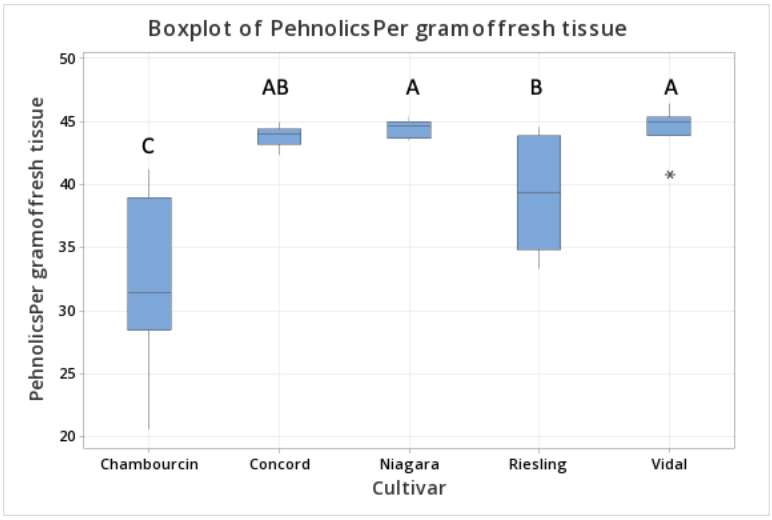

Figure 3. Concentration of phenolics per gram of fresh weight in grapes at pre-veraison. Different letters indicate significant differences among treatment means obtained with Tukey at alpha=0.05.

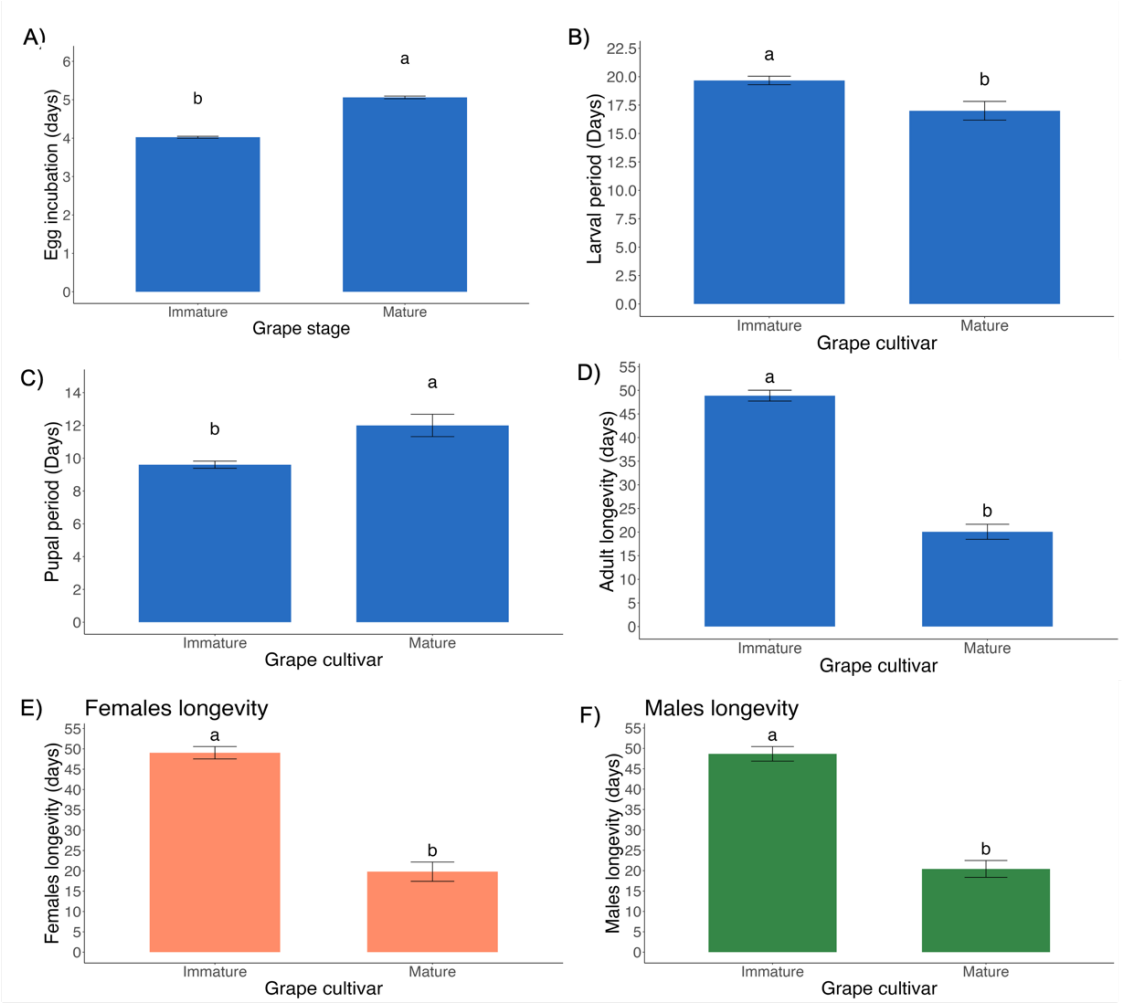

Figure 4. Development time of GBM immature stages reared on two ripening grape stages at 25 ± 1°C, 70 ± 2% relative humidity, photoperiod 16:8 h (L:D). (A) Egg incubation, (B) larval period, (C) pupal period, (D) adult longevity, (E) female longevity, (F) male longevity. Bars represent mean ± standard error (SE). Different letters refer to significant differences among the cultivars (Two Sample t-test / Wilcoxon test, $P < 0.05$).

Table 1. Grape berry moth development on different grape cultivars

Cultivar	Initial number of eggs (n)	% eggs hatched	% larvae that penetrated the berries	Number of larvae that developed into pupa	Number of emerged adults from pupa
Chambourcin	51	98.04	98.04	82.35	76.47
Concord	51	86.27	86.27	76.47	74.51
Niagara	60	95	95	85	73.33
Riesling	60	90	85	71.67	70
Vidal	63	87.3	82.54	76.19	71.43

Table 2. Survival of GBM development stages when reared on two ripening grape stages at 25 ± 1°C, 70 ± 2% relative humidity, photoperiod 16:8 h (L:D).

Rippening stage	n	% Hatched eggs	% Bored larvae	% Developed pupae	% Emerged adults
Mature	81	60.49	58.02	27.16	22.22
Immature	51	86.27	86.27	76.47	74.51

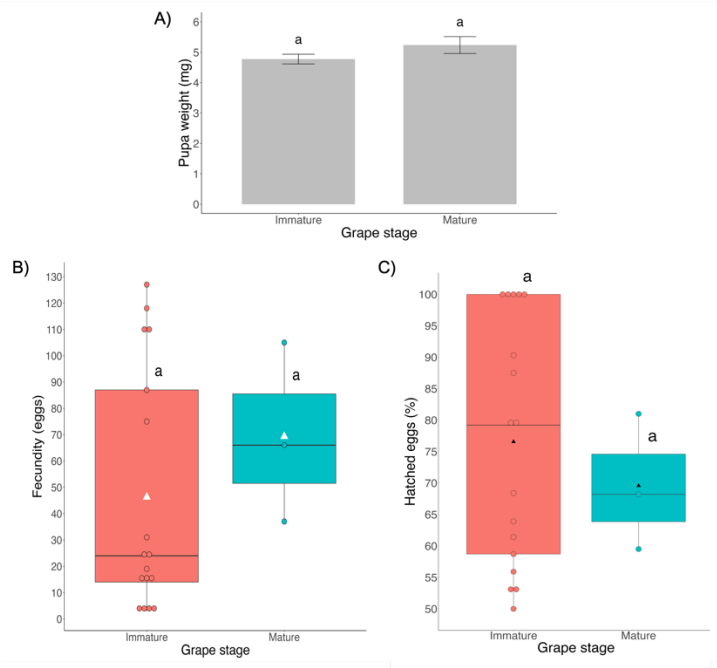

Figure 5. Pupal and fecundity parameters of GBM individuals reared on two ripening grape stages at 25 ± 1°C, 70 ± 2% relative humidity, photoperiod 16:8 h (L:D). (A) Pupal weight by ripening stage, (B) Total fecundity (number of eggs laid) per female, (C) Percentage of eggs hatched per female. Grape ripening stages with the same letter are not significantly different (Two Sample t-test / Wilcoxon test, $p > 0.05$). Means in (B) and (C) are represented as white/black triangles.

SECTION 3:

Project summary and objectives:

Grape berry moth is a pest of grape that causes loses in yield, juice and wine quality. This insect attacks grapes at different ripening stages and from different cultivars. The goal of this study was to investigate differences in grape berry moth survival on different grape cultivars with contrasting chemical composition.

Importance of research to the NY wine industry:

New York grape growers grow different grape cultivars that may have different susceptibility to grape berry moth infestations. Understanding the effect that different grape cultivars may have on grape berry moth survival and development can help improve management.

Project Results/next steps:

We grew grape berry moth on immature grape clusters (harvested the first week of August) from Concord and Niagara (*Vitis labrusca*), Chambourcin and Vidal Blanc (*Vitis vinifera*, French-American hybrids), and Riesling

(*Vitis vinifera*) grapes in laboratory conditions. We found that on average 87% of the eggs laid hatched successfully; from these, approximately 73.1% became adults (results are summarized in table 1). Larvae developed faster when reared on Niagara (17.71 days) and Riesling (17.49 days) compared with those reared on the Chambourcin (19.02), Concord (19.67), and Vidal (18.81) cultivars. The cultivars tested did exhibit small differences in pH at the time they were used for the experiments; the average pH of immature grape juice was 2.37 across treatments, with Chambourcin having the lowest pH (average = 2.33) and Riesling having the highest (average= 2.43). The cultivars also had some differences in the concentration of sugars, Riesling and Niagara had higher degrees Brix (average = 5,53 and 5.21, respectively), and Chambourcin had the lowest (average = 3.9) when compared with Vidal and Concord (4.5 and 5). We also found a lower concentration of total phenolics in unripe Chambourcin compared with other cultivars. We conclude that grape berry moth development is slightly affected by the grape cultivar they feed on; these differences are likely associated with differences in berry chemistry and nutritional quality. Further studies on the effects of different cultivars and weather changes on grape berry moth development and fecundity will help with timing management practices.

Supporting attachments:

Table 1. Grape berry moth development on different grape cultivars

Cultivar	**Initial number of eggs (n)**	**% hatched eggs**	**% larvae that penetrated the berries**	**Number of larvae that developed into pupa**	**Number of emerged adults from pupa**
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