NYWGF RESEARCH - FINAL REPORT TEMPLATE

Please fill in by typing over the red directions in each section and change font to black.

Funding for fiscal year: 2023-2024

SECTION 1:

Project title: Use of a high throughput assay to detect insecticide resistance in *Drosophila melanogaster*

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New Research ☒ **Continued Research** ☐ (CHECK APPROPRIATE BOX)

Amount Funded **$** 57,361

SECTION 2: (This section should be in depth and akin to an academic report)

Project Summary Impact Statement: Sour rot is an economically important disease of wine grapes in NY. Management is achieved primarily by late season control of vectors (vinegar flies) of the disease along with antimicrobial pesticides targeting yeast and bacteria. Insecticide resistance in *Drosophila melanogaster* is severe and widespread in NY. Current assays for resistance are time consuming and of low resolution. We seek to develop a rapid, high throughput assay to detect resistance providing the knowledge needed to better protect NY vineyards.

Objectives: Our goal is to improve control of *D. melanogaster,* and hence sour rot, in NY vineyards. To achieve our goal, we pursued two objectives that focus on the only insecticide to which resistance is not yet widespread (spinetoram, the active ingredient in Delegate).
1. Modify our existing allele specific PCR to detect resistance in field-trapped *D. melanogaster*. This assay will differentiate homozygous resistant, homozygous susceptible and heterozygous resistant individuals.
2. Examine if there is a connection between use of Delegate in vineyards or Counties, and the levels of resistance detected.

Materials & Methods:

2.1. Drosophila.

Adult *Drosophila* species (vinegar flies) were collected in September and early October 2023 (harvest period) from multiple vineyard sites in NY including two sites on Long Island (Suffolk county), three sites in the Lake Erie region (Chautauqua county), and four sites in the Finger Lakes (Yates, Ontario, and Schuyler counties) (Table 1). At the Long Island sites, flies were captured in traps baited with spotted wing drosophila (SWD, *D. suzukii*) Scentry lures plus a drowning solution comprised of water and ethanol and a drop of unscented soap. Traps were deployed in vineyards for a maximum of 48 hours after which flies were filtered and moved to vials with 100% ethanol and returned to Cornell AgriTech (Loeb lab) for sorting into male *D. melanogaster* and female *D. melanogaster/D. simulans*. At all the Finger Lakes sites flies were collected from pomice piles near wineries and vineyards using an insect vacuum system, sorted, and preserved in 100% ethanol within 24 hours, separating out male *D. melanogaster* and female *D. melanogaster/D. simulans*. At all the Lake Erie area sites a sweep net was used to collect adult vinegar flies attracted to bins of ripe grape clusters set out in the vineyard. Flies were placed into 100% ethanol and returned to Cornell AgriTech within 2 days where they were separated out into male *D. melanogaster* and female *D. melanogaster/D. simulans.* We used characteristic morphological features to distinguish male *D. melanogaster* from other species of Drosophila. However, morphology is not sufficient to separate out female *D. melanogaster* from female *D. simulans.* Vials with flies in 100% ethanol were placed in a -80°C freezer until transported to Ithaca (Scott lab) for DNA isolation and genotyping. SpR is the homozygous resistant strain resulting from selection of field-caught with spinetoram, and it was used as a control in genotyping (Scott et al., 2023).Canton-S was the laboratory susceptible *D. melanogaster* strain used in all experiments. *D. melanogaster* were reared on standard fly medium under a standard laboratory environment (~23 ℃) with a photoperiod of about 12L: 12D as previously described (Sun et al., 2019). *D. simulans* were from the National Drosophila Species Stock Center (Ithaca, NY). At the end of the season, we requested insecticide spray records for the past 4 to 5 years for all sites used to collect vinegar flies for analyses.

Table 1. Locations of *D. melanogaster* collections for 2023 and the number of individuals genotyped for the spinetoram resistance allele.

Code location	City	County	Males genotyped	Freq of R allele (%)
A	Irving	Chautauqua	51	0
B	Hector	Schuyler	49	8.2
C	Sheridan	Chautauqua	47	0
D	Ovid	Seneca	45	5.6
E	Geneva	Ontario	44	0
F	Penn Yan	Yates	38	1.3
G	Portland	Chautauqua	32	0
H	Cutchogue	Suffolk	15	10
I	Cutchogue	Suffolk	10	0

2.2. DNA Extraction.
DNA was extracted from *Drosophila* as follows. Individual flies were put in 10 µL 0.2 M NaOH along with three or four zircon beads in individual wells of a 96-well PCR plate. Plates were vortexed at top speed for one minute. Lysis was performed by incubating at 70°C for 10 minutes. Finally, 90 µL of TE buffer and ddH_2O was added to neutralize the solution. DNA was stored at -20 °C.

2.3. Molecular methods to differentiate *D. melanogaster* and *D. simulans* females.
Common species of field collected *Drosophila* can be separated based on their morphology. However, female *D. melanogaster* and *D. simulans* cannot be differentiated morphologically. Therefore, in order to be able to use females that were collected, we needed a molecular method to distinguish these species. There are two published methods that have been proposed for differentiating *D. melanogaster* and *D. simulans* (Lee et al., 2012; Raquin et al., 2018) and we investigated these, using our laboratory strains of *D. melanogaster* and the *D. simulans* from the National Drosophila Species Stock Center.

2.4. Detection of the resistance allele by allele-specific PCR.
A multi-primer amplification refractory mutation system (ARMS) was used to determine the genotype of individual flies by PCR (Little 1995). Allele-specific primers were designed to amplify an allele-specific 674-676 bp fragment of *alpha 6* that includes parts of exons 8 and 9. The external (allele-specific) reverse primers were complementary to either the resistant or susceptible allele at their 3' base. The internal control primers were designed to amplify a 445 bp fragment of *Ace* fully complementary to either strain's sequence. Primers were ordered from IDT DNA (Morrisville, NC). The primer sequences were previously reported (Scott et al., 2023). The reaction mixture was 12.5 µL GoTaq Green (Promega, Madison WI), 9.5 µL H_2O, 0.5 µL Dace-F1, 0.5 µL Dace-R1, 0.5 µL of either Da6-R12 (resistant) or Da6-R11 (wild type), 0.5 µL of either Da6-F14 (resistant) or Da6-F12 (wild type), and 1 µL of DNA extraction. Empirically optimized PCR conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, 58°C for 30 seconds, and 72°C for 30 seconds, with a final extension time of 2 minutes at 72°C. After amplification, 3 µL of each reaction were electrophoresed on a 2% agarose-TBE gel stained with ethidium bromide. Each gel contained controls (DNA from two susceptible, two resistant and two heterozygous individuals).

Results/Outcomes/Next Steps:

3.1 Collections.
Our collection methods worked well. Each collection generated a sufficient number of flies, although at the Long Island locations, most of the collected flies were *D. simulans* (Table 1). The flies collected by our methods allowed for extraction of DNA from nearly all samples.

3.2 Differentiating *D. melanogaster* and *D. simulans.*
We were unable to replicate the two previously published molecular methods for differentiating *D. melanogaster* and *D. simulans* (Lee et al., 2012; Raquin et al., 2018). Thus, all of our results from 2023 were based on males.

3.3. Genotyping.
We genotyped 331 males from our 2023 collections. These ranged in number from 51 to 10 per site (Table 1). The resistance allele was detected in four of the collections, ranging in

frequency from 1.3-10%. There were no homozygous resistant individuals observed. The frequency of the resistance allele was slightly increased in 2023 from what was seen in 2019/2020, although this comparison could only be made at two locations and the changes were 6% or less.

3.4 Correlating frequency of resistance alleles with use of spinosyns by vineyard and by County.
We were able to obtain spray records from five of the vineyards from which we collected flies, and we used the New York State Department of Environmental Conservation 2022 database to acquire use reports for each of the counties from which we collected flies. These correlations suggest that vineyard use of spinosyns is a greater predictor of allele frequency than county wide use (Figure 1)

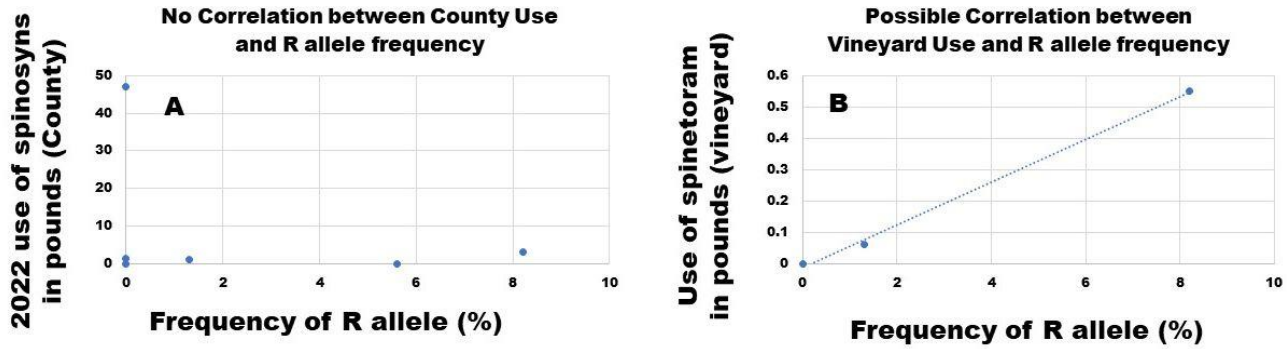

Figure 1. Results from 2023 suggest vineyard use of spinetoram (B) is a greater predictor of resistance than County wide use (A) of spinosyns.

3.5 Outlook and next steps.
Our preliminary results suggest that the frequency of the spinetoram resistance allele is low in field collected populations, but that it may be on the rise. Our next steps are to conduct a survey in 2024, to bolster the data with which we can make recommendations. It appears that a resistance management strategy could be implemented, and that annual monitoring may not be needed, at least in the short term.

Technology Transfer Plan:
Presentations
Wise, A. and Gilrein, D. 2023. Long Is Agriculture Forum Viticulture Session our Viticulturist Alice Wise spoke about these topics in an update on the berry cuticle enhancer project.
Loeb, G. 2023. Update on spotted lanternfly and sour rot. Tailgate meeting with Finger Lakes Grape Program on 22 August, 2023 at Fox Run winery, near Penn Yan, NY. Approx. 30 growers in attendance. Meeting was 1.5 hours, contact hours = 45.
Loeb, G. 2023. Update on spotted lanternfly and sour rot. Tailgate meeting with Finger Lakes Grape Program on 8 August, 2023 at Wickhams Tango Oaks Farm near Hector NY. 25 growers in attendance. Meeting was 1.5 hours, contact hours = 37.5.

Loeb, G. 2023. Understanding late-season grape berry moth damage and other ongoing entomology research. 45 minute presentation at 2023 LERGP Winter Grape Grower Conference held on 16 march, 2023 in Fredonia, NY. Approx. 110 in audience. Contact hours = 82.5.
Loeb, G. 2023. Update on managing pests of grapes: both the new and the old. 45 minute in-person presentation in the Viticulture session at the 2023 Long Island Ag Forum. Session was held in Riverhead, NY on 12 January 2023. Approx. 30 in attendance. Mostly growers but some extension educators. Contact hours = 22.5.

Publications

Scott, J. G., R. H. Norris, R. W. Mertz, A. E. Dressel, and G. Loeb. 2023. Selection and characterization of spinetoram resistance in field collected *Drosophila melanogaster*. Pestic Biochem Physiol 194: 105508.
Loeb, G. 2023. Grape and Mite Pests, 2023 Field Season. Published in newsletters from Finger Lakes grape program, Lake Erie grape program, and Long Island grape program.

Attachments: None

SECTION 3: (The goal of this research is to benefit growers and producers across New York State. Result summaries will be shared on the NYWGF website and via email newsletters. To that end, this section should be brief and written in terms understandable for the average grower and producer, as well as consumers and trade interested in our industry.)

Project summary and objectives:
Spinetoram (Delegate) is the only registered insecticide (for control of *Drosophila melanogaster* in vineyards) to which vinegar flies have not yet evolved high levels of resistance. However, low levels of resistance have been found in vineyard populations, and we were able to select (in the laboratory) a highly resistant strain. We identified the mutation responsible for the resistance and have developed a rapid, high-throughput assay for resistance. Surveys of collections made in 2023 show low levels of the resistance allele in three populations. Although these are still low levels, at two sites the frequency of the resistance allele appears to be increasing.

Importance of research to the NY wine industry:
Sour rot is a devastating disease of wine grapes in NY. Management is primarily achieved by late season control of vectors (fruit flies) of the disease. Insecticide resistance in *Drosophila melanogaster* is severe and widespread in NY, limiting our ability to control flies and sour rot. Current assays for resistance are time consuming and of low resolution. We have developed a new, rapid, high throughput assay to monitor resistance to spinetoram (Delegate). Results will provide the information needed to better manage the use of Delegate, prolong its efficacy, and protect NY vineyards. Our initial results suggest that resistance management strategies at individual vineyards may be an appropriate strategy to delay resistance.

Project Results/next steps: Our next steps are to develop a molecular method to differentiate between *D. melanogaster* and *D. simulans* females, and to conduct a final year of resistance surveys. Our goal is to provide resistance management strategies to growers for the 2025 season and onward. We will also propose how often monitoring for the resistance allele is necessary.

Supporting attachments: (None)

References Cited

Lee, S.F., White, V.L., Weeks, A.R., Hoffmann, A.A., Endersby, N.M. 2012. High-throughput PCR assays to monitor Wolbachia infection in the dengue mosquito (Aedes aegypti) and Drosophila simulans. Appl Environ Microbiol 78, 4740-4743.

Little, S. 1995. Amplification-refractory mutation system (ARMS) analysis of point mutations. Current Protocols in Human Genetics 7, 9.8.1-9.8.12.

Raquin, V., Henri, H., Vallat, M., Leulier, F., Gibert, P., Kremer, N. 2018. Development of a PCR-RFLP assay to identify Drosophila melanogaster among field-collected larvae. Ecol Evol 8, 10067-10074.

Scott, J.G., Norris, R.H., Mertz, R.W., Dressel, A.E., Loeb, G. 2023. Selection and characterization of spinetoram resistance in field collected *Drosophila melanogaster*. Pestic Biochem Physiol 194, 105508.

Sun, H., Loeb, G., Walter-Peterson, H., Martinson, T., Scott, J.G. 2019. Insecticide resistance in *Drosophila melanogaster* is associated with field control failure of sour rot disease in a New York vineyard. J. Econ. Entomol. 112, 1498-1501.