FRONTISPIECE : Roof timber split longitudinally to reveal decay caused by the brown-rot fungus *Coniophora puteana*.

A STUDY OF VARIATION IN WOOD-DECAY ABILITY AMONG ISOLATES OF *CONIOPHORA PUTEANA* (SCHUM. EX FR.) KARST.

A thesis submitted to the University of Glasgow

for the degree of Doctor of Philosophy

by

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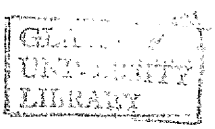

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SUMMARY

An investigation was carried out to examine the variation in decay-potential existing among a range of isolates of the brown-rotting basidiomycete *Coniophora puteana*. Consideration was given to possible factors which might contribute to such differences.

Levels of decay of *Pinus sylvestris* sapwood test-pieces, produced by 37 isolates of *C. puteana*, were measured after 10 weeks incubation under controlled conditions. Decay was assessed by measuring the percentage weight losses and the cross-grain breaking strengths of the wood test-pieces.

A wide variation in decay-potential was found among the isolates tested, and it was possible to identify two distinct populations; namely, one which produced high levels of decay, and another which showed low decay-potential.

Rates of radial growth on agar were measured, and it was found that these varied substantially between strains. These variations did not, however, show a consistent correlation with decay-potential. There was also variation in the influence of temperature on growth by three representative strains.

The extracellular cellulase activities of a number of isolates were measured after growing them in static liquid culture on native cellulose and on sodium carboxymethylcellulose (a soluble substituted form of cellulose). Carboxymethylcellulase (C_x), C_1 (see Reese *et al.*, 1950), and cellobiase activities were assayed in the culture filtrates.

There was little variation in either C_x or C_1 activities, and it did not correlate with the decay-potential variation; there was no detectable cellobiase activity. C_1 activity was not present in filtrates from cultures grown on sodium carboxymethylcellulose, but was present in those from cultures grown on cotton wool, so it appears to be induced. Conversely, C_x activity was present in both sets of culture filtrate.

A partial separation of the components of the cellulase complex, was carried out by gel filtration on Sephadex G-75; two C_x enzymes were identified, with approximate molecular weights of 32000 and less than 10000. The filtrates from a number of isolates were further analysed by polyacrylamide gel electrophoresis, and it was

found that the cell-free protein profile will vary in accordance with the substrate used to grow the fungus; strain differences were also indicated.

The effect which different concentrations of the two wood preservatives pentachlorophenol (PCP) and tri-n-butyl tin oxide (TBTO), exert on growth by a range of isolates of *C. puteana*, was examined. A diversity of response to low concentrations of both fungicides was found.

GENERAL INTRODUCTION

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The study of timber decay began in earnest in the early nineteenth century, as a consequence of serious losses which were occurring among ships of the line in the Royal Navy through timber degradation (Ainsworth, 1976). This problem reached a climax during the Napoleonic wars when an increase in shipbuilding necessitated the use of incompletely seasoned timber, and even sapwood for large structural members (Cartwright and Findlay, 1946). James Sowerby, the notable botanical illustrator, and the Reverend Berkeley, were two early mycologists who were involved with the survey of the decay. One of the first people to establish the true relationship of fungi to wood-decay, was Robert Hartig who, in 1878, was able to distinguish two major groups of decay fungi, namely, those which cause brown rots and those which produce white rots. A third major group of wood-decay fungi was identified more recently (Savory, 1954) and comprised those fungi which produce a soft rot of wood. The macromorphological, micromorphological, chemical and physical characteristics of decay by these three groups of fungi, have been well documented (Seifert, 1968; Liese, 1970; Amburgey, 1972; Carey, 1975).

The brown-rotting basidiomycete *Coniophora puteana* (Schumacher ex Fries) Karsten. (Syn. *Coniophora cerebella* (Persoon) Duby.), is one of the most important fungi causing decay of timber in buildings in Europe (Cartwright and Findlay, 1946), and it has been suggested that 95% of all fungal wood-decay in the United Kingdom can be attributed to *C. puteana* and another brown-rot fungus, *Serpula lacrymans* (Hickin, 1963). *Coniophora* is also found in the United States of America (Silverborg, 1953), Australia (Cartwright and Findlay, 1946) and has been recorded in building timber in Uttar Pradesh, India, at an altitude of 6,500 ft. (Bakshi *et al.*, 1957).

This dissertation describes a study of the variation in decay-potential which exists among a range of isolates of *C. puteana*; it goes on to consider possible factors which might contribute to such differences, and consideration is also given to differences in sensitivity of different isolates to fungicides commonly used in wood preservation.

A knowledge of the variation existing within this species, should be of fundamental importance to those industries which are involved with the prevention, treatment and eradication of timber decay in buildings. Firstly, to the treatment companies who could use such information to aid in the optimisation of their approach to specific problems of decay by

this fungus, and secondly, to the preservative manufacturing industry in the design of adequate laboratory and field tests for new products.

Many fungi have been examined for intra-specific variation, and wood-decaying fungi are no exception. Variation has been found in their colony characteristics, growth rates, temperature responses, decay abilities, nuclear status and in their response to wood-preservatives (Childs, 1937; Aoshima, 1954; Cowling and Kelman, 1964; DaCosta and Kerruish, 1965; Amburgey, 1967; 1970). A study has recently been carried out in this Department on the 'Dry Rot' fungus *S. lacrymans* (Abu-Heilah, 1975), in which a comprehensive survey was made of the decay-potential among a large number of monokaryotic and heterokaryotic isolates. A wide variation was found in their ability to cause decay of Scots pine sapwood and of four more commonly used building timbers (Abu-Heilah and Hutchinson, 1977). Factors which might be important in contributing to some of the differences found, were later examined (Abu-Heilah and Hutchinson, 1978). The investigation reported in this thesis is designed largely to complement this previous study of *S. lacrymans*.

Previous workers have included *Coniophora* in general surveys of various aspects of wood-decay by a range of representative fungi, or they have looked at specific features of the growth and physiology of the organism; very few have statistically analysed their results, or used more than one strain.

A preliminary study of the variation between seven isolates of *C. puteana* was carried out by Masser (1975); she found great variation in a number of characters, and concluded by suggesting that a more comprehensive study would be highly desirable.

Gross characteristics of decay by *C. puteana*

Coniophora produces a brown rot of wood by degrading the cellulose component whilst leaving the lignin relatively intact. It has, however, been shown that a ligninase is present, since during decay, the lignin content is reduced by 10%, p-hydroxybenzaldehyde being a decomposition product (Seifert, 1962). Similarly, microspectrophotometric studies have indicated that there is some depolymerisation of the lignin after decay by *C. puteana* (Bauch *et al.*, 1976).

During decay, the wood becomes darkened and shrinkage cracks occur

which run mainly parallel with the grain. The timber will commonly display advanced deterioration internally and this only becomes apparent on the removal of the thin skin of intact wood from the surface (see frontispiece).

C. puteana may not be purely saprophytic, as it has been implicated as a causal organism of butt rot in standing conifers (Macdonald, 1939; Jorstad and Juul, 1939; Etheridge, 1956), and in black cherry (Davidson and Campbell, 1943). This produces a structural weakening of the tree base, making the tree more prone to windfall.

Morphology of the fungus

The hyphae vary in width (2-10 µm), but are generally wider than is normal for basidiomycetes. The young, growing hyphae at the advancing zone of a colony will produce clamp connections in the heterokaryon (usually after about 3 days growth on agar); these develop into multiple clamps which are whorled about a single septum and are a diagnostic feature of *Coniophora*. Kemper (1937) studied the development and cytology of a number of isolates of *C. puteana*; the clamp connections are formed predominantly, on aerial hyphae, and from one to several nuclei may pass into each pocket, which fuses with the cell below the septum and discharges all the nuclei into that cell. Alternatively, a lateral branch may be formed, into which the nuclei pass. Kemper suggested that the clamps had lost their original task and their main responsibility nowwas to produce a general mixing of nuclei. *C. puteana* is very similar to *Coniophora arida*, and it has been suggested that the best method of distinguishing them is by the appearance of their sporophores (Kemper, 1937). *C. puteana* produces sporophores out of doors in the autumn, in northern countries, but rarely does so in buildings. There are reports of the production of sporophores of *C. puteana* in the laboratory, but the logic behind the methods used remains obscure (Badcock, 1941; 1943; Tamblyn and DaCosta, 1958). The sporophore consists of a plate-like hymenium which may vary in diameter from a few centimetres, up to half a metre. At first, it is light yellow in colour, but with spore development, it becomes olive coloured, though often with a light margin.

Colony characteristics have been described (Kaarik , 1973), but these can vary widely between strains (Cartwright and Findlay, 1946; Davidson and Lombard, 1953; Matters, 1955; Gersonde, 1958b). There are probably

two basic growth forms; in the first type, the culture is at first smooth, soft and even, and only when older do fine strands appear, and in the second type, numerous fine strands are formed as the culture develops, and it has a distinctly 'wooly' appearance. Colours vary from white to yellow to brown, and the agar may be discoloured to different degrees.

Growth and environment

(a) Temperature: The growth of four species of brown-rot fungi on agar was measured over a range of temperatures from 18 to 32°C, and it was found that each had a distinct optimum (*C. puteana* : 24-28°C); it was suggested that such differences could be a useful aid to their identification (Davidson and Lombard, 1953). In a comprehensive survey of the effect of temperature on the growth of 64 species of wood-destroying fungi on agar, it was found that *C. puteana* produced most growth at 24°C (Humphrey and Siggers, 1933), this was later supported in a similar study by Cartwright and Findlay (1934).

The optimum temperature for the decay of wood does not necessarily correspond with that for mycelial growth on agar (Grinda, 1976); Wälchli (1977) found that an isolate of *C. puteana* produced greatest decay levels at 28°C, but most growth on agar at 26°C. Optimum wood decay by *C. puteana* has been obtained at 28°C (Schulze, 1942), 26°C and 20°C (Gersonde, 1958b). These differing values indicate that strain differences may have an effect on the response to temperature.

Coniophora can be reasonably tolerant to extremes of temperature, being able to survive an hour at 40°C and twenty minutes at 60°C; it will also remain viable after three hours exposure to -30°C (Vanine and Vladimirskaya, 1932). However, growth of *C. puteana* will stop at temperatures below -2°C (Bocharova *et al.*, 1958).

The possible influence of temperature on the ecology of the fungus is discussed in a study of the decay fungi present in logging residues of lodgepole pine in Alberta (Loman, 1965). Two populations of fungi were identified; a group which grew in the top 2 inches and which had a high temperature optimum with wide tolerances, and a second group which included *Coniophora*, these growing at depths below 2 inches. This group showed a low temperature optimum and a narrower tolerance range. It was found that *C. puteana* was killed within ten minutes at 52°C in dry wood, and within 40 minutes in wet wood. On occasion, *Coniophora* was

found in the upper layer and Loman attributed this to reinvasion and colonisation during overcast, wet periods.

(b) Wood water content: Perhaps one of the most striking physiological traits of *Coniophora* is its ability to grow at relatively high substrate water contents (decay by this fungus is popularly termed 'Wet Rot'). A study of the growth of *C. puteana* in birch wood, under a range of water contents, revealed very good growth at a water content of 50-60%; good growth at 45%; poor growth at 35-40% and no significant growth below a wood water content of 30% (*S. lacrymans* did not grow in wood with a water content greater than 40%) (Lehmann and Scheible, 1923). These values were supported during work on wood preservatives, when it was found that optimum growth of *Coniophora* occurred in wood with water contents of 40-50%, but beyond 60%, growth began to be inhibited (Flerov and Popov, 1933). Bjoerkmann (1946) produced an optimal range for growth of *C. puteana* on pine of 35-85% wood water content; this is rather too broad to allow an optimal value to be identified. However, in an extensive study of the growth produced by a number of wood-destroying fungi at different wood water contents, a well defined optimum of 60% was revealed for decay by *C. puteana* (Ammer, 1963), a higher water content producing a reduction in the decay rate. It is accepted that a very high wood water content will inhibit the destructive ability of wood-destroying fungi (Theden, 1941), possibly due to a reduction in the level of available oxygen. A considerable portion of the increase in wood water during decay may be attributed to fungal respiration; experiments with *C. puteana*, *S. lacrymans* and *Poria vaporaria*, showed that the amount of free water formed by them in breaking down wood cellulose, varied little from the theoretical amount (55.6% of cellulose dry weight), calculated from the equation

$$\underset{(162)}{C_6H_{10}O_5} + \underset{(192)}{6O_2} \rightarrow \underset{(90)}{5H_2O} + \underset{(264)}{6CO_2}$$

representing the degradation of cellulose to water and carbon dioxide by these fungi (Miller, 1932). The author suggested that this self-wetting would be favourable to *S. lacrymans* in allowing it to colonise new wood in the absence of external moisture; *C. puteana* may not be able to do this as it has a much greater demand for water, and is more sensitive to drying effects.

Theden (1941), Bjoerkmann (1946) and Ammer (1963) each conducted extensive studies on the behaviour of wood-destroying fungi in wet wood, using essentially the same procedure, and their results agree. However, it is pointed out that the resolution of their results might have been improved had the wood water levels been more sharply delimited. Intrinsic difficulties in controlling wood water contents were exacerbated by the aforementioned self-wetting effect of fungal respiration.

A further aspect to be considered is the extent to which atmospheric humidity affects fungal growth. Studies on the growth of *C. puteana* on malt agar, indicated that growth is inhibited below a relative humidity (R.H.) of 77% (Ferdinandsen and Buchwald, 1937) or, in the case of Bavendamm and Reichelt (1938), below an R.H. of 85.6%. However, recent work in this Department has shown that strains vary in their response to R.H. (Croall, 1978), and this may account for the different values. Both these values are low and may be due to their being obtained from agar cultures; as mentioned earlier, fungal growth will vary in response to the substrate water content, and R.H. will affect growth by affecting the substrate water content. Miller (1932), suggested that the build-up of water is a function of three main factors, namely, the initial moisture content of the wood, the rate of flow of the air, and the decay-potential of the fungus; one should also add the humidity of the air to this list. So agar, being merely a gelled form of water, will be able to lose moisture to an atmosphere of low R.H. whilst retaining sufficient for the support of fungal growth. In contrast, wood equilibrates to a water content of 30.5% at an R.H. of 96.5% and to 25% at an R.H. of 92.4% (Theden, 1941), the lower limit for fungal growth being about 27%, namely, the fibre saturation point of pine. Nevertheless, it has been shown that *C. puteana* will produce weight losses in wood up to an R.H. of 93%, but this can be termed 'terminal growth', being due to the lag period between the insertion of a wet piece of wood at a particular R.H. and the achievement of its equilibrium moisture content (Ammer, 1963). Miller (1932) found that *S. lacrymans* produced a luxuriant aerial mycelium which produced many water droplets, and he suggested that this might aid in the colonisation of dry regions, by actually raising the humidity of the ambient air.

The level of substrate water in relation to growth, will vary in accordance with specific properties of the substrate. It has been shown that the limiting water content for the development of a 3% weight loss was approximately 26% for decay of pine sapwood by *C. puteana* as

opposed to 24% for decay of spruce sapwood and 18% for Athy Mill Bowater standard hardboard and Swedish Tempered Masonite. Also, decay of tempered hardboard was prevented at an initial water content of 66%, but that of standard board was hardly checked at a water content of 214% (Grant and Savory, 1975); this may be due to differences in porosity and available oxygen.

Toxic chemicals and growth by *C. puteana*

A wide range of chemicals have been tested in an attempt to prevent colonisation by the fungus, to stop its growth or to kill it, although the logic behind some of the early tests is not immediately clear. Certainly, a common denominator is that they employed noxious chemicals with strong smells, for instance : acetic acid vapour, chlorine, chloropicrin, sulphur dioxide, formalin, carbon disulphide, benzol, benzene and sulphuric ether (Vanine *et al.*, 1932a). These were used, possibly in an attempt to fumigate infected wood, and a further paper (Vanine *et al*, 1932b) describes tests of protectant treatments; creosote performed very well against *S. lacrymans*, *C. puteana* and *Fomes pinicola*, and protected the wood for the three month duration of the test. Commercial oil paints and varnishes were tested, but these gave the wood only partial protection, which was due not to chemical inhibition, but to the prevention of water uptake by the wood and to their action as mechanical barriers to the fungus. At that time in Russia, reed bundles were used as a constructional filling material, and these were very susceptible to attack and decay by *C. puteana*. It was found that soaking the reeds in a 5% solution of zinc chloride preserved them from decay, even under optimum conditions for fungal growth, but copper sulphate was only partially effective (Vladimirskaya, 1932). Metallic salts prevent the normal development of the cell by acting as protein precipitants; the heavy metals (Hg, Ag) are generally more effective as fungicides than those with low atomic weights, which in many cases are essential for growth and metabolism (e.g. Na, K, Ca and Mg), although others of higher atomic weight may be equally important (e.g. Cu, Zn). Each metal, however, has a limited range of tolerance by the fungus beyond which it becomes increasingly toxic, exhibiting first inhibitory, and then lethal properties (Sykes, 1965).

Gersonde (1958a and b) tested seven isolates of *C. puteana* and a number of strains of three other brown-rot fungi, against four water soluble and three water insoluble wood preservatives. The strains of *C. puteana* displayed a diversity of growth and form, and a range of

sensitivity to sodium arsenate, mercurous chloride, pentachlorophenol, coal-tar oil and sodium fluoride. *Coniophora* showed greatest sensitivity to sodium arsenate and mercurous chloride. The behaviour of the individual strains towards any particular preservative was not uniform, their sensitivies to that chemical varying from one strain to another. Greatest differences between strains were with sodium arsenate, mercurous chloride and pentachlorophenol; no differences in response to magnesium silicofluoride and α-chloro-naphthalene were found. This varying range of response might be connected to the mode of operation of the individual preservatives and to the physiological differences between strains.

In another survey using the agar-plate method, a number of chemicals were tested for toxicity against an isolate of *C. puteana*, and it was found that sodium pentachlorophenate, sodium fluoride and arsenic pentoxide were the most effective (Henningson and Nilsson, 1976). The use of copper-chrome-arsenate (CCA) was tested by Sorkoh and Dickinson (1976), and they found differences in activity according to the type of wood used as a substrate. The same compound was tested by Levi (1976) and it was found that *C. puteana* colonised treated wood, but produced no decay; the fungus, however, remained viable and it actually solubilised some components of the fungicide. This led to the proposal of a 'suicide' theory to account for the action of copper based preservatives; if present in high concentrations, the preservative will be 'recognised' by the fungus, and will not be absorbed, but with low concentrations of CCA, the fungus will solubilise and absorb the preservative and eventually succumb to a build-up of toxin. Aspects of fungicide action are further considered in Part 4 of this thesis.

Consideration of the extracellular secretions produced by *Coniophora*, and their possible role in decay of wood, has been reserved until Part 3, where it is dealt with fairly extensively.

PART 1

GENERAL METHODS

GENERAL METHODS

This section describes the conditions and basic methods which were employed generally throughout a large part of the work. Techniques which were used in more specific studies, are described individually within the relevant sections.

1.1 Origin of cultures

The origins of the 37 isolates studied are presented in Appendix 1.

Those cultures which were obtained from the field were isolated in the following way: gross debris was removed from the surface of the affected wood sample, which was then sawn longitudinally to expose the decay front inside. Using a flamed scalpel, pieces of wood were removed from the region just in front of the area of brown discoloration, and placed on 2% malt agar incorporating 50 ppm Benlate (Du Pont). Fast growing Fungi Imperfecti, which would have swamped the slower growing Basidiomycete, were inhibited by the Benlate in the medium (Coggins and Jennings, 1975; Hale and Savory, 1976). The cultures were incubated and monitored for the production of a white mycelium bearing multiple clamp connections; when present, this was subcultured onto an agar slope.

1.2 Culture media and culture maintenance

Unless otherwise stated the cultures used in this work were grown on 2% malt extract agar [Malt extract (Oxoid) 20 g, Agar agar (Oxoid) 20 g, both dissolved in 1 litre of distilled water and autoclaved at 120°C for twenty minutes]. Stock cultures were maintained on slopes of this medium in test tubes stored at 4°C in a refrigerator; they were subcultured at approximately six monthly intervals. Inocula for experiments were prepared by subculturing from the stock cultures onto 2% malt agar in 9 cm glass petri dishes and incubating for 14 days. Eight millimetre diameter discs, cut with a sterile cork borer from the colony margins, were used to inoculate the experimental vessels.

1.3 Conditions of incubation

1.3.1 Temperature and light

The cultures were grown at 24°C in the dark. This temperature was chosen as the result of a preliminary investigation which is

reported in Appendix 2.

It has been shown that very small periods of low intensity light from the visible spectrum (possibly the shorter wavelengths) can have a stimulating effect on the rates of decay by a number of wood-destroying Basidiomycetes, and this has been identified as a source of large variation in wood-decay experiments, even under well-controlled conditions (Duncan, 1967). This, combined with a consideration of the available facilities, made it convenient for all growth experiments to be carried out in the dark.

1.3.2 Substrate water content

The control of this important factor is discussed in section 1.3.3, and a series of investigations relating to wood water content, is described in Appendix 5.

1.3.3 Preparation of culture vessels

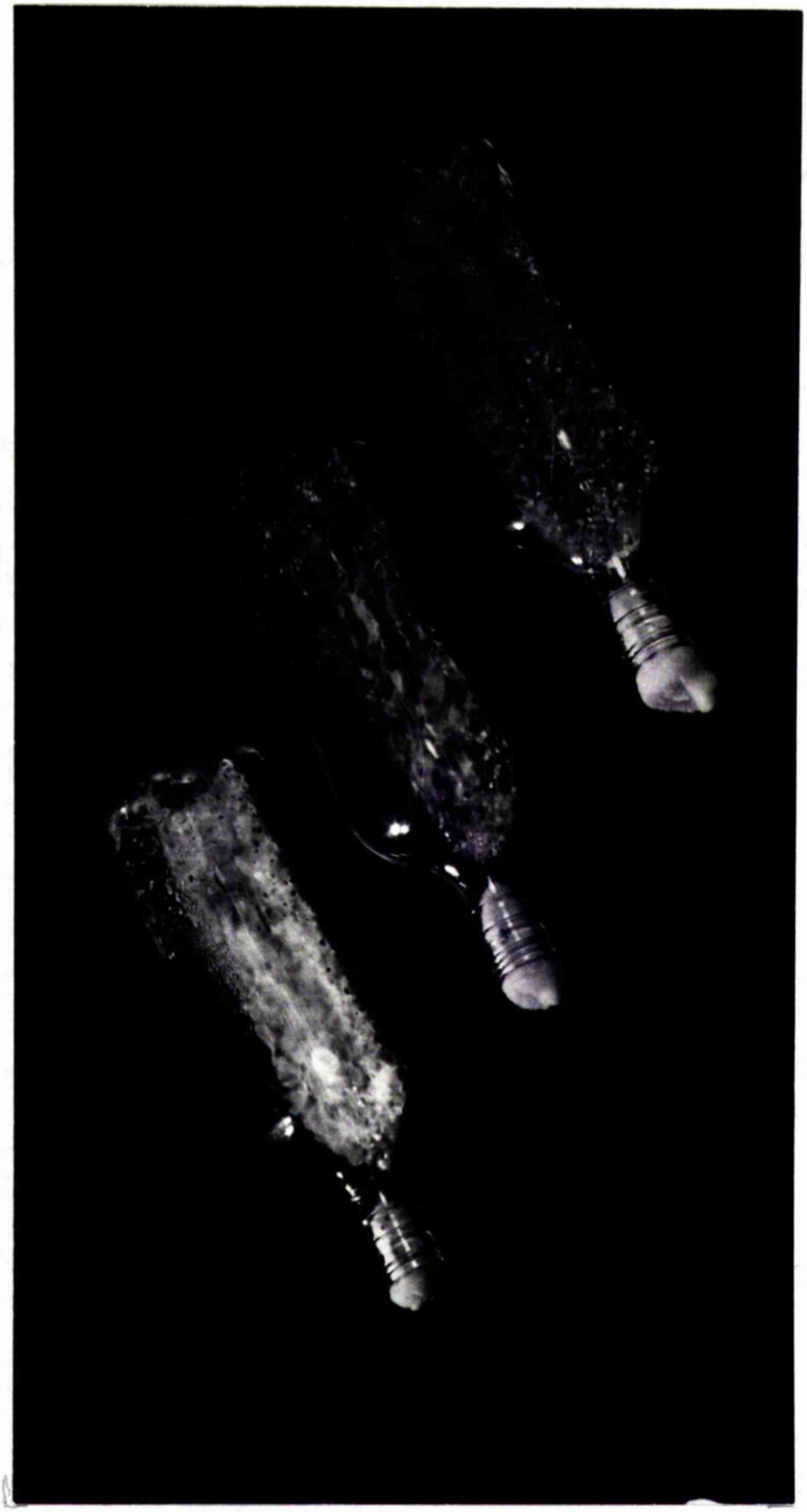
All decay experiments were carried out in rectangular (60 x 60 x 190 mm) glass bottles as shown in plate 1.

Our early decay studies involved the inoculation of 2% malt agar within these bottles in order that a vigorous mycelium might be produced. Wood test pieces were then placed directly onto the fungal mat in each of the bottles which were then incubated for a number of weeks. However, the amount of decay obtained using this method was small, and the water contents of the wood test pieces on harvest, were correspondingly high (see Appendix 4); this suggested that water-logging of the wood may have been inhibiting fungal growth. Consequently, an alternative method was sought which would replace the agar as a major source of water, and which might also control any increase in the wood water during incubation, resulting from fungal respiration.

An examination of the use of moist vermiculite to replace agar was carried out, and the possibility of raising the wood above the agar on glass supports, was also considered; these investigations are recorded in Appendix 5. The vermiculite was adopted as it provided good control of wood water content, so allowing substantial decay to occur. However, both the agar method and the vermiculite method were used in the survey of wood-decay ability, and are described fully in Part 2.

PLATE 1

Rectangular glass bottles (60 x 60 x 190 mm) used as culture vessels in all the decay experiments.



1.4 Measurement of wood decay

1.4.1 Choice of test material

The weight losses of a wide range of hardwoods and softwoods, resulting from decay by *C. puteana*, have been measured, and a correspondingly wide range of susceptibilities were found (Kemper, 1937; Timberlab Papers No. 50, 1972; Wälchli, 1976).

However, it was decided that for the present study, it would be most profitable to look at the decay of one species of wood only. *Pinus sylvestris* sapwood was chosen for its relative susceptibility to decay, and because it is a commonly used and readily available building timber.

The wood used throughout this work had been taken from a large diameter tree felled in the New Forest, and after ponding for six months followed by air drying, it had been cut into planks at the Princes Risborough Laboratories of the Building Research Establishment. Our material was from one plank which had been cut into strips, each having a cross-sectional area of 10 x 5 mm and with their annual rings parallel to the 5 mm face. These strips were cut into 60 mm lengths which constituted our test pieces.

It was appreciated that structural variation in the wood might cause substantial experimental error. Abu-Heilah (1975) carried out a study of the influence which grain angle variation might have on the cross-grain breaking strengths of the test pieces of wood. It was found that selection for similarity in grain angle did not affect experimental variation sufficiently to warrant the waste of material and labour which would be involved. An *ad hoc* selection was recommended, and this we did by selecting for gross similarity in grain angle and in the number of annual rings.

1.4.2 Parameters to be measured

Levels of decay were assessed by measurement of the loss in wood dry weight and of the alteration in cross-grain breaking strength of the wood after incubation with the fungus.

Loss in wood dry weight has been a widely used criterion for decay assessment, being a relatively simple method requiring no special equipment. However, this method may not necessarily produce results which can be correlated with any alteration in structural properties

of the wood. Breaking strength measurements will be of direct relevance to the building situation, and will complement the weight loss measurement.

Standard strength tests have been reviewed by Lavers (1969). It was decided to use a static bending test because this has been used successfully for the measurement of wood-decay by isolates of *S. lacrymans* (Abu-Heilah, 1975), and because the necessary equipment was already available. Although measurement of cross-grain breaking strength is by a single test to destruction, adequate replication reduces the experimental variation to an acceptable level.

1.4.3 Measurement of cross-grain breaking strength

This was carried out using a Hounsfield Tensometer type W (Monsanto), which was fitted with a timber static bend attachment for three point loading (see plate 2).

The applied load is transmitted by the deflection of a spring beam, to a piston, via a series of levers. The piston displaces mercury into a capillary tube, the degree of displacement being directly proportional to the load.

Consideration was given to the influence which the water content of a piece of wood might have upon its breaking strength. Below a wood water content of 27% (fibre saturation point), water is present only within the cell walls, so that with further loss of water the microfibrils are brought closer together, the timber shrinks, and there is a corresponding increase in strength. At wood water contents greater than 27%, water is present both within the cell wall and within the lumen. On drying down to 27%, no dimensional changes occur, and there is no effect on strength (Lavers, 1969; Dinwoodie, 1975). Consequently, by measuring the cross-grain breaking strengths of test pieces while still moist, their strength properties should be relatively unaltered. There would, however, be some advantage in measuring dry test pieces, because there would be less crushing at the three load points, and although dry wood is highly hygroscopic, causing differential creep, if the test pieces are stored dry and broken fairly rapidly, then this effect should be minimised.

Abu-Heilah (1975) tested the cross-grain breaking strengths of a population of wet wood test pieces and of dry test pieces. An obvious difference was found, that the breaking strengths of the wet samples

PLATE 2

Hounsfield Tensometer type W (Monsanto) fitted with a timber static bend attachment; used to measure the cross-grain breaking strengths of *P. sylvestris* test pieces.

were significantly lower than those of the dry, but the difference in variance between the two populations was not significant.

It was decided to test the strength properties of the wood pieces in the dry state. They were maintained in a vacuum desiccator prior to fracture, this being carried out in less than thirty seconds on the tensometer at room temperature. To reduce any possibility of crushing, each piece of wood was sandwiched between two thin strips of rubber during the test.

It was appreciated that variation in the rate of application of load to the test pieces might introduce an additional source of error: such variation might be reduced if the tensometer were driven by motor, and this was tested in an experiment which is described in Appendix 3. No significant difference was found between those results from the manually driven tensometer and those from the motor driven instrument, consequently, the manual drive was retained.

PART 2

SURVEY OF DIFFERENCES IN WOOD-DECAY ABILITY

2.1 Analysis of decay-potential among isolates of *C. puteana* using the direct agar method

2.1.1 Introduction

A survey of saprophytic ability of strains of *S. lacrymans* was carried out recently in this department using a simple agar culture technique (Abu-Heilah, 1975). In the early stages of planning, it seemed obvious that it would be convenient to use this same technique and that there was a good chance, although no certainty, of its being suitable for the *Coniophora* work. A preliminary test of a number of strains was therefore set up using this method. At the same time, a supplementary experiment was set up to determine a suitable time for harvesting the wood test pieces; this was carried out using the same method, and is described in Appendix 4.

The possible loss of effort and material through this simultaneous approach was regarded of less importance than the loss of time involved in doing the investigations consecutively.

2.1.2 Method

Sixteen strains of *C. puteana* were taken at random from our collection, and inoculated onto 2% malt agar in petri dishes in order to provide inocula for the decay vessels. 100 ml of 2% malt agar were poured into each of the bottles being used as decay vessels, and after stoppering with cotton wool, these were autoclaved at 120°C for twenty minutes and allowed to cool on their sides. Each bottle was then inoculated with six 8 mm diameter discs of fungus taken from the margins of the 14-day-old petri dish cultures. Five bottles were inoculated per strain, and they were then incubated for 14 days at 24°C in the dark.

Pieces of *P. sylvestris* sapwood which had been labelled, dried to constant weight at 105°C, and allowed to cool in a vacuum desiccator, were immersed for thirty minutes in distilled water at room temperature in order to provide them with water contents greater than 27% (fibre saturation point). They were then autoclaved at 120°C for thirty minutes, and five pieces were placed onto the fungal mat growing in each bottle; incubation continued for a further eight weeks (see Appendix 4).

At the end of this period, the test pieces were removed, cleaned of adhering mycelium and weighed immediately. They were then dried to constant weight at 105°C, and after cooling, their cross-grain breaking strengths were measured on the Hounsfield tensometer.

FIGURE 1

The mean percentage weight losses of *P. sylvestris* sapwood pieces decayed by 9 strains of *C. puteana* after eight weeks incubation in the dark at 24°C. Each vertical line represents the mean of five values per bottle with the corresponding mean wood water content in the lower histogram. Each block represents the total mean of values from five bottles per strain.

In the upper histogram, those means which are connected by a single straight line are not significantly different at the 5% level.

The means in the lower histogram were tested, and were not found to be significantly different at the 1% level.

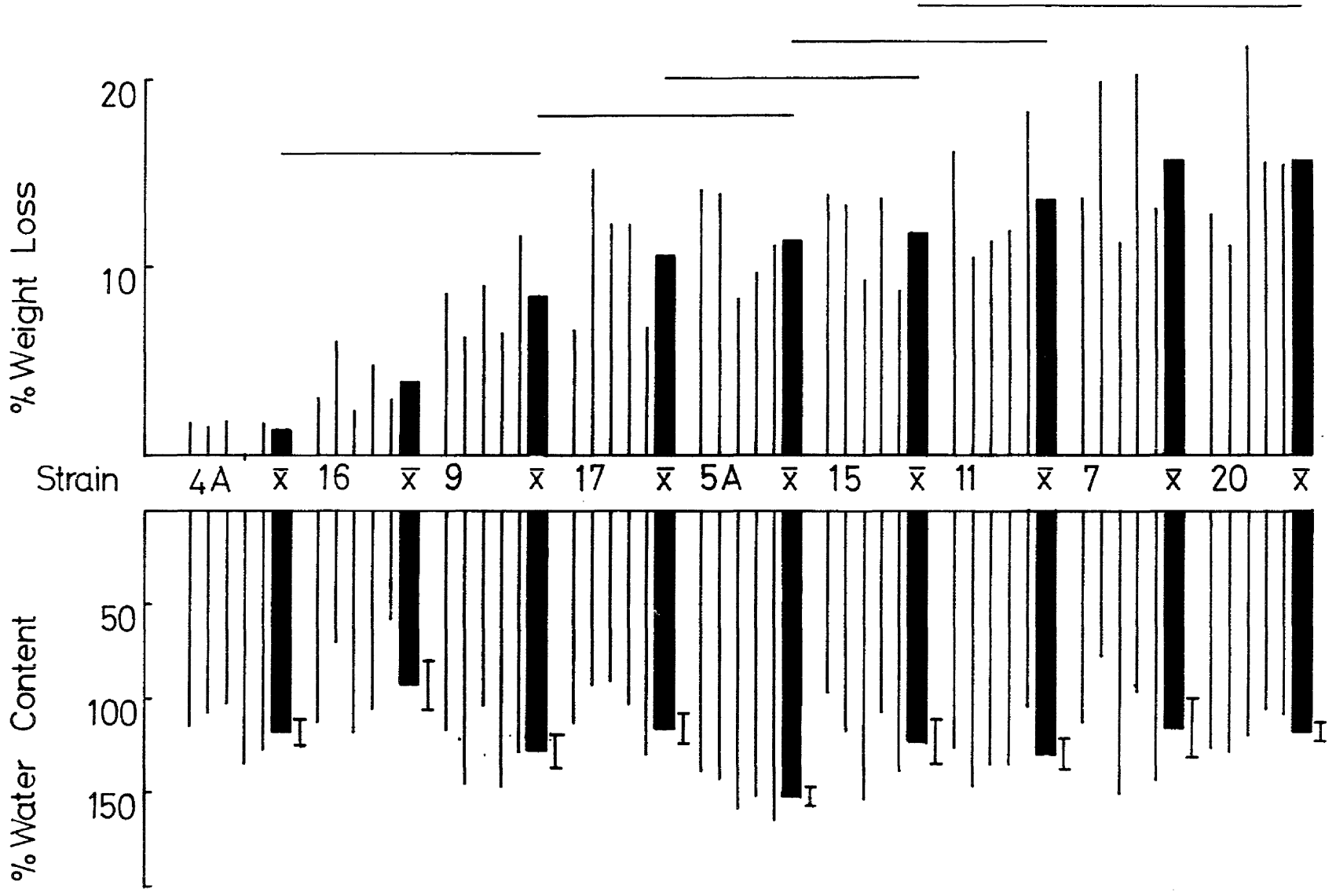

% Weight Loss
20
10
Strain
4A
x̄
16
x̄
9
x̄
17
x̄
5A
x̄
15
x̄
11
x̄
7
x̄
20
x̄
% Water Content
50
100
150

TABLE 1

Results of the significance test carried out on the mean percentage weight losses of *P. sylvestris* sapwood pieces decayed by 9 strains of *C. puteana*.

Isolate	Mean % wt. loss	Significant differences
4A	1.34	
16	3.78	
9	8.32	
17	10.54	
5A	11.32	
15	11.64	
11	13.52	
7	15.56	
20	15.56	

Means connected by a single straight line are not significantly different at the 5% level.

2.1.3 Results

These are recorded in Appendix 6, table 7 , and summarised in text figure 1. After analysis of variance, the weight loss results were analysed by Duncans Multiple Range F test (Duncan, 1955), the results of which are presented in table 1.

Seven strains produced no measurable decay under these conditions, and were therefore not included in the statistical analysis; these were 3, 13, 12, 14, 2A, 1 and 6.

2.1.4 Discussion

There are significant differences between the amounts of decay produced by different strains, even though the levels are low. Figure 2 shows that in most of the bottles, the wood water contents were high, and mean water contents varied little between strains, so the different mean percentage weight losses produced by the strains (ranked in order of increasing decay-potential) are likely to be due to true inter-varietal differences.

Looking at the mean values from each bottle, there is a suggestion that where wood water contents are higher, levels of decay are lower, and *vice versa*. Correlation of wood water contents with percentage weight losses achieved by each strain produced extremely low negative correlation coefficients (table 8 , Appendix 6), but this may be due to most of the water contents being reasonably high. Nevertheless, results from the decay experiment which was run at the same time as this survey (Appendix 4), indicate that the low levels of decay achieved using the direct agar method are due to wood waterlogging. This method is therefore unsatisfactory for work on *Coniophora* and it is necessary to find an alternative which will provide more control over wood water content.

2.2 Analysis of decay-potential among isolates of *C. puteana* using the vermiculite method

2.2.1 Introduction

A series of investigations was performed on the use of vermiculite, to replace agar as a method of providing better control of wood water content during incubation, and these are reported in Appendix 5. The method provided good control of wood water and has been used for this survey, which examines the range of decay-potential existing within the majority of our collection of isolates.

This work was carried out in two sections, an initial survey covering the majority of isolates, followed by another smaller survey. This second survey was designed to allow a further appraisal of a number of particularly interesting points arising from the earlier survey; in addition, three newly acquired strains were tested.

2.2.2 Method

Survey A

2% malt extract solution was added to 30 g of dry vermiculite in each of 204 bottles. Sufficient solution was added to wet the vermiculite to 225% of its water holding capacity (see Appendix 5), the bottles were laid on their sides and the vermiculite spread out evenly after mixing. Five test pieces of *P. sylvestris* sapwood, previously marked, and dried to constant weight, were inserted into the vermiculite within each bottle. The bottles were stoppered with cotton wool and autoclaved at 120°C for thirty minutes.

The bottles were left at room temperature for three days to allow the wood to achieve its equilibrium moisture content, then two replicate bottles were inoculated in each of three successive weeks. Similarly, the bottles were harvested in each of three successive weeks after ten weeks incubation at 24°C in the dark.

At harvest, the wood was removed and the vermiculite and fungus adhering to the surface of each piece was removed with minimum damage to the wood. The pieces were dried to constant weight at 105°C, allowed to cool in a vacuum desiccator, and the cross-grain breaking strength of each was measured on the tensometer.

In all, 34 isolates were tested in this survey.

Survey B

The bottles were prepared as described for survey A, with vermiculite. Five replicate bottles were inoculated for each isolate and all were incubated at 24°C in the dark for ten weeks.

Nine isolates were tested in this survey, three new strains and six isolates from survey A. Decay assessment was carried out as described previously.

2.2.3 Results

These are recorded in table 9 , Appendix 6, and summarised in text figures 2 and 3. Statistical analysis was by Duncan's Multiple Range F test.

2.2.4 Discussion

There is a wide variation within our collection of isolates in their ability to decay *P. sylvestris* sapwood under these conditions. Two distinct populations are recognisable from the results of survey A as seen in figure 2, namely 16 isolates which produced weight losses within the range of 2-7% and which do not differ from one another significantly, and a second population which produced weight losses within the wider range of 14-37%.

The cross-grain breaking strength values shown in figure 3, closely mirror the weight loss values. With increasing loss in weight, there is a corresponding decrease in the load required to break the wood. The statistical analysis confirms the presence of two populations, 16 isolates which left the wood with high strength properties, requiring loads of 400-500 newtons to fracture, and a population which left the wood with poor strength properties, requiring loads within the low range of 20-250 newtons for breakage.

The less pronounced significant differences which were found on analysis of the weight losses produced by the high decaying isolates, were not detected in the breaking strength analysis. This perhaps indicates that although breaking strength measurement does provide a useful estimate of the effect which *Coniophora* exerts upon some structural components of the timber, it is not, however, a comprehensive indicator; the fact that weight loss measurement provided a greater resolution, reinforces the decision to use both parameters as criteria for decay.

A further point which is of interest in survey A is that the decay-potential of isolate 5D is significantly greater than that of sister isolates 5A and 5C. Strain 5 is the British standard test strain 11E, and any differences between isolates of this strain are important, with the possibility of repercussions within the preservative manufacturing industry. It was for that reason that the three isolates of this strain were tested again in survey B; as can be seen from figures 2 and 3, the differences have not recurred in this survey. The

FIGURE 2

The mean percentage weight losses of *P. sylvestris* sapwood pieces decayed by *C. puteana* after 10 weeks incubation in the dark at 24°C.

A. Each column represents the mean of 30 measurements.

B. Each column represents the mean of 25 measurements.

Means which are connected by straight lines are not significantly different at the 5% level.

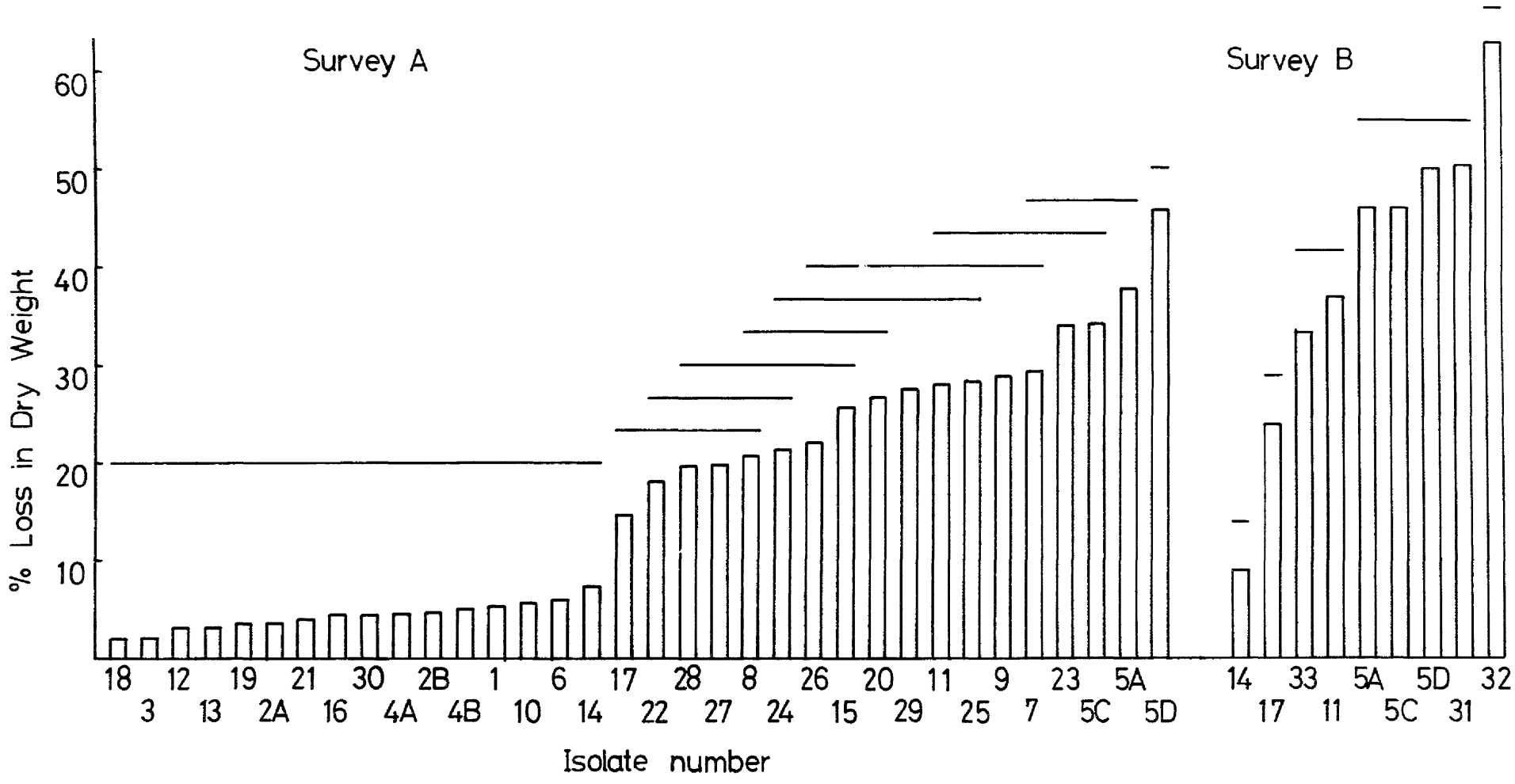

Survey A
Survey B
% Loss in Dry Weight
60
50
40
30
20
10
18 12 19 21 30 2B 1 6 17 28 8 26 20 11 9 23 5A
3 13 2A 16 4A 4B 10 14 22 27 24 15 29 25 7 5C 5D
14 33 5A 5D 32
17 11 5C 31
Isolate number

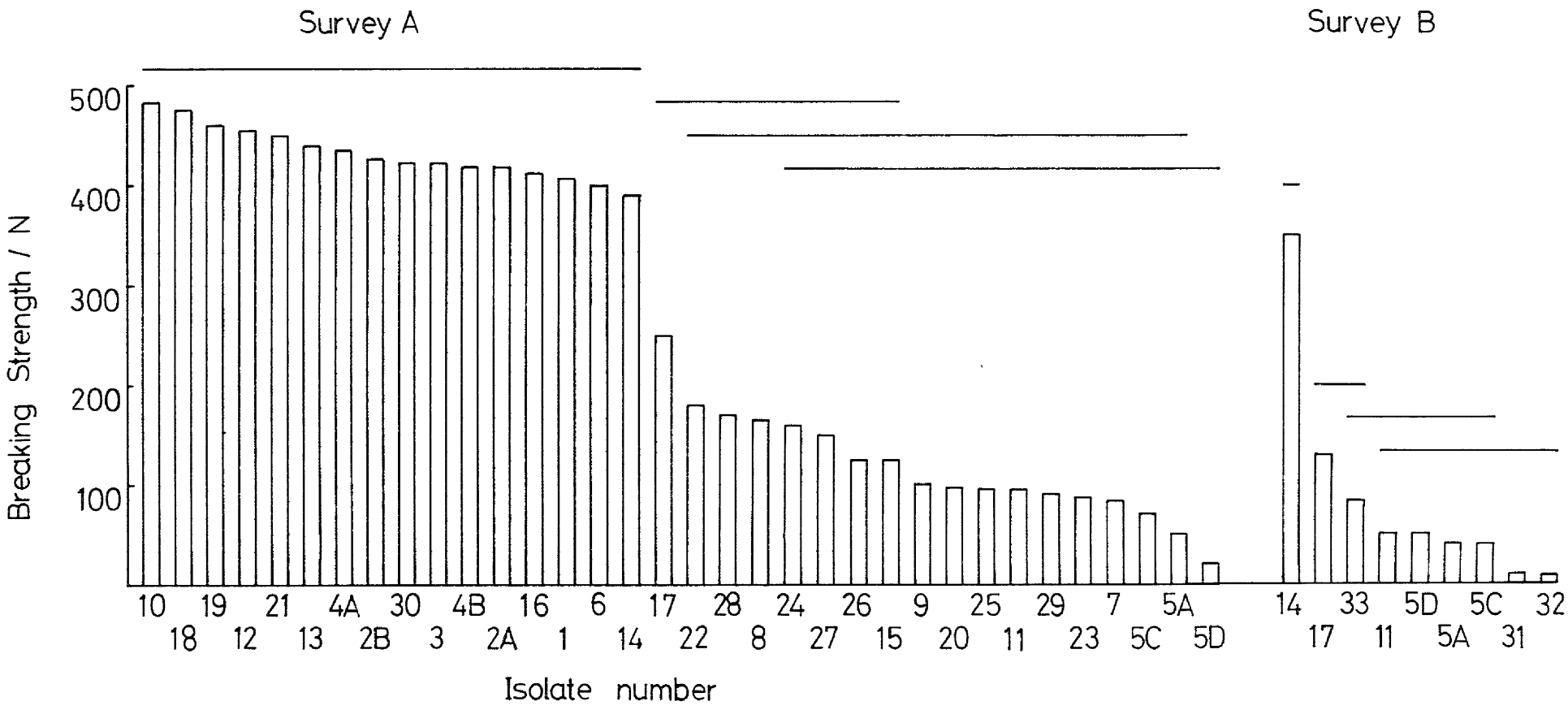
FIGURE 3

The mean cross-grain breaking strengths of *P. sylvestris* sapwood pieces decayed by *C. puteana* after 10 weeks incubation in the dark at 24°C.

A. Each column represents the mean of 30 measurements.

B. Each column represents the mean of 25 measurements.

Means which are connected by straight lines are not significantly different at the 5% level.


Survey A
Survey B
Breaking Strength / N
500
400
300
200
100
10 19 21 4A 30 4B 16 6 17 28 24 26 9 25 29 7 5A
18 12 13 2B 3 2A 1 14 22 8 27 15 20 11 23 5C 5D
14 33 5D 5C 32
17 11 5A 31
Isolate number

amount of decay produced by isolates in survey B is generally greater than that produced by the same isolates in survey A; this is possibly due to slight differences in conditions of incubation such as temperature. 5D is represented by a mean percentage weight loss which is still slightly higher than those of 5A and 5C, and the fact that they are not significantly different may be because isolate 5D, having destroyed 50% of the wood by the time of harvest, will possibly have been retarded in any further decay activity by the lack of α-cellulose, this being the main substrate for brown-rot fungi, forming 40-50% of the wood cell wall (Jane, 1970). Meanwhile, isolates 5A and 5C will continue to degrade available α-cellulose and so allow a reduction in the gap between the isolates which was manifested as a significant difference in survey A.

The two strains which exhibited the greatest decay-potential in the low decaying population and the lowest in the high decaying population, namely 14 and 17 respectively, were tested again in survey B, and the significant difference between the population has been maintained. Strain 11 is important and was included in survey B because it is the European standard test strain. Apart from the discrepancy produced by the three isolates of strain 5, the pattern of differences is similar, and the three new isolates 31, 32, and 33 all show high decay-potential.

2.3 Geographical distribution

The mean percentage weight losses, produced by each of the 36 strains tested, have been grouped according to their countries of origin, and are presented in text figure 4.

The number of strains tested is not large enough to allow conclusions to be made, but it may be noted that the North American, German, British and Swedish groups all contain strains with decay-potentials representing the complete spectrum of high to low decay.

2.4 Age of cultures

Text figure 5 presents the mean percentage weight losses produced by a number of strains, together with the date of isolation of each strain. They are ranked chronologically from left to right.

Again, the number of strains tested is too small to allow any conclusions to be made. However, it is interesting that those five strains which were isolated between 1928 and 1947 all produced low

FIGURE 4

36 strains of *C. puteana*, represented by the mean percentage wood weight losses which they produced, grouped under their countries of origin.

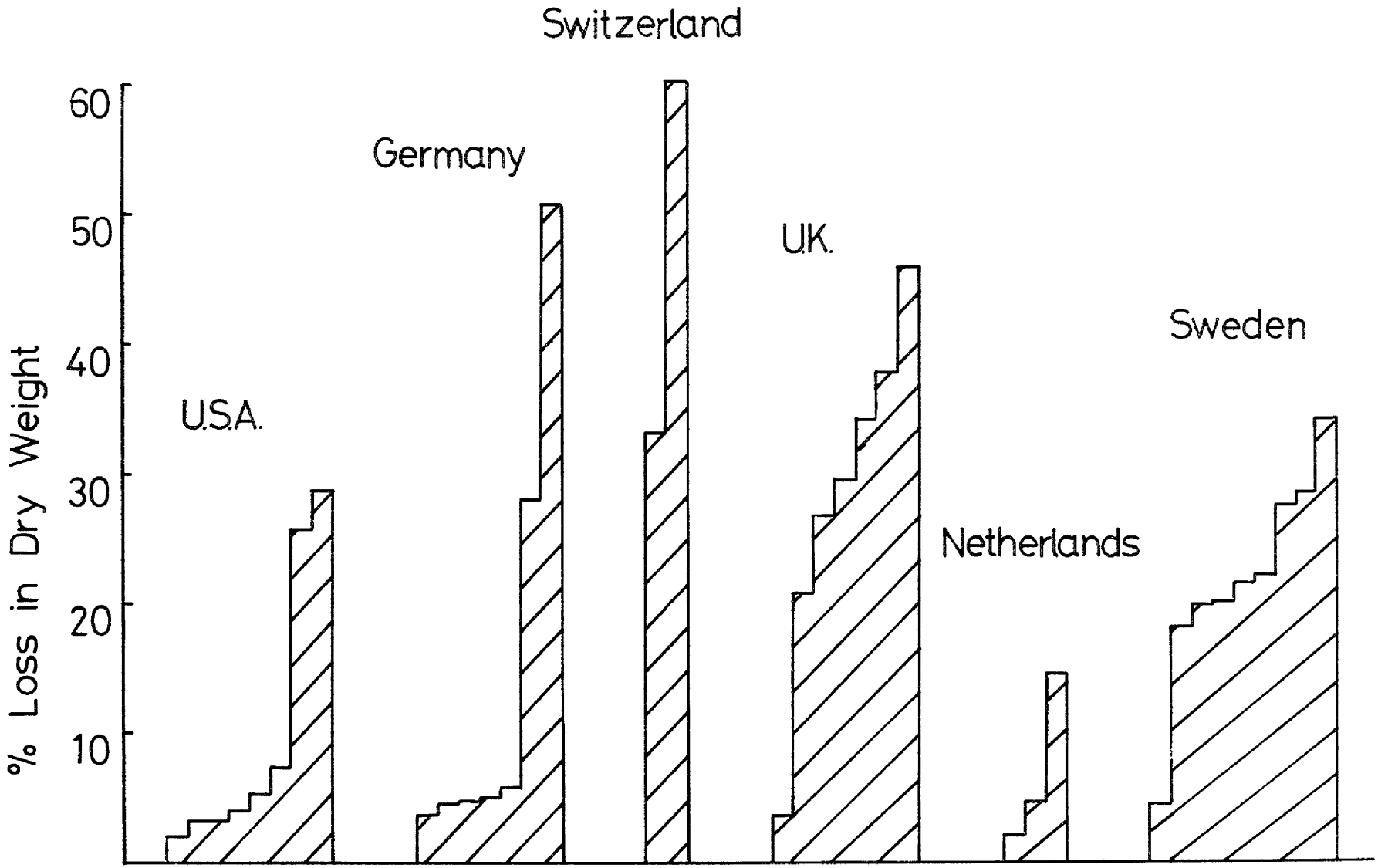
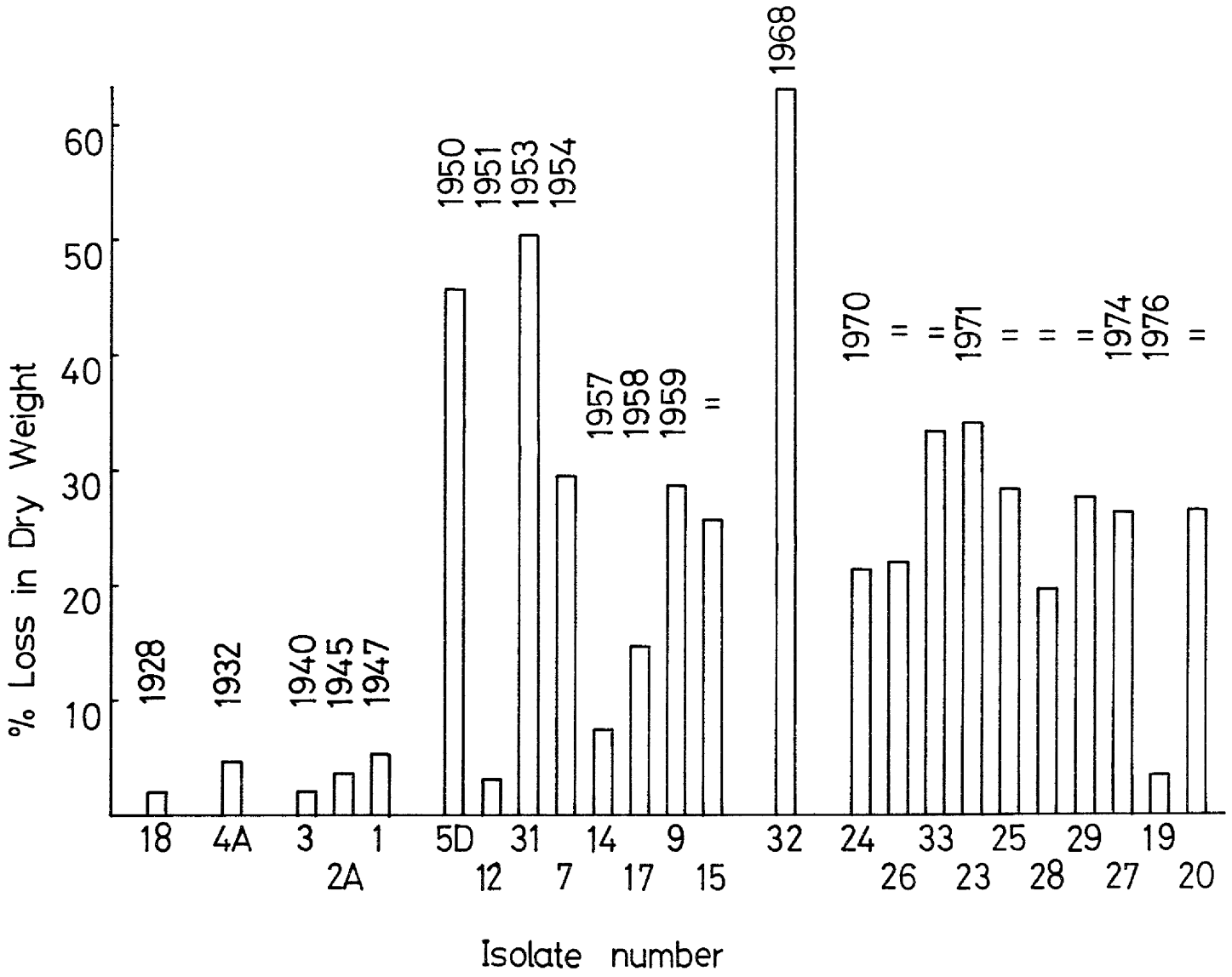

Switzerland
60
50
40
30
20
10
% Loss in Dry Weight
Germany
U.K.
Sweden
U.S.A.
Netherlands

FIGURE 5

Mean percentage weight losses produced by a number of strains of *C. puteana*, presented in chronological order from left to right according to the date of isolation of the strain.


% Loss in Dry Weight
60
50
40
30
20
10
1928
1932
1940
1945
1947
1950
1951
1953
1954
1957
1958
1959
=
1968
1970
=
=
1971
=
=
=
1974
1976
=
18
4A
3
2A
1
5D
12
31
7
14
17
9
15
32
24
26
33
23
25
28
29
27
19
20
Isolate number

levels of decay. This might indicate a loss of saprophytic activity with age, but the fact that strain 19 isolated in 1976 also shows a very low decay potential, makes such a statement merely speculative.

PART 3

ANALYSIS OF FACTORS WHICH MIGHT CONTRIBUTE TO VARIATION IN DECAY-POTENTIAL

Analysis of factors which might contribute to variation in decay-potential

This part describes an analysis of the growth rates of a number of isolates, and a correlation of these with their decay-potentials. It goes on to describe a study of the extra-cellular secretions of isolates of *C. puteana*, which was carried out to look for differences which might relate to decay-potential.

3.1 Rates of growth on agar

3.1.1 Introduction

It is reasonable to suppose that decay ability may be related to the growth rate of an isolate, this characteristic itself, possibly correlating with an increase in the substance(s) causing timber degradation. The concept of 'growth rate' is rather loose, being the sum of a number of properties, and in order to measure this comprehensively, a wide range of different characters would have to be considered (e.g. weight increase, chitin synthesis, DNA synthesis, nitrogen levels, *etcetera*). Such detail is not practicable or necessary for our purpose, which is to obtain an easy and convenient measure of the gross metabolic vigour of an isolate which will allow comparison between a number of them. A correlation between rate of increase in colony radius and decay-potential, would be informative.

Abu-Heilah (1975) compared the growth rates of isolates of *S. lacrymans* with their decay-potentials, and found no consistent correlation. Of particular relevance to the present study is work by Gersonde (1958a), who looked at the variation between 7 isolates of *C. puteana*, and found that these did not correlate with their decay-potentials. This study will complement that by Gersonde by examining a greater number of isolates.

3.1.2 Method

Five discs (8 mm in diameter) were cut from the margin of a 14-day-old colony of each of twenty isolates of *C. puteana* growing on 2% malt agar. Each disc was placed centrally on 2% malt agar in a 9 cm diameter plastic petri dish. All cultures were incubated in the dark at 24°C and growth was measured daily as the increase in colony radius along two transects marked at right angles to each other on the underside of each dish. (Measurement on day 6 was omitted.)

3.1.3 Results

These are recorded in Appendix 7, table 10 , and summarised in text figure 6. Text figure 7 compares growth after 7 days with the decay-potential of each isolate.

3.1.4 Discussion

Figure 6 shows clearly that there are wide differences in rates of growth on agar, between isolates; the strains presented here provide examples which are representative of the range of growth rates produced. When the radial growth achieved after 7 days, is compared with the decay-potential of each isolate (Figure 7), there is some indication that those strains which produce high decay levels also show vigorous growth on agar. However, there is a poor correlation coefficient ($r = 0.73$), and discrepancies such as those provided by isolates 8, 1 and 12 are interesting and suggest that if growth rate is a factor contributing to decay-potential, then there appear to be other factors involved also. Good growth on agar may well signify a vigorous isolate which would have an early advantage on wood, but there is no *a priori* reason why it should; growth on simple sugars will not select for those characteristics which allow good growth on cellulose, and may actively suppress such factors. Nevertheless, this basic analysis has exposed a further example of the variation between isolates and useful information has been gained.

FIGURE 6

The rates of radial growth of 6 representative strains of *C. puteana* on 2% malt agar incubated at 24°C in the dark.

The upper broken line represents the maximum radius of 37 mm.

(Vertical bars represent the standard error of each value which is the mean of ten measurements.)

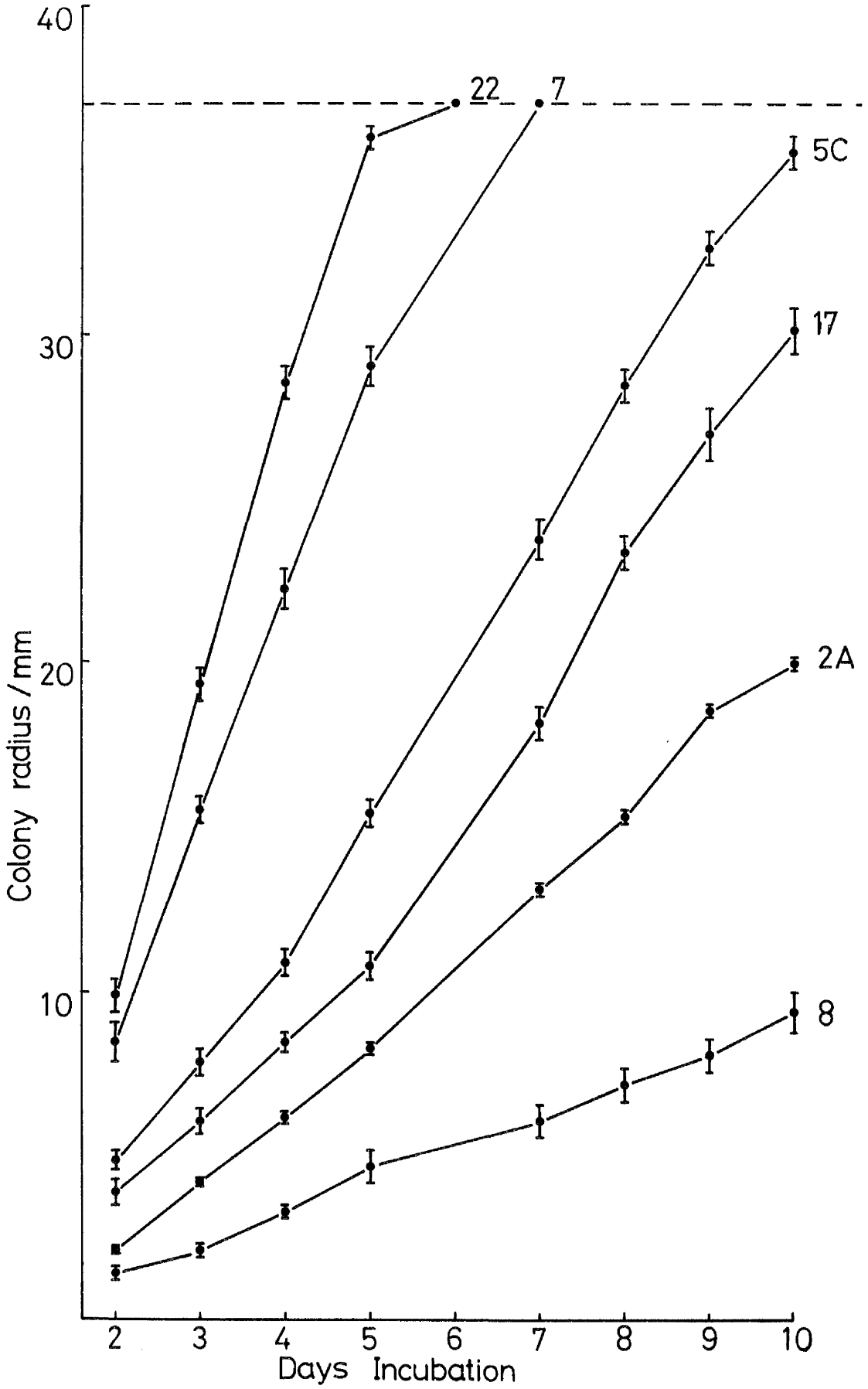
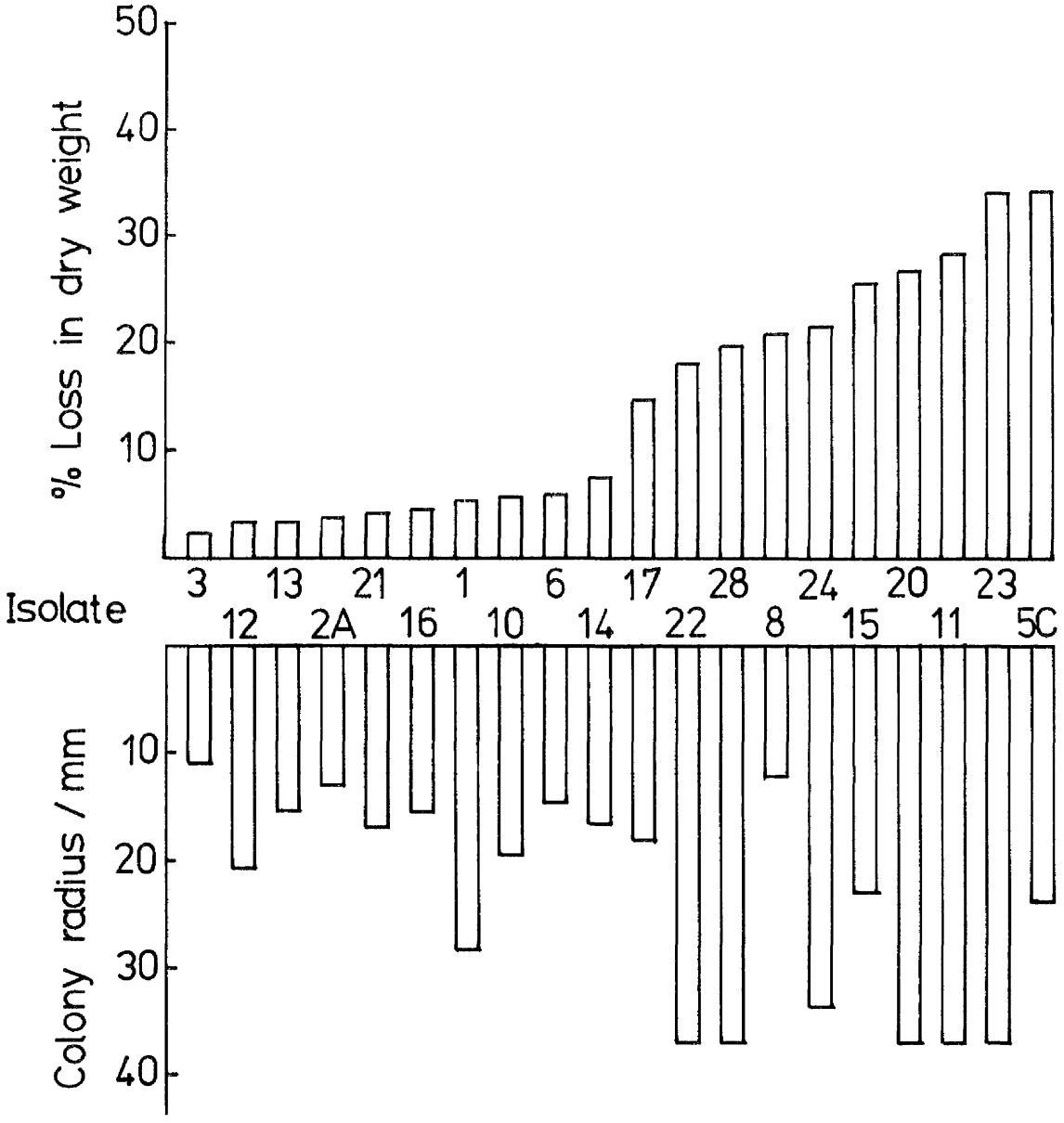

40
30
20
10
Colony radius / mm
22
7
5C
17
2A
8
2
3
4
5
6
7
8
9
10
Days Incubation

FIGURE 7

The decay-potentials of 20 strains of *C. puteana* represented by percentage loss in dry weight of *P. sylvestris* sapwood, compared with their growth on 2% malt agar after 7 days' incubation at 24°C in the dark.

(r = correlation coefficient)


% Loss in dry weight
50
40
30
20
10
3 13 21 1 6 17 28 24 20 23
Isolate
12 2A 16 10 14 22 8 15 11 5C
Colony radius / mm
10
20
30
40

r = 0·73

3.2 Extracellular secretions

3.2.1 Introduction

Brown rot fungi carry out their initial colonisation of wood through the largest spaces available, resin canals and early wood tracheids in pine, as well as through the ray parenchyma (Liese, 1970; Wilcox *et al.*, 1974; Bravery, 1975). Hyphae grow from cell to cell mainly through bordered pits, although in advanced stages of colonisation, they may grow directly through cell walls. Once inside the lumen, they grow against the cell wall and often produce distinct, sharply bordered but shallow traces (Necesany, 1974). The wood tracheids are therefore degraded from the inside to the outside, and deep failure of the S_3 and S_2 layers of the secondary wall may be seen in electron micrographs of colonised tissue (Liese, 1970; Necesany, 1974). Such damage to the cell wall structure will be produced by those extracellular substances which the fungus secretes into its environment; damage can often be seen some distance from fungal hyphae. These secretions will hydrolyse high molecular weight polysaccharides and proteins within the cell wall, so allowing their constituent sub-units to enter the fungal cell where they are assimilated as a nutrient source. The composition of the extracellular material, produced by the fungus, is therefore likely to be of importance in providing information about the mechanisms by which a complex and stable material like wood is degraded and used as an energy source by invading micro-organisms. In addition, the differences in decay-potential between isolates may be partly due to differences in the quality and/or quantity of the extracellular secretions. Consequently, this section describes a study which was carried out on the proteins released into liquid culture medium by a number of isolates of *C. puteana*.

Much work has been done on the extracellular enzymes of *C. puteana* over the past thirty years; Norkrans (1950) was the first to show that an extracellular cellulase is produced which can degrade a precipitated cellulose.

Subsequent work on the cellulolytic enzymes produced by brown-rot fungi in general, has been succinctly reviewed by Nilsson (1974). Text table 2 presents the enzymes which have been detected in culture filtrates from *Coniophora* cultures, and it is clear that the fungus produces a reasonably formidable battery of extracellular proteins. All these are carbohydrases and act on holocellulose which makes up 70-80% of wood cell wall material; holocellulose is itself made up of α-cellulose (40-50% of

TABLE 2

Exo-enzymes detected in *C. puteana* culture filtrates.

Enzyme activity	Reference
Cellulase (C_x)*	Norkrans (1950)
Amylase, xylanase, pectinase, cellulase (C_1)	Lyr (1959(a & b), 1960, 1963)
Arabinase, xylanase, cellulase (C_1)	Jurasek and Sopko (1962)
Cellulase (C_1)	Johansson (1966)
Cellulase (C_1), laminarinase, glucoamylase, maltase, xylanase	King (1966, 1968 (a & b))
Polygalacturonase, α-galactosidase, xylosidase	King and Fuller (1968)
Xylanase, cellulase (C_1)	Nilsson (1974)
Cellulases (C_x)	Eidsa (1974)
Cellulase (C_x), xylanases	Lewis (1975)

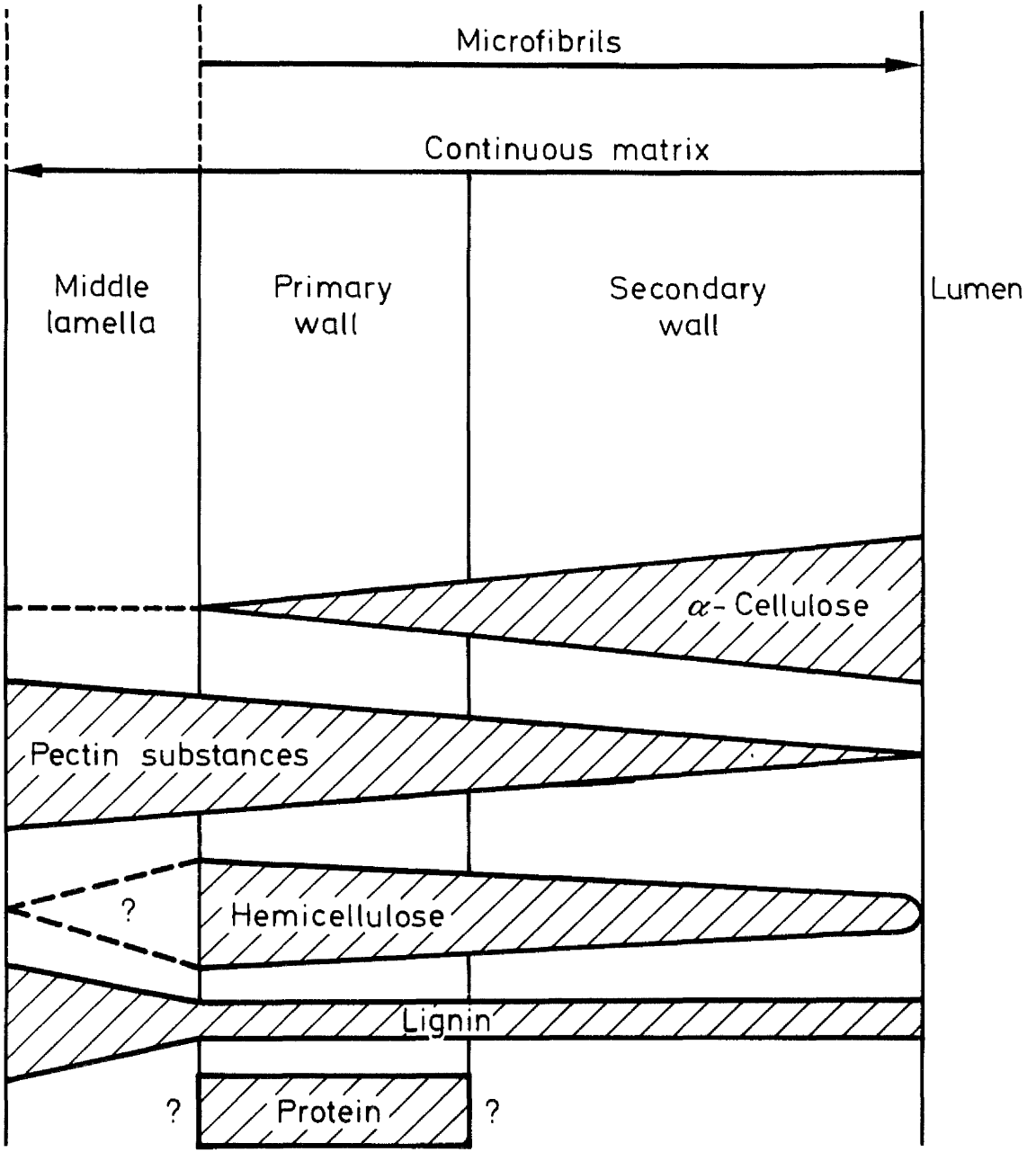
* C_1 indicates activity against native cellulose

C_x indicates activity against soluble (substituted) cellulose

FIGURE 8

Distribution in the plant cell wall of the major wall constituents.

(From: Bateman and Basham, 1975)


Microfibrils
Continuous matrix
Middle lamella
Primary wall
Secondary wall
Lumen
α- Cellulose
Pectin substances
?
Hemicellulose
Lignin
?
Protein
?

cell wall) and hemicellulose (Jane, 1970; Dinwoodie, 1974). Text figure 8 has been taken from Bateman and Basham (1975) and represents the distribution in plant cell walls of their major constituents: the three major regions are shown, middle lamella, primary wall and secondary wall. In mature wood, lignin is most abundant in the middle lamella and comprises 60-90% of the dry weight of that region; it is least abundant in the secondary wall, where it rarely exceeds 10% of the dry weight. The proportion of α-cellulose is greatest in the most recent wall layers. The interrelationship of hemicellulose, α-cellulose and lignin, is complex and Jane (1970) suggests that two interlocking systems may be visualised; truly cellulosic microfibrils which are mainly crystalline, and that of the spaces between them which are occupied by amorphous hemicelluloses, lignin and water.

Brown rot fungi in general and *C. puteana* in particular, do produce a form of 'ligninase', but in contrast to the white rotting fungi, their main carbon source is α-cellulose and, to a lesser extent hemicellulose. It is for this reason, combined with the fact that the fungus attacks the secondary wall from the lumen so exploiting a major source of α-cellulose, that we decided to pay some attention to the production of extracellular cellulases by *Coniophora* in order to look for differences which might be correlated with decay-potential.

Mechanism of the enzymic degradation of cellulose

The mechanism of enzymic cellulose degradation is not yet completely understood, although there has been a lot of research effort devoted to it. For this reason, this section has been included to outline the current thinking on the mode of action of cellulases, and to introduce the enzymes which might be relevant to this investigation.

Gascoigne and Gascoigne (1960) provide a review of the very early work on cellulases. The most popular and widely cited hypothesis for the action of cellulase enzymes has been that proposed by Reese *et al.* (1950, 1952), who suggested that there must be at least two components to the cellulase system. This was based upon the observation that some micro-organisms can attack native cellulose whereas many can only degrade soluble substituted celluloses such as sodium carboxymethyl cellulose (NaCMC). Consequently, there was thought to be a component present in the cellulase produced by native cellulose degrading organisms which is missing in that produced by the soluble cellulose degraders. This

FIGURE 9

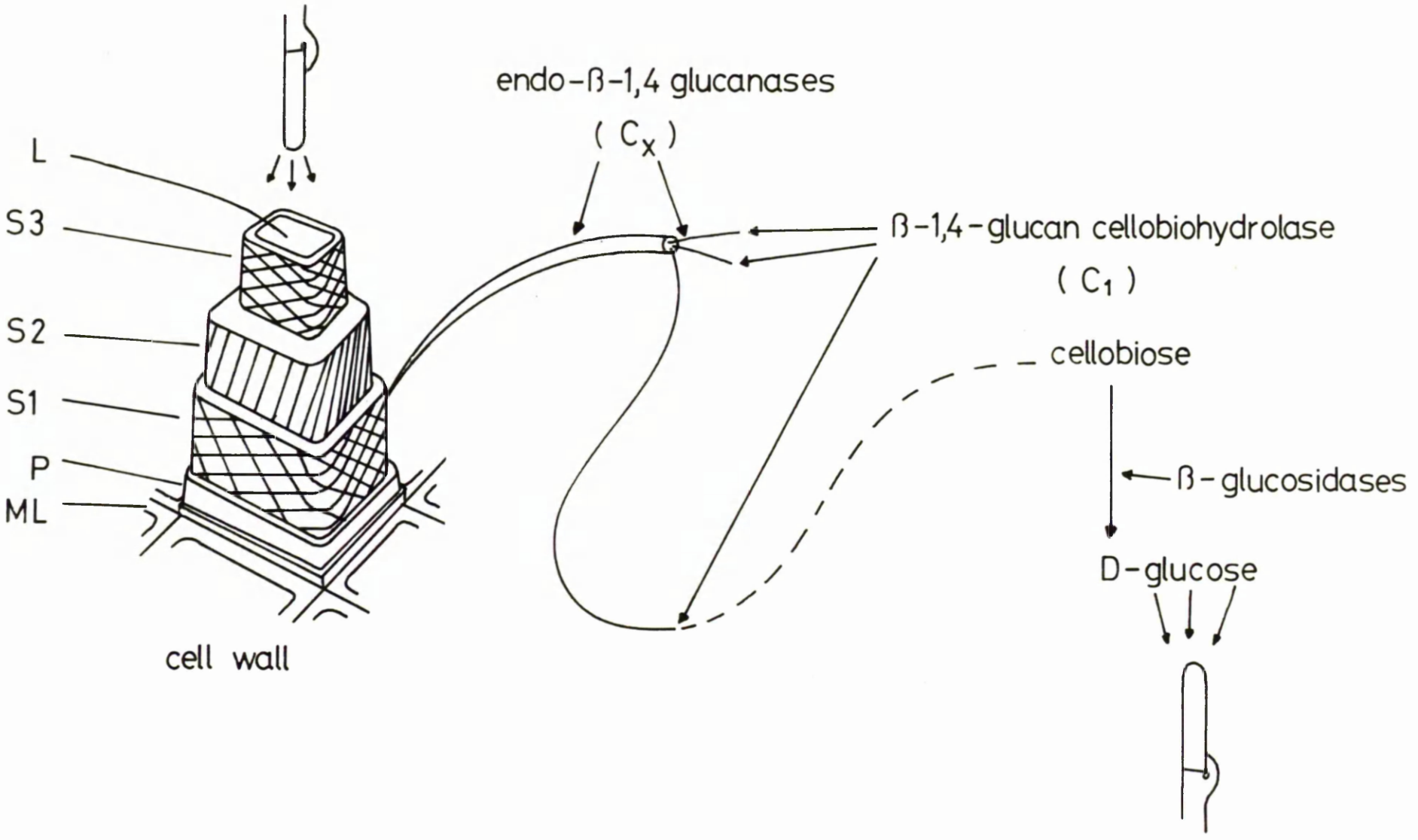
A diagrammatic summary of the possible mode of action of extracellular enzymes of the cellulase complex on a cell wall microfibril.

L = Lumen

$S_3S_2S_1$ = Secondary wall layers

P = Primary wall

ML = Middle lamella


endo-ß-1,4 glucanases
(C_x)
L
S3
S2
S1
P
ML
ß-1,4-glucan cellobiohydrolase
(C_1)
cellobiose
ß-glucosidases
D-glucose
cell wall

component was termed C_1, its function being to open up the crystalline cellulose polymer and so make the glucopyranose chains accessible to hydrolysis by C_x enzymes.

It is now well established that cellulase is a multi-component enzyme system, and using various chromatographic and electrophoretic techniques, the complex has been resolved into a number of component parts (Mandels and Reese, 1964; Selby, 1968; Gould, 1969; Whitaker, 1971; Wood, 1975; Enari and Markonnen, 1977). Most of this work has been carried out on the enzymes produced by the ubiquitous soil-inhabiting fungi *Trichoderma viride* and *T. koningii*, precisely because they produce large amounts of highly active enzyme which contains all the components required for hydrolysis of insoluble cellulose (this enzyme can also be obtained commercially).

Until relatively recently, the C_1 component remained something of an enigma, although Reese had suggested that it might be a nonhydrolytic enzyme. However, results from fractionation studies on *T. viride* (Berghem and Pettersson, 1973) and *T. koningii* (Wood and Macrae, 1972; Halliwell *et al.*, 1972; Halliwell and Griffin, 1973) have shown that the C_1 enzyme from these two organisms is a β-1,4-glucan cellobiohydrolase acting as an exocellulase (E.C.3.2.1.74). The C_x enzymes are endo β-1,4-glucan glucanohydrolases (E.C.3.2.1.4) which attack β-1,4-D-glucopyranose chains randomly; these vary in number, but there may be at least five of them (Enari and Markonnen, 1977). The third activity involved in the breakdown of cellulose is β-glucosidase (E.C.3.2.1.21) which hydrolyses cellobiose and cellotriose to glucose. The interaction of these three groups of enzyme in the degradation of native cellulose, is summarised in text figure 9 . Reese *et al.* (1950) suggested that the C_1 component initiated the attack on native cellulose and allowed the C_x enzymes access; however, it now seems likely that the endo β-1,4-glucanases (C_x) initiate the attack and produce an increase in the number of free end groups available to the β-1,4-glucan cellobiohydrolase (C_1), which can then release cellobiose units successively. β-glucosidase finally completes the process by hydrolysing cellobiose to glucose (Wood and Macrae, 1972; Berghem, 1974; Berghem *et al.*, 1975; Wood, 1975; Enari and Markonnen, 1977).

The present study

Abu-Heilah (1975) working on *Serpula*, found a close correlation between cellulase activity and decay-potential. However, those investi-

gations were confined solely to the production of carboxymethylcellulase which is generally recognised as being of the C_x type: as mentioned earlier, it is thought that the factor which confers true celluloytic activity upon an organism is the production of C_1 enzyme. Referring back to text table 2 , Jurasek and Sopko (1962) followed Norkrans (1950) in demonstrating that *C. puteana* could degrade holocellulose, and in 1966, Johansson compared the cellulolytic activity of a great number of white- and brown-rot fungi and found plenty of C_1 activity among the white-rots, but none in the brown-rots, with the exception of *Coniophora* and *Serpula*. Nilsson (1974) carried out a similar survey and found that out of 31 species of brown-rot fungi tested, only 7 produced C_1 activity, and of these, 3 species belonged to the genus *Coniophora*. Work by Koenigs (1974, 1975) suggested that the brown-rot fungi, as a group, may rely upon an $H_2O_2Fe^{++}$ complex rather than a C_1 enzyme to allow the utilisation of native cellulose. However, it appears that *C. puteana* and *S. lacrymans* may be anomalous among brown-rot fungi in their ability to produce a C_1-type activity with little or no evidence of hydrogen peroxide production.

Therefore, in addition to the C_x enzymes, a key factor contributing to differences in decay-potential between isolates, might be the C_1 component of the cellulase complex, consequently, it was decided to look at the production of both by a number of isolates.

As well as assaying for C_x and C_1 activities, a preliminary purification and fractionation of culture filtrates from a few of the isolates was carried out in order to attempt to separate components of the cellulase complex. It was hoped that this would allow detection of any variation in the pattern of cellulases produced and in the general profiles of extracellular proteins secreted by the isolates.

The culture filtrates, from a number of isolates grown on three different carbon sources, were examined by electrophoresis in order to see if there was any variation between isolates in their complements of cell-free proteins.

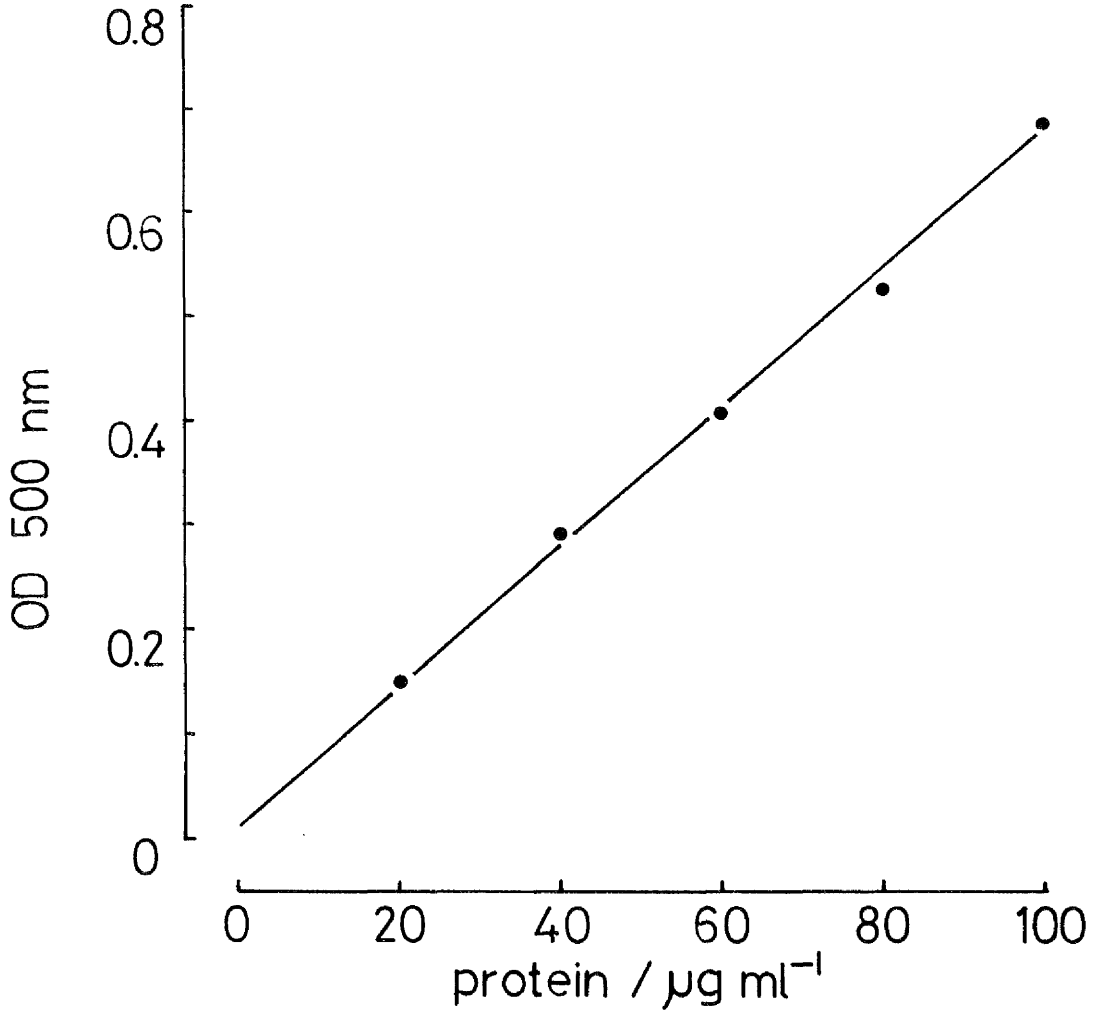
3.2.2 General methods

Assay of protein

Protein was assayed by a modification of the method of Mejbaum-Katzenellenbogen and Drobryszycka (1959). The following stock solutions were made up, filtered before use, and kept at room temperature.

FIGURE 10

A typical protein calibration curve obtained on Bovine serum albumin standards after assaying by the tannic acid precipitation technique.


0.8
0.6
0.4
0.2
0
OD 500 nm
0
20
40
60
80
100
protein / µg ml⁻¹

A) Tannin reagent: 982 ml of 1N HCl were heated to 80°C and 2 ml of phenol added followed by 10 g tannic acid, the solution was stirred and kept warm until the tannic acid had dissolved.

B) Gum arabic (gum acacia): 0.4 g of gum arabic was dissolved in 200 ml of distilled water heated to 30°C.

To measure protein concentration, 1 ml of the protein solution was warmed to 30°C for 5 minutes. 1 ml of pre-warmed tannin reagent was added and the tube was incubated at 30°C for 10 minutes, after which 1 ml of gum arabic solution was added. The tube was mixed and allowed to cool to room temperature and the turbidity was measured on a spectrophotometer at 500 nm. As shown in text figure 10 the assay is suitable for protein estimation at least over the range of 10 to 100 µg. The standard curve, which was performed routinely for each assay, was constructed using bovine serum albumin.

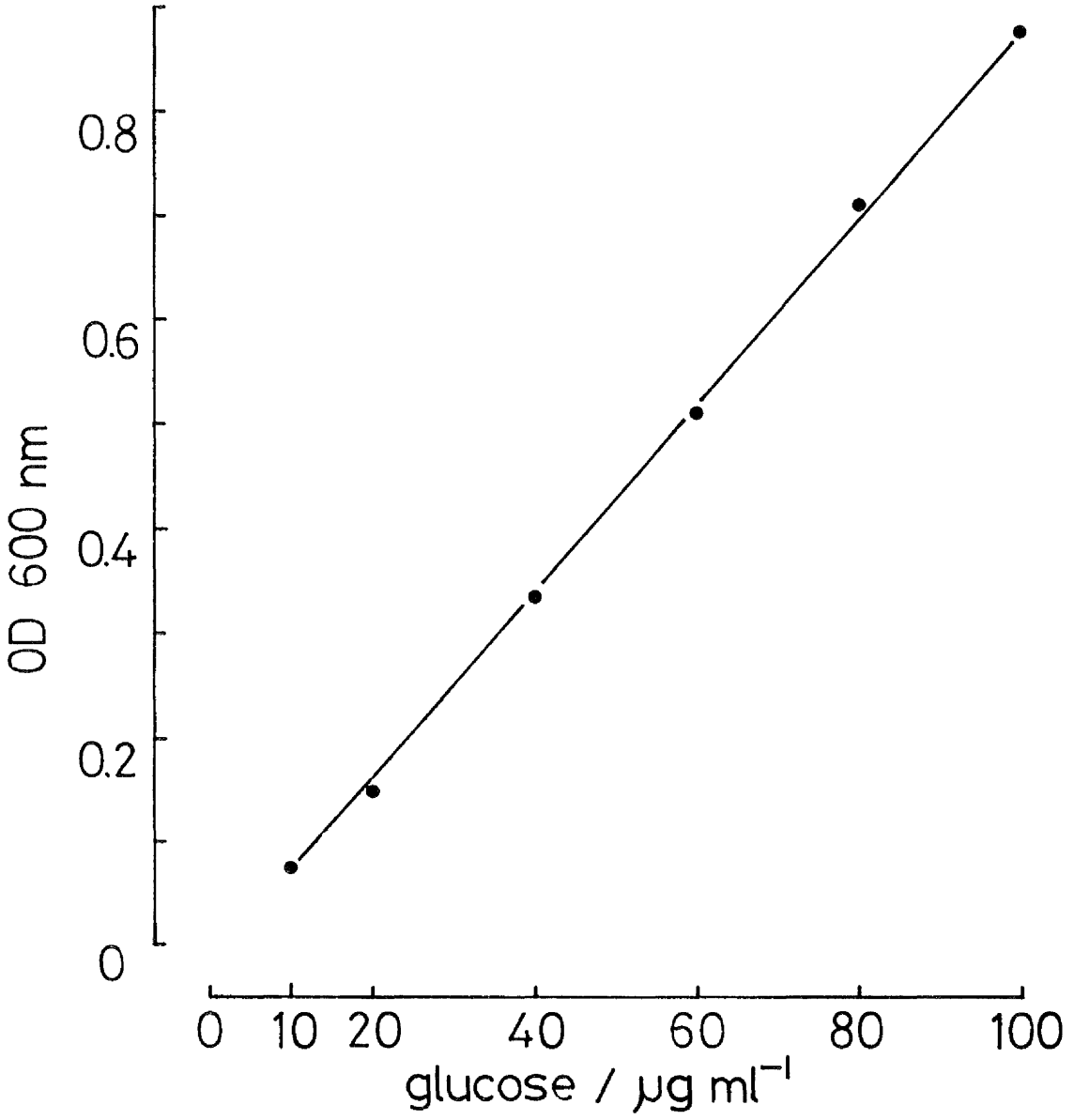
Assay of reducing sugars

Reducing sugars were assayed by a modification of the method of Nelson (1944) and Somogyi (1952). The following stock solution was prepared and kept at room temperature. Copper reagent: 24 g of anhydrous Na_2CO_3 were dissolved in 250 ml of distilled water with 12 g of sodium potassium tartrate. 40 ml of 1% $CuSO_4 \cdot 5H_2O$ were added with stirring followed by 16 g of $NaHCO_3$. 18 g of anhydrous Na_2SO_4 were dissolved in 500 ml of hot distilled water which was then boiled. After cooling, this was added to the previous solution and the whole was diluted to 1 litre with distilled water; the reagent was filtered after 2 days.

To measure reducing sugar levels, 1 ml of copper reagent was added to the sugar solution which was then capped with foil and boiled for 20 minutes. After cooling, 1 ml of arsenomolybdate reagent (BDH) was added, mixed, and 7 ml of distilled water added. After thorough mixing, the optical density was measured on a spectrophotometer at 600 nm. As shown in text figure 11, the assay is suitable for reducing sugar estimation at least over the range 10 to 100 µg under these conditions. The standard curve was repeated routinely for each assay, and was prepared using D-glucose.

FIGURE 11

A typical reducing sugar calibration curve obtained on D-glucose standards, after assay by the Nelson-Somogyi method.


0.8
0.6
0.4
0.2
0
OD 600 nm
0
10
20
40
60
80
100
glucose / µg ml⁻¹

Estimation of total nitrogen

Nitrogen levels were determined by the micro-Kjeldahl method. (A) Digestion: The material to be analysed was boiled with 1.5 ml of conc. H_2SO_4 (nitrogen-free incorporating 3% (w/v) salicylic acid) plus a small amount of catalyst (8 g K_2SO_4, 1 g HgO, 1 g $CuSO_4$), until the digest cleared, the digestion was then continued for a further 30 minutes. Each digest was diluted to 25 ml with distilled water. (B) Distillation: 5 ml of diluted digest was distilled for 4 minutes with 4 ml of 40% (w/v) NaOH in a Markham still, and the distillate was collected in 5 ml of 2% (w/v) H_3BO_3 which contained 4 drops of mixed indicator solution (methyl red + bromo-cresol green). (C) Titration: The boric acid was titrated to neutrality with 0.01 N. HCl and the relationship 1 ml 0.01 N HCl ≡ 0.14 mg N was used to determine the level of nitrogen. The distillation and titration was first tested by determining the nitrogen content of samples of a known concentration of NH_4Cl solution.

3.2.3 Experimental

Production of enzyme

Initial work was performed employing a culture method described by King (1966) in which agar disc inoculae were supported over liquid media on glass-wool pads; this produced poor growth which was very variable. Therefore the main survey presented here was carried out using a modification of the method of King and Smith (1973). The fungus was grown in stationary culture on a medium consisting of a carbon source and a basal salts solution containing (per litre):

KH_2PO_4	(1.5 g)	$FeCl_3$	(145 µg)
$MgSO_4.7H_2O$	(0.5 g)	$CoCl_2.6H_2O$	(40 µg)
NH_4NO_3	(0.7 g)	$CuSO_4.5H_2O$	(60 µg)
$CaCl_2$	(0.1 g)	$MnCl_2.4H_2O$	(30 µg)
Thiamine hydrochloride	(1.0 mg)	$ZnSO_4.7H_2O$	(310 µg)
		$(NH_4)_6Mo_7O_{24}.4H_2O$	(20 µg)
H_3BO_3	(570 µg)		

dissolved in distilled water and adjusted to pH 5.0 with 4N NaOH.

The isolates were grown on 2% malt agar in 9 cm diameter petri dishes and after 14 days incubation, 8 mm diameter discs were taken from the colony margins and used to inoculate 100 ml of 2% malt agar in rectangular bottles, these were then incubated for 14 days in the dark

at 24°C. Three bottles were inoculated per isolate and at the end of this period, the mycelial mats in each were gently scraped off the agar and homogenised in 100 ml of salts solution in an MSE Ato-mixer at half speed for 5 seconds. 1 ml aliquots of mycelial suspension were then used to inoculate 20 ml of basal salts solution plus the appropriate carbon source in 100 ml wide-necked Erlenmeyer flasks (these had previously been stoppered and autoclaved at 120°C for 20 minutes).

The following carbon sources were used: (1) 3 flasks per isolate contained 1% sodium carboxymethylcellulose (NaCMC) (Koch-Light Laboratories); (2) 3 flasks per isolate contained 0.2 g BPC cotton wool; (3) A number of flasks were set up with *P. sylvestris* sapwood sawdust as carbon source; this had been seived through a 2.0 mm mesh.

After inoculation, the flasks were incubated in the dark at 24°C for 5 weeks and they were then harvested. Their contents were filtered through pre-weighed, oven-dry Whatman glass-fibre filter paper (GF/A grade) under partial vacuum in a Buchner funnel. 2 ml of the filtrate from each flask were stored at 4°C for immediate enzyme assay and the remainder was bulked with that from other flasks of the same isolate; these were also stored at 4°C after the addition of 0.02% sodium azide to prevent microbial contamination.

The mycelia from the NaCMC cultures were washed, on the filter papers, with distilled water, and dried to constant weight at 90°C; the hyphal dry weights were then estimated. The cotton wool and mycelium was removed from the filter papers, dried to constant weight at 90°C, and stored in a vacuum desiccator for later total nitrogen determination (see Section 3.2.2). The nitrogen content of 0.2 g cotton wool which had been soaked in the basal salts solution, was estimated and subtracted from the sample values.

Assay of cellulase activity

Cellulase activity was measured by incubating 0.1 ml of filtrate with 1 ml of the following substrates made up in acetate buffer (47.6 g sodium acetate, 8.6 ml glacial acetic acid, made up to 2.5 litres with distilled water and adjusted to pH 5.0; 0.02% sodium azide was added routinely).

a) 0.1% sodium carboxymethylcellulose; incubated for 1 hour at 40°C. Used for the assay of endo β-1,4-glucanases (C_X).

b) 0.1% cellobiose; incubated for 1 hour at 40°C. Used for the assay of β-glucosidases.

Each sample was duplicated and after incubation, the reducing sugars produced were measured on the total digest by the Nelson-Somogyi method (see Section 3.2.2). A blank was prepared in the same way, but using 0.1 ml of boiled filtrate.

c) 0.02 g of cotton wool (BPC) in 1 ml of acetate buffer; incubated for 24 hours at 40°C. Used for the assay of endo β-1,4-glucan cellobiohydrolase (C_1) activity. After incubation the contents of each tube were mixed and 0.5 ml of the digest buffer was pipetted out and added to 0.5 ml of distilled water, the reducing sugars in the diluted buffer were then assayed as described previously.

C_x activity over a range of pH values was determined, and the time courses of both C_x and C_1 activities were also measured. The activities in the filtrates from 16 isolates were measured after growth on NaCMC and cotton wool; each replicate flask being assayed.

Filtrate fractionation

Before fractionation it was necessary to reduce the volume of each filtrate in order to produce a concentrated protein solution. An initial attempt to do this was made using ammonium sulphate precipitation as used successfully by King and Smith (1973), however, the precipitated protein could not be re-dissolved.

Instead, the culture filtrate was desalted by dialysis for 24 hours in Visking tubing (which had previously been boiled for 20 minutes with the tetra sodium salt of ethylenediaminetetra-acetic acid [EDTA]) against 2 litres of distilled water, at 4°C. The protein concentration was measured before and after dialysis (see section 3.2.2) and the filtrate was then freeze-dried, resulting in a brown powder. This was re-dissolved in 2.5 ml of acetate buffer pH 5.0, tested for enzyme activity, and stored at 4°.

Gel filtration

10 g of Sephadex G-75 (Pharmacia) were allowed to swell in excess acetate buffer (pH 5.0) for 24 hours and packed into a glass column 29 cm long and with a volume of 110 ml^3. It was connected to a peristaltic pump in a cold room at 4°C and buffer was run through for 24 hours to

allow equilibration (the flow rate settled to 25 ml hr^{-1}). The column was calibrated with blue dextran (M.W. 2,000,000), bovine serum albumin (M.W. 67,000), Myoglobin (M.W. 18,750), cytochrome-c (M.W. 12,800) and potassium chromate (M.W. 194) and Kav values plotted for each, enabling a molecular weight calibration curve to be prepared.

A 1 ml aliquot of the concentrated filtrate was applied to the top of the column and eluted with acetate buffer (pH 5.0) incorporating 0.02% sodium azide. The eluate passed through an LKB monitor which measured absorption at 280 nm, and was collected in 2.5 ml fractions on an LKB fraction collector. 0.1 ml aliquots of each fraction were then assayed for cellulase activity by the method described earlier. The Kav of each peak was calculated, and the molecular weight estimated from the calibration curve.

Polyacrylamide-Gel Electrophoresis

This was carried out on disc electrophoresis apparatus.

(a) Non-denaturing conditions

The following stock solutions were prepared and stored at $4^{o}C$; (A) running buffer: 0.6% Tris, 2.88% glycine pH 8.3, (B) acrylamide stock: 30% acrylamide and 1% methylene-bis-acrylamide (filtered and stored in a dark bottle), (C) Gel buffer: 3.63% Tris, 0.23% (v/v) N,N,N^{1},N^{1}-tetramethyl-ethylenediamine (TEMED) and HCl to pH 8.9, (D) ammonium persulphate: 14% ammonium persulphate freshly prepared prior to each polymerisation. (E) Staining solution: 45.4% methanol (v/v), 9.2% acetic acid (v/v) and 0.25% Coomassie brilliant blue (w/v); (F) destaining solution: 5% methanol (v/v) and 7.5% acetic acid (v/v).

To prepare 10% gels all constituents were warmed to room temperature and 8 ml of solution B, 6 ml of solution C and 10 ml of water were mixed. After degassing by vacuum pump, 120 µl of solution D were added to polymerise the gels. The gels were poured in glass tubing (80 x 2.5 mm internal diameter) which had been sealed at the bottom with parafilm. All gels were poured to the same height and a few drops of 50% methanol added to produce a flat surface at the top of the gels. Samples were added to 50 µl of a mixture of 5 drops of running buffer, 5 drops of glycerol and 50 µl 0.05% bromophenol blue (tracking dye) and applied to the gel using a Hamilton micro-syringe. The total sample volume never exceeded 150 µl and was usually between 50 and 100 µl. The gels were run at 3-4 m amp per gel. The position of the dye front was

marked by the insertion of a piece of fine wire and the gels stained for 60 minutes at room temperature in solution E. The destaining time varied but was not less than 48 hours.

(b) Denaturing conditions

Sodium dodecyl sulphate (SDS) electrophoresis was performed. The following stock solutions were prepared; (A) running buffer: 50 mM Tris, 1% SDS pH 8.0; (B) gel buffer: 9.1% Tris, 0.2% SDS, 0.05% (v/v) TEMED pH 8.8. (C) Acrylamide: 45% acrylamide, 1.2% methylene-bis-acrylamide; (D) ammonium persulphate: 0.15% ammonium persulphate. (E) Boiling solution: 2% SDS, 10% (v/v) glycerol, 50 mM Tris pH 8.0; (F) denaturing solution: 1 ml solution E and 20 µl mercaptoethanol (prepared just prior to use). Staining and destaining solutions were as described in section (a).

Protein samples were mixed with equal volumes of denaturing solution and 5 µl of bromophenol blue. They were boiled for 2 minutes and applied to the gels (sample volume never exceeded 150 µl), which were run at 3 m amp each for 20 minutes, after which the current was increased to 5 m amp per gel. The dye front was marked with a piece of thin wire and staining and destaining performed as described previously.

The molecular weights of the stained bands were estimated by the inclusion to each set of gels of a set of protein standards, namely, bovine serum albumin (M.W. 68,000); alcohol dehydrogenase (M.W. 43,000); trypsin (M.W. 23,500); cytochrome-c (M.W. 12,800) and catalase (M.W. 60,000).

The stained gels were scanned on a Gilford recording spectrophotometer, model 2000, fitted with a gel-scanning attachment; Rf values were then calculated for each peak.

3.2.4 Results

Enzyme activities

Carboxymethylcellulase activity of the culture filtrates was assayed after incubation at different pH values, and the results are presented graphically in text figure 12. A classic bell-shaped curve was obtained with optimum activity at pH 4.5-5.0, therefore pH 5.0 was used routinely for all enzyme assays.

Carboxymethylcellulase activity was measured over a time course and text figure 13 shows the linear curve obtained, which means that the enzyme activity was not limited during the period tested. A 60-minute incubation period was selected, as it is within the linear section of this activity/time plot, and because measurable levels of reducing sugar were produced. The activity of the filtrate was also tested against cotton wool, and reducing sugars assayed after a range of incubation periods. This activity (C_1) was not linear (text figure 14), and although a 2 hour incubation period was tested with a number of filtrates, reducing sugar levels were very low and a 24 hour incubation was chosen to give measurable levels.

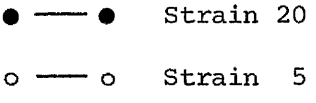
Results of enzyme assays on the crude filtrates are presented in tables 3 to 8. No cellobiase activity was detected in any of the samples, and those filtrates from cultures grown on the soluble cellulose (NaCMC) as carbon source, showed no detectable C_1 activity.

The enzyme activities in each table have been correlated with the equivalent decay-potentials of the strains of origin, and as the r values show, the correlations were very poor, except for those values in table 7, for which a correlation coefficient of 0.89 was obtained. This indicates that the decay-potentials of strains in that table are related to their levels of C_x activity; however, table 4 presents C_x activities of culture filtrates from strains grown under the same culture conditions, and the correlation coefficient was found to be -0.56. So the results in table 7 are at odds with those in other tables and will consequently be disregarded.

Tables 3-5 and tables 6-8 present the results obtained from two different batches of culture filtrates. The enzyme values obtained from the cotton grown cultures cannot be directly compared between batches, as the nitrogen values representing total protein were obtained by different sampling procedures: those from the first batch being obtained from representative samples of the cotton + fungus,

FIGURE 12

Carboxymethylcellulase (C_x) activity at different pH values, measured as reducing sugar produced after 1 hour's incubation at 40°C.



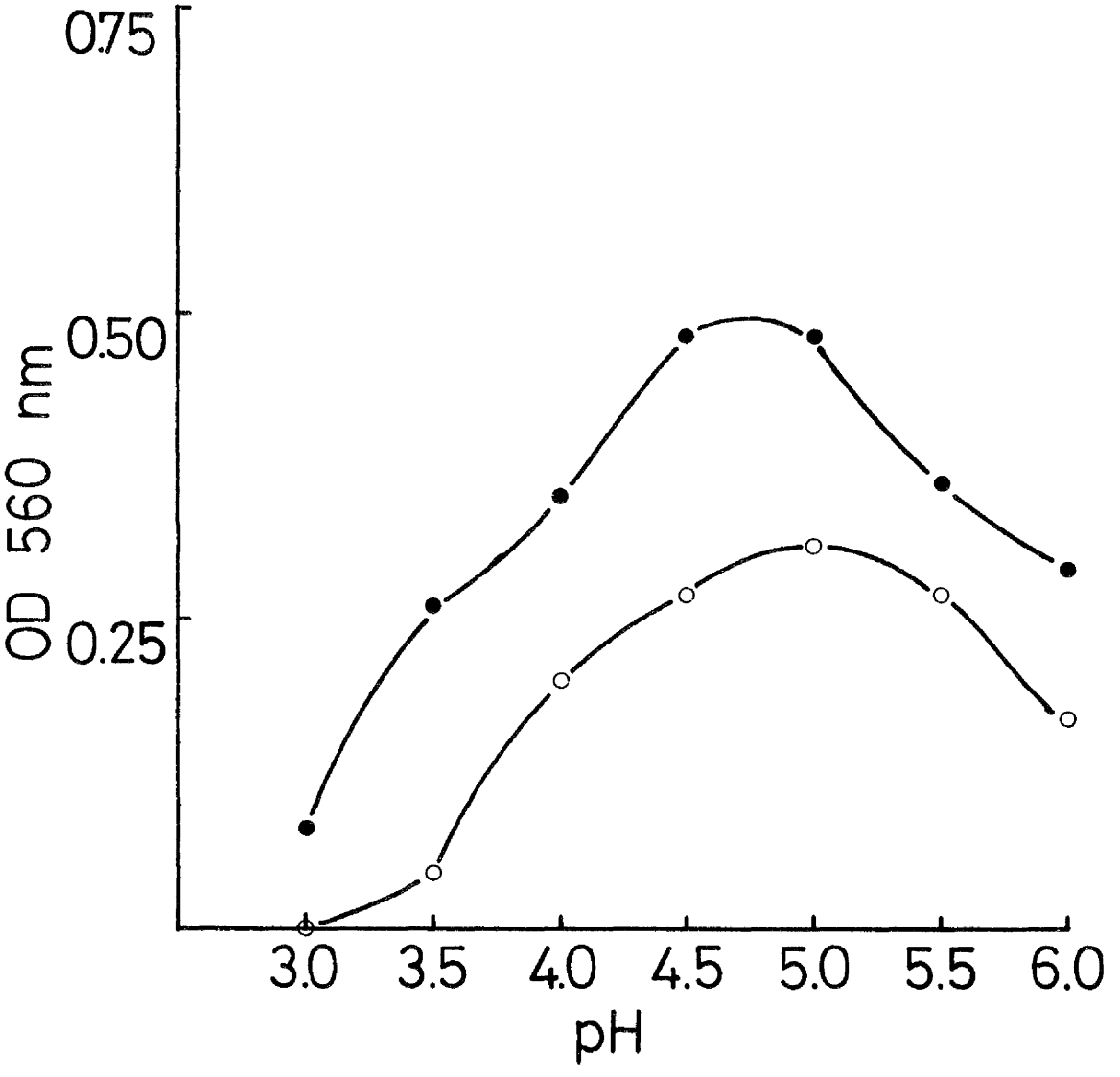
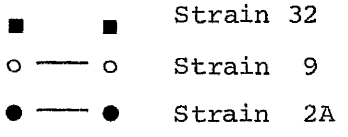

0.75
0.50
0.25
OD 560 nm
3.0
3.5
4.0
4.5
5.0
5.5
6.0
pH

FIGURE 13

Carboxymethylcellulase (C_x) activity after a range of incubation periods at 40°C.



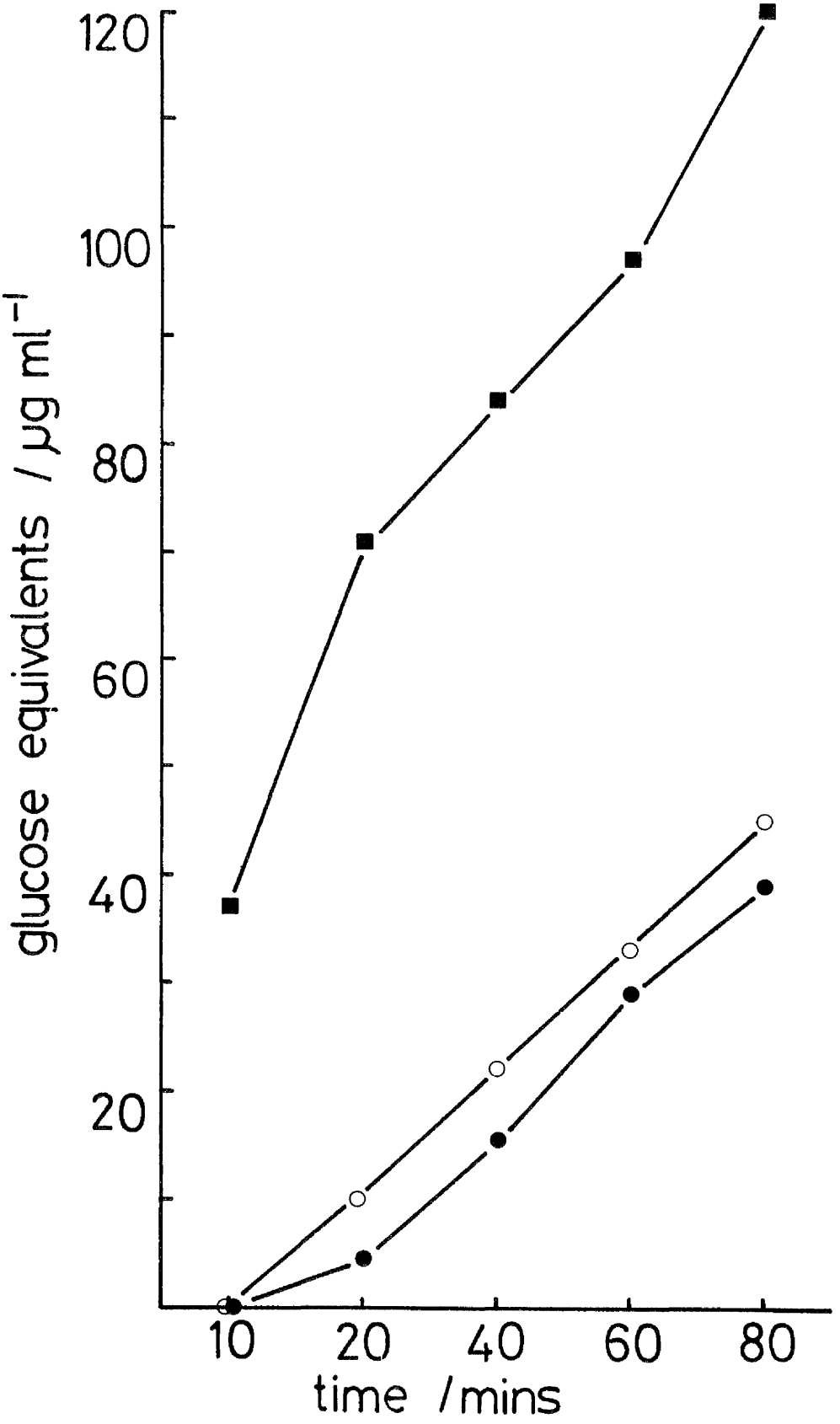

120
100
80
60
40
20
glucose equivalents / µg ml⁻¹
10
20
40
60
80
time / mins

FIGURE 14

C_1 activity with time. 0.1 ml of culture filtrate incubated with cotton wool; digest harvested after different periods of incubation at 40°C, and assayed for reducing sugars.

■ —— ■ Strain 1

o —— o Strain 20

● —— ● Strain 5C

▲ —— ▲ Strain 17

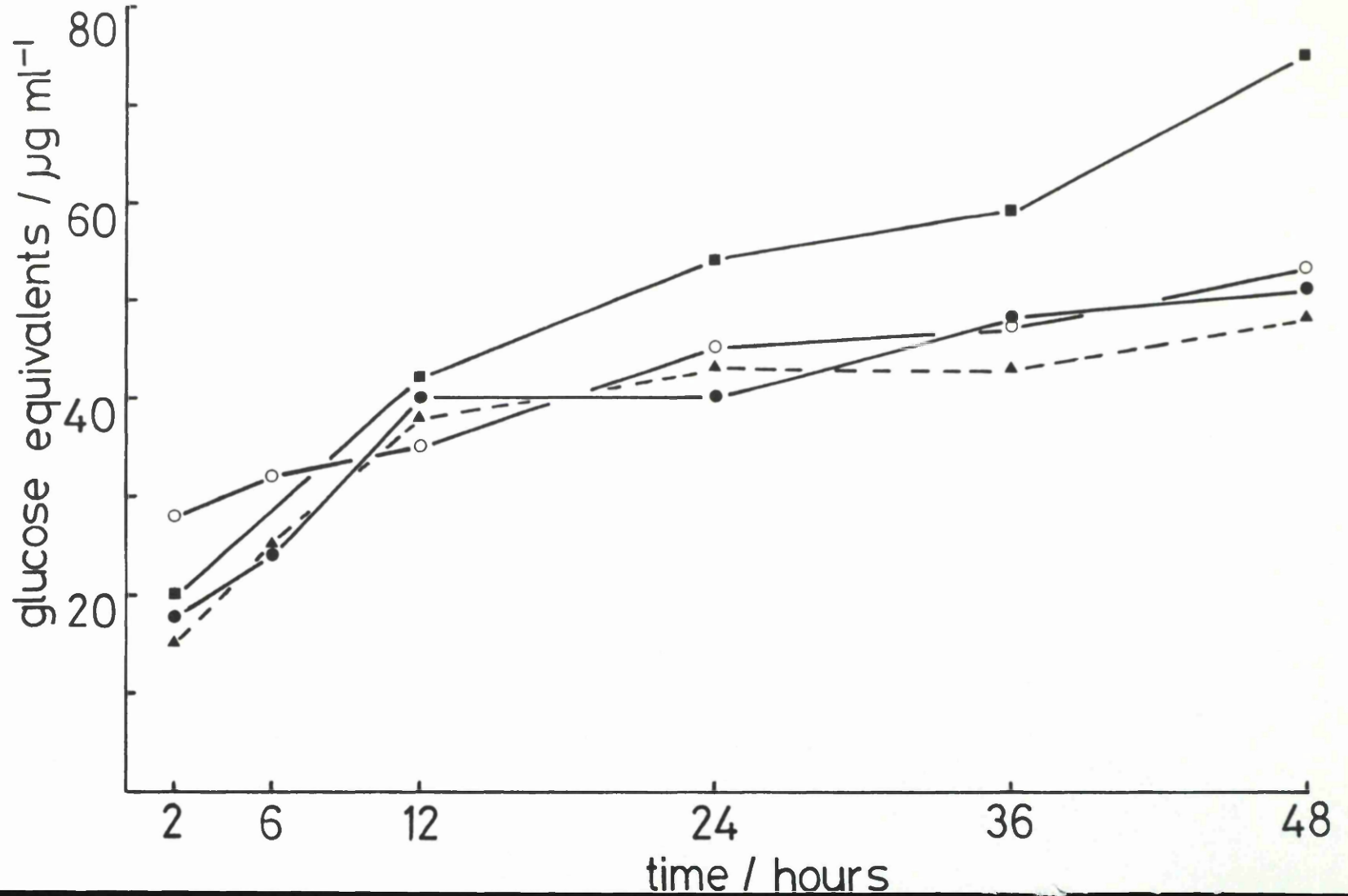

glucose equivalents / µg ml⁻¹
80
60
40
20
2
6
12
24
36
48
time / hours

Table 3. C_x activity of filtrates from cultures grown on sodium carboxymethylcellulose as the carbon source.

Isolate	Flask	Hyphal Dry wt. (mg)	Glucose Equivalents ($\mu g\ ml^{-1}\ hr^{-1}$)	µg Glucose per mg Fungus	$\bar{x}$	S.E.	Decay-Potential (% wt. loss)
	1	43	143	3.33			
12	2	51	142	2.78	2.87	0.30	3.2
	3	46	115	2.50			
	1	57	113	1.98			
1	2	60	125	2.08	1.87	0.20	5.2
	3	59	92	1.56			
	1	62	163	2.63			
17	2	37	92	2.49	2.82	0.32	14.6
	3	50	167	3.34			
	1	46	217	4.72			
15	2	42	190	4.52	4.44	0.24	25.5
	3	45	183	4.07			
	1	71	90	1.27			
20	2	66	180	2.73	2.46	0.76	26.6
	3	60	202	3.37			
	1	55	137	2.49			
7	2	56	165	2.95	2.96	0.34	29.2
	3	51	176	3.45			
	1	52	169	3.25			
5C	2	42	210	5.00	5.23	1.50	34.1
	3	31	231	7.45			
	1	53	>200	>3.77			
5D	2	56	213	3.80	3.63	0.19	45.7
	3	60	>200	>3.33			

$r = .58$

Table 4. C_x activity of filtrates from cultures grown on a cotton carbon source.

Isolate	Flask	Nitrogen Total (µg)	Glucose Equivalents (µg $ml^{-1}hr^{-1}$)	µg Glucose per µg Nitrogen	$\bar{x}$	S.E.	Decay-Potential (% wt. loss)
	1	246	560	2.28			
12	2	154	510	3.31	2.37	0.64	3.2
	3	303	460	1.52			
	1	628	800	1.27			
1	2	418	800	1.91	1.77	0.32	5.2
	3	383	820	2.14			
	1	350	560	1.60			
17	2	350	500	1.43	1.59	0.11	14.6
	3	280	490	1.75			
	1	336	800	2.38			
15	2	341	720	2.11	2.35	0.16	25.5
	3	332	850	2.56			
	1	357	590	1.65			
20	2	387	680	1.76	1.66	0.07	26.6
	3	405	630	1.56			
	1	452	700	1.55			
7	2	483	650	1.35	1.61	0.21	29.2
	3	354	680	1.92			
	1	427	620	1.45			
5C	2	390	630	1.62	1.54	0.12	34.1
	3						
	1	438	720	1.64			
5D	2	452	820	1.81	1.42	0.38	45.7
	3	982	790	0.80			

$r = -.56$

Table 5. C_1 activity of filtrates from cultures grown on a cotton carbon source.

Isolate	Flask	Total Nitrogen (µg)	Glucose Equivalents ($\mu g\ ml^{-1} hr^{-1}$)	µg Glucose per µg Nitrogen	$\bar{x}$	S.E.	Decay Potential (% wt. loss)
	1	246	14	0.057			
12	2	154	12	0.078	0.058	0.012	3.2
	3	303	12	0.040			
	1	628	38	0.061			
1	2	418	40	0.096	0.092	0.021	5.2
	3	383	46	0.120			
	1	350	24	0.069			
17	2	350	30	0.086	0.085	0.011	14.6
	3	280	28	0.100			
	1	336	34	0.100			
15	2	341	32	0.094	0.099	0.00	25.5
	3	332	34	0.103			
	1	357	14	0.039			
20	2	387	12	0.031	0.033	0.00	26.6
	3	405	12	0.030			
	1	452	38	0.084			
7	2	483	38	0.079	0.094	0.016	29.2
	3	354	42	0.119			
	1	427	48	0.112			
5C	2	390	134	0.344	0.228	0.164	34.1
	3						
	1	438	54	0.123			
5D	2	452	52	0.115	0.098	0.026	45.7
	3	982	54	0.055			

$r = .38$

Table 6. C_x activity of filtrates from cultures grown on sodium carboxymethylcellulose as the carbon source.

Isolate	Flask	Hyphal Dry Weight (mg)	Glucose Equivalents ($\mu g\ ml^{-1} hr^{-1}$)	µg Glucose per mg Fungus	$\bar{x}$	S.E.	Decay-Potential (% wt. loss)
	1	78	83	1.15			
2A	2	64	68	1.02	0.97	0.15	3.6
	3	32	66	0.75			
	1	69	62	0.89			
4A	2	60	60	1.00	0.94	0.04	4.5
	3	73	68	0.93			
	1	80	108	1.35			
22	2	72	140	1.94	1.65	0.30	18.0
	3	73	-				
	1	75	90	1.20			
26	2	81	120	1.48	1.48	0.20	21.9
	3	77	135	1.75			
	1	72	97	1.35			
11	2	68	100	1.47	1.44	0.05	28.1
	3	71	106	1.49			
	1	78	120	1.54			
9	2	66	110	1.67	1.52	0.11	28.8
	3	81	110	1.36			
	1	61	93	1.53			
32	2	82	91	1.11	1.44	0.21	63.1
	3	65	110	1.69			

$r = .55$

Table 7. C_x activity of filtrates from cultures grown on a cotton carbon source.

Isolate	Flask	Total Nitrogen (µg)	Glucose Equivalent (µg $ml^{-1}hr^{-1}$)	µg Glucose per µg Nitrogen	$\bar{x}$	S.E.	Decay Potential (% wt. loss)
	1	205	300	1.46			
2A	2	265	190	0.72	0.92	0.33	3.6
	3	290	170	0.59			
	1	665	520	0.78			
4A	2	65	→ no growth		0.88	0.14	4.5
	3	550	540	0.98			
	1	640	640	0.98			
22	2	435	650	1.49	1.24	0.18	18.0
	3	545	680	1.25			
	1	505	630	1.25			
26	2	770	720	0.94	1.08	0.11	21.9
	3	700	740	1.06			
	1	485	560	1.16			
11	2	475	560	1.18	1.14	0.04	28.1
	3	525	560	1.07			
	1	665	640	0.96			
9	2	535	620	1.16	1.06	0.14	28.8
	3	765	-				
	1	370	600	1.62			
32	2	410	560	1.37	1.44	0.11	63.1
	3	440	590	1.34			

$r = .89$

Table 8. C_1 activity of filtrates from cultures grown on a cotton carbon source.

Isolate	Flask	Total Nitrogen (µg)	Glucose Equivalents (µg $ml^{-1}h^{-1}$)	µg Glucose per µg Nitrogen	$\bar{x}$	S.E.	Decay Potential (% wt. loss)
	1	205	0	0			
2A	2	265	0	0	0	-	3.6
	3	290	0	0			
	1	665	24	0.036			
4A	2	65	n o g r o w t h		0.038	0.00	4.5
	3	550	22	0.040			
	1	640	32	0.050			
22	2	435	28	0.064	0.058	0.00	18.0
	3	545	32	0.059			
	1	505	24	0.048			
26	2	770	28	0.036	0.041	0.00	21.9
	3	700	28	0.040			
	1	485	22	0.045			
11	2	475	32	0.067	0.046	0.014	28.1
	3	525	14	0.027			
	1	665	18	0.027			
9	2	535	16	0.030	0.029	0.00	28.8
	3	765	-	-			
	1	370	18	0.049			
32	2	410	16	0.039	0.040	0.00	63.1
	3	440	14	0.032			

$r = .33$

and those from the second being obtained on the total cotton + fungus. Therefore the values in the first batch may be lower, so producing final enzyme values which are higher. A point which should be noted is that in both sets of tables, the C_x activities of the filtrates from the cotton grown cultures are much higher than those from the cultures grown on soluble cellulose; this difference was also seen in their protein contents.

Gel filtration

Two representative culture filtrates were taken and their volumes reduced in order to concentrate their protein contents. They were then fractionated on Sephadex G-75 in order to gain some information about the component enzymes of their cellulase complexes. Text figures 15 and 16 show the peaks of activity found after assaying for reducing sugars obtained after incubating each fraction with carboxymethyl cellulose; C_1 activity was assayed by incubating with cotton wool plus a 0.1 ml aliquot of eluant from the C_x peaks. No C_1 activity was detected, nor were there differences between their protein profiles as measured by absorbance at 280 nm. However, one peak of C_x activity (A) was found in the fractions from the filtrate of strain 5C grown on cotton, and peak A was also detected in the fractions from the filtrate of strain 1 grown on NaCMC, in addition to another peak of C_x activity (B). The approximate molecular weights of A and B were estimated by measuring their K_{av} values and relating these to molecular weight from a calibration curve (see table 9 and text figure 17). Peak A had an approximate molecular weight of 32,000 and peak B of less than 10,000.

Polyacrylamide gel electrophoresis

PAG electrophoresis was employed as it allows a much more detailed separation of protein to be achieved. Plate 3 shows the gels obtained after electrophoresis of native filtrates from a number of filtrates. Some of the protein has stayed on the top of the gels, but all the gels have bands 5, 6 and 9 in common (identified from Rf values); the filtrates from the cotton grown cultures also have bands 7 and 8 in common, but that filtrate from strain 22 grown on carboxymethyl cellulose (cmc), does not appear to have these two. Other faint bands are also visible in some of the gels (see black triangles), but these may be due to impurities.

Denatured samples were also run in order to gain information

FIGURE 15

Carboxymethylcellulase (C_x) activity of fractions after fractionating 1 ml of filtrate from a culture of strain 5C grown on cotton wool (BPC), on Sephadex G-75 in acetate buffer pH 5.0, at 4°C. The sample contained 1300 µg protein.

——— absorbance at 280 nm

●——● C_x activity

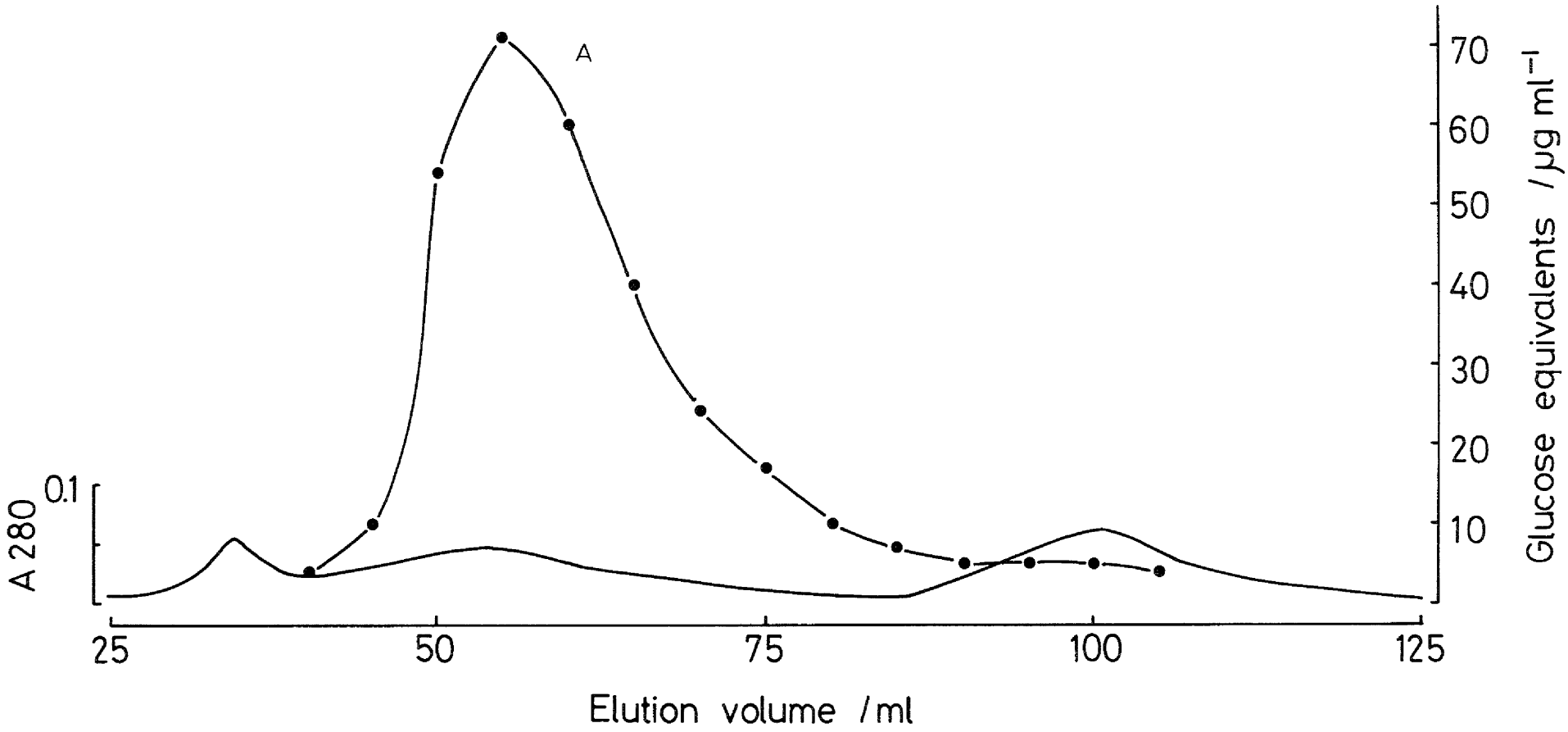

A
A 280
0.1
25
50
75
100
125
Elution volume /ml
70
60
50
40
30
20
10
Glucose equivalents /μg ml⁻¹

FIGURE 16

Carboxymethylcellulase (C_x) activity of fractions after fractionating 1 ml of filtrate from a culture of strain 1 grown on sodium carboxymethylcellulose, on Sephadex G-75 in acetate buffer pH 5.0, at 4°C. The sample contained 800 µg protein.

——— absorbance at 280 nm

● —— ● C_x activity

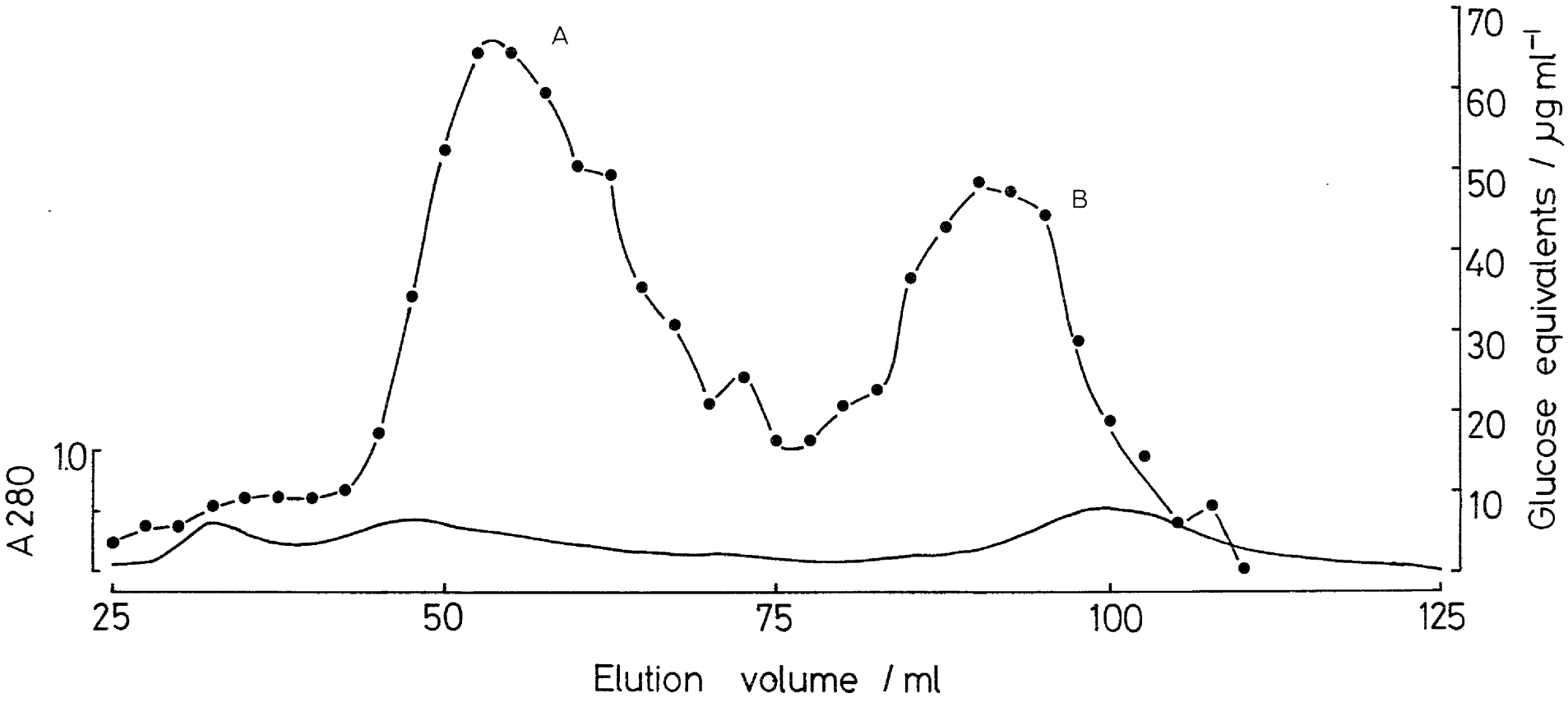

A
B
A 280
1.0
25
50
75
100
125
Elution volume / ml
70
60
50
40
30
20
10
Glucose equivalents / µg ml⁻¹

Table 9. Molecular weight estimates of peaks from G-75 gel filtration.

Peak	Peak fraction	Ve-Vo	K_{av}	M.W.
Blue Dextran	15.3			
Bovine Serum Albumin	17.3	2.0	0.07	67,000
Myoglobin	25.0	9.7	0.32	18,750
Cytochrome-c	26.2	10.9	0.35	12,800
A	21.5	6.2	0.20	32,000
B	37.0	21.7	0.71	<10,000
K_2CrO_4	46.1	$30.8(V_t-V_o)$		

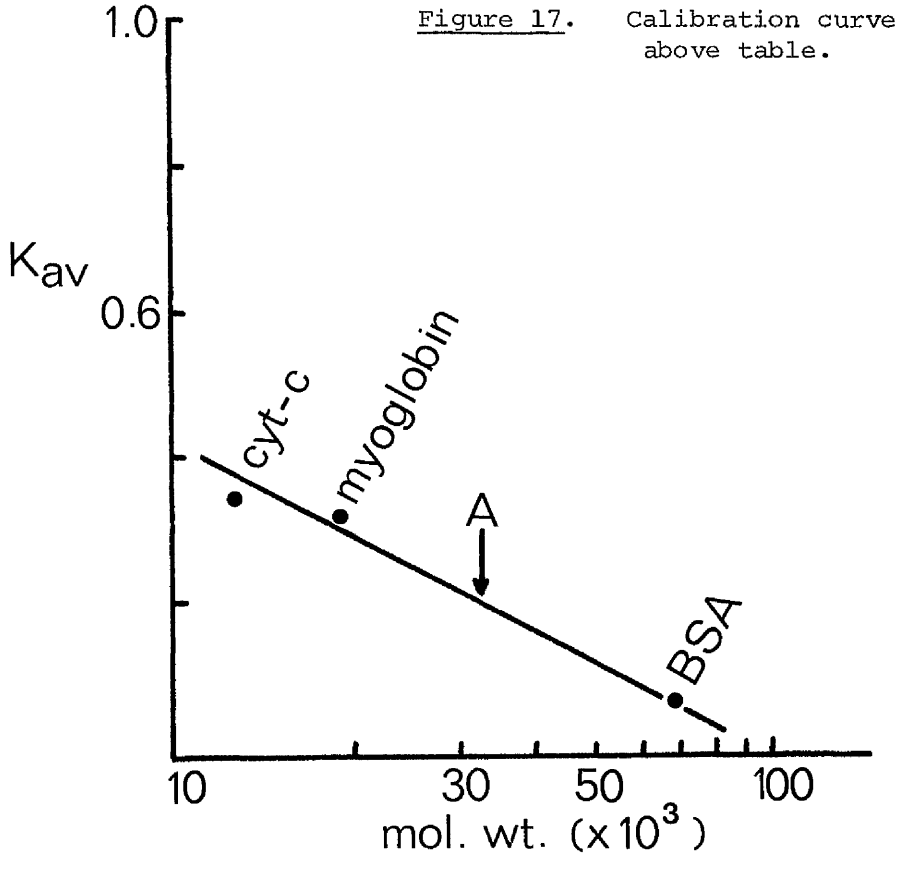
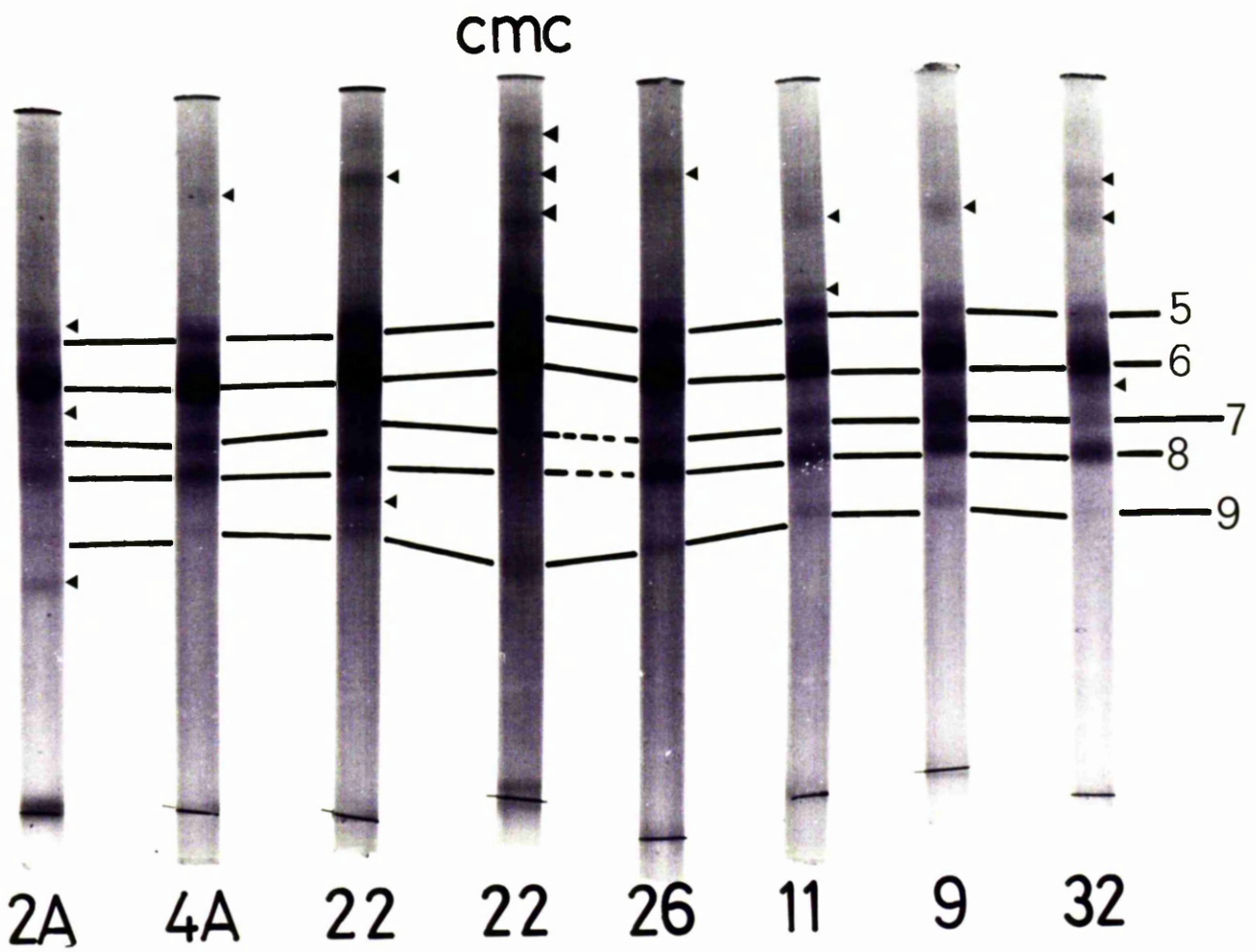


Figure 17. Calibration curve from above table.

PLATE 3

Stained gels obtained after PAG electrophoresis of native culture filtrates (10% gels). All the isolates were grown on cotton wool except for 22 cmc which was grown on sodium carboxymethylcellulose.

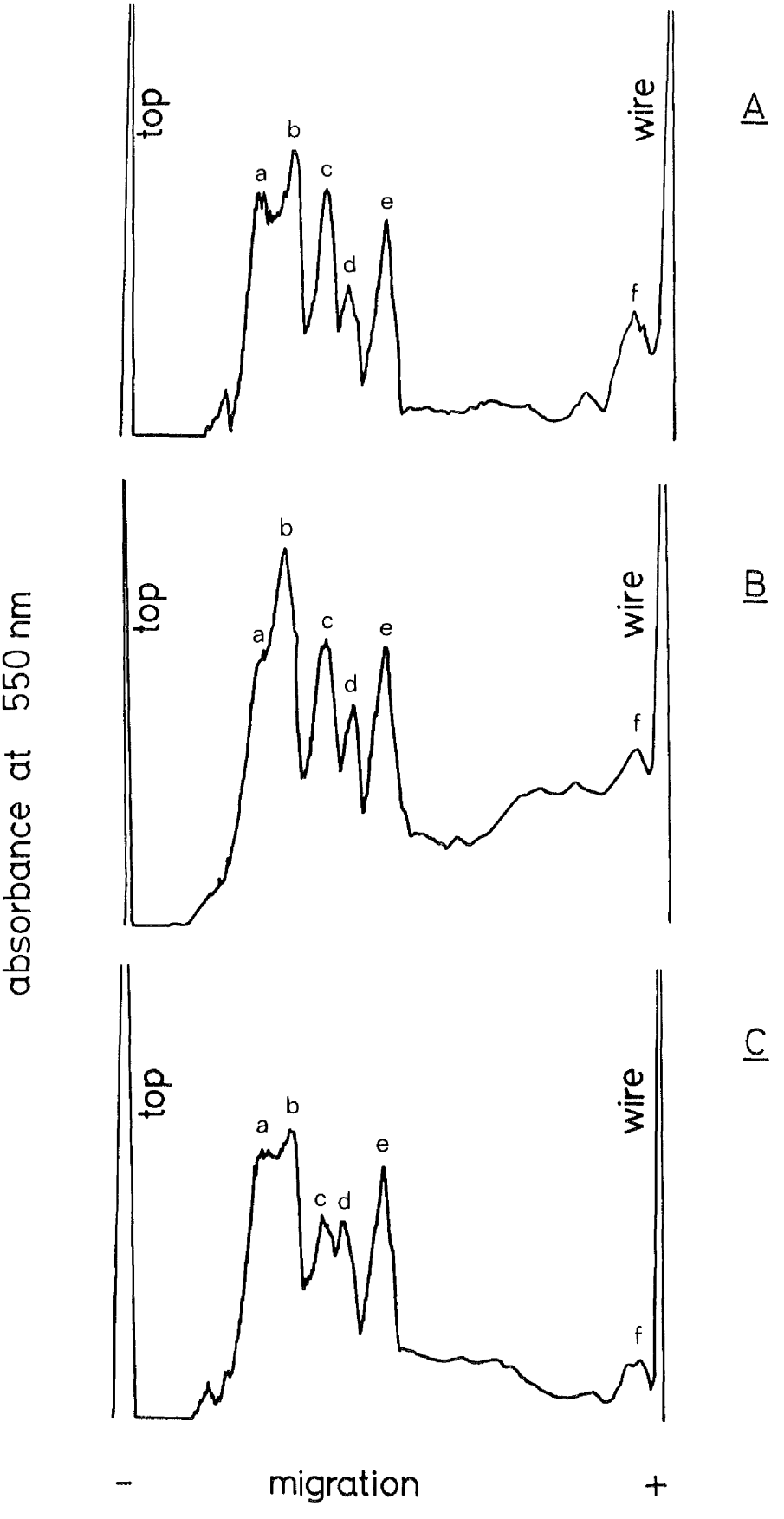

cmc
5
6
7
8
9
2A
4A
22
22
26
11
9
32

FIGURES 18 & 19

Gel scans obtained after PAG electrophoresis of a number of concentrated, denatured culture filtrates:

			[protein]
A	filtrate from strain	32	1868 µg ml^{-1}
B	" " "	9	1856 " "
C	" " "	11	2304 " "
D	" " "	26	2156 " "
E	" " "	22	2622 " "
F	" " "	4A	2520 " "

All grown on cotton and presented in order of their decay-potential.


absorbance at 550 nm
top
wire
a
b
c
d
e
f
A
B
C
-
migration
+

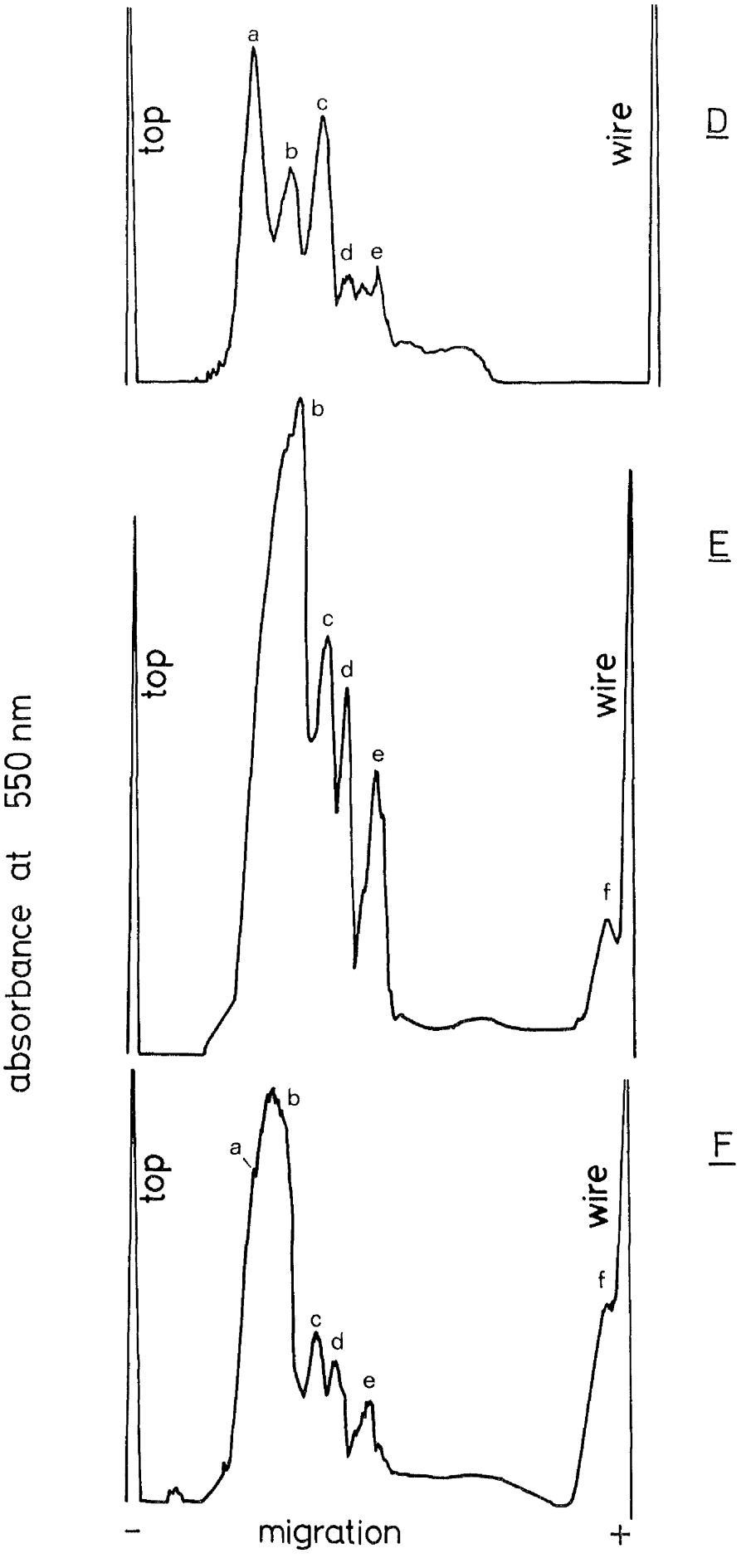

absorbance at 550 nm
top
wire
a
b
c
d
e
D
top
wire
b
c
d
e
f
E
top
wire
a
b
c
d
e
f
F
–
migration
+

Table 10. Molecular weight estimates of peaks from SDS gel scans.

Standards

	Distance Run (cm)	Rf	M.W.
Dye Front	14.00		
Catalase	3.45	0.25	60,000
Alcohol dehydrogenase	5.00	0.36	43,000
Cytochrome-c	10.50	0.75	13,000

Figure 20. Calibration curve from above table.

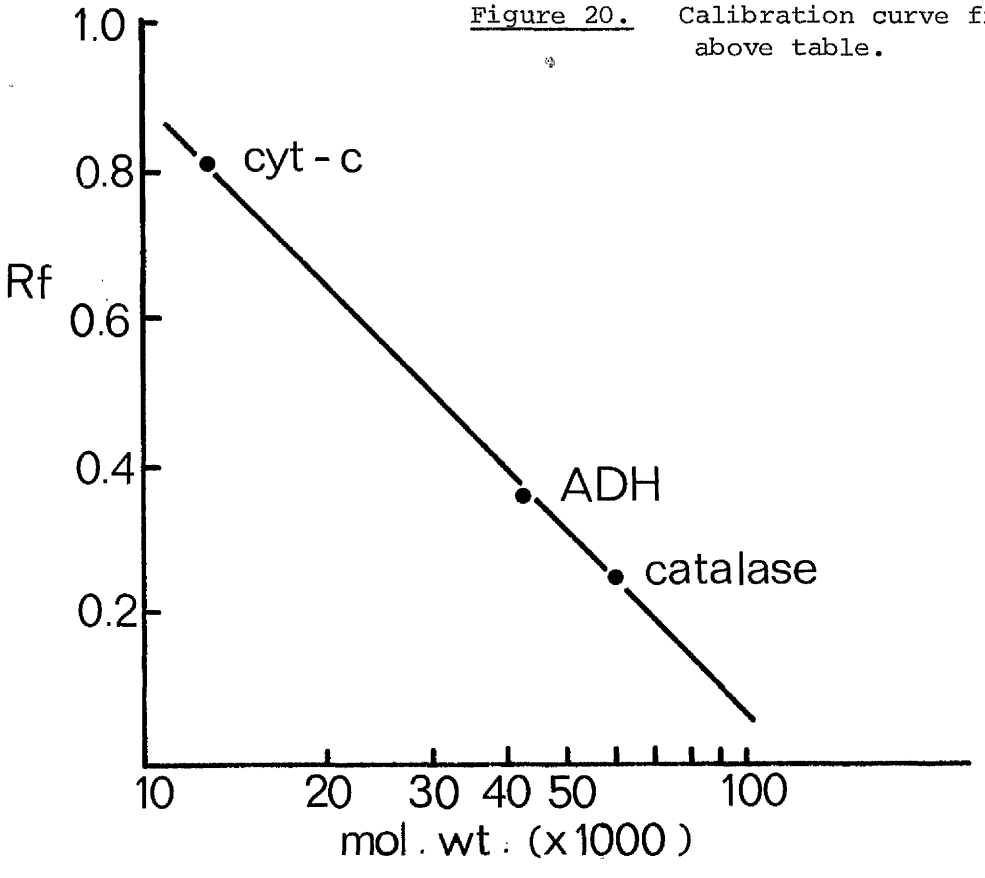
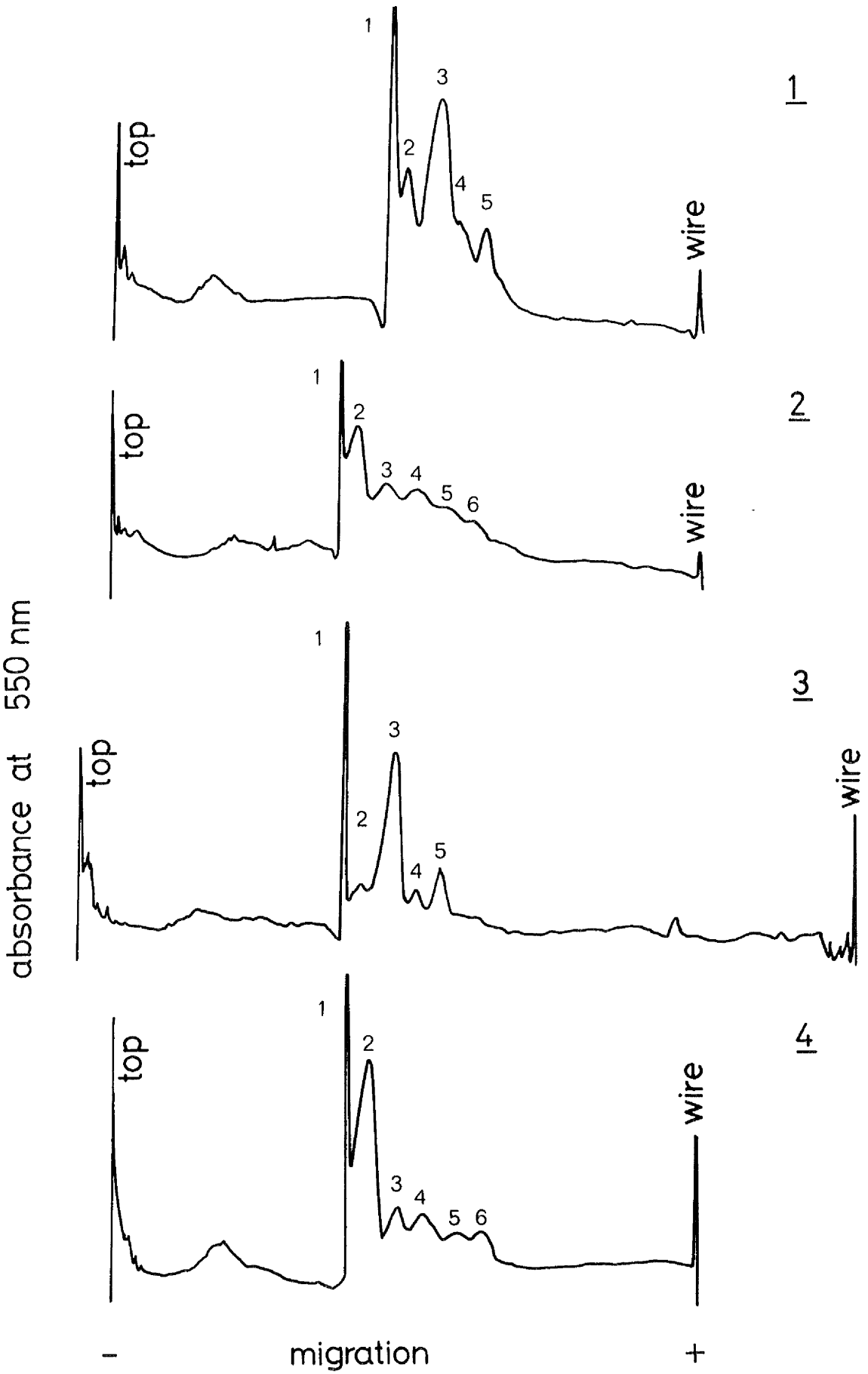


Table 11. Samples: Molecular weight estimates of bands from gel scans.

	Peak	Distance Run (cm)	Rf	M.W.
	Dye Front	13.70		
Strain 32	a	3.30	0.24	61,000
Scan A	b	4.20	0.31	50,000
	c	5.05	0.37	43,000
	d	5.60	0.41	39,000
	e	6.60	0.48	32,000
	f	12.90	0.94	<10,000
	Dye Front	13.50		
Strain 9	a	3.45	0.26	59,000
Scan B	b	4.00	0.30	51,000
	c	5.00	0.37	43,000
	d	5.65	0.42	38,000
	e	6.50	0.48	32,000
	f	13.00	0.96	<10,000
	Dye Front	13.40		
Strain 11	a	3.50	0.26	59,000
Scan C	b	4.20	0.31	50,000
	c	5.00	0.37	43,000
	d	5.50	0.41	39,000
	e	6.50	0.49	31,000
	f	12.90	0.96	<10,000
	Dye Front	13.90		
Strain 26	a	3.30	0.24	61,000
Scan D	b	4.30	0.31	50,000
	c	5.10	0.37	43,000
	d	5.80	0.42	38,000
	e	6.50	0.47	33,000
	Dye Front	13.40		
Strain 22	b	4.40	0.33	48,000
Scan E	c	5.20	0.39	41,000
	d	5.70	0.43	37,000
	e	6.50	0.49	31,000
	f	12.45	0.93	<10,000
	Dye Front	13,00		
Strain 4A	a	3.20	0.25	60,000
Scan F	b	3.70	0.29	52,000
	c	4.85	0.37	43,000
	d	5.30	0.41	39,000
	e	6.20	0.48	32,000
	f	12.50	0.96	<10,000

FIGURE 21

Gel scans obtained after PAG electrophoresis of denatured culture filtrate from strains 5C (1 & 2) and 1 (3 & 4) grown on *P. sylvestris* sapwood sawdust (2 & 4) and cotton wool (1 & 3).


absorbance at 550 nm
top
wire
1
2
3
4
5
6
1
2
3
4
− migration +

on molecular weights of component proteins, and these were scanned spectrophotometrically. Text figures 18 and 19 present the results obtained and molecular weight estimates are given in table 11 taken from a calibration curve (table 10 and text figure 20).

Five major bands are evident in all and some contain six (a band may be absent either because the background absorbancy made the base-line too high, or because it is hidden behind a dense band). The molecular weight values in table 11 vary, within narrow limits, between strains but are essentially the same. These fall within the range 61,000 to less than 10,000. Band e decreases in concentration in relation to the other bands, with the decrease in decay ability of the isolate.

The pine sawdust cultures grew very slowly and most were lost through drying out. However, some were harvested, concentrated, denatured and run on SDS gels, and the scans from cultures of strain 5C and 1 after growth on cotton wool and wood, are shown in text figure 21. The pattern of bands between equivalent cultures of the two strains is similar, but the filtrates from the sawdust grown cultures of both strains (scans 2 and 4) have one extra band (6). Also band 2 constitutes the prominent peak in the wood filtrates, whereas band 3 is prominent in the scans of the cotton filtrates (1 and 3). These differences suggest that the substrate will affect the pattern of proteins secreted by the fungus, and it may be that bands 6 and 2 are enzymes (for example hemicellulases) which would not be required for the breakdown of cotton wool. Certainly, it is likely that band 6 represents a protein which is induced by the substrate.

3.2.5. Discussion

Carboxymethylcellulases (C_x) are probably constitutive enzymes, but the C_1 component was not detected in filtrates from cultures grown on soluble cellulose, so this indicates that it is inducible. The assays for C_x activity revealed very little correlation with the decay-potentials of the isolates.

Cellobiase was not detected; this lack of activity may have been due to a number of reasons: (1) the assay itself is ineffective; the test substrate will respond to the reducing sugar assay and produce a chromogen. This increase in colour of the blank, may have reduced the sensitivity of the assay to small levels of reducing sugar. (2)

The enzyme may not be extracellular but may be located, as suggested by Hofsten (1975), in the periplasmic region between the cell wall and the cell membrane. (3) The enzyme may be extracellular, but bound to the walls and not released into the medium (Berg and Pettersson, 1977). (4) Cellobiase may not be produced.

The lack of linearity of C_1 activity when plotted against incubation time, indicates that the method of assay may be unsatisfactory. It may be due to the most reactive substrate being converted at the beginning of the reaction period (Enari and Markannen, 1977), or it may have been due to some form of enzyme inhibition. For instance, it has been found that cellobiose acts as a product inhibitor of β-1,4-glucan cellobiohydrolase (Halliwell, 1975; Maguire, 1977); cellobiase enhancing the activity of this enzyme. As mentioned earlier, we found no cellobiase activity, so this would allow a build-up of cellobiose.

Cotton is the purest form of naturally occurring α-cellulose (Gascoigne and Gascoigne, 1960), but it is highly crystalline and solubilised only with great difficulty. Therefore the linearity of this assay cannot be increased by reducing the reaction time. Leisolo *et al.* (1975) and Leisolo and Linko (1976) recommend the use of dyed substrate for the assay of C_1 activity. They obtained a linear relationship between dye production and substrate solubilisation. This was tested in our laboratory, but the quantity of substrate (cellulose azure (Calbiochem)) required for each assay, made the method prohibitively expensive for our purposes.

Alternatively, the results obtained may reflect a genuine lack of correlation between C_1 activity and decay-potential. Using wood as a substrate, other enzymes may be induced which may correlate with decay ability. Eriksson *et al.* (1974) found that *Sporotrichum pulverulentum* produces an enzyme, cellobiose:quinone oxidoreductase, which participates in the degradation of wood cellulose. This enzyme needs a quinone (from lignin) as a co-substrate, and therefore cannot function in the degradation of pure cellulose.

Results from the gel filtration of filtrates, indicated that this degree of concentration and purification of the enzyme system could be achieved with the maintenance of activity. The fact that only one peak of C_x activity was obtained from the fractions of strain 5C filtrate, whilst two were detected in those from the filtrate of

strain 1, may reflect a true difference between strains or substrates. However, further replication on a number of different strains grown on different carbon sources, will be necessary before further useful comment can be made. The molecular weights of the peaks A and B were 32,000 and less than 10,000 respectively, and these differ from those peaks of carboxymethylcellulase activity found by Eidså (1974). He fractionated *C. puteana* culture filtrate by gel filtration and ion-exchange chromatography, and isolated a peak of activity A (M.W. 42,000) and another, B (M.W. 38,500). So it seems likely that *Coniophora* may produce four C_x enzymes, which would conform with the findings on other celluloytic fungi, where five have been isolated. Gel filtration is a useful preliminary fractionation procedure, but its resolution is strictly limited, and ion-exchange chromatography would certainly be useful in allowing a more detailed fractionation to be carried out.

Polyacrylamide-gel electrophoresis of the culture filtrates, produced results which indicate possible differences between strains in their cell-free proteins. The method might be used to separate and to assay for more of the enzymes mentioned in table 2 of the introduction (section 3.2.1). If gels were run in the cold room, then enzymatically active bands could be located by cutting unstained gels into thin slices and extracting each with buffer, the extracts could then be assayed for enzymic activity. An electrophoretic method which would be most specific, is that of isoelectric focussing which could be used to further separate proteins after PAG electrophoresis, on the basis of their isoelectric points.

These methods, if used in logical sequence, could be used to resolve some of the differences which our results have indicated exist between strains. Such strain differences could, in themselves, be used to provide information on the process of cellulose degradation. This may be compared with the use of mutants such as those of *T. viride* employed by Montenecourt and Eveleigh (1977).

PART 4

RESPONSE TO WOOD PRESERVATIVES

RESPONSE TO WOOD PRESERVATIVES

4.1 Introduction

This study so far has been concerned with the variation among isolates of *C. puteana* under conditions which have approached the optimum for vegetative growth and wood decay by the fungus. However, growth will be affected by stress factors present in the variety of sub-optimal conditions to be found in the field situation. With this consideration in mind, we have extended the investigation to look at differences in tolerance among a number of our isolates, to the wood preservatives pentachlorophenol (PCP) and tri-n-butyl tin oxide (TBTO): the structural formulae of both these compounds are presented in text figure 22. A number of workers have looked at the response of *C. puteana* to various chemicals, the results of which have been reviewed in the General Introduction.

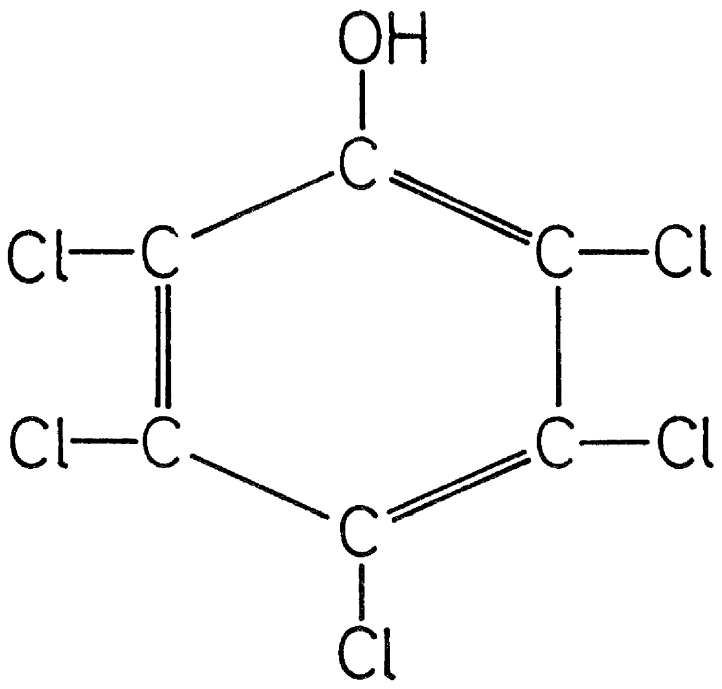
PCP, first produced commercially by Monsanto in the United States, has been a popular chemical in the remedial treatment of wood decay for some time, whereas TBTO, produced by Albright and Wilson, is a more recent treatment chemical and is used mainly in joinery work; both act by the inhibition of oxidative phosphorylation (Lyr, 1961; Johanssen and Hagerby, 1974). Despite the popularity of these two chemicals, there are a number of disadvantages in their use; they are toxic to mammals and therefore require extreme care in handling, they are both degraded with time, by ultra-violet light and they may also be lost from treated timber by volatilisation, although this would be reduced in painted wood. Both are susceptible to biodegradation; PCP will be broken down by soil bacteria (Stranks and Hulme, 1976) and it may also be degraded in the timber by oxidising exo-enzymes produced by fungi, such as laccase, tyrosinase and peroxidase (Lyr, 1962). Actively growing basidiomycetes can debutylate TBTO to its di- and monobutyl derivatives; both these have fungistatic activity, but not so pronounced as that of the tributyl tin. *Coniophora* will degrade TBTO to its dibutyl derivative, whereas *Coriolus versicolor* (a white rot fungus) will reduce TBTO to its monobutyl derivative; this is possibly due to different oxidase systems. Indeed, the tri-, di-, and monobutyl forms have been isolated from the hyphae of *C. puteana*, so breakdown may not be solely extracellular (Henshaw *et al.*, 1978).

Nevertheless, both these compounds are used widely for the preservation of many biodegradable materials, so it is of value to gain some

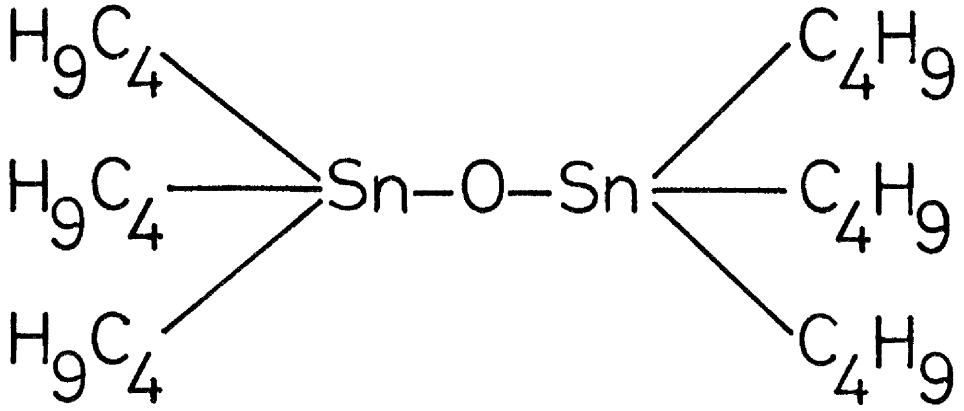
FIGURE 22

Structural formulae of pentachlorophenol (PCP) and tri-n-butyl tin oxide (TBTO).

Pentachlorophenol (PCP)



Tributyltin Oxide (TBTO)



information on the range of toxicity tolerance within the species.

We are interested in intra-specific variation in response, so in order to monitor this, we must standardise the conditions of growth within an easily reproducible test, into which the test chemicals may be introduced at different concentrations, whilst ensuring a homogenous dispersal within the substrate.

In the field situation, extraneous factors may modify the expression of a particular chemical treatment, but we have adopted the approach of measuring the potential effects of these fungicides in controlled conditions which are unlikely to limit their action.

Study of the degree to which fungicidal potential will be limited by other environmental factors, would follow as a logical progression. Such a development would introduce certain difficulties into the system; a laboratory test of great complexity would be needed to mimic the 'in-timber' situation, let alone the field situation, and having achieved such a system, the difference between the natural and the simulated might still be so great as to preclude any useful extrapolation.

Hutchinson (1976), in similar work on *S. lacrymans*, compared radial growth from single inoculae over the surface of 2% malt agar incorporating known quantities of PCP: this method will be used for this work on *Coniophora*.

4.2 Method

Appropriate aliquots of a solution of 0.1% w/v PCP in ethanol were added to 500 ml volumes of 2% malt agar held at 60°C to give final PCP concentrations within the range 0-20 ppm. The same was done with a solution of 0.2% w/v TBTO in acetone, to produce final concentrations within the range 0-35 ppm.

Each batch of treated agar was stirred thoroughly to ensure complete dispersal of the preservative with maximum evaporation of the solvent, and the agar was then dispensed, in 20 ml aliquots, into 9 cm diameter plastic petri dishes. Control plates were prepared which incorporated solvent alone. The plates were inoculated with a number of isolates of *C. puteana* by taking an 8 mm diameter disc from the margin of a growing colony of the fungus (14 days old), and inserting it into an 8 mm diameter well which had been cut in the centre of the treated agar. The plates were incubated at 24°C in the dark, and growth was measured as the increase in colony diameter along two transects drawn at right angles to each other

Table 12(a)

Diameters of colonies grown on agar incorporating PCP, presented as percentages of their equivalent control colony diameters. (Each value represents the mean of 10 measurements.)

Isolate	Days incub-ation	Control colony diameter /mm	Pentachlorophenol/ppm				
			1	5	10	15	20
	3	28	71	0			
11	7	47	83	54	38	28	22
	14	83	93	71	54	40	28
	3	33	77	42	0		
20	7	63	79	51	23	0	
	14	83	100	83	51	34	21
	3	27	64	0			
25	7	54	59	19	25	18	
	14	83	80	39	53	27	0
	3	26	87	52	0		
24	7	53	87	63	29	0	
	14	83	99	90	59	32	0
	3	20	84	58	0		
16	7	35	90	71	53		
	14	51	90	76	59	0	
	3	26	62	0			
17	7	45	72	29	34		
	14	83	87	39	12	0	
	3	19	85	50			
14	7	34	81	59			
	14	68	77	46	0		
	3	26	66	45			
5D	7	45	71	49			
	14	83	76	60	0		
	3	26	64				
5C	7	46	68				
	14	83	76	0			
	3	24	64				
5A	7	43	63				
	14	83	64	0			

Table 12(b)

Diameters of colonies grown on agar incorporating PCP, presented as percentages of their equivalent control colony diameters. (Each value represents the mean of 6 measurements.)

Isolate	Days incub-ation	Control colony diameter /mm	Pentachlorophenol/ppm				
			1	5	10	15	20
	3	14	83	87	0		
2B	7	29	91	72	43	29	
	14	62	95	72	47	35	0
	3	42	91	22	0		
7	7	N.A.	-	-	-	-	-
	14	83	100	89	66	49	0
	3	14	75	0			
6	7	30	80	56	33	0	
	14	62	80	60	34	25	0
	3	12	85	0			
4A	7	22	81	61	43		
	14	40	90	66	45	0	
	3	14	70	0			
4B	7	30	73	44	0		
	14	62	86	60	31	0	
	3	20	73	0			
9	7	57	73	36	0		
	14	83	100	79	0		
	3	15	61	0			
3	7	30	70	38	0		
	14	65	85	57	0		
	3	14	0				
1	7	43	74	31	0		
	14	83	96	60	0		
	3	13					
8	7	22					
	14	43	0				

Table 13(a)

Diameters of colonies grown on agar incorporating TBTO, presented as percentages of their equivalent control colony diameters. (Each value represents the mean of 10 measurements.)

Isolate	Days incub-ation	Control colony diameter /mm	Tri-n-butyl tin oxide/ppm					
			10	15	20	25	30	35
	3	48	21	0				
11	7	83	17	14	12	0		
	14	83	26	18	14	12	13	0
	3	34	0					
24	7	72	14	13				
	14	83	18	15	0			
	3	48	0					
20	7	83	13	12				
	14	83	15	12	0			
	3	21	0					
14	7	42	26	0				
	14	83	18	13	0			
	3	23						
17	7	42	0					
	14	77	13	0				
	3	35						
25	7	70	0					
	14	83	12	0				
	3	30						
5A	7	55						
	14	83	0					
	3	30						
5C	7	52						
	14	83	0					
	3	35						
5D	7	60						
	14	83	0					
	3	25						
16	7	43						
	14	64	0					

Table 13(b)

Diameters of colonies grown on agar incorporating TBTO, presented as percentages of their equivalent control colony diameters. (Each value represents the mean of 6 measurements.)

Isolate	Days incub-ation	Control colony diameter /mm	Tri-n-butyl tin oxide/ppm						
			5	10	15	20	25	30	35
	3	16	79	70	0				
6	7	34	71	59	50	42	39	36	32
	14	71	63	52	44	39	33	30	27
	3	11	100	0					
4A	7	24	74	62	54	49	44	40	0
	14	44	71	59	53	44	42	35	27
	3	15	71	70	0				
2B	7	31	57	52	45	37	34	31	0
	14	61	42	35	33	29	28	25	19
	3	29	52	0					
7	7	81	27	21	14	13	13	12	0
	14	83	49	35	25	20	20	17	15
	3	13	89	0					
4B	7	29	66	57	44	41	37	0	
	14	64	48	39	34	31	27	22	21
	3	15	0						
3	7	40	34	27	24	23	0		
	14	82	25	20	15	15	14	16	15
	3	25	40	0					
9	7	80	26	14	0				
	14	83	39	20	15	0			
	3	14	0						
8	7	31	42	30					
	14	63	32	22	0				
	3	14	0						
1	7	38	28	0					
	14	82	20	15	0				

on the underside of each dish; the measurements were made after 3, 7 and 14 days incubation.

4.3 Results

These are recorded in Appendix 8, Table 11 and summarised in text tables 12 (a and b) and 13 (a and b) where growth on the treated plates is presented as a percentage of that on the control plates.

4.4 Discussion

There is a wide variation in response to TBTO which does not appear to be related to growth rate, as slow growing isolates such as 2B, 4A and 4B are tolerant, whereas 8 and 16 are not; likewise, vigorous growers like 11, 20, 24, 25 and 14 are susceptible to low concentrations of TBTO. It is interesting to note that strain 5 (British standard test strain) as represented by isolates A, C and D, shows very poor tolerance, whilst strain 11, the European standard strain, shows an average tolerance to TBTO.

Pentachlorophenol is less effective at lower concentrations on most of the isolates tested, and variation in response between isolates, is much reduced. Strain 11 stands out in its tolerance of all the concentrations presented to it, but strain 8 is completely susceptible.

Plates 4 and 5 show the colony characteristics of two strains after growth under a range of treatment conditions. It may be noted at this point that the prominent sector formation which is characteristic of *S. lacrymans* in stress conditions (Hutchinson, personal communication; Coggins, 1976), was not to be found in any of the *Coniophora* cultures. We did, however, observe an inhibition of radial growth with a promotion of aerial growth which, in some cases, particularly in the PCP treatments, led to further radial growth. The result of this mode of growth is clearly visible on the treated plates as seen in Plate 4 where a series of very marked concentric rings is evident; the control plates of both series lack this pattern. This peculiar form of growth may be a consequence of the fungus combatting the toxin; aerial growth representing a transient 'holding phase' while the PCP is partially degraded, to allow further radial growth. Liese and Schmidt (1976) carried out some work on *Coniophora* using their ring-dish test (Ringschalentest) and found that the fungus was able to grow over treated

PLATE 4

Growth of two strains of *C. puteana* on agar containing a range of concentrations of pentachlorophenol, after 4 weeks incubation at 24°C in the dark.

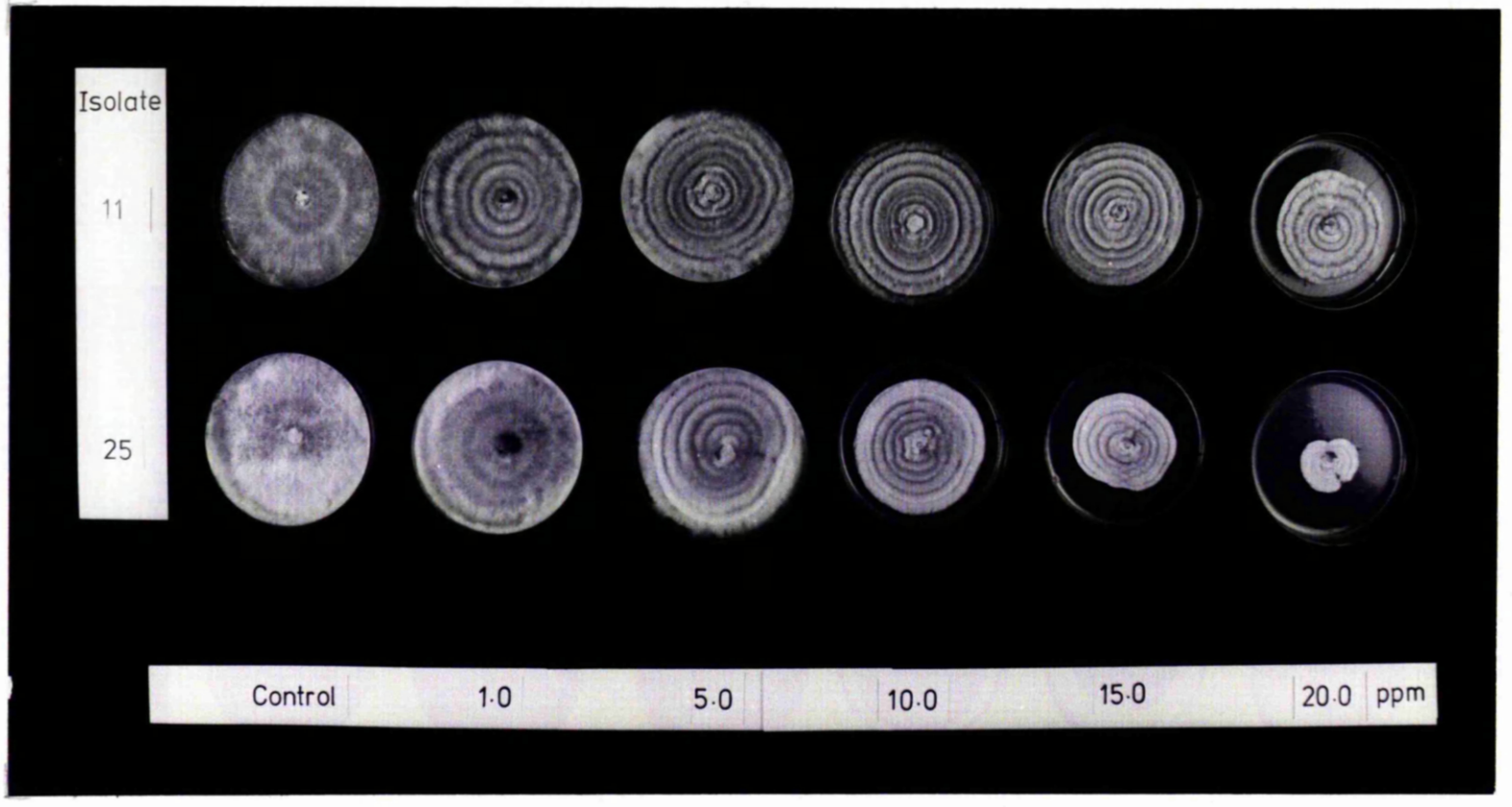
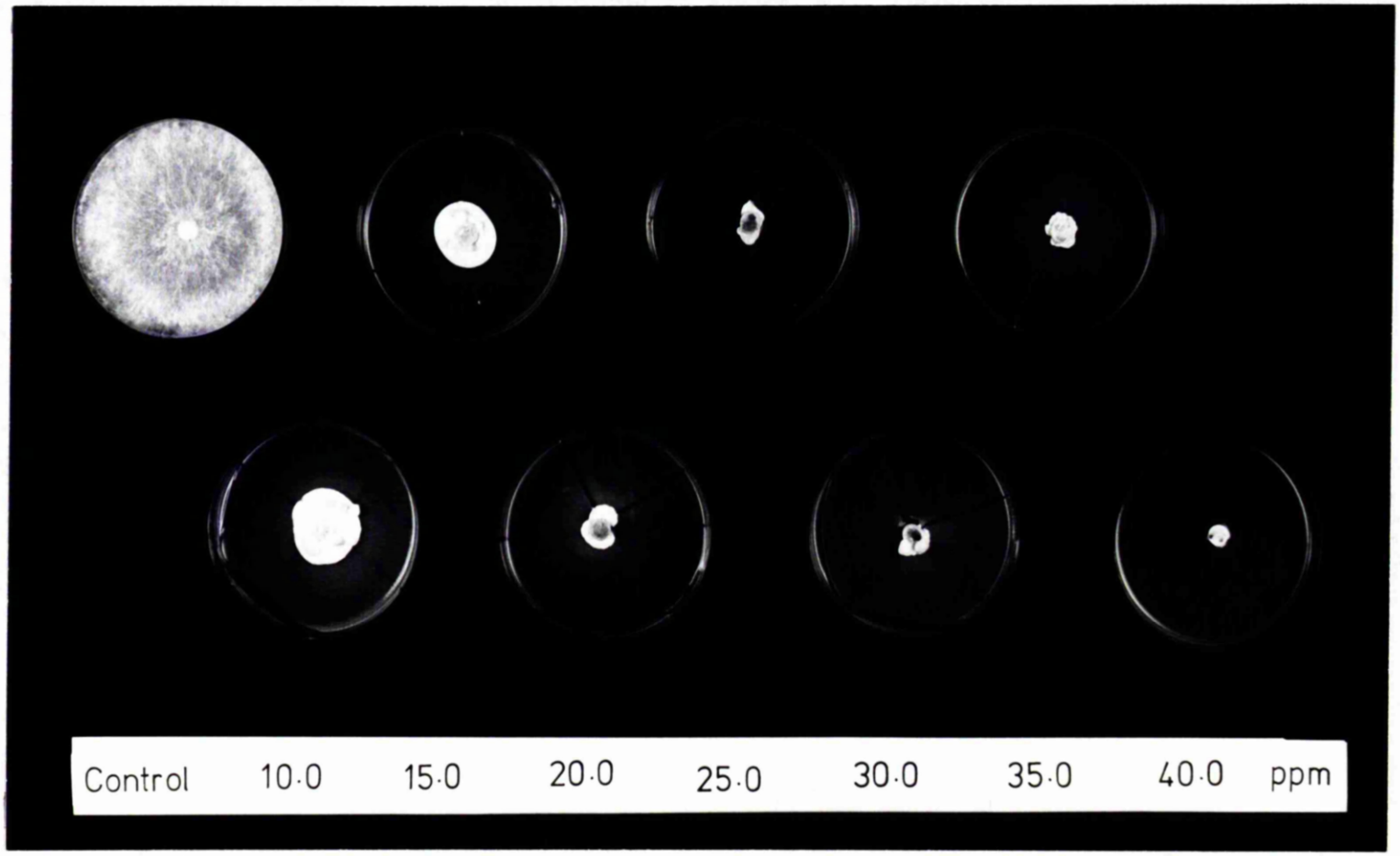

Isolate
11
25
Control
1·0
5·0
10·0
15·0
20·0
ppm

PLATE 5

Growth of strain 11 on agar containing a range of concentrations of tri-n-butyl tin oxide, after 4 weeks incubation at 24°C in the dark.


Control
10·0
15·0
20·0
25·0
30·0
35·0
40·0
ppm

areas of substrate from one untreated area to another, by the formation of strands; evidently the fungus may adapt in a number of ways when encountering adverse conditions.

The colony characteristics on TBTO treated plates (Plate 5) are perhaps similar to those on PCP, but taken to an extreme. The colony produced is very compact, with often an area of diffusion visible in the agar around the colony. Where no growth is possible from the disc of inoculum, rounded-off aerial projections may form which, in many cases, actually overhang the toxic substrate. Often, brown pigmented droplets are produced on the mycelial surface, and in all the cultures, a yellow pigmentation was soon evident; perhaps indicative of a stress situation.

In the most severely inhibited cultures, no growth was possible on the treated agar, but the inoculum was not killed, and by means of these aforementioned aerial growths, a *status quo* appeared to be maintained; longer incubation periods may have revealed some breakdown of the inhibition, but drying out of the agar prevented this.

This type of test may not, as mentioned in the Introduction, have much relevance to the in-timber situation, for instance the energy content of the substrate used in preservative tests can increase the tolerance of a fungus to the chemical (Schmidt and Ziemer, 1976; Liese and Schmidt, 1976), but it has been a quick and relatively straightforward method of revealing a wide diversity in response within the species. Gersonde (1958a) was aware of the inherent dangers in the use of standard strains, and our results prompt the question: with strain 5 showing such a low tolerance to both PCP and TBTO, does its use as a standard strain in preservative tests provide a representative estimate of the susceptibility of the species.

CONCLUSIONS

CONCLUSIONS

The work described in this thesis permits the following conclusions to be made:-

(1) There is a wide variation among isolates of *C. puteana* in their ability to decay *P. sylvestris* sapwood under the test conditions described.

(2) The rate of increase in colony radius of the fungus, varies substantially, between strains, but this variation does not show a consistent correlation with the variation in decay-potential.

(3) Inter-strain variation is shown in the effect of temperature on radial growth.

(4) The pattern of cell-free proteins, varies in response to the substrate, and possibly also between strains; the C_1 component of the cellulase complex is inducible.

(5) The fungus produces at least two C_x enzymes.

(6) Carboxymethylcellulase (C_x) activity does not correlate with decay-potential.

(7) There is a diversity of response to low concentrations of tri-n-butyl tin oxide (TBTO) and pentachlorophenol (PCP).

These studies might be profitably continued along the following lines:-

(1) Walchli (1976) found differences in the levels of decay of a range of wood species by *C. puteana*, and this may be extended to look at the decay produced by a number of isolates on a range of commercially important woods. The patterns of variation obtained, may be quite different to that obtained on *P. sylvestris* sapwood.

(2) It would be of value to establish the flexibility of the species in response to temperature. Preliminary studies described in this thesis, have shown that there are appreciable differences between strains in their growth at different temperatures.

(3) Croall (1978) found significant differences between strains in their growth on wood held at different relative humidities (RH). RH is an important environmental factor, and a knowledge of its effect on variations in decay-potential, would be most useful. Closely

associated with RH, is substrate water content, and the effect of this on growth by strains of *C. puteana*, might be further examined. Any differences between strains in their ability to 'self-wet' the wood, will be of particular importance.

(4) Although we found no differences in C_x production, and no correlation with decay-potential, the cellulase analysis could be improved by a revision of the assay for C_1 activity. Also, the resolution of separation of the cellulase components would be vastly improved by gel-filtration on a wider range of Sephadex types, followed by ion-exchange chromatography.

As mentioned at the end of section 3.2.5, the work on extracellular protein secretions indicated strain differences, and this is likely to be where significant factors contributing to decay-potential, will be. Therefore, it would be profitable to assay for a wider range of enzymes (see text table 2). Analysis of proteins produced by isolates, would be greatly enhanced by the use of PAG electrophoresis followed by isoelectric focussing.

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APPENDICES

APPENDIX 1

Cultural data and origins of isolates of *C. puteana* used in this study

Isolate No.	Origin	Locality	Date of Collection
1	From Madison via Rabanus. Received from Building Research Establishment, Bucks. No. 11A.	U.S.A. (Wisconsin)	Isolated pre 1947 Received 15.7.75
2A	From J. Liese (his race No. 15). Received from B.R.E., Bucks. No. 11B.	Germany	Isolated Nov. 1945 Received 15.7.75
2B	From J. Liese (his race No. 15) (see also 2A above). Received from Centraalbureau voor Schimmelcultures, Baarn. Acc. no. CBS 126.45.	Germany	Isolated Nov. 1945 Received 24.2.76
3	Rot isolate, on Black Cherry (*Prunus serotina*) log, Westline, Camp Run, Kane, Pennsylvania. Collected by Campbell and Davidson. Received from U.S.D.A. Forest Products Lab., Madison. Acc. no. FP-94042-R.	U.S.A. (Pennsylvania)	Isolated 12.9.40 Received 21.1.76
4A	Idaweiche strain from R. Falck. Received from B.R.E., Bucks. No. 11D.		Isolated June 1932 Received 6.8.75
4B	'Idaweiche' strain from R. Falck. Received from C.B.S., Baarn. Acc. no. CBS 148.32.	W. Germany (Hann-Münden)	Isolated June 1932 Received 24.2.76
5A	From sporophore on *Cupressus* pole, Princes Risborough, Bucks. British Standard Test Strain since 1953. Received from B.R.E., Bucks. No. 11E.	U.K. (Bucks.)	Isolated 1950 Received 15.7.75
5C	B.S.I. test strain 11E, held by 'Cuprinol'. Received from B.R.E., Bucks.	U.K. (Bucks.)	Isolated 1950 Received Jan. 1976

Isolate No.	Origin	Locality	Date of Collection
5D	B.S.I. test strain 11E, held by Penarth Research Labs. Received from B.R.E., Bucks.	U.K. (Bucks.)	Isolated 1950 Received Jan., 1976
6	Lohwag. Received from B.R.E., Bucks. No. 11F.	Unknown	Received Jan. 1976
7	From decayed board, Princes Risborough, Bucks. Received from B.R.E., Bucks. No. 11G.	U.K. (Bucks.)	Isolated 1954 Received 15.7.75
8	From sporophore in house. Received from B.R.E., Bucks. No. 11H.	Unknown	Received Jan. 1976
9	On Redwood (*Sequoia sempervirens*) post size test specimen. Madison, Wisconsin. Collected by Pane. K. Blew. Received from U.S.D.A. Forest Products Lab., Madison. Acc. no. MD-382.	U.S.A. (Wisconsin)	Isolated 2.11.59 Received 21.1.76
10	From G. Becker as BAM (Ebw) No. 15. CEN Test Strain. Received from B.R.E., Bucks. No. 11J.	E. Germany (Eberswalde)	Received 24.6.75
11	From G. Becker as BAM (Ebw) No. 15 to replace B.R.E. 11J which had changed. No. 11K.	E. Germany (Eberswalde)	Received 7.7.75
12	Polysporous isolate, on Aspen (*Populus* sp.) log, White River National Forest, Colorado. Collected by Davidson, Hawksworth and Hines. Received from U.S.D.A. Forest Products Lab. Acc. no. FP-100264-Sp.	U.S.A. (Colorado)	Isolated 5.8.51 Received 21.1.76
13	Received from U.S.D.A. Forest Products Lab. Acc. no. FP-104419-Sp.	U.S.A.	Received 21.1.76
14	Polysporous isolate, from sporophore on Chestnut (*Castanea* sp.) board in used lumber pile, Lombard Farm, Etchison, Montgomery County, Maryland. Collected by F.F. Lombard.	U.S.A. (Maryland)	Isolated 11.11.57 Received 21.1.76

Isolate No.	Origin	Locality	Date of Collection
15	Polysporous isolate, on standing dead Pine (*Pinus* sp.), Fox State Park, Hillsborough, New Hampshire. Collected by Davidson, Mook and Eno. Received from U.S.D.A. Forest Products Lab. Acc. no. FP-105438-Sp.	U.S.A.	Isolated 25.9.59 Received 21.1.76
16	Received from Centraalbureau voor Schimmelcultures, Baarn.	Netherlands	Received 24.2.76
17	From floorboards in Leiden. Received from C.B.S., Baarn. Acc. no. CBS 132.71.	Netherlands	Isolated 3.6.58 Received 24.2.76
18	Isolated by M.A. van Beyma from a cellar in Wageningen. Received from C.B.S., Baarn. Acc. no. CBS 154.28.	Netherlands	Isolated Feb. 1928 Received 24.2.76
19	Isolated from decayed roof timber in whisky bond warehouse, 244 Speres Wharf North, Glasgow.	U.K. (Glasgow)	Isolated 25.2.76
20	Isolated from decayed skirting board underneath paint. 57 Rosebery St., Glasgow G5.	U.K.(Glasgow)	Isolated 12.3.76
21	Isolated, F.F. Lombard from oak (*Quercus* sp.) planking. Strain Madison 515. American Type Culture Collection No. 12675. B.R.E. No. 11M.	U.S.A.	Received 8.4.76
22	Received from Royal College of Forestry, Stockholm. Acc. no. 74450-2.	Sweden	Received May 1976
23	Polysporous isolate from a sporophore on dead wood of *Alnus incana*. Böda, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 71 535.	Sweden (Öland)	Isolated 23.10.71 Received May 1976

Isolate No.	Origin	Locality	Date of Collection
24	Polysporous isolate from a sporophore on dead wood of *Betula alba*. Ottenby, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 70 444.	Sweden (Öland)	Isolated 29.9.70 Received May 1976
25	Polysporous isolate from a sporophore on dead wood of *Corylus avellana*. Ismanstorp, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 71 454.	Sweden (Öland)	Isolated 20.10.71 Received May 1976
26	Polysporous isolate from a sporophore on dead wood of *Betula alba*. Ottenby, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 70 432.	Sweden (Öland)	Isolated 29.9.70 Received May 1976
27	Polysporous isolate from a sporophore on dead wood of *Quercus robor*. Kullen, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 74 479.	Sweden (Öland)	Isolated 11.11.74 Received May 1976
28	Polysporous isolate from a sporophore on dead wood of *Quercus robor*. Homrevet, Grankullaviken, Öland. Received from Royal College of Forestry, Stockholm. Acc. no. 71 428.	Sweden (Öland)	Isolated 19.10.71 Received May 1976
29	Polysporous isolate from a sporophore on dead wood of *Picea abies*. Vännäs, Västerbotten. Received from Royal College of Forestry, Stockholm. Acc. no. 71 353.	Sweden (Västerbotten)	Isolated 9.10.71 Received May 1976
30	Received from Royal College of Forestry, Stockholm. Acc. no. 72 079.	Sweden	Received May 1976.
31	Isolated by Prof. Zycha of Inst. f. Angew. Mykologie und Holzschutz, Hannover. Received from Eidgenössische Materialprüfungs und Versuchsanstalt, St. Gallen, Switzerland. Acc. no. EMPA 62.	Germany	Isolated 1953 Received Jan. 1977

Isolate No.	Origin	Locality	Date of Collection
32	Isolated by drilling decayed wood. Received from Eidgenössische Materialprüfungs und Versuchsanstalt, St. Gallen. Acc. no. EMPA 214.	Switzerland	Isolated 1968 Received Jan. 1977
33	Isolated by Frau. Vetsch from wood slat. Received from Eidgenössische Materialprüfungs und Versuchsanstalt, St. Gallen. Acc. no. EMPA 252.	Switzerland	Isolated 8.10.70 Received Jan. 1977

APPENDIX 2

Determination of a convenient incubation temperature for *C. puteana*

Introduction

Previous workers have described different temperatures for optimal growth of *C. puteana* (see General Introduction); some of these differences may be due to their use of different strains. The following experiment was done to determine an incubation temperature which will be convenient for this study, using three representative strains.

Method

Eight millimetre diameter discs were cut from the margins of 14 day old colonies of strains 1, 5A and 11 growing on 2% malt agar in 9 cm diameter petri dishes, and placed centrally on the surface of 20 ml aliquots of 2% malt agar in petri dishes. Each strain was used to inoculate sixty dishes, and ten dishes of each strain were incubated in the dark at the following temperatures: 18°, 20°, 22°, 24°, 26° and 28°C. Radial growth was measured on the sixth day of incubation, along a transect which had been marked on the underside of each dish, prior to incubation; thereafter, growth was measured daily for a further eight days.

Results

These are recorded in Appendix table 1, the mean growth at three of the temperatures tested, is summarised in Appendix figure 1.

Discussion and Conclusion

The results show that all three strains grow well at a temperature of 24°C, and that this will be convenient for our purposes.

It can also be seen that there are differences between the strains in their growth at different temperatures. For instance, the growth of strain 11 is retarded more by lowering the temperature than by raising it, but the other two strains show the opposite response; this may be worth pursuing at some later stage.

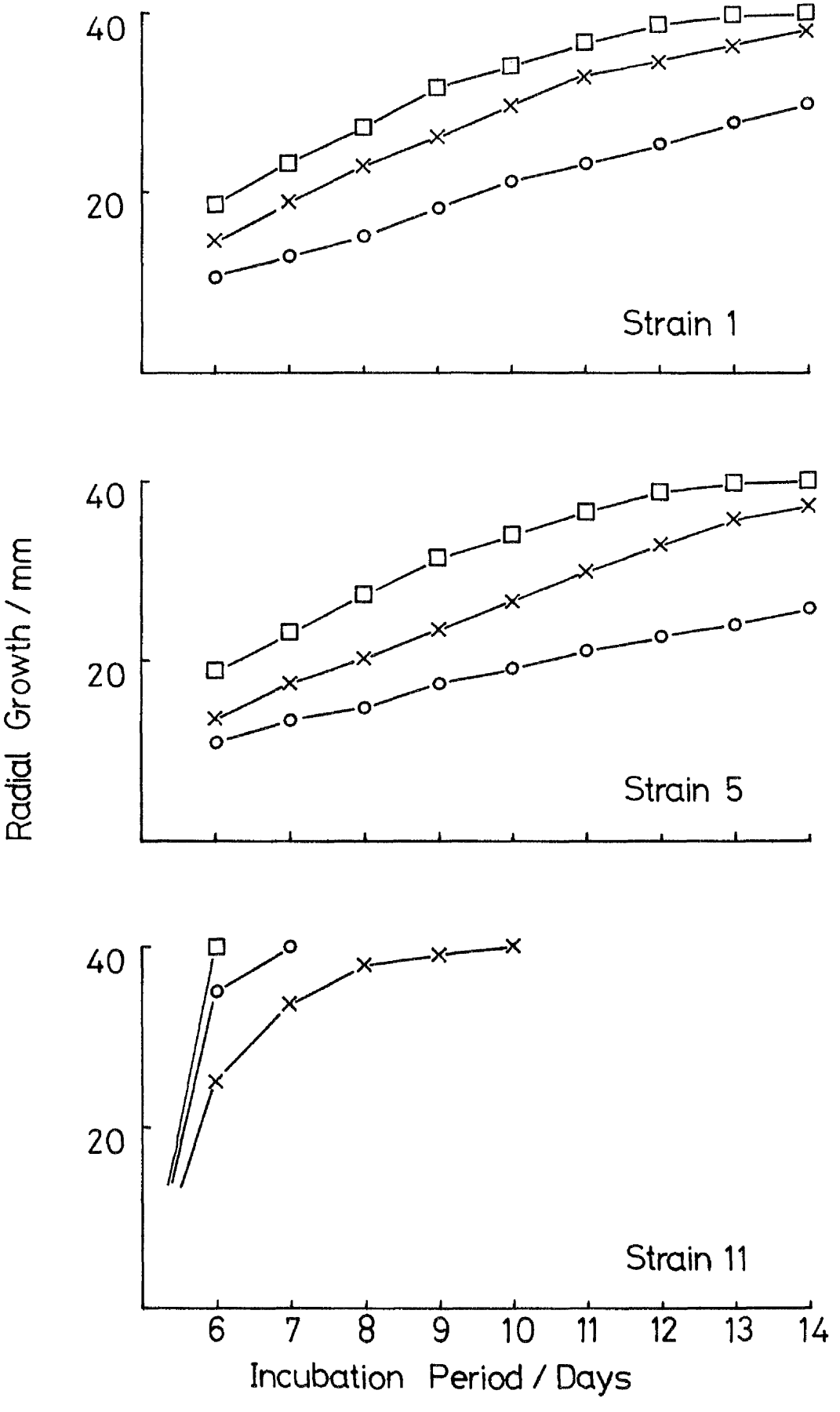
Table 1. Increase in colony radius (mm) of 3 strains of *C. puteana* after incubation under a range of temperatures in the dark. (Each value represents the mean of 10 measurements with its standard error beneath it.)

		Days Incubation								
Strain	T°C	6	7	8	9	10	11	12	13	14
1	18	12.9 0.76	17.1 0.94	20.9 1.07	24.6 1.50	28.2 1.67	31.8 1.78	34.4 1.53	36.0 1.16	38.2 0.75
	20	14.6 1.15	19.0 1.16	22.9 1.50	26.3 1.56	29.6 1.67	32.8 1.71	34.4 1.51	36.3 1.30	38.0 1.06
	22	17.5 0.98	21.9 1.24	26.3 1.28	31.1 1.61	34.4 1.28	37.1 0.98	39.1 0.51	40 -	40 -
	24	18.8 1.33	23.3 1.52	27.4 1.79	31.6 2.05	34 1.67	36.6 1.25	38.8 0.79	39.5 0.36	40 -
	26	16.5 0.93	20.5 1.17	23.5 1.40	26.9 1.69	32.9	32.9 1.62	34.9 1.20	36.9 0.86	38.8 0.46
	28	10.5 0.28	12.9 0.29	15.1 0.37	18.1 0.41	20.9 0.41	23.3 0.46	25.2 0.52	27.7 0.47	29.9 0.54
5A	18	13.5 0.63	17.6 0.98	20.5 1.0	23.4 1.16	26.4 1.28	30.4 1.34	33.2 1.36	36 1.41	37.8 1.11
	20	13.4 0.50	17.4 1.01	20.2 1.15	23.6 1.20	26.6 1.40	30 1.50	33.3 1.71	35.7 1.85	37.2 1.65
	22	17.9 0.78	21.8 0.86	25.5 0.91	28.8 1.0	32.3 1.09	36.0 1.20	38.2 0.81	40 -	40 -
	24	18.8 1.33	23.2 1.52	27.4 1.79	31.6 2.1	34 1.67	36.6 1.25	38.8 0.79	39.6 0.36	40 -
	26	15.6 0.66	20.4 0.61	23.4 0.75	27.3 0.98	33 1.14	35.4 1.31	37.1 0.96	38.9 0.55	40 -
	28	10.7 0.46	13.2 0.66	14.8 0.66	17.4 1.01	21 0.97	19 0.96	22.6 1.10	24 1.18	25.9 1.23
11	18	23.5 0.48	28.8 0.64	33.2 0.83	38.8 0.54	39.8 0.21	40 -	(Max growth)		
	20	25.5 1.60	33.7 2.10	37.9 1.48	38.9 0.78	40 -				
	22	35.3 0.70	40 -							
	24	40 -								
	26	40 -								
	28	35.1 1.02	40 -							

APPENDIX FIGURE 1

Mean growth rates of three strains of *C. puteana* at the following temperatures:

X—X	20°C
□—□	24°C
O—O	28°C


40
20
Strain 1
40
20
Strain 5
40
20
Strain 11
Radial Growth / mm
6
7
8
9
10
11
12
13
14
Incubation Period / Days

APPENDIX 3

An examination of the mode of operation of the Tensometer

Introduction

The Hounsfield tensometer may be operated by hand or by motor; this experiment was performed in order to compare the results obtained by each method of operation and to ascertain whether one has an advantage over the other.

Method

Sixty *P. sylvestris* sapwood test pieces (6.0 x 1.0 x 0.5 cm) were chosen for their grain uniformity, dried to constant weight at 105°C and allowed to cool in a vacuum desiccator. They were split randomly into two batches of 30 pieces each, and the cross-grain breaking strengths of one batch were measured on the manually driven tensometer, and those of the other batch were measured on the motor driven tensometer. The results were analysed by Student's t test.

Results

Table 2. Cross-grain breaking strengths/Newtons

Manual Drive	Motor Drive
390, 350, 340, 300, 320	340, 380, 360, 455, 320
410, 365, 295, 375, 345	335, 355, 335, 340, 380
400, 410, 325, 345, 385	335, 340, 360, 335, 305
430, 340, 340, 350, 355	375, 365, 390, 360, 400
315, 410, 355, 345, 355	365, 425, 320, 355, 425
360, 460, 375, 355, 345	375, 295, 355, 320, 360
$\bar{x}$ = 361.5	$\bar{x}$ = 358.7
S.E. = 6.96	S.E. = 6.61

With 58 degrees of freedom t = 0.18 is not significant at the 5% level.

Conclusion

There is no significant difference between the two methods of operation.

APPENDIX 4

Determination of a suitable incubation period for wood decay by *C. puteana*

Introduction

The following experiment was carried out to determine a convenient incubation period for the main survey of decay-potential.

Method

Sixty rectangular bottles (60 x 60 x 190 mm) were prepared by the method described in Part 2, section 2.1.2. They were all inoculated with *C. puteana* strain 5A, and incubated for 14 days at 24°C in the dark. Five strips of *P. sylvestris* sapwood were then inserted into each after wetting beyond 27% of their dry weights, and incubation was continued under the same conditions. After four weeks, ten bottles were harvested at convenient intervals. The wood pieces were removed, cleaned, dried to constant weight, and their cross-grain breaking strengths were measured.

Results

These are recorded in Appendix table 3 and summarised in Appendix figure 2.

Discussion

Although there is some weight loss, correlating with loss in cross-grain breaking strength, the alteration in value of both parameters is very small, even after a substantial incubation period. It appears from Graph A in figure 2, that decay was delayed after 8 weeks' incubation until a sudden surge after 11 weeks. These results, however, are subject to fairly wide variation, and if the water contents of the wood pieces after decay are analysed, two groups can be separated, namely, those with water contents below 100%, and those with water contents greater than 100%. The mean decay levels for each group are plotted in Graph B, and it is clear that those pieces of wood with low water contents have been subjected to a steady increase in decay over the twelve weeks of incubation. Conversely, those samples in the high water content group have suffered much less decay. These correlations are consistent throughout

Table 3. % loss in dry weight (Wt. Loss), cross-grain breaking strength (BS) and % water content (WC) of *P. sylvestris* sapwood test pieces after decay by *C. puteana* strain 5A. (Each value is the mean of 5 measurements per bottle.)

Weeks Incubation	Parameter	Bottle Number										$\bar{x}$	S.E.
		1	2	3	4	5	6	7	8	9	10		
4	Wt. loss	2.1	2.5	5.0	1.1	3.3	2.2	5.8	0.9	4.9	2.4	3.02	0.56
	B.S.	393	440	370	417	427	403	354	429	382	384	399.9	9.37
	W.C.	136	135	108	149	119	141	123	151	108	140	131	5.23
6	Wt. loss	2.4	1.0	4.2	3.8	4.4	4.5	2.2	2.9	5.0	4.6	3.5	0.44
	B.S.	368	394	360	354	374	345	468	338	368	323	369.2	13.34
	W.C.	154	151	125	138	135	128	129	138	107	128	133	4.48
8	Wt. loss	4.7	3.9	11.0	5.0	7.5	3.0	7.2	5.1	5.8	5.6	5.9	0.75
	B.S.	326	411	258	322	279	387	324	342	342	332	332.3	14.89
	W.C.	129	142	113	137	125	136	118	137	129	136	130	3.09
11	Wt. loss	6.2	4.4	3.7	9.0	8.4	6.0	6.4	4.3	3.0	5.9	5.73	0.65
	B.S.	338	403	348	298	296	295	366	409	374	308	344	14.60
	W.C.	148	144	151	136	130	137	131	131	142	143	139	2.48
12	Wt. loss	8.5	8.1	9.3	7.1	5.2	19.0	5.6	10.0	10.8	5.7	8.93	1.34
	B.S.	291	276	337	310	382	257	334	309	235	400	313.1	17.37
	W.C.	145	143	114	138	144	86	149	125	131	135	131	6.33
14	Wt. loss	6.8	13.0	5.3	5.7	10.0	9.4	8.7	5.4	-	-	8.04	1.03
	B.S.	319	341	343	330	330	323	281	305	-	-	321.5	7.69
	W.C.	147	132	146	144	140	137	150	145	-	-	142.6	2.23

APPENDIX FIGURE 2

Graph A

Mean % weight loss and reduction in cross-grain breaking strength of test pieces of *P. sylvestris* after decay by *C. puteana* (5A) for different incubation periods.

O——O Mean % weight loss

O---O Cross-grain breaking strength

Graph B

Mean % weight loss and mean water contents of test pieces of *P. sylvestris* after decay by *C. puteana* for different incubation periods.

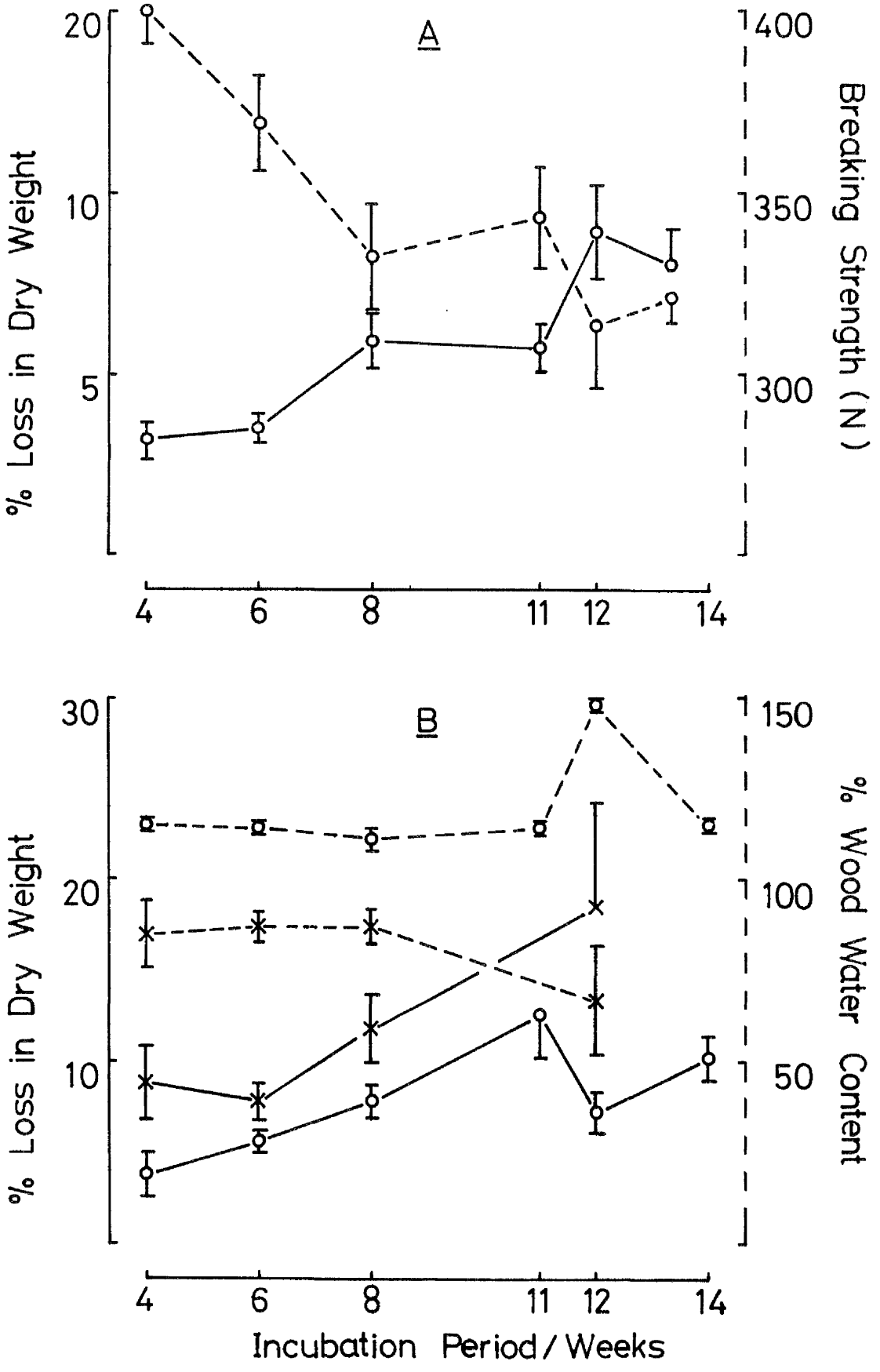
O——O Mean % weight loss of test pieces with water contents greater than 100%

X——X Mean % weight loss of test pieces with water contents less than 100%

O---O Mean wood water contents greater than 100%

X---X Mean wood water contents less than 100%

[The bars in both graphs, represent standard error; on occasion, one side only is shown in order to avoid overlap.]


A
20
10
5
% Loss in Dry Weight
1400
1350
1300
Breaking Strength (N)
4
6
8
11
12
14
B
30
20
10
% Loss in Dry Weight
150
100
50
% Wood Water Content
4
6
8
11
12
14
Incubation Period / Weeks

University of Glasgow

To Dr. Hutchinson & Dr. Elliott ✓ Date 6.11.78

From Prof. Wilkins

I have arranged for the oral examination of Alistair McPhee's thesis to be held at 2 p.m. on Monday 27 November 1978 in the Conference Room.

pp M. B. Wilkins.

26th October, 1978

Application by: Alistair Barlow McPHEE

A copy of the thesis has been sent to the Additional Examiner, who is:

Dr. F.T. Last,
Institute of Terrestrial Ecology,
Penicuik

The Special Committee is : Professor M.B. Wilkins (Convener)
Dr. C.G. Elliott
Dr. S.A. Hutchinson

both groups. It therefore appears probable that the low decay values are at least partly due to waterlogging of the wood.

Conclusion

These results indicate that this technique does not support good growth and decay by *C. puteana*. It is unlikely that useful information can be obtained in less than eight weeks' incubation, and even after this period, the decay levels may not be sufficient to reveal small differences between isolates.

APPENDIX 5

Modification of the method used for the decay of wood, in order to provide increased control of wood water

Introduction

The low levels of decay which were obtained in the work described in Appendix 4 emphasise the need for a modification of the agar method used in order to provide a better control of wood water content during incubation.

Improvement was sought in two ways; (a) by examining the effect of raising the wood test pieces above the agar surface on glass rods, so eliminating direct absorption of water from the agar, and (b) by the use of vermiculite.

Vermiculite is a heat processed porous mineral mica which is used for insulation purposes; it has also been used to wet Scots pine sapwood test pieces to various equilibrium moisture contents (Carey and Grant, 1975), and having no nutritional value, it is a useful substitute for soil in standard decay tests (Henningsson, 1977). The use of vermiculite might overcome the waterlogging problem in two ways: by replacing the agar as a water and nutrient holding material and by absorbing excess water produced during incubation as a result of fungal respiration. Both these requirements could be met by using vermiculite which has a very high water holding capacity (WHC) and will absorb and retain water readily. The WHC is defined as that volume of water which is required to saturate the particles of material completely; substantial water uptake occurs beyond WHC, presumably by inter-grain capillarity which will involve the retention of water effectively outside the vermiculite particles.

(1) The use of agar with glass supports

Method

100 ml of 2% malt agar were poured into each of ten bottles (60 x 60 x 190 mm), and after stoppering with cotton wool, these were autoclaved at 120°C for twenty minutes and allowed to cool on their sides. Each bottle was then inoculated with six 8 mm diameter discs of *C. puteana*, strain 5A, and incubated for 14 days at 24°C in the dark.

At the end of this period two 15 cm lengths of glass rod, previously flamed, were placed onto the fungal mat growing in each bottle, so that

Table 4. Weight losses and water contents of *P. sylvestris* test pieces supported on glass rods over agar, after attack by *C. puteana*.

Incubation period	Bottle	% wt. loss of test pieces	$\bar{x}$	S.E.	% water content of test pieces after decay	$\bar{x}$	S.E.
6 weeks	1	17, 26, 25, 23, 4	18.7	4.5	69, 64, 66, 76, 114	77.4	9.3
	2	11, 4, 10, 14, 0	7.6	2.8	66, 76, 71, 68, 61	68.4	2.5
	3	2, 22, 12, 11, 0	9.4	4.4	60, 71, 65, 67, 65	65.6	1.8
	4	4, 12, 6, 0, 5	5.3	2.2	65, 67, 66, 67, 73	67.6	1.4
	5	0, 16, 0, 11, 0	5.4	3.8	70, 73, 68, 77, 60	69.6	2.8
			9.28	2.47		69.7	2.0
12 weeks	6	40, 37, 45, 36, 28	37.2	2.8	88, 91,100, 87, 84	90.0	2.7
	7	31, 39, 36, 35, 42	36.6	1.9	80, 87, 84, 81, 85	83.4	1.3
	8	39, 36, 46, 32, 36	37.8	2.3	92, 87, 94, 90, 88	90.2	1.3
	9	48, 34, 20, 35, 27	32.8	4.7	98, 91, 86, 83, 92	90.0	2.6
	10	47, 37, 62, 41, 50	47.4	4.3	128, 78, 96, 76, 83	92.0	9.6
			38.4	2.41		89.1	1.5

they were parallel to each other with roughly a 4 cm distance between them. Five pieces of *P. sylvestris* sapwood, moistened and autoclaved as described previously were then placed across the glass rods in each bottle so that they were supported above the agar. The bottles were then incubated under the same conditions, and five were harvested after six weeks, the remainder being harvested after twelve weeks. Decay assessment was as described previously.

Results

These are presented in Appendix table 4.

Discussion

Reasonably high levels of decay were obtained after twelve weeks' incubation, but not after six weeks. Wood water levels increased, but only gradually, and were mostly below 100%. The small increase may have been due to respiratory water, over which there would be little control, or by a wick action to the wood from the agar via connecting fungal strands.

There was considerable practical difficulty in the arrangement of the glass rods and in the manipulation of the test pieces on them; this was compounded by the danger of the test pieces being dislodged when handling the bottles during incubation.

(2) The use of vermiculite

(i) An examination of the effect of vermiculite water content on the wetting of wood

Method

There are several types of vermiculite available and the water holding property of each differs, therefore a reference standard must be established to permit comparison of results and repetition of experiments. The water holding capacity (WHC) was chosen as it has commonly been used by other workers, and the water content of the material can be adjusted to various percentages of its WHC.

Three replicate 15 g samples of dry vermiculite (Hobden Davis Ltd), were soaked in an excess of distilled water. The water was then filtered out through Whatman filter paper (GP grade) under partial vacuum in a Büchner funnel for ten minutes. The remaining water contents of the

vermiculite samples were determined, and the mean of the three values was taken to be the water content of the vermiculite at its water holding capacity.

Batches of vermiculite were prepared by adding sufficient quantities of water to produce water contents of 25%, 50%, 75%, 100%, 125%, 150% and 225% of the WHC. Five Erlenmeyer flasks (250 ml) were prepared for each of the water levels, each containing vermiculite with two pieces of *P. sylvestris* sapwood embedded in it (these had previously been dried to constant weight and cooled in a desiccator). The flasks were stoppered with cotton wool and autoclaved at 120°C for thirty minutes. The experiment using the 225% level was carried out later, in bottles containing five pieces of wood embedded in vermiculite. After being left to equilibrate for three days at 24°C, the test pieces were removed, cleaned of adhering vermiculite, and weighed immediately, their equilibrium water contents were then calculated.

Results

These are recorded in Appendix tables 5a and 5b and summarised in Appendix figure 3.

Discussion

These results show a steady, almost linear rise in wood water content with increasing vermiculite water content, with the values at each level being suitably consistent.

The broken line in figure 3 represents those results which were obtained by Carey and Grant (1975), using *P. sylvestris* sapwood and vermiculite. The wood water contents which they obtained up to a vermiculite water level of 100% WHC, are similar to those from this experiment, but beyond 100%, the wood they used increased its water content dramatically. They suggest that this sudden increase is due to free water being available in the vermiculite above 100% WHC. However, the results which they obtained with standard hardboard test pieces, resemble our results with no rapid rise in wood water content.

Our results may differ from theirs for a number of reasons: differences in wood samples, or in incubation temperature, or it may be due to differences in test piece orientation in the vermiculite (our pieces were buried horizontally whereas theirs were buried vertically). The ratio of volume to surface area is similar between the two sets of test pieces, but the

Table 5a. % water contents of *P. sylvestris* test pieces after embedding in vermiculite at various water contents.

% WHC	% water content of test pieces	$\bar{x}$	S.E.
25	25, 27, 27, 25, 27, 27, 25, 25, 24, 26	25.8	0.36
50	30, 31, 31, 30, 30, 32, 32, 28, 31, 32	30.7	0.40
75	32, 36, 34, 34, 35, 32, 38, 35, 33, 33	34.2	0.59
100	37, 35, 41, 36, 42, 42, 45, 43, 40, 39	40.0	1.02
125	43, 50, 42, 44, 42, 44, 38, 47, 45, 53	44.8	1.36
150	54, 53, 61, 57, 54, 54, 51, 56, 57, 56	55.3	0.87

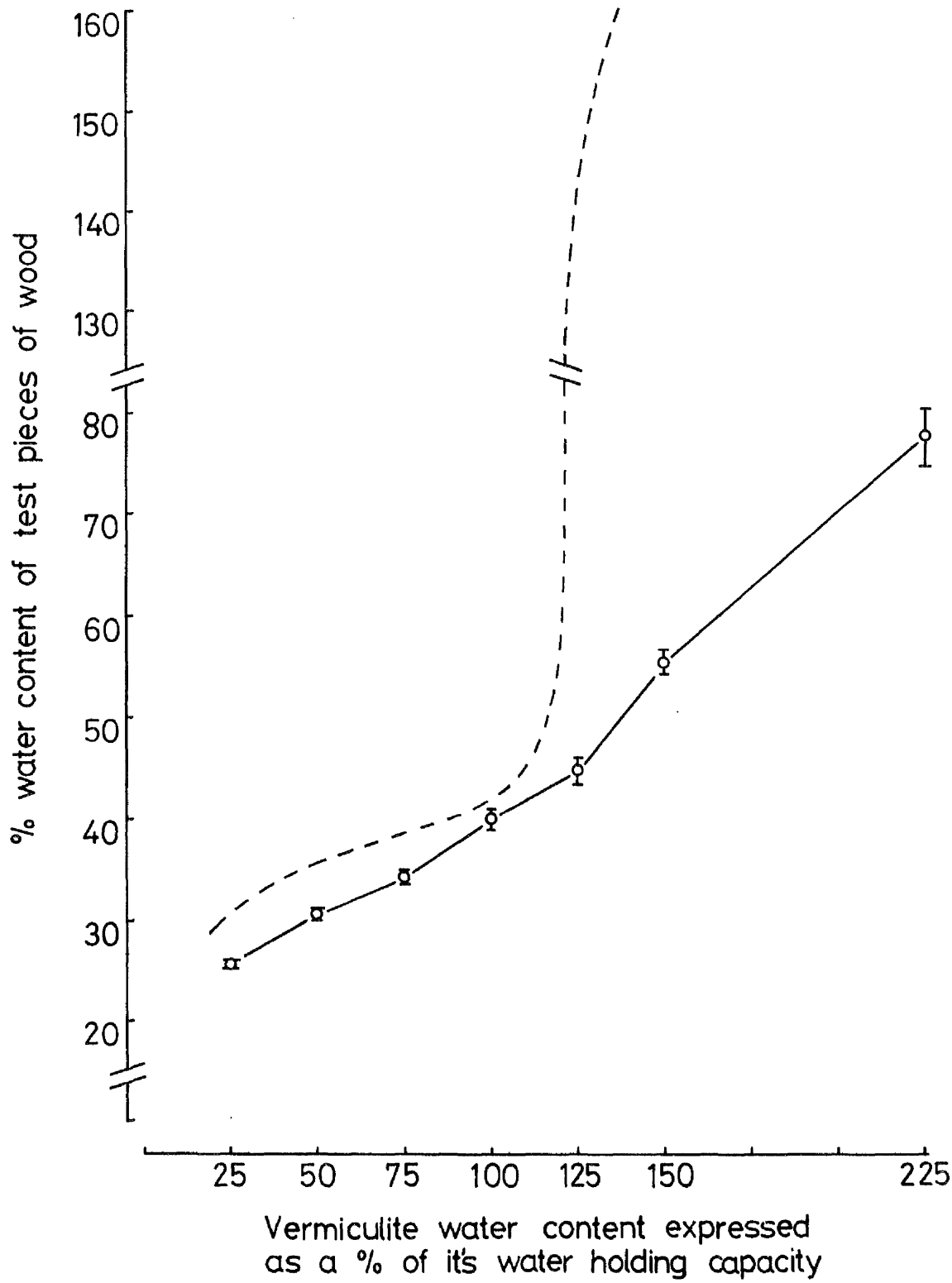
Table 5b. % water contents of *P. sylvestris* test pieces after embedding in vermiculite at 225% WHC.

Bottle	% water content of test pieces	$\bar{x}$	S.E.
1	70, 80, 66, 61, 76	70.6	3.4
2	68, 87, 96, 69, 85	81.0	5.4
3	96, 74, 74, 89, 73	81.2	4.7
4	92, 79, 96, 116, 60	88.6	9.3
5	81, 62, 81, 57, 78	71.8	5.1
		78.6	3.33

APPENDIX FIGURE 3

O——O The mean equilibrium water content of *P. sylvestris* sapwood test pieces after burial in vermiculite, at different moisture levels, for 3 days at 24°C. Each point represents the mean of the replicates with associated standard error.

--- The results of a similar experiment adapted from Carey and Grant (1975).


160
150
140
130
80
70
60
50
40
30
20
% water content of test pieces of wood
25
50
75
100
125
150
225
Vermiculite water content expressed
as a % of it's water holding capacity

fact that our pieces presented a larger area nearer the surface of the vermiculite, may have led to reduction in water uptake or an increase in water loss.

(ii) Decay of wood in vermiculite

Method

30 g of dry vermiculite were poured into each of ten bottles (60 x 60 x 190 mm), and a volume of 10% malt extract solution was added to provide the vermiculite with a water content of 150% WHC; the bottles were laid on their sides, and the vermiculite spread out evenly. After being stoppered with cotton wool, the bottles were autoclaved at 120°C for twenty minutes, after which each was inoculated with six 8 mm diameter discs of *C. puteana* strain 5A and incubated for 14 days at 24°C in the dark.

Pieces of *P. sylvestris* sapwood, previously dried to constant weight, were allowed to equilibrate for three days in vermiculite soaked to 150% WHC; they were then removed, placed in a stoppered conical flask and autoclaved at 120°C for thirty minutes. Five pieces of wood were inserted into the vermiculite in each bottle and the incubation was allowed to continue under the same conditions. Five bottles were harvested six weeks later, and the remainder after twelve weeks. The wood was cleaned of fungus and vermiculite adhering to the surface, weighed and dried to constant weight, and the percentage weight losses estimated.

Results

These are presented in Appendix table 6.

Discussion

High levels of decay were obtained after both six and twelve weeks' incubation. Again wood water contents increased only gradually, possibly because any permanent increase in the equilibrium moisture content of the wood would have to be accompanied by a permanent increase in that of the vermiculite. Variation in both the weight losses and the water contents of the wood is low.

Table 6. Weight losses and water contents of *P. sylvestris* sapwood test pieces after attack by *C. puteana* in vermiculite.

Incubation period	Bottle	% wt. loss of test pieces	$\bar{x}$	S.E.	% water content of test pieces after decay	$\bar{x}$	S.E.
6 weeks	1	36, 35, 49, 33, 32	37.1	3.1	66, 75, 73, 70, 67	70.2	1.7
	2	34, 31, 33, 37, 37	34.5	1.1	67, 62, 69, 74, 66	67.6	2.0
	3	41, 41, 33, 40, 46	40.2	2.1	75, 70, 65, 78, 79	73.4	2.6
	4	41, 35, 37, 31, 39	36.7	1.7	81, 70, 73, 65, 75	72.8	2.7
	5	39, 42, 32, 29, 31	34.6	2.5	69, 70, 64, - , 63	66.5	1.8
			36.6	1.04		70.1	1.4
12 weeks	6	67, 54, 38, 67, 61	57.4	3.4	76, 99, 78, 81, 90	84.8	4.3
	7	65, - , - , - , -	65.0	-	94, - , - , - , -	94.0	-
	8	54, 65, 58, - , 51	57.0	3.0	89, 65, 88, - , 83	81.3	5.6
	9	67, 59, 61, - , 56	60.8	2.3	96, - , 83, 97, 96	93.0	3.3
	10	- , 55, - , 54, -	54.5	0.5	- , 91, - , 88, -	89.5	1.5
			58.9	1.80		88.5	2.4

The results from weight loss determinations after the twelve week incubation, show a number of values missing; this was due to badly decayed test pieces crumbling on drying. This could be a significant problem in a major survey, and perhaps an eight week incubation period might be more suitable. With this degree of attack, the brown discoloration of the wood is very marked, consequently, identification of each piece of wood becomes exceedingly difficult, and this could also lead to problems.

Nevertheless, the vermiculite method does offer a number of advantages over the use of glass supports on agar: it is easy to set up and need not involve pre-wetting of the wood; it allows ease of handling and perhaps above all, a considerably shorter incubation period is required for the same level of decay. For these reasons, it was decided to adopt this technique for the present investigation.

APPENDIX 6

Table 7 (a). Percentage weight losses of *P. sylvestris* sapwood test pieces after decay by *C. puteana* during 8 weeks incubation at 24°C in the dark using the direct agar method.

Strain	Replicate Test Pieces										Mean of Total
	Bottle 1	$\bar{x}$	Bottle 2	$\bar{x}$	Bottle 3	$\bar{x}$	Bottle 4	$\bar{x}$	Bottle 5	$\bar{x}$	
4A	1.4, 3.3, 2.0 1.0, 0.8	1.7	1.5, 0, 1.4, 0 1.7	1.5	1.6, 1.5, 2.2, 0,0	1.8	0, 0, 0, 0, 0	0	1.6, 0, 2.4, 1.1	1.7	1.34
16	5.7, 3.0, 0.4, 0,0	3.0	6.5, 8.1, 3.7, 3.3, 8.3	6.0	0.5, 4.1, 4.0, 0.4, 0	2.3	4.6, 5.5, 0, 4, 0	4.7	3.6, 0.9, 3.9, 1.6, 4.5	2.9	3.78
9	3.6, 9.3, 2.4, 13.9, 13.3	8.5	0.8, 11.0, 7.2 4.8, 7.3	6.2	10.7, 14.9, 1.6, 9.5, 8.3	8.9	4.8, 5.7, 8.2, 5.0, 8.2	6.4	3.6, 0, 0, 26.9, 4.3	11.6	8.32
17	6.1, 3.8, 0, 9.5, 6.4	6.5	10, 0, 20.5, 3.9, 25.9	15.1	0, 12.9, 6.2 6.1, 23.7	12.2	3.4, 25.4, 3.0, 22.3, 6.7	12.2	0, 2.6, 6.7, 7.6, 9.9	6.7	10.54
5A	15.1, 17.4, 2.0, 26.1, 9.6	14	17.9, 13.2, 9.3 20.2, 8.2	13.8	7.7, 4.3, 14.5, 9.7, 5.0	8.2	14.6, 9.3, 4.3, 12.0, 8.0	9.6	11, 12.3, 12, 12, 7.6	11	11.32
15	13.9, 14.2, 14, 13.7, 12.8	13.7	8.8, 15.2, 16.2, 14.7, 11.1	13.2	14.5, 9.7, 4.8, 11.5, 5.4	9.2	5.8, 18, 11.8, 7.3, 24.8	13.5	14.5, 18.4, 1.1 0, 8.9	8.6	11.64
11	15, 16.3, 29.2, 14.3, 5.4	16	21.8, 3.8, 9.4, 10.9, 6.2	10.4	10.6, 6.4, 24.4, 9.8, 4.6	11.2	19.5, 4.4, 21, 6.0, 8.3	11.8	34.7, 10.7, 29, 13.5, 3.3	18.2	13.52
7	6.4, 33.6, 10.2, 7.2, 10.4	13.6	24.7, 30.4, 15.6 21.2, 7.2	19.8	12.4, 5.3, 8.8, 3.5, 26.2	11.2	16.9, 17.7, 13.9, 35.3, 16.9	20.2	5.9, 5.6, 22.9, 24.7, 6.0	13.0	15.56
20	12.2, 2.3, 12.3, 27.0, 9.9	12.7	2.3, 0, 13.1, 20, 8.5	11	26.5, 14.8, 43 17, 14.7	23.2	16.6, 22.8, 7.4, 21.1, 9.7	15.5	12.2, 5.2, 6.5, 34.6, 18.6	15.4	15.56

Table 7 (b). Percentage water contents of *P. sylvestris* test pieces on harvesting after 8 weeks incubation with *C. puteana* at 24°C in the dark using the direct agar method.

Strain	Replicate Test Pieces										Mean of Total
	Bottle 1	$\bar{x}$	Bottle 2	$\bar{x}$	Bottle 3	$\bar{x}$	Bottle 4	$\bar{x}$	Bottle 5	$\bar{x}$	
4A	99, 136, 125, 111, 103	115	95, 110, 123, 117, 94	108	89, 86, 126, 109, 106	103	129, 128, 138, 156, 124	135	115, 132, 114, 133, 144	128	118
16	75, 139, 109, 96, 144	113	62, 45, 96, 58, 91	70	111, 87, 125, 145, 121	118	74, 115, 153, 91, 98	106	53, 43, 78, 69, 48	58	93
9	122, 116, 114, 107, 126	117	148, 133, 163, 131, 149	145	100, 101, 115, 112, 94	104	161, 155, 146, 156, 117	147	141, 152, 121, 148, 85	129	128
17	126, 78, 127, 134, 102	113	93, 117, 70, 111, 72	93	133, 95, 72, 92, 62	91	133, 62, 121, 64, 136	103	144, 145, 101, 129, 130	130	106
5A	120, 164, 160, 131, 129	139	135, 148, 154, 133, 143	143	170, 144, 168 165, 146	159	141, 167, 148, 146, 158	152	165, 154, 158, 192, 156	165	152
15	100, 91, 94, 112, 90	97	127, 128, 96, 126, 107	117	133, 131, 156, 144, 165	154	117, 108, 112, 109, 90	107	116, 133, 149, 137, 160	139	123
11	120, 110, 123, 127, 150	126	149, 155, 137, 148, 145	147	150, 135, 82, 151, 159	135	130, 117, 138, 153, 138	135	66, 119, 74, 134, 132	105	130
7	129, 63, 93, 139, 139	113	72, 79, 60, 80, 100	78	157, 147, 132 143, 174	151	118, 91, 124 69, 83	97	146, 125, 135, 162, 152	144	117
20	150, 147, 120, 86, 133	127	130, 144, 145, 124, 100	129	129, 110, 104, 136, 123	120	96, 104, 119, 98, 114	106	113, 132, 126, 58, 118	109	118

Table 8. Correlation of mean % weight losses (of 5 test pieces) with the mean % wood water contents (of 5 test pieces) from appendix tables 7 (a) and (b).

Strain	Mean % wt. loss	Mean % water content	Correlation coefficient
4A	1.34	118	- .0034
16	3.78	93	- .00044
9	8.32	128	- .00065
17	10.54	106	- .00071
5A	11.32	152	- .0013
15	11.64	123	- .00077
11	13.52	130	- .0009
7	15.56	117	- .0004
20	15.56	118	- .0003

Tables 9 (a), (b), (c) & (d). Percentage weight losses of *P. sylvestris* sapwood test pieces after incubation with isolates of *C. puteana* for 10 weeks at 24°C in the dark. Survey A (by the vermiculite method).

Isolate	1	2A	2B	3	4A	4B	5A	5C
	5.6	4.8	3.2	5.0	6.1	6.2	35.6	37.9
Bottle	5.2	4.0	4.3	4.0	5.7	5.8	47.1	38.0
1	7.9	4.5	3.4	3.9	5.5	5.3	20.6	48.3
	6.0	4.6	2.6	3.8	5.3	5.6	41.2	37.8
	7.0	3.7	2.2	3.4	4.9	6.3	29.8	27.4
$\bar{x}$	6.3	4.3	3.1	4.0	5.5	5.8	34.9	37.9
	2.2	4.7	5.1	4.6	4.5	6.1	36.8	44.7
Bottle	8.6	3.5	5.3	4.5	4.7	5.5	43.7	32.0
2	2.4	3.6	6.0	5.1	4.9	5.6	39.9	21.7
	7.4	4.2	5.2	4.3	5.1	6.6	21.1	44.1
	2.3	3.9	5.5	4.8	4.0	5.3	47.7	37.1
$\bar{x}$	4.6	4.0	5.4	4.7	4.6	5.8	37.8	35.9
	0.8	1.4	3.2	3.1	2.2	4.9	36.6	34.1
Bottle	1.1	1.4	9.1	1.5	2.2	2.3	51.9	48.4
3	1.6	1.5	3.0	1.6	2.0	2.4	38.9	45.1
	1.7	1.8	2.7	1.8	2.0	2.2	35.6	36.6
	1.8	1.3	5.0	1.9	2.1	2.3	32.4	43.3
$\bar{x}$	1.4	1.5	4.6	2.0	2.1	2.8	39.1	41.5
	1.8	2.2	4.6	1.2	2.1	3.0	33.3	29.2
Bottle	1.3	1.9	2.5	1.9	1.9	3.0	38.6	36.0
4	1.6	2.1	2.0	1.8	2.4	2.8	33.5	27.4
	1.9	2.3	1.8	1.9	2.2	3.0	35.7	32.8
	1.5	1.6	1.5	1.5	7.7	2.7	31.6	27.7
$\bar{x}$	1.6	2.0	2.5	1.7	3.3	2.9	34.5	30.6
	14.1	4.8	6.6	4.1	6.8	6.5	31.9	33.8
Bottle	7.6	4.3	7.3	4.9	5.0	6.4	45.2	31.1
5	12.6	4.6	5.8	5.4	5.6	6.1	26.5	42.5
	8.2	5.0	6.7	5.1	6.5	7.3	50.5	46.5
	4.8	4.5	3.3	8.9	6.6	7.8	37.8	30.8
$\bar{x}$	9.5	4.6	5.9	5.7	6.1	6.8	38.4	36.9
	7.9	5.6	5.4	5.4	5.4	5.1	43.4	35.4
Bottle	6.3	5.6	5.7	4.0	5.2	5.4	42.7	7.0
6	9.0	5.5	11.4	6.3	5.9	-	47.7	33.8
	6.1	4.7	6.0	5.4	6.0	4.7	27.1	15.8
	8.5	5.5	5.3	5.0	5.2	4.6	45.7	17.6
$\bar{x}$	7.6	5.4	6.8	5.2	5.5	5.0	41.3	21.9
Mean of Total	5.2	3.6	4.7	2.2	4.5	4.9	37.7	34.1

Table 9 (b).

Isolate	5D	6	7	8	9	10	11	12
	48.7	17.2	27.6	20.0	32.1	1.4	34.4	3.2
Bottle	49.3	6.0	29.3	31.0	34.3	3.8	13.4	3.4
1	35.3	7.4	31.0	-	31.6	10.5	35.5	3.2
	47.6	5.4	38.6	25.6	28.7	5.0	18.0	4.0
	26.6	5.4	14.6	30.4	11.3	1.4	37.5	3.7
$\bar{x}$	41.5	8.3	28.2	26.8	27.6	4.4	27.8	3.5
	50.1	6.3	9.0	24.4	33.0	12.1	32.7	1.5
Bottle	44.2	5.0	41.1	25.2	10.3	8.3	19.1	1.7
2	53.1	8.1	42.3	16.7	3.0	8.3	12.4	1.0
	45.8	4.2	33.4	10.4	11.4	7.0	18.7	2.0
	54.2	4.2	28.6	19.2	6.6	8.1	15.6	0.8
$\bar{x}$	49.5	5.6	30.9	19.2	12.9	8.8	19.7	1.4
	39.8	4.6	40.9	22.8	32.7	0	37.6	0.8
Bottle	50.1	2.8	31.6	14.8	33.7	0	19.0	1.5
3	43.7	2.5	18.8	13.8	33.3	0	36.8	1.2
	48.2	2.4	12.5	21.7	31.0	0	18.4	1.5
	41.0	2.3	30.3	22.2	35.1	0	34.8	1.6
$\bar{x}$	44.6	2.9	26.8	19.1	33.2	0	30.0	1.3
	44.9	1.7	11.9	17.0	27.9	0	18.3	1.8
Bottle	46.5	1.8	8.0	11.7	29.1	0	32.1	2.3
4	43.8	2.2	24.2	16.7	31.0	0	24.3	1.9
	33.7	2.5	35.0	11.3	32.4	0	17.3	1.1
	47.6	2.6	27.9	25.9	32.3	0	14.8	1.8
$\bar{x}$	43.3	2.2	21.4	16.5	30.5	0	21.4	1.8
	49.6	8.3	36.2	14.9	33.2	4.1	46.1	5.0
Bottle	48.1	8.3	21.1	24.4	36.9	5.3	45.9	10.2
5	46.0	11.7	46.6	18.4	31.3	4.9	44.0	4.4
	-	6.2	19.0	17.3	34.5	4.9	47.5	4.9
	41.4	6.1	42.2	22.8	28.6	4.4	17.3	4.9
$\bar{x}$	46.3	8.1	33.0	19.6	32.9	4.7	40.1	5.9
	51.8	6.2	33.7	26.5	40.3	4.5	39.4	5.2
Bottle	53.2	6.1	36.5	22.8	28.9	4.3	15.4	5.5
6	54.2	7.8	38.2	20.7	31.4	4.5	13.2	4.1
	41.7	13.3	37.5	21.9	41.5	4.4	37.7	5.9
	45.1	6.0	30.1	23.6	35.2	4.5	41.7	4.6
$\bar{x}$	49.2	7.9	35.2	23.1	35.5	4.4	29.5	5.1
Mean of Total	45.7	5.8	29.2	20.7	28.8	5.6	28.1	3.2

Table 9 (c).

Isolate	13	14	15	16	17	18	19	20
	4.5	6.1	18.1	2.9	27.7	4.7	4.5	14.8
Bottle	4.4	5.7	20.5	3.2	17.2	4.4	4.2	32.1
1	5.8	5.1	15.8	2.9	4.3	3.8	4.2	11.3
	4.9	5.2	23.0	3.1	17.6	4.9	3.9	10.7
	4.3	8.1	36.2	3.2	15.7	5.1	4.3	34.7
$\bar{x}$	4.8	6.0	22.7	3.1	16.5	4.6	4.2	20.7
	4.1	8.1	26.5	4.1	14.8	3.8	1.6	23.1
Bottle	3.7	7.7	41.7	3.6	26.7	4.0	2.3	30.8
2	4.1	7.7	13.1	4.1	14.0	3.4	2.3	28.2
	4.2	7.8	43.1	3.9	23.4	3.9	2.3	31.2
	3.9	7.7	36.7	4.2	12.9	4.3	2.5	32.8
$\bar{x}$	4.0	7.8	32.2	4.0	18.4	3.9	2.2	29.2
	1.1	1.3	9.6	1.3	9.6	0	1.1	23.3
Bottle	0.5	1.8	20.1	2.0	1.2	0	1.1	28.9
3	1.0	2.5	10.2	1.8	5.1	0	0.5	26.6
	1.1	1.7	11.0	1.5	26.1	0	1.3	15.1
	1.3	1.7	5.1	<1.0	1.8	0	1.2	22.8
$\bar{x}$	1.0	1.8	11.2	1.5	8.8	0	1.0	23.4
	1.1	3.3	13.8	8.3	1.2	0	0.9	26.7
Bottle	1.3	4.6	21.4	16.3	1.3	0	1.2	25.3
4	1.4	15.9	22.4	7.1	<1.0	0	0.9	30.6
	1.3	4.6	33.5	3.7	27.7	0	0.9	17.9
	1.2	4.8	41.2	8.3	9.6	0	0.7	25.5
$\bar{x}$	1.3	6.6	26.5	8.7	10.0	0	0.9	25.2
	3.5	9.6	24.8	6.6	11.7	-	6.9	16.5
Bottle	4.8	6.9	20.9	6.6	14.0	-	7.0	32.6
5	3.8	14.7	15.2	5.7	19.4	-	6.0	32.9
	3.9	6.3	24.7	4.6	27.4	-	7.5	37.1
	4.6	14.9	18.6	-	5.5	-	5.3	27.3
$\bar{x}$	4.1	10.5	20.8	4.7	15.6	-	6.5	29.3
	3.8	6.8	38.4	4.5	19.0	-	5.3	24.7
Bottle	4.1	11.9	48.6	4.1	26.3	-	4.7	21.7
6	4.6	7.2	45.4	4.1	35.0	-	5.3	41.8
	4.2	14.6	41.1	4.0	5.5	-	5.4	36.3
	4.3	15.3	22.9	4.3	5.8	-	9.9	34.7
$\bar{x}$	4.2	11.2	39.3	4.2	18.3	-	6.1	31.8
Mean of Total	3.2	7.3	25.3	4.4	14.6	2.1	3.5	26.6

Table 9 (d).

Isolate	21	22	23	24	25	26	27	28	29	30
	3.2	21.0	14.0	35.1	24.1	30.0	28.7	25.1	33.3	4.7
Bottle	2.5	14.3	34.2	-	19.6	16.5	28.2	24.9	18.9	4.9
1	2.9	10.5	48.5	26.0	8.7	28.6	17.7	30.2	19.6	5.0
	1.9	13.1	37.4	5.8	37.0	23.4	15.0	30.9	15.8	4.4
	2.6	10.4	23.4	23.6	30.5	21.5	27.3	14.3	9.3	5.0
$\bar{x}$	2.6	13.9	31.5	22.6	24.0	24.0	23.4	25.1	22.4	4.8
	7.4	15.2	47.6	31.8	38.2	8.7	18.5	9.1	29.3	4.8
Bottle	7.2	23.5	35.1	21.2	25.6	11.1	25.5	33.8	24.7	4.3
2	7.2	14.5	43.3	25.2	43.0	10.2	25.0	32.9	22.2	4.1
	7.5	20.5	45.8	36.5	47.5	19.1	21.1	20.4	23.6	4.6
	7.5	24.0	46.2	16.6	47.9	14.3	16.5	34.6	24.7	4.6
$\bar{x}$	7.4	19.5	43.6	26.3	40.4	12.7	21.3	26.2	24.9	4.5
	1.4	11.5	15.3	2.9	9.8	8.0	14.3	16.5	27.7	1.9
Bottle	1.6	12.9	37.9	23.2	9.8	25.7	13.7	16.5	19.8	1.7
3	1.7	8.8	48.3	30.8	8.9	16.5	15.1	15.2	29.7	1.8
	1.2	15.8	10.2	21.6	14.3	17.5	13.3	10.1	27.1	6.5
	1.3	6.0	19.4	16.8	8.7	28.1	24.1	15.1	29.6	1.5
$\bar{x}$	1.4	11.0	26.2	19.1	10.3	19.2	16.1	14.7	26.8	2.7
	1.5	11.3	34.0	14.7	32.7	23.1	17.4	7.7	29.1	2.7
Bottle	2.0	9.7	22.7	25.8	35.7	19.5	22.8	11.4	21.2	2.6
4	2.8	17.1	39.6	15.4	32.3	28.7	12.5	11.1	22.8	2.5
	1.6	14.1	39.8	12.9	35.9	16.2	25.5	6.5	24.9	2.5
	2.9	10.4	43.2	16.1	34.2	19.3	11.6	8.4	24.0	2.2
$\bar{x}$	2.2	12.5	35.9	17.0	34.2	21.4	18.0	9.0	24.4	2.5
	5.8	17.7	26.7	19.6	30.3	23.9	14.1	22.6	40.7	6.2
Bottle	4.6	33.6	54.7	17.0	30.6	22.5	12.5	14.3	29.7	6.0
5	5.4	16.5	56.3	-	35.2	19.0	44.8	25.6	42.9	5.4
	5.7	33.1	-	-	20.5	19.8	11.5	30.0	41.7	7.8
	5.7	24.2	25.5	-	25.1	17.6	23.9	20.1	39.7	5.6
$\bar{x}$	5.4	25.0	40.8	18.3	29.7	20.6	21.4	22.5	32.5	6.2
	4.8	26.6	25.5	32.6	37.8	41.8	18.5	7.9	23.4	6.5
Bottle	5.0	25.9	18.8	27.0	25.6	38.3	13.7	18.9	42.6	5.2
6	4.6	28.2	19.8	28.0	32.1	22.2	14.3	23.2	27.6	4.9
	4.8	14.5	-	30.1	19.1	37.8	24.8	18.9	46.3	5.0
	4.3	35.5	-	8.0	37.0	26.5	17.2	32.1	31.3	5.5
$\bar{x}$	4.7	26.1	25.7	25.1	30.3	33.3	17.7	20.2	34.2	5.4
Mean of Total	4.0	18.0	34.0	21.4	28.2	21.9	19.7	19.6	27.5	4.4

Tables 9 (e), (f), (g) & (h). Cross-grain breaking strengths of *P. sylvestris* sapwood test pieces (Newtons) from survey A after decay by *C. puteana* in vermiculite.

Isolate	1	2A	2B	3	4A	4B	5A	5C
	275	325	400	295	300	280	45	30
Bottle	205	300	380	335	320	280	10	40
1	310	270	510	315	370	430	160	20
	320	355	380	340	350	310	25	20
	310	400	500	355	295	280	50	90
$\bar{x}$	284	330	434	328	327	316	58	40
	490	310	300	355	380	305	25	20
Bottle	270	320	370	355	345	275	15	60
2	440	365	285	350	355	340	20	80
	230	390	400	365	330	315	110	40
	470	285	330	350	375	295	10	95
$\bar{x}$	380	334	337	355	357	306	36	59
	395	430	550	570	610	505	30	35
Bottle	275	580	130	500	500	430	15	20
3	530	555	480	580	570	460	50	15
	525	430	610	575	365	290	50	45
	525	370	495	510	555	410	90	45
$\bar{x}$	450	473	453	547	520	419	47	32
	535	575	455	540	525	500	70	70
Bottle	505	530	530	530	620	565	45	35
4	600	520	530	570	590	580	55	70
	500	570	620	530	480	650	50	65
	535	530	560	475	490	600	55	65
$\bar{x}$	535	545	539	529	541	579	55	61
	235	460	270	560	-	480	55	45
Bottle	310	560	305	310	-	420	20	75
5	225	430	550	370	-	360	100	10
	390	430	350	310	-	465	10	30
	380	495	300	450	-	430	35	60
$\bar{x}$	308	475	355	400	-	431	44	44
	370	310	460	310	-	470	25	80
Bottle	495	420	498	560	-	500	25	460
6	420	305	340	330	-	480	25	40
	450	405	460	340	-	430	220	210
	350	320	445	375	-	420	20	185
$\bar{x}$	417	352	440	383	-	460	63	195
Mean of Total	408	418	426	424	436	419	51	72

Table 9 (f).

Isolate	5D	6	7	8	9	10	11	12
	10	270	55	95	40	350	45	435
Bottle	10	500	35	30	40	570	270	390
1	35	570	40	-	20	325	15	375
	10	410	40	90	45	420	245	320
	90	540	180	45	150	540	20	400
$\bar{x}$	31	458	70	65	59	441	119	384
	20	320	175	75	45	320	60	390
Bottle	10	450	30	90	250	350	45	245
2	5	320	10	230	370	300	115	390
	30	295	60	230	200	320	115	400
	5	390	60	195	340	330	110	300
$\bar{x}$	14	355	67	164	243	324	89	345
	35	410	40	80	90	500	50	515
Bottle	15	550	100	185	60	390	170	545
3	20	500	115	390	70	485	50	515
	25	530	250	120	80	545	115	620
	30	375	50	130	60	600	40	520
$\bar{x}$	25	473	111	181	72	504	85	543
	25	395	235	190	85	535	130	620
Bottle	20	460	275	350	70	410	50	395
4	25	500	70	250	90	450	145	415
	40	535	10	390	70	620	100	460
	20	420	70	20	65	555	155	520
$\bar{x}$	26	462	132	240	76	514	116	482
	5	375	30	270	60	575	25	500
Bottle	10	445	150	160	80	450	5	360
5	10	170	20	190	110	630	5	550
	-	420	170	220	75	595	5	470
	30	410	30	120	120	550	200	470
$\bar{x}$	11	364	80	192	89	560	48	470
	20	265	25	135	50	480	40	530
Bottle	15	350	55	130	100	550	255	450
6	10	395	40	180	60	605	220	510
	20	235	35	145	65	600	30	500
	25	270	40	115	70	570	10	530
$\bar{x}$	18	283	39	141	69	561	111	504
Mean of Total	21	399	83	164	101	484	95	455

Table 9 (g)

Isolate	13	14	15	16	17	18	19	20
	340	400	195	350	70	310	350	205
Bottle	240	430	110	390	85	330	400	55
1	415	500	180	335	330	420	330	135
	320	470	60	370	105	380	345	170
	235	460	30	315	180	315	375	40
$\bar{x}$	310	452	95	352	154	351	360	121
	340	300	60	310	90	390	370	98
Bottle	415	345	10	325	40	350	515	80
2	350	270	115	370	60	430	550	30
	430	380	10	290	60	380	370	65
	360	210	40	350	175	300	490	85
$\bar{x}$	379	301	47	329	85	370	459	71
	470	600	310	300	290	530	320	30
Bottle	480	435	140	450	390	570	450	70
3	400	370	140	600	280	595	620	110
	560	550	325	570	100	600	575	270
	495	530	470	535	470	520	630	90
$\bar{x}$	481	497	277	491	306	563	519	114
	610	575	185	455	550	640	605	80
Bottle	570	430	230	175	500	630	555	120
4	560	280	55	495	495	640	500	80
	525	520	45	200	100	575	585	170
	505	590	45	390	350	595	430	30
$\bar{x}$	554	479	112	343	399	616	535	96
	510	215	165	395	370	-	320	165
Bottle	340	315	155	210	355	-	370	50
5	560	320	310	560	195	-	510	75
	515	340	120	510	180	-	300	55
	340	240	215	540	530	-	450	110
$\bar{x}$	453	286	193	443	326	-	390	91
	520	275	40	475	120	-	360	110
Bottle	475	300	15	600	50	-	585	130
6	450	420	25	540	80	-	455	110
	450	325	20	430	430	-	535	75
	465	280	40	510	460	-	545	75
$\bar{x}$	472	320	28	511	228	-	496	100
Mean of Total	442	389	125	412	250	475	460	99

Table 9 (h).

Isolate	21	22	23	24	25	26	27	28	29	30
	435	100	190	75	100	60	65	90	55	360
Bottle	520	115	35	-	85	135	65	120	145	330
1	500	185	20	120	185	70	165	90	150	290
	460	170	20	350	30	75	170	100	200	355
	570	155	145	60	55	70	50	180	235	310
$\bar{x}$	485	145	82	151	91	82	103	116	157	329
	320	180	10	35	30	195	120	330	75	330
Bottle	350	90	45	80	45	200	80	50	80	380
2	335	170	10	90	20	155	80	40	110	400
	345	160	20	5	5	100	75	85	55	355
	280	100	10	180	10	165	100	50	95	365
$\bar{x}$	326	140	19	78	22	163	91	111	83	366
	455	280	220	460	235	265	165	170	125	400
Bottle	365	150	60	100	300	20	210	190	160	475
3	620	295	15	60	320	200	200	110	55	555
	565	255	210	95	195	165	265	225	95	580
	440	380	170	200	260	110	120	140	55	435
$\bar{x}$	489	372	135	183	262	152	192	167	98	489
	520	300	50	110	70	95	175	320	60	520
Bottle	470	360	65	70	50	155	120	245	150	540
4	455	190	35	340	30	65	290	270	155	580
	525	280	40	350	60	185	110	400	130	600
	480	205	15	170	60	150	260	290	110	540
$\bar{x}$	490	267	41	208	54	130	191	305	99	556
	400	120	155	160	70	160	210	220	20	350
Bottle	555	80	20	205	95	170	240	255	155	355
5	605	210	5	-	60	130	30	105	50	370
	425	65	-	-	95	175	190	95	40	420
	430	120	185	-	80	170	95	235	60	350
$\bar{x}$	463	119	91	182	80	161	153	182	65	369
	430	150	135	85	25	60	100	110	190	365
Bottle	380	195	220	145	65	50	230	190	30	445
6	535	100	95	205	40	95	140	160	115	400
	430	130	-	80	190	50	140	150	30	500
	510	60	-	320	35	85	230	30	95	470
$\bar{x}$	457	127	150	167	71	68	168	128	48	436
Mean of Total	452	178	86	157	97	126	150	168	92	424

Table 9 (i). Percentage weight losses of *P. sylvestris* sapwood test pieces after decay by isolates of *C. puteana* (10 weeks incubation at 24°C in the dark). Survey B (by the vermiculite method).

Isolate	5A	5C	5D	11	14	17	31	32	33
	31.6	35.4	42.2	20.4	10.0	31.0	17.2	67.1	34.3
Bottle	31.6	37.2	45.7	51.0	12.0	17.9	51.6	66.8	39.8
1	32.2	57.4	60.9	38.0	15.4	28.6	53.3	65.1	40.8
	58.5	49.4	47.0	41.0	5.3	31.1	43.8	64.0	30.6
	54.3	42.1	54.7	16.0	8.7	32.2	59.7	68.4	6.9
$\bar{x}$	41.7	49.2	50.1	33.3	10.3	28.2	51.1	66.3	30.5
	29.7	42.0	49.9	23.7	10.8	32.2	51.3	65.4	36.3
Bottle	50.4	49.9	36.2	47.9	9.1	45.7	53.5	67.2	23.8
2	51.0	60.2	48.6	39.1	6.0	33.8	54.3	26.9	18.4
	54.9	56.6	52.4	15.1	11.1	13.0	53.9	64.6	16.4
	50.7	42.8	50.9	46.1	4.3	8.4	46.5	62.4	37.2
$\bar{x}$	47.3	50.3	47.6	34.3	8.3	25.0	51.9	57.3	26.4
	41.5	51.0	55.3	27.3	7.3	24.9	51.4	67.6	28.8
Bottle	49.6	32.8	49.7	35.9	17.1	32.0	50.4	68.5	40.6
3	54.9	46.2	-	41.2	8.3	33.7	44.2	69.0	37.7
	59.7	29.7	55.6	30.1	5.7	20.5	45.2	43.7	36.0
	47.1	27.2	50.1	39.7	7.0	20.0	47.0	69.1	36.8
$\bar{x}$	50.5	37.4	53.0	34.8	9.8	26.2	47.6	63.6	36.0
	39.0	37.3	62.6	37.9	4.5	6.5	46.1	72.7	36.2
Bottle	32.7	62.1	40.9	19.2	2.9	8.3	53.1	40.9	39.1
4	32.0	19.6	38.9	44.9	7.2	19.8	34.0	68.9	37.9
	59.8	45.6	38.4	28.9	4.1	24.0	55.9	67.9	42.5
	50.2	32.3	45.6	39.4	5.0	12.5	49.8	64.2	35.4
$\bar{x}$	42.7	39.4	45.3	34.1	4.7	14.2	47.8	62.9	38.2
	38.9	54.5	56.9	45.7	4.4	26.8	52.3	66.1	30.7
Bottle	34.3	54.9	52.5	54.3	4.9	35.9	54.3	62.8	41.2
5	53.2	53.9	47.2	44.2	14.9	16.0	55.4	66.6	43.5
	44.8	52.3	51.7	44.2	13.2	13.7	56.4	66.2	27.8
	59.4	51.6	42.0	57.9	13.6	27.8	48.6	66.3	34.3
$\bar{x}$	46.1	53.4	50.1	49.3	10.2	24.1	53.4	65.6	35.5
Mean of Total	46.0	45.9	50.3	37.2	8.7	23.5	50.4	63.1	33.3

$\frac{246.1}{5} = 49.2$

$\frac{1175.9}{24} = 49.0$

Table 9 (j). Cross-grain breaking strengths of *P. sylvestris* sapwood test pieces (Newtons) from survey B after decay by *C. puteana* in vermiculite.

Isolate	5A	5C	5D	11	14	17	31	32	33
	50	75	100	65	260	45	20	0	45
Bottle	130	40	50	10	490	180	20	0	35
1	45	5	125	55	310	60	15	0	40
	5	15	65	65	580	60	10	0	70
	10	55	15	270	420	25	5	0	350
$\bar{x}$	48	38	71	93	412	74	14	0	108
	15	45	15	145	440	90	10	0	35
Bottle	25	10	80	5	350	20	5	5	225
2	20	0	15	10	340	60	10	110	270
	5	5	20	85	300	205	0	0	255
	15	20	15	15	320	-	20	0	40
$\bar{x}$	20	16	29	52	350	94	9	23	165
	30	0	5	90	405	330	20	5	130
Bottle	15	65	20	30	275	40	10	10	25
3	10	5	140	20	420	50	30	0	45
	15	20	15	25	410	130	10	70	50
	25	135	20	25	315	185	20	0	50
$\bar{x}$	19	45	40	44	365	147	18	17	60
	120	140	5	15	370	310	10	0	60
Bottle	45	0	140	140	310	340	15	10	25
4	160	180	160	10	275	160	55	0	35
	0	70	145	60	280	170	10	0	35
	15	125	20	25	330	265	10	0	25
$\bar{x}$	68	103	94	50	313	249	20	2	36
	20	5	5	15	265	65	5	0	75
Bottle	60	5	10	0	370	45	5	0	20
5	10	10	25	25	300	140	10	0	5
	30	5	20	15	280	165	5	0	65
	120	5	20	0	320	55	10	0	65
$\bar{x}$	49	6	16	11	307	94	7	0	46
Mean of Total	41	42	50	50	349	132	12	8	83

One-way analysis of variance of data in tables 9 (a-j)

SURVEY A

(i) Percentage weight loss

	Sum of squares	D.o.f.	F ratio
Isolates	155070.4	34	89.4
Error	49102.4	962	
Total	204172.8	996	

(ii) Cross-grain breaking strength

	Sum of squares	D.o.f.	F ratio
Isolates	27190998.4	34	84.3
Error	9090513.4	962	
Total	36281511.8	996	

SURVEY B

(iii) Percentage weight loss

	Sum of squares	D.o.f.	F ratio
Isolates	50599.5	8	71.1
Error	19041.2	214	
Total	69640.7	222	

(iv) Cross-grain breaking strength

	Sum of squares	D.o.f.	F ratio
Isolates	2240488.9	8	70.2
Error	857860.7	215	
Total	3098349.6	223	

For each table, differences between isolates are significant at the 5% level.
Differences between replicates are not significant.

APPENDIX 7

Tables 10 (a) and (b): Growth of thirty isolates of *C. puteana* on 2% malt agar at 24°C in the dark, measured by increase in colony radius (mm). Each value represents the mean of 10 measurements with the standard error beneath. (37.0 mm = maximum possible growth.)

Isolate:-	1	2A	2B	3	4A	4B	5C	6	7	8	9	10	11	12	13
Day 1															
2	4.5 0.28	2.1 0.1	2.0 0.22	1.2 0.13	2.3 0.21	2.5 0.18	4.9 0.33	2.6 0.28	8.5 0.59	1.4 0.17	6.7 0.15	4.4 0.55	7.0 0.45	2.4 0.22	3.6 0.37
3	8.9 0.33	4.2 0.14	3.3 0.16	2.3 0.23	4.4 0.23	4.9 0.11	7.9 0.43	4.9 0.25	15.5 0.25	2.1 0.24	14.2 0.60	6.8 0.31	17.5 0.48	5.5 0.53	4.9 0.60
4	12.9 0.33	6.2 0.14	4.5 0.24	4.2 0.38	6.9 0.19	7.0 0	10.9 0.37	7.1 0.43	22.2 0.64	3.3 0.22	20.3 0.92	10.0 0.39	24.3 0.45	9.0 0.39	7.4 0.59
5	17.9 0.48	8.3 0.16	6.6 0.17	6.3 0.72	9.4 0.17	9.0 0	15.4 0.42	10.0 0.35	29.0 0.63	4.7 0.52	27.9 2.11	12.8 0.31	32.1 0.55	12.7 0.47	9.7 0.74
6															
7	28.2 0.61	13.1 0.19	11.7 0.41	11.3 0.57	13.5 0.36	13.7 0.16	23.7 0.61	14.7 0.47	37.0 0	6.1 0.51	37.0 0	19.5 0.63	37.0 0	20.7 0.47	15.4 1.19
8	32.9 0.66	15.3 0.16	14.7 0.31	13.4 0.73	15.6 0.28	15.9 0.10	28.4 0.47	17.6 0.36		7.2 0.54		23.6 0.23		25.5 0.42	18.8 1.22
9	37.0 0	18.5 0.24	17.7 0.50	15.7 1.07	17.7 0.42	18.5 0.18	32.6 0.53	20.1 0.50		8.1 0.53		27.7 0.22		30.1 0.50	22.4 1.52
10		19.9 0.19	19.6 0.47	17.9 1.09	20.4 0.45	20.6 0.23	35.5 0.53	22.9 0.48		9.4 0.61		30.4 0.36		33.4 0.69	25.4 1.61

Table 10 (b)

Isolate:-	14	15	16	17	20	21	22	23	24	25	26	28	31	32	33
Day 1															
2	4.1 0.23	4.3 0.15	2.8 0.25	3.9 0.37	6.8 0.21	2.6 0.17	9.9 0.52	8.3 0.47	6.8 0.31	5.5 0.22	9.5 0.31	6.7 0.45	8.6 0.34	7.2 0.13	9.9 0.28
3	6.1 0.25	7.2 0.14	5.3 0.23	6.1 0.40	17.2 1.0	5.0 0.22	19.3 0.53	17.9 0.85	11.4 0.42	10.1 0.25	19.0 0.39	13.9 0.71	16.6 0.39	12.3 0.16	14.3 0.39
4	9.0 0.39	10.5 0.23	7.9 0.33	8.5 0.32	26.5 0.97	7.5 0.36	28.5 0.49	27.0 0.50	15.0 0.57	14.9 0.33	26.1 0.37	18.1 1.0	23.8 0.38	16.6 0.23	19.2 0.34
5	12.0 0.69	14.6 0.23	9.9 0.29	10.8 0.38	33.3 0.61	10.3 0.45	35.9 0.32	35.4 0.42	22.0 0.47	20.7 0.47	33.3 0.47	23.4 1.07	32.2 0.44	22.2 0.56	24.6 1.58
6															
7	16.6 0.79	22.9 0.29	15.5 0.39	18.13 0.51	37.0 0	16.9 0.85	37.0 0	37.0 0	33.6 0.83	32.8 0.56	37.0 0	37.0 0	37.0 0	32.9 0.90	37.0 0
8	21.8 1.46	27.4 0.23	18.8 0.41	23.3 0.49		19.5 0.92			37.0 0	37.0 0				37.0 0	
9	25.1 1.66	31.6 0.23	21.3 0.55	26.9 0.79		23.1 1.05									
10	- -	35.5 0.36	24.2 0.56	30.1 0.74		25.6 1.48									

Tables 11 (a) and (b) Mean diameters (mm) of colonies of *C. puteana* isolates grown on agar incorporating pentachlorophenol and tri-n-butyl tin oxide. (Each value represents the mean of 10 measurements, and the standard deviation is presented beneath.)

Isolate	Incubation (days)	Pentachlorophenol (ppm)						Tri-n-butyl tin oxide (ppm)						
		C	1	5	10	15	20	C	10	15	20	25	30	35
11	3	27.6 0.84	19.7 0.48	0 0	0 0	0 0	0 0	48.0 1.3	9.9 0.57	0 0	0 0	0 0	0 0	0 0
	7	46.5 1.43	38.8 1.55	25.2 2.53	17.7 1.49	13.0 1.76	10.1 0.99	83.0 0	14.3 1.5	11.7 1.9	10.1 1.1	0 0	0 0	0 0
	14	83.0 0	76.8 3.29	59.1 2.6	44.6 1.9	33.5 2.76	23.4 4.60	83.0 0	21.9 1.66	15.2 1.8	11.6 1.4	9.8 0.71	11 1.41	0 0
20	3	32.5 1.18	25.0 1.49	13.6 2.41	0 0	0 0	0 0	48 4.7	0 0	0 0	0 0			
	7	63.0 3.43	49.5 2.42	31.9 3.84	14.8 2.74	0 0	0 0	83.0 0	10.6 1.08	9.6 0.84	0 0	No growth		
	14	83.0 0	83.0 0	68.8 3.94	42.3 3.16	27.9 10.2	17.6 8.2	83.0 0	12.8 2.53	9.5 0.97	0 0			
25	3	27.3 1.16	17.5 1.27	0 0	0 0	0 0	0 0	35.0 1.79	0 0	0 0				
	7	54.4 3.78	31.9 2.73	13.6 2.5	10.3 0.5	9.5 0.58	0 0	69.4 3.97	0 0	0 0	No growth			
	14	83.0 0	66.5 5.52	43.6 2.95	32.5 1.92	22.5 3.11	0 0	83.0 0	10.2 0.92	0 0				
24	3	26.4 1.3	23.0 1.24	13.6 1.96	0 0	0 0	0 0	34.0 1.6	0 0	0 0	0 0			
	7	52.5 1.93	45.7 1.57	33.2 2.78	15.4 2.6	0 0	0 0	72.0 2.64	10.4 0.70	9.5 0.63	0 0	No growth		
	14	83.0 0	83.0 0	74.8 2.25	49.3 3.2	26.2 3.43	0 0	83.0 0	15.1 1.66	12.3 1.8	0 0			
5A	3	23.6 0.97	15.2 2.1	0 0	0 0			30.4 1.07	0 0					
	7	43.2 1.62	27.1 3.5	12.2 2.1	0 0	No growth		55.0 1.41	0 0	No growth				
	14	83.0	52.8	30.3	0			83.0	0					

Table 11 (b)

Isolate	Incubation (days)	Pentachlorophenol (ppm)						Tri-n-butyl tin oxide (ppm)						
		C	1	5	10	15	20	C	10	15	20	25	30	35
16	3	20.1 1.2	16.9 2.47	11.7 1.64	0 0	0 0		25.2 1.69						
	7	35.3 1.06	31.7 3.06	25.1 1.60	18.7 1.86	0 0	No growth	43.3 2.26	No growth					
	14	51.1 1.60	46.0 5.10	38.7 2.21	30.0 2.45	0 0		64.0 1.33						
17	3	25.9 0.88	16.1 1.73	0 0	0 0	0 0		23.4 0.52	0 0	0 0				
	7	45.3 1.16	32.6 2.07	13.2 3.19	9.6 0.84	0 0	No growth	41.9 0.84	0 0	0 0	No growth			
	14	83.0 0	71.8 3.23	32.5 7.88	15.4 2.63	0 0		76.7 1.16	10.1 1.45	0 0				
14	3	18.7 0.68	15.9 0.74	9.4 0.52	0 0			20.9 0.99	0 0	0 0	0 0 0			
	7	34.0 1.33	27.6 1.35	16.2 1.69	0 0	No growth		41.9 0.88	10.7 0.68	0 0	0 0	No growth		
	14	68.0 1.56	52.6 4.2	31.1 6.89	0 0			83.0 0	14.5 0.53	10.6 0.84	0 0			
5D	3	26.4 2.37	17.5 1.18	11.9 1.78	0 0			35.0 0.42	0 0					
	7	44.9 3.67	31.7 2.11	22.0 4.06	0 0	No growth		60.0 0.95	0 0	No growth				
	14	83.0 0	62.8 2.62	50.0 5.74	0 0			83.0 0	0 0					
5C	3	25.5 2.4	16.2 0.79	0 0	0 0			30.0 2.4	0 0					
	7	46.2 4.9	31.3 3.16	18.1 1.45	0 0	No growth		52.0 3.0	0 0	No growth				
	14	83.0 0	62.7 3.34	44.9 2.56	0 0			83.0 0	0 0					

Tables 11 (c) and (d) Mean diameters (mm) of colonies of *C. puteana* isolates grown on agar incorporating pentachlorophenol and tri-n-butyl tin oxide. (Each value represents the mean of 6 measurements, and the standard deviation is presented beneath.)

Isolate	Incubation (days)	Pentachlorophenol (ppm)						Tri-n-butyl tin oxide (ppm)							
		C	1	5	10	15	20	C	5	10	15	20	25	30	35
2B	3	13.8 1.17	12.0 0	0 0	0 0	0 0		14.8 0.75	10.5 1.05	10.3 0.52	0 0	0 0	0 0	0 0	0 0
	7	29.3 0.52	21.0 2.0	12.7 2.94	8.5 0.58	0 0	No growth	31.0 1.79	17.7 0.82	16.2 0.75	13.8 0.75	11.5 1.23	10.5 0.55	9.7 0.52	0 0
	14	62.3 2.42	4.50 4.69	29.5 8.53	21.5 2.07	0 0		60.7 8.9	25.5 1.64	21.0 1.67	19.8 0.41	17.5 1.23	17.2 0.41	15.3 1.21	11.7 1.97
7	3	42.2 1.17	38.3 2.4	9.3 0.52	0 0	0 0	0 0	28.5 0.84	14.8 1.60	9.0 0	0 0	0 0	0 0	0 0	0 0
	7	-	-	29.8 2.64	18.7 1.03	13.0 1.67	0 0	81.3 2.58	22.3 0.52	17.0 0.63	11.5 1.05	10.7 0.52	10.3 0.52	10.0 0	0 0
	14	83.0 0	83.0 0	72.8 2.23	53.7 1.03	40.17 0.98	0 0	83.0 0	40.7 1.21	29.2 0.98	20.3 0.52	16.2 1.60	16.2 1.33	14.0 0	12.7 1.37
6	3	14.3 0.52	10.7 0.52	0 0	0 0	0 0	0 0	15.5 1.05	12.2 0.98	10.80 0.75	0 0	0 0	0 0	0 0	0 0
	7	30.2 0.41	24.0 0.89	17.0 1.10	9.8 1.71	0 0	0 0	34.2 2.32	24.3 0.82	20.2 1.72	17.0 0	14.5 1.05	13.2 1.17	12.2 0.75	10.8 0.41
	14	62.3 2.25	49.8 2.56	37.3 3.62	21.30 3.62	15.8 1.47	0 0	71.3 3.01	45.0 0.89	37.0 1.10	31.2 0.41	26.5 1.38	23.2 1.84	21.5 1.38	19.5 0.55
4A	3	11.8 0.41	10.0 0	0 0	0 0	0 0		10.7 0.52	10.7 0.82	0 0	0 0	0 0	0 0	0 0	0 0
	7	21.7 1.51	17.5 1.87	13.3 1.21	9.3 0.82	0 0	No growth	24.3 1.21	18.0 1.10	15.0 0.63	13.2 0.75	11.8 0.41	10.7 1.37	9.7 0.52	0 0
	14	40.0 3.16	35.8 4.36	26.3 1.37	18.0 2.28	0 0		43.7 1.75	30.8 0.75	25.7 1.51	23.9 1.41	19.2 0.41	18.3 1.21	15.2 0.98	11.8 1.47
4B	3	14.2 0.75	10.0 0	0 0	0 0	0 0		12.7 0.82	11.3 0.52	0 0	0 0	0 0	0 0	0 0	0 0
	7	30.2 0.41	22.0 1.90	13.3 1.03	0 0	0 0	No growth	28.7 1.37	19.0 0.63	16.3 0.52	12.7 1.51	11.7 0.82	10.5 0.55	0 0	0 0
	14	61.8 1.17	53.3 2.34	37.3 2.16	19.3 2.87	0 0		63.5 2.07	30.50 0.84	24.70 1.03	21.3 3.08	19.8 1.33	17.3 1.03	14.0 1.10	13.5 1.38

Table 11 (d)

Isolate	Incubation (days)	Pentachlorophenol (ppm)						Tri-n-butyl tin oxide (ppm)							
		C	1	5	10	15	20	C	5	10	15	20	25	30	35
9	3	20.2 0.41	14.5 0.84	0 0	0 0			25.3 2.07	10.2 0.41	0 0	0 0	0 0			
	7	56.8 3.87	41.2 2.64	20.7 5.13	0 0	No growth		80.2 3.6	21.0 0.63	11.2 0.41	0 0	0 0	No growth		
	14	83.0 0	83.0 0	65.0 10.14	0 0			83.0 0	32.7 1.86	16.7 0.52	12.7 0.52	0 0			
3	3	15.0 0	12.5 1.05	9.2 0.41	0 0	0 0		15.2 0.75	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	7	30.3 0.52	27.7 1.75	21.2 1.94	11.5 0.71	0 0	No growth	39.5 1.05	13.3 1.51	10.8 0.41	9.5 0.55	9.2 0.41	0 0	0 0	0 0
	14	64.5 1.23	61.0 5.52	55.0 4.77	36.5 0.71	0 0		81.5 2.36	20.3 1.75	16.0 1.27	12.5 1.52	11.83 1.17	11.3 1.75	13.0 0.89	11.8 0.75
1	3	13.8 0.75	0 0	0 0	0 0			13.5 0.84	0 0	0 0	0 0				
	7	42.7 1.97	31.7 2.25	13.3 1.50	0 0	No growth		38.2 1.94	10.7 0.82	0 0	0 0	No growth			
	14	83.0 0	79.3 3.45	49.5 1.0	0 0			82.0 1.67	16.5 0.84	12.0 1.27	0 0				
8	3	13.0 1.67	0 0					14.0 0.63	0 0	0 0	0 0				
	7	21.8 1.17	0 0	No growth				30.8 0.98	12.8 0.98	9.3 0.52	0 0	No growth			
	14	42.8 5.67	0 0					62.8 0.75	20.2 0.98	13.7 0.52	0 0				