

TEAK (*TECTONA GRANDIS*) DECAY ASSOCIATED WITH *VERTICILLIUM* SP. AND *FOMES* SP. WITHIN REFORESTATION AREAS IN COTE D'IVOIRE

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Teak (*Tectona grandis*) occupies a significant place for reforestation in Cote d'Ivoire. Recently, there has been a resurgence of withering in different forestry areas, which represents a serious threat to the profitability and survival of forest plantations. A study has been undertaken to resolve this problem. Plant samples were collected from the trunk (bark, sapwood and central cylinder) and the roots for fungal microflora analysis through *in vitro* cultures to identify the causative agent of the decay of healthy trees. Sixteen different fungal genera were selected, including two species responsible for teak disease: *Verticillium* sp. with 82% and 98% frequencies and *Fomes* sp. with 79% and 60% frequencies, respectively in the roots and trunks of the diseased teaks. The presence of fungi was recorded as *Verticillium* sp. in 30% of diseased trees, *Fomes* sp. in 7.5% and all species combined in 70.5% of the cases. The inoculation of young seedling teaks in semi-controlled environment with isolates of different species of fungi allowed the restoration of the infection over 73.3% of the plants with *Verticillium* sp. and 16.4% with *Fomes* sp. The study recorded, for the first time, *Verticillium* sp. responsible for teak decay in plantation and reforestation (semi deciduous dense forest environment) in Cote d'Ivoire. Very often the causes of teak and forest species decay associated with fungi are attributed by error to *Fomes* sp., ignoring a formidable decay agent in forestry, the *Verticillium* sp. The lack of knowledge and information on the real causality of teak decay, associated with fungi, could make fungicidal treatments, for control of wood tree diseases, difficult and unsuccessful.

Keywords: Fungal microflora, fungal identification, fungicidal, withering, tree disease

INTRODUCTION

In Cote d'Ivoire, six main tree species are cultivated in the reforestation environment by the Société de Développement des Forêts (SODEFOR). They are *Tectona grandis*, *Terminalia superba*, *Terminalia ivorensis*, *Cedrela odorata*, *Triplochiton scleroxylon* and *Gmelina arborea*. Among them, teak (*Tectona grandis*) occupies a significant place with 39.4% of reconstituted surface and 35% volume of wood sold each year. Originating from Asia, this sun-loving plant of semi deciduous dense forests was introduced to Cote d'Ivoire in 1926 (FAO 1957). With a strong vegetative growth and adaptability to various ecological zones, teak represents a remarkable asset in the fight against desertification and for the reforestation of depleted forests. Teak has long been known for its hardness (stable, durable, pest-resistant) and for its highly remarkable technological qualities. These are positive aspects that make the wood

noble and highly sought in many woodworks, led to its growth over wide areas in African Countries. In Cote d'Ivoire forestry research programs, teak has been long subjected to numerous studies in silviculture, genetic improvement and wood technology (Wencellius et al. 1975).

The reaction of plants against parasites and pests have been generally studied, but little attention has been paid to phytosanitary. In 1972, cases of grounded teak decay were observed, particularly in the forestry sector of Segouie. Resistance to fungi and insects is an important characteristic to be taken into account in the varietal trials conducted in Cote d'Ivoire. Diverse and large families of fungi, widely distributed throughout the world, have high diversity in tropics, existing on decayed wood (Lee et al. 2002, Koyani et al. 2016). Different genera of various fungal groups are associated with cultivated plants

and forestry species, such as teak (Nagadesi and Arya 2014). In parasite attacks on forest strains, the insect path is often been sought according to the agents (Attignon et al. 2005, Joshi 2006). In Malaysia, the first case of teak root decay caused by *F. noxius* was reported by Farid et al. (2005). Wencellis et al. (1975) reported *F. lignosus* attacks on many forest plantation strains in Segue Cote d'Ivoire, making reference to damages caused by insects. In Nigeria, dramatic withering cases in teak plantations (60–70% of decaying plots) due to *F. lignosus* attack has been reported (Momoh, 1993). Recently in Cote d'Ivoire, the resurgence of withering was experienced in different forestry areas. Due to the extent of damage, this phenomenon is a serious threat to the profitability and survival of forest plantations. Moreover, the causative agent is not sufficiently known, in order to seek appropriate control measures to safeguard the forestry sustainability. This study aims to determine the cause of grounded teak withering.

MATERIALS AND METHODS

Area of study

The study was conducted in a forest area, mid-southern part of Cote d'Ivoire, located above the equator, 6° 30' N, 5° 25' W, 100–250 m above sea level, on teak reforestation plots of SODEFOR in Segue, Agboville Tene, Oume and Bouafle. It is a degraded and semi-deciduous dense forest. The soil is ferralitic and averagely unsaturated with derivatives of granite bedrock. At indurations, it is gravel-like or top of slope sesquioxides, deep and less gritty, with chemically richer hillside and less advanced and poor in low slope. The hydromorphy is sometimes absent, but more permanent down the slope. The climate is subequatorial-like with two rainy seasons and two dry seasons. The average temperature is 27 °C, with a maximum of 32 °C and minimum of 25 °C, and a capacity of reaching 15 °C during harmattan.

Parasite detection in the field

In each area of study, a survey was conducted on the plot, considering four categories of teak based on their health status. The categories were: (1) trees apparently healthy with normal

foliage (2) sick trees at the stage of leaf symptoms (3) trees partially withered up with defoliation or partial drying up of the branches and (4) trees completely withered with total defoliation and dried up trunk and branches. The survey was conducted on teak plantations distributed in three reforestation centres namely Tene (20,550 ha), Segue (9,300 ha) and Bouafle (9,669 ha). The roots, trunk and leaves of the affected trees were observed. Initially the land was cleared to expose the roots, and when the disease was detected, cuttings were done on the bark, sapwood and central cylinder all around the trunk, about 40 cm from the ground. Plant samples were collected for each of the 4 types of trees from the trunk, bark, sapwood, central cylinder and roots at 0–20 cm pivot. The samples were labelled and transported to the phytopathology laboratory, Centre National de Recherche Agronomique (CNRA), Bimbresso, for mycological analysis.

In vitro tissue culture

Thirty pieces of root cuttings and stem cuttings were randomly selected from the samples issued from each of the affected teak category. They were washed in a bleach solution and blazed with benzene in a laminar flow hood. Tissue fragments of 1 cm² were cut using a sterilised scalpel. The fragments were washed in 100 ml sterile water and 250 ml of Erlenmeyer for 24 h, subjected to rotational movements. From the suspension, serial dilutions were prepared. A 1 ml aliquot of dilution (1/1000) was pipetted and introduced into Petri dishes containing Agar Potato Dextrose (APD) medium. The medium, composed of 200 g potato, 20 g dextrose, 20 g agar, 1 L distilled water and chloramphenicol (100 ml/L), was sterilised by autoclaving at 103 °C for 45 min. It was then cooled up to 45 °C and poured into Petri dishes. The medium, inoculated in Petri dishes with the fungal suspensions, were incubated for 1–2 weeks in 25 + 2 °C rooms for a photoperiod of 12 h. All fungal colonies obtained were separated and transplanted anew, with isolation of the identified spores using a binocular stereomicroscope. The frequency of fungi was determined by calculating the percentage of samples in which fungal colonies were recorded with regard to the number of samples analysed.

Artificial infection of young teaks

For the reconstitution of the infection, young teak plants were grown in concrete tanks (1.50 m × 0.60 m × 0.50 m) filled with sea sand, with 6 plants per tank. The seedlings were watered 2 times a day using a watering can. Mineral feeding was carried out using NKP fertilisers supplemented with nutrient solution trace elements (Co, Mn, Cu, B, Zn, I, Br, Mo, V, Cr, Be, Ni, Ag, Sn, Pb, As, Ba, Sr, Ti), twice at three month intervals during vegetative growth of seedlings. Young plants at 12 months of age were artificially inoculated with the 3 main fungal strains isolated from tissues fragments of the diseased trees. They were *F. lignosus*, *F. noxius* and *Verticillium* spp. The inoculation consisted in applying substrate fungal issued from the root tissue fragments and the sapwood of the diseased trees. The tissue fragments were applied and maintained by the installation of compresses, moistened with distilled water and secured with a tape. For confirmation of fungi, a simple laboratory technique commonly used in plant disease clinics, such as culture medium with streptomycin water agar, was applied and incubated for 4 days. Ten plants were subjected to repetition for each treatment. Observations on both roots and stems, with phytosanitary data, were conducted a year after the inoculation of the young teak plants.

The frequency (%) evaluation of parasites was done by calculating the ratio of infected trees per particular fungi over the number of plants per treatment, as per inoculation at both root and trunk level. Data was subjected to variance analysis, followed by SAS linear regression with average comparison using Newman-Keuls test.

RESULTS

Teaks dieback in the field

The easily noticeable symptoms of withering teaks were yellowing, wilting and foliaceous organ desiccation (Figure 1). At the first stage of the disease, the parasite entered through wounds around the neck or the roots (Figure 2). Attacked tissues underwent deep decay (Figure 3). The decaying of woody tissues increased along the axis of the rods to the edge of the plants. When the plants were young and less lignified, the degradation invaded the trunk, and the tree decayed faster. On lignified trees, rot extended laterally on the same side of the tree trunk, along the affected root. Trunk contamination of adult teaks resulted in the appearance of small dark dimpled areas that evolved into a vertical and unilateral necrotic band (Figure 4). The disease revealed itself on the pivot, deep into the ground, and moved into the strain of the teak (Figure 5).

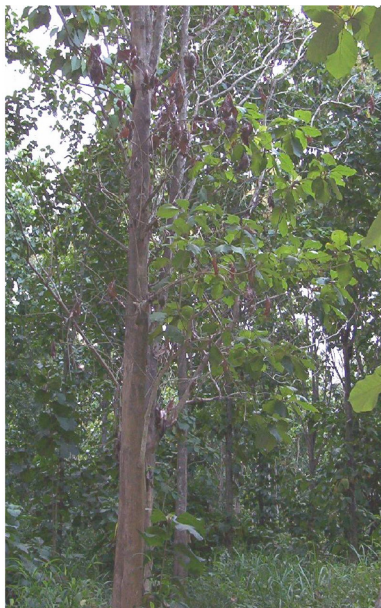


Figure 1 Foliar symptoms of dieback teaks



Figure 2 Roots of teaks at early stage of decomposition by rot attack



Figure 3 Extension of rot attack effects on roots and teak trunk

In the case of regeneration section (Figure 6), the infection reached the strains.

The consequences of the pathologic attacks spread up throughout the plant and led to the withering of the tree (Figure 7). At this stage, certain trees carried the germs, the fruiting bodies (carpophores) of *Fomes* sp. (Figure 8).

Determination of fungal microflora roots and trunks of teak

Sixteen different fungal species isolated from root fragments and trunks, cultured *in vitro*, as well as their frequencies on diseased or healthy trees are reported in Table 1. The occurrence frequencies of *Fomes* sp. and *Verticillium* sp. in the roots of the majority of diseased teaks were 79% and 60% for *Fomes* sp., and 82% and 98% for *Verticillium* sp., respectively in the roots and trunks of diseased teaks.

These fungal species rarely showed up in the organs of healthy trees, unlike other fungal species whose presence were found without impact on the teaks. Both fungi, *Fomes* sp. and *Verticillium* sp., were present in almost all diseased trees (Table 2), and was significantly associated to the pathological condition of teaks. In some cases, they separately induced phytopathological action leading to teak withering, represented

by 30% of *Verticillium* sp. and 7.5% of *Fomes* sp. However, they were not observed on 0.5% of decaying trees. A large number of fungi did not appear on diseased teaks. Some others, such as *Acremonium* sp., *Cunninghamella* sp., *Mucor* sp. and *Trichoderma* sp. occurred on healthy as well as diseased teak trees. Through a microscopic observation, *Fomes* sp. had a fronds morphogenesis characterised by a mycelia shape. The aggregation of hyphae, however, produced some typical rhizomorphs. In *Verticillium* sp. the presence of mycelia filaments, composed of thin colourless hyphae, were observed. The conidiophores had small isolated ovoid conidia at the edges. The spores viewed in bulk were tinted. Many microsclerotia, formed by small brown areola aggregates, were also observed. In culture medium, incubated after fungus growth, vascular tissues were examined microscopically. The proportions of fungi genera detected in different cross layers of teaks were variable, i.e. 56% shallow penetration in the bark by *Acremonium*, *Aspergillus*, *Cardona*, *Cunninghamella*, *Cylindrocladium*, *Humicola*, *Penicillium*, *Trichoderma* and sterile mycelium, 25% in the region of cambium by *Cladosporium*, *Fusarium*, *Mucor* and *Scytalidium* and 18% deep penetration in the sapwood by *Cylindrocarpon*, *Fomes* and *Verticillium*.



Figure 4 Diseased strain with infection on teak pivot



Figure 5 Extension of disease on infected teak strain



Figure 6 Formation of a necrotic band in the length of the diseased teak trunk



Figure 7 Completely dried and decayed tree



Figure 8 Occurrence of carpophores *Fomes lignosus* at the neck of the teak

Artificial infection of young teaks

The percentage of diseased teak trees according to type and state of health of the inoculated organ by three pathogenic species, *F. lignosus*, *F. noxius* and *Verticillium* sp., are shown in Table 2. The infection was carried out on 83.6%, 66.6% and 85.2% of the plants through *F. lignosus*, *F. noxius* and *Verticillium* sp., respectively in the case of inoculation at root level. It was absent in inoculation performed on the trunk. Withering symptoms, similar to those observed in the field (root attack and climbing necrosis on

the stem) were performed on 73.3% of plants, inoculated with *Verticillium* sp., against 16.4% of plants inoculated with *F. noxius*. However, the progression of necrotic front was faster, about two times, in *Verticillium* sp. compared with *F. noxius*. After one year, the necrosis band was about 15–20 cm long with *Verticillium* sp. against 5–10 cm with *F. noxius*. In both cases, the infection reached the sapwood on one side of the stem and the soft parts of young diseased trees, thus forcing them into sooner wilting from the age of 6 months. In the case of *F. lignosus*, there was no remarkable increase in necrosis of the stem, but rather a uniform drying of the plant after one year.

DISCUSSION

In this study, the phenomenon of teak decay occurred identically across all the surveyed reforestation sites. It included yellowing, wilting and leafy organs desiccation, leading to the widespread drying of the tree. Field investigations followed by laboratory analysis highlighted two major fungal pathogenic agents, i.e. *Fomes* sp. and *Verticillium* sp., responsible for teak decay, among several fungi identified. The decline of forest tree species in plantations and reforestation of terminalia and teak has been reported by many authors, indicating the causality of either fungi or insects (Arya et al. 2008, Lachat et al. 2006, Attignon et al. 2005, Swaminathan and Srinivasan 2006, Joshi et al. 2008, Wencellus et al. 1975). In Cote d'Ivoire forestry development, fungal attacks on teak have been attributed mainly to two root rot agents, namely *F. lignosus* and *F. noxius*, which are now the targets of phytosanitary treatment measures in forestry operations. The study highlighted *Fomes* sp. occurrence frequencies about 79% and 60%, respectively in the roots and trunks of diseased teaks.

For *Fomes* sp., phytosanitary plantation surveys were generally limited to macroscopic examinations on the presence of fruiting bodies (mycelium, rhizomorph and carpophores) which were easily recognisable with foliar symptoms. The rhizomorphs were flattened mycelia strands of 1–2 mm thick that grew firmly attached to the surface of infected roots. The rhizomorphs grew rapidly at the head of the rot and extended many meters through the soil, free from woody substrate. When roots of infected trees were exposed, profusely branched white rhizomorphs were readily seen. Attacks by *Fomes* sp. polyphage

Table 1 Frequency (%) of fungi identified in isolates from roots and teak trunks

Fungal species	Roots of the teak		Trunks of the teak	
	Healthy	Diseased	Healthy	Diseased
<i>Trichoderma</i>	7	26	2	10
<i>Penicillium</i>	3	22	0	30
<i>Fusarium</i>	4	36	1	38
<i>Cunninghamella</i>	12	25	4	7
<i>Scytalidium</i>	3	3	0	17
<i>Acremonium</i>	8	6	0	3
<i>Verticillium</i>	0	82	0	98
<i>Humicola</i>	1	2	2	5
<i>Fomes</i>	2	79	0	60
<i>Mucor</i>	11	6	1	0
<i>Cordona</i>	0	2	0	12
<i>Cylindrocarpon</i>	0	2	0	8
<i>Cylindrocladium</i>	1	1	0	6
<i>Aspergillus</i>	7	6	3	2
<i>Mycelium stérile</i>	-	20	2	13
<i>Cladosporium</i>	1	7	0	11
Others	2	5	5	6

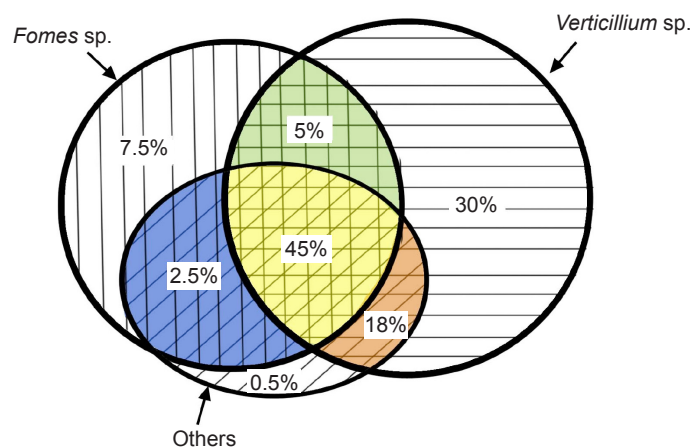


Figure 9 Diagram of fungi frequencies on sick teaks

Table 2 Percentage (%) of diseased teak plants depending on type and condition of the organ inoculated by three fungi species

Inoculated organ	Root			Trunk		
	A	B	C	A	B	C
Health status of the infected organ						
<i>Fomes lignosus</i>	83.6 a	0	0	0	0	0
<i>Fomes noxius</i>	66.6 b	0	16.4 b	0	0	0
<i>Verticillium sp.</i>	85,2 a	0	73,3 a	0	0	0

The values are averages of 6 repetitions; within a column, figures followed by the same letter are not significantly different, $p < 0.05$, according to Duncan test classification; A = roots infection, B = necrosis on trunk only, C = root infection and extension of necrotic band on the trunk

on many woody species were widely reported in the forest plantations of Cote d'Ivoire. The main reason being, *F. lignosus* and *F. noxiu* were economically important and appeared as major causes of rubber tree losses in plantation, causing the decay of lignified root tissues, degrading both the lignin and polysaccharide fractions of the wood by enzymes such as cellulose, pectinase and laccase (Lindsey and Gilbertson 1978, Nicole et al. 1982, Geiger et al. 1986, Geiger et al. 1989, Liyanage 1997, Lachat et al. 2006, Nagadesi and Arya 2013, Oghenekaro et al. 2015). Different fungi causing white rot in teak wood, mostly by *Lenzites stereoides*, *Lenzites* sp., *Ganoderma lucidu* and *Hexagonia apiaria*, showed delignification and different pattern of wood decay through microscopic observation, for the first time in India.

In Cote d'Ivoire, due to lack of knowledge and information on real causes of teak decay associated with fungi, the responsibility was commonly attributed to *Fomes* sp. Thus, the fungicidal treatments were unsuccessfully applied to the erroneous target, *Fomes* sp., ignoring a formidable decay agent in forestry represented by *Verticillium* sp. The occurrence frequencies of *Verticillium* sp. were 82% and 98%, respectively in the roots and trunks of diseased teaks. Berlangier and Powelson (2000) indicated that *Verticillium* sp., ascomycete of *Plectosphaerellaceae* family, is a tellurique fungus found in soil. It's symptoms varied among hosts and none were absolutely diagnostic. Premature foliar chlorosis, necrosis and vascular discoloration in stems and roots, however, were characteristics found in all hosts. Since fungal structures were not visible on most diseased specimens, confirmation of *Verticillium* sp. required the use of laboratory techniques. The pathogens persisted in soil for many years in the absence of a susceptible crop. Infection is through the roots, thus diagnosis and management of the disease is difficult. *Verticillium* fungus blocks the xylem vascular tissue of the host tree, causing a reduction of water and nutrients to the crown or foliage.

On withering teak trees observed during the surveys in the reforestation areas of Côte d'Ivoire, *Verticillium* sp. was noted in 98% of cases, with 30% in solo action. The symptoms were decaying and browning of the attacked root tissues along the extension on one side of the tree trunk. There was no particular structure

appearances, such as mycelium, rhizomorphes or carpophores reported in the case of *Fomes* sp. *Verticillium* sp., a mushroom from the whorled family, was extremely polypohyte with a highly phytopathological role in producing the wilting of attacked trees. The wilting of tree seedlings under the attack of *Verticillium* sp. were reported by Harsh et al. (1992). It usually entered through wounds in the neck and roots, and limited its extension to woody tissues. It can remain confined from the collar to the upper part within the same group of vessels. Hence, the quite often sharp, unilateral localisation of the external symptoms were observed on the beams of the overgrown tree, on both the root and trunk.

As far as *Verticillium* sp. was concerned, the sapwood necrosis occurred under the bark of the trunk. As the vessels of the bark were still alive and functional, despite the affection of the sapwood, the teak tree did not show signs of distress. When the sapwood tissue degradation reached the bark, where many conducting vessels of the metabolic products resided, the deregulation of the vital functions of the tree occurred. This resulted in the appearance of teak withering leaf symptoms, corresponding to a very advanced stage of the disease progression. The bark's affection on the attacked side of the teak became noticeable while observing the tree closely. Fourteen other fungi of the genus *Trichoderma*, *Penicillium*, *Cunninghamella*, *Acremonium*, *Humicola*, *Cardona*, *Cylindrocladium*, *Aspergillus*, *Fusarium*, *Scytallidium*, *Mucor*, *Cladosporium*, *Cylindrocarpon* and sterile mycelia were observed on both the diseased teaks and those free from disease. The mushrooms were absent in many diseased trees and therefore not associated with teak withering. Lindsey and Gilbertson (1978) identified more than 260 species of basidiomycete fungi linked to *Populus tremuloides* and *P. grandidentata* wood decay and staining.

The inoculation of young teak plants with *Verticillium* sp. allowed to reproduce the withering symptoms observed in the farm, e.g. the disintegration and browning of attacked root tissues, with extension on one side of the tree trunk. With *Fomes* sp. strains, the inoculation had effects on the roots and pivot of young teaks. In both cases, drying of the trees were evident. The inoculation carried out at the root level induced some effects, in contrast to the one conducted in the trunk which had no impact on the teak trees.

CONCLUSIONS

Teak withering occurred the same way across all reforestation centres in forest areas. Sixteen different fungal species were observed on the diseased teak trees. Two species, *Verticillium* sp. and *Fomes* sp. were found to be mainly involved, either individually or simultaneously, in the pathological events responsible for withering. The attack associated with *Verticillium* sp. seemed to be more spectacular in its intensity and speed than *Fomes* sp., both remaining harmful to teak. Very often, teak decay was attributed by error to *Fomes* sp. The lack of knowledge and information on the real causes of teak decay associated with fungi, had consequences on fungicidal treatments which made the control of wood tree diseases difficult and unsuccessful.

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