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Induced Systemic Resistance Triggered by *Clonostachys rosea* Against *Fusarium circinatum* in *Pinus radiata*

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Abstract

Clonostachys rosea (teleomorph Bionectria ochrouleuca) is a powerful biological control agent (BCA), and has been categorized as a broad-spectrum agent against several phytopathogens affecting different crops and forest species. One possible way by which *C. rosea* can reduce the disease incidence is the Induced Systemic Resistance (ISR), an event associated to several biochemical changes conditioning plants to resist the attack of pathogens. Several studies have found that *C. rosea* induces resistance against pathogens in legumes, cereals and other crops, but there is a lack of information about the situation in forest species. Therefore, the main goal of this study was to evaluate the behavior of different *C. rosea* strains as inductors of resistance against the pathogen *Fusarium circinatum* Niremberg and O`Donnell in two contrasting genotypes of *Pinus radiata* D. Don. Ten *C. rosea* strains were applied to the substrate at 8 and 1 days before confronting *P. radiata* plants with *F. circinatum*, which was inoculated into 5 µL droplets at a previously cut shoot. The lesion length produced by the pathogen was measured at 60 days post inoculation. It was found that only the resistant *P. radiata* genotype showed evidence of ISR, with two *C. rosea* strains, Cr7 and Cr8, triggering resistance and decreasing lesion length to 48.7% and 47.4%, respectively, when compared to pathogen control. These results demonstrate the potential of some *C. rosea* strains to produce ISR on *P. radiata*, but at least for this particular pathosystem, this protection appears to be both dependent on the genotype of the host and the inducer *C. rosea* strain. This is the first report indicating that *C. rosea* can act as an inducer of resistance on the *P. radiate-F. circinatum* pathosystem.

Keywords: Induced resistance; Biocontrol; Pitch canker; *Radiata pine*

Introduction

Pinus radiata D. Don is the most important species in the Chilean forestry plantations with an area of 1.4 million hectares, representing 64% of the country forest plantations. Nevertheless, *P. radiata* has been considered as one of the most susceptible species to *Fusarium circinatum* Niremberg and O`Donnell, a fungus that causes the disease known as pitch canker [1,2]. Due to the potential damage that this pathogen could cause if is spread in commercial plantations in Chile, the Servicio Agrícolay Ganadero (SAG), has declared *F. circinatum* as a quarantine disease and several control measures have been implemented in nurseries to prevent its spread to plantations. A notorious control of the disease by using a biological control strategy based on *Clonostachys rosea* (teleomorph Bionectria ochrouleuca) have shown a reduction of the pathogen incidence and an increase of the survival of *P. radiata* seedlings up to 69%, when the substrates were pre-treated with selected strains of *C. rosea* [3]. More recently, new surveys have been done in order to find new microorganisms with antagonism against *F. circinatum*, with some isolates (mainly of Clonostachys spp and Trichoderma spp) have showed more than 80% of biocontrol against *F. circinatum* in *P. radiata* seedlings.

The non-pathogenic and worldwide distributed fungus *C. rosea* has the ability to act as a saprophyte on a wide range of soils, or as an endophyte or epiphyte on live plants, and is even recognized as a mycoparasite in some studies [4-6]. The antagonist activity of *C. rosea* is of wide spectrum, and is currently identified as a strong biological control agent (BCA) against pathogenic fungi affecting varied crops of agronomic and forest importance [6-11]. In the forestry field in Chile, studies aimed at the evaluation of this BCA against important diseases such as the gray mold caused by Botrytis cinerea on seedlings of *Eucalyptus globulus* [12,13], and the damping-off caused by *F. circinatum* on *P. radiata* seedlings [3], demonstrated a reduction on both diseases. Although it has been determined that the principal mechanism of biocontrol employed by *C. rosea* is the parasitism of *F. circinatum* hyphae, there are no studies investigating the role of *C. rosea* as a potential systemic resistance inductor in *P. radiata*.

Induced resistance has been defined as the increased resistance exhibited by plants appropriately stimulated by an inducer agent, leading to physical or chemical responses that allow plant protection when is challenged with the pathogen [14-16]. Inducer agents can include, but are not restricted to, pathogens, non-pathogenic microorganisms (i.e., endophytic fungi and bacteria), pathogenic strains with incompatibility for the host, and chemical agents. It is possible that some inducing agents trigger some pathways involving multiple polygenic response, in this case, when the ISR has been activated, a prolonged resistance against multiple pathogens can be achieved [14]. The information available about the influence of the genotype on induced resistance is very reduced and apparently depends on the pathosystem on which the resistance is induced, while

in barley and cucumber the most resistant varieties have a strong induced resistance [17,18], in soy and wheat the opposite is observed, with the susceptible varieties being induced [19,20]. In the case of *Arabidopsis thaliana*, the resistance was induced for most of the ecotypes when elicited by *Pseudomonas fluorescens* [21]. Due to the lack of information about any possible induced resistance on the *P. radiate- F. circinatum* pathosystem elicited with this BCA, the objective of this study was to evaluate the effect of different *C. rosea* strains on the generation of ISR against *F. circinatum*, in two genotypes of *P. radiata* contrasting on its susceptibility to the pathogen.

Methodology

Clonostachys rosea and *Fusarium circinatum* strain culture conditions

Ten *C. rosea* strains belonging to the collection of Forest Pathology Lab at University of Concepción were included in this study. These strains were isolated from different tissues and plantation soils of *P. radiata* and were previously selected for their BCA activity, providing protection over 80% against the damping-of disease caused by *F. circinatum* under greenhouse conditions. Additionally, an aggressive strain of *F. circinatum* (Pr 44-4641), isolated from symptomatic *P. radiata* hedges was also included [3]. The antagonistic fungi and pathogen were stored in tubes containing Potato Dextrose Agar (PDA) as culture medium at 4°C. Prior to the assays, the fungal strains were replicated in Petri dishes containing PDA and incubated at 25°C for seven days to obtain fresh inoculum.

Plant material

Two previously characterized *P. radiata* genotypes, contrasting in susceptibility to *F. circinatum* were used, a susceptible (S) genotype, and a resistant (R) genotype. Both genotypes were originated from a controlled cross-pollination and cryo-preserved embryos, and were facilitated by Bioforest S.A. The plants were maintained under controlled conditions of 80% RH, 25°C and 12/12 photoperiod from two weeks before the first application of *C. rosea* strains to the end of the assay.

Induced systemic resistance assay

Both *P. radiata* clones were eighteen months old. The assay consisted in twelve treatments, corresponding to ten different *C. rosea* strains (Cr1 to Cr10) applied to the substrate in a volume of 15 mL (1×10^7 conidia $\times$ mL^{-1}), and two controls, the cut control (mechanical damage only, CC), and pathogen treatment (Pr44-4641 strain only, PT). The *C. rosea* treatments were applied two times, at eight and one days before proceeding to inoculate the plants with *F. circinatum*. The pathogen was inoculated at the cut apex of each plant by depositing a micro-drop (5 µL) containing a final concentration of 1×10^5 conidia per mL^{-1}. The damage caused by the pathogen was evaluated at 60 days post inoculation and was measured as a lesion length in millimeters.

Experimental design and data analysis

A completely random design with 12 treatments and 10 replicates was used. Statistical data analysis was performed by ANOVA with a significance level of 0.05. All data were subjected to variance homogeneity analysis and normality assumptions and pooled accordingly. Multiple comparisons were made using Tukey test. Analyses were performed with Statistical Analysis System program (SAS Institute).

Results

The disease development was evident externally by two different symptoms, dark brown color of shoot and/or dehydration of affected zone. The removal of the external tissue allowed a better visualization of the xylem necrosis and was used to evaluate the lesion length precisely.

As shown by Figure 1, in the case of the susceptible genotype S, the lesion lengths were substantially larger when compared to the cut control CC (1.4 mm), nevertheless, none of the treatments were statistically different from the pathogen treatment PT (35.0 mm), even when some treatments such as Cr10 showed a smaller lesion length of 24.4 mm, this size was still not statistically different when compared with PT.

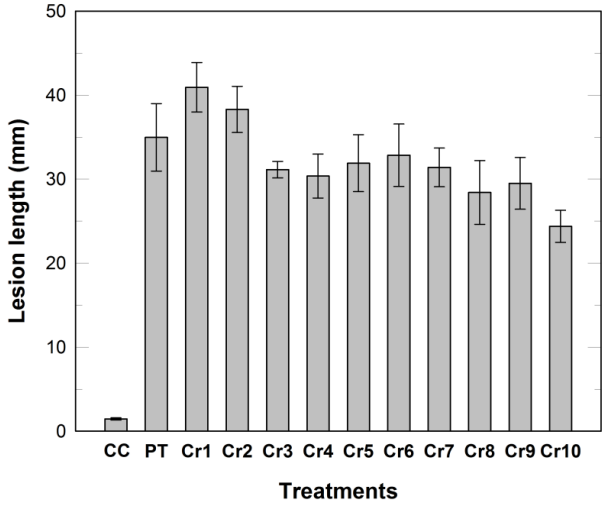

Figure 1: Average lesion length of *Pinus radiata* (susceptible genotype S) inoculated with *Fusarium circintaum*. Each bar represents different treatments. CC, cut control treatment, without pathogen inoculation. PT, pathogen control treatment, without *Clonostachys rosea*. Cr1 to Cr10, different strains of *C. rosea* applied to the substrate previous to pathogen inoculation. Statistical differences assessed by Tukey test at 95% confidence, error bar represents SD.

The situation is different when considering the resistant genotype R, with smaller lesion lengths when compared to S genotype, with the only exception of cut control who presents the same length on both S and R genotypes, thus excluding any genotype-specific tendency to present different lesion lengths when subjected to a mechanical damage. Both Cr7 (7.5 mm) and Cr8 (7.7 mm) treatments from R plants showed significantly smaller lesion lengths when they were compared to PT (14.6 mm), reducing the damage caused by the pathogen in a 48.7% and 47.4%, respectively, as shown on Figure 2.

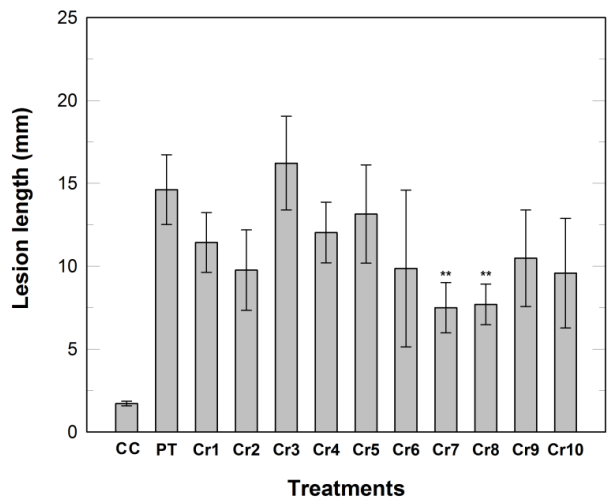

Figure 2: Average lesion length of *Pinus radiata* (resistant genotype R) inoculated with *Fusarium circintaum*. Each bar represents different treatments. CC, cut control treatment, without pathogen inoculation. PT, pathogen control treatment, without *Clonostachys rosea*. Cr1 to Cr10, different strains of C. rosea applied to the substrate previous to pathogen inoculation. *Asterisks indicate significant differences between treatments compared to PT (Tukey test, 95% confidence). Error bar represents SD.

Discussion

Accordingly, only R genotype showed evidence of ISR, with Cr7 and Cr8 strains inducing this resistance, indicating that ISR is genotype-dependent on *P. radiata*. This phenomenon of host genotypes affecting the manifestation of ISR was previously reported for other pathosystems, studies on spring varieties of barley, using different elicitors, showed that ISR against foliar pathogens varied strongly depending on the host genotype [22]. Similar effect was reported by Tucci et al. [23], where several but not all tomato lines tested showed ISR against *B. cinerea*.

Our results demonstrate the potential for two *C. rosea* strains, Cr7 and Cr8, to elicit ISR in *P. radiata*, displaying a dependence on the *C. rosea* strain to induce the effect. Previous studies have showed that BCA such as strains of Trichoderma are capable to elicit ISR, and furthermore, the colonized roots appeared to be primed for an increased defensive response when confronted with pathogens [23-25]. Additionally it has been observed that ISR can be influenced by the pathogen, as determined in a study using different tomato genotypes that displayed different level of BABA-mediated resistance against *Phytophthora infestans*, with the induction levels strongly more related to the pathogen strain than by the tomato genotype tested [26]. Since this study used a *F. circinatum* strain previously selected by its high aggressiveness [3], we consider that the ISR effect determined here can be attributed to both the *C. rosea* strain used and the *P. radiata* genotype tested.

Even when the induced resistance is a well-known phenomenon on herbaceous plants and short-lived perennial agricultural crops [27,28], it is just recently studied on trees. Enebak and Carey [29] reported the first evidence of ISR in trees, finding that four strains of plant growth promoting rhizobacteria (PGPR) had an ISR effect against *Cronartium uercquum* f. sp. fusiforme in loblolly pine. Reglisnki et al. [25] tested four isolates of Trichoderma atroviride for growth promotion, finding that one isolate (R33), elicited ISR on stem inoculation with *Diplodia pinea* in *P. radiata* seedlings. Another study tested ten different inducers, including biotic and abiotic agents, to enhance the tolerance to F. circinatum in *P. patula*, showing that the most promising treatment was chitosan at a concentration of 10 mg/ml, resulting in a significant reduction in lesion length [30].

Blodgett et al. [31] showed that induced resistance in *Pinus nigra* is bidirectional (acropetally and basipetally) and proceeds if the induction is produced on the stem base and the challenge with the pathogen is on the upper stem or if the scheme is reversed, nevertheless, the response is not elicited when the induction is made on the stem base and the challenging is on the shoots, showing an organ-dependent nature for the induction. While in *P. radiata* it has been demonstrated that the induced resistance can be elicited as a response to the pitch canker pathogen (*F. circinatum*) on trees previously infected in the field. When previously infected trees presenting signs of natural disease remission were confronted with pitch canker, 89% showed a very limited lesion length, indicating some resistance to the pathogen. Furthermore, it was evident that trees from areas where pitch canker was established long ago tended to be more resistant than trees from areas with recent colonization of the disease [32]. Even when these studies demonstrated the phenomenon of resistance induction, a pathogen agent was the responsible for the elicited resistance; therefore some authors have named this phenomenon as systemic induced resistance or SIR [28,31,32], in order to separate it from ISR, which is triggered or elicited by a non-pathogenic agent.

Our results indicate that ISR is present in *P. radiata* and could be used to enhance the phytosanitary status of the trees by the application of Cr7 and Cr8 strains, allowing the host to respond faster to the pathogen attack, and also to develop a prolonged and wide range defense response as reported for other ISR responses [14,30]. This information will be the base for a bioproduct formulation based on a consortium of microorganisms possessing different strategies of biocontrol, including Cr7 and Cr8 strains, previously selected by its ability to control damping-off on *P. radiata* seedlings, and that also showed ISR induction against the pathogen on this study, thus reducing the severity of the symptoms on stem and helping to control the disease. This strategy will represent an environmentally friendly solution amenable to be included into the integrated disease management of *F. circinatum* on greenhouses of *P. radiata*, in order to avoid the secondary dissemination of the pathogen on plantations of this species, especially considering that currently there are no products or measures for the efficient control of this pathogen.

To the best of our knowledge, this is the first report indicating that *C. rosea* can act as an inducer of resistance on the pathosystem *P. radiate-F. circinatum*. Additionally, this study demonstrates that the elicited resistance in *P. radiata* is dependent on both the *P. radiata* genotype and the *C. rosea* strain.

Conclusion

In this study, the priming phenomenon associated to ISR and used to activate defense pathways against pathogen attacks was studied on *P. radiata* against *F. circinatum* on, selecting two strains of *C. rosea* that

showed defense elicitation on a resistant R genotype of *Radiata pine*. The application of Cr7 and Cr8 strains previous to *F. circinatum* innoculation reduced the severity of the disease in a period of two months, showing resistance induction on the host. Even when the defense response elicited by *C. rosea* was not analyzed deeply and requires much more research, this work constitutes the base for future studies of the elucidation of the molecular mechanism of ISR triggered by *C. rosea* on *P. radiata*.

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References

1. Gordon TR, Kirkpatrick SC, Aegerter BJ, Wood DL, Storer AJ (2006) Susceptibility of Douglas fir (Pseudotsuga menziesii) to pitch canker, caused by Gibberella circinata. Plant Pathol 55: 231-237.
2. Wikler K, Gordon TR, Storer AJ, Wood DL (2003) Pitch Canker. Integrated pest management for home gardeners and landscape professionals. Pest Note 741707, pp: 1-5.
3. Moraga-Suazo P, Opazo A, Zaldúa S, Gonzalez G, Sanfuentes E (2011) Evaluation of Trichoderma spp. and Clonostachys spp. strains to control Fusarium circinatum in Pinus radiata seedlings. Chilean Journal of Agricultural Research 71: 412-417.
4. Hoopen GM, Rees R, Aisa P, Stirrup T, Krauss U (2003) Population dynamics of epiphytic mycoparasites of the genera Clonostachys and Fusarium for the biocontrol of black pod (Phytophthora palmivora) and moniliasis (Moniliophthora roreri) on cocoa (Theobroma cacao). Mycological research 107: 587-596.
5. Lübeck M, Knudsen IMB, Jensen B, Thrane U, Janvier C, et al. (2002) GUS and GFP transformation of the biocontrol strain Clonostachys rosea IK726 and the use of these marker genes in ecological studies. Mycological Research 106: 815-826.
6. Sutton JC, Liu W, Huang R, Owen-Going N (2002) Ability of Clonostachys rosea to establish and suppress sporulation potencial of Botrytis cinerea in deleafed stems of hydroponic greenhouse tomatoes. Biocontrol Sci Technol 12: 413-425.
7. Lahoz E, Contillo R, Porrone F (2004) Induction of systemic resistance of Erysiphe orontii Cast in tobacco by application on roots and isolate of Gliocladium roseum. Bainier J Phytopath 152: 465-470.
8. Morandi MAB, Maffia LA, Sutton JC (2001) Development of Clonostachys rosea and interactions with Botrytis cinerea in rose leaves and residues. Phytoparasitica 29: 103-113.
9. Nobre SAM, Maffia LA, Mizubuti ESG, Cota LV, Dias APS (2005) Selection of Clonostachys rosea isolates from Brazilian ecosystem effective in controlling Botrytis cinerea. Biological control 34: 132-143.
10. Molina G, Zaldua S, Gonzalez G, Sanfuentes E (2006) Selección de hongos antagonistas para el control biológico de Botrytis cinerea en viveros forestales. Bosque 27: 126-134.
11. Zaldúa S, Sanfuentes E (2010) Botrytis cinerea control in Eucalyptus globulus moni-cuttings using Clonostachys and Trichoderma strains. Chilean Journal of Agricultural Research 70: 576-582.
12. Rodriguez MA, Cabrera G, Gozzo FC, Eberlin MN, Godeas A (2011) Clonostachys rosea BAFC3874 as a Sclerotinia sclerotitum antagonist: mechanism involved and potential as a biocontrol agent. Journal of Applied Microbiology 110: 1177-1186.
13. Tarantino P, Caiazzo R, Carella A, Lahoz E (2006) Control of Rhizoctonia solani in a tobacco-float system using low rates of iprodione- and iprodione-resistant strains of Gliocladium roseum. Crop Protection 26: 1298-1302.
14. Ganley RJ, Sniezko RA, Newcombe G (2008) Endophyte-mediated resistance against White pine blister rust in Pinus monticola. Forest ecology and Management 255: 2751-2760.
15. Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology 103: 753-765.
16. Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology 95: 1368-1373.
17. Hijwegen T, Verhaar MA (1995) Effects of cucumber genotype on the induction of resistance to powdery mildew, Sphaerotheca fuliginea, by 2,6-dichloroisonicotinic acid. Plant Pathol 44: 756-762.
18. Martenelli JA, Brown JKM, Wolfe MS (1993) Effects of barley genotype on induced resistance to powdery mildew. Plant Pathol 42: 195-202.
19. Dann E, Diers B, Byrum J, Hammerschmidt R (1998) Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzo- thiadiazole (BTH) on seed yields and the level of disease caused by Sclerotinia sclerotiorum in field and greenhouse studies. Eur J Plant Pathol 104: 271-278.
20. Stadnik MJ, Buchenauer H (1999) Effects of benzothiadiazole, kinetin and urea on the severity of powdery mildew and yield of winter wheat. Z. Pflanzenkrankh Pflanzenschutz 106: 476-489.
21. Van Wees SCM, Pieterse CMJ, Trijssenaar A, Van't Westende YAM, Hartog F, et al. (1997) Differential induction of systemic resistance in Arabidopsis by biocontrol bacteria. Mol. Plant-Microbe Interact 10: 716-724.
22. Walters DR, Havis ND, Paterson L, Taylor J, Walsh DJ (2011) Cultivar effects on the expression of induced resistance in spring barley. Plant Disease 95: 595–600.
23. Tucci M, Ruocco M, Masi L, Palma M, Lorito M (2011) The beneficial effect of Trichoderma spp. on tomato is modulated by the plant genotype. Molecular Plant Pathology 12: 341-354.
24. Hanson LE, Howell CR (2004) Elicitors of plant defense responses from biocontrol strains of Trichoderma virens. Phytopathology 94: 171-176.
25. Reglinski T, Rodenburg N, Taylor JT, Northcott GL, Chee AA, et al. (2012) Trichoderma atroviride promotes growth and enhances systemic resistance to Diplodia pinea in radiata pine (Pinus radiata) seedlings. Forest Pathology 42: 75-78.
26. Sharma K, Butz AF, Finckh MR (2010) Effects of host and pathogen genotypes on inducibility of resistance in tomato (Solanum lycopersicum) to Phytophthora infestans. Plant Pathology 59: 1062–1071.
27. Gatehouse JA (2002) Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist 156: 145-169.
28. Eyles A, Bonello P, Ganley R, Mohammed C (2010) Induced resistance to pests and pathogens in trees. New Phytologist 185: 893-908.
29. Enebak SA, Carey WA (2000) Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. Plant Disease 84: 306-308.
30. Fitza K, Myburg A, Steenkamp E, Payn K, Naidoo S (2011) Induced resistance and associated defence gene responses in Pinus patula. BMC Proceedings 5: 82.
31. Blodgett JT, Eyles A, Bonello P (2007) Organ-dependent induction of systemic resistance and systemic susceptibility in Pinus nigra inoculated with Sphaeropsis sapinea and Diplodia scrobiculata. Tree Physiology 27: 511-517.
32. Gordon TR, Kirkpatrick SC, Aegerter BJ, Fisher AJ, Storer AJ, et al. (2010) Evidence for ocurrente of induced resistance to pitch canker, caused by Gibberella circinata (anamorph Fusarium circinatum), in populations of Pinus radiata. Forest Pathology 41: 227-232.