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RESEARCH ARTICLE

FUNGI ASSOCIATED TO *TERMINALIA SUPERBA*, *TERMINALIA IVORENSIS* AND *TECTONA GRANDIS* DIEBACK IN IROBO AND BOUAFLÉ FORESTS IN CÔTE D'IVOIRE

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ABSTRACT

The dieback of reforested plantations is a pathology with various biotic and /or abiotic causes. In Cote d'Ivoire, this pathology affects *Tectona grandis*, *Terminalia ivorensis* and *Terminalia superba* in the reforestation plantation. The involvement of macroscopic and microscopic fungi has been studied to investigate the causes of the decline of these forest species. Assessments of land in classified forests of Irobo and Bouaflé and sampling of carpophores, roots, stems and soil were done. The fungi were isolated on standard Potato Dextrose Agar (PDA) and specific P5PARH media. Our results showed that the decline is more pronounced in the classified forest of Irobo (78.51%) than that of Bouafle and also showed a biotic kind of fungal disease. Various fungi have been identified, including pathogens (*Fusarium*, *Cylindrocarpon*, *Verticillium*, *Cladosporium*, *Sclerotinia*, *Botryodiplodia*, *Pestalotia*, *Phytophthora*, *Phoma*), parasites of weakness (*Aspergillus*, *Penicillium* and *Fusarium*) and hyperparasites (*Trichoderma*). The fungi differ from one site to another and between types of samples. Fungal species by their nature actively participate in this complex phenomenon feared for these reforestation silvicultural consequences.

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INTRODUCTION

Forest dieback is a recurrent disease in forest plantations in Africa, Europe and Asia. It is a process involving multiple causes acting in synergy (Nageleisen, 2006). It is perceptible by the loss of vitality of the trees and by the death of twigs and branches. Following reduction of branching and leaf mass, the crown becomes sparse. The ultimate stage of withering, but not obligatory, is the death of the tree. The death of trees leads to huge losses for forestry and timber exporting countries. In Côte d'Ivoire, teak (*Tectona grandis*) and *Terminalia* sp. constitute the bulk of the timber resources of the Forestry Development Corporation (SODEFOR). These species cover about 60 percent of the planted area and provide SODEFOR with over 70 percent of its income (ITTO, 2006).

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In recent years, a phenomenon of dieback of these species is observed, but the causes of this pathology remain difficult to define. The first one's symptoms of *Terminalia* dieback were observed in a plot aged 20 years in the Banco forest (Brunk and Malagnoux, 1976). This disease is characterized by yellowing of the leaves, followed by their falls. The drying of the tree is observed more later. In teak, dieback affects on average in young and adults 1 to 25 p.c trees and in plantations over 30 years 50 to 80 p.c trees (CNRA, 2005). Given the importance of these species in the Ivorian economy, the search for the causes of withering is essential in order to find adequate solutions. Different works were done to explain the decline of these species. Concerning teak, the work carried out by the CNRA involved mushrooms of the genus Fomes spp and *Verticillium* spp. These pathogens appear in the roots of most of the diseased teak, with high frequencies (79 to 98% of cases). The studies of Bernard Reversat (1976) on *T. superba* and *T. ivorensis* as well as those of Didier de Saint Armand (1979) showed that dieback was related to nutritional

disturbances. According to the model of the withering mechanism proposed by Abendi (2003), fungi are an incentive for tree dieback. For example, in Ecuador, the tropical Rostraureum mushroom (Diaporthales) has been associated with *Terminalia ivorensis* dieback (Gryzenhout *et al*. 2005). However, the involvement of these pests in this pathology remains little explored. The purpose of this work is to contribute to the research of fungi associated with dieback of these tree species. More specifically, it will be to:

- evaluate the decline rates in the Irobo and Bouaflé classified forests,
- isolate and characterize mushrooms from bark, roots and soil by different isolation techniques and,
- compare the diversity of fungi between the two forests.

MATERIALS AND METHODS

Study sites

Irobo classified forest: The Irobo classified forest is in the Sikensi sub-prefecture 90 km north-west of the city of Abidjan between 5 ° 25 and 5 ° 48 north latitude and 4 ° 40 and 4 ° 50 of west longitude (Fig 1.). It covers an area of 41250 ha (SODEFOR, 1996). The relief is very little rugged with many small hills of slopes between 5 p.c. and 10 p.c. The Irobo classified forest is subject to an Attiean climate regime forest with 4 modalities: a big rainy season (April to June), a small dry season (July to August), a small rainy season (September to October) and a long dry season (November to March). The average annual rainfall is 1284.3 mm of rain (SODEFOR, 2002). Soils in Irobo are shallow and chemically poor (Bernhard-Reversat, 1976). Three large soil families are found in Irobo: alluvial soils, soils resulting from the degradation of granitic rocks and soils derived from shale.

Bouaflé classified forest: It is located 320 km from the city of Abidjan between the departments of Daloa and Bouaflé (Fig. 1). The Bouaflé classified forest covers an area of 20,350 ha. It lies between 6 ° 50 N latitude and 6 ° 10 W longitude. The climate is Guinean subequatorial type. The average annual rainfall is about 1107.2 mm of rain (SODEFOR, 2002). The average annual temperature is 26.5°C. Granite layers are observed at more than 300 meters high. In the lowest, a thin alluvial layer of fine texture comes to cover the sands.

Evaluation of the dieback rate: At both sites, plots were set up to determine the rate of tree decline in each forest. This consisted of delimiting shape surfaces square or rectangular then count the number of trees (Fraké and Framiré) healthy as withered. Thus, 3 plots of 400 m^2 each have been set up.

Prospecting and sampling: The observation in the forest focused on the trees of *T. ivorensis* or Framiré, *T. superba* or Fraké and *Tectona grandis* or Teck. Samples of bark, roots and soil (at the rhizosphere depth of 20 cm) were collected from healthy and withered trees of Framire and Fraké. For teak, only withered and healthy tree bark samples were collected. These different samples were used for isolation in the laboratory. The carpophores on the trunks of the *Terminalia* sp trees were also sampled. Bark removal was done with a sterile machete. Nicks have been made on the trunks of healthy and withered trees. These cuts were made 1.5 m above ground level. The bark collected was bagged and then on each bag were given the indications relating to the sampled tree (forest of sampling, state). To remove the roots, the soil was cleared near the foothills and then of a clean machete, samples were taken and then bagged as in the case of barks. The soil was dug to a depth of 20 cm near the roots of withered trees and healthy with a chisel. Samples taken were labeled indicating the name of the the tree and its situation.

Microbiological analyzes: Microbiological analyzes consisted of isolating fungi from different root, bark and soil samples on culture media of different composition based on their nutrient requirements.

Isolation from bark and roots of *Tectona grandis* and *Terminalia* spp: Potato Dextrose Agar (PDA) was used for the isolation of fungi from root and bark samples. Bark and root samples were washed under running water and then wiped on sterile blotting paper. Then they were cut into small pieces or explants. The explants were disinfected with sodium hypochlorite (4%.) for 10 min and then rinsed twice with sterile distilled water for the same duration (5 min by rinsing). They were dried on sterile blotting paper. Using sterilized forceps, under the hood and near the flame, the explants were inoculated into Petri dishes containing PDA media at a rate of 5 explants per box. Inoculated boxes were sealed and incubated in a room culture. The temperature and photoperiod were 27 ± 2 ° C and 12 hours, respectively. Two to 3 days after seeding, purifications were made on new media to isolate fungal colonies. Repetition of the explants of each sample in 4 Petri dishes allowed to calculate the isolation rates of each fungus by sample.

Study of the telluric mycoflore of *Terminalia* sp: PDA and P5ARPH culture media were used for the isolation of soil fungi. P5ARPH medium is a selective medium that is used to isolate *Phytophthora* or *Pythium*. The suspension-dilution method was used for the isolation of soil fungi. 25 g of each soil sample was put into an Erlenmeyer flask and then made up to 250 ml with water agar. The resulting solution (S1) was stirred for 30 min on a magnetic stirrer. A volume of 10 mL of this solution S1 was removed using a sterile pipette, made up to 100 mL with water agar and then stirred for 20 min. A volume of 1 mL of this latter solution was taken and then deposited on the P5ARPH and PDA media. Using a pasteur pipettor bent, the suspension was spread on the culture media. Petri dishes were incubated in a culture room at a temperature of 27 ± 2 ° C. After 2 to 3 days of incubation, the colonies (fungal species) appeared were counted and the purifications were made to isolate the fungal colonies. The number of colonies formed (colony forming unit or CFU) is expressed for each gram of soil in CFU / g of soil considering the dilutions.

Identification of fungi: The identification of fungi was made from macroscopic and microscopic observations. At the macroscopic level, the description of the strains was made based on the color and appearance of the thallus in a petri dish. Microscopic observations have consisted of a description of the mycelium and conidia according to their aspects and according to whether the mycelium is partitioned or not. The strains were identified using a key of "Illustrated Genera of Fungi" determination (Barnett and Barry, 1960).

Statistical Analyzes: The data obtained was analyzed using the Statistica 7.1 software. The test of chi-2 was used to compare fungi isolation and dieback rates.

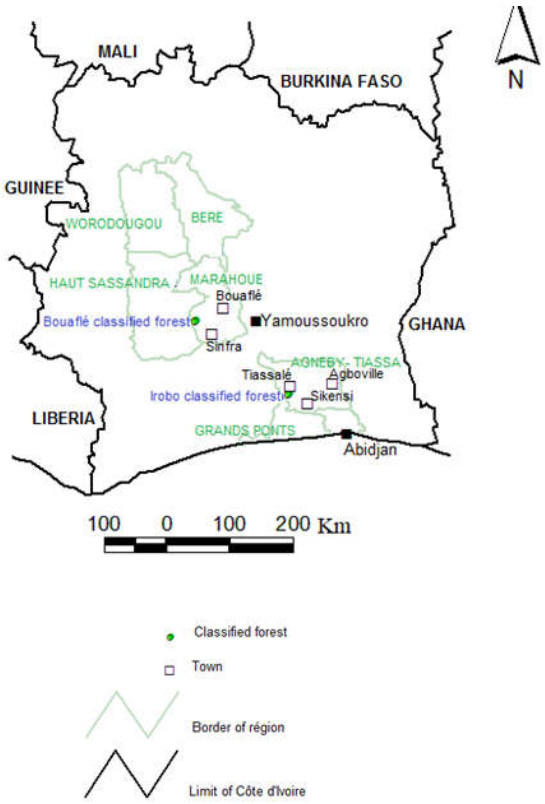

Fig 1. Map of Côte d'Ivoire showing Irobo and Bouaflé classified forests

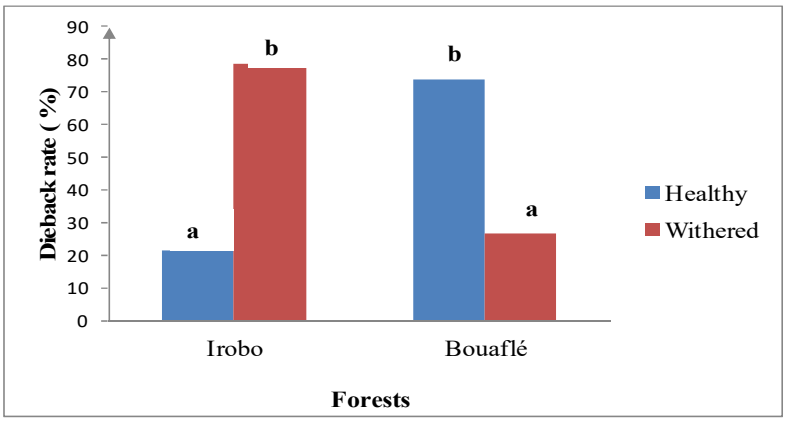

Fig 2. Rate of dieback of species according to classified forests Bars surmounted by different letters are significantly different at the threshold of 5% (Khi- 2; $\chi^2 = 3.84$).

Fig 3. Carpophores of upper fungi collected on *Terminalia* sp A : *Phellinus* sp ; B : *Ganoderna* sp

After ANOVA I, the Newman-Keuls comparison test at the 5-point threshold was used to compare the average number of fungal colonies in soils under trees healthy and that obtained in the soils below the withered trees. The fungi obtained made it possible to compare the fungal diversity between the 2 sites. This study was based on a list of strains isolated from stem, root and soil (healthy and withered) by species (teak, Fraké, Framiré) and by site. The presence of a species in a site is noted + and - if the species is absent. From Ecological Software Methodology version 6.1 (Krebs, 2002), the Sorensen index was calculated for each species and for the sites. This index makes it possible to compare the similarity or not of fungal communities. If Cs are greater than 50, then the fungal communities are similar. If Cs is less than 50 p.c., fungal communities are not similar.

RESULTS

Dieback rate in Bouaflé and Irobo classified forest: The evaluation of the dieback of *T. superba* and *T. ivorensis* in the Irobo and Bouaflé classified forests shows the effective presence of the disease with a variable and more marked intensity at Irobo (Fig 2.). The die-back rate varies from 26.42% (Bouaflé Classified Forest) to 78.51% (classified forest Irobo). The differences observed are statistically significant at the 5% threshold (chi-square test, $\chi^2 = 3.84$). Macroscopic aspects of the superior fungi of the trunks of *Terminalia* spp: The carpophores of mushrooms harvested on *Terminalia* belong to the genera *Ganoderma* and *Phellinus* (Fig 3.). *Phellinus* sp (Fig 3A.) shows a brown-colored carpophore less than 5 mm thick with irregular contours. *Ganoderma* sp (Fig 3B.) shows red-brown spheres, thicker than 5 mm and the underside has a smooth appearance while the upper side has concentric half-circles. The point of insertion of *Ganoderma* sp is clearly more visible whereas in *Phellinus*, the whole basal part of the carpophore is inserted into the tree.

Frequency of isolation of fungi on teak trunks: The isolation of fungi on teak in both forests revealed that the genus *Aspergillus* was the most recovered. In the Irobo classified forest, twelve strains were isolated. These strains belong to 3 identified genera and 4 other unidentified fungi, one to sterile mycelium and the other 3 to spores called strain A, strain B and oomycete. Isolation rates ranged from 1 to 12% A brown-colored, shallow-colored strain A isolated at 12%. in withered trees was absent in healthy plants. The *Aspergillus* and *Trichoderma* genera were present in healthy trees as decay (Table I). In the Bouaflé forest, seven strains were isolated. The isolation rates varied from 1 to 27.08 pp. In addition to *Verticillium* sp. (17%), *Cylindrocarpon* (2%), strain A from brown color was present at a rate of 12% only in withered trees (Table 1).

Frequency of isolation of fungi on the trunks and roots of *Terminalia ivorensis*: On *T. ivorensis*, the genus *Aspergillus* was the most isolated on both withered and healthy trees in both forests. In the Irobo forest, ten strains were isolated from roots and trunks including a sphaeropsidale and an unidentified sterile mycelium fungus. The genus *Aspergillus* represents 45.45% of isolated strains. Isolation rates ranged from 1 to 62%. On the roots, the genus *Fusarium* sp. (5%) and the sphaeropsidales strain (2%) were found only in withered trees (Table 2). On the trunk, *Aspergillus* and *Trichoderma* genera were more common with more diversity of *Aspergillus* fungi in deciduous trees and high *Trichoderma*. In the Bouaflé classified forest, ten strains were isolated from roots and trunks. The isolation rates ranged from 1 to 61.67 pp. Among the inventoried fungi, the *Aspergillus* genus represents 40 % On the roots, *Aspergillus* and *Trichoderma* are present in healthy trees as well as in withered trees. On trunks, in addition to the genera *Aspergillus* and *Trichoderma*, and a sterile mycelium fungus were found only in withered trees (Table 2).

Frequency of isolation of fungi on the trunks and roots of *Terminalia superb*: In Irobo, thirteen strains belonging to 5 identified and 3 unidentified genera were isolated. Rates ranged from 1 to 31%. On the roots, in addition to the *Aspergillus* and *Trichoderma* genera isolated from healthy trees such as withered trees, the presence of *Fusarium* sp. (6%), *Penicillium* (2%) and Sphaeropsidale 2 (5%) only in the withered (Table 3). On the trunks, isolation rates ranged from 1 to 35% and were higher for *Aspergillus* and *Trichoderma*. The genus *Pestalotia* and sterile mycelium fungi were isolated from the withered trees at relatively low frequencies (1.67 to 6.67%). In the Bouaflé classified forest, 13 strains were found on the roots and stems of Fraké. Rates ranged from 0 to 28.33 pp. *Aspergillus* and *Trichoderma* genera were found in both healthy and deciduous trees. On the roots, the strains of *Fusarium* sp and unidentified strain 1 isolated at 20 and 8.33% respectively were found only in the withered trees. On the trunks, *Pestalotia* and a sterile mycelium fungus respectively isolated at 10 and 3.33% were present in withered trees (Table 3). Thus, *Pestalotia* is observed in Irobo as Bouaflé on the roots and stems of *Terminalia superba*.

Telluric mycoflora of *Terminalia* sp

Fungi encountered in the rhizosphere of *Terminalia* sp: Several genera of fungi have been isolated from the *Terminalia* rhizosphere. These are *Acremonium* sp, *Cylindrocarpon* sp, *Sclerotinia* sp, *Curvularia* sp, *Phoma* sp, *Diplodia* sp, *Phytophthora* sp, *Fusarium*, *Penicillium* and 6 strains of the order Sphaeropsidales (Fig 4 & 5.).

- **Number of fungal colonies on PDA and PARPH media:** In general, the number of fungal colonies of soils under healthy trees is high compared to that of fungal colonies of soils under withered species in both types of forest (Irobo and Bouaflé). However, in the Bouaflé forest, this number is high in PDA medium in the withered Framiré and in PARPH medium in the Fraké withered compared to healthy trees (Fig 6.).
- **2-7- Comparison of fungal diversity of fungi Comparison of fungi between soil samples and roots and stems of sites:** The results showed low values for similarity indices between soil and root and stem samples (Ss <0.5). These indices ranged from 0.105 to 0.207 for the Framiré and 0.330 and 0.333 for the Fraké respectively in Irobo and Bouaflé (Table 4.). This reflects a difference in the fungi found in the soil and those on the roots and stems.
- **Comparison of fungi between sites:** The similarity indices obtained were less than 0.5 except for the roots and stems (Table 5). At ground level, these indices were 0.080 for Framiré and 0.333 for Fraké. For roots and stems, they were 0.526 for Framiré, 0.385 for Fraké and 0.353 for teak. These results show that the soil fungi in the Irobo and Bouaflé forests are not similar, as are those in the roots and stems.

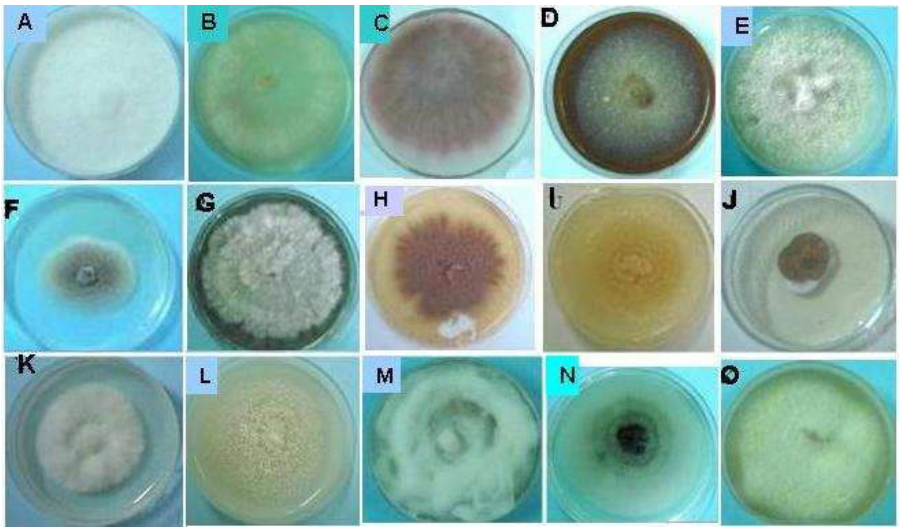

Fig 4. Isolated fungal colonies in the *Terminalia superba* rhizosphere by the suspension-dilution method A: *Phytophthora* sp.; B, C, D, E: *Fusarium* sp.; F: *Phoma* sp.; G: *Acremonium* sp.; H, I: *Penicillium* sp; J: *Diplodia* sp.; K, L, M, N: Fungi with sterile mycelium; O: *Cylindrocarpon* sp.

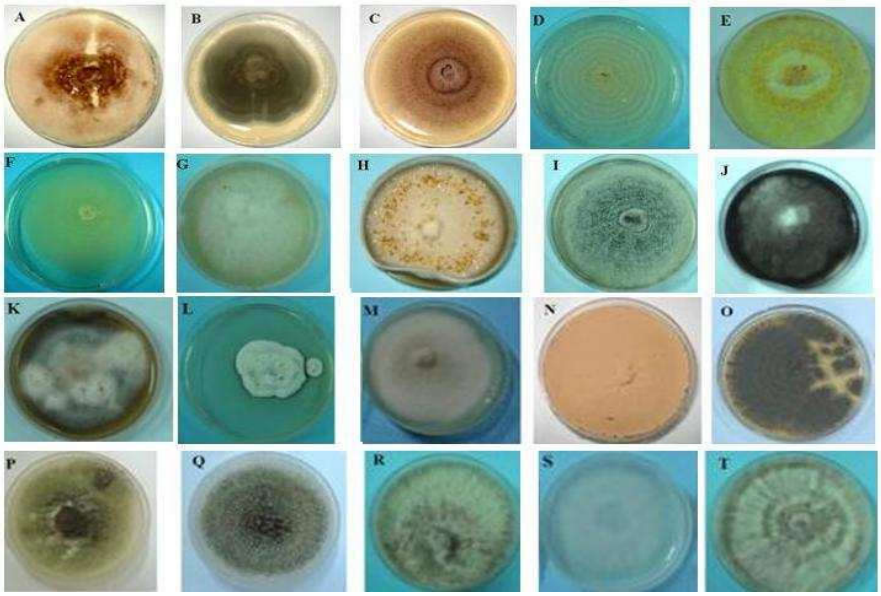

Fig 5. Isolated fungal colonies in the rhizosphere of *Terminalia ivorensis* by the suspension-dilution method A, B, C D et E: Sphaeropsidale; F, G, H: *Fusarium* sp; I: *Verticillium* sp; J: *Curvularia* sp. K: *Acremonium*; L : Non Identified fungi; M : *Phytophthora* sp. ; N, O, P, Q : *Penicillium* sp. ; R, S, T : Fungi with sterile mycelium.

Table 1. Relative frequencies of isolated fungi on *Tectona* trunks grown in the classified forests of Irobo and Bouaflé

Fungi	Tree trunks in Irobo		Tree trunks in Bouaflé	
	Healthy	Withered	Healthy	Withered
Aspergillus sp A	0 a	2 a	-	-
Aspergillus sp B	5 a	11 a	2,08 a	1 a
Aspergillus sp C	0 a	10 b	-	-
Aspergillus sp D	1 a	2 a	-	-
Aspergillus sp E	6 a	1 a	-	-
Trichoderma sp	2 a	7 a	10,41 a	3 b
Penicillium sp A	2 a	3 a	-	-
Penicillium sp B	0 a	1 a	-	-
Non identified strain A	0 a	12 b	0 a	12 b
Non identified strain B	0 a	2 a	2,08 a	0 a
Non identified strain C	-	-	27,08 a	0 a
Non identified Oomycete	1 a	0 a	-	-
Sterile Mycélium	0 a	1 a	-	-
Verticillium sp	-	-	0 a	17 b
Cylindrocarpon sp	-	-	0 a	2 a

On a same line, the letters a and b indicate the differences between the isolation rates of healthy and withered trees, Not isolated (-)

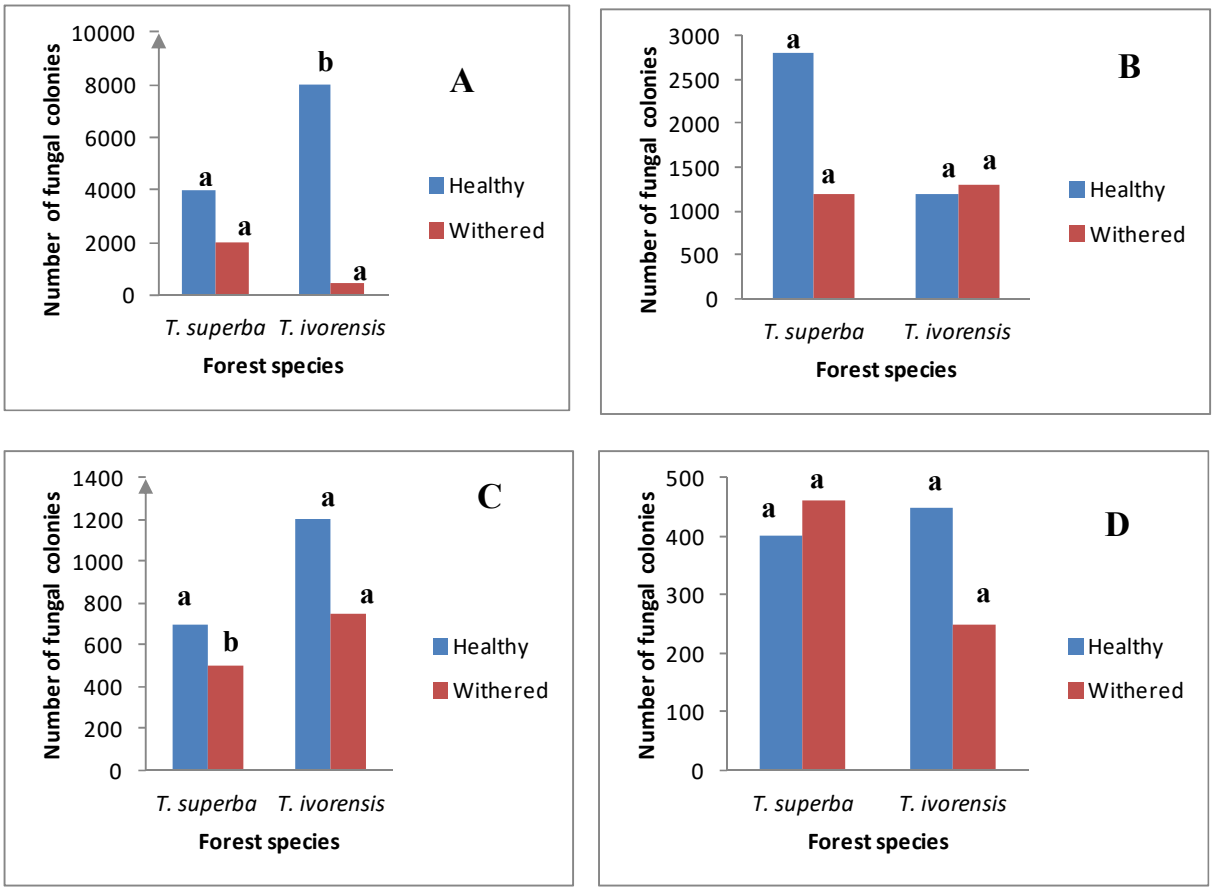

Fig 6. Number of fungal colonies on PDA (A and B) and PARPH (C and D) media in the soils of the *T. superba* rhizosphere and *T. ivorensis* of the Irobo (A and C) and Bouaflé (B and D).

DISCUSSION

The study of the involvement of pests in the decline of trees has made it possible to highlight the variability of this pathology which was more severe in Irobo compared to Bouaflé. Several factors could explain this difference, among others, edaphic and climatic factors. Indeed, the spatiotemporal distribution as well as the proliferation of the diseases are influenced by the temperature, the light and the humidity. The two forests are in different ecological zones where the rainfall conditions are not the same. Microbiological analyzes focused on macroscopic and microscopic fungi. At the macroscopic level, the fungi encountered belong to the genus *Ganoderma* sp and *Phellinus* sp. These genera are responsible for significant losses of forest species. This is the case of *Ganoderma boninense*, which is responsible for oil palm stem rot in Papua New Guinea (Pilotti, 2005). In Pakistan, perennial crop diseases caused by *Ganoderma* species have also been reported. (Nasreen, 2003). These genera are at their first observations in Côte d'Ivoire on the species of *Terminalia* sp. At the microscopic level, several fungi of the genus *Aspergillus*, *Trichoderma* and *Penicillium* have been isolated on teak. We also note the presence of the unidentified strain A with a rate of 12 % and *Verticillium* sp with a rate of 17% on rotten trunks. Strain A and *Verticillium* sp could be the main agents of infection. Strains of *Aspergillus* and *Penicillium* may be secondary parasites or weakness that will aggravate the disease. The presence of *Verticillium* sp was reported by Wahonou (CNRA; 2005), which reported attacks of teak against the roots caused by this fungus and resulting in the death of certain trees in association with *Fomes*.

In the Irobo forest, 8 fungal genera were found on the roots and trunks of *T. superba* including *Fusarium*, *Penicillium*, *Pestalotia* and sphaeropsidale only on withered trees. On *T. ivorensis*, there are four genera including *Fusarium* at the level of withered roots. In Bouaflé, the presence of a sterile mycelium fungus and an undetermined strain only on the roots and trunks of the withered Framiré was observed. On the Fraké, the presence of 12 strains including *Fusarium*, *Pestalotia* was noted. The isolation of *Fusarium* and *Pestalotia* corroborates the work of Panconesi *et al*. (1999) on declining cypress trees where these fungi were also isolated. The presence of these fungi on teak, Framiré and Fraké could be explained by their ability to use the simple carbohydrates of wood and by their ability to secrete certain enzymes able to hydrolyze the wood polymers that are lignin, cellulose, hemicellulose. During their growth, these fungi secrete hydrolytic enzymes for their follow-up. It was demonstrating the production of these enzymes in *Cylindrocarpon*, *Phoma*, *Ganoderma australe*, *Phellinus* sp, *Trichoderma virens* on the species of *Ocotea* sp (Nsolomo *et al*., 2000). The presence of ligninolytic enzymes (laccase and tyrosinase) in these fungi facilitates the decomposition of lignin. Thus, by their lignivorous activity, these organisms are also capable of degrading cellulose and are considered as white rot fungi (Blanchette, 1995). At ground level, microbiological analysis revealed the presence of a high number of fungal colonies in soils below healthy *Terminalia* species compared to withered trees on both growing media. This would demonstrate significant microbial activity of parasites under healthy trees compared to diseased trees.

Table 2. Inventoried fungi on healthy and withered roots and trunks of *Terminalia ivorensis* and their relative isolation frequencies in the Irobo and Bouaflé classified forests

Fungi	Irobo				Bouaflé			
	Roots		Trunks		Roots		Trunks	
	healthy	Withered	healthy	Withered	healthy	Withered	healthy	Withered
Aspergillus sp A	12 a	0 b	0 a	12,5 b	-	-	0 a	5 b
Aspergillus sp B	15 a	0 b	36 a	2,5 b	16,67 a	31,37 a	28,33 a	17 a
Aspergillus sp C	62 a	8 b	0 a	2,5 a	23,33 a	5 b	10 a	34 b
Aspergillus sp D	9 a	2 b	0 a	3 a	-	-	-	-
Aspergillus sp E	4 a	1 a	-	-	-	-	-	-
Aspergillus sp F	-	-	-	-	61,67 a	50 a	21,67 a	0 b
Trichoderma sp A	2 a	13 b	10 a	30 b	1,67 a	0 a	15 a	23 a
Trichoderma sp B	-	-	3 a	2,5 a	0 a	3,33 a	-	-
Sterile mycelium	-	-	6 a	0 b	-	-	0 a	1 a
Sphaeropsidale A	0 a	2 a	-	-	-	-	-	-
Fusarium sp	0 a	5 b	-	-	-	-	-	-
Non identified strain 1	-	-	-	-	-	-	0 a	1 a
Botryodiplodia sp	-	-	-	-	-	-	3,33 a	0 a
Penicillium sp	-	-	-	-	-	-	0 a	1 a

On a same line, the letters a and b indicate the differences between the isolation rates of healthy and withered trees

Table 3. Inventoried fungi on healthy and withered of roots and trunks and *Terminalia superba* and their relative isolation frequencies in the Irobo and Bouaflé classified forest

Fungi	Irobo				Bouaflé			
	Roots		Trunks		Roots		Trunks	
	Healthy	Withered	Healthy	Withered	Healthy	Withered	Healthy	Withered
Aspergillus sp. A	2 a	4 a	4 a	0 a				
Aspergillus sp. B	0 a	7 b	31 a	35 b	0 a	1,67 a	6,67 a	20 b
Aspergillus sp. C	19 a	11 a	16 a	1.67 b	-	-	1,67 a	11,67 b
Aspergillus sp. E	0 a	1 a	-	-				
Aspergillus sp. F	-	-	-	-	1,67 a	0 a	-	-
Penicillium sp	0 a	2 a	-	-				
Trichoderma sp. A	0 a	1 a	5 a	10 b	1,67 a	21,67 b	11,67 a	8,33 a
Trichoderma sp. B	1 a	4 a	2 a	0 a	0 a	3,33 a	1,67 a	5 a
Sphaeropsidale 1	31 a	7 b	-	-	-	-	-	-
Sphaeropsidale 2	0 a	5 b	-	-				
Fusarium sp. A	0 a	6 b	-	-	0 a	8,33 b	-	-
Fusarium sp. B	-	-	-	-	1,67 a	0 a	-	-
Pestalotia sp.	-	-	0 a	1,67 a	-	-	0 a	10 b
Sterile mycélium A	-	-	0 a	1,67 a	20 a	0 b	-	-
Sterile mycélium B	-	-	0 a	6,67 b	-	-	0 a	3,33 b
Non identified strain 1	-	-	-	-	0 a	20 b	-	-
Non identified strain 2	-	-	-	-	28,33 a	0 b	-	-
Acremonium sp	-	-	-	-	-	-	1,67 a	0 a

On a same line, the letters a and b indicate the differences between the isolation rates of healthy and withered trees

It has been shown that under stress, withered trees accumulate certain antifungal compounds such as phytoalexins, phenolic compounds that diffuse into the soil and may have antifungal activity (Puch-Ceh *et al*., 2005).

Table 4. Similarity indices of fungi between soil and root and stem samples of species based on sites

Forests	*T. ivorensis*	*T. superba*
Irobo	0,105	0,303
Bouaflé	0,207	0,333

Table 5. Fungus Similarity Indices between Sites Based on Soil and Root / Stem Samples

Species	*T. ivorensis*	*T. superba*	*T. grandis*
Soil	0,080	0,333	-
Roots and Stems	0,526	0,385	0,353

The species found in soils under healthy trees are dominated by the genera *Penicillium*, *Aspergillus* and *Trichoderma*. The action of these biological control agents in the soil will consist, on the one hand, in an increase in the density of propagules (Hadar *et al*., 1984) and, on the other hand, in a production of diffusible and toxic substances in soil (Papavizas, 1985).

These secreted substances will hinder the development of certain fungi recognized as pathogenic on forest tree species, including *Fusarium*, *Diplodia*, *Cylindrocarpon* and *Verticillium*. At ground level below the declining *Terminalia*, several genera have been isolated (*Fusarium*, *Cylindrocarpon*, *Verticillium*, *Diplodia*, *Acremonium*, *Phoma* and Sphaeropsidales fungi). These fungi have also been isolated on the trunks and branches of some *Quercus* species (Ragazzi *et al*., 2003). Sorensen similarity index values showed a difference in soil fungi, roots and stems. Indeed, all the fungi found in the soil do not attack the species. Several genera were isolated from soil samples. In addition to the genera *Trichoderma* and certain *Aspergillus* that are hyperparasites and therefore may be secondary colonizers, the genus *Fusarium* sp is found both in the soil and in the roots of *Terminalia*. Its pathogenic action has already been reported (Daami-Remadi and Mahjoub, 1996). Similarity indices between sites are also low except for Framiré roots and stems. The fungi met at Irobo were different from those of Bouaflé. The distribution of microorganisms by site is governed by several factors. At ground level, the physical and chemical characteristics of the soil are decisive factors in the distribution of microorganisms.

Briones *et al*. (1995) found that organic matter, pH, and exchangeable cations are important factors in establishing ecological differences in earthworms.

Conclusion

This study has shown that the decline of forest tree species is a phenomenon that can cause significant losses depending on ecological zones and tree species. Microbiological analysis of root, stem and soil samples revealed the presence of macromycetes and micromycetes. The macromycetes observed are lignivorous fungi of the class Basidiomycetes belonging to the genera *Ganoderma* sp and *Phellinus* sp. Several genera of micromycetes have been isolated. These fungi encountered are not similar in the ecological zones of Bouaflé and Irobo. Among these fungi are plant pathogens, parasites of weaknesses, saprophytes and hyperparasites.

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