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Ceratocystis Fimbriata: A risk for the GMelina Arborea Roxb. (Melina) forests in Ecuador

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Abstract--In Ecuador, GMelina arbórea (Melina) faces the disease “vascular wilt and stem rot”, with no known etiology so far. The aim was to describe the symptomatology and to know the cause of the disease. Three plantations (2.0, 3.5, and 5.0 years old) were studied in the Ecuadorian Humid Tropics, where three plots of 500 m² were delimited, and the incidence and severity of the disease were evaluated using an arbitrary scale of five categories (1=healthy tree, and 5=dead tree). Three trees were dissected per plot and their tissues were analyzed in the laboratory where the fungi were isolated, morphologically identified, and inoculated (pathogenicity tests) on healthy Melina plants. The detection of initial disease symptoms at the field level was complex since trees have a high resilience capacity. In trees with easily visible symptoms, chlorosis, loss of turgor, dead growth buds, epicormic bud emission, and stem rot, the release of exudates from wounds and wilting was observed. Disease incidence was 24.1%, 7.1%, and 21.3% for 2.0-, 3.5-, and 5.0-year-old plantations, respectively. Disease severity detected in the Melina plantations ranged from 2 to 5 on a scale. The following were isolated and identified *Fusarium* spp. and *Ceratocystis fimbriata* Ellis & Halst.

from diseased trees to *G. arborea*. Pathogenicity tests allowed determining that *C. fimbriata* is the causal agent of the disease.

Keywords---diseased trees, fusarium spp, phytopathogenic fungi, forest pathology, pathogenicity tests.

Introduction

GMelina arborea Roxb. (Melina) is a fast-growing tropical forest species, selected by developing countries for medium-term timber supplies from commercial plantations. In Ecuador, it found excellent edaphoclimatic conditions for its development, despite being native to Southeast Asia (Moya *et al.*, 2008; Rojas *et al.*, 2004). According to the Undersecretariat of Forestry Production of the Ministry of Agriculture and Livestock (MAGAP) of Ecuador, the production of *G. arborea* is concentrated in the tropical and subtropical coastal region, where the “Incentive Program for Reforestation for Commercial Purposes” was implemented, boosting the productivity, competitiveness, and sustainability of the forestry sector, with several species, among them Melina (MAGAP, 2016).

Since the introduction of *G. arborea* in Ecuador, it has become an important item for the country's economy, with a significant planted area, which as of 2015 was 11458 hectares (ha), representing 21.9% of the 52395-ha planted with other forest species of economic importance (teak, balsa, pine, others) registered, contributing approximately with an equal percentage of direct and indirect jobs (MAGAP, 2016). However, Melina, like other forest species, is prone to diseases that can seriously compromise its survival and, therefore, generate significant economic losses by directly affecting its productivity (Arguedas, 2004; Arguedas *et al.*, 2018, Belezaca-Pinargote *et al.*, 2021a). In the last decade, a complex and aggressive disease characterized by vascular wilt and stem rot is affecting commercial plantations of the species in the Ecuadorian Humid Tropics (THE), manifesting itself with a premature and gradual detriment of tree vigor, accompanied by discoloration of the leaf system (chlorosis) and stunted growth. Some trees excrete dark brown exudates from the trunk, with a strong odor of decomposing organic matter, indicating internal rotting of the trunk (Belezaca-Pinargote *et al.*, 2021a; Belezaca-Pinargote *et al.*, 2021b; Belezaca-Pinargote *et al.*, 2021c).

The disease detected in *G. arborea* seems to be associated with fungal microorganisms, due to the characteristics of the disease and the presence of signs at the field level. Currently, the disease “vascular wilt and stem rot” is distributed in almost all Melina plantations in THE, but its etiology, predisposing factors, and even worse, efficient prevention and control mechanisms are still unknown. Therefore, it was proposed to diagnose the fungal agents present in diseased trees, describe the symptoms of the disease at the field level, and carry out pathogenicity tests, inoculating Melina plants at the nursery level with the isolated fungi.

Materials and Methods

Location of research

The study was carried out in three Melina plantations in Ecuador, two of which were located in the canton of Valencia, Los Ríos province, aged 2 years and 3.5 years, respectively, and one plantation in the canton of Pangua, Cotopaxi province, aged 5 years. All plantations received silvicultural management consisting of thinning and pruning, using shears, saws, and chainsaws. Weed management was done manually and chemically. The initial density at the time of the evaluations was different among the plantations (Table 1).

Table 1
Initial density and density detected at the time of the evaluations in three plantations of *G. arborea* of different ages

No.	Plantation (age)	Initial density (trees ha ⁻¹)	Density at time of evaluation (trees ha ⁻¹)
1	2 years	1111	1080
2	3,5 years	1111	840
3	5 years	1111	940

According to the Holdridge & Tosi (1967) life zone classification, the three plantations are located in the “Tropical Rainforest” (bh-T) life zone, at an altitude between 427 - 504 meters above sea level, with a warm and humid climate, temperature, precipitation and average annual relative humidity of 21 °C, 2700 mm, and 88 - 95%, respectively, with predominantly clay loam soils.

Symptomatologic description, incidence, and severity of the disease

For this purpose, the methodology used by Belezaca-Pinargote *et al.* (2018) and Belezaca-Pinargote *et al.* (2021a) was followed, consisting of a detailed description of the disease symptomatology, considering external and internal morphological differences between diseased and healthy trees. Three rectangular plots of 500 m² were delimited in each plantation, within which a census was carried out to establish the total number of trees present, the number of trees with disease symptoms, dead trees, and healthy trees. Disease severity was estimated using an arbitrary scale of five categories, proposed by Salas-Rodríguez *et al.* (2016) and applied by Belezaca-Pinargote *et al.* (2021a), which considers visible morphological differences of branches, leaves, and trunk, and contrasted with healthy trees (Table 2).

Table 2
Arbitrary five-category scale proposed by Salas-Rodríguez *et al.* (2016) to assess the severity of the disease “vascular wilt and stem rot” in *G. arborea*

Severity (Criteria)	Symptoms
1	A healthy tree, with no evidence of visible symptoms.
2	Initial yellowing of the crown (evident foliar wilting), the stem may

	have small necrotic wounds and black exudation in places other than where pruning has occurred, and resprouting 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, bark exposure and bulging, prominent oozing, loss of more than 50% of the leaf area in a progressive pattern, and developed resprouts.
4	Total affectation of the individual, total absence of foliage, there is loss and evident detachment of branches, and resprouts are still observed in some sectors of the trunk, the apparent external rotting reaches 75% of the trunk, where the cancerous zone (canker) is manifested.
5	The tree is completely dry, and rotten, the wood has already lost its commercial value.

Collection of tissue from diseased trees

Following the methodology used by Belezaca-Pinargote *et al.* (2018) and Belezaca-Pinargote *et al.* (2021a), 3 trees ($7\pm 0.5\%$) with disease symptoms were sampled in the plots of all the plantations studied, which were felled with the help of a chainsaw. Natural and anthropogenic wounds (branch pruning, machete wounds, etc.) were inspected through tangential and transversal cuts in the trunk (every 70 cm). Tissues with evidence of necrosis were transferred to the Environmental and Plant Microbiology Laboratory of the State Technical University of Quevedo (UTEQ) for phytopathological analysis.

Isolation and identification of fungal microorganisms

In the laboratory, necrotic tissues were conditioned and analyzed using three methodological strategies (wet chamber, carrot sandwiches, and potato-dextrose-agar (PDA) culture medium), following the guidelines proposed by Belezaca-Pinargote *et al.* (2018) and Belezaca-Pinargote *et al.* (2021a). The fungal microorganisms were identified with the help of dichotomous taxonomic keys, based on morphology (Von Arx, 1981; Barnett & Hunter, 1987; Hanlin, 1992).

Preparation of inoculum

Using a punch, segments (approximately 0.5 cm in diameter) of colonies of the previously isolated fungi were obtained and replicated in Petri dishes, containing 10 mL of PDA culture medium, plus 0.2 mL of an antibiotic mixture (50 $\mu\text{g}/\text{mL}$ penicillin and 25 $\mu\text{g}/\text{mL}$ streptomycin), under aseptic conditions, and then incubated for 8 days at 24 ± 2 oC (Parkinson, 1994; Suryanarayanan, 2013).

Pathogenicity tests

Three-month-old Melina plants in good health, with a stem diameter of 1 cm and 60 cm in height were used. Inoculation was performed on the stem of the plants at 5 cm above ground level, for the effect with a sterile scalpel was made an inclined cut that compromised the bark and xylem of the plant in the previously disinfected area. A colony segment (0.5 cm disk) of the selected phytopathogen,

previously cut in the Petri dish with a punch, was deposited inside the wound and the wound was covered with parafilm tape. As a control, Melina plants were inoculated under the same conditions as above, with the difference that instead of inoculating the pathogen, a segment of agar-agar (innocuous, without nutrients) was applied inside the wound, and the wound was closed with parafilm tape (Belezaca-Pinargote *et al.*, 2021b; Belezaca-Pinargote *et al.*, 2021c; Belezaca-Pinargote *et al.*, 2021c).

The plants were periodically irrigated with water according to their needs. The experiment was established for 60 days, during which time systematic observations were made on the health status of the plants, to detect the appearance of symptoms of the disease “vascular wilt and stem rot”, and recording in detail the development of the symptomatology. Sixty days after inoculation, the plants were dissected by longitudinal and transverse cuts to estimate necrotic lesions in the vascular tissues, both upward and downward, taking as a reference point the central part of the wound made at the time of inoculation. The areas of necrosis were measured in three dimensions (height, width, and depth) to estimate the apparent area of necrosis, expressed in cm³ (Zauza *et al.*, 2004).

Treatments and Experimental Design

A Completely Randomized Design (CRD) was used, consisting of four treatments: T1 = Melina plants inoculated with *Ceratocystis fimbriata*, T2 = Melina plants inoculated with *Fusarium* sp., T3 = Melina plants inoculated with *C. fimbriata* + *Fusarium* sp., T4 = uninoculated Melina plants (control). For each treatment, 35 Melina plants were used (replicates).

Statistical analysis

Quantitative data obtained at the field and laboratory levels were analyzed using descriptive statistical tools. To establish the existence or not of significant statistical differences between treatments, the data were analyzed under the analysis of variance (ANOVA) scheme 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 SYSTAT 11 statistical package for Windows was used for this purpose.

Results and Discussion

Results

Symptomatology of the disease

Loss of turgor was detected in leaves of upper branches with signs of wilting, accompanied by slight chlorosis in trees with initial stages of the disease. Due to vascular blockage and gradual progression of the disease, the growth apices of the branches die progressively. Vigor in diseased trees decreases significantly, compared to healthy neighboring trees, mainly due to the loss of photosynthetic

area. As a survival mechanism, diseased trees emit numerous epicormic shoots on the stem, stimulated by the need for photosynthesis. However, as vascular plugging continues to impede the ebb and flow of nutritional substances, the tree eventually dies. In diseased trees, it is common to observe the presence of dark-colored fluids released from wounds generated by silvicultural management activities such as pruning, thinning, weed control, and natural wounds. In diseased trees, the presence of bark and/or wood-boring insects is generally not observed.

When transverse cuts were made at the base of the trees (5 - 10 cm above ground level), in most cases no symptoms of necrosis or plugging of vascular tissues were observed. However, when transverse and longitudinal cuts were made above 50 cm above ground level, damage caused by the pathogen(s) in vascular tissues, necrosis, and plugging of conductive vessels that prevent the normal flow and reflux of nutrient solutions and photoassimilates were observed. It was evident that the entry of the phytopathogen(s) occurs mainly through mechanical wounds of anthropogenic origin resulting from silvicultural activities, such as the pruning of branches and accidental cuts during weed control activities. Many wounds are healed; however, they mask the previous entry of the pathogen, which is spread upwards, downwards, and radially under the bark, colonizing the sapwood and heartwood, causing necrosis and clogging of vascular tissues, which finally culminates in the death of the tree. On some occasions, the wounds were not found healed, but exposed, from where brown fluids constantly emanate with an odor of decomposing organic matter (Figure 1).





Figure 1. Healed and unhealed mechanical wounds as sites of entry and dissemination of phytopathogen(s) associated with “vascular wilt and stem rot” disease in Melina trees.

The behavior of *G. arborea* trees against the disease is not uniform, generally, the manifestation of the initial symptoms is difficult to detect, since chlorosis and loss of turgor occur when the xylem and vascular tissues of the trees are irretrievably compromised and necrotic, both radially and tangentially. Defoliation and death of growth buds and branches can be observed in the advanced stages of the disease. The presence of wounds of natural and/or anthropogenic origin on the trunk is common, with abundant exudation of dark brown fluids, with a strong odor of decomposing organic matter.

Disease incidence and severity

An average of 10 diseased trees and 2 dead trees per plot (500 m²) were detected in the two-year plantation, which allowed inferring the existence of 200 diseased trees and 40 dead trees per ha⁻¹. In the 3.5-year plantation, an average of 3 diseased trees and no dead trees were found, resulting in 60 diseased trees and zero dead trees per ha⁻¹. While in the 5-year plantation, 80 diseased trees and 40 dead trees per ha⁻¹ were detected. These results show that the incidence of the disease was 17.9%, 7.9%, and 8.5% for the 2-, 3.5- and 5-year-old Melina plantations, respectively (Table 3).

Table 3

Number of apparently healthy, diseased, and dead trees and incidence of vascular wilt and stem rot disease per ha⁻¹ in three plantations of *G. arborea* in the Ecuadorian Humid Tropics. Values represent the average of three replicates, with their respective standard error

Plantation (age)	Trees ha ⁻¹ (Density)	Trees apparently healthy ha ⁻¹	Diseased trees ha ⁻¹	Dead trees ha ⁻¹	Incidence (%) *
2 años	1080	820 ± 0,67	215 ± 0,25	45 ± 0,80	24,1 ± 1,15
3,5 años	840	780 ± 0,75	60 ± 0,61	0,0 ± 0,00	7,1 ± 0,95
5 años	940	740 ± 0,90	140 ± 0,40	60 ± 0,28	21,3 ± 0,87

Most of the diseased trees in the 2-year-old plantation were found on scales 2 and 4, representing 9.52% of trees in medium disease progress and 7.14% dead standing (scale 5). In the 3.5-year-old plantation, diseased trees were located on a scale 2 with 7.14%. In the 5-year-old plantation, trees with different levels of

disease were found on scales 2, 3, 4, and 5, with 8.51%, 4.25%, 2.13%, and 6.38%, respectively (Table 4).

Table 4
Disease severity of “vascular wilt and stem rot” per ha-1, in plantations of *G. arborea* of 2, 3.5, and 5 years of age

Plantation (age)	Árboles ha ⁻¹ (Densidad)	No. árboles por escala *				
		1	2	3	4	5
2 years	1080	820	150	45	20	45
3,5 years	840	780	60	0	0	0
5 years	940	740	80	40	20	60

Isolated phytopathogenic fungi

In 33% of the necrotic woody tissues incubated in a humid chamber, the presence of black globular ascocarps of perithecial morphology covering the necrotic areas of the wood was detected. Carrot sandwiches containing necrotic tissues after incubation showed the presence of mycelium and perithecia in 55% of the diseased trees. Sowings of small sections of necrotic wood in the PDA culture medium generated black fungal colonies and the presence of globose perithecia, with several appendages (fimbriae) at the end of the neck, showing an accumulation of spherical masses of pale yellow ascospores. Pure colonies of the fungus replicated on a PDA culture medium generated young hyaline perithecia at 72 hours, which turned dark when mature until they became completely black. The perithecia presented a globose base, elongated neck with lengths between 800-900 µm, and presence of fimbriae at the upper end of the neck surrounding the ostiole. On the fimbriae there were masses of hyaline or creamy appearance, constituted by mature ascospores released by lysis of the asci inside the perithecium. The ascospores were unicellular (non-septate), hyaline, elliptical in shape, hat-like, and 4-8 x 2-5 µm in size. These characteristics allowed the fungus to be identified as *Ceratocystis fimbriata* Ellis & Halst. In addition, white fungal colonies with a cottony appearance were isolated and identified by morphological taxonomic keys as *Fusarium* spp.

Significant statistical differences were detected in the number of fungal colonies isolated in the PDA culture medium from necrotic tissues of diseased trees. *Fusarium* spp. showed a higher number of colonies, while *C. fimbriata* showed a lower presence. In the two-year-old plantation (F=7.05; P=0.001), an average of 15 colonies of *Fusarium* spp. and 5 colonies of *C. fimbriata* were obtained, while in 3.5-year-old trees (F=19.3; P=0.000) 17 and 3 colonies were obtained, and in 5-year-old trees (F=17.4; P=0.000) 6 and 14 colonies of *Fusarium* spp. and *C. fimbriata* were obtained, respectively (Figure 2).

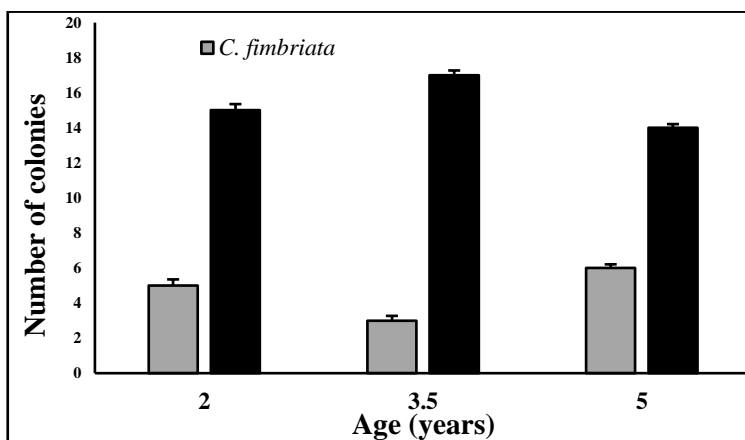


Figure 2. Number of colonies of *C. fimbriata* and *Fusarium* spp. isolated from diseased trees in three plantations of *G. arborea*. Values correspond to the average number of colonies obtained per tree, with their respective standard error.

Apparent volume of necrosis (cm³) in inoculated plants

Significant statistical differences ($F=30.56$; $P=0.000$) were detected between the apparent volumes of necrosis generated by the inoculated phytopathogens (treatments) on Melina plants. The treatments *C. fimbriata*, and *C. fimbriata* + *Fusarium* sp., generated the highest apparent necrosis volumes, with 0.31 cm³ and 0.18 cm³, respectively, being statistically different from each other, and different from the *Fusarium* sp. and Control treatments, which reached lower volumes, of 0.038 cm³ and 0.014 cm³ respectively (Figure 3).

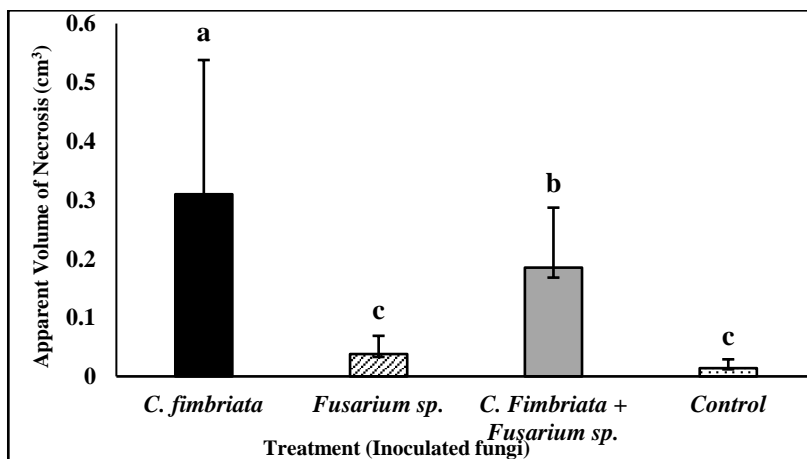


Figure 3. Apparent volume of necrosis (cm³) generated by phytopathogenic fungi (treatments) inoculated on 3-month-old *GMelina arborea* (Melina) plants, 60 days after inoculation at greenhouse level. Values correspond to the average apparent volume of necrosis of 35 Melina plants, with their respective standard deviation and standard error

Total necrosis length (cm)

Significant statistical differences ($F=87.98$; $P=0.000$) were detected between the necrosis lengths generated by the fungi. The treatments *C. fimbriata*, and *C. fimbriata*+ *Fusarium sp.*, produced the greatest necrosis lengths, with 6.76 cm and 5.79 cm, respectively, being statistically different from each other, and different from the treatments *Fusarium sp.*, and the Control that reached smaller lengths, of 1.74 cm and 0.68 cm, respectively (Figure 4).

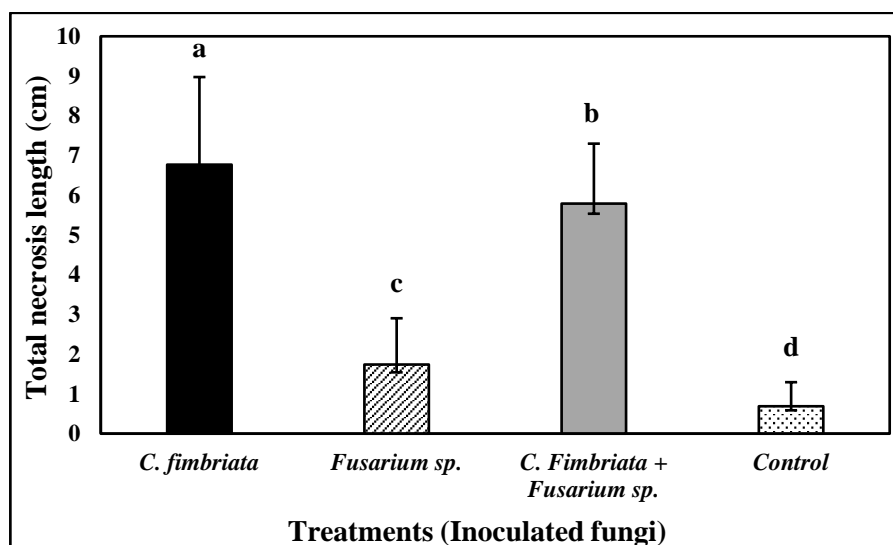


Figure 4. Total length of necrosis (cm) generated by phytopathogenic fungi (treatments) inoculated on 3-month-old *GMelina arborea* (Melina) plants, 60 days after inoculation at greenhouse level. Values correspond to the average apparent volume of necrosis of 35 Melina plants, with their respective standard deviation and standard error

Ascending and descending length of necrosis (cm)

Significant statistical differences were detected between the lengths of ascending ($F=42.86$; $P=0.000$) and descending ($F=130.65$; $P=0.000$) necrosis caused by the inoculated phytopathogens (treatments) in Melina plants. The treatments *C. fimbriata*, and *C. fimbriata* + *Fusarium sp.*, generated the greatest ascending lengths of necrosis, with 4.16 cm and 3.48 cm, respectively, and maintained the same trend, although with lower values for the descending length, with 2.60 cm and 2.19 cm, respectively. The *Fusarium sp.* and Control treatments reached lower ascending lengths of 1.14 cm and 0.43 cm, and descending lengths of 0.59 cm and 0.25 cm, respectively (Table 5).

Table 5

Ascending and descending length of necrosis (cm) generated by the inoculation of phytopathogenic fungi (treatments) in 3-month-old *G. arborea* (Melina) plants, 60 days after inoculation at greenhouse level. Values correspond to average ascending and descending necrosis lengths of 35 Melina plants, with their respective standard error

Treatments	Ascending length	Standard error	Descending length	Standard error
<i>C. fimbriata</i>	4.16 a	0.31	2.60 a	0.11
<i>Fusarium</i> sp.	1.14 c	0.16	0.59 c	0.06
<i>C. fimbriata</i> + <i>Fusarium</i> sp.	3.48 b	0.23	2.19 b	0.09
Control	0.43 d	0.08	0.25 d	0.03

Description of the symptomatology of inoculated plants

Between 15 and 20 days after inoculation, plants showed loss of coloration (yellowing) and turgor in their foliar system, and epicormic shoots appeared approximately 2 cm below the inoculation point. At 22 days after inoculation, the foliar tissues of 2 plants inoculated with the *C. fimbriata* treatment and 10 plants of the *C. fimbriata* + *Fusarium* sp. treatment with disease symptoms died and were completely defoliated. At the time of evaluation (60 days) the epicormic shoots emitted under the inoculation site were still alive (Figure 5).

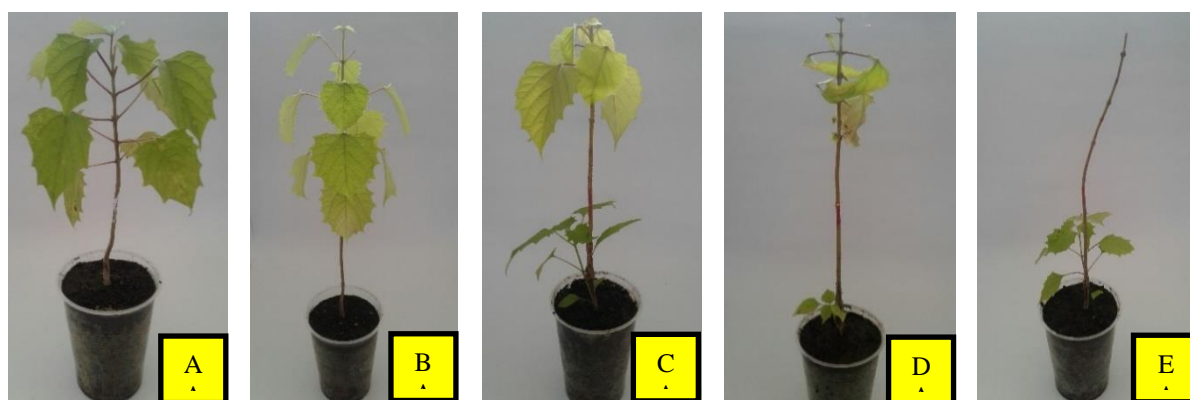


Figure 5. Progressive symptomatology generated by inoculation of fungal microorganisms on Melina plants at greenhouse level. Treatments: *C. fimbriata*, and *C. fimbriata* + *Fusarium* sp. A = Freshly inoculated (healthy) plant, B = Leaf system with onset of chlorosis, C = Leaf system with loss of turgor and emission of epicormic shoots below the inoculation point, D = Leaf system strongly affected by vascular wilt, E = Leaf system dead, totally defoliated, epicormic shoots below the inoculation point

Plants inoculated with *Fusarium* sp. at the time of evaluation (45 days of incubation) showed no visible symptoms of the disease. There was an absence of

discoloration, wilting, loss of turgor, etc., and they looked like healthy plants, similar to the control plants (Figure 6).

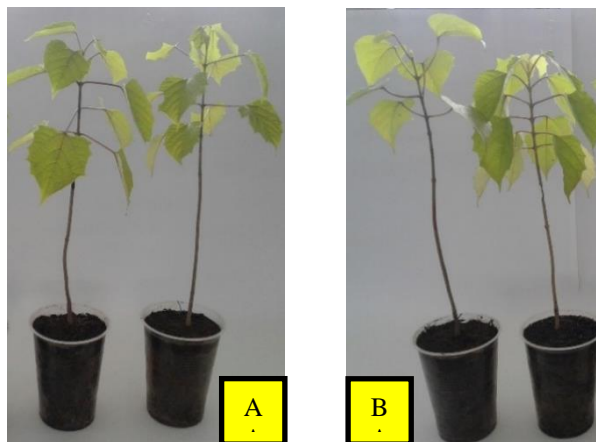


Figure 6. Melina plants inoculated with (A) *Fusarium* sp. at 60 days of incubation, with no visible presence of disease symptoms, looking similar to (B) control plants (without inoculation of phytopathogens)

Reisolation of inoculated fungi. After the evaluations, and in compliance with Koch's fourth postulate, the fungi inoculated on Melina plants were recovered from necrotic tissues. *C. fimbriata* was recovered by means of the carrot sanding technique, and *Fusarium* sp. in PDA culture medium.

Discussion

Since the introduction of *G. arborea* into THE production systems in the mid-1980s, the species had not presented significant phytosanitary problems, but in the last decade, due to the increase in planted area, phytosanitary problems have become recurrent as mentioned by Belezaca-Pinargote *et al.* (2021a), Belezaca-Pinargote *et al.* (2021b) and Belezaca-Pinargote *et al.* (2021c). The occurrence of diseases in monospecific plantations is related to massification, increase in planted area, susceptibility of the species and time, factors that, from the point of view of forest pathology, predispose trees to disease (Haas *et al.*, 2011; Bostock *et al.*, 2014; Hughes *et al.*, 2015). The disease “vascular wilt and stem rot” in Melina, presented similar symptomatology to that reported in other economically important forest species in the region, such as *Schizolobium parahybum* (Geldenhuis *et al.*, 2004; Belezaca-Pinargote *et al.*, 2011), *Acrocarpus fraxinifolius* (Belezaca-Pinargote *et al.*, 2012) that destroyed entire plantations between the 1990s and 2000s, and *Tectona grandis* (Belezaca-Pinargote *et al.*, 2018; Belezaca-Pinargote *et al.*, 2020), whose disease appeared at the beginning of the last decade and continues to eliminate thousands of trees in THE, to the present days.

Field observations show that phytopathogens enter the trees through mechanical wounds, generated by pruning branches, and injuries caused by sharp tools (machetes) during cultural work, such as weeding. However, abiotic factors such as wind, which can generate micro-injuries due to twisting of the trunks, which are easily colonized by phytopathogenic fungi, should not be ruled out (Nkuekam

et al., 2010; Agler *et al.*, 2016). Trees of *G. arborea* in the early stages of the disease showed high resilience since at the time of evaluation they did not show visible symptoms of disease, however, when they were cut transversally and longitudinally in the trunk, the internal tissues were completely necrotic in all directions. This natural resilience makes it difficult to identify Melina trees in the early stages of the disease. Field observations show the existence of trees to which the disease does not seem to cause major damage, which leads to suspecting the presence of individuals with acceptable levels of tolerance/resistance to the phytosanitary problem. This behavior would be due to the genetic variability of individuals obtained from open-pollinated seeds (Bräutigam *et al.*, 2013; Inza *et al.*, 2018), which can be used in future genetic improvement programs for the species.

The incidence of the disease in the three plantations (24.1%, 7.1%, and 21.3%) could be considered high, and a warning for the national Melina industry, if adequate strategies for prevention and management of the disease are not considered. Although diseased trees were found at all levels of the proposed scale, most of them were detected in the initial stage of the disease, but it is a matter of time before the symptomatology progresses in them. Based on the apparent volumes of necrosis, total lengths of necrosis, and the manifestation of symptoms similar to those observed at the field level in Melina plants inoculated with the fungi *C. fimbriata*, *Fusarium* sp. and their combinations, it is suggested that the pathogen causing the disease “vascular wilt and stem rot” in *G. arborea* is *C. fimbriata*.

The pathogenicity of *C. fimbriata* is reported in the scientific literature as a highly aggressive pathogen, with a wide host range, from angiosperms to gymnosperms (Herrera-Isla *et al.*, 2015). This pathogen is of great importance for the Ecuadorian forestry sector, as it is known to be a pathogen of woody species globally: *Theobroma cacao* (Baker & Harrington, 2005), *GMelina arborea* (Melina) (Ferreira *et al.*, 2011; Belezaca-Pinargote *et al.*, 2021a), *Schizolobium parahybum* (pachaco) (Geldenhuis *et al.*, 2004; Belezaca-Pinargote *et al.*, 2011), *Acrocarpus fraxinifolius* (pink cedar) (Belezaca-Pinargote *et al.*, 2012), *Eucalyptus* spp. (Alves-Ferreira *et al.*, 2006), *Carapa guianensis* (tangaré) (Halfeld-Vieira *et al.*, 2012), *Tectona grandis* (teak) (Belezaca-Pinargote *et al.*, 2018; Belezaca-Pinargote *et al.*, 2020). Although the genus *Fusarium* has highly pathogenic species (Landeras *et al.*, 2005), it did not generate significant pathogenesis in Melina plants during the 60 days of the experiment. This does not mean that the phytopathogen plays no role in the development of the disease; on the contrary, it can be theorized that the presence of *Fusarium* sp. in diseased Melina trees would have a secondary role, and the fact that it is present in weakened/affected trees would probably be due to a non-pathogenic saprophytic activity.

Conclusions

The incidence of the disease “vascular wilt and stem rot” in the Melina plantations studied was between 7% and 24%, with trees in a medium stage of disease progress. The results obtained from the pathogenicity tests lead to the conclusion that the phytopathogenic fungus causing the disease in *G. arborea* is *C. fimbriata*. It is theorized that *Fusarium* sp. plays a secondary or saprophytic role in the

development of the disease. Wounds resulting from silvicultural activities (pruning, thinning, weed control, etc.) and natural wounds (twisting, splitting, etc.) are the gateway for *C. fimbriata* to enter and colonize the internal tissues of trees, causing vascular wilt and stem rot.

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