RESEARCH ARTICLE

Gmelina arborea "death disease" in fast-growth plantations: Effects of soil and climatic conditions on severity and incidence and its implications for wood quality

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Abstract

Aim of study: Plantations are threatened by an emerging disease called "Gmelina death disease". The objective of this study was measured the incidence and severity of this disease and were correlated with the characteristics of the plantations, micro- and macronutrients in the soil and climatic parameters.

Area of study: The present study evaluated 16 symptomatic fast-growth plantations of different age in Costa Rica.

Material and methods: Fungi were identified from xylem of infected trees. Incidence and severity was measured and correlated with the characteristics of the plantations, micro- and macronutrients in the soil and climatic parameters. Root condition and the quality (specify gravity, mechanical and decay resistance and chemical compositions), of the wood of symptomatic and asymptomatic trees were compared.

Main results: Three fungal species *(Chaetomella raphigera, Fusarium solani* and *Rhizomucor variabilis)* were identified from diseased samples. Clay content in the soil profile from 10-20 cm deep explained a significant proportion of the variation in the incidence and severity of the disease, and stand density was related to severity. Although two climatic variables, Holdridge's potential annual evapotranspiration and Thornthwaite's potential evapotranspiration, showed a relationship between the incidence in the trees and symptoms of the disease. Infected wood turned black in symptomatic trees. Specify gravity and mechanical resistance of infected wood decreased, whereas its natural durability was unaffected. Changes were observed in the quantities of Mg, Fe, Ca, K and Zn in infected wood.

Research highlights: Gmelina plantations established in sites with high stand densities and high contents of clay increase susceptibility to this disease.

Additional keywords: death syndrome; soil management; tropical species; pathogen.

Abbreviations used: DBH (diameter at 1.3 m height); h (dominant height); ECEC (effective cation exchange capacity); PDA (potato-dextrose-agar); SG (specific gravity)

Introduction

Gmelina arborea Roxb. (Gmelina) is a fast-growing forest species that produces high quality timber used for furniture manufacturing (Vallejos *et al.*, 2015). This species is widely used in commercial programs in tropical countries, for lumber, pulp and energy production (Dvorak, 2004; Tenorio *et al.*, 2016). In Central America (Costa Rica, Nicaragua and Guatemala), approx. 40,000 ha have been planted for commercial purposes (Dvorak, 2004). From those, approx. 18,000 ha correspond to Costa Rica (Moya & Tomazello, 2008). Since the 90's, Costa Rica has begun to develop wood pallet industries for agriculture products and all pallets are made with *G. arborea* wood from commercial plantations (Barrantes & Ugalde, 2017).

A series of problems has been observed in Gmelina plantations related to diseases and insect pests (Wingfield & Robinson, 2004; Umana *et al.*, 2015). In particular, several studies have reported *Gmelina arborea* "death

disease". This disease impairs the development of trees, limiting their girth and height growth and thus hampering commercial plantations. The incidence of the disease in commercial plantations can reach up to to 40% (Murillo *et al.*, 2014). Generally, some trunk rot is also observed, called canker. Canker lesions appear with bulging, frequently together with exudation and sprouting in the stem.

Duke (1987) found that seven pathogens were present at the seedling stage. Julian (1982) reported damping-off, mold and stem rot in beds of seedlings caused by several fungi. Wingfield & Robinson (2004) performed an extensive revision and classified the diseases and insect pests of Gmelina according to their association with the foliar system, the trunk or the roots. Maringoni & Furtado (1997) and Umana *et al.* (2015) associated stem gall or stem canker in trees from Gmelina plantations with several fungi and showed canker development with tree death and foliage decrease. Lastly, one of the most severe pathogens attacking Gmelina is *Ceratocystis fimbriata*, which causes stem and branch canker and vascular wilt disease (Muchovej *et al.*, 1978; Ribeiro, 1982; Harrington *et al.*, 2011; Umana *et al.*, 2015).

Furthermore, problems of mortality in gro-ups of trees between 2 and 8 years old in Gmelina plantations has been reported in Costa Rica (Arguedas, 2004; Salas, 2015). Until recently, the death of trees in Gmelina plantations had been known as the "Nectria disease" in Costa Rica and many parts of tropical America (Arguedas, 2004; García-Díaz *et al.*, 2011). The name Nectria comes from the fact that Nectria species' anamorphs include Fusarium species, thus forming complexes of species (Samuels & Nirenberg, 1989; O'Donnell, 2000; Samuels *et al.*, 2009). However, this problem has recently been referred to as "Gmelina death disease" (García-Díaz *et al.*, 2011; Murillo *et al.*, 2014; Chavarría-Vega & Carmona-Solís, 2016).

According to Arguedas (2004), in early stages of the disease, the foliage withers, becomes dry and then falls. Trees often sprout at the bottom of the stem and have sap "weeping holes" at the pruning point, which later become blackish. In general, it is not possible to detect canker clearly during the initial stages of the disease. Rather, it can be observed in cuts made above the sprouts, where depressions of the bark can be detected. The affected cortical tissue shows dark brown necrosis, and thicker trees crack at the canker region. When the disease has progressed, a canker or anthracnose encircles the stem at some point, thus causing death of the apical part.

An important aspect of the disease that causes the greatest loss of trees in Gmelina plantations, with concomitant economic loss, is the scarce amount of knowledge regarding the cause of the damage (Muchovej *et al.*, 1978; Arguedas & Quirós, 1997; Arguedas, 2004; Umana *et al.*, 2015; Chavarría-Vega & Carmona-Solís, 2016). Several researchers have indicated the economic problems caused by Gmelina death disease. However, few studies have focused on evaluating the quality of the wood in stems damaged by the disease.

Recent observations of the disease symptoms show that it is also associated with a widespread black stain of wood (Murillo *et al.*, 2014). Regarding this issue, an extensive study performed by Moya *et al.* (2009) analyzed black stained wood − denominated "wetwood" −, a technical term that refers to wood with high moisture content, darker color after exposure to air, a distinctive odor, low permeability, a higher pH compared to normal wood, slow drying, and the development of drying defects (Ward & Pong, 1980).

In view of the importance of *G. arborea* to the forestry sector in Costa Rica and other countries in tropical America, and considering the lack of knowledge about Gmelina death disease, its causes and implications for the quality of wood, the aim of the present study was to determine the incidence and severity of the disease in juvenile plantations of *Gmelina arborea*. Then, the incidence and severity of the disease were correlated with (i) tree morphology, tree age and stand density (ii) the presence and quantity of micro- and macronutrients in the soil and (iii) the climatic variables of the zones where Gmelina grows. Additionally, fungi were identified from six isolates of the xylem of infected trees from six plantations. Lastly, the root development and wood quality in symptomatic and asymptomatic trees were compared.

Material and methods

Severity and incidence measurements of the disease and stand variables

— **Sampled plantations:** Based on the information provided by the *Oficina Nacional Forestal* (National Forestry Office) and the *Fondo Nacional de Financiamiento Forestal* (National Forestry Financing Fund) of the government of Costa Rica regarding the disease in plantations of *Gmelina arborea*, sampling was conducted in 16 sites between 2 and 6 years old, distributed in various regions of the country (Fig. 1). Two of these plantations showed no symptoms of the disease (sites 2 and 4), whereas diseased trees were evident in the remaining 14 plantations.

— **Sampling within the plantations:** A 14 m radius circular plot (615 m²) was established. Trees in that area were counted and the projection per hectare was
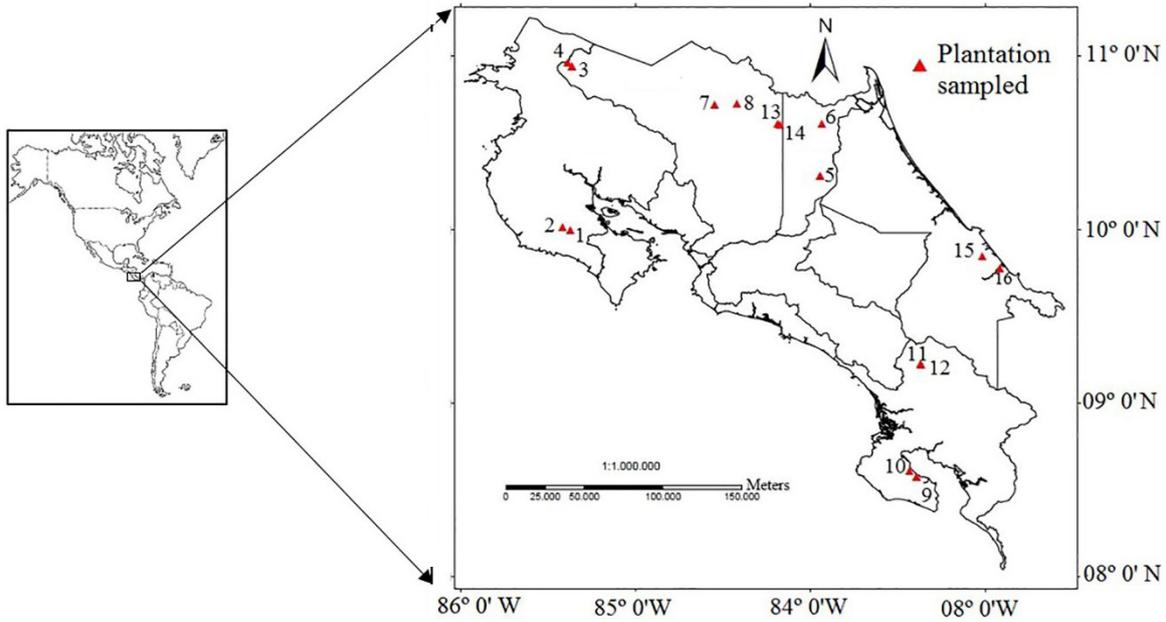
Figure 1. Geographic location of the 16 plantations of *Gmelina arborea* in Costa Rica.

performed (stand density). In addition, the diameter at 1.3 m height (DBH) and the dominant height (h) represented by the three highest trees within the plot, were measured. Healthy trees were identified within the plot, as were trees showing any symptom of the disease, the progress of which was evaluated. According to Arguedas (2004) and Salas (2015) the symptoms of the disease in *G. arborea* are leafless crown or foliar withering (Fig. 2a), canker occurrence (Fig. 2b), sprout occurrence (Fig. 2c) and black exudation in the stem (Fig. 2d). Then, four classes were defined (Table 1).

— **Determination of incidence and severity:** The incidence of the disease and the average index of severity were determined in each plot, following the methodology proposed by Araúz (1998) and Salas (2015).

— **Soil evaluation:** Ten sampling points were randomly selected within each assessment plot. Two soil samples were obtained at each point, employing a manual drill from depths of 0 to 10 cm (profile A) and 10 to 20 cm (profile B). The ten soil portions were mixed, and then the samples per layer were divided into four equal portions and one portion was randomly selected. The soil was characterized according to order and texture. The samples were measured for their pH in water at a 1:2 ratio, and for their proportions of Ca, Mg and K; the effective cation exchange capacity (ECEC), which corresponds to the sum of pH+Ca+Mg+K; the percent base saturation calculated as (pH/ECEC)×100; and the amounts of P, Zn, Cu, Fe and Mn. In addition, the bulk density (g/cm³), clay (< 2 μm), silt (<2-50 μm) and sand (>50 μm) proportions of the soil in the sampled plantations

were determined. For this purpose, soil samples of 80 cm³ were gathered at two depths: from 5 cm to 10 cm and from 10 cm to 15 cm; these samples were dried at 110 °C for 24 hours to reach dry weight. Then, the density, which corresponds to the ratio of
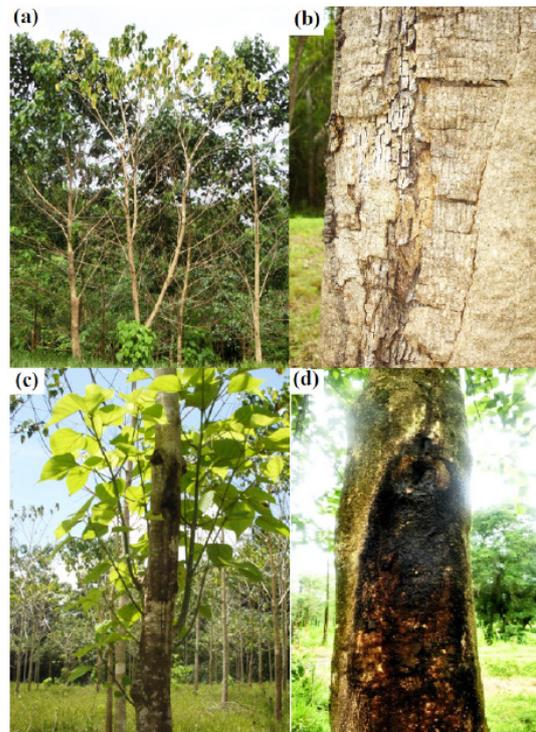
Figure 2. Main symptoms assessed to classify the effects of the Gmelina death disease in plantations of *Gmelina arborea*: (a) foliar wilting, (b) presence of cankers, (c) sprouting and (d) black exudation in the stem.

Table 1. Classes established to evaluate the degree of severity of the Gmelina death disease in symptomatic trees in forest plantations of *Gmelina arborea*

Class of severity	Symptoms
A	Early foliar wilting; the stem may exhibit small necrotic wounds with mild blackish exudation generally at the pruning points. Sprouting may start. Not all symptoms are present in this class.
B	The tree is visibly affected. The foliage wilts, leaves are smaller and over 50% are lost. Canker lesions may appear (with bulging). Frequent exudation and sprouting in the stem.
C	Tree is almost dead. No foliage, evident bark detachment and loss due to canker, and sprouting.
D	Tree completely dry and rotten.

Source: Salas (2015)

the dry weight (g) and volume of the cylinder (cm³), was calculated.

— **Climatic characterization:** First, the climate in the regions where plantations are located was characterized following Holdridge's world life zones classification (Holdridge, 1967). Then, the geographic coordinates and elevation (masl) were obtained for each sampled plantation. This was performed by means of the Global Positioning System (Garmin GPS). In parallel, the permanent meteorological stations of the *Instituto Meteorológico Nacional* closest to the sampled plantations were located. Information was gathered about average temperature and monthly precipitation within a period of 10 years (2004-2014).

A vertical temperature gradient of 0.6 °C per 100 m and the difference in elevation between each plantation based on the elevation of each meteorological station were used to adjust the temperature of each plantation. To adjust the precipitation, an interpolation of the data was performed using the QGis® Analysis of Spatial Interpolation.

Later, the hydric balance was calculated for each plot following the Thornthwaite & Mather (1957) method. Data on the annual potential evapotranspiration, actual evapotranspiration and water deficit in the soil were gathered by means of Thornthwaite's table for soil moisture retention, according to the effective depth and soil texture in each plot. Additionally, Holdridge's annual potential evapotranspiration was calculated as (annual biotemperature * 0.58), and the ratio of potential evapotranspiration and average annual temperature equal to Holdridge's annual biotemperature (Holdridge, 1967) were also calculated.

— **Fungal identification:** Wood with symptoms is presents a dark color (Fig. 3a-b), whilst healthy wood does not (Fig. 3c). Trees affected by the Gmelina death disease were cut in six plantations (Fig. 3d). Cross sections were extracted from the stems one meter from the base of the tree (Fig. 3e-g). During cutting, black areas with a strong odor were observed on the stems, perhaps because of a tree pathogen (Fig. 3a-b). A cross section of each tree was cut at breast height for later identification of the pathogen. The cross sections were washed with distilled water and then three small sections from the heartwood were cut from the periphery of the affected areas (black, strong smell), with the help of a sterile scalpel (Fig. 3h-i).

The three samples per tree were placed on separate Petri dishes containing potato-dextrose-agar (PDA; Difco laboratories, Detroit) and incubated for 22 days at 25 °C under continuous darkness. Several colonies developed during that period. Mycelia were collected from the periphery of the colonies and again placed on a Petri dish to grow into PDA. Then, fungal purification was again performed by hyphal tip transfer to Petri dishes with PDA, which were stored in the dark at 25 °C for 22 days. The mycelial samples of each purified culture for phylogenetic analysis were identified by extracting the DNA employing the Murray & Thompson's method (White *et al.*, 1990). Then, a PCR was conducted and the product was sequenced for one sample for each Petri dish. A total of 18 sequences were obtained (3 trees × 6 plantation or sites × 1 Petri dish). Each sample was added to a 50 μL solution containing 25 pmol of each
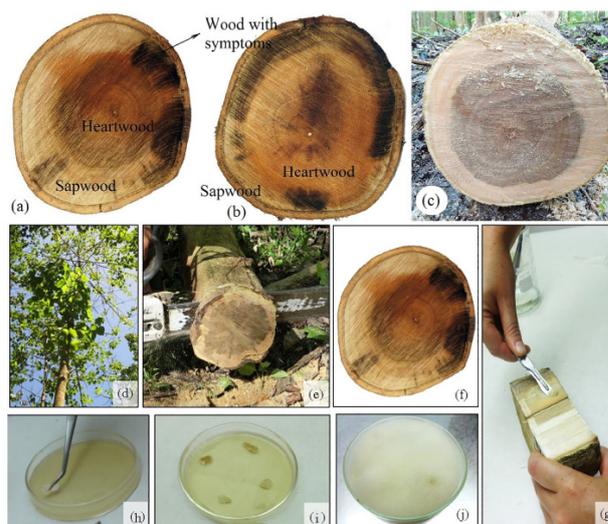
Figure 3. Cross sections of *Gmelina arborea* trees showing areas associated to the Gmelina death disease (a and b) and normal cross section without disease (c). Fungal insulation: tree with disease (d), cross-section extractions (e), cross-section infected (f), and process of insolation (g-j).

primer. The primers used were ITS4 and ITS5. PCR products were sequenced by Macrogen. To analyze the obtained sequences, the NCBI Blast tool was employed for comparison with the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) to identify the isolated fungi.

Comparative study of healthy and infected trees

— **Root morphology evaluation:** Two trees were chosen in each of the plantations where the disease was present, one visibly healthy and the other infected. For each selected tree, two points were located for sampling at the edge of the dripping area (the edge of the crown). At these points, 20 cm³ of soil with roots was extracted at ground level. The samples were washed with distilled water to eliminate the organic matter and then placed in glass containers with ethanol (70%) to prevent deterioration. Then, two representatives 5 cm long subsamples were extracted from the terminal parts of the absorption roots. Using an Epson Expression 1680 scanner, a digital image was captured of the roots and was processed afterwards with WinRhizo Basic V 2005-b® software. The following variables resulted from the image processing: root total length, root average diameter, number of tips, number of bifurcations and number of crossings.

— **Evaluation of the wood properties:** For the present study, we followed the methods proposed by Jeremic *et al.* (2004) and Moya *et al.* (2009), where 9 trees with symptoms of the disease were selected from a 3-year-old plantation in Penshurst, Limón, Costa Rica. The average annual temperature at this location is 26 °C and the annual precipitation is 3078 mm. The average DBH of the trees was 21 cm and the average total height was 19 m. One-meter-long logs were cut from each selected tree, until reaching the commercial height (13 cm diameter). A cross section approximately 4 cm thick was extracted at the end of each log. Five one-meter-long logs with stained and unstained regions were chosen. Then, the samples for determining wood properties were extracted first from zones affected by the disease, corresponding to wood infected by Gmelina death disease. Later, samples of unaffected wood were extracted from the same log or cross section, at the same distance from the heartwood. The properties evaluated were as follows:

a) Physical properties: Green wood density (green weight/green volume), specific gravity according to ASTM D-143 (ASTM, 2003a) and moisture content according to ASTM D-4442 (ASTM, 2003b). A total of 30 samples of dimensions 2.5 cm × 2.5 cm × 2.5 cm were extracted from the discs of infected and non-infected wood. The infected and non-infected wood samples were collected at the same distance from

the heartwood. Each sample was measured for its green weight and green volume (their condition at the moment of extraction of the sample) and then placed in an oven for 24 hours at 105 °C to determine its final weight. This information was used to determine the physical parameters.

b) Mechanical properties: From the 2.7 cm thick boards obtained from the 1 m long logs, approx. 30 samples of dimensions 2.7 cm × 2.7 cm × 1 m were obtained and dried at 22 °C and 66% relative humidity to reach an equilibrium moisture content of 12%. The mechanical properties evaluated were maximum stress in compression, modulus of rupture and modulus of elasticity in static bending. All tests were performed following the ASTM D-143 standard (ASTM, 2003a).

c) Resistance to fungal decay: 240 wood samples measuring 2.5 cm × 2.5 cm × 2.5 cm were cut and divided into three groups: 80 with infected sapwood, 80 with unstained sapwood and 80 with infected heartwood. The samples were inoculated by two strains of the fungi, *Trametes versicolor* (white decay) and *Lenzites acuta* (brown decay), following the procedure set in the ASTM-D 2017-81 standard (ASTM, 2003c). This test yields the percentage of weight loss of the samples after 16 weeks of exposure to fungal attack.

d) Inorganic ash composition of wood: Chips of wood were collected from healthy and infected samples from the cross sections of the sampled trees and then ground and sieved through 40- and 60-sized meshes. Ash analysis was performed using the methods of Sparks (1996) and included determination of the mass of N, P, Ca, Mg, K and S, whose concentrations were reported as percentages, and Fe, Mn, Cu, Zn and B, whose concentrations were reported as milligrams of inorganic element per gram of ash.

e) Wood color: Color was measured in the samples to measure the mechanical properties of the infected and uninfected wood. These samples were conditioned to 12% moisture content and were evaluated in the tangential face. To measure color, the standardized color measuring system CIE L*a*b* was used with a miniScan XE Plus spectrophotometer, following the procedure described by Moya *et al.* (2012).

Statistical analysis

A Pearson correlation matrix was used for determining the most correlated incidence and severity for further analysis. Next, incidence and severity were correlated with the tree morphology (DBH and h), stand density, tree age, soil and climatic characteristics. A forward stepwise analysis was applied to establish the effect that tree morphology, tree age, climate characteristics and soil characteristics

in the profile from 10-20 cm had on the percentage of incidence and severity of the disease and on the various damage classes. The analysis of variance, ANOVA, was also performed to determine the level of significance of symptomatic and asymptomatic wood; it was applied to characteristics of the roots and the physical and mechanical characteristics, content of macro- and microelements, loss of weight during fungal attack and different color parameters. Afterwards, the presence of significant differences among the media of these parameters of normal wood and symptomatic wood was verified by means of the Tukey test ($p<0.05$). The statistical software SAS 8.0 was used to perform the statistical analysis of the data.

Results

Incidence and severity of the disease in the plantations

The incidence of the disease varied from 0% to 58% of the total number of trees in the plantation. When sorted the 16 sampled plantations from highest to lowest incidence and severity, three different inflection points could be observed. According to this, four groups were established: (i) plantations where incidence was equal to 0 (plantations 2 and 4); (ii) a second group with values of incidence from 3% to 4% (plantations 13, 7 and 9); (iii) a third group with values between 17% and 35% (plantations 1, 8, 6, 15, 12, 16, 10 and 11) and (iv) a

fourth group with values of incidence between 48% and 58% (plantations 3, 5 and 14) (Fig. 4a). For severity, it ranged between 0% and 28% (Fig. 2b) and four groups were determined as well: (i) the first group includes healthy plantations (plantations 2 and 4), (ii) the second group showed severities between 1% and 4% (plantations 13, 7, 9, 8 and 6), (iii) the third group showed severities between 10% and 13% (plantations 1, 12, 16, 3, 15 and 10) and (iv) the last group showed values between 21% and 28% (plantations 5, 11 and 14) (Fig. 4b).

The percentage of the damage class A (wilting, exudation and small sprouting) was higher in plantations 1, 3, 6, 8, 12 and 16, while the percentage of damage class B (wilting, small leaves, 50% loss of foliage, canker or swelling, exudation and sprouting) was higher in plantations 5 and 10. For damage class C, the percentage was higher in plantations 14 and 15. Finally, plantation 11 showed the highest number of trees with damage class D (Table 2).

Fungal identification

The DNA sequence analysis of the six types of wood from samples collected from symptomatic trees revealed the presence of three species of fungi: *Fusarium solani* was present in 3 sites (2, 3 and 4) showing a BLAST match of 99% in the GenBank database (AF178407.1, KF255997.1 and AF178407.1, respectively). *Rhizomucor variabilis* was present in sites 5 and 6 with a BLAST match of 99% with HM623314.1 of GenBank database. Finally, *Chaetomella raphigera*, with a 98% BLAST match with AY487076.1 of the GenBank database, was present only in the xylem of the tree in site 1.

Effect of the parameters of the plantations, environmental conditions and soil fertility.

The amount of clay in the soil layer between 10 cm and 20 cm accounted for 66% of the variation of incidence of the disease, followed by K in the same layer explaining 22% of the variation (Table 3). The amount of clay was positively correlated with the incidence of the disease (Fig. 5a). In the case of K, contrariwise, the correlation with the incidence was negative (Fig. 5b). However, this relation was influenced by two points, 0.5 and 0.6 in K content. If these two points were removed, then the relationship would become non-significant ($R^2=0.02$). Altogether, clay and K content explained 87% of the total variation of the incidence (Table 3).

With respect to severity, it was first related to stand density, which explained 33% of the variation, after which the amount of clay in segment 10-20 cm explained 27% of the variation (Table 3). Once more, K appeared in the 10-20 cm profile and explained 21% of the variation.
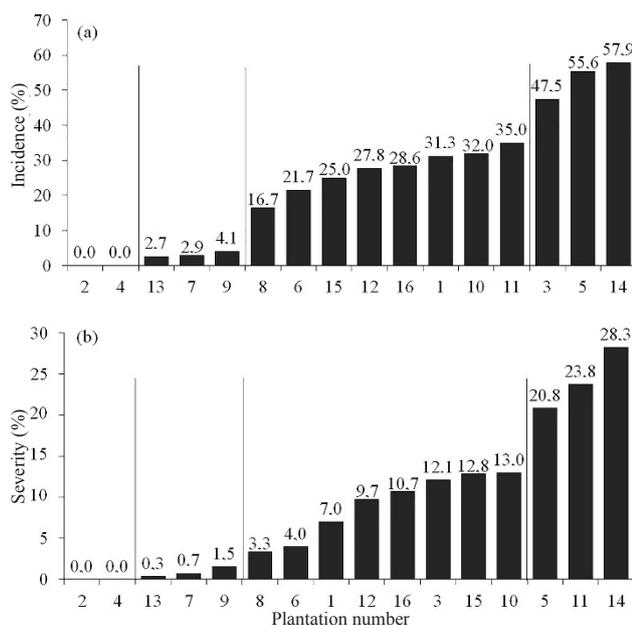
Figure 4. (a) Incidence and (b) severity in *Gmelina arborea* plantations infected with the Gmelina death disease in Costa Rica.

Table 2. Percentage of trees in the different classes of damage severity in *Gmelina arborea* plantations in Costa Rica.

Plantation No.	Density (N/ha)	Age (years)	Healthy trees (%)	Damage class (%)			
				A	B	C	D
1	520	3	68.75	21.88	6.25	3.13	0.00
2	585	2	100.00	0.00	0.00	0.00	0.00
3	991	2	52.46	34.43	3.28	8.20	1.64
4	1104	2	100.00	0.00	0.00	0.00	0.00
5	585	3	44.44	19.44	25.00	2.78	8.33
6	974	2	78.33	20.00	0.00	0.00	1.67
7	1104	2	97.06	1.47	1.47	0.00	0.00
8	974	2	83.33	13.33	1.67	1.67	0.00
9	796	4	95.92	2.04	0.00	2.04	0.00
10	406	4	68.00	8.00	16.00	4.00	4.00
11	650	4	65.00	5.00	2.50	7.50	20.00
12	585	6	72.22	16.67	2.78	2.78	5.56
13	601	5	97.30	2.70	0.00	0.00	0.00
14	309	2	42.11	15.79	10.53	21.05	10.53
15	585	3	75.00	5.56	0.00	19.44	0.00
16	682	2	71.43	11.90	7.14	7.14	2.38

All these variables explained 81% of the variation of severity (Table 3). The stand density was negatively correlated with severity (Fig. 5c); the amount of clay in 10-20 cm profile was again positively correlated with severity (Fig. 5d).

Root morphology

The average number of tips, forks and crossings

Table 3. Multiple regression analysis for the relationship between incidence, severity scores and classes established for severity grades for 16 plantations of *Gmelina arborea* infected with Gmelina death disease in Costa Rica.

Incidence (%)	Severity (%)
Clay between 10-20 cm deep** (R_p^2=0.66; R_T^2=0.66)	Stand density** (R_p^2=0.33; R_T^2=0.33)
K between 10-20 cm deep** (R_p^2=0.22; R_T^2=0.87)	Clay between 10-20 cm deep * (R_p^2=0.27; R_T^2=0.60)
	K between 10-20 cm deep * (R_p^2=0.21; R_T^2=0.81)

R_p^2: coefficient of partial correlation. R_T^2: coefficient of total correlation. **Statistically significant at 99% confidence, * statistically significant at 95% confidence.

and the root length was statistically lower in infected trees than in healthy trees. Meanwhile, no significant difference was observed between both conditions of sampled trees regarding root diameter (Fig. 6).

Wood quality

— **Appearance:** symptoms of the disease in *Gme-lina arborea* trees were evident in both the sapwood and the heartwood (Fig. 3). The affected region was differentiated from the surrounding tissue by its darker color and occasional sap secretion (Fig. 3). Fermentation was another characteristic of these dark regions as it causes a rancid to fetid rumen-like odor. Affected wood showed light (L*) tonality, statistically lower than unaffected wood, in both green and dry wood (Fig. 7a). A higher tonality was also observed in redness (a*), although this was statistically higher in the dry condition (Fig. 7b) compared to the wet condition. Finally, less yellowish tonality (b*) was observed in green wood in relation to normal wood (Fig. 7c).

— **Wood properties:** it was observed that affected wood presented a statistically higher value of density in green condition, higher moisture content, less resistance to compression stress, less modulus of elasticity and module rupture in the bending test compared to unaffected wood
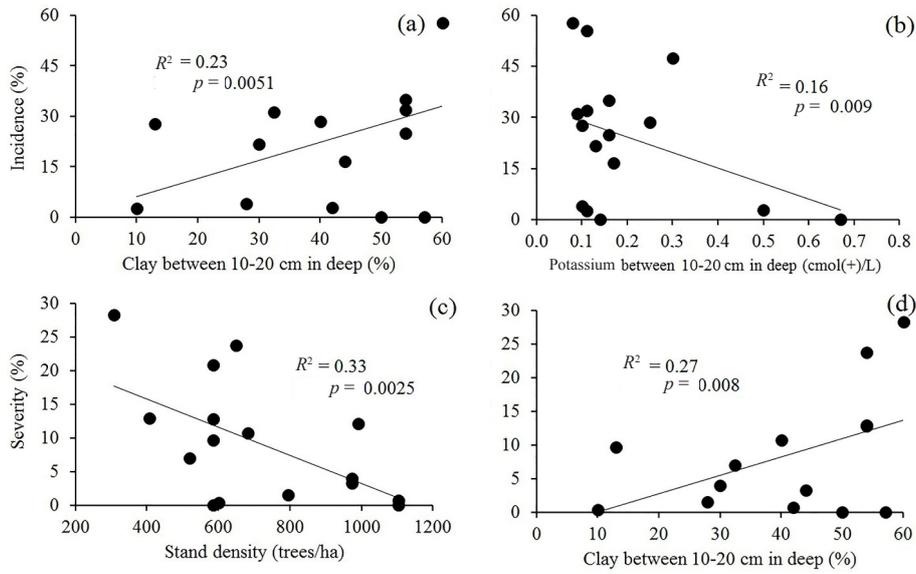
Figure 5. Correlation between incidence of Gmelina death disease and quantity of clay and potassium in the second profile 10-20 cm deep (a-b); correlation between severity and stand density (c) and quantity of clay in the second profile 10-20 cm deep (d).
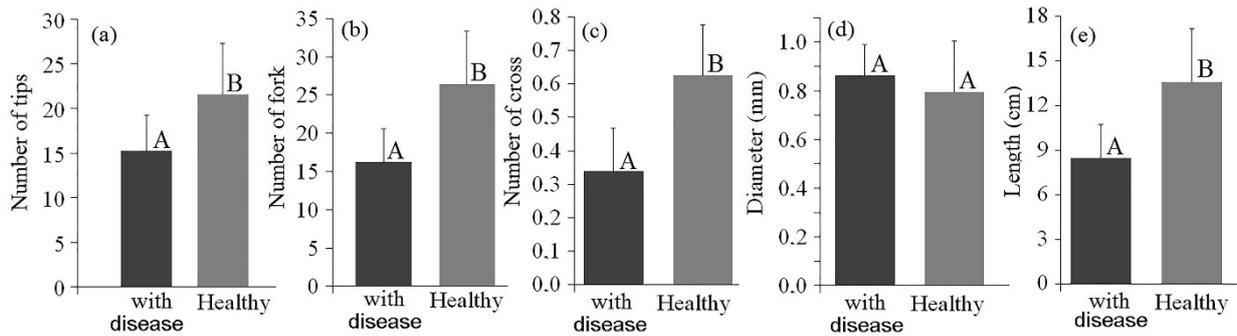
Figure 6. Number of tips (a), forks (b) and crossings (c), diameter (d) and length (e) of roots in trees infected with Gmelina death disease in plantations of *Gmelina arborea* in Costa Rica. Different capital letters indicate a statistical significance of 99% and bars indicate confidence intervals. The averages were derived from root samples in 16 sampled plantations.

(Table 4). The specify gravity was not statically different in affected and unaffected wood (Table 4). Regarding the durability of the wood to white-decay (*T. versicolor*) and brown-decay (*L. acuta*) fungal attack, there was no statistical evidence that symptomatic wood was less durable than normal wood (Fig. 7d-e).

On the other hand, the evaluation of micro- and macronutrients of wood ash showed that the amount of Mg and Fe is statistically higher and quantities of Ca, K and Zn are statistically lower in symptomatic wood compared to asymptomatic wood (Table 4). The amounts of remaining nutrients were not statistically different between symptomatic and asymptomatic wood. For other chemical characteristics, the pH and electrical conductivity were statistically lower in wood from trees with "Gmelina death disease" than in normal wood (Table 4).

Discussion

The incidence (0 to 58%) and severity (0 to 28%) found in this study show the variation in the presence of the Gmelina death disease in *G. arborea* plantations in Costa Rica (Fig. 4). Currently, it is possible to observe juvenile plantations with such variations throughout the country. These values are higher than those from the study by Murillo *et al.* (2014) of three-year plantations in a wet tropical climate region also in Costa Rica, which found incidence rates of 23.5 to 26.2% and a severity of 8.54 to 17.55%. Meanwhile, Singh *et al.* (2003) and Soni *et al.* (2005) recorded 8 to 15% incidence of wilt disease in *Gmelina arborea* and other species such as *Dalbergia sissoo*, *Tectona grandis* and *Buchnania lanzan*, but their measurements were performed during the seedling stage.

Trees in C and D classes could pose pro-blems regarding their use because of their darker tonality
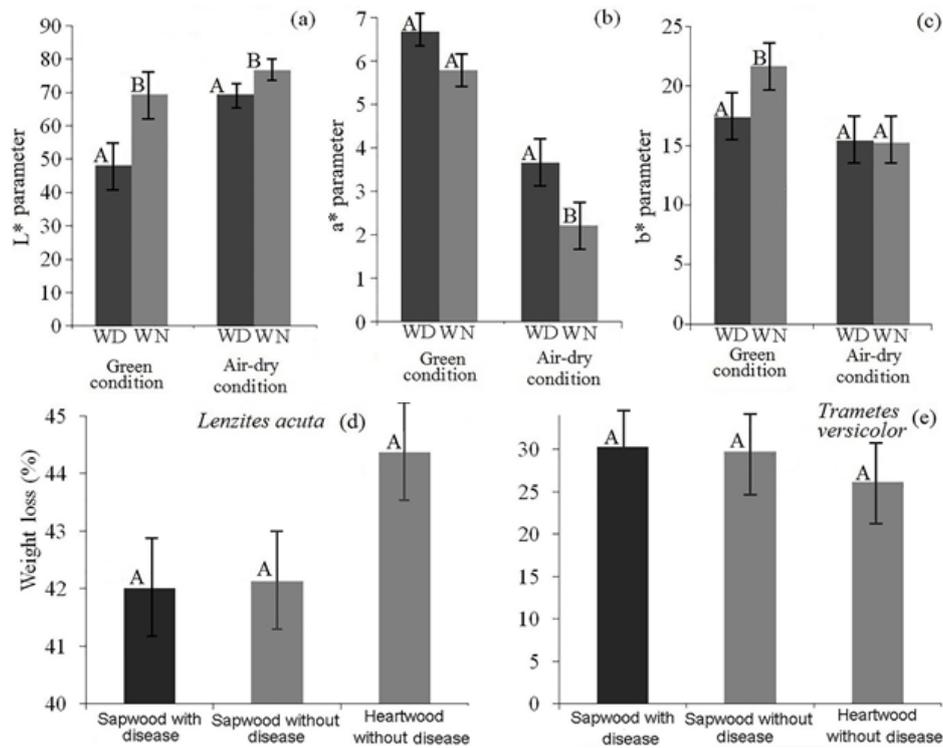
Figure 7. Colour parameters in green and air-dry condition in wood with diseased (WD) and without diseased or normal wood (WN) (a-c), and weight loss in wood for *Lenzites acuta* and *Trametes versicolor* (d) in normal wood and wood from trees with Gmelina death disease growing in *G. arborea* plantations. Different capital letters indicate a statistical significance of 99%

Table 4. Physical and mechanical properties, and micro- and macronutrients in normal wood and in wood showing symptoms of Gmelina death disease in *Gmelina arborea* plantations in Costa Rica.

Property	Variable	Normal wood	Symptomatic wood	Percentage of difference
Physical	Density (g/cm³)	1.11[A]	1.02 [B]	-8.82
	Specific gravity (g/cm³)	0.46[A]	0.44 [A]	4.35
	Moisture content (%)	123.7[A]	154.7 [B]	-25.06
Mechanical	Compression stress (MPa)	0.24[A]	0.13 [B]	45.83
	Compression density (g/cm³)	0.48[A]	0.48 [A]	0.00
	Flexural modulus of elasticity (MPa)	11166[A]	8792 [B]	21.26
	Flexural modulus of rupture (MPa)	63.84[A]	52.76 [B]	17.36
Ash content	Nitrogen (%)	0.23[A]	0.25[A]	-8.70
	Phosphorus (%)	0.02[A]	0.02 [A]	0.00
	Calcium (%)	0.19[B]	0.14[A]	26.32
	Magnesium (%)	0.04[B]	0.03 [A]	25.00
	Potassium (%)	0.54[B]	0.30[A]	44.44
	Sulphur (%)	0.01[A]	0.01[A]	0.00
	Iron (mg/kg)	27.17[B]	63.00 [A]	-131.87
	Copper (mg/kg)	4.00[B]	3.17 [A]	20.75
	Zinc (mg/kg)	13.33[B]	11.83 [A]	11.25
	Manganese (mg/kg)	0.00[A]	0.00[A]	0.00
	Boron (mg/kg)	3.67[A]	3.33 [A]	9.26
Other	pH in water	6.47[B]	5.80 [A]	10.36
	Electrical conductivity (mS/cm)	1.93[B]	1.03 [A]	46.63
	Carbon (%)	46.90[A]	46.42 [A]	1.02
	Ratio C/N	211.24[A]	187.83 [A]	11.08

compared to normal wood (Fig. 3), their higher moisture content and their lower resistance in some mechanical properties (Table 4). Although the percentages of incidence and severity (58% and 28%) can be considered high, the highest severity classes (C and D) did not appear in all plantations. Approximately 20% of damage class C appeared in sites 14 and 15 and damage class D in site 11 (Table 2). These results can be somewhat confounded by tree age, as plantations varied in age (2-6 years old range) and each one was taken as a single point in the analysis, *i.e.* within-plantation variation was not considered. However, age was weakly correlated with damage class, as the oldest plantations, 6 and 5 years (plantations 12 and 13, respectively) old, had low severity damage (Table 2).

Although three species of fungi were found in stained wood (*Fusarium solani, Chaetomella raphigera* and *Rhizomucor variabilis*), only *F. solani* has been reported by other researchers as being present in infected trees of *G. arborea* (García-Díaz *et al.*, 2011; Murillo *et al.*, 2014; Umana *et al.*, 2015; Chavarría-Vega & Carmona-Solís, 2016). On the other hand, *Chaetomella raphigera* is reported to occur together with fruit rot pathogens, and can be linked to fungi of the *Nectria* and *Fusarium* species (Gajbhiye *et al.*, 2016). However, it is necessary to know the actual association of *C. raphigera* and Fusarium species in Gmelina trees in a plantation. Finally, *Rhizomucor variabilis* was renamed after *Mucor irregularis* (Voigt *et al.*, 1999) and many reports indicate that this pathogen can cause plant disease.

On the other hand, the *Fusarium solani* species complex (FSSC) may embrace approx. 50 phylogenetic species (O'Donnell, 2000; O'Donnell *et al.*, 2008; Coleman, 2016) that dwell in soil, plants and animals (Nalim *et al.*, 2011). In trees, this fungus is considered a problem since it reportedly affects timber production in certain species, such as *Dalbergia sissoo* (Basak & Basak, 2011) and *Terminalia nigrovenulosa* (Seo *et al.*, 2013). The presence the *Fusarium solani*, the anamorph of *Nectria*—now Neocosmospora—*haematococca* (Nalim *et al.*, 2011; Coleman, 2016), agreed with several studies that identified the same tree fungi as "Gmelina death disease" (García-Díaz *et al.*, 2011; Murillo *et al.*, 2014; Chavarría-Vega & Carmona-Solís, 2016). However, we only isolated strains from 6 sites and other important tests must be performed. Therefore, we cannot infer that this fungus or FSSC is a causal agent of "Gmelina death disease".

An additional important effect relative to the development of root rot disease in other agricultural crops occurs in root development (Román-Avilés *et al.*, 2004). It was demonstrated that symptomatic Gmelina trees exhibit different root characteristics (Fig. 6), that is, the number of tips, forks and crossings and root length were reduced relative to asymptomatic trees. This is because pathogen infection reduces root density by killing roots and may thus attenuate the functional efficiency of the remaining roots (Román-Avilés *et al.*, 2004). Root disease becomes more severe when roots are unable to escape the pathogen due to edaphic factors, such as high clay content (Barros *et al.*, 2014). In fact, clay content was positively correlated with incidence and severity of *Gmelina* death disease (Table 4). Soils with a high clay content tend to affect the roots of many agricultural crops (Leslie & Summerell, 2006) and probably the xylem of woody plants too because high clay content implies a high moisture storage capacity and poor drainage of the soil (Belete *et al.*, 2013).

Stand density, on the contrary, did not contribute to the development of disease symptoms, but high stand density decreased the severity in Gmelina trees (Table 3). This result disagreed with the findings of Arguedas (2004), who mentioned that trees growing in high density are unable to develop appropriately and create mechanisms of protection against pathogens. Most likely, if high density is present in a juvenile plantation, then either trees do not develop the disease, or two to six years is not enough time for juvenile trees develop the disease. On the contrary, the low stand density present in adult plantations was associated with high severity levels and some trees probably died (Fig. 5c). A positive correlation ($p<0.0001$) between severity and tree age confirmed that mature plantations present trees with high severity, due to adult trees that had developed the disease.

In relation to the negative relationship between K and the incidence of Gmelina death disease, no clear conclusions could be drawn. The reason is that even though a statistically significant relationship was found (Fig. 5b), this was strongly influenced by two observations. Therefore, more research is necessary on this point.

As noted, wood quality was impaired (Table 4), color being the most negatively affected parameter (Fig. 7) in regard to appearance. Color change is likely produced by changes in the content of extractives in the wood, which give color to Gmelina (Moya *et al.*, 2009; Moya *et al.*, 2012). Other affected characteristics (Table 4) relate to wood structure, lower density and mechanical resistance. As explained by Xu *et al.* (2001), the lower specific gravity (SG) of infected wood in *Quercus rubra* and *Q. falcata* is possibly due to wood mass loss resulting from degradation of infected wood associated with bacteria and wood decaying fungi. They also stated that volatile constituents such as methane, acetic acid, propionic acid and butyric acid present in infected wood are lost during oven drying, resulting in a lower SG and reduction of wood resistance.

However, a positive aspect of symptomatic infected wood is that the wood's resistance to fungi is not impaired. Symptomatic wood is characterized by brown stains in heartwood (or sapwood) that are susceptible to decay. The decreased pH of infected wood in some species may make it less susceptible to acid-forming brown-rot fungi, but offers no protection from white-rot fungi (Moya *et al.*, 2009).

Inorganic elements are essential for tree growth and are measured as micro- and macronutrients in ash (Xu *et al.* 2001). An increasing amount of inorganic elements is associated with the presence of infected wood (Jeremic *et al.*, 2004). The present study showed that the amounts of Ca, Mn, Fe, Cu, K and Zn differ between infected wood and normal wood. Murdoch *et al.* (1987) suggested that elevated levels of inorganic elements in infected wood resulted from the selective degradation of cell membranes by bacteria, leading to the accumulation of unused micronutrients.

Gmelina death disease management

Gmelina plantations have been established in Costa Rica in sites with high stand densities and high contents of clay in the profile 10 to 20 cm deep, which are inappropriate conditions that increase susceptibility to Gmelina death disease. Different solutions may be implemented for this problem. One solution is related to the selection of suitable sites where the amount of clay is below 40%, to allow the soil to conveniently drain the levels of rainfall characteristic of reforestation sites in Costa Rica. The second solution refers to managing infected trees at early ages to grow healthy trees capable of combating pathogens.

In summary, the quantity of clay present in the 10-20 cm layer offers conditions for the development of the Gmelina death disease. Lack of management of plantations also promotes the development of the disease (high severity) though increased stand density. The characteristics of climate had no effect on the disease. Although two climatic variables, Holdridge's potential annual evapotranspiration (PAEH) and Thornthwaite's potential evapotranspiration (PET), showed a relationship with the incidence in the trees and symptoms of the disease, it was not statistically significant. The visual quality of the wood was affected by Gmelina death disease. The affected area turned black, and mechanical resistance was also reduced. The natural durability of the wood, however, was not affected. There were changes in ash composition, specifically in the levels of Mg, Fe, Ca, K and Zn. An appropriate selection of sites, with the amount of clay below 40%, allows adequate growing of trees in commercial plantations in Costa Rica, and adequate management at early ages allows to grow healthy trees capable of combating pathogens.

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