BEYOND IMIDACLOPRID AND CHLORPYRIFOS. EVALUATING NOVEL INSECTICIDES FOR GRAPE MEALYBUG MANAGEMENT, QUANTIFYING IMIDACLOPRID RESISTANCE IN GRAPE MEALYBUGS, AND DEVELOPING PRELIMINARY DATA FOR A MATING DISRUPTION PROGRAM

Principal Investigator: Doug Walsh, PhD
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1. Summary: In this 2-year project we completed springtime (2022) and summertime (2021) insecticide efficacy studies. In both trials, several candidate insecticides provided significant control of grape mealybugs. Neonicotinyl-based insecticides for the most part failed to provide effective control of mealybugs. Controlled laboratory bioassay studies were inconclusive since we had trouble developing a consistent methodology with systemic insecticides and maintaining laboratory colonies of field collected mealybugs in the greenhouse proved to be difficult. The molecular biologist from the University of Idaho upon whom we were depending to complete our molecular marker-based analysis of imidacloprid resistance resigned in 2022 and we were unable to complete these studies. Our greatest successes in this two-year project were with the several pilot studies we completed with pheromone-based mating disruption. Twist tie pheromone dispensers were provided by Pacific Biocontrol and Cide-Trak dispensers were provided by provided by Trece, Inc. and both products provided effective shut-down of male capture in pheromone-baited sentinel traps at rates of 60 twist tie and 32 Cide-Trak dispensers per acre. Unfortunately, supply issues enabled us to complete only small scale 5-acre test plots with both of these dispenser types.

2. FINAL REPORT 2021-2023

3. Title: BEYOND IMIDACLOPRID AND CHLORPYRIFOS. EVALUATING NOVEL INSECTICIDES FOR GRAPE MEALYBUG MANAGEMENT, QUANTIFYING IMIDACLOPRID RESISTANCE IN GRAPE MEALYBUGS, AND DEVELOPING PRELIMINARY DATA FOR A MATING DISRUPTION PROGRAM.

4. Principal Investigators:

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5. Objective(s) and Experiments Conducted to Meet Stated Objective(s):

Objective 1. ***Conduct a delayed dormant mealybug insecticide efficacy trial with horticultural spray oils and candidate insecticides.*** Our original intention was to find a high sulfur dormant oil for use in these trials. High sulfur oils are no longer commercially available. We had a grower collaborator apply buprofezin (Applaud) at 12 fluid oz per acre in 2 water volumes of 50 and 100 gallons per acre on May 23, 2022. Post treatment evaluations noted no differences in the abundance of mealybugs among the two treatments and areas nearby that were not treated with Applaud. Our results were inconclusive.

Objective 2. ***Conduct a mid-spring insecticide trial.***

Plots were established in a grower collaborator vineyard near Whitstran, WA in May 2022. In this vineyard a series of 13 insecticide treatment regimes were established. Several treatments were applied by chemigation and others were applied as foliar sprays with an echo duster mister gas powered backpack sprayer. Treatments and application dates are detailed in Table1.

Treatments designated as foliar were applied with a gas-powered Echo Duster Mister air assisted backpack sprayer in the equivalent of 100 gallons per acre. Treatments designated as drip application were applied through plastic cups hung beneath drip irrigation system drip emitters. The irrigation set was approximately 8 hours and applications were made 3 hours after the irrigation set had begun. Plots comprised 5 vines in length in row at a 6-foot spacing. Only the 3 middle vines were sampled for mealybug abundance and rated for honeydew.

Plots were evaluated on 30 and 31 August 2022. The grape clusters on the 3 center vines of each plot were visually scanned and the number of clusters with mealybug infestation was counted.

Following this cluster analysis the 3 center vines in each plot were visually scanned for 3 minutes (1 minute per vine) and the number of mealybugs observed was counted and recorded.

One week later on 8 September 2022, vines were rated for honeydew on the lower foliage on the 3 center vines of each plot. Rates were subjective with 5 meaning that the leaves had a substantial amounts of honeydew on them while 1 had no honeydew on the leaves. Ratings of 2, 3, and 4 represented that roughly 25%, 50%, and 75% of the leaves had honeydew on them, respectively. No sooty mold was observed on any of the vines.

Table 1.

Treatment	Rate/acre	Timing	Infested clusters±SE	3-minute counts ± SE	Honeydew rating ± SE
MS Treatment *df*=12			113.9**	21,280**	2.59**
Error *df*=39			5.3	776	0.49
1. Untreated Control	n/a		17.50±1.71	201.25±36.76**	3.2±0.25
2. GWN-12030[1]	18.0 fl oz	May 25	7.05±1.47**	65.75± 9.67**	1.00**
3. GWN-12030[1]	18.0 fl oz	June 29	5.75±1.11**	43.50±11.79**	1.00**
4. GWN-12030[1]	18.0 fl oz	July 12	11.50±1.32**	63.75±14.13**	1.00**
5. Platinum[1]	17 fl oz	May 25	17.00±1.58[ns]	191.00±20.49[ns]	3.00±0.41
6. Movento[2]	8 fl oz	June 29	5.25±0.85**	28.50± 5.74**	2.00±0.41*
7. Applaud2 70DF + Silwet L-77[1]	12 oz 0.1% v/v	May 25	16.75±0.85 [ns]	165.25± 8.61[ns]	2.75±0.63
8. Senstar[2] + Silwet L-77	16 fl oz 0.1% v/v	June 29	5.75±0.84**	43.50±12.94**	1.50±0.50**
9. GWN-12030[2]	9.0 fl oz	May 26	6.25±0.84**	7.25± 1.65**	1.50±0.50**
10. Proprietary 75G[1]	81g/acre	May 25	6.50±1.04**	7.50± 2.90**	1.25±0.25**
11.Proprietary 22.2WG[2]	120 g	May 26	7.00±0.91**	8.00± 3.29**	1.25±0.25**
12. Proprietary75G[1] + Movento 240[2]	281g/acre 8 fl oz	May 25 June 29	2.25±0.85**	7.25± 2.39**	1.00**
13. Transform WG[2]	2.75	May 26	2.75±1.11**	2.75± 1.49**	1.50±0.29**

[1]/ Chemigation

[2]/ Foliar application

*/ Treatment mean is significantly lower than untreated control at $p<0.05$ in pairwise t-tests

**/ Treatment mean is significantly lower than untreated control at $p<0.01$ in pairwise t-tests

Insecticide treatment with all the various GWN-12030 treatments and timings, the June 29 Movento and Senstar treatments, both formulations of the proprietary treatment including the treatment with a June 29 bump application and the May 29 Transform application significantly ($p<0.01$) reduced the number of mealybug infested clusters, the abundance of mealybugs in timed vine counts, and the quantity of honeydew produced. Platinum was largely ineffective and the application timing of Applaud was likely too early in the life-cycle of the mealybugs and it too was ineffective. There appears to be some new chemistry in the regulatory pipeline that may soon become available for mealybug suppression in the future. Unfortunately, no insecticide completely eliminated mealybugs, but Senstar + Silwet and GWN-12030, the proprietary insecticide and Transform did have specific replicate plots that had no mealybugs in them following treatment.

Objective 3. ***Conduct an early summer insecticide efficacy trial.*** Candidate and registered insecticides were applied on 10 August 2021. Treatments designated as foliar were applied with a gas-powered Echo Duster Mister air assisted backpack sprayer in the equivalent of 100 gallons per acre. Treatments designated as drip application were applied through plastic cups hung beneath drip irrigation systems drip emitters. The irrigation set was approximately 8 hours and applications were made 3 hours after the irrigation set had begun. Plots comprised 5 vines in length in row at a 6-foot spacing. Only the 3 middle vines were sampled for mealybug abundance and rated for honeydew. Plots were evaluated on 9 September 2021, thirty days post treatment. The grape clusters on the 3 center vines of each plot were visually scanned and the number of clusters with mealybug infestation was counted. Following this cluster analysis, the 3 center vines in each plot were visually scanned for 3 minutes (1 minute per vine) and the number of mealybugs observed was counted and recorded.

One week later, on 17 September 2021, vines were rated for honeydew on the lower foliage on the 3 center vines of each plot. Rates were subjective with 5 meaning that the leaves had a substantial amounts of honeydew on them while 1 had no honeydew on the leaves. Ratings of 2, 3, and 4 represented that roughly 25%, 50%, and 75% of the leaves had honeydew on them, respectively. No sooty mold was observed on any of the vines.

Collected data were analyzed by analysis of variance. All three measurements taken passed the *F*-test ($p<0.01$) and treatment means of the insecticide treated plots were compared to the treatment mean of the untreated control plots by Fisher's protected least significant difference test. The insecticide treatments GWN-12030 applied by drip at 9 fl oz/acre, Platinum applied by drip, and the Applaud treatment applied by foliar spray did not reduce the infestation of clusters by mealybugs or the abundance of mealybugs on the vines. All the other treatments provided significant reduction ($p<0.01$) of mealybug infestation of clusters and the abundance of mealybugs on vines. Movento and Senstar applied as foliar sprays and GWN-12030 applied by drip irrigation at 18 and 27 oz per acre provided the most superior control of mealybugs.

Unfortunately, no insecticide completely eliminated mealybugs, but Senstar + Silwet and GWN-12030 drip irrigated at 27 fl oz did have replicate plots that had no mealybugs in them following treatment.

Table 2. Treatment	Rate/acre	Infested clusters per 3 vines ± SE	3-minute counts ± SE	Honeydew rating ± SE
MS Treatment df=10		255.3**	26707**	10.22**
Error df=33		18.3	1180	0.15
1. Untreated Control	n/a	18.25±3.59	167.25±20.14	5.00±0
2. GWN-12030[1]	6.0 fl oz	9.25±2.39**	98.00±18.78**	3.25±0.25**
3. GWN-12030[1]	9.0 fl oz	6.75±0.85**	61.50± 8.81**	3.00±0**
4. GWN-12030[2]	9.2 fl oz	17.25±1.65	169.75±12.04	5.00±0
5. Platinum[1]	17 fl oz	18.75±3.57	201.75±43.15	5.00±0
6. Movento*	8 fl oz	4.00±1.47**	32.25±14.09**	1.75±0.25**
7. Applaud* 70DF + Silwet L-77[1]	12 oz 0.1% v/v	24.50±2.25	226.00± 7.43	5.00±0
8. Senstar* + Silwet L-77[2]	16 fl oz 0.1% v/v	3.00±1.29**	19.25± 6.88**	1.50±0.29**
9. Senstar& + V-10487[2]	16 fl oz 1% v/v	5.25±1.49**	42.75±14.62**	2.25±0.25**
10. GWN-12030[1]	18 fl oz	2.50±1.19**	10.00± 4.14**	1.25±0.25**
11. GWN-12030[1]	27 fl oz	2.50±1.66**	13.50± 6.03**	1.25±0.29**

[1/] Foliar Application
[2/] Drip Application

Objective 4. ***Conduct a comprehensive survey of mealybug populations in Washington State vineyards and other crops grape mealybug infest***. Mealybug populations have been collected from 5 conventional vineyards and a pear orchard in organically certified production. These insects are in the freezer in 95% ethanol waiting for a molecular biologist.

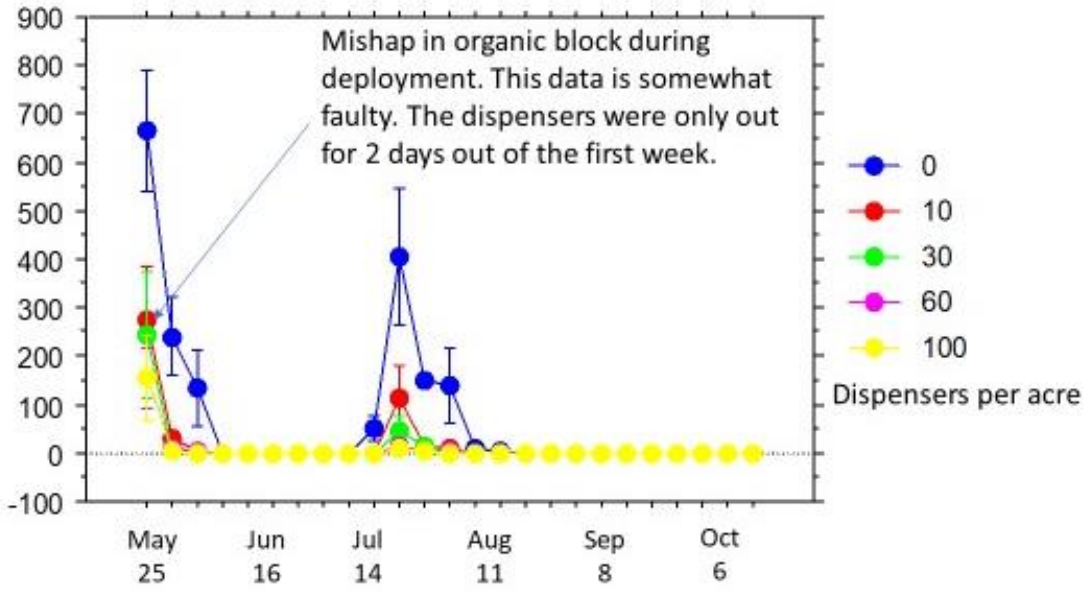
Objective 5. ***Conduct whole leaf bioassays utilizing test tubes as arenas***. We initiated studies with these whole-leaf bioassays in August 2021. Increasing the abundance of mealybugs in our colony turned out to be difficult. Our methodology proved to be problematic and after 2 summers of attempting to get these bioassays initiated we have failed.

Objective 6. ***Validate presence of resistance to imidacloprid via molecular methods (Year 2).*** The molecular toxicologist with whom we planned to complete these studies separated from the University of Idaho and took another position with private industry. He was, unfortunately, no longer able to assist us with this particular study.

Objective 7. ***Test and validate pheromone-based trapping of males with subsequent hatch and movement of crawlers with sticky tape***. Over the summer of 2022 we placed two-sided sticky tape out in cordons in the control plots in our mating disruption program (detailed later in this report) for a 4-week period following the egg hatch of mealybugs and captured no mealybug crawlers on this tape. Two-sided sticky tape is not an efficient monitoring tool for observing the movement of mealybug crawlers.

Objective 8. ***Conduct a pilot study on a mating disruption program for grape mealybugs based on available commercial technology*****.** In 2021 Pacific Biocontrol provided us with 2,000 pheromone dispensers imbibed with grape mealybug pheromone. We initiated a pilot study with the pheromone dispensers in 2 replicates of 5 acres for each deployment at 0, 10, 30, 60, and 100 dispensers per acre. We monitored each 5-acre replicate block with 2 sentinel delta-traps baited with grape mealybug lures from Trece Inc. One of the replicated blocks was in an organically certified vineyard and the second was in a conventional vineyard. We were a bit late in deploying the dispensers, but our results were immediate and outstanding. During both mating flights we observed that as few as 10 dispensers per acre significantly reduced trap capture in the sentinel traps and in the 2 blocks in which 60 and 100 dispensers were deployed we had nearly a complete shut down in grape mealybug male capture. These results were heartening (Figure 1.).

Figure 1. Trap capture in sentinel traps of male mealybugs in vineyard blocks in which pheromone dispensers were deployed at 0, 10, 30, 60, and 100 per acre.

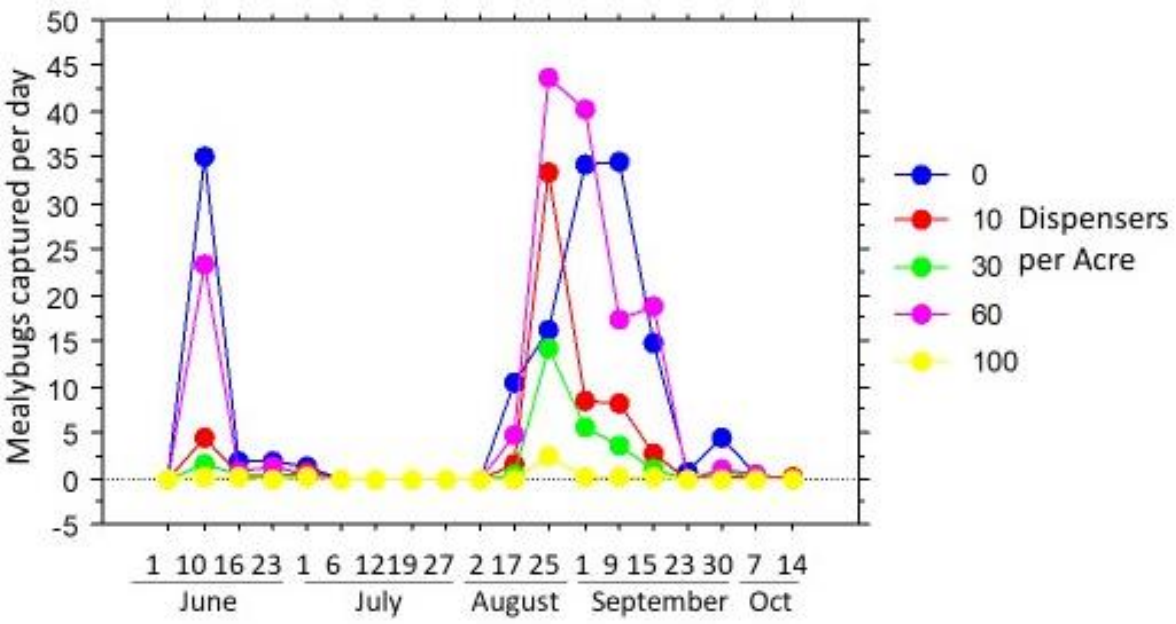


We had a mishap in the organic vineyard block in that we had deployed the Sentinel traps when the spray rig came through spraying sulfur. The REI for sulfur is 1 day. Due to time constraints, we did not get the pheromone dispensers out for another 5 days. However, with the second flight we had a complete shut down of trap capture at 60 and 100 dispensers per acre.

In 2022 we had Pacific Biocontrol again provide us with a total of 2000 twist tie dispensers. Additionally, we had Trece Inc. provide us with 1,500 of their Cide-Trak dispensers. We deployed the Pacific Biocontrol dispensers in 2 blocks at Columbia Crest in Paterson, WA. One block was in the same WSDA Organic Certification program as the block we used in 2021. The second block was in a conventional vineyard but this vineyard was substantially younger than the

block we used in 2021 and had fewer mealybugs and less virus. (The conventional block we used in 2021 was pulled due to poor production that was associated with leafroll infection.) We deployed the Pacific Biocontrol twist tie pheromone imbibed dispensers in the vineyard blocks in late April. These dispensers were again deployed at 0, 10, 30, 60 and 100 emitters per acre in 5-acre plots. We monitored with 2 sentinel traps per block and we checked these traps weekly from early May through mid-October. These trap captures are detailed in Figures 2 and 3, respectively.

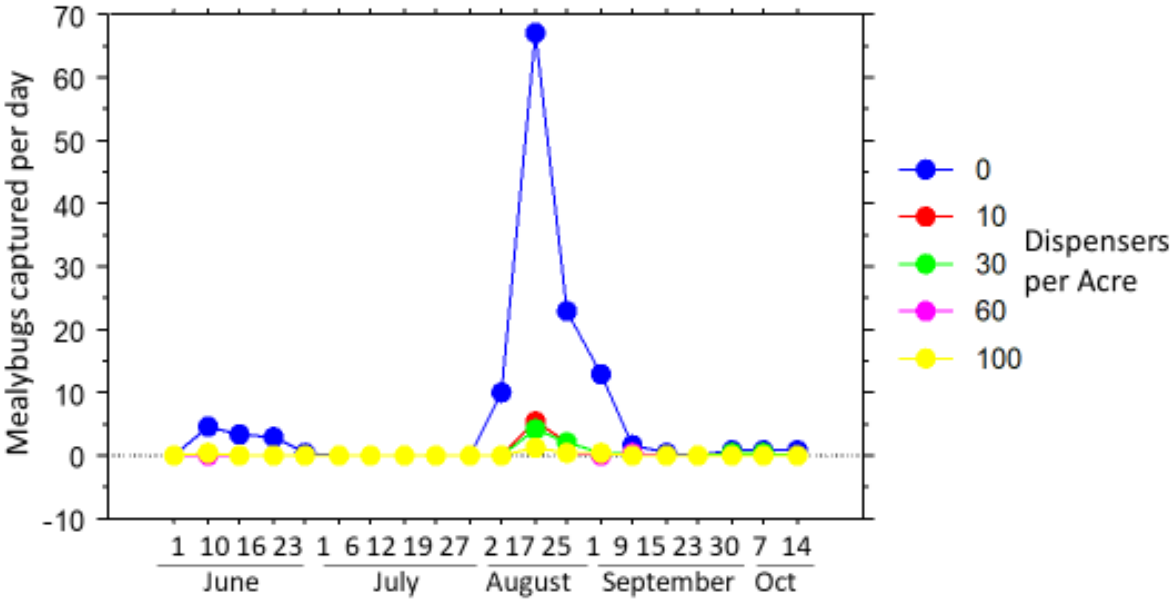
Figure 2. Mealybug males captured per day for the prior week in baited sentinel traps in a WSDA-Certified Organic Vineyard near Paterson, WA- Pacific Biocontrol Dispensers



We placed our dispensers out in late April and did not capture any male mealybugs until late May/ early June so we are confident that we captured the first males to fly in this vineyard. We are somewhat perplexed by our results in this vineyard. We have no clue as to what happened with the 60 dispenser per acre treatment in this organic vineyard. This 60 dispenser per acre treatment failed for both the first and second mating flights. The 100 dispenser per acre treatment was very consistent and male mealybug trap capture in our sentinel traps was effectively zero throughout the 2 flights with the exception of capturing a few males at the peak flight in August. The 10 dispenser treatment and 30 dispenser treatment per acre greatly reduced sentinel trap capture for the first flight. The 30 dispenser rate per acre reduced male mealybug trap capture for the second flight in August but the 10 dispenser treatment appears to have had a real significant impact on the second flight.

With the second conventional vineyard in which we deployed the Pacific Biocontrol Dispensers our results were much simpler and trap capture was substantially lower (Figure 3).

Figure 3. Mealybugs captured per day for the prior week in baited sentinel traps in a Conventional Vineyard near Paterson, WA- Pacific Biocontol Dispensers



The trap capture again demonstrates that, for the 2 mating flights over the growing season, all of the dispenser treatments substantially reduced sentinel trap capture and the 60 and 100 dispenser per acre treatments completely shut down trap capture. Given this is a younger vineyard this study demonstrates that continued mating disruption does look like it could substantially aid in the management of grape mealybug.

In 2022, Trece Inc. provided us with 1,600 of their CideTrak pheromone dispensers baited with grape mealybug pheromone. We deployed these dispensers in 2 blocks in younger vineyards near Patererson, WA and a third site was in a Concord juice grape vineyard that was located near Whitstran, WA. These dispensers were distributed at 0, 32, and 50 dispensers per acre in 5-acre blocks. The dispensers were deployed in early May and sentinel traps were deployed as described above and were monitored weekly from mid-May through early November. We captured our first male mealybug during the first week of June and our last mealybug at the end of September. In the wine grape blocks we had a near complete shut down in the sentinel trap capture for the first (June) mating flights with both the 32 and 50 dispensers per acre deployment rates compared to the untreated control plots with 0 dispensers per acre (Figure 4). For the second (August) flight we observed some breakdown in both the 32 and 50 dispensers per acre deployment treatment rate at a net peak capture of 15 mealybug males per day. This was substantially lower than the 0 dispensers deployed per acre treatment at 40 male mealybugs per day, but these data are somewhat concerning (Figure 4).

Trap capture was quite different in the lone Concord juice grape block. Again, in the blocks in which 32 and 50 dispensers were deployed per acre we observed a nearly complete shut down in

male mealybug sentinel trap capture compared with the substantially greater male mealybug sentinel trap capture that peaked during the 3rd week of June in which over 65 males were caught on average per day (Figure 5). From early July through October very few male mealybugs were captured in any of the 3 treatments. Even in the plot with 0 dispensers only a relatively low number of males were captured in late August (Figure 5).

Figure 4. Mealybugs captured per day for the prior week in baited sentinel traps in a Conventional Vineyard near Paterson, WA- Trece Dispensers

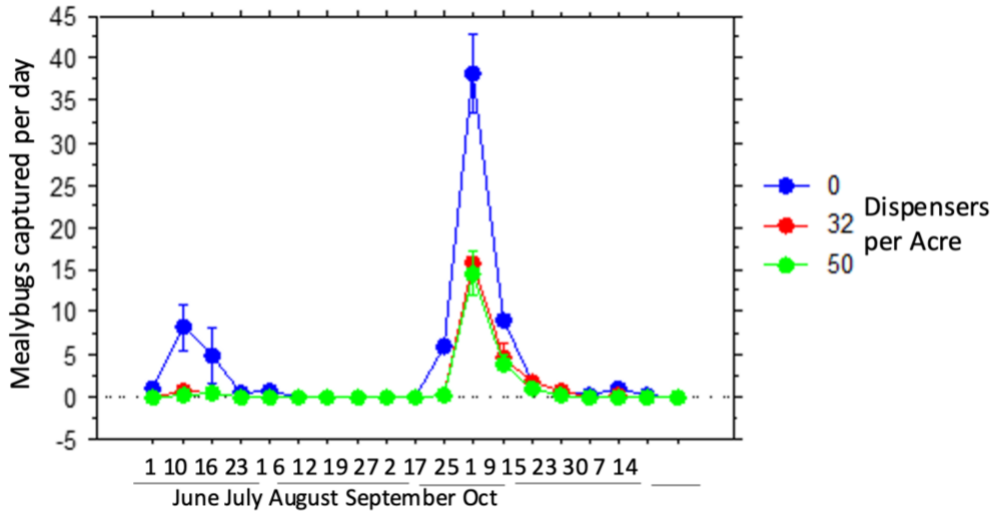
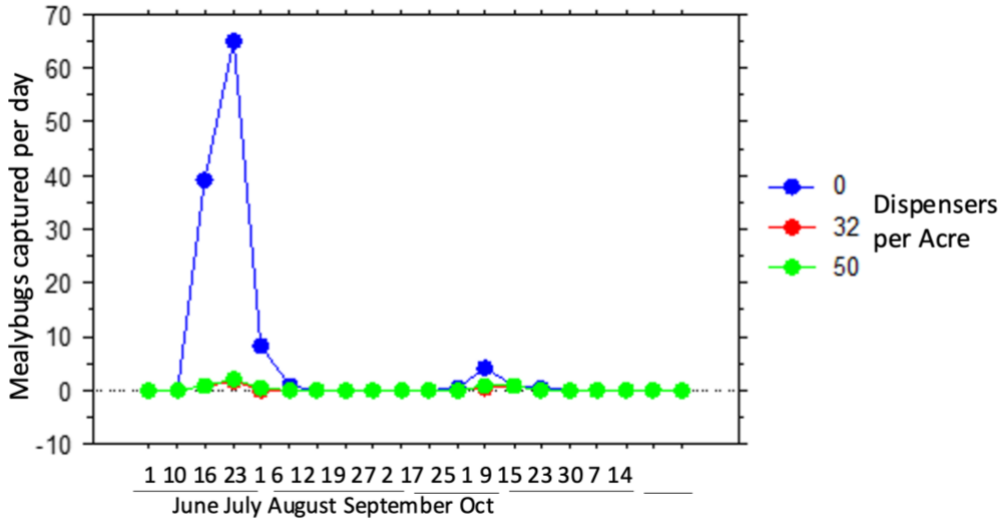


Figure 5. Mealybugs captured per day for the prior week in baited sentinel traps in a Concord Vineyard near Whitstran, WA- Trece Dispensers



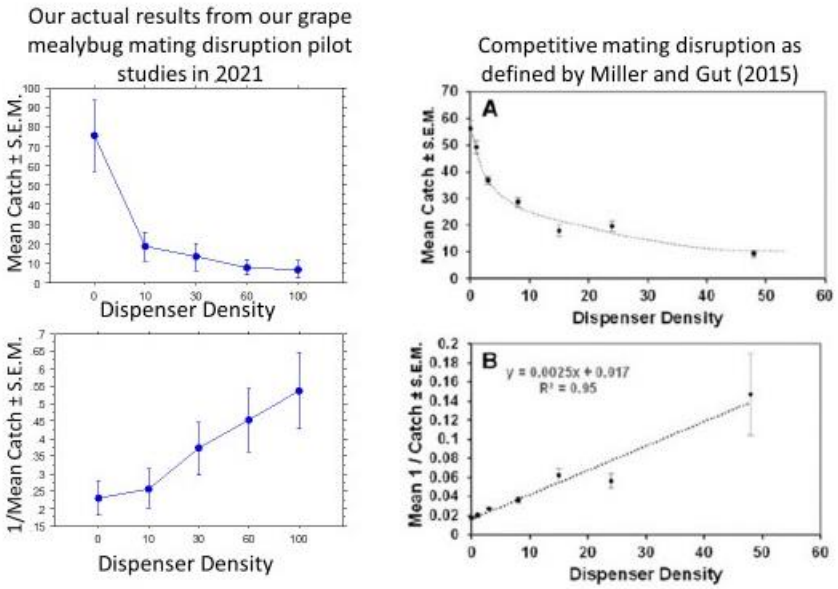
I have communicated with several pheromone chemists who have, to date, assumed that that the grape mealybug pheromone might be to difficult to produce in quantities great enough for

commercial application. Fortunately, a research group working in Israel has discovered that the essential oils from Spanish lavender can be used to serve as the precursor for grape mealybug pheromone. This discovery may increase the possibility of producing grape mealybug pheromone in quantities great enough for commercial mating disruption or mass trapping in vineyards for grape mealybug management.

Unfortunately, there is a shortage of magnesium and magnesium is important in synthesizing grape mealybug pheromone. Pacific Biocontrol has only committed to providing us with 2,000 pheromone dispensers. However, Trece will be providing us with an additional type of dispenser, a sachet format that we will test as well.

Objective 9. ***Determine if mating disruption of grape mealybug is competitive or noncompetitive while concurrently quantifying the minimum density of emitters required per acre to achieve mating disruption*****.** From the results of our small pilot studies completed detailed in Objective 8, above, it appears that grape mealybug mating disruption is competitive. Under competitive disruption, no impairments are experienced by males, females, or the signal of females. Therefore, males can respond to females and traps. Competitive disruption is entirely a numbers game where the ratio of dispensers to females and traps is highly consequential. This renders the control provided is pest-density-dependent. Again, this means that as the abundance of females increases there is an increased likelihood that some may get mated. And it looks like mating disruption is achieved at about 60 pheromone dispersers per acre with Pacific Biocontrol's dispensers and 32 with Trece's dispensers.

Figure 6. Comparing our 2021 results with models developed by Miller and Gut (2015)



It sure looks like grape mealybug mating disruption is competitive.

6. Summary of Major Research Accomplishments and Results by Objective

Objective 1. ***Conduct a delayed dormant mealybug insecticide efficacy trial with horticultural spray oils and candidate insecticides.*** We were unable to find a high-sulfur horticultural oil. This objective was not completed as originally envisioned. The buprofezin trial described under Objective 1 in the previous section was inconclusive.

Objective 2. ***Conduct a mid-spring insecticide trial.*** Insecticide treatment with 2 unregistered candidate treatments and several Movento, Transform, and Senstar treatments provided effective control of mealybugs. Platinum was largely ineffective and the application timing of Applaud was likely too early in the life-cycle of the mealybugs and it too was ineffective. Unfortunately, no insecticide completely eliminated mealybugs, but Senstar + Silwet and GWN-12030, the proprietary insecticide and Transform did have replicate plots that had no mealybugs in them following insecticide treatment.

Objective 3. ***Conduct an early summer insecticide efficacy trial.*** The insecticide treatments GWN-12030 applied by drip at 9 fl oz/acre, Platinum applied by drip, and Applaud applied by foliar spray did not reduce the infestation of clusters by mealybugs or the abundance of mealybugs on the vines. All the other treatments provided significant reduction ($p<0.01$) of mealybug infestation of clusters and the abundance of mealybugs on vines. Movento and Senstar applied as foliar sprays and GWN-12030 applied by drip irrigation at 18 and 27 oz per acre provided the most superior control of mealybugs.

Objective 4. ***Conduct a comprehensive survey of mealybug populations in Washington State vineyards and other crops grape mealybug infest.*** This objective is partially completed, with mealybug populations collected but not yet classified due to the unplanned absence of a molecular biologist on the project.

Objective 5. ***Conduct whole leaf bioassays utilizing test tubes as arenas*****.** This is a failed objective as we were unable to increase the abundance of mealybugs we have in colony.

Objective 6. ***Validate presence of resistance to imidacloprid via molecular methods***. As explained in the previous section, this was a failed objective due to the departure of a key cooperator. That being said, there appears to be enough observational information regarding recurring field failures with imidacloprid and other neonicotinyl insecticides for us to strongly believe that grape mealybug populations in Washington State vineyards have developed tolerance to being killed by exposure to imidacloprid or other neonicotinoids.

Objective 7. ***Test and validate pheromone-based trapping of males with subsequent hatch and movement of crawlers with sticky tape.*** Through completion of this objective we learned that two-sided sticky tape is not an efficient monitoring tool for observing the movement of mealybug crawlers.

Objective 8. ***Conduct a pilot study on a mating disruption program for grape mealybugs based on available commercial technology*****.** We have demonstrated that pheromone dispensers

deployed at sufficient rates depending on product can effectively shut down trap capture of male mealybugs in pheromone baited traps during mating flights. We can only assume that this is reducing the males to locate calling females and subsequently reduce mating and egg laying by the females. Our longer-term goals include larger plots and quantifying if reducing the spread of leafroll can be correlated with successful mating disruption.

Objective 9. ***Determine if mating disruption of grape mealybug is competitive or noncompetitive while concurrently quantifying the minimum density of emitters required per acre to achieve mating disruption*****.** We have concluded that mating disruption of grape mealybug is competitive and that with our present technology that it will take between 30 to 60 Pacific Biocontrol dispensers and around 32 Trece Inc. dispensers to achieve successful mating disruption.

7. Outreach and Education Efforts - Presentations of Research

Abstracts

Onayemi, S. & D. Walsh. 2021. Pilot studies on mating disruption for grape mealybug, Pseudococcus (Ehrhon) in Washington State. Entomological Society of America. Denver, CO November 1, 2021

Extension Presentations

March 8, 2023	Washington State Juice Grape Insect Update, 2023. Grandview, WA
February 7, 2023	Leafroll "its not about you it's about us" WineVit. Kennewick, WA
December 8, 2022	The Progression of Mealybug Research in Washington State, USA Vineyards. Hemipteran-Plant Interactions Symposium. Melbourne, Australia.
April 12, 2022	Insecticide resistance management in specialty crops in the Yakima Valley. Pacific Branch Entomological Society of America, Santa Rosa, CA.
April 12, 2022	Twenty years of grape mealybug mismanagement and the resulting rampant spread of leafroll virus in Washington State vineyards Pacific Branch Entomological Society of America, Santa Rosa, CA.
February 22, 2022	Managing mealybugs in vineyards. Virtual, WA
November 17, 2021	Pilot Studies on Mating Disruption of Grape Mealybugs in WA. State Wine Grape Vineyards

September 15, 2021	IPM in Grape and Hop Production in the Pacific Northwest. Entomological Society of America Webinar Series in conjunction with the US Environmental Protection Agency.
July 16, 2020	Managing Mealybugs: Life Cycle, Control Tactics, and Insecticide Resistance Management. Washington Grape Society. Virtual, WA
February 25, 2020	Monitoring and Management of Grape Phylloxera in Washington State
February 19, 2020	Latest in Vector Management. Washingtton Advancement in Viticulture and Enology. Prosser, WA

8. Research Success Statements: The development of a successful pheromone-based mating disruption program that is not cost prohibitive is the most sustainable method for controlling mealybugs and consequently slowing the leafroll virus mealybugs vector.

9. Fund Status: The funds received for this project have been used in partial support of PhD student Stephen Oneyemi.