THE EFFECTS OF NIGERIAN FUNGI ON NIGERIAN WOOD

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SUMMARY

The effects of Nigerian fungi on Nigerian wood are studied. The fungal flora of Nigerian soil samples, wood veneers and the aerial environment where the soil samples were collected was investigated. The isolates included many fungal biodeteriogens. There was a close relationship between the fungal flora of the three environments.

The effects of Nigerian soil fungi on Nigerian wood were determined by the soil burial method using Nigerian soil, by direct incubation in the laboratory of the factory-colonised test wood pieces, and by the inoculation of sterilised test wood pieces with test fungi.

The criteria used to assess wood decay were weight loss and loss in bending strength. Bending strength losses were measured on a tensometer with specially designed three-point loading jaws. There was a close correlation between strength and weight loss.

The ability of the test fungi to decay wood at various biocidal concentrations was examined. These fungi were discovered to decay wood treated with the lower concentrations of biocides used.

Some of the fungal isolates investigated were found to utilise amino acids in wood as sole nitrogen and sole carbon sources. The fungal isolates were found to utilise the same amino acids as sole nitrogen sources for cellulolytic activity.

The effects of irradiation on the germination and growth of thermophilic and thermotolerant fungi on simulated wooden joints and varnished timber surfaces were investigated under laboratory conditions using infra-red rays. The heat produced by the infra-red rays raised the wood temperature to a level which supported the germination and growth of these fungi.

The economic implications of wood decay are reviewed and the preventive methods of wood decay are discussed. As a result of a pilot experiment, methods of harnessing the activities of these fungi for man's advantage in the decomposition of waste products such as wood bark are suggested.

Nigerian soil fungi: ecology, Physiology.

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CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

Nigeria is one of the world's most important producers of tropical timbers. Timber production and exports have steadily grown since the beginning of the century. The local consumption is also increasing at a fast rate - Okigbo, (1964).

The distribution of timber in Nigeria include mangrove swamps, dense forests in the coastal areas, inland rainforest, and dry savanna with scattered trees. The vegetational distribution pattern is shown in Figure 1. The total area of forest reserves in Nigeria is about 96,000 km^2, that is about 7% of the area of Nigeria of which about 20,000 km^2 are dense virgin rainforest (high forest). The detailed distribution of the forest reserves is shown in Figure 2.

Forest reservation is most complete in the western states of Nigeria, which were the earliest of the Nigerian states to receive forestry attention. Some 15% of the total area of these states have been set aside as reserves, (Buchanan and Pugh, 1958) and forestry has assumed its place as a profitable element in the permanent economic structure of these states. The biggest blocks of reserves occur in the rain-forest zone of Bendel state, and it is from these that a large proportion of Nigeria's export timber is today derived. Another group of reserves is located in the thinly populated north-western sector of Western Nigeria, and these consist for the most part of savanna woodlands and are of minor economic importance. In the heavily populated Eastern states of Nigeria, forest reserves occupy 10.5% of the area and this figure is considered to represent the practical limit of reservation there.

Fig. 1

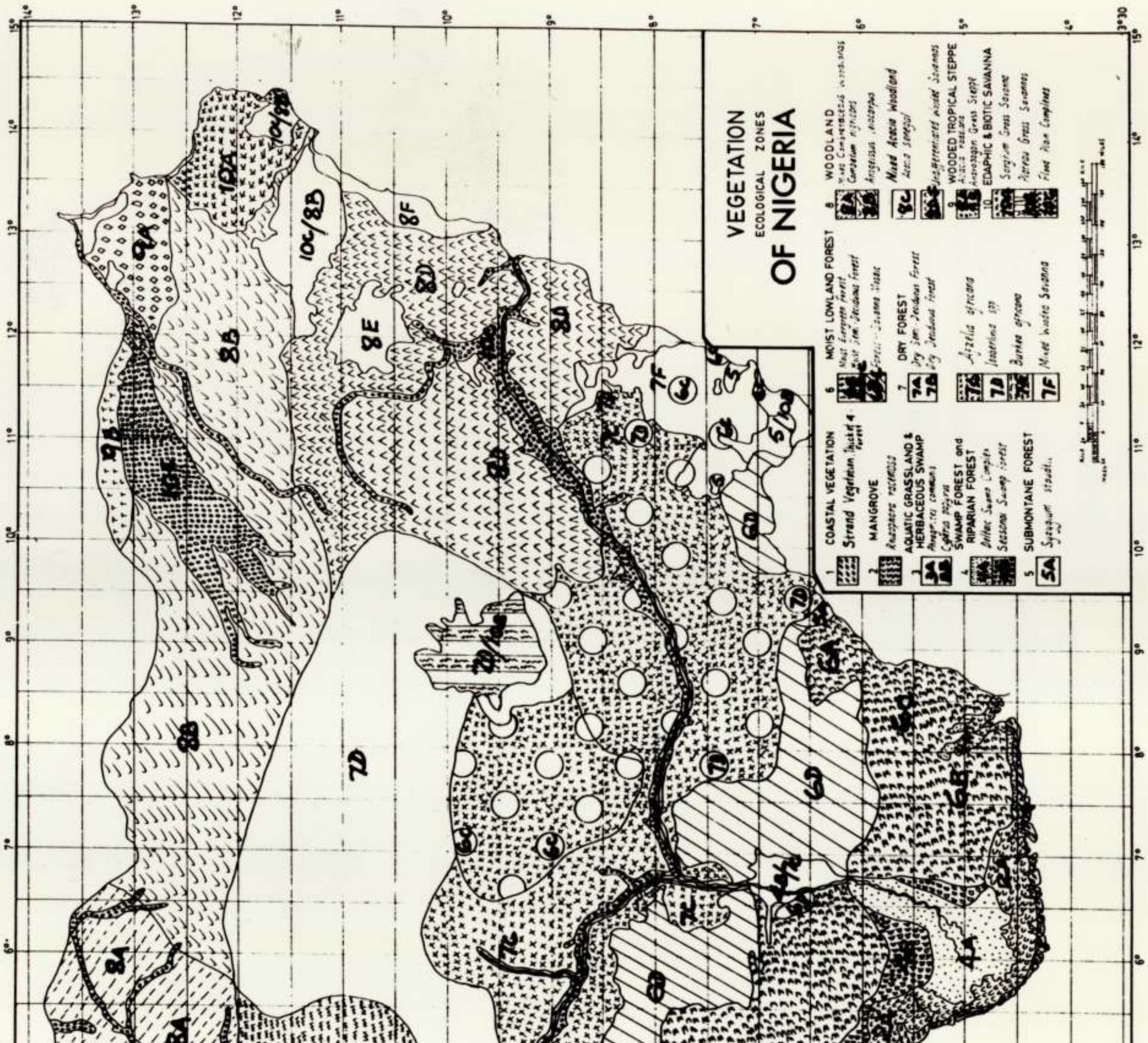
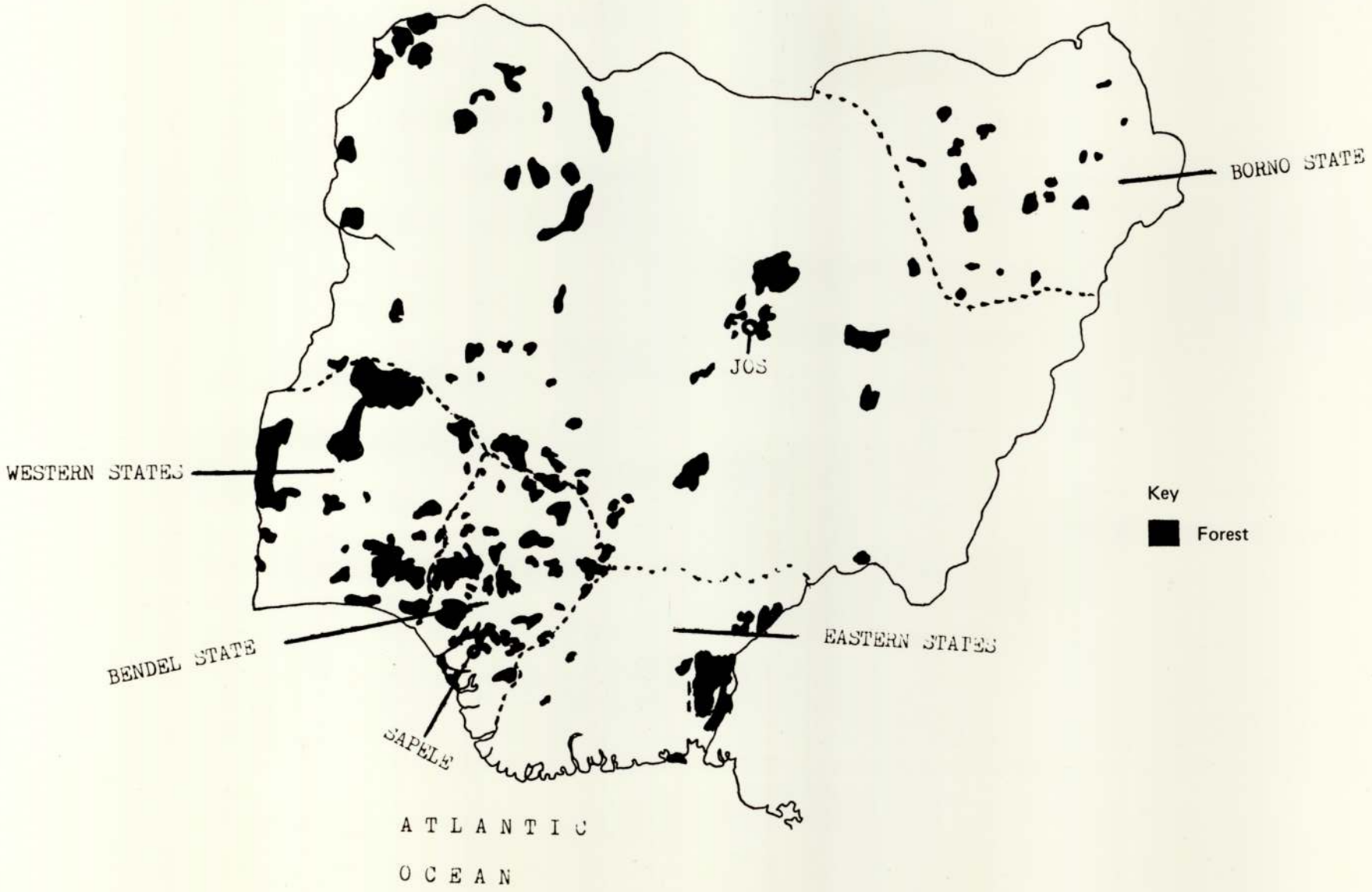


Fig 2 Map of Nigeria Showing Nigerian Forest Reserves



In these states, more especially Imo and Anambra states, the heavy pressure of population and resultant scarcity of agricultural land make reservation virtually impossible and forestry is of negligible importance.

In the savanna zone of Northern Nigeria, there are remains of the former virgin forest, in addition, forest estates are being established, but this process is still in its initial stages. Only 5.5% of the area is set aside as reserves, and of this the greater proportion is in the Borno state of Nigeria. The development of forests in the northern savannas presents problems quite different from those of the southern rain-forests. Export production is ruled out at the moment because there are not many commercially valuable timbers. The major problem is the development of these fire-scarred woodlands as a source of constructional timbers for the growing population of the northern states. Unfortunately this programme is fraught with difficulties. These areas of sparsely inhabited savanna woodlands which offer great potential as forest reserves equally play a vital role in the grazing economy of the northern peoples. A compromise between the forester and the grazier is essential, for unless these grazing rights are recognised, approval for a reserve would rarely be forthcoming from the Northern Nigerian state administrations.

The high forest zone of Nigeria used to be replete with tree species of outstanding natural durability. But with the increase in consumption of wood both locally and through export, the well known species of timber were grossly overcut. Research efforts into the establishment of man-made plantations of these valuable species have so far met with serious pathological set-backs. Research emphasis

was thus moved to the study of the strength properties of the secondary species to explore the possibility of finding more economic uses for them. As a direct result of investigations into their strength properties, the number of lesser used Nigerian tree-species being utilised by the timber industry has increased more than threefold. However, commercial exploitation of Nigerian timber is still confined largely to about 80 tree species out of about 600 different species of trees that can attain commercially exploitable sizes for the production of lumber and plywood.

Wood in Nigeria is used in buildings, in boats and ship construction, in drug production, for transmission poles, fences, mining, packaging, pulp and pulp products. It is used in a variety of less readily recognisable ways, such as in chemicals, plastics, transparent films, and fabrics. These uses are beset with many problems which include:

1. The high cost of wood.

2. Lack of knowledge about the properties of Nigerian timber species and inadequate information on their end uses in consumer countries.

3. Wood decay caused by fungi and insects.

Of these problems, wood decay is the most crucial to the Nigerian wood industry. The short service life of wooden structures in Nigeria is due to damage caused by biodeteriorating and mechanical agents such as wind-blown sand. These agents are aided by extremely favourable conditions of temperature of $25^{o}C$ - $35^{o}C$ and high relative

humidity of about 40 - 100% which prevail in Nigeria throughout the year. Thus the large scale use of wood has been hindered by the damage which these agents can cause.

One of the first series of reports pointing to the serious nature of deterioration of materials in Nigeria by microorganisms was that of the British Royal Air Force Scientific Mission, which visited Lagos and Port-Harcourt in 1946. They discovered that the chief cause of damage to most of the materials was fungal growth. Termites were later discovered to be posing another serious problem which was documented by Butterworth, Kay and MacNulty in 1966. They observed that in Southern Nigeria the main damage to structural wood which is out of contact with the ground, and to manufactured wooden articles, was caused by the dry-wood termite Cryptotermes havilandi (Sjost). They found the damage to be widespread in the coastal belt and more serious than that caused by timber-attacking Coleoptera. This termite completes the whole of its life cycle in dead or seasoned tim ber and never makes contact with the soil. Butterworth and MacNulty (1966), tested Obeche stakes for resistance to termite attack in grave-yards. The results they obtained showed that a number of stakes appeared to be unattacked after two months but all showed attack after four months, mostly of a severe nature. Dhanarajan, cited by King (1975) reported that insects are the main agents of deterioration in the tropics while fungi are the main agents of deterioration in more temperate climates.

While both fungi and termites are important in tropical areas, the work reported in this thesis is concerned primarily with fungi.

Hueck (1968) classified biological interaction with materials into two groupings, respectively termed "Biodegradation" and

"Biodeterioration". Biodegradation refers to an enhanced economic value of materials as a result of organism interaction, whereas Biodeterioration refers to a loss in economic value after such activity. Fungal colonisation of wood may be allowed to produce biodegradation of the wood such as in pole ponding, cattle food, although there are no economic processes to date (Lindgren, 1952; Ricard and Bullen, 1967; Hulme and Shields, 1970, 1972; Henningsson, Henningsson and Nilsson, 1972; King and Eggins, 1973; Levy, 1975); but more generally produces a loss in economic value (Biodeterioration). Wood deterioration is defined as a loss of the physical or structural characteristics of wood when these qualities are desirable from the view-point of wood utilisation. As wood is of increasing economic value, considerable research is being undertaken to provide satisfactory systems to preserve it against decay.

Fungi are important agents causing the decay of wood, and wood may be regarded as consisting of a large number of conveniently orientated holes surrounded by food. Four different major types of fungal attack have been recognised, and classified as brown-rots, white-rots, soft-rots and wood stain attack. The first three have been studied extensively and several reports have been published on them, (Campbell and Booth 1929; Hubert, 1931; Campbell 1931, 1932; Cartwright and Findlay, 1950; Corbett and Levy, 1963; Swift, 1964; Levy 1965; Duncan and Lombard, 1965; Eslyn, 1970; and Kaarik, 1974).

As far as wood stain is concerned, Humbert (1931) noted that attempts to solve the sapstain problem, dating back to 1888 when lime in various forms was used on boards and under lumber piles. Scheffer and Lindgren (1940), published their work "Stains of

sapwood and sapwood products and their control", in which they described chemical tests which have subsequently been used as the standard in work on wood stains. Gadd (1949), described the stains produced by certain of the wood-rotting fungi as follows, Coniophora cerebella, light brown; Polyporus abietinus, dark-brown; Lentinus lepideus, yellow-white; Lenzites saepiaria, yellow-white; Fomes annosus, brown and Stereum sanguinolentum, brown. Umezuruike (1968) investigated the effect of Botryodiplodia theobromae on the wood of Bombax buonopozense, a large tropical forest tree which becomes heavily stained by the fungus under humid conditions after removal of the bark from the felled tree. Olafinboba and Lawton (1968) investigated the biology of blue stain organisms in Antiaris africana and demonstrated that Botrodiplodia theobromae generally considered to be the most important fungal species in the staining of Antiaris africana, utilised a considerable range of carbohydrates. Normally, stained wood is rejected mainly because of consumer resistance to the discolouration. However, the wood strength can also be affected by stain fungi.

Findlay and Pettifer (1939) reported a loss in toughness and bending strength of 45% and 20% respectively in the wood of Obeche (Triplochiton scleroxylon) heavily stained by Botryodiplodia theobromae. These observations implied that the fungus had broken down structural components of the cell walls of the wood. Wilcox (1968) reported on the changes in wood microstructure through progressive stages of decay.

The knowledge of the biochemistry of decay processes has been greatly expanded by research on the role of diffusible enzymes that catalyse the dissolution of cell walls, (Cowling, 1961), and by

studies on the changes in the properties of wood following attacks by brown, and white rot fungi (Wilcox 1968).

Greaves (1972) carried out an investigation on the microbial ecology of chip piles after two and four months storage in equatorial New Guinea. He observed biodeterioration in the form of discolouration and loss in wood substance. Discolouration by dematiaceous fungi, hyaline phenoloxidase-producing microorganisms, and non-microbial reactions affected 33% of the pile after two months storage and 85% after four months.

Fougerousse (1975) published his results of an investigation on the spectrum of fungi involved in the soft-rot process in treated stakes tested in Ivory Coast and in France. It appeared to him that several fungal species were involved in the decay process but two genera Phialophora and Rhinocladiella seemed to have a particular importance because of their frequency of occurrence together.

There are other reports on wood decay by fungi in West Africa. Asiedu (1974) carried out a survey in Ghana to collect and identify wood decay fungi from the forest, sawmills, wooden structures and on billets. Most of the common wood decay fungi collected belonged to the family Polyporaceae, and the most notable among them were Coriolopsis polyzona, Pycnoporus sanguineus, Trametes cingulata, Trametes scabrosa and Schizophyllum commune. He found that these fungi have a wide host range and are evenly distributed throughout the vegetational zones of Ghana.

In Nigeria prior to this time, losses due to wood decay by fungi were not of much concern, however it has now been recognised

that decay losses are now no longer negligible but rather need to be identified and quantitatively determined. As far as the literature shows there has not been any major research on this crucial problem of wood decay in Nigeria. The nearest work to this was the survey carried out at Ibadan by the Forest Research Institute of Nigeria (Odeyinde, 1977). The survey revealed that leakages in roofs of buildings were responsible for fungal attack of building rafters and purlins. He showed that the most prevalent decay fungus of wood rafter and purlins was Trametes sp. and that this was followed closely by Phellinus contiguus. He estimated that at least 70% of wood produced in Nigeria is lost due to biological decay. Since enough data on fungal decay of wood and wood products have not been obtained, it has been very difficult to apply prophylactic measures against decay in Nigeria effectively. Hence there is much need for detailed research in this field in order to save Nigeria from the great economic losses caused by these microorganisms.

The present research was undertaken therefore to investigate the effects of Nigerian fungi on Nigerian wood.

Any fungi, from any source could colonise the timber used in buildings, for example. When the timber is inserted into the soil, either as posts or in foundations for buildings, then the fungi which live in the soil can readily come in contact with it. The spores of these soil fungi, as well as of fungi growing on wood above ground can also be deposited on timber from the air spora. It is therefore a logical beginning for all research programmes connected with Biodeterioration and Biodegradation to adopt the hypothesis that in addition to the direct attacks on the particular substrate under investigation, the soil and the aerial environment are the potential sources of fungal biodeteriogens.

Since it has been assumed that in addition to the direct attack on the particular substrate under investigation, the soil and the aerial environment are the sources of fungal biodeteriogens, the first aspect of the research study therefore, was to investigate the mycoflora of Nigerian soil samples, the air-spora from where the soil samples were collected and the mycoflora of Nigerian timber veneers. It was found more convenient to divide the investigation into two parts, "thermophilic and thermotolerant fungi", and "mesophilic fungi".

Subsequently work was carried out on the effects of these fungi on Nigerian wood. These experiments on wood decay together with some studies on wood preservation formed the major objectives of the present study.

CHAPTER 2

THE ISOLATION OF THERMOPHILIC, THERMOTOLERANT AND MESOPHILIC FUNGI FROM NIGERIAN SOIL SAMPLES

2.1 THE ISOLATION OF THERMOPHILIC AND THERMOTOLERANT FUNGI FROM NIGERIAN SOIL SAMPLES

2.1.1 MATERIALS AND METHODS

Soil samples were collected at monthly intervals from a selected area within the campus of University of Jos, Nigeria; the area being chosen because the University of Jos sponsored this research. African Timber and Plywood Factory Sapele (a division of United African Company of Nigeria Limited (U.A.C)) agreed to forward wood veneers for the research. The soil within the selected area supported the growth of some tree species which are also found in the rainforest zone of Nigeria.

The soil samples were obtained with sterilised stainless steel corers. The samples were packed in sterile polyethylene bags and finally enclosed in 8cm x 8cm x 2.50cm wooden boxes and forwarded to Birmingham. It took about 4-5 days for each soil sample to arrive.

The soil pH of the sampling site was measured each month, just before a sample was collected. This was necessary in order to check any variation that might occur as a result of the activities of the microorganisms in the soil samples during transit. The surface layer of the soil (the humus layer) was collected on each occasion. Procedures for microbial analysis were begun as soon as the samples were received.

(a) Culture media

Five standard media were used:

1. Cellulose agar (Eggins and Pugh, 1962) (C A)
2. Malt extract agar (MEA)
3. Yeast-starch agar (Emerson, 1941) (YSA)
4. Littman's agar medium (LAM)
5. Czapek's agar (Raper and Thom, 1949) (CzA)

The compositions of media are presented in Appendix 13.

CA and MEA were employed for statistical analysis of the fungal microflora of the Nigerian soil samples. YSA was used for the purification of the isolated species and for routine isolations, while LAM and CzA were used only for routine isolations.

(b) Isolation techniques

Three major isolation techniques were used. These were soil plate method of Warcup 1950, soil dilution method and soil enrichment method (Tansey, 1976).

(i) Soil plate method

In this method, 0.015gm of soil sample was transferred into each of a set of 20 petri dishes. 10ml. of cooled (45^{o}C) CA was added into each of the dishes. The soil particles were dispersed throughout the agar by gently swirling the dishes. After the solidification of the agar, the plates were incubated at 45^{o}C for

one week, and re-examined a week later for the presence of thermophilic and thermotolerant fungi. Monthly scorings were made for each of the fungal species that appeared on the plates.

The same procedures were carried out using MEA, where the plates were examined after five days and a week later for the presence of thermophilic and thermotolerant fungi.

Soil plates were prepared for the other media used, for routine isolations. Five plates were incubated at 45°C, and five at 50°C for both LAM and CzA before examination for the presence of thermophilic and thermotolerant fungi.

Plates were packed in sterile polythene bags prior to incubation, and beakers containing sterile distilled water were left in the incubators to minimise drying of the agar.

(ii) Soil dilution method

The soil samples were first sifted through a sieve with a 2mm mesh. 1gm of the sifted soil sample was then dispersed in 10ml. of sterile distilled water in a sterile McCartney bottle. 1ml. of the resultant soil suspension was transferred into a 9ml. sterile water blank in a sterile McCartney bottle and throughly mixed using (Horizontal) mechanical shaker for 30 minutes. 1ml. of this suspension was then transferred immediately through successive 9ml. sterile water blanks until the desired final dilution was reached. Each suspension was shaken by hand for a few seconds and was in motion while being drawn into the pipette.

The preceding method for making soil dilutions yielded dilutions of soil in water of 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000. A control bottle was equally prepared which contained 10ml. of sterile distilled water. Dilutions were stopped at 1:100 ,000 because below this, only actinomycetes were isolated as was observed during preliminary experiments. 1ml. of the desired dilution was transferred aseptically into each of several Petri dishes and 10ml. of the agar medium, cooled to just above solidifying temperature, was then added to each dish. The dishes were rotated by hand in a gentle swirling motion so that the diluted soil could be dispersed in the agar medium. Normally five Petri dishes were set up for each dilution. 1ml. of the control experiment was equally plated out. CA and MEA were employed for the soil dilution experiment. The plates were incubated at 45^{o}C and examined daily for the presence of thermophilic and thermotolerant fungi.

(iii) Soil enrichment method

The monthly soil samples were supplemented with 1% (w/w) of autoclaved cellulose powder (Whatman CFII). The same procedure was repeated using 1% w/w of autoclaved lignosite ordered from Georgia Pacific Corporation, Bellingham, Washington, U.S.A., as the enriching substance. Three plates were set up for each soil sample. The plates were adequately moistened with sterile distilled water and incubated at 45^{o}C for 28 days, with periodic addition of sufficient sterile distilled water to keep the soil damp. Twenty plates were used to plate out each of the cellulose and lignin enriched monthly soil samples. The plates were incubated at 45^{o}C and examined after seven days for the presence of thermophilic and thermotolerant fungi. The plates were re-examined a week

later for the presence of additional thermophilic and thermotolerant fungi. The effects of soil enrichment on the isolation of these fungi was assessed.

(iv) Isolation of fungi from soil using an amino acid as a sole nitrogen source

This experiment was designed to find out whether some of the amino acids inherent in wood can act as sole nitrogen sources for fungal growth and hence lead to fungal decay of wood.

The standard CA was modified by replacing ammonium sulphate with an amino acid as the sole nitrogen source. Thiamine hydrochloride was employed in place of yeast extract and asparagine as source of vitamin. Nitrogen inherent in agar and thiamine hydrochloride was considered to be negligible when compared to the nitrogen level added.

The nitrogen sources investigated included Glutamic Acid, Aspartic Acid, Threonine and Leucine. These amino acids are among the amino acids known to occur in Scots pine sapwood (Baker, Laidlaw and Smith, 1970).

The medium was then used to plate out the Nigerian soil samples. Twenty plates were used for each amino acid. The plates were incubated at 45^{o}C for one week and re-examined a week later for the presence of thermophilic and thermotolerant fungi. The result obtained was compared with that obtained when the standard CA is employed for fungal isolation.

(v) Isolation from root pieces

Root pieces from all the monthly soil samples were picked up with the aid of forceps. The recovered root pieces were then divided into seven portions. The first portion was washed once with sterile distilled water. The second portion was washed twice in two changes of sterile distilled water. The third portion in three changes of sterile distilled water. The fourth portion in four changes and fifth portion in five changes of sterile distilled water. The sixth portion was left unwashed.

The root pieces of each portion were then plated out on CA and MEA. Twenty plates were set up for each portion of root pieces. The plates were incubated at 45°C and observed daily for the presence of thermophilic and thermotolerant fungi.

2.2 ISOLATION OF THERMOPHILIC AND THERMOTOLERANT FUNGI FROM TEMPERATE SOIL SAMPLES

Soil samples collected from Handsworth Village, University of Aston, were examined for the presence of thermophilic and thermotolerant fungi. This was done for the purpose of comparison of the isolates with those of Nigerian tropical soil samples.

The temperate soil samples were then irradiated with Philips 300W infra-red lamp (12 hours a day for 14 days, sterile distilled water being added at intervals to avoid drying up of the soil samples). These soil samples were again plated out in order to find the effect of heating on the soil mycoflora.

2.3 THE ISOLATION OF MESOPHILIC FUNGI FROM NIGERIAN SOIL SAMPLES

2.3.1 MATERIALS AND METHODS

(i) Soil-plate and soil dilution methods

The procedures were exactly the same as were described for thermophilic and thermotolerant fungi but instead of incubating the plates at 45°C, they were incubated at 25°C. The MEA plates were incubated for four days and re-examined a week later for the presence of mesophilic fungi. CA plates were incubated for one week and re-examined a week later for the presence of mesophilic fungi. Scorings were subsequently made for each fungal species that was isolated on monthly basis.

(ii) Milk deteriogens

Five sterile Petri dishes were set up. 100gm of soil samples were weighed out into each of the plates. 10ml. of pasteurised Unigate milk was measured out into each plate. The plates were incubated at 25°C for two weeks and then examined for mesophilic fungal deteriogens.

(iii) Isolation of Thielaviopsis basicola

Particles of soil sample were spread over the surface of 5mm thick carrot root discs in Petri dishes. Enough water was added to make the soil quite moist but with no free water present. After four days at room temperature, the discs were washed to free them of soil. The washed carrot discs were then incubated in sterile Petri

dishes in moist chamber for seven days and re-examined a week later for the presence of Thielaviopsis basicola as was described by Yarwood, (1946).

(iv) The creosoted matchstick method for isolating Amorphotheca resinae from soil (Parbery, 1967)

By adding creosote, which is biocidal to most organisms, most potential competitors of Amorphotheca resinae are removed, thus allowing this organism to grow not withstanding its low competitive saprophytic ability.

An even layer of soil was placed in five 9cm diameter Petri dishes to one-third of their depths. The soil was firmed without being compacted so as to allow good aeration. Two beheaded matchsticks, freshly soaked in creosote for seven minutes and allowed to drain off the free liquid, were placed centrally in each Petri dish 2cm apart. The Petri dish lids were then replaced.

They were then incubated at 25^{o}C for two weeks, and examined daily for the presence of Amorphotheca resinae.

(v) The soil-steaming method for the isolation of fungi from soil

125gm samples of soil in glass beakers were placed in a steamer at 100^{o}C for periods of 4-10 minutes. The steamed soil was later plated out and the plates were incubated at 25^{o}C. These were examined daily for the presence of ascomycetes.

(vi) Isolation of mesophilic fungi from root pieces

The procedure was the same as already described for thermophilic fungi. However, the isolation plates for this present experiment were incubated at 25°C and observed daily for the presence of mesophilic fungi.

(vii) Other methods

The other specialised methods employed for the isolation of mesophilic fungi from the Nigerian soil samples together with the keratinophilic fungal isolates are given in Appendix 12.

2.4 ESTIMATION OF PERCENTAGE FREQUENCY OF OCCURRENCE

To estimate the percentage frequency of occurrence of the fungal isolates, the results of the soil-plate method of both the thermophilic and mesophilic fungi were used. The estimation was carried out for each monthly soil sample. If a fungus occurred on X plates out of 20 plates, the percentage frequency of occurrence of that fungus in that soil sample $= \frac{X}{20} \times \frac{100}{1}$

$= 5X\%$

2.5 PURIFICATION OF CULTURES

The culture dishes were examined under the microscope to determine the presence of thermophilic, thermotolerant and mesophilic forms. When such fungi were found the spores or the active growing hyphae were transferred to agar plates by means of a sterile inoculating needle. The agar contained streptomycin 3g/lit. and aureomycin 2g/lit. (two drops of the mixture of the two types of antibiotic for each agar plate). In the case of the thermophilic and thermotolerant fungi, the sub-cultures were incubated at 50°C until good growth was established. Such plates were then removed to a 40°C incubator to prevent too rapid drying out of the agar. The subjection of the plates to higher temperatures made it possible to suppress many common saprophytic forms. It was evident that fungi appearing at this temperature were either thermophilic or thermotolerant.

Most of the mesophilic types were subcultured several times before they were purified. Aspergillus fumigatus gave trouble occasionally, but it was usually possible to avoid this mould if transfers of the desired fungi were made before the Aspergillus

had fruited and dispersed its spores. Thermophilic bacteria presented the greatest problem with regards to thermophilic and thermotolerant fungi and to ensure their complete absence, single spore isolation of some of the fungi were necessary. Isolates from some of the dilution plates were at first incubated for 24 hours at 40°C for the thermophiles but this proved to be too long a time.

Since most thermophilic fungi have rapid growth, single sporelings had to be isolated in 12 hours. The germlings were located by observing the inverted Petri dish under the low power objective of the compound microscope. After location, suitable sporelings were marked by small circles of India ink on the surface of the Petri dishes. The dishes were placed under the dissecting microscope and the germinating spores were removed by means of a flamed meedle to sterile agar slants. These were then incubated at 45°C or 50°C until growth was complete. Transfers of these cultures were later made to agar plates for further purifications. YSA was employed for the purification of the thermophiles.

2.6 METHODS OF MEASURING THE ECOLOGICAL PARAMETERS OF THE SOIL SAMPLES

Estimates were made of the pH, water content, organic content and water holding capacity of the monthly soil samples.

2.6.1 DETERMINATION OF SOIL pH

5gm of a soil sample was suspended in 10ml. of distilled water and stirred thoroughly. The soil suspension obtained was

allowed to stand for 30 minutes. The pH of the 1:2 soil: water suspension was measured using a Pye Unicam glass electrode pH meter. The meter was calibrated at regular intervals using pH 7.0 solution prepared by dissolving a buffer tablet (B.D.H.) in 100ml. distilled water. The mean pH of five replicates of each soil sample was taken.

2.6.2 WATER CONTENT OF SOIL SAMPLES

20gm of sieved soil was weighed out in an evaporating basin. The soil was then placed in an oven at 105°C and dried to constant weight. It was then cooled in a desiccator and weighed. Then the percentage fresh moisture was calculated from the loss in weight. The mean result of five replicate samples was taken.

Calculation

$$\text{Moisture (\%)} \qquad \frac{\text{loss in wt. on drying (g)} \times 100}{\text{initial sample wt. (g)}}$$

Since the air-dry moisture is frequently used for later correction of results to a dry weight basis, the percentage dry matter was calculated as follows:

$$\text{Dry matter (\%)} = \frac{\text{oven-dry wt. (g)} \times 100}{\text{initial sample wt. (g)}}$$

2.6.3 WATER HOLDING CAPACITY OF THE SOIL SAMPLES

Small amounts of air-dried sieved soil samples were put in water holding capacity trays which had earlier been weighed. The trays were tapped gently to obtain loose and even distribution of the soil samples. When the trays were full, they were then placed in Petri dishes containing water so that the perforated bases on the trays were in contact with water. The trays were then left overnight. They were then weighed and dried to constant weights in an oven at $105^{o}C$. On cooling in a desiccator, the trays plus soil samples were weighed and the percentage losses in weights were determined. Replicate trays were set up too and the mean value of the results was taken.

2.6.4 DETERMINATION OF SOIL MINERAL CONTENTS

The presence of non-metallic elements essential for microbial growth in the soil samples, was determined by the use of "Perkin-Elmer elemental analyser, model 240B" and the method described by Belcher, Natten and MacDonald, (1970).

2.7 CLIMATIC FACTORS

The meteorological data of Jos-Nigeria was collected in the Geography Department of University of Jos during the period of the investigation.

2.8 RESULTS

FUNGI ISOLATED DURING THE SURVEY

THERMOPHILIC AND THERMOTOLERANT FUNGI

Twenty-nine thermophilic and fifteen thermotolerant fungi were isolated during the survey. These are listed in Appendices 1 and 2 respectively.

Nine of the species isolated Chaetomium thermophile var. coprophile Cooney and Emerson, C. thermophile var. dissitum Cooney and Emerson, Mucor pusillus Lindt, Rhizomucor sp, Talaromyces emersonii Stolk, Thermoascus aurantiacus Miehe sensu Apinis, T. crustaceus (Apinis and Chesters) Stolk, Thermomyces ibadanensis Apinis and Eggins and T. Lanuginosus Tsiklinski have been previously reported in Nigeria while thirty-one listed in Table 1 are new records for Nigeria.

MESOPHILIC FUNGI

One hundred and forty six mesophilic fungi were isolated from the Nigerian soil samples. This number does not represent the total number of fungi present in the soil samples. The details of the mesophilic fungal isolates are given in Appendix 3. Aspergillus, Chaetomium, Fusarium, Penicillium and Trichoderma were the commonest genera in the Nigerian soil samples. Aspergillus and Fusarium had the greatest incidence of individual species.

TABLE 1

THE NEW NIGERIAN RECORDS OF THERMOPHILIC AND THEMOTOLERANT FUNGI

THERMOPHILIC FUNGI

ASCOMYCETES

Corynascus thermophilu s (Fergus and Sinden) v. Klopotek

Melanocarpus albomyces (Cooney and Emerson) v. Arx

Talaromyces thermophilus Stolk

Thermoascus thermophilus (Sopp) v. Arx

Thielavia australiensis Tansey and Jack

FUNGI IMPERFECTI

Acremonium alabamensis Morgan-Jones

Calcarisporiella thermophila (Evans) de Hoog

Humicola grisea var. thermoidea Cooney and Emerson

H. insolens Cooney and Emerson

Malbranchea pulchella var. sulfurea (Miehe) Sigler and Carmichael

Myceliophthora thermophilum van Oorschot

Myrioconium thermophilum (Fergus) v.d. Aa (=Papulaspora thermophila Fergus)

Scytalidium thermophilum (Cooney and Emerson) Austwick (=Torula thermophila Cooney and Emerson)

Stibella thermophila Fergus

Thermomyces stellatus (Bunce) Apinis

Continued ...

PHYCOMYCETES

Mucor miehei Cooney and Emerson

THERMOTOLERANT FUNGI

ASCOMYCETES

Chaetomium rectopilum Fergus and Amelung

C. virginicum Ames

Corynascus sepedonium (Emmons) v. Arx

Emericella nidulans (Eidam) Wint

BASIDIOMYCETES

Coprinus cinereus (Schaeff. ex Fr.) S.F. Gray (=C. delicatulus Apinis sensu Kemp)

FUNGI IMPERFECTI

Aspergillus candidus Link ex Fr.

A. fumigatus Fres

A. terreus Thom

Paecilomyces variotii Bain

Penicillium argillaceum Stolk et al.

Sporotrichum pulverulentum Novobranova

Continued ...

PHYCOMYCETES

Absidia corymbifera (Cohn Sacc-and Trotter (=Absidia ramosa (Lindt) Lendner sensu Nottebrock et al.)

Mortierella wolfii Mehrotra and Baijal

Rhizopus microsporus van Tieghem

R. sp.

Most of the ascomycetes isolated were collected through the soil plate and soil-steaming methods of fungal isolation. Two basidiomycete species were isolated during the survey. All attempts to make them fruit proved futile and because of this, their individual species could not be determined. They have been name sp. 1 and sp. 2 respectively. Some of the sterile hyphae isolated could be those of basidiomycetes or another class of fungi.

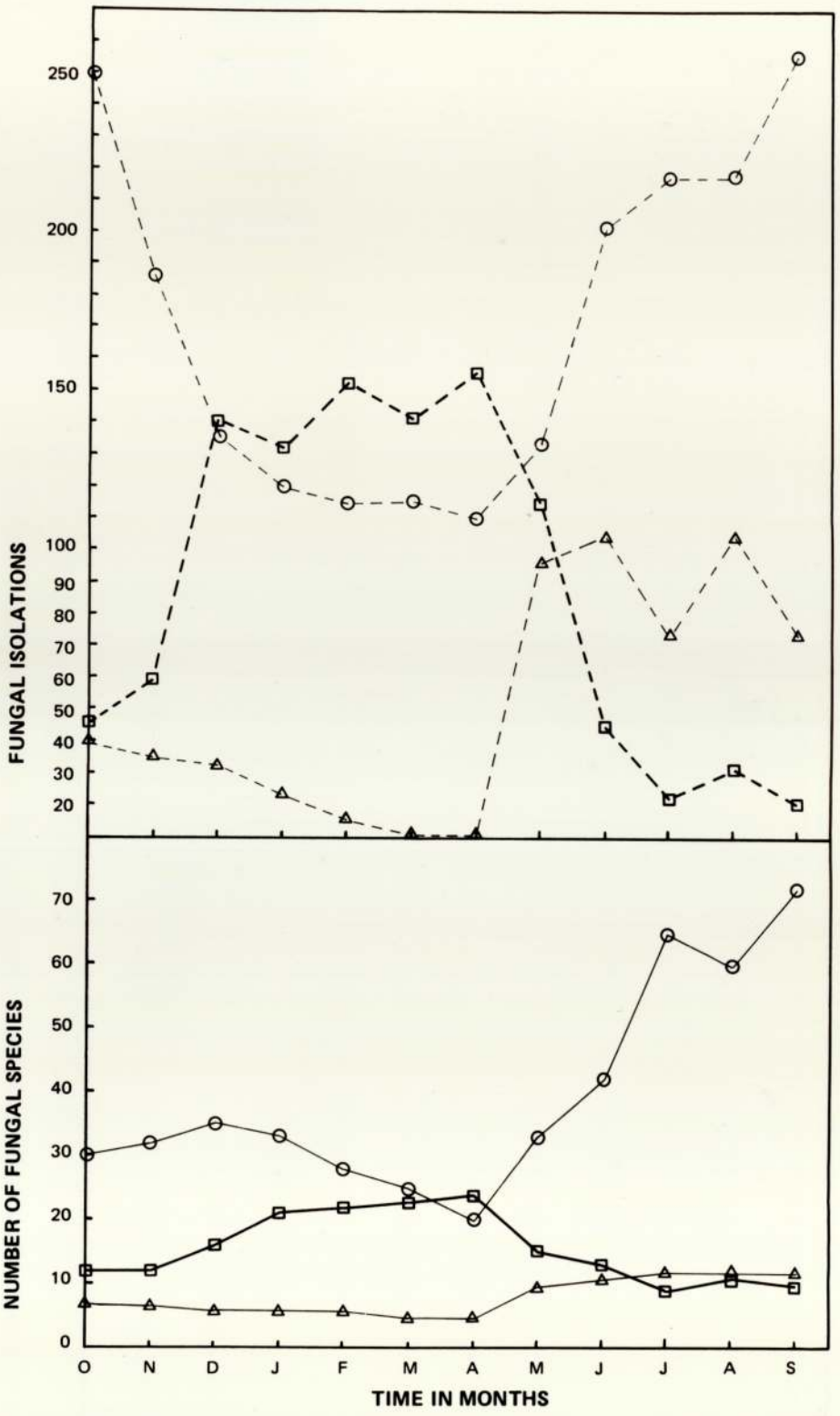
The soil samples were found to contain phycomycetes too and Mucor was the genus that had the highest number of phycomycete species.

A majority of the fungal isolates belong to litter hyphomycetes. Helminthosporium sativum, Nigrospora oryzae, Papularia sphaerosperma, Pithomyces chartarum and Torula herbarum were among the numerous dematiaceous hyphomycetes isolated. These fungi contain melanin pigments which might help in the absorption of ultraviolet radiation and so confer some degree of protection on the fungal propagules from the tropical sun. Amorphotheca resinae could not be isolated from the soil samples using the creosoted matchstick method. Thielaviopsis basicola was isolated through the carrot disc method.

SEASONAL VARIATIONS OF FUNGI IN THE SOIL SAMPLES

The fungi isolated from the Nigerian soil samples were found to show seasonal variation (Figure 3). The dry season (November to April) soil samples were comparatively found to have more thermophilic fungi than the wet season (May to October) soil samples. Thermophilic fungal species like Corynascus thermophilus, Humicola

Fig.3 **Monthly occurrence of Thermophilic, Thermotolerant and Mesophilic Fungi in Nigerian Soil Samples.**



grisea var. thermoidea, Malbranchea pulchella var. sulfurea, Myceliophthora thermophilum. Myrioconium thermophilum, Thermoascus aurantiacus and Thermomyces lanuginosus were found to be present in all the monthly soil samples investigated. Mucor miehei was isolated only during the wet season.

The thermophilic fungal isolations had three peaks, in December, February and April, all within the dry season. The thermophilic fungal species isolation reached peak in April and declined. It rose slightly again in August when there was a break in the amount of rainfall in Nigeria (otherwise known as August break). The thermophilic fungal isolations equally rose slightly in August.

The thermotolerant fungal isolations had two peaks one in June and the other in August. Their persistence in August (during August break) when there was a slight rise in temperature could be due to their ability to withstand high temperatures. The isolation of individual thermotolerant species reached peak in the wet season. Absidia corymbifera, Aspergillus fumigatus. A. terreus, Emericella nidulans, Paecilomyces variotii and S. pulverulentum were common in most of the soil samples.

There was a decline in the mesophilic fungal isolations and in the number of individual species during the dry season. Mesophilic fungal isolations reached peak in the rainy season (June and September). The slight depression noticed in August in the isolation of individual mesophilic species could be attributed to the August break. There was a predominance of mesophilic dematiaceous hyphomycetes during the dry season.

The fact that both thermotolerant and mesophilic fungi were abundant during the wet season shows that there is a relationship between them.

THE EFFECTS OF SOIL ENRICHMENT ON THE ISOLATION OF THERMOPHILIC AND THERMOTOLERANT FUNGI FROM THE NIGERIAN SOIL SAMPLES

Appendices 4 and 5 give the details of fungi isolated from the cellulose and lignin enriched soil samples respectively, while the effects of soil enrichment on thermophilic and thermotolerant fungal isolations are presented in Figure 4.

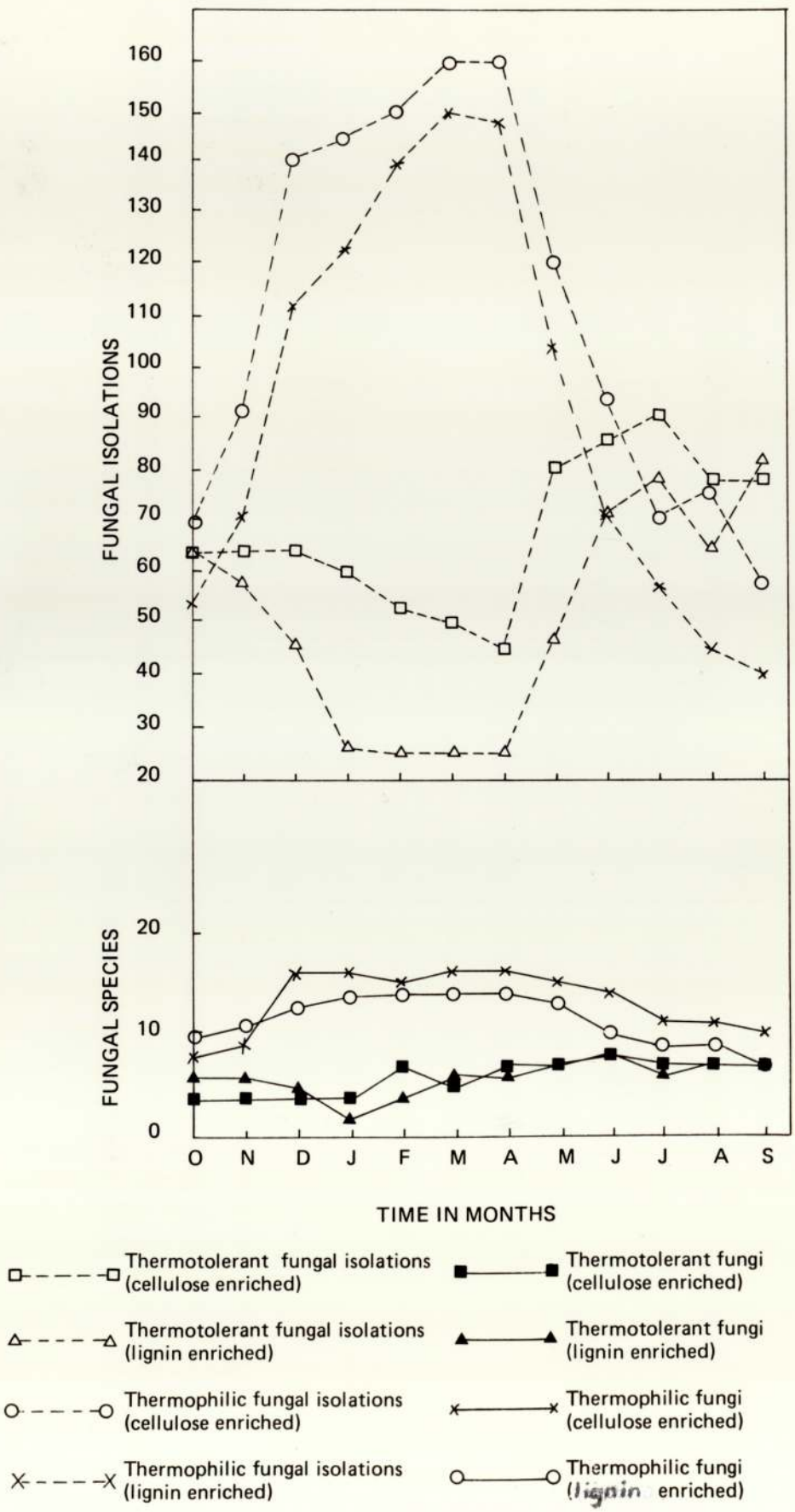
More thermophilic and thermotolerant fungal isolations were made from the enriched soil samples than the non-enriched ones. However, there was a higher incidence of individual species in the non-enriched soil samples.

The occurrence of species like Chaetomium thermophile var. coprophile, C. thermophile var. dissitum, Myceliophthora thermophilum, Myrioconium thermophilum, Paecilomyces variotii and Sporotrichum pulverulentum were increased by enriching the soil with cellulose and lignin. Cellulose and lignin are two major components of wood. The fact that these fungi showed special affinity for cellulose and lignin means that they may be capable of degrading these two major components of wood if they ever came in contact with them.

FUNGI ISOLATED FROM THE SOIL SAMPLES USING AMINO ACID AS THE SOLE NITROGEN SOURCE AND THIAMINE HYDROCHLORIDE AS SOURCE OF VITAMIN

The fungal species isolated from the Nigerian soil samples

Fig.4 **The Effects of Soil Enrichment on the Isolation of Thermophilic and Thermotolerant Fungi from Nigerian Soil Samples**



using modified CA (containing an amino acid as the sole nitrogen source and thiamine hydrochloride as source of vitamin) are given in Appendix 6. More thermophilic and thermotolerant fungi were isolated from the soil samples when normal CA was employed than when modified CA was used. The fact that the fungal isolates could utilise amino acids as sources of nitrogen means that they are capable of attacking any material like wood which contains numerous amino acids.

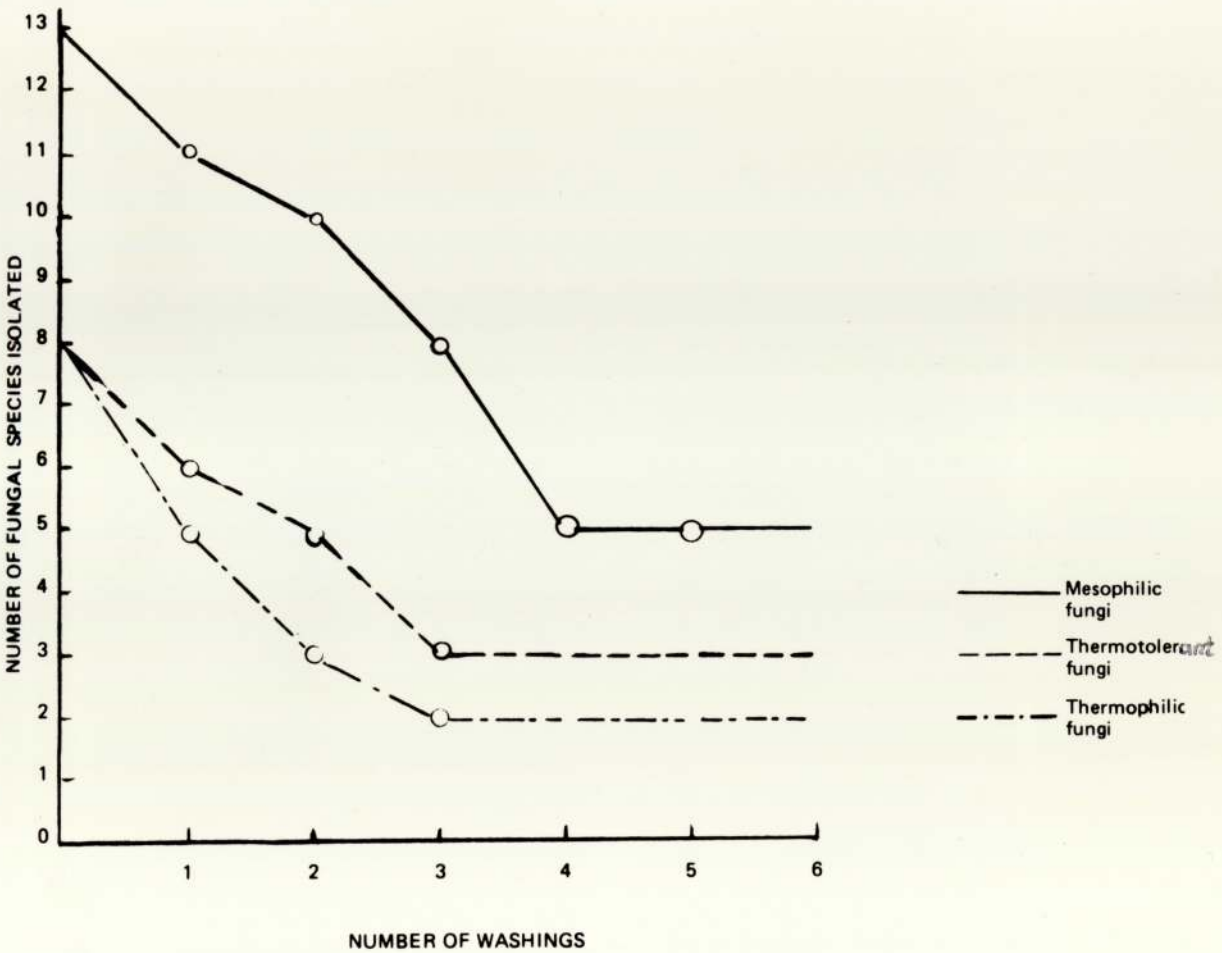
THE EFFECTS OF SOIL DILUTION ON FUNGAL ISOLATION

The effects of soil dilution on the number of fungi isolated from the soil samples are shown in Appendix 7. There was a reduction in the number of fungal isolates as the soil suspension became more dilute. The results obtained from the dilution experiments reveal equally that mesophilic fungi are more abundant during the wet season while thermophilic fungi are more abundant during the dry season.

THE EFFECTS OF WASHING ON THE NUMBER OF FUNGI ISOLATED FROM THE ROOT PIECES

Figure 5 shows the effects of washing of the root pieces on the number of fungi isolated from them. The more the number of washing the fewer the number of fungi isolated. Appendix 8 shows the details of the fungal species isolated from the unwashed root pieces. Thermophilic fungi were the least number of fungi isolated from the washed root pieces.

Fig.5 The Effects of Number of Washings on Fungal Isolations from Root Pieces Collected from the Nigerian Soil Samples



COMPARISON OF THE THERMOPHILIC FUNGAL ISOLATES FROM THE NIGERIAN SOIL SAMPLES AND OTHER SOURCES OF ISOLATION OF THERMOPHILIC FUNGI

The results obtained from the soil isolations show that the Nigerian soil samples have yielded more thermophilic fungi than have been reported from other sources by other workers (Appendix 9).

COMPARISON OF THE THERMOPHILIC AND THERMOTOLERANT FUNGAL ISOLATES FROM THE TROPICAL SOIL SAMPLES AND TEMPERATE SOIL SAMPLES

Thermophilic and thermotolerant fungal isolates from the temperate soil samples are presented in Appendix 10. Comparatively more thermophilic and thermotolerant fungi were isolated from the tropical soil samples (Nigerian soil samples) than the temperate soil samples (British soil samples). Fungal species like Acremonium alabamensis and Myceliophthora thermophilum were isolated after the soil samples had been irradiated with an infra-red lamp.

MICRO-ORGANISMS OTHER THAN FUNGI

Bacterial and actinomycete species were found to be among the numerous micro-organisms present in the Nigerian soil samples. A list of the identified bacterial and actinomycete species is given in Appendix 11. Two of the actinomycete species, Actinomyces thermophilus and Actinomyces thermofuscus were isolated at 50^{o}C.

CHARACTERISTICS OF THE EXPERIMENTAL SOIL SAMPLES AND THE VEGETATIONAL COVER CONTRIBUTING TO THE EXPERIMENTAL SOIL-SITE ORGANIC MATTER

Table 2 shows the characteristics of the soil samples and the

TABLE 2

CHARACTERISTICS OF THE EXPERIMENTAL NIGERIAN SOIL SAMPLES

TYPE OF SOIL - Humus

MOISTURE CONTENT - (presented in Figure 6)

RANGE OF SOIL pH - (✓)

RANGE OF % CARBON CONTENT - (✓)

RANGE OF % Cl^- CONTENT - 2.10 - 3.60

RANGE OF % NITROGEN CONTENT - 0.40 (non-detectable in most of the samples.

RANGE OF % PHOSPHORUS CONTENT 1 - 1.80

% SULPHUR CONTENT - could not be determined.

LAND USE - Agriculture and Grazing.

SETTLEMENT - University of Jos Faculty of Science blocks, nearby.

VEGETATIONAL COVER CONTRIBUTING TO THE EXPERIMENTAL SITE ORGANIC MATTER

Acacia

Afzelia africana

Andropogon tectorum

Annona senegalensis

Burkea africana

Cassia siamea

Cassia sp.

Commelina sp.

Cussonia barteri

Daniellia oliveri

Eupatorium odoratum

Imperata cylindrica

Continued...

Nauclea latifolia

Parkia clappertoniana

Sida aureus

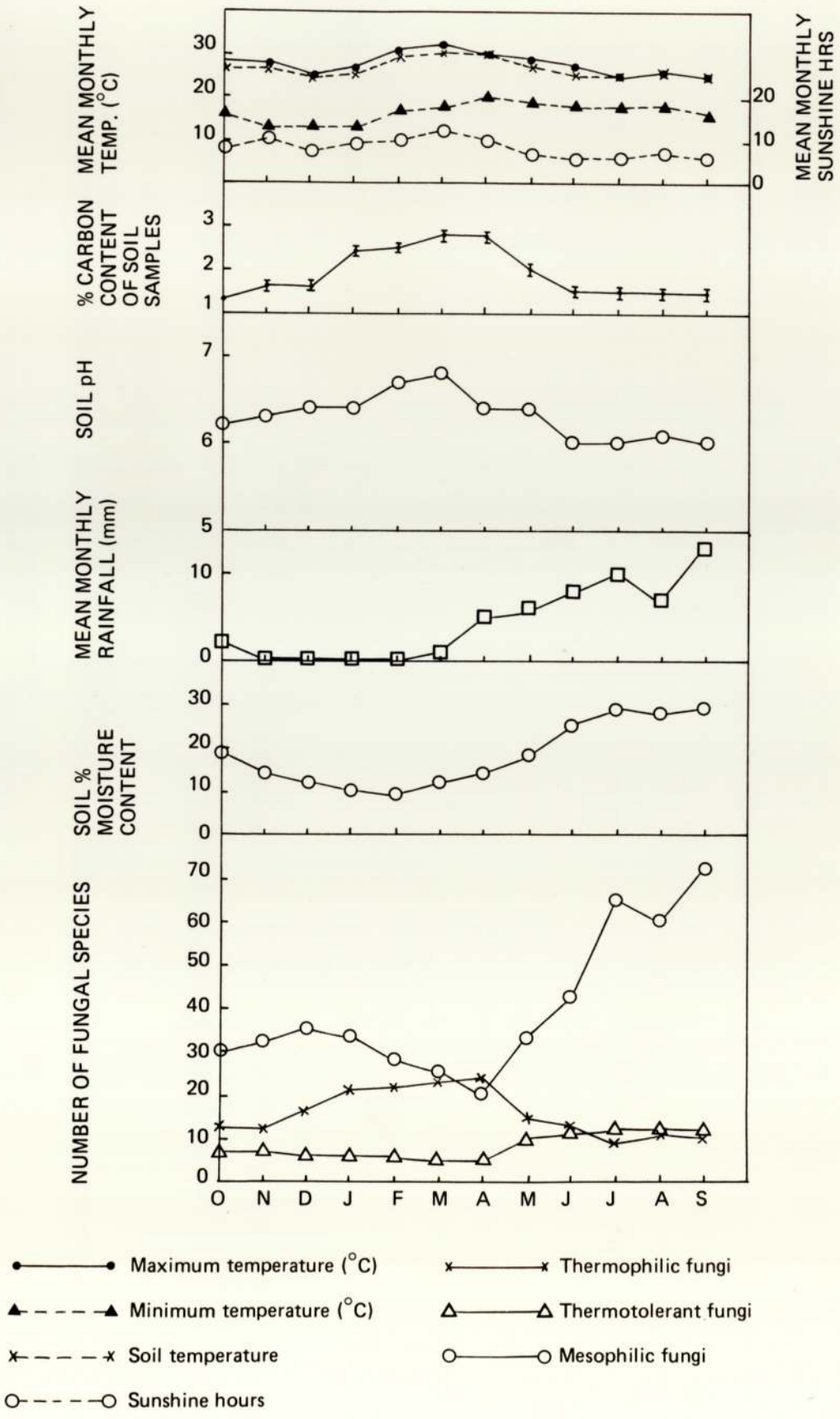
vegetational cover contributing to the organic matter of the sampling site. Non-metallic elements like carbon, chlorine, nitrogen and Phosphorus were detected in the soil samples. The presence of Sulphur was blocked by the presence of Mercuric ion and because of this it could not be detected.

THE RELATIONSHIP BETWEEN THE PHYSICAL-CHEMICAL FEATURES OF THE NIGERIAN SOIL SAMPLES PLUS OTHER CLIMATIC FACTORS (SUNSHINE HOURS AND TEMPERATURE) AND THE NUMBER OF FUNGI ISOLATED

The relationship between the soil physical-chemical features and climatic factors like rainfall, sunshine hours and temperature are shown in Figure 6. The mean atmospheric temperatures, mean soil temperatures and the number of thermophilic fungal isolates seem to be tied up. The more sunshine hours, the higher the atmospheric temperature and hence the soil temperature and the more the number of thermophilic fungi isolated. The higher the organic content of the soil samples the more the thermophilic fungal isolates.

Rainfall seems to be directly linked up with the moisture content of the soil samples and hence the number of thermotolerant and mesophilic fungal isolates. The more the rainfall, the more moisture content and the more the thermotolerant and mesophilic fungal isolates. The pH range recorded for the soil samples was well within the range that would give good growth of thermophilic fungi in pure culture. pH 6 and a little above seem to favour the growth of mesophilic fungi because most of the mesophilic fungal isolates were made within this range.

Fig.6 **The Relationship between the Physical-Chemical Features of the Nigerian Soil Samples plus other Climatic Factors (Sunshine Hours and Temperature) and the Number of Fungi Isolated**



2.9 DISCUSSION

The results of the survey have shown the occurrence of mesophilic, thermophilic and thermotolerant fungi in the Nigerian soil samples. The one hundred and forty-six mesophilic fungal isolates belong to 54 genera while the twenty-nine thermophilic and fifteen thermotolerant fungi belong to 18 and 10 genera respectively.

Of the twenty-nine thermophilic fungal species, six, Chaetomium thermophile var. coprophile, C. thermophile var. dissitum, Talaromyces emersonii, Thermoascus aurantiacus, T. crustaceus and Thermomyces ibadanensis have been isolated from palm kernel stacks in Nigeria (Eggins and Coursey, 1964; Apinis and Eggins, 1966; Oso, 1974) while two, Mucor pusillus and Thermomyces lanuginosus have been isolated from maize stacks in Nigeria (Okafor 1966). The remaining thermophilic fungal isolates are therefore new records for Nigeria.

The isolation of the same thermophilic fungi from different monthly samples indicates that these fungi were widespread in the soil samples and were not deposited in the culture plates as contaminants.

Comparatively more thermophilic fungi were isolated from tropical soil samples than from the temperate soil samples investigated. In temperate soils insolation has been shown by Eggins, von Szilvinyi and Allsopp (1972) to provide sufficient heat for fungal growth. Hedger (1974) recorded high populations of thermophiles equalling the populations of mesophilic fungi in the soils of Teak forest in East Java which has a dry monsoon climate. He therefore assumed that it is in the open forest soils of Java that insolation causes sufficiently high temperatures to induce growth of thermophilic fungi. This effect

of insolation on fungal growth was confirmed by Tansey (1976) when he reported that a diverse population of thermophilic and thermotolerant fungi is present throughout the entire year in temperate soils, with species present at greater frequency in sun-heated soil than in comparable shaded soils. In the current work with infra-red light, the irradiation of the temperate soil samples prior to sampling led to the recovery of two more species of thermophilic fungi. These species are, Myceliophthora thermophilum and Acremonium alabamensis. This increase in the number of thermophilic fungi recovered from temperate soil samples due to infra-red lamp heating might be a direct proof that solar heating is sufficient for growth and successful competition of thermophilic fungi in the soil. The results justify the earlier suggestion put forward by Cooney and Emerson (1964), that tropical soils with their higher insolation and therefore temperature values, might well support a higher population of thermophilic fungi.

Most of the thermophilic fungi isolated from the Nigerian soil samples have earlier been reported from different sources, including the soil. A comparison of the present thermophilic fungal isolations and earlier reports on thermophilic fungi from other sources show that more thermophilic fungi have been isolated from the Nigerian soil samples.

Fungal growth with enrichment cultures showed that the procedures used in the monthly survey of the soil samples detected the major components of the populations of thermophilic fungi present in the soil. The enrichment of the soil samples led to the increase in the number of thermophilic and thermotolerant fungal isolations but in the recovery of fewer number of fungal species. The enrichment of the soil might have led to the increase in the number of propagules of the fungi that could utilise cellulose and lignin. The fact that similar fungi were isolated

from both the cellulose and lignin enriched soils suggest that similar thermophilic and thermotolerant fungi could degrade both substances, and hence wood in ground contact.

Most of the thermophilic fungal isolates have been known to produce enzymes capable of causing wood decay. Chapman, Evans, Jacobelli and Logan (1975) reported of cellulolytic and amylolytic activity of Papulaspora thermophila. They observed that this fungus utilised soluble carboxymethyl cellulose and was able to degrade filter paper. The cellulases produced by the fungus were found to be induced enzymes, because P. thermophila cannot produce them in a medium lacking cellulose. They equally found that this fungus utilised starch and also produced amylolytic enzymes. Adams and Deploey (1978) reported of pectate lyase production by Papulaspora thermophila, Sporotrichum thermophile and Thermoascus aurantiacus. Oso (1978) recorded the ability of Talaromyces emersonii to synthesize extracellular cellulase in stationary liquid medium. He found that while this organism could not degrade filter paper, its cell-free filtrate hydrolyzed soluble carboxymethyl cellulose to reducing sugars. He therefore suggested T. emersonii was able to synthesize the Cx, but not the C_1 enzyme. The optimum temperature for the synthesis of the Cx enzyme was found to be $45^{o}C$.

Of the fifteen thermotolerant fungi isolated, Paecilomyces variotii has been isolated from deteriorating Nigerian palm oil (Eggins 1963), while Rhizopus sp. has been isolated from rotting maize (Okafor 1966).

Sporotrichum pulverulentum which is one of the thermotolerant fungal isolates has been found to cause wood decay. The endo- and exo-glucanases produced by this fungus have been carefully investigated (Eriksson and Peterson, 1957a, b).

Paecilomyces variotii has been used extensively for the manufacture of "PEKILO", the new animal feedstuff derived from wood. It is therefore useful in the utilisation of cellulosic and agricultural wastes but is also an agent of wood deterioration.

The most common genera among the mesophilic fungal isolates are Aspergillus, Chaetomium, Fusarium and Trichoderma. The high number of Aspergilli isolated during the survey could be related to the soil temperature. Waksman (1916) regarded the Aspergilli as being more prevalent in tropical soils and the Penicillia as more abundant in temperate regions. A picture of the response of the soil mycoflora to soil temperature has been built up by the accumulated results of many experiments. Thus Saksena (1955) showed the prevalence of Aspergillus over Penicillium in India, and Nour (1956) showed a similar picture in the Sudan. Borut (1960) working on the soil in Northern Negev found both Aspergillus and Penicillium frequent, but his work is unusual in attempting to measure the response of his isolates to temperature variation. Curves of growth at different temperatures were plotted for four species of Aspergillus, five species of Penicillium and a number of other isolates. All the species of Penicillium had a peak of optimum growth at 26^{o}C. The Aspergilli were more variable but the majority had high optima, the values being 36, 30, 30, and 26^{o}C.

The general abundance of Aspergillus as compared to Penicillium in the Nigerian soil samples may reflect the view quoted by

Griffin (1972) that Aspergillus is a more xerothermic genus than Penicillium.

Farrow (1954) investigated the tropical mycobiota of soil samples collected from Panama and Costa Rica. He isolated one hundred and thirty-five species, of which one hundred were Fungi Imperfecti. His isolates including seventeen species of Aspergillus, a number higher than that previously recorded from soil studies of temperate regions. Species of Chaetomium were also found to have a wide range of distribution in the soil samples while Mucor and Rhizopus were rarely recorded.

The results obtained during the current survey confirmed the abundance of species of Aspergillus and Chaetomium in tropical soils. The importance of A. fumigatus, in the degradation of wood has been reported by Flannigan and Sago (1977). Its high frequency of occurrence in the Nigerian soil samples may be pointing to the fact that it is playing an active role in biodeterioration in that country.

Some of the mesophilic fungal isolates have earlier been reported in Nigeria. Smith (1956) described Aspergillus funiculosus isolated by R.M. Jackson from Ibadan soil and sent to the Commonwealth Mycological Institute in 1954. Huang and Raper (1971) described a new species of the Aspergillus clavatus group, A. longivesica isolated from Nigerian soil during a microfungal survey of Nigerian soils.

Mesophilic fungal species like Fusarium oxysporum lycopersici, Helminthosporium sp, Trichoderma viride, Cladosporium sp, and

Myrothecium verrucaria have been known to produce amylase (Hankin and Anagnostakis, 1975). They are therefore potentially capable of utilising the starch in wood and may promote wood decay. Aspergillus niger has been known to produce pectinases while Aspergillus oryzae produces amylases and amyloglucosidase (Beckhorn, 1967). Reese and Mandel (1963) reported cellulase production in Trichoderma viride. The significance of these reports is that these fungi are capable of deteriorating wood.

The results obtained revealed that most of the fungal propagules originally associated with the root surfaces were washed out during the serial washing. The remaining fungi associated with the root pieces were firmly attached to the roots. Some of these might be mycorrhizal fungi. Guttay (1975) suggests that mycorrhizae grow on the roots of nearly all plants and behave almost like a filamentous extensions of the roots. The root surface is a region of varied habitats which have their own specialised floras. Many species of phycomycetes were isolated from the unwashed root pieces. These fungi are frequently associated with the utilisation of sugars which are known to be exuded more abundantly at the seedling stage of plant growth. Odunfa and Oso (1979) reported that as the plant matures exudation decreases and the sugar fungi are replaced by cellulose decomposing fungi which degrade sloughed off cell debris.

The employment of different methods of isolation and different media led to the isolation of many species of fungi from the Nigerian soil samples. Jensen (1934), Chesters (1948) and others have stressed the fact that different methods of soil microbiology are complementary rather than mutually exclusive. However, most of

the fungi were isolated by the dilution plate method and the soil plate method. The other specialised methods employed helped to recover the fungal species that are rarely recorded in either the dilution plate method or the soil plate method.

Fungal occurrence in the Nigerian soil samples showed a definite seasonal incidence. The seasonal variation might have been caused by changes in certain environmental factors, such as rainfall, soil moisture content, soil pH, sunshine hours, temperature and organic carbon content.

During the dry season when the soil moisture was low mesophilic and thermotolerant fungal isolates were low. Conversely, more thermophilic fungal isolates were obtained during this period. During the rainy season, the soil moisture content increased and there was an increase in the mesophilic and thermotolerant fungal isolates. There was a decrease in the number thermophilic fungal isolates within this period. This may be related to a somewhat lower soil temperature, but could also reflect an increase in competition by the increased number of mesophilic and thermotolerant species. Terrestrial fungi, regardless of their habitat are micro-aquatic organisms. Not only is water necessary for intracellular metabolism, but an adequate layer of external water is necessary for diffusion of extracellular enzymes and of substrates as well as of toxic products, for the maintenance of turgor for hyphal development. Water also plays an important role in the discharge of spores, and is essential for the dispersal of some spores. Fungi may persist for long periods of time in the absence of water, usually by virtue of spores or other dormant structures. Limitations in available water may also select for genetic types capable of persisting under drought

conditions. The thermophilic fungal species are good examples of this. Falk, Hartman and Lord (1962, 1963 a, b) demonstrated that the DNA molecule is highly hydrated until potential decreases to - 115 bar and that with further reduction water molecules are progressively removed. Between about - 400 and - 800 bar the helical structure is reversibly distorted and the base pairs become disordered. However, Seidel (1965) showed that soil fungistasis increased in intensity with increases in soil water content. He interpreted his results as indicating enhanced diffusion of an inhibitor, however they might also indicate enhanced diffusion of nutrients away from the spore. Ko and Lockwood (1967) put up the hypothesis that fungistasis apparently is a consequence of the availability in soil or loss from spores of nutrients required for the spore germinations.

Many thermophilic fungi have been known to produce antibiotics. The antibiotics production by some of these fungi under soil conditions which allowed their growth may have helped to keep down the population of mesophilic and thermotolerant fungi during the dry season.

Fungal species like Corynascus thermophilus, Humicola grisea var. thermoidea, Malbranchea sulphurea, Myceliophthora thermophilum, Thermoascus aurantiacus and Thermomyces lanuginosus were isolated during both seasons but with higher incidence during the dry season. Aspergillus fumigatus was the only thermotolerant fungus that was isolated from all the soil samples although it had a higher frequency of occurrence during the wet season. The common mesophilic fungal genera (Aspergillus, Chaetomium, Fusarium and Trichoderma) that had a wide range of distribution in the soil samples were equally found

to have higher incidence during the wet season. The pH range of the soil (6.0 - 6.80) cannot be used to give any indication of pH preference for thermophilic fungi. However, the pH values were well within the ranges required to give good growth in pure culture. The soil pH reached its peak in March and gradually decreased with the coming of the rains. The decrease noticed in the soil pH during this period might have been caused by leaching of the soil by rain water. Leaching is a major problem in Nigerian soils. Leaching removes bases from the soil and therefore tends to lower the pH with time.

The pH value of any soil depends on a variety of factors. These include the soil forming factors plus the season of the year, cropping practices, the soil horizon sampled, the water content at sampling time and the way the pH was determined. Soil pH affects the availability of nutrients in the soil. It is possible that it also influences the physiological functioning of the fungal thallus, possibly through its effect on the reactions occurring on the cell surfaces rather than through a direct influence on the internal processes of the cell. However, the small pH range recorded during this study is unlikely to have affected the patterns of fungal distribution which were found.

Judging from the meteorological data obtained, there is a relationship between the sunshine hours, the mean maximum temperature and the mean soil temperature (2-4cm below surface).

The highest atmospheric temperature was recorded in March during the dry season. Sunshine hours and soil temperature equally reached their peak during this month.

Several factors influence soil temperature. These are, angle of the sun's rays, any cover on the soil, the colour of the soil to some extent, soil water content, the depth and the time of measurement.

The more squarely the sun's rays strike the soil surface, the warmer its temperature will be. Wet soil tends to be cooler than dry soils in part especially in the wet season. This is because evaporation helps cool the moist soil by dissipating heat. The high heat capacity of water reduces the temperature change from the heat that is absorbed by the soil. These factors can account for the higher soil temperatures recorded during dry season than wet season and hence the more thermophilic fungi isolated during the dry season. The presence of water and the reduced temperature of wet soil would have favoured the growth and isolation of mesophilic and thermotolerant fungi during this season. Other factors being equal, microbial activity increases with increasing temperature. The soil temperatures recorded during the dry season were high enough to support the growth of thermophilic and thermotolerant fungi. The soil temperatures as were recorded during the wet season were within the mesophilic fungal growth range.

The results have revealed a correlation between the organic carbon content of the soil and the number of thermophilic fungi present in the soil during the dry season. Most of the thermophilic fungal species were found to be more abundant in the soil in January - April when the soil was richer in organic carbon. Trees normally shed their leaves during the dry season in Nigeria. A build up in the number of leaves at the sampling site within this period would bring about the increase in organic carbon content of the soil. Species like Stibella thermophile known to be found in

compost was abundant in the April soil sample. The decrease in organic carbon content of the wet season soil samples could be due to soil erosion washing out organic matter content from the site within this period. Chloride ion, nitrogen and phosphorus were detected in the Nigerian soil samples. Nitrogen is an essential element used by fungi for functional as well as structural purposes. The cell wall of many species, with the exception of the Oomycetes and yeasts appears to be composed of chitin (Brian, 1949). Chitin is a linear polymer, similar to cellulose, of D-glucosamine. The amino group of glucosamine in chitin is acetylated. Protein, the basis of protoplasm, is composed of nitrogenous substances. Some of the vitamins are also nitrogen-containing compounds. The isolation of soil fungi with CA having an amino acid as a sole nitrogen source and thiamine hydrochloride as source of vitamin confirms the utilisation of nitrogen by soil fungi. However, more fungi were isolated with normal CA than the CA having an amino acid as a sole nitrogen source and thiamine hydrochloride as a source of vitamin. The fungal species isolated with the modified CA (having an amino acid as sole nitrogen source and thiamine hydrochloride as a source of vitamin) might have been those that can utilise thiamine hydrochloride while the non-thiamine hydrochloride utilisers were excluded. Normal CA medium contains asparagine and yeast-extract and therefore could offer a wider choice of vitamins to soil fungi.

Phosphorus compounds play an important role in the functions of chemical transformations and energy transfer in all forms of life. Raulin (1869) found phosphorus to be an essential element for Aspergillus niger. Omission of phosphate from his synthetic medium reduced the yield approximately 50 per cent. Fungi utilise phosphorus in the form of phosphate salts and esters. The presence of this

element in the Nigerian soil samples confirms the suitability of the soil samples for the growth of fungi.

It is not known whether fungi require non-metallic elements other than hydrogen, oxygen, sulphur, phosphorus, and nitrogen. Sodium chloride is frequently added to media, but neither sodium nor chlorine, so far as is known, is essential for the fungi. Fungi may come in contact with many non-essential elements, some of these may be metabolised while others may modify the life processes of the fungi by their action or by other means. It is difficult to explain the role of chlorine with regards to the Nigerian soil fungi but chlorine is found in various compounds synthesised by fungi for example, non-ionic chlorine is found in chloramphenicol, one of the newer antibiotics (Lilly and Barnett, 1951).

Nine bacterial species and three actinomycete species were identified during the survey. Over the past few years, interest has been increasing in the effects of bacteria on wood. Studies have been made on their ability to increase the permeability of wood (Greaves 1966a, 1968a). Reports and observations on their incidence in wood have been increasing.

Organisms concerned in biodeterioration are usually a selection of those found in the soil. Results from soil work are therefore relevant to deterioration studies. Pugh (1965) in his publication on some problems in the classification of soil fungi mentioned that fungi in soil have complex relationships with their substrates and with each other. There is no single easy way to isolate a complete mycoflora.

Chapters 4 and 5 of the Thesis will be dealing with the effects these fungi from soil have, on wood in ground contact.

CHAPTER 3

THE EARLY FUNGAL COLONISATION OF WOOD

3.1 INTRODUCTION

Wood may be regarded as a series of conveniently orientated holes surrounded by food to the micro-organisms colonising it. The colonisation sequence is purely an ecological one and the organisms concerned can change the wood in several ways, and their activities can take a number of forms: Nitrogen fixation by bacteria has been shown by Baines and Millbank (1975); competition for nutrients will occur and this could well involve scavenging for nitrogen; synergism can also occur(Hulme and Shields, 1975); there could be destruction of the pit membranes(Dunleavy and McQuire, 1970); various aspects of changes in the fine structure of wood can also be seen.

The colonisation sequence is the initial part of the process of wood decay. During this process there are a number of ecological niches to be filled and for timber in ground contact, the soil provides an abundance of organisms to fill these niches. The exact species which fill a particular niche will depend on a number of environmental factors such as the type of wood involved, temperature, moisture content and the wood pH value. What is important is not the exact fungal species, which may differ with time and place, but the particular physiological action it may have on the wood in a given habitat. There is a spectrum of interaction between fungus and wood.

The investigation of wood colonisation by fungi has been the subject of research for many wood scientists. Bailey and Vestal (1937) found hyphae of fungi, thought to be Pyrenomycetes in the central layer of secondary wall of wood elements of 114 species from thirty-six families of Gymnosperms and Angiosperms. The hyphae lay in cavities with pointed ends, and such cavities often occurred

in chains. Tamblyn (1937) noted similar attacks on Jarrah (Eucalyptus marginata) and found that the cavities were connected by extremely fine hyphae.

Barghoorn and Linder (1944) isolated Fungi Imperfecti and Pyrenomycetes from wood blocks and cordage immersed in the sea. Infected timber showed hyphae of the sap-stain type and also colourless hyphae attacking the wood, as described by Bailey and Vestal, which caused a surface decay. Findlay and Savory (1950) briefly noted similar attacks on wood.

Savory (1954) investigated the breakdown of timber by Ascomycetes and Fungi Imperfecti. He reported thirty-three species of fungi which he isolated from timber showing soft rot. He showed that soft rot of wood is caused by certain species of Ascomycetes and Fungi Imperfecti. He found that the fungal hyphae typically ran spirally in the central zone of the secondary xylem walls, where they gave rise to cavities with pointed ends. He also found that the fungi causing the soft-rot were cellulose-attacking species which initiate attack in the less heavily lignified parts of the walls of the wood elements. The rate of attack was found to be increased when inorganic nutrient salts were added to the wood. He observed that C. globosum can cause severe decay of hardwoods under laboratory conditions.

Duncan (1960) published a list of fungi capable of producing soft-rot on preservative-treated poles and piling, and suggested that these might facilitate the entry of other decay fungi.

Kaarik (1967) investigated the fungal-colonisation of pine and spruce poles in contact with the soil, on three different soil types in Southern Sweden. She isolated fungal mycelia from different levels and from different wood layers on the poles. She observed that after six months the poles showed attack by different Basidiomycetes on several restricted patches. She found that the dominant decay fungi were different in different stands, but all belonged to the common forest flora. Besides the decay fungi, she equally isolated numerous mycelia of Fungi Imperfecti and Ascomycetes. She found the fungal flora on the three sites to be rather similar but quite marked differences in the frequency of a number of species was found in the different stands.

King (1972) considered that many microfungi which colonise wood possess a potential wood-decay ability. This was confirmed by Nilsson (1973), who showed that the majority of the 169 common-found wood-colonising microfungi, some of which were used by King (1972), produced soft rot in wood under laboratory conditions.

King and Eggins (1973) showed that many microfungi, noted for early colonisation of wood after felling, possessed the ability to produce a spectrum of enzymes which would enable them to deteriorate wood. These enzymes, suggested to be both the Ci and Cx components of cellulase along with polygalacturonase and amylase, were associated with production of an enhanced permeability of wood, frequently observed after wood was colonised by these organisms.

Butcher (1975) investigated the colonisation of Pinus radiata sapwood by soft-rot fungi by means of sequential light microscopy. He exposed the wood under various conditions of both natural infection

and infection by pure culture. Soft-rot was more pronounced in wood exposed to undisturbed, unsterile soils than in unsterile, sieved soil or sterilised soil inoculated with pure cultures of Chaetomium globosum Kunze. When pure cultures of Chaetomium globosum were grown in a synthetic soil (acid-washed sand plus nutrients), soft-rot development in the exposed timber was as good as, if not better than, that in undisturbed, unsterile soil.

3.2 THE SUCCESSION OF MICRO-ORGANISMS DURING WOOD DECAY

Wood decomposition has been under investigation for a long period of time mainly by sporophore studies. Garrett (1963) recognised three stages in the decomposition of woody tissues in and above the soil. The first stage involves the invasion of the wood tissues by primary saprophytic fungi, which live on sugar and carbon compounds simpler than cellulose. The second stage involves cellulose decomposers associated with secondary saprophytic sugar fungi, which utilise the products of cellulose decomposition. Stage three consists of lignin decomposers and associated fungi. As an exception to the general succession described, Garrett mentions the colonisation of fresh coniferous stumps, where the lignin-decomposing fungi appear before the cellulolytic fungi. This general scheme is often accepted in literature. The details of primary infection and subsequent colonisation depend on the manner in which the wood is killed and exposed to micro-organisms. It also depends on the seasoning process to which it is subjected. In some cases the succession of micro-organisms during wood decay has been found to follow another pattern different from that described by Garrett.

The first stages of infection of Ponderosa pine were studied in the U.S.A. by Merrill and French (1966); while Greaves (1972), investigated the first stages of infection of stakes of Pinus radiata and Eucalyptus regnans in Australia. In the investigations of Merrill and French, certain changes in the microflora of the stakes were found during the early invasion (between 1 week and 3 months). The stakes in Australian soil showed a very rich flora, especially of Penicillium species, under 1 to 7 months but it is difficult to

ascertain any clear successional changes. This test showed that the wood substrate did not exert a significant effect on the invading populations and that the main influence was exercised by the time of exposure.

Butcher (1968) believed that in addition to characterising the changing fungal flora, it is equally important to know whether the fungi isolated were in physiologically active or inactive state within the wood and continued lack of this essential information would mean that a particular organism might be assigned more importance than is warranted. More attention should therefore be placed on the build-up of activities of micro-organisms in wood rather than on their apparent continued presence. Therefore, in addition to periodic isolations and anatomical studies on the distribution of micro-organisms in wood, it is desirable to obtain measurements of either oxygen uptake or carbon dioxide release to provide further information on the build-up of activities of micro-organisms in wood cells.

Banerjee and Levy (1971) studied the succession of micro-organisms in birch and Scots -pine fence posts in England over a period of ten months. The fungus flora was studied above and at the soil line. Fungi belonging to 20 genera colonised the surface but less than half that number penetrated to a depth of 5mm and only three of the surface colonisers penetrated to a depth of 25mm and two to 45mm after ten months. Basidiomycetes, on the other hand, were found at each depth, except the surface layer. Succession of the fungi at the soil line below the surface layer was summarised as follows: sap stain fungi - sap stain fungi with soft rot characteristics - Basidiomycetes.

Rayner (1977), investigated the fungal colonisation of freshly cut stumps of Fagus sylvatica, Betula pendula and Quercus robur by fungi other than basidiomycetes for periods of up to 2½ years. He found that chemical stump treatments especially with 40% (w/v) ammonium sulphate solution (AMS) strongly influenced the succession. Within 1-3 months of exposure the cut surface of AMS-treated stumps was extensively colonised, especially by Botrytis cinerea while stumps treated with water as controls remained virtually uncolonised. Subsequently AMS-treated stumps became extensively colonised by a number of other fungi including Corynesarcoides. Pezizella sp. and Hypoxylon serpens (on beech only) while water-treated stumps were more often colonised by Xylaria hypoxylon. He found that as decay became more advanced, which happened particularly rapidly and extensively with AMS-treated stumps, other fungi appeared.

3.3 FACTORS AFFECTING INITIAL COLONISATION AND BUILD-UP OF MICRO-ORGANISMS IN WOOD

3.3.1 NUTRIENTS IN WOOD AND INITIAL COLONISATION

Nutrients play a major role in the susceptibility of different timbers to microbial attack.

Olofinboba and Lawton (1968), for instance, identified maltose, raffinose, glucose and sucrose in the sapwood of Antiaris africana and noted that these substances were highly utilised by the blue-stain fungus, Botryodiplodia theobromae which rapidly colonises logs after felling. Banerjee and Levy (1971) reported that the presence or absence of surface nutrients is an important factor which affects colonisation of the surface and eventually the layers below. They found that the number of fungal species present on the surface of posts reached a climax by the fourth and fifth months of exposure and thereafter declined. They explained this observation by saying that, with the depletion of nutrients, not only is the activity of the existing 'sugar' fungi in the surface layers greatly curtailed, but also, the surface layers rendered uninhabitable for other 'sugar' fungi, which generally colonise the wood by deposition of spores.

Takahashi and Nishimoto (1973) in Japan, have found that xylose, which occurs to a greater extent as part of the hemicellulose content of hardwoods, and mannose, occurring in the hemicellulose of softwoods, were equally utilised as carbon sources by Chaetomium globosum, a fungus known to cause considerable soft rot in hardwoods.

King, Oxley and Long (1974) have shown that during drying of wood, soluble nutrients move towards the surface and this enhanced the colonisation and decay of this layer by soil micro-organisms. Long (1975) showed by paper chromatography that there was an accumulation of larger quantities of simple sugars in the surface layer cells of laboratory dried wood planks. He also found smaller quantities in the interior cells.

Teyegaga (1976) observed from his investigation that simple sugars as well as amino acids present in sapwoods enhanced colonisation by soil micro-organisms.

These reports have shown the importance of nutrients in wood and its initial colonisation by fungi.

3.3.2 MOISTURE CONTENT OF WOOD AND ITS COLONISATION BY FUNGI

Wood decay occurs when its moisture content exceeds 20% of its oven dry weight. There may be severe damage of wood, when the moisture content is above the fibre saturation point, that is about 30%. Water, apart from its function in hydrolytic reactions, is also required for creating a continuum between the colonising organism and the wood substrate. It also helps in the diffusion of enzymes to the site of reaction. Water swells wood polymers and enlarges the openings that are almost closed in the dry wood. The exclusion of water from wood has been used as a means of controlling wood decay. The use of water repellent additives in wood preservation has also received attention (Belford, 1968).

It has been found from laboratory and field observation that for rapid attack of wood, many soft rot fungi require more moisture than do most decay fungi (Savory, 1955 and Duncan, 1960). Butcher (1968) investigating the ecology of fungi infecting untreated sapwood of Pinus radiata noticed that the course and speed of the succession of fungi in the experimental zones of the stakes (above ground, ground line and below ground) was influenced by the moisture content of the wood. He explained that timber above ground was too dry for the establishment of soft rot fungi and basidiomycetes whereas the high moisture content in the below ground zones favoured the development of soil borne moulds and soft rot fungi. Yazawa (1960) (cited by Ueyama 1966) has pointed out that Ceratocystis moniliforme can tolerate a high moisture content (up to 80% of the oven dry weight) in the timber it invades, while the slow growing decay fungi are limited by a moisture content of 65 - 70%. Thus moisture

and available nutrients are very necessary for fungal colonisation of wood. Teyegaga (1976) found that the distribution of moisture in his test wood blocks showed that all the layers were at sufficient moisture content to promote decay. He explained that the high moisture content in the surface layers of all the test blocks might be due to the softening of these layers as a result of decay which consequently increased the permeability and perhaps the hydrophilic nature of the wood.

3.3.3 pH RELATIONS AND WOOD COLONISATION

The role of pH in the colonisation of wood by fungi has attracted the attention of wood scientists. Cartwright and Findlay (1958) wrote that fungi may change the pH of their substrate possibly in the directions most suited to their growth and the extent of this change varies according to the species of fungus. Duncan (1960) noted that generally soft rot organisms grow at pH 4-5 and reach their maximum growth at pH 6-7. pH 9 was found to have a retarding effect on their growth.

Butcher (1968) found that comparative studies on the effect of pH on the growth rate of the various fungi he isolated from wood were of little value in explaining the broad bases for the successional trends. Good, Basham and Kadzielawa (1968) associated the stain reaction in wood caused by certain members of the Fungi Imperfecti and certain bacteria, with high pH, high moisture content and relatively high respiratory activity. They observed that as the stain is destroyed the pH and the water content fell and the primary decay organisms became dominant. Teyegaga (1976) in his investigation on the changes in pH of wood blocks at different depths after different periods of incubation noted that the pH of the wood fell to a slightly more acid state. This he found to be more evident in the outer layer than in the inner layer where the change did not appear to be significant.

3.3.4 TEMPERATURE AND FUNGAL COLONISATION OF WOOD

Information on the effect of temperature on the rate of fungal colonisation of wood in soil contact is almost lacking. Duncan (1960) noted that soft rot organisms tolerate a fairly wide range of temperature optima. In the tropics where the soil temperature is usually high especially during the dry season, the activities of thermophilic and thermotolerant fungi invading wood in ground contact are probably quite high.

3.4 AIM OF THE PRESENT EXPERIMENT

While much work has been done on the ecology of micro-organisms colonising wood in contact with soil in the temperate regions, there is no published information on the Nigerian fungal colonisation of Nigerian wood in ground contact. It has already been established in section one of this thesis that the soil is a reservoir of biodeteriogens. The aim of the present experiment is to investigate the fungal colonisation of Nigerian timber veneers exposed at the factory. It was hoped that such investigation would provide data for a comparative study of the early colonisation of plywood surfaces produced in Nigeria and in the U.K.

3.5 TECHNIQUES FOR THE ISOLATION OF FUNGI FROM WOOD

Grant and Savory (1968) have described some of the methods of obtaining fungal inocula from wood. These methods include the use of the Pressler borer, the two-chisel technique, the cut-block technique, the split-billet technique, the saw-cut technique and the drill technique. The development and modification of the drill technique was outlined by Levy (1968). In addition there are the grinding-wheel technique described by (Eggins, Malik and Sharp, 1968) and the split-block technique of Kaarik (1967).

Such techniques are of value when the most active fungus is to be isolated from a region of decay or where an indication of species colonising the wood is required. Methods which result in inocula of small size (saw-cut, grinding-wheel and drill techniques) are usually regarded as being superior to the remainder because species are more often obtained in almost pure culture and a greater range of fungal species is encountered.

All of the techniques employed for obtaining wood inocula have limitations because there is no standardisation of the unit volume of wood sampled. Where techniques such as the saw-cut or grinding-wheel method are used, it is possible either to control the size of the zone sampled, or to regulate the number of inocula landing on exposed agar plates. The drill technique goes some way in ensuring that the sampling zone remains of constant size.

The pattern of sampling is an important factor which is often overlooked, and can have a marked influence on results because it may be subjective (selection of typical zones) or

objective (samples taken at random or systematically). Subjective sampling is of value where the main causes of stains and decays in wood are to be determined. Objective sampling has been used in ecological studies where knowledge is required of all the fungi colonising wood, but sampling has been strictly systematic.

Techniques for the present survey have been adopted with two objects in view:

i) To obtain pure cultures of individual species.

ii) To obtain the maximum number of different species.

3.6 MATERIALS AND METHODS

The wood veneers for the present experiment were obtained from African Timber and Plywood Limited (AT & P) factory at Sapele, Nigeria. The veneers included Khaya ivorensis (Lagoswood), Triplochiton scleroxylon (Obeche), Terminalia superba (White Afara), Daniellia ogea, Canarium schweinfurthii and Sterculia oblonga (sterculia, yellow).

3.6.1 THE CUT BLOCK TECHNIQUE

"1cm" × "1cm" pieces of wood were removed from the peripheral portions of each species of the wood samples. The wood pieces were divided into two sets. One set of samples were washed in seven changes of sterile distilled water with the help of a horizontal mechanical shaker. From preliminary experiments the number of washings was found that removed surface fungal contaminants leaving real fungal colonisers in the wood samples. The other set of wood pieces were left unwashed.

The wood pieces from each set were then plated out on CA and MEA. Five plates of each set of wood pieces (either washed or unwashed) of each wood veneer were incubated at 25°C, another five at 35°C and five at 45°C. Each agar plate contained five pieces of the "1cm" × "1cm" wood pieces. The procedure was carried out for both CA and MEA media. The plates were left for daily observation and any fungus appearing was subcultured several times until pure culture was established. The fungal isolates were identified as described in section one. Scorings were made for the number of wood veneers being colonised or surface contaminated by the same fungus.

3.6.2 THE PRESSLER INCREMENT BORER

The borer together with the associated plug extractor were sterilised by flaming in alcohol. The borer was again dipped in alcohol and the adhering alcohol was then ignited and the flame issuing from the end of the borer was used to sterilise the surface of the area of wood veneer being sampled. The borer was driven into the wood and the plug inoculum was transferred directly to a slope of a medium (both CA and MEA were employed). The plug was loosened from the extractor by forcing it against the inner surface of the test tube with a rolling movement.

Some of the slopes with their inocula were incubated at 25°C, some at 35°C and some at 45°C. The slopes were left for daily observation and the resultant fungi from the inocula were purified and then identified.

3.6.3 THE TWO-CHISEL TECHNIQUE

A sterilised 13mm chisel was used to remove the superficial layer of a stained portion of the wood veneer. The superficial layer so removed was plated out on a culture medium. The underlying wood was then sampled with the aid of a 6mm sterilised chisel. The resultant shavings were placed on agar with a pair of sterilised forceps. The shavings were stuck on edge so that half of their cross sections were embedded in the agar. The culture plates were divided into three. The first portion was incubated at 25°C, the second at 35°C and the third at 45°C. The plates were observed daily for the presence of fungal species.

3.6.4 THE SPLIT-BILLET TECHNIQUE

Wood inocula were taken from the freshly split surface of a sample billet by cutting samples of matchstick-size longitudinally from the freshly exposed surface, with the aid of a sterilised scribe. Sterilised forceps were used to transfer the inoculum to the culture media (CA and MEA). A dozen small samples were planted around the edge of each petri dish. The resultant culture plates were divided into three. One portion were incubated at 25°C, the second portion at 35°C and the third at 45°C. The plates were observed daily for fungal presence. The developing fungal colonies were subsequently purified and identified.

3.6.5 THE SAW-CUT TECHNIQUE

This method provided much smaller inocula than others. Small particles of sawdust were obtained by cutting the wood veneers with a sterilised hack-saw blade. The particles were allowed to fall over the surface of petri dishes of CA, MEA, sawdust agar (SDA) and Russell's orthophenyl phenol medium (ROPM). Planting of too many sawdust particles on any one dish of the nutrient media was avoided.

The resultant plates were again divided into three. One set of plates were incubated at 25°C, another at 35°C and the third set at 45°C. The plates were observed daily for the presence of thermophilic, thermotolerant and mesophilic fungi, which were then subcultured and identified.

3.6.6 THE DRILL TECHNIQUE

A sterilised 13mm twist drill was inserted in a standard carpenter's brace. The drill was then introduced into a wood veneer whose surface has been sterilised prior to the experiment by flaming. The wood particles obtained from the holes made by the drill were plated out in the same manner as in the sawdust technique. Particles were taken from the drill by tapping the drill on the base of a sterilised petri dish and subsequently overlaid with agar at a temperature of 45°C. CA, MEA, SDA and ROPM were employed. The resultant culture plates were treated as described in the saw-cut technique.

3.6.7 ISOLATION FROM FUNGAL SPORES

Spore suspensions were prepared from the stained areas of the wood veneers. The spore suspension was then plated out on CA, MEA and SDA.

The culture plates were divided into three portions incubated as previously described and then identified.

3.6.8 THE ISOLATION OF BASIPHILIC FUNGI FROM THE TEST WOOD VENEERS

Plates of SDA with pH value of 8.2 were prepared. These plates were inoculated with sawdust from the wood veneers. The plates were then incubated at 25°C and examined daily for the development of fungal species. The plates were left in the incubator for six weeks in order to allow the slow-growing fungal species to develop. The fungi isolated by this method were subcultured

several times in order to get them into pure culture.

3.6.9 VENEERS pH AND % MOISTURE CONTENT

The pH and percentage moisture contents of the factory exposed wood samples were determined using the same methods described for the soil samples in Chapter 2 of this thesis.

3.7 RESULTS

Eighty species of fungi belonging to forty-nine genera were isolated from the wood veneers and they are presented in Appendix 15a. Table 3 shows the number of thermophilic, thermotolerant and mesophilic fungi isolated from each wood veneer.

More fungi were isolated from the unwashed wood veneers than the washed ones. This shows that some of the fungal isolates from the wood samples, might have been there as surface contaminants. The fungal isolates from the washed samples might be real wood colonisers.

Most of the fungi isolated from the wood veneers have been reported earlier from the Nigerian soil samples and the Nigerian aerial environment. (The details of the fungal isolations from the aerial environment where the soil samples were collected are presented in Appendix 14). It is likely that the fungal colonisers and surface contaminants might have reached the wood veneers in ground contact from the soil and aerial environment. Some of the wood-veneers showed severe fungal colonisation (PLATE 1).

Some of the wood veneers were found to be heavily stained (PLATE 2). The fungal species isolated from the stained areas are given in Appendix 15b.

The results of the other ecological parameters of the wood samples are given in Appendix 15a.

TABLE 3

NUMBER OF THERMOPHILIC, THERMOTOLERANT AND MESOPHILIC FUNGI ISOLATED FROM THE UNWASHED AND WASHED WOOD VENEERS

	NO. OF THERMOPHILIC FUNGI ISOLATED						NO. OF THERMOTOLERANT FUNGI ISOLATED						NO. OF MESOPHILIC FUNGI ISOLATED						TOTALS
UNWASHED	5	7	13	12	13	8	3	6	10	6	9	6	10	21	33	36	32	15	245
WASHED	4	6	12	8	10	6	2	5	9	6	6	5	8	11	21	17	15	8	159
*WOOD VENEERS	1	2	3	4	5	6	1	2	3	4	6	6	1	2	3	4	5	6	

*WOOD VENEERS

Botanical Names	Trade Names
1 = Khaya ivorensis	Mahogany, Lagos
2 = Triplochiton scleroxylon	Obeche
3 = Terminalia superba	Afara
4 = Daniellia ogea	Ogea
5 = Canarium schweinfurthii	Canarium
6 = Sterculia oblonga	Sterculia, Yellow

PLATE 1: A - a wood veneer showing signs of severe fungal colonisation after one month exposure at the factory site - Sapele, Nigeria.

PLATE 2: stains developed on the wood veneers as a result of fungal colonisation (after exposing the wood veneers at the factory for one month).

3.8 DISCUSSION

The results of this study have shown that wood veneers exposed at the factory are vulnerable to infection by stain and other decay fungi. It is assumed that the heat treatment given to wood during veneer production kills the initial fungal colonisers. The fungal species isolated from the wood veneers must have reached the veneers from the soil or aerial environment because most of the fungal isolates from the veneers have already been reported from the Nigerian soil samples and air-spora (details of fungal isolations from the aerial environment where the soil samples were collected are presented in Appendix 14).

Out of the eighty fungal species isolated from the veneers, fifteen are thermophilic, thirteen are thermotolerant and the rest are mesophilic. There were more fungal isolates from the unwashed wood pieces than the washed ones. The fungi from the washed wood pieces could be real wood colonisers capable of causing wood decay, while the rest of the fungi from the wood pieces could have been there as ordinary surface contaminants. There were more fungal isolates from Canarium schweinfurthii, Daniellia ogea and Terminalia superba than the rest of the wood veneers. These results supplement the classification of von Wendorff and Okigbo (1964) in which they placed Canarium schweinfurthii, and Daniellia ogea in their list of perishable Nigerian wood species. However, in their classification, Terminalia superba was listed as non-durable but judging from the extent of fungal attack I think it should be regarded as perishable too. Khaya ivorensis (Lagoswood or African Mahogany) was regarded by them as a durable wood species. This is justified by the fact that this particular timber species had the least number of fungal isolates.

The isolation of similar fungal species from the wood veneers could be due to the fact that the veneers were tightly stacked in the wooden package and there was a possibility of fungal contamination between the veneers. However, there is the possibility that similar fungal species colonised the wood veneers when they were exposed at the factory.

No one technique of fungal isolation will give a complete picture of what is occurring at the site of a biological attack. The picture can be built up piece by piece from the results obtained from a wide range of isolation experiments. The employment of many isolation techniques offered most of the fungal colonisers of the wood veneers the chance of being detected.

The increase in the number and concentration of thermophilic and thermotolerant fungi in the wood veneers exposed at the factory and the ability of most of them (for example, Chaetomium thermophile, Coprinus cinereus, Myceliophthora thermophilum, Myrothecium verrucaria and Sporotrichum pulverulentum) to degrade cellulose suggest that they contribute to the physical and chemical changes in exposed wood veneers. There have been earlier reports of thermophilic, thermotolerant and mesophilic fungi from wood. Shields (1969) isolated Aspergillus fumigatus, Allescheria terrestris, Byssochlamys sp. Humicola lanuginosa, Paecilomyces variotii, Sporotrichum thermophile and most of the mesophilic fungi reported in the present study, during his survey of microflora of Eastern Canadian wood chip piles. Tansey (1971) isolated Chaetomium thermophile var. coprophile, C. thermophile var. dissitum, Humicola grisea var. thermoidea, H. lanuginosa, Sporotrichum thermophile, Talaromyces emersonii, T. thermophilus. Thermoascus aurantiacus

and Aspergillus fumigatus from self-heated wood chips stored at a paper factory. Smith and Ofosu-Asiedu (1972) examined the distribution of fungi in a spruce-pine wood chip pile in Prince George, British Columbia, and concluded that thermophilic and thermotolerant fungi were causing decay of the chips. The same authors in 1973 carried out experiments to determine the decay of three softwoods by thermophilic and thermotolerant fungi. They observed that some thermophilic and thermotolerant fungi can cause the decay of softwood species normally stored in outside chip piles in the British Columbia interior.

Sharp and Levy (1973) studied the ecology of some wood colonising microfungi using a perfusion culturing technique. Their isolates were similar to those of the present study and included Chaetomium thermophile var. coprophile, Humicola grisea var. thermoidea, Malbranchea pulchella, Talaromyces duponti and Torula thermophila. They found that colonisation started with non-cellulolytic species and continued with invasion by fungi having greater cellulolytic capability until either the mesophilic Humicola or thermophilic Torula were present. They explained that the irregularities in the appearance of late colonisers was probably due to a discontinuous distribution in the soil as well as an uneven pattern of penetration through wood.

Morton (1975) found that almost a quarter of the fungi he isolated during his investigation to establish the spectrum of fungal flora of timber coming into Britain via the docks at Preston and Liverpool, were microthermophilic. These species contained cellulolytic and known soft rot organisms. Savory (1967, 1974) reported that newly imported timber is often fungally infected

on arrival into Britain. The extent to which contamination has occurred will depend upon the date of felling, the treatment that the timber receives prior to shipment and the method of shipment employed.

The isolation and identification of basidiomycetes from the wood veneers exposed at the factory was one of the tasks of the present study. The basidiomycete fungal isolates included the thermotolerant fungus, Coprinus cinereus, a Coprinus sp. and a basidiomycete sp. Most of the sterile mycelia observed could be those of basidiomycete sp. The Coprinus sp. has a wide range of occurrence in Nigeria. It is often found growing on dead palm trees and palm products refuse . Probably this fungus takes part in the degradation of these waste palm products.

Warcup and Talbot (1962, 1963, 1965 and 1966) have shown that basidiomycetes are abundant in the soil, but few of the species recorded have been associated with the decay of wood.

Butcher (1968) isolated five basidiomycetes from stakes. In his study, it appeared that the primary basidiomycete colonisers of wood in contact with the soil were not usually those fungi first to be isolated from fallen branches, logging slash, or timber stored above ground.

Carruthers and Rayner (1979) surveyed the fungal communities in decaying hardwood branches. They found Phlebia merismoides and Hypholoma fasciculare to be the most important species in terms of frequencies and volume occupied in fungal communities in oak and ash wood. There was a clear relationship between degree of exposure of the substrate and the fungal species present. Aerially exposed wood was occupied predominantly by species which probably colonise by spores while wood on ground contact more usually contained species colonising by mycelium. They suggested that the fungal community pattern in wood-piles develops initially as a result of difference in the type of inoculum available in each part of the pile. The initial pattern may be stable for a period of time, but eventually more vigorous fungi, replace others leading to a situation in which the majority of the wood is occupied by only one or two species.

Fungal colonisation of standing trees has been studied by Liese (1975). He pointed out that micro-organisms that inhabit wood in living trees have the greatest survival advantage when they attack wounds in a sequential manner. Each invading micro-organisms exerts its specific force against the dynamic protective barriers formed by the wounded tree. The pioneer micro-organism first alter the substrate to their advantage and then digest the cell contents. As the pioneers advance, the substrate is altered further to the advantage of other organisms that follow a succession.

The West African reports of fungal colonisation of wood include those of Fougerousse (1975) and Ofosu-Asiedu (1975). Fougerousse in his investigation of the soft rot fungi occurring in Field Tests in Ivory Coast isolated eleven soft rot fungi from seventy-five-stakes. All his isolates were found to belong to the family Moniliales (Hyphomycetes) except Allescheria boydii which is an ascomycete of the family Eurotiales. Ofosu-Asiedu in his survey to collect and identify wood decay fungi from the forest, sawmills, wooden structures and on billets found that most of the common wood decay fungi he collected belonged to the family Polyporaceae. Most notable among his isolates were Coriolopsis polyzona, Pycnoporus sanguineus, Trametes cingulata, Trametes scabrosa and Schizophyllum commune. He reported that these fungi had a wide host range and to be evenly distributed throughout the vegetational zones of Ghana. On this basis he concluded that Coriolopsis polyzona, Schizophyllum commune, Trametes cingulata, Pycnoporus sanguineus and Gloeophyllum striatum could be selected and used for the evaluation of natural durability of Ghanaian lesser known timber through accelerated tests and for the evaluation of preservatives.

Terminalia superba was found to have 23% moisture content while Canarium schweinfurthii and Daniellia ogea were found to have 22% moisture content each. The percentage moisture content recorded for these wood veneers were within the range that would support the growth of decay fungi. There were more fungal isolates from these veneers than the rest of the wood veneers. The 11%, 15% and 18% moisture content recorded for Khaya ivorensis, Sterculia oblonga and Triplochiton scleroxylon respectively might not have been high enough to support the growth of many fungal species.

The build up in the moisture content of some of the wood veneers might have been due to their exposure at the factory.

Fungal staining was one of the commonest forms of attack observed on the experimental wood veneers. The stains were either blue, dark, red or yellow in colour. Many fungal species were isolated from the stained areas of the wood. The staining might have been as a result of the proliferation of hyphae of dark coloured microfungi through the interstices and-cell walls of the wood. Gadd (1965) and Norkrans (1967) have suggested that deep or surface staining of wood could be as a result of the colonisation of wood by fungi with either hyaline or dematiaceous hyphae, by whose metabolism the sap of green wood is discoloured due to iron reactions. Norkrans (1967) suggested that wood staining could be as a result of the production of pigmented spores and hyaline hyphae by fungal species colonising wood surface. Turner (1976) explained that tropical hardwoods rich in sugars and other soluble solids support the growth throughout the wood of the black-pigmented Botryodiplodia theobromae. He suggested that the red staining of African hardwoods may arise from infection by species of Fusarium and Cylindrocarpon. Fusarium was among the genera of fungi isolated during the present survey. The red stain observed on the wood veneers might have been caused by the Monilia sp. isolated from the veneers or by Oidiodendron rhodogenum. The Monilia sp. was found to produce pink conidia while Oidiodendron rhodogenum was observed to produce red pigment which diffused into the culture agar medium. Turner (1967) also mentioned that yellow stain on oak is caused by Paecilomyces variotii one of the isolates in the present study. He also reported that Cladosporium herbarum and Aureobasidium pullulans could be responsible for the blue stain.

These fungi he explained, have been found to contribute to sapstaining in Poland.

Karkanis (1966) showed that the initial colonisation of wood by blue-staining fungi occurred in ray tissue. He considered blue-staining fungi to be intermediate between mould fungi which apparently only colonise the surface of wood and do not cause decay, and the Basidiomycetes, which penetrate deeply into wood, and cause serious decay. However, such consideration based on wood penetration is unacceptable because many fungal species like Trichoderma sp. and Gliocladium sp. penetrate deeply into wood (Butcher 1968; Hulme and Shields 1970). Liese and Schmid (1964) Liese (1965) and Liese and Schmid (1966) have shown that the hyphae of the blue-staining fungi, Aureobasidium pullulans, Ceratocystis piceae, Ceratocystis pilifera and Phialocephala phycomyces, all exhibit transpressoria, by means of which lignified cell walls are penetrated. Butcher (1968) observed that the blue-staining fungal penetration from cell to cell lay through the bordered pits or through bore holes produced by mechanical pressure.

pH is one environmental factor that could affect the growth of fungi significantly. The pH values of the wood veneers (6.1 - 6.4) were within ranges that could support the growth of fungal species in pure cultures. Wolpert (1924) and Edgecombe (1941) have emphasised the importance of pH in the growth of fungi. They discovered that acidic levels favoured Basidiomycete growth while alkalinity caused inhibition.

Siu and Sinden (1951) found Aspergillus flavipes, Curvularia lunata and Myrothecium verrucaria (also isolated in the present study) to have a pH optimum between 6 and 7; whereas Gliomastix convoluta was able to tolerate a hydroxyl ion concentration at pH 11 Duncan (1960) showed 40% of thirty-two isolates to fail at pH 3, 50% to grow at pH 7, 25% at 8 and for only a few to survive at pH 9. The optima occurred at pH 6. Henningsson (1967) found that the optimal growth of basidiomycetes occurred at pH 5 to 6 during his study of pH tolerance by basidiomycetes using birch and aspen. Sharp and Eggins (1970) investigated the effects of pH on soft rot mircrofungi. They found that most of their fungal isolates favoured acidic conditions with optimal frequency of isolation and cellulolytic activity tending to between pH 6 and 7.

Bacteria were among the micro-organisims isolated from the Nigerian wood veneers. They have been known to play an active part in the colonisation of wood by fungi. Seidler, Aho, Raju and Evans, (1972) and Sharp And Millbank, (1973) have reported the presence of nitrogen fixing activity in wood. They suggested that the nitrogen fixing organisms could be of significance in decaying timber as they may provide the fungi concerned with nitrogen. Baines and Millbank (1975) investigated the penetration of nitrogen fixing bacteria into partially sealed oriented blocks of sapwood of Scots pine and beech in ground contact by the acetylene reduction technique. The results indicated that bacteria primarily penetrated along the rays in Scots pine but do not penetrate into beech. Levy (1975) in his preliminary experiments on bacteria associated with wood in ground contact discovered that under the conditions of his experiment, the ray parenchyma pit membranes were destroyed first and subsequently the membranes of the bordered pits. He found

that bacterial isolates from wood in soil contact proved capable of increasing the permeability of spruce sapwood.

The results have shown that the biodeterioration of wood in the form of discolouration occurs in wood veneers stored at the factory. The isolation of fungi identical to those reported in the temperate regions means that the preservative treatments employed in these regions will also work in tropical Nigeria. The use of preservative treatments to inhibit biodeterioration should be considered as soon as the veneers are produced in the factory.

CHAPTER 4

WOOD DECAY STUDIES

4.1 INTRODUCTION

Many methods of determining fungal decomposition of wood have been employed in wood technology research. A lot of factors may influence the choice of a particular method or how an existing method is to be modified to fulfil the aims of a study. In certain cases the optimal rate of wood attack is aimed at and certain extra nutrients are therefore introduced into the wood. In some other cases, the progressive changes in the chemical composition of the wood during decay are to be studied and foreign substances are consequently not added to the wood. In some studies, heat sterilisation of wood is not needed, more especially if the wood has been impregnated with heat-unstable preservative. In such cases wood sterilisation by means of an inert gas or non-destructive radiation is then preferred, provided the preservative or the wood substance is not affected and there are no secondary effects on the fungus either.

A number of national and international standard methods have been established in the standardisation of laboratory methods for determining fungal decomposition of wood. These standard methods include ASTMD 1413 (American Society for Testing and Materials 1956), BS 838 (British Standards Institution), DIN 52176 (Deuscher Normenausschuss 1939), and NWPC 1.4.1.1/70 (Nordic Wood Preservation Council 1970).

Loss of dry weight is widely used as a convenient measure of wood decay, although, as it is well known, its relationship to other changes may be inconsistent (Pechman and Shaile, 1950; Richards, 1954). This method was therefore employed in the present study but

it was supported by direct measurement of change in bending strength.

4.2 MATERIALS AND METHODS

4.2.1 CHOICE OF WOOD

Canarium schweinfurthii (other names: Abel-Cameroons, Aiele - French West Africa, Mwafu-Uganda, Elemi - Nigeria) was chosen for the soil burial studies because it is a tree native to Nigeria and other West African countries.

(i) Description

The genus Canarium is widely distributed throughout all the tropical regions of the world except South America. As far as it is known, this is the species of Canarium that is of economic importance in the tropics.

(ii) The Timber

It is moderately hard, with a broad sapwood and light pinkish yellow heartwood. Its interlocked grain gives a decorative broad stripe when quarter-sawn, giving it a slightly coarse texture. It is strong, with even grain and good texture. It also seasons well. It is easy to saw especially with vanadium-tipped saws. Although it can be readily peeled and planed, it blunts veneer knives if insufficietnly steamed or boiled (AT & P, Sapele).

(iii) Resistance to Insect Attack

It is susceptible to damage by Ambrosia (pinhole borer) beetles, Lyctus africanum and Minthea rugicollis (dry wood insect attack, Ifebueme, 1979). The sapwood is liable to attack by powder-post beetles (Bostrychidae). It is classified in Nigeria as non-resistant to termites.

(iv) Natural Durability

It is perishable (von Wendorff and Okigbo, 1964).

(v) Mechanical Properties

The timber has an approximate density of 477.03 kg/m^3 and is slightly weak in bending. It is rather less tough and has an appreciably lower shock resistance. It possesses equal hardness and resistance to splitting. It has slightly greater stiffness, resistance to compression and shear strength (AT & P, Sapele - Nigeria).

(vi) Uses

Its light coloured timber makes an agreeable match with mahoganies for parquetry, interior decoration, and marquetry. It stains and polishes well. It can be used as a substitute for mahogany in flooring, interior decoration, doors, joinery, furniture, etc. It is available in logs of 66.04cm to 127.00cm diameter (von Wendorff and Okigbo, 1964).

4.2.2 PREPARATION OF THE CANARIUM WOOD VENEER FOR DECAY EXPERIMENTS

The Canarium wood veneer supplied by AT & P Nig. Limited was converted to yield test pieces measuring 6.50cm x 1.0cm x 0.50cm (length x width x thickness) with grain running parallel to the long axis (Figure 7).

4.2.3 THE EFFECTS OF THE EARLY FUNGAL COLONISERS ON THE WOOD VENEER

Sixty pieces of the test pieces were tied up in six sets of ten pieces each. These sets of wood blocks were then incubated in a jar of 75% relative humidity (average humidity in Nigeria for twelve months) (Figure 8) at a temperature of 30^{o}C (an ideal temperature in Nigeria). The effects of the fungi which have colonised the wood at the factory (Plate 3) were investigated by measuring the reduction in both the bending strength and wood weight caused by them every month, for six months. The details of bending strength and weight losses are given later in this section.

4.2.4 THE EFFECTS OF SOIL STORAGE ON SOIL FUNGI

Before the Nigerian soil samples were used for the wood burial studies, the effects of soil storage on the soil microfungi were investigated. This was done by plating out the Nigerian soil samples as was done in the first section of this thesis. The effects of soil physio-chemical features were equally assessed.

4.2.5 THE WOOD BURIAL EXPERIMENTS

(i) Ten sterilised test pieces were buried in each of the monthly

Fig. 7 **A Test Wood Piece (measurements)**

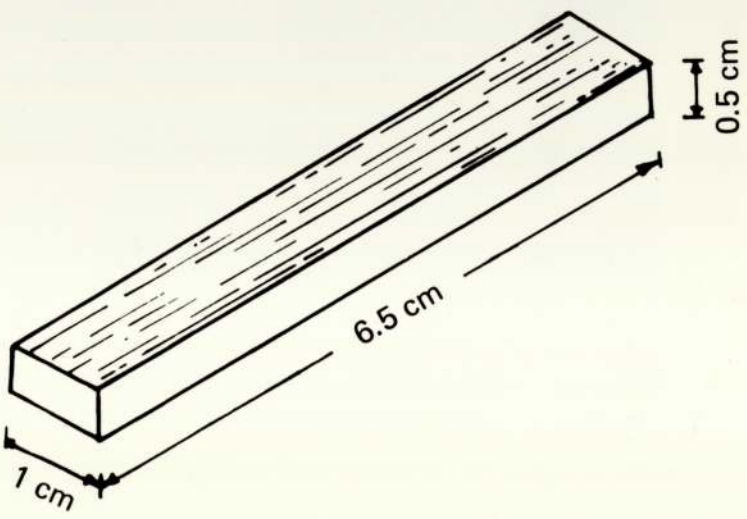
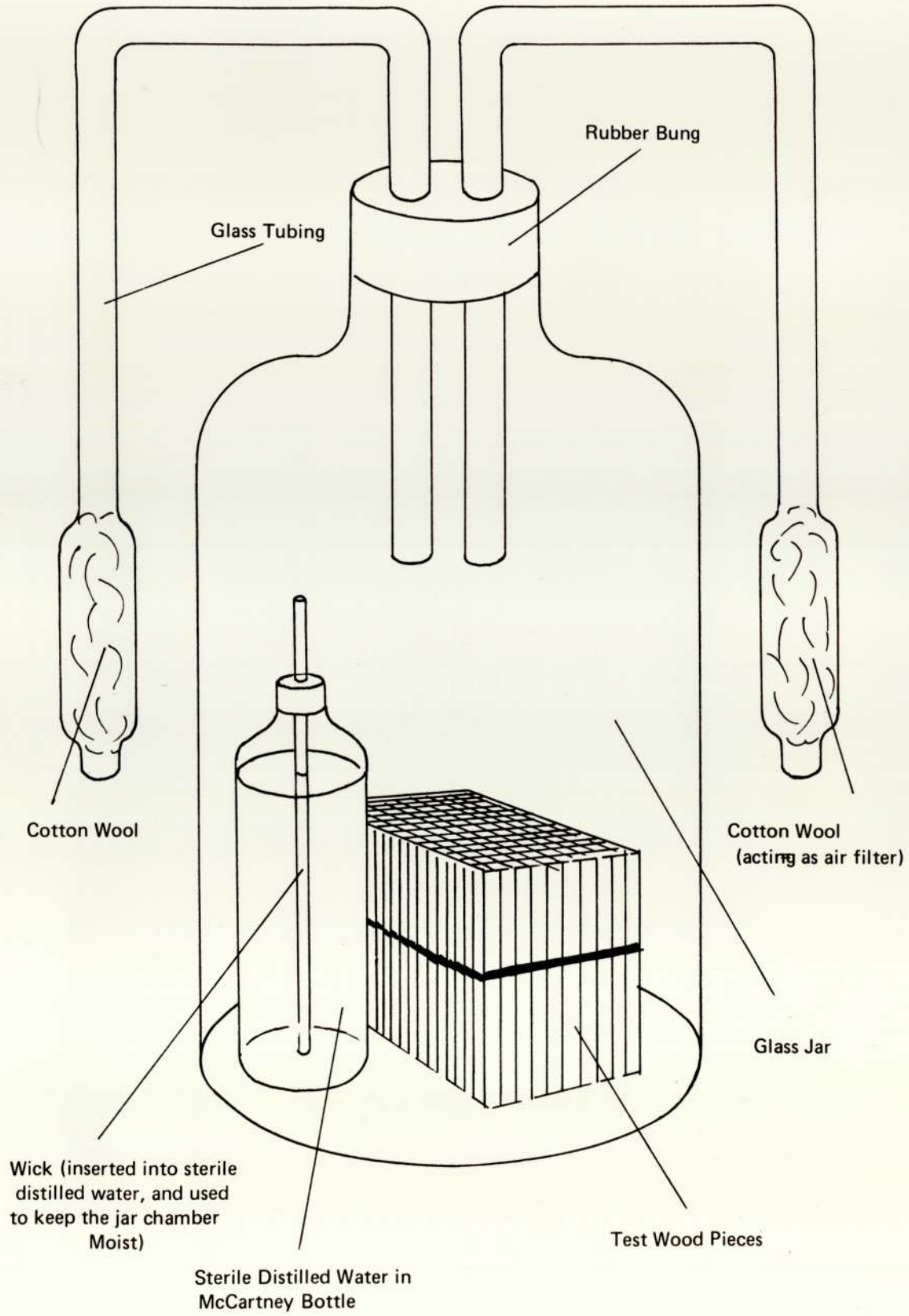


Fig. 8


Rubber Bung
Glass Tubing
Cotton Wool
Cotton Wool
(acting as air filter)
Glass Jar
Wick (inserted into sterile distilled water, and used to keep the jar chamber Moist)
Sterile Distilled Water in McCartney Bottle
Test Wood Pieces

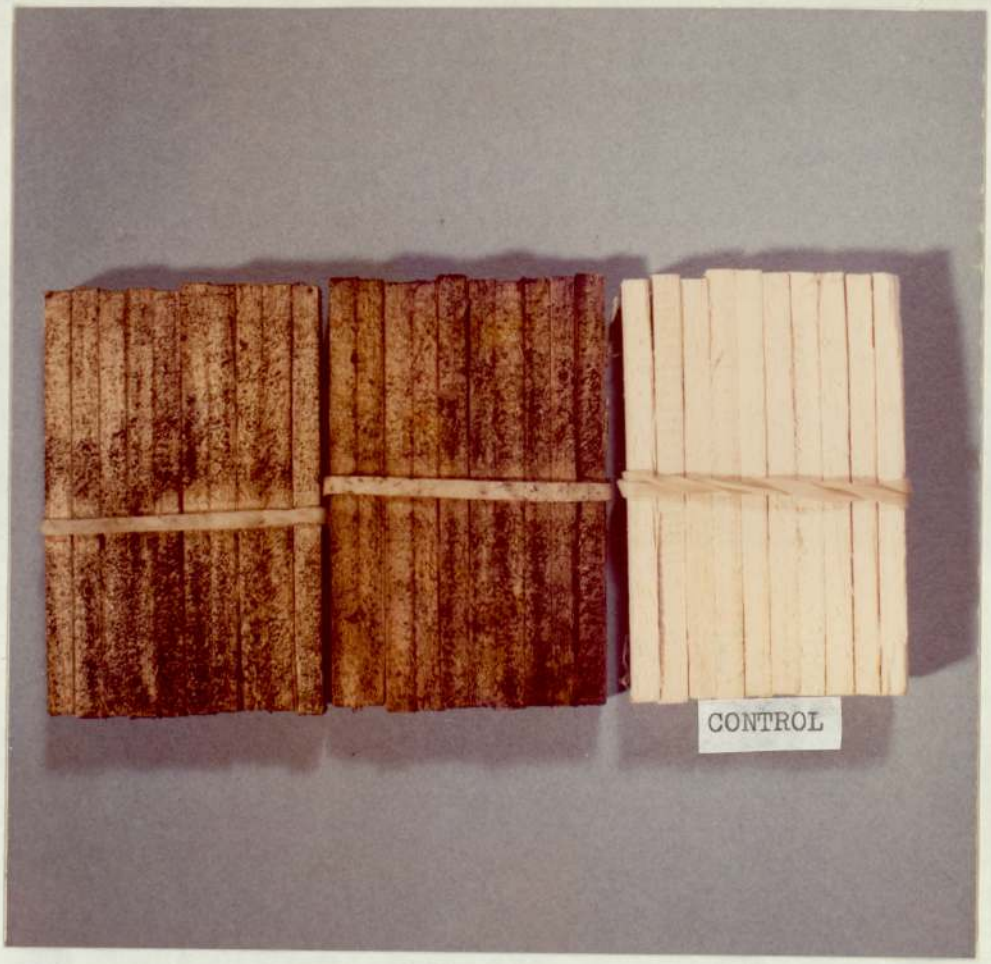


PLATE 3: test wood pieces prepared from the factory exposed wood veneers and incubated in a jar at 30°C.

soil samples. This was done in order to find out if there is any correlation between the fungal species isolated from the monthly soil samples and wood decomposition by the microfungi. Each of the monthly soil samples was sifted through a 5mm gauge sieve before it was used for the burial studies. Heat-resistant glass jars with a capacity of 1,300ml. were employed for all the soil burial experiments. The jars were filled with lightly packed soil samples to just over half their capacities. The soil moisture content of each jar was raised to 80% of its water holding capacity prior to the introduction of the wood pieces. The jars were then fitted with cotton plugs and incubated at $30^{o}C$ for six months, after which the wood test pieces were removed and the reductions in bending strength and weight recorded. The jars were kept under observation during the incubation period with sterile water being added to the burial soil samples at intervals in order to avoid the drying out of the soil samples.

(ii) Sixty of the test pieces (fungal infected at the factory) were buried in non-sterile mixed and sifted Nigerian soil samples (the twelve monthly soil samples). Equal weights of the monthly soil samples were taken.

(iii) Sixty fungal infected wood pieces were buried in sterilised mixed and sifted Nigerian soil samples.

(iv) Sixty sterilised wood pieces were buried in non-sterile mixed and sifted Nigerian soil samples.

(v) Sixty sterilised wood pieces were buried in sterilised mixed

and sifted Nigerian soil samples and inoculated with selected fungi (reasons for the selection of the test fungi are given in Chapter 5 of this thesis).

In this experiment, spores of the selected fungi were introduced into the sterilised soil sample mixture. The spores were mixed with the soil aseptically in the burial jar prior to the introduction of the wood pieces.

Spore suspensions from fourteen days old fungal cultures were used for the inoculation. Sterile distilled water (10ml.) was added to each of the culture dishes and the surface of the fungus culture was carefully scraped and squeezed with a sterile glass rod. The resultant spore suspension was transferred under sterile conditions to a flask containing 100-200ml. of sterile distilled water to give the final spore suspension. The jar was inoculated with 2ml. of the final spore suspension of each of the selected fungi making use of sterile pipettes. Fungal re-isolations were made at the end of the experiment to make sure that the test fungi were still present in the soil and that there has not been other fungal contaminants.

(vi) Sixty sterilised wood pieces were buried in sterilised mixed and sifted soil samples. These pieces acted as the control experiment and helped to correct for changes in the bending strength and weight that were not due to fungal attack.

(vii) Sixty sterilised wood pieces were pressure creosoted according to British Standards Institution test (BS 913: 1973) and buried in non-sterile soil.

The burial soil in each jar was moistened to 80% of its water holding capacity (w.h.c.) prior to the introduction of the wood pieces. All the jars were incubated at 30^{0}C and observed periodically, with sterile distilled water being added to avoid drying out of the soil.

4.2.6 DECAY CRITERIA

The criteria used to assess wood decay in the present investigation were loss in bending strength and loss in weight.

(i) Bending Strength Determinations

The method used for measuring bending strength in this investigation has evolved from the nail-head pull-through principle of Merrill and French (1964). Similar methods of strength assessment have been used by Liese and Pechmann (1959) on birchwood, Sharp (1970) on veneers and Hardie (1979) on pine, lime and beech.

A Monsanto tensometer with specially designed 3-point loading jaws (Plates 4a, 4b &4c)was used for breaking the test pieces. Bending strength was measured under an evenly increasing strain (jaw separation of 4.17×10^{-2} cm/s) to breaking point. The tensometer was used in a room maintained at 80% relative humidity.

The test wood pieces were saturated with steam before bending (this was done in order to bring the test pieces to the same condition). Complete saturation of the test pieces was determined when the samples sank to the bottom of the water in the steaming

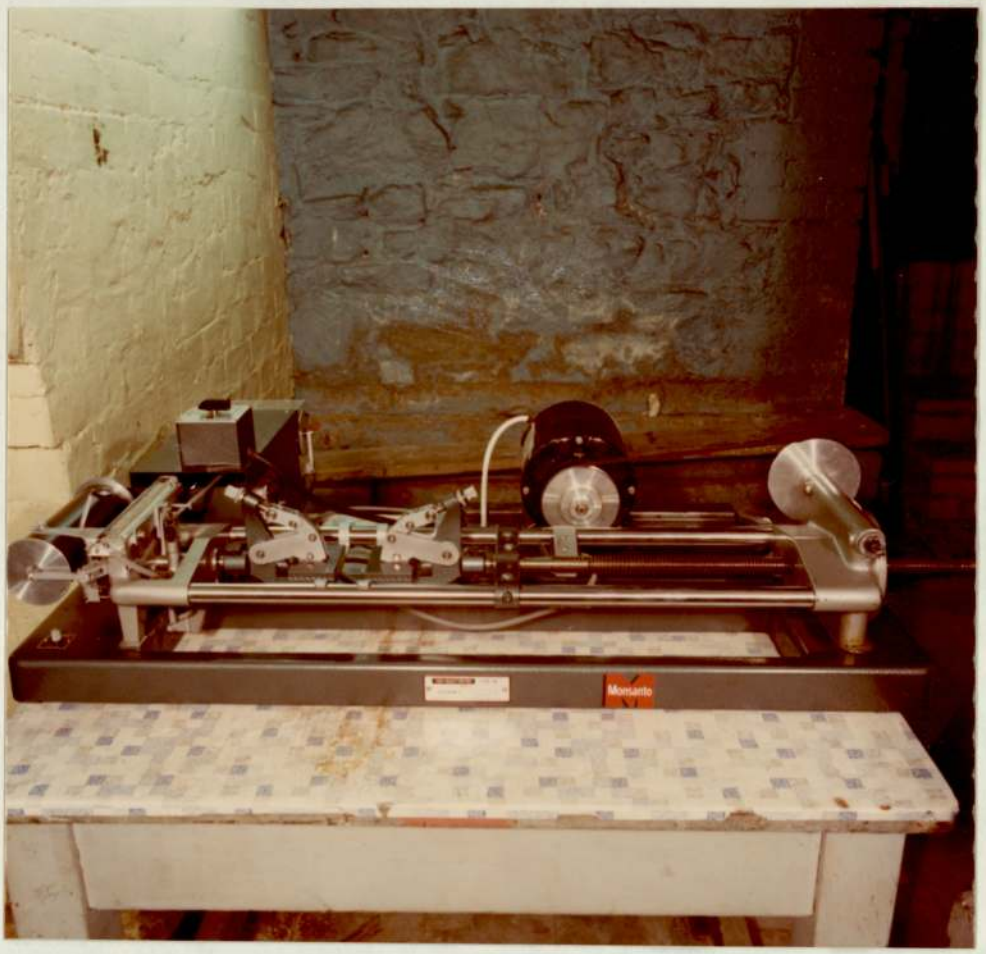


PLATE 4A: A Monsanto tensometer employed for the wood bending strength tests.

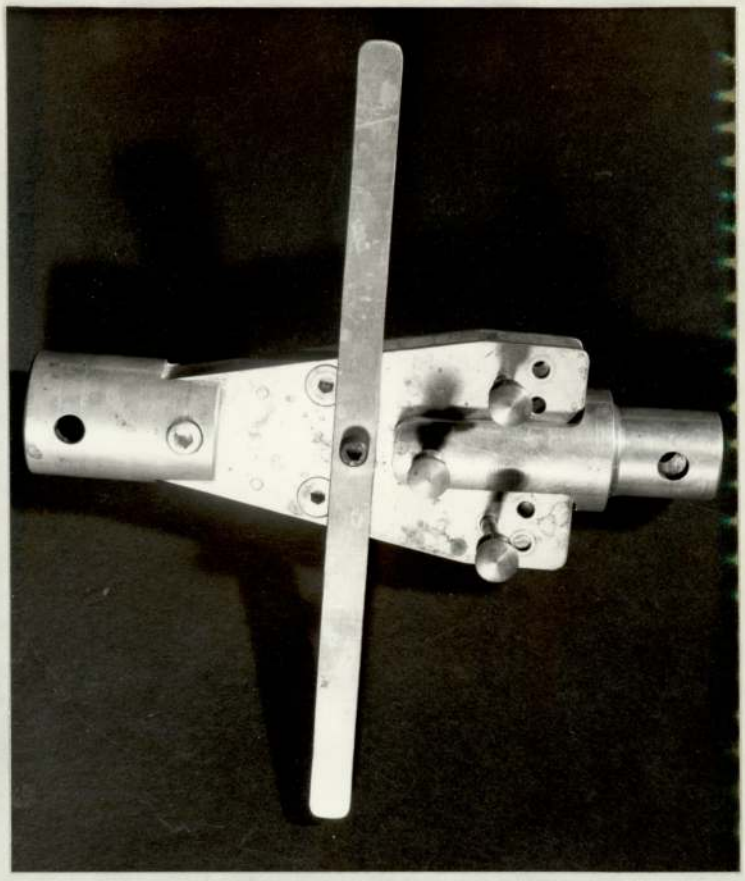


PLATE 4B: Bending strength jaws for use with the tensometer.

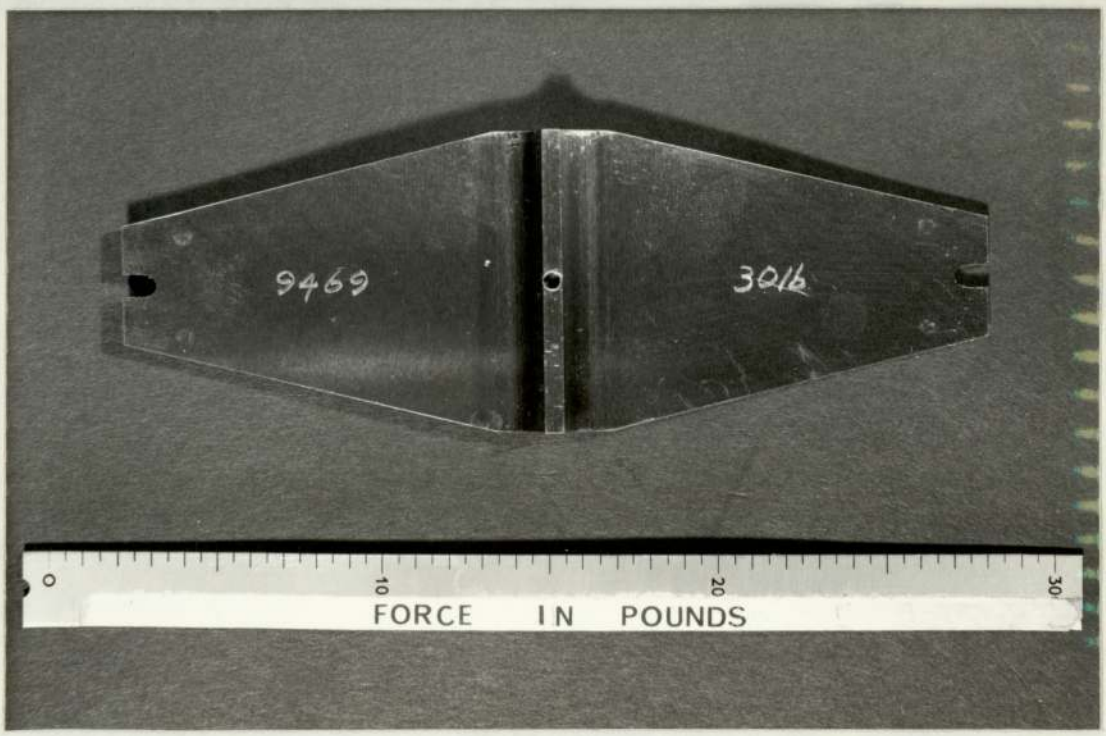


PLATE 4C: The tensometer beam employed for the bending strength tests.

chamber instead of floating on top of it.

Percentage Bending Strength Loss

This was calculated by finding the mean bending strength of ten freshly cut test pieces. This mean was regarded as the standard. The difference between this standard and those subsequently measured each month was regarded as due to fungal decay of the test pieces.

Calculation

If the standard wood bending strength is M (mean of ten test pieces). If the mean bending strength of ten test pieces broken at the end of a month is N.

$$\therefore \%\text{ age bending strength loss} = \frac{M - N}{M} \times \frac{100}{1}$$

(ii) Weight Loss Determinations

Percentage Weight Loss

The ten freshly cut out and bent test pieces used in the standard bending strength determination were dried to constant weight and their mean dry weight taken. Weight loss of the buried or non-buried wood blocks due to fungal decay was regarded as the difference between this initial mean dry weight and the mean dry weight of each batch of ten decayed test pieces.

Calculation

If the original mean dry weight of the test pieces = X. If the mean dry weight of the decayed wood blocks = Y. Weight loss due to fungal decay = X - Y

$$\% \text{ age weight loss due to fungal decay} = \frac{X - Y}{X} \times \frac{100}{1}$$

(iii) The Buried Wood Pieces

Ten wood pieces were removed from each jar, each month and the effects of the microfungi on their strength tested. The samples were then dried to constant weight at 105°C in order to determine the losses in weight of the wood pieces caused by the microfungi. The adherent mycelium and soil were carefully brushed off before drying, making sure that no wood particles were lost in the process.

4.2.7 SOIL STERILISATION

The jars containing the soil samples to be sterilised were plugged with cotton wool and then covered with aluminium foil. The soil samples were then sterilised by autoclaving them for one hour at approximately 120°C. The jars were then left until the next day to allow any living heat-resistant spores in the soil to germinate. The soil samples were then autoclaved for a second time for one hour at 120°C. This second autoclaving sterilised the soil samples and killed any germinated, heat-resistant spores remaining in the soil. After cooling the wood blocks (sterilised or unsterilised depending on the wood burial investigation) were

introduced into the sterilised soil, vertically, the soil covering the entire wood except the areas of the wood blocks containing the wood numbers. The areas of the wood pieces carrying the numbers faced the open end of the jar. The whole manipulation was carried out aseptically.

4.2.8 STERILISATION OF THE TEST WOOD PIECES

The wood blocks to be sterilsed were heated to 80^{o}C and cooled to -18^{o}C. This procedure was repeated seven times. Preliminary experiments revealed that such treatments killed off the initial fungal flora of the wood samples.

4.3 RESULTS

THE EFFECTS OF SOIL STORAGE ON THE MICROBIAL POPULATION OF THE SOIL SAMPLES

There was no major change in the microbial population of the experimental Nigerian soil samples as a result of their storage in the refrigerator. All the microbes reported from them earlier, were found to be present. However, there was an increase in the frequencies of occurrence of Penicillium argillaceum Stolk et al. and Thermomyces stellatus Apinis. There were no changes in the soil physio-chemical features.

SOIL BURIAL STUDIES

The appearance of the decayed wood pieces

The surfaces of the decayed wood veneers were completely discoloured, very soft and looked completely eroded. They could easily be squeezed with the hands, thus depicting serious soft rot attack. When dry, the rotted surfaces were crumbly and showed many fine cracks and fissures. Some of the decayed wood pieces shrunk abnormally when dried, showed a characteristic brown colour, depicting brown rot. Most of the sixth month buried wood pieces looked completely charred, also depicting brown rot attack. Some of the decayed wood pieces had a bleached appearance with "zone lines", were soft and linty and showed normal shrinkage properties thus depicting white rot. Plate 5 shows Coprinus sp. growing out from the jar inoculated with test fungi while Plate 6 shows the detailed appearance of the decayed wood pieces when compared with

PLATE 5: Coprinus sp growing out from the soil burial jar inoculated with the test fungi.

PLATE 6: shows the stages in the fungal decay of test wood pieces.

KEY: 0 = Non-decayed wood pieces

1 = What the wood pieces looked like after one month burial in the soil samples

2 = What the test pieces looked like after 2 months burial

3 = What the test pieces looked like after 3 months burial

4 = What the test pieces looked like after 4 months burial

5 = What the test pieces looked like after 5 months burial

6 = What the test pieces looked like after 6 months burial

* The fungus at the middle is an early stage of development of a Coprinus sp found growing in the jar inoculated with test fungal species.

the non-decayed ones.

Strength and weight reductions of the decayed wood pieces

The bending strength and weight losses of the decayed wood pieces are presented graphically in Figure 9. The non-sterile wood pieces buried in non-sterile Nigerian soil were found to have had the highest strength and weight reductions. They were followed by the sterilised wood pieces buried in non-sterile soil and then the non-sterile wood pieces buried in sterile soil. Comparatively, the least reductions were recorded for the sterile wood pieces buried in sterile soil and inoculated with selected fungi.

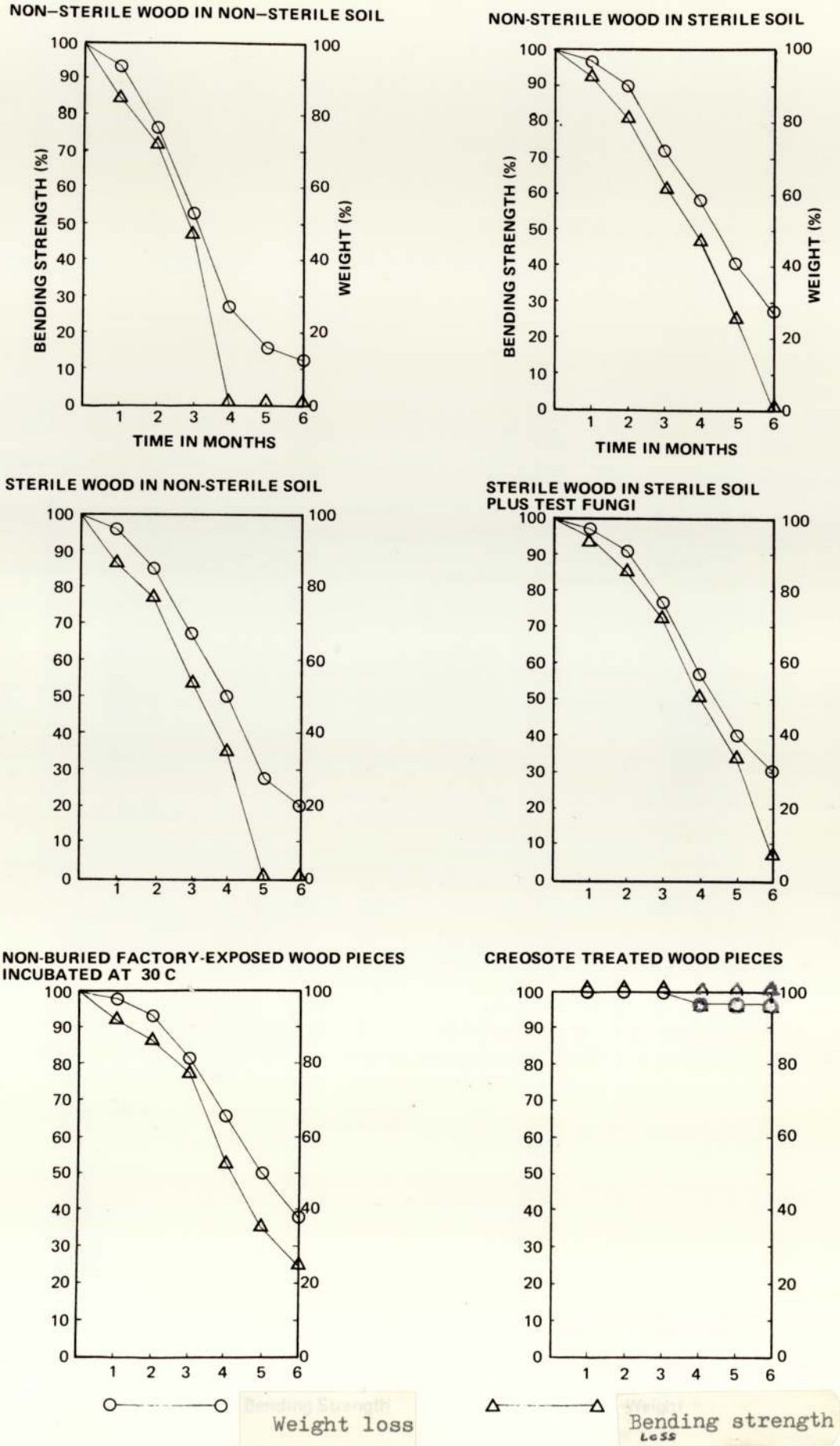
Microscopic appearance of the decayed wood pieces

The decayed wood pieces showed an intensive soft rot cavity formation on the cell walls when sections cut from the decayed wood pieces were observed under the microscope.

Permeability of the decayed wood pieces

The decayed wood pieces were found to be more permeable and more absorptive than comparable sound wood pieces. When introduced into water in a beaker the decayed wood pieces sank to the bottom of the beaker quicker than the sound wood pieces indicating increased permeability in the decayed pieces. The wood pieces were dried to constant weights prior to wetting.

Fig.9 Bending strength and weight losses caused by the Nigerian Fungi.



The monthly soil samples

For the monthly soil samples, the highest bending strength and weight reductions were recorded for the wood pieces buried in the dry season soil samples. The least were recorded for the wood pieces buried in the wet season soil samples. The results followed the same trend as was observed for the fungal isolations, soil samples with a greater number of thermophilic fungi giving greater wood decay than soil samples with a lesser number of thermophilic fungi. The detailed result is presented in the form of a histogram (Figure 10).

The non-buried factory exposed wood pieces

The non-buried wood pieces cut out from the factory exposed wood veneers and then incubated at $30^{o}C$ did not undergo drastic reductions in bending strength and weight like the buried wood pieces. However, they were found to have suffered from bending strength and weight reductions too. Many of them showed signs of soft rot. The detailed results are presented in Figure 9.

The creosoted wood pieces

There were no bending strength reductions for the creosoted wood pieces throughout the duration of the soil burial experiments. There were slight weight reductions for the creosoted wood pieces (Figure 9).

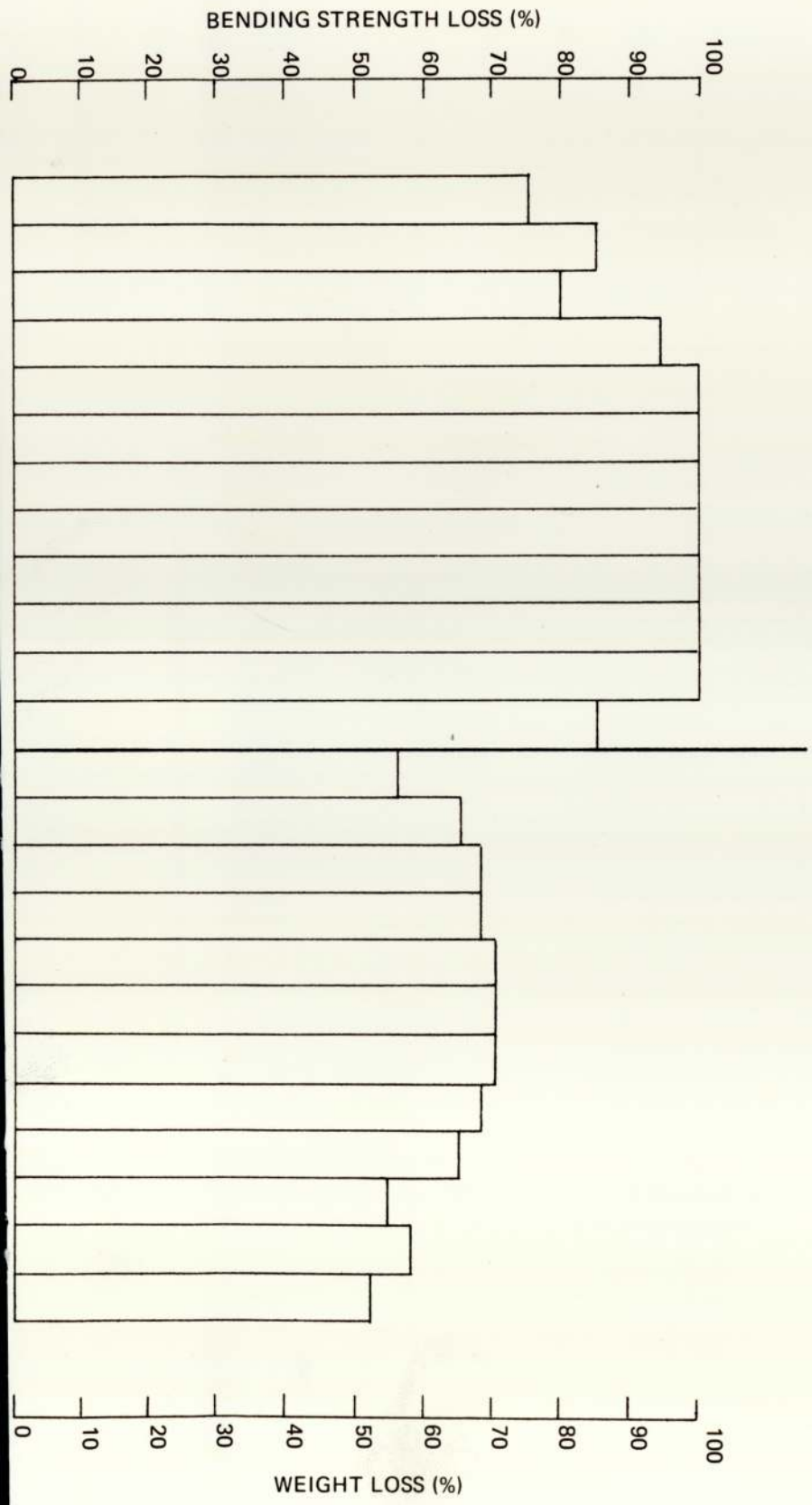


Fig.10 The Effects of the Monthly Soil Samples on the Bending Strength and Weight of the Wood Pieces after Six Months

Differences in alkaline solubility

The wood pieces which were suspected to have undergone either white or brown rot were introduced into 1% aqueous sodium hydroxide. The solubility of the white rotted wood suspects were slightly greater than that of sound wood. Brown rotted wood suspects however, were much more soluble than sound wood. Cowling (1961) reported that the differences in the solubility of white and brown rotted wood in 1% aqueous sodium hydroxide provide a reliable chemical means for distinguishing the two types of decay. He reported that the solubility of white rotted wood is only slightly greater than that of sound wood, and brown rotted wood has a greater solubility than sound wood.

4.4 DISCUSSION

The results of the soil burial studies have shown that wood in ground contact is liable to being decayed by soil fungi. The level of attack achieved for the non-sterile wood pieces buried in the non-sterile soil might have resulted from the action of micro-organisms in the soil and wood. The organisms likely to have been active in this particular case include bacteria, Basidiomycetes, cellulolytic and ligninolytic microfungi.

The sterilised wood pieces buried in the non-sterile soil were found to have undergone greater decay than the non-sterilised wood pieces buried in sterile soil. The difference might have resulted from the more and varied micro-organisms present in the soil than in the wood pieces as was observed during the isolation studies. A combined aggressive action by the varied micro-organisms in the non-sterile soil might have led to the greater decay rate observed in the sterile wood pieces in non-sterile soil. Secondly, the sterilisation of the soil by heat might have led to the evaporation of some volatile substances in the soil thus leading to the alteration of the soil.

Lawrence (1956) reported that the amount of each of a number of water soluble chemical compounds in the soil is made soluble by heat in larger amounts than others hence nutrient unbalance commonly ensues. The degree of chemical unbalance ranges from small to very large, depending on the nature of the soil and the amount of heat applied. Crumb structure (tilth), aeration and drainage are all improved. These factors may have contributed to the lower decay rate observed in the non-sterilised wood

pieces buried in sterile soil as compared to the sterilised wood pieces buried in non-sterile soil.

The lower decay rate observed for the non-buried factory exposed wood pieces could have been due to the wood pieces being non-buried, having been removed from the soil mineral nutrients which usually aid microbial growth. Baines and Millbank (1975) have shown that nitrogen fixation takes place in wood in ground contact. The non-buried pieces were therefore removed from this advantage. However, the exclusion of some other soil ecological factors from these wood pieces must have contributed to the reduction in the extent of wood decay observed - as compared to the buried pieces.

The increase in the absorptive rate noticed in the decayed wood pieces might have been as the result of openings and enlargements in its microstructure by the invading fungi as they moved from cell to cell. Pit membranes might have been enlarged through the removal of portions of the pit membrane and surrounding substances. In addition, cells comprising the wood rays must have been destroyed, thus promoting radial flow of liquids into the wood. As decay progressed, wood substances might have been removed which correspondingly enlarged or even created void spaces and thereby increased the liquid holding capacity of the wood.

There was a good correlation between bending strength and weight losses. Hartley (1958) reviewed the scattered literature on strength properties of wood decayed by white and brown rotted fungi. He concluded that at comparable stages of decay, as measured

by weight loss, brown rot fungi reduce the strength of wood more than do white rot fungi. Kennedy (1958) confirmed the above generalisation. He demonstrated a very close correlation between the extent of strength reduction and the solubility of several wood species decayed by Poria monticola Murr. in 1% sodium hydroxide.

The close relationship between loss in bending strength and loss in weight due to fungal decay of wood has been demonstrated by (Armstrong and Savory, 1959; Abou Heilah and Hutchinson, 1977 and Hardie, 1979).

The bending strength of the test wood pieces reduced gradually but diminished considerably before major signs of decay became detectable by loss of weight. The reduction of the bending strength of the non-sterile wood pieces buried in non-sterile soil and sterile wood pieces buried in non-sterile soil to zero within six months showed the aggressive nature of the decaying fungi involved.

The strength and weight losses observed from the decay experiments are likely to have resulted from enzymatic degradation of cellulose and lignin. Henningsson (1967) suggested that early reductions in impact bending strength may be caused by the splitting of linkages between lignin and carbohydrates.

The disadvantage in the use of weight loss as a criterion for determining wood decay is that a long incubation period is needed to attain suitable weight losses and corrections must be made to allow for weight changes caused by other factors, such as uptake of mineral salts from the test medium. The involvement

of another factor, bending strength loss, eliminated the need for weight corrections but introduced the need for particular care in the selection of samples and for the use of expensive testing equipment, "The Monsanto tensometer".

The buried wood pieces were observed to show strong signs of soft rot attack. Savory (1954) reported that soft rot of woods is caused by certain species of Ascomycetes and Fungi Imperfecti, with the fungal hyphae typically running spirally in the central zone of the secondary xylem walls, where they give rise to cavities with pointed ends. He also reported that the fungi which cause soft rot are cellulose attacking species which initiate attack in the less heavily lignified parts of the walls of the wood elements. He observed that the rate of attack is increased when inorganic nutrient salts are added to the wood.

Duncan (1960) observed that soft rot fungi are more prevalent in hardwoods than softwoods.

The signs of brown rot shown by the decayed wood pieces might have been due to fungal enzymes which destroyed the cellulose and left the lignin largely unaltered so that the wood acquired a distinct brown colour with its structural strength almost lost as explained by Richardson (1978). He reported that as decay progresses the wood becomes very dry and shrinkage cracks appear both across and along the grain, the size and shape of the resulting triangles often being useful feature in identification. Kirk(1973) reported that in brown rot, fungi depolymerize and metabolize the cellulose and hemicelluloses. They also alter the lignin. Thus brown rot involves progressive removal of carbohydrates, leaving

lignin behind.

In white rot as was observed from some of the decayed wood pieces it is believed that both the cellulose and lignin are destroyed leaving the wood largely unaltered but giving a soft felty or stringy texture. Kirk (1973) reported that white rot fungi degrade and metabolise all the structural polymers in wood - the cellulose, the hemicelluloses, and lignin. The components remaining in wood at any stage of decay are relatively intact. He suggested that cellulose and hemicellulose depolymerisation probably occur by the splitting apart of monomeric units. He suggested that the enzymes from white, brown, and soft rot fungi depoly merise isolated polysaccharide molecules hydrolytically and usually in an apparently random manner, producing oligosaccharides and causing rapid depolymerisation.

The molecular association of lignin with the cellulose and hemicellulose in wood is not entirely clear. Kirk (1973) reported that evidence points, however to the following picture, the lignin and hemicellulose form a three-dimensional, inter-penetrating matrix that surrounds the cellulose microfibrils, the lignin and hemicellulose being bonded to each other via covalent linkages. If this proposition is correct, it seems likely that wood-destroying fungi may have to alter the lignin before they can decompose the cellulose. That could explain why Myrothecium verrucaria and Trichoderma viride, which were among the fungal species isolated during the present study and many other fungi and bacteria which were virulently cellulolytic were unable to significantly affect the cellulose in wood. This was detected during the preliminary experimental trials of wood decay activities by these fungi.

The increase in alkali solubility of the brown rotted wood pieces as was observed from the soil burial experiments has been attributed largely to carbohydrate degradation products by Pechman and Schaile (1950), Campbell (1950) and Kennedy 1958.

The wood decay capacity of the micro-organisms in the Nigerian soil might have depended on the complex interactions of all the soil characteristics, moisture content, water holding capacity, organic content, the soil mineral status and pH.

Soil moisture is one of the important ecological factors that affect wood decay. Microbiological degradation of wood can occur only if the wood has a moisture content exceeding 20% of its oven dry weight. This factor was achieved in the buried wood pieces by the fact that the burial soil was moistened to 80% of its water holding capacity before the wood pieces were introduced. The addition of water to the burial jars periodically helped to maintain adequate moisture level in the buried wood pieces thus enabling them to be decayed by soil fungi. The water holding capacity of the Nigerian soil was high enough to promote wood decay. Becker and Kaune (1966) demonstrated that the decisive factor for fungal attack of wood is the resulting wood moisture. They observed that its lower limit for wood destruction is about 30-35%, its upper limit for pine sapwood is about 60-80% and for beechwood about 80-120%. Kerner-Gang (1970) however, demonstrated that soft rot fungi show range of moisture optima. Carey and Grant (1975) showed that over a wide range of moisture levels both soil and vermiculite control the moisture content of buried test blocks at levels suitable for decay to take place.

The amount of organic matter content of the Nigerian soil was not high enough as was revealed by the soil organic carbon content, to have affected the rate of wood decay by the Nigerian fungi. Savory and Bravery (1970) suggested a broad trend of weight losses inversely proportional to the organic matter content of the soil. Their results with sterile soil suggested if anything a relationship of weight losses directly proportional to the soil organic matter content.

The Nigerian soil samples were found to contain essential mineral elements like Chlorine, Nitrogen and Phosphorus which are essential for microbial growth. The presence of these mineral nutrients might have affected the rate of dec a y of the buried wood pieces. Becker and Kaune (1966) observed that soils which are sufficiently enriched with nitrogen and minerals and are adequately aerated under moisture conditions yielded, independently of their particular composition and kind of soil - almost identical optimum values for the destruction of wood by soft rot fungi.

The optimal nutritional needs of wood-damaging micro-organisms might vary, but it seems all species obviously are able to get along with what is available in wood. Energy and most of the cell-building materials for the decaying fungi are supp l ied mainly by the carbohydrate fraction, consisting of holocellulose, starches, and sugars, and for some organisms by the lignin fraction.

The pH values of both the Nigerian soil samples and wood samples were well within the ranges that would give good fungal growth in pure culture. This might have pro moted the rate of decay of the buried wood pieces by the Nigerian fungi. Cartwright and Findlay

(1958) reported a lower limit of pH 2.0 for growth of decay fungi but concluded that the optimum for most species lies between 4.5 and 5.5, which is within the normal range for wood. They noted further that fungi may change the PH of their substrate, possibly in the direction most suited to their growth, by the acids they produce. The direction and extent of the fungus-induced change in pH varied according to the species of the fungus. Duncan (1965) considered that soils with pH between 5 and 7 should be suitable for wood decay experiments while Becker and Kaune (1966) found the presence of lime and an alkaline pH to be beneficial. Sharp and Eggins in their investigation of the ecology of soft rot fungi, discovered that nearly all the fungi they isolated were capable of some degree of clearing of cellulose agars and decomposing wood. Most of their fungal isolates favoured acidic conditions with optimal frequency of isolation and cellulolytic activity tending to be between pH 6 and 7.

The wood pieces buried in the dry season soil samples were found to be comparatively more decayed than the wet season soil samples. This result suggests that wood in ground contact like electric poles, railway sleepers and telephone transmission poles are likely to be decayed if they are untreated. With the coming of the first rains the soil moisture content and hence the moisture content of the wood in ground contact are raised. This renders the wood in ground contact conducive for the thriving of biodeteriogens which have been proved to be most abundant in the soil at this period, and hence lead to their eventual decay. Similar situation is likely to arise at the end of the rains and with the setting in of the dry season. Kay and MacNulty (1968) made a comparison of the ease with which termites attack wood with

and without bark using one mangrove species found in the Mangrove forest of Nigeria. They found that attack on timber from which bark had been removed might be only half as rapid as on wood with bark still attached. They found however, that attack was concentrated toward the portion of the structure nearest ground presumably because of the difficulty termites have in building runs on surfaces as smooth as wood. Again, they found that increase in the intensity of attack to coincide with the beginning and end of the rainy season.

The resistant nature of the preservative treated wood pieces to decay must have been due to the toxic nature of the preservative to decay fungi. This also shows that the wood pieces received a good amount of preservative through the vacuum impregnation method used. The slight weight reductions observed for the creosoted wood pieces might have been due to the diffusion of creosote into the soil.

Richardson (1978) reported that creosote does not perform simply as a toxic preservative but that the residue and other heavier components tend to protect the lighter, more toxic components from loss by leaching or volatilisation. In addition, impregnation with creosote provides extra protection against moisture content changes so that treated wood is stable and very resistant to splitting. Creosote thus possesses a variety of advantageous properties, which are not all readily imitated in alternative preservatives.

CHAPTER 5

THE EFFECTS OF THE TEST FUNGI ON NIGERIAN WOOD

5.1 TEST FUNGI

The test fungi used were:

THERMOPHILIC

Chaetomium thermophile var. *coprophile*

Myceliophthora thermophilum

THERMOTOLERANT

Coprinus cinereus

Sporotrichum pulverulentum

MESOPHILIC

Chaetomium globosum

Coprinus sp.

5.2 REASONS FOR CHOOSING THESE FUNGAL SPECIES

(i) Most of them were found to have a wide distribution in Nigeria, having been isolated from the soil, the aerial environment and from Nigerian timber.

(ii) They are of potential economic importance, as some are soft-rot micro-organisms.

(iii) They are convenient and active laboratory organisms.

5.3 AMYLOLYTIC ACTIVITY OF THE TEST FUNGI

The amylolytic, and cellulolytic activities of the test fungi were determined before they were used to inoculate the wood pieces.

5.3.1 MATERIALS AND METHODS

Using a sterile 6mm corkborer, inocula were removed from actively growing cultures of the test fungi, maintained on Potato Dextrose agar, and transferred to the centres of petri dishes which contained 15ml. of solidified starch agar. The inocula were transferred to the agar plates in such a way that the inoculum surface bearing fungal growth, was in contact with the medium surface. The petri dishes were incubated at 30^{o}C for ten days.

Duplicate petri dishes for each species were tested at intervals of 3, 5, 7 and 10 days. The disc of a fungal inoculum and the surface mycelium of the organism were carefully scraped from the agar plate before the starch agar was flooded with potassium iodide solution at the end of each growth period. The iodide solution was allowed to stand in the plate for 45 seconds before the solution was decanted, and the hydrolised area measured. Non-hydrolised starch was indicated by its deep blue-black coloration, while hydrolised starch was indicated by the unstained areas.

5.3.2 RESULTS

All the test fungi produced clear unstained zones. They were therefore considered to show good starch hydrolysis which they

completed within seven to ten days. The details of the amylolytic activity of the test fungi are given in Figure 11.

5.4 CELLULOLYTIC ACTIVITY OF THE CHOICE FUNGI

5.4.1 MATERIALS AND METHODS

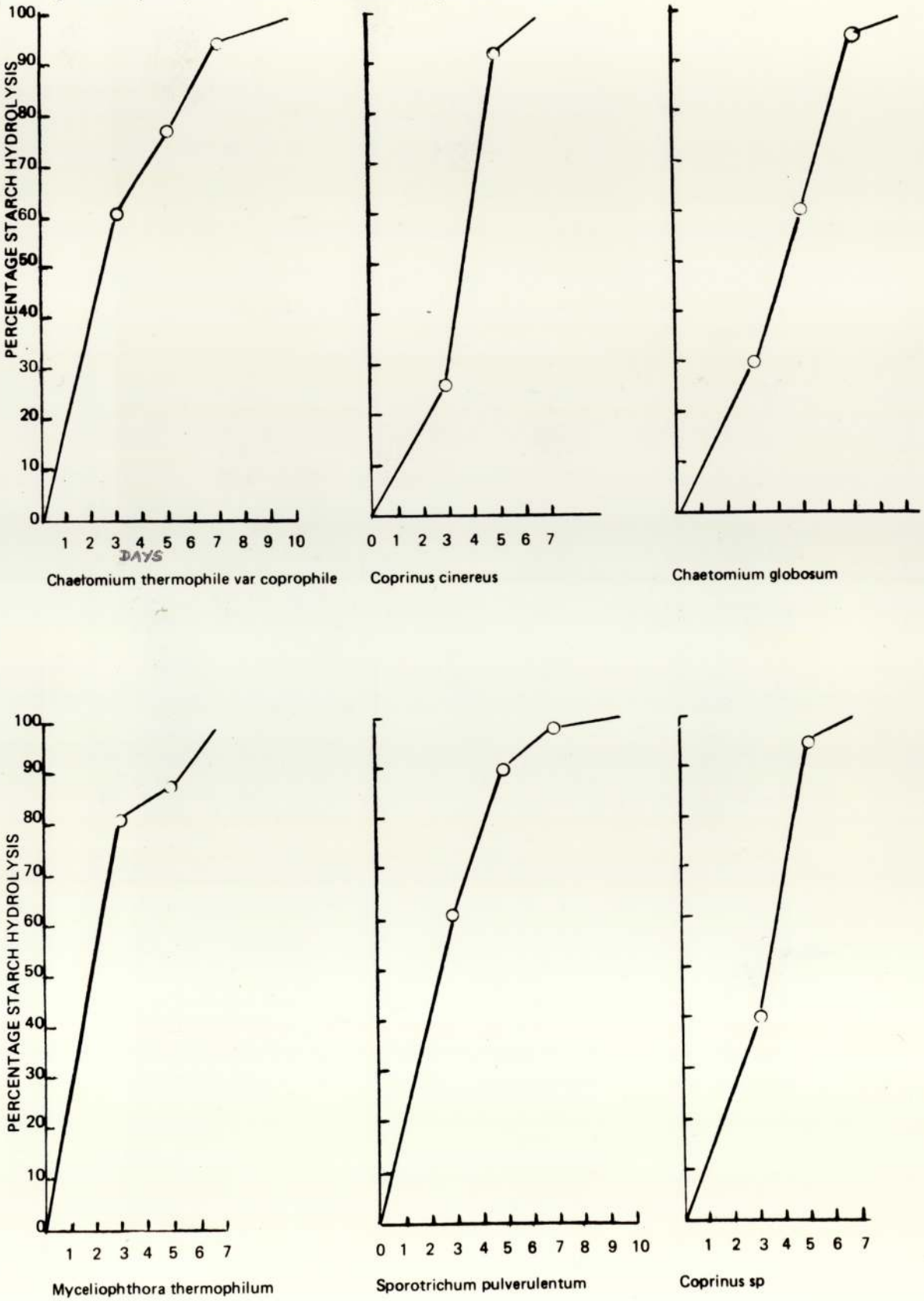
0.25% E & P CA was employed for the determination of the cellulolytic activities of the test fungi. The technique employed was that described by Rautella and Cowling (1966).

Columns of CA were prepared by transferring molten cellulose agar into sterile 15mm diameter boiling tubes to a depth of 8cm. The molten CA was cooled down to 40°C before it was poured into the boiling tubes and care was taken not to allow the CA touch the sides of the tubes. Care was taken to make sure that there was an even dispersion of the cellulose particles in the column. The tubes were immediately plunged into cold water after pouring in order to obtain a quick solidification of the agar and to avoid the precipitation of the cellulose particles.

10mm diameter fungal discs were removed from actively-growing cultures of the test fungi, which were maintained on P.D.A., and transferred to the surface of the CA columns in such a way that the surface of the agar inocula bearing the mycelium was in contact with the surface of the test medium.

The test was prepared in triplicate for each organism. The tubes were then incubated at 30°C (average room temperature in Nigeria) for twenty-eight days. Measurement of cellulose clearing

Fig. 11 Hydrolysis of Starch by the Test Fungi at 30°C



Chaetomium thermophile var coprophile

Coprinus cinereus

Chaetomium globosum

Myceliophthora thermophilum

Sporotrichum pulverulentum

Coprinus sp

was undertaken at the end of the experiment.

The height of the agar column was marked on the side of each boiling tube at the time of inoculation. Measurement of depth of clearing, transpiration and evaporation were taken from this marked point. Control samples were included, from which evaporation levels were calculated over the test period, and from which results were adjusted.

Fungal species which produced clearing were considered to be cellulolytic. According to the clearing of cellulose obtained, fungi were classified as being moderately cellulolytic, cellulolytic and strongly cellulolytic.

5.4.2 RESULTS

All the test fungi showed good cellulolytic activity. Myceliophthora thermophilum and Sporotrichum pulverulentum showed the best cellulolytic activity. The details are presented in Table 4. Plate 7 shows what cleared cellulose column looks like.

TABLE 4

CELLULOLYTIC ACTIVITY OF THE TEST FUNGI

FUNGAL SPECIES	Depth of Cellulose clearing	CELLULOLYSIS: Moderately cellulolytic	Cellulolytic	Strongly cellulolytic	Further comments
Chaetomium globosum	25mm	-		+	
C. thermophile var coprophile	20mm	-	+		
*Coprinus cinereus	20mm	-	+		*There was hyphal pentration into the agar column
C. sp.	25mm	-		+	
Myceliophthora thermophilum	27mm	-		+	
Sporotrichum pulverulentum	27mm	-		+	

PLATE 7: What cleared cellulose column looks like.

Key

S.p. = Sporotrichum pulverulentum
M.t. = Myceliophthora thermophilum
C.g. = Chaetomium globosum
C.t. = Chaetomium thermophile var. coprophile

5.5 GROWTH OF THE TEST FUNGI IN A MEDIUM CONTAINING LIGNIN AS SOLE CARBON SOURCE

5.5.1 MATERIALS AND METHODS

A slight modification was made in E & P CA. 2% Lignosite received from Georgia Pacific Corporation, Bellingham, Washington was substituted for the usual cellulose content. Yeast extract and Asparagine were excluded. 0.001gm thiamine hydrochloride was added as source of vitamin. The medium was sterilised by autoclaving and dispensed in petri dishes. The plates were then inoculated with the test fungi. Three plates of the lignin medium were used for each fungus. The plates were incubated at 30^{o}C and observed daily.

5.5.2 RESULTS

The test fungi grew well in this medium.

5.6 THE EFFECTS OF THE TEST FUNGI ON CANARIUM WOOD PIECES

5.6.1 MATERIALS AND METHODS

Some of the Canarium wood pieces measuring (6.50cm x 1.00cm x 0.50cm) were numbered and weighed. They were then vacuum impregnated with water. The blocks were then steam and cold sterilised (Chapter 4) and placed on thin "S" shaped glass rods on seven day old fungal cultures in 9cm petri dishes. Seven culture plates were set up for each test fungus. Three wood samples were placed on each "S" shaped glass rod in each culture

plate. Twenty-one wood blocks were therefore set up for each test fungus. Twenty-one control blocks were also set up but placed in petri dishes with no fungal cultures. The plates were then incubated at 30°C (average room temperature in Nigeria). When the wood pieces had been sufficiently colonised by the test fungi, they were removed from the culture plates into sterile damp jars containing previously sterilised bottles of water with wicks inserted into them (Figure 8). Seven jars were thus set up, each containing twenty-one colonised wood pieces belonging to a particular test fungus. One of the jars contained the twenty-one control pieces. The bottles of water with wicks helped to keep the jar chambers moist. All the jars were then incubated at 30°C for twenty-four weeks. In all the experiments the fungus was cleaned carefully from each specimen on completion of period of incubation. Final wet weights were determined and the samples were then subjected immediately to bending strength tests. Subsequently oven-dry weights were determined and final moisture contents and average percentage weight losses were calculated. A correction based on the change in dry weight of the controls not exposed to fungus attack was applied to the average weight losses of the bent wood specimens.

5.6.2 RESULTS

The test fungi caused considerable bending strength and weight losses. The details are given in Figure 12. Among the test fungi, Sporotrichum pulverulentum, Coprinus sp. and Chaetomium globosum were the most aggressive wood decomposers.

There was a good correlation between bending strength and

Fig. 12 The Effects of the Test Fungi on Canarium

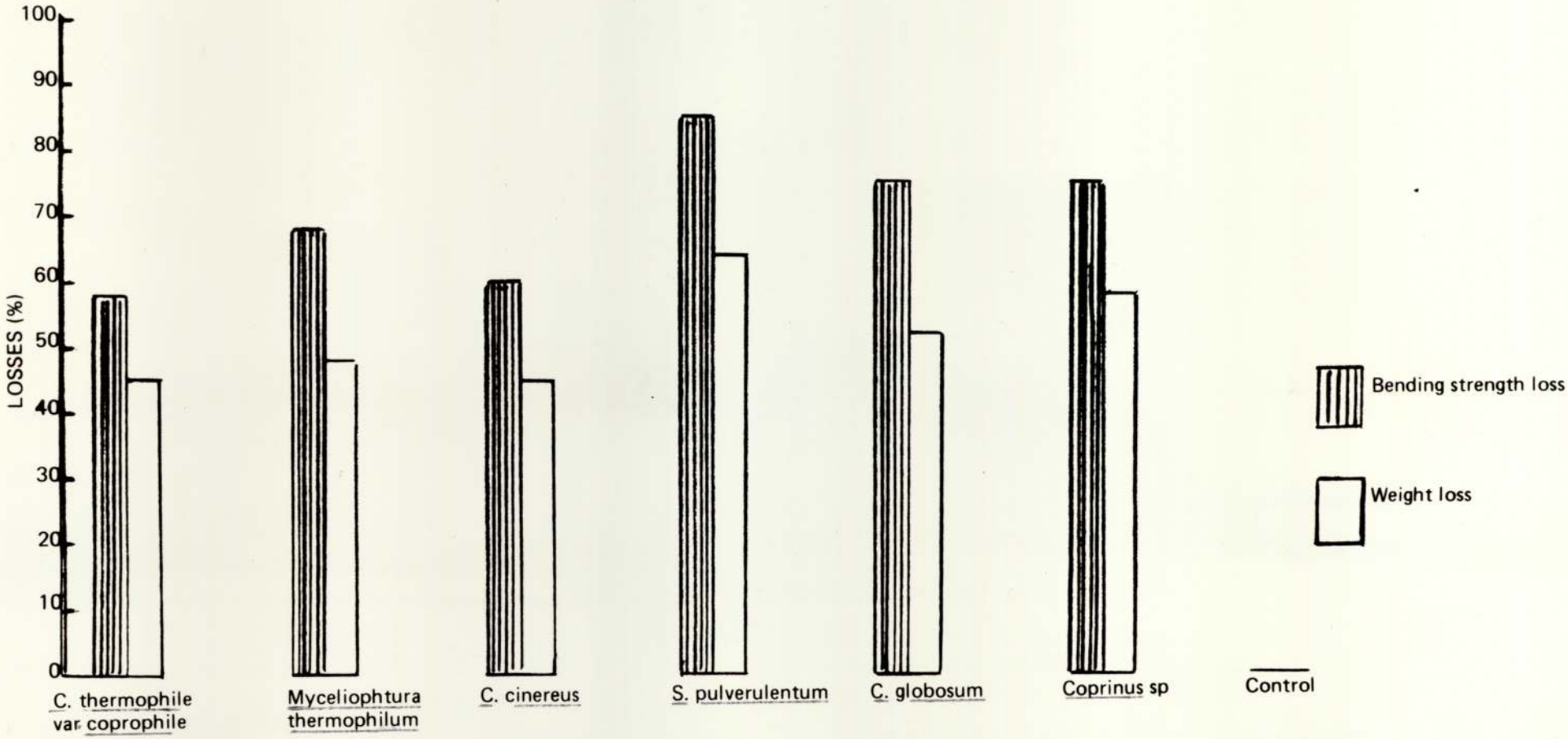


PLATE 8: "B" shows the compressible nature of a test wood piece decayed by Sporotrichum pulverulentum

A = control

weight losses. The wood pieces colonised and decayed by the two species of Coprinus were stained black, probably due to the ink-like substance produced by them. S. pulverulentum reduced most of the wood pieces it was growing on to a highly compressible state (Plate 8).

5.7 DISCUSSION

The results obtained from the amylolytic and cellulolytic experiments have shown that the test fungi are capable of utilising the starch and cellulose fractions in wood. Myceliophthora thermophilum, Coprinus cinereus and Coprinus sp. were the most active starch utilisers, while Myceliophthora thermophilum and Sporotrichum pulverulentum showed most cellulolytic activity. The growth of the test fungi in a medium containing lignin as sole carbon source depicts that these organisms are capable of utilising lignin. However, this needs a further investigation.

The bending strength and weight loss experiments have also shown that the individual soil fungi tested are capable of decomposing wood. The results obtained from the soil burial studies seem to have been the sum-total of the activities of individual soil fungi. The increase in loss of bending strength and weight caused by the test fungi as compared with the losses in mixed cultures may have been due to fungal antagonism as could happen in mixed soil fungal population.

S. pulverulentum was the most aggressive wood decay fungus amongst the test fungi. The compressible nature of the wood pieces decayed by S. pulverulentum could be due to the degradation

of lignin which surrounds hemicellulose and cellulose in wood as this species grew most quickly of the test fungi on the lignin agar.

Ofosu - Asiedu & Smith (1973) investigating the degradation of three softwoods by thermophilic and thermotolerant fungi found that at 45°C, weight losses caused by Chrysosporium pruinosum were low for this potentially destructive fungus. The low result they obtained might have been due to the high temperature employed. This fungus has been proved to be the same as the Sporotrichum pulverulentum used in the present experiment. The difference in the results obtained in the two experiments using the same fungus could have been due to the employment of different strains of the same fungus. Ander and Eriksson (1975) in their studies on the degradation of lignin with wildtype and mutant strains of the white rot fungus Sporotrichum pulverulentum found that the cellulose-less mutant could degrade both kraft lignin and lignin in wood although no cellulose was degraded. In the wildtype lignin degradation was stimulated by the presence of cellulose, while the cellulose-less mutant degraded lignin to an extent which was almost independent of the presence of cellulose. The enzyme cellobiose-quinone oxidoreductase is important for lignin degradation but not absolutely necessary.

In the laboratory tests of natural decay resistance of timber conducted in Princes Risborough laboratory in 1972, it was discovered that Polystictus sanguineus produced an average weight loss of 45.4% of Canarium schweinfurthii within four months. Polystictus versicolor produced an average weight loss of 40.5% of the same timber while Coniophora cerebella caused a loss of 28.9%

of the same wood, all within the same period.

Flannigan and Sagoo (1977) investigated the degradation of wood by Aspergillus fumigatus isolated from self-heated wood chips. They confirmed from their experiment that the A. fumigatus can cause loss of wood substance and showed that the organism produced enzymes involved in the degradation of polysaccharides in wood. However, they reported that extensive studies would be necessary to understand the nature and mode of action of these enzymes, and particularly to determine whether there is any significant degradation of cellulose.

Hardie (1979) recorded over 25% bending strength loss in beech sapwood supplemented with 0.11% inorganic nitrogen due to decay by Chaetomium globosum within eight weeks. She also recorded over 5% weight loss in lime sapwood supplemented with 0.11% inorganic nitrogen as a result of decay by the same fungus. The strain of C. globosum used in the present study was an active decomposer. It caused bending strength and weight losses of 75% and 52% respectively of the experimental wood pieces within six months.

CHAPTER 6

THE ABILITY OF THE NIGERIAN FUNGAL DETERIOGENS TO DECAY NIGERIAN WOOD AT DIFFERENT BIOCIDAL CONCENTRATIONS

6.1 MATERIALS AND METHODS

The technique employed in the present experiment was a modification of that described by the British Standards Institution (B.S. 838: 1961). The determination of the toxic limits of the wood preservatives used could not follow a closely graded series of concentrations because of the shortage of the experimental wood pieces.

Seven dilutions (80%, 75%, 50%, 40%, 25%, 10% and 5%) of the creosote wood preservative were prepared using white spirit (turpentine) as the solvent. This solvent was chosen because preliminary experiments showed that it had no inhibitary effect on the growth of fungal isolates investigated. Eight sets of the experimental wood pieces each measuring 6.5cm x 1cm x 0.5cm (length x width x depth) were prepared. Each set contained ten of the experimental test pieces. Each wood preservative dilution was used to treat a set of the wood pieces, using the vacuum impregnation method (British Standards Institution 913: 1973). This resulted in seven sets of wood pieces treated with the seven preservative dilutions. The eighth set of wood pieces were treated with full strength (100% concentration) of the creosote wood preservative.

Eight burial jars were set up, each containing lightly packed mixed and sieved Nigerian soil samples to just over half its capacity. The soil moisture content of each jar was raised to 80% of its water holding capacity. The jars were then numbered, each numbering coinciding with one of the dilution factors.

Each set of treated wood pieces was buried in the jar bearing a number corresponding to the dilution factor in which the wood pieces were treated.

The jars were incubated at 30°C for six months. The treated wood pieces were washed in twelve changes of sterile water and fungal isolations were made from them at the end of the experiment. The buried wood pieces were then subjected to bending strength tests. The bent wood pieces were finally dried to constant weights and losses due to fungal decay were recorded.

The experiment was repeated with "Cuprinol Wood Preservative Clear" (an organic solvent preservative containing 1.8% zinc as zinc naphthenate (approximately 20% zinc naphthenate) plus 2% pentachlorophenol).

6.2 RESULTS

No strength and weight losses were recorded for the wood pieces treated with 100% concentration of creosote. Similar results were obtained for the wood pieces treated with 100% concentration "cuprinol clear". Greater wood decay losses were recorded at lower biocidal concentrations.

There were also more fungal isolates from the buried wood pieces at lower concentrations of biocidal treatment. The highest number of fungal isolates was recorded from 5% concentration "cuprinol clear" - treated wood pieces. The detailed results are shown in Figure 13 and Table 5. Plate 9 shows the behaviour of some of the fungal isolates to filter paper discs treated with some of the "cuprinol wood preservative clear" dilutions.

Fig. 13

The Decay of Wood at Different Biocidal Concentrations by Nigerian Microfungi

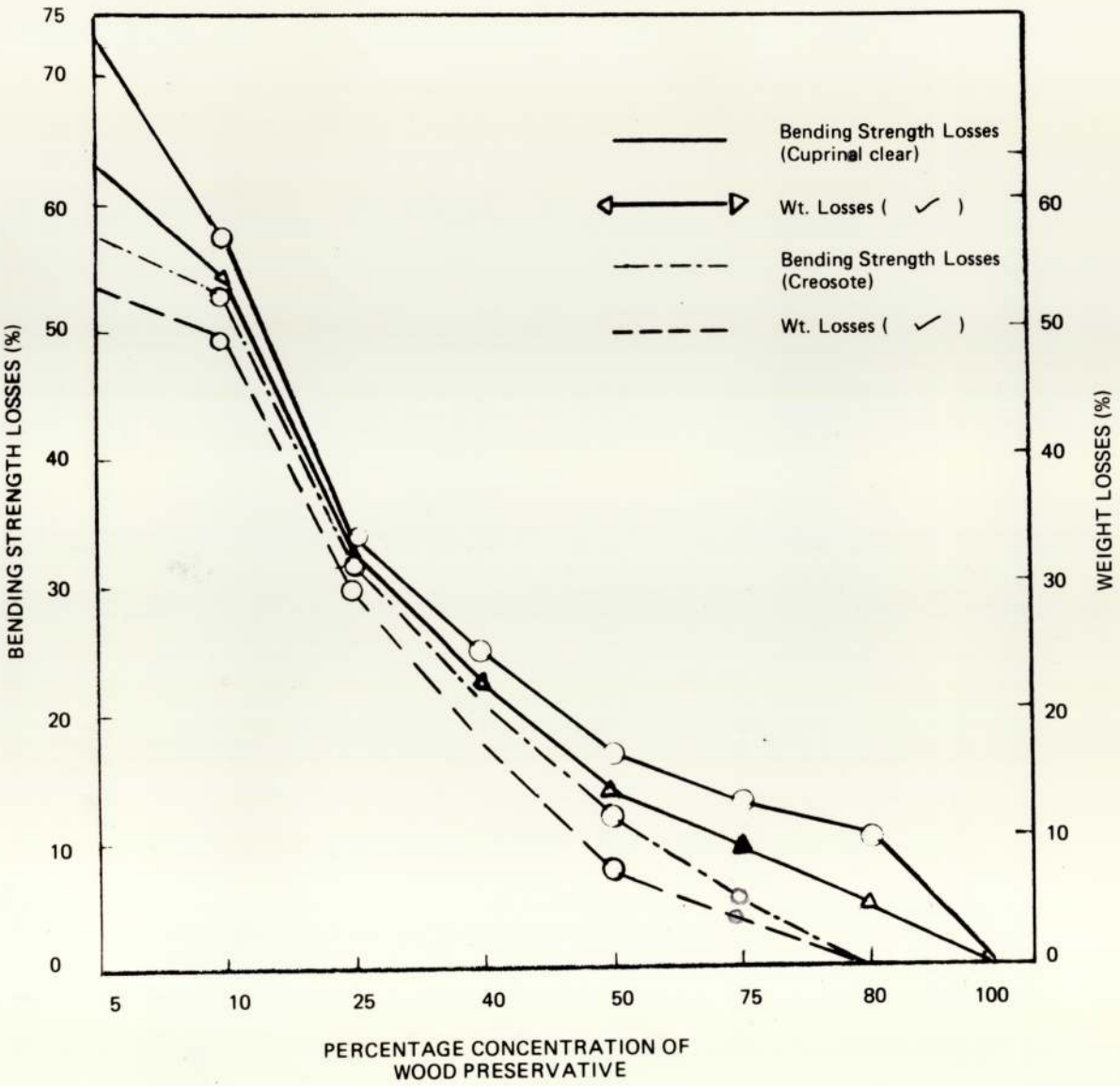


TABLE 5

DISTRIBUTION OF FUNGAL ISOLATES FROM THE WOOD PIECES TREATED WITH DIFFERENT CONCENTRATIONS OF BIOCIDES

	CREOSOTE								CUPRINOL CLEAR							
FUNGAL ISOLATES	100%	80%	75%	50%	40%	25%	10%	5%	100%	80%	75%	50%	40%	25%	10%	5%
Alternaria alternata	*-	-	-	-	-	*+	+	+	-	-	-	-	+	-	-	+
A. tenuis	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-
Amorphotheca resinae	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus longivesica	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
A. niger	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+
Chaetomium globosum	-	-		+	+	+	+	+	-	-	+	+	+	+	+	+
Coprinus cinerea	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
C. sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Corynascus thermophilus	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+
Fusarium sp.	-	-	-	+	-	-		+	-	-	+	+	+	-	+	+
Graphium sp.	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-
Humicola grisea	-	-	+	-	-	-	-	+	-	-	-	+	+	-	+	+
Monilia sitophila	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
Myceliophthora thermophilum	-	-		+	+	+	+	+	-	-	-	+	+	+	+	+
Oidiodendron sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Paecilomyces variotii	-	-	-	-	+	+	+	+	-	-	+	+	-	+		
Papulaspora sp.	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+
Scytalidium thermophilum	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Sporotrichum pulverulentum	-	-	+		+	+	+	+	-	+	+	+	+	+	+	+
TOTALS	-	-	4	4	5	6	8	11	-	4	5	8	9	11	12	15

+ = fungus present

- = fungus absent

PLATE 9A: The behaviour of Trichoderma viride to filter paper discs treated with some of the "Cuprinol wood preservative clear" dilutions (after 5 weeks incubation).

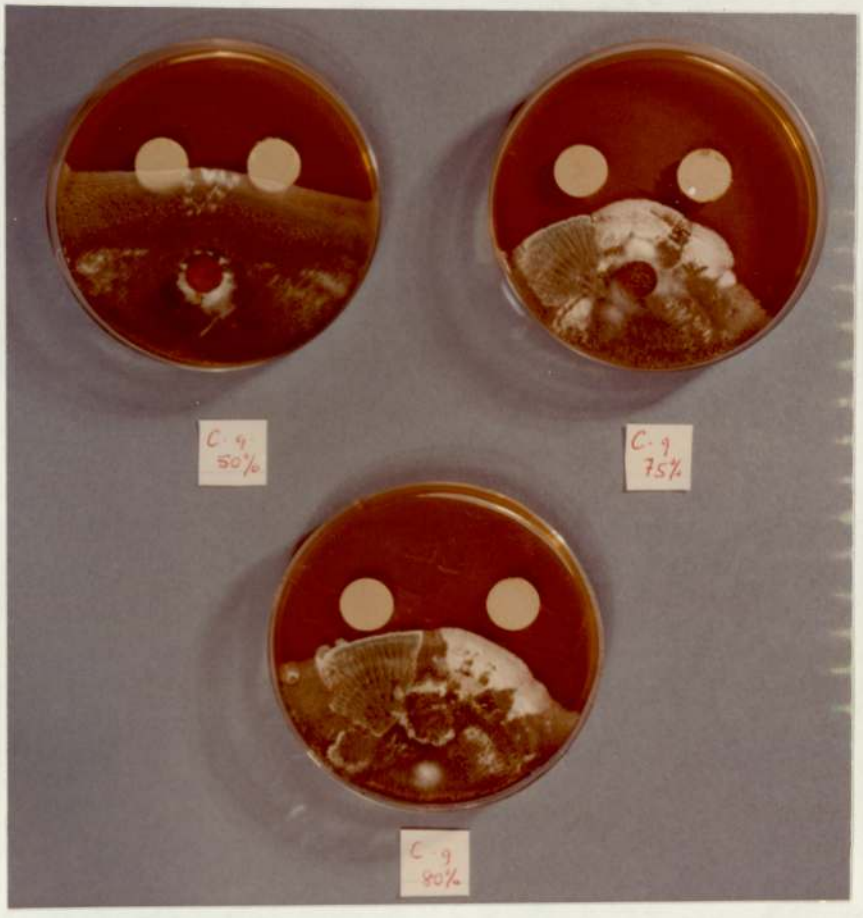


PLATE 9B: Behaviour of Chaetomium globosum to filter paper discs treated with some of the "Cuprinol wood preservative clear" dilutions (after 5 weeks incubation).

6.3 DISCUSSION

The results obtained from this experiment have shown the ability of soil micro-organisms to decay wood treated with biocidal concentration lower than that prescribed by the makers. The results have also shown that "Cuprinol Wood Preservative Clear" dilutions are more susceptible to attack by soil micro-organisms than creosote. 80% creosote concentration proved to be toxic to the soil biodeteriogens and at 75% concentration the percentage wood decay was minimal. Creosote is one of the most effective of wood preservatives. Its wide spectrum of chemical constituents enables it to control the growth of a range of different fungi and only a few fungi show any degree of tolerance to it. Mayfield (1951) gave an excellent survey of the work done on the fungicidal value of the individual toxic elements of high temperature coal tar creosote and concluded that the toxicity of creosote is not due to one or a few highly effective materials, but is due to the many and varied compounds which occur throughout its boiling range. Smith and Savory (1964) examined the toxicity of aged creosote to Lentinus lepideus and concluded that whatever physical or chemical changes occur in creosote during its service life in posts, it still retains its toxicity to wood-destroying fungi.

The highest bending strength and weight losses were recorded at both 5% and 10% biocidal concentrations (both preservatives), losses being greater in "Cuprinol Wood Preservative Clear". There were also more fungal isolates from wood pieces which had been treated at these levels of biocidal concentration. It appears that, these levels of biocidal concentration are within the range of tolerance by the soil micro-organisms, and it could be that at these

levels of biocidal concentration, the soil micro-organisms are much able to degrade and metabolise the products of biocides.

CHAPTER 7

STUDIES ON THE AMINO ACID NUTRITION OF SOME OF THE FUNGAL ISOLATES

7.1 THE UTILISATION OF AMINO ACIDS AS THE SOLE NITROGEN SOURCE FOR CELLULOLYTIC ACTIVITY BY SOME OF THE FUNGAL ISOLATES

The literature on the use of amino acids as a nitrogen source by fungi is extensive. Leonian and Lilly (1938) tested the relative value of twenty-four amino acids for fourteen fungi. They found none of the amino acids best for these species. Steinberg (1942) studied the growth of Aspergillus niger on twenty-two amino acids. He observed that alanine, arginine, aspartic acid, glutamic acid, glycine, proline and hydroxyproline were excellent sources of nitrogen for A. niger. He was of the opinion that these seven amino acids are synthesised first by this fungus and that the other amino acids are normally formed from them.

Stocks and Ward (1962) compared the growth and sporulation cycles of Rhizopus nigricans using twenty-three amino acids and two inorganic compounds as nitrogen sources. They found that all monoamino-monocarboxylic acids supported growth when supplied individually. They discovered that most rapid germination occurred when the amino acids were supplied as a mixture by a process of single elimination. However, they observed that this happened in the absence of valine or aspartic acid. They also found that the earliest sporulation took place when aspartic acid, tyrosine, threonine or dihydroxyphenylalanine were omitted.

Leal, Gallegly and Lilly (1971) studied the value of twenty-one amino acids as nitrogen sources for Phytophthora cactorum and P. heveae. They found L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, and L-tryptophan to be poor sources of

nitrogen for both species. L-phenylalanine was a good amino acid for P. cactorum and a poor amino acid for P. heveae. They noticed that L-cysteine and L-valine were good nitrogen sources for P. cactorum. Their 'study' showed L-α-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, glycine, L-histidine, L-proline, L-serine, L-threonine and L-tyrosine to be good nitrogen sources for both species.

Oso (1974) examined the organic nitrogen requirements of Chaetomium thermophile var coprophile, C. thermophile var. dissitum, Mucor pusillus, Thermoascus aurantiacus, T. crustaceus and Thermomyces lanuginosus. He found that all the organisms grew well on casein hydrolysate. He noticed that M. pusillus grew well on all the amino acids, while Thermomyces lanuginosus grew well on all but six of the amino acids he used. He also observed that T. aurantiacus and T. crustaceus gave a fair yield of mycelium on about 50% of the single amino acids tested while the other 50% served as poor sources of nitrogen. All his single amino acids were poorly utilised by C. thermophile var. coprophile and C. thermophile var. dissitum.

So far, most of the work on amino acid-nitrogen utilisation has concentrated on the effect of amino acids on fungal growth. The effect of amino acid as a sole nitrogen source for cellulolytic activity by fungi has been neglected. This area of work is important in studies dealing with wood decay, since most of the amino acids have been known to occur freely in wood (Baker, Laidlaw and Smith 1970; Hardie 1979). Hardie (1979) investigated the effect of amino acids on the cellulolytic activity of Alternaria tenuissima,

Chaetomium globosum, Coriolus versicolor and Trichoderma viride. She found that the majority of the amino acids she investigated supported less cellulolytic activity than the inorganic sources. Her results confirmed previous evidence of reduced cellulose production in the presence of alternative carbon sources. However, her test fungi never included thermophilic and thermotolerant fungi many of which are known to be strongly cellulolytic. She also never mixed her amino acids to find out the effects of amino acid mixtures on the cellulolytic activities of her test fungi. Since most of these amino acids occur freely in timber the fungal utilisation of each is likely to be affected by the presence of others. The present experiment was designed to answer some of these questions.

7.2 MATERIALS AND METHODS

A modification of the method originally described by Rautella and Cowling was employed. This involved the measurement of the depth of clearance of an opaque column of cellulose agar contained in a tube.

A basal cellulose agar medium, without a substantial nitrogen source was prepared. The medium was composed of:

KH_2PO_4	1.0g
KCL	0.5g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.1g
Thiamine hydrochloride	0.001g
Agar	20g
Distilled water	1000ml.

Whatman cellulose powder was ball-milled for seventy-two hours to reduce the particle size and then used as cellulose source. It was prepared as a 4% suspension in distilled water. The cellulose suspension was added into the basal medium in such a way that the final medium had 1% cellulose in it.

The nitrogen sources investigated were alanine, aspartic acid and glutamic acid. These three amino acids were among the amino acids reported to be present in Scots pine sapwood by Baker, Laidlaw and Smith (1970). According to their report these acids were comparatively higher in proportion than others.

Each amino acid under investigation was added to supply nitrogen equivalent to that in $(NH_4)_2SO_4$ in the CA formulated by Eggins and Pugh (1962). The medium was buffered to pH 5.0. This pH has been shown to be within the range of optimal cellulase activity (King 1966).

The medium without thiamine hydrochloride was then autoclaved at 15psi (120°C) for twenty minutes. On removal, the medium was transferred to a 40°C water bath and allowed to cool. When the medium had cooled sufficiently, thiamine hydrochloride was added. Columns of agar were prepared by transferring molten cellulose agar into sterile 15mm diameter boiling tubes to a depth of 8cm approximately. The temperature of pouring was approximately 40°C and the medium was poured in such a fashion that the molten agar did not touch the sides of the tubes. The cellulose particles were evenly dispersed in the medium so that uniform opacity of cellulose agar columns was obtained. The tubes

were immediately plunged into cold water after pouring in order to solidify the agar and to prevent the precipitation of the cellulose.

Discs of fungal inocula were removed from actively-growing cultures of the test fungal species and also from Malbranchea pulchella var sulphurea and Talaromyces thermophilus, which were maintained on P.D.A. The fungal discs were transferred to the surface of the cellulose agar columns in such a way that the surface of the agar inocula bearing the mycelium was in contact with the surface of the agar column. Triplicate test specimens were prepared for each test fungal species and all test specimens were incubated at 30°C (average room temperature in Nigeria) for twenty-eight days. Measurement of cellulose clearance was taken at seven-day intervals.

The height of the agar column was marked on the side of each test tube at the time of fungal inoculation. Measurement of depth of cellulose clearing, transpiration and evaporation were taken from this point. Control samples were included, from which evaporation levels were calculated and from which results were corrected. Fungal species which produced clearing were considered to be cellulolytic.

The experiment was repeated using a mixture of the amino acids. The acids were mixed in the same proportion as they occur in Scots pine sapwood. The mixture yielded the same level of nitrogen as when single amino acids were employed.

Eggins and Pugh (1962) CA formulation was equally prepared, having ammonium sulphate as the main nitrogen source but without asparagine and yeast extract. This was inoculated with the eight fungal species and used for comparison.

7.3 RESULTS

Cellulose clearing was obtained with each of the amino acids and the modified E & P CA. The best cellulose clearing was obtained with the mixture of amino acids. The detailed results are given in Figure 14.

7.4 DISCUSSION

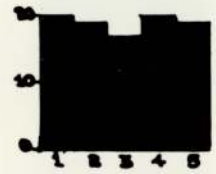
The results of this experiment have shown that thermophilic, thermotolerant and mesophilic fungi can utilise amino acid as nitrogen sources for cellulolytic activity.

A significant amount of cellulose clearing was obtained for each of the amino acids employed in the experiment. The E & P CA medium without Asparagine and Yeast Extract proved to be as good as that with an amino acid as a sole nitrogen source. The best cellulose clearing was observed in the medium containing a mixture of amino acids. These results are significant in wood deterioration because free amino acids are known to occur in timber (Baker, Laidlaw and Smith 1970; Hardie 1979). Wood decay fungi can therefore meet their nitrogen requirement for cellulolytic activity in wood by utilising the wood amino acids.

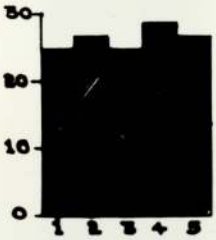
Fig.14 Fungal Species **Depth of Cellulose Clearing in (mm)**

Chaetomium thermophile var. coprophile

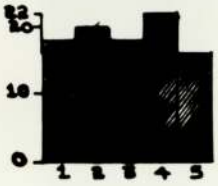
Malbranchea pulchella var. sulphurea



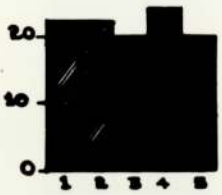
Myceliophthora thermophilum



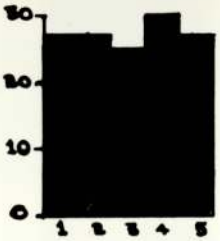
Talaromyces thermophilus



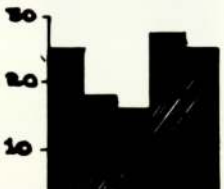
Coprinus cinereus



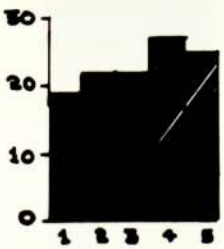
Sporotrichum pulverulentum



Chaetomium globosum



Coprinus sp.



Key to Nitrogen Sources

1. = Alanine
2. = Aspartic acid
3. = Glutamic acid
4. = Amino acid mixtures
5. = E α p C A

Another significance of the results is that it shows that the nitrogen level in the cellulose medium formulated by E & P to be high. Two significant sources of nitrogen (Ammonium sulphate and Asparagine) have been included. Park (1973) suggested that Asparagine should be removed from that medium. He felt the presence of Asparagine in CA was disadvantageous in work connected with cellulolysis. Hardie (1979) obtained similar results.

Since a significant result was obtained with a mixture of amino acids and since micro-organisms seem to have different nitrogen requirements, a substitution of the original nitrogen source in CA medium with a mixture of amino acids is likely to produce better results in studies connected with cellulolysis.

7.5 THE UTILISATION OF AMINO ACIDS AS THE SOLE NITROGEN AND CARBON SOURCE BY SOME OF THE FUNGAL ISOLATES

Sorenson and Hesseltine (1966) investigated carbon and nitrogen utilisation by Rhizopus oligosporus.

They found that common sugars such as glucose, fructose, galactose, maltose and xylose supported excellent growth. They found ammonium salts and such amino acids as proline, glycine, aspartic acid and leucine to be excellent sources of nitrogen. Arginine was the only amino acid they tried that supported significant growth without another carbon source. Keratinophilic fungi have also been shown to have the ability to utilise the carbon in amino acids (Ibbotson, 1974).

Hardie (1979) investigated the utilisation of amino acids as the sole nitrogen and carbon source by Chaetomium globosum, Coriolus versicolor and Trichoderma viride. C. globosum and T. viride are fungi capable of causing soft rot decay of timber, while Coriolus versicolor causes white rot of timber. She found that growth on glutamic and aspartic acids without extra carbon source was similar to that on the two inorganic nitrogen sources plus glucose for all three fungi. She observed that threonine supported relatively good growth of C. globosum and C. versicolor while it supported only poor growth of T. viride. She also observed that Leucine supported mediocre growth of C. globosum, C. versicolor and only poor growth of T. viride. She discovered

that growth on asparagine followed a similar pattern; C. versicolor produced relatively good growth, T. viride poor growth and C. globosum mediocre growth. C. versicolor and T. viride produced very little growth on histidine although C. globosum again produced mediocre growth.

Wood is known to contain many amino acids, (Baker, Laidlaw and Smith, 1970; Hardie, 1979). Hardie (1979) also reported the presence of nineteen amino acids in Chaetomium globosum. These amino acids were the same amino acids she extracted from Beech, Lime and Pine. This similarity in the amino acid constituents of wood and Chaetomium globosum was significant.

Some of the fungal isolates in the present study, (test fungi) have been shown to cause wood decay. The present experiment was designed to find out whether some of the thermophilic, thermotolerant and mesophilic fungal isolates are capable of utilising some of the amino acids in wood as the sole nitrogen and carbon source.

7.6 MATERIALS AND METHODS

A basal medium was prepared which omitted major carbon and nitrogen sources. The medium was made up of:

KH_2PO_4	1.0g
KCL	0.5g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.1g
Thiamine hydrochloride	0.001g
Distilled water	1000ml.

Each amino acid under investigation was added to supply N. equivalent to that in $(NH_4)_2 SO_4$ in normal E & P CA. The nitrogen inherent in thiamine hydrochloride was considered to be negligible when compared to the nitrogen levels subsequently added.

The basal medium excluding thiamine hydrochloride was sterilised by autoclaving. Thiamine hydrochloride was added aseptically after the basal medium had been cooled down to room temperature.

The medium was then dispensed aseptically into 100ml. sterile conical flasks at 30ml. per flask. The flasks had cotton wool plugs. The flasks were inoculated with mycelium from the growing edge of seven day old fungal cultures. The fungi investigated included Chaetomium thermophile var. coprophile, Malbranchea pulchella var. sulphurea, Myceliophthora thermophilum, Talaromyces thermophilus, Coprinus cinereus, Sporotrichum pulverulentum, Chaetomium globosum and Coprinus sp. Each flask was replicated three times for each fungus. The amino acids investigated included alanine, aspartic acid and glutamic acid. These were among the most abundant amino acids in Scots pine sapwood (Baker et al. 1970). Hardie (1979) found alanine and aspartic acid to form a significant proportion of her amino acid extracts from timber.

The flasks were then incubated at 30°C on a shaker table for twenty-one days.

Mycelium was harvested by filt ration under vacuum through preweighed No. 1 Whatman filter paper (7.0cm diameter) in a Buchner funnel. The mycelium of each flask was rinsed with 100ml. sterile distilled water to remove all traces of the medium. The filter papers were then dried to constant weight at 90°C in an oven for 24 - 48 hours, cooled in a desiccator over anhydrous $Cacl_2$ for one hour and weighed.

The experiment was repeated with a mixture of the amino acids. The amino acids were mixed in the same proportion as they occur in Scots pine sapwood. Care was taken to make sure that the mixed amino acids yielded the same N level as when single amino acids were employed.

7.7 RESULTS

The details of the results are shown in Figure 15. The growth in the individual amino acids was not as good as growth in the E & P CA. The mixture of amino acids proved better than the individual amino acids. Of the amino acids, Alanine produced the best growth with Myceliophthora thermophilum, Talaromyces thermophilus, Coprinus cinereus, Sporotrichum pulverulentum, Coprinus sp; Aspartic acid with Malbranchea pulchella var. sulphurea, Talaromyces thermophilus, Coprinus cinereus and Glutamic acid with Malbranchea pulchella var. sulphurea, Myceliophthora thermophilum, Coprinus cinereus.

Fig.15 Fungal Species — Mycelial Dry Weight in MGM

Chaetomium thermophile var. coprophile

Malbranchea pulchella var. sulphurea

Myceliophthora thermophilum

KEY

⟷ (ie. 5 mm) = 10 mgm

1. = Alanine
2. = Aspartic acid
3. = Glutamic acid
4. = Amino acid mixtures

5 = Exp CA

Talaromyces thermophilus

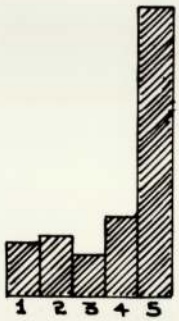
Coprinus cinereus

Continued...

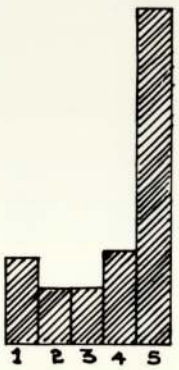
Fig. 15 continued

Sporotrichum pulverulentum

Chaetomium globosum



Coprinus sp.



7.8 DISCUSSION

The results obtained from the present investigation have demonstrated the ability of thermophilic, thermotolerant and mesophilic fungal species to utilise the carbon and nitrogen of amino acids. The significance of the result is that the presence of a more readily utilisable carbon source may suppress the production of cellulases required for cellulose decomposition.

The results agree with the first stage in the scheme of decomposition of wood tissues in and above the soil recognised by Garrett (1963). In his scheme he stated that in stage one of wood decomposition, the tissues are invaded by primary saprophytic fungi which live on sugar and carbon compounds simpler than cellulose. The amino acids could be among these simpler substances.

There was a better fungal growth in the medium containing a mixture of amino acids than those containing single amino acids. A mixture of amino acids may be better utilised than single amino acids. The amino acids may not be of equal value in fungus nutrition. The effect of one amino acid on the utilisation of another might vary with the amino acids involved and the specific fungus used.

Of the vast number of organic compounds which contain nitrogen, the ones of interest in fungus nutrition are those which occur naturally. Barnett (1951) reported that the amides of aspartic and glutamic acids are found free in many plants and are thus available to the fungi in nature. Foster (1949) hinted the

direct assimilation of organic nitrogen by fungi. Henningsson (1968) found that a number of Basidiomycete fungi, capable of attacking birch and aspen pulp-wood, were able to utilise the amino acids found in aspen wood by Merrill and Cowling (1966). Hardie (1979) observed that the composition of nitrogen fraction of the fungus is, in general terms, similar in ratio, though not in order of magnitude, to the timbers. She also found aspartic acid, glutamic acid, glycine and alanine (three out of the four were employed in the present study) amongst the most abundant amino acids of the fungus, as they were in the timbers. The ability of the fungi to utilise amino acids of wood shows the versatility of these fungi.

Comparatively, Chaetomium thermophile var. coprophile showed poor growth in the amino acids, and its growth in the amino acids mixture was even lower than that of other fungi. The relatively poor growth exhibited by this fungus in a medium containing an amino acid or amino acids may be due to the formation of toxic substance or substances by this fungus. The concentration of this substance or these substances might have reached the level that was toxic to the growth of this fungus. Leal, Gomez-Miranda and Nicolas (1966) obtained a similar result in their investigation of the formation of toxic substances by fungi in the degradation of some amino acids. Their results suggested that certain amino acids are poor sources of nitrogen for certain fungi because of the inability of the fungi to catabolise them completely after deamination. Coll and Leal (1972) investigated the utilisation of L-leucine by three fungi. They observed that the maximum amount of mycelium was produced by Fusarium culmorum which used glucose and leucine

rapidly without accumulation of acid. They also observed that Penicillium italicum which reached a lower dry weight, consumed glucose and leucine rapidly, but part of the carbon skeleton of leucine accumulated as isovaleric acid. At the end of their experiment they found that 50% of the leucine had been consumed.

CHAPTER 8

THE EFFECTS OF IRRADIATION ON THERMOPHILIC FUNGAL SPORE GERMINATION AND GROWTH ON A VARNISHED WOODEN SURFACE

8.1 INTRODUCTION

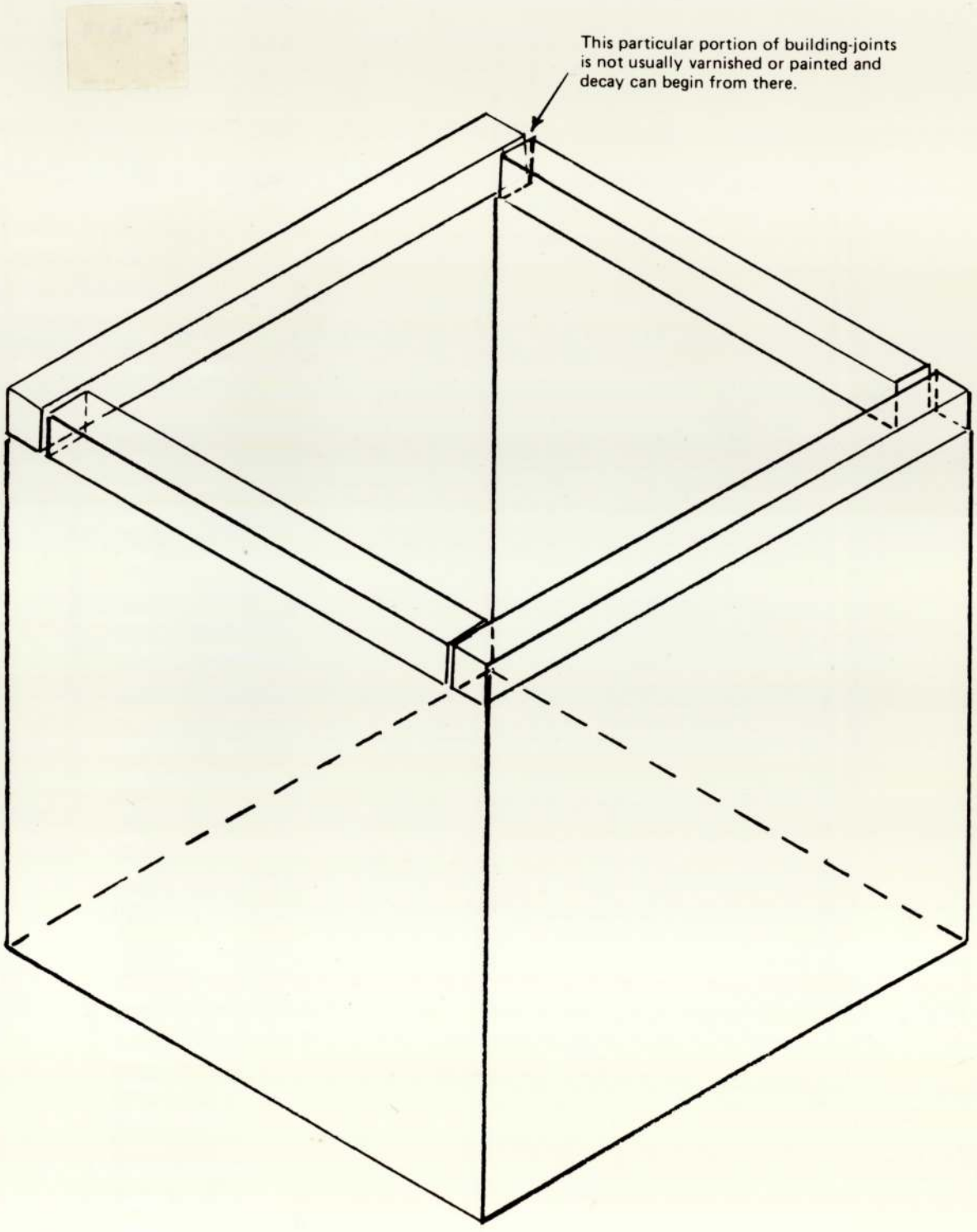
Generally, protective coatings are used on wooden surfaces because they act as barriers to the free access of gases, vapours and liquids. Growth of micro-organisms with substantial oxygen requirements will be inhibited when the partial pressure of oxygen is low. Under such conditions, reduced levels of water activity Aw, would be expected to produce additional inhibition.

However, the end surfaces of wood forming parts of wooden joints, for example, those used in buildings and some other wooden constructions are not usually painted (Figure 16). Water molecules evaporating from the interior parts of the wood through the unpainted end surfaces could accumulate within the small spaces created by the two wood pieces forming each joint. The heat supplied by the sun's irradiation could help in increasing the water activity of the molecules in these spaces. Troller and Christian (1978) have demonstrated that fungal germination under favourable temperature, pH and oxygen concentration are water activity (Aw) dependent. They equally reported that on nutritionally limiting substances, the inhibitory effect of reduced water activity will usually be enhanced.

In Nigeria, thermophilic and thermotolerant fungal spores have been shown to form part of the Nigerian aerial mycoflora. Some of these spores could land on the unpainted wood surfaces forming the joints in timber used for building construction and other wooden constructions. Under conditions of increased water

Fig. 16

A Diagram Showing the First Layer of Wood-Lining in a Typical Wood-Roofing in an Ideal Nigerian House



activity, favourable temperature and pH, the thermophilic and thermotolerant fungal spores could germinate and grow on the unpainted wood surfaces.

The obvious solution to enclose wood within a protective film is to stabilise and maintain a low moisture content. However, while these coatings give good protection against rainfall, they are unable to prevent moisture content changes resulting from slow seasonal fluctuations in atmospheric relative humidity. This results in the surface coatings fracturing wherever a joint involves stable side grain in contact with unstable end grain. Rain could be absorbed by capillarity into the crack, yet the remaining paint coatings could restrict evaporation so that the moisture content steadily increases. Although water is not a good solvent for most organic polymers, it does interact physically with polar groups present in the polymer and is absorbed. The activity of the absorbed water increases with concentration, thus affecting permeation (Yaseen and Ashton, 1978).

With this build up in water content of the wood, the germinated thermophilic fungal spores could then grow with ease through the wood and could cause considerable decay despite the protective coating.

The present experiment was therefore devised in order to find the effects of irradiation on thermophilic and thermotolerant fungal spore germination. And secondly to find the effect of irradiation on the germination and growth of thermophilic and thermotolerant fungal spores on a varnished wood surface.

8.2 THE EFFECTS OF INFRA-RED LAMP IRRADIATION ON THERMOPHILIC AND THERMOTOLERANT FUNGAL SPORE GERMINATION

Spore suspensions of Myceliophthora thermophilum and Sporotrichum pulverulentum were prepared from fourteen day old cultures grown on 2% malt agar. A haemacytometer was used to assess the density of spores in each fungal suspension. Each fungal suspension was then diluted to give approximately 10,000 spores/ml.

The surface of a thin layer of wood shaving (transparent when viewed under the microscope) was placed horizontally on well moistened sterile petri dish (100% relative humidity) and inoculated with a spore suspension of Myceliophthora thermophilum by using a sterile hypodermic syringe. The same procedure was repeated using a Sporotrichum pulverulentum spore suspension. Each of the experiments was replicated three times. The plates were then irradiated with an infra-red lamp (Plate 10) at a dose of 400 cal cm^{-2} day^{-1}. Small amounts of sterile distilled water was added at two hour intervals into each of the plates to avoid their drying out due to the lamp's heat.

The transparent wood shavings in the petri dishes were examined under "x 10 and x 20" microscope objectives and the germinated fungal spores were counted. The counting was carried out at five hour intervals. Usually a spore was considered germinated if the length of the germ tube was one-half the larger diameter of the spore. Five microscope fields were counted.

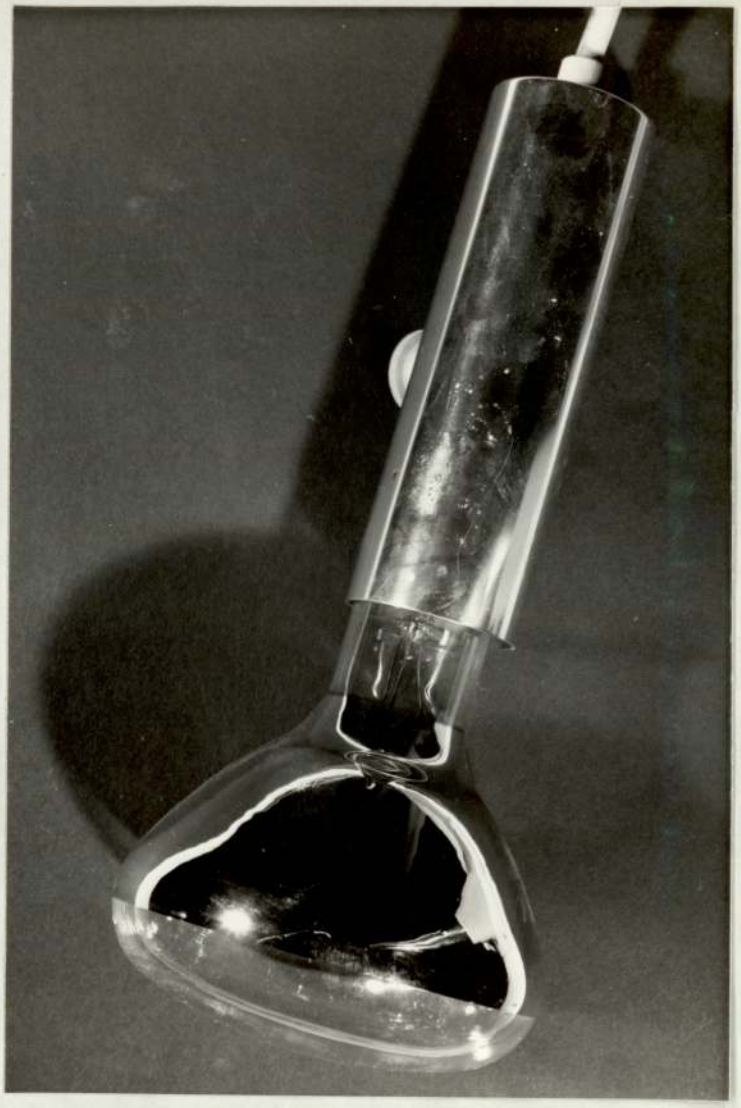


PLATE 10: An infra-red lamp employed for the irradiation experiments.

Countings were made from the replicate plates. The results are presented in Figure 17.

8.3 THE EFFECTS OF INFRA-RED LAMP IRRADIATION ON THE GERMINATION AND GROWTH OF FUNGAL SPORES ON VARNISHED WOOD SURFACES, NON-VARNISHED WOOD SURFACES AND VARNISHED WOOD SURFACES WITH CRACKS

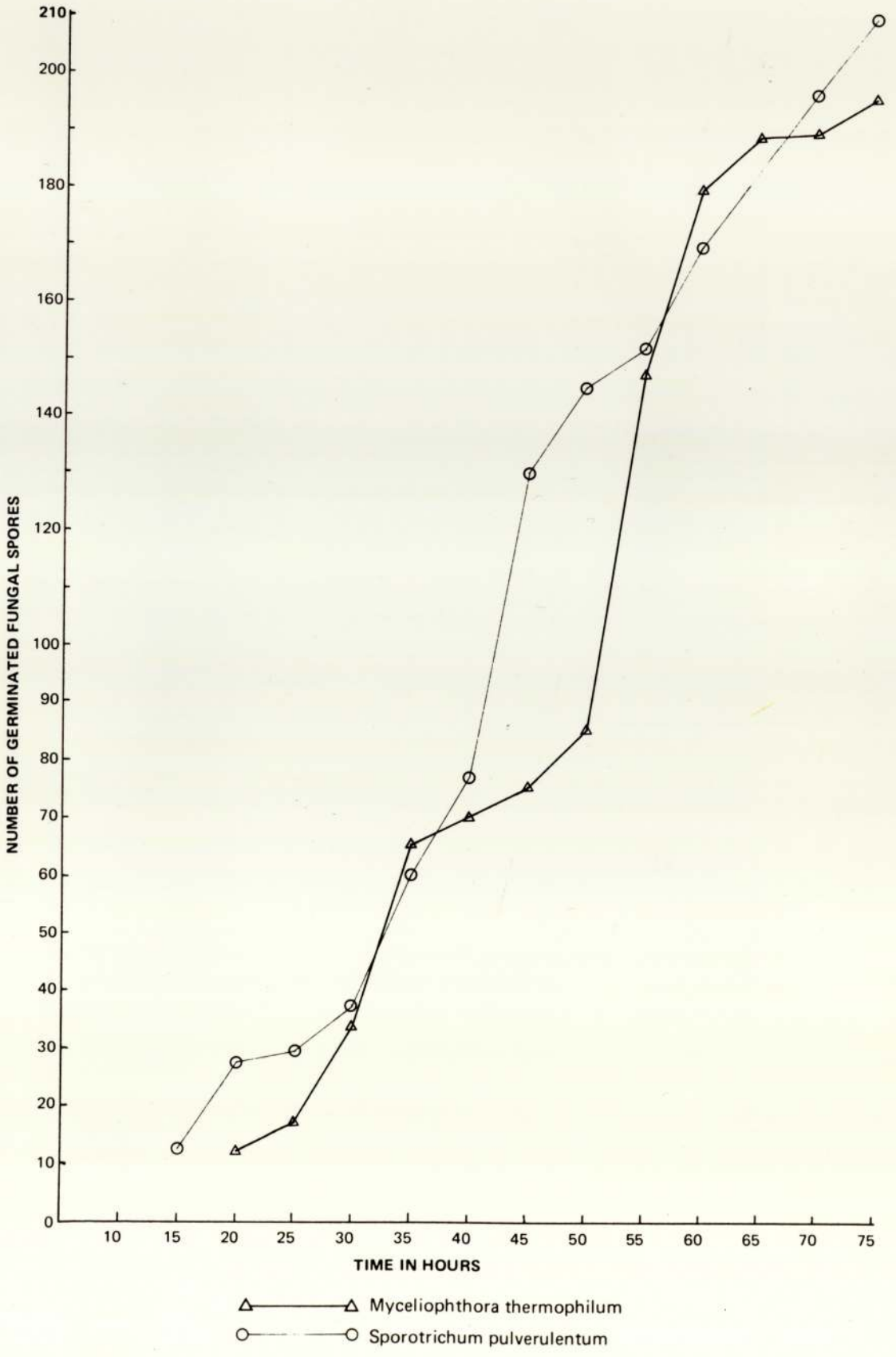
Five sets of wood blocks, each measuring 6.50cm x 6.50cm x 6.50cm (length x width x depth) were cut out from Canarium wood. Each of the blocks had another block measuring 6.50cm x 6.50cm x 2cm (length x width x depth) placed on top of it. The space created in between the two blocks of wood represented the spaces seen in timber-roofing joints.

The first set of wood blocks were impregnated with Cuprinol clear (an organic solvent preservative containing 1.8% zinc as zinc naphthenate) (approximately 20% zinc naphthenate plus 2% pentachlorophenol), and finally varnished (all surfaces varnished).

The second set of wood blocks were not treated with wood preservative but had all the surfaces varnished. The third set of wood blocks had all surfaces varnished except the two meeting surfaces (ie. the lower surface of the upper block of wood and the top surface of the lower block of wood).

The fourth set of wood blocks had all surfaces varnished but had cracks of various shapes designed on them. The fifth set of wood blocks were neither treated with wood preservative nor varnished. All the wood blocks were sterilised prior to

The Germination of Thermophilic and Thermotolerant Fungal Spores on Canarium Wood Surface under Infrared Light Irradiation.



the experiment. Each set of wood blocks were replicated three times.

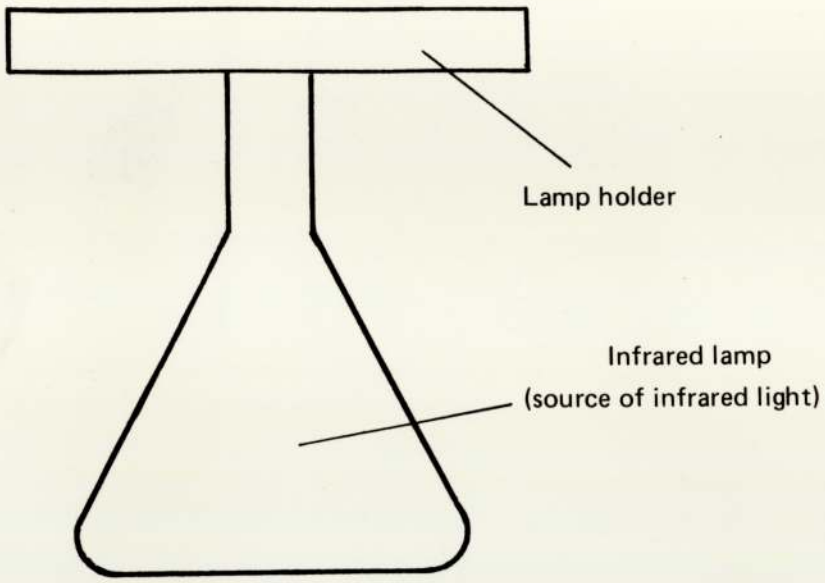
Discs of Sporotrichum pulverulentum were cut out from four-day old cultures. Each fungal disc was placed in between the upper and lower blocks of wood. Each pair of inoculated wood blocks (ie. top block on lower block) were placed in a deep glass petri dish. The wood pieces were perfused with sterile distilled water with the aid of sterilised lamp wicks which were fitted into holes drilled into the lower blocks of wood prior to the wood sterilisation (Figure 18). The whole manipulation was carried out aseptically in the inoculating chamber.

The petri dishes containing the inoculated wood blocks were finally irradiated with an infra-red lamp at a level which gave the wood blocks a dose of 400 cal cm^{-2} day^{-1}. At this level of irradiation the wood "surface and internal" temperatures were raised to a range of $37^{o}C$ - $42^{o}C$. The wood temperatures were recorded with the aid of a thermocouple. The experiment was left for observation for eight weeks. The temperature of the room where the experiment was conducted was maintained at a range of $27^{o}C$ - $30^{o}C$ throughout the duration of the experiment. The experiment was repeated using Myceliophthora thermophilum.

8.4 RESULTS

The non-treated and non-varnished wood blocks were completely colonised by the test fungi (Plate 11). When the water activity

Fig. 18 **The Colonisation of Wood by Fungi on Infrared Light Irradiated Wood**



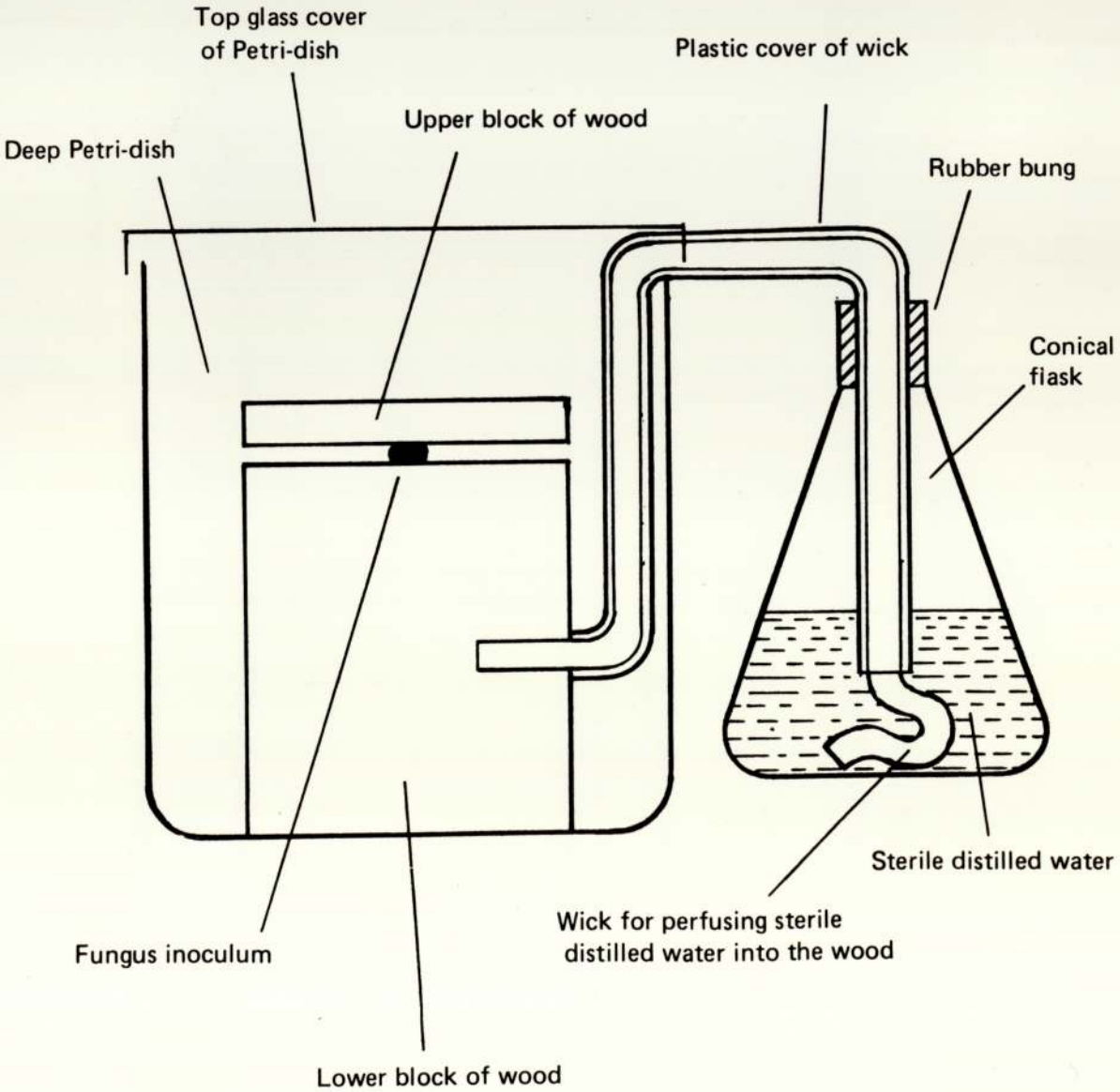


PLATE 11: The colonisation of simulated wood joints (non-treated and non-varnished) by Sporotrichum pulverulentum under infra-red rays irradiation.

in between the wood blocks was assessed it gave a range of 0.85 - 1.0.

For the wood block with all surfaces varnished except the meeting surfaces, the test fungi grew out in between the wood blocks and colonised the wood blocks. The water activity in between the top and lower blocks of wood in this case ranged from 0.75 - 0.85.

The wood blocks with cracks designed on them were colonised too but not as much as was observed in the two sets of wood blocks described already. The all-surface varnished wood blocks together with the preservative treated and varnished wood blocks were not colonised by the test fungi.

8.5 DISCUSSION

Solar radiation can heat up wood to the level that can support the germination and growth of thermophilic and thermotolerant fungi (Morton, 1975). Some of these fungi like *Myceliophthora thermophilum* and *S. pulverulentum* have been shown in the present study to cause wood decay. Temperature influences the germination of spores as has been shown in the present experiment. It might be that temperature affects the basic biochemical reactions of germination. It could also limit or control the production of germ tubes. Since the reactions in spores are enzymatic, it might be the effect of temperature on the specific protein involved in germination that limits or promotes the germination process.

Wood has a low thermal conductivity when compared to other structural materials. The thermal conductivity becomes greater with increase in the moisture content of the wood (Browning, 1971). In addition to its low conductivity wood possesses low thermal capacity which means that it will warm up rapidly when exposed to sources of heat. Morton (1975) investigated the effect of insolation on the temperature ranges and moisture content in wood. He observed that the effect of insolation was most marked in direct sunlight, in wood dyed black when temperatures in excess of 45^{o}C were recorded, not only at the surface of wood but within wood, representing a value of 16^{o}C above ambient temperature. He recorded values in the order of 10^{o}C above ambient as a result of insolation for wood painted white and in varnished wood. Similar temperature rises were obtained during the present study at the level of irradiation.

The high temperature developed by the heated wood blocks in the experiment must have given rise to the evaporation of water molecules from the interior parts of the wood through the unvarnished wood surfaces into the spaces between the wood blocks. This might have resulted in the increased water activity observed in these spaces. The water activity of a material is the ratio of the vapour pressure of water over the substance to the vapour pressure over pure water at the same temperature. It is the most useful expression of water requirements for, or water relations of microbial growth (Scott, 1957).

The lower limit for the continued growth of any fungus in the laboratory is about 0.65 Aw and in practice materials kept below this level are not damaged by fungi although many materials are known to be attacked at 0.7 and 0.75 Aw. The water activities recorded in the spaces between the colonised blocks of wood were within ranges that will support fungal germination and growth (Ayerst, 1969).

If similar water activities result in roofing joinery, thermophilic, thermotolerant and mesphilic fungal spores which have landed on them from the aerial mycoflora could germinate, grow out from them and colonise the entire wood used for the roofing despite their protective coating. This might even become worse if the painted surfaces have dirt retention because dirt will supply extra nutrients for the invading fungi. The invasion could also be aided by the weathering of the painted surface due to unfavourable environmental conditions as known to exist in the tropics. Ultra-violet light can pass through the film and bleach or degrade the underlying wood surface. In the case of varnished surfaces, there could be premature varnish failure due to detachment of degraded wood surface layer brought about by water getting behind the film where end grain is exposed as could be seen in joints. A fungus like Merulius lacrymans which can attack timber with a moisture content as low as about 20% and which is capable of conducting water from one part of the timber to another could gain access to such unvarnished roofing joints.

All timber joinery meant for Nigerian house roofs and other wooden constructions should be treated where possible before being

put together. All cracks on varnished wood surfaces should be sealed off. The essence of varnishing is to cut off oxygen from the internal parts of the wood. Decay fungi will not develop in the absence of oxygen. If cracks result in varnished wood surfaces, oxygen gains access into the wood, and any fungal spore that germinates in such a situation propagates itself, other conditions being favourable. This was seen in the present study.

CHAPTER 9

THE FUNGI OF NIGERIAN WOOD BARK INCLUDING COLONISATION EXPERIMENTS BY THERMOPHILIC AND THERMOTOLERANT FUNGI FROM NIGERIAN MUNICIPAL COMPOST

9.1 THE FUNGAL FLORA OF NIGERIAN WOOD BARK

INTRODUCTION

Bark fungi are of interest to ecologists and taxonomists who are interested in the decay of wood tissues.The wood bark is protective in function and since it is in contact with the aerial enviroment there is every possibility that aerial spores can get deposited on it in standing trees. Some of these spores could colonise the wood bark in cut timbers when conditions become favourable and help in its eventual decompostion. A specialised mycoflora mainly of Pyrenomycetes and Aphyllophorales are involved in the early stages of bark decomposition in logs and there has been much speculation about the exact role of some of these fungi (Chesters, 1950). Cappacrt, Verdonck and Boodt (1976) reported that the influences of the moisture content on the decomposition of bark is very important. They found that there was a higher decomposition rate at 60% than at 40% moisture. They found that the addition of a mineral nitrogen fertiliser to the bark increased its rate of decomposition. As excess of nitrogen on the contrary decreased the decomposition of the bark. The optimal addition of mineral nitrogen was respectively 0.5% for bark with a moisture content of 40% and 0.8% for bark with 60% moisture content. Their report did not make any mention of the fungal species involved in the bark decomposition.

Bark is currently one of the most puzzling residue problems facing the wood conversion industries in Nigeria. The amount of produced bark, almost 6% of the wood, is so high that a continuous

removal from the mill sites is required. Different outlets of utilisation of the bark have been proposed such as tannin extraction, production of fire brickets and burning of bark as fuel. With the increasing interest in recycling of waste products in Nigeria, the trend for using of bark as a substrate for horticultural purposes has become more important.

If wood bark is left lying about on the soil there will be a tendency for the heavy rain that falls in Nigeria to leach the mineral nutrients it contains to the sub-soil. Such mineral nutrients will therefore be inaccessible to the land plants. But if the bark is composted, there will be a tendency for the mineral nutrients to get incorporated into the fungal mycelia and gradually get released to the soil for the plants' use.

The aim of the first part of the experiment therefore was to investigate the fungal flora of Nigeria wood bark while the second part dealt with the colonisation of the wood bark by some of the bark fungi plus thermophilic and thermotolerant fungi from Nigerian municipal compost.

9.2 MATERIALS AND METHODS

The bark of Canarium schweinfurthii was employed in the present experiment. The bark was obtained from AT & P, Sapele, Nigeria. It was grey in colour and was found to be rough and fissured. It was cut into 1cm x 1cm pieces with the aid of a hack saw which had its blade sterilised with 95% alcohol prior to cutting. One portion of the wood bark was washed in five changes of sterile

distilled water with the aid of a horizontal mechanical shaker and the remaining portion was left unwashed.

The unwashed pieces were plated out on malt extract agar (MEA) and cellulose agar (CA), each plate containing five pieces of the 1cm x 1cm bark pieces. Twenty of such plates (10 MEA and 10 CA) were incubated at 25°C, 35°C and 45°C to encourage the growth of mesophilic, thermotolerant and thermophilic fungi respectively. The plates were examined after seven and fourteen days. The same procedures were followed for the washed bark pieces.

9.3 RESULTS

Twenty-eight fungi were isolated from the wood bark and the isolates are listed in Appendix 16. More fungi were isolated from the unwashed bark pieces than the washed pieces. Most of the isolates might have been ordinary surface contaminants from the aerial environment. Fungal species like Absidia corymbifera, Aspergillus fumigatus, Alternaria sp. Cladosporium herbarum, Curvularia lunata and Mucor pusillus have been reported from the aerial environment (Appendix-14). The thirteen fungal species isolated from the washed bark pieces might be real bark colonisers which could play an active role in its eventual decomposition.

9.4 THERMOPHILIC AND THERMOTOLERANT FUNGI FROM NIGERIAN MUNICIPAL COMPOST

Fungi, especially thermophilic species have often been reported from composts. Waksmann, Umbreit and Cordon (1939) were the first to demonstrate the importance of a thermophilic fungus in the decomposition of stable manure. Henssen (1957) successfully isolated five thermophilic fungi from stable manure, and demonstrated the ability of Humicola insolens to decompose cellulose and Sporotrichum thermophile to decompose hemicellulose and pectin. Gregory, Lacey, Festenstein and Skinner (1963) carried out an extensive investigation on the microbial and biochemical changes in mouldy timothy and fescue grass hay. They concerned themselves with the changes in the major carbohydrate constituents of the composting material. Chang and Hudson (1967) employed three methods, direct observation, plating out of washed particles and dilution plate to study the occurrence of fungi in experimental wheat straw compost. They considered the distribution of the thermotolerant and thermophilic fungi in detail and found it to be related in particular to variations in temperature recorded in the composts. Kane and Mullins (1973) systematically sampled a high-rate municipal compost system during the digestion period for the presence of thermophilic fungi. They established pure cultures of 304 isolates which included Aspergillus fumigatus, Chaetomium thermophile, Humicola lanuginosa, Mucor pusillus, Thermoascus aurantiacus and Torula thermophila. Their representative isolates demonstrated the relationship between temperature and growth, the requirement for aerobic growth

conditions and that Chaetomium thermophile could utilise cellulose as a carbon source.

One of the fundamental properties of composting is thermogenesis resulting from microbial activity. It is this property that is of primary importance to the acceptibility of composting as a sanitary method of waste disposal. Temperatures in excess of 60°C are common and they have been recommeded as being essential to the production of a stable and sanitary product. Eastwood (1952), for example, showed that cut barley straw compost attained a temperature of 64°C in the centre on the fifth day of composting. This development of a high temperature phase makes it difficult for mesophilic fungi to survive in composting systems.

The present experiment was carried out to find out (1) the thermophilic and thermotolerant mycoflora of Nigerian municipal compost, (2) the relationship between such mycoflora and those isolated from the soil samples, (3) to investigate the colonisation of Nigeria wood bark by some of the isolated thermophilic and thermotolerant fungi, and (4) to see if wood bark could be composted and decomposed.

9.5 MATERIALS AND METHODS

Compost samples were obtained from Jos, Nigeria Municipal compost system. These samples were collected at a depth of 15cm from the surface of the compost. The temperature, pH and percent moisture content were recorded at sampling points.

Portions of the compost samples were plated onto Emerson YpSs agar, malt extract agar, E & P cellulose agar and Littmans agar medium. Drops of streptomycin sulphate solution (3g/litre) were added to suppress the growth of bacteria. The plates were then incubated at 45°C for seven days and examined for the presence of thermophilic and thermotolerant fungi. The plates were re-examined a week later for additional species of these fungi. The pure cultures obtained were grown on YSA and were identified.

9.6 RESULTS

Thirty species of thermophilic and thermotolerant fungi were isolated, from the Jos municipal compost samples and these are given in Appendix 17. All the species have been reported from the Nigerian soil samples in Chapter 2 of this thesis. Some have also been reported from the aerial enviroment (Appendix 14). These fungi must have colonised the municipal compost from the soil or the aerial environment. Most of the fungi are active decomposers and many are capable of degrading cellulose. The ecological parameters of the compost samples are presented in Table 6. The pH value recorded in the compost is well within the range that will give good growth in laboratory cultures. The temperature recorded in the compost system was high enough to support the growth of thermophilic and thermotolerant fungi.

TABLE 6

ECOLOGICAL MEASUREMENTS OF THE MUNICIPAL COMPOST SAMPLES

MUNICIPAL COMPOST SAMPLES	ECOLOGICAL PARAMETERS RECORDED
pH value	6.4 - 6.7
Water content	40%
Colour of sample	Black
Temperature at depth of sample collection	$53^{o}C$

9.7 THE COLONISATION OF NIGERIAN WOOD BARK BY THERMOPHILIC AND THERMOTOLERANT FUNGI ISOLATED FROM NIGERIAN MUNICIPAL COMPOST

Bark is resistant to decay and because of this it was considered necessary to employ fungal strains found in a composting system.

50gm of the wood bark were weighed out into each of three 500ml. sterile conical flasks. Another 50gm portion which had been pulverised was weighed into three further 500ml. sterile conical flasks. The two portions were heated to 80°C and then cooled down to -18°C. This procedure was repeated seven times. Preliminary experiments revealed that such treatments killed off the initial fungal flora of the bark sample.

Each flask was then inoculated with 1ml. of equal dilutions of Chaetomium thermophile var. coprophile, Myceliophthora thermophilum, Aspergillus fumigatus and Sporotrichum pulverulentum. These fungi were chosen because of their strong cellulolytic activities.

The wood bark in the flasks was adequately moistened with sterile distilled water (65% moisture content) and incubated at 40°C (the optimum growth temperature for many thermophilic fungi). The samples were left for observation for their eventual colonisation by the test fungi. The observation period lasted for six months. Reisolation experiments were carried out at the end of composting to make sure that the test fungi were still there and that there had not been other fungal contaminants.

Loss in weight by the bark samples was regarded as due to colonisation by the test fungal species. The completion of composting was determined by the production of mass which did not decompose any

further or produce offensive odours as readily as the original material. The completion of composting was equally determined by taking note of the pH changes that take place in organic material during composting.

9.8 RESULTS

There were considerable weight reductions in the two sets of composted bark. There was more weight reduction in the pulverised bark than the non-pulverised bark.

The fungal re-isolation experiments carried out at the end of the experiment showed that the test fungi were still present at the end of the experiment. There were no fungal contaminants. In both pulverised and non-pulverised bark samples, the pH was higher after composting. The composting process seemed to have gone far. The details of the results obtained from the bark composting are given in Table 7.

TABLE 7

WEIGHT LOSSES OF COMPOSTED NIGERIAN WOOD BARK

	INITIAL WEIGHT	FINAL WEIGHT	% WEIGHT LOSS	INITIAL pH	FINAL pH
PULVERISED BARK	50.0gm	10.50gm	79	6.2	7.2
NON-PULVERISED BARK	50.0	17.30	65.40	6.2	6.8

9.9 DISCUSSION

The results of the experiment have shown that wood bark can be composted. The pH of the pulverised bark was tending towards the alkaline side, depicting that the bark composting was nearing completion. Jann, Howard and Salle (1959) reported that organic material if fully composted or stabilised will maintain an alkaline pH for twenty-four hours under these conditions. They reported that such a compost will not undergo anaerobic fermentation on storage.

Bark is a difficult material to compost probably because of its high carbon/nitrogen ratio which makes it unattractive to saprophytic micro-organisms and it could also be because two of its main constituents, cork and lignin are resistant to decay. The greater weight loss observed for the pulverised bark might have been due to the more surface area created for colonisation by the microfungi by reducing the bark to fine particles.

Probably the best outlet for bark is as a compost for use in horticulture. It is possible that such composted wood bark might be suitable for mushroom culture in Nigeria. Apart from mushroom cultivation, bark makes a long-lasting mulch for horticultural crops, giving good weed suppression and soil moisture retention. If bark is first pulverised, it has a tidier appearance and is probably less resistant to penetration by rain. It is often claimed that if granulated bark is dug into the soil it can improve fertility by increasing aeration or by assisting the retention of moisture. The former property might well be

useful on heavy soils and the latter on sandy soils, but so far as is known no well-designed experiments have been carried out to determine the extent to which this might be true.

Apart from composting, a possible alternative use of wood-bark in Nigeria will be in leather manufacture. So far, the value placed on bark by tanners in Nigeria is not sufficient and the quality of their leather products can be improved by the use of this material.

Bark can be used also as a deep litter medium for poultry, but it should be delivered dry, although the cost of artificial drying would make it uneconomic.

CHAPTER 10

GENERAL DISCUSSION

The quality of the plywood produced by the AT & P Sapele, Nigeria is below standard because of the use of already fungal infected sheets (Ifebueme 1979). This is particularly true of the cores or inner layers of plywood that are made from non-durable or perishable timber species. It has been reported that there are many problems with the decorative veneers and interior plywood sheets due to dry wood insects.

The present study was designed to find the effects of Nigerian fungi on Nigerian wood and to provide fundamental information on the effects of fungal colonisation of wood veneers exposed at the factory.

A hypothesis was put forward that in addition to the direct attacks on the particular substrate under investigation, the soil and the aerial environment are the potential sources of fungal biodeteriogens. In order to prove this hypothesis, a survey of the Nigerian soil samples, aerial environment and timber was carried out. The results showed the occurrence of mesophilic, thermotolerant and thermophilic fungi in the three environments examined. Among the isolates were potential fungal biodeteriogens.

Most of the fungal isolates had already been reported from other parts of the world. A comparison of the thermophilic fungal isolates from the Nigerian soil samples and temperate soil samples showed that the Tropical Nigerian soil samples contained more thermophilic fungal species than have been reported from any other single source. The dry season proved favourable for the collection of thermophilic fungi while the wet season was found favourable

for the isolation of thermotolerant and mesophilic fungi. There was a direct relationship between the mycoflora of the Nigerian soil samples, aerial environment and wood samples.

The soil-wood fungal inter-relationship provided valuable information which helped in the understanding of the complex process of wood decay in the soil. The results of the survey showed that wood exposed at the factory was liable to colonisation by both aerial and soil fungi. The fungal isolates from the wood included isolates from stained areas of the wood. Momoh (1966) showed that logs are blue-stained more quickly in the wet season than in the dry season. He therefore suggested that the more susceptible hosts such as Antiaris and Pterygota should be felled in the dry season.

Apart from fungi, actinomycetes and bacteria were also isolated.

The work described in Chapter 5 showed the effects of these fungi on Nigerian wood. The bending strength and weights of the buried wood pieces were drastically reduced by the Nigerian soil fungi. The decayed wood pieces showed intensive soft rot cavity formation on the cell walls when sections were cut from them. Proctor (1941) concluded from his microscopical study of bore-hole formation that the holes result from enzymatic dissolution of the wood substance rather than by mechanical forces imposed by advancing hyphal tips.

The major environmental factors which affected wood transformation by fungi in the Nigerian soil may have been the availability of mineral nutrients in the soil samples (Table 2). temperature (soil incubated at 30°C), good aeration, moisture content (soil raised to 80% of its water holding capacity before wood pieces were buried), soil pH (value found to be within the range that could give good growth of fungi in pure culture) and the presence or absence of carbohydrates in the soil.

The fungi which colonised the test veneer pieces at the factory were found to have reduced the bending strength of the pieces to 25% and the original weight to 38% after incubation for six months in the laboratory. No major losses in either bending strength or weight were recorded in the preservative-treated wood pieces. The study also revealed the ability of the test fungi to decay wood. Sporotrichum pulverulentum was found to be the most aggressive of the test fungi. It reduced most of the wood pieces to compressible states. The test fungi were found to hydrolyse starch. Starch serves the plant as a storage product and as such is a major reserve carbohydrate. This product is produced in large amounts in leaves carrying out photosynthesis and is present in the xylem parenchyma, phloem, cortex and pith of the woody plants. Plant starches usually contain two components, amylose and amylopectin. Starch decompostion producing glucose, is likely to proceed at a greater rate than the microbiologically induced losses of cellulose, hemicelluloses and lignin because it is simpler in form. Starch-hydrolysing enzymes, the amylases, are characteristically

extracellular and remain in the culture fluid after the removal of the micro-organisms.

Cellulose and lignin comprise the bulk of any wood. The test fungi were found to be cellulolytic. They were also found to grow in a medium containing lignin as sole carbon source. Alexander (1964) found that forty-four of a total of forty-six soil inhabiting Basidiomycetes could degrade both lignin and cellulose. Wood-destroying fungi have been divided into the brown rotting group which effects the cellulose of wood forming reducing sugars but these fungi do not attack the lignin, and a group of white rotting fungi which attack both lignin and cellulosic constituents. Both brown rot and white rot were observed in the decayed wood pieces. Lignin is probably depolymerised to give simple aromatic substances such as vanillin and vanillic acid or possibly other methoxylated aromatic structures. The enzyme systems of the fungi which attack the lignin component of wood are likely to be extracellular.

In Chapter 7, the ability of the Nigerian soil fungi to decompose wood at different biocidal concentrations was shown. While these fungi were able to decay wood at the lower preservative concentrations used they were unable to decay wood at full preservative concentration within the six months of exposure. Few studies have reported on the detoxification of preservative treated wood. The observed tolerances of some wood decay fungi to copper-chrome-arsenate (CCA) treated wood have been linked to their ability to solubilise the copper, thereby allowing it to leach out of the wood (Levi, 1969). Greaves (1973) reported that CCA affects the permeability of bacterial cell membranes. Other

workers have also reported on the accumulation of the components of the preservative in the hyphal cells thereby killing the fungi (Chou, Preston and Levi 1974).

Chapter 8 showed the ability of some of the fungal isolates to utilise amino acids as sole nitrogen and carbon source. The medium containing a mixture of amino acids proved to be a better-growth medium than that containing a single amino acid. However, the amount of mycelial growth observed in the medium where an amino acid (or amino acids) had been employed as sole nitrogen source and sole carbon source was smaller than that obtained in the E & P CA. In the same chapter, the ability of some of the fungal isolates to utilise amino acids as the sole nitrogen source for cellulolytic activity was shown. Again there was greater cellulolysis in a medium containing a mixture of amino acids than in that containing one amino acid. The significance of these amino acid experiments is that it showed that the Nigerian fungi had the ability to utilise amino acids in the Nigerian wood during the wood decay. The amino acids specifically associated with wood have been reported by Baker et al. (1970) and Hardie (1979).

The irradiation of wood with infra-red rays showed that this was able to raise the wood temperature to the level in which it supported the germination and growth of thermophilic and thermotolerant fungal isolates.

The rotting of timber and the growth of fungi on wooden joints and painted surfaces result from the deposition of fungal

spores of many kinds which have been shown to be widely distributed in the Nigerian aerial environment and which would propagate when the wood temperature is favourable and when the moisture content exceeds about 22%. The principal agents of wooden joint decay in Nigeria especially in house roofings are likely to be soft rot fungi. The sapwood of all species is regarded as perishable but it cannot be economically excluded from joinery grade timber. Since it cannot be ensured that the moisture content of joinery remains at a safe level, even when properly painted, preservative treatment is necessary. The economic considerations of wood decay, the part it plays in Nigerian economy and the need to preserve timber are reviewed in Appendix 18.

The most inexpensive methods to preserve wood in Nigeria will be to use naturally durable timber, better seasoning and storage, control of moisture levels, better design and construction practices. It may not always be possible to obtain naturally durable timber and in fact it is becoming more expensive to obtain as the stock of our naturally durable species diminishes. Lesser known and plantation grown trees may become the timber of the future. Cleanliness in sawmills can only lead to a reduction in the inoculum potential of wood deteriorating organisms but will not prevent infection of wood completely.

Chemical wood preservatives are available for the control of wood decay but successful control depends upon the strict adherence to details such as wood specification, treatability of

wood and strict adherence to the prescribed timber species.

In the selection of a wood preservative or preservatives, end-product performance should take into account three main points, the species of wood, the method of application and the environment in which the timber will be ultimately used. Savory (1980) has recommended the sap-displacement method for preservative-treatment of wood.

Creosote proved successful in the present study but it is not advisable to use it for the treatment of indoor timber because of health hazards and creosote-treated wood cannot be painted easily. Many wood preservatives which have proved successful in the prevention of wood deterioration are available in the market and they should be sought. Though this will increase the cost of the wood, it has been pointed out (Watson, 1975) that chemical preservation should be regarded as an insurance premium resulting in direct saving in money, and that treated timber was an inherently superior material on a cost/performance basis rather than just a more costly improved commodity. By preserving perishable timber a maximum advantage of Nigeria's natural forest resources can be taken by reserving the supply of the primary species for export and ensuring that local requirements are met by indigenous secondary species.

The purpose of this study was to investigate the presence of fungi in the soil and on wood in Nigeria, and to assess the part that these fungi can play in the decay of Nigerian timber. The fungi isolated have been shown to be very capable of causing

economic losses: however, it has been suggested that their activities can be harnessed for man's advantage in the decomposition of waste products such as bark. There is great scope for further work in this field.

APPENDIX 1

APPENDIX 1

THERMOPHILIC AND THERMOTOLERANT FUNGI ISOLATED DURING THE SURVEY

THERMOPHILES

ASCOMYCETES

Chaetomium thermophile La Touche *var. coprophile* Cooney and Emerson

C. thermophile La Touche *var. dissitum* Cooney and Emerson

Corynascus thermophilus (Fergus and Sinden) v. Klopotek (=*Thielavia thermophila* Fergus and Sinden)

Melanocarpus albomyces (Cooney and Emerson) v. Arx (=*Myriococcum albomyces* Cooney and Emerson)

Talaromyces emersonii Stolk

T. thermophilus Stolk (=*Talaromyces dupontii* (Griffon and Maublanc) Emerson)

Thermoascus aurantiacus Miehe

Thermoascus crustaceus (Apinis and Chesters) Stolk (=*Dactylomyces crustaceus* Apinis and Chesters)

T. thermophilus (Sopp) v. Arx (=*Dactylomyces thermophilus* Sopp)

continued

Thielavia australiensis Tansey and Jack

T. terrestris (Apinis) Malloch and Cain (=Allescheria terrestris Apinis)

Unidentified sp. Y (241431) suspected to be a Coniochaetidium sp. The sporulation is very poor. Its spore size suggest the species is undescribed - Dr. Hawksworth, Commonwealth Mycological Institute.

HYPHOMYCETES (Fungi Imperfecti)

Acremonium alabamensis Morgan-Jones

Calcarisporiella thermophila (Evans) de Hoog

Humicola grísea var. thermoidea Cooney and Emerson

H. insolens Cooney and Emerson

Malbranchea pulchella var. sulphurea (Miehe) Sigler and Carmichael

Myceliophthora thermophilum (Apinis) v. Oorschot

Myrioconium thermophilum (Fergus) v.d. Aa (=Papulaspora thermophila Fergus)

Scytalidium thermophilum (Cooney and Emerson) Austwick (=Torula thermophila Cooney and Emerson)

Continued ...

Stilbella thermophila Fergus

Thermomyces ibadanensis Apinis and Eggins

T. lanuginosus Tsiklinski

T. stellatus (Bunce) Apinis

Unidentified sp Y^2 =Thielavia sp. (Is being examined further - Dr. R. A. Samson - Centraalbureau voor schimmelcultures - Baarn (Netherlands)

Unidentified sp Z = (Suspected to be a variety of Thermomyces ibadanensis Apinis and Eggins)

Sterile hyaline mycelium

PHYCOMYCETES

Mucor miehei Cooney and Emerson

M. pusillus Lindt

APPENDIX 2

APPENDIX 2

THERMOTOLERANT FUNGI

ASCOMYCETES

Chaetomium rectopilium Fergus and Amelung

C. virginicum Ames

Corynascus sepedonium (Emmons) v. Arx (=Thielavia sepedonium Emmons)

Emericella nidulans (Eidam) Wint

FUNGI IMPERFECTI

Aspergillus candidus Link ex Fr

A. fumigatus Fres

A. niger series

A. terreus Thom

Paecilomyces variotii Bain

Penicillium argillaceum Stolk et al

Sporotrichum pulverulentum Novobranova

Continued ...

PHYCOMYCETES

Absidia corymbifera (Cohn) Sacc. and Trotter (=Absidia ramosa (Lindt)) Lender sensu Nottebrock et al

Mortierella wolfii Mehrotra and Baijal

Rhizopus microsporus van Tieghem

R. sp.

APPENDIX 3

APPENDIX 3

MESOPHILIC FUNGI ISOLATED FROM THE NIGERIAN SOIL SAMPLES

ASCOMYCETES

Chaetomium bostrychodes Zopf

C. cancroideum Tschudy, Am

C. cochliodes Palliser

C. globosum Kunze

C. homopilatum Omvik

C. nigricolor Ames

C. senegalensis sp. nov. Ames

C. spirale Zopf

Fimetaria fimicola (Roberge) Griffiths and Seaver

Gymnoascus reesii Baranetzky

Myxotrichum chartarum Kunze

Continued ...

BASIDIOMYCETES

Basidiomycete sp. 1 (with clamp connections)

Basidiomycete sp. 2 (✓)

FUNGI IMPERFECTI

Acremoniella atra Sacc

Aspergillus awamori Nakazawa

A. candidus Link

A. flavus Link

A. funiculosus Smith

A. giganteus Wehmer

A. glaucus Link

A. japanicus Saito

A. longivesica Huang and Raper

A. niger van Tieghem

A. niger series

A. ochraceus Wilhelm

Continued ...

A. ornatus Raper, Fennell, and Tresner

A. oryzae Cohn

A. quadrilineatus Thom and Raper

A. repens de Bary

A. restrictus Smith

A. ruber Thom and Church

A. rugulosus Thom and Raper

A. sclerotiorum Huber

A. stellatus Curzi

A. tamarii Kita

A. ustus Thom and Church

A. versicolor Tiraboschi

A. wentii Wehmer

A. zonatus Kwon and Fennell

Alternaria alternata Keissler

Continued ...

Aureobasidium pullulans

Balanium sp

Botrytis cinerea Pers. ex Pers.

Cladosporium herbarum Link

C. sp. 1

C. sp. 2

Curvularia lunata Beodijn

C. tetramera Boedijn

Epicoccum purpurascens Ehrenberg

Fusarium argillaceum Saccardo

F. avenaceum Saccardo

F. bostrycoides Wollenweber and Reinking

F. culmorum Saccardo

F. fusarioides Booth comb. nov.

F. heterosporum Nees ex Fr.

Continued ...

F. javanicum Koorders

F. moniliforme Sheldon

F. oxysporum Schlechtendahl

F. poae Wollenweber

F. roseum Link

F. semitectum Berkeley and Ravenel

F. solani Appel and Wollenweber

F. sporotrichioides Sherbakoff

Geotrichum candidum Link

Gliocladium atrum Gilman and Abbott

G. roseum Link

G. vermoeseni Thom

G. sp. 1

G. sp. 2

Graphium sp.

Continued ...

Helminthosporium sativum Pammel, King and Bakke

H. sp.

Memnoniella echinata Galloway

Nigrospora oryzae Molliard

N. sphaerica Mason

Olpitrichum sp.

Papularia sphaerosperma von Hohnel

Penicillium chrysogenum Thom

P. citrinum Thom

P. funiculosum Thom

P. javanicum van Beyma

P. lapidosum Raper and Fennell

P. nigricans Bainier

P. notatum Westling

P. thomii Maire

Continued ...

P. sp. 1

P. sp. 2

Pleospora herbarum Rabenh

Pithomyces chartarum Ellis

Sepedonium chrysospermum Fries

Stachybotrys atra Corda

S. lobulata Berkeley

Thielaviopsis basicola Zopf

Torula herbarum Gray

T. graminis Desm.

Trichocladium asperum Harz

T. pyriforme Dixon

Trichoderma viride Rifai aggr.

T. hamatum Bain. aggr.

T. harzianum Rifai aggr.

Continued ...

T. koningii Oud. aggr.

T. longibrachiatum Rifai aggr.

T. polyspor um Rifai aggr.

T. piluliferum Webster and Rifai aggr.

Ulocladium atrum Preuss

U. botrytis Preuss

Volutella piriformis Gilman and Abbott

V. ciliata Fries

Wardomyces anomalus Brooks and Hansford

PHYCOMYCETES

Absidia blakesleeana Lendner

A. capillata van Tieghem

A. repens van Tieghem

A. spinosa Lendner

Achlya dubia Coker

Continued ...

A. racemosa Hildebrand

Allomyces arbuscula Butler

Blakeslea trispora Thaxter

Choanephora cucurbitarum Thaxter

Circinella simplex van Tieghem

C. spinosa van Tieghem et Le Monnier

C. tenella Zycha

Cunninghamella echinulata Thaxter

C. elegans Lendner

C. verticillata Paine

Isoachlya anisospora Coker

Mortierella bainieri Costantin

M. elongata Linnemann

M. nigrescens van Tieghem

M. ramanniana Linnemann

Continued ...

Mucor hiemalis Wehmer

M. mucedo Brefeld

M. plumbeus Bonorden

M. spinosus van Tieghem

Pythium sp.

Rhizopus arrhizus Fischer

R. nigricans Ehrenberg

R. oryzae Went and Gerlings

Syncephalastrum racemosum Schroeter

Thamnidium elegans Link

Zygorhynchus heterogamus Vuillemin

Z. moelleri Vuillemin

MYCELIA STERILIA

Papulaspora sp. 1

P. sp. 2

Continued ...

Sterile dematiaceous mycelium 1

Sterile dematiaceous mycelium 2

Sterile hyaline mycelium

APPENDIX 4

APPENDIX 4

THERMOPHILIC AND THERMOTOLERANT FUNGAL SPECIES ISOLATED FROM THE CELLULOSE ENRICHED SOIL SAMPLES

ASCOMYCETES

THERMOPHILIC

Chaetomium thermophile var. *coprophile*

C. thermophile var. *dissitum*

Corynascus thermophilus

Thermoascus aurantiacus

Thielavia terrestris

THERMOTOLERANT

Emericella nidulans

FUNGI IMPERFECTI

THERMOPHILIC

Humicola grisea var. *thermoidea*

H. insolens

Malbranchea pulchella var. *sulphurea*

Myceliophthora thermophilum

Scytalidium thermophilum

Thermomyces lanuginosus

Unidentified sp. 1

Continued ...

THERMOTOLERANTS

Aspergillus candidus

A. fumigatus

A. terreus

Paecilomyces variotii

Sporotrichum pulverulentum

PHYCOMYCETES

THERMOPHILIC

Mucor pusillus

THERMOTOLERANT

Absidia corymbifera

APPENDIX 5

APPENDIX 5

FUNGI ISOLATED FROM THE LIGNIN ENRICHED SOIL SAMPLES

ASCOMYCETES

THERMOPHILIC

Chaetomium thermophile var. *coprophile*

Corynascus thermophilus

Talaromyces thermophilus

Thermoascus aurantiacus

THERMOTOLERANTS

Chaetomium virginicum

Corynascus sepedonium

FUNGI IMPERFECTI

THERMOPHILIC

Humicola grisea var. *thermoidea*

Malbranchea pulchella var. *sulphurea*

Myceliophthora thermophilum

Myrioconium thermophilum

Scytalidium thermophilum

Stilbella thermophila

Thermomyces ibadanensis

T. lanuginosus

Continued ...

THERMOTOLERANTS

Aspergillus fumigatus

A. niger series

A. terreus

Paecilomyces variotii

Sporotrichum pulverulentum

PHYCOMYCETES

THERMOPHILIC

Mucor miehei

M. pusillus

THERMOTOLERANTS

Absidia corymbifera

Rhizopus sp.

APPENDIX 6

APPENDIX 6

FUNGI ISOLATED FROM THE SOIL SAMPLES USING AMINO ACID AS THE SOLE NITROGEN SOURCE AND THIAMINE HYDROCHLORIDE AS SOURCE OF VITAMIN

ASCOMYCETES

THERMOPHILIC

Chaetomium thermophile var. *coprophile*

Corynascus thermophilus

Melanocarpus albomyces

Talaromyces thermophilus

Thermoascus aurantiacus

Thielavia terrestris

THERMOTOLERANT

Emericella nidulans

FUNGI IMPERFECTI

THERMOPHILIC

Humicola grisea var. *thermoidea*

Malbranchea pulchella var. *sulphurea*

Myceliophthora thermophilum

Myrioconium thermophilum

Scytalidium thermophilum

Thermomyces ibadanensis

Continued...

T. lanuginosus

THERMOTOLERANTS

Aspergillus fumigatus

A. niger series

A. terreus

Paecilomyces variotii

Penicillium argillaceum

Sporotrichum pulverulentum

PYCOMYCETES

THERMOPHILIC

Mucor miehei

M. pusillus

THERMOTOLERANTS

Absidia corymbifera

Mortierella wolfii

Rhizopus sp.

APPENDIX 7

APPENDIX 7

MONTHLY FUNGAL COLONIES PER DILUTION PLATE

METHOD OF PLATING	TEMPERATURE OF INCUBATION	
	25°C	45°C
Soil Dilution	Monthly Samples	Monthly Samples
	O N D J F M A M J J A S	O N D J F M A M J J A S
10^{0}	8 8 7 6 6 5 5 6 9 9 8 8	5 7 8 8 7 7 6 5 5 4 4 3
10^{-1}	6 7 5 4 5 4 4 6 7 7 7 7	4 5 7 7 6 6 5 4 4 3 3 3
10^{-2}	5 4 4 4 3 2 4 4 5 6 6 7	4 4 6 6 6 5 5 4 3 3 4 3
10^{-3}	5 3 4 3 2 1 1 4 4 5 5 5	3 4 4 2 3 4 3 2 3 2 2 2
10^{-4}	3 1 2 2 2 1 - 2 3 3 4 3	1 2 2 1 2 3 3 1 - 1 2 -
10^{-5}	2 1 1 1 - - - 2 2 3 2 1	- 1 2 - 1 1 1 1 - - 1 -
CONTROL	- -	

APPENDIX 8

APPENDIX 8

FUNGI ISOLATED FROM THE ROOT PIECES

UNWASHED ROOT PIECES

FUNGAL SPECIES

THERMOPHILIC

ASCOMYCETES

Chaetomium thermophile var. *coprophile*

C. thermophile var. *dissitum*

Corynascus thermophilus

Thermoascus aurantiacus

FUNGI IMPERFECTI

Humicola grisea var. *thermoidea*

Malbranchea pulchella var. *sulphurea*

PHYCOMYCETES

Mucor miehei

M. pusillus

THERMOTOLERANTS

ASCOMYCETES

Continued ...

Chaetomium virginicum

Emericella nidulans

FUNGI IMPERFECTI

Aspergillus fumigatus

A. terreus

Paecilomyces variotii

PHYCOMYCETES

Absidia corymbifera

Mortierella wolfii

Rhizopus sp.

MESOPHILIC

ASCOMYCETES

Chaetomium globosum

C. cochliodes

C. spirale

FUNGI IMPERFECTI

Aspergillus flavus

A. oryzae

Fusarium culmorum

F. oxysporum

Continued ...

PHYCOMYCETES

Absidia repens

Choanephora cucurbitarum

Cunninghamella echinulata

Rhizopus nigricans

Syncephalastrum racemosum

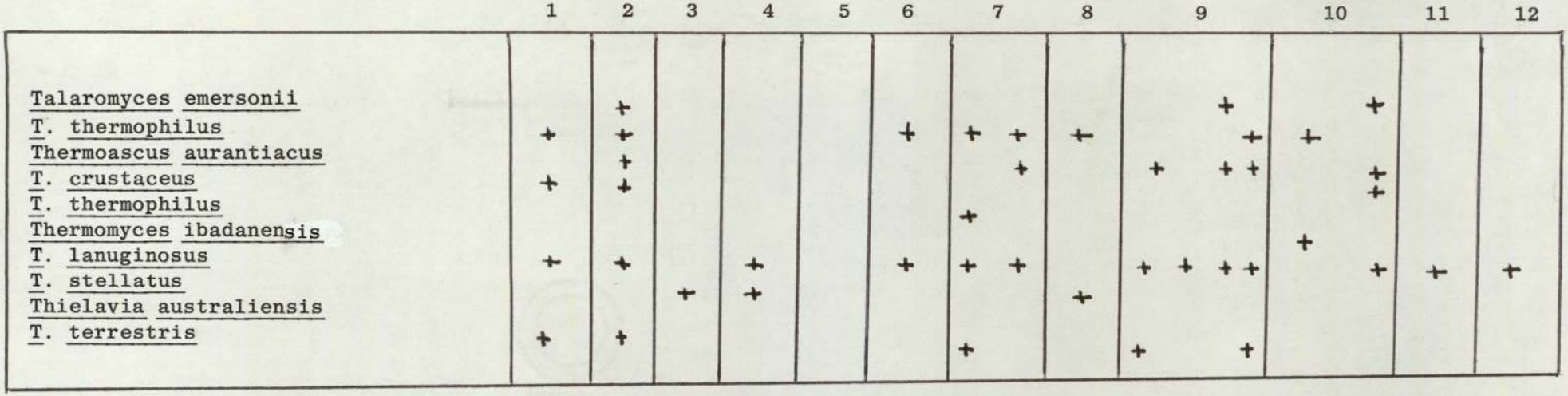
Zygorhynchus heterogamus

APPENDIX 9

APPENDIX 9

THERMOPHILIC FUNGI FROM NIGERIAN SOIL SAMPLES	OTHER SUBSTRATES FROM WHERE THE SAME FUNGI HAVE BEEN ISOLATED BY OTHER WORKERS											
	1 Air Spora (Hudson 1973)	2 Coal Spoil Tips (Evans 1971)	3 Hay (Bunce 1961)	4 Hay & Grass (Gregory & Lacey 1963)	5 Hot Spring (Tansey & Brock 1971)	6 Mushroom Compost (Fergus 1964)	7 Nesting Materials (Aphnis & Pugh 1967) (Tansey 1973)	8 Peat (Kuster & Locci 1964)	9 Soil (Aphnis 1963a) (Eggins et al 1972) (Mills 1973) (Tansey 1976)	10 Stacks Oil Palm Kernels (Aphnis & Eggins 1966) (Oso 1974)	11 Straw (Mills 1973)	12 Town Waste (Mills 1973)
Acremonium alabamensis												
Calcarisporiella thermophila		+ +										
Chaetomium thermophile var coprophile							+		+ +	+	+	+
C. thermophile var dissitum	*					+	+		+	+		
Corynascus thermophilus	+ +											
Humicola grisea var thermoidea		+ +				+ +			+			
Humicola insolens		+				+	+	+	+		+	+
Malbranchea pulchella var sulfurea	+						+		+			+
Melanocarpus albomyces		+										
Mucor miehei	+ +			+		+	+ +	+	+ +	+	+	+
M. pusillus		+										
Myceliophthora thermophilum									+			
Myrioconium thermophilum									+			
Scytalidium thermophilum							++		+			
Stibella thermophila						+			+			

... Continued ...



* means fungus is present

APPENDIX 10

APPENDIX 10

THERMOPHILIC AND THERMOTOLERANTS FUNGI ISOLATED FROM THE TEMPERATE SOIL SAMPLES

THERMOPHILIC FUNGI

FUNGI IMPERFECTI

* *Acremonium alabamensis*

Humicola grisea var. *thermoidea* Cooney and Emerson

Malbranchea pulchella var. *sulfurea*

* *Myceliophthora thermophilum* (Apinis) v. Oorschot

Thermomyces lanuginosus Tsiklinski

PHYCOMYCETES

Mucor pusillus Lindt

THERMOTOLERANT FUNGI

PHYCOMYCETES

Absidia corymbifera (Cohn) Sacc. and Trotter

Mortierella wolfii Mehrotra and Baijal

* Isolated after irradiation of the soil samples.

APPENDIX 11

APPENDIX 11

BACTERIA SPECIES ISOLATED FROM THE NIGERIAN SOIL SAMPLES

1. *Bacillus cereus* var. *mycoides* (Flügge) Smith, Gordan
2. *Bacilus cereus* Frankland and Frankland
3. *Bacillus coagulans* Hammer
4. *Bacillus megaterium* de Bary
5. *Bacillus sphaericus* Meyer and Neide
6. *Bacillus stearothermophilus* Donk
7. *Flavobacterium capsulatum* Leifson
8. *Micrococcus roseus* Flugge
9. *Micrococcus* sp (not viable)
10. *Sporocytophaga myxococcoides* (with yellow pigment) (Krzemieniewska) Stainier

ACTINOMYCETE SPECIES FROM THE NIGERIAN SOIL SAMPLES

1. *Actinomyces thermophilus* (isolated at 50^{0}C)

Continued ...

2. *Actinomyces* sp.

3. *A. thermofuscus* (isolated at 50°C)

APPENDIX 12

APPENDIX 12

SOIL-BAITING METHOD

10gm of each monthly soil sample was weighed out into 100ml. of sterile distilled water in a sterile conical flask. The flask was then swirled for about fifteen minutes in order to obtain a soil suspension. The soil suspension so obtained, was dispensed into ten sterile petri dishes, each petri dish containing 15ml. of the soil suspension. Three drops of streptomycin sulphate solution (3gm 1 litre) were added to each plate in order to suppress bacterial contamination. Five sterilised boiled hemp seeds which had been shelled were added to each petri dish containing the soil suspension. The plates were incubated at 25°C. The baits were subsequently examined for the presence of aquatic phycomycete species.

SURFACE INOCULATION OF TOMATO FRUITS WITH SOIL PARTICLES

Healthy tomato fruits were surface sterilised using 95% alcohol and inoculated with soil particles from the soil samples. These fruits were then incubated at 25°C and examined daily for the presence of fungal pathogens.

(The results have been included in the list of fungi isolated from the Nigerian soil samples).

ISOLATION OF KERATINOPHILIC FUNGI FROM THE NIGERIAN SOIL SAMPLES

10gm of each monthly soil sample was weighed out into each of a set of five petri dishes. Sterile distilled water was added

and sterilised human hair were sprinkled on the surface. The plates were wrapped in paper, incubated at 25°C and regularly examined for eight weeks for the presence of keratinophilic fungi.

RESULTS

Four fungal species were isolated with the hair baiting technique. These are:

Microsporum gypseum

Trichophyton sp.

Chrysosporium keratinophilum

Trichophyton ajelloi

APPENDIX 13

APPENDIX 13

COMPOSITION OF THE CULTURE MEDIA

(i) CELLULOSE AGAR (E x P CA)

Potassium dihydrogen phosphate	1.0g
Ammonium sulphate	0.5g
Potassium chloride	0.5g
D.L. Asparagine	0.5g
Yeast extract	0.5g
Magnesium sulphate	0.2g
Calcium chloride	0.1g
Agar	20.0g
Distilled water	1 litre

Ball-milled cellulose added to yield 2% suspension in the final mixture.

(ii) Czapek-Dox Agar

Agar	15.0g
$NaNO_3$	2.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.5g
KCL	0.5g
$FeSO_4.7H_2O$	10.0mg
Sucrose	30.0g
Distilled water	1000.0ml

(iii) LITTMAN'S AGAR MEDIUM (LAM)

Dextrose	10.0g
Peptone	10.0g
Oxgall	15.0g
Crystal violet	0.01g
Agar	20.0g
Distilled water	1000ml

Medium cooled down to 46°C after sterilization and sufficient strptomycin in sterile saline then added to produce a final concentration of 30 units/cc medium. Final pH 7.0.

(iv) LIGNIN AGAR

The same composition as cellulose agar except that lignin was substituted for ball-milled cellulose.

(v) MALT EXTRACT AGAR (MEA)

Agar	25.0g
Malt extract (Difco)	20.0g
Dextrose	20.0g
Peptone	1.0g
Distilled water	1000ml

(vi) POTATO-DEXTROSE AGAR

Agar	17.0g
Potatoes (peeled and sliced)	200.0g
Dextrose	20.0g
Distilled water	1000ml

(vii) RUSSELL AGAR MEDIUM FOR THE ISOLATION OF BASIDIOMYCETES

'Oxoid' desiccated malt extract	3.0g
'Oxoid' mycological peptone	0.5g
Agar	2.5g
o-Phenyl phenol	0.006g
Distilled water	100ml

The required amount of o-phenyl phenol was added from a stock solution prepared by dissolving 1gm in 50ml industrial alco hol. The solution was diluted to 100ml. with distilled water.

(viii) STARCH AGAR (SA)

Potassium dihydrogen phosphate	1.0g
Ammonium sulphate	0.5g
Potassium chloride	0.5g
D.L Asparagine	0.5g
Yeast Extract	0.5g
Magnesium sulphate	0.2g
Calcium chloride	0.1g
Agar	20.0g

Starch	10.0g
Distilled water	1000ml

(ix) SAWDUST AGAR (SDA)

The same composition as cellulose agar except that ball-milled cellulose was replaced with ball-milled sawdust (the final mixture contained 2% ball-milled sawdust in it).

(x) YEAST-STARCH AGAR (YSA)

Difco powdered yeast extract	4.0g
Dipotassium hydrogen phosphate	1.0g
Magnesium sulphate	0.5g
Soluble starch	15.0g
Agar	20.0g
Water ($\frac{1}{4}$ tap, $\frac{3}{4}$ distilled)	1000ml

APPENDIX 14

APPENDIX 14

THE FUNGAL SPORA OF THE AIR AT UNIVERSITY OF JOS, NIGERIA WHERE THE SOIL SAMPLES WERE COLLECTED

INTRODUCTION

The constitution of the air-spora is studied chiefly because of its importance in biodeterioration, respiratory allergy or diseases of plants.

While the composition of the air-spora at several localities in the temperate regions has been studied in some detail, very little information exists on the types of fungi in the air-spora of tropical regions.

The few tropical reports include that of Meredith (1962) who sampled the air in several Jamaican banana plantations with an automatic volumetric spore trap. His isolates included the following genera, Alternaria, Cladosporium, Curvularia, Deightoniella, Glomerella, Mycosphaerella, Nigrospora, Periconiella and Zygosporium. He found that in dry weather Cladosporium accounted for 23% of the total catch; coloured fusiform spores for 18%; coloured basidiospores for 16%; unclassified minute spores (including Sporobolomyces) for 14%; and Deightoniella only 0.2%.

Upsher (1968) recognising that fungal growth is copious at all times and that it is a major factor in the deterioration of materials exposed to hot/wet conditions at the Joint Tropical

Research Unit, Innisfail, North Queensland (Latitude $17\frac{1}{2}^{o}$S), carried out a survey to determine the nature and magnitude of the spore load of the air. He found Monilia sitophila to be the dominant airborne spore throughout the day during the dry season. It was also most abundant during the night. In the wet season, he found Geotrichum sp. to be dominant and also found it to be most abundant during the night. Other than the heterogeneous group of fungi failing to sporulate, he did not find any spore abundant throughout the year. But from the daily exposures at 0830 some frequency patterns were apparent. Cladosporium spp. were abundant from June to January and Leptosphaerulina australiae from April to November. Monilia sitophila and Epicoccum nigrum were regularly trapped from August to December, while Geotrichum sp. was trapped from March to May.

The few Nigerian reports include that of Cammack (1955) who investigated the seasonal changes in three common constituents of the air-spora of Southern Nigeria with the aid of an automatic volumetric spore trap. He found that the absolute values of spore concentrations illustrated that the wet season numbers of Puccinia polysora Underw, a leaf rust of maize, were much higher than those of the dry season. He found Cladosporium sp. concentration to be high in both seasons, while Nigrospora sphaerica (Sacc.) Mason, a dry rot of maize was higher in the dry season. He assumed that variations in spore numbers depended largely on the abundance of hosts in the two seasons as well as climatic factors.

Dransfield (1966) extended this work by carrying out a survey of the fungal air-spora at Samaru, in Northern Nigeria.

He found that the tropical genus Curvularia replaced Alternaria, which is more typical of cooler climates. The genera he recorded most frequently were Cladosporium (36.8%), Curvularia (25.1%), Fusarium (8.2%), Epicoccum (5.8%), Nigrospora (4.3%) and Pullularia (4%). He found that heavy rain-storms scrubbed the air relatively free from spores during the final weeks of the rainy season, but spore numbers increased when the dry season began. He equally discovered that variations in temperature and wind speed did not affect the numbers of spores deposited, but grass-cutting operations markedly increased the amount of Cladosporium, Aspergillus and Penicillium.

However, not one of the Nigerian reports has taken into account the thermophilic and thermotolerant fungal content of the air. Many of these thermophilic and thermotolerant fungal components of the air are active biodeteriogens and are capable of causing decay of wood if they land on exposed wooden surfaces, under favourable conditions of temperature and moisture content. The present study had four objectives. First to compare the air-spora of Jos where the soil samples were collected, with the fungal isolates from the soil samples. Secondly, to compare such air-spora with other Nigerian reports of air-spora. Thirdly, to find out if there are any seasonal variations in fungal air-spora of Jos and fourthly to discover if there is any relationship between the air-spora and the fungi isolated from wood veneers exposed at the Timber and Plywood Factory, Sapele, Nigeria.

MATERIALS AND METHODS

A simple spore trap was designed consisting of wooden platform containing apertures wide enough to hold 60 9cm diameter petri dishes (Figure 1 of Appendix 14). The platform was constructed in such a way that it could be adjusted to different angles in order to face directional winds, and to avoid direct flooding by rain.

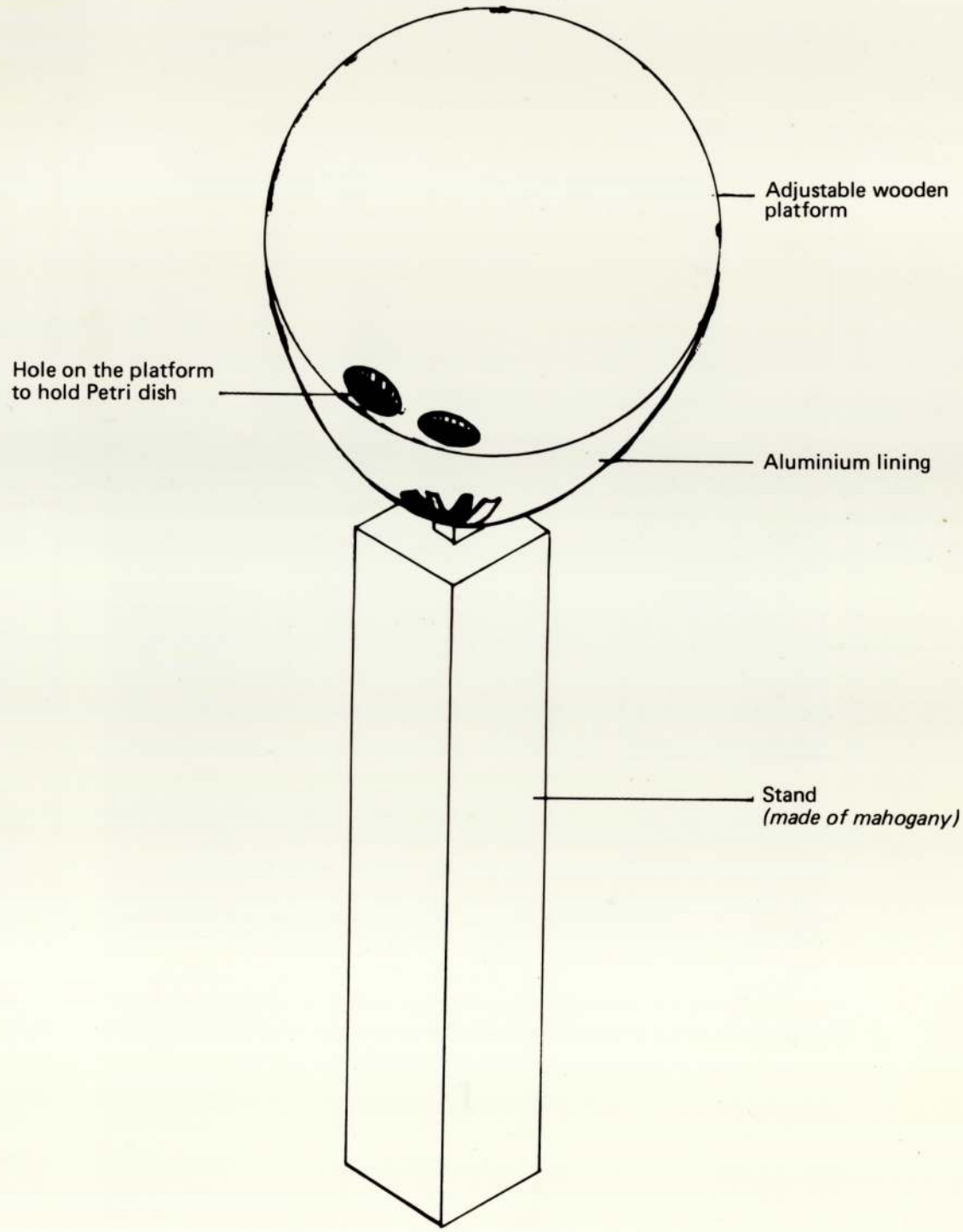
Trial petri plate exposures were made to determine suitable exposure durations and heights. It was found that one hour was long enough to accumulate as many as eight different fungal colonies. Sampling was carried out approximately 2.40m above ground level within the area of the University of Jos where the monthly soil samples were collected.

The sampling platform was swabbed with 95% ethanol prior to plate exposure time. Sixty plates of malt extract agar medium were laid out on the wooden platform. Forty of these plates were left open for one hour while twenty were left covered to act as control. The plates were laid out at noon on each day the soil samples were collected. Midday was chosen for exposing the plates because it was one of the least turbulent periods during daylight. A record of the relevant meteorological data like rainfall, relative humidity (R.H.), sunshine hours and temperature of the sampling site was kept throughout the period of the experiment.

In order to prevent excessive drying of the agar plates, very deep plates were poured. Twenty of the exposed plates were incubated at 25% for four days and examined for the presence of

Fig. 1 of Appendix 14

A diagram of the wooden platform employed for the sampling of the Aerial Mycoflora.



mesophilic fungi. The plates were re-examined a week later for the presence of additional mesophiles.

The remaining twenty of the exposed plates were incubated at 45°C for seven days and examined for the presence of thermophilic and thermotolerant fungi. The plates were re-examined a week later for the development of additional thermophilic and thermotolerant fungal colonies. Scorings were subsequently made for each fungal species that was isolated on a monthly basis.

Ten of the control plates were incubated at 25°C while the remaining ten were incubated at 45°C. These plates were then left for observation. It was assumed that any fungus colony developing in the culture plate must have originated from a single spore or spores from the aerial environment.

RESULTS

The species of fungi isolated from the agar plate exposure are listed in Table 1 of Appendix 14. The changes in the concentration of air-spora observed during the survey are shown in Figure 2 of Appendix 14 which equally shows the changes in the relevant meteorological data during the survey.

The isolates included seven thermophilic fungi, five thermotolerant fungi and seventeen mesophilic fungi. Aspergillus fumigatus was the most abundant fungus in the air-spora followed by Curvularia lunata.

TABLE 1 OF APPENDIX 14

THERMOPHILIC FUNGI

ASCOMYCTES

Chaetomium thermophilum La Touche

Corynascus thermophilus (Fergus and Sinden) v. Klopotek (= Thielavia thermophila Fergus and Sinden)

FUNGI IMPERFECTI

Humicola insolens Cooney and Emerson

Malbranchea sulphurea (Miehe) Sigler and Carmichael

Myceliophthora thermophilum (Apinis) v. Oorschot

Thermomyces lanuginosus Tsiklinski

PHYCOMYCETES

Mucor pusillus Lindt

THERMOTOLERANTS

ASCOMYCETES

Emericella nidulans (Eidam) Wint

Continued ...

FUNGI IMPERFECTI

Aspergillus fumigatus Fres.

Penicillium argillaceum Stolk et al.

Sporotrichum pulverulentum Novobranova

PHYCOMYCETE

Absidia corymbifera (Cohn) Sacc. and Trotter (=Absidia ramosa sensu Nottebrock et al)

MESOPHILIC FUNGI

FUNGI IMPERFECTI

Alternaria solani Sorauer

Aspergillus flavus Link

A. niger van Tiegh

Botryodiplodia theobromae Patouillard

Cladosporium herbarum Fr.

Curvularia lunata (Wakker) Boedijn

Epicoccum purpurascens Ehrenb.

Fusarium oxysporum sensu Snyder and Hansen

F. solani sensu Snyder and Hansen

Helminthosporium sp.

Myrothecium verrucaria (Alb. and Schw.) Ditm. ex Fr.

Continued ...

Monilia sitophila (Montagne) Saccardo

Nigrospora sacchari (Speg.) Mason

Trichoderma viride Fr.

Torula herbarum (Pers.) Link ex S.F. Gray

PHYCOMYCETES

Choanephora sp.

Rhizopus stolonifer (Fr.) Lind.

Fig.2 of Appendix 14

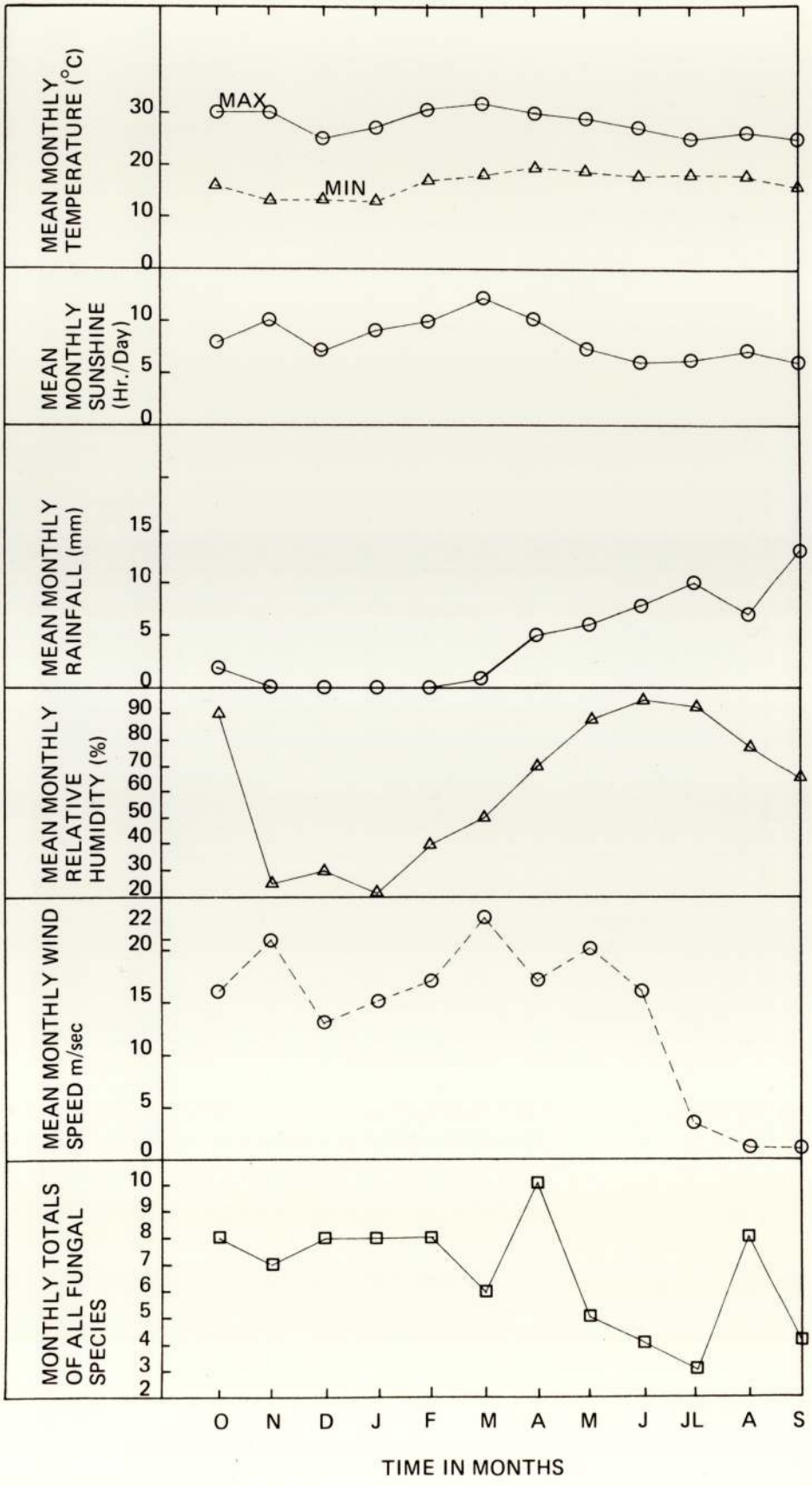


Fig.7

Sporotrichum thermophile was the most common thermophilic species. Details of all the isolations are given in Table 2 of Appendix 14.

Fungal isolations reached a peak in April when there was less wind, no rain, high relative humidity and less sunshine hours. The fungal spora declined in March when there was high relative humidity, high wind velocity and high temperature. Judging from the results obtained there seem to be an inverse relationship between the number of fungal isolates and rainfall.

All the fungi isolated from the air-spora have been reported earlier from the Nigerian soil samples. Fungal genera like Aspergillus, Cladosporium Curvularia, Epicoccum, Fusarium, Helminthosporium, Monilia, Myrothecium, Nigrospora, Penicillium, Rhizopus and Thielavia which are among the genera isolated during the present study have been reported earlier from Samaru, Northern Nigeria by (Dransfield, 1966). The others are new records of the Nigerian air-spora.

More fungi were isolated from the air during dry season (November - April) than during rainy season (May - October). There was no fungal colony development in any of the control plates. This confirmed the aerial origin of the fungi in the exposed plates.

TABLE 2 OF APPENDIX 14 FUNGI ISOLATED FROM JOS AIR-SPORA

FUNGAL SPECIES	MONTHS												
	O	N	D	J	F	M	A	M	J	J	A	S	
Aspergillus fumigatus	*+	+	+	+	+	+	+	+			+	+	10
A. flavus				+							+		2
A. niger							+			+	+		3
Absidia corymbifera	+			+						+			3
Alternaria solani	+												1
Botryodiplodia theobromae		+			+								2
Chaetomium thermophile			+			+			+				3
Choanephora sp.	+										+		2
Cladosporium herbarum		+			+		+						3
Curvularia lunata	+	+	+	+			+	+					6
Emericella nidulans							+						1
Epicoccum sp.		+			+								2
Fusarium oxysporum											+	+	2
F. solani	+												1
Helminthosporium sp.				+	+								2
Humicola insolens				+									1
Malbranchea pulchella			+				+						2
Monilia sitophila		+		+							+		3
Mucor pusillus			+		+				+				3
Myrothecium verrucaria			+				+					+	3
Nigrospora sacchari						+							1
Penicillium argillaceum	+								+				2
Rhizopus stolonifer								+			+		2
Sporotrichum pulverulentum						+	+				+		3
S. thermophilum (= Myceliophthora thermophilum		+	+		+		+						4
Thermomyces lanuginosus					+	+	+						3
Thielavia thermophila (= Corynascus thermophilus				+				+					2
Torula herbarum	+		+										2
Trichoderma viride						+		+				+	3
Sordaria sp.									+	+			2
* means fungus is present TOTAL	8	7	8	8	8	6	10	5	4	3	8	4	79

DISCUSSION

The results of the survey have shown that fungal spores are present in the Nigerian atmospheric environment and are capable of being deposited on exposed surfaces.

Myceliophtora thermophilum (Sporotrichum thermophilum) was found to be the most abundant thermophilic fungus in the Jos air-spora. This fungus accounted for 5.1% of the total number of fungal colonies. Following it were M. pusillus and T. lanuginosus. Each constituted 3.8% of the total fungal colonies. A. fumigatus was the most common thermotolerant fungus isolated from the air-spora and also the most abundant fungus in Jos air-spora. It formed about 12.7% of the entire fungal colonies.

Sporotrichum pulverulentum and Absidia corymbifera constituted 3.8% each, of the fungal colonies.

Evans (1972) investigating the thermophilous fungi in the air surrounding coal spoil tips in Britain, found the thermophilous fungus A. fumigatus to be the most frequent, comprising almost 50% of his total catch. He equally found T. lanuginosus and M. pusillus to be well represented. He pointed out that these three fungi are extremely common members of the thermophilous fungal populations in a variety of habitats. The dry nature of these spores accounts for their prevalence in air. The term 'thermophilous' as used by Apinis (1963a, b) denoted fungi growing at elevated temperatures but in Evans's (1971) context it embraced all those fungi appearing on soil isolation plates incubated at 45 - 48^{o}C.

Hudson (1973) surveying the thermophilous and thermotolerant fungi in the air-spora at Cambridge, encountered twelve fungi. Of these A. fumigatus was by far the most common and it made up 69.0% of the total count. He equally found T. lanuginosus and M. pusillus to be frequent.

In the present study T. lanuginosus and M. pusillus were present in the atmosphere but were not very common. Curvularia lunata, second in abundance to A. fumigatus, and the most common mesophile in this survey, is rarely reported in European work. It is common in warm climates, occurring as a facultative or secondary parasite on monocotyledons such as grasses, sorghum, maize, millet and sugar-cane (Dickson, 1956- Lam-Quang-Back, 1964). Dransfield (1966) found Curvularia to show a rapid rise at the start of the rainy season because it was able to parasitise the flush of grasses which occurred soon after the first rains. However, this observation is quite contrary to the results obtained during the present survey. Most of the Curvularia isolates were recorded during the dry season.

The build up of fungal spores in April (last month of the dry season) as shown in Figure 2 of Appendix 14 could reflect the lower foliage availability during this period when most of the plants have shed their leaves. So there are not enough leaves to serve as natural filter for the spores and many of them remain in the atmosphere. The second peak noticed in August could be as a result of the fact that the weather was less turbulent during this time.

The lower number of isolates recorded during the rainy season could stem from the fact that spores were washed from the air by

the heavy rains during this season. Pady and Kapica (1953) found that in winter, a season similar to rainy season in the tropics, the air is practically sterile, at least as far as fungal spores were concerned. Hirst and Stedman (1963) described rain as influencing the air-spora both by removing spores from the air and by dislodging spores from conidiophores.

An Ascomycete like Sordaria sp. was present in the air during the rainy season. It is possible that the presence of the ascospores of this fungus in the air at this time of the year could have resulted from the fact that they were being forcibly ejected from mature asci after wetting. Meredith (1962) observed that rain, dew, and high humidity favoured liberation of many ascospore types.

It was observed that the number of isolates recovered from the air each month depended on wind-speed. The greater the wind-speed the fewer the number of isolates. The air currents may have had more eddies at the higher wind-speeds, and these would interfere with spore deposition.

This is contrary to the results obtained by Gregory (1950). In his wind tunnel test experiments, he observed that at wind speeds approaching ten metres per second as many Lycopodium clavatum spores (approximately 32μ in diameter) were deposited on the under surface of a horizontal slide as on the upper. He assumed that sedimentation under gravity must therefore play a minor part except at low wind speeds. He suggested that deposition is brought about largely by turbulent flow over the trap surface. He discovered that the number of spores deposited on the trap surface was in fact, nearly

proportional to the number of spores passing over the surface in a given time irrespective of the amount of air they were diluted with. That is irrespective of concentration.

However, an abundance of basidiospores under conditions of low atmospheric turbulence and high humidity has been observed on several occasions in England (Hirst, 1953; Gregory and Hirst, 1957; Gregory and Sreeramulu 1958). The number of fungal spores in the air is probably correlated with the particular type of air mass and may indeed give a clue to identity of air masses (either N.E. trade wind or S.W. trade wind as it applies to Nigeria).

There were minor atmospheric temperature changes during the course of the experiment, and these changes might not have greatly influenced the number of isolates.

APPENDIX 15

APPENDIX 15a

FUNGI ISOLATED FROM THE WOOD VENEERS

FUNGAL SPECIES	WOOD VENEERS											
	UNWASHED						WASHED					
PHYCOMYCETES	*1	2	3	4	5	6	1	2	3	4	5	6
Absidia corymbifera			+		+	+			+	+		
A. sp.				+	+							
Choanephora cucurbitarum		+	+	+	+							
Cunninghamella echinulata				+	+							
Mortierella wolfii			+		+							
Mucor miehei					+	+					+	+
M. pusillus		+		+	+					+	+	
Rhizopus arrhizus			+	+								
R. microsporus				+		+				+		+
R. nigricans	*+	+	+	+	+	+	+	+	+	+	+	+
R. oligosporus			+		+				+			
R. oryzae		+										
Zygorhynchus sp.	+											
Z. moelleri			+	+	+					+	+	
ASCOMYCETES												
Chaetomium cellulolyticum		+	+	+	+	+		+	+	+	+	+
C. cochlioides	+	+	+	+	+	+	+	+	+	+	+	+
C. funicola			+			+			+			
C. globosum	+	+	+	+	+	+	+	+	+	+	+	+
C. thermophile var coprophile	+	+	+	+	+	+	+	+	+	+	+	+

Continued ...

	*1	2	3	4	5	6	1	2	3	4	5	6
C. virginicum			+		+				+		+	
Corynascus sepedonium		+		+								
C. thermophilus	+	+	+	+	+	+	+	+	+	+	+	+
Emericella nidulans			+			+			+			+
Sordaria fimicola		+			+			+				
Talaromyces thermophilus		+	+	+	+			+	+		+	
Thielavia sp.				+								
FUNGI IMPERFECTI												
A. fumigatus	+	+	+	+	+	+	+	+	+	+	+	+
A. giganteus		+			+							
A. niger	+	+	+	+	+	+	+	+	+	+	+	+
A. terreus		+		+	+			+		+	+	
A. versicolor			+		+				+			
Aureobasidium pullulans				+	+	+						
Alternaria alternata			+						+			
Botrytis cinerea		+		+	+							
Cladosporium oxysporum				+								
Curvularia leonensis					+							
C. lunata			+	+					+			
Diplococc um asperum			+		+							
Epicoccum purpurascens		+		+								
Fusarium moniliforme			+	+	+							
F. oxysporum		+	+	+	+	+			+			
F. roseum	+	+				+						
F. solani				+	+					+	+	
Gliocladium roseum			+	+		+			+	+		
Graphium sp.		+		+	+							
Helminthosporium sp.			+			+						

Continued ...

	*1	2	3	4	5	6	1	2	3	4	5	6
Humicola grisea		+		+								
H. grisea var thermoidea	+	+	+	+	+	+		+	+	+	+	+
H. insolens			+	+					+	+		
Malbranchea sulphurea	+	+	+	+	+	+	+	+	+	+	+	+
Monilia sitophila	+	+	+	+	+	+	+	+	+	+	+	+
Myceliophthora thermophilum 1	+	+	+	+	+	+	+	+	+	+	+	+
M. thermophilum 2			+	+	+				+	+	+	
Myrioconium thermophilum			+	+	+	+						
Myrothecium verrucaria	+	+	+	+	+	+	+	+	+	+	+	+
Oidiodendron rhodogenum			+	+	+				+	+	+	
Papulaspora sp			+	+	+				+	+	+	
Paecilomyces variotii	+	+	+	+	+	+		+	+	+	+	+
Penicillium argillaceum			+		+				+			
P. citrinum				+								
P. foniculosum				+								
Periconia sp			+									
Scytalidium thermophilum			+	+	+				+			
Sporotrichum pulverulentum	+	+	+	+	+	+	+	+	+	+	+	+
Stachybotrys atra			+	+	+				+	+	+	
Thermomyces ibadanensis			+		+				+			
T. lanuginosus			+	+	+	+			+		+	
T. stellatus			+						+			
Torula herbarum		+	+	+		+			+			
Trichoderma koningi	+	+	+				+	+				
T. lignorum		+	+	+				+				
T. viride	+	+	+	+	+	+	+	+	+	+	+	+
Trichocladium canadense			+									
Ulocladium atrum			+	+	+							
Blue stain fungus 1			+	+	+					+	+	
Blue stain 2			+	+	+				+	+		
Sterile dematiaceous fungus			+		+				+			
BASIDIOMYCETES												
Basidiomycete sp				+						+		
Coprinus cinereus		+	+		+			+	+		+	
C. sp			+		+				+		+	
Total no. of fungal species isolated	18	34	56	54	54	29	14	22	42	31	31	19
ACTINOMYCETE												
Thermophilic actinomycete			+		+			+	+		+	
BACTERIA												
Unidentified bacterial sp 1	+	+	+	+	+	+						
2	+	+	+	+	+	+						
3	+	+	+	+	+	+						
TOTAL no. of micro-organisms isolated	21	37	60	57	58	32	14	23	43	31	32	19
pH values of wood veneers	6·1	6·2	6·6	6·4	6·4	6·2						
% moisture content of wood veneers	11	18	23	22	22	15						

* + means micro-organism is present

* KEY

1. = *Khaya ivorensis*

2. = *Triplochiton scleroxylon*

3. = *Terminalia superba*

4. = *Daniellia ogea*

5. = *Canarium schweinfurthii*

6. = *Sterculia oblonga*

APPENDIX 15b

FUNGI ISOLATED FROM STAINED AREAS OF WOOD VENEERS

FUNGAL SPECIES	WOOD VENEERS					
	1	2	3	4	5	6
PHYCOMYCETES						
Absidia corymbifera			+			
Choanephora cucurbitarum			+	+		
Rhizopus arrhizus			+	+		
R. nigricans	+	+	+	+	+	
Zygorhynchus moelleri			+	+	+	
ASCOMYCETES						
Chaetomium cochlioides		+	+	+	+	+
C. globosum		+	+	+	+	+
C. thermophile var coprophile		+	+	+	+	
Corynascus thermophilus			+			
Sordaria sp.			+	+	+	
FUNGI IMPERFECTI						
A. fumigatus	+	+	+	+	+	+
A. giganteus				+	+	
A. niger	+	+		+	+	
A. terreus			+		+	
A. versicolor				+	+	
Alternaria alternata			+		+	
Botrytis cinerea		+		+	+	
Cladosporium oxysporum				+		
Curvularia leonensis					+	
C. lunata			+	+		
Diplococcium asperum			+		+	

Continued ...

	1	2	3	4	5	6
Epicoccum purpurascens		+		+		
Fusarium solani				+	+	
F. sp.				+		
Gliocladium roseum			+	+		+
Humicola grisea		+	+		+	
Humicola grisea var thermoidea			+			
Malbranchea sulphurea		+	+		+	
Monilia sitophila			+	+	+	
Myceliophthora thermophilum (S. thermophilum)		+	+	+	+	+
Myrioconium thermophilum			+	+	+	
Myrothecium verrucaria			+	+	+	
Oidiodendron rhodogenum			+	+	+	
Papulaspora sp.			+	+	+	
Paecilomyces variotii		+	+	+		
Periconia sp.			+	+		
Scytalidium thermophilum					+	
Sporotrichum pulverulentum		+	+	+	+	+
Stachybotrys atra			+	+	+	
Trichoderma koningi			+			
T. lignorum						
T. viride			+		+	
Trichocladium canadense					+	
Ulocladium atrum			+		+	
BASIDIOMYCETES						
Coprinus cinereus		+	+		+	
C. sp.			+		+	
Basidiomycete sp. (hyphae with clamp connections)				+		
TOTAL	3	14	35	30	32	6

KEY

1 = Khaya ivorensis

2 = Triplochiton scleroxylon

3 = Terminalia superba

4 = Daniellia ogea

5 = Canarium schweinfurthii

6 = Sterculia oblonga

APPENDIX 16

APPENDIX 16

FUNGAL SPECIES	WOOD BARK					
	UNWASHED BARK PIECES			WASHED BARK PIECES		
	TEMPERATURES OF INCUBATION			TEMPERATURES OF INCUBATION		
	25°C	35°C	45°C	25°C	35°C	45°C
Absidia corymbifera Sacc & Trotter	+*	+				
A. fumigatus Fres.	+	+	+	+	+	+
A. nidulans Wint.		+	+			
A. niger van Tieghem	+			+		
A. terreus Thom		+			+	
Alternaria sp.	+					
Botrytis cinerea Pers ex Fr.	+					
Chaetomium globosum Kunze	+			+		
Cladosporium herbarum Link	+					
C. sp.	+					
Curvularia lunata Boedijn	+			+		
Epicoccum sp.	+					
Monilia sitophila Saccardo	+			+		
Mucor pusillus Lindt		+	+			
Penicillium sp. 1	+					
P. sp. 2	+					
Myceliophthora thermophilum v. Oorschot		+	+		+	+
Stachybotrys atra Corda	+			+		
Thielavia thermophila = Corynascus thermophilus v. Klopotek		+	+		+	+

Continued

	25°C	35°C	45°C	25°C	35°C	45°C
Trichoderma viride Pers	+			+		
T koningi Oudemans	+			+		
T. lignorum Harz	+			+		
Ulocladium sp.	+					
Fusarium solani Appel and Wollenweber	+					
Fusarium sp. 1	+					
F. sp. 2	+					
F. sp. 3	+					
Malbranchea pulchella var. *sulphurea* Sigler & Carmichael		+	+		+	+
TOTAL	22	8	6	10	5	4

* Means fungus is present

APPENDIX 17

APPENDIX 17

FUNGI ISOLATED FROM JOS MUNICIPAL COMPOST SAMPLES

THERMOPHILIC FUNGI

ASCOMYCETES

Chaetomium thermophile var. *coprophile* Cooney and Emerson

C. thermophile var. *dissitum* Cooney and Emerson

Corynascus thermophilus (Fergus and Sinden) v. Klopotek (=*Thielavia thermophila* Fergus & Sinden)

Talaromyces thermophilus Stolk (=*Talaromyces dupontii* (Griffon and Maublanc) Emerson)

Thermoascus aurantiacus Miehe

Thermoascus thermophilus (Sopp) v. Arx (=*Dactylomyces thermophilus* Sopp)

Thielavia terrestris (Apinis) Mallock & Cain (=*Allescheria terrestris* Apinis)

FUNGI IMPERFECTI

Humicola grisea var. *thermoidea* Cooney and Emerson

H. insolens Cooney and Emerson

Malbranchea sulphurea (Miehe) Sigler & Carmichael

Myceliophthora thermophilum (Apinis) v. Oorschot

Scytalidium thermophilum (Cooney & Emerson) Austwick *Torula thermophila* Cooney & Emerson)

Continued...

Stilbella thermophila Fergus

Thermomyces ibadanensis Apinis & Eggins

T. lanuginosus Tsiklinski

T. stellatus (Bunce) Apinis

Myrioconium thermophilum (Fergus) v.d. Aa (=Papulaspora thermophila Fergus)

PHYCOMYCETES

Mucor miehei Cooney and Emerson

M. pusillus (Lind) Schipper

THERMOTOLERANT FUNGI

ASCOMYCETES

Corynascus sepedonium (Emmons) v. Arx (=Thielavia sepedonium Emmons)

Emericella nidulans (eidam) Wint

FUNGI IMPERFECTI

Aspergillus fumigatus Fres

A. nidulans (eidam) Wint

A. terreus Thom

Paecilomyces variotii Bain

Sporotrichum pulverulentum Novobranova

Continued...

PHYCOMYCETES

Absidia corymbifera (Cohn) Sacc. & Trotter (=Absidia ramosa (Lindt) Lendner sensu Nottebrock et al.

Mortierella wolfii Mehrotra & Baijal

Rhizopus microsporus van Teighem

R. oligosporus Saito

APPENDIX 18

APPENDIX 18

ECONOMIC CONSIDERATIONS

Microbiological deterioration of timber causes large monetary losses which can have a heavy impact on a national economy.

Of the many types of micro-organisms responsible for widespread damage of fabricated materials, the wood-rotting fungi is the class that is of the greatest economic importance. Hueck-van der Plas (1965) made a one year (1959 - 1960) global evaluation for biodeterioration based on the 21 O.E.C.D. (organisation for economic co-operation and development) countries including North America. Her evaluation placed global damage at just over £357 million (one billion dollars).

Boats have received attention with the U.S.A. Department of Agriculture, (Anon 1953) showed that the majority of 500 boats examined in U.S.A. were deteriorated.

Boocock (1965) reported that in 1929 U.S.A. lost £4 million due to fungal stain of timber. An Australian report (Anon 1966) stated that U.S.A. lost £83 million every year as a result of wood decay. Turner (1967) quoted a 1951 estimate of 10% of the U.S.A. total annual production of timber as being used to replace deteriorated wood. This was equivalent to £94 million. Norkrans

(1967) gave a much increased value of £1.25 billion for U.S.A. due to wood decay. Sharp (1970) reckoned that the differences in estimates was probably due to different wood products considered and that some of the reports might include the loss from incomplete or superficial decay of timber.

Hatton, Smith and Rogers (1968) predicted that financial loss due to the biodeterioration of stored wood-chip piles in British Columbia would amount to £42 million by 1987.

Financial estimates have equally been made for the European countries. Gad (1957) estimated that 1.75 million cubic metres of timber were deteriorated every year in Finland. Goksoyr (1965) gave a Norwegian value of constructional timber destroyed by rot-fungi as £20 million. This was four times that destroyed by fire. Norkrans (1967) gave an estimate of attack on Spruce by Polyporus annosus as costing about £5.8 million to £11.6 million.

Tyrer (1961) found 27% of 13,000 dwellings surveyed in the U.K. during 1960 to have wet rot and 17% revealed dry rot. The expenditure from decay was put at about £10 million per year. Findlay (1967) estimated the cost of repairing houses damaged by dry rot in the U.K. built before 1939 to be £1 million per year and £10 million per year after 1945. Sharp (1970) put the cost of repairs at £20 million per year for many of the post-war years. Findlay (1967) put the cost of decay of various water craft in the U.K. to be £2 million. Scott (1968) reported that the value of wood imported into Britain each year was in the order of

£2,200 million. He considered home-grown timber to be less than 10% of the value of the wood imported and that very little of the home-grown material was used in the actual fabric or structure of buildings. Hardie (1979) estimated that U.K. imported 85% of her total timber consumption from overseas. She suggested that such imported timber should therefore be used as efficiently and economically as possible and wastage caused by biodeterioration should be minimised.

Skolman (1964) found 39.6% of Eucalyptus lumber decayed through bad seasoning in Hawaii. Miller (1979) estimated that losses of Douglas fir, hemlock, and true fir logs stored in dry decks in the Pacific Northwest ranged from 0% - 5% during the first year and 5% - 15% during the second. Estimates of loss during a year were 0% - 10% for Ponderosa pine and 0% for Douglas fir logs. Comparative value losses reported after a year in dry decks (Circa) (1953) were $13 to $14 per thousand board feet for Ponderosa pine and $6.67 per thousand board feet for (1954) for Idaho white pine logs. Losses after one summer were $4.55 per thousand board feet (1966) for white spruce and an estimated potential loss of $25 per thousand board feet (1971) in unprotected high grade hemlock logs.

Wood preservation has helped to minimise losses. Turner (1967) estimated that towards the end of the last century annual sleeper replacements in U.S.A. were 339 per mile, while later use of preserved sleepers reduced replacements to only 67 per mile at an estimated saving in labour and material cost of $900,000 daily. Wilkinson (1979) reported that 100 million

transmission poles scattered across U.S.A. would normally need to be replaced after five years, but when preserved last six times as long. Over a period of thirty years this means a saving of 50 million poles or 16.5 million trees each year. Turner (1967) mentioned that by the adoption of creosote as a preservative for railway sleepers in Germany, the replacement rate was cut by much more than 50%, the service life resulting from treatment increased from an average of five years to one of twenty-seven years.

Apart from its money-earning potential as an industry, timber preservation has provided another economic benefit. In Malaysia cheap non-durable species can be treated for use in the country, thus allowing expensive durable timbers to be exported. In Papua, New Guinea, preservation has allowed traditionally designed buildings to be constructed using local non-durable timber thereby keeping investment capital within the country rather than using it to import alternative building materials.

The West African estimates include that of Martinka (1968) who found Wawa timber, Triplochiton scleroxylon to be badly damaged by 33% to 50% after six months outdoor storage in Ghana when no preservative was used. Ofosu-Asiedu and Cannon, (unpublished report) during a preliminary study conducted in Kumasi between (1973/74) discovered that the service life of wood houses in Ghana varied from a few years to as long as fifty years depending upon the type of structure, the species of wood used in the construction, and care and maintenance of the building. They found that the parts of the building which were more badly decayed were the roof trusses, the

outer walls and in the case of barns the supporting poles.

Ofosu-Asiedu (1975) reported that as a result of the rapid exploitation of economic timber species from the forest of Ghana and the difficulty involved in the natural and artificial regeneration of some of these species, the country would have to rely more in the future on the lesser known timber species to supply the wood needs of the country and for export.

Boateng (1980) reported that the demand for Ghana's wood products has grown in the MIddle East with firm orders placed for $12 million worth of Ghanaian timber. He equally mentioned that the timber industry was Ghana's second most important foreign exchange earner after cocoa.

The importance of biodeterioration in economic terms especially in Nigeria, is immense yet it is only recently that biodeterioration technology has attracted the attention of the Nigerian Government. It affects all Nigerian industries in one way or another, but the wood industry is the most affected. Timber is one of the oldest building materials in Nigeria and is still used in huge quantities for structural, semi-structural and other purposes in building. Lightness, rapidity of construction and versatility in use are three of its greatest assets. Unfortunately this large scale use of timber as a building material is being hampered by fear of it being deteriorated by fungi and insects. The purchase of a house in Nigeria represents a large and important capital investment and a building owner can ill afford to pay costly bills for replacement of timber in a short period of time because of decay.

Bamboos though intrinsically strong and used in many Nigerian countryside houses as substitutes for building timbers, have a short life of one to two years owing to attack by fungi and insects. Data collected from the Ministry of Trade and Industry, Imo State, Nigeria has shown that a community council area (Etiti) of about 5 million people spend about £3 million annually on house repairs as a result of microbial and insect deterioration of wood, bamboos and raffia mats used in building construction. This amount will run into many millions of pounds if calculated for the entire Nigerian population of about 95 million people. This large sum has been mostly unrecognised because the cost to individual homes (totalling several million occupied housing units) is not large enough to stimulate concerted complaints.

Wood has been used in the construction of many bridges across many rivers and deep streams in Nigeria. The maintenance of such bridges is expensive, and presents an expanding problem. When such bridges collapse as a result of fungal decay of the wood used in their construction an untold hardship is created for people who use them.

Wood used for farm fences and barns has to be replaced annually because of fungal or termite decay. If this was not done, animal livestock would find it very easy to break through the deteriorated wood into the farms and barns causing further economic losses through their destruction of the agricultural crops. Telegraph and electric poles have a short life span due to fungal decay. The use of asbestos-cement or plastic alternatives

to wood have not proved any great advantage because tests have shown that these have poorer performance than treated timber which could last for over thirty years. Besides, the high strength-to-weight ratio inherent in wood and its flexural rigidity (modulus of elasticity X moment of inertia), ensure its continued use.

Apex mill in Lagos, Nigeria, which deals with both pulp and paper products is faced with fungal decay problems. It is known that the use of pulp which is badly infected with fungi and bacteria can result in poor quality sheet and in a loss of production time. The strength of the sheet is impaired in some cases where decay fungi are present. If the fibre cannot be completely dispersed, small knots or masses of fibre and fungal hyphae will cause spots, and holes in the sheet, lowering its quality.

Sawmillers who were interviewed in Nigeria during the present research have confirmed that wood stain is a major problem in the Nigerian wood industry. It was even observed that the experimental wood veneers exposed at the factory were badly stained. Momo and Olujide (1967) recommended that logs should be removed from the field after felling and dipped immediately into anti stain solution in order to prevent blue stain attack. Ofosu-Asiedu (1973) suggested that a change in the season in which logging takes place can minimise blue stain attack of perishable logs. Momo (1966) demonstrated in Nigeria that there is less blue stain attack of wood in the dry season than in the rainy season. It has also been shown

from the present research that most of the stain fungi which belongs to soil Hyphomycetes are more predominant during the rainy season.

Lumber stacks are often prepared without suitable foundations in some Nigerian sawmills. These stacks touch the ground because of insufficient clearance. The stacking of the lumber in the open leads to its colonisation by soil fungi and aerial spores and its eventual deterioration.

Martinka (1967) has pointed out that such lumber stacks had not more than twenty-five layers on the average and the upper planks representing 4% of the stored lumber degraded badly and were rendered fit only for firewood. Losses due to wood deterioration during storage have been estimated by Nigerian sawmillers and lumber dealers. They have given a reject factor of about 3 - 7%.

Biodeteriorating agents of wood in Nigeria are aided by the extremely favourable conditions of temperature and high relative humidity which prevail in parts of the country all the year round.

Odeyinde (1977) has estimated that at least 70% of the timber produced in Nigeria is lost due to unbridled biological degradation and deterioration.

Owing to the high cost of cement, many people in Nigeria are now resorting to the use of wood for building construction as an alternative to cement. Unless such building timbers are adequately preserved, there will be need to replace them after

a short period. It must be realised that the cutting of forest stands to replace unserviceable wood buildings entails a costly commitment of labour and of timber approaching critically short supply.

Nigeria has exported very large quantities of wood to the European countries and other parts of the world and she will unlikely meet the demand for this commodity. The distribution of Nigerian timber exports between 1946 and 1974 is shown in Table 1 of Appendix 18.

FAO (1976) yearbook of forest products showed that Nigeria earned about $846307 between 1961 and 1976 from wood products (industrial roundwood, sawlogs and veneer logs, pulpwood and particles, pulpwood, other industrial roundwood, sawnwood and sleepers, sawnwood, wood-based panels, other paper plus paperboard, forest products, veneer sheets, plywood, paper and paperboard). It must be stressed that the values for many other industrial wood products were not represented and it is difficult to explain how Fao conducted their survey. However, this amount did not include the timber trade between AT & P (U.A.C. Nigeria Limited) Sapele, one of the world's largest timber establishments, and other parts of the world. It also did not include the value of trade between many other Nigerian wood companies and other parts of the world.

The importance of timber in the Nigerian economy cannot be overemphasised. Palmer (1979) stated that in 1974 the U.K. imported 12000m^3 of plywood from Nigeria. The equivalent of

TABLE 1 OF APPENDIX 18

THE DISTRIBUTION OF NIGERIAN TIMBER EXPORTS 1946-74

Commodity	1946 m	1946 % *	1950 m	1950 %	1960 m	1960 %	1965 m	1965 %	1970 m	1970 %	1974 m	1974 %
Timber (Logs & Sawn)	0.7	1.4	4.9	2.7	14.1	4.1	12.5	2.3	6.2	0.7	11.2	0.2

* percentage of total export commodities.

Source: Federal Office of Statistics (Nigeria), Annual Abstract of Statistics and Review of External Trade. Central bank of Nigeria, Annual Reports.

this financially is enormous. A Nigerian timber trade expert has estimated that the Nigerian Government derives several million pounds from the timber trade between it and other parts of the world and according to him this forms a major part of the total royalties derived from Nigerian natural resources.

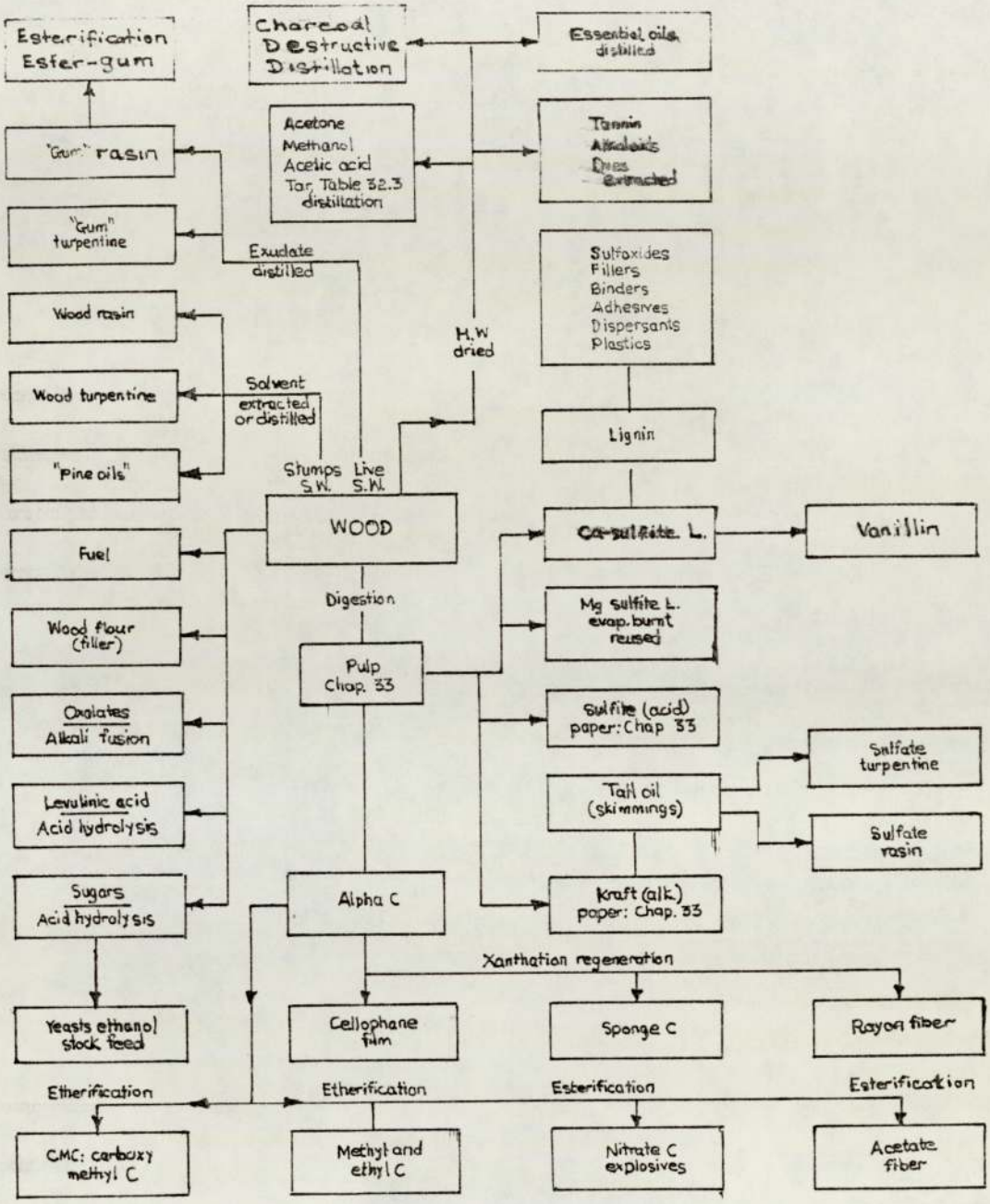
Wood has innumerable uses and should therefore be protected from decaying. As President Theodore Roosevelt of the U.S.A. said in 1905, "Wood is an indispensable part of the material structure upon which civilisation rests....". It is a good source of many industrial chemicals (Figure 1 of Appendix 18) and it is because of this that the Nigerian Government is setting up a multi-million pound wood factory in collaboration with the Romanian State owned company, Forexim. The factory named "The Nigerian Romania Wood Industries" would produce wood chemicals apart from its other products.

Since timber plays such an important role in Nigeria's economy, it should be protected from both fungal and insect attack through preservation. It must be realised that our customers in different parts of the world do not accept stained or decayed wood. The problems involved are deserving not only of increased research directed at improved means of timber protection but also of much better extension of the existing information on the subject.

Making timber last longer decreases the demand on the Nigerian forests and plays an important part in the conservation

Fig.1 of Appendix 18

WOOD CHEMICALS



Sources: Chemicals from the Other Half of the Tree, C&EN, Feb. 11, 1963, p. 83; I&EC, 50, 570 (1958); ibid., 44, 3808 (1952); CMC: of Chemical Economics Handbook, Stanford Research Institute; Hercules Powder Co., Wilmington, Del., for rosin and derivatives; Dickey, Chemistry of Cellulose, J. Chem. Educ., 41 (4). 196 (1964); ECT, 2d ed., vol. 4, p. 616, 1964. Abbreviations: L-liquor, Alk-alkaline, C-cellulose, S.W.-soft wood, H.W.-hard wood.

of the world's forests. The amount of wood converted each year continues to rise and Wilkinson (1979) has estimated that the world's demand for pulp and paper alone requires harvesting of a forest, the size of the U.K.

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