ECOLOGICAL STUDIES OF FUNGI GROWING ON INSOLATED WOOD

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SUMMARY

The widespread occurrence of thermophilous microfungi on wood is reported. Imported timber, in-service timber joinery and wood employed in colonization experiments were sampled and a list of cellulolytic fungi including thermophilous and soft rot fungi is submitted.

The influence of insolation on the temperature and moisture ranges in wood was investigated. Temperatures up to 16°C above ambient were recorded as a result of insolation and data is submitted on the diurnal temperature cycle in a block of wood undergoing insolation. It is recorded that colour had no significant effect upon the moisture content of insolated wood in soil contact.

Using a simple moisture gradient apparatus the surface growth and penetration of thermophilous fungi were investigated at above ambient temperatures, showing that thermophilous fungi could grow at the surface and within beech veneers at moisture levels below the 20% minimum recorded in the literature, it is considered that the biological activity of water is increased at above ambient temperatures.

The effect of constant, alternating and fluctuating temperatures on the growth of microfungi was investigated. A temperature cycle simulator was devised producing a diurnal cycle similar to that occurring in insolated wood. Addition, stimulation and retardation of growth were recorded as a result of temperature alternation, whilst evidence is submitted that some thermophilous wood inhabiting microfungi were better adapted to temperature fluctuations than mesophilic forms.

Interaction studies between pairs of wood inhabiting microfungi were undertaken at above ambient temperature and under conditions of fluctuating temperature. Evidence that metabolic products influence interactions is offered, and that under conditions of fluctuating temperature thermophilous fungi can play a significant role in ecological sequences.

Preliminary experiments into the tolerance of cellulolytic thermophilous micro-fungi to wood preservatives indicate that they were more tolerant to preservatives at above ambient temperature than at 25°C.

To my wife, who supplied the midnight oil, and for my daughters,

Janet, Susan and Ann

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Glyn Morton
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CHAPTER I

GENERAL INTRODUCTION

It is now recognized that timber especially worked timber can be damaged not only by brown rot and white rot fungi but also by microfungi causing soft rot and staining of the wood.

Most investigations so far have been at mesophilic temperatures and it was considered that an investigation of the colonization of unworked and worked timber, particularly insolated timber such as timber-joinery, would be of importance to show whether fungi able to grow at such markedly above ambient temperatures may play a significant part in the deterioration of timber. Organisms capable of colonizing wood can be divided into seven major groups (Levy, 1969, 1971; Leise, 1970) namely: bacteria, moulds, staining fungi, staining fungi capable of causing soft rot in hard woods, soft rot fungi, brown rot fungi and white rot fungi. Gorschin and Krapivina, (1969) however, consider that the arbitrary distinction between mould, staining fungi and soft rot fungi should be discarded and for the future microfungi colonizing wood should be considered as micromycetes with basidiomycetes referred to as macromycetes.

The grouping together of mould, staining and soft rot fungi into a single category, the micromycetes, seems expedient since microfungi colonizing wood and causing discolouration may be described in the literature as 'blueing fungi', 'sapstain fungi', 'blue staining fungi', and 'mould staining fungi' with little differentiation between the terms (Eslyn 1967) whil e many microfungi causing discolouration as described above may also cause soft rot. (Savory, 1954b, Duncan and Eslyn, 1963, Levy, 1969 and Nilsson, 1973). Furthermore, mould and staining fungi colonizing wood are reported as being essentially non degradative (Savory, 1954a; Duncan, 1960, 1963; Leise and Schmid, 1962; Karkanis, 1966; Norkrans, 1967; Olofinboba and Lawton 1968; Butcher, 1968; Gorschin and Krapivina, 1969), however,

under circumstances which inhibit basidiomycete colonization of wood a number of microfungi may act as soft rot fungi. There is also evidence that many mould and staining microfungi may produce significant soft rotting and degradation of wood and wood products under circumstances presumably not unconducive to basidiomycete colonization (Krapivina, 1960; Corbett, 1965; Merrill and French, 1966; Ofosu-Asiedu and Smith, 1973).

In the summaries of Levy (1967), De Groot, (1972), and Liska (1971) the principal decay fungi can be grouped according to the relative rates at which they attack the cellulose or lignin components of the walls of the structural cells in woody tissue.

Soft rot is caused by fungi growing within the less lignified walls of tracheary cells and fibres producing elongated cavities that run longitudinally and follow the cellulose fibrils (Duncan, 1960). The degradation of cellulose in wood by soft rot organisms was noted earlier (Savory and Pinion, 1958). Soft rot is reported to develop in wood too wet for typical brown and white rotting fungi. Soft rot, so termed by Savory, in 1954, is generally recognized by the macroscopic appearance of the wood, (Corbett, 1965) superficial layers being extremely soft when wet and cracking across the grain when dry. The characteristic cavity formation in the secondary cell wall was first observed by Schacht in 1850, though it was not studied extensively until 1937 (Bailey and Vestal) and subsequently by many other workers (Duncan, 1960; Corbett, 1965; Corbett and Levy, 1963a; Greaves and Levy, 1965; Greaves, 1966; Levy and Stevens, 1966; Meier, 1955; Curtois, 1963; Levi, 1965; Levy, 1965; Lundstrom, 1972; Jutte and Wardrop, 1970). Comprehensive lists of soft rot organisms are presented by Rosche and Leise (1968), and by Nilsson (1973).

Brown rotting fungi rapidly decompose cellulose with little alteration of the lignin (Cowling, 1961). Since lignin is brown this pattern of attack gives the wood a corresponding colour.

White rot fungi attack lignin as well as cellulose (Wilcox, 1968; Kirk and Moore, 1972) and destruction of the lignin gives the wood a whitish appearance. White rot fungi attack the various layers of the cell wall in sequence from the innermost layer adjacent to the lumen towards the outermost layer of cellulose (Wilcox, 1968).

Timber joinery will become infected either from contact with soil or by airborne spores (Corbett and Levy, 1963; Findlay, 1965; Butcher, 1968; Toole, 1971), and thereby a succession will begin. Banerjee and Levy (1971) suggested that the sequence of succession of organisms colonizing wood in soil contact was generally, firstly bacteria, then moulds, followed by staining and soft rot fungi and then finally basidiomycetes. This succession is confirmed for timber above ground by Kääri k (1971); however, other workers (Corbett and Levy, 1963; Merrill and French, 1966; Butcher, 1968; Toole, 1971; Shigo, 1962) record moulds staining and soft rot fungi only above ground.

Most fungi grow between the limits of 10°C and 40°C and have an optimum somewhere around 25-35° (Cochrane, 1958). These can be thought of as mesophilic. Thermophilic fungi grow at elevated temperatures (50°C and above) but not below ordinary laboratory temperatures (20°C). Thermotolerant fungi are those with maxima near 50°C but minima below 20°C (Cooney and Emerson, 1964). The term thermophilous may be used to include thermophilic, psychrotolerant and microthermophilic fungi (Apinis and Pugh, 1967). Psychrotolerant thermophiles compare with the thermotolerant fungi whilst the microthermophiles are considered as those with an optimum between 25° and 35°C and a maximum exceeding 40°C but not 45°C.

Studies of thermophilic fungi first started with Miehe (1907) who studied the mycoflora of self-heating plant materials and the important role thermophilic fungi play in the decomposition of plant materials was shown by Rege (1927); Waksman and Gerretsen (1931); Waksman et al (1939) and Hensen (1957). The widespread nature of this group became more apparent after the work of Huber (1937); Vaartaja (1954); Waterhouse (1955) and Ansari and Loomis (1959), who studied various types of vegetation and temperature conditions in Northern latitudes.

Thermophilic fungi are most frequently found in self-heating environments including stored hay (Chang and Hudson, 1967; Resz, 1968), grains (Flannigan, 1969; Mulinge and Apinis, 1969; Okafor, 1966), bagasse (Lacey, 1967; Seabury et al, 1968) and peat (Küster and Locci, 1964; Fergus, 1964 and Stolk, 1965). They are common in herbivore dung (Cooney and Emerson, 1964; Crisan, 1964 and Henson, 1957), birds nests (Apinis and Pugh, 1967), and have been isolated from leaf litter (Pugh, 1958).

The biodeterioration of stored products, other than stored grain, by thermophilic organisms is reported by Eggins and Coursey (1964) working on Nigerian palm oil produce whilst the ability of thermophiles to utilize other types of substrate was shown by Mills and Eggins (1970) using oxidation products of polyethylene.

The widespread occurrence of thermophilic fungi in soils from cool climates has been shown by Mishoustin (1950); Apinis (1963, 1965); Allsopp (1968) and Eggins and Malik (1969). The cellulolytic activity of thermophilic fungi was noted by Fergus (1969) whilst Eggins et al (1972) have provided evidence that cellulolytic thermophiles are active in soils whose upper temperature limits are less than those of self-heating organic systems and that although thermophilic fungi are widespread in soil, they are not active under shaded and, therefore, cooler conditions.

Tansey (1971) reported that thermotolerant and thermophilic fungi are abundant in self-heating wood chips which contribute to heating and biodeterioration. Thermophilic and thermotolerant fungi have been reported from wood chip piles in Sweden (Bergman and Nilsson, 1966, 1967, 1968) and in Canada (Shields, 1969; Shields and Unligil, 1968).

Loman (1962) reported that microthermophiles are responsible for decay in the upper and central regions of insolated logging slash where temperatures develop which restrict the development of low temperature fungi.

Extemal timber joinery will absorb solar energy and heat up. The effect of solar energy in providing a temperature sufficient for the growth of thermophilous fungi as a separate source of heat from that of decaying matter has been mentioned by Loman (1962); Apinis and Pugh, (1967); Mulinge and Apinis (1969) and Eggins et al (1972). Henningson (1968) and Jensen (1968) have provided field observations on the effects of insolation on the temperatures in wood and there is evidence that with an increase in temperature there is an increase in the biological activity of water (Ayerst, 1965), since it has been recorded that thermophilous fungi are often cellulolytic (Fergus, 1969; Eggins et al, 1972) and can cause weight loss in wood (Ofosu-Asiedu and Smith, 1973), it would seem that worked and unworked timber, subject to insolation could provide suitable substrates for such thermophilous fungi. This primary hypothesis initiated the present investigation which proceeded along the following lines of research, firstly by identifying the cellulolytic microfungi of imported softwoods and recording those thermophilous species encountered, thus checking whether new timber was already contaminated by such fungi. Thermophilous fungi were then sought on in-service timber and a comparison made of the flora isolated with that of imported timbers. A study was then carried out of the effect of insolation on the moisture content and temperature

ranges in wood with various surface treatments. The effect of such moisture contents in wood was then investigated on the surface growth and penetration of microfungi at higher than ambient temperatures, and a similar investigation made on the effect of constant, alternating and fluctuating temperatures on the growth of selected microfungi. Investigations were also made of some of the interactions between thermophilous and mesophilic microfungi which colonize wood. The effect of surface treatment and insolation was then studied in relation to the early colonization by thermophilous fungi of -

(i) the above ground regions of untreated pine stakes,

(ii) beech veneers suspended in air.

Finally a study was made of the effects of wood preservatives on the growth of selected thermophilous and mesophilic microfungi which colonize wood. To these ends the following seven chapters are submitted dealing in turn with each project.

Chapter II, presents an account of sampling work undertaken on imported softwoods. A sampling technique is described and a list of fungi isolated from a wide range of incubation temperatures is submitted. Over a wide range of temperatures, the cellulolytic activity and temperature tolerance range of each isolate is recorded.

Chapter III, presents a survey of the thermophilous microfungi of painted and unpainted external timber joinery. A list of the isolates is submitted and this flora is compared with that of the imported timber. Cellulolytic activity and temperature tolerance ranges of the taxa are recorded.

Chapter IV, records the effects of insolation on the temperature at the surface and within painted wood. A model three-colour sill is described which was employed to provide the environments for the thermocouple estimations of temperature.

An account of the effect of insolation on the moisture content of unpainted blocks in soil contact is submitted.

Chapter V, presents an account of experiments using a model system devised to produce a moisture gradient, at constant temperature, in discs of timber veneer. The system was employed to determine the minimum moisture content necessary in wood to support the growth and active growth of selected fungi at above ambient temperatures.

Chapter VI, presents an account of the effects of constant, alternating and fluctuating temperatures on the growth of selected wood inhabiting fungi. An apparatus is described which was devised to simulate a cycle of temperatures recorded in insolated wood. This apparatus was employed to investigate the effects of fluctuating temperatures on the growth of fungi. Data was submitted on the additive, stimulative, and retardative effects of temperature variations.

Chapter VII, presents an account of some interaction experiments between wood colonizing microfungi. Three series of experiments are described :

1. between pairs of fungi inoculated onto opposite sides of agar plates,
2. between pairs of fungi inoculated onto separate veneers within perfusion systems,
3. between pairs of fungi inoculated in close proximity on strips of veneer.

Records of interactions are submitted.

In Chapter VIII the effects of insolation and of surface treatment on the colonization of wood by thermophilous fungi are investigated. Pine stakes and beech veneers were the substrates employed and the study was confined to the above ground regions of the stakes whilst the veneers were suspended in air.

The surface treatment consisted of the application of a black dye; treated and untreated samples were then placed in situations so as to receive either, all available sunshine, or, none at all. Colonization sequences at the surface and at depth within the substrates are recorded. Selected colonizers, designated thermophilous or mesophilic on the results of temperature tolerance and cellulose clearing experiments, were grown on agar containing wood preservative preparations. The performance of isolates at near ambient and above ambient temperatures is recorded.

CHAPTER II

THE CELLULOLYTIC FUNGI OF IMPORTED SOFTWOODS

Contents

2.1 Introduction

Newly imported timber is often fungally infected on arrival in this country (Savory 1967, 1974; Savory et al 1971). 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.

Savory (1967) provided information on imported timbers. He recorded that it is normal practice to ship loose timber from Scandanavia in the shipping dry condition (20 - 25% moisture) and that experience has shown that this is sufficiently low to prevent the growth of fungi on the wood during the normal short sea voyages from Scandanavia. In packaged timber,however, drying should be 20% or below because no drying can occur in packaged wood. Particular care should be taken with packaged redwood from Russia as it is not always sufficiently dried before packaging to prevent blue stain.

It has been the practice since the war to ship loose timber, green, from Western Canada; a certain amount of rot has always been found in such timber, (Cartwright and Findlay, 1948) the infection being already present in over-mature trees (Roff, 1962).

Kiln dried spruce is being imported from Canada in packages wrapped in polythene. Condensation within the polythene has been recorded, but this does not cause problems if the timber was dried adequately beforehand.

In Scandanavia and in Canada it is the practice to give superficial anti-stain treatments to prevent the development of blue stain, they are not, however, of a type to give long term protection (Savory and Cockroft, 1961).

The practice of shipping green timber from Canada has been extended to the packaging trade, which means that during transit fungi can develop (Smith, 1966).

Wood destroying fungi may be present in the package :

1. As a result of unsuspected infection.
2. As infection introduced by pin-hole borers present in certain grades of carcassing timber.
3. In pieces of No. 3 Commons, which may be included in the package together with better quality timber.

The investigation which follows was conducted in order to establish :

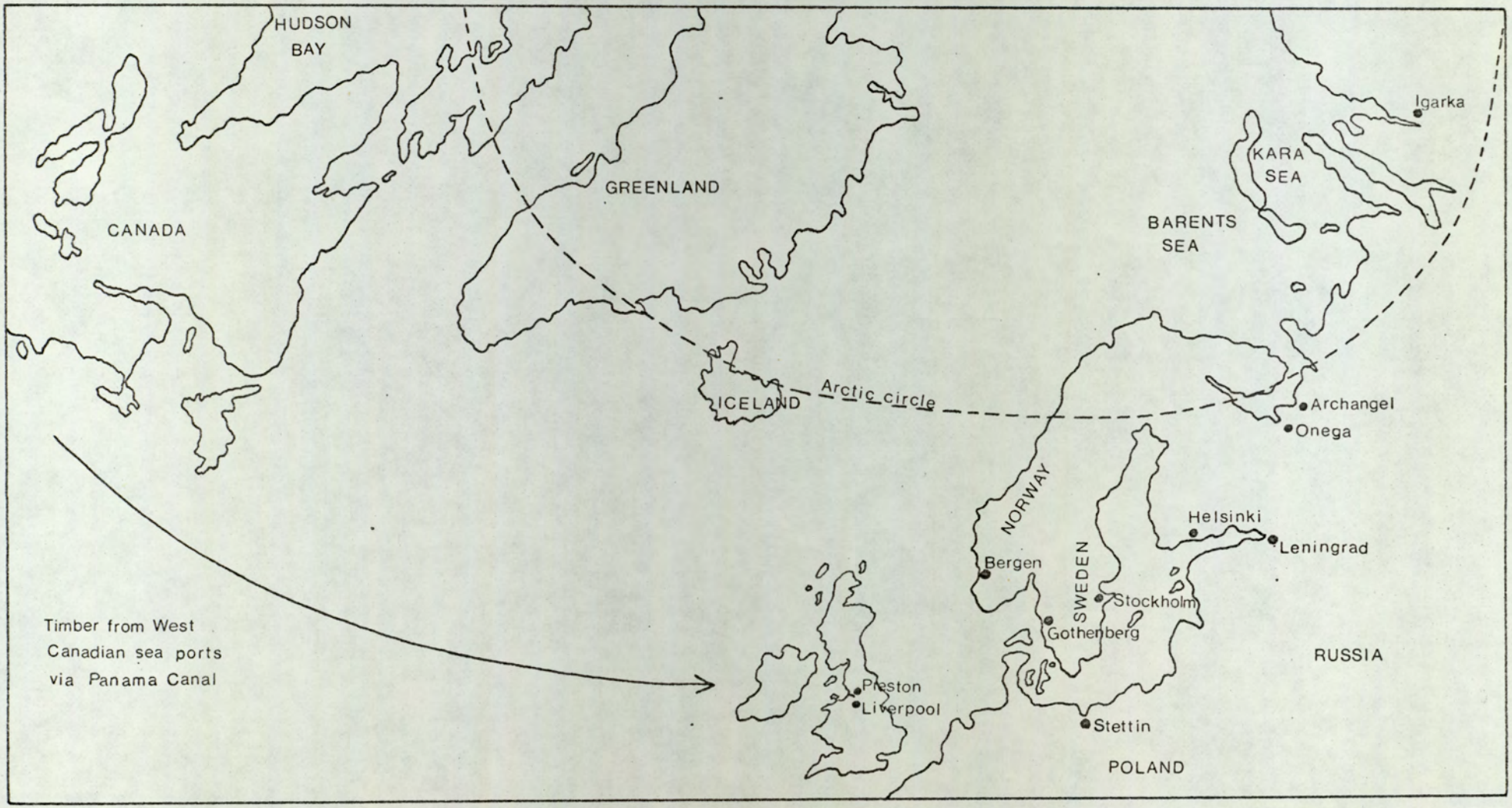
1. The spectrum of fungal flora of timber coming into this country via the docks at Preston and Liverpool.
2. The effect of temperature upon the growth ranges and in particular any cellulolytic activity of isolates.
3. Whether such infection included thermophilous fungi.

Temperatures suitable for the growth of thermophilous fungi were included in the range of temperatures employed during the experimental work because timber may reach above ambient temperatures during shipment as a result of insulation in packages, or as a result of insolation during shipment or whilst stacked in the timber yards.

Timber is imported directly into Preston Dock from Russia, Finland, Sweden and Czechoslovakia. The timber yards of the local importers are situated on the dockside and readily accessible for the collection of samples after the timber has been unloaded. The yards also contain timbers from Canada and from Brazil, but these are brought in by road from the docks at Liverpool and Manchester. (Fig 2·1).

During the course of this investigation six commercial softwoods were available for study :

FIG.2.1 PRINCIPAL EXPORTING PORTS OF TIMER RECEIVED AT PRESTON AND LIVERPOOL, LANCASHIRE, U.K.



Russian Redwood, Swedish Redwood	-	Pinus sylvestris
European Whitewood, (Czechoslovakia)	-	Picea abies
Western Hemlock (Canada)	-	Tsuga heterophylla
Western Red Cedar (Canada)	-	Thuja plicata
Western White Spruce (Canada)	-	Picea alba

Timber from Russia

The Russian Redwoods arriving in Preston are termed Kara Sea Redwood, Onega Redwood, Leningrad Redwood and Archangel Redwood. Kara Sea Redwood is the only one floated down river from the felling sites. It arrives at Igarka for milling and shipment. It is air dried only and all shipments are sent out loose. Onega, Leningrad and Archangel Redwoods travel to their ports of export by road, where they are then milled, air dried and once again shipped loose. These three latter ports are now shipping small quantities of timber in package form, but such imports into Preston are, as yet, rare. During this investigation Redwood from Onega was available for sampling.

Timber from Canada

Increasing quantities of packaged softwoods are being brought into the yards at Preston. In their country of origin the timbers are pushed into rivers in log form and floated to mills downstream. All timber is packaged mainly to length, in regular sized packs, (c. 3' 6" x 2' 6" - end section) driven by straddle carriers through anti-stain treatment tanks and then placed on to rail sidings for overland transport to Western Coast ports. During this investigation Western Hemlock, Western White Spruce, and Western Red Cedar were available for sampling.

Timber from Finland and Sweden

Timbers imported into Preston from these countries arrive as random length packages. The timber is milled, kilned and anti-stain treated before it is exported. No standard sizes of packages are in use at the moment, although

during the past year an increasing number of packages of approximately 3.5 cubic meters have been arriving in Preston.

Timber from Czechoslovakia

The timber is sent out loose from Stettin in Poland. The timber is converted near to the forest sites and air dried only. It arrives in Stettin by rail. During the investigation European Whitewood was available for sampling.

The problem of sampling and examining fungi in any timber is basically four-fold (Levy, 1967), a) to isolate the organism at depth, b) to use a suitable range of media for growing the organisms present and bringing them into pure culture, c) to identify and classify them and d) to determine whether they are simply wood inhabiting fungi or whether they can cause active decay.

In order to meet the first of these problems, a sampling technique was devised based on the experience gained by other workers in this field. The methods available for such an undertaking are :

1. The Pressler borer (Cartwright and Findlay, 1958; Corbett and Levy, 1963; Greaves and Savory, 1965). The cores are inoculated onto agar slopes.
2. The two chisel technique (Cartwright and Findlay, 1958; Greaves and Savory, 1965). One tool is used to remove the surface layer and the other to obtain the sample.
3. The sterile block technique (Cartwright and Findlay, 1958; Greaves and Savory, 1965). The material is generally split into convenient boards or sticks and small blocks are then chiselled from them. The blocks are lightly flamed before being placed on agar slopes.
4. The split technique (Shigo, 1965) Material is split longitudinally using a chisel or saw and specially modified forceps are used to pick out fragments for inoculation.

5. The saw cut technique (Greaves and Savory, 1965). The small particles of dust obtained by employing a sterilized hacksaw are allowed to fall over the surface of a petri-dish. This technique allows for rapid diffusion of nutrients into the dust, it enables one to subculture pure isolates at the first attempt and it allows the slower growing colonizers to develop free from competing fungi.

6. The drill technique (Greaves and Okigbo, 1966). A 5/8" drill-bit is employed to sample at depth; the particles from the drill hole are plated out. Levy, Stephens and Asmah (1967) used a $\frac{1}{4}$" drill for the primary drilling to depth and a 1/8" drill for obtaining the fine particle inocula.

   The technique used in this investigation uses the drill technique and makes use of an "Abrafile", a circular file, which lends itself well to obtaining dust for inoculation onto the surfaces of petri-dishes.

7. Surface isolation techniques (Lloyd, 1965; Okigbo, 1966). Surfaces may be sampled by applying adhesive tapes which are removed, cut up and plated onto agar. Okigbo (1966) devised a flattened grinding modification of the drill that ground the wood into fine sawdust.

   During this investigation the "Abrafile" was used for surface sampling.

More recently, workers interested in obtaining samples from within wood, have included Toole (1971) who used a technique similar to the split technique (Shigo, 1965), Käärick (1971), who cut cross sectional discs from poles and then removed small sample blocks from each disc and Butcher (1972) who employed a borer to obtain cores for his analysis of the fungal population in wood.

In order to meet the second of the problems, that of using a suitable medium for growing organisms isolated and bringing them into pure culture, the cellulose medium of Eggins and Pugh (1962) was used in this work; this medium was considered suitable since Savory (1965) compared eleven media when making isolations from wood and the results showed that sugar rich media do not favour the isolation of more fungi than do cellulose containing media. Furthermore, the cellulose media were more selective for slower developing fungi.

In order to meet the third of the problems, to identify and classify fungi, the taxonomic classifications of Gilman (1966); Barnett (1962), and Smith (1969) were used and isolates whenever possible were sent to the Commonwealth Mycological Institute for verification of taxonomic status and comments of interest.

Organisms isolated from wood and capable of clearing cellulose may be considered as possible soft rot organisms, it was decided, therefore, to use this criterion to distinguish wood inhabiting fungi from those which may cause active decay.

2.2 Experimental

2.2(i) Materials and methods

2.2(ii) Sampling

From the ends of newly imported planks pieces of timber three inches long were sawn, placed in clean polythene bags and brought into the laboratory. Plain sawn planks were chosen which were free from heart. Plank sizes varied in section but were uniform for a particular type of timber. The percentage moisture content of each sample was determined and then each block was prepared for sampling as shown in Fig. 2.2.

(a) Surface sampling

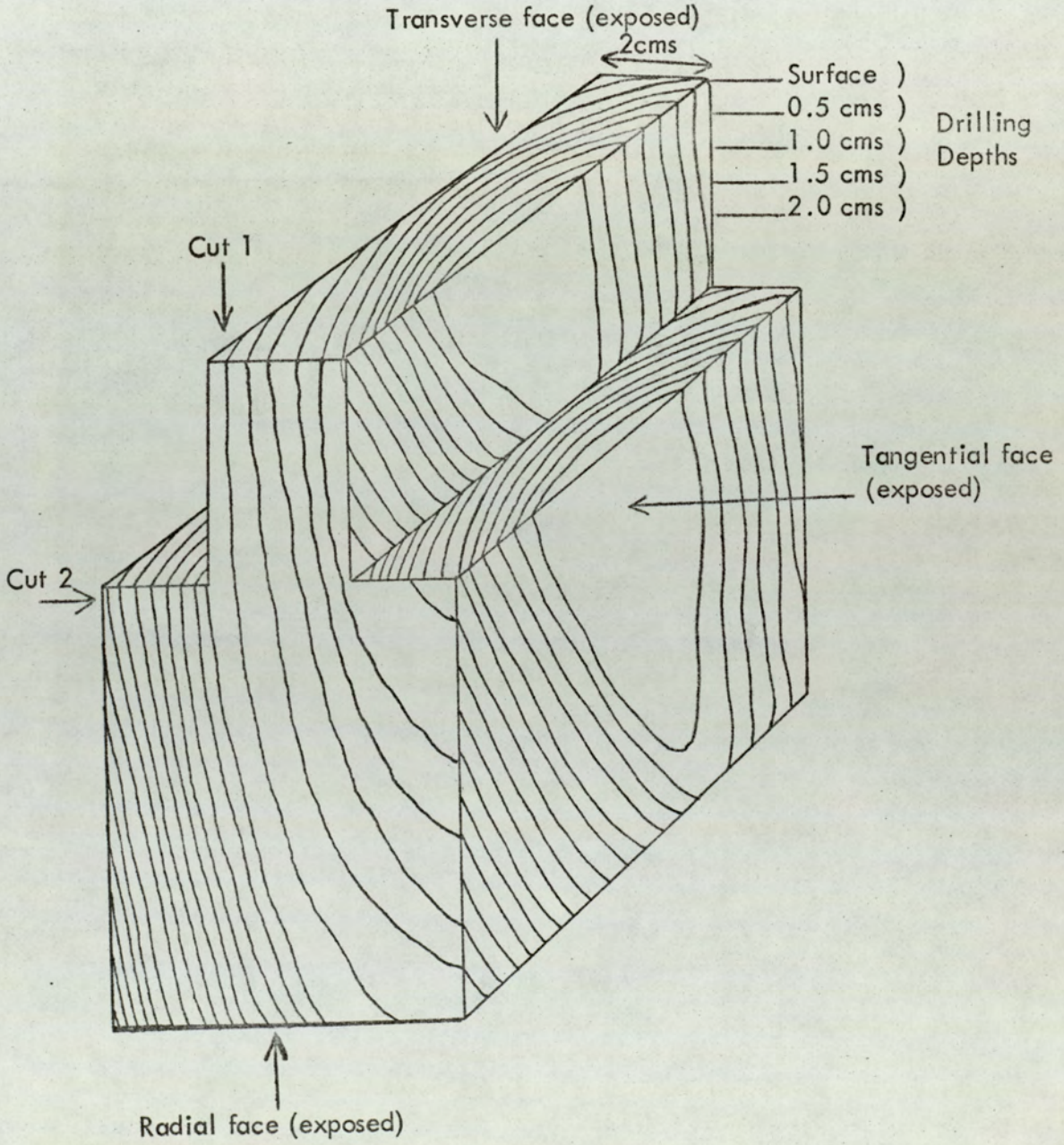
An 'Abrafile' was applied to the exposed surface of the wood and the filings were evenly distributed over the surfaces of plates containing Eggins and Pugh cellulose medium (Appendix I).

(b) Sampling at depth

A 3/32" bit was fitted to a power drill. Before each drilling the bit was alchohol flamed. A hole 3/32" in diameter allows a flamed 'Abrafile' to be inserted and manipulated so that filings can be evenly distributed over the surface of the cellulose medium. After each drilling and sampling the hole was plugged with plasticine to prevent filings escaping and contaminating subsequent sites. Between each sampling procedure the timber was flamed thoroughly to remove surface dust. Holes were drilled at depths of 0.5, 1.0, 1.5 and 2.0 cms.

FIG. 2.2

BLOCK OF WOOD PREPARED FOR SAMPLING



Filings from four equidistant sites on the surface of the wood were inoculated onto separate plates of the cellulose medium. Four holes were drilled at each level in the wood below the surface sites and filings from each hole were inoculated onto separate plates of the medium.

For each of the six timber samples available for investigation separate plates containing cellulose medium were inoculated with dust from each of the four surface sampling sites and each of the sixteen sites below the surface of each timber sample. The incubation temperatures employed were 5°, 15°, 25°, 35° and 45°C so as to obtain a representative cross section of the flora. The incubation period was two weeks during which time fungal colonies were identified and sub-cultured. Initially, sampling was undertaken in January, 1971 with incubations at 25°C. In May, samples were incubated at 35°C, in June at 5° and 15°C and in July at 45°C. Two sets of timber samples were available for the January investigation while one set of the six samples was available for each of the May, June and July investigations.

2.2 (iii) Determination of temperature tolerance ranges and the effect of temperature on cellulolytic activity

The ability of fungi to grow at temperatures other than those at which they were isolated was investigated. The temperatures employed were 0° to 60°C, by increments of 5 degrees. Each isolate was grown in monoculture, hyphal tips were then transferred to freshly prepared plates of Eggins and Pugh medium. Each isolate was then incubated at each of the temperatures. The plates were inspected at regular intervals and a record of growth, when it occurred, was noted.

In order to investigate the effects of temperature on the cellulolytic activity of the isolates the clearing tube technique was used (Rautella and Cowling, 1964). Tubes with a 25mm bore were plugged and autoclaved. 50cm^3 of sterile Eggins and Pugh medium was poured into each tube. The tubes were partially immersed in cold water to hasten gelling and to maintain the level of suspended cellulose. The tubes were inoculated with 1cm^2 pieces of fungal mycelium taken from near their colony perimeter. The height of the cellulose in the tube was marked and the plugged tubes were incubated for 30 days. The tubes were inspected at regular intervals and a record of the amount of clearing was kept. A high level of humidity was maintained to prevent shrinkage. Temperatures ranging through the minimum to maximum temperatures recorded for the growth of each isolate were used.

2.3 Results

A list of all fungi isolated is submitted (Appendix II). In all thirty-eight different species were isolated which included mould, stain and soft rot fungi. No Basidiomycetes were isolated. Table 2.1 shows the fungi isolated at the surface and at depth in the timbers investigated; all fungi except Trichoderma viride, Phoma glomerata, Graphium album, Scopulariopsis brevicaulis, Trichoderma harzianum, Chaetomium trilaterale and Aspergillus ochraceous were obtained at depth within the timbers during this investigation. Eight fungi were isolated at 5°C, of which, three species are known to cause soft rot (Table 2.1a). Eleven fungi were isolated at 15°C, of which, five species are known to cause soft rot (Table 2.1b). Twenty-two species were isolated at 25°C, of which, four are known to cause soft rot (Table 2.1c). Thirteen isolates were obtained at 35°C, of which, four species are known to cause soft rot (Table 2.1d). The single isolate obtained at 45°C was Aspergillus fumigatus, (Table 2.1e), an organism known to cause soft rot, it was also obtained at the 25° and 35°C incubations. Twelve of the isolates occurred at two or more of the incubation temperatures (Figure 2.3).

The moisture contents of the timbers at the time of collection ranged from -

13 to 67% in Picea alba
13 to 56% in Picea abies
12 to 55% in Thuja plicata
11 to 28% in Tsuga heterophylla - and from
11 to 20% in Pinus sylvestris

The isolation of fungi from timber with low moisture content points, possibly, to the ability of fungi to grow at moisture contents below those previously recorded in the literature.

It is presumed that a micro-organism grows most rapidly at that temperature at which the various metabolic processes that contribute to growth operate optimally (Farrell and Rose, 1967). Figure 2.3, shows the temperature growth range for each fungus, together with the temperature(s) at which it was isolated and a temperature at which maximum clearing of cellulose was recorded. Figure 2.4, submits a record of the amount of clearing of cellulose by each isolate at temperatures within its growth range. Of the thirty-eight isolates obtained during this investigation twenty-two produced measurable clearing of cellulose, and sixteen failed to clear cellulose. Liberation of pigment occurred in both cellulolytic and non cellulolytic fungi. The results indicate that the majority of fungi isolated would seem to be mesophilic (Cochrane, 1958) and micro-thermophilic (Apinis and Pugh, 1967) since of the twenty-two isolates which cleared cellulose, eleven produced maximum clearing of cellulose at 20° or 25°C (mesophiles), nine produced maximum clearing of cellulose at 30° or 35°C (microthermophiles), whilst two produced maximum clearing of cellulose at 15°C (psychrophiles).

The temperature tolerance ranges of the organisms considered as thermophilous (Figure 2.3) indicate their ability to remain viable between periods of insolation, whilst the data in Figure 2.4 shows their continued cellulolytic activity during these cooler periods.

Of the thirty-eight isolates obtained during this investigation nine are known to cause soft rot (Table 2.1), of which, three are considered to be thermophilous, namely : Aspergillus fumigatus, Chaetomium indicum, and Cephalosporium acremonium; these fungi were isolated from samples taken from below the surface of timber and would probably be protected from subsequent surface-only treatments. It is felt, therefore, that thermophilous fungi could well contribute to the early decay of imported wood which is subject to insolation.

TABLE 2.1(a)

FUNGI ISOLATED FROM IMPORTED SOFTWOODS

INCUBATION AT 5° SAMPLED JUNE, 1971

Species	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Aureobasidium * pullulans	+					Picea abies
	+					Picea alba
	+					Pinus sylvestris (S)
	+					Tsuga heterophylla
Cephalosporium sp. A			+			Picea alba
	+					Pinus sylvestris (S)
		+				Tsuga heterophylla
Cladosporium * herbarum	+		+			Picea abies
	+			+		Picea alba
	+					Pinus sylvestris (R)
	+	+	+	+	+	Pinus sylvestris (S)
				+		Thuja plicata
	+	+	+	+		Tsuga heterophylla
Penicillium brevicompactum	+					Picea alba
	+	+				Pinus sylvestris (R)
	+	+				Pinus sylvestris (S)
	+					Thuja plicata
	+	+	+			Tsuga heterophylla
Penicillium cyclopium		+	+			Pinus sylvestris (S)
Phoma herbarum		+	+			Tsuga heterophylla
Trichoderma viride *	+					Picea abies
	+					Pinus sylvestris (S)
Ulocladium atrum				+		Tsuga heterophylla

Recorded moisture contents:

Thuja plicata	12%	Pinus sylvestris (Russian)	13%
Picea alba	13%	Pinus sylvestris (Swedish)	14%
Picea abies	13%	Tsuga heterophylla	20%

* Organisms known to cause soft rot.

TABLE 2.1 (b)

FUNGI ISOLATED FROM IMPORTED SOFTWOODS cont.....

INCUBATION AT 15°C SAMPLED June, 1971

Species	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Amorphotheca resinae	+	+				Tsuga heterophylla
Aureobasidium pullulans *	+	+	+	+	+	Picea abies
	+					Picea alba
	+	+		+	+	Pinus sylvestris (S)
			+		+	Thuja plicata
	+	+	+	+	+	Tsuga heterophylla
Cephalosporium sp. A		+	+			Tsuga heterophylla
Cladosporium herbarum *	+					Pinus sylvestris (R)
	+	+	+	+	+	Tsuga heterophylla
Geotrichum candidum			+			Tsuga heterophylla
Penicillium crustosum	+					Picea alba
	+					Pinus sylvestris (R)
		+	+	+		Pinus sylvestris (S)
	+	+				Thuja plicata
	+		+	+		Tsuga heterophylla
Phialophora bubakii	+	+				Picea abies
	+					Picea alba
	+					Pinus sylvestris (R)
	+	+		+	+	Pinus sylvestris (S)
Phialophora fastigiata *	+	+		+		Tsuga heterophylla
Phoma glomerata *	+					Pinus sylvestris (S)
	+					Tsuga heterophylla
Phoma herbarum	+	+	+			Tsuga heterophylla
Trichoderma viride *	+					Pinus sylvestris (S)
	+					Tsuga heterophylla

Recorded Moisture Contents:

Thuja plicata	16%	Pinus sylvestris (Swedish)	19%
Picea abies	16%	Tsuga heterophylla	18%
Picea alba	17%	Pinus sylvestris (Russian)	19%

* Organisms known to cause soft rot.

TABLE 2.1(c)
FUNGI ISOLATED FROM IMPORTED SOFTWOODS cont.....

INCUBATION AT 25°C SAMPLED JANUARY, 1971

Species Isolated	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Absidia corymbifera			+	+	+	Pinus sylvestris (S)
					+	Thuja plicata
Alternaria alternata	+					Picea abies
	+					Picea alba
	+					Pinus sylvestris (S)
	+	+	+			Tsuga heterophylla
Aureobasidium pullulans *	+	+	+	+		Picea alba
	+	+	+			Picea abies
	+	+	+	+	+	Pinus sylvestris (S)
	+		+			Pinus sylvestris (R)
	+	+	+	+	+	Thuja plicata
	+	+	+	+	+	Tsuja heterophylla
Aspergillus fumigatus *	+		+		+	Pinus sylvestris (S)
Aspergillus nidulans			+		+	Pinus sylvestris (S)
Botrytis cinerea			+			Picea abies
	+					Pinus sylvestris (R)
		+				Thuja plicata
					+	Tsuga heterophylla
Cephalosporium acremonium *	+					Pinus sylvestris (S)
				+	+	Thuja plicata
					+	Tsuga heterophylla
Chaetomium bostrychodes					+	Picea abies
	+					Picea alba
	+					Pinus sylvestris (R)
	+					Thuja plicata
	+					Tsuga heterophylla
Cladosporium cladosporioides	+		+	+		Picea alba
				+	+	Tsuga heterophylla
Epicoccum purpurascens	+					Picea alba
	+					Pinus sylvestris
			+			Tsuga heterophylla

continued.....

TABLE 2.1(c)

INCUBATION AT 25°C SAMPLED JANUARY, 1971 continued.....

Species Isolated	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Geomyces vulgare			+			Tsuga heterophylla
Graphium album	+					Tsuga heterophylla
Mortierella sp.					+	Picea alba
Penicillium brevicompactum	+	+	+			Picea abies
	+	+			+	Picea alba
	+	+				Pinus sylvestris (S)
	+					Thuja plicata
	+	+				Tsuga heterophylla
Penicillium decumbens	+	+		+		Picea abies
Phoma herbarum	+					Picea abies
	+	+		+	+	Picea alba
Scopulariopsis brevicaulis	+					Pinus sylvestris (R)
Syncephalastrum racemosum				+		Pinus sylvestris (S)
Trichoderma viride *	+					Picea abies
	+					Picea alba
	+					Pinus sylvestris (R)
	+					Thuja plicata
	+					Tsuga heterophylla
Ulocladium atrum	+					Picea alba
	+					Pinus sylvestris (S)
Verticillium intertextum	+					Picea abies
	+					Pinus sylvestris (S)
	+					Pinus sylvestris (R)
				+	+	Thuja plicata
Verticillium latertitium	+					Thuja plicata

Recorded moisture contents: (two samples)

Picea alba	13 - 20%	Tsuga heterophylla	20 - 28%
Pinus sylvestris (Russian)	12 - 16%	Thuja plicata	18 - 20%
Picea abies	24 - 56%	Pinus sylvestris (Swedish)	11 - 18%

* Organisms known to cause soft rot.

TABLE 2.1 (d)
FUNGI ISOLATED FROM IMPORTED SOFTWOODS cont.....
INCUBATION AT 35°C SAMPLED MAY, 1971

Species Isolated	Depth of samples in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Amorphotheca resinae	+					Picea abies
Aspergillus fumigatus *	+		+			Picea alba
	+		+			Pinus sylvestris (R)
	+		+			Tsuga heterophylla
Aspergillus ochraceous	+					Picea abies
Aureobasidium pullulans *	+	+				Picea abies
	+	+	+	+	+	Picea alba
	+	+	+	+	+	Pinus sylvestris (S)
	+	+	+	+		Pinus sylvestris (R)
	+			+		Thuja plicata
	+	+	+	+		Tsuga heterophylla
Cephalosporium sp. A	+	+	+			Picea abies
		+				Pinus sylvestris (R)
Cephalosporium sp. B	+					Picea alba
			+			Tsuga heterophylla
Cephalosporium acremonium *	+					Picea alba
Chaetomium indicum *				+		Pinus sylvestris (R)
	+					Pinus sylvestris (S)
	+					Thuja plicata
Chaetomium trilaterale	+					Pinus sylvestris (S)
Geotrichum candidum	+	+	+			Picea abies
	+		+			Picea alba
	+	+	+	+	+	Pinus sylvestris (R)
	+	+				Pinus sylvestris (S)
	+	+	+	+	+	Tsuga heterophylla
Paecilomyces variotii		+				Picea abies
	+					Pinus sylvestris (S)
	+			+		Tsuga heterophylla

continued.....

TABLE 2.1(d)

INCUBATION AT 35°C SAMPLED MAY, 1971 continued.....

Species Isolated	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Penicillium citrinum	+	+				Picea abies
	+	+	+			Picea alba
	+	+				Pinus sylvestris (S)
	+					Thuja plicata
Trichoderma harzianum	+					Picea alba
	+					Pinus sylvestris (R)

Recorded moisture contents :

Picea alba	67%	Pinus sylvestris (Swedish)	20%
Thuja plicata	55%	Pinus sylvestris (Russian)	18%
Tsuga heterophylla	11%		
Picea abies	13%		

* Organisms known to cause soft rot

TABLE 2.1 (e)
FUNGI ISOLATED FROM IMPORTED SOFTWOODS cont.....
INCUBATION AT 45°C SAMPLED JULY, 1971

Species Isolated	Depth of sample in cms.					Type of Timber
	0	0.5	1.0	1.5	2.0	
Aspergillus fumigatus *	+	+	+	+		Picea abies
	+			+	+	Picea alba
	+	+	+	+	+	Pinus sylvestris (R)
	+					Pinus sylvestris (S)
			+			Thuja plicata
	+	+		+	+	Tsuga heterophylla

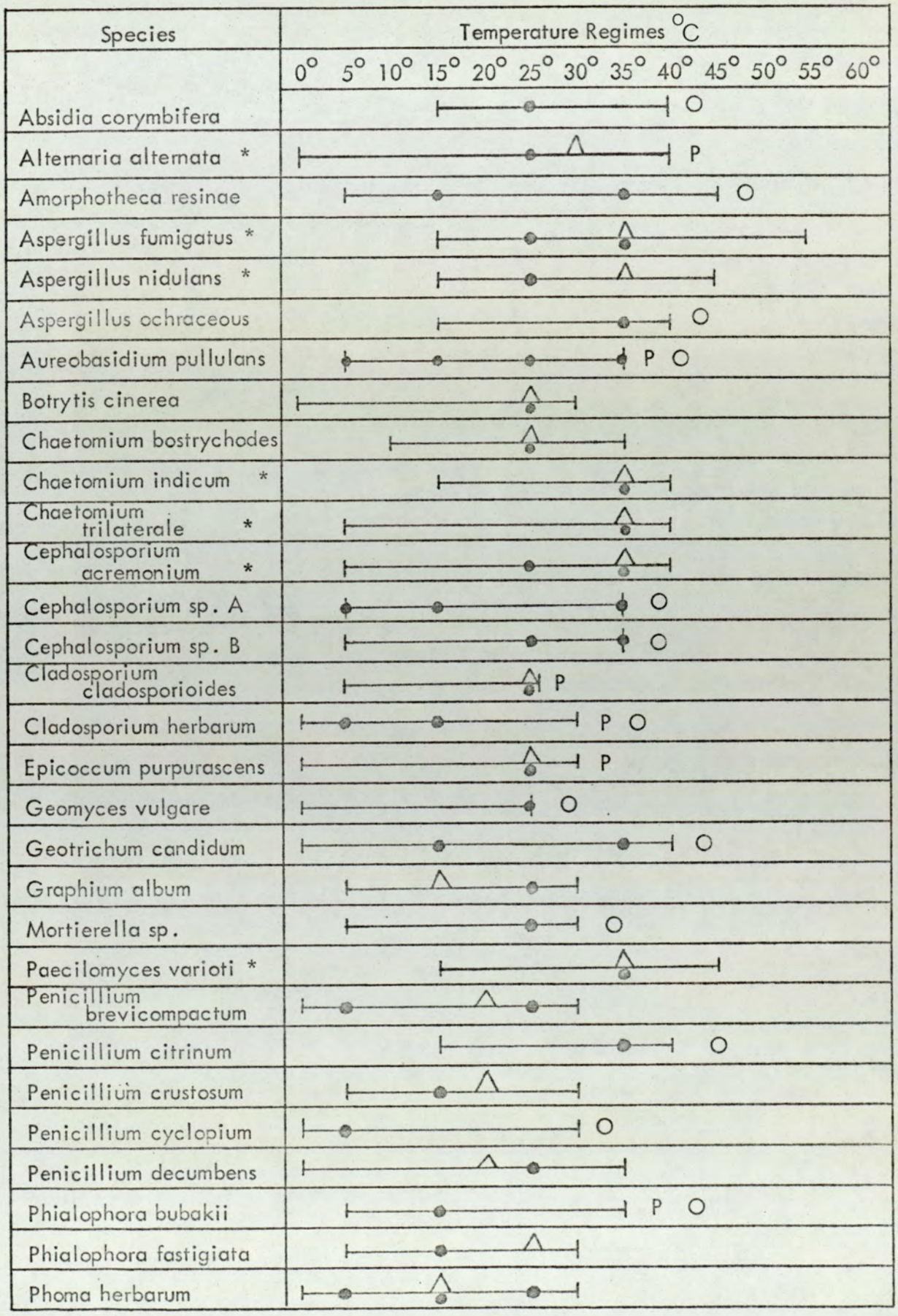
Recorded moisture contents :-

Pinus sylvestris (Russian)	10%	Pinus sylvestris (Swedish)	10%
Picea alba	12%	Tsuga heterophylla	13%
Thuja plicata	11%	Picea abies	11%

* Organisms known to cause soft rot

FIG. 2.3

GROWTH RANGE ISOLATION TEMPERATURES AND TEMPERATURES OF MAXIMUM CLEARING OF CELLULOSE



continued.....

FIG. 2.3

GROWTH RANGE ISOLATION TEMPERATURES AND TEMPERATURES OF MAXIMUM CLEARING OF CELLULOSE continued.....

Species	Temperature Regimes °C
	0° 5° 10° 15° 20° 25° 30° 35° 40° 45° 50° 55° 60°
Phoma glomerata	P O
Scopulariopsis brevicaulis	
Syncephalastrum rascemosum	O
Trichoderma harzianum *	
Trichoderma viride	
Ulocladium atrum *	P
Verticillium intertextum	O
Verticillium latertitium	

* Microthermophiles

Key:

- ● = isolation temperatures
- △ = maximum clearing of cellulose
- P. = pigment production
- O = fungi which gave no measurable clearing of cellulose

FIG. 2.4

CLEARING OF CELLULOSE BY FUNGI AT TEMPERATURES WITHIN THEIR TEMPERATURE TOLERANCE RANGES

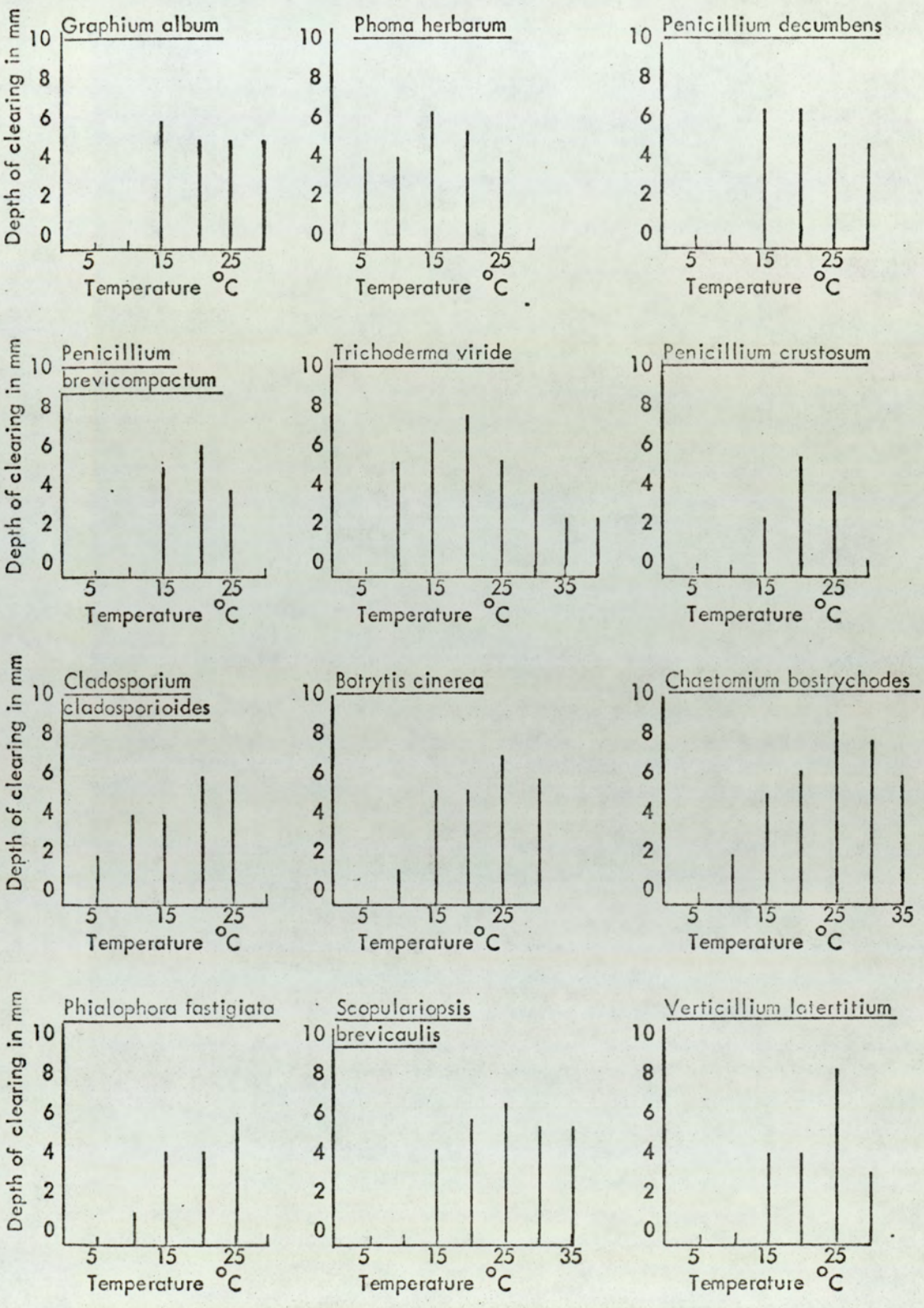


FIG. 2.4

CLEARING OF CELLULOSE BY FUNGI AT TEMPERATURES WITHIN THEIR TEMPERATURE TOLERANCE RANGES cont.....

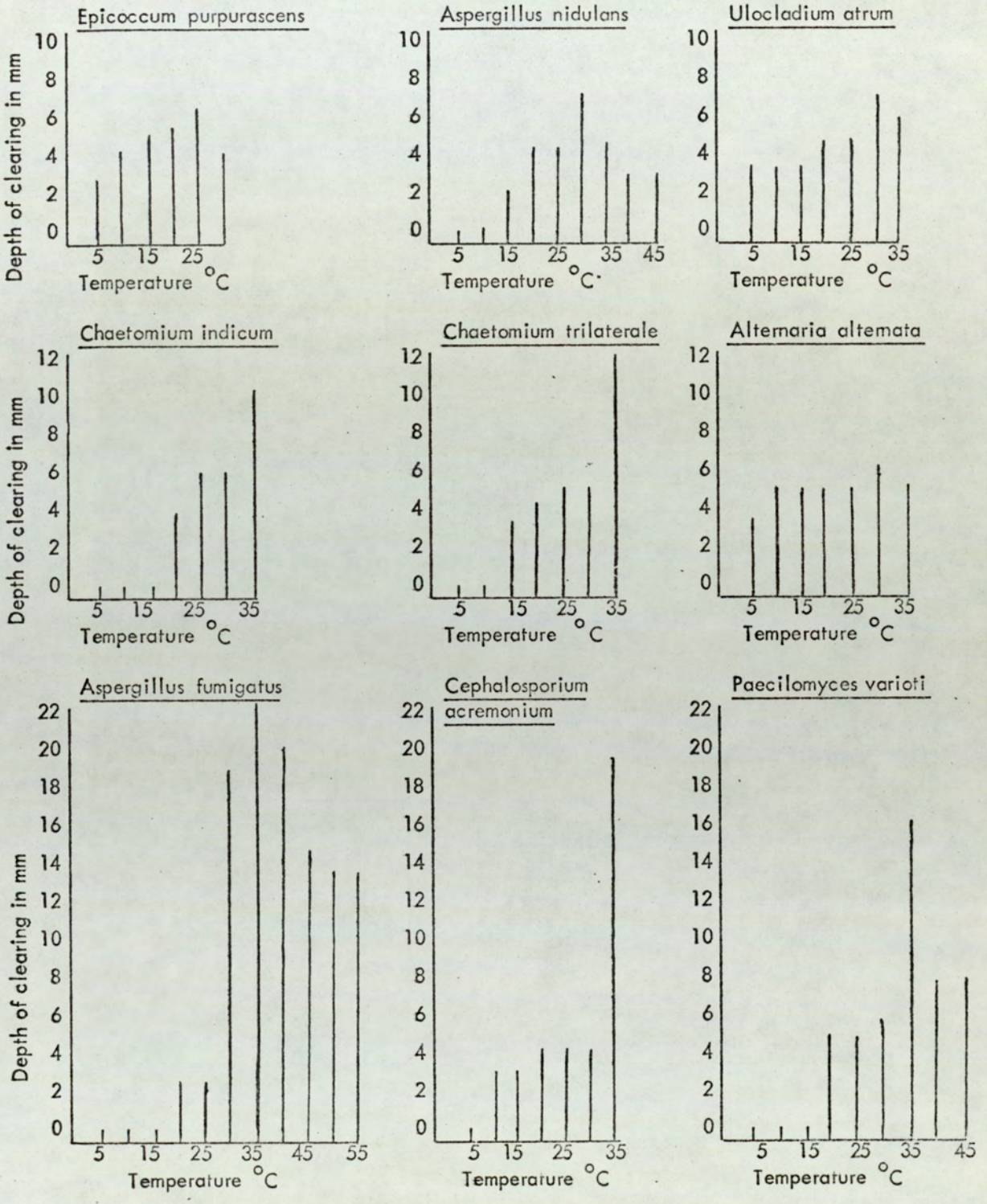
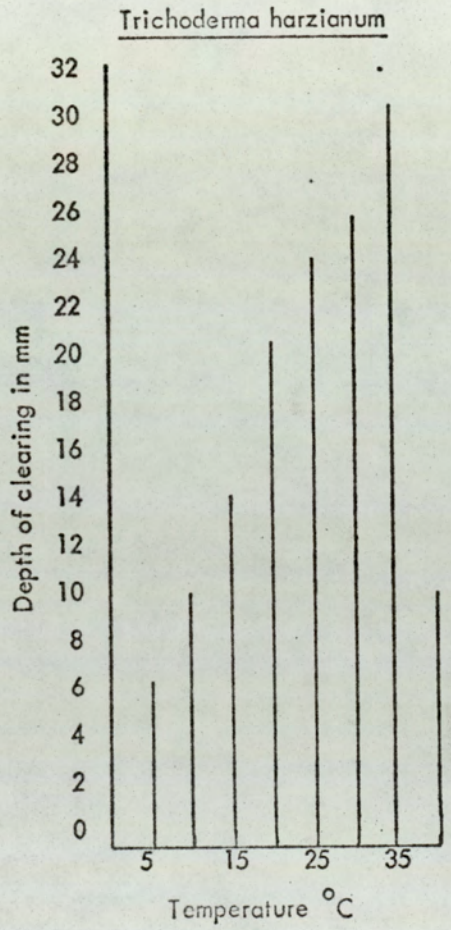


FIG. 2.4

CLEARING OF CELLULOSE BY FUNGI AT TEMPERATURES WITHIN THEIR TEMPERATURE TOLERANCE RANGES continued.....



2.4 Discussion

Whether infection of the timbers investigated during this survey occurred in the country of origin, during shipment or even in this country is a matter for speculation (Cartwright and Findlay, 1958), as is the source of infection which could be from water, soil or from spores in the air (Corbett and Levy, 1963b; Findlay, 1965; Butcher, 1968; Toole, 1971; Hudson, 1973). However, Savory (1967, 1974) reported the presence of mould, staining, Basidiomycete and occasionally soft rot fungi in imported timbers and although no statistical significance is implied to the occurrence or seasonal availability of the fungi in the results of this investigation they do record the presence of mould, staining and soft rot fungi in imported timbers. No Basidiomycetes were isolated during this survey.

Moisture contents of timber recorded during this survey were often very low, due in part to the weather conditions at the time of collection and due, no doubt, to the fact that it was the ends of planks which were the most convenient and practical portions to remove at the yards for subsequent sampling in the laboratory. The isolation of fungi, however, from timber with low moisture content points to the ability of fungi to survive at low moisture contents. This may be due to the viability of their propagules during low moisture conditions, or possibly to increased biological activity of water at higher temperatures (Ayerst, 1965) resulting from the insulation of timber in package (Savory, 1967) or possibly as a result of insolation. The ability of insolation to raise the temperature of wood above ambient has been recorded by Henningson,(1968) and Jensen, (1968) and in logging slash by Loman, (1962); the cellulolytic nature of thermophilous fungi has been reported by Eggins et al (1972) and their ability to cause weight loss in wood has been reported by Ofosu-Asiedu and Smith (1973). It is considered then

to be of particular significance that almost a quarter of the fungi isolated during this investigation may be termed microthermophilic containing cellulolytic and known soft rot organisms (Table 2.1, Figure 2.3, Appendix X, XI) consistent with the flora expected from a wood substrate which may have been subject to insolation (Loman 1962).

Of considerable interest are the results (Figure 2.4) which show the continued cellulolytic activity of those fungi, designated thermophilous, at temperatures below those of their optimal activity. These results complement the report of Henningson (1968) that many fungi with higher optima are more active at low temperatures than the low temperature fungi themselves. It is considered possible that as in soils (Apinis 1963, 1965; Allsop, 1968; Eggins and Malik, 1969; Eggins et al 1972) thermophilous fungi are widespread in wood but they are at their most active during periods of insolation.

Although imported timber will be planed and probably treated prior to being put into service, the isolation of cellulolytic fungi from beneath the surface of the imported timber may provide a case for considering that some timber, particularly that which is surface treated only, may be infected when it goes into service.

CHAPTER III

A SURVEY OF THE THERMOPHILOUS FUNGI OF IN-SERVICE TIMBER

Contents:

3.1 Introduction

Although surveys of mesophilic microfungi growing on timber joinery have been undertaken (Tack, 1968; Richardson, 1969; Dooper, 1970), the higher growth temperature fungi, shown to be of frequent occurrence on imported timber have not been investigated. This survey was undertaken to isolate thermophilous microfungi from timber joinery in the Preston area and to compare the results with those obtained from the dock survey (Chapter II). A comparison of the two flora was considered important because much of the timber joinery going into service in thePreston area is constructed from timber obtained from the dockside timber yards of local importers, and since thermophilous cellulolytic fungi have been isolated from below the surface of such timbers, there may be a case for considering that some infected wood is put into service. It was decided to concentrate on the isolation of thermophilous fungi because it was considered that the insolation of timber joinery, together with the possible increase in the biological activity of water at higher temperatures, could lead to the establishment in wood of thermophilous fungi.

Nine sites were investigated (Table 3.1). Apart from two unpainted hardwood fence posts (Sites 7 and 9) the other sites were painted softwood comprising window joinery, weather boarding and a door.

In unprotected wood the moisture content of the outer fibres fluctuates with changes in atmospheric conditions. The resultant repeated swelling and shrinkage loosens these fibres and surface degradation occurs. Further, the ultra violet part of sunlight and changes in temperature cause physical and chemical changes to occur (Desai and Clarke, 1972). Painting of external joinery retards weathering by providing a barrier against the entry of water and by reducing the amount of light energy reaching the wood (Heebink, 1970). Painting, up to fairly recently

TABLE 3.1

NATURE, LOCATION AND DESCRIPTION OF SITES

Nature of sites	Location	Condition of surface
1. Weather-board facing South. Softwood	The quadrangle, Preston Polytechnic	Paint flaking off timber rotting
2. Window sill facing West. Softwood	Biodeterioration Laboratory, Preston Polytechnic	Surface weathered, paint flaking off, but no signs of rot
3. Weather-board facing West. Softwood	Greenhouse, Freckleton, Lancs.	Paint flaking off, timber rotting
4. Window-frame facing North. Softwood	Inorganic Chemistry Laboratory, Preston Polytechnic	Surface weathered, paint flaking off, but but no signs of rot
5. Window sill facing West. Softwood	Students Common-room, Preston Polytechnic	Paint flaking off, timber rotting
6. Door-base facing North. Softwood	Private house Forton, Lancs.	Paint film intact, timber rotting
7. Fence post, facing East. Hardwood	Private house Great Harwood, Lancs.	Timber rotting
8. Window-frame, facing North. Softwood	Private house, Woolton, Lancs.	Paint flaking off, timber rotting
9. Fence post, facing East. Hardwood	Railway siding, Leyton Street, Preston.	Timber rotting

was the most commonly used method of protecting wood. In recent years preservation followed by painting or staining has come into prominence.

Paradoxically, paint provides a barrier against the entry of moisture, but may also act as a seal keeping moisture in thuscreating an environment suitable for wood decaying organisms. Nash-Wortham and Savory (1968) report that wood dries very slowly through an intact paint system and this is taken to indicate that moistening of joinery either prior to painting or subsequently through an unsealed joint, can lead to sustained risk of development of decay.

Moisture content of wood has always appeared to be of primary importance in the failure of paint film on wood (Van Loon, 1966). The explanation seems to be that wood with a high moisture content will shrink when it dries out, and as wood is not homogeneous, shrinkage will be irregular resulting in an irregular surface with stress in the paint layer and consequently cracking and peeling occur. Shrinkage also promotes movement of wood, opening up joints and letting water in (Dooper, 1970). The results of a recent survey of window joinery (Tack, 1968), have demonstrated the serious damage that exists. Of the windows examined 66.4% had areas with moisture contents in excess of 21%, usually at the lower joints, and were considered to be susceptible to decay or had been repaired or replaced as a result of decay. Richardson's (1969) survey of external joinery in houses which were only four years old, reported decay present in many window frames and doors, even after two years' service, and in all cases this was associated with joints in timber.

White (1971) commented that the changes in climate both indoors and outdoors determine not only the rate and extent of dimensional change but also the direction in which moisture is taken up and released by the wood. He made the point that warmer air indoors has the capacity to hold a much higher amount of water vapour

than air outside. Dooper (1970) made the point that an accumulation of moisture in painted wood can only be avoided if the permeability of the interior paint system to water vapour is less than that of the exterior one thus balancing out the amount of water entering from the inside to that being lost on the outside. This has been called the "principle of relative moisture isolation" and its validity has been demonstrated by Van Loon (1966, 1968). However, the problem of moisture in in-service timber has proved rather more difficult to resolve than by simply applying one more coat of the same paint on the inside than on the outside. The result of Dooper's (1970) experiments and calculations indicate that at a relative humidity of 100% (inside) four coats of paint on the inside to one coat on the outside would give favourable permeability rates. At relative humidities up to, but not exceeding 70%, three coats on the inside to two on the outside would seem acceptable. Both of these experimental findings have short-comings, firstly a one coat-external system would give inadequate protection, and secondly the 70% relative humidity limit is too restricting. There are also obvious economic disadvantages to both systems. Dooper (1970) and Richardson (1969) stress that the development of fungal decay in external timber joinery is an indication of neglect in design, specification, construction or maintenance.

3.2 Experimental

3.2 (i) Materials and Methods

At each site the surface temperature and moisture content of the timber were determined before samples were taken. The temperatures were recorded with a portable copper/constantan thermocouple and the moisture contents were estimated with a Protimeter moisture meter. Flamed scalpels, forceps and needles were used to detach material from the surfaces at each site and to inoculate it onto the surface of plates containing an agar medium of ball-milled redwood and the salts of the Eggins and Pugh modified cellulose medium (Appendix I).

Samples were taken from exposed timber by removing small fragments, some 1 or 2mm thick, and inoculating them onto the agar. Similar fragments were removed from below the intact paint films and samples of intact and flaking paint films were taken. Three plates were inoculated with material from each surface and incubation was carried out at 30°, 35° and 40°C, temperatures suitable for the growth of micro-thermophilous fungi (Chapter II). As an upper limit to the incubation temperatures, 40° rather than 45°C was employed, as only one isolate was obtained at this latter temperature during the investigation of imported timber. Sub-cultures were taken from the master plates during the next two weeks and each isolate was brought into pure culture. The cellulolytic activity of each isolate was determined using the clearing tube technique (Rautella and Cowling, 1964), and the temperature tolerance range of each isolate on ball-milled cellulose agar was determined. The incubation temperatures for these experiments ranged from 0°C to 60°C by 5 degree increments.

3.3 Results

The results show the presence of cellulolytic thermophilous fungi in painted and unpainted timber in service. A taxonomic list of isolates is included. (Appendix III).

Table 3.2, lists the fungi isolated at the sites investigated. Information on the surfaces sampled and the temperatures of incubation is included. All sites investigated produced at least one organism which was both thermophilous and cellulolytic and on record as being capable of producing soft rot in timber.

Table 3.3, shows the overall occurrence of isolates in terms of the surfaces sampled. Thirteen isolates were obtained from paint films, fifteen from wood samples taken from below paint films and twenty from the samples of exposed wood. Twelve species were common to the wood substrates sampled, of which nine were cellulolytic including six microthermophiles, two of which are on record as being capable of producing soft rot; they were, Aspergillus fumigatus and Penicillium funiculosum. Of the isolates obtained from the paint films all species except Penicillium caryophilum, a non-cellulolytic fungus isolated from Sites 4 and 9, were also isolated from wood.

Table 3.4, records the incubation temperatures at which the fungi were isolated. Twenty isolates were obtained at 30°C, fifteen at 35°C and nine at 40°C. Fourteen isolates were common to two or more of these temperatures, eleven of which were cellulolytic. Five isolates were common to all three incubation temperatures of which four were cellulolytic including the two thermophilous soft rot organisms Aspergillus fumigatus and Penicillium funiculosum.

Table 3.5, presents a summary of these results together with a record of the moisture content and temperature of wood at the times of sampling which were between September, 1972 and February, 1973. Two of the sites investigated

(Table 3.2, Sites 2,3) had moisture contents below 20% at the time of collection. On no occasion was the temperature of wood recorded above 22°C (Table 3.2, Site 1) and was recorded as low as 4°C (Table 3.2, Site 4).

In all twentyfive fungi were isolated during this investigation of which eighteen cleared cellulose. Of those fungi which cleared cellulose thirteen produced maximum clearing at 30°, 35° or 40°C and may be considered as microthermophiles (Table 3.6). Six of the fungi isolated are on record as being capable of causing soft rot in timber (Table 3.2; Appendix X) of which four are microthermophiles namely, Aspergillus fumigatus, Cephalosporium acremonium, Fusarium solani and Penicillium funiculosum.

TABLE 3.2 FUNGI ISOLATED AT THREE INCUBATION TEMPERATURES

Site No. 1: Weather-board, The Quadrangle, Preston Polytechnic. 12.9.72

Surface temperature: Paint Film 21°C, exposed wood and wood below paint film 22°C

Moisture content wood: 22%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Trichoderma koningii	+			+					+
Aspergillus fumigatus *			+	+			+		+
Aureobasidium pullulans*				+					
Fusarium culmorum							+		
Cephalosporium acremonium							+		
Rhinocladiella mansonii					+		+		
Nectria inventa		+			+			+	

Site No. 2: Window sill, Biodeterioration Laboratory, Preston Polytechnic. 22.9.72

Surface temperature: Paint film, exposed wood and wood below paint film 16.5°C

Moisture content wood: 18%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Acremonium sp.	+	+		+	+		+		
Mucor racemosus	+			+					
Aspergillus fumigatus *		+	+	+	+		+	+	+
Trichoderma koningii							+		
Chrysosporium pruinosum							+		

* Organisms capable of causing soft rot.

TABLE 3.2 FUNGI ISOLATED AT THREE INCUBATION TEMPERATURES cont....

Site No. 3: Weather-board, Greenhouse, Freckleton, Lancs. 7.10.72

Surface Temperatures: Paint film, exposed wood and wood below paint film 17°C

Moisture content wood: 15%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Rhinocladiella mansonii	+								
Alternaria alternata	+						+		
Aspergillus fumigatus *		+	+	+		+		+	+
Penicillium funiculosum *				+			+		
Absidia corymbifera				+					
Mucor racemosus				+			+		
Acremonium kiliense		+		+	+	+	+		
Aspergillus nidulans							+		
Aspergillus ochraceous							+		
Phoma sp.						+			
Coprinus sp.				+	+	+	+	+	+

* Organisms capable of causing soft rot.

TABLE 3.2 FUNGI ISOLATED AT THREE INCUBATION TEMPERATURES cont....

Site 4: Window Frame, Inorganic Chemistry Laboratory, Preston Polytechnic. 21.11.72

Surface Temperature: Paint film, exposed wood and wood below paint film 4° C

Moisture content wood: >30%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Acremonium curvulum	+			+			+		
Rhinocladiella mansonii	+								
Penicillium caryophilum	+								
Aspergillus fumigatus *		+	+		+	+	+		
Aureobasidium pullulans *							+		
Trichoderma viride *		+						+	

Site 5: Window sill, Students' Common Room, Preston Polytechnic. 21.11.72

Surface temperature: Paint film, exposed wood and wood below paint film 4°C

Moisture content wood: >30%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Trichoderma viride *	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus *			+			+			+

* Organisms capable of causing soft rot.

TABLE 3.2 FUNGI ISOLATED AT THREE INCUBATION TEMPERATURES cont.....

Site No. 6: A door, private house, Forton, Lancs. 4.2.73

Surface temperature: Paint film, wood below paint film 8°C

Moisture content wood: > 30%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C					
	Paint Film			Wood Below Paint		
	30	35	40	30	35	40
Aspergillus fumigatus *	+	+	+	+	+	+
Absidia corymbifera	+	+	+	+	+	+
Phoma sp.	+					
Verticillium psalliotae				+		
Trichoderma viride *		+	+		+	

Site No. 7: Fence Post, Great Harwood, Lancs. 12.2.73

Surface temperature: 6°C (exposed wood only)

Moisture content wood: 26%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C		
	Exposed Wood		
	30	35	40
Trichoderma viride *	+	+	
Fusarium solani *	+		
Alternaria alternata	+	+	
Aspergillus fumigatus *		+	
Absidia corymbifera			+
Scopulariopsis brevicaulis			+
Paecilomyces varioti			+

* Organisms capable of causing soft rot.

TABLE 3.2 FUNGI ISOLATED AT THREE INCUBATION TEMPERATURES cont....

Site No. 8: Window sill, Private House, Woolton, Lancs. 19.9.72

Surface temperature: Paint film, exposed wood 17°C

Moisture content wood: 23%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperatures °C								
	Paint Film			Wood Below Paint			Exposed Wood		
	30	35	40	30	35	40	30	35	40
Cephalosporium sp		+		+			+	+	
Aspergillus fumigatus *		+		+			+		
Fusarium solani							+		
Mucor racemosus							+		
Absidia corymbifera						+	+		+
Scopulariopsis brevicaulis								+	

Site No. 9: Fence Post, Railway Siding, Leyton Street, Preston. 24.10.73

Surface temperature: 18°C (exposed wood only)

Moisture content wood: > 30%

Fungi isolated on a medium of ball-milled wood and salts	Sample/Incubation Temperature °C		
	Exposed Wood		
	30	35	40
Trichoderma viride *		+	+
Aspergillus fumigatus *		+	+
Penicillium coryophilum		+	

* Organisms capable of causing soft rot.

TABLE 3.3 OCCURRENCE OF ISOLATES AT SURFACES SAMPLED

Sample	M. racemosus	A. corymbifera	N. inventa (C)	A. kiliense (C)	Acremonium sp. (C)	A. fumigatus (C)	A. alternata (C)	Cephalosporium sp. (C)	P. caryophilum	R. mansonii	Phoma sp. (C)	T. viride (C)	T. koningii (C)	V. psalliotae	P. funiculosum (C)	C. macrocephalus	A. pullulans	A. ochraceous	A. nidulans (C)	S. brevicaulis (C)	C. acremonium (C)	C. pruinosum (C)	F. culmorum (C)	F. solani (C)	P. varioti (C)
Paint Film	+	+	+	+	+	+	+	+	+	+	+	+	+												
Wood below paint film	+	+	+	+	+	+		+		+	+	+	+	+	+	+	+								
Exposed Wood	+	+	+	+	+	+	+	+		+		+	+		+	+		+	+	+	+	+	+	+	+

C = cellulolytic fungi

TABLE 3.4 INCUBATION TEMPERATURES AND FUNGI ISOLATED

Incubation Temperatures ^0C	M. racemosus	A. corymbifera	N. inventa (T)	A. kiliense (T)	Acremonium sp.	A. fumigatus (T)	A. alternata (T)	Cephalosporium sp. (T)	P. caryophilum	R. mansonii	Phoma sp.	T. viride	T. koningii (T)	V. psalliotae	P. funiculosum (T)	C. macrocephalus	A. pullulans	A. ochraceous	A. nidulans (T)	S. brevicaulis	C. acremonium (T)	C. pruinosum (T)	F. culmorum (T)	F. solani (T)	P. varioti (T)
30		+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	
35	+	+	+	+	+	+	+	+		+	+	+	+		+	+				+					
40	+	+				+	+				+				+	+				+					+

T = microthermophilous fungi

TABLE 3.5 SUMMARY OF RESULTS: THERMOPHILOUS FUNGI OF NATURALLY DETERIORATING TIMBER

Fungi Isolated	SAMPLE/INCUBATION TEMPERATURES									Ranges of moisture % and temperature of wood at time of collection	
	Paint Film			Wood Below Paint			Exposed Wood				
	30	35	40	30	35	40	30	35	40	Moisture %	Temp. °C
Mucor racemosus	+	+		+			+			15.0 - 23.0	16.5 - 17.0
Absidia corymbifera	+	+	+	+	+	+	+		+	15.0 -> 30.0	8.0 - 17.0
Nectria inventa		+			+			+		22.0	21.0
Acremonium kiliense		+		+			+			15.0	17.0
Acremonium sp.	+	+		+	+		+			16.5	18.0
Aspergillus fumigatus *	+	+	+	+	+	+	+	+	+	15.0 -> 30.0	4.0 - 22.0
Aspergillus ochraceous							+			15.0	17.0
Aspergillis nidulans							+			15.0	17.0
Aureobasidum pullulans *				+						22.0	22.0
Alternaria alternata	+							+	+	15.0 - 26.0	6.0 - 17.0
Cephalosporium acremonium *								+		22.0	22.0
Cephalosporium sp.		+		+			+	+		23.0	17.0
Chrysosporium pruinosum							+			18.0	16.5
Fusarium culmorum							+			22.0	22.0
Fusarium solani *							+			23.0 - 26.0	6.0 - 17.0
Paecilomyces varioti									+	23.0 - 26.0	6.0 - 17.0

continued.....

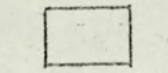
TABLE 3.5 SUMMARY OF RESULTS: THERMOPHILOUS FUNGI OF NATURALLY DETERIORATING TIMBER continued.....

Fungi Isolated	SAMPLE/INCUBATION TEMPERATURES									Ranges of moisture % and temperature of wood at time of collection	
	Paint Film			Wood Below Paint			Exposed Wood				
	30	35	40	30	35	40	30	35	40	Moisture %	Temp. °C
Penicillium caryophilum	+									> 30.0	4.0
Penicillium funiculosum *				+					+	15.0	17.0
Rhinocladiella mansonii	+				+		+			22.0 -> 30.0	4.0 - 22.0
Phoma sp.	+					+				15.0 -> 30.0	8.0 - 17.0
Trichoderma viride *	+	+	+	+	+	+	+	+	+	26.0 -> 30.0	4.0 - 18.0
Trichoderma koningii	+			+			+	+	+	18.0 - 22.0	16.5 - 22.0
Verticillium psalloitae				+						> 30.0	8.0
Scopulariopois brevicaulis								+	+	23.0 - 26.0	6.0 - 17.0
Caprinus macrocephalus				+	+	+	+	+	+	15.0	17.0

* Organisms capable of causing soft rot.

TABLE 3.6

CELLULOLYTIC ACTIVITY AND TEMPERATURE TOLERANCE RANGES



Fungi	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°
Nectria inventa		2	3	3	7	7	7	8	7				
Acremonium kiliense							3	5	3				
Acremonium sp.			3	7	9	11	10	10	8				
Aspergillus fumigatus					3	3	19	23	20	15	14	14	
Aspergillus nidulans				2	4	4	7	4	8	3			
Alternaria alternata		3	5	5	5	5	6	5					
Cephalosporium acremonium			3	3	4	4	4	16	4				
Cephalosporium sp.						3	9	13	7	7			
Fusarium culmorum			2	2	3	5	5	5					
Fusarium solani			5	6	7	10	13	8	6				
Paecilomyces varioti					5	5	6	16	8	8			
Chrysosporium pruinosum			4	4	9	10	11	13	15	4			
Phoma sp.			3	6	2								
Trichoderma viride			5	6	7	5	4	2	2				
Trichoderma koningii			5	7	10	12	15	14	14	12	5		
Verticillium psalliotae													
Scopulariopsis brevicaulis				4	6	6	5	5					
Coprinus macrocephalus				2	5	4	4	4	3	2			
Mucor racemosus													
Absidia corymbifera													
Aureobasidium pullulans			Penetration and pigmentation										
Rhinocladiella mansonii			Penetration and pigmentation										
Aspergillus ochraceous													
Penicillium caryophillum			Penetration and pigmentation										
Penicillium funiculosum				2	3	3	6	11	8				

TABLE 3.6

CELLULOLYTIC ACTIVITY AND TEMPERATURE TOLERANCE RANGES

□ Clearing of Cellulose in mm

Temperature Tolerance Range °C

Fungi	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°
Nectria inventa		2	3	3	7	7	7	8	7				
Acremonium kiliense							3	5	3				
Acremonium sp.			3	7	9	11	10	10	8				
Aspergillus fumigatus					3	3	19	23	20	15	14	14	
Aspergillus nidulans				2	4	4	7	4	3	3			
Alternaria alternata		3	5	5	5	5	6	5					
Cephalosporium acremonium			3	3	4	4	4	16	4				
Cephalosporium sp.						3	9	13	7	7			
Fusarium culmorum			2	2	3	5	5	5					
Fusarium solani			5	6	7	10	13	8	6				
Paecilomyces varioti					5	5	6	16	8	8			
Chrysosporium pruinosum			4	4	9	10	11	13	15	4			
Phoma sp.			3	6	2								
Trichoderma viride			5	6	7	5	4	2	2				
Trichoderma koningii			5	7	10	12	15	14	14	12	5		
Verticillium psalliotae													
Scopulariopsis brevicaulis				4	6	6	5	5					
Coprinus macrocephalus				2	5	4	4	4	3	2			
Mucor racemosus													
Absidia corymbifera													
Aureobasidium pullulans			Penetration and pigmentation										
Rhinocladiella mansonii			Penetration and pigmentation										
Aspergillus ochraceous													
Penicillium caryophillum			Penetration and pigmentation										
Penicillium funiculosum					2	3	3	6	11	8			

3.4 Discussion

As in the surveys of Tack (1968) and Richardson (1969) this survey demonstrates the presence of fungi in painted and unpainted timber joinery in service capable of causing decay. Whilst these two former workers cite high moisture content in timber as the prime cause for decay the present survey provides evidence of the occurrence of soft rot from wood with moisture content below 21%. (Table 3.2, Sites 2, 3). The isolation of thirteen thermophilous cellulolytic fungi, of which four commonly occurring taxa (Aspergillus fumigatus, Cephalosporium acremonium, Fusarium solani and Penicillium funiculosum) are known to cause soft rot in timber point to the presence of a population of fungi in in-service timber which under conditions of insolation could contribute significantly to decay. The results show not only the widespread distribution of such isolates but their persistence even during the colder months of the year, suggesting that such a population in wood (Henningson, 1968) as in soil (Eggins et al 1972) may be most active during periods of insolation throughout the year.

Microfungi were isolated from timber where paint films were in tact and where they were flaking off, pointing to the contamination of wood by exogenous (Corbett and Levy, 1963b; Findlay, 1965; Butcher, 1968; Toole, 1971; Hudson, 1973) and endogenous (Smith, 1966) agents. The survey records the isolation of fungi from paint films. Whether biodegradation of the films occurred is beyond the scope of this investigation, however, cases of mould growth on painted surfaces are also reported by Hendy (1962), Hoffman, (1967, 1969), Kühne et al (1970) and Nigam et al (1970).

There seems to be evidence of correlation between the flora of the dock survey (Chapter II) and the present one in that ten species common to both locations were recorded: Absidia corymbifera; Aspergillus fumigatus*; Aspergillus nidulans*;

Aspergillus ochraceous; Cephalosporium acremonium*; Paecilomyces varioti*; Scopulariopsis brevicaulis; Trichoderma viride; Alternaria alternata* and Aureobasidium pullulans. Of particular significance, however, is that five of these fungi may be considered to be microthermophiles (*), whilst four of the organisms namely Aspergillus fumigatus, Cephalosporium acremonium, Trichoderma viride and Aureobasidium pullulans can cause soft rot in timber. These findings are offered as evidence of the occurrence of microfungi, including thermophilous forms, capable of causing decay in in-service timber.

CHAPTER IV

THE EFFECT OF INSOLATION ON THE TEMPERATURE RANGES AND MOISTURE CONTENT IN WOOD

Contents: Pages:

4.1 Introduction

The rate at which a material heats up is called the thermal conductivity or diffusivity. This is dependent not only upon the rate of conduction of heat through the material but also upon its thermal capacity (Tiemann, 1951). Macfadyen (1968) discussing soil and solar radiation explains that the higher the thermal capacity and the lower the conductivity of the surface layers, the more effectively will energy be contained and restricted within soil. These principles will apply to some extent to wood undergoing insolation, heat which penetrates below the surface will be stored until the thermal gradient is reversed. The cyclic reversals of temperature gradient result in corresponding reversals in the direction of heat flow and these may be an important element in the environment of wood inhabiting organisms.

Little information is available on the effects of insolation on temperature or moisture ranges in wood. Therefore, the present investigation was undertaken to record the ranges of temperature which occurred when wood, painted various colours, was subject to insolation, and to record the effect of insolation on the moisture content of unpainted wood in contact with soil.

Ludwig and Harper (1958) have shown that experimental alteration of soil colour can modify absorption of solar energy, whilst Eggins et al (1972) have recorded temperatures 2.0cm down in the soil on sunlit sites higher than air temperature and make the point that at the surface they would be even higher. Macfadyen (1968) relates that the most important factors determining heat exchange at the soil surface are components of regional climate, of these water and carbon dioxide contents of the air are conspicuously important. In addition the general level of air movement and humidity which are largely determined by geographical location, control the evaporative and conductive cooling of the soil and are

balanced by solar radiation. Such factors are acknowledged to be of importance in the colonization of above ground regions of wooden stakes (Corbett and Levy, 1963; Okigbo, Greaves and Levy, 1966; Butcher, 1968; Banerjee and Levy, 1971). It would seem that to organisms attacking wood at the surface, like soil organisms, the most potent influences are truly micro-climatic ones involving steep gradients of temperature and air movements that occur very close to the surface.

Henningson (1968) observed that even in winter temperatures could be high enough in wood to allow decay fungi to be active. This worker also recorded that many fungi with high temperature optima were more active at low temperatures than the low temperature fungi themselves. Jensen (1968) recorded that the internal temperatures of red oak trees fluctuated diurnally following a pattern established by the air temperature; as the air temperature increased the internal tree temperature also increased, at a slower rate but got as high as air temperature after several hours. A similar pattern was observed with a decrease in air temperature, in addition, with an increase in depth within the tree changes became progressively slower.

Asiedu and Smith (1973) comment that many factors affect the ability of fungi to attack wood, but with thermophilous fungi temperature is very important because the required temperatures are only available in certain restricted and self-heating habitats (Bergman and Nilsson, 1966, 1967, 1968, 1971; Asiedu and Smith, 1973; Shields and Unligil, 1968; Tansey, 1971).

The effect of sun in providing a temperature sufficient for the growth of thermophilous fungi as a separate source of heat from that of decaying matter has been mentioned by Apinis and Pugh (1967) and Melinge and Apinis (1969). Eggins et al

(1972) have shown that thermophilous fungi are active in soils whose upper limits are less than those of self-heating organic systems and that although thermophilous fungi are widespread in soil they are not active under shaded and, therefore, cooler conditions. The widespread occurrence of thermophilous fungi in soils from cool climates has been shown by Mishoustin (1950), Apinis (1963, 1965), Allsop (1968), and Eggins and Malik (1969) and there is now some evidence that thermophilous fungi may be equally as widespread in timbers (Chapters II and III). It was considered, therefore, to be of great importance to obtain some information on the temperature and moisture levels in insolated wood.

4.2 Experimental I

4.2 (i) Materials and methods I

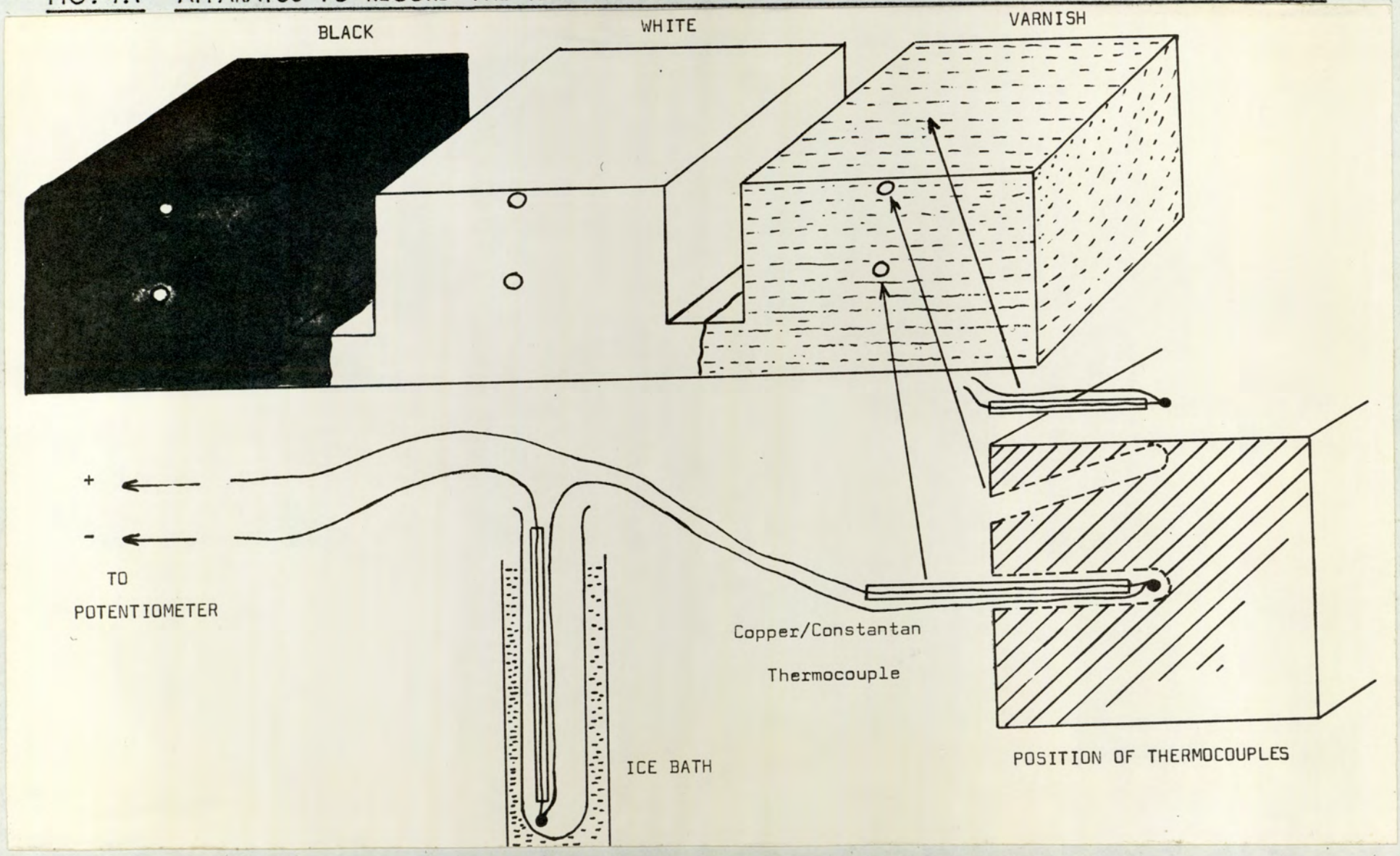
A block of red pine measuring 58 x 9 x 4.5cms was prepared in the following manner :

Three sections each 15 x 9cm were marked on the surface of the block and separated by grooves 3cm wide and 2.5cm deep. Into the side of each section of the block two holes were drilled, one horizontally into the centre of the block and the other at an angle of approximately 30° to the surface of the block so that the bore-hole ended 1mm under the surface (Figure 4.1). Two sections of the block were given one coat of lead based primer, one section was then painted white and the other black by the application of one coat of 'Dulux' undercoating and gloss paint. The third section received three coats of clear polyurethane varnish.

The block was placed outside on a window sill on the third floor of the chemistry block at Preston Polytechnic facing South so that it received all available sunshine in the morning and was in the shade in the afternoon.

Four sets of copper/constantan thermocouples were assembled and calibrated. On a number of occasions in May, June, July and August 1972 readings were taken, one at midday and one at 4.00 p.m. One thermocouple was used to estimate the temperature of the air, the other three were used to estimate the temperature at the surface, 1mm below the surface and at the centre of each section of the block. The technique provides information about the temperatures in the regions of the block on the occasions of recording only.

FIG. 4.1 APPARATUS TO RECORD THE TEMPERATURES AT THE SURFACE AND WITHIN A BLOCK OF WOOD



4.2 (ii) Results I

The results show that all sections of the block both at the surface and within the wood were recorded at temperatures above air temperature as a result of insolation. As would be expected the effect of insolation was most marked at midday, but the data also show that wood was recorded at above ambient temperatures when no longer in direct sunlight.

Appendix IV contains the mean, maximum and minimum temperatures recorded during the survey for air and all three levels in each section of the block, whilst Figure 4.2, presents these data so that comparisons can be made between the temperatures recorded in the sections of the block. The results indicate that the section of the block painted black was that most obviously influenced by insolation. In July, 1972 temperatures in excess of 45°C were recorded at all three levels monitored in this section of the block representing values in the order of 16°C above ambient occurring at all three levels (Figure 4.2c). Temperatures in excess of 40°C were also recorded in this section of the block in May and August,1972 (Figure 4.2a and d). The effect of insolation on the recorded temperatures of the other two sections were not always as marked, although temperatures in excess of 40°C were recorded in the varnished section of the block in July, 1972 (Figure 4.2c); nevertheless values representing an increase of 10°C above ambient were recorded (Figure 4.2a, and c).

Insolation then had an effect on the surface and internal temperatures of wood. The effect was most marked in direct sunlight in wood painted black. Wood was also recorded at above recorded ambient temperatures when no longer in direct sunlight.

FIG.4.2a A COMPARISON OF THE TEMPERATURES RECORDED AT THE SURFACE AND WITHIN A BLOCK OF WOOD

MAXIMUM, MEAN AND MINIMUM TEMPERATURES FOR MAY, 1972

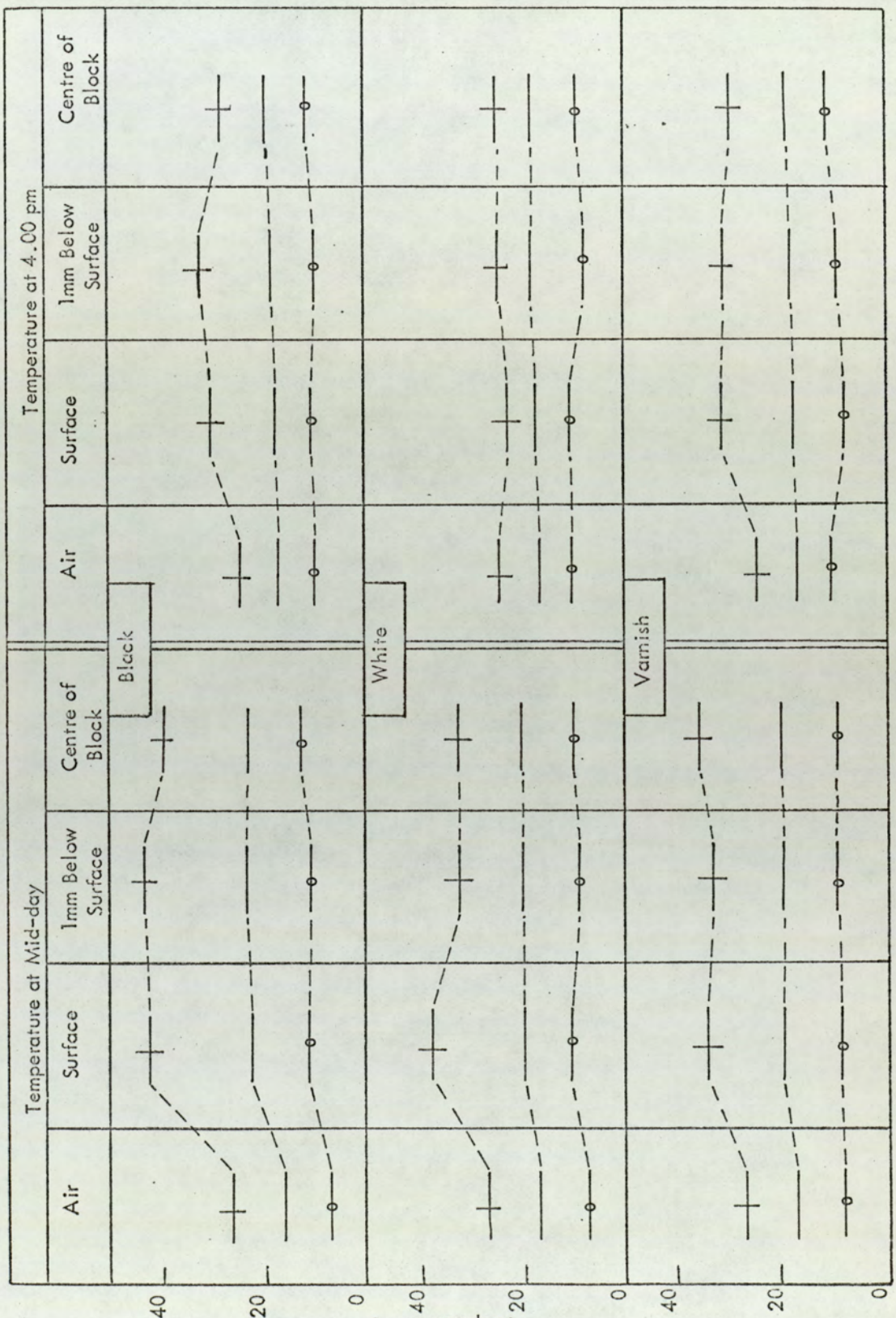


FIG.4.2b A COMPARISON OF THE TEMPERATURES RECORDED AT THE SURFACE AND WITHIN A BLOCK OF WOOD cont....

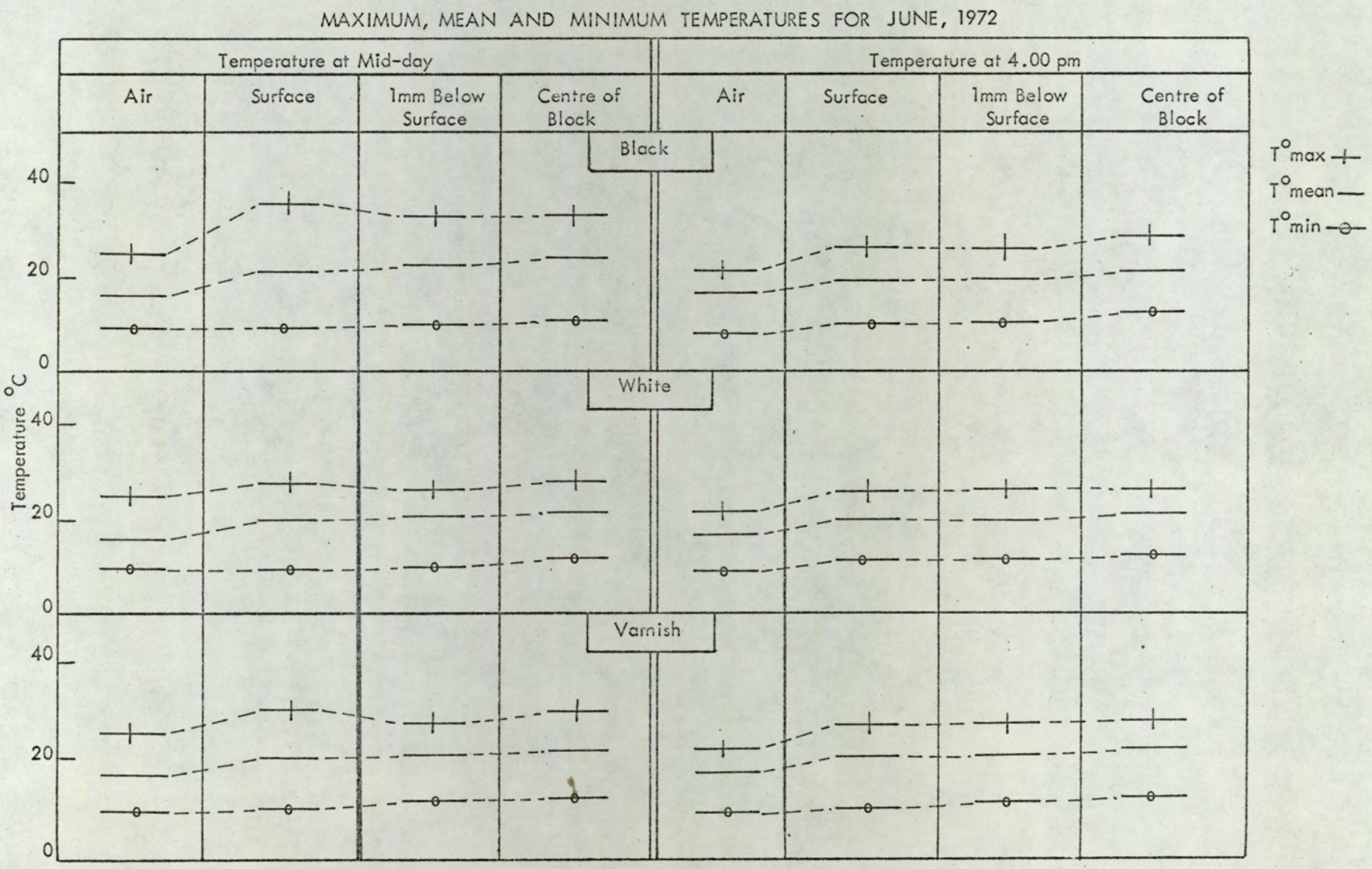


FIG.4.2c A COMPARISON OF THE TEMPERATURES RECORDED AT THE SURFACE AND WITHIN A BLOCK OF WOOD cont.....

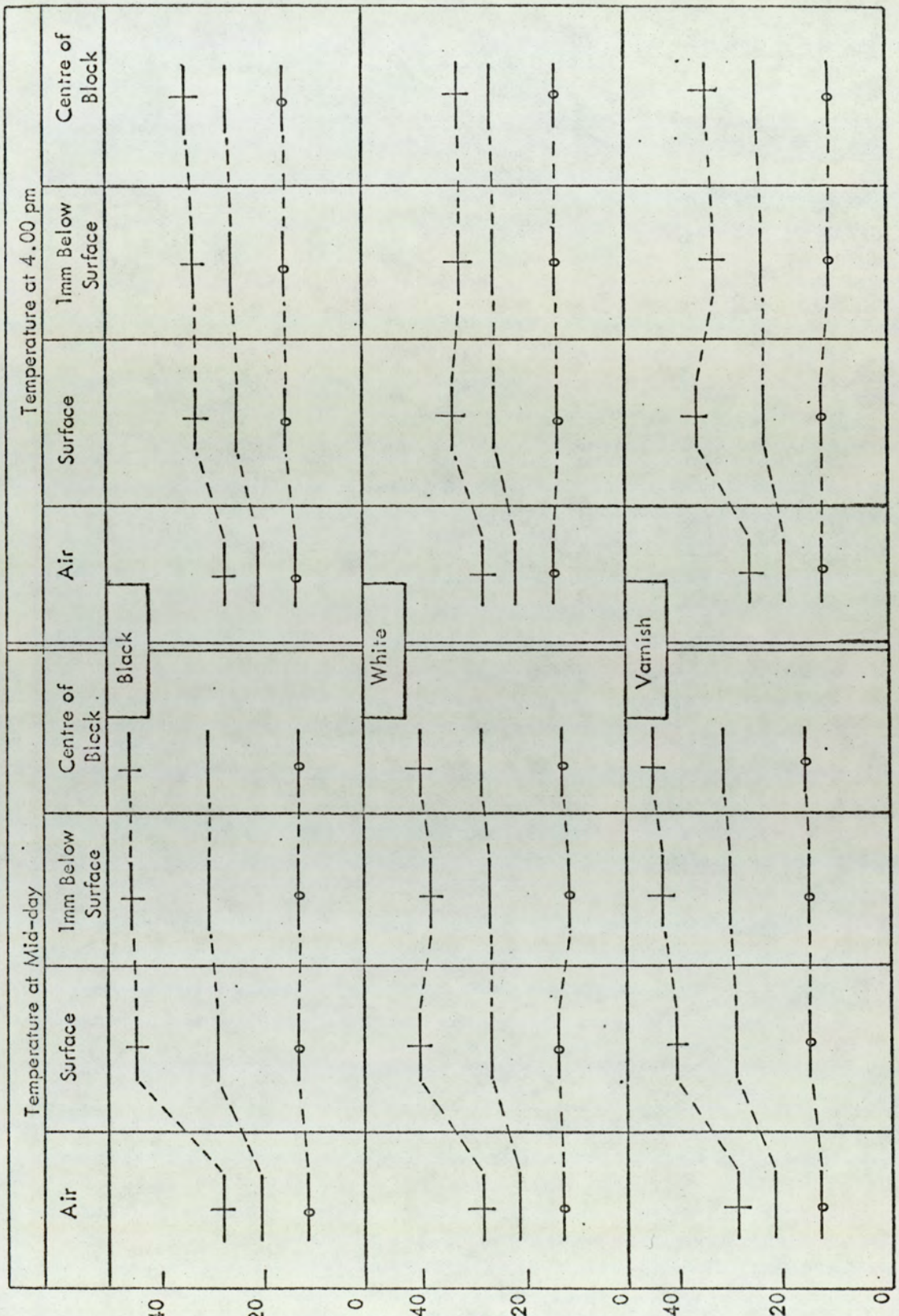
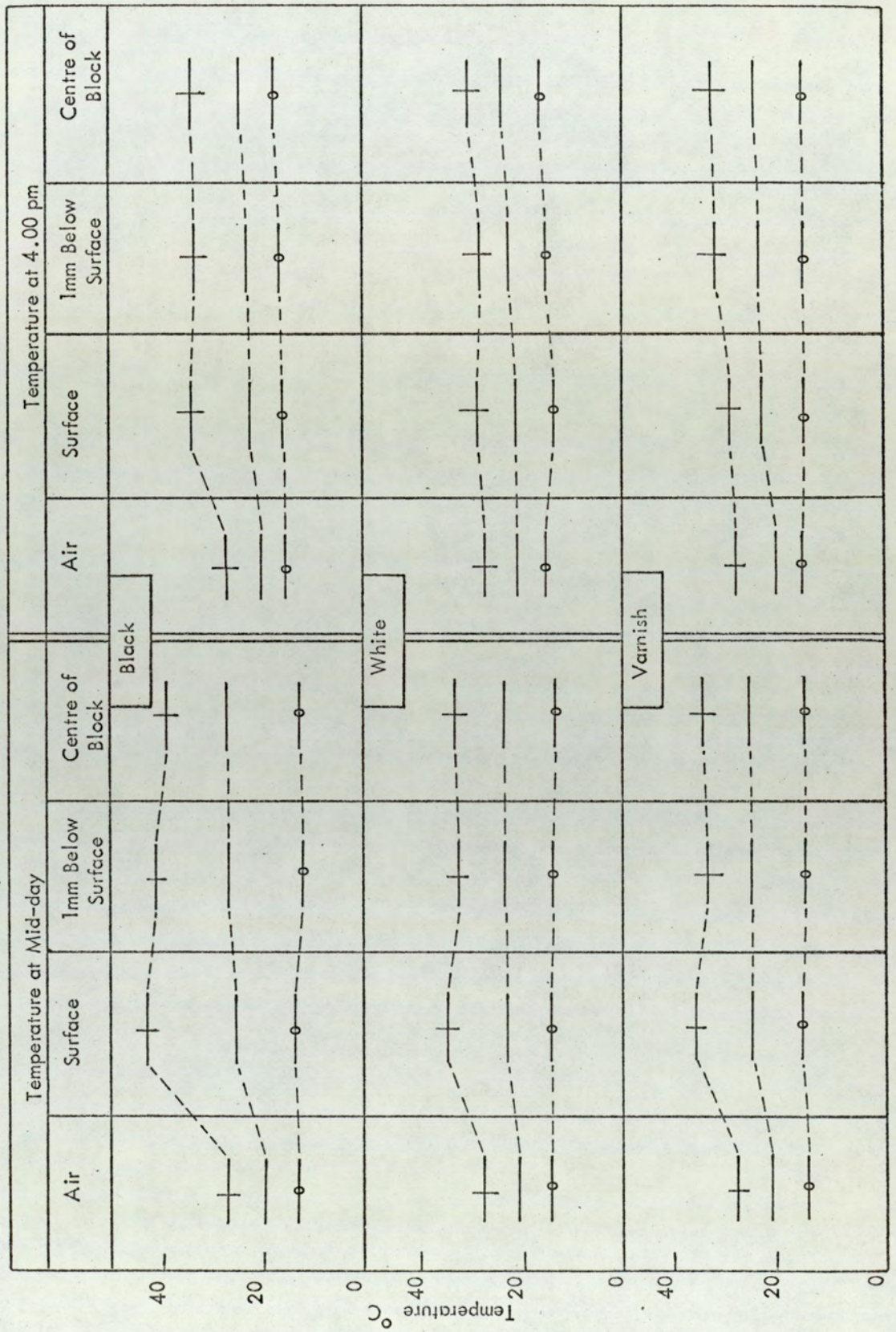


FIG.4.2d A COMPARISON OF THE TEMPERATURES RECORDED AT THE SURFACE AND WITHIN A BLOCK OF WOOD cont.....

MAXIMUM, MEAN AND MINIMUM TEMPERATURES FOR AUGUST, 1972



4.3 Experimental II

4.3 (i) Materials and methods II

Eight blocks of pine measuring 4 x $1\frac{3}{4}$ x $2\frac{3}{4}$ were placed out of doors in plastic trays measuring $14\frac{1}{2}$ x $9\frac{1}{2}$ x $2\frac{1}{2}$" containing $1\frac{1}{2}$" of soil. The trays had holes drilled in their bases to ensure good drainage. Four of the blocks were coated with black indian ink, the other four were left untreated. All the blocks were oven dried to around 12% moisture content. Four trays each containing two blocks were prepared, two trays contained black blocks and two trays contained plain untreated blocks. Two trays, one containing black blocks and one containing plain blocks were placed in a situation so as to receive all available sunshine, two similar trays were placed in constant shade. The blocks in each tray were positioned so that one had its transverse grain surface in contact with the soil (designated T.S. grain) and the other had its tangential grain surface in contact with the soil (designated T.L.S. grain). The experiment was conducted during the months of August, September and October, 1972. A record was kept of the moisture content at the top surface, one side and the base of each block, the readings were taken with a "Protimeter" moisture meter. The surface temperature of the blocks and air temperature were recorded using a copper/constantan thermocouple. All readings were taken at midday. A record was kept of the rainfall and of the hours of sunshine during the period of the experiment.

4.3 (ii) Results II

During the months of August, September and October, 1972 the records show that there were periods of intermittent rain during a period which had sufficient hours of sunshine to provide conditions suitable for an investigation of this nature (Figure 4.3).

The effect of insolation on the surface temperature of the wood was most marked in direct sunlight in those blocks which were dyed black. Under conditions of constant shade the recorded wood temperatures followed more closely those of the air (Figures 4.4 - 4.6).

The graphs (Figure 4.7) record the moisture values obtained from the 'top' (green trace on the graphs) 'side' (red trace on the graphs) and 'base' (blue trace on the graphs) surfaces of the blocks. Data were assembled in balanced sets and a series of t-tests on two groups of unpaired data were computed (Lucas, 1974: Appendix V). The order in which the data were considered is shown in Table 4.1 ; where Group 1 tests for levels of significance between all the moisture content data of blocks situated in sunshine with those of blocks situated in shade, where Group 2 tests for levels of significance between all the moisture content data of black blocks with those from untreated blocks in the same situation (sun or shade) and where Group 3 tests for levels of significance between the moisture data from 'top', 'side', and 'base' surfaces, considered independently between blocks similarly treated (black or plain) in the same situation (sun or shade) but with either T.S. or T.L.S. grain in contact with soil. In such a situation 'top', 'side', and 'base' surfaces will be represented in turn by the transverse grain and longitudinal grain of the blocks.

Those results based on readings taken from three surfaces of the blocks, where the data were considered as a whole, indicate that over the period of the experiment the average moisture content of the blocks situated in sunshine was significantly lower than the average moisture content of the blocks situated in the shade (Table 4.1 : Group 1; Figure 4.7, a cf b, c cf d, e cf f, g cf h). The results also indicate that in those blocks similarly positioned within the trays which were situated in sunshine, there was no significant difference between the average moisture content of the blocks dyed black and the plain or untreated blocks. In the shade, however, there was a significant difference, the plain blocks recorded a higher average moisture content; the difference was most marked between black and plain blocks which had their transverse grain in contact with the soil (Table 4.1 : Group 2; Figure 4.7, a cf e, c cf g, b cf f, d cf h).

Those results based on readings taken from three surfaces of the blocks where the data for each surface were considered independently (Table 4.1 : Group 3) indicate that over the period of the experiment blocks similarly treated and in the same situation but with either their transverse grain or longitudinal grain in contact with the soil recorded highly significant differences between the 'top' surfaces of the black blocks, the average moisture content was higher in the longitudinal than in the transverse grained 'top' surfaces. No significant differences were recorded for the 'top' surfaces of plain blocks.

The results show that no significant difference in moisture content was recorded between 'side' surfaces of black blocks situated in sunshine,

however, in the shade the longitudinal grain 'side' surface of black blocks recorded a higher moisture content than the transverse grain 'side' surface. No significant difference was recorded between the 'side' surfaces of plain blocks in sunshine or in the shade.

The 'base' surfaces, those in contact with soil, recorded the highest moisture levels throughout the experiment (Figure 4.7 ; blue trace). There was no significant difference in the average moisture content of 'base' surfaces between black blocks in sunshine, plain blocks in sunshine or plain blocks in shade. Records from black blocks in shade, however, show that the transverse grain in soil contact had a higher moisture content than the longitudinal surface in soil contact. The traces of 'top', 'side' and 'base' and the computed data are shown (Table 4.1 : Group 3, Figure 4.7 a cf c, e cf g, b cf d, f cf h).

There is evidence from the results of this investigation that in wood including wood subject to insolation the direction of the grain in contact with soil and the colour of the timber may influence the moisture content.

FIG. 4.3 RAINFALL AND HOURS OF SUNSHINE RECORDED DURING THE INVESTIGATION

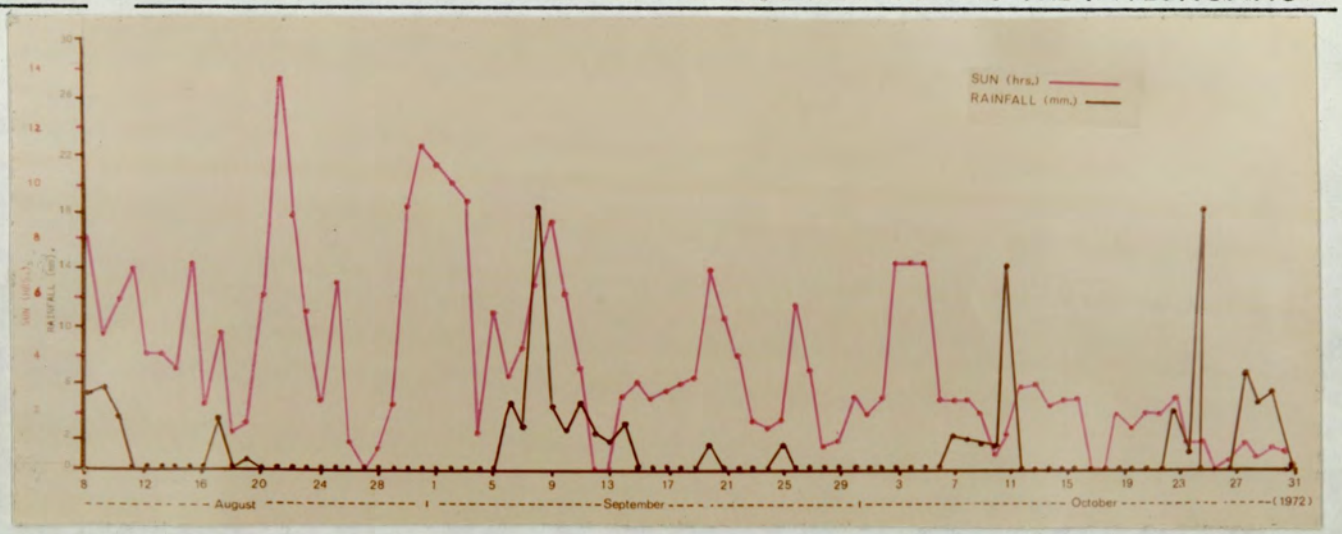


FIG. 4.4 AIR TEMPERATURES RECORDED DURING THE INVESTIGATION

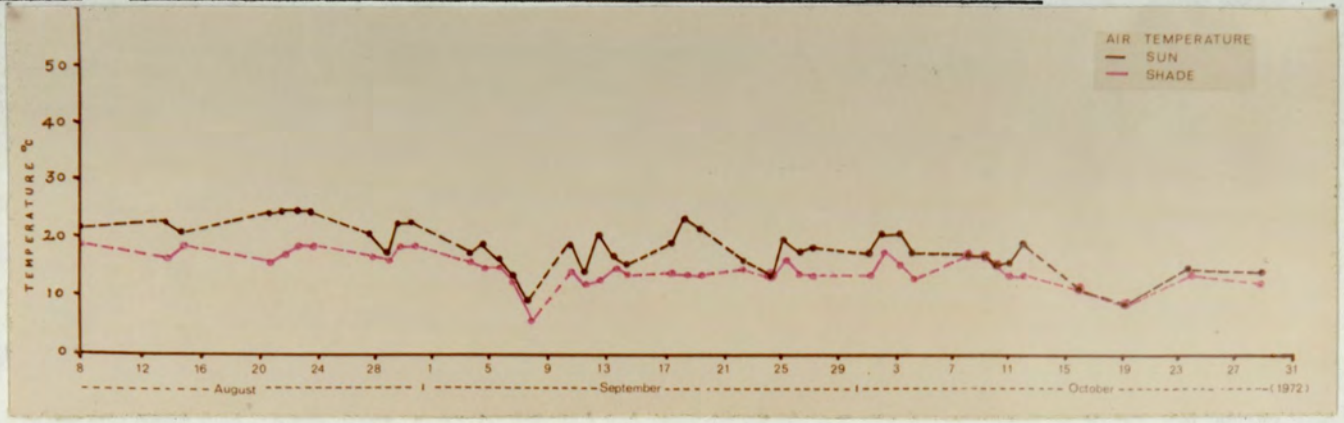


FIG. 4.5 SURFACE TEMPERATURES OF WOOD DYED BLACK

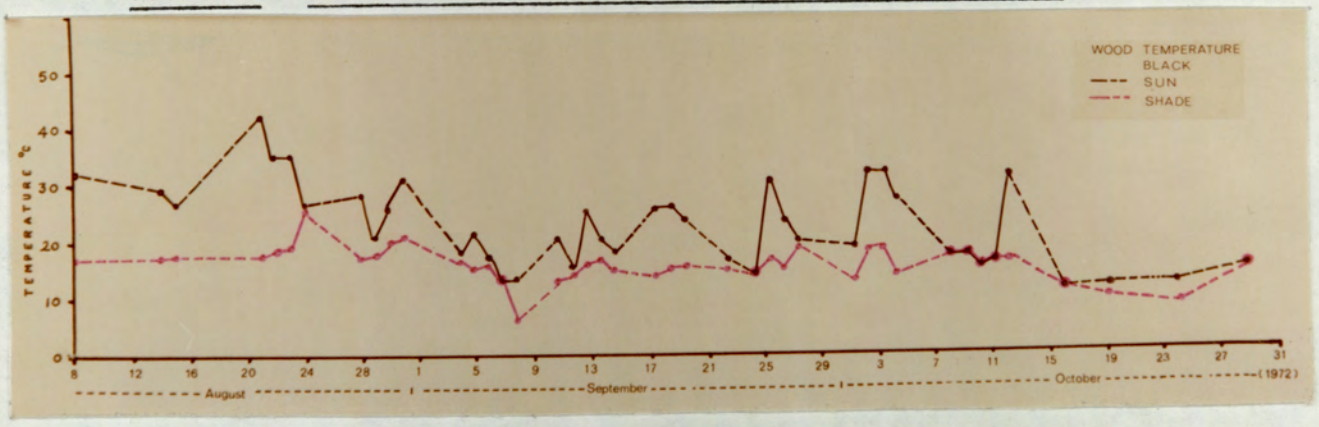


FIG. 4.6 SURFACE TEMPERATURES OF UNTREATED WOOD

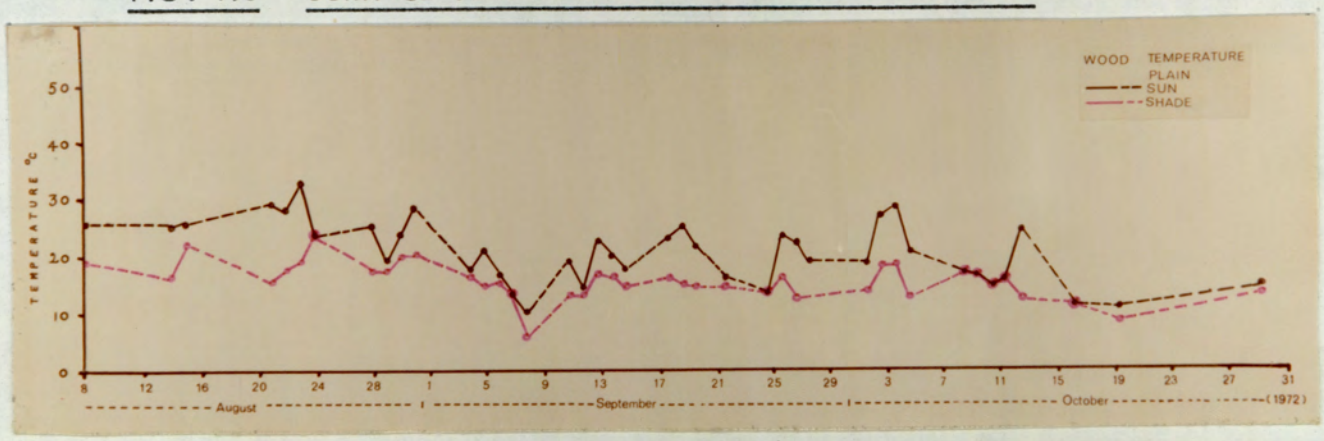
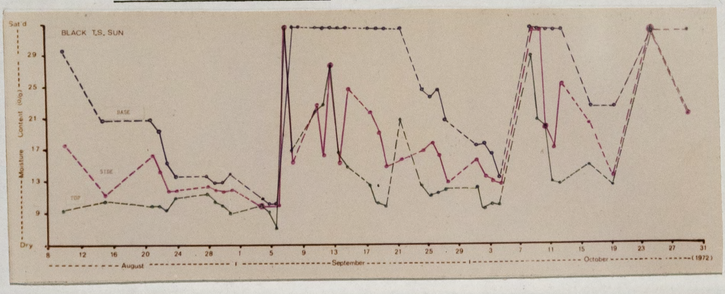
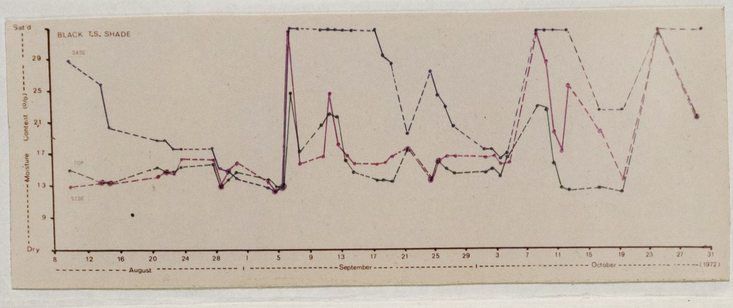


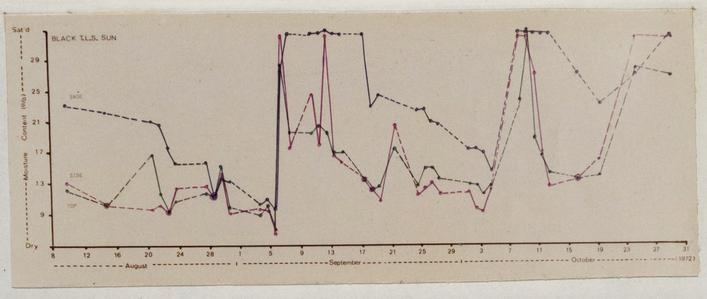
FIG. 4.7 THE EFFECT OF INSOLATION ON THE MOISTURE CONTENT OF WOODEN BLOCKS IN CONTACT WITH SOIL



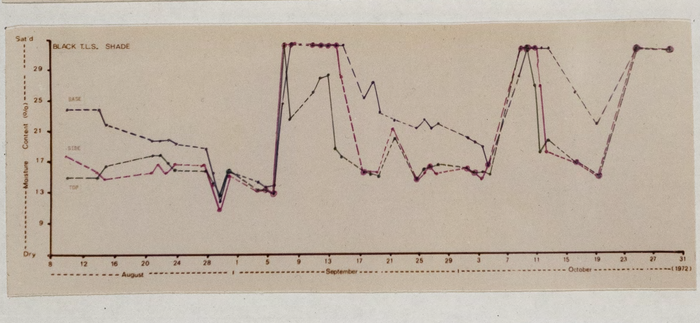
(a) Colour: Black
Grain in soil contact: T.S.
Situation: Sun



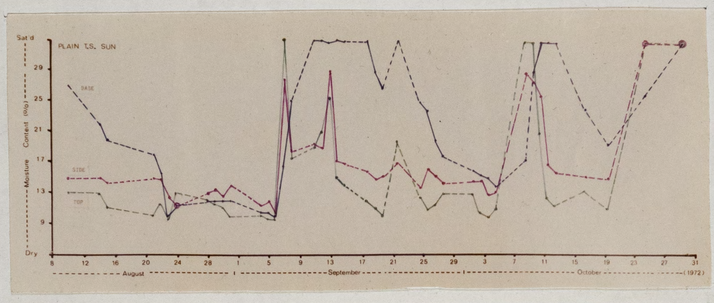
(b) Colour: Black
Grain in soil contact: T.S.
Situation: Shade



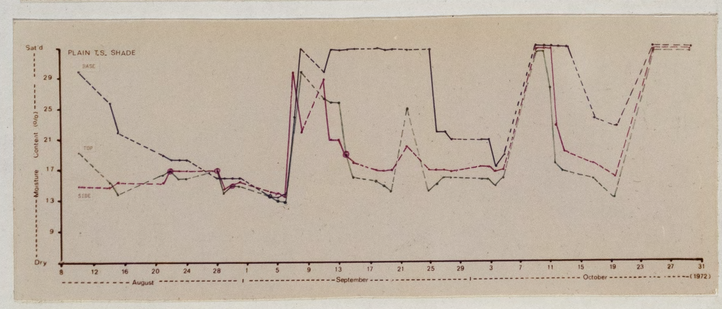
(c) Colour: Black
Grain in soil contact: T.L.S.
Situation: Sun



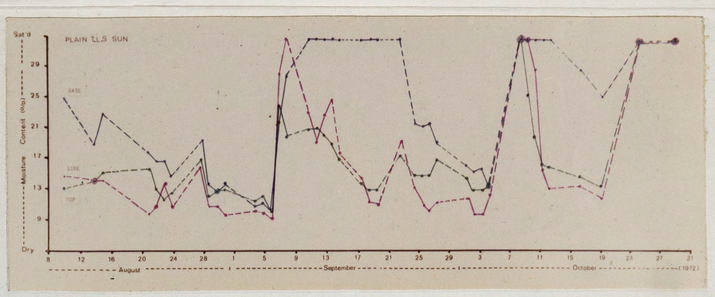
(d) Colour: Black
Grain in soil contact: T.L.S.
Situation: Shade



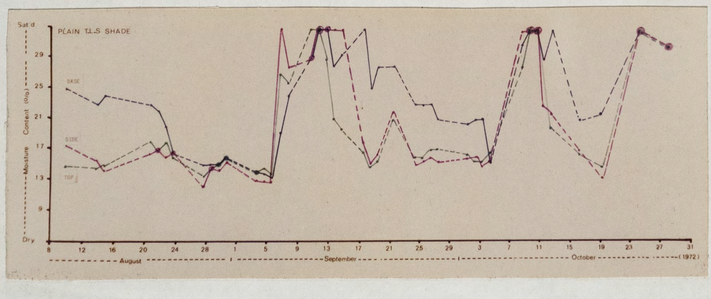
(e) Colour: Plain
Grain in soil contact: T.S.
Situation: Sun



(f) Colour: Plain
Grain in soil contact: T.S.
Situation: Shade



(g) Colour: Plain
Grain in soil contact: T.L.S.
Situation: Sun



(h) Colour: Plain
Grain in soil contact: T.L.S.
Situation: Shade

TABLE 4.1 ORDER OF PRESENTATION OF BALANCED SETS OF DATA FOR UNPAIRED t TESTS

Grain in soil contact	Colour	Situation	Surfaces of Block Monitored		Grain in soil contact	Colour	Situation	Surfaces of Block Monitored	Magnitude of A and B	Value of t	Significance Levels α = p(Ho)
Group 1 Data Set A					Group 1 Data Set B						
TS	Black	Sun	Top + Side + Base	v	TS	Black	Shade	Top + Side + Base	B > A	3.284	0.1% > α >0.05% VHS
TS	Plain	Sun	Top + Side + Base	v	TS	Plain	Shade	Top + Side + Base	B > A	2.963	0.5% > α >0.1% HS
TLS	Black	Sun	Top + Side + Base	v	TLS	Black	Shade	Top + Side + Base	B > A	1.460	10.0% > α >5.0% PS
TLS	Plain	Sun	Top + Side + Base	v	TLS	Plain	Shade	Top + Side + Base	B > A	4.119	α < 0.05% VHS
Group 2 Data Set A					Group 2 Data Set B						
TS	Black	Sun	Top + Side + Base	v	TS	Plain	Sun	Top + Side + Base	-	0.924	α >10.0% NS
TLS	Black	Sun	Top + Side + Base	v	TLS	Plain	Sun	Top + Side + Base	-	0.874	α >10.0% NS
TS	Black	Shade	Top + Side + Base	v	TS	Plain	Shade	Top + Side + Base	B > A	1.745	10.0% > α > 2.5% S
TLS	Black	Shade	Top + Side + Base	v	TLS	Plain	Shade	Top + Side + Base	B > A	1.435	10.0% > α > 5.0% PS
Group 3 Data Set A					Group 3 Data Set B						
TS	Black	Sun	Top	v	TLS	Black	Sun	Top	B > A	2.530	1.0% > α > 0.5% HS
			Side	v				Side	-	0.577	α >10.0% NS
			Base	v				Base	-	0.276	α >10.0% NS
TS	Plain	Sun	Top	v	TLS	Plain	Sun	Top	-	0.354	α >10.0% NS
			Side	v				Side	-	0.444	α >10.0% NS
			Base	v				Base	-	1.080	α 10.0% NS
TS	Black	Shade	Top	v	TLS	Black	Shade	Top	B > A	2.607	1.0% > α > 0.5% HS
			Side	v				Side	B > A	1.667	10.0% > α > 5.0% PS
			Base	v				Base	A > B	1.595	10.0% > α > 5.0% PS
TS	Plain	Shade	Top	v	TLS	Plain	Shade	Top	-	1.152	α >10.0% NS
			Side	v				Side	-	0.966	α >10.0% NS
			Base	v				Base	-	0.708	α >10.0% NS

NS = not significant PS = possibly significant S = significant HS = highly significant VHS = very highly significant
Ho = null hypothesis

4.4. Discussion

Substances with a high thermal capacity and low thermal conductivity store heat energy, soil and wood are two such substances. Eggins et al (1972) recorded temperatures in soil higher than air temperatures, similar results have been obtained for wood during this investigation. Ludwig and Harper (1958) showed that experimentally altered soil colour can modify the absorption of solar energy, the results of this investigation show that this is also the case with wood. During the period of this investigation the effect of insolation was most marked in direct sunlight in wood dyed black when temperatures in excess of 45°C were recorded, not only at the surface of wood but within wood, representing a value of 16°C above ambient temperature. Values in the order of 10°C above ambient were recorded as a result of insolation for wood painted white and in varnished wood. There is also evidence that wood retains the heat energy absorbed during periods of insolation.

The effect of sun in providing a temperature sufficient for the growth of thermophilous fungi has been mentioned by Apinis and Pugh (1967); Melinge and Apinis (1969), it has been demonstrated that thermophilous fungi may be as widespread in wood (Chapters II and III) as they are in soil (Mishoustin, 1950; Apinis, 1963, 1965; Allsop, 1968; Eggins and Malik, 1969) however, for thermophilous fungi to be active in insolated wood suitable moisture levels must be available. Table 4.2 compiled from data available as a result of this investigation shows that at above ambient temperatures (33-42°C) moisture levels of 17.5 - 21.5% were recorded, levels which with the increase in water activity which may come with elevated temperatures (Ayerst, 1965) could support the activity of thermophilous fungi in wood.

The findings of this investigation suggest that unpainted wood subject to insolation

TABLE 4.2

MAXIMUM MOISTURE LEVELS RECORDED AT MAXIMUM AND MINIMUM TEMPERATURES

AIR		BLOCKS: PLAIN		BLOCKS: BLACK	
Shade	Exposed	Shade	Exposed	Shade	Exposed
Max °C 19	Max °C 25	Max °C 24 Max. Moisture 17%	Max °C 33 Max. Moisture 17.5%	Max °C 25.5 Max. Moisture 19.5%	Max °C 42 Max. Moisture 21.5%
Min °C 5.6	Min °C 9	Min °C 5.6 Max. Moisture 30%	Min °C 11 Max. Moisture 25.5%	Min °C 5.6 Max. Moisture 30%	Min °C 11.9 Max. Moisture 30%

which is in contact with soil has a lower average moisture content than wood situated in the shade (Table 4.1 : Group 1).

There is also evidence that in timber undergoing insolation which is in soil contact there may be a flow of water from soil through wood to the air. Timber dyed black and untreated timber both subject to insolation recorded no significant difference in moisture content which pointed to a higher evaporation rate from the black timber with an increased flow of water through the wood from soil to compensate for the higher rate of water loss. Without the high evaporative force of the sun, however, timbers in shade recorded significant differences in moisture content indicating a build up of water in wood especially when transverse grain was in soil contact giving a higher capillarity uptake (Table 4.1 : Group 2).

There is some evidence that transverse grain surfaces may dry out more rapidly than longitudinal grain surfaces of wood painted black, this seemed to be the case in sunshine and in shade, however, this was not found to be the case with plain wood in sunshine or in shade which points to high evaporation from black surfaces. There was also further evidence of increased capillarity when the transverse grain of black wood in contact in soil recorded a significantly higher moisture content than its longitudinal grain counterpart (Table 4.1 : Group 3).

As a result of this investigation it is considered that further information is required concerning the diurnal temperature fluctuations in wood which is subject to insolation and the effect that such temperatures have upon the growth of thermophilous fungi. It is also considered important to obtain information on the limiting moisture content necessary in wood to support the growth of thermophilous fungi. Both of these topics are included in later chapters.

CHAPTER V

THE EFFECT OF MOISTURE CONTENT IN WOOD ON THE SURFACE GROWTH AND PENETRATION OF FUNGI

5.1 Introduction

The presence of moisture in wood is significant in two ways, firstly changes in moisture content cause swelling or shrinkage, secondly high moisture levels allow the development of decay (Nash-Wortham and Savory, 1968). This latter condition is generally accepted and the literature shows a firm agreement that below 20% moisture content fungal decay does not occur (Savory, 1967; Nash-Wortham and Savory, 1968; Liska, 1971; De Groot, 1972). However, during the sampling work undertaken on newly imported and in-service timber, fungi, including thermophilous cellulolytic organisms capable of causing soft rot, were isolated from timbers which were shown to have low moisture contents (Chapters II and III). The occurrence of soft rot fungi in situations of extreme dryness has also been recorded by Duncan and Eslyn (1966).

The increase in the biological activity of water at higher temperatures (Ayerst, 1965) could be responsible for the growth of fungi, especially thermophi lous fungi, in wood with low moisture content which is subject to insolation. It was considered, therefore, that an investigation to establish the limiting moisture content necessary to support the growth of higher temperature fungi on wood would be of considerable interest and importance.

In order that such an investigation could be undertaken an apparatus was devised which would establish and maintain a gradient of moisture content in discs of timber veneer so that the low moisture/growth relationships of selected thermophilous fungi could be studied. An apparatus which could give a moisture gradient was considered desirable because there is growing opinion that a moisture gradient exists in window joinery (Van Loon, 1964, 1965; Seifert, 1967), indicative of uptake of water from the interior of buildings and loss to the exterior through paint films. Unpainted wood dries out more rapidly than painted

wood (Nash-Wortham and Savory, 1968) and although a recent survey (Tack, 1968) indicated that the amount of moisture in painted window joinery in service is surprisingly high and drying out probably very slow, the fact that fungi might grow actively at low moisture contents where a gradient exists would seem to be an important factor in wood colonization by fungi of both painted and unpainted wood.

A simple apparatus, within which a natural moisture gradient develops in beech veneers, was considered adequate for this work rather than a series of constant relative humidity desiccators (Wink, 1946; Rockland, 1960; Gur Arieh, 1965 a and b; Bosin and Easthouse, 1970) since the range of moisture contents required, that is, 20% and below were readily obtained in the apparatus devised.

The infection of wood by wood rotting fungi results at least in part from spores (Toole, 1971). The germination of spores on wood has been investigated by a few workers (Ferguson, 1902; Bayliss, 1908; Price, 1913; Zeller, 1920; Rishbeth, 1951 and 1958; Morton and French, 1966; Toole, 1971), whilst others (Dayal et al, 1972) have recorded the viability of spores for long periods in sterile distilled water. The ability of spores to resume their activity after periods of dessication has also been studied (Theden, 1972). The information produced by these workers prompted the use of spore suspensions in sterile distilled water as the inocula to be employed in this work.

5.2 Experimental I

An apparatus was devised to establish a gradient of moisture content in discs of beech veneer in order to find the minimum moisture content necessary in wood to support the surface growth of fungi.

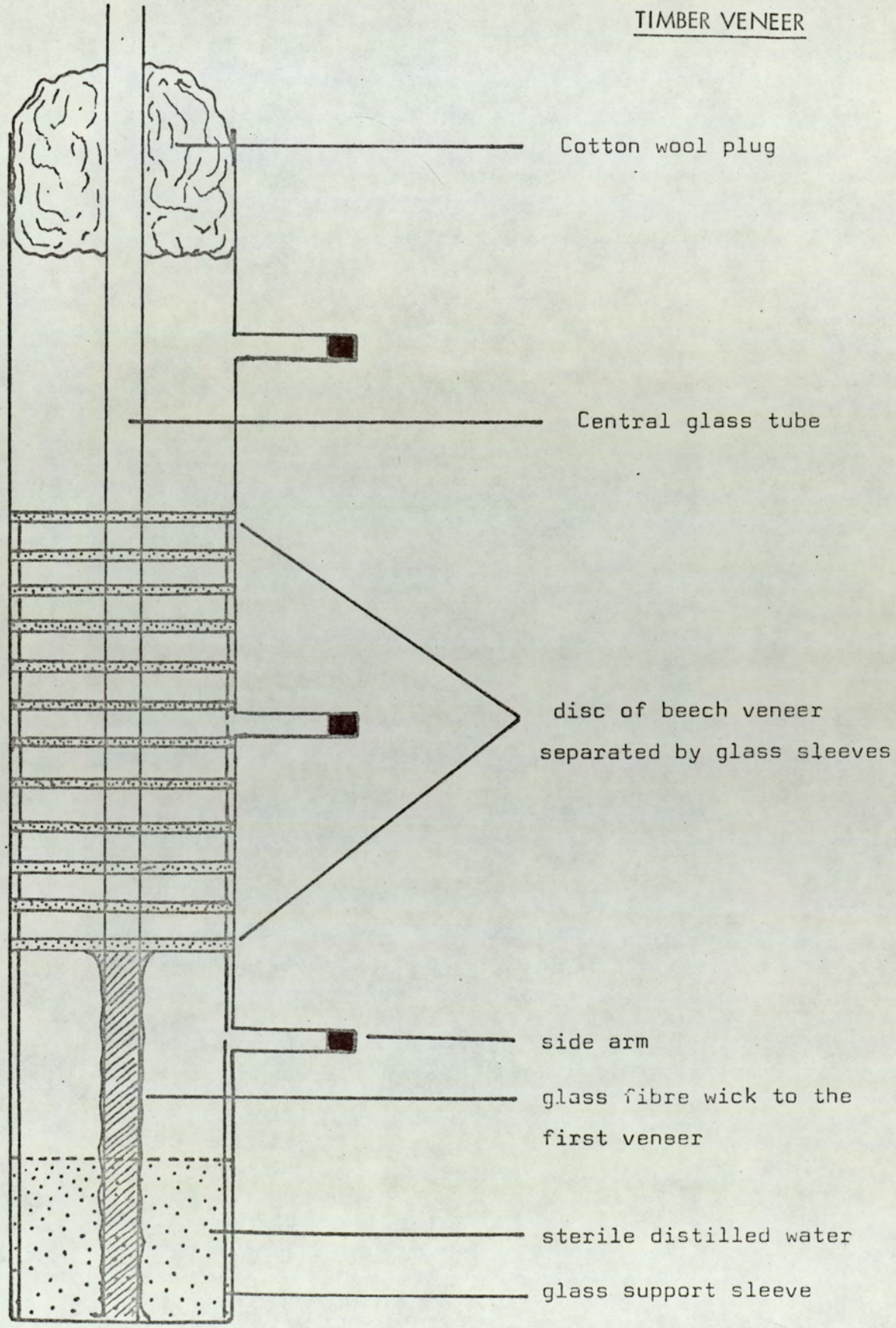
5.2 (i) Materials and methods I

The apparatus consists of a tube 30cm long, made out of 4cm bore glass tubing fitted with three side arms, a 35cm length of 0.4cm bore glass tubing, eleven glass sleeves or separators made from 1.0cm lengths of 3.6cm bore glass tubing, twelve beech veneer discs, a 10cm length of 3.6cm bore glass tubing, a 10cm length of glass-fibre wick and a plug of non-absorbent cotton wool. The apparatus was assembled in the following manner :-

Holes were punched in the centres of the veneer discs and these were fitted at 1cm intervals along the 0.4cm bore glass tube. The first veneer was placed at a point 10cm along the tube and held in position by a washer of glass-fibre tape, a glass sleeve was then placed between each of the subsequently fitted veneers. The glass-fibre wick was then fitted over the lower 10cm portion of the axial glass tube and the entire assemblage was lowered onto the 10cm length of 3.6cm bore tubing lying within the 4cm bore glass tubing and acting as a supporting platform for the veneers and glass sleeves (Figure 5.1). A non-absorbent cotton wool plug was placed in the neck of the tube and the entire apparatus was sealed in a 'Sterilin' autoclavable bag and autoclaved for 30 minutes at 15 lbs./sq. inch. When the apparatus had cooled, $30cm^3$ of sterile distilled water was introduced into the apparatus through the axial glass tube. The glass-fibre wick takes water to the first veneer, the only disc in the system directly supplied with water. The veneer discs form a tight but flexible seal against the side of the cylinder and water is lost to the atmosphere through the cotton wool

FIG. 5.1

AN APPARATUS TO ESTABLISH A GRADIENT OF MOISTURE IN DISCS OF TIMBER VENEER



plug, thus establishing a gradient in humidity from the first to the last veneer. Control experiments were set up and left for periods of seven and twenty days at temperatures of 30°, 35° and 40°C.

Initially three test organisms were employed in experiments of twenty days duration at each of the temperatures. The test organisms were cellulolytic microthermophiles, Trichoderma harzianum, Chaetomium trilaterale and Chrysosporium pruinosum.

Inoculation was carried out by withdrawing the central assemblage of the apparatus and placing a loopful of spore suspension of the test fungus on each level of veneer. Each apparatus was inspected daily and finally after twenty days the veneers were removed, their moisture content determined using a 'Protimeter' moisture meter and each veneer was examined carefully for the presence of fungal mycelium.

Further experiments each of twenty days duration were conducted at 30°, 35° and 40°C with the following four test organisms :

Absidia corymbifera, a non cellulolytic Phycomycete;

Ulocladium atrum, a cellulolytic microthermophile;

Alternaria alternata, a cellulolytic microthermophile, and

Fusarium solani, a cellulolytic microthermophile known to cause soft rot in timber.

Absidia corymbifera was included among the test organisms because it was frequently isolated at the higher temperature incubations of wood samples.

The side arms on the apparatus allowed gas samples to be withdrawn from within it. These were plugged with 'Silastoseal B' after the apparatus had been assembled and prior to autoclaving. The side arms are so positioned

that gas samples could be withdrawn from the region above the veneers, from the central region of the veneers and from the region below the veneers above the water level (Figure 5.1). After twenty days $1cm^3$ air samples were drawn from an inoculated apparatus and passed through a Perkin Elmer gas chromatograph calibrated to register oxygen levels in gas samples. The oxygen content of these samples was compared with that of the air in the laboratory.

5.2 (ii) Results I

The temperatures employed during these experiments represent above ambient levels which can be achieved in insolated wood (Chapter IV) and at which the biological activity of water may be increased.

The data in Table 5.1 indicates that a measurable moisture gradient can be set up in an apparatus of the type devised. The results show that the gradient was established within seven days and was maintained for the three week duration of the experiment. Table 5.2, shows the way in which the moisture gradient in stacked veneers altered with the temperatures employed, these data show that as temperature increased the veneers registered a lower moisture content throughout the stack.

The chromatograph traces (Appendix VI) indicated that there was no difference between oxygen content of air within the apparatus and the air in the laboratory.

The minimum moisture requirements for visible growth to take place varied with temperature in most organisms (Table 5.1). Table 5.3, presents data which shows that at 30°C all seven test organisms were observed growing on veneers whose moisture content was below 20%. At 35°C there were five test organisms and at 40°C there were four test organisms observed growing on veneers whose moisture content was below 20%. These results also indicate that in the case of some of the test organisms the biological activity of water was maintained or increased with an increase in temperature, this was the case in Ulocladium atrum, Alternaria alternata, Trichoderma harzianum and Chaetonium trilaterale; in the case of Absidia corymbifera, Fusarium solani and Chrysosporium pruinosum however, no apparent increase in the biological activity of water with increased temperature was recorded.

These results demonstrate the ability of microthermophilic mould stain and soft rot fungi to grow, at above ambient temperatures, on wood with low moisture content. Such observations point to the possibility that other micromycetes (Gorschin and Krapivina, 1969) especially thermophilous forms may be able to grow on timber with low moisture content which is subject to insolation, and the widespread occurrence of soft rot fungi in situations of extreme dryness (Duncan and Eslyn, 1966) may be a result of increased water activity at higher temperatures brought about by insolation.

Corbett's (1965) terms have been used to describe the types of attack on wood by microfungi causing soft rot, where Type 1 indicates cavity formation and Type 2 indicates a form of cell wall erosion. Both types of attack will only take place if the fungi are able to grow within the substrate and this point is taken up in the second section of this chapter.

TABLE 5.1 (a)

THE RELATIONSHIP BETWEEN SURFACE GROWTH AND MOISTURE CONTENT AT ABOVE AMBIENT TEMPERATURES

MOISTURE CONTENT OF VENEERS AT 30°C

EXPERIMENTS CONDUCTED AT 30°C	% MOISTURE CONTENT AT VENEER LEVELS											
	1	2	3	4	5	6	7	8	9	10	11	12
No. 1 Control duration 7 days	10.0	10.0	10.5	10.5	11.5	12.5	13.5	14.0	17.0	17.25	19.0	>30.0
No. 2 Control duration 20 days	9.5	9.5	10.0	10.5	11.0	12.5	13.5	14.0	16.75	17.0	18.75	>30.0
INOCULUM: No. 3 Trichoderma harzianum, duration 20 days	9.5	9.5	10.0	10.5	11.0	12.0	13.5	14.0	15.5	16.5	18.5	>30.0
INOCULUM: No. 4 Chrysosporium pruinosum, duration 20 days	9.5	10.0	10.0	10.5	12.0	12.0	14.0	15.0	16.0	17.0	18.5	>30.0
No. 5 Chaetomium trilaterale, duration 20 days	9.5	9.5	10.0	10.5	11.5	12.0	14.25	15.5	16.6	17.5	19.0	>30.0

____ = lowest recorded moisture level with visible fungal growth.

TABLE 5.1 (b)

THE RELATIONSHIP BETWEEN SURFACE GROWTH AND MOISTURE CONTENT AT ABOVE AMBIENT TEMPERATURES cont....

MOISTURE CONTENT OF VENEERS AT 35°C

EXPERIMENTS CONDUCTED AT 35°C	% MOISTURE CONTENT AT VENEER LEVELS											
	1	2	3	4	5	6	7	8	9	10	11	12
No. 1 Control duration 7 days	<9.0	10.0	10.0	10.5	11.0	11.75	12.5	12.5	13.75	15.5	18.0	> 30.0
No. 2 Control duration 20 days	<9.0	9.0	10.0	10.5	11.5	11.75	12.25	12.5	14.5	15.5	17.0	> 30.0
INOCULUM: No. 3 Trichoderma harzianum, duration 20 days	<9.0	9.0	9.5	10.0	10.5	12.0	12.0	13.0	14.0	16.0	18.0	30.0
INOCULUM: No. 4 Chrysosporium pruinosum, duration 20 days	<9.0	9.5	10.0	10.5	10.5	11.0	12.5	12.5	13.25	14.0	16.5	29.0
No. 5 Chaetomium trilaterale, duration 20 days	<9.0	9.0	9.5	10.0	10.0	11.0	11.5	12.5	14.0	15.0	17.0	30.0

_____ = lowest recorded moisture level with visible fungal growth

TABLE 5.1 (c)

THE RELATIONSHIP BETWEEN SURFACE GROWTH AND MOISTURE CONTENT AT ABOVE AMBIENT TEMPERATURES cont...

MOISTURE CONTENT OF VENEERS AT 40°C

EXPERIMENTS CONDUCTED AT 40°C	% MOISTURE CONTENT AT VENEER LEVELS											
	1	2	3	4	5	6	7	8	9	10	11	12
No. 1 Control duration 7 days	<9.0	<9.0	9.0	10.0	10.0	10.75	11.5	11.75	13.5	16.0	17.0	> 30.0
No. 2 Control duration 20 days	<9.0	<9.0	10.0	10.0	10.0	10.5	11.75	12.0	13.5	15.5	17.0	> 30.0
No. 3 Trichoderma harziamum, duration 20 days	<9.0	<9.0	9.0	9.0	9.5	9.5	11.5	13.0	13.5	15.0	17.5	> 30.0
INOCULUM: No. 4 Chrysosporium pruinosum, duration 20 days	<9.0	<9.0	<9.0	9.0	9.5	10.5	11.85	12.25	14.0	16.0	17.5	> 30.0
No. 5 Chaetomium trilalerale, duration 20 days	<9.0	<9.0	9.0	9.0	9.0	10.0	11.0	12.5	13.5	16.0	17.0	> 30.0

____ = lowest recorded moisture level with visible fungal growth

TABLE 5.2

THE EFFECT OF TEMPERATURE ON THE MOISTURE CONTENT OF STACKED VENEERS

Temperature °C	MEAN MOISTURE CONTENT % AT VENEER LEVELS											
	1	2	3	4	5	6	7	8	9	10	11	12
30°C	9.6	9.7	10.1	10.5	11.4	12.2	13.8	14.5	16.4	17.0	19.0	> 30.0
35°C	9.0	9.3	9.8	10.5	11.25	11.5	12.13	12.6	13.9	15.8	17.3	> 30.0
40°C	< 9.0	<9.0	9.2	9.4	9.6	10.5	11.4	12.3	13.6	15.2	17.2	> 30.0

TABLE 5.3

LOWEST RECORDED MOISTURE LEVELS OF FUNGAL GROWTH

Isolate	TEMPERATURE/MOISTURE CONTENT		
	30°C	35°C	40°C
Absidia corymbifera	15.5%	> 30.0%	> 30.0%
Ulocladium atrum	18.25%	17.0%	15.5%
Fusarium solani	19.0%	> 30.0%	-
Alternaria alternata	15.0%	17.5%	13.5%
Trichoderma harzianum	16.5%	18.0%	14.75%
Chrysoporium pruinosum	17.0%	29.0%	> 30.0%
Chaetomium trilaterale	17.5%	17.0%	17.0%

5.3 Experimental II

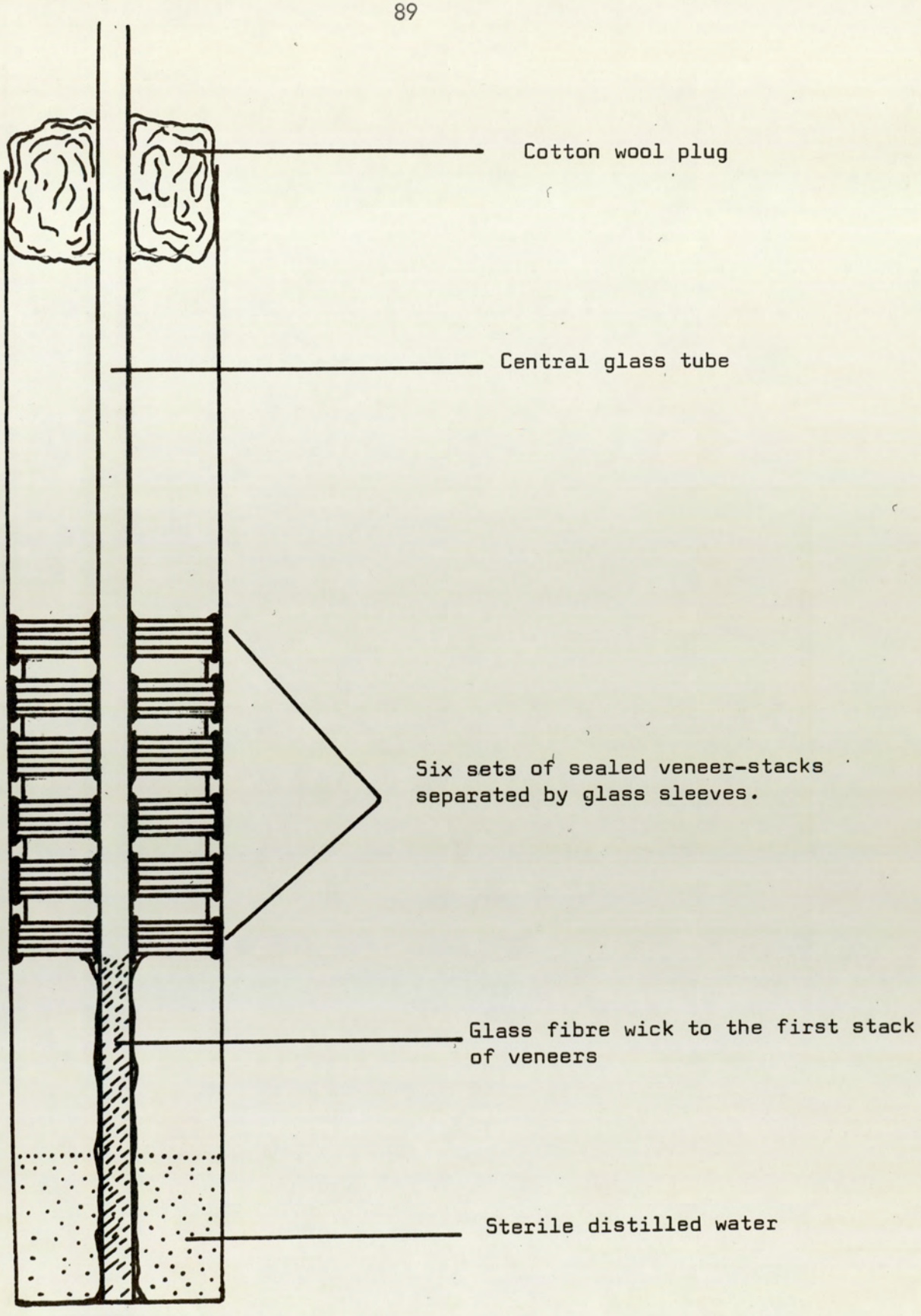
In order to investigate the active growth or penetration into the veneers by the test organisms the basic moisture gradient apparatus was modified.

5.3 (i) Materials and methods II

Small stacks, each of four veneer discs, were prepared as follows :

The veneers were stacked one on top of the other, 'Silastoseal B' was smeared around the outer edges of the discs to form a seal and the stacks were clamped for 24 hours to ensure a tight stack. Six of these stacks were placed on the central glass tube of the moisture gradient apparatus and 'Silastoseal B' was used once again to seal the space between the central hole in the veneer discs and the glass tube. Glass separators 1cm wide were placed between each of the stacks during assembly and finally the whole was allowed to stand for 24 hours so that the silicone rubber adhesive could set. The supporting platform used in the previous experiments was not needed in this system as it was sufficiently rigid once the Silastoseal had set. Seven sets of the apparatus were assembled (Figure 5.2) and each was inoculated with the spores of one of the seven test organisms. In each case a loopful of spore suspension was inoculated onto a spot marked on the uppermost veneer of the stacks at the six levels within the apparatus. Incubation was at 35°C for a period of three weeks.

After incubation each stack of veneers was carefully removed and using a flamed No. 8 cork borer discs were cut from each veneer in the stack by cutting upwards from the base of the stack through to the uppermost marked veneer. The discs were inoculated onto plates of ball milled wood agar and incubated for five days at 35°C. The moisture content at the top and base of each stack was recorded using a Protimeter moisture meter.



MOISTURE GRADIENT APPARATUS CONTAINING STACKS OF VENEERS

FIG. 5.2

5.3 (ii) Results II

Timber may contain xylophilous (wood inhabiting) as well as xylophagus (wood decaying) fungi (Etheridge, 1971). The ability of xylophagus micromycetes to penetrate into wood is important from the point of view of decay (Corbett, 1965; Nilsson, 1973) whilst the ability of xylophilous fungi to grow within wood may play an important role in interactions which occur between members of these two groups of fungi (Etheridge, 1971).

The results demonstrate the ability of thermophilous cellulolytic fungi to penetrate into veneers with low moisture content at above ambient temperatures. The ability of a non cellulolytic organism to grow within wood is also recorded.

Table 5.4, shows the effect of moisture content in discs of beech veneer on the growth and active growth or penetration of test organisms. The data show the moisture contents at the top and base of veneer stacks at each of the six levels within the moisture gradient apparatus, the levels at which fungal growth was visible and the extent of fungal penetration into the veneers. It can also be seen that discs cut from the uppermost veneers of stacks with low moisture contents, below the values required for growth and penetration, often produce growth on agar, indicating the varying degrees of viability of the spores of the test organisms under conditions of low moisture content.

Penetration below the top veneer of the stacks occurred for all test organisms including the non cellulolytic fungus Absidia corymbifera. The results also show that there could be penetration without growth being visible at the surface, this was seen in Absidia corymbifera, Fusarium solani, Trichoderma harzianum, and Chaetomium trilaterale.

The moisture contents of veneers where growth and active growth or penetration took place at 35°C are shown in Table 5.5. It can be seen that in Ulocladium atrum and Alternaria alternata surface growth only was recorded at moisture levels below 20%, higher moisture levels above 20% were required for active growth to take place. The remaining five isolates, however, showed growth within the veneer stacks at levels below 20% moisture content. The moisture levels recorded at 35°C for fungal growth to be detected visually at the surfaces of veneers showed a high degree of correlation in both sections of the work (Table 5.3 cf Table 5.4). The only exception was the case of Absidia corymbifera which was not detected visually at moisture levels below 20% on a single veneer but which was detected growing at the surface of a stack of veneers.

The results offer evidence that with increased biological activity of water at higher temperatures thermophilous micromycetes can remain active in timbers with low moisture contents which are subject to insolation.

TABLE 5.4

THE EFFECT OF MOISTURE CONTENT IN DISCS OF BEECH VENEER ON THE SURFACE GROWTH AND PENETRATION OF THE TEST ORGANISMS AT 35°C

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
MOISTURE %	9·0			10·0	10·0			10·6	12·0			12·0	13·5			14·0	19·0			21·0	23·0			28·0

Absidia corymbifera

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+
MOISTURE %	10·0			10·0	11·0			11·0	12·0			12·5	12·5			13·0	17·0			20·0	28·0			>30·0

Ulocladium atrum

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
MOISTURE %	<9·0			<9·0	10·0			10·0	11·0			11·5	13·0			13·0	17·0			17·0	28·0			>30·0

Fusarium solani

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+
MOISTURE %	<9·0			<9·0	11·0			11·0	13·5			14·0	14·0			14·0	17·0			18·0	25·0			30·0

Alternaria alternata

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	+	-	-	-	+	-	-	-	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+
MOISTURE %	10·0			11·0	12·0			12·0	13·0			13·0	15·0			15·5	19·0			22·0	>30·0			>30·0

Trichoderma harzianum

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+
MOISTURE %	9·0			9·0	10·0			10·0	12·5			14·0	14·0			14·25	17·0			17·5	28·0			30·0

Chrysosporium pruinosum

LEVEL	1				2				3				4				5				6			
VENEER	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
GROWTH ON AGAR	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+
MOISTURE %	10·0			11·5	11·5			11·5	12·5			13·0	14·0				14·0			17·0	25·0			>30·0

Chaetomium trilaterale

— fungal growth visible

\+ fungal growth detected

TABLE 5.5

MOISTURE LEVELS REQUIRED FOR GROWTH AND PENETRATION AT 35° C

Fungus	Minimum % moisture content necessary for:	
	Surface Growth	Penetration of veneers in stack
Absidia corymbifera	14%	14% veneers 1 to 4
Ulocladium atrum	17%	28% veneers 1 to 4
Fusarium solani	13%	13% veneers 1 to 4
Alternaria alternata	17%	25% veneers 1 to 4
Trichoderma harzianum	15%	a) 15% veneers 1 and 2 only b) 19% veneers 1 to 4
Chrysosporium pruinosum	17%	17% veneers 1 to 4
Chaetomium trilaterale	14%	a) 14% veneers 1 and 2 only b) 17% veneers 1 to 4

5.4 Discussion

Previous workers including Corbett (1963), Corbett and Levy (1963b), Shigo (1962), Merrill and French (1966), Käärick (1967), Butcher (1968), Toole (1971) and Banerjee and Levy (1971) have shown that mould stain and soft rot fungi are successful colonizers of the above-ground regions of wood.

The test organisms employed in this investigation included fungi from each of these groups which collectively have been termed the micromycetes (Gorschin and Krapivina, 1969). With the exception of Absidia corymbifera the test organisms are considered to be microthermophiles (Apinis and Pugh, 1967), so designated because of their ability to clear cellulose most markedly at elevated temperatures (30°, 35° and 40°C).

All the experiments in this investigation were conducted at temperatures well above normal ambient temperatures consistent with those encountered in insolated wood (Chapters IV and VI), and the results show that at such temperatures thermophilous micromycetes can grow on and within wood even at moisture levels below 20%, the accepted minimum quoted in the literature (Savory, 1967; Nash-Wortham and Savory, 1968; Liska, 1971; De Groot, 1972). Such results point positively to an increase in the biological activity of water at these higher temperatures (Ayerst, 1965) and correlate well with the findings of field studies undertaken (Chapters II, III, and VIII) which record fungi isolated from timber with moisture contents below 20% at the time of sampling. Of the test organisms employed in this investigation, Chaetomium trilaterale was isolated from a timber sample which recorded a moisture content of 20%, whilst all the other test organisms were isolated from samples with a moisture content below this value (Chapters II, III, and VIII). The reports of Duncan and Eslyn (1966) that soft rot organisms occurred in situations of extreme dryness are, therefore, noted with

interest. The findings of King and Eggins (1973) that mould and stain fungi possess enzymatic degradative mechanisms other than those of cellulosis are particularly pertinent, because if thermophilous micromycetes are present in wood, even wood with low moisture content which is subject to insolation, then they may constitute a group capable of contributing to the decay of such timbers. The results of previous experiments (Chapters II and III) show that microthermophiles can remain physiologically active at lower temperatures which indicates that they may constitute a group of fungi particularly well adapted to inhabit timber which is subject to insolation.

An important effect of temperature is the differentiation of the mycoflora within wood (Loman, 1962; Nilsson, 1965; Bergman and Nilsson, 1966, Henningson, 1968) and it must be appreciated that wood inhabiting fungi will be exposed to fluctuations in temperature and their response to such fluctuations will affect the extent to which they will colonize or cause decay in wood (Wagener and Davidson, 1954; Cartwright and Findlay, 1958; Savory, 1967; Jensen, 1968, Henningson, 1968). Interactions between wood inhabiting fungi will also be affected by variations in temperature. These points are the subjects of later chapters.

CHAPTER VI

THE EFFECT OF CONSTANT, ALTERNATING AND FLUCTUATING TEMPERATURES ON THE GROWTH OF SELECTED FUNGI

Contents :

6.1 Introduction

Fungi inhabiting timber, especially timber subject to insolation, may be exposed to a wide range of temperatures above and below their optima and possibly, on occasions, outside their normal range of activity.

It was decided, therefore, to devise a series of experiments using fungi, isolated from imported and in-service timbers to look more closely at the effects of constant, alternating and particularly diurnal fluctuating temperatures on their growth.

Perhaps the most severe test of fungal tolerance to temperature variations was conducted by Curtis (1966) who exposed fungi to near Martian diurnal temperature ranges ($-94^{\circ}C$ to $23 \pm 2^{\circ}C$). Some Ascomycetes and Fungi imperfecti survived and grew under such conditions.

A more restricted series of experiments conducted by Jensen (1968) using wood decaying fungi indicate that constant and alternating temperatures affect dry weight production of fungi grown in liquid culture. The fungi employed by Jensen were in the class of intermediate temperature forms (Humphrey and Siggers, 1938) producing the greatest amount of dry weight in liquid culture between 27° and $30^{\circ}C$. Using alternating temperatures which gave a mean of $21^{\circ}C$ he found that dry weight increased as the fluctuations changed from 0 - 6 or $12^{\circ}C$, but decreased as they increased to $22^{\circ}C$, data similar to Smith's (1964) data on soil borne organisms. Jensen commented that change in dry weight production may be due either to a stimulation of growth or to the additive effect of separate constant temperatures. Henningson (1968) studying the growth and decay activity of pure cultures of birch and aspen fungi showed that low temperature fungi (optima below $25^{\circ}C$) have a comparatively low decay activity even at their optimal temperatures and that many fungi with higher optima are more active at low temperatures ($10^{\circ}C$) than the low temperature fungi themselves. The

implications of such findings, when one considers that the temperature of the environment in which decay fungi are actively growing is constantly changing, are very important. Henningson (1968) observed that even in winter temperatures could be high enough to allow decay fungi to be active. The survey work conducted in Preston recorded that temperatures in wood ranged from below 10°C to above 45°C (Chapter IV).

Two series of experiments were undertaken, the first to investigate the influence of alternating temperatures upon the growth of selected fungi and the second to investigate the growth response of thermophilous fungi to fluctuating diurnal temperature cycles.

6.2 Experimental I

6.2 (i) Materials and methods I

Thirteen test organisms were grown on plates of the Eggins (1964) medium containing ball-milled redwood (Appendix I).

Eighteen plates were inoculated with disc inocula of each test fungus and three plates were placed at each of the six temperature regimes chosen for the investigation. The constant temperatures employed were 25°C, 35°C and 5°C. The alternating temperatures, in operation for periods of twenty-four hours were $\frac{25}{35}$ °C, $\frac{25}{5}$ °C and $\frac{35}{5}$ ° C. The times taken for the plates to adjust from one temperature to another was noted. An alchohol flamed cork-borer was used to obtain the 3mm diameter disc inocula.

The experiments were conducted over a period of ten days using 9cm diameter petri-dishes. . The diameter of the developing culture was measured daily by using a scale etched onto the lid of a plastic petri-dish. In the case of Trichoderma harzianum it was necessary to use 14cm diameter glass petri-dishes over a period of eight days as this fungus grew very rapidly on the Eggins ball-milled medium.

Based on the results of clearing tube experiments (Chapter II) the test organisms used in the following experiments included four mesophiles and five microthermophiles. Four fungi which did not clear cellulose in the earlier experiments were also included. The thirteen test organisms were :

1. Mesophiles, Penicillium decumbens, Scopulariopsis brevicaulis, Trichoderma viride and Chaetomium bostrychodes.
2. Microthermophiles, Paecilomyces varioti, Ulocladium atrum, Trichoderma harzianum, Chaetomium trilaterale, and Alternaria alternata.

3. Non cellulolytic fungi, Amorphotheca resinae, Cephalosporium sp.A, Cephalosporium sp. B, and Phoma glomerata. Of these four fungi Phoma glomerata produced pigment during the clearing tube experiments which made the detection of clearing difficult to assess; this fungus is on record as being capable of producing soft rot in timber (Appendix X).

An analysis of variance (Steel and Torrie, 1960) was carried out on the results and the significance (at the 5% level) of the differences between individual treatments was examined using the Duncan multiple range test. Further consideration was given to the expected growth against actual growth obtained in the alternating temperature experiments. An analysis of variance (Brown, 1974; Appendix VII) was carried out on these data to determine whether there was any evidence for additivity, stimulation or retardation of growth resulting from temperature alternation. Since the time taken for the plates to adjust from one temperature to another ranged from 20 to 30 minutes only, such short durations of time are not considered sufficient to affect the statistical significance of the results.

6.2 (ii) Results I

The results indicate that alternating temperatures influence the growth of fungi.

Figure 6.1, records the diameters (in millimeters) of colonies at each of the constant and alternating temperatures employed. Their order in ranking means is displayed and expected values, where significantly different (at the 5% level) from actual results, are also indicated. Maximum growth at the constant temperatures employed was at 25° or 35°C, minimum growth at 5° or 35°C.

Table 6.1, shows the means of expected linear growth against actual growth obtained for alternating temperatures. These data suggest that the growth of fungi in response to alternating temperatures may be :

1. Additive, where the amount of growth obtained is equal to that expected at each constant temperature. This effect was recorded for Paecilomyces varioti at $\frac{25}{35}$°C; Trichoderma harzianum at $\frac{25}{5}$, $\frac{25}{35}$, °C; Phoma glomerata at $\frac{25}{5}$°C; Chaetomium trilaterale at $\frac{25}{5}$, $\frac{35}{5}$, $\frac{25}{35}$°C; Amorphotheca resinae at $\frac{25}{5}$, $\frac{35}{5}$, $\frac{25}{35}$°C; Chaetomium bostrychodes at $\frac{25}{5}$ °C; Scopulariopsis brevicaulis at $\frac{25}{5}$, $\frac{35}{5}$ °C; and Penicillium decumbens at $\frac{25}{5}$ °C.

2. Stimulative, where the amount of growth obtained is greater than that expected at each constant temperature. This effect was recorded for Paecilomyces varioti at $\frac{25}{5}$ °C; Ulocladium atrum at $\frac{35}{5}$ °C; Cephalosporium sp. A and B at $\frac{25}{5}$, $\frac{35}{5}$, $\frac{35}{25}$ °C; Scopulariopsis brevicaulis at $\frac{25}{35}$ °C; and Alternaria alternata at $\frac{25}{5}$, $\frac{35}{5}$ °C.

3. Retardative, where the amount of growth obtained is less than that expected at each constant temperature. This effect was recorded for Paecilomyces varioti at $\frac{35}{5}$ °C; Ulocladium atrum at $\frac{25}{5}$, $\frac{25}{35}$°C; Trichoderma harzianum at $\frac{35}{5}$ °C; Phoma glomerata at $\frac{35}{5}$, $\frac{25}{35}$°C; Trichoderma viride at $\frac{25}{5}$, $\frac{35}{5}$, $\frac{25}{35}$ °C; Chaetomium bostrychodes at $\frac{35}{5}$, $\frac{25}{35}$ °C; Alternaria alternata at $\frac{25}{35}$ °C; Penicillium decumbens at $\frac{35}{5}$, $\frac{25}{35}$ °C.

For Amorphotheca resinae and Chaetomium trilaterale the effect of alternating temperatures was additive only, whilst for both species of Cephalosporium temperature alternations stimulated their growth, in Trichoderma viride, however, growth was retarded by the temperature alternations (Table 6.1). Addition and stimulation were recorded for Scopulariopsis brevicaulis (Table 6.1).

Addition and retardation were recorded for Trichoderma harzianum, Penicillium decumbens, Phoma glomerata, and Chaetomium bostrychodes. (Table 6.1).

Stimulation and retardation were recorded for Ulocladium atrum and Alternaria alternata (Table 6.1).

Addition, stimulation and retardation were recorded in Paecilomyces varioti only (Table 6.1)

Such results prompted further investigations into the response of fungi, particularly thermophilous fungi to the temperature regimes which might be encountered in timber subject to insolation.

FIG. 6.1
THE EFFECT OF CONSTANT AND ALTERNATING TEMPERATURES ON THE GROWTH OF FUNGI

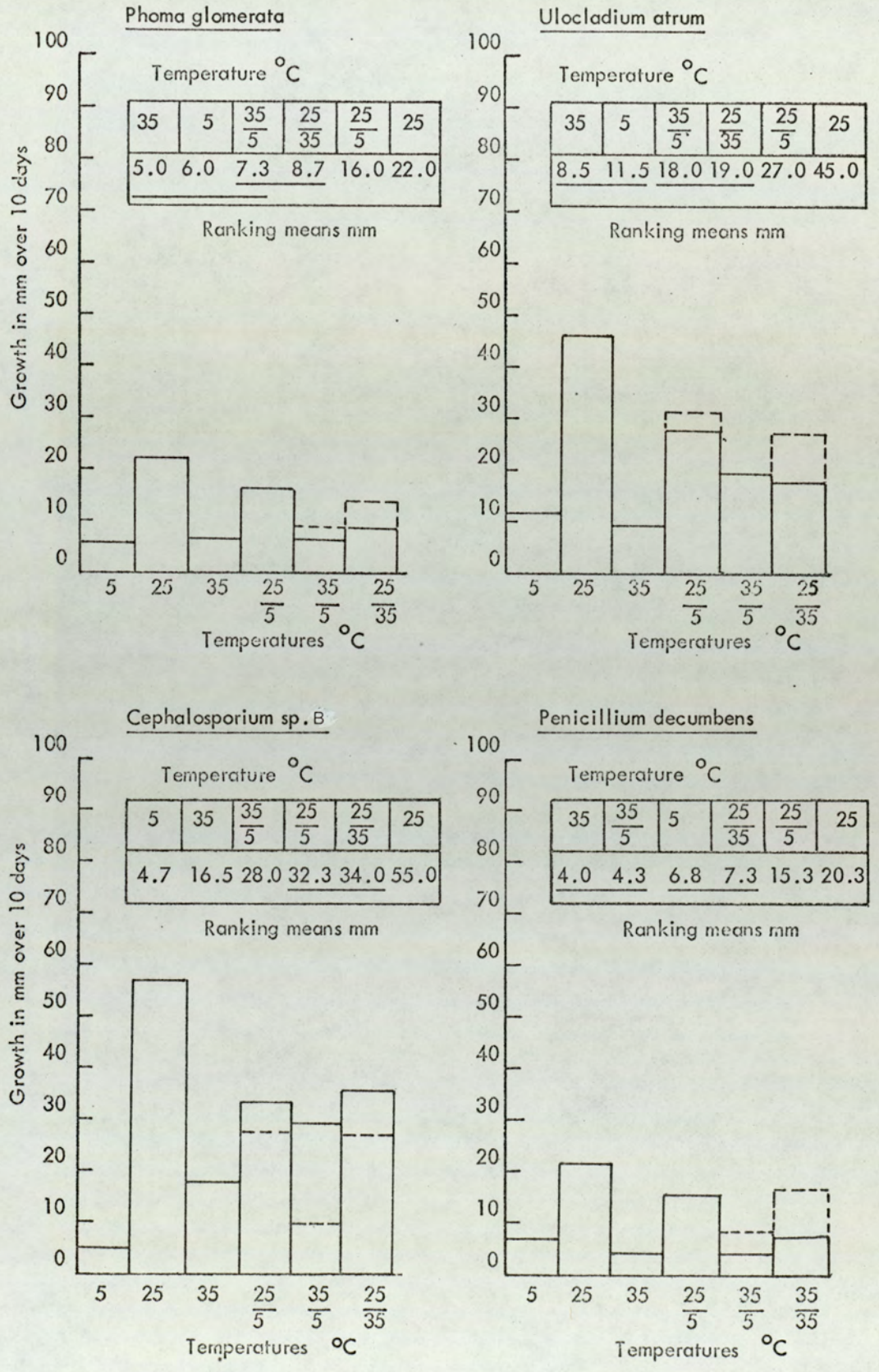


FIG. 6.1

THE EFFECT OF CONSTANT AND ALTERNATING TEMPERATURES ON THE GROWTH OF FUNGI cont.....

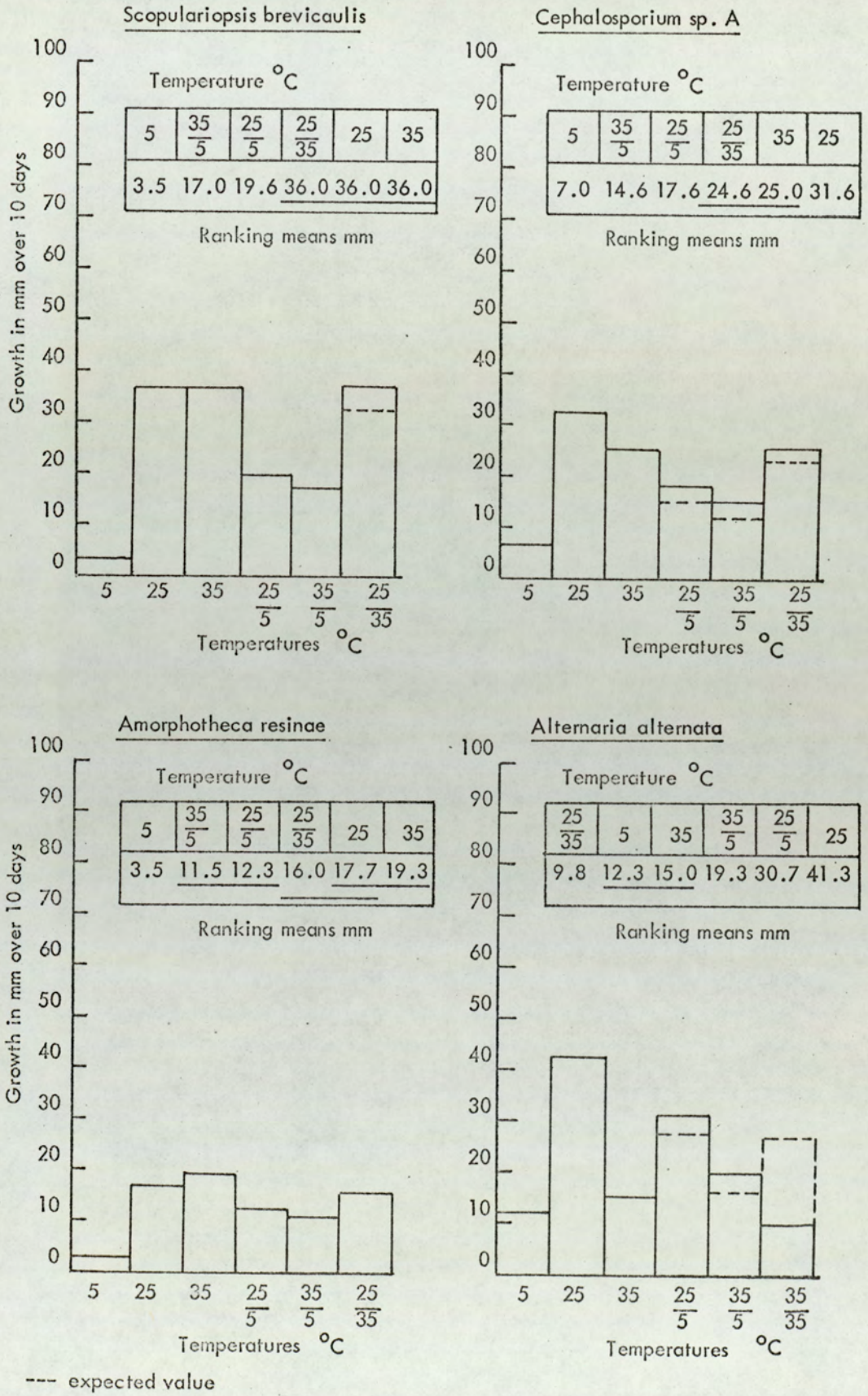


FIG. 6.1

THE EFFECT OF CONSTANT AND ALTERNATING TEMPERATURES ON THE GROWTH OF FUNGI cont.....

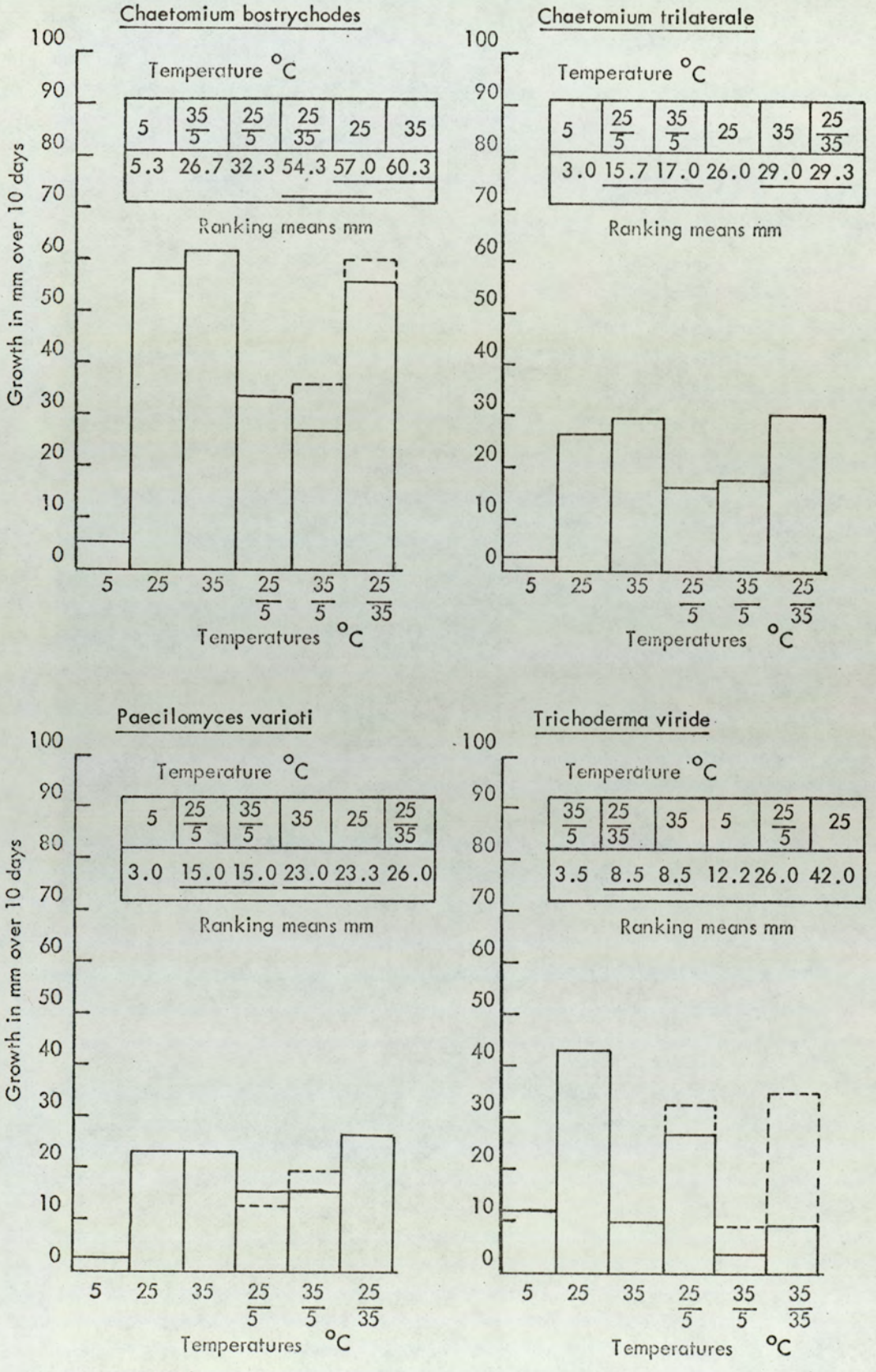


FIG. 6.1

THE EFFECT OF CONSTANT AND ALTERNATING TEMPERATURES ON THE GROWTH OF FUNGI cont.....

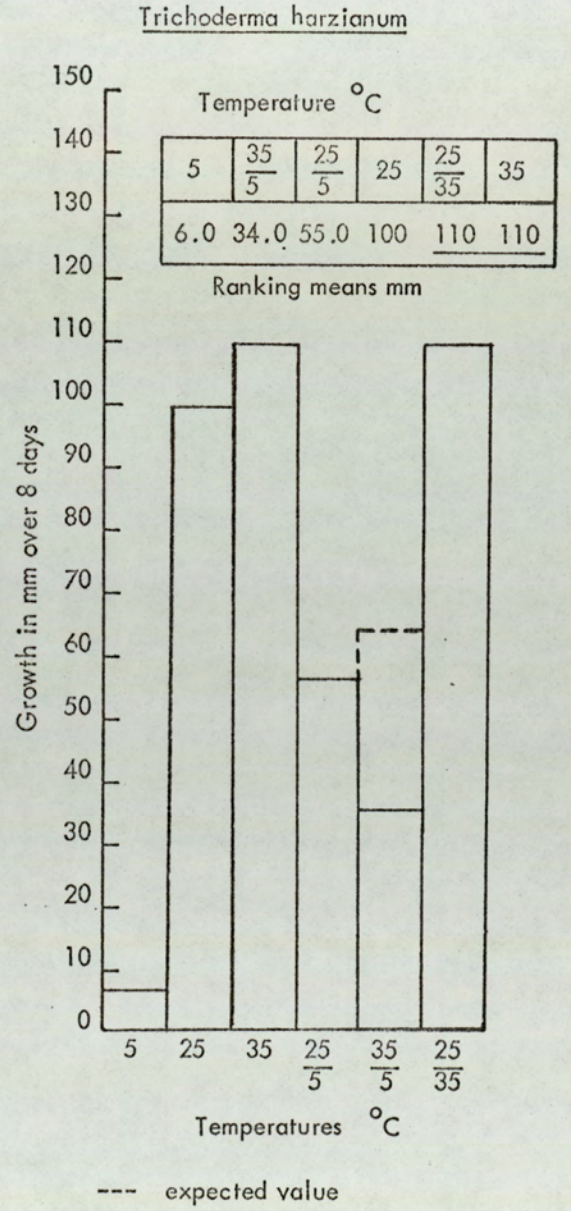


TABLE 6.1

ALTERNATING TEMPERATURES:

EXPECTED AND ACTUAL GROWTH IN mm, EXPRESSED AS THE MEANS OF THREE REPLICATES

	Temperatures: 25/5			Temperatures: 35/5			Temperatures 25/35		
Fungus	Expected	Actual		Expected	Actual		Expected	Actual	
P. varioti *	13.0	15.0	+	18.6	15.0	-	25.6	26.0	
U. atrum *	30.0	27.0	-	10.0	19.0	+	26.0	18.0	-
T. harzianum *	54.0	55.0		63.0	34.0	-	107.0	110.0	
P. glomerata ∅	15.0	16.0		9.0	7.3	-	13.6	8.6	-
C. trilaterale *	16.0	15.6		17.0	17.0		27.0	29.3	
T. viride o	31.3	26.0	-	8.5	4.0	-	34.3	8.5	-
Cephalosporium ∅ sp. A	15.6	17.6	+	12.8	15.0	+	23.0	25.0	+
Cephalosporium ∅ sp. B	26.0	33.0	+	9.0	28.0	+	26.6	34.0	+
A. resinae ∅	11.6	12.3		11.0	11.0		14.0	16.0	
C. bostrychodes o	31.0	32.3		34.5	27.6	-	58.8	54.3	-
S. brevicaulis o	21.0	19.6		17.0	17.0		32.0	35.6	+
A. alternata *	26.6	30.6	+	15.6	19.3	+	26.0	9.8	-
P. decumbens o	16.6	15.3		7.0	4.3	-	15.6	7.3	-

_____ no significant difference: addition

\+ stimulation

\- retardation

* microthermophiles

o mesophiles

∅ non cellulolytic organisms

6.3 Experimental II

An actual cycle of temperatures recorded in a block of wood was used as the basis for a simulated temperature cycle to study the effect of fluctuating temperatures on the growth of fungi. Selected fungi were grown under these conditions and their growth compared to that obtained at four constant temperatures within the range of the temperature cycle employed.

6.3 (i) Materials and methods II

A constantly recording copper/constantan thermocouple was placed at the centre of the black section of the pine block employed in Chapter IV. A record was kept of the temperature ranges encountered during a 24 hours cycle in June, 1973. The block was in sun from about 8.15 a.m. to 4.00 p.m.

A large water bath was constructed measuring 24 x 24 x 10" using $\frac{1}{4}$" glass. Within this a central well measuring 12 x $8\frac{1}{2}$ x 10" was constructed using 32 oz. window glass. A silicone rubber adhesive was used to secure all glass. Four thermostatically controlled temperature units were used together with a cooling coil (Figure 6.2). The heaters were set at 8°, 20°, 35° and 47°C respectively against the cooling coil which ran continuously. The 20°, 35° and 47°C heaters were operated by time clocks and the sequence was as follows :

20°C : 8.30 a.m. to 11.00 a.m.
35°C : 10.15 a.m. to 12.20 a.m.
47°C : 12.00 a.m. to 2.35 p.m.

The 8°C unit operated when the time switches were all at 'off' preventing the cooling coil from lowering the water temperature below this level. In order to prevent evaporation expanded polystyrene 'worms' were floated on the surface of the water in the tank.

FIG. 6.2 THE TEMPERATURE CYCLE SIMULATOR

Ten fungi were selected and grown in monoculture on the medium containing ball-milled redwood and the salts of the modified Eggins and Pugh cellulose medium. Three replicates of each fungus were grown on this medium at the constant temperatures of 8°, 20°, 35°, and 47°C, and three replicates of each fungus were grown in the simulator. The experiments ran for ten days at the end of which time colony diameters were recorded. Disc inocula (3mm diameter) were used in these experiments. All data were subjected to an analysis of variance (Appendix VII). The fungi employed were :

Epicoccum purpurascens, Cephalosporium acremonium,
Chaetomium trilaterale, Alternaria alternata,
Scopulariopsis brevicaulis, Aspergillus fumigatus,
Chaetomium indicum, Trichoderma koningii,
Chrysosporium pruinosum and Paecilomyces varioti.

The first four of these test organisms are proven soft rot fungi (Appendix I), two of which are mesophilic (Epicoccum purpurascens and Alternaria alternata) and two are thermophilous (Cephalosporium acremonium and Chaetomium trilaterale). Of the remainder all but Scopulariopsis brevicaulis may be considered thermophilous. The term thermophilous is a designation based on their ability to clear cellulose most markedly at higher temperatures (Chapters II and III).

6.3 (ii) Results II

The results show that when wood is subjected to insolation it heats up quite rapidly but cools much more slowly. Two traces of actual temperature cycles in wood over twenty-four hours are submitted, recorded on suitable sunny days in June, 1973 and April, 1974 (Figure 6.3). The trace obtained in June showed that as a result of insolation temperatures in excess of 40°C were recorded whilst the maximum values on the April trace approached 30°C, both traces, however, show the rapid heating and slower cooling processes. The simulator was adjusted so as to produce a temperature cycle which resembled that recorded in the June trace (Figure 6.3).

Figure 6.4, records the means of the diameters of each test organism grown on the ball-milled redwood medium at each of the constant temperatures employed and as a result of being subjected to the simulated daily temperature cycle for a period of 10 days. Their order in ranking means is displayed so that the performance of each fungus under conditions of fluctuating temperature can be compared with its performance at each of the four constant temperatures.

All test organisms produced measurable amounts of growth under the fluctuating temperature conditions. In all cases except Paecilomyces varioti, and Epicoccum purpurascens (Figure 6.4) this amount was significantly greater than the minimum amount of growth recorded on agar at the constant temperatures employed. In no case did the amount of growth produced under conditions of fluctuating temperature exceed the maximum values recorded at the constant temperatures employed. The results show that Trichoderma koningii and Aspergillus fumigatus produced quantitatively the greatest amounts of growth under conditions of fluctuating temperature

whilst Paecilomyces varioti produced the least. The values obtained for growth under the fluctuating temperature conditions presented in Figure 6.4 represent the overall response of the fungi to the changing temperature environment.

The results of this investigation show that thermophilous fungi can produce significant amounts of growth under the conditions of fluctuating temperature which occur in insolated wood.

FIG. 6.3 ACTUAL AND SIMULATED TEMPERATURE CYCLES

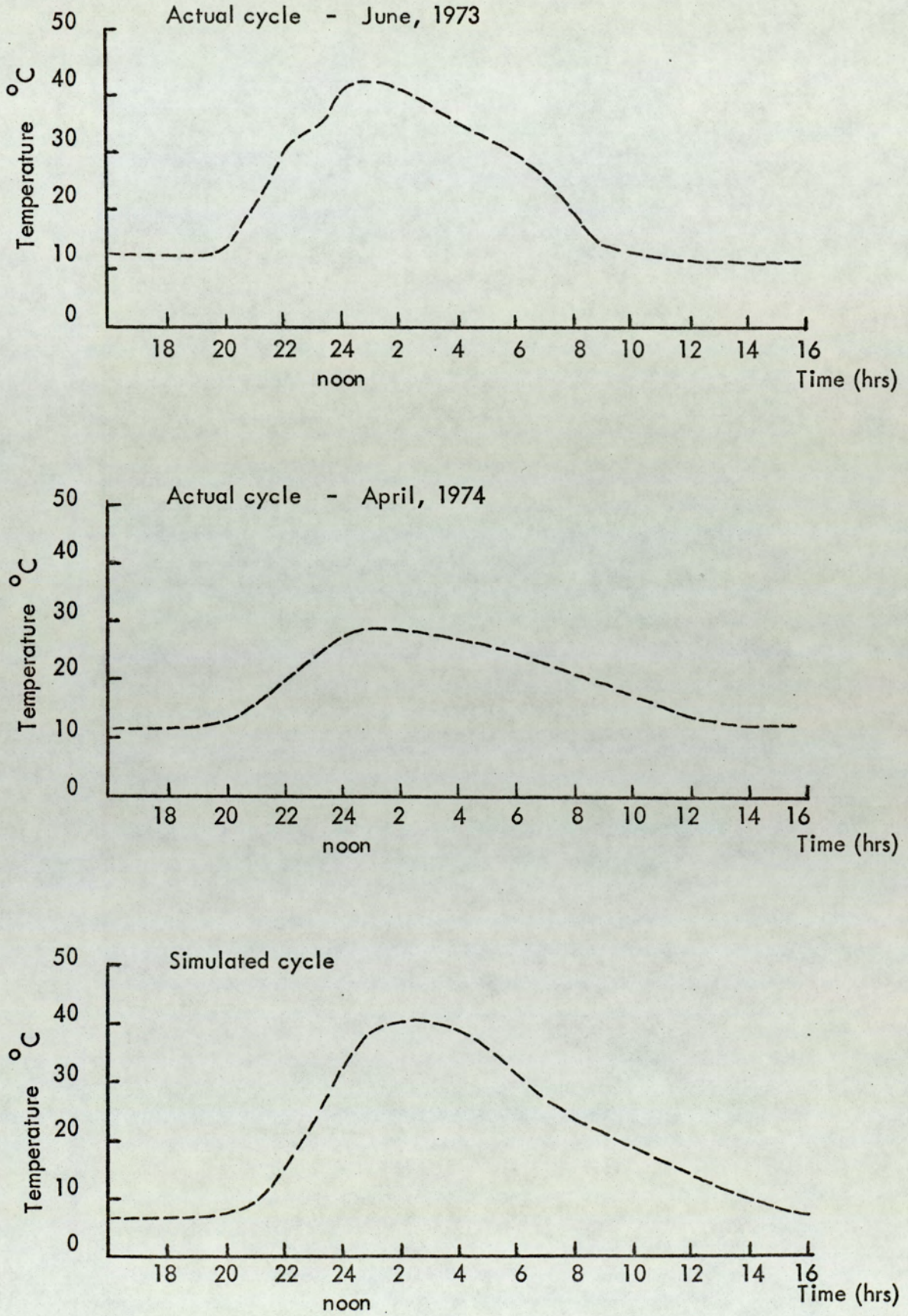


FIG. 6.4

THE EFFECT OF DIURNAL TEMPERATURE FLUCTUATIONS ON THE GROWTH OF FUNGI

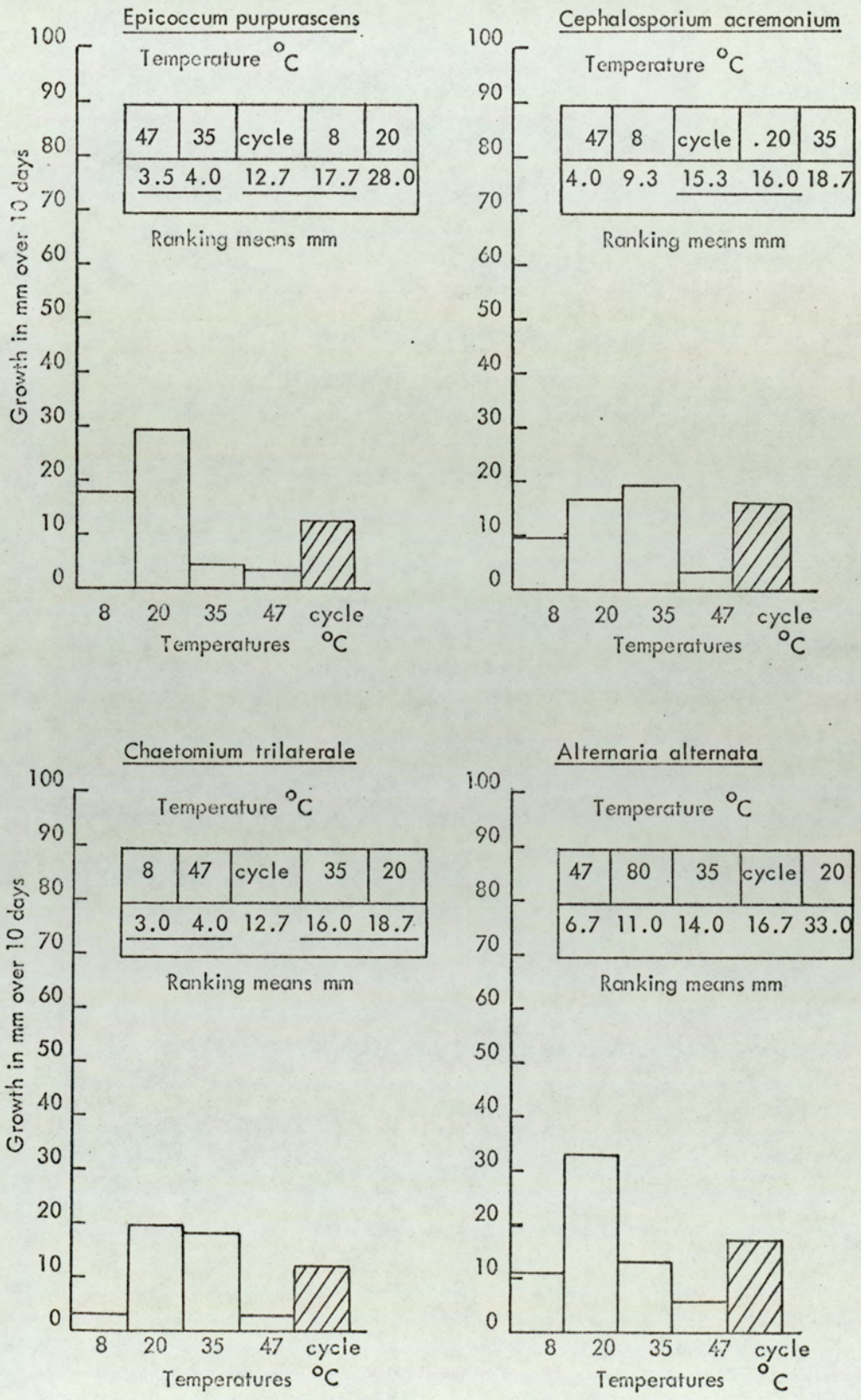


FIG. 6.4

THE EFFECT OF DIURNAL TEMPERATURE FLUCTUATIONS ON THE GROWTH OF FUNGI cont.....

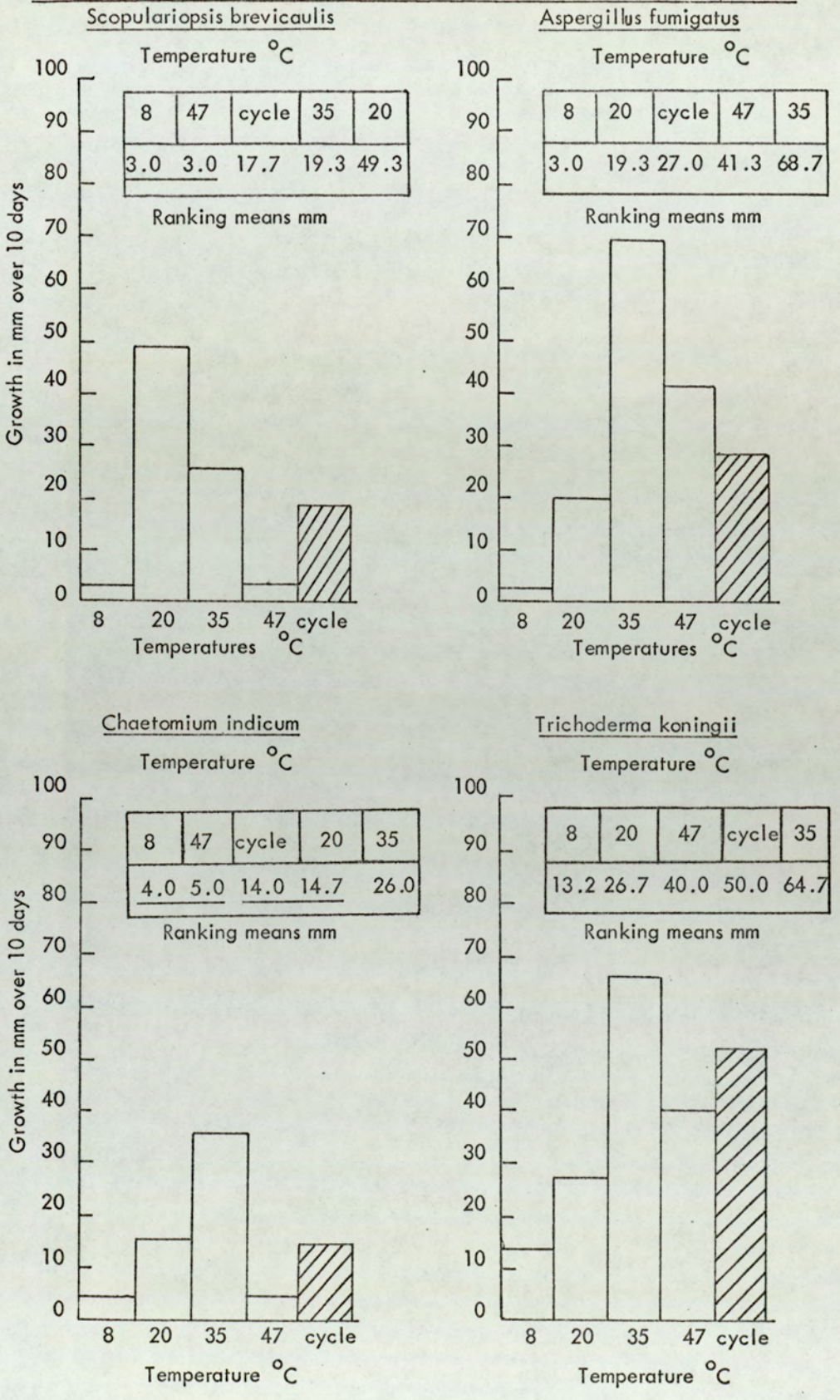
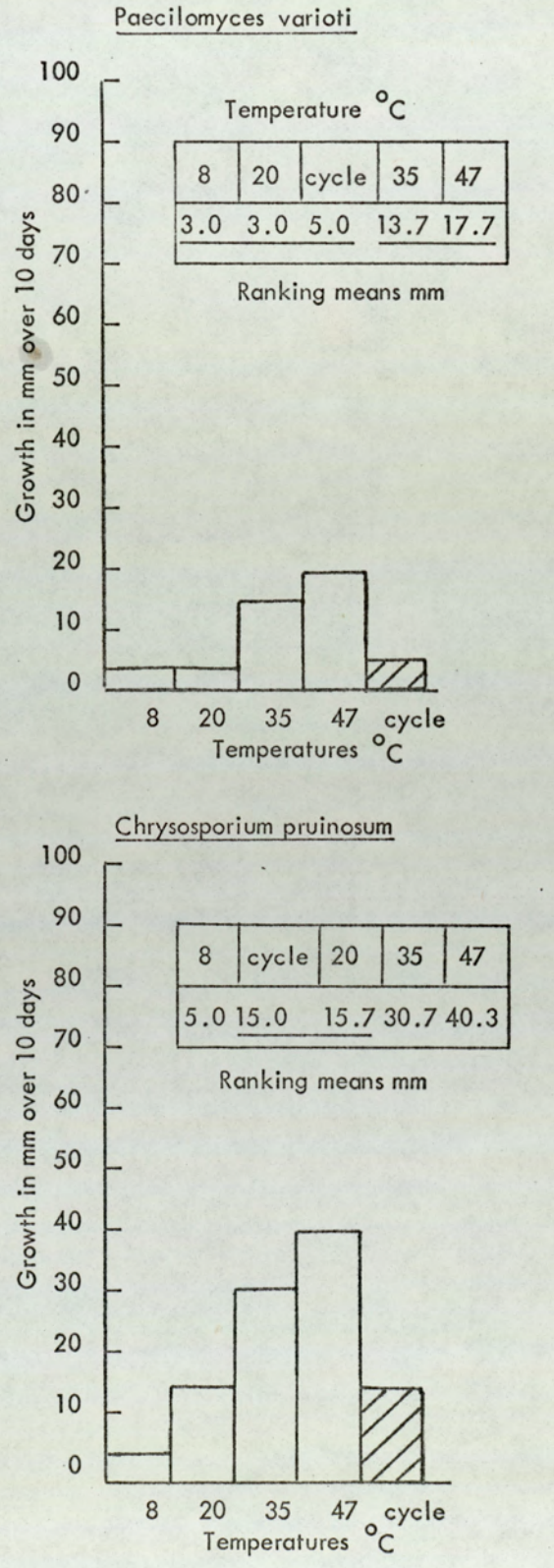


FIG. 6.4

THE EFFECT OF DIURNAL TEMPERATURE FLUCATIONS ON THE GROWTH OF FUNGI cont.....



6.4 Discussion

Disagreement exists in the literature concerning the appropriate parameter to choose to obtain a linear plot of growth rate over the surface of agar (Brancato and Golding, 1953; Cochrane, 1958) and suggestions include radius, diameter, area various reciprocals of these and other data plotted against time. Data from mesophilic fungi often give a linear plot when diameter of colony growth is plotted against time. The data of Tansey (1972) show that this is also the case for thermophilic fungi. Evans (1971) used diameter of colonies to determine the cardinal temperatures for the growth of thermophilic fungi isolated from coal tips. The results of this investigation are based upon the growth of fungi over the surface of agar where the diameters of colonies were used to measure the amount of growth produced.

The first section of the results are a comparison of actual growth values obtained when fungi were grown under conditions of alternating temperatures against those which would be expected as a result of linear growth at constant temperatures of appropriate duration. Evidence is offered of addition, stimulation and retardation of growth resulting from such treatment :

1. Alternation of the higher temperatures $\frac{25}{35}$ °C showed that the thermophilous cellulolytic fungi responded with addition or retardation of growth, no stimulation of growth was recorded. The growth of one mesophilic cellulolytic organism, Scopulariopsis brevicaulis, was stimulated by this treatment, the remaining three cellulolytic mesophiles recorded a retardation of their growth.
2. Alternations of the higher temperatures (25° and 35°C) with the low temperature (5°C) resulted in cases of addition, retardation and stimulation amongst the cellulolytic thermophilous fungi. The

cellulolytic mesophiles produced cases of addition and retardation but no stimulation of growth was recorded.

The test organisms considered as being non cellulolytic in culture which were employed during this investigation may be designated tentatively as mesophilic (Cephalosporium spp. and Phoma glomerata;) or thermophilous (Amorphotheca resinae) on the basis of their growth over the surface of agar (Figure 6.1). Stimulation of growth resulted at all three temperature alternations for the two species of Cephalosporium, whilst addition was recorded at all three temperature alternations for Amorphotheca resinae. Phoma glomerata responded with addition $\frac{25}{5}$ °C and retardation $\frac{35}{5}$, $\frac{25}{35}$ °C.

The influence upon growth of the alternating temperatures employed during this investigation are clearly not purely additive. Stimulation and retardation are recorded as a result of alternating an above ambient temperature with a low temperature in thermophilous and mesophilic fungi of both a cellulolytic and non cellulolytic nature.

The results are in agreement with those for changes in dry weight production resulting from temperature alternations described by Jensen (1968), he did not, however, report any retardation of growth.

In wood undergoing insolation it has been shown that temperature may be constantly changing rather than there being fixed periods of alternating temperature. The growth response to such fluctuating temperature regimes will be an important factor influencing wood inhabiting fungi.

The cycles of temperature used during the second series of experiments simulate the rapid heating up and slower cooling down that can occur when wood is subject to insolation. The results obtained offer an indication of the total response of wood inhabiting fungi in terms of their growth to such conditions.

The results apply to the growth of the test organisms over the surface of agar rather than on wood, nevertheless, there is evidence that wood inhabiting fungi produce significant amounts of growth under such conditions. A comparison of the amount of growth produced by each test organism under the conditions of fluctuating temperature with the maximum growth value recorded at constant temperature in each case, shows that of the nine test organisms employed, the thermophilous fungi Chaetomium trilaterale, Cephalosporium acremonium and Trichoderma koningii (Figure 6.3) produced by far the greatest growth responses under conditions of fluctuating temperature.

CHAPTER VII

INTERACTION STUDIES

7.1 Introduction

Fungal succession in wood is a complex process. When one fungus inhibits the growth of a competitor, then in general terms this is likely to be due to either nutrient depletion, production of toxic metabolites, mycoparasitism or any combination of these (Hulme and Shields, 1970; 1972a and b; Park, 1968; Banergee and Levy, 1971).

Hulme and Shields (1970, 1972a and b) explain fungal antagonism in wood as being related to the course of wood colonization and to the speed with which fungi develop on traces of simple more accessible nutrients thus depleting their supply to later colonizers. The results of their work favour competition for simple nutrients as the dominant mechanism of antagonism between fungi when colonizing wood in field conditions rather than antibiotics or mycoparasitism.

It is difficult to obtain positive evidence that antibiotics are responsible for inhibition since extrapolations of findings from laboratory media to the natural habitat are not necessarily valid (Käärick, 1968). Often no antibiotic can be detected even in artificial culture (Hulme and Shields, 1972a; Aluko and Hering, 1970; Webster and Lomas, 1974).

The growth and decay activity of wood inhabiting microbes and the interactions between them will be influenced by the temperature and moisture content of wood (Humphrey and Siggers, 1933; Bjorkman, 1946; Ammer, 1964; Henningson, 1968). The temperature of the environment in which decay fungi are actively growing is constantly changing (Tansey, 1972) and information is now available on the order of these temperature changes in insolated wood (Chapter VI).

The present investigation was undertaken to study interactions between pairs of wood inhabiting microfungi. Interactions between fungi isolated at above ambient temperatures from wood samples were studied at a constant temperature of 35°C and interactions between mesophilic and thermophilous wood inhabiting fungi were studied under conditions of fluctuating temperature similar to those encountered in insolated wood. Three series of experiments were undertaken :

1. The growth of selected fungi over the surface of agar was recorded for monocultures and for pairs of fungi.

2. Perfusion experiments were devised employing timber veneer as the substrate to investigate the effects of fungal perfusants upon growth.

3. The interactions of selected fungi on the surface of timber veneer was investigated by inoculating the test organismsin close proximity to each other on the substrate.

When the inter-relationships of two or more organisms have been studied, antagonistic activity has usually been recorded (Etheridge, 1957, 1971; Persoon-Huppel, 1963; Shields and Atwell, 1963; Glaser, Tarocinski and Bouza, 1959; Koblinska, 1961; Klingstrom and Beyer, 1965; Shigo, 1965; Basham, 1966; Leise and Eckstein, 1967; Butcher, 1972; Bergman and Nilsson, 1967; Eggins, Malik and Sharp, 1968; Henningson, 1968; Kerner-Gang, 1970; Toole, 1971; Hulme and Shields, 1970, 1972a and b). Reports of stimulation, however, are much rarer, such effects among wood inhabiting microbes have been demonstrated by Freis (1938); Bouchier (1961); Pentland (1964); Eggins, Malik and Sharp (1968); and Mikhiln and Ulezo (1970).

In the following work the amount of growth obtained for a fungus in monoculture compared with that obtained for the fungus as one of an interacting pair is used

to decide the nature or type of interaction. Interactions between pairs of fungi may be considered to fall into one or two of three major categories :

1. Stimulation, where the presence of one fungus increases the amount of growth of the other.

2. Synergism, where there is a mutual stimulation of the growth of the interactants.

3. Retardation (inhibition or supression), where the amount of growth of one organism is reduced by the presence of the other.

This latter category may also be referred to as antagonism, however, in the present work it is felt that this term should constitute a fourth category where there is mutual inhibition or retardation of the growth of the interacting fungi.

7.2 Experimental

7.2 (i) Materials and methods

7.2 (ii) Experiments using agar plates

(a) at 35°C

Four test organisms were grown in monoculture for ten days on ball-milled wood agar. After incubation the radii of the colonies were recorded. Each test fungus was then grown against each of the others by inoculating the pairs of fungi one on either side of an agar plate. After ten days the radii of the colonies were determined. Three replicates of each plate were prepared and 3mm disc inocula were employed.

The fungi chosen for this work were isolated from wood at incubation temperatures of 35° or 40°C, they were :

Aspergillus fumigatus,	Trichoderma koningii,
Alternaria alternata,	Scopulariopsis brevicaulis.

(b) Using a temperature cycle

Three thermophilous fungi and three mesophilic fungi were grown in monoculture for ten days in the temperature cycle simulator (Chapter VI). The radii of resulting colonies were recorded. Each thermophilous fungus was then grown with each mesophile for ten days at the temperature cycle. Three replicates of each plate were prepared and disc inocula were used.

The fungi chosen for this work were :

Aspergillus fumigatus)
Chrysosporium pruinosum) Thermophilous fungi
Trichoderma koningii)

Verticillium intertextum)
Epicoccum purpurascens) Mesophilic fungi
Chaetomium trilaterale)

7.2 (iii) Perfusion experiments, using timber veneer

An apparatus was devised based on the principle of the interaction perfusion apparatus of Eggins, Malik and Sharp (1968). The modified apparatus makes use of autoclavable, polycarbonate, Gilson macro-snap tubes. Three tubes are employed together with glass fibre wick, veneer strips measuring 7.0 x 1.0cms, silicone rubber tubing and bungs made from moulded Silastoseal B. The apparatus is assembled as shown in Figure 7.1. Tube C acts as the reservoir for the system while tubes A and B each house a veneer taped to exposed portion of wick. When assembled, 25cm^3 of sterile distilled water is placed in the reservoir and the apparatus is autoclaved for 20 minutes at 15 lbs./sq. inch. pressure.

The 3mm disc inocula are positioned so that growth along the veneer is against the perfusion stream. The direction of the perfusion flow within the apparatus is from tube C (reservoir) $\xrightarrow{\text{to}}$ B $\xrightarrow{\text{to}}$ A (Figure 7.1). By setting up duplicate sets of apparatus for each pair of fungi to be studied, and by inoculating each fungus in turn onto the veneer in tubes B and A, the effect of the perfusant of each fungus upon the other can be investigated.

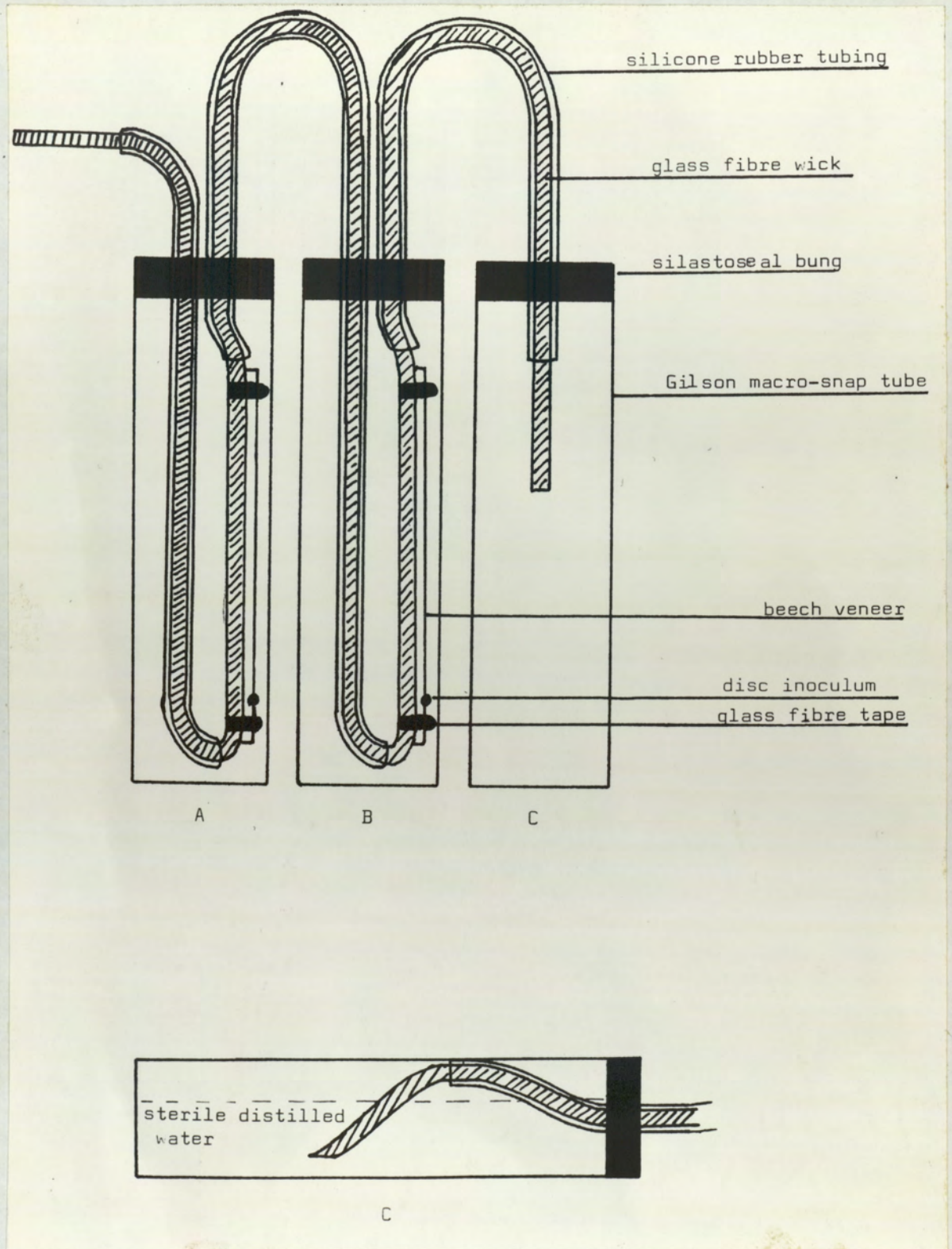
The pairs of fungi used in this work were those which, when grown together on agar, gave a significantly different result from that obtained for their growth in monoculture. The fungi used were:

Aspergillus fumigatus $\rightleftharpoons$ Alternaria alternata)
Trichoderma koningii $\rightleftharpoons$ Scopulariospsis brevicaulis) at 35°C
Trichoderma koningii $\rightleftharpoons$ Alternaria alternata)
Trichoderma koningii $\rightleftharpoons$ Aspergillus fumigatus)

Chaetomium trilaterale	⇌	Aspergillus fumigatus	)
Chaetomium trilaterale	⇌	Chrysosporium pruinosum	)
Verticillium intertextum	⇌	Chrysosporium pruinosum	)
Epicoccum purpurascens	⇌	Chrysosporium pruinosum	) At the temperature cycle
Epicoccum purpurascens	⇌	Trichoderma koningii	)
Verticillium intertextum	⇌	Trichoderma koningii	)
Verticillium intertextum	⇌	Aspergillus fumigatus	)
Epicoccum purpurascens	⇌	Aspergillus fumigatus	

All experiments ran for four days at 35°C and for ten days at the temperature cycle.

FIG. 7.1 PERFUSION APPARATUS FOR INTERACTION STUDIES



7.2 (iv) Close proximity inoculation experiments using timber veneer

The apparatus employed to study the interactions of fungi growing in close proximity on timber veneer consisted of glass petri-dishes, the bases of which contained small glass rings. Each petri-dish housed a piece of beech veneer measuring 7cm x 3 cm which lay on top of the glass rings. Into each petri-dish $20cm^3$ of distilled water was introduced and each apparatus was autoclaved for twenty minutes at 15 lbs. pressure. When the apparatus was cool, disc inocula of the selected pairs of fungi were placed 5mm in from one narrow edge of the veneer strip, 15mm apart. The test organisms employed were those of the perfusion experiments and here again the experiments were conducted at $35^{\circ}C$ and at the temperature cycle. The distance travelled by each fungus along the veneer was measured in each case. The fungi were grown in monoculture and as interacting pairs.

7.3 Results

The interactionsrecorded in the following results are based upon comparisons of the growth of a fungus in monoculture at 35°C or under conditions of fluctuating temperature with the growth recorded when it was grown as one of an interacting pair under the same temperature conditions.

Table 7.1 (a), shows the growth of the test organism grown in monoculture at 35°C for ten days.

Table 7.1 (b), shows the growth after ten days at 35°C for pairs of fungi inoculated at opposite sides of agar plates.

The data were subjected to statistical analysis (Appendix VII) and there was evidence of interaction. Stimulation, retardation and antagonism were recorded :

Stimulation of Aspergillus fumigatus by Alternaria alternata and Alternaria alternata by Trichoderma koningii was evident; inhibition of Trichoderma koningii by Scopulariopsis brevicaulis and Alternaria alternata was recorded, whilst antagonism between Trichoderma koningii and Aspergillus fumigatus was noted.

Table 7.2 (a), records the growth in monoculture produced by the test organisms grown on agar under conditions of fluctuating temperature.

Table 7.2 (b), shows the interactions between these organisms.

The data when subjected to analysis indicated that stimulation, retardation and synergism occurred :

Aspergillus fumigatus was stimulated by the presence of Chaetomium trilaterale and Epicoccum purpurascens. Chrysosporium pruinosum was stimulated by the presence of Chaetomium trilaterale and Verticillium intertextum. Trichoderma koningii was stimulated by the presence of Epicoccum purpurascens and Verticillium

intertextum. Synergism between Epicoccum purpurascens and Chrysosporium pruinosum was noted.

Table 7.3, shows the results of the perfusion experiments conducted at 35°C. The trends agree with the results outlined in Table 7.1(b). An additional record is the retardation of Alternaria alternata by the perfusants of Aspergillus fumigatus.

Table 7.4, records the results of perfusion experiments conducted at the temperatures of the fluctuating cycle. The trends agree only in part with the results in Table 7.2(b). There is broad agreement on the perfusant effects of :

Verticillium intertextum ⇄ Chrysosporium pruinosum,

Epicoccum purpurascens ⇄ Chrysosporium pruinosum,

Verticillium intertextum ⇄ Aspergillus fumigatus,

Epicoccum purpurascens ⇄ Aspergillus fumigatus, and

Chaetomium trilaterale ⇄ Chrysosporium pruinosum.

However, interaction studies with Trichoderma koningii on agar show a marked stimulation of its growth by the presence of Epicoccum purpurascens and Verticillium intertextum (Table 7.2 (b); on veneer, however, the converse seemed the case, its growth being retarded by the perfusants of these two fungi. An additional observation was the stimulation of Chaetomium trilaterale by the perfusants of Aspergillus fumigatus.

Table 7.5, shows the results of the close proximity interaction experiments which were conducted at 35°C for four days using timber veneer. These results are in broad agreement with those obtained from interaction experiments using agar and with those obtained from perfusion experiments using beech veneer (Tables 7.1(b) and 7.3).

Table 7.6, records the results of the close proximity interaction experiments conducted at the temperature cycle for ten days using beech veneer. There is no complete correlation with either set of results from previous experiments carried out under these conditions (Tables 7.2(b) and 7.4). The degree of correlation and otherwise is shown in Table 7.7.

TABLE 7.1

THE GROWTH OF FUNGI ON AGAR AS MONOCULTURES AND AS INTERACTING PAIRS AT 35°C

MONOCULTURES GROWN ON AGAR AT 35°C FOR 10 DAYS

(a)

Fungus	Colony radius in mm 3 replicates		
A. fumigatus	35.0	34.0	34.0
T. koningii	60.0	60.0	59.0
A. alternata	6.5	7.0	7.5
S. brevicaulis	10.0	9.5	9.5

INTERACTION EXPERIMENTS CONDUCTED ON AGAR AT 35°C FOR 10 DAYS

(b)

Fungus	Colony radius in mm 3 replicates			Interaction
A. fumigatus	34.0	35.0	35.0	-
v				
S. brevicaulis	10.0	10.0	10.0	-
A. fumigatus	38.0	38.0	37.0	> S.D.
v				
A. alternata	7.5	7.5	7.5	-
T. koningii	50.0	47.0	50.0	< S.D.
v				
S. brevicaulis	10.0	10.0	10.0	-
T. koningii	45.0	45.0	45.0	< S.D.
v				
A. alternata	9.0	9.0	10.0	> S.D.
T. koningii	40.0	36.0	36.0	< S.D.
v				
A. fumigatus	17.0	18.0	17.0	< S.D.
S. brevicaulis	9.5	9.0	10.5	-
v				
A. alternata	7.0	7.5	7.0	-

\- no interaction

\> stimulation

< inhibition/antagonism

S.D. significantly different from monoculture at 5% level

TABLE 7.2

THE GROWTH OF FUNGI ON AGAR AS MONOCULTURES AND AS INTERACTING PAIRS AT THE TEMPERATURE CYCLE

MONOCULTURES GROWN ON AGAR AT THE DAILY TEMPERATURE CYCLE FOR 10 DAYS

(a)

Fungus	Colony radius in mm 3 replicates		
A. fumigatus	13.5	14.0	13.0
C. pruinosum	7.5	7.5	7.5
T. koningii	30.0	31.5	32.5
V. intertextum	7.0	7.0	8.0
E. purpurascens	6.5	6.0	6.5
C. trilaterale	6.5	6.0	6.5

INTERACTION EXPERIMENTS CONDUCTED ON AGAR AT THE DAILY TEMPERATURE CYCLE FOR 10 DAYS

(b)

Fungus	Colony radius in mm 3 replicates			Interaction	
C. trilaterale	7.0	7.0	7.0	-	
v					
A. fumigatus	15.0	15.0	15.0	>	S.D.
C. trilaterale	10.0	8.0	7.5	-	
v					
T. koningii	32.0	34.0	34.0	-	
C. trilaterale	7.0	7.0	7.0	-	
v					
C. pruinosum	12.5	12.5	12.5	>	S.D.
V. intertextum	6.5	7.0	7.0	-	
v					
C. pruinosum	12.5	12.0	11.0	>	S.D.
E. purpurascens	7.0	8.0	8.0	>	S.D.
v					
C. pruinosum	12.0	11.5	11.5	>	S.D.
E. purpurascens	6.5	7.0	7.0	-	
v					
T. koningii	36.0	35.0	34.0	>	S.D.
V. intertextum	6.0	6.0	7.0	-	
v					
T. koningii	36.0	35.0	37.0	>	S.D.
V. intertextum	5.0	6.0	6.5	<	S.D.
v					
A. fumigatus	13.0	15.0	15.0	-	
E. purpurascens	6.5	6.5	6.0	-	
v					
A. fumigatus	15.0	16.0	15.0	>	S.D.

\- no interaction

\> stimulation/synergism

< inhibition

S.D. significantly different from monoculture at 5% level

TABLE 7.3

EFFECTS OF PERFUSANTS ON LINEAR GROWTH AT 35°C

Tube B		Tube A
A. fumigatus 70.0 mm +	→	A. alternata 3.0 mm
A. alternata 25.0mm	→	A. fumigatus 70.0 mm +
T. koningii 70.0 mm +	→	S. brevicaulis 7.0 mm
S. brevicaulis 5.0 mm	→	T. koningii 45.0 mm
T. koningii 70.0 mm +	→	A. alternata 70.0 mm +
A. alternata 30.0 mm	→	T. koningii 36.0 mm
T. koningii 70.0 mm +	→	A. fumigatus 34.0 mm
A. fumigatus 70.0 mm +	→	T. koningii 56.0 mm

TABLE 7.4

EFFECTS OF PERFUSANTS ON LINEAR GROWTH AT THE TEMPERATURE CYCLE

Tube B		Tube B
C. trilaterale 6.0mm	→	A. fumigatus 4.0mm
A. fumigatus 5.0mm	→	C. trilaterale 10.0mm
C. trilaterale 7.0mm	→	C. pruinosum 12.0mm
C. pruinosum 5.0mm	→	C. trilaterale 4.0mm
V. intertextum 5.0mm	→	C. pruinosum 22.0mm
C. pruinosum 5.0mm	→	V. intertextum 5.0mm
E. purpurascens 12.0mm	→	C. pruinosum 11.0mm
C. pruinosum 5.0mm	→	E. purpurascens 13.0mm
E. purpurascens 15.0mm	→	T. koningii 18.0mm
T. koningii 70.0mm +	→	E. purpurascens 13.0mm
V. intertextum 6.0mm	→	T. koningii 14.0mm
T. koningii 70.0mm +	→	V. intertextum 6.0mm
V. intertextum 7.0mm	→	A. fumigatus 1.0mm
A. fumigatus 4.0mm	→	V. intertextum 8.0mm
E. purpurascens 15.0mm	→	A. fumigatus 8.0mm
A. fumigatus 4.0mm	→	E. purpurascens 15.0mm

TABLE 7.5

INTERACTION ON VENEER AT 35°C FOR 4 DAYS

Fungi	Linear growth in mm	Linear growth in mm
	Monoculture	Interaction
A. fumigatus	70.0 +	70.0 +
A. alternata	27.0	5.0
T. koningii	70.0 +	15.0
S. brevicaulis	5.0	5.0
T. koningii	70.0 +	60.0
A. alternata	30.0	50.0
T. koningii	70.0 +	60.0
A. fumigatus	70.0 +	10.0

TABLE 7.6

INTERACTIONS ON VENEER AT THE TEMPERATURE CYCLE FOR 10 DAYS

Fungi	Linear growth in mm	Linear growth in mm
	Monoculture	Interaction
C. trilaterale	6.5	10.0
A. fumigatus	4.5	4.0
C. trilaterale	6.5	5.0
C. pruinosum	5.0	5.0
V. intertextum	5.0	37.0 *
C. pruinosum	5.0	13.0
E. purpurascens	14.0	6.0
C. pruinosum	5.0	70.0 +
E. purpurascens	14.0	2.0 *
T. koningii	70.0 +	20.0
V. intertextum	6.0	4.0 *
T. koningii	70.0 +	20.0
V. intertextum	6.0	2.0
A. fumigatus	4.5	10.0
E. purpurascens	14.0	3.0
A. fumigatus	4.5	7.5

* hyphae intermingling

TABLE 7.7

CORRELATIONS BETWEEN TABLES 7.2(b) and 7.4

Interacting Pairs	Stimulation/Synergism			Retardation/Antagonism		
	Agar	Perfusion	Proximity	Agar	Perfusion	Proximity
C. trilaterale ⇆ A. fumigatus	→	←	←			
C. trilaterale ⇆ C. pruinosum	→	→			←	
V. intertextum ⇆ C. pruinosum	→	→	⇇			
E. purpurascens ⇆ C. pruinosum	⇇	→	→			←
E. purpurascens ⇆ T. koningii	→				→	⇇
V. intertextum ⇆ T. koningii	→				→	→
V. intertextum ⇆ A. fumigatus			→	←	→	←
E. purpurascens ⇆ A. fumigatus	→	→	→			←

7.4 Discussion

There is evidence from the results of all three experimental treatments of interaction between the selected pairs of microfungi.

The interaction studies conducted on agar during this investigation exclude the effects of hyphal proximity since the opposed cultures did not grow close enough towards each other during the ten day period of the experiment. It is considered that the influence of metabolic products was transmitted by diffusion through the agar or in the moisture vapour within the petri-dishes. In the perfusion experiments the effects of hyphal proximity were excluded by the design of the apparatus and in this system the effects of the perfusant of each partner fungus upon the other was recorded independently by the 'one way' flow system in operation. Only in the close proximity inoculation experiments were fungal hyphae free to approach one another and as in the experiments on agar metabolic products were free to diffuse within the substrate.

The records of the experiments employing early colonizers of wood conducted at a constant temperature of 35°C show a high degree of correlation. They show near total agreement as to the nature of the interaction between pairs of fungi at all three treatments. These interactions included :

1. Stimulation or retardation of one partner with no effect upon the other.
2. Stimulation of one partner with retardation of the other.
3. Mutual retardation or antagonism of both partners.

(Tables 7.1(b), 7.3, 7.5 and 7.7)

Where selected thermophilous and mesophilic fungi were grown together in pairs under conditions of fluctuating temperature, there was some variation in the individual results obtained at each treatment. (Table 7.7).

Results obtained from interaction studies of fluctuating temperatures on agar show a marked stimulation of the growth of thermophilous fungi by the presence of mesophilic fungi. Six out of eight cases were recorded where thermophilous fungi were stimulated by the presence of mesophiles, the mesophiles being unaffected by the presence of their thermophilous partners. One case of synergism was recorded and one case of the retardation of a mesophilic fungus by the presence of its unaffected thermophilous partner (Table 7.2(b).

Results of perfusion experiments at fluctuating temperatures recorded four out of eight cases where stimulation of the growth of thermophilous fungi resulted from the effects of the perfusant of their mesophilic partners, in two of these cases the perfusants of the thermophilous fungi had no effect upon the mesophiles, in one case synergism resulted and in one case the growth of the mesophile was retarded. Three cases were recorded where the growth of the thermophilous fungus was retarded by the perfusant of the mesophilic partner which remained unaffected by the perfusants of the thermophilous fungus. One case was recorded where the perfusant of the thermophilous partner stimulated the growth of the mesophilic fungus which was unaffected by the perfusant of the thermophile. (Table 7.4).

The results of the close proximity inoculation experiments at fluctuating temperatures record four cases of the stimulation of the growth of thermophilous fungi by the presence of mesophilic partners. These cases included an example of synergism and three cases where the growth of the mesophilic partner was retarded. Two cases of antagonism were recorded, one case where the thermophilous partner while remaining unaffected stimulated the growth of the mesophile and one case where no interaction occurred with this treatment.(Table 7.6). The intermingling of hyphae was recorded in the cases of synergism and antagonism

only, indicating that the influence of hyphal contact upon interaction may be separate from metabolic effects.

As a result of this investigation evidence is submitted of interactions between mesophilic and thermophilous microfungi growing under conditions of fluctuating temperature similar to those occurring in insolated wood. The nature of individual reactions is shown to vary as a result of experimental treatment. There is evidence that metabolic products may be a factor influencing interactions between wood inhabiting fungi where the distribution of such products in wood, whether freely diffusing or transported in a water flow through wood (Chapter IV) may also be of significance. There is evidence that the surface growth of fungi on wood is influenced by hyphal responses.

The results of this investigation suggest that in wood which is subject to insolation thermophilous fungi will form a viable competitive portion of the fungal community.

CHAPTER VIII

THE INFLUENCE OF INSOLATION ON COLONIZATION SEQUENCES, AND THE EFFECTIVENESS OF SOME BIOCIDAL PREPARATIONS

Contents:

8.1 Introduction

Factors which influence the attack of wood by fungi include the presence of residual cell contents or other food material in parenchyma, the proportions in which cellulose or hemicellulose are present in the cell wall, the extent to which they are protected by lignin or by a wide range of minor non structural constituents. (Scheffer and Cowling, 1966). The growth and decay activity of wood inhabiting microbes and the interactions between them are also influenced by the temperature and moisture of wood (Bjorkman, 1946; Ammer, 1964; Henningson, 1967) and orientation to environment (Okigbo, Greaves and Levy, 1966).

The presence or absence of surface nutrients appears to be an important factor which can affect the colonization of the surface and eventually below surface layers of wood (Banerjee and Levy, 1971). Fungal activity of the surface layers may reach a climax within a few months of exposure and with the depletion of nutrients the activity is curtailed and surface layers are rendered uninhabitable for new fungi.

Timber joinery will become infected from either contact with soil or by air borne spores (Corbett and Levy, 1963b; Findlay, 1965; Okigbo, Greaves and Levy, 1966; Butcher, 1968; Toole, 1971; Banerjee and Levy, 1971) and thereby a succession will begin. Butcher (1972) suggested that during the initial phase of colonization, invading organisms had little effect on each other, later, however, during the early stages of spread and establishment some groups of fungi exerted an influence on the resident flora and once fungi became established they tended to develop independently and away from the influence of other organisms.

Corbett and Levy (1963b) have suggested that a correlation exists between the intensity of decay and moisture/air conditions of different zones along the height of posts in the ground. Considering their "hydrological zones" they found that infection was heaviest at the top and at ground level of the posts, least below ground and half way up the posts. Butcher (1968) records that timber above ground was too dry for the establishment of fungi other than moulds.

Corbett and Levy (1963b) have suggested the following general pattern of colonization at ground level :

Moniliales group I (Penicillium spp, Trichoderma viride, Botrytis sp.)
↓
Sphaeropsidales (Soft rot fungi)
↓
Moniliales group II (Gliocladiopsis sp. Cylindrocarpon sp. Memoniella sp.)
↓
Basidiomycetes (Coprinus sp. and unidentified species)

Banerjee and Levy (1971) proposed that the sequence of succession of organisms colonizing wood in soil content was generally, bacteria, moulds, staining and soft rot fungi and then basidiomycetes. This basic succession pattern is agreed by Käärik (1971) for timber above ground, but other workers record the succession as being incomplete above ground finding moulds only or mould, staining and soft rot fungi above ground (Corbett and Levy, 1963b; Merrill and French, 1966; Shigo, 1962; Käärik, 1967; Butcher, 1968; Toole, 1971; Banerjee and Levy, 1971).

The distribution and succession of fungi on debarked wood described by early workers were mostly based on results of periodic isolation of fungi from below the surface layers (Corbett and Levy, 1963b; Merrill and French, 1966; Käärick, 1967, 1971; Butcher, 1968, 1972b, c; Toole, 1971). Banerjee and Levy (1971) have paid particular attention to surface colonization. Considering their

patterns of isolations obtained from above ground regions they found that in birch stakes Penicillium spp. and Cladosporium herbarum were primary colonizers followed by Fusarium sp. , Botrytis sp. and Paecilomyces varioti which were among the dominant and sub-dominant species. Fungal colonization was never beyond the surface layers in the ten months of sampling. In pine stakes the colonization was again confined to the surface and did not follow any definite pattern. However, Cladosporium herbarum was the primary colonizer and Fusarium spp. a dominant species over six months. The colonization by further species of fungi and those which were earlier eliminated continued throughout (Aspergillus sp., Alternaria sp., Trichoderma viride, Phoma sp.) but the number of fresh species colonizing the posts declined with exposure time.

Despite the economically acceptable techniques which are available for the application of preservatives to new timber, (Wallace 1967) in-service external joinery is becoming increasingly susceptible to early decay (Purslow, 1965; Tack, 1968; Richardson, 1969; Abankwah, 1970; McQuire, 1971). Furthermore, the literature provides evidence of the ineffectiveness of certain wood preservative preparations against some basidiomycete and soft rot organisms (Price, 1957; Scholles, 1957; Savory, 1955; Duncan, 1960; Madhosingh, 1961; Corbett and Levy, 1963; Duncan and Deverall, 1963; Da Costa and Osborne, 1968; Levi, 1969). There is general agreement that soft rot organisms have a higher group tolerance for most preservatives than do basidiomycetes.

The mechanisms of toxicity of preservatives has received some attention. Bravery, (1970, 1971), suggests that the toxic effect may depend to some extent on the concentration of the preservative in wood and that the distribution of the preservative within the cell wall may have a marked effect upon the toxic limits of the fungal types. The mechanisms of these effects are still under investigation,

but preliminary observations seem to indicate that some preservatives generally act by slowing down the rate of colonization by the fungus. Greaves and Savory (1965) recorded that there are creosote utilizing fungi and similar mechanisms have been suggested for other preservatives (Madhosingh, 1961; Duncan and Deverall, 1964).

The present investigation was undertaken to study whether insolation influenced the early colonization of above ground regions of untreated pine stakes by microfungi. The effect of insolation on the early colonization of veneers via air spora was also studied. Timber prior to being put into service is invariably treated with a preservative preparation. Since in-service timber has been shown to contain cellulolytic microfungi and since there is evidence that soft rot fungi have a high tolerance to wood preservatives it was considered important to investigate the effectiveness of wood preservatives against thermophilous and mesophilic fungi at near ambient and above ambient temperatures. Three preservatives in common use were chosen for these experiments, being pentachlorophenol, amine-pentachlorophenate, and copper naphthenate.

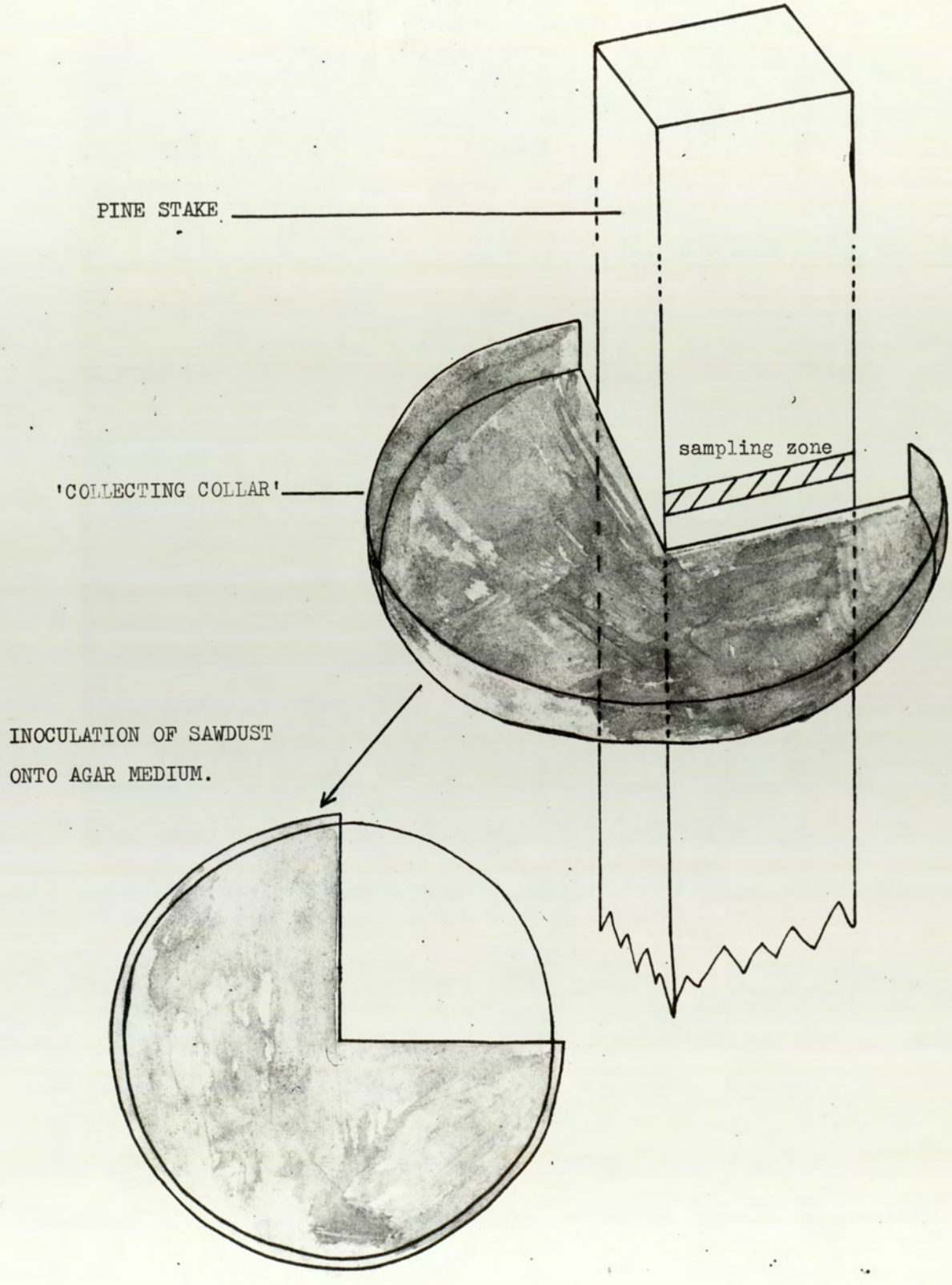
8.2 Experimental

8.2 (i) Materials and methods I : Pine Stakes

Four pine stakes measuring 24 x $1\frac{1}{2}$ x $1\frac{1}{2}$" were oven dried to around 20% moisture content. The surfaces of the stakes were cleaned by planing and then sampled for the presence of thermophilous microfungi. Two of the stakes were dyed black using Indian ink and two were left untreated. The stakes were placed in soil to a depth of six inches, the soil surface having been cleared of all surface vegetation. Two stakes, one black and one untreated, were situated in constant shade while two similar stakes were placed in a situation so as to receive all available sunshine. The location of the experiment was the garden of the author's home in Freckleton, Lancashire.

The experiment was set up at the beginning of June, 1973. The first samples were taken after two weeks exposure and then at monthly intervals for the next seven months. Samples from the surface of the stakes were taken from zones $\frac{1}{4}$" wide on opposite sides of the stake, the zones being six inches above ground. An 'Abrafile' was used to obtain dust from the sampling zones. The dust was collected in a glass 'collar' devised for this purpose, made from the lid of a glass petri-dish by removing a quarter section from it. The dust inoculum is transferred to agar when this 'collar' is placed over an exposed plate of ball-milled wood agar. (Figure 8.1). Samples from each stake were incubated at 35°C and 40°C for two weeks during which time subcultures of the isolates were transferred onto an Eggins and Pugh cellulose medium. At each time of sampling the surface temperature and moisture content of the stakes were determined.

FIG. 8.1 COLLECTING COLLAR IN POSITION ON A STAKE



After the eight month period in the field the stakes were removed from the ground and brought into the laboratory where a transverse section $\frac{1}{2}$" wide, which included the $\frac{1}{4}$" sample zones, was removed using a flamed saw. By employing the technique developed for sampling timber at depth (Chapter II), dust from four equidistant holes at 2.0, 5.0 and 10.0mm below the surface of the sampling zones was inoculated onto plates of cellulose medium and incubated at 35° and 40°C for two weeks.

8.2 (ii) Materials and methods II : Timber Veneers

Six pieces of beech veneer each measuring $1\frac{1}{2}$ x $1\frac{1}{2}$" were stacked one on top of the other. Five faces of the stack were sealed with 'Silastoseal' leaving one exposed veneer. Twelve such stacks were prepared, six were dyed black with Indian ink and six were left untreated. The stacks were placed inside a 'Sterilin' bag and autoclaved for 30 minutes. They were then taken into the field and suspended from a rope some 4 feet above the ground. The stacks were suspended in the same situations as the stakes in the previous experiment; three dyed and three untreated stacks were suspended in each situation. The experiment was conducted for three months, commencing in February, 1974. Two stacks of veneers, one dyed and one untreated were sacrificed from each situation after 3, 6 and 12 weeks. They were brought into the laboratory and treated as follows :

1. All sealed surfaces were wiped with alcohol and flamed.
2. The surface of the exposed veneer was sampled using an Abrafile, the dust was evenly distributed over the surfaces of two plates containing ball-milled wood agar.
3. The exposed veneer was flamed and then removed to enable the second veneer in the stack to be sampled. The procedure was repeated for each veneer in the stack.

4. Incubation of the plates was at 35° and 40°C, subcultures were prepared on the cellulose medium of Eggins and Pugh.

8.2 (iii) Materials and methods III : Biocides

Three biocides were used in this investigation :

1. Pentachlorophenol, powder
2. Formula 681, O/S copper naphthenate
3. Formula 682, O/S amine pentachlorophenate

The two latter preparations were supplied by Imperial Chemical Co, Preston Ltd., and appear in the Wood Preservatives and Fire Retardation Register (1973).

The biocides were added to molten malt agar at around 60°C, the preparations were then shaken continuously (to disperse the biocides) until the temperature was around 45°C when the plates were poured. In this way the pentachlorophenol was evenly distributed throughout the medium as it set and the emulsions of the oil soluble biocides gelled successfully.

The concentrations employed were :

1g. P.C.P./100cm^3 malt agar

10cm^3 O/S preservatives/500cm^3 malt agar

Mesophilic and thermophilous fungi were chosen to investigate the effectiveness of the biocide preparations. Disc inocula of the test organisms were inoculated centrally on the plates of the biocide preparations in malt agar and each isolate was incubated at 25° and 40°C. Control experiments with inocula on malt agar only, were also set up. Two replicates of each fungus were prepared. The diameters of the fungal colonies were measured at 2, 5 and 12 days.

The fungi employed were :-

Mesophiles	Thermophilous Fungi (micro-thermophiles)
Trichoderma viride	Chrysosporium pruinosum
Scopulariopsis brevicaulis	Trichoderma koningii
Aureobasidium pullulans	Aspergillus fumigatus
Phoma sp.	Penicillium sp.
Coprinus macrocephalus	Paecilomyces varioti
	Basidiomycete 157

8.2 (iv) Materials and methods IV : Cellulolytic activity and temperature tolerance ranges

The temperature tolerance range of each isolate was determined and the cellulolytic activity of each isolate on cellulose agar was investigated using the Rautella and Cowling (1964) method.

8.3 Results

Table 8.1, records the monthly sequence of fungi isolated at incubation temperatures of 35° and 40°C from the dyed and untreated stakes in shade and sunlight.

Table 8.2, shows the overall distribution of each isolate in terms of the situation and treatment of the stakes.

Table 8.3, shows the fungi isolated as a result of sampling beneath the surface of the stakes after their period in the field.

Table 8.4, shows the occurrence of fungi at the surface and beneath the surface of stacked veneers which had been exposed to contamination by air spora for 12 weeks.

Table 8.5, records the results of experiments conducted to investigate the effect of three biocides upon the growth of mesophilic and thermophilic fungi at 25° and 40°C.

Table 8.6, records the cellulolytic activity and temperature tolerance range of each isolate. There is some repetition of the information presented in this table with that included in Figures 2.3, 2.4 of Chapter II and Table 3.6 of Chapter III, as eight isolates were common to imported, in-service and colonized timber; these fungi are listed in Table 8.7.

A taxonomic list of all fungi isolated during this investigation is submitted in Appendix VIII.

Colonization of stakes

Primary colonizers of the untreated stakes situated in sunshine were Penicillium spp, Cephalosporium sp. and Fusarium solani. The dominant species were Aspergillus fumigatus and Fusarium solani. Sub-dominants appearing later in the succession were Alternaria alternata, Acremonium strictum and Trichoderma harzianum. Species colonizing but becoming eliminated were Chaetomium sp, Coprinus macrocephalus and Fusarium semitectum (Tables 8.1 a,b,; 8.2)

Primary colonizers of stakes treated with black dye and situated in sunshine were Penicillium sp, Cephalosporium sp, Scopulariopsis brevicaulis, Fusarium semitectum and Aspergillus fumigatus. The dominant species was Aspergillus fumigatus. Sub-dominants appearing later in the succession were Alternaria alternata, Acremonium strictum and Trichoderma harzianum. Species colonizing but becoming eliminated included Coprinus macrocephalus, Aureobasidium pullulans and Absidia corymbifera (Tables 8.1c, d; 8.2).

Primary colonizers of untreated stakes situated in shade were Paecilomyces varioti, Thermomyces stellatus and a Penicillium sp. The dominant species was Aspergillus fumigatus. Sub-dominants appearing later in the succession were Alternaria alternata and Trichoderma harzianum. Species colonizing but becoming eliminated included Penicillium funiculosum, Absidia corymbifera, Scopulariopsis brevicaulis, Fusarium solani, Aspergillus nidulans and Acremonium strictum. Fungi which re-appeared after early elimination were Chaetomium sp., Paecilomyces varioti and the Penicillium sp (Tables 8.1 e, f; 8.2)

Primary colonizers of stakes treated with black dye and situated in shade were Penicillium sp, Acremonium sp. Trichoderma viride, Scopulariopsis brevicaulis Fusarium solani and Aspergillus fumigatus. Dominant species were Fusarium solani, and Aspergillus fumigatus. Sub-dominants were Scopulariopsis brevicaulis,

Acremonium strictum and Alternaria alternata. Species colonizing but becoming eliminated included Penicillium cyclopium, Cochliobolus sativus, Ulocladium atrum, Aspergillus nidulans, Phoma sp., Coprinus macrocephalus, Chrysosporium pruinosum and a Basidiomycete (157). Trichoderma viride re-appeared after early elimination but failed to persist (Tables 8.1g, h; 8.2).

Active growth within stakes

When samples taken from within the stakes were incubated at 35° and 40°C, two fungi only were isolated, namely, Aspergillus fumigatus and Fusarium solani (Table 8.3).

Infection via air spora

Veneers dyed black and suspended in a sunny situation were infected by spores of Aspergillus fumigatus, Alternaria alternata and Aureobasidium pullulans. No active growth beneath the surface veneer was recorded. (Table 8.4).

Untreated veneers suspended in a sunny situation were infected by spores of Aspergillus fumigatus, Trichoderma harzianum, Cephalosporium sp, Fusarium solani, Aureobasidium pullulans, Chaetomium sp. and Scopulariopsis brevicaulis. Active growth to level 1 in the stack was recorded for Aspergillus fumigatus after 3 weeks exposure, for Scopulariopsis brevicaulis and Fusarium solani 6 weeks exposure and for Trichoderma harzianum after 12 weeks exposure (Table 8.4). Veneers dyed black and suspended in shade were infected by spores of Aspergillus fumigatus, Chrysosporium pruinosum, Penicillium sp., Trichoderma harzianum and Fusarium solani. Active growth beneath the surface veneer at level 1 was recorded for Aspergillus fumigatus and Chrysosporium pruinosum after 6 weeks exposure and for Trichoderma harzianum and Aspergillus fumigatus after 12 weeks exposure. Fusarium solani was the only fungus recorded at level 2 and this occurred after a 12 week exposure period.

Untreated veneers suspended in the shade were infected by Aspergillus fumigatus and Ulocladium atrum. Active growth at level 1 was recorded for Aspergillus fumigatus after 6 and 12 week exposure periods.

The veneers suspended in a sunny situation recorded penetration below the surface by Aspergillus fumigatus, Fusarium solani, Trichoderma harzianum and Scopulariopsis brevicaulis; whilst the veneers suspended in shade recorded penetration below the surface by Aspergillus fumigatus, Fusarium solani, Trichoderma harzianum and Chrysosporium pruinosum. All of these fungi with the possible exception of Scopulariopsis brevicaulis are considered to be microthermophiles and such observations demonstrate the ability of these fungi to be physiologically active at ambient and above ambient temperatures.

Biocides

On the Formula 681 preparation, the thermophilous fungi Chrysosporium pruinosum, Aspergillus fumigatus and Paecilomyces varioti grew better at the higher incubation temperature (40°C) than at the lower one (25°C). Trichoderma koningii, however, grew better at 25°C, the higher temperature of 40°C was possibly too high above its optimum which was established at 30°C on the cellulose medium.

Aspergillus fumigatus grew on the P.C.P. preparation at both 25° and 40°C. Aspergillus fumigatus and Chrysosporium pruinosum grew on the Formula 682 preparation at 40°C but not at 25°C (Table 8.5(2).

The mesophilic fungi Trichoderma viride, Aureobasidium pullulans and the Phoma sp. grew on the Formula 681 preparation at 25°C. At 40°C only the Phoma sp. was able to grow and at a greater rate than at 25°C (Table 8.5(2).

In all twenty-five fungi were isolated, nineteen of which were able to clear cellulose. Of these cellulolytic fungi fourteen could be considered as being microthermophiles, and five as mesophiles, no psychrophiles were encountered.

TABLE 8.1

FUNGI ISOLATED FROM WOOD STAKES SAMPLED AT MONTHLY INTERVALS

(a) Location: Sun Surface treatment: Plain Wood

Incubation Temperature: 35°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Cephalosporium sp.	+							+
F. solani	+	+	+	+	+			
A. fumigatus		+	+		+	+	+	+
F. semitectum		+						
Penicillium sp.		+	+	+				
Chaetomium sp.				+				
A. alternata				+	+	+	+	+
C. macrocephalus				+				
T. harzianum						+	+	+
% moisture	12/28	20/19	18/23	28/22	>30/14	>30/0	>30/6	22/13

Temperature °C

(b) Location: Sun Surface treatment: Plain Wood

Incubation temperature: 40°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Penicillium sp.	+	+	+	+				
A. fumigatus		+	+		+	+	+	
A. strictum				+			+	+
C. macrocephalus					+			
T. harzianum								+
% moisture	12/28	20/19	18/23	28/22	>30/14	>30/0	>30/6	22/13

Temperature °C

TABLE 8.1

FUNGI ISOLATED FROM WOOD STAKES SAMPLED AT MONTHLY INTERVALS

cont....

(c) Location: Sun Surface treatment: Black Dye

Incubation temperature: 35°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Cephalosporium sp.	+							
S. brevicaulis	+							
F. semitectum	+	+						
A. corymbifera		+						
Penicillium sp.		+						
A. alternata		+	+	+	+			
A. pullulans				+				
A. fumigatus				+	+			
T. harzianum						+	+	+
% moisture	12/35	19/20	16/25	26/27	>30/16	>30/0	>30/6	20/15

Temperature °C

(d) Location: Sun Surface treatment: Black Dye

Incubation temperature: 40°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Penicillium sp.	+							
A. fumigatus	+	+	+	+	+		+	+
A. strictum					+	+	+	+
C. macrocephalus					+			
F. solani								+
A. corymbifera								+
T. harzianum								+
% Moisture	12/35	19/20	16/25	26/27	>30/16	>30/0	>30/6	20/15

Temperature °C

TABLE 8.1

FUNGI ISOLATED FROM WOODEN STAKES SAMPLED AT MONTHLY INTERVALS

cont.....

(e) Location: Shade Surface treatment: Plain Wood

Incubation temperature: 35°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
P. varioti	+	+						
A. fumigatus		+	+	+	+		+	+
Chaetomium sp.		+				+		
A. kiliense			+			+		
F. solani			+	+	+			
S. brevicaulis			+					
A. alternata			+			+	+	+
A. nidulans				+				
T. harzianum					+	+	+	+
% moisture	14/22	21/18	18/19	> 30/20	>30/12	>30/0	>30/6	25/11

Temperature °C

(f) Location: Shade Surface treatment: Plain Wood

Incubation temperature: 40°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
T. stellatus	+							
Penicillium sp.	+			+				
P. varioti		+	+		+			
A. fumigatus		+	+		+	+	+	+
A. strictum						+	+	
P. funiculosum						+	+	
A. corymbifera						+		
T. harzianum								+
% moisture	14/22	21/18	18/19	>30/20	> 30/12	> 30/0	> 30/6	25/11

Temperature °C

TABLE 8.1

FUNGI ISOLATED FROM WOODEN STAKES SAMPLED AT MONTHLY INTERVALS

cont.....

(g) Location: Shade Surface treatment: Black Dye

Incubation temperature: 35°C

Fungi Isolated	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
A. fumigatus	+	+	+	+	+	+	+	+
T. viride		+		+				
A. kiliense		+	+					
S. brevicaulis		+	+	+	+			
F. solani		+	+	+	+		+	+
P. cyclopium			+					
C. sativus			+					
A. alternata				+	+	+	+	
U. atrum				+				
A. nidulans				+				
Basidiomycete 157					+			
% moisture / Temperature °C	14 / 23	19 / 19	18 / 19	> 30 / 20	> 30 / 12	> 30 / 0	> 30 / 6	26 / 11

TABLE 8.1

FUNGI ISOLATED FROM WOODEN STAKES SAMPLED AT MONTHLY INTERVALS

cont.....

(h) Location: Shade Surface treatment: Black Dye

Incubation temperature: 40°C

Fungi Isolated	Jun.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
A. fumigatus	+		+	+	+	+	+	+
Penicillium sp.	+	+	+					
A. strictum				+	+	+	+	
C. macrocephalus				+				
S. brevicaulis				+				
Phoma sp.				+				
A. alternata				+				
F. solani				+		+	+	
U. atrum				+				
C. pruinosum					+			
T. stellatus					+			
A. corymbifera						+	+	+
T. harzianum								+
% moisture / Temperature °C	14/23	19/19	18/19	>30/20	>30/12	>30/0	>30/6	26/11

TABLE 8.2

DISTRIBUTION OF THE ISOLATES

A	SUN		SHADE	
Primary Colonizers	Black	Plain	Black	Plain
Penicillium sp.	+	+	+	+
Cephalosporium sp.	+	+		
Fusarium spp.	+	+	+	
S. brevicaulis	+		+	
A. fumigatus			+	
P. varioti				+
T. stellatus				+
A. kiliense			+	
T. viride			+	
B Dominant Species				
A. fumigatus	+	+	+	+
F. solani		+	+	
Sub-Dominants				
A. alternata	+	+	+	+
A. strictum	+	+	+	
S. brevicaulis			+	
T. harzianum	+	+		+
C Further Species/ Eliminated Colonizers				
Chaetomium sp.		+		
C. macrocephalus	+	+		
A. corymbifera	+			+
Phoma sp.			+	

continued.....

TABLE 8.2

DISTRIBUTION OF THE ISOLATES continued.....

C Further Species/ Eliminated Colonizers	SUN		SHADE	
	Black	Plain	Black	Plain
A. pullulans	+			
S. brevicaulis				+
F. semitectum		+		
F. solani				+
A. nidulans			+	+
A. kiliense				+
A. strictum				+
P. cyclopium			+	
C. sativus			+	
U. atrum			+	
C. pruinosum			+	
P. funiculosum				+
Basidiomycete 157			+	
Penicillium sp.				+

TABLE 8.3

FUNGI OCCURRING AT DEPTH WITHIN STAKES

Colour/Situation	Depth		
	0..2mm	0.5mm	1.0cm
Black : Sun	-	F. solani	-
Black : Shade	-	A. fumigatus	-
Untreated : Sun	-	A. fumigatus	-
Untreated : Shade	A. fumigatus F. solani	A. fumigatus	A. fumigatus

TABLE 8.4

ISOLATION OF FUNGI FROM SURFACES AND AT DEPTH IN VENEER STACKS

Colour/Situation	SURFACE		LEVEL 1		LEVEL 2	
	35°	40°	35°	40°	35°	40°
Exposed: 3 weeks						
Black : Shade	-	-	-	-	-	-
Black : Sun	-	A. fumigatus	-	-	-	-
Untreated : Shade	-	A. fumigatus	-	-	-	-
Untreated : Sun	-	A. fumigatus T. harzianum		A. fumigatus		
Exposed: 6 weeks						
Black : Shade	A. fumigatus	-	A. fumigatus C. pruinosum	-	-	-
Black : Sun	A. fumigatus A. alternata A. pullulans	-	-	-	-	-
Untreated: Shade	A. fumigatus U. atrum	A. fumigatus	-	-	-	-
Untreated : Sun	Cephalosporium sp F. solani A. Pullulans Chaetomium sp	A. fumigatus	S. brevicaulis F. solani	-	-	-
Exposed: 12 weeks						
Black : Shade	A. fumigatus Penicillium sp.	T. harzianum	A. fumigatus	-	F. solani	-
Black : Sun	-	A. fumigatus	-	-	-	-
Untreated : Shade	A. fumigatus	-	A. fumigatus	-	-	-
Untreated : Sun	S. brevicaulis T. harzianum	T. harzianum	-	-	-	-

TABLE 8.5

GROWTH OF FUNGI ON BIOCIDE PREPARATIONS : 1, THERMOPHILOUS FUNGI

Incubation temperature: 25°C

Medium:	Basic Malt			Malt & P.C.P.			Malt + Formula 681			Malt + Formula 682		
Incubation/Days	2	5	12	2	5	12	2	5	12	2	5	12
C. pruinosum	90+	90+	90+	-	-	-	6	17	23	-	-	-
T. koningii	41	90+	90+	-	-	-	13	27	39	-	-	-
A. fumigatus	14	28	90+	-	4	5	6	7	12	-	-	-
Penicillium sp.	8	10	13	-	-	-	-	-	-	-	-	-
P. varioti	46	90+	90+	-	-	-	5	12	25	-	-	-
Basidiomycete 157	-	4	6	-	-	-	-	-	-	-	-	-

Incubation temperature: 40°C

Medium:	Basic Malt			Malt + P.C.P.			Malt + Formula 681			Malt + Formula 682		
Incubation/Days	2	5	12	2	5	12	2	5	12	2	5	12
C. pruinosum	90+	90+	90+	-	-	-	6	21	51	-	-	5
T. koningii	90+	90+	90+	-	-	-	8	16	16	-	-	-
A. fumigatus	36	90+	90+	-	5	6	6	10	23	-	5	6
Penicillium sp.	15	45	90+	-	-	-	6	6	11	-	-	-
P. varioti	90+	90+	90+	-	-	-	7	15	36	-	-	-
Basidiomycete 157	25	72	90+	-	-	-	-	-	-	-	-	-

TABLE 8.5 cont.....

GROWTH OF FUNGI ON BIOCIDE PREPARATIONS: 2, MESOPHILIC FUNGI

Incubation temperature: 25°C

Medium:	Basic Malt			Malt & P.C.P.			Malt + Formula 681			Malt + Formula 682		
Incubation/Days	2	5	12	2	5	12	2	5	12	2	5	12
T. viride	32	90+	90+	-	-	-	6	18	37	-	-	-
S. brevicaulis	4	9	17	-	-	-	-	-	-	-	-	-
A. pullulans	5	26	50	-	-	-	8	15	27	-	-	-
Phoma sp.	6	20	44	-	-	-	-	-	2	-	-	-
C. macrocephalus	4	7	60	-	-	-	-	-	-	-	-	-

Colony diameters in mm

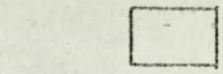
Incubation temperature: 40°C

Medium:	Basic Malt			Malt & P.C.P.			Malt + Formula 681			Malt + Formula 682		
Incubation/Days	2	5	12	2	5	12	2	5	12	2	5	12
T. viride	-	-	-	-	-	-	-	-	-	-	-	-
S. brevicaulis	-	-	5	-	-	-	-	-	-	-	-	-
A. pullulans	-	-	-	-	-	-	-	-	-	-	-	-
Phoma sp.	48	90+	90+	-	-	-	-	13	23	-	-	-
C. macrocephalus	8	22	90+	-	-	-	-	-	-	-	-	-

Colony diameters in mm

TABLE 8.6

CELLULOLYTIC ACTIVITY AND TEMPERATURE TOLERANCE RANGES



Fungi	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°
Absidia corymbifera													
Acremonium kiliense							3	5	9				
Acremonium strictum					2	2	4	6	13	5	1		
Altemaria altemata		3	5	5	5	5	6	5					
Aspergillus fumigatus					3	3	19	23	20	15	14	14	
Aspergillus nidulans				2	4	4	7	4	8	3			
Aureobasidium pullulans													
Cochliobolus sativus		Penetration and pigmentation											
Coprinus macrocephalus				2	6	4	4	4	3	1			
Cephalosporium sp.					2	2	8	8	9	2			
Chaetomium sp.		1	1	2	4	5	3	3					
Chrysosporium pruinosum			4	4	9	10	11	13	15	4			
Fusarium solani			5	6	7	10	13	8	6				
Fusarium semitectum						3	5	4					
Paecilomyces varioti					5	5	6	16	8	8			
Penicillium sp.						3	7	10	20	16	7		
Penicillium cyclopium													
Penicillium funiculosum					2	3	3	6	11	8			
Thermomyces stellatus							Penetration and Pigmentation						
Trichoderma harzianum													
Trichoderma viride			5	6	7	5	4	2	2				
Scopulariopsis brevicaulis				4	6	6	5	5					
Uclocladium atrum		3	3	3	4	4	7	6					
Phoma sp.			3	3	5								
Basidiomycete 157						3	9	13	7	7	3		

TABLE 8.6

CELLULOLYTIC ACTIVITY AND TEMPERATURE TOLERANCE RANGES

□ Clearing of Cellulose in mm

Temperature Tolerance Range °C

Fungi	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°
Absidia corymbifera													
Acremonium kiliense							3	5	3				
Acremonium strictum					2	2	4	6	13	5	1		
Altemaria altemata		3	5	5	5	5	6	5					
Aspergillus fumigatus					3	3	19	23	20	15	14	14	
Aspergillus nidulans				2	4	4	7	4	3	3			
Aureobasidium pullulans													
Cochliobolus sativus		Penetration and pigmentation											
Coprinus macrocephalus				2	6	4	4	4	3	1			
Cephalosporium sp.					2	2	8	8	9	2			
Chaetomium sp.		1	1	2	4	5	3	3					
Chrysosporium pruinosum			4	4	9	10	11	13	15	4			
Fusarium solani			5	6	7	10	13	8	6				
Fusarium semitectum						3	5	4					
Paecilomyces varioti					5	5	6	16	8	8			
Penicillium sp.						3	7	10	20	16	7		
Penicillium cyclopium													
Penicillium funiculosum					2	3	3	6	11	8			
Thermomyces stellatus							Penetration and Pigmentation						
Trichoderma harzianum													
Trichoderma viride			5	6	7	5	4	2	2				
Scopulariopsis brevicaulis				4	6	6	5	5					
Uclocladium atrum		3	3	3	4	4	7	6					
Phoma sp.			3	3	5								
Basidiomycete 157						3	9	13	7	7	3		

8.4 Discussion

The results indicate that it was the situation of the stakes rather than their colour which determined the nature of the colonizing flora.

Thermophilous fungi considered here as primary colonizers of the stakes included species common to both sunny and shaded situations, species isolated only from stakes in the shade and a species isolated only from stakes in the sunshine (Table 8.2 A). All dominant and sub-dominant thermophilous species were common to both situations (Table 8.2 B), whilst further colonizing thermophilous species which were frequently eliminated were isolated from stakes in the shade (Table 8.2 C). Such results show the widespread occurrence of thermophilous fungi on wood in sunny and shaded situations.

It is interesting to note that the Basidiomycetes were recorded as unsuccessful colonizers which were eliminated (Table 8.1b, d, g).

Of the fourteen fungi considered as primary colonizers, dominant and sub-dominant species, thirteen were shown to be cellulolytic and should be considered as cellulolytic micromycetes (Gorschin and Krapivina, 1969).

The flora of the stakes showed some correlation with the flora of the dock survey (Chapter II) and the survey of in-service timber (Chapter III). Table 8.7 is submitted to show the correlations and it can be seen that eight fungi, namely, Absidia corymbifera, Aspergillus fumigatus, Aspergillus nidulans, Paecilomyces varioti, Scopulariopsis brevicaulis, Trichoderma viride, Alternaria alternata, and Aureobasidium pullulans were common to all three investigations.

Active growth or penetration beneath the surface layers of the stakes was recorded for Aspergillus fumigatus and Fusarium solani only, whilst in the exposed beech veneers these two fungi were again encountered beneath the surface veneer

TABLE 8.7

COMMON FUNGI IN THE TIMBER SAMPLED

Fungi isolated	Colonized Stakes	Imported Timber	In-service Timber
Absidia corymbifera	+	+	+
Aspergillus fumigatus	+	+	+
Aspergillus nidulans	+	+	+
Aspergillus ochraceous	-	+	+
Cephalosporium acremonium	-	+	+
Paecilomyces varioti	+	+	+
Penicillium cyclopium	+	+	-
Scopulariopsis brevicaulis	+	+	+
Trichoderma viride	+	+	+
Trichoderma harzianum	+	+	-
Chrysosporium pruinosum	+	-	+
Fusarium solani	+	-	+
Penicillium funiculosum	+	-	+
Alternaria alternara	+	+	+
Aureobasidium pullulans	+	+	+
Ulocladium atrum	+	+	-
Coprinus macrocephalus	+	-	+

together with Scopulariopsis brevicaulis, Trichoderma harzianum, Chrysosporium pruinosum and a species of Penicillium.

The results indicate that some thermophilous fungi have a higher tolerance to copper naphthenate and amine pentachlorophenate, particularly the former compound, at 40°C than at 25°C; whilst in some mesophilic fungi the increase in temperature reduced their tolerance to copper naphthenate. Four thermophilous fungi (Chrysosporium pruinosum, Aspergillus fumigatus, Penicillium sp. and Paecilomyces varioti) had a higher tolerance to copper naphthenate at 40°C than at 25°C; two thermophilous fungi (Chrysosporium pruinosum and Aspergillus fumigatus) recorded a higher tolerance to amine pentachlorophenate at 40°C than at 25°C. Two mesophilic fungi (Trichoderma viride and Aureobasidium pullulans) showed a reduction in their tolerance to copper naphthenate at 40°C compared to that at 25°C. Only Aspergillis fumigatus was able to grow on the pentachlorophenol preparation, growth was recorded at 25° and 40°C but was, in each case, very sparse. The thermophilous and mesophilic Basidiomycetes recorded no tolerance to the preservative preparations whatsoever.

It would seem then that in preservative treated timber which is subject to insolation, where above ambient temperatures around 40°C occur (Chapters IV and VI), thermophilous microfungi may have a distinct advantage over mesophilic microfungi and Basidiomycetes in that they may be more tolerant to certain preservative preparations.

CHAPTER IX

GENERAL DISCUSSION

The situation in Preston, Lancashire, where timber is imported directly into the docks and is then removed for use by local contractors, presented an opportunity to investigate the nature of the flora of newly imported and in-service timber in the Preston area and to isolate and prepare cultures of wood inhabiting microfungi.

External timber joinery is subject to fluctuations of temperature and to changes in moisture content. The position of wood whether in sun or shade together with any surface treatment received in the form of paint, stain or even preservative coatings will influence the response of wood to changes in the environment. Despite these variations in environmental conditions, most investigations which have been conducted so far into the degradation of timber by microfungi have been in the main at mesophilic temperatures and to a lesser extent at thermophilic temperatures only, and have tended not to include the effects of temperature fluctuations or the varying availability of water upon the growth of wood inhabiting microfungi. In particular, information on the effect of insolation upon temperature and moisture levels in wood is sparse and it was felt strongly that the response of mesophilic and thermophilous fungi to such conditions should receive attention. Therefore, an attempt has been made to answer these and other fundamental questions which apply to the colonization of wood by microfungi.

Into which taxonomic, temperature and degradative categories do the microfungi of imported and in-service timber fall, and how do the respective flora compare with one another? What are the effects of insolation on the temperature and moisture levels in wood when surface treatment varies? What effects do diurnal temperature fluctuations, similar to those which occur in insolated wood, have upon the growth of wood inhabiting fungi and what levels of moisture content are

necessary in wood to support surface growth and penetration of microfungi into wood at above ambient temperatures? When interactions occur between wood colonizing fungi how are they affected by temperature? Is insolated wood a selective environment for thermophilous fungi and does surface treatment influence colonization patterns? How effective are the biocidal preparations currently in use against wood inhabiting microfungi at above ambient temperatures?

As a result of this investigation a list of cellulolytic microfungi isolated from timber is available (Appendix IX), furthermore twelve of these species are known to cause soft rot in wood (Rosch and Leise, 1968; Nilsson, 1973), (Appendix X). The numbers of thermophilous or more specifically microthermophiles (Apinis and Pugh, 1967) isolated during this investigation (Appendix XI) indicate that wood has a flora which may well contribute to the development of soft rot, especially if optimal conditions are made available by insolation. The sampling of imported and in-service timbers produced ten common species. This does not represent a true comparison because the incubation temperatures employed during the sampling of in-service timbers were confined to 30°C, 35°C and 40°C for the isolation of thermophilous fungi rather than at temperatures ranging from 5° to 45°C as was the case for in-service timbers, however, correlation is established between imported and in-service timber and points possibly to infected wood from the dock being used in construction work locally, the contamination of in-service timber by the air spora of the fungi of imported timber, or possibly the contamination of both substrates by the air spora of indigenous species. Since much of the timber used by local contractors is obtained directly from the importers on the dock there would seem to be a case for considering that infected timber may be employed in local timber joinery. Whilst it is appreciated that during this investigation the imported timbers were sampled before planing and

prior to the application of preservative, which is usual before timber joinery is put into service, the isolation of thermophilous cellulolytic fungi from below the surface of the imported timbers strengthens the case for suspecting that some infected timber is put into service.

Data obtained during this investigation show that insolation can raise the temperature of wood above ambient, whilst experimentally altered surface colour modifies the extent to which solar energy is absorbed. Temperatures up to 16°C above ambient are recorded at the surface and within wood; these results make a quantitive addition to the observations of previous workers. Data is now also available as a result of this investigation on the diurnal temperature ranges in wood undergoing insolation. They show that whereas wood heats up fairly quickly it cools down much more slowly. It is also recorded for the first time that colour has no significant effect upon the moisture content of insolated wood in soil contact, which indicates that there may be a movement of water from soil through wood to the air.

Subjecting microfungi to alternating temperatures influences their growth. Results obtained during this investigation confirm the additive and stimulative effects reported by Jensen (1968), but retardation of growth is also recorded. Microfungi growing on insolated wood will be exposed to diurnal temperature fluctuations, data obtained on the growth response of thermophilous fungi to such fluctuations demonstrates for the first time their adaptability and reflect not only on their competence as wood colonizers but indicate that they can be better adapted to such fluctuating conditions than mesophilic fungi. It is quite conceivable then that thermophilous fungi may have the ability to create niches on insolated timber where they may be sufficiently competitive to withstand elimination by blanket fungi. Such findings point to the need for more work to

be undertaken on the response of timber rotting fungi to fluctuating temperatures rather than at constant mesophilic or thermophilic temperatures.

Results obtained from experiments conducted at the fairly high temperatures of 35° and 40°C record microfungi growing on and within wood at moisture levels below the 20% minimum moisture content quoted in the literature. This points to an increase in the biological activity of water at higher than ambient temperatures (Ayerst, 1965) and may in part, explain the isolation of microfungi from very dry samples during this investigation and the reported occurrence of soft rot fungi in extremely dry situations. It seems curious then that workers in the field of timber decay have as yet, failed to appreciate that the biological activity of water may be increased at above ambient temperatures, a factor which may be highly significant in the role of thermophilous fungi as agents of decay.

A consideration of the results of the interaction experiments between pairs of microfungi show the need for some standardization of terminology. It is suggested that mutual stimulation between interactions should be termed synergism, whilst mutual retardation should be termed antagonism. There is evidence from the experiments conducted on agar, perfused veneers and unperfused veneers that metabolic products are, in part, responsible for interaction both at constant and under conditions of fluctuating temperature similar to those encountered in insolated wood. It would seem that the physical forces operating within the substrate, which govern the distribution of metabolites, may also influence interaction. Interactions between thermophilous and mesophilic fungi show that under conditions of fluctuating temperature each may influence the growth of the other. In terms of retardation there was near equality in the number of recorded cases where mesophiles retarded thermophiles and where thermophiles retarded the growth of mesophiles. There were, however, considerably more

cases which recorded the stimulation of thermophiles by the presence or perfusants of mesophiles than the stimulation of mesophiles by thermophilous species. Since under natural conditions the interactions between microfungi contribute to the early colonization of wood and determine to some extent the succession of fungi on wood, it is considered that thermophilous fungi may play a significant role in ecological sequences.

The results of experiments into the early colonization of above-ground regions of untreated pine stakes suggest that primary colonizers, dominant and sub-dominant species are common to both sunny and shaded situations. These observations agree with the findings of Eggins et al (1972) for soil organisms and suggest that thermophilous fungi are more widespread in wood than has been previously acknowledged. The isolation of thermophilous further colonizing species from wood in the shade offers further evidence of their widespread distribution. Ten fungi isolated from the above-ground regions of untreated stakes were common with those of the dock survey, thirteen with those of in-service timber, whilst eight fungi were common to all three investigations.

The results of preliminary experiments conducted into the tolerance of cellulolytic thermophilous wood inhabiting microfungi to wood preservatives indicate that they are more tolerant to amine-pentachlorophenate and copper naphthenate at 40°C than at 25°C. Such results point to the possible occurrence of cellulolytic thermophiles in preserved timber which is subject to insolation and stress the need for testing the effectiveness of preservative preparations at above ambient temperatures.

Timber, like all other raw materials is increasing in price, however, unlike metallic and plastic structures which are being offered as substitutes for timber joinery, it remains a material with an unmatched versatility in that it has the

ability to expand and contract as an integral part of a building. There has always been, and there still is growing opinion that if sound timber is handled correctly at the construction stage, then its life span in service will be very satisfactory.

As a result of this research programme it has been shown that insolation has a marked effect upon the diurnal temperature ranges and the moisture content of wood. Thermophilous fungi have been shown to be far more widespread in wood than has been previously acknowledged and this together with their ability to grow well under diurnal fluctuating conditions of temperatures suggests that they should receive more attention as organisms contributing to the decay of wood. The possible increase in the biological activity of water at above ambient temperatures is a field which needs further exploration, especially in relation to thermophilous fungi with their ability to remain physiologically active at above and below ambient temperatures. It is felt that interaction studies under conditions of fluctuating temperature should be extended to include low moisture conditions since the performance of fungi under these conditions may play a significant part in the colonization sequences which lead to the establishment of fungi in wood. Finally it is suggested that preservative preparations should be tested under conditions of fluctuating temperatures similar to those encountered in insolated wood.

APPENDICES :

APPENDIX 1

MEDIA

1. Eggins and Pugh modified cellulose medium:

	Constituents in grams per litre
$KH_2\ PO_4$	1.0
KCl	0.5
$Mg\ SO_4\ 7H_2O$	0.2
$Ca\ Cl_2$	0.1
Agar	20.0
Cellulose	10.0
$(NH_4)_2SO_4$	0.543
Thiamine hydrochloride	0.001

The cellulose preparation is a 4% suspension of Whatman cellulose in distilled water, the standard grade cellulose powder having been ball-milled for 72 hours.

Ingredients are steamed for 15 minutes without the cellulose which is then mixed in before autoclaving at 10 p.s.i. for not more than 20 minutes.

2. Eggins ball-milled timber medium:

(a) Sawdust is ball-milled dry for 120 hrs., passed through a 90 mesh sieve and ball milled for a further 24 hrs. as a 4% suspension in distilled water.

(b) The mineral salt constituents are the same as those used in the cellulose medium, as is the method of preparation.

APPENDIX II

FUNGI ISOLATED FROM IMPORTED TIMBERS

Phycomycetes

Mucorales

Absidia corymbifera (Cohn) Sacc. & Trott.
Syncephalastrum racemosum (Cohn) Schroet.
Mortierella sp.

Ascomycetes

Chaetomiales

Chaetomiaceae

Chaetomium bostrychodes Zopf.
Chaetomium indicum Corda
Chaetomium trilaterale Chivers

Fungi Imperfecti (Deuteromycetes)

Moniliales

Moniliaceae

Amorphotheca resinae Parbery
Aspergillus fumigatus Fres.
Aspergillus nidulans (Eidam) Wint.
Aspergillus ochraceous Wilhelm
Botrytis cinerea Pers. ex Pers.
Cephalosporium acremonium Corda
Cephalosporium spp.
Cladosporium cladosporioides(Fresen.) De Vries
Cladosporium herbarum (Pers.) Link ex Gray
Geotrichum candidum Link ex Pers.
Paecilomyces varioti Bainer
Penicillium brevicompactum Dierckx
Penicillium citrinum Thom.
Penicillium crustosum Thom.
Penicillium cyclopium Westling
Penicillium decumbens Thom.
Phialophora bubakii (Laxa) Schol-Schwarz
Phialophora fastigista (Lagerb. & Melin) Conant.
Scopulariopsis brevicaulis (Sacc.) Bain.
Trichoderma viride Pers. ex Gray
Trichoderma harzianum Rifai agg.
Verticillium intertextum Isaac & Davies
Verticillium latertitium (Fr.) Rabenh.

Dematiaceae

Alternaria alternata (Fr.) Keissler
Aureobasidium pullulans (de Bary) Arnaud
Ulocladium atrum Preuss

Stilbacaea

Graphium album (Corda) Sacc.

Tuberculariaceae

Epicoccum purpurascens Ehrenb. ex Schlecht.

Sphaeropsidales

Sphaeropsidaceae

Phoma glomerata (Corda) Wr. & Hochapf.
Phoma herbarum Westend

APPENDIX III

FUNGI ISOLATED FROM IN-SERVICE TIMBERS

Phycomycetes
- Mucorales
  - Absidia corymbifera (Cohn) Sacc. & Trott.
  - Mucor racemosus Fres.

Ascomycetes
- Hypocreales
- Nectriaceae
  - Nectria inventa Pethybr

Fungi imperfecti (Deuteromycetes)
- Moniliales
- Moniliaceae
  - Acremonium kiliense Gütz
  - Acremonium sp.
  - Aspergillus fumigatus Fres.
  - Aspergillus nidulans grp.
  - Aspergillus ochraceous Wilhelm
  - Cephalosporium acremonium Corda
  - Cephalosporium sp.
  - Chrysosporium pruinosum (Gilman & Abbott) Carmichael
  - Fusarium culmorum (W.G.Sm.) Sacc.
  - Fusarium solani (Mart.) Sacc.
  - Paecilomyces varioti Bainer
  - Penicillium coryophilum Diesckx
  - Penicillium funiculosum Thom.
  - Scopulariopsis brevicaulis (Sacc.) Bain
  - Trichoderma viride Pers. ex Gray
  - Trichoderma koningii Oud. aggr.
  - Verticillium psalliotae Treschow
- Dematiaceae
  - Alternaria alternata (Fr.) Keissler
  - Aureobasidium pullulans (de Bary) Arnaud
  - Rhinocladiella mansonii (Castell) Scholl-Schwartz
- Sphaeropsidales
- Sphaeropsidaceae
  - Phoma sp.

Basidiomycetes
- Agaricales
- Agaricaceae
  - Coprinus macrocephalus (Berk.) Berk

APPENDIX IV

TEMPERATURE READINGS FOR THE MONTH OF MAY, 1972
(42 readings 2/day on 21 occasions)

		TEMPERATURE IN °C			
COLOUR	TIME	AIR	SURFACE	1mm BELOW SURFACE	CENTRE
Black	MD	Mean: 16.37	22.61	23.04	23.02
		Max. Min. 26.25 7.50	41.75 11.5	42.5 11.0	39.5 12.5
	PM	Mean: 16.06	17.55	17.67	18.74
		Max. Min. 24.00 9.75	29.75 10.75	32.00 9.30	27.75 11.5
White	MD	Mean: 16.37	19.70	19.30	19.83
		Max. Min. 26.25 7.50	37.50 10.0	31.75 8.75	33.00 9.25
	PM	Mean: 16.06	16.80	17.39	18.21
		Max. Min. 24.00 9.75	22.25 10.25	24.50 7.50	25.00 9.00
Varnish	MD	Mean: 16.37	19.15	19.17	19.49
		Max. Min. 26.25 7.50	33.75 8.00	32.50 8.75	35.00 9.00
	PM	Mean: 16.06	17.00	17.70	18.29
		Max. Min. 24.00 9.75	30.75 7.50	30.25 9.00	28.75 11.00

APPENDIX IV continued.....

TEMPERATURE READINGS FOR THE MONTH OF JUNE, 1972
(38 readings 2/day on 19 occasions)

		TEMPERATURE IN °C			
COLOUR	TIME	AIR	SURFACE	1mm BELOW SURFACE	CENTRE
Black	MD	Mean: 16.80	22.01	23.62	24.33
		Max. Min. 26.00 10.00	35.75 10.50	33.00 10.75	32.75 11.50
	PM	Mean: 17.18	19.13	19.53	20.95
		Max. Min. 22.00 8.75	26.00 10.00	25.50 10.50	20.75 12.25
White	MD	Mean: 16.80	20.38	20.87	21.49
		Max. Min. 26.00 10.00	28.25 10.50	26.25 10.15	28.00 12.00
	PM	Mean: 17.18	19.30	19.57	20.41
		Max. Min. 22.00 8.75	25.25 10.75	25.75 11.00	26.00 11.75
Varnish	MD	Mean: 16.8	20.91	21.47	22.32
		Max. Min. 26.0 10.0	30.75 10.50	28.00 11.50	29.75 12.25
	PM	Mean: 17.18	19.75	20.07	21.30
		Max. Min. 22.00 8.75	26.25 9.25	26.76 11.00	27.25 11.25

APPENDIX IV continued.....

TEMPERATURE READINGS FOR THE MONTH OF JULY, 1972
(26 readings 2/day on 13 occasions)

		TEMPERATURE IN °C			
COLOUR	TIME	AIR	SURFACE	1mm BELOW SURFACE	CENTRE
Black	MD	Mean: 21.17	29.58	31.12	31.06
		Max. Min. 28.25 12.00	45.50 13.50	46.50 13.50	46.00 13.00
	PM	Mean: 21.54	25.15	26.13	27.05
		Max. Min. 27.25 14.00	32.75 15.50	33.00 15.25	34.25 15.25
White	MD	Mean: 21.17	26.02	26.46	27.35
		Max. Min. 28.25 12.00	40.00 12.75	38.00 11.00	39.25 12.25
	PM	Mean: 21.54	24.91	25.04	25.85
		Max. Min. 27.25 14.00	33.80 12.50	31.75 12.75	32.50 12.75
Varnish	MD	Mean: 21.17	27.32	28.27	28.81
		Max. Min. 28.25 12.00	39.60 13.25	41.25 13.00	42.75 13.50
	PM	Mean: 21.54	25.68	26.19	27.29
		Max. Min. 27.25 14.00	38.00 13.25	35.00 12.75	36.75 12.75

APPENDIX IV continued.....

TEMPERATURE READINGS FOR THE MONTH OF AUGUST, 1972
(36 readings 2/day on 18 occasions)

		TEMPERATURE IN °C			
COLOUR	TIME	AIR	SURFACE	1mm BELOW SURFACE	CENTRE
Black	MD	Mean: 20.29	26.42	26.44	27.60
		Max. Min. 27.00 14.00	43.00 13.5	41.00 13.25	39.00 13.00
	PM	Mean: 20.05	22.53	23.18	24.47
		Max. Min. 27.00 14.75	33.5 15.5	33.00 16.50	33.00 17.25
White	MD	Mean: 20.29	22.53	22.29	22.67
		Max. Min. 27.00 14.00	34.00 13.50	32.00 12.75	33.00 12.50
	PM	Mean: 20.05	19.96	21.33	22.55
		Max. Min. 27.00 14.75	28.00 13.50	27.00 15.00	29.00 15.50
Varnish	MD	Mean: 20.29	24.18	24.65	24.90
		Max. Min. 27.00 14.00	35.00 14.25	33.00 14.25	33.00 14.25
	PM	Mean: 20.05	22.27	22.7	23.68
		Max. Min. 27.00 14.75	28.50 14.50	31.00 14.25	32.00 14.50

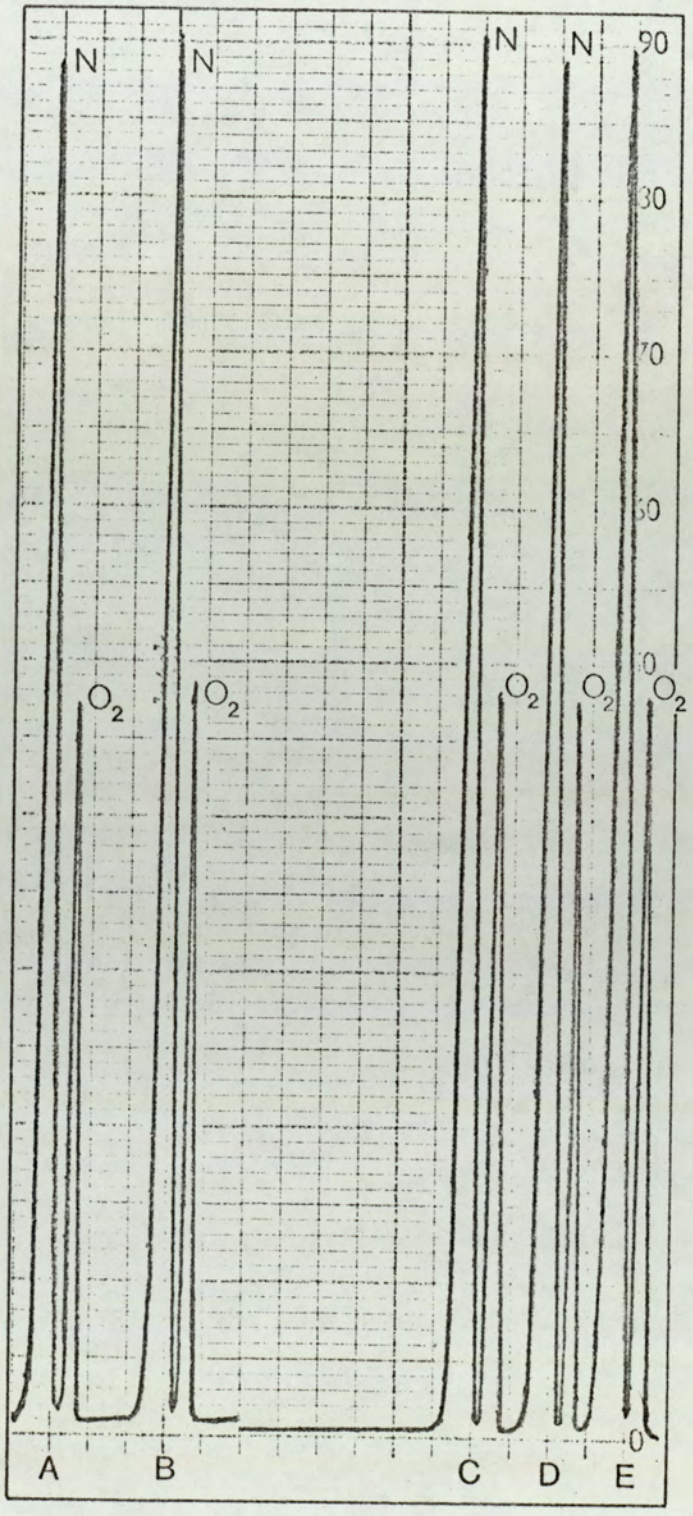
APPENDIX V

COMPUTER PROGRAM FOR UNPAIRED T-TESTS

```
                                        FORTRAN  COMPILATION BY XFAE  DATE  10/10/74
      MASTER MC03RE TTEST
      DIMENSION X(100,3),Y(100,3)
       REAL M1,N1
      INTEGER L1,F1,F2
26     N = 0
      M=0
      N1=0
      M1=0
      READ(1,2)REF1,REF2,REF3,REF4
2     FORMAT(4A8)
3     N=N+1
      READ(1,4) X(N,1),X(N,2),X(N,3)
      IF(X(N,1).NE.99..AND.X(N,2).NE.99..AND.X(N,3).NE.99.) GO TO 3
5     M=M+1
      READ(1,4) Y(M,1),Y(M,2),Y(M,3)
      IF(Y(M,1).NE.99..AND.Y(M,2).NE.99..AND.Y(M,3).NE.99.) GO TO 5
4     FORMAT(3F0.0)
      L1=0
      M=M-1
      N=N-1
      READ(1,6)K,L
      IF(K.EQ.0)GO TO 14
      L1=K
6     FORMAT(2I1)
14    S1=0
      S2=0
      T1=0
      T2=0
      DO 7 I=1,N
      IF(L1.NE.0) GO TO 8
      DO 9 K=1,3
8     S1=S1+X(I,K)
      S2=S2+X(I,K)*X(I,K)
      N1=N1+1
      IF(L1.NE.0)GO TO 7
9     CONTINUE
7     CONTINUE
      DO 11 I=1,M
      IF(L1.NE.0)GO TO 12
      DO 13 L=1,3
12    T1=T1+Y(I,L)
      T2=T2+Y(I,L)*Y(I,L)
      M1=M1+1
      IF(L1.NE.0)GO TO 11
13    CONTINUE
11    CONTINUE
      S3=S1/N1
      T3=T1/M1
      S4 =1./(N1-1.)*(S2-S1*S1/N1)
      T4 = 1./(M1-1.)*(T2-T1*T1/M1)
      IF(T4.GT.S4)GO TO 24
      F=S4/T4
      GO TO 16
24    F=T4/S4
16    S5=((N1-1)*S4+(M1-1)*T4)/(N1+M1-2)
      S6=SQRT(S5/N1+S5/M1)
      T=(S1-T1)/S6
      F1=N1-1
      F2=M1-1
      WRITE(2,17)
17    FORMAT(1H1 //40X,39HT TEST WITH TWO GROUPS OF UNPAIRED DATA/)
      WRITE(2,18)REF1,REF2,REF3,REF4
18    FORMAT(10X,13HREFERENCE  - ,4A8/20X,10HX GROUP   /)
      DO 19 I=1,N
19    WRITE(2,20)X(I,1),X(I,2),X(I,3)
20    FORMAT(/10X,F20.9,10X,F20.9,10X,F20.9)
      WRITE(2,21)
21    FORMAT(//10X,10HY GROUP   /)
      DO 22 I=1,M
22    WRITE(2,20)Y(I,1),Y(I,2),Y(I,3)
      WRITE(2,23)F1,S3,F2,T3,F,T,S6
      WRITE(2,30)S1,S2,S3,S4,N1,T1,T2,T3,T4,M1
30     FORMAT(10F10.3/)
23    FORMAT(//22HDEGREE OF FREEDOM X = ,I3,2X,9HMEAN X = ,1P1E13.5,25H
     1 DEGREES OF FREEDOM Y = ,I3,2X,9HMEAN Y = ,2X,1P1E13.5 /14H VALUE
     2OF F = ,F10.3,10X,14H VALUE OF T = ,F10.3,10X,17HSTANDARD ERROR =
     3,1P1E13.5)
      READ(1,25)IZ
25     FORMAT(I2)
      IF(IZ.EQ.0)GO TO 26
      STOP
      END

END OF SEGMENT, LENGTH   657, NAME  MC03RETTEST

     FINISH

END OF COMPILATION -  NO ERRORS

S/C SUBFILE:     16 BUCKETS USED
```

APPENDIX VI

GAS CHROMATOGRAPHS SHOWING A COMPARISON OF THE OXYGEN CONTENT OF LABORATORY AIR WITH THAT OF AIR SAMPLES FROM WITHIN THE MOISTURE GRADIENT APPARATUS



Specifications:

$1cm^3$ air sample

$8cm^3$ He/min (Carrier Gas)

Molecular sieve 5A, baked for 12 hrs. at 200°C

A. and B: Samples of laboratory air

C: Air sample from within the apparatus taken from the region above the veneers

D: Air sample taken from the centre of the veneer stack

E: Air sample taken from below the veneer stack above the water level

APPENDIX VII

PROGRAM : MCO4RE M. E. BROWN 1.1.1.

This program uses a t-test to test if there is a significant difference between samples.

A is the control group and B is the test group.

The three values A are read in and the standard deviation calculated in the following way :-

SIG A = the sum of the three values of A

SIG A2 = the sum of the squares of values A

S1 (standard deviation of A) uses the formula :

$$S1 = \sqrt{\frac{SIG\ A2 - \frac{(SIG\ A)^2}{3}}{3 - 1}}$$

The first test group is then read in and the standard deviation, S2, is calculated in the same way as S1, in this case the sum and sum of squares are called SIG B and SIG B2 respectively.

An F-test is then done to see if it is valid to do a t-test. The larger of the standard deviations S1 and S2 is divided by the smaller i.e. F equals either S1/S2 or S2/S1. For 2 x 2 degrees of freedom F must be less than 19.0 for a t-test to be valid. If F is greater than 19.0 TEST NOT VALID is printed.

The combined standard deviation, SIG is calculated by :-

$$SIG = \sqrt{\frac{(S1)^2 + (S2)^2}{2}}$$

$$t = \frac{\frac{SIG\ A}{3.0} - \frac{SIG\ B}{3.0}}{SIG} \times 1.2247$$

. continued.....

If the value of t is greater than 2.776 there is a significant difference between the control and the test groups, if the value of t is less than or equal to 2.776 there is no significant difference.

The program then loops back either to read in more test data or to read control data and test data as appropriate.

This program is on file in the Department of Computer Studies at the Polytechnic, Preston.

APPENDIX VIII

FUNGI ISOLATED DURING THE ABOVE-GROUND COLONIZATION SEQUENCE OF UNTREATED PINE STAKES

Phycomycetes
- Mucorales
  - Absidia corymbifera (Cohn) Sacc. & Trott.

Ascomycetes
- Chaetomiales
- Chaetomiaceae
  - Chaetomium sp.

Deuteromycetes
- Moniliales
- Moniliaceae
  - Aspergillus fumigatus Fresenius
  - Aspergillus nidulans (Eidam) Wint.
  - Acremonium strictum W. Gams.
  - Acremonium kiliense Gütz
  - Acremonium sp.
  - Cephalosporium sp.
  - Chrysosporium pruinosum (Gilman & Abbott) Carmichael
  - Fusarium semitectum Berk & Ravenel
  - Fusarium solani (Mart.) Sacc.
  - Paecilomyces varioti Bainer
  - Penicillium sp.
  - Penicillium funiculosum Thom.
  - Penicillium cyclopium Westling
  - Scopulariopsis brevicaulis (Sacc.) Bain
  - Trichoderma harzianum Rifai agg.
  - Trichoderma viride Pers. ex Gray

- Dematiaceae
  - Alternaria alternata (Fr.) Keissler
  - Aureobasidium pullulans (de Bary) Arnaud
  - Cochliobolus sativus (Ito & Kuribayashi) Deschler ex Dastur
  - Thermomyces stellatus (Bunce) Apinis
  - Ulocladium atrum Preuss.

- Sphaeropsidales
- Sphaeropsidaceae
  - Phoma sp.

Basidiomycetes
- Agaricales
- Agaricaeae
  - Coprinus macrocephalus (Berk.) Berk.

APPENDIX IX

CELLULOLYTIC FUNGI ISOLATED DURING THIS INVESTIGATION

Fungi	Imported timber	In-service timber	Colonized stakes	Air spora
Acremonium kiliense		+	+	
Acremonium strictum			+	
Acremonium sp.		+		
Alternaria alternata	+	+	+	+
Aspergillus fumigatus	+	+	+	+
Aspergillus nidulans		+	+	
Botrytis cinerea	+			
Cephalosporium acremonium	+	+		
Cephalosporium sp.			+	+
Cephalosporium sp.		+		
Chaetomium bostrychodes	+			
Chaetomium indicum	+			
Chaetomium trilaterale	+			
Chaetomium sp.			+	
Chrysosporium pruinosum		+	+	+
Cladosporium cladosporioides	+			
Epicoccum purpurascens	+			
Fusarium culmorum		+		
Fusarium solani		+	+	+
Fusarium semitectum			+	
Graphium album	+			
Nectria inventa		+		
Paecilomyces varioti	+	+	+	

continued.....

APPENDIX IX

CELLULOLYTIC FUNGI ISOLATED DURING THIS INVESTIGATION cont.....

Fungi	Imported timber	In-service timber	Colonized stakes	Air spora
Penicillium brevicompactum	+			
Penicillium crustosum	+			
Penicillium decumbens	+			
Penicillium funiculosum			+	
Penicillium sp.			+	+
Phialophora fastigiata	+			
Phoma herbarum	+			
Phoma sp.		+		
Phoma sp.			+	
Scopulariopsis brevicaulis	+	+	+	+
Trichoderma harzianum	+		+	+
Trichoderma koningii		+		
Trichoderma viride	+	+	+	
Ulocladium atrum	+		+	+
Verticillium lateritium	+			
Coprinus macrocephalus		+	+	
Basidiomycete 157			+	

APPENDIX X

MICRO-FUNGI ISOLATED DURING THIS SURVEY WHICH ARE KNOWN TO CAUSE SOFT ROT

Fungi	Imported timber	In-service timber	Colonized stakes	Air spora
Aspergillus fumigatus	+	+	+	+
Aureobasidium pullulans	+	+		
Cephalosporium acremonium	+	+	+	
Chaetomium indicum	+			
Cladosporium herbarum	+			
Epicoccum purpurascens	+			
Fusarium solani		+	+	+
Phialophora fastigiata	+			
Phoma glomerata	+			
Penicillium funiculosum			+	
Trichoderma viride	+	+	+	

APPENDIX XI

MICRO-FUNGI CONSIDERED TO BE THERMOPHILOUS (MICRO-THERMOPHILES) BASED ON THE RESULTS OF CELLULOSE CLEARING EXPERIMENTS

Fungi	Imported timber	In-service timber	Colonized stakes	Air spora
Acremonium kiliense		+	+	
Acremonium strictum			+	
Acremonium sp.		+		
Altemaria alternata	+	+	+	+
Aspergillus fumigatus	+	+	+	+
Aspergillus nidulans	+	+	+	
Chaetomium indicum	+			
Chaetomium trilaterale	+			
Cephalosporium acremonium	+	+		
Cephalosporium sp.	+	+		
Chrysosporium pruinosum		+	+	
Fusarium culmorum		+		
Fusarium solani		+	+	+
Fusarium semitectum			+	
Paecilomyces varioti	+		+	
Penicillium funiculosum			+	
Penicillium sp.			+	+
Trichoderma harzianum	+		+	+
Trichoderma koningii		+		
Ulocladium atrum	+		+	+

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