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Research Article

Susceptibility of *Pericopsis elata* (Assamela) to heartwood decay and identification of micro and macro fungi associated with the disease in Cameroon

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Abstract: Heartwood decay is one of the major pathological constraints affecting the quality and marketable volume of tropical timber. A study on its behavior was conducted on *Pericopsis elata* in order to contribute to its sustainable management in Cameroon. Tree susceptibility to heartwood decay was evaluated using three diameter classes, based on allometric equations between decayed and healthy trees. Wood samples and visible macromycetes were taken from living and felled trees with an auger. Isolation of microfungi was performed on potato dextrose agar medium and their identification was based on the morphological and microscopic characteristics of the mycelium and conidia with reference to identification keys of mycology. Identification of macromycetes was based on their morphological characteristics as described in reference books on macromycetes identification. Results show that diameter class]110, 120[presented highly significant ($P<0.05$) volume loss (3755.96 cm^3), followed by diameter class]100, 110[. Microfungi frequently associated with heartwood decay of *P. elata* were *Cercospora* sp (24.57%), *Fusarium oxysporum* (12.64%) and *Penicillium* sp (12.58%) in living decay trees and *Aspergillus niger* (25.19%), *Cercospora* sp (22.21%), *Penicillium* sp (17.69%) and *Phoma* sp (15.05%) in felled decay trees. Macrofungi associated with living trees were *Inonotus* sp and *Ganoderma* sp. This is the first time that these fungal species are reported on *P. elata* wood. This study provides baseline information for the study of heartwood decay and management of *P. elata* in Cameroon.

Keywords: Heartwood decay; Macrofungi; Microfungi; Diameter class, *Pericopsis elata*; Wood loss.

Introduction

The Congo Basin forests represent a rich floristic diversity thatneeds to be managed sustainably. Logging constitutes a vibrant economic activity for the financial performance of governments and forest companies (Belinga, 2009). In Cameroon, *Pericopsis elata* (Harms) Meeuwen (*Fabaceae* family) which has as trade name Assamela or Afrormosia is an economically important timber species. It isclassified by the International Union for the Conservation of Nature (IUCN) as an endangered species (Bourland *et al*., 2010). Since 2005, the International Tropical Timber Organization (ITTO) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have been working together to develop a broad capacity-building project for countries on the sustainable trade of this species. It is limited in distribution (Betti, 2008; Ouédraogo, 2014) and its exploitation is confronted with biotic constraints such as its natural regeneration and susceptibility to heartwood decay. *Pericopsis elata* is a valuable timber species in the world tropical timber market due to its varied uses (ITTO, 2007). In Cameroon, a cubic meter is 252 US $ (Ambara, 2009; MINFI, 2009) and the felling tax in zone 3 of the country (East Region) according to decree N° 000533/CF/A/MINFI/DGD of 02/09/2016 is 6 US $ per cubic meter. The ITTO/CITES Workshop on Timber Trade in *Afrormosia* which held in Cameroon (Kribi) in 2008 was one of the most important steps for the establishment of the basis of sustainable management of the species. During this workshop, forest companies representing the timber industry raised, among other concerns, the need for its systematic regeneration, a review of its minimum exploitable diameter (100 cm) and heartwood decay problem. In Cameroon and within the Congo basin, the

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productivity of *P. elata* is confronted with heartwood decay, which results in significant though undetermined wood fibre and financial losses. Heartwood decay is an enzymatic alteration of wood by fungi capable of producing various enzymes for wood polysaccharide destruction such as cellulases, which degrade cellulose and oxidases that degrade lignin (Lekounoungou, 2008; Durand and Simard, 2011). For conservation measures, Cameroon sets the minimum exploitable diameter (MED) of *P. elata* at 100 cm (Amougou *et al.*, 2009). Due to the relationship between the Minimum Exploitable Diameter (MED) of the species, heartwood decay and senescence, MED has to be re-evaluated based on scientific data. Due to the numerous biotic threats (low natural regeneration, heartwood rot and decrease in stand density), against insufficient data on its sustainable management and restricted knowledge about the heartwood decay of *P. elata* (Belinga, 2009), this study was embarked upon to identify mycetes that are associated with the heartwood decay of the species. It is envisaged that the results will provide additinal knowledge that can be used in prescribing a better management strategy for the species.

Materials and Methods

Study area and sampling

Samples of wood decay and visible macromycetes on tree trunks were collected from April to May 2016, using a simple random sampling design, in an annual cutblock of concession 10-010 (66,688ha), owned by SEFAC (*Société d'Exploitation Forestière et Agricole du Cameroun*) group. The concession is found in Libongo, Moloundou Sub-division of Boumba and Ngoko Division of the East Region of Cameroon. Specifically, it is located between latitudes 2° 30' 08"and 2° 47' 14" North and longitudes 15° 36' 00" and 16° 01' 10" East. Samples on symptoms of heartwood decay were collected both from standing and fallen trees. A random data collection by diameter class was carried-out on the trees. Infected tree cores as well as visible fruiting bodies of macromycetes were collected with a manual auger and introduced into plastic sachets that were labeled by type (living or felled trees) and taken to the laboratory for culture and identification of fungi.

Culture, isolation and identification of micro and macromycetes

Tree cores were disinfected in a 2% sodium hypochlorite solution for 5 minutes, followed by a triple rinse with sterilized distilled water and thereafter, the fragments were deposited on Hydrophilic paper to absorb excess water. Small core fragments were aseptically introduced into sterilized Petri dishes containing potato dextrose agar medium, in 4 replicates per type of lesion. The Petri dishes were then sealed and incubated at 21°C (Djeugap *et al.*, 2017a). After the development of microscopic fungi around the decayed wood fragments, they were purified and identified based on their morphological characteristics using identification keys in mycology (Barnett and Hunter, 1972; Champion, 1997; Warharm *et al.*, 2008). During isolation, the frequency of occurrence (FO) of each fungus was computed using the following formula: FO = (NF/NT) x 100, where NF = Highest number of samples from which a particular fungus was isolated, NT = Total number of samples from which isolations were carried out (Iqbal and Saeed, 2012). Identification of macromycetes was based on the morphological characteristics of their carpophores with respect to the identification keys available in related literature (Camaioni, 1989; Courtecuisses and Duhem, 2007).

Determination of wood loss volume by diameter class

Allometric parameters such as tree diameter and wood length, and disease parameters such as decay diameter and decay depth were used to estimate the initial wood volume (V_i) and the wood loss volume (V_o) based on the following equations developed by the French National Organization for Standardization (Cailliez, 1980; AFNOR, 1985):

$V_i = \pi L [d_o^2 + (d_o x d_i) + d_i^2]/12$, where L = log length, d_o = Log diameter at the base and d_i = log diameter at small-end. $V_o = (\pi d_i^2) D/4$, Where D = Wood decay depth and d_i = Log diameter.

For each diameter class, data was collected from 10 randomly selected individuals in a cutblock of concession 10-010. The difference between the two volumes ($V_i - V_o$) was calculated to obtain the useful or merchantable timber volume (Vu). Collected data was grouped into three diameter classes,]90-100],]100-110] and]110-120] cm. Sensitivity to heartwood decay was assessed based on a study of the correlation between heartwood diameter (x) and tree diameter (y). The Pearson's correlation coefficient (r) between the two variables x and y was calculated by dividing the covariance of the variables by the product of their standard deviation. It is a statistical index that expresses the linear relationship between two quantitative variables and helps to predict a variable by another using a linear model (Artusi *et al.*, 2002).

Data analysis

Data was collected on the initial wood volume, wood lost, merchantable volume and the isolation frequencies of the fungi. The data was subjected to analysis of variance and data in percent (isolation frequency) was previously transformed (arcsin) before being subjected to statistical analysis. SAS version 9.1 software was used for the analysis and the means were separated using Duncan's multiple separation tests at 5% probability.

Results

Identification and description of micromycetes associated with *P. elata* wood

Several microscopic fungi were identified on infected wood fragments of *P. elata* trees on both standing and felled trees. The most frequently identified microscopic fungi were *Cercospora* sp (24.57%) and *Aspergillus niger* (25.19%) in standing or living trees and *Cercopora* sp (22.21%) in felled trees (Table 1). The least identified was *Trichoderma harzianum* (4.18%). Microscopic fungi like *Colletotrichum* sp, *Fusarium oxysporum* and *T. harzianum* were absent in felled trees. The fungal colony on culture medium and microscopic characteristics of mycelium and conidia are shown on Figure 1. The physical aspect of both mycelium and conidia varied from one fungal species to another. After 14 days of culture on PDA medium, mycelium of *A. flavus* turned greenish and purplish when mature while *A. niger* was black, *Cercospora* sp and *Rhizoctonia* sp were greyish, *Colletotrichum* sp, *Phoma* sp and *Phomopsis salicina* were whitish and *Trichoderma harzianum* was green. The conidia size (morphology) and the number of cells per conidia also varied from one species to another (Figure 1).

Table 1. Isolation frequencies of microfungi associated with heartwood decay of *P. elata*.

Microfungi	Isolation frequencies* (%)	
	Living trees	Felled trees
Aspergillus flavus	6.91^{c*}	11.09^{b*}
Aspergillus niger	9.20^{c}	25.19^{a}
Cercospora sp	24.57^{a}	22.21^{a}
Colletotrichum sp	7.97^{c}	0^{d}
Fusarium oxysporum	12.64^{b}	0^{d}
Penicillium sp	12.58^{b}	17.69^{b}
Phoma sp	7.41^{c}	15.05^{b}
Phomopsis salicina	6.10^{c}	4.17^{c}
Rhizoctonia solani	8.43^{c}	4.6^{c}
Trichoderma harzianum	4.18^{c}	0^{d}

*Means with the same letter in the column are not significantly different using the Duncan multiple range test at 5%.

Table 2. Healthy and decay wood volumes of *Pericopsis elata* by diameter class.

Class diameter (cm)	Initial wood volume (cm³)	Healthy wood volume (cm³)	Wood decay volume (cm³)
]90-100]	31409.82 ± 761.85^{b}	31035.31 ± 442.68^{a}	374.52 ± 34.6^{c}
]100-110]	40474.87 ± 832.77^{b}	38548.26 ± 276.12^{a}	1926.61 ± 128.7^{b}
]110-120]	58170.49 ± 893.41^{a}	45414.53 ± 678.01^{a}	3755.96 ± 221.73^{a}

*Means with the same letter in the column are not significantly different using the Duncan multiple range test at 5%.

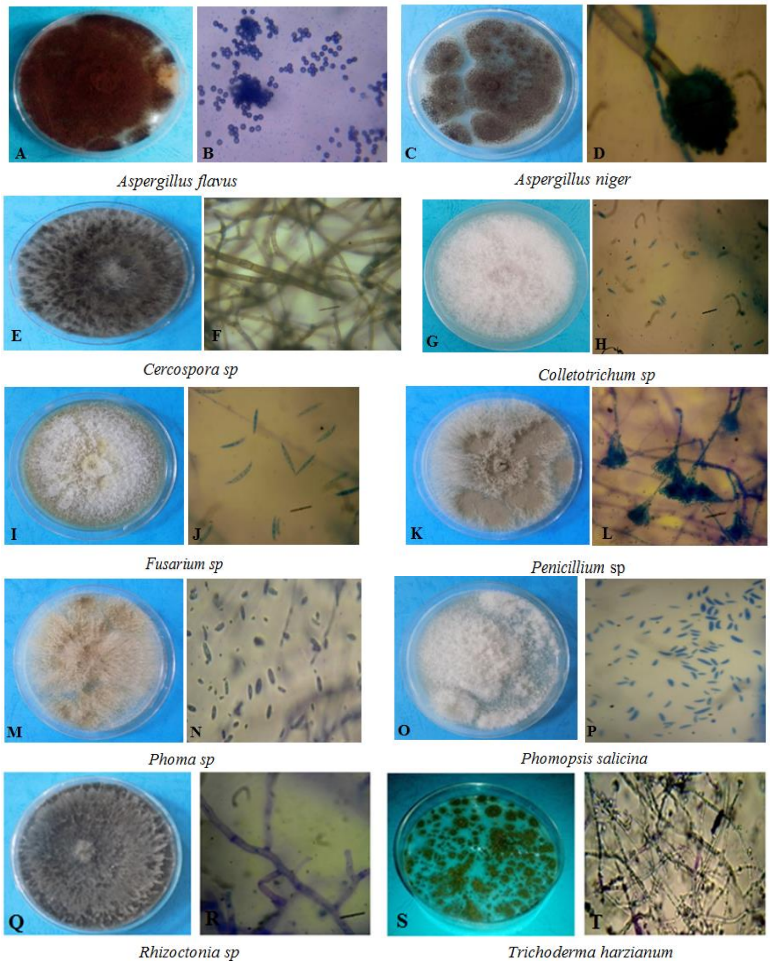

Figure 1. Pure culture in Petri dishes, mycelium and conidia of microfungi associated with heartwood decay of *P. elata* after 14 days of culture.

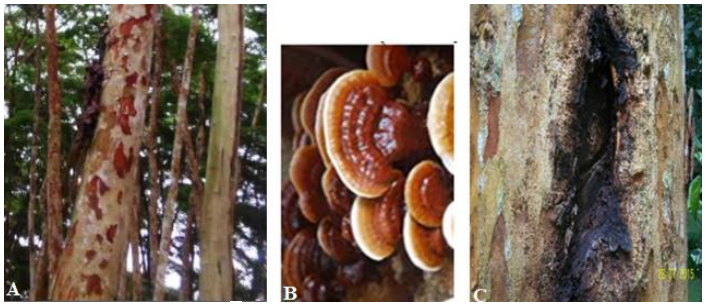

Figure 2. Fruiting bodies of macromycetes *Ganoderma* sp. (A and B) and senescent fruiting bodies of *Inonotus* sp. (C) on the trunk of *P. elata.*

Description of the macromycetes identified on *Pericopsis elata* wood

The macromycetes identified on *P. elata* wood were *Ganoderma* sp and *Inonotus* sp. On *P. elata* wood, *Ganoderma* sp has the appearance of varnished wood of reddish brown color and honey. The carpophore is thick, tough, rounded and slightly wrinkled radially with margins covered with a whitish pruin. *Inonotus* sp presents itself in the form of a soft and fleshy mass to touch, yellowish to golden, without the hymenal layer and becoming grayish in the internal parts disintegrating into a mass of chlamydospores (Figure 2).

Evaluation of impact of heartwood decay by diameter class

Highly significant (P≤0.05) wood volume loss was obtained from individuals within diameter class] 110,120 [followed by individuals within diameter class]100,110[. The lowest wood volume loss was obtained from individuals within diameter class]90,100[. No significant difference (P>0.05) was observed in merchantable wood volume among the three diameter classes that were investigated (Table 2).

Discussion

This study reveals that several micro fungi and two macro fungi are associated with heartwood decay of *P. elata* both on standing and felled trees. Based on literature review, most isolated micromycetes would be endophytic, molds or parasitic fungi on forest trees or wood. For example, the species *Fusarium oxysporum* cause Fusarium wilt, a common and lethal disease of *Albizia julibrissin* in the USA (Stipes, 1999), dieback disease symptoms on *Acacia koa* in Hawaii (James *et al*., 2007). It was reported that *Fusarium* spp. and *Penicillium* spp. are superficial wood colonizing fungi; which first became noticeable as green, yellow, brown, or black, fuzzy or powdery surface growths on the wood surface (Kaarik, 1980). The species *T. harzianum* was reported as responsible for primary staining and superficial discoloration of wood which becomes bluish and blacken (Colling, 2002; Smith *et al*., 1979); the species is also known to be responsible for weight loss of holocellulose in intermediate and well decayed wood in Japan (Yu *et al.,* 2011). On open pored hardwoods however, these molds may cause stains too deep to be easily removed. However, molds do not reduce the wood strength, but they can increase the capacity of wood to absorb moisture, thus increasing the potential of attack by decay fungi (Zabel and Morrell, 2012). Wood discoloration/staining observed in *P. elata* could be due to these fungi. Further studies should be conducted to confirm or deny their responsibility for the coloring of *P. elata* wood. The high moisture conditions in the forest where samples were collected (humid bimodal rainfall forest zone) could also favor wood colonization by these molds. Indeed, it is clearly established that moisture promotes mold growth (Johansson, 2012). However, the species belonging to the mold groups do not participate in wood degradation, but their presence on the wood surface either accelerates wood decomposition or creates favorable conditions for wood degradation fungi (Colling, 2002; Marthur and Olga, 2003). Given the that some of these molds produce mycotoxins on foodstuff (Colling, 2002; Okigbo and Osuinde, 2003; Abras *et al*., 2008; Djeugap *et al.,* 2017b), it would be necessary to test their capacity to secrete toxic metabolites in *P. elata* wood in order to take appropriate biosafety measures by employees during logging and wood processing. The species *Colletotrichum* sp and *Cercospora* sp are generally found on tree leaves where they cause anthracnosis and leaf spot respectively (Douglas, 2009). Some species of the genus *Phomopsis* sp have been shown to be directly involved in wood decay. *Phomopsis* sp was reported as wood decay fungus on the trunk of almond trees in Spain (Gramaje *et al*., 2012) and as pathogenic on almond trees in Portugal (Diogo *et al.,* 2010). Moreover, Abras *et al*., (2008) show that *P. salicina* provokes necrosis and gray, brown circular necrosis which favors wood rot in willows in Wallonia. Therefore, *Phomospis salicina* could be associated with heartwood decay both in standing and felled trees of *P. elata.*

Macromycetes *Inonotus* sp and *Ganoderma* sp. identified on the trunks of *P. elata* had been reported in previous studies as true wood decomposition Basidiomycetes. Indeed, these wood decay fungi have a set of degradation enzymes (oxidases, cellulases) which degrade lignin and

cellulose. The species *Inonotus rickii*, *I. hispidus* and *I. dryophilus* were reported as the causal agents of canker, wood decay and white rot in various tropical hardwoods (Binion *et al.*, 2008). It was the same with *Ganoderma applanatum*, *G. lucidum* causing white stem rot and root rot in *Acacia confusa* and *Ficus macrocarpa* in China (Melo *et al.*, 2002; Dai *et al.*, 2007). However, the rate of decomposition of these polysaccharides varies widely, depending on the fungal species and environmental conditions within the wood (Abras *et al.*, 2008). It is the first time that these fungal species are reported on *P. elata* wood.

The study also shows that heartwood decay causes wood losses of 3755.96 cm^3 in *P. elata*. This volume is high for only 30 trees sampled in the Annualcutblock (ACB 4-1) where the study was carried out. It was observed that heartwood decay in *P. elata* increases with increase in diameter class. This could be explained by the fact that the tree loses its ability to defend itself naturally against wood decay fungi with age. Although the tree has various natural defense mechanisms during active growth such as anatomical barriers, biochemical molecules (phytoanticipin and phytoalexin) and compartmentalization of decay in trees (Shigo and Tippett, 1981, Pearce, 1996), it loses the ability to produce defense tools with age and becomes vulnerable to wood decay fungi. It would therefore be wise to extend the study to all other ACB of the SEFAC group forest concessions (10-008, 10-009, 10-012, 10-064) in order to have complete estimates of the wood loss volume resulting from heartwood decay fungi in the concessions.

Heartwood decay has negative impacts on timber yield of the species. On the other hand, it was noted that individuals belonging to the diameter class] 110-120] recorded the highest wood loss volume and these losses gradually decreased as the diameter class decreased to]90-100]. This variation shows that wood decay increases with senescence of the tree. It also suggests reduction of the minimum exploitable diameter (MED) of *P. elata* by the Cameroon government to 90 cm. Indeed, Bourland *et al.* (2012) raise the ambiguity between MED of the species, tree senescence and heartwood decay. From this point of view, it would be preferable to harvest wood with a low MED and a high volume of healthy wood than an aging wood (with high MED) with low volume of healthy wood.

Conclusion

The objective of this work was to contribute to the improvement of the management of *P. elata* through the identification of mycetes associated with heartwood decay of the species. In Cameroon forests, *P. elata* is colonized both by micro and macromycetes which are responsible for a total wood loss volume of 3755.96 cm^3 in individuals within a diameter class ranging from 110 to 120 cm. The volume of decayed wood increases with increase in diameter class. Micromycetes frequently associated with the disease were *Cercospora* sp, *Fusarium oxysporum* and *Penicillium* sp for standing trees and *Aspergillus niger* and *Cercospora* sp for felled trees. Wood discoloration fungi identified were *Phomopsis salicina* and *Trichoderma harzianum* while wood decay fungi were *Inonotus*sp and *Ganoderma* sp. It is necessary to complete their identification through the use of molecular biology tools.

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