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"Decay and descending death" new disease of *Schizolobium parahybum* (Vell.) S.F. Blake (pachaco) in Ecuador

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***Abstract*---**Plantations of *Schizolobium parahybum* (pachaco) in the Ecuadorian Humid Tropics are being affected by the disease "decay and descending death", whose etiology is unknown so far. The aim was to know the incidence, severity, and symptomatology of the disease and to identify morphologically the fungi associated with diseased trees. A 5-year-old plantation of *S. parahybum* was studied in the province of Los Ríos, where three rectangular plots of 500 m2

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were established. Disease severity was evaluated with a 5-category scale (1=apparently healthy tree, and 5=tree with more than 50% dead tissue). For each plot, three trees were dissected and their tissues were analyzed under three methodological strategies (wet chamber, carrot sandwiches and potato-dextrose-agar (PDA) culture medium). Disease incidence was low at 1.7% (13 diseased trees ha-1). Diseased trees were found at scale 2, which indicates that they are at an early stage of the disease. Diseased trees showed loss of turgor, paleness and yellowing of leaflets, with young leaves showing reduced size and poor formation. Longitudinal areas of necrosis were observed in the upper third of the stem. The fungi *Fusarium* sp. 1, *Fusarium* sp. 2, and *Botryodiplodia* sp. were isolated and identified, all causing pathogenesis in plants. The incidence, severity, and symptomatology detected differed from those reported for other diseases in *S. parahybum*, so the "decay and descending death" disease is a new report for this forest species in Ecuador.

***Keywords*---**Botryodiplodia sp., Fusarium sp., pathogenesis, forest pathology.

Introduction

Schizolobium parahybum (Vell.) S.F. Blake (pachaco) is a species native to the tropical rainforests of Central and South America that was introduced into the production systems of the Ecuadorian Humid Tropics (EHT) in the 1960s and 1970s, becoming one of the promising species for afforestation and reforestation projects in Ecuador. Beginning in 1980, a complex disease with characteristics of "vascular wilt and stem rot" appeared that caused the death of thousands of *S. parahybum* trees and the almost total disappearance of the species in the production systems of the region (Belezaca-Pinargote *et al.*, 2011, Belezaca-Pinargote *et al.*, 2012a).

At that time, studies conducted by Ramírez (1990) and later by Belezaca-Pinargote and Suárez-Capello (2003), Belezaca-Pinargote *et al.* (2011) established that the symptoms associated with the disease were caused by phytopathogenic fungal species of the *Ceratocystis* genus (*C. paradoxa*, *C. moniliformis*, and *C. fimbriata*).

Currently, *S. parahybum* has reawakened the interest of the timber industry, so plantations have been established in small areas (≤ 15 ha^{-1}) to know the behavior of the species against the disease that decimated plantations in EHT in past decades. However, recent field observations show that several young trees of *S. parahybum* are affected by a disease whose symptoms are characterized by decay and generation of necrotic zones in the apex or middle third of the trees until they die downwards. This symptomatology differs from that previously reported by Ramírez (1990), Belezaca-Pinargote and Suárez-Capello (2003), Belezaca-Pinargote *et al.* (2011), and Belezaca-Pinargote *et al.* (2012a), which leads to the suspicion that the causal agents are not those previously known.

For this reason, it was proposed to describe the symptomatology and morphologically identify the fungal microorganisms associated with *S. parahybum* trees with symptoms of the new disease.

Methodology

Study area, soil and climatic conditions, plot establishment, and planting density

The research was carried out in a 5-year-old experimental plantation of *S. parahybum*, located at 120 m above sea level in the Buena Fe canton, province of Los Ríos, Ecuador, belonging to the forestry company PLANTABAL S.A., whose coordinates are 0°48'53.13" South latitude and 79°30'42.04" West longitude (Figure 1). The area where the *S. parahybum* plantation is located has typical edaphoclimatic conditions, as shown in Table 1. Three rectangular plots of 500 m2 were delimited, within which the initial density was determined at the time of the evaluations (Table 2).

Figure 1. Geographical location of the *S. parahybum* plantation studied in the central zone of the Ecuadorian Humid Tropics.

Table 1. Values of predominant edaphoclimatic variables in the area where the *S. parahybum* plantation under study is located.

Parameter	**Average**
Climate	Warm and Humid Tropical
Life Zone	Tropical Rainforest

Average annual temperature	24.5 °C
Average annual precipitation	1,500 - 3,200 mm
Relative Humidity	85 - 95 %
Soil	Loamy, clayey
Altitude	100 - 300 masl

Table 2. Initial density and density detected at the time of the evaluations in three plots within a plantation of S. parahybum.

Plot No.	Initial planting density (árboles ha^{-1})	Density at time of evaluation (trees ha^{-1})
1	840	800
2	840	780
3	840	820
$\overline{x}$	840	800

Disease incidence

In each plot, a tree-by-tree census was conducted, to establish the total number of trees present, the number of trees with disease symptoms, and dead and healthy trees (Belezaca-Pinargote *et al.*, 2018; Belezaca-Pinargote *et al.*, 2021a), [Equation 1].

$$\text{Incidence (\%)} = \frac{\text{Nº of diseased trees}}{\text{Total trees}} * 100 \qquad \text{[Equation 1]}$$

Severity of the disease

An arbitrary scale of five categories was used according to the visible morphological differences of the branches, leaves, and trunk, to be compared with healthy trees (Belezaca-Pinargote *et al.*, 2021b), as shown in Table 3. In addition, a detailed description of the disease symptomatology was made.

Table 3. Arbitrary scale used to determine the severity of the "decay and descending death" disease in *S. parahybum*.

Scale	Criteria
1	Tree apparently healthy, no evidence of visible symptoms.
2	Initial yellowing of the crown, the trunk may have small necrotic wounds with black exudation in different places or where there was natural pruning; the appearance of resprouts may begin. Not all symptoms are expressed.
3	The tree is visibly diseased. There are canker-like lesions on the bark with signs of rot, presence of exudation; loss of more than 50% of the leaf area in a progressive pattern; resprouts developed.
4	The individual is completely affected; total absence of foliage; there is

	evident loss and detachment of branches; resprouts are still observed in some sectors of the trunk; rotting and exudation in the cancerous zone (canker) is clearly evident.
5	The tree is completely dead, the wood has already lost its commercial value.

Evaluation of dasometric variables

The following variables were recorded for the trees present in each plot: diameter at breast height (DBH; 1.30 m above ground level), height (m), number of epicormic shoots (m), number of epicormic buds (m), and number of epicormic shoots.

Sampling and collection of tissue from diseased trees

Within each plot, 3 trees (7±0.5%) with disease symptoms were sampled and cut at ground level with the aid of a chainsaw. Subsequently, transverse cuts were made in the trunk every 70 cm, to determine the site of entry of the pathogen or pathogens and their dissemination within the tissues. The observation of internal symptoms (tissue necrosis) was used for the symptomatological description of the disease.

Sections of wood with evidence of necrosis were selected, stored in plastic bags, labeled (date of collection, origin, tree number, age of plantation, etc.), and transferred to the phytopathology laboratory of PLANTABAL S.A. for subsequent analysis. The samples of necrotic tissues were conditioned under three methodological strategies detailed below:

Wet chamber

To provide conditions of high relative humidity and constant temperature (22±2 oC) at the laboratory level, wood samples with necrotic tissues were placed in plastic bags containing moistened paper and incubated for 96 hours. After this time and with the assistance of a stereomicroscope, the samples were analyzed to detect the development of signs (mycelium, fruiting bodies, etc.) present on the necrotic tissues (Belezaca-Pinargote *et al.*, 2018; Belezaca-Pinargote *et al.*, 2021a). When signs of microorganisms growing on the wood were detected, they were transferred to the Potato-Dextrose-Agar culture medium (PDA).

Sowing in carrot sandwiches

To stimulate the growth and development of fungal microorganisms that are difficult to grow initially in synthetic culture media, sowings were made with necrotic tissue segments of approximately 2 x 2 x 0.5 cm (length, width, and thickness) between two carrot slices, tightened with paper tape, forming a kind of sanduche (Li *et al.*, 2014; Piveta *et al.*, 2016). For each tree, 10 sanduches were formed and placed in sterile plastic containers, covered, and incubated for 120 hours (5 days). Subsequently, with the aid of a stereomicroscope, the sanduches were analyzed to detect the development of signs (mycelium, fruiting bodies, etc.) growing on the carrot, and when present, they were transferred to PDA.

Direct sowing in PDA culture medium

For each selected tree, from fresh samples of necrotic wood, small pieces of wood of approximately 0.5 x 0.5 cm were cut with a sharp knife and deposited in a sterile Petri dish, from which, without prior disinfection with any antiseptic, four pieces of necrotic wood were seeded in five Petri dishes containing 10 mL of PDA culture medium + 0.2 mL of an antibiotic mixture (50 µg/mL penicillin and 25 µg/mL streptomycin), (Belezaca-Pinargote *et al.*, 2018; Belezaca-Pinargote *et al.*, 2021b), and were left to incubate for 96 hours at laboratory temperature (22±2 oC). After this time, the fungi that grew in the culture medium were identified. The identification was carried out with the help of taxonomic keys (Von Arx, 1981; Barnett and Hunter, 1987).

Description of symptoms at field level

Within the plantation, a detailed description of the disease symptomatology was made, considering external visible morphological differences (branches, leaves, stem) and internal differences (necrosis, lesions) between diseased and healthy trees (Belezaca-Pinargote *et al.*, 2021a, 2021a).

Response of fungi to two temperatures

An experiment was prepared where each isolated phytopathogenic fungus (treatments) was seeded in five Petri dishes (replicates) containing the culture medium and incubated for six days (144 hours) under two temperatures (24 oC and 30 oC). The radial growth (cm) of each fungus was recorded in the Petri dishes at 24, 48, 72, 96, 120, and 144 hours after seeding.

Statistical analysis

The quantitative data obtained were analyzed using descriptive statistical tools: mean, standard deviation, standard error, coefficient of variation, etc. To establish the existence or not of significant statistical differences between treatments, the data were analyzed under the analysis of variance scheme (ANOVA) with a significance level of 95% ($P < 0.05$), after checking the assumptions of normality and homoscedasticity of variances. Subsequently, the LSD (least significant difference) test was applied, with a significance level of 95% ($P < 0.05$). The statistical package *SAS 9.0* version for Windows was used for this purpose.

Results

Disease incidence and severity

Table 4 shows the dasometric variables, the number of diseased trees, and disease incidence in each of the three plots evaluated. On average, 13 diseased trees ha-1 were detected, which allowed inferring that the incidence of the disease was 1.7%. In plots 1 and 2, 20 diseased trees ha-1 were detected on scale 2 of disease progress, while in plot 3 no diseased trees were found.

Table 4. Dasometric variables, incidence, and severity of "decay and dieback" disease in trees of a plantation of *S. parahybum* in the Ecuadorian Humid Tropics.

Plot No.	Total height (m)	Commercial height (m)	DAP (m)	Volume (m^3)	Incidence (%)	Severity (No. Of trees ha^{-1} according to scale)				
						1	2	3	4	5
1	9,5 ± 2,8	7,4 ± 2,6	0,11 ± 0,02	0,06 ± 0,04	2,5	780	20	0	0	0
2	11,7 ± 1,5	10,1 ± 1,7	0,13 ± 0,02	0,11 ± 0,04	2,5	760	20	0	0	0
3	12,6 ± 2,7	11,3 ± 2,5	0,15 ± 0,03	0,16 ± 0,07	0,0	820	0	0	0	0
$\overline{x}$	11,3 ± 2,3	9,6 ± 2,3	0,13 ± 0,02	0,11 ± 0,05	1,7	787	13	0	0	0

Symptomatologic description of the disease of "decay and descending death"

The initial symptoms evident in diseased *S. parahybum* trees are loss of turgor accompanied by paleness and yellowing of leaflets, especially in young leaves of upper branches. In many cases, young leaves are small in size compared to those of healthy trees, very yellow and curled up (shrunken and poorly formed). In heavily attacked trees, longitudinal zones of necrosis are visible, located mainly in the upper third of the stem, where the tissues are succulent and not yet fully lignified. These zones of necrosis have the appearance of dark brown spots, and when the infection is more accentuated, necrosis envelops the entire stem, impeding (blocking) the flow and reflux of mineral and organic substances to all the tissues of the tree, until it reaches a black coloration. When the infection is generalized, the diseased trees lose all their leaves and the growth zones become necrotic (die), giving the trees a dull cigar-shaped appearance. When the upper part of the trees dies, they emit epicormic buds to generate new photosynthetic areas, however, the total death of the standing tree is only a matter of time.

Isolation and identification of fungi in a humid chamber

It was determined that in 100% of the samples from all the plots studied, using the three methodological strategies, the presence of two types of cottony mycelium was observed, one of whitish color with the presence of hyaline conidiophores and two types of conidia: septate macroconidia with a morphology similar to a banana finger, and unicellular microconidia. The other fungus observed presented black pigmented mycelium with hard globose pycnidial structures, inside which hyaline (young) and dark (mature) globose conidia were detected. These characteristics allowed the identification of the first fungus as *Fusarium* sp. and the second as *Botryodiplodia* sp.

When the isolated colonies were seeded and incubated in the PDA culture medium, it was noted that Fusarium presented two morphologically distinct species, which were designated as *Fusarium* sp.1 and *Fusarium* sp.2. For *Botryodiplodia* sp. only a single species was detected (Figure 2).

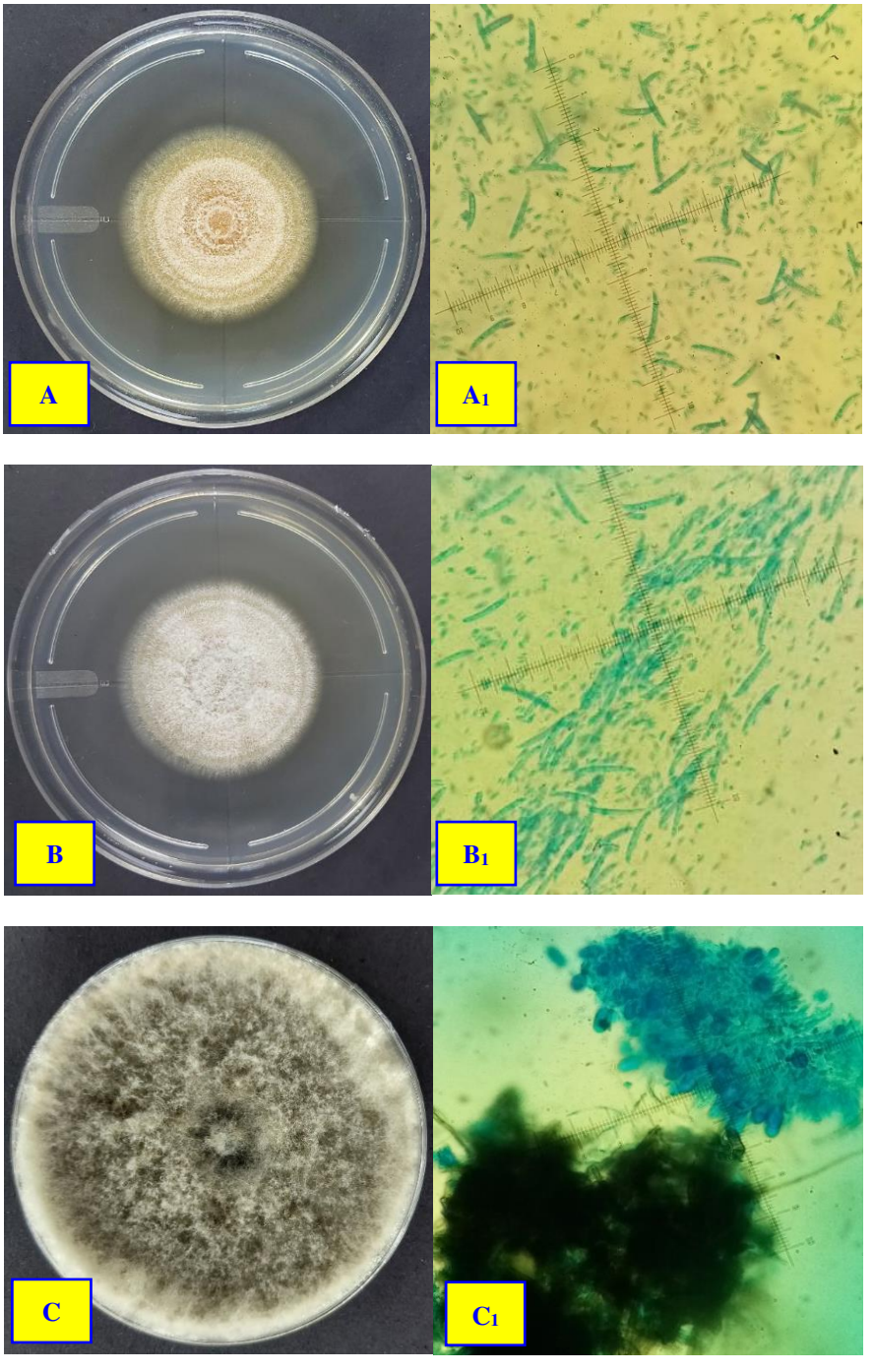

Figure 2. Colonies and signs of fungi isolated from diseased *S. parahybum* trees with "decay and descending death": A and A[1]) Colony, macro- and microconidia of *Fusarium* sp. 1. B and B[1]) Colony, macroconidia, and microconidia of *Fusarium* sp. 2. C and C[1]) Colony and lysed pycnidium releasing a mass of juvenile and mature *Botryodiplodia* sp. conidia.

Influence of temperature on phytofungi

Significant statistical differences were detected between the growth rate of the three fungi studied for the two time periods evaluated, both at 24 oC (*F=3.05; P=0.001*) and 30 oC (*F=2.95; P=0.001*). In both cases, *Botryodiplodia* sp. covered the entire Petri dish 72 hours after sowing, while *Fusarium* sp. 1 and *Fusarium* sp. 2 grew much more slowly and gradually, reaching approximately 4 cm in diameter at 144 hours after sowing (Figures 3 and 4).

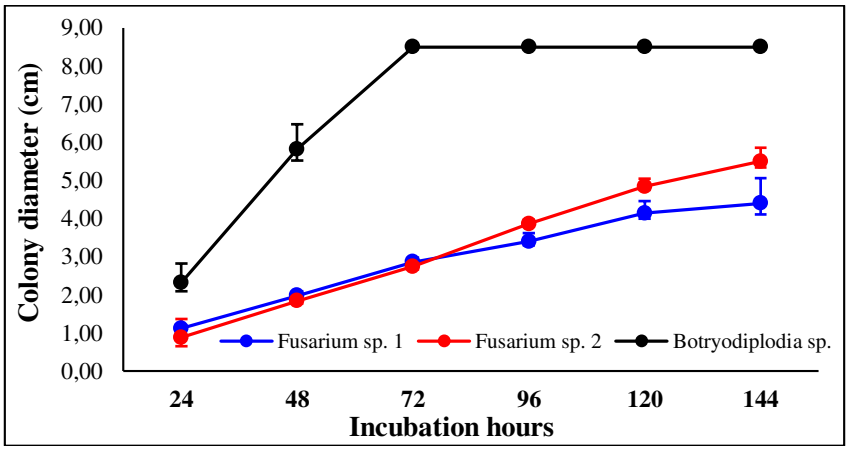

Figure 3. Growth kinetics of phytoparasitic fungi isolated from diseased *S. parahybum* trees and incubated at 24 oC. Values correspond to the average of five Petri dishes, with their respective standard deviation and standard error.

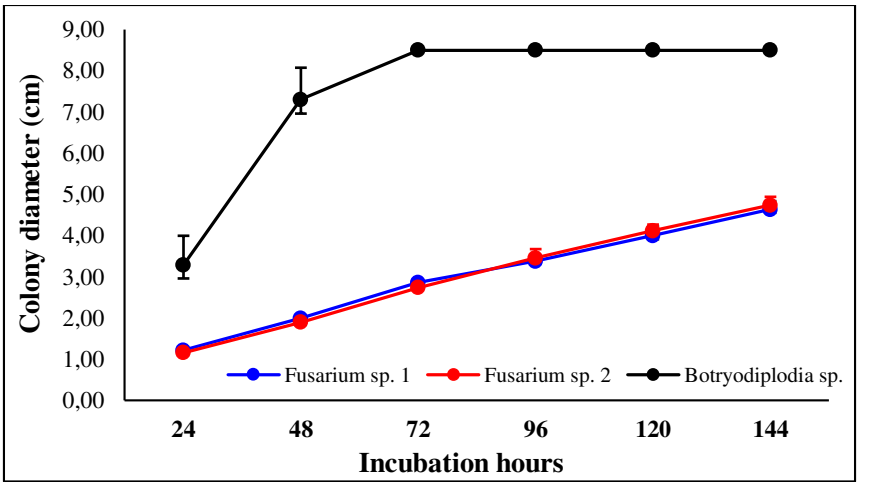

Figure 4. Growth kinetics of phytoparasitic fungi isolated from diseased *S. parahybum* trees and incubated at 30 oC. Values correspond to the average of five Petri dishes, with their respective standard deviation and standard error.

Discussion

Since the beginnings of Plant Pathology, it has been known that the massification of monocultures causes losses in biodiversity and ecological imbalances that can have diverse impacts on agroecosystems (Iezzi *et al.*, 2018). One of the effects of the imbalance is the appearance of several pathologies (diseases) of biotic or abiotic origin. This situation is evident in *S. parahybum* plantations, whose surface area increased between the 1980s and 1990s, periods that coincided with the appearance of the disease known as "vascular wilt and stem rot" that killed thousands of trees throughout Ecuador (Belezaca-Pinargote *et al.*, 2011; Belezaca-Pinargote *et al.*, 2012b).

In the last five years, small experimental plantations of *S. parahybum* began to be established, but again phytosanitary problems were detected, whose characteristics differ from those already known in past decades, without previous evidence of the causes of the new problem. However, the studies carried out in the present study show that the "decay" disease of pachaco would be of biotic origin since fungal phytopathogens associated with necrotic tissues were found in the diseased trees.

The values of incidence (1.7%) and severity (13 diseased trees on scale 2) of the "decay and descending death" disease in the evaluated plantation of *S. parahybum*, although low and not representing a serious phytosanitary problem at present, do highlight the presence of new pathogenesis in this forest species, so periodic evaluations of the evolution of the disease at the field level are necessary. When comparing the incidence values of "vascular wilt and stem rot" (36%) reported by Belezaca-Pinargote *et al.,* 2011, with those of "decay and descending death" (1.7%), the difference in aggressiveness between the two diseases present in the *S. parahybum* plantations of EHT.

Three phytopathogenic fungi, *Fusarium* sp. 1, *Fusarium* sp. 2, and *Botryodiplodia* sp., microorganisms reported in the scientific literature as associated with vascular diseases and stem rot in agricultural and forestry species of global economic importance, were detected, isolated, and identified from necrotic tissues of diseased trees of *S. parahybum* (Sowmya *et al.*, 2018; Zhu *et al.*, 2020). In this regard, Belezaca-Pinargote *et al.* (2021b) reported *Fusarium* spp. associated with diseased trees of *Gmelina arborea* (melina) in Ecuador, however, its direct involvement in the pathology of the forest species is still inconclusive.

Fusarium sp. is an imperfect fungus, cosmopilta, whose genus consists of a large number of species, generally lives in the soil and some of its species are pathogenic to plants. Although commonly, colonies of *Fusarium* sp. are isolated in microbiological analysis of necrotic plant tissues, they are not necessarily causal agents of pathogenesis, due to the saprophytic behavior of most species of this fungal genus (Salerno, 2000).

The symptomatology presented by trees with the disease "decay and descending death" has different characteristics from those generated by other diseases in other tropical forest species, such as *A. fraxinifolius*, and *T. grandis* (Belezaca-

Pinargote *et al.*, 2020), *G. arborea* (Belezaca-Pinargote *et al.*, 2021a), and even within *S. parahybum* itself (Belezaca-Pinargote *et al.*, 2011).

It is known that every phytopathogen needs an entry gate to infect and disseminate in the internal tissues of plants (Savatin *et al.*, 2014). However, although in the present study no natural or anthropogenic wounds were found to act as entry points for phytopathogenic microorganisms, it could be theorized that the young, succulent, poorly lignified tissues of the last third of *S. parahybum* trees could facilitate the entry of pathogenic fungi into the xylem, as evidenced by the longitudinal necrotic lesions observed in these areas of the diseased trees (Savatin *et al.*, 2014).

Conclusions

Although the presence of two fungal microorganisms causing plant diseases (*Fusarium* sp. 1, *Fusarium* sp. 2, *Botryodiplodia* sp.) associated with diseased trees of *S. parahybum* was detected, it is still not possible to be sure if the disease of "decay and descending death" is caused by any of them, or if they act together, so it is necessary to carry out pathogenicity tests (Koch's postulates). Finally, since the identification of the microorganisms was based on morphology, molecular studies are recommended to carry out a more precise characterization of the possible phytopathogens causing the disease.

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