Washington State Grape and Wine Research Program

FINAL REPORT
2020-21 FUNDING CYCLE

MONITORING MEALYBUGS FOR POTENTIAL IMIDICLOPRID RESISTANCE AND BUFFALO TREEHOPPERS FOR IMIDICLOPRID SUSCEPTIBILITY

1. **Summary**:

Objective 1. *Collect different populations of grape mealybugs*. Two distinct populations of grape mealybugs were collected in 2019. The first was from Ste Michelle Wine Estates Vineyard 10 location near Paterson, WA. The second population was collected from a pear orchard in a WSDA certified organic production system. It appears that the grape population was developing resistance to imidacloprid while the pear population appears to still be susceptible to imidacloprid. We made considerable attempts to find populations in Concord vineyards and failed. We tried to work with crop consultants from the cherry industry in finding sites. We were for the most part ignored by these orchardists.

Objective 2: Develop and validate a whole leaf bioassay technique utilizing test tubes as arenas. We did validate that we could observe treatment effects with our whole-leaf bioassay method. With this technique we can chemigate a vineyard with a systemic insecticide like imidaclorprid by hanging a plastic cup under a drip emitter putting a dose of insecticide in the cup and then irrigating the vineyard to deliver the insecticide. Chemigation was completed on June 10, 2019. Rates of imidaclopid were at the equivalent of 0 (control), 50, 100, and 200% of the maximum labeled field rate. Leaves were collected for bioassay arena construction on 13 June, three days after chemigation. Arenas consisted of individual excised leaves with their petioles placed in large test-tubes full of water. These arenas sat for one week to determine viability of leaves. On leaves that were successful in establishing arenas small rootlets developed on the petioles.

On June 20, 2019, ten mealybug crawlers were placed on ten replicate leaves from each of the treatments detailed above. Our initial study was with the mealybug population collected from the vineyard near Paterson, WA. The mealybugs were permitted to settle and feed for one week. On June 27, 2019 these leaves were scanned and the number of surviving mealybugs quantified. Mealybug crawlers were collected from the organic pear block near Wenatchee, WA on July 10, 2019. On July 11, 2019 arenas were constructed as detailed above. This was 31 days post chemigation of the vineyard. Crawlers were placed on the leaves on July 18, 2019. These were scanned after one week.

Results:

There were substantial differences in the mortality observed between the two mealybug populations (Table 1.). Although it is statistically impossible to compare these two populations against one another the differences in mortality between these populations is of great concern. Additionally, the grape population was bioassayed on leaves between 10 and 17 days following chemigation. The population from the organic pear orchard was completed on leaves between 38 to 45 days following chemigation. The simple lack of mortality in the vineyard collected mealybug population leads us to believe that grape mealybugs are developing tolerance to imidacloprid in Washington State

vineyards. We are still working on our bioassay method. These studies will initiate after we develop a bioassay method that provides us with consistent results.

2. **This is a Final Report**

3. **Project Title**:

4. **Principal Investigator/Cooperator(s)**:
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5. **Objective(s) and Experiments Conducted to Meet Stated Objective(s)**:
Objective 1. Collect different populations of grape mealybugs.
Two distinct populations of grape mealybugs were collected in 2019. The first was from Ste Michelle Wine Estates Vineyard 10 location near Paterson, WA. The second population was collected from a pear orchard in a WSDA certified organic production system. As our subsequent data points out the grape population was developing resistance to imidacloprid while the pear population appears to still be susceptible to imidacloprid. We made considerable attempts to find populations in Concord vineyards and failed. We tried to work with crop consultants from the cherry industry in finding sites. We were for the most part ignored by these orchardists.
Objective 2: Develop and validate a whole leaf bioassay technique utilizing test tubes as arenas.
We did validate that we could observe treatment effects with our whole-leaf bioassay method. With this technique we can chemigate a vineyard with a systemic insecticide like imidaclorprid by hanging a plastic cup under a drip emitter putting a dose of insecticide in the cup and then irrigating the vineyard to deliver the insecticide. Replicate size was 5 vines in row. Leaves were only collected from the middle 3 vines for use in bioassay.

Mealybug bioassay results with imidacoprid.
Chemigation with imidacloprid using the method described above was completed on June 10, 2019. Rates of imidaclopid were at the equivalent of 0 (control), 50, 100, and 200% of the maximum labeled field rate on 0.5 lb active ingredient per acre. Leaves were collected for bioassay arena construction on 13 June, three days after chemigation. Arenas consisted of individual excised leaves with their petioles placed in large test-tubes full of water. These arenas sat for one week to determine viability of leaves. On leaves that were successful in establishing arenas small rootlets developed on the petioles.
On June 20, 2019, ten mealybug crawlers were placed on ten replicate leaves from each of the treatments detailed above. Our initial study was with the mealybug population collected from the vineyard near Paterson, WA. The mealybugs were permitted to settle and feed for one week. On June 27, 2019 these leaves were scanned and the number of surviving mealybugs quantified. Mealybug crawlers were collected from the organic pear block near Wenatchee, WA on July 10, 2019. On July 11, 2019 arenas were constructed as detailed above. This was 31 days post chemigation of the vineyard. Crawlers were placed on the leaves on July 18, 2019. These were scanned after one week.

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There were substantial differences in the mortality observed between the two mealybug populations (Table 1.). Although it is statistically impossible to compare these two populations against one another the differences in mortality between these populations is of great concern. Additionally, the grape population was bioassayed on leaves between 10 and 17 days following chemigation. The population from the organic pear orchard was completed on leaves between 38 to 45 days following chemigation. The simple lack of mortality in the vineyard collected mealybug population leads us to believe that grape mealybugs are developing tolerance to imidacloprid in Washington State vineyards.

Table 1. Number of grape mealy bugs surviving out of 10 one week following placement on leaves that were from an imidacloprid chemigated vines. The mealybugs from the grape vineyard were collected near Paterson, WA. The mealybugs from the organic pear orchard were collected near Wenatchee, WA.

Percent		Survived out of 10 mealybugs per leaf ± standard error	
Field rate		grape vineyard	organic pear orchard
Mean square	df=3	51.92**	204.18**
Error	df=36	5.05	1.036
0% (control)		9.33±0.37	9.60±0.22
50%		5.50±0.79	0.70±0.39
100%		4.50±0.65	0.60±4.40
200%		4.20±0.89	0.40±0.22

Objective 4. Validate presence of resistance to imidacloprid via molecular methods.

No work has been completed on this objective. As detailed in this recent proposal this was an objective that will be included in year 2 of this project if actual resistance was documented by bioassay.

Objective 5: Determine susceptibility for buffalo treehoppers to imidacloprid.
Buffalo treehoppers Stictocephala bisonii were collected on 22 July and 10 potted grapevines were drench treated with a low field rate of imidacloprid equivalent to 50% of the maximum label rate of 0.5 pounds active ingredient per acre. Treehoppers were immediately placed on Red Blotch infected vines and permitted to feed for one week. On 29 July, the 10 treated potted grapevines were placed individually in 10 cages, and 10 treehoppers were placed on each of the vines. The treehoppers were to feed for one week. On 5 August, vines and cages were checked for surviving treehoppers.

In 2019 we completed transmission assays with buffalo treehoppers in which buffalo treehoppers were permitted to feed on leafroll

On 1 August, 10 ten potted grapevines were treated with the high field rate of imidacloprid equivalent to 0.5 pounds active ingredient per acre and treehoppers were collected and placed on Red Blotch infected grapevines and permitted to feed for one week. On 8 August, the treated grapevines were placed individually in 10 cages and 10 treehoppers were placed on each vine. The treehoppers were permitted to feed for one week and then vines and cages were checked for surviving treehoppers.
On 12 August, a final cohort treehoppers were collected and placed on Red Blotch infected vines for one week to serve as control. On 19 August, treehoppers were transferred to 10 non-treated vines in cages and allowed to feed for one week.
Results for all three bioassay runs are detailed in Table 2.

Table 2. Total number of treehoppers out of 10 that survived in cages in which potted grape vines that were untreated with imidaclorid or treated with 50 or 100% of the maximum label rate of imidacloprid.

Percent field rate	Survived out of 10 treehoppers per cage ± standard error
0% (control)	9.80±0.13
50%	2.30±0.33
100%	0.40±0.16

Given these preliminary results it appears that buffalo treehoppers would likely prove susceptible to being killed by imidacloprid under vineyard conditions.

FYI. The grapevines in this study were held for 2 years and were tested for redblotch to determine if imidacloprid reduced transmission of redblotch disease. In both 2020 and 2021 all vines including the untreated vines came back from the laboratory as negative for redblotch. Although I cannot prove a negative, it appears that adult buffalo treehoppers do not vector redblotch.

6. **Summary of Major Research Accomplishments and Results by Objective:**
Mealybugs in our test vineyard was more tolerant of exposure to imidacloprid then our test population from a WSDA Organically Certified pear orchard. Resistance may be developing in mealybugs infesting vineyards.

Buffalo treehoppers are readily killed by imidacloprid treatment and they did not vector redblotch in the transmission assays we completed and followed through with in 2020 and 2021.

7. **Outreach and Education Efforts - Presentations of Research**:

November 19, 2020 Thrips, Flea Beetles and Cutworms. Pest Management for Insects We Don’t Always spray for. Washington Grape Society. Virtual, WA

Spray For. Washington Grape Society. Virtual, WA

July 16, 2020	Managing Mealybugs: Life Cycle, Control Tactics, and Insecticide Resistance Management. Washington Grape Society. Virtual, WA
February 19, 2020	Latest in Vector Management. Washington Advancement in Viticulture and Enology. Prosser, WA
November 14, 2019	Grape Phylloxera. Washington Grape Society. Grandview, WA
February 12, 2019	Mealybugs, Leafrollers and Grape Flea Beetles. Washington Wine Growers Annual Meeting. Kennewick, WA
January 17, 2019	Monitoring Mealybugs for Potential Imidicloprid Resistance and Buffalo Treehoppers for Imidicloprid Susceptibility. Washington Wine Commission. Prosser, WA
January 17, 2019	Why are We Having Spider Mite Outbreaks in Washington Vineyards? Washington Wine Commission. Prosser, WA

8. **Research Success Statements**: Two publications were published based on this project. The Washington State Grape and Wine Research Programwas acknowledged in both publications.

O"Hearn, J. & D. Walsh. 2020. GLRaV-3 Vectored by Grape Mealybug, Pseudococcus maritimus (Hemiptera: Pseudococcidae), at low population levels. J Entomol Sci doi.org/10.18474/0749-8004-56.1.106.

O"Hearn, J. & D. Walsh. 2020. Effectiveness of imidacloprid, spirotetramat, and flupyradifurone to prevent spread of GLRaV-3 by grape mealybug, Pseudococcus maritimus (Hemiptera: Pseudococcidae). J Plant Disease and Protection. DOI https://doi.org/10.1007/s41348-020-00359-1

9. **Funds Status**: The funds were expended