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FULL RESEARCH PAPER

Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by in vitro and in planta tests

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Abstract One isolate each of *Trichoderma viride*, *Epicoccum nigrum*, *Fusarium tricinctum*, *Alternaria alternata*, *Sclerotinia sclerotiorum* and *Cytospora* (teleomorph: *Valsa* sp.) present in epigeous declining oak tissues was evaluated for its ability to control *Diplodia corticola* (isolate 79). This fungus is the causal agent of cankers, vascular necrosis and dieback on various oak species. Among the isolates tested, *T. viride* and *F. tricinctum* showed maximum in vitro inhibition of mycelial growth of *D. corticola* (isolate 79). Species were also evaluated for their ability to reduce mortality caused by *D. corticola* (isolate 79) of *Quercus cerris* and *Q. pubescens* seedlings under controlled conditions. Two series of inoculations were carried out through wounds in the stem; in the first, the distance between the point of inoculation of the antagonist and the pathogen was 6 cm, whereas in the second series the distance was shortened to 3 cm. In seedlings of *Q. cerris* and *Q. pubescens* at a distance of 3 cm, inoculation with *F. tricinctum* and *A. alternata* significantly reduced mortality caused by *D. corticola* (isolate 79). Inoculation of *T. viride* through artificial cuticular wounds in the stem of seedlings prevented the proliferation of *D. corticola* (isolate 79) only on seedlings of *Q. cerris*. All *Q. pubescens* seedlings treated with *T. viride* manifested pathological symptoms subsequent to proliferation of *D. corticola* (isolate 79). These observations indicate that the interactions between endophytes in planta and *D. corticola* (isolate 79) are complex and merit further study.

Keywords Biocontrol agents · Culture filtrates · Dual culture · *Fusarium tricinctum* · *Quercus cerris* and *Q. pubescens* seedlings · *Trichoderma viride*

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Introduction

Since the early 1980s, numerous *Quercus* species, including Turkey oak, *Q. cerris* and downy oak, *Q. pubescens* stands in the Mediterranean area have been affected by frequent and widespread dieback of trees. On the basis of the observed symptomatology and subsequent related etiological investigations this phenomenon has been referred to as 'oak decline' (Anselmi, Mazzaglia, & Vannini 2000; Delatour, 1983; Sicoli, de Gioia, Luisi, & Lerario, 1998). Several distinguishing factors based on origin (abiotic, biotic, anthropic) or role (predisposing, inciting, contributing) have

been implicated in oak decline (Belisario, Motta, & Scortichini, 1990; Houston, 1992). Biotic factors include the endophytic community associated with oak trees consisting of numerous saprophytic fungi belonging to the genera *Acremonium*, *Cytospora*, *Epicoccum*, *Fusarium, Alternaria, Sclerotinia* and *Trichoderma* (Anselmi et al., 2002; Ragazzi, Morricca, Vagniluca, & Dellavalle 1996) and pathogens such as *Diplodia corticola*, *Apiognomonia quercina* and *Phomopsis quercina* (Thomas, Blank & Hartmann, 2002). *Diplodia corticola* causes dieback and canker disease of the apical twigs and branches of *Quercus* spp. that are subjected to stress, mainly in the western mediterranean area; in the context of oak decline it must be considered a contributing factor to mortality (Franceschini, Corda, Maddau, & Marras 1999; Luisi, Lerario, & Bianco 1996; Ragazzi et al., 1996). The symptoms caused by *D. corticola* are discoloration or yellowing of leaves, dieback of branches in the upper crown and progressive necrosis of bark, cambium and epicormic shoots. The symptoms do not necessarily occur simultaneously and may vary in degree of expression. In the progressive proliferation of *D. corticola* part of the crown or whole tree dies. Many epidemiological and pathogenic aspects of its relation with the host still need to be clarified, in addition to which little information is available on the biological control of this fungus (Franceschini, Linaldeddu, & Marras 2005; Maddau, Linaldeddu, & Franceschini, 2005). The use of fungicides and, more generally, of chemicals in forestry has mostly been restricted to nurseries and arboriculture stands due to environmental safety reasons more than to technical and economical constraints. Because large-scale application of such chemicals is neither desirable nor feasible, there had been a move to adopt biological control strategies capable of ensuring the protection of forest ecosystems (Covassi, 1996; Nicolotti & Gonthier, 2005). The aim of this study was to assess the antagonistic activity of *Trichoderma viride*, *Epicoccum nigrum*, *Fusarium tricinctum*, *Alternaria alternata*, *Sclerotinia sclerotiorum* species and one *Cytospora* (teleomorph: *Valsa* sp.) genus toward *D. corticola* (isolate 79). Experiments were performed both in vitro and in planta.

Materials and methods

Fungal isolates

Diplodia corticola (isolate 79) used in this study was recovered from branches of *Q. cerris* in Difesa Grande, Apulia. One isolate each of *T. viride*, *E. nigrum*, *F. tricinctum*, *A. alternata*, *S. sclerotiorum* and *Cytospora* (teleomorph: *Valsa* sp.) was taken from leaves, bark and buds of *Q. cerris* and *Q. pubescens* of four oak woods in southern Italy showing symptoms of oak decline: Difesa Grande (Apulia), Brindisi di Montagna (Basilicata), Dragoni (Campania) and San Donato di Ninea (Calabria) (R. Ubaldo, Department of Biology and Plant Pathology, University of Bari, Italy, pers. comm.) and evaluated for antagonism towards the pathogen *D. corticola* (isolate 79). The fungi were identified by Dr. A.W.A.M. de Cock from the Centraalbureau voor Schimmelcultures (CBS), The Netherlands, using morphological and molecular methods.

Antagonism tests in vitro

Growth of D. corticola and antagonistic fungal mycelium in dual culture

Petri dishes (9 cm) containing 20 ml of sterile PDA were inoculated with a 0.5 cm sterile plug of a 3 day-old pure culture of potential antagonists *T. viride*, *E. nigrum, F. tricinctum*, *A. alternata*, *S. sclerotiorum*, *Cytospora* sp. and the pathogen *D. corticola* (isolate 79). The distance between the pathogen and antagonist was 4 cm. Each combination of pathogen/antagonist was repeated 15 times, and as negative controls 15 Petri dishes were inoculated with *D. corticola* (isolate 79) and a water agar plug. All Petri dishes were incubated at 25°C in the dark and randomly distributed. The experiment was replicated three times. Radial growth was recorded by measuring colony diameter at 1-day intervals for the time required to reach the margin of the dish in controls. The Antagonism Index (AI) was assessed according to the following formula: $AI = (RM - rm)/RM \times 100$, where rm = ray of the colony towards the antagonist and RM = average of the three rays of the colony in the other directions. The

significance of the main effects and interaction effects of the antagonistic fungi was determined by analysis of variance (ANOVA; SAS Institute Inc. Cary, North Carolina). The antagonistic effects were compared using Duncan's multiple range test ($P \leq 0.05$).

Interactions between D. corticola and antagonistic fungi in dual culture

The antagonistic ability of the selected endophytic fungi towards *D. corticola* (isolate 79) was tested using the dual-culture method described by Badalyan, Innocenti, & Garibyan, (2002, 2004). A plug (0.5 cm diam.) of *D. corticola* (isolate 79) and an antagonistic fungus was cut from the margin of a 3 day-old culture and placed respectively on opposite sides (4 cm from the margin) of Petri dishes containing PDA. Each combination of pathogen/antagonist was repeated 15 times and plates were randomly placed in the dark and incubated at 25°C for 30 days. As negative controls 15 Petri dishes were inoculated with *D. corticola* (isolate 79) and a water agar plug. Interactions were examined daily. Antagonism towards *D. corticola* (isolate 79) was scored using the Badalyan rating scale (2002) according to 3 types (A, B and C) and 4 subtypes (CA1, CA2, CB1 and CB2), where: A = deadlock with mycelial contact, B = deadlock at a distance, C = replacement, overgrowth without initial deadlock; CA1 and CA2 = partial and complete replacement after initial deadlock with mycelial contact, CB1 and CB2 = partial and complete replacement after initial deadlock at a distance. The following scores were assigned to each type or subtype of reaction: $A = 1.0$; $B = 2.0$; $C = 3.0$; CA1 = 3.5; CB1 = 4.0; CA2 = 4.5; and CB2 = 5.0. The AI was calculated for each species using the formula: $AI = \Sigma N \times I$, where N = number (frequency) of each type or subtype of reaction and I = corresponding score.

Growth of D. corticola in the presence of antagonistic culture filtrates

Monax flasks (100 ml) containing 40 ml of 2% sterile malt extract (ME) were inoculated with two 12 mm disks antagonistic fungus cut from the margin of a 3 day-old culture. The flasks were placed in a Gallenkamp orbital incubator (150 rpm) at 25°C for 10 days. The liquid cultures were filtered and sterilized through Stericup and Steritop (millipore) to remove hyphal fragments and conidia and to collect antagonist metabolites. To test the antagonistic activity, 10 ml of sterile PDA with a pH of 6.5 was poured into Petri dishes (Dennis & Webster, 1971a, b). The pH was adjusted before autoclaving with sodium hydroxide. Before solidification, 5 ml of antagonist culture filtrate was added. After solidification, a 0.5 cm disk cut from the margin of 3 day-old cultures of *D. corticola* was placed on the centre of each plate. Control plates were prepared with only PDA. Each combination of pathogen/antagonist culture filtrate and control was repeated 15 times. All plates were incubated at 25°C in the dark and randomly distributed. Radial growth was recorded by measuring mean colony diameter at 1-day intervals for the time required to reach the margin of the dish in controls. The significance of the main effects and interaction effects of the antagonistic fungi was determined by ANOVA (SAS Institute Inc. Cary, North Carolina). The antagonistic effects were compared using Duncan's multiple range test ($P \leq 0.05$).

Pathogenicity tests of antagonistic fungi in planta

An assay to test the pathogenicity of antagonistic fungi was carried out by inoculating stems of 2 year-old *Q. cerris* and *Q. pubescens* seedlings that did not show any foliar symptoms. The artificial cuticular wounds cut in the stem of the seedlings were inoculated with a 0.5 cm diam. mycelium plug of a 3 day-old pure culture of an antagonistic fungus. A control test was performed using inoculum consisting of only agar under the same experimental conditions. After inoculation all wounds were covered with parafilm. Thirty seedlings of each oak species were used for each antagonistic fungus. The test was replicated twice and conducted in controlled conditions at 25°C and with 90–100% humidity under natural light. After a 10-day

inoculation period disease severity (DS) was rated on a scale of 0–3, where: 0 = no foliar symptoms; 1 = medium infection (50% of leaves with necrosis); 2 = severe infection (>50% of leaves with necrosis); and 3 = dead plant (Bianco, Mannerucci, Coricello, & Luisi 2002).

Antagonism tests between *D. corticola* and antagonistic fungi in planta

The antagonistic fungi were inoculated on 2 year-old *Q. cerris* and *Q. pubescens* seedlings without foliar symptoms. Two series of inoculations were carried out through artificial cuticular wounds cut in the stems. After inoculation all wounds were covered with parafilm. In the first, the distance between the two points of inoculation of the antagonists was 12 cm, whereas in the second series the distance was shortened to 6 cm. *Diplodia corticola* (isolate 79) was inoculated 10 days after the antagonist and placed equidistant at 6 and 3 cm in the first and second inoculations, respectively, between the two points of antagonist inoculation. For each combination of antagonistic fungi/pathogen and sterile water agar/pathogen (control), 30 *Q. cerris* and 30 *Q. pubescens* seedlings were inoculated. Tests were conducted in a randomized block in controlled conditions at 25°C and 90–100% humidity under natural light. To confirm results the tests were repeated twice. The parameter observed was crown dieback. To assess DS a scale was used comprised of 4 classes: 0 = no crown symptoms; 1 = dieback of several crown branches; 2 = dieback of the outer crown portion; and 3 = dead plant. Symptoms were observed 10 days after inoculation of the pathogen; these observations were repeated at 10-day intervals for 2 months. Subsequently, antagonistic fungi and *D. corticola* were isolated from *Q. cerris* and *Q. pubescens* seedlings. The significance of the main effects and interaction effects of the antagonistic fungi was determined by ANOVA (SAS Institute Inc. Cary, North Carolina). The antagonistic effects were compared using Duncan's multiple range test ($P \leq 0.05$).

Results

Growth of *D. corticola*, antagonistic fungal mycelium and competitive interactions in dual culture

Varying degrees of mycelial growth inhibition of *D. corticola* were observed with antagonistic fungal isolates (Table 1). *Trichoderma viride* had the maximum inhibitory effect on mycelial growth of *D. corticola* (isolate 79) with a reduction of 28.5% compared to control. The isolates of *Cytospora* sp., *E. nigrum, A. alternata* and *S. sclerotiorum* expressed significantly lower mycelial growth inhibition with reductions in the range of 5.7–19.2% ($P \leq 0.05$, compared to

Table 1 Effect of antagonistic fungal isolates on the growth of *Diplodia corticola* (isolate 79)

Fungal isolate	Mycelial growth of *D. corticola* (% reduction over control)*
Trichoderma viride	28.5 ^{A}
Cytospora sp.	19.2 ^{AB}
Epicoccum nigrum	16.7 ^{AB}
Alternaria alternata	9.5 ^{AB}
Sclerotinia sclerotiorum	5.7 ^{AB}
Fusarium tricinctum	4.2 ^{BC**}
Control†	0.0 ^{C**}

* Mean of three replicates

** Values followed by the same letter(s) are not statistically significantly different ($P \leq 0.05$) according to Duncan's multiple range test

† Growth of *D. corticola* (isolate 79) in the absence of antagonistic fungal isolates

Table 2 Antagonism Index (AI) and type/subtype of interaction between antagonistic fungal isolates and *Diplodia corticola* (isolate 79) scored on Badalyan's scale

Fungal isolate	AI	Type/Subtype
Trichoderma viride	22.5	CA2
Fusarium tricinctum	20.0	CB1
Cytospora sp.	5.0	A
Epicoccum nigrum	5.0	A
Alternaria alternata	5.0	A
Sclerotinia sclerotiorum	5.0	A

A = deadlock with mycelial contact; CB1 = partial replacement after initial deadlock at a distance; CA2 = complete replacement after initial deadlock with mycelial contact

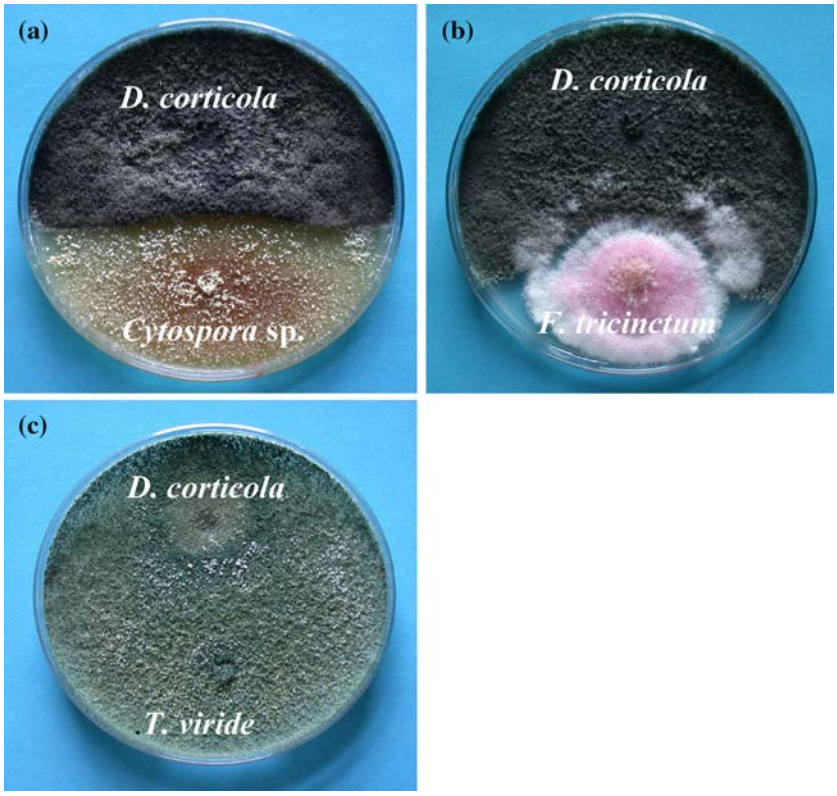

Fig. 1 Competitive interactions between *Cytospora* sp., *Trichoderma viride*, *Fusarium tricinctum* and *Diplodia corticola* (isolate 79) on PDA at 25°C 30 days after inoculation. (**a**) deadlock with mycelial contact; (**b**) partial replacement after initial deadlock at a distance; (**c**) complete replacement after initial deadlock with mycelial contact

controls and other antagonists). *Fusarium tricinctum* was ineffective in reducing radial growth of *D. corticola* (isolate 79) (4.2%), and the results were not statistically different from control. The interactions between antagonistic fungi and *D. corticola* (isolate 79) in dual cultures on PDA are shown in Table 2. One type and two subtypes of competitive interactions were observed: A, CA2 and CB1 (Fig. 1). For *F. tricinctum*, initial deadlock was observed 4 days after inoculation followed by partial replacement on *D. corticola* (isolate 79). On the basis of AI values, antagonistic fungi were divided into two groups: active (*T. viride* and *F. tricinctum*) and weakly active (*A. alternata*, *S. sclerotiorum, E. nigrum*, and *Cytospora* sp.).

Growth of *D. corticola* in the presence of antagonistic culture filtrates

The activity of antagonistic culture filtrates on the growth of *D. corticola* (isolate 79) was low (Table 3). *Fusarium tricinctum* culture filtrates inhibited the growth of *D. corticola* (isolate 79) by 16.6%; in the culture filtrates of *A. alternata*, *Cytospora* sp., *S. sclerotiorum* and *E. nigrum* the activity was largely similar (1.5–6.4%). In contrast, *T. viride* culture filtrates did not inhibit the

Table 3 Activity of antagonistic culture filtrates obtained from 2% sterile ME on the growth of *Diplodia corticola* (isolate 79)

Antagonistic culture filtrate	Mycelial growth of *D. corticola* (% reduction over control)*
Fusarium tricinctum	16.6 ^{A}
Alternaria alternata	6.4 ^{B}
Cytospora sp.	4.3 ^{B}
Sclerotinia sclerotiorum	3.5 ^{B}
Epicoccum nigrum	1.5 ^{BC**}
Trichoderma viride	0.0 ^{C**}
Control†	0.0 ^{C**}

* Mean of three replicates

** Values followed by the same letter(s) are not statistically significantly different ($P \leq 0.05$) according to Duncan's multiple range test

† Growth of *D. corticola* (isolate 79) in the absence of antagonistic culture filtrates

growth of *D. corticola* isolates. Statistical analysis showed that growth of *D. corticola* (isolate 79) was significantly reduced only by *F. tricinctum*, *A. alternata*, *Cytospora* sp. and *S. sclerotiorum*.

Pathogenicity tests of antagonistic fungi and antagonism tests in planta

The pathogenicity tests of antagonistic fungi conducted on *Q. cerris* and *Q. pubescens* seedlings did not show any symptoms and the antagonistic fungi were re-isolated from each inoculated seedling. Antagonism tests in planta showed significant differences compared to the two treatment groups (3 and 6 cm); all control seedlings inoculated with *D. corticola* (isolate 79) were dead at both distances of inoculation (Table 4). In the 3 cm treatment group, no *Q. cerris* seedlings treated with *F. tricinctum* manifested disease symptoms subsequent to proliferation of *D. corticola* (isolate 79). All other antagonistic fungi, except for *Cytospora* sp., reduced DS compared with controls. At the same distance on *Q. pubescens* seedlings only, *F. tricinctum* and *A. alternata* significantly reduced infection of the pathogen, however, inoculation with *T. viride* had no effect on *D. corticola* (isolate 79). In the 6 cm treatment group, the DS score was significantly high compared to the 3 cm treatment group. On *Q. cerris* seedlings the presence of *F. tricinctum* and *A. alternata* reduced mortality caused by *D. corticola* (isolate 79), while the other antagonists exhibited weak activity towards the pathogen. At the same distance on *Q. pubescens* seedlings of the six antagonistic fungi, only *E. nigrum* and *T. viride* were not effective in reducing DS. At 3 cm, only *F. tricinctum* and *A. alternata* were successfully re-isolated from *Q. cerris* and *Q. pubescens* seedlings, whereas *T. viride*, *E. nigrum* and *S. sclerotiorum* were re-isolated from *Q. cerris* seedlings only. In the 6 cm treatment group, no inoculated antagonistic fungi could be re-isolated at the end of the experiment.

Discussion

Identification and selection of effective antagonistic organisms is the first and foremost step in biological control (Kamalakannan et al., 2004). Our findings show that *F. tricinctum* and *T. viride*

Table 4 Disease severity of 30 *Quercus cerris* and 30 *Q. pubescens* seedlings 60 days after inoculation with *Diplodia corticola* (isolate 79) between the points of antagonist inoculation

Distance of inoculation	Antagonistic fungi	Disease severity	
		Q. cerris	*Q. pubescens*
3 cm	Control**	3.0^{A*}	3.0^{A*}
	Cytospora sp.	$2.4^{A–E*}$	$2.7^{A–C*}$
	Trichoderma viride	1.1^{IJ}	3.0^{A*}
	Alternaria alternata	0.9^{JK}	0.3^{KL}
	Epicoccum nigrum	0.8^{JK}	$2.1^{C–G}$
	Sclerotinia sclerotiorum	0.8^{JK}	$2.1^{C–G}$
	Fusarium tricinctum	0.0^{L}	0.8^{JK}
6 cm	Control**	3.0^{A*}	3.0^{A*}
	S. sclerotiorum	2.9^{AB*}	$1.8^{E–H}$
	E. nigrum	$2.7^{A–D*}$	$2.5^{A–D*}$
	T. viride	$2.4^{A–E*}$	2.8^{AB*}
	Cytospora sp.	$2.3^{B–F}$	$1.6^{G–I}$
	A. alternata	$1.7^{F–I}$	$2.0^{D–G}$
	F. tricinctum	$1.2^{H–J}$	$2.0^{D–G}$

* Values followed by the same letter(s) are not statistically significantly different ($P \leq 0.05$) according to Duncan's multiple range test

** *Q. cerris* and *Q. pubescens* seedlings inoculated with *D. corticola* (isolate 79) and sterile water agar

Disease severity scale: 0 = no crown symptoms; 1 = dieback of several crown branches; 2 = dieback of the outer crown portion; and 3 = dead plant

demonstrated significant antagonistic activity towards *D. corticola* (isolate 79) in in vitro dual cultures. However, the results observed were inconsistent. In the first dual-culture method, based on the growth of *D. corticola* (isolate 79) in the presence of antagonistic fungal mycelium, *T. viride* produced the highest inhibitory effect on mycelial growth of *D. corticola* (isolate 79). In the second method, based on competitive hyphal interactions both *T. viride* and *F. tricinctum* scored highly on the Badalyan Antagonism Index. These results may be explained upon consideration of the experimental conditions. In the first dual-culture method, which assessed the growth of fungal mycelium, the pathogen/antagonist interaction was obtained by measuring the radial growth of the two colonies. The most important parameter determining antagonistic activity was speed of colony growth. Specifically, the growth rate of *T. viride* was equal to that of *D. corticola* (isolate 79) demonstrating effective antagonism in contrast with *F. tricinctum* which was characterized by a slower growth rate. In the second dual-culture method, high containment levels of *T. viride* and *F. tricinctum* isolates were shown despite the varying growth rates. Until now, no antagonistic activity for *F. tricinctum* has been cited in the literature. Logrieco, Mulè, & Bottalico (1994) reported antagonistic activity for *Fusarium acuminatum*. These authors demonstrated that 10 of 19 *F. acuminatum* isolates from different geographical origins and sources showed in vitro antagonistic activity (inhibition at a distance) toward mycelial growth of *Fusarium moniliforme*. On the other hand, several studies (Bell, Wells, & Markham 1982; Elad & Kapat 1999; Howell, 2003; McSpadden Gardner & Fravel 2002; Weindling, 1934; Yedidia, Benhamou, & Chet 1999) have demonstrated that *Trichoderma* species are highly effective biocontrol agents of plant pathogens (e.g., *Sclerotium rolfsii*, *Rhizoctonia solani*, *Ceratobasidium cornigerum*, and *Phytophthora parasitica* f. *nicotianae*.

Our study also showed that inhibition of the growth of *D. corticola* (isolate 79) in vitro by antagonistic culture filtrates was maximum for *F. tricinctum*, moderate for *E. nigrum*, *A. alternata*, *S. sclerotiorum* and *Cytospora* sp. and non-existent for *T. viride*. The metabolites involved in biological control are strongly influenced by the substrate on which the fungus is grown, by temperature and by pH (Dennis & Webster, 1971a, b). In this study we used a defined substrate, 2% sterile ME with a pH of 6.5, which might have negatively influenced the biocontrol activity of *T. viride*. Further studies are necessary to evaluate the metabolites produced by fungi tested and their role in antagonistic activity toward *D. corticola* (Logrieco, Mulè, Moretti, & Bottalico, 2002; Schulz et al., 1995; Schulz, Guske, Dammann, & Boyle 1998; Schulz, Römmert, Dammann, Aust, & Strack 1999). Burmeister and Plattner (1987) demonstrated that *F. tricinctum* produces enniatins, which are a group of cyclohexadepsipeptide antibiotics produced by various *Fusarium* species, including *F. acuminatum* (Deol, Ridley, & Singh 1978; Logrieco, Altomare, Moretti, & Bottalico, 1992, 1994) and *F. oxysporum* f.sp. *lycopersici* (Gäumann, 1951). Previous studies (Dennis & Webster 1971a, b; Ghisalberti and Sivasithamparam, 1991; Harman et al., 1996) have demonstrated that *T. viride* produces volatile and non-volatile antibiotics that inhibit the growth of other pathogenic (e.g., *R. solani*, *Heterobasidion annosum* and *F. oxysporum*) and non-pathogenic fungi (e.g., *Mucor hiemalis* and *Pyronema domesticum*). It would be opportune to verify in future studies whether or not *F. tricinctum* and *T. viride* isolates produce antibiotics that may inhibit the growth of *D. corticola* (isolate 79).

Of the two inoculation distances used in planta, consistent and significant antifungal activity was observed when antagonist and pathogen were placed 3 cm apart. Inoculation of *F. tricinctum* significantly reduced the growth of *D. corticola* (isolate 79) as confirmed by in vitro tests. In particular the presence of this fungus on *Q. cerris* seedlings prevented the proliferation of *D. corticola* (isolate 79); none of the plants showed any foliar symptoms. *Alternaria alternata* also demonstrated considerable antagonism toward *D. corticola* (isolate 79) contrasting with the results obtained *in vitro*. *Trichoderma viride*, *E. nigrum* and *S. sclerotiorum* reduced the activity of *D. corticola* (isolate 79) only on *Q. cerris* sedlings. The discreet antagonistic activity of *T. viride*, but not *E. nigrum* and

S. sclerotiorum, on *Q. cerris* seedlings confirmed in vitro results. These results demonstrated that significant complex interactions occur between distances of inoculation, host species and antagonistic fungi. The interactions between angiosperms and fungal endophytes that grow asymptomatically within the healthy aerial tissues of plants are little known (Petrini, 1991; Saikkonen, Helander, & Rousi, 2003). Among the best-studied endophytes are the intercellular symbionts of the ascomycotan family *Clavicipitaceae*, which grow within the above-ground tissues of many cool season grasses in temperate zones (e.g., *Poaceae* family) (Arnold et al., 2003; Clay and Schardl, 2002; Saikkonen, Faeth, Helander, & Sullivan, 1998; Schardl, Leuchtmann, Spiering, 2004). Moreover, recent studies demonstrated that integrating the biocontrol agents *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* with soil caused an early and increased activity of defence-related enzymes leading to the synthesis of defence chemicals in plants, such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and total phenol (Benhamon, Gagne, Quere, & Dehbi, 2000; Chen, Belanger, Benhamou, & Paulitz, 2000; Daayf, Bel-Rhlid, & Belange, 1997; Howell, Hanson, Stipanovic, & Puckhaber, 2000; Kamalakannan et al., 2004; M'Piga, Belanger, Paulitz, & Benhamou, 1997; Ramamoorthy & Samyappan, 2001). We hypothesize that in *Q. pubescens* seedlings the presence of *T. viride*, *E. nigrum* and *S. sclerotiorum* failed to increase the level of enzymes involved in the defence reaction toward *D. corticola* (isolate 79). Further studies are necessary to test both this hypothesis and also to evaluate the ability of *T. viride* in *Q. cerris* seedlings, and *F. tricinctum* and *A. alternata* in both in *Q. pubescens* and *Q. cerris* seedlings to increase the activity of defence enzymes. Moreover, we would like to conduct further studies using additional *F. tricinctum* isolates from different geographic origins and sources to better understand its potential role as an agent of biological control.

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