Berbegal Martinez, M.; Ramón-Albalat, A.; León Santana, M.; Armengol Fortí, J. (2020). Evaluation of long-term protection from nursery to vineyard provided by Trichoderma atroviride SC1 against fungal grapevine trunk pathogens. Pest Management Science. 76(3):967-977. https://doi.org/10.1002/ps.5605

Evaluation of long-term protection from nursery to vineyard provided by *Trichoderma atroviride* SC1 against fungal grapevine trunk pathogens

Mónica Berbegal, Antonio Ramón-Albalat, Maela León and Josep Armengol*

**Correspondence to: J Armengol, Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022 - Valencia, Spain. E-mail: jarmengo@eaf.upv.es*

Abstract

BACKGROUND: Fungal grapevine trunk diseases (GTD) represent a threat for viticulture, being responsible of important economic losses worldwide. Nursery and vineyard experiments were set up to evaluate the ability of *Trichoderma atroviride* SC1 to reduce infections of GTD pathogens in grapevine planting material during the propagation process and to assess the long-term protection provided by this biocontrol agent on grapevine plants in young vineyards during two growing seasons.

RESULTS: Reductions of some GTD pathogens incidence and severity were found on grapevine propagation material after nursery application of *T. atroviride* SC1 during the grafting process, and also after additional *T. atroviride* SC1 treatments performed during two growing seasons in young vineyards, when compared with untreated plants.

CONCLUSION: *Trichoderma atroviride* SC1 showed promise to reduce infections caused by some GTD pathogens in nurseries, and also when establishing new vineyards. This biological control agent could possibly be a valuable component in an integrated management approach where various strategies are combined to reduce GTD infections.

Keywords: Biocontrol, Black-foot, Botryosphaeria dieback, Petri disease, *Vitis vinifera* L.

1 INTRODUCTION

Despite the efforts done since the early ’90s to investigate the etiology and epidemiology of fungal grapevine trunk diseases (GTD), they still represent a threat for viticulture, being responsible of important economic losses worldwide.[1-3] Petri and Black-foot diseases are considered as the main GTD affecting young vineyards, and Eutypa, Botryosphaeria and Phomopsis diebacks, and Esca and Grapevine Leaf Stripe diseases, as the prevalent GTD in adult vineyards.[1,3-5]

GTD are complex diseases due to several factors: i) they are caused by up to 133 wood-infecting fungal species belonging to 34 genera; ii) several GTD can infect grapevines at the same time; iii) they have similar internal or external symptomatology, or different symptomatologies overlap when multiple infections occur. Moreover, GTD on young and adult plants are intimately connected, because they share common pathogens and because grapevines infected by GTD when they are young can show symptoms when they are adult, after a more or less extended latency period.[1,3-5]

Altogether, these factors can explain why the management of GTD is so difficult, but other additional aspects to consider are: the cultural practices that favor fungal infection, such as high density spur-pruned trellised vineyards, often mechanically pruned, which present a high number of pruning wounds and multiple additional infection opportunities; and the banning in the early 2000s of effective fungicides against GTD, such as sodium arsenite or benzimidazoles.[1,3]

Grapevine producers are aware of the high likelihood of infection and substantial yield losses from GTD but, in general, they do not adopt management practices until disease symptoms appear in the vineyard, frequently when the vines are at around 10 years old.[6] Viticulturists are currently assuming that they must face GTD throughout the entire life of the vine, integrating different control measures that can contribute to mitigate the economic impact of the diseases, but taking into account that complete eradication of these diseases is not possible.[1,3] In this sense, the most important objectives to be covered by a GTD integrated management program are the improvement of the phytosanitary

quality of the vines produced in the nursery,[4] and the prevention of infections of pruning wounds in the vineyard from the moment of planting. [1,7]

In nurseries, grapevine planting material is very prone to infection by GTD pathogens due to the large number of cuts and wounds made during the different steps of the propagation process.[1,4] Gramaje and Di Marco (2015)[8] conducted a wide questionnaire survey in grapevine nurseries of 13 European countries, Israel and Algeria, and identified a clear need for further research into the effects of treatments including hot water treatment, biological control agents (BCA) and other strategies such as ozonation, to reduce infection by GTD pathogens during grapevine propagation. The incorporation of all available control measures in an integrated management program has been indicated as the best approach to improve the phytosanitary quality of grapevine planting material.[1,4]

In established vineyards, pruning wounds have been identified as the most relevant infection pathway for aerial inoculum of many GTD pathogens.[9,10] The importance of this pathway is enhanced by the long susceptibility period of grapevine pruning wounds, which can last up to 4–6 months, even with decreasing susceptibility.[10-14] Different fungicides have been reported to be effective for pruning wound protection, but they have a short-term efficacy and, in Europe, the current range of authorized active ingredients for this purpose is very limited.[1-3] New chemical and physical protection formulations are under development, aiming to a long-lasting protection against GTD infection,[14,15] but BCAs preventively colonizing the fresh wounds may also serve for this purpose.[3]

Trichoderma species have been investigated extensively as biocontrol agents of plant pathogens, including GTD. They currently represent one of the most important fungal based BCAs used in agriculture.[16-20] Depending on the strain, *Trichoderma* species present several benefits for crop plants: i) they can stimulate plant growth by enhancing nutrient mobilization and uptake; ii) they can suppress pathogens by competition for nutrients and space, mycoparasitism and antibiosis; and iii) they can suppress plant diseases by inducing systemic resistance.[20-23]

In grapevine, the species *T. atroviride* strains USPP-T1 and USPP-T2,[24,25] I-1237,[26,27] and SC1,[28] have shown a good performance for the protection of grapevine against GTD. Soil applications of *T. atroviride* T11 + *T. asperellum* T25, combined with

white mustard cover crop residue amendment and chemical fumigation with propamocarb + fosetyl-Al, were evaluated to reduce Black-foot pathogens infection in grapevine grafted plants, but no added benefit was obtained when biofumigation was used with *Trichoderma* spp..[29] Other *Trichoderma* spp. for which research against GTD have been reported are different *T. harzianum* strains,[30-34] and *T. asperellum* + *T. gamsii*.[35]

Most of the research works dealing with the control of GTD in grapevine nurseries with BCAs, have evaluated their efficacy in a short-term period, showing a disconnection between the development of management strategies in nurseries and the subsequent protection of the plants once they are planted in vineyards.[28,31,32,34] For this reason, the objectives of this work were: i) to evaluate the ability of the BCA *T. atroviride* SC1 to reduce infections of GTD pathogens in grapevine planting material during the propagation process in a commercial nursery, and ii) to assess the long-term protection provided by this BCA during two growing seasons in two commercial vineyards stablished using the planting material previously treated with *T. atroviride* SC1 in the nursery.

2 MATERIALS AND METHODS

2.1 Grapevine scion and rootstock materials used

The experiment was carried out using two stocks of each Tintorera cv. scions and 110 Richter rootstocks in a nursery located in Aielo de Malferit, Valencia province (eastern Spain) in 2015.

Prior to the grafting process and the *T. atroviride* SC1 applications, 25 scion fragments with buds and 25 rootstock cuttings were randomly selected from the stocks and analyzed for the presence of GTD pathogens. For this purpose, in each material type, isolations were performed from sections of 1 cm long. These sections were washed under running tap water, surface-disinfested for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Then, 10 internal wood fragments per section were placed on malt extract agar (MEA) supplemented with 0.5 $g.L^{-1}$ of streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA) (MEAS) (five fragments per two Petri dishes). Plates were incubated for 10-15 days at 25ºC in the dark, and all emerging colonies were transferred to PDA. Preliminary morphological identification of

the colonies was conducted by observation of cultural and microscope characters for Botryosphaeriaceae, *Cadophora luteo-olivacea*, *Cylindrocarpon*-like asexual morphs, the genus *Phaeoacremonium* and *Phaeomoniella chlamydospora*.[36-41]

For species identity confirmation, fungal mycelium and conidia from pure cultures grown on PDA for 2 to 3 weeks at 25ºC in the dark were scraped and mechanically disrupted using FastPrep-24™5G (MP Biomedicals, Santa Ana, CA, USA). Total DNA was extracted using the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek, Doraville, USA) following manufacturer's instructions. The quality and integrity of the DNA was visualized on 1% agarose gels stained with REALSAFE (Durviz S.L., Valencia, Spain). All DNA samples were stored at −20°C.

The identification of all isolates was performed by analysis of the ITS region amplified using the fungal universal primers ITS1F and ITS4.[42,43] Then, further molecular identification was conducted for specific groups of pathogens. *Cadophora* and *Phaeoacremonium* species were identified by sequence analysis of the β-tubulin gene. For *Cadophora* the primers used were BTCadF and BTCadR,[44] and for *Phaeoacremonium* they were T1 and Bt2b.[45,46] Identification of Botryosphaeriaceae species was confirmed by analysis of elongation factor 1-α gen amplified using EF1F and EF2R primers.[47] Identification of *Cylindrocarpon*-like asexual morphs was confirmed by sequencing part of the histone H3 gene with primers CYLH3F and CYLH3R.[48]

2.2 Nursery experiment

A water suspension of formulated *T. atroviride* SC1 (Vintec ®, Belchim Crop Protection; 2 x 10^{10} conidia g^{-1} formulated product) at 2 g l^{-1} was used. The viability of the conidia in the commercial product was checked to be at a minimum of 85 % before each trial as described by Pertot *et al*. (2016).[28] A serial dilution of the conidia suspension was plated on potato dextrose agar (PDA) (Biokar-Diagnostics) and the colony forming units were counted after 24–48 h incubation at room temperature.

Grapevine propagating material (cuttings of 110 R rootstock subsequently grafted with Tintorera cultivar) were treated with *T. atroviride* SC1 at three stages during the grapevine propagating process: i) a 1-day soak in *T. atroviride* SC1 suspension prior to grafting; ii) the application of *T. atroviride* SC1 suspension in sawdust at stratification;

and iii) a 1-h soak of the basal parts of the plants in *T. atroviride* SC1 suspension before planting in the rooting field. The untreated control involved treatments with water at each of the three stages. For *T. atroviride* SC1 treatments and the untreated controls, there were three replicates of 120 plants, which were managed separately.

Grafted plants were planted in a nursery rooting field in May 2015, and were arranged in a randomized complete block design with three replicates (120 plants) per treatment. Cultural practices were performed according to the common Integrated Pest Management (IPM) guidelines and only copper compounds and wettable sulphur were applied at label dosages to control downy and powdery mildew, respectively, if required. Plants were uprooted in October 2015 and wrapped in individual perforated plastic bags to avoid cross contamination, but also to prevent oxygen deprivation and fermentation, without exposing the cuttings to dehydration.[49] Twenty five plants per treatment and replicate were selected randomly and taken to the laboratory for fungal isolation analyses. The remaining plants were cold stored at 2-3 ºC and 90% humidity until the next growing season.

Isolations were made from 3 cm long sections cut from three different areas: the grafting point, the basal end of the rootstock cuttings, and the root system. These materials were washed, surface-sterilized and isolations were performed as described before. Ten wood fragments per each type of area (five fragments per two Petri dishes) were analyzed (30 wood fragments per plant). Molecular identification of GTD pathogens was also performed as described before. In addition, the identity of the *T. atroviride* SC1 colonies recovered was confirmed on a random sample of 10 % of the colonies isolated using the real-time PCR method described by Savazzini *et al*. (2008).[50]

2.3 Vineyard experiments: first growing season.

In May 2016 the remaining *T. atroviride* SC1 treated and the untreated grafted plants from the nursery experiment were planted in two field sites located in Campo Arcís (Field 1) and Requena (Field 2), both in Valencia province, and separated 10 km. Before plantation, basal parts of treated plants were soaked during 1-h in *T. atroviride* SC1 suspension, while untreated plants were soaked in water.

In each field, grafted plants were spaced 50 cm from center to center, and with an interrow spacing of 150 cm. Each field plot included three rows, each with two groups of 30 grafted plants. In both sites, the experimental design consisted of three randomized blocks (corresponding to the rows), each containing a group (replicate) of treated or untreated plants spaced 200 cm. In total, there were 180 grafted plants per field. Standard cultural practices were employed in both sites during the grapevine growing season, and the control of downy and powdery mildews were performed using only copper compounds and wettable sulphur applied at label dosages and following IPM guidelines, respectively, if required. In Field 2, the plants were protected from rabbits with plastic shelters, but not in Field 1.

In February 2017, at the pruning moment, shoots of each plant without the leaves were collected and immediately assessed for undried shoot weight. In addition, in each vineyard ten plants per treatment and repetition were uprooted and taken to the laboratory for fungal isolation, which was performed on the grafting point, the basal end of the rootstock cuttings, and the root system, as described before. Molecular identification of GTD pathogens and *Trichoderma* SC1 was also performed as described before.

2.4 Vineyard experiments: second growing season

In February 2017, twenty four hours after pruning, *T. atroviride* SC1 suspension was applied to pruning wounds of the remaining treated plants in each field (20 plants per replicate), and untreated plants were sprayed with water. During the 2017 growing season, cultural practices, and downy and powdery mildews management, were performed as described before. In February 2018, at the pruning moment, dormant plants were pruned, immediately assessed for undried shoot weight, and ten plants per repetition analyzed for fungal isolation as described for the first growing season.

2.5 Data analyses

The incidence and severity of each of the GTD pathogens detected were determined as the mean percentage of infected plants and mean percentage of positive fungal isolation from wood fragments, respectively. For each experiment, incidence and severity were also calculated for each type of GTD detected: i) Botryosphaeria dieback (BOT) including

fungi belonging to the family Botryosphaeriaceae; ii) Petri disease (PETRI) including *C. luteo-olivacea*, *Phaeoacremonium* spp. and *Pa. chlamydospora*, and iii) Black-foot disease (BF) including *Cylindrocarpon*-like asexual morphs. The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *T. atroviride* SC1 treatment. In the vineyard experiments, mean values of undried shoot weight (in g/plant) were calculated for the *T. atroviride* SC1 treatment and the untreated control at each experimental vineyard after the first and the second growing seasons.

Significance levels for mean percentages of incidence and severity corresponding to each type of GTD (BOT, PETRI and BF) and mean values of undried shoot weight were determined by the Kruskal-Wallis one-way analysis of variance on ranks and mean separation was conducted for Fisher´s least significant differences (LSD) at $P<0.05$. The analysis were performed using R package agricolae.[51, 52]

3 RESULTS

The analysis of 25 scions with buds of cv. Tintorera and 25 cuttings of 110 Richter rootstocks prior to nursery experiment showed that their initial infection level was low. Specifically, only six infected rootstock cuttings were detected: two with *Diplodia seriata,* two with *Cadophora luteo-olivacea*, and two with *Neofusiccoum parvum*. Regarding the scions, only one of them was found infected by *C. luteo-olivacea*.

Conidia viability of the *T. atroviride* SC1 suspensions prior to the applications was confirmed in both nursery and vineyard trials and was at least 85%. Moreover, in both nursery and vineyard experiments percentage recovery of this strain was high: over 80% of the treated plants (incidence) or wood fragments (severity) were colonized by *T. atroviride* SC1, and it was not recovered from untreated plants.

In general, at the end of all trials, the GTD pathogens associated with BF were preferably found with the highest incidences and severities on the roots, those associated with BOT were preferably found on the grafting point and, finally, those associated with PETRI were found in all the three isolation areas considered in this study. Thus, the statistical comparison of *T. atroviride* SC1 treated and untreated plants was performed

taking into account these three diseases, and grouping the results of the different associated pathogens associated with each disease and their preferred areas for infection.

3.1 Nursery experiment

At the end of the grafting process, the GTD pathogens isolated were *Dactylonectria torresensis*, *D. seriata*, *C. luteo-olivacea*, *N. parvum*, *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. Their incidence and severity in the three isolation areas of the grafted plants was highly variable (Table 1).

On *T. atroviride* SC1 treated plants, there were highly variable reductions in GTD pathogens incidence when compared with untreated controls, which ranged from 100% for *N. parvum* on the grafting point to 16.6 % for *Pm. minimum* on the rootstock basal end. There was no reduction in *C. luteo-olivacea* incidence, but its percentage on untreated plants was very low (1.3%). Regarding the severity of the GTD pathogens, the reductions ranged from 100% for *N. parvum* on the grafting point to 33.3% for *Pa. chlamydospora* on the roots, with no reduction for *C. luteo-olivacea* on the rootstock basal end.

Data from the different pathogens were grouped according to the three main GTD considered. Percentages of incidence for BOT, PETRI and BF diseases in untreated plants were 33.3, 30.2 and 1.3, respectively; while in *T. atroviride* SC1treated plants they were 1.3, 12.4 and 0, respectively. Regarding, severity, percentages for BOT, PETRI and BF were 8.6, 6.3 and 1.1, respectively; while in treated plants they were 0.4, 2.1 and 0, respectively. The analyses of variance revealed significant reductions in both incidence and severity for BOT (P=0.04 and P=0.04, respectively) and only in severity for PETRI (P=0.109 and P=0.03, respectively) in the *T. atroviride* SC1 treated plants (Figure 1). For BF, there was not a statistically significant reduction in both incidence and severity (P=0.32 and P=0.32, respectively). In this case, the pathogens associated with this disease were detected at very low levels on untreated plants and were not detected on treated plants.

3.2 Vineyard experiments

Results of undried shoot weight (g/plant) at the end of the two growing seasons for Fields 1 and 2 are shown in Figure 2. At the end of the first growing season, undried shoot weight in Field 1 were 42.2 and 55.0 g/plant for untreated and treated plants, respectively; and in Field 2, they were 17.9 and 22.7 g/plant, respectively. At the end of the second growing season, undried shoot weight in Field 1 were 273.3 and 245.7 g/plant for untreated and treated plants, respectively; and in Field 2, they were 127.2 and 125.3 g/plant, respectively.

A significant increase of the undried shoot weight was observed in the *T. atroviride* SC1 treated plants in both fields at the end of the first growing season (2016-17) (P=0.00009 and P=0.0024, for Fields 1 and 2 respectively), but not at the end of the second (2017-18) (P=0.12 and P=0.85, for Field 1 and Field 2 respectively) (Figure 2).

In Field 1 at the end of the first growing season (2016-17), the GTD pathogens isolated were *D. torresensis*, *D. seriata*, *Ilyonectria liriodendri*, *N. parvum*, *Pm. minimum* and *Pa. chlamydospora*, all with variable incidence and severity percentages in the three isolation areas of the grafted plants (Table 2). At the end of the second growing season (2017-18), the GTD pathogens that were isolated were the same indicated before, but also *Pm. iranianum* was detected on the grafting point (Table 3).

In this field in both growing seasons, there were variable reductions in GTD pathogens incidence and severity when *T. atroviride* SC1 treated plants were compared with untreated controls. In the first growing season (Table 2), the highest reductions (100%) were obtained for *Pa. chlamydospora* on the grafting point, *D. torresensis* on the rootstock basal end, and for *D. seriata* and *Pa. chlamydospora* on the roots. In the second growing season (Table 3), 100% reduction was obtained for *D. seriata*, *Pm. iranianum* and *Pa. chlamydospora* on the grafting point, *Pa. chlamydospora* on the roostock basal end, and for *D. torresensis*, *I. liriodendri* and *Pa. chlamydospora* on the roots. Nevertheless, in both seasons the percentages of reduction achieved for some GTD pathogens were low (e.g. *Pm. minimum* incidence at the rootstock basal end was reduced only 16.6% and 28.5% in seasons 2016-17 and 2017-18, respectively) (Tables 2 and 3).

In Field 1, percentages of incidence for BOT, PETRI and BF diseases at the end of the first growing season in untreated plants were 26.6, 24.4 and 10.0, respectively; while in *T. atroviride* SC1 treated plants they were 10.0, 8.8 and 3.3, respectively. Regarding, severity, percentages for BOT, PETRI and BF were 9.5, 5.6 and 1.6,

respectively; while in treated plants they were 2.3, 1.3 and 0.3, respectively. The statistical analyses showed significant reductions in both incidence and severity of PETRI (P=0.028 and P=0.039, respectively) at the end of the first growing season (Figure 3). For BOT and BF, there was not a statistically significant reduction in both incidence (P=0.184 and P=0.796, respectively) and severity (P=0.126 and P=0.796, respectively).

At the end of the second growing season, percentages of incidence for BOT, PETRI and BF diseases in untreated plants were 56.6, 40.0 and 16.6, respectively; while in *T. atroviride* SC1treated plants they were 23.3, 14.4 and 0, respectively. Regarding, severity, percentages for BOT, PETRI and BF were 16.0, 8.3 and 2.0, respectively; while in treated plants they were 5.3, 2.1 and 0, respectively. A significant reduction in both incidence and severity of BOT (P=0.046 and P=0.049, respectively) and PETRI (P=0.031 and P=0.039, respectively) diseases was observed (Figure 3). For BF, there was not a statistically significant reduction in both incidence and severity (P=0.32 and P=0.31, respectively), although the pathogens associated with this disease were not detected on treated plants.

Field 2 presented the highest GTD pathogens species diversity, because at the end of the first growing season (2016-17), the GTD pathogens that were isolated were *Botryosphaeria dothidea*, *C. luteo-olivacea*, *D. torresensis*, *D. seriata*, *Eutypa lata*, *Ilyonectria alcacerensis, I. robusta*, *N. parvum*, *Pm. minimum* and *Pa. chlamydospora* (Table 4), and at the end of the second growing season (2017-18) the species found were *D. novozelandica*, *D. torresensis*, *I. robusta*, *N. parvum*, *Pm. iranianum*, *Pm. minimum, Pm. parasiticum* and *Pa. chlamydospora* (Table 5). In both seasons their incidence and severity in the three isolation areas of the grafted plants was variable.

In this field, reductions in GTD pathogens incidence and severity in both growing seasons were also variable when *T. atroviride* SC1 treated plants were compared with untreated controls. In the first growing season (Table 4), the highest reductions (100%) were obtained for *E. lata and N. parvum* on the grafting point, *B. dothidea, C. luteo-olivacea* and *I. alcacerensis* on the rootstock basal end, and for *B. dothidea, I. liriodendri* and *Pa. chlamydospora* on the roots. In the second growing season (Table 5), 100% reduction was obtained for *Pm. iranianum* on the grafting point, *N. parvum* on the roostock basal end, and for *D. novozelandica*, *I. liriodendri* and *Pm. parasiticum* on the roots. Also in this field, in both seasons the percentages of reduction for some GTD

pathogens were low (e.g. *Pm. minimum* incidence was reduced only 20.8% and 23.3% the grafting point and the rootstock basal end, respectively) (Table 5).

In Field 2, percentages of incidence for BOT, PETRI and BF diseases at the end of the first growing season in untreated plants were 40.0, 36.3 and 16.6, respectively; while in *T. atroviride* SC1 treated plants they were 23.3, 13.3 and 16.6, respectively. Regarding, severity, percentages for BOT, PETRI and BF were 18.0, 10.4 and 5.3, respectively; while in treated plants they were 5.3, 1.8 and 5.3, respectively. The statistical analysis showed significant reductions in both incidence and severity of PETRI (P=0.002 and P=0.003, respectively) at the end of the first growing season (Figure 4). For BOT and BF, there was not a statistically significant reduction in both incidence (P=0.368 and P=0.8166, respectively) and severity (P=0.076 and P=1, respectively).

At the end of the second growing season percentages of incidence for BOT, PETRI and BF diseases in untreated plants were 56.6, 61.1 and 33.3, respectively; while in *T. atroviride* SC1 treated plants they were 30.0, 42.2 and 10.0, respectively. Regarding, severity, percentages for BOT, PETRI and BF were 16.6, 16.3 and 4.0, respectively; while in treated plants they were 12.0, 10.5 and 1.0, respectively. The reductions observed on BOT, PETRI and BF incidence (P=0.126, P=0.230 and P=0.177, respectively) or severity (P=0.512, P=0.144 and P=0.07, respectively) were not significant.

4 DISCUSSION

In the nursery experiment after the application of *T. atroviride* SC1, variable values of the incidence and severity reduction percentages of GTD pathogens were observed, being in some cases very low. Thus, when data from the different pathogens were grouped according to the three main GTD considered, only reductions in Botryosphaeria dieback incidence and severity, and Petri disease severity were statistically significant.

In grapevine nurseries, it is well known that the grafting process increases the risk of contamination by GTD pathogens, being the rooting phase the one in which the infections are most likely to occur.[4] In our nursery experiment, prior to grafting, both scions with buds and rootstock cuttings showed low initial infection levels by only three pathogens, *D. seriata*, *C. luteo-olivacea* and *N. parvum*. However, at the end of the grafting process, the diversity of GTD fungal species detected and its incidence and

severity levels increased noticeably. Black-foot, Botryosphaeria dieback and Petri disease pathogens were found, confirming that these three GTD are the most frequent affecting young grapevines worldwide.[1,4,39,53]

Fourie et al. (2001)[30] evaluated different strains of *T. harzianum* to control Petri disease and Black-foot pathogens in South African grapevine nurseries and obtained low isolation of fungal species associated with these two GTD from the treated vines. Nursery treatments with *T. atroviride* SC1 had already been reported as effective to control grapevine plants infections by *Pa. chlamydospora* and *Pm. minimum*, fungal species associated with Petri disease, during the grafting process.[28] But, our results also suggest that, in addition to this pathogens, this BCA may be used to control some Botryosphaeria dieback pathogens, such as *D. seriata*, in the grapevine propagation process in an integrated approach for the management of GTD.[1,4]

Regarding vineyard experiments, similar to what happened in the nursery experiment, our results were somewhat inconsistent. After additional *T. atroviride* SC1 treatments performed during two growing seasons in vineyards planted with these grafted plants, some GTD pathogens incidence and severity reductions were found. But, when data from the different pathogens were grouped according to the three main GTD considered, only some of them were statistically significant. When the grafted plants were planted in the experimental vineyards, *T. atroviride* SC1 was applied only by immersion of the roots and then, in the second growing season, this BCA was applied early in this season to the pruning wounds immediately after pruning. These preventive strategies aimed to maintain the better phytosanitary quality of the treated planting material obtained at the end of the nursery experiment, when compared with the untreated one. After the first growing season, all evaluated plants showed infections by diverse GTD pathogens, some of them not previously detected in the nursery, evidencing that grapevine plants from nurseries are continuously exposed to new GTD pathogens infections immediately after plantation.[1,4] In the two vineyards, *T. atroviride* SC1 treated plants still showed lower levels, to various degrees, of incidence and severity of Black-foot, Botryosphaeria dieback and Petri diseases, when compared with the untreated ones, but the infection levels were again very variable and, in some cases, too low to be conclusive regarding the percentage reductions obtained.

It is interesting to note that at the end of the first growing season the undried shoot weight of *T. atroviride* SC1 treated plants in both experimental vineyards was

significantly higher than the untreated ones. It is well known that *Trichoderma* BCAs can exert positive effects on plants with an increase in plant growth and the stimulation of plant-defense mechanisms.[54] Fourie et al. (2001),[30] already reported a significantly higher root mass for *T. harzianum* treated vines in nursery experiments. Moreover, a better phytosanitary state of the plants could also have contributed to a better plant establishment and development in the vineyards after plantation. Nevertheless, in the second growing season this plant growth effect was not detected, probably due to the absence of a *T. atroviride* SC1 root application, because *Trichoderma* strains must colonize plant roots prior to stimulation of plant growth,[54] and only the beneficial reduction of some GTD incidence and severity was observed.

Trichoderma atroviride SC1 showed high levels of reisolation from all plant parts of treated plants in both nursery and vineyard experiments. In fact, at the end of the first growing season these levels were high even in the grafting point, which was not treated with the BCA at planting moment or later, and in the second growing season from the roostock basal end and the roots, also not treated in this season. The real-time PCR developed by Savazzini et al. (2008)[50] for the detection and quantification of *T. atroviride* SC1 was very useful for the quick confirmation of the identity of the *Trichoderma* colonies recovered. These results reinforce that this BCA, which was isolated from decayed hazelnut wood in northern Italy, is very efficient in the colonization of different plant substrates as already demonstrated by Longa et al., (2008) and Pertot et al. (2016),[28,55] and it also can survive in nurseries during the storage of grafted plants at low temperatures in the period comprised between plant harvest and their sale to the farmers.

Currently, *Trichoderma* strains are gaining importance as BCAs to manage GTD in grapevine nurseries and vineyards with many new registered products becoming available in Europe and other grapevine regions worldwide.[2,24-28,33,35] Moreover, *Trichoderma* based treatments or other BCAs can be combined with other management strategies in a nursery IPM management program including: fungicides, hot-water treatments (HWT) and biofumigation to prevent or at least reduce the development of GTD.[1,29] For example, Halleen and Fourie (2016)[34] evaluated several integrated strategies for the proactive management of GTD infections during the grapevine grafting process, including fungicides, BCAs and HWT, and recommended a combination with Benomyl, Sporekill and *T. harzianum* treatments for its use in South African nurseries.

5 CONCLUSIONS

In this context, our results indicated that *T. atroviride* SC1 showed promise to reduce infections caused by some GTD pathogens in nurseries, and also when establishing new vineyards. This is a GTD management priority, due to the restrictions with the use of chemicals and the total absence of curative treatments once the grapevine plants are already infected.[1] Although there is no information yet available about the GTD pathogens infection thresholds that could lead to disease development and subsequent economic losses,[1,56] any reduction in the contamination levels in nurseries and at early stages of plant development after plantation will be beneficial for the long term sustainability of the vineyards. *Trichoderma atroviride* SC1 could possibly be a valuable component in a more integrated approach where various management strategies are combined to reduce GTD infections.[1,4]

ACKNOWLEDGEMENTS

Part of this study was financially supported by Belchim Crop Protection. We also acknowledge the availability of nursery staff and vineyard farmers to facilitate nursery and field work, respectively.

CONFLICT OF INTEREST

Trichoderma atroviride SC1 is commercialized by the company Belchim Crop Protection, that partially financed the research.

REFERENCES

1 Gramaje D, Urbez-Torres JR and Sosnowski MR, Managing Grapevine Trunk Diseases With Respect to Etiology and Epidemiology: Current Strategies and Future Prospects. *Plant Dis* **102**:12–39 (2018).

2 Mondello V, Larignon P, Armengol J, Kortekamp A, Vaczy K, Prezman F, Serrano E, Rego C, Mugnai L and Fontaine F, Management of grapevine trunk diseases:

knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathol Mediterr* **57**:369-383 (2018a).

3 Mondello V, Songy A., Battiston E, Pinto C, Coppin C, Trotel-Aziz P and Fontaine F, Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Dis* **7**: 1189–1217 (2018b).

4 Gramaje D and Armengol J., Fungal Trunk Pathogens in the Grapevine Propagation Process: Potential Inoculum Sources, Detection, Identification, and Management Strategies. *Plant Dis* **95**:1040–1055 (2011).

5 Bertsch C, Ramírez-Suero M, Magnin-Robert M, Larignon P, Chong J, Abou-Mansour E and Fontaine F, Grapevine trunk diseases: Complex and still poorly understood. *Plant Pathol* **62**:243-265 (2013).

6 Kaplan J, Travadon R, Cooper M, Hillis V, Lubell M and Baumgartner K, Identifying economic hurdles to early adoption of preventive practices: the case of trunk diseases in California winegrape vineyards. *Wine Economics and Policy* **5**:127-141 (2016).

7 Úrbez-Torres JR and WD Gubler, Susceptibility to grapevine pruning wounds to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum*. *Plant Pathol* **60**:261-270 (2011).

8 Gramaje D and Di Marco S, Identifying practices likely to have impacts on grapevine trunk disease infections: a European nursery survey. *Phytopathol Mediterr* **54**:313–324 (2015).

9 Rolshausen PE, Úrbez-Torres JR, Rooney-Latham S, Eskalen A, Smith RJ and Gubler WD, Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. *Am J Enol Vitic* **61**:113–119 (2010).

10 van Niekerk JM, Halleen F and Fourie P, Temporal susceptibility of grapevine pruning wounds to trunk pathogen infection in South African grapevines. Phytopathol Mediterr **50**:S139–S150 (2011).

11 Eskalen A, Feliciano J. and Gubler WD Susceptibility of grapevine pruning wounds and symptom development in response to infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. *Plant Dis* **91**:1100-1104 (2007).

12 Serra S, Mannoni MA and Ligios V, Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy. *Phytopathol Mediterr* **47**:234–46 (2008).

13 Elena G and Luque J, Seasonal susceptibility of grapevine pruning wounds and cane colonization in Catalonia, Spain following artificial infection with *Diplodia seriata* and *Phaeomoniella chlamydospora*. *Plant Dis* **100**:1651-1659 (2016).

14 Díaz G A and Latorre BA, Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. *Crop Prot* **46**:106–112 (2013).

15 Kühn A, Zappata A, Gold RE, Zito R and Kortekamp A, Susceptibility of grape pruning wounds to grapevine trunk diseases and effectiveness of a new BASF wound protectant. *Phytopathol Mediterr* **56**:536 (abstract) (2017).

16 Compant S and Mathieu F, Biocontrol of Major Grapevine Diseases: Leading Research, 231 pp., CABI, Wallingford, UK, pp. 160-170. (2017).

17 Harman GE and Bjorkman T, Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. In: Harman, G.E., Kubicek, C.P. (eds.), *Trichoderma & Gliocladium*, Vol. 2, pp. 229–265. Taylor & Francis, London (1998).

18 Harman GE, Myths and dogmas of biocontrol changes in perceptions derived from Research on *Trichoderma harzianum* T-22. *Plant Dis* **84**:377–393 (2000).

19 Muckherjee M, Muckherjee PK, Horwitz BA, Zachow C, Berg G and Zeilinger S, Trichoderma–plant–pathogen interactions : advances in genetics of biological control. *Indian J Microbiol* **52**:522–529 (2012).

20 Waghunde RR, Shelake RM and Sabalpara AN, *Trichoderma*: A significant fungus for agriculture and environment. *Afr J Agric Res* **11**:1952–1965 (2016).

21 Harman GE, Overview of Mechanisms and Uses of *Trichoderma* spp. *Phytopathol* **96**:190–194 (2006).

22 Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM and Bakker PAHM, Induced Systemic Resistance by Beneficial Microbes. *Annu Rev Phytopathol* **52:**347–375 (2014).

23 Van Wees SC, Van der Ent S and Pieterse CM, Plant immune responses triggered by beneficial microbes. *Curr Opin Plant Biol* **11**:443–448 (2008).

24 Kotze C, Van Niekerk J, Mostert L, Halleen F and Fourie P, Evaluation of biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. *Phytopathol Mediterr* **50**: S247–S263 (2011).

25 Mutawila C, Fourie PH, Halleen F and Mostert L, Grapevine cultivar variation to pruning wound protection by *Trichoderma* species against trunk pathogens. Phytopathol Mediterr **50**:S264-S276 (2011).

26 Pajot E, Mounier E, Blal B, Cortes F and Coutant A, Selection steps of the *Trichoderma atroviride* strain i-1237, and some practical results of the development of the fungi within the cankers diseases of grapevine and soil borne diseases of vegetables. In: Barker EA and Clement C, IOBC, Working Group, Biological Control of Plant Pathogens: Biocontrol of Plant Pathogens in Sustainable Agriculture. Book of Abstracts. Universite de Reims, Reims, Champagne-Ardenne, France, p. 40 (2012).

27 Reis P, Letousey P and Rego C, *Trichoderma atroviride* strain I-1237 protects pruning wounds against grapevine wood pathogens. *Phytopathol Mediterr* **56**:580 (2017).

28 Pertot I, Prodorutti D, Colombini A and Pasini L, *Trichoderma atroviride* SC1 prevents *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* infection of grapevine plants during the grafting process in nurseries. *Biocontrol* **61**:257-267 (2016).

29 Berlanas C, Andrés-Sodupe M, López-Manzanares B, Maldonado-González MM and Gramaje D, Effect of white mustard cover crop residue, soil chemical fumigation and *Trichoderma* spp. root treatment on black-foot disease control in grapevine. *Pest Manag Sci* **74**:2864-2873 (2018).

30 Fourie PH, Halleeen F, van der Vyver J and Schereuder W, Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. *Phytopathol Mediterr* **40**:S473-S478 (2001).

31 Di Marco S and Osti F, Applications of *Trichoderma* to prevent *Phaeomoniella chlamydospora* infections in organic nurseries. *Phytopathol Mediterr* **46**:73–83 (2007).

32 Fourie PH and Halleen F, Chemical and biological protection of grapevine propagation material from trunk disease pathogens. *Eur J of Plant Path* **116**:255–265 (2006).

33 Halleen F, Fourie PH and Lombard PJ, Protection of grapevine pruning wounds against *Eutypa lata* by biological and chemical methods. *S Afr J Enol and Vitic* **31**:125–132 (2010)

34 Halleen F and Fourie P, An integrated strategy for the proactive management of grapevine trunk disease pathogen infections in grapevine nurseries. *S Afr J Enol and Vitic* **37**:104-114 (2016).

35 Bigot G, Freccero A, Montermini A, Bortolotti P and Nannini R, Impiego di *Trichoderma* contro il mal dell'esca della vite. *L'Informatore Agrario* **6**:62-66 (2015).

36 Dissanayake AJ, Phillips AJL, Li XH and Hyde KD, Botrysophaeriaceae: Current status of genera and species. *Mycosphere* **7**:1001-1073 (2016).

37 Gams W, *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. *Stud Mycol* **45**:187-199 (2000).

38 Harrington TC and McNew DL, Phylogenetic analysis places the phialophora-like anamorph genus *Cadophora* in the Helotiales. *Mycotaxon* **87**:141-151 (2003).

39 Agustí-Brisach C and Armengol J, Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. *Phytopathol Mediterr* **52**:245-261 (2013).

40 Mostert L, Groenewald JZ, Summerbell RC, Gams W and Crous PW, Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium* anamorphs. *Stud Mycol* **54**:1-115 (2006).

41 Crous PW and Gams W, *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathol Mediterr* **39**:112-188 (2000).

42 Gardes M and Bruns TD, ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. *Mol Ecol* **2**:113-118 (1993).

43 White TJ, Bruns TD, Lee SB and Taylor JW, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications **18**:315-322 (1990).

44 Travadon R, Lawrence DP, Rooney-Latham S, Gubler WD, Wilcox WF, Rolshausen, PE and Baumgartner K, *Cadophora* species associated with wood-decay of grapevine in North America. *Fungal Biol* **119**:53-66 (2015).

45 O'Donnell and Cigelnik E, Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylog Evol* **7**:103–116 (1997).

46 Glass NL and Donaldson GC, Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous infection due to *Phaeoacremonium* spp. *J of Clin Microbiol* **41**:1332-1336 (1995).

47 Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE and Wingfield BD *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles *Mycol Res* **108**:411–418 (2004).

48 Crous PW, Groenewald JZ, Risede JM and Hywel-Jones N L, *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Stud in Mycol* **50**:415–429 (2004).

49 Waite H, May P and Bossinguer G, Variations in phytosanitary and other management practices in Australian grapevine nurseries. *Phytopathol Mediterr* **52**:369-379 (2013).

50 Savazzini F, Longa CMO, Pertot I and Gessler C, Real-time PCR for detection and quantification of the biocontrol agent *Trichoderma atroviride* strain SC1 in soil. *J Microbiol Methods* **73**:185–194 (2008).

51 R Core Team, R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. [http://www.R-project.org] (2017)

52 Mendiburu F, agricolae: Statistical Procedures for Agricultural Research. R package Version 1.2-3. [http://CRAN.R-project.org/package=agricolae] (2015).

53 Úrbez-Torres JR, The status of Botryosphaeriaceae species infecting grapevines *Phytopathol Mediterr* 50:S5–S45 (2011).

54 Benitez T, Rincon AM, Limon MC and Codon AC, Biocontrol mechanisms of *Trichoderma* strains. *Int Microbiol* **7**:249–260 (2004).

55 Longa CMO, Pertot I and Tosi S, Ecophysiological requirements and survival of a *Trichoderma atroviride* isolate with biocontrol potential. *J Basic Microbiol* **48**:269-277 (2008).

56 Úrbez-Torres JR, Haag P, Bowen P, Lowery T and O'Gorman DT, Development of a DNA macroarray for the detection and identification of fungal pathogens causing decline of young grapevines. *Phytopathol* **105**:1373-1388 (2015).

Table 1. Mean percentage of fungal grapevine trunk pathogens incidence and severity on untreated plants, and pathogen reduction achieved by *Trichoderma*-treatment in the grapevine nursery at the end of the propagation process.

	Incidence %		Severity %	
Isolation area / Fungi	**Control[a]**	**Reduction[b]**	**Control[c]**	**Reduction**
Grafting point				
Diplodia seriata	26.6±5.9	96.3±3.8	7.5±1.7	96±4.1
Neofusicoccum parvum	6.6±1.4	100±0	1.1±0.1	100±0
Phaeoacremonium minimum	26.6±4.9	76.6±5.2	5.8±1.5	82±5.1
Phaeomoniella chlamydospora	21.3±9.5	66.6±33.9	3.2±1.3	66.6±33.9
Rootstock basal end				
Dactylonectria torresensis	1.3±0.5	33.3±30.2	0.8±0.5	50±41.5
Cadophora luteo-olivacea	1.3±0.5	0±0	0.1±0.1	0±0
Phaeoacremonium minimum	18.6±3.6	16.6±9.8	5.3±0.9	52.5±19.1
Phaeomoniella chlamydospora	16.0±8.4	92.8±5.9	3.3±1.8	93.3±8.3
Roots				
Dactylonectria torresensis	1.3±0.5	100±0	1.1±0.5	100±0
Phaeoacremonium minimum	2.6±1.3	33.3±30.4	0.6±0.2	50±29.4
Phaeomoniella chlamydospora	4.0±2.3	50±41.6	0.7±0.5	33.3±30.1

[a]At each isolation area, the percentages of incidence are the mean of 75 plants analyzed (25 plants per replicate) ± Standard error of the mean.

[b]The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *Trichoderma*-treatment.

[c]At each isolation area, the percentages of severity are the mean of 250 wood fragments (10 wood fragments per plant and isolation area).

Table 2. Mean percentage of fungal grapevine trunk pathogens incidence and severity on untreated plants, and pathogen reduction achieved by *Trichoderma*-treatment in the Field 1 at the end of the first growing season (2016-17).

	Incidence %		Severity %	
Isolation area / Fungi	**Control[a]**	**Reduction[b]**	**Control[c]**	**Reduction**
Grafting point				
Diplodia seriata	10±5.8	75±20.8	3±1.7	83.3±13.9
Neofusicoccum parvum	16.6±8.9	50±29.4	8±4.1	58.9±30.7
Phaeoacremonium minimum	36.6±12.2	61.1±20.4	10.3±2.9	77.3±12.6
Phaeomoniella chlamydospora	3.3±3.3	100±0	0.3±0.3	100±0
Rootstock basal end				
Dactylonectria torresensis	6.6±6.6	100±0	11.6±1.1	100±0
Ilyonectria liriodendri	3.3±3.3	0±0	0.6±0.6	50±0
Phaeoacremonium minimum	10±5.8	16.6±16.5	2±1.5	26.6±20
Roots				
Dactylonectria torresensis	10±10.1	22.2±22.6	1.6±1.6	26.6±21.5
Diplodia seriata	3.3±3.3	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	20±5.8	83.3±16.9	4±1.7	91.6±8.5
Phaeomoniella chlamydospora	3.3±3.3	100±0	0.3±0.3	100±0

[a]At each isolation area, the percentages of incidence are the mean of 30 plants analyzed (10 plants per replicate) ± Standard error of the mean..

[b]The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *Trichoderma*-treatment.

[c]At each isolation area, the percentages of severity are the mean of 100 wood fragments (10 wood fragments per plant and isolation area).

Table 3. Mean percentage of fungal grapevine trunk pathogens incidence and severity on untreated plants, and pathogen reduction achieved by *Trichoderma*-treatment in the Field 1 at the end of the second growing season (2017-18).

	Incidence %		Severity %	
Isolation area / Fungi	**Control[a]**	**Reduction[b]**	**Control[c]**	**Reduction**
Grafting point				
Diplodia seriata	36.6±8.90	100±0	8±2.3	100±0.32
Neofusicoccum parvum	20±5.8	33.3±33.9	8±4.6	56.8±30.2
Phaeoacremonium iranianum	3.3±3.4	100±0	0.6±0.6	100±0
Phaeoacremonium minimum	46.6±8.9	41.1±4.9	10±0.6	58.3±15.9
Phaeomoniella chlamydospora	3.3±3.4	100±0	1±1.1	100±0
Rootstock basal end				
Dactylonectria torresensis	3.3±3.4	50±41.6	0.3±0	0±0
Phaeoacremonium minimum	33.3±20.6	28.5±23.7	7.3±5.6	47.2±39.3
Phaeomoniella chlamydospora	10±5.8	100±0	1.6±1.2	100±0
Roots				
Dactylonectria torresensis	13.3±13.5	100±0	1.6±1.6	100±0
Ilyonectria liriodendri	3.3±3.4	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	16.6±8.9	83.3±13.9	3.6±2.4	93.7±5.2
Phaeomoniella chlamydospora	10±5.8	100±0	1.3±0.8	100±0

[a]At each isolation area, the percentages of incidence are the mean of 30 plants analyzed (10 plants per replicate) ± Standard error of the mean.

[b]The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *Trichoderma*-treatment.

[c]At each isolation area, the percentages of severity are the mean of 100 wood fragments (10 wood fragments per plant and isolation area).

Table 4. Mean percentage of fungal grapevine trunk pathogens incidence and severity on untreated plants, and pathogen reduction achieved by *Trichoderma*-treatment in the Field 2 at the end of the first growing season (2016-17).

	Incidence %		Severity %	
Isolation area / Fungi	**Control[a]**	**Reduction[b]**	**Control[c]**	**Reduction**
Grafting point				
Botryosphaeria dothidea	26.6±12.2	36.6±18.9	10.6±3.7	78.2±4.2
Diplodia seriata	10±5.8	66.6±33.9	3±1.7	66.6±33.9
Eutypa lata	3.3±3.3	100±0	0.3±0.33	100±0
Neofusicoccum parvum	3.3±3.3	100±0	4.3±4.4	100±0
Phaeoacremonium minimum	40±11.7	55.5±29.9	18.3±3.4	85.4±11.1
Rootstock basal end				
Botryosphaeria dothidea	3.3±3.3	100±0	0.3±0.3	100±0
Cadophora luteo-olivacea	3.3±3.3	100±0	0.3±0.3	100±0
Dactylonectria torresensis	10±5.8	50±41.6	1±0.6	50±29.4
Ilyonectria alcacerensis	3.3±3.3	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	40±10.2	49.9±16.9	9±3.6	66.7±15.8
Roots				
Botryosphaeria dothidea	3.3±3.3	100±0	0.3±0.3	100±0
Dactylonectria torresensis	30±5.8	16.6±16.6	3.6±0.3	16.6±16.9
Ilyonectria alcacerensis	13.3±8.9	22.2±22.6	1.3±0.9	33.3±27.7
Ilyonectria robusta	3.3±3.3	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	20±5.8	66.6±33.9	2.6±0.8	66.6±33.9
Phaeomoniella chlamydospora	6.6±6.6	100±0	1±1.1	100±0

[a]At each isolation area, the percentages of incidence are the mean of 30 plants analyzed (10 plants per replicate) ± Standard error of the mean.

[b]The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *Trichoderma*-treatment.

[c]At each isolation area, the percentages of severity are the mean of 100 wood fragments (10 wood fragments per plant and isolation area).

Table 5. Mean percentage of fungal grapevine trunk pathogens incidence and severity on untreated plants, and pathogen reduction achieved by *Trichoderma*-treatment in the Field 2 at the end of the second growing season (2017-18).

	Incidence %		Severity %	
Isolation area / Fungi	**Control[a]**	**Reduction[b]**	**Control[c]**	**Reduction**
Grafting point				
Neofusicoccum parvum	56.6±8.9	45.6±19.2	16.6±5.1	33.1±24.6
Phaeoacremonium iranianum	3.3±3.3	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	56.6±12.2	20.8±21.2	17.6±0.3	41.6±23.1
Phaeomoniella chlamydospora	20±10.2	33.3±33.9	4.3±2.4	33.3±33.9
Rootstock basal end				
Ilyonectria liriodendri	3.3±3.3	0±0	0.3±0.3	0±0
Neofusicoccum parvum	3.3±3.3	100±0	0.3±0.3	100±0
Phaeoacremonium minimum	56.6±3.4	23.3±5.2	12.6±1.7	9.2±5.5
Phaeomoniella chlamydospora	26.6±14.8	63.3±2.7	7.3±5.1	49.4±7.8
Roots				
Dactylonectria novozelandica	13.3±13.5	100±0	1.3±1.3	100±0
Dactylonectria torresensis	3.3±3.3	50±41.6	0.6±0.6	50±41.6
Ilyonectria liriodendri	13.3±8.9	100±0	1.6±1.2	100±0
Phaeoacremonium minimum	16.6±8.9	83.3±13.9	5.6±3.2	90.9±7.6
Phaeoacremonium parasiticum	3.3±3.3	100±0	0.3±0.3	100±0
Phaeomoniella chlamydospora	10±5.8	75±20.8	1.3±0.9	83.3±13.9

[a]At each isolation area, the percentages of incidence are the mean of 30 plants analyzed (10 plants per replicate) ± Standard error of the mean.

[b]The percentage of reduction (Pr) of the pathogens detection at each isolation area and for each fungal species was calculated as Pr=100(Pc-Pt)/Pc, in which Pc is the mean pathogen incidence or severity in the control and Pt is the mean pathogen incidence or severity in the *Trichoderma*-treatment.

[c]At each isolation area, the percentages of severity are the mean of 100 wood fragments (10 wood fragments per plant and isolation area).

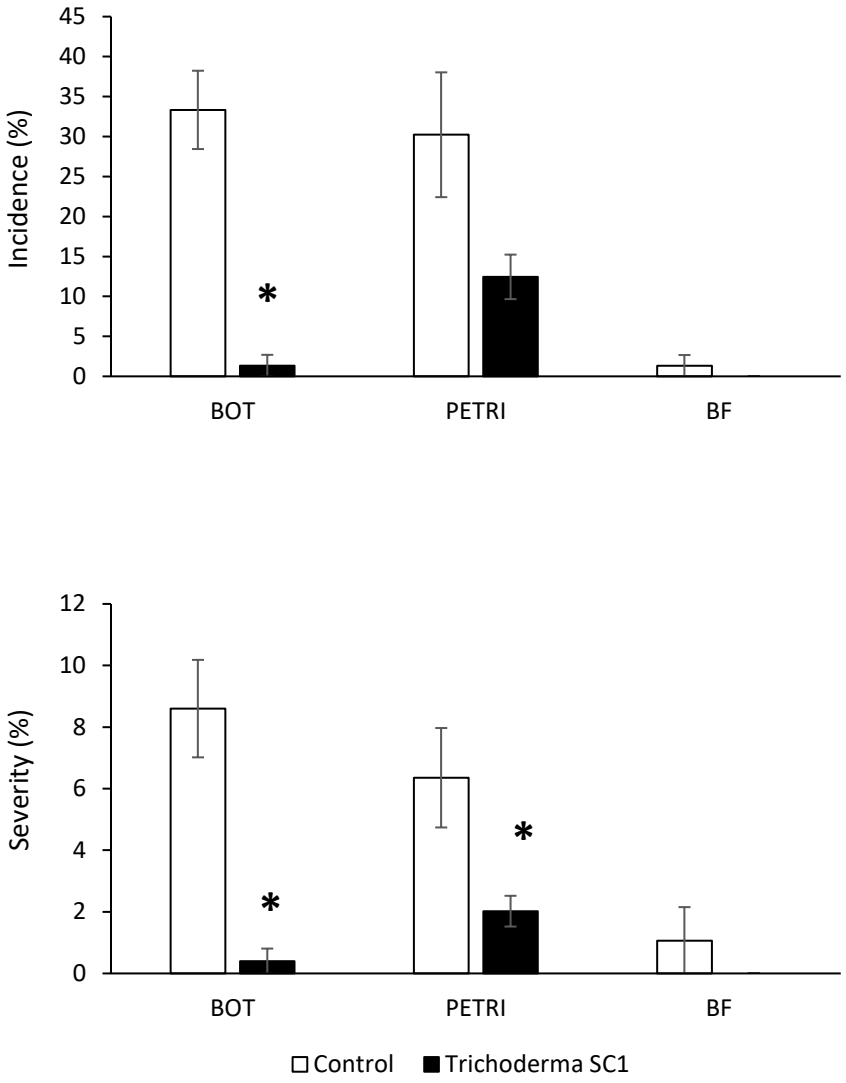

Figure 1. Percentages of incidence and severity observed in *Trichoderma atroviride* SC1 treated and untreated control plants in the nursery experiment for each type of grapevine trunk disease: Botryosphaeria dieback (BOT) including *Diplodia seriata* and *Neofusicoccum parvum*, Petri disease (PETRI) including *Cadophora luteo-olivacea*, *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, and black-foot disease (BF) including *Cylindrocarpon*-like asexual morphs. Mean percentages are based on three replicates of 25 plants per treatment. Asterisks indicate significant difference between treatments and control at the level of $P<0.05$. Bars represent the standard errors of the means.

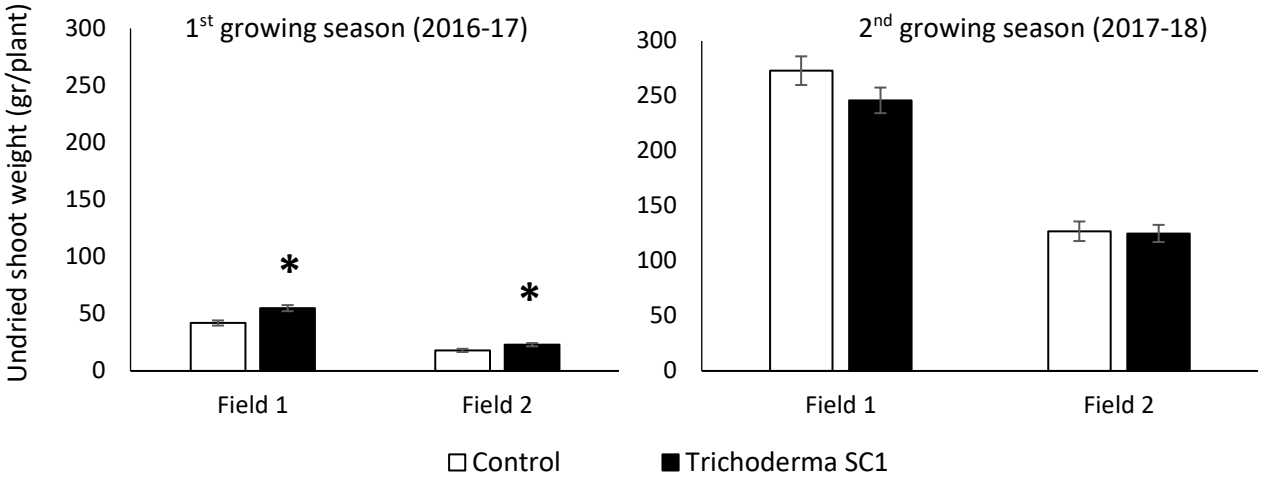

Figure 2. Undried shoot weight (gr/plant) observed in *Trichoderma atroviride* SC1 treated and untreated control plants at the end of the first and second growing seasons in the vineyard experiment at each experimental vineyard. Mean values are based on three replicates of 30 and 20 plants per treatment at the first and second growing seasons, respectively. Asterisks indicate significant difference between treatments and control at the level of $P<0.05$. Bars represent the standard errors of the means.

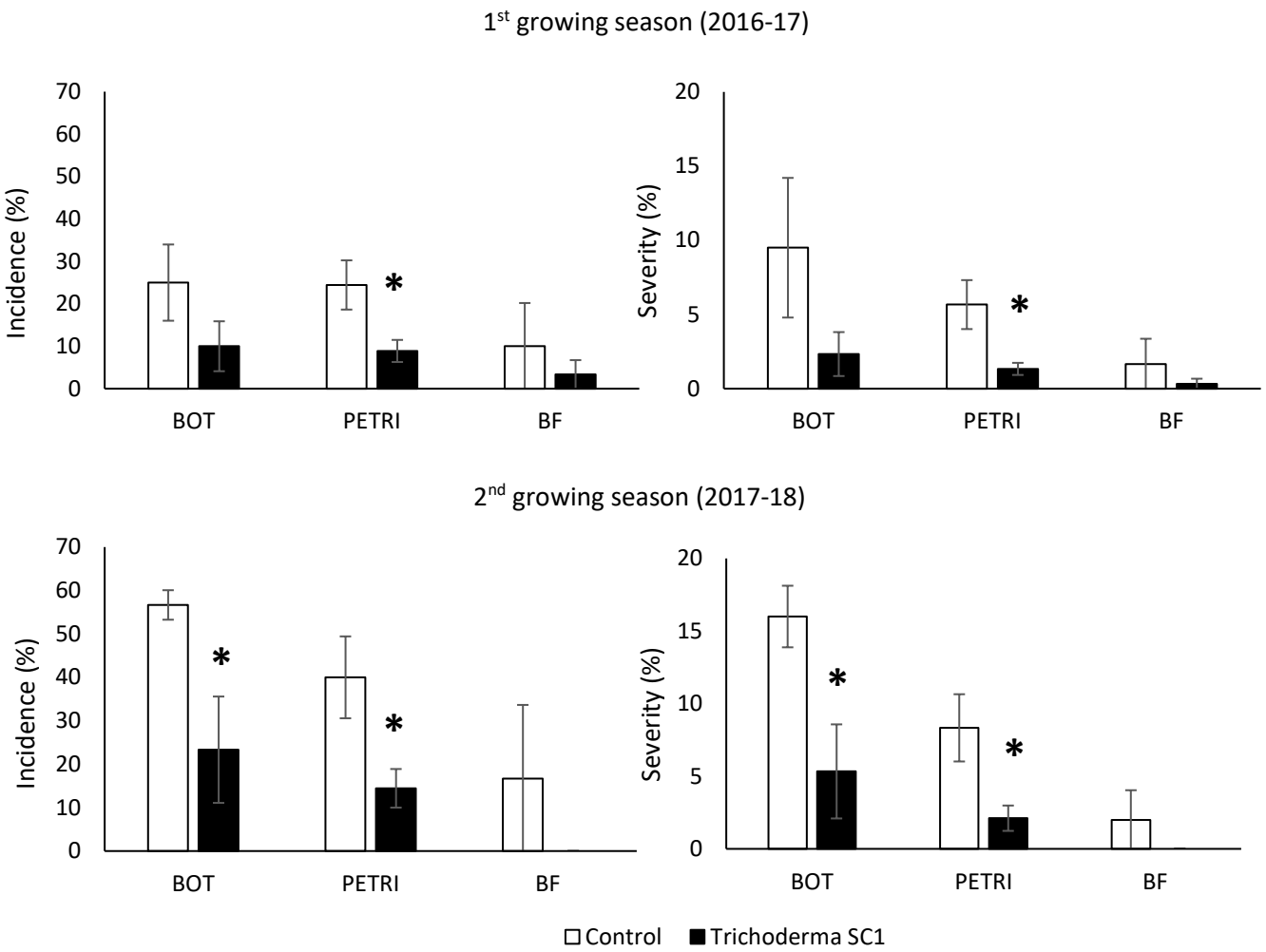

Figure 3. Percentages of incidence and severity observed in *Trichoderma atroviride* SC1 treated and untreated control plants in Field 1 at the end of the first and second growing seasons, for each type of grapevine trunk disease: Botryosphaeria dieback (BOT) including *Diplodia seriata* and *Neofusicoccum parvum*, Petri disease (PETRI) including *Cadophora luteo-olivacea*, *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, and black-foot disease (BF) including *Cylindrocarpon*-like asexual morphs. Mean percentages are based on three replicates of 10 plants per treatment. Asterisks indicate significant difference between treatments and control at the level of $P<0.05$. Bars represent the standard errors of the means.

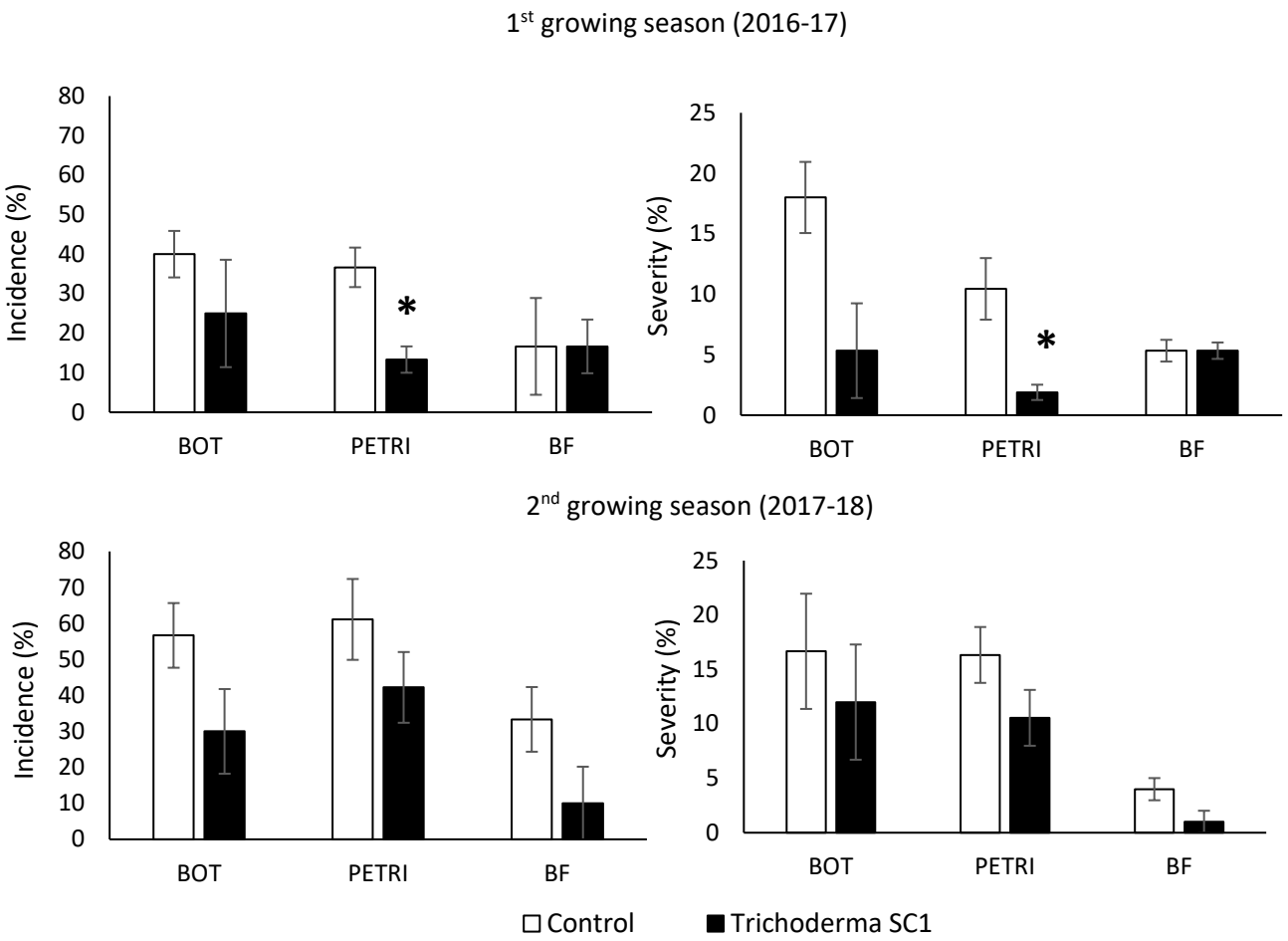

Figure 4. Percentages of incidence and severity observed in *Trichoderma atroviride* SC1 treated and untreated control plants in Field 2 at the end of the first and second growing seasons, for each type of grapevine trunk disease: Botryosphaeria dieback (BOT) including *Botryosphaeria dothidea*, *Diplodia seriata* and *Neofusicoccum parvum*, Petri disease (PETRI) including *Cadophora luteo-olivacea*, *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, and black-foot disease (BF) including *Cylindrocarpon*-like asexual morphs. Mean percentages are based on three replicates of 10 plants per treatment. Asterisks indicate significant difference between treatments and control at the level of $P<0.05$. Bars represent the standard errors of the means.