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Pathogenic Characterization of *Pestalotiopsis grandis-urophylla* Isolates Using Mycelial Suspension

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Abstract

Eucalyptus species are among the most important forest crops in the world and can be affected by several pathogens, mainly by fungi of the genus *Pestalotiopsis*, which cause leaf spots. Studies aimed at the pathogenic characterization of *Pestalotiopsis* spp. from lesions of eucalyptus leaves in Brazil are still limited. The objective of this work was to evaluate the pathogenic potential of *Pestalotiopsis grandis-urophylla* isolates. For that, healthy leaves of adult *Eucalyptus grandis* 'GG 100' plants were inoculated with mycelial suspension of different *P. grandis-urophylla* isolates. The leaves inoculated with the pathogen were submitted to controlled conditions in a humid chamber in transparent acrylic gerbox boxes. Disease severity assessments were performed at 4, 6, 8 and 10 days after inoculation. Isolate E 72-04 had the highest area under the disease progress curve (AUDPC). Regarding the development of lesions, isolates E-72-02 and E-72-03 fit the polynomial model of the second degree, while isolate E-72-04 fit a linear model. The methodology tested reproduced typical symptoms of *Pestalotiopsis* and can be used as a parameter for new pathogenicity tests with this fungal genus.

Keywords: mycology, epidemiology, forest pathology

1. Introduction

Eucalyptus species are among the most important forest crops in the world, due both to the versatility that allows their cultivation in several countries, and to their rapid growth, giving them characteristics of great economic importance (Cerveira Junior et al., 2020). However, the cultivation of *Eucalyptus* spp. can be harmed by the occurrence of diseases. *Eucalyptus* is affected by several pathogens, mainly fungi, from the nursery stage to adult plantations, occurring in the most varied places, species and times of the year (Auer et al., 2016).

Among the diseases that affect eucalyptus, leaf spots caused by *Pestalotiopsis* spp. have assumed great importance, especially in adult plantations (Suwannarach et al., 2012). *Pestalotiopsis* spp. have already been reported as responsible for lesions on the bark of *E. globulus* plants (Alonso et al., 2009), as an endophytic and epiphytic fungus in leaves of *E. citriodora* (Kharwar et al., 2010), and causing leaf spot in *E. citriodora* leaves (Kharwar et al., 2010) and in *E. camaldulensis*, *E. globulus* and *E. botryoides* (Morales-Rodríguez et al., 2019). There are reports of this pathogen in a variety of hosts, causing damage with different symptoms (Joshi et al., 2009; Reddy et al., 2016), but leaf spots are relevant because they decrease the photosynthetic area of the leaves,

reducing energy absorption and, consequently, the decrease in the accumulation of biomass, which leads to a reduction in yield (Taiz & Zeiger, 2013).

The spread of *Pestalotiopsi*s spp. is favored because the spores are easily air transported and penetrate plant tissues through wounds or natural openings (Alonso et al., 2009; Reddy et al., 2016; Morales-Rodríguez et al., 2019). In addition, several species of *Pestalotiopsis* produce thermotolerant spores (Suryanarayanan et al., 2011). In this context, knowing the infective potential and virulence of pathogen isolates is important when considering the adoption of integrated management measures and increasing productivity.

The ability of a pathogen to cause diseases in the host is proven through its pathogenic potencial in wich the degree of pathogenicity can vary between fungal isolates as well as the tipe of host (Milan et al., 2021). It is worth to mention that, in terms of pathogenic potencial of a plant pathogen, some metrics can to be obtained as well as evaluated, so that, the area under the disease progresse curve (AUDPC) has been most used and the one that allows a better understanding of the pathogenic potential of fungal isolates (Silva et al., 2021).

There are few studies aimed at the pathogenic characterization of *Pestalotiopsis* spp. from lesions of eucalyptus leaves in Brazil. Therefore, the objective of this work was to evaluate the pathogenic potential of *P. grandis-urophylla* isolates, by means of a pathogenicity test using mycelial suspension for inoculations in healthy leaves of adult *Eucalyptus grandis* 'GG 100' plants.

2. Method

2.1 Pathogenic Potential

Healthy leaves of adult plants of *E. grandis* 'GG 100' (18 to 24 months old) were washed in running water and left to dry in a laminar flow chamber for 10 min. For inoculations, five holes were made on the left side and five on the right side of the leaf blade with the aid of a sterilized needle. Then, plates containing mycelium (without spores and after 10 days of culture) of *P. grandis-urophylla* (isolates E-71-02, E-71-03 and E-71-04) had 10 mL of sterilized distilled water (SDW) added to obtain a mycelial suspension with the aid of a Drigalski loop. Soon after, a volume of 25 µL of *P. grandis-urophylla* mycelial suspension was applied to the region containing the five holes. The leaves inoculated with the pathogen were submitted to controlled conditions in a humid chamber in transparent acrylic gerbox boxes, containing a sheet of germination paper with constant humidity maintained only on the paper. Disease severity assessments were performed at 4, 6, 8 and 10 days after inoculation (DAI), measuring the diameter (mm) of the lesions with the aid of a caliper (average of two diametrically opposed measurements). Leaf area injured at 10 DAI was obtained using the formula: $A = \pi (d1 \times d2)/4$. The design was completely randomized (DIC), with five replications (gerbox) per *P. grandis-urophylla* isolate. The control consisted of the application of 25 µL of SDW in the region of the leaf blade holes.

2.2 Statistical Analysis

The results were submitted to the Kolmogorov-Smirnov test to check the normality of the data and, later, for the analysis of variance, to the Scott-Knott test ($P < 0.05$) and to regression analysis to obtain models for the development of injuries, with the help of the SISVAR 5.3 program (Ferreira, 2011). Severity was calculated as the area under the disease progress curve (AUDPC), using the formula $AUDPC = \Sigma\{[(y1 + y2)/2] \times (t2 - t1)\}$, where, y1 and y2 are two consecutive assessments performed at times t1 and t2, respectively.

3. Results

Isolates E-72-02, E-72-03 and E-72-04 did not differ in relation to the injured leaf area, having a variation of 44.04 mm^2 to 51.15 mm^2 at 10 DAI. Isolate E-72-04 obtained the greatest area under the disease progress curve (AUDPC) (Table 1), followed by isolates E-72-02 and E-72-03, which did not differ statistically.

Table 1. Leaf area of *Eucalyptus grandis* 'GG100' injured by *P. grandis-urophylla* at 10 DAI, regression models for the increase in lesions from 2 to 10 DAI and area under the disease progress curve (AUDPC)

Isolate	Injury to 10 DAI (mm^2)	Regression model	r^2 (%)	(P ≤ X)	AUDPC
E-72-02	44.04 a	$Y = 0.9231x^2 – 7.6762x + 28.2791$	99.84	0.05	138.98 b
E-72-03	47.44 a	$Y = 1.1142x^2 – 10.1180x + 37.2410$	99.99	0.01	150.59 b
E-72-04	51.16 a	$Y = 5.6393x – 8.1378$	95.70	0.01	182.96 a
CV (%)	19.43	-	-	-	17.87

Note. Values followed by the same letter in the column do not differ statistically, according to the Scott-Knott test ($P \leq 0.05$).

Regarding lesion development, isolates E-72-02 and E-72-03 fitted the second-degree polynomial model, while isolate E-72-04 fitted a linear model (Figure 1). All models were significant and had a high coefficient of determination (r^2).

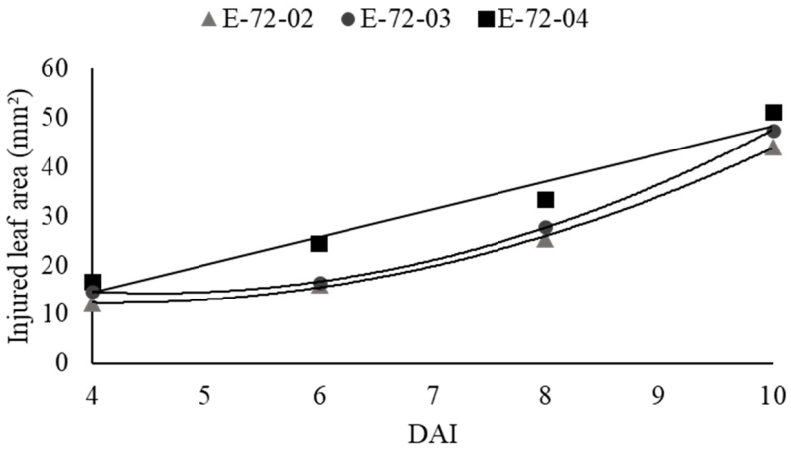

Figure 1. Injured leaf area (mm^2) of *Eucalyptus grandis* 'GG100' leaves after inoculation with mycelial suspension of *P. grandis-urophylla* (isolates E-71-02, E-71-03 and E-71-04)

After inoculations with mycelial suspension, the typical symptoms of the disease caused by *Pestalotiopsi*s were reproduced, which was characterized by dark brown necrotic lesions. At 10 DAI, black spots corresponding to the development of acervulli of the fungus were also observed (Figure 2).

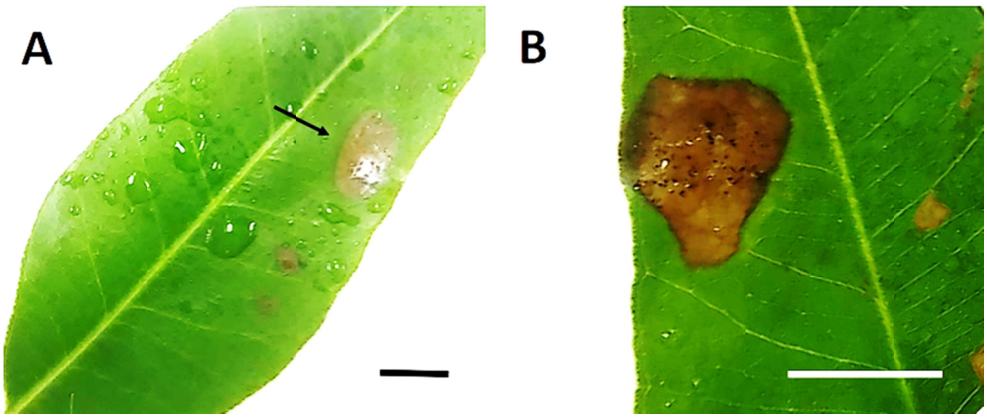

Figure 2. *Eucalyptus grandis* 'GG100' leaves inoculated with *P. grandis-urophylla* at 10 DAI, showing symptoms and lesions. (A) Arrow shows brown coalesced necrotic lesion, typical of the genus *Pestalotiopsis*. Isolate E-72-03. (B) Dark brown necrotic lesion in greater detail. Isolate E-72-04. Bars correspond to 5.5 and 6.5 mm for figures 2A and 2B, respectively

4. Discussion

The pathogenicity of *P. grandis-urophylla* in the present study is in agreement with other works that indicated the diversity of species of this genus in infecting *Eucalyptus* spp. plants. To exemplify, one can cite the work of YUAN (1996), which demonstrated that the pathogenicity of *P. disseminata* and *P. neglecta* are associated with leaf spot of *Eucalyptus alba* and *Eucalyptus pellita*. However, the pathogenicity of *P. virgatula* (Suwannarach et al., 2012) and *P. biciliata* (Morales-Rodríguez et al., 2019) has been confirmed on *Eucalyptus camaldulensis* plants and recently, Carvalho et al. (2019) and Silva et al. (2021) reported *P. grandis-urophylla* pathogenicity on *E. urograndis*.

In addition to eucalypt species, the genus *Pestalotiopsis* has already been identified causing foliar diseases in other plant species. Maciel et al. (2012), verified the pathogenicity of *Pestalotiopis* causing necrotic lesions on

leaves of red mastic (*Schinus terebinthifolius*) and with subsequent coalescence and leaf fall. Similarly, to the present study, Wu et al. (2009) also reported *P. microspora* on *Reineckea carnea*, a medicinal herbaceous plant, causing dark brown, oval, 20-50 mm^2 lesions on the leaf lamina due to their coalescence. El-Argawy (2015) used pathogenicity tests with species of *P. psidii*, *P. microspora*, *P. neglecta*, *P. clavispora* and *Pestalotiopsis* spp. and observed symptoms on the leaves of guava (*Psidium guajava* L.), a tropical fruit plant, with similar characteristics to those observed in the present study. These authors reported lesions between 18.2 and 36.4 mm^2.

The pathogenicity found (Table 1 and Figure 2) was possibly due to the fact that these isolates were obtained from injured leaves, ruling out the possibility of being endophytes or only secondary pathogens. The fact that there is no difference between isolates of *P. grandis-urophylla* (E-71-02, E-71-03 and E-71-04) regarding the size of the injured leaf area may be related to the way in which they were obtained, *i.e.*, the fact that they belong to the same population and, therefore, are descendants of a common ancestor (Jeewon et al., 2002). Although laboratory conditions cannot perfectly simulate the conditions of the natural environment (Keith et al., 2006), the reaction of isolates of phytopathogens to different physical factors, in vitro cultivation or the strength of their pathogenicity provide support for studies related to the epidemiology of diseases caused by them in field conditions (Teramoto et al., 2013).

Another important aspect to be addressed is the difference found for the AUDPC of isolate E-72-04 in relation to E-72-02 and E-72-03. One explanation for this event may be the rapid mycelial growth and initial colonization of the leaf parenchyma by E-72-04 compared to the other isolates. Corroborating the results of this study, Carvalho et al. (2019) also reported divergence in behavior among isolates of *P. grandis-urophylla* on eucalyptus plants.

In a similar study, mycelial growth was shown to be a useful variable in separating isolates of *Pestalotiopsis* (Lazarotto et al., 2014). The reaction of phytopathogens isolates to different physical factors, such as in vitro culture or any other factor that can influence the pathogenicity power, are the main tools that subsidies studies related to epidemiology of diseases under field conditions (Silva et al., 2021). Thus, we believe that studies directed to the pathogenic behavior of different species and isolates of *Pestalotiopsis* should be conducted in order to help the understanding of its pathogenic strength and also the behavior of its pathogenicity.

Finally, the results about the AUDPC were similar with the results obtained by Silva et al (2021), on what the isolates E-72-02, E-72-03 and E-72-04 shown results of AUDPC vary from 128 to 188. Such data concordance sugest that the final size of lesions was indifferent to the inoculation method, since Silva et al (2021) used agar plugs containing fungal mycelium which was depositet on leaf surface.

4. Conclusion

The methodology tested reproduced typical symptoms of *Pestalotiopsis* and can be used as a parameter for new pathogenicity tests with this fungal species. Regarding the leaf area injured, there was no statistical difference among the isolates of *P. grandis-urophylla* tested. Isolate E-72-04 obtained the highest AUDPC, showing itself to be more virulent than the others.

References

Alonso, R., Tiscornia, S., Alfenas, A. C., & Bettucci, L. (2009). Fungi associated to bark lesions of *Eucalyptus globulus* stems in plantations from Uruguay. *Revista Árvore, 33*, 591-597. https://doi.org/10.1590/S0100-67622009000400001

Auer, C. G., Santos, A. F., & Furtado, E. L. (2016). Doenças do Eucalipto. In L. Amorim, A. Bergamim Filho, J. A. M. Rezende, & L. E. A. Camargo (Eds.), *Manual de Fitopatologia: Doenças de plantas cultivadas* (5th ed., Vol. 2, pp. 359-372). Ouro Fino-MG. Agronômica Ceres.

Carvalho, D. D. C., Oliveira, R. M., Marques, M. G., Milan, M. D., Pinho, D. B., & Dianese, E. C. (2019). Molecular, morphophysiological and pathogenic characterization of eucalypt *Pestalotiopsis grandis-urophylla* isolates, a new species. *Tropical Plant Pathology, 44*, 132-139. https://doi.org/10.1007/s40858-019-00277-0

Cerveira Junior, W. R., Costa, Y. K. S., Carbonari, C. A., Duke, S. O., Alves, P. L. C. A., & Silva, L. B. C. (2020). Growth, morphological, metabolic and photosynthetic responses of clones of eucalyptus to glyphosate. *Forest Ecology and Management, 470-471*, 118218. https://doi.org/10.1016/j.foreco.2020.118218

El-Argawy, E. (2015). Characterization and control of *Pestalotiopsis* spp. the causal fungus of guava scabby canker in el-beheira governorate, Egypt. *International Journal of Phytopathology, 4*(3), 121-136. https://doi.org/10.33687/phytopath.004.03.1403

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

Jeewon, R., Liew, E. C. Y., & Hyde, K. D. (2002). Phylogenetic relationships of *Pestalotiopsis* and allied genera Inferred from ribosomal DNA sequences and morphological characters. *Molecular Phylogenetics and Evolution, 25*(3), 378-392. https://doi.org/10.1016/s1055-7903(02)00422-0

Joshi, S. D., Sanjay, D., Baby, U. I., & Andal, A. K. A. (2009). Molecular characterization of *Pestalotiopsis* spp. associated with tea (*Camellia slnensis*) in Southern India using RAPD and ISSR markes. *Indian Journal of Biotechnology, 8*(4), 377-383.

Keith, L. M., Velasquez, M. E., & Zee, F. T. (2006). Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava*, in Hawaii. *Plant Disease, 90*(1), 16-23. https://doi.org/10.1094/PD-90-0016

Kharwar, R. N., Gond, S. K., Kumar, A., & Mishra, A. (2010). A comparative study of endophytic and epiphytic fungal association with leaf of *Eucalyptus citriodora* Hook., and their antimicrobial activity. *World Journal of Microbiology and Biotechnology, 26*, 1941-1948. https://doi.org/10.1007/s11274-010-0374-y

Lazarotto, M., Bovolini, M. P., Muniz, M. F. B., Harakawa, R., Reiniger, L. R. S., & Santos, A. F. (2014). Identification and characterization of pathogenic *Pestalotiopsis* species to pecan tree in Brazil. *Pesquisa Agropecuária Brasileira, 49*(6), 440-448. https://doi.org/10.1590/S0100-204X2014000600005

Maciel, C. G., Ferrera, T. S., Bovolini, M. P., Gonzatto, R., Muniz, M. F. B., & Buriol, G. A. (2012). Transmissão de fungos via semente e patogenicidade de *Pestalotiopsis* sp. em mudas de *Schinus terebinthifolius* Raddi. *Semina: Ciências Agrárias, 33*(1), 2767-2774. https://doi.org/10.5433/1679-0359.2012v33Supl1p2767

Milan, M. D., Souza, D. P., Muniz, P. H. P. C., Peixoto, G. H. S., Ahmad, S., Guimarães, G. R., ... Carvalho, D. D. C. (2021) Circadian Rhythms, Such as Light Regimes Influencing *in vitro* Growth of *Pestalotiopsis mangiferae* from Mango Tree. *Plant Pathology Journal, 20*(1), 23-28. https://doi.org/10.3923/ppj.2021.23.28

Morales-Rodríguez, C., Valle, M. D., Aleandri, M. P., & Vannini, A. (2019). *Pestalotiopsis biciliata*, a new leaf pathogen of *Eucalyptus* spp. recorded in Italy. *Forest Pathology, 49*(12492), 1-7. https://doi.org/10.1111/efp.12492

Reddy, M. S., Murali, T. S., Suryanarayanan, T. S., Rajulu, M. B. G., & Thirunavukkarasu, N. (2016). *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. *Fungal Ecology, 24*, 70-75. https://doi.org/10.1016/j.funeco.2016.09.002

Silva, L. R., Marques, M. G., Muniz, P. H. P. C., Oliveira, T. A. S., Duarte, E. A. A., Rodrigues, F., Santos, S. X., & Carvalho, D. D. C. (2021). Re-Isolation methodologies for recovering sporulation of eucalyptus *Pestalotiopsis grandis-urophylla* isolates after 14 months storage. *Journal of Scientific Research & Reports, 27*(1), 68-77. https://doi.org/10.9734/jsrr/2021/v27i130350

Suryanarayanan, T. S., Govindarajulu, M. B., Thirumalai, E., Reddy, M. S., & Money, N. C. (2011). Agni's fungi: heat-resistant spores from the Western Ghats, southern India. *Fungal Biology, 115*, 833-838. https://doi.org/10.1016/j.funbio.2011.06.011

Suwannarach, N., Kumla, J., Bussaban, B., & Lumyong, S. (2012). New report of leaf blight disease on eucalyptus (*Eucalyptus camaldulensis*) caused by *Pestalotiopsis virgatula* in Thailand. *Canadian Journal of Plant Pathology, 34*, 306-309. https://doi.org/10.1080/07060661.2012.680501

Taiz, L., & Zeiger, E. (2013). *Plant Physiology* (5th ed., p. 952) Sinauer, Sunderland.

Teramoto, A., Parisi, M. C. M., & Cunha, M. G. (2013). Caracterização fisiológica de isolados de *Corynespora cassiicola*. *Tropical Plant Pathology, 38*(4), 313-322. https://doi.org/10.1590/S1982-56762013005000012

Wu, M. D., Li, G. Q., & Jiang, D. H. (2009). First report of *Pestalotiopsis microspora* causing leaf blight of *Reineckea carnea* in Central China. *Plant Disease, 93*(6), 667. https://doi.org/10.1094/PDIS-93-6-0667A

Yuan, Z. Q. (1996). Fungi and associated tree diseases collected from Melville Island, Northern Territory, Australia. *Australian Systematic Botany, 9*, 337-360. https://doi.org/10.1071/SB9960337

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