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ORIGINAL RESEARCH ARTICLE

Impact of water stress on *Phaeomoniella chlamydospora* abundance and Petri disease symptom development in young grapevines

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ABSTRACT

Phaeomoniella chlamydospora (*Pch*) is one of the main pathogens causing Petri disease, a grapevine trunk disease responsible for the decline and mortality of grapevines within a few years after planting. *Phaeomoniella chlamydospora* has been shown to be prevalent in asymptomatic grapevine nursery material, leading to the hypothesis that it may act as a latent pathogen, transitioning from an endophytic to a pathogenic phase under grapevine stress. To investigate this hypothesis, a two-year greenhouse and a four-year field experiment were conducted on young self-rooted 'Merlot', and 'Merlot' grafted onto 'SO4' rootstock, artificially inoculated with different spore concentrations of *Pch* and subjected to water stress. Additionally, the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* was inoculated in the soil in the greenhouse experiment to investigate its effects on abiotic stress mitigation and thus, disease development in water stressed and non-stressed grapevines. DNA was extracted from the grapevine wood, and Droplet Digital™ PCR was conducted to determine *Pch* abundance before and after the experiments. In the greenhouse, *Pch* abundance in inoculated grapevines was greater in water stressed grapevines treated with AM than in stressed grapevines without AM or in non-stressed grapevines. Basal necrosis was greater in grapevines inoculated with *Pch*. In the field, *Pch* abundance was not affected by water stress, but basal necrosis was greater in grapevines inoculated with a high spore concentration of the fungus. Symptoms resembling Petri disease developed in the third year of the field experiment, where water stress increased grapevine mortality. This study shows that water stress may increase *Pch* abundance and mortality in young grapevines within the first few years after planting.

KEYWORDS: Droplet Digital™ PCR, water stress, Petri disease, *Phaeomoniella chlamydospora*, *Vitis vinifera*, young vine decline

INTRODUCTION

Young vine decline, comprised by the grapevine trunk diseases black foot and Petri disease, is caused by several taxonomically unrelated xylem-colonizing fungi, including *Phaeomoniella chlamydospora*. These fungi induce vascular necrosis leading to poor grapevine vigour, lower fruit yield, and eventual grapevine mortality within the first few years after planting (Gramaje & Armengol, 2011). Young vine decline fungi have been shown to be prevalent in both asymptomatic and symptomatic wood of ready-to-plant nursery material, with significant variation in pathogen abundance between grapevines, different parts of the grapevine, and among nurseries (Berlanas *et al.,* 2020; Billones-Baaijens *et al*., 2013; Hrycan *et al.*, 2023; Maldonado-González *et al.*, 2020). In addition, many young vine decline fungi are commonly found in asymptomatic grapevine tissue in mature grapevines in vineyards (Gainza-Cortés *et al.*, 2020; González & Tello, 2011; Nerva *et al.*, 2019).

Most grapevines harbor young vine decline fungi, and if their presence always resulted in symptom development and grapevine mortality, viticulture would not exist in its current form today. Studies conducted in British Columbia (BC) showed that approximately 8 % of young grapevines were symptomatic for young vine decline across the province (Úrbez-Torres *et al.*, 2014), yet up to 100 % of ready-to-plant nursery grapevines sold in Canada are infected with young vine decline fungi (Hrycan *et al.*, 2023). It has been hypothesized that some Petri disease fungi may act as latent pathogens, living as endophytes within the grapevine microbiome until a trigger in the form of grapevine stress occurs, resulting in a transition to a pathogenic lifestyle (Hrycan *et al.*, 2020; Sieber, 2007).

Several observations have led to the hypothesis of an interaction between grapevine stress and disease development in grapevines affected by grapevine trunk diseases. For instance, Esca symptoms, in which *Pch* is thought to be involved, can vary yearly, and that variation has been correlated with climatic conditions (Marchi *et al.*, 2006; Péros *et al.*, 2008; Surico *et al.*, 2000). Esca foliar symptom development has also been correlated with higher internal necrosis (Maher *et al.*, 2012). A combination of pathogen infection level and grapevine stress is hypothesized to increase pathogen growth in the grapevine, leading to an increase in internal necrosis and mortality (Hrycan *et al.*, 2020; Sieber, 2007). Investigations into Esca symptom development and water stress found that Esca foliar symptoms were inhibited in water stressed grapevines (Bortolami *et al.*, 2021a), contrary to the hypothesis that grapevine stress results in symptom development and grapevine mortality. However, Esca is a complex disease with two forms, characterized as chronic foliar symptom development, which can vary year to year, and apoplexy, resulting in grapevine wilting and sudden collapse (Gramaje *et al.*, 2018). Esca-related apoplexy has been associated with high temperature and low rainfall conditions (Surico *et al.*, 2006). The differing conditions leading to chronic and apoplectic symptoms of Esca suggest the mechanisms for chronic symptom development may be different than apoplexy, which could be the result of grapevine stress. Like the apoplectic form in Esca, Petri disease in younger grapevines can result in sudden grapevine collapse (Gramaje & Armengol, 2011). Both diseases share some of the same fungi, which could respond similarly to stress in both young and older grapevines.

The effect of water stress on *Pch* growth in grapevines has been a research focus. *Phaeomoniella chlamydospora* can grow at osmotic water potentials as low as -8.3 MPa on potato dextrose agar, indicating the fungus is able to grow under severe water deficit (Whiting *et al*., 2001). Xylem sap and nutrient concentrations increase in infected grapevines subjected to water stress (Lima *et al*., 2017), which may provide the fungus with increased carbohydrates under water stress conditions. *Phaeomoniella chlamydospora* can also impact grapevine-water relations. 'Cabernet-Sauvignon' and 'Zinfandel' vines infected with *Pch* and subjected to water stress displayed lower leaf water potential compared with non-infected water stressed grapevines, indicating that these fungi may increase susceptibility to water stress (Edwards *et al.,* 2007a; Edwards *et al.*, 2007b). Effects of *Pch* on grapevine-water relations may be due to the formation of tyloses in the xylem, which can affect stem hydraulic conductivity and may impact grapevine survival during periods of stress (Bortolami *et al.*, 2021b). In combination, these studies indicate *Pch* can grow during periods of severe drought, increase grapevine susceptibility to water stress, and may gain access to increased resources for pathogen growth. While conditions for pathogen growth may improve under water stress, the implications of *Pch* abundance on grapevine health are not well understood.

Long-term or severe water stress may also limit grapevine defense responses to pathogen infection. Plant defense against biotrophic plant pathogens is thought to be mediated by salicylic acid pathways while defense against necrotrophic plant pathogens is activated by jasmonic acid pathways (Glazebrook, 2005). In grapevines subjected to long-term water stress, jasmonate content was slightly decreased (Zhan *et al.*, 2023). Water stress may also induce stomatal closure, limiting photosynthesis, and under severe water stress, xylem embolism may occur (Gambetta *et al.*, 2020), which may create more favourable conditions for pathogen growth and limit the amount of carbohydrates available to produce defense compounds.

Arbuscular mycorrhizal (AM) fungi are root symbionts that mitigate abiotic and biotic stresses in plants, including grapevine (Trouvelot *et al*., 2015). Under drought conditions, AM-colonized grapevines maintain greater leaf water content, chlorophyll content, and root and shoot dry weights (Ye *et al.*, 2022). Colonization of AM fungi may also increase jasmonic acid levels in the roots (Hause *et al.,* 2007), which may improve plant defense against necrotrophic pathogens. It has been shown that AM fungi can reduce black foot disease severity in grapevines, suggesting a potential use in disease management (Petit & Gubler, 2006). However, recent work

has reported increased internal necrosis and abundance of black foot disease fungi in mycorrhizal grapevines compared to non-mycorrhizal grapevines (Holland *et al.*, 2019; Vukicevich *et al.*, 2018), thus further investigation is required to understand the benefits or detriments of AM colonization in grapevine trunk disease infected grapevines.

Understanding how grapevine stress factors affect the growth of young vine decline fungi is essential in limiting the impact of the disease in vineyards, especially as water stress management in vineyards has other goals that need to be considered. Previous studies on *Pch* infection of grapevines have primarily focused on foliar symptom development, lesion length and phenotypical measurements to record the effect of water stress on disease development. Droplet Digital PCR™ technology has been used to quantify *Pch* in grapevines (Hrycan *et al.*, 2023) and can be used to help further our understanding of how pathogen abundance affects grapevine health. Accordingly, the main objectives of this study were to investigate if water stress increases *Pch* abundance and disease development in both the greenhouse and field, and to determine if AM fungi can mitigate water stress under greenhouse conditions, thus reducing disease development.

MATERIALS AND METHODS

1. Greenhouse experiment

1.1. Preparation of inoculated, rooted grapevines

Dormant 'Merlot' canes were collected in February 2020, from an experimental vineyard block located at the Summerland Research and Development Centre (SuRDC), British Columbia, Canada. The canes were placed in bags and stored in the dark at 2.5 °C until used. *Phaeomoniella chlamydospora* isolate SuRDC-1070, obtained from grapevines showing Petri disease symptoms in BC and kept in the SuRDC plant pathology fungal collection was used for this study (Úrbez-Torres *et al.*, 2014). Mycelial plugs from the isolate were retrieved from the collection and plated onto potato dextrose agar (PDA; Sigma-Aldrich, St Louis, MO) Petri plates and incubated at 22 °C in the dark for three weeks until mycelial colony developed. Sterile distilled water (2 mL) amended with a drop of Tween 80, a non-ionic surfactant to reduce clumping of spores, was pipetted onto the fresh mycelial colony and gently scraped with a sterile spoonula. The resulting spore suspension was vortexed and adjusted to 25,000 (25k), 5,000 (5k), and 1,000 (1k) spores per 10 μL with a haemocytometer. Viability of *Pch* was determined by plating a 10 μL suspension from each inoculum concentration in triplicate immediately following the vacuum inoculation procedure. Plates were incubated at 22 °C in the dark for seven days until fungal colonies were observed.

A total of 280 'Merlot' canes were pruned to three nodes (approximately 22 cm in length) and fifty-six canes were allocated to each of the five inoculum treatments: 25k, 5k, 1k, a water inoculated control which contained Tween 80 as in spore suspensions, and a non-inoculated control. Each vine was vacuum inoculated by pipetting 10 μL of allocated inoculum suspension onto the freshly cut base of each cutting and then applying vacuum at the other end of the cane for 1 s. Vacuum pressure was 71.5 mmHg measured with a manometer (Model 06-664-19, Fisher Scientific, Waltham, MA). Dormant canes were transferred to the greenhouse and rooted in five 2 L buckets per treatment containing 750 mL of water, changed twice weekly. Canes were placed in separate buckets by treatment. Buckets were placed on rooting mats set to 20 °C for six weeks. Upon budbreak, greenhouse lights were turned on and the greenhouse temperature was set to 20 °C.

On March 31, 2020, twenty-eight rooted cuttings from each treatment were randomly selected for planting. Rooted cuttings were planted in 3.78 L pots containing 3,000 g of pasteurized soil and with saucers underneath to retain water. The loamy sand y soil was collected from a field at SuRDC and pasteurized twice in a LANSA soil pasteurizer (Model LA, Johnson Machine Company LTD, Burlington, Ontario) set at a temperature reaching 72 to 88 °C (Trevors, 1996) following manufacturer's instructions. Half of the pots (14) were amended with 0.25 g of AGTIV specialty crops powder (Premier Tech Growers and Consumers, Quebec, Canada) containing 3,000 *Rhizophagus irregularis* spores following manufacturer's instructions.

1.2. Experimental design and grapevine health measurements

The 5 x 2 x 2 factorial combination of treatments was arranged in a completely randomized design and consisted of five levels of inoculum: 25,000 (25k), 5,000 (5k), and 1,000 (1k) *Pch* spores, water-inoculated (WIC), and non-inoculated (NIC); two levels of AM fungi: AM inoculated (AM+), and non-inoculated (AM-); and two levels of irrigation: fully irrigated (NWD) and 50 % irrigation (WD). Leaf water potential measurements were used to adjust irrigation volume based on WD measurements to maintain a minimum leaf water potential of -1.2 to -1.4 MPa and NWD above -1.0 MPA. There were seven replicate pots of each of the 20 treatment combinations with 140 grapevines total. In 2020, newly emerging shoots were removed to retain one shoot. As a result of pandemic restrictions during 2020, irrigation treatments were not applied and the potted grapevines were watered with 500 mL twice weekly until July 10, and three times a week onward until mid-September when water was gradually reduced to 50 mL three times a week in preparation for dormancy. Grapevines were placed in cold storage in the dark at 2.5 °C on December 18, 2020, and returned to the same greenhouse compartment on March 5, 2021, and equally watered until start of irrigation treatments on June 18, 2021. In 2021, two shoots were retained, and the grapevines were watered every second day starting June 18. NWD grapevines received 500 ml at each irrigation event from June 18 to August 14; 400 ml from August 18 to August 28; and 300 ml from September 1 until experiment concluded on September 13, 2021. WD grapevines were watered on the same days as NWD grapevines but with one-half the volume.

All grapevines were fertilized with the same amount of 20-20-20 NPK fertilizer bi-weekly.

Leaf water potential, leaf chlorophyll content, and soil matric potential were measured in 2021. Mid-day leaf water potential was measured bi-weekly between 1:00 pm to 3:00 pm from June 29 to September 9, 2021, with a pressure chamber (Model 610, PMS Instrument Co. Corvallis, OR). Leaf chlorophyll content was measured between 9:00 am to 11:00 am bi-weekly from May 25 to September 9, 2021, with a leaf chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Aurora, IL). Soil matric potential was measured using soil tensiometers (Model T5, Meter Group, Pullman, WA) inserted in the soil of six each of NWD and WD pots randomly distributed in the greenhouse. Measurements were taken every 15 minutes and averaged daily from June 8 to September 9, 2021. On December 18, 2020, and at the end of the experiment in 2021, the number of grapevine nodes were counted, and grapevines were pruned to retain two buds before the prunings were weighed as a measure of growth. Root weight was measured at the end of the experiment. The prunings and roots were dried in an environmental chamber (Model 217502, Hotpack, Anaheim, CA) at 65 °C until dry weights were consistent over a 24 h period.

1.3. Trunk measurements

Total basal surface area (whole cross-section) and necrosis measurements were conducted upon conclusion of the experiment at one cm from the base of each grapevine as described in Hrycan *et al.* (2023) with the following alterations: Images were set at 8-bit colour and a threshold analysis was conducted in Fiji ImageJ version 1.53t. Threshold numbers were set values for the degree of shading from black to white (0-255). Black wood discolouration (Black necrosis) was set at 5-45, brown wood discolouration (Brown necrosis) was set at 46-80, and black and brown wood discolouration (total necrosis) was 5-80 (Figure 1).

1.4. Phaeomoniella chlamydospora absolute quantification

In order to verify presence of *Pch* in the grapevine and possible *Pch* growth between inoculation and planting, three grapevines from each treatment were randomly selected 24 h after vacuum inoculation and at the time of planting (six weeks post-inoculation). Grapevines were cut into 2 cm sections from 0-18 cm from the base. At the end of the experiment, a 2 cm section of wood was cut 2 cm above the base. Individual sections were surface sterilized via submersion in 70 % ethanol for 30 s, 10 % bleach for 1 min, and sterile water 2 x 1 min washes. Sections were cut in half longitudinally, finely chopped with a razor blade, and 0.2 g were placed in Qiagen DNeasy PowerSoil DNA extraction tubes (Qiagen, Hilden, Germany). The other half of each section was finely cut and plated on PDA amended with 0.1 mg/mL tetracycline hydrochloride (PDA-tet) to re-isolate *Pch* as described in Hrycan *et al*. (2023). A Qubit 1.0 Fluorometer dsDNA HS Qubit assay (Thermofisher, Waltham, MA) was used to calculate DNA concentration.

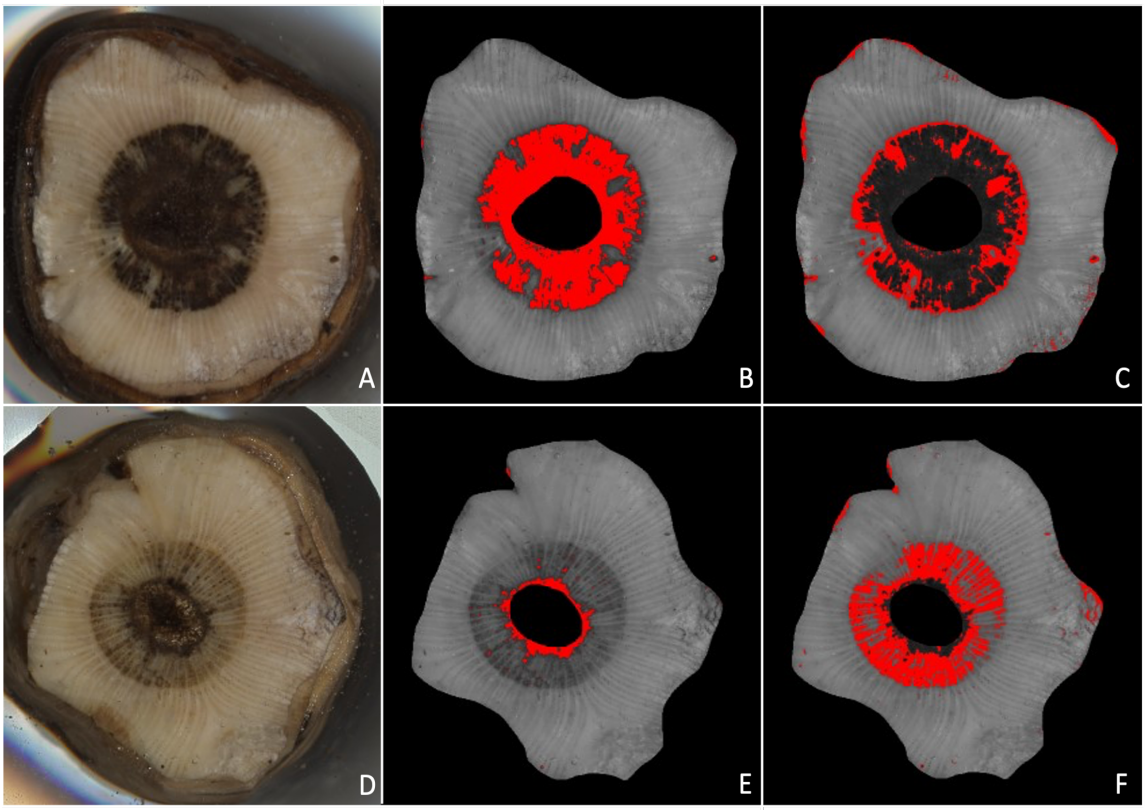


FIGURE 1. Scan showing basal necrosis of a *Phaeomoniella chlamydospora* inoculated grapevine (A, B, C) and non-inoculated grapevine (D, E, F). Threshold analysis of black necrosis set at 5-45 (B, E) and brown necrosis set at 46-80 (C, F) using ImageJ software.

Quantification of *Pch* was conducted with a Droplet Digital™ PCR (ddPCR) system (Bio-Rad Laboratory, Hercules, CA) using ITS primers (Martín *et al.*, 2012) with the addition of a probe (Hrycan *et al.*, 2023). Reactions and analysis were conducted as described in Hrycan *et al.* (2023). *Pch* abundance quantification results were standardized via calculation of gene copies/ng of DNA (Hrycan *et al.*, 2023). *Pch* abundance analysis was conducted on grapevine sections at time of inoculation, time of planting (Figure S1) and at the end of the experiment.

1.5. Arbuscular mycorrhizal fungal colonization

Fresh roots were collected from one replicate of each treatment at the end of the experiment. Roots were frozen in liquid nitrogen, crushed with a pestle and mortar, and 0.2 g of crushed root material was placed in Lysing Matrix A (MP Biomedicals Irvine, CA) tubes and extracted using the Qiagen DNeasy Powerlyzer Powersoil kit according to manufacturer's specifications. The Qiagen DNeasy Powerlyzer Powersoil kit was used due to manufacturer discontinuation of the DNeasy Powersoil kit. DNA concentration was calculated using a Qubit 1.0 fluorometer as previously described and ddPCR was conducted on each sample to determine presence or absence of the AM fungi using primers and probe described in Kokkoris *et al.* (2019). Black Sheaffer ink root staining was conducted to visually confirm presence of AM fungi via microscope (Vierheilig *et al.*, 1998).

2. Field experiment

2.1. Grapevine material, inoculum preparation and vacuum inoculation

Dormant 'Merlot' and dormant 'SO4' canes were collected from vineyard blocks at SuRDC in February 2020 and stored in bags in the dark at 2.5 °C. Mycelial plugs containing *Pch* (SuRDC-1070) obtained from the SuRDC Plant Pathology fungal collection were plated, incubated, and used to create spore suspensions containing 25k and 2,500 (2.5k) spores as previously described. Canes were pruned to retain three nodes (22 cm) and 10 μl aliquots were vacuum inoculated (Rooney & Gubler, 2001) from the base with WIC, 2.5k, or 25k as previously described. *Phaeomoniella chlamydospora* viability was determined by plating 10 μL of each suspension in triplicate onto PDA as previously described.

Inoculated dormant 'SO4' canes, inoculated 'Merlot' canes and non-inoculated 'Merlot' canes used for grafting were dipped in 10 % bleach for 1 s. 'SO4' canes were entirely disbudded while inoculated 'Merlot' canes were disbudded below the top bud. Non-inoculated 'Merlot' canes were cut to retain one bud and were omega grafted onto 'SO4' grapevines. The grafted and non-grafted grapevines were then dipped in Stim-Root rooting hormone (Premier Tech Home and Garden, Brantford, ON) and layered in bins containing moist perlite, separated by treatment, and stored at room temperature in the dark for four weeks. On April 6, 2020, callused grapevines were removed from perlite, the top bud was dipped in wax, and planted in individual cartons measuring 35 x 8.5 x 8.5 cm containing 50 % perlite and 50 % Sunshine Mix #4 (Sun Gro Horticulture, Agawam, MA). The grapevines were placed in the greenhouse at 20 °C and watered evenly 3x per week. The rooted grapevines were planted on July 9, 2020, in an experimental field plot at the SuRDC.

2.2. Experimental design

The experiment was conducted in a field site at the SuRDC containing loamy sand y soil with row x grapevine spacing of 2.5 m x 0.93 m. Treatments were allocated in a blocked split-plot design as follows: Each block consisted of twelve vines and each block was replicated eight times. Whole-plot treatments of fully irrigated (NWD, no water deficit) or 50 % irrigation (WD, water deficit) were randomly applied to half of each block. The six vine-treatment combinations of vine type: self-rooted or grafted, and inoculum: WIC, 2.5k, and 25k were randomly allocated within each irrigation whole-plot. NWD plots had two Toro 0.5 GPH pressure compensating emitters (Toro, Bloomington, MI) on each vine while WD plots had one emitter per vine. Grapevines were irrigated Mon-Wed-Fri with equal timing between treatments with the duration of each irrigation run adjusted according to leaf water potential measurements. Grapevines were fertilized each spring equally with 15 g of 14-14-14 slow-release fertilizer placed under each grapevine. Grapevines were shoot thinned to one shoot in 2020, and three shoots in 2021 and 2022. The grapevines were removed in the spring of 2023.

2.3. Grapevine physiology and growth

No measurements were taken in 2020 due to pandemic restrictions. Mid-day leaf water potential was measured with a pressure chamber (Model 610, PMS Instrument Co. Corvallis, OR) in 2021 and 2022. In 2021, mid-day leaf water potential was measured on one leaf per grapevine on September 9. In 2022, leaf water potential was measured bi-weekly on Tuesday or Thursday from July 19 to August 16, and then weekly until September 15.

Leaf chlorophyll content was measured on ten leaves per grapevine and averaged, on August 17 and September 8 in 2021 due to poor growth in 2021, weekly from July 11 to September 26 in 2022, and on May 29 in 2023 with a chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Aurora, IL). Pruned shoots were collected and node numbers were counted each growing season. The shoots were dried at 65 °C and weighed as previously described. Development of young vine decline-like foliar symptoms and grapevine mortality were recorded each year. On May 30, 2023, grapevines were uprooted and placed in cold storage in the dark at 2.5 °C for analysis. Roots were cleaned in fresh water, removed from the grapevine, and dried at 65 °C until weights were consistent over two days.

2.4. Trunk measurements

Total basal surface area (entire cross-section) and necrosis measurements were taken 1 cm from the base as previously described. Trunk diameter was measured below the primary bud of the initial cane with a digital caliper (Model CD-6" C, Mitutoyo Corp. Japan).

2.5. *Phaeomoniella chlamydospora* absolute quantification

To verify presence of *Pch*, three grapevines from each treatment were randomly selected at time of planting, 13 weeks post-vacuum inoculation. The Qiagen DNeasy Powersoil kit was used for DNA extractions and DNA concentration and *Pch* abundance was analyzed as previously described. At the end of the experiment, grapevines were drilled at 0-1 cm, 2-4 cm, and 4-6 cm locations from the base using a sterile 1/8" drill-bit for re-isolation of *Pch*. 0.1 g of wood obtained from each drilling was used, placed in Lysing Matrix A tubes, frozen in liquid nitrogen, and macerated in a FastPrep-24™ at 6.0 m/s x 40 s x3. DNA was extracted using the Qiagen DNeasy Plant Mini kit according to manufacturer's instructions. The DNA extraction kit was changed due to manufacturer discontinuation of the Qiagen DNeasy Powersoil kit. The DNA concentration and ddPCR were conducted as previously described. Each grapevine section was quantified separately and the sum of copies/ng DNA for 0-1, 2-4, and 4-6 cm was used for analysis to compare *Pch* abundance between grapevines.

2.6. Statistical analysis

For the greenhouse experiment, a three-way analysis of variance (ANOVA) was used to assess individual main-factor and interaction effects of *Pch* inoculum concentration, irrigation level and AM inoculation on necrosis, pruning and root weights, node number, chlorophyll, leaf water potential and soil matric potential. Tukey's Honest Significant Difference test set at 5 % significance was used for post-hoc comparison of inoculum level x treatment combinations. Residuals were tested for normality with the Shapiro-Wilk test. An ANOVA was used to assess the effects of WIC and NIC on all measurements. Spearman's coefficients of correlation were used to assess relationships between inoculum level and response variables.

Field experiment data (pruning weights, node number, chlorophyll, leaf water potential) were analyzed with a linear mixed effect ANOVA model considering the blocked split-plot design and fit with REML with the package nlme. Fixed effects were irrigation treatment, inoculum and grafting treatment (self-rooted or grafted), while block was considered as a random effect. Irrigation treatments were designated whole-plot experimental units, and the inoculum x rootstock vine treatment combinations were sub-plot experimental units nested within whole-plots. Significance was tested with a Type 3 sums of squares. Residuals were inspected visually. Tukey's Honest Significant Difference test set at 5 % was conducted on significant interactions using the emmeans package in R Version 4.3.0 (Lenth, 2023; Pinheiro & Bates, 2000; R Core Team, 2023). Correlations between inoculum level and other response variables were assessed using a Spearman correlation coefficient. A chi-squared test was used to determine effects of grafting treatment (self-rooted *vs*. grafted), inoculum, and irrigation treatment on proportions of grapevines expressing symptoms and proportional grapevine mortality.

RESULTS

1. Greenhouse experiment

1.1. Grapevine physiology and growth measurements

In 2021, leaf chlorophyll content differed with irrigation x date ($p < 0.001$). Leaf chlorophyll content was 4.1 % and 2.5 % higher in WD than NWD grapevines on July 29 and September 9 respectively (Table S1), while no other treatment effects were observed. Mid-day leaf water potential differed

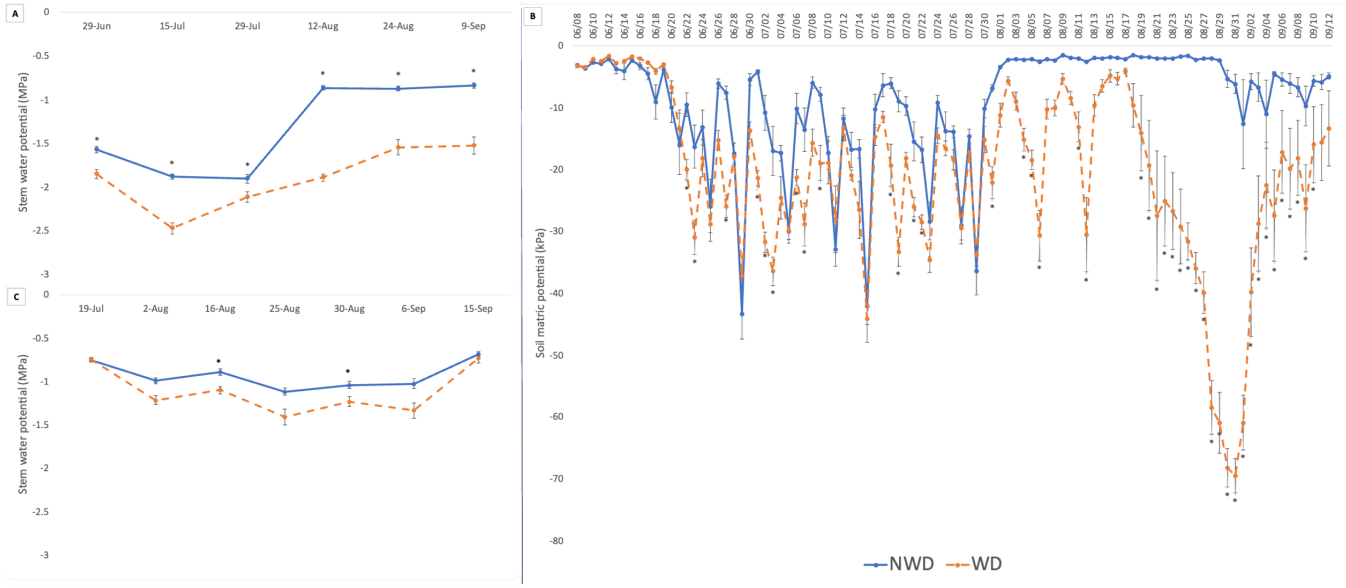


FIGURE 2. Mean greenhouse leaf water potential (A) and greenhouse soil matric potential (B) measured over time in 2021 and mean field leaf water potential (C) measured over time in 2022 in 100% irrigated grapevines (NWD) and 50 % irrigated grapevines (WD). Significant difference at p = 0.05 according to ANOVA. Error bars represent standard error of the mean for each date.

TABLE 1. Analysis of variance summary of effects of inoculum level, irrigation level, AM (+/-), and their interactions on *Phaeomoniella chlamydospora* abundance, black, brown, and total necrosis, and basal surface area at the end of the experiment in the greenhouse.

		Inoculum	Irrigation	AM	Inoculum x Irrigation	Inoculum x AM	Irrigation x AM	Inoculum x Irrigation x AM
Pch abundance	Df	2	1	1	2	2	1	2
	F value	0.989	23.373	4.416	2.463	0.120	5.857	0.401
	Pr (> F)	0.377	< 0.001	0.039	0.092	0.887	0.018	0.671
Black necrosis	Df	4	1	1	4	4	1	4
	F value	240.757	10.094	3.452	1.221	1.802	1.127	0.315
	Pr (> F)	< 0.001	0.002	0.066	0.306	0.133	0.291	0.868
Brown necrosis	Df	4	1	1	4	4	1	4
	F value	66.728	1.576	1.673	2.182	2.144	0.378	1.443
	Pr (> F)	< 0.001	0.212	0.199	0.075	0.080	0.540	0.225
Total necrosis	Df	4	1	1	4	4	1	4
	F value	10.473	4.758	1.172	0.434	2.120	0.682	0.940
	Pr (> F)	< 0.001	0.031	0.281	0.784	0.082	0.411	0.444
Basal surface area	Df	4	1	1	4	4	1	4
	F value	3.980	10.740	1.402	1.028	2.360	0.691	1.476
	Pr (> F)	0.004	0.001	0.239	0.396	0.058	0.408	0.214

based on irrigation treatment ($p < 0.001$), date ($p < 0.001$), and irrigation treatment x date ($p < 0.001$). Mid-day leaf water potential was lower in WD than NWD grapevines at each time point (Figure 2A) and overall (WD: -1.9 MPa; NWD: -1.32 MPa), but no effects of inoculum ($p = 0.817$) or AM fungi were observed. ($p = 0.709$). Irrigation treatment x AM had no effects ($p = 0.547$). Mean leaf water potential of all measurement dates was NWD/AM-: -1.30 MPa, NWD/AM+: -1.34 MPa, WD/AM-: -1.90 MPa, WD/AM+: -1.89 MPa. Soil matric potential differed based on irrigation x date ($p < 0.001$), with soil matric potential lower in response to WD treatment (-19.75 kPa) compared with NWD (-7.58 kPa) overall. Soil matric potential daily average was lower in response to WD treatment compared with NWD over time, reaching a minimum of -72.35 kPa (Figure 2B) in the soil of WD treated pots.

Pruning weights differed based on irrigation x year ($p < 0.001$), with dry pruning weight reduced by 10.4 % due to WD treatment in 2021. Node number also differed with irrigation x year ($p < 0.001$) with a 22.4 % reduction in node number in WD treated grapevines. Node number also different based on irrigation x AM inoculation ($p = 0.047$) with mean node number higher in NWD/AM+ grapevines and NWD/AM- than both WD/AM+ and WD/AM- grapevines in 2021 (Table S2). Dry root weight differed based on irrigation treatment ($p < 0.001$) at the end of the experiment with WD treated grapevine dry root weight 17.7 % lower than in NWD treated grapevines (Table S2). Inoculation with AM had no effect on pruning weight, node number, or root weight in either year.

27 % of AM+ grapevines had roots colonized whereas no AM- grapevines were colonized with AM fungi. Visual confirmation via compound microscope confirmed the presence of AM fungi in the roots.

1.2. Quantification of Phaeomoniella chlamydospora

Phaeomoniella chlamydospora was not detected with ddPCR at 2-4 cm from the base at time of inoculation; however, re-isolations confirmed the presence of *Pch* in 100 % of inoculated grapevines. Abundance of *Pch* at 2-4 cm from the base was correlated with inoculum levels (Spearman r = 0.91), while WIC and NIC grapevines were negative for *Pch*. Re-isolations confirmed the presence of *Pch* at time of planting

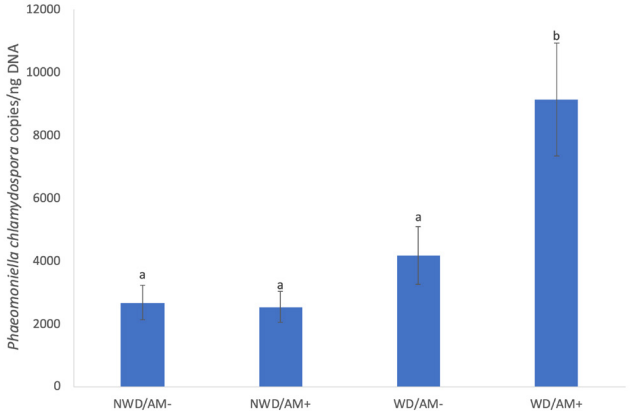


FIGURE 3. Interaction effect of fully irrigated grapevines (NWD), 50 % irrigated grapevines (WD), arbuscular mycorrhizal fungal inoculated (AM+) and non-inoculated (AM-) on mean abundance of *Phaeomoniella chlamydospora* (copies/ng DNA) in *Pch* inoculated grapevines at the end of the greenhouse experiment. Treatments with the same letter are not significantly different ($p = 0.05$) according to Tukey's significance test. Error bars represent standard error of the mean.

TABLE 2. Mean + standard error percentage black, brown, and total necrosis, and mean basal surface area of grapevines receiving each level of inoculum and irrigation treatment in the greenhouse.

Treatment[a]	Black necrosis	Brown necrosis	Total necrosis	Basal surface area (mm^2)
NIC	1.3% ± 0.2 a	17.8% ± 1.3 a	19.1% ± 1.3 a	419.1 ± 22.2 a
WIC	1.0% ± 0.1 a*	16.1% ± 0.9 a*	17.1% ± 0.9 a*	352.9 ± 15.5 a*
1k	15.9% ± 1.0 *	8.1% ± 0.4 *	24.0% ± 1.1 *	385.1 ± 16.7 *
5k	17.9% ± 1.0 *	7.7% ± 0.5 *	25.6% ± 1.3 *	362.5 ± 17.0 *
25k	15.3% ± 1.0 *	6.4% ± 0.4 *	21.7% ± 1.1 *	431.2 ± 18.8 *
NWD	9.5% ± 0.9 B	11.0% ± 0.6 A	20.4% ± 0.7 B	416.1 ± 12.5 B
WD	12.0% ± 1.1 A	10.9% ± 0.9 A	22.9% ± 0.9 A	364.2 ± 10.7 A

[a] NIC: non-inoculated control, WIC: water inoculated control, 1k: 1,000 spores, 5k: 5,000 spores, 25k: 25,000 spores, NWD: no water deficit (100% irrigation), WD: water deficit (50% irrigation).

Same lowercase and uppercase letter in each column represent no significant differences ($p = 0.05$) according to Tukey's significance test. * indicates effect of inoculum ($p < 0.05$) according to Spearman correlation test.

TABLE 3. Analysis of variance summary of effects of grafting treatment, inoculum, irrigation treatment, and their interactions on *Phaeomoniella chlamydospora* abundance, black, brown, and total necrosis, and basal surface area at the end of the field experiment.

		Cultivar	Inoculum	Irrigation	Grafting treatment x Inoculum	Grafting treatment x Irrigation	Inoculum x Irrigation	Grafting treatment x Inoculum x Irrigation
Pch abundance	Df	1	2	1	2	1	2	2
	F value	3.105	8.737	0.055	1.951	0.425	0.165	0.069
	Pr (> F)	0.085	< 0.001	0.824	0.155	0.518	0.849	0.933
Black necrosis	Df	1	2	1	2	1	2	2
	F value	15.828	7.287	0.899	2.291	0.876	0.149	0.444
	Pr (> F)	< 0.001	0.002	0.387	0.114	0.355	0.862	0.644
Brown necrosis	Df	1	2	1	2	1	2	2
	F value	2.319	1.540	0.011	0.353	0.792	3.930	0.241
	Pr (> F)	0.135	0.226	0.921	0.705	0.378	0.027	0.787
Total necrosis	Df	1	2	1	2	1	2	2
	F value	14.266	6.606	2.309	0.198	2.382	1.464	1.502
	Pr (> F)	< 0.001	0.003	0.189	0.821	0.130	0.243	0.235
Basal surface area	Df	1	2	1	2	1	2	2
	F value	9.642	7.289	1.355	2.876	1.929	0.557	3.067
	Pr (> F)	0.003	0.002	0.297	0.068	0.172	0.577	0.057
Trunk diameter	Df	1	2	1	2	1	2	2
	F value	0.926	1.918	0.839	0.547	1.221	0.235	2.676
	Pr (> F)	0.341	0.160	0.402	0.583	0.276	0.792	0.081

in 100 % of inoculated grapevines while the fungus was not isolated from WIC or NIC grapevines. At the end of the experiment, *Pch* was not detected in WIC or NIC grapevines with ddPCR or traditional plating, but it was isolated from 96 % of 25k, 93 % of 5k, and 90 % of 1k grapevines. Initial inoculum quantity was also correlated with *Pch* abundance at the end of the experiment (Spearman r = 0.63). Mean *Pch* abundance for 1k was 3,300 copies/ng, 5k was 5,614 copies/ng, 25k was 4,996 copies/ng. WD treatment and AM fungi had a positive effect on pathogen growth in *Pch* inoculated grapevines (Table 1); *Pch* abundance was greater in WD/AM+ grapevines than in WD/AM-, NWD/AM+ and NWD/AM- grapevines (Figure 3).

1.3. Trunk measurements

Basal necrosis measurements indicated main-factor effects of inoculum and irrigation on black and brown necrosis, total necrosis, and basal surface area (Table 1). Coefficients of correlation between inoculum level and black necrosis ($p < 0.001$), brown necrosis ($p < 0.001$), total necrosis ($p = 0.002$), and basal surface area ($p = 0.027$) were 0.53, -0.67, 0.29 and 0.21, respectively, showing a correlation between *Pch* inoculated and WIC/NIC grapevines (Table 2). Black necrosis,

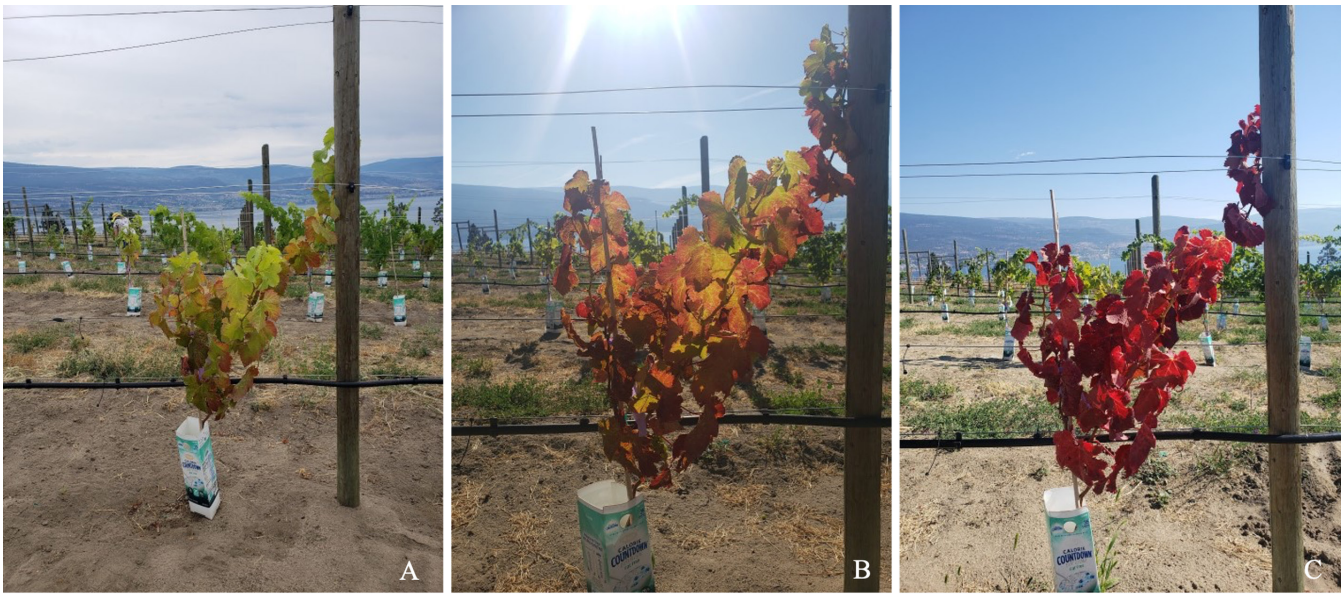


FIGURE 4. Vine decline symptom progression in 2022 on August 9 (A), August 30 (B), and September 27 (C) for a grapevine infected with 25k spores of *Phaeomoniella chlamydospora* subject to 50 % irrigation treatment.

total necrosis, and basal surface area were impacted by irrigation level (Table 1). Black and total necrosis were greater in grapevines containing *Pch* and in WD grapevines, while grapevines containing *Pch* had less brown necrosis compared with WIC grapevines (Table 2). Basal surface area was greater in NWD grapevines compared with WD grapevines regardless of inoculum level (Table 2).

2. Field experiment

2.1. Grapevine physiology and growth measurements

Leaf chlorophyll content was affected by inoculum x grafting treatment ($p = 0.022$) and irrigation treatment ($p = 0.013$) in 2021. Spearman correlation r was -0.27 for the effect of inoculum on grafted leaf chlorophyll content in 2021 and was not significant for self-rooted ($p = 0.481$). NWD grapevines showed overall a 4.8 % lower leaf chlorophyll content than WD grapevines. In 2022, grafting treatment had main-factor effects ($p = 0.003$) on leaf chlorophyll and was 2.7 % greater in self-rooted than grafted. Inoculum had no impact on leaf chlorophyll in 2022 or 2023 (Table S3).

Mid-day leaf water potential was affected by irrigation treatment ($p = 0.020$) in 2021. In 2022 the irrigation x date ($p = 0.003$) and irrigation x grafting treatment ($p = 0.001$) interactions affected mid-day leaf water potential. Mid-day leaf water potential was lower in WD grapevines (-0.85 MPa) than NWD grapevines (-0.79 MPa) on September 9, 2021, and overall, in 2022 (WD = -1.11 MPa, NWD = -0.93 MPa) according to a repeated measures ANOVA. In 2022, WD grapevines had lower leaf water potential on August 25 and September 6 (Figure 2C). Mean leaf water potential did not differ between self-rooted (-0.84 MPa) and grafted (-0.80 MPa) grapevines in 2021 but self-rooted leaf water potential (-0.99 MPa) was greater than grafted (-1.11 MPa) in 2022.

Dry pruning weights ($p < 0.001$) and node numbers ($p = 0.002$) had main factor effects of grafting treatment x year. Self-rooted dry pruning weight was 41.4 % lower than grafted in 2021, 44.5 % in 2022, and 64.7 % in 2023 (Table S4). Self-rooted node number was 30.2% lower in 2022 (self-rooted: 26.6, grafted 38.1), and 38.8 % lower in 2023 (self-rooted: 42.9. grafted: 70). Dry root weight differed due to grafting treatment ($p = 0.007$) and was 45% lower in self-rooted than grafted upon conclusion of the experiment, but irrigation treatment and inoculum had no effect (Table S4).

2.2. Quantification of *Phaeomoniella chlamydospora*

Phaeomoniella chlamydospora abundance was correlated with inoculum at time of planting ($r = 0.73$). However, ddPCR analysis showed WIC grapevines to be infected with *Pch* at time of planting. 25k abundance was 69,253 ± 15,370, 2.5k was 22,055 ± 9,967, WIC was 8,685 ± 5,758. Re-isolations confirmed the presence and viability from the first 0-6 cm of *Pch* in 66 % of 25k and 2.5k grapevines, and 0 % of WIC grapevines.

Phaeomoniella chlamydospora abundance was impacted by initial inoculum only ($p < 0.001$; $r = 0.46$). At the end of the experiment, 25k grapevines contained 65,605 ± 14,669 copies/ng, 2.5k contained 44,227 ± 9,889 copies/ng and WIC contained 21,167 ± 4,233 copies/ng.

2.2. Trunk measurements

Black necrosis was positively correlated with inoculum for self-rooted ($p = 0.036$; $r = 0.40$), grafted ($p < 0.001$; $r = 0.54$), and for mean of self-rooted and grafted ($p = 002$; $r = 0.37$) [Table 4]. Brown necrosis was not different between treatments (Table 4). Total necrosis was positively correlated with inoculum for self-rooted ($p = 0.021$; $r = 0.43$), grafted ($p = 0.07$; $r = 0.43$), and mean of self-rooted and grafted ($p < 0.001$; $r = 0.43$) [Table 4]. Basal surface area was negatively correlated with inoculum for the mean of self-rooted and grafted ($p = 0.033$; $r = -0.26$), while trunk diameter was not different between treatments (Table 4).

TABLE 4. Mean percent black, brown, total necrosis, and mean basal surface area and standard error of the mean of each grafting treatment containing each level of inoculum and in each level of irrigation in the field.

Grafting treatment	Inoculum[a]	Irrigation[b]	Black necrosis	Brown necrosis	Total necrosis	Basal surface area (mm^2)
Self-rooted	WIC	NWD	9.14 ± 2.52 a	9.57 ± 1.46 a	18.71 ± 3.70 a	189.52 ± 25.50 a
		WD	13.10 ± 1.93 a	12.41 ± 2.62 a	24.63 ± 3.71 a	164.41 ± 9.55 a
		Mean	11.12 ± 1.69 *	10.86 ± 1.52	21.40 ± 2.75 *	178.11 ± 14.53
	2.5k	NWD	6.65 ± 1.86 a	8.94 ± 2.22 a	15.60 ± 4.08 a	219.22 ± 54.95 a
		WD	28.98 ± 11.21 a	18.94 ± 6.86 a	47.91 ± 17.82 a	171.66 ± 28.97 a
		Mean	17.82 ± 6.92 *	13.94 ± 4.01	31.75 ± 10.78 *	195.44 ± 32.18
	25k	NWD	21.81 ± 5.53 a	17.59 ± 3.19 a	39.41 ± 8.54 a	151.16 ± 18.62 a
		WD	37.19 ± 11.80 a	13.67 ± 3.49 a	50.87 ± 12.79 a	107.09 ± 20.61 a
		Mean	28.65 ± 6.59 *	15.85 ± 2.44	44.50 ± 7.64 *	131.57 ± 15.63
Grafted	WIC	NWD	0.91 ± 0.23 a	8.42 ± 1.06 a	9.32 ± 1.06 a	155.58 ± 14.41 a
		WD	12.71 ± 8.84 a	12.95 ± 3.14 a	25.66 ± 11.91 a	107.20 ± 7.17 a
		Mean	6.81 ± 4.87 *	10.68 ± 1.82	17.49 ± 6.60 *	131.39 ± 10.33
	2.5k	NWD	7.23 ± 1.64 a	7.39 ± 0.72 a	14.62 ± 1.97 a	133.83 ± 16.73 a
		WD	5.24 ± 1.88 a	8.27 ± 0.63 a	13.51 ± 1.74 a	129.09 ± 3.47 a
		Mean	6.24 ± 1.28 *	7.83 ± 0.50	14.07 ± 1.32 *	131.46 ± 8.57
	25k	NWD	7.72 ± 1.17 a	21.34 ± 8.56 a	30.69 ± 9.59 a	111.14 ± 10.76 a
		WD	9.58 ± 3.23 a	7.81 ± 0.87 a	17.39 ± 2.91 a	129.60 ± 15.77 a
		Mean	8.74 ± 1.86 *	14.57 ± 4.59	24.04 ± 5.22 *	120.37 ± 10.10
Mean	WIC	NWD	5.02 ± 1.74 a	8.99 ± 0.92 a	14.01 ± 2.35 a	172.55 ± 15.44 a
		WD	12.58 ± 4.51 a	12.17 ± 2.06 a	24.34 ± 6.44 a	133.36 ± 9.41 a
		Mean	8.96 ± 2.65 *	10.77 ± 1.20	19.36 ± 3.70 *	153.74 ± 9.96 *
	2.5k	NWD	7.00 ± 1.24 a	8.01 ± 1.02 a	15.01 ± 2.02 a	167.98 ± 27.55 a
		WD	14.74 ± 5.91 a	12.54 ± 3.22 a	27.27 ± 8.96 a	146.12 ± 13.49 a
		Mean	10.87 ± 3.14 *	10.28 ± 1.76	21.14 ± 4.79 *	157.05 ± 15.53 *
	25k	NWD	14.77 ± 3.60 a	19.64 ± 4.76 a	34.65 ± 6.60 a	129.33 ± 12.25 a
		WD	20.63 ± 6.66 a	10.15 ± 1.74 a	30.78 ± 7.49 a	120.60 ± 13.03 a
		Mean	17.70 ± 3.84 *	15.12 ± 2.79	32.81 ± 5.00 *	125.17 ± 8.98 *

[a] WIC: water inoculated control, 2.5k: 2,500 spores, 25k: 25,000 spores

[b] NWD: no water deficit (100% irrigation), WD: water deficit (50% irrigation)

Same lowercase and uppercase letter within each grafting treatment in each column represent no significant differences ($p = 0.05$) according to Tukey's significance test. * indicates effect of inoculum ($p < 0.05$) according to Spearman correlation test. WIC = water-inoculated control.

Total necrosis was higher in self-rooted compared with grafted and basal surface area was higher in self-rooted than grafted.

2.3. Grapevine symptom development and mortality

Grapevine mortality was observed in the winter of 2021, followed by young vine decline-like symptom development and grapevine mortality in the summer of 2022 and grapevine mortality in the winter of 2022. In 2021, grapevine mortality was greater in self-rooted grapevines inoculated with 25k and 2.5k compared with 100 % survival of WIC grapevines (Table 5). In 2021, 100 % of grafted grapevines survived. In 2022, WIC grapevines under 50% irrigation had higher mortality compared with fully irrigated WIC grapevines (Table 5). In 2022 and cumulatively, 50 % irrigation treatment increased mortality compared with fully irrigated grapevines (2022: 47 % compared with 13 %; Cumulatively: 62 % compared with 28 %) in self-rooted grapevines but not for grafted grapevines (Table 6). Across all years, self-rooted grapevine mortality was 46.2 % which was greater than grafted grapevine mortality of 18 %.

Six grapevines were symptomatic for young vine decline in 2022 (Figure 4). Leaf water potential and leaf chlorophyll content were measured on symptomatic leaves due to lack of asymptomatic leaves in symptomatic grapevines. The leaf water potential of symptomatic grapevines averaged -0.71 MPa compared with -1.04 MPa in asymptomatic grapevines. Leaf chlorophyll content readings were lower in symptomatic grapevines, averaging 18.81 in SPAD units compared with 27.25 in asymptomatic grapevines in 2022. Three of the six symptomatic grapevines died prior to the conclusion of the experiment in 2023, when most grapevine mortality occurred, thus these three grapevines were excluded

TABLE 5. Chi-squared analyses of percent mortality and percentage of grapevines with symptoms for each year and cumulatively across years for each grafting treatment, level of inoculum, and irrigation treatment.

			2021			2022			Cumulative	
Grafting treatment	Inoculum[a]	Irrigation[b]	Reps	% Mortality	% Symptoms	Reps	% Mortality	% Symptoms	Reps	% Mortality
Self-rooted	WIC	NWD	6	0 a	N/A	6	0 a	0 a	6	0 a
		WD	7	0 a	N/A	7	43 a	14 a	7	43 a
		Overall	13	0 B	N/A	13	23 A	8 A	13	23 B
	2.5k	NWD	6	33 a	N/A	4	25 a	0 a	6	50 a
		WD	7	43 a	N/A	4	75 a	0 a	7	86 a
		Overall	13	38 A	N/A	8	50 A	0 A	13	69 A
	25k	NWD	6	17 a	N/A	5	20 a	0 a	6	33 a
		WD	7	43 a	N/A	4	25 a	0 a	7	57 a
		Overall	13	31 A	N/A	9	22 A	0 A	13	46 A
Grafted	WIC	NWD	6	0 a	N/A	6	0 a	0 a	6	0 a
		WD	7	0 a	N/A	7	14 a	14 a	7	14 a
		Overall	13	0 A	N/A	13	8 A	8 A	13	8 A
	2.5k	NWD	6	0 a	N/A	6	0 a	0 a	6	0 a
		WD	7	0 a	N/A	7	29 a	29 a	7	29 a
		Overall	13	0 A	N/A	13	15 A	15 A	13	15 A
	25k	NWD	6	0 a	N/A	6	50 a	40 a	6	50 a
		WD	7	0 a	N/A	7	14 a	14 a	7	14 a
		Overall	13	0 A	N/A	13	31 A	25 A	13	31 A
Mean	WIC	NWD	12	0 a	N/A	12	0 b	0 a	12	0 b
		WD	14	0 a	N/A	14	29 a	14 a	14	29 a
		Overall	26	0 A	N/A	26	15 A	8 A	26	15 A
	2.5k	NWD	12	17 a	N/A	10	10 a	0 a	12	25 a
		WD	14	21 a	N/A	11	45 a	18 a	14	57 a
		Overall	26	19 A	N/A	21	29 A	10 A	26	42 A
	25k	NWD	12	8 a	N/A	11	36 a	18 a	12	42 a
		WD	14	21 a	N/A	11	18 a	9 a	14	36 a
		Overall	26	15 A	N/A	22	27 A	14 A	26	38 A

[a] WIC: water inoculated control, 2.5k: 2,500 spores, 25k: 25,000 spores

b NWD: no water deficit (100% irrigation), WD: water deficit (50% irrigation)

For each column within each grafting treatment, percent mortality and symptoms with the same lowercase or upper-case letter are not significantly different (p = 0.05) according to Chi-squared test.

from overall grapevine health and *Pch* abundance analysis. The three grapevines that died were 1) self-rooted WIC/WD, 2) grafted 2.5k/WD, and 3) grafted WIC/WD. *Pch* abundance was 1) 33,700, 2) 11,384, and 3) 17,466 copies/ng DNA. For the three grapevines mean dry shoot weight was 2.82 g, mean node number was 32, and mean dry root weight was 11.05 g. Mean black necrosis was 25.8 %, brown necrosis was 42.2 %, and total necrosis was 68 %. Mean basal surface area was 129.04 mm.

DISCUSSION

This is the first study to investigate the interactive effects of grapevine water stress and *Phaeomoniella chlamydospora* inoculum levels on Petri disease development in grapevines. The results of this study suggest water stress influences *Pch* growth and grapevine mortality within the first few years after planting.

The main objective of this study was to understand whether water stress increases *Pch* growth within the grapevine. *Phaeomoniella chlamydospora* can grow under severe water deficit and may have better access to carbohydrates under drought conditions (Lima *et al.*, 2017; Whiting *et al.*, 2001), indicating water stress may favor pathogen growth and colonization (Hrycan *et al.*, 2020). Recently, in grapevines naturally infected with *Pch,* water stress reduced microbiome diversity and increased *Pch* abundance (Leal *et al.*, 2024). In our greenhouse experiment, *Pch* abundance was greater in inoculated grapevines under 50 % deficit irrigation, confirming the results of Leal *et al.* (2024). However, in

TABLE 6. Chi-squared analysis of grapevine total (Reps), percent mortality and symptom expression for each year and cumulatively for each grafting treatment under each irrigation treatment.

	Self-rooted				Grafted				Mean			
	NWD		WD		NWD		WD		NWD		WD	
	Reps	%	Reps	%	Reps	%	Reps	%	Reps	%	Reps	%
2021 mortality	18	17 a	21	29 a	18	0 a	21	0 a	36	8 a	44	14 a
2022 mortality	15	13 b	15	47 a	18	17 a	21	19 a	33	15 a	33	31 a
Cumulative mortality	18	28 b	21	62 a	18	17 a	21	19 a	36	22 a	44	40 a
Cumulative symptoms	15	0 a	15	7 a	18	12 a	21	19 a	33	6 a	33	14 a

NWD: no water deficit (100% irrigation), WD: water deficit (50% irrigation).

For each row within each grafting treatment, percent mortality and symptoms with the same lowercase letter are not significantly different ($p = 0.05$) according to Chi-squared test.

our study, *Pch* abundance was higher only when AM fungi were also present. Arbuscular mycorrhizal fungi can increase grapevine growth and improve resistance to abiotic and biotic stress factors (Trouvelot *et al.*, 2015; Ye *et al.*, 2022). It was hypothesized that AM fungi would reduce grapevine water stress and decrease disease development in grapevines infected with *Pch.* However, AM treatment had no impact on plant growth measurements in this study and our results indicate AM fungi are beneficial to *Pch* growth under grapevine water stress. These findings are in accordance with Holland *et al.* (2019) and Vukicevich *et al.* (2018) who observed an increase in fungal abundance and necrosis in grapevines infected with black foot fungi and colonized by AM fungi. In our study, no link between AM fungal colonization and increased internal necrosis was observed in *Pch* inoculated grapevines, however, due to the shorter length of the greenhouse experiment, it may not have been enough time for greater *Pch* abundance to result in greater internal necrosis.

The mechanism behind the influence of AM colonization and drought on *Pch* fungal abundance is unclear, but an interactive effect of AM fungi and drought on grapevine defenses may explain *Pch* growth. Water stress alone and in combination with AM fungi has been reported to increase salicylic acid production in other hosts (Choudhary & Senthil-Kumar, 2022; Sanchez-Romera *et al.*, 2018). An increase in salicylic acid, which is antagonistic to jasmonic acid, involved in induced systemic resistance (Thakur & Sohal, 2013), could result in reduced grapevine resistance against necrotrophic pathogens (El Rahman *et al.*, 2012). Additionally, *Pch* showed increased access to carbohydrates in the xylem under water stress (Lima *et al.*, 2017), which could further promote pathogen growth in grapevines with suppressed induced systemic resistance. If *Pch* is a latent pathogen, increased *Pch* growth under water stress conditions will reach the colonization threshold in which grapevine mortality occurs sooner (Hrycan *et al.*, 2020). Overall, our greenhouse *Pch* abundance results support the hypothesis of increased *Pch* growth under grapevine water stress, but other factors may be involved in *Pch* growth, as combined water stress and AM+ treatment increased *Pch* abundance but water stress alone did not. It is unclear whether greater *Pch* abundance is detrimental to grapevine health, but pathogen abundance has been correlated with symptom development in other hosts such as Swiss Needle Cast disease in Douglas-Fir and brown stem rot of soy (Impullitti *et al.*, 2009; Manter *et al.*, 2003).

In contrast to the greenhouse experiment, *Pch* abundance was not impacted by water stress under natural field conditions. Differences between findings in the greenhouse and field may be due to the much higher levels of water stress obtained in the greenhouse compared with field grapevines. In the field, mid-day leaf water potential indicated mild to moderate grapevine water stress under the WD irrigation regime compared with the NWD, while in the greenhouse, mid-day leaf water potential indicated high to severe grapevine water stress under the WD irrigation regime (Deloire *et al.*, 2020). While *Pch* abundance was not affected by water stress in the field, initial inoculum quantity influenced *Pch* abundance at the end of the experiment. In both the greenhouse and field, *Pch* abundance was positively correlated with initial inoculum level. However, *Pch* abundance also varied widely among individual grapevines. Variation in *Pch* abundance is in accordance with the findings of Hrycan *et al.* (2023) in ready-to-plant nursery material, however, while variation in nursery material could be attributed to natural infection, it is unclear why we obtained such high *Pch* variation in both greenhouse and field grapevines inoculated with known *Pch* spore quantities, but may be the result of variations in spore distribution within the grapevine in nursery grapevines and vacuum inoculated grapevines. Further work is required to understand the underlying cause of this variation between grapevines. The microbiome of individual grapevines plays a role in protection of wood against pathogens (Xiong & Yang, 2023). Variation in the microbiomes of individual grapevines could similarly influence *Pch* establishment and growth within the grapevine, but further investigation is required to determine if the individual grapevine microbiome is the cause of this variation in *Pch* abundance.

Infection of *Pch* in the WIC grapevines in the field was observed at time of planting suggesting infection occurred during the propagation process. Infection was not observed in greenhouse grapevines, but the propagation process was different between the greenhouse and field and contamination or natural infection may have spread during the callusing stage which was not completed for the greenhouse experiment.

A second objective of the present study was to determine whether initial *Pch* abundance had an impact on internal necrosis within the grapevine after planting. Previous work has reported no correlation between internal necrosis and pathogen abundance in ready-to-plant nursery grapevines (Hrycan *et al.*, 2023). In both greenhouse and field experiments, *Pch* abundance was correlated with internal necrosis. In the greenhouse and field, black and total necrosis were positively correlated with inoculum level. Brown necrosis in the greenhouse was negatively correlated with inoculum level, while in the field inoculum level had no effect on brown necrosis. However, internal necrosis was similar between *Pch* inoculated grapevines, but was higher than WIC/NIC grapevines. Recent nursery surveys have reported a correlation between necrosis and individual nurseries and rootstocks rather than pathogen abundance or infection (Carbone *et al.* 2022; Gramaje & Armengol, 2011; Hrycan *et al.*, 2023; Lade *et al.*, 2022). However, previous studies have primarily investigated total necrosis without separating black necrosis from browning of the wood (Hrycan *et al.*, 2023). Necrosis from both greenhouse and field grapevines suggests browning of the wood should be excluded from pathogen necrosis analysis for *Pch.*

In the field, black necrosis and total necrosis were positively correlated with inoculum level, while basal surface area negatively correlated with inoculum level, however, differences were primarily observed between WIC/2.5k and 25k. Water stress alone was mild to moderate and unlikely to induce grapevine death, instead, increased necrosis and lower basal surface area could have impacted grapevine symptom development and mortality in combination with water stress (Maher *et al.*, 2012; Oliva *et al.*, 2014). In addition, grapevine water stress was higher in the greenhouse than the field, and stress induced higher necrosis in the greenhouse, potentially due to xylem cavitation (Gambetta *et al.*, 2020). Interestingly, different effects were observed in self-rooted grapevines on basal surface area, increasing in the greenhouse and having no effect on the self-rooted grapevines in the field experiment. *Phaeomoniella chlamydospora* infection can result in xylem blockage due to necrosis or tylose formation (Bortolami *et al.*, 2021b), which the grapevine could respond to by the growth of new xylem vessels as observed in other host responses to xylem dysfunction after water stress (Gauthey *et al.*, 2022). A decrease of basal surface area in field grapevines could be due to increased *Pch* growth in the field over the longer-term.

Increased internal necrosis and decreased basal surface area could have influenced symptom development and grapevine mortality. Foliar symptoms developed in the field in August and September of 2022, primarily in 25k and 2.5k inoculated grafted grapevines, leading to sudden vine collapse, a characteristic symptom of severe Petri disease (Gramaje *et al.*, 2018). Symptoms included reddening of the leaves, lower leaf chlorophyll content, and greater leaf water potential in 2022, indicating leaf dysfunction, expected of sudden vine collapse. In older grapevines with Esca, in which *Pch* is also involved, water stress has been correlated with the inhibition of symptoms (Bortolami *et al*., 2021a), in contrast to the findings of this study. However, Esca is a separate and complex disease, and has two forms, chronic and apoplectic (Gramaje *et al.*, 2018), and symptom development in this study was followed by sudden vine collapse, like the Esca apoplectic form. Chronic Esca symptom development may be unrelated to internal necrosis, as observed in other grapevine trunk diseases (Travadon *et al.*, 2013; Sosnowski *et al.*, 2011) and instead could be due to leaf vascular occlusions (Bortolami *et al.*, 2023). On the other hand, Esca apoplexy has been associated with high temperatures and lack of rainfall (Surico *et al.*, 2006).

In our study, self-rooted grapevine mortality was higher in WD than NWD grapevines. In contrast, symptom development and grapevine mortality did not occur in the greenhouse, which is unexpected due to the greater *Pch* abundance observed in WD *Pch* inoculated grapevines in the greenhouse. However, the greenhouse experiment was shorter in time than the field experiment and it was conducted under controlled conditions in pasteurized soil, which may have resulted in more favourable conditions for grapevine survival due to exposure to fewer external factors than field grapevines. Irrigation treatment was applied for a longer period in the field and external abiotic and biotic stress factors in the field may have weakened the grapevines further compared with the greenhouse. The findings of this study suggest *Pch* abundance is correlated with symptom development, which has been observed in other diseases in other hosts (Impullitti *et al.*, 2009; Manter *et al.*, 2003). *Phaeomoniella chlamydospora* has often been isolated from asymptomatic grapevines and nursery material, indicating it can have a limited impact on grapevine growth (Gainza-Cortes *et al.*, 2020; Nerva *et al.*, 2019).

Variations in rootstock susceptibility to *Pch* infection have been previously reported (Eskalen *et al.*, 2001), but rootstock tolerance to infection under stress has not been investigated. In this study, self-rooted grapevines had lower dry pruning weight, node numbers, dry root weight, higher *Pch* abundance, total necrosis, and overall grapevine mortality than grafted grapevines, suggesting self-rooted has higher susceptibility to *Pch* infection compared with grafted rootstock under natural field conditions. However, this study found grafted grapevines had lower leaf water potential compared with self-rooted indicating a higher level of stress in grafted, which may have been due to the increased growth of grafted grapevines. Murolo and Romanazzi (2014) observed increased disease incidence in 'SO4' compared with '1103P' rootstock, which is thought to be more drought tolerant, but our results indicate grapevine water stress may not be the only factor. Xylem diameter can also play a role in pathogen abundance and grapevine susceptibility to *Pch* (Pouzoulet *et al.*, 2017). In grapevines infected with *Phaeoacremonium minimum*, another Petri disease associated fungus, smaller xylem diameter was associated with lower pathogen abundance (Ramsing *et al.*, 2021). Xylem diameter in combination with grapevine water stress

should be investigated to determine how pathogen growth and symptom expression are impacted.

Several Petri disease fungi have been hypothesized to be latent pathogens, in part due to isolating them from both asymptomatic grapevine wood tissue and their ability to grow under low osmotic water potentials, which affect nutrient levels in the xylem (Lima *et al.*, 2017; Hrycan *et al.*, 2020; Whiting *et al.*, 2001). This study revealed a correlation between water stress and AM fungal inoculation with increased *Pch* abundance in the greenhouse. In the field, all grapevines contained *Pch* and overall, water stressed grapevines had greater mortality than non-stressed grapevines. These results support the hypothesis that *Pch* is a latent pathogen and increased *Pch* abundance leads to mortality in young grapevines under stress (Hrycan *et al.*, 2020; Sieber, 2007). To our knowledge, this is the first study to induce young vine decline-like foliar symptom expression, and sudden vine collapse, with a link between *Pch* abundance and grapevine mortality within the first few years after planting. However, the underlying mechanisms for symptom development and *Pch* growth remain unclear. Increased initial *Pch* inoculation was correlated with an increase in internal necrosis which could lead to heightened risk of severe grapevine water stress. Grapevines under severe stress could upregulate anthocyanin biosynthesis genes resulting in anthocyanin production in the leaves, and sudden vine collapse during periods of high stress (Dabravolski & Isayenkov, 2023; Gramaje *et al.*, 2018; Haider *et al.*, 2017). This study suggests that grafted grapevines may be less susceptible to symptom expression and mortality compared with self-rooted in natural environments, but further investigation into the factors' influencing susceptibility is required to further understand the relationship between *Pch* infection, grapevine water stress, and self-rooted or grafted grapevine susceptibility on symptom expression and grapevine mortality.

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REFERENCES

Berlanas, C., Ojeda, S., López-Manzanares, B., Andrés-Sodupe, M., Bujanda, R., Del Pilar Martínez-Diz, M., Díaz-Losada, E., & Gramaje, D. (2020). Occurrence and diversity of black-foot disease fungi in symptomless grapevine nursery stock in Spain. *Plant Disease, 104*(1), 94-104. https://doi.org/10.1094/PDIS-03-19-0484-RE

Billones-Baaijens, R., Ridgway, H. J., Jones, E. E., Cruickshank, R. H., & Jaspers, M. V. (2013). Prevalence and distribution of *Botryosphaeriaceae* species in New Zealand grapevine nurseries. *European Journal of Plant Pathology, 135*(1), 175-185. https://doi.org/10.1007/s10658-012-0076-4

Bortolami, G., Farolfi, E., Badel, E., Burlett, R., Cochard, H., Ferrer, N., King, A., Lamarque, L. J., Lecomte, P., Marchesseau-Marchal, M., Pouzoulet, J., Torres-Ruiz, J. M., Trueba, S., Delzon, S., Gambetta, G. A., & Delmas, C. E. L. (2021b). Seasonal and long-term consequences of Esca grapevine disease on stem xylem integrity. *Journal of Experimental Botany, 72*(10), 3914-3928. https://doi.org/10.1093/jxb/erab117

Bortolami, G., Ferrer, N., Baumgartner, K., Delzon, S., Gramaje, D., Lamarque, L. J., Romanazzi, G., Gambetta, G. A., & Delmas, C. E. L. (2023). Esca grapevine disease involves leaf hydraulic failure and represents a unique premature senescence process. *Tree Physiology, 43*(3), 441-451. https://doi.org/10.1093/treephys/tpac133

Bortolami, G., Gambetta, G. A., Cassan, C., Dayer, S., Farolfi, E., Ferrer, N., Gibon, Y., Jolivet, J., Lecomte, P., & Delmas, C. E. L. (2021a). Grapevines under drought do not express Esca leaf symptoms. *Proceedings of the National Academy of Sciences - PNAS, 118*(43)https://doi.org/10.1073/pnas.2112825118

Carbone, M. J., Gelabert, M., Moreira, V., Mondino, P., & Alaniz, S. (2022). Grapevine nursery propagation material as source of fungal trunk disease pathogens in Uruguay. *Frontiers in Fungal Biology, 3*, 958466-958466. https://doi.org/10.3389/ffunb.2022.958466

Choudhary, A., & Senthil-Kumar, M. (2022). Drought attenuates plant defence against bacterial pathogens by suppressing the expression of CBP60g/SARD1 during combined stress. *Plant, Cell and Environment, 45*(4), 1127-1145. https://doi.org/10.1111/pce.14275

Dabravolski, S. A., & Isayenkov, S. V. (2023). The role of anthocyanins in plant tolerance to drought and salt stresses. *Plants (Basel), 12*(13), 2558. https://doi.org/10.3390/plants12132558

Deloire, A., Pellegrino, A., & Rogiers, S. (2020). A few words on grapevine leaf water potential. *IVES Technical Reviews : Vine & Wine,* https://doi.org/10.20870/IVES-TR.2020.3620

Edwards, J., Salib ,S., Thomson, F., & Pascoe, I. G. (2007b). The impact of *Phaeomoniella chlamydospora* infection on the grapevine's physiological response to water stress part 2: Cabernet sauvignon and chardonnay. *Phytopathologia Mediterranea, 46*(1), 38-49. https://doi.org/10.14601/Phytopathol_Mediterr-1856

Edwards, J., Salib, S., Thomson, F., & Pascoe, I. G. (2007a). The impact of *Phaeomoniella chlamydospora* infection on the grapevine's physiological response to water stress - part 1 : Zinfandel. *Phytopathologia Mediterranea, 46*(46, 1), 26-37. https://doi.org/10.1400/68064

El Rahman, T. A., Oirdi, M. E., Gonzalez-Lamothe, R., & Bouarab, K. (2012). Necrotrophic pathogens use the salicylic acid signaling pathway to promote disease development in tomato. *Molecular Plant-Microbe Interactions, 25*(12), 1584-1593. https://doi.org/10.1094/MPMI-07-12-0187-R

Eskalen, A., Gubler, W. D., & Khan, A. (California Univ., Davis (USA). Dept. of Plant Pathology). (2001). Rootstock susceptibility to *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. [*Vitis vinifera* L. - California]. *Phytopathologia Mediterranea, 40*(3), S433-S438. https://doi.org/10.14601/phytopathol_mediterr-1636

Gainza-Cortes, F., Roa-Roco, R., Arrano-Salinas, P., Rodriguez-Herrera, P., Lolas, M. A., Caris-Maldonado, J. C., Silva-Flores, P., & Gonzalez, A. (2020). Distribution of three grapevine trunk pathogens in Chilean vineyards, determined using molecular detection from asymptomatic woody pruning material. *Phytopathologia Mediterranea, 59*(3), 503-513. https://doi.org/10.14601/Phyto-11274

Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U., & Castellarin, S. D. (2020). The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. *Journal of Experimental Botany, 71*(16), 4658-4676. https://doi.org/10.1093/jxb/eraa245

Gauthey, A., Peters, J. M. R., Lòpez, R., Carins-Murphy, M. R., Rodriguez-Dominguez, C. M., Tissue, D. T., Medlyn, B. E., Brodribb, T. J., & Choat, B. (2022). Mechanisms of xylem hydraulic recovery after drought in Eucalyptus saligna. *Plant, Cell and Environment, 45*(4), 1216-1228. https://doi.org/10.1111/pce.14265

Glazebrook, J. (2005). Contrasting mechanisms of defense against Biotrophic and Necrotrophic Pathogens. *Annual Review of Phytopathology*, *43*(1), 205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923

González, V., & Tello, M. L. (2011). The endophytic mycota associated with *Vitis vinifera* in central Spain. *Fungal Diversity, 47*(1), 29-42. https://doi.org/10.1007/s13225-010-0073-x

Gramaje, D., & Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: Potential inoculum sources, detection, identification, and management strategies. *Plant Disease, 95*(9), 1040-1055. https://doi.org/10.1094/PDIS-01-11-0025

Gramaje, D., Úrbez-Torres, J. R., & 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. https://doi.org/10.1094/PDIS-04-17-0512-FE

Haider, M. S., Zhang, C., Kurjogi, M. M., Pervaiz, T., Zheng, T., Zhang, C., Lide, C., Shangguan, L., & Fang, J. (2017). Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-seq analysis. *Scientific Reports, 7*(1), 13134-15. https://doi.org/10.1038/s41598-017-13464-3

Hause, B., Mrosk, C., Isayenkov, S., & Strack, D. (2007). Jasmonates in arbuscular mycorrhizal interactions. *Phytochemistry (Oxford)*, *68*(1), 101–110. https://doi.org/10.1016/j.phytochem.2006.09.025

Holland, T., Bowen, P., Kokkoris, V., Urbez-Torres, J. R., & Hart, M. (2019). Does inoculation with arbuscular mycorrhizal fungi reduce trunk disease in grapevine rootstocks? *Horticulturae, 5*(3), 61. https://doi.org/10.3390/horticulturae5030061

Hrycan, J., Hart, M., Bowen, P., Forge, T., & Urbez-Torres, J. R. (2020). Grapevine trunk disease fungi: Their roles as latent pathogens and stress factors that favour disease development and symptom expression. *Phytopathologia Mediterranea, 59*(3), 395-424. https://doi.org/10.14601/Phyto-11275

Hrycan, J., Theilmann, J., Mahovlic, A., Boulé, J., & Úrbez-Torres, J. R. (2023). Health status of ready-to-plant grapevine nursery material in Canada regarding young vine decline fungi. *Plant Disease, 107*(12), 3708-3717. https://doi.org/10.1094/PDIS-05-23-0900-SR

Impullitti, A. E., Malvick, D. K., & Grau, C. R. (2009). Characterizing reaction of soybean to *Phialophora gregata* using pathogen population density and DNA quantity in stems. *Plant Disease, 93*(7), 734-740. https://doi.org/10.1094/PDIS-93-7-0734

Kokkoris, V., Li, Y., Hamel, C., Hanson, K., & Hart, M. (2019). Site specificity in establishment of a commercial arbuscular mycorrhizal fungal inoculant. *The Science of the Total Environment, 660*, 1135-1143. https://doi.org/10.1016/j.scitotenv.2019.01.100

Lade, S. B., *Štraus*, D., & Oliva, J. (2022). Variation in fungal community in grapevine (*Vitis vinifera*) nursery stock depends on nursery, variety and rootstock. *Journal of Fungi (Basel), 8*(1), 47. https://doi.org/10.3390/jof8010047

Leal, C., Bujanda, R., Carbone, M. J., Kiss, T., Eichmeier, A., Gramaje, D., and Maldonado-Gonzalez, M. M. (2024). Drought influences the fungal community structure, diversity, and functionality inhabiting the grapevine xylem and enhances the abundance of *Phaeomoniella chlamydospora*. *Phytobiomes Journal*, https://doi.org/10.1094/PBIOMES-02-24-0027-R

Lenth, R. (2023). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.5, https://CRAN.R-project.org/package=emmeans.

Lima, M. R. M., Machado, A. F., & Gubler, W. D. (2017). Metabolomic study of Chardonnay grapevines double stressed with Esca-associated fungi and drought. *Phytopathology, 107*(6), 669-680. https://doi.org/10.1094/PHYTO-11-16-0410-R

Maher, N., Piot, J., Bastien, S., Vallance, J., Rey, P., & Guérin-Dubrana, L. (2012). Wood necrosis in Esca-affected vines: Types, relationships and possible links with foliar symptom expression. *OENO One, 46*(1), 15-27. https://doi.org/10.20870/oeno-one.2012.46.1.1507

Maldonado-González, M. M., del Pilar Martínez-Diz, M., Andrés-Sodupe, M., Bujanda, R., Díaz-Losada, E., & Gramaje, D. (2020). Quantification of *Cadophora luteo-olivacea* from grapevine nursery stock and vineyard soil using droplet digital PCR. *Plant Disease, 104*(8), 2269-2274. https://doi.org/10.1094/PDIS-09-19-2035-RE

Manter, D. K., Winton, L. M., Filip, G. M., & Stone, J. K. (2003). Assessment of Swiss needle cast disease: Temporal and spatial investigations of fungal colonization and symptom severity. *Journal of Phytopathology, 151*(6), 344-351. https://doi.org/10.1046/j.1439-0434.2003.00730.x

Marchi, G., Peduto, F., Mugnai, L., Di Marco, S., Calzarano, F., & Surico, G. (2006). Some observations on the relationship of manifest and hidden Esca to rainfall. *Phytopathologia Mediterranea, 45*, S117-S126. https://doi.org/10.36253/phyto-5195

Martín, M. T., Cobos, R., Martín, L., & López-Enríquez, L. (2012). Real-time PCR detection of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. *Applied and Environmental Microbiology, 78*(11), 3985-3991. https://doi.org/10.1128/AEM.07360-11

Murolo, S., & Romanazzi, G. (2014). Effects of grapevine cultivar, rootstock and clone on Esca disease. *Australasian Plant Pathology, 43*(2), 215-221. https://doi.org/10.1007/s13313-014-0276-9

Nerva, L., Turina, M., Zanzotto, A., Gardiman, M., Gaiotti, F., Gambino, G., & Chitarra, W. (2019). Isolation, molecular characterization and virome analysis of culturable wood fungal endophytes in esca symptomatic and asymptomatic grapevine plants. *Environmental Microbiology, 21*(8), 2886-2904. https://doi.org/10.1111/1462-2920.14651

Oliva, J., Stenlid, J., Martínez-Vilalta, J., & Sveriges lantbruksuniversitet. (2014). The effect of fungal pathogens on the water and carbon economy of trees: Implications for drought-induced mortality. *The New Phytologist, 203*(4), 1028-1035. https://doi.org/10.1111/nph.12857

Péros, J. -., Berger, G., & Jamaux-Despréaux, I. (2008). Symptoms, wood lesions and fungi associated with Esca in organic vineyards in Languedoc-Roussillon (France). *Journal of Phytopathology, 156*(5), 297-303. https://doi.org/10.1111/j.1439-0434.2007.01362.x

Petit, E., & Gubler, W. D. (2006). Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. *Plant Disease, 90*(12), 1481-1484. https://doi.org/10.1094/PD-90-1481

Pinheiro, J. C., & Bates, D. M. (2000). SpringerLINK eBooks - English/International Collection (Archive), Ebook Central, and SpringerLink (Online service). Mixed-effects models in S and S-PLUS 1st ed. Springer. https://doi.org/10.1007/b98882

Pouzoulet, J., Scudiero, E., Schiavon, M., & Rolshausen, P. E. (2017). Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. *Frontiers in Plant Science, 8*, 1442-1442. https://doi.org/10.3389/fpls.2017.01442

R Core Team. (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramsing, C. K., Gramaje, D., Mocholí, S., Agustí, J., Cabello Sáenz de Santa María, Félix, Armengol, J., & Berbegal, M. (2021). Relationship between the xylem anatomy of grapevine rootstocks and their susceptibility to *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. *Frontiers in Plant Science, 12*, 726461-726461. https://doi.org/10.3389/fpls.2021.726461

Rooney, S. N., Eskalen, A., & Gubler, W. D. (2001). Recovery of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from soil and grapevine tissues [*Vitis vinifera* L. - California]. *Phytopathologia Mediterranea, 40*, S351-S356.

Sanchez-Romera B., Calvo-Polanco M., Ruiz-Lozano J. M., Zamarreno A. M., Arbona V., Garcia-Mina J. M., Gomez-Cadenas, A., & Aroca, R. (2018). Involvement of the def-1 mutation in the response of tomato plants to arbuscular mycorrhizal symbiosis under well-watered and drought conditions. *Plant and Cell Physiology, 59*(2), 248-261. https://doi.org/10.1093/pcp/pcx178

Sieber, T. N. (2007). Endophytic fungi in forest trees: Are they mutualists? *Fungal Biology Reviews, 21*(2), 75-89. https://doi.org/10.1016/j.fbr.2007.05.004

Sosnowski, M. R., Luque, J., Loschiavo, A. P., Martos, S., Garcia-Figueres, F., Wicks, T. J., & Scott, E. S. (2011). Studies on the effect of water and temperature stress on grapevines inoculated with *Eutypa lata*. *Phytopathologia Mediterranea, 50*(4), S127-S138. https://doi.org/10.14601/Phytopathol_Mediterr-8959

Surico, G., Marchi, G., Braccini, P., & Mugnai, L. (2000). Epidemiology of Esca in some vineyards in Tuscany (Italy). *Phytopathologia Mediterranea,* (39, 1)https://doi.org/10.1400/57844

Surico, G., Mugnai, L., & Marchi, G. (2006). Older and more recent observations on Esca: A critical overview. *Phytopathologia Mediterranea, 45*(4), S68-S86. https://doi.org/10.14601/PHYTOPATHOL_MEDITERR-1847

Thakur, M., & Sohal, B. S. (2013). Role of elicitors in inducing resistance in plants against pathogen infection: A review. *ISRN Biochemistry, 2013*, 762412-10. https://doi.org/10.1155/2013/762412

Travadon, R., Rolshausen, P. E., Gubler, W. D., Cadle-Davidson, L., & Baumgartner, K. (2013). Susceptibility of cultivated and wild vitis spp. to wood infection by fungal trunk pathogens. *Plant Disease, 97*(12), 1529-1536. https://doi.org/10.1094/PDIS-05-13-0525-RE

Trevors, J. T. (1996). Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods, 26*(1), 53-59. https://doi.org/10.1016/0167-7012(96)00843-3

Trouvelot, S., Bonneau, L., Redecker, D., van Tuinen, D., Adrian, M., & Wipf, D. (2015). Arbuscular mycorrhiza symbiosis in viticulture: A review. *Agronomy for Sustainable Development, 35*(4), 1449-1467. https://doi.org/10.1007/s13593-015-0329-7

Úrbez-Torres, J. R., Haag, P., Bowen, P., & O'Gorman, D. T. (2014). Grapevine trunk diseases in British Columbia: Incidence and characterization of the fungal pathogens associated with Esca and Petri diseases of grapevine. *Plant Disease, 98*(4), 469-482. https://doi.org/10.1094/PDIS-05-13-0523-RE

Vierheilig, H., Coughlan, A. P., Wyss, U., & Pichie, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology, 64*(12), 5004-5007. https://doi.org/10.1128/aem.64.12.5004-5007.1998

Vukicevich, E., Lowery, D. T., Úrbez-Torres, J. R., Bowen, P., & Hart, M. (2018). Groundcover management changes grapevine root fungal communities and plant-soil feedback. *Plant and Soil, 424*(1/2), 419-433. https://doi.org/10.1007/s11104-017-3532-2

Whiting, E. C., Khan, A., & Gubler, W. D. (2001). Effect of temperature and water potential on survival and mycelial growth of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. *Plant Disease, 85*(2), 195-201. https://doi.org/10.1094/PDIS.2001.85.2.195

Xiong, Q., Yang, J., & Ni, S. (2023). Microbiome-mediated protection against pathogens in woody plants. *International Journal of Molecular Sciences, 24*(22), 16118. https://doi.org/10.3390/ijms242216118

Ye, Q., Wang, H., & Li, H. (2022). Arbuscular mycorrhizal fungi improve growth, photosynthetic activity, and chlorophyll fluorescence of *Vitis vinifera* L. cv. ecolly under drought stress. *Agronomy (Basel), 12*(7), 1563. https://doi.org/10.3390/agronomy12071563

Zhan, Z., Wang, N., Chen, Z., Zhang, Y., Geng, K., Li, D., Wang, Z., & Chaves, M. (2023). Effects of water stress on endogenous hormones and free polyamines in different tissues of grapevines (*Vitis vinifera* L. cv. 'Merlot'). *Functional Plant Biology: FPB, 50*(12), 993–1009. https://doi.org/10.1071/FP22225