Washington State Grape and Wine Research Program

FINAL REPORT
Funding July 2020-June 2024 (3 years + 1 NCE)
30 May 2024

Project Title: Fungicide Resistance Monitoring and Alternative Management Strategies for Grape Powdery Mildew

Principle Investigator: Dr. Michelle M. Moyer, Washington State University

Summary: In 2020, we began a three-year project that had two main goals: 1) To evaluate the use of UV-C light as an alternative management strategy for grape powdery mildew; and 2) To improve capacity for fungicide resistance testing / monitoring.

The 2020-2022 field seasons provided distinct weather patterns under which we could evaluate the effectiveness of UV-C. In 2020, the growing season saw temperatures that were slightly above average; in 2021, we experienced a heat dome event. For these two vintages, disease pressure was low (2020) to non-existent (2021), meaning very little disease was seen even in our untreated controls. The 2022 vintage, however, was "average" and we were able to see some clear disease management differences between our UV-C, standard fungicide, and untreated controls. Given the nature of how UV-C works, we saw improved disease control with increased frequency of application. We also saw that UV-C worked well when used as a part of a regime, rather than that sole management option. The UV-C system can easily be integrated into early-season spray programs to reduce pesticide inputs and allow for management during periods when sprays would not be allowed (i.e., windy or rainy conditions). UV-C may be able to provide season-long disease control as a stand-alone solution when applied weekly to twice weekly, but canopy management will also play a critical role in it's effectiveness; future studies are focusing on this. In laboratory studies, we determined that UV-C is effective against young, developing powdery mildew colonies, indicating that it has curative properties. This emphasizes why its effectiveness is likely best early season, where it can kill early-established mildew prior to the critical window for fruit disease control. There were only minor impacts on grape mealybug crawlers in laboratory assays. But given these results were seen after only a single treatment, we expect that the repeated treatments that crawlers would be exposed to when UV-C is used as a powdery mildew control tactic, might make it a useful combination tool in grape mealybug control (i.e., helpful when also combined with mating disruption).

Despite continued restrictions due to COVID-19 from 2020 through 2021, we were able to establish a fungicide resistance qPCR testing process at WSU Prosser IAREC. In 2021, our average sample turn-around time was four days. We have also developed preliminary cost-sheets (price valid as of 2021), which have allowed us to determine actual cost per test reaction. This information can be used to assist both private and public laboratories in determining the feasibility of integrating this test into their offered services. There are several private companies which can currently offer the testing service, however, at the actual cost per unit to meet appropriate profit margins is still higher than what most growers are willing to pay; these costs can only go down if sufficient volume of samples are received to help offset the fixed costs of PCR processing.

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PRINCIPAL INVESTIGATOR / COOPERATORS:

PI Name:	Michelle M. Moyer		
Organization	Washington State University		
Address	24106 North Bunn Rd., Prosser, WA 99350		
Telephone	509-786-9234		
Email	michelle.moyer@wsu.edu		
Cooperator Name:	Walt Mahaffee	**Cooperator Name:**	David Gadoury
Organization	USDA-ARS	Organization	Cornell University
Description of participation:	Co-PI on USDA-SCRI FRAME. Assist in resistance testing, and west-coast UV testing across multiple sites.	Description of participation:	UV-C technology originator. Provided base funding for equipment build. Will assist in tech questions and machine maintenance and operation.

OBJECTIVES & EXPERIMENTS CONDUCTED TO MEET STATED OBJECTIVES:

Challenges associated with reducing on-farm chemical inputs, along with consumer demands for transparency in the food production pathway, will change how modern agriculture works. Achieving high-quality food production with the bare minimum pesticide input needed to achieve a quality crop will likely require an increase in inputs in other aspects of production (e.g., labor, alternative plant genetic selection). Fortunately, with the advancements in understanding pest and pathogen biology, as well as past research on alternative management strategies for plant pests and diseases, there are viable technologies coming to the market that can help with the two aforementioned challenges: (1) Technologies that use little to no actual chemicals, reducing potential residues on fresh and processed produce; and (2) Technologies that affect the very basic aspects of pest and disease biology, thus having a significantly reduced risk (to essentially no-risk) for pesticide resistance development. **UV-C light for grape powdery mildew control** is one technology that is rapidly approaching broad commercialization. In Florida, several commercial strawberry producers have adopted this tool for powdery mildew management, and engineers have created autonomous solutions for UV-C delivery that were evaluated on the east coast in 2020 and expanded to evaluation in private operations on the west coast in 2022. However, with every new technology comes new challenges in optimizing its use and adoption. The benefit of differing management approaches that target fundamental biological responses, is the cross-over role they can play in the efforts to extend the functional life of our available chemical approaches. Combined, cultural practices, alternative approaches (e.g., UV-C), and chemical approaches, can be combined for effective disease management.

Finally, **we can no longer rely on just rotating chemistries (FRAC groups) as our primary means of fungicide resistance management.** We must take a holistic approach that includes understanding the local extent of fungicide resistance prior to product application, as well as alternative management solutions that allow us to "break the cycle" of resistance. Technology has kept pace with these requirements, and we now have tools for rapid detection of certain types of fungicide resistance in grape powdery mildew. We need to adopt the practice of routine testing for fungicide resistance throughout the growing season to adjust spray programs accordingly.

The following two objectives in this project address these core principles of informed pest management, through the evaluation of non-chemical approaches as an alternative or supplemental to standard disease management programs, and the development and implementation of rapid resistance testing tools to take the guess work out of fungicide program alteration in response to a disease control failure.

Objective 1 – Evaluation of UV-C light for integration into powdery mildew management programs in Washington State (Years 1-3).

Background. UV-C light is a short wavelength light between 100-280 nm that is damaging to cells. This highly energetic wavelength light does not reach Earth's surface due to ozone in our stratosphere (Stapleton 1992). Much of the original work on the use of UV-C light in agricultural settings has been done on *Botrytis* control in strawberries. While effective at killing the pathogen, original research trials also resulted in phytotoxic responses. That changed with the discovery that lower doses of UV-C could be fungicidal if applied at night, when UV-C treatments were followed by 4 hours of darkness (Janisiewicz et al. 2016). Since powdery mildew grows on the surface of the plant, it has evolved a way to repair damage done to its DNA as a result of UV light exposure. That repair is triggered by blue light. But at night, blue light is not available, and thus, if UV damage occurs it cannot be repaired. Since this discovery, a proliferation of commercial and custom-built "UV light" applicators have emerged across Northern Europe and the USA, with a focus on strawberry and hop mildew management.

While this technology has seen more wide-spread use in other crops, only a few preliminary trials have been conducted in grapes – much of this is simply due to the stage of technology development. Since the original proposal, additional commercial interest in this technology has a resulted in the development of a fully autonomous unit, which is improving the adoptability of this technology by allowing continual application overnight without the need for an extensive labor pool. We were contacted by Cornell University to lead field trials due to our expertise in whole-program design and efficacy evaluations. Washington State offered a unique environment that results in genuinely low disease pressure (relative to other regions), making it a likely good candidate for successful adoption of this tool. In addition, our standard canopy architecture is different than that of where this technology was vetted; canopies in eastern Washington tend to be larger to compensate for our climate conditions. Wine grape canopies in New York tend to be heavily manipulated to a strict VSP. Given UV-C applications will function like contact fungicides, these viticulture management differences could play a significant role in how such a tool can be regionally adopted. In other words, we need to better understand application timing, frequency, and potential limitations to optimize the use of this technology in Washington winegrape vineyards.

Approach. Our hypothesis is that UV-C will serve as a functional alternative for early season contact pesticides, and it will positively improve early-season disease management due to lack of environmental restrictions on application (e.g., rain, wind, temperature). Supplemented by existing funding, **we built a functioning pull-behind "UV-C applicator"** in 2020, and then redesigned the system for a 3-pt hitch in 2021**.** We evaluated UV-C applications at our WSU Prosser IAREC 2-acre *V. vinifera* 'Chardonnay' block (known as the "pathology vineyard"). Two main experiments were conducted – the first looked at the integration into an early-season spray program (*timing trial*), and the second looked at efficacy of a season-long program that only consisted of UV-C treatments (*interval trial*). In all cases, UV-C treatments started 30 minutes after sunset. UV-C dose used was 200 J/m^2. Operational speeds were between 1 and 2 mph, depending on the unit. Note, when it comes to the appropriate application of UV-C, the ultimate factor is dose; our slow application speeds were a function of the research unit we are using. Commercially available units use larger lighting arrays, which allows them to achieve the same dose at a faster application speed. While described in this report, all the UV-C work has either been published, or accepted for publication, with citations to that published (and open-access) work in the **Outreach and Education** section.

Field Experiments

*Design: Field Experiment 1 – Early-season integration with a standard spray program (**timing trial**)*. We evaluated early-season applications (6-inch shoot growth to rachis elongation; replacement of the first 3 to 4 sprays) on 7-day interval, switching to a standard spray program at bloom. In year 2 and 3 we added a twice-weekly UV-C treatment. Treatments were applied in a randomized design, with applications covering half-rows (i.e., 30 + vines), replicated 4 times. Actual application dates (and in the case of fungicide controls, products), are listed in **Table 1**.

Table 1 – Treatment application dates and rates for the **early-season** field evaluation of ultraviolet-C (UV-C; 200 J/m^2) on *Erysiphe necator* management in a *Vitis vinifera* 'Chardonnay' vineyard in Prosser, WA. Fungicides were applied at maximum label rates unless listed. (*Table from McDaniel et al. 2024a)*

Treatments		2020	2021	2022
Fungicide program	**Pre-bloom**	**8 May** (*BBCH 15*) – Microthiol Disperss (5.6 kg/ha) **14 May** (*BBCH 55*) – Microthiol Disperss (4.4 kg/ha) **21 May** (*BBCH 60*) – Vivando + Microthiol Disperss (2.2 kg/ha)	**5 May** (*BBCH 15*) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex **12 May** (*BBCH 19*) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex **19 May** (*BBCH 55*) – Vivando + Complex	**19 May** (*BBCH 15*) - Microthiol Disperss (4.4 kg/ha) + Complex **25 May** (*BBCH 19*) - Microthiol Disperss (4.4 kg/ha) + Complex **1 June** (*BBCH 55*) - Vivando + Complex
	Post-bloom	**4 June** (*BBCH 68*) – Quintec + Cinnerate **18 June** (*BBCH 71*) – Torino + Cinnerate **2 July** (*BBCH 75*) – Gatten + Cinnerate	**1 June** (*BBCH 68*) – Quintec + PureSpray Green (0.25%) **15 June** (*BBCH 71*) – Torino + PureSpray Green (0.25%)	**15 June** (*BBCH 60*) – Quintec + Microthiol Disperss (2.2 kg/ha) + Complex **29 June** (*BBCH 71*) - Torino + PureSpray Green (0.25%) **13 July** (*BBCH 75*) – Aprovia + Complex
Early 2x-wk UV-C	**Pre-bloom**		2x weekly 6 to 27 May	2x weekly 19 May to 9 June

Early 1x-wk UV-C	**Pre-bloom**	1x weekly 7 to 28 May	1x weekly 6 to 27 May	1x weekly 19 May to 13 June
Early unsprayed	**Pre-bloom**	Unsprayed		
Season long unsprayed				

*Design: Field Experiment 2 – Evaluation of UV-C treatment intervals for season long disease control (**interval trial**).* In Year 1, we evaluated a season-long management program using UV-C applied at 7- or 14-day intervals, relative to a standard sprayed control and an unsprayed control. In year 2 and 3, we opted for tighter UV-C intervals of twice a week versus once a week. The experimental design is as described above and listed in **Table 2**.

Table 2. Treatment application dates and rates for the **season-long** field evaluation of ultraviolet-C (UV-C; 200 J/m^2) on *Erysiphe necator* management in a *Vitis vinifera* 'Chardonnay' vineyard in Prosser, WA. UV-C treatments and controls were applied from 10 cm shoot growth (BBCH[a] 15), until 3 weeks post fruit set (BBCH 75). Fungicides were applied at maximum label rates unless listed. (*Table from McDaniel et al. 2024a).*

Treatments	2020	2021	2022
Fungicide program	**8 May** (*BBCH 15*) – Microthiol Disperss (5.6 kg/ha) **14 May** (*BBCH 55*)– Microthiol Disperss (4.4 kg/ha) **21 May** (*BBCH 60*) – Vivando + Microthiol Disperss (2.2 kg/ha) **4 June** (*BBCH 68*) – Quintec + Cinnerate **18 June** (*BBCH 71*) – Torino + Cinnerate **2 July** (*BBCH 75*) – Gatten + Cinnerate	**5 May** (*BBCH 15*) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex **12 May** (*BBCH 19*) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex **19 May** (*BBCH 55*) – Vivando + Complex **1 June** (*BBCH 68*) – Quintec + PureSpray Green (0.25%) **15 June** (*BBCH 71*) – Torino + PureSpray Green (0.25%)	**19 May** (*BBCH 15*) - Microthiol Disperss (4.4 kg/ha) + Complex **25 May** (*BBCH 19*) - Microthiol Disperss (4.4 kg/ha) + Complex **1 June** (*BBCH 55*) - Vivando + Complex **15 June** (*BBCH 60*) – Quintec + Microthiol Disperss (2.2 kg/ha) + Complex **29 June** (*BBCH 71*) - Torino + PureSpray Green (0.25%) **13 July** (*BBCH 75*) – Aprovia + Complex
2x-wk UV-C		2x weekly from 6 May to 24 June	2x weekly from 19 May to 29 July
1x-wk UV-C	1x weekly from 7 May to 9 July	1x weekly from 6 May to 24 June	1x weekly from19 May to 29 July
Season long unsprayed			

Evaluation: Field Experiments – Disease management. We evaluated treatment efficacy based on control of powdery mildew. Powdery mildew incidence and severity was visually rated on 40 leaves per treatment replicate, and 20 clusters per treatment replicate in 2020. We increased cluster observation numbers to 40 clusters per treatment replicate in 2021 and 2022.

To monitor the potential impact of UV-C on the powdery mildew fungus population (e.g., QoI resistance), samples were collected biweekly from treated tissues and untreated control tissues using the cotton swab method (Lowder et al. 2019) beginning from 10-inch shoot growth until harvest. One swab per treatment replicate was collected. The DNA from samples was extracted with a 5% Chelex method (Thiessen et al. 2016) and stored until

processing. The presence of the G143A mutation was measured using a quantitative PCR protocol modified from previous papers (Thiessen et al. 2016, Baudoin et al. 2008).

Evaluation: Field Experiments – Vine Growth, Phenology, and Fruit Quality. There have been studies in ornamental plants that suggest UV-C promotes changes in physiological growth and the timing of flowering (Bridgen 2016). To monitor the physiological responses of the plant to UV-C treatment, we tracked in fruit cluster morphology and cluster size, focusing on treatments in Experiment 2. After two seasons, we saw absolutely no difference on cluster size or return fruitfulness, so did not continue to track that in year 3 (except for fruit quality). In addition, eight clusters per treatment replicate in year 1, and twelve clusters in year 2 and 3 were used for cluster weight determination. From those clusters, 50 berries were randomly selected to measure average berry weight, Brix, titratable acidity, and pH. For further analysis of fruit quality, 30 berries were randomly selected from those same clusters to determine phenolics and tannins using the Adam-Harbertson method (Harbertson et al. 2002; Harbertson et al. 2003).Additional phenolic compounds were evaluated through HPLC analysis in year 3; this data is not presented as we are still in the process of developing protocols that can handle grape juice for the analyses we are interested in doing. Phenolic compounds of interest are stilbenes and flavonols, which can increase in pre-harvest UVB treatments and post-harvest UV-C treatments (Cantos et al 2000; Del-Castillo-Alonso et al. 2020; Keller et al. 2000). Methods will be adapted from Keller et al. (2000).

Greenhouse Experiments

Greenhouse Experiments – Powdery Mildew. We conducted a series of greenhouse and growth chamber experiments to directly observe the effect of UV-C on powdery mildew growth and development (*see McDaniel et al. 2024a* for more details). For these experiments, we custom-build a laboratory-scale UV-C machine on a conveyor belt to assist with rapid treatment. In these experiments, detached leaves were inoculated with *Erysiphe necator* conidia. These colonies were then allowed to develop for a set period (24, 72, or 144 hours) before being exposed to UV-C at 100 or 200 J/m^2. Colonies were also treated with a 2% oil solution at those same times as a positive control. The colonies were then allowed to continue to develop until they were 8 or 13 days old (from the time of initial inoculation), when they were rated to see if they survived the UV-C treatment. This information will help determine the primary role of UV-C in vineyard management – whether it should be considered a curative (early pathogen establishment) or eradicant (established pathogen) management tool.

Greenhouse Experiments – Insects. The limited studies on UV-C for insect management show variable efficacy at different insect developmental stages (Beard 1972). Preliminary data from the east coast on spider mites (Gadoury, *personal communication*) indicates that while UV-C does not kill adults, it reduces their ability to lay eggs, resulting in a second-generation population crash. Currently, no one has investigated whether UV-C could be a viable option for grape mealybug (*Psuedococcus maritimus*) management, killing crawlers without the requirement of feeding on treated tissue. We directly measured the effects of UV-C on grape mealybug crawlers, using a similar detached leaf assay as described above

for powdery mildew. We exposed mated *P. maritimus* adult females in a lab study to UV-C light doses of 100, 200, 500 and 1,000 J/m^2. UV-C was applied during darkness and was followed by an additional 4 hr dark period post-treatment to enhance possible suppressive effects of UV-C. Positive (2% horticultural oil) and negative (untreated) controls were included. Adult female *P. maritimus* oviposition was observed two weeks after treatment and hatching of eggs was enumerated four weeks after treatment. The UV-C dose gradient was designed to allow us to determine the lower-dose threshold for effective treatment (i.e., if the same dose used for early-season mildew control is also effective against crawlers, or if lower doses can be used, which would allow for more rapid applications).

Objective 2 – Establishment of local laboratory for fungicide resistance testing (Year 1). As a part of the national FRAME SCRI Project (framenetworks.wsu.edu), we had access to a rapid qPCR test to determine the presence or absence of the G143A mutation in *Erysiphe necator* that confers resistance to FRAC 11 fungicides. Most of the testing costs were covered by the FRAME grant, and the funds requested here were to supplement additional testing costs that allowed us to process more Washington-based samples. Testing funded by this proposal was prioritized in the following way: (1) In-season Washington-based grower requests for FRAC 11 fungicide resistance; (2) WSU-based research evaluations; and (3) overflow from other FRAME-associated testing sites. Testing (up to 500 samples annually) was free to participating Washington growers. We provided growers with packets containing sterile cotton swabs, shipping instructions and rapid glove sampling instructions which were developed and tested by Washington growers during the 2019 growing season. In 2020, packet availability was publicized through extension newsletters (i.e., VEEN), social media posts, but physical general distribution was severely limited due to COVID-19 travel and interaction restrictions. In 2021 and 2022, packets were available for pick-up through WSU Prosser IAREC, and handed out at various meetings. After samples were received, powdery mildew DNA was extracted with a 5% Chelex method (Thiessen et al. 2016) and G143A presence (resistance mutation) was determined using a quantitative PCR (qPCR) protocol modified from previous papers (Thiessen et al. 2016, Baudoin et al. 2008). Extracted DNA has been cataloged and stored at -80 ºC until additional tools become available for FRAC 3, FRAC 7, and FRAC 13 tests. Results were sent to participating growers along with a decision tree developed by FRAME for interpreting testing results. Cost for sampling and testing were tracked in batches of 100 samples as supplies for testing cannot be purchased on an individual basis and key components, such as the qPCR probe, have a minimum purchase volume.

In addition to providing this temporary testing service, we used the process to develop baseline budgets, equipment lists, and establishment protocols. This information will be compiled and made available for public and private diagnostic labs, so they can better prepare for incorporation of this test into their existing testing services. We do not intend to establish a long-term fungicide-resistance only testing service through this project, but rather, develop the information infrastructure necessary for existing entities to more easily adopt the tests.

SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS AND RESULTS BY OBJECTIVE:

Objective 1 – Evaluation of UV-C light for integration into powdery mildew management

programs in Washington State. Disease pressure was very low in 2020 and 2021 at WSU Prosser IAREC, but was average in 2022. Low disease pressure can make the separation of treatment responses very challenging. Rather than compare end-of-season disease ratings, we used a technique called "Area Under the Disease Progress Curve" (AUDPC; Simki and Piepho, 2012), which not only factors in final disease severity, but also duration of time visible disease was present. This method is a good tool for comparing season-long programs, in order to discern if a certain program loses management efficacy earlier than another.

Field Experiment 1 - Early-season integration with a standard spray program ***(timing trial).*** As a reminder, the basic design of this experiment was to replace the earliest fungicide treatments of the season with either UV-C, or no treatment, and compare that to a standard early-season program. Once the vines reached bloom, all treatments (including the early-season unsprayed) converted to the standard spray program as previously described in **Table 1.**

In 2020 and 2021, there was no real treatment effect, other than the season-long unsprayed control having more foliar disease than vines that transitioned to a fungicide program at bloom (**Fig. 1, A-C**). In 2020, end-of-season foliar disease severity was moderate (ranging from 15-22% severity). Fruit disease severity was very low (less than 1% of the cluster surface infected). In 2021, end-of-season foliar severity was even less than 2020, ranging from 8.2 to 12.4%. As a reminder, there are two types of unsprayed controls in this experiment – one that was unsprayed until bloom then transitioned to sprayed for the rest of the season, and one that was unsprayed season-long. This control was in place to emphasize that most of the season-long disease control in a program happens during the bloom period; that if "weaker" treatments were to be applied or tested, the lowest risk to a grower would be to integrate them in for the earlier sprays. Alternatively, one could think of it in this fashion – if any sprays were to be skipped, it could be those pre-bloom sprays. We expected to potentially see a lack-of-differences in those experiments receiving some form of treatment starting at bloom. This is timing is the critical window for fruit disease management. It is also a key time in foliar disease epidemics – if disease is kept low through this bloom period it significantly reduces population build up for the rest of the season, particularly in years characterized by hot, dry summers. In both years of this study, early-season conditions (cold nights in 2020 and 2021; heat dome and lack of any in-season precipitation in 2021) were not conducive for initial disease establishment; the heat dome especially in 2021 likely killed off any nascent mildew colonies.

In 2022, however, we had environmental conditions that were conducive for disease development, and this was readily visible in disease control responses **(Fig. 1 D&E).** Interestingly, the trends in treatment responses (i.e., which treatments did well, and which did not) where the same as 2020 and 2021, with the only difference of there being sufficient disease for statistical separation. Once again, the season-long untreated control had the highest level of foliar and fruit disease, followed by the early season untreated control, early season weekly UV-C, early season 2x weekly UV-C, and finally, the full-season sprayed control. Note, though, that the early-season UC-C treatments had end of season cluster disease severity that was statistically the same as the full-season fungicide spray.

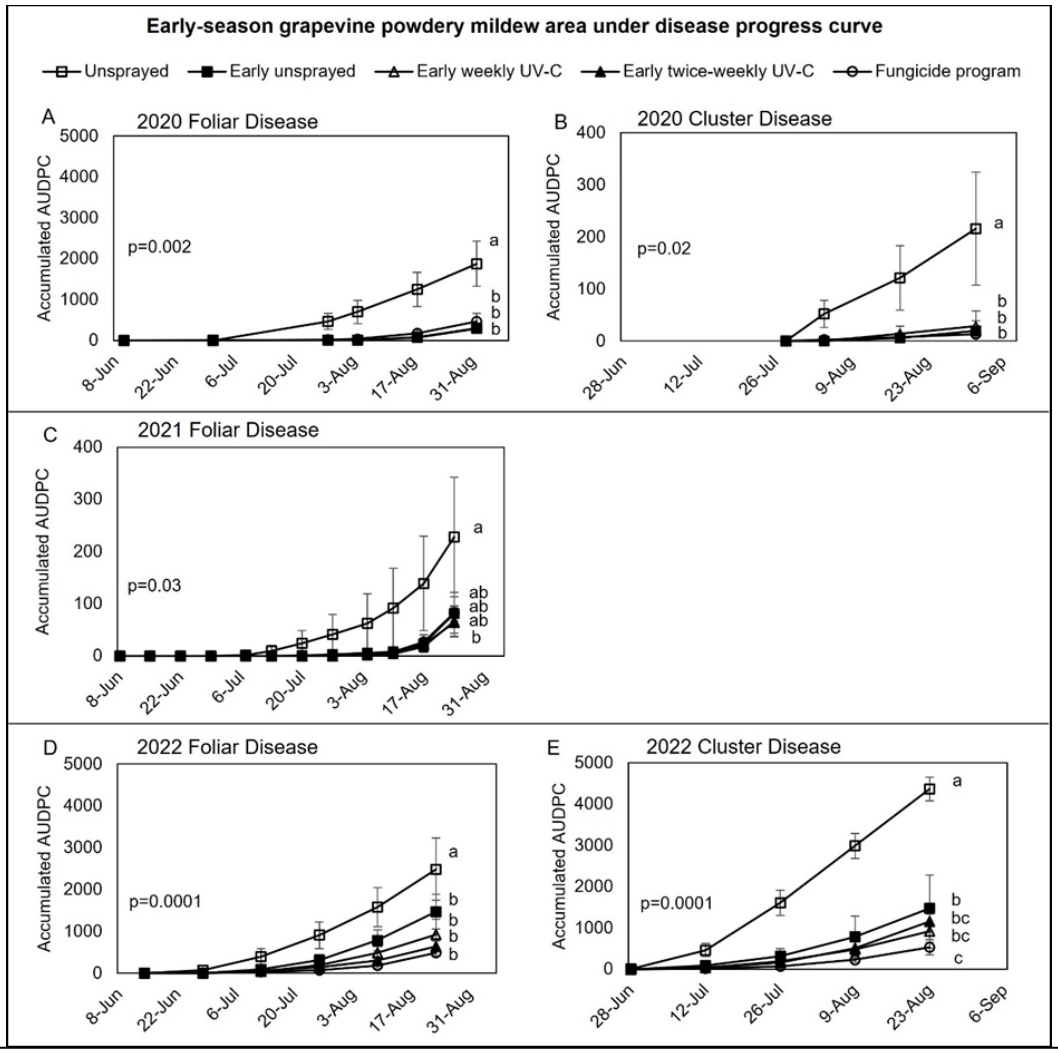

Figure 1. Foliar and cluster disease severity ratings represented as accumulated area under disease progress curve (AUDPC) for early season disease management treatments including an unsprayed controls (early season and all-season), a full fungicide program, or weekly or twice weekly UV-C treatments. (A) 2020 foliar disease AUDPC, (B) 2020 cluster disease AUDPC, (C) 2021 foliar disease AUDPC, (D) 2022 foliar disease AUDPC, and (E) cluster disease AUDPC. There is no data for 2021 cluster disease AUDPC as there was no recorded disease on the fruit in the field. Error bars are standard error (n = 4). *From: McDaniel et al. (2024a).*

The use of early-season UV-C did not impact yield, cluster weight, berry weight, or total number of berries per cluster in any years of the study. The only real difference seen was un the untreated fruit in 2022, whereas diseased fruit was so diseased that the fruit was unmarketable. Additional details on fruit quality can be found in McDaniel et al. (2024a).

Field Experiment 2 – Evaluation of UV-C treatment intervals for season long disease control ***(interval trial).*** Larger differences between UV-C treatments and control treatments were noticeable in our season-long treatment approaches. While 2020 and 2021 still provided very low disease, the trend was higher disease in the untreated controls, moderate levels of disease in longer-interval UV-C, less disease in shorter interval UV-C treatments, and the least disease in the fungicide control treatments (**Fig. 2**). In 2020, foliar

disease in the weekly UV-C treatments were statistically the same as the untreated control, but there was no statistical separation of treatment for impact on cluster disease severity (**Fig. 2 A&B).** The typical range in foliar disease severity was 24.6% in the grower control to 43.6% in the unsprayed control. On fruit, typical disease severity ranged from 0.3% (grower control) to 4.8% (14-day UV-C; data not shown). We saw no statistical separation in foliar disease severity in 2021, and there was no disease on fruit across all treatments in that year. The lowest observed disease was in the 2x wk UV-C treatment (5.9%), and highest observed disease was in the unsprayed control (16.5%). In 2022, treatments were statistically separated, where the untreated control had the highest foliar disease, the UV-C treatments had intermediate levels of foliar disease, and the fungicide treatment hand the lowest foliar disease. This same trend was seen on fruit, but where there was greater resolution between UV-C treatments, where more frequent UV-C applications had lower cluster disease severity (**Fig. 2 D&E).**

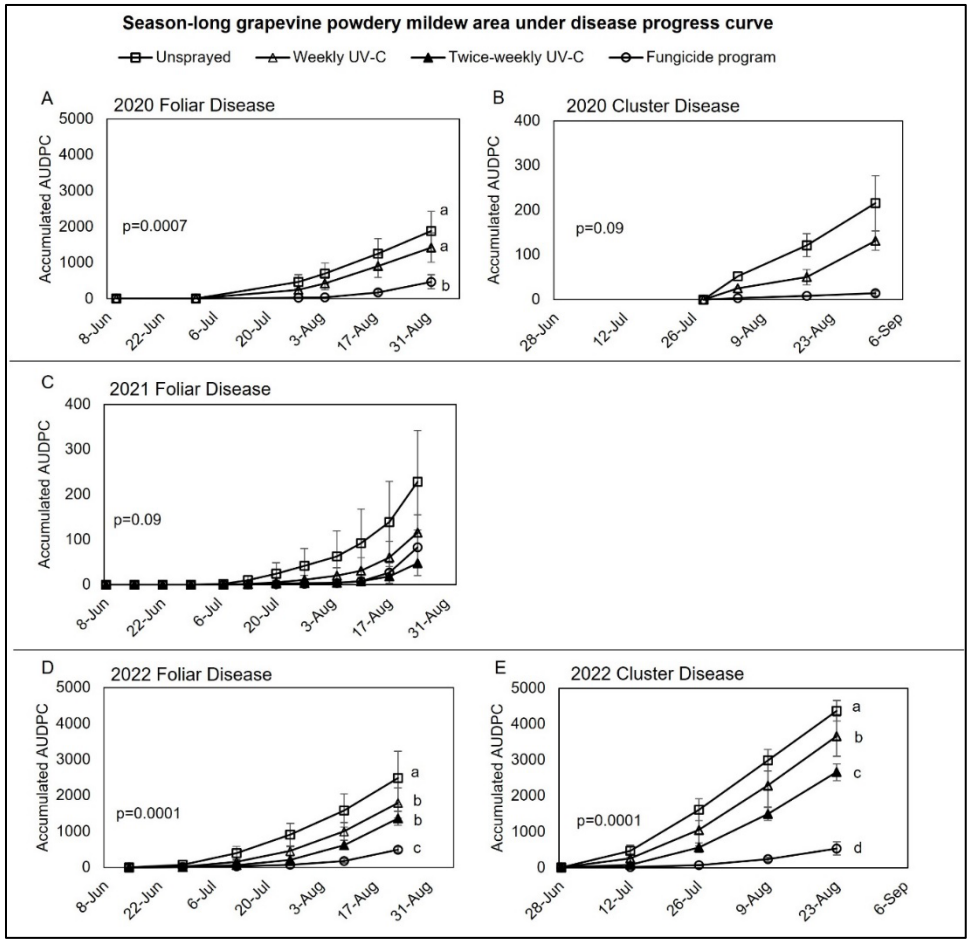

Figure 2. Foliar and cluster disease severity ratings represented as accumulated area under disease progress curve (AUDPC) for season-long treatments including an unsprayed control, a full fungicide program, or weekly or twice weekly UV-C treatments. (A) 2020 foliar disease, (B) 2020 cluster disease, (C) 2021 foliar disease, (D) 2022 foliar disease, and (E) cluster disease. There is no data for 2021 cluster disease AUDPC as there was no recorded disease on the fruit in the field. Error bars are standard error (n = 4). *From: McDaniel et al. (2024a).*

Season-long UV-C had no impact on fruit quality in 2020 and 2021. In 2022, the season-long fungicide program and twice-weekly UV-C had higher average yield per vine, cluster weight, and more berries per cluster than the unsprayed treatment (due to high levels of disease in the unsprayed control). Fruit quality was also starkly different, where the fungicide treated and twice-weekly UV-C had a lower juice pH and Brix compared to the unsprayed control – heavily infected fruit was beginning to dehydrate.

Total phenolics and tannins of berry skins were inconsistently affected by season-long UV-C treatments in all three years. In 2020, total phenolics and tannins were increased with weekly UV-C treatments compared to the fungicide program and unsprayed control. In 2021, the twice-weekly UV-C and fungicide program resulted in berries with higher phenolic and tannin concentration compared to the unsprayed control. In 2022, there were no differences between all treatments for both total phenolics and tannins in Washington. This tells us that vintage and disease control effects are stronger influencers on fruit quality then the actual use of UV-C light.

UV-C and E. necator *Monitoring.* Using UV-C light did not result in a change in detection frequency of the G143A mutation in *Erysiphe necator* (*data not show*). This is likely due to the nature of our smaller plots size and the potential movement of *E. necator* between plots. But to show the difference in disease pressure between the three years, below is a graph (**Fig. 3**) of when we had positive detections of *E. necator* using the glove-swab method. Note in 2022, we were able to detect *E. necator* even on our first monitoring pass. We didn't see *E. necator* until our second pass in 2020, and in 2021, it took until our last pass before 100% of our samples came back positive for *E. necator.*

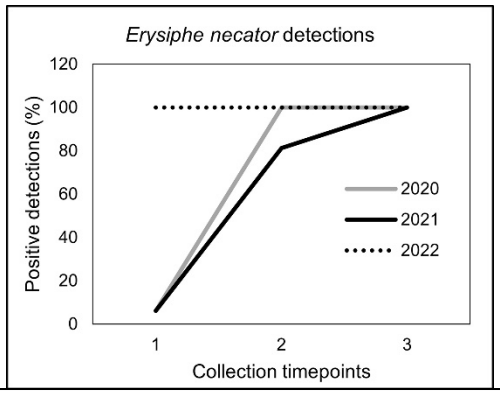

Figure 3. *Erysiphe necator* DNA detections throughout the season. Collection timepoint 1 was on 10 June 2020 (BBCH 71), 10 June 2021 (BBCH 71), and 14 June 2022 (BBCH 65) falling before visual powdery mildew was seen. Collection timepoint 2 was on 8 July 2020 (BBCH 81), 22 July 2021 (BBCH 79), and 11 July 2022 (BBCH 79) when disease was first recorded. Collection timepoint 3 was on 19 August 2020 (BBCH 85), 19 August 2021 (BBCH 85), and 18 August 2021 (BBCH 83) when berries were softening or almost ripe. *E. necator* glove swab samples were collected with the methods described in Lowder et al. (2023).

Greenhouse Experiments

Greenhouse Experiments – Powdery Mildew. The effects of UV-C light on developing mildew colonies does appear to be dose-dependent (i.e., stronger effect at higher doses), and appears to be more effective when colonies are around 3 days old, with milder efficacy at 6 days old, and reduced efficacy when the colonies are just starting (i.e., 1 day old) (**Fig. 4A**). This means that UV-C light has some curative properties, and can be used to help kill young, developing powdery mildew colonies. The fact that UV-C is most effective on younger, but not brand-new, colonies fits well with the hypothesis that UV-C is damaging the fungus' DNA – there needs to be enough colony surface area (hyphae / mycelium) exposed to UV-C for there to be an effect.

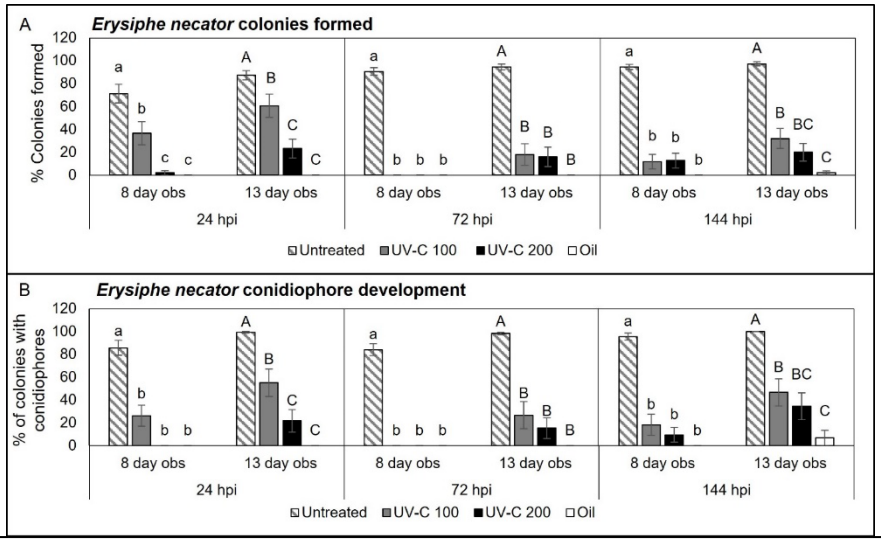

Figure 4. Ultraviolet-C light (UV-C) effects on laboratory-grown *Erysiphe necator* after UV-C and oil treatments at 24, 72, and 144 hpi. Untreated = no UV-C or oil; UV-C 100 = UV-C dose of 100 J/m^2; UV-C 200 = UV-C dose of 200 J/m^2; oil = 2% v/v horticultural oil. (A) Percent of colonies visible at 8 or 13 days post treatment. (B) Of the colonies that did form after treatment, the percent of colonies that developed conidiophores. Error bars are standard error (n = 10). Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference. *From: McDaniel et al. (2024a).*

Greenhouse Experiments – Insects. Regardless of UV-C dose, adult female grape mealybugs were not affected by UV-C exposure. They were all able to successfully lay eggs. When we looked at the impacts of UV-C on nymphs we noticed an interesting trend – while UV-C did not perform as good as the positive control (i.e., treatment with 2% oil), when compared to each other only, we saw that there was a positive trend of increased nymph mortality with increasing UV-C dose **(Fig. 5)**.

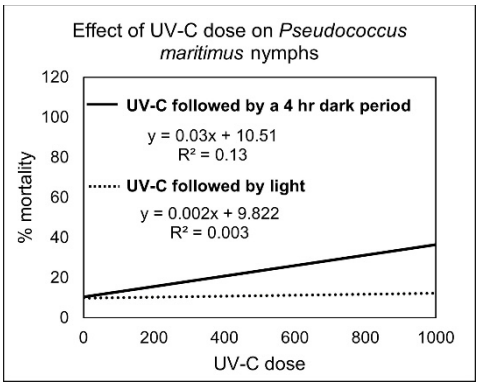

Figure 5. Relationship between dose of UV-C treatment and nymph mortality in *Pseudococcus maritimus*. UV-C was applied to first instar nymphs in a laboratory setting either with a 4 hr dark period following treatment, or without a dark period following treatment. *From: McDaniel et al. (2024b).*

While our sample sizes were small in this study (nymph survival is difficult in growth chamber conditions), we did find this encouraging. It was even more encouraging because this was slightly increased nymph mortality after only 1 UV-C treatments; in the field, the nymphs would likely be exposed to repeated treatments, which could enhance the efficacy of UV-C in a comprehensive management program (i.e., coupled with mating disruption). While the goal in the development and deployment of any new insect management strategy is often complete control, sublethal effects can play an important role in IPM programs.

Objective 2 – Establishment of local laboratory for fungicide resistance testing (Year 1 only). In summer 2020, despite COVID restrictions, we were able to process 74 grower-submitted powdery mildew samples and 180 research samples for FRAC 11 fungicide resistance testing, as well as complete a series of test optimization procedures. All early grower-submitted samples (fruit set and earlier) were negative for powdery mildew. This was expected, as many of our early-season grower samples were submitted as self-tests, to see if the sampling procedures could detect powdery mildew before field scouts could visually detect the disease. The rates of positive powdery mildew samples increased between post-fruit set and harvest, as well as the rate of FRAC 11 resistance. At post-fruit set, of the samples with powdery mildew, 75% were still FRAC 11 sensitive. By bunch closure, that changed to 57% sensitive, and by véraison, all submitted samples with powdery mildew were resistant to FRAC 11 fungicides. This trend, of increasing resistance as the season progresses, is commonly seen across our sampling regions in the United States. In our research samples, we saw a similar trend, but a higher percentage of early-season samples with powdery mildew (likely due to more intensive sampling within a block and choosing blocks with extensive histories of powdery mildew), but that number of powdery mildew positive samples increased as the season progressed. Most of the bloom and earlier samples were FRAC 11 sensitive, but that quickly transitioned after bloom to where most of our samples were either mixed (contained both FRAC 11 sensitive and resistant individuals), or fully FRAC 11 resistant.

In 2021, we processed 260 grower submitted samples, and over 500 research samples for FRAC 11 fungicide resistance. Of the submit samples, only 5.4% came back as either resistant, or mixed (sensitive + resistant). These resistant samples were submitted in early June and again

in early July and were typically groups of samples from the same or nearby vineyards. Interestingly, 85% of the total submitted samples *had no mildew whatsoever.* We attribute this to the fact that many of those who were using the glove swab technique for FRAC 11 resistance testing also realized that this technique could be used for powdery mildew monitoring. This means that samples were being collected for presence / absence sampling for powdery mildew (with the added benefit that if mildew was present, we could then test for resistance status). This approach to glove-swabbing could provide a much faster decision support tool (relative to spore trapping) for determining whether to continue with spray programs after the period of ontogenic resistance has set in and provide additional value beyond simple resistance testing.

Establishment of this lab in 2020 and its continued use in 2021 was especially helpful, given the restrictions our partnering groups had due to COVID-19 (i.e., the USDA-ARS laboratory was severely limited in processing for several months due to access restrictions to laboratory spaces). It also meant that result turn-around time was much faster – by 2021, our average time of sample receipt to result to grower was 4 days.

After developing and evaluating testing protocols and procedures, we were able to develop a preliminary budget / start-up cost estimate for laboratories wishing to adopt the ability to test for FRAC 11 fungicide resistance. While our start-up budgets due not include the purchasing and depreciation of a qualitative PCR (qPCR) machine, we were able to determine the what the costs per test would be (includes fixed reagent costs, and variable disposable products and labor costs), and at what charged price and total processed reactions a testing facility would be profitable. This preliminary budget is shown below in **Table 3**. If only 200 samples are processed annually, a processing fee of $60 is needed for FRAC 11 fungicide testing to be profitable for the business entity. If the processing fee were reduced to $50, then a minimum of 300 samples would be needed to be profitable.

Table 3 – Economics of FRAC 11 resistance testing. Cost per reaction (RXN) include fixed equipment costs (excl. qPCR machine), fixed reagent costs, and variable supply and labor costs.

No. Of RXNs		**50**	**100**	**150**	**200**	**250**	**300**	**350**	**400**
Cost per RXN		$151.20	$88.70	$67.87	$57.46	$51.21	$47.04	$44.06	$41.83
Profit / loss per RXN									
RXN Fee	**$40.00**	-$111.20	-$48.70	-$27.87	-$17.46	-$11.21	-$7.04	-$4.06	-$1.83
RXN Fee	**$50.00**	-$101.20	-$38.70	-$17.87	-$7.46	-$1.21	**$2.96**	**$5.94**	**$8.17**
RXN Fee	**$60.00**	-$91.20	-$28.70	-$7.87	**$2.54**	**$8.79**	**$12.96**	**$15.94**	**$18.17**
Total Profit / Loss									
RXN Fee	**$40.00**	-$5,559.84	-$4,870.28	-$4,180.72	-$3,491.16	-$2,801.60	-$2,112.04	-$1,422.47	-$732.91
RXN Fee	**$50.00**	-$5,059.84	-$3,870.28	-$2,680.72	-$1,491.16	-$301.60	**$887.96**	**$2,077.53**	**$3,267.09**
RXN Fee	**$60.00**	-$4,559.84	-$2,870.28	-$1,180.72	**$508.84**	**$2,198.40**	**$3,887.96**	**$5,577.53**	**$7,267.09**

OUTREACH AND EDUCATION EFFORTS - PRESENTATIONS OF RESEARCH:

All of the research presented in this report has been either formally published, or presented, as highlighted below. In addition, much of our Extension efforts on fungicide resistance testing were done through the FRAME network platform (framenetworks.wsu.edu). The program

values information through a variety of formats, but we were trying to be careful with sharing UV-C results until we had a better understanding of its potential and drawbacks in our climate.

Research Papers (open-access)

- McDaniel, A.L. , D. Gadoury and M.M. Moyer. 2024. Effects of germicidal ultraviolet-C light on grape mealybug (*Pseudococcus maritimus*). Crop Protec. 178:106584. DOI: 10.1016/j.cropro.2024.106584
- McDaniel, A.L., M. Mireles, D. Gadoury, T. Collins and M.M. Moyer. 2024. Effects of ultraviolet-C light on grapevine powdery mildew and fruit quality in *Vitis vinifera* 'Chardonnay'. Am. J. Enol. Vitic. *(In press at time of report)*
- Lowder, S.R., T. M. Neill, A.B. Peetz, T.M. Miles, M.M. Moyer, C. Oliver, I. Stergiopoulos, S. Ding, and W. Mahaffee. 2023. A Rapid Glove-Based Inoculum Sampling Technique to Monitor *Erysiphe necator* in Commercial Vineyards. Plant Dis. 107: 3096-3105. DOI: 10.1094/PDIS-02-23-0216-RE
- McDaniel, A., M. Mireles, D. Gadoury, and M.M. Moyer. 2023. Managing Grapevine Powdery Mildew with Ultraviolet-C Light in Washington State. 22nd International Meeting of Viticulture G*i*ESCO. 17-21 July, 2023. Ithaca, NY, USA. https://ives-openscience.eu/34212/
- McDaniel, A.L, L. Khot, and M.M. Moyer. Alternative Disease Management Approaches: Best Timed Before the "Critical Window" for Erysiphe necator Management in Eastern Washington. *Proceedings of the 9th International Workshop on Grapevine Downy and Powdery Mildew*, 20-22 Jul 2022. Cremona, Italy. https://doi.org/10.1051/bioconf/20225004004
- Oliver, C.L. and M.M. Moyer. 2022. Influence of Sustainability Programs on Fungicide Stewardship Practices in Pacific Northwest United States Vineyards. *Proceedings of the 9th International Workshop on Grapevine Downy and Powdery Mildew*, 20-22 Jul 2022. Cremona, Italy. https://doi.org/10.1051/bioconf/20225003012

Posters:

- McDaniel, A., M. Mireles, and **M. Moyer.** 6 Feb 2023. "Ultraviolet-C Radiation in Grapevine Powdery Mildew Management." Poster. 2023 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 3rd Graduate Student*).**
- McDaniel, A. and **M. Moyer.** 7 Feb 2022. "Illuminating Alternative Powdery Mildew Management." Poster. 2022 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 3rd Graduate Student*).**
- Oliver, C. and **M. Moyer.** 8 Feb 2022. "FRAME Network Research Update." Poster. 2022 Washington WineVit Conference, Kennewick, WA, USA.
- Oliver, C. and **M. Moyer.** 18 Nov 2021. "FRAME Network Research Update." Poster. 2021 Washington State Grape Society Annual Meeting, Grandview, WA, USA.
- McDaniel, A. and **M. Moyer.** 18 Nov 2021. "Illuminating Alternative Powdery Mildew Management." Poster. 2021 Washington State Grape Society Annual Meeting, Grandview, WA, USA.

- McDaniel, A. and M.M. Moyer. 17 Mar 2021. "The Brighter Side of Alternative Pest Management." Poster. WineVit2021 (Formerly Washington Winegrowers Association Annual Meeting). Virtually presented.
- Oliver, C. and M.M. Moyer. 17 Mar 2021. "FRAME Network Research Update." Poster. WineVit2021 (Formerly Washington Winegrowers Association Annual Meeting). Virtually presented.

Presentations:

- "A Pre-Season Checklist for Powdery Mildew Management." 30 Mar 2023. LIVE Annual Grower Meeting. Chehalem, OR. (M. Moyer)
- "Grape Powdery Mildew: As Predictable as the Weather!" 14 Feb 2023. Growers Supply Annual Horticulture Show, Oliver, BC, Canada. Invited presentation (ZOOM pre-recorded with live Q&A) (M. Moyer)
- "Cool vs. Warm Years: Disease and Pest Management." 8 Feb 2023. WineVit2023 Trade Show and Convention. Kennewick, WA. (M. Moyer)
- "Illuminating Ultraviolet-C Light for Grapevine Powdery Mildew Management". 22 Jun 2022. 73rd American Society for Enology and Viticulture National Conference, San Diego, California USA. (M. McDaniel)
- "Grape Powdery Mildew: Understanding the Plant, Pathogen and People for Better Management." 4 May 2022. Department of Viticulture and Enology, Fresno State Lecture Series. (M. Moyer)
- "Illuminating Alternative Grapevine Powdery Mildew Management". 8 Apr 2022. Honoring Undergraduate and Graduate Scholars Symposium, Yakima, WA. **2nd Place Graduate Student – Oral Presentation**. (A. McDaniel)
- Grape Disease Management: Best Practices for Disease and Fungicide Resistance Management." 24 Feb 2022. University of Wisconsin and University of Minnesota Grape Webinar Series. Virtual. (Moyer)Grape Powdery Mildew and Wine Quality." 15 Feb 2022. Oregon Wine Symposium. Virtual (Pre-recorded + "live" panel discussion) (M. Moyer)
- Powdery Mildew Management: Fighting the Resistance." 2 Feb 2022. VinCo (Colorado). Virtual (ZOOM, Live). (M. Moyer)
- "Powdery Mildew Biology and Control in Grapes." October 2021. California Association of Pest Control Advisors Conference and Agri-Expo. (M. Moyer)
- "New Scouting and Sampling Techniques for Grape Powdery Mildew and Fungicide Resistance." 29 Jul 2021. WA State Viticulture Field Day 2021. Prosser, WA USA. (M. Moyer)
- Lake Chelan Grower Technical Group Meeting – A Regional Viticulture Discussion. 11 Aug 2021. Manson, WA. (M. Moyer)
- "New Scouting and Sampling Techniques for Grape Powdery Mildew and Fungicide Resistance." 29 Jul 2021. Washington State Viticulture Field Day 2021. Prosser, WA. (M. Moyer)
- "Fungicide Resistance Update and Alternative Powdery Mildew Management." 27 May 2021. WAVE Meeting. Webinar. (M. Moyer)
- "Grape Disease Management – Powdery Mildew & Botrytis Bunch Rot." 9 Jun 2020. Wine Island Growers Association. ZOOM Webinar. (M. Moyer)

Websites:

- All of our fungicide resistance outreach efforts are routinely posted on the FRAME Networks website: https://framenetworks.wsu.edu/grower-information/. This also includes a new interactive dashboard for real-time observation of national testing results.
- We have developed a UV-C Informational Website, which includes technical sheets for building a UV-C array: https://wine.wsu.edu/2021/11/11/grapevine-powdery-mildew-UV-C-information

Trade Publication Interviews / Mentions:

- Good Fruit Grower – *Shinning the light on collaboration.* 3 Nov 2021
- "Wine Minute: How to Address Powdery Mildew." 30 July 2021. Washington Ag Network Radio Show and Webcast. https://www.pnwag.net/2021/07/30/wine-minute-how-to-address-powdery-mildew/
- "Wine Minute: Alternatives When Looking At Mildew Control." 16 July 2021. Washington Ag Network Radio Show and Webcast. https://www.pnwag.net/2021/07/16/wine-minute-alternatives-when-looking-at-mildew-control/
- "Wine Minute: The Latest On Fungicide Resistant Powdery Mildew." 11 June 2021. Washington Ag Network Radio Show and Webcast. https://www.pnwag.net/2021/06/11/wine-minute-the-latest-on-fungicide-resistant-powdery-mildew/

RESEARCH SUCCESS STATEMENTS:

This research project tackled to major factors in sustainable powdery mildew management in wine grapes: access to non-chemical or alternative disease management approaches and enhancing fungicide stewardship by building a better testing infrastructure in order to identify fungicide resistance development before widespread disease control failure. We saw that newer technology is not a silver bullet for disease but could become an additional tool in our ever-evolving tool kit for disease management. This new tool can be used in many weather extremes and provides curative effects against powdery mildew and potential sublethal effects against grape mealybug. However, the use of UV-C light does require strict interval adherence, good canopy management, and appropriate timing (i.e., night application). We also saw that these additional tools will become more and more necessary in the future, and resistance to our most used fungicides becomes common in grape diseases. While silver bullets are always the desired solution, options provide us more practical approach for sustainable disease management.

FUNDS STATUS:

We had a requested a 1 year no-cost-extension, due to expenditure challenges that had propagated from slow award set up and COVID restrictions. We have now fully spent those funds.

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Harbertson JF, Picciotto EA, Adams DO. 2003. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic 54:301-306.

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McDaniel, A.L. , D. Gadoury and M.M. Moyer. 2024b. Effects of germicidal ultraviolet-C light on grape mealybug (*Pseudococcus maritimus*). Crop Protec. 178:106584. DOI: 10.1016/j.cropro.2024.106584

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