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Fungal diseases of trees in Tanzania with emphasis on the stem decay of the East African camphor tree, *Ocotea usambarensis* Engl.

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DEDICATION

To my parents, my father Joseph Rubuye and my beloved late mother Crispina Nditije for bringing me up and for encouraging me to go to school.

To my wife Christina and my children Timotheo, Angelina and Gabriel, who suffered the consequences of my absence from home but absorbed them with great understanding.

ABSTRACT

This study presents the current situation and gives a background on forest disease research and knowledge in Tanzania. Some important disease epidemics are discussed and known fungi causing diseases to indigenous and exotic tree species have been tabulated to show the hosts, the pathogens and the parts of the host affected. The review on the disease situation also provides some information on the stem decay of *Ocotea usambarensis*, which is reported in detail under the current study.

The stem decay of *O. usambarensis* has been studied in the Usambara and Kilimanjaro mountain rain forests and causative fungi isolated from standing trees and identified. The decay, characterized by butt rot and main stem decay, and which may attack both the heart wood and sapwood, has been diagnosed basing on the Koch's postulates. The symptoms, signs, infection courts and the ways by which the decay is transmitted from mother trees to young regeneration are also reported. Trees of all age classes and size are susceptible to the stem decay regardless of whether they have developed heartwood or not. Also, sporophores of 14 larger fungi from the families *Hymenochaetaceae*, *Polyporaceae*, *Ganodermataceae*, *Schizophylaceae*, *Corticiaceae* and *Xylariaceae* were collected from various wood material of the tree species. 72 species of fungi were isolated from decay of standing trees, 12 of which were basidiomycetes and 60 were non-basidiomycetes. It was found that the decay is attributed to a number of fungi which infect and colonize the tree through a series of succession stages defined by the niches available in the decaying stems and by the roles of the fungi in such niches.

Pioneering fungi of living sapwood were dominated by parasitic and facultative species which include *Ophiostoma* spp, *Ceratocystis* spp, *Botryosphaeria ribis*, *Cylindrocarpon destructans*, *Cylindrodendrum album*, *Pestalotiopsis* sp, *Nodulisporium* sp, *Leptodontidium* sp, some basidiomycetes and other fungi which could not be properly grouped because they had sterile mycelium among which was an important pathogenic fungus known here as 'Sterile mycelium sp 3'. Possible pioneering fungi of the heartwood were the *Alternaria* sp, *Paecilomyces lilacinus*, *Phoma* sp (*Coniothyrium insigne*), *Penicillium* spp and other unidentified conidial species. The secondary and climax fungi were dominated by basidiomycetes including *Phellinus senex*, and other conidial and sterile mycelium species. *Phellinus senex*, which has been widely reported earlier as a primary decay fungus of *O. usambarensis*, participates in the decay and becomes part of the decay-climax flora of the tree. However, it is not a primary decayer because it can not infect sapwood or heartwood which is un colonized by other fungi.

Some selected fungi were used in pathogenicity tests and inoculated in sapwood and heartwood of healthy trees. The rate of infection of some fungi was variably affected by the moisture content and pH of the sapwood. Determination of the decay ability of some fungi was also conducted *in vitro* using wood blocks of *O.usambarensis*, and the fungi were also tested for the possession of phenoloxidase enzymes that can degrade gallic acid, tannic acid or lignin. Basidiomycetes were the most aggressive in terms of the rate and magnitude of decay but some non-basidiomycetes were also able to degrade the wood significantly. About 70% of the fungi tested possessed enzymes capable of degrading lignin which means they are white rot fungi. As most of these fungi have pH optima similar to that in the trees, this shows they function optimally in the stems and hence explains the formation of hollow stems in decay-affected trees.

Factors which may affect fungal growth and their ability to cause decay in standing trees, such as temperature, pH and oxygen stress were evaluated in culture. It was found that environmental temperature and the pH in trees favour the growth of the fungi, while oxygen stress seems to act as a factor imposing a selection pressure on fungi during succession by favouring tolerant species. Most fungi are mesophilic and the optimum temperature for most of them was within the average found in the forest environment, while their optimum pH was similar to that found in trees. It was also argued that the ability of *Phellinus senex* to tolerate anaerobic conditions was a major factor making it the main climax species of the decay and which later fruitify on standing trees with heartrot or butt rot. Comparing the flora of fungi attacking trees in the Usambara and Kilimanjaro forests, trees in the Usambara are infected by relatively more fungi. This is likely due to the climatic and edaphic conditions which are less optimal in the Usambara and hence predispose the trees to infection.

Key words: Tanzania, forest diseases, check list, *Ocotea usambarensis*, stem decay, decay fungi, fungal pathogenicity, wood degradation, phenoloxidase enzymes, fungal growth, temperature, pH, oxygen stress.

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CONTENTS

LIST OF PAPERS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I. Nsolomo, V.R. and Venn, K., 1994. Forest fungal diseases of Tanzania: background and current status. **Norwegian Journal of Agricultural Sciences, 8:** 189-201.

II. Nsolomo, V.R. and Venn, K., 1996. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests. **Manuscript.**

III. Nsolomo, V.R. and Venn, K., 1996. The pathogenicity of some fungi of *Ocotea usambarensis* Engl. trees. **Manuscript.**

IV. Nsolomo, V.R. and Venn, K., 1996. Decay ability of some fungi from *Ocotea usambarensis* Engl. trees. **Manuscript.**

V. Nsolomo, V.R., Solheim, H. and Venn, K., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. I. Temperature requirements. **Manuscript.**

VI. Nsolomo, V.R., Venn, K. and Solheim, H., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. II. pH requirements. **Manuscript.**

VII. Nsolomo, V.R., Solheim, H. and Venn, K., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. III. Effect of oxygen stress. **Manuscript.**

1. INTRODUCTION AND BACKGROUND

1.1 Background on forest diseases in Tanzania

Fungal diseases of trees in Tanzania are due to both indigenous pathogens and exotic pathogens. The indigenous pathogens have been attacking numerous trees in natural forests and among the most important ones include the root and butt rot pathogen, *Armillaria mellea* sl. and the heart rot agent of *Ocotea usambarensis* tree, *Phellinus senex* (Gibson, 1962; Willan, 1965a; Dick, 1969). Examples of pathogens which were introduced together with their hosts are the *Helicobasidium compactum*, a *Poria* sp and *Ustulina deusta* (Gibson, 1967, 1975; Griffin, 1968). Both the indigenous and exotic pathogens started to attack hosts which were previously unknown to them (e.g., Gibson, 1975; Diwani *et al.*, 1984) and this brought about a more complex disease situation as nothing was yet known on the kind of interactions that were taking place between the new hosts and pathogens. Some introduced pathogens became more aggressive to their natural hosts in the new environment than when they were in their native provenances. Such introduced fungal pathogens were the *Monochaetia unicornis* which caused severe canker in *Cupressus macrocarpa* and the *Dothistroma pini* which caused a severe needle blight of *Pinus radiata* (Olembo, 1969; Diwani *et al.*, 1984). The information on such epidemics and indeed on most diseases of trees is in most cases obscured and difficult to find. Hence, putting such information into a single document would benefit forest managers and researchers alike.

Research on forest diseases in Tanzania, and indeed in the whole of East Africa, started after the second world war and was prompted by an increase in diseases of the exotic trees (Gibson, 1965). The research was undertaken by the East African Agriculture and Forest Research Organisation (EAAFRO) which catered for Tanzania, Kenya and Uganda. A number of reports show that emphasis was on diseases of the exotic trees while very little was done on diseases of indigenous trees. Moreover, the various information on diseases of trees growing in Tanzania was scattered in various literature and so it was not easily accessible for reference or for possible use in the management of the trees. As regards to indigenous trees, diseases of few important timber trees such as the stem decay of *Ocotea usambarensis* were reported, and initiatives were started to find ways of regenerating the tree to minimize the transmission of the decay to young regeneration (Willan, 1965a; Kimaryo, 1971). After the disintegration of the East African Community in 1977, the EAAFRO also collapsed and due to the lack of local forest pathologists, no substantial research on tree diseases in the country was done. Research on the decay of *O.usambarensis* came to an end as well.

1.2 Distribution, ecology, uses and the stem decay of *Ocotea usambarensis*

Ocotea usambarensis Engl. (Family: Lauraceae; commercial name: East African camphor tree; common names: camphor, *Ocotea*) is an important tree species growing naturally in mountain rain forests of Tanzania and Kenya, and also is reported in Uganda, Malawi and Zambia (Mwamba, 1986; Renvall and Niemelä, 1993). In Tanzania, it grows on the southern slopes of Kilimanjaro volcanic mountain and on the crystalline mountains of Usambara, Nguru, Ukaguru, Uluguru and mountains of the southern high plateau. Mountain rain forests occupy the largest area of catchment forest reserves of the country and are managed for water and gene pool conservation, control of soil erosion and to some extent, for timber exploitation (Pòcs, 1988).

Ocotea usambarensis is a large evergreen tree which grows up to 40 m height and may attain a diameter of 1.5 m (Kimaryo, 1971). It occurs between 900-2740 m altitude (Bryce, 1967) with an average annual rainfall ranging between 1150 - 3050 mm (Mugasha, 1978). Temperatures are never very high in the camphor zone and range between 7°C to 27°C. Soils are generally acidic loams which may be shallow or deep, derived from volcanic or igneous rocks and the tree prefers well drained areas such as steeper slopes of small watershed ridges separating streams (Pitt-Schenkel, 1938; Mugasha, 1978). The pH of soils in forests where camphor grows ranges from 3.5-5.5 (Pitt-Schenkel, 1938; Lundgren, 1978;

Mwamba, 1986). It regenerates by seed (but this is sporadic and unreliable), coppicing and through root suckers (Mugasha, 1978). Harvesting is by selection and the wood is one of the best utility hardwoods used mainly as furniture wood, for panelling, vehicle building, boat ribs, flooring, acid vats, fittings in shops and laboratories and the logs are peeled for veneers. Seasoned timber is resistant to borers and the heartwood is also resistant to decay. The wood has a strong but impermanent aromatic scent caused by the volatile oil cineol, but unlike oil of the true camphor, *Cinnamonum camphora*, cineol gives no protection against insects (Bryce, 1967).

Reports indicate that for the past 40 years or more, a heartrot disease has been the major defect affecting mature trees of 50 years and above (Gibson, 1962; Willan, 1965a; Dick, 1969; Kimaryo, 1971). Such trees may contain a hollow core in the stem, and a survey in the Kilimanjaro forest indicated the decay averaging to 12% of total volume of affected trees and hence rendering the trees useless for peeler logs (Dick, 1969). Studies carried out in 1961 indicated also that both decay and stain entered mostly through stem wounds and branch stubs and to a limited extent originated from the parent root system (Willan, 1965a). Recent reports still mention the heartrot as the major problem affecting the survival of camphor which also reduces its potential as a commercial tree species (Mwamba, 1986; Forest Division, 1991).

Fungi which are implicated to cause the heartrot were identified basing on field observations of basidiocarps occurring on living and dead trees, fallen decaying trunks and stumps of camphor trees (e.g., Gibson, 1962; Renvall and Niemelä, 1993). The fungus widely reported as the main heartrot agent is *Phellinus senex* (syn. *Fomes senex*; *Polyporus senex*), but there are other fungi also implicated as causatives of the decay and are mentioned by Ebbels and Allen (1979) and Renvall and Niemelä (1993).

A survey by Dick (1969) confirmed an earlier report by Willan (1965a) that the decay originated in the tree quite early in life because trees with a diameter (at breast height) of less than 20 cm and hence which had not formed any substantial amount of heartwood were also infected. It was due to these observations on the infection of young trees and that the infection was less frequent in root sucker regeneration than in coppice regeneration (Willan, 1965a), that prompted the modification of silvicultural practices in the regeneration of *O. usambarensis* during the early 1950s. In that period, experiments were established to regenerate the tree by promoting sprouting from root suckers because they were less susceptible to the heartrot disease. This method of regeneration involved killing of large mature trees (which definitely contained the heartrot) by girdling and poisoning and then promoting sprouting by wounding surface roots (Kimaryo, 1971; Mugasha, 1978). Although some good coppice regenerations were allowed to survive after felling, this practice was not encouraged because it was correctly thought that the decay could easily be transmitted from infected stumps into the coppice trees. However, Willan (1965a) and Dick (1969) acknowledged also that butt rot entering suckers from the parent roots was a more difficult problem on which there was limited information. As such, there are more fungi which are involved in the decay of *Ocotea usambarensis* which have not been identified because no fungal isolations were done from the decay in stems of living trees.

1.3 Concepts of succession, pathogenicity and ecology of decay in living trees.

A standing tree is said to consist of a heterogenous assemblage of micro environments which pose distinctive constraints on the growth and modes of establishments of fungi and other microorganisms that invade it. Only stress tolerant fungi are characterized by their ability to colonize wood under conditions which other fungi can not tolerate. Wood micro environments which impose stress are unfavourable moisture and aeration conditions, temperature, pH, allelopaths (extractives and volatiles) and restrictions on nutrient availability (Highley *et al.*, 1983; Rayner and Boddy, 1988). Tolerance to the micro environmental stresses in the trees determines the patterns of colonization. These patterns are diverse and may involve active pathogenesis of living tissues in the sapwood or invasion of the heartwood and other dead parts of the xylem. However, it is mentioned that the distribution of water and

its reciprocal relationship to aeration are the primary determinants of the colonization patterns, either directly or through their effects on living wood cells (Rayner and Boddy, 1988). According to Shortle and Cowling (1978), succession of microorganism in stem sapwood upon wounding is characterized by the process where bacteria and certain non-hymenomycetous fungi develop rapidly in the discoloured sapwood and then decay-causing hymenomycetes become abundant in later stages of the succession.

The mode of nutrition of some fungi determines the niches which they will occupy in the stem of living trees. Some fungi are only able to live in the living tissues of sapwood and these are obligate parasites which in decay columns are normally found at the decay margin in sapwood. The second group is composed of fungi which are able to live in both living and dead tissues (that is, in living sapwood and in the heartwood or dead parts of sapwood) and these are known as facultative fungi. The third group consists of fungi which can only live in dead parts of sapwood or in the heartwood, and these are obligate saprotrophs. Fungal ability to colonize living sapwood tissues or intact heartwood in standing trees are further attributes which may define their roles in decay of standing trees. Depending on the individual capability of the fungi, some may be pathogenic, able to kill living tissues in sapwood or invade the heartwood and cause decay or stain which will be exhibited by certain characteristic symptoms. But also, studies have revealed that some fungi are able to live in living sapwood or in healthy-looking heartwood as endophytes without affecting the tree's physiological or structural status and hence do not cause any visible symptoms in the wood (Roll-Hansen and Roll-Hansen, 1979; Huse, 1981; Barklund and Kowalski, 1996). It has also been reported that such heartwood endophytes may not interfere with the phase of infection by other wound fungi although they may influence further extension and composition of the wound flora (Huse, 1978). Hence, the patterns of colonization of the various niches in trees vary between various species of fungi and the roles that they assume in wood. Characterization of such roles during colonization and decay of tree species can be assisted by the identification of the types of niches the fungi occupy. According to Rayner and Boddy (1988), individual fungi are capable of combining one or more of the colonization strategies and hence occupy more than one niche. This also means that certain fungi may combine saprotrophism with active pathogenesis to get access to the food resources they require and by so doing they may also assume roles which overlap with those of other fungi during the decomposition of stem wood. There are fungi which are capable of degrading the cell wall and cause decay while also there are non-decay fungi which rely on simple sugars, starch and proteins or may form associations with decay species to get nutrients (Rayner and Boddy, 1988) and these two types may occupy the same niches. Testing the fungi for pathogenic ability further contributes to their categorization into those which can pioneer the invasion of intact tissues, while decay ability tests will identify fungi which are able to degrade wood polymers.

As already discussed, fungi colonizing the heartwood are mostly saprotrophs which live on dead wood and must have the ability to hydrolyse the cellwall polymers in order to survive. In most cases, fungi attacking the heartwood usually form the climax wood decay flora and have the capacity to exit inner decays through cracks in stems, butts or through exposed branch stubs and then form fruitbodies on the surface of trees (Rayner and Boddy, 1988). However, the heartwood is said to contain allelopathic extractives which inhibit some fungi (Rayner and Boddy, 1988) and hence only those fungi which are able to detoxify these extractives can establish themselves directly in the heartwood. Hence, the pioneering fungi in the heartwood encounter allelopathic substances impregnated in dead xylem tissues and have to detoxify them. Fungi which are not able to detoxify the extractives but are obligate saprotrophs will occupy the heartwood after the toxins have been neutralized by the other fungi. This may explain why some strong wood decay basidiomycetes are said to enter the heartwood only when it has already been colonized by other fungi (see Mercer, 1982). The pioneers in the sapwood also encounter phenolic compounds which they have to tolerate or detoxify. These compounds are formed in the vessel elements as a response to fungal invasion or wounding (Shigo, 1967; Sharon, 1973) and may act as chemical barriers to wood decay fungi (Shortle, 1979). It is also reported that the successful infection and colonization of living sapwood tissues is dependent on the ability of the fungi to overcome the constitutive and inducive defence responses such as the formation of the physico-chemical barriers (Shain, 1971, 1979; Shigo and Marx, 1977; Shortle, 1979).

When conditions are not favourable for the fungi in the stem, they move out (or get removed out) of the competition and those which can tolerate the stresses remain while at the same time others may come in as well. As decay is an oxidation process, the elevated CO_2 levels and moisture in wood tissues make conditions unfavourable to some fungi (Highley *et al.*, 1983). Reduced aeration may also be brought by the increase in moisture content which has a reciprocal relationship with aeration, meaning that as moisture increases inside the trunk, less air may not favour the growth of some fungi (Rayner and Boddy, 1988). The relationship of these factors to the pathogenicity or decay ability of fungi is important in understanding how fungi may succeed one another because it clarifies the circumstances which restrict or enable fungi to colonize wood in stems of living trees.

Yeasts and bacteria also play significant roles in decay of wood. They are pioneers and as well occupy advanced decay zones in wood (Rayner and Boddy, 1988) but are infrequent when decay fungi are active (Shigo and Sharon, 1970; Van Der Kamp, 1975). Yeasts and bacteria are thought to form associations with mycelial fungi in order to get access to inner tissues and the pioneering fungi are usually found together with bacteria which are also involved in the process (Shigo, 1966; Blanchette and Shaw, 1978; Blanchette *et al.*, 1981). Results of an experiment by Shortle *et al.* (1978) showed that pioneer bacteria found in sapwood and discoloured wood contribute to wood discolouration, alter the rate of cellulase activity of decay fungi, interact with wood parenchyma to alter the rate of wood colonization by decay fungi and help to provide growth factors and soluble nitrogen.

1.4 Stem decay in trees and decay ability of fungi

The decay of sapwood is commonly referred to as saprot while the central decay in trees which usually attacks the heartwood, is often referred to as heartrot. Two distinctive classes of heartrot are often recognized in trees, and are the top rot which originates in the crown and upper parts of the stem and spreads downwards, and the butt rot which originates in the roots or root collar and spreads upwards (Rayner and Boddy, 1988). A wide range of microorganisms are associated with decay in trees (e.g., Shigo and Hillis, 1973; Shigo, 1976; Blanchette and Shaw, 1978) but according to Rayner and Boddy (1988), it is only a relatively small selection of Ascomycotina and Basidiomycotina that are known to cause significant decay. Basidiomycetes are described as the main causes of decay and form the climax of the succession of colonising microorganisms (Levy, 1982; Mercer, 1982). The ability of fungi to decay wood is attributed to their possession of enzymes capable of digesting the cell-wall polymers which are lignin, cellulose and hemicelluloses (Nobles, 1965; Taylor, 1974; Stalpers, 1978; Rayner and Boddy, 1988) although it has been reported that some rots may, in addition, also involve a non-enzymatic mechanism (Highley, 1975). The determination of the types of enzymes that decay fungi possess may assist in grouping the fungi according to the type of rots they cause. On the other hand, the occurrence of such fungi in decays may help to explain why certain types of rots are formed in some trees.

Three types of decay namely white, brown and soft rots are recognized. White rots are decays in which the wood acquires a bleached appearance and lignin and as well as cellulose and hemicellulose are broken down (Rayner and Boddy, 1988). Two types of white rots are further described by Liese (1970) which are the simultaneous removal of both lignin and the polysaccharides, and a second one is the selective removal of lignin prior to the polysaccharide degradation. Brown rot on the other hand, is a decay in which hemicelluloses and cellulose have been selectively removed leaving a modified lignin which discolours the wood brown, and becomes friable, ultimately powdery, and cracks cubically. A third type of decay known as soft rot is recognized and differs from the other two types in that cell-wall breakdown occurs in the immediate vicinity of hyphae and lignin removal is absent or slow or partial (Rayner and Boddy, 1988). In all the three types of decay, enzymes are involved in the breakdown of the cell wall polymers although some reports suggest that in addition, brown rots may also involve a non-enzymatic mechanism (e.g., Highley, 1975). However, the decay ability of fungi is also dependent on various micro environmental factors which affect their growth as living organisms and which provide a conducive environment for the decay to take place. The determination of the effect of such factors on

the growth of fungi may explain their behaviour and indicate why certain fungi favour certain tree species or occur in certain habitats or why they tend to occupy certain niches in the decay columns.

1.5 Micro environmental factors affecting growth and ability of fungi to colonize and decay wood

Factors affecting the growth of fungi and their ability to colonize and decay wood have already been mentioned and are temperature, extractives and volatiles (allelopaths), pH, moisture and aeration (Highley *et al.*, 1983; Rayner and Boddy, 1988).

1.5.1 Temperature

It is reported that generally wood decay fungi are mesophilic, with a growth range within 0-45 °C and an optimum temperature between 20-30 °C (Wagner and Davidson, 1954; Rayner and Boddy, 1988). Temperature affects nutrient uptake in fungi by increasing mobility of ions and molecules and hence accelerating uptake by passive diffusion, and to a point, facilitated diffusion and active transport. Also temperature influences fungi through its effect on enzyme catalysed reactions. The overall response of a fungus to different temperatures represents the combined effect on numerous different chemical reactions, each of which exhibits its own characteristic relationship to temperature (Rayner and Boddy, 1988). It has also been found that there is a relationship between the optimum temperature requirements of a fungus for its growth on agar medium and that for rapid decay of wood that it causes, whereby rapid decay of wood occurs 2-3 °C below the optimum for extension on agar (Gaüman,1939; Henningsson, 1968).

1.5.2 Extractives and volatile compounds

Various extractives and volatile compounds do exist in plants and they are produced as secondary metabolites. Based on biosynthetic criteria, they are divided into three main groups which are terpenes (lipids synthesized from acetyl CoA via the mevalonic acid pathway), phenolic compounds (aromatic substances formed via the shikimic acid pathway) and the aromatic nitrogen-containing compounds such as alkaloids (Taiz and Zeiger, 1991). Terpenes and phenolic compounds are well documented as compounds involved in defence of plants against infection by microorganisms (Gibbs, 1968; Alcubilla *et al.*, 1971; Shain and Hillis, 1971; Forrest, 1980; Woodward and Pearce, 1988; Salisbury and Ross, 1992). Terpenoids are also called essential oils because they are volatile and contribute to the essence (odour) of certain species (Salisbury and Ross, 1992). One monoterpenoid, cineol, has been reported in the East African camphor tree, *Ocotea usambarensis* (Bryce, 1967). Simple phenolic compounds such as cinnamic, p-coumaric, caffeic, ferulic, chlorogenic, protocatechuic and gallic acids are derivatives of the shikimic pathway and are important because they are converted into proteins, phytoalexins, coumarins, lignin and various flavonoids (Salisbury and Ross, 1992). Lignin is known to offer physical defence to plant tissues against fungal infection (Ladejtschikova and Pasternak, 1980; Ride, 1983). According to Taiz and Zeiger (1991), a second type of phenolic polymer with defensive properties, besides lignin, is tannin which is formed by flavonoid units (condensed tannin) or gallic acid (hydrolysable tannin).

In trees or wood, toxic extractives are usually more abundant in heartwood and they account for the durability of heartwood of felled timber against attack by decay fungi (Scheffer and Cowling, 1966). In living sapwood they accumulate mainly as a result of the induction caused by wounding or due to infection by microorganisms (Shain and Hillis, 1971; Forrest, 1980; Johansson and Stenlid, 1985; Woodward and Pearce, 1988; Taiz and Zeiger, 1991). Many naturally occurring allelopaths are phenolic in nature and their inhibitory mechanisms to fungal action during colonization and decay of wood is due to their interference with enzyme-mediated reactions (Rayner and Boddy, 1988). The toxicity of tannins

has also been attributed to their ability to bind proteins thus inactivating enzymes (Taiz and Zeiger, 1991). The neutralization process of these chemicals by fungi is regarded as an integral part of the colonization process and is an important determinant of the decay community in structure and development in standing trees. The mechanisms underlying detoxification is also said to involve phenol-oxidizing enzymes such as laccase, tyrosinase and peroxidase (Rayner and Boddy, 1988). There is also considerable variation in tolerance to phenolics and other aromatic compounds by different fungal species (Rudman, 1963; Hintikka, 1971; Popoff *et al.*, 1975). Some blue stain fungi have been found to be inhibited by volatile extracts (Shrimpton and Whitney, 1968) while some non-decay fungi were found to be tolerant and could detoxify polyphenolic extractives (Shortle *et al.*, 1971). It has also been observed that the toxicity of phenolic compounds varies with pH with the effect varying between different enzymes (Popoff *et al.*, 1975).

1.5.3 Wood pH

Cultural studies have shown that pH optimum of between 4 and 6 is common for the growth of wood decay fungi, but such laboratory results provide difficulties in understanding how they relate to conditions in wood (Rayner and Boddy, 1988). In trees, there can be considerable variations in pH between different tissues or between infected and uninfected tissues (Hartley *et al.*, 1961). Variations occur also between different tree species, and in the family *Lauraceae* in which *Ocotea usambarensis* tree belongs, pH is recorded to range between 3.65 to 4.9 (Gray, 1958). The pH state between the heartwood and sapwood differs within and between tree species whereas some species have a lower pH in heartwood than the sapwood while in other species the sapwood may have a lower pH than the heartwood (Rayner and Boddy, 1988). Extremes of pH have been implicated in the selectivity of fungi for particular timber types (Gray, 1958; Packman, 1960). Many decay fungi are reported to lower the pH of the medium in which they are growing in order to suit their requirements. In wood, lowering pH is partly due to the formation of carbonic acid which is formed as carbon dioxide is formed during decomposition (Rayner and Boddy, 1988). It has also been observed that pH may influence the toxicity of phenols whereby toxicity of some phenols was found to decrease with increasing pH (Popoff *et al.*, 1975).

It has also been established for most wood decay fungi that the optimum pH for lignin breakdown is between 4 and 4.5 (Rayner and Boddy, 1988) while brown rot species are reported to show a greater tolerance to acidic pH and a greater sensitivity to high pH, correlating with the susceptibility to pH of their cellulase and hemicellulase enzymes (Henningsson, 1967). Most blue stain fungi tolerate pH values of up to 9 (Butcher, 1968).

1.5.4 Moisture content and aeration

Moisture influences decay rate both when it is insufficient to meet metabolic requirements and when it is in excess, resulting in conditions of reduced aeration (Rayner and Boddy, 1988). The absence of decay and stain due to fungi in dry timber is an indication of the susceptibility of fungi to desiccation. It has been estimated that the lower water potential limits for the growth of some decay fungi was between -4 to -6 MPa corresponding to wood moisture content of less than 30% (Griffin, 1971; Boddy, 1983; Rayner and Boddy, 1988). Development of decay in wood is also limited by high moisture content which in functional sapwood in standing trees is thought to limit decay of inner tissues (Boddy and Rayner, 1983). Various mechanisms underlie the effect of water saturation on decay and non-decay fungi and include the inhibition of enzyme activity and the interference with hyphal cell wall interactions, but most of all, water inhibits growth by limiting oxygen supply and altering the gaseous regime in wood (Rayner and Boddy, 1988).

Carbon dioxide has also an effect on growth of fungi and there is considerable variation in tolerance to

high carbon dioxide concentration whereby some heartrot species are said to grow significantly above 70% carbon dioxide. It is also reported that slightly elevated carbon dioxide levels of up to 10% often stimulate growth of wood inhabiting fungi (Hintikka and Korhonen, 1970). The basis for the stimulation is described as due to the ability of some wood decay fungi to fix carbon dioxide into organic acids (Tachibana, 1968) and due to its effect on the activity of the extracellular oxidase enzymes of laccase and peroxidase (Rayner and Boddy, 1988). When carbon dioxide concentration increases in wood there is a concomitant decrease in oxygen concentration and it has been observed that wood decay basidiomycetes are tolerant to high carbon dioxide concentrations and to as low as 1% oxygen (Jensen, 1967). At low oxygen concentrations, anaerobic respiration may occur with the build-up of products such as ethanol, methanol, formate, acetate, lactate, and propionate (Rayner and Boddy, 1988). Studies by Highley *et al.* (1983) and Hall and Leben (1985) observed that the decay rate of wood by heart rot and sap rot basidiomycetes was significantly inhibited by high carbon dioxide and low oxygen concentrations. Wood decay fungi are said to be primarily aerobic although they do have some capacity for anaerobic metabolism and require oxygen as a terminal electron acceptor for oxidative phosphorylation. Also, about 40-60% oxygen is said to be optimal for lignin metabolism in culture (Rayner and Boddy, 1988). Apart from its influence on ligninolysis, oxygen also stimulates cellulose hydrolysis partly due to its effect on some enzymes (Highley *et al.*, 1983).

2. OBJECTIVES OF THE STUDY

The major objective of the study was to expose research knowledge available on tree diseases of Tanzania as a basis for reference and future research, and to elucidate on the stem decay of *Ocotea usambarensis* tree species. This objective is intended to provide the bench-mark information on what is known about fungal diseases of trees and disease research in the country so that such knowledge can be useful for management of the diseases and for further research purposes. It is also intended to answer questions which have not been fully answered for a long time now on the decay of *Ocotea usambarensis* tree. Such questions are on the causes of damage to the tree, the symptoms of trees with decay, the fungi involved in the decay, the ability of the fungi to colonize and cause decay of the tree, and what the characteristics of the fungi are when subjected to certain controlled micro environmental factors. As the tree grows on different habitats characterized by different edaphic and climatic conditions, it would be of interest to compare if there are different fungi attacking the tree in such locations.

The specific objectives were the following:-

1. To collect and compile information on diseases of both indigenous and exotic trees growing in the country into a checklist (I).

2. To record the causes of damage, the infection courts, the symptoms and signs of decay of living trees, the transmission of decay from one tree to another, and to record the flora of larger fungi causing decomposition of wood of *Ocotea usambarensis* trees in the forest (II).

3. To identify fungi causing stem decay in living trees of *Ocotea usambarensis* and to elucidate on their possible succession pattern during colonization and decay of the stem (II).

4. To compare the flora of fungi causing decay of the tree in the Usambara and Kilimanjaro forest habitats (II).

5. To determine which of the fungi are pathogenic to *O.usambarensis* and to explore if the rate of spread of infection in the stem is related to the moisture content and pH of trees (III).

6. To determine the ability of the fungi to decompose wood of *O.usambarensis* and to investigate if they possess certain phenoloxidase enzymes (IV).

7. To determine the cardinal levels of temperature and pH of the fungi and to examine how the fungi grow under oxygen stress (V, VI, VII).

3. MATERIALS AND METHODS

In order to bring together the scattered knowledge relevant to tree diseases in Tanzania, literature search was conducted and compiled from various documents. Then tables were developed to show the host trees affected, the pathogenic fungi involved and the parts of the hosts affected (I).

For the investigation on the stem decay of *Ocotea usambarensis* and the fungi involved, the general methodology was based on the Koch's postulates as outlined in Holliday (1989). The symptoms were studied and recorded; fungi were isolated from stem of trees with decay; pathogenicity tests were carried out by inoculating fungi into healthy trees and later re-isolated into pure culture and re-identified; and the characteristics of the fungi were studied *in vitro*. This means that there were experiments which were established in the forest and others which were conducted in the laboratory (II-VII).

Field studies were carried out in the mountain rain forests of the Usambara and Kilimanjaro between 1993 and 1995. Causes of damage to trees, symptoms and signs of decay, and infection courts of fungi on the trees were recorded. Sporophores of larger fungi were collect from various decaying wood material of the tree species and fungi involved in the stem decay of living trees were isolated from 48 disks cross-cut from 14 trees with decay. Fresh wounds were also inflicted in the sapwood of a few trees and fungi were isolated from them after one month and after one year. Identification of the fungi was done using the sporophores directly, or by comparative methods using mycelia or by using other cultural diagnostic tests (II).

Selected fungi were used for pathogenicity tests by inoculating them in sapwood and heartwood of healthy trees selected from the study areas. After the inoculation, the trees were left for one year after which they were cut and fungi re-isolated. During cutting, the trees were sampled at various diameters and heights for moisture content and pH in both the sapwood and the heartwood (III).

In the laboratory, fungi were tested for their ability to cause decay of wood blocks of *Ocotea usambarensis* during a period of 4 months. The fungal cultures were also tested for possession of phenoloxidase enzymes capable of degrading gallic acid, tannic acid and lignin (IV). Studies on the growth of the fungi on malt agar were also conducted by subjecting them to various temperature and pH levels and to oxygen stress (V, VI, VII). The pH was fixed in the growth medium using organic buffers. For the oxygen stress test, nitrogen was used to replace normal air in the tubes into which the test fungi had been inoculated.

4. RESULTS AND DISCUSSION

4.1 Tree diseases of Tanzania and their management

Diseases of 36 indigenous and 45 exotic trees growing in Tanzania have been reported and are now tabulated in a checklist. Owing to the fact that Tanzania has a vast forest area with about 1,200 indigenous tree species (Willan, 1965b), there are more diseases attacking many trees than those reported here. The *Armillaria* root rot has been attacking 15 % of all the trees reported and it is the only diseases which attacks a wide variety of host species (I).

Disease outbreaks have been significant and severe. The *Dothistroma* blight of *Pinus radiata* (caused by *Dothistroma pini*) and the *Monochaetia* canker of *Cupressus macrocarpa* (caused by *Monochaetia unicornis*) are examples of diseases which have caused enormous economic loss because the two tree

species were abandoned as commercial plantation trees in the whole of East Africa (Olembo, 1969; Diwani *et al.*, 1984; Zulu, 1991).

In plantation forestry where a single species is usually planted over large areas, the impact of the disease is usually enormous and it is evaluated in terms of the investment and the expected revenues lost. However, the decision to replace the trees is also usually easy where resources are available and where alternative species do exist. But as the plantation forests continue to suffer from further disease outbreaks, choices of alternative species may later become limited, and other methods such as breeding for resistance may therefore be more important. Efforts to breed *Pinus radiata* resistant to the *Dothistroma* needle blight in the country had been started (Ivory and Paterson, 1970) but were later abandoned due to lack of experts after the collapse of the East African Agriculture and Forest Research Organisation (EAAFRO). Elsewhere, such as in New Zealand, success has been achieved through gradual selection for healthy trees to develop clones of *P.radiata* resistant to the *Dothistroma* needle blight (Ivory and Speight, 1993).

In natural forests where individual species occur in a mixture with many others and where the management objectives are usually different from those of the plantation forests, immediate solutions such as replacement of affected species may not be feasible. One alternative in natural forests, as is the case with *Ocotea usambarensis*, would therefore be to amend management practices so as to reduce losses to a minimum and prevent future decay losses as much as possible. As far as *O.usambarensis* was concerned, some amendments on the regeneration techniques were carried out by promoting root suckers and discouraging coppice sprouts to limit transmission of decay to young regeneration Kimaryo, 1971; Mugasha, 1978). However, it was also observed that butt rot was another problem, and as confirmed in this study (II), many fungi including *Phellinus senex* which cause stem decay are as well responsible for butt rot of both the root sucker and coppice regenerations. So, the regeneration methods applied earlier were less effective in limiting the spread of the fungi from mother trees to young regeneration. This therefore means that more studies need to be done to identify ways of protecting young regeneration from infection, for example, by protecting stumps after harvesting or protecting roots during the promotion of root suckers. This can be achieved through the use of non-polluting chemicals or antagonistic fungi if they are known. Another way would be to change the method of promoting regeneration, or by doing enrichment plantings in the forests using nursery stock raised from seed.

Many other indigenous and exotic trees suffer from diseases and therefore further surveys are necessary to determine their extent. The current study on the decay of *O.usambarensis* is only the beginning of a wider process aiming at providing more information on major diseases of important tree species of Tanzania.

4.2 The stem decay of *Ocotea usambarensis*

The study on the decay of living trees of *O.usambarensis* has found out that the tree species suffers from both butt rot and main stem decay (II). As earlier reported (e.g., Gibson, 1962; Willan, 1965a; Dick, 1969; Kimaryo, 1971), heartrot is the major type of decay affecting trees which have developed heartwood. The infection takes place through wounds which expose either the heartwood or the sapwood, and this may happen on the stem, butt or root. Infection through dead branch stubs is also another method of fungal entry in the stem. The causes of damage to the tree are due to human activities in the forests, animal damage and due to the silvicultural practice of wounding roots to promote sprouting. However, although the presence of heartrot in most tree species is sometimes associated with wounds, other modes of establishment of fungi which are referred to as specialized opportunism (Rayner and Boddy, 1988) do exist, whereby some fungi establish in the sapwood of intact branches and enter the heartwood (Toole, 1961; Boddy and Rayner, 1982).

Young and mature trees which have developed heartwood and those which have not developed it, are

both susceptible to the stem decay. The susceptibility of both types of trees is due to the type of fungi infecting the trees because some of them are parasitic, others are facultative, while others are saprotrophs (II). Young regeneration trees of *O.usambarensis* are infected directly from mother trees or get infected through contaminated stumps or roots from which they are regenerated. The infection from stumps and roots has been the main problem facing the regeneration of the tree species (Dick, 1969; Mugasha, 1978).

Fourteen species of larger fungi forming sporophores on decaying wood of *O.usambarensis* were collected in the forests of the study areas (II). They were 13 basidiomycetes from the families *Hymenochaetaceae*, *Polyporaceae*, *Ganodermataceae*, *Schizophylaceae* and *Corticiaceae*, and one ascomycetes from the family *Xylariaceae*. Most of these fungi seem to be only involved in the decomposition of dead wood in the forest and apart from *Phellinus senex*, others were not isolated in the stem decay of the sample trees. Pathogenicity tests also showed that some of them were unable to infect intact sapwood or heartwood of standing tress (III). This was due to fact that most of the fungi are secondary colonizers of stem and even the *Phellinus senex* was only isolated from wood which was already occupied by other fungi (I). Also it was later found that most fungi which cause decay of dead wood in the forest may not be favoured by one or a combination of the conditions which affect growth of fungi in living standing trees such as temperature, pH and oxygen stress (V, VI, VII). The above factors are widely reported to affect fungal growth in trees and in addition, allelopathic substances and moisture conditions are as well very important (see, Highley *et al.*, 1983; Rayner and Boddy, 1988).

The decay in *O.usambarensis* is attributed to a number of fungi which colonize the stem through a series of succession processes defined by the niches available and their state, and also by the roles of the fungi involved (II). The roles of the fungi in the decay are defined by their modes of nutrition which indicate whether they are parasitic, facultative or saprotrophic. About 72 species of fungi were isolated from the various niches in the stem, and among them were 12 basidiomycetes (forming 17%) and 60 non-basidiomycetous species (forming 83%). In both sapwood and heartwood there were pioneering fungi which started the colonization of wood. Such fungi encounter the sapwood's defence responses (Shain, 1971; 1979; Shain and Hillis, 1971), allelopathic compounds, different pH, moisture and aeration conditions (Rayner and Boddy, 1988) and in *O.usambarensis*, they may need to detoxify the oil cineol which exists in the tree (Bryce, 1967). Fungi infecting fresh wounds of *O.usambarensis* are mostly the parasitic and facultative species dominated by *Ophiostoma* spp, *Ceratocystis* spp, *Botryosphaeria ribis*, *Cylindrocarpon destructans*, *Cylindrodendrum album*, *Pestalotiopsis* sp, *Nodulisporium* sp, some basidiomycetes and some fungi which could not be properly grouped because they had sterile mycelium among which was an important one referred to as 'Sterile mycelium sp 3' (II, III). Some of the pioneering fungi tested positive for the possession phenoloxidase enzymes capable of detoxifying simple phenolic compounds such as gallic acid and tannic acid while others had enzymes capable of degrading lignin as well (IV). The pH optima for these fungi were in the acidic region and ranged between 2.7-4.8 (IV), suiting their role to detoxify acidic allelopathic compounds. Most of the pioneering fungi were also quite sensitive to oxygen stress and stopped growing within two weeks (VII).

Fungi which pioneer the colonization of heartwood, dead sapwood or branch stubs are mostly saprotrophic, unable to colonize living wood tissues, but facultative parasites or facultative saprotrophs are also involved. These fungi were *Alternaria* sp *Paecilomyces lilacinus*, *Phoma* sp (*Coniothyrium insigne*), *Penicillium* spp and other unidentified conidial species (II). Some of these fungi had phenoloxidase enzymes against the simple phenols and lignin and are therefore capable of causing decay of wood as well (IV).

Some fungi were found to be facultative and were able to colonize intact sapwood or heartwood and could occupy all niches in the stem (II, III). These included *Cylindrocarpon destructans*, a *Leptodontidium* sp, *Phialophora parasitica*, a *Nodulisporium* sp, *Trichoderma harzianum*, some other conidial and sterile mycelium species including sterile mycelium sp 3, and some unidentified basidiomycetes and yeasts. Like the other groups above, some of these fungi possessed phenoloxidase enzymes (IV).

The third group involved in the decay of *O.usambarensis* consisted of fungi which were only isolated in niches occupied by other fungi. This group consisted of the species which mostly occupied the heartwood (II) and which were later found to possess a high ligninolytic capacity and were able to cause significant loss in weight of wood blocks of the tree species when tested (IV). This flora was dominated by *Phellinus senex*, a *Phellinus* sp, other 5 unidentified basidiomycetes, *Pseudomorfea coffeae*, *Periconia* sp, *Gliocladium roseum*, *Penicillium* spp, *Cladosporium* sp, *Acremonium* sp, *Pseudallescheria boydii*, *Auresporium* sp, some other conidial spp and sterile mycelium species. About 58% of all the isolated basidiomycetes were found in this group (II). Apart from possessing phenoloxidase enzymes, some members of this group such as *Phellinus senex* were also found to be tolerant to oxygen stress (VII), a factor which is likely to contribute significantly to its ability to became a decay climax species such that basidiocarps of the fungus are usually more abundant on standing trees which have the decay (see Gibson, 1962; Renvall and Niemelä, 1993).

About 70% of the decay fungi of *O.usambarensis* which were tested for phenoloxidase enzymes possessed ligninolytic enzymes and mostly the laccase and some had the tyrosinase enzyme (IV). The decay flora of the tree species seems to be suited to the conditions within the forests and within the tree species itself. The temperature optima for 94% of the fungi was between 21-28 °C which is within the average annual temperature of the forests in which *O.usambarensis* grows (V). The growth range of the fungi extended from the lowest minimum of 3 °C to the highest maximum of 40 C and can be categorized as mesophilic. The pH optima for 68% of the fungi was between 4 and 4.8 (VI) which corresponds to that of 4.2 and 5.0 found in the heartwood and sapwood of standing trees, respectively (III). This optimum pH of the fungi also corresponds to that of 4.0-4.5 which has been reported for optimum ligninolytic activity (Rayner and Boddy, 1988). This implies that most fungi of *O.usambarensis* are favoured by the pH in the trees and as such pH is not a limiting factor to their growth in the stems. Also, the pH of the soils in the forests where the tree grows ranges between 3.5 to 5.5 (Pitt-Schenkel, 1938; Lundgren, 1978; Mwamba, 1986) indicating that it is favourable to fungi which may decompose or live on wood or other plant material in the soil. Some of the fungal species isolated from *O.usambarensis* are known from other locations in the world and have been isolated from the soil (e.g. Domsch *et al.*, 1993).

Decay ability tests of the fungi showed that, in terms of the rate and magnitude of decay, basidiomycetes were more aggressive than non-basidiomycetes (IV). However, it was also argued that considering their numerical advantage over basidiomycetes, their ability to degrade wood due to their possession of phenoloxidase enzymes, and their pioneering role in the colonization of wood in living trees, then cumulatively, the role of non-basidiomycetes in the decomposition of the tree species may be equal or more in magnitude to that of the basidiomycetes. The possession of ligninolytic enzymes by most decay fungi of *O.usambarensis* also suggests that the fungi cause white rot in the tree, which according to Nobles (1965), Stalpers (1978), Mercer (1982), Rayner and Boddy (1988) and Blanchette (1995) such enzymes are also capable of degrading both lignin and cellulose of the cell walls. Coupled by the fact that these fungi are favoured by pH in the trees and hence function optimally, this may explain why many *O.usambarensis* trees with decay contain hollow stems (see, Willan, 1965; Dick, 1969; Kimaryo, 1971; Mugasha, 1978).

When the trees have been infected and developed the decay, some of the most conspicuous symptoms are, the presence of wounds and scars; presence of dead branches; dead branch stubs; stem bumps; stem cracks (which are also associated with butt rot); epicormic shoots or sprouts along the stem; and black exudates along the lower stem (also associated with butt rot). The most common sign on butts and stems of trees with decay is the presence of sporophores of *Phellinus senex* (II).

Trees in the Usambara forests are infected by relatively more fungal species than trees in the Kilimanjaro forests. Out of the total fungi isolated from the trees, 21% of them were common to both forests, 43% were only isolated from Usambara and 36% from Kilimanjaro (II). This susceptibility of *O.usambarensis* trees in the Usambara is most likely due to the sub-optimal growing conditions brought by climatic and

edaphic factors in the area. However, these differences can be verified further by more isolations of fungi from more sample trees than used in the current study.

5. CONCLUSION

The study has indicated that there may be more un reported diseases attacking many trees in the country than currently known. More surveys are therefore necessary to expose the diseases of both the indigenous and the exotic trees. *Armillaria* root rot disease seems to be the only one which is widely spread among various tree species, but apart from reporting its occurrence, no detailed studies have been carried out to determine its behaviour in relation to its hosts. Also, the identity of *Armillaria* species causing the root rots need to be known because the pathogens have been always reported as *Armillaria mellea* although many pathogenic *Armillaria* species do exist. Serious diseases have occurred in the country causing significant losses to plantation forestry. Further research should assist in the selection of resistant genotypes for breeding purposes because abandoning the affected species may only provide short term solutions as the alternative species may later be susceptible to other pathogens as well. However, the present review of tree diseases in the country has provided bench-mark information which will benefit further research and guide the management of the affected trees.

As far as *Ocotea usambarensis* is concerned, the stem decay of the tree species is not due to a single fungal species but rather it is a result of colonization by a number of fungi infecting the tree through a series of succession stages. The decay manifests itself as butt rot or decay of the main stem, and trees of all age classes are infected as the fungi may attack both the sapwood and the heartwood. Pioneering fungi in living sapwood are dominated by parasitic and facultative species which include *Ophiostoma* spp, *Ceratocystis* spp, *Botryosphaeria ribis*, *Cylindrocarpon destructans*, *Cylindrodendrum album*, *Pestalotiopsis* sp, *Nodulisporium* sp, *Leptodontidium* sp, some basidiomycetes and other fungi which could not be properly grouped because they had sterile mycelium including an important pathogen known in this study as 'Sterile mycelium sp 3'. The heart wood is dominated by saprotrophic and as well as some facultative fungi some of which act as pioneering fungi of the heartwood. The secondary and climax fungi are dominated by saprotrophic species which occupy the heartwood or dead sapwood and most of them (especially the basidiomycetes) are characterized by their inability to infect uncolonized heartwood. *Phellinus senex* which has been widely reported earlier as a primary decay fungus of *O. usambarensis* participates in the decay and forms a decay climax flora of the tree, but is not a primary decayer because it can not infect sapwood or heartwood which is uncolonized by other fungi. Although most of the fungi which can not infect intact sapwood or heartwood possess enzymes capable of detoxifying simple phenolic compounds such as gallic and tannic acids, it is most likely that the oil cineol which is present in *O. usambarensis* also contributes to the inhibitory effect on the fungi, although this needs to be tested.

Basidiomycetes form a small percentage of the decay fungi of the tree species, amounting to only 17% while non-basidiomycetes were the most abundant, forming up to 83% of the isolated fungi. Most of the fungi involved in the decay of the tree cause white rot because of their possession of phenoloxidase enzymes. White rot fungi are able to degrade both lignin and cellulose and hence this explains the presence of hollow stems in trees with advanced decay. Although basidiomycetes are numerically few, they cause significant decay in the trees in terms of the rate and magnitude of decomposition. However, non-basidiomycetes have also a significant contribution to the decay which may be equal or more to that of the basidiomycetes. This is due to their numerical advantage over basidiomycetes, their pioneering role in decay, and most important is the fact that some of them also possess enzymes capable of degrading the cell wall lignin.

The decay flora of *Ocotea usambarensis* is favoured by the environment in the forests and in the trees. The temperature of the forests where the tree grows allows for the optimal growth of the fungi while the pH in the trees allows for optimum ligninolytic activity to take place. Oxygen stress may also play a

significant role during succession of the fungi in decay columns by exerting a selection pressure to favour the stress tolerant species. *Phellinus senex* which is an important climax fungus and whose fruitbodies dominate on trees with decay, is significantly tolerant to the oxygen stress.

REFERENCES

Alcubilla von M., Diaz-Palacio, M.P., Kreutzer, W.L., Rehfuess, K.E. and Wenzel, G., 1971. Relationship between nutrition and heartrot attack of Norway spruce (*Picea abies* Karst.) and the fungistatic effect of its inner bark. **European Journal of Forest Pathology, 1:** 100-114.

Barklund, P. and Kowalski, T., 1996. Endophytic fungi in branches of Norway spruce with particular reference to *Tryblidiopsis pinastri*. **Canadian Journal of Botany, 74:** 673-678.

Blanchette, R.A., 1995. Degradation of the lignocellulose complex in wood. **Canadian Journal of Botany, 73(Suppl. 1):** S999-S1010.

Blanchette, R.A. and Shaw, C.G., 1978. Associations among bacteria, yeasts and basidiomycetes during wood decay. **Phytopathology, 68:** 631-637.

Blanchette, R.A., Sutherland, J.B. and Crawford, D.L., 1981. Actinomycetes in discoloured wood of living silver maple. **Canadian Journal of Botany, 59:** 1-7.

Boddy, L., 1983. The effect of temperature and water potential on growth rate of wood-rotting basidiomycetes. **Transactions of the British Mycological Society, 80:** 141-149.

Boddy, L. and Rayner, A.D.M., 1982. Population structure, intermycelial interactions and infection biology of *Stereum gausapatum*. **Transactions of the British Mycological Society, 78:** 337-351.

Boddy, L. and Rayner, A.D.M., 1983. Origins of decay in living deciduous trees: the role of moisture content and re-appreisal of the expanded concept of tree decay. **New Phytologist, 94:** 623-641.

Bryce, J.M., 1967. **The Commercial Timbers of Tanzania.** Forest Division, Utilisation Section. Ministry of Natural Resources and Tourism. Dar es Salaam, Tanzania. Pp 138.

Butcher, J.A., 1968. The ecology of fungi which infected untreated sapwood of *Pinus radiata*. **Canadian Journal of Botany, 46:** 1577-1589.

Dick, J.H., 1969. Heartwood development and heartrot in East African camphorwood, *Ocotea usambarensis* Engel. Tanzania Silviculture Research Note, No.9. Division of Forestry, Dar-es-Salaam. Unpublished.

Diwani, S.A., Kumburu, O., Mshiu, E.N. and Kisaka, E.Z., 1984. Preliminary report on the survey of forest tree diseases and pests in Sao Hill Forest Plantation. Ministry of Lands, Natural Resources and Tourism. Division of Forestry, Dar-es-Salaam. Unpublished.

Domsch, K.H., Gams, W. and Anderson, T., 1993. **Compendium of Soil Fungi, Vol. 1.** IHW-Verlag, Germany. Pp 859.

Ebbels, D.L. and Allen D.J., 1979. A supplementary and annotated list of plant diseases, pathogens and associated fungi in Tanzania. **Phytopathological Paper No. 22.** Commonwealth Agricultural Bureau. Kew, Surrey. England. Pp 89.

Forest Division, 1991. Magamba Forest Project Management Plan, 1991-1996/97. Ministry of Natural Resources and Tourism. Dar-es-Salaam, Tanzania. Unpublished.

Forrest, G.I., 1980. Preliminary work on the relation between resistance to *Fomes annosus*n and the monoterpene composition of sitka spruce resin. In: **Resistance to Diseases and Pests in Forest Trees.** Proceedings of the 3rd international workshop on genetics of host parasite interactions in forestry. Wageningen. Pp 194-197.

Gaümann, E., 1939. Über die Wachstums- und Zerstörungs-intensität von *Polyporus vaporarius* und von *Schizophyllum commune* bei verschiedenen temperaturen. **Angew. Bot. 21:** 59-69.

Gibbs, J.N., 1968. Resin and the resistance of conifers to *Fomes annosus*. **Annals of Botany, 32:** 649-665.

Gibson, I.A.S., 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February - 3rd March, 1962. Kenya Forest Division. Unpublished.

Gibson, I.A.S., 1965. Forest pathology in East Africa. **East African Agricultural and Forestry Journal, 31(2):**194-198.

Gibson, I.A.S., 1967. The present world situation in regard to the spread of internationally dangerous forest diseases. **East African Agricultural and Forestry Journal, 32(4):**478-483.

Gibson, I.A.S., 1975. **Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part One.** Important members of the Myritaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Forestry Institute. Oxford. pp 51.

Gray, V.R., 1958. The acidity of wood. **Journal of the Institute of Wood Science, 1:** 58-64.

Griffin, H.D., 1968. Forest tree diseases. Report to the Government of Tanzania. Report No.TA 2533. UNDP/FAO, Rome.

Griffin, D.M., 1971. Water potential and wood decay fungi. **Annual Review of Phytopathology, 15:** 319-329.

Hall, T.J. and Leben, C., 1985. A new method for evaluating decay: the effect of oxygen on *Polyporus compactus* in red oak. **Canadian Journal of Forest Research, 15:** 1021-1024.

Hartley, C., Davidson, R.W. and Crandall, B.S., 1961. Wetwood, bacteria and increased pH in trees. **US Forest Service. Forest Products Laboratory Report, 2215: 1-34.**

Henningsson, B., 1967. Physiology of fungi attacking birch and aspen pulpwood. **Studia Forestalia Suecia, No.52.**

Henningsson, B., 1968. Ecology of decomposition of birch and aspen. In: Walters, A.H. and Elphic, J.J. (eds), **Biodeterioration of materials.** Elsevier, Amsterdam. Pp 408-423.

Highley, T.L., 1975. Can wood-rot fungi degrade cellulose without other wood constituents? **Forest Products Journal, 25:**38-39.

Highley, T.L., Bar-Lev, S.S., Kirk, T.K. and Larsen, M.J., 1983. Influence of O_2 and CO_2 on wood decay by heartrot and saprot fungi. **Phytopathology, 73:** 630-633.

Hintikka, V., 1971. Tolerance of some wood-decomposing basidiomycetes to aromatic compounds related to lignin degradation. **Karstenia, 12:** 46-52.

Hintikka, V. and Korhonen, K., 1970. Effects of carbon dioxide on the growth of lignicolous and soil-inhabiting Hymenomycetes. **Communicationes Instituti Forestalia Fenniae, 62:** 1-22.

Holliday, P., 1989. **A Dictionary of Plant Pathology.** Cambridge University Press. Pp 369.

Huse, K.J., 1978. Discolouration and microflora in wounds due to thinning operations in stands of Picea abies (L.) Karst. A report to the Norwegian Forest Research Institute. Pp 54.

Huse, K.J., 1981. The distribution of fungi in sound-looking stems of *Picea abies* in Norway. **European Journal of Forest Pathology, 11:** 1-6.

Ivory, M.H. and Paterson, D.N., 1970. Progress in breeding *Pinus radiata* resistant to *Dothistroma* needle blight in East Africa. **Silvae Genetica, 19:**38-42.

Ivory, M.H. and Speight, M.R., 1993. Pest Management. In: Pancel, L. (ed.); **Tropical Forestry Handbook, Volume 2.** Springer-Verlag. Berlin, London, New York. Pp 1142-1219.

Johansson, M. and Stenlid, J., 1985. Infection of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*. I. Initial reaction in sapwood by wounding and infection. **European Journal of Forest Pathology, 12:** 346-357.

Kimaryo, P.E., 1971. Regeneration of *Ocotea usambarensis* Engl. At Sungwi, West Usambaras. Tanzania Silviculture Research Note, No. 21. Division of Forestry, Dar es Salaam Unpublished.

Ladejtschikova, E.I. and Pasternak, G.M., 1980. Biochemical aspects of the resistance of *Pinus sylvestris* to *Fomes annosus*. In: **Resistance to Diseases and Pests in Forest Trees.** Proceedings of the 3rd international workshop on genetics of host parasite interactions in forestry. Wageningen. Pp 198-200.

Levy, J.F., 1982. The place of basidiomycetes in the decay of wood in contact with the ground. In: Frankland *et al.* (Eds), **Decomposer Basidiomycetes, their Biology and Ecology.** British Mycological Society Symposium 4. Cambridge University Press, Cambridge, London. Pp 161-178.

Liese, W., 1970. Ultrastructural aspects of wood tissue disintegration. **Annual Review of Phytopathology, 8:** 231-257.

Lundgren, B., 1978. Soil conditions and nutrient cycling under natural and plantation forests in Tanzania highlands. **Report in Forest Ecology and Forest Soils, No.31.** Swedish University of Agricultural Sciences.

Mercer, P.C., 1982. Basidiomycete decay of standing trees. In: Frankland *et al.* (Eds), **Decomposer Basidiomycetes, their Biology and Ecology.** British Mycological Society Symposium 4. Cambridge University Press, Cambridge, London. Pp 141-160.

Mugasha, A.G., 1978. Tanzania natural forests' silvicultural research review report. Tanzania Silvicultural Technical Note (New Series) No. 39. Division of Forestry, Dar es Salaam. Unpublished.

Mwamba, B.K., 1986. The ecology and distribution of *Ocotea usambarensis* in the Uluguru mountains. A special project report. Faculty of Forestry, Sokoine University of Agriculture. Morogoro, Tanzania. Unpublished.

Nobles, M.K., 1965. Identification of cultures of wood-inhabiting hymenomycetes. **Canadian Journal of Botany, 43:**1097-1139.

Olembo, T.W., 1969. The incidence of cypress canker disease in E. Africa. **East African Agricultural and Forestry Journal, 35(2):**166-173. Jensen, K.F., 1967. Oxygen and carbon dioxide affect the growth of wood-decay fungi. **Forest Science, 13:**384-389.

Packman, D.F., 1960. The acidity of wood. **Holzforschung, 14:** 178-183.

Pitt-Schenkel, C.J.W., 1938. Some important communities of warm temperate rain forest at Magamba, West Usambara, Tanganyika Territory. **Journal of Ecology, 26**: 50-81.

Popoff, T., Theander, O. and Johansson, M., 1975. Changes in sapwood of roots of Norway spruce attacked by *Fomes annosus*. Part II. Organic chemical constituents and their biological effects. **Physiologia Plantarum, 34:** 347-356.

Pòcs, T., 1988. The importance of catchment forests to Tanzania. Professorial inaugural lecture, Sokoine University of Agriculture. Unpublished.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Renvall, P. and Niemelä, T., 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. **Bulletin Jardin Botanique National de Belgique, 62:** 403-414.

Ride, J.P., 1983. Cell walls and other structural barriers in defence. In: Callow, J.A., (ed). **Biochemical Plant Pathology.** Wiley Interscience Publication. Chichester, New York, Toronto. Pp 215-236.

Roll-Hansen, F. and Roll-Hansen H., 1979. Microflora of sound-looking wood in *Picea abies* stems. **European Journal of Forest Pathology, 9:** 308-316.

Rudman, P.,1963. The causes of natural durability in timber. XI. Some tests on the fungi toxicity of wood extractives and related compounds. **Holzforschung, 17:** 54-57.

Salisbury, F.B. and Ross, C.W., 1992. **Plant Physiology**. Fourth edition. Wadsworth Publishing Company. Belmont, California. Pp 682.

Scheffer, T.C. and Cowling, E.B., 1966. Natural resistance of wood to microbial deterioration. **Annual Review of Phytopathology, 4:** 147-170.

Shain, L., 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. **Phytopathology, 61:**301-307.

Shain, L., 1979. Dynamic responses of differenciated sapwood to injury and infection. **Phytopathology, 69:** 1143-1147.

Shain, L. and Hillis, W.E., 1971. Phenolic extractives in Norway spruce and their effects on *Fomes annosus*. **Phytopathology, 61:** 841-845.

Sharon, E.M., 1973. Some histological features of Acer saccharum wood formed after wounding. **Canadian Journal of Forest Research, 3:** 83-89.

Shigo, A.L., 1966. Decay and discolouration following logging wounds on northern hardwoods. **U.S Forest Service Research Paper NE-47**. Forest Service, Department of Agriculture. Northeast Forest Experiment Station. Upper Darby, Pa. Pp 43

Shigo, A.L., 1967. Succession of organisms in discolouration and decay of wood. **International Review of Forest Research, 2: 238-299.** Academic Press Inc., New York.

Shigo, A.L., 1976. Microorganisms isolated from wounds inflicted on red maple, paperbirch, American beech, and red oak in winter, summer, and autumn. **Phytopathology, 66:** 559-563.

Shigo, A.L. and Hillis, W.E., 1973. Heartwood, discoloured wood and microorganisms in living trees. **Annual Review of Phytopathology, 11:** 197-222.

Shigo, A.L. and Marx, H.G., 1977. Compartmentalization of decay in trees. **US Department of Agriculture, Forest Service Agricultural Information Bulletin, No. 405.**

Shigo, A.L. and Sharon, E.M., 1970. Mapping columns of discoloured and decayed tissues in sugar maple, *Acer saccharum*. **Phytopathology, 60:** 232-237.

Shortle, W.C., 1979. Mechanisms of compartmentalization of decay in living trees. **Phytopathology, 69:**1147-1151.

Shortle, W.C. and Cowling, B.E., 1978. Development of discolouration, decay, and microorganisms following wounding of sweetgum and yellow-poplar trees. **Phytopathology, 68:** 609-616.

Shortle, W.C., Menge, J.A. and Cowling, B.E., 1978. Interaction of bacteria, decay fungi, and live sapwood in discolouration and decay in trees. **European Journal of Forest Pathology, 8:** 293-300.

Shortle, W.C., Tattar, T.A. and Rich, A.E., 1971. Effects of some phenolic compounds on the growth of *Phialophora melinii* and *Fomes connatus*. **Phtopathology, 61:** 552-555.

Shrimpton, D.M. and Whitney, H.S., 1968. Inhibition of growth of blue stain fungi by wood extractives. **Canadian Journal of Botany, 46:** 757-761.

Stalpers, J.A., 1978. Identification of wood inhabiting Aphyllophorales in pure culture. **Studies in Mycology No. 16.** CBS Institute of the Royal Netherlands Academy of Arts and Sciences. Pp 248.

Tachibana, S., 1968. On the mechanisms of L-malic acid fermentation using *Schizophyllum commune* Fries. **Mushroom Science, VII:** 273-280.

Taiz, L. and Zeiger, E., 1991. **Plant Physiology**. Benjamin / Cummings Publishing Co. Inc., New York, Amsterdam. Pp 565.

Taylor, J.B., 1974. Biochemical tests for identification of mycelial cultures of basidiomycetes. **Annals of Applied Biology, 78:** 113-123.

Toole, E.R., 1961. Rot entrance through dead branches of southern hardwoods. **Forest Science, 7:** 218-226.

Van der Kamp, B.J., 1975. The distribution of microorganisms associated with decay of western red cedar. **Canadian Journal of Botany, 5:** 61-67.

Wagner, W.W. and Davidson, R.W., 1954. Heartrots of living trees. **Botanical Review, 20:** 61-134.

Willan, R.L., 1965a. Natural regeneration of high forest in Tanganyika. **East African Agricultural and Forestry Journal, 31:**43-53.

Willan, R.L., 1965b. A master plan for silvicultural research in Tanzania. Forest Division, Ministry of Agriculture, Forests and Wildlife, Dar-es-Salaam. 26 pp.

Woodward, S. and Pearce, R.B., 1988. The role of stilbenes in resistance of Sitka spruce (*Picea stichensis* (Bong.) Carr.) to entry of fungal pathogens. **Physiological and Molecular Plant Pathology, 33:** 151-162.

Zulu, T.S., 1991. Introduction to the major forest diseases in Malawi. In: Chilima, C.Z.(ed.), **Proceedings of the 1991 Forest Pest and Disease Monitoring course.** Forestry Research Institute of Malawi. Pp 15-19.

PAPER I

Forest fungal diseases of Tanzania: background and current status

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A review of the background and current status of forest diseases in Tanzania is presented in this article. Outbreaks of the most destructive exotic and indigenous diseases are addressed and currently known diseases of both indigenous and exotic trees, including ornamental and agroforestry trees, are tabulated to form a preliminary checklist. It is concluded that more knowledge on forest diseases is still required and therefore further research is necessary to reveal the full extent of the diseases in both natural and plantation forests.

Key words: Check list, forest diseases, fungal pathogens, Tanzania.

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Tanzania has a vast forest area which covers 44 million ha (about half the country's total area) of which 80,000 ha comprises plantations. These forests have about 10,000 species of indigenous higher plants (Polhill 1968) of which more than 1,200 are tree species (Wilan 1965). If conservation and productivity are to be sustained, the natural and plantation forests will have to be protected against fire, indiscriminate cutting, encroachment and, equally importantly, against pests and diseases. Whilst the protection of the forests against hazards which are related to human activities can partly be achieved through law enforcement and use of extension services, protection against diseases requires, in addition, a much more integrated approach incorporating specialized knowledge on the types and nature of the diseases. Full utilization of such an approach has not yet been achieved, due to insufficient knowledge about diseases of many tree species growing in the country. This deficiency is also one of the major problems facing forest managers in the monitoring and reporting of diseases which prevail in their forests. This implies also that if such knowledge were available it would be incorporated in formulating management programmes involving protection of forests against potential diseases. Therefore, the role of research in providing such knowledge is of utmost importance.

Any information on tree diseases in Tanzania (for both indigenous and exotic trees, including ornamental and agroforestry trees) which is available today tends to be diffuse,

and the aim of this review is therefore to explain how the existing knowledge about forest diseases came about and to compile a list of the diseases that are currently scattered in various literature as a basis for reference and future work. This is achieved by presenting the background to forest disease research and knowledge in the country, discussing the most important disease outbreaks which have occurred, and then tabulating the currently known diseases of both indigenous and exotic trees, including ornamental and agroforestry trees.

BACKGROUND TO FOREST DISEASE RESEARCH AND KNOWLEDGE

In East Africa, research in plant diseases including forest trees was conducted after the World War II by the then East African Agriculture and Forestry Research Organization (EAAFRO) which came under the auspices of the now defunct East African Community. EAAFRO catered for Tanzania, Kenya and Uganda. The section in EAAFRO dealing with forest diseases was formed as a result of an increase in disease outbreaks in exotic tree plantations (Gibson 1965a) which were established to supplement timber production from the natural forests. Research reports which are currently available indicate that the emphasis was on diseases of exotic trees such as pines, cypress, eucalyptus, teak, and others grown in E. Africa. Some diseases of indigenous trees of Tanzania are mentioned in some checklists (e.g. Riley 1960; Peregrine & Siddigi 1972; Ebbels & Allen 1979) which give the names of the host and pathogen and the kind of disease caused. Research reports by EAAFRO provide details on the infection biology, spread, economic impact of the diseases and the limitations to most pathogens in the case of exotic plantation trees while very few indigenous trees were covered in such detail. Sometimes pathological defects of indigenous trees of economic importance were mentioned in other fields of forestry, such as mensuration (e.g. Paterson 1965).

After the departure of most expatriate staff from EAAFRO and the break-up of the East African Community in February 1977, very little research was conducted in both the plantations and the natural forests due to the lack of/or very few local forest pathologists. The bulk of knowledge on forest diseases of indigenous and plantation forests and in related fields such as mycology is thus currently limited to reports by the EAAFRO and a few researchers who either visited the country or worked in the government departments of agriculture or forestry. Most of the work is chiefly acknowledged to the invaluable contribution by researchers from Britain, Nordic/Scandinavian and North American countries and the FAO who worked in, or visited E. Africa in the past. The researchers were plant pathologists or mycologists and some of the most important reports are those by Gibson (1956, 1957, 1960, 1962, 1965a, 1965b, 1966a, 1966b, 1967, 1968, 1975), Gibson & Corbett (1964), Procter (1965, 1967), Ivory (1967), Griffin (1967, 1968), Hocking & Jaffer (1967), Hocking (1968), Howland & Gibson (1969), Olembo (1969, 1972), Ryvarden (1972), Allen (1975a, 1975b), Ebbels & Allen (1979) and Ryvarden & Johansen (1980). Since then, only a very small amount of research has been accomplished by local and visiting researchers (e.g. Waring 1982; Diwani et al. 1984; Canon 1985; Tangwa et al. 1988; Renvall & Niemela 1993), and therefore, the gap in knowledge on diseases of indigenous trees and fungi in general is still enormous.

FOREST DISEASE OUTBREAKS

The introduction of exotic tree species provided an opportunity for the emergence of new diseases which were previously only found in the native countries (Gibson 1967; Griffin 1968). Root pathogens such as *Poria* sp., *Helicobasidium compactum* and *Ustulina deusta* were introduced in E. Africa with the exotics (Griffin 1968). Today, there is a considerable risk of loss from diseases in many tree species due to the increase in the number of pathogens.

Among the most serious diseases which were "imported" are the *Dothistroma* blight of pines caused by the ascomycete fungus *Mycosphaerella pini* (syn. *Scirrhia pini*, imperfect stage: *Dothistroma pini*), the cypress canker caused by *Rhynchosphaeria cupressi* (syn. *Leptentypa cupressi*; imperfect stage: *Monochaetia unicornis*) and, recently, a severe leaf spot disease of *Eucalyptus maidenii* caused by *Mycosphaerella molleriana* (imperfect stage: *Sphaeropsis molleriana*).

The *Dothistroma* blight, first observed in northern Tanzania in 1958 at Shume forest plantations (Ivory & Paterson 1970), spread vigorously and virtually wiped out the young plantations of *Pinus radiata* in E. Africa and Malawi within 20 years. The disease led the governments of E. Africa to abandon further planting of *P. radiata* in 1964 (Diwani et al. 1984) and the government of Malawi to clearfell its last compartment of *P. radiata* in 1978 (Zulu 1991). *P. radiata* was a superior conifer tree in terms of wood quality and was comparable to the most durable indigenous timber trees growing in the region. Similarly, the planting of *Cupressus macrocarpa* (which was very susceptible to the *Monochaetia* canker) had been stopped earlier in E. Africa in the early 1950s and replaced with *C. lusitanica*, which is less susceptible to the pathogen (Olembo 1969).

The leaf spotting fungus *M. molleriana* (first observed in Tanzania in 1991) has attacked *Eucalyptus maidenii* throughout the country causing severe necrotic spots leading to foliage drying and defoliation in nursery seedlings, coppice sprouts and in young plantation trees which have not acquired their mature foliage form. Mature foliage is also attacked in some trees but the damage is mild when compared to the juvenile foliage and no defoliation occurs. There is a possibility that the pathogen has spread throughout E. Africa due to similarity in the climate throughout the region. The fungus was previously reported in Brazil as "unusually severe" (Gibson 1975) and as "serious" in Malawi (Zulu 1991). In South Africa, a mycosphaerella leaf disease was first reported on an unspecified *Eucalyptus* sp. as early as 1923, but a few years later it was reported as "very serious" on *E. maidenii* and *E. globulus* to the extent that the two eucalyptus species were abandoned as commercial forest species of South Africa (Lundquist 1987). Three species of this fungal genus, namely *M. molleriana*, *M. heimii* and *M. nubilosa*, have been reported to attack foliage of eucalyptus trees in Africa (Gibson 1975). In South Africa the *Mycosphaerella* leaf disease has been found on 10 eucalyptus species and pathogens were identified as being *M. molleriana* and *M. nubilosa* (Lundquist 1987). This therefore implies that Tanzania is facing yet another serious disease outbreak which is capable of causing great damage to the many eucalyptus species growing in the country.

Serious indigenous diseases also exist which have caused an unquantified amount of loss to economically important timber trees. Among these diseases is the heart rot of stem of *Ocotea usambarensis* (East African camphor tree) which has been attributed to a number

of basidiomycete fungi with the most widely reported pathogen being *Phellinus senex* (syn. *Fomes senex*; *Polyporus senex*). There are many fungi that attack the tree species and the ones that are most likely to cause decay are mentioned by Ebbels & Allen (1979) and Renvall & Niemelä (1993). Damage to the tree by the pathogens is enormous and in most localities where this species grows, for example the Uluguru mountains, its regeneration capacity has been affected because many stumps which could have had coppice or root sucker regeneration are simply rotting away (Mwamba 1986). In its favourite habitats in the Usambara and Kilimanjaro mountains the species is also in decline, which is manifest in some trees of all age classes as dieback of leading branches and stem-decay symptoms. Hamilton et al. (1989) described the virtual total lack of regeneration of *O. usambarensis* in the East Usambara mountains as a "remarkable feature", suggesting the severity of the problem. The species used to be a potential commercial tree which provided round and sawn timber for local and export markets, but now its supply has been greatly affected by the decay diseases.

Another native disease is the root rot of numerous tree species caused by *Armillaria mellea* (s.l). It has been reported that *A. mellea* destroyed 66% of a large compartment of pines and *Grevillea robusta* trees (specific size unspecified) at Usa River in the Mount Meru Forest Project, northern Tanzania (Diwani et al. 1984).

The presence of indigenous and exotic trees in the same ecosystem creates possibilities for the exotic pathogens to attack indigenous hosts and the indigenous pathogens to attack the exotic hosts. This interaction brings about a complex combination of disease problems which could probably be easily controlled in the native ecosystems. For example, the tropical trees are said to be only slightly susceptible to infection by *A. mellea* root disease, but when a conifer plantation is established on a site with contaminated stumps or other plant debris from a cleared natural forest, heavy damage to the conifers is normally expected (Gibson 1960; Olembo 1972). This also means that new strains of disease agents (which could be more virulent) could have evolved to attack new hosts as a result of changes in climate and food characteristics. Gibson & Corbett (1964) found that *A. mellea* in Malawi existed in various forms while the same situation has been found also in Europe (e.g., Raabe 1980). Today, *Armillaria* is the most widely reported disease found on 15% of all the tree species reported to have one or more diseases (see Tables 1 and 2). The extent to which the exotic pathogens have spread and infected the indigenous hosts in Tanzania has yet to be determined because no comprehensive surveys have been conducted in the natural forests.

KNOWN DISEASES OF INDIGENOUS AND EXOTIC TREES

Research during the past 50 years has shown an increase in the number of forest disease outbreaks in Tanzania. This fact is partly explained by Tables 1 and 2 which present the currently known diseases of indigenous and exotic trees, respectively. The tables form a preliminary checklist into which new diseases (formerly unidentified or unreported) can be added. The information has been gathered from various reports written since the introduction of forest disease research in East Africa.

Table 1. Known fungal diseases of indigenous tree species in Tanzania

Tree species	Pathogen	Disease/part infected	
ADANSONIA DIGITATA	*Leveillula taurica* (mildew)	Leaves	(6)[1])
AFZELIA QUANZENSIS	*Microstoma* sp.	White leaf spot	(2)
ALBIZIA VERSICOLOR	*Phomopsis mendax*	Dieback	(6)
ALBIZIA PETERSIANA	*Phomopsis mendax*	Dieback	(6)
ANTHIOCLEISTA ORIENTALIS	*Pucciniosira mitragynes* (Rust)	Leaves	(6)
ARUNDINARIA ALPINA	*Engleromyces goetzii*	Stem canker	(6)
BRACHYSTEGIA SPICIFORMIS	*Oidium* sp. (mildew)	Leaves	(2)
	Phyllachora brachystegiae	Leaf spot	(2)
BRACHYSTEGIA SP.	*Perisporiopsis brachystegiae*	Black spots on leaves	(6)
CALODENDRUM CAPENSE	*Phloeospora* sp.	Leaf spot	(6)
CASSIA SINGUEANA	*Ravenelia baumiana* (Rust)	Leaves	(2)
CEPHALOSPHAERA USAMBARENSIS	*Armillaria mellea* s.l.	Root rot	(30)
COMBRETUM MOLLE	*Uredo combreticola* (Rust)	Leaves	(1)
COMBRETUM PURPUREIFLORUM	*Aecidium* sp. (Rust)	Leaves	(2)
DALBERGIA NITIDULA	*Mycosphaerella dalbergiae*	Leaf spot	(6)
	Phomopsis dalbergiae	Leaf spot	(6)
	Phyllachora dalbergiae	Leaf spot	(2)
	Uredo sp. (rust)	Covers foliage	(2)
ELAEIS GUINEENSIS (oil palm)	*Cercospora elaeidis*	Leaf freckle	(6)
	Pestalotiopsis palmarum	Leaf spot	(6)
EUPHORBIA TIRUCALLI	*Sphaeropsis euphorbiae*	Stem canker	(6)
HARUNGANA MADAGASCARIENSIS	*Pestalotia harongae*	Leaf spot	(6)
JUNIPERUS EXCELSA (PROCERA)	*Antrodia juniperina*		
	(syn. *Agaricus juniperina*)	Cubical stem rot	(43)
	Calisopsis nigra	Galls	(6)
	Daedalea juniperina	Stem rot	(6)
	Daedalea quercina	Cubical stem rot	(6)
	Omphalotus olearius	Stump decay	(6)
	Pyrofomes demidoffii		
	(syn. *Polyporus demidoffii*)	Branch/stem rot(Pers. Comm.) [2])	
	Ganoderma lucidum	Stem rot (Pers. Comm.)	
KHAYA ANTHOTHECA (NYASICA)	*Meliora khayae*(Sooty mildew)	Premature defoliation	(6)
MACARANGA KILIMANDSCHARICA	*Englerula macarangae*	Leaves	(2)
MAESOPSIS EMINII	*Fusarium solani*	Stem canker	(6)
MARKHAMIA OBTUSIFOLIA	*Cladosporium oxysporum*	Leaf blight	(2)
	Mycosphaerella sp.	Leaf blight	(2)
MILICIA (CHLOROPHORA) EXCELSA	*Armillaria mellea* s.l.	Root rot	(20)
	Helicobasidium brebissonni		
	(syn. *H.purpureum, Rhizoctonia crocorum*)	Purple butt/root rot	(20)
NUXIA CONGESTA	*Phellinus punctatus*	Stem rot (Pers. Comm.)	
	Oxyporus populinus	Stem rot (Pers. Comm.)	
OCOTEA USAMBARENSIS	*Armillaria mellea* s.l.	Root rot (Pers. Comm.)	
	Loweporus inflexibilis	Root and butt rot	(40)
	Pestalotiopsis sp.	Leaf blotch (Pers. Comm.)	
	Phellinus (Fomes) allardii	Stem dacay	(40)
	Phellinus apiahynus	Root and stem decay	(40)
	Phellinus senex	Heart rot	(10)
	Stereum hirsutum	Streaked white rot	(6)
OLEA CAPENSIS (WELWITSCHII)	*Alternaria porri*	Seedling leaf spot	(6)
	Alternaria tenuissima	Seedling collar rot	(6)
	Cladosporium oxysporum	Seedling leaf spot	(2)
	Macrophomina phaseolina	Seedling collar rot	(6)
	Meliora petiolaris (Sooty mildew)	Leaf spot/defoliation	(2)
	Myrothecium verrucaria	Seedling collar rot	(6)

Table 1. Cont.

Tree species	Pathogen	Disease/part infected	
PACHYSTELA MSOLO	*Helminthosporium pachystelae*	Leaf spot	(6)
PODOCARPUS USAMBARENSIS	*Ganoderma australe*	Stem rot	(Pers. Comm.)
RAPANEA SP.	*Stereum hirsutum*	Timber decay	(6)
STERCULIA AFRICANA	*Macrophyllosticta sterculiae*	Leaf net-spot	(6)
STOEBE KILIMANDSCHARICA	*Aecidium elytropappi* (Rust)	Leaves	(6)
STRYCHNOS POTATORUM	*Cercospora strychni*	Leaf blight	(6)
	Mycosphaerella sp.	Leaf spot	(6)
	Sirosporium sp.	Leaf blight	(6)
STRYCHNOS STUHLMANII	*Phyllosticta strychni*	Leaf spot	(6)
SYNADENIUM GRANTII	*Phyllosticta* sp.	Leaf spot	(2)
TAMARINDUS INDICA	*Gloesporium tamarindi*	Leaf spot	(6)
	Mycosphaerella tamarindi	Leaf spot	(6)
TECLEA NOBILIS	*Puccinia tecleae (rust)*	Leaves	(6)
TECLEA SIMPLICIFOLIA	*Puccinia tecleae*	Leaves	(6)
TRICHILIA EMETICA (ROKA)	*Cercospora* sp.	Leaf spot	(6)

[1]) Numbers in parentheses correspond to the numbers given in the list of references to indicate the source of the information. For convenience, only one reference per disease is provided
[2]) Pers. Comm. = Personal Communication

Table 2. Known fungal diseases of exotic tree species in Tanzania

Tree species	Pathogen	Disease/part infected	
ACACIA MEARNSII (MOLLISMA)	*Poria vincta* var. *cinerea*	Root rot	(6)[1])
	Stereum hirsutum	Timber decay	(6)
ACACIA MELANOXYLON	*Poria vincta* var. *cinerea*	Root rot	(6)
ACACIA SPP.	*Ravenelia volkensii*	Witches broom	(6)
	Armillaria mellea s.l.	Root rot	(6)
ALBIZIA LEBBECK	*Phomopsis mendax*	Dieback	(2)
	Uredo ngamboensis (rust)	Defoliation	(6)
ANACARDIUM OCCIDENTALE (cashew)	*Gliocladium roseum*	Dieback	(6)
	Oidium anacardii (mildew)	Kills inflorescence	(Pers. Comm.[2])
BAUHINIA SP.	*Oidium* sp.	Pods	(6)
CAMELLIA SINENSIS	*Phomopsis theae*	Collar and branch canker	(6)
CASSIA ABSUS	*Ravenelia berkleyi* (Rust)	Leaves	(2)
CASSIA ALATA	*Phomopsis cassiae*	Wilt and Dieback	(6)
CASSIA FLORIBUNDA	*Macrophomina phaseolina*	Black root rot	(18)
CASSIA LAEVIGATA	*Macrophomina phaseolina*	Black root rot	(18)
CASSIA OBTUSIFOLIA	*Aecidium cassiae* (Rust)	Leaves	(6)
	Fusarium sp. (?*F. avenaceum*)	Stem canker	(6)
	Oidium sp.	Leaves	(6)
CASSIA OCCIDENTALIS	*Oidium* sp.	Leaves	(6)
	Pseudoperonospora sp.	Disc-spot of leaves	(6)
CASSIA SENNA	*Corticium rolfsii*	Wilt	(18)
	Fusarium oxysporum	Seedling death	(6)
CASSIA SIAMEA	*Cercosporidium cassiae*	Defoliation	(18)
	Oidium sp.	Defoliation	(1)
	Polyporus baudoni	Root rot	(35)

Table 2. Cont.

Tree species	Pathogen	Disease/part infected	
CASSUARINA MONTANA	*Armillaria mellea* s.l.	Root rot	(6)
CEDRELLA SPP.	*Armillaria mellea* s.l.	Root rot	(18)
	Ceratocystis moniliformis	Dieback	(18)
CINNAMOMUM CAMPHORA	*Armillaria mellea* s.l.	Root rot	(6)
CINNAMOMUM ZEYLANICUM	Colletotricum cingulata	Leaf spot	(6)
	Phyllosticta sp.	Leaf spot	(6)
CITRUS AURANTIFOLIA (lime)	*Gloeosporium limetticola*	Withertip	(6)
CITRUS LIMON (lemon)	*Alternaria citri*	Leaf spot	(6)
	Fusarium sp.	Rough lemon of nursery seedlings	(6)
CITRUS PARADISI (grape fruit)	*Fusarium solani*	Root gummosis	(6)
CITRUS SINENSIS (orange)	*Phytophthora nicotianae* var.*parasitica*	Gummosis	(6)
COCOS NUCIFERA (coconut)	*Asteridium ferrugineum*	Sooty mould	(6)
	Ganoderma sp.	Stem rot	(6)
	Gloesporium sp.	Nut fall and calyx end rot	(6)
	Lasmeniella cocoes	Leaf spot	(6)
	Marasmiellus coccophilus	Lethal bole rot	(6)
	Phytophthora palmivora	Nut fall and Calyx end rot	(6)
	Pseudoepicoccum cocos	Zonate leaf spot	(6)
	Zukalia stuhlmanniana (Sooty mould)	Leaves	(6)
CUPRESSUS ARIZONA	*Rhynchosphaeria cupressi*	Stem canker	(32)
CUPRESSUS LINDLEY	*Rhynchosphaeria cupressi*	Stem canker	(5)
CUPRESSUS LUSITANICA	*Armillaria* s.l.	Butt rot	(33)
	Coriolus versicolor	White sap rot of timber	(6)
	Fusicoccum tingens	Associated with death of young trees	(6)
	Peniophora ceberella	Butt rot	(6)
	Poria vincta var. *cinerea*	Root rot	(6)
	Poria vaillantii	Brown cubical rot of timber	(6)
	Rhynchosphaeria cupressi (syn. *Leptentypa cupressi*)		
(Imperfect stage:	*Monochaetia unicornis*)	Stem canker	(6)
DELONIX (PONCIANA) REGIA	*Inonotus ochroporus*	Wood rot	(6)
EUCALYPTUS MAIDENII	*Mycosphaerela molleriana*		
(Imperfect stage:	*Sphaeropsis molleriana*)	Leaf spot & defoliation	(Pers.Comm.)
EUCALYPTUS SPP.	*Armillaria mellea* s.l.	Root rot	(18)
EUGENIA AROMATICA (clove)	*Armillaria mellea* s.l.	Root rot	(18)
	Botryodiplodia sp.	Seedling death	(6)
	Endothia eugeniae	Dieback	(6)
	Valsa eugeniae	Sudden death of trees	(6)
EUGENIA JAMBOS	*Endothia eugeniae*	Dieback	(6)
FICUS ELASTICA	*Colletotricum cingulata*		(6)
	(Perfect state: *Glomerella cingulata*)	Leaves	(6)
	Phoma atrocincta	Petiole	(1)
GMELINA ARBOREA	*Irpex flavus*	White sap rot of logs	(35)
	Polyporus baudoni	Root rot	(35)
	Xylosphaera (Xylaria) multiplex	Butt rot and death	(6)
GREVILLEA ROBUSTA	*Armillaria mellea* s.l.	Root rot	(5)
MANGIFERA INDICA	*Capnodium mangiferae*	Leaf spot	(6)
	Dimerosporium mangiferae	Sooty mould	(6)
	Phyllostica mangiferae	Leaf spot	(6)
PARKINSONIA ACULEATA	*Ganoderma vanmeelii*	Wood rot	(6)
PERSEA AMERICANA	*Curvularia intermedius*	Leaves	(6)

Table 2. Cont.

Tree species	Pathogen	Disease/part infected	
PHOENIX DACTYLIFERA	*Zukalia stuhlmanniana*	Leaves	(6)
PINUS SPP.	*Alternaria* sp.	Tip dieback, *P. patula*	(6)
	Armillaria mellea s.l.	Root rot	(9)
	Botryodiplodia theobromae (syn. *Diplodia natalensis*)	Needle blotch	(24)
	Cercospora pini-densiflorae	Needle blight	(6)
	Cladosporium sp.	Seedling browning and dieback in *P. patula*	(6)
	Fusarium oxysporum	Tip blight of *P. patula*	(6)
	Fusarium sp.	Damping off	(7)
(Perfect stage:	*Fusicoccum tingens* *Botryosphaeria ribis*)	Dead top of *P. patula* & *P.radiata*; Dieback of *P. caribaea*	(25)
	Mycosphaerella pini (syn. *Scirrhia pini*)		
(Imperfect stage:	*Dothistroma pini*)	Needle blight in *P. radiata*, *P. caribaea* & *P. montezumae*	(6)
	Mycosphaerella pinicola	Needle blight	(6)
	Naemacyclus niveus	Needle cast, *P. radiata*	(6)
	Pestalotiopsis cruenta	Needle blotch and cast	(6)
	Phytophthora spp.	Damping off	(8)
	Pythium spp.	Damping off	(7)
	Sphaeropsis sapinea (syn. *Diplodia pinea*)	Shoot dieback	(24)
	Stereum sanguinolentum (Syn *Haematostereum sanguinolentum*)	Stem and log decay	(19)
	Thanatephorus cucumeris (Imperfect stage: *Rhizoctonia solani*)	Damping off	(7)
TECTONA GRANDIS	*Armillaria mellea* s.l.	Root rot	(20)
	Cephaleuros sp. (algae fungus)	Leaf spot	(6)
	Fusarium semitectum	Root	(22)
	Fusarium solani	Canker, wood pink stain.	(23)
	Helicobasidium compactum	Violet root rot	(22)
	Nectria haematococca	Stem canker in nurseries	(6)
	Poria sp.	Root rot	(22)
	Rhizoctonia sp.	Root	(6)
	Ustulina deusta	Stem	(22)
TERMINALIA CATAPPA	*Cercospora catappae*	Leaf spot	(6)
TERMINALIA IVORENSIS	*Mycosphaerella* sp.	Leaf blotch	(6)
THEOBROMA CACAO	*Calonectria rigidiuscula*		(6)
(Imperfect state:	*Fusarium decemcellulare*)	Dieback	(6)
	Cercospora sp.	Leaf spot	(6)
	Fusarium solani	Roots	(6)
	Phomopsis folliculicola	Dieback	(6)
TOONA CILIATA	*Pestalotiopsis disseminata*	Stem canker	(6)
	Thyronectria pseudotrichia	Stem necrosis and twig dieback	(6)

[1]) Numbers in parentheses correspond to the numbers given in the list of references to indicate the source of the information. For convenience, only one reference per disease is provided

[2]) Pers. Comm. = Personal Communication

CONCLUSION

The list of diseases presented in Tables 1 and 2 shows the existence of indigenous and exotic pathogens which can cause severe damage to trees. Some pathogens are capable of attacking more than one host species and may therefore be difficult to control. The list also shows that only 36 indigenous and 45 exotic tree species have been covered so far. However, owing to the fact that Tanzania has a vast forest area and an enormous species diversity, it is justifiable to speculate that there must be many more diseases attacking more tree species than are presented in this report. Moreover, the tables report more diseases of exotic trees than those of indigenous trees although indigenous species in the country are far more numerous than exotic species. The reason for this is that in the past the emphasis was on exotic trees as timber supplements of the indigenous forest trees and also because many of the exotics are grown as plantation and ornamental trees in areas that are easily accessible to foresters and researchers.

Outbreaks have also been significant and severe. Consequently, future prevention of such epidemics should be given priority in forest management programmes. Some initiatives to address the problems through promoting resistance in susceptible species can be taken. For example, some pioneer research was carried out to select resistant genotypes of *P. radiata* against the *Dothistroma* blight (Ivory & Paterson 1970). Although this work was not continued, due to the paucity of experts and other resources which faced forest disease research, it was a good starting-point towards the revival of the conifer in East Africa.

Successful results in research on breeding for disease resistance in some susceptible species in other parts of the world have added impetus to tree breeding. For example, in New Zealand clones of *Pinus radiata* resistant to the *Dothistroma* needle blight are already under development through gradual selection for healthy trees (Ivory & Speight 1993). In the USA it was possible to establish resistant varieties of chestnut trees (*Castanea dentata*) through hybridization of the native survivors of the chestnut blight fungus, *Endothia parasitica* syn. *Cryphonectria parasitica* with the more resistant members of the genus from Europe and Asia (Beattie & Driller 1954). This means that tree breeding in Tanzania can also make use of the common hybridization principles used in tree improvement in order to establish disease resistant forests.

An effort has also been taken to investigate how silvicultural and cultural methods in forest management could limit disease incidence. For example, trials of *O. usambarensis* were established in natural forests of the Usambara and Kilimanjaro mountains in the late 1950s to determine the best treatments in regenerating the species in order to reduce the incidence of transmitting the heart rot of stem and butt to the next regeneration (Kimaryo 1972; Mugasha 1978). Again, owing to lack of forest disease experts and other research resources, this research has had to be abandoned. As a result of insufficient research projects, there has been a stagnation in knowledge on forest diseases and the subsequent efforts to control them. Given that resources were available, possible areas of emphasis would be to carry out more surveys covering all forest types; to study how breeding techniques can be used to develop resistance in susceptible species; to study the effect of diseases on the regeneration capacity of forests; to study the ecological factors and management techniques that might limit the establishment and spread of disease; and to study the effect of diseases on wood quality. Such studies will provide information which

will help forest managers to include certain measures in the protection of forests against potential diseases when formulating management programmes.

REFERENCES

1. Allen, D.J. 1975a. Additions to the fungi and plant diseases of Malawi. Society of Malawi Journal 28: 35-44.

2. Allen, D.J. 1975b. A further supplementary checklist of Tanzania plant diseases. Ministry of Agriculture, Dar-es-Salaam. Unpublished.

3. Beattie, R.K. & J.D. Driller 1954. Fifty years of chestnut blight in America. American Journal of Forestry 52: 323-329.

4. Canon, P. 1985. Studies in fire protection, stumpage and dieback. Technical Cooperation Programme - United Republic of Tanzania, FAO, Rome. Document No. 2. Pp 3-24.

5. Diwani, S.A., O. Kumburu, E.N. Mshiu & E.Z. Kisaka 1984. Preliminary report on the survey of forest tree diseases and pests in Sao Hill Forest Plantation. Ministry of Lands, Natural Resources and Tourism. Division of Forestry, Dar-es-Salaam. Unpublished.

6. Ebbels, D.L. & D.J. Allen 1979. A supplementary and annotated list of plant diseases, pathogens and associated fungi in Tanzania. Phytopathological Paper No. 22. Commonwealth Agricultural Bureau. Kew, Surrey. England. Pp 89.

7. Gibson, I.A.S. 1956. Sowing density and damping off in pine seedlings. East African Agricultural and Forestry Journal 21: 183-188.

8. Gibson, I.A.S. 1957. Saprophytic fungi and destroyers of germinating pine seeds. East African Agricultural and Forestry Journal 22: 203-206.

9. Gibson, I.A.S. 1960. *Armillaria* root rot in Kenya pine plantations. Commonwealth Forestry Review 39: 94-99.

10. Gibson, I.A.S. 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February-3rd March, 1962. Kenya Forest Division. Unpublished.

11. Gibson, I.A.S. & D.C. Corbett 1964. Variation in isolates from *Armillaria* root disease in Nyasaland. Phytopathology 54(1): 122-123.

12. Gibson, I.A.S. 1965a. Forest pathology in East Africa. East African Agricultural and Forestry Journal 31(2): 194-198.

13. Gibson, I.A.S. 1965b. A note on *Stereum sanguinolentum* (Alb. et. Schw.) Fr., a new record for Kenya forests. East African Agricultural and Forestry Journal 32(1): 38-40.

14. Gibson, I.A.S. 1966a. Cercospora blight of pines. EAAFRO Mycological Paper No. 30. Muguga, Kenya. Unpublished.

15. Gibson, I.A.S. 1966b. A note on fungicides for use on forest stations. East African Agriculture and Forestry Research Organization. Mycological note No. 47.

16. Gibson, I.A.S. 1967. The present world situation in regard to the spread of internationally dangerous forest diseases. East African Agricultural and Forestry Journal 32(4): 478-483.

17. Gibson, I.A.S. 1968. The changing role and needs of forest pathology in the Commonwealth. Proceedings of the 9th British Commonwealth Forestry Conference. Commonwealth Forestry Institute, Oxford. Pp. 1-3.

18. Gibson, I.A.S. 1975. Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part One. Important Members of the Myritaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Forestry Institute, Oxford. Pp. 51.

19. Griffin, H.D. 1967. Further studies on *Stereum sanguinolentum* Alb. & Schw. ex Fries in Kenya forest plantations. EAAFRO Mycological Note No. 49. Muguga, Kenya. Unpublished.

20. Griffin, H.D. 1968. Forest tree diseases. Report to the Government of Tanzania. Report No. TA 2533. UNDP/FAO, Rome.

21. Hamilton, A.C., C.K. Ruffo, I.V. Mwasha, C. Mmari, P. Binggeli & A. Macfadyen 1989. Profile diagrams of the East Usambara forests. In: A.C. Hamilton & R. Bensted-Smith, (eds.); Forest Conservation in the East Usambara Mountains, Tanzania. The IUCN Forest Programme. Man Graphics Ltd., Nairobi, Kenya. Pp. 241-254.

22. Hocking, D. & A.A. Jaffer 1967. Field observations on root rot of teak in Tanzania. FAO Plant Protection Bulletin 15(1): 10-14.

23. Hocking, D. 1968. Stem canker and pink stain on teak in Tanzania associated with *Fusarium solani*. Plant Disease Reporter 52(8): 628-629.

24. Howland, A.K. & I.A.S. Gibson 1969. A note on *Diplodia* spp. on pines in East Africa. East African Agricultural and Forestry Journal 35(1): 45-48.

25. Ivory, M.H. 1967. *Fusicoccum tingens* Goid: A wound pathogen of pines in East Africa. East African Agricultural and Forestry Journal 32(3): 341-343.

26. Ivory, M.H. & D.N. Paterson 1970. Progress in breeding *Pinus radiata* resistant to *Dothistroma* needle blight in East Africa. Silvae Genetica 19: 38-42.

27. Ivory, M.H. & M.R. Speight 1993. Pest Management. In: L. Pancel (ed.), Tropical Forestry Handbook, Volume 2. Springer-Verlag. Berlin, London, New York. Pp. 1142-1219.

28. Kimaryo, P.E. 1972. Initial intensive and medium thinnings increase Dbh growth in second growth camphor regeneration. Tanzania Silviculture Research Note, No. 26. Division of Forestry, Dar es Salaam.

29. Lundquist, J.E. 1987. A history of five forestry diseases in South Africa. South African Forestry Journal 140: 51-59.

30. Mugasha, A.G. 1978. Tanzania natural forests' silvicultural research review report. Tanzania Silvicultural Technical Note (New Series) No. 39. Division of Forestry, Dar es Salaam. Unpublished.

31. Mwamba, B.K. 1986. The ecology and distribution of *Ocotea usambarensis* in the Uluguru mountains. A special project report. Faculty of Forestry, Sokoine University of Agriculture. Morogoro, Tanzania. Unpublished.

32. Olembo, T.W. 1969. The incidence of cypress canker disease in E. Africa. East African Agricultural and Forestry Journal 35(2): 166-173.

33. Olembo, T.W. 1972. Studies on *Armillaria mellea* in East Africa. Effect of soil chelates on penetration and colonization of *Pinus patula* and *Cupressus lusitanica* wood cylinders by *Armillaria mellea* (Vahl. ex Fr.) Kummer. European Journal of Forest Pathology 2(3): 134-140.

34. Paterson, D.N. 1965. The determination of log volume errors due to methods of measurements and other defects for four important indigenous Kenya tree species. East African Agricultural and Forestry Journal 31: 125-131.

35. Peregrine, W.T.H. & M.A. Siddigi 1972. A revised and annotated list of plant diseases in Malawi. Phytopathological Paper No. 16. Commonwealth Mycological Institute Kew, England.

36. Polhill, R.M. 1968. Tanzania vegetation. In: Hedberg & Hedberg (eds.), Conservation of Vegetation of Africa South of Sahara. Proceedings of a symposium on AETFAT. Uppsala, Sweden. Pp. 166-170.

37. Procter, J.E.A. 1965. Diseases of pines in the Southern Highlands Province Tanganyika. East African Agricultural and Forestry Journal 31: 203-209.

38. Procter, J.E.A. 1967. A nutritional disorder of pines. Commonwealth Forestry Review 46: 145-152.

39. Raabe, R.D. 1980. Variation in pathogenicity and virulence in *Fomes annosus* (Fr.) Karst and *Armillaria mellea* (Vahl. ex. Fr.) Kummer. In: Resistance to Diseases and Pests in Forest Trees. Proceedings of the 3rd international workshop on genetics of host-parasite interactions in forestry. Wageningen. Pp. 251-259.

40. Renvall, P. & T. Niemelä 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. Bulletin Jardin Botanique National de Belgique 62: 403-414.

41. Riley, E.A. 1960. A revised list of plant diseases in Tanganyika Territory. Mycological Papers No. 75. Commonwealth Mycological Institute. Kew, England. Pp. 28.

42. Ryvarden, L. 1972. A critical checklist of the *Polyporaceae* in tropical East Africa. Norwegian Journal of Botany 19: 229-238.

43. Ryvarden, L. & I. Johansen 1980. A Preliminary Polypore Flora of East Africa. Fungiflora, Oslo. Pp. 636.

44. Tangwa, J.L., S.A.O. Chamshama & V.R. Nsolomo 1988. Dieback disorder in *Pinus patula*, *P. elliottii* and *P. caribaea* at Sao Hill, southern Tanzania. Commonwealth Forestry Review 67(3): 263-268.

45. Waring, H.D. 1982. Dieback in Pines and Eucalyptus. Sao Hill Forest Protection. Technical Cooperation Programme, United Republic of Tanzania. FAO, Rome. Document No. 2.

46. Wilan, R.L. 1965. A master plan for silvicultural research in Tanzania. Forest Division, Ministry of Agriculture, Forests and Wildlife, Dar-es-Salaam. Pp. 26.

47. Zulu, T.S. 1991. Introduction to the major forest diseases in Malawi. In: C.Z. Chilima (ed.), Proceedings of the 1991 Forest Pest and Disease Monitoring course. Forestry Research Institute of Malawi. Pp. 15-19.

PAPER II

DECAY FUNGI OF *OCOTEA USAMBARENSIS* ENGL. TREES IN THE USAMBARA AND KILIMANJARO MOUNTAIN RAIN FORESTS.

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SUMMARY

A study was conducted in the mountain rain forests of Usambara and Kilimanjaro to determine symptoms, signs, causes and sources of infection, and to isolate and identify fungi which cause stem decay of standing *Ocotea usambarensis* trees. Surveys were made in the forests and sporophores of larger fungi were also collected on trees, stumps, dead trunks and branches of the tree species. Sample trees were felled, disks cross-cut out and fungi isolated into pure culture from various decay zones in heartwood and sapwood, and later identified.

The symptoms of standing trees were characteristic of the main stem decay and butt rot and were the presence of wounds on stems, dead branch stubs, dead branches, stem bumps, stem cracks, presence of epicormic sprouts along the main trunk or at the butt, black exudations along the lower stem, and lastly, trees had thin crowns. The main sign of decay was the presence of fruitifications of the fungus *Phellinus senex* on trunks of standing trees. Fungi infect trees mainly through wounds on stems or roots and through branch stubs. The coppice regeneration is infected directly from the stumps on which they are growing and these stumps get contaminated by the infection which was present in mother trees or from the fungi which infect the stumps when the trees are cut. The root-sucker regeneration is infected during its early period of growth through wounded roots of mother trees from which the regeneration was derived. Many causes of injury on trees are due to human activities in the two forests and due to resident wild animals.

The decay in the tree species is attributed to the infection by a number of fungi in the sapwood and the heartwood and hence causing sap rot and heart rot. As about 46% of the fungi were isolated from the heartwood only, 30% in sapwood only and 24% in both sapwood and heartwood, this shows that heart rot is still a major problem although sap rot is also significant. Butt rot is another major problem of the tree and it is as well the source of decay of the main stem because 78% of the trees sampled had butt rot in addition to the decay of main stem. Trees of all sizes and of all age classes may be affected by the decay.

A total of 72 fungal taxa were isolated from various decay zones in 15 sample trees from both sapwood and heartwood and about 17% of the fungi were basidiomycetes while 83% were non-basidiomycetes. Among the fungi isolated was *Phellinus senex* which, for the past 40 years or more, has been implicated to cause heartrot of the tree but no isolations were made to connect it to the disease. *P.senex* is now confirmed to cause decay of the main stem and that of the butt, and its transmission through the root system is as well confirmed. In general, the flora of fungi causing decay in camphor was heterogenous and consisted of soil inhabiting fungi, saprotrophs, facultative or pathogenic fungi and mycoparasites.

The succession pattern during colonization and decay of stem has been defined and described basing on the various niches from which the fungi were isolated. For most fungi, it was likely that occupation of certain niches was determined by their nutritional strategies supported by their inherent ability to survive. Pioneering, secondary and climax fungi of the tree species are also identified. A large number of basidiomycetes including *Phellinus senex*, and other non-basidiomycetes come later in the heartwood after it has been colonized by other fungi. But it was most likely that these secondary fungi form the major component of the decay climax flora of standing trees.

From various wood material of the tree species, sporophores of 14 larger fungal species belonging to the families *Corticiaceae*, *Ganodermataceae*, *Hymenochaetaceae*, *Polyporaceae*, *Schizophylaceae* and *Xylariaceae*, were collected. Seven of them are reported on *O. usambarensis* for the first time. These fungi, together with those collected earlier, make a total of 19 species of larger fungi reported so far on the tree species.

Comparing the two forests on the crystaline Usambara and the volcanic Kilimanjaro mountains (300 km apart),

Usambara had relatively more fungi invading *O. usambarensis* than Kilimanjaro. Climatic and edaphic factors may be responsible for this difference although further isolations of fungi from more sample trees are suggested for better comparative results. Nevertheless, it is important to note that out of the total fungal species isolated from the trees, 21% of them were common to both forests, 43% were only isolated from Usambara and 36% from Kilimanjaro.

Key words: *Ocotea usambarensis*, stem decay, decay fungi, fungal succession.

1. INTRODUCTION

Ocotea usambarensis Engl. (Family: Lauraceae; commercial name: East African camphor tree; common names: camphor, *Ocotea*) has been suffering from stem decay for a long time. Reports indicate that for the past 40 years or more, a heartrot disease has been the major defect affecting mature trees of 50 years and above, and such trees may contain a hollow stem (Gibson, 1962; Willan, 1965; Dick, 1969; Kimaryo, 1971). Studies carried out in 1961 indicated also that both decay and stain entered mostly through stem wounds and branch stubs and to a limited extent originated from the parent root system (Willan, 1965). Recent reports still mention the heartrot as the major problem affecting the survival of camphor which also reduces its potential as a commercial tree species (Forest Division, 1991).

Fungi which are implicated to cause the heartrot were identified basing on field observations of basidiocarps occurring on living and dead trees, fallen decaying trunks and stumps of camphor trees (e.g., Gibson, 1962; Renvall and Niemelä, 1993). The fungus widely reported as the main heartrot agent is *Phellinus senex* (syn. *Fomes senex*; *Polyporus senex*) although, there are other fungi also implicated (see Ebbels and Allen, 1979; and Renvall and Niemelä, 1993).

A survey by Dick (1969) confirmed an earlier report by Willan (1965) that the decay originated in the tree quite early in life because young trees which had not formed any substantial amount of heartwood were also infected. Willan (1965) acknowledged also that butt rot entering suckers from the parent roots was a more difficult problem on which there was limited information.

Available research information on the heartrot disease reveals a deficiency in the existing knowledge concerning the decay. For example, previous reports show that the identification of fungi implicated to cause the heartrot was done by directly associating fungal fruitifications (found on dead or living trees) with the decay inside them without actually proving the level at which the observed fungi participated in the inner decay. Such ways of associating the signs with the symptoms form only a preliminary part of the diagnostic process and indeed confirm the presence of decay, but do not tell the level of participation of the implicated fungi. Some fungi incite the decay, others come later in the decay process while some others enter later after the host has already been killed. Therefore, isolation of heartrot fungi into pure culture from various decay zones in the stem and the subsequent comparative identification to confirm their participation in the decay, are important. In so doing, the niche and the role of the involved fungi can also be identified.

Hence, the objectives of the current study were to identify fungi causing stem decay in living trees of *Ocotea usambarensis* and to elucidate on their possible succession pattern during colonization and decay of the stem. It was also important to record the causes of damage, the infection courts, the symptoms and signs of decay of living trees, the transmission of decay from one tree to another; and to record the flora of larger fungi causing decomposition of wood of *O. usambarensis* trees in the forest. As camphor grows on both volcanic and igneous originated soils, it was also the aim of the study to compare the decay fungal flora between the two forest habitats.

2. MATERIALS AND METHODS

2.1 Study areas

The study was conducted in the mountain rain forests of Usambara (crystalline mountains) and Kilimanjaro (volcanic mountain) which are a distance of about 300 km apart. In the Usambara mountains, the study area was located at Magamba at an altitude of 2,200 m above sea level within the 28,262 ha Magamba catchment forest reserve (Forest Division, 1991). The study area in the Kilimanjaro forest was located in the Barankata range on the southern slopes of the mountain forming part of the 107,800 ha Kilimanjaro catchment forest reserve, and altitude ranges between 2000-2500 m above sea level (Forest Division, undated). In general, the Kilimanjaro forest receives more rainfall than the West Usambaras and soils are more fertile and hence camphor grows better in the area. The Usambara study area receives an average of 1250 mm of rain per year while the Kilimanjaro study area receives an average of 1900 mm of rain per year (Forest Division, undated and 1991).

2.2 Collection and culturing of fungal fruit bodies from *Ocotea usambarensis*.

In each study area, a survey was first made to collect any basidiocarps or visible ascocarps from wood and trees of *O. usambarensis* in order to get an idea on the flora of larger fungi which are commonly associated with the tree species and to later use them for various studies and for comparative identification of fungi to be isolated in trees. Living and dead trees, decaying logs, fallen trunks, branches and stumps were checked for any visible fungal fruitifications. Each fungal specimen collected was given a collector's number. Information such as the location where the specimen was collected and the type of wood on which it was growing (logs, stumps, dead or living trees) was also recorded. In the laboratory, pure cultures were isolated from the fruit bodies onto a growth medium (1.25% malt and 2% agar, w/v). For each fungal species, many replicate cultures were taken in order to make sure that there was consistence in similarity of the resulting mycelia and that they actually represented the fungus collected. Some mycelia of certain fruit bodies were later verified by comparing them with mycelia of the same species which were available in the laboratory culture collections. After the isolations, the fruit bodies were sterilized by drying at 105°C for 48 h and then stored.

2.3 Cutting of trees with decay and sampling for fungal isolation

Trees with decay were selected by looking at various symptoms and signs which became familiar after making preliminary surveys in the forests. A total of 14 trees were cut (7 from each study area) during the sampling period between 1993 - 1995. The number of trees cut was within the restricted total number of 30 trees (from both forest reserves) allowed for this research by the Division of Forestry Headquarters (Dar es Salaam, Tanzania). The trees had an average age of 40 years, a diameter range of 22 - 53 cm (measured at breast height) and an average height of 16 m. They were coppice and root suckers regenerations which were initiated during the 1950s. However, the selection was guided by the observed symptoms and signs and did not bias on the type of regeneration. As a result, five trees of root sucker regeneration and two trees of coppice regeneration were cut in the Usambara forest while two trees of root sucker regeneration and five trees of coppice regeneration were cut in the Kilimanjaro forest. In total, 7 trees from root sucker regeneration and another 7 trees from the coppice regeneration were cut from both study areas.

Trees were felled using hand tools (axes and a two-man cross-cut saw) and sampling involved cutting of stem disks at certain points along the trunk which had decay. Whenever decay was found at the butt after felling, a disk would be taken for fungal isolation. All disks were cut at some distance away from the infection courts. Height from the ground to the point where each disk was taken was measured and the entrance point of infection recorded. The number of disks taken from a tree depended on the size of a decay column and the number of infection courts. This meant that if a tree had a single source of infection (e.g., one wound) the number of disks would be determined by the extension (length) of the decay column, but if the sources of infection were many then at least one disk would be cut for each source. Isolation of fungi took place within two days of field sampling.

2.4 Isolation of fungi from stem disks and separation into individual species

Isolation of fungi from sample stem disks was done in an improvised laboratory. An isolation chamber was established in a well cleaned room in which air movement was restricted. The chamber consisted of a wooden frame (1 m horizontal length, 0.5 m width and 0.5 m height) which was put on top of a table and covered by sterilized muslin cloth on the top, bottom and on three sides. The cloth was sterilized by soaking in tap-water containing the domestic 3-5% Sodium hypochlorite in an approximate volume ratio of 3 (NaHOCl) : 100 (water). One side of the chamber was left uncovered to provide access into it. Isolation equipment were; an alcohol lamp, scalpels, a chisel and test tubes containing malt-agar. During the isolation and culturing process, the muslin cloth was continually kept moist by re-soaking into the sterilizing solution.

Prior to fungal isolation, decay was mapped on cards (as it appeared on the disks), to show the arrangement of various decay zones. Lines were drawn across the disk maps and marked at certain points where samples for culturing would be taken, as dictated by the decay pattern. Disks were then split according to the pattern shown on the cards and culture samples were taken from the inner wood of the split pieces and put in tubes under the shelter of the tent. The culture medium in tubes was made up of 1.25% (w/v) malt and 3% (w/v) agar. The cultured wood pieces were taken from the various decay types (advanced decay, incipient decay, decay margin with heathy wood) and also from healthy wood itself. After replacing the cap, each tube (containing one sample), was sealed with laboratory film (parafilm) and properly labelled by assigning it a NISK (Norwegian Forest Research Institute) number. Other information recorded on cards showed the type of decay, colour of decay, type of wood (sap or heart wood), the disk number, height from ground where the disks was cut, and the possible source of infection (roots, stem wound or branch stub). After culturing, tubes were initially kept at room temperature (about 23°C) until growth from wood was visible and later (depending on the availability of a refrigerator) stored in a cool place at about 4°C until transported back to Norway.

More often, one cultured wood sample would produce more than one fungal species, so separation into single species was done in the laboratory by first reculturing the fungi in Petri-dishes containing malt-agar and then separating the fungi using the single spore or individual hyphal tip techniques. In some cases it was difficult to separate species in a mixture, but under a microscope, each of the species could be traced, sketched and provisionally categorized and later compared to isolates of the same species which were successfully isolated.

2.5 Isolation of fungi from artificial wounds on the stem

Two trees, one from each study area, were selected and artificial wounds of size 10 x 10 cm wide and 2 cm deep, inflicted in the sapwood. Due to time constraint, the experiment was not extended to many trees and only two wounds per tree were made at the same diameter on opposite sides. After one month, wood samples (up to 5cm from the wound surface) were taken for fungal isolation, and after one year, it was possible to cut only the tree from the Usambara for further fungal isolations. The aim was to get an idea on the type of fungi which were the first to colonize fresh wounds and which may later be displaced completely by other fungi or persist in the succession process.

2.6 Identification of fungi and analysis of their succession and occurrence

In the laboratory, all fungal cultures from wood were scanned in the compound microscope and important features were recorded. These included the type of spores present (conidia, clamydospores), arrangement of spores on hyphae, conidiophores or on conidiogenous cells; size and colour of spores; and other important features like the presence and number of clamps on hyphae. A sketch of the appearance of each isolate was drawn so that similar isolates could be easily recognized and grouped as single species. Other features which were useful and recorded were the growth characteristics on malt-agar medium, such as the colour of mycelium, the colour imparted on the medium, the general growth rate of the isolate and the nature of the advancing zone of the mycelium on malt-agar (whether smooth or indented margin). These features assisted in the placing of various isolates into similar groups or even species.

The fungal fruit bodies collected from camphor, were identified by Professor Leif Ryvarden of the University of Oslo who has worked extensively with the East African fungi (see Ryvarden, 1972; Ryvarden and Johansen, 1980). Also, 50 isolates of fungi from the stem decay cultures were sent to the International Mycological Institute (IMI) in Britain for identification. All of them were given IMI numbers and most of them identified except those which did not sporulate in culture. Yeast fungi were also recorded and presented as one group. Similarly, no attempt was made to classify bacteria which appeared on the culture medium from wood cultures, so they were presented as one group. All the other information similar to that recorded for the fungi was also presented for the yeasts and bacteria, for example, the type of rot, type of wood and infection courts through which they entered their hosts. The mycelia of the identified fruit bodies and of the fungi identified by the IMI, were used for comparison with the mycelia obtained from other wood cultures for further verification of their identity. The fungal isolates which were different from the known cultures and which could not be traced to their family, generic or species names were presented by their wider taxa such as 'Basidiomycetes' sp., 'Sterile mycelium' sp. or 'Conidial' sp. Some groups of fungi which could not be located their right species names were referred to by their generic names e.g., *Penicillium* sp 1 or 2, etc.

After the identification of the fungi and by using the information from the field notes and the isolation record cards, it was eventually possible to analyse their occurrence. This involved determination of how many times a particular species was isolated, which trees each fungal species was isolated from, what part of the tree, or the type of the decay, location in the decay (at margin with healthy wood or close to pith, etc.), type of wood (sap or heartwood).The information was used to determine the possible succession pattern of the fungi during colonization and decay of *O.usambarensis* trees. The distribution of the fungi according to the infection courts which they used to pass through into the stem was also determined and then grouped according to the location from which the trees were sampled from.

3. RESULTS

3.1 Symptoms and signs of trees with stem decay

The symptoms and signs shown by *Ocotea usambarensis* trees suffering from internal stem decay are many and variable and the expression of any one of them depends on the part of the tree infected, the nature of the infection courts and the species of fungi involved. However, the most common symptoms observed were the following:-

- The presence of wounds and scars on the stem which exposed either the sapwood or the heart wood.
- The presence of dead side branches or dead leading branches.
- Dead branch stubs on the trunk or branches.
- Stem bumps. These are swellings on the trunk visible at points where new wood and bark (formed during the healing process) have grown over wounds or dead branch stubs.
- Stem cracks. These were common on trees with extensive butt rot that covered the sapwood as well.
- Epicormic shoots / sprouts along the stem are also an indication of stem decay.
- Thin and pale crowns were associated with trees with stem decay.
- Black exudates along the lower stem also indicated stem decay. This was mostly associated with butt rot.

The main signs of decay in camphor were the presence of fungal fruit bodies. By far the most abundant basidiocarps were those of *Phelinus senex*. The fruitbodies were seen on trunks of living trees or on large surface roots and butts of older trees and on dead stumps. The fungus *Trametes versicolor* was seen on dead branches of living trees but was most abundant on dead wood lving in the forest.

3.2 Infection courts, causes of damage to trees and sources of inocula

The most visible infection courts were stem and butt wounds and dead branches including branch stubs. Stem wounds are caused by injury due to animals, human activity in the forests and due to naturally falling trees. Branch wounds are caused in various ways but mostly by monkeys (blue, velvet and colobus monkeys) and by falling trees. Some branches die naturally due to shading out and become infection courts for the wood decay fungi which may grow further into the inner main stem. Root injuries are caused by human activity during harvesting and during the deliberate wounding done to promote root sucker regeneration. Both forests had experienced heavy mechanical logging (mainly for camphor) after the second world war which had caused damage to both young regeneration and mature trees. Old road tracks, rusting machinery and remains of sawmills can still be seen in these forests today. Also animals such as wild pigs dig and injure roots. Injured surface roots are as vulnerable to the various types of aerial inocula as are injured stems and branches, but roots in the soil are prone to infection by soil dwelling fungi and those living in decomposing plant material in the soil. A number of inoculum sources do exist in the camphor forests at both study areas. These include sporophores and mycelia of fungi on various substrates such as dead trees, stumps, logs, fallen trunks, branches and twigs, foliage, dead parts of living trees, dead roots in the soil and on litter.

3.3 Fungal sporophores collected on *Ocotea usambarensis*

A total of 14 fungal species producing large sporophores were collected from both study areas of the West Usambara and South Kilimanjaro forests. These were collected on various camphor wood including living and dead trees, decaying or abandoned logs, stumps, twigs and branches. They included 13 Basidiomycetes and one Ascomycetes species (Table 1).

Table 1. Fungal sporophores collected on *Ocotea usambarensis* in the West Usambara and South Kilimanjaro catchment forests, Tanzania.

Species of fungus	NISK number	Camphor material from which collected
Daldinia concentrica	93-169	Decaying logs.
Ganoderma australe	93-127B	Fallen trunks and dead stumps.
Gloeocystidiellum wakullum	93-295	Decaying branches.
Grammothele sp	93-113B	Decaying logs
Loweporus inflexibilis	93-103	Surface roots, dead trunks and stumps.
Phellinus gilvus	93-126B	Decaying logs, dead stumps and trunks.
Phellinus senex	93-102	Trunks of living and dead trees, stumps and fallen trunks.
Phellinus sp. 1	93-104	Dead stumps, fallen trunks
Phlebia chrysocreas	93-113A	Decaying logs
Schizophyllum commune	93-115B	Logs (1-3 years since felling).
Schizopora flavipora	93-118	Decaying logs.
Stereum hirsutum	93-119B	Decomposing logs.
Stereum ostrea	93-119	Decomposing logs.
Trametes versicolor	93-108	Decaying logs and dead branches.

The most common basidiocarps in both forests were those of *Phellinus senex*, *Ganoderma australe*, *Trametes versicolor* and *Phellinus gilvus*. Another unidentified *Phellinus* species called here as *Phellinus* sp 1 was also collected, but less frequently. *Phellinus senex* was common on standing trees, decomposing trunks and on dead stumps. *Loweporus inflexibilis* was collected on dead trunks or at the base of large surface roots at the butts of living trees or dead stumps. *Schizophyllum commune* was also common on felled trunks (felled 1-3 years) which did not show any advanced decomposition. The ascomycetes

fungus *Daldinia concentrica* was collected on decomposing logs in the forest and this was the only large ascocarp which was found on camphor during the survey.

Out of the 14 larger fungi collected, pure cultures of only 10 species grew successfully on malt-agar. These were *D. concentica*, *G. australe*, *Loweporus inflexibilis*, *Phellinus gilvus P. senex*, *Phellinus* sp. 1, *Schizophyllum commune*, *Stereum hirsutum S. ostrea*, and *Trametes versicolor*. When the mycelia from the fruit bodies were compared to those from the wood cultures, it was found that only *Phellinus senex* was isolated from the heartrot of living trees. An unidentified *Phellinus* species (*Phellinus* sp. 2, also isolated in stem decay of a standing tree, and whose mycelium was culturally different from the collected *Phellinus* sp.1) was another basidiomycetes which could be identified to the genus level using the cultures from the basidiocarps.

3.4 The representativeness of the field sampling and isolation methods

Taking into consideration the relatively rough field conditions encountered, the cleanliness of the fungal cultures which grew from the wood samples onto the culture medium was an indication of the effectiveness of the isolation chamber and the use of test tubes (as growth medium containers and not Petri-dishes) in limiting contamination. However, the total number of cultures which had fungi growing from them was very low when compared to the samples which did not show any growth at all. A total of 783 cultures were taken from various decay zones and healthy wood in 48 stem and butt disks. Out of 674 cultures taken from decay zones alone (a total of 89 were taken from healthy wood, in the sapwood and heartwood), only 265 had fungi growing from them and this was about 34% of the 783 total cultures taken. The distribution of the cultures which resulted into fungal growth according to infection courts was 35%, 35% and 30% for the butt rot, stem wounds and branch stubs respectively.

3.5 The taxa of fungi isolated from artificial wounds and the decay in trees

A total of 72 distinct fungal taxa were isolated from the wounds and stem decay of trees, and also some unidentified bacteria were isolated as well. There were a total of 12 Basidiomycetes species (constituting 17% of the total number of fungal species isolated), 60 species of non-basidiomycetes (constituting 83% of total fungi isolated). The non-basidiomycetes were mostly ascomycetes, other conidial fungi and some fungi presented only as 'Sterile mycelium species' because they could not be placed in any known groups as they did not sporulate in culture or show any distinct features such as the presence of clamps which are essential for proper grouping. There was no growth from the cultures taken from healthy wood either in the sapwood or hertwood.

3.5.1 Fungi isolated from artificial stem-wounds

Fungi which were isolated from artificial stem wounds one month and then one year after wounding are presented in Table 2. A total of 15 species were isolated from the two trees when the whole period of one year is considered. However, during the one month period, the trees were mostly infected by blue stain and wound pathogens. These were *Trichoderma harzianum*, *Pestalotiopsis* sp., *Botryosphaeria ribis* (anamorph: *Fusicoccum tingens*), *Cylindrodendrum album*, two *Ophiostoma* species (anamorphs of *Graphium* and *Sporothrix* types), *Penicillium* sp 1, *Cylindrocarpon destructans* (teliomorph: *Nectria radicicola*), Basidiomycetes sp 4, *Ceratocystis* spp 1 and 2. After one year, most of the blue stain and wound fungi were not isolated from the continued decay in the inner stem of the wounded tree (Table 2). However, it was found that four fungi (which previously were not isolated after one month) had infected the wounds as well and these included Basidiomycetes sp 1, a *Nodulisporium* sp and two fungi which did not sporulate in culture (Sterile mycelium spp 4 and 5).

Table 2. Fungi isolated from four artificial stem wounds in the sapwood of two trees, each from Usambara and Kilimanjaro forests, one month and one year respectively, after wounding.

Fungal species	NISK number	IMI number	Isolated after one month	Isolated after one year	Tree code
Basidiomycetes sp.1	93-105/70-1	367837	-	+	93-106*
Basidiomycetes sp. 4	93-106/10	-	+	+	93-106
Botryosphaeria ribis	93-106/3-2-1	367239	+	-	93-106 & 93-129
Ceratocystis sp.1	93-129/1-1-1	367075	+	-	93-129**
Ceratocystis sp. 2	93-129/4-2-1	367076	+	-	9 3 - 1 2 9
Cylindrocarpon destructans (teliomorph: *Nectria radicicola*)	93-106/7-1	366376	+	+	93-106
Cylindrodendrum album	93-106/4-1	367069	+	-	93-106
Nodulisporium sp	93-127/28-1	366379	-	+	93-106
Ophiostoma sp (anamorph: *Graphium* type)	93-106/4-1-1	367240	+	-	93-106
Ophiostoma sp (anamorph: *Sporothrix* type)	93-129/2-1	367244	+	-	93-129
Penicillium sp.1	93-106/4-2	-	+	-	93-106
Pestalotiopsis sp	93-106/3-1	-	+	-	93-106
Sterile mycelium sp. 4	93-110/50-2	-	-	+	93-106
Sterile mycelium sp. 5	93-111/28-1-1	-	-	+	93-106
Trichoderma harzianum	93-105/88-1	367446	+	-	93-106

+ = These fungi were isolated after one month or after one year.
* = This tree was from the Usambara and was the only one cut among the two trees, a year later.
** = This tree was from the Kilimanjaro forest and was not cut down.

Out of the 15 species which infected the tree, only 6 species were isolated in the inner decay after one year and among the six species, four entered the wounds after the one month period and the other two were among the pioneers which had been isolated one month after wounding. Thus the species which persisted in the wounds from the first month of wounding and participated in the prolonged decay of inner stem were *Cylindrocarpon destructans* and Basidiomycetes sp 4. These were later joined by the *Nodulisporium* sp, Basidiomycetes sp 1, and Sterile mycelium spp 4 and 5.

3.5.2 Fungi isolated from decay of sample trees

The total number of fungal species isolated from the sample trees including those from artificial stem wounds were 72 and this number included 12 basidiomycetes and 60 non-basidiomycetes. The decay in standing trees was of two main types. First there was the heartrot which affected the heartwood, and second there was the saprot which affected the sapwood. Some decay columns also originated from the root system through the butt causing butt rot and decay of the main stem. In some trees, there was no distinct demarcation of the two types of decay because the origin of heartrot was from the saprot and that of saprot was sometimes from the heartrot. Also, some of the fungi were isolated from both of the two types of decay. Fungi with a high frequency of isolation were the *Nodulisporium* sp, *Phialophora parasitica*, Conidial sp 2, Sterile mycelium sp 3, *Leptodontidium* sp, *Glioladium roseum* and Sterile mycelium sp 4. About 71% of all the fungi were isolated in single trees while 29% were found in at least two trees (Table 3). The isolated Basidiomycetes were found in single trees although more than one basidiomycetes species could be isolated from the same sample tree. Only one of them, Basidiomycetes sp 1 was found in two trees and this fungus is also unique because it produces distinct conidia in culture and initially has a white mycelium which after about one week turns yellowish.

Table 3. Fungal species isolated from decay of standing *Ocotea usambarensis* and their frequency of isolation in various decay zones according to the type of infection courts, and the number of infected trees in each study area.

Fungal species	NISK Culture number	IMI No.	Location in the stem		Type of decay or location in decay			Infection courts			Trees infected in each location		Total trees infected
			Heart wood	Sap wood	Advanced decay	Incipient decay	Decay margin	Butt or root	Stem wound	Branch stub	Usambara	Kilimanjaro	
Acremonium sp	93-105/118-1	367487	1	0	1	0	0	0	1	0	1	0	1
Alternaria sp	94-1113/37-1-1	367248	1	0	0	0	1	1	0	0	0	1	1
Aureobasidium sp	94-1113/15	367492	2	0	2	0	0	2	0	0	0	1	1
Bacterial species	-	-	9	5	14	0	0	6	8	0	1	2	3
Basidiomycetes sp 1	93-105/70-1	367837	8	8	6	6	4	2	14	0	2	0	2
Basidiomycetes sp 2	93-105/93	-	0	3	1	2	0	0	0	3	1	0	1
Basidiomycetes sp 3	93-105/103-1	-	0	1	1	0	0	0	1	0	1	0	1
Basidiomycetes sp 4	93-106/10	-	0	9	0	8	1	0	9	0	1	0	1
Basidiomycetes sp 5	93-110/27-1	-	3	0	3	0	0	0	3	0	1	0	1
Basidiomycetes sp 6	93-110/28-2	-	3	2	5	0	0	0	5	0	1	0	1
Basidiomycetes sp 7	93-120/1	-	5	2	4	2	1	0	6	1	0	1	1
Basidiomycetes sp 8	93-121/19	-	6	0	4	1	1	2	4	0	0	1	1
Basidiomycetes sp 9	93-122/18-1	-	0	3	3	0	0	0	3	0	0	1	1
Basidiomycetes sp 10	93-127/16-2	367490	1	7	1	6	1	0	8	0	1	1	2
Botryosphaeria ribis	93-106/3-2-1	367239	0	5	0	5	0	0	5	0	1	1	2
Ceratocystis sp 1	93-129/1-1-1	367075	0	1	0	1	0	0	1	0	0	1	1
Ceratocystis sp 2	93-129/4-2-1	367076	0	1	0	1	0	0	1	0	0	1	1
Cladosporium sp	94-1115/3	367077	5	0	0	5	0	5	0	0	1	0	1
Conidial sp 1	93-110/30-2	367838	1	0	1	0	0	0	1	0	1	0	1
Conidial sp 2	93-110/36-1-1	366377	11	6	8	5	4	5	7	5	2	5	7
Conidial sp 3	93-110/45	367241	0	4	4	0	0	0	4	0	1	0	1
Conidial sp 4	93-110/52-1	-	0	1	1	0	0	0	1	0	1	0	1
Conidial sp 5	93-111/46-1	367488	2	0	1	1	0	1	1	0	2	0	2

Fungal species	NISK Culture number	IMI No.	Location in the stem		Type of decay or location in decay			Infection courts			Trees infected in each location		Total trees infected
			Heart wood	Sap wood	Advanced decay	Incipient decay	Decay margin	Butt or root	Stem wound	Branch stub	Usambara	Kilimanjaro	
Conidial sp 6	93-120/6-2	367842	2	0	2	0	0	0	2	0	0	1	1
Conidial sp 7	93-120/50-1	367843	0	5	0	4	1	0	1	4	0	2	2
Conidial sp 8	93-121/2-1-1	-	0	1	0	1	0	1	0	0	0	1	1
Conidial sp 9	93-122/21-1-1	-	0	1	1	0	0	0	1	0	0	1	1
Conidial sp 10	93-123/30-1	367074	0	1	0	0	1	0	1	0	0	1	1
Conidial sp 11	93-127/12-1	-	1	0	1	0	0	1	0	0	1	0	1
Conidial sp 12	93-127/49-3	367491	1	0	0	0	1	0	1	0	1	0	1
Conidial sp 13	94-1114/17	366382	1	0	1	0	0	1	0	0	0	1	1
Conidial sp 15	95-1005/10	-	1	0	1	0	0	1	0	0	1	0	1
Conidial sp 16	95-1005/15	-	13	0	6	2	5	10	3	0	1	1	2
Conidial sp 17	95-1005/20	-	1	0	0	0	1	0	1	0	1	0	1
Conidial sp 18	95-1006/1	-	4	0	3	1	0	4	0	0	0	1	1
Conidial sp 19	95-1006/4	-	2	0	1	0	1	2	0	0	1	1	2
Conidial sp 20	95-1006/21	-	1	0	0	0	1	1	0	0	0	1	1
Cylindrocarpon destructans	93-106/7-1	366376	3	3	0	4	2	0	6	0	1	1	2
Cylindrodendrum album	93-106/4-1	367069	0	3	0	3	0	0	3	0	1	0	1
Gliocladium roseum	93-111/84-1	367070	27	1	18	9	1	6	9	13	2	2	4
Gliocladium viride	94-1115/14-1	367495	11	0	0	8	3	11	0	0	1	0	1
Leptodontidium sp	93-105/17	367485	12	6	1	10	7	18	0	0	3	2	5
Nodulisporium sp	93-127/28-1	366379	24	27	8	25	18	13	21	17	5	3	8
Ophiostoma sp (Graphium type)	93-106/4-1-1	367240	0	1	0	1	0	0	1	0	1	0	1
Ophiostoma sp (Sporothrix type)	93-129/2-1	367244	0	1	0	1	0	0	1	0	0	1	1
Paecilomyces lilacinus	94-1114/10	367846	2	0	0	1	1	2	0	0	1	1	2
Penicillium janczewskii	93-111/5-1-1-2	367840	2	1	2	1	0	0	3	0	1	0	1
Penicillium sp 1	93-106/4-2	-	0	1	0	1	0	0	1	0	1	0	1

Fungal species	NISK Culture number	IMI No.	Location in the stem		Type of decay or location in decay			Infection courts			Trees infected in each location		Total trees infected
			Heart wood	Sap wood	Advanced decay	Incipient decay	Decay margin	Butt or root	Stem wound	Branch stub	Usambara	Kilimanjaro	
Penicillium sp 2	93-120/1-1-1	-	8	1	5	4	0	0	5	4	0	1	1
Penicillium sp 3	93-121/2	-	0	4	1	3	0	4	0	0	0	1	1
Penicillium sp 4	93-122/16-1	-	0	1	0	1	0	0	1	0	0	1	1
Penicillium sp 5	93-127/56-1	-	1	0	1	0	0	0	0	1	1	0	1
Penicillium sp 6	94-1115/7	-	10	0	0	8	2	10	0	0	1	0	1
Penicillium sp 7	95-1005/6	-	1	0	0	0	1	1	0	0	1	0	1
Periconia sp	94-1115/2-2	367493	3	0	0	3	0	3	0	0	1	0	1
Pestalotiopsis sp	93-106/3-1	-	0	1	0	1	0	0	1	0	1	0	1
Phellinus senex	95-1005/4	-	2	0	2	0	0	2	0	0	1	0	1
Phellinus sp 1	93-127/74-1	-	7	0	7	0	0	0	0	7	1	0	1
Phialophora parasitica	93-105/51-1	367068	17	10	17	5	5	1	16	10	2	5	7
Phoma sp (Coniothyrium insigne)	94-1114/12	366381	1	0	0	0	1	1	0	0	0	1	1
Pseudallescheria boydii	94-1113/1-2	367247	1	0	1	0	0	1	0	0	0	1	1
Pseudomorfea coffeae	94-1114/20	366383	1	0	0	1	0	1	0	0	0	1	1
Sterile mycelium sp 1	93-105/100-1	-	0	1	1	0	0	0	0	1	1	0	1
Sterile mycelium sp 2	93-110/20-1-1	-	1	0	1	0	0	0	1	0	1	0	1
Sterile mycelium sp 3	93-110/48-1	367839	9	19	8	17	3	1	23	3	3	3	6
Sterile mycelium sp 4	93-110/50-2	-	5	4	4	3	2	3	6	0	3	1	4
Sterile mycelium sp 5	93-111/28-1-1	-	1	1	0	2	0	0	2	0	2	0	2
Sterile mycelium sp 6	93-121/3	-	0	1	1	0	0	1	0	0	0	1	1
Sterile mycelium sp 7	93-122/1-1	366378	0	1	0	1	0	0	1	0	0	1	1
Sterile mycelium sp 8	94-1113/12	366380	3	0	0	1	2	3	0	0	0	1	1
Sterile mycelium sp 9	94-1114/3-2	367845	1	0	0	1	0	1	0	0	0	1	1
Trichoderma harzianum	93-105/88-1	367446	1	5	1	3	2	1	1	4	2	1	3
Yeast species	-	-	8	5	5	3	5	7	2	4	3	3	6

3.6 The succession of fungi in the colonization and decay of stem of *O. usambarensis*

3.6.1 Distribution of fungi according to niches in decay of the trees

The niches occupied by the decay fungi were variable. Some fungi were found in either the heartwood or the sapwood while others were found in both types of wood. Classifying the niches according to their state as defined by the type of decay in the trees, some fungi were found either in advanced, incipient decay or at the decay margin with healthy wood, while others occupied all decay types (Table 3). About 46% of all the species were isolated in the heart wood only, 30% in sapwood and 24% in both sapwood and heart wood (Table 4). In the heartwood, basidiomycetes constituted 15% while non-basidiomycetes were 85% of the fungi isolated from it. Also, about 29 species (40% of the total) had, at one time, been found at the margin of decay with healthy wood (Table 4).

Table 4. Number of fungal species isolated in various niches in the decay of living trees of *Ocotea usambarensis*.

Fungal group	Number of species from each type of wood			Number of species in the various decay zones						
	Sap wood	Heart wood	Sap & heart wood	Advanced decay	Incipient decay	Decay margin	Advanced & incipient decay	Advanced & decay margin	Advanced, incipient & decay margin	Incipient & decay margin
Basidiomycetes	3	5	4	6	0	0	1	0	4	1
Non-basidiomycetes	19	28	13	16	16	7	4	1	10	6
Total number of species	22	33	17	22	16	7	5	1	14	7

Looking at individual groups of fungi, about 50% of the basidiomycetes species lived only in advanced decay in sapwood or heartwood and the other 50% in all three types of decay. About 27% of the non-basidiomycetes fungi were found in the advanced decay only, another 27% in the incipient decay, 12% at the decay margin and 34% in all types of decay including the decay margin. Among the fungi, yeasts occurred in the sapwood as well as in the heart wood and were isolated from advanced decay, incipient decay and decay margin. They also occurred in butt rots and stem decays and were isolated from 40% of the sampled trees. Bacteria also participated in the decay of standing trees but were only isolated in advanced decay in both sapwood and heart wood in the butt rot or stem decay and were found in 20% of the sampled trees.

3.6.2 The possible succession pattern of fungi in decay as illustrated by the isolated species

The succession pattern of fungi causing stem decay in living *Ocotea usambarensis* trees is explained by the niches they occupied, and also, their roles are classified according to such niches. Many fungi occupied several niches and hence they had several roles which overlapped during the colonization and decay process. This makes it difficult to give a chronological sequence of succession of the fungi involved because they enter or exit the decomposition process depending on their role in a particular niche. The niche here is described in terms of the type and condition of wood in standing trees and hence the various niches are the living or dead sapwood, heartwood, colonized or un colonized wood, incipient decay, advanced decay and the reaction zone or the decay margin with healthy wood. So the succession pattern which exists, tends to group fungi according to their ability to occupy those niches at either the same time (when niches overlap between fungi) or at different times.

The first group of fungi consisted of those which were the first to infect living wood tissues in sapwood and are regarded as pioneering species. The artificial wounds which were made on two trees of *O.*

usambarensis gave a picture on the type of such fungi that invade healthy sapwood in the initial days of injury. These pioneering fungi were mostly ascomycetes and other conidial fungi which are known to cause wood staining and canker in trees. They were dominated by *Ophiostoma* spp, *Pestalotiopsis* sp, Basidiomycetes sp 4, *Ceratocystis* spp, *Penicillium* sp, *Cylindrodendrum album*, *Cylindrocarpon destructans* (*Nectria radicicola*) and *Botryosphaeria ribis* (Table 2). All of the above fungi were isolated one month after wounding. However, after one year, most of the pioneering fungi had vanished and were not re-isolated but only *Cylindrocarpon destructans* and Basidiomycetes sp 4 were re-isolated again. More fungi had joined in the succession after the one month period and these were Basidiomycetes sp 1, Sterile mycelium spp 4 and 5 and a *Nodulisporium* species. Basidiomycetes sp 1 and the *Nodulisporium* sp, were found at the decay margin with healthy sapwood and they showed an ability to compete actively as facultative saprotrophs in this case. Other facultative fungi such as *Cylindrocarpon destructans* and Basidiomycetes sp 4 and the parasites mentioned earlier (such as the *Ophiostoma* spp, *Pestalotiopsis* sp and *Botryosphaeria ribis*, to mention a few) were the pioneering fungi in the decay of *O. usambarensis* as well.

For all the other sample trees studied, fungi which can be regarded as the pioneers were those which were at one period isolated at the decay margin, either in sapwood or in heartwood (refer to Table 4) and total to 40% of all the fungi isolated. Other than those mentioned from the artificial wounds, pioneering species which also were found at the decay margin were Conidial sp 4 and *Penicillium* sp 4. The most abundant decay pioneering fungi were those which were found in the heart wood, that is, those found at the decay margin adjacent to healthy heartwood. These fungi were the *Alternaria* sp, *Cladosporium* sp, Conidial spp 17, 19 and 20, *Gliocladium viride*, Sterile mycelium sp 9, *Paecilomyces lilacinus*, *Penicillium* spp 6 and 7 and the *Phoma* sp (*Coniothyrium insigne*).

The second group after the pioneers is composed of fungi which are more versatile. These were isolated in both the sapwood and heartwood and were also found in all types of decay zones (advanced, incipient and at the decay margin) and hence can sometimes be pioneering fungi as well. This group constitutes the facultative parasites and facultative saprotrophs and was composed of *Leptodontidium* sp, *Phialophora parasitica*, *Nodulisporium* sp, Basidiomycetes spp 1, 7 and 10, *Trichoderma harzianum*, *Cylindrocarpon destructans*, Conidial spp 2, 10, 12 and 16, Sterile mycelium spp 3 and 4 and the yeasts (Table 3). These fungi are able to explore a broader spectrum of niches and can invade uninfected wood (dead and living sapwood or heartwood) or occupy wood colonized by other fungi. With this group it is difficult to classify them according to the type of niche they occupy because they may enter the decay process at any stage of the succession and are not specific to one type of niche, hence it suffices to classify them as facultative fungi capable of invading dead and living wood.

The third group consists of fungi which were never isolated in uncolonized wood and so did not live in fresh un-inhabited wood. Fungi in this group were isolated mostly from heartwood, both in incipient and advanced decay zones where other fungi shared the same niche. This group consists of obligate saprophytes and other fungi with a greater saprotrophic tendency judged by the frequency of isolation in heartwood or in advanced decay as presented in Table 3. They were Basidiomycetes spp 2, 3, 5, 6, and 8, *Phellinus* sp 2, *Phellinus senex*, Conidial spp 1, 3, 4, 5, 6, 8, 9, 11, and 13, *Pseudomorfea coffeae*, *Periconia* sp, *Gliocladium roseum*, *Cladosporium* sp, Sterile mycelium spp 1, 2, 5, 6 and 7, *Penicillium* spp 2, 3, 4 and 5, *Acremonium* sp, *Pseudallescheria boydii* and *Aureobasidium* sp. The group consisted 58% of all the Basidiomycetes and only 28% of all the ascomycetes and conidial fungi isolated from camphor. It is likely that this group constitutes the 'climax' decay fungi of camphor which gradually displaces the pioneers and other secondary fungi until the inner stem ultimately decomposes completely. All the isolated bacteria shared as well the same niches as this group. Therefore, to summarize, the succession of fungi in the decay of living *Ocotea usambarensis* can only be explained by the relationship of fungi to particular niches which they occupy at a particular time, because as already observed, niches overlap between and within the various species of fungi.

3.7 Distribution of the fungi according to infection courts

The infection courts of the fungi were the wounds on either the stem, butt or roots or the branch stubs. Fungi isolated from the butt were those which entered through wounds on the lower parts of the trunk from infected stumps to coppice regeneration or those associated with root rot. Out of the 14 trees cut, 11 of them (about 79%) had butt rot in addition to the main stem decay. Also, about 50% of the root sucker trees cut had butt rot, while 28% of the coppice trees also had butt rot. The remaining 22% of the trees suffered from the decay of the main stem only. The fungi which were isolated from the main trunk entered the trees through stem and branch wounds, through the butt or roots, or through dead branch stubs. The distribution of the fungi according to the infection courts which they used to get access into the trees is presented in Table 5.

Fungi which entered the tree through the butts or roots were 35% of the total fungi isolated from the tree species while those which passed through stem wounds and branch stubs were 39% and 5%, respectively. Some fungi infected trees through more than one infection court and these were 10% of the total fungi (Table 5). The most important infection courts are the stem wounds and through the butt/roots, which together accounted for more than 74% of the total infections (Table 5). Most basidiomycetes infected trees through stem wounds but few infected trees through the butt/roots or branch stubs as well. Among the fungi isolated from the butt rot was *Phellinus senex* (Table 3) which is widely distributed in the camphor forests. Butt rots were also caused by direct infection coming from stumps on which coppice regeneration was derived but some originated from the root system, especially that which infected the root-sucker regenerations.

The non-basidiomycetes entered the trees mostly through the butts/roots and stem wounds. The butt rot of one tree was exclusively dominated by non-basidiomycetous fungi dominated by a *Cladosporium* sp and *Gliocladium viride*. Fungi species which infected the trees through the branch stubs were relatively few but also included a *Phellinus* sp 2 (Table 3). One fungus, a *Leptodontidium* species, did not infect trees through any other courts than the butts or roots, and it had infected one-third of all the trees sampled and also occurred in both locations of Usambara and Kilimanjaro. The most abundant fungal species which infected trees through all courts and which were isolated from more than four trees were *Phialophora parasitica*, Conidial sp 2, Sterile mycelium sp 3, the *Nodulisporium* sp and the yeast fungi.

Table 5. Distribution of the isolated fungi species according to infection courts

Infection court	Basidiomycetes	Non-basidiomycetes	Total	% of the total
Butt or root	1	24	25	35
Stem wounds	6	22	28	39
Branch stub	2	2	4	5
Butt/root and stem wounds	2	3	5	7
Stem wound and branch stub	1	2	3	4
Butt/root, stem wound and branch stub	-	7	7	10

3.8 Distribution of the decay fungi between the Usambara and Kilimanjaro forests

Fungi isolated from sample trees in the Usambara and Kilimanjaro camphor forests were heterogenous but 15 species (about 21% of the total number of fungi isolated) appeared in trees of both forests. About 43% of the total fungi were only isolated in the Usambara forest while 36% were isolated in the Kilimanjaro forest only (Table 6). Among the fungi which were common to both forests (see Table 3) were the *Leptodontidium* sp, *Phialophora parasitica*, *Trichoderma harzianum*, *Botryosphaeria ribis*, *Cylindrocarpon destructans*, Conidial sp 2 , 7, 16 and 19, Sterile mycelium sp 3 and 4, *Gliocladium roseum*, Basidiomycetes sp 10, *Nodulisporium* sp, *Ophiostoma* species and *Paecilomyces lilacinus*.

Table 6. Distribution of decay fungi between the Usambara and Kilimanjaro mountain rain forests.

Location	Basidiomycetes	Non-basidiomycetes	Total	% of the total
Usambara	8	23	31	43
Kilimanjaro	3	23	26	36
Usambara and Kilimanjaro	1	14	15	21

4. DISCUSSION

4.1 Symptoms and signs of decay in trees, causes of damage and the sources and transmission of inocula.

The symptoms observed on *Ocotea usambarensis* confirm earlier reports which had described branch stubs and stem wounds as the main features of trees containing heart rot (Gibson, 1962; Willan, 1965; Dick, 1969). The symptoms associated with butt rot such as stem cracks, lower stem exudates and the trees having thin pale crowns could also be expressing root rot. Some of these butt rot symptoms in camphor have also been described for butt rot of conifers (e.g., Vollbrecht and Eric, 1995). Decay of camphor through roots and butts has been recognized as a difficult problem on which little information was available and was reported as part of the heart rot problem (Dick, 1969). The term heartrot as it was adopted in early reports does not satisfy the general terminology describing decay in *O. usambarensis* because most camphor trees now suffer from decay of both the heartwood and the sapwood. The observed decay symptoms are not specific to the decay of the heartwood alone, but also to a number of disorders associated with decay of the sapwood, including the butt and root rots.

The current study also agrees with the observation by Manion and Zabel (1979) that it is no longer accepted that infection of heartwood occurs only through branch stubs and wounds that expose the heartwood. Some infections of the heartwood of *O. usambarensis* originated from sapwood, and some, such as that by Basidiomycetes sp 10 extended from the inner bark, through sapwood to the heartwood. This could be possible if the inner bark was previously injured or exposed by the removal of the dead outer bark. This strategy of infection has been reported for other fungi such as *Stereum gausapatum* which may penetrate intact branches of oak into sapwood and then enter the heartwood (Boddy and Rayner, 1982). Etheridge and Craig (1976) also showed that infection can pass through branches or small twigs which have not developed heartwood.

The infection to the young regeneration of *O. usambarensis* is transmitted from mother trees through stumps or roots on which the regeneration are derived and hence they both have butt rot as well. The colonization pattern of live stumps which support regeneration has been described to be fundamentally

similar to that following wounding of standing trees (Rayner and Boddy, 1988). This suggests that the infection of the coppice and root sucker regenerations is due to fungi which were present in the cut mother-trees and those which infect the fresh stumps after felling or roots after wounding. But saprotrophic fungi such as *Phellinus senex* which colonize dead stumps can also eventually find their way into heartwood of the regeneration when conditions are favourable. However, infection through the roots can also be by active pathogenesis whereby fungi infect intact roots (Rayner and Boddy, 1988) although this was not established during the current study. The current decay infections of camphor trees in the two forests are also due physical wounding by heavy logging operations carried out after the second world war for export timber. Injuries due to logging operations serve as infection courts to many fungal pathogens and stain fungi which infect stems, butts or roots in many forests around the world (e.g., Huse, 1978b; 1983).

When roots and butts are extensively infected to include the sapwood, water transport is disrupted and this may result into the trees developing thin crowns. Some decay fungi spread radially quite fast and block water transport to the upper parts of the tree. If only a part of the cross-section of the main stem is blocked, the healthy side will normally show the stress by the formation of sprouts along its side. If the fungi cover the whole cross section of the upper stems or branches, the symptoms are the occurrence of dead branches or dieback of leading branches. Stem cracks are a result of internal wounds (Shigo, 1966) or due to water stress as well, while exudations along the stem are a result of the interaction between the sapwood tissues and the pathogens. When side branches die and then fall off due to decay, the trees may grow fresh wood and bark around the area of detachment and stem bumps appear. Bumps also appear when the trees have embedded stubs (Rayner and Boddy, 1988).

Branch stubs become inoculum foci from which fungi infect inner parts of stems as they become embedded in the main trunks or branches. In one tree sampled, a branch stub was recovered inside the stem and it was estimated (by counting growth rings) to have stayed in the tree for 33 years, that is to say the embedding started when the tree was about 7 years old. It has also been reported in western hemlock (*Tsuga heterophylla*) that stubs less than 1 cm diameter become infected and are embedded progressively more deeply in the wood as the tree increases in girth, and act as foci from which mycelial outgrowth can take place when heartwood formation occurs (Etheridge and Craig, 1976; Rayner and Boddy, 1988). The inocula may be transmitted either through the air as spores, mycelial fragments or when the fungi grow physically towards healthy trees through the soil or by contact between the infected substrates with vulnerable parts of living trees. Transmission through rain drops carrying inocula from sources in the higher parts of the canopy to the lower stem and roots is also a possibility in these rain forests. Canopy animals such as monkeys can not be ruled out as they may be in contact with sporophores and mycelium in the branches and stems and hence carry the inocula on their bodies directly to stem wounds or branches which they break.

4.2 Fungal sporophores collected from *Ocotea usambarensis*

The sporophores collected on *Ocotea usambarensis* wood material during this study enrich the list of larger fungi currently known to be involved in the decomposition of the tree species. Renvall and Niemelä (1993) collected ten polypore species on camphor in the Usambara forests, five of which have also been collected during this study. Other five polypores collected by the two authors were not collected during this study and these are *Loweporus roseoalbus*, *Phellinus allardii*, *Phellinus apiahynus*, *Spongipellis pachyodon* and *Wrightoporia avellanea*. One of the species collected on camphor, *S. pachyodon*, was being reported for the first time from Africa (Renvall and Niemelä 1993). The species collected during the current study and which are now being reported for the first time on *O. usambarensis* are *Schizophyllum commune*, *Stereum ostrea*, *Schizopora flavipora*, *Gloeocystidiellum wakullum*, *Phlebia chryosocreas*, *Grammothele* sp. and the ascomycetous fungus, *Daldinia concentrica*. The collection makes so far a total of 19 larger fungi from camphor in Tanzania (18 basidiomycetes from the families *Hymenochaetaceae*, *Polyporaceae*, *Ganodermataceae*, *Schizophylaceae* and *Corticiaceae*, and the ascomycetes from the family *Xylariaceae*).

The occurrence of some of the sporophores on dead wood material shows their participation in the decomposition of camphor wood but does not necessarily indicate their participation in decay of standing trees. Lack of re-isolation of the collected fungi from the decay in trees also suggests that they are only decayers of dead wood in the forest and not in standing trees. Also, their occurrence on camphor wood does not indicate a special preference to it as they have already been collected on other wood species as well (see Eriksson and Ryvarden, 1975; Ryvarden and Johansen, 1980). Some species such as *S. commune* are described as unspecialized opportunists which are favoured when a tree experiences stress or when a tree has just recently died (Rayner and Boddy, 1988). This confirms its occurrence on recently felled trees and logs (1-3 years) which have not decomposed.

Among the macro fungal species collected, only *Phellinus senex* (*Hymenochaetaceae*) was isolated from decay in standing trees confirming earlier suspicions that it participated in the decay of standing *Ocotea usambarensis* trees (e.g., Gibson, 1962; Willan, 1965; Dick, 1969). The participation of the other fungi in decay of standing trees is not ruled out because only few trees were cut and also pathogenicity tests have not been done to confirm their ability to infect wood of standing trees. However, fungi causing decay in standing trees are said to rarely be responsible for decay in felled or fallen timber, probably due to the lack of appropriate conditions for establishment (Rayner and Boddy, 1988).

4.3 Representativeness of the field sampling and isolation methods

The selection of trees for felling was guided by the symptoms of the trees and signs of decay found on them. However, apart from the trees which were selected, there were many other trees which had similar symptoms and signs but were not cut due to the restriction on the number of trees allowed to be cut for this study. The 15 trees sampled (and only 14 of them cut) were numerically not a good representative sample of the camphor population suffering from decay. But, due to the strong selectivity of most decay fungi to particular tree taxa (Rayner and Boddy, 1988), the fungi isolated in the trees would be expected to give a fair picture on the type of decay flora affecting standing *O. usambarensis*. Looking at the high number of wood cultures which did not result into any fungal growth, it is difficult to give a satisfactory explanation why some decay zones did not have any fungi in them even when some samples taken were substantially decomposed. A nearest hypothesis would be due to the competitiveness between the decay fungi whereby once some fungi colonize a niche they produce antagonistic chemical substances which may inhibit other fungi from establishing in that niche even when the original fungus has moved out of it. Also some secondary fungi are slow growing and may not cover the deserted niche fast enough as the primary fungus leaves. Another cause for lack of growth from wood cultures could be due to the growth medium which might not have favoured some groups of fungi. Hence future work should consider using various kinds of artificial growth media to compare results.

4.4 The taxa of fungi isolated from artificial wounds and the decay in trees

4.4.1 Fungi isolated from artificial stem wounds

Fungi isolated from artificial wounds included those which are known to be pioneers in many sapwood infections and cause wood staining or canker in many trees. *Ophiostoma* and *Ceratocystis* species are known pathogens and stain fungi in conifers and in broad leaved trees (Venn, 1972; Roll-Hansen and Roll-Hansen, 1980). *Penicillium* species have also been associated with blue staining of many species including tropical trees (Masuka and Kariwo, 1992). *Botryosphaeria ribis* (anamorph: *Fusicoccum tingens*) is a known pathogen of many tropical and subtropical plants including tree species and causes diebacks and cankers in trees (Ivory, 1967; Ebbels and Allen, 1979; Punithalingham and Holliday, 1973). *Cylindrocarpon destructans* (teliomorph: *Nectria ridicicola*) has also been reported to cause diseases of pine seedlings in temperate forests (Unestam *et al.*, 1989). The genus *Pestalotiopsis* consists weak pathogens which cause leaf spots and cankers in many tree species in Tanzania, including camphor (see, Ebbels and Allen, 1979, Nsolomo and Venn, 1994) and a species called *Pestalotia ocoteae* (possibly now a *Pestalotiopsis* sp because the genus has been revised) is known to have been isolated from an *Ocotea* species in South Africa (Dr. Mordue, IMI, Pers. Comm., 1993).

In most wounds, bacteria also participate in the primary invasion of living sapwood tissues and hence pioneer the decay in association with fungi (Shigo, 1965, 1967; Shortle and Cowling, 1978). But the period of one month which it took for the first isolations to be carried out from the artificial wounds was too long to get a picture on the type of bacteria involved because bacteria appear during the early days of injury in fresh wounds (Shigo, 1967; Venn, 1972).

4.4.2 Fungi isolated from decay of sample trees

The taxa of fungi isolated from camphor trees represent most categories of fungi which live on wood at various periods and their diversity reflects the types of trophic levels that may exist during wood decomposition. They include parasites and wood staining fungi which pioneer the decomposition process. Then there are those fungi which are known to degrade the cell wall polymers (hemicelluloses, cellulose and lignin) and these are mostly basidiomycetes and some ascomycetes including other conidial fungi. Also there were mycoparasites which attack the decomposers and hence complete the food chain. No fungi were isolated from living sapwood indicating that *O. usambarensis* has no endophytic fungal flora. As 46% of the fungi were isolated only in the heartwood while 30% were isolated only from the sapwood and the remaining 24% in either sapwood or heartwood, this indicates that the heartrot is still a major problem of the tree species, although saprot is as well another significant problem.

The 72 species of fungi isolated from the stem and butt disks are not many when compared to the number of fungi which may occur in a tropical forest environment. The main reason is that the environment around the trees in the forests is quite different from that occurring inside the trees. Also, wood decay fungi are said to be special because they have to overcome heterogenous microenvironments in the trees, which pose distinctive constraints to growth and modes of establishment (Rayner and Boddy, 1988). In *O. usambarensis*, the presence of the oil cineol (Bryce, 1967) may have some selective effect on the fungi colonizing living trees and wood, thus exerting a pressure which favours a flora which can overcome this compound. On the other hand the outside environment usually supports more fungi in the soil, litter and on other woody substrates. Lodge and Cantell (1995) report on a study by Cornejo *et al.* (1994) who identified 500 species of fungi from leaf litter of a moist tropical forest. However, as 71% of the fungi were isolated in single trees, this suggests that if more trees were sampled most likely more fungal species would have been isolated. Also, 29% of the species isolated appeared in at least two trees which shows that some fungi have a specialized preference to *Ocotea usambarensis*.

Among the fungi isolated, *Phellinus* spp are well known as wood decayers possessing enzymes which degrade cellulose and lignin (Stalpers, 1978; Rayner and Boddy, 1988). *Phellinus senex* has been a subject of many reports in East Africa since it was implicated to be the cause of the heartrot disease of *O. usambarensis*. The successful isolation of the fungus during this study therefore confirms its participation in the heartrot of camphor. Also, its isolation from the butt rot of a root sucker regeneration confirms that it can be transmitted through the root system, apart from the stem. Other important fungi were the Sterile mycelium sp 3 and the *Nodulisporium* sp which had the highest frequency of isolation and had infected many trees. These two fungi were mostly associated with decays whose symptoms included dead leading branches, epicormic shoots on the stem, thin crowns and stem wounds. The fact that they were isolated from all decay types and at the decay margin suggests their capability as potential pathogens.

The diversity of species causing decay in camphor included fungi which are known to be antagonistic or parasitic to other fungi (mycoparasites). These were *Trichoderma harzianum* (Elad *et al.*, 1980; Tsuneda and Thorn, 1995) and *Gliocladium roseum* (Shigo, 1967; Zhang *et al.*, 1996). These two mycoparasites were mostly isolated in wood occupied by other species and occasionally at the decay margin indicating their ability to invade healthy tissues. *G. roseum* has also been reported to cause dieback in cashew-nut trees in Tanzania (Ebbels and Allen, 1979). Another fungus isolated from the heartwood and which is known to inhabit soil and water was *Pseudallescheria boydii* which is also a well known opportunistic human pathogen (Washburn, 1996). *Penicillium* species are also known to inhabit wood in standing trees, however, it has also been observed that many *Penicillium* species derive from

contaminations during the isolation and may not belong to the natural flora of the wood (Huse, 1978a). In this study, *Penicillium* spp were isolated from the decay of the trees in heartwood and sapwood, and in both advanced and incipient decays, suggesting that they can participate in the decay by occupying various niches and assuming various roles. Yeasts also play a significant role in wood decay by pioneering decay and by occupying advanced decay zones in the wood (Rayner and Boddy, 1988).

A number of the ascomycetes and other conidial fungi are known to be soil inhabiting fungi indicating soil as a potential reservoir of inoculum. These are such as *Penicillium janczewskii* (Pitt, 1979), *Gliocladium roseum*, *G.viride*, *Acremonium* sp., *Cylindrocarpon destructans* and *Paecilomyces lilacinus* (Domsch *et al.*, 1993). The *Leptodontidium* sp which was only involved in butt rots and isolated from discs taken at the stump and mostly at the decay margin, showed specialization to that particular part of the stem. It is mentioned that certain species of *Leptodontidium* show a remarkable propensity for occupation of the relatively undecayed interaction zones in wood (Rayner, 1976). The special anatomy of the wood in roots is said to influence colonization by certain fungi although the mechanisms involved are not known (Rayner and Boddy, 1988). One fungus, the *Phoma* sp, whose anamorphic state fits the description of *Coniothyrium insigne* (Dr. Punithalingam, IMI, Pers. Comm., 1995) had been also isolated from an *Ocotea* sp in South Africa (Sydow, 1924).

4.5 The succession of fungi in the colonization and decay of stem of *O. usambarensis*

The infection of either sapwood or heartwood by fungi is determined by their nutritional strategies which, as identified in *O.usambarensis*, put them into four groups. First are those which invade the sapwood first and fight their way inwards by active pathogenesis but can not survive beyond the living sapwood (obligate wood parasites); second, are fungi which get directly in contact with heartwood through large wounds or broken branches and can only live in heart wood or dead sapwood (obligate saprotrophs); third are those which can live in both dead and living sapwood and in the heartwood (facultative parasites or facultative saprotrophs); and fourth are the fungi which come later and occupy colonized wood (these may include both the obligate or facultative saprotrophs). Fungi which are mycoparasites, can be found in all niche types and in wood inhabited by any of the above categories of fungi. Hence the patterns of colonization of various niches in *O. usambarensis* overlap and according to Rayner and Boddy (1988), individual fungi are capable of combining one or more of the colonization strategies and hence occupy more than one niche. This overlapping of niches and roles of fungi during the colonization of the trees suggests that there is no straight forward sequence of succession by the various fungal species but only the presence of the niches may explain the type of fungi which can occupy them.

The distribution of the decay fungi according to the niches they occupy gives a better picture on how they succeed one another in the decay of stems of standing trees. The heartwood is said to contain allelopathic extractives which inhibit some fungi (Rayner and Boddy, 1988) and hence only those fungi which are able to detoxify these extractives can establish themselves directly in the heartwood. This may explain why some strong wood decayers will only enter the heartwood when it has already been colonized by other fungi. *Phellinus senex* seems to behave like such fungi which can not infect heartwood which is uncolonized, although this observation needs further investigation by inoculating it in uncolonized heart wood of living trees. Fungi which are not able to detoxify the extractives but are obligate saprophytes will occupy the heartwood after the toxins have been neutralized by the other fungi.
The pioneers in the sapwood also encounter phenolic compounds which they have to tolerate or detoxify. These compounds are formed in the vessel elements as a response to fungal invasion or wounding (Shigo, 1967; Sharon, 1973) and may act as chemical barriers to wood decay fungi (Shortle, 1979). When conditions are not favourable for the pioneering fungi, they move out (or get removed out) of the competition and those which can tolerate the stresses remain while at the same time others may come in as well. An example was shown by the fungi isolated from artificial wounds such as the *Ophiostoma* spp, *Ceratocystis* spp, *Cylindrodendrum album*, *Pestalotiopsis* sp and *Botryosphaeria ribis* (to mention

just some) which were not favoured by the inner micro environment of the trees and hence were eliminated from the competition within one year. Following invasion of inner tissues, most pioneering fungi find the environment inside the tree more different from that on or close to the surface of wounds. Wood micro environments which impose stress include unfavourable moisture and aeration conditions, temperature, pH, allelopaths and restrictions on nutrient availability (Rayner and Boddy 1988). Solheim (1991) demonstrated how oxygen deficiency was able to inhibit the growth of some *Ophiostoma* species, and concluded that the ability to tolerate low oxygen pressure was one of the important attributes for a successful primary invasion of sapwood.

The heartwood pioneer fungi can also be regarded here as a group which is different from the pioneer fungi of the sapwood because the conditions in healthy sapwood can only be tackled by fungi with parasitic tendencies while healthy heartwood will be favoured by fungi with saprotrophic tendencies. Pioneering fungi of the heartwood can probably also initiate a decay process in un colonized dead sapwood. However, it has been observed that some species attached to pith and heartwood may not interfere with the phase of infection when sapwood is wounded (Huse, 1978a). Both groups of pioneers have a similar task which is to initiate and facilitate a process of decay and colonization of wood in trees involving other fungi which could not start the process on their own.

Some non-decay fungi enter wood as well and rely on simple sugars, starch and proteins or may form associations with decay species to get nutrients (Rayner and Boddy, 1988). However, most decay fungi colonizing the heart wood must have the ability to hydrolyse the cellwall polymers in order to survive and these usually form the climax wood decay flora having the capacity to exit inner decays through cracks in stems, butts or through exposed branch stubs and then form fruitbodies on the surface of trees (Rayner and Boddy, 1988). This strengthens earlier observations and those verified during this study about the association of *Phellinus senex* with the heartrot of camphor because its basidiocarps are frequently found on trees with heartrot. Another fungus reported by Renvall and Niemelä (1993) and also by this study whose basidiocarps are found on large surface roots of older trees (but not isolated from trees during this study) was *Loweporus inflexibilis*.

Basidiomycetes are strong wood decayers, and although they numbered only 13% of the total fungi isolated in the heartwood, they could be responsible for most of the decay observed. Rayner and Boddy (1988), report that although a range of micro-organisms are associated with heartrots of living trees, only relatively few ascomycetes and basidiomycetes cause significant decay. Also yeasts and bacteria play significant roles in decay of wood. They are pioneers and occupy advanced decay zones in wood as well (Blanchette *et al.*, 1981; Rayner and Boddy, 1988) but are infrequent when decay fungi are active (Shigo and Sharon, 1970; Van Der Kamp, 1975). Yeasts and bacteria are thought to form associations with mycelial fungi in order to get access to inner tissues (Shigo, 1966; Blanchette and Shaw, 1978). Results of an experiment by Shortle *et al.* (1978) showed that pioneer bacteria found in sapwood and discoloured wood contribute to wood discolouration, alter the rate of cellulase activity of decay fungi, interact with wood parenchyma to alter the rate of wood colonization by decay fungi and help to provide growth factors and soluble nitrogen. Although the purpose of the current study was not to study wood decay bacteria, it was important to report their presence and possible importance in decay of the tree species.

4.6 Distribution of fungi according to infection courts

The distribution of the fungi which entered through the various courts indicate possible ways in which the infections took place. Fungi causing butt rot can also cause upper-stem decay and those causing upper-stem decay can also cause butt rot as has been found with *Phellinus senex*. Due to the small sample of trees cut, the percentages on the extent of the butt rot may not be representative of the camphor population but it serves to show that both the coppice and root sucker trees are vulnerable to butt rot fungi as well. Another unspecialized fungus is *Cylindrocarpon destructans* which has been described as a root surface saprophyte (Evans *et al.*, 1967) or as a soil inhabiting fungus (Matturi and Stenton,

1964; Domsch *et al.*, 1993), but in this study it was isolated from stem wounds on camphor, and also it has been reported as a cause of root death in *Pinus sylvestris* seedling (Unestam *et al.*, 1989). So the various fungi may assume various roles in order to satisfy their nutritional needs. Hence, the distribution of fungi from camphor according to infection courts should only be a guide to indicate the courts which are most vulnerable to infection and also the parts of trees which are most frequently injured. They should not be used as criteria to classify fungi according to their preferences or specialization to any parts of the trees, because such implications may need further investigation. In *O.usambarensis* therefore, stem wounds and stumps or roots are the major infection courts followed by branch stubs.

4.7 Distribution of the decay fungi between Usambara and Kilimanjaro forests

The differences in the decay fungal flora between the Usambara and Kilimanjaro can not explicitly be regarded as significant because the results on some species are based on single isolations and also on a limited number of trees sampled during this study. However, the two forests differ in many attributes such as the climate and soils which affect the vigour of the trees. Favourable climate and fertile soils will result in healthy forests which are less susceptible to diseases. As is the case, the South Kilimanjaro forests receive more rainfall than the Usambara forests and the volcanic soils of the Kilimanjaro are said to be more fertile and well drained than those of igneous origin found on the Usambara mountains (Forest Division, undated and 1991). Also, it has been recorded that the growth rate of camphor in Kilimanjaro is higher than in the Usambara forests (Dick, 1969). Hence, the trees in the Usambara forest are growing under less optimal conditions than those in the Kilimanjaro forest. Trees experiencing stress are more susceptible to infection by many fungi (including weaker pathogens) than are trees which are growing optimally. More fungal species were isolated in the Usambara mountains than in the Kilimanjaro. About 21% of the isolated fungi belonged to both locations which indicates the possibility of the existence of a specialized flora on *Ocotea usambarensis*. But the full extent of the fungal diversity between the two locations could have been clearer if more sample trees had been cut from each area.

5. CONCLUSION

This study has confirmed and clarified a number of observations previously reported on the decay of standing *Ocotea usambarensis* trees (mostly referred to as the heartrot of camphor). The symptoms of decay are many and involve those expressing decay of sapwood in the stem and of butt rot. The main signs are the presence of sporophores, mainly of *Phellinus senex* and other *Phellinus* species on dead parts of living trees. The main infection courts are the stem wounds and stumps or injured roots and dead branch stubs.

The causes of wounding are related to human activities in the two forests (as the surrounding villages acquire their forest-based needs from them) and also by the resident animals of the forests mostly monkeys and wild pigs. The sources of fungal inocula are many and include fungi living on other plant material in the forests such as dead trees, branches, fallen trunks, forest litter and those living in the soil. Infections to young regeneration from mother trees is transmitted through stumps to coppice regeneration or through roots to root suckers. Both the root sucker and coppice regenerations are infected by decay fungi and develop butt rot. Root sucker regeneration was thought to be less vulnerable to infection than coppice regeneration, however, it seems likely that the method of wounding roots to promote the suckers contributed enormously to their subsequent infection by decay fungi. Hence, it is likely that any method of regeneration which involves wounding of stem or roots will result in root rot and the butt rot which may extend further into the main stem.

Young trees (as young as 7 years old) which have not developed heartwood, and medium aged and mature trees which have developed heartwood, may all suffer from various degrees of decay. The decay can develop from the heartwood to sapwood or from sapwood to heartwood or be confined to the

heartwood only, depending on the type and the nature of the fungi involved. So the decay problems of camphor can not be described solely by the term 'heartrot' alone, but rather by a comprehensive terminology such as 'stem decay' which would include heartrot as well. Trees can stay with the decay for many years before symptoms or signs are expressed and it may take even more years for the trees to die if the sapwood is not infected. However, as the majority of the fungi were isolated in heartwood, this indicates that heartrot is still a major decay problem of camphor, although saprot is as well significant. Butt rot is also a major problem of the tree and is also the source of decay of the main stem.

Some fungi participate in the decomposition of stem of standing trees while others decompose dead camphor wood in the forest. It is likely that macro fungi whose sporophores appear on standing camphor trees participate in the decay of inner wood as has been observed with *Phellinus senex*. However, most fungi found on camphor wood other than standing trees, may just be wood decomposing saprophytes which may not participate in the internal stem decay of standing trees. During this study, 7 species of macro fungi (6 basidiomycetes and 1 ascomycetes) were collected and reported for the first time on camphor. These were *Schizophyllum commune*, *Stereum ostrea*, *Daldinia concentrica*, *Schizopora flavipora*, *Gloeocystidiellum wakullum*, *Phlebia chrysocreas* and a *Grammothele* sp. This collection together with collections made earlier by other researchers make a total of 19 species of larger fungi so far reported on *O. usambarensis*.

Basidiomycetes constituted 17% of the total number of fungi isolated while non-basidiomycetes were 83%.The fungus *Phellinus senex*, widely implicated as the main causative agent of heartrot by earlier reports, was also isolated from the heartwood of a root sucker regeneration tree and hence its participation in the decay has now been confirmed. Other important fungi which occurred in the decay most frequently were the *Leptodontidium* sp (which specialized in butt rots), *Nodulisporium* sp and Sterile mycelium sp. 3 (whose decays were associated with death of branches or trunks as they spread rapidly across the stems).

The pattern of succession of fungi in the decay of camphor is dependent on the niches available but for most species, the niches and also the roles of the fungi in the niches do overlap. Fungi which invade living sapwood are mainly the wood stain fungi and the wound rot / canker fungi and these are regarded as sapwood pioneers. They are dominated by *Ophiostoma* spp, *Penicillium* spp, *Pestalotiopsis* sp, *Cylindrodendrum album*, *Botryosphaeria ribis* and some basidiomycetes. Other fungi can also be pioneers and these are the facultative parasites or facultative saprotrophs and are *Cylindrocarpon destructans*, *Nodulisporium* sp, *Leptodontidium* sp, Sterile mycelium sp 3, Conidial sp 2 , *Phialophora parasitica* and some basidiomycetes fungi. Some obligate saprotrophs could be pioneers of decay in the heartwood and these were dominated by *Paecilomyces lilacinus*, *Phoma* sp (*Coniothyrium insigne*), *Penicillium* spp, *Alternaria* sp and other conidial fungi. The climax group consists of obligate saprotrophs which mostly occupy wood already inhabited by other fungi either in the heartwood or sapwood. This group included basidiomycetes (including *Phellinus senex*), some unidentified non-basidiomycetes, *Penicillium* spp, *Periconia* sp, *Gliocladium roseum*, *Cladosporium* sp, *Acremonium* sp, *Pseudomorfea coffeae*, *Aureobasidium* sp and *Pseudallescheria boydii*. Also most bacteria occurred in the niches occupied by this last group. The niche-preference indicated by most basidiomycetes (including *Phellinus senex*) suggests a strong possibility that certain heartwood decomposers can not initiate the decay of healthy heartwood of standing trees without the pioneering fungi which detoxify the impregnated allelopaths in the heartwood.

There could be differences in the flora of decay fungi attacking camphor between the Usambara and Kilimanjaro forests because growing conditions for the tree in the two forests are different. However, this study can not certify these differences conclusively due to the few number of trees sampled and due to the fact that some fungi were only isolated once. But, of the 72 fungal taxa isolated, 43% occurred in the Usambara, 36% in Kilimanjaro and 21% from both locations.

REFERENCES

Blanchette, R.A. and Shaw, C.G., 1978. Associations among bacteria, yeasts and basidiomycetes during wood decay. **Phytopathology, 68:** 631-637.

Blanchette, R.A., Sutherland, J.B. and Crawford, D.L., 1981. Actinomycetes in discoloured wood of living silver maple. **Canadian Journal of Botany, 59:** 1-7.

Boddy, L. and Rayner, A.D.M., 1982. Population structure, intermycelia interactions and infection biology of *Stereum gausapatum*. **Transactions of the British Mycological Society, 78:** 337-351.

Bryce, J.M., 1967. **The Commercial Timbers of Tanzania.** Forest Division, Utilisation Section. Ministry of Natural Resources and Tourism. Dar es Salaam, Tanzania. Pp 138.

Cornejo, F.D., Varela, A. and Wright, S.J., 1994. Tropical forest litter decomposition under seasonal drought: nutrient release, fungi and bacteria. **Oikos, 70:** 183-190.

Dick, J.H., 1969. Heartwood development and heartrot in East African camphorwood, *Ocotea usambarensis* Engel. Tanzania Silviculture Research Note, No.9. Division of Forestry, Dar-es-Salaam. Unpublished.

Domsch, K.H., Gams, W. and Anderson, T., 1993. **Compendium of Soil Fungi, Vol. 1.** IHW-Verlag, Germany. Pp 859.

Ebbels, D.L. and Allen D.J., 1979. A supplementary and annotated list of plant diseases, pathogens and associated fungi in Tanzania. **Phytopathological Paper No. 22.** Commonwealth Agricultural Bureau. Kew, Surrey. England. Pp 89.

Elad, Y., Chet, I. and Katan, J., 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. **Phytopathology, 70:** 119-121.

Eriksson, J. and Ryvarden, L., 1975. **Corticiaceae of Northern Europe Vol. 3:** 405. Fungiflora. Oslo, Norway.

Etheridge, D.E. and Craig, H.M., 1976. Factors influencing infection and initiation of decay by the Indian paint fungus (*Echinodontium tinctorium*) in western hemlock. **Canadian Journal of Forest Research, 6:** 299-318.

Evans, G., Cartwright, J.B. and White, N.H., 1967. The production of a phytotoxin, necrolide, by some root surface isolates of *Cylindrocarpon radicicola* Wr. **Plant and Soil, 26:**253-260.

Forest Division, undated. Description of the Kilimanjaro Catchment Forest Reserve. Hai, Moshi and Rombo districts, Kilimanjaro region. The Kilimanjaro catchment forest office, Moshi, Tanzania. Unpublished.

Forest Division, 1991. Magamba Forest Project Management Plan, 1991-1996/97. Ministry of Natural Resources and Tourism. Dar-es-Salaam, Tanzania. Unpublished.

Gibson, I.A.S., 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February - 3rd March, 1962. Kenya Forest Division. Unpublished.

Huse, K.J., 1978a. The fungal flora in uninjured stems of *Picea abies* (L.) Karst. A report to the Norwegian Forest Research Institute. Pp 42.

Huse, K.J., 1978b. Discolouration and microflora in wounds due to thinning operations in stands of *Picea abies* (L.) Karst. A report to the Norwegian Forest Research Institute. Pp 54.

Huse, K.J., 1983. Frequency of butt rot in stands of Picea abies (L.) in Norway. **Research paper from The Norwegian Forest Reseach Institute, No. 3/83. ISSN 0333-001X.** Ås, Norway.

Ivory, M.H., 1967. *Fusicoccum tingens* Goid: A wound pathogen of pines in East Africa. **East African Agricultural and Forestry Journal, 32(3):** 341-343.

Kimaryo, P.E., 1971. Regeneration of *Ocotea usambarensis* Engl. At Sungwi, West Usambaras. Tanzania Silviculture Research Note, No. 21. Division of Forestry, Dar es Salaam. Unpublished.

Lodge, D.J. and Cantrell, S., 1995. Fungal communities in wet tropical forests: Variation in time and space. **Canadian Journal of Botany, 73 (Suppl. 1):** S1391-S1398.

Lundgren, B., 1978. Soil conditions and nutrient cycling under natural and plantation forests in Tanzania highlands. Report in Forest Ecology and Forest Soils, No.31. Swedish University of Agricultural Sciences.

Manion, P.D. and Zabel, R.A., 1979. Stem decay perspectives - An introduction to the mechanisms of tree defence and decay patterns. **Phytopathology, 69:** 1136-1138.

Masuka, A.J. and Kariwo, P., 1992. Sapstain and mould in pine logs in Zimbabwe. **Commonwealth Forestry Review, 71 (3/4):**193-196.

Matturi, S.T. and Stenton, H., 1964. Distribution and status in the soil of *Cylindrocarpon* species. **Transactions of the British Mycological Society, 47:** 577-587.

Mugasha, A.G., 1978. Tanzania natural forests' silvicultural research review report. Tanzania Silvicultural Technical Note (New Series) No. 39. Division of Forestry, Dar es Salaam. Unpublished.

Mwamba, B.K., 1986. The ecology and distribution of *Ocotea usambarensis* in the Uluguru mountains. A special project report. Faculty of Forestry, Sokoine University of Agriculture. Morogoro, Tanzania. Unpublished.

Nsolomo, V.R. and Venn, K., 1994. Forest Fungal Diseases of Tanzania: Background and current status. **Norwegian Journal of Agricultural Sciences, 8:**189-201

Pitt, J.I., 1979. **The Genus *Penicillium* and its Teliomorphic States *Eupenicillium* and *Talaromyces*.** Academic Press, London. Pp 634.

Pitt-Schenkel, C.J.W., 1938. Some important communities of warm temperate rain forest at Magamba, West Usambara, Tanganyika Territory. **Journal of Ecology, 26:** 50-81.

Pòcs, T., 1988. The importance of catchment forests to Tanzania. Professorial inaugural lecture, Sokoine University of Agriculture. Unpublished.

Punithalingam, E. and Holliday, P., 1973. **CMI Description of Pathogenic Fungi and Bacteria. No.395.** Commonwealth Mycological Institute. Kew, England.

Rayner, A.D.M., 1976. Dematiaceous hyphomycetes and narrow dark zone in decaying wood. **Transactions of the British Mycological Society, 67:** 546-549.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Renvall, P. and Niemelä, T., 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. **Bulletin Jardin Botanique National de Belgique, 62:** 403-414.

Roll-Hansen, F. and Roll-Hansen H., 1980. Microorganisms which invade *Picea abies* in seasonal stem wounds II. Ascomycetes, Fungi imperfect and Bacteria. General discussion, Hymenomycetes included. **European Journal of Forest Pathology, 10:** 396-410.

Ryvarden, L., 1972. A critical checklist of the *Polyporaceae* in tropical East Africa. **Norwegian Journal of Botany, 19:** 229-238.

Ryvarden, L. and Johansen I., 1980. **A Preliminary Polypore Flora of East Africa.** Fungiflora, Oslo. Pp 636.

Shigo, A.L., 1965. Organism interactions in decay and discolouration in beech, birch, and maple. **U.S Forest Service Research Paper NE-43.** Forest Service, Department of Agriculture. Pp 23.

Shigo, A.L., 1966. Decay and discolouration following logging wounds on northern hardwoods. **U.S Forest Service Research Paper NE-47.** Forest Service, Department of Agriculture. Northeast Forest Experiment Station. Upper Darby, Pa. Pp 43

Shigo, A.L., 1967. Succession of organisms in discolouration and decay of wood. **International Review of Forest Research, 2: 238-299.** Academic Press Inc., New York.

Shigo, A.L. and Sharon, E.M., 1970. Mapping columns of discoloured and decayed tissues in sugar maple, *Acer saccharum*. **Phytopathology, 60:** 232-237.

Shortle, W.C. and Cowling, B.E., 1978. Interaction of live sapwood and fungi commonly found in discoloured and decayed wood. **Phytopathology, 68:** 617-623.

Shortle, W.C., Menge, J.A. and Cowling, B.E., 1978. Interaction of bacteria, decay fungi, and live sapwood in discolouration and decay in trees. **European Journal of Forest Pathology, 8:** 293-300.

Solheim, H., 1991. Oxygen deficiency and spruce resin inhibition of growth of fungi associated with *Ips typographus*. **Mycological Research, 95:** 1387-1392.

Stalpers, J.A., 1978. Identification of wood inhabiting Aphyllophorales in pure culture. **Studies in Mycology No. 16.** CBS Institute of the Royal Netherlands Academy of Arts and Sciences. Pp 248.

Sydow, H., 1924. Deschereibungen neuer südafrikanisker Pilze-V. **Annales Mycologic, 22:** 431.

Tsuneda, A. and Thorn, R.G., 1995. Interaction of wood decay fungi with other microorganisms, with emphasis on the degradation of cell walls. **Canadian Journal of Botany, 73 (Suppl.1):** S1325-S1333.

Unestam, T., Beyer-Ericson, L. and Strand, M., 1989. Involvement of *Cylindrocarpon destructans* in root death of *Pinus sylvestris* seedlings: Pathogenic behaviour and predisposing factors. **Scandinavian Journal of Forest Research, 4:** 521-535.

Van der Kamp, B.J., 1975. The distribution of microorganisms associated with decay of western red cedar. **Canadian Journal of Botany, 5:** 61-67.

Venn, K., 1972. Discolouration and microflora in stored pulpwood of birch (*Betula pubescens* Ehrh.) in Norway. **Communications from the Norwegian Forest Research Institute, No. 121, Bind. 30.** Ås, Norway.

Vollbrecht, G. and Eric, A., 1995. Identifying butt roted Norway spruce trees from external signs. **Forest and Landscape Research, 1:**241-254.

Washburn, R.G., 1996. Opportunistic mold infections. In: **The Mycota, VI. Human and Animal Relationships.** Springer Verlag, Berlin. Pp 148-158.

Willan, R.L., 1965. Natural regeneration of high forest in Tanganyika. **East African Agricultural and Forestry Journal, 31:**43-53.

Zhang, P.G., Sutton, J.C. and Hopkin, A.A., 1996. Inoculum concentration and time of application of *Gliocladium roseum* in relation to biocontrol of *Botrytis cinerea* in black spruce seedlings. **Canadian Journal of Forest Research, 26:** 360-367.

PAPER III

THE PATHOGENICITY OF SOME FUNGI OF *OCOTEA USAMBARENSIS* ENGL. TREES

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ABSTRACT

Based on the Koch's postulates, a study was carried out to determine the pathogenicity of 24 decay fungi of *Ocotea usambarensis* tree species. The fungi had been previously collected on standing trees and other wood material of the same tree species and some had been isolated from stem decay of living trees. The trees were inoculated in healthy sapwood and heartwood and were left for one year before they were cut. Re-isolation of the fungi from the sapwood confirmed the pathogenicity of a *Leptodontidium* sp, *Cylindrocarpon destructans*, a *Nodulisporium* sp, two species with sterile mycelium (Sterile mycelium spp 3 and 6) and three basidiomycetes species (Basidiomycetes spp 1, 3 and 10). In the heartwood, *Trametes versicolor*, Sterile mycelium sp 3 and 6, the *Nodulisporium* sp and Basidiomycetes spp 2 and 8, were able to infect intact heartwood. Among the important decay fungi of *O. usambarensis* commonly found on trees, dead trunks or stumps and which failed to infect intact sapwood or heartwood were *Phellinus senex*, *Loweporus inflexibilis*, *Phellinus gilvus*, *Ganoderma australe*, *Stereum ostrea*, *Stereum hirsutum* and *Schizophyllum commune*. This was an indication that some decay basidiomycetes of *O. usambarensis* are unable to attack intact sapwood or heartwood in standing trees and can only colonize wood which has been inhabited by other fungi.

The relationship between moisture content and pH of the trees with the pathogenicity of the fungi was also determined. Average moisture content of sapwood of *O. usambarensis* was 81.8% while that of heartwood was 80.8%, and the two types of wood did not differ significantly in moisture content. However, the two types of wood differed significantly in pH as that of heartwood was 4.2 while that of sapwood was 5.0. One fungus, Sterile mycelium sp 3 showed that an increase in sapwood moisture content slows its rate of infection although infection also increases with increasing pH up to levels found in sapwood. This relationship was an indication that the fungus would mostly prefer sapwood of moisture-stressed trees although it was also able to infect trees with higher moisture content as well. The pathogenicity of other fungi which infected the sapwood was not influenced by either the moisture content or pH of the trees and this indicated that fungi which infect sapwood of *O. usambarensis* are able to tolerate the levels of the two physiological factors.

Key words: *Ocotea usambarensis*, wood pathogenic fungi, fungal pathogenicity, wood moisture content, wood pH.

1. INTRODUCTION

Ocotea usambarensis Engl. (commonly referred to as camphor or Ocotea)suffers from stem and butt rots which have significantly reduced its potential as a commercial timber tree. The fungi implicated to cause the disease have been reported earlier (Gibson, 1962; Willan, 1965; Renvall and Niemelä, 1993), and recently, 72 species of fungi which include 12 basidiomycetes and 60 non-basidiomycetes, have been isolated from decay in standing trees (Nsolomo and Venn, 1996). Among the reported fungi *Phellinus senex* had been associated with the heartrot of the tree species basing on the occurrence of its basidiocarps on the stems or butts of standing trees (Gibson, 1962; Dick, 1969; Renvall and Niemelä, 1993). Only recently have isolations of fungi from stem decay of standing trees been done and confirmed the participation of *Phellinus senex* in the heartrot (Nsolomoand Venn, 1996). However, in all the studies done so far, no pathogenicity tests were carried out to verify if the fungi observed or isolated from the trees were actually able to initiate decay of intact sapwood or heartwood or whether they only colonized and decomposed the trees after the stems had been infected by pioneering fungi.

Basidiomycetes are recognized as major decay fungi of standing trees and wood in general but also ascomycetes, fungi imperfecti and other conidial fungi are important decay organisms (Mercer, 1982; Rayner and Boddy, 1988). In a study which involved isolation of fungi from wounds and stem decay in *O. usambarensis*, a few basidiomycetes were able to infect sapwood wounds of standing trees while most of them were found in the heartwood in advanced or incipient decay (Nsolomo and Venn, 1996). The observations from the above study seem to correspond with earlier reports which mentioned that the majority of basidiomycetes involved in decay of standing trees colonize wood which has been already inhabited by other microorganisms while a small minority of them required freshly created wounds for entry (Etheridge, 1973). In *O. usambarensis*, these observations can only be verified if pathogenicity tests were carried out by inoculating fungi in healthy tissues of standing trees. Pathogenicity tests, based on the Koch's postulates, can prove if the reported fungi are able to infect and cause decay of intact sapwood or heartwood.

Several studies involving the verification of fungi as potential pathogens causing decay and discolouration have been carried out in many tree species (e.g., Solheim, 1988; Christiansen and Solheim, 1990; Horntvedt and Solheim, 1991; Robin, 1992). The determination of such relationships between fungi and decay provides the understanding of how they succeed one another in the colonization of stem wood in living trees and lead to further investigation into the circumstances which restrict or enable the fungi to colonize the stems. It has been suggested that in order to understand such circumstances leading to wood colonization, characterization of the physiological requirements of fungi is important (Rayner and Boddy, 1988). This is further emphasized because the roles of sapwood and heartwood fungi in standing trees is related to their adaptation to environmental constraints in the wood such as extractives, volatiles and pH (Highley *et al.*, 1983), including moisture and aeration (Rayner and Boddy, 1988).

In this study, several species of fungi collected in the forest on wood or isolated from stem decay of living *O. usambarensis* trees were inoculated in healthy trees in sapwood and heartwood to verify if they were able to attack living wood or initiate decay of uncolonized heartwood. It was thus important to determine also if the rate of colonization was related to the physiological status of the trees and specifically to determine the effect of moisture content and pH in the stems on the rate of fungal colonization.

2. MATERIALS AND METHODS

2.1 Study areas and the selection of healthy trees and fungi

The study was carried out between 1994 and 1995 in the mountain rain forests of Usambara and Kilimanjaro which have been described previously (Nsolomo and Venn, 1996). In each area, a total of 6 healthy trees were selected, 4 trees for sapwood inoculations and 2 trees for the heartwood inoculations. Healthy trees were those with dense green crowns; had no dead braches; no wounds, scars or swellings on stems; had straight trunks with no epicormic sprouts along the stem and no fungal fruitifications or exudations on the stem. The number of trees selected for the inoculations was influenced by the total number of 30 trees (15 trees from each study area) which were allowed by the Division of Forestry to be cut for the whole research on camphor (see also Nsolomo and Venn, 1996) and hence the number of trees selected for sapwood or heartwood inoculations did not reflect any statistical design but rather was intended to maximize the use of the allowed trees in each study area to obtain results which would confirm pathogenicity.

Pure cultures of 24 species of fungi were selected from those previously isolated from the decay of standing trees and from sporophores collected on the various wood material of *Ocotea usambarensis* in the two study areas. Among the 24 species, 17 of them were basidiomycetes and the rest were either ascomycetes, other conidial fungi or fungi with sterile mycelium which could not sporulate in culture.

The criteria used in the selection of the fungi which were isolated from standing trees were that either they had to be basidiomycetes or had to be those isolated from the decay margin in the sapwood or heartwood or those which had occupied all niches in the decay of the trees. However, it was only possible to use fungi collected or isolated from trees during the year 1993 because these were the only fungi which were known at the time of the inoculations (although other fungi were as well collected or isolated from trees during 1994 and 1995). The same 24 fungal species were used for the inoculation of sapwood and heartwood.

2.2 Inoculation of fungi in sapwood and heartwood of trees

The inoculation of trees was done between June and August, 1994. Pure cultures of actively growing mycelium of the selected fungi were inoculated in the trees through holes drilled using sterile increment borer (first dipped in 96% alcohol and flamed over a burning alcohol lamp). Controls were inoculated with sterile malt-agar blocks of the same size as those containing fungi.

For each tree, dead bark was first scrapped off the stem around the diameter where inoculations would take place. Inner bark was exposed without causing wounds to it and tape was put around the tree at that diameter and then points were marked 10 cm apart. At each mark, a clean slash in the bark was made and a hole, 7 mm in diameter, was drilled using the increment borer to a depth of approximately 5 cm in the sapwood, or as deep as it was reasonably thought the heartwood had been reached. Fungi and the controls were picked for inoculation in a random sequence so that adjacent inocula were not the same around each diameter. A 5 mm diameter malt-agar block colonized by an actively growing fungus or without a fungus (for controls) was picked and inserted using a sterile bark borer immediately after removing the wood core from the hole. The wood core was then rapidly re-inserted and the hole sealed with sterile vaseline oil (Nycomed Pharma As, Oslo). The whole inoculation process was done as rapidly as possible to reduce the risk of contamination.

For the sapwood, each fungus or control was inoculated once in each tree and the size of the tree determined the number of inoculation points around a certain diameter. Hence, in total, each fungus was inoculated in 8 trees for the sapwood experiment (once in each tree) and for the heartwood experiment, each fungus was inoculated in 4 trees (but replicated twice in each tree). For both the sapwood and heartwood experiments, the arrangement of the inoculated fungi around the stem of each tree was recorded and mapped and permanent labels put at each inoculation point (without wounding the stem) for future identification. The 12 trees were then left to grow for one year and were cut for fungal re-isolation between June and August, 1995.

2.3 Re-isolation of the fungi and measurement of pathogenicity

Disks containing the inoculated fungi and full longitudinal extension of the lesions were cross-cut. Fungal isolations from the disks took place in a sterile tent as described before (Nsolomo and Venn, 1996) and cultures were taken in the middle of the dark part of the discoloured wood around the malt-agar block. The discolouration often consisted of two different zones, one of which was a dark coloured area enclosing the fungus and an outer lighter area similar to the reaction zone described by Shain (1967), which spread ahead of the dark zone. Hence, cultures were taken in the dark coloured area (for those infections showing it) or in the lighter area for those showing no dark zones. For each inoculation point, five cultures were taken, two in the longitudinal direction (lower and upper side of inoculation point), another two in the tangential direction (right and left sides) and one in the radial direction (in front of the inoculation point). The growth on malt-agar medium (1.25% malt, 3% agar) of the re-isolated fungi from at least one of the five wood cultures was regarded as a positive qualitative measure of pathogenicity while lack of growth from any of the cultures was regarded as a measure of their inability to grow in healthy wood of standing trees. The total extension of the discolouration caused by wound and fungal infection was measured longitudinally, tangentially and radially around the inoculum focus and this was regarded as the quantitative measure of pathogenicity.

2.4 Measurement of moisture content and pH in trees

Immediately after felling a tree and while the cross-cutting of disks was also taking place, five pair samples of sapwood and heart wood were taken at the same diameters and heights along the main trunk. These fresh (green) wood samples were immediately weighed using a kitchen scale, and for better estimations of moisture content, larger samples (>100 g) were cut off the trunk. Later at the station, the wood blocks were oven dried at 105°C for at least 48 h until they attained a constant weight after three consecutive measurements, also weighed using the same kitchen scale. Also, at the same diameters and heights on the trunk, pH measurements were estimated using pH-indicator strips (Acilit Merk, Germany). A chisel was first hammered in freshly exposed sapwood or heartwood and the xylem sap squeezed out. The pH strip was then held in the sap until it was wet and changed colour, and the pH value later read on a scale by matching the colour of the wet indicator with that on the scale. Trees sampled for the moisture content and pH were the same trees in which the inoculations of fungi were carried out and hence a total of 12 trees were sampled, 6 from each study site.

2.5 Data analysis

For data analysis, trees from the two study sites were treated as belonging to the same population of *Ocotea usambarensis* and no site differences were considered. Hence, 8 trees were analysed for the sapwood inoculations and 4 trees for the heartwood inoculations. For each fungus, pathogenicity was qualitatively confirmed when the fungi from the wood cultures grew on the malt-agar medium and then re-identified by comparative methods with original cultures present in the laboratory. The sizes of wood discolouration were regarded as the quantitative measures of pathogenicity and were compared between those resulting from fungal infection and those induced by the controls, using the paired t-test. The t-test was conducted only if the fungi had infected at least 3 trees, using longitudinal, radial and tangential measurements.

Moisture content of the wood samples was calculated using the formula:-

$$MC = \frac{GW - ODW}{ODW}$$

Where:- MC = Moisture content
GW = Green weight
ODW = Oven dry weight

Using all the 12 sample trees, moisture content and pH were estimated for the sapwood and heartwood and these factors were individually tested if they were related to each other or if they differed with diameter or height of the trunk, using simple linear regression analysis. For the trees in which fungi were re-isolated, the relationship between the moisture content or pH or the combination of the two factors with the size of the longitudinal discolouration were determined, using simple or multiple linear regression analysis. The regression analysis was only performed for fungi which had infected at least 5 trees.

3. RESULTS

3.1 The pathogenicity of fungi in the sapwood and heartwood

Out of the 24 fungi inoculated, seven of them were able to infect the sapwood during the one year period. These were the *Leptodontidium* sp., Basidiomycetes spp 1, 3 and 10, *Cylindrocarpon destructans*, Sterile mycelium sp 3 and the *Nodulisporium* sp (Table 1). The seven fungi were not re-isolated in each tree in which they were inoculated, but four of them, namely Basidiomycetes sp 1, *Cylindrocarpon destructans*, Sterile mycelium sp 3 and *Nodulisporium* sp were able to infect sapwood in at least 4 trees.

The heartwood inoculations were partly successful because most fungal inocula did not reach the heartwood and instead ended up in the sapwood. Out of the 192 inoculations (representing all 24 fungal species in the four trees), only 81 of them made contact with the heartwood (Table 1). One tree had no single inoculum of any fungus reaching the heartwood (tree KT6) and another one (tree UT6) had only 27% of the inoculum making contact. However, some of the few inoculations which reached the heartwood were infectious while some did not grow in the heartwood at all. Six fungi which infected the heartwood were Basidiomycetes spp 2 and 8, *Trametes versicolor*, Sterile mycelium spp 3 and 6, and the *Nodulisporium* sp (Table 1). Two of the fungi were also among those which infected the sapwood and these were the Sterile mycelium sp 3 and the *Nodulisporium* sp. The size of the discoloured zones in the heartwood was smaller than that observed in the sapwood (for the same fungi) as there were no reaction zones in the heartwood. Some of the known fungi which reached the heartwood but were not re-isolated from it were *Loweporus inflexibilis, Cylindrocarpon destructans, Phellinus senex, P. gilvus, Schizophyllum commune, Stereum ostrea, S. hirsutum* and *Ganoderma australe*. The discoloured zones due to these fungi were small and mostly confined around the wound caused by the ring borer.

Table 1. Fungi inoculated in sapwood and heartwood of standing *Ocotea usambarensis* trees and their qualitative pathogenicity.

Fungal species	Nisk No.	IMI No.	Sample trees inoculated in sapwood and the results								Sample trees inoculated in heartwood and the results							
			UT1	UT2	UT3	UT4	KT1	KT2	KT3	KT4	UT5		UT6		KT5		KT6	
Basidiomycetes sp 1	93-105/70-1	367837	+	+	-	-	+	+	-	-	-	-	nc	nc	-	-	nc	nc
Basidiomycetes sp 2	93-105/93	none	-	-	-	-	-	-	-	-	-	+	nc	nc	-	nc	nc	nc
Basidiomycetes sp 3	93-105/ 100-1	none	-	-	+	-	-	-	-	-	-	-	-	-	-	-	nc	nc
Basidiomycetes sp 4	93-106/10	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nc	nc
Basidiomycetes sp 6	93-110/28-2	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	-	nc	nc
Basidiomycetes sp 8	93-121/19	none	-	-	-	-	-	-	-	-	+	+	nc	nc	-	nc	nc	nc
Basidiomycetes sp 9	93-122/18-1	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	-	nc	nc
Basidiomycetes sp 10	93-127/16-2	367490	+	-	-	-	+	+	-	-	-	-	nc	nc	-	nc	nc	nc
Cylindrocarpon destructans	93-106/7-1	366376	+	+	+	+	-	-	+	+	-	-	nc	nc	-	nc	nc	nc
Ganoderma australe	93-127B/4-1	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nc	nc
Leptodontidium sp	93-105/17	367485	+	-	-	+	-	-	-	-	-	-	nc	nc	-	nc	nc	nc
Loweporus inflexibilis	93-103/2-2	none	-	-	-	-	-	-	-	-	-	nc	-	nc	-	nc	nc	nc
Nodulisporium sp	93-127/28-1	366379	+	+	+	+	-	+	+	-	-	-	+	nc	-	-	nc	nc
Phellinus gilvus	93-126B/3	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	nc	nc	nc
Phellinus senex	93-102/2	none	-	-	-	-	-	-	-	-	-	-	-	nc	-	-	nc	nc
Phellinus sp 2	93-127/74-1	none	-	-	-	-	-	-	-	-	-	-	-	nc	-	nc	nc	nc
Schizophyllum commune	93-115B/3	none	-	-	-	-	-	-	-	-	-	-	-	nc	-	nc	nc	nc
Stereum hirsutum	93-119B/6-1	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	-	nc	nc
Stereum ostrea	93-119/7-1	none	-	-	-	-	-	-	-	-	-	-	-	nc	-	nc	nc	nc
Sterile mycelium sp 1	93-105/100-1	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	-	nc	nc
Sterile mycelium sp 3	93-110/48-1	none	+	-	+	+	+	+	+	+	+	-	nc	nc	+	nc	nc	nc
Sterile mycelium sp 4	93-110/52-2	none	-	-	-	-	-	-	-	-	-	-	nc	nc	-	-	nc	nc
Sterile mycelium sp 6	93-121/3	none	-	-	-	-	-	-	-	-	+	-	nc	nc	-	-	nc	nc
Trametes versicolor	93-108/1	none	-	-	-	-	-	-	-	-	-	-	+	nc	-	-	nc	nc

Key to Table 1.

UT 1-6 = Sample trees from the Usambara forest, No. 1-6
KT 1-6 = Sample trees from the Kilimanjaro forest, No. 1-6
- = Not re-isolated
+ = re-isolated
nc = No contact with heartwood / did not contact heartwood
NISK No. = Culture numbers given by The Norwegian Forest Research Institute
IMI No. = Identification number given by The International Mycological Institute, England

3.2 Longitudinal, tangential and radial infection by the sapwood pathogenic fungi

In the trees in which fungi were re-isolated, the amount of virulence was determined by the amount of discolouration observed in the longitudinal (axial), tangential and radial planes of the stems. Table 2 compares the extent of sapwood discolouration in trees due to fungal infection (for fungi which were able to infect at least three trees) with that caused by wounding alone as shown by the control. The rate of spread in the longitudinal direction was much higher than that in the tangential or radial directions. Longitudinal spread of Basidiomycetes spp 1 and 10 was not significantly different ($p < 0.05$, paired t-test) to that caused by wounding alone (as shown by the control). However, wounds inoculated with *Cylindrocarpon destructans*, Sterile mycelium sp 3 and the *Nodulisporium* sp caused significantly more longitudinal discolouration than the controls.

Infection of sapwood in the tangential direction was significant in wood infected by Basidiomycetes sp 1, Sterile mycelium sp 3 and *Nodulisporium* sp while infection by *Cylindrocarpon destructans* and Basidiomycetes sp 10 did not spread significantly along the tangential plane. In the radial direction, only Basidiomycetes sp 10 did not cause any significant discolouration in that direction while the other four fungi were able to cause highly significant amount of infection when compared to the control (Table 2).

Looking at the average size of discolouration in the longitudinal direction caused by the fungi during the one year period (in descending order) the most aggressive fungi are *Nodulisporium* sp, *Cylindrocarpon destructans*, Sterile mycelium sp 3, Basidiomycetes sp 1 and then Basidiomycetes sp 10.

Table 2. Extent of longitudinal, tangential and radial discolouration of sapwood due to some pathogenic fungi re-isolated from *Ocotea usambarensis* trees (fungi arranged in decreasing order of pathogenicity).

Fungal species or Control	Size of sapwood discolouration (cm) in each tree according to the direction of fungal spread.									Mean, ± std. error (n = total trees)	T-value, pair formula, compared to the Control	Significance ($p < 0.05$) of the difference with Control
	Direction of spread	UT1	UT2	UT3	UT4	KT1	KT2	KT3	KT4			
Nodulisporium sp	Longitudinal	8.1	6.8	10.8	13.5	-	7.2	11.0	-	9.6 ± 1.1	3.218	Significant
	Tangential	1.0	1.1	1.1	1.6	-	1.7	1.5	-	1.33±0.1	3.322	Significant
	Radial	0.5	0.2	0.4	0.2	-	0.2	0.4	-	0.32±0.0	4.000	Significant
Cylindrocarpon destructans	Longitudinal	6.7	7.7	8.6	10.8	-	-	7.3	5.5	7.8 ± 0.7	2.640	Significant
	Tangential	0.8	1.2	1.2	1.3	-	-	1.7	1.4	1.27±0.1	1.806	Not Significant
	Radial	0.5	0.3	0.4	0.3	-	-	0.3	0.2	0.33±0.0	4.000	Significant
Sterile mycelium sp 3	Longitudinal	6.4	-	7.5	8.5	6.7	8.0	7.8	5.4	7.2 ± 0.4	3.700	Significant
	Tangential	0.9	-	1.2	1.4	1.5	2.2	1.5	1.4	1.44±0.1	2.931	Significant
	Radial	0.3	-	0.5	0.4	0.2	0.2	0.4	0.4	0.34±0.0	4.076	Significant
Basidiomycetes sp 1	Longitudinal	4.3	6.7	-	-	5.2	8.7	-	-	6.2 ± 1.0	0.718	Not Significant
	Tangential	1.0	1.1	-	-	1.5	2.0	-	-	1.4 ±0.23	3.873	Significant
	Radial	0.7	0.3	-	-	0.1	0.1	-	-	0.4±0.14	1.321	Not Significant
Basidiomycetes sp 10	Longitudinal	4.9	-	-	-	4.2	5.5	-	-	4.9 ± 0.4	0.6003	Not Significant
	Tangential	0.9	-	-	-	1.4	1.5	-	-	1.27±0.19	0.756	Not Significant
	Radial	0.5	-	-	-	0.1	0.1	-	-	0.23±0.1	1.000	Not Significant
Control	Longitudinal	3.9	8	7.1	6.5	5.2	4.2	4.5	5.3	5.6 ± 0.5	-	-
	Tangential	0.7	1.0	0.6	1.5	1.3	1.6	1.0	1.3	1.13±0.13	-	-
	Radial	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.18±0.03	-	-

3.3 Relationship between fungal pathogenicity with moisture content and pH of trees

3.3.1 Moisture content and pH of the inoculated trees

The average moisture content percentage of the 12 trees was 81.8 ± 7.0 in the sapwood and about 80.8 ± 5.1 in the heartwood (Table 3). There was no significant difference in moisture content between the sapwood and the heartwood ($p = 0.05$) of the trees. The pH of sapwood was 5 ± 0.04 (less acidic) while that of heartwood was 4.24 ± 0.04 (more acidic). The difference in pH between sapwood and heartwood was highly significant ($p > 0.001$), (Table 3).

Table 3. Average moisture content and pH in sapwood and heartwood of twelve *O. usambarensis*sample trees used in the pathogenicity tests.

Variables described and compared (n = 60 samples from 12 trees)		Sapwood	Heartwood	T-value for pairs, sapwood vs heartwood
Moisture content (percentage)	Mean	81.8	80.8	0.184782 NS
	Minimum	50.1	59.4	
	Maximum	131.7	111.1	
	Std. Error	6.97	5.14	
pH	Mean	4.99	4.24	13.3044***
	Minimum	4.80	4.05	
	Maximum	5.19	4.5	
	Std. Error	0.04	0.04	

NS = Not Significant
*** = Significant at $p < 0.001$

The relationship between moisture content and pH, and between the two factors with the size or height of tree stems are shown in Table 4. It was found that, for the trees used in this study, there was no linear relationship between moisture content and pH in either sapwood or heartwood. However, sapwood moisture content was weakly related to height ($r = 0.419$) but not to the diameter of the stems. The pH was also not dependent on either the moisture content, the diameter or the height of the stems (Table 4).

Table 4. The relationship between moisture content, pH, diameter and height of the stem as shown in sapwood and heartwood of *Ocotea usambarensis* trees.

Variables tested for relationship	Type of stem wood	No. of samples compared	Simple linear regression equation	Correlation coefficient (r)
MC vs pH	Sapwood	60	%MC = 225.15 - 28.74 pH	- 0.205 nr
	Heartwood	60	%MC = 33.65 + 11.13 pH	0.075 nr
MC vs HT	Sapwood	60	%MC = 66.04 + 5.1 HT	0.419 Wr
	Heartwood	60	%MC = 73.54 + 2.36 HT	0.204 nr
MC vs DM	Sapwood	60	%MC = 91. 77 - 0.23 DM	- 0.057 nr
	Heartwood	60	%MC = 86.51 - 0.13 DM	- 0.035 nr
pH vs HT	Sapwood	60	pH = 4.91 + 0.03 HT	0.261 nr
	Heartwood	60	pH = 4.23 + 0.003 HT	0.029 nr
pH vs DM	Sapwood	60	pH = 5.27 - 0.007 DM	- 0.215 nr
	Heartwood	60	pH = 4.39 - 0.004 DM	- 0.127 nr

MC = Moisture content (%)
HT = Height (m)
DM = Diameter (cm)
nr = No linear relationship
Wr = Weak linear relationship

3.3.2 Relationship between moisture content and pH with fungal pathogenicity in sapwood

The relationship between fungal pathogenicity with the moisture content or pH in *O. usambarensis* as determined in some trees in which fungi were re-isolated is shown in Table 5. Results show that the amount of discolouration in the sapwood of the trees caused by mechanical wounding alone (as indicated by the control) is neither related to moisture content, pH or to the combination of the two factors. However, the discolouration caused by *Cylindrocarpon destructans* is correlated to the combination of the two factors (R = 0.638) but not to only one of the factors.

Table 5. The relationship between moisture content and pH with the amount of longitudinal sapwood-discolouration caused by some pathogenic fungi inoculated in *O. usambarensis*

Control or Fungal species	Samples tested per variable	Simple or Multiple linear regression equations and their correlation coefficients relating longitudinal discolouration (LD, in **cm**) to moisture content (%MC) and pH		
		Moisture content (MC)	pH	Combined effect of Moisture content and pH
Control	40	LD = 5.76 - 0.002 MC r = - 0.032	LD = 9.71 - 0.83 pH r = 0.064	LD = 21.8-0.01 MC - 3.035 pH R = 0.149
Cylindrocarpon destructans	30	LD = 6.23+0.017 MC r = 0.264	LD= -3.45 +2.26pH r = 0.145	LD= -71.7+0.066MC+14.81pH R = 0.638
Sterile mycelium sp 3	35	LD = 8.94 - 0.016 MC r = - 0.487	LD= 26.56+ 6.85pH r = 0.933	LD = -37.7+0.013MC+8.86 pH R = 0.969
Nodulisporium sp	30	LD = 14. 4 - 0.04 MC r = - 0.239	LD= - 54.2+13.1pH r = 0.355	LD = - 65.2+0.01MC+15.1pH R = 0.358

Sterile mycelium sp 3 has a fairly conspicuous negative linear relationship (r = - 0.487) with moisture content and the discolouration decreasing in trees with more moisture content. The fungus also prefers sapwood pH (less acidic) than heartwood pH (more acidic) and infection increases with increasing pH (r = 0.933) between the two wood types. The combined effect of moisture content and pH had a much stronger influence on the amount of infection it caused (R = 0.969). *Nodulisporium* sp had a poor linear relationship with moisture content, pH or the combination of the two factors.

4. DISCUSSION

4.1 Pathogenicity of fungi in the sapwood and heartwood.

The seven fungal species which were pathogenic to sapwood are now also proved to be pioneers in the succession to colonize the tree stems. All of the fungi were previously isolated from decay of standing trees and none of them belonged to the fungi whose fruit bodies were collected on decomposing camphor wood or standing trees (see Nsolomo and Venn, 1996). This indicates that most basidiomycetes whose fruitifications appear on standing trees or on dead camphor wood in the forest are mostly saprotrophic and hence colonize non-living wood. However, the ability to grow in intact heartwood by some fungi such as *Trametes versicolor* whose basidiocarps are as well found on standing trees (see Nsolomo and Venn, 1996), indicates also their pioneering ability to initiate decay and later become part of the climax decomposing flora of camphor. However, it is known that most basidiomycetes lack the ability to grow in heartwood which has not been colonized by other fungi (Mercer, 1982). *Phellinus senex*, which has been asssociated with the heart rot of *Ocotea usambarensis* for a long time (Gibson,1962; Willan, 1965; Dick, 1969), together with other fungi were unable to infect living sapwood or intact heartwood of standing trees. This was probably due to their inability to detoxify allelopaths in the tree which are dominated by the presence of the oil cineol (Bryce, 1967).

The failure of some inocula replicates to make contact with the heartwood during inoculation was mainly due to the small size of heartwood in the selected trees and as they were relatively young (about 40 years) the distinction between the sapwood and heartwood by only looking at the wood core from bored holes during inoculation could be difficult. Most of the trees had more sapwood than heartwood and hence could not be reached by the ring borers. Bryce (1967) also described the heartwood of camphor as

sometimes indistinct and merging with sapwood and only recognizable when trees are cut because the exposure causes the heartwood to darken.

Although some fungi could not be re-isolated from more than one or two trees, it was significantly important that they were able to infect *Ocotea usambarensis* as pioneering fungi, a factor which proves their pathogenicity. As pioneering fungi, they contribute to the decay of the trees by directly attacking the wood polymers or by giving way to other decay fungi which can not infect healthy wood. On the other hand, the ability to infect many trees shows that a fungus is a potential pathogen capable of causing extensive damage to trees due to its ability to overcome defences of various individual genotypes. These potential pathogens were fungi such as Sterile mycelium sp 3 and the *Nodulisporium* sp (which are able to infect both sapwood and heartwood), *Cylindrocarpon destructans* and Basidiomycetes spp1 and 10.

4.2 Longitudinal, tangential and radial growth of the pathogenic fungi

The discolouration in the radial direction was statistically significant in wounds inoculated by some fungi than in those inoculated with control. This observation however, should not give an impression that there was remarkable extension of the fungi in the radial direction as they did in longitudinal or tangential directions. For a fungus like *Nodulisporium* sp, the average extension of the infection in the longitudinal axis was 30 times more than the radial infection while tangential infection was about 4 times more than the radial extension (Table 2). Hence by far, fungi infecting the sapwood spread in the longitudinal axis faster than in the tangential or radial directions. This phenomenon demonstrates the theory of compartmentalization of decay in trees (CODIT) by Shigo and Marx (1977). According to the theory, spread of infection in the longitudinal axis is restricted by a barrier called wall no.1 which is made of occlusions and tyloses in vessels and this is regarded as the weakest wall, and hence infection spreads easily in that direction. Tangential spread is restricted by wall 3 made of ray parenchyma cells and is stronger than wall 1 hence restricts infection better. Radial spread encounters the strongest barrier walls, wall 2 (made of the physical presence of the growth rings) and the strongest of all, called wall 4, made of wood deposited immediately after wounding or fungal infection by the wood present at the time of wounding or infection. Hence the differences in the spread of infection in various planes in the sapwood of camphor trees corresponds well with the CODIT theory.

4.3 Relationship between moisture content and pH with fungal pathogenicity in the sapwood

4.3.1 Moisture content and pH of the inoculated trees

The average values for moisture content and pH in *O. usambarensis* found by this research are strikingly similar to values already given in previous reports on camphor or other members of the family to which it belongs. Bryce (1967) gives moisture content of camphor when green as 80% while Gray (1958) gives pH range for the family Lauraceae to which camphor belongs as 3.65 to 4.9. This range corresponds to the present results whereby pH of heartwood was 4.24 and that of sapwood 5. Rayner and Boddy (1988) report that generally measurements of wood pH are in the range of 4 to 6. There was lack of dependence between moisture content and pH in trees which is an indication that the fluctuation in one of the two factors does not affect the other. However, the lack of relationship between moisture content or pH with the diameter and height of stems indicates that the two factors are evenly distributed within various parts regardless of tree size or height from the ground. This also means that fungi inoculated in trees at different diameters or heights on the stem have similar growth conditions as far as the two factors are concerned. Within trees, differences exist in pH between the sapwood and heartwood and hence this may affect the type of fungi which can infect or grow in the two wood types. Fungi preferring acidic conditions would tend to establish in the heartwood while those preferring less acidic conditions would tend to establish in the sapwood. In the trees studied, moisture content was not different between sapwood and heartwood and it is likely that the heartwood was still functioning by conducting water like the sapwood. Also, it has been reported that some species of angiosperms have higher moisture content

in heartwood than in sapwood (Skaar, 1972) and that some trees may have a condition known as wetwood whereby heartwood is saturated with water (Etheridge and Morin, 1962). These observations may apply to the heartwood of *Ocotea usambarensis* because green timber (normally heartwood is harvested) is usually known to have a moisture content of 80% (Bryce, 1967).

4.3.2 Relationship between moisture content and pH with fungal pathogenicity in sapwood

Different responses have been shown by the fungi tested on the effect of moisture content or pH on the extent to which they can spread in sapwood. For most of the fungi, the two factors had no effect on their pathogenicity. However, Sterile mycelium sp 3 was sensitive to an increase in moisture content and this might be an indication that it grows faster in moisture-stressed trees. Also, it was highly sensitive to fluctuations in pH and although it was also re-isolated from heartwood (which has average pH of 4.24) it would prefer a less acidic environment such as that found in the sapwood (which has a pH of 5). This shows that the rate of infection by the fungus is highly dependent on the balance between moisture content and pH. However, as the fungus was able to establish itself in 75% of the trees in which it was inoculated (and in both the sapwood and heartwood), these factors are not bindingly limiting but only influence the rate of its spread as it remains one of the most important camphor pathogens. *Cylindrocarpon destructans* and *Nodulisporium* sp were not influenced by moisture content, pH or the combination of the two factors in sapwood and hence these factors are not limiting to their infection. Extremes of pH have been implicated in the selectivity of certain fungi for particular tree species or between heartwood and sapwood within a tree species while high moisture content is said to act firstly by becoming a physical barrier to fungi when spaces are filled with water and secondly by restricting aeration (Rayner and Boddy, 1988).

5. CONCLUSION

Various fungi have been proved to be pathogens of *Ocotea usambarensis* which establish in sapwood or heartwood. Out of the 24 species of fungi inoculated in the tree, 5 species were able to infect the sapwood, 4 species infected the heartwood and 2 species infected both the sapwood and heartwood. The most infectious fungi which were re-isolated from the sapwood and which infected at least 50% of the trees in which they were inoculated were the Basidiomycetes sp 1, *Cylindrocarpon destructans*, Sterile mycelium sp 3 and the *Nodulisporium* sp. Other fungi which infected the sapwood but were re-isolated in fewer trees were the *Leptodontidium* sp, Basidiomycetes sp 3 and Basidiomycetes sp 10. Fungi which infected the heartwood were Basidiomycetes sp 2 and 8, *Trametes versicolor*, Sterile mycelium sp 3 and 6, and the *Nodulisporium* sp. This also confirms that *T. versicolor* whose fruit bodies are frequently found on dead parts of standing trees, is able to initiate decay of intact heartwood.

Some of the fungi previously thought to be primary decayers of camphor such as *Phellinus senex* and a number of basidiomycetes which are common on dead camphor wood (stumps and trunks) such as *Loweporus inflexibilis, Phellinus gilvus, Ganoderma australe, Stereum ostrea, Stereum hirsutum* and *Schizophyllum commune* failed to infect sapwood or heartwood. This is an indication that most decay fungi of *Ocotea usambarensis* are unable to attack intact sapwood or heartwood in standing trees and can only colonize wood which has been inhabited by other fungi. However, further tests involving heartwood inoculations need to be made in order to give results which are based on equal numbers of observations for each fungus because during this study some fungi did not make contact with the heartwood in certain replicates.

Fungi which are able to infect sapwood of *O. usambarensis* are adapted to the levels of moisture content and pH of the trees and for a few fungi which were tested, infection of the sapwood was not influenced by the two physiological factors. Average moisture content of the sapwood was 81.8% and that of heartwood was 80.8% and there were no significant differences in moisture content of the two types of

wood. Heartwood pH was 4.24 and that of sapwood was pH of 5.0 and the two types of wood differed significantly as far as pH was concerned.

REFERENCES

Bryce, J.M., 1967. **The Commercial Timbers of Tanzania.** Forest Division, Utilization Section. Ministry of Natural Resources and Tourism. Dar es Salaam, Tanzania. Pp 138.

Christiansen, E. and Solheim, H., 1990. The bark beetle associated blue-stain fungus *Ophiostoma polonicum* can kill various spruce and Douglas fir. **European Journal of Forest Pathology, 20:** 436-446.

Dick, J.H., 1969. Heartwood development and heartrot in East African camphorwood, *Ocotea usambarensis* Engl. Tanzania Silviculture Research Note, No.9. Division of Forestry, Dar es Salaam. Unpublished.

Etheridge, D.E., 1973. Wound parasites causing tree decay in British Columbia. **Forest Pest Leaflet of the Pacific Forest Research Center, No. 62.** Victoria, British Columbia.

Etheridge, D.E. and Morin, L.A., 1962. Wetwood formation in balsam fir. **Canadian Journal of Botany, 40:**1336-1345.

Gibson, I.A.S., 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February - 3rd March, 1962. Kenya Forest Division. Unpublished.

Gray,V.R., 1958. The acidity of wood. **Journal of the Institute of Wood Science, 1:**58-64.

Highley, T.L., Bar-Lev, S.S., Kirk, T.K. and Larsen, M.J., 1983. Influence of O_2 and CO_2 on wood decay by heartrot and saprot fungi. **Phytopathology, 73:** 630-633.

Horntvedt, R. and Solheim, H., 1991. Pathogenicity of *Ophiostoma polonicum* to Norway spruce: The effect of isolate age and inoculum dose. **Communications of the Norwegian Forest Research Institute, No. 44.4.** Ås, Norway.

Mercer, P.C., 1982. Basidiomycete decay of standing trees. In: Frankland, J.C., Hedger, J.N. and Swift, M.J. (eds.), **Decomposer Basidiomycetes: Their Biology and Ecology**. Cambridge University Press. Cambridge. Pp 355.

Nsolomo, V.R. and Venn, K., 1996. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests, Tanzania. PhD thesis, Paper II. Agricultural University of Norway, Ås. Unpublished.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Renvall, P. and Niemelä, T., 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. **Bulletin Jardin Botanique National de Belgique, 62:** 403-414.

Robin, C., 1992. Trunk inoculations of *Phytophthora cinnamomi* in red oaks. **European Journal of Forest Pathology, 22:** 157-165.

Shain, L., 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. **Phytopathology, 57:**1034-1045.

Shigo, A.L. and Marx, H.G., 1977. Compartmentalization of decay in trees. **Agriculture Information Bulletin of United States Department Agriculture, Forest Service No.405.** Washington, D.C.

Skaar, C., 1972. **Water in Wood.** Syracus University Press. NewYork.

Solheim, H., 1988. Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway Spruce. **Communications of the Norwegian Forest Research Institute, No. 40.10.** Ås, Norway.

Willan, R.L., 1965. Natural regeneration of high forest in Tanganyika. **East African Agricultural and Forestry Journal, 31:**43-53.

PAPER IV

DECAY ABILITY OF SOME FUNGI FROM *OCOTEA USAMBARENSIS* ENGL. TREES

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ABSTRACT

Laboratory tests were conducted using decay fungi of *Ocotea usambarensis* tree on their ability to degrade wood and to determine if they possessed certain phenoloxidase enzymes. Sapwood blocks of the tree species were subjected to a four-month period of degradation by 21 fungal species while a total of 47 fungi were tested for phenoloxidase activity against gallic and tannic acids and for the laccase and tyrosinase enzymes.

Results showed that all fungi tested were able to cause a significant loss in dry weight of the wood blocks ($p = 0.05$; paired t-test), and it was later found that the fungi possessed phenoloxidase enzymes including those capable of degrading lignin. One-way analysis of variance and the comparison of the means in weight loss using the Duncan's Multiple Range Test, showed significant variation in the ability of the fungi to degrade wood. Basidiomycetes showed a greater ability to decay wood in terms of the rate and magnitude of the decomposition than non-basidiomycetes. Ranking them by their capability showed that *Trametes versicolor* caused the largest loss in weight amounting to 28%. Other strong decayers (with their respective percentage loss in weight they caused in brackets) were *Ganoderma australe* (16%), an unidentified *Phellinus* sp 2 (15%), *Phellinus senex* (10%), *P. gilvus* (7%), *Loweporus inflexibilis* (9%), *Stereum hirsutum* (8%) and *Stereum ostrea* (10%). Some non-basidiomycetous fungi such as *Cylindrocarpon destructans* (2%), *Cylindrodendrum album* (3%), *Daldinia concentrica* (2%) and a *Nodulisporium* sp (2%) were comparable in their decay ability to some basidiomycetes such as *Schizophyllum commune* (2%).

About 77% and 85% of the tested fungi showed phenoloxidase activity against gallic acid and tannic acid respectively, while 70% had the laccase enzyme and 13% had the tyrosinase enzyme. Most fungi which produced enzymes against the simple phenolic compounds of gallic acid or tannic acid only, were those belonging to groups of blue stain fungi. However, the possession of enzymes against both the simple phenolics and lignin indicated the ability of the fungi to detoxify wood allelopaths and to live on the more complex cell wall polymers. Basing on the presence of the ligninolytic enzymes, it has been found that about 70% of the tested fungi are able to cause white rot decay and this may explain the formation of hollow trunks in heartrot-affected *O.usambarensis* trees.

Considering their numerical advantage over basidiomycetes, their ability to degrade wood due to the possession of phenoloxidase enzymes, and their pioneering role in the colonization of wood in living *Ocotea usambarensis* trees, it is concluded that cumulatively, the role of non-basidiomycetous fungi in the decomposition of the tree species may be more or equal in magnitude to that of the basidiomycetes.

Key words: *Ocotea usambarensis*, decay fungi, wood degradation, phenoloxidase enzymes.

1. INTRODUCTION

Numerous fungi inhabiting dead wood or decay in standing *Ocotea usambarensis* trees have now been documented and some of them tested for pathogenicity. They include both saprotrophic and necrotrophic species capable of infecting fresh wounds in sapwood or heartwood. A total of 19 species of larger fungi have been collected on standing trees, stumps or dead trunks of the tree species (Renvall and Niemelä, 1993; Nsolomo, 1996a) and about 72 taxa which include 12 basidiomycetes and 60 non- basidiomycetes, have been isolated from stem decay of standing trees (Nsolomo and Venn, 1996a). Some of the fungi isolated from standing trees were secondary wood colonizers involved in the decomposition of the heartwood or dead sapwood while few of them were capable of infecting fresh wounds in the sapwood

(Nsolomo and Venn, 1996b). Necrotrophic and saprotrophic fungi are able to utilize the simple carbohydrates in the wood and some of them may be capable of breaking down the complex wood polymers (lignin and cellulose) as their food source. The determination of the ability of wood inhabiting fungi to utilize simple sugars or the wood polymers is important for classifying their role in decay either as blue stain fungi or as wood decayers able to cause structural disability of the wood. In addition to an improved understanding of decay processes in living trees and forest products, knowledge of decomposition is thought to be important in the utilization of decay fungi in new industrial bio-processing technologies (Blanchette, 1995; Reid, 1995). In order to determine the decay ability of wood inhabiting fungi, laboratory tests are usually carried out whereby wood is subjected to pure cultures of a fungus under aseptic conditions and then the determination of loss in dry weight is regarded as the index of the state and rate of decay caused by the fungus (Building Research Establishment, 1972; Rayner and Boddy, 1988).

During their growth, fungi also produce a wide variety of enzymes which they use to exploit their food sources. Some of these enzymes can be detected by simple biochemical tests and the tests are as well helpful for diagnostic purposes (e.g., Nobles, 1948, 1965; Stalpers, 1978). The most useful enzyme tests are those which show the ligninolytic ability of the fungi as it is commonly found with white rot fungi (Rayner and Boddy, 1988). White rot fungi are able to degrade lignin together with the cellulose (Liese, 1970) and hence the determination of the lignolytic ability of fungi encompass the degradation of the two cell wall polymers.

In this study, some fungi previously collected or isolated from *Ocotea usambarensis* trees were tested for their ability to cause decay of wood blocks of the tree species under aseptic conditions, while a larger number of them were used to determine if they possess some ligninolytic enzymes. The aim was to get an indication of the fungi which are able to decompose cellwall lignin (and hence cellulose) and those which are predominantly sapstain fungi during the decay in trees or decay of wood. The information would as well be useful for diagnostic purposes of the decay fungi of the tree species.

2. MATERIALS AND METHODS

2.1 Selection of fungi and preparation of culture medium

About 21 species of fungi were used to test for their ability to degrade wood blocks of *Ocotea usambarensis* and a total of 47 species (including those used for the wood-block test) were used to test if they possessed certain lignin degrading enzymes. Fungi previously isolated in the decay of standing *O. usambarensis* trees or the fungi whose sporophores were collected on standing trees, stumps or dead trunks of the same tree species (see Nsolomo and Venn, 1996a) were used. Basidiomycetes were given priority but also other fungi were selected basing on a number of factors including the position they occupied in the decay column of standing trees. For the wood-block test, the number of fungi was limited by the number of blocks which could be sawn from wood available for the study. In all cases, the fungi selected were those with good growth rates and able to form aerial mycelia (especially those used for the wood degradation test).

The malt-agar medium used to grow fungi for the wood-block decay test was made according to the specifications given by the British Standards Institution (1961), whereby 40 g of malt and 20 g of agar were used in a litre of distilled water. About 30 ml of the sterilized medium was put in each of the rectangular screw-top bottles of size 10.5 x 5 x 5 cm with a mouth diameter of 3.5 cm. While the medium was still molten and hot, the bottles were laid down on their long axes so that the medium could cover the longitudinal sides of the bottles and allowed to cool. Test fungi were inoculated on the medium within 6 days of its preparation and allowed to grow until they covered the malt-agar surface before the wooden blocks were introduced.

2.2 Preparation of wood blocks and inoculation with fungi

Stem wood from a single *O. usambarensis* tree was taken, and due to the lack of adequate heartwood, inner sapwood (taken at least 2 cm from the wood cambium) was used. The wood was processed according to the procedure described in British Standards Institution (1961) and the Building Research Establishment (1972). Planed wood was sawn into blocks of size 2.5 x 2.5 x 1.5 cm and for each fungus, 6 blocks were prepared and then permanently marked to display the fungus species that would be used and the replicate number so that subsequent measurements would be designated to that particular block. They were then oven dried at 105°C for 18 h, then immediately weighed, later autoclaved at 121°C for 20 min at 15 atm and then re-weighed again after cooling. Autoclaving was intended to kill any fungi which might be present in the wood prior to the test, and also to impregnate the blocks with some moisture.

Sterile glass frames were put first on top of the mycelial mat so that wood blocks were not in direct contact with the medium (to avoid getting wet), but only in contact with the aerial mycelium. The wood blocks were introduced in the bottles under aseptic conditions in such a way that the long axes of the bottles were parallel to the grain of wood. Bottles were closed and then labelled to show the species of fungus and the replicate number of the wood block inside, and afterwards incubated for 4 months in the dark at 22°C ±1°C and 70% relative humidity. After the incubation period, the mycelia were scrapped off the wood (without removing any wood fragments) and the blocks oven-dried at 105°C for 18 h and then weighed.

2.3 Enzyme tests

Biochemical assays were employed to detect phenoloxidase enzymes capable of degrading simple or complex phenolic compounds. According to Nobles (1965) and Rayner and Boddy (1988), the Bavendamm test was used to detect enzymes against gallic or tannic acids which are simple phenols, while lignin degrading enzymes such as the laccase (a partial enzyme of phenoloxidase) and tyrosinase (also a partial enzyme of phenoloxidase responsible for hydroxylation of monophenols) were as well tested.

For the Bavendamm test, fungi were grown on a medium containing either tannic or gallic acid prepared as described by Nobles (1948). To make a litre of the medium, 15 g of malt and 20 g of agar were first added to 850 ml of distilled water and the remaining 150 ml being placed in a separate flask. The contents of both flasks were autoclaved and while the sterile water was still hot, 5 g of gallic or tannic acid were dissolved in it. The dissolved acid was then added to the slightly cooled malt-agar and thoroughly mixed before pouring about 20 ml of the medium in Petri-dishes. Each fungal species for the test had been grown on normal malt-agar medium (made of 12.5 g malt and 20 g agar in a litre of distilled water) for at least one week. A colonized malt-agar block of size 4 mm diameter was transferred to the medium containing the acid and left to grow for up to one week before assessment was made. Three replicates (Petri-dishes containing the acid medium) were prepared for each fungus. Indication of positive results was the presence of brown discolouration of the medium under or about the mycelial mat.

For the laccase and tyrosinase enzyme tests, fungi were grown on normal laboratory medium (made up of 12.5 g malt and 20 g agar per litre of distilled water) and left to grow until a satisfactory colony developed. The chemicals were prepared according to Stalpers (1978) and were applied on actively growing marginal hyphae. For the laccase enzyme, a drop of 0.1 M α-naphthol (1.44 g) in 96% ethanol (100 ml) was applied to a fungal colony and a positive result gave a purplish colour after 4-24 h. For the tyrosinase enzyme, a drop of 0.1% p-cresol (1.08 g) in 96% ethanol (100 ml) was added to a colony and a positive result gave orange-brown colour after about 4-48 h. For each fungus and for each enzyme tested, two replicate Petri-dishes were prepared and in each, drops of α-naphthol or p-cresol were applied on at least three places on the actively growing marginal hyphae.

2.4 Analysis of the decay ability of fungi and assessment of cultures for enzymes

The amount of loss in dry weight of the wood blocks was the measure of the ability of the fungi to decompose wood. The loss in dry weight was calculated as the difference between the oven dry weight before fungal degradation and the oven dry weight after fungal degradation. Within each fungal treatment, the loss in dry weight of the 6 replicates was compared for significance, using the paired t-test. Also for each fungus, average percentage weight loss of the oven-dry weight before degradation, was calculated and subjected to the arcsine transformation. The various fungi were then compared for their decomposition ability by the one-way analysis of variance and differences in means were compared for significance using the Duncan's Multiple Range Test. Moisture content percentage of the blocks was as well calculated to see if it was favourable during the decay by using the oven-dry weight before fungal degradation and the weight measured immediately after autoclaving.

For the enzyme tests, the intensity of the colour produced on the growth medium or on the mycelial mat was qualitatively assessed as absent, weak, strong or very strong to give and indication of the amount of wood degrading enzymes the fungi produced.

3. RESULTS

3.1 Fungal degradation of *O.usambarensis* wood blocks

Analysis of the loss in dry weight of the wood blocks after the 4-month period of fungal degradation showed that each fungal species tested was able to cause a significant loss in weight of the wood when dry weight before degradation was compared with that after degradation ($p = 0.05$, paired t-test). Before being subjected to degradation, the wood blocks had an average wood moisture content range of 40.4 to 65.5%. Both basidiomycetes and non-basidiomycetes were able to degrade the wood blocks and all caused significant loss in weight. There was no loss of weight in the wood blocks which were not subjected to any fungi (i.e., the control wood blocks). Although all fungi were able to cause significant loss in weight of the blocks, there were considerable variations between the fungi in the amount of degradation caused by each of them. The ranking of the fungi according to their decomposition ability showed that *Trametes versicolor* (which caused an average weight loss of 28%) was by far the strongest decomposer followed by *Ganoderma australe* and *Phellinus* sp 2. Other strong decomposers were *Loweporus inflexibilis*, *Phellinus gilvus*, *Phellinus senex*, *Stereum hirsutum* and *Stereum ostrea* (Table 1 and Figure 1).

Some non-basidiomycetes such as *Cylindrocarpon destructans*, *Cylindrodendrum album*, *Daldinia concentrica*, *Nodulisporium* sp. and Sterile mycelium spp 3 and 6 had the ability to decompose wood which was comparable to that of some basidiomycetes such as *Schizophyllum commune* and Basidiomycetes spp 1, 3 and 4. Overall, basidiomycetes were the most effective decomposers which caused higher weight losses of the wood blocks (Table 1 and Figure 1).

Table 1. Mean percentage loss in dry-weight of wood blocks of *O.usambarensis* subjected to a 4-month period of fungal degradation and the ranking of the fungi according to their decomposition ability.

Fungal species	NISK No.	IMI No.	Origin of fungal cultures	% Average moisture content of wood blocks (± Std. deviation; n = 6)	% Mean loss of dry-weight (± Std. deviation; n = 6)	Ranking of degradati-on ability [1)]	T-values for pair samples
Basidiomycetes sp 1	93-105/70-1	367837	Stem decay	57.0 ± 12.2	3.2 ± 0.9	5	9.1*
Basidiomycetes sp 3	93-105/103-1	-	Stem decay	59.1 ± 20.6	1.1 ± 0.5	5	5.2*
Basidiomycetes sp 4	93-106/10	-	Stem decay	59.4 ± 5.9	1.6 ± 0.7	5	5.5*
Basidiomycetes sp 6	93-110/28-2	-	Stem decay	64.2 ± 10.0	5.1 ± 1.2	4	9.9*
Basidiomycetes sp 9	93-122/ 18-2	-	Stem decay	52.8 ± 7.8	4.2 ± 2.0	4	5.4*
Basidiomycetes sp 10	93-127/16-2	367490	Stem decay	60.2 ± 11.3	6.2 ± 3.2	4	4.8*
CONTROL	-	-	-	51.5 ± 4.1	0.22 ± 0.17	6	0.09 NS
Cylindrocarpon destructans	93-106/7-1	366376	Stem decay	62.0 ± 8.1	2.2 ± 0.4	5	13.2*
Cylindrodendrum album	93-106/4-1	367069	Stem decay	62.9 ± 4.7	3.1 ± 0.8	5	8.8*
Daldinia concentrica	93-169/2	-	Fruitbody	58.6 ± 17.9	2.3 ± 0.6	5	10.5*
Ganoderma australe	93-127B/4-1	-	Fruitbody	51.6 ± 11.7	15.5 ± 3.1	2	12.4*
Loweporus inflexibilis	93-103/2-2	-	Fruitbody	40.4 ± 11.1	8.7 ± 2.2	3	8.5*
Nodulisporium sp	93-127/28-1	366379	Stem decay	53.6 ± 13.1	2.2 ± 0.2	5	16.9*
Phellinus gilvus	93-126B/3	-	Fruitbody	53.5 ± 10.8	6.8 ± 4.1	3	3.9*
Phellinus senex	93-102/2	-	Fruitbody	45.2 ± 12.3	9.5 ± 2.6	3	8.8*
Phellinus sp 2	93-127/74-1	-	Stem decay	44.5 ± 12.5	14.6 ± 5.1	2	6.0*
Schizophyllum commune	93-115B/3	-	Fruitbody	58.9 ± 13.4	2.4 ± 1.1	5	4.7*
Stereum hirsutum	93-119B/6-1	-	Fruitbody	52.7 ± 11.2	8.1 ± 0.9	3	21.4*
Stereum ostrea	93-119/9-1	-	Fruitbody	65.5 ± 7.4	10.0 ± 4.3	3	5.7*
Sterile mycelium sp 3	93-110/48-1	367839	Stem decay	50.3 ± 12.5	2.3 ± 0.5	5	11.3*
Sterile mycelium sp 4	93-110/50-2	-	Stem decay	52.1 ± 9.0	1.9 ± 1.7	5	2.8*
Trametes versicolor	93-108/1	-	Fruitbody	54.0 ± 7.8	28.4 ± 11.0	1	6.0*

Key for Table 1.

[1] Ranking of the degradation ability of fungi is based on the percentage mean loss of dry weight of wood caused. The smaller the number, the greater the decomposition ability of the fungus. Means not having the same ranking number differ significantly at p = 0.05, Duncan's Multiple Range Test.

NISK No. = Culture numbers of the Norwegian Forest Research Institute

IMI No. = Identification numbers of the International Mycological Institute, England

* = Significant at p = 0.05, T-test for paired samples

NS = Not Significant at p = 0.05, T-test for paired samples

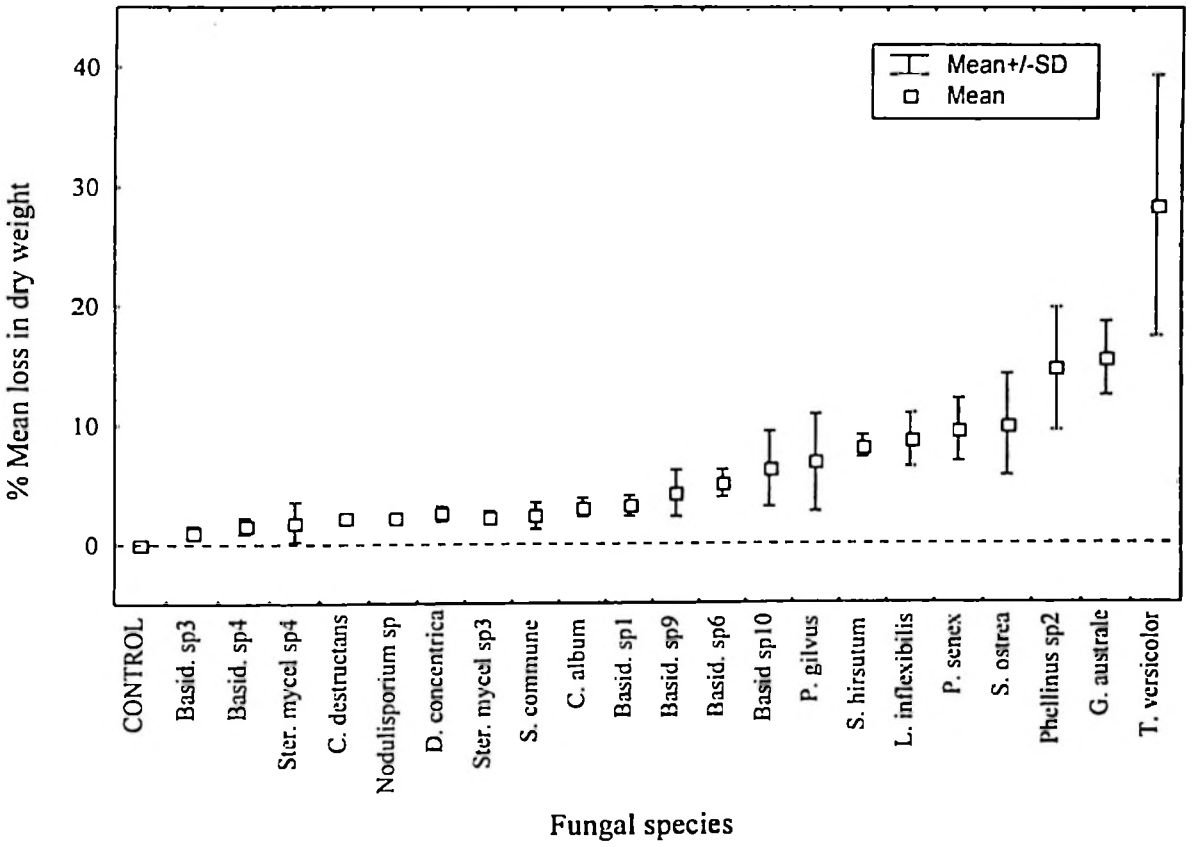

Figure 1. Mean percentage loss in dry-weight of wood blocks of *O.usambarensis* caused by various decay fungi during a 4-months period (arranged in ascending order of mean values).

3.2 Some enzymes present in decay fungi of *Ocotea usambarensis* tree

Many of the fungi tested were able to produce phenoloxidase enzymes against the simple phenolic compounds, gallic acid or tannic acid, but a large number were also able to produce phenoloxidases (mainly the laccase enzyme) which can decompose lignin. Out of the 47 different fungi tested, 77% of them secreted enzymes against gallic acid, 85% had enzymes against tannic acid, 70% possessed the laccase enzyme and 13% had the tyrosinase enzyme (Table 2). Of the 18 basidiomycetes tested, 89% of them had enzymes against gallic acid, 100% had enzymes against tannic acid, 83% had the laccase enzyme and 28% had the tyrosinase enzyme. Of the 29 non-basidiomycetes, about 69% of them produced enzymes against gallic acid, 76% against tannic acid, 62% had the laccase enzyme and only one of them, *Cylindrocarpon destructans* had also the tyrosinase enzyme. All fungi which were previously used in the wood block decay test have also shown to possess at least two enzymes against the phenolic compounds.

Table 2. Reaction of some enzymes of the decay fungi of *Ocotea usambarensis* tree on various enzyme-detecting media and biochemicals.

Fungal species	NISK No.	IMI No.	Reaction on growth media or with enzyme testing biochemicals			
			Gallic acid medium	Tannic acid medium	α-naphthol (Laccase)	ρ-cresol (Tyrosinase)
Alternaria sp	94-1113/37-1-1	367248	+++	+++	++	-
Basidiomycetes sp 1*	93-105/70-1	367837	+	+	+++	+++
Basidiomycetes sp 2	93-105/93	-	-	+++	-	-
Basidiomycetes sp 3*	93-105/103-1	-	+++	+++	++	-
Basidiomycetes sp 4*	93-106/10	-	+++	++	+++	-
Basidiomycetes sp 5	93-110/27-1	-	+++	+++	+++	-
Basidiomycetes sp 6*	93-110/28-2	-	+++	+++	++	-
Basidiomycetes sp 8	93-121/19	-	+++	+++	++	+++
Basidiomycetes sp 9*	93-122/18-1	-	+	+	+++	+++
Basidiomycetes sp 10*	93-127/16-2	367490	++	++	+++	++
Botryosphaeria ribis	93-106/3-2-1	367239	+++	++	-	-
Ceratocystis sp 1	93-129/1-1-1	367075	++	+++	-	-
Ceratocystis sp 2	93-129/4-2-1	367076	+++	++	+	-
Cladosporium sp	94-1115/3	367077	+++	+++	++	-
Conidial sp 2	93-110/36-1-1	366377	-	++	+	-
Conidial sp 10	93-123/30-1	367074	+++	+++	-	-
Conidial sp 13	94-1114/17	366382	-	+	-	-
Cylindrocarpon destructans*	93-106/7-1	366376	++	++	+++	+++
Cylindrodendrum album*	93-106/4-1	367069	+	+++	+	-
Daldinia concentrica*	93-169/2	-	+++	+++	+	-
Ganoderma australe*	93-127B/4-1	-	+++	+++	+++	-
Gliocladium roseum	93-111/84-1	367070	+++	+++	+++	-
Gliocladium viride	94-1115/14-1	370464	++	+++	++	-
Leptodontidium sp	93-105/17	367485	-	-	-	-
Loweporus inflexibilis*	93-103/2-2	-	+++	+++	+++	-
Nodulisporium sp *	93-127/28-1	366379	+++	+++	++	-
Ophiostoma sp (Graphium type)	93-106/4-1-1	367240	+++	+++	++	-
Ophiostoma sp (Sporothrix type)	93-129/2-1	367244	+++	++	-	-
Pestalotiopsis sp	93-106/3-1	-	+++	+++	+	-
Phellinus gilvus*	93-126B/3	-	+++	+++	+++	-
Phellinus senex*	93-102/2	-	+++	+++	+++	-
Phellinus sp 2*	93-127/74-1	-	+++	+++	+++	-
Phialophora parasitica	93-105/51-1	367068	+++	++	-	-
Phoma sp (Coniothyrium insigne)	94-1114/12	366381	-	-	+++	-
Pseudallescheria boydii	94-1113/1-2	367247	-	-	-	-
Pseudomorfea coffeae	94-1114/20	366383	-	-	-	-
Schizophyllum commune*	93-115B/3	-	-	+	-	-
Stereum hirsutum*	93-119B/6-1	-	+++	+++	+++	+
Stereum ostrea*	93-119/9-1	-	+	+	-	-
Sterile mycelium sp 1	93-105/100-1	-	-	+	-	-
Sterile mycelium sp 3*	93-110/48-1	367839	++	+++	++	-

Fungal species	NISK No.	IMI No.	Reaction on growth media or with enzyme testing biochemicals			
			Gallic acid medium	Tannic acid medium	α-naphthol (Laccase)	ρ-cresol (Tyrosinase)
Sterile mycelium sp 4*	93-110/50-2	-	-	+++	++	-
Sterile mycelium sp 6	93-121/3	-	+++	+++	++	-
Sterile mycelium sp 7	93-122/1-1	366378	++	-	-	-
Sterile mycelium sp 8	94-1113/12	366380	-	-	+++	-
Sterile mycelium sp 9	94-1114/3-2	367845	+	-	+	-
Trametes versicolor*	93-108/1	-	+++	+++	+++	-

* = These fungi were also used in the wood-block decay test. + = weak reaction; ++ = strong reaction; +++ = very strong reaction. Other abbreviations are as defined in Table 1.

A number of non-basidiomycetes fungi have ligninolytic enzymes similar to most basidiomycetes. However, some of them also did not react with the gallic or tannic acid media and neither did they react positively for laccase nor the tyrosinase (Table 2). Some basidiomycetes like *Schizophyllum commune* reacted only poorly with tannic acid and had none of the other tested enzymes. Some known fungi such as *Botryosphaeria ribis*, *Ophiostoma* spp and *Phialophora parasitica*, were able to react against the simple phenolics and did not produce the laccase or tyrosinase enzymes.

4. DISCUSSION

4.1 Fungal degradation of *O.usambarensis* wood blocks

The fungi which have been tested for their ability to degrade wood blocks seem to be adapted to surviving on camphor wood as they were able to cause significant loss of weight, meaning that they were able to utilize wood carbohydrates and the complex cell wall polymers. However, the use of sapwood as the test material (as done in this study) could have contributed to the easiness with which fungi degraded it because sapwood is known to lack the natural resistance to decay such as that found in heartwood (Building Research Establishment, 1972). The impregnation of the wood blocks with moisture to between 40.4 and 65.5% tried to simulate conditions that would exist in dead wood in a moist forest and although it was lower than that found in standing trees (see Nsolomo and Venn, 1996b), fungi previously isolated from decay of standing trees and used in this study would as well find it non-inhibiting. Similar additions of water have been done directly to wood or to the test medium (Building Research Establishment, 1972; Jones and Worrall, 1995) but moderate levels of moisture content have to be estimated and maintained during the test period as excessive moisture inhibits the degradation of wood (Venn, 1972; Rayner and Boddy, 1988).

The presented results on the loss of weight of camphor wood are actually an underestimation of the genuine amount of degradation because the mass of fungal hyphae in the wood blocks was not subtracted from the measurements. For *Trametes versicolor* it has been found that an error in weight loss of up to 45% can be cased by mycelium inside the wood (Jones and Worrall, 1995). However, in most experiments done previously, fungal biomass in wood was considered as having a negligible effect on weight measurements (Swift, 1973).

In this study, fungi such as *Trametes versicolor*, *Ganoderma australe* and *Phellinus* sp 2 are more aggressive in decomposing wood than others and this may partly depend on their ability to grow fast and colonize wood, apart from their ability to produce wood degrading enzymes. *Trametes versicolor* was also found to be pathogenic to intact heartwood of living *O. usambarensis* trees (Nsolomo and Venn, 1996b) and this proves that it is an important decay fungus of the tree species. *Stereum hirsutum* is recorded to cause streaked white rot of camphor wood (Ebbels and Allen, 1979) while *Phellinus senex*,

Loweporus inflexibilis, *P. gilvus*, *G. australe*, and *Stereum ostrea* are associated with decay of standing trees or dead camphor wood (Gibson, 1962; Dick, 1969; Renvall and Niemelä, 1993; Nsolomo and Venn, 1996a).

Other important pathogenic fungi capable of infecting healthy sapwood or heartwood of living *O.usambarensis* (see Nsolomo and Venn, 1996b) and which were able to degrade the wood blocks were the Basidiomycetes spp 1 and 10, *Cylindrocarpon destructans*, the *Nodulisporium* sp and the Sterile mycelium sp 3. This may show that apart from causing infection to living or intact tissues in standing trees, these fungi can also cause significant decomposition of the tissues they occupy in trees, and in addition, are also able to survive on dead wood of the tree species in the forest. Generally, fungi which were reported to occupy sapwood wounds or as pioneering fungi during stem decay of the tree species (Nsolomo and Venn, 1996a) are moderate decomposers of dead wood and hence seem to be mostly facultative parasites.

4.2 Enzymes present in the decay fungi of *O.usambarensis* tree

Most of the fungi studied have proved to possess phenoloxidase enzymes capable of degrading phenolic compounds such as gallic acid, tannic acid and lignin. Both basidiomycetes and non-basidiomycetes have such enzymes and this may verify the active participation of the non-basidiomycetous fungi in the degradation of the cell wall polymers such as lignin. Gallic acid and tannic acid are simple phenolic compounds which are involved in resistance against intruding microorganisms (Taiz and Zeiger, 1991). Gallic acid is important in plants because of its conversion to gallotannins which are known to have allelopathic properties (Salisbury and Ross, 1992). Hence fungi able to produce enzymes against these simple phenols have the ability to detoxify them and thus creating a better environment for other decay fungi which may have no such ability. Neutralization of the plants defence substances such as phenols is also among the first steps in the colonization of wood before fungi are able to utilize wood carbohydrates or before they degrade cellulose or lignin of cell walls. But fungi possessing enzymes against gallic and tannic acids only are more likely to be blue stain fungi or wound pathogens (as is the case with *Botryosphaeria ribis*, *Ophiostoma / Ceratocystis* spp and *Phialophora parasitica*) than being strong decayers of *O.usambarensis* because they may be unable to degrade the complex lignin which requires various ligninolytic phenoloxidases. Fungi not possessing enzymes against any of the phenolic compounds may be regarded as wood inhabitants utilizing simple carbohydrates or other compounds released by other fungi during decomposition of the complex polymers. The *Leptodontidium* sp which was able to infect sapwood (Nsolomo and Venn, 1996b) does not possess any of the enzymes tested. Although it can infect living tissues, its role may only be that of pioneering colonization of sapwood. As such, possession of phenoloxidases against gallic and tannic acids may not mean ability to infect or survive in *O.usambarensis* which is rich in the oil cineol (Bryce, 1967), a non-phenolic compound (Salisbury and Ross, 1992).

The presence of ligninolytic enzymes (such as laccase and tyrosinase) in some of the fungi tested indicates their ability to decompose lignin and hence this qualifies them as white rot fungi. Laccase is one of the many oxidative and reductive extracellular enzymes secreted by fungi capable of degrading lignin (Reid, 1995). The only organisms known to extensively degrade lignin are fungi and white rot fungi are the most effective lignin degraders which also are able to degrade cellulose (Gilbertson, 1980; Kirk and Farrell, 1987; Rayner and Boddy, 1988; Blanchette, 1995). In this study, non-basidiomycetous fungi such as the *Alternaria* sp, *Cylindrocarpon destructans* (teliomorph: *Nectria radicicola*), *Cylindrodendrum album*, *Cladosporium* sp, *Gliocladium roseum*, *Gliocladium viride*, *Nodulisporium* sp, *Ophiostoma* sp, *Pestalotiopsis* sp, *Phoma* sp (*Coniothyrium insigne*) and Sterile mycelium sp 3, 4, 6, 8 and 9 possess lignin degrading enzymes and hence qualify as white rot fungi. Domsch *et al.* (1993) report the ability of *Gliocladium roseum* to utilize tannins and cellulose in culture as well. Nsolomo and Venn (1996a) reports butt-rot of an *O. usambarensis* tree which was exclusively caused by non-basidiomycetous fungi whereby *Gliocladium viride* and the *Cladosporium* sp were the most dominant. These two non-basidiomycetes fungi have tested positive for the possession of phenoloxidase enzymes

against both simple and complex phenolic compounds. During the same study (Nsolomo and Venn, 1996a), the *Alternaria* sp, *Cladosporium* sp, *Gliocladium viride* and the *Phoma* sp were identified as pioneering fungi in the decay of heartwood.

Basidiomycetes are well documented as the main wood decayers capable of degrading the cell wall polymers although some ascomycetes and deuteromycetes are as well reported to have the ability to decompose wood (Rayner and Boddy, 1988). In some decay ecosystems such as those found in stems of standing *O. usambarensis* trees where basidiomycetes accounted for only 17% of all the decay fungi isolated and hence were out-numbered by the other fungal groups (Nsolomo and Venn, 1996a), the role of the non-basidiomycetous fungi in decay may be more significant than that of the basidiomycetes. This is strengthened by the fact that the non-basidiomycetes studied in *O.usambarensis* dominate as pioneering fungi during sapwood and heartwood colonization. They also have pathogenic abilities (see Nsolomo and Venn, 1996 a & b) and now have proved to possess enzymes capable of detoxifying allelopaths and digesting wood cellwalls.

Phellinus senex, the widely reported heartrot decay fungus of *O.usambarensis* (Gibson, 1962; Willan, 1965; Dick, 1969) and which was also isolated from decay of standing trees (Nsolomo and Venn, 1996a), is confirmed as one of the strong white rot fungi. The abundance of its sporophores on tree-trunks, stumps and dead wood of the tree species indicates its potential to compete effectively as a climax decay fungus (Nsolomo and Venn, 1996a) which is able to regenerate, spread and cause decay in many standing trees. Mature trees (above 50 years old) which are affected by heartrot, usually contain hollow trunks. This can now be explained by the fact that most decay fungi of the tree species (about 70%) are able to cause white rot decay which degrades all the wood components (cellulose, lignin and hemicelluloses) and leave hollow stems.

The enzyme tests have also confirmed that all fungi used in the wood block test possessed phenoloxidase enzymes some of which are capable of breaking down the cellwall, and hence that was the reason they were able to cause significant loss in dry-weight of the wood blocks. Some fungi were not able to express any of the enzymes tested. This lack of expression should not be taken as their inability to live in wood or utilize wood components because several other possible tests to show their capability to degrade lignin, cellulose or hemicellulose (see Stalpers, 1978; Rayner and Boddy, 1988) were not conducted. Also, according to Rayner and Boddy (1988), in studies such as this one, a fungal isolate may not exhibit a certain characteristic due to several factors and hence it is suggested that only positive attributes need to be acknowledged. For example, in this study, *Stereum ostrea* did not test positive for any of the ligninolytic enzymes (laccase or tyrosinase) but it was able to degrade the wood blocks and caused a significant 10% loss in dry-weight of the wood. In addition, Stalpers (1978) shows that *S. ostrea* has the laccase enzyme and another strong ligninolytic enzyme, the peroxidase, which was not tested here. As for *Schizophyllum commune*, the lack of ligninolytic ability should also not mean its inability to degrade the cell wall because it was able to cause weight loss in wood blocks, and a report by Nevalainen and Penttilä (1995) shows it has at least two cellulolytic enzymes.

5. CONCLUSION

The results of this study have shown that non-basidiomycetes play a significant role in the decay of *Ocotea usambarensis*, although basidiomycetes remain the most aggressive fungi in terms of the rate and magnitude of the decomposition. Ranking of the fungi according to their decomposition ability showed that *Trametes versicolor* was the strongest decomposer of wood of the tree species followed by *Ganoderma australe* and *Phellinus* sp 2. Other strong decomposers were *Stereum ostrea, Phellinus senex, Loweporus inflexibilis,Stereum hirsutum* and *Phellinus gilvus* (Table 1 and Figure 1). It has also been clarified that most necrotrophic or saprotrophic pathogenic fungi of the tree species reported by Nsolomo and Venn (1996b) such as the Basidiomycetes spp 1 and 10, *Cylindrocarpon destructans*, the *Nodulisporium* sp, Sterile mycelium sp 3 and *Trametes versicolor* have both the enzymes capable of

detoxifying wood allelopaths and digesting the lignin. Fungi able to produce enzymes only against simple phenolic compounds such as gallic acid or tannic acid were those belonging to groups of blue stain fungi.

Phellinus senex, the most reported heartrot fungus of *O.usambarensis* is confirmed as one of the strong white rot fungi. Basing on the possession of ligninolytic enzymes, about 70% of the fungi which have been tested during this study are able to cause white rot decay, and this may explain the formation of hollow trunks in heartrot-affected trees. Also, considering their numerical advantage over basidiomycetes, their ability to degrade wood due to their possession of phenoloxidase enzymes, and their pioneering role in the colonization of wood in living *Ocotea usambarensis* trees, it can be concluded that cumulatively, the role of non-basidiomycetous fungi in the decomposition of the camphor trees may be more or equal in magnitude to that of the basidiomycetes.

REFERENCES

Blanchette, R.A., 1995. Degradation of the lignocellulose complex in wood. **Canadian Journal of Botany, 73(Suppl. 1):** S999-S1010.

British Standards Institution, 1961. **Methods of Test for Toxicity of Wood Preservatives to Fungi.** British Standard 838. Waterlow and Sons Ltd. London. Pp 28.

Building Research Establishment, 1972. Laboratory tests of natural decay resistance of timber. **Timberlab Papers No. 50.** Princes Risborough Laboratory. Department of the environment, UK.

Dick, J.H., 1969. Heartwood development and heartrot in East African camphorwood, *Ocotea usambarensis* Engel. Tanzania Silviculture Research Note, No.9. Division of Forestry, Dar-es-Salaam. Unpublished.

Domsch, K.H., Gams, W. and Anderson, T., 1993. **Compendium of Soil Fungi, Vol. 1.** IHW-Verlag, Germany. Pp 859.

Ebbels, D.L. and Allen D.J., 1979. A supplementary and annotated list of plant diseases, pathogens and associated fungi in Tanzania. **Phytopathological Paper No. 22.** Commonwealth Agricultural Bureau. Kew, Surrey. England. Pp 89.

Gibson, I.A.S., 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February - 3rd March, 1962. Kenya Forest Division. Unpublished.

Gilbertson, R.L., 1980. Wood-rotting of North America. **Mycologia, 72:** 1-49.

Jones, H.L. and Worrall, J.J., 1995. Fungal biomass in decayed wood. **Mycologia, 87 (4):** 459-466.

Kirk, T.K. and Farrell, R.L., 1987. Enzymatic "combustion"; the microbial degradation of lignin. **Annual Review of Microbiology, 41:** 465-505.

Liese, W., 1970. Ultrastructural aspects of wood tissue disintegration. **Annual Review of Phytopathology, 8:**231-257.

Nevalainen, H. and Penttilä, M., 1995. Molecular biology of cellulolytic fungi. In: **The Mycota II. Genetics and Biotechnology**. Spinger-Verlag, Berlin. Pp 303-319.

Nobles, M.K., 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. **Canadian Journal of Research, Section C, 26:** 281-431.

Nobles, M.K., 1965. Identification of cultures of wood-inhabiting hymenomycetes. **Canadian Journal of Botany, 43:**1097-1139.

Nsolomo, V.R. and Venn, K., 1996a. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests, Tanzania. PhD thesis, Paper II. Agricultural University of Norway, Ås. Unpublished.

Nsolomo, V.R. and Venn, K., 1996b. The pathogenicity of some decay fungi of *Ocotea usambarensis* Engl. trees. PhD thesis, Paper III. Agricultural University of Norway, Ås. Unpublished.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Reid, I.D., 1995. Biodegradation of lignin. **Canadian Journal of Botany,73(Suppl.1):**1011-1018.

Renvall, P. and Niemelä, T., 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. **Bulletin Jardin Botanique National de Belgique, 62:** 403-414.

Salisbury, F.B. and Ross, C.W., 1992. **Plant Physiology**. Fourth edition. Wadsworth Publishing Company. Belmont, California. Pp 682.

Stalpers, J.A., 1978. Identification of wood inhabiting Aphyllophorales in pure culture. **Studies in Mycology No. 16.** CBS Institute of the Royal Netherlands Academy of Arts and Sciences. Pp 248.

Swift, M.J., 1973. The estimation of mycelial biomass by determination of the hexosamine content of wood decayed by fungi. **Soil Biological Biochemistry, 5:** 321-332.

Taiz, L. and Zeiger, E., 1991. **Plant Physiology**. Benjamin / Cummings Publishing Co. Inc., New York, Amsterdam. Pp 565.

Venn, K., 1972. Discolouration and microflora in stored pulpwood of birch (*Betula pubescens* Ehrh.) in Norway. **Communications from the Norwegian Forest Research Institute, No. 121, Bind. 30.** Ås, Norway.

Willan, R.L., 1965. Natural regeneration of high forest in Tanganyika. **East Africa Agricultural and Forestry Journal, 31:** 43-53.

PAPER V

GROWTH STUDIES ON SOME FUNGI OF *OCOTEA USAMBARENSIS* ENGL. TREES IN CULTURE. I. TEMPERATURE REQUIREMENTS

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ABSTRACT

Growth of 47 fungal species (18 basidiomycetes and 29 non-basidiomycetes) from *Ocotea usambarensis* tree was determined at various temperatures in culture. The results are presented to show the cardinal and the minimum lethal temperatures for a 16-days growth period. They are useful for identification of the fungi and for reference during seasoning of timber infected by them. Generally, the fungi can be classified as mesophilic, with a possibility that among the 53% of them which were able to grow at 3°C, some may be capable of growing under temperatures close to freezing point. Some fungi started to grow only around 6, 9 or 12°C. The optimum temperature for the majority (94%) ranged from 21 to 28°C while the highest value of maximum temperature reached was 40°C, by *Schizophyllum commune*. Most basidiomycetes such as *Ganoderma australe*, *Loweporus inflexibilis*, *Phellinus gilvus*, *P.senex*, a *Phellinus* sp, *Stereum hirsutum*, *S.ostrea*, *Trametes versicolor* and about 9 other unidentified basidiomycetes had an optimum temperature from 21 to 25°C. The known pioneering fungi in the colonization of stem wounds of the tree species such as *Cylindrocarpon destructans*, *Botryosphaeria ribis*, *Ophiostoma* spp, *Ceratocystis* spp, *Nodulisporium* sp, *Pestalotiopsis* sp, and the 'Sterile mycelium sp 3' had optimum temperature ranging from 21 to 28°C. *Daldinia concentrica* and *Schizophyllum commune* had the highest optimum temperature at 34°C which may partly explain their occurence on dead wood in exposed areas of the forest. Also, 91% of the fungi which tested positive for possession of ligninolytic enzymes in a previous experiment, had an optimum temperature at 21 or 25 °C. The minimum temperatures at which most fungi could be killed (lethal temperatures) within 16 days varied between 31-44°C and there was no fungus able to grow again at room temperature (21±2 °C) after previously being subjected at 44°C for the 16 day period. Generally, both the basidiomycetes and the non-basidiomycetes showed similar temperature requirements and the two groups could not be separated basing on their cardinal or lethal temperatures or by their growth rates.

The results are discussed in relation to the decay ecology and the utilization aspects of timber from the tree and also on the applicability of the results for the identification of the fungi studied. It is also concluded that the decay, pathogenic and wood inhabiting fungi of *O.usambarensis* have adapted to the prevailing temperatures of the mountain rain forests and that their growth during colonization and decay of standing trees or dead wood, is regulated by the forest temperature.

Key words: *Ocotea usambarensis*, decay fungi, fungal growth, temperature.

1. INTRODUCTION

Between 1993 and 1995 a total of 72 taxa of fungi were isolated from decay of standing *Ocotea usambarensis* trees and 14 species were collected in the forest as sporophores from decomposing wood of the tree species (Nsolomo and Venn, 1996a). Out of the 72 fungi from stem decay, 13 have been identified to species level and 20 to generic level, 10 to larger taxa (basidiomycetes) and 29 species are yet to be identified. Of the 14 species of sporophores, 12 have been identified to species level and 2 to generic level. A total of 81 isolates (10 from sporophores and 71 from stem decay) are now maintained on artificial growth medium while some are as well preserved as sporophores. Together, they form a special mycological collection of decay, pathogenic and wood inhabiting fungi from the tree species.

After isolation of fungi, studies on their growth behaviour in pure culture are usually done as part of identification work and their responses to various growth conditions are among the most important

diagnostic parameters (Robak, 1942; Nobles, 1965; Stalpers, 1978). Some of the fungi from *O.usambarensis* are well known from other hosts and substrates as they have been identified from different geographical locations of the world and also their growth characteristics in culture have also been determined (e.g., Stalpers, 1978; Domsch *et al.*, 1980; Rayner and Boddy, 1988). However, it is also a known fact that a tree decay or pathogenic fungus may exist in various distinct races which are different in behaviour and mostly influenced by the type of the host tree species (e.g., Gibson and Corbett, 1964; Korhonen, 1978; Courtois, 1980). Hence it is also of scientific interest to determine whether some of the known fungal isolates from *O.usambarensis* behave similarly to those isolated from other hosts.

Most studies on temperature requirements of fungi aim to determine the cardinal points (the minimum, optimum and maximum temperatures) for their growth (Venn, 1983; Rayner and Boddy, 1988; Domsch *et al.*, 1993) and such knowledge is important in the identification and classification of the fungi. Also, the importance of temperature lies in its influence to the occurrence of various fungi, and Shigo (1976) has reported that it has an effect on the type of fungi and their growth in wounds inflicted during different seasons of the year. In this study fungi were grown at various temperatures to define their growth range and also to determine the minimum temperatures at which they get killed. These observations form the basis of a general discussion relating temperature requirements of the fungi with the decay ecology and the utilization aspects of timber from *O. usambarensis* and also on the applicability of such temperature studies for the identification of the fungi involved.

2. MATERIALS AND METHODS

The fungi used in this experiment were those previously isolated in the decay of standing trees or the fungi whose sporophores were collected on trees, stumps or dead trunks of *O. usambarensis* (see Nsolomo and Venn, 1996a). Fungi from decay of standing trees were selected basing on a number of factors including the role they played in the decay such as whether they were pioneering fungi or heartwood decayers or their frequency of isolation in trees. The ability of a fungus to grow on malt-agar medium was also considered.

Selected fungi were cultured in 9 cm diameter Petri-dishes on 20 ml malt-agar medium (1.25% malt and 2% agar, w/v). A block of malt agar of size 4 mm diameter containing an actively growing mycelium of a fungus was placed at the edge of each Petri-dish containing the growth medium. For each fungal species tested, 4 replicates were made and a total of 47 fungi (18 basidiomycetes and 29 non-basidiomycetes) were tested. The cultures were then incubated in the dark at 15 levels of pre-set temperatures ranging from 3- 44°C (± 1°C). Starting at day 0 (day of inoculation), growth was measured at a 4-day interval for a total of 16 days. Measurements were taken along a line which divided the inoculum block of malt-agar and the Petri-dish into two halves, that is, across their diameters.

At certain temperatures, some fungi were growing faster and could cover the Petri-dishes before the 16-day period, and so estimation of the total growth for the whole period was done basing on average growth of the previous 4-day intervals. At each temperature and for each fungus, average total growth for the 16-day period was calculated from the 4 replicates. The minimum temperature was that at which a fungus showed some growth and below which it was expected not to grow, while the maximum temperature was that at which a fungus would show some growth but above which there would be no growth. The optimum temperature was defined as that at which a fungus attained a maximum growth rate. For some fungi, however, optimal growth was similar at more than one or two temperatures and so the growth had to be confirmed for no significant differences between those temperatures using the t-test or one-way analysis of variance. At certain higher temperatures fungi would not grow, but after the termination of the experiment (that is, after the 16 days period) they were incubated in the dark at room temperature (21°C ±2°C) for 2 weeks, to determine whether they were still viable or were killed at the tested temperatures. The temperature (after the maximum temperature) at which fungi were incubated

for 16 days and all 4 replicates failed to grow, then put at room temperature for 14 days and again failed to grow, was called the lethal temperature (that is, the minimum temperature at which a fungus was killed).

3. RESULTS

The minimum, optimum, maximum and lethal temperatures, together with the mean growth rate of each fungus tested are presented in Table 1. About 53% of the fungi have the minimum temperature at which growth was measurable at or close to 3°C, while 24% had minimum temperature slightly below or at 6°C and 23% at 9 °C. Only *Daldinia concentrica* had the minimum temperature at 12 °C and did not grow at the other lower temperatures. Generally, most of the fungi had their optimum growth between 21-28°C and for some fungi there were no significant differences ($p < 0.05$) in optimal growth rates between certain temperature levels, for example, between 18 and 21°C, between 21 and 25 °C between 25 and 28 °C or over a wider range of temperatures such as from 21-28 C (see Table 1). The strong decay-basidiomycetes of the tree species such *Ganoderma australe*, *Loweporus inflexibilis*, *Phellinus gilvus*, *P.senex*, a *Phellinus* sp, *Stereum hirsutum*, *S.ostrea*, *Trametes versicolor* and about 9 other unidentified basidiomycetes have an optimum temperature between 21 and 25 °C. The pioneering fungi which are mostly the pathogenic and blue stain species such as *Cylindrocarpon destructans*, *Botryosphaeria ribis*, *Ophiostoma* spp, *Ceratocystis* spp, *Nodulisporium* sp, *Pestalotiopsis* sp, and a 'Sterile mycelium sp 3' had optimum temperature ranging from 21 to 28°C (Table 1). The maximum temperatures varied between fungi but about 60% of them had a maximum temperature range between 28-31°C, and 34% had a range between 34-40°C while three species (Basidiomycetes spp 2 and 5, and a *Leptodontidium* sp) had a maximum temperature of 25°C (Table 1). On the other extreme, *Daldinia concentrica* and *Schizophyllum commune* had maximum temperatures at 37 and 40°C, respectively.

The minimum lethal temperatures at which the fungi from *O.usambarensis* were killed after exposure for 16 days ranged between 31 to 44°C. About 43% were killed at temperatures between 31- 34°C while 57% were killed at temperatures between 37-44°C. After being exposed at 44°C for 16 days, no fungus was able to grow again when put at room temperature. Some fungi such as Basidiomycetes spp 1 and 10, *Leptodontidium* sp, Sterile mycelium sp 7 and *Trametes versicolor* had their lethal temperatures far beyond their maximum temperatures (Table 1). For example, Basidiomycetes sp 1 had a maximum temperature at 31°C but its lethal temperature was 44 C. It was also observed that the maximum temperature for the *Pestalotiopsis* sp had an effect on its later growth because after removing it from its maximum temperature of 31°C and putting it at room temperature, it did not recover its fast growth which it would have attained at 21°C if it had not been subjected first to the maximum temperature.

There was a great deal of variation in terms of the growth rates of the fungi. Fungi with the highest growth rate such as Basidiomycetes sp10 and *Botryosphaeria ribis* had an average daily growth of 10 mm while *D.concentrica*, *Nodulisporium* sp, *Stereum hirsutum*, Sterile mycelium sp 3 and *Trametes versicolor* had an average of at least 7 mm per day. The *Phellinus* species, mainly *Phellinus senex* and *Phellinus* sp 2 had a relatively slow growth rate of 1.5 and 1 mm per day, respectively while *Ganoderma australe* and *Loweporus inflexibilis* were growing at a rate of 3.4 and 1.4 mm per day, respectively. About 66% of all the fungi had a growth rate of 3 mm or less per day (Table 1).

Table 1. Temperature range and the mean growth rate of various fungi of *O. usambarensis* tree

Fungal species	NISK No.	IMI No.	Temperature (°C)				Mean growth rate (mm) at the optimum temperature (n = 4)	
			Minimum	Optimum	Maximum	Lethal **	For 16 days (± Std. dev.)	Per day
Alternaria sp*	94-1113/37-1-1	367248	3	31	34	37	78.5 ± 1.3	4.9
Basidiomycetes sp 1*	93-105/70-1	367837	3	18-21	31	44	48.7 ± 0.6	3.0
Basidiomycetes sp 2	93-105/93	-	3	18-21	25	31	13.3 ± 2.3	0.8
Basidiomycetes sp 3*	93-105/103-1	-	3	21	28	34	63 ± 1.7	3.9
Basidiomycetes sp 4*	93-106/10	-	3	21	28	31	55 ± 1.7	3.4
Basidiomycetes sp 5*	93-110/27-1	-	9	25	25	31	58 ± 1	3.6
Basidiomycetes sp 6*	93-110/28-2	-	9	25	34	41	90 ± 5.6	5.6
Basidiomycetes sp 8*	93-121/19	-	6	25	31	34	14.3 ± 2.3	0.9
Basidiomycetes sp 9*	93-122/18-1	-	6	21	28	34	44 ± 1	2.8
Basidiomycetes sp 10*	93-127/16-2	367490	6	25-28	31	44	155.3 ± 4.5	9.7
Botryosphaeria ribis	93-106/3-2-1	367239	6	25	34	37	158.5 ±10.5	9.9
Ceratocystis sp 1	93-129/1-1-1	367075	3	28	34	37	41.3 ± 1.7	2.6
Ceratocystis sp 2*	93-129/4-2-1	367076	3	25-28	31	37	43.3 ± 0.5	2.7
Cladosporium sp*	94-1115/3	367077	3	21	31	34	40.5 ± 0.6	2.5
Conidial sp 2*	93-110/36-1-1	366377	6	25	34	37	33.8 ± 0.5	2.1
Conidial sp 10	93-123/30-1	367074	6	21	31	37	21.5 ± 1	1.3
Conidial sp 13	94-1114/17	366382	3	21	28	31	19.5 ± 0.6	1.2
Cylindrocarpon destructans*	93-106/7-1	366376	3	21	28	34	46.3 ± 1.5	2.9
Cylindrodendrum album*	93-106/4-1	367069	3	21	28	31	35.5 ± 0.6	2.2
Daldinia concentrica*	93-169/2	-	12	34	37	41	129.8 ± 8.3	8.1
Ganoderma australe*	93-127B/4-1	-	3	25	31	37	53.7 ± 6.4	3.4
Gliocladium roseum*	93-111/84-1	367070	3	25	31	34	21.3 ±1	1.3
Gliocladium viride*	94-1115/14-1	370464	9	21-25	28	37	28.3 ± 0.5	1.8

Fungal species	NISK No.	IMI No.	Temperature (°C)				Mean growth rate (mm) at the optimum temperature (n = 4)	
			Minimum	Optimum	Maximum	Lethal **	For 16 days (± Std. dev.)	Per day
Leptodontidium sp	93-105/17	367485	9	18-21	25	31	14.3 ± 5.8	0.9
Loweporus inflexibilis*	93-103/2-2	-	9	21	28	34	22.7 ± 1.5	1.4
Nodulisporium sp *	93-127/28-1	366379	3	21-25	28	37	127.7 ± 8.7	7.8
Ophiostoma sp (Graphium type)*	93-106/4-1-1	367240	3	25	34	37	41 ± 0.8	2.6
Ophiostoma sp (Sporothrix type)	93-129/2-1	367244	3	28	34	37	37.8 ± 3.9	2.4
Pestalotiopsis sp*	93-106/3-1	-	6	28	31	34	86.5 ± 1	5.4
Phellinus gilvus*	93-126B/3	-	6	25-28	34	41	44.7 ± 2.1	2.8
Phellinus senex*	93-102/2	-	9	21	31	34	24 ± 1	1.5
Phellinus sp 2*	93-127/74-1	-	9	21-25	31	37	14.7 ± 1.5	0.9
Phialophora parasitica	93-105/51-1	367068	6	21-28	31	37	30.3 ± 1	1.9
Phoma sp (Coniothyrium insigne)*	94-1114/12	366381	3	21-25	28	31	17.8 ± 1	1.1
Pseudallescheria boydii	94-1113/1-2	367247	9	25-28	34	37	46.3 ± 1	2.9
Pseudomorfea coffeae	94-1114/20	366383	9	25-28	31	34	20.8 ± 0.5	1.3
Schizophyllum commune	93-115B/3	-	3	31-34	40	44	87.3 ± 10.8	5.5
Stereum hirsutum*	93-119B/6-1	-	3	25	34	37	134.3 ± 1.2	8.4
Stereum ostrea	93-119/9-1	-	3	21	31	34	39.7 ± 0.6	2.5
Sterile mycelium sp 1	93-105/100-1	-	9	25-28	34	37	15.7 ± 1.5	1.0
Sterile mycelium sp 3*	93-110/48-1	367839	3	25	28	31	115.3 ± 6.8	7.2
Sterile mycelium sp 4*	93-110/50-2	-	3	25	31	37	60 ± 7	3.8
Sterile mycelium sp 6*	93-121/3	-	3	21	31	37	32.3 ± 0.6	2.0
Sterile mycelium sp 7	93-122/1-1	366378	6	25-28	34	41	26.8 ± 0.5	1.7
Sterile mycelium sp 8*	94-1113/12	366380	6	21-25	34	37	23 ± 1.6	1.4
Sterile mycelium sp 9*	94-1114/3-2	367845	3	21	28	34	22 ± 1.4	1.4
Trametes versicolor*	93-108/1	-	3	25	34	41	138.3 ± 5	8.6

Key for Table 1.
* These fungi tested positive for some ligninolytic enzymes (see Nsolomo, 1996b)
**Lethal temperature is only effective if the fungi are exposed to it for 16 days.

4. DISCUSSION

4.1 The temperature requirements of the fungi

The fungi of *Ocotea usambarensis* tree can be classified, according to Rayner and Boddy (1988), as mesophilic as the minimum temperatures are above freezing point and the maximum points up to 40°C. Most fungi have shown to be able to grow at low temperatures close to freezing point which is an indication of their ability to grow under colder temperatures which sometimes occur in the camphor forests at higher altitudes. As the average annual temperature in the forests varies from 7-27°C (Pitt-Schenkel, 1938; Mugasha, 1978), most fungi seem to have adapted to the forest temperatures as 94% of them have optimum growth at temperatures from 21 to 28°C. Some fungi such as *Daldinia concentrica* and *Schizophyllum commune* whose optima temperatures are 34 and 31-34°C respectively, may not grow optimally at the forest temperatures and may tend to colonize exposed wood which is inclined to experience high temperatures at some periods than the wood inside stems of standing trees or dead wood in well sheltered places. But, temperature within wood is less conditioned by the intrinsic properties of the material and more depends on the surrounding microclimatic conditions (Rayner and Boddy, 1988). Hence, fungi infecting or growing on exposed surfaces of wood (including wounds or fresh stumps) will be subject to variation in temperature of the environment, and this also means that temperature of a forest habitat influences the type of fungi which are likely to dominate in colonizing living or dead wood. This has been observed by Shigo (1976) that seasonal variations in temperature influence the type of microorganisms that would infect wounds inflicted on trees at different times of the year.

Some fungi such as Basidiomycetes spp 5 and 10, *Leptodontidium* sp, Sterile mycelium sp 7 and *Trametes versicolor* retain viability at temperatures far beyond their maximum temperatures. This is an indication that they can resist certain higher temperatures and remain dormant for some period of time until favourable temperatures are restored, as part of their survival strategy. After being subjected to certain higher temperatures some fungi (such as the *Pestalotiopsis* sp) do not recover their optimal growth rates probably due to the inactivation effect by high temperatures on certain enzymes which are essential for growth. Exposure to temperatures above the growth maximum are said to be lethal, but killing of fungi depends also on the length of exposure and on humidity (Rayner and Boddy, 1988). The cardinal and the lethal temperatures or the growth rates for the various fungi can not be used to distinguish between basidiomycetes and non-basidiomycetes as distinct groups because some individual fungi from either groups behaved similarly as far as temperature was concerned.

The growth rates of fungi at optimal temperatures in pure culture normally would not reflect the growth rate of the same fungi in dead wood or living tissues of trees because in wood, the food resources are not readily available like in the laboratory nutrient medium and also they are subjected to competition from other microorganisms colonizing the wood. In addition, the fungi have to overcome a number of stresses including allelopaths, moisture, pH and aeration conditions and have to produce enzymes which can break down the complex cell wall polymers (Rayner and Boddy, 1988). However, Gaüman (1939) and Henningsson (1968) have reported a relationship between growth in agar and that in wood whereby the most rapid decay of wood is said to often occur 2-3°C below the optimum for growth on agar medium.

As far as the application of these results for diagnostic purposes is concerned, there are similarities and differences in temperature requirements between some of the *O.usambarensis* isolates and those from other hosts or substrates. For example, the optimum temperature of *Cylindrocarpon destructans* is reported by Domsch *et al.* (1993) as 20-21°C which is similar to that in this study (which is 21°C). But, there is great variation in maximum temperature which is reported as 37 °C while results of this study show the maximum temperature as 28 °C. Some variations may exist between strains of the same fungus

depending on the growth medium used, the period which the fungi have been maintained in culture since isolation and also may be due to differences in original habitats and hosts (Rayner and Boddy, 1988). Also, it has been known that a tree decay or pathogenic fungus may exist in various distinct races which are different in behaviour and mostly influenced by the type of the host tree species (e.g., Gibson and Corbett, 1964; Korhonen, 1978; Courtois, 1980). However, the growth rates for *Stereum hirsutum* and *S. ostrea* achieved during this study agree with those given for the two species by Stalpers (1978) and hence confirm the usefulness of these results for diagnostic purposes.

4.2 Temperature requirements of fungi in relation to the decay ecology and utilization of *Ocotea usambarensis*.

Temperature is regarded as an ecological parameter which determines the decomposition of wood in the forest and it influences colonization by different fungi (Loman, 1962; 1965). It is also regarded as an environmental stress which may favour or eliminate certain species of fungi in the competition for resources during decay and hence affects the succession process in wood. The rates of decay by different fungi also differ with temperature (Rayner and Boddy, 1988). Fungi which can tolerate a wider range of temperature fluctuations are therefore more likely to compete well when temperature is the only microenvironmental factor imposing stress. In this study, 91% of the fungi which tested positive for possession of ligninolytic enzymes (Nsolomo and Venn, 1996b) have an optimum temperature of 21 or 25 °C. As an important environmental condition, temperature also regulates the sporulation of various fungi (e.g., Alderman and Nutter, 1994; Petrie, 1994; Fravel *et al.*, 1996) and as such, it is an important variable which is used in disease prediction models of natural epidemics (e.g., Van der Plank, 1963). In wood utilization, the importance of the knowledge on the temperature requirements of a fungus is illustrated by the temperate dry-rot fungus, *Serpula lacrimans*, whereby temperature in a house may determine the location in which the fungus is likely to be active (Rayner and Boddy, 1988). Also, for some fungi which cause blue stain of timber, kiln drying at known lethal temperatures may eliminate the fungi from the wood. As shown by the results of this study, at 44°C no fungus was able to grow again when put at room temperature and this may indicate that blue-stain or decay fungi of *O.usambarensis* can be controlled in timber if seasoning is maintained at that temperature for a period of at least 2 weeks. Large boards of timber from the tree species of about 2.5 cm thick were normally dried in the kiln for 20 days (Bryce, 1967).

5. CONCLUSION

The decay fungi of *Ocotea usambarensis* tree are mesophilic as the growth was observed at 3°C and no fungus could grow above 40°C. However, there is a possibility that among the 53% of the fungi which were able to grow at 3 °C some may be capable of growing under temperatures close to freezing point. All fungi from the tree species can be killed when exposed at 44°C for up to 16 days and this information may be important in seasoning timber infected by these fungi. Most basidiomycetes such as *Ganoderma australe*, *Loweporus inflexibilis*, *Phellinus gilvus*, *P.senex*, a *Phellinus* sp, *Stereum hirsutum*, *S.ostrea*, *Trametes versicolor* and about 9 other unidentified basidiomycetes had an optimum temperature from 21 to 25°C. The known pioneering fungi in the colonization of stem wounds of the tree species such as *Cylindrocarpon destructans*, *Botryosphaeria ribis*, *Ophiostoma* spp, *Ceratocystis* spp, *Nodulisporium* sp, *Pestalotiopsis* sp, and a 'Sterile mycelium sp 3' had optimum temperature ranging from 21 to 28°C. *Daldinia concentrica* and *Schizophyllum commune* had the highest optimum temperature at 34°C which may partly explain their occurence on dead wood in exposed areas of the forest. Also, 91% of the fungi which tested positive for possession of ligninolytic enzymes in a previous experiment had an optimum temperature at 21 or 25 °C. The temperature requirements of the fungi fall within the prevailing mean annual temperature range of 7-27°C of the mountain rain-forests in which *O.usambarensis* grows, and hence, their growth during colonization and subsequent decay of standing trees or dead wood is regulated by the forest temperature.

REFERENCES

Alderman, S.C. and Nutter, F.W. Jr., 1994. Effect of temperature and relative humidity on development of *Cercosporidium personatum* on peanut in Georgia. **Plant Disease, 8:690-694.**

Bryce, J.M., 1967. **The Commercial Timbers of Tanzania.** Forest Division, Utilization Section. Ministry of Natural Resources and Tourism. Dar es Salaam, Tanzania. Pp 138.

Courtois, H., 1980. About the occurrence of ecotypes of the species *Fomes annosus* (Fr.) Cooke. **Proceedings of the 5th International Conference on Root and Butt Rot in Conifers.** Kassel, Germany.

Domsch, K.H., Gams, W. and Anderson, T., 1993. **Compendium of Soil Fungi, Vol. 1.** IHW-Verlag, Germany. Pp 859.

Fravel, D.R., Stosz, S.K. and Larkin, R.P., 1996. Effect of temperature, soil type, and matric potential on proliferation and survival of *Fusarium oxysporum* f. sp. *erythroxyli* from *Erythroxylum coca.* **Phytopathology, 86:**236-240.

Gaümann, E., 1939. Über die Wachstums- und Zerstörungs-intensität von *Polyporus vaporarius* und von *Schizophyllum commune* bei verschiedenen temperaturen. **Angew. Bot. 21:** 59-69.

Gibson, I.A.S. and Corbett, D.C., 1964. Variation in isolates from *Armillaria* root disease in Nyasaland. **Phytopathology, 54(1):**122-123.

Henningsson, B., 1968. Ecology of decomposition of birch and aspen. In: Walters, A.H. and Elphic, J.J. (eds), **Biodeterioration of materials.** Elsevier, Amsterdam. Pp 408-423.

Korhonen, K., 1978. Intersterility groups of *Heterobasidion annosum.* **Communicationes Institute Forestalis Fenniae, 94:** 1-25.

Loman, A.A., 1962. The influence of temperature on the location and development of decay fungi in lodgepole pine logging slash. **Canadian Journal of Botany, 40:** 1545-1559.

Loman, A.A., 1965. The lethal effect of periodic high temperatures on certain lodgepole pine slash decaying basidiomycetes. **Canadian Journal of Botany, 43:** 334-338.

Mugasha, A.G., 1978. Tanzania natural forests' silvicultural research review report. Tanzania Silvicultural Technical Note (New Series) No. 39. Division of Forestry, Dar es Salaam. Unpublished.

Nobles, M.K.,1965. Identification of cultures of wood-inhabiting hymenomycetes. **Canadian Journal of Botany, 43:**1097-1139.

Nsolomo, V.R. and Venn, K., 1996a. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests, Tanzania. PhD thesis, Paper II. Agricultural University of Norway, Ås. Unpublished.

Nsolomo, V.R. and Venn, K., 1996b. Decay ability of some fungi from *Ocotea usambarensis* Engl. trees PhD thesis, Paper IV. Agricultural University of Norway, Ås. Unpublished.

Petrie, G.A., 1994. Effects of temperature and moisture on the number, size and septation of ascospores produced by *Leptosphaeria maculans* (blackleg) on rapeseed stubble. **Canadian Plant Disease Survey, 74:** 141-151.

Pitt-Schenkel, C.J.W., 1938. Some important communities of warm temperate rain forest at Magamba, West Usambara, Tanganyika Territory. **Journal of Ecology, 26:** 50-81.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Robak, H., 1942. Cultural Studies in some Norwegian wood-rotting fungi. **Meddelelser fra Vestlandets Forstlige Forsøksstation, No.25.** Pp 248.

Shigo, A.L., 1976. Microorganisms isolated from wounds inflicted on red maple, paper birch, American beech, and red oak in winter, summer and autumn. **Phytopathology, 66:** 559-563.

Stalpers, J.A., 1978. Identification of wood inhabiting Aphyllophorales in pure culture. **Studies in Mycology No. 16.** CBS Institute of the Royal Netherlands Academy of Arts and Sciences. Pp 248.

Van der Plank, E., 1963. **Plant Diseases. Epidemics and Control.** Academic Press. NewYork.

Venn, K., 1983. Winter vigour in Picea abies (L.) Karst. IX. Fungi isolated from mouldy nursery stock held in overwinter cold storage. **Reports of the Norwegian Forest Research Institute. No.38.7.**

PAPER VI

GROWTH STUDIES ON SOME FUNGI OF *OCOTEA USAMBARENSIS* ENGL. TREES IN CULTURE. II. pH REQUIREMENTS

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ABSTRACT

A study on the pH requirements of 47 fungi (18 basidiomycetes and 29 non-basidiomycetes) from *Ocotea usambarensis* trees was carried out in culture. Various pH levels ranging from 2.2 to 8.0 were fixed in malt-agar medium using buffer solutions. Basidiomycetes had pH range within the acidic region (except for *Ganoderma australe* whose maximum pH value was 7.7), while most non-basidiomycetes had a much wider range starting around 2.2 and going above 8.0. The optimum pH for 98% of the fungi was in the acidic region (below pH 6.6) corresponding to the acidic environment in the trees and the soils of the mountain rain forests in which the tree species grows. Also, 68% of the fungi had optimum pH between 4.0-4.8 which corresponds to the pH range of 4.2 and 5.0 previously reported in the heartwood and sapwood, respectively of standing *O.usambarensis* trees, and also to that of 4.0-4.5 reported for optimum ligninolytic activity of fungi. Among the fungi with pH optima corresponding to that in standing trees, were the important pathogenic and decay fungi of the tree species which included 7 species of unidentified basidiomycetes, a *Cladosporium* sp, *Cylindrocarpon destructans*, *Ganoderma australe*, *Gliocladium viride*, *Loweporus inflexibilis*, *Nodulisporium* sp, *Phellinus gilvus*, *P.senex*, *Stereum hirsutum*, *S.ostrea*,the Sterile mycelium sp 3, and *Trametes versicolor*. Some known pioneering fungi of the tree species such as *Ophiostoma* spp, *Ceratocystis* spp, *Botryosphaeria ribis* and few unidentified conidial fungi had pH optima at 3.4 which is below the levels found in trees, and this may correspond to their role of detoxifying acidic allelopaths. As 98% of the fungi had their optima pH in the acidic region below 6.6, this also implies that the flora of fungi of *O.usambarensis* is suited to the conditions within the trees, and hence pH is not a limiting factor to their growth in stems and may contribute significantly to the decay of the trees.

The results are discussed in relation to the ecology of *O.usambarensis* and to the succession of fungi in the colonization and decay of the tree species.

Key words: *Ocotea usambarensis*, decay fungi, fungal growth, pH

1. INTRODUCTION

The ecology of *O.usambarensis* (camphor) is related to its preference for acidic pH of soils (Pitt-Schenkel, 1938; Willan, 1965), and recently, it was found that pH of wood in standing trees was as well acidic with the heartwood pH averaging 4.2 and that of sapwood being around 5.0 (Nsolomo and Venn, 1996b). The above information suggests also that pH has an ecological significance on the fungi inhabiting camphor forest ecosystems, and as well as on fungi that cause stem decay of the tree species. Some of the non-basidiomycetous fungi isolated from the stem decay of the tree (Nsolomo and Venn, 1996a) had been isolated from decomposing plant material in the soil (Domsch *et al.*, 1993), thus making soil pH a factor influencing the type of fungi forming inocula reservoirs in the soil. The pH is also regarded as a predisposing environmental factor to plant diseases (Colhoun, 1979) and it has an influence on the ligninolytic activity of wood decomposing fungi (Rayner and Boddy, 1988). Hence, pH can be regarded as an ecological and as well as a physiological factor influencing fungal growth and behaviour. Many wood decay fungi are reported to have the ability to lower the pH of the growth media in which they grow to suit their requirements (Cartwright and Findlay, 1958) although brown rot species lower

the pH much more than do white rot species (Rayner and Boddy, 1988). In wood, lowering of pH is partly due to the production of carbon dioxide during decomposition which forms carbonic acid (Rayner and Boddy, 1988). As wood decomposition is an enzyme dependent reaction, it directly depends on favourable pH for specific enzymes to function. Also, it has been found that the toxicity of phenols to fungi is dependent on pH, with the effect varying between different fungal enzymes (Popoff *et al.*, 1975).

Similar to a previous study done to determine the temperature requirements of decay fungi from *Ocotea usambarensis* (Nsolomo and Venn, 1996d), this study on their pH requirements is a part of the same attempt to determine the characteristics of the fungi in pure culture and to determine if pH has any influence to the decay process of the tree species by the fungi. The results will also be discussed in relation to the decay and ecology of *Ocotea* trees, and to their applicability for identification of the fungi. These objectives will be accomplished by determining the cardinal pH levels (minimum, optimum and maximum) of the fungi.

2. MATERIALS AND METHODS

A total of 47 species of fungi were used in this experiment and were those previously tested for their phenoloxidase enzyme activity and for their temperature requirements (see Nsolomo, 1996c & d). They were originally isolated from decay of standing trees or collected as sporophores on trees, stumps or dead trunks of *O.usambarensis*. They include 18 basidiomycetes and 29 non-basidiomycetes.

The fungi were cultured in 9 cm diameter Petri-dishes each containing 20 ml of pH-buffered malt-agar medium (1.25% malt and 2% agar, w/v). Ten pH levels were fixed in the medium at 2.2, 2.7, 3.4, 4.0, 4.8, 5.7, 6.6, 7.0, 7.7 and 8.0 using various buffer solutions prepared according to Gomori (1955). To fix pH in the medium at 2.2, the hydrochloric acid-potassium chloride buffer was used, and from pH 2.7 to 7.7 the citrate-phosphate buffer was used while at pH 8.0 the phosphate buffer was used. After the buffer stock solutions had been prepared, the subsequent dilutions with distilled water (done to reach desired pH-levels - according to Gomori, 1955) were not carried out directly and instead, the distilled water was first used to prepare the malt-agar medium. The medium was then autoclaved as usual while the buffer stock solutions were only heated up to 70 ^{0}C in the oven, and were later mixed thoroughly when they had cooled to about 45-50 ^{0}C. Usually, there would be some deviations in pH levels from those expected if only distilled water was used as described by Gomori (1955), and this was due to the effect of the malt-agar. So the final pH was recorded when the buffered medium was still warm at 45-50 ^{0}C before it solidified and the above pH values used in this study were derived in this way. It was also observed that if the stock buffer solutions are diluted first with distilled water and then used to prepare the malt-agar medium and autoclaved together, the resulting buffered medium does not solidify after cooling and this happens at pH levels less than 4.5. However, using this improvised method it was possible to prepare sterile solid medium with a pH as low as 2.2 if the malt-agar was not autoclaved together with the buffer solutions.

For each of the 47 species of fungi, 4 replicates were prepared at each of the 10 pH levels. Fungi were inoculated on the buffered medium using a colonized block of malt-agar of size 4 mm diameter containing an actively growing mycelium, placed close to the edge of the Petri-dish. The Petri-dishes were then incubated in the dark at 22 ^{0}C ± 1 ^{0}C for a total of 16 days and growth monitored at 4-day intervals. Measurements were taken along a line which divided the inoculum block of malt-agar and the Petri-dish into two halves, that is, across their diameters. At certain pH-levels, some fungi were growing faster and could cover the Petri-dishes before the 16-day period, and so estimation of the total growth for the whole period was done basing on average growth of the previous 4-day intervals. At each pH level and for each fungus, average total growth for the 16-day period was calculated from the 4 replicates. For some fungi, however, optimal growth was similar at more than one pH-levels tested and

so it had to be confirmed for no significant differences between those pH-levels using the t-test or one-way analysis of variance.

3. RESULTS

The results on the pH requirements of the various fungi are presented in Table 1. About 98% of the fungi tested had optimum pH in the acidic region below 6.6. The growth range of most fungi starts from slightly below pH 2.2 to above 8.0 and about 45% of the fungi were able to grow under highly acidic pH levels at 2.2, while 47% started to grow at pH around 2.7 and 8% started to grow at pH of 3.4. Some fungi, amounting to 21% of the total fungi, had optimum pH below 4.0 and these included pioneering and fungi such as *Botryosphaeria ribis*, *Ceratocystis* spp, *Ophiostoma* spp and other conidial species. About 66% of the fungi had optimum pH between 4.0 to 5.7 and this group included fungi such as *Ganoderma australe*, *Gliocladium viride*, *Loweporus inflexibilis*, a *Nodulisporium* sp, *Phellinus gilvus*, *P. senex*, *Stereum hirsutum*, *S. ostrea*, seven species of unidentified basidiomycetes, Sterile mycelium sp 3, a *Cladosporium* sp and *Trametes versicolor*.

The distribution of the fungi according to their pH optima does not necessarily reflect whether they were isolated from sapwood or heartwood (Table 1). But, regardless of whether they were isolated from sapwood or heartwood, about 83% of the basidiomycetes had pH optima between 4 - 4.8. Except for *Ganoderma australe* which had a wider pH range (from 2.7 to 7.7) compared to other basidiomycetes, most of them had their maximum pH levels at or below 7.0 and all their optimum levels were within the acidic region. Also, about 79% of the fungi which had earlier tested positive for the possession of ligninolytic enzymes, have pH optima between 4.0 - 4.8 (Table 1).

Table 1. The pH range and the mean growth rate (at optimum pH and 22 °C) of various fungi of *Ocotea usambarensis* tree species.

Fungal species	NISK No.	IMI No.	Origin of fungus	Cardinal pH values			Mean growth rate (mm) at Optimum pH and 22 °C	
				Minimum	Optimum	Maximum	For 16 days	Per day
Alternaria sp *	94-1113/37-1-1	367248	H	2.7	4.0	> 8.0	69.8 ± 4.6	4.4
Basidiomycetes sp 1 *	93-105/70-1	367837	S H	3.4	4.8	7.0	31.8 ± 2.4	2.0
Basidiomycetes sp 2	93-105/93	-	S	2.7	3.4 - 4.8	7.0	11.8 ± 0.5	0.7
Basidiomycetes sp 3 *	93-105/103-1	-	S	2.2	3.4	7.0	42 ± 3.9	2.6
Basidiomycetes sp 4 *	93-106/10	-	S	2.2	4.8	7.0	111.3 ± 6.7	7.0
Basidiomycetes sp 5 *	93-110/27-1	-	H	2.7	3.4 - 4.0	5.7	46.3 ± 4.0	2.9
Basidiomycetes sp 6 *	93-110/28-2	-	S H	2.2	3.4	5.7	60.5 ± 1.3	3.8
Basidiomycetes sp 8 *	93-121/19	-	H	2.7	4.0	7.0	9.3 ± 1.3	0.6
Basidiomycetes sp 9 *	93-122/18-1	-	S	3.4	4.0	7.0	28.3 ± 0.5	1.8
Basidiomycetes sp 10 *	93-127/16-2	367490	S H	2.7	4.8	6.6	106.5 ± 2.6	6.7
Botryosphaeria ribis	93-106/3-2-1	367239	S	2.7	3.4	7.7	83.5 ± 12.3	5.2
Ceratocystis sp 1	93-129/1-1-1	367075	S	2.2	2.7 - 3.4	> 8.0	31.5 ± 0.6	2.0
Ceratocystis sp 2 *	93-129/4-2-1	367076	S	2.2	3.4	> 8.0	31.5 ± 0.6	2.0
Cladosporium sp *	94-1115/3	367077	H	2.2	4.0 - 4.8	> 8.0	37.3 ± 3.6	2.3
Conidial sp 2 *	93-110/36-1-1	366377	S H	2.2	3.4 - 4.0	> 8.0	30 ± 1.2	1.9
Conidial sp 10	93-123/30-1	367074	S	2.7	3.4 - 4.8	> 8.0	18.8 ± 0.5	1.2
Conidial sp 13	94-1114/17	366382	H	2.2	3.4	> 8.0	14.3 ± 1	0.9
Cylindrocarpon destructans *	93-106/7-1	366376	S H	2.2	4.0 - 5.7	> 8.0	40.5 ± 0.6	2.5
Cylindrodendrum album *	93-106/4-1	367069	S	2.7	4.8	> 8.0	33.3 ± 1.5	2.1
Daldinia concentrica *	93-169/2	-	F	2.7	5.7	> 8.0	78.5 ± 1.9	4.9
Ganoderma australe *	93-127B/4-1	-	F	2.7	4.0	7.7	43.3 ± 3.8	2.7
Gliocladium roseum *	93-111/84-1	367070	H	2.7	4.8 - 6.6	> 8.0	18.8 ± 0.5	1.2
Gliocladium viride *	94-1115/14-1	370464	H	2.2	3.4 - 4.8	> 8.0	35.8 ± 1	2.2
Leptodontidium sp	93-105/17	367485	S H	2.2	3.4	7.0	14.3 ± 0.5	0.9

Fungal species	NISK No.	IMI No.	Origin of fungus	Cardinal pH values			Mean growth rate (mm) at Optimum pH and 22 °C	
				Minimum	Optimum	Maximum	For 16 days	Per day
Loweporus inflexibilis *	93-103/2-2	-	F	2.7	4.0 - 4.8	6.6	12 ± 0.8	0.8
Nodulisporium sp *	93-127/28-1	366379	S H	2.7	4.0	7.7	137 ± 18.1	8.6
Ophiostoma sp (Graphium type) *	93-106/4-1-1	367240	S	2.2	3.4	> 8.0	31.8 ± 0.5	2.0
Ophiostoma sp (Sporothrix type)	93-129/2-1	367244	S	2.2	3.4	> 8.0	32.8 ± 2.5	2.1
Pestalotiopsis sp *	93-106/3-1	-	S	2.2	4.8	> 8.0	102.8 ± 2.6	6.4
Phellinus gilvus *	93-126B/3	-	F	2.2	4.0	5.7	41.5 ± 3.1	2.6
Phellinus senex *	93-102/2	-	H	2.2	3.4 - 4.8	5.7	23.3 ± 1.5	1.5
Phellinus sp 2 *	93-127/74-1	-	H	2.7	5.7 - 6.6	7.0	10 ± 0.8	0.6
Phialophora parasitica	93-105/51-1	367068	S H	2.7	4.8 - 5.7	> 8.0	20.8 ± 1.9	1.3
Phoma sp (Coniothyrium insigne) *	94-1114/12	366381	H	2.7	5.7	> 8.0	17.0 ± 0.0	1.1
Pseudallescheria boydii	94-1113/1-2	367247	H	3.4	5.7	> 8.0	30.3 ± 1.5	1.9
Pseudomorfea coffeae	94-1114/20	366383	H	2.2	4.0	7.0	14.3 ± 1	0.9
Schizophyllum commune	93-115B/3	-	F	2.7	3.4 - 4.8	7.0	39.8 ± 3.1	2.5
Stereum hirsutum *	93-119B/6-1	-	F	2.2	4.8	7.0	116.3 ± 2.5	7.3
Stereum ostrea	93-119/9-1	-	F	2.7	4.0	5.7	56 ± 0.8	3.5
Sterile mycelium sp 1	93-105/100-1	-	S	2.2	4.0	4.8	12.0 ± 0.0	0.8
Sterile mycelium sp 3 *	93-110/48-1	367839	S H	2.7	4.0	7.7	70.3 ± 3.8	4.4
Sterile mycelium sp 4 *	93-110/50-2	-	S H	2.7	4.8	> 8.0	56 ± 2.3	3.5
Sterile mycelium sp 6 *	93-121/3	-	S	2.2	4.0	7.0	11.3 ± 1.3	0.7
Sterile mycelium sp 7	93-122/1-1	366378	S	2.2	4.0 - 5.7	> 8.0	13.8 ± 0.5	0.9
Sterile mycelium sp 8 *	94-1113/12	366380	H	3.4	7.7 - 8.0	> 8.0	20.8 ± 1	1.3
Sterile mycelium sp 9 *	94-1114/3-2	367845	H	2.7	8.0	> 8.0	21.5 ± 1.3	1.3
Trametes versicolor *	93-108/1	-	F	2.7	4.8	6.6	72.8 ± 3.8	4.6

Key for Table 1.

* = These species possess ligninolytic enzymes (from Nsolomo and Venn, 1996c)
H = Isolated in Heartwood decay in standing trees
S = Isolated in Sapwood decay in standing trees
S H = Isolated from decay of both sapwood and heartwood in standing trees
F = Collected as Fruitbody from various *Ocotea usambarensis* wood.

4. DISCUSSION

4.1 The pH requirements of the fungi

Results have shown that most basidiomycetes have the pH range within the acidic or neutral region except for *Ganoderma australe* whose maximum level goes to 7.7. This shows that most fungi especially the basidiomycetes, are sensitive to high pH levels as most of them are specialized white rot fungi (Nsolomo and Venn, 1996c) and because high pH levels do not favour ligninolysis or cellulolysis (Rayner and Boddy, 1988). Most non-basidiomycetous fungi have pH requirements ranging from 2.2 to above 8.0 which also indicates the heterogeneity of their role in colonization and decay of the tree species. As the pH in standing *O. usambarensis* trees ranges from 4.2 in uninfected heartwood to 5.0 in the sapwood (Nsolomo and Venn, 1996b), and as 68% of the fungi have pH optimum between 4 and 4.8, this is an indication that the majority of the fungi are more favoured by the conditions in the trees. Hence, in living trees, pioneering fungi would be expected to tolerate the levels found in healthy sapwood or heartwood while secondary fungi are expected to tolerate pH levels in colonized wood which according to Rayner and Boddy (1988) may be quite different from those found in intact wood as decay fungi are able to alter the pH. This heterogeneity in tolerance of pH may also be an indication of the different capabilities of fungi in colonizing a wide range of hosts and substrates. The fungi which are known to pioneer the colonization of sapwood wounds in standing trees such as *Botryosphaeria ribis*, *Ceratocystis* spp, *Ophiostoma* spp and other conidial species (Nsolomo and Venn, 1996b) have lower pH optima of around 3.4 which could reflect their role in detoxifying various allelopaths in wood which, according Rayner and Boddy (1988) are mostly acidic. These fungi had also been found to possess phenoloxidase enzymes against gallic and tannic acids (Nsolomo and Venn, 1996c).

As 98% of all the fungi have optimum pH levels in the acidic region below 6.6, this is a further indication that most fungi have adapted to living in the tree or its wood because they can tolerate such pH levels. However, the distribution of the fungi according to their pH optima does not reflect whether they were isolated from sapwood or heartwood. Some fungi which were isolated in sapwood had pH optima similar to that found in heartwood while some of those isolated from heartwood had pH optima similar to that in sapwood of standing trees. This may mean that during decay, the fungi will tend to adjust the pH to suit their optimum requirements. It has been reported that wood decay fungi tend to acidify their growth medium, a process which may be facilitated by the formation of carbonic acid when carbon dioxide is released during decomposition (Rayner and Boddy, 1988). However, this tends to suggest also that pH is not the only factor governing fungal distribution in the stem during colonization and decay, but also most probably their roles in the stem as dictated by their nutritional requirements.

About 83% of the basidiomycetes had pH optima between 4.0 - 4.8 which corresponds to that found in intact stem of *O.usambarensis*, indicating the suitability of the stem to their establishment. A previous study (Nsolomo and Venn, 1996c) using the same fungi as used here, showed that the majority of the fungi which had ligninolytic enzymes have pH optima in the range of 4-4.8 and according to Rayner and Boddy (1988), the range also coincides with that of 4-4.5 described for optimum ligninolytic activity. Therefore findings of this study as strengthened by previous work, indicate that the decay fungi of *O. usambarensis* are favoured by the microenvironmental conditions in the tree such as pH. Given such suitable conditions and their ability to degrade lignin, the decay fungi are able to cause optimum decay

which then leads to the formation of hollow stems in trees. It has been reported earlier that most *O.usambarensis* trees affected with heart rot, develop hollow stem in the later stages of the decay (Gibson, 1962; Willan, 1965; Dick, 1969).

It should also be noted that, a fungus can have an optimum pH for ligninolytic activity which is different from its growth optimum (Rayner and Boddy, 1988), and so the values reported under this study showing the optimum pH for growth, should not be regarded as the only indicators for ligninolytic efficiency of the fungi. However, when the two properties coincide, they may greatly increase the efficiency of a fungus to degrade wood. For example, *Phellinus* sp 2 has a pH optimum between 5.7-6.6 which is beyond the reported optimum for ligninolysis, but a study on its decay ability (Nsolomo and Venn, 1996c) showed that it tested strongly for the laccase enzyme and was able to cause a significant loss in weight of wood blocks, becoming third to *Trametes versicolor* and *Ganoderma australe*. A previous decay test categorized 70% of all fungi from *O.usambarensis* as white-rot basing on their possession of ligninolytic enzymes (Nsolomo and Venn, 1996c). The current study has shown that 79% of those possessing ligninolytic enzymes have pH optima in the range of 4 - 4.8 which corresponds to that found in living trees (Table 1), and which is also said to support optimum ligninolytic activity (Rayner and Boddy, 1988).

4.2 The pH requirements of fungi in relation to the ecology of *Ocotea usambarensis*

The ecology of *O.usambarensis* is characterized by acidic soils of pH 3.5 to 5.0 (Pitt-Schenkel, 1938; Willan, 1965; Lundgren, 1978). The soil pH range is quite close to the pH range of 4.2 to 5.0 found in standing *O.usambarensis* trees (Nsolomo and Venn, 1996b) although the influence of the soil pH to that in the trees has not been investigated. Soil dwelling fungi and those attacking roots or decomposing wood material in the soil, are affected by the soil pH. Decomposition of wood material in the soil would be expected to be high because the soil pH range corresponds to the optimum levels of most fungi in this study. For example, some fungi such as *Cylindrocarpon destructans*, *Gliocladium viride* and *Gliocladium roseum*, to mention just afew, have been isolated from the soil in other places around the world (Domsch *et al.*, 1993) but have also been isolated from decay of living *O.usambarensis* trees (Nsolomo and Venn, 1996a) and possess ligninolytic enzymes (Nsolomo and Venn, 1996c; Table 1). This is a futher indication that the ecosystem in which the tree species grows favours also fungi which invade and cause its decay. However, there could be a possibility that races of the fungi have evolved to tolerate conditions in the tree and these fungi may behave differently from those found in other ecosystems. As already observed in this study, 98% of the fungi studied have pH optima in the acidic region and hence fit well in the *Ocotea* ecosystem and therefore, the possibility that the soil may act as a reservoir for fungi attacking *O.usambarensis* can not be ruled out.

Results of this study on some known fungal species correspond with results obtained from other studies using same species, but some differences exist in precise pH values. For example, Domsch *et al.* (1993) report the pH optimal of *Cylindrocarpon destructans* as 4.0 while this study gives the optimum as 4.0-5.7; *Gliocladium roseum* is reported to prefer alkaline or neutral pH while this study shows its optimum pH is from 4.8-6.6 which is in the acidic region. Also, *Gliocladium viride* is reported to have a range from 2.0-9.0 which corresponds well with results of this study, but the optimum pH is reported as 5.7-6.0, which is less acidic than the one found in this study (3.4-4.8). These variations in pH values could be due to differences in origin of the fungal isolates used in the different studies because various fungal strains of the same species usually develop when tree host-species are different (e.g., Courtois, 1980). The pH in *Ocotea usambarensis* tree is acidic (Nsolomo and Venn, 1996b) and the soils in the *Ocotea* forests are as well acidic (Pitt-Schenkel, 1938; Willan, 1965; Lundgren, 1978) and hence strains of fungi suited to acidic pH may have evolved to survive in the acidic wood and soils. This also demonstrates the specificity of the flora in *O.usambarensis* although other factors more than pH may be responsible for their adaptation to the *Ocotea* ecosystem.

5. CONCLUSION

The individual fungi of *Ocotea usambarensis* differ in specific pH requirements, but generally, most fungi have a preference to acidic pH levels, and those performing similar roles in the colonization and decay of the tree species have similar pH ranges. Basidiomycetes have pH range within the acidic region (except for *Ganoderma australe* whose upper pH value was 7.7), while most non-basidiomycetes have a much wider range starting around 2.2 and going above 8.0. As the basidiomycetes have been previously found to be stronger decayers of *Ocotea* wood, both in terms of the rate and magnitude of the process, their acidic pH requirements satisfy the decay role. This study has added a clue which, together with other previous knowledge, may explain the conditions governing the decay taking place in *O.usambarensis* trees. It is now clear that the majority of the fungi that possess ligninolytic enzymes and which therefore cause white rot, have pH optimum range corresponding to that found in standing trees, and that such pH range also corresponds to that known to support maximum ligninolytic activity. This therefore consolidates the hypothesis on the formation of hollow stems in decay affected trees because the fungi are likely to function optimally. Some pioneering fungi of the tree had more acidic pH optima than other fungi, which suggests they can tolerate and possibly detoxify acidic allelopaths during colonization of stem.

For some fungi reported in this study which are world wide distributed and on which pH studies have been carried out elsewhere, there are some similarities in optimum pH requirements but also some differences exist. This is an indication that wood inhabiting fungi may be able to evolve and adjust their pH requirements to suit the growth environment found in the new hosts or in different habitats in which they exist. In this case, the decay fungi of *O.usambarensis* are suited to the pH conditions in the trees and as such, this adaptation seems to be linked to their various roles in colonization and decay of stems, and hence makes them a special flora of the tree species.

REFERENCES

Cartwright, K.G. and Findlay, W.P.K., 1958. **Decay of Timber and its Prevention.** 2nd edition. HMSO. London.

Colhoun, J., 1979. Predisposition by the environment. In: Horsfall, J.G. and Cowling, E.B. (eds), **Plant Disease. An Advanced Treatise. Vol IV.** Academic Press, NewYork, London. Pp75-96.

Courtois, H., 1980. About the occurrence of ecotypes of the species *Fomes annosus* (Fr.) Cooke. **Proceedings of the 5th International Conference on Root and Butt Rot in Conifers**. Kassel, Germany.

Dick, J.H., 1969. Heartwood development and heartrot in East African camphorwood, *Ocotea usambarensis* Engel. Tanzania Silviculture Research Note, No.9. Division of Forestry, Dar-es-Salaam. Unpublished.

Domsch, K.H., Gams, W. and Anderson, T., 1993. **Compendium of Soil Fungi, Vol. 1.** IHW-Verlag, Germany. Pp 859.

Gibson, I.A.S., 1962. Report on a tour of plantation and forest areas in Tanganyika, Nyasaland and Southern Rhodesia, 3rd February - 3rd March, 1962. Kenya Forest Division. Unpublished.

Gomori, G., 1955. Preparation of buffers for use in enzyme studies. In: Colowick, P.S. and Kaplan, N.O. (eds), **Methods in Enzymology, Vol. 1.** Academic Press Inc., NewYork. Pp 138-146.

Lundgren, B., 1978. Soil conditions and nutrient cycling under natural and plantation forests in Tanzania highlands. **Report in Forest Ecology and Forest Soils, No.31.** Swedish University of Agricultural Sciences.

Nsolomo, V.R., Solheim, H. and Venn, K., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. I. Temperature requirements. PhD thesis, Paper V. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996a. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests, Tanzania. PhD thesis, Paper II. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996b. The pathogenicity of some decay fungi of *Ocotea usambarensis* Engl. trees. PhD thesis, Paper III. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996c. Decay ability of some fungi from *Ocotea usambarensis* Engl. trees. PhD thesis, Paper IV. Agricultural University of Norway, Ås. Manuscript.

Pitt-Schenkel, C.J.W., 1938. Some important communities of warm temperate rain forest at Magamba, West Usambara, Tanganyika Territory. **Journal of Ecology, 26**: 50-81.

Popoff, T., Theander, O. and Johansson, M., 1975. Changes in sapwood of roots of Norway spruce attacked by *Fomes annosus*. Part II. Organic chemical constituents and their biological effects. **Physiologia Plantarum, 34:** 347-356.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Willan, R.L., 1965. Natural regeneration of high forest in Tanganyika. **East Africa Agricultural and Forestry Journal, 31:** 43-53.

PAPER VII

GROWTH STUDIES ON SOME FUNGI OF *OCOTEA USAMBARENSIS* ENGL. TREES IN CULTURE. III. EFFECT OF OXYGEN STRESS

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ABSTRACT

The effect of oxygen stress on the growth of 46 fungal species (17 basidiomycetes and 29 non-basidiomycetes) of *Ocotea usambarensis* tree was determined in culture during a 24 days period. Results have shown that linear growth of mycelia from the fungi can take place under anaerobic conditions and will continue for at least one week. However, the fungi behaved differently under prolonged exposure to such conditions and growth was affected in four different ways which were; inhibition of growth, retardation of growth, normal growth, and the promotion of growth. About 72% of the fungi were inhibited within 20 days, 8% had a significantly retarded growth, 13% were not affected by the lack of oxygen and growth of 7% of them was promoted. The growth of pioneering or pathogenic fungi of sapwood wounds such as *Ceratocystis* spp, *Ophiostoma* spp, *Cylindrocarpon destructans*, *Botryosphaeria ribis*, Basidiomycetes sp 4, *Cylindrodendrum album*, *Pestalotiopsis* sp, *Nodulisporium* sp and Sterile mycelium sp 3, was inhibited within 8-16 days under anaerobic conditions. Growth of 91% of the fungi previously found to possess ligninolytic enzymes was significantly inhibited or retarded in the absence of oxygen. *Trametes versicolor* was inhibited within 8 days and the other decay fungi such as *Daldinia concentrica*, *Ganoderma australe*, *Stereum hirsutum*, *S.ostrea* and *Schizophyllum commune* were inhibited within 16-18 days. The growth of *Phellinus senex* was retarded but not inhibited within the 24 days period while the growth of *Loweporus inflexibilis*, *Phellinus gilvus* and *Phellinus* sp 2 was not significantly affected.

The behaviour of the fungi under such oxygen stress conditions is discussed in view of their roles in colonization and decay of stem in standing *O.usambarensis* trees. It is hypothesized that oxygen stress exerts a selection pressure during succession of the fungi and that tolerance to it is an important characteristic which, among other factors, favours *Phellinus* species and *Loweporus inflexibilis* to become the major decay climax fungi, the species whose fruitbodies are usually found on standing trees with heartrot.

Key words: *Ocotea usambarensis*, decay fungi, fungal growth, oxygen stress

1. INTRODUCTION

Studies on the effect of aeration to decomposition of wood have found that the growth of decay fungi is reduced by low oxygen or by high carbon dioxide concentrations (Jensen, 1969) and also that decay organisms in decay columns in trees may become inactive if the entry points have healed (Jensen, 1967; Highley *et al.*, 1986). In trees or wood, moisture content is inversely related to the availability of oxygen and hence fungal growth is inhibited by both the physical presence of moisture which obstructs passage of hyphae and also by imposing anaerobic conditions (Van der Kamp and Gokhale, 1979; Rayner and Boddy, 1988). In *Ocotea usambarensis* (with average moisture content of 80%), one pathogenic fungus, a 'Sterile mycelium sp. 3', was found to be less aggressive in trees with higher moisture content (Nsolomo and Venn, 1996b). Hence, this reciprocal relationship between moisture content and oxygen availability or aeration in trees affects the capacity of either the pathogenic or decay fungi to colonize stems. Such observations also indicate that oxygen, as a stress factor, controls the growth and survival of fungi and hence is capable of exerting a selection pressure during succession of the fungi in decay of wood or trees. The importance of aeration to the colonization of living wood tissues by fungi has already been emphasized and it has been observed that rapid growth and the ability of fungi to tolerate low

oxygen are important attributes for primary invaders to overcome tree resistance mechanisms (Solheim, 1991).

In this study, fungi previously isolated from decay of standing *O.usambarensis* trees or collected on various decaying wood of the tree species are tested *in vitro* for their tolerance to the absence of oxygen. The results are intended to characterize the fungi for identification purposes and also to give clues to their capacity to colonize and participate in decay of trees or wood under oxygen stress. The information is important in understanding the ability of some fungi to infect or cause decay of *O.usambarensis* trees which usually have a high moisture content averaging to 80% in both the sapwood and the heartwood.

2. MATERIALS AND METHODS

A total of 46 species of fungi (17 basidiomycetes and 29 non-basidiomycetes) were used. They were originally isolated from decay of standing trees or collected as sporophores on trees, stumps or dead trunks of *Ocotea usambarensis*. Test tubes which are 'dammed' (Scheffer, 1935) were used and each contained 20 ml of malt-agar medium (1.25% malt and 2% agar, w/v) slanting along the entire lower side of their longitudinal axis and forming about 4 mm thick solid layer as described by Scheffer (1967). A colonized block of malt-agar of diameter 4 mm containing actively growing mycelium of a fungus was inoculated on the medium a few centimetres from the open end of the tube. The fungi were left to grow for a maximum of one day before nitrogen gas was introduced to replace the air, under aseptic conditions. There were two treatments, one in which tubes were filled with nitrogen gas (100% N_2) and another in which tubes contained normal atmospheric air (20% O_2). For each fungus a total of 8 tubes were inoculated, 4 of which were late filled with the N_2 gas and the other 4 left with the atmospheric air. The tubes filled with atmospheric air were regarded in this experiment as the controls. Both the tubes containing N_2 and those with sterile normal air were tightly sealed with rubber stoppers and a film of sterile vaseline applied around the sealed mouths. In order to check the effectiveness of the experimental system, two species of fungi, *Ophiostoma penicillatum* (which is inhibited in N_2) and *O.polonicum* (which continues to grow in N_2) were used as controls of the system (Solheim, 1991). The fungal cultures were then incubated in dark at 22 °C ± 1°C and growth monitored after every 2 or 4 days for a total of 24 days, which were arbitrarily pre-determined as the duration of the experiment.

For each fungal species, the experiment was stopped before the 24 days period if it had grown to reach the end of the tube and total growth would be calculated up to that day when it reached the end of the tube. Also the experiment was stopped if the fungus stopped growing, that is, after two consecutive assessments showed no increment. In this case total growth was also calculated up to that day when it stopped. But such growth was later compared to the growth of the other treatment (which did not reach the end of the tube or did not stop growing) attained within the 24-day period. For each fungus, average total growth was compared between the atmospheric air and N_2 treatments using the t-test. When statistical analysis showed no significant growth differences between the treatments but in one of them the fungus had stopped growing, the growth was nevertheless judged as significantly different between the treatments.

3. RESULTS

During the first week, fungi were able to growth in nitrogen, that is, in the absence of oxygen. Later, the fungi showed four distinct characteristics which were; the inhibition of growth (i.e., stopped growing), retardation of growth, normal growth and promotion of growth. During the 24-days, growth of 37 (about 80%) of the fungi was significantly affected (stopped or retarded) in the absence of oxygen ($p < 0.05$). About 33 (72%) of the total fungi stopped growing within 20 days, 4 (8%) had growth being significantly reduced but continued to grow, 6 (13%) were not affected by the absence of oxygen while the growth of 3 (7%) of the fungi was significantly promoted (Table 1).

Some fungi such as *Trametes versicolor* and the *Ophiostoma* sp (*Graphium* type) were highly sensitive to lack of oxygen and stopped growing within 8 days. Fungi which stopped within two weeks of growing without oxygen were *Alternaria* sp, Basidiomycetes sp 4 and 10, *Ceratocystis* sp 2, *Cylindrocarpon destructans*, *Gliocladium viride*, *Nodulisporium* sp, *Ophiostoma* sp (*Sporothrix* type), *Pestalotiopsis* sp, and Sterile mycelium spp 3, 7 and 9. Fungi which were inhibited between 16-20 days were Basidiomycetes sp 2, 3, and 9, *Botryosphaeria ribis*, *Ceratocystis* sp 1, *Cladosporium* sp, Conidial spp 2 and 10, *Cylindrodendrum album*, *Daldinia concentrica*, *Ganoderma australe*, *Pseudomorfea coffeae*, *Schizophyllum commune*, *Stereum hirsutum*, *Stereum ostrea* and Sterile mycelium spp 1 and 6.

The growth of some fungi was retarded but did not stop in the absence of oxygen during the 24 days period. These fungi were *Phellinus senex*, Basidiomycetes sp 1 and 6, *Gliocladium roseum*, and Sterile mycelium spp 4 and 8. Fungi whose growth was not affected by the absence of oxygen during the test period were Conidial sp 13, *Leptodontidium* sp, *Loweporus inflexibilis*, *Phellinus gilvus*, *Phellinus* sp 2 and *Pseudallescheria boydii*. The growth of some fungi however, was significantly promoted in the absence of oxygen during the 24 days period and these were the Basidiomycetes sp 6, the *Phoma* sp (*Coniothyrium insigne*) and the Sterile mycelium sp 8. The growth of *Ceratocystis* sp 1 in nitrogen within the 16 days before it stopped was significantly higher than its growth in oxygen for the 24 days. Out of the 46 fungi, 32 of them previously tested positive for the possession of enzymes capable of degrading lignin and 91% of these ligninolytic fungi had their growth significantly affected (stopped or retarded) in the absence of oxygen (Table 1).

Table 1. Growth of various fungi of *Ocotea usambarensis* tree in sterile atmospheric air (with oxygen) and in nitrogen (without oxygen).

Fungal species	NISK No.	IMI No.	Total growth (mm) at 22 °C (± Std. Deviation)		Days of growth in Nitrogen	T - value and significance
			Oxygen (n = 4)	Nitrogen (n = 4)		
Alternaria sp +	94-1113/37-1-1	367248	58.3 ± 1.5	53.0 ± 4.1	14	2.41 NS"
Basidiomycetes sp 1 +	93-105/70-1	367837	74.5 ± 1	68.3 ± 3.8	24	3.2*
Basidiomycetes sp 2	93-105/93	-	19.5 ± 1.7	12.3 ± 1	16	7.33*
Basidiomycetes sp 3 +	93-105/103-1	-	102.3 ± 3.3	39.3 ± 1.5	20	34.72*
Basidiomycetes sp 4 +	93-106/10	-	80.5 ± 5.3	20.0 ± 2.7	12	20.45*
Basidiomycetes sp 6 +	93-110/28-2	-	89.5 ± 11.4	107.0 ± 7.9	24	2.53*
Basidiomycetes sp 8 +	93-121/19	-	18.5 ± 1.3	12.5 ± 0.6	16	8.49*
Basidiomycetes sp 9 +	93-122/18-1	-	68.5 ± 3.1	44.0 ± 2.2	20	12.94*
Basidiomycetes sp 10 +	93-127/16-2	367490	169.0 ±12.3	71.5 ± 7.9	14	13.3*
Botryosphaeria ribis	93-106/3-2-1	367239	176.3 ± 30.7	155.3 ± 24.9	20	1.06 NS"
Ceratocystis sp 1	93-129/1-1-1	367075	23.3 ± 3.8	34.3 ± 4.2	16	3.90*
Ceratocystis sp 2 +	93-129/4-2-1	367076	48.3 ± 1.3	22.8 ± 2.9	12	16.26*
Cladosporium sp +	94-1115/3	367077	50.5 ± 1	39.0 ± 5.9	16	3.85*
Conidial sp 2 +	93-110/36-1-1	366377	33.5 ± 0.6	32.5 ± 0.6	16	2.45*
Conidial sp 10	93-123/30-1	367074	27.0 ± 0.8	25.3 ± 1	16	2.78*
Conidial sp 13	94-1114/17	366382	18.5 ± 1	20.0 ± 1.8	24	1.44 NS
Cylindrocarpon destructans +	93-106/7-1	366376	66.5 ± 2.7	31.5 ± 7	12	9.35*
Cylindrodendrum album +	93-106/4-1	367069	47.0 ± 1.4	38.8 ± 1.3	16	8.72*
Daldinia concentrica +	93-169/2	-	101.0 ± 3.4	67.3 ± 3.1	16	14.76*
Ganoderma australe +	93-127B/4-1	-	62.8 ± 10.1	29.0 ± 2.6	16	6.45*
Gliocladium roseum +	93-111/84-1	367070	14.0 ± 2.2	11.5 ± 1.7	24	1.81*
Gliocladium viride +	94-1115/14-1	370464	33.8 ± 1.3	13.5 ± 2.1	14	15.04*
Leptodontidium sp	93-105/17	367485	16.0 ± 1.4	17.5 ± 1	24	1.73 NS
Loweporus inflexibilis +	93-103/2-2	-	30.0 ± 3.7	31.8 ± 5.1	24	0.56 NS

Fungal species	NISK No.	IMI No.	Total growth (mm) at 22 °C (± Std. Deviation)		Days of growth in Nitrogen	T - value and significance
			Oxygen (n = 4)	Nitrogen (n = 4)		
Nodulisporium sp +	93-127/28-1	366379	179.0 ± 6.7	63.3 ± 3.5	14	30.69*
Ophiostoma sp (Graphium type) +	93-106/4-1-1	367240	44.5 ± 2.9	11.3 ± 1.5	8	20.44*
Ophiostoma sp (Sporothrix type)	93-129/2-1	367244	39.8 ± 0.5	23.5 ± 1.7	14	18.03*
Pestalotiopsis sp +	93-106/3-1	-	89.8 ± 3	56.8 ± 13.6	14	4.75*
Phellinus gilvus +	93-126B/3	-	50.3 ± 3.6	49.0 ± 4.1	24	0.46 NS
Phellinus senex +	93-102/2	-	17.5 ± 2.1	7.3 ± 3.4	24	5.14*
Phellinus sp 2 +	93-127/74-1	-	27.5 ± 2.4	24.0 ± 3.4	24	1.70 NS
Phialophora parasitica	93-105/51-1	367068	34.3 ± 0.5	30.5 ± 3.7	20	2.01 NS"
Phoma sp (Coniothyrium insigne)	94-1114/12	366381	20.3 ± 1	26.5 ± 1.7	24	6.32*
Pseudallescheria boydii	94-1113/1-2	367247	44.3 ± 2.5	47.5 ± 4.4	24	1.29 NS
Pseudomorfea coffeae	94-1114/20	366383	20.8 ± 1.3	15.5 ± 2.1	16	4.32*
Schizophyllum commune	93-115B/3	-	72.5 ± 2.7	26.3 ± 7.4	16	11.82*
Stereum hirsutum +	93-119B/6-1	-	176.3 ± 8.7	89.8 ± 17.9	16	8.70*
Stereum ostrea	93-119/9-1	-	62.0 ± 1.4	41.3 ± 7.9	18	5.15*
Sterile mycelium sp 1	93-105/100-1	-	18.0 ± 2.6	14.0 ± 1.2	16	2.83*
Sterile mycelium sp 3 +	93-110/48-1	367839	78.3 ± 4.8	37.0 ± 2.3	12	15.52*
Sterile mycelium sp 4 +	93-110/50-2	-	92.3 ± 1.7	53.3 ± 7	24	10.83*
Sterile mycelium sp 6 +	93-121/3	-	41.8 ± 3.4	37.5 ± 3.11	16	1.84 NS"
Sterile mycelium sp 7	93-122/1-1	366378	30.0 ± 2.9	14.8 ± 0.5	14	10.21*
Sterile mycelium sp 8 +	94-1113/12	366380	28.5 ± 1.3	33.3 ± 1	24	5.91*
Sterile mycelium sp 9 +	94-1114/3-2	367845	20.3 ± 2.1	15.3 ± 1	12	4.40*
Trametes versicolor +	93-108/1	-	192.3 ± 1.9	45.5 ± 1	8	137.09*

Key to Table 1.

+ = These fungi tested positive for the possession of ligninolytic enzymes (From Nsolomo and Venn, 1996c).

* = The differences in growth of fungi in O_2 and N_2 are significant at p = 0.05 or above; NS = not significant.

NS" = Growth in O_2 and N_2 is statistically insignificant but was judged significant because growth stopped in N_2 within the 24 days.

4. DISCUSSION

The the methodology used in this study may allow a small amount of atmospheric air to enter the tubes containing nitrogen during the plugging of rubber stoppers. However, the variations in the results due to this situation were assumed insignificant because replicates of the same fungi behaved similarly, and also within the whole treatment there was a general similarity in the way groups of fungi were affected. This method also allows for the build-up of carbon dioxide in the sealed tubes as fungi grow, and hence the amount of oxygen in the tubes containing atmospheric air decreases progressively. But, the advantage of this method is that it gives comparable conditions to fungi growing in both treatments in terms of the volume of air or gas in the tubes and on the possible effect of carbon dioxide produced during growth. A different approach, whereby the tubes containing atmospheric air are plugged with cotton wool to allow air movement, has been used previously to study the behaviour of blue stain fungi infecting living trees (Solheim, 1991).

The results of the current study have demonstrated that under oxygen stress, the growth of various pathogenic, decay and wood inhabiting fungi is variably affected. Also, it has been shown that the fungi of *Ocotea usambarensis* can grow under such anaerobic conditions for at least one week before some of them are inhibited or retarted. In wood lying in a moist forest (such as the mountain rain forests in which *O.usambarensis* grows) and indeed in living trees, moisture content is an important factor which imposes oxygen stress. Assuming that oxygen stress was the only limiting factor to the growth of fungi in water saturated wood, it could be possible that mycelia of most of the fungi of *O.usambarensis* would only be able to grow in such wood for a short period. According to Rayner and Boddy (1988), water saturation affects both the decay and non-decay fungi by inhibition of enzyme activity and by interference with hyphal-cell-wall interactions, but most of all, it inhibits growth by limiting the oxygen supply.

In a previous study (Nsolomo and Venn, 1996b), moisture content of artificially infected trees was found to have a negative correlation with the aggressiveness of the pathogenic fungus 'Sterile mycelium sp 3' which in this study, has also been inhibited within 12 days of growing under anaerobic conditions. Sterile mycelium sp 3 is an example of a fungus whose behavior in tissues of trees in relation to oxygen stress, can be explained or demonstrated by its behaviour in the laboratory.

Oxygen stress in wood is also imposed by high concentrations of carbon dioxide gas during decomposition. In tree trunks, oxygen concentrations have been found to be lower while carbon dioxide concentrations were much higher than levels in the atmosphere (Jensen, 1969). An increase in oxygen levels promotes growth of fungi and the decomposition activity of wood while an increase in carbon dioxide reduces the growth and the rate of decomposition (Jensen, 1967). It is reported that when entry points of decay organisms in tree trunks have healed and sealed off, the resulting high carbon dioxide concentrations tend to suppress the activity of decay organisms and may inactivate decay columns (Jensen, 1967). Also, it has been obseved that at low oxygen tensions anaerobic respiration may occur with the buid up of products such as ethanol, methanol, formate, acetate, lactate and propionate, empounds which may inhibit growth (Rayner and Boddy, 1988).

Most pioneering and pathogenic fungi that colonize fresh wounds of *Ocotea usambarensis* tree such as Basidiomycetes sp 4, *Botryosphareria ribis*, *Ceratocystis* spp, *Nodulisporium* sp, *Ophiostoma* spp, *Pestalotiopsis* sp, *Cylindrocarpon destructans*, *Cylindrodendrum album* and Sterile mycelium sp 3 (Nsolomo and Venn, 1996a) were among the most sensitive fungi to the absence of oxygen and were inhibited within 8-16 days. Some of these pioneering fungi were not present in inner tissues of the tree trunks after one year of wounding (Nsolomo and Venn, 1996a) and this could have been contributed by their inability to tolerate oxygen stress imposed by the high moisture content in *O.usambarensis* trees which averages 80% (Nsolomo and Venn, 1996b). Van der Kamp and Gokhale (1979) observed that heart rot fungi could not decay wetwood of cottonwood apparently due to anaerobic conditions existing in the wetwood. Past studies have also suggested the importance of tolerance to oxygen stress during the colonization of living wood tissues by fungi as an important attribute for primary invaders to overcome

tree resistance mechanisms (Solheim, 1991). Such pioneering fungi would tend to exploit a habitat fast enough before conditions become limiting and will give way to other species which are most capable of exploiting the habitat.

Other decay fungi of *O.usambarensis* trees have also shown different levels of tolerance to oxygen stress. *Trametes versicolor* was inhibited within 8 days while *Ganoderma australe*, *Daldinia concentrica*, *Stereum hirsutum*, *S.ostrea*, *Schizophyllum commune* and some few unidentified basidiomycetes stopped within 16-18 days (Table 1). This also may suggest their inability to compete for prolonged periods in wood of high moisture content such as that found in standing trees or in wet wood. But the longer the tolerance period under total oxygen stress the more the indication that the fungus can survive in decay columns in wood where conditions may not be as absolute as those found in experimental systems such as in this study. The current experiment studied the fungi at two extreme levels of oxygen availability and can not give results on those fungi that tolerate intermediate levels of oxygen stress because such extreme conditions may not apply completely in decay columns in trees. So, while the current results can give an indication of the behaviour of the fungi under extreme conditions, they may not explain why some fungi can still grow in highly moisture saturated wood such as that found in standing trees or in some decay columns, although they have been found to be inhibited under near-absolute anaerobic conditions of this study. Therefore a combination of factors may be responsible for the ability of fungi to grow in wood, although tolerance to at least one of them is a positive indication to survive in wood.

On the other hand, the heartrot fungi of the tree species such as *Phellinus senex* and *Phellinus* sp 2 which were isolated in standing trees (Nsolomo and Venn, 1996a), were able to grow continuously in absence of oxygen for 24 days although the growth of *P.senex* was significantly reduced. Other strong decay fungi of the tree species such as *Loweporus inflexibilis* and *Phellinus gilvus* continued to grow normally without oxygen for the 24 days period. This tolerance to oxygen stress may be an important characteristic which, among others, qualifies *Phellinus* species and *Loweporus inflexibilis* to become the major climax decay fungi of the trees and whose basidiocarps usually dominate later on heartrot affected *O.usambarensis* (see Renvall and Niemelä, 1993; Nsolomo and Venn, 1996a). In addition to tolerance to oxygen stress, these species are favoured by the forest temperature which is close to their optimal requirements, and also by the pH in standing trees which is in the range of their optimal requirements and especially that in the heartwood, and in addition, they have various wood decomposing phenoloxidase enzymes (Nsolomo, Solheim and Venn, 1996; Nsolomo and Venn, 1996a, Nsolomo, Venn and Solheim, 1996). In heart rots and other decays in standing trees, moisture content and high levels of carbon dioxide are major factors imposing oxygen stress which affects the growth of decay organisms (Jensen, 1967; Rayner and Boddy, 1988). There have been suggestions that heartrot fungi are more tolerant to the chemical and physical environment within heartwood of living trees (Highley and Kirk, 1979) but other constraints are the levels of O_2 and CO_2 (Highley *et al.*, 1983). It is therefore likely that, the oxygen stress imposes a selection pressure during succession which eliminates some fungi from the decay columns but favours those capable of tolerating it, such as the *Pellinus* species and *Loweporus inflexibilis*. Several heart rot species have been reported to be highly tolerant and could grow significantly well at 70% carbon dioxide and are thought to have some capacity for anaerobic metabolism (Rayner and Boddy, 1988).

As far as ligninolysis is concerned, the effect of oxygen stress on the process is reported to be significant and the rate of ligninolysis increases with increasing oxygen supply up to certain optimum levels (Jensen, 1967; Highley *et al.*, 1983; Rayner and Boddy, 1988). Also the growth of fungi is said to be limited by the rate of lignin degradation if oxygen is in short supply because as the degradation consumes oxygen, this condition imposes stress on the growth of fungi as well (Reid and Seifert, 1982). The fact that growth of 91% of the fungi of *O.usambarensis* previously found to possess ligninolytic enzymes (Nsolomo and Venn, 1996c) have been significantly inhibited or retarded in the absence of oxygen, is an indication that factors imposing oxygen stress in wood such as high moisture content and CO_2 levels not only do they adversely affect the linear growth of some fungi, but also may interfere with the chemistry of the decay process. This tends to support the observation by Rayner and Boddy (1988) that

the phenoloxidase activity is correlated with ligninolytic ability of fungi.

Fungi whose growth was not affected and those whose growth was promoted during the 24 day period indicated strong tolerance to very low oxygen supply such as that which would be found in moisture saturated wood or in decomposing wood in decay columns of standing trees. Also, it has been obesrved that growth of some wood rotting basidiomycotina can be stimulated at carbon dioxide concentrations of up to 10% and the production of the ligninolytic enzymes of laccase and peroxidases is also increased (Schäfel, 1976). However, such tolerance or stimulation are most likey to be temporary because oxygen is essential for respiration of the fungi. Although some fungi have been found to grow well at concentrations of oxygen below 1% (Jensen, 1967), the existence of obligate anaerobes has been questioned and it is unclear whether anaerobism is a permanent or temporary condition (Scheffer, 1986). As if to emphasize the importance of oxygen, one fungus, *Ceratocystis* sp 1 grew initially fast in nitrogen such that the growth was significantly higher than that in oxygen, but it was later inhibited and stopped growing within 16 days due to lack of oxygen.

5. CONCLUSION

The study has shown that most of the fungi from decay of *Ocotea usambarensis* trees can grow for at least some limited period under anaerobic conditions. However, under prolonged exposure to oxygen stress, a large proportion of fungi (about 80%) are inhibited or retarded. Also, it was found that 91% of the fungi previously found to possess ligninolytic enzymes are among those inhibited. This indicates that oxygen stress inhibits both the linear growth of fungi and the subsequent capacity to decompose wood through its interference with their ligninolytic activity. In standing *Ocotea usambarensis* trees where moisture content averages 80%, oxygen stress is likely to be experienced and will tend to favour fungal species which can tolerate it. Although pathogenic and decay fungi were affected similarly in the absence of oxygen, some decay fungi such as *Phellinus senex* and *Loweporus inflexibilis* which are decay-climax fungi of the tree and which show their dominance on suffering trees by fruitifying on them later, showed continuous growth under anaerobic conditions although growth of *P.senex* was retarded significantly. This is an indication that among other factors which influence succession of fungi in decay columns in trees, oxygen stress also exerts a selection pressure which enables tolerant species such as *P.senex* and *L.inflexibilis* to form a climax flora once they are established in the decay columns.

REFERENCES

Jensen, K.F., 1967. Oxygen and carbon dioxide affect the growth of wood-decay fungi. **Forest Science, 13:**384-389.

Jensen, K.F., 1969. Oxygen and carbon dioxide concentrations in sound and decaying red-oak trees. **Forest Science, 15:** 246-251.

Highley, T.L. and Kirk, T.K., 1979. Mechanisms of wood decay and the unique features of heartrots. **Phytopathology, 69:** 1151-1157.

Highley, T.L., Bar-Lev, S.S., Kirk, T.K. and Larsen, M.J., 1983. Influence of O_2 and CO_2 on wood decay by heartrot and saprot fungi. **Phytopathology, 73:** 630-633.

Nsolomo, V.R., Solheim, H. and Venn, K., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. I. Temperature requirements. PhD thesis, Paper V. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996a. Decay fungi of *Ocotea usambarensis* Engl. trees in the Usambara and Kilimanjaro mountain rain forests, Tanzania. PhD thesis, Paper II. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996b. The pathogenicity of some decay fungi of *Ocotea usambarensis* Engl. trees. PhD thesis, Paper III. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R. and Venn, K., 1996c. Decay ability of some fungi from *Ocotea usambarensis* Engl. trees. PhD thesis, Paper IV. Agricultural University of Norway, Ås. Manuscript.

Nsolomo, V.R., Venn, K. and Solheim, H., 1996. Growth studies on some fungi of *Ocotea usambarensis* Engl. trees in culture. II. pH requirements. PhD thesis, Paper VI. Agricultural University of Norway, Ås. Manuscript.

Reid, I.D. and Seifert, K.A., 1982. Effect of an atmosphere of O_2 on growth, respiration, and lignin degradation by white-rot fungi. **Canadian Journal of Botany, 60:**252-260.

Rayner, A.D.M. and Boddy, L., 1988. **Fungal Decomposition of Wood. Its Biology and Ecology.** John Wiley & Sons. Chichester, New York. Pp 587.

Renvall, P. and Niemelä, T., 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. **Bulletin Jardin Botanique National de Belgique, 62:** 403-414.

Schäňel, L., 1976. Role of carbon dioxide in growth and decaying activity of wood-rotting fungi. **Folia Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis, 17:** 5-54.

Scheffer, T.C., 1935. Scientific apparatus and laboratory methods. A tube for culturing fungi. **Science, 82:** 467-468.

Scheffer, T.C., 1967. Effect of oxygen deficiency on wood-inhabiting fungi. In: Kelman *et al* (eds), **Sourcebook of Laboratory Exercises in Plant Pathology.** Freeman & Co., London. Pp 228-229.

Scheffer, T.C., 1986. O_2 requirements for growth and survival of wood-decaying and sapwood-staining fungi. **Canadian Journal of Botany, 64:**1957-1963.

Solheim, H., 1991. Oxygen deficiency and spruce resin inhibition of growth of fungi associated with *Ips typographus*. **Mycological Research, 95:** 1387-1392.

Van der Kamp, B.J. and Gokhale, A.A., 1979. Decay resistance owing to near anaerobic conditions in black cottonwood wetwood. **Canadian Journal of Forest Research, 9:**39-44.