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SHORT NOTE

Trichoderma spp. and *Bacillus subtilis* for control of *Dactylonectria macrodidyma* in grapevine

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Summary. Black foot disease, which is primarily caused by *Dactylonectria* species, affects young and mature grapevines, causing loss of productivity and reduction of longevity of vineyards. Because it is a soil-borne fungus, this pathogen offers limited control possibilities. This study aimed to assess the biocontrol activity of products based on *Trichoderma* spp. and *Bacillus subtilis*, *in vitro* and *in vivo*, against *D. macrodidyma*. *In vitro* assays were carried out to evaluate inhibition of mycelial growth of the pathogen through volatile metabolites and paired cultures assays. An *in vivo* experiment evaluated the potential biocontrol of the pathogen in plants of *Vitis vinifera* cv. Merlot, grafted on 'Paulsen 1103'. In the volatile metabolites assay, mean inhibition of mycelial growth in treatments using *Trichoderma* spp. was 59%. In the paired cultures experiment, treatment with *B. subtilis* (Rizolyptus®) gave 41% inhibition of the pathogen. In the *in vivo* assay, the best results for length of the primary shoots, total numbers of nodes and root dry weight were achieved with the *B. subtilis* treatments. This study demonstrates that *B. subtilis* has potential for biocontrol of *D. macrodidyma*.

Key words: *Vitis vinifera*, black foot disease, soil-borne pathogen, biological control.

Introduction

Since 1990, symptoms of young grapevine decline and deaths of planting material have affected the production of table grapes, raisins and wine grapes (Gramaje and Armengol, 2011). Black foot is one of the major grapevine wood diseases (Santos *et al.*, 2014a), associated mainly with young grapevine decline caused by soil-inhabiting fungi (Halleen *et al.*, 2006). Fungi of the genus *Ilyonectria* (anamorph: "*Cylindrocarpon*" Wollew) are soil inhabitants, saprophytes, root colonizers or plant pathogens often associated with herbaceous and woody plants (Brayford, 1993). Recently, multi-gene studies of this species complex indicated that the genus *Ilyonectria* is paraphyletic. Thus, the genus *Dactylonectria* was introduced with ten new combinations including *D. macrodidyma* (Halleen, Schroers and Crous) L. Lombard and Crous, comb. nov., several of which were previously included in *Ilyonectria* (Lombard *et al.*, 2014).

In Brazil, death of young grapevines caused by black foot disease has been observed since 1999 (Garrido *et al.*, 2004). Symptomatic plants show dark brown to black discolorations at the bases of the

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rootstock, root rot, dark discolouration of the vascular tissues, reduced vigour, sudden wilting of the foliage, decline and death (Garrido *et al*., 2004). In addition, Santos *et al.* (2014b) reported reductions in root biomass, necrosis of leaf ribs and high incidence of the *D. macrodidyma* in Southern Brazil.

Due to the worldwide importance of black foot disease of grapevine, control methods have been investigated. Biological control with species of *Bacillus* (Nagórska *et al*., 2007; Lugtenberg and Kamilova, 2009) and *Trichoderma* (Harman, 2000) is one possible control strategy. *Trichoderma harzianum* has been reported as a potential biocontrol agent against grapevine wood fungi, including *Phaeomoniella chlamydospora, Phaeoacremonium* spp., *"Cylindrocarpon"* spp., *Botryosphaeria* spp. and *Phomopsis* spp. (Di Marco and Osti, 2007; Fourie *et al*., 2001). *Bacillus subtilis* inhibited the growth of *Botrytis cinerea* and *Colletotrichum gloeosporioides* in *in vitro* experiments with grapevine, and reduced the development of *Plasmopara viticola* symptoms in fruits and leaves (Furuya *et al*., 2011). No information is available about the control efficacy of these biocontrol agents against *D. macrodidyma*.

The aim of the present study was to evaluate the potential of *Trichoderma* spp. and *B. subtilis, in vitro* and *in vivo,* as biocontrol agents against *D. macrodidyma* in grapevine plants of cv. Merlot grafted on 'Paulsen 1103' rootstock.

Materials and methods

Isolates of *D. macrodidyma* were collected in 2012 from grapevines showing symptoms of black foot disease in different vineyards cultivated at Rio Grande do Sul State, Brazil (Table 1). Symptomatic plants showed reduction of the root biomass, necrotic roots and crowns, delayed sprouting, reduced vigour, wilting of the foliage and plant death. The isolates were stored on potato dextrose agar (PDA; Difco) in slants at 5ºC, in the fungal collection of the Plant Pathology Laboratory of the Federal University of Santa Maria (Universidade Federal de Santa Maria) and also at Embrapa Grape and Wine (Embrapa Uva e Vinho), in Brazil. In preliminary studies to confirm identification, the histone H3 region of all *D. macrodidyma* isolates was sequenced and deposited in GenBank (Santos *et al*., 2014b). The commercial formulations of biocontrol agents based on *Trichoderma* spp. and *B. subtilis* (Table 2) were tested in *in vitro* and *in vivo* assays.

The influence of volatile metabolites produced by biocontrol agents in inhibiting the mycelial growth of *D. macrodidyma* was assessed using the two faces of Petri dishes, fixed on one another and sealed with flexible film to prevent leakage of the metabolites. For this assay, 90 mm diam. Petri dishes containing PDA were used. Colonies of *Trichoderma* spp. were obtained from aliquots of the products (Table 2) and plated onto PDA 10 d prior of the beginning of the experiment. A mycelial disc (8 mm diam.) of the pathogen, which had grown for 10 d at 25ºC in the dark, was transferred to the center of one face of each dish and an identical *Trichoderma* spp. mycelial disc was transferred to the center of the second face of the dish. To evaluate the biocontrol activity of *B. subtilis*, the treatment followed the method of parallel grooves for bacteria suspensions in the dishes (Monteiro *et al*., 2013). In control dishes, only the pathogen

Table 1. Isolates of *Dactylonectria macrodidyma* used in this study.

Isolate	Origin (Region of Brazil)	Rootstock/cultivar	GenBank access numbers (Histone H3)
Cy4UFSM	Erechim	VR 043-43/Isabel	KF633167
Cy5UFSM	Garibaldi	Niágara Branca*	KF633168
Cy7UFSM	Garibaldi	16149/ Isabel	KF633170
Cy11UFSM	Bento Gonçalves	Gravesac/Merlot	KF633155
Cy15UFSM	Flores da Cunha	Niágara Branca*	KF633159
Cy16UFSM	Flores da Cunha	Bordô*	KF633160

* Own-rooted cultivar

Table 2. Characteristics of six biocontrol products studied.

Treatment	Species	Colony forming units (CFU mL^{-1})	Formulation	Trade name	Manufacturer
T1	*Trichoderma harzianum*	2×10^9	Concentrated suspension	Trichodermil SC1306®	Itaforte Bio Produtos, Brazil
T2	*Trichoderma asperellum*	1.5×10^{10}	Emulsifiable concentrate	TrichoderMax EC®	Novozymes BioAg, Brazil
T3	*Trichoderma asperellum*	1×10^{10}	Water-dispersible granules	Quality WG®	Grupo Farroupilha, Brazil
T4	*Trichoderma harzianum*, *Trichoderma viride/atroviride*, *Trichoderma viride*.	1×10^9	Concentrated suspension	Trichodel®	Empresa Caxiense de Controle Biológico Ltda, Brazil
T5	*Bacillus subtilis*	5×10^9	Concentrated suspension	Rizos®	Grupo Farroupilha, Brazil
T6	*Bacillus subtilis*	1×10^8	Concentrated suspension	Rizolyptus®	Grupo Biosoja, Brazil

was used. The dishes were incubated at 25ºC in the dark and assessments were performed after 14 d incubation by determining the average diameter of the pathogen growth, measured in two perpendicular directions with a digital caliper.

In the paired culture assay for *Trichoderma*, two 8 mm diam. mycelial discs, one of *Trichoderma* and other of the pathogen, were deposited at different times into 90 mm diam. Petri dishes containing PDA, 5 mm from the periphery of the dish. The *D. macrodidyma* mycelial disc was placed on one side of the dish, and 14 d later a *Trichoderma* spp. disc was placed on the opposite side of the dish. To assess the effects of *B. subtilis*, one pathogen mycelial disc (8 mm diam.) was placed in the center of the dish and after 4 d, 10 μL of the bacterial suspension (1×10^8 cfu mL^{-1}) obtained from each of the products (Table 2) were symmetrically placed at four equidistant sites (30 mm) from the center of the plate. As controls, only the pathogen was used. The dishes were incubated at 25ºC in the dark and assessments were performed 14 d after the transfer of the antagonist, by determining diameters of the pathogen mycelial colonies.

In all assays the percentage of pathogen mycelial growth inhibition was calculated by the formula used by Erdogan and Benlioglu (2010): [(treatment - control) / control × 100]. For each treatment, five replicates were carried out, and for each replication, the mycelial growth inhibition was evaluated in two replicates.

In vivo biocontrol potential against *D. macrodidyma* was assessed using different *Trichoderma* spp. and *B. subtilis*-based products (Table 2). Ten replicates of 1 y old plants of cv. Merlot grafted on 'Paulsen 1103' rootstock were subjected to each treatment. One liter plastic bags containing plant growth substrate (80% of Mec Plant® commercial substrate and 20% of sand) were drenched with the biocontrol agents (1×10^4 cfu g^{-1} of substrate), 14 d prior to the beginning of the experiment. After that period, plants were transplanted and, simultaneously, the substrate was inoculated with the highly aggressive isolate of *D. macrodidyma*, Cy5UFSM (Table 1) (Santos *et al.*, 2014b), at a concentration of 5×10^5 cfu g^{-1} of substrate (Alaniz *et al.*, 2011). Control replicate treatments were set up, one treatment was used only with the pathogen (positive control) and the other without the pathogen and without the antagonist (negative control). Thirty days after the beginning of the experiment, the treatments with *Trichoderma* spp. and *B. subtilis* were repeated with a new inoculation of biocontrol agents, as described above. After this, plants were maintained for more 3 months in a greenhouse at 25ºC and were irrigated each day to near field capacity.

After 4 months, the plants were uprooted and the roots were washed under running water. The following growth parameters were assessed for each plant: length of the longest root formed (LR, cm),

length of the primary shoot (LPS, cm), number of nodes in the primary shoot (NNPS), total number of nodes (TNN), total number of shoots (TNS), shoot dry weight (SDW, g), root dry weight (RDW, g), and the pathogen re-isolation (RI, %). Dry matter was obtained by drying the plant material in a forced ventilation oven at 60°C, until constant weight was reached. For pathogen re-isolation, ten fragments of wood from the basal end of each rootstock (at least 2 cm above the base) were superficially disinfected by the immersion in alcohol (70%), sodium hypochlorite (1%) followed by three immersions in sterilized distilled water, each for 1 min. Tissue fragments were dried on sterilized filter paper and transferred to Petri dishes containing PDA medium amended with 0.5 g L^{-1} of streptomycin sulfate (Sigma-Aldrich). The plates were incubated at 25ºC for 14 d. in the dark. After this period, the percentages of re-isolation of *D. macrodidyma* were recorded in relation to the total number of fragments obtained from each plant.

The experimental designs used in the *in vitro* and *in vivo* assays were completely randomized. For the *in vitro* experiment, bi-factorial analysis was used, Factor A (*D. macrodidyma* isolates) and Factor D (antagonist commercial products). Separate statistical analyses were performed for *B. subtilis* and *Trichoderma* spp. treatments due to the different experiments and microorganism behaviours. For the *in vivo* experiment, unifactorial analysis of variance was performed followed by a Scott-Knott test ($P \leq 0.05$) whenever a significant effect was observed. All data were transformed prior to analyses. The SISVAR 5.3 (Sistema de Análise de Variância Para Dados Balanceados [Variance Analysis System for Balanced Data]) software package was used for all statistical analysis (Ferreira, 2010).

Results

The assay of volatile metabolites showed that all treatments with *Trichoderma* spp. and *B. subtilis* inhibited mycelial growth of the six *D. macrodidyma* isolates by producing volatile metabolites (Table 3). Treatment with Trichodermil SC1306® was significantly different from the other treatments, showing the greatest reduction of mycelial growth of the pathogen, followed by the treatments with TrichoderMax EC® and Trichodel®. The least inhibition percentage, differing significantly from the other treatments was recorded for Quality WG®. The least mean inhibition

Table 3. Mean inhibition of mycelial growth (%) of isolates of *Dactylonectria macrodidyma* exposed to volatile metabolites of *Trichoderma* spp. and *Bacillus subtilis in vitro*.

Treatment[1]	Inhibition of mycelial growth of *D. macrodidyma* (%)						
	Cy4UFSM	Cy5UFSM	Cy7UFSM	Cy11UFSM	Cy15UFSM	Cy16UFSM	Mean
T1	66.2 aA[2,3]	71.5 aA	66.2 aA	62.7 aA	68.8 aA	65.6 aA	66.8 a
T2	61.1 aA	63.1 bA	50.5 cB	65.6 aA	59.0 bA	61.2 aA	60.1 b
T3	45.8 bB	44.4 dB	58.6 bA	47.7 bB	50.4 cB	46.5 cB	48.8 c
T4	67.8 aA	53.1 cB	51.1 cB	61.6 aA	63.5 bA	52.7 bB	58.3 b
Mean	60.2 A	58.0 A	56.6 A	59.3 A	60.4 A	56.5 A	58.5
T5	40.9 aA	50.5 aA	32.9 aA	50.9 aA	47.2 aA	68.8 aA	48.6 a
T6	45.5 aA	48.5 aA	41.0 aA	44.2 aA	29.5 aA	69.1 aA	46.3 a
Mean	43.2 B	49.5 B	37.0 B	47.6 B	38.3 B	69.0 A	47.4

[1] Treatment: T1, *Trichoderma harzianum* (Trichodermil SC1306®); T2, *Trichoderma asperellum* (TrichoderMax EC®); T3, *Trichoderma asperellum* (Quality WG®); T4, *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (Trichodel®); T5, *Bacillus subtilis* (Rizos®); T6, *Bacillus subtilis* (Rizolyptus®).

[2] Means within each column followed by the same lowercase letter and within each row followed by uppercase letter within are not different by the Scott-Knott test ($P \leq 0.05$).

[3] Transformed data ($\sqrt{(x + 0.5)}$).

percentage was obtained for the Cy16UFSM isolate. For the *B. subtilis*-based treatments, there were only differences between the mean inhibition values of the two tested products. The greatest mean mycelial growth inhibition was obtained for Cy16UFSM isolate, and this differed from all the other treatments. The least mean inhibition percentage was obtained for the Cy7UFSM isolate (Table 3).

In the paired cultures assay, *Trichoderma* spp. and *B. subtilis* gave interactions among the treatments and *D. macrodidyma* isolates (Table 4). The best *Trichoderma*-based treatment was with Trichodel®, differing significantly from the other treatments. The greatest mean percentage inhibition of the pathogen was recorded for the Cy15UFSM isolate, followed by the isolate Cy7UFSM. The least mean percentage inhibition was obtained from the Cy11UFSM isolate. In the assessment of *B. subtilis*, for Rizolyptus®, the pathogen mycelial growth was reduced by 41%, while Rizos® inhibited mycelial grown by only 29%. The greatest mean control by both products was observed for the Cy11UFSM isolate, which was significantly different from the others.

At the end of the *in vivo* experiment period, black foot symptoms were visible in the plants inoculated with the *D. macrodidyma* (Cy5UFSM), in contrast with control plants, which were asymptomatic. The disease symptoms included reduced vigour, sudden wilting of the foliage, root rots, reduction in root biomass and vascular necrosis. Plants treated with *Trichoderma* spp. and *B. subtilis* presented less disease symptoms than positive control plants infested only with the pathogen. However, for plants growing in the substrate treated by *B. subtilis*, fewer disease symptoms were observed than for the plants growing in the substrate treated with *Trichoderma* spp. (Table 5).

The LR did not show statistically significant differences amongst the assessed treatments. For LPS, treatments with the two products containing strains of *T. asperellum* and *B. subtilis* gave the greatest mean values, and these differed from the positive control. For NNPS, treatments with the two products containing strains of *T. asperellum* and Rizos® gave the greatest mean values and they did differ from positive and negative control. The TNN and the TNS were greater in the negative control treatment, which was significantly different from the other treatments (Table 5). For SDW and RDW, there were decreases in the plants inoculated with *D. macrodidyma*, even in

Table 4. Mean inhibition of mycelial growth (%) of isolates of *Dactylonectria macrodidyma* in *in vitro* paired culture with isolates of *Trichoderma* spp. and *Bacillus subtilis*.

Treatment[1]	Inhibition of mycelial growth of *D. macrodidyma* (%)						
	Cy4UFSM	Cy5UFSM	Cy7UFSM	Cy11UFSM	Cy15UFSM	Cy16UFSM	Mean
T1	33.9 bB[2,3]	37.1 aA	34.5 bB	38.6 aA	41.7 bA	35.0 bB	36.8 c
T2	34.2 bB	37.5 aB	44.0 aA	35.2 aB	40.9 bA	35.5 bB	37.9 c
T3	44.2 aA	38.2 aB	40.9 aA	37.2 aB	40.6 bA	41.3 aA	40.4 b
T4	44.2 aA	40.9 aA	42.8 aA	40.9 aA	46.0 aA	43.7 aA	43.1 a
Mean	39.1 B	38.4 B	40.6 A	38.0 B	42.3 A	38.9 B	39.5
T5	26.8 bC	26.5 bC	20.9 bD	36.1 bA	34.0 bA	29.5 bB	29.0 b
T6	50.6 aA	37.4 aC	29.8 aD	45.8 aA	40.5 aB	42.9 aB	41.2 a
Mean	38.7 B	31.9 C	25.4 D	40.9 A	37.3 B	36.2 B	35.1

[1] Treatment: T1, *Trichoderma harzianum* (Trichodermil SC1306®); T2, *Trichoderma asperellum* (TrichoderMax EC®); T3, *Trichoderma asperellum* (Quality WG®); T4, *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (Trichodel®); T5, *Bacillus subtilis* (Rizos®); T6, *Bacillus subtilis* (Rizolyptus®).

[2] Means within each column followed by the same lowercase letter and within each row followed by uppercase letter within are not different by the Scott-Knott test ($P \leq 0.05$).

[3] Transformed data ($\sqrt{(x + 0.5)}$).

Table 5. Mean growth parameters for grapevine plants treated with different potential biocontrol products, including length of the longest root formed (LR, cm), length of the primary shoot (LPS, cm), nodes number in the primary shoot (NNPS), total number of nodes (TNN), total number of shoots (TNS), shoot dry weight (SDW, g), root dry weight (RDW, g), as well as , and the frequency of re-isolation (RI, %) in cv. Merlot grapevines inoculated with *Dactylonectria macrodidyma*.

Treatment[1]	LR	LPS	NNPS	TNN	TNS	SDW	RDW	RI
T1	31.8 a[2,3]	53.5 b	16.6 b	29.0 c	2.8 b	8.5 c	4.9 c	58.9 b
T2	24.8 a	80.2 a	27.0 a	34.2 c	3.1 b	10.8 b	4.0 c	52.2 b
T3	29.6 a	71.6 a	24.1 a	41.4 b	2.8 b	11.4 b	4.4 c	61.1 b
T4	28.6 a	55.3 b	16.9 b	27.9 c	2.3 b	5.6 c	3.5 c	64.4 b
T5	38.7 a	80.6 a	22.4 a	36.6 b	3.1 b	13.7 a	6.4 b	61.1 b
T6	33.2 a	67.2 a	18.7 b	40.3 b	3.8 b	10.1 b	6.8 b	44.4 b
T7	27.4 a	47.7 b	13.9 b	24.0 c	2.4 b	6.1 c	4.1 c	74.4 b
T8	31.4 a	66.1 a	19.1 b	56.9 a	5.6 a	17.7 a	9.4 a	0 a

[1] Treatment: T1, *Trichoderma harzianum* (Trichodermil SC1306®); T2, *Trichoderma asperellum* (TrichoderMax EC®); T3, *Trichoderma asperellum* (Quality WG®); T4, *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (Trichodel®); T5, *Bacillus subtilis* (Rizos®); T6, *Bacillus subtilis* (Rizolyptus®); T7, pathogen (positive control); T8, negative control.
[2] Means within the column followed by the same are not different by the Scott-Knott test ($P \leq 0.05$).
[3] Transformed data ($\sqrt{(x + 0.5)}$).

the presence of the biocontrol agents. Plants treated with Rizos® showed mean values of SDW not significantly different from the control treatment, but it was greater than those recorded for the other treatments. Regarding RDW, the negative control gave a mean value significantly greater than the other treatments, followed by the *B. subtilis*-based treatments, in which there was a weight increase when compared to the *Trichoderma* spp.-based treatments. The greatest pathogen re-isolation was achieved from the treatment where only *D. macrodidyma* was used (positive control), not differing significantly from the infested treatments, with the exception of the negative control treatment.

Discussion

This study has demonstrated the potential of biological control agents against *D. macrodidyma* in grapevine. Lopes *et al.* (2012) found a positive correlation between the production of volatile metabolites and the antagonist ability of different species of *Trichoderma* against *Sclerotinia sclerotiorum*. In our experiments, the average inhibition percentage achieved was reached 59%, which was greater than the values found by Lopes *et al.* (2012), who recorded inhibition percentages varying from 8 to 56% with a mean value of 24.9%.

Fiddaman and Rossall (1993) suggested the production of antifungal volatiles by a *B. subtilis* strain, which are active against fungi. Several *Bacillus* strains produce antibiotic substances (iturin A, fengycin and surfactin) (Romero *et al.*, 2007), which play key roles in the biological control of phytopathogenic fungi. *In vitro* experiments have shown that heat-stable metabolites of *Bacillus subtilis* AG1 can inhibit the growth of *Lasiodiplodia theobromae*, a causal agent of trunk and roots rot in grapevines (Alfonzo *et al.*, 2009). In our experiment, two commercial formulations of *B. subtilis* inhibited the growth of *D. macrodidyma* by 46 and 49%. Monteiro *et al.* (2013), using the same method, reported a reduction of 84% of the mycelial growth of *S. sclerotiorum* subjected to volatile metabolites of *B. subtilis*.

In paired cultures of *Trichoderma* spp. against *D. macrodidyma* mycelial growth inhibition of 40% was observed. Bomfim *et al.* (2010), using paired cultures, reported that *T. viride* and *T. harzianum* yielded the best results in the control of *Rhizopus stolonifer*, causal agent of passionflower rot. Sharfuddin and Mohanka (2012) reported 71 to 83% growth inhibition of *Fusarium oxysporum* f. sp. *lentis*, comparing different

isolates of *Trichoderma* spp.

Bacillus subtilis KS1 inhibited the mycelial growth of *Colletotrichum gloeosporioides* and *Botrytis cinerea*, resulting in large inhibition zones in the Petri dishes (Furuya *et al.*, 2011), which was similar to the results obtained in the present study with two *B. subtilis* commercial formulations (Rizolyptus® and Rizos®) against *D. macrodidyma*. Compant *et al.* (2013) reported that some biocontrol bacterial strains and their metabolites have direct effects on the growth of fungi in grapevine wood, either by inhibiting the mycelial growth or by fungal enzymatic activities.

In the *in vivo* experiment, *B. subtilis* gave promising results for biological control of *D. macrodidyma*. Growth parameters for plants treated with *B. subtilis* were greater than the negative and positive experimental controls. Similar results were obtained by Erdogan and Benlioglu (2010), who evaluated isolates of *Pseudomonas* spp. for control of Verticillium wilt on cotton crops naturally infested with *Verticillium dahliae*. Kotze *et al.* (2011) observed that *B. subtilis* causes malformation of the hyphae of pathogens associated with grapevine trunk diseases, which could be due to the production of antibiotic substances.

Regarding the dry weight of roots, the *Trichoderma* spp. treatments showed similar values when compared with positive control, probably due to lower competence in rhizosphere colonization, which may be one of the factors of low biocontrol efficiency. Biological control agents that are able to effectively colonize host rhizospheres provide root protection by eliminating propagules of pathogens, or pathogens competing for the existing nutrients (Gava and Menezes, 2012). Halleen *et al.* (2007) and Di Marco and Osti (2007) assessed the effects of products based on *T. harzianum* in soil as potential biological control agents of grapevine trunk associated pathogens. Halleen *et al.* (2007) also found that the incidence of pathogens associated with black foot in grapevine commercial nurseries did not decrease. Teixeira *et al.* (2012), using the same *Trichoderma* spp. products used in the present study, reported that there were increases in the emergence of bean seedlings, and reduced severity of dry root rot caused by *Fusarium solani* f. sp. *phaseoli*, although incidence of the disease was not affected when compared to the experimental control.

Regarding re-isolation percentages of the pathogen, there were no significant differences among the biocontrol treatments applied. In commercial nurseries, low percentages of "*Cylindrocarpon*" were isolated when *Trichoderma* treatments were carried out, which showed that *Trichoderma* applications might result in a production of more healthy plants (Fourie *et al.*, 2001). Fourie and Halleen (2006) dipped grapevine propagation material in a *T. harzianum* suspension, and found inconsistent results regarding the efficacy of this method for control of trunk disease pathogens, probably due to short periods of immersion.

The present study is a first step towards further research involving *Trichoderma* spp. and *B. subtilis* biocontrol agents, to suppress *D. macrodidyma* in grapevine. Further studies must be carried out to confirm these results, and also evaluate efficacy of biocontrol treatments against natural infections in vineyards and nurseries.

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