NYWGF RESEARCH -FINAL REPORT

SECTION 1:

Project title: Insecticide resistance is limiting control of sour rot in New York vineyards

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New Research ☐ **Continued Research** ☒

Amount Funded **$** 161,861 (over three years)

SECTION 2:

Project Summary Impact Statement: Sour rot is a devastating disease of wine grapes in NY and management is achieved by late season control of vectors (fruit flies) of the disease. Insecticide resistance in *Drosophila melanogaster* is severe and widespread in NY, present even in the early season, and attempts to identify new insecticides for control of *D. melanogaster* have been only partially successful. Resistance detected in *D. melanogaster* is driven by insecticide use across many commodities, not just use in vineyards. Resistance to zeta-cypermethrin, malathion and spinetoram appears to be due to independent resistance mechanisms.
(Maximum 5 sentences)

Objectives:

Our goal is to improve control of *Drosophila melanogaster*, and hence improve management of sour rot, in NY vineyards.

In 2019-2020 our objective was:

Evaluate resistance to 15 populations of *D. melanogaster* collected from grape producing regions across the state.

In 2020-2021 our objectives were:

1. Evaluate 14 new insecticides for future use in *D. melanogaster* control)

2. Examine the stability, genetics and mechanisms of zeta-cypermethrin and malathion resistance*.

In 2021-2022 our objectives were:

1. Determine if zeta-cypermethrin and malathion resistance are genetically linked.

2. Examine the mechanisms of zeta-cypermethrin and malathion resistance.*

3. Quantify the levels of resistance to zeta-cypermethrin and malathion in early season *D. melanogaster*.

Our objective for 2019 was fully met. Objective #1 for 2020 was fully met. The second objective for 2020 partially completed, but we fell short of fully completing this objective because of the pandemic and the 2020 budget cut. Objectives #2 and 3 for 2021 were fully met. Objective #1 for 2021 was met, but via indirect evidence from the mechanisms research.

*This objective was part of the proposed research for 2020, but we were unable to fully complete it due to the budget cut.

Materials & Methods:

Drosophila spp. were collected, from August through October, from nine vineyards and one orchard across NY in 2019 and in two vineyards in 2020 (one from NY and one from MO, USA). Collected flies (or infested clusters of grapes) were sent to the laboratory at Cornell AgriTech in Geneva, NY where they were collected with an aspirator and placed on a cold plate for initial species identification. The majority of collected adult flies across the different sites appeared to be *D. melanogaster* based on morphological traits, although other fruit-feeding *Drosophila* species were observed as well, including *D. suzukii* and *D. simulans*. These other species were not evaluated for insecticide resistance. To ensure correct identification, a subset of female flies (N = 30-50) were individually isolated in small vials with food and placed in a walk-in growth chamber (25 °C, 14:10 light/dark cycle at 55% relative humidity). They were maintained until the F_1 adult generation at which time males were checked for species identification based on diagnostic morphology of the genital arch.[11] Greater than 90% of the isolated females produced a F_1 adult generation and all were confirmed as *D. melanogaster* except at one site (Seneca #2) where 25% were identified as *D. simulans*. The *D. melanogaster* adults from each site were combined into a single colony to represent the vineyard population and moved to the Ithaca campus for further rearing and analysis of resistance. In 2021 we also made three early season collections (made from the same locations as were made in 2019). These flies were collected in live traps baited with mashed ripe banana plus Baker's yeast (24 g yeast per 8 bananas[11]. To ensure correct identification, female flies (N = 30-50 per site) were individually isolated in small vials with food and placed in a walk-in growth chamber (25 °C, 14:10 light/dark cycle at 55% relative humidity). They were maintained until the F_1 adult generation at which time males were checked for correct species identification.[11] As a control we used a laboratory susceptible strain (Canton-S). We also evaluated the resistant population (NY18) we collected in 2018 and held under laboratory conditions (without insecticide exposure) since,[10] to determine if the resistance levels were stable over time. *D. melanogaster* were reared on standard fly medium under a standard laboratory environment (~23 ℃) with a photoperiod of about 12L: 12D as previously described.[10]

For our evaluations of resistance in each of the collected populations we tested four registered insecticides for *Drosophila* control: acetamiprid, malathion, spinetoram and zeta-cypermethrin. These represent four classes of insecticides (neonicotinoids, organophosphates, spinosyns and pyrethroids), each with a different mode of action. The field collected populations were tested at one or more diagnostic concentrations relative to Canton-S (LC_{95}, 4 × LC_{95} or 16 × LC_{95}) as previously described.[10] Insecticides were dissolved in acetone (ACS grade, VWR, Radnor, PA, USA) and 0.5-1.0 mL was applied evenly to the inside of a scintillation vial (Wheaton Scientific, Millville, NJ, USA) with an internal surface area of 38.6 cm^2 and allowed to evaporate on a hot dog rolling machine at room temperature (Gold Medal Products Co., Cincinnati, OH, USA) for at least 30 min before flies were placed inside. Controls were treated with acetone only. Stoppers were made with a piece of cotton covered by white nylon tulle and 10% sugar water was applied with a syringe to saturate the stoppers. Each treated vial containing 20 female flies (3 to 7 day-old) was laid on its side and held in a chamber at 25 ℃ with a

photoperiod of 16L: 8D. Mortality was assessed after 24 h of exposure for all insecticides, and flies were considered dead if they were ataxic. For all concentrations tested, a minimum of 100 flies from each strain were tested over a minimum of two days. For statistical analysis, the percentage survival was arcsine transformed and analyzed by one-way analysis of variance (ANOVA) with a post-hoc Tukey's test. Canton-S females (3 to 7 day-old) were used side-by-side with the field collected flies as a control.

We evaluated the potential of 19 additional insecticides for control of *D. melanogaster* by determining the toxicity of each to the insecticide susceptible Canton-S strain using the residual method described above. For a subset (15) of these insecticides, we also examined the levels of cross-resistance in one of our field collected populations (Ulster) using concentrations of 1×, 4× and 16× of the susceptible strain LC_{95}. Sugar water was replenished every 24 hours as needed. Bioassays were replicated at least 4 times over a two-week period. Bioassay data were pooled, corrected for control mortality using the method of Abbott,[13] and analyzed by standard probit analysis using an R script (https://github.com/JuanSilva89/Probit-analysis). Most insecticides were evaluated after 24 hr, but for those that were found to be more slow acting, endpoints of 48 or 72 hr were used.

Results/Outcomes/Next Steps:

Evaluation of the field collected populations indicated that there was widespread resistance to all registered insecticides, except spinetoram. There was resistance to zeta-cypermethrin evident at all three diagnostic concentrations in all populations. The percent survival ranged from 66-92, 18-73 and 1.7-18% at the LC_{95}, 4 ×LC_{95} and 16 × LC_{95}, respectively. The percent survival for malathion in the field collected populations ranged from 93-100, 25-83 and 0-7.5% at the LC_{95}, 4 × LC_{95} and 16 × LC_{95} respectively. Based on the high levels of acetamiprid resistance observed in 2018, we examined patterns of acetamiprid, but only at the 4 × LC_{95} diagnostic concentration. Acetamiprid resistance was widespread with 61-97% survival at all locations. In 2018, spinetoram was the only insecticide to which resistance was not detected.[10] Therefore, we tested only the LC_{95} for this insecticide. Overall, the populations were still largely susceptible to spinetoram. However, the Ulster population showed 20% survival at the LC_{95} diagnostic concentration (statistically higher survival than Canton-S ($P<0.05$). Due to this surprising result, we also tested the Ulster population with the 4 × LC_{95} concentration and 9.2% of the flies survived (compared to 0% survival in Canton-S). This suggests that resistance is evolving to spinetoram. Generally, the field collected population with the highest percent survival across the four insecticides was Ulster Co., NY, but there was no apparent geographic association of resistance beyond that population.

The stability of resistance to zeta-cypermethrin, malathion and acetamiprid was evaluated over time in the NY18 strain. There was no significant change in survival of the NY18 strain (NY18 (2018)) after being reared under lab conditions for 33 months (NY18 (2021)) to zeta-cypermethrin or malathion, indicating the resistance is relatively stable. Conversely, survival of the NY18 strain to acetamiprid (at the Canton-S 4 × LC_{95}) significantly ($P>0.05$) decreased in 2021 (27%) compared to 2018 (83%). The low level of resistance to spinetoram in the NY18 population (5.8% ± 3.3% survival at the Canton-S LC95) decreased significantly ($P < 0.05$) to 0% in Sept. 2020.

Early season field collections were made in 2021 at three locations we had sampled in 2019 to check whether or not early season resistance levels were different from those we had observed at the end of the field season. These field collections (Seneca #1, Ontario #2, and Schuyler #1) were tested for resistance against zeta-cypermethrin, malathion, and acetamiprid in the same manner as the 2019 and 2020 field collections. The % survival (SE in parentheses) against zeta-cypermethrin at the 1×, 4×, and 16× concentrations for Seneca #1 was 88.3% (2.5), 73.3% (2.8), and 32.5% (4.2), respectively; for Ontario #2, 84.2% (2.7), 63.3% (4.2), and 15.0% (5.2); and for Schuyler #1, 94.2% (3.7), 76.7% (3.8), and 33.3% (4.6). The % survival (SE in parentheses) against malathion at the 1×, 4×, and 16× concentrations for Seneca #1 was 99.2% (0.8), 38.3% (4.9), and 0% (0.0), respectively; for Ontario #2, 100% (0.0), 46.7% (3.6), and 0.8% (0.8); and for Schuyler #1, 99.2% (0.8), 50.8% (3.0), and 0.8% (0.8). The % survival (SE in parentheses) against acetamiprid at the 4x concentration was 44.2% (11.4) for Seneca #1, 58.3% (11.9) for Ontario #2, and 39.2% (8.9) for Schuyler #1. These results from the early season collections show similar levels of resistance to the late season collections indicating that the levels of resistance detected in these populations was not due to a full season of spraying in vineyards.

The geographically widespread resistance seen for zeta-cypermethrin, malathion and acetamiprid appears to be the result of insecticide use both within and outside of vineyards. The widespread zeta-cypermethrin resistance may be due to the over-reliance on this insecticide for control of late season *Drosophila* in vineyards. Acetamiprid, and several other neonicotinoid insecticides, are used occasionally in NY vineyards, although not to as great an extent as pyrethroid insecticides. However, neonicotinoid insecticides are widely used on other crops in the vicinity of vineyards.

Our evaluation of the toxicity of 19 insecticides revealed activity over many orders of magnitude, with LC_{50} values from 0.65 (deltamethrin) to 15,000 ng/cm^2 (carbaryl). We tested a subset (15) of these insecticides against the Ulster population and found high levels of cross-resistance (>50% survival at 4 × the Canton-S LC_{95}) to all, except broflanilide, fipronil and flumethrin. High levels of resistance were found to cyclaniliprole, even though this insecticide was only labeled for use in vineyards in 2019. In fact, most of the insecticides for which we detected high levels of resistance have never been labeled for use against *D. melanogaster* in vineyards, suggesting the levels of resistance seen are likely due to insecticide selection in other crops or selection by insecticides used against different grape pests (including direct selection, cross-resistance and/or multiple resistance). The malathion LC_{50} we obtained was similar to what was previously reported for Canton-S.[14]

A major limitation to our understanding of insecticide resistance in *D. melanogaster* is that the detection of resistance is time consuming, slow and of low resolution (cannot distinguish heterozygous and homozygous resistant individuals reliably). Currently, detection of resistance requires weeks (because of rearing and evaluation using a residual contact assay) and provides limited information (just phenotype). What is urgently needed is a rapid and high throughput assay for detection of resistance. Such an assay would provide more timely assessments of resistance, more information, and aid establishment of resistance management strategies.

The number of registered and effective (i.e., those to which there is not widespread resistance) insecticides remaining for control of *Drosophila* in vineyards is very limited (e.g., only two in NY: spinetoram and cyclaniliprole). This presents numerous problems. First, the days to harvest label restriction for spinetoram and cyclaniliprole is relatively long (3 and 7 days, respectively) making it difficult to use for end of season *Drosophila* control. Second, cyclaniliprole is not allowed for use on Long Island, NY, where a significant amount of high-value premium wine grapes are grown. Third, there is now evidence that resistance to spinetoram is starting to evolve in at least one NY population and all populations have resistance (or cross-resistance) to cyclaniliprole. It is critically important that new insecticides be evaluated and registered for *Drosophila* control in vineyards and used only when justified to reduce selection pressure for resistance. The biological *Chromobacterium subtsugae* strain PRAA4-4 (Grandevo WDG®) is also labeled for use against *Drosophila* in grapes, although it is only moderately effective.[16] In addition, new non-insecticide control strategies, such as use of exclusion netting,[17] are needed to achieve sustainable production.

Technology Transfer Plan:

The results of this research were presented (at least as part of) the following talks:

- Annual meeting of NY BEV (Business. Enology. Viticulture) in Rochester, NY in the spring of 2019 by T. Martinson. (150 in attendance)
- Loeb, G. and Scott, J. 2020. New developments in the management of arthropod pests of grapes. 45-minute talk at the Long Island Ag Forum Viticulture Session on 9 January 2020. Approximately 40 growers and others in the session. Contact hours = 30 contact hours.
- Loeb, G. and Scott, J. 2020. What are we learning about fruit fly resistance to insecticides in the Finger Lakes as related to sour rot? 30-minute talk at B.E.V. NY 2020, February 28, 2020, in Rochester, NY. Approximately 130 in attendance. Contact hours = 65.
- Walter-Peterson, H. 2020 – B.E.V. NY 2020 [Business. Enology. Viticulture]: “Evaluation of berry cuticle supplements to reduce cluster rots in vineyards”. February 28. About 175 growers.
- Loeb, G. 2020. Entomology review for 2020. I participated in an online extension meeting as part of the Finger Lakes Program on May 5, 2020. I spoke for about 15 minutes and then participated

in the remainder of the program, including answering questions, for another 1.75 hours. Approximately 150 participants for virtual meeting, including many who received DEC pesticide credits. Contact hours = 2 X 150 = 300.

- Loeb, G. 2020. Insect Management. Virtual coffee pot meeting organized by CCE in western NY and PA. I presented pest management information for DEC and PA pesticide credit. 20 May 2020. Approximately 31 participants. Spoke and answered questions for about 1.75 hr. Credit hours = 31 X 1.75 = 54.25.
- Loeb, G. 2020. Insects and post-veraison fruit rots. Online extension meeting organized by Cornell and CCE grape programs on 11 August, 2020. 20-minute presentation. Credit hours = .33 X 80 participants = 26.5 contact hours.
- Loeb, G. 2021. Finger Lakes Grape Program Virtual Tailgate meeting. Held on 17 August 2021. Discussed aspects of sour rot management.
- Loeb, G. 2021. Finger Lakes grape spring meeting. Virtual meeting where I presented for about 30 minutes on issues relevant to the grape industry, including sour rot and insecticide resistance. April 28, 2021. About 90 participants were online for most of the session. Contact hours = 45.
- 2021 Loeb, G. Grape and Mite Pests, 2021 Field Season. Posted online at https://ecommons.cornell.edu/bitstream/handle/1813/103685/2021-Insects-Grapes-Review-FINAL.pdf?sequence=2&isAllowed=y. Also published in Finger Lakes Vineyard Update, May 12, 2021 at https://nygpadmin.cce.cornell.edu/pdf/newsletter_update/pdf611_pdf.pdf. Includes update on sour rot and insecticide resistance issues for Drosophila.
- Loeb, G. 2022, Entomology update: insects and grape disease and managing new invasive species. 35 minute talk (in person) for the Lake Erie Regional Grape Program Winter Grape Growers' Conference held in Fredonia, NY on 16 March 2022. Approximately 100 in attendance. Contact hours = 58.3.
- Loeb, G. 2022. Program update: management of arthropod pests of grapes. 45 minute talk (virtual) for the Long Island Hort Show, Viticulture Session. Approximately 70 in attendance. Contact hours = 52.5.

In addition, the following research paper was published (see attachment):
Mertz RW, Hesler SP, Pfannenstiel LJ, Norris RH, Loeb G and Scott JG, Insecticide resistance in *Drosophila melanogaster* in vineyards and evaluation of alternative insecticides. *Pest Man Sci*; **78**:1272-1278 (2021).

Attachments:

S#217 Mertz - Insecticide resistanc