Progress Report
NYW&GF 88466 Evaluation of Vinegar Flies

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Sour rot is an economically important disease of wine grapes in NY and other grape producing areas around the world (McFadden-Smith and Gubler 2015). 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) (Barata et al. 2012a, Barata et al. 2012b, Hall et al. 2018b, Ioriatti et al. 2018). 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 (Hall et al. 2018b).

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 (Bisiach et al. 1986, McFadden-Smith and Gubler 2015, Hall et al. 2018a). 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 (Hall et al. 2018a). 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 (Weigle et al. 2020). 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 to be highly resistant to zeta-cypermethrin, malathion and acetamiprid (Sun et al. 2019). However, how widespread insecticide resistance was in *D. melanogaster* in NY was not known.

In 2019 we collected *D. melanogaster* from 11 NY vineyards across 7 counties an examined the levels of resistance to zeta-cypermethrin, malathion, acetamiprid and spinetoram. 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. There were high levels of resistance to zeta-cypermethrin, malathion and acetamiprid found in all populations sampled. Results from two vineyards also suggested that resistance to spinetoram, the only other registered insecticide, at the time, for *D. melanogaster* control in grapes, was starting to evolve. The venom toxin had low toxicity to *D. melanogaster*

suggesting it would not be an effective insecticide to control this pest. We also tested the population we had collected from 2018 and found that the levels of resistance to zeta-cypermethrin, malathion and acetamiprid were stable. Given these dire results there was a need to identify alternative insecticides for *D. melanogaster* control.

In 2020, with NYSW&G funding, we first examined the toxicity of 14 insecticides against a susceptible strain of *D. melanogaster* in the lab (to learn which insecticides had greatest potential against this species) and next examined the levels of cross resistance, using one of the populations collected in 2019. Eight insecticides were identified that had high levels of toxicity against susceptible *D. melanogaster.* However, there was high levels of cross resistance to six of these insecticides (even though they have never been labeled for use in vineyards). There was significant, but more modest cross-resistance to two insecticides that appear to have the greatest potential: broflanilide and fipronil. We also selected a strain that now is homozygous for resistance to zeta-cypermethrin and another strain that is homozygous resistant to malathion. These strains, however, are still resistant to a wide range of other insecticides (because they originated from multi-resistant populations).

Preliminary field studies with broflanilide were undertaken (based on the laboratory studies detailed above) to compare this novel insecticide with zeta-cypermethrin (Mustang Maxx) and to determine if we can reduce chemical control from weekly (3-4 sprays), to start and close to harvest (2 sprays). The experiments were conducted at Cornell AgriTech using Vignoles grapes, which are very susceptible to sour rot. Both zeta-cypermethrin and broflanilide reduced fly numbers compared to the unsprayed control. The insecticide timing study indicates that two pesticide applications may be as effective at controlling sour rot as four, thereby reducing selection for insecticide resistance, although environmental conditions during late season in 2020 were not conducive to sour rot development.

The results from the 2020 field studies, combined with no reports of zeta-cypermethrin control failures in 2019 and 2020, contrast with the laboratory results that indicate resistance is stable. One possibility is that the resistance levels decrease under field conditions, but simply rise to the same levels each year (or can be pushed to levels that cause control failures in year like 2018). Nothing is known about the variation in resistance in *D. melanogaster* in NY vineyards over time and this is a major data gap in our knowledge.

Materials and Methods

Two strains of *Drosophila* were used. Canton-S is an insecticide susceptible strain. RMV is a multi-resistant strain collected form Ulster Co. NY in 2019. *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 ℃) with a photoperiod of 12L:12D.

Insecticides were obtained from the following suppliers with purity given in parentheses. Abamectin (95.5%), carbaryl (98.1%), chlorfenapyr (99.4%), cyfluthrin (99.2%), fipronil (98%), flubendiamide (99.5%), phosmet (97.0%) and S-indoxacarb (99.5%) were purchased from Chem Service (West Chester, PA). Broflanilide was obtained from BASF (Florham Park, NJ). Chlorantraniliprole (97.8%) was obtained from DuPont (Wilmington, DE). Cyclaniliprole (96.6%) was obtained from LGC Labor GmbH (Augsburg, Germany). Fenpyroximate (99%) was purchased from Sigma-Aldrich (St. Louis, MO). Flupyradifurone (99.4%) was obtained from

Bayer (Kansas City, MO). The Vestaron insecticide was obtained as a formulation (Spear-T liquid concentrate, 2% GS-omega/kappa-Hxtx-Hv1a) from Vestaron Corporation (Kalamazoo, MI).

Bioassays were carried out by residual contact application method as previously described (Sun et al. 2019). 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 1 hr 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 °C with a photoperiod of 16L: 8D. Mortality was assessed after 24 h of exposure for all insecticides (except for slow acting 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. 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 (Silva 2018) (https://github.com/JuanSilva89/Probit-analysis). The LC_{50} determined for Canton-S was used as a single concentration assay to assess levels of cross-resistance in the RMV strain. Insecticides which killed none of the RMV flies were labeled as having "high levels of cross-resistance" while those that killed some of the flies (none of them killed 50% of RMV) were labeled as having "low levels of cross-resistance".

There is a lack of knowledge on whether weekly pesticide applications are necessary to achieve adequate control. We conducted field studies aimed to: 1) determine if we can reduce chemical control from weekly (3-4 sprays) to start and close to harvest (2 sprays) and 2) to evaluate the efficacy of alternative insecticides in controlling Drosophila in vineyards. The experiments were conducted at Cornell AgriTech using Vignoles grapes, which are very susceptible to sour rot. For the insecticide timing trial, as vines approached 15 Brix, we established three treatments: 1) no insecticides or microbial pesticide, 2) weekly applications of insecticide plus oxidate 2.0 (industry standard), and 3) two applications of insecticide + oxidate; one at around 15 Brix, and within a week of harvest (around 21 Brix). For the insecticide efficacy trial, starting at about 17 Brix, we established the following treatments: 1) water control; 2) Mustang Maxx (grower standard), weekly until harvest at a rate of 4 fl oz/A; 3) broflanilide weekly until harvest at a rate of 1.71 fl oz/A rate, and 4) Verdypryn weekly until harvest at a rate of 11 fl oz/A. For each experiment, the efficacy of the treatments was evaluated by 1) clear sticky cards checked weekly for Drosophila, 2) rearing adult flies from a subset of fruit collected near harvest, and 3) assessing incidence and severity of sour rot near harvest.

Results and Discussion

Starting with the field collected flies from 2019, we selected a strain (ZCR) that was homozygous resistant to zeta-cypermethrin and a strain that was homozygous resistant to malathion (MalR). The ZCR strain is 500-fold resistant to zeta-cypermethrin and the MalR strain is 160-fold resistant to malathion. We propose to further study these strains in 2021 (see research proposal).

The most toxic insecticide against the insecticide susceptible Canton-S strain of *D. melanogaster* was broflanilide with an LC_{50} of 1.6 ng/cm^2 [for reference the zeta-cypermethrin to

Canton-S is 56 ng/cm^2]. Assuming that compounds of suitable efficacy for field control of *D. melanogaster* would have an LC_{50} of <100 ng/cm^2, this indicates eight insecticides with suitable toxicity to *D. melanogaster* (Table 1). Our examination of cross-resistance in the RMV population from 2019 indicated high levels of cross-resistance to all compounds, except broflanilide and fipronil. While the compounds to which we detected cross-resistance are not used for control of *D. melanogaster* in vineyards, many of these have been used in other fruit production systems and it appears that this may be the reason for the cross-resistance levels we observed.

Table 1. Toxicity of 14 insecticides to susceptible (Canton-S) vinegar flies and relative levels of cross-resistance in a population collected in 2019 from a NY vineyard

Insecticide	**Canton-S LC_{50} (ng/cm^2)**	**RMV19** [e]
broflanilide	1.6 (1.3-2.0)	r
fipronil	2.2 (1.8-2.8)	r
cyclaniliprole	2.2 (1.8-2.8)	R
cyfluthrin	16 (14-20)	R
phosmet	17 (15-18)	R
chlorfenapyr	26 (24-28)	R
chlorantraniliprole	36 (34-41)	R
S-indoxacarb [b]	54 (52-57)	R
flupyradifurone [a]	390 (310-490)	R
flubendiamide [b]	540 (440-650)	R
abamectin [b]	1300 (1100-1400)	ND
Vestaron [c]	3900 (3200-4700)	ND
carbaryl [bd]	15000 (13000-16000)	R
fenpyroximate [b]	>> 1300	ND

[a] mortality measured at 48 hr
[b] mortality measured at 72 hr
[c] mortality measured at 96 hr [d] RMV19 tested at only the Canton-S LC_{50} dose
[e] R = high levels of cross-resistance, r = low levels of cross-resistance
ND= Not determined

Our field studies did not find differences in the number of *Drosophila* species on sticky cards nor from rearing between weekly sprays (4) and at 15 Brix and close to harvest (2 sprays)

but both were significantly reduced compared to unsprayed control. Similarly, no difference was observed in sour rot severity between the weekly and start and near harvest treatments but both treatments had reduced sour rot compared to control. We observed fewer Drosophila on sticky cards in Broflanilide and Mustang Maxx relative to the water control in the insecticide efficacy trial. A fewer number of Drosophila adults were reared from Mustang Maxx-treated vines as well as Verdypryn-treated vines relative to control. We observed a lower percentage of sour rot in Mustang Maxx treated berries than the rest of the insecticide efficacy trial treatments. The insecticide timing study indicates that two pesticide applications may be as effective at controlling sour rot as four, thereby reducing selection for insecticide resistance, although environmental conditions during late season in 2020 were not conducive to sour rot development.

Two small components of the proposed research were not accomplished in 2020 due to the pandemic: the genetic analyses of resistance and the synergist bioassays. We invested eight months into the analysis of the genetics of zeta-cypermethrin resistance. Unfortunately, with just a few weeks to go in the project the undergrad who had been doing the analyses was required to leave Ithaca because of the pandemic. The lab became horribly understaffed (all the undergrad help left town) and we struggled just to keep our different strains of insects alive. Unfortunately, there were just not enough hours in the day at that point to finish the experiments on the genetic analysis and we were banned from conducting research at that point. The budget cut that came along in the fall forced us to cut back and we were not able to complete the insecticide synergist bioassays.

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* (Battlay et al. 2018, Duneau et al. 2018) have been implicated in resistance, but mutations in the *voltage sensitive sodium channel* (*Vssc*) have not been reported (Scott 2019). For malathion, resistance has been associated with mutations in *Ace* (Menozzi et al. 2004, Battlay et al. 2018) and CYP-mediated detoxification (Houpt et al. 1988), perhaps due to *Cyp6g1* (Battlay et al. 2018). In the case of acetamiprid (a neonicotinoid), nAChR genes (such as *Dα1* or *Dβ2* (Perry et al. 2008)) or *Cyp6g1* (Daborn et al. 2001) have been implicated in resistance to other neonicotinoids such as imidacloprid (Perry et al. 2008). Thus, understanding the mechanisms for decreased toxicity of these insecticides will require further study.

Outreach Activities

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

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.

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