PREVENTIVE AND CURATIVE CONTROL OF *Oidium eucalypti* IN *Eucalyptus benthamii* CLONAL SEEDLINGS[1]

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ABSTRACT – One of the main diseases occurring in clonal nurseries of *Eucalyptus* spp. is powdery mildew caused by the fungus *Oidium eucalypti*. This work evaluated the efficiency of biological products based on *Trichoderma* spp. and *Bacillus* spp. applied as preventive and curative treatments for the control of *Oidium eucalypti* in clonal seedlings of *E. benthamii* Maiden et Cambage. Treatments were: *Trichoderma harzianum* (THP), *T. atroviride* (TAI), *T. harzianum* (THE), *Trichoderma* spp. (TTA), *Bacillus* spp. (BNE), Sulfur (KUM), Difenoconazol (SCO) and distilled water (AD). Treatments were applied preventively by spraying seven days before inoculation of the pathogen, and in curatively, ten days after inoculation. Weekly evaluations of the incidence and severity of the disease were done. The analyzed variables were: Disease Index (ID) and Incidence (I), calculating the area under the disease curve (AUDPC) for each treatment. The results pointed BNE (*Bacillus* spp.) and TAI (isolate *T. atroviride*) as effective preventive treatments and BNE (*Bacillus* spp.), applied curatively for the control of *O. eucalypti* in *E. benthamii* seedlings, proving that treatments based on biological products may be effective for controlling eucalyptus powdery mildew in clonal nurseries.

Keywords: *Bacillus* spp.; Forest production; Powdery Mildew.

CONTROLE PREVENTIVO E CURATIVO DE Oidium eucalypti *EM MUDAS CLONAIS DE* Eucalyptus benthamii

RESUMO – Uma das principais doenças que ocorrem nos viveiros clonais de **Eucalyptus** *spp. é o oídio causado pelo fungo* **Oidium eucalypti**. *O objetivo deste trabalho foi avaliar a eficiência de produtos biológicos baseados em* **Trichoderma** *spp. e* **Bacillus** *spp. aplicados na forma de tratamentos preventivos e curativos para o controle de O.* **eucalypti** *em mudas clonais de* **E. benthamii** *Maiden et Cambage. Os tratamentos foram:* **Trichoderma harzianum** *(THP), T.* **atroviride** *(TAI), T.* **harzianum** *(THE),* **Trichoderma** *spp. (TTA),* **Bacillus** *spp. (BNE), Enxofre (KUM), Difenoconazol (SCO) e água destilada (AD). Estes foram aplicados preventivamente por pulverização sete dias antes da inoculação do patógeno, e, de forma curativa, dez dias após a inoculação. Foram realizadas avaliações semanais da incidência e severidade da doença. As variáveis analisadas foram: Índice de Doença (ID) e Incidência (I), calculando-se a área abaixo da curva de progresso da doença (AACPD) para cada tratamento. Os resultados evidenciaram BNE (***Bacillus** *spp.) e TAI (***T. atroviride** *isolado) como efetivos quando aplicados preventivamente, e o tratamento BNE (***Bacillus** *spp.) também efetivo em aplicação curativa para o controle biológico de O. eucalypti em mudas clonais de* **E. benthamii**, *comprovando-se que tratamentos baseados em produtos biológicos podem ser eficazes para controlar o oídio no eucalipto nos viveiros clonais.*

Palavras-Chave: **Bacillus** *spp.; Produção florestal; Oídio.*

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1. INTRODUCTION

Eucalypt plantation in Brazil is intensive, and, in the state of Rio Grande do Sul, it has occupied significant areas of production. The plantations are established mainly with cuttings from selected mother trees, using criteria that express high productivity. According to a report by the Indústria Brasileira de Árvores (IBÁ, 2017), the total area planted with trees was 7.84 million ha in 2016, a growth of 0.8% in relation to the previous year. The report also states that eucalypt plantations occupy 5.6 million ha in the country and, in the last five years, the growth of the eucalypt area was 2.8% per year.

Among the species planted in the country, *Eucalyptus benthamii* Maiden et Cambage presents as peculiar characteristics its resistance to frost, good stem shape, rusticity and fast growth, predominating in clonal forest plantations in southern Brazil. Its characteristics intensified the demand for seedlings, demanding for in-depth studies on aspects relevant to the species production in nursery conditions (Schultz, 2011). During the production of eucalypt seedlings in nurseries, pathogen attacks are frequent, especially those caused by fungi, which reduce the production of seedlings, causing economic losses, depending on the species attacked, time of the year (Santos et al., 2001) and the stage of seedling development.

One of the main diseases causing damage in *E. benthamii* clonal mini gardens is powdery mildew, caused by the fungus *Oidium eucalypti*. This pathogen has greater incidence in young leaves and shoots, occurring mainly in greenhouse and clonal mini garden, causing distorted leafs and over-sprouting of the plants, causing, in more severe cases, death of the seedlings (Krugner and Auer, 2005). Due to the high conidia dissemination capacity, this fungus rapidly infects healthy ministumps, causing infestation throughout the clonal mini garden.

The fungicide Priori Top©, composed of azoxystrobin and difenoconazole, was recently registered in the Ministry of Agriculture, Livestock and Supply for the control of *O. eucalypti* in eucalypt plantation. However, according to Campanhola and Bettiol (2003), alternatives have already been proposed with cultural control, genetic and natural products. Thus, other forms of control can also be used, alternatively or in joint use, since the best way to maintain a controlled pathogen population is the combination of techniques, namely integrated management.

An alternative to chemical control, for example, is the use of biological control agents such as *Trichoderma* spp. and *Bacillus* spp., which have been recommended as potential biocontrollers of several plant pathogens, especially in the last decades. The capacity of *Trichoderma* spp. of acting as a biocontrol agent has been reported for more than 60 years, and many isolates are classified as plant symbionts and actuators in the control of phytopathogens (Brotman et al., 2010).

The species of the *Trichoderma* genus are among the most studied antagonists and are found naturally in different types of soil. They act against phytopathogens by different mechanisms, such as: antibiosis, nutrient and substrate competition, mycoparasitism, cell wall degrading enzymes production, plant growth promotion and resistance inducers (Harman et al., 2004; Shoresh et al., 2005; Viterbo et al., 2005; Vinale et al., 2008). *Trichoderma* spp. are used to control important diseases such as those caused by *Botrytis cinerea* (Sanz et al., 2005), *Rhizoctonia solani* (Ruocco et al., 2009), *Pythium ultimum* (Montero et al., 2011), *Fusarium oxysporum* (Carvalho et al., 2011) and *Moniliophthora perniciosa* (Simões et al., 2012).

Bacillus species are antagonists of phytopathogenic fungi and can be used in biological control (Angonese et al., 2009). According to Bettiol and Morandi (2009), within the genus *Bacillus*, the species *Bacillus subtilis* and *B. pumilus* are able to inhibit phytopathogenic bacteria and fungi. *Bacillus* spp. have the ability to control diseases caused by *Fusarium moniliforme* (Bacon et al., 2001), *Colletotrichum acutatum* (Kupper et al., 2003), *Erwinia carotovora* (Dong et al., 2004), *Botrytis cinerea* (Hang et al., 2005) and *Puccinia psidii* (Raasch et al., 2012), which cause great damage to the affected crops.

The objective of this work was to evaluate the efficiency of biological products based on *Trichoderma* spp. and *Bacillus* spp. applied preventively and curatively to control *Oidium eucalypti* in tree cuttings of *E. benthamii*.

2. MATERIAL AND METHODS

2.1 Place of experiments, seedlings origin and inoculum

The experiments were done from January to March of 2016 in a greenhouse belonging to the Laboratory of Plant Bacteriology of the Plant Protection Department of the Federal University of Rio Grande do Sul (UFRGS), in Porto Alegre, RS.

Cuttings of *Eucalyptus benthamii* with approximately 90 days old were provided by CMPC - Celulose Riograndense nursery's company, located in Barra do Ribeiro, RS (Geographical coordinates: 30°20'38.3" S 51°14'43.9" O). The seedlings were immediately brought to the greenhouse, where they were put in trays with 50 cm^3 containers and maintained with daily manual irrigation, moistening only the substrate during the experiment.

The *Oidium eucalypti* isolate was obtained from the clonal mini garden, a natural source of the pathogen occurrence, belonging to the same company that supplied the seedlings. The inoculum was kept in ministumps that remained in a temperature-controlled room (23 ± 2ºC) and 12 h photoperiod. For the inoculation procedure, in the ministumps and in the seedlings that received the treatments, powdery mildew were collected from leaves infected naturally in the clonal mini garden and removed by surface scraping with the aid of a brush, and were inoculated in the upper third of healthy seedlings.

2.2 Preventive and curative treatments

Five biological control agents were tested as treatments for preventive and curative control: Predatox® (*Trichoderma* spp.); Ecotrich® (*Trichoderma harzianum*); Trichodel aéreo ® (*Trichoderma harzianum*); Nemathel® (*Bacillus* spp.) and *Trichoderma atroviride* liquid suspension of conidia. The latter was isolated from *Pinus* sp. (Lazarotto et al., 2016). For all biocontrol agents, the concentration of 10^7 conidia mL^{-1} or CFU mL^{-1} was used. Two fungicides were used as positive controls to compare the efficacy of biocontrol agents: Kumulus®, (sulfur at 0.3 g 100 mL^{-1} of distilled water); and Score®, (difenoconazole at 0.03 mL 100 mL^{-1} of distilled water), as well as a control treatment using water (Table 1). Preventive treatments were composed of 4 replicates of 6 seedlings for each treatment, while curative treatments were composed of 4 replicates of 10 seedlings for each treatment. Both experiments were designed in a completely randomized design.

The same volume of suspension was used at the concentration of 10^7 conidia or cells mL^{-1}, and one drop of Tween 80 was added. The dilution of each product was determined from the concentration established on the labels, except for the suspension of conidia of *T. atroviride*, which was counted in a Neubauer chamber. For fungicide Score® the recommended dose for rose powdery mildew (*Sphaerotheca panossa*) of the label of the commercial product was used (30 mL 100 L^{-1} of water), and for fungicide Kumulus®, Alfenas et al. (2009) recommendation for eucalypt powdery mildew (3 g L^{-1} of water) was used.

Table 1 – Description of the biological and chemical treatments used for the control of powdery mildew in *Eucalyptus benthamii*. Porto Alegre, RS, 2016.
***Tabela 1** – Descrição dos tratamentos biológicos e químico utilizados para controle do oídio em **Eucalyptus benthamii**. Porto Alegre, RS, 2016.*

Treatment	Acronym	Composition	Commercial Brand
	THP	*Trichoderma harzianum*	Predatox®
	THE	*Trichoderma harzianum*	Ecotrich®
Biological	TTA	*Trichoderma* spp.Trichodel	aéreo®
	TAI	*Trichoderma atroviride*	Collection isolated
	BNE	*Bacillus* spp.	Nemathel®
Chemical	KUM	Sulfur	Kumulus®
	SCO	Difenoconazol	Score®
Control	AD	Distilled water	-

®Trade mark.

The application of preventive and curative treatments was performed by spraying with hand sprayers for each product tested. Treatments were sprayed preventively seven days before pathogen inoculation, in the adaxial and abaxial faces of all leaves until runoff. One week later, the pathogen was inoculated with a brush by scraping conidia present on infected leaves, followed by transferring them to the upper third of the seedlings. The seedlings remained in a growth chamber with a temperature of 23 ± 2°C and 12 h photoperiod, for 56 days.

The curative treatments followed the same procedure, but were applied 10 days before the inoculation. Treatments were applied in the adaxial and abaxial faces of all the leaves until runoff, and the seedlings remained in greenhouse at ambient temperature conditions for 37 days.

The severity of the disease in eucalypt seedlings was evaluated weekly. The descriptive scale presented in Table 2 was used for severity evaluation. The notes of this descriptive scale were used to calculate the disease index (DI, ranging from 0 to 1), expressed by the equation:Eq.1

$$DI = \frac{\Sigma(Y * Xy)}{Xt * h}$$

in which Y is the note obtained in the scale, X_y is the number of plants with Y note, X_t is the total number of plants and h is the maximum value of the scale (Mckinney, 1923).

Disease incidence was determined by the number of infected seedlings weekly. Severity and incidence values of each treatment were used to calculate the area under the disease progress curve (AUDPC) (Campbell and Madden, 1990), according to the formula below:Eq.2

$$AUDPC = \sum\left[\left(\frac{y_1 + y_2}{2}\right) * (t_2 - t_1)\right]$$

y_1: evaluation note in time t_1

y_2: consecutive evaluation note in time t_2

$t_2 - t_1$: interval in days between two consecutive evaluations

For each variable analyzed, the average of the data was calculated and the normality of the data was confirmed. The analysis of variance was performed, and the averages compared by Tukey's test at 5% probability using SAS 9.4.

3. RESULTS

A significant reduction of the DI with the treatments TAI (*Trichoderma atroviride*) and BNE (*Bacillus* spp.) was observed with the preventive treatments (Table 3). All treatments reduced the incidence of the disease, except TTA for AUDPC (DI), and TTA and THP treatments that were statistically equal for AUDPC (I).

Regarding the Final incidence, evaluated 56 days after inoculation, the control treatment (AD) presented high disease incidence (83.3%), and was statistically equal to the treatments SCO (87.5%) and TTA (95.8%). The treatments with the lowest Final incidence were TAI (29.2%) (*T. atroviride*), and BNE (41.7%) (*Bacillus* spp.). This demonstrates that even if the fungicide SCO is recommended for the control of other powdery mildew species, it was not effective for the preventive control of eucalypt powdery mildew. However, KUM, at the recommended dose in the literature for the control eucalypt powdery mildew, reduced disease incidence in relation to the control. Nonetheless, it was less effective than the previously mentioned biological treatments.

Table 2 – Descriptive scale used to evaluate the severity of powdery mildew in cuttings of *Eucalyptus benthamii*. Porto Alegre, RS, 2016.

***Tabela 2** – Escala descritiva utilizada para avaliar a severidade de oídio em mudas clonais de **Eucalyptus benthamii**. Porto Alegre, RS, 2016.*

Score	Description of disease severity	Visual symptoms
0	No symptoms	Absent
1	Low infection	Presence of mycelium of the fungus on the leaves, without sporulation
2	Medium infection	Sporulation on the first pairs of leaves
3	High infection	Deformation, shrouding and leaf fall

Table 3 – Evaluation of the final McKinney Disease Index (final DI), Final Incidence (Final I) and Area Under the Disease Progress Curve (AUDPC) of DI and I, data in percentage (%) of preventive treatments applied by foliar spray in cuttings of *Eucalyptus benthamii* for the control of *Oidium eucalypti*. Porto Alegre, RS, 2016.

***Tabela 3** – Avaliação do Índice de Doença de McKinney final (DI final), da Incidência final (I final) e das Áreas Abaixo da Curva de Progresso da Doença (AUDPC) de DI e I, valores em porcentagem (%) dos tratamentos preventivos aplicados via foliar em mudas clonais de **Eucalyptus benthamii** para o controle de **Oidium eucalypti**. Porto Alegre, RS, 2016.*

Treatment	DI final	AUDPC (DI)	AUDPC (I)	I final
AD	49.2A	694.7AB*	1560.4A	83.3A
KUM	45.5A	566.8ABC	1137.5AB	66.7AB
SCO	51.4A	422.9BCD	918.8ABC	87.5A
THP	46.4A	771.5AB	1647.9A	70.8AB
THE	44.4A	481.3ABCD	1108.3ABC	75.0AB
BNE	24.0B	278.5CD	612.5BC	41.7BC
TTA	53.6A	800.1A	1677.1A	95.8A
TAI	12.2B	126.4D	335.4C	29.2C
p Value	< 0.0001	<0.0001	<0,0001	<0.0001

*Averages followed by the same letter in the columns are not different by Tukey's test at 5% significance. AD: Control; KUM: fungicide Kumulus®; SCO: fungicide Score®; THP: Predatox® (*T. harzianum*); THE: Ecotrich® (*T. harzianum*); BNE: Nemathel ® (*Bacillus* spp.); TTA: Trichodel Aéreo® (*Trichoderma* spp.); TAI: *T. atroviride*.

The TTA treatment (*Trichoderma* spp.) obtained the highest AUDPC values (800.1% and 1677.1%) for disease severity and incidence (53.6% and 95.8%, respectively), with no significant difference compared to the others *Trichoderma* biological preventive treatments, except TAI (*Trichoderma atroviride*). This show that probably then could have different effects on the action and effectiveness of biological agents even if they are antagonists of the same genus. The lowest values of AUDPC, severity and incidence, observed in the TAI treatment (*Trichoderma atroviride*), confirm its efficiency in the control of eucalypt powdery mildew, when used preventively (Table 3).

Curative treatments to control *Oidium eucalypti* showed that BNE (*Bacillus* spp.) had greater efficiency (Table 4). This treatment showed the lowest values of AUDPC for severity (394.2%) and incidence (798.8%). For this last variable, it was statistically equal to the chemical treatment KUM. In addition, it presented lower final incidence of disease (10%) differing significantly from the control and THP, TTA and TAI treatments (Table 4). It is highlighted here difference in the behavior of TAI (*Trichoderma atroviride*), since this treatment (TAI) was similar in terms of efficiency with BNE in a preventive way, which was not observed in the curative treatments.

Table 4 – Evaluation of the final McKinney Disease Index (final DI), Final Incidence (Final I), and Area Under the Disease Progress Curve (AUDPC) of DI and I, data in percentage (%) of curative treatments applied by foliar spray in cuttings of *Eucalyptus benthamii* for the control of *Oidium eucalypti*. Porto Alegre, RS, 2015.

Tabela 4 – *Avaliação do Índice de Doença de McKinney final (DI final), da Incidência final (I final) e das Áreas Abaixo da Curva de Progresso da Doença (AUDPC) de DI e I, valores em porcentagem (%), dos tratamentos curativos aplicados via foliar em mudas clonais de Eucalyptus benthamii para o controle de Oidium eucalypti. Porto Alegre, RS, 2015.*

Tratamento	DI final	AUDPC (DI)	AUDPC (I)	I final
AD	19.2ns	789.6*AB	1743.8AB	52.5A
SCO	16.7	685.0AB	1355.0ABC	30.0ABC
KUM	12.5	512.1AB	913.8C	17.5BC
THP	20.8	790.4AB	1981.3AB	57.5A
THE	16.7	654.6AB	1300.0BC	35.0ABC
BNE	6.7	394.2B	798.8C	10.0C
TTA	18.3	846.3AB	1953.8AB	45.0AB
TAI	24.2	966.3A	2011.3A	52.5A
p Valor	0.1774	0.0228	<0.0001	<0.0002

* Averages followed by the same letter in the columns are not different by Tukey's test at 5% significance. AD: Control; KUM: fungicide Kumulus®; SCO: fungicide Score®; THP: Predatox® (*T. harzianum*); THE: Ecotrich® (*T. harzianum*); BNE: Nemathel ® (*Bacillus* spp.); TTA: Trichodel Aéreo® (*Trichoderma* spp.); TAI: *T. atroviride*.

Fungicide treatments, SCO and KUM, also reduced the incidence and, or, severity of powdery mildew in eucalypt seedlings, although, they did not present a significant difference compared to the biological treatments.

4. DISCUSSION

The significant preventive and curative effects of the biological treatments TAI and BNE applied to the control of *Oidium eucalypti* make it possible to establish alternatives for the control of this pathogen in *Eucalyptus benthamii* seedlings. The efficiency of BNE (*Bacillus* spp.) in preventive and curative control of eucalypt powdery mildew may be associated to the antimicrobial production, such as lipopeptides, which may be of three types: fengicins, iturin and surfacins (Ongena and Jacques, 2008) and several substances that inhibit other microorganisms (Bettiol et al., 2005).

Bacillus subtilis acts directly or indirectly in the control of plant diseases (Ongena et al., 2007; Leelasuphakul et al., 2008). The direct antagonism exerted against phytopathogens occurs through antibiosis, synthesis of antimicrobial substances, parasitism, competition for space and nutrients, and the synthesis of volatile compounds (Leelasuphakul et al., 2008). This may explain the action of BNE in the preventive control of eucalypt powdery mildew. *Bacillus* species have the ability to produce endospores that are resistant to high temperatures and survive in adverse environmental conditions (Collins and Jacobsen, 2003), favoring their action as agents of biological control. According to Kupper et al. (2003), generally, microorganisms that act by antibiosis have broad spectrum of action, so that the production of toxic substances is more effective than any other mechanism of action involved in the inhibition of fungi.

For eucalypt species, the reduction in disease incidence caused by *Puccinia psidii* in two hybrids of *E. urophylla* and *E. grandis* was significant for one of the clones using treatments with inoculation of Rizolyptus® (*Bacillus subtilis*) in minicuttings and in the substrate with reduction of 28.1 and 40.1% of the disease compared to the control treatment. Reduction of disease severity was obtained for the two clones, by 45.9% for one clone and from 65.7 to 70.9% for the other one (Raasch et al., 2012).

A study by Romero et al. (2004) reported four strains of *Bacillus subtilis* as potential biocontrol agents against powdery mildew caused by *Podosphaera fusca*, a pathogen of cucurbitaceae. The relevant role of iturins and fengicins in the antifungal activity and in the control of the cucurbit powdery mildew has been demonstrated (Romero et al., 2007b). In addition, these strains controlled powdery mildew under greenhouse conditions and demonstrated their fermentation potential for large-scale production (Romero et al., 2007a). According to Ashwini and Srividya (2014), *Bacillus subtilis* not only produces antibiotics but also produces different types of enzymes such as glucanase, chitinase, and cellulase, which also play a very important role in the antagonistic property through the mechanism mediated by lytic enzymes.

The forms of action of antagonistic microorganisms could be attributed to the different mechanisms of action regarding biocontrol agents. *Trichoderma* species are capable of acting through competition for space and nutrients, production of volatile antibiotics and hydrolytic enzymes such as chitinase and â-1,3-glucanase (Chutrakul et al., 2008; Sharma et al., 2009). Hydrolytic enzymes partially degrade the cell wall of the pathogen and lead to parasitism (Kubicek et al., 2001). There may also be a direct interaction between the pathogen itself and the biocontrol agent, in the case of mycoparasitism, which involves physical contact and synthesis of hydrolytic enzymes, toxic compounds and, or, antibiotics that act synergistically with the enzymes. Fungi of the genus *Trichoderma* can even exert positive effects on plants, with an increase in their growth, in the case of application by biofertilization, and also by stimulating plant defense mechanisms (Benitéz et al., 2004).

Biological control agents are living organisms and their activities depend mainly on the different physicochemical conditions that are subjected. The understanding of *Trichoderma* intraspecific genetic diversity and its biocontrol mechanisms result in better implementation of the selected isolates as biocontrol agents (Benitéz et al., 2004). Also, the use of *Trichoderma* spp., isolated *in vitro* and *in vivo*, is considered an excellent biocontrol agents and has the advantage of being less harmfull to humans (Melo, 1996) and does not cause negative impacts on the environment (Patrício et al., 2001), contrasting with the toxic action caused by chemical fungicides. These studies reiterate the results obtained with the present study in which the treatment TAI (*T. atroviride*) showed a greater efficiency in the control of eucalypt powdery mildew when preventively applied.

Although the biological treatments THE, TTA, TAI, THP and the chemical fungicide SCO did not show significant differences from the control, the former are still more advantageous, since these microorganisms can promote plant growth, as already evidenced in several studies (Amaral et al., 2017; Chagas et al., 2017; Gonçalves et al., 2018). Carvalho Filho et al. (2008) concluded that the isolate CEN 262 of *Trichoderma* spp. provided greater index of development of shoots in eucalypt seedlings. Some strains of *Trichoderma* have the ability to endophytically colonize different organs of the plant (Rubini et al., 2005; Silva et al., 2006). In addition to controlling disease, it would be possible to increase the production of minicuttings in clonal eucalypt mini gardens, an effect that does not occur with the use of chemical fungicides.

All the biological treatments applied both preventively and curatively, reduced AUDPC. A study by Belan et al. (2013) using alternative treatments for cucumber (*Cumumis sativus* L.) powdery mildew control, observed that the treatment with distilled water used as negative control obtained the lowest reduction of the fungus population, except when compared to the alcoholic extract of propolis treatment.

Several studies point out that chemicals are not as effective in controlling diseases caused by *Oidium eucalypti*. Picinini et al. (2003) using different fungicides for the treatment of wheat seeds, observed that difenoconazole, applied at the dose of 30 g a.i 100 kg^{-1} seeds showed greater severity of powdery mildew (*Blumeria graminis* f. sp. *tritici*) at 60 days after plant emergence when compared to another active principle, such as fluquinconazole. This demonstrates that a chemical fungicide based on difenoconazole is not efficient in the control of *Oidium eucalypti* and other species of the fungus. As for Pavanello et al. (2015), the fungicide difenoconazole controlled brown rot, caused by the fungus *Monilinia fructicola*, until harvest, presenting a 0.84% of rot incidence caused by the fungus, whereas the control presented 10.2%.

The results of the present study suggest that it is possible to opt for the use of preventive and curative treatments based on biological antagonists rather than a chemical treatment, which may favor the increase of disease incidence during growth of seedlings. Biological treatments by spraying biological control agents could be considered a viable alternative for the control of powdery mildew, such as *Oidium eucalypti* in replacement for the chemical fungicides that cause genetic resistance to pathogens due to their frequent use in the combat against a certain microorganisms, besides leading to residual harmful effects for workers' health and to the environment.

5. CONCLUSIONS

Biological treatments with *Trichoderma atroviride* and *Bacillus* spp. can be applied preventively, and the treatment based on Bacillus spp. can be applied after the appearance of disease symptoms to control *Oidium eucalypti* in cuttings of *Eucalyptus benthamii* under controlled environment and greenhouse conditions.

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7. REFERENCES

Alfenas AC, Zauza EAV, Mafia RG, Assis TF. Clonagem e doenças do eucalipto. 2ª ed. Viçosa, MG: Editora UFV; 2009.

Amaral PP, Steffen GPK, Maldaner J, Missio EL, Saldanha CW Promotores de crescimento na propagação de caroba. Pesquisa Florestal Brasileira. 2017;37:149-57

Angonese MT, Della-Giustina J, Paim LH, Pansera MR, Pagno RS, Mezzomo F, et al. Fungistatic effect of *Bacillus* spp. on plant pathogenic fungi. Revista Brasileira de Agroecologia. 2009;4:97-100.

Ashwini N, Srividya S. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. 3 Biotech 2014;4:127-136.

Bacon CW, Yates IE, Hinton DM, Meredith F. Biological control of *Fusarium moniliforme* in maize. Environmental Health Perspectives. 2001;109:325-32.

Belan LL, Pereira AJ, Oliveira MJV, Barbosa DHSG, Jesus Junior WC, Alves FR. Manejo alternativo do oídio na cultura do pepino em ambiente protegido. Revista Acadêmica Ciências Agrárias e Ambientais. 2013;11:S103-S112.

Benitéz T, Ricón AM, Limón MC, Códon AC Biocontrol mechanisms of Trichoderma strains. International Microbiology. 2004;7:249-60.

Bettiol W, Ghini R, Morandi MAB. Alguns métodos alternativos para o controle de doenças de plantas disponíveis no Brasil. In: Venzon M, Paula Júnior TJ, Pallini A, editores. Controle alternativo de pragas e doenças. Viçosa, MG: EPAMIG/CTZM; 2005. p.163-83.

Bettiol W, Morandi MAB. Biocontrole de doenças de plantas: uso e perspectivas. Jaguariúna: Embrapa Meio Ambiente; 2009.

Brotman Y, Gupta, KJ, Viterbo A. Trichoderma. Current Biology. 2010;20:R390 R391.

Campbell CL, Madden LV. Introduction to plant disease epidemiology. New York: Wiley-Interscience; 1990.

Campanhola C, Bettiol W. Métodos alternativos de controle fitossanitário. Jaguariúna: Embrapa Meio Ambiente; 2003.

Carvalho Filho MR, Mello SCM, Santos RP, Menêzes JE. Avaliação de isolados de Trichoderma na promoção de crescimento, produção de ácido indolacético in vitro e colonização endofítica de mudas de eucalipto. Embrapa Recursos Genéticos e Biotecnologia; 2008. (Boletim de Pesquisa e Desenvolvimento, 226).

Carvalho DCD, Mello SCM, Lobo Júnior M, Silva MC. Controle de *Fusarium oxysporum* f.sp. *phaseoli in vitro* e em sementes, e promoção do crescimento inicial do feijoeiro comum por *Trichoderma harzianum*. Tropical Plant Pathology. 2011;36:28-34.

Chagas LFB, Chagas Junior AF, Soares LP, Fidelis RR. Trichoderma na promoção do crescimento vegetal. Revista de Agricultura Neotropical. 2017;4:97-102.

Chutrakul C, Alcocer M, Bailey K, Peberdy JF. The production and characterization of Trichotoxin peptaibols by *Trichoderma asperellum*. Chemistry and Biodiversity. 2008;5:1694-706.

Collins C, Jacobsen BJ. Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet cercospora leaf spot. Biological Control. 2003;26:153-61.

Dong YH, Zhang XF, Xu JL, Zhang LH. Insecticidal *Bacillus thuringiensis* silences *Erwinia carotovora* virulence by a new form of microbial antagonism, signal interference. Applied and environmental microbiology. 2004;70:954-60.

Gonçalves AH, Chagas LFB, Santos GR, Fidelis RR, C Filho MR, Miller LO, Chagas Junior AF Trichoderma efficiency in the maintenance and productivity of soybean plants in producing savanna regions, Tocantins, Brazil. Revista de Ciência Agrárias. 2018;41:175-81.

Hang NTT, Oh SO, Kim GH, Hur JS, Koh YJ. *Bacillus subtilis* S1-0210 as a biocontrol agent against *Botrytis cinerea* in strawberries. The Plant Patology Journal. 2005;21:59-63.

Harmam GE, Howell CR, Viterbo A, Chet I, Lorito M. Trichoderma species opportunistic, avirulent plant symbionts. Nature Reviews Microbiology. 2004;2:43-56.

Indústria Brasileira de Árvores - IBÁ. Relatório Anual 2017. [acessado em: 01. out. 2018] https://iba.org/datafiles/publicacoes/pdf/iba-relatorioanual2017.pdf.

Krugner TL, Auer CG. Doenças do eucalipto. In: Kimati H, Amorim L, Bergamin Filho A., Camargo LEA, Rezende JAM, editores. Manual de fitopatologia: doenças das plantas cultivadas. 4th ed. São Paulo: Agronômica Ceres; 2005.

Kubicek CP, Mach RL, Peterbauer CK, Lorito M. Trichoderma: From genes to biocontrol. Journal of Plant Pathology. 2001;83:11-23.

Kupper KC, Gimenes-Fernandes N, Goes A. Controle biológico de *Colletotrichum acutatum*, agente causal da queda prematura dos frutos cítricos. Fitopatologia Brasileira. 2003;28:251-7.

Lazarotto M, Oliveira LS, Harakava R, Zanatta P, Farias CRJ. Identificação de fungos emboloradores em madeira de *Pinus* spp. em laboratório. Floresta e Ambiente. 2016;23:602-5.

Mckinney HH. Influence of soil, temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Researc. 1923;26:195-217.

Leelasuphakul W, Hemmanee P, Chuenchitt S. Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. Postharvest Biology and Technology. 2008;48:113-21.

Melo IS. Trichoderma e Gliocladium como bioprotetores de plantas. Revisão Anual de Patógenos de Plantas. 1996;4:261-95.

Montero MB, Hermosa R, Cardoza RE, Gutiérrez S, Monte E. Functional analysis of the *Trichoderma harzianum* nox1 gene, encoding an NADPH oxidase, relates production of reactive oxygen species to specific biocontrol activity against *Pythium ultimum*. Applied and Environmental Microbiology. 2011;77:3009-16.

Ongena M, Jacques P. Bacillus lipopeptides: Versatile weapons for plant disease control. Trends in Microbiology. 2008;16:115-25.

Ongena M, Jourdan E, Adam A, Paquot M, Brans A, Joris B, Arpigny JL, Thonart P. Surfactin and fengycin lipopeptides of Bacillus subtilis as elicitors of induced systemic resistance in plants. Environmental Microbiology . 2007;9:1084-1090.

Pavanello EP, Brackmann A, Thewes FR, Both V, Santos JRA, Schorr MRW. Eficiência de fungicidas no controle da podridão parda do pessegueiro e sua relação com parâmetros fisiológicos dos frutos. Semina: Ciências Agrárias. 2015;36:67-76.

Patrício FRA, Kimati H, Barros BC. Seleção de isolados de *Trichoderma* spp. antagônicos a *Pythium aphanidermatum* e *Rhizoctonia solani.*

Summa Phytopathologica. 2001;27:223-9.

Picinini EC, Fernandes JMC. Efeito do tratamento de sementes com fungicida sobre o controle de doenças na parte aérea do trigo. Fitopatologia Brasileira. 2003;28:515-20.

Raasch LD, Bonaldo SM, Oliveira AAF. Rizolyptus® na proteção de miniestacas de eucalipto contra *Puccinia psidii*. Enciclopédia Biosfera. 2012;8:854-64.

Romero D, Pérez-García A, Rivera ME, Cazorla FM, De Vicente A. Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. Applied Microbiology Biotechnology. 2004;64:263-9.

Romero D, De Vicente A, Rakotoaly RH, Dufour SE, Veening JW, Arrebola E, et al. The iturin and fengycin families of lipopeptides are key factors in antagonism of Bacillus subtilis toward *Podosphaera fusca.* Molecular Plant-Microbe Interactions. 2007b;20:430-40.

Romero D, De Vicente AD, Zeriouh H, Cazorla FM, Fernández-Ortuño D, Torés JA, et al. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. Plant Pathology. 2007a;56:976-86.

Rubini MR, Silva-Ribeiro RT, Pomella AWV, Maki CS, Araújo WL, Santos DR, et al. Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. International Journaul Biology Science. 2005;1:24-33.

Ruocco M, Lanzuise S, Vinale F, Marra R, Turrà D, Woo SL, et al. Identification of a new biocontrol gene in *Trichoderma atroviride*: the role of an ABC transporter membrane pump in the interaction with different plant-pathogenic Fungi. Molecular Plant Microbe Interactions. 2009;22:291-301.

Santos AF, Auer CG, Grigoletti Júnior A. Doenças do eucalipto no sul do Brasil: identificação e controle. Colombo: Embrapa Florestas; 2001.

Sanz L, Montero M, Redondo J, Llobell A, Monte E. Expression of an a-1,3-glucanase during mycoparasitic interaction of *Trichoderma asperellum*. The FEBS Journal. 2005;272:493-9.

Schultz B. Levantamento de doenças bióticas e abióticas em *Eucalyptus benthamii* Maiden nos estados do Paraná e Santa Catarina [dissertação] Curitiba: Universidade Federal do Paraná; 2011.

Silva RLO, Luz JS, Silveira EB, Cavalcante UMT. Fungos endofíticos em *Annona* spp.: isolamento, caracterização enzimática e promoção de crescimento em mudas de pinha. Acta Botânica Brasileira. 2006;23:649-55.

Sharma K, Kumar M, Misra RM. Morphological, biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as biocontrol agents. Journal of Phytopathology. 2009;157:51-6.

Shoresh M, Yedidia I, Chet I. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. Phytopathology. 2005;95:76 84.

Simões MLG. Evaluation of Trichoderma spp. for the Biocontrol of *Moniliophthora perniciosa* Subgroup 1441. Journal of Biology and Life Science. 2012;3:18-36.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, et al. A novel role for Trichoderma secondary metabolites in the interactions with plants. Physiological and Molecular Plant Pathology. 2008;72:80 6.

Viterbo A, Harel M, Horwitz BA, Chet I, Mukherjee PK. Trichoderma mitogen activated protein kinase signaling is involved in induction of plant systemic resistance. Applied and Environmental Microbiology. 2005;71:6241 6.