Unified Grant Management for Viticulture and Enology
FINAL REPORT FORMAT 2021-2022 FUNDING CYCLE
OREGON WINE BOARD (OWB)

1. Summary

Project Title: Grapevine Trunk Diseases (GTDs) in Oregon Vineyards: A Pilot Project on Epidemiology and Management

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Grapevine trunk diseases (GTDs) are found in vineyards worldwide and can be caused by different fungal pathogens. To characterize types of GTDs in Oregon vineyards, and how the different geographical regions have affected the GTD pathogens' prevalence, a survey was conducted in which grapevine trunk samples were collected from 15 and 14 wine grape vineyards in southern and northern Oregon respectively. Fungal species were identified through culture and PCR based methods. GTD pathogens that were identified included Botryosphaeriaceae spp. and *Phaeoacremonium* spp. from 72 and 21% of the surveyed vineyards respectively; *Phaeomoniella chlamydospora*, *Cryptovalsa ampelina, Truncatella angustata, Seimatosporium lichenicola, Hormonema viticola* from 7% of the surveyed vineyards; and *Ilyonectria macrodidyma*, and *Pestaloptiopsis* sp. from 3% of the surveyed vineyards. Pathogens were identified in both regions and in young and mature vineyards. A test was performed to determine if the age and/or location of the vineyards had a significant effect on the presence of GTD pathogens from the complexes: Botryosphaeria dieback, Esca, Eutypa dieback, and Blackfoot. The presence of GTD pathogens from the Esca disease complex was significantly affected by vineyard age (P=0.02) with pathogens being significantly more abundant in older vineyards compared to younger vineyards. However, no significant effect of age and/or location was observed for pathogens from other three complexes.

In order to determine the dynamics of spore release at two climatically distinct regions in Oregon, two Burkard volumetric spore traps (Burkard Scientific, U.K.) were set up in vineyard blocks in Willamette valley in Northern Oregon and Applegate Valley in Southern Oregon. The selection of vineyard block in both regions followed the history of trunk disease in the vineyard as communicated by vineyard manager. In order to understand the spore availability between an old vineyard block and a young block, each spore trap was set up in an old block and a young block in each vineyard in both locations. The spore traps were installed at the beginning of December 2019 and continued until March 2021. Between these timeframe, 475 spore trap samples were collected from each block in Willamette Valley and 477 spore trap samples were collected from each block in Applegate Valley. DNA extraction was performed from individual day samples and followed by qPCR analysis of Botryosphaeriaceae spores trapped in each tape sample. In Willamette Valley, at the younger block, the detection occurred between January and February. At

the older block, it occurred between December and February. Similarly in Applegate Valley, at the younger block, the detection occurred between November and January. However, at this site, no detections occurred in the older block. The spore trap was installed in between the vines in a row at this block and at the end of the rows at other blocks. We suspect that the spore trap location could have contributed to no detection at the older block in Applegate site.

This study provides insight on common GTD pathogens in Oregon vineyards, which is important in implementing management practices to prevent and mitigate disease. Furthermore, detection of GTD pathogens in young vineyards suggests that adoption of preventative disease management practices is crucial for thousands of acres of newly planted vineyards in Oregon.

2. Final report for the 2020-2021 funding cycle

3. Project Title and UGMVE proposal number: Grapevine trunk diseases (GTDs) in Oregon vineyards: A pilot project on epidemiology and management (2020-2338)

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5. Objectives and Experiments Conducted to Meet Stated Objectives

Objectives

The objectives of this research are to identify fungal pathogens associated with grapevine trunk disease in Oregon as well as to understand if the different climatic regions in Oregon influence the spore dispersal of most common trunk disease pathogens in Oregon vineyards. Eventually, we desire to compare different cultural practices while pruning to minimize availability of primary inoculum to cause grapevine trunk disease.

Experiments

Objective 1: Surveying for disease incidence and sample collection. For surveying trunk disease, 15 vineyards were identified in Southern Oregon and 14 vineyards in Northern Oregon. Vineyards were chosen based on a history of trunk disease and vine age. A mixture of old and young vines were surveyed in both the Rogue Valley and Willamette Valley with ages ranging from 2 to more than 40 years. Based on size, vineyard plots were divided into a representative number of strata. At least 5 vines were flagged per stratum and symptoms were recorded. The vines that were flagged were every 5^{th}, 10^{th}, or 20^{th} vine in a row starting from row 1, vine 1 in each stratum. The number of vines flagged depended on the row length. Each flagged vine was examined and any symptoms it exhibited were recorded. Symptoms were varied and included brown and yellow leaves, red blotch, shriveled berries, and stunted growth. Some vines did not exhibit any symptoms (Figure 1).

Each flagged vine was sampled in order to identify pathogens. Using an electric drill (Black+Decker) with 1/8 inch drill bits, three holes were drilled into each flagged vine (Figure 2). One hole was towards the top of the vine, another was near the graft union, and the last hole was

at the bottom of the vine, near soil level. Wood tissue was collected off the drill and put into a labeled tube indicating the stratum number and the position of the hole from which tissue was collected. In order to prevent contamination, drill bits were sterilized with ethanol before and after each use and each drilled hole was filled with petroleum jelly. After collection, samples were stored in the -80^{o}C freezer. DNA was extracted from 100 mg of tissue sample. To identify fungi, primers designed to detect grapevine trunk disease pathogens were used for PCR. In addition, approximately 150 mg of tissue sample collected from each vineyard was sprinkled onto potato dextrose agar (PDA) medium. Fungi grown on PDA were isolated. DNA was extracted and sequenced in order to identify all isolated fungi.

Objective 2: Using spore traps for spore collection. In order to determine what fungal spores are present in the vineyards, as well as the conditions that promote spore release and dispersal, two Burkard spore traps were set up in an old vineyard block and a young vineyard block in the Applegate Valley on December 5, 2019 (Figure 3). Two spore traps were also set up in an old vineyard block and a young vineyard block in Willamette Valley on the same day. The traps remained in the Applegate Valley until March 25, 2021 and in Willamette Valley until March 23, 2021. Samples were collected weekly. Each spore trap contains a drum which rotates 2 mm every hour. Clear tape was added to each drum and an adhesive known as Tangle Trap was brushed onto the tape. Tape was changed from each drum every seven days. The tape was cut into eight pieces with each piece representing a day and the eighth piece representing part of the eighth day. Collected tapes were used to quantify the number of spores trapped every day in each of these vineyard blocks. There were some modifications to the original proposal on quantifying spores trapped on these tapes. Instead of microscope-based quantification, we used qPCR based quantification measures. Recent studies have developed primers and protocols for quantifying spores using molecular tools (Billones-Baaijens et al., 2018). These methods are more robust and less time consuming. We followed these molecular protocols for quantifying spores released by most common pathogens identified from objective 1. DNA was extracted from all samples and then quantified using quantitative polymerase chain reaction (qPCR). The primers available for major trunk disease pathogens were used to quantify the number of spores trapped on each individual spore trap sample. 475 spore tapes were collected from each site in Willamette Valley and 477 spore tapes were collected from each site in Applegate Valley. Samples were quantified using *Botryosphaeriaceae* primers.

Objective 3: Disease management

To evaluate the effectiveness of different practices for controlling diseases in grapevine three treatments including 1) complete removal of vine prunings from the vineyard; 2) leave the prunings in the alleyway then mechanically mow with a flail mower; and 3) leave the prunings in the alleyway, mechanically mow, and spray with Bio-Tam (both wound and soil application) were tested in the older vineyard block where spore samplers were installed for objective 2. Twelve samples from the pruning wounds were collected each time on the following dates: 2/25/21, 3/11/21, 3/24/21, 4/8/21, 4/22/21, and 5/6/21. Eight samples from the vine prunings were collected

each time on the following dates: 3/11/21, 3/24/21, 4/8/21, 4/22/21, and 5/6/21. One vine pruning sample was collected per row in which either treatment 2 or treatment 3 had been applied. DNA was extracted from all samples and then quantified using qPCR. Primers designed for *Trichoderma asperellum* and *Trichoderma gamsii*, the two species of *Trichoderma* used in Bio-Tam, were used to detect these species from the pruning wound and vine prunings samples.

6. Summary of Major Research Accomplishments and Results by Objective:

Objective 1: Grapevine disease symptoms

For surveying trunk diseases, 15 vineyards were identified in the Rogue Valley and 14 vineyards were identified in Willamette Valley. Vineyards were primarily chosen based on a history of trunk disease and vine age. A mixture of old and young vines were surveyed in both regions with ages ranging from 2 to at least 40 years. Within these sites, at least eight different wine grape varieties were included. Each identified vine was flagged, and any symptoms it exhibited were recorded. Between the two regions, 760 vines were examined for GTD symptoms such as dead arm, stunted internodes, striped and necrotic leaves, and shriveled berries. The number of vines examined for symptoms per vineyard ranged from 15 to 50 depending on the size of a block. Some symptoms that may indicate trunk disease included vines with short internodes, dead arms, necrotic leaf margins, tiger striped leaves, shriveled berries, and low vigor (Figure 1). Other vines had necrotic leaf tissues which may also indicate an unhealthy plant. Some vines appeared symptomless.

Fungal species were identified through culture and PCR based methods. GTD pathogens that were identified included Botryosphaeriaceae spp. and *Phaeoacremonium* spp. from 72 and 21% of the surveyed vineyards respectively; *Phaeomoniella chlamydospora, Cryptovalsa ampelina, Truncatella angustata, Seimatosporium lichenicola, Hormonema viticola* from 7% of the surveyed vineyards; and *Ilyonectria macrodidyma,* and *Pestaloptiopsis* sp. from 3% of the surveyed vineyards (Table 1). Pathogens were identified in both regions and in young and mature vineyards. The Mann-Whitney U test was performed to determine if the age and/or location of the vineyards had a significant effect on the presence of GTD pathogens from the complexes: Botryosphaeria dieback, Esca, Eutypa dieback, and Blackfoot. The presence of GTD pathogens from the Esca disease complex was significantly affected by vineyard age (P=0.02) with pathogens being significantly more abundant in older vineyards compared to younger vineyards. However, no significant effect of age and/or location was observed for pathogens from other three complexes.

Figure 1: Severity of trunk disease symptoms observed while surveying at Willamette Valley and Rogue Valley.

Figure 2: Grapevine trunk wood sample collected using Black and Decker 20-volt cordless drill with DEWALT 1/8 in. x 6 in. Black Oxide Twist Drill Bit.

Table 1. Summary of grapevine trunk disease pathogens detected in Oregon vineyards. Sites A-J were in Rogue Valley and represents the southern region. Sites K-W were in Willamette Valley and represents the northern region.

Region	Sites	Parts of a grapevine trunk	Isolated Pathogens[y]
Rogue Valley	H-2	Top	-
		Middle	-
		Base	-
Rogue Valley	H-3	Top	-
		Middle	-
		Base	-
Rogue Valley	F-1	Top	*Phaeoacremonium* sp., *Phaeomoniella chlamydospora*
		Middle	Botryosphaeriaceae sp.
		Base	*Phaeoacremonium* sp., Botryosphaeriaceae sp.
Rogue Valley	F-2	Top	-
		Middle	-
		Base	*Truncatella angustata*
Rogue Valley	J	Top	*Seimatosporium lichenicola*
		Middle	*Seimatosporium lichenicola*
		Base	Botryosphaeriaceae sp., *Seimatosporium lichenicola*
Rogue Valley	B	Top	Botryosphaeriaceae sp.
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.
Rogue Valley	A	Top	-
		Middle	-
		Base	-
Rogue Valley	D	Top	-
		Middle	-
		Base	-
Rogue Valley	G-1	Top	Botryosphaeriaceae sp., *Seimatosporium lichenicola*

		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp., *Pestaloptiopsis* sp.
Rogue Valley	I	Top	-
		Middle	Botryosphaeriaceae sp.
		Base	-
Rogue Valley	G-2	Top	-
		Middle	-
		Base	*Truncatella angustata*
Rogue Valley	H-1	Top	-
		Middle	-
		Base	-
Rogue Valley	C-1	Top	-
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.
Rogue Valley	C-2	Top	Botryosphaeriaceae sp., *Hormonema viticola*
		Middle	-
		Base	-
Rogue Valley	E	Top	-
		Middle	-
		Base	Botryosphaeriaceae sp.
Willamette Valley	U	Top	*Phaeoacremonium* sp., Botryosphaeriaceae sp.
		Middle	*Phaeoacremonium* sp., Botryosphaeriaceae sp.
		Base	*Phaeoacremonium* sp., Botryosphaeriaceae sp.
Willamette Valley	M	Top	Botryosphaeriaceae sp.
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.
Willamette Valley	Q	Top	-
		Middle	-

		Base	-
Willamette Valley	K	Top	-
		Middle	*Phaeoacremonium* sp., *Phaeomoniella chlamydospora*, Botryosphaeriaceae sp.
		Base	*Phaeoacremonium* sp., *Phaeomoniella chlamydospora*
Willamette Valley	L	Top	Botryosphaeriaceae sp.
		Middle	-
		Base	*Hormonema* sp.
Willamette Valley	S	Top	-
		Middle	Botryosphaeriaceae sp., *Cryptovalsa ampelina*
		Base	
Willamette Valley	W	Top	Botryosphaeriaceae sp.
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.
Willamette Valley	T-2	Top	Phaeoacremonium sp.
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp., *Ilyonectria macrodidyma*
Willamette Valley	O	Top	-
		Middle	*Phaeoacremonium* sp.
		Base	Botryosphaeriaceae sp.
Willamette Valley	R	Top	Botryosphaeriaceae sp.
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.
Willamette Valley	P	Top	-
		Middle	Botryosphaeriaceae sp.
		Base	*Phaeoacremonium* sp.
Willamette Valley	T-1	Top	-
		Middle	-

		Base	Botryosphaeriaceae sp.
Willamette Valley	N	Top	Botryosphaeriaceae sp.
		Middle	-
		Base	Botryosphaeriaceae sp.
Willamette Valley	V	Top	Botryosphaeriaceae sp., *Cryptovalsa ampelina*
		Middle	Botryosphaeriaceae sp.
		Base	Botryosphaeriaceae sp.

[y] - Designates no pathogens were isolated from the grapevine trunk.

Objective 2: Spore trap monitoring

Burkard volumetric spore traps were installed in an old vineyard block and in a young vineyard block in the Applegate Valley as well as in Willamette Valley in early December, 2019 until March 2021 (Figure 3). Tape containing the trapped fungal spores was collected weekly from each of the four traps. Between these timeframe, 475 days of spore samples were collected from each site in Willamette Valley and 477 days of spore samples were collected from each site in Applegate Valley. DNA extraction was performed from individual day samples and followed by qPCR analysis of Botryosphaeriaceae spores trapped in each tape sample. In Willamette Valley, at the younger block, the detection occurred between January 17 and February 4. At the older block, it occurred between December 2 and February 3. Similarly in Applegate Valley, at the younger block, the detection occurred between November 14 and January 26. However, at this site, no detections occurred in the older block. The spore trap was installed in between vines in a row at this block and at the end of the rows at other blocks. We suspect that the spore trap location could have contributed to no detection at the older block in Applegate site.

The number of spores detected ranged from 13 to 26 for Willamette Valley-younger block, 22 to 102 for Willamette Valley-older block, and 5 to 30 for Applegate Valley-younger block. During the days of collection, precipitation occurred more frequently and at a higher amount in Willamette Valley than in the Rogue Valley (Figure 5) For both regions, precipitation appeared to occur more frequently from late fall to late spring. Average temperature seemed to be similar in both regions with temperatures peaking from late spring to late summer (Figure 5).

Figure 3: A Burkard volumetric spore trap set up in a vineyard in the Applegate Valley.

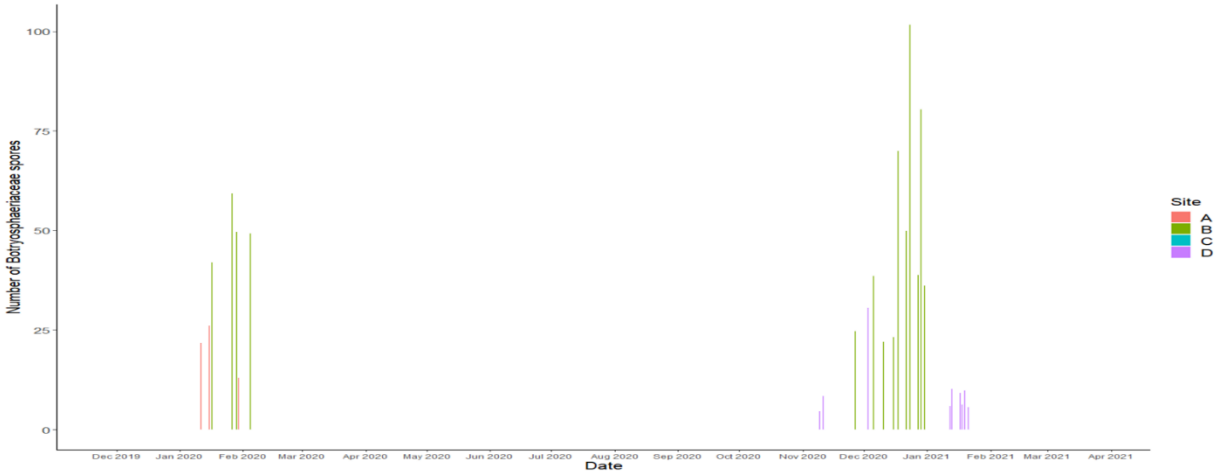

Figure 4: Number of Botryosphaeriaceae spores trapped in a young vineyard block in Willamette Valley (Site A), an old vineyard block in Willamette Valley (Site B), an old vineyard block in Applegate Valley (Site C), and a young vineyard block in Applegate Valley (Site D) from December 2020 to March 2021.

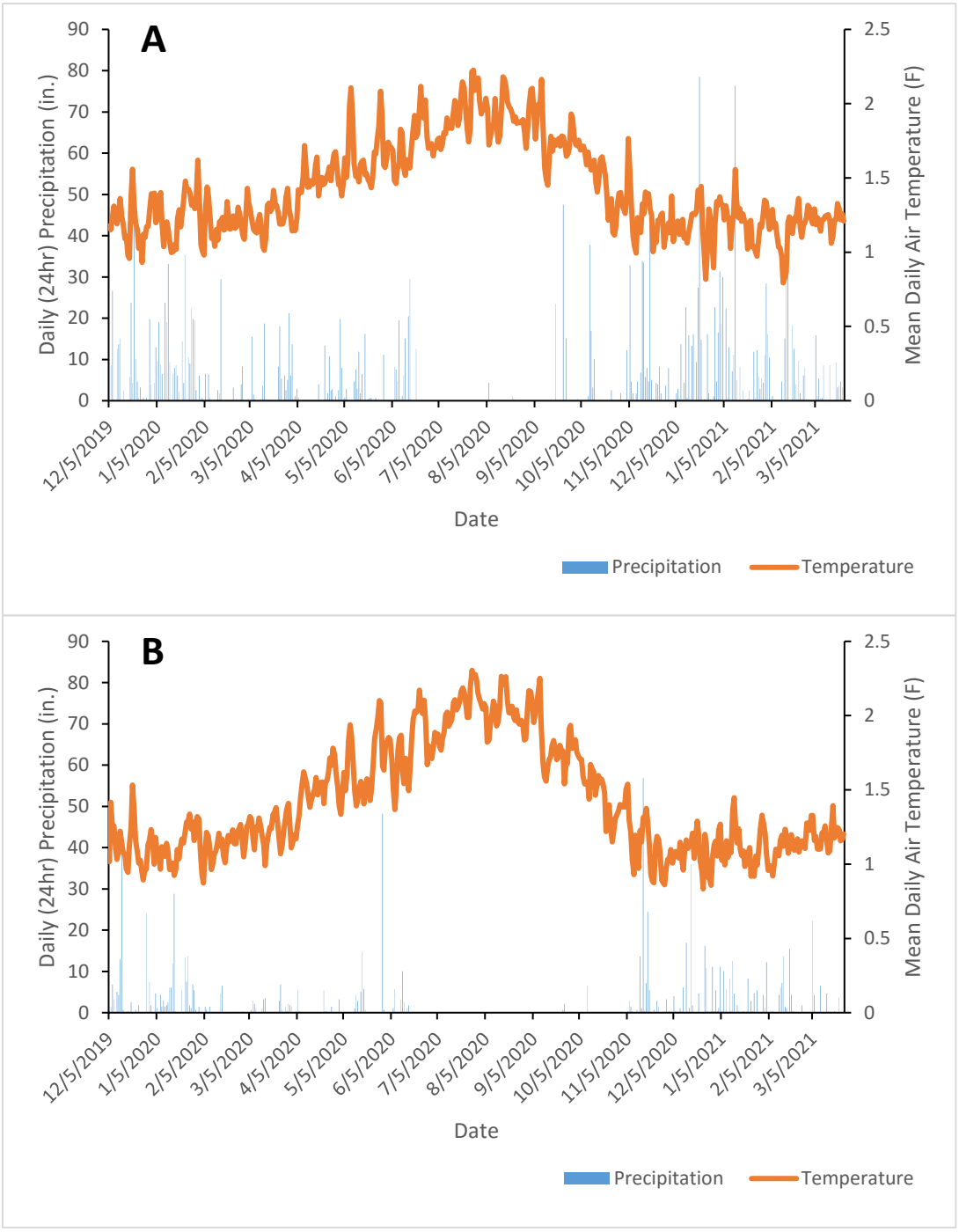

Figure 5: Daily (24hr) precipitation (in.) and mean daily air temperature (F) from December 2019 to March 2021 in (A) Willamette Valley and (B) Rogue Valley.

Objective 3: Disease management

Following the pruning in 2021, three treatments including 1) complete removal of vine prunings from the vineyard; 2) Leave the prunings in the alleyway then mechanically mow with a flail mower; and 3) Leave the prunings in the alleyway, mechanically mow, and spray with Bio-Tam (both wound and soil application) were tested in the older vineyard block where spore samplers were installed for objective 2. The pruning wound treatments were applied on 2/25/2021 and vine prunings in the alleyway were treated with Bio-Tam on 3/5/2021. Samples from the pruning wounds were collected every other week from 2/25/2021 to 5/6/2021. Samples from the vine prunings were collected every other week from 3/11/2021 to 5/6/2021. The biocontrol fungus, *Trichoderma*, was detected in 5 pruning wound samples collected on 2/25/2021 and from 1 vine pruning sample collected on 3/11/2021 (Figure 6). It is interesting to find that the biocontrol agents were detected only during the first sampling period. At the subsequent sampling periods after the first one, the *Trichoderma* spp. were not detected in any of the samples. We suspect two possibilities for this result. First, when wounds were treated we observed active sap flow in the pruning wounds. Second, few days after the application we observed significant amount of rain in Applegate Valley (Figure 7). Both of these may have contriuted to the washing off of the applied treatments. We expect to repeat this trial again in 2022 while pruning the vines earlier than 2021 to rule out the possibility of vine bleeding.

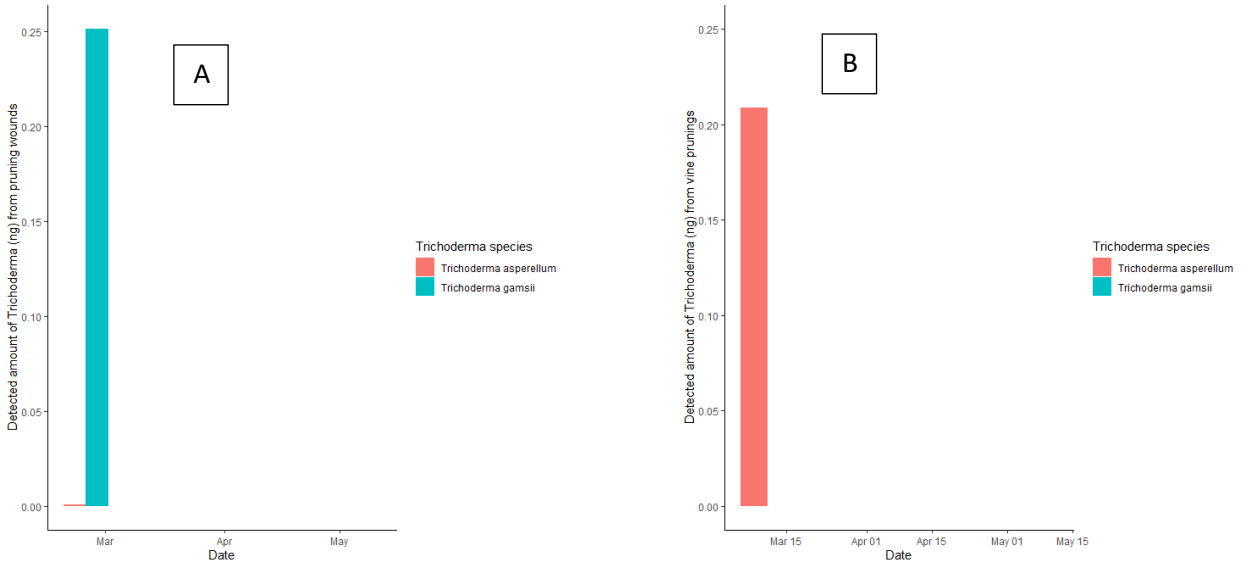

Figure 6: Amount in nanograms of *Trichoderma asperellum* and *Trichoderma gamsii* detected from (A) pruning wounds and (B) vine prunings from February 2021 to May 2021.

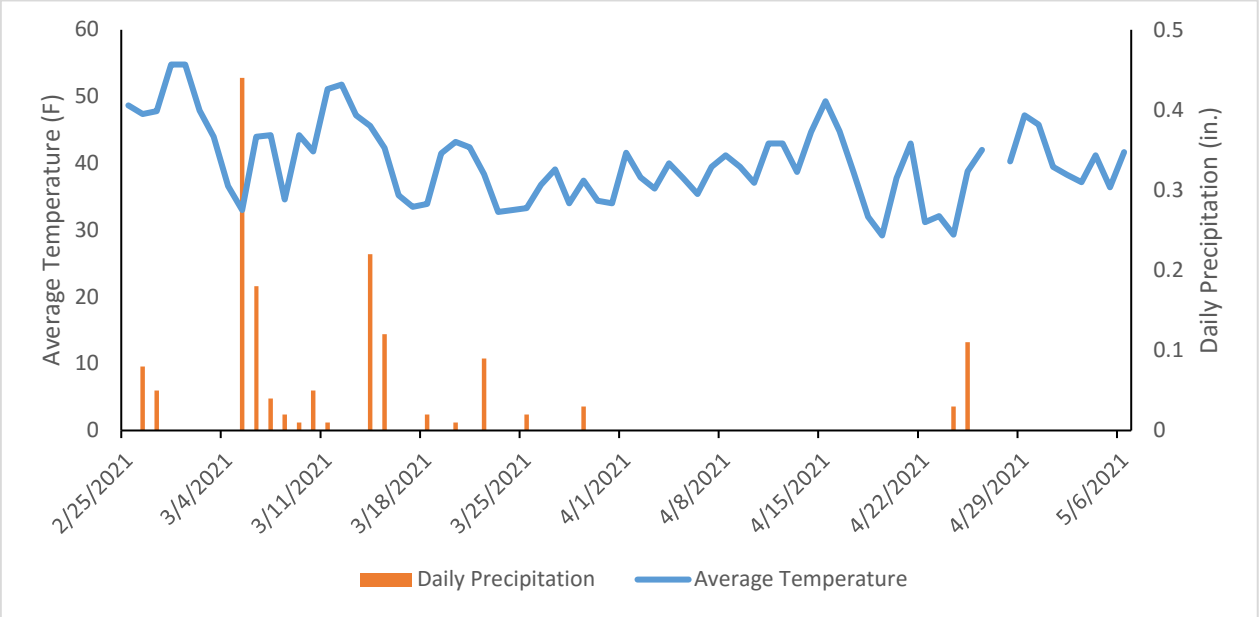

Figure 7: Daily (24hr) precipitation (in.) and minimum daily air temperature (F) from February 25, 2021 to May 6, 2021 in the Applegate Valley.

7. Outside Presentations of Research:

A talk on Grapevine Trunk Disease was presented by Dr. Achala KC in a *Willamette Valley Wine Growers Group* meeting on September 3, 2019, and in a viticulture session at *Oregon Wine Symposium*, Portland, OR, on February 11, 2020. Two online recorded sessions were presented in February 2021 and 2022 at *Oregon Wine Symposium virtual events* where more than 100 industry representatives and growers attended the session. Similarly, other talks on Grapevine Trunk Disease Epidemiology was presented at 6th and 7th Annual *Southern Oregon Grape Symposium* on March 2020 and 2022, Central Point, OR by Dr. Monica Hernandez, Postdoctoral Research Associate (hired by partial funding from this research). One Oregon Wine Research Institute's online *Vine to Wine Newsletter* was published in September 2021 issue and one *Grapevine Newsletter* is being prepared for spring 2022 issue. Similarly, one abstract from this research has been published in *American Phytopathological Society* and one manuscript has been submitted to the journal of *Plant Disease*.

8. Research Success Statements:

We made significant progress on the research objectives and activities as planned throughout the research period. In order to make the survey more representative of the regions, we surveyed 15

vineyards in Rogue Valley and 14 vineyards in Willamette Valley as opposed to the two commercial vineyards in each location proposed in original proposal. These activities happened at the midst of COVID-19 in 2020 field season. However, the participating grower collaborators made this possible by collecting samples from their vineyards and shipping the samples to us. We were able to identify the most prevalent trunk diseases in OR vineyards. This information is useful in designing future research trials while targeting the most important GTD pathogens management. Similarly, it will be important for the growers to keep abreast of the knowledge that Botryosphaeriaceae spp. are the most prevalent GTD pathogens and both newer and older vineyard are susceptible to this disease. Disease management, specially pruning wound protection is crucial in all age vineyards. A manuscript summarizing the finding of this research has been submitted to Plant Disease journal. In addition, this information has led to the funding of another project by Northwest Center for Small Fruit Research, where a holistic microbiome approach will be used to understand the depth of fungal microorganisms present in these samples.

Another challenges of this project was to quantify the spore traps based on microscopic examinations. With one full year of spores trapped by four traps, we would have been looking at 1,460 total daily pieces of tapes. However, recent research advancement on molecular quantification of trunk disease pathogens allowed us to perform more robust quantification with less time and effort. We extracted DNA from the collected spore tapes which was then subjected to quantification using qPCR methods. We were able to detect the spores of Botryosphaeriaceae pathogens during the rainy months of December, January, and February in both southern and northern Oregon. This information is useful while planning grapevine pruning in winter. It would be necessary to protect pruning wounds if grapevines are pruned earlier during December or January. It may be required to provide repeated protection if it rains a lot after the application. However, if we manage to prune later in February, we need lesser protection of the pruning wounds. This finding has led to the development of another research project while aiming at the management of Botryosphaeria die back in different combinations of pruning and application timings. From the third objective of this research we found that timing of Bio-Tam (biological control product) application is critical for the two *Trichoderma* spp. to be active in the pruning wounds. If it rains after the application and/or vines are in active sap flow stage the biological control agent may be washed off and they are no more effective. However, this result was summarized from one year of study and we plan on repeating this trial again for the confirmation.

9. Funds Status:

A postdoctoral research associate and undergraduate research assistant were hired by partial funding from this research (50%). Four Burkard 7 day volumetric samplers (average cost of $5,855 per sample) were purchased through Burkard Mfg. Co. Ltd., Woodcock Hill Industrial Estate, England. The remaining fund (~ $30,000) will be used to repeat the second year trial of *Trichoderma* testing and to continue supporting postdoctoral research associate to prepare reports, presentations, and manuscripts until the contracted date in June 2022.