Stipes of a Coprinus sp. on a Perfusion Device filled with a selective medium.

SOME ASPECTS OF THE BIODETERIORATION OF SILICONE TREATED WOOD BY SOFT-ROT FUNGI.

by

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SUMMARY

An economic evaluation of wood biodeterioration confirmed the need for improved efficient preservation. An historical survey illustrated a trend of specialisation in timber technology with enhanced selectivity of preservative action and increased accuracy of measuring their performance.

A series of model analytical techniques was devised for facilitating the study of cellulolytic microfungal ecology. A novel perfusion device allowed continuous nutrient supplementation of beechwood veneers, a grinding method produced rapid reliable fungal isolation and a tensiometric technique provided precise strength data for monitoring deterioration.

Different perfused pH values gave varying responses with fungal communities and monocultures. Some fungi were tolerant, others displayed pH specificity. Behaviour depended on the cellulosic substrate used.

Hyphal mixing of bi-cultures followed a pattern capable of formularisation and this reflected cellulolytic activity. Antagonistic, synergistic and neutralistic relationships were recognised.

A consistent wood penetration sequence was delineated and found to be a function of hyphal growth rate independent of substrate changes.

Moisture affected deterioration but not fungal incidence. Drying did not hasten deterioration. Glucose initially reduced deterioration and species isolated. Asparagine accelerated deterioration but yeast-extract had less effect. Wood extractives allowed decay whilst distilled, rain and soil water gave poor deterioration.

Short light wavelengths favoured deterioration, other spectra had less effect. Variable light intensities were uninfluential.

Oxygen deficiency prevented deterioration but concentrations above ambient increased deterioration and decreased fungal colonization. Carbon dioxide induced deterioration but did not affect colonization. Substrate oxidation reduced growth and deterioration whereas reduction increased them.

Water-repellent silicones were examined for natural preservative qualities. They were screened alone for biodegradeability, toxicity, hydrophobicity and metabolic activity and proved to be inert and stable in all respects to the applied conditions. These investigations initiated the design of a percolator for enrichment culture of potential biodeteriogens. Silicones aided penetration of other toxicants.

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I am once more appreciative of my father for helping to construct various pieces of apparatus.

"It is certain that all bodies have perception; for when one body is applied to another, there is a kind of election to embrace that which is agreeable, and to exclude or expel that which is ingrate; and whether the body be alterant or altered, evermore a perception precedeth operation; for else all bodies would be like one to another."

Francis Bacon 1623.

Chapter 1.

A Research Assessment

Biodeterioration has been defined as "any undesirable change in the properties of a material caused by the vital activities of organisms" (Hueck 1968). Substrates or materials, organisms and environments are all intimately concerned in biodeterioration (Eggins 1968).

The purpose of biodeterioration research is to overcome or reduce deterioration and to anticipate future cases with proper prophylactic treatment. Cellulose is the most widely occurring natural source of utilizable carbon for assimilation by microorganisms and therefore this carbohydrate polymer is a prominent material researched on. Its presence in wood accounts for the rapid, global and costly fungal decay of wood.

Most of the work on the ecology of wood-inhabiting fungal species has been carried out on the standing tree or on unbarked logs and branches. Little has been done with debarked timber in ground contact (Levy 1968). It is imperative to undertake research and correct this situation so that a better understanding of fungal decay of worked timber can be obtained. The incentive originates in the monetary loss incurred by such deterioration and the extensity of this can only be gauged by an economic evaluation.

Economic Evaluation

Owing to the paucity of statistics it is difficult to evaluate the economics of biodeterioration. Any assessment is obscured by over emphasis of recent cases, reluctance of certain manufacturers to admit their problems, ignorance of certain complications having biological origins and a lack of industrially orientated biologists (Eggins 1967).

All published estimations of the monetary loss through fungal attack on timber are either generalisations or highly specific cases that are quoted. Most of these estimations result from national surveys. Turner (1967) in his book requotes a 1951 estimate of 10% of the U.S.A. total annual production of timber as being used to replace deteriorated wood. This costs in dollars the equivalent of £94 million. Boocock (1965) has reported many economic facts including that in 1929 the loss of timber in the U.S.A. to sap stain fungi was equal to about £4 million. Heart rots destroyed 3,400 million cubic feet of wood per year. Untreated railway sleepers would last for 5 to 6 years whereas creosoted ones lasted 20 to 25 years. Similarly transmission poles would normally stand for 4 to 6 years but for treatment with creosote prolonging life to 30 years. He reckoned one forest acre in ten was being grown for fungus consumption.

An Australian report (Anon. 1966) stated that the U.S.A. every year lost £83 million in wood decay. Norkrans (1967) has recently given a much increased version of wood loss in the U.S.A. as £1·25 billion. The difference in estimations is probably due to the wood products considered and the second report including the loss from incomplete or superficial decay of timber.

An impressive evaluation of biodeterioration for stored wood-chip piles in British Columbia has been made by Hatton, Smith and Rogers (1968) by estimating the financial loss for the future. By 1987 £42 million will be lost and this means the wastage of 100 to 500 thousand

tons of usable cellulosic fibre per year. If 10% of these losses were to be recovered by research the cost saved over 20 years would be a hundred times greater than the research investment.

Workers in other countries have not been slow to justify research by numerating national losses. Gadd (1957) estimated that 1·75 million cubic metres of timber were deteriorated every year in Finland. Norkrans (1967) repeated a Swedish estimation of attacks on spruce by Polyporus annosus as costing about £5·8 million to £11·6 million. An interesting comparison has been made by Goksoyr (1965) of a Norwegian estimation by setting the value of constructional timber destroyed by rot (£20 million) as four times that destroyed by fire. Skolmen (1964) found 39·6% of Eucalyptus lumber to decay through bad seasoning in Hawaii. Martinka (1968) found Wawa timber, Triplochiton scleroxylon, to be badly damaged by 33 to 50% after six months outdoor storage in Ghana when no preservation was used. No similar estimations for this country have been encountered.

National statistics for the extent of preservation with attendant costs are infrequently given. One set of estimations has been provided by Mann (1959) who concludes that wood preservation is still an art. He quotes 1956 statistics of 325 wood preserving plants in the U.S.A. with over 250 equipped for pressure treatment. 10% of the annual cut of sawn lumber is preservatively treated and this amounts to 275 million cubic feet per year. Harrow (1966) from New Zealand reveals that with the introduction of wood preservatives the timber usage rose from being less than 10 million feet in 1945 to 160 million feet in 1960. This illustrates the changes in timber markets that can accompany improved preservation.

A global evaluation has been made for biodeterioration (Hueck-van der Plas 1965) based on the 21 O.E.C.D. countries including North

America during 1959 to 1960. She valued the annual production or consumption of selected perishable materials for these countries as :-

£10^6 x 2,750 for wood.
£10^6 x 1,750 for paper.
£10^6 x 542 for rayon.
£10^6 x 58 for jute.
£10^6 x 1,166 for cotton.

From these figures the total global damage was placed at just over £400 thousand million (one billion dollars) which is one fiftieth of the world's annual turnover of materials. Hendey (1967) requotes this data but unfortunately decreases each item by about fifty times to obtain estimates of damage to each material. He also takes the original estimate of 2% loss in world materials and applies them to the U.K. without considering the heavier losses occurring in developing countries.

In contrast to these overall evaluations many specific accounts for particular fabrications have been cited. One favourite subject is deterioration in buildings. Tyrer (1961) found 27% of 13,000 dwellings surveyed in the U.K. during 1960 to have wet rot and 17% revealed dry rot. The expenditure from decay was put at above £10 million per year.

Findlay (1967) has given many estimations in his book. In the U.K. before 1939 the cost of repairing houses damaged by dry rot was £1 million per year, £10 million per year after 1945 and according to one expert £20 million per year for many of the post-war years. Few reports appear in western literature of biodeterioration in the eastern countries but 38 basidiomycetes have been isolated from houses in the Ukraine (Demikhovska 1959) and the same number from 1,000 buildings throughout the U.S.S.R. (Demidova 1960).

Another well documented subject is decay of water cooling towers. Baechler, Blew and Duncan (1961) found repairs to a redwood tower after

fungal damage over 34 months operation to cost about £4,000.

Boats have received attention with the U.S.A. Department of Agriculture (Anon. 1953) finding that the majority of 500 boats examined to be deteriorated. Findlay (1967) put the decay of various water-craft in the U.K. to be £2 million per annum.

There are many economic facts given in the book, "Wood Preservation" by Hunt and Garratt (McGraw-Hill book company, 1967) but all relate to specific cases of timber fabrications without presenting an overall account of decay. An interesting comparison is made of the replacement of "crossties" on railway track from 1911. With prolongation of service life by preservation 262 new ties were replaced per mile of track in 1911 and only 47 in 1965. Without preservation 78 million new ties would have been needed in 1964 instead of the 13·5 million actually used.

It is therefore apparent that there are many fragmentary pieces of statistical evidence attempting to quantify the damage rendered to timber by fungi. Overall estimates are intangible and therefore insensate whilst specific cases although more realistic are unrepresentative and difficult to view in perspective. Perhaps to communicate this wastage more effectively it would be better to assess the percentage occurrence of basidiomycete and microfungal growth on integral timber fabrications. Then together with a survey of fungal penetration in timbers under natural conditions a more understandable probability of owning biodeteriorating and depreciating woodwork could be realised.

If it is possible to estimate the extent of wood decay once the colonizing fungi have been determined and knowing their usual substrate penetration and wood degradative activity it ought to be simple to translate this volume of damage into monetary terms. Then from this index the cost of treatment could be assessed to find whether it is best to preserve or replace the affected timber.

The compilation of many examples of monetary loss caused by wood fungal decay is deceptive in that it suggests little effort has been made to prevent decay. The history of timber studies, however, is an account of attempts to achieve satisfactory wood preservation and the following enumeration illustrates such historical developments.

DATE	AUTHORITY	EVENT
2,000B.C.	Archeology	Dowel pins in Egyptian temples treated with bitumen.
77A.D.	Pliny	Recorded use of olive, cedar, larch, juniper and hardbrush (valeriana) oils for wood protection.
1540	West Indians	Used arsenite and sublimate against wood lice.
1657	J.R.Glauber	Used charring followed by dipping in wood tars.
1665	R.Hooke	Blue, white and hairy mouldy spots seen on rotten sappy wood.
1705	Homberg	Used mercuric chloride for preservation.
1716	W.Crook	American patent on "Oyle or Spirit of Tarr".
1767	Boissieu and Bordenave	Used copper sulphate for preservation.
1772	J.A.Scopoli	Described 75 fungal species found in caves and mines.
1793	F.A.von Humboldt	Described 18 wood fungi.
1815	T.Wade	Used zinc chloride for preservation.
1817	W.Chapman	Wrote on timber preservation and concluded that almost every chemical had been suggested for preservation.
1832	J.Kyan	British patent on use of mercuric chloride.
1836	F.Moll	British patent on use of coal-tar creosote.
1837	J.J.L.Margary	British patent on use of copper sulphate.
1838	Sir W.Burnett	British patent on use of zinc chloride.
1838	J.Bethell	British patent on use of creosote for pressure injection into wood.

DATE	AUTHORITY	EVENT
1838	J.R.Bréant	French patent for creosote treatment.
1838	Boucherie	Preservation of freshly felled trees by sap displacement.
1844	T.Hartig	Believed fungal hyphae in wood resulted from decay.
1853	A.de Bary	Showed fungi cause plant diseases.
1861	L.Pasteur	Disproved the Spontaneous Generation of Life.
1863	H.Schacht	Described cavities in wood caused by microfungi.
1864	J.Wiesner	Observed hyphae in wood cell lumens.
1866	M.Willkomm	Studied hyphae in wood.
1867	C.A.Seely	U.S.A. patent for hot and cold soaking for applying preservatives.
1874	R.Hartig	Showed fungi cause wood decay.
1878	R.Hartig	Showed blue stain in conifers to be caused by Ceratostoma sp. (Ceratocystis sp.)
1878	T.J.Burrill	Showed bacteria cause plant diseases.
1883	F.Hoppe-Seyler	Probably the first to record the decay of cellulose.
1886	A.de Bary	Showed a Sclerotinia sp. to destroy the middle lamellae of plant cells.
1889	von Wolniewicz	Used metallic naphthenates the first organic preservatives.
1898	L.Dippel	Observed fungal hyphae growing along cell lumens.
1899	V.L.Omelyanskii	Studied the anaerobic decomposition of cellulose.
1900	H.von Schrenk	Described a blueing process in conifers.
1902	M.Rüping	U.S.A. patent for pressure preservative impregnation.
1906	C.B.Lowry	U.S.A. patent for a simple pressure treatment.
1906	G.C.Hedgcock	Increased the list of stain fungi.
1906	J.B.Card	U.S.A. patent for use of a zinc chloride/creosote mixture.

DATE	AUTHORITY	EVENT
1926	G.Gunn	British patent for copper/chromium/arsenic preservative mixtures.
1927	T.Lagerberg, G.Lundberg and T.Melin	Extended the list of microfungi causing decay.
1927	Forest Products laboratory	Set up to investigate timber problems.
1928	W.Iwanowski and J.Turski	British patent for use of di-, tri- and polychlorinated phenols.

For a historical development of other aspects of wood science the eleven contributions in Wood Science and Technology (volume 1, p.161-190, 1967) should be consulted.

It can be seen that by 1930 the basis of modern wood preservation and mycological understanding was established. Many toxic chemicals had been tested for preservative activity and different methods of applying them to wood had been tried. Many wood inhabiting fungi had been isolated and apart from the microfungi their abilities to degrade wood was normally known.

It is considered that since 1930 advances in timber technology have been more concerned with the specialisation of particular aspects of the subject. Progress in preservation, preservative formulation, testing of preservative performance and ecological research has occurred along particular lines of work with emphasis of some aspects and neglect of others.

The pursuit of preservation has been devoted to intensive investigation into those cases where treated wood will maintain a commercial market in the face of competition from alternative materials. Belford (1968) has drawn attention to the natural product industries being gradually superceded by synthetic substitution and cites the exceptions

of only wool and particular wood markets remaining. Five flourishing wood markets obtained after preservation success are packing for water cooling towers, motorway fencing, softwood telegraph and transmission poles in Australia, structural timber in low cost Malaysian housing and external window joinery. The preservation of joinery has recieved much attention due to the growing concern at the extent of fungal attack which can occur during the first few years after installation. Oliver (1963) has discussed the current interest in water-repellent treatments of external joinery for achieving some measure of moisture stabilisation in the wood, whilst Purslow (1965) has found some water-repellent preservatives to give acceptable protection to joinery. Finally Hilditch (1969) has reviewed all the attendant problems of preserving joinery. Similar specialisation of research has occurred with other kinds of timbering where for some reason a particular consideration is needed.

Newer preservatives include special formulations in which the toxicant reacts with the cellulose or other components of the wood. Baechler (1956) has mentioned some such treatments that have been recently developed as well as promising orthodox toxicants that might give a clean, odourless, paintable wood product. The importance of solvents on preservative efficacy has been explained in a British wood preserving association leaflet (number 9).

Methods for measuring the performance of preservatives have become specialised. Techniques may be described but industrial test data often remains undisclosed. Reviews for preservative testing include those of Colley (1953), Hartley (1958), Russell (1961), Hof (1962), Smith (1965) and Kyte (1968). Becker (1968) has discussed thoroughly the protection of timber and its problems including those of testing. Gibson (1969) has recently listed nine categories of timber environments in which any preservative process ought to be exposed for a thorough evaluation and also announced a scheme for standardising evaluations by the formation of a technical panel to assess treatments. Lloyd (1968) has raised some objections to textile preservative testing.

Even distinctive trends in fundamental mycological research have revealed themselves. Thus with the revelation by Findlay and Savory (1950) of active wood degradation by microfungi a spate of studies was initiated. Savory (1954a and b) termed these organisms soft-rot fungi and so demarcated them from those basidiomycetes and few ascomycetes already known for their wood degradation activities. The advent of these fungi has inspired examination of their hyphal growth characteristics using special microscopical techniques (Levy 1967) and also the determination of ecological features in various environments for which Duncan's (1960) work is foremost.

Alongside soft-rot studies there has been a steady interest in two further lines of research. One is the ecology of selective cellulosic materials which has stemmed from Siu and Sinden's (1951) paper marking the beginning of zealous physiological and ecological investigation of cellulolytic microfungi. Such cellulose derivatives used for substrates include Tribe's (1960) buried cellulose films, the lens tissue of Griffiths and Jones (1963), jute and cotton by Ghosh, Bose and Basu (1968), the screened and supported cellulose paper of Eggins and Lloyd (1968) and conveyor belting by Stewart (1968). Kendrick and Burges (1960) followed later by Hayes (1965) examined pine leaf litter, Caldwell (1963) used beech litter, Hering (1965) oak litter and Macauley and Thrower (1966) carried on the series with decomposing leaf litter of Eucalyptus.

The second line is concerned with the enzymes of organisms. Cellulolysis from an enzymatic standpoint has been reviewed by Norkrans (1967) and discussed by Selby (1968).

Biodeterioration is a multiscience combining qualities from all the lines of research involving materials and organisms. Thus to produce a novel treatment for preserving wood it is necessary to assess the effect of environmental conditions including preservatives and their solvents on a model non-durable wood with fungi that cause an economically significant loss by decay.

Therefore with the realisation of the economic losses from different kinds of wood deterioration, consideration of past experimentation and trends in timber technology and mindful of problems requiring active investigation guided the direction for the subject of this thesis to be the examination of the environmental influence on the soft-rot deterioration of beechwood veneers using model culturing techniques and strength measurements for determining decay. Soil was chosen for the primary source of fungi and water-repellents for the attempted accomplishment of wood preservation.

Justifications for these determinants were :-

a Whilst basidiomycetes are known to have a high substrate specificity soft-rot fungi have been isolated from a wide range of habitats (Savory 1954b). Soft-rot fungi might therefore be ubiquitous.

b Beechwood is known to have little resistance to decay and possesses a uniform grain for strength testing. Gäumann (1935) has determined the mineral salt content of Fagus sylvatica. Breazzano (1934) contended that thin transverse sections of wood gave more rapid decay than small blocks, and thus veneers were chosen.

c Field tests or service trials of timber are time-consuming and so there is an obvious need for accelerated laboratory tests for the quick evaluation of timber decay. It soon became obvious from early experimentation that existing culture systems were inadequate for controlled long-term incubation observations. Thus the new design of model systems that allowed optimal microbial growth became part of the exercise which resulted in the design of a perfusion culturing apparatus. The perfection of isolation and strength determination techniques also became necessary.

d The use of soil instead of monocultures for obtaining fungi follows from Duncan's (1953) work where five advantages were found with soil.

e Water-repellents are important components of textile and wood preservatives. Wallace (1967) has praised the use of water-repellents for external joinery and Norman (1967) has discussed the recent interest

in wood water-repellents with mention made of the demand for the British wood preserving association leaflet (number 13) on "Water-repellent wood preservatives" published in 1966 indicating the need for such information. Silicones were chosen since they might effectively deprive deteriogens of water, aid preservative penetration, ensure minimum preservative volatilization and leaching and perhaps chemically link with the wood providing a stable barrier to fungal penetration.

The use of silicones for rendering surfaces water-repellent was discovered in 1940 by Patnode and made commercially available in the U.K. in 1951. Their usage as water-repellents has been discussed thoroughly by Bass and Porter (1963) including the four possible mechanisms that silicones might bond with materials,

1 Hydrogen bonding between surface hydroxyls and the siloxane oxygen. 2 Chemical reaction between the Si-H of siloxanes and the -OH in the substrate.

3 Condensation of surface hydroxyls with siloxane hydroxyls.

4 Adsorption to the substrate via the polar siloxane oxygen.

A large amount of information is known about these reaction mechanisms with many materials, particularly cellulosic ones, but no attempt has been made to treat wood for a purpose other than water-repellency. A comparison of timber waterproofing agents has been made by Gray and Wheeler (1959) for the timber development association with the Midland Silicone preparation M.S.2202 being used with success for a short period of time.

f Accumulation of relevant ecological data by experimentation with one environmental factor varied each time is important for understanding the nature of fungal growth so as to devise preservatives or foresee preservative limitations.

After delineation of the research programme the sequence of work

began with an initial screening of the biological properties of silicones as is usual with development of any potential biocides. This was followed by the development of wood fungi culturing, isolation and deterioration measurement techniques. Using these techniques the ecology of isolated soft-rot fungi was examined and finally an assessment made of selected preservative treatments. The order of research only differs slightly from that presented in this thesis.

Throughout the research the following endeavourments were pursued :-

1 A precise determination of the effect of some silicones on the activities of microorganisms.

2 Simplification and acceleration of culturing, isolating and material testing techniques for replicated fungal cultures on silicones and wood.

3 Rationalisation of the taxonomy and ecology of colonising microfungi on wood veneers.

4 Accurate assessment of possible preservation from various silicone preparations and some environmental conditions.

5 Clarification of some known timber problems with suggestions as to their cause or possible means of resolving them.

Chapter 2

SILICONE STUDY

"It is probably not unscientific to suggest that somewhere or other some organism exists which can, under suitable conditions, oxidise any substance which is theoretically capable of being oxidised".

E.F.Gale 1952.

"The hypothesis that there are indeed recalcitrant molecules, substances inherently largely or totally resistant to biological decomposition under all circumstances, gains credibility if one considers the long life of many synthetic chemicals in soil and water ecosystems that represent a variety of ecological extremes ".

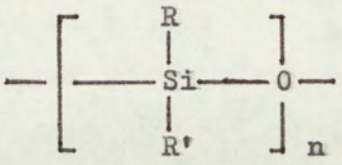
M.Alexander 1965.

Introduction

The evaluation of a polymeric material for possible applications is a complex task. Selection of potential preservatives requires a realisation of a chemical's suitability as regards its chemical, physical, economical and biological attributes.

A biological evaluation demands many considerations including the chemical's resistance to biodegradation or detoxification, its toxicity or inhibitory limits against microorganisms and humans, its constancy of properties when compounded with other chemicals and its ease of application. Only after such research has vindicated a chemical can its value in some aspect of biodeterioration be determined.

Silicones are organo-polysiloxanes with chemical properties resembling both organic polymers and mineral silicates. Their molecular configuration is characterised by alternate silicon and oxygen atoms with hydrocarbon radicals directly attached to the silicon atom,



They have a variety of unique chemical and physical properties and this is reflected in their numerous technological applications. They occur in such forms as fluids, lubricants, greases, elastomers and resins.

The silicone's chemical inactivity, stable physical qualities and remarkable physiological inertness (Levin 1958) have already made them important materials for some biological uses. Their ability to disperse and form thin films offers a possible means of protecting economically important materials against biodeteriogens and with low concentrations this could offset their weakness of high cost (Hardy 1947). Such a potential application warrants a microbiological assessment to see if silicones affect microorganism growth and permit preservation.

Assessment of Biodegradeability

History

The first report on the biodeterioration of silicones stated that silicone resins and rubbers were resistant to fungal decay (Greathouse, Wessel and Shirk 1951).

Glazer (1954) found silicone varnishes to be fungal resistant because of their low percentage of organic material, their water-repellency and their good film continuity. It was suggested they were of especial use at high operating temperatures where there is deterioration of orthodox varnishes. Investigation showed that curing preponderantly methyl and phenyl silicone resins at 85°C. for 100 hours gave unsatisfactory fungal resistance, 140°C. was not much better but a curing temperature of 200°C. gave excellent fungistatic properties.

By treating textiles with polyalkyl hydrosiloxanes, alkyl acyloxysilones and polyalkyl siloxanolates of certain metals in conjunction with impregnation by copper and chromium salts has resulted in water-repellent

finishes which are resistant to weathering, chemical cleaning and biological attack (Voronkov and Kalugin 1959).

When the vulnerability of polymers to deterioration by microorganisms was listed (Hueck 1960) silicones were given good decay resistance. Later work (Olson, Langston and Rainey 1962) showed silicones to increase the resistance to microbiological attack of cotton fabrics without radically altering the properties of the material. Stotzky, Culbreth and Mish (1961) found silastic R.T.V. (room temperature vulcanising) silicones to be effective sealing compounds for biological work and unlike other chemicals tested they were resistant to microbial and environmental deterioration and caused no physical damage when used with plants.

Cellulose textiles are described as being made water-repellent, weather, flame and rot resistant in a patent by Bullock and Welch (1962) when treated with polyvinyl chloride polymers, zirconium acetate and methyl hydropolysiloxane.

It has been shown (Ross 1963) that a silicone rubber containing a dibutyl tin laurate catalyst supports fungal growth after one week of incubation at 29°C. and 95% humidity. A surface coating of copper-8-quinolinolate suppressed fungal growth as did p-chloro-m-xylenol over 2% concentration. Similar results, however, were obtained using polyurethane instead of silicone.

R.T.V. 102 and 108 silicone rubber was attached to glass slides by Calderon and Staffeldt (1965) and then buried in various soils for up to 107 days at 30°C. and 95% R.H. Both rubbers were similarly colonised. Streptomycetes were particularly prevalent in sandy soil and included Streptomyces albus, S. globisporus, S. rochei, S. novaecaesarea and S. acidophilus. Fungi involved were Fusarium spp., Aspergillus fumigatus, Spondylocladium sp., Cunninghamella echinulata, Phoma pigmentivora and Chaetomella sp. It was concluded that silicone rubber is resistant to fungal and bacterial degradation but allows surface growth. The streptomycetes,

however, appeared to utilise the silicone under certain conditions but in a private communication (Calderon 1967) it was stated that the acetate catalyst probably acts as the nutrient.

When a silicone rubber was tested (Muraoka 1966) as an electrical insulant in a deep marine environment it was found to perform satisfactorily for twelve months and then insulation resistance declined rapidly. It is stated that this was caused by slight bacterial attack and water absorption without further explanation.

A patent of Dow Corning Corporation (1966) claims that a sealing preparation containing organopolysiloxanes and phenylmercuric carboxylic acids is resistant to fungi.

A review (Fessenden and Fessenden 1967) of the biological properties of silicon compounds mentions a private communication from MacDiarmid and Brown (1965) about a Pseudomonas sp. capable of using phenylsilane and toluene as a nutrient.

Bengson and Gillis (1968) report that although an unspecified silicone rubber was amonst the least penetrable materials tested there was some evidence of bacterial penetration and slight penetration by a Penicillium sp.

Therefore, it appears that past literature indicates that pure silicones are stable towards microbiological deterioration but that any preparation appears capable of supporting surface growth. To confirm these impressions a series of investigations were undertaken whereby different silicones were subjected to varying environmental conditions.

Experimental

Four methods for culturing microorganisms that might possibly degrade silicones were used.

1 Isolation from naturally occurring silicone enriched soils:

Soil that had been accidently splashed for some time with silicones was obtained from the Midland Silicones Plant, Barry, Wales. Soil particles were introduced into petri-dishes and then covered with glucose and glucose

plus silicone agars following Warcup's method (1950). This agar, and all nutrients used in this thesis, was composed of the mineral salts from Eggins and Pugh cellulose agar (1962). It is hereinafter referred to as E. and P. salts or E. and P. cellulose or E. and P. glucose if these carbon sources are added.

Glucose was added as a 4% concentration and methyl silicone fluid was vigorously shaken into a just molten agar in the concentration of 2·5mls. per 10mls. of medium. Incubation of the pour plates was at 25°C. for 20 days.

Isolated fungi were Aspergillus fumigatus, A. niger, A. repens and Geotrichum candidum. Since all these fungi grew on the non-silicone agar as well as the silicone agars and no observable changes in the silicone were seen it appears that the fungi are incidental colonisers of the nutrient media and not colonisers of the silicone.

2 Isolation from artificially enriched silicone soils:

a 120 glass slides were smeared with R.T.V. silicone rubber and allowed 7 days to cure. They were each weighed, autoclaved and buried in soil for 184 days at a temperature fluctuating between 20 and 25°C. Every 30 to 40 days a few slides were recovered, gently washed and reweighed. They were examined microscopically as well as particles being cut off and plated onto glucose agar, glucose plus methyl silicone (viscosity 20 centistokes) agar, mineral salts plus 20cs. methyl silicone agar and the bacterial basal agar of Lochead and Chase (1943). Isolated organisms were :-

Aspergillus fumigatus	Fusarium solani
Zygorhynchus moelleri	Eurotium vermiculatum
Paecilomyces varioti	Gliocladium roseum
Penicillium sp.	Chaetomium globosum
Cladosporium sp.	Trichoderma viride
Sordaria sp.	Bacteria

No degradation of the silicone rubber or medium silicone was seen.

No weight loss was detected. Similar fungi grew on all the agars, and these were uninhibited where silicone was present. Sparse phialophores of Penicillium sp. and Aspergillus fumigatus and zygospores of Zygorhynchus moelleri were found growing over the silicone surface but not penetrating. No streptomycetes were found.

b Pure methyl silicone rubber was ground up into fine pieces and buried in soil at 20 to 25°C. for 172 days. After recovery the pieces were gently washed in water and placed on to the surface of 0·65cs. and 20cs. silicone plus glucose agar.

No fungi were seen under the microscope to be penetrating the rubber particles but the following fungi were isolated from them :-

Aspergillus fumigatus	Fusarium sp.
A. niger	Trichoderma viride
A. ochraceus	Penicillium sp.

These fungi had been isolated before without any real indication that they were utilising the silicone in a way other than as a supporting substratum.

3 Isolation from percolated soils.

To obtain deterioration of a particular material it is usual to add suitable amounts of the material to an appropriate substrate and provide an environment with suitable biological, nutritional and physical conditions. One way of achieving these conditions is to use the soil percolator system first described by Lees and Quastel (1944) in which nutrients and air are repeatedly cycled through a substrate containing a material. Thus any microorganisms present in the soil able to tolerate or utilise the material will be able to grow more effectively than other organisms and so these species can be elected for preferential culture. Afterwards by using selective and demonstrative agars the elected organisms can be isolated.

There have been many designs of percolators, each being devised for a different need. Audus (1946) used a simply constructed apparatus for studying sulphur metabolism. Lees (1947) simplified and improved his

previous design, whilst Temple (1951) adapted this version to provide aseptic conditions and reduce operational adjustments. Collins and Sims (1956) modified the Audus perfusion system to provide a stronger and more compact apparatus.

Other percolator designs include those of Gundersen (1960), Morrill and Dawson (1964), Singh and Hanna (1965) and Traxler (1965). Smith (1962) eliminated air pumps and operated a percolator by mechanical rotation.

Elective culture of organisms requires a simply constructed percolator that can be efficiently operated in quantity. It must be easily assembled, cleaned and capable of being autoclaved without dismantling. It is with these considerations in mind that the following percolator has been developed (see plate 1, and diagram 1).

Experimental

The percolator operates by blowing air down a glass tube into a nutrient reservoir held in a milk-bottle. This air tube fits inside another glass tube leaving only a small space between them. When the air escapes from the inner tube into the nutrient solution, it rises up the outer lift tube and carries droplets of nutrient solution. These droplets pass upwards between the tubes as far as the top of the lift tube where they pass out into the soil. Another glass tube, the same diameter as the lift tube, rests on top of the lift tube and is held in position by the air tube. This upper half of the lift tube supports a 25 mm. diameter boiling tube which has its base opened up so that the glass tubing can pass through. The base of this test- tube is plugged with glass wool, soil is laid on top and then the tube is attached to a heat resistant bung fixed to the air tube.

The arrangement is such that the gap between the two halves of the lift tubes is less than two inches from the surface of the nutrient reservoir so that the height of lift of liquid is small.

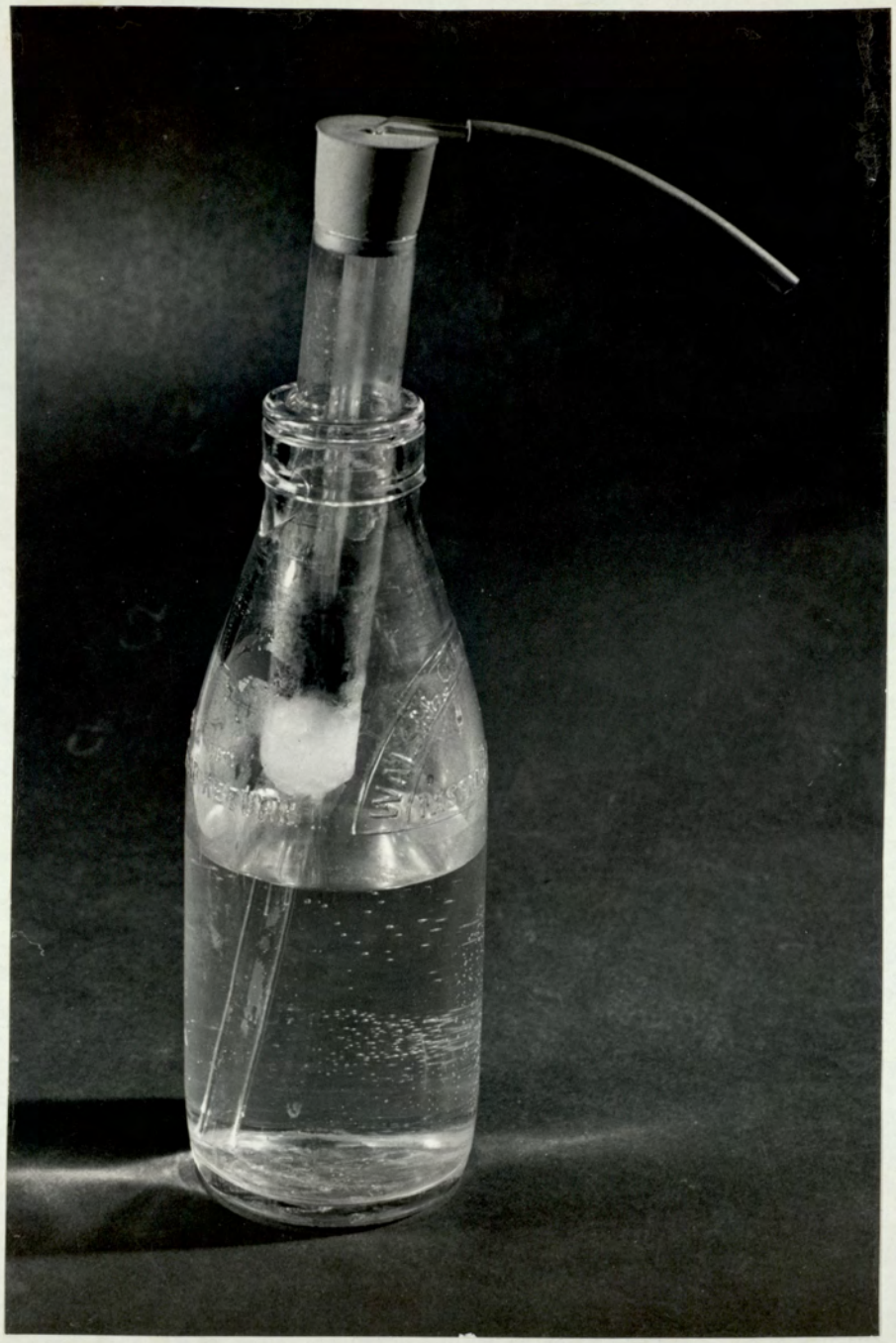


Plate 1, The assembled percolator. The skirt is omitted.

The nutrient reservoir is thoroughly mixed by the percolation since the solution is taken up at the bottom of the reservoir by the lift tube and drains back after percolation through the soil to the reservoir surface.

The air must permeate the soil and glass-wool plug before leaving the apparatus. Therefore, the air flow prevents the nutrient solution

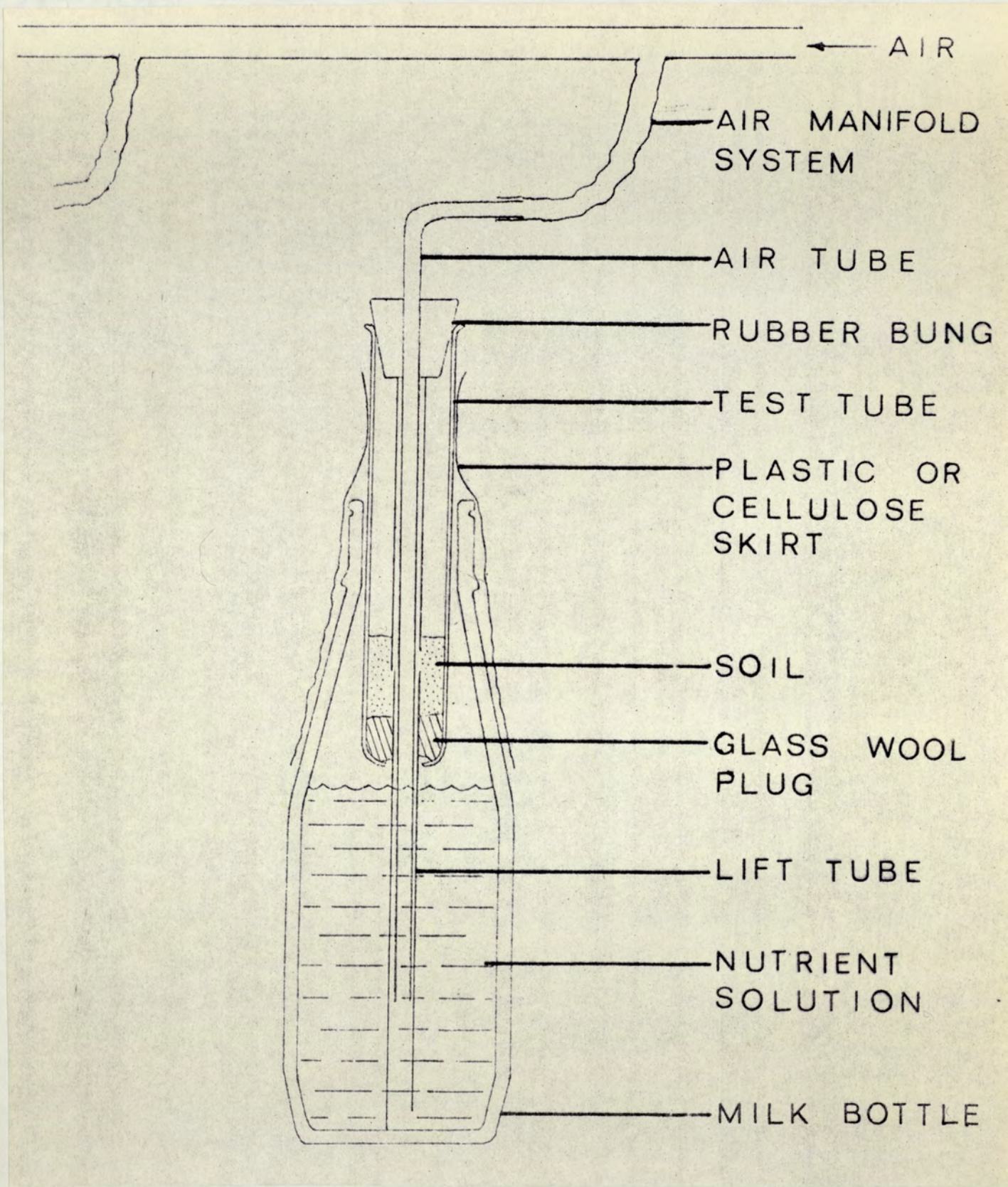


Diagram 1, Diagram of the percolator system.

from compacting the soil. Any water-logging will immediately divert the air passing through the soil to escape through the base of the lift tube. This allows the soil to drain and percolation to restart.

The mouth of the milk-bottle is skirted with a heat-resistant plastic or cellulose sheet held in position by glass-adhesive tape so that airborne contamination is lessened.

Operation of the percolators.

A number of these percolators were set up with various nutrients for percolation. Banks of fifteen percolators were operated from one piston-type aquarium air pump. The pump was connected to a sterilised cotton-wool air filter and then to an air manifold system with screw clamps to equalise the air flow to each percolator as seen by the rate of bubble formation in the nutrient solution. The percolators were kept at constant temperature in a thermostatic water bath with the air tubes immersed to preheat the air supply.

The percolators were autoclaved normally when completely assembled, with only the rubber bung being left loose in the boiling tube. The soil was introduced through the top of the boiling tube and the air system then connected.

Experimental.

32 percolators were constructed and operated at 25°C. for 30 to 40 days with a mineral salts solution as the cycling nutrient and various methyl silicone fluids added as the soil enrichment. Afterwards soil particles were inoculated onto the surface of various agars. The isolated organisms for each kind of isolation medium are shown in table 1.

Another 39 percolators were similarly set up and percolated for periods up to 27 days. The cycling nutrient, however, was given a 4% glucose additive. The results are seen in table 2.

Finally 20 more percolators were installed with 10 having glucose and the 10 others being without but this time no silicone was incorporated

Eggins and Pugh mineral salts .+2·5 mls silicone per 10 mls agar. Silicone viscosities	FUNGAL SPECIES: Aspergillus fumigatus	Aspergillus niger	Aspergillus funiculosus	Trichoderma viride	Fusarium sp	Cladosporium sp	Penicillium sp	Eurotium vermiculatum	Paecilomyces varioti	Gliocladium sp	Sordaria sp	Ceratocystis sp	Mucor sp	Rhodotorula sp	Bacteria	Ciliates
− GLUCOSE																
0·9cs	+	+		+	+										+	
1·0cs	+			+	+	+	+	+							+	
3·0cs	+					+									+	+
20cs	+				+	+		+							+	
+ GLUCOSE																
0·65cs	+	+	+	+	+	+	+	+	+	+	+				+	
3·0cs	+			+	+	+	+	+	+		+	+	+	+	+	
20cs	+			+	+	+	+	+		+		+	+		+	

Table 1. Organisms isolated onto silicone agars from percolated soils. Soils were given silicones and mineral salts.

Eggins and Pugh mineral salts .+2·5 mls silicone per 10 mls agar. Silicone viscosities	FUNGAL SPECIES: Aspergillus fumigatus	Aspergillus niger	Trichoderma viride	Fusarium sp	Penicillium sp	Zygorhynchus moelleri	Eurotium vermiculatum	Chaetomium globosum	Ceratocystis sp	Mucor sp	Bacteria	Ciliates	Nematodes
− GLUCOSE													
0·65cs			+	+	+	+	+		+		+	+	+
2·0 cs			+	+		+				+	+	+	+
3·0 cs	+	+	+	+		+		+		+	+	+	+
+ GLUCOSE													
0·65 cs			+	+	+	+	+						
2·0 cs			+		+	+	+			+			
3·0 cs	+		+	+	+	+							

Table 2. Organisms isolated onto silicone agars from percolated soils. Soils were given silicones, mineral salts and 4% glucose.

Eggins and Pugh mineral salts +2·5 mls silicone per 10 mls agar. Silicone viscosities	FUNGAL SPECIES: Aspergillus fumigatus	Aspergillus niger	Trichoderma viride	Fusarium sp	Penicillium sp	Eurotium vermiculatum	Paecilomyces varioti	Zygorhynchus moelleri	Mucor sp	Absidia glauca	Papulaspora sp	Nematodes
− GLUCOSE												
3·0cs	+	+	+	+	+			+			+	+
10 cs	+	+	+	+	+			+			+	+
20 cs	+	+		+				+			+	+
+ GLUCOSE												
1·0cs	+		+	+	+	+	+	+		+		
3·0cs	+	+	+	+		+		+	+	+		
10cs	+		+	+	+			+		+		
20cs	+	+	+	+	+	+		+				

Table 3. Organisms isolated onto silicone agars from percolated soils. Soils were given mineral salts with 10 having glucose and 10 percolators having no glucose additive. No silicones were percolated.

into the soil . The percolators were left for 20 days at 25°C. before the usual isolation procedure. The results appear as table 3.

It was concluded that the environment circumscribed by the percolation allows the growth of common soil organisms irrespective of the presence of silicones. The silicones must also be non-inhibitory. There was no effect on growth due to the differing degrees of silicone polymerization, as reflected by their viscosities.

Glucose in the isolation plates increased the species isolated and their mycelial growth. Glucose within the percolators decreases the number of species isolated and this is probably due to the thorough percolation of staling substances or toxicants produced in high glucose

conditions. Cellulolytic organisms tended to appear when there was no extra carbon source present.

The elective culture of common, highly sporing fungi without any concomitant physical change of the silicones means that the intended silicone degradation proved to be abortive.

4 Isolation from perfused soils.

In order to enrich a substrate with silicones and supply nutrients in a gradual manner a perfusion system was employed. Perfusion permits the slow supplementation of a substrate with ancillary nutrients and the passage away from the substrate of waste fungal substances. A more detailed description of the principle is dealt with in the wood studies but essentially the system consists of a glass sleeving composed of finely woven glass capillaries which when dipped into nutrients at one end it will transport liquids by capillarity to a substrate attached to the other end. A tailpiece conveys waste away.

Two types of perfusion systems were operated.

a Various methyl silicone fluids were mixed with soil and held in a test-tube through which a perfusing glass sleeving was passed. This glass sleeving passed a mineral salts nutrient solution along by capillarity from a reservoir to the soil and then away to a tailpiece held in a dry atmosphere. Incubation was at 25°C. for 140 days. Afterwards soil particles next to the perfusion sleeving were plated out onto various agars. After incubation fungal growths were identified as :-

Aspergillus fumigatus	Fusarium sp.
A. niger	Eurotium vermiculatum
Penicillium sp.	Graphium sp.
Paecilomyces varioti	Chaetomium globosum
Zygorhynchus moelleri	Trichoderma viride
Rhizopus sp.	Arthrobotrys sp.

Nematodes and soil ciliates were also found. No silicone degradation was again witnessed.

b 24 coverslips were cured with a thin film covering of R.T.V. silicone rubber and weighed. They were then placed next to a nutrient wick which had been inoculated with one of five fungi which previous experiments had shown were capable of forming large amounts of growth on silicone. These fungi were Aspergillus fumigatus, Penicillium sp., Eurotium vermiculatum, Zygorhynchus moelleri and Trichoderma viride.

After incubation at 25°C. for 45 days microscopic examination revealed no pitting of the silicone film. Reweighing the samples after drying at 160°C. for three hours indicated no weight loss and hence no decay.

Assessment of toxicity

History

Much has been written on the toxicity of silicones to mammals but there is little information pertaining to microorganisms.

Pady (1949) relied on the unreactivity of silicone greases by using them to trap air-spora. The grease was coated onto a plate, heat sterilised and then exposed to the air. After exposure the grease was covered with growth media.

It has been found (Speier 1952) that bis (hydroxyphenyl) silanes formed with hexamethylenetetramine thermosetting resins are active bacteriocides. Thus 0·025wt. % is more effective than 0·125% phenol towards Staphylococcus aureus.

Organosilylmethyl thiocyanates were found to be useful for modifying silicone resins and also as mould inhibitors by Midland Silicones Limited (1957).

When a number of microorganisms were grown on several medicinal silicone rubbers by Riley and Winner (1960) it was found that the rubber's toxicity was low.

Bailey and Pike (1960) in a patent describe organosilicon compounds containing an organo-substituted sulfonamido group attached to the silicon

atom to be useful insecticides and fungicides.

A silicone-substituted penicillin has been shown to have similar activity to benzyl penicillin (Voronkina, Strukov and Shostakovsku 1964).

To clarify the position as regards the toxicity of pure silicones towards fungi the following investigation was made.

Experimental

Many randomly selected fungal species representing many taxonomic classes were inoculated onto petri-dishes containing various silicone agars (8ml. of viscosities 0·65cs, 1·0cs. and 5cs. of methyl silicone fluid together with 50cs, 125cs. and two 500cs. with different specific gravities of phenyl methyl silicone fluid per 100ml. of medium). Two plates were inoculated for each fungal species.

After two or four days incubation at 25°C., depending on the rate of hyphal growth, the cross-diameters of the fungal colonies were measured. Size of growths on pure glucose media acted as a standard.

The results are:- 1 Methyl Silicone Fluid

Fungal species	Days of incubation	Colony size in centimetres with each silicone viscosity.			
		0·65cs.	1·0cs.	5·0cs.	Control
Phycomycetes					
Pythium aphanidermatium	4	9x9	9x9	9x9	9x9
Zygorhynchus moelleri	4	4·5x3·5	5x4	5x5	7·5x8
Rhizopus stolonifer +	2	9x9	9x9	8x8·5	4·5x4
Absidia glauca -	4	2·5x2·5	2·5x2·5	2·5x2·5	3x2
Mucor hiemalis	4	5x5·5	4x3	6x3	3·5x3
Ascomycetes					
Yeast	4	2x3	2·5x3·5	2·5x3·5	2x1·5
Endothia parasitica	4	1x1·5	2·5x2	3·5x4	3x3
Carpenteles javanicum	4	5x3·5	3·5x3	4·5x2·5	4x3
Byssochlamys nivea	4	4x3·5	4·5x 4	3·75x3·5	5x4·5

Fungal species	Days of incubation	Colony size in centimetres with each silicone viscosity.			
		0·65cs.	1·0cs.	5·0cs.	Control
Gymnoascus subumbrinus	4	4x3·5	4x3·5	3·5x2	1·25x1
Chaetomium globosum	4	7x7	5x4	5x3·5	7·5x5
Eurotium vermiculatum	4	7x6	3x3	4x3·5	3x3
Fungi Imperfecti					
Trichoderma viride	2	7·5x7	7·5x7	8x7	9x9
Penicillium expansum	4	1·5x1	2·5x1·5	2x2·5	1·5x1·5
Aspergillus amstelodami	4	3x2	3·5x2·5	3·5x3	5x3·5
A. tamarii	4	1·5x1·5	3·5x3	4x3·5	3x2
A. niger	4	9x9	6x5	5x5	7x5
A. giganteus	4	2x2	3x2	3x2·5	2·5x2·5
A. fumigatus	2	7x7	9x9	6x6	7x8
Paecilomyces varioti	4	4·5x4	7x7	7x6	7·5x6·5
Papulaspora sp.	4	2·5x2	2x1·5	2x1·5	3x1·5
Cladosporium resinae	4	3·5x3	3·5x3	5x4	4·5x4
Nigrospora sp.	4	1·5x1·5	2x1·5	2x1·5	2x1
Periconia sp.	4	1·5x1·5	3x3	2x1·5	1·5x1·5
Torula nigra	4	4x4	3·5x3	4x4	5x4
Graphium sp.	4	1x1	1·5x1·5	2x1	1x1
Phoma sp.	4	1·5x1·5	3x1·5	3x2	3·5x2·5
Gliocladium sp.	4	2·5x3	3x2	3x2·5	3x2·5
G. roseum	4	2·5x2	2x1·5	3x2·5	4x4
Fusarium oxysporum	4	3x2·5	2·5x2·5	4x3	3·5x3
Myrothecium verrucaria	4	2x2	2·5x2	1·5x1·5	3x3
Geotrichum candidum	4	3x3	2·5x2	3x1·5	3·5x3
Sclerotium rolfsii	4	9x9	4x3·5	2·5x2·5	9x9
Basidiomycetes					
Peniophora gigantea	4	1·5x1	1x1	3x3	4x4

Fungal species	Days of incubation	Colony size in centimetres with each silicone viscosity.			
		0·65cs.	1·0cs.	5·0cs.	Control
Polystictus sanguineus	4	4x3·5	4x3	7x6	6x6
Poria vaillantii	4	1·5x1·5	1x1	1·5x1·5	2x1·5
Polystictus versicolor	4	3x2·5	2x1·5	3x2·5	6·5x6·5
Shizophyllum commune	4	2·5x2·5	4·5x4	5·5x5·5	4x2·5

2 Phenyl methyl silicone fluid.

Fungal species	Colony size in centimetres with each silicone viscosity after 4 days incubation.			
	M.S.510/50	M.S.510/500	M.S.550	M.S.710
Phycomycetes				
Pythium aphanidermatium	9x9	9x9	9x9	9x9
Rhizopus stolonifer +	6x6·5	7x7·5	7x6	6x6
Zygorhynchus moelleri	5x4·5	5·5x4·5	5·5x5	5x4·5
Absidia glauca	9x9	9x9	9x9	9x9
Mucor racemosus	4x4	2·5x2	4·5x3·5	2·5x1·5
Ascomycetes				
Yeast	1x1	1x1	2·5x1·5	2x1
Carpenteles javanicum	5x3	5x3	5x3	4·5x3
Byssochlamys nivea	4x3·5	4·5x3·5	6x5	5x5
Gymnoascus subumbrinus	1x1	0·5x0·5	1x1	1x1
Chaetomium funiculum	2x1	2·5x1·5	2x2·5	2x1
Chaetomium globosum	3·5x1·5	2·5x1·5	2·5x2·5	3x2
Eurotium vermiculatum	3·5x3	3x2	3x2·5	3x3
Gelasinospora cerealis	9x9	9x9	9x9	9x9
Sordaria sp.	4x1	2·5x2	2x2	3·5x2·5
Fungi Imperfecti				
Aspergillus fumigatus	9x9	9x9	9x9	9x9
A. giganteus	3x2·5	2·5x1·5	2·5x2	2·5x1·5
A. amstelodami	5x5	4·5x2	2·5x2	9x9

Fungal species	Colony size in centimetres with each silicone viscosity after 4 days incubation.			
	M.S.510/50	M.S.510/500	M.S.550	M.S.710
Penicillium cyclopium	4x4	4·5x4	4x3	4·5x4
Trichoderma viride	9x9	9x9	9x9	9x9
Gliocladium sp.	3x1·5	3·5x2	2·5x1·5	3x2
G. roseum	4x2·5	3x2	3·5x2	3·5x2·5
Humicola sp.	5x3·5	4x4	4·5x4	3·5x3·5
Papulaspora sp.	3·5x2·5	2·5x2	3x1·5	2·5x1·5
Geotrichum candidum	3·5x2·5	2x1·5	3·5x2	3x2·5
Paecilomyces varioti	6x5·5	9x9	7·5x6·5	7x6·5
Cephalosporium acremonium	3x1·5	2·5x2	2·5x2	3x2
Myrothecium verrucaria	3x2·5	2x2	1·5x1·5	2x1·5
Fusarium sp.	3·5x2·5	5·5x4	5x4	3·5x3·5
Phoma sp.	2·5x2	3x2·5	3x2	3x2·5
Sclerotium rolfsii	5·5x5	7x6·5	7x6·5	7·5x7
Basidiomycetes				
Polystictus sanguineus	5·5x5	5x4·5	6x5	5x4·5
Poria vaillantii	2x1	1x1	1x1	1x0·5
Shizophyllum commune	2·5x2·5	4x3·5	9x9	2·5x1·5

No inhibition or stimulation resulted and therefore the degree of silicone polymerization was of no significance. Examination of the mycelia showed normal pycnidia, perithecia, sclerotia, chlamydospores etc. and normal hyphal ramifications with occasional penetration through silicone droplets within the medium.

Assessment of hydrophobic attraction

History

Dow Corning's "D.C. 200" silicone caused Mycobacterium tuberculosis var. hominis to adhere tenaciously to a silicone film spread over a glass surface (Fisher 1954). In the presence of polyoxyethylene sorbitan

monooleate ("Tween 80") the hydrophobic environment was destroyed and the bacterium became unattached.

Experimental

To see whether or not silicones provide an hydrophobic environment favourable for hyphal growth the previous experiment was repeated.

30 fungal species were inoculated and 0·02% (volume to volume of the medium) of "Tween 80" was introduced into the agars causing a much greater dispersal of finer silicone droplets. Two plates were inoculated for each species with measurement of the cross-diameters for the best of the two growths recorded. The results were:-

Fungal species	Colony size in centimetres on agar with 0·02% "Tween 80" incubated for 4 days with different silicones.			
	1·0cs.	5·0cs.	1·0cs.+glucose	5·0cs.+glucose
Phycomycetes				
Zygorhynchus moelleri	9x9	6·5x6	6x5·5	5·5x5·5
Pythium aphanidermatium	9x9	9x9	9x9	9x9
Rizopus stolonifer +	9x9	9x9	9x9	9x9
Ascomycetes				
Gymnoascus subumbrinus	1x1	1·5x1·5	1x0·5	1x1
Carpenteles javanicum	6x4	7x4·5	4·5x3·5	5x3·5
Eurotium vermiculatum	5x4	5x4	2·5x2·5	6x4·5
Gelasinospora cerealis	6x5	9x9	9x9	9x9
Chaetomium globosum	2x2	2x1·5	2x1·5	2·5x2
Sordaria sp.	3x2	3x2·5	2x1·5	2·5x2
Fungi Imperfecti				
Paecilomyces varioti	3·5x3	5·5x5	4·5x4·5	6x6
Gliocladium sp.	2x2	6x2·5	2·5x2	3x2
G. roseum	4x3·5	5x3	5x3·5	4·5x3·5
Cytospora sp.	2·5x2·5	2·5x2	3x2·5	3·5x3
Fusarium sp.	4x3	4·5x3·5	4x4	4·5x3·5

Fungal species	Colony size in centimetres on agar with 0·02% "Tween 80" incubated for 4 days with different methyl silicones.			
	1·0cs.	5·0cs.	1·0cs.+glucose	5·0cs.+glucose
Trichoderma viride	9x9	9x9	9x9	9x9
Cladosporium resinae	5x4·5	6x3·5	5·5x4	5·5x3
Papulaspora sp.	2·5x1	3x2	1·5x1·5	2·5x2
Penicillium cyclopium	2·5x2·5	4x3	5x3·5	4x3·5
Phoma sp.	3x2·5	3x2·5	3x1·5	3x2·5
Diplodia sp.	4x3·5	4·5x4·5	4x4	5x4·5
Geotrichum candidum	2x1·5	3x2·5	2·5x2	2·5x1·5
Periconia sp.	5x5	5x4·5	6x5·5	6·5x5
Cephalosporium acremonium	3x2·5	2·5x2·5	2·5x1·5	3·5x3
Aspergillus giganteus	1·5x1	4x3	2·5x2	3x2·5
A. fumigatus	9x9	8x5	9x9	9x9
A. amstelodami	2x2	8x6	7x5	9x9
Sclerotium rolfsii	9x9	7x7	8x5·5	9x9
Basidiomycetes				
Polystictus sanguineus	4x4	4x4	3·5x2·5	4x4
P. versicolor	5x4·5	5x3·5	6x5	4x3
Peniophora gigantea	6x6	6x6	6x6	6x6

It was found that similar sized growths occurred as before showing no reaction to the "Tween 80" or the silicone. There was normal fungal morphology and commensurate hyphal branching in relation to the redistributed silicone as before.

Assessment of incidental action on fungal metabolism.

History

Although silicones appear to have no toxicity towards fungi it is possible that they have a subordinate action on fungal metabolism that is not growth limiting. Thus even though silicones have been exhaustively proved to possess inertness towards mammalian tissues there

is one report of its association with tumours in mice (Kulkarni 1965) and this implicates the possibility of silicones being capable of stimulating in an indeterminate manner a response in tissues. Little (1969) has discussed the instability of polymers used for medical implantations. She mentions that silicones in contact with blood having a high cholesterol level may take up the cholesterol and other fats to the detriment of their mechanical properties. This has happened with some aortic heart valves.

To assess whether silicones upset the growth of fungi by physical means instead of affecting metabolism physiologically their effect on the cellulase enzyme secreted by two microfungi was determined.

Experimental

The effect of silicone on cellulase activity was measured in terms of the rate of clearing in depth against time, of cellulose agar with a silicone additive. This was performed using Rautela and Cowling's assay tubes which are described in more detail later when used during the wood study. Essentially they are boiling tubes one third filled with E. and P. cellulose agar and after inoculation with a monoculture there is a clearing in depth gradually of the agar.

Prior to filling the tubes with agar the medium was mixed with different concentrations by volume of 20cs. methyl silicone. After inoculation with Trichoderma viride, a fungus capable of rapid clearing of cellulose agar, and Humicola sp., a rapid wood decaying fungus, the tubes were incubated at 30°C. for 32 days. Every two days of incubation the extent of clearing in agar depth was measured. The results were averaged (two tubes per concentration being used) and compared with controls. They are seen in fig. 1.

The graphs show only slight differences between the silicone supplemented agar and the non-siliconed controls. This indicates the absence of any profound or subtle influence from the silicone on the microfungal deterioration of cellulose. There is no pattern with either

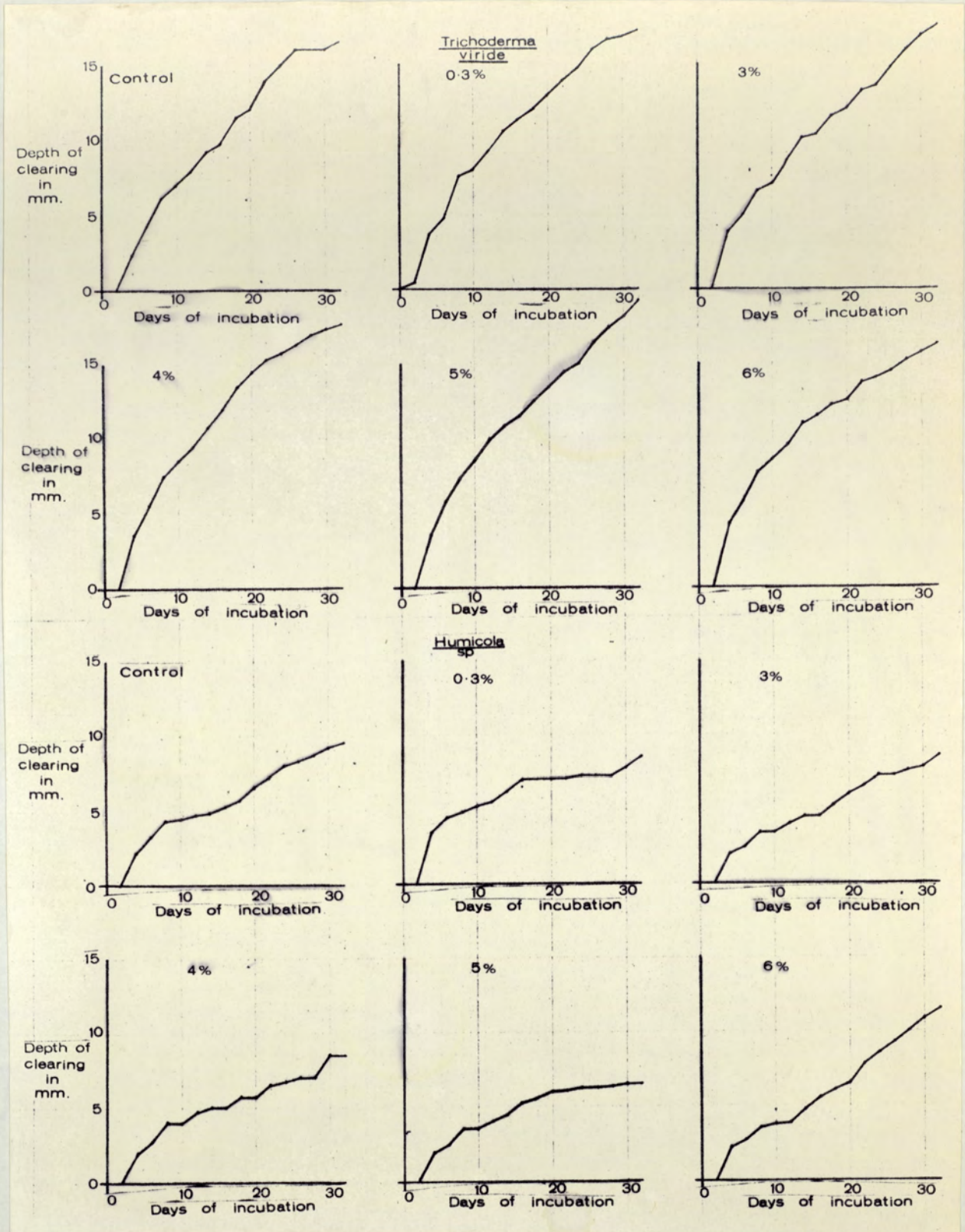


Fig.1, Graphs of clearing rates of two microfungi on cellulose agar with a silicone additive.

species of an effect on cellulose clearing as the concentration of silicone increases suggesting that the water-repellent silicone does not hinder by physical means the cellulose degradation rate.

These analyses have therefore shown the silicones tested to be resistant to deterioration by common soil fungi, possess no biocidal properties and to be inactive towards fungi as regards hydrophobic attraction and other physical characteristics they have. They might be true recalcitrant chemicals with a high degree of biological inertness under most conditions.

Chapter 3

"Disease will be extirpated; the causes of decay will be removed; immortality will be invented. Finally, men will become master forces of nature; they will become themselves architects of systems, manufacturers of worlds".

An evolutionary theory by Winwood Reade in "Martyrdom of Man",1872.

WOOD STUDY

Introduction

In order to examine the soft-rot deterioration of wood and analyse the effect of preservatives it is necessary to design precise techniques for providing reliable data. Furthermore, the efficiency of each technique must be assessed to take into account any defects.

1 Assessment of culturing systems.

Initial cellulose studies were concerned with the isolation of cellulolytic fungi from soil using a paper baiting technique described by Eggins and Lloyd (1968). This technique has an almost pure cellulose chromatography paper covered with a thin film of polythene for support in cases of severe deterioration. The paper laminate is then wrapped around a test-tube with the paper surface outwards and covered with fine weave glass cloth to prevent spore or particle contamination of the paper after autoclaving. The tubes are finally buried in soil and incubated. Plate 2 illustrates the construction of these tubes.

When the culture tubes were first used they were recovered after incubation periods up to 20 days at 25°C. and the colonizing fungi isolated. Isolation was made by exposing the paper and cutting it up into minute fragments which were then aseptically placed onto sterile agar media in a scattered distribution of four per plate. E. and P. cellulose agar was used for the secondary isolation with incubation at 25°C. for seven days before fungal identification. With this technique Trichoderma viride, Rhizopus sp., Aspergillus sp., Chaetomium globosum and Coniothyrium fuckelii were isolated.

Nematodes were also present. These organisms were isolated from sixteen tubes buried in pairs in soil contained in 600ml. beakers.

The soil was taken from pastureland on a farm near Clent, Worcestershire, England, and is the soil used throughout this research. It is a Permian to Triassic lower Keuper red and brown sandstone producing a ferritic brown earth containing fine muscovite micas. The top six inches is a dark reddish-brown clay-loam of fine blocky peds texture. Below this the soil is more compacted into coarse blocky peds. There is therefore a high iron content, little humus and no crumb structure. There are known pebbly bands in the area but only occasional quartzites were encountered. The top layers had not received artificial fertilizers for at least twenty-five years but fragments of leached lime about one foot down were suggestive of past treatment. Pieces of drainage pipe ceramic also indicated previous management.

The flora of the soil consisted of the grasses Agrostis tenuis Sibth. and Dactylis glomerata L. together with Trifolium repens L., Ranunculus repens L., Bellis perennis L., Taraxacum officinale Weber, Plantago lanceolata L., P.media L. and Rumex spp.

The soil was found to have a moisture content of 20-24% when analysed by the oven drying method and a pH of 6•2 to 6•6 using both a meter and indicator solutions.

The soil was collected with a three inch diameter soil borer having a piston to expel the six inch long plug of earth. These soil plugs were later dissected so that only soil from beneath the grass roots to four inches depth was used as inoculum.

It was deduced from the early experiments that the addition of extra mineral nutrients would increase growth on the primary isolation substrate since the enrichment of substrates with a paucity of nutrients is an established microbiological practice. For increasing fungal growth on wood a variety of methods for supplying nutrients have been devised.

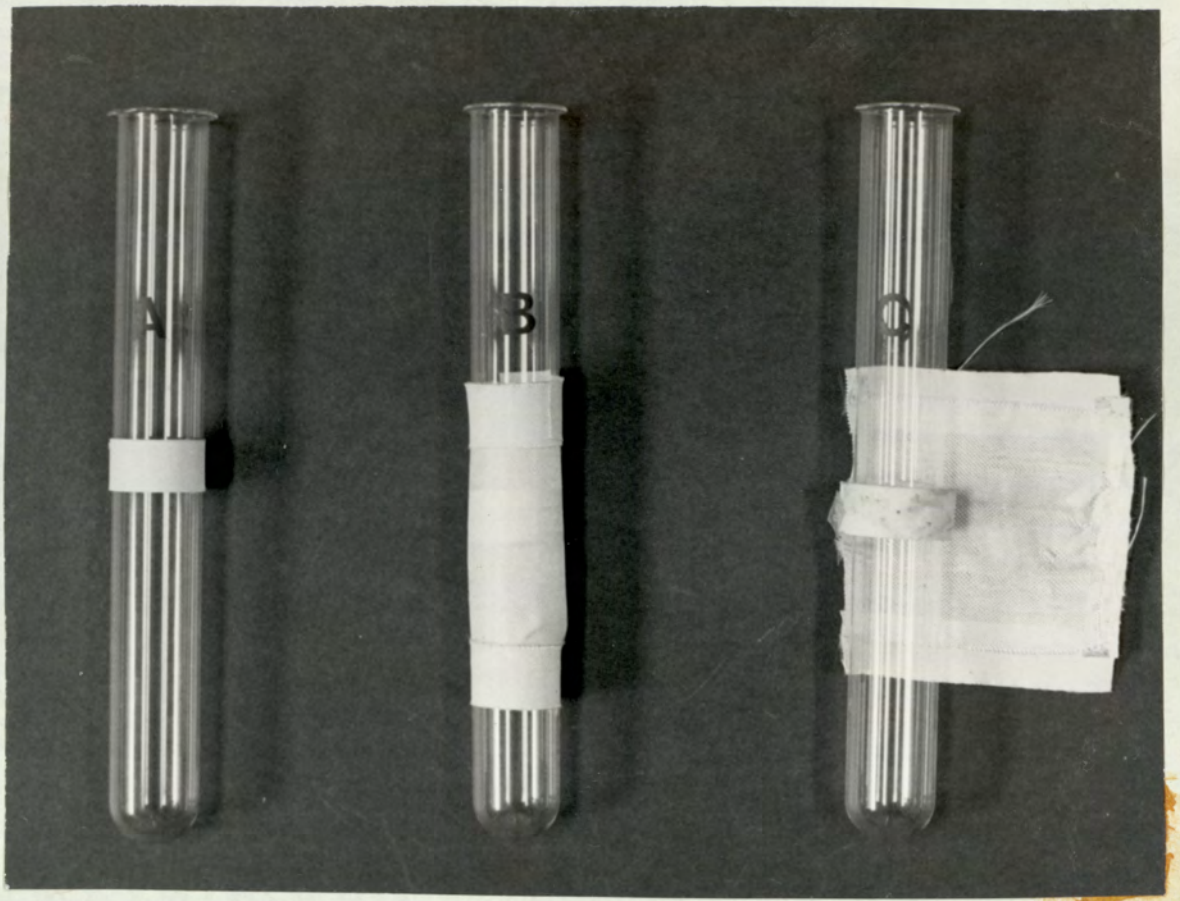


Plate 2. Screened soil burial tubes with the paper wrapped around the test-tube (A), covered with the glass cloth (B), deteriorated (C).

Savory (1954a) used wooden blocks on Abrams medium, Merrill and French (1964) saturated veneers with water using a vacuum, whilst Duncan (1960) used shake cultures and Lloyd (1960) used a modification of the percolator described by Audus (1946). Corbett (1963) devised a "Tide" and a "Wheel" system for keeping woods wet.

Two methods were applied for the enrichment with specific nutrients of the cellulose paper substrate. The first was to increase the nutrient status of the soil by addition of E. and P. mineral salts solution. Thus to each of eight beakers of Clent soil was added 100mls. of E. and P. salts and two screened cellulose tubes. Incubation was for 14 days after which the colonizing fungi were isolated and identified. They were found to be Trichoderma viride, Aspergillus spp., Humicola grisea, Fusarium sp. and Chaetomium globosum. Nematodes were again evident.

The second method was to increase the nutrient status of the cellulose paper by addition of E. and P. mineral salts solution before burial. Sixteen paper strips were left to soak in the nutrient salts for 24 hours and then they were incorporated into the burial tubes. Incubation was as for the previous test at 25°C. The fungi isolated were Trichoderma viride, Aspergillus sp., A. niger, Fusarium sp., Chaetomium globosum and Coniothyrium fuckelii. This isolation is a small improvement on the results found with untreated soil and paper in terms of the number of species isolated.

It was observed during this experiment that the faint yellow colouration given to the paper by the mineral salts solution disappeared within two days of incubation and that brown soil spots appeared on the paper with increasing incubation. This strongly suggested the movement of nutrients and soil water between the substrate and soil. To test these observations a simple investigation was conducted whereby nine paper strips were soaked in E. and P. salts for 24 hours and finally given a quick wash with potassium dichromate solution. They were dried for ten minutes at 100°C. and weighed. These yellow coloured papers were wrapped around test-tubes, shielded with glass cloth and buried in wetted soil for four days at 25°C. Afterwards the papers were recovered, redried and reweighed. The results were :-

Initial Dry Weight	Final Dry Weight	Difference in Weights.
0·14	0·16	+0·02
0·14	0·15	+0·01
0·15	0·18	+0·02
0·15	0·16	+0·02
0·39	0·93	+0·54
0·4	1·17	+0·77
0·43	1·2	+0·77
0·37	1·16	+0·79
0·39	1·35	+0·96

Data is expressed in gram weight.

It can be seen that there is an increase in paper weight suggesting an adherence of soil substances. Furthermore the yellow colouration was washed out indicating a loss by diffusion outwards into the soil of paper chemicals. This rapid equalisation of concentrations of soil and paper substances illustrates the difficulty of maintaining a constant and desired micro-nutrient environment over a protracted growth period.

To overcome these shortcomings a new model culturing system was developed in which it is possible to provide continuous enrichment and constant maintainance of specific nutrients within a porous substrate. This development is implemented by a slow controlled perfusion of supplementary nutrients along a glass sleeving material that is able to transport liquids by capillarity. The nutrient transmission passes from a reservoir to a simulated cellulosic substrate and then away so that not only are fresh ancillary nutrients supplied to the growth medium but stale nutrient residues and waste metabolic products are also removed.

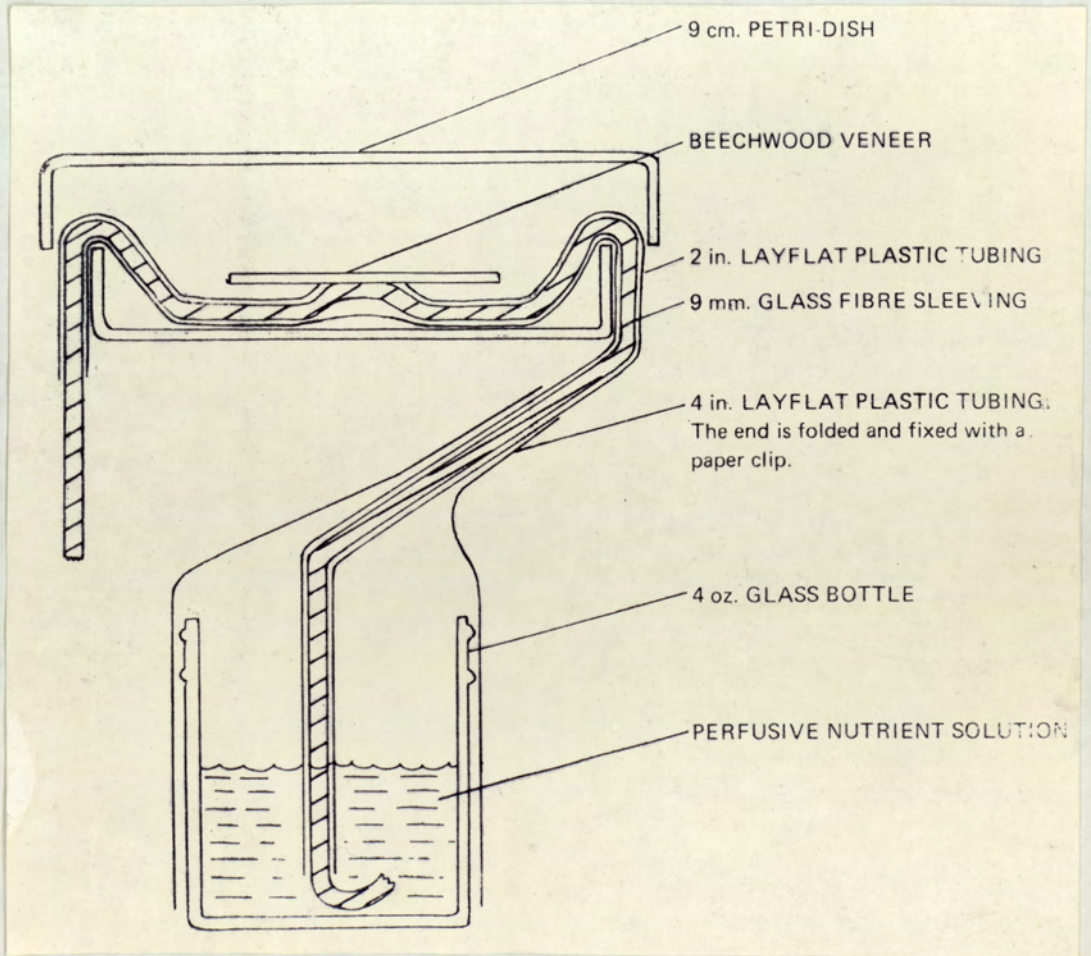
This new nutrient perfusion system therefore depends entirely on the property of electrical insulating glass sleeving to act as a capillatory conductor. If one end of the sleeving is dipped into nutrients then the fine glass fibres of the sleeving pass the nutrients between them by capillarity. This process becomes continuous if the other end of the sleeving is held to dry in the atmosphere causing the water solvent to be evaporated.

Thus a strip of beech veneer when made to lie on the sleeving is repeatedly wetted from the lateral diffusion of the fresh perfusing nutrients. The upper veneer surface remains damp and therefore not oxygen deficient so that any inoculum will give rapid fungal growth.

The arrangement can be seen in plate 3 and diagram 2.

In practice a 9mm. wide sleeving is used in two inch wide layflat, thermostable, plastic tubing with one end dipped in the nutrient salts of the E. and P. formulation contained in four ounce wide-necked bottles.

Plate 3 and Diagram 2. The Wood Perfusion System.



Around the open-necked bottle is wrapped a 7 inch long, 4 inch wide piece of layflat tubing and the end turned over and held with a paper-clip. The veneer is attached to the sleeving with non-toxic, adhesive glass tape through a small window cut in the 2 inch wide layflat tubing. The veneer is housed in a petri-dish above the nutrient bottle.

It is important to have the"tail" held higher than the nutrient level to prevent a too rapid flow due to an automatic siphoning action. Water logging is not usual since the rate of evaporation and fungal utilization of the nutrients governs the rate of flow along the sleeving.

The whole system is built from plastic and glass so that it is non-toxic and autoclavable.

The wood is wetted prior to normal autoclaving so that a water film spans the internal pores of the wood and allows diffusion of the nutrients. The device will perfuse unattended for at least forty days with eighty millilitres of nutrient solution in an atmosphere at 25°C. and 65% R.H.

The outer 4 inch wide plastic tubing successfully prevented airborne contamination of the nutrients for about the first fifteen days of incubation. After this the lowering of the level of nutrients in the bottle sucked into the system contaminated air. This contamination problem was overcome by applying to the surface of the nutrients immediately after sterilization a water immiscible covering of a mixture containing methyl silicone and magnesium stearate with 1% pentachlorophenol. The water-repellents formed an inert emulsion physically protecting the nutrients and the biocide which preferentially dissolved and remained in the hydrophobic layer prevented fungal growth down the walls of the bottle. This nutrient preservation was used extensively and no observable affects on the fungal growths occurred.

The perfusion system therefore repeatedly supplies fresh ancillary nutrients so that it is possible to have a nutrient status at the end of incubation as supplied at the beginning.

Previous perfusion systems for culturing microorganisms include those

of Moor (1945), Fries (1956), Seim (1966) and Plunkett (1966).

The perfusion system, whether it is used with beechwood veneers as described or modified for use with a polythene backed cellulose paper, is only a model technique for laboratory analysis and can only suggest possible explanations for the ecology of natural habitats.

With the adoption of wood and paper substrates it was decided early on to compare the effectiveness of each material for the isolation of cellulolytic fungi.

Experimental

For this comparison twelve E. and P. perfusion devices were set up with each containing a single piece of 0·6mm. thick veneer. Around the wood was wrapped a $\frac{1}{4}$ inch wide strip of cellulose paper with the polythene back against the wood. Thus both materials were made to touch the perfusion sleeving and be exposed on the upper surface for the soil inoculum. Incubation was for 36 days at 25°C. with one device being sacrificed every 3 days.

The colonizing fungi were isolated from the wood by a grinding technique (to be described later) and from the cellulose strip by cutting the paper into small pieces and inoculating four pieces per plate onto E. and P. cellulose agar. The results are shown in table 4.

Conclusions

It can be seen that there are few differences between the substrates and these differences might be due to the isolation technique. Chaetomium globosum, Dicoccum sp., Paecilomyces elegans and Aspergillus niger tended to be found on the wood only whilst Eurotium vermiculatum and Dactylella sp., occurred exclusively on the cellulose.

There was a higher incidence of some species on the wood and they are Humicola grisea, Fusarium sp., Gliocladium roseum and Penicillium sp. Arthrobotrys sp. and Zygorhynchus moelleri tended to favour the paper.

Days of incubation	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Papulaspora sp	Dicoccum sp	Eurotium vermiculatum	Arthrobotrys sp	Dactylella sp	Ceratocystis sp	Paecilomyces elegans	Piricauda sp	Gelasinospora cerealis	Aspergillus niger	Zygorhynchus moelleri	Streptomycete sp	Ciliates	Nematodes
Paper																				
3		+		+			+										+			
6	+	+		+													+			
9		+		+													+			+
12	+	+	+						+	+										+
15	+			+			+		+	+		+								
18	+	+					+			+				+						+
21	+	+								+							+			
24	+	+		+																+
27	+	+		+	+	+		+	+	+										
30	+	+		+									+					+		+
33	+	+									+								+	+
36	+				+				+								+			+
Wood																				
3	+	+	+	+		+	+										+			
6	+	+	+	+												+				
9	+	+	+	+				+												
12	+	+	+	+			+			+						+				
15	+	+		+	+		+			+			+							
18	+	+				+									+		+			+
21	+	+	+	+		+		+		+			+			+	+			
24	+	+		+									+							
27	+	+	+	+	+	+		+												
30	+	+	+	+				+					+			+				+
33	+	+		+	+								+							+
36	+	+		+	+															+

Table 4. A comparison of fungi isolated from a paper and wood substrate.

Generally, those fungal species that were most prevalent colonized the wood more frequently than the paper and this indicated the beechwood veneers to be a better model substrate for electing the growth of cellulolytic fungi.

2 Assessment of Isolation techniques.

Alongside the production of nutrient supplemented substrates by perfusion there needs to be a similar maturation of isolation procedures so as to give the maximum recovery of colonizing fungi. The problems of isolation have been stated clearly by Levy (1967) and shown to need the development of new techniques before it becomes a relatively simple and routine task. Reviews of isolation techniques have been published by Levy (1968) and Grant and Savory (1968).

One hindrance to maximum isolation with soil inoculated substrates is the interference soil fungi cause when only wood colonizing fungi want to be isolated. Separation of viable propagules of soil and substrate fungi is an often neglected aspect of biodeterioration studies.

One method for removing surface growths was suggested by Merrill and Cowling's (1966) idea of surface sterilising wood blocks by dipping them into boiling water for five seconds. When this was tried on four 15 day E. and P. perfused veneers with subsequent isolation onto E. and P. cellulose agar incubated at 25°C. it was found that Fusarium sp., Mucor ramosus and Paecilomyces varioti were growing from the wood splinter inocula. Thus the method killed or prevented the growth of dematiaceus fungi from the veneers but allowed these highly sporing, presumably surface growers, to be isolated.

The use of serial washings in repeated aliquots of sterile water of material in which the surface mycelia is to be examined is a well established technique (Simmonds 1930, Chesters 1948) that has found particular use for plant material (Harley and Waid 1955) and has induced the design of a special apparatus (Mahiques 1966). The method can be extended for use with soiled veneers but first a comparison is necessary to find if it is anymore efficient than the usual method of simply scraping

or brushing off the soil from the veneers.

Experimental

Seventeen perfused veneers were incubated for 34 days with a soil inoculum and one veneer was sampled every two days. Each sacrificed veneer was split longitudinally. One half was washed free of soil by gentle rubbing under a water tap with a soft-haired paint brush. Then the colonizing fungi were isolated by grinding (described later).

The other half was washed in ten changes of sterile water by shaking vigorously in a mechanical shaker for five minutes the aliquot of water and veneer. The washing waters were plated out onto the surface of E. and P. agar and the cleansed wood ground up for normal isolation.

After seven days incubation at 25°C. the fungi were identified and the number of species counted. The results appear as table 5.

Conclusions

Thorough removal of soil was possible with the brush washing but soil adhered to the wood for up to eight changes of water.

There was no progressive decrease in the number of species isolated with successive bottle washes and since these species were predominantly Zygorhynchus moelleri, Trichoderma viride, Penicillium sp. and Aspergillus niger it appears that they either have a tenacious growth or an ubiquitous nature. It is improbable that a more vigorous shaking would dislodge these species since the oscillation rate employed was vigorous enough to loosen the surface wood fibres.

The number of species isolated from the veneers after bottle and brush washing is similar suggesting that the tedious serial washing has little to commend it in this case with veneers used.

All the species isolated from the veneers after bottle washing occurred in at least one change of water. It seems therefore that all the colonizing fungi are capable of leaving a viable propagule behind in the washing waters.

Days of incubation	Number of species with each change of water.										Number of species isolated from veneers	
	1	2	3	4	5	6	7	8	9	10	after bottle washing	after brush washing
2	5	6	5	5	4	5	5	4	3	2	3	4
4	2	4	6	3	3	6	5	4	3	2	9	8
6	5	7	6	4	6	6	6	6	6	5	7	6
8	4	4	6	6	5	7	6	7	5	5	6	6
10	3	3	3	3	3	3	3	3	3	3	4	5
12	3	4	4	4	4	4	4	4	4	4	4	5
14	2	2	3	2	4	4	3	2	3	3	5	5
16	2	4	4	5	3	3	4	3	5	5	5	6
18	3	4	3	4	4	4	2	4	4	3	5	4
20	5	3	3	4	4	4	4	4	4	4	9	7
22	4	6	5	4	3	4	3	3	4	5	7	7
24	3	5	6	5	6	5	5	5	6	5	6	4
26	4	1	2	3	1	3	3	3	3	2	6	6
28	4	4	6	5	4	2	5	3	3	5	8	6
30	1	2	5	4	5	5	4	4	6	3	8	7
32	4	6	4	5	6	3	5	4	6	3	7	7
34	4	5	4	5	4	6	6	5	5	6	5	6

Table 5. Comparison of the number of species isolated after bottle and brush washing off the adhering soil.

These observations together with the objection that serial washing samples the lower uninoculated surface as well as the upper one persuade the use of brush washing for future studies.

The next uncertainty in technique arises in the actual sampling of material. Greaves and Savory (1965) have examined intensively four methods for isolating fungi from large pieces of preservative-treated timber.

They are a borer which extracted wood cores, a chisel technique giving shavings, a sterile block technique using wooden cubes and a saw-cut method producing sawdust. They found the last method to be most efficient and reasoned this to be due to easier nutrient diffusion in the small particles, low residual preservative in the small volume of inoculum and the possibility of one species occurring in one particle and being unaffected by the widely disseminated fungi of other species. Okigbo (1966) used a flattened grinding modification of a drill that ground the wood surface into fine sawdust for inoculation. The trend in isolation techniques for large timber has therefore been to produce particularized inocula which is spatially separated over the growth media in the belief that there is separation of fast and slow growing fungi. It is likely that such principles apply equally well to micro-substrates and that it only requires the determination of optimum inoculum size for successful adaptation.

Experimental

Veneer strips were perfused at 25°C. for seven days and then used for isolating their colonized fungi by five different isolation methods which gave different sized inocula. The short incubation period enabled fast growing early colonizing fungi to be sampled from the soil inoculated veneers alongside any slower growing fungi.

The five methods of isolation onto E. and P. cellulose agar were :-

1. A scalpel was dipped into 75% alcohol and flamed. Splinters of wood were taken and placed onto the agar surface.

2. $\frac{1}{4}$ inch diameter discs were extracted using an alcohol flamed cork borer and the discs then aseptically placed onto the agar surface.

3. 1/16 inch diameter discs were plated out after being drilled using a punch-card drill with an attachable drill-head that had been autoclaved.

4. A junior hack-saw blade was autoclaved and then fitted to its frame. After initial saw-cuts the saw-dust was picked up with a flamed nichrome

wire and plated out.

5. A veneer was clamped vertically in a metal frame such that the wood could be swivelled onto a horizontally revolving wheel which had small grooves cut into its perimeter, see plate 4.

Plate 4. The grinding wheel with cutting grooves along the edge.

The wheel was sterilised by dipping in alcohol and flaming. When the wood contacted the high-speed wheel wood particles flew off in one direction. These particles were caught in a momentarily opened petri-dish containing sterile media. Since this method allows a rapid sampling of all the wood substrate it is a dilution technique with all the diluent used as inocula.

The species isolated with each technique were :-

Isolation technique	Isolated species
Scalpel	Trichoderma viride
	Zygorhynchus moelleri
	Penicillium sp.
Cork borer	Trichoderma viride
	Zygorhynchus moelleri
	Penicillium sp.
Electric drill	Zygorhynchus moelleri
Saw	Zygorhynchus moelleri
	Penicillium sp.
Grinder	Trichoderma viride
	Zygorhynchus moelleri
	Penicillium sp.
	Paecilomyces varioti
	Gliocladium roseum
	Graphium sp.
	Fusarium sp.
	Humicola grisea

Conclusions

It can be seen that by dissecting the substrate into particles by grinding there is maximum isolation of fungi unsurpassed by the other techniques.

The final parameter requiring consideration when screening isolation methods is the choice of culture medium. Greaves and Savory (1965) favoured the use of both sugar-rich and cellulosic media for obtaining the best isolation results.

A small investigation was undertaken to compare the isolation from 17 day perfused veneers using E. and P. glucose, cellulose and ball-milled

beech sawdust (Eggins 1965) in conjunction with the grinding technique at 25°C. The results were :-

Fungal species isolated from five heavily decayed veneers by isolation onto 20 plates for each medium.

E. and P. salts + 4% glucose	E. and P. cellulose	E. and P. beechwood
Trichoderma viride	Trichoderma viride C.	Trichoderma viride
Fusarium sp.	Fusarium solani C.	Fusarium solani
Zygorhynchus moelleri	Zygorhynchus moelleri	Aspergillus niger
Nigrospora sp.	Nigrospora sp.C.	Coniothyrium sp.
	Chaetomium globosum C.	Humicola grisea
	C. funiculum C.	Stysanus sp.
	Alternaria sp. C.	Chaetomium funiculum
	Verticillium sp. C.	
	Papulaspora sp. C.	
	Penicillium sp.	
	Gliocladium roseum C.	
	Sordaria sp. C.	
	Humicola grisea C.	
	Rhizopus sp.	

C. denotes clearing of the agar.

It was concluded that efficient isolation resulted with the use of ball-milled cellulose agar and that glucose and beech agars allowed the growth of similar but fewer species. Only clearing was seen with the cellulose agar and the absence of clearing of the beechwood agar suggests the non-utilization of colouring substances.

3. Measurement of Deterioration

It is essential in any ecological study to assess quantitatively the extent of substrate breakdown. Any method must have a high sensitivity especially where decay is insidious and must be capable of great and rapid

replication.

The state of beech veneer decay is observed by determining the loss of strength; for which special jaws have been devised for a tensiometer.

Strength loss determinations of wood have been actively investigated, particularly since Cartwright et al's 1931 publication. Many kinds of strength tests have been employed and almost an equal number of testing devices for evaluating the strengths have been used. Hartley (1958) in his review describes hardness, crushing, tensile andvarious bending and toughness tests.

The method proposed has evolved from the nail head pull-through principle of Merrill and French (1964) and is similar to the static bend testing used by Armstrong and Savory (1959) on beechwood and by Liese and Pechmann (1959) on birchwood.

In essence, the technique consists of two clamps for a tensiometer which supports horizontally a thin strip of beechwood veneer, so that when the clamps move apart the veneer is made to bend until fracture occurs. The veneer is pressed or cut into strips 6mm. wide and 5 to 8 cms. long. Control strips are cut tangentially to the strips that will be exposed to decay. This close proximity between control and exposed veneer strips ensures minimal strength variation. All veneers are cut so that the grain runs the length of the strip for maximum strength initially.

The clamps are each 7•5cms. long and are constructed to fit onto the type "E" tensiometer of Tensometer Ltd. The veneer strip after subjection to decay is placed on the clamps so that the point of severest decay is under the $\frac{1}{8}$ inch round steel bar of the lower clamp. The strip is supported horizontally by being held underneath the ends by two loops of the upper clamp. The arrangement is seen in plates 5, 6 and 7.

The round bar of the lower clamp can slide between the two loops of the upper clamp so that when the clamps are parted the round bar over the

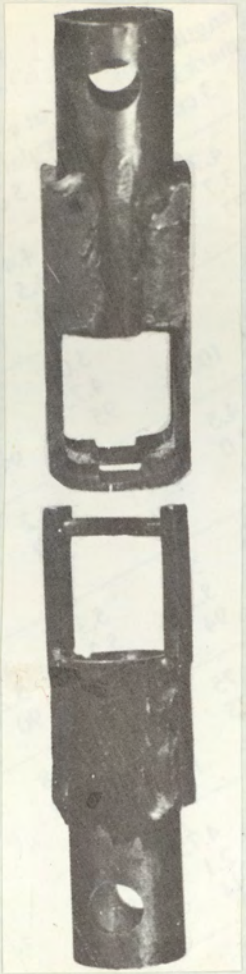


Plate 5. Tensiometer jaws.

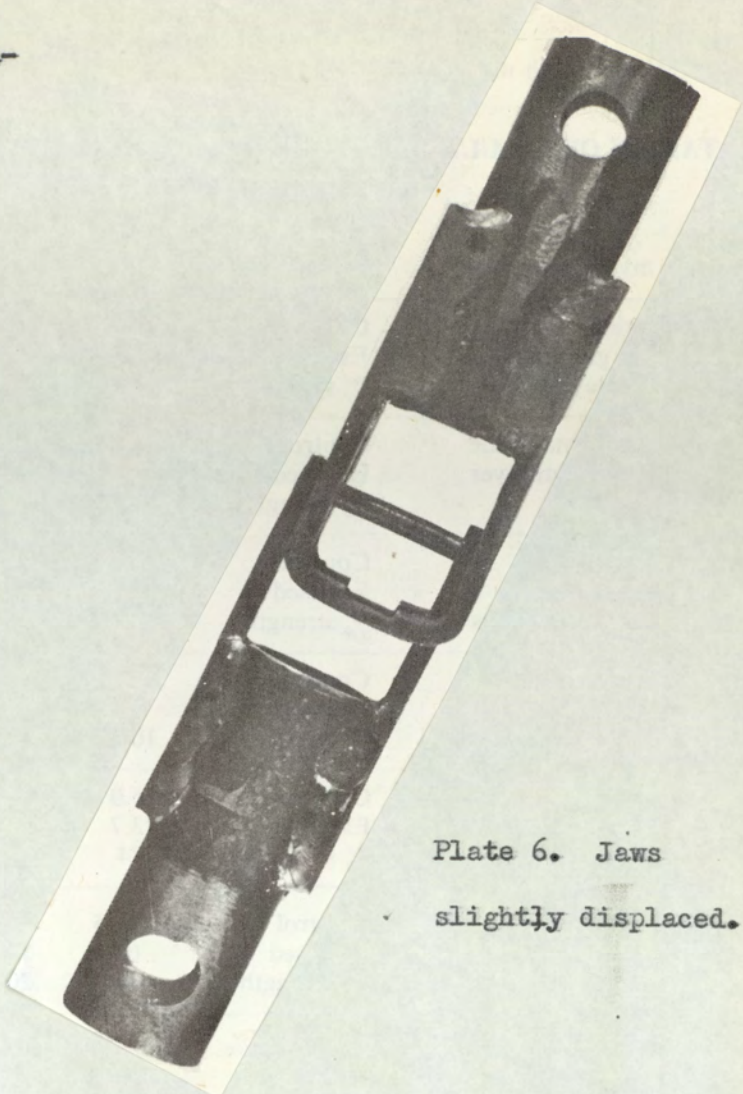


Plate 6. Jaws slightly displaced.

Plate 7. Jaws with wood before test.

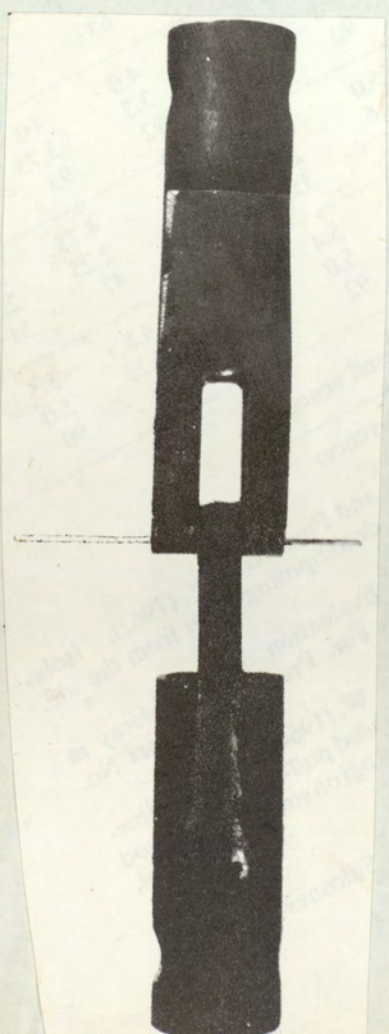
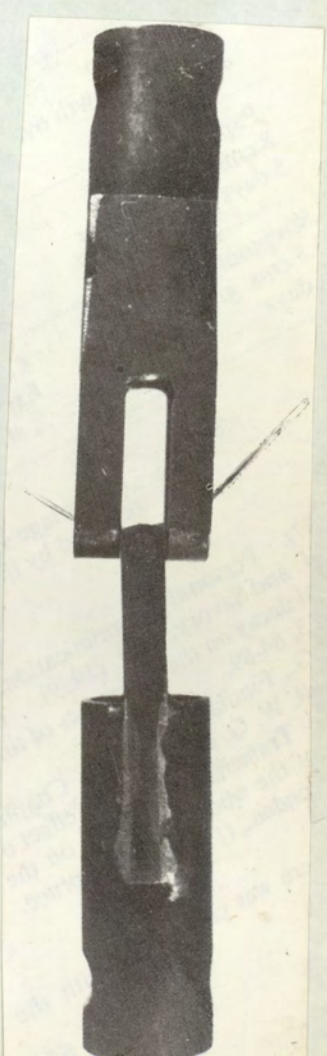


Plate 8. Jaws after fracture.



veneer will be brought downwards to bear on the top of the wood strip, this being shown in plate 8. On bending downwards the upper surface of the veneer is compressed and the lower surface undergoes tension. As bending continues the central round bar slides further downwards between the two outer loops. Finally fracture of the wood fibres of the lower veneer surface occurs at which point the strength recorded on the tensiometer is suddenly reduced. The greatest resistance to bending is noted.

The tensiometer is set up with a 50lb. load cell and the pen recorder at a chart speed of $\frac{1}{4}$ inch of paper per minute. The two clamps are parted at a cross-head speed of 1 inch per minute.

The technique is rapid since it takes less than one minute to test each veneer strip. It is sensitive for even disintegrating wood will still give a reading of 0·25lb. p.s.i. and diffuse decay shows up as a gradient of strength loss along the veneer. It is easy to use and there is quick resetting. Since the veneer can be moved to any position for testing it is possible to measure the exact spot of decay and no freak points of unusual strength are recorded. Furthermore the culturing of uniform decay is not necessary.

The use of veneer as the test material means that it quickly succumbs to decay and this hastens strength determinations.

One variable that is well known to affect wood strength is its moisture content. However, this can be overcome by placing all the veneer strips over saturated sodium chloride solution gelled by agar and held in universal bottles (Ayerst 1967). When left for at least seven days the veneers obtain a moisture content corresponding to 75% R.H. which when determined for a small number of veneers was found to vary from 160 to 180%, average 174%, water content using the oven drying method for moisture measurement.

To assess the sensitivity of this technique a statistical analysis has

been conducted. It is impossible to assess the sensitivity of such a test technique without also accounting for the variation of the culturing organism producing different degrees of deterioration and the wood substrate having inherent strength variation due to anatomical peculiarities. Three precautions have been taken to minimise these extra sources of variation and so help to demonstrate only that variation due to the technique.

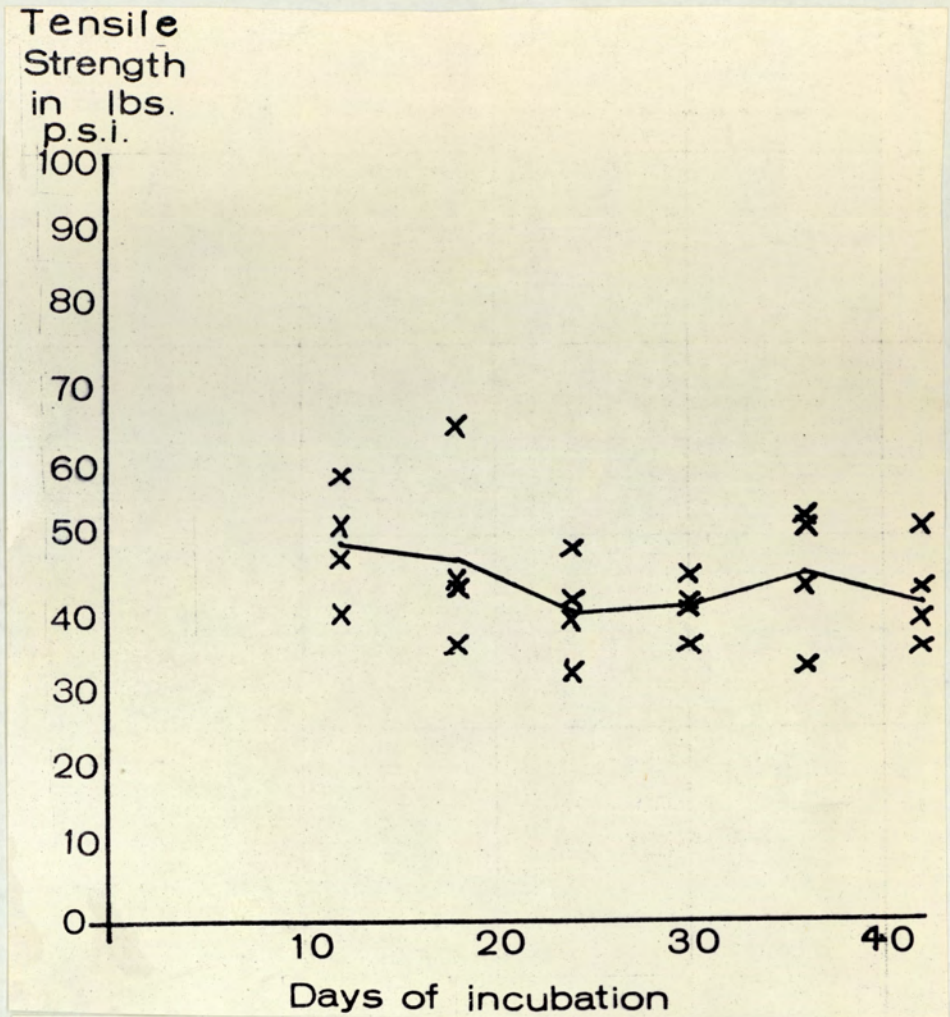
1 The bending technique is compared with a tensile test. Thus the analysis is a relative one with comparison of experimental performances. The tensile stress involves simple tension which causes a linear increase in wood dimension in the direction of the applied force, whereas bending gives a lateral displacement combined of the effects from tension, compression and shear forces. The tensile stress therefore provides a basis against which the three forces of bending can be assessed.

2 Reducing experimental error by selecting straight grained woods, equalising wood moisture contents, carefully controlling the fungal culturing systems and using a test technique for comparison that can be repeated exactly each time it is applied.

3 Randomising the sampling by taking veneer test pieces from the log in an irregular fashion. This prevents the appearance of strength patterns that might be present if the veneers were taken systematically. The deteriorated veneers were also sacrificed after incubation in a random way by taking any four veneers every six days. This gave six separated blocks of results and allowed the comparison of data within each block.

Experimental

48 veneer strips were randomly cut and autoclaved in E. and P. salts. They were then inoculated onto E. and P. agar in petri-dishes by using alcohol flamed forceps. One centimetre square pieces of the edge of a Papulaspora sp. growth were cut with a flamed nichrome wire and inoculated onto the middle of each wood strip with the inoculum being placed upside down.



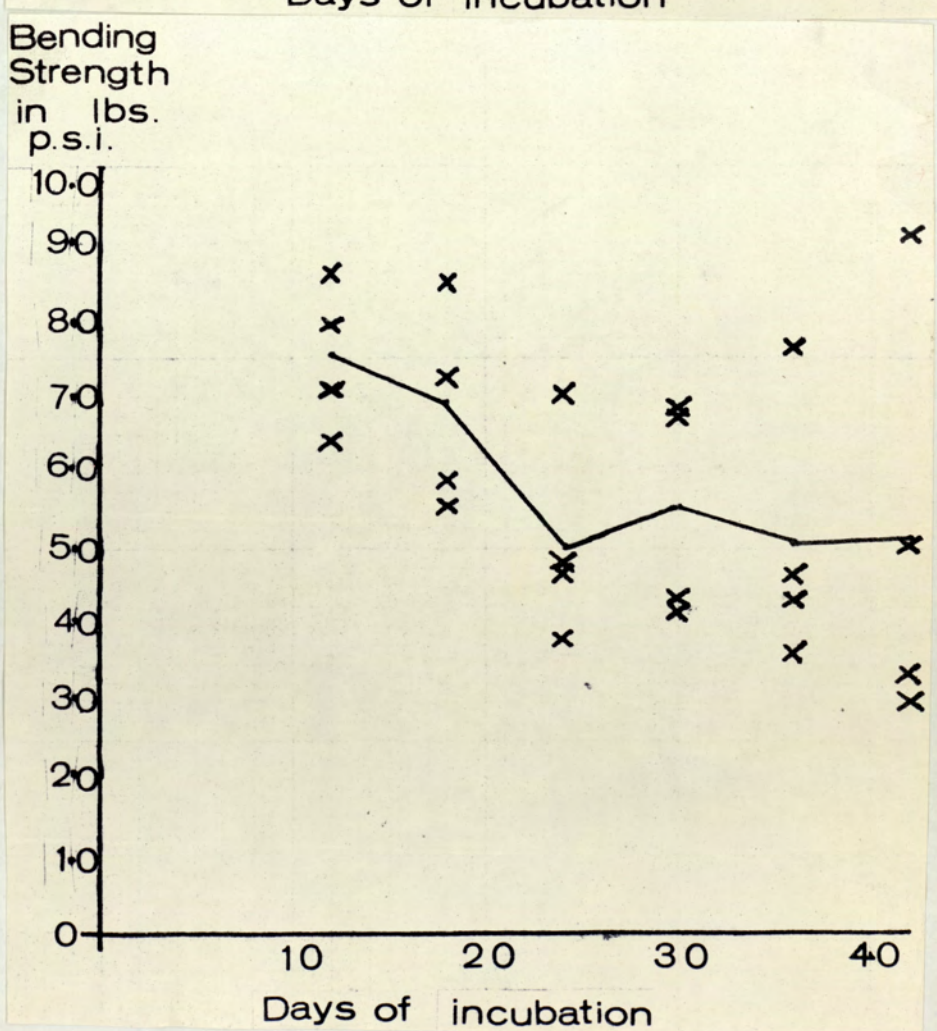


Fig 2. Graphs of veneer strengths determined by tensile and bending tests.

Incubation of the plates at 25°C. was for 42 days with eight plates being sacrificed every six days, there being no sampling before the first six days. The veneers were moisture equalised over salt agar for seven days and then four of each sacrificed group of veneers were broken by bending and four by the tensile test. The tensile test used eccentric roller jaws to grip the woods. The bending test was applied twice to each veneer and the average of the two results taken.

The results appear in fig. 2.

The graphs reveal a slight decline in strength with the tensile test and a greater strength loss with bending. There is, however, more scatter with the bending results. Both these observations could be anomalous since the two tests have data of different magnitude; the tensile test measuring higher strength values. Therefore to rescale the results and provide parity between the tests a process of "normalisation" has been applied. This is done by altering each result with the formula :-

$$\text{New scaled result} = \frac{Xe - Xl}{Xh - Xl}$$

where Xe = old experimental result

Xl = lowest mean result

Xh = highest mean result

The mean results are the lines in fig. 2 and are calculated by averageing the four results of each block of sampling.

The new scaled results appear in fig. 3, together with the mean results found as before.

These results reveal the bending test to have a more constant decrease in strength than the tensile test as before. They also show the tensile test to have more variation than the bending test and since this is a different conclusion to that reached previously it illustrates the misleading ways such data can be interpreted if statistics are not involved.

Therefore the bending test as compared to the tensile test is more

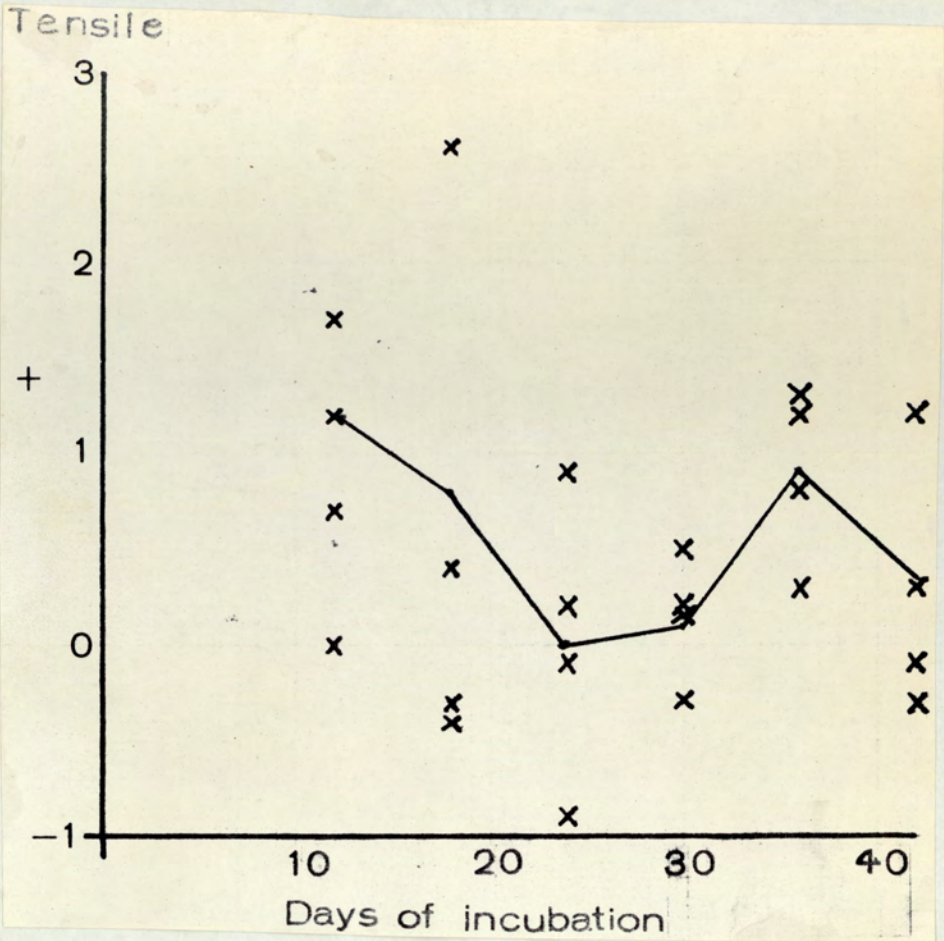
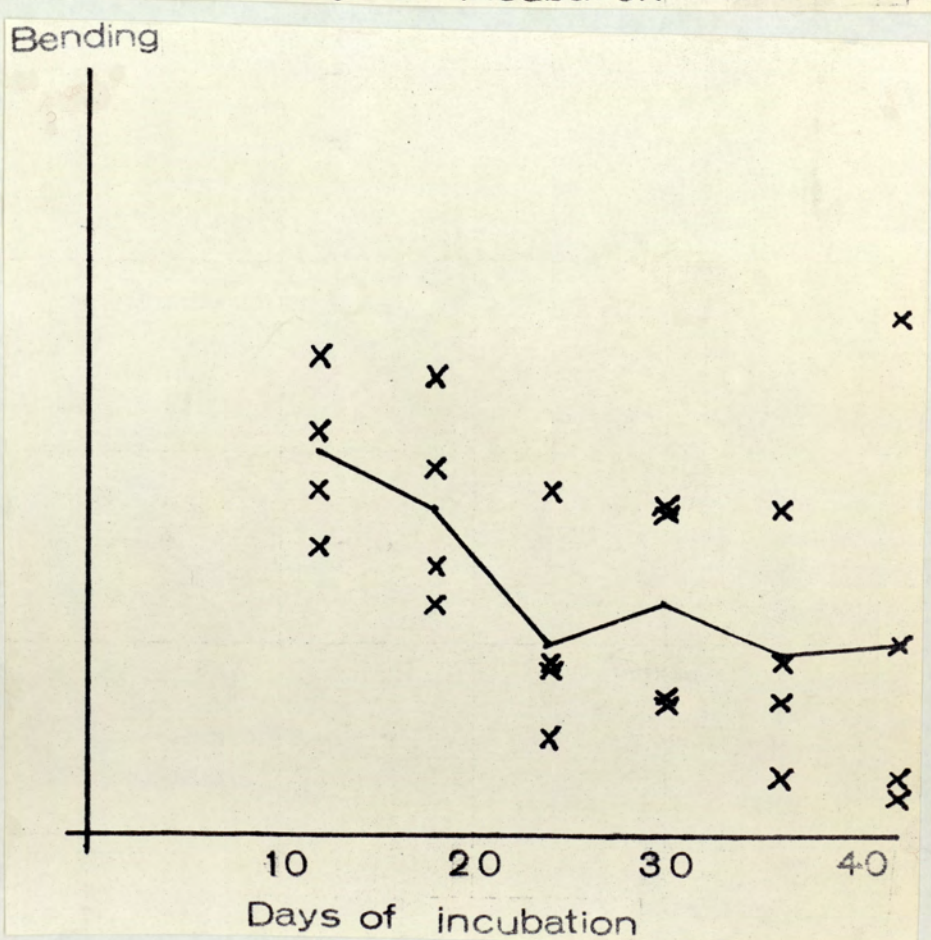


Fig. 3. Veneer strengths after normalisation.



sensitive as seen by the progressive strength loss and also gives less variation as reflected by the scatter so affording reliability.

To thoroughly examine these conclusions it is necessary to undertake further investigations using a variety of woods, culturing systems, fungi, other tests for comparison and different methods of statistical analysis.

Chapter 4

Taxonomy of isolated organisms.

The taxonomic classifications of Gilman (1957), Barnett (1962) and Smith (1960) were used throughout this thesis study. References were made to Brown and Smith (1957) on the genus *Paecilomyces*, to Ames (1963) for the Chaetomiaceae, to Cooney and Emerson (1964) for the *Humicola* spp. and to Warren (1948) for information on the genus *Papulaspora*.

Verification of soft-rot status for the isolates was made by consultation of such published lists for these fungi as Levy and Lloyd (1960), Duncan and Eslyn (1966) and Rosch and Liese (1968). Fungi isolated were :-

Phycomycetes:

Mucorales

Zygorhynchus moelleri Vuillemin

Mucor racemosus Fresenius

Mucor spinosus van Tieghem

Ascomycetes:

Eurotiales

Eurotiaceae

Eurotium vermiculatum Dangeard

Microascales

Ophiostomataceae

Ceratocystis sp.

Chaetomiales

Chaetomiaceae

Chaetomium funiculum Cooke

Chaetomium globosum Kunze ex Fr.

Sphaeriales

Sordariaceae

Sordaria sp.

Fimetariaceae

Gelasinospora cerealis Dowding

Fungi Imperfecti (Deuteromycetes):

Moniliales

Moniliaceae

Arthrobotrys sp.

Aspergillus fumigatus Fresenius

Aspergillus niger von Tiegh

Aspergillus ochraceus Wilhelm

Aspergillus repens (Corda) Saccardo

Cladosporium sp.

Fusidium sp.

Geotrichum candidum Link

Gliocladium roseum (Link) Thom

Monilia brunnea Gilman and Abbott

Monosporium olivaceum Cooke and Massee

Paecilomyces elegans (Corda) Mason and Hughes

Paecilomyces marquandii (Massee) Hughes

Paecilomyces varioti Bain

Penicillium sp.

Trichoderma viride Persoon ex Fries

Verticillium sp.

Dematiaceae

Dicoccum sp.

Humicola grisea Traaen

Humicola sp.

Nigrospora sp.

Periconia sp.

Piricauda sp.

Pullularia pullulans (De Bary and Low) Berkhout

Stachybotrys atra Corda ex Fries

Torula sp.

Stilbaceae

Graphium sp.

Stysanus sp.

Tuberculariaceae

Fusarium solani (Martius) Saccardo

Fusarium sp.

Mycelia Sterilia

Papulaspora sp.

Sphaeropsidales

Sphaeropsidaceae

Coniothyrium sp.

Cytospora sp.

Phoma sp.

Notes on the isolates.

There was no exact match of *Arthrobotrys* , *Torula* , *Papulaspora* and *Graphium* against species contained in the herbarium of the Commonwealth Mycological Institute and therefore no species identification was made. *Graphium* is deposited at Kew as I.M.I. 136612.

Humicola sp. was found to be similar to *Humicola fusco-atra* Traaen but dark spored. *Nigrospora* sp. was identified as *Humicola grisea* Traaen but differences occur as regards colony colouration, slower hyphal growth, lower wood degradation ability and small and swollen conidiophore cells appearing more frequently than with *Humicola* all of which give uncertainty. It is deposited at Kew as I.M.I.136613.

Monosporium olivaceum was identified as a heavily sporulating species which is possibly a conidial stage of *Allescheria boydii*. It gave no synnemata.

More than one *Fusarium* sp. was evident amongst isolates with *Fusarium solani* probably being the predominantly occurring species. Difficulty was encountered in recognition and identification of this genus so that

its frequency of isolation is perhaps lower than it ought to be and throughout this thesis it is considered in generic detail only.

The publication of a revision of the genus Trichoderma (Rifai 1969) came too late to be used. In this paper nine "species aggregates" are recognised based on the types of conidiophore branching, the manner of phialide disposition and the characters of the phialospores. For this research all Trichoderma isolates were considered to be Trichoderma viride. Similarly, the taxonomic study on the genus Arthrobotrys (Haard 1969) was read well after it could be applied to the isolates.

Zygorhynchus moelleri was invariably recognised as the dominant phycomycetous fungus occurring on wood although it is possible that other mucoraceous species were mistakenly included in these identifications.

The inclusion of Penicillium spp. follows a long tradition of reporting this genus in association with timber (Gerry 1923, Merrill and French 1964 and Rajderkar 1966). No real attempt has yet been made to determine which species are responsible for wood degradative activity. In this thesis the fungi of this genus are described under Penicillium sp. with the understanding that a heterogeneous assemblage might be the subject of study.

In addition to the examination of these microfungi attempts were made to isolate other organisms at mesophilic temperatures.

To culture basidiomycetes twelve veneers were perfused with Russell's medium (1956) and incubated for up to 36 days with Clent soil. Six veneers were found to be infected with a Coprinus sp. and this was isolated at 12, 18, 21, 30, 33 and 36 days of incubation indicating the development of fructifications at comparatively late incubation stages. The stipes of the fungus are seen growing on wood in the frontispiece.

Sub-culturing the Coprinus isolate on to Russell's agar medium was successful only twice illustrating the imperfections of the isolation

technique for basidiomycetes. No determinations were made to measure its cellulolytic ability although visual estimations of the wood indicated some loss of strength after incubation.

An effort was made to isolate bacteria and actinomycetes colonizing the wood. For this twelve veneers were perfused with E. and P. salts solution containing Nystatin antifungal antibiotic substance (8 units per millilitre of medium). After inoculation with Clent soil and incubation for 36 days any growths were isolated from one veneer every three days. Isolation was onto E. and P. cellulose agar with the antibiotic substance. Only Streptomycete species were found to "clear" the cellulose agar. Of the few bacteria observed as discrete colonies the black "iron" and "sulphur" bacteria were prominent. These same bacteria were also seen to colour the wood immediately underneath the soil inoculum with a black ring. This ring did not spread out as a nigrescent fungal colouration does and is therefore indicative of slow or no bacterial passage through the wood.

Chapter 5

The Ecology of Soft-rot Fungi

Influence of pH.

Introduction

It is essential for the timber user and preserver to know under what conditions fungal growth is inevitable. One environmental factor that could significantly influence growth is pH. Both Wolpert (1924) and Edgecombe (1941) emphasised the importance of pH and found acidic levels favoured basidiomycete growth whilst alkalinity caused inhibition. Recently Henningsson (1967) has revised the study of pH on basidiomycetes by using ten wood fungi on birch and aspen over a pH range of 1·5 to 8. Optimal growth occurred at pH 5 to 6 with general tolerance for the complete range; brown rot species grew best at pH 1·5 and were more sensitive to higher pH values. During incubation acidification by the fungi of the substrate occurred necessitating continuous adjustment of the pH.

As regards microfungi Siu and Sinden (1951) have found Curvularia lunata, Aspergillus flavipes and Myrothecium verrucaria to have a pH optimum between 6 and 7 whereas Gliomastix convoluta was able to tolerate a hydroxyl ion concentration at pH 11. Fusarium oxysporum Schlecht. em. Snyd. and Hansen grew well between pH 5·5 to 9·5 according to Madhosingh (1962) and was less sensitive than three basidiomycetes used.

The research confined to soft-rot fungi and pH (Duncan 1960) showed 40% of thirty-two isolates to fail at pH 3, 50% to grow at pH 7, 25% at 8 and for only a few to survive pH 9. The optima occurred at pH 6.

Wessels and Adema (1968) have found the fungicidal activity of many toxicants to be pH dependent; sodium pentachlorophenol for example decreased in activity by one hundred times from pH 5 to pH 8.

Any investigation of pH requires a technique that could counteract substrate acidification if it occurs. Moreover it must be active under all

circumstances since Wazny (1960) found for twenty basidiomycetes the mycelial pH to vary under similar medium conditions, with different strains, time, temperature, nutrients, pH conditions and concentration of sodium fluoride biocide. To overcome this possibility and provide a constant pH micro-environment was considered feasible if a perfusion system was employed. A perfusion system ought to supply a nutrient flow of desired pH to a wood substrate and transport away waste substances including secreted acids which might alter the pH.

A series of experiments was arranged with continuous analysis of pH over an extended incubation period using soil and monoculture inocula. The influences of different pH values were recorded and comparisons made with pH altered selective agars.

Synecological Study

Experimental

Perfusion devices were set up consisting of rotary-cut beechwood veneer pieces supplied with E. and P. nutrient salts. The hydrogen ion concentration was varied with 0·5N hydrochloric acid and the hydroxyl ion concentration with 0·5N sodium hydroxide producing media with pH values of 3·7, 4·5, 5·4, 6·4, 7·0 and 8·6. Each pH level was buffered using McIlvaine's formulations with citric acid and disodium orthophosphate such that the phosphate content of the medium equalled 0·1M concentration.

This pH range was chosen because agars below pH 3·5 do not gel and above pH 7·5 phosphates have a poor buffering capacity (Good *et al* 1966). The phosphate concentration was arranged to be high enough for fungal utilisation with sufficient remaining to buffer effectively but not so concentrated as to be inhibitory.

After autoclaving at ten pounds pressure for twenty minutes the beechwood veneers were inoculated with Clent soil taken from a depth of

two inches. An alcohol flamed spatula was used for inoculation.

Incubation was undertaken at 25°C. for at least 34 days during which on every two days one veneer for each pH was sacrificed. The colonizing fungi were isolated by the grinding technique with inocula plated out onto sterile cellulose medium altered to the respective pH of perfusion and buffered. These plates were incubated at 25°C. for seven days after which the cultured fungi were identified, subcultured on to the same medium and any "clearing" of the medium noted.

From each veneer a strip was pressed out and its moisture content stabilised. The bending strength of the strip was measured and these measurements compared with control strips that had been cut as near laterally as possible to the exposed veneers and treated similarly.

Using this methodology the microfungi were induced to grow from the soil on to the wood at particular pH levels, the isolates were identified and the frequency of isolation for each species at each pH found by calculation using the formula:-

$$\text{Percentage frequency of isolation.} = \frac{\text{Number of veneers a species is isolated from}}{\text{Total number of veneers used for each pH.}} \times 100$$

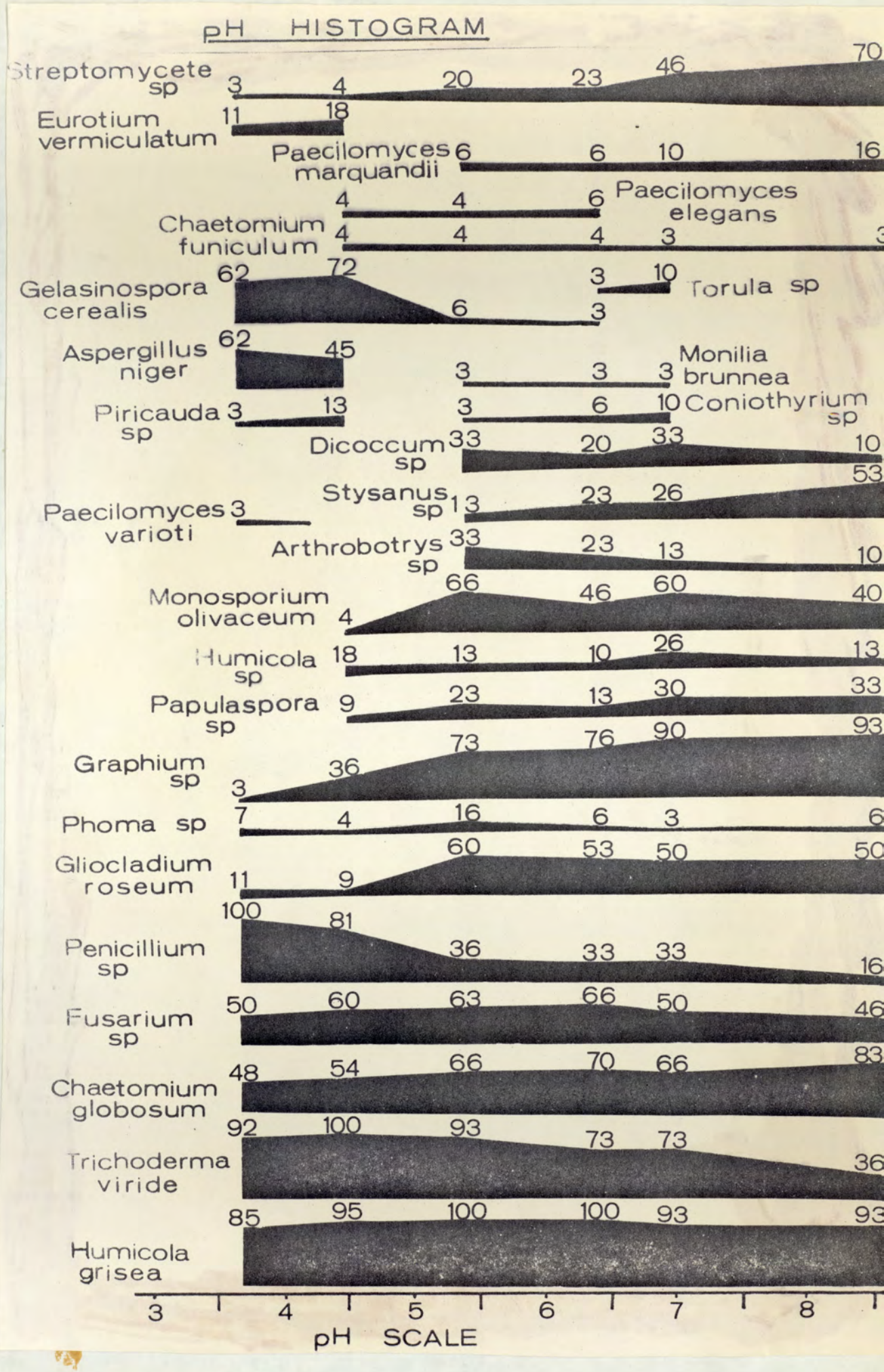
This data is expressed as a histogram with the percentage frequency of isolation shown at each pH.

Also the strength measurements reflect the wood degradative activity for all species at each pH and the results are seen in fig. 4.

Conclusions

Most of the species isolated tolerated a wide pH spectrum. Those increasing in frequency of isolation with increasing alkalinity include Chaetomium globosum, Papulaspora sp., Stysanus sp., Graphium sp., Paecilomyces marquandii and the Streptomycete sp.

Those species preferring acidic conditions include Gelasinospora cerealis, Dicoccum sp., Arthrobotrys sp., Trichoderma viride, Penicillium sp.


pH HISTOGRAM
Streptomycete sp
3 4 20 23 46 70
Eurotium vermiculatum
11 18
Paecilomyces marquandii
6 6 10 16
Paecilomyces elegans
4 4 6
Chaetomium funiculum
4 4 4 3
Gelasinospora cerealis
62 72 6
Torula sp
3 10
3
Aspergillus niger
62 45
Monilia brunnea
3 3 3
Piricauda sp
3 13
Coniothyrium sp
3 6 10
Dicoccum sp
33 20 33 10
Stysanus sp
13 23 26 53
Paecilomyces varioti
3
Arthrobotrys sp
33 23 13 10
Monosporium olivaceum
4 66 46 60 40
Humicola sp
18 13 10 26 13
Papulaspora sp
9 23 13 30 33
Graphium sp
3 36 73 76 90 93
Phoma sp
7 4 16 6 3 6
Gliocladium roseum
11 9 60 53 50 50
Penicillium sp
100 81 36 33 33 16
Fusarium sp
50 60 63 66 50 46
Chaetomium globosum
48 54 66 70 66 83
Trichoderma viride
92 100 93 73 73 36
Humicola grisea
85 95 100 100 93 93
3 4 5 6 7 8
pH SCALE

and Aspergillus niger. The remaining fungi have a growth little affected by pH.

The greatest preponderance of fungal growth in terms of highest frequency of isolation occurs between pH 6·4 and 7.

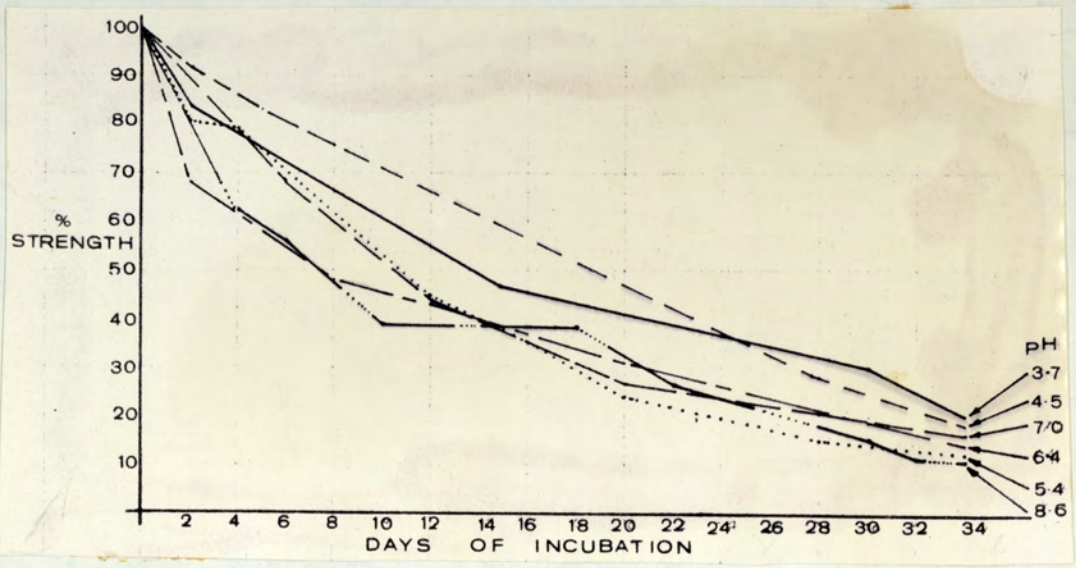


Fig. 4. Strength losses of perfused veneers at different pH levels.

The strength measurements show that with each pH there is a similar loss of strength. This suggests a regular threshold level of strength deterioration is reached irrespective of pH or colonizing species. This effect might be due to a few fungi being resistant to pH changes and being responsible for most of the wood degradation. The strength graph indicates strength loss almost immediately on incubation with only a decrease in the rate of decay after 80% of the strength has been lost.

There was some indication that the acids of pH 3·7 caused slight cellulose hydrolysis, as Seifert (1967) found, but the effect on wood strength was nullified by similar activity occurring on the control veneers.

Autecological Study

Experimental

Two methods were used for culturing the isolated species on cellulosic substrates at the pH values used previously. One method uses the natural

wood substrate and perfusion whilst the other uses synthetic cellulose agar.

The first method to be described is a slight modification of Rautela and Cowling's (1964) cellulose clearing tubes with ball-milled chromatography cellulose used as the incorporated carbon source.

Eighty millilitres of E. and P. cellulose medium plus Rose Bengal bacteriostat (3µg. per millilitre of medium) was aseptically poured into autoclaved 25mm. diameter boiling tubes plugged with cotton wool. The medium has prior to sterilization been adjusted and buffered to a particular pH. Four tubes were filled for each species at each pH value.

After pouring, the tubes were immediately immersed in cold water to hasten gelling and maintain the level of suspended cellulose in the agar. The tubes were then inoculated with one centimetre square pieces of fungal mycelium taken from near their colony perimeter. The isolates were cut and inoculated with flamed nichrome wires.

The height of the cellulose medium was marked on the side of the tubes before incubation at 25°C. for 36 days. Upon growth the secreted cellulase of each species produced a clearing zone below the mycelium. The depth of this zone where the cellulose had been utilised was measured every two days of incubation from the original mark. Thus the increase in depth of clearing over time was measured and taken to be a reflection of cellulolytic activity. It was found necessary to undertake all incubation in conditions of high humidity to prevent the agars from drying and shrinking.

Conclusions

Different types of clearing were observed with different fungi. *T. viride*, *Fusarium* sp. (Plate 9.), *C. globosum* and *M. olivaceum* gave a transparent clearing. *Penicillium* sp. and *Streptomycete* sp. (Plate 10.) gave a partial clearing. *Paecilomyces varioti* gave no clearing at all.

Species with dark hyphae such as *Humicola* sp., *Humicola grisea* (Plate

11.), Papulaspora sp., Piricauda sp. (Plate 12.), Torula sp., and Dicoccum sp. gave a black area of growth with a narrow zone of clearing underneath. Gliocladium roseum gave a red pigmentation to the agar at pH 7.

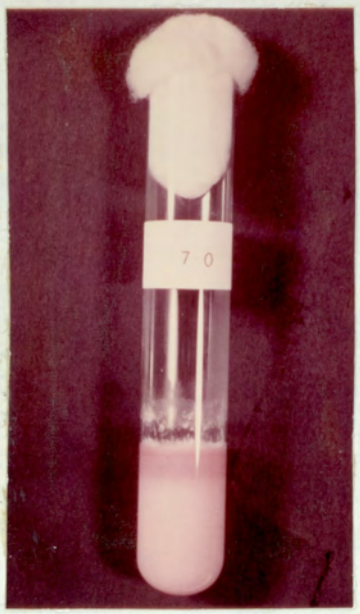


Plate 9. Fusarium sp. at pH 7

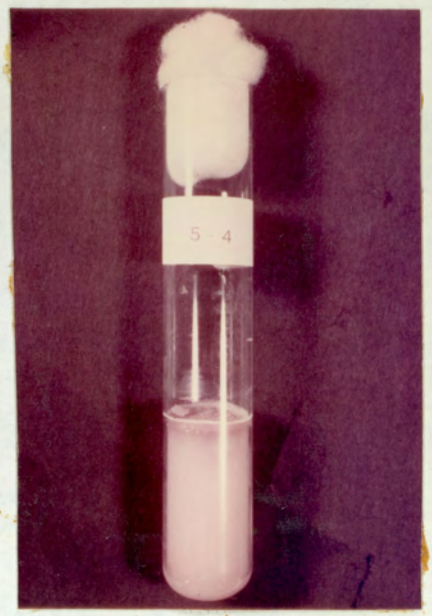


Plate 10. Streptomycete sp. at pH 5.4

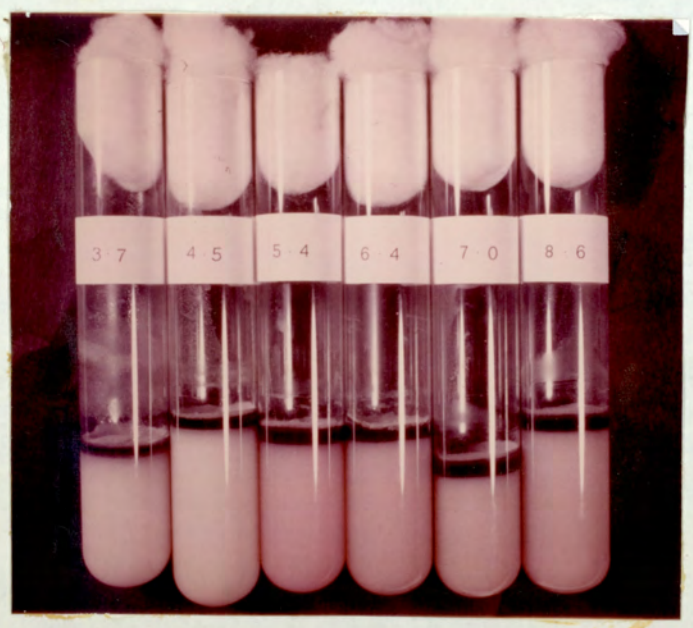


Plate 11. Humicola grisea at all pH values tested.

Plate 12. Piricauda sp. at pH 8.6

The results of the clearing rates are expressed in a series of graphs, figs. 5 and 6.

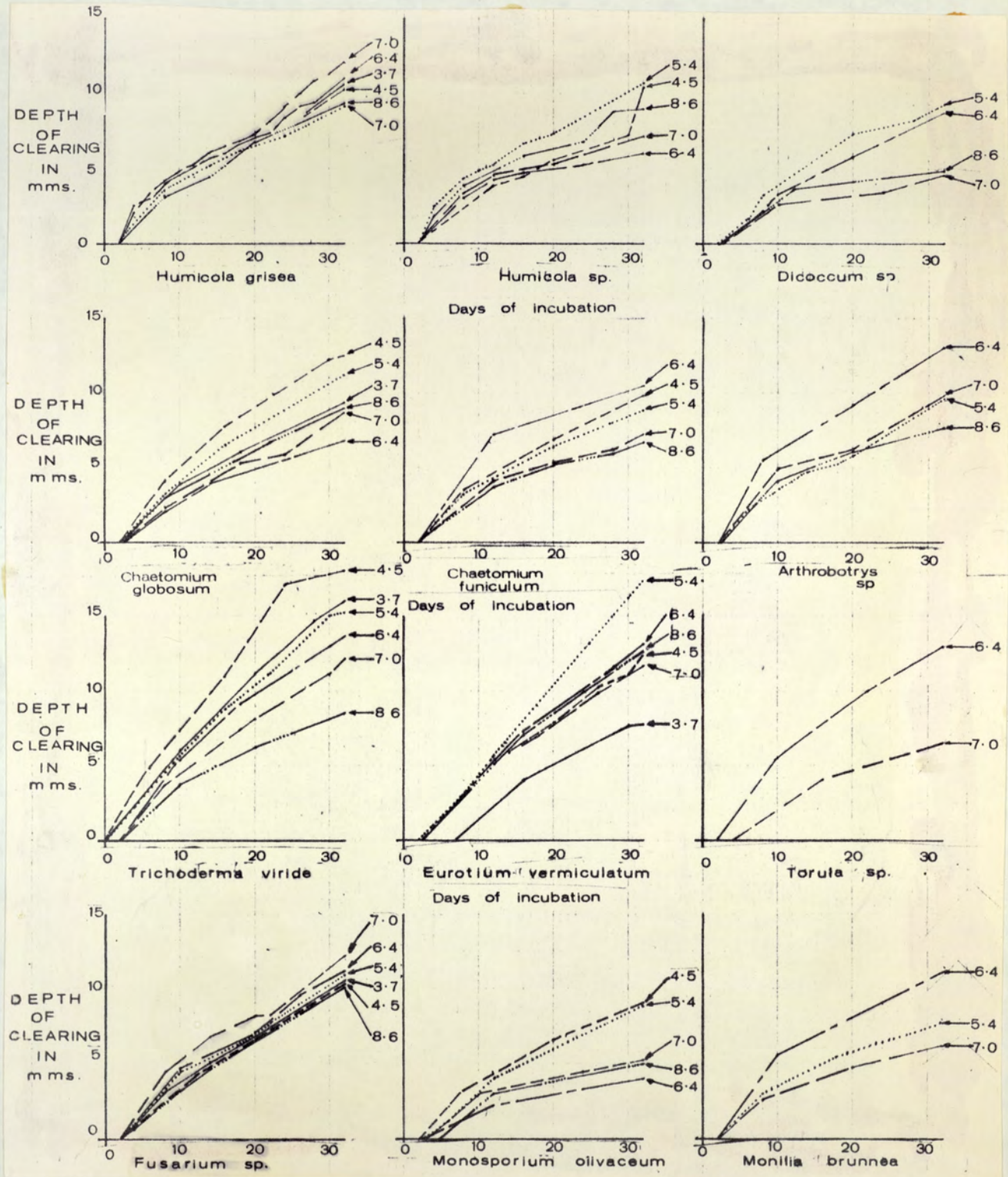


Fig. 5. Clearing rates of cellulose agar by various isolates.

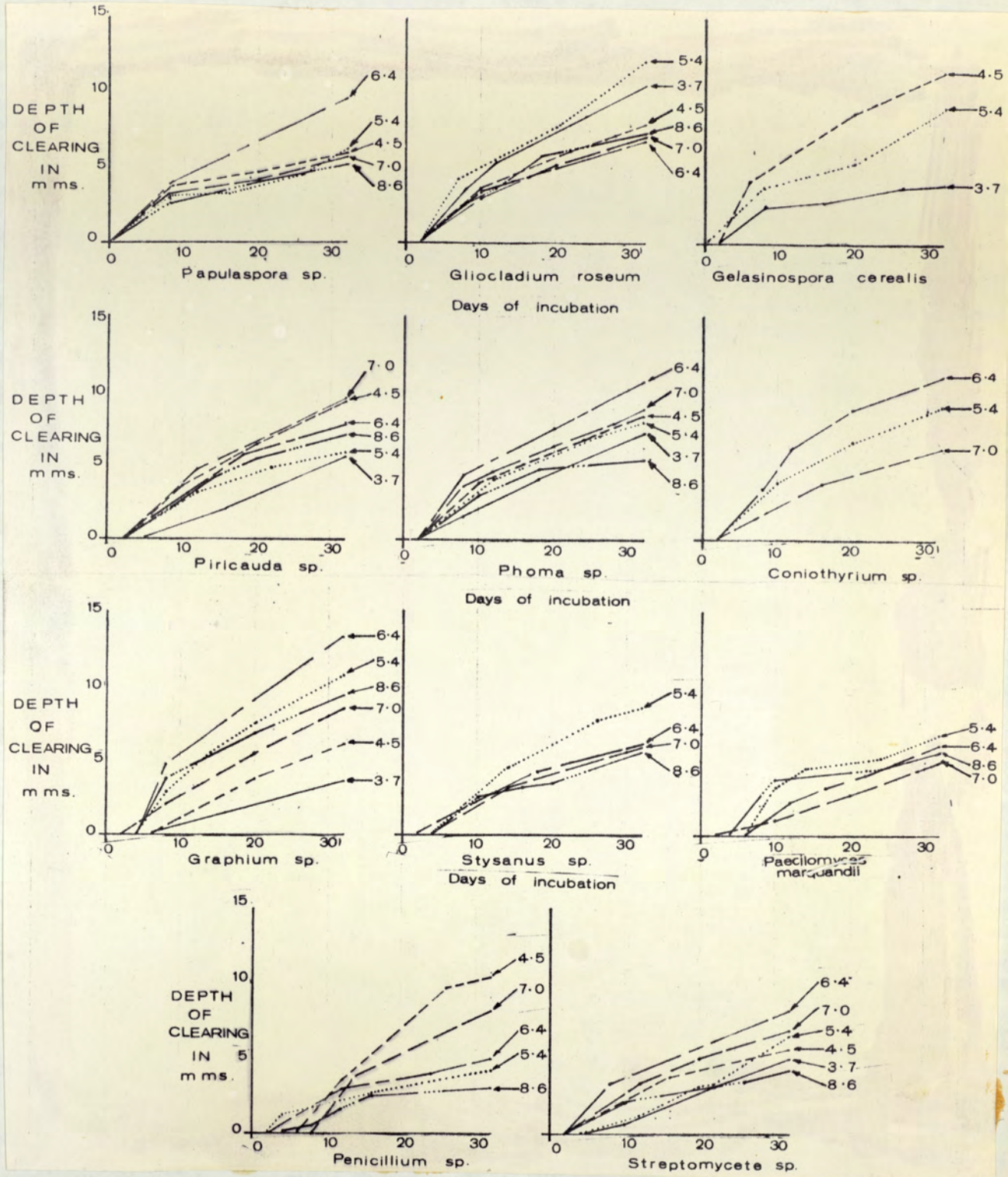


Fig. 6. Clearing rates of cellulose agar by various isolates.

The twenty-three species inoculated preferred acidic to neutral media for maximum cellulolytic activity. Five species cleared best below pH 5, the rest had optima between pH 5 and 7.

At their optimum pH twenty species cleared a zone of at least 9mm. by the thirty-second day of incubation. T.viride, E. vermiculatum, Fusarium sp., Chaetomium funiculum and C. globosum displayed the greatest clearing activity of similar magnitude to one another.

Those species least affected by pH were Humicola spp., Fusarium sp., Phoma sp. and G. roseum. This agrees with their frequency of isolation results. The rest exhibit sensitivity to at least one pH value.

T. viride gave consistent results with frequency of isolation, cellulolysis activities and in addition produced differing amounts of hyphal growth in relation to the pH. Thus luxuriant growth was formed at pH 4·5, moderate growth at pH 3·7 and 5·4, sporadic growth at pH 6·4 and 7 and only sparse growth at pH 8·6. Since this fungus has a frequent occurrence this suggests that it might be an "indicator" fungus with the amount of hyphal growth reflecting the substrate pH. It is, however, necessary to know whether other environmental factors upset this behaviour before any application.

Pronounced differences were noted between the clearing of the cellulose in the tubes and in the petri-dishes. Papulaspora sp. cleared petri-dishes rapidly as the hyphae extended outwards but cleared the tubes only where hyphae were able to penetrate into the respiration limiting agar. T.viride, Arthrobotrys sp. and occasionally Graphium sp. and Stysanus sp. colonized the agar surface in a petri-dish first and only after a certain amount of growth had formed did they clear the cellulose. In the tubes they cleared the agar in depth before much growth occurred.

The second analysis for determining cellulolytic activity of the monocultures involved inoculation on to the perfusion devices set up as before. A flamed nichrome wire was used for inoculation and incubation was allowed for 36 days at 25°C. One beechwood veneer for each species at each pH was sacrificed every three days. Its strength was measured and compared with

controls. The results are seen in the graphs of figs. 7 and 8.

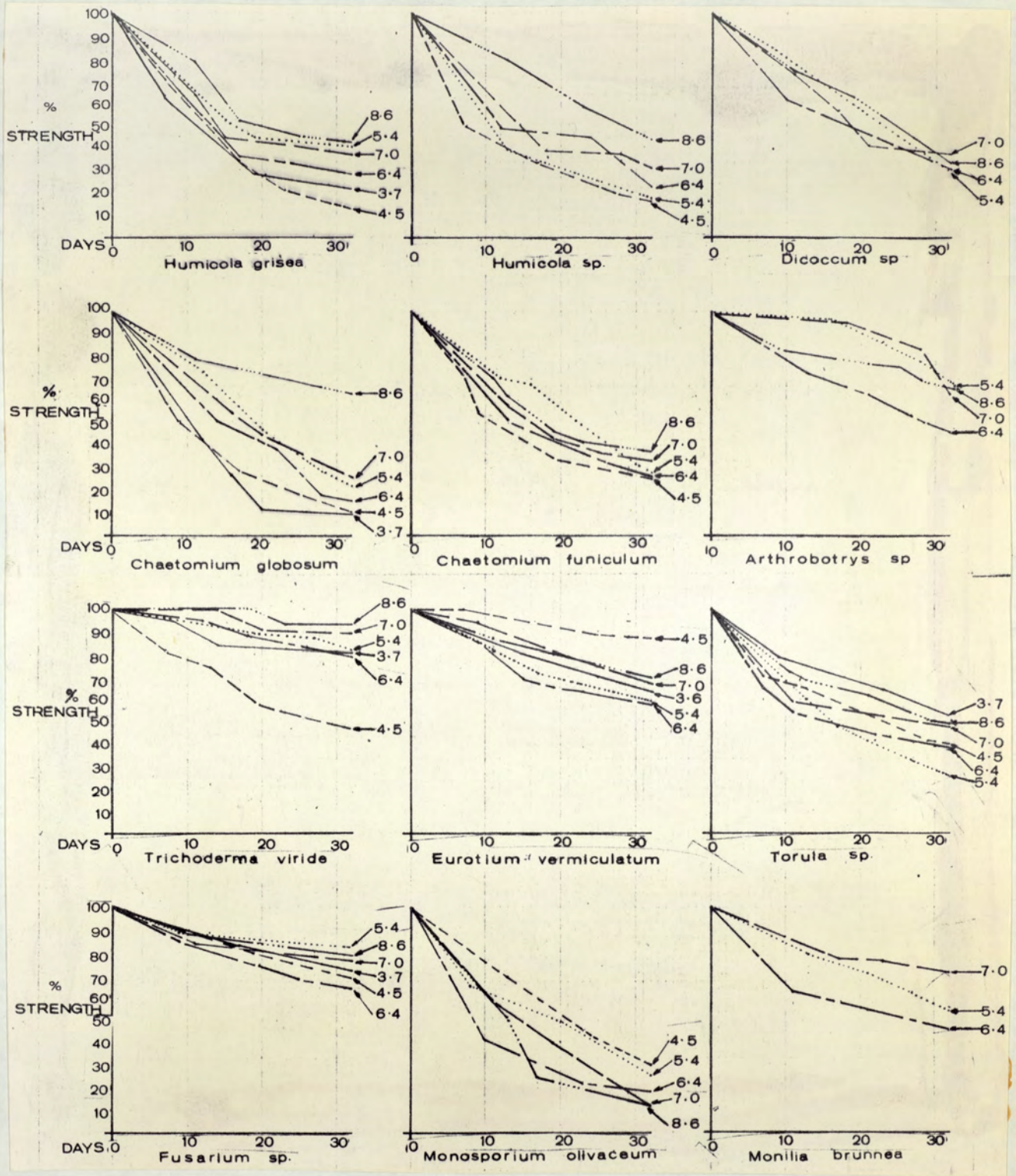


Fig. 7 Strength losses caused by various isolates at different pH levels.

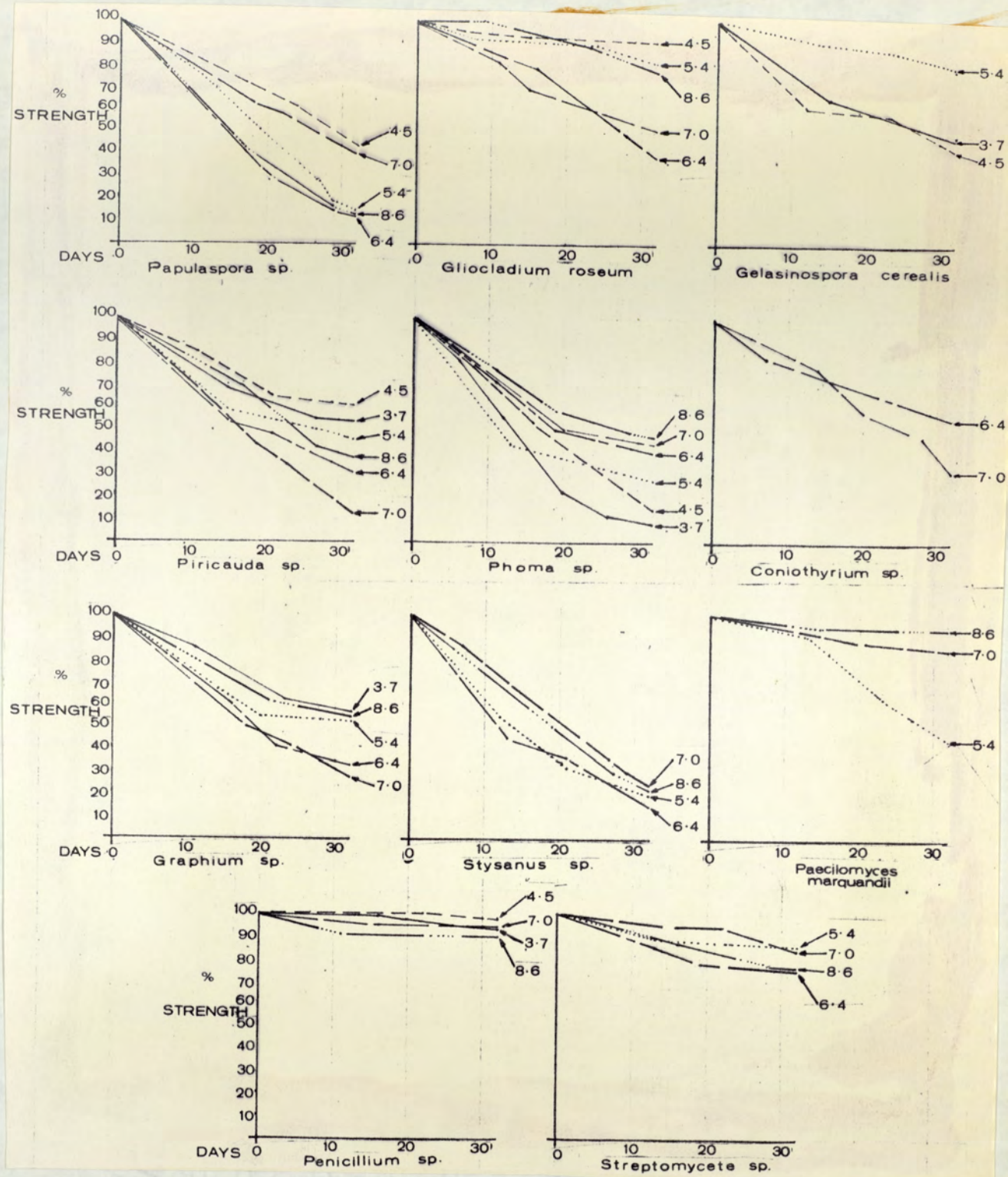


Fig. 8. Strength losses caused by various isolates at different pH levels.

Conclusions

The extent of wood deterioration from any of the isolates is less than

that obtained with the soil studies implying that polycultures are more efficient in causing degradation of wood.

Of the twenty-three species examined eighteen showed maximum decay in acidic conditions. M.olivaceum gave a definite preference for alkaline conditions whilst Piricauda sp., Coniothyrium sp. and Graphium sp. deteriorated the wood best at neutrality and Penicillium sp. and Streptomycete sp. gave so little decay as to confuse distinguishing the pH optima.

There is general agreement between the influence of pH on these results and with the cellulose clearing activities. Where there are differences it involves the favouring of more acidic conditions when cultured on the wood substrate. This arises with the Humicola spp., Chaetomium spp., Fusarium sp. and Phoma sp. The exception is M. olivaceum which gave an alkaline optimum on wood and acidic optimum on cellulose agar.

There are a few anomalies as regards the amount of clearing and wood deterioration. Fusarium sp., T. viride, E.vermiculatum, G.roseum , Penicillium sp. and the Streptomycete species produced greater clearing in proportion to their slight wood degradation. Conversely, Papulaspora sp. and Stysanus sp. caused considerable wood decay and yet were only moderate clearers of the cellulose agar. These two species warrant further investigation particularly from a lignolytic viewpoint. These differences in reaction to pH and variable activity on the two cellulosic substrates indicate the irregularities that will occur if preservatives are tested using single species, substrates or means of measuring performance.

Suprisingly T.viride at pH 4·5 and Paecilomyces marquandii at pH 5·4 reduced considerably the strength of the veneers, when at other pH values they had little effect. In the case of T. viride this could explain conflicting results of its soft-rot activity from different laboratories. Thus Greaves and Savory (1965) found no activity whereas Merrill (1965) reports 47% strength loss after twelve weeks of incubation.

There was little correlation between the frequency of isolation and

and the wood degradation ability of species. The influence that pH had , however, on varying the frequency of isolation was similar to the influence on loss of wood strength.

Discussion

It is apparent that nearly all the fungi isolated were capable of some degree of clearing cellulose agars and decomposing wood. Extreme pH conditions do not affect soft-rot growth in any extreme way but there are pH specific reactions for some of the fungi.

The elective isolation from soil of fungi at different pH's gave a pattern similar to that found by other workers. Warcup (1951) found a Penicillium sp. to be associated with acid soils, Trichoderma viride to occur over a wide range although it was only rarely found above pH 4·6 and Humicola grisea and a Paecilomyces sp. to grow at pH 6·4 to 8·4. The rarely encountered Stysanus sp. and Paecilomyces marquandii were isolated between pH 7 and 8·4. Chesters and Thornton (1956) isolated Dicoccum asperum at pH 6·8 but not pH 4·8. Isaac (1954) found Gliocladium roseum to maintain optimal cellulolytic activity between pH 6·4 and 8. Griffiths and Jones (1963) isolated Monilia brunnea and a Streptomycete sp. from soil of pH 5·5 with a potassium nitrate additive and Humicola grisea at pH 4·2 with phosphate. Latter, Cragg and Heal (1967) found Penicillium sp. and Trichoderma sp. in moorland of pH 3 to 5·6. Walsh (1968) places the optima of Humicola grisea above pH 7 and also mentions that past pH data is limited and unreliable.

Studies of cellulases have yielded results in accordance with the obtained data. The cellulases of Trichoderma viride have been shown to have a maximum activity at pH 5 by Cappellini and Peterson (1966), at pH 4·1 by Hanstein (1960) and pH 4·2 to 4·6 for Trichoderma koningii by Halliwell (1966). Other determinations are pH 4·8 to 5·2 for Chaetomium globosum (Agarwal, Verma, Verma and Sahgal 1963), pH 3·5 for Aspergillus niger (Simpson and Marsh 1964), pH 4 for commercial Aspergillus enzymes (Eriksson

1967) and two Penicillium spp. to have optima at pH 4 with a third at pH 5·5 (Garber, Beraha and Shaeffer 1965). Mandels and Reese (1965) concluded that cellulases were remarkably stable to pH.

The strength results show good correlation with the textile strength loss data of White, Darby, Stechert and Sanderson (1948). They found Chaetomium globosum, C.funiculum, Coniothyrium sp., Phoma sp., Fusarium spp., all Humicola spp. and most isolates of Trichoderma viride to be strongly cellulolytic. Gliocladium roseum gave 53% strength loss after twelve days whereas little decay occurred with the Penicillium isolates and Paecilomyces varioti. Merrill, French and Hossfeld (1965) found Trichoderma viride, Stysanus sp., Humicola grisea, Coniothyrium sp. and Papulaspora sp. to cause weight and strength loss of wood fibreboard but suprisingly Phoma sp. was inactive.

Therefore comparison with other researches using different isolates, substrates and culturing conditions reveals a constancy of reaction between pH and particular species and almost uniform strength losses produced each time by the majority of fungi.

Corbett (1963) suggested that since beechwood has a pH of 4·4 to 4·6 the initial colonizers need to withstand acidic conditions. The initial colonizing fungi isolated from the soil inoculated veneers were Humicola grisea, Fusarium sp., Trichoderma viride and the Chaetomium spp. all of which gave maximum wood degradation at about pH 4·5 and therefore have no need to overcome any inherent buffering capacity of the wood.

Any test method for the sensitive determination of a preservative's performance that used monocultures needs to meet certain requirements. The fungal species must be tolerant of wide pH conditions especially since certain preservatives change the pH of the wood to extreme levels. The fungus must also produce rapid decay and be easy to culture. These results indicate Papulaspora sp., Humicola grisea or Phoma sp. to be the most suitable soft-rot representatives.

Species Interaction

Introduction

Soft-rot fungi are pioneer colonizers of wood and have been shown to alter the wood substrate so as to permit other organisms to follow (Shigo 1967).

In the search to determine the sequential pattern of fungi colonizing wood it is necessary to consider mechanisms of fungal interactions. Interaction has been defined as "to include any activity of one organism which in some way adversely affects another growing in association with it" (Wood and Tveit 1955). It consists of parasitism, mycophagy, secretion of toxins (including antibiotics) and competition all dependent on environmental conditions.

The activities that constitute interaction and contribute towards succession are complex and best studied individually as previous investigations have done. Thus parasitism by wood microfungi was observed (Warren 1948) when Papulaspora parasitised Rhizoctonia solani Kuhn. Shigo (1958) noted Trichothecium roseum and Gliocladium roseum to be mycoparasites growing on mycelial mats of firstly Ceratocystis fagacearum and later C. coerulescens (Shigo 1962). Shigo (1960) has also found that most species of Ceratocystis, Graphium and Leptographium are parasitised by Gonatobotryum fuscum and under certain laboratory conditions Graphium can reverse the roles and attack the parasite.

An example of mycophagy was demonstrated (Tribe 1960) when nematodes, mites, collembolans and enchytraeid worms were seen to complete decomposition of fungal colonised cellulose film.

Antibiosis has been inferred in the numerous inhibitions Trichoderma sp. has caused on Rhizoctonia solani (Weindling 1934), on wood rotting fungi (Lindgren and Harvey 1952), on Fomes annosus (Persson-Hüppel 1963) and on

four storage rot fungi (Shields and Atwell 1963). A growth inhibitor produced by Cryptosporiopsis sp. will inhibit a variety of fungi (Stillwell 1966). Of the fungi isolated from conveyer belting by Stewart (1968) the slightly cellulolytic species were demonstrated to be less liable to inhibition when tested together on cotton yarn. Many of the fungi able to resist inhibition were producers of antibiotic substances but Stewart could not establish whether such a mechanism was important in this ecosystem.

Competition has been defined by Donald (1963) as when two or more organisms seeks the measure each wants of any factor or thing and when the immediate supply of the factor or thing is below the combined demand of the organisms. Barlund (1950) suggested primary colonizers might utilise wood substances and prevent later colonization of secondary colonists. Fast growing fungi will therefore have an advantage. Studying natural habitats Ueyama (1965) found Ceratocystis moniliforme to be an early coloniser of beech logs but with the decline of nutrients and moisture the active wood deteriorating Shizophyllum commune grew.

The environmental influence was exemplified by Liese and Eckstein's (1967) study of ten soft-rot species with varied cellulolytic ability. They obtained no significant inhibition or stimulation with water saturated wood but lower weight losses occurred with air-dried samples.

It is with this background of information that an interaction study between soft-rot fungi has been made so that the influence of this ecological factor on wood deterioration can be assessed. Such a study can consider interaction in the soil, on a host or on artificial media but it is difficult to relate observations between the three environments. The environment selected for this study consists of sterile beechwood veneers supplied with supplementary nutrients, constant temperature and high moisture conditions. This model system might not properly simulate colonization of virgin beechwood timber in a natural climate but it will allow reaction between known species

and diffusion of fungal secretions. It will not incur interference from dead mycelia, other organisms or drastic chemical and physical changes of the substrate that otherwise complicate field studies.

Experimental

Strips of beechwood veneer 6cm. x 6mm. x 0·6mm. were cut and marked off with lines spaced apart at one centimetre intervals. They were autoclaved in the mineral salts of E. and P. cellulose medium, pH 5·4, before being place on agar medium of this formula held in a petri-dish. The wood was aseptically manipulated with alcohol flamed forceps.

Soft-rot species and other microfungi isolated from deteriorated beechwood were inoculated on to the veneer strips in pairs simultaneously. One species was placed at one end of the strip and another species at the other end. Inoculation was performed using a flamed nichrome wire and the inocula were taken by cutting approximately one centimetre square pieces from the colony edge of growing fungal cultures on cellulose agar. Controls were arranged with inoculation of individual species at one end only of the strip.

Incubation was undertaken at 25°C. for fourteen days. The arrangement can be seen in diagram 3.

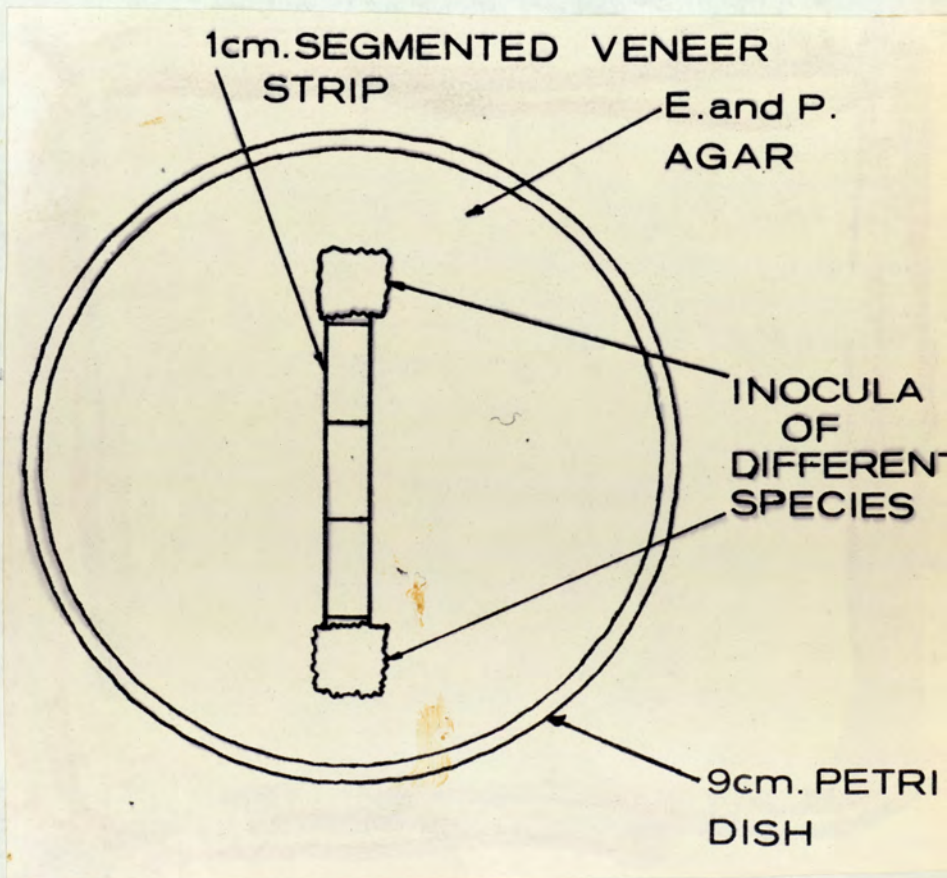


Diagram 3. Interaction culture system with two fungi inoculated on to a veneer strip.

Afterwards the extent of growth along the strips was measured and any hyphal intermingling noted. This absence or appearance of intermingling was taken to be a reflection of interaction. If two species intermingled mutually then this was considered as compatability whilst mixing of colony edges or no mixing was regarded as grades of inhibition. Porter (1924) used five growth criteria for classifying reactions and these were:- mutually intermingling, overgrowing, slight inhibition, growth around and inhibition at a distance. He found that the richer the medium in nutrients the less marked were the inhibitions, that slight variations occurred with amount of inoculum, time of inoculation and depth of medium and that inhibition qualities of a fungus might aid in its identification.

For this exercise only the absence or presence of hyphal intermingling were considered but these reactions were recorded for five points each one centimetre apart between the two inocula. At these five points the strength retained after the fourteen days deterioration was also recorded so that the decay rate could be correlated with the hyphal mixing. This model arrangement therefore allowed hyphal reactions to be compared with wood degradative activities at equidistant points between two different microfungi growing towards each other.

The bending strength at each centimetre mark was measured using the tensiometric technique so that any influence on the wood deterioration along the strips for each species will be revealed.

The extents of hyphal mixing and the strength results are enumerated as a series of graphs, figs. 9 to 14.

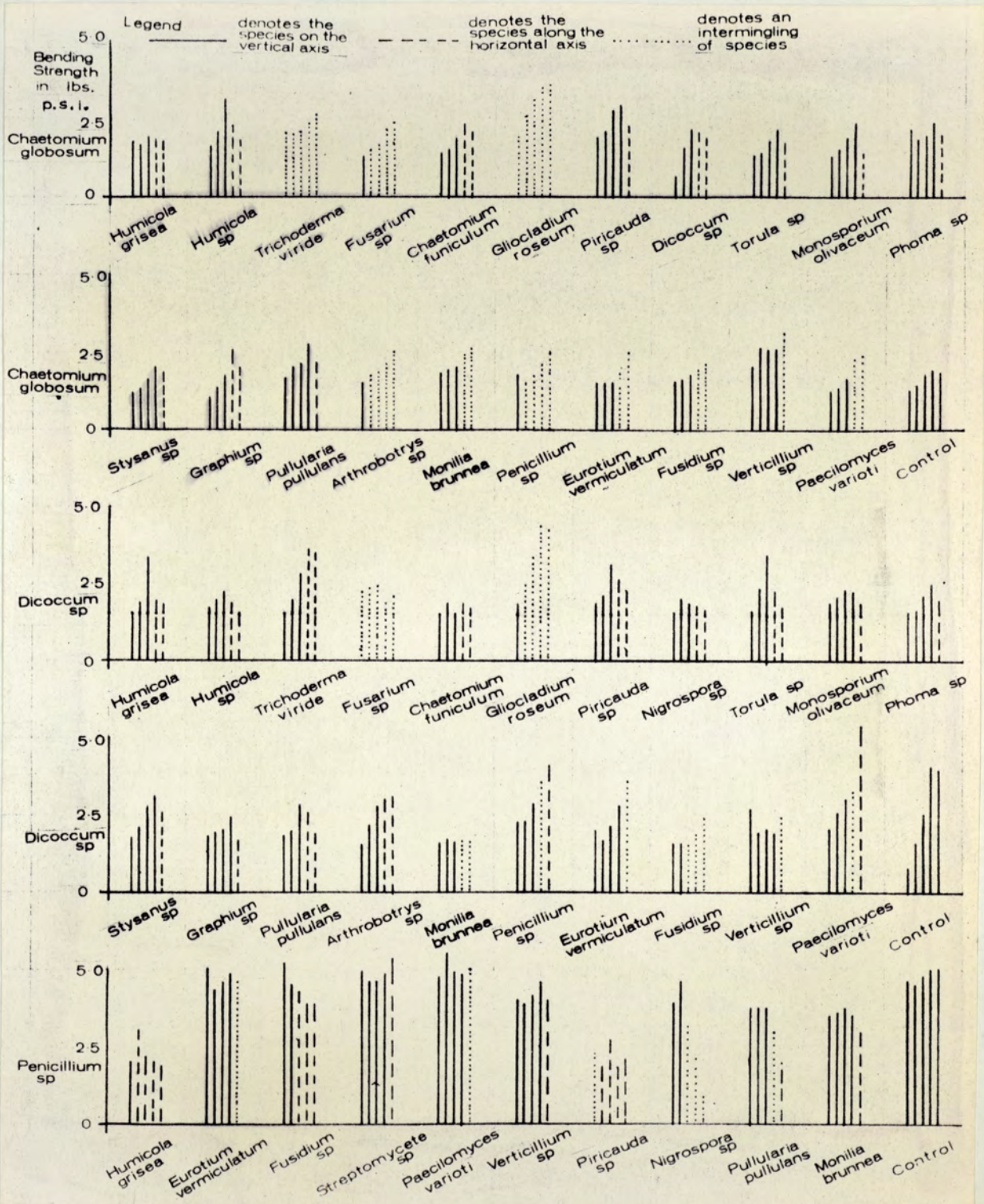


Fig. 9. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

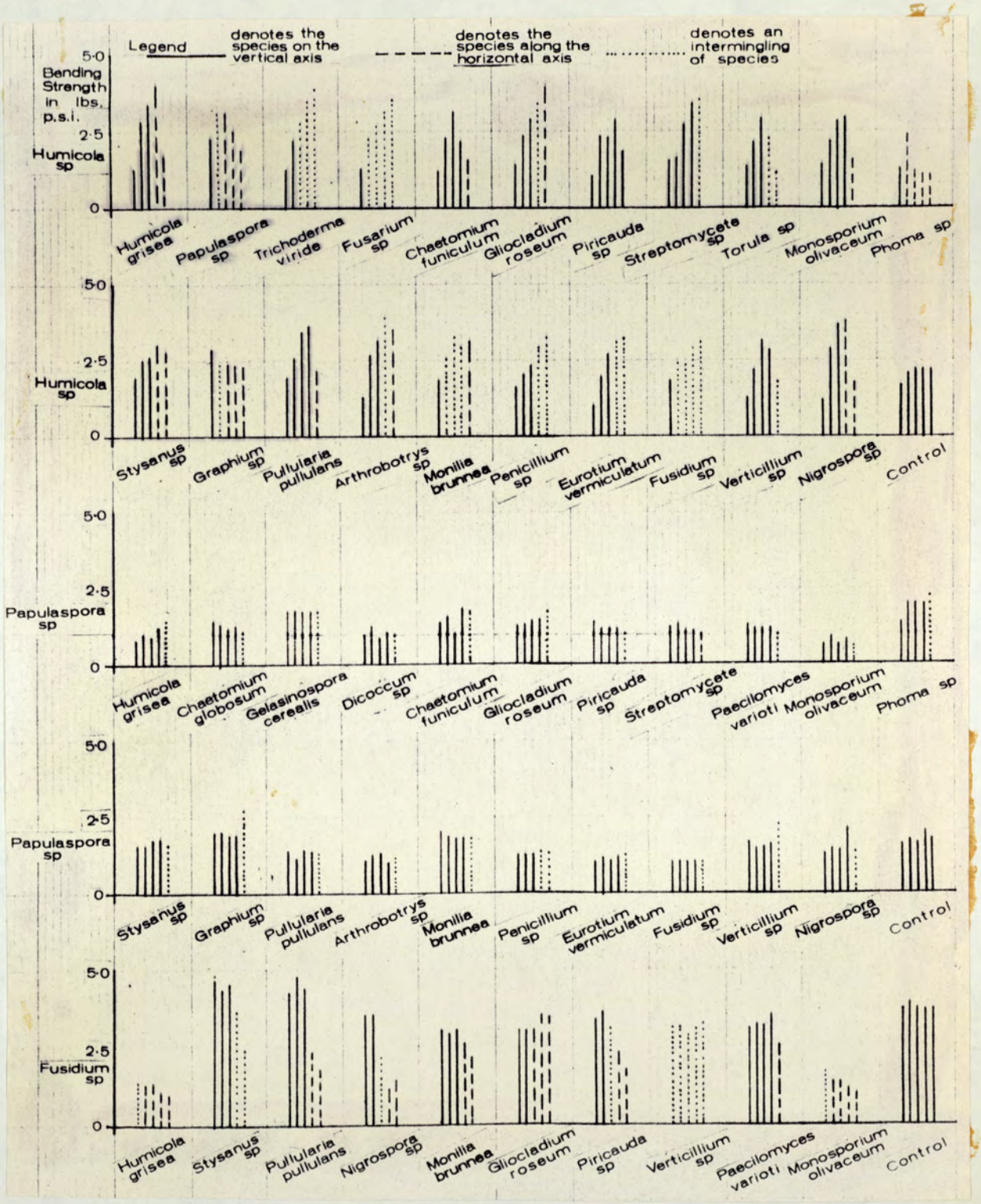


Fig. 10. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

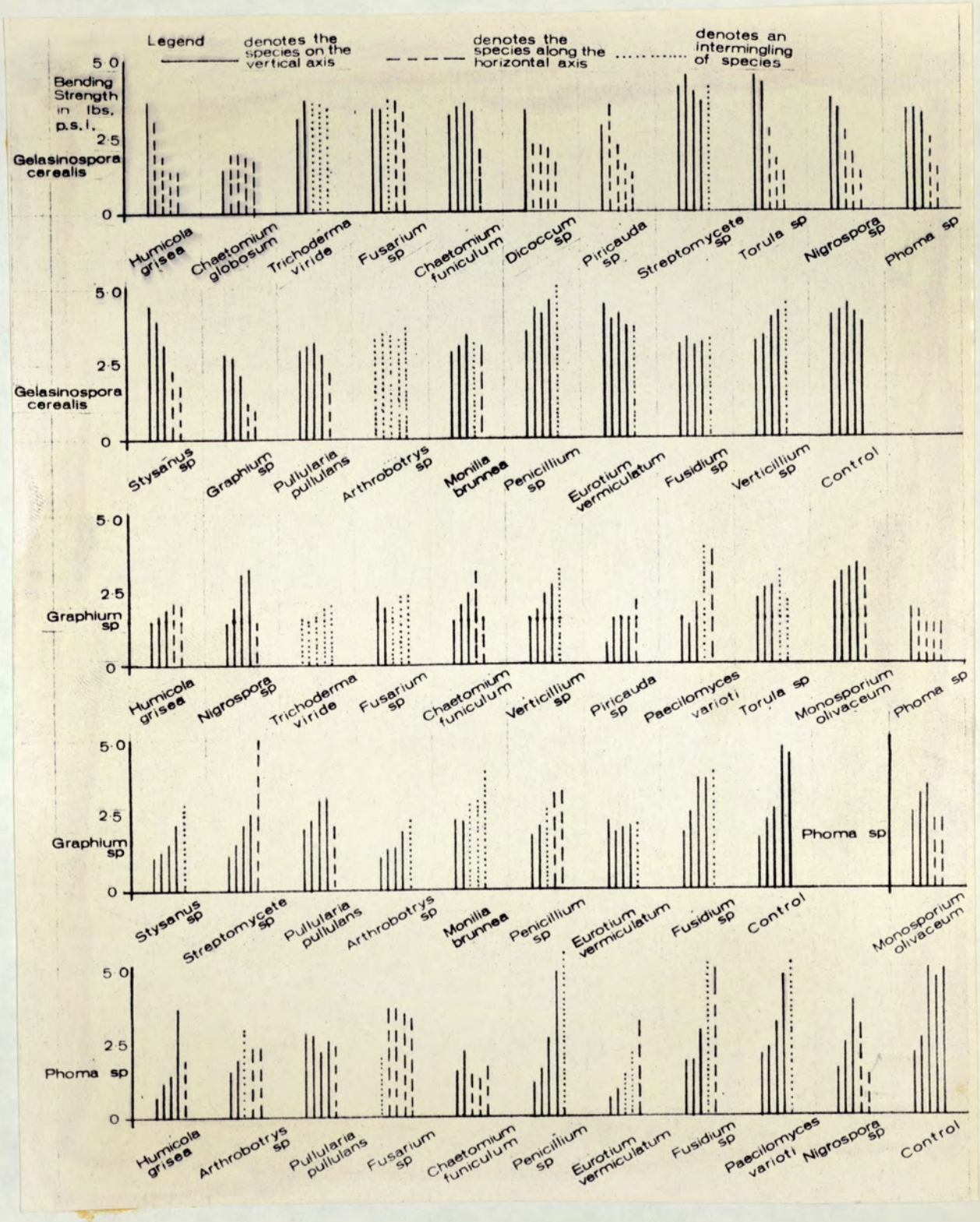


Fig. 11. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

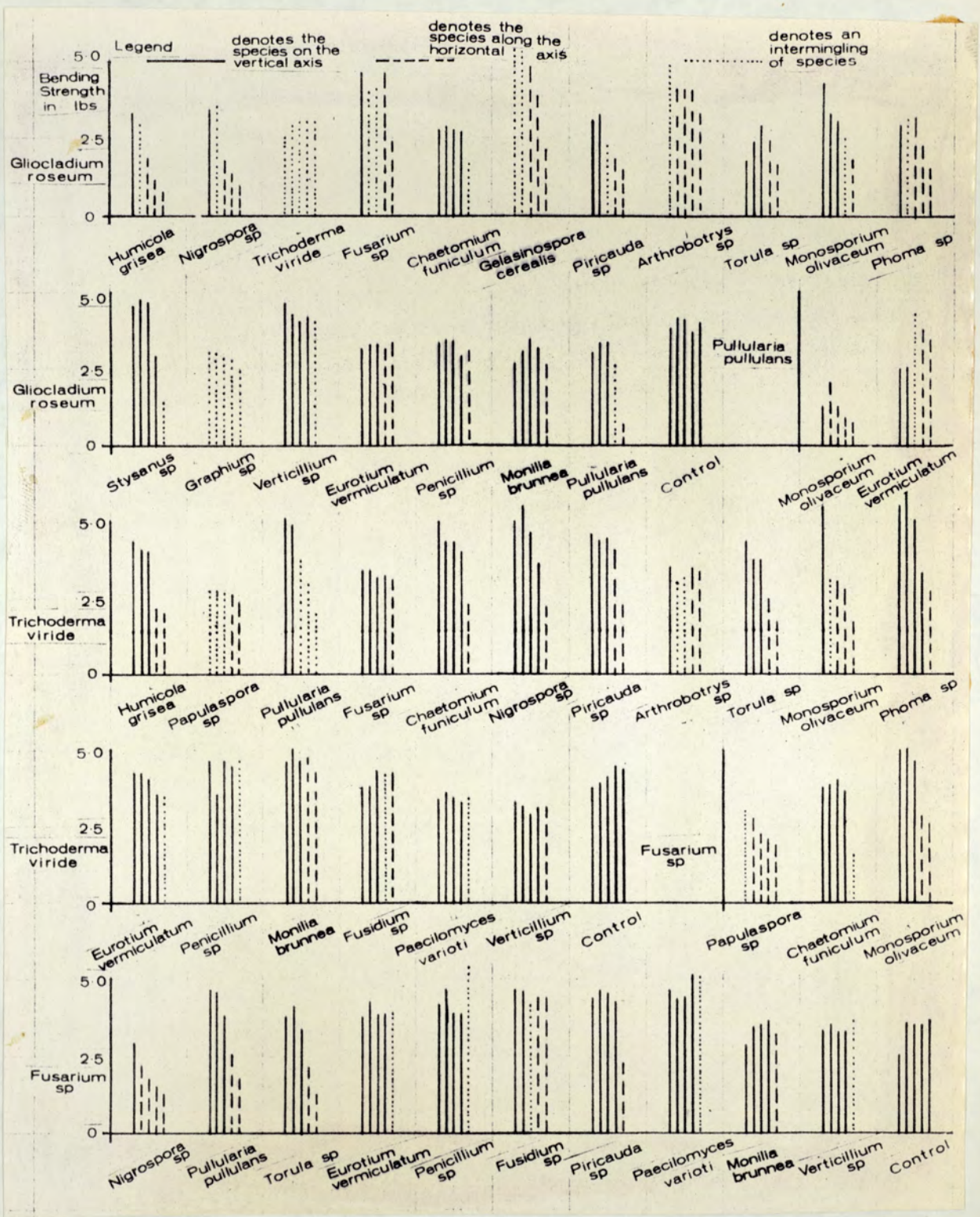


Fig.12. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

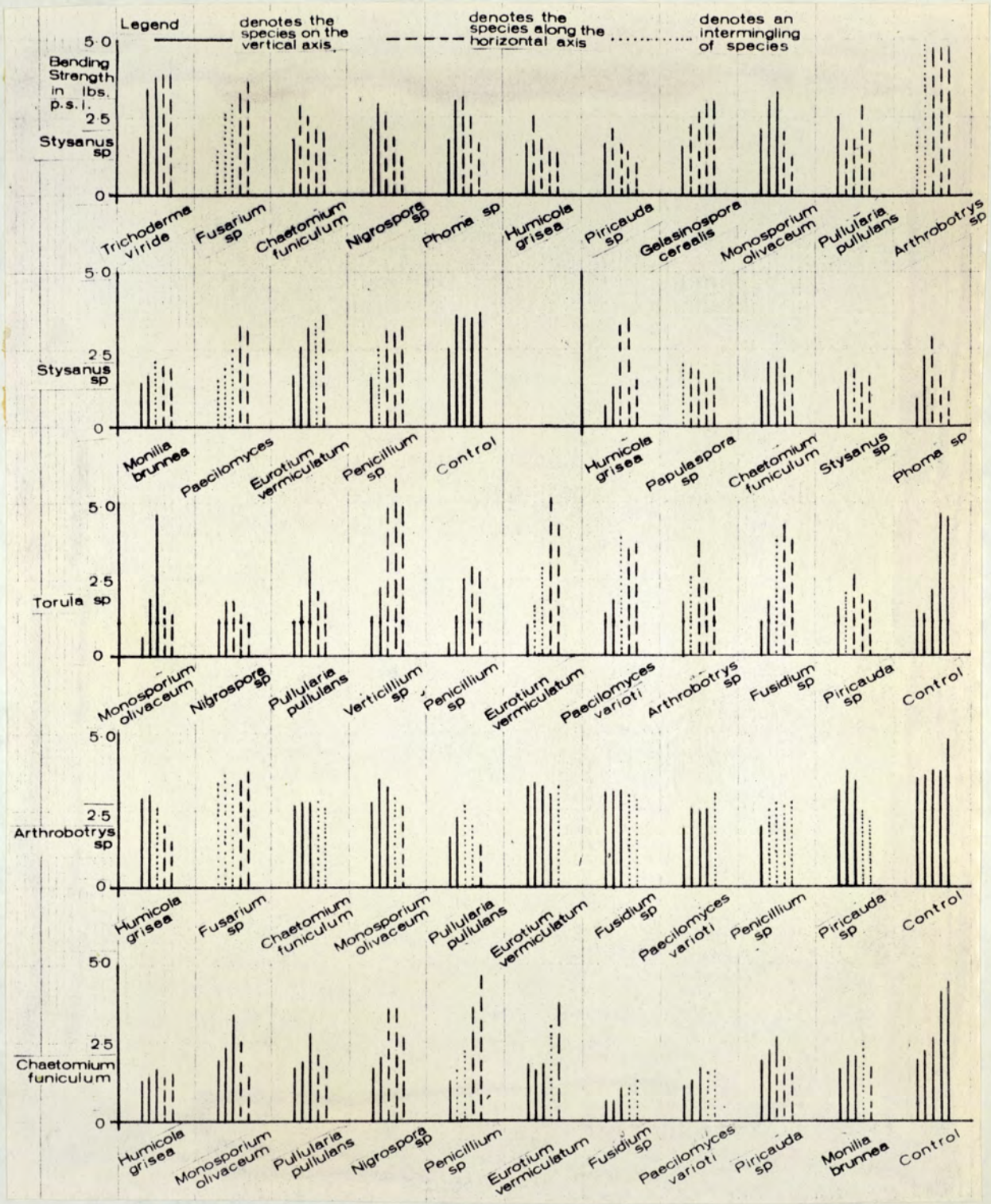


Fig.13. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

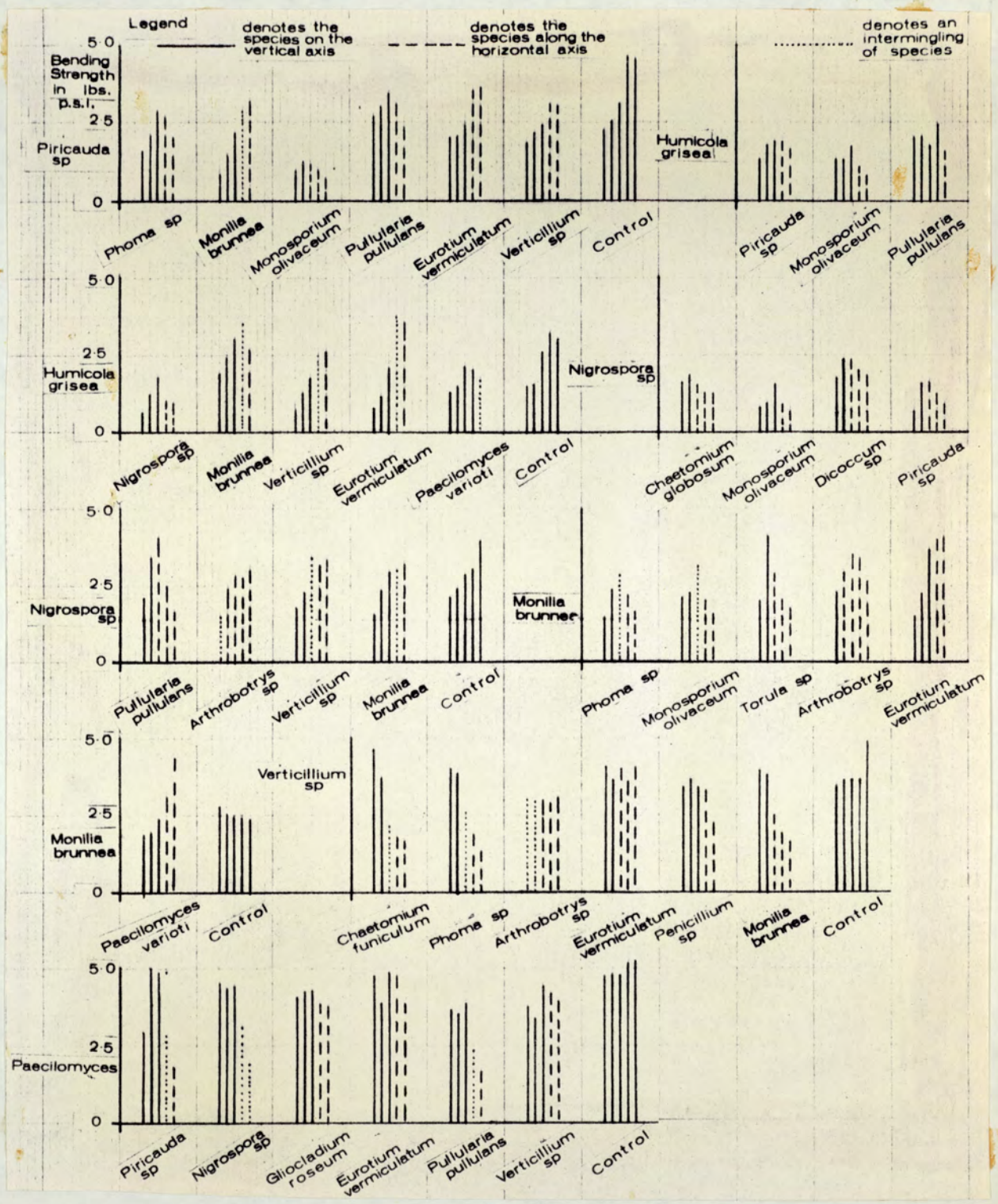


Fig. 14. Strength loss measurements taken along the veneer strips for each interacting dual culture with indication of any hyphal intermingling.

Conclusions

Of the twenty-six isolates analysed it was found that if the two inoculated species did not mix the normal level of deterioration these fungi usually give was repeated here. If the two species mixed then without exception the amount of decay in the area of mixing was equal to that normally produced by the most cellulolytic species of the two.

No distinct antagonism whereby the decay activity of a species was completely prevented occurred and similarly no one fungus enhanced or inhibited all the fungi it encountered. There are, however, examples of partial inhibition and enhancement of one fungus on another but since there is no pattern to these results they are probably manifestations of varying "inoculum potential", incubation conditions etc.

The outstanding inhibitions include Gliocladium roseum reducing the activity of Chaetomium globosum but being affected itself by Gelasinospora cerealis. Pullularia pullulans reduced the activity of Humicola sp. The reaction between Graphium sp. and Monosporium olivaceum was a mutually inhibiting effect.

There were many more examples of synergism with one species increasing the wood degrading efficiency of another species on comparison with controls. This suggests bicultures are more degradative than monocultures.

Few macroscopical morphological aberrations were observed and most of these involved the formation of different amounts of aerial hyphae where two growths met.

The ability to mix or not was found to be related to the degree of cellulose activity. The pattern of mixing allowed arrangement of species into groups of equal cellulolytic status. The grouping is illustrated in diagram 4. All fungi of the same group do not mix with each other. Fungi of different groups intermingle and this is represented by the arrows in the diagram.

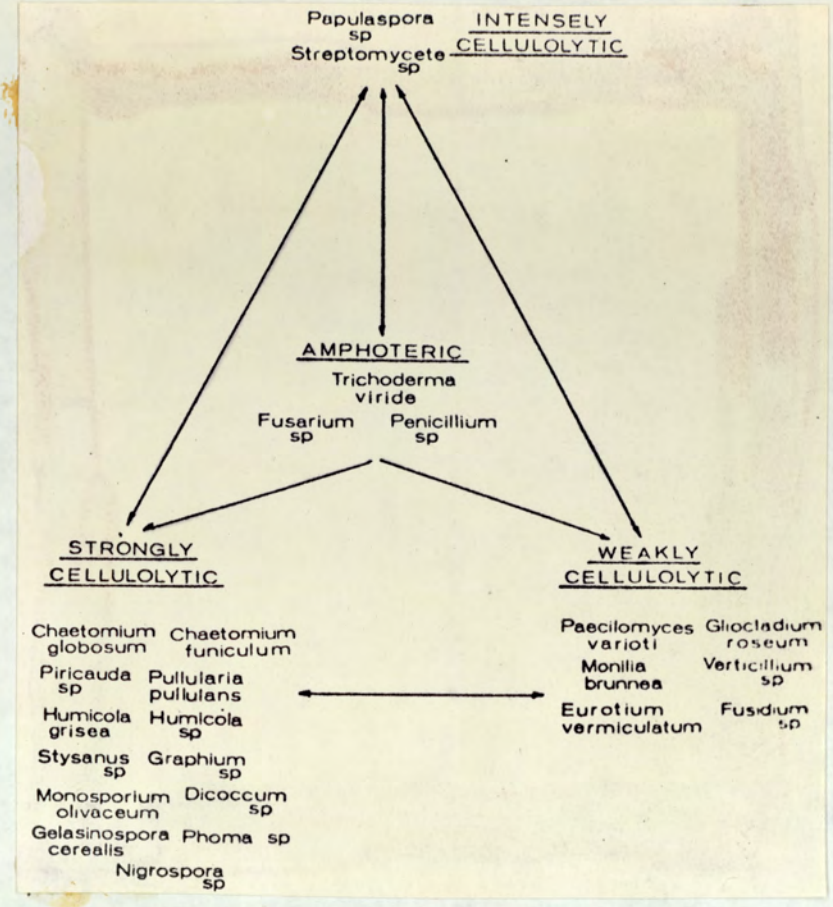


Diagram 4. Fungal groupings based on the ability to intermingle or not with other fungi. The interconnecting arrows represent those interactions which allow mixing. Each group consists of fungi unable to mix with one another.

Some species mixed at their colony perimeters only and this was considered an incomplete reaction of not mixing. Some species like Fusarium sp. and some mixtures like Humicola grisea and Humicola sp. proved impossible to detect their edges of growth so estimations were made based on the extent of growth across the agar surface.

The only exceptions to these groupings were Graphium sp. mixing with Stysanus sp. and Humicola sp. Verticillium sp. mixed with Fusidium sp. G. roseum and Penicillium sp. mixed with E. vermiculatum and P. varioti.

Arthrobotrys sp. tended to mix with all but six fungi. This suggests

a metabolism that is different and unaffected by the activities of other species. This might be fungal neutralism.

Penicillium sp., Fusarium sp. and Trichoderma viride gave no ordered pattern and probably react amphoterically according to the prevailing conditions of substrate and environment. These fungi are known to actively secrete toxins and antibiotic substances and so might mix only when these substances have reached a threshold of concentration.

Torula sp. tended to mix with the weakly or non-cellulolytic group and not with the strongly cellulolytic fungi. However, there were exceptions causing doubt as to its exact placement.

Papulaspora sp. and Streptomycete sp. were ostensibly capable of mixing but microscopical examination revealed that they grew respectively under and over other species.

Discussion

The model culturing system has allowed some aspects of the behaviour of interacting fungi to be characterised. The information obtained might provide a basis for predicting behaviour in natural systems. Thus with a plentiful supply of cellulose the intensely and strongly cellulolytic groups of fungi might secure a maximum utilization of the substrate resources without mixing and encroaching upon the preserve of each other. The spatial separation of these primary wood degraders would allow rapid and widespread deterioration. Individual species of the weakly cellulolytic group of fungi might mix freely with the previous group and have a more satiable rate of cellulose decomposition. In this way there might be complete utilisation of available cellulose. The amphoteric fungi might be responsible for primary colonization or be evident with a change of sere. They would allow mixing initially but perhaps upon exhaustion of the substrate become competitive. Such postulates might be fanciful or satisfy the stability within each group of no intermixing and diversity between groups of differing cellulolytic

activity. It does not explain the sequence of succession nor the highly competitive interactions reported by Tribe (1966).

One possible outcome is to rapidly define the cellulolytic activity of newly isolated species by their ability or inability to mix with neighbouring fungi of known cellulolytic capability when seen on cellulose agar cultures.

Colonization and Penetration

Introduction

The ability of a fungus to colonize, penetrate and ramify throughout timber demarcates it as a specialised organism adapted to a lignicolous habitat. Those fungi able to rapidly colonize and penetrate thoroughly in wood will have an advantage over others. Therefore colonization patterns and penetration behaviour are decisive factors for successful fungal growth and important aspects of wood fungi ecology.

The study of colonization patterns of microfungi on wood in contact with soil has only recently invoked interest. Observations on decomposing leaf litter have been made with beech (Caldwell 1963; Hogg and Hudson 1966), oak (Hering 1965), Scots pine (Hayes 1965) and Eucalyptus (Macauley and Thrower 1966). The dominant species for each substrate appears to be host-specific and succession occurs with a few initial colonizers followed by a larger number of secondary species.

The first study involving timber by Corbett (1963) found the succession of fungi on various wood stakes to be in the order, Moniliales group 1. with Penicillium spp. and Trichoderma viride; Sphaeropsidales; Moniliales group 2. with Gliocladiopsis sp. and Cylindrocarpon sp. and finally invasion by basidiomycetes. Merrill and French (1966) described a similar sequence with soft-rot decay on stakes of Pinus ponderosa Laws. Fusarium solani (Mart.) Appel and Wr. emend. Snyd. and Hans. was the first fungus to colonize the wood and was dominant for five weeks until replacement by Trichoderma viride Pers. Together with species of Aspergillus and Penicillium they accounted for 92% of all isolates. Jones (1963) has demonstrated a succession of marine fungi on wood in the sea.

Butcher (1968) found Epicoccum nigrum Link to be the primary colonist of pine stakes in the above-ground zone and together with Fusarium spp. to

be the primary colonizers at the ground-line. Below ground Zygorhynchus moelleri Vuillemin was initially dominant to be replaced after one month with Trichoderma viride Pers ex. Fr. Secondary colonizers above-ground were members of the Dematiaceae to be followed by T. viride and Penicillium spp. Secondary infections at and below the ground-line included T.viride, Gliocladium spp., Penicillium spp. and Verticillium sp.

Penetration behaviour has never been studied in detail with soft-rot fungi on wood. They are considered to cause shallow deterioration unlike the deep penetration of basidiomycetes. Price (1961) has shown the rate of progression into louvre packing in water cooling towers to be 1/30 inch per year on all surfaces. Butcher (1968) observed $\frac{1}{8}$inch penetration in preservative-treated stakes but 1/16 inch to $\frac{1}{8}$inch penetration in untreated ones. Savory (1955) found soft-rot to a depth of 1/16inch on creosoted telegraph poles and railway sleepers and expressed belief that with no oxygen shortage there would be no limit to the depth of penetration. However, Boutelje and Kiessling (1964) isolated microfungi from considerable depths within shipwreck timber recovered from the Baltic sea. Verticillium malthousei was isolated 0·5cm. beneath the surface and a Penicillium sp. together with other unidentified fungi were found up to 10cm. below the surface.

It is therefore apparent that a beginning has been made for defining the order of fungal succession on cellulosic substrates with further work needed on additional environments including artificial laboratory models. More understanding is also required on the extent of fungal penetration of all worked timber and the relation of rates of penetration to succession times. Some of these shortcomings are the object for this experimental study with reference to the colonization and penetration of model beechwood veneers by soil microfungi.

The first aspect studied was the growth rates of isolated microfungi. This was because if observations are to be made on the temporal nature of

colonizing and penetrating fungi it is necessary to obtain controls whereby the fungi are inoculated on to a wood substrate and allowed to grow over the surface at their own growth rates. These speeds are then an indication of probable colonization and penetration rates.

Experimental

The rate of hyphal growth of twenty microfungi along a beechwood surface was determined by cutting veneer strips 8cm. x 6mm. and marking off one centimetre lines along the strip. The pieces were autoclaved in E. and P. salts and aseptically placed on E. and P. mineral agar using alcohol flamed forceps. One species was inoculated on to the end of a strip with a flamed nichrome wire. Four strips were inoculated for each species and then the petri-dishes were incubated at 25°C. for varying times. The length of mycelial spread for each veneer per day was noted until the growth had reached the end of the strip.

The results are seen as graphs of hyphal growth distance against days of incubation, figs.15 and 16. Although the veneers were 8cms. long the centimetre square pieces of fungal inoculum occupied the first centimetre marked on the wood and therefore the length of permitted fungal coverage was only 7cms.

Conclusions

There are definite differences in growth rates between the species examined and this allows a grouping into at least three divisions.

Fast growing fungi	Intermediate fungi	Slow growing fungi
Trichoderma viride	Humicola spp.	Phoma sp.
Fusarium sp.	Gliocladium roseum	Dicoccum sp.
Chaetomium spp.	Penicillium sp.	Monosporium olivaceum
Papulaspora sp.	Arthrobotrys sp.	Stysanus sp.
Gelasinospora cerealis	Periconia sp.	Streptomycete sp.
Coniothyrium sp.	Paecilomyces varioti	
Eurotium vermiculatum		

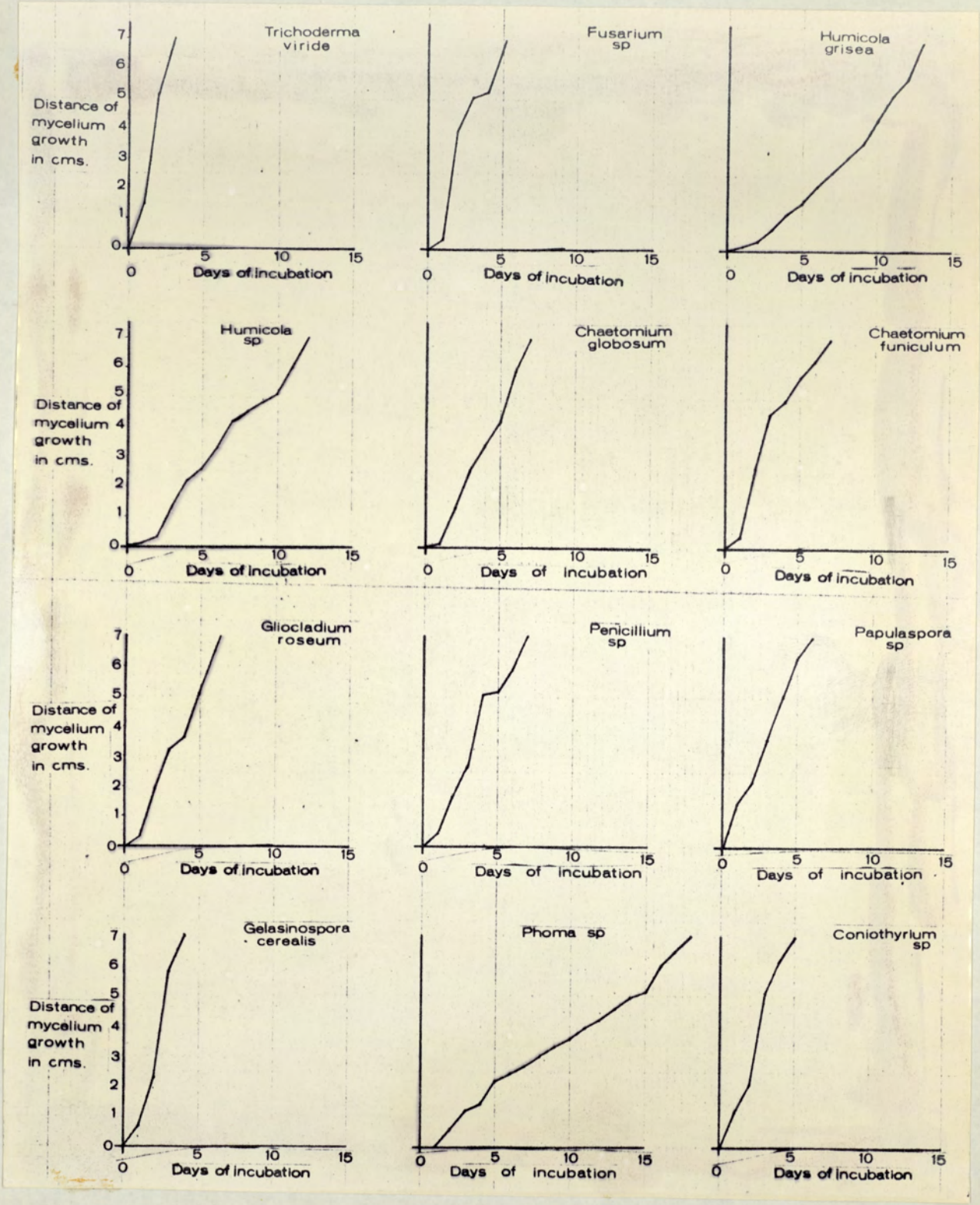


Fig. 15. Graphs of hyphal growth rates along a veneer strip surface.

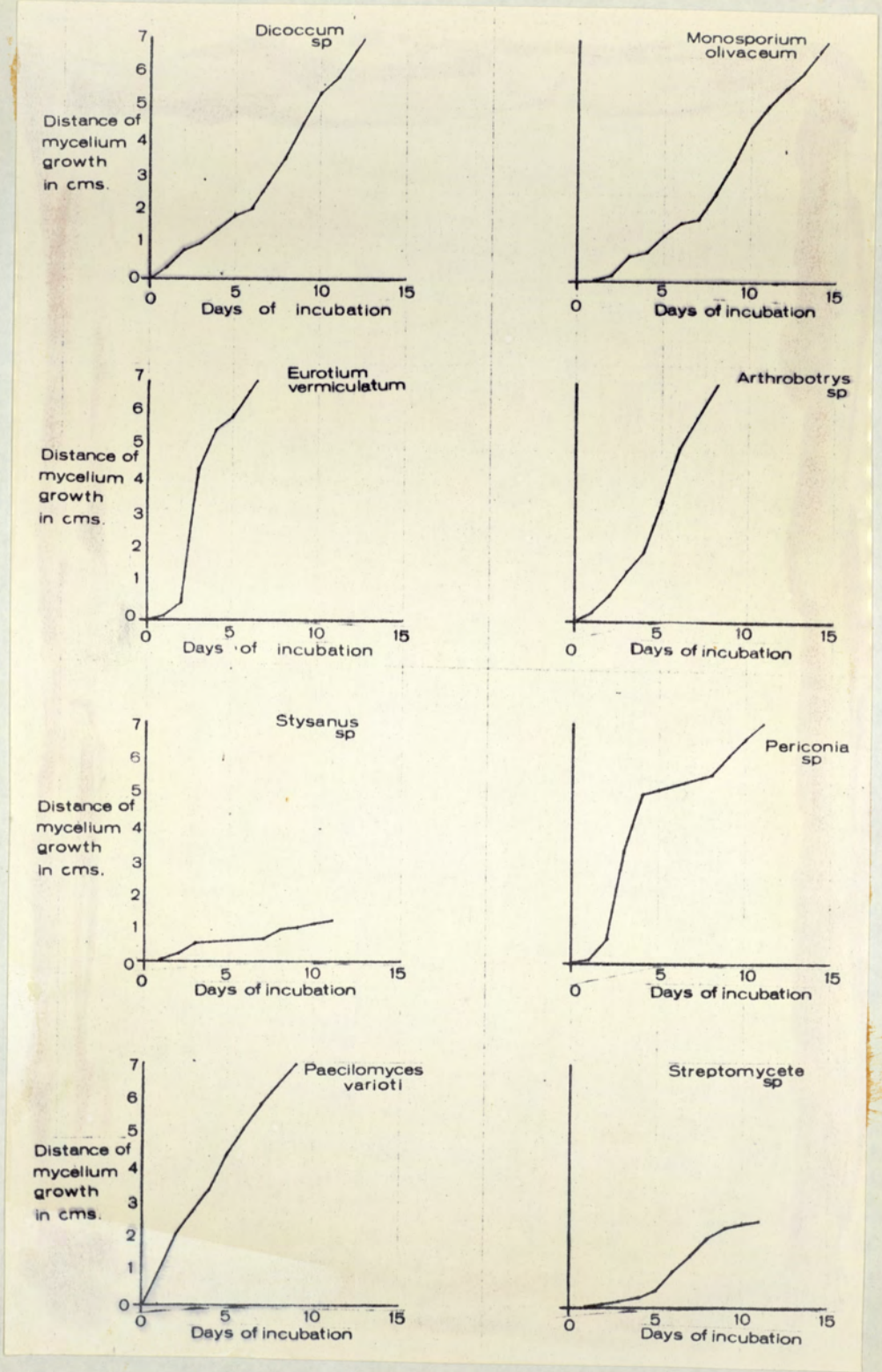


Fig. 16. Graphs of hyphal growth rates along a veneer strip surface.

There were small variations between cultures of the same species due to variable "inoculum potentials " and culture conditions. The species displaying this variation were Eurotium vermiculatum, Gliocladium roseum and Phoma sp.

Most species grew in a radiating manner along the wood and across the agar surface. Fusarium sp. and Humicola sp. tended to grow more along the wood than on the agar. The perithecia of the Chaetomium spp., pycnidia of Phoma sp. and the coremia of Stysanus sp. were found to prefer to grow on the wood surface rather than the agar surface. These observations might be the result of physical, nutrient or chemical stimulation by the wood.

Establishment of individual growth differences leads to the next determination of the relationship between these growth characteristics and wood strength deterioration patterns.

Experimental

To investigate this relationship the same experimental method as used previously was repeated so that along each sacrificed veneer there occurred a range of growth with comparatively old mycelia next to the inoculum and new growth some length along the veneer. The extent of mycelial growth was measured as before but this time, however, the loss of strength at each of the one centimetre marks was determined as well. Thus the gradient of decay in relation to the range of growth was found.

The results were:-

Species inoculated and their mycelial growth.		Strength in lbs. p.s.i. at each centimetre mark from the inoculation point						
		1cm.	2cms	3cms	4cms	5cms	6cms	7cms
Trichoderma viride	Control	4·75	4·2	4·8	5·2	5·0	5·3	5·0
7cms. growth over	Exposed	5·0	4·75	4·3	5·2	4·75	5·25	5·5
5 days.	% Strength	105	113	89	100	95	99	110

Species inoculated and their mycelial growth.		Strength in lbs. p.s.i. at each centimetre mark from the inoculation point						
		1cm	2cms	3cms	4cms	5cms	6cms	7cms
Fusarium sp.	Control	5·25	4·6	4·75	4·75	4·4	4·75	4·8
7cms. growth	Exposed	3·2	3·4	3·7	3·25	3·5	3·5	3·5
over 5 days.	% Strength	60	73	77	68	79	73	72
Humicola grisea	Control	4·7	4·3	5·1	4·75	4·7	4·2	4·1
3·8cms. growth	Exposed	1·2	1·75	2·5	4·4	4·4	4·4	4·4
over 14 days	% Strength	25	40	49	92	93	104	107
Humicola sp.	Control	4·75	5·0	4·75	4·75	4·5	4·75	5·0
5·8cms. growth	Exposed	0·8	1·0	0·75	0·85	1·4	2·1	5·0
over 18 days	% Strength	16	20	15	17	31	44	100
Chaetomium globosum	Control	6·25	5·3	4·0	5·0	4·9	5·2	5·0
7cms. growth	Exposed	2·5	2·25	2·5	3·0	3·1	3·2	4·0
over 8 days	% Strength	40	42	62	60	63	61	80
Chaetomium funiculum	Control	4·8	4·9	4·75	4·75	4·9	4·7	4·7
7cms. growth	Exposed	1·75	1·75	1·75	2·3	2·5	2·5	3·75
over 12 days	% Strength	3 6	35	36	48	51	54	79
Gliocladium roseum	Control	5·5	5·5	5·4	5·3	5·5	5·3	5·75
7cms. growth	Exposed	5·5	5·5	5·2	5·2	5·2	5·2	5·2
over 14 days	% Strength	100	100	96	99	94	99	90
Papulaspora sp.	Control	4·5	5·0	5·0	4·3	4·75	5·25	4·75
7cms. growth	Exposed	1·75	1·6	1.75	2·25	2·25	2.7	2.9
over 5 days	% Strength	38	32	35	52	47	51	61
Gelasinospora cerealis	Control	4·5	4·75	4·75	4·9	5·0	5·0	5·0
5·9cms. growth	Exposed	4·2	4·2	4·2	4·5	4·4	4·4	4·4
over 4 days	% Strength	93	88	88	91	89	88	88
Phoma sp.	Control	4·5	5·0	5·2	5·0	4·75	5·0	5·5
6·5cms growth	Exposed	0·25	0.4	0·5	0.75	1·0	1·6	3·25
over 18 days	% Strength	5	8	9	15	21	32	59

Species inoculated and their mycelial growth.		Strength in lbs. p.s.i. at each centimetre mark from the inoculation point						
		1cm	2cms	3cms	4cms	5cms	6cms	7cms
Coniothyrium sp.	Control	5·0	5·3	5·3	5·3	5·25	4·75	4·7
7cms. growth	Exposed	4·0	4·0	4·25	4·25	4·25	4·5	4·5
over 5 days	% Strength	80	75	80	80	80	94	95
Dicoccum sp.	Control	5·0	4·75	5·0	4·7	4·75	4·4	4·8
2·5cms. growth	Exposed	3·7	2·9	3·5	4·75	4·75	4·9	5·0
over 8 days	% Strength	74	61	70	101	100	111	104
Monosporium olivaceum	Control	4·5	4·2	4·0	4·5	4·5	5·2	5·0
1cm. growth	Exposed	2·0	4·25	4·75	4·0	4·1	4·0	4·1
over 4 days	% Strength	44	101	118	88	91	76	82
Eurotium vermiculatum	Control	4·0	4·25	4·5	4·25	4·2	4·8	5·0
7cms. growth	Exposed	3·9	3·5	3·4	3·6	3·75	3·6	4·2
over 5 days	% Strength	97	82	75	84	89	75	84
Arthrobotrys sp.	Control	4·75	4·85	5·3	5·5	5·1	5·0	5·0
4·5cms. growth	Exposed	3·7	4·0	3·75	3·5	3·75	4·0	4·5
over 6 days	% Strength	77	82	70	63	73	80	90
Stysanus sp.	Control	4·0	4·0	4·0	4·0	4·0	3·75	3·75
0·5cms. growth	Exposed	2·5	3·6	3·75	3·7	3·75	3·85	3.75
over 4 days	% Strength	62	90	93	92	93	102	100
Graphium sp.	Control	4·1	3·7	3·8	4·3	4·4	4·0	4·3
4cms. growth	Exposed	1·5	1·8	2·5	3·75	4·6	5·25	5·0
over 14 days	% Strength	36	48	65	87	104	131	116
Piricauda sp.	Control	4·25	4·25	4·15	4·25	4·25	4·4	4·1
4·1cms. growth	Exposed	1·9	2·25	2·6	3·1	3·5	4·2	4·1
over 14 days	% Strength	44	52	62	72	82	95	102
Pullularia pullulans	Control	4·1	4·25	4·25	4·3	4·35	4·25	4·1
3cms. growth	Exposed	1·9	2·25	3·5	3·75	3·8	3·9	4·25
over 14 days	% Strength	46	52	82	87	87	91	103

Species inoculated and their mycelial growth.		Strength in lbs. p.s.i. at each centimetre mark from the inoculation point						
		1cm	2cms	3cms	4cms	5cms	6cms	7cms
Ceratocystis sp.	Control	4·8	4·8	4·5	4·25	4·3	4·2	3·75
4·5cms. growth	Exposed	2·25	2·5	2·75	3·3	4·1	4·75	4·3
over 14 days	% Strength	46	52	61	77	95	113	114
Nigrospora sp.	Control	4·1	4·25	4·25	4·5	4·7	4·15	4·25
3·2cms. growth	Exposed	2·2	2·2	3·5	4·4	4·3	4·25	3·8
over 14 days	% Strength	53	51	82	97	91	102	89
Torula sp.	Control	3·9	3·75	3·25	3·8	3·5	3·25	3·6
3·5cms. growth	Exposed	1·75	1·9	2·2	3·7	3·8	4·0	4·0
over 14 days	% Strength	44	50	67	97	108	123	111
Monilia brunnea	Control	3·8	3·75	4·25	3·9	4·2	4·0	4·25
3·5cms. growth	Exposed	2·5	3·0	2·6	4·2	4·0	4·5	3·8
over 14 days	% Strength	65	80	61	107	95	112	89
Fusidium sp.	Control	4·0	4·25	4·3	4·5	4·3	4·8	4·75
7cms. growth	Exposed	3·5	3·8	3·7	3·25	3·75	3·4	3·25
over 14 days	% Strength	87	89	86	72	87	70	68
Verticillium sp.	Control	5·0	5·3	5·2	5·2	5·75	5·2	5·4
5·2cms. growth	Exposed	4·1	3·7	3·8	4·0	3·9	4·0	3·75
over 14 days	% Strength	82	69	73	76	67	76	69
Paecilomyces marquandii	Control	4·5	4·7	4·9	4·25	4·0	3·5	3·75
7cms. growth	Exposed	3·5	3·25	3·6	3·25	3·25	3·25	3·25
over 14 days	% Strength	77	69	73	76	81	92	86
Streptomycete sp.	Control	5·0	5·2	5·4	5·25	4·8	5·5	5·5
2·8cms. growth	Exposed	4·0	4·25	5·0	5·25	4·25	5·0	5·5
over 12 days	% Strength	80	81	92	100	88	90	100

$$\text{Percentage strength retained by the veneer} = \frac{\text{Strength of exposed veneer}}{\text{Strength of control veneer}}$$

Conclusions

The mycelial spread along the veneer as seen by the surface growth corresponds to the spread of decay caused by internal penetration of hyphae. There are no distinct areas of great decay at particular points in the fungal colony suggesting that the wood decay is gradual and its rate is a function of time and is unrelatable to fungal morphology. The distribution of vegetative hyphae and not the perithecia of Chaetomium spp. and pycnidia of Phoma sp. and Coniothyrium sp. reflects their area of decay.

Six arbitrary groupings can be formed according to the rate of growths and rate of decay.

	Fungi which grow quickly	Fungi which grow slowly
Fungi which decay quickly.	Chaetomium globosum	Humicola grisea
	Chaetomium funiculum	Humicola sp.
	Fusarium sp.	Phoma sp.
	Papulaspora sp.	Dicoccum sp.
		Monosporium olivaceum
		Stysanus sp.
		Graphium sp.
		Piricauda sp.
		Pullularia pullulans
		Ceratocystis sp.
		Nigrospora sp.
		Torula sp.
		Monilia brunnea
Fungi which decay slowly.	Gelasinospora cerealis	Arthrobotrys sp.
	Eurotium vermiculatum	Verticillium sp.
	Coniothyrium sp.	
	Fusidium sp.	
	Paecilomyces marquandii	
Fungi which gave slight or no decay.	Trichoderma viride	Gliocladium roseum
		(Streptomycete sp.)

Determination of the growth rates of individual microfungi now allows comparison with the rates of colonization and penetration on to a thickened model wood from a soil inoculum.

Experimental

Perfusion devices were set up using the mineral salts solution of E. and P. They wereslightly modified by using five beechwood veneer pieces, 0·6mm. thick, place on top of one another as the model wood. The perfusion glass sleeving which passed the nutrients along by capillarity was placed between the fourth and fifth veneers. The veneer pile was held together with non-toxic silicone glue spread around the edges of the pile. The arrangement can be seen in diagram 5.

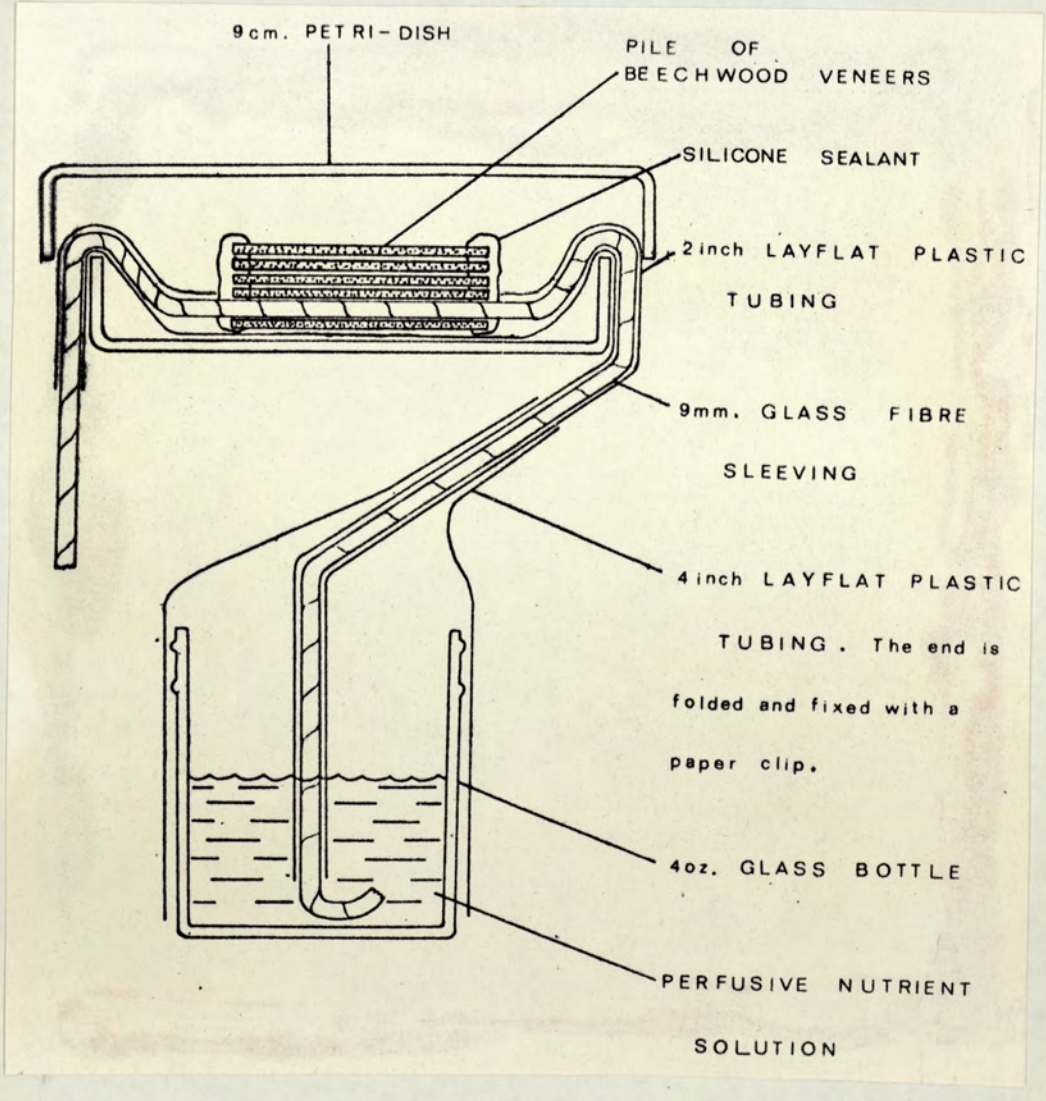


Diagram 5. The model wood perfusion system for penetration studies. The adhesive glass tape for fastening the glass sleeving to the veneer has been omitted.

The devices were autoclaved and then inoculated with soil taken from near Clent. They were incubated at 25°C. for 48 days with two sets being sacrificed every fourth day. A few sets were left for 72 days to complete some results. The colonizing fungi and the strength loss were determined for each of the 180 veneers. The isolation results are simplified, as table 6, with numerical indication of the days of incubation before a species was isolated from each of the five veneers so as to illustrate the penetration rates.

FUNGAL SPECIES.	Days of incubation until the first isolation from each veneer				
	1st	2nd	3rd	4th	5th
Trichoderma viride	4	4	4	4	4
Graphium sp	4	4	4	4	4
Chaetomium globosum	4	8	8	8	8
Papulaspora sp	8	8	8	8	8
Gliocladium roseum	4	4	12	16	16
Gelasinospora cerealis	8	8	8	8	16
Fusarium sp	8	12	12	12	24
Penicillium sp	8	16	16	4	4
Humicola grisea	8	16	20	24	24
Paecilomyces elegans	4	8	36	16	16
Arthrobotrys sp	4	8	16	52	72
Monosporium olivaceum	16	16	16	20	20
Dicoccum sp	20	12	36	36	36
Stysanus sp	—	20	—	44	44
Streptomycete sp	8	—	—	20	20
Phoma sp	68	52	56	52	56
Pullularia pullulans	—	—	—	56	56

Table 6. Penetration sequence and time through a veneer pile by microfungi.

Results for some fungi do not show a logical sequence of isolation day with increasing penetration and this is due to either imperfect isolation or because some fungi have penetrated completely through one veneer without leaving sufficient hyphae for successful inoculation on to agar. Only after further ramification might the inoculation potential be reached.

Conclusions

All the fungi isolated were capable of penetration through the 6mm. thick pieces of veneers forming a $\frac{1}{4}$inch deep pile. Therefore no "surface colonizers" were isolated.

Penetration was rapid for Trichoderma viride, Chaetomium globosum, Papulaspora sp. and Graphium sp. with complete penetration within eight days. The first two species are usual pioneer colonisers and together with the slightly slower penetrating Humicola grisea and Fusarium sp. are probably co-dominants. The incidence of Papulaspora sp. and Graphium sp. was low enough to be of no numerical importance but since they are highly cellulolytic they are active wood deteriogens.

Following the pioneer species came a gradual secondary colonization by Gliocladium roseum, Gelasinospora cerealis and Penicillium sp. with such species as Stysanus sp., Pullularia pullulans and Streptomycete sp. in evidence only after prolonged incubation.

Monosporium olivaceum and Paecilomyces elegans were of infrequent occurrence.

There is therefore an evenly distributed range of penetration sequence and rate with no separation into distinct groups. This indicates a dynamic interchange of species and not a saltatory succession interrupted by radical substrate alterations.

It is apparent that most of the species have their own "penetration time" with a regular penetration rate at a particular point in the colonization sequence. A few have a recondite pattern of occurrence.

Since the dominant fungi were isolated with similar intensity after long incubation as at the onset they must remain dominant for the duration of the experiment.

The unusually late occurrence of Phoma sp. and the slow penetration of Arthrobotrys sp. on comparison with previous studies serve to show the small variation that can occur in growth patterns.

The isolation of a Streptomycete sp. at some depth in the compound wood requires further investigation. It is difficult to visualise its penetration by growth and easy to conclude its spores being carried by the numerous soil ciliates and nematodes that were seen permeating the wood at all levels. Heal (1962) has observed soil amoebae moving fungal spores and Tribe (1957) found fungal spores amongst various excretions from the micro-fauna with nematodes occurring abundantly in acidic soils. Tracey (1955),(1958) has found cellulases in soil amoebae and those nematodes which feed on fungi or higher plants.

The comparative rates of each species to penetrate through the veneers in a radial direction appears to be similar to the growth rates along veneers in a longitudinal direction as recorded previously.

It is evident from this data that it is necessary to establish whether the differing penetration times are the result of differing rates of hyphal growth or consequences of micro-successions on a subtly changing wood substrate. To investigate this further a study of penetration times through an inert substrate was undertaken so as to eliminate any influence from the wood.

The loss of strength incurred by each veneer is shown in fig. 17. and is compared with the average decay of a single perfused veneer. It can be seen that the multiple veneer pile deteriorated as a single unit and because of its greater volume it deteriorated much slower than a single veneer does.

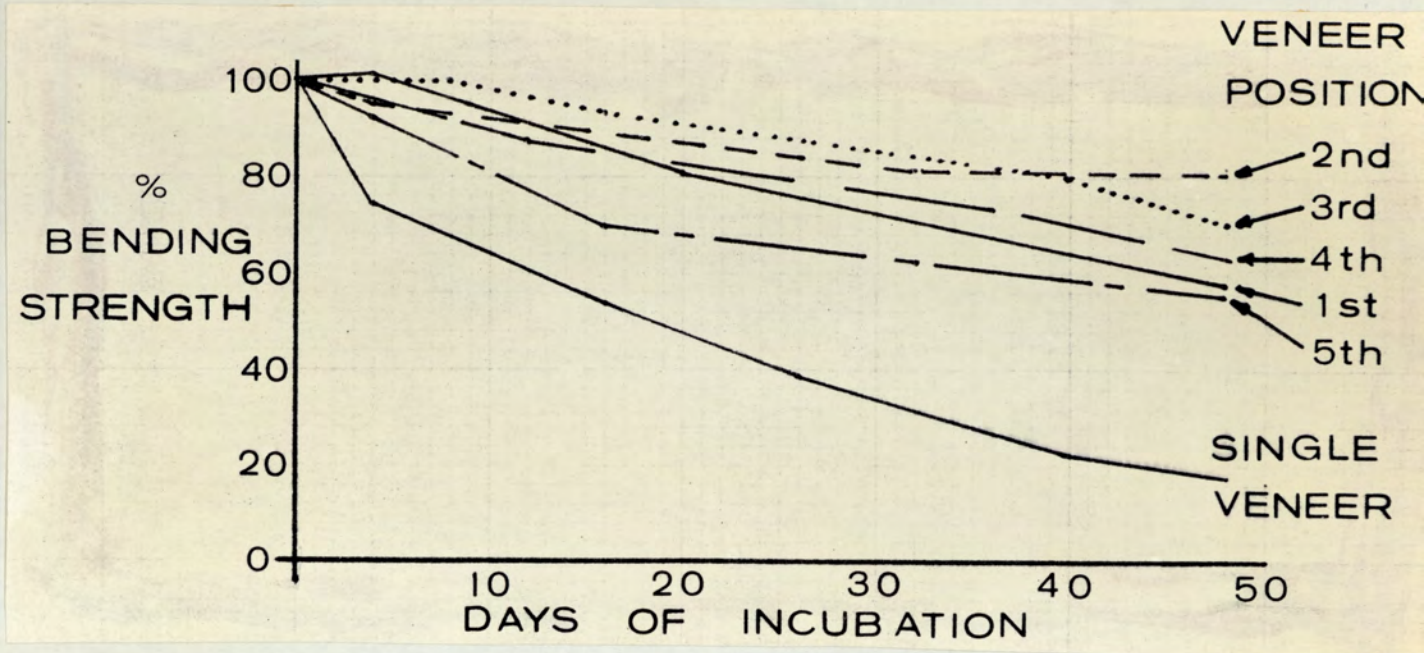


Fig. 17. Graph of loss in strength of each veneer in a wood pile.

This suggests that these microfungi penetrate through that area of wood conducive for growth before causing significant degradation.

The uppermost (1st.) and lowermost (5th.) veneers of the pile have the strongest decay but the level of decay is not much more than with the other veneers. This effect must be the result of fungal hyphae concentrating in the first and last veneers causing more deterioration. It could be due to either a peculiarity of growth or because more suitable oxygen conditions exist at the two surfaces of the veneer pile. Only microscopic examination of hyphal branching will reveal whether more decay occurs where the microfungi initiate and terminate their penetration growth.

Penetration through an Inert Substrate.

Experimental

A number of perfusion sets were constructed and filled with E. and P. mineral salts solution, as before. Each set was given a single beechwood veneer and one to five layers of glass cloth placed on top of the veneers. A soil inoculum was placed on top of the cloths after autoclaving the sets.

Inoculation was made using an alcohol flamed spatula.

Thus between the soil and wood was positioned an inert substrate of up to five increments of glass cloth each of fine weave and 0·08mm. thickness.

One perfusion set for each amount of cloth coverings was sacrificed every three days for a period of 36 days incubation at 25°C. Afterwards those fungi which had grown from the soil, through the cloths and on to the wood were isolated from the veneer and identified using the techniques described previously.

The percentage frequency of isolation and the first appearance on the veneer of each species was found and is expressed in table 7. Again the loss of strength for each veneer was determined.

Conclusions

There was a progressive decrease in frequency of isolation of most species with increasing thickness of glass cloths. Those species able to grow well on non-cellulosic media penetrated best and this applied particularly to T. viride and Penicillium sp.

Five coverings of glass cloth giving a thickness of 0·4 to 0·5mm. markedly reduced the colonization of Fusarium sp. and H. grisea. It prevented growth of G.cerealis, Dicoccum sp. and Arthrobotrys sp. The prevention of penetration is partly due to a dearth of nutrients but since it probably affects equally all the cellulolytic species it appears there are similar penetration patterns as found before. This similarity of penetration rate and sequence through the inert cloths and wood pile indicates that penetration behaviour is a function of hyphal growth rate and is independent of any changes that might occur in the wood substrate under the conditions of incubation.

The cloths prohibited any spore germination on the wood surface so that colonization was entirely by hyphae. Even a Streptomycete sp. was able to penetrate the cloths.

Strength measurements reflected a progressive reduction in wood

FUNGAL SPECIES	% Frequency of Isolation and Days of incubation until the first isolation from the veneer. No. of cloths									
	1		2		3		4		5	
	%	Days	%	Days	%	Days	%	Days	%	Days
Trichoderma viride	83	3	83	9	91	3	58	3	91	3
Chaetomium globosum	41	3	66	3	66	3	32	15	58	3
Papulaspora sp	8	12	8	30	—	—	8	18	—	—
Fusarium sp	100	3	91	3	100	3	100	3	17	9
Humicola grisea	100	3	91	3	100	3	66	3	17	18
Gliocladium roseum	41	12	41	12	58	6	25	15	25	12
Penicillium sp	33	3	50	3	75	3	25	15	66	3
Gelasinospora cerealis	8	24	—	—	—	—	8	27	—	—
Dicoccum sp	33	3	8	30	8	33	8	36	—	—
Piricauda sp	8	3	—	—	—	—	—	—	—	—
Arthrobotrys sp	8	12	8	36	8	30	—	—	—	—
Graphium sp	—	—	—	—	—	—	—	—	8	15
Streptomycete sp	—	—	—	—	—	—	—	—	8	15

Table 7. Frequency of microfungi isolation and penetration times through an inert substrate.

deterioration with each additional cloth layer. Since at no time was decay prevented any wood preservation treatment using an inert film to cover and

protect the wood would need to be unbroken or at least 0·5mm. thick to be effective.

Discussion

A realisation of the nature of fungal colonization and penetration through such model woods is necessary if model culturing systems are to be used for wood preservative testing.

Obtaining a true appreciation of the decay process for untreated wood is equal in importance to understanding the ecology of treated wood so that comparisons can be made. If, as these results suggest, the model woods are vulnerable to rapid and thorough penetration and this is in excess of that observed in timbers placed in a natural environment then to extrapolate data from model tests to field situations requires information on the limiting factors of fungal growth. The use of veneers and the artificial nutrient augmentation could explain the enhanced colonization and interpenetration of the model woods. The low oxygen diffusion, water permeation or action of some undefined contingency might possibly prevent considerable growth in natural timbers and equally likely widespread fungal decay might have been overlooked.

Most of the microfungi isolated displayed their own characteristic "penetration time" which was found to be a manifestation of differences in growth rate. Therefore the penetration patterns are probably due to growth differences and are perhaps unaccompanied by any change in environment or substrate. With Levy's (1967) suggestion that somewhere in the chain of succession or association of microorganisms causing decay there could be a weak link that can be exploited for preservation it might best be sought in growth patterns rather than subtle environment alterations or wood chemistry.

Finally this work demonstrates the possibility of determining the depth of soft-rot penetration by measuring the extent of Trichoderma viride growth or assessing the stage of wood decay by identifying which of the late penetrators have begun incipient growth into the wood.

Influence of Water

Introduction

Two factors which selectively favour soft-rot attack on wood have been singled out by Price (1961) as insufficient preservation and high moisture conditions.

Hyphae have limited means of conserving water and most fungi are obliged to exist in water, moist or humid conditions (Brown and Wood 1953). The aqueous condition having most influence on wood fungi is normally the intrinsic moisture content of the substrate since few ecosystems exist with timber fully immersed in water and atmospheric moisture probably only affects surface fungal growths. The effect of soaking spores of Polyporus squamosus with water has been reported by Buller (1906) and on Phoma herbarum by Renfro and Wilcoxson (1963). The effect of humidity on those microfungi that colonize cellulosic substrates has been reviewed by Panasenko (1967) and the influence of humidity on basidiospores of Lenzites species investigated by Zeller (1920) and Morten and French (1966).

Two of the earliest papers on the relation of wood moisture contents on decay (Snell 1925 and 1929) used different fungi in an attempt to classify the basidiomycetes on the basis of their air-moisture reactions. Other work with basidiomycetes includes that of Ammer (1964) which found that "metabolic water" augments wood moisture and that the lowest moisture limit for five fungi examined lay at 30-31%. Lenzites sepiaria was inhibited above 158% moisture content and Hypholoma fasciculare needed higher moisture levels. Using various amounts of water mixed with vermiculite Henningsson (1967) embedded wood samples into the mixture and found maximum decay of birch to occur at 60-120% moisture content and 60-100% for aspen. The range of moisture allowing decay extended from 35% to 160%.

Björkman (1946) found decay to occur within a wide moisture gradient. Ghosh, Bose and Basu (1968) decomposed jute fibre with Corticium sp. and found the deterioration to be closely dependent on moisture levels with a reduction in wet conditions. Osborne (1967) observed the denser and less water-absorbent species of Fijian trees to be more decay resistant.

Few researches have examined the affect of moisture on wood microfungi. Björkman and Haeger (1963) investigated the fungi colonizing a chip-wood stack and found them to inhabit a moisture quotient of 28-120%. Liese and Ammer (1964) used two ingenious methods for producing graduated zones of water content in wood blocks and showed Chaetomium globosum and Paecilomyces sp. to increase decay with increasing moisture content. Ceratocystis needed 100% and Trichoderma viride favoured a high water content coupled with sufficient aeration. The lower limit for any fungal activity occurred between 32 to 35% moisture content. The rate of drying out of the wood did not influence fungal survival.

Corbett (1963), realising the need to augment wood substrates with nutrients and maintain a high moisture content for rapid fungal deterioration, devised a "Tide" and a "Wheel" system for supplying these growth factors in an intermittent manner.

The effect that the water content of beechwood has on the colonizing microfungi was investigated by adapting the perfusion principle to produce moisture gradients.

Experimental

Thirty perfusion sets were constructed and filled with mineral salts. Four veneer pieces were arranged around the perimeter of each petri-dish. The end of each piece was taped to the end of another veneer and one end of these four linked veneers was attached to the perfusion sleeving. The arrangement is illustrated in diagram 6. The limited contact between each veneer caused a limited diffusion of nutrient solution through the train of four veneers and so formed a gradient of moisture contents.

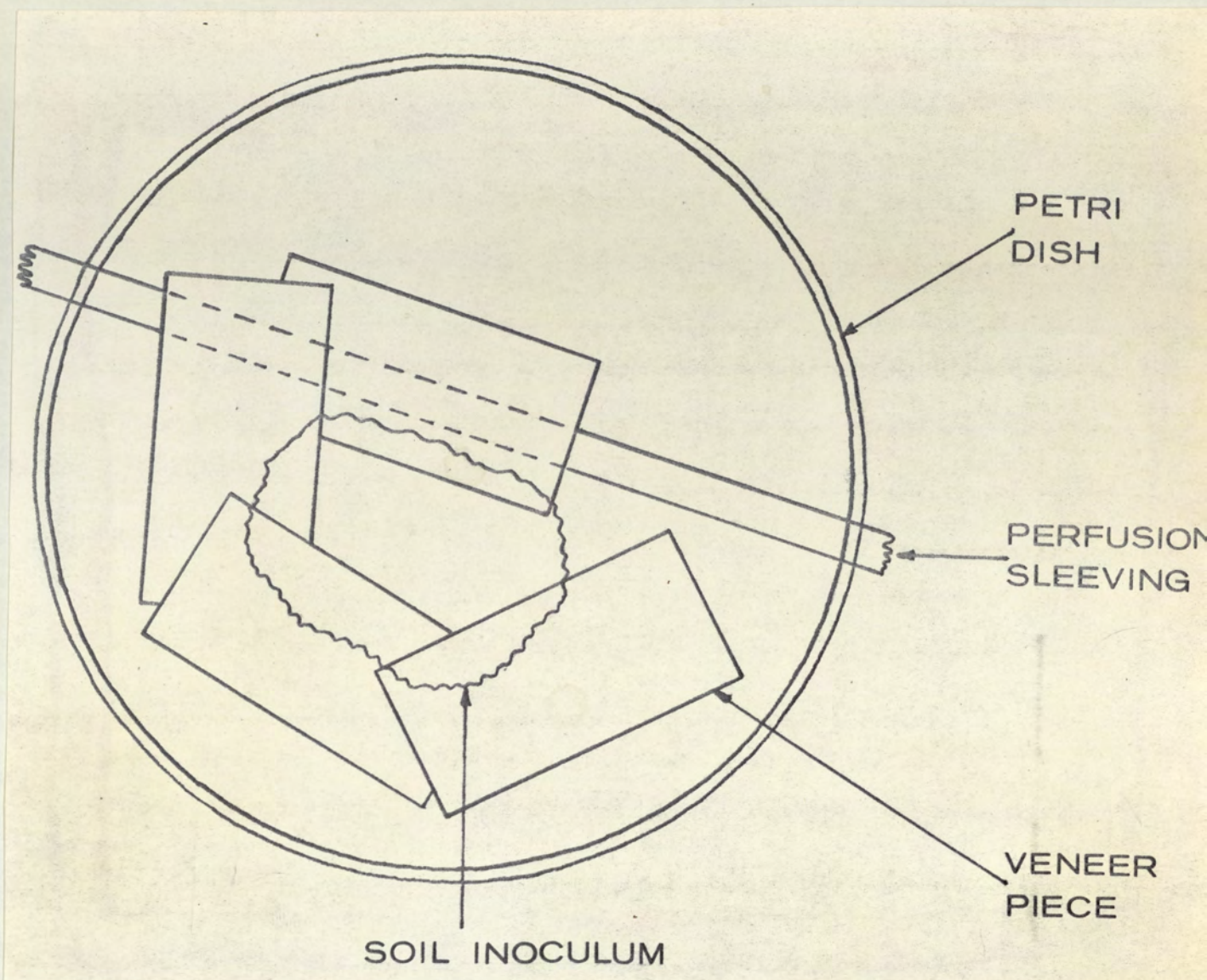


Diagram 6. The model perfusion system for producing a graduated water content through a series of four veneers.

A common inoculum of Clent soil was given to the four veneers. After incubation at 25°C. one perfusion set was sampled every two days and its colonizing fungi, strength loss and moisture content determined. The moisture content was found by using the oven drying method of heating the woods to 105°C. for 20 minutes and calculation with the formula :-

$$\text{Percentage moisture content.} = \frac{\text{Initial wet weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$

A range of moisture contents resulted with some sets having wide and some narrow differences in moisture. Those sets of veneers with wide moisture gradients are shown in tables 8 and 9 together with veneer strengths and colonizing fungi.

Position of veneer and Days of incubation		% Moisture content	% Bending strength	FUNGAL SPECIES: Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Graphium sp	Gelasinospora cerealis	Eurotium vermiculatum	Arthrobotrys sp	Dicoccum sp	Monosporium olivaceum	Stysanus sp	Phoma sp	Papulaspora sp	Zygorhynchus moelleri	Streptomycete sp
2	1st	72	94		·	+	+		+											
	2nd	81	89	+	+	+			+											
	3rd	127	92	+	+	+														
	4th	123	84		+	+	+													
4	1st	41	68		+	+			+			+								
	2nd	72	83	+	+	+	+		+											
	3rd	100	56		+	+	+		+											
	4th	180	71		+	+														
10	1st	33	94	+	+		+	+	+										+	
	2nd	30	100	+	+	+	+	+	+										+	
	3rd	30	99		+	+	+		+	+									+	
	4th	92	98	+	+	+	+	+			+								+	
12	1st	23	88	+		+	+	+	+											
	2nd	23	92	+		+	+	+	+	+									+	
	3rd	23	100				+	+	+											
	4th	33	80	+	+	+	+	+	+										+	
16	1st	133	67	+	+	+		+	+	+									+	
	2nd	133	78	+		+	+		+	+									+	
	3rd	133	87	+		+	+	+	+	+									+	
	4th	136	58	+	+	+		+	+	+			+	+					+	
20	1st	125	82	+	+	+	+		+	+				+					+	
	2nd	116	85	+	·		+	+		+			+							
	3rd	125	66	+	+		+	+		+								+	+	
	4th	154	58	+	+	+			+	+								+		
24	1st	145	67	+	+		+	+	+										+	
	2nd	115	66	+	+		+	+	+										+	
	3rd	125	84	+	+	+	+	+	+	+					+					
	4th	133	45		+		+			+					+				+	
34	1st	116	66	+	+			+		+										
	2nd	136	53	+	+		+		+	+			+		+					+
	3rd	160	52	+	+					+					+	+				
	4th	188	25	+	+					+					+					

Table 8. The moisture content, retained strength and colonizing fungi of veneers with a graduated water supply.

Position of veneer and Days of Incubation		% Moisture content	% Bending strength	FUNGAL SPECIES: Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Graphium sp	Gelasinospora cerealis	Eurotium vermiculatum	Arthrobotrys sp	Dicoccum sp	Monosporium olivaceum	Stysanus sp	Phoma sp	Papulaspora sp	Zygorhynchus moelleri	Streptomycete sp
36	1st	153	55	+	+		+	+	+	+					+		+			
	2nd	136	53	+	+	+		+	+	+					+		+	+		
	3rd	133	60	+	+		+	+							+		+			
	4th	170	40		+	+		+		+			+		+		+			+
40	1st	23	92	+		+	+	+	+	+							+			+
	2nd	36	100	+		+	+	+	+	+						+	+			+
	3rd	41	89	+			+	+	+	+										+
	4th	140	39	+	+	+		+		+										+
46	1st	115	61	+	+	+		+		+					+	+				
	2nd	133	65	+	+	+	+	+		+					+	+				+
	3rd	146	76	+	+		+	+							+		+	+		
	4th	170	15	+	+			+		+			+		+		+			+
48	1st	123	44	+	+			+						+			+				+
	2nd	115	60			+	+	+							+		+				+
	3rd	133	39	+	+	+		+		+					+			+			+
	4th	190	29	+	+	+	+	+													+
50	1st	23	56	+	+	+	+	+		+											+
	2nd	23	100	+		+	+	+	+	+					+	+					+
	3rd	30	89	+		+	+	+	+	+					+						+
	4th	45	27	+	+	+	+	+	+	+					+						+
56	1st	125	50	+	+	+	+	+									+				
	2nd	125	54	+	+	+	+	+		+							+				
	3rd	125	51	+	+	+	+	+									+				
	4th	160	23	+	+	+	+	+									+	+			+
60	1st	160	27		+	+	+														
	2nd	154	36	+	+		+	+													
	3rd	163	39	+	+	+			+					+							
	4th	180	17	+	+	+				+				+							

1st veneer is farthest from the perfusion sleeving, 4th is nearest and 2nd and 3rd are in between.

Table 9. The moisture content, retained strength and colonizing fungi of veneers with a graduated water supply.

Conclusions

The range of moisture contents produced extended from 23 to 190%. The majority of species isolated survived both extreme levels. The exceptions were Arthrobotrys sp., Dicoccum sp., Papulaspora sp. and Phoma sp. occurring only in woods with moisture contents above 115% and Penicillium sp. tending to be found only on the least wetted woods. Any election of particular species at specific moisture ranges causing frequent isolation was not evident and therefore moisture appears to have little influence on which species of fungi colonize the wood.

The moisture conditions markedly affected the loss of bending strength with none or slight decay in veneers below 30-33% moisture content. When high levels of deterioration occurred there were high levels of moisture. Thus the rate of decay is closely associated with the moisture content.

One consideration that became apparent in this experiment for needing further investigation was the factor of cyclical water movement. Thus a transient period of high moisture in a dry veneer (less than 30% moisture content) might explain the isolation of many fungi from them. This effect depends on the ability of fungi to survive a dry period.

Influence of drying on wood strength loss

Experimental

A number of perfusion sets with single veneers were inoculated with soil and incubated at 25°C. for 20 days. Thereafter two sets were sacrificed every two days until the thirtieth day of incubation. One test strip for strength measurement was taken from each sampled veneer and stored. Then the remaining wood of each sample was kept for a further two days in an anhydrous calcium chloride desiccator at 25°C. after which another test strip was pressed out. The normal strips and the strips which had recieved a further two days incubation in a dry atmosphere were moisture equalised and then broken on the tensiometer. Therefore it was possible to observe the effect of a two day drying period on the rate of decay by comparing strengths with untreated

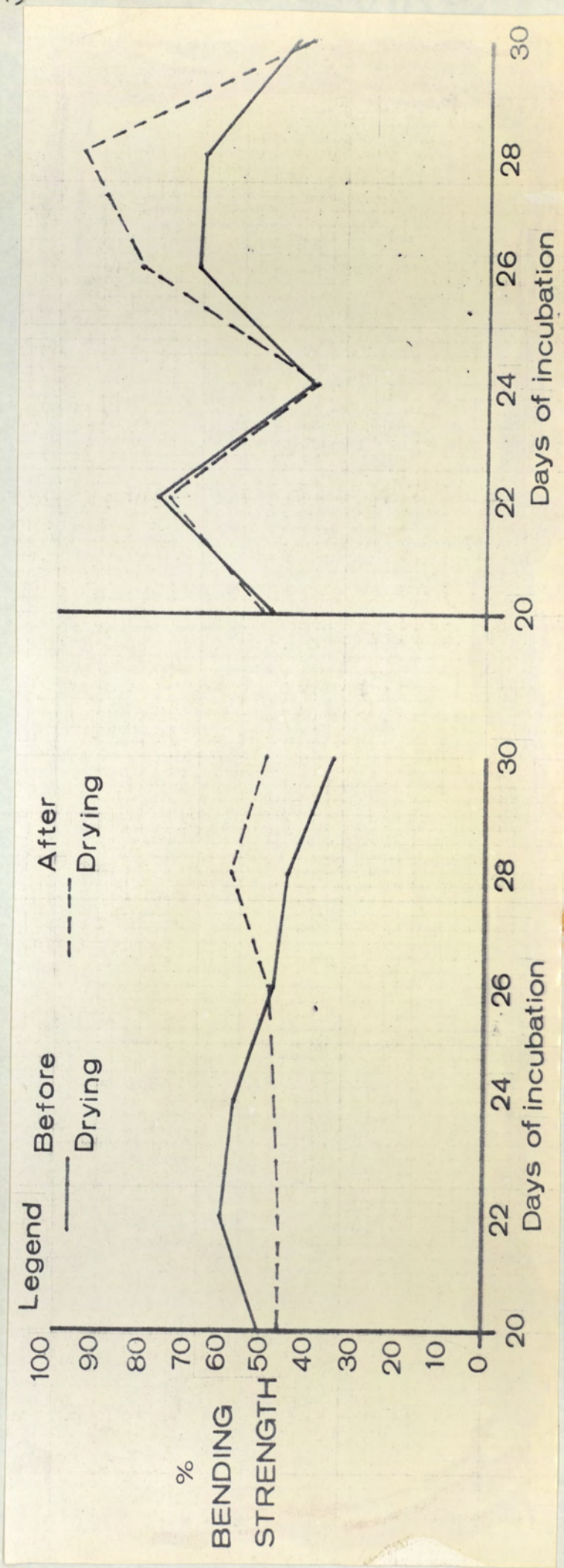


Fig. 18. Graphs of two identical experiments showing the effect on strength of a further two days deterioration in a dry atmosphere.

control veneers. The experiment was performed twice and the two graphs of results are seen in fig. 18.

Conclusions

It was difficult to obtain uniform levels of deterioration in the many wood samples and with also the variations in strength inherent in the wood because of a variform anatomy this disorder is increased. Generally, however, there is no marked decrease or increase in strength of the veneers with a drying moisture phase. Therefore these results suggest that a two day drying period does not affect the activity of colonizing fungi causing a change in wood strength but from the previous data the fungi can survive such transient periods.

Discussion

Adaptation of these findings to worked timber in a natural environment suggests that in climatic periods of high moisture levels the wood decay is hastened and in low moisture periods the microfungal growth is in abeyance.

For testing preservative treated timber an environment producing moisture contents in the wood of 40-50% appears ideal since at this level rapid decay would occur with minimum leaching or dilution of the preservative.

Influence of some Nutrients

Introduction

The factor which most often limits growth of an organism is the supply of energy source (Waid 1968). From the onset of soft-rot studies it was recognised that ancillary nutrients stimulated wood degradation and this contrasted with the lower proportional increase in decay by basidiomycetes when supplied with nutrients (Savory 1955).

No detailed investigations have been made on the nutrient requirements of soft-rot fungi although Duncan (1960) has found minerals, vitamins and additional organic matter in the soil to increase soft-rot attack.

Nutrients can be divided into carbon, nitrogen and accessory nutrient sources.

1 Carbon compounds :

It is well substantiated that ancillary carbon sources freely available for fungal utilization inhibits cellulolytic activity. Siu and Sinden (1951) found glucose, sucrose, xylose and cellobiose to inhibit cellulose utilization by Myrothecium verrucaria. Talboys (1958) found dextrose, sucrose, starch and lactose to inhibit secretion of cellulase from Verticillium albo-atrum. Walseth (1952) found a similar phenomenon with enzymes and Johansson (1966) with basidiomycetes.

To extend such investigations of this diauxie action a comparison was made between glucose supplied and deficient veneers supporting a mixed fungal community.

2 Nitrogen compounds :

Merrill and Cowling (1966) have demonstrated that there is a good correlation between the nitrogen content of wood and its rate of decay. Duncan (1960) increased the rate of decay by soft-rot fungi with addition of an extra nitrogen source.

Schmitz and Kaufert (1938) showed dextrose and asparagine increased

the wood decay by Lenzites trabea in low concentration but decreased the activity of Lentinus lepideus by being utilized in preference to the wood. Bravery (1968) insinuated that any D.L.asparagine and yeast-extract incorporated into E. and P. cellulose nutrient medium will act as as alternative carbon source and inhibit utilization of cellulose by cellulolytic fungi. Despite this Levi, Merrill and Cowling (1968) found that asparagine was the fourth most suitable nitrogen compound tested of 26 for growth of wood basidiomycetes and Siu and Sinden (1951) showed that increasing the concentration of yeast-extract from 0·00625% to 0·2% gave a progressive strength loss of cloth decayed by Gliomastix convoluta. To clarify these conflicting results an experiment was performed in which the effect of the presence or absence of the two compounds was measured in terms of loss of wood strength.

3 Accessory compounds :

Water soluble extractives can be obtained from wood on autoclaving. The effect of these extractives have been studied on Lenzites saepiaria (Anderson 1931), on basidiospores of Fomes pini (De Groot 1965) and Fomes roseus (Morton and French 1966). Shrimpton and Whitney (1968) found extracts from resinous and non-resinous sapwood of lodgepole pine to inhibit blue-stain fungal growth. To determine whether the soluble extractives of beechwood have a significant effect on microfungal growth a number of veneers were perfused with the extracted solution after they had been autoclaved in water.

In addition the water extractives from Clent soil, rainwater and distilled water were perfused through veneers.

Experimental

Sixteen perfusion sets were constructed for nine different perfusive solutions. They were :-

1. E. and P. mineral salts solution at pH 5·4.

2. E. and P. mineral salts solution + 4% glucose.

3. E. and P. mineral salts solution minus asparagine.

4. E. and P. mineral salts solution minus yeast-extract.

5. E. and P. mineral salts solution minus asparagine and yeast-extract.

6. Distilled water.

7. Rainwater.

8. Soilwater obtained by autoclaving Clent soil in distilled water at 10 lbs. p.s.i. for 20 minutes.

9. Soluble wood extractives obtained by autoclaving beechwood veneer pieces in distilled water at 10lbs. p.s.i. for 20 minutes.

After autoclaving, the perfusion sets were incubated for 32 days at 25°C. with sampling of one veneer for each treatment every two days.

The colonizing fungi were inoculated on to E. and P. cellulose medium for all except the glucose perfused veneers which were inoculated on to the cellulose and an E. and P. mineral salts plus 4% glucose medium. After seven days incubation at 25°C. the isolation plates were examined.

The results are seen in tables 10 to 18.

The strength loss for each veneer was measured and the data is expressed in a series of graphs in fig. 19.

Conclusions

No radical changes were observed with each nutrient perfusant on the kind of species isolated and their frequency of isolation.

The glucose supplementation gave no differences in incidence of fungi on the veneers but less fungal species were seen on the glucose isolation plates in comparison with the cellulose ones. Eurotium vermiculatum was seen only on the glucose isolation media. These glucose results largely imitate those of Greaves and Savory (1965) except for one difference that might prove to be fundamental and that is they found that rapid growing early colonizers were obtained more often on the sugar-rich media whereas the reverse is true

E & P salts

FUNGAL SPECIES	Days of incubation: 2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Trichoderma viride	+	+		+		+	+	+	+	+	+	+	+			+
Humicola grisea		+	+		+	+	+	+	+	+	+	+	+	+	+	+
Chaetomium globosum	+	+	+	+	+		+		+	+			+	+		+
Fusarium sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gliocladium roseum		+		+	+	+		+	+	+		+	+			+
Penicillium sp		+	+	+	+					+		+				+
Gelasinospora cerealis		+														
Graphium sp						+										
Papulaspora sp						+										+
Arthrobotrys sp								+								
Dicoccum sp													+			
Phoma sp														+		
Fusidium sp																
Monosporium olivaceum																
Eurotium vermiculatum																
Stysanus sp																
Piricauda sp																
Torula sp																
Pullularia pullulans																
Ceratocystis sp									+				+			
Paecilomyces elegans						+										
Paecilomyces marquandii														+		
Aspergillus niger							+	+	+				+			+
Zygorhynchus moelleri		+	+	+	+		+	+		+	+				+	
Streptomycete sp			+													
Ciliates														+		
Nematodes		+												+		+

Table 10. Colonizing organisms on veneers perfused with basal mineral salts.

E & P salts + GLUCOSE

FUNGAL SPECIES

c = CELLULOSE g = GLUCOSE

Days of incubation	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Trichoderma viride	cg	c	cg	c	g	c g	c g	c g	c g	c g	c	c g	c g	c g	c	g c
Humicola grisea	c	c	c	c	c	c	c	c	cg	c	c	c	c	c	c	c
Chaetomium globosum	c	c	c	g	c		c		cg				c			
Fusarium sp	c	c	cg	cg	cg	c	c	c	cg	c	g	c		c	cgc	c
Gliocladium roseum	c	c	c	c			c			c			c	c	c	
Penicillium sp				c	c		cg	c		cg		cg		cg	c	
Gelasinospora cerealis																
Graphium sp			•			cg										
Papulaspora sp						c										
Arthrobotrys sp				c		c		c								
Dicoccum sp																
Phoma sp																
Fusidium sp																
Monosporium olivaceum		c			c											
Eurotium vermiculatum												g	g	g		g
Stysanus sp																
Piricauda sp																
Torula sp																
Pullularia pullulans																
Ceratocystis sp							c			g	c	g	cg			
Paecilomyces elegans				c												
Paecilomyces marquandii														c		
Aspergillus niger							cg	c	c					c		
Zygorhynchus moelleri	g	cg	cg	cg	cg	g	cg	g	c	c				g		
Streptomycete sp	g	g			c		g	g				c		c		
Ciliates																
Nematodes																

Table 11. Colonizing organisms on veneers perfused with mineral salts plus an extra carbon source.

E & P salts minus ASPARAGINE

FUNGAL SPECIES	Days of incubation: 2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Trichoderma viride	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Humicola grisea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chaetomium globosum	+			+		+	+		+		+			+		
Fusarium sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gliocladium roseum	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Penicillium sp	+	+	+		+				+		+	+	+	+		+
Gelasinospora cerealis	+			+												
Graphium sp																
Papulaspora sp																
Arthrobotrys sp		+								+						
Dicoccum sp				+							+		+			
Phoma sp																+
Fusidium sp																
Monosporium olivaceum																
Eurotium vermiculatum																
Stysanus sp	+											+				
Piricauda sp						+		+				+				
Torula sp																
Pullularia pullulans																
Ceratocystis sp																
Paecilomyces elegans																
Paecilomyces marquandii	+	+	+	+	+							+	+	+		
Aspergillus niger	+	+				+			+		+	+	+	+		+
Zygorhynchus moelleri	+						+	+	+	+	+	+	+	+	+	+
Streptomycete sp									+							
Ciliates																
Nematodes																

Table 12. Colonizing organisms on veneers perfused with mineral salts minus one nitrogen source.

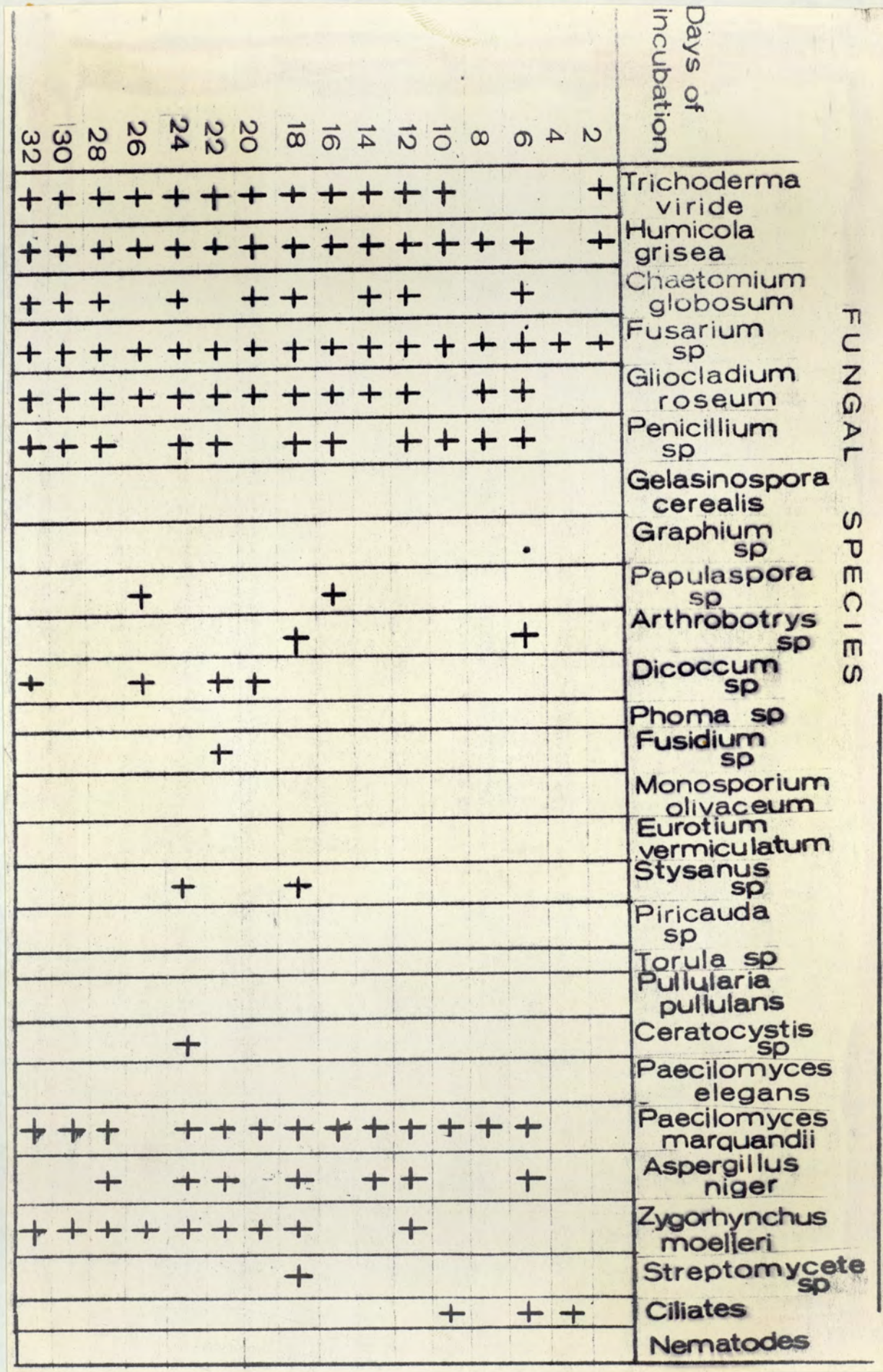


Table 13. Colonizing organisms on veneers perfused with mineral salts minus one nitrogen source.

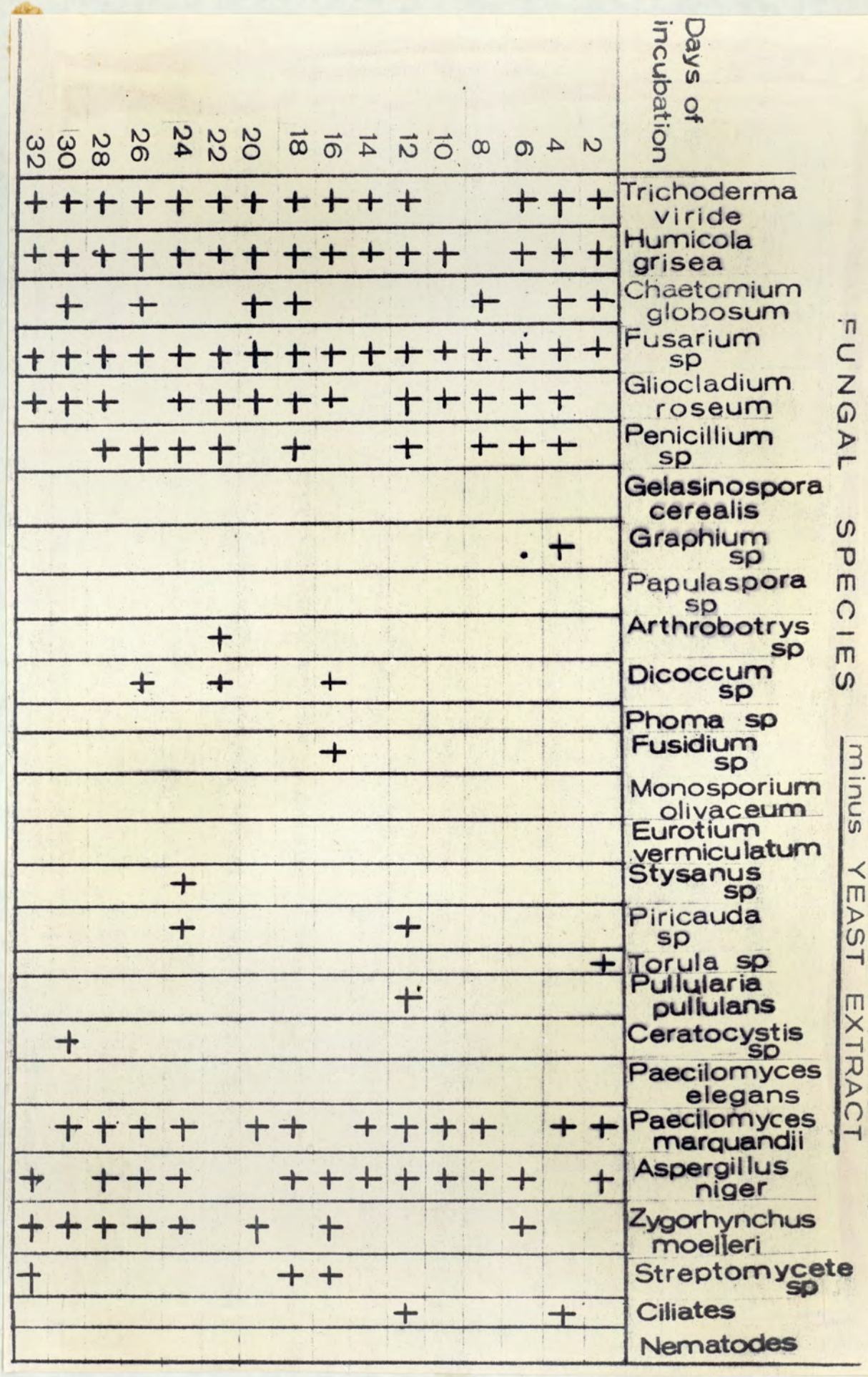


Table 14. Colonizing organisms on veneers perfused with mineral salts minus two nitrogen containing compounds.

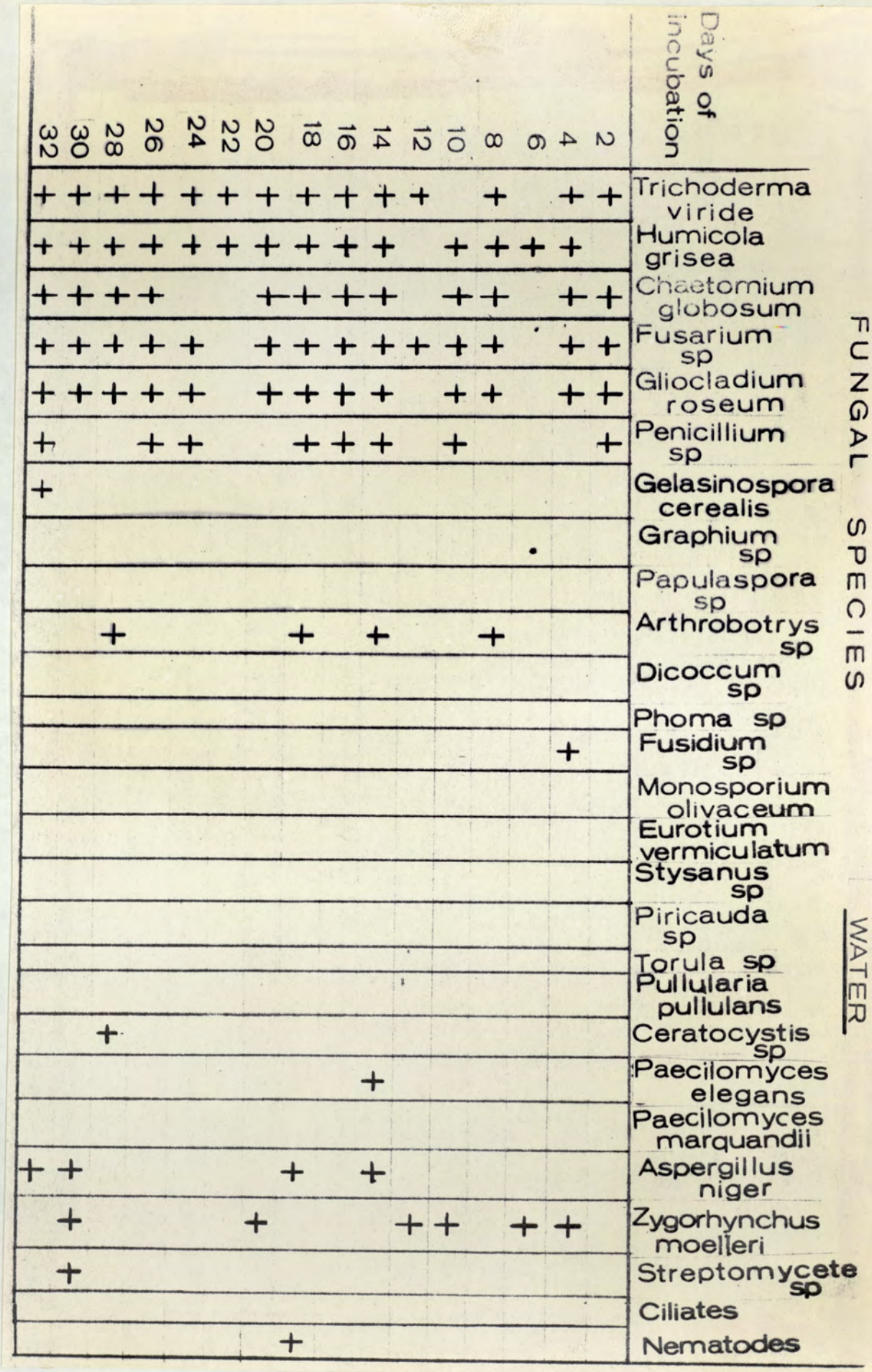


Table 15. Colonizing organisms on veneers perfused with distilled water.

FUNGAL SPECIES RAINWATER

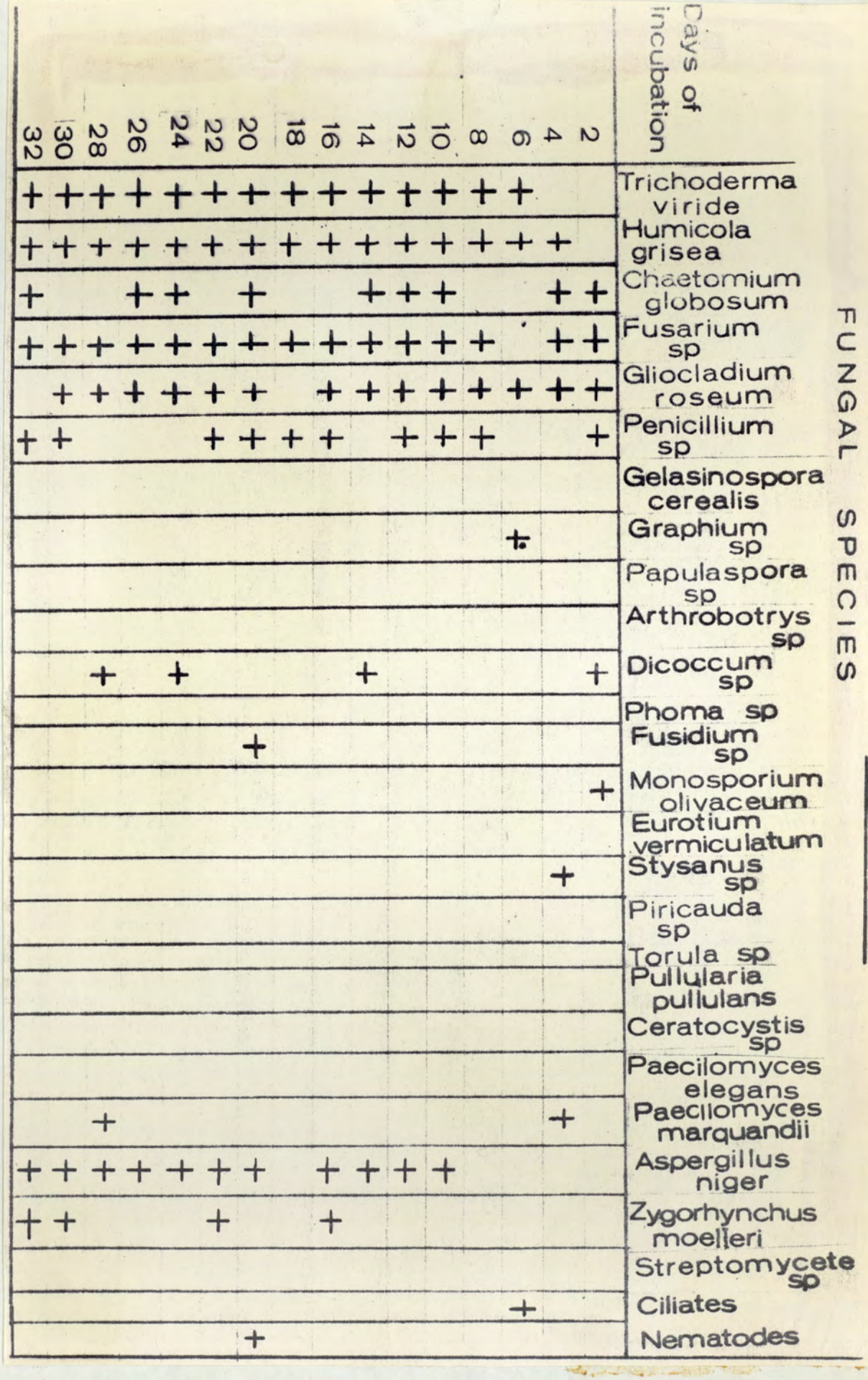


Table 16. Colonizing organisms on veneers perfused with rainwater.

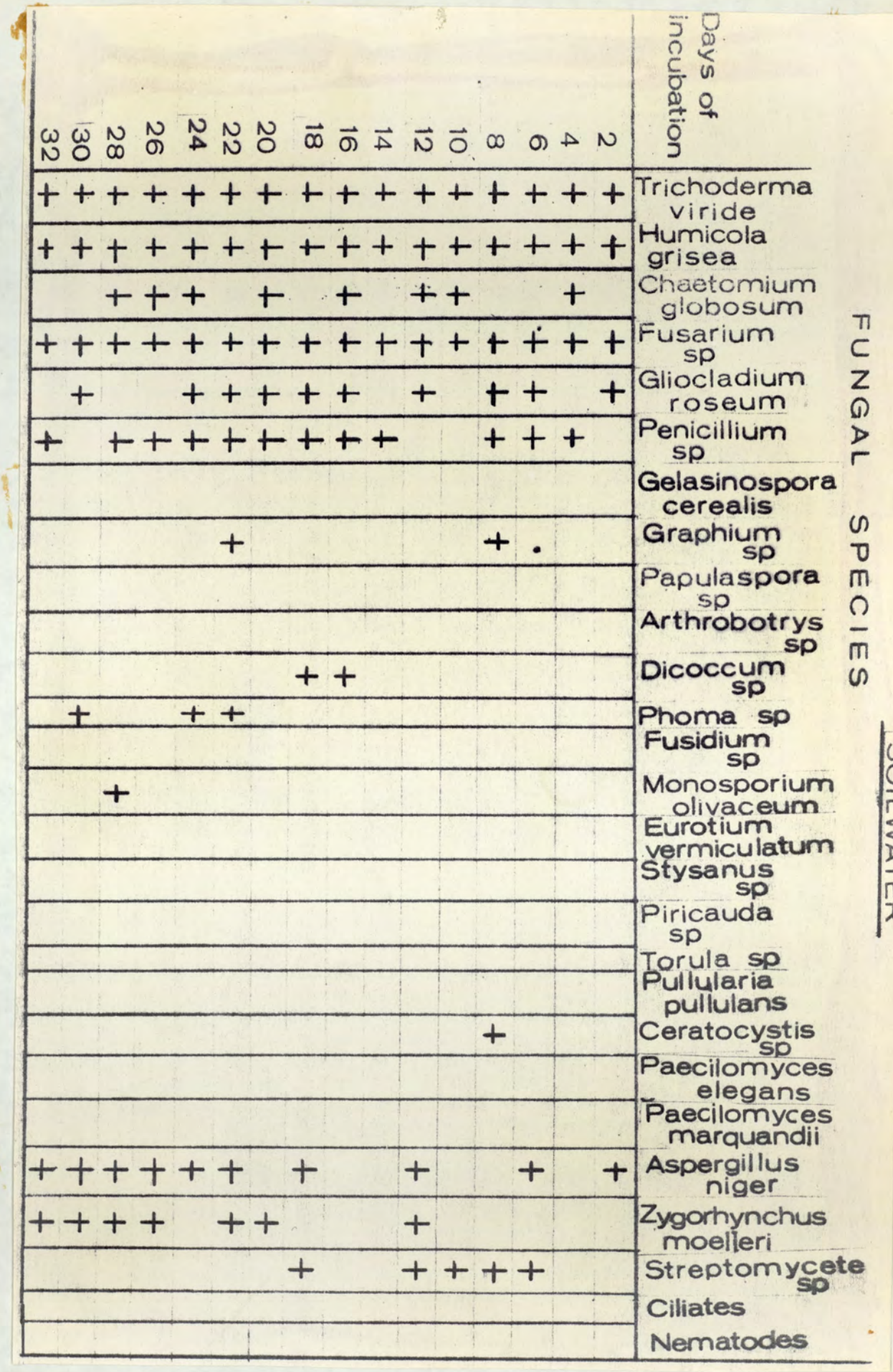


Table 17. Colonizing organisms on veneers perfused with soluble soil extracts.

WOOD EXTRACTIVE

FUNGAL SPECIES

Days of incubation	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Trichoderma viride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Humicola grisea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chaetomium globosum		+		+	+	+		+	+	+			+			
Fusarium sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gliocladium roseum			+		+			+		+	+	+				+
Penicillium sp			+	+			+	+	+	+	+	+	+	+	+	+
Gelasinospora cerealis																
Graphium sp			•													
Papulaspora sp																
Arthrobotrys sp				+												
Dicoccum sp						+										
Phoma sp																
Fusidium sp																
Monosporium olivaceum				+		+										
Eurotium vermiculatum																
Stysanus sp																
Piricauda sp																
Torula sp																
Pullularia pullulans																
Ceratocystis sp																
Paecilomyces elegans																
Paecilomyces marquandii																
Aspergillus niger	+		+			+		+	+			+				+
Zygorhynchus moelleri					+			+		+			+	+	+	+
Streptomycete sp			+													
Ciliates																
Nematodes																

Table 18. Colonizing organisms on veneers perfused with soluble wood extracts.

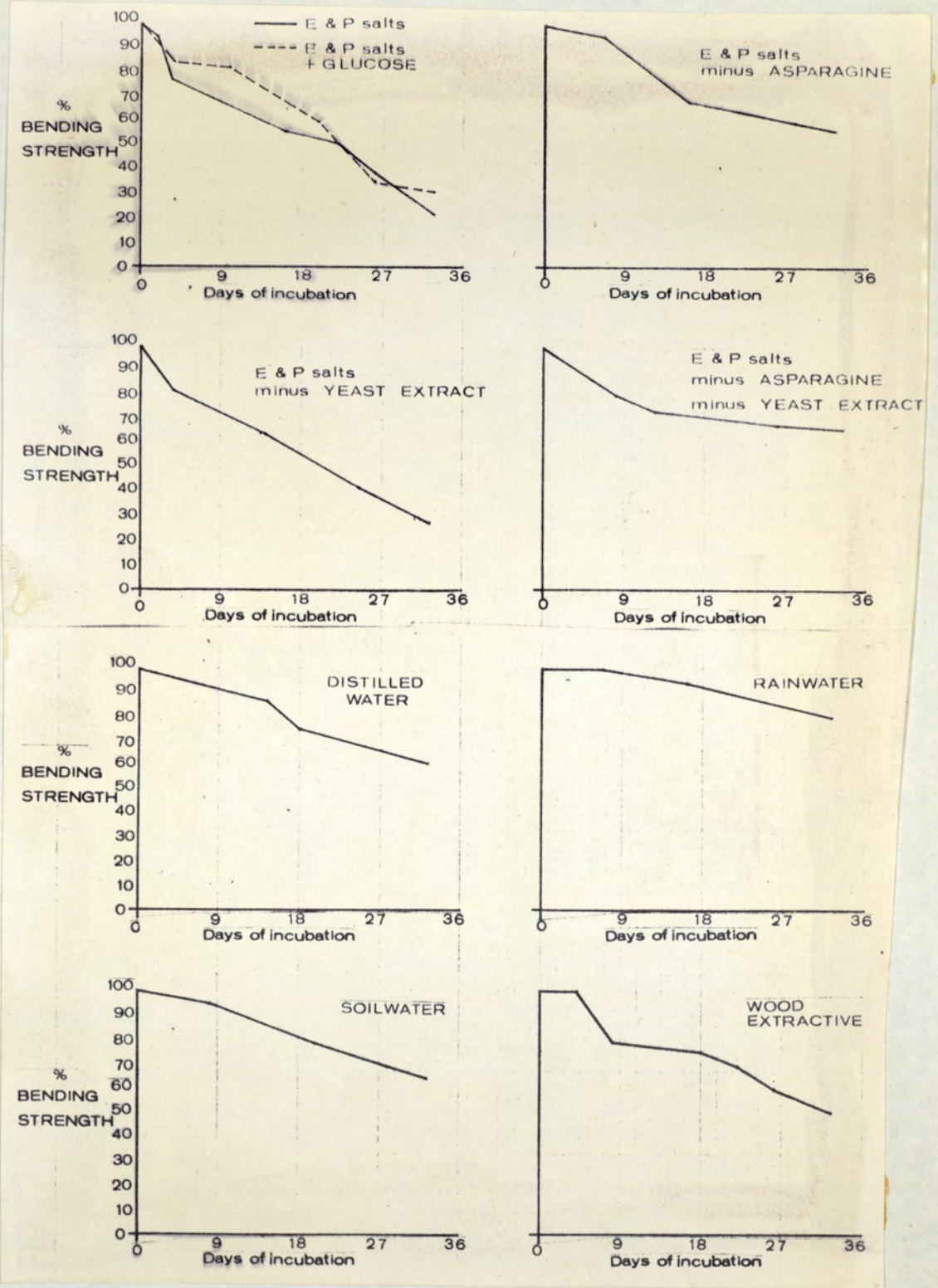


Fig. 19. Strength loss graphs of veneers perfused with some nutrient mixtures.

for these results.

The loss of strength data reveals that slightly less decay occurs with added glucose but although this inhibition is obvious early on it becomes less marked with further incubation. This could be due to a gradual toleration of the extra carbon source as the hyphae become established within the cellulose substrate.

The differences in nitrogen content of the nutrient mixtures perfused caused great changes in the rate of deterioration. Asparagine accelerated decay, as seen from the graph of E. and P. salts minus yeast-extract, whilst yeast-extract had less effect. Absence of both nutrients produces slightly less decay but this is equal to that found with distilled water and so this rate of decay is probably the lowest obtainable without resorting to inhibitors. Since the nutrients minus both nitrogen containing compounds still contains ammonium sulphate which has some available nitrogen then it seems that this third nitrogen source adds little to the rate of decay. The only time Pullularia pullulans was isolated occurred with both nitrogen compounds being absent and this implies confirmation of Metcalfe and Chayen's (1954) demonstration that this fungus fixes isotopic nitrogen as seen by mass-spectrometric methods.

The various water perfusants illustrate that soilwater and distilled water have an equal effect on the rate of decay whilst rainwater is slightly repressive. This might be due to a higher acidity. The water soluble extract from beechwood promotes decay suggesting that these aqueous chemicals act as metabolites and do not include any toxins.

Discussion

Timber in a natural environment subject to washings by rain and soilwater will probably succumb less to decay because of the repressive action of rainwater and the leaching of water soluble wood compounds that aid decay.

The low rate of decay seen with asparagine omitted and with distilled

water could be the basic rate of unadulterated model veneers and therefore be the amount of decay expected with ineffective preservatives.

Action of Light

Introduction

The influence of visible light on the growth and reproduction of microfungi has received much attention. A literature guide has been prepared by Marsh, Taylor and Bassler (1959) and reviews by Carlile (1965) and Page (1965).

Light has been shown to inhibit mycelial growth and stimulate conidial formation for Aspergillus and Penicillium species (Tatarenko 1954), Fusarium (Reid 1958) and Penicillium herquei (Riedhart and Porter 1958).

Light inhibited mycelial growth of Coprinus lagopus (Fries)Fries (Page 1965), inhibited conidial formation in Alternaria, Stemphylium and Helminthosporium (Aragaki 1964) and inhibited sclerotia but encouraged the formation of Aspergillus japonicus conidia (Heath and Eggins 1965).

Light slightly stimulated perithecial development of Chaetomium globosum and conidiophore formation in Alternaria solani (Lukens 1963). It considerably affected sporulation of Alternaria porri (Fahim 1966). Different wavelengths of light had different growth and sporulation responses with Verticillium albo-atrum (Kaiser 1964) and Trichoderma lignorum (Miller and Reid 1961).

Christensen (1961) after evaluation of photobiological investigations has concluded light to be a secondary factor in fungal development since species grow equally well in light and darkness.

Few correlations exist between the influence of light and fungal metabolic activities particularly those connected with wood decay. Duncan (1967) has shown that light from the visible spectrum stimulated decay most with Lenzites trabea, Poria monticola and Lentinus lepideus growing on Pinus palustris. She found a close relationship between the amount of decay and the intensity of light and that shorter wavelengths were more effective in promoting decay.

A similar investigation was undertaken to see whether such responses by basidiomycete fungi apply to soft-rot species. Light could be an important aspect of the environment for soft-rot fungi since wood will have a high light intensity at the surface and darkness in the interior, partly buried timber has different light intensities over its surface and preservative treated timber might have a chemical covering that only allows certain wavelengths of light to pass through.

Experimental

Examination of the response to light wavelengths was measured by wrapping petri-dishes containing perfused veneers with different coloured plastic films that allow the transmission of specific wavelengths only. These colour filters ("Cinemoid" acetate sheeting of the Strand Electric and Engineering company, London) allow passage of particular light spectra and the wavelengths used are shown in fig.20.

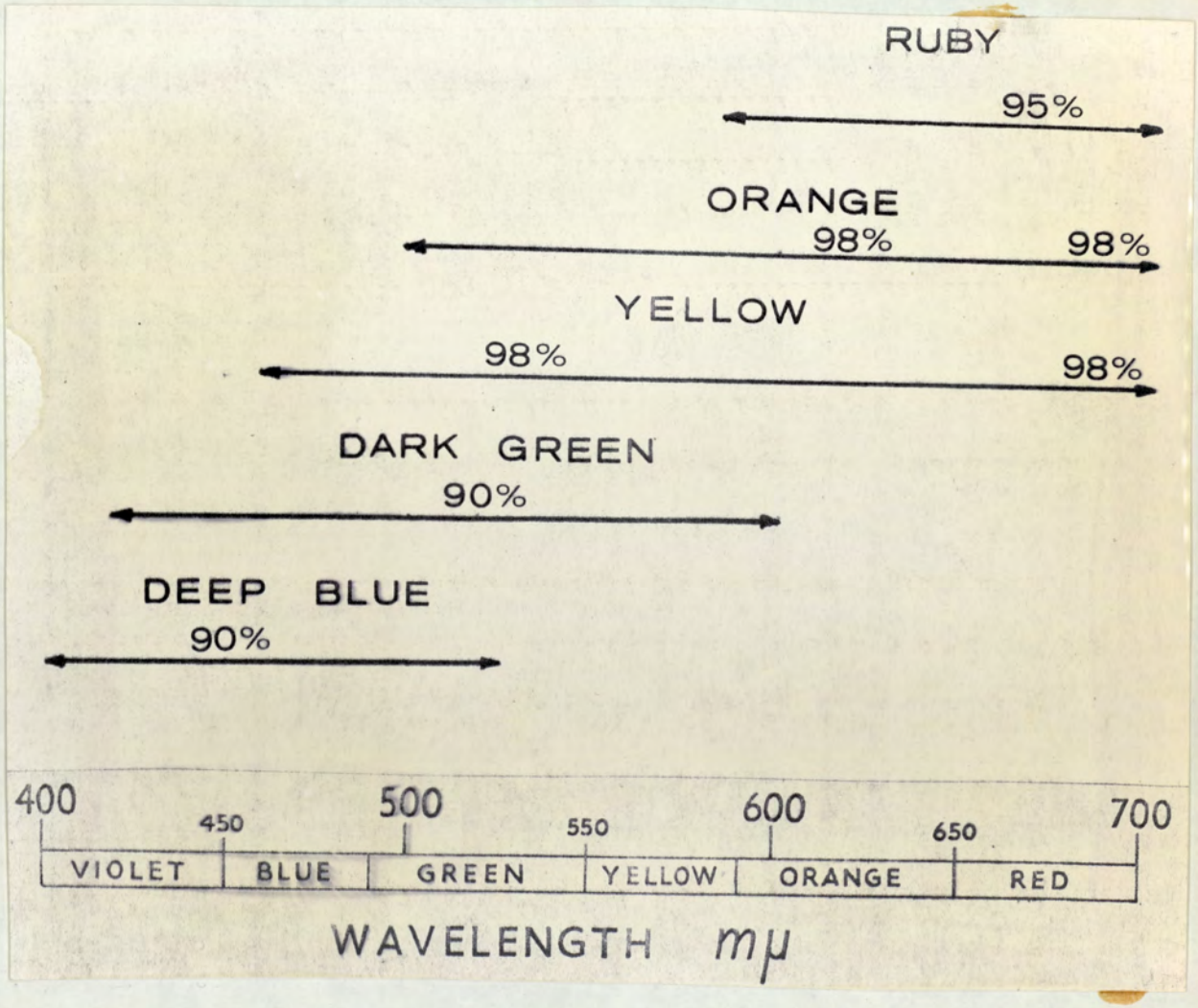


Fig.20. The spectra produced by each filter together with numerical indication of percentage transmission at the peak of maximum transmission.

One set of perfusion devices was covered with aluminium foil to completely occlude the light whilst another set received a covering with tissue paper to simulate translucent conditions and act also as a control for the slightly translucent plastic filters.

All the perfusion sets were given E. and P. salts solution, inoculated with Clent soil and incubated at 25°C. for 36 days. Perspex incubators were used and set at a north facing window to catch even light during the day. At night a 200watt. lamp was used with precautions taken so as not to increase the incubator temperature.

Every six days one veneer per treatment was sampled and the colonizing fungi identified, the results appearing in tables 19 and 20. The veneer strengths were determined and these are seen in fig. 21.

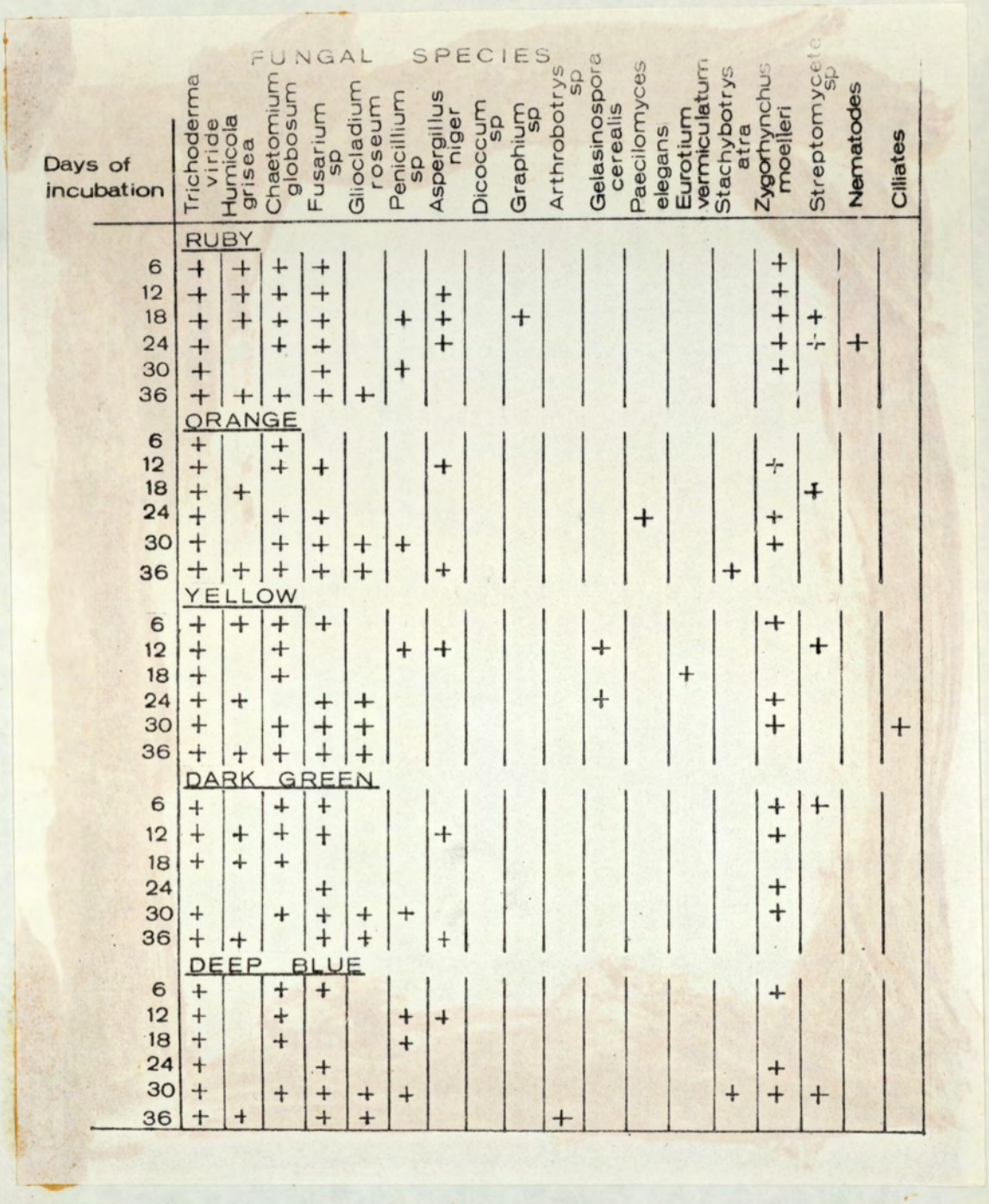
Conclusions

The different wavelengths of illumination were unselective as regards the species isolated and their frequency of isolation. There was slightly greater strength loss with the shorter deep blue wavelengths and less but similar activity to each other from the other spectra. Since the range of wavelengths analysed have unavoidably considerable overlap it is not unexpected for only slight trends to be observed. This bias for the shorter wavelengths of light to promote decay agrees with Duncan's (1967) results.

There was no increase in deterioration with increasing light intensity the response to translucent and opaque filters being similar to that found with normal light. This disagrees with Duncan's work.

Walchli (1968) has recently found Stachybotrys atra and Penicillium funiculosum to attack textiles best in daylight whilst Chaetomium globosum and Trichoderma viride preferred darkness and Stemphylium verruculosum was light indifferent. When some of these species were isolated these responses were not observed. Perhaps these responses are the result of the interplay of light with some other environmental factor that was absent in this research.

A positive phototropic response was seen to occur with a mucoraceous



Days of incubation	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Aspergillus niger	Dicoccum sp	Graphium sp	Arthrobotrys sp	Gelasinospora cerealis	Paecilomyces elegans	Eurotium vermiculatum	Stachybotrys atra	Zygorhynchus moelleri	Streptomycete sp	Nematodes	Ciliates
RUBY																		
6	+	+	+	+											+			
12	+	+	+	+			+								+			
18	+	+	+	+		+	+		+						+	+		
24	+		+	+			+								+	+	+	
30	+			+		+									+			
36	+	+	+	+	+													
ORANGE																		
6	+		+															
12	+		+	+			+								+			
18	+	+														+		
24	+		+	+								+			+			
30	+		+	+	+	+									+			
36	+	+	+	+	+		+							+				
YELLOW																		
6	+	+	+	+											+			
12	+		+			+	+				+					+		
18	+		+										+					
24	+	+		+	+						+				+			
30	+		+	+	+										+			+
36	+	+	+	+	+													
DARK GREEN																		
6	+		+	+											+	+		
12	+	+	+	+			+								+			
18	+	+	+															
24				+											+			
30	+		+	+	+	+									+			
36	+	+		+	+		+											
DEEP BLUE																		
6	+		+	+											+			
12	+		+			+	+											
18	+		+			+												
24	+			+											+			
30	+		+	+	+	+								+	+	+		
36	+	+		+	+					+								

Table 19. Colonizing organisms on veneers wrapped with different coloured filters for transmission of different light wavelengths.

Days of incubation	FUNGAL SPECIES: Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Aspergillus niger	Dicoccum sp	Graphium sp	Arthrobotrys sp	Gelasinospora cerealis	Paecilomyces elegans	Eurotium vermiculatum	Stachybotrys atra	Zygorhynchus moelleri	Streptomycete sp	Nematodes	Ciliates
TRANSLUCENT																		
6	+	+	+	+											+			
12	+	+	+	+		+		+										+
18	+	+	+	+	+	+	+					+	+					
24	+	+	+		+										+			
30	+			+	+												+	
36	+	+	+	+	+		+											
OPAQUE																		
6		+		+											+			
12	+	+	+	+													+	
18	+	+				+							+					
24	+	+	+	+	+										+		+	
30	+			+	+												+	
36	+																	

Table 20. Colonizing organisms on veneers given two intensities of light.

species, probably Zygorhynchus moelleri, with a darkened veneer that had a broken covering allowing entrance of light from one direction. The sporangiophores curved towards this unilateral light.

Discussion

It therefore appears that light is a secondary factor involved in wood decay by microfungi to the extent that even in darkness fungi will effectively deteriorate wood. Possibly small changes in the rate of wood deterioration occur with different light quantities and qualities and this might be linked with other ecological factors. For the timber preserver the modification of light conditions offers little hope as a means of preventing fungal decay. However, light could become significant with the concoction of copper based preservatives where apart from their toxicity their blue colouration permits optimum deterioration.

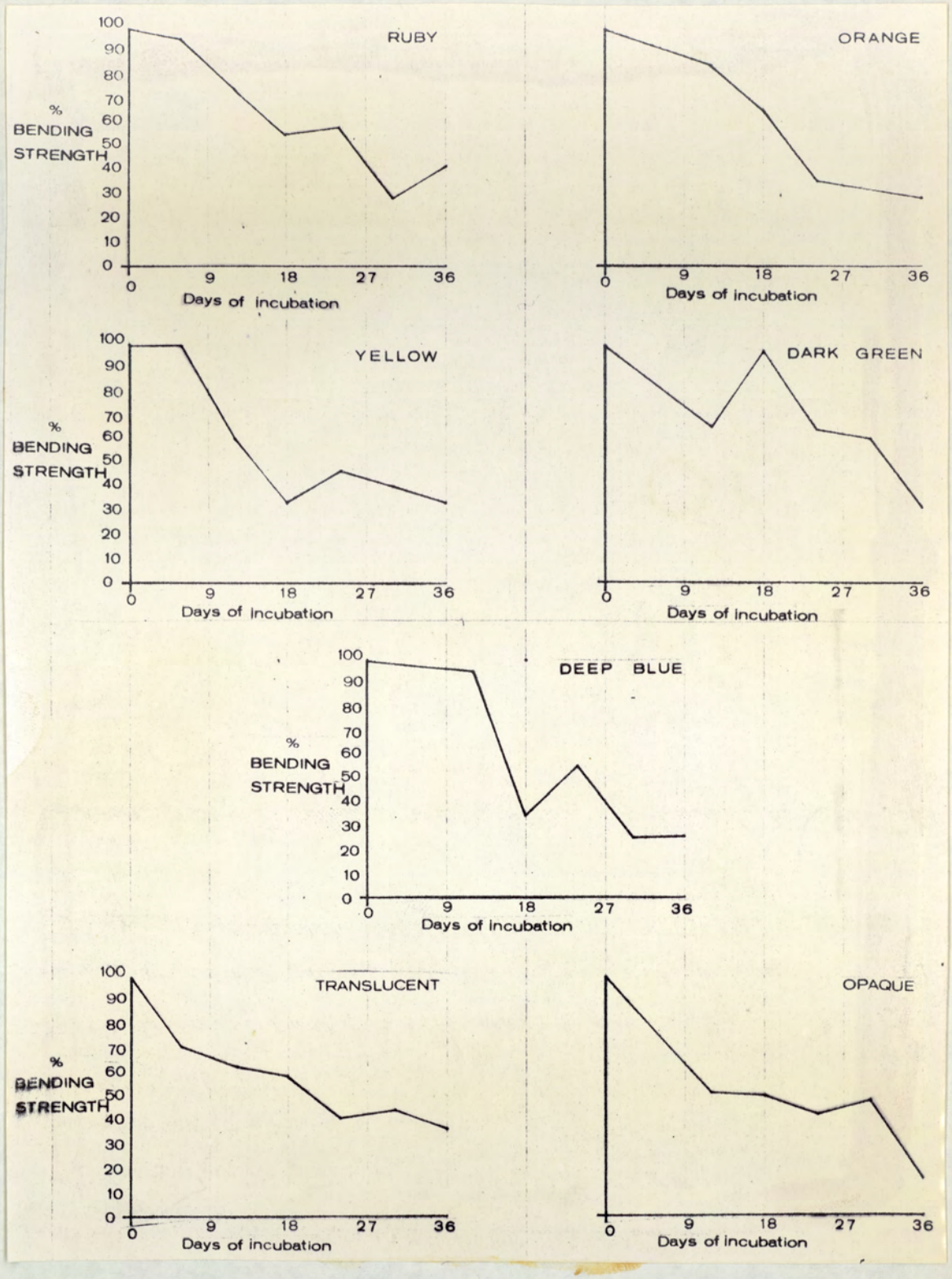


Fig. 21. Strength losses of veneers exposed to various light treatments.

Influence of Aeration and some gas conditions

Introduction

Prolonged microbial utilization of a substrate leads to the decline of energy sources, fall of oxygen levels and a concurrent rise in carbon dioxide, bicarbonate, ammonia, hydrogen sulphide, antibiotics and other toxic materials (Waid 1968). The ability to grow at low oxygen pressures could therefore be a major factor governing the specialisation of wood decaying organisms (Gundersen 1961).

Information on aeration has been attained from a variety of sources. Panasenko (1944) cultured sixteen fungi under sterile vaseline oil in a hydrogen atmosphere and found many morphological abnormalities resulting. After 35 days treatment the Penicillium species survived but the Aspergillus species did not. Waid (1962) isolated Trichoderma viride and Fusarium culmorum from the root surface of Lolium perenne and Periconia sp. from the inner cortex. Periconia sp. was more tolerant of low oxygen. Björkman and Haeger (1963) noted good growth of Trichoderma up to 40-50% carbon dioxide concentrations and little inhibition until almost zero oxygen was reached.

Forest pathology has provided some insight into the gas conditions within trees. Chase (1934) found carbon dioxide up to 6% and oxygen below 5% in red oak. Thacker and Good (1952) measured carbon dioxide in excess of 15% and oxygen below 5% in decaying maple trees with maximum fungal decay at 10% oxygen.

Pure studies with monocultures tend to be concerned with either increasing carbon dioxide or decreasing oxygen but rarely both.

Carbon dioxide has been shown to be essential for spore germination, perithecial production and probably vegetative growth for Chaetomium globosum by Buston, Moss and Tyrrell (1966). Aqueous extracts of jute, hops, malt and yeast were found to substitute for the carbon dioxide. Nyiri (1967) found 1%

carbon dioxide to be optimal for spore germination of Penicillium chrysogenum.

When carbon dioxide levels were increased above 5% by Burges and Fenton (1953) they inhibited Penicillium nigricans but had less effect on Zygorhynchus vuillemini. When carbon dioxide levels were raised to very high values and mixed with small concentrations of nitrogen and oxygen (Stotzky and Goos 1965) some species of Fusarium developed under all gas mixtures except 100% carbon dioxide. Trichodermaviride developed under all gases except 100% carbon dioxide and 95% carbon dioxide with 5% nitrogen. Actinomycetes did not develop except with 100% nitrogen.

The rate of carbon dioxide evolution from wood destroying fungi has been used as an index to measure the rate of wood decay (Good and Darrah 1967) and for determining the efficiency of preservatives (Smith 1967).

Oxygen has been studied by Stewart (1968) when he found that low oxygen tensions reduced the cellulolytic activity of microfungi isolated from mine conveyor belting. Three fungi, Fusarium solani, F. culmorum and Trichoderma koningi, were tolerant of 5% oxygen with the Trichoderma species giving 10% strength loss of cotton yarn at 1% oxygen. Chaetomium globosum, Fusarium moniliforme and F. solani were stimulated appreciably by high levels of carbon dioxide. Duncan (1961) found that dry weight measurements of soft-rot and basidiomycete fungi grown in atmospheres of varying degrees of gas exclusion showed the superiority of soft-rot fungi to withstand low oxygen tensions.

Jensen (1967) studied both oxygen and carbon dioxide levels on four basidiomycetes and found a decrease in growth with oxygen concentrations below atmospheric levels and carbon dioxide concentrations above zero.

The intensive interest in this subject indicates the high selectivity various workers have found gas conditions to exert on the growth of fungi. Successful wood preservation might be achieved by extension of such selective influences. With these past experiences as a guide it was decided to

investigate the influence of gases on the thin permeable veneers having a continual perfusion of fresh nutrients.

The first step was to ascertain any capability the microfungi colonizing the veneers have to actively degrade wood under a zero oxygen atmosphere. This would also determine the effect, if any, of dissolved oxygen in the perfusing liquid. Later experimentation was concerned with the neglected aspect of oxygen levels above ambient and small increases in carbon dioxide on a mixed fungal population. In addition a fourth parameter was considered and this was the gas state at a localised level in the form of an oxidised, or the complementary reduced, substrate condition. There is a paucity of quantitative data on this aspect although such oxidising agents as dichromates, permanganates and chlorine liberating compounds have been widely used as preservatives.

Experimental

1 Affect of zero oxygen :

Numerous piles of four veneers were glued around the edges with silicone glue. The arrangement is the same as that used for the penetration work and is shown in diagram 5. The veneers were incorporated into perfusion sets and incubated with Clent soil at 25°C. for 30 days. Afterwards the veneer pieces of one set were analysed for strength loss and to the rest a thick film of petroleum jelly was applied to all the surfaces of every pile. Then the sets were re-incubated with sampling of one of these treated sets every day.

Strength loss determinations were made for every sacrificed veneer and the results are displayed in fig.22.

Thus by enclosing veneers with an established fungal colonization in an inert atmosphere it was possible to see the effect of zero oxygen on the progressive wood decay.

Conclusions

Even though there is little decay during the 30 days incubation prior to coverage by the jelly, which is characteristic for veneer piles, there is

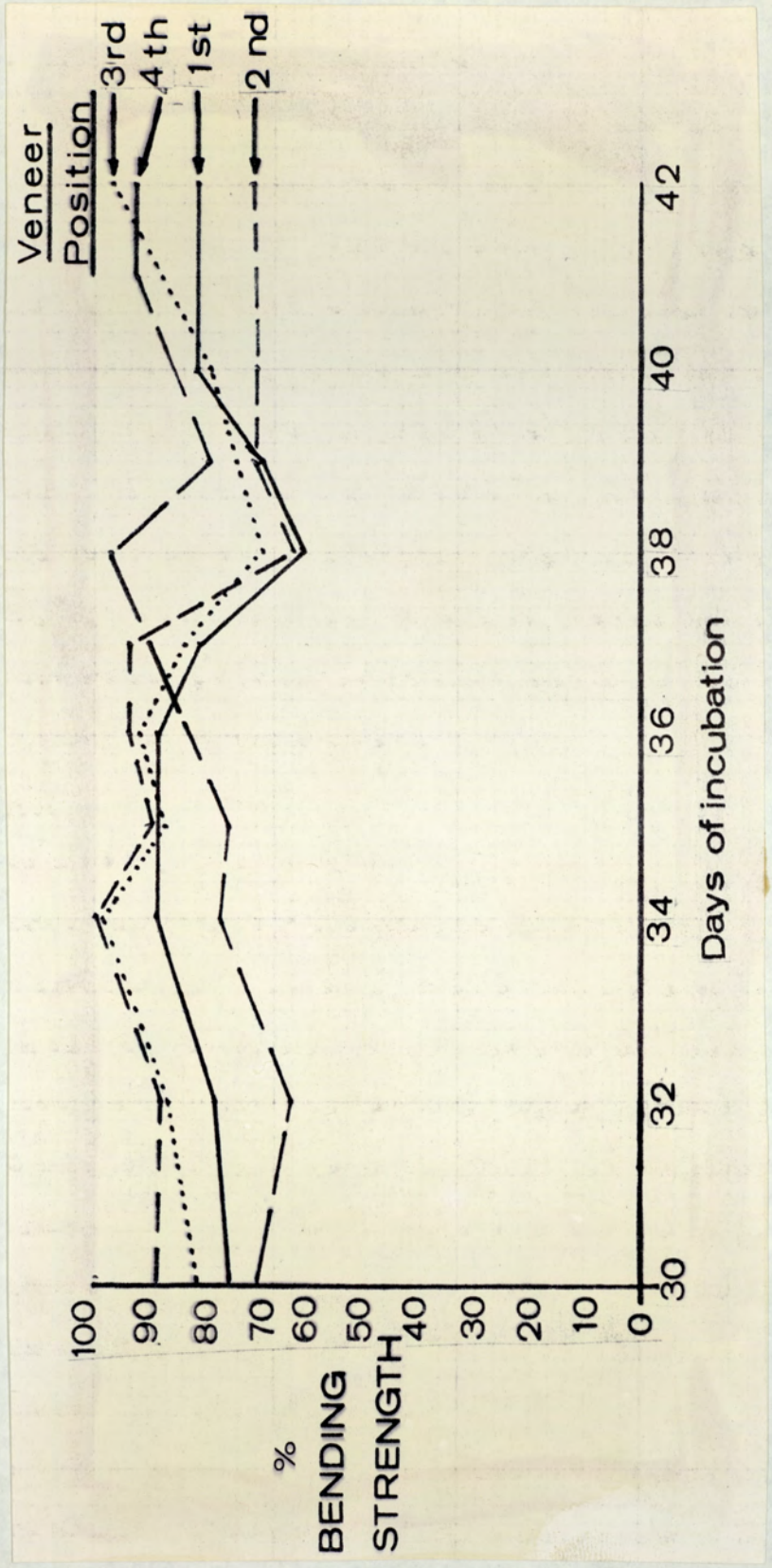


Fig. 22. Strength loss graph for a compound veneer with an established mixed fungal population under an inert atmosphere.

no further decrease afterwards. Thus the gas barrier halted the blackening and decay of the wood. With the last sacrificed veneers there was a hydrogen sulphide odour emanating from them indicating anaerobiosis. Obviously the oxygen contribution from the gas dissolved in the perfusing liquid was negligible.

2 Oxygen concentrations above ambient :

Experimental

The composition of the atmosphere is :-

Gas	Percentage proportion by volume
Nitrogen	77·32
Oxygen	20·8
Water-vapour	0·92
Carbon dioxide	0·03

To produce oxygen levels above those of the atmosphere 60 perfusion devices were set up with an extra 6mm. wide perfusion sleeving contained in silicone rubber tubing passing underneath the veneers at right angles to the nutrient perfusion sleeving. The wick beneath the veneer was exposed and covered with manganese dioxide. Five different concentrations of hydrogen peroxide were perfused along this sleeving and on reaching the manganese dioxide it catalytically decomposed liberating oxygen in amounts proportional to its concentration. The arrangement is illustrated in plate 13.

Clent soil was inoculated on to the veneers which were then incubated for 36 days. One veneer for each oxygen level was sampled every three days and its colonizing fungi and strengths analysed.

The frequencies of isolation are shown in table 21 and the strength results in fig. 23.

Conclusions

Over the 36 days incubation period about 20mls. of peroxide was perfused and therefore the amounts of oxygen liberated were :-

Plate 13. The perfusion device for increasing the oxygen level around veneers. The side bottle contains hydrogen peroxide which perfuses through the extra sleeving.

Oxygen liberated in cc.	FUNGAL SPECIES																	
	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Arthrobotrys sp	Dicoccum sp	Graphium sp	Papulaspora sp	Eurotium vermiculatum	Stachybotrys atra	Paecilomyces elegans	Aspergillus niger	Zygorhynchus moelleri	Streptomycete sp	Nematodes	Ciliates
50	100	66	33	33	25	41	17	17		8	17	17	17	17	50	8	41	
100	100	75	41	50	25	58	17	25		8	8	8	25		33		25	
200	83	83	41	50	33	58	8	8			8	8	8	8	41	8	33	
300	100	75	33	58	25	58		25	8		8	8	8	8	33	17	25	8
400	100	66	50	33	25	58	17					8	8	17	50		25	

Table 21. Frequencies of organism isolation in some oxygen atmospheres.

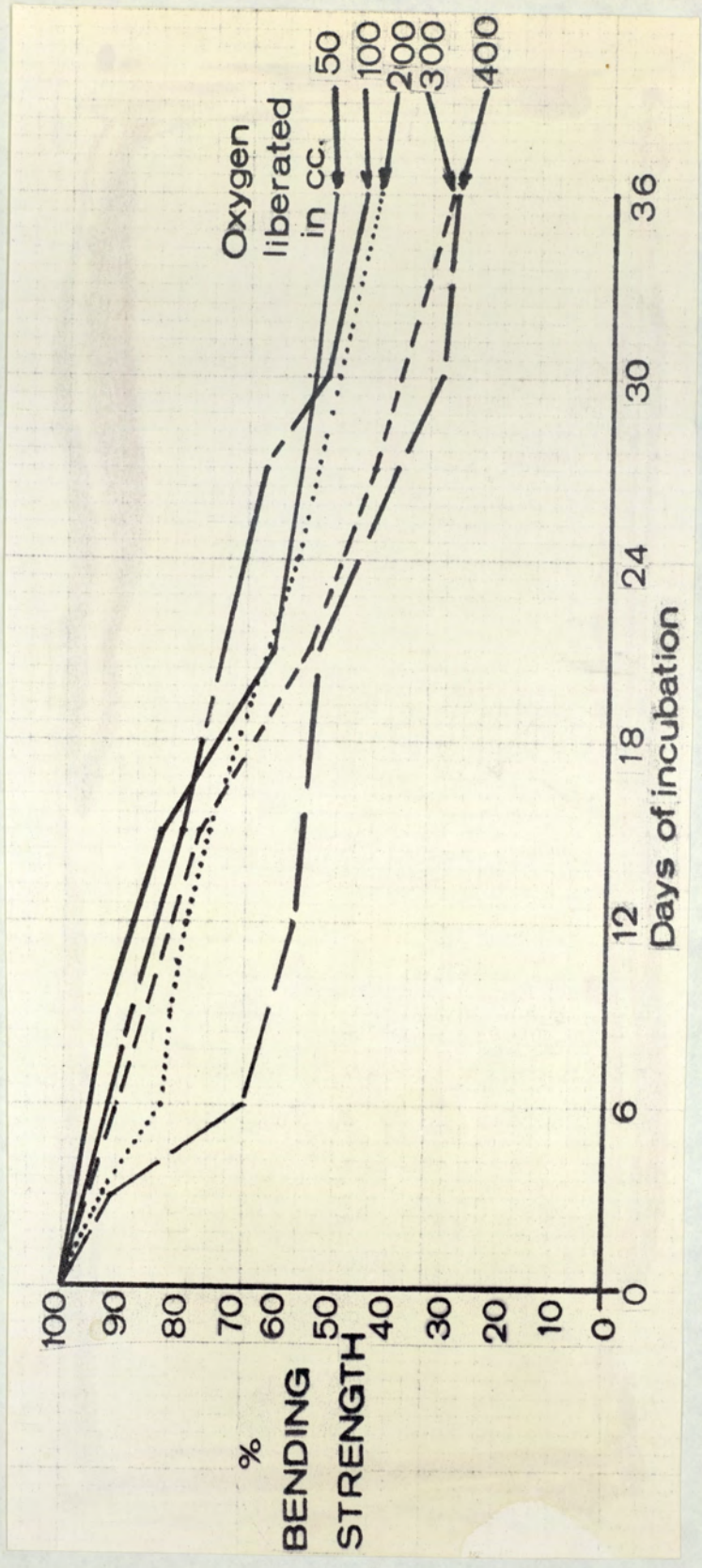


Fig.23. Strength loss graph of veneers in atmospheres of different oxygen contents.

Hydrogen peroxide concentration	Oxygen volume liberated
20 vols.	400cc.
15 vols.	300cc.
10 vols.	200cc.
5 vols.	100cc.
2·5 vols.	50cc.

The various oxygen concentrations do not influence the species isolated nor their frequencies of isolation. There was no induction of rapid colonization or development of extensive hyphal growth. There are slight differences in the rate of strength decrease proportionate with the different oxygen concentrations. This indicates that by increasing the oxygen level around the wood substrate there is an increase in wood decay.

3 Carbon dioxide concentrations above ambient :

Experimental

Similar devices were set up as for the oxygen study but in this case a 9mm. wide wick was used for perfusing different concentrations of hydrochloric acid into 10grams. of sodium bicarbonate contained in the petri-dish. On incubation at 25°C. for 36 days different amounts of carbon dioxide were evolved corresponding to the acid concentration perfused.

After the fungal growths were identified their frequencies of isolation were calculated and these appear in table 22. The strength graph is seen as fig. 24.

Conclusions

The loss of weight of bicarbonate by the 36 day of incubation for each acid concentration was :-

$\frac{N}{40}$ = 1·8g. $\frac{N}{20}$ = 2·8g. $\frac{N}{10}$ = 2·5g. $\frac{N}{5}$ = 3·1g. $\frac{N}{2}$ = 4·2g.

which by calculation from the equation

$$Na\,H\,CO_3 + H\,Cl = Na\,Cl + H_2O + CO_2$$

gives theoretical amounts of carbon dioxide gas evolution as

$\frac{N}{40}$ =480cc. $\frac{N}{20}$ =746cc. $\frac{N}{10}$ =666cc. $\frac{N}{5}$ =826cc. $\frac{N}{2}$ 1,120cc.

FUNGAL SPECIES	Carbon dioxide concentration in cc					
	480 ($\frac{N}{40}$)	746 ($\frac{N}{20}$)	666 ($\frac{N}{10}$)	826 ($\frac{N}{5}$)	1120 ($\frac{N}{2}$)
Trichoderma viride	91	100	91	100	91
Humicola grisea	75	100	75	75	91
Chaetomium globosum	75	83	66	75	91
Fusarium sp	58	75	58	41	75
Gliocladium roseum	50	50	17	41	83
Penicillium sp	17	17	8	8	25
Papulaspora sp	33	25	25	33	25
Eurotium vermiculatum				8	17
Dicoccum sp	25			8	8
Arthrobotrys sp	8	8	8	8	8
Stachybotrys atra					8
Gelasinospora cerealis				8	
Piricauda sp				8	
Torula sp	8		8		
Paecilomyces elegans	33	17		17	41
Paecilomyces marquandii				8	
Aspergillus niger	41	58	33	33	50
Zygorhynchus moelleri	41	58	33	50	41
Streptomycete sp	25	25	25	8	
Ciliates	17				8
Nematodes	8		8	8	8

Table 22. Frequencies of isolation of organisms colonizing veneers in atmospheres with different carbon dioxide contents.

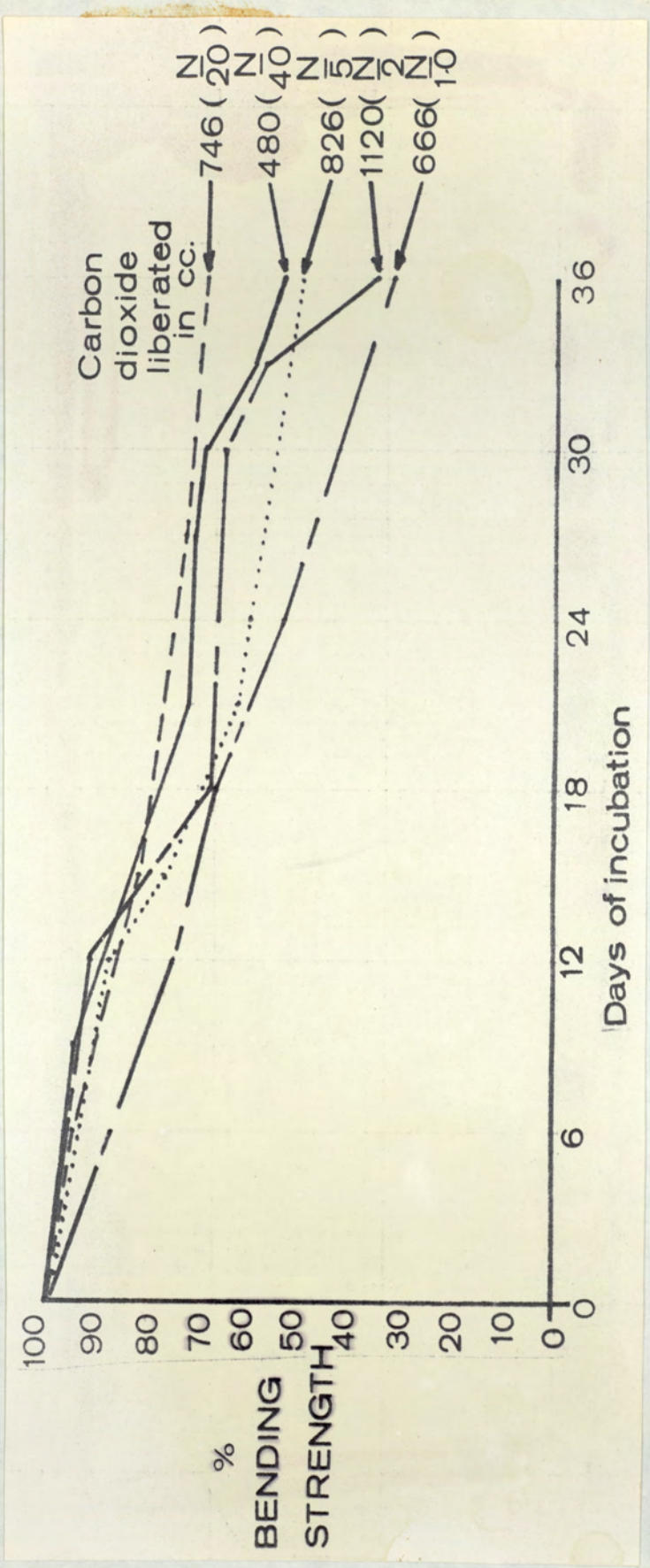


Fig.24. The strength loss graph of veneers in atmospheres of different carbon dioxide contents.

No obvious differences are apparent between the elected fungi for each carbon dioxide tension as shown by the frequencies of fungal isolation. This is similar to the oxygen data. There is one marked difference, however, between the oxygen and carbon dioxide results and that is that there is a higher frequency of fungal occurrence with the carbon dioxide atmosphere. An above ambient concentration of carbon dioxide might therefore provide gas conditions nearer the optimum for these wood microfungi. It is conceivable that the fibrous structure of wood normally behaves as a barrier to gaseous exchange and so raises the carbon dioxide levels by containment of respired gases.

The graph for strength losses gives few clues as to the effect of carbon dioxide on the decay rate, indeed the lack of any logical pattern is more indicative of carbon dioxide not affecting the rate of decay at all. The liberation of 746ccs. of gas for some unknown reason caused a reduced rate of decay.

4 Oxidising the substrate :

Experimental

Oxidation in a narrow sense means the addition of oxygen to a substance or the complementary removal of hydrogen from it.

Forty-eight perfusion sets were set up and their veneers oxidised with low concentrations of four oxidising agents of relatively low toxicity. Sodium metaperiodate and sodium nitrite turned the wood pale brown, alkaline sodium hypochlorite (alkaline from potassium dihydrophosphate and sodium hydroxide) turned the wood white and a mixture of potassium dichromate and sulphuric acid gave an ochre brown colouration. These colour changes indicate that a reaction with the wood had occurred.

After inoculation with Clent soil and incubation at 25°C. for 36 days one veneer for each treatment was sampled every three days and its colonizing fungi and strength loss determined. The isolation pattern appears as table 23 and the loss of strength incurred is shown in fig. 25.

Days of incubation	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Graphium sp	Dicoccum sp	Ceratocystis sp	Gelasinospora cerealis	Papulaspora sp	Eurotium vermiculatum	Monilia brunnea	Paecilomyces elegans	Aspergillus niger	Zygorhynchus moelleri	Streptomycete sp	Nematodes
Sodium metaperiodate																		
3																+		
6	+	+		+	+	+												
9	+		+	+	+													
12	+		+	+	+										+			
15	+	+				+			+							+		
18	+			+														
21	+		+	+			+											
24	+		+	+														
27	+		+	+	+	+							+		+	+	+	
30	+	+		+		+								+	+			
33	+			+											+		+	
36	+			+	+													
Sodium nitrite																		
3	+																	
6	+	+	+	+	+	+										+		
9	+		+	+	+										+			
12	+		+	+											+			
15	+	+	+	+		+		+										
18	+																	
21	+		+	+		+												
24	+		+	+			+											
27	+		+	+		+												
30	+		+	+														
33	+			+													+	
36	+			+	+	+												
alkaline Sodium hypochlorite																		
3	+	+		+														
6	+	+	+	+	+					+				+				
9	+	+	+	+		+												
12			+															
15	+	+	+	+												+		
18	+	+	+	+	+	+												
21	+		+	+	+	+									+			
24	+		+	+							+					+	+	+
27	+			+	+													
30	+	+		+														
33	+			+													+	
36	+			+			+			+				+			+	
Potassium dichromate–Sulphuric acid																		
3																+		
6	+	+	+	+	+	+												
9	+					+										+		
12	+		+			+												
15	+		+		+	+									+	+		
18	+	+	+	+	+	+												
21	+	+	+			+	+											
24	+		+			+												
27	+		+		+	+												
30	+	+		+	+	+												
33	+	+	+	+		+									+			
36	+		+	+	+	+	+			+								

Table 23. Organism isolation pattern from oxidised veneers.

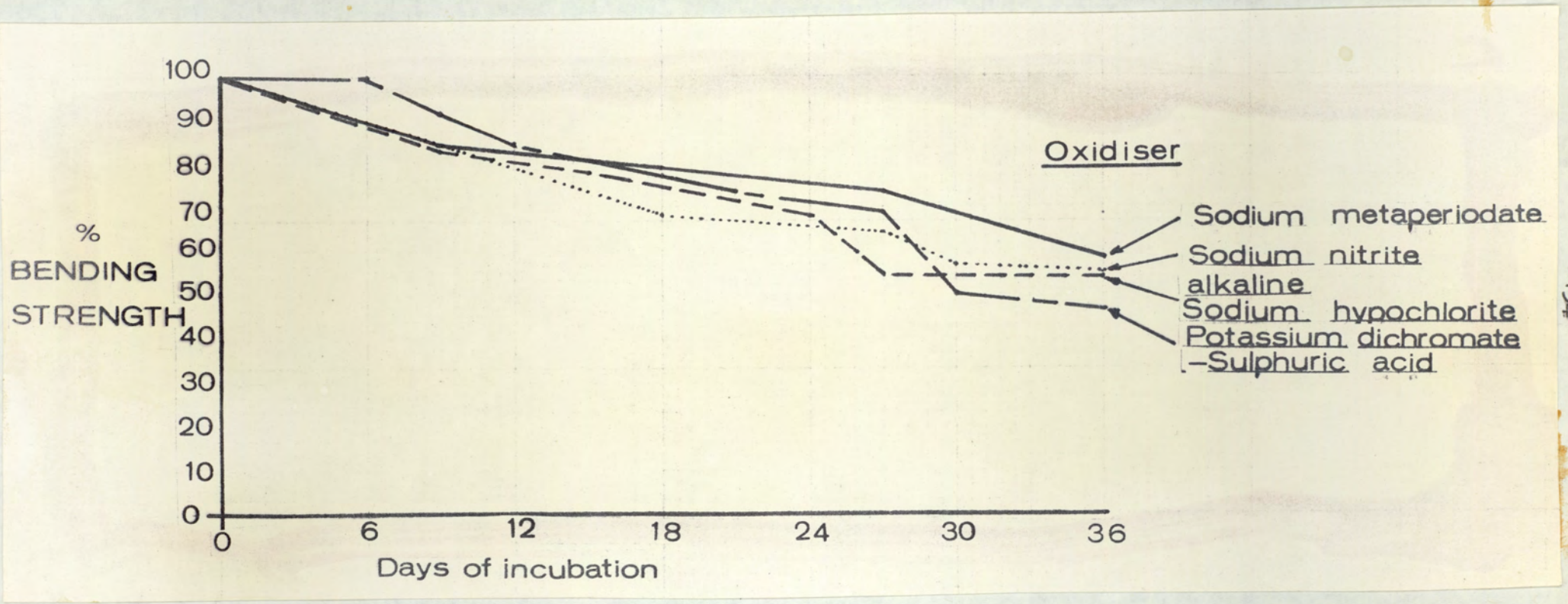


Fig. 25. Strength loss graph of oxidised veneers inoculated with soil.

Conclusions

There are no differences in frequencies of fungal isolation between the treatments but overall there is a reduction in fungal colonization particularly of the dark hyphal species. The strength graph shows a less than normal strength loss. If any of the oxidising agents are toxic then its potency is no more than the other agents since similar inhibition of growth and reduced strength loss occurs with each.

Oxidising the wood chemical constituents therefore results in a decrease in hyphal growth and in the rate of decay.

5 Reducing the substrate :

Experimental

Reduction means the addition of hydrogen to a substrate or the complementary removal of oxygen.

Like the oxidation study reduced wood veneers were inoculated with soil, perfused for 36 days and the fungi and their decay of the wood analysed. Reduction was achieved by four treatments. The wood turned black with ferrous sulphate but there was no colour change with stannous chloride dissolved in acetone, sodium thiosulphate (30% weight to volume in water) and hydrogen peroxide made alkaline with sodium hydroxide.

The results are seen in table 24. and fig. 26.

Conclusions

A wide selection of fungi were regularly isolated with each treatment including a Coprinus sp. basidiomycete.

Therefore a reduced substrate elects the colonization of more fungal species at higher frequencies of isolation than an oxidised substrate does. There is also enhancement of decay with sharp reductions in the strength of the veneers. It should be noted that the stannous chloride treatment is anomalous because this powerful reducing agent suprisingly removed those

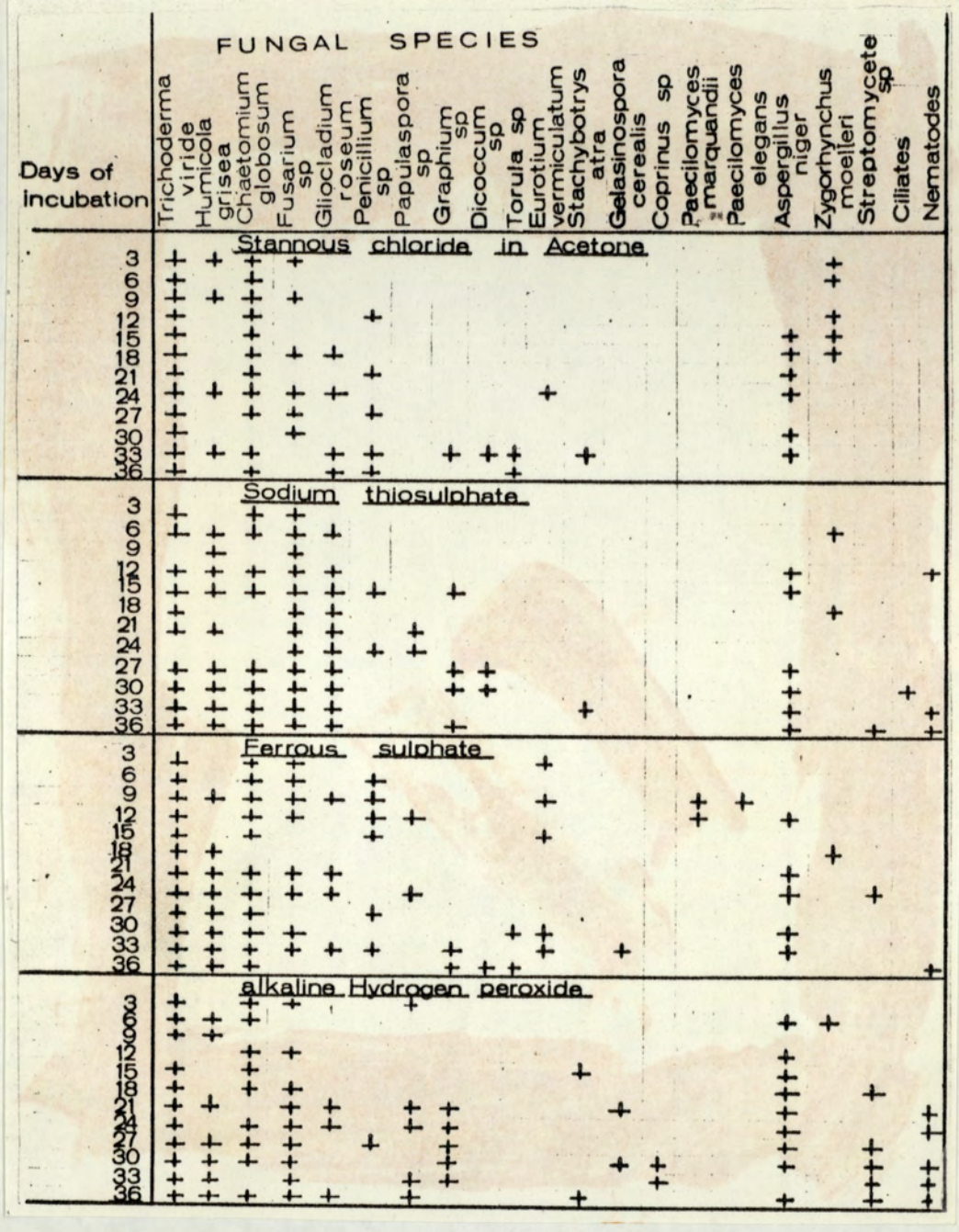


Table 24. Organism isolation pattern on reduced veneers with a soil inoculum.

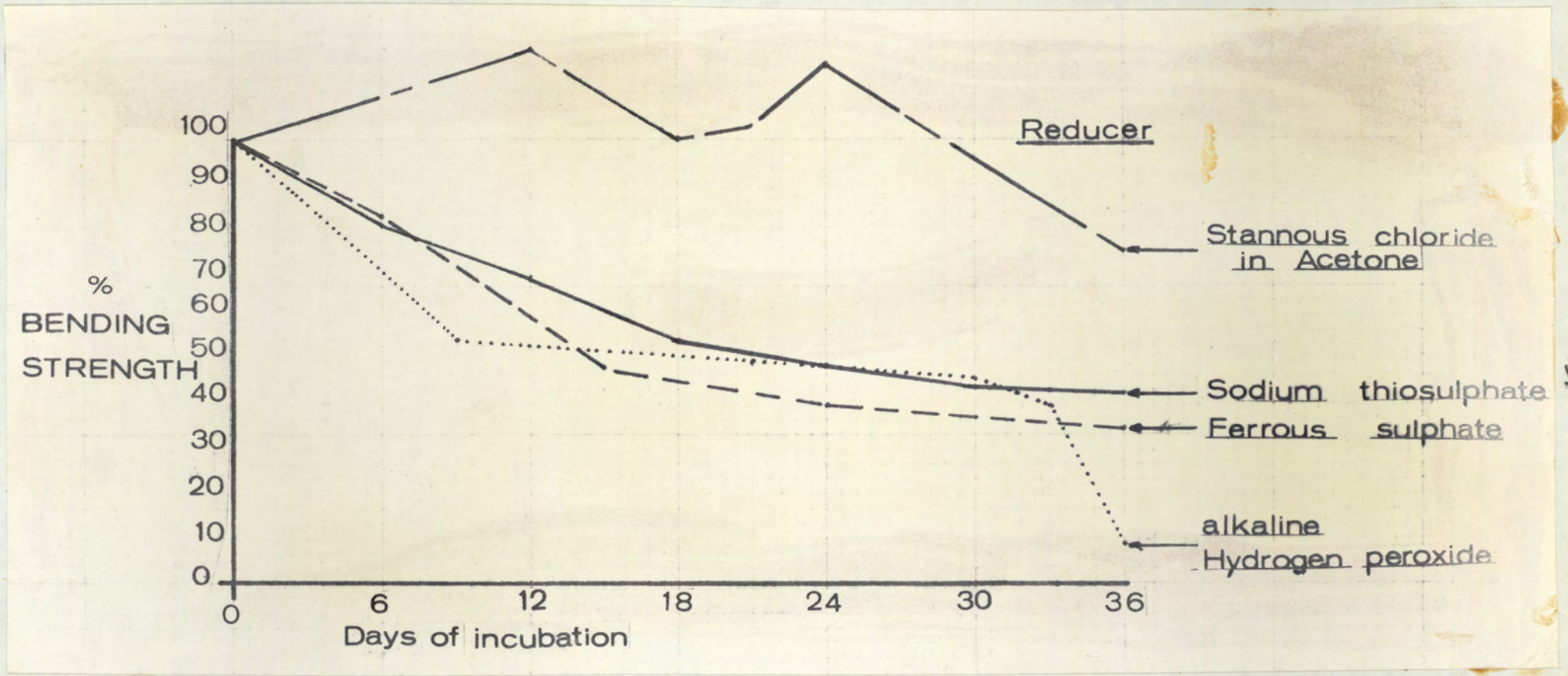


Fig. 26. Strength loss graph of reduced veneers with a soil inoculum.

elements of the wood responsible for its strength and therefore any strength loss of the exposed veneers was impossible.

Discussion

These studies show that a lack of oxygen prevents wood deterioration and confirms the widely held belief that the microfungi are obligately aerobic.

Introduction of higher oxygen concentrations into the wood atmosphere slows fungal colonization but increases decay proportionately with the increased oxygen levels used in the experiment. Oxidation of the wood substance reduces fungal growth and also partially inhibits decay.

Carbon dioxide stimulates fungal colonization but indistinct evidence prevented any detection of whether the fungi produced a correspondingly high amount of decay. Reduction of the wood gave both extensive fungal growth and rapid decay.

As regards the influence of oxygen or hydrogen linked to the substrate it is obvious that quantitative experiments conducted with known oxidation-reduction potentials of a wood will determine the significance of this environmental aspect in a way these cursory studies cannot do. However, these studies so strengthen the opinion that the omission of such precise gas considerations from wood biodeterioration research is a mistake.

For preserving wood it appears that oxidising agents are better than reducing ones and for wood ecological studies where fungi need to be cultured a reduction of the substrate seems desirable.

The results raise the question of whether the mass impregnation of timber by heavy oil preservatives initially prevents decay by total exclusion of oxygen and then with the formation of shakes in the wood due to weathering there occurs a permeation inwards of oxygen. The occurrence of any microorganism growth afterwards will produce respired carbon dioxide which is subsequently retained internally because of the oil barrier. This could then encourage extensive fungal colonization as seen with carbon dioxide concentrations above ambient levels.

Chapter 6

"These also shall be unclean unto you among the creeping things that creep upon the earth; the weasel, and the mouse, and the tortoise after his kind, And the ferret, and the chameleon and the lizard, and the snail, and the mole. These are unclean to you among all that creep; whosoever doth touch them, when they be dead, shall be unclean until the even. And upon whatsoever any of them, when they are dead, doth fall, it shall be unclean; whether it be any vessel of wood, or raiment, or skin, or sack, whatsoever vessel it be, wherein any work is done, it must be put into water, and it shall be unclean until the even; so it shall be cleansed.

Leviticus, chapter 11, verses 29 to 32.

Preservation

Introduction

The silicone properties so far gleaned as relevant to biodeterioration problems include being unbiodegradeable, water immiscible, inert biologically, low surface tension and having a low amount of organic material that could be nutriment to microorganisms. These properties suggest the suitability of silicones for preventing biodeterioration.

Previous work includes a report from Hoffman (1964) of a fungicidal wash containing a silicone compound which controls moulds infesting paintwork. The silicone renders the surface water-repellent and at the same time fixes the fungicide onto the surface.

A preservative treatment for electrical cables consisting of a polyester varnish, trimethyl siloxane, ethyl acetate and pentachlorophenol has been described by Vincent (1966).

Midland Silicones (1966) hold a patent that describes certain chemical products from alkylchloro-silanes which on hydrolysis gives a solid material that may be incorporated into cements, paper etc., to improve water resistance or added to a biocide to regulate the release of active ingredients.

Silicones are often cited as suitable water-repellents for wood and there is a description (Hyde 1967) of various polysiloxane resins curable at room temperature which give a wood finish. Cutler (1957) has patented a thixotropic paste containing preservative, 5% pentachlorophenol and 2% silicone for preventing wood decay.

Silicones can therefore be important additives to various mixtures such as water-repellent solvents. Turner (1967) stated that with formulations of organic preservatives becoming increasingly more complex there is a greater need to understand the influence of the solvent on the extent of preservative penetration and retention. Thus there is a need to understand the microbiological properties of a silicone solvent with silicone containing preservatives to ensure effective toxicity over a long service life. For this aim a few experiments were carried out with silicone solvated preservatives on perfused wood model systems. These include a protection assessment of silicone dissolved sodium pentachlorophenol and tri-butyl tin oxide treatments of wood and an evaluation of various silicone preparations for preventing fungal growth on wood without adulteration by mixture with other compounds.

Sodium pentachlorophenol has a mixed reputation as a preservative. It is mostly converted to pentachlorophenol when the pH drops below 6·8 (Hunt and Garratt 1953). It has a solubility of 79p.p.m. at pH 5, 640p.p.m. at pH 6·08, less than 4,000p.p.m. at pH 6·99 and over 4,000p.p.m. at pH 8·03 (Meyling and Pitchford 1966). Wessels and Adema (1968) found the activity of sodium pentachlorophenol to decrease one hundred times from pH 5 to pH 8. It penetrated less than one centimetre into pinewood (Johnson and Cowling 1965) but remained effective when applied to calcium containing wall materials (Findlay and Savory 1960).

It has been shown to have limitations against decay of unseasoned lumber (Roff and Cserjesi 1965), to be similar to other treatments for combating blue-stain in Scots pinewood (Savory, Pawsey and Lawrence 1965) and to inhibit basidiomycetes better than Chaetomium globosum (Savory 1955).

Duncan and Deverall (1964) found 43% loss of pentachlorophenol from wood inoculated with Trichoderma. Unligil (1968) found Trichoderma viride, Gliocladium viride and Pullularia pullulans to be tolerant of sodium pentachlorophenol. Sharp and Taylor (1969) showed laurylpentochlorophenol to be affected in some undefined way when incorporated into agars inoculated with particular fungi.

Tri-butyl tin oxide in comparison is a more recently discovered and less known wood preservative. Van der Kerk (1954) first pointed out its potential, Brown (1963) found the compound to be fifteen times more effective than pentachlorophenol and Nishimoto and Fuse (1965) placed the toxic limit at 0·001 to 0·002% against Coriolellus palustris. Richardson (1968) estimated the toxic limit as 0·08 to 0·1% with no evidence for volatilization or leaching loss and later (Richardson 1968) has discussed action mechanisms of triorganotin compounds.

There are no reports with quantitative data presented on the use of silicones as or in association with wood preservatives.

1 Silicone solvated sodium pentachlorophenol.

Experimental

Various concentrations between 0·005% to 5% of sodium pentachlorophenol were mixed with Dri-Sil.29 silicone fluid (a 49% active resin in xylene) on a volume to volume basis. Six different concentrations were applied to beech veneers and twelve perfusion sets constructed for each concentration. Application was by coating the upper veneer surface with a thin film.

E. and P. salts were perfused at 25°C. for 36 days with one set for each concentration being sampled every three days. The colonizing fungi were isolated and the "coefficient of protection" for each species calculated according to the formula :-

$$\text{Coefficient of Protection} = 100 - \frac{\text{Number of veneers each species was isolated from}}{12 \text{ (total veneers used for each concentration)}}$$

The results are expressed as percentages in table 25.

% Concentration of preservative	pH of wood	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Dicoccum sp	Ceratocystis sp	Graphium sp	Phoma sp	Gelasinospora cerealis	Papulaspora sp	Humicola sp	Chaetomium funiculum	Eurotium vermiculatum	Aspergillus niger	Zygorhynchus moelleri	Streptomycete sp	Nematodes	Ciliates
·005	6·8	8·7	0	33	8·7	42	17	83			92	92					66	50	92		
·05	7·2	8·7	0	42	0	50	17	92	92	92	75						75	50			
·1	7·5	0	8·7	50	17	50	17	75	92		92						50	75	83	92	
·5	7·8	0	8·7	25	8·7	59	17	92					92	92			42	59	92	92	
1·0	8·2	0	17	42	0	59	17	92							92		66	66			
5·0	8·5	17	50	50	50	75	50				92					92	83	83			92

Table 25. Percentage coefficient of protection results of organisms isolated from veneers coated with sodium pentachlorophenol in silicone.

The strength loss for each veneer was determined and the results are shown in fig.27. There are six graphs for each concentration together with a copy of the tensiometer pen recordings for the exposed veneers only. There are two peaks recorded for each veneer with sampling every third day of incubation; the thirty-sixth day being the right-hand double peak.

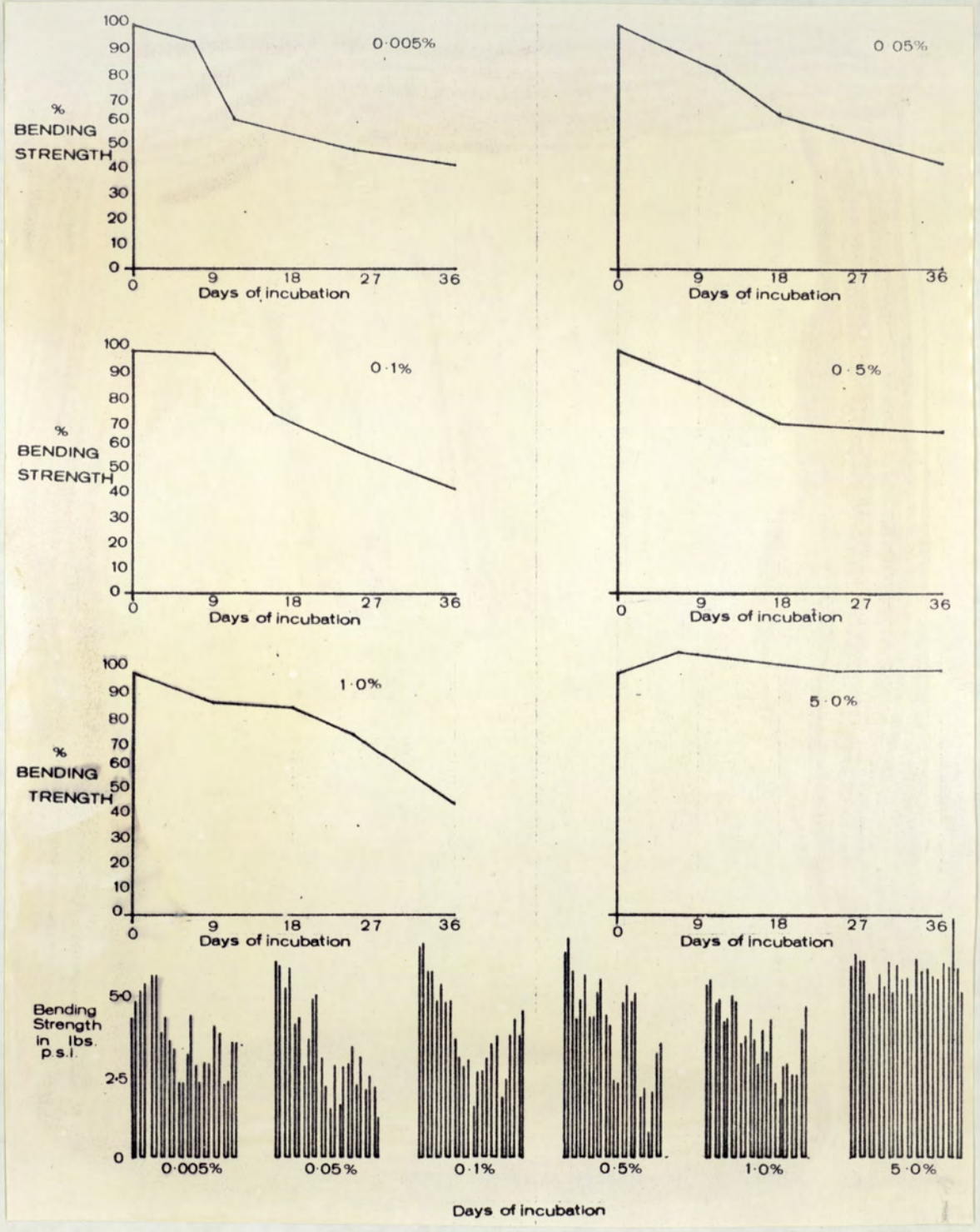


Fig. 27. Strength loss graphs and exposed veneer pen recordings of veneers coated with sodium pentachlorophenol in silicone.

Conclusions

Neither the preservative, silicone or pH of the wood were selective for particular species.

The immediate (three days of incubation) isolation of fungi with all concentrations suggests that with a gradual loss of preservative toxicity with time will eventually allow these fungi to deteriorate the wood. The experimental period of thirty-six days being too short to permit substantial decay. There was no complete eradication of the fungi at high concentrations merely an abeyance of their growth.

The coefficients of protection generally increased as the toxicant concentration increased. The proportional increase in the coefficient of protection for Humicola grisea with rising preservative concentration suggests its use as an "indicator" fungus to assay pentachlorophenol activity.

Where gaps appear in the table this represents a coefficient of protection of 100 and signifies no isolation. This frequency of no isolation for each species was seen to be similar to that encountered with normal isolation from untreated wood.

Only at 5% concentration was no strength loss experienced and this corresponds to the recommended 5% concentration for effective wood preservation as suggested by the pentachlorophenol suppliers.

The silicone carrier did not deter fungal colonization nor prevent decay. Its possible pre-eminence as an aid to thorough preservative penetration did not enhance the toxicity of the pentachlorophenol.

2 Silicone solvated tri-butyl tin oxide.

Experimental

Twelve perfusion sets were set up for five different concentrations of tri-butyl tin oxide in 10centistokes viscosity methyl silicone fluid. The mixtures were wiped over the upper surface of perfused veneers and incubated

as with the pentachlorophenol. Every three days the colonizing fungi and strength loss of one veneer for each concentration was determined. The coefficient of protection results expressed as percentages appear in table 26. The strength losses appear in fig.28. together with pen recordings of exposed veneers arranged as before.

FUNGAL SPECIES	% Concentration of preservative					
	.05	.1	.5	1.0	5.0
Trichoderma viride	8.7	17	0	0	25
Humicola grisea	33	33	17	66	83
Chaetomium globosum	59	50	66	66	92
Fusarium sp	0	0	0	8.7	33
Gliocladium roseum	66	75	42	59	
Penicillium sp	17	42	87	25	66
Gelasinospora cerealis			83		
Phoma sp		83		92	
Dicoccum sp	92	83			
Piricauda sp			92	92	
Stysanus sp			92		
Arthrobotrys sp		92			
Fusidium sp				92	
Paecilomyces marquandii		92	75	83	92
Aspergillus niger	66	75	33	50	92
Zygorhynchus moelleri	66	83	50	83	92
Streptomycete sp	75	83		75	
Ciliates	92				
Nematodes		92	83	92	92

Table 26. Percentage coefficient of protection results of organisms isolated from veneers coated with tri-butyl tin oxide in silicone.

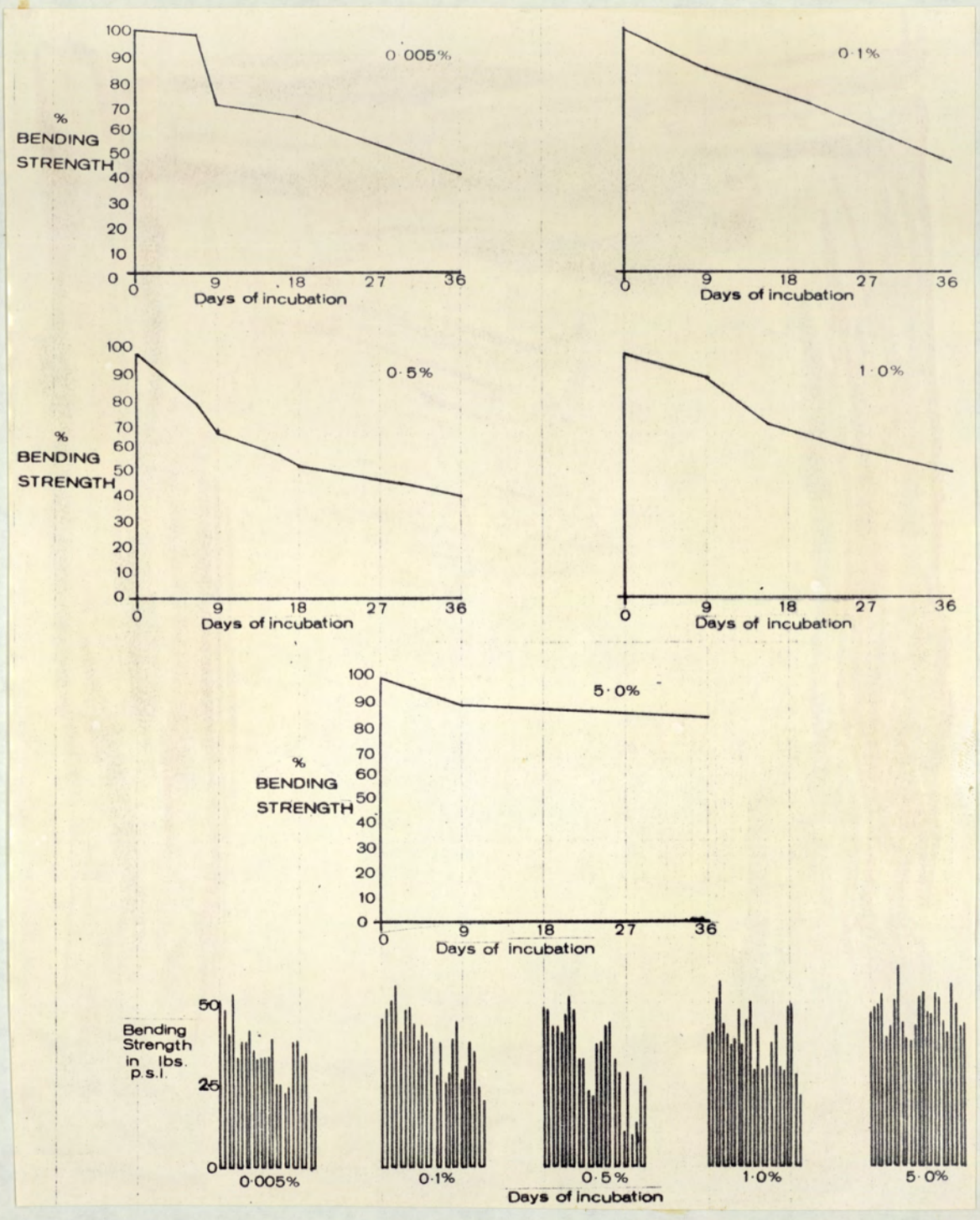


Fig. 28. Strength loss graphs and exposed veneer pen recordings of veneers coated with tri-butyl tin oxide in silicone.

Conclusions

The toxicity limit of 1 to 5% concentration was similar to that found for pentachlorophenol but much above that quoted earlier by other workers. Ignoring the results for a 5% concentration gives an isolation pattern for each species resembling that normally found in the absence of a preservative. There are only slight increases in the coefficient of protection as the concentration of toxicant increases. At the 5% level there were no fungi isolated before the fifteenth day of incubation and then there was a gradual adaptation or toleration to the biocide with their appearance thereafter.

Five percent concentration completely inhibited Gliocladium roseum and almost completely prevented the growth of Humicola grisea. Again Humicola grisea reflected the biocide's effectiveness .

The strength loss measurements reveal equal decay for those concentrations less than 5% and little decay with 5%. This substantiates the data for the coefficient of protection.

3 Silicones

Experimental

Three kinds of preservative action were considered using thirteen types of silicone treatment and these are summarised in table 27.

Thirteen veneers were set up for each treatment having their silicone coating on the upper surface only and being perfused with a mineral salts solution on their lower surface. The veneers were inoculated with Clent soil and incubated at 25°C. for 39 days with one veneer of each treatment being sacrificed every three days.

The percentage frequencies of isolation for each fungal species were found and are shown in table 28. The strength loss of each veneer is depicted in the series of graphs of fig.29.

No.	Treatment	pH on wood	Type of preservation
1	M.S. 200/50 Dimethylpolysiloxane.	4·2	Mass impregnation
2	M.S. 200/1000 Dimethylpolysiloxane.	4·2	
3	M.S.510/50 Phenylmethylpolysiloxane.	3·8	
4	M.S.710 Phenylmethylpolysiloxane.	3·8	
5	M.S.1107 – SiH active polysiloxane. Water-repellent for glass and ceramic.	3·8	
6	Dri-Sil 29. 49% solution of active resin in xylene.	3·4	
7	Dri-Sil 29 + a catalyst.	4·2	
8	Dri-Sil 29 + an emulsifier.	4·0	
9	R.T.V.(room temperature curing) rubber.	4·0	Surface film coating
10	M.S. 2202 a 50% solution of air-drying silicone water-repellent in white spirit.	4·2	
11	Methyltrichlorosilane.	3·0	Chemical linkage with the wood
12	Trimethylchlorosilane.	3·0	
13	Mixed chlorosilanes.	2·8	

Table 27. The treatments selected for attempted preservation of wood by silicone preparations.

Treatment	FUNGAL SPECIES																								
	Trichoderma viride	Humicola grisea	Chaetomium globosum	Fusarium sp	Gliocladium roseum	Penicillium sp	Dicoccum sp	Monosporium olivaceum	Stysanus sp	Gelasinospora cerealis	Phoma sp	Eurotium vermiculatum	Piricauda sp	Fusidium sp	Monilia brunnea	Paecilomyces elegans	Arthrobotrys sp	Aspergillus niger	Zygorhynchus moelleri	Streptomycete sp					
1	85	100	85	69	62	31	38	15					8						54	15					
2	69	100	92	62	69	38	23	8	8									8	77						
3	92	100	92	62	69	54	23	8	8									15	85	8					
4	77	85	85	62	62	69	46	15				8							85	15					
5	92	92	85	69	77	54	23	15		8								8	38						
6	77	92	85	69	69	31	46	15	8	8	15			8	8			8	46	15					
7	77	100	69	69	69	46	62	8			8								62	31					
8	77	92	69	77	77	54	38		31	23	15		8			8	8	15	54	8					
9	85	100	85	77	69	46	38	8	15		8							8	62	15					
10	77	100	85	85	62	31	38	8	15	8								8	54	8					
11	92	92	92	62	62	54	23	23	8									8	69						
12	92	100	100	54	54	100	15	8	15			15						8	77						
13	85	92	85	54	54	77	31	15	15		8							8	85						

Table 28. Percentage frequencies of isolation of organisms colonizing veneers treated with various silicone preparations.

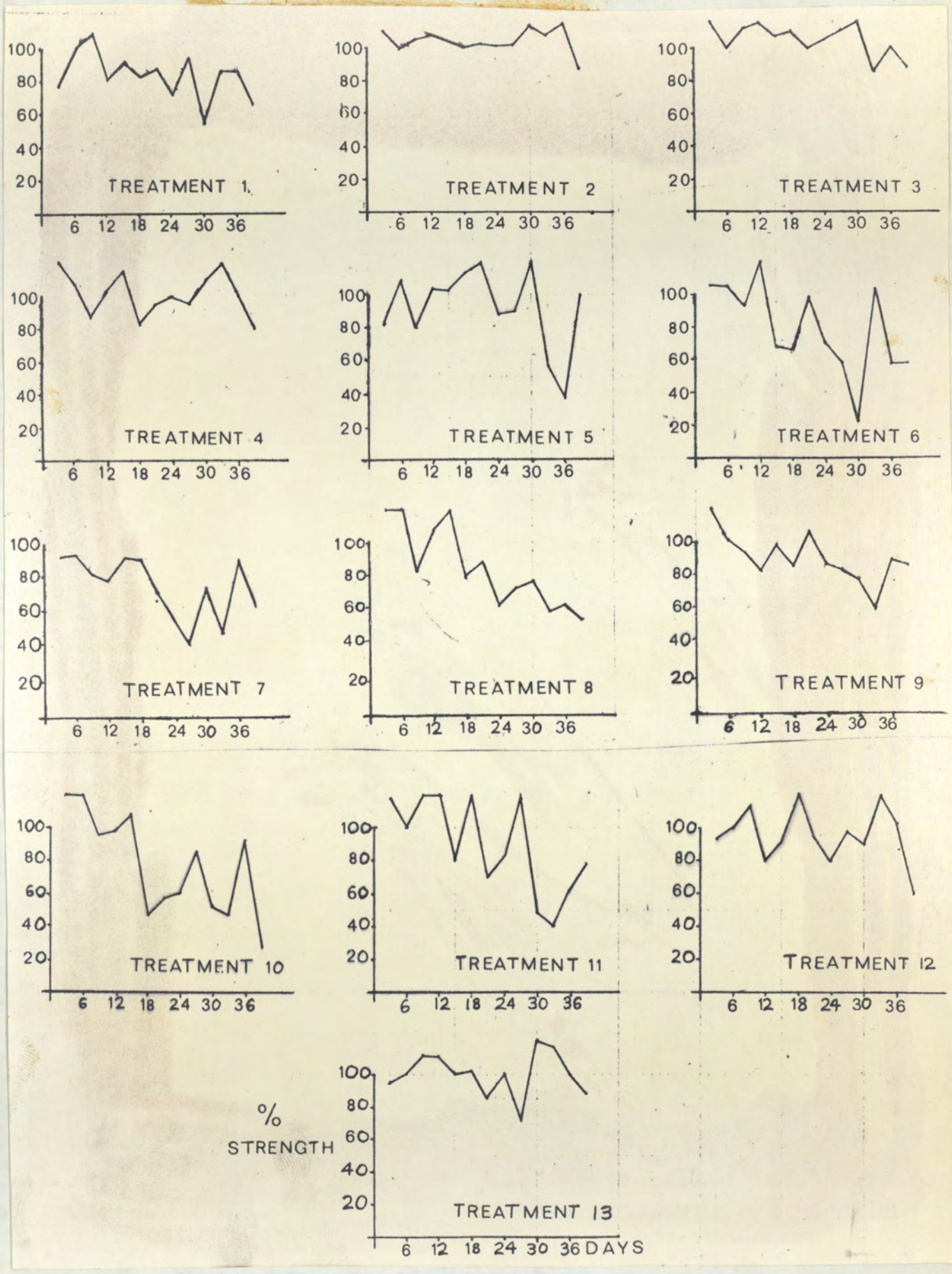


Fig. 29. Strength loss graphs for deterioration of silicone treated veneers.

Conclusions

These results show that the silicones and their high acidities have not hindered the growth of fungi nor have they elected their own mycoflora. The number of species isolated is that normally found on untreated wood. The loss of strength is less rapid than usually expected of deterioration in perfused veneer and it appears that the silicones have slowed down the quantity of fungal growth.

Apart from the mixed chlorosilanes there is some decay with each treatment. The chlorosilane acids partially hydrolysed the wood cellulose during the heat sterilization prior to incubation. Thus the wood strength was reduced from the onset and this prevented any loss of strength occurring until there had been extensive incubation and also precludes its use as a preservative.

The greatest decay was associated with those treatments having least effect on the wood, namely, the three Dri-Sil 29 preparations and the surface rubber film coatings.

The lowest viscosity dimethyl fluids allowed more deterioration than the others and the dimethyl fluids gave less protection than the phenylmethyl fluids. This suggests that greater protection is afforded by supplying large amounts of high viscosity silicones that are able to disperse nearest to the cellulose.

Discussion

Of the two widely used commercial biocides and the silicone solvents and preparations none gave satisfactory preservation of the wood under the conditions arranged as being optimal for fungal growth. Only with high concentrations of biocide and drastic changes in the wood by the silicones was there any resistance to deterioration. This raises the question of whether such methods of preservation are counter to economic considerations.

A method of preservation better than the use of highly toxic compounds would appear to be forthcoming only if a greater selectivity of action

against these biodeteriogens could be achieved. This probably means a mechanism based on biological control or subtle alteration of the wood chemistry producing a recalcitrant cellulose incapable of degradation.

Chapter 7

Discussion

Although the implications of the research data have been detailed throughout the thesis it must now be reassessed and mention made of any probable future developments arising from it.

After recognition had been made in the economic evaluation of the difficulty in assessing correctly the monetary loss caused by timber decay it was apparent from the examples cited that the wastage is considerable and universal. A plea was given for more assessments of damage to particular timber fabrications and estimates of national monetary losses by more precise methods of surveyance.

An historical compilation illustrated that the basis of timber technology was established by 1930 and an assessment of research since then suggested that the last forty years have been concerned in the specialisation of particular aspects including that of microfungal ecology.

To speculate on the pattern of future progress is precarious. There is a growing awareness that the conservation of trees is an essential practice for preventing a deforested countryside. There is a lesser awareness of an impending shortage of timber. As early as the onset of the First World War more than 90% of British timber was being imported and with the later blockade of shipping during the war David Lloyd George was to admit that the war was almost lost because of no timber rather than an expected lack of food. Elliott (1969) states that since the creation of the Forestry Commission after the war (1919) only £320 million has been invested up to 1967 with £130 million of this being compound interest. The current annual grant of £15 million is described as nugatory particularly since the Commission harvests over seven million tons of timber each year. If successive governments tolerate the third largest imported item as being timber, worth in 1968 £640 million, then credence can be attached to a

forecast (Rooke 1969) of a world timber shortage by A.D. 2,000. The present domestic effort of British foresters produces 1·3 million tons of timber yearly and this could only double by 1980, treble by 1990, be five times the volume at 2,000 and still only represent 20% of U.K. requirements.

Prevention of wood biodeterioration appeared possible by the application of silicones after consideration of their many chemical and physical properties and this led to diverse investigations for precisely assessing their potential. A literature survey showed silicones to be inert and stable and experimentation proved them to be unbiodegradeable. Of four culture systems used one was a new soil percolator device that enabled replication without troublesome construction and operation of the apparatus. This inertness suggests the silicones could be useful in environments where deterioration is probable. They might also convert a readily biodegradeable chemical into a resistant one on reaction or prevent detoxification of a toxicant within the silicone's hydrophobic vicinity.

A toxicity study using a wide spectrum of fungi revealed no inhibition or stimulation by various silicones. They would thus constitute an ideal medium for organism culture in biochemical processes. Examination of the effect of silicone hydrophobicity on fungal growth only showed normality. The general conclusion was that silicones might be recalcitrant chemicals with possible complete biological inertness under most conditions.

The need to provide a constant environment in which enhanced deterioration of a material is encouraged was realised early on in the wood study. Enrichment of the soil used for inoculation and of a cellulose paper bait with ancillary nutrients permitted efficient isolation of cellulolytic fungi. However, a novel perfusion method using glass sleeving to convey nutrients by capillarity provided the best results. Brush washing deteriorated wood was found to enable isolation from the wood and not the adhering soil in a superior way to that achieved by surface sterilisation and serial washes with water. Grinding the wood for producing inocula and using E. and P. cellulose agar for isolation plates gave maximum isolation of fungal species.

Measuring the rate of deterioration by determining the strength of strips of veneers proved an easy operation with some specially designed tensiometer jaws. Preliminary statistical tests showed this bending technique of breaking wood to give more reliable results than a tensile test used in comparison. Such model methods of investigating the biodeterioration of wood produce an increase in complexity of the subject but allow a more precise control and analysis of fungal growth.

It is plainly evident that the perfusion principle will undergo modification to serve a variety of culturing systems for different organisms and environments. Whether it can be adapted for large volume culture systems is conjectural. The use of perfusion to provide graduated moisture and gas regimes needs further elaboration for greater control but this should not be too difficult.

Such physical phenomena as measuring the rate of preservative leaching from wood could be undertaken if the perfusion system is arranged to be the leaching mechanism. Different widths of the perfusion sleeving will supply different amounts of leachate to the wood and so give variable degrees of leaching. Similarly a chemical use would arise if perfused metabolites from organisms cultured on the capillary sleeving could be isolated and identified whilst on the glass tape.

There may be a future desire to standardise the methods of comminution of substrates by grinding and monitoring decay by tensiometry. Certainly simpler and robust versions of such apparatus will be an advantage for field use and automated designs with equal accuracy of operation could be constructed for routine laboratory purposes.

The many reactions occurring when nutrients of different pH values were perfused to a soil inoculated veneer underlies the importance of understanding the ecology of fungi before successful inhibition of growth can be obtained. The individual species displayed specific reactions but collectively the highest frequency of isolation occurred at a pH between 6·4

and 7 even though strength losses were almost the same for each pH perfused. An autecological study with monocultures clearing cellulose agar in depth showed five species with highest activity below pH 5 and the rest with optima between pH 5 and 7. Monocultures inoculated onto perfused veneers also gave species-specific reactions. Polycultures caused more strength loss than monocultures and a few anomalies arose between differences in activity of species growing on agar when compared with their activity on wood.

An interaction study with simultaneous inoculation of two species onto the same veneer strip revealed a pattern of mixing that was related to the species cultured and reflected their cellulolytic activities. It is conceivable that such an interaction with a lignicolous species might produce behaviour which symptomatises lignolytic activity far better than the many dubious experiments with chemical preparations of lignin show. In the study few inhibitions and stimulations occurred.

Different rates of fungal growth were detected in cultures on veneer strips and it was possible to erect three groups of activity. A similar study with strength measurements subdivided these groups into three more divisions. The results gave a basis for understanding penetration rates through thickened model woods. No permanent surface colonizers were isolated since all the fungi penetrated the wood pile in a sequence at a rate peculiar to the species and so formed the view that the fungi have their own "penetration time" based on the growth rate. The veneer pile lost its strength as a unit suggesting that maximum colonization occurs before any appreciable deterioration results.

When a graduated moisture system was arranged for a series of perfused veneers the majority of species survived the extreme levels of 23 to 190% moisture content. Four species occurred on wet woods and one tended to be found on dry woods only. No strength loss occurred below 30 to 33% moisture but much deterioration was found with high moisture levels. A two day drying period did not alter the rate of decay.

Various perfusants gave no marked changes in species isolated nor their

frequency of isolation. Glucose temporary reduced the initial loss of strength whilst asparagine accelerated decay and yeast-extract had less effect. Distilled and soilwater permitted some decay but rainwater allowed less decay than this. A beechwood aqueous extract gave almost normal decay. Lack of time prevented a more detailed study of pure nutrients by their incorporation as crystallites or coloured compounds into agars to measure the rate of clearing of the opacity or colour intensity.

Light reactions were seen to be a secondary influence on wood decay. Slightly greater strength loss occurred with the shorter deep blue wavelengths and no response to variations in light intensity was witnessed.

No deterioration of wood was experienced when it was surrounded by an inert gas barrier and a zero oxygen atmosphere. Increasing the atmospheric oxygen above ambient had no effect on species isolated but caused higher strength losses with greater oxygen levels. Higher carbon dioxide levels similarly unaffected the kinds of species isolated but it did increase their frequency of isolation.

Oxidation of the wood reduced fungal colonization and the rate of decay. Reduction elected the colonization of more fungal species and caused greater decay. It was considered that such analyses must be continued with greater quantification.

Adaptation of the results accumulated from silicone and wood deterioration experimentation finally enabled an analysis to be made of fungal colonization on silicone treated wood. Sodium pentachlorophenol dissolved in silicone only prevented wood decay at a 5% concentration and below this level it had little effect. Silicone solvated tri-butyl tin oxide gave much the same response with silicone having no enhancement on the preservation. Thirteen silicone preparations gave differing responses to fungi colonizing the wood but none hindered their growth. Apart from one anomalous case there was some deterioration with each treatment. It is therefore apparent that silicones will only prevent the decay of wood if the material is completely enveloped by a solid film of silicone. They could, however, aid preservative penetration

because of their low surface tension and this particularly applies to those preservatives requiring high temperature treatment for injection into the wood.

After discussing possible research that directly arises from the collected data it is now necessary to mention some long term lines of work. One favoured venture is to examine the effect of various chemical, physical and some biological actions that change the physical structure of wood, without affecting the chemical composition and wood strength, and simultaneously prevents the penetration or degradative activity of fungi. The use of silicone water-repellents, chemical oxidation and penetration by non-cellulolytic fungi are examples from the research of such possible actions and similar mechanisms ought to be tried with regard to changing the availability of wood cellulose or moisture for fungi by physical treatments.

Another recommendation is to investigate the relationship between "humus" molecules and the chemicals of intact and deteriorated wood. The study of humus is usually made on extracts from well decayed material with no attention to the initial stages of its formation. Similarly the few studies of chemicals from wood in advanced stages of deterioration take little account of humic compounds. The two subjects ought to be brought more together since cellulolytic fungi might form substantial amounts of humus and humic compounds might influence the rate of growth and enzymic activity of wood fungi.

The sooner such researches are undertaken and basic information obtained the earlier can effective measures be implemented to conserve wood.

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