## Table of Contents

# Application Summary

## Competition Details

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| **Competition Title:** | 2021 Request for Applications: NCSFR |
| **Category:** | Open Funding Opportunities |
| **Cycle:** | 2021-2022 |
| **Submission Deadline:** | 04/1/2021 10:00 PM |

## Application Information

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| **Submitted By:** | Achala KC |
| **Application ID:** | 34 |
| **Application Title:** | Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards |
| **Date Submitted:** | 04/1/2021 8:16 PM |

## Personal Details

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| **Applicant First Name:** | Achala |
| **Applicant Last Name:** | KC |
| **Email Address:** | achala.kc@oregonstate.edu |
| **Phone Number:** | (541) 772-5165 |
| **Principle Investigator (PI):** | Achala KC |
| **University/Institution:** | Oregon State University |
| **Department:** | BPP |
| **Is a USDA-ARS PI or Cooperator(s) part of this project?:** | No |

**If USDA-ARS PI and other cooperator(s), list names and organization.**

N/A

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| **Suggest first potential scientist review panelist based on relevant discipline or working group your project falls under:** | Kendra Baumgartner, kbaumgartner@ucdavis.edu, plant pathology, USDA-ARS |
| **Suggest second potential scientist review panelist based on relevant discipline or working group your project falls under:** | Akif Eskalen, aeskalen@ucdavis.edu, plant pathology, UC Davis |

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| **Suggest first industry review panelist based on relevant discipline or working group your project falls under:** | David Beck, david@crawfordbeck.com, Crawford Beck vineyard |
| **Suggest second industry review panelist based on relevant discipline or working group your project falls under:** | Leigh Bartholomew, leigh@resultspartners.com, Results Partners |

## Application Details

**Proposal Title**
Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards

**Budget Requested (AMOUNT MUST BE BELOW $50 000)**
49,705

**Are you submitting a new proposal?**
Yes

**Are you submitting a continuing project proposal?**
No

**Which year of funding are you requesting for this project (if continuing project)?**
N/A

**Project length in years from start to finish**
2 year project

**Are you applying to other groups for funding this project?**
No

**If you are applying for funding from other groups, please list how much money for each request.**
N/A

**Technical Working Group**
Pest Management

**Commodity Group**
Grape Viticulture

**What are the primary and secondary NCSFR Research Priorities being addressed?**
Primary: Wine and juice viticulture research

Secondary: Biology and management of trunk disease

**Project Summary (1-page)**

Many researches in California and other wine grape growing regions have studied the type of Grapevine Trunk Diseases (GTDs) that are present as well as the yield and economic losses caused by them. Botryosphaeria dieback and Eutypa dieback have been reported to cause yield losses of 30% to 50% and up to 94% respectively in severely infected vineyards (Gramaje et al., 2018). The economic impact of this has been estimated to be $260 million per year (Gramaje et al., 2018; Gubler et al., 2005; Mondello and Songy, 2018; Úrbez-Torres et al., 2006). GTDs have a significant impact in Washington as well with Eutypa dieback accounting for 20-50% yield loss in moderate cases and 60-95% yield loss in severe cases (OSU Plant Clinic, 2020). Through samples submitted to the Oregon State University (OSU) Plant Clinic, GTDs such as Esca, Eutypa dieback, Blackfoot, and Botryosphaeria dieback have been shown to be present in Oregon as well.

In this study we propose to provide data on which types of GTDs are most prevalent in Oregon by performing next-generation sequencing (NGS) on wood tissue samples collected from vineyards in the Rogue Valley, located in Southern Oregon, and in Willamette Valley, located in Northern Oregon. Traditionally, fungi have been identified via culture based methods. However, culturing and identifying fungi based on morphology can be very time intensive. Furthermore, some fungal isolates may outgrow others in culture while other fungi cannot be cultured at all. This leads to an inaccurate account of what types of fungi may be present in a particular system. In contrast, NGS will be able to capture the fungi present in the grapevine system since it can detect the DNA of even fungi that cannot be cultured.

Since grapevine pathogens typically infect the trunk by entering through pruning wounds, we also aim to evaluate different pruning management practices by performing NGS on wound tissue and soil samples following the management practices. We have begun collecting samples from a vineyard in the Rogue Valley where different pruning wound treatments have been implemented. Samples are currently being stored at 4 oC. One treatment is the application of Bio-Tam 2.0 to both pruning wounds and soil. This product contains two strains of the fungus Trichoderma. This fungus is commonly used as a biocontrol agent as it can outcompete and antagonize fungal pathogens. Traditionally, the effect of GTDs management has been evaluated as culture based identification of targeted pathogens, and/or spore trapping of the targeted pathogens at the management trial site. This method is still applicable; however, there are several challenges associated with pathogen identification as explained earlier. With the application of microbiome-based analysis of these samples, we can explore the big picture of changes in the microbiome as a result of different management practices. We can also evaluate the effect on targeted pathogens without compromising the dubious results of culture-based methods.

**Literature References**

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J.F., Sagües, A., and Gramaje, D. 2019. The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Frontiers in Microbiology 10(1142): 1-16.

Gramaje, D., Úrbez-Torres, J.R., and Sosnowski, M.R. 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: Current strategies and future prospects. Plant Disease 102(1):12-39.

Gubler, W.D., Rolshausen, P.E., Trouillase, F.P., Úrbez, J.R., Voegel, T., Leavitt, G.M., and Weber, E.A. 2005. Grapevine trunk diseases in California. Practical Winery & Vineyard pp. 1-9.

Martínez-Diz, M.P., Andrés-Sodupe, M., Bujanda, R., Díaz-Losada, E., Eichmeier, A., and Gramaje, D. 2019. Soil-plant compartments affect fungal microbiome diversity and composition in grapevine. Fungal Ecology 41: 234-244.

Mondello V., Songy, A., Battistion, E., Pinto, C., Coppin, C., Trotel-Aziz, P., Clément, C., Mugnai, L., and Fontaine, F. 2018. Grapevine trunk diseases: A review of fifteen years of trials for their control with chemicals and biocontrol agents. Plant Disease 102(7):1189-1217.

Niem, J.M., Billones-Baaijens, R., Stodart, B., and Savocchia, S. 2020. Diversity profiling of grapevine microbial endosphere and antagonistic potential of endophytic Pseudomonas against grapevine trunk diseases. Frontiers in Microbiology 11(477): 1-19.

OSU Plant Clinic. https://bpp.oregonstate.edu/main/grapevine-trunk-diseases. Accessed online: March 2020.

Úrbez-Torres, J.R., Leavitt, G.M., Voegel, T.M., and Gubler, W.D. 2006. Identification and distribution of Botryosphaeria spp. associated with grapevine cankers in California. Plant Disease 90(12): 1490-1503.

**Cooperators: Provide details on the contribution each Cooperator will make to your proposed project.**

co-PI Dr. Posy Busby has many years of experience working on microbiome of different environmental samples. Dr. Posy will co-advise the graduate research assistant who will be hired full time from this funding. It is expected that the GRA will complete their first year of study while housed at Dr. Busby's lab in Corvallis. In second year, they will be housed at Dr. KC's lab in SOREC for the second year of project, data analysis, and thesis preparation.

co-PI Dr. Monica Hernandez is a post-doctoral research associate with Dr. KC and is leading a grapevine trunk disease project funded by Oregon Wine Board. She will lead sample collection and preparation efforts while supervising the undergraduate research assistant.

Cooperator Dr. Patty Skinkis is involved in many collaborative research projects with Willamette Valley wine grape growers and is aware of vineyards with history of trunk diseases. Her cooperation will be instrumental in connecting with growers in the region. In addition, she will cooperate on extension and outreach activities relating to this project.

Grower cooperators, Leigh Bartholomew from Results Partners and Jason Cole from Pacific Crest Vineyard Services will assist in identifying vineyards and trial sites in Willamette and Rogue valley. They will keep us informed on the regular activities in the vineyard sites while the trials are running actively.

# Progress Report

## 2021 Request for Applications: NCSFR

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| **Progress Report Title:** | Final Report |
| **Applicant Name:** | Achala KC |
| **Application Title:** | Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards |
| **Application ID:** | 34 |
| **Review Deadline:** | 10/27/2023 4:00 PM |

# Final Report

Please fill out the following information by inserting text directly onto the space provided. Please do not write your report on a separate sheet. There are files that need to be filled out and uploaded in the last sections (this includes the 2023 Conference proceedings form). NOTE: Material from this final report will be used to create a NCSFR Factsheet circulated to the PNW industry.

Please email info@nwberries.org if you have received this final reporting request in error. Provide proof of No Cost Extension or other timeline in your email.

Deadline for submission is Oct. 27, 2023 at 4pm PST.

Oral presentations of findings will take place during the NCSFR Confrerence on Nov. 13-15, 2023 in Corvallis, OR. You will be receiving a mailing with more conference information shortly.

**Please note: Failure to submit final project reports and research impact statements, as well as to present a final oral presentation at the NCSFR conference, may result in disqualification for future proposals considered for funding through this program. PI is required to present in person during the conference.**

# Research Reporting

| | |
|---|---|
| **Project Title:** | Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards |
| **Principle Investigator & Cooperator(s): :** | Dr. Achala KC, Oregon State University, Southern Oregon Research and Extension Center, 569 Hanley Road, Central Point, OR 97502. Tel: 541-772-5165 ext. 222. email: achala.kc@oregonstate.edu; Dr. Monica Hernandez, Oregon State University, Southern Oregon Research and Extension Center, 569 Hanley Road, Central Point, OR 97502. Tel: 909-732-6883. email: monica.hernandez@oregonstate.edu; Dr. Posy Busby, Oregon State University, 4575 SW Research Way, Corvallis, OR 97333. Tel: 541-737-1533. email: posy.busby@oregonstate.edu |
| **Full Project Reporting Period Covered (M/D/Y-M/D/Y):** | Start Date: 10/27/2023 — End Date: 10/31/2023 |
| **Is this a final report? (Y/N):** | Yes |

**Abstract:**

Grapevine trunk disease (GTD) is a disease complex that consists of the largest group of fungal pathogens causing disease and progressive vine decline. Due to the complex nature of GTD pathogens, understanding the disease as a phenomenon of single species or multiple species infection is always challenging. In addition, the environmental variables play a greater role in the dominance of one species over another. It is important to understand the species that are predominant in a region and their causal role in GTD development so that targeted disease management programs can be developed. In this research project, we proposed to study the diversity of GTD pathogens in grapevines of Northern and Southern Oregon; as well as to compare the different disease management practices including vineyard floor management and pruning wound protection. For the first objective, we explored how fungal communities vary along gradients of GTD disease incidence, vineyard age, and geography in

Oregon and conducted a molecular field study, amplifying the ITS1 region of fungal rDNA extracted from vine stem tissue sampled from 29 vineyards in the Rogue and Willamette Valleys (n= 396). In total, we found over 2000 Operational Taxonomic Units (OTUs) in stem tissues, predominantly in the Ascomycota (85%) and Basidiomycota (14%). The most abundant genera, Cladosporium (30%), Penicillium (8%), Alternaria (5%), and Aureobasidium (5%), were not associated with core GTD genera, regardless of disease incidence. The GTD associated genera, Seimatosporium (7%), Truncatella (5%), Phaeomoniella (1.7%) and Phaeoacremonium (1.1%) were the most abundant. Other genera associated with GTD were least abundant ranging from <0.1 to 0.6%. We also found significant correlations between the most abundant OTUs and GTD associated genera suggesting interactions within core GTDs and between core GTDs and other genera.

For second objective, we identified a vineyard in southern Oregon to implement both pruning wound protection and soil treatments. For the pruning wound treatments, we applied three treatments with four replicate vines per treatment. The first treatment was the wound treatment with VitiSeal, the second treatment was with Bio-Tam 2.0, and the third was non-treated control. All wound treatments were applied within 24 hrs of pruning in early February 2022. For the soil treatment, vineyard rows were divided to include three treatments with four replicate rows per treatment. For the first treatment, we removed all pruning debris from the row. For the second treatment, we left all the pruning debris in the row and mowed with a flail mower. For the third treatment, we left all the pruning debris in the row, mowed with a flail mower, and applied Bio-Tam 2.0 to the soil. The treatments were applied in late February, 2022 after the pruning debris were mowed. The second year trial could not be completed as the entire vineyard block was pulled out to replace with new vines. Wood tissue samples from treated wound tissues and pruned brushes from soil application were collected in biweekly basis until the beginning of May 2022. Culture based analysis of treated pruning wound tissues suggested no significant difference between treatments on fungal diversity. No known GTD pathogens were isolated from pruning wound samples or brush samples. Trichoderma spp. from Bio-Tam application were recovered in average of 23% of the samples suggesting their adaptability and establishment in specific environments require further investigation.

**Objectives:**

1. Use microbiome-based studies to understand the diversity of grapevine trunk disease pathogens and how factors such as the age and location of a vineyard affect their distribution.
2. Explore how different pruning wound and soil treatments affect the fungal microbiome on wound and soil samples.

**Accomplishments:**

Within two years of proposed project period, we completed all activities related first objective and partial activities related to second objective. For the first objective, we completed DNA extraction of 456 trunk tissue samples collected from 15 vineyards in Southern Oregon and 14 vineyards in Northern Oregon. The numbers of samples processed per vineyard ranged from 9 to 30 depending on size of the vineyard block. Out of these, 396 DNA extracts from 29 vineyards were processed for next-generation sequencing using ITS primers. Data analyzed from this objective suggested that the most prevalent GTD associated genera were Seimatosporium and Truncatella, which were found in virtually all vineyards, and almost all strata within those vineyards. The role these taxa play in GTD is not well understood, and they are not associated with any known GTD complexes, yet previous work has shown they can exacerbate symptoms of GTD. Considering their prevalence in Oregon vineyards, we suggest further investigation into their role in the greater fungal community.

Furthermore, our findings confirmed the prevalence of Botryosphaeria, Dothiorella, Neofusicoccum, and Diplodia, the genera in the Botryosphaeriaceae in 79, 57, 18, and 4% of the vineyards respectively. Neonectria, Thelonectria, Ilyonectria, and Dactylonectria the genera causing Black Foot disease in 71, 32, 29, and 29% of the vineyards respectively. Phaeomoniella, Phaeoacremonium, Cadophora, Fomitiporella, Pleurostoma, the genera causing Esca disease in 93, 93, 79, 4, and 4% of the vineyards respectively (Table 1).

Within vineyards, Seimatosporium and Truncatella were detected in the highest percentage of strata in both valleys. On average, GTD associated genera were detected in a higher percentage of strata in the Willamette Valley than in the Rogue Valley (Willamette mean = 12%, Rogue mean = 6%, $p < 0.001$). This trend was mostly consistent across GTD associated genera, with only three exceptions (Dactylonectria 3% higher in the Rogue Valley, Diplodia 0.5%, and Eutypa 0.5%), none of which were statistically significant. Phaeoacremonium, Phaeomoniella, Botryosphaeria, and Neonectria were detected in notably more strata in the Willamette Valley (Fig. 1).

Vineyards in the Rogue Valley were on average 20 years old, compared to 11 years in the Rogue Valley (t-test $p = 0.046$). Of the GTD associated genera we examined, seven showed a statistically significant positive correlation with vineyard age (Benajamini-Hochberg corrected $p< 0.05$). Spearman's correlation coefficients ranged from 0.14 for Botryosphaeria and Stereum, and reached as high as 0.26 for Phaeomoniella (Fig. 2), which supports our hypothesis that

relative abundance would increase with vineyard age. However, our hypothesis that disease incidence would also relate to GTD relative abundance was not supported. There were no genera that correlated positively with disease incidence; the only two significant correlations were negative (Diaporthe and Truncatella).

For the second objective, we identified a vineyard in southern Oregon to implement both pruning wound protection and soil treatments.

Pruning treatments. To identify what effect different pruning practices have on the fungal microbiome, a vineyard was divided into rows representing three treatments with four replicates per treatment. The first treatment was to remove all pruning debris from the row. The second treatment was to leave the pruning debris in the row and to mow with a flail mower. The third treatment was to leave the pruning debris in the row, mow with a flail mower, apply Bio-Tam 2.0. Similarly, for the pruning wound treatments, three treatments were tested with four replicate vines per treatment. The first treatment was the wound treatment with VitiSeal, the second treatment was with Bio-Tam 2.0, and the third was non-treated control. All wound treatments were applied

within 24 hrs of pruning in early February 2022 and the soil applications were made on late February.

Collecting pruning wound tissue samples. Samples from pruning wounds were collected every other week starting on February 8, 2022 and ending on May 3, 2022. Pruning shears were used to cut a piece of a pruning wound from a trunk. The sample was placed in a sterile 15 mL tube. Pruning shears were sterilized with ethanol between each cut that was made. One cut was made in each treatment vine resulting in 12 samples per sampling period. All samples were taken to the lab where they were placed in the fridge at 4C until further processing.

Collecting soil samples. Samples from the soil were collected every other week starting on February 24, 2022 and ending on May 2, 2022. A soil auger was used to collect soil at a depth of 2 inches. The sample was placed in a plastic bag. The soil auger was sterilized with ethanol between each sample that was taken. Two samples were made in each row of each treatment resulting in 24 samples in total. For each row, one sample was taken at the 5th vine and the other sample was taken at the 17th vine. All samples were taken to the lab where they were placed in the fridge at 4C until DNA extraction.

Collecting brush samples. Samples of pruning brush were collected every other week starting on February 24, 2022 and ending on May 3, 2022. Samples were collected in each row of the second and third treatments resulting in 8 samples in total. For each row, brush was collected at the 5th vine and also at the 17th vine. The brush from both locations was combined and placed into a plastic bag. All samples were taken to the lab where they were placed in the fridge at 4C until further processing.

Extracting DNA from pruning wound tissue samples. The sample tissue was homogenized using a sterile mortar and pestle. It was then frozen in liquid nitrogen and further homogenized using a bead mill. Samples were grinded in the bead mill for homogenization, and DNA was extracted following a standard laboratory protocol.

Plating pruning wound samples. To monitor the fungal populations, each homogenized pruning wound sample was plated onto GI media by sprinkling the wood chips from the sample onto the plate. Plates were stored at room temperature under a cycle of 12 hours of light and 12 hours darkness until fungi had grown. Fungi were then analyzed and identified.

PCR to detect Trichoderma spp. Species specific primers for Trichoderma asperellum, and T. gamsii were used to amplify the DNA extracted from both pruning wound tissues and brush samples using qPCR. Data on Trichoderma spp. detection were recorded and converted into percentage detection per treatment sampling date.

From the culture based assessment of both pruning wound tissues and brush samples, many saprophytic fungal species such as Altermaria, Fusarium, Aureobasidium, Penicillium, and Cladosporium, were isolated from treated tissues. The isolations were random among the treatments and no significant effect of the treatments were observed on both wound and soil applications. No significant pathogenic species were isolated from any of the treatments including non-treated control. The PCR based detection resulted 20% detection of T. asperellum on the day of application, 10% detection on eight and ten weeks after application. Similarly, T. gamsii was detected on 10%, 30%, and 50% of the samples at two, eight, and ten weeks after application respectively (Fig. 3). All these detections occurred on Bio-Tam applied pruning wounds. On the brush samples, only T. asperellum was detected from 25% of the samples on the third treatment rows where Bio-Tam was applied after pruning brushes were mowed (Fig. 4).

These results suggest the applied treatments do not affect fungal microorganisms naturally present in the vines. The Bio-Tam application increased the chances of establishing biocontrol population in the vine tissues, however, their low isolation percentage suggests their adaptability and establishment in specific environments require further investigation. Unfortunately, the treatments effect on pathogen population could not be determined due to their absence in the selected vineyard. We could not artificially inoculate the pathogens to understand the treatment effect because it was commercially established vineyard. Furthermore, the trial was terminated in second year because the vineyard block was removed.

**Reasons why goals and objectives were not met (when applicable): :**

We met both objectives goals, however we could not follow all the methods that we proposed to obtain the second goal. We proposed to use microbiome based methods to analyze samples collected from second objective, similar to first objective. However, the timeline of two years for a Master's student did not allow to complete both analysis. The student graduated when the project funding was ended. Similarly, the post-doc who was working on the culture based analysis on second objective moved onto new position in late summer 2022.

**Industry Significance:**

In recent decades we have begun to appreciate the complex nature of GTD, particularly the number of associated taxa, and the diversity that can be seen in a given region. Our study sought to investigate how GTD associated taxa relate to visually observed disease incidence, vineyard age, and geography in Oregon. As of 2018 there were 133 known GTD associated species globally, yet each region reports its own subset of species, and new species are not a rarity. In previous study, Hernandez and KC isolated 45 fungal cultures associated with GTD from the samples collected in Oregon vineyards. From the study, they identified Botryosphaeriaceae, Phaeoacremonium as the most common genera. In comparison, using the same samples in present study, ITS metabarcoding identified 102 OTUs in 20 different GTD associated genera, although Phaeoacremonium and Botryosphaeriaceae species were still highly represented.

In the present study, the genera Seimatosporium and Truncatella were by far the most prevalent GTD associated taxa, not only in terms of relative abundance, but they were detected in all vineyards, and at least 82% of strata. This indicates that not only are these taxa in every vineyard, but they are widespread throughout each vineyard. Both genera have been reported in association with GTD around the world, including Iran, Chile, and France, and Northern California.

Between the two valleys, GTDs were detected in a higher number of strata in the Willamette than in the Rogue Valley. This aligns with the culture based results, but also with the broader trends of higher pathogen pressure in the Willamette Valley. With the exception of taxa that were found in every vineyard (Truncatella and Seimatosporium), the next 8 most abundant GTD associated taxa were detected in more vineyards, and a higher average percentage of strata in Willamette Valley vineyards. This indicates that GTD associated genera are not only more widespread in the Willamette Valley, but also that they likely inhabit more plants per vineyard than in the Rogue Valley.

Metabarcoding allows us to examine the fungal community as a whole, and thus think about GTD in ecological terms. With so many implicated taxa, not only is it likely that there are more species to be found, but also that known GTDs may not act as pathogens all of the time. In this study we have shown that GTD associated taxa make up less than 25% of the relative abundance in the average Oregon vineyard and their presence were not correlated with disease incidence, which means that the pathogenic community do not always cause disease but rather inhabit the grapevine environment. However, the sources of this community and the environment that favors these community to become pathogens require further investigation.

Placing GTDs in the context of their fungal community is one of the biggest benefits of using a Metabarcoding based approach. Perhaps most striking is that GTD associated taxa were not the dominant community members. Among the top 20 most abundant OTUs, only 4 were associated with GTD, and the combined relative abundance of all GTD associated taxa was less than 25% of the total in all but 5 vineyards. Of the 5 OTUs that significantly correlated with NMDS axes, only one was associated with GTD, suggesting that GTD taxa do not strongly shape community composition overall. The dominant genus was by far Cladosporium, representing 30% of the total relative abundance in the whole dataset, while Seimatosporium being the only GTD associated genus in the top 5 most abundant genera. Despite the lower relative abundance of most GTD genera, significant correlations between the most abundant OTUs and GTD genera indicate these taxa are likely interacting. Verifying and determining the nature of those interactions is beyond the scope of this study, however it does support using a community-based approach to better manage GTD.

A major motivator for studying GTDs as a community is the hope of effectively applying biocontrols, especially considering the complexity of the vine-associated microbiome. Strains of Aureobasidium have previously been shown to have antagonistic effects towards Diplodia, Eutypa lata, and Neofusicoccum parvum. One negative correlation in particular, between an Aureobasidium spp and Phaeomoniella, indicates that Aureobasidium based biocontrols could be a fruitful starting point for further research on how to implement these new products in Oregon. Results from second objective further support a need of identifying locally adapted biological species to explore their biocontrol potential. The fact that two biocontrol species of Trichoderma were not recovered from applied tissues suggest that these species need adaptation to the environmental conditions where they are applied. Since one of the most common mode of action of biocontrol agents is by competition, they have to be established in the plant tissues to be effective.

**Changes to standard production practices :**

- Awareness in finding planting materials from nurseries that prioritize sanitation of both rootstock and scion materials, and prevent contamination by GTD causing pathogens
- Pruning wound protection to prevent infection by GTD pathogens whose aerial/splash spore dispersal are primary source of infection
- Limit movement of vine cuttings within and between vineyards
- Adjust the timing of bio-fungicide application so that the biocontrol agents are exposed to environment favoring their development in applied tissues

**New grower recommendations:**

- Prioritize on cleanliness of the planting material when obtaining from nurseries.
- Older vineyards inhabit more GTD pathogens. If a vineyard has a mix of old and new vines, the newer vines need to be protected by more effective treatments.
- New pathogenic species have been identified that are less aggressive but can be pathogenic when vines undergo stress environments. Keeping vines healthy and less stressed is important

**Provide a list of all scientific citations and papers that have been published because of the funding you received from NCSFR :**

Zimmerman J, 2023. Fungal microbiome associated with grapevine trunk disease in Oregon vineyards. MS Thesis, Oregon State University.

Zimmerman J, Busby PE, KC A, 2023. Grapevine Trunk Disease: Using Amplicon Sequencing to Examine GTD in Western Oregon, USA. Abstract, 4th Plant Microbiome Symposium. August 2023. Quito, Ecuador.

Zimmerman J, Busby PE, Hernandez, M, KC A 2022. Fungal microbiome associated with grapevine trunk disease in Oregon vineyards. Abstract, Plant Microbiome Symposium. December 2022. Oregon State University, USA

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|---|---|
| **All Funding Sources:** | Three years of funding was provided by Oregon Wine Board (OWB) with a project period of 07/01/2019 to 06/30/2022. The objectives of this project was to understand the most common GTD present in Oregon vineyards and understand their epidemiology of spore release as affected by weather variables such as temperature and precipitation. One main difference between the OWB funded project and NCSFR funded project is the way we analyzed data. The OWB funded project gathered the preliminary information on GTD fungal species using traditional approach of culture and identification, while the current project will identify the fungal species based on DNA and Next-generation sequencing based analysis. Since Grapevine Trunk Diseases are complex of multiple fungal pathogens, the traditional culture based identification of pathogens is very challenging. As a result, many pathogenic spp. that may play a bigger role in GTD development could have been overlooked. Whereas microbiome based approach is reportedly more informative than traditional approaches and will capture a larger diversity of microbes present in grape vine trunks. The preliminary result from OWB funded project prompted us to propose the current NCSFR project.<br><br>A graduate student was hired from the funding through NCSFR that supported student's annual salary. Tuition (Graduate Teaching Assistant, approximately $20,000/year) is supported by OSU-department of Botany and Plant Pathology. |
| **Project Keywords:** | Microbiome<br><br>Grapevine Trunk Disease<br><br>Biological Control<br><br>Oregon |
| **Please list the individual number of Post Docs, Docs, Masters Students supported by this research project funding:** | PI - 1 (0.16 FTE)<br><br>Masters Students - 1 (0.8 FTE)<br><br>Undergraduate Student - 1 (0.25 FTE) |

**Table 1.** Detected GTD associated fungal genera

| Family | Genus | Primary GTD Association | % Vineyards Detected | % Relative Abundance | Citations |
|---|---|---|---|---|---|
| Bartaliniaceae | *Truncatella* | None | 100 | 4.7 | Úrbez-Torres 2009 |
| Botryosphaeriaceae | *Botryosphaeria* | Botryosphaeria | 78.6 | 0.5 | Bertsch 2013 |
| | *Dothiorella* | Botryosphaeria | 57.1 | 0.1 | Bertsch 2013 |
| | *Neofusicoccum* | Botryosphaeria | 17.9 | < 0.1 | Bertsch et al., 2013; Yan et al., 2013 |
| | *Diplodia* | Botryosphaeria | 3.6 | < 0.1 | Bertsch 2013 |
| Diaporthaceae | *Diaporthe* | Phomopsis | 57.1 | < 0.1 | Guarnaccia et al., 2018; Úrbez-Torres et al., 2013 |
| Diatrypaceae | *Eutypa* | Eutypa | 3.6 | < 0.1 | Kenfaoui et al., 2022 |
| Hymenochaetaceae | *Inonotus* | Esca | 46.4 | 0.3 | Bertsch 2013; Cloete et al., 2015 |
| | *Fomitiporella* | Esca | 3.6 | < 0.1 | Cloete et al., 2015 |
| Nectriaceae | *Neonectria* | Black foot | 71.4 | 0.6 | Agustí-Brisach & Armengol, 2013 |
| | *Thelonectria* | Black Foot | 32.1 | < 0.1 | Agustí-Brisach & Armengol, 2013 |
| | *Ilyonectria* | Black Foot | 28.6 | < 0.1 | Agustí-Brisach & Armengol, 2013 |
| | *Dactylonectria* | Black Foot | 28.6 | < 0.1 | Lombard et al., 2014; Pintos et al., 2018 |
| Phaeomoniellaceae | *Phaeomoniella* | Esca | 92.9 | 1.4 | Bertsch 2013 |
| Pleurostomataceae | *Pleurostoma* | Esca | 3.6 | < 0.1 | Fontaine et al., 2016 |
| Ploettnerulaceae | *Cadophora* | Esca | 78.6 | 0.4 | Úrbez-Torres 2014 |
| Sporocadaceae | *Seimatosporium* | Non-specific | 100 | 7.8 | Kanetis 2022 |
| Stereaceae | *Stereum* | Non-specific | 21.4 | 0.4 | Bertsch 2013; Cloete et al., 2015 |
| Togniniaceae | *Phaeoacremonium* | Esca | 92.9 | 1.1 | Bertsch 2013 |

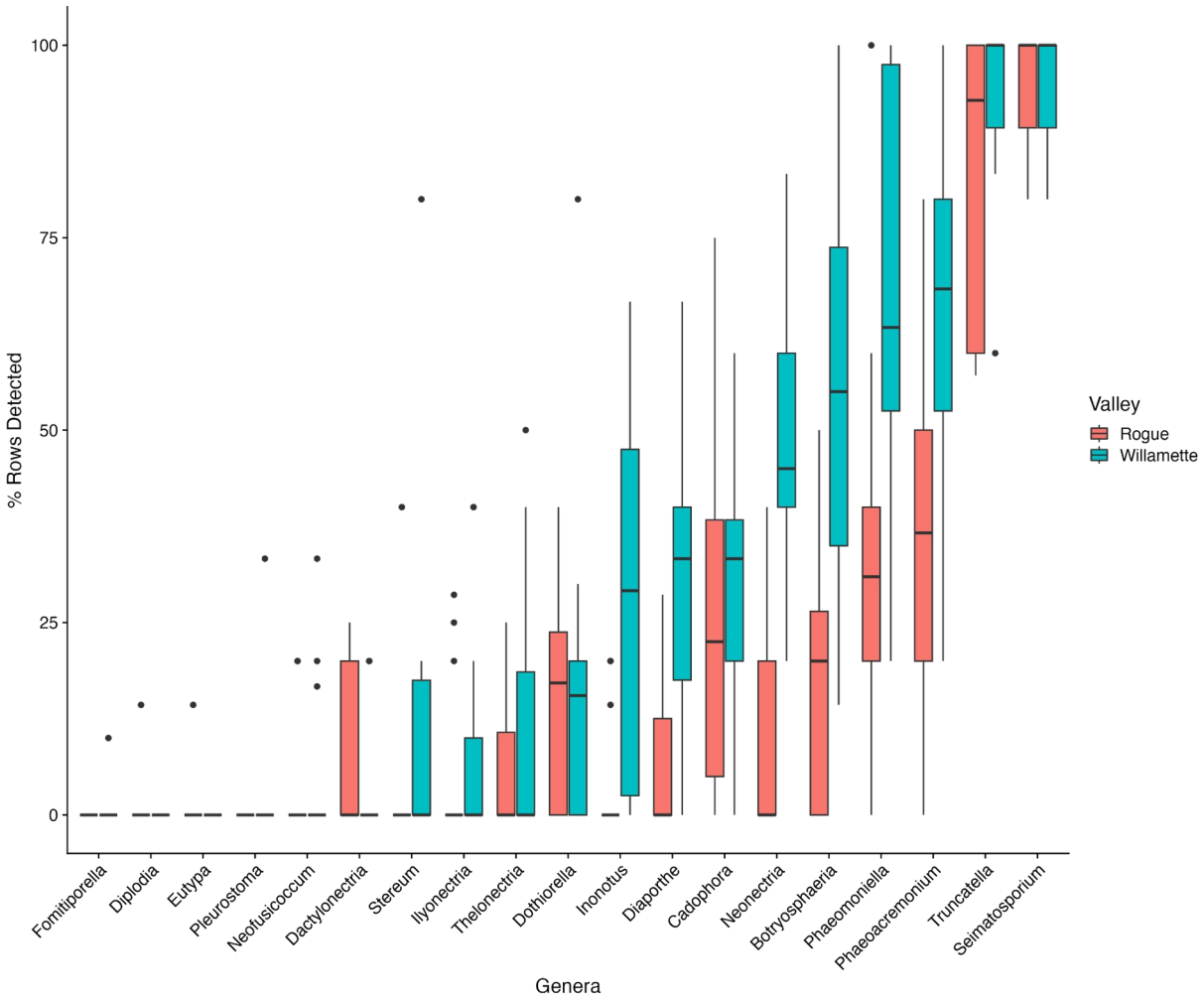


Figure 1. Detection barplot. The percentage of rows detected indicates rows per vineyard in which a given genus was detected in the post-filtering ITS1 metabarcodes. Color indicates valley.

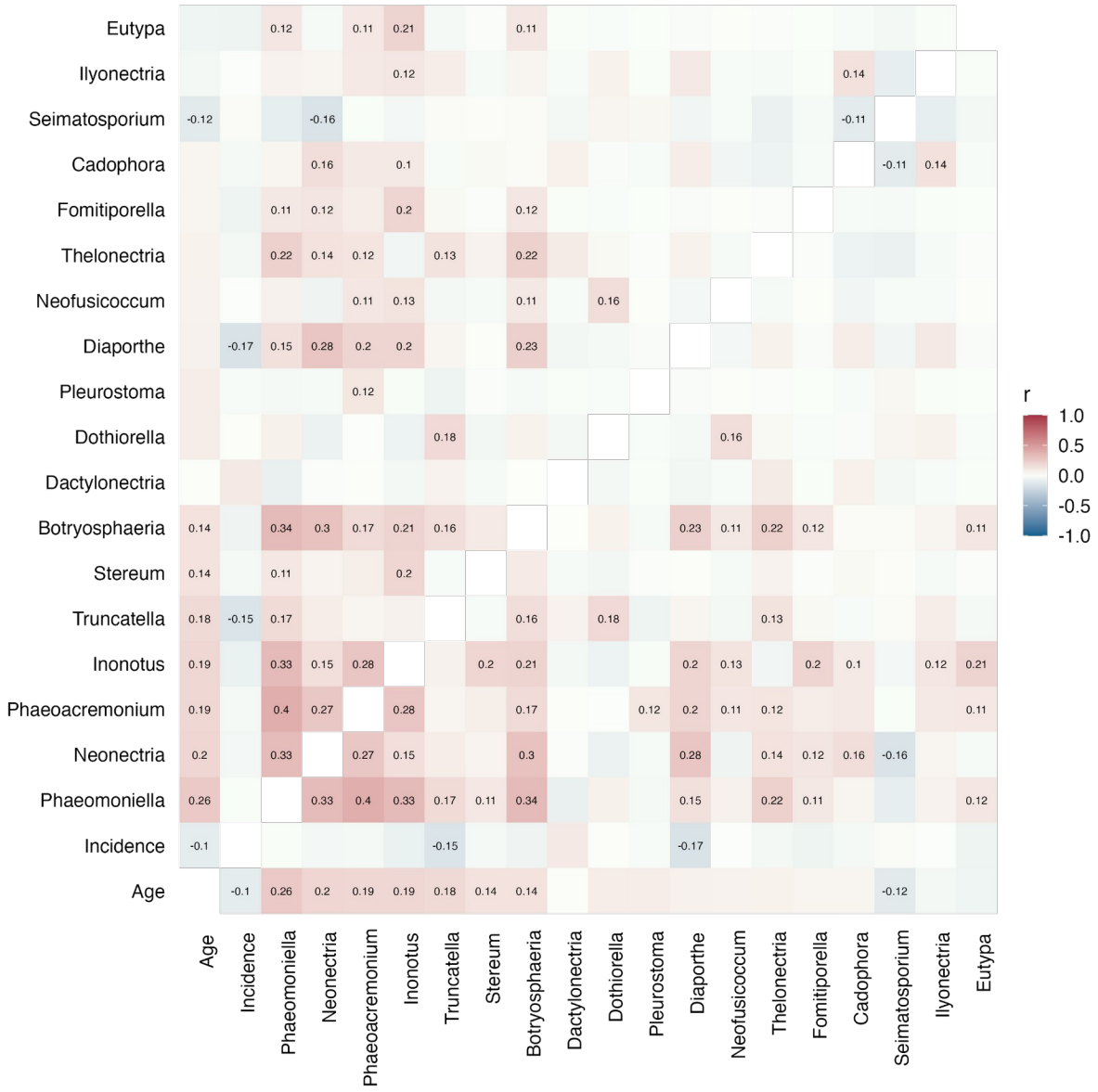


Figure 2: Spearman correlation heatmap of GTD genera. Age refers to vineyard age. Incidence refers to stratum level disease incidence. Included genera are associated with grapevine trunk disease. Significant (Benjamini-Hochberg corrected $p < 0.05$) are labeled with their $R^2$ value. Negative correlations are indicated by blue colors, positive correlations by red.

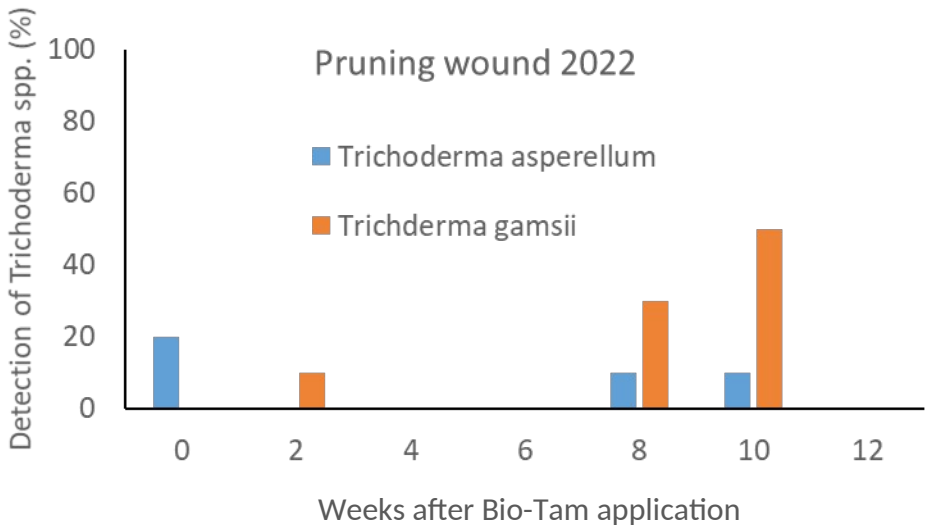


Figure 3: *Trichderma* spp. detection on pruning wound samples treated with Bio-Tam on pruning wounds on February 8, 2022.

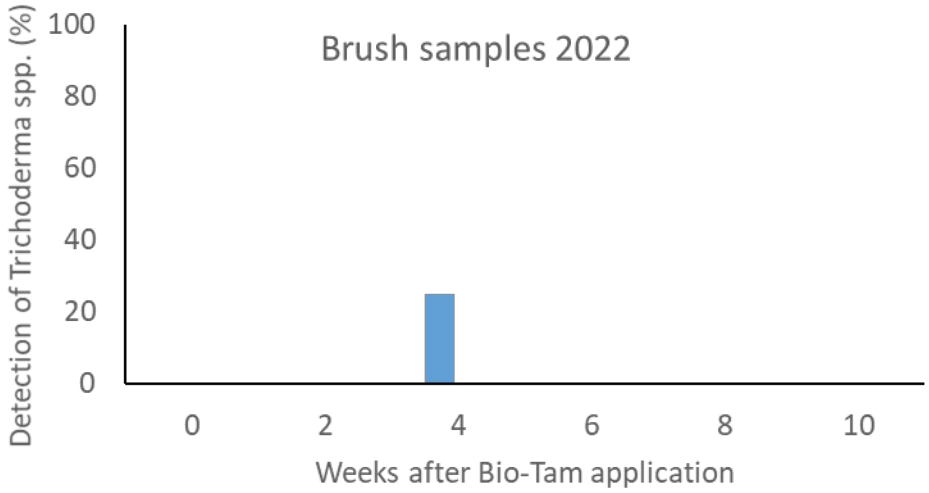


Figure 4: *Trichderma* spp. detection on brush samples treated with Bio-Tam as soil application on February 24, 2022.

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## Research Impact Statement

Please use layperson language when completing this form. This document will be read by members of Congress and it is important to be specific with details. Congress must understand the value of your work.

1. **Project Title**: Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards

2. **Principle Investigator & Cooperator(s)**:
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   **Cooperators:**
   Dr. Patricia Skinkis, Oregon State University, Corvallis, OR 97333
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   Leigh Bartholomew, Results Partners, 10525 OR-99W, McMinnville, OR 97128

3. **Research Objectives & Procedures**:
   **Objectives**:
   i. Use microbiome-based studies to understand the diversity of grapevine trunk disease pathogens and how factors such as the age and location of a vineyard affect their distribution.
   ii. Explore how different pruning wound and soil treatments affect the fungal microbiome on wound and soil samples.

   **Procedures**:
   *Objective 1: Use microbiome-based studies to understand the diversity of grapevine trunk disease pathogens and how factors such as the age and location of a vineyard affect their distribution.*
   Surveying vineyards. For surveying trunk diseases, 16 vineyards were identified in Southern Oregon (Rogue Valley) and 14 vineyards in Northern Oregon (Willamette Valley). 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 35 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 5th, 10th, or 20th 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.

Collecting wood tissue samples. Each flagged vine were sampled in order to identify pathogens. Using an electric drill, three holes were drilled into each flagged vine. One hole was towards the top of the vine, another was where the graft union is, 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. 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°C freezer.

Processing samples. The sample tissue were flash frozen in liquid nitrogen and homogenized using a bead mill. After homogenization, DNA were extracted from 100 mg of tissue sample. All samples were stored at -20°C until ready to use.

Next-generation sequencing. NGS was used to identify fungi in the samples. ITS primers were used to generate amplicons and Illumina indexing was performed to create indexed amplicon libraries. Amplicon libraries were pooled and sequenced on an Illumina MiSeq with a V3, 600 cycle kit (2 x 300 base pair, pair-ended reads).

*Objective 2: Explore how different pruning wound and soil treatments affect the fungal microbiome on wound and soil samples.*

Pruning treatments. To identify what effect different pruning practices have on the fungal microbiome, a vineyard was divided into rows representing three treatments with four replicates per treatment. The first treatment was to remove all pruning debris from the row. The second treatment was to leave the pruning debris in the row and to mow with a flail mower. The third treatment was to leave the pruning debris in the row, mow with a flail mower, apply Bio-Tam 2.0. Similarly, for the pruning wound treatments, three treatments were tested with four replicate vines per treatment. The first treatment was the wound treatment with VitiSeal, the second treatment was with Bio-Tam 2.0, and the third was non-treated control. All wound treatments were applied within 24 hrs of pruning in early February 2022 and the soil applications were made on late February.

Collecting pruning wound tissue samples. Samples from pruning wounds were collected every other week starting on February 8, 2022 and ending on May 3, 2022. Pruning shears were used to cut a piece of a pruning wound from a trunk. The sample was placed in a sterile 15 mL tube. Pruning shears were sterilized with ethanol between each cut that was made. One cut was made in

each treatment vine resulting in 12 samples per sampling period. All samples were taken to the lab where they were placed in the fridge at 4◦C until further processing.

Collecting soil samples. Samples from the soil were collected every other week starting on February 24, 2022 and ending on May 2, 2022. A soil auger was used to collect soil at a depth of 2 inches. The sample was placed in a plastic bag. The soil auger was sterilized with ethanol between each sample that was taken. Two samples were made in each row of each treatment resulting in 24 samples in total. For each row, one sample was taken at the 5th vine and the other sample was taken at the 17th vine. All samples were taken to the lab where they were placed in the fridge at 4◦C until DNA extraction.

Collecting brush samples. Samples of pruning brush were collected every other week starting on February 24, 2022 and ending on May 3, 2022. Samples were collected in each row of the second and third treatments resulting in 8 samples in total. For each row, brush was collected at the 5th vine and also at the 17th vine. The brush from both locations was combined and placed into a plastic bag. All samples were taken to the lab where they were placed in the fridge at 4◦C until further processing.

Extracting DNA from pruning wound tissue samples. The sample tissue was homogenized using a sterile mortar and pestle. It was then frozen in liquid nitrogen and further homogenized using a bead mill. Samples were grinded in the bead mill for homogenization, and DNA was extracted following a standard laboratory protocol.

Plating pruning wound samples. To monitor the fungal populations, each homogenized pruning wound sample was plated onto GI media by sprinkling the wood chips from the sample onto the plate. Plates were stored at room temperature under a cycle of 12 hours of light and 12 hours darkness until fungi had grown. Fungi were then analyzed and identified.

PCR to detect *Trichoderma* spp. Species specific primers for *Trichoderma asperellum*, and *T. gamsii* were used to amplify the DNA extracted from both pruning wound tissues and brush samples using qPCR. Data on *Trichoderma* spp. detection were recorded and converted into percentage detection per treatment sampling date.

4. **Total $ Funding through NCSFR**: $99,073

5. **Describe the Economic Impact and Benefits**. We believe that the potential long-term economic impact of this project is increased return from vineyards that will be productive for longer duration than vineyards infected with grapevine trunk diseases. Once the infection by GTD pathogens is established in a vineyard, age of the vineyard is significantly reduced due to vine death and yield losses. With our first objective, we have a better understanding on the group of microbes associated with grapevine trunks that are reported to cause GTD. Based on this research we identified that pathogens

belonging to Botryosphaeria die back and Esca disease complex are most common among already reported species in Oregon vineyards. We also identified two newer pathogens in the genera *Seimatosporium* and *Truncatella* were by far the most prevalent GTD associated taxa, not only in terms of relative abundance, but they were detected in all vineyards, and at least 82% of strata. This indicates that not only are these taxa in every vineyard, but they are widespread throughout each vineyard. Both genera have been reported in association with GTD around the world, including Iran, Chile, and France, and Northern California. These results help us to prioritize management practices targeted to these most common and aggressive pathogens. For GTD management, it is important that growers start preventative practices as early as possible to delay the infection. With thousands of acres of newly planted vineyards every year in Oregon, information from this objective is crucial in adapting preventative management practices for these vineyards.

In addition, with a microbiome based approach there is a potential that we will identify beneficial microbes that can be further studied for their benefit as a biological control agents. Strains of *Aureobasidium* have previously been shown to have antagonistic effects towards *Diplodia*, *Eutypa lata*, and *Neofusicoccum parvum*. In our study we identified *Aureobasidium* spp. was one of the most abundant fungal genera in grapevines and based on its negative correlation in particular, between an *Aureobasidium* spp and *Phaeomoniella*, indicates that *Aureobasidium* based biocontrols could be a fruitful starting point for further research on how to implement these new products in Oregon. Results from second objective further support a need of identifying locally adapted biological species to explore their biocontrol potential. The fact that two biocontrol species of *Trichoderma* were not recovered from applied tissues suggest that these species need adaptation to the environmental conditions where they are applied. This result could prevent the cost of biofungicide application without prior knowledge on their efficacy.

6. **Describe the Environmental Impact and Benefits**. This study has identified most abundant genera of fungal species present in grapevine environment. Except for one genera, the top ten abundant genera were saprophytic fungal species, some of which have already been in commercial use for their biofungicidal potential. Isolation of these fungal species and understanding their beneficial impact as fungal community to suppress pathogenic species provides significant environmental impact. If we can isolate organisms from nature and utilize their potential to manage diseases, this practice reduces the use of chemical fungicides and directly impacts the environment.

7. **Describe Other Impact and Benefits**. Other long-term benefit of this project is potential development of new products. As explained earlier, if we identify the microorganisms that are adapted to grapevine trunks and offer a competitive advantage over pathogenic species, there is a possibility that they can be further explored as a biological control agent and subsequent development of bio-control product.

8. **Concluding statement**. Since Grapevine Trunk Diseases are complex of multiple fungal pathogens, a traditional culture based identification of pathogens is very challenging. As a result, many pathogenic spp. that may play a bigger role in GTD development could have been overlooked. This study is important as we are using microbiome based approach in understanding these fungal species which is DNA based and reportedly more informative than traditional approaches. We captured a larger diversity of microbes present in grape vine trunks and reported the most commonly present species. It has a dual benefit of identifying the pathogenic species from which we can develop targeted disease management programs; and identifying beneficial species which can be further explored for their potential as a bio-fungicide for managing pathogenic species.

Northwest Center
FOR SMALL FRUITS RESEARCH

# NCSFR Conference Proceedings Form

*This form is used to gather project information to complete the conference proceedings document. Please do not change any of the formatting.*

**Project Title:** *Fungal microbiome associated with grapevine trunk diseases in Oregon vineyards*


**Authors:**
*Achala KC[1,2], Jessie Zimmerman[2], Posy Busby[2], and Monica Hernandez[1]*

**Institution:**
*[1]OSU Southern Oregon Research and Extension Center, Central Point, OR; [2] OSU Department of Botany and Plant Pathology, Corvallis, OR*



**Abstract/ Summary:**
Grapevine trunk disease (GTD) is a disease complex that consists of the largest group of fungal pathogens causing disease and progressive vine decline. Due to the complex nature of GTD pathogens, understanding the disease as a phenomenon of single species or multiple species infection is always challenging. In this research project, we proposed to study the diversity of GTD pathogens in grapevines of Northern and Southern Oregon; as well as to compare the different disease management practices including vineyard floor management and pruning wound protection. We explored how fungal communities vary along gradients of GTD disease incidence, vineyard age, and geography in Oregon and conducted a molecular field study, amplifying the ITS1 region of fungal rDNA extracted from vine stem tissue sampled from 29 vineyards in the Rogue and Willamette Valleys (n= 396). The most abundant genera, *Cladosporium* (30%), *Penicillium* (8%), *Alternaria* (5%), and *Aureobasidium* (5%), were not associated with core GTD genera, regardless of disease incidence. The GTD associated genera, *Seimatosporium* (7%), *Truncatella* (5%), *Phaeomoniella* (1.7%) and *Phaeoacremonium* (1.1%) were the most abundant. Other genera associated with GTD were least abundant ranging from <0.1 to 0.6%. At vineyard level, their prevalence ranged from 4 to 93% of the surveyed vineyards. The prevalence of *Botryosphaeria*, *Dothiorella*, *Neofusicoccum*, and *Diplodia*, the genera in the Botryosphaeriaceae was 79, 57, 18, and 4% of the vineyards respectively; *Neonectria*, *Thelonectria*, *Ilyonectria*, and *Dactylonectria* the genera causing Black Foot disease was 71, 32, 29, and 29% of the vineyards respectively; *Phaeomoniella*, *Phaeoacremonium*, *Cadophora*, *Fomitiporella*, *Pleurostoma*, the genera causing Esca disease was 93, 93, 79, 4, and 4% of the vineyards respectively. For the second objective, we implemented both pruning wound protection and soil treatments. From the culture based assessment of both pruning wound tissues and brush samples, many saprophytic fungal species such as *Altermaria*, *Fusarium*, *Aureobasidium*, *Penicillium*, and *Cladosporium*, were isolated from treated tissues. The isolations were random among the treatments and no significant effect of the treatments were observed on both wound and soil applications. No significant pathogenic species were isolated from any of the treatments including non-treated control. The PCR based detection resulted 20% detection of *Trichoderma asperellum* on the day of application, 10% detection on eight and ten weeks after application. Similarly, *T. gamsii* was detected on 10%, 30%, and 50% of the samples at two, eight, and ten weeks after application respectively. All these detections occurred on Bio-Tam applied pruning wounds. On the brush samples, only *T. asperellum* was detected from 25% of the samples where Bio-

Tam was applied after pruning brushes were mowed. These results suggest the applied treatments do not affect fungal microorganisms naturally present in the vines. The Bio-Tam application increased the chances of establishing biocontrol population in the vine tissues, however, their low isolation percentage suggests their adaptability and establishment in specific environments require further investigation. Unfortunately, the treatments effect on pathogen population could not be determined because none of the pathogenic species were isolated in culture-based media.