Progress Report
NYW&GF 88466 Evaluation of Vinegar Flies

Robert Mertz[1], Greg Loeb[2], Tim Martinson,[3] and Jeffrey G. Scott[1]
[1] Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853 USA
[2] Department of Entomology, Cornell AgriTech, Cornell University, Geneva New York 14456 USA
[3] Section of Horticulture, School of Integrative Plant Science, Cornell AgriTech, Geneva, NY 14456 USA

Sour rot is an economically important disease of wine grapes in NY and other grape producing areas around the world [1]. Sour rot is caused by interacting microorganisms, in particular several different species of acetic acid bacteria and yeast, but also frequently associated with presence of vinegar flies (*Drosophila* species) [2-5]. The role of *Drosophila* in the etiology of sour rot is two-fold. First, they are a source of the bacteria and yeasts involved in disease symptoms. In addition to providing a source of microbes, however, recent research shows that the presence of *Drosophila*, beyond their contribution of microbes, is required for full development of sour rot symptoms, although the mechanisms are not understood [4].

Sour rot is managed through a combination of cultural practices to limit injury to grape clusters as well as chemical control targeting both causal microbes and vinegar flies [1, 6, 7]. Indeed, a field study conducted over three-years found that insecticides targeting *Drosophila* species contributed more to successful control of sour rot than antimicrobial pesticides [7]. Consequently, grape producers in NY and other regions frequently apply insecticides targeting vinegar flies near harvest to manage sour rot in susceptible wine grape cultivars [8]. One of the most commonly used insecticides is the pyrethroid zeta-cypermethrin (Mustang Maxx®) due to its relatively low costs and short days to harvest label restriction of 1 day.

During the harvest period of 2018 we observed a devastating control failure of sour rot in *Vitis vinifera* in a vineyard in the Finger Lakes region of central NY. In this vineyard block the grower had applied zeta-cypermethrin along with antimicrobial pesticides three times in mid- to late-September prior to the expected harvest date. Immediately following the third application of pesticides we observed high populations of vinegar flies (later identified mostly as *D. melanogaster*) and high levels of sour rot. With the support of NYSW&G we found this population was found to be highly resistant to zeta-cypermethrin, malathion and acetamiprid [9]. However, how widespread insecticide resistance was in *D. melanogaster* in NY was not known. Therefore, in 2019 we expanded our work to include nine vineyards across NY.

The goal of this study was to determine the geographic extent of insecticide resistance in *D. melanogaster* in NY vineyards and to evaluate the toxicity of a new and novel (venom toxin) insecticide. We collected flies from nine different vineyards across NY. There were high levels of resistance to zeta-cypermethrin, malathion and acetamiprid found in all populations sampled. The implications of these results to insecticide resistance monitoring and management are discussed.

Materials and Methods

Drosophila were collected in the fall of 2019 from nine vineyards and one orchard across NY State as shown in Table 1 using a D-vac vacuum system (Echo ES-230 Shred 'N' Vac®, Lake Zurich, IL, USA) and by hand collection of infested clusters. Adult flies were returned 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. To ensure correct identification, a subset of female flies (50) were individually isolated in small vials and placed in a walk-in growth chamber (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 [10]. Greater than 90% of the isolated females produced a F_1 adult generation and all were confirmed as *D. melanogaster*. 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. A laboratory susceptible strain (Canton-S) was used as a control. *D. melanogaster* were reared on standard fly medium (sucrose, cornmeal, yeast, tegosept, acid mix and agar, see https://cornellfly.wordpress.com/protocols/s-food/) under standard laboratory environment (~23 °C) with a photoperiod of 12L:12D.

Table 1. Locations from which *D. melanogaster* were collected and tested for insecticide resistance in 2019.

Farm	Variety	City	County	Collection Date
Robbins	Mixed	Geneva	Ontario	8/5/19
Loomis	V. labrusca	Geneva	Ontario	8/5/19-8/12/19
Hazlitt	Vignoles	Hector	Schuyler	9/10/19
Wickham	peaches	Hector	Schuyler	9/11/19
Clerel	vignoles	Portland	Chautauqua	9/19/19
Stormville	Table grapes	Stormville	Dutchess	9/19/19
Sheldrake	Pinot Gris	Ovid	Seneca	10/4/19
Hosmers	Riesling	Ovid	Seneca	10/2/19
Sawmill	Sauvignon blanc	Hector	Schuyler	10/4/2019
Red Maple Vineyards	Baco noir	West Park	Ulster	9/10/19

The five insecticides registered for *Drosophila* control in NY vineyards were used for bioassays: acetamiprid (99%, Chem Service, West Chester, PA, USA), malathion (99.2%, Chem Service), spinetoram (96.4%, Chem Service) and zeta-cypermethrin (98.7%, Chem Service). These represent four different classes of insecticides (neonicotinoids, organophosphates, spinosyns and pyrethroids), each with a different mode of action [11]. A new spider venom insecticide, Spear®, was also tested against the susceptible strain.

Bioassays were carried out by residual contact application method for four insecticides on Canton-S (spinetoram and spinosad are very similar insecticides so only spinetoram was tested). The field collected populations were tested at one or more diagnostic concentrations relative to Canton-S (LC_{95}, 4 x LC_{95} or 16 x LC_{95}) as previously described [9]. Insecticides were dissolved in acetone 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 (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 ball covered by white nylon tulle and applied with 10% sugar water with a syringe. Each treated vial containing 20 female flies (3-7-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. 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 (3-7-day-old) were used side-by-side with the field collected flies as a control. The LC_{50} of Spear insecticide was determined for the susceptible Canton-S strain using at least five concentrations. All bioassays were replicated a minimum of three times over a minimum of two days. Bioassay data were pooled and analyzed by standard probit analysis using R [12] (https://github.com/JuanSilva89/Probit-analysis).

Results and Discussion

The resistance patterns were similar in all of the collections from across New York State. There was resistance to zeta-cypermethrin evident at all three diagnostic concentrations in all populations (Fig. 1). There was only a very modest decrease in survival of the NY18 strain after being reared under lab conditions for nine months. There was survival to malathion at the LC_{95}, 4X LC_{95} and 16 x LC_{95} in all populations, except the susceptible (Canton-S) strain (Fig. 1). There was no change in survival of the NY18 strain (2018) after being reared under lab conditions for nine months (2019). Based on the high levels of acetamiprid resistance observed in 2018, we examined patterns of acetamiprid at only the 4 x LC_{95} diagnostic concentration. Again, resistance was widespread with 79-97% survival at all locations (compared to 0% in the controls). In 2018 spinetoram was the only insecticide to which we did not detect widespread resistance. Therefore we tested only the LC_{95} for this insecticide. Overall the populations were still largely susceptible to spinetoram (Fig. 4). However, the RMV population showed 20% survival at the diagnostic concentration. This population had the highest levels of resistance to all of the insecticides tested and the 20% survival in this population is potential evidence that resistance is evolving to the sole insecticide class for which resistance is not yet widespread in vineyards.

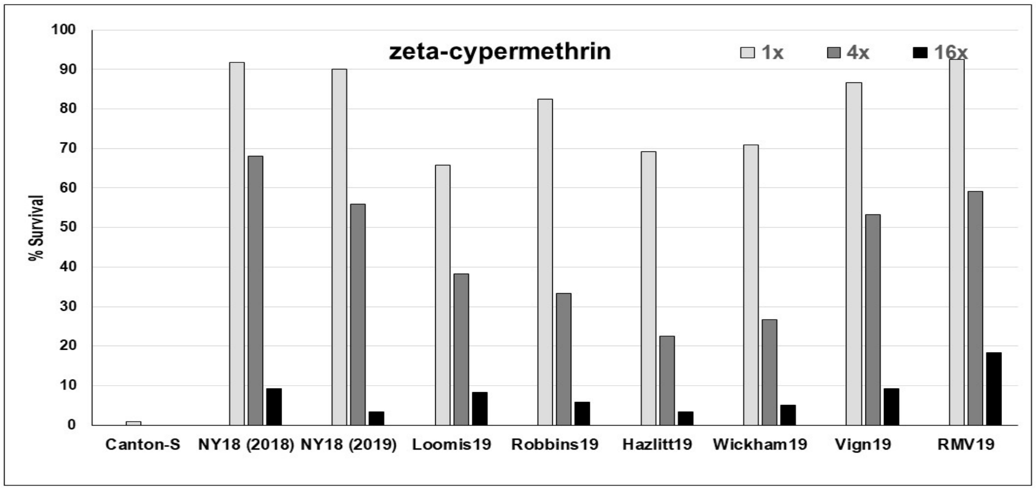

Figure 1. Survival of susceptible (Canton-S) and six populations collected from New York in 2019 tested against three diagnostic concentrations of zeta-cypermethrin. The survival previously reported for a field collected population from NY (2018) and that same population after being reared in the lab for nine months (2019).

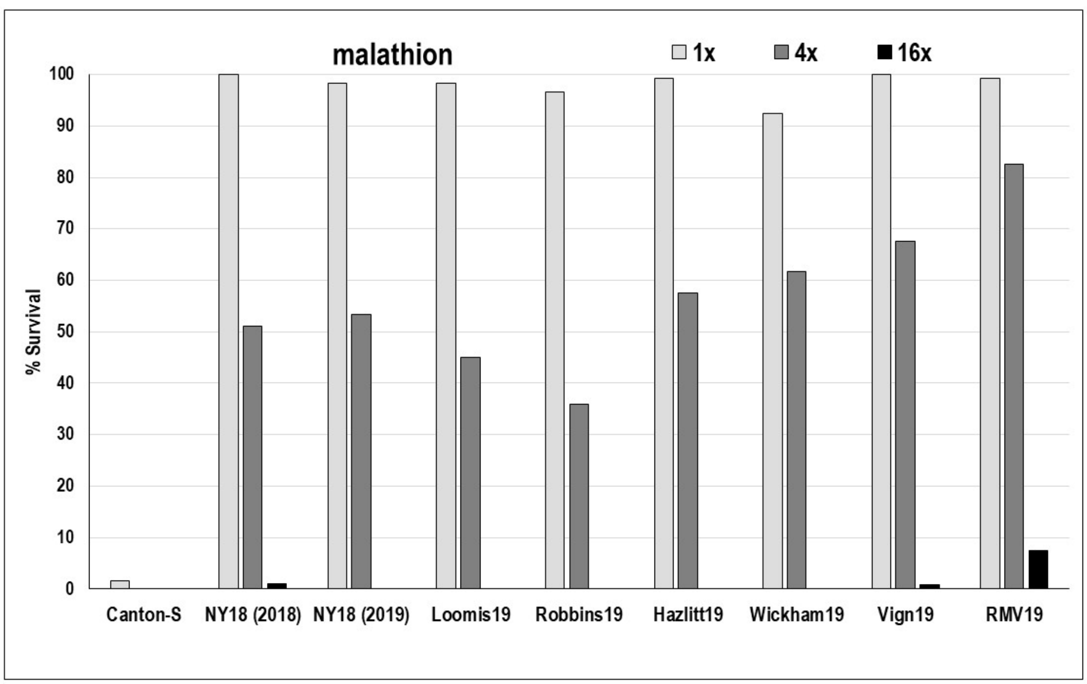

Figure 2. Survival of susceptible (Canton-S) and six populations collected from New York in 2019 tested against three diagnostic concentrations of malathion. The survival previously reported for a population from NY (2018) and that same population after being reared in the lab for nine months (2019).

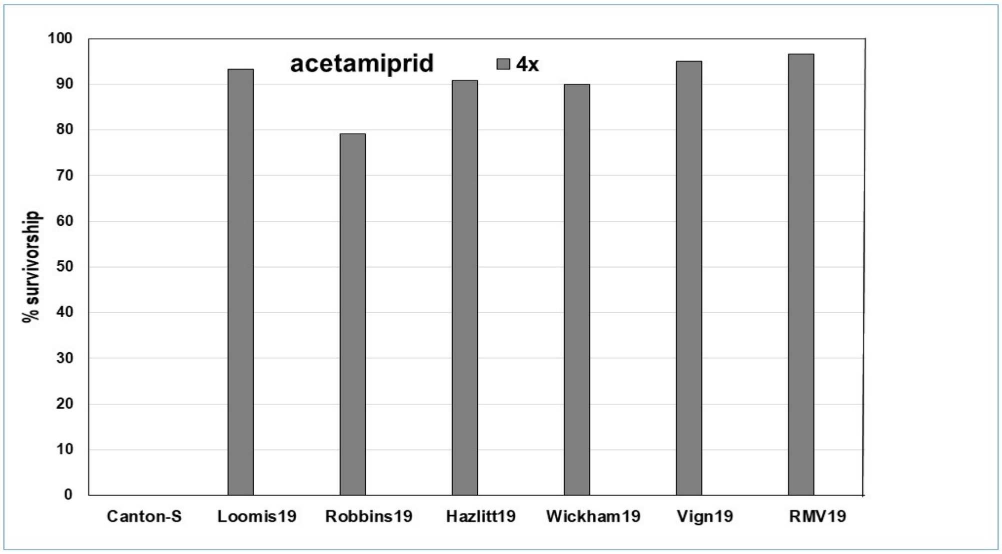

Figure 3. Survival of susceptible (Canton-S) and six populations collected from New York in 2019 tested against a diagnostic concentration of acetamiprid.

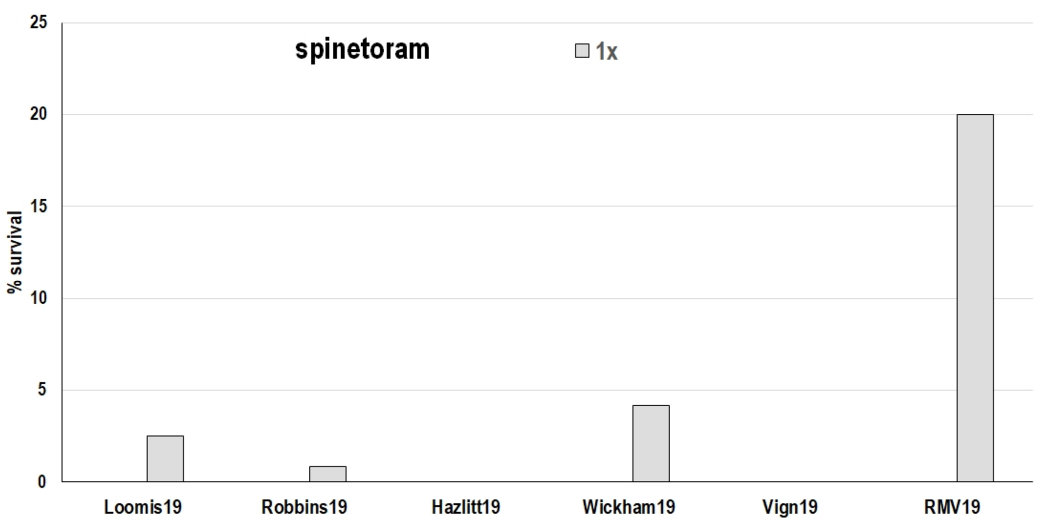

Figure 4. Survival of susceptible (Canton-S) and six populations collected from New York in 2019 tested against a diagnostic concentration (susceptible strain LC_{95}) of spinetoram.

Our results indicate that insecticide resistance in *D. melanogaster* from NY vineyards is widespread, with no susceptible populations detected against zeta-cypermethrin, malathion or acetamiprid. The field collected flies remained susceptible to only one class of insecticides tested (spinosyns). However, our results also suggest that spinetoram resistance is starting to evolve. There is an increasing urgency to find new insecticides that can be used against *Drosophila*, so that effective resistance management strategies can be implemented. The widespread resistance to zeta-cypermethrin, acetamiprid and malathion is likely causing problems for control of *D. melanogaster*, and thus sour rot.

As of the writing of this progress report we had completed analysis of six of the populations we have collected. We will finish our analyses of the remaining three strains and anticipate submitting these results for publication by late spring 2020.

We also tested a novel insecticide, Spear. This insecticide was 70- and 580-fold less toxic than zeta-cypermethrin and malathion, respectively [9], with an LC_{50} of 3.9 ug/cm^2. The relatively low toxicity of Spear suggests this is not likely to be effective for control of *D. melanogaster* in vineyards.

As a complement to insecticide bioassays, it would be valuable to have high throughput DNA-based molecular assays to monitor the frequencies of the resistance alleles. There are several reports from the literature that suggest possible genes/mutations to examine. For zeta-cypermethrin, or at least for other pyrethroids, *Cyp6a17*, *Cyp6a23* [13, 14] have been implicated in resistance, but mutations in the *voltage sensitive sodium channel* (*Vssc*) have not been reported [15]. For malathion, resistance has been associated with mutations in *Ace* [14, 16] and CYP-mediated detoxification [17], perhaps due to *Cyp6g1* [14]. In the case of acetamiprid (a neonicotinoid), nAChR genes (such as *Dα1* or *Dβ2* [18]) or *Cyp6g1* [19] have been implicated in resistance to other neonicotinoids such as imidacloprid [18]. Thus, understanding the mechanisms for decreased toxicity of these insecticides will require further study.

Outreach Activities

The results of this research were presented at the viticulture session of the Long Island Agricultural Forum in January 2020 by G. Loeb and at the annual meeting of NY BEV (Business. Enology. Viticulture) in Rochester, NY in the spring of 2019 by T. Martinson.

Presentation:
Greg Loeb[1], Tim Martinson[2], Jeffrey Scott[1], Haina (Tina) Sun[1], and Hans Walter-Peterson. UPDATE ON INSECTICIDE RESISTANCE IN FRUIT FLIES RESULTING IN FAILURE TO CONTROL SOUR ROT IN A FINGER LAKES VINEYARD. Presented at BEVNY conference, Feb 28, 2019. (150 in attendance)

We also directly met with the vineyard owners where the insecticide-resistant flies were collected to discuss these results and provide guidance on management options.

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