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# Antagonistic potential of *Trichoderma harzianum* and *Purpureocillium lilacinum* on *Ceratocystis fimbriata* in soil cultivated with eucalyptus

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**ABSTRACT**

Antonio, G.L.; Firmino, A.C. **Potencial Antagônico de *Trichoderma harzianum* e *Purpureocillium lilacinum* contra *Ceratocystis fimbriata* em solo cultivado com eucalipto.** ***Summa Phytopathologica,*** v.50, p.1-6, 2024.

Cultivation of eucalyptus is of great importance in Brazil. *Ceratocystis* wilt is a disease of great economic relevance in the country, affecting eucalyptus, cacao and mango crops. New alternatives are needed for the management of this disease because infected plants frequently have to be removed from the area, causing losses to the farmers. The present study aimed to evaluate the efficiency of biological control agents (*Trichoderma harzianum* and *Purpureocillium lilacinum*), applied by different modes, against *Ceratocystis fimbriata* in the soil. The experiment was conducted in a greenhouse in Dracena – São Paulo State, in two phases: one phase of 4 months with 90-day-old seedlings, and another phase of 2 months with 60-day-old seedlings. The following treatments were performed: Infested soil and soaking (IS + S); Infested soil and hydrogel (IS + H); Infested soil and irrigation (IS + IR); Infested soil and powder (IS + P); Negative Control (NC); Positive Control (CP). Results indicated that plants inoculated with the pathogen and treated with the control agents tended to have a higher chlorophyll index. Treatments with both biological agents applied by irrigation and powder in the soil (IS+IR and IS+P) presented the best and most consistent results for the two tested seedling ages.

**Keywords:** biological control, antagonistic fungi, wilt.

**RESUMO**

Antonio, G.L.; Firmino, A.C. **Potencial Antagônico de *Trichoderma harzianum* e *Purpureocillium lilacinum* contra *Ceratocystis fimbriata* em solo cultivado com eucalipto.** ***Summa Phytopathologica,*** v.49, p.1-6, 2024.

O cultivo de eucalipto tem grande importância no Brasil. A murcha-de-ceratocystis, é uma doença com grande importância econômica no país nas culturas do eucalipto, cacau e manga. São necessárias novas alternativas para o manejo da doença, pois frequentemente plantas infectadas precisam ser removidas, causando prejuízo aos produtores. Esse estudo teve o objetivo de avaliar a eficiência dos agentes de controle biológico (*Trichoderma harzianum* e *Purpureocillium lilacinum*) em diferentes modos de aplicação contra *Ceratocystis fimbriata* no solo. O experimento foi conduzido em casa de vegetação em Dracena – SP em duas fases, uma durante 4 meses com mudas de 90 dias e outra durante 2 meses com mudas de 60 dias. Os seguintes tratamentos foram realizados: Solo infestado e imersão (IS + S); Solo infestado e hidrogel (IS + H); Solo infestado por irrigação (IS + IR); Solo infestado com pó (IS + P); Controle negativo (NC); e Controle positivo (CP). Os resultados indicaram que plantas inoculadas com o patógeno e tratadas com os agentes de controle tendem a apresentam maior índice de clorofila. Os tratamentos com ambos agentes biológicos de controle aplicados em forma de irrigação ou pó no solo (IS+IR e IS+P) apresentaram os melhores e mais consistentes resultados nas duas idades de mudas avaliadas.

**Palavras-chave:** controle biológico, fungos antagonistas, murcha.

*Eucalyptus* genus encompasses about 730 species and had its origin in Australia and other islands of Oceania (1). These species have as characteristics good adaptation to various edaphoclimatic conditions, rapid growth and high production rates (2).

*Ceratocystis* wilt is a disease which clogs the plant xylem, blocking the translocation of water through these conductive vessels. Its major symptom is xylem darkening, progressing from the extremity to the center of the vessel, in the form of darkened radial striae (3, 4).

The first report of *Ceratocystis* wilt in *Eucalyptus* in Brazil occurred in southeastern Bahia in 1997, affecting clonal reforestation areas (5).

Since then, the disease has spread to other Brazilian states such as Espírito Santo, São Paulo, Mato Grosso do Sul, Minas Gerais, Pará and Maranhão (6, 7). In addition to Brazil, the pathogen has been reported causing this disease in eucalyptus in Uruguay (8) and Africa (9).

This pathogen is capable of causing losses in eucalyptus crops, both quantitatively and qualitatively. In southern Bahia, hybrid eucalyptus (*E. grandis* x *E. urophylla*) clones showed a 58% decrease in volumetric increment and a 13.7% reduction in purified pulp yield. (10). The disease progression became a concern since losses were not greater than 20-25% in the first months but increased over time, and the mortality rate found in monoclonal plots was 40% superior to that in southeastern Bahia (11).

Management methods vary according to the presence or absence of the pathogen in the area. If the pathogen is not installed in the area yet, its entry should be avoided, since the presence of the vector insect is endemic in regions where there are susceptible plants. In case the pathogen is present, cutting and burning of infected tree trunks and branches is recommended, but this measure is only effective if the root system of the plants is not contaminated yet (12).

Absence of effective control methods leads to the search for new alternatives such as biological control, which consists in reducing inoculum or disease-determining activities by one or more organisms other than humans (13). Among the biological control agents used on a commercial scale are fungi of the genera *Trichoderma* and *Purpureocillium*.

*Trichoderma* as a biological control agent was first reported in Brazil by Foster (14) in 1950. Some species of this genus have the ability to colonize plant roots and cause changes in the plant metabolism, inducing the production of plant defense substances such as phenols and phytoalexins, and increasing plant tolerance to stress (15, 16, 17). In addition, they can act as mycoparasites, compete for biological niches and sites of infection with phytopathogenic fungi, produce chemicals that are toxic to pathogens, and act as resistance elicitors (15, 16, 18, 19, 20, 21, 22). In the management of *Eucalyptus* diseases, *Trichoderma* has been successfully used to control pathogens such as *Ralstonia solanacearum* and *Cylindrocladium candelabrum*, besides acting as growth promoter by colonizing different plant organs (23, 24).

*Purpureocillium* can also parasitize fungi, compete for nutrients, inhibit the growth of fungal pathogens, produce toxic metabolites against other microorganisms and act as a resistance inducer, reducing the levels of fungal diseases (25, 26, 27, 28, 29, 30). Due to these characteristics, species of this genus are successfully used in the biological control of phytopathogenic fungi (31).

The current study aimed to evaluate the efficiency of biological control agents (*Trichoderma harzianum* and *Purpureocillium lilacinum*), using different application modes, against *Ceratocystis fimbriata* in the soil.

## MATERIAL AND METHODS

### Experimental design

The experiment was conducted in a greenhouse at FCAT (UNESP Dracena) and divided into two phases. The first phase occurred between December 2016 and March 2017 with 6-month-old seedlings, while the second phase occurred between April and May 2017 with 2-month-old seedlings.

*Eucalyptus* seedlings were transplanted to 3L pots containing a mixture of sterile substrate and peat at 2:1 ratio. For treatments in which the pathogen was present, inoculation was performed right before transplantation. The pots were daily irrigated and weeds manually removed. There were no fertilizers or application of phytosanitary products during the experiments.

### Source of fungal isolates and *Eucalyptus* clones

*Ceratocystis* isolate was obtained from the phytopathogenic fungal collection maintained at UNESP Dracena. Isolates of *T. harzianum* and *P. lilacinum* were supplied by Ballagro Company, and *Eucalyptus* clones (*E. urograndis*) were produced and provided by "Fazenda Santa Vírginia", owned by Brochmann Pollis group.

### Inoculation of *Ceratocystis fimbriata*

The soil, contained in pots, was incorporated with 25 mL suspension of $10^6$ endoconidia.mL$^{-1}$ *C. fimbriata*, which was grown in liquid Yeast Malt Broth Himedia™ (5g/L Peptic Digest of Animal Tissue; 3g/L yeast extract; 3g/L malt extract; 10 g/L dextrose) for 10 days under constant agitation. The suspension with *C. fimbriata* was manually homogenized in each pot to achieve good inoculum distribution. After the soil infestation with this fungus, the following treatments were performed and *Eucalyptus* seedlings were immediately placed in the pots:

Infested soil and soaking **(IS+S)**: seedlings had their roots immersed in 1 L sterile water containing the biological agent (3 g commercial product per L water) for 15 min and were planted in soil infested with *C. fimbriata*;

Infested soil and hydrogel **(IS+H)**: seedlings were planted in pots with *C. fimbriata* infested soil and 25 mL hydrogel (1 g hydrogel per L sterile water), and the biological control agent was incorporated into the hydrogel solution (3 g commercial product per L hydrogel).

Infested soil and irrigation **(IS+IR)**: seedlings were planted in pots with infested soil and irrigated with 25 mL biological control agent (3 g commercial product per L sterile water).

Infested soil and powder **(IS+P)**: seedlings were planted in pots with infested soil; however, unlike the previous treatment, the biological control agent was distributed directly into the seedling planting hole before planting (3 g commercial product per pot).

Negative control **(NC)**: seedlings were planted in pots with soil without *C. fimbriata* infestation and without any biological treatment.

Positive control **(PC)**: seedlings were planted in pots with soil infested by *C. fimbriata*.

Six treatments were performed for each biological control agent (*T. harzianum* and *P. lilacinus*) separately. Each treatment had 8 repetitions. The concentration of *T. harzianum* and *P. lilacinus* conidia used in the study was $10^{10}$ CFU/g and $7.5\times10^9$ CFU/g (commercial product), respectively.

### Experiment Evaluation

In both phases of the experiment, the following parameters were evaluated:

Stem diameter – measured with a caliper.

Shoot fresh mass (SFM) – obtained with 0.01g precision balance.

Shoot dry mass (SDM) – seedlings previously weighed for SFM were placed in a greenhouse with air circulation at 65 ºC for 48 h and then weighed on the same balance.

Chlorophyll index – measured in the laboratory using the SPAD-502 (Soil Plant Analysis Development) device. Measurements were obtained from the second highest leaf, second newest leaf of the middle branch, and second newest leaf of the lower branch. This was conducted at a constant temperature of 25°C, prior to the drying out of seedlings.

Mortality – occurrence of plant death was observed during the experiment.

The two phases were conducted following a completely randomized design, and each experimental plot consisted of a pot. All data underwent analysis of variance and the averages were compared according to Tukey's test at 5% probability level, using the software SISVAR (32).

***Ceratocystis fimbriata* reisolation**

For pathogen isolation, carrot discs were used as bait, a technique considered selective by Moller & De Vay (33); however, in this case, soil of each treatment was sprayed on the discs. After 7 days, with the aid of a stereoscope microscope, *C. fimbriata* perithecia with ascospores could be observed, which are characteristic structures of this fungus. Antagonistic fungi were also observed in the reisolation.

## RESULTS AND DISCUSSION

Regarding stem diameter, no considerable difference could be observed between treatments (Tables 1 and 2) during the experimental period. This parameter is probably more relevant in larger plants installed in the field, since it is directly influenced by the climate and the management (34). Therefore, as the experiment was conducted in pots, stem diameter development was not an expressive parameter. This factor does tend to exhibit less variation in experiments conducted in pots; in addition, the high coefficient of variation may have contributed to the lack of statistical difference observed for this parameter. An experimental design with a larger number of replicates could potentially reveal statistical differences.

**Table 1**: Evaluated parameters of 6-month-old *Eucalyptus* seedlings treated with *Trichoderma harzianum* (TRI) and *Purpureocillium lilacinus* (PUR) and cultivated in soil infested with *Ceratocystis fimbriata.*

| Treatments | Stem diameter (mm) | | SDM* (g) | | SFM* (g) | |
|---|---|---|---|---|---|---|
| | TRI | PUR | TRI | PUR | TRI | PUR |
| IS+S | 6.33 b | 6.82 a | 22.08 ab | 23.96 b | 54.12 a | 64.81 ab |
| IS+H | 7.32 a | 7.35 a | 21.78 b | 23.15 b | 57.13 a | 58.58 ab |
| IS+IR | 7.00 ab | 7.39 a | 23.69ab | 24.49 b | 61.43 a | 63.91 ab |
| IS+P | 6.86 ab | 6.94 a | 27.42 a | 25.13 a | 66.60 a | 68.87 a |
| NC | 7.02 b | 6.14 a | 23.26 ab | 21.80 b | 61.61 a | 54.40 ab |
| PC | 7.09 b | 6.47 a | 22.11b | 17.85 b | 56.67 a | 50.31 b |
| CV* | 17.45 | 14.66 | 38.00 | 48.43 | 34.49 | 46.04 |
| CVT* | 11.64 | 9.07 | 9.89 | 23.8 | 20.69 | 22.75 |

*(IS+S): seedling roots immersed in solution containing the biological agents for 15 minutes before transplantation to pots infested with the pathogen; (IS+H): biological agents incorporated in hydrogel solution applied to the soil before seedling transplantation to pots infested with the pathogen; (IS+IR): soil irrigated with biological agent solution before seedling transplantation to pots infested with the pathogen; (IS+P): biological agents directly distributed as powder within the soil prior to seedling transplantation to pots infested with the pathogen; (NC): soil without pathogen infestation and biological treatment; (PC): soil infested with the pathogen and without biological treatment.
*SDM: shoot dry mass; SFM: shoot fresh mass; CV: Coefficient of variation; CVT: Coefficient of variation of the data transformed into neperian logarithm of Y - Ln(Y). Values followed by the same lowercase letter do not differ from each other in the columns, according to Tukey's Test at 5% probability.

**Table 2**: Evaluated parameters of 2-month-old *Eucalyptus* seedlings treated with *Trichoderma harzianum* (TRI) and *Purpureocillium lilacinus* (PUR) and cultivated in soil infested with *Ceratocystis fimbriata.*

| Treatments* | Stem diameter (mm) | | SDM* (g) | | SFM* (g) | |
|---|---|---|---|---|---|---|
| | TRI | PUR | TRI | PUR | TRI | PUR |
| IS+S | 4.37 a | 4.70 a | 3.37 ab | 3.50 a | 12.12 ab | 12.99 a |
| IS+H | 4.87 a | 4.65 a | 4.12 a | 3.41 a | 13.50 ab | 12.33 a |
| IS+IR | 4.75 a | 4.52 a | 4.37 a | 3.53 a | 14.5 a | 13.15 a |
| IS+P | 4.87 a | 4.52 a | 3.75 a | 2.50 a | 13.00 ab | 8.40 a |
| NC | 4.50 a | 4.50 a | 3.12 ab | 2.97 a | 9.12 b | 9.08 a |
| PC | 4. 00 a | 4.89 a | 2.00 b | 2.07 a | 7.44 c | 7.39 a |
| CV* | 9.63 | 12.91 | 15.43 | 23.33 | 18.38 | 20.39 |
| CVT* | 5.25 | 6.75 | 5.50 | 7.87 | 4.84 | 5.11 |

* (IS+S): seedling roots immersed in solution containing the biological agents for 15 minutes before transplantation to pots infested with the pathogen; (IS+H): biological agents incorporated in hydrogel solution applied to the soil before seedling transplantation to pots infested with the pathogen; (IS+IR): soil irrigated with biological agent solution before seedling transplantation to pots infested with the pathogen; (IS+P): biological agents directly distributed as powder within the soil prior to seedling transplantation to pots infested with the pathogen; (NC): soil without pathogen infestation and biological treatment; (PC): soil infested with the pathogen and without biological treatment.
*SDM: shoot dry mass; SFM: shoot fresh mass; CV: Coefficient of variation; CVT: Coefficient of variation of the data transformed into neperian logarithm of Y - Ln(Y). Values followed by the same lowercase letter do not differ from each other in the columns, according to Tukey's Test at 5% probability.

Shoot fresh mass (SFM) evaluation presented important results. As shown in Tables 1 and 2, positive control (PC) presented lower numbers, compared to most treatments, indicating that the biological control of the disease was effective. Since this pathogen obstructs the plant xylem, so that the flow of water and nutrients is impaired, the plants suffer water stress, which in turn leads to impaired metabolism, causing their development to be reduced.

According to Costa and Marenco (35), the water flow gradient in the plant directly influences the plant water potential, so that any variation in the leaf water potential can affect carbon uptake by the plant since it closes its stomata and reduces photosynthesis. In order to favor cell turgidity under water stress situations, the plant adjusts its cellular metabolism, which can cause energy expenditure, which in turn causes the plant to lose fresh mass, for example.

Still in relation to SFM, PC had the lowest numerical value, except in the treatment with *Trichoderma* in 6-month-old seedlings, for which infested soil and soaking (IS+S) had inferior values and statistically differed from IS+H, IS+IR and IS+P, also showing inferior values in 2-month-old seedlings treated with *Trichoderma*. These results were confirmed in the evaluation of shoot dry mass (SDM), for which PC again had the lowest value, except in the treatment with *Trichoderma* in 6-month-old seedlings plus IS+S; moreover, except for the treatment with *Purpureocillium* in 2-month-old seedlings, PC showed significant difference from at least one treatment in the three other evaluations.

Based on SFM and SDM data, IS+IR and IS+P were the best treatments for both fungi used in the biological control of *Ceratocystis*, even when compared to the negative control − NC (Table 1 and 2). The highest numerical value was obtained for plants treated with biological agents at both plant ages.

Studies developed by Mafia et al. (36) and Takada (37) with *Trichoderma* spp. in *Eucalyptus* showed the effectiveness of using this biological control agent against *Rhizoctonia solani,* together with lower plant mortality in soils treated with *Trichoderma*. Maciel et al. (24) found that some species of *Trichoderma*, including *T. harzianum,* reduced the severity of symptoms caused by *C. candelabrum* by 35.35% in *Eucalyptus*. *Trichoderma longibrachiatum* and *T. inhamatum* also reduced the presence of sclerotia of *Ralstonia solanacearum* in infested leaves of *Eucalyptus* (23).

*Purpureocillium lilacinum* has already been shown to: be effective against *Drechslera tritici-repentis*, a pathogen in which it caused conidial plasmolysis (30); reduce the development of *Moniliophtora roreri in vitro*, and promote hypha lysis in this pathogen (26).

Analysis of SFM and SDM confirms the beneficial effect of biological agents on *Eucalyptus* seedlings, indicating that *P. lilacinum* and *T. harzianum* acted on different aspects of biological control: they effectively controlled the fungus, as evidenced by the poorer performance in the PC, and served as growth promoters, since treatments with these agents generally outperformed the NC.

This effect has already been described by Chet et al. (38) for *Trichoderma*; the association between *Purpureocillium* and *Trichoderma* also promotes growth, as reported by Fragoso & Custódio (39). Prates *et al.* (40) observed that, under field conditions, application of the antagonistic fungus on citrus seedling substrate reflected in better shoot production and better development of the pivoting root and rootlets.

According to Lucon (41), plant growth promotion by the application of isolates of *Trichoderma* spp. was initially related to the control of harmful microorganisms present in the rhizosphere and soil. More recently, it has been related to the production of hormones or growth factors, greater efficiency in the use of some nutrients, and increased availability and absorption of nutrients by the plant. It is also known that microorganisms used in biological control can cause substantial changes in the plant metabolism in order to stimulate the plant defense mechanisms, such as phenols and phytoalexins, and increase nutrient availability and stress tolerance (16).

Regarding chlorophyll content, numerically lower values were found for NC in 6-month-old plants in relation to all other treatments (Table 3 and 4). In the experiment with 2-month-old seedlings, the ratios between NC and PC were the same, while the relationship between NC and the best performing treatments for SFM and SDM was also maintained. There was a case where the best treatment using *Purpureocillium* (IS+IR) had a lower leaf chlorophyll index in the upper leaf, compared to NC, but continued with higher values in the middle and lower leaves (Tables 3 and 4). These results indicate a tendency of plants treated with biocontrol agents or infected with *Ceratocystis* to have higher chlorophyll index, since the plants infected by the phytopathogenic fungus that were most influenced by biological agents had, in general, the highest rates.

**Table 3**: Chlorophyll index of 2-month-old *Eucalyptus* seedlings treated with *Trichoderma harzianum* (TRI) and *Purpureocillium lilacinus* (PUR) and cultivated in soil infested with *Ceratocystis fimbriata.*

| Treatments | Clo. H. L* | | Clo. M.L* | | Clo. L.L* | |
|---|---|---|---|---|---|---|
| | TRI | PUR | TRI | PUR | TRI | PUR |
| IS+S | 29.63 a | 29.55 ab | 45.21 a | 47.31 a | 47.16 a | 46.80 a |
| IS+H | 30.04 a | 29.24 ab | 46.89 a | 46.34 a | 42.55 a | 44.23 a |
| IS+IR | 29.79 a | 28.76 b | 47.13 a | 48.12 a | 44.46 a | 44.48 a |
| IS+P | 31.73 a | 30.61 ab | 48.80 a | 48.95 a | 46.13 a | 43.21 a |
| NC | 28.28 a | 28.08 ab | 46.05 a | 45.29 a | 43.63 a | 42.02 a |
| PC | 30.44 a | 32.73 a | 47.16 a | 47.10 a | 45.96 a | 42.67 a |
| CV* | 18.63 | 12.12 | 12.47 | 11.57 | 6.59 | 8.12 |
| CVT* | 10.77 | 3.35 | 3.91 | 3.30 | 1.69 | 2.14 |

*Clo. H. L: chlorophyll index of the second highest leaf; Clo. M.L: chlorophyll index of the second newest leaf of the middle branch; Clo L.L: chlorophyll index of the second newest leaf of the lower branch; CV: Coefficient of variation; CVT: Coefficient of variation of the data transformed into neperian logarithm of Y - Ln(Y). Values followed by the same lowercase letter do not differ from each other in the columns, according to Tukey's Test at 5% probability.

**Table 4**: Chlorophyll index of 6-month-old *Eucalyptus* seedlings treated with *Trichoderma harzianum* (TRI) and *Purpureocillium lilacinus* (PUR) and cultivated in soil infested with *Ceratocystis fimbriata.*

| Treatments | Clo. H. L* | | Clo. M.L* | | Clo. L.L* | |
|---|---|---|---|---|---|---|
| | TRI | PUR | TRI | PUR | TRI | PUR |
| IS+S | 38.75 a | 34.92 a | 43.75 ab | 42.11 a | 53.50 a | 51.85 a |
| IS+H | 32.62 a | 36.31 a | 38.37 b | 40.36 a | 50.75 ab | 50.09 a |
| IS+IR | 36.00 a | 33.91 a | 43.50 ab | 41.24 a | 50.75 ab | 50.59 a |
| IS+P | 32.37 a | 45.60 b | 45.00 ab | 45.60 a | 48.37 b | 54.26 a |
| NC | 36.87 a | 36.37 a | 41.00 ab | 40.53 a | 49.62 ab | 49.75 a |
| PC | 37.33 a | 37.18 a | 46.00 a | 46.03 a | 52.33 ab | 52.27 a |
| CV* | 13.83 | 17.48 | 11.9 | 8.47 | 9.70 | 9.33 |
| CVT* | 4.35 | 5.36 | 3.29 | 2.20 | 2.63 | 2.55 |

*Clo. H. L: chlorophyll index of the second highest leaf; Clo. M.L: chlorophyll index of the second newest leaf of the middle branch; Clo L.L: chlorophyll index of the second newest leaf of the lower branch; CV: Coefficient of variation; CVT: Coefficient of variation of the data transformed into neperian logarithm of Y - Ln(Y). Values followed by the same lowercase letter do not differ from each other in the columns, according to Tukey's Test at 5% probability.

The pattern observed in the present study has already been found by other authors such as Shi *et al.* (42), who reported that beet plants had higher chlorophyll indices when inoculated with *Bacillus pumilus*, *Chryseobacterium indologene* and *Acinetobacter johnsonii*, in relation to plants not inoculated with biological agents, and also had increased carbohydrate content. Chlorophyll variation was also observed by Kahn *et al.* (43) in plants treated with endophytic fungi. In that study, eight strains of endophytic fungi were inoculated in bell pepper plants; the strains had been isolated from the roots of the same plants. At the end, the plant chlorophyll content, compared to the control, was increased for five of these strains, showed no difference for two strains and was lower for one strain.

Difference in chlorophyll content in plants infected with a pathogen and treated with a biological control agent has already been observed by Viecelli et al. (44), who tested *Pycnoporus sanguineus* filtrate as resistance elicitor in bean plants infected with *Pseudocercospora griseola*; at the final evaluation, the filtrate led to a higher chlorophyll content compared to the control treated with water.

Gomes et al. (45) found that corn plants inoculated with the pathogenic fungus *Fusarium verticilioides* showed variations in chlorophyll a and b level in relation to the control: chlorophyll a was higher in inoculated plants, while chlorophyll b was higher in controls. Those authors consider that it can be advantageous for the plant, since chlorophyll b is responsible for the less intense light absorption and its degradation increases the relative level of chlorophyll a. According to Shukla et al. (17), colonization of rice seedling roots with *Trichoderma* delayed negative effects of hydric stress such as stomatal conductance, photosynthetic rate and greenness, besides increasing phenolic concentration and membrane stability index. Stargarlin & Pascholati (46) verified chlorophyll content varying in bean cultivars infected by *Uromyces appendiculatus*: it was increased for the cultivar Rosinha but did not change for the cultivar Carioca. Those authors also observed that bean cultivars infected by *Phaeoisariopsis griseola* did not show any variation in relation to the control.

In carrot baits and *Ceratocystis* reisolation tests, perithecia (sexual structure of the pathogen) were observed in all treatments, except the NC which had not been inoculated with the pathogen. It is noteworthy that mycelium from the fungi used in biological control was also present, pointing out that they were also active. Thus, the presence of pathogen perithecia in the current study does not mean that there was no control of the pathogen, but rather a balance between the microorganisms in the soil. It should also be considered that the employed reisolation methods create a favorable environment for pathogen growth, which may not occur in the soil.

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