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ORIGINAL ARTICLE

Management of *Ralstonia solanacearum* in eucalyptus seedlings: initial studies with *Trichoderma harzianum* and *Purpureocillium lilacinum*

Manejo de *Ralstonia solanacearum* em mudas de eucalipto: estudos iniciais com *Trichoderma harzianum* e *Purpureocillium lilacinum*

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Abstract

The study evaluated the effectiveness of *Trichoderma harzianum* and *Purpureocillium lilacinum* in the management of *Ralstonia solanacearum* on eucalyptus seedlings. For each biological control agent, the following treatments were conducted: plants with their roots immersed in a cell suspension of the biological control agent (SI+I); plants in pots containing the biological control agent in hydrogel (SI+H); plants in pots previously drenched with the biological control agent (SI+IR), and plants in pots where the biological control agent was applied directly into the planting hole (SI+PO). Control treatments consisted of plants in pots infested and pots not infested with *R. solanacearum* (PC and NC, respectively). All treatments were carried out with two eucalyptus clones (SR and 144). The analyzed variables were mortality rate of eucalyptus seedlings and dry weight of plants. The most effective treatment had the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase (PAL) measured at 7, 14 and 21 days after inoculation. The method of incorporating the biological control agent influenced *R. solanacearum* control. Results revealed that SI+H and SI+IR were the most effective treatments. Using *T. harzianum,* mortality was only 12% for clone SR and 0% for clone 144 in the treatments SI+H and SI+IR, respectively. In the treatment SI+H, using *P. lilacinum*, 25% and 20% dead seedlings were obtained for clone SR and clone 144, respectively. Both biological agents increased the dry mass of plants. The activity of peroxidase, polyphenol oxidase and PAL was higher at 14 days after inoculation with *T. harzianum* in the treatments SI+IR and SI+H, for clones SR and 144, respectively. Using *T. harzanium* can serve as a support for the sustainable management of areas where cases of *R. solanacearum* have previously occurred.

Keywords: Biological control; Resistance induction; Forestry.

Resumo

O estudo avaliou a eficácia de *Trichoderma harzianum* e *Purpureocillium lilacinum* no manejo de *Ralstonia solanacearum* em mudas de eucalipto. Para cada agente de controle biológico foram realizados os seguintes tratamentos: plantas com raízes embebidas na suspensão celular do agente biológico (SI+I); plantas colocadas no vaso contendo o agente de controle biológico dentro de hidrogel (SI+H); plantas transferidas para

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vasos previamente embebidas com o agente de controle biológico (SI+IR); plantas colocadas em vasos com agente de controle biológico colocados diretamente nas mudas (SI+PO). Os tratamentos usados como controle consistiram de mudas em vasos infestadas e não infestadas com *R. solanacearum* (PC e NC, respectivamente). Todos os tratamentos foram realizados com dois clones de eucalipto (clones SR e 144). As variáveis analisadas foram a mortalidade de mudas de eucalipto e a massa seca das plantas. O tratamento mais eficaz teve atividade de peroxidase, polifenoloxidase e fenilalanina amônia-liase (FAL) medida aos 7, 14 e 21 dias após a inoculação. O método de incorporação do agente de controle biológico influenciou o controle de *R. solanacearum*. Os resultados revelaram que os tratamentos SI+H e SI+IR foram os mais eficazes no controle de *R. solanacearum*. *T. harzianum* apresentou apenas 12% de mortalidade do clone SR e 0% de mortalidade do clone 144 nos tratamentos SI+H e SI+IR, respectivamente. Quando aplicado o tratamento SI+H, *P. lilacinum* apresentou 25% e 20% de plântulas mortas (clone SR e clone 144, respectivamente). Os dois agentes biológicos aumentaram a massa seca das plantas. A atividade da peroxidase, polifenoloxidase e fenilalanina FAL se mostrou maior após 14 dias após a inoculação nos tratamentos SI + IR e SI + H com *T. harzianum*, nos clones SR e 144, respectivamente. O uso de *T. harzanium* pode servir de suporte no manejo sustentável de áreas onde já ocorreram casos de *R. solanacearum*.

Palavras-chave: Controle biológico; Indução de resistência; Silvicultura.

INTRODUCTION

According to the Brazilian Tree Industry (Indústria Brasileira de Árvores, 2019), the area dedicated to eucalyptus cultivation in Brazil represents 7.83 million hectares. However, several plant pathogens have reduced the production, and bacterial wilt caused by *Ralstonia solanacearum* (Alfenas et al., 2009) is one of the most important constraints of eucalyptus tree crops.

In 2005, bacterial wilt led to great losses in several producing states in Brazil, which were estimated at approximately U$ 1 million. Such losses increase to approximately U$ 3 million if the discarded material, e.g., mini-stumps, and the delays in the companies' schedule, are considered. Other countries have also reported bacterial wilt incidence in eucalyptus areas, including the world's largest producers like China, Taiwan, Indonesia, Australia, Venezuela and South Africa (Alfenas et al., 2006).

Ralstonia solanacearum is the causal agent of bacterial wilt, a vascular disease. Symptoms of darkening and collapse of vascular tissues can lead the host to wilting and death (Amorim et al., 2016). The pathogenesis mechanisms of *R. solanacearum* are related to extracellular polysaccharide (EPS) production, biofilm formation and type III secretion system (T3SS) variability (Schell, 2000; Mori et al., 2016; Murthy & Srinivas 2015; Valls et al., 2006). Therefore, this bacterium has a complex of regulatory genes that detect different environments and trigger physiological changes during its life cycle. This sensory matrix, which is linked to the expression of different genes, also controls virulence and pathogenicity (Schell, 2000). Such survival and pathogenicity-related features, added to the fact that it is pathogenic to several important species (Yabuuchi et al., 1995; Lebeau et al., 2011; Genin & Denny, 2012), evidence the difficulties in controlling *R. solanacearum*.

Thus, alternative and environment-friendly measures have to be found and integrated in the management of *R. solanacearum*. In this context, induced resistance has recently emerged as an effective strategy to manage plant pathogens. According to Kuhn (2007), induced resistance consists in using abiotic or biotic agents that have low environmental impact and trigger the expression of defense-related mechanisms in plants. For example, acibenzolar-s-methyl is an abiotic resistance-inducing agent that has already shown effective results in controlling *R. solanacearum* in different hosts, including tomato (Anith et al., 2004).

The resistance induction process involves the activation of plant defense-related enzymes, such as phenylalanine ammonia lyase, peroxidase and β-1,3-glucanase, important components in the response to pathogens (Pascholati et al., 2008; Romeiro & Garcia, 2009). Some fungi, like *Trichoderma* species, have the ability to induce resistance, i.e., they colonize root surfaces, causing changes in the plant metabolism in order to stimulate defense mechanisms, such as production of phenols and phytoalexins, and increase nutrient availability and stress tolerance (Nachtigal, 2012). Although the fungal genus *P. lilacinum,* formerly classified as *Paecilomyces lilacinus* (Luangsa-Ard et al., 2011) is known to provide greater nematode control, Parveen et al. (1998) demonstrated that cucurbit and legume root

infection caused by soilborne fungi decreased with the use of *Purpureocillium.* According to those authors, such a reduction was a result of the nematode control which diminished the lesions and the routes for pathogens to enter the plant roots.

Based on the above-mentioned data and considering that farmers have easy access to *T. harzianum* and *P. lilacinum*, the present study aimed to evaluate the potential of these microorganisms for *R. solanacearum* control in the soil and to verify possible resistance induction in eucalyptus plants treated with these agents.

MATERIAL AND METHODS

Experimental site, plant material and biological control agents

The experiment was conducted in a greenhouse located at FCAT (UNESP/ Dracena/Brazil), between March and April 2018. Three-month-old eucalyptus seedlings of "urograndis" hybrid (*Eucalyptus grandis* x *Eucalyptus urophylla*), donated by a multinational paper and cellulose company, were kept in containers and transplanted into the pots for treatment application. The two adopted eucalyptus clones were SR (susceptible to *R. solanacearum*) and 144 (one of the clones most planted by eucalyptus companies in Brazil). During the trials, the plants were kept in 10-L pots containing sterile clayey soil and peat at a 2:1 ratio (w/w). The pots were irrigated with automated irrigators twice every day.

The fungi *T. harzianum* and *P. lilacinum* were commercially obtained as Wettable Powder (WP); they are sold at concentrations of 10^{10} CFU/g and 10^{9} CFU/g commercial product, respectively. The amount of biological control agent used in the experiment was adapted according to the manufacturer's recommendations for perennial crops.

Acquisition of R. solanacearum inoculum

Ralstonia solanacearum isolate was obtained from the Phytopathogenic Microorganisms Collection maintained at FCAT - UNESP/Dracena/Brazil. The identity of the disease causal agent was confirmed through PCR analyzes of the rDNA16S part, using oligonucleotides PS1 and PS2, specific to detect this bacterial species (Pastrik & Maiss, 2000).

The isolate, which had been preserved in sterile water for approximately six months, was grown in TZC culture medium in Petri dishes (Kelman, 1954). Two days after cultivation, irregularly round, fluidal, white, pink-centered colonies (described as virulent by Kelman, 1954) were selected and multiplied to obtain a bacterial suspension (10^{7} CFU.mL^{-1}/OD540 nm = 1.15). For the experiment, 1 mL bacterial suspension was added to Kelman medium and incubated at 28°C ± 1 under constant agitation (65RPM) and 12-hour photoperiod for 24 hours. Then, the liquid medium with *R. solanacearum* was calibrated to 10^{7} CFU.mL^{-1} cell suspension (OD 540 nm = 1.15) with 0.85% NaCl solution.

Experimental design and plant treatment

Before the planting of eucalyptus seedlings, a 50-mL suspension containing 10^{7} CFU.mL^{-1} *R. solanacearum* (OD540 nm = 1.15) was incorporated into the substrate in the pots. After infestation, the following treatments were set:

- Infested soil and soaking **(SI+S)**: Eucalyptus seedlings had their roots immersed in 1 mL sterile water containing the biological control agent for 15 minutes and planted in pots infested with *R. solanacearum*;
- Infested soil and hydrogel **(SI+H)**: Eucalyptus seedlings were planted in pots infested with *R. solanacearum* and containing 25 mL hydrogel 0.1% incorporated with the biological control agent;
- Infested soil and irrigation **(SI+IR)**: Eucalyptus seedlings were planted in pots infested with *R. solanacearum* and irrigated with 25 mL biological control agent suspension.
- Infested soil and powder **(SI+PO)**: Eucalyptus seedlings were planted in pots infested with *R. solanacearum*, and the biological control agent was directly applied into the planting hole previous to planting;

- Negative control **(NC)**: Eucalyptus seedlings were planted in pots without *R. solanacearum* infestation and without any biological treatment;
- Positive control **(PC)**: Eucalyptus seedlings were planted in pots infested with *R. solanacearum*.

Six treatments were performed for each biological control agent (*T. harzianum* and *P. lilacinum*) and for each eucalyptus clone (SR and 144) separately. Each treatment had 15 biological replicates and experiments were conducted twice.

At the end of each experiment, shoot dry weight and plant mortality rate were assessed. A completely randomized design was adopted, and results were obtained as average values which underwent Tukey's honest test at 5% probability level using SISVAR software (Ferreira, 2011).

Determination of induced resistance based on the activity of the enzymes peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase

To determine the activity of some defense-related enzymes, samples were collected from the third apical leaf (1g fresh sample) of each treatment at 7, 14 and 21 days after the application of resistance-inducing agents. The leaves were ground in liquid nitrogen and mixed with 2mL sodium acetate buffer (0.1 M, pH 5.0) containing 1.0 mM EDTA and 0.3 g polyvinylpyrrolidone (PVP). The extracts were stored in a freezer (-20°C) for 12 hours and, after this period, centrifuged at 14000 rpm for 25 minutes (4ºC). The supernatant was transferred to fresh microtubes and stored at -80ºC. Crude plant extract from each treatment was used to determine all the following enzymes.

Peroxidase activity was analyzed according to the methodology described by Boava et al. (2010). To determine the activity of polyphenol oxidase, the methodology described by Duangmal & Apenten (1999) was adopted. The activity of phenylalanine ammonia lyase (PAL) was analyzed according to Pierozzi (2013). All enzymatic assays were performed in triplicate for each treatment. The obtained average values were adjusted to regression equations with a higher R^2 so that the response trends of the evaluated characteristics could be obtained as a function of time.

RESULTS AND DISCUSSION

The mean values of mortality rate and dry mass obtained for eucalyptus seedlings of clone SR are shown in Table 1. The best results were found for treatments SI+IR and SI+H with *T. harzianum*, since they showed lower mortality and higher dry mass of plants, compared to PC. The percentage of dead plants was also lower for SI+IR and SI+H with *P. lilacinum* as the biocontrol agent, compared to PC; using *T. harzianum,* those treatments had the lowest mortality rate. The data obtained for clone 144 are presented in Table 2. The treatment SI+H was better with *T. harzianum*, as it did not present dead plants during the evaluation period. Associated with *P. lilacinum*, SI+IR provided a drop in mortality for this clone.

Table 1. Mean mortality (%) and dry weight (g) of eucalyptus seedlings, clone SR, inoculated with *Ralstonia solanacearum* and treated with *Trichoderma harzianum* and *Purpureocillium lilacinum*.

Treatments	Percentage of dead plants		Dry weight	
	T. herzianum	*P. lilacinum*	*T. herzianum*	*P. lilacinum*
SI+S	62.5%	75%	5.25 b	6.35 ab
SI+IR	12.5%	25%	7.01 ab	9.39 a
SI+H	25%	37.5%	10.19 a	9.88 a
SI+PO	50%	25%	7.98 ab	10.18 a
CP	75%	75%	6.26 b	5.84 ab
CN	12.5%	12.5%	4.78 b	4.78 b
CV (%)	-	-	6.91	7.74

CV: Coefficient of variation (values followed by the same lowercase letter in the column do not differ between treatments according to Tukey's test at 5% confidence interval).

Table 2. Mean mortality (%) and dry weight (g) of eucalyptus seedlings, clone 144, inoculated with *Ralstonia solanacearum* and treated with *Trichoderma harzianum* and *Purpureocillium lilacinum.*

Treatments	Percentage of dead plants		Dry weight *	
	T. herzianum	*P. lilacinum*	*T. herzianum*	*P. lilacinum*
SI+S	60%	20%	2.0	1.4
SI+IR	40%	20%	2.5	3.8
SI+H	0%	60%	3.8	2.9
SI+PO	40%	60%	2.2	1.7
CP	40%	60%	2.7	2.0
CN	0%	0%	2.0	2.0

***** Non-significant difference according to F test at 0.01% and 0.05%.

The activities of peroxidase, polyphenol oxidase and PAL were analyzed for the treatments SI+H and SI+IR using *T. harzianum* in clones 144 and SR, respectively, since they were most promising for the disease management. Increased activity was detected for the three evaluated enzymes at 14 days after the product application (Figure 1A, Figure 1B; Figure 2A, Figure 2B; Figure 3A and Figure 3B). However, it is important to highlight that for SI+IR only peroxidase had higher activity at all assessed periods (Figure 1A and Figure 1B).

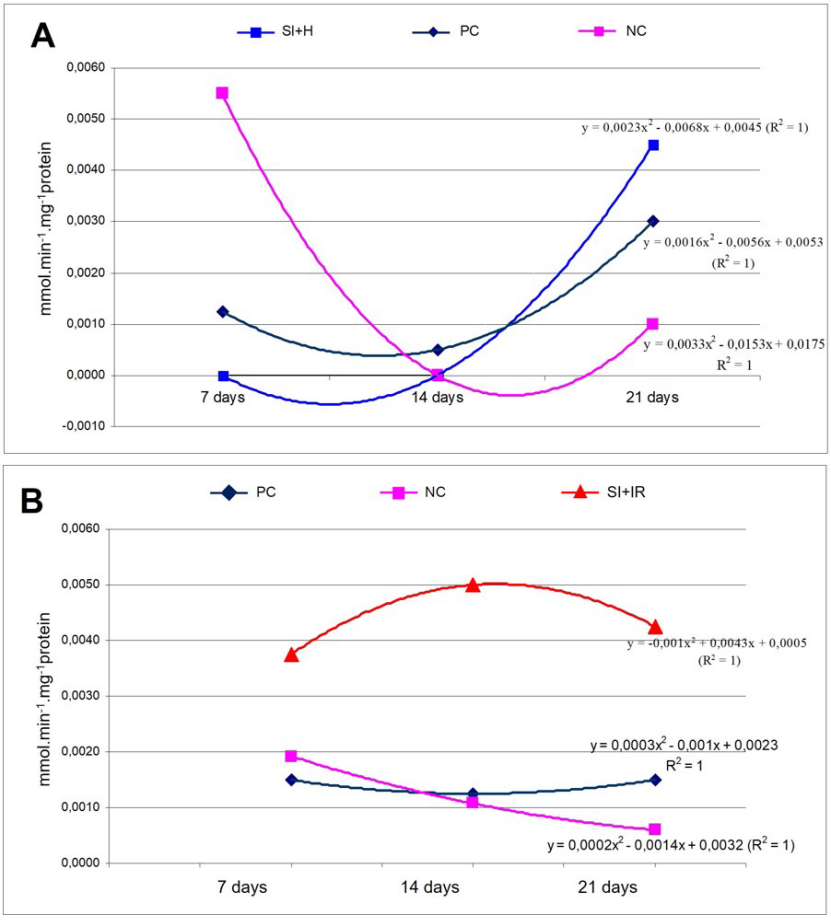

Figure 1: Peroxidase activity (mmol.min^{-1}.mg^{-1}protein) in eucalyptus seedlings, clones 144 **(A)** and SR **(B)**, subjected to different treatments with *Trichoderma harzianum* and planted in soils infested with *Ralstonia solanacearum.* **PC:** Positive control - soil infested with *R. solanacearum*; **NC:** Negative control - sterilized soil; **SI+H:** Soil infested with *R. solanacearum* and treated with *T. harzianum* embedded in hydrogel; **SI+IR:** Soil infested with *R. solanacearum* and irrigated with *T. harzianum* suspension.

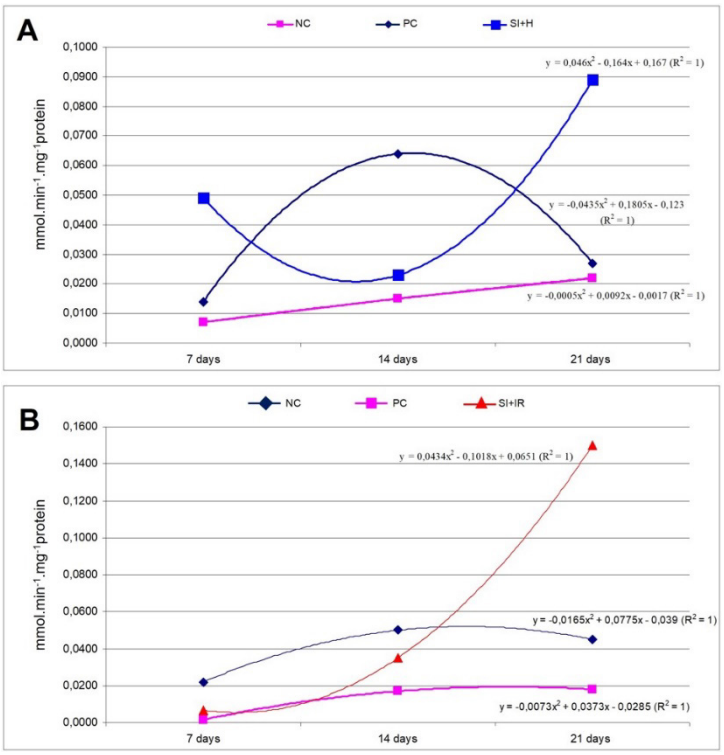

Figure 2: Polyphenol oxidase activity (mmol.min^{-1}.mg^{-1}protein) in eucalyptus seedlings, clones 144 **(A)** and SR **(B),** subjected to different treatments with *Trichoderma harzianum* and planted in soils infested with *Ralstonia solanacearum.* **PC:** Positive control - soil infested with *R. solanacearum*; **NC:** Negative control - sterilized soil; **SI+H:** Soil infested with *R. solanacearum* and treated with *T. harzianum* embedded in hydrogel; **SI+IR:** Soil infested with *R. solanacearum* and irrigated with *T. harzianum* suspension.

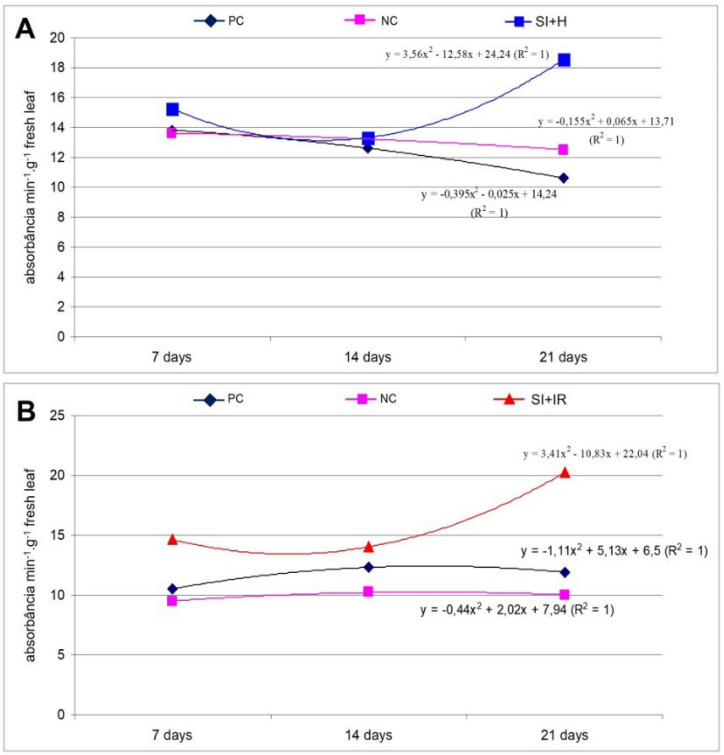

Figure 3: PAL activity (absorbance min^{-1}.g^{-1} fresh leaf) in eucalyptus seedlings, clones 144 **(A)** and SR **(B)**, subjected to different treatments with *Trichoderma harzianum* and planted in soils infested with *Ralstonia solanacearum.* **PC:** Positive control - soil infested with *R. solanacearum*; **NC:** Negative control - sterilized soil; **SI+H:** Soil infested with *R. solanacearum* and treated with *T. harzianum* embedded in hydrogel; **SI+IR:** Soil infested with *R. solanacearum* and irrigated with *T. harzianum* suspension.

The ability of biocontrol agents to reduce plant mortality caused by *R. solanacearum* has already been reported. Lwin & Ranamukhaarachchi (2006) screened antagonists as potential biocontrol agents against bacterial wilt. Those authors emphasized the importance of time, frequency and application method on the performance of the control agent, since changing these factors may improve the results. Indeed, the current data highlight the importance of such variables, since the percentage of dry plants depended on the adopted application method.

Both tested microorganisms showed potential for the disease management. A biological control agent can limit plant pathogens through several mechanisms, including induced resistance. It can cause substantial changes to the metabolism of plants while interacting with them, promoting not only the activation of defense mechanisms, such as oxidative burst, but also the synthesis of phenols and phytoalexins. In addition, it can increase nutrient availability and tolerance to biotic and abiotic stress (Nachtigal, 2012).

Treatments with *T. harzianum* led to a greater reduction in plant mortality, especially when applied through irrigation and incorporation into hydrogel. Antibiosis is a major component of *T. harzianum*-mediated control, suppressing the development of plant pathogens through secreted metabolites. These metabolites include jasmonic acid/ethylene, which constitute important hormones that regulate the plant immune system (Pascholati et al., 2019). The present data also showed induced resistance, which has been reported for different pathosystems, as well as a significant versatility of this fungus, acting through various mechanisms (Harman et al., 2004; Hoyos-Carvajal et al., 2009; Nachtigal, 2012).

Improved plant metabolism, due to the use of *T. harzianum*, was found in the present study based on the activity of the evaluated enzymes which increased with SI+IR in clone SR and SI+H in clone 144. Peroxidase, polyphenol oxidase and PAL are involved respectively in cell lignification, transformation of phenols into quinones that are toxic to microorganisms, and synthesis of phenylpropanoids which are involved in the plant defense (Stangarlin et al., 2011, Lorenzetti et al., 2018). This may explain the good performance of those treatments when compared to NC and PC.

Peroxidase activity was highlighted for remaining constant and high with treatment SI+IR. This can contribute to disadvantage the disease establishment since such an enzyme is capable of eliminating certain hydrogen atoms from hydroxycinnamic alcohol groups, forming lignin which, added to cellulose and other polysaccharides, constitutes a physical barrier that hinders the penetration of pathogens into the plant (Cavalcanti et al., 2005; Lorenzetti et al., 2018).

The treatment SI+IR can be a good option for controlling this bacterium both in the field and in the greenhouse, since the used products are highly soluble in water. In the field, the biological control agent can be adopted through irrigation while planting the seedlings in infested areas. In the greenhouse, the agent can be used in the irrigation systems since it does not increase the production costs.

Trichoderma species have been shown as a viable alternative to control plant pathogens in orchards or agroforestry. Maciel et al. (2012) found satisfactory results for *Cylindrocladium candelabrum* control with the application of Trichodel® (*Thichoderma* spp.); seedlings treated with this product had a significant decrease in pathogens (32%), compared to control plants (67.35%). Testing *Trichoderma inhamatum* for *Rhizoctonia solani* control in eucalyptus clonal propagation, Mafia et al. (2003) demonstrated the suppressive effect of *T. inhamatum* on the pathogen's saprophytic activity.

In treatments involving *T. harzianum* and clone 144, the positive influence of the fungus was noticeable; the dry mass of plants was much higher, compared to the other treatments. According to Costa & Marenco (2007), the water flow gradient in the plant, which is prevented by pathogenic bacteria, directly influences the water potential of the plant so that any variation in the water potential of the leaf can affect the carbon assimilation by the plant as soon as it closes its stomata and reduces photosynthesis. To favor cell turgor under water stress situations, the plant adjusts its cell metabolism, which can cause energy expenditure and consequently lead to fresh mass loss.

CONCLUSIONS

The two biotic agents were effective in controlling *R. solanacearum*, but the fungus *T. harzianum* stood out as a potential collaborative biological agent for both seedling development improvement and *R. solanacearum* control in eucalyptus culture.

Considering the present results, the application form of biocontrol agents directly influences their effectiveness.

All three evaluated enzymes had increased activity with the treatments involving the biological control agents, which suggests that the plant's biochemical defense was activated.

REFERENCES

Alfenas, A. C., Mafia, R. G., Sartório, R. C., Binoti, D. H., Silva, R. R., Lau, D., & Vanetti, C. A. (2006). *Ralstonia solanacearum* em viveiros clonais de eucalipto no Brasil. *Fitopatologia Brasileira*, *31*(4), 357-366. http://dx.doi.org/10.1590/S0100-41582006000400005.

Alfenas, A. C., Zauza, E. A. V., Mafia, R. G., & Assi, T. F. (2009). *Clonagem e doenças do eucalipto* (2. ed.). Viçosa: UFV.

Amorim, L., Rezende, J. A. M., Bergamin Filho, A., & Camargo, L. E. A. (2016). *Manual de fitopatologia: doenças das plantas cultivadas* (5. ed.). Ouro Fino: Agronômica Ceres.

Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S. M., & Jones, J. B. (2004). Efficacy of plant growth promoting rhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato. *Plant Disease*, *88*(6), 669-673. PMid:30812591. http://dx.doi.org/10.1094/PDIS.2004.88.6.669.

Boava, L. P., Kuhn, O. J., Pascholati, S. F., Di Piero, R. M., & Furtado, E. L. (2010). Efeito de indutores bióticos e abióticos na atividade de quitinase e peroxidase e no controle da ferrugem causada por *Puccinia psidii* em eucalipto. *Summa Phytopathologica*, *36*(2), 168-172. http://dx.doi.org/10.1590/S0100-54052010000200012.

Cavalcanti, L. S., Brunelli, K. R., & Stangarlin, J. R. (2005). Aspectos bioquímicos e moleculares da resistência induzida. In L. S. Cavalcanti, R. M. Di Piero, P. Ci.a, S. F. Pascholati, M. L. V. Resende & R. S. Romeiro (Eds.), *Indução de resistência em plantas a patógenos e insetos* (pp. 81-124). Piracicaba: FEALQ.

Costa, G. F., & Marenco, R. A. (2007). Fotossíntese, condutância estomática e potencial hídrico foliar em árvores jovens de andiroba (*Carapa guianenssi*). *Acta Amazonica*, *37*(2), 229-234. http://dx.doi.org/10.1590/S0044-59672007000200008.

Duangmal, K., & Apenten, R. K. O. (1999). A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum*). *Food Chemistry*, *64*(3), 351-359. http://dx.doi.org/10.1016/S0308-8146(98)00127-7.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001.

Genin, S., & Denny, T. P. (2012). Pathogenomics of the *Ralstonia solanacearum* species complex. *Annual Review of Phytopathology*, *50*(1), 67-89. PMid:22559068. http://dx.doi.org/10.1146/annurev-phyto-081211-173000.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. E., & Lorito, M. (2004). *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nature Reviews. Microbiology*, *2*(1), 43-56. PMid:15035008. http://dx.doi.org/10.1038/nrmicro797.

Hoyos-Carvajal, L., Orduz, S. E., & Bissett, J. (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma. Biological Control*, *51*(3), 409-416. http://dx.doi.org/10.1016/j.biocontrol.2009.07.018.

Indústria Brasileira de Árvores – IBÁ. (2019). *Relatório Industria Brasileira de Árvores.* Retrieved in 2019, November 30, from https://www.iba.org/datafiles/publicacoes/relatorios/iba-relatorioanual2019.pdf

Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, *44*, 693-695.

Kuhn, O. J. (2007). *Indução de resistência em feijoeiro (Phaseolus vulgarSl) por acibenzolar S-metil e Bacillus cereus: aspectos fisiológicos, bioquímicos e parâmetros de crescimento e produção*. Retrieved in 2019, November 30, from https://teses.usp.br/teses/disponiveis/11/11135/tde-05042007-140014/publico/OdairKuhn.pdf

Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J., Chiroleu, F., Wicker, E., & Prior, P. (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology*, *101*(1), 154-165. PMid:20795852. http://dx.doi.org/10.1094/PHYTO-02-10-0048.

Lorenzetti, E., Stangarlin, J. R., Kuhn, O. J., & Portz, R. L. (2018). Induction of resistance to *Macrophomina phaseolina* in soyben treated with rosemary extract. *Summa Phytopathologica*, *44*, 45-50. http://dx.doi.org/10.1590/0100-5405/176895.

Lwin, M., & Ranamukhaarachchi, S. L. (2006). Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. *International Journal of Agriculture and Biology*, *8*, 657-660.

Luangsa-Ard, J., Houbraken, J., van Doorn, T., Hong, S. B., Borman, A. M., Hywel-Jones, N. L., & Samson, R. A. (2011). *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus. FEMS Microbiology Letters*, *321*(2), 141-149. http://dx.doi.org/10.1111/j.1574-6968.2011.02322.x.

Maciel, C. G., Lazarotto, M., Mezzomo, R., Poletto, I., Muniz, M. F. B., & Lippert, D. B. (2012). Control of *Cylindrocladium candelabrum* by *Trichoderma* spp in *Eucalyptus saligna* seedlings. *Revista Árvore*, *36*, 825-832. http://dx.doi.org/10.1590/S0100-67622012000500004.

Mafia, R. G., Alfenas, A. C., Maffia, L. A., Ventura, G. M., & Sanfuentes, E. A. (2003). Encapsulamento de *Trichoderma inhamatum* para o controle biológico de *Rhizoctonia solani* na propagação clonal de Eucalyptus. *Fitopatologia Brasileira*, *28*(1), 101-105. http://dx.doi.org/10.1590/S0100-41582003000100016.

Mori, Y., Inoue, K., Ikeda, K., Nakayashiki, H., Higashimoto, C., Ohnishi, K., Kiba, A., & Hikichi, Y. (2016). The vascular plant pathogenic bacterium *Ralstonia solanacearum* produces biofilms required for its virulence on the surfaces of tomato cells adjacent to intercellular spaces. *Molecular Plant Pathology*, *17*(6), 890-902. PMid:26609568. http://dx.doi.org/10.1111/mpp.12335.

Murthy, K. N., & Srinivas, C. (2015). Extraction and characterization of extracellular polysaccharide from *Ralstonia solanacearum* causing wilt of tomato. *International Journal of Agricultural Science and Research (IJASR)*, *5*, 81-92.

Nachtigal, G. F. (2012). *Espécies de Trichoderma: fungos benéficos a serem favorecidos por práticas adequadas de manejo*. Retrieved in 2020, July 28, from http://www.infobibos.com/Artigos/2012_1/Trichoderma/index.htm

Parveen, S., Ehteshamul-Haque, S., & Ghaffar, A. (1998). Efficacy of *Pseudomonas aureginosa* and *Paecilomysces lilacinus* in the control of Root Rot-Root knot disease complex of some vegetables. *Nematologia Mediterranea*, *26*, 209-212.

Pascholati, S. F., Leite, B., Stangarlin, J. R., & Cia, P. (2008). *Interação planta- patógeno: fisiologia, bioquímica e biologia molecular.* Piracicaba: FEALQ. 627p.

Pascholati, S. F., Souza, V. H. M., & Cardoso Filho, J. A. (2019). Indução de resistência por *Trichoderma*. In C. M. Meyer, S. M. Mazaro & J. C. Silva (Eds.), *Trichoderma*: uso na agricultura. Brasília, DF: Embrapa.

Pastrik, K. H., & Maiss, E. (2000). Detection of *Ralstonia solanacearum* in potato tubers by polymerase chain reaction. *Journal of Phytopathology*, *148*(11-12), 619-626. http://dx.doi.org/10.1046/j.1439-0434.2000.00567.x.

Pierozzi, C. G. (2013). *Fungos sapróbios do semiárido nordestino: aspectos fisiológicos, ação no controle da ferrugem e indução de enraizamento em mudas de eucalipto* (Dissertação de mestrado). Retrieved in 2019, September 1, from https://repositorio.unesp.br/handle/11449/99787.

Romeiro, R. S., & Garcia, F. A. O. (2009). Indução de resistência em plantas a patógenos por eliciadores de natureza bacteriana. In W. Bettiol & M. A. B. Morandi (Eds.), *Biocontrole de doenças de plantas: uso e perspectivas*. Jaguariúna: Embrapa Meio Ambiente.

Schell, M. A. (2000). Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annual Review of Phytopathology*, *38*(1), 263-292. PMid:11701844. http://dx.doi.org/10.1146/annurev.phyto.38.1.263.

Stangarlin, J. R., Kuhn, O. J., Toledo, M. V., Portz, R. L., Schwan-Estrada, K. R. F., & Pascholati, S. F. (2011). A defesa vegetal contra fitopatógenos. *Scientia Agrária Paranaensis*, *10*, 18-46.

Valls, M., Genin, S., & Boucher, C. (2006). Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum. PLoS Pathogens*, *2*(8), 82-99. PMid:16933989. http://dx.doi.org/10.1371/journal.ppat.0020082.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., & Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Douderoff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. & *Ralstonia eutropha* (DavSl 1969) comb. nov. *Microbiology and Immunology*, *39*(11), 897-904. PMid:8657018. http://dx.doi.org/10.1111/j.1348-0421.1995.tb03275.x.

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