https://doi.org/10.17221/94/2023-PPS

The control of soil-borne fungal pathogens in grapevine nurseries in Türkiye and their impact on sapling quality

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Citation: Savas Gungor N. (2024): The control of soil-borne fungal pathogens in grapevine nurseries in Türkiye and their impact on sapling quality. Plant Protect. Sci., 60: 241–257.

Abstract: In the production of grafted vines, losses are caused by fungal pathogens during callus forming or after planting in the soil. To control or reduce natural soil-born fungal infections in nurseries, certain applications were conducted in the sapling cultivation stage to analyse the efficacy of cyprodinil + fludioxonil, fluopyram + tebuconazole active substances, and *Trichoderma harzianum* biological preparation: 1103 Paulsen rootstock and *Vitis viniferea* L. cv. In the study, Sultana cultivars were stored in fungicide suspensions for 60 min before and after grafting. After grafting, the saplings were divided into *(i)* cutting + sawdust *(ii)* cutting + sawdust + soil application groups and transferred to the callus room. After nine months in the nursery, the plants were uprooted, classified as diseased or healthy, and analysed for morphological and molecular diagnosis of fungal species, isolation incidence, and sapling quality and yield. *Boeremia exigua* var. *exigua* was isolated for the first time from cuttings during grapevine sapling production and was first registered in NCBI Genbank. After callus development, *Fusarium solani* was most frequently isolated pathogen in the roots (21.5%); cyprodinil + fludioxonil reduced the *Ilyonectria* sp. isolation rate in both shoots and roots. *Botryosphaeria dothidae* and *I. liriodendri* pathogens were not detected in disease and healthy cyprodinil + fludioxonil treated saplings. The highest sapling yield was observed in fludioxonil + cyprodinil, cutting + sawdust + soil (78.75%) and cutting + sawdust (70.63%) applications.

Keywords: bio-fungicide; chemical control; sapling quality; soil-borne pathogens; vine

Vine (*Vitis vinifera* L.) is a commonly cultivated plant globally since it is not very sensitive to climate and soil, can be reproduced easily, is used in several industries, and is economically significant. In Türkiye, 4.2 mil. t grapes were cultivated in about 400 000 ha in 2020, and 246 000 t were exported with a revenue of 514 mil. USD (FAOSTAT 2021). Despite the established viticulture in Türkiye, there is a significant need for sapling nursing due to ageing and fungal lignum diseases and new vines. The economic life of a vineyard is about 40 years (Ilter & Uzun 1991; Celik et al. 1991; Santos et al. 2020). Thus, several authors indicated that every year, 7.5–15.0 mil. saplings are required in Türkiye (Şen & Yağcı 2016).

As in every sector in Türkiye, there are some problems in the vineyard nurseries. Vine cultivation, the most cultivated fruit in Türkiye, is sustainable with a healthy sapling supply (Cangi et al. 2019). In the last few decades, the interest in the impact of dieback on vine saplings and studies to develop control strategies against them have increased (Hallen et al. 2003; Lade et al. 2022). Investigations are generally limited to diagnosing disease pathogens and detecting susceptibility of the nurseries, cultivars, and rootstock (Cruz et al. 2014; Lade et al. 2022; Tan & Rodríguez

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López 2023). Besides that, it is unclear when fungal pathogens started to be a problem in grapevine nurseries and which fungicides can be used to control at various cultivation stages.

Grafted vine sapling cultivation includes the stages of graft and cutting procurement, cold storage, grafting, post-grafting callus development in the germination chamber, and planting. A temperature of 25 °C and 90% relative humidity in post-grafting germination chambers are suitable for pathogen development. Particularly at the pre-grafting stage, there might be problems in pathogen control with fungicides. It was reported that soil-borne fungal species and other known vineyard pathogens (i.e., *Diaporthe ampelina*, *Phoma exigua* var. *exigua*, *Botrytis cinerea*, etc.) were observed on healthy main branches in vineyards (Machowicz-Stefaniak & Król 2006; Koycu et al. 2006; Król 2006). It was reported that vine cuttings were mostly infected with soil-borne polyphagous pathogens such as *Cylindrocarpon* spp., *Fusarium oxysporum*, *Phytophthora* spp. and *Rhizoctonia solan*i after planting in nurseries (Hallen et al. 2003; Król et al. 2006). In previous studies on controlling fungal infections that cause low sapling yields, fungicides were not applied before and during rooting. At the same time, practices to control both soil-borne and transmitted fungal lignum disease pathogens were ineffective before and after planting the saplings in the nursery. In such studies, pre- and post-grafting fungicide applications were also found to be inadequate (Becker & Hiller 1977; Koycu et al. 2006).

To reduce the losses caused by soil-borne pathogens during sapling production, fungicides were applied in this study at the post-planting and pre-planting soil stages before grafting and callus formation. After the applications, fungal isolations were made by taking plant samples. The frequency of isolation of soil-borne pathogens in saplings at each stage was determined, and morphological and molecular identification of pathogens was also carried out. In addition, the effects of fungicide applications on sapling yield, quality and root development were analysed.

MATERIAL AND METHODS

Plant material. The study was conducted at a commercial nursery in Manisa Province in the Aegean Region, where vine nurseries are a common occupation, between 2017 and 2018. The nursery where the experiments were conducted has cultivated vine saplings for several years. The nursery has adopted crop rotation, where vine saplings are produced every two years, and barley is planted for rotation in the remaining years.

Sultan 7 seedless grape variety grafted on 1103 Paulsen American vine rootstocks with deep root structure and good compatibility with grape varieties were employed in the experiment. Both rootstock and cultivar were considered susceptible to soil-borne pathogens. Sultan 7 seedless grape variety was selected since it is the most exported variety from the Aegean region for fresh and dried consumption.

Fungicide applications in the production of grapevine planting material. In the study, pre-grafting fungicides were applied to cuttings and grafts in two stages. In Türkiye, an isolated and approved bio-preparation of *Trichoderma harzianum* Rifai KRL-AG2 was used in this experiment. The fungicides presented in Table 1 were mixed separately in plastic tanks containing 20 L of water based on manufacturer-recommended doses. During pre-grafting storage, the cuttings were blunted except for the bottom buds, and the cuttings and rootstocks were kept in the fungicide solution for 60 min to regain the water lost.

The trials were conducted in five replicates, and each replicate included 50 cuttings and grafts, and 1 800 cuttings and grafts were placed in polyethylene bags and stored at 0 °C (Figure 1). All rootstocks and cuttings were treated with the fungicides/bio-

Table 1. Employed fungicide and bio-fungicide active ingredients, commercial names, and doses

Active ingredient	Short nomenclature given during the trial	Trade name/company	Product rate (recommended dose/100 L water)
Fluopyram 200 g/L + tebuconazole 200 g/L	A	Luna Experience SC 400/Bayer	25 mL
Cyprodinil % 37.5 + fludioxanil % 25	B	Switch 62.5/WG Syngenta	50 g
Trichoderma harzianum Rifai KRL-AG2	C	T-22 Planter box/ Bioglobal	50 g
Control	D	water	–

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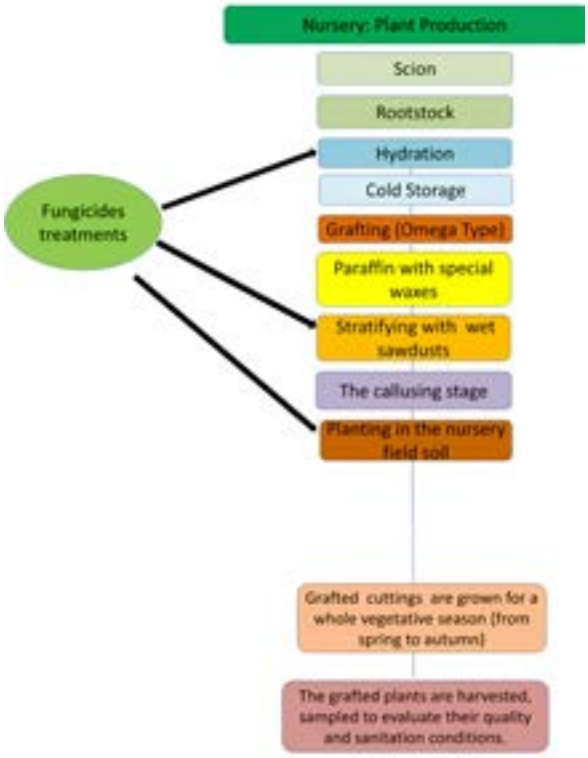

Figure 1. Main steps in vine sapling cultivation applications

fungicides (fluopyram + tebuconazole, cyprodinil + fludioxanil, *Trichoderma harzianum* Rifai KRL-AG2), listed in Table 1, before inoculation. Grafting was performed in March using an omega grafting machine. Paraffin was applied to the grafted cuttings to cover the scion, graft site and top of the rootstock (Köse et al. 2023). The grafted cuttings were then placed in plastic crates using fine, damp sawdust, and at this stage, the sawdust was soaked with water prepared with the same fungicides/bio-fungicides. The grafted grapevine cuttings were kept at 25 °C for the first three weeks to ensure healthy callus formation at the graft site. During the last week, the callusing chamber was kept at a temperature of 26 °C and a humidity level of 85–90%. At the end of 30 days, the grafted grapevine cuttings were acclimatised to the environment by keeping them in room conditions for 3–4 days. To determine the success of the treatments, *(i)* cutting and sawdust treatment evaluations were carried out during this period. Before planting, the raised bed nursery pilots were mulched to control weeds and water loss. A drip irrigation system was installed. The grafted cuttings were planted on a raised bed in early April in double rows with 8–10 cm spacing (designated as cuttings + sawdust) (Figure 1). *(ii)* For the cuttings, sawdust and soil treatment, one day after planting, each fungicide/bio-fungicide was prepared according to the application doses indicated in Table 1 in 10 L of water and applied to the soil in the root area. One week after the application, fungicide applications to the root areas were repeated (designated as cuttings + sawdust + soil). *(iii)* No fungicide was applied to the control group; only water was used (designated as control). The following describes how the applications were evaluated.

The following three treatments were carried out at the same time. The treatments were compared: *(i)* cuttings + sawdust treatments: after callus formation, fungal isolations were made from the shoots and roots of 10 randomly selected saplings of each fungicide treatment and fungal infection rates were determined; *(ii)* cuttings + sawdust + soil treatments: saplings harvested from the nursery in December were separated into diseased and healthy looking saplings, and the fungal incidence rate was determined by isolation from the root; and *(iii)* control: only water was applied. The commercial products and the doses used in the treatments are shown in Table 1.

To evaluate the effect of fungicide applications on saplings developing after callus formation, isolations were carried out from the shoot and root regions of randomly taken samples. Symptomatic and asymptomatic 0.5 cm^2 samples were cut from roots and shoots, and superficial disinfection was performed by immersion in 5% (*v/v*) sodium hypochlorite for 2 min. Disinfected plant tissues were sown on potato dextrose agar (PDA, Darmstadt, Germany) and incubated at 25 °C in the dark for 10 days. Mycelial structures were taken from fungal colonies growing around the tissues after inoculation, transferred to new Petri dishes containing PDA, and purified according to colony characteristics and morphological structures. The isolation incidence was calculated by counting the fungal colony growth in wood chips placed in Petri dishes, and the isolation frequencies of pathogenic fungi (%) were calculated based on the ratio of 100 sawdusts collected from 10 vines in each application.

The grafted cuttings were irrigated weekly with a drip irrigation system until September in both applications and maintained based on a regular maintenance schedule throughout the year. The saplings grew up for nine months.

Evaluation of the efficacy of the fungicides applied to root zones. After the saplings were up-

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rooted in December, two methods were used in the analysis. In the first method, samples were collected from the disease and healthy saplings into plastic bags in five replicates (four saplings in each replicate for each application). Fungi were isolated from the root regions of the samples. After the surfaces were sterilised in 5% *(v/v)* sodium hypochlorite in the laboratory, the samples were planted in Petri dishes that included PDA medium in four replicates, and each replicate included seven pieces. After the samples were incubated for an average of 7–10 days, fungal development was identified, and the isolation incidence of the fungi in naturally infected saplings was determined as a percentage. Isolation incidence was calculated by counting the fungal colony growth in wood chips placed in Petri dishes, and the isolation frequencies of pathogenic fungi (%) were calculated as a proportion of a total of 400 wood chip number (coated on PDA) taken in total 40 vine root from which 10 vine roots for each treatment.

In the second method, after the saplings were removed from the experimental area, they were counted and classified based on the TS 3981 standard (Kara et al. 2023). The number of saplings/planted cuttings was calculated as the total sapling yield (%), and the number of 1st size saplings/total saplings was accepted as the 1st size sapling yield (%). Furthermore, the mean shoot length (cm), root length (cm) and callus development level (0–4 scale) were determined for the saplings (Korkutal et al. 2011) [0 – no callus, 1 – unilateral callus formation, 2 – bilateral callus formation, 3 – three-sided callus formation, 4 – circumferential (four-sided) callus formation]. Considering the condition of the callus tissue surrounding the graft site, these figures are presented as ratios (Korkutal et al. 2011) and analysed statistically.

Pathogenicity test. To determine the virulence of the fungal species obtained as a result of isolations, 35 cm long cuttings of 1103 Paulsen grapevine rootstock with 4–5 buds were used. For disease inoculation, 14-day-old fungal cultures of these species were grown in a PDA medium at 20 ± 2 °C. Each isolate was inoculated on six test plants, and the experiment was conducted using a randomised experimental design with six replications. Each cutting was considered as one replicate. Firstly, the cuttings were surface disinfected with 95% ethanol. Inoculation was carried out following the method of Úrbez-Torres et al. (2013). A 4 mm wound was made under the bark with a cork-bore tool, and 4 mm mycelial agar disks were placed in this part, which were covered with Vaseline and parafilm. In control plants, only PDA agar disks were placed in the wounds. The inoculated cuttings were planted in pots containing sterile perlite and peat in a 1:1 ratio. The pots were incubated in climate chambers (25 °C temperature, 70% humidity, 16 h of daylight and 8 h of darkness) for two months, and growth was checked every morning.

At the end of this period, colour changes in the wood tissue and lesions in the vascular system were examined. After the incubation period, each test plant was cut longitudinally from the point of inoculation with a knife, and the lesion lengths were measured from the bottom to the top of the inoculation point. The most virulent isolates causing the longest lesion length (mm) were identified. Finally, six rooted rootstock cuttings were used for each species, and the top and bottom of the inoculation points on each test plant were measured. Re-isolation results were evaluated according to the fungal colonies growing on seven pieces 1 cm^2 pieces taken from six test plants for each isolate. Placed on PDA medium with streptomycin sulphate to re-isolate the inoculated pathogen and complete Koch's postulates. At the end of these procedures, fungal identity was confirmed by morphological and microscopic examination.

Identification of fungal pathogens. The fungal pathogens were isolated from 10 randomly selected sapling roots and shoots in the fungicide/bio-fungicide application crates transferred from the grafting chamber, and vine roots and shoots were uprooted after 9 months using the method proposed by Rego et al. (2006). The morphological character of post-isolation fungal pathogen infections was employed for the preliminary identification of fungi. The most isolated soil-borne pathogen species were analysed in the study. Morphological and molecular identifications of one or more of these isolates, which represented colony morphology and conidial structure determined under the microscope, were conducted at the species level. The morphological identification of the species was conducted based on the microscopic features and colony morphology specified in the literature (Koike et al. 2006; Mirzaei et al. 2009; Úrbez-Torres et al. 2013; Al-Fadhal et al. 2019).

Molecular diagnosis was conducted on pure and morphologically diagnosed isolates. DNAs were extracted based on the method Cenis (1992) suggested, using 50 mg of fresh mycelium collected from iso-

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lated colonies. The concentration and purity of the isolated DNAs were determined with the Multi-scan GO μ-drop plate (Thermo Scientific, USA). PCR was determined with internal transcribed spacers (ITS) rDNA region ITS1 (5' TCCTCCGCTTATTGATATGC 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primer pairs (White et al. 1990). A partial sequence of the β-tubulin nuclear gene was amplified using the Bt2a (5' GGTAACCAAATCGGTGCTGCTTTC3') and Bt2b (5' ACCCTCAGTGTAGTGACCCTTGGC 3') (Glass & Donaldson 1995) primer pairs respectively. In real-time (RT)-PCR reactions, 0.3 μL 20 μM forward primer, 0.3 μL 20 μM reverse primer, 2 μL DNA, and 10 μL 2 × FastStart Essential DNA Green Master Mix were added into sterile PCR tubes and the volume was completed to 20 μL with DNase/RNase distilled water. For ITS and β-tubulin were as follows; RT-PCR (Roche Light Cycler® Nano) amplification conditions were determined as follows: Initial denaturation at 95 °C for 10 min and denaturation for 30 s at 95 °C, annealing temperature at 56 °C (ITS1-ITS4) for 10 s, 54 °C for 10 s (Bt2a-Bt2b), 72 °C for 1 min and 35 cycles. Real-time PCR was conducted with universal ITS, Beta-tubulin gene primers and SYBR Green fluorescent dye. MBAE321, MBAE322, MBAE329, MBAE241, MBAE259, MBAE77 and MBAE249 isolated from the root zones peaked over the threshold (Ct) level the earliest for ITS primer, and the mean figures were 16.75, 17.14, 17.21, 18.20, 18.50, 19.67, and 22.20, respectively. This figure was 0.0 for the negative control reaction with sterile distilled water. The SYBR melting curve was conducted at the ramp rate of 0.2 °C/s at 56 (for ITS1-4) and 54 (for Bt2a-2b) –95 °C temperatures to determine the amplification products of Real-Time PCR. The melting curve reaction continued at elevated temperatures after the normal Real-Time PCR reaction was completed. MBAE322, MBAE329, MBAE241, MBAE259 and MBAE77 produced melting peaks at 80.44 °C, 83.34 °C, 84.22 °C, 86.65 °C and 89.34 °C, respectively for ITS primer. This confirmed the single fragment recorded in previous PCR tests.

PCR sequence data were obtained with bidirectional genome sequencing at a Sanger sequencing laboratory (TiroGen, Türkiye). The sequence data chromatogram files were analysed with the ChromasPro 1.7.6 chromatogram analysis software, and consensus sequences were obtained by combining the forward and reverse sequence data. Blast analyses of the fungi species and consensus sequences for each gene region were conducted using the National Center for Biotechnology Information (NCBI) Gene Bank database. The identified isolates were submitted to the NCBI, and accession numbers were assigned. The sequenced PCR products were aligned, and BLAST analyses were conducted using the BLASTn software. They confirmed ≥ 99% similarities of the species for Bt2a-Bt2b gene primers and 99–100% of the species for ITS1-4 primers. NCBI GenBank access numbers parts of both ITS and Beta-tubulin were obtained for isolates other than the *Trichoderma afroharzianum* and *T. gamsii* isolates based on gene sequencing and nucleotide BLAST analysis findings (Table 2). As a result of sequence data analysis of *T. afroharzianum* and *T. gamsii*, no consensus sequences were obtained for the beta-tubulin gene region. Therefore, NCBI GenBank accession numbers could not be obtained.

Based on the Blast search results, a sequence dataset was prepared for phylogenetic analysis using representative sequences from previous publications (Table 2). *Xenogliocladiopsis cypellocarpa* (CBS133814) and *T. strictipile* (CBS:338.93) were used as outgroups. The nucleotide alignment was fulfilled with the ClustalW algorithm (Thompson et al. 1994) included within the MEGA X software package (Kumar et al. 2018), and manual adjustments were made where necessary. The study constructed the phylogenetic tree using Maximum Likelihood (ML) in MEGAX. Bootstrap values for the Maximum Likelihood tree (ML) were calculated for 500 replicates. Phylogenetic trees were drawn with the models that best fit the data set obtained from ITS and β-tubulin combined gene sequences and ITS gene sequences for *Trichoderma* species. The edited ITS and β-tubulin sequences were compared with reference species sequences in GenBank for the species in Table 2. In addition, the sequences of these species were downloaded from GenBank and used to construct a tree. The ITS region and beta-tubulin gene pairs, including those for phytopathogen species, were drawing a combined phylogenetic tree. For phylogenetic analysis, 20 taxa were included in the combined dataset. For the phylogenetic analysis of the ITS gene region of *Trichoderma* species, 6 taxa, including references, were used, as shown in Table 2. The diagnosis of the species was confirmed by considering the positions of the isolates obtained as a result of the sequence with the reference isolates.

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Table 2. GenBank accession numbers of partial sequence of β-tubulin and ITS region of references species used for the phylogenetic analyses and obtained species in this study

Species	GenBank Accession No.		Host	Country
	ITS1-4	β-tubulin (tub)		
Boeremia exigua (MBAE77) in this study	OQ392363	OR908877	*Vitis vinifera*	Türkiye
B. exigua var. *exigua* CBS:534.75	MH860950	KY484746	–	Belgium
Fusarium solani (MBAE 236) in this study	OQ392364	OR908881	*V. vinifera*	Türkiye
F. solani (MBAE 237) in this study	OQ392365	OR908882	*V. vinifera*	Türkiye
F. solani (MBAE 259) in this study	OQ392370	OR908883	*V. vinifera*	Türkiye
F. cf. solani CBS 115660	MH855035	HE648029	–	France
Diaporthe ampelina (MBAE 239) in this study	OQ392366	OR908879	*V. vinifera*	Türkiye
D. ampelina CBS 111888	KC343016	KC343984	*V. vinifera*	USA
D. ampelina CBS 267.80	KC343018	KC343986	*V. vinifera*	Italy
Rhizoctonia solani (MBAE241) in this study	OQ392367	OR948564	*V. vinifera*	Türkiye
R. solani AG-2-1 strain S2222	MN160705	MZ945487	–	USA
Botryosphaeria dothidea (MBAE242)	OQ392368	OR908878	*V. vinifera*	Türkiye
B. dothidea YCNSZ-01	OR879311	OR885808	*Choerospondias axillaris*	China
B. dothidea CBS:110484	MT587330	MT592466	*V. vinifera*	Argentina
Ilyonectria liriodendri (MBAE 322) in this study	OQ392371	OR908880	*V. vinifera*	Türkiye
I. liriodendri CBS 112602	AY997532	AY677242	–	Netherlands
I. liriodendri CBS 110.81	NR_119565	DQ178170	*Liriodendron tulipifera*	USA
Alternaria alternata (MBAE329) in this study	OQ392372	OR948563	*V. vinifera*	Türkiye
A. alternata CBS 115152	KP124348	KT920430	*Psychotria serpens*	Italy
Trichoderma gamsii (MBAE374) in this study	OQ359568	–	*V. vinifera*	Türkiye
T. gamsii CBS 120072	KT028599	–	–	–
T. afroharzianum (MBAE377NS) in this study	ON819609	–	*V. vinifera*	Türkiye
T. afroharzianum CBS 124620	NR_137304	–	*Moniliophthora roreri*	Peru
T. afroharzianum CBS 466.94	KP009262	–	Vegetable	Netherlands
T. strictipile CBS:338.93	MH862408	–	–	Netherlands
Xenogliocladiopsis cypellocarpa CBS 133814	KM231760	KM232017	*Eucalyptus cypellocarpa*	Australia

Statistical analysis. All trials were set up based on random plot design, and the post-callus development fungal isolation rate was determined through SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) ($P < 0.01$) and two-way ANOVA. Sapling isolation finding analysis after nursery soil treatment, the pathogenicity test and analysis of the impact of the treatments on sapling yield and analysis of variance for quality (ANOVA) were conducted on SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) software and Duncan's multiple range test ($P < 0.05$).

RESULTS

Identification of fungi that cause rot during callus development. After fungicide/biofungicide treatments, the most frequently isolated fungal genus was *Fusarium* in shoots (D: control, 30.10%; B: cyprodinil + fludioxanil, 13.53%; C: *Trichoderma harzianu*m Rifai KRL-AG2, 12.21%; and A: floupyram + tebuconazole, 9.84%), followed by *A. alternata* complexed this genus in sapling shoots (Figure 2). In control, the most frequently isolated fungal genus in both shoots and roots ($P < 0.001$) was also *Fusarium* (30.10% and 27.19%, respectively) (Figures 2 and 3). The most frequently isolated fungi was *Fusarium* spp. in roots (21.55%). It was determined that the reason for the high isolation of *Fusarium* spp. in vine saplings after grafting and cutting and sawdust applications was the neutralisation of the protective effects of fungicides by the grafting chamber temperature and humidity (25 °C and 90% humidity) and almost provided control group condi-

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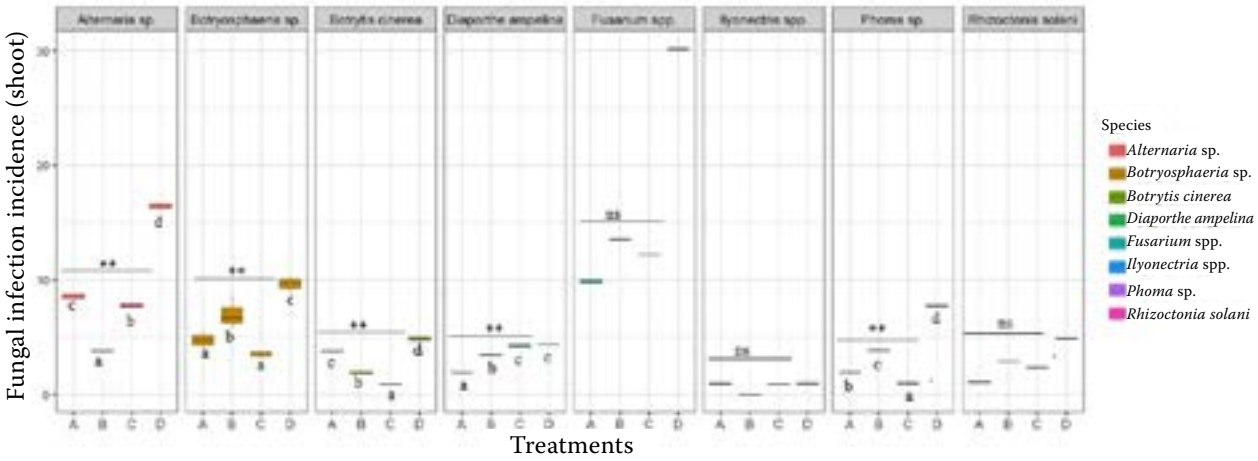

Figure 2. Fungal infection incidences in isolations from the shoots of the grafted vine cuttings, where fungicide was applied to the cuttings + sawdust, after the callus chamber

Shortcodes for fungicides/bio-fungicide applications representing different chemicals; A: Floupyram 20 g/L + Tebuconazole 200 g/L; B: Cyprodinil 37.5% + Fludioxanil 25%; C: *Trichoderma harzianum* Rifai KRL-AG2; D: Control; NS: not significant, a—d: results with different letters in the same column were found to be statistically significant. Bars with two asterisks indicate significant differences by control (ANOVA test, two one-way $P < 0.01$). Error bars symbolise ± mean standard error (SE) ($n = 3$)

tions. *Botryosphaeria* spp. was isolated from shoots and roots (9.67%, 9.70%), leading to dieback in the control group (Figures 2 and 3). However, *Botryosphaeria* spp. was isolated in the B treatment group (cyprodinil + fludioxanil) from the shoot (7.04%) and in the C treatment group (*Trichoderma harzianum*) from the root (4.87%) asymptomatic tissues (Figure 3). *Diaporthe ampelina* isolation rate was 3–5% from the shoots of the vine saplings after the grafting chamber at 25 °C and 90% relative humidity. It was the highest dieback pathogen, and its isolation rate was 11.67% from the roots in the control group. In A (fluopyram + tebuconazole), C (*T. harzianum*) and control applications, *Ilyonectria* spp. was isolated and led to black foot disease. In the B treatment (cyprodinil + fludioxanil) callus stage, black foot

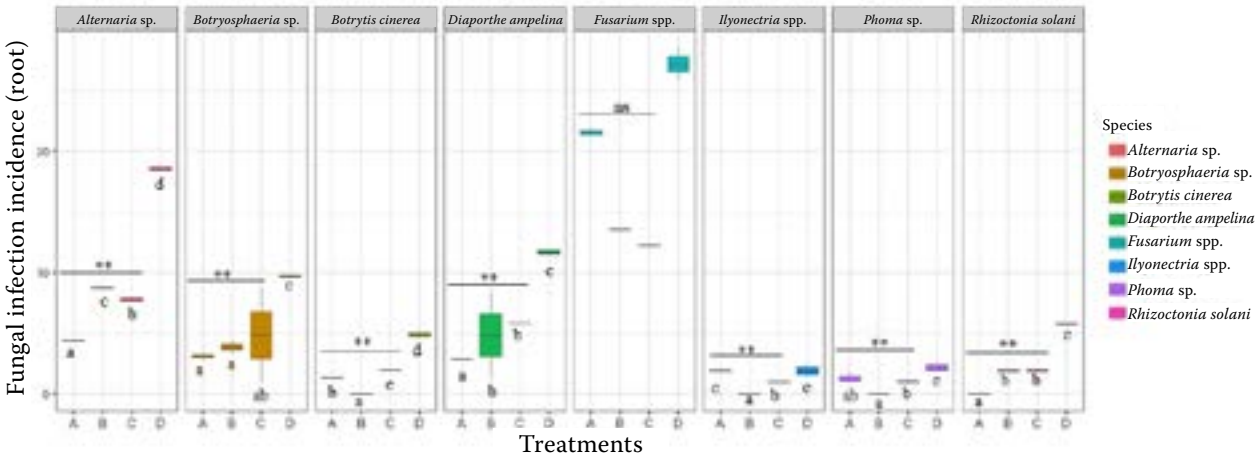

Figure 3. Fungal infection incidences in isolations from the roots of the grafted vine cuttings, where fungicide was applied to the cuttings + sawdust, after the callus chamber

Short codes for fungicides/bio-fungicide applications representing different chemicals; A — Floupyram 20 g/L + Tebuconazole 200 g/L; B — Cyprodinil 37.5% + Fludioxanil 25%; C — *Trichoderma harzianum* Rifai KRL-AG2; D — Control. NS — not significant, and a-d — results with different letters in the same column were found to be statistically significant. Bars with two asterisks indicate significant differences by control (ANOVA test, two one-way $P < 0.01$). Error bars symbolise ± mean standard error (SE) ($n = 3$). Error bars symbolise ± mean standard error (SE) ($n = 3$)

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development was controlled in shoots and roots. Eight fungal genera were isolated after callus development. Among these genera, *Botrytis*, *Botryosphaeria*, *Rhizoctonia*, *Ilyonectria,* and *Diaporthe* were known vineyard pathogens. *Phoma* spp. was detected in all applications, including control (with an isolation rate of 7.78%), especially in shoots.

Morphological and molecular identification of fungal pathogens. A total of 515 fungi isolates were obtained from the treated sapling shoot and root samples. Isolates were classified into five groups (A–E) according to colony colour: white, green, grey/black, brown and others (orange, yellow, purple and violet), respectively. Thirty species that were considered to be pathogenic were selected and subjected to molecular identification. Among the selected species, microconidia of *F. solani* complex species were reniform and ellipsoid with 1–2 septa of 3.5–12.5 × 2.5–4.4 µm and developed in false heads on short monophialides. Macroconidia were nearly flat, with 3–4 segments and measured 21.0–51.7 × 3.0–4.6 µm. All *R. solani* isolates were multinucleated (5–10 nuclei per cell). Colonies on PDA were light brown to brown and had a mycelial growth of 2.2 cm on day 1. Hyphae showed a right-angled branching pattern with a constriction at the base and adjacent septa. *Ilyonectria liriodendri,* the isolate, produced 1 to 3-septate, straight or slightly curved, cylindrical macroconidia (31–36 × 4.8–5.5 µm) and a few microconidia. Chlamydospores were brown, ovoid (12–16 × 11–18 µm), and mostly in short and intercalary chains. The colony growth of *Alternaria alternata* isolates on PDA is initially in the form of a white-greyish overhead mycelium with light to dark green interiors emerging

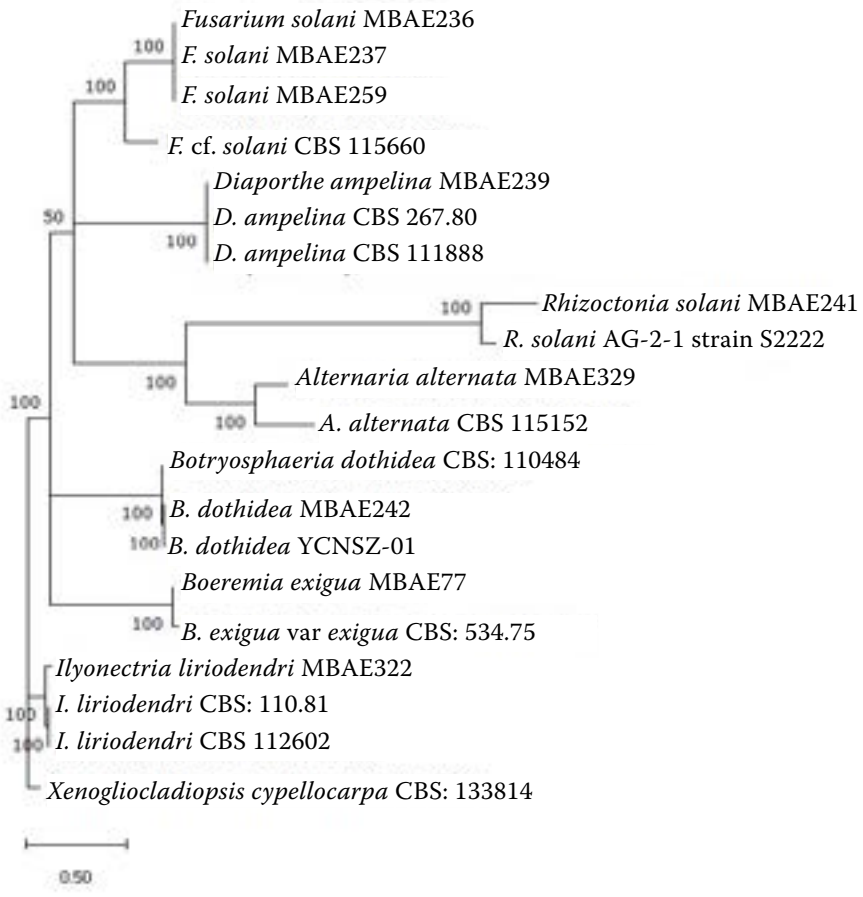

Figure 4. Maximum likelihood (ML) phylogenetic tree based on rDNA ITS and β-tubulin combined sequences of soil-borne fungal isolates and fungal ITS and β-tubulin sequences from the GenBank

ML tree was constructed using the substitution model Hasegawa-Kishino-Yano model (HKY) model and gamma distributed (+G) with invariant sites (+I) (= GTR + G + I). All positions containing gaps and missing data were included for analysis. Group supports were calculated based on 500 bootstrap re-samplings. Bootstrap values below 50% are not shown. The scale bar shows the number of nucleotide changes. The tree was rooted to *Xenogliocladiopsis cypellocarpa* (CBS 133814)

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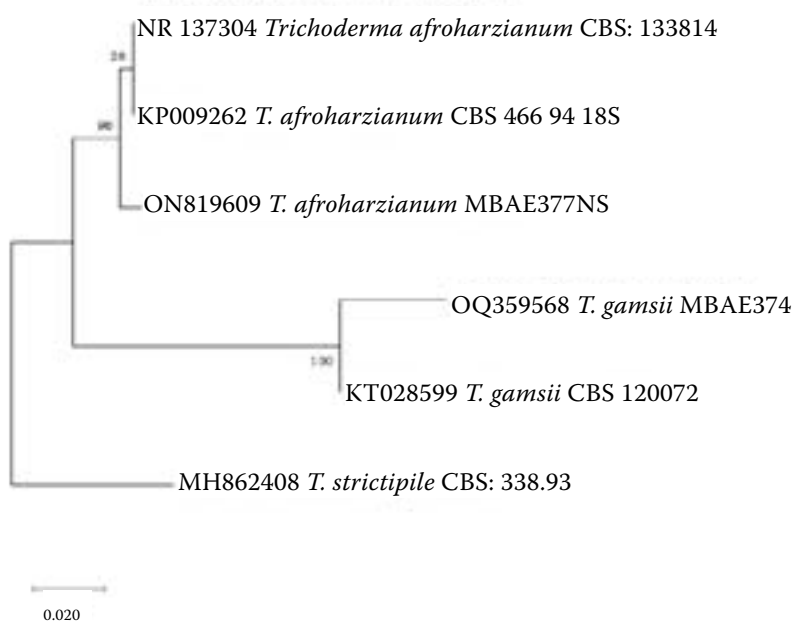

Figure 5. Maximum likelihood (ML) phylogenetic tree based on rDNA ITS sequences of *Trichoderma* isolates and fungal ITS sequences from the GenBank

ML tree was constructed using the substitution model Jukes-Cantor model (JC). All positions containing gaps and missing data were included for analysis. Group supports were calculated based on 500 bootstrap resamplings. The scale bar shows the number of nucleotide changes. The tree was rooted to *Trichoderma strictipile* (CBS 338.93)

from a common centre. Conidiophores are approximately straight-walled; conidia are sometimes one-third as long and 24.7 x 13.5 µm long and 4–6 µm thick, with a beak with 3–8 transverse and 1–3 longitudinal or oblique septa.

Based on colony morphology and microscopic examination, a total of 11 isolates were selected for molecular identification: three from the *F. solani* complex, two from *Trichoderma* spp. and one from each of the other species. The tree construct-

Figure 6. Pathogenicity of fungal species isolated from treatments (A) during pathogenicity measurements (B) control treatment on rootstock cuttings (C) *Ilyonectria liriodendri* infected vascular tissues on rootstock cuttings (D) root zone discolouration caused by *Fusarium solani*

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ed from the combined ITS regions and β-tubulin dataset supported the previously inferred group 2 (Figure 4). The species *I. liriodendri* inferred from the combined ITS and β-tubulin sequences was separated from the other species and identified as *I. liriodendri* with a bootstrap value of 100%. The different fungal genera isolated from the roots and shoots of the saplings represented six different species in two groups, each of which was related to the reference species with a bootstrap value of 100% (Figure 4). In the phylogenetic analysis, the β-tubulin gene region in *T. gamsii* and *T. afroharzianum* species could not be sequenced with the universal primers (βt2a-2b) used in PCR reactions, and the phylogenetic separation of this species could not be made together with other species. This is because the same gene region of all isolates must be amplified and aligned when creating a combined tree. The genetic links of *Trichoderm*a species were found to be very close to each other. The bootstrap values of *T. gamsii* species and *T. afroharzianum* species were found to have 100% and 98% bootstrap values, respectively, in the family tree made with the nucleotide sequences of the area amplified by ITS1-ITS4 primers (Figure 5). The reference isolates (*T. gmasii* CBS 120072 and *T. afroharzianum* CBS 466.94 – CBS 124620) showed a close similarity. The diagnosis of the species was confirmed by considering the positions of the isolates obtained in the study with the reference isolates.

Pathogenicity test. At the end of the pathogenicity trials, all isolates were pathogenic on rooted cuttings of 1103 Paulsen rootstock. After a two-month incubation period under greenhouse conditions, the leaves of the inoculated cuttings showed visible symptoms compared to the leaves of the controls. In particular, the leaves of the cuttings inoculated with *I. liriodendri* and *Botryosphaeria dothidae* isolates showed yellowing and brown necrosis at the tips. In addition, the pathogenicity test showed it was determined that all inoculated isolates caused discolourations (of mean lengths from 6.0 to 40.0 mm) of the vascular tissue above and below the inoculation points. However, severe root discolouration was observed in the root zone of inoculated cuttings compared to controls (Figure 6). For each species, ANOVA analysis showed differences between the virulence of the isolates according to the pathogenicity assay ($P > 0.05$).

On average, *F. solani* isolates MBAE259 produced the largest lesion (43.67 mm) among all fungi tested, followed by *B. dothidea* (41.33 mm), *I. liriodendri* (38.17 mm), *F. solani* MBAE237 (36.33 mm) and *R. solani* (35.00 mm) (Table 3). The value of lesion length caused by the inoculated *B. exigua var. exigua* isolate ranged from 12.00 mm and statistically showed the lowest virulence value in Table 3. Fungal recovery from inoculated plants ranged from 52.20% to 82.50%, with mean recovery rates above 60% for *Botryosphaeria,* including *B. dothidea*, *D. ampeline* and above 70% for *I. liriodendri*.

Fungicide efficacy and quality of saplings uprooted from nursery. Soil-borne fungi are the main cause of loss in vine nurseries during the sapling cultivation stage. It was determined that there was a statistical difference between the isolation incidence of fungi based on the treatment applied to disease and healthy saplings re-

Table 3. Pathogenicity of isolates on 1103 Paulsen rootstock cuttings

Species	Isolate	Mean lesion length ± SE (mm)*	Re-isolation rate (%)
Boeremia exigua	MBAE77	12.00 ± 2.52^{b}	54.20
Fusarium solani	MBAE236	27.00 ± 2.60^{bc}	62.80
F. solani	MBAE237	36.33 ± 7.53^{cd}	76.50
F. solani	MBAE259	43.67 ± 4.32^{d}	82.50
Diaporthe ampelina	MBAE239	28.33 ± 2.33^{bc}	75.60
Rhizoctonia solani	MBAE241	35.00 ± 3.01^{cd}	54.30
Botryosphaeria dothidea	MBAE242	41.33 ± 2.74^{d}	68.50
Ilyonectria liriodendri	MBAE322	38.17 ± 3.77^{cd}	74.20
Alternaria alternata	MBAE329	25.33 ± 3.09^{bc}	80.00
Control		6.00 ± 0.42^{a}	0.00

*Means in the column are significantly different at 0.05 level according to Duncan multiple comparison test; means with the same letter were included in the same statistical group; SE — standard error

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moved from the nursery soil (Table 4). While the fungal incidence rate of *Trichoderma* spp. was 56.25% in the healthy-looking C (*T. harzianum*) (cutting + sawdust + soil) treatment, the incidence rate of the same treatment was found to be 10.63% in the diseased looking saplings, which was lower and statistically different. *Botryosphaeria dothidea*, *I. liriodendri*, *Diaporthe ampelina*, *Alternaria alternata*, *Rhizoctonia solani*, *Boeremia exigua* and *Botrytis cinerea* are known as grapevine nursery pathogens. Furthermore, *Acremonium*, *Aspergillus*, *Aureobasidium* are known as epiphytic fungi. *Cladosporiu*m, *Clonostachys*, *Epicoccum*, *Penicillium* species are known as endophytic species. In addition to *Aspergillus Penicillium* is also known saprotrophic fungi. Moreover, *Clonostachys rosea* is a mycoparasitic fungi. *Trichoderma* species were identified as mycoparasitic and saprotrophic fungi. Since *T. harzianum* bio-preparation was included in the applications, this fungus was included in the analyses conducted after soil applications (Table 4).

As presented in Table 4, *Fusarium solani* was isolated at a higher rate from the roots of both disease and healthy-looking saplings in the control (D cuttings + sawdust 25.99%) and (D cuttings + sawdust + soil 36.86%) treatments than in the other fungicide/bio-fungicide treatments. *Botryosphaeria dothidae* pathogen incidence was 18.75% and

Table 4. Isolation incidences of the fungi isolated from the roots of looking disease and healthy saplings uprooted from the nursery (%)

Treatment	Fungal incidence rate (%)						
	Alternaria alternata complex	*Botryospheria dothidae*	*Fusarium solani* complex	*Diaporthe ampelina*	*Rhizoctonia solani*	*Ilyonectria liriodendri*	*Trichoderma* spp.
Looking disease							
A (cuttings + sawdust + soil)	3.125^{ab}	6.51^{bc}	9.08^{c}	1.92^{a}	15.625^{d}	1.46^{a}	9.56^{c}
A (cuttings + sawdust)	0.00^{a}	5.08^{ab}	15.31^{b}	6.25^{ab}	0.00^{a}	3.125^{a}	15.54^{b}
B (cuttings + sawdust + soil)	3.14^{b}	0.00^{a}	9.16^{c}	3.13^{b}	0.00^{a}	0.00^{a}	0.00^{a}
B (cuttings + sawdust)	12.53^{c}	0.00^{a}	4.79^{ab}	3.25^{a}	9.46^{bc}	0.00^{a}	6.25^{ab}
C (cuttings + sawdust + soil)	0.00^{a}	0.00^{a}	10.88^{b}	9.43^{b}	15.625^{c}	0.00^{a}	18.28^{c}
C (cuttings + sawdust)	6.25^{ab}	3.16^{ab}	14.88^{c}	9.38^{bc}	6.25^{ab}	0.00^{a}	10.63^{bc}
D (cuttings + sawdust + soil)	15.68^{a}	15.63^{a}	36.86^{b}	12.52^{a}	6.25^{a}	6.25^{a}	15.65^{a}
D (cuttings + sawdust)	6.25^{a}	12.25^{a}	25.99^{b}	12.50^{a}	6.50^{a}	3.13^{a}	6.25^{ab}
Looking healthy							
A (cuttings + sawdust + soil)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	3.125^{b}
A (cuttings + sawdust)	0.00^{a}	0.00^{a}	6.50^{b}	0.00^{a}	0.00^{a}	0.00^{a}	10.75^{b}
B (cuttings + sawdust + soil)	0.00^{a}	0.00^{a}	6.50^{b}	0.00^{a}	0.00^{a}	0.00^{a}	8.67^{b}
B (cuttings + sawdust)	0.00^{a}	0.00^{a}	3.75^{a}	0.00^{a}	0.00^{a}	0.00^{a}	18.13^{c}
C (cuttings + sawdust + soil)	0.00^{a}	0.00^{a}	6.25^{b}	3.25^{b}	0.00^{a}	0.00^{a}	56.25^{d}
C (cuttings + sawdust)	15.63^{c}	12.25^{c}	12.65^{c}	6.25^{b}	0.00^{a}	0.00^{a}	34.36^{c}
D (cuttings + sawdust + soil)	3.25^{a}	12.50^{b}	31.25^{c}	6.17^{ab}	0.00^{a}	0.00^{a}	31.25^{c}
D (cuttings + sawdust)	3.13^{ab}	8.49^{c}	18.68^{d}	6.25^{bc}	0.00^{a}	0.00^{a}	28.79^{d}

Fungicides/bio-fungicides representing different chemicals were applied at certain stages of sapling production and then planted in the nursery. The efficacy of the fungicides/bio-fungicides was evaluated on the saplings that completed their development.

Shortcodes for fungicides/bio-fungicide applications representing different chemicals: A — Floupyram 20 g/L + Tebuconazole 200 g/L, B — Cyprodinil 37.5% + Fludioxanil 25%, C — *Trichoderma harzianum* Rifai KRL-AG2; D — control

Cutting — application of fungicides/bio-fungicides at the stage of watering the cuttings; sawdust — moistening sterile sawdust with fungicides/bio-fungicide during the callus formation phase; soil — application of fungicides/biofungicide for 60 min before planting in the nursery and one day after planting

*Means with the same letter are not significantly different ($P \leq 0.05$) according to Duncan multiple compares test. They are the average of twenty cuttings per experiment

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21.84% in A (fluopyram + tebuconazole) (cutting + sawdust + soil); (cutting + sawdust) applications, and these figures were higher when compared to control (D) (15%, 63% and 12.25%). *I. liriodendri* isolations were low in B (cyprodinil + fludioxanil) (cutting + sawdust + soil), C (cutting + sawdust) and C (*T. harzianum*) (cutting + sawdust + soil) applications. It was observed that *Trichoderma* spp. isolation in all applications except B (cyprodinil + fludioxanil) (cutting + sawdust + soil) from disease saplings demonstrated that the fungicides did not affect *Trichoderma* spp.

As shown in Table 4, in the isolations carried out on healthy-looking saplings taken from the nursery in autumn, six different pathogenic fungal species and Trichoderma spp were found. grew. *F. solani* was isolated in all applications except for A (fluopyram + tebuconazole) (cutting + sawdust + soil). The least infected saplings were observed in the healthy saplings in the A (fluopyram + tebuconazole) (cutting + sawdust + soil) application group. It could be suggested that applying these two fungicides to the soil led to a significant decrease in soil-borne fungal pathogens. Pathogenic strains *F. solani* complex (12.67%), *A. alternata* complex (15.63%), *B. dothidae* (12.25%), *D. ampelina* (6.25%) were isolated in the C (*T. harzianum*) (cutting + sawdust) application group, demonstrating that these species were asymptomatic in the inner tissues of healthy vines. The high *Trichoderma* spp. Incidences in both bio-fungicide application groups [C (*T. harzianum*): 56.25%, 34.36%, respectively] and control groups (D; 31.25%, 28.79%, respectively) demonstrated that its antagonistic effect was promoted in the soil over time, and it was effective in controlling soil-borne pathogens. This was explained by the rapid colonisation of *Trichoderma* bio-fungicide in the soil, limiting pathogen development due to antagonistic properties. It was determined that there was a positive correlation between the isolation incidences of *F. solani* (36.86%), *B. dothidae* (15.63%), and *D. ampelina* (12.52%) pathogens in control (D; cutting + sawdust) application and the development of certain pathogens in the moisture generated by soaking the saplings with water during initial planting.

The impact of fungicide and biological preparation applications on sapling quality and yield was statistically significant at 1% and 5% confidence levels (Table 5). B (cutting + sawdust + soil) application was statistically significant in total sapling yield (78.75%) and first-size grafted sapling yield (61.25%), and it was the most effective treatment. It was determined that the fungicides that included fluopyram + tebuconazole and cyprodinil + fludioxonil improved sapling yield and quality. It was determined that the C (cutting + sawdust) application promoted callus development and shoot length by 3.90% and 28.70%, respectively. It was determined that bio-fungicide application during

Table 5. The treatment effect on total sapling yield, first-size grafted vine sapling yield, callus development and shoot length

Treatments	Total sapling yield (%)	I. length grafted vine sapling yield (%)	Callus development level (scale 0–4)	Shoot length (cm)
A (cuttings + sawdust + soil)	69.38^{ab**}	40.625^{ab*}	3.75^{ab**}	23.60^{ab*}
B (cuttings + sawdust + soil)	78.75^{a}	61.25^{a}	3.80^{b}	22.90^{ab}
C (cuttings + sawdust + soil)	64.38^{abc}	44.25^{ab}	3.60^{ab}	16.40^{bc}
D (cuttings + sawdust + soil)	57.50^{bc}	34.38^{b}	3.45^{ab}	16.00^{bc}
A (cuttings + sawdust)	61.25^{abc}	43.75^{ab}	3.65^{ab}	21.00^{ab}
B (cuttings + sawdust)	70.63^{ab}	50.00^{ab}	3.85^{b}	20.70^{ab}
C (cuttings + sawdust)	60.00^{abc}	43.13^{ab}	3.90^{a}	28.70^{a}
D (cuttings + sawdust)	45.00^{c}	25.00^{ab}	3.60^{ab}	11.20^{b}

Fungicides/bio-fungicides representing different chemicals were applied at certain stages of sapling production and then planted in the nursery. The efficacy of the fungicides/bio-fungicides was evaluated on the saplings that completed their development.

The data were analysed using the Duncan multiple comparison test; *: significant at 1% level; **: significant at 5% level. Lowercase letters on the same line indicate statistically significant differences between applications.

Shortcodes for fungicides/bio-fungicide applications representing different chemicals: A — Floupyram 20 g/L + Tebuconazole 200 g/L, B — Cyprodinil 37.5% + Fludioxanil 25%, C — *Trichoderma harzianum* Rifai KRL-AG2; D — control

https://doi.org/10.17221/94/2023-PPS

the sapling cultivation stage was effective on callus and shoot development.

DISCUSSION

So far, only pre- and post-planting fungicide treatments have been carried out in the soil to identify and reduce symptoms associated with stem, Black food, and Esca disease in grapevine saplings. A study demonstrated that *Botryosphaeria dothidae, Diaporthe ampelina,* and *I. liriodendr*i species significantly threatened the grapevine nursery industry (Gramaje et al. 2018). Furthermore, it demonstrated that *F. solani* complex and *A. alternata* complex species were the most frequently isolated fungi in grapevine nurseries and, thus, the main reason for the decline in young grapevine (Lade et al. 2022). Certain studies reported *A. alternata* as an endophyte and saprophyte species in grapevine saplings (Halleen et al. 2003); however, in this study, isolations conducted in all stages, pathogenicity tests and molecular analyses showed that it is pathogenic. During the isolation steps, it was observed that *F. solani* and *A. alternata* fungi were the most common species in shoots and roots during callus development, *Botrytis cinerea* pathogen was found in low amounts during callus development and was not detected after planting. Although *B. exigua* was detected at the shoots and roots during callus development, it was not found in uprooted saplings. In a previous study, Phoma negriana Thum was shown. pathogen led to black rot and necrosis in the infected vine stems and leaves (Davari & Hajieghrari 2008), demonstrating that the pathogen remained effective during callus development under high humidity. The fungi *Phoma koolunga*, *P. herbarum*, *Boeremia exigua* var. *exigua* (Cakir et al. 2021) and *P. glomerata* are necrotrophic, generalist and polyphagous species, and these traits facilitate the colonisation of new environments. These pathogens can use synergism to infect a wide range of plants and may explain why some pathogens are more aggressive than others in a given environment or plant host (Lamichhane & Venturi 2015). The fungus *Boeremia exigua*, isolated during the production process of grapevine cuttings and registered for the first time in the NCBI Genbank, is thought to synergise with the root rot pathogen *F. solani*, which may explain the increase in the frequency of *F. solani* isolated that during the callus development post. Because, in this study, *B. exigua* could not be isolated from the saplings planted in the nursery after cutting + sawdust + soil treatments, and the frequency of *F. solani* isolation decreased with the effect of the treatments. A comparable study was conducted by Yildiz & Gursoy (1994) on a grapevine nursery in the Aegean Region in Türkiye, and *B. cinerea*, *Pythium* sp. and *Fusarium* sp. were reported as the most frequently isolated species. Numerous species in *Fusarium* genus have been isolated from the sapling phyllosphere and *F. oxysporum* was reported to be responsible for the vineyard sapling decline (Omer 1999; Król 2006; Cruz et al. 2014). In a study where the fungal species associated with healthy vine cuttings were identified in South African nurseries, *Alternaria*, *Epicoccum*, *Cladosporium* were the most frequently isolated fungal species during the callus development stage and before planting. Although *Fusarium* spp. was isolated during callus development, it was the most frequently isolated species three months after planting the grapevine saplings (Hallen et al. 2003; Van Coller et al. 2004). In this study, *F. solani* complex was the most isolated species after fungicide and bio-fungicide applications and during folding. *F. solani* complex was responsible for losses in sapling cultivation and isolated from saplings with symptoms in transmission bundles such as the young grapevine decline disease. The high incidence of *F. solani* complex in healthy saplings during sapling cultivation (Table 3) demonstrated its asymptomatic character. In the isolations conducted after folding, *D. ampelina*, the root and leaf spot disease pathogen, was isolated from shoots and roots. It was proved that the disease was transmitted to the vineyards due to the employment of *Phomopsis viticola* contaminated cuttings and grafts in nurseries, increasing the incidence and early yield losses in vines and evidencing the latency of the pathogen in vine shoots (Król 2006). Similar findings that revealed the damages induced by *P. viticola* in grapevine cuttings in various parts of the world were reported previously (Scheper et al. 1997; Pearson & Goheen 1988; Mostert et al. 2000). One of the fungal trunk disease agents, *Botryosphaeria* spp., and the black foot disease agent *Ilyonectria* spp. were isolated from shoots and roots in certain applications, albeit with low incidence. It was claimed that *Cylindrocarpon* spp., the black foot disease pathogen, was rarely observed in propagation materials during this period in isolations from callused cuttings before planting (Halleen et al. 2003). In the study, *I. liriodendri* isolation incidence was 1.93% in roots before planting, while the isolation incidences of *I. liriodendri* were 6.25%

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and 3.13% in the roots of the diseased vines in the control group uprooted from the nursery. According to Halleen et al. (2003), *Cylindrocarpon* spp. incidence was less than 1% before planting and more than 50% in the planted saplings at the end of the season (June). The spread of the black foot disease in nurseries indicates that a soil-born pathogen causes the disease and could spread rapidly based on the soil and via water (Petit et al. 2011; Berlanas et al. 2020). Certain active substances, such as tebuconazole, exhibited different behaviors in various grapevine stem diseases in the triazoles group. It was reported that these differences were due to the differences between the tested vine tissues, pathogens, strains and vine varieties. Tebuconazole was reported to be effective against the cancer induced by *Botryosphaeria* sp. (Pitt et al. 2012) and was effective against *Diplodia seriata*, *Eutypa lata*, *Inocutis* sp. and *Phaeomoniella chlamydospora* when rubbed on fresh pruning wounds (Di Marco et al. 2000; Diaz & Lattore 2013). It was reported that fluopyram, an SDHI (Succinate Dehydrogenase Inhibitor) fungicide, was effective against soil-borne *Sclerotinia* spp., *Rhizoctonia* spp., *Fusarium* spp., and *Phoma* spp. and *Alternaria solani* fungi in certain plant tissues (Pitt et al. 2012). It was also reported that, when applied on pruning wounds, fluopyram was successful against wood tissue cancers caused by various pathogens such as *D. seriata*, *D. mutila*, *Botryosphaeria* spp., *Lasiodiplodia theobromae* (Delen 2008). In the present study, the application of fungicide that included a mixture of fluazinam + tebuconazole solution to soil decreased the incidence of soil pathogens such as *R. solani*, *F. solani* complex, *A. alternata* complex and *I. liriod*endri in both disease and healthy saplings when compared to controls. Also, it was determined that both callus and soil period applications of this fungicide were successful against *B. obtusa* and *D. ampelina*.

Cyprodinil + fludioxonil solution significantly reduced the incidence and latency of *B. obtusa*, *D. ampelina* and *I. liriodendri* in healthy plants after soil application under high and very high inoculum pressures. The incidences of *A. alternata* complex and *R. solani* fungi were also significantly reduced. This fungicide solution did not eradicate these pathogens in cuttings but significantly reduced their incidence and severity compared to the water-treated control. The spread of endophyte and root rot pathogens in transported grafted vine saplings was reduced, preventing further contamination during grafting, callusing and storage and positively affecting the health and quality of the vines (Rego et al. 2009). It was determined that cyprodinil + fludioxonil fungicide was effective against *Ilyonectria* spp. *in vitro*. It was confirmed that cyprodinil + fludioxonil, employed in controlled trials and pots against *I. liriodendri* during the rooting of 1103 Paulsen rootstock cuttings, reduced the incidence by 63.4% in 2020 and 69.6% in 2021 in the roots (Yildiz & Tosun 2022).

In the present study, *Trichoderma* was the second most frequently isolated genus in both isolation points (shoots and roots) after soil applications. It was observed that *T. afroharzianum* and *T. gamsii* species were beneficial endophytes in wood and stimulated sapling growth after the applications. Selected *T. harzianum* strains were applied during layering, soil improvement and irrigation in the nursery on Petri and blackfoot disease pathogens, and the findings were compared with (standard) grapevines treated with quintozene/procymidone fungicides. The bioactivity of *T. harzianum* Rifai KRL-AG2 strain was tested in pot trials to prevent *I. liriodendri* infections during the rooting of 1103 Paulsen vine rootstock in Aegean Region nurseries in Türkiye. In pot trials, *T. harzianum* Rifai KRL-AG2 strain significantly increased the vine root biomass when compared to the control and reduced the root disease severity by 60.8% by reducing necrosis in the roots (Yildiz & Tosun 2022). In our study, the *T. harzianum* Rifai KRL-AG2 biopreparation applied to cuttings + sawdust and nursery soil reduced fungal disease incidence and increased the total sapling yield as much as the fludioxonil + cyprodinil application.

It was observed that shoot growth was noticeably better in vines treated with *Trichoderma* than in the control group. Significantly fewer fungi were isolated from the roots of the *Trichoderma*-treated vines. The Petri disease incidence was similar in the roots treated with *Trichoderma* and the standard application, but *Cylindrocarpon* spp. was isolated less in *Trichoderma*-treated vines (Fourie et al. 2001). These findings demonstrated that *Trichoderma* treatment would contribute to strong vine growth with lower *Botryopshaeria* and *Ilyonectria* infection levels. *Trichoderma* treatment could produce varying findings based on the sapling cultivation stage. *Trichoderma* applications in the rooting stage were the most effective, while the applications in callus development or the rooting and callus stages were inconsistent but generally produced negative results. *Trichoderma* treatment also reduced necrotic areas induced

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by *Botrytis cinerea* in leaves and the degree of cutting necrosis in plants inoculated with *Phaeomoniella chlamydospora.* These reductions in necrosis were significantly higher 15 months after inoculation (Di Marco & Osti 2007).

Planting could stress vines in the nurseries, and grafting could lead to detrimental changes in plant physiology (Bavaresco & Lovisolo 2000). Among the bench grafting techniques, omega grafting is the most common grafting method in grapevine nurseries due to its high success rate. Little research has been conducted on grafting and grafting quality to date. Further studies are required to confirm the significance of GTDs in developing graft quality (Gramaje & Di Marco 2015). Soil fungicide applications and rooting and callus stages further improved the quantitative and qualitative properties of the root system and increased the rate of certified vine production. It was confirmed that *Trichoderma* application only during rooting had positive effects on the morphophysiological properties of the vine. It increased the tolerance to stress-related diseases such as Esca forms in nurseries (Di Marco & Osti 2007).

CONCLUSION

This study was conducted to determine the effects of fungicide and antagonist applications on controlling soil-borne fungal pathogens during sapling production. The effects of these applications on sapling yield, quality and root development were determined. In the study, *F. solani* and *A. alternata* species complex were the most frequently isolated taxa in the nursery at every stage. *B. exigua* was isolated for the first time from cuttings during the production of grapevine saplings and was registered in the NCBI Genbank. It was found that there was a synergism between *B. exigua MBAE77* and *F. solani* complex of MBAE236, MBAE 237 and MBAE259 strains and that it caused an increase in the frequency of isolation of *F. solani* strains after callus formation. Bio-preparation of *T. harzianum* Rifai KRL-AG2 race and cyprodinil + fludioxanil active substances applied to cuttings + sawdusts and nursery soil both reduced the incidence of fungal trunk tissue and root rot diseases such as *B. obtusa*, *D. ampelina* and *I. liriodendri* and increased the total yield of saplings. This study recommends controlling root rot and wood diseases for nursery producers in Türkiye who do not use fungicides or bio-preparation in the production of vine saplings.

Acknowledgement: Many thanks to the Manisa Viticulture Research Institute for their support.

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Received: September 1, 2023
Accepted: April 4, 2024
Published online: July 1, 2024