Research, Society and Development, v. 11, n. 3, e55211326975, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i3.26975

Etiology, transmission and pathogenicity of fungi associated with Cagaita (*Eugenia dysenterica*) and Tingui (*Magonia pubescens*)

Etiologia, transmissão e patogenicidade de fungos associados a sementes de Cagaita (*Eugenia dysenterica*) e Tingui (*Magonia pubescens*)

Etiología, transmisión y patogenicidad de hongos asociados a semillas de Cagaita (*Eugenia dysenterica*) y Tingui (*Magonia pubescens*)

Received: 02/14/2022 | Reviewed: 02/21/2022 | Accept: 02/25/2022 | Published: 03/07/2022

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Abstract
Cagaita (*Eugenia dysenterica*) and Tingui (*Magona pubescens*) are two species of trees native to the Cerrado, with sociological, economic, and medicinal importance, besides to being used in the recovery of degraded areas. The incidence of plant diseases, as well as the mycoflora associated with their seeds, may represent a possible threat to the survival of the species. The cultivation of trees in the Cerrado Biome, as well as the diseases associated with them, are still poorly studied. The present work aimed to evaluate the mycoflora associated with Cagaita and Tingui seeds, the transmission and pathogenicity of these fungi to plants. The seeds were collected from mother trees located in the municipality of Gurupi and Figueirópolis in the state of Tocantins, Brazil. The blotter-test method was used to evaluate the mycoflora associated with the seeds of both species, with and without asepsis. The fungal incidence per seed was evaluated with the aid of a stereoscopic and optical microscope. The potentially pathogenic fungi identified in the sanitary analysis of the seeds and in the transmission, test were inoculated in the plants in order to confirm Koch's postulates. The transmission test was carried out from non-disinfested seeds until the appearance of symptoms in the seedlings. A total of 11 fungi associated with the seeds of both species were identified. The genus *Curvularia* showed pathogenicity, causing spots on Cagaita leaves, being also transmitted from seeds to seedlings. The *Fusarium* genus showed pathogenicity in Tingui seedlings, causing damping-off and wilting. *Colletotrichum* sp. caused leaf spots on both species and fruit spots on Cagaita.
Keywords: Seed pathology; Cerrado trees; *Curvularia* sp.; *Fusarium* sp.; *Colletotrichum* sp.

Resumo
Cagaita (*Eugenia dysenterica*) e Tingui (*Magona pubescens*) são duas espécies de árvores nativas do Cerrado, com importância sociológica, econômica e medicinal, além de serem utilizadas na recuperação de áreas degradadas. A incidência de doenças de plantas, bem como a micoflora associada às suas sementes, pode representar uma possível ameaça à sobrevivência da espécie. O cultivo de árvores no Bioma Cerrado, bem como as doenças a elas associadas,

ainda são pouco estudados. O presente trabalho teve como objetivo avaliar a micoflora associada às sementes de Cagaita e Tingui, a transmissão e patogenicidade desses fungos às plantas. As sementes foram coletadas de árvores matrizes localizadas no município de Gurupi e Figueirópolis no estado do Tocantins, Brasil. O método blotter-test foi utilizado para avaliar a micoflora associada às sementes de ambas as espécies, com e sem assepsia. A incidência fúngica por semente foi avaliada com auxílio de microscópio estereoscópico e óptico. Os fungos potencialmente patogênicos identificados na análise sanitária das sementes e no teste de transmissão foram inoculados nas plantas para confirmar os postulados de Koch. O teste de transmissão foi realizado a partir de sementes não desinfestadas até o aparecimento de sintomas nas mudas. Foram identificados 11 fungos associados às sementes de ambas as espécies. O gênero *Curvularia* apresentou patogenicidade, causando manchas nas folhas de Cagaita, sendo também transmitido de sementes para mudas. O gênero *Fusarium* apresentou patogenicidade em mudas de Tingui, causando tombamento e murcha. *Colletotrichum* sp. causou manchas foliares em ambas as espécies e manchas frutíferas em Cagaita.
Palvras-chave: Patologia de semente; Árvores do Cerrado; *Curvularia* sp.; *Fusarium* sp.; *Colletotrichum* sp.

Resumen
Cagaita (*Eugenia dysenterica*) y Tingui (*Magona pubescens*) son dos especies de árboles nativos del Cerrado, con importancia sociológica, económica y medicinal, además de ser utilizados en la recuperación de áreas degradadas. La incidencia de enfermedades de las plantas, así como la micoflora asociada a sus semillas, pueden representar una posible amenaza para la supervivencia de la especie. El cultivo de árboles en el Bioma Cerrado, así como las enfermedades asociadas a ellos, aún están poco estudiados. El presente trabajo tuvo como objetivo evaluar la micoflora asociada a semillas de Cagaita y Tingui, la transmisión y patogenicidad de estos hongos a las plantas. Las semillas se recolectaron de árboles madre ubicados en el municipio de Gurupi y Figueirópolis en el estado de Tocantins, Brasil. Se utilizó el método blotter-test para evaluar la micoflora asociada a las semillas de ambas especies, con y sin asepsia. La incidencia fúngica por semilla se evaluó con la ayuda de un microscopio estereoscópico y óptico. Los hongos potencialmente patógenos identificados en el análisis sanitario de las semillas y en la prueba de transmisión fueron inoculados en las plantas para confirmar los postulados de Koch. La prueba de transmisión se realizó desde semillas no desinfestadas hasta la aparición de síntomas en las plántulas. Se identificaron once hongos asociados a las semillas de ambas especies. El género *Curvularia* mostró patogenicidad, provocando manchas en las hojas de Cagaita, siendo también transmitida de semilla a plántula. El género *Fusarium* mostró patogenicidad en plántulas de Tingui, provocando marchitez y marchitez. *Colletotrichum* sp. causó manchas en las hojas en ambas especies y manchas en los frutos en Cagaita.
Palabras clave: Patología de semillas; Arboles cerrados; *Curvularia* sp.; *Fusarium* sp.; *Colletotrichum* sp.

1. Introduction

The Cerrado is a savanna formation that occupies most of Brazil's Central Plateau. With a surface of approximately 2 million km², equivalent to 22% of the Brazilian territory (Ministério do Meio Ambiente, 2019). Although it is still a little studied biome, compared to the Amazon, it is internationally considered a biodiversity hotspot (Critical Ecosystem Partnership Fundation, 2017). Most plant species have high economic and ecological potential. In addition to its social importance, it often complements the diet and serves as a source of medicines, fiber, wood for construction and energy for the local inhabitants. The cerrado is considered the richest savanna in the world in terms of biodiversity, besides having a high level of endemism (Klink & Machado, 2005).

Eugenia dysenterica DC., known as Cagaita or Cagaiteira is a tree in the Myrtaceae family. It is a characteristic tree of the cerrado, being found in Bahia, Minas Gerais, Goiás, Tocantins, São Paulo and Mato Grosso do Sul. Its distribution is irregular, being abundant in certain places and absent in others (Lorenzi, 1998). The flowering of Cagaita occurs in September, together with the recovery of the foliage. Fruiting coincides with the first rains, that is, from October to November (Camilo *et al.*, 2013). Cagaita is indicated for the recovery of degraded areas due to the following characteristics: heliophilous, xerophytic (Lorenzi, 1998) and zoochorous (Sano *et al.*, 1995), melliferous (Simeão *et al.*, 2015), low nutritional requirements (Duboc & Guerrini, 2007). In addition, Cagaita represents a high economic potential for the Cerrado regions, however it is poorly developed due to lack of consumer acceptance and difficulty in seedling production (Ministério do Meio Ambiente, 2016).

Magonia pubescens St. Hill, commonly called Tingui, Timbó, Tingui-do-Cerrado, Cuitê, Timbopeba, or Tangui, belongs to the family of Sapindaceae. Tingui is found in the cerrado, both in primary and secondary formations, from Ceará to Minas Gerais, passing through Goiás, Tocantins, Mato Grosso and Mato Grosso do Sul. Flowering occurs from August to

September, and fruit maturation can take a year, so seed production will occur in August, September of the following year. It is a heliophilous, pioneer, deciduous, selective xerophytic tree species (LORENZI, 2008). However, the seedlings support several levels of shading, from full sun to 70% shading (Oliveira *et al.*, 2016). This species is recommended in the recovery of degraded areas, in particular due to its colonization by arbuscular mycorrhizal fungi (Scabora et al., 2011).

It is known that seed germination, survival and mortality rate are the first indicators evaluated in a process of recovery of degraded areas (Palma & Laurance, 2015). The basis of successful planting of native species depends on several factors, including the sanitary quality of the seeds (Lima et al., 2014). Due to the seasonality of fruiting (Kuhlmann, 2012) and the difficulty of monitoring and harvesting (Oliveira *et al.*, 2016), seeds from native trees are still poorly studied. Furthermore, the incidence of plant diseases, as well as the mycoflora associated with their seeds, may represent a possible threat to the survival of native species, which must be multiplied before being used in degraded areas.

Given the above, this study aimed to evaluate the mycoflora associated with Cagaita and Tingui seeds, the transmission and pathogenicity of these fungi to these plants intended for the recovery of degraded areas.

2. Methodology

The experiments were conducted at the Phytopathology Laboratory of the Federal University of Tocantins, Gurupi-TO campus.

2.1 Origin of seeds and place of experiment

The seeds of both species were collected (Table 1) between September and October 2018, at the UFT de Gurupi campus and its surroundings. According to the species, a collection method was chosen. The seeds were removed from the fruits, dried in the shade in a dry and ventilated place, and later placed in a paper bag and stored in a refrigerator at 5 ºC.

Table 1 - Information on the locations and types of seed collection in Cagaita and Tingui.

Common name / Species	**Place of collection**	**Collection typ**	**Beneficiation**
Cagaita (*Dysenteric eugenics* DC.)	12°25'46” S 49°16'42” W	In ground	The seeds were removed from the fruits and sanitized with water and dried in the open air.
Tingui (*Magonia* pubsecens A.St.-Hil.)	11°44'49” S 49°03'01” W	On the tree	The seeds were removed from the fruits and sanitized.

Source: Authors.

2.2 Transport of fungi associated with seeds

The method used to survey fungal populations associated with the seeds was the filter paper method or “Blotter Test” (BRASIL, 2009). An acrylic Gerbox box, disinfected with 50% alcohol and 1% Sodium Hypochlorite, was used, containing two sheets of filter paper sterilized by autoclave and moistened with sterile distilled water.

The seeds were subjected to treatments, with and without asepsis, performed in the following sequence: seeds immersed in 50% alcohol solution for 40 seconds, followed by 1% sodium hypochlorite for 40 seconds. After disinfestation, they were washed twice in sterile distilled water and left to dry at room temperature.

200 seeds per species were used and divided into 5 repetitions of 40 seeds. Due to the unequal size of the seeds, each repetition was composed of four gerboxes for Cagaita and eight for Tingui. Subsequently, the boxes were placed in a growth chamber, type BOD, at a temperature of 25 °C and a photoperiod of 12 hours for a period of five to seven days. After this period, each seed was examined with the aid of a magnifying glass and optical microscope, where slides were prepared for

visualization of fungal structures (conidia and conidiophores). The initial identification of the genres was made with the help of specialized literature (Watanabe, 2010). The identified fungi were isolated and cultivated in potato-dextrose-agar (PDA) culture medium with antibiotic (Amoxilin) to limit the growth of bacteria.

The experimental design used was completely randomized, with five replications. The treatments were arranged in a 2x2 factorial scheme (seed and species treatment). For variance analysis, the incidence values of each fungal genus were transformed by the following formula: f(x) = arcsine (square root (x)), which: x = incidence of the genera in the seeds and f(x) = Transformed variable.

The transformed variable of each fungal genus was submitted to the ANOVA test for the model that considered the effect of the treatments. The means were compared by the Scott-Knott Test at 5% significance. For data analysis, the R Statistical Analysis System version 3.5.2 was used.

2.3 Transmissibility of fungi from seeds to seedlings

The seeds were sown without asepsis and without fungicide treatment, in order to evaluate the natural incidence of the fungi and their transmission to the plants. After sowing, it was sought to maintain soil moisture, so that the environment was favorable for transmission. During the growth of the seedlings, the appearance of the first symptoms of diseases were observed. To confirm Koch's Postulates, plant fragments that showed symptoms were detached for isolation. The plant material was collected and disinfected in the following sequence: a 50% alcohol solution for 40 seconds, followed by 1% sodium hypochlorite for 40 seconds and finally washed in sterile water. Then, the tissues were incubated in petri dishes with PDA medium. Subsequently, the petri dishes were placed in a growth chamber, type BOD, at a temperature of 25 °C and a photoperiod of 12 hours for a period of five to seven days. The plant material was observed with a magnifying glass and an optical microscope to verify the presence of fungal structures and aided by specialized literature.

2.4 Pathogenicity of fungi on seedlings and fruits

According to the physiology of each species, different treatments were made to overcome dormancy. For Cagaita, there was seed dormancy of tegumentary and biochemical origin. According to some authors, the integument of Cagaita seeds does not prevent water absorption, but limits gas exchange (Martinotto *et al.*, 2008; Silveira *et al.*, 2013). With scarification or complete suppression of the integument, the percentage of germination reaches 88%, therefore, the integument is removed with the help of a sterile stylet. Tingui seeds have a high germination rate without treatment, therefore, they were sown without any treatment. The seeds were sown in a black plastic bag (9 x 15 cm) containing a mixture of sterilized sand mixed with organic compost. The seedlings were irrigated daily. For each species, the fungi isolated during the sanitary analysis were inoculated in the aerial parts or/and in the soil according to the mode of infection of each fungal species. When the seedlings presented more than two definitive leaves, sprays were made with conidia solutions at a concentration of 2x106 spores mL^{-1}. Suspensions were prepared from colonies of fungi previously isolated in PDA medium. The inoculated seedlings were placed in a moist and dark chamber for 72 h. After this period, the seedlings were removed from the moist chamber and observed until the appearance of the first symptoms. To confirm pathogenicity, Koch's Postulates were performed.

3. Results and Discussion

3.1 Transport of fungi associated with seeds

Table 2 shows the average incidence of fungal genera found in Cagaita and Tingui seeds. According to the results obtained, a total of nine genders were identified.

There was a significant difference between the species of fungi in general (Pr = 0.0010 < 0.05), for Cagaita (Pr = 0.0000 < 0.05) and for Tingui (Pr = 0.0000 < 0.05). 05).

Table 2 - Mean incidence (%) of fungal genera associated with Cagaita and Tingui seeds submitted to treatments with asepsis (CA) and without asepsis (SA) [1].

Gender	Cagaita			Tingui			
	SA	CA	Average	SA	CA	Average	Average[3]
Trichoderma	0.0	0.0	0.0 a	0.0	2.0	1.0 aB	0.5 B
Alternaria	2.0	0.5	1.2 aB	0.0	0.0	0.0 a	0.6 B
Curvalaria	2.5	0.5	1,5 aB	0.0	0.0	0.0 a	0.7 B
Periconia	0.0	0.0	0.0 a	8.0	0.0	4.0 aB	2.0 B
Rhizopus	10.0	1.0	5.5 aB	0.0	0.0	0.0 a	2.7 B
Fusarium	3.5	2.5	3.0 aB	9.5	13.5	11.5 aA	7.2 B
Aspergillus	1.5	2.0	1.7 aB	59.0	0.0	29.5 aA	15.6 B
Cladosporium	81.0	45.0	42.7 aA	0.0	0.5	2.5 aB	21.5 B
Aspergillus niger	70.0	1.5	43.7 aA	35.0	2.0	18.5 aA	31.1 A
Penicillium sp	83.5	12.5	48.0 aA	45.0	46.5	45.7 aA	46.9 A
Average2		18.4 a			12.7 a		12,9

[1]Means followed by the same letter has no significant differences according to the Scott-Knott Test (P < 0.05), with capital letters relating to each column and lowercase letters relating to each row. [2]Average fungal incidence per species. [3]Average incidence per fungal gender. Source: Authors.

Of the fungal genera identified, four of them were common associated with the two species of trees: *Cladosporium*, *Aspergillus*, *Penicillium* and *Fusarium.* These same fungi were also found in Cerrado plant species in studies developed by Pinheiro et al. (2016). According to studies developed by Watanabe (2010), these fungi are very frequent because they are associated with plants. It is known that some fungi found associated with seeds can be considered as being external saprophytes).

There were no significant differences between Cagaita and Tingui in terms of total fungal incidence (Pr = 0.5212 > 0.05). But there were differences between the associated fungal genders. Eight fungi associated with Cagaita seeds were found, including *Curvularia* sp., *Rhizopus* sp. and *Alternaria* sp. that have been identified exclusively in this species. Other genders, not observed in this study, have already been found associated with Cagaita seeds, namely: *Helminthosporium* sp., *Asterinella sublibera*, *Cercospora* sp., *Colletotrichum* sp., *Didymella glacialis*, *Drechslera* sp., *Johansonia pandani*, *Mucor* sp., *Nigrospora* sp., *Phloeosporella* sp., *Puccinia psidii*, *Schizothyrium coutareae* and *Stigmopeltis* sp. (EMBRAPA, 2010).

Nine genera associated with Tingui seeds were found. In addition to the common genera already mentioned, *Periconia* and *Trichoderma* were also identified.

The asepsis had a significant effect on the elimination of some fungi, decreasing the incidence in seeds (Pr = 0.0146 < 0.05). Whereby, in the seeds of the forest species studied, the treatment without asepsis had an average incidence of 24.96% and, in the treatment with asepsis, there was an incidence of 6.20%. Thus, it can be affirmed that the asepsis of the seeds significantly reduced the fungal incidence in the seeds of Cagaita and Tingui (Table 2).

For Cagaita seeds, the fungal incidence in seeds with asepsis was 5.12%, and 31.75% without asepsis. It can be observed the same fungi associated with the seeds in both treatments, that is, the asepsis did not eliminate the fungi, however, there was a decrease in their incidence. The asepsis can eliminate part of the inoculum (spores, hyphae fragments) and thus limit the development of fungi. According to Pinheiro (2016), this method acts on the surface of the seeds, protecting them from some pathogens. However, we have to consider that the treatment with alcohol and sodium hypochlorite does not present systemic action on the seeds, so that some more resistant fungi or those located in the more internal layers of the seeds can survive to the treatment.

Likewise, the fungal incidence in Tingui seeds was lower with asepsis (7.2%) than without asepsis (18.16%). However, unlike Cagaita, the specific diversity has also changed. Three fungi, two of them *Aspergillus* sp. and *Periconia* sp., were no longer found after asepsis. Some studies had similar results with other forestry species, where asepsis eliminated fungal species (Muniz et al., 2007). However, two other genera, absent in the treatment without asepsis, were found in the seeds with asepsis, being them, *Cladosporium* and *Trichoderma*. The Tingui seeds have external protective layers that, in contact with water, produce a gel composed of cellulosic oligosaccharides (Gorin *et al.*, 1996), divided into five regions of several composition (Oliveira *et al.*, 2001). This gel has potential antifungal activity, in particular against *Penicillium*, *Aspergillus* and *Hormodendrum* sp. Studies demonstrate the potential use of powder covering Tingui seeds as products for pelleting commercial seeds, as their characteristics are similar to HPMC (hydroxypropylmethylcellulose), in addition to having an antifungal activity (Teixeira, 2007). Thus, the alcohol and sodium hypochlorite, used in asepsis, may have denatured, or altered the protective gel of the seeds, which could explain these differences in the fungal population.

The fungi with larger frequency in Cagaita and Tingui were *Aspergillus niger* and *Penicillium* sp., with an incidence of 31.11% and 46.8% on seeds, respectively. Furthermore, it was observed that the asepsis did not eliminate these fungi that were associated with the seeds of both species, even after the disinfestation. These two fungi are usually found in closed environments of seed storage, so they are called storage fungi. Consequently, they may come from external contamination. The so-called storage fungi may not be phytopathogenic to plants, however, they can cause damage to the seeds (Dhingra *et al.*, 2003). Under not suitable conditions, such as humidity and high temperature, the "storage" fungi can quickly degrade seeds and impair the seedling germination and emergence, in addition to producing toxic substances.

In particular, the seeds with a high-water content and sensitive to desiccation suffer more from saprophytic fungi. Despite being generalist molds, these fungi can impair the seed storage, especially in tropical climates. The Cagaita seeds are recalcitrant, that is, they cannot withstand a long period of storage, desiccation and very low temperatures (Delgado & Barbedo, 2012). They present a high-water content, between 47 and 53%. When the water content of the seeds dropped below 18-22%, the seeds lost their viability. The ideal for its storage is inside plastic bags in a cold chamber at 10 °C with 60% humidity (Andrade *et al.*, 2003). These conditions favor the development of fungi associated with the seeds and their deterioration, as shown in species close to Cagaita, *Eugenia brasiliensis*, *E. pyrifomis* and *E. uniflora* (Oliveira *et al.*, 2011). Preferably, the Cagaita seeds should be collected in the soil than in the tree, for a better germination rate, however, this can increase the risk of contamination by fungi. Certain fungi may be associated with the seeds, and remain dormant until the fruit maturation (Singh, 2011).

The orthodox species tend to have a low water content, which limits the fungi development and attack. Unlike to the recalcitrant species that need to be kept with high water content, presenting greater colonization by so-called field fungi, such as *Fusarium* (Schmidt, 2007). The Tingui is an orthodox species (Consolaro *et al.*, 2019). Its seeds have an average water content of 6% (Arantes *et al.*, 2017). However, storage for up to 90 days is recommended, as it is after this period that the germination rate decreases and the seeds lose vigor (Jeromini *et al.*, 2015).

The fungi found in the sanitary analysis of Cagaita and Tingui seeds can seriously compromise the storage of these species. Most Cerrado tree species produce seeds once a year and usually at the end of the dry season. These characteristics, together with the need to wait for the rainy season to the planting of species, require a storage period, even short, which can be compromised by the fungi identified in this work. The tropical climatic condition can, however, favor the degradation of seeds and, consequently, compromise their planting.

3.2 Transmission

In the transmission study, from seeds without disinfestation, arise in Cataiga seedlings, initially, yellow-brown leaf spots, which evolved into dark necrosis (Figure 1). It is worth noting, that in the evaluated period, during four months, these lesions were restricted to adult leaves that, over time, did not cause the death of the plants. This symptom type has already been reported by other authors, in nursery conditions (Martinotto *et al.*, 2008).

From the leaf lesions, the isolations of necrotic tissues showed the presence of several associated fungi (Table 3).

Table 3 - Fungi isolated from different parts of Cagaita seedling plants in the transmissibility test.

Specie	Plant part	Symptoms
Phoma sp.	Leaves	Dark spot with necrosis
Colletotrichum sp.	Leaves	Dark spot with necrosis
Cladosporium sp.	Leaves	Dark spot with necrosis
Pestalotia sp.	Leaves	Dark spot with necrosis

Source: Authors.

Among the fungi found, only the genus *Cladosporium* was found during the sanitary analysis of Cagaita seeds. The other fungi, *Colletotrichum*, *Pestalotia* and *Phoma* were not observed in the seeds. In this case, they may have been transmitted from the environment to the seedlings, by ways: plant-plant or soil-plant. Other hypotheses are that these fungi were already present in the seeds, at low frequencies, in the dormant hyphae form, so they were not previously found. There is also the possibility that other faster growing fungi such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* may have limited the development of these fungi.

Figure 1 - Cagaita leaf (*Eugenia dysenterica*) presenting necrotic lesions during the transmission test.

Source: Authors.

The Tingui seedlings showed different symptoms, including stains on the edges of the leaves that progressed to the necrosis. In addition, some plants wilted with the dry leaves until death (Figure 2).

After isolating the lesions from different plant tissues, some associated fungi were found, as shown in Table 4.

Table 4 - Fungi isolated from different diseased tissues of Tingui plants in the transmissibility test.

Specie	Diseased tissues	Symptoms
Cladosporium sp.	Leaves	Leaf spots
Colletotrichum sp.	Leaf and Stem	Leaf spots
Fusarium sp.	Stem, Roots, Cotyledons, Leaves	Total wilting - dead plant

Source: Authors.

Figure 2 - Tingui seedlings with symptoms of leaf spot (left) and wilting (right) during the transmissibility test.

Source: Authors.

Of the fungal genera found, *Cladosporium* and *Fusarium* had already been detected associated with Tingui seeds. Only the *Colletotrichum* had not been observed in the seeds. In the same way that it appeared in the Cagaita seeds, this genus may have been transmitted by seeds, but with a very low incidence, or it was transmitted by the environment to the plant. Although this genus was found associated with leaf spots, this fungus was not detected in the respective species seeds.

3.3 Pathogenicity

Of the eight fungal genera detected during the sanitary analysis of the seeds, or isolated from lesions on seedlings, in the transmission test, some of them were tested for pathogenicity in Cagaita plants, as can be seen in Table 5.

Table 5 - Pathogenicity of fungi associated to Cagaita seeds or leaves.

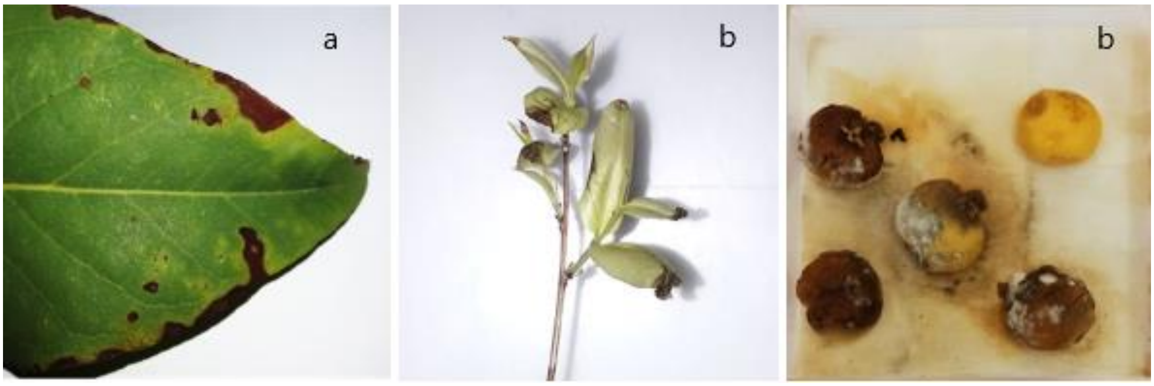
Specie	Tissue	Pathogenicity[1]
Curvularia sp	seed	+
Cladosporium sp	seed	-
Phoma sp	leaf	-
Colletotrichum sp	leaves	+
Cladosporium sp	leaves	-
Pestalotia sp	leaves	-

[1] (+) Pathogenic fungus (-) Non-pathogenic fungus (NT) Not tested. Source: Authors.

The seedlings inoculated with *Curvularia* showed leaf spots after one month. The spots, irregularly shaped, started at the edges of the leaves and progressed to the center (Figure 3a). From these spots, the *Curvularia* were isolated again, which confirms the Koch's postulates. Even being pathogenic and associated with Cagaita seeds, the *Curvularia* was not observed during the transmissibility test. It is thus noted that for transmission, even for potentially pathogenic fungi, the inoculum transported via seed depends, fundamentally, on the cultivated species (resistance), environmental conditions (ambient and soil humidity, temperature, wind, rain and light), inoculum (viability, location in the seed), cultivation practices (soil type, pH, plant population, planting depth and planting season, fertilization), inoculum survival, soil and seed microflora, among others (Mayer et al., 2001).

The seedlings inoculated with *Colletotrichum* showed leaf spots, starting at the periphery, leading to total necrosis, after 4 days (Figure 3b). From these spots, *Colletotrichum* was isolated again, which allowed the confirmation of the Koch's postulates. In addition to the leaves, *Colletotrichum* was also inoculated in healthy Cagaita fruits. The pathogen caused rot symptoms on the fruits, 3 days after inoculation.

Figure 3 - Symptoms details associated with the pathogenic fungi *Curvularia* sp. (a.) and *Colletotrichum* sp. (b.) in Cagaita seedlings during the pathogenicity test.



Source: Authors.

Therefore, spores of *Colletotrichum* sp. were inoculated in healthy Cagaita fruits in order to test its pathogenicity. After three days, rot symptoms with spots appeared on the fruits (Figure 3b). From these spots, the genus *Colletrotrichum* was identified under the microscope.

Colletotrichum gloesporioides is a pathogen of species close to Cagaita, such as Araça-boi (*Eugenia stipitata*), causing lesions on the fruits (Sterling-Cuellar *et al.*, 2005). The *Colletotrichum* species are recognized as pathogens of a broad spectrum, that is, they can attack different plants, in particular their fruits (Phoulivong, 2012).

Of the fungi isolated, only the genera *Colletotrichum* and *Fusarium* will cause symptoms of diseases in seedlings of Tingui, confirming their pathogenicity, as can be seen in Table 6. The pathogenicity was confirmed through the Koch's Postulates. *Colletotrichum* caused leaf spots that appeared four days after inoculation (Figure 4a), while *Fusarium* sp. created the wilting and death of the seedlings (Figure 4b). According to the literature consulted, there were no records of these fungi as being pathogenic to the *Magonia pusbescens* plant.

Table 6 - Pathogenicity to seedlings of fungi transmitted horizontally and vertically associated with the Tingui specie.

Specie	Tissue	Pathogenicity[1]
Periconia sp.	Seeds	-
Fusarium sp.	Seeds	+
Fusarium sp.	Roots	-
Cladosporium sp.	Leaves	-
Colletotrichum sp.	Leaves	+

[1] (+) Pathogenic fungus (-) Non-pathogenic fungus. Source: Authors

Figure 4 - Symptoms details associated with the pathogenic fungi *Colletotrichum* sp. (a.) and *Fusarium* sp. (b.) in Tingui seedlings.

Source: Authors.

The seedlings inoculated with *Colletotrichum* showed leaf spots after four days. The spots, of round shape and brown, started at the edges of the leaves and progressed to the center (Figure 4a). From these spots, the *Colletotrichum* sp. was isolated again, which confirmed Koch's postulates.

These results show the importance of studying tree species native from the Cerrado. In particular, the nursery diseases can quickly spread and compromise the final planting.

4. Conclusion

There was diversity of microflora associated with Cagaita and Tingui seeds. The fungi with the highest incidence in the seeds of these species are *Penicillium* sp. and *Aspergillus niger*, which were not eliminated by asepsis.

The asepsis can be used to reduce the incidence of fungi in Cagaita seeds. However, in Tingui seeds, even with a decrease in fungal incidence with this treatment, it is not recommended, because Tingui seeds have protective and antifungal layers, and the asepsis may compromise these protections.

The fungi identified as a pathogen for Cagaita were *Curvularia* sp., that caused leaf spot. The fungus *Colletotrichum* sp. also caused pathogenicity in Cagaita, both in leaves and fruits.

For Tingui, the fungi *Colletotrichum* sp., causing leaf spots, and *Fusarium* sp., causing the seedlings to wilt to death, were pathogenic.

More studies are also needed on the health, storage and alternative control of fungi associated with forest seeds and seedlings. Fungi present in seeds can cause lesions that result in the weakening or death of plant species used in the recovery of degraded areas such as Cagaita and Tingui.

Acknowledgments

The Federal University of Tocantins, Campus de Gurupi, for the conditions to carry out this work. To the Postgraduate Program in Forestry and Environmental Sciences (PPGCFA/UFT) for the resources to translate this article.

References

Andrade, A. C. S., Cunha, R., Souza, A. F., Reis, R. B. & Almeida, K. J. (2003). Physiological and morphological aspects of seed valbility of neotropical savannah tree, *Eugenia dysenterica* DC. *Seed Science & Technology*, 31 (1), 125-137.

Arantes, C. R. A., Fava, C. L. F., Camili, E. C. & Scaramuzza, L. M. P. (2017). Germination and vigor of seeds of *Magonia pubescens* A. St.-Hil. submitted to storage and pre-imbibition in water. *Journal of Seed Science*, 39 (4), 344–352.

Brasil. MAPA. *Manual de análise sanitária de sementes.* (2009). Mapa/ACS.

Camilo, Y. M. V., Souza, E. R. B., Vera, R. & Naves, R. V. (2013). Fenologia, produção e precocidade de plantas de *Eugenia dysenterica* visando melhoramento genético. *Revista de Ciências Agrárias*, 36 (2), 192-198.

Consolaro, H., Alves, M., Ferreira, M. C. & Vieira, D. L. M. (2019). *Sementes, plântulas e restauração no sudeste goiano*. Catalão.

Critical Ecosystem Partnership Fundation. (2017). *Perfil do Ecossistema Hotspot de Biodiversidade do Cerrado*. https://www.cepf.net/our-work/biodiversity-hotspots/cerrado.

Delgado, L. F. & Barbedo, C. J. (2012). Water Potential and Viability of Seeds of *Eugenia* (Myrtaceae), a tropical tree species, based upon different levels of drying. *Brazilian Archives of Biology and Technology*, 55 (4), 583-590.

Dhingra, O., Lustosa, D. C., Maia, C. & Mesquita, J. B. (2003). Seedborne fungal pathogens of jacaranda (*Dalbergia nigra*) tree. *Seed Science and Technology*, 31 (2). 341–349.

Duboc, E. & Guerrini, I. A. (2007). *Desenvolvimento inicial e nutrição da cagaita em áreas de Cerrado degradado*. Embrapa Cerrados ed. Planaltina.

Embrapa. (2010). *Banco de Dados - Fungos Relatados em Plantas no Brasil*. http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fichafg.asp?id=2602.

Pinheiro, C. G., Lazarotto, M., Muniz, M. F. B., Redin, C. G. & Santos, M. V. (2016). Efeito da assepsia superficial na germinação e incidência de fungos em sementes de espécies florestais. *Pesquisa Florestal Brasileira*, 36 (87), 253-260.

Gorin, P. A. J., Teixeira, A. Z. A., Travassos, L. R., Labouriau, M. L. S. & Lacomini, M. (1996). Characterization of carbohydrate components of an unusual hydrogel formed by seed coats of *Magonia pubescens* (Tingui). *Carbohydrate Research*, 282 (2), 325–333.

Jeromini, T. S., Scalon, S. P. Q., Perreira, S. T. S., Fachinelli, R. & Filho, H. S. (2015). Armazenamento de sementes e sombreamento na emergência e crescimento inicial das mudas de *Magonia pubescens* A. St.-Hil. *Revista Árvore*, 39 (4), 683–690.

Klink, C. & Machado, R. (2005). A conservação do Cerrado brasileiro. *Megadiversidade*, 1, (1), 147–155.

Kuhlmann, M. (2012). *Frutos e Sementes do Cerrado Atrativos para Fauna: Guia de Campo*. Rede de Sementes Cerrado ed. Brasília.

Lima, Y. B. C., Durigan, G. & Souza, F. M. (2014). Germinação de 15 espécies vegetais do cerrado sob diferentes condições de luz. *Bioscience Journal*, 30 (6), p. 1864-1872.

Lorenzi, H. (1998). *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. (2a ed.), Instituto Plantarum de Estudos da Flora.

Lorenzi, H. (2008). *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. (5a. ed.), Instituto Plantarum de Estudos da Flora.

Martinotto, C., Paiva, R., Soares, F. P., Santos, B. R. & Nogueira, R. C. (2008). *Cagaiteira (Eugenia dysenterica DC.)*. Editora UFLA.

Mayer, A. M., Staples, R. C. & Gil-Ad, N. L. (2001). Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. *Phytochemistry*, 58 (1), 33–41.

Ministério do Meio Ambiente. (2016). *Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial - Plantas para o Futura - Região Centro Oeste*. Brasília.

Ministério do Meio Ambiente (2019). *O Bioma Cerrado*. https://www.mma.gov.br/biomas/cerrado.

Muniz, M. F. B., Silva, L. M. & Blume, E. (2007). Influência da assepsia e do substrato na qualidade de sementes e mudas de espécies florestais. *Revista Brasileira de Sementes*, 29 (1), 140–146.

Oliveira, C. M. R., Lacomine, M., Alquini, Y. & Gorin, P. A. J. (2001). Microscopic and NMR analysis of the external coat from seeds of *Magonia pubescens*. *New Phytologist*, 152 (3), 501–509.

Oliveira, M. C., Ogata, R. S., Andrade, G. A., Santos, D. S., Guimarães, T. G., Silva Junior, M. C., Pereira, D. J. S. & Ribeiro, J. F. (2016). *Manual de viveiro e produção de mudas: espécies arbóreas nativas do Cerrado*. Editora Rede de Sementes do Cerrado. Brasília.

Oliveira, B., Reis, S. M., Morandi, P. S., Valadão, M. B. X., Oliveira, E. A., Marimon, B. S. & Marimon-Junior, B. H. (2016). Germinação das sementes e desenvolvimento de mudas de *Magonia pubescens* A.St.-Hil. (Sapindaceae) sob diferentes intensidades de sombreamento. *Scientia Forestalis*, 44 (112), 905-916.

Oliveira, C. F., Oliveira, D. C., Parise, J. J. D. & Barbedo, C. J. (2011). Deterioração de sementes de espécies brasileiras de Eugenia em função da incidência e do controle de fungos. *Revista Brasileira de Sementes*, 33 (3), 520–532.

Palma, A. C. & Laurance, S. G. W. (2015). A review of the use of direct seeding and seedling plantings in restoration: what do we know and where should we go? *Applied Vegetation Science*, 18 (4), 561–568.

Phoulivong, S. (2012). Cross infection of Colletotrichum species, a case study with tropical fruits. *Current Research in Environmental & Applied Mycology*, 2 (2), 99–111.

Sano, S. M., Fonseca, C. E. L., Riberio, J. F., Oga, F. M. & Luiz, A. J. B. (1995). Folhação, floração, frutificação e crescimento inicial da cagaiteira em Panaltina, DF. *Pesquisa Agropecuária Brasileira*, 30 (1), 5-14.

Scabora, M. H., Maltoni, K. L. & Cassiolato, A. M. R. (2011). Associação micorízica em espécies arbóreas, atividade microbiana e fertilidade do solo em áreas degradadas de cerrado. *Ciência Florestal*, 21 (2), 289-301.

Schmidt, L. H. (2007). *Seed Storage*. In: Tropical Forest Seed. Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg.

Silveira, C. E. S., Palhares, D., Pereira, L. A. R. & Pereira, K. D. B. (2013). Strategies of plant establishment of two Cerrado species: *Byrsonima basiloba* Juss. (Malpighiaceae) and *Eugenia dysenterica* Mart. ex DC (Myrtaceae). *Plant Species Biology*, 28 (2), 130–137.

Simeão, C. M. G., Silveira, F. A., Sampaio, I. B. M. & Bastos, E. M. A. F. (2015). Pollen analysis of honey and pollen collected by Apis *mellifera* linnaeus, 1758 (Hymenoptera, Apidae), in a mixed environment of *Eucalyptus* plantation and native cerrado in Southeastern Brazil. *Brazilian Journal of Biology*, 75 (4), 821-829.

Singh, S. P. (2011). Guava (*Psidium guajava* L.). In: *Postharvest Biology and Technology of Tropical and Subtropical Fruits*. Elsevier.

Sterling-Cuellar, A., Vinasco-Sandoval, G. T., Martínez-Cotacio, C. A., Garzón-Gómes, M. T., Perdomo-Rojas, L. T., Parra-Ramos, N. C. & Calderón-Rosas, B. M. (2015). Caracterización de daños histológicos ocasionados por hongos patógenos aislados del fruto de arazá (*Eugenia stipitata*). *Momentos de Ciencia*, 2 (2), 70-74.

Teixeira, A. Z. A. (2007). Evaluation of powder properties from the seed coat of tingui (*Magonia pubescens*) as an excipient for seed pelleting. *Estudos de Biologia*, 29 (67), 171-178.

Watanabe, T. (2010). *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species*, (3a ed.), CRC Press.