Saprophytic, leaf endophytes, and orchid mycorrhizal fungi as biocontrol agents of the phytopathogenic fungi *Rhizoctonia solani and Fusarium circinatum*

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Abstract

Pathogenic fungi cause approximately 80 % of crop diseases worldwide. Among these, *Rhizoctonia solani* and *Fusarium circinatum* have a significant economic impact, affecting major crops and *Pinus* species. Traditional fungicides pose risks to health and the environment when applied improperly, therefore, sustainable approaches such as biocontrol are necessary. This work explores the biocontrol potential of saprophytic, endophytic, and orchid mycorrhizal fungi (OMF), including *Coriolopsis rigida*, *Pleurotus ostreatus* and *Epicoccum nigrum,* on the pathogenic fungi *Fusarium circinatum* and *Rhizoctonia solani*. Additionally, the volatile organic compound (VOC) profiles of biocontrolling fungi and pathogens during the biocontrol process were also examined. All fungi exhibited an *in vitro* biocontrol effect on both pathogenic fungi, similar to that of the known biocontroller fungi *Trichoderma harzianum*, except for the OMF isolated from the orchid *Chloraea lamellata*. A representative amount of the VOCs corresponded to terpenes, present in most individual and dual cultures. Dual cultures presented specific volatile, which may be related to the inhibitory effect. This research highlights the biotechnological potential of saprophytic fungi and OMFs in the control of agriculturally important pathogenic fungi. Moreover, the preliminary study of VOCs provided insight into one of the possible mechanisms underlying the biocontrol process, but further studies are needed.

Keywords: Antagonistic microorganisms. Biological control agents. Soil-borne Pathogenic Fungi. Volatile Organic Compounds. Biotrophic Fungi.

Introduction

Pathogenic fungi are the cause of close to 80 % of crop diseases worldwide (Abbas *et al.*, 2021). They are responsible for 10-20 % of pre and post-harvest crop losses (Steinberg, Gurr, 2020), caused by damage due to rot and seed and plant overall deterioration (Lamichhane *et al.*, 2017). *Rhizoctonia solani* Kühn and *Fusarium circinatum* Nirenberg & O'Donnell are among the most common pathogenic fungi.

R. solani is a cosmopolitan species that causes root rot and damping off in several crops, with serious negative economic impact in agriculture (Abbas *et al.*, 2022). On the other hand, *F. circinatum* causes pine pith canker, which affects key forest conifer species of the genera *Pinus* spp., and *Pseudotsuga* spp. (Aloi *et al.*, 2021). Its control, based mainly on the use of agrochemicals, is no longer a viable strategy since the European Union has established a directive of standards to maintain a sustainable

use of these products to reduce negative effects on human health and the environment (European Parliament & Council, 2019). This strategy also reduces the risk of developing greater resistance to these compounds (Ons *et al.*, 2020). *R. solani* is treated with a wide range of fungicides, such as benzimidazole, external quinone inhibitors, succinate dehydrogenase inhibitors, and some multisite contact fungicides (Lee *et al.*, 2023). However, these have also been shown to have negative effects on the environment and have generated resistance in some strains (Ons *et al.*, 2020). Additionally, some countries continue to use methyl bromide or bromomethane to control *R. solani* (Abbas *et al.*, 2022), a compound toxic to humans (Koutros *et al.*, 2016) that also damages the ozone layer and has already been restricted in developed countries (U.S. Environmental Protection Agency, 2025). *F. circinatum* is treated with commercial formulations based on phosphate that increase *Pinus* resistance to the fungal infection (Cerqueira *et al.*, 2017). Additionally, phosphites or their derivatives can cause a mild foliar phytotoxic effect on some conifers (Scott *et al.*, 2016).

It has been proposed that biological control of pathogenic fungi is an effective method that also has low impacts on human health and the environment (Steinberg, Gurr, 2020). The biocontrol of *R. solani* has proven successful, using the commercially available fungi *T. harzianum* Rifai (Rajani *et al.*, 2021). There is also evidence of biocontrol of pathogenic *Fusarium* species. For example, Wang *et al.* (2021) combined the edible saprotrophic fungus *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm. with the antagonistic bacterium *Bacillus amyloliquefaciens* (Priest *et al.*, 1987) as a dual biocontrol strategy against *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *cucumerinum* in infected cucumber plants. *Epicoccum nigrum* Link, a leaf endophytic fungus, also had biocontroller potential against *Fusarium graminearum*, anamorph of *Gibberella zeae* (Schwein.) Petch (Li *et al.*, 2022), and *Botrytis cinerea* Pers. (Sanhueza *et al.*, 2023).

Although saprophytic fungi have shown to have biocontrol potential over phytopathogenic fungi (Yao *et al.*, 2023), some such as *T. harzianum* also improve plant growth and nutrition (Yao *et al.*, 2023). *C. rigida* (syn. *Coriolopsis floccosa* (Bull.) Murrill), a saprophytic fungus, has been shown to have a potential bioremediation effect (Pereira *et al.*, 2025), increasing plant growth in combination with mycorrhizal fungi and *T. harzianum* (Pereira *et al.*, 2025). Its biocontroller potential, however, has not been evaluated. To date, no evidence has been reported on the biocontrol of *F. circinatum* using other fungal species.

Some isolated strains of non-pathogenic Rhizoctonia-like fungi have shown to have a biocontroller effect on *R. solani* (Pereira *et al.*, 2021). These biocontroller fungi include orchid mycorrhizal fungi (OMF, or simply orchid fungi) isolated from native Chilean orchids (Pereira *et al.*, 2021). The OMF are naturally found in symbiosis with orchids (Pereira *et al.*, 2018) and participate in seed germination (Chamara *et al.*, 2024) and in the growth and development of embryos and adult plants (Pereira *et al.*, 2020). In Chile, most studies mainly focus on describing OMF diversity and presence in native orchid species (Atala *et al.*, 2015; Pereira *et al.*, 2021). These OMF could hold an interesting biocontroller potential of pathogenic fungi (Pereira *et al.*, 2021). This is especially relevant, considering that the currently known OMF diversity in Chile greatly exceeds the experimentally tested species (Herrera *et al*., 2019, 2022; Pereira *et al.*, 2021).

The mechanisms behind the biocontroller effect of fungal species on pathogenic fungi are not fully understood. However, some studies associated with the *Trichoderma* genus propose parasitism, competition for resources, antibiosis, induction of plant resistance and/or defenses,

preventing or hindering the proliferation of the pathogen (Rajani *et al.*, 2021), producing growth regulators, cell wall-degrading enzymes, and secondary metabolites as possible mechanisms (Abbas *et al.*, 2022; Guo *et al.*, 2022). Among the secondary compounds that have been identified in *T. harzianum* are polyketides, alkaloids, and terpenoids, the latter being one of the most diverse groups of known volatile organic compounds (Guo *et al.*, 2022). In *T. harzianum* it has been proposed that organic volatile compounds (VOCs) could be responsible for their antifungal effect (Rajani *et al.*, 2021; Rubio *et al.*, 2023).

In this study, it was hypothesized that the saprophytes *C. rigida* and *P. ostreatus*, the leaf endophyte *E. nigrum,* and two OMF isolated from native Chilean orchids will exhibit biocontrol activity against the phytopathogenic fungi *R. solani* and *F. circinatum*. Furthermore, it is hypothesized that when cultivated together, these fungi (whether saprophytic, endophytic, or orchid mycorrhizal) will alter their VOC profiles compared to monocultures, which could contribute to their antagonistic effects. Therefore, the objective is to determine the biocontrol potential of the aforementioned fungi against the phytopathogenic fungi *R. solani* and *F. circinatum*, and how these fungi might alter their VOC profile in dual cultures.

Material and methods

Microorganisms

Strains of *C. rigida, P. ostreatus, T. harzianum,* and *E. nigrum* were obtained from the fungal culture collection of the Bioremediation Laboratory, Faculty of Agricultural and Environmental Sciences, Universidad de la Frontera, Temuco, Chile. *Ceratobasidium* D.P. Rogers strains were isolated from the orchids *C. lamellata* Lindl. and *C. virescens* (Willd.) Lindl. (Pereira *et al.*, 2018; 2020). Strains of the phytopathogenic fungi *R. solani* and *F. circinatum* were provided by the Fungal Biotechnology Laboratory, Department of Plant Sciences and Technology, Los Angeles Campus, University of Concepción, Los Angeles, Chile. Isolation of *R. solani* was done *in vitro* in the laboratory directly from an infected potato (*Solanum tuberosum* L.) (Pereira *et al.*, 2021). Table 1 summarizes the

Table 1. Summary on the origin, identity and abbreviation of the fungal strains to be used in the study.

Type	Identity	Code	Isolation source	GeneBank Accession	Reference
Pathogen	*Rhizoctonia solani*	Rs	Infected potato sclerotia	-	Pereira *et al.*, 2021
Pathogen	*Fusarium circinatum*	Fc	Previous isolate	-	-
Positive control	*Trichoderma harzianum*	Th	Previous isolate	HG940501	Pereira *et al.*, 2021
Biocontroller	*Ceratobasidium* sp.	Cer-1	*Chloraea lamellata* roots	KT003600	Pereira *et al.*, 2018
Biocontroller	*Ceratobasidium* sp.	Cer-2	*Chloraea virescens* roots	MN199626	Pereira *et al.*, 2020
Biocontroller	*Coriolopsis rigida*	Cr	Previous isolate	-	Pereira *et al.*, 2025
Biocontroller	*Epicoccum nigrum*	En	Previous isolate	OK000990	Sanhueza *et al.*, 2023
Biocontroller	*Pleurotus ostreatus*	Po	Previous isolate	-	-

Source: authors (2026).w

key information regarding each biocontrol agent evaluated in this study.

Dual growth and dual culture experiments

To evaluate the *in vitro* biocontrol capacity of *C. rigida*, *P. ostreatus*, *E. nigrum*, and *Ceratobasidium* sp. strains isolated from the orchids *C. lamellata* and *C. virescens*, the dual culture technique described by Pereira *et al.*, (2021) was employed. First, 5-mm agar discs were excised from plates containing actively growing fungal mycelia and placed in 9-cm Petri dishes containing Potato-Dextrose Agar (PDA). The cultures were incubated at 24°C ± 1°C in the dark for 20 days. After incubation, 5 mm diameter discs were taken from the edge of each fungal colony, and two fungal species were co-cultured on a new PDA plate, each disc placed 1 cm from opposite edges of the dish. One disc corresponded to a potential biocontrol strain, while the other corresponded to a pathogenic strain (*R. solani* or *F. circinatum*). *T. harzianum* was included as a positive control due to its well-documented biocontrol activity. In addition, monocultures of each biocontrol and pathogenic fungus were prepared (one fungus per plate) and used as growth controls. Each treatment consisted of four replicates (n=4). All plates were incubated at 24 °C ± 1 °C in the dark for 20 days. Radial growth (in mm) was measured at the end of the incubation period, and the percentage inhibition of relative growth (PIRG) was calculated by comparing the growth of the pathogen in dual culture with the growth in the individual culture (Pereira *et al.*, 2021).

Statistical analysis

For each response variable, a one-way ANOVA with six levels (five treatments and one control) was applied, with four independent replicates per group (n = 4; total N = 24). Assumptions of normality (Shapiro–Wilk test) were verified prior to analysis. When assumptions were met, one-way ANOVA was performed, followed by Dunnett's post hoc test to compare each treatment against the control. Additionally, Tukey's honestly significant difference (HSD) test was used for multiple comparisons among all groups when pairwise differences between treatments were evaluated. Statistical significance was considered at $p < 0.05$. Analyses were performed using GraphPad Prism 8.0.1, and data were presented as mean ± SD.

Volatile organic compound profile of pathogenic and biocontrol fungi

For the analysis of VOCs produced by biocontrolling fungi in the presence and absence of the pathogens *R. solani* and *F. circinatum*, part of the methodology used by researchers in previous works (Rajani *et al.*, 2021) was used, but with some modifications. The same growth conditions used for the dual culture described above were maintained.

The dual-culture and single-growth plates were opened, and a 100 μm SPME fiber coated with polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was directly exposed for 5 min to the headspace, i.e. the zone between the PDA medium and the lid, of the Petri dish. The adsorbed volatiles were desorbed directly in the injector port of a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Ultra, Shimadzu, Kyoto, Japan). The GCMS was equipped with a fused-silica capillary column coated with 0,25 μm 95 % polydimethylsiloxane-5 % polydiphenylsiloxane (SLB-5ms, Supelco). The oven temperature was set at 40 °C for 3 min and then increased to 270 °C at a rate of 10 °C/min and finally held for 10 min. Helium was used as carrier gas at 35 cm/s. The ion source temperature was 200 ºC and mass spectra were acquired at 70 eV in scanning mode (m/z 35–500). Three samples were analyzed for each treatment. The volatiles were tentatively identified by comparison of

their mass spectra and retention indices (RI) calculated using an *n*-alkane standard (C7–C33). The mass spectra and calculated RI values for the VOCs were compared with the spectra and RI values available in the NIST database (Wallace, 2025a,b). To recognize compounds emitted by the substrate, headspace sampling was also performed in Petri dishes filled with sterile, mold-free PDA medium.

Results and discussion

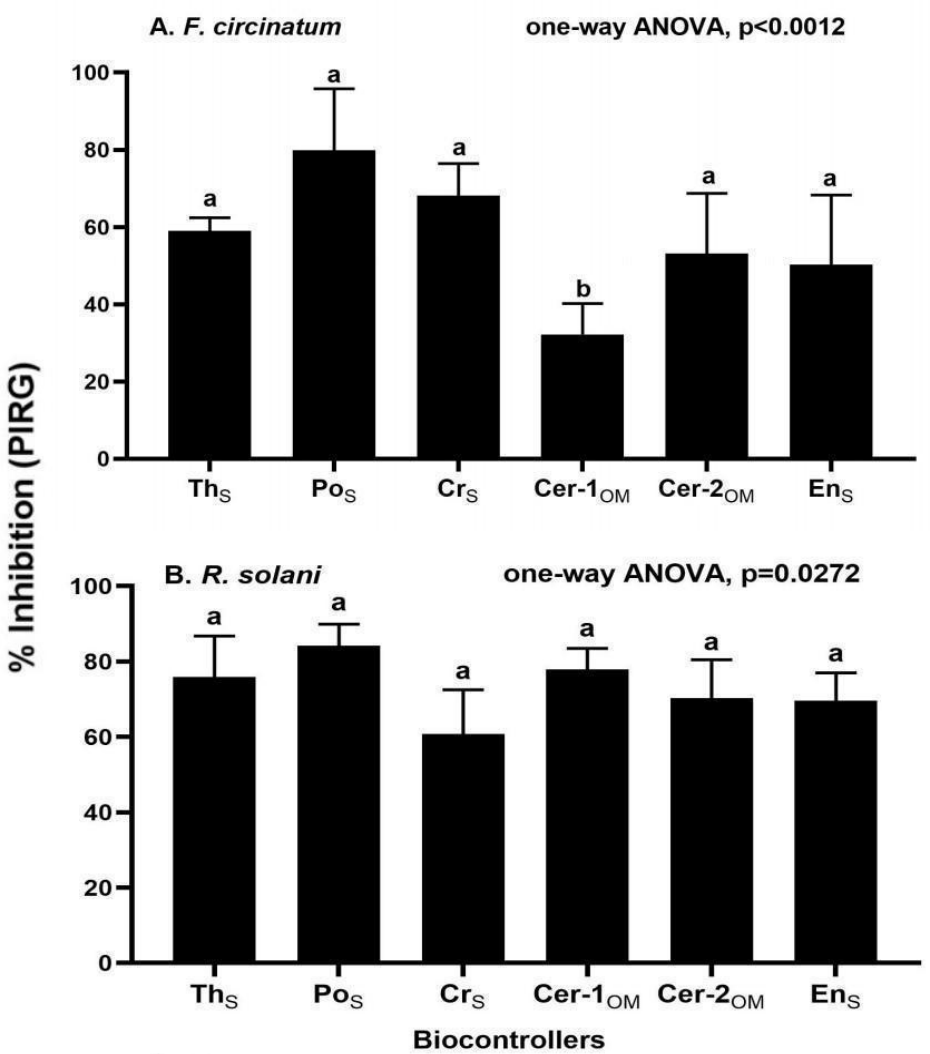
Biocontrol activity and growth inhibition of R. solani and F. circinatum

The saprophytic fungi *P. ostreatus*, *C. rigida*, the leaf endophytic fungus *E. nigrum*, and the Rhizoctonia-type OMF isolated from *C. lamellata* exhibited significant *in vitro* biocontrol activity against the phytopathogen *F. circinatum* (Figure 1A; Shapiro–Wilk test, W = 0.7647–0.9831, p = 0.0526–0.92; Tukey test $p<0.05$; Dunnett's test p=0.1179, p=0.7720, p=0.9426 and p=0.7845 with respect to *T. harzianum*, respectively). Their inhibition levels were comparable to that of the well-known biocontrol fungus *T. harzianum* (Figure 1A). Furthermore, among the tested fungi, *P. ostreatus* exhibited the highest inhibition against *F. circinatum,* while *Ceratobasidium* sp. isolated from *C. virescens,* reached a significantly lower biocontrol effect (Figure 1A, Tukey test $p<0.05$). The antagonistic effect of these strains can be qualitatively observed in the dual cultures shown in Figure 2A-2C. In the case of *R. solani*, the saprophytic fungi *P. ostreatus* and *C. rigida,* the leaf endophyte *E. nigrum*, the Rhizoctonia-type OMF from *C. lamellata,* and the *Ceratobasidium* sp. strain from *C. virescens* exhibited high *in vitro* biocontrol potential, with inhibition values statistically similar to that of *T. harzianum* (Figure 1B; Tukey test, $p>0.05$). Notably, *P. ostreatus* again showed the highest inhibition value against this pathogen (Figure 2B; Tukey test $p<0.05$). These effects were also evident in the corresponding dual cultures plates (Figure 2D-2F).

The observed biocontrol effect is consistent with previous studies showing that *P. ostreatus* exhibits *in vivo* biocontrol activity against *F. oxysporum* f. sp. *cucumerinum* (Wang *et al.*, 2021), and that *E. nigrum* demonstrates *in vitro* biocontrol efficacy against *F. graminearum* (Li *et al.*, 2022). Additionally, the data provide new insights, as there is no prior research on the biocontrol potential of *C. rigida* and OMFs against *F. circinatum*. The bioremediation and plant growth promoting effects reported for *C. rigida* (Pereira *et al.*, 2025) complement these findings and underscore the importance of further investigating the biotechnological potential of this species. *T. harzianum* has been widely used as a biocontrol agent against a broad range of phytopathogens, as well as bioremediator (Guo *et al.*, 2019). To the best of current knowledge, this is the first report of the *in vitro* biocontrol potential of *T. harzianum* against *F. circinatum*.

Regarding *R. solani*, the saprophytic fungi *P. ostreatus* and *C. rigida*, the leaf endophyte *E. nigrum*, and the two OMF strains studied exhibited *in vitro* biocontrol activity against this pathogen. The results for *Ceratobasidium* sp., isolated from the orchid *C. virescens*, are comparable with those obtained by Pereira *et al.* (2021), where a similar biocontrol effect was observed. Similarly, Yin *et al.* (2023) reported that the saprophytic fungus *Ceriporia lacerata* suppressed the growth of *R. solani* under *in vitro* conditions. Additionally, Muthu Narayanan *et al.* (2024) evaluated the antagonistic potential of 14 mangrove endophytic fungi (MEF) against *R. solani* using dual culture assays, with four of the MEF showing promising antagonism, with inhibition percentages ranging from 52 % to 64 %. This study contributes by identifying *E. nigrum, C. rigida*, and *P. ostreatus* as potential new biocontrol agents for *R. solani.* However, *in vitro* results remain preliminary, and further *in*

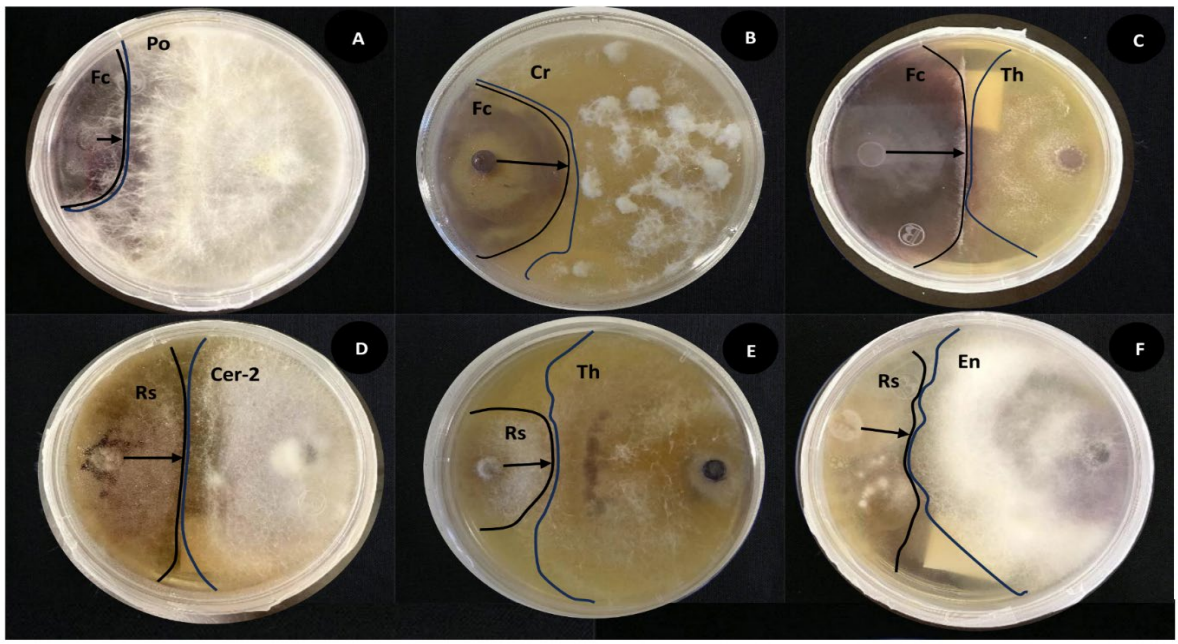
Figure 1. Percentage of inhibition of relative growth (PIRG) of different saprophytic fungi and orchid mycorrhizal fungi (OMF) species on the phytopathogens *F. circinatum* (A) and *R. solani* (B). The columns represent the mean of the inhibition values (PIRG) and the bars the standard deviation of these values. The different letters represent statistically significant differences with a $p<0.05$ between the PIRG value of the different biocontrol agents with respect to the control *T. harzianum*. The subscripts 'S' in the abbreviations correspond to saprophytic fungi, and the subscripts 'OM' to orchid mycorrhizal fungi. Th: *T. harzianum*; Po: *P. ostreatus;* Cr: *C. rigida;* Cer-1: *Ceratobasidium* sp. of *C. lamellata;* Cer-2: *Ceratobasidium* sp. of *C. virescens;* En: *E. nigrum*.



Source: authors (2026).

vivo biocontrol studies are necessary before these fungi can be considered viable biocontrol agents against *F. circinatum* and *R. solani*. Additionally, OMFs from native Chilean orchids also demonstrated *in vitro* biocontrol activity against both *R. solani* and *F. circinatum,* underscoring the importance of studying native fungal species for their biotechnological potential. These fungi may serve as biocontrol agents for pathogenic fungi and may have significant potential ecological relevance. This is particularly important as some fungal pathogens have developed resistance to traditional fungicides (Lee *et al.*, 2023), making biocontrol a sustainable and environmentally-friendly alternative (Tyagi *et al.*, 2024).

Figure 2. Representative sample of dual cultures of the pathogens *F. circinatum* against the biocontrollers *P. ostreatus* (A) and *C. rigida* (B) and *T. harzianum* (C), *R. solani* against the biocontrollers *Ceratobasidium* sp. of *C. virescens* (D), *T. harzianum* (E) and *E. nigrum* (F).



Source: authors (2026).

Volatile organic compound profile of pathogenic and biocontrol fungi

Analysis of the fungal cultures by SPME-GC/MS revealed that all samples, both from fungi growing individually (absence of pathogen) and from dual cultures (presence of pathogen), presented volatile organic compound (VOC) emissions (Tables 2 and 3). A total of 82 compounds, including alcohols, ketones, esters, lactones, aldehydes, alkanes, and terpenes, were detected (main compounds in Table 2 and 3). Terpenes were represented mainly by sesquiterpenes, however, some monoterpenes like limonene, β-pinene, and γ-terpinene were also identified. Comparison of the chromatograms of individual pathogen and biocontrol cultures with their respective dual cultures revealed the presence of volatiles in most dual cultures that were not present when fungi were cultured individually. For example, 3-methyl-2(5 H)-furanone was identified from a dual culture (and not in single culture) of *R. solani* against *P. ostreatus*, together with an unknown sesquiterpene and another unknown compound (Figure 3A). Another example is the dual culture of *F. circinatum* against *C. rigida*, where limonene and an unknown sesquiterpene were not present in the individual cultures (Figure 3B). The chromatograms of other species are shown in Figure 4.

The mechanisms associated with the biocontrol process of the studied fungi are still unknown. However, preliminary results of the present study suggest that VOCs could play a role in the biocontrol process. The groups of volatiles tentatively identified were mainly terpenes, but also included non-terpenoid alkanes, alcohols, and ketones. Terpenes were the most representative volatiles in the analyzed samples, since all biocontrol and the phytopathogen *F. circinatum* produced at least one terpene (Tables 2 and 3). Terpenes have been shown to be involved in biocontrol mechanisms. For example, Gualtieri *et al.* (2022) studied the

Table 2. Volatile compounds (VOCs) released by pathogenic and biocontrol fungi in individual cultures. (N°) VOCs are listed according to their increasing retention time. Their retention time (Rt), retention indices (Ri), and compound type are shown. (+) trace compound; (++) minor compound; (+++) major compound; (n.d.) not determined. Fc: *F. circinatum*; Rs: *R. solani*; Po: *P. ostreatus;* Cr: *C. rigida;* Cer-2: *Ceratobasidium* sp. of *C. virescens;* Th: *T. harzianum*; En: *E. nigrum*.

				Pathogens			**Biocontrollers**			
N°	Rt [min]	RI	Compound	Fc	Rs	Po	Cr	Cer-2	Th	En
1	4,85	n.d.	3-Methyl-1-butanol	+			+		+	
2	4,92	n.d.	2-Methyl-1-butanol		+	+	+			
3	9,79	983	β –Pinene					+		
4	10,52	1023	Alkane					+		+
5	11,21	1063	γ-Terpinene					+		

Source: authors (2026).

VOC profile of fungi of the genus *Trichoderma* spp. and highlighted the antibacterial, antiviral and antifungal activities of the terpene *p*-cymene of *Trichoderma longibrachiatum* and its relationship with the biocontrol process. Other research highlights β-caryophyllene produced by the endophytic fungus *Talaromyces wortmannii,* which induced plant resistance to the pathogen *Colletotrichum higginsianum* (Werner *et al.*, 2016). The volatiles 3-methyl-1-butanol and 2-methyl-1-butanol, tentatively identified in this study (Tables 2 and 3), have been shown in other studies to inhibit vegetative growth of the phytopathogenic fungus *Guignardia citricarpa* (Werner *et al.*, 2016) and could be related to the mechanisms that explain the biocontrol process.

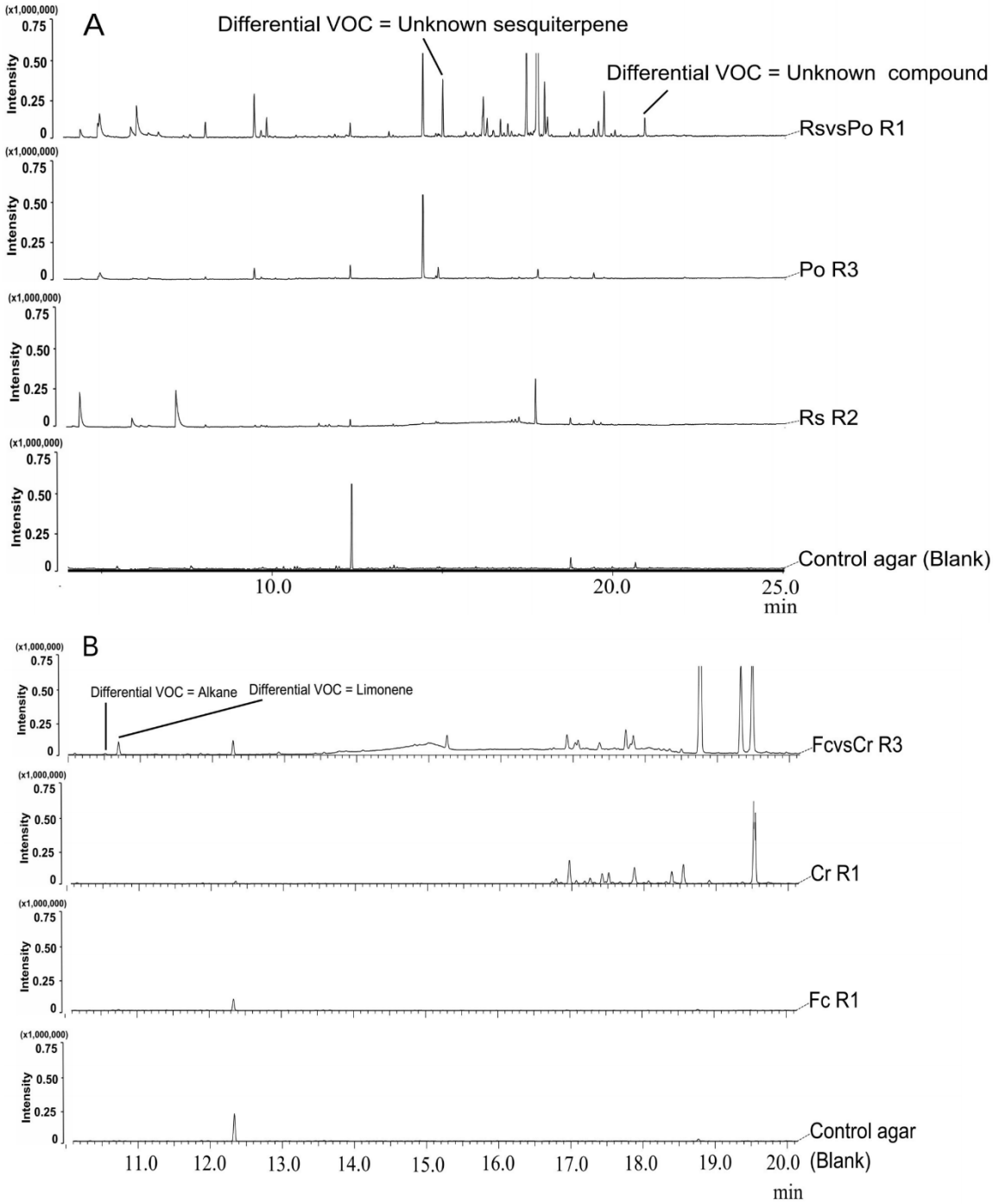
Specific volatiles from dual cultures were present in most dual cultures against both pathogens, as detailed in Figure 3 and Figure 4. However, only some of the VOCs were present in larger amount and in more than one of the samples from each dual culture. Of these distinctive compounds, only limonene and 3-methyl-2(5 H)-furanone could be tentatively identified. The production of different compounds in dual cultures could be related to the response of some of the biocontrol agents to the plant

Table 3. Volatile compounds (VOCs) released by pathogenic and biocontrol fungi in dual cultures. (N°) VOCs are listed according to their increasing retention time. Their retention time (Rt), retention indices (Ri), and compound type are shown. (+) trace compound; (++) minor compound; (+++) major compound; (n.d.) not determined. Po: *P. ostreatus;* Cr: *C. rigida;* Cer-1: *Ceratobasidium* sp. of *C. lamellata;* Cer-2: *Ceratobasidium* sp. of *C. virescens;* Th: *T. harzianum*; En: *E. nigrum*.

				Biocontrollers with *F. circinatum*				**Biocontrollers with *R. solani***				
Nº	Rt [min]	RI	Compound	Po	Cr	Cer-1	Cer-2	Po	Cr	Cer-2	Th	En
1	4,85	n.d.	3-Methyl-1-butanol					+				
2	4,92	n.d.	2-Methyl-1-butanol					+				
3	9,64	976	3-Methyl-2(5 *H*)-furanone	+				++				
4	10,52	1023	Alkane		+				+	+	+	+
5	10,70	1034	Limonene		+				+			
6	16,51	1417	α-Bergamotene			+	+					

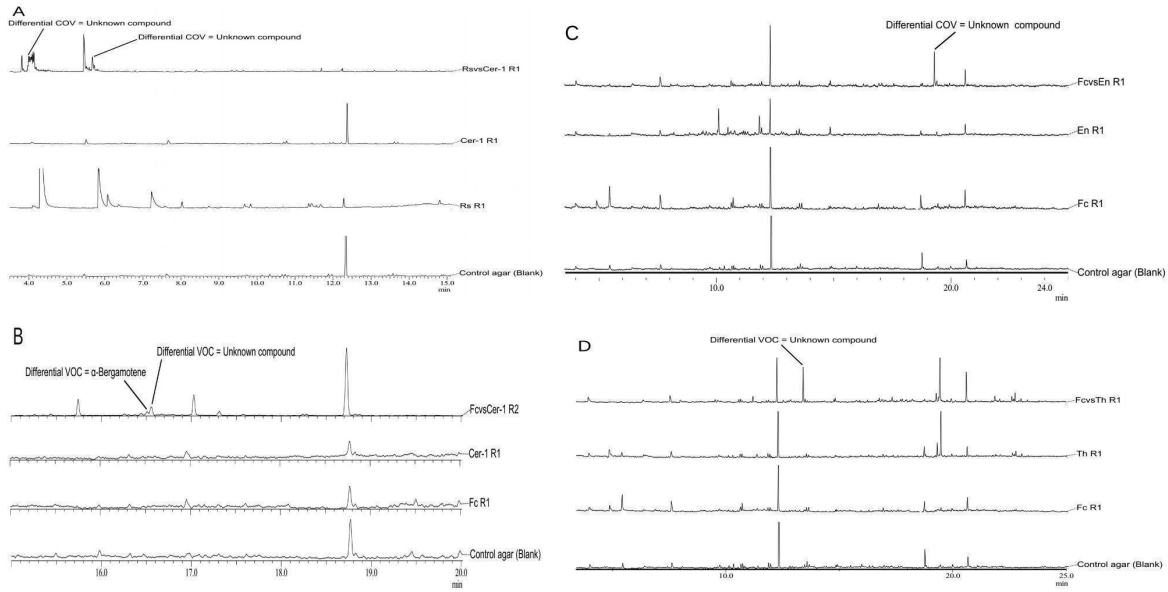
Source: authors (2026).

Figure 3. Representative comparisons of the chromatograms of the pathogens *R. solani* versus *P. ostreatus* (RsvsPo) (A) and *F. circinatum* versus *C. rigida* (FcvsCr) (B). Chromatograms of the pathogen, the biocontroller, the respective dual culture, and the agar blank without fungi are shown in each comparison. Tentative identification of differential volatiles from the dual culture are indicated. Po: *P. ostreatus*; Rs: *R. solani*; Fc: *F. circinatum*; Cr: *C. rigida*.



Source: authors (2026).

Figure 4. Representative comparison of the chromatograms of the pathogens *R. solani* versus *Ceratobasidium* sp. isolated from *C. lamellata* (RsvsCer-1) (A), *F. circinatum* versus *Ceratobasidium* sp. isolated from *C. lamellata* (FcvsCer-1) (B), *F. circinatum* versus *E. nigrum* (FcvsEn) (C) and *F. circinatum* versus *T. harzianum* (FcvsTh) (D). Chromatograms of the pathogen, the biocontroller, the respective dual culture, and the agar blank without fungi are shown in each comparison. Tentative identification of differential volatiles from the dual culture are indicated. Cer-1: *Ceratobasidium* sp. of *C. lamellata;* Rs: *R. solani*; Fc: *F. circinatum*; Th: *T. harzianum*.



Source: authors (2026).

pathogens under study. However, given the preliminary nature of these results, and the fact that not all compounds were identified, it is necessary to thoroughly investigate the volatile profile behavior of the fungi studied in the biocontrol process.

These findings provide valuable insights into the control of two major plant pathogens, highlighting the biotechnological potential of the tested fungal species with contrasting ecological strategies, namely saprophytic, endophytic and orchid mycorrhizal fungi. Furthermore, the study underscores the importance of exploring the biotechnological potential of native Chilean orchid species. Many Chilean species are threatened (Novoa *et al.*, 2015; Atala *et al.*, 2017), emphasizing the need for their conservation as valuable sources of biotechnologically-relevant fungi.

Conclusions

It was demonstrated the *in vitro* biocontrol activity of the saprophytic fungi *P. ostreatus* and *C. rigida*, the foliar endophyte *E. nigrum*, and the Rhizoctonia-like mycorrhizal fungus isolated from *C. lamellata* against the phytopathogen *F. circinatum*. Furthermore, the saprophytic fungi *P. ostreatus* and *C. rigida*, the foliar endophyte *E. nigrum*, the Rhizoctonia-like fungus associated with *C. lamellata*, and the *Ceratobasidium* sp. strain isolated from *C. virescens* also showed antagonistic activity *in vitro* against the phytopathogen *R. solani*.

Volatile organic compounds (VOCs) were detected in all the evaluated treatments, including individual fungal cultures and dual-culture assays. Terpenes were the predominant chemical group. Comparative analysis of volatile compound profiles revealed qualitative differences between single and dual cultures,

including the exclusive detection of specific VOCs in dual-culture interactions.Overall, these results support the role of these fungal isolates as sources of bioactive VOCs and biological control agents against *F. circinatum* and *R. solani* under *in vitro* conditions.

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