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Short Communication

First Record of Leaf Spot in Clonal Garden of *Corymbia torelliana* × *C. citriodora* in Brazil

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Abstract

In recent years, forestry companies have increasingly invested in research focused on hybrids resulting from the species crossing from genus *Corymbia*, because they present desirable wood quality for energy purposes while presenting high rusticity and high resistance to pests and diseases. In December 2019, leaf spots were observed in mini-stumps of the hybrid *Corymbia torelliana* × *C. citriodora* established in a clonal mini-garden in Alta Floresta, Mato Grosso, Brazil. After morphological and molecular analyses, it was found that leaf spots were caused by *Stigmina eucalypti* Alcorn and *Sulcosporium thailandicum* Phook. & K.D. Hyde. Thus, this work reports the first occurrence of *S. eucalypti* and *S. thailandica* causing leaf spots in *C. torelliana* × *C. citriodora* in Brazil, as well as being the first record of disease associated with this hybrid in clonal mini-garden in Brazil. These findings are likely to have great importance for promoting *Corymbia torelliana* × *C. citriodora* forestation in Brazil. © 2023 Friends Science Publishers

Keywords: Diseases; Clonal forestry; *Corymbia*; *Pseudocercospora; Stigmina*

Introduction

Most of the seedlings used in Eucalyptus plantations are produced through vegetative propagation, with emphasis on mini-cutting techniques in clonal mini-gardens, which can result in greater susceptibility to diseases. This is mainly due to the homogeneity of the seedlings, as well as these systems having very favorable environmental conditions, such as high humidity and temperature, for diseases development. Therefore, special attention should be paid to the management and diseases control also in the clonal mini-gardens, especially because the occurrence of diseases has increased even in this phase of the cloned seedlings production, with new records being increasingly frequent.

Currently in Brazil, hybrids of the genus *Corymbia*, have gained importance as an alternative to conventional species and hybrids of genus *Eucalyptus*, which are being used as raw material for various segments of industrial activity such as coal production, biomass energy production, wood for sawmill and preservative treatment. Its use occurs mainly for its high growth potential, resistance to quite a few pests and diseases, tolerance to water deficit, wind resistance and the high density of its wood (Wang *et al.* 2019). It is also noteworthy from different studies that have been carried out for the use of *Corymbia* hybrids industrially, in the cellulose pulp and paper producing sector (Assis 2020).

The hybrids resulting from the crossing between the species *Corymbia torelliana* (F. Muell.) K.D. Hill & L.A.S. Johnson and *C. citriodora* (Hook.) K.D. Hill & L.A.S. Johnson are the ones that have been most explored in research and received more investments from forestry companies. This is being pointed out as the most promising, for presenting high basic wood density and good drought resistance (Lee *et al.* 2009). However, the use of this hybrid in large forest plantations has run into the difficulty of large-scale seedling production, since vegetative propagation via cloning has not been successful in forest nurseries.

Diseases caused by fungi that attack the leaves of commercial species such as *S. eucalypti* and *S. thailandicum* can lead to total defoliation of trees directly influencing the productivity of plantations. Also in this case, in which the occurrence of pathogens occurred in a mini clonal garden, the losses can be even greater because the plants are more susceptible and the environment, which is favorable to the pathogen making the production of clonal seedlings unfeasible.

In this sense, the objective of this work was to report the occurrence of leaf spot disease caused by *Stigmina eucalypti* Alcorn and *Sulcosporium thailandicum* Phook. &

K.D. Hyde in mini-stumps of *Corymbia torelliana* × *C. citriodora* in a clonal mini-garden in Alta Floresta, Mato Grosso, Brazil.

Materials and Methods

Leaf spots were observed in the mini stumps of the hybrid *Corymbia torelliana* × *C. citriodora* established in a clonal mini garden in December 2019 (Fig. 1A) located in the Forest Nursery of the Forest Engineering Department from UNEMAT, in the municipality of Alta Floresta, Mato Grosso, Brazil (9° 52' 32" S 56° 05' 10" O).

Leaf spots were incident in 100% of the mini-stumps (Fig. 1A–B). Thus, in order to identify the leaf spots causal agents, leaf samples from the attacked mini-stumps were sent to the Agronomic Laboratory, specialized in phytosanitary diagnosis, and accredited by MAPA (Ministry of Agriculture, Livestock and Supply) for phytosanitary diagnosis analysis and biological products control for agronomic use.

In the laboratory, Eucalyptus leaves with leaf spot symptoms were examined under a light microscope with an adhesive tape preparation, where the conidia characteristic of *Stigmina* were visualized (Fig. 1B–C). The isolate was identified as *S. eucalypti* (Fig. 1D) according to Crous *et al.* (2007, 2019). The conidia were transferred to a microscopy slide containing 100 µL of sterilized deionized water using a histological needle. Subsequently the conidia were transferred to half agar dextrose potato (BDA) supplemented with chloramphenicol (50 mg L^{-1}). The plates were maintained at 25°C, photoperiod 12 h for 14 days. The colonies of the isolated fungus presented slow growth, dark brown coloration at the edges and gray to dark gray in the center (Fig. 1E). The asci were bitunicate and the ascospores were fusiform or slightly clavate, with rounded ends, 1 septum and thick walls similar to that of *S. thailandica* (Fig. 1F–G).

For molecular characterization, three mycelium discs were transferred to sterilized Erlenmeyer (250 mL) containing 50 mL of 2% liquid malt medium. The sample was maintained at 25°C under agitation at 110 rpm for 4 days. The internal transcribed spacer (ITS) region, using ITS1 and ITS4 oligonucleotides, was sequenced. The sequences obtained were edited in the BioEdit 7.0.5.3 program and the consensus sequences were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA 7.0) software (Kumar *et al.* 2016), constructed with the ClustalW algorithm and compared in the NCBI GenBank database. The similarity of the nucleotide sequence from the isolate was calculated using the Basic Local Alignment Search Tool (BLAST) program. In the phylogenetic analysis, using Maxima Parsimony, the isolate from sample 5501 was grouped with the reference isolate *S. thailandica* (Fig. 2).

Results

In the material sent for analysis, it was found that leaf spots were caused by *S. eucalypti* and *S. thailandica*. *Stigmina* is a *Pseudocercospora* synamorph, thus being considered a *Pseudocercospora* synonym, which is a large cosmopolitan genus of phytopathogenic fungi that are usually associated with leaf and fruit spots in several host plants. It occurs in arid and humid environments in both cold temperate and subtropical and tropical regions.

The generic epithet "*Sulcosporium*" refers to the striated ascospores, this genus being composed of a single species currently described, *S. thailandica*, first reported in Thailand in leaves of *Axonopus compresus* (Poaceae). Colony development of this species occurred at a temperature of 25 to 30°C (Ariyawansa *et al.* 2015). Thus, considering the environmental characteristics for the development of the species found in the clonal mini-garden of *C. torelliana x C. citriodora*, it is noteworthy that these pathogens can be considered as extremely relevant for the region where they are reported (north of Mato Grosso), which presents high temperatures throughout the year and also high humidity between November and April.

Discussion

Pseudocercospora is now treated as a genus although previously recognized as a *Mycosphaerella* anamorphic state (Crous *et al.* 2013). The first report of *S. eucalypti* causing damage to eucalyptus was performed by Alcorn (1973) reporting damage of this species in *Eucalyptus tessellaris* F. Muell. in north-eastern Australia. Some species of *Pseudocercospora* have already been reported causing leaf spots in eucalyptus in Brazil, however the available information is scarce, being only reports. It is therefore noteworthy that this genus presents little information available on symptomatology, occurrence and forms of control.

Associated with *S. eucalypti*, the occurrence of *S. thailandica* was found (Fig. 2). Ariyawansa *et al.* (2015) described the genus *S.* Phookamsak in 2015 as a grass pathogen, causing necrotic spots on the leaves. *Sulcosporium* was introduced as a monotypic genus to accommodate a species (*S. thailandica*). Phylogenetic analyses showed that *Sulcosporium* forms a well-supported, basal clade to the Mauritian clade in the Halotthiaceae family.

It is a species of Dothideomycetes (Ascomycota) that has bicellular, striated and thick-walled ascospores, being a species more similar to *Sulcispora* (Senanayake *et al.* 2015), which does not belong to the Phaeosphaeriiaceae. The symptoms caused by *Sulcosporium* are very similar to those caused by *Mixtura* in Poaceae, with pseudoparaphyses with saccate asci (Phookamsak *et al.* 2014). However, *Sulcosporium* has bicellular ascospores, while *Mixtura* has muriform ascospores (Ariyawansa *et al.* 2015). This is the first report of occurrence of *S. thailandica* in eucalyptus, and this pathogen, until then, commonly associated with necrotic spots in grasses, a symptom also found in this study associated with eucalyptus (Fig. 1). In the phylogenetic tree of this study (Fig. 2), *S. thailandica* appeared near

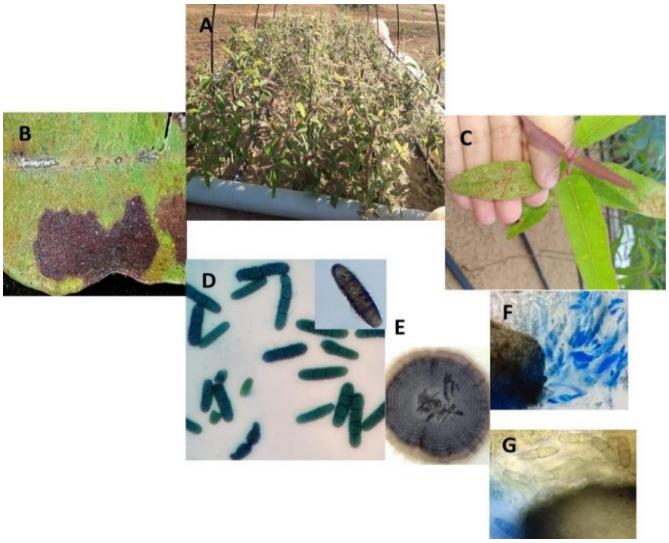

Fig. 1: (A) Clonal mini-garden of the "canaletão" type. (B) Leaf spots on the mini-stumps of *Corymbia torelliana* × *C. citriodora*. (C) Approximate image of lesions observed in the mini-stumps of *Corymbia torelliana* × *C. citriodora*. (D) Conidia of *Stigmina eucalypti*. (E) *Sulcosporium thailandica* colony grown in BDA medium. (F) Bitunicate asci and (G) fusiform ascospores

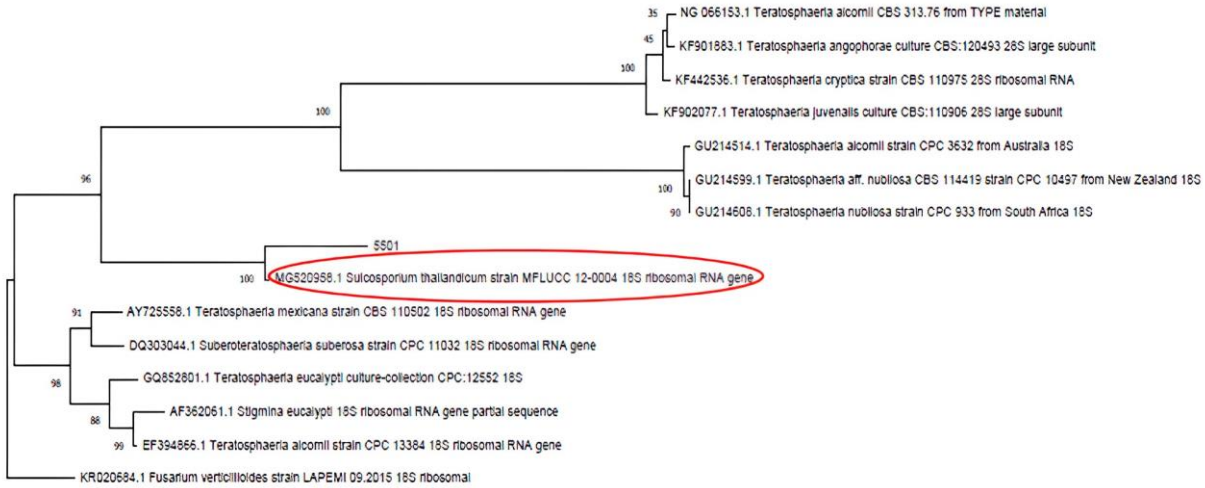

Fig. 2: Maximum Parsimony of the isolate from eucalyptus leaves with leaf spot symptoms

Teratosphaeria mexicana, of the family Teratosphaeriaceae, while *S. eucalypti*, of the family Mycosphaerellaceae, appeared near *T. alcornii*. Like *S. eucalypti*, *S. thailandica* presented little information available even for hosts, and regions of occurrence.

Conclusion

This work reports the first occurrence of *S. eucalypti* and *S. thailandica* causing leaf spots in *Corymbia torelliana* × *C. citriodora* in Brazil, as well as being the first record of diseases occurrence associated with this hybrid in clonal mini-garden in Brazil. The findings may have great implications for forestation of *Corymbia torelliana* × *C. citriodora* hybrids.

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Author Contributions

MSFP, LBB and ESM planned the experiments, JG, LE and MSFP interpreted the results, JG and MSFP made the write up and JG and LE analyzed the data and made the illustrations

Conflicts of Interest

All authors declare no conflict of interest among themselves

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable to this paper

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