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Cultivar-specific effects of physical and biological treatments on grapevine trunk disease control and plant vigour

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Summary. Grapevine trunk diseases (GTDs), including black foot and Petri disease, pose threats to young grapevine establishment. Efficacy of hot water treatment (HWT), *Trichoderma atroviride* SC1 (TCH), and their combination (HWT + TCH) was assessed for control of GTDs and promotion of early plant development in nine Galician grapevine cultivars. Treatments were applied either prior to grafting or before planting in the field. The treatments were more effective against Petri disease than black foot, with the HWT + TCH combination reducing Petri disease incidence and severity in several cultivars, particularly when applied at the pre-grafting stage. In contrast, limited efficacy was observed against black foot, indicating that post-planting strategies are likely to be required to manage root-infecting pathogens. Plant performance responses were cultivar- and timing-dependent: early treatments generally improved root biomass, whereas late applications occasionally reduced shoot length and root weight. These results highlight the importance of tailoring integrated disease management strategies to specific grapevine cultivars and propagation stages, to optimize nursery outcomes and grapevine health.

Keywords. Biological control, black foot, hot water treatment, Petri disease, *Trichoderma*, *Vitis vinifera*.

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INTRODUCTION

Over the past two decades, there has been a marked increase in decay and progressive mortality among young grapevine plants (Gramaje *et al*., 2018). This situation has been primarily driven by fungal grapevine trunk diseases (GTDs), which pose significant threats to the sustainability of grapevine cultivation (Bertsch *et al*., 2013). GTDs cause substantial economic losses due to reduced

yields, increased costs for disease control, and the premature decline of vineyards (Bertsch *et al*., 2013; Gramaje *et al*., 2018). These diseases, caused by fungal infections, typically originate during growing seasons through the release of pathogen conidia, which germinate to colonize pruning wounds. While most infections occur in vineyards, some plants may be infected prior to planting (Gramaje and Armengol, 2011), because mother plants can harbour GTD pathogens, contributing to disease transmission (Fourie and Halleen, 2004). Nurseries, therefore, may act as reservoirs of infected plant material, and grapevine propagation processes can present increased risks of infection for cuttings (Gramaje and Armengol, 2011).

The most prevalent diseases in nurseries and young grapevines are Petri disease (PD), black foot (BF), and Botryosphaeria dieback. PD is primarily caused by *Cadophora luteo-olivacea*, *Phaeomoniella chlamydospora*, and *Phaeoacremonium* spp., which enter grapevines through wounds or pruning cuts (Gramaje and Armengol, 2011). This disease is characterized by black discolourations of host xylem and accumulation of phenolic compounds in the vessels (Mugnai *et al*., 1999). External symptoms of PD include interveinal leaf chlorosis, leaf necrosis, stunted plant growth and decline, and, in severe cases, dieback (Fourie and Halleen, 2004).

The disease black foot is caused by fungi in the genera *Dactylonectria*, *Cylindrocladiella*, *Ilyonectria*, *Neonectria*, and *Thelonectria*. These pathogens typically infect young grapevines through root wounds or injuries (Agustí-Brisach and Armengol, 2013), leading to black necrotic lesions on roots and brown discolourations at trunk bases (Fourie and Halleen, 2006).

Botryosphaeria dieback, caused by multiple species within *Botryosphaeriaceae* (Úrbez-Torres, 2011), manifests as cordon dieback, characterized by spur losses and wedge-shaped internal necroses in cordon and trunk cross-sections (Gramaje *et al*., 2018). Eradication of GTDs is not possible, so control of these diseases is primarily focused on prevention and mitigation (Gramaje *et al*., 2018). In nurseries, integrated management programmes including physical, chemical, and/or biological controls have been previously implemented to reduce GTD infections (Halleen and Fourie, 2016). In recent years, the number of chemicals authorized for use during grapevine propagation have reduced (Gramaje and Di Marco, 2015), and biological control has then been proposed as a sustainable alternative in grapevine nurseries (Gramaje *et al*., 2018).

Biocontrol agents offer several direct mechanisms of action against pathogens, including competition for space and nutrients, production of hydrolytic enzymes, and parasitism or antibiosis (Thambugala *et al*., 2020). Indirect mechanisms of these agents are associated with plant defense responses after pathogen colonization (Legein *et al*., 2020). *Trichoderma atroviride* SC1 (TCH) has been the most used and effective biocontrol agent against GTD fungi in grapevine nurseries (Pertot *et al*., 2016; Berbegal *et al*., 2020; Leal *et al*., 2023). This *Trichoderma* strain was especially effective in reducing incidence of PD (Pertot *et al*., 2016; Berbegal *et al*., 2020; Leal *et al*. 2023) and Botryosphaeria dieback (Berbegal *et al*., 2020; Leal *et al*., 2021) when applied during the hydration stage of propagation material. Hot-water treatment (HWT) has also been proposed as an environmentally-friendly strategy to reduce GTD infections in grapevine nurseries (Halleen and Fourie, 2016; Eichmeier *et al*., 2018; Lade *et al*., 2022). However, anecdotal reports of unacceptably high rates of vine mortality after HWTs have sometimes resulted in reluctance by nurseries to use them (Gramaje and Di Marco, 2015).

Given that GTD control options are limited in grapevine nurseries, and that TCH and HWT are considered the most effective strategies, the present study aimed to investigate and compare the efficacy of both treatments, applied either individually or in combination, for the control of GTDs. The treatments were applied to two types of plant material at two different time points: (i) rootstock and scion cuttings before grafting, and (ii) grafted plants after the rooting phase in nursery fields. The study also aimed to assess effects of biological and physical treatments on the viability of the plant material. The hypotheses assessed in the study were: (i) that physical and biological treatments reduce the incidence of GTDs compared to untreated controls; (ii) that efficacy of these treatments depends on application timing; (iii) the combination of HWT and TCH enhances effectiveness of disease management, reducing GTD incidence through propagation processes; and (iv) there is variability in susceptibility of rootstock/cultivar combinations to biological and physical treatments.

MATERIALS AND METHODS

Planting material

The experiments described here were carried out in a nursery in O Barco de Valdeorras and a vineyard in Leiro, both located in Ourense, Galicia, Northwestern Spain. Nine native Galician grapevine cultivars were used, including three red grape ('Brancellao', 'Mencía', and 'Merenzao') and six white grape cultivars ('Albariño', 'Branco Lexítimo' 'Dona Branca', 'Loureira' 'Torrontés', and 'Treixadura'). All the cultivars were grafted onto the 110 Richter rootstock (110 R).

Treatments and fungal isolations

The effectiveness of the following treatments was evaluated: HWT at 53°C for 30 min; TCH (Vintec®, Certis Belchim; 2×10^{10} CFU g^{-1} of formulated product) at a dose of 2 g L^{-1}, by immersion in an aqueous suspension for 24 h at room temperature; and a combination of treatments (HWT + TCH), commencing with HWT application followed by maintenance of at 20°C for 24 h before TCH inoculation. The viability of conidia from the fungus TCH was verified to be at least 85% before the trial (Pertot *et al*., 2016). A serial dilution of the conidial suspension was carried out, and the diluted suspensions were plated onto potato dextrose agar (PDA), with colony-forming units counted after 24-48 h of incubation at room temperature.

Before application of treatments and the onset of propagation processes in a nursery, 25 rootstock cuttings and ten scion cuttings of each cultivar were randomly selected and analyzed for the presence of fungal pathogens associated with GTDs. Isolations were carried out from 1 cm-long sections of the cutting stems. These sections were washed with water, surface-disinfected for 1 min in 1.5% sodium hypochlorite solution, and then rinsed twice with sterile distilled water. Wood fragments were then placed on malt extract agar (MEA) supplemented with 0.5 g L^{-1} streptomycin sulfate (Sigma-Aldrich) (MEAS) (five fragments per two Petri dishes). The isolation plates were then incubated at 25°C in darkness for 10-15 d. All developng colonies were transferred to PDA. Preliminary morphological identification of colonies was carried out by observing their macroscopic characteristics to identify potential pathogens.

Identities of fungal species were confirmed using molecular methods. Fungal mycelium from pure cultures grown on PDA for 2-3 weeks at 25°C in darkness was mechanically disrupted using the FastPrep-24™5G system (MP Biomedicals). Total DNA was then extracted following the manufacturer's instructions using the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek). Identification of all isolates was carried out through analyses of Internal Transcribed Spacer (ITS) regions by amplification with universal fungal primers ITS1F and ITS4. Additional molecular identifications were then carried out for specific fungi. Cylindrocarpon-like asexual morphs were confirmed by sequencing part of the histone H3 gene with primers CYLH3F and CYLH3R (Crous *et al*., 2004). The beta-tubulin (tub2) region was amplified using the T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) primer set for *Phaeoacremonium* spp., and the BTCadF/BTCadR primer set for *Cadophora* spp. (Travadon *et al*., 2015). Identity of TCH was revealed by using the specific primers developed by Savazzini *et al*. (2008). All PCR products were visualized in 1% agarose gels (agarose D-1 Low EEO, Conda Laboratories) and sequenced in both directions by Eurofins GATC Biotech.

Disease incidence (DI) caused by BF and PD pathogens was determined as the mean percentage of grafted plants infected by these fungi. Disease severity (DS) was assessed as the mean percentage of wood segments (ten segments per stem section) colonized by these fungi, after use of the standard pathogen isolation protocol described for GTDs (Berlanas *et al*., 2020). The wood segments were placed vertically on the agar medium.

Assessments of treatments to cuttings or grafted plantss

Treatments assessed were applied at two critical points in traditional nursery plant production. It is recognized that there is increased risk of contamination by GTD pathogens during pre-grafting hydration (Experiment 1), or after the rooting of plants in a nursery field prior to planting in commercial vineyards (Experiment 2). Two experiments were each repeated for two consecutive years (2021 and 2022).

Experiment 1: Application of treatments to rootstock and scion cuttings prior to grafting. Propagation material was subjected to HWT, TCH, or a combination of both (HWT + TCH) during pre-grafting hydration of the grapevine propagation process, in April 2021. HWT was applied exclusively to rootstock cuttings, while TCH was applied to both rootstock and scion cuttings. Control cuttings (C) underwent the standard nursery hydration using water at room temperature (Gramaje and Armengol, 2011). Grafting and stratification were carried out in May 2021 and repeated in May 2022, followed by field planting in the nursery in, resectively, June 2021 and June 2022. For each trial, the planting material was arranged in a randomized complete block design with three replicates, each of 30 plants per treatment and per cultivar. Standard cultural practices were followed throughout the two growing seasons, and crop maintenance included use of integrated pest management (IPM) strategies.

In March 2022 and March 2023, rooted plants were removed from the nursery field, their roots were trimmed to a uniform length (10 cm), and they were then stored at 4–6°C. In April 2022 and April 2023, the plants were transplanted into an experimental vineyard, maintaining a spacing of 50 cm between plants and 2.5 m between rows. At the end of August 2022 and August 2023, plant viability was assessed as percentage of

sprouted plants (SP), while vegetative development was evaluated by measuring main shoot lengths (SLs). During vegetative dormancy in December 2022 and December 2023, plants were uprooted and transported to the laboratory for fungal isolations and evaluation of the root weight (RW). Three segments (each 1 cm length) were excised from three distinct zones: the graft union, the basal end of the rootstock, or the root system. These segments were surface-washed, disinfected, and processed following the fungal isolation protocol described above. Ten wood fragments from each zone were analyzed (five per Petri dish), totalling 30 fragments per plant. Molecular identification of GTD pathogens and TCH were carried out as described above.

Experiment 2: Application of treatments to grafted plants prior to planting in a commercial vineyard. Grafted plants were produced using 110R rootstock cuttings and scions of the nine grapevine cultivars listed above, following the standard procedure of the production nursery. This included hydration of cuttings for 24 h, grafting using an omega grafting machine, and stratification at 25°C with 75% relative humidity (Gramaje and Armengol, 2011). After stratification, shoots were pruned, plants were waxed, and subsequently planted in the nursery field in June 2021 and June 2022. A total of 90 grafted plants per cultivar were established. Crop maintenance in the nursery followed standard techniques for managing plant growth.

In March 2022 and March 2023, plants were uprooted from the nursery field, roots were trimmed, and shoots were pruned to two buds before being waxed again. The plants were then subjected to HWT, TCH, or HWT + TCH and immediately transplanted into a commercial vineyard. A randomized complete block design was used, with three replicates of ten plants per treatment and cultivar, totalling 360 plants per treatment. The planting arrangement was 0.5 m spacing between plants and 2.5 m between rows. Standard cultural practices were followed throughout the growing season.

At the end of August 2022 and end of August 2023, plant viability was assessed as described in Experiment 1. In December 2022 and December 2023, during vegetative dormancy, plants were uprooted and processed for fungal isolations and RW analysis, as described for Experiment 1.

Data analyses

Before conducting statistical analyses, all data were tested for normality and homogeneity of variances. Since no significant differences ($P > 0.05$) were observed between the two years of experiments (2021 and 2022) for any of the variables analyzed (DI, DS, SP, SL, and RW), and no significant year × treatment interactions were detected, data from both years were pooled for final analyses. Transformations were applied when necessary, with percentage data being converted using the arcsine square root transformation, expressed as arcsin $(DI \text{ or } DS/100)^{1/2}$. Treatment means for variables such as DI, DS, SP, SL, and RW were calculated based on their values at each sampling point. Two-way ANOVA was employed to analyze the experimental results, considering blocks and treatments as independent variables, and DI (%), DS (%), SP (%), SL (cm), and RW (g) as dependent variables. Treatment means were compared using the Student's t-test with the least significant difference (LSD) method, at $P < 0.05$. All analyses were carried out using XLSTAT v24.3 software (Addinsoft).

RESULTS

Analysis of the plant material prior to application of treatments and the onset of nursery propagation processes showed that no fungal pathogens associated with GTDs were present. Specifically, no fungi linked to PD or BF were isolated from 25 rootstock cuttings or ten scion cuttings randomly selected from each cultivar.

Experiment 1: Incidence and severity of fungal grapevine trunk diseases when treatments to rootstock and scion cuttings were applied prior to grafting

No statistically significant effect ($P > 0.05$) year was observed for any of the variables analyzed (DI and DS for both BF and PD); all, and no interactions between year and treatment were detected. Data from both years (2021 and 2022) were therfore pooled for final analyses. Analysis of the plant material at the end of the experiment showed that fungi associated with GTDs were present. For BF, the fungi *Dactylonectria torresensis*, *Dactylonectria macrodidyma* and *Ilyonectria liriodendri* were identified. For PD, the isolated species included *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Cadophora luto-olivacea*. These results are presented by disease category, grouping the fungi associated with each disease.

Effectiveness of HWT, TCH, and the combined HWT + TCH treatments for reducing BF and PD incidence and severity varied across the different grapevine cultivars (Table 1). Disease incidence was greater for BF than for PD. For BF incidence, no statistically significant differences ($P > 0.05$) were observed among treatments for

Table 1. Incidence and severity of Black Foot and Petri disease in grafted plants of nine grapevine cultivars following HWT, TCH, and HWT+TCH treatments prior to planting in a commercial vineyard (Experiment 1).

Treatments	Albariño	Branco Lexítimo	Brancellao	Dona Blanca	Loureira	Mencía	Merenzao	Torrontés	Traixadura	LSD
BF incidence (%)[a]										
Control	34.0[b] ± 5.9	36.1 ± 9.8	31.9 ± 5.8	38.8 ± 5.5	20.8 ± 12.1	20.1 ± 7.2	42.3 ± 11.6	43.7 ± 12.8	25.0 ± 11.4	0.61
HWT	54.8 ± 15 AB	58.4 ± 16.4 A	29.8 ± 6.7 AB	40.2 ± 10.2 AB	25.6 ± 5.7 B	31.9 ± 7.6 AB	49.3 ± 9.1 AB	44.4 ± 12.5 AB	57.6 ± 10.4 A	0.01
TCH	38.1 ± 16.7	37.5 ± 4.7	27.7 ± 8.6	32.6 ± 8.9	27.0 ± 7.3	26.3 ± 1.4	36.1 ± 9.8	34.7 ± 11.7	39.5 ± 8.4	0.96
HWT+TCH	27.7 ± 10.4	49.3 ± 11.6	27.0 ± 3.0	33.3 ± 7.8	25.0 ± 9.4	33.3 ± 10.8	50.0 ± 10.8	42.3 ± 8.5	34.0 ± 13.8	0.57
LSD[c]	0.5	0.4	0.9	0.9	0.9	0.6	0.7	0.9	0.2	
BF severity (%)										
Control	12.5 ± 7.55	9.5 ± 3.3	5.6 ± 1	8.4 ± 2.3	3.6 ± 2.4	2.3 ± 0.6 b	10.9 ± 3	12.6 ± 3.8	6.2 ± 3.4 b	0.39
HWT	10.9 ± 3.76 ABC	18.6 ± 7.8 AB	6.6 ± 1.6 C	11.3 ± 4.1 ABC	4.7 ± 0.7 C	7.9 ± 2.7 abBC	9.5 ± 2.6 BC	9.7 ± 2.6 BC	21.8 ± 5.3 aA	0.01
TCH	8 ± 3.63 BC	15.8 ± 3.1 A	5.1 ± 2 BC	5.9 ± 2 BC	4 ± 0.7 C	5.1 ± 0.7 abBC	6.5 ± 1.6 aBC	11.3 ± 3.3 AB	7.9 ± 2.8 bBC	0.03
HWT+TCH	4.7 ± 1.67	14.8 ± 6.6	7.6 ± 2.3	8.1 ± 2.6	6.9 ± 2.8	9.4 ± 3 a	12.2 ± 2.2	10.4 ± 2.2	11.5 ± 5.5 ab	0.79
LSD	0.8	0.7	0.7	0.6	0.6	0.02	0.4	0.9	0.04	
PETRI DISEASE incidence (%)										
Control	6.9 ± 4.53 BC	30.5 ± 8.0 aABC	27.7 ± 12 aAB	0 ± 0 C	13.8 ± 9 abBC	8.3 ± 5.2 BC	4.1 ± 4.1 BC	43 ± 14.5 aA	12.5 ± 8.5 BC	0.02
HWT	4.1 ± 4.16	4.1 ± 2.8 b	0 ± 0 b	5.5 ± 5.5	0 ± 0 b	0 ± 0	0.5 ± 0.4	4.1 ± 4.1 b	0 ± 0	0.70
TCH	13.8 ± 9.02 AB	11.1 ± 6.0 bAB	5.5 ± 5.5 abAB	2.7 ± 2.7 AB	18 ± 7.5 aA	6.9 ± 4.5 AB	4.8 ± 3.1 AB	8.3 ± 8.3 bAB	0 ± 0 B	0.02
HWT+TCH	0 ± 0 B	4.1 ± 4.2 bAB	9 ± 4.3 abAB	0 ± 0 B	0 ± 0 bB	2.7 ± 2.7 AB	0 ± 0 B	0 ± 0 bB	12.5 ± 8.5 A	0.03
LSD	0.5	0.009	0.01	0.5	0.04	0.3	0.4	>0.001	0.2	
PETRI DISEASE severity (%)										
Control	1.2 ± 0.86 B	12.2 ± 5.3 aA	9.1 ± 4.2 aAB	0 ± 0B	8.8 ± 7.6AB	2.9 ± 2.4B	0.4 ± 0.4 abB	17 ± 6.5A	5.8 ± 4.1AB	0.01
HWT	2.5 ± 2.49	1.6 ± 1.1 b	0 ± 0 b	3.8 ± 3.8	0 ± 0	0 ± 0	0 ± 0 b	0.8 ± 0.8	0 ± 0	0.62
TCH	7.7 ± 4.98 AB	1.5 ± 0.8 bAB	0.5 ± 0.5 bAB	0.8 ± 0.8 AB	9.2 ± 8.1 AB	1.1 ± 0.8 AB	1.9 ± 1.2 aAB	33.3 ± 33.3 A	0 ± 0 B	0.04
HWT+TCH	0 ± 0	0.4 ± 0.4 b	4.1 ± 2.3 ab	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0 b	0 ± 0	4.1 ± 3.2	0.14
LSD	0.3	0.02	0.02	0.4	0.4	0.4	0.05	0.4	0.3	

[a] HWT = Hot Water Treatment; TCH = *Trichoderma atroviride* SC1.

[b] Values expressed as the mean ± SE (Standard error of the mean).

[c] Least significant difference: means followed by the same letter do not differ significantly ($P < 0.05$). Capital letters are for comparison of means in the same row. Small letters are for comparison of means in the same column.

any of the nine cultivars, although a reduction trend was noted for some combinations. For example, the lowest BF incidences were recorded for 'Albariño' (27.7 ± 10.4%) and 'Brancellao' (27.0 ± 3.0%) after the HWT + TCH treatment, compared to the experimental controls (34.0 ± 5.9% for 'Albarino', 31.9 ± 5.8% for 'Brancellao'). However, none of these differences reached statistical significance. For BF severity, significant differences were observed in 'Mencía' (P = 0.02) and 'Treixadura' (P = 0.04). In 'Mencía', BF severity was less in the control (2.3 ± 0.6%) than from the HWT + TCH treatment (9.4 ± 3.0%). Similarly, in 'Treixadura', BF severity was less in the controls (6.2 ± 3.4%) than from the HWT (21.8 ± 5.3%).

For PD incidence, statistically significant differences were observed in several cultivars, including 'Branco Lexítimo' (P = 0.009), 'Brancellao' (P = 0.01), 'Loureira' (P = 0.04), and 'Torrontés' (P = 0.008). The HWT + TCH treatment completely suppressed PD in 'Albariño', 'Loureira', and 'Torrontés, and markedly reduced PD in 'Branco Lexítimo' (4.1 ± 4.2%), compared to the controls (30.5 ± 8.0%). In 'Brancellao', the HWT treatment was particularly effective, fully suppressing detection of the pathogen. For PD severity, significant reductions were recorded in 'Branco Lexítimo' and 'Brancellao' (P = 0.02 in both cases). In 'Branco Lexítimo', the HWT + TCH treatment resulted in the lowest severity (0.4 ± 0.4%) compared to the control (12.2 ± 5.3%). Similarly, in 'Brancellao', both TCH and HWTs led to less severity (0.5 ± 0.5% from TCH, 0 ± 0% from HWT) relative to the control (9.1 ± 4.2%). Statistically significant differences were observed between cultivars for particular treatments and pathogen (Table 1). For BF, HWT gave differences (P < 0.01) between cultivars. Incidence and severity of BF were high in 'Treixadura' compared to those in 'Loureira'. For PD, the TCH treatment showed dependence on the cultivar factor, with differences were detected between cultivars for incidence (P = 0.02) and severity (P = 0.04) (Table 1). 'Loureira' developed greatest incidence and severity, in contrast to these parameters in 'Treixadura'. In addition, intravarietal variations were observed after the HWT + TCH treatment, specifically for incidence of PD.

Experiment 1: Effects of treatments applied prior to grafting on grapevine viability

Effects of the experimental treatments on plant viability and vegetative growth were evaluated, based on the percentage of SP, SL, and RW in grafted grapevine plants following one growing season in the field (Table 2).

Sprouting varied between the cultivars for the control treatments, indicating that this parameter was strongly influenced by genotype (Table 2). The cultivars that showed the highest budburst values were 'Treixadura', 'Torrontés', 'Merenzao', 'Mencía', 'Brancellao', and 'Albariño', in comparison with 'Blanco Lexítimo', which showed lower values. This genotype-dependent behavior was also observed under the HWT, where differences between cultivars persisted. In particular, the 'Dona Branca' cultivar exhibited the lowest budburst after the application of this treatment.

Sprouting rates were generally high across all cultivars and treatments, ranging from 83.3% to 100%. In most cultivars, no statistically significant differences were detected among treatments (P > 0.05). However, in 'Branco Lexítimo', significant differences were observed (P = 0.03), with the control showing a lower sprouting percentage (83.3 ± 4.5%) compared to HWT (100%) and TCH (100%). In 'Loureira', the TCH treatment significantly reduced sprouting (93.3 ± 1.6%) compared to control and other treatments, which all achieved 100% (P = 0.01).

Shoot length proved to be a trait highly dependent on genotype, both in the control treatment and under each of the applied treatments (P < 0.05) (Table 2). In all cases, the 'Brancellao' produced the longest shoots, compared to the cultivars 'Loureira', 'Torrontés', and 'Treixadura', which produced shorter shoots. No differences (P > 0.05) were observed among treatments in any of the cultivars. Nevertheless, trends were noted: for example, in 'Brancellao', the longest shoots were observed after HWT + TCH (67.6 ± 5.3 cm), and less from the control (61.5 ± 6.0 cm). Similar trends favouring HWT + TCH were observed in 'Albariño' mean length = 43.6 ± 0.3 cm) and 'Mencía' (45.9 ± 5.0 cm).

Root weight was not a genotype-dependent trait (P > 0.5) (Table 2). For the treatments, statistically significant differences in RW were found in five cultivars: 'Albariño' (P = 0.03), 'Branco Lexítimo' (P < 0.01), 'Dona Branca' (P < 0.01), and 'Mencía'. The HWT gave the greatest RW, in 'Albariño' (60.2 ± 7.2 g), which was greater than from the control and most of the other treatments. In 'Branco Lexítimo', HWT + TCH gave the greatest RW (72.2 ± 8.3 g), which was greater than the control (50.4 ± 6.3 g). For 'Dona Branca', HWT increased RW to 91.3 ± 8.3 g compared to 67.0 ± 6.5 g from the control. Similarly, the TCH treatment gave the greatest RW in 'Mencía' (92.2 ± 7.8 g), which was greater than from the control and most of the other treatments.

Experiment 2: Incidence and severity of fungal grapevine trunk diseases when treatments were applied to grafted plants prior to planting in a commercial vineyard

Analysis of the plant material at the end of the experiment yielded fungal species associated with GTDs.

Table 2. Average percentage of sprouting (%), shoot length (cm), and root weight (g) of plants from nine grapevine varieties following the treatment of rooted plants with HWT, TCH, or HWT+TCH and cultivation in a commercial vineyard (Experiment 1).

Treatment	Albariño	BrancoLexítimo	Brancellao	DonaBranca	Loureira	Mencía	Merenzao	Torrontés	Treixadura	LSD
SP(%)[a]										
Control	100 A	83.3 ± 4.5 bB	96.6 ± 1.3 A	93.3 ± 2.7 AB	100 aA	100 A	100 A	96.6 ± 1.3 A	100 A	<0.001
HWT	100 A	100 aA	100 A	90.0 ± 2.8 B	100 aA	100 A	100 A	96.6 ± 1.3 AB	100 A	<0.001
TCH	96.6 ± 1.3	100 a	96.6 ± 1.3	96.6 ± 1.3	93.3 ± 1.6 b	96.6 ± 1.3	100	96.6 ± 1.3	100	0.76
HWT+TCH	99.5 ± 0.0	96.6 ± 1.3 ab	100	100	100 a	100	100	100	100	0.09
LSD[c]	0.44	0.03	0.73	0.49	0.01	0.46	0.50	0.66	0.5	
SL(cm)										
Control	28.9 ± 3.5 B	25.5 ± 3.3 B	61.5 ± 6.0 A	35.1 ± 3.0 B	24.3 ± 1.3 B	41.9 ± 4.2 AB	41.7 ± 5.8 AB	27.8 ± 1.3 B	35.2 ± 2.3 B	0.04
HWT	36.9 ± 4.3 AB	31.6 ± 4.2 AB	53.4 ± 4.0 A	29.0 ± 2.2 B	23.7 ± 1.6 B	39.9 ± 3.5 AB	39.1 ± 4.2 AB	28.7 ± 1.3 B	28.3 ± 1.5 B	0.01
TCH	26.8 ± 3.5 B	26.9 ± 4.0 B	57.8 ± 5.2 A	35.7 ± 2.8 AB	22.1 ± 0.8 B	40.2 ± 4.3 AB	40.0 ± 4.3 AB	30.1 ± 2.3 B	28.8 ± 2.0 B	0.04
HWT+TCH	43.6 ± 0.3 AB	34.2 ± 3.6 B	67.6 ± 5.3 A	28.5 ± 2.7 B	23.1 ± 0.8 B	45.9 ± 5.0 AB	41.6 ± 4.0 B	28.2 ± 2.5 B	31.2 ± 2.3 B	0.01
LSD	0.10	0.47	0.44	0.19	0.84	0.14	0.76	0.57	0.15	
RW(g)										
Control	48.0 ± 5.3 b	50.4 ± 6.3 b	86.8 ± 8.0	67.0 ± 6.5 b	70.2 ± 5.8	70.3 ± 6.7 c	62.3 ± 6.2	68.1 ± 7.0	81.2 ± 7.5	0.92
HWT	60.2 ± 7.2 a	49.8 ± 5.8 b	78.9 ± 7.3	91.3 ± 8.3 a	64.0 ± 6.7	83.8 ± 6.8 ab	73.0 ± 8.2	83.7 ± 7.7	73.7 ± 7.0	0.95
TCH	54.1 ± 7.2 ab	55.1 ± 6.8 ab	84.2 ± 7.3	71.1 ± 7.8 b	70.7 ± 6.7	92.2 ± 7.8 a	76.4 ± 7.7	72.2 ± 7.0	80.8 ± 7.7	0.96
HWT+TCH	54.0 ± 0.0 b	72.2 ± 8.3 a	87.5 ± 8.3	71.0 ± 7.2 b	65.4 ± 6.7	79.2 ± 7.3 bc	75.9 ± 7.7	77.6 ± 7.5	81.4 ± 8.0	0.99
LSD	0.03	<0.01	0.81	<0.01	0.61	<0.01	0.19	0.48	0.37	

[a] SP, Sprouting; SL, Shoot Lenght; RW, Root Weight; HWT, Hot Water Treatment; TCH, *Trichoderma atroviride* SC1.
[b] Values expressed as the mean ± SE (Standard error of the mean).
[c] Least significant difference: means followed by the same letter do not differ significantly ($P < 0.05$). Capital letters are for comparison of means in the same row. Small letters are for comparison of means in the same column.

As causes of BF, *D. torresensis*, *Dactylonectria pauciseptata*, and *Dactylonectria novozelandica* were identified. For PD, the isolated species included *Pa. chlamydospora*, *Pm. minimum*, and *C. luteo-olivacea*. These results are presented by disease category, grouping the fungi associated with each category.

In *Experiment 2*, application of HWT, TCH, and HWT + TCH to grafted grapevine plants prior to planting in a commercial vineyard gave variable effects on incidenc and severity of BF and PD across the nine grapevine cultivars (Table 3). For BF incidence and severity, differences among treatments were only observed in 'Branco Lexítimo' (P = 0.01 for incidence; P = 0.03 for severity), and in 'Torrontés' (P = 0.008 and P = 0.03). In 'Branco Lexítimo', HWT (5.5 ± 5.6%) and TCH (4.0 ± 11.3%) both reduced incidence compared with the the control (36.1 ± 9.8%). For BF severity in this cultivar, HWT reduced incidence (1.6 ± 1.7%) compared to the control (9.5 ± 3.3%). In 'Torrontés', none of the treatments significantly reduced DI or DS compared to the control.

Petri disease incidence was different in six of the grapevine cultivars, including 'Albariño' (P = 0.04), 'Branco Lexítimo' (P = 0.005), 'Dona Branca' (P = 0.002), 'Mencía' (P = 0.04), and 'Torrontés' (P = 0.01). In 'Albariño', 'Dona Branca', and 'Mencía', none of the treatments reduced DI compared to the control. In 'Branco Lexítimo', all three treatments reduced incidence from 30.5 ± 8.0% in the control to 2.7 ± 1.8% after HWT. Similarly, for 'Torrontés', HWT (7.5 ± 2.2%), TCH (5.8 ± 2.4%), and HWT + TCH (6.7 ± 3.1%) reduced DI relative to the control (43.0 ± 14.5%). For PD severity, significant treatment effects were observed in 'Albariño' (P = 0.04), 'Branco Lexítimo' (P = 0.005), 'Dona Branca' (P = 0.002), 'Mencía' (P = 0.04), and 'Torrontés' (P = 0.01). However, only in 'Torrontés', all three treatments reduced incidence from 17.0 ± 6.6% in the control to as little as no fungal infection from the TCH. Treatment.

When the effects of each treatment was compared among cultivars, PD exhibited a marked genotype-dependent response (Table 3). From the control treatment, large differences were observed among cultivars, with 'Torrontés' showing high incidence, while 'Dona Branca' displayed no symptoms. Application of HWT reduced disease incidence in some susceptible cultivars (e.g. 'Branco Lexítimo'), but was counterproductive in others, such as 'Albariño', where incidence increased. Similarly, disease severity responded variably: while 'Torrontés' had less severe disease after HWT, disease increased in 'Loureira'.

Experiment 2: Effects on grapevine viability of treatments applied to grafted plants prior to planting in a commercial vineyard

Effects of the experimental treatments on plant viability and growth performance were assessed by measuring the SP, SL, and RW of grafted plants after one growing season in the commercial vineyard (Table 4).

Statistically significant differences in SP were observed between genotypes in some cases, particularly under after the control and TCH treatments (Table 4). The cultivars 'Albariño', 'Loureira', 'Mencía', and 'Treixadura' maintained 100% survival under all conditions, while 'Branco Lexítimo' showed reduced survival after some of the treatments, indicating a specific varietal sensitivity. SP rates were generally high across all cultivars and treatments, ranging from 83.3% to 100%. No differences (P > 0.05) were detected among treatments within each cultivar. However, some trends were observed. In 'Branco Lexítimo', sprouting increased from 83.3 ± 4.5% in the control to 100% after HWT. Conversely, in 'Merenzao', there was a small sprouting reduction after HWT + TCH (83.3 ± 6.9%) compared to the control (100%), although this was not statistically significant.

There were a clear genotype-dependent responses in SL from the treatments (Table 4). 'Brancellao' consistently had the longest shoots, while 'Loureira' and 'Torrontés' had the shortest shoots. Response to the HWT + TCH treatment was variable depending on the cultivar: in 'Albariño', this treatment led increased shoot lengths, whereas for other cultivars (e.g. 'Treixadura' and 'Merenzao'), this effect was less pronounced. This variability reflected a strong interaction between genotype and the applied treatments.

Shoot length was affected by treatments in several of the cultivars (Table 4). In 'Albariño', plants receiving with HWT + TCH developed longer shoots (60.1 ± 4.5 cm) than those in the control group (28.9 ± 3.5 cm) (P < 0.01). In 'Branco Lexítimo', HWT alone resulted in the greatest shoot length (41.1 ± 4.9 cm), which was greater than from the control (25.5 ± 3.3 cm). In contrast, in 'Brancellao' and 'Mencía', the control plants (means, respectively, 61.5 ± 6.1 cm and 41.9 ± 4.1 cm) had longer shoots than those after HWT + TCH (48.8 ± 5.3 cm for 'Brancellao', and 33.5 ± 3.8 cm for 'Mencía') (P < 0.01), indicating a possible negative effect of the combined treatment in these cultivars.

For RW, there was no clear trend of exclusive genotype dependence, as responses varied between treatments within each cultivar (Table 4). Only HWT + TCH caused severe reduction in root weight in 'Albariño' (16.8 g), whereas in other cultivars, including 'Mencía' and 'Branco Lexítimo', effects were less severe or nil.

Table 3. Incidence and severity of Black Foot and Petri disease in grafted plants of nine grapevine cultivars following HWT, TCH, and HWT+TCH treatments prior to planting in a commercial vineyard (Experiment 2).

Treatment	Albariño	Branco Lexítimo	Brancellao	Dona Branca	Loureira	Mencía	Merenzao	Torrontés	Traixadura	LSD
BF incidence (%)[a]										
CONTROL	34.0[b] ± 5.9	36.1 ± 9.8 a	31.9 ± 5.9	38.3 ± 2.8	20.8 ± 12.1	20.1 ± 7.2	42.3 ± 11.6	43.7 ± 12.8 ab	25 ± 11.3	0.61
HWT	33.3 ± 8.3 AB	5.5 ± 5.6 bB	27 ± 7.1 AB	30.5 ± 9.0 AB	20.8 ± 12.1 AB	11.1 ± 5.1 B	26.3 ± 12.4 AB	56.9 ± 4.5 aA	34.7 ± 11.1 AB	0.01
TCH	20.1 ± 5.1	4.0 ± 11.3 b	21.5 ± 5.3	36.1 ± 9.6	19.4 ± 9.0	26.3 ± 12.2	28.5 ± 10.2	36.1 ± 13.8 ab	52.7 ± 8.5	0.50
HWT+TCH	30.5 ± 10.7	20.1 ± 9.6 ab	16.6 ± 5.7	30.5 ± 10.0	27 ± 7.6	27 ± 11.3	29.7 ± 8.5	25 ± 6.8 b	43.7 ± 4.2	0.63
LSD[c]	0.5	0.01	0.3	0.9	0.9	0.6	0.7	0.008	0.4	
BF severity (%)										
CONTROL	12.5 ± 7.6	9.5 ± 3.3 a	5.6 ± 1.0	8.4 ± 1.6	3.6 ± 2.4	2.3 ± 0.7	10.9 ± 3.1	12.6 ± 3.8 ab	6.2 ± 3.4	0.39
HWT	12.5 ± 4.3 AB	1.6 ± 1.7 bB	6.9 ± 3.4 AB	9.5 ± 3.4 AB	3.7 ± 2.0 AB	2.7 ± 1.4 B	4.1 ± 2.1 AB	17.7 ± 3.8 aA	15 ± 4.0 AB	<0.001
TCH	4.5 ± 1.8 BC	10.2 ± 3.6 aC	3.1 ± 0.9 C	9.5 ± 2.2 ABC	3.4 ± 1.6 C	4.5 ± 2.4 BC	5.2 ± 1.7 BC	5.8 ± 2.4 bABC	11.6 ± 3.1 A	0.04
HWT+TCH	12.5 ± 8.0	4.8 ± 2.2 ab	4.0 ± 1.3	6.3 ± 2.2	4.7 ± 2.1	6.9 ± 2.9	9.5 ± 2.8	4.8 ± 1.4 b	10.5 ± 2.2	0.59
LSD	0.7	0.03	0.5	0.8	0.6	0.3	0.1	0.03	0.3	
PETRI incidence (%)										
CONTROL	6.9 ± 4.5 bBCD	30.5 ± 8.0 aAB	27.7 ± 12.2 ABC	0 ± 0.0 cD	13.8 ± 9.0 BCD	8.3 ± 5.3 abBCD	4.1 ± 4.2 CD	43.0 ± 14.5 aA	12.5 ± 8.5 BCD	0.02
HWT	22.7 ± 5.4 aA	2.7 ± 1.8 bD	18.6 ± 6.6 AB	11.3 ± 3.0 abBCD	8.4 ± 2.2 BCD	7.9 ± 1.6 bCD	13.2 ± 3.6 ABC	7.5 ± 2.2 bCD	11.5 ± 2.9 BCD	0.01
TCH	10.3 ± 3.3 b	11.4 ± 2.0 b	13.1 ± 5.5	19.4 ± 4.4 a	9.3 ± 4.1	9.7 ± 2.0 ab	11.9 ± 3.1	5.8 ± 2.4 b	15 ± 3.1	0.63
HWT+TCH	10.6 ± 2.4 bBC	15.2 ± 4.5 bAB	8.6 ± 2.2 C	9.5 ± 2.5 bC	11.9 ± 4.2 BC	18.6 ± 4.0 aA	13.7 ± 3.5 B	6.7 ± 3.1 bC	15.4 ± 2.8 AB	0.01
LSD	0.04	0.005	0.3	0.002	0.8	0.04	0.2	0.01	0.2	
PETRI severity (%)										
CONTROL	1.2 ± 0.9 bB	12.2 ± 5.3 bAB	9.1 ± 4.2AB	0 ± 0.0 bB	8.8 ± 7.6 AB	2.1 ± 2.5 bB	0.4 ± 0.4 B	17 ± 6.6 aA	5.8 ± 4.2 AB	0.01
HWT	7.9 ± 3.7 aABC	15.4 ± 8.1 aAB	15.4 ± 3.8 AB	1.4 ± 1.0 bC	17.8 ± 7.3 A	3 ± 1.6 bC	4.1 ± 2.4 BC	0.4 ± 0.2 bC	1.5 ± 1.0 C	0.01
TCH	3.4 ± 1.6 b	11.5 ± 3.8 b	9 ± 4.4	9.5 ± 3.6 a	2 ± 1.2	2.8 ± 1.6 b	2.5 ± 1.3	0 ± 0.0 b	0 ± 0.0	0.06
HWT+TCH	3.5 ± 1.7 a	8.8 ± 3.8 b	8.1 ± 5.1	5.9 ± 4.0 ab	3.8 ± 1.7	10.5 ± 2.8 a	5.6 ± 3.1	2.9 ± 1.9 b	7.6 ± 3.0	0.83
LSD	0.04	0.005	0.3	0.002	0.8	0.04	0.2	0.01	0.2	

[a] HWT = Hot Water Treatment; TCH = *Trichoderma atroviride* SC1.

[b] Values expressed as the mean ± SE (Standard error of the mean).

[c] Least significant difference: means followed by the same letter do not differ significantly ($P < 0.05$). Capital letters are for comparison of means in the same row. Small letters are for comparison of means in the same column.

Table 4. Average percentage of sprouting (%), shoot length (cm), and root weight (g) of plants from nine grapevine varieties following the treatment of rooted plants with HWT, TCH, or HWT+TCH and cultivation in a commercial vineyard (Experiment 2).

Treatment	Albariño	BrancoLexítimo	Brancellao	DonaBranca	Loureira	Mencía	Merenzao	Torrontés	Traixadura	LSD
SP (%)[a]										
CONTROL	100[b] A	83.3 ± 4.5 B	96.66 ± 1.3 A	93.33 ± 2.7 AB	100 A	100 A	100 A	96.6 ± 1.3 A	100 A	0.01
HWT	100	100	100	100	100	100	100	100	100	1
TCH	100 A	86.6 ± 4.0 B	100 A	90.0 ± 1.8 AB	100 A	100 A	100 A	100 A	96.6 ± 1.3 AB	0.03
HWT+TCH	100	96.6 ± 1.3	100	100	100	100	83.3 ± 6.9	100	100	0.54
LSD[c]	0.52	0.06	0.43	0.06	0.35	0.75	0.43	0.14	0.43	
SL (cm)										
CONTROL	28.9 ± 3.5 bB	25.5 ± 3.3 bB	61.5 ± 6.1 aA	35.1 ± 3.0 B	24.3 ± 1.3 B	41.9 ± 4.1 aAB	41.7 ± 5.3 AB	27.8 ± 1.3 B	35.2 ± 2.3 B	0.01
HWT	37.6 ± 4.5 bAB	41.1 ± 4.9 aAB	56.9 ± 5.3 abA	39.2 ± 4.5 AB	28.7 ± 2.3 AB	42.4 ± 4.5 aAB	39.6 ± 5.3 AB	21.3 ± 2.3 B	29.0 ± 1.5 AB	<0.001
TCH	37.9 ± 4.5 bAB	31.4 ± 3.7 abAB	54.8 ± 5.3 abA	28.9 ± 2.5 B	28.1 ± 2.3 B	40.6 ± 3.8 abAB	39.1 ± 4.5 AB	28.2 ± 1.8 B	31.6 ± 2.0 AB	<0.001
HWT+TCH	60.1 ± 4.5 aA	29.1 ± 4.1 abAB	48.8 ± 5.3 bAB	31.4 ± 9.0 AB	22.7 ± 2.3 B	33.5 ± 3.8 bAB	28.2 ± 4.9 AB	26.9 ± 2.2 B	28.4 ± 2.3 AB	0.02
LSD	<0.01	<0.01	<0.01	0.14	0.06	<0.01	0.14	0.12	0.26	
RW (g)										
CONTROL	48.0 ± 5.3 b	55.4 ± 6.5 a	86.8 ± 8.2	67.0 ± 6.5	70.2 ± 5.7	70.3 ± 6.5	62.3 ± 6.1	68.0 ± 6.9 a	81.2 ± 7.5 a	0.77
HWT	67.5 ± 7.3 a	61.8 ± 6.9 a	85.8 ± 7.3	71.7 ± 6.5	65.3 ± 5.7	72.9 ± 6.9	62.0 ± 6.5	49.4 ± 7.3 b	60.4 ± 7.0 b	0.93
TCH	61.3 ± 6.9 a	36.3 ± 4.9 b	79.2 ± 7.3	69.2 ± 6.9	67.8 ± 6.5	81.9 ± 6.5	68.6 ± 7.8	74.0 ± 6.9 a	68.0 ± 7.7 b	0.74
HWT+TCH	16.8 ± 7.3 cB	55.3 ± 5.7 aAB	74.6 ± 7.3 A	67.8 ± 7.3 AB	64.5 ± 7.3 AB	73.9 ± 7.3 AB	67.5 ± 6.5 AB	72.6 ± 7.3 aAB	59.3 ± 8.0 bAB	0.01
LSD	<0.01	<0.01	0.56	0.91	0.15	0.09	0.96	<0.01	<0.01	

[a] SP: Sprouting; SL: Shoot Lenght; RW: Root Weight; HWT = Hot Water Treatment; TCH = *Trichoderma atroviride* SC1.
[b] Values expressed as the mean ± SE (Standard error of the mean).
[c] Least significant difference: means followed by the same letter do not differ significantly ($P < 0.05$). Capital letters are for comparison of means in the same row. Small letters are for comparison of means in the same column.

Root weight responses to the treatments varied across cultivars. In 'Albariño', Treatments of HWT and TCH increased RW compared to the control (48.0 ± 5.3 g), with the greatest RW after HWT (67.5 ± 7.3 g; $P < 0.01$). In contrast, treatments resulted in reductions in RWs ($P < 0.01$) in 'Branco Lexítimo', 'Torrontés', and 'Treixadura' compared to the control, indicating potentially detrimental effects of these treatments on root development in these cultivars.

DISCUSSION

This study evaluated the potential effectiveness of different treatments (HWT, TCH, and their combination, HWT + TCH for controlling GTDs, specifically black foot (BF) and Petri disease (PD), and their influence on plant viability and early development for nine Galician grapevine cultivars. Results obtained indicated variable responses to the treatments, depending on cultivar, particular pathogen, and timing of applications.

The treatments were more effective against PD than BF. In Experiment 1 (pre-grafting host stage), the combination of HWT + TCH reduced PD incidence and severity in several cultivars, including complete suppression in 'Albariño', 'Loureira', and 'Torrontés'. Reductions in severity were also observed in 'Branco Lexítimo' and 'Brancellao' following the combined or individual treatments. These results are consistent with previous research showing that the early application of HWT and/or biocontrol agents can effectively suppress internal wood-colonizing pathogens such as *Pa. chlamydospora* and *Pm. minimum* during pre-planting hydration and stratification phases (Gramaje *et al*., 2009; Pertot *et al*., 2016; Berbegal *et al*., 2020). This reinforces the concept of combining physical and biological control strategies can enhance disease suppression, as previously reported by Fourie and Halleen (2006), Halleen and Fourie (2016), and Martínez-Diz *et al*. (2021). While HWT physically reduces inoculum loads by eliminating fungal propagules (Gramaje *et al*., 2009; Pertot *et al*., 2016; Lade *et al*., 2022), TCH provides long-term protection through competitive colonization, induction of host defenses, and antifungal metabolite production (Pertot *et al*., 2016; Leal *et al*., 2024).

In contrast, the treatments had limited efficacy against BF in both experiments, with differences observed only in specific cultivars such as 'Branco Lexítimo' and 'Torrontés' in Experiment 2 (post-rooting stage). These results align with the known biology of BF pathogens, which primarily infect host plants through root systems, and may not be fully reached by treatments applied prior to or shortly after grafting (Fourie and Halleen, 2006; Leal *et al*., 2023). This indicates that post-planting interventions may also be necessary to more effectively target BF pathogens during the early grapevine establishment in the field, when the risks of soilborne infections are is greatest (Mártinez-Diz *et al*., 2021; Labarga *et al*., 2025). Similar limited efficacy of TCH against BF pathogens was reported by Berbegal *et al*. (2020), who observed that although TCH reduced incidence and severity of PD and Botryosphaeria dieback in nursery and vineyard conditions, the reduction in BF was not statistically significant despite decreased pathogen recovery from treated plants.

For plant development, treatment effects were most evident on root weights (RW), with differences observed among cultivars. In Experiment 1, treatments, particularly HWT or HWT + TCH, gave increased RW in 'Albariño', 'Branco Lexítimo', 'Dona Branca', and 'Mencía'. This indicates potential stimulatory effects of these treatments on root development, when applied early. However, in Experiment 2, the same treatments led to reductions in RW in several cultivars, including 'Branco Lexítimo', 'Torrontés', and 'Treixadura'. These contrasting results indicate that treatment timing is likely to play a crucial role in determining plant responses, and late application may exert stress on developing root systems, particularly in sensitive cultivars. Shoot length (SL) responses also varied with timing and cultivar. While in Experiment 1 SL remained largely unaffected by the treatments, in Experiment 2, differences emerged. For example, 'Albariño' showed increased SL after HWT + TCH, but 'Brancellao', 'Mencía', and 'Treixadura' had short shoots following HWT + TCH treatment. These result indicate that combined treatments applied at advanced plant developmental stages can adversely affect vegetative growth in some genotypes, likely due to additive stress effects (Waite and May, 2005; Waite and Morton, 2007). Sprouting percentages (SP) were generally unaffected by treatments in both experiments, although minor cultivar-specific variations were noted. This is consistent with previous results showing that HWT, when adequately managed, does not impair grapevine bud viability (Waite and Morton, 2007; Gramaje *et al*., 2014; Soltekin and Altindisli, 2017).

The differential cultivar responses highlight the importance of considering genotype-specific tolerance and physiological traits. For example, 'Branco Lexítimo' benefitted from all treatments, in terms of PD suppression and root biomass in Experiment 1, but had showed reduced growth parameters under the same treatment in Experiment 2. Conversely, 'Albariño' was less affected by treatment stress overall, and had increased vegetative

vigour in some cases. These results are similar to those of previous studies, indicating that grapevine varietal differences influence tolerance to HWT, and colonization success by biological agents (Waite and May, 2005; Mutawila *et al*., 2011; Eichmeier *et al*., 2018; Işçi *et al*., 2019). These differences may be due to variations among cultivars in tissue tolerance, metabolic recovery, and hydration status. Waite and Morton (2007), Gramaje *et al*. (2009), Gramaje and Armengol (2012), and Soltekin and Altindisli (2017) emphasized that effects of HWT on rooting and sprouting can vary according to the rootstock–scion combination and the timing of HWT application. The reduced root biomass observed in some cultivars following HWT + TCH, particularly in Experiment 2, could reflect additive stress effects when thermally treated plants are subsequently exposed to microbial colonization during later metabolically demanding growth phases. These results underline the importance of optimizing timing and combinations of treatments to avoid unintended negative impacts on plant vigour in sensitive genotypes.

Use of TCH showed promise as a biostimulant for some cultivars, increasing RW and SL, particularly when applied early. This indicates potential biostimulatory effects in the cultivars consistent with previous results demonstrating that *Trichoderma* spp. can promote plant growth through endophytic colonization, production of phytohormones such as indole-3-acetic acid (IAA), and improved nutrient uptake (Mutawila *et al*., 2011; Leal *et al*., 2021; Leal *et al*., 2024). However, in other cases, especially following HWT in Experiment 2, TCH had neutral or even negative effects. These results support previous observations that efficacy of *Trichoderma*-based products is context-dependent, influenced by plant physiological status, environmental conditions, and timing of applications (Leal *et al*., 2021; Lade *et al*., 2022).

While HWT + TCH can offer an effective strategy for managing PD, especially during early grapevine propagation phases, its application must be tailored to each cultivar and propagation stage, to avoid unintended impacts on plant vigour. For BF, post-rooting interventions may be more appropriate, though additional complementary strategies may be needed to enhance disease control. From a practical standpoint, integrating physical and biological treatments into nursery practices requires genotype-informed protocols. Early application of HWT + TCH may be recommended for cultivars with high susceptibility to PD and greater resilience to stress, while alternative approaches may be preferable for more sensitive genotypes, or for managing BF pathogens. These results reinforce the value of flexible and adaptive nursery management, as well as the need for continued research on cultivar-specific physiological responses to integrated disease management strategies.

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