THE MECHANISM OF INFECTION AND DECAY OF WINDOW JOINERY

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by

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To John G Savory and John F Levy

In recognition of the
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ABSTRACT

The mechanism of infection and decay of window joinery

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Samples of joinery from five sites, were examined either in service or soon after removal; none had received preservative treatment. Design defects allowing entry of water into joints were primary causes of decay and it was concluded that the preservative treatments currently advocated will not be adequate to provide protection unless improvements are also made in design. A high proportion of the decay was of the white rot type. The decay fungi isolated were slow acting, were shown to be tolerant to tri-n-butyltin oxide (TnBTO) and pentachlorophenol (PCP) and were not those normally associated with decay within buildings.

The process of colonisation of joinery has been investigated using simulated joinery units (L-joints) exposed out of contact with the ground. Untreated samples, and replicates treated with one per cent TnBTO or five per cent PCP by three minute immersion were destructively examined after periods of up to three years exposure. Moisture content, CO_2 production and permeability were recorded and the progress of bacterial and fungal colonisation was monitored.

Uptake of moisture from the unsealed joint was followed closely by microbial colonisation in the sequence bacteria, mould fungi, bluestains, soft rots and Basidiomycetes. Permeability assessment provided a sensitive measurement of the earliest effects of microbial activity. The majority of bluestain organisms were identified as Aureobasidium pullulans. A detailed study of the cultures showed they could be divided into sub-groups based on a combination of morphological and physiological features. PCP was more effective than TnBTO in retarding colonisation and attack; this may provide an indication of comparative efficacy in service.

Colonisation and attack of the simulated joinery units and, by inference, of joinery in service, is a complex process involving interactions between climatic influences, a wide range of micro-organisms, the timber substrate and, when present, the wood preservative.

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SECTION 1

GENERAL INTRODUCTION

1.1 HISTORICAL

The first reports of decay in exterior softwood joinery originated in the United States of America in the early 1930s. Hubert (1934) concluded that the number of failures due to decay was small and that, since it would be very difficult to change the design of the joint or to use waterproof glues, the use of wood preservatives or water repellents offered the best possibilities for decay control. This recommendation was followed, the National Woodwork Manufacturers Association "Seal of Approval" being inaugurated in 1938 and a water repellent preservative standard was added in 1950 (Lance, 1958). Treating solutions were required to contain a minimum of five per cent pentachlorophenol, 2-chloro-ortho-phenyl-phenol or tetrachlorophenol or mixtures thereof. Lance estimated that in 1957 over 85 per cent of all stock sash was treated according to the Standard.

In the United Kingdom, the major problem of joinery decay did not occur until much later; Cartwright and Findlay (1946) only considered the problem was noteworthy in high risk situations such as greenhouses. By 1958, there had been an increase in advisory enquiries, on joinery decay, received by the Princes Risborough Laboratory (then the Forest Products Research Laboratory); this was communicated to the Timber Research and Development Association (then the Timber Development Association) when they took over such enquiries (Savory, personal communication). After investigation, Oliver (1961) reported three noteworthy cases of premature failure although the eleven local authority architects contacted reported they were experiencing no real problems of decay. Further evidence accumulated, leading to various publications on the repair of defective joinery and preservative pretreatment of new or replacement joinery (Anon, 1966, 1967, 1968). Attempts were made to determine

the extent of the problem. Tack (1968) surveyed 10 estates, up to 14 years old, in the relatively dry south east of England and found that more than half of the frames examined had one or more lower joints sufficiently moist to allow the development of decay. In a postal survey of doors, in 1973, local authority maintenance departments were almost unanimously of the opinion that the amount of decay in external doors was such that preservative pretreatment would be desirable (PRL, unpublished data). Subsequently, 2 200 unprotected external doors were examined on 18 sites, $2\frac{1}{2}$ to 18 years old, ranging from the English south coast to Scotland; overall 38 per cent were decayed but in the 6-8 year age bracket, over 60 per cent were decayed on some sites (Soane, 1978). Detailed examination of a sample of the doors revealed a high incidence of moisture entry into centre and bottom rail joints as a result of bad design and a lack of sealing of the joints (Savory and Carey, 1975). They concluded that low cost panelled doors, as currently manufactured, were not suitable for external use in unprotected situations.

Prophylactic preservative treatment has been introduced gradually. As early as 1965 one major construction firm and one joinery manufacturer were pretreating joinery. Two years later Technical Note No 24 (Anon, 1967) recommended a treating concentration toxicity equivalent to that of five per cent pentachlorophenol (based on American experience) with suitable penetrating power and applied by specified processes. In 1969, the National House Builders Registration Council made preservative treatment mandatory for softwood window joinery of houses constructed by its members, and later (1975) extended the requirement to include non-flush softwood external doors. In 1971 the Property Services Agency made mandatory the preservative treatment of all external joinery for both new work and replacements, and various local authorities also specified preservative treatment. There is, however, still no national requirement for pretreatment for although recommendations were published by the British Standards Institution in CP 153:Part 2 (1970) and quoted in the revision of BS 1186:Part 1 (BSI, 1971), they were not made mandatory.

In Europe, joinery decay has also become a problem. Korf (1973) quoted German statistics showing that approximately 7 per cent of frames fitted between 1950 and 1965 were decayed; the figure was

probably higher in Holland, where mandatory pretreatment of non-durable species was introduced at the beginning of 1971, for all State subsidised housing. Henningsson (1977) concluded that houses in Sweden, less than 7 years old, were decayed to a great extent and quoted figures from Harmsen (1966, 1972 and 1976) showing the increasing importance of joinery decay in Denmark. Between 1946 and 1966 only 3.5 per cent of enquiries concerned joinery; during the period 1966 to 1971 the figure had risen to 19 per cent and for 1974 and 1975 stood at 28 per cent. Most recently, Fougerousse (1979) reveals a similar situation in France.

Decay has been attributed to the action of Basidiomycetes and various species have been listed by Duncan and Lombard (1965), Shields and Krzyzewski (1976) and Harmsen (cited by Henningsson, 1977). Lenzites trabea and Lenzites saepiaria are shown to be important in both North America and Europe. However, L. trabea has not been listed as occurring in the United Kingdom (Rea, 1922) and although L. saepiaria is not uncommon it is rare in buildings and when it does occur, the infection can usually be traced to imported timber infected before its arrival (Cartwright and Findlay, 1958). Alternative fungi are therefore likely to be responsible. Examination of decaying exterior hemlock doors (Savory and Carey, 1975) showed a high proportion of the fungi were of the white rot type; Phellinus contiguus, Coriolus versicolor, Sistotrema brinkmannii and isolates belonging to the genera Hyphoderma, Hyphodontia and Stereum were identified. Evidence showed infection had taken place after installation, decay due to the use of infected timber was a rare occurrence, and it must therefore occur as a result of the germination of air-borne spores.

Evaluation of the effectiveness of preservative treatments for external joinery has been attempted in various ways. The treating concentration of chemicals listed as acceptable in Technical Note No 24 (Anon, 1967) was derived by comparison of toxic values, determined in laboratory tests to BS 838:1961, with those of pentachlorophenol. This test method uses test blocks fully penetrated by preservative whereas, in practice, an envelope treatment is used in which the central portion remains untreated. Other test methods have been developed using test specimens treated in this way

(Becker and Starfinger, 1971; Dickinson, 1976; Savory and Carey, 1976). It was concluded (Baker et al, 1977) that the initial toxic efficacy of most of the recommended British joinery preservative formulations, when applied by 3 minute immersion, conferred, to pine sapwood, a decay resistance at least equivalent to that of pine heartwood and that double-vacuum treatments could be better still. Savory and Carey (1976) using the germination of basidiospores of Lenzites trabea (FPRL No 108E) showed them to be as tolerant, to the range of preservatives used in joinery treatments, as the mycelium of the same organism growing from a malt agar substrate.

Many exposure trials of actual windows or test units containing a single joint have been undertaken; the methodology has been summarised by Fougerousse (1976a). The evaluation of these trials has been based on the external condition of the units and, in some cases, their internal condition as revealed by deep sawing (eg Verrall, 1961; Scheffer et al, 1963; Sedziak et al, 1970; Shields and Krzyzewski, 1975 and 1976; Feist and Mraz, 1978; Purslow and Williams, 1978; Amburgey and Johnson, 1979). These studies are providing valuable information on the comparative performance of many preservative treatments.

1.2 THE CURRENT STUDY

The historical account given above indicates that much research effort has been devoted to the problem of joinery decay and to the evaluation of preservative treatments to prevent decay. Consideration of the ecology of joinery decay is an area which has been neglected although an ecological approach has been adopted with timber in service in many other situations, for example, fence posts and stakes (Kaarik, 1967 and 1968; Butcher, 1968 and 1971; Banerjee and Levy, 1971; Clubbe, 1980a), water cooling towers (Eaton 1969 and 1972; Eaton and Jones, 1971a and 1971b), mines (Levy and Lloyd, 1960) and the marine environment (Jones, 1963 and 1968). The colonisation of timber in natural habitats such as logs and branches (Chesters, 1950), softwood stumps (Meredith, 1959 and 1960; Halleksala, 1977) and hardwood trunks (Mangenot, 1952) and stumps (Rayner, 1977a and 1977b) has received similar attention.

Work by Morton (1975) and Morton and Eggins (1976a, 1976b, 1976c, 1977, 1979) has been concerned with the ecology of joinery but unfortunately the direct relevance of much of this work to joinery in service is in doubt. Material has been exposed without a surface finish, the aerial parts of stakes have been equated with an out of ground contact situation and most work has been concentrated on the thermophilic microfungal flora and therefore has neglected the Basidiomycetes. The large scale isolation of the flora of exposure trial units has been attempted in one series of experiments (Sedziak et al, 1970; Shields and Krzyzewski, 1975 and 1976) but only after many years exposure; another study (Savory et al, 1977) involved destructive examination, after $4\frac{1}{2}$ years exposure, using both biological and physical methods. This investigation demonstrated a potential method for evaluating preservative performance after relatively short periods of exposure and indicated the need for further more detailed work.

The present study of the mechanism of infection and decay of window joinery is divided into two major parts. First, a detailed examination has been carried out on prematurely decayed window joinery from various sites. The cause of the decay has been investigated and many of the causative organisms isolated and characterised. This work is reported in Section 2. Secondly, following Savory et al (1977), two series of simulated joinery components (L-joints) have been exposed, out of contact with the ground, and examined in detail by a variety of methods. These studies are reported in Sections 3 and 4. Details of the fungi isolated from the second series L-joints are contained in Section 5.

As a result of queries raised during the course of the work, ad hoc experiments were carried out. These are recorded in Section 6 and referred to in other parts of the text, where appropriate.

The development of methodology for monitoring changes in permeability of the test samples and for assessing carbon dioxide production and bacterial numbers is presented in Appendices A and B respectively.

Tables, followed by figures, are presented together, in numerical order, at the end of each section.

SECTION 2

OBSERVATIONS ON WINDOW JOINERY IN SERVICE

2.1 INTRODUCTION

Although extensive decay of window joinery has been reported for many housing developments in the United Kingdom, no systemmatic study of a significant sample of decay frames has been undertaken. Observations at the Princes Risborough Laboratory (PRL) have been confined, largely, to single samples received through the post; information gleaned from them (PRL, unpublished data) may be summarised as follows:

a Coniophora puteana, the most common decay fungus elsewhere in buildings (Cartwright and Findlay, 1958) and the organism used for the comparison of the toxicity of joinery preservatives in Technical Note 24 (Anon, 1967), has rarely been positively identified in joinery.

b A significant amount of white rot occurs.

c The situation in the United Kingdom would appear to be different from Denmark where Lenzites trabea is a major cause of decay of softwood joinery largely constructed of Norway spruce (Picea abies (L) Karst) (Harmsen, cited by Henningsson, 1977).

There was an obvious need to validate these observations by examining significant numbers of samples from sites where premature failure had occurred. This was undertaken in the present study which has been divided into the following three aspects.

First (section 2.2), observations were made on joinery, in service or recently removed from service at five sites, none of which had received preservative treatment prior to use. The sites varied with respect to age, type of housing, location and exposure as detailed in Table 2.1.

Much of the joinery was examined in detail, recording, where possible, features such as the location of decay, details of the construction and signs of water entry. However, the joinery was not always examined under ideal conditions, so the approach had to allow for this; details of the methods employed are, therefore, given with the report from each site.

Secondly (section 2.3), attempts were made to isolate the Basidiomycetes causing active decay, at the time of the examination, in the joinery from four sites. The isolates obtained were examined closely in an attempt to identify them, and subjected to a series of experiments to characterise their activity in terms of decay type, decay capacity and preservative tolerance.

Thirdly (section 2.4), experiments were carried out on a single window frame to examine the changes which had occurred during service. These included any predisposition to decay as a result of the action of non-wood rotting organisms, monitored using basidiospore germination, and changes in the permeability of the timber. It is essential that checks such as this be made on joinery in service to allow comparison with similar data from simulated joinery units (L-joints) and thus aid the assessment of the relevance of these units to the practical situation.

2.2 EXAMINATION OF DECAYED JOINERY

2.2.1 Hayes

2.2.1.1 Site conditions

Joinery on this site was inspected in June 1975. The estate, owned by Hillingdon Borough Council, was completed in 1968 and consisted of tower blocks with metal window frames which did not need attention together with 234 flats and maisonettes in three and four storey blocks (fig 2.1) in which wooden joinery was used. The extent of decay in first, second and third floor windows, which was worse on the south and west aspects, had led to the decision that all exterior joinery on these floors needed replacement. Ground floor joinery, protected by a 12 inch overhang (fig 2.2) was less affected and was being replaced only where necessary. Staircase window joinery units, having no opening lights, were also giving less trouble.

2.2.1.2 Method of examination

Observations were made on twenty 4ft 8 in wide centre pivot hung opening lights, removed in a group from the building by the contractors and subsequently stored under cover, with a view to recording features associated with the failure, and the incidence of different types of decay.

2.2.1.3 Results

All the twenty windows were constructed from redwood (Pinus sylvestris L) with comb joints fixed with a single aluminium star dowel. Most of the beading was ramin (Gonystylus macrophyllum (Miq) Airy Shaw) though one utile (Entandrophragma utile (Dawe and Sprague) Sprague) bead was noted. The beading, with a T-shaped cross-section was screwed into rebates cut into the outside of the stiles below the hinges and along the underside of the bottom rail to act as a "stop" on closure of the light and to provide a throated weather bar along the bottom rail (fig 2.3).

The beading was fixed to the frame by screws through the throating. Neither the rebate nor the joint surface of the beading had been primed, and there was no indication that the joint had been glued. Accumulation of dirt in the horizontal joint gave indications of its openness and that water penetration had occurred on a considerable scale.

Two of the twenty windows showed no decay; in the remainder decay was confined to the lower rail region and the stiles jointed into it (fig 2.4). Examination of some less decayed material indicated decay initiated at the interface between the frame and the lower beading. Spread of decay along the grain of the stile was markedly greater than that across the grain of the rail. Decay was less marked within the rail/stile joint possibly indicating less water penetration at this point; in the joints examined the putty was still sound at the corner.

Many frames showed evidence of attack by more than one fungus; the incidence of the various types is given in Table 2.2.

2.2.1.4 Discussion

The need to replace all of the external joinery on the upper floors of the flats and maisonettes contrasts markedly with the relatively sound condition of the ground floor external joinery and gives an indication of the extent of protection given by the 12 inch overhang (fig 2.2).

The rapid and extensive decay in the opening lights (there was less problem with fixed lights) can be related directly to the mode of insertion of the beading which, along the lower rail, served to trap moisture (fig 2.3). No trace of glue was found in the joint; any glue used must therefore have failed to maintain the integrity of the joint. It is, however, unlikely that a watertight seal could be maintained along the entire length of such a joint by use of glue alone.

The incidence of white rot was at least twice the incidence of brown rot. This may be due to the use of ramin, a perishable species (Farmer, 1972) for much of the beading; it is known that many hardwoods are more susceptible to white rot than to brown rot (Anon,1972a). The selection of ramin may not have increased the overall incidence of decay since one severely attacked frame was supplied with utile beading, a durable species (Farmer, 1972), which had remained sound.

2.2.2 Gosport

2.2.2.1 Site conditions

The site, inspected in October 1975,was selected following contact during a survey of decay in doors (Soane, 1978). The site, approximately one mile from the coast and on level ground, consisted of both two storey terraced houses and three to four storey blocks of flats (fig 2.5), aged 8-9 years at the time of inspection. A number of windows were observed and photographed in situ (fig 2.6 and 2.7). Twelve frames selected at random from an uncovered pile of those already removed from the buildings were transported to the laboratory for detailed examination.

2.2.2.2 Method of examination

The complete frames were examined and any visible defects noted. The upper half of each window was then sawn off and retained for future use. The lower halves were dissected, splitting where necessary to ensure thorough examination. Details of construction, timber species, water

penetration, glue failure and decay were recorded on specially designed sheets (fig 2.8).

2.2.2.3 Results

Two different designs of frame were represented; type A (10 frames) consisted of a two piece sill, with additional weather strips on the lower part of both stiles. The timber was a mixture of Scots pine (Pinus sylvestris L) and commercial hemlock {composed of western hemlock (Tsuga heterophylla (Rof) Sarg) and fir (Abies sp)} apparently selected at random. Type B (2 frames) consisted of a single piece bottom rail (there was no projection to form a sill); the timber was Douglas fir (Pseudotsuga menziesii (Mirb) Franco). Sections through the bottom rail of both types can be seen in Fig 2.9. The glazing beads were hardwood (red meranti (Shorea spp) where checked) and all were on the outside of the windows; many were almost devoid of paint. To help prevent moisture penetration, the major vertical and horizontal join in each lower joint of type A windows had been drilled out and filled with mastic (fig 2.10) which sometimes penetrated the glazing rebate; beading along the sill was also bedded in mastic.

There was evidence of water penetration via the backfill putty, under the glazing beads, into the lower joints of the frames, and into the horizontal joint in the sill (type A only) of all the windows examined.

Details of glue failure and the distribution and type of decay are given in Table 2.3.

2.2.2.4 Discussion

The windows were selected at random as representative of the type of failure present in the joinery on this site. The two designs represented both showed failure. Type A was a complex design (fig 2.10). The manufacturer was aware of the need to prevent moisture penetration into the joints and had attempted to seal these with plugs of mastic; this had not been successful. The beading along the sill had also been bedded in mastic; again water penetration had occurred. No attempt had been made to seal the horizontal joint in the two part sill. The horizontal collecting surface on the top of the lower member served to funnel water into this joint and the effect is clearly demonstrated by eight of the ten windows observed showing decay within this joint.

Type B was a much simpler design; no attempt had been made to seal the joints to prevent ingress of moisture. One frame (marked No 1) had failed principally around the glazing rebate of the sill. Water entry at this point was probably accentuated by the horizontal upper surface of the sill (fig 2.9) which would not shed water readily. The other frame (marked No 12) only showed pockets of decay in the sill remote from the normal paths of water entry. It is suggested that this decay was the result of standing tree infection, sometimes present in Douglas fir shipments (Roff et al, 1974), which was not eradicated during drying and was activated by wetting in service.

Glue failure had occurred throughout nine joints and through the greater part of a further 13 joints; only one was largely sound. The glue type was most probably UF type and therefore not greatly resistant to the effects of moisture.

Decay of both white rot and brown rot types was present in most windows; if the window marked No 12 is omitted (since decay was not due to infection in service) the frequency of occurrence of white rot and brown rot is the same (8 out of 12 windows).

2.2.3 Larkhill

2.2.3.1 Site conditions

This site is characterised by being very exposed on top of a rise in the centre of Salisbury Plain. The site, inspected in February 1976, was first visited during a survey of decay in doors (reported later by Soane, 1978) during which the problem of decay in window joinery was revealed. Major problems of decay were only encountered in the newest houses, two storey terraced houses 6-8 years old, while the older houses, two storey semi-detached houses, with a front porch, up to 46 years old were causing much less concern. The opportunity was therefore taken to examine both types of joinery in situ and to obtain samples for more detailed observations in the laboratory.

2.2.3.2 Examination of old doors in situ

2.2.3.2.1 Method

Interest centred on houses in Bidolph Road, constructed in 1930 and Bingham Road, constructed in 1931. Due to the extremely cold conditions at the time of inspection most observations were made on single unoccupied houses in each road. It was found that porches had been added, shortly after construction, over the front doors of houses in Bingham Road, and both front and back doors of houses in Bidolph Road (a more exposed situation). In all cases the porches were provided with an additional door set at right angles to the line of the outer wall.

2.2.3.2.2 Observations

The doors were constructed with wedged mortise and tenon joints, centre and bottom rails having a double tenon. The paint film had broken along the line of the wide centre rail joints, even in recently redecorated houses. It could not be ascertained if joints had been primed before assembly.

Wood samples taken from the hinge stile of four doors yielded three samples of pine sapwood and one of pine heartwood.

Despite long standing paint loss, established by the considerably weathered surface of the exposed wood, on the north facing back door of No 11 Bingham Road, the highest recorded moisture content in the stile was 17 per cent adjacent to the lower rail joints. There was some decay present in the centre rail of this door (fig 2.11). Similar measurements on the west facing front door yielded 13 per cent at upper joints, 16 per cent at centre joints and 19/20 per cent at lower joints. Measurements in the upper part of the bottom rail near the joints yielded 22 per cent on the hinge side and 28 per cent on the lock side. Slight decay was present in this zone. Both doors were fitted with rim locks.

2.2.3.2.3 Discussion

Although a recent inspection revealed a proportion of these old doors are decayed, some to an extent which warrants their replacement, it is known that there had been no previous major replacement over their 45/46 years of service. The reason for this longevity was not elucidated during examination. Breaks in the paintwork were evident thus giving moisture access to the timber. Perishable sapwood was present in three of the doors tested although the proportion cannot be established using such a small sample. Moisture contents were not high in both doors of the one house examined although some decay was present. This may be indicative of sealing of the joints at the time of manufacture but this cannot be confirmed until doors become available for more detailed examination. Alternatively it may reflect on the inadequacy of the moisture meter for detecting internal moisture contents.

2.2.3.3 Examination of modern joinery in situ

2.2.3.3.1 Method

A number of windows were examined in the two storey terraced houses (Jesperson Houses) completed in 1968, and orientated facing north/south or east/west. A few doors showing decay were also examined; however the extent of decay had been the subject of a recent survey (BRL, unpublished report) and an extensive programme of replacement was already underway.

2.2.3.3.2 Observations

Extensive decay was found in numerous windows often associated with joints (fig 2.12), but sometimes in the sill (fig 2.13) where the centrally positioned butt joint appeared to adversely influence the situation. A significant proportion was due to white rot organisms. Decay in the doors followed the pattern established previously (Savory and Carey, 1975).

2.2.3.3.3 Discussion

No detailed examination of this joinery was possible on site due to the occupancy of the dwellings and the inclement weather. Since replacement of numerous windows was due to take place in the near future, a supply of defective frames was arranged for laboratory examination.

2.2.3.4 Laboratory examination

2.2.3.4.1 Method

Fifteen frames from the Jesperson Houses (completed 1968) were examined in the laboratory. Since the frames were destined for testing the efficacy of preservative treatments applied in situ to joinery, no destructive testing could be undertaken at this time. Cross-sections through the stile and bottom rail/sill are shown in fig 2.14.

The moisture content at numerous positions was measured using a Protimeter Timbermaster. A small groove was chiselled across the 'wall' face of each member at approximately mid-length, to expose fresh timber. A mixture of 1 part 0.5 per cent w/w o-anisidine in 0.65 per cent hydrochloric acid and 1 part 10 per cent aqueous sodium nitrite was applied to each groove to detect the presence of heartwood and sapwood. All measurements were recorded on specially designed record sheets (fig 2.15). The presence of decay and other defects was also noted. Random samples were sent for identification of timber species.

2.2.3.4.2 Results

Details of moisture content readings are recorded in Table 2.4. The extent of decay and proportion of sapwood in the main members are recorded in Table 2.5. All timber samples proved to be Scots pine type.

2.2.3.4.3 Discussion

A large number of moisture content readings were in excess of twenty per cent and therefore the frames were susceptible to decay in these areas. These elevated readings were largely confined to the lower parts of the windows but a few were recorded in the upper parts. This pattern of moisture reflects the relative exposure of the various parts of the windows; the small overhang at the top of the window gives some protection while the horizontal surface of the sill serves to collect water.

Decay appeared to be confined to the sapwood, but since the frames had to remain intact, for future use in other work, the extent of internal decay could not be ascertained. High moisture contents did not correlate with either the presence of sapwood or of decay. This is a problem encountered when a survey is made; the conditions found on a single occasion may not be representative of the situation over a period of time.

2.2.4 Cambridge

2.2.4.1 Site conditions

The development situated in one of the drier parts of the country consisted of two three-storey blocks of privately owned flats (fig 2.16) then aged between seven and ten years. One softwood window frame was brought to the laboratory; the causative organism of an active brown rot in one joint was isolated and tentatively identified as Poria calcea. The surveyors kindly agreed to supply further samples of decayed joinery during the replacement programme. To ensure the correct type of sample was being selected the site was visited in April 1976. The opportunity was taken to carry out a partial survey of existing joinery to establish the extent of failure and the type of decay.

2.2.4.2 Examination in situ

2.2.4.2.1 Method

Ten window frames or French door/window units were examined on each elevation (orientated north, south, east and west) of one of the blocks; only ground floor units were examined. Several windows set back from the main faces of the building were not included as it was felt they occupied a more protected situation.

Using a bradawl, the lower joints of window frames and the centre and lower rail joints of French door/window units were probed for the presence of decay and the extent of spread. Since the windows were all being replaced it was possible to probe deeply to detect decay without fear of causing unnecessary damage. Where possible the type of decay was recorded and any special features noted. Top joints were not examined; they were physically out-of-reach and experience has established failure though decay is rare in these joints.

2.2.4.2.2 Results

A summary of results is presented in Table 2.6. More detailed results are presented in Table 2.7 for the window frames (including the centre rail joints of French windows) and in Table 2.8 for the complete French window/door units.

2.2.4.2.3 Discussion

Observations in this survey were made only on the ground floor joinery. From the ground, it appeared that the joinery on the upper floors may have been decayed to a greater extent; the results therefore may be a slight underestimate of the situation as a whole.

Of the 80 joints included in Table 2.7, 42 were badly decayed (52.5%) and an additional 18 were recorded as having slight decay (22.5%). For practical purposes these data have been summarised in Table 2.6 in terms of the numbers of windows affected in one or both joints. This summary shows only six windows (15 per cent) to be free from decay and a further six (15 per cent) with slight decay in one or both joints and not therefore in need of immediate replacement. The remaining 70 per cent of the windows were in need of replacement after 7-10 years service. There was some variation with elevation; of the 12 windows not requiring immediate replacement, 5 faced north, 3 south and 2 each east and west. However, with this small sample little can be concluded from this distribution.

As recorded in Table 2.7 a number of the window sills showed advanced decay sometimes for their entire length. Observation suggested this may have been due to a water channel cut into the sill towards each end (fig 2.17). The sharp corners around the channel would induce failure of the paint film and enable easy access of water into the sill.

The presence of white rot was detected in 40 joints and brown rot in 17 joints; in a further 11 cases, often the early stages of decay, the rot type could not be identified. This high incidence of white rot has also been detected in exterior doors (Savory and Carey,1975) but is in marked contrast to the almost exclusive brown rot of softwood elsewhere in buildings (Cartwright and Findlay, 1958). On this site where the joinery is entirely softwood, with the exception of hardwood sills in French door/window units, the influence of hardwood in selecting white rots can be discounted.

2.2.4.3 Laboratory examination

2.2.4.3.1 Method

Eleven samples, some collected at the time of the site visit and some subsequently made available, were examined in the laboratory. They consisted of parts of softwood frames to metal window units or timber French door/window units. Each sample was examined, after splitting where necessary to improve observation; timber species, water penetration, glue failure and the type and extent of decay plus any other relevant features were recorded on data sheets (fig 2.8).

2.2.4.3.2 Results

A brief description of each sample together with the type of decay present is given in Table 2.9. Details of the construction are shown in Figs 2.18 and 2.19. The timber was all pine (Scots pine type) with the exception of the hardwood sills to the French door/window units (samples 1 and 2), which were keruing (Dipterocarpus sp). There were indications of water penetration into all joints though to a lesser extent in top joints. The glue used had failed throughout all the joints examined although in some cases traces remained but not in sufficient quantity to permit identification of the type.

2.2.4.3.3 Discussion

The construction used for the French door/window units (fig 2.18) created various water traps which resulted in the pattern of decay observed in both samples examined. Major trouble centred on the joints between the wide bottom rail and the uprights; the bottom rail/sill joint also contributed to the failures and in places resulted in decay of the more durable hardwood sill. Decay occurred around the glazing rebate in one sample.

Detailed observations of the softwood frames to metal window units (samples 3-11) confirm those made on site, that the advanced decay of the sills, particularly along the front edge, is apparently associated with the water channels near either end. Less advanced decay was also present in the majority of bottom joints but not in any of the five top joints examined.

Three of the samples bore fruitbodies of the brown rot Dacryomyces deliquescens; this fungus has not previously been encountered so frequently. Most samples contained both brown rot and white rot giving an incidence of 10 out of 11 for both types. This is somewhat different from the figures of 40 white rot to 17 brown rot recorded in the site survey, but may only indicate the presence of more brown rot in the less accessible portions of the frames.

Three samples showed carpenter bee (Megachile sp) activity in decayed areas; these bees can only excavate in previously decayed wood and therefore do not constitute any hazard to the frames.

2.2.5 Chatham

2.2.5.1 Site conditions

During a site visit to Chattenden Barracks in January 1977 advantage was taken of the opportunity to make observations on the general condition of the joinery. The site, on a slight hill, was situated some 2 miles from the coast. The dwellings were 2 storey terraced houses and 3 storey blocks of flats 10 years old when inspected (fig 2.20).

2.2.5.2 Observations

Decay had occurred in the lower joints of a large proportion of the centre pivot hung opening lights examined although there was wide variation in the extent of the damage. Decay had also occurred in the centre of the lower rail of a proportion of the windows (fig 2.21); examination in the laboratory showed the problem was associated with the rebate cut to house the closing mechanism. In some frames this penetrated the glazing rebate (fig 2.22). Since the glazing beads were not adequately weathered and were not sealed there was an easy path for the entry of moisture into the bottom rail, facilitated by the end grain surfaces in the closing mechanism rebate (fig 2.23).

2.2.5.3 Discussion

The pattern of attack of these frames, with decayed lower rail joints and sound top joints, conforms to the pattern previously established. The decay associated with the closing mechanism serves to emphasise

the need for careful consideration of design details. Use of this complicated closing mechanism, requiring a large proportion of the cross-section of the lower rail to be cut away to house the mechanism, has introduced a further path for moisture entry as well as reducing initial strength. More simple closing mechanisms already in common use on this type of window do not have these drawbacks and have lower initial and maintenance costs.

2.2.6 Discussion on decayed joinery observations

The observations on joinery after 7 to 10 years service provide a depressingly monotonous picture of water penetration and decay. Considering the detailed observations first, the high incidence of decay of the white rot type is of great interest. Data on the incidence of the decay types recorded on the various sites have been summarised in Table 2.10. The two surveys conducted on site (Hayes and Cambridge) show a higher proportion of white rot than brown rot (approximately 2.5 to 1) while the surveys conducted in the laboratory (Gosport and Cambridge) show equal amounts of both. This suggests much of the brown rot is concealed within the timber or is less severe than the accompanying white rot. It was evident from samples examined in the past, that a significant amount of white rot was present in window joinery, but it was not appreciated that the proportion was so high. A similar situation has been shown to exist in softwood exterior doors (Savory and Carey, 1975) but here the use of hardwood as plywood, dowels or beading, could have had an influence. It is well documented (Anon, 1972a) that hardwoods are more susceptible to attack by white rots. In two sites covered in detail by the present survey (Gosport and Cambridge) there was no hardwood component (except keruing sills in French door/window units at Cambridge) but the proportion of white rot remained high.

Considering the observations in general, there is certain evidence to suggest that the problem of decay is worse among properties in the seven to ten year age group than among older properties; a similar situation has been noted in Sweden (Henningsson, 1977). The obvious conclusion that the quality of joinery has recently sharply deteriorated requires further consideration.

It has been suggested (PRL Technical Note 28, Anon 1972b) that certain modern building practices cause greater exposure of exterior joinery, for example the omission of eaves and the insertion of frames in the outer leaf of brickwork. In addition higher standards of heating coupled with lower standards of ventilation have increased the incidence of condensation, which has been implicated in joinery decay (Tack, 1968). However, the major cause of failure appears to be the joinery itself.

Traditionally joinery in the United Kingdom has been constructed from redwood (Scots pine) heartwood. Over the years the quality of joinery grade timber has deteriorated as virgin forests have been cleared and second growth trees utilised. This is not a problem peculiar to the UK or to our generation; Hubert (1934) also bemoaned the declining standards of timber used for joinery in the USA. The inclusion of sapwood in joinery quality redwood is a fact of life (fig 2.24) and in the current studies some large section material examined contained the entire heartwood produced by a tree. This accounted for no more than 50 per cent of the cross-section and the less durable juvenile heartwood in the region around the pith was, therefore, also included (fig 2.25). The overall natural durability of joinery constructed from this material is reduced to that of the included sapwood. The time has come to consider utilisation of alternative timbers; many have been documented by Webster (1978). One timber readily available and being grown and imported in increasing amounts is whitewood (spruce). Although this timber would never have been used in the past because of its inferiority to redwood heartwood, it should now (Morgan, 1975) be considered as being superior to redwood sapwood, since it absorbs water less readily, and with modern technology can be adequately treated with preservatives.

Over the period of increasing problems, the production of joinery has become increasingly automated, a fact which in itself, should not have reduced quality. However, it seems probable that the craft skills involved in hand production of joinery have not been appreciated and therefore have not been translated into modern technology. For example the practice of coating all joint surfaces with a lead based primer before assembly probably effectively sealed the joint against entry of moisture and therefore prevented decay. The toxicity of the lead

itself may have played a rôle. In modern joinery UF glue, supplemented by metal fixing pins is commonly used to maintain the integrity of joints. However, when the paint film across the joint fails water entering the joint will lead to glue failure which will facilitate further entry of water. In many cases the quantity of glue applied is insufficient to seal the joint, even prior to glue failure. This is particularly evident in doors with dowelled joints (Savory and Carey, 1975) where the only glue between the main frame components is that which squeezes out from around the dowels (fig 2.26). We cannot return to the use of lead primer on health and safety grounds but use of a waterproof glue eg a PF or resorcinol type, in sufficient quantities to effectively seal the joint could duplicate its effect. The commercial considerations of cost of the glue, problems with the shorter "pot-life" and the additional process of cleaning glue squeezed out on assembly of the joints should not be allowed to outweight the improved performance of such joinery in service and hence the lower maintenance costs.

Various design details have been shown to accentuate the problems of decay eg the weatherbars at Hayes, the two-part sills at Gosport and the closing mechanism rebates at Chatham. With the advantages of hind-sight, such details which produce very obvious water traps should never have left the drawing board; they show their designers had insufficient understanding of the necessity to keep wood lacking natural durability dry, to ensure good service life, or where this is impractical, to provide adequate preservative treatment. It is however much easier to point out the faults in joinery designs that have failed, than it is to create good designs anew; in one case examined, the water channels at Cambridge, a feature aimed at reducing water entry had apparently had the reverse effect and resulted in increased decay.

All the opening lights examined were of the centre pivot hung type. This was not intentional; the sites were examined because their problems had been brought to the attention of PRL. This could indicate that this type of window is particularly susceptible to decay. On theoretical grounds (fig 2.27) it is obvious that surfaces must be "weathered" to an impossible degree to prevent puddle formation in the glass/bottom rail area when the window is open. If there are no small opening lights suitable to provide ventilation, and this often appears

to be the case, the large windows will probably be left open for considerable periods and the importance of such "weathering" or rather lack of it, becomes obvious. Further observations are required but if decay is found to be generally higher in this type of window, consideration should be given to the use of alternative designs.

Inevitably consideration of the problems associated with the joinery examined leads to the question "what next?". Preservation of joinery has been used increasingly over the years; the introduction of treatment has recently been documented (Savory and Carey, 1978, 1979). Undoubtedly adequate preservative treatment will provide protection but looking at the severity of exposure introduced by bad designs one must question whether treatments currently in use are adequate. The period over which wide scale treatments have been carried out (NHBC regulations were introduced in 1969) barely exceeds the most common age of failure of untreated joinery (7-9 years) and therefore treatments providing even marginal improvement in performance are unlikely to have failed by the present time. A destructive survey of the present condition of treated joinery would be required before further comment can be made on this point.

Recently published work (Feist and Mraz, 1978) shows that over twenty years of exposure trial conditions, the use of water repellent alone has provided external protection equivalent to that of a water repellent preservative containing five per cent pentachlorophenol and better physical protection than five per cent PCP alone. No internal examination was carried out.

In summary, it appears that the future should lie in protection as much as in preservation, the protection coming from good design, effective sealing of joints and the use of improved water repellents.

2.3 ISOLATION AND CHARACTERISATION OF BASIDIOMYCETES FROM DECAYED JOINERY

2.3.1 Materials

2.3.1.1 Benomyl/malt agar (Ben 10)

40 g powdered malt extract (Oxoid L39)
20 g agar
50 ml benlate suspension (containing 200 ppm ai benomyl)
deionised water to 1 litre

The ingredients were mixed and autoclave sterilised for 20 minutes at 121°C. 20 ml aliquots were dispensed, aseptically, into 90 mm diameter disposable plastic petri dishes or approximately 10 ml aliquots were dispensed into 19 mm diameter test tubes, plugged, reautoclaved and allowed to set, whilst sloped to provide the maximum surface area.

2.3.1.2 Five or two per cent malt agar

40 or 16 g powdered malt extract (Oxoid L39)
20 g agar
deionised water to 1 litre

The malt agars were prepared by the same method as the Ben 10 agar.

2.3.1.3 Sawdust medium (Badcock, 1943)

100 g spruce sawdust
3 g maize meal
2 g bone meal
deionised water to mix

The water was gradually added to the dry ingredients; when the sawdust ~~moisture~~ just stuck together when gently squeezed (approximately 200per cent moisture content) it was used to fill 20-30 mm diameter glass boiling tubes. The mixture was compacted by gently tapping the base of the tube on a hard surface at intervals during filling. The tubes were plugged with cotton wool and autoclave sterilised for 30 minutes at 121°C.

2.3.1.4 Gallic acid agar (Bavendamm, 1928)

15 g powdered malt extract (Oxoid L39)
20 g agar
5 g gallic acid
deionised water to 1 litre

The gallic acid was dissolved in a small quantity of the water; the remaining ingredients were mixed. Both parts were autoclave sterilised for 20 minutes at 121°C then mixed and dispensed, aseptically, in 20 ml aliquots into 90 mm disposable plastic petri dishes. The gallic acid was autoclaved separately to prevent acid hydrolysis of the agar.

2.3.2 Method

2.3.2.1 Isolations

After the samples of window joinery had been examined (2.2) match-stick size samples were cut, using a 6 mm wide U-shaped gouge, from the positions selected as most likely to yield the causative organism ie the advancing edge of the decay (Carey, 1975). With joinery from Larkhill a number of positions were selected on a fixed plan basis (fig 2.28) with additional positions "of choice". The samples were initially placed, four per dish of Ben 10 agar (2.3.1.1), partly submerged in the agar to give the greatest chance of growth by the organisms (Carey, 1975). Isolates were purified by sub-culturing on dishes of 5 or 2 per cent malt agar (2.3.1.2). At this stage micro-scopic observations were made which allowed definite non-Basidiomycete cultures to be rejected. Pure cultures of the remainder were transferred to tubes of 5 or 2 per cent malt agar for maintenance, and sawdust tubes (2.3.1.3) to detect white rot or brown rot reactions, followed by the possible development of fruitbodies to aid in identification (Carey, 1975).

2.3.2.2 Growth rate on malt agar

A number of the organisms isolated from the Gosport joinery were inoculated onto 2 dishes each of 5 per cent malt agar (2.3.1.2) and incubated at 20°C. The growth was measured on two diameters, at 90 degrees to one another, after 6 and 11 days.

2.3.2.3 Growth on gallic acid agar

A number of the organisms isolated from the Gosport joinery were inoculated onto dishes of gallic acid agar (2.3.1.4) and incubated at 22°C. Observations were made after 2 and 7 days.

2.3.2.4 Decay capacity

Blocks of Scots pine sapwood measuring 30 x 10 x 5 mm were numbered, oven dried for 18 hours at 103°C and weighed, then autoclave sterilised for 20 minutes at 121°C. Three blocks were planted, on previously sterilised Netlon mesh, over each of two cultures of each fungus, actively growing on 5 per cent malt agar (2.3.1.2) in petri dishes. New, named isolates from various sources were included as control organisms. All dishes were incubated for 6 weeks at 22°C. The overgrowth of the blocks by the fungi was noted; the blocks were then cleaned, weighed, oven dried for 18 hours at 103°C and reweighed. The final moisture content and percentage loss in weight of each block was calculated.

2.3.2.5 Preservative tolerance

Solutions of tri-n-butyltin oxide (TnBTO) were prepared at 0.01, 0.03, 0.1 and 0.3 per cent w/w in 60-80 petroleum ether, and solutions of pentachlorophenol (PCP) at 0.03, 0.1, 0.3 and 1.0 per cent w/w in acetone. Black filter papers (Whatman No 29) were dipped in preservative solution, blotted to remove excess liquid and allowed to dry flat, supported by Netlon mesh, to aid even drying. Each filter paper was sterilised by exposure to propylene oxide vapour for 24 hours, ventilated in a stream of sterile air for 48 hours then placed in a 90 mm sterile plastic petri dish and moistened with 0.75 ml sterile deionised water. One 6 mm diameter plug cut from an actively growing petri dish culture of each fungus was placed, mycelium side up, on one paper treated at each of the preservative/concentrations used plus an untreated paper as a control. Three such plugs could be placed on each paper. The plates were incubated, in polythene bags to prevent drying out, at 22°C for 3 weeks. The growth was then measured across two diameters at 90 degrees to one another. Coniophora puteana (FPRL No 11E) and Coriolus versicolor (FPRL No 28A) were used as control fungi.

2.3.3 Results

The numbers of attempts and the rates of successful isolations of Basidiomycetes have been calculated for each of the four sites and are presented in Table 2.11. Details of the organisms isolated are presented in Tables 2.12 to 2.15 for Hayes, Gosport, Cambridge and Larkhill respectively, and the rot types summarised in Table 2.16.

Individual measurements of colony diameter together with the mean values after each period of incubation and the calculated growth rate in the intervening period are presented in Table 2.17.

Observations after 2 and 7 days growth on gallic acid agar are presented in Table 2.18, together with the rot types determined by growth on sawdust medium.

The mean and the range of values obtained in the weight loss tests are presented in Table 2.19. The final moisture contents were suitable for fungal attack.

The toxic values established in the filter paper toxicity tests are presented in Table 2.20.

2.3.4 Discussion

2.3.4.1 Isolations

The number of isolations attempted varied between the samples; the success rate was also variable. These data have been summarised in Table 2.11. One interesting feature is that the isolations from chosen positions at Larkhill were twice as successful as those taken using the set pattern. However, a number of successful isolates from the set pattern were taken from areas of apparently sound wood; these would have been missed using positions of choice alone and show the benefits of using the system.

Details of the organisms isolated are presented in Tables 2.12 to 2.15; the types of decay produced by most isolates have been characterised and are summarised in Table 2.16. Although there is variation between the sites, the totals show more white rots than brown rots which correlates with the incidence of these types of decay revealed by detailed laboratory examination (table 2.10). The high incidence of Sistotrema brinkmannii at Larkhill is

interesting; ten of the isolates grew from apparently sound timber while the others were associated with discoloration or decay. The exact role of this organism is unknown but is discussed further in Section 3.5.4.

Fomes pinicola, which does not occur naturally in the United Kingdom, was isolated from five pockets of brown rot in a single Douglas fir bottom rail. When it was first examined (section 2.2.2.4), the distribution of the decay suggested infection present prior to use; this is confirmed by the identity of the causative organism.

Relatively few isolates have been positively identified, with the exception of Sistotrema brinkmannii and Dacryomyces deliquescens, which both fruit readily in culture, and Fomes pinicola. Identification of Basidiomycete cultures is difficult. Until recently only the works of Nobles (1948 and 1965) gave keys covering a wide range of organisms, unfortunately those occurring in North America. Stalpers (1978) has now published a key to 550 European species which gives hope for the future but unfortunately time has not permitted identification of these isolates at present. The fact that few isolates have been identified indicates they are not among the group of organisms which have become familiar through their common occurrence within buildings and which have received considerable attention because of their economic importance. It seems likely that the joinery decay fungi are normally associated with the decay of wood in natural habitats such as stumps, fallen logs or fence posts.

2.3.4.2 Growth rate on malt agar

The normal pattern of growth of a fungus on a solid nutrient substrate begins with a period of acclimatisation to the new environment, the lag phase, followed by a period of growth at a steady rate, the accepted growth rate, and finally a reduced rate of growth associated with, for example, a build up of toxic waste products near the edge of the dish (Cartwright and Findlay, 1934). Measurements of growth are usually made daily, so that each phase can be distinguished, but in this pilot study measurements were made less frequently to reduce effort. Unfortunately many plates became

contaminated and so only results after 6 and 11 days growth are available. The growth rate based on these data should equal the steady rate of growth provided the lag phase was passed by day 6 and the reduced phase had not started by day 11; at this time most cultures were well short of the edge of the dish and should not therefore have started to slow down.

In Table 2.17, there is more than one culture for each FPRL No, since the study was carried out during the isolation process prior to matching of cultures and the allocation of FPRL Herbarium numbers. There was a wide variation in rates of growth from the 0.5 mm per day of culture 6Ra to the greater than 7.5 mm per day of culture 7Fa. There was also variation by a factor of up to 2.3 by the cultures 12G, 12H, 12I, 12J and 12L which were all isolated from the same member and subsequently identified as Fomes pinicola. Such variation may reflect considerable differences between isolates, each was isolated from a separate pocket of brown rot, or the shortcomings of the test method.

Consideration of the time required to produce more reliable data, and the limited practical importance of such data led to the conclusion that growth rate studies should be discontinued in favour of other methods of characterisation.

2.3.4.3 Growth on gallic acid agar

This method of detecting white rot organisms was first published by Bavendamm (1928) and later used extensively by Davidson, Campbell and Blaisdell (1938) and Nobles (1948, 1965) in her work on the characterisation of wood decay fungi in culture. A selection of organisms isolated from the Gosport joinery was inoculated onto gallic acid agar to compare this method with that more commonly used at PRL, growth on sawdust medium (Table 2.18).

Culture 9Eb gave a strong white rot reaction on both media whilst culture $11Nb_1$ gave a moderate reaction on gallic acid and a delayed white rot reaction on sawdust; both methods therefore gave the same result. On sawdust four cultures (7Fa, 7Fb, 11Na and 11 0) gave

the "no rot" reaction exhibited by, for example, Sistotrema brinkmannii; on gallic acid, these cultures were not distinguishable from those giving a brown rot reaction on sawdust.

Although the gallic acid method can detect white rot organisms in a slightly shorter time than the sawdust method, it is known not to be completely reliable (Davidson et al, 1938) and cannot differentiate between true brown rots and those exhibiting no rot reaction on sawdust medium. Additionally, the sawdust tubes can be used subsequently in an attempt to induce the fungi to fruit (Carey, 1975); it was therefore concluded that the use of gallic acid agar should be discontinued.

2.3.4.4 Decay capacity

The isolates tested for their decay capacity (table 2.19) proved less active than four recent isolates from other sources. The most active (B795) produced a mean weight loss of only 6 per cent with the highest weight loss of an individual block at 10.8 per cent.

These data could indicate that those fungi occurring in joinery are generally slow acting; even premature failure of joinery takes in the order of 7-8 years. Alternatively they may indicate that the organisms have not been provided with the optimum conditions for their activity although the conditions suited the new isolates of Poria monticola and Coniophora puteana and are known to suit most of the fungi commonly used for testing the toxicity of wood preservatives (PRL, unpublished data). The importance of the test method is exemplified by work with Gloeocystidium lactescens (FPRL, unpublished data) which, in an agar/block method produced only 1.0 per cent weight loss of Scots pine sapwood blocks in 3 months while in a soil/block method a weight loss of 36.6 per cent was achieved in the same period. Evidence from small scale trials (PRL, unpublished data) suggests Dacryomyces deliquescens may be more active at a higher moisture content than that achieved in this test method.

Whatever the cause of their low activity, the results indicate the Basidiomycetes isolated from joinery are different from those present elsewhere in buildings.

2.3.4.5 Preservative tolerance

The filter paper method of assessing the toxicity of wood preservatives to fungi was proposed by Dickinson (1974) and has been compared extensively with other cellulosic substrates (Bravery and Carey, 1977). Most white rot fungi grew well but none of the brown rots produced sustained growth. Growth of the isolates giving no rot reaction on sawdust was variable and one isolate (B783) grew in one test but not the other.

The lack of growth by the brown rots is explained by recent work of Nilsson and Ginns (1979). They have shown that the ability of brown rot fungi to degrade non-associated cellulose is limited to the family Coniophoraceae and two other organisms, Paxillus panuoides (Fr)Fr and Hygrophoropsis aurantiacus (Fr) Maire both of which they suspect as being placed wrongly among the Paxillaceae. The isolates from joinery used in the present study are therefore not members of the Coniophoraceae, which contains many of the fungi commonly associated with decay inside buildings.

The variable ability to grow on filter paper shown by the isolates giving no rot reaction on sawdust (table 2.20) suggests they are a heterogeneous group. The organisms producing growth could be related to the white rots or the brown rots in the Coniophoraceae while those not producing growth are probably associated with other brown rots. Further investigation of this group is desirable but could not be undertaken in the present study.

In the test for tolerance to TnBTO (table 2.20), both control fungi give toxic values of 0.06-0.12 per cent w/w of the treating solution. Of the eight new isolates for which toxic values were established, three were apparently more tolerant than the control fungi and a further three were of similar tolerance. In the test with PCP the situation is less clear since the control fungi gave very different toxic values; this difference is not encountered in wood block tests using pine sapwood although C. versicolor is tolerant of high loadings in beech (Savory and Carey, 1976). One isolate (B804) gave toxic values approaching those of C. versicolor and exceeding those of

C. puteana; two further isolates gave toxic values similar to those of C. puteana.

The two control fungi are accepted as being tolerant to the two preservatives used; the high proportion of the new isolates showing similar tolerance, particularly to TnBTO, must therefore be regarded as being significant.

2.4 SPORE GERMINATION ON, AND THE PERMEABILITY OF THE SAPWOOD OF A PINE WINDOW AFTER TEN YEARS' SERVICE

2.4.1 Methods

A window approximately 1450 x 1200 mm, manufactured from Baltic redwood and with a large proportion of sapwood in each member, was selected from those obtained for examination from the Chatham site.

The four component members were labelled A (top rail), B (left stile), C (right stile) and D (bottom rail) when viewed from the exterior face. A sapwood stick, 38 x 12 mm in cross-section, was cut from the entire length of each member, its exact position being dictated by the location of the sapwood (fig 2.29). Each stick was cross-cut into 20 mm lengths which were numbered starting at the joint and working towards the centre; it was not possible to cut blocks from within the joints due to the joint detail. Blocks from the two ends of a single member were differentiated by the code letter in parentheses thus D (B) numbers signify they were cut from the bottom rail, D, at the end jointing with the left stile, B (fig 2.30).

The amount of bluestain in each block was assessed visually. The blocks were then steeped in distilled water for 4 hours, placed in sets of four on moist filter paper in glass petri dishes and autoclave sterilised for 30 minutes at 121°C. A suspension of basidiospores of Lenzites trabea (FPRL No 108E) containing 10^7 spores per ml was prepared according to the method described in section 6.3.2.7. One drop was added to a central, premarked point on each block; the dishes were placed in polythene bags to reduce drying then incubated at 25°C for 72 hours. One drop of 0.05 per cent aniline blue in lactophenol was placed on each block at the spore application site; the blocks were examined, using a binocular microscope, for germination and growth of the L. trabea basidiospores.

After completion of the spore germination test, the blocks were dried to constant weight at 50°C then weighed and dipped in dekalin for 10 seconds, with the transverse face horizontal. Each block was then blotted to remove excess solvent, and reweighed.

2.4.2 Results

The location of sample sticks within the window members is shown in Fig 2.29 and the system of block numbering in Fig 2.30. The amount of stain present in each block on conversion and the results of the spore germination test are presented in Table 2.21, together with the absorption of dekalin by each block, expressed as a percentage of its initial weight. These latter data are also presented in Fig 2.31. The mean absorption of dekalin for each of the various "stain" categories is presented in Table 2.22 and details of the statistical analysis in Table 2.23

2.4.3 Discussion

The window selected for this study was in service on the site at Chatham for some 10 years; the site has known decay problems (section 2.2.5) although, on conversion this particular window did not show evidence of decay.

The location of the sample sticks within each member was dictated by the position of the sapwood. In each case the sticks were cut from the inner face (fig 2.29) and were, therefore, remote from moisture entering via the glazing rebate but subject to end-grain penetration of moisture from the joint surfaces.

The pattern of stain in the window is interesting. The top rail (A) showed no stain for its entire length; this correlates with the greater protection afforded to the upper parts of a window by even a very slight overhang of the wall. The vertical members (B and C) showed some stain at their top ends which may indicate timber stained prior to utilisation. C showed only minor stain at the bottom end whilst B was stained for its entire length, although more heavily at the bottom. The lower rail was heavily stained at the end jointing with B but not at the other end. A portion in the middle was stained; this appeared to be caused by water entering via the glazing rebate into the closing mechanism rebate which has been shown to be a cause of failure (section 2.2.5).

During preparation of Table 2.21 there appeared to be a correlation between the various "stain" categories and the permeability measurements; mean values were therefore calculated for each category (table 2.22) and do, indeed, show differences. Statistical analysis using an F test (table 2.23) has shown that there is no significant difference between "none" and "very little" but that "very little" is significantly different at the 1 per cent level from "light" which, in turn, is different from "moderate" at the 5 per cent level, while "moderate" is different from "heavy" at the 0.01 per cent level.

Various authors have shown increased permeability following the growth of moulds or bluestain (Lindgren and Scheffer, 1939; Bellmann and Francke-Grosmann, 1952; Panek, 1957) sometimes when associated with bacteria (Tranina and Konstantnaja, 1965; Konstantnaja, 1968). Saling (1930) showed uptake of zinc chloride and creosote increased with increasing bluestain in Pinus ponderosa sapwood. A similar trend has been noted with other fungus/timber combinations (Lindgren, 1952; Lindgren and Harvey, 1952; Lindgren and Wright, 1954; Schulz, 1956). The effect of non-decay fungi on permeability is therefore well established. The distribution of the effect within the window is of interest. Major increases in permeability occurred in the timber on either side of the joint between B and D (fig 2.31); such an increase in permeability will result in an increased rate of wetting and probably, therefore, put the joint at a greater risk of colonisation by decay fungi. The data also show the inclusion of timber, bluestained prior to use, to be undesirable because of its greater permeability. However, with refractory species the increase in uptake of preservative during treatment probably out-weighs the disadvantages; this is the basis of much of the work cited above together with the more recent work on bacterial pretreatments (Unligil, 1969; Bergman, 1973).

In the L-joint trials (sections 3 and 4) the data finally led to the conclusion that the fungi were probably responsible for the increases in permeability although originally the bacteria had been implicated, at least in the early stages of exposure. The observation from the Chatham window, that increases in permeability are correlated with increasing intensity of stain, supports the rôle of the fungi in the practical situation.

Lenzites trabea does not occur naturally in the UK although it sometimes develops from infection present in imported timber. It has been used in spore germination studies because it is possible to induce fructification in culture when required (Morton and French, 1966) and it is an important cause of decay of joinery in both Europe (Harmsen cited by Henningsson, 1977) and North America (Shields and Krzyzewski, 1976). Germination was recorded only on four blocks, two either side of the joint between B and D (table 2.21). Outgrowth was considerable on the "end" blocks but was much reduced on the adjacent blocks indicating very localised detoxification of the timber but sufficient to allow infection to become established. Such detoxification, by a range of bluestain organisms and T. viride, had been effected in the laboratory (Savory and Carey, unpublished data); it is gratifying to show its occurrence under natural exposure conditions.

The observations on stain, spore germination and permeability all point to gross water penetration having taken place at the joint between B and D resulting in heavy bluestain, major increases in permeability and detoxification of the timber to allow basidiospore germination. These data give corroborative evidence that effects produced in the laboratory or in exposure trials do occur in joinery. Although this window showed no gross signs of decay one joint was obviously at a considerable risk and would, no doubt, have failed before long.

It is of interest to consider why only one of the lower joints was affected. The distribution of sapwood does not appear to have been contributory. In both stiles, sapwood surrounds the glazing rebate, a common path of moisture entry, and C, the less affected member, has a higher proportion of sapwood. It is possible that, due to the siting of the window within the building, the right side was afforded more protection than the left; unfortunately this cannot be confirmed. Alternatively, one joint may have been glued more effectively during manufacture perhaps resulting in the paint film across the joint remaining intact over a longer period. Whatever the cause, the difference is probably only slight and yet one joint appears on the verge of failure while the other remains relatively sound. This

suggests that even minor improvements in the physical protection afforded to joinery could lead to a considerable increase in service life.

2.5 GENERAL DISCUSSION

Examination of samples of prematurely failed joinery from five sites has shown that, in general, the failures can be attributed to designs which encourage water penetration and methods of construction which, having abandoned traditional craft practices, facilitate water entry.

Considering the detailed observations, the high incidence of decay of the white rot type is of great interest despite the variation between observed decay (table 2.10) and the isolated fungi (table 2.16). This situation had not been documented until a detailed observation of decay in softwood doors was carried out (Savory and Carey, 1975). The present observations on windows confirm the position; early doubts that the white rot was associated with the presence of hardwoods, which are more susceptible to this type of attack (Anon, 1972a), can now be dispelled as frames solely of softwood follow the same pattern. Many frames show visible signs of extensive brown rot from the exterior; it is only when the individual members are probed deeply, or deep sawn, that the presence of white rot is revealed. In numerous cases of less advanced decay, the white rot is widespread and contains distinct pockets of brown rot. In the early stages of failure white rot is often present without any brown rot. These observations lead to the hypothesis that among the decay fungi, the white rots colonise first and may cause quite extensive decay. This is, however, not so readily detected, particularly by the layman, because there is little associated cracking or shrinkage. Brown rots generally colonise at a later stage, initially forming pockets of rot within the area affected by the white rot, but later causing the extensive decay which is so easy to detect due to the friable nature of the decayed wood. Unless carefully examined, the white rot may not be detected. There are, of course, a proportion of cases where decay is solely of the brown rot type.

Much of the work with the fungi isolated from decayed joinery indicates the causative organisms are not those normally associated with decay within buildings. This new group are only slow decayers of wood under test conditions which promote active decay by the within-building fungi. However, the destruction of joinery that they cause over prolonged periods of exposure undoubtedly means that they are of economic importance in untreated joinery.

Due to a lack of evidence to the contrary, Coniophora puteana was regarded as being a major causative organism and results with this fungus were therefore employed in the comparisons of toxicity used in Technical Note No 24 (Anon, 1967) as the basis of evaluating joinery preservatives. Although it is now known that C. puteana is not the most applicable organism since it has not been isolated in any recent work (Savory and Carey, 1976; Suhirman, 1978; section 2.3 of this thesis) the value of such a comparison is not destroyed because of the known tolerance of this organism to most types of preservatives. The reference in Technical Note No 24, that no other test organism shows particular tolerance to a given preservative, assumes greater importance. Coriolus versicolor, which has been isolated from joinery is more tolerant of TnBTO in pine sapwood than C. puteana (Savory and Carey, 1976) although the white rot Phellinus contiguus, common on the Hayes site has been shown to be less tolerant of TnBTO and PCP than C. versicolor (PRL, unpublished data). Further investigations must be made of the apparent tolerance, as indicated by the filter paper test results (section 2.3.4.5), of a high proportion of the organisms isolated from the joinery examined. Their tolerance should be assessed using a wood block decay system so the results can be compared with the bank of background data available. However, the low levels of attack achieved in the decay tests (section 2.3.4.4) mean that new methodology must be developed. If tolerant organisms of economic importance are identified, they should be used in the assessment of the potential of fungicides for use in joinery.

The detailed examination of a single window frame (section 2.4) has indicated the sensitivity of the permeability test in detecting changes due to microbial activity and hinted that increases in physical protection should lead to increased service life. Additionally, tests using the germination of basidiospores of Lenzites trabea indicate the action of non-decay organisms predisposes the timber to attack.

This study has given a valuable introduction to the process of joinery decay and highlights the need to study the ecology of the organisms colonising joinery in greater detail.

TABLE 2.1 SUMMARY OF SITE CONDITIONS

Site	Age in years at time of inspection	Dwelling type	Material examined	Remarks
Hayes	7-9	Three and four storey blocks of council owned flats and maisonettes	20 opening lights (on site)	Urban site; no climatic extremes
Gosport	8-9	Two storey semi-detached houses, three and four storey flats; Navy married quarters	12 opening lights (laboratory)	Near coast which may increase exposure
Larkhill	6-8	Two storey terraced houses; army married quarters	15 window frames (laboratory)	Very exposed site in the centre of Salisbury Plain
	>40	Two storey semi-detached houses; army married quarters	4 doors (on site)	
Cambridge	7-10	Two three storey blocks of privately owned flats	40 window frames in situ (on site) 11 parts of window frames (laboratory)	Dry area
Chatham	10	Two storey terraced houses; three storey blocks of flats army married quarters	Numerous frames and opening lights (on site)	Near coast which may increase exposure.

TABLE 2.2 INCIDENCE OF DECAY TYPES IN WINDOW JOINERY FROM HAYES

Decay type	Total number	Remarks
White rot	15	7* instances of Phellinus contiguus, the worst having caused decay of the whole bottom rail and the base of each stile. 1 instance of Coriolus versicolor 7 instances of unidentified white rot
Brown rot	6	No species identified
Untypified attack	2	Characterless early decay

*in one case adjacent utile beading was not decayed.

TABLE 2.3 GOSPORT JOINERY DETAILS OF CONDITION

Window No	Type*	Glue failure		Decay distribution						Decay type	
		Left joint	Right joint	Left joint		Right joint		Bottom rail		White rot	Brown rot
				Rail	Stile	Rail	Stile	Glazing rebate	Horizontal joint		
1	B	Most of joint	Most of joint	+	-	+	-	+	NA	+	+
2	A	Most of joint	Throughout joint	+	-	+	+	+	+	-	+
3	A	Most of joint	Throughout joint	-	-	-	-	-	-	-	-
4	A	Most of joint	Most of joint	-	-	-	+	-	-	-	-
5	A	Throughout joint	Throughout joint	+	-	-	-	-	+	+	-
6	A	Throughout joint	Mostly sound	+	+	+	-	-	+	+	+
7	A	Throughout joint	Very decayed ∴not assessed	+	+	+	+	-	+	+	+
8	A	Throughout joint	Most of joint	+	+	slight	slight	-	+	+	+
9	A	Most of joint	Throughout joint	-	-	+	+	-	+	+	+
10	A	Most of joint	Most of joint	-	-	-	-	-	+	+	+
11	A	Most of joint	Most of joint	+	-	+	+	-	+	+	+
12	B	Throughout joint	Most of joint	-	-	-	-	+**	NA	-	+

*See text
**Pockets of brown rot remote from points of water penetration suggesting prior infection
NA Not applicable

TABLE 2.4 MOISTURE CONTENTS AT TIME OF RECEIPT FROM LARKHILL

Window No	Bottom part								Top part				Presence of decay
	Left joint			Centre of rail		Right joint			Left joint		Right joint		
	Stile	Sill	Beading	Sill	Beading	Stile	Sill	Beading	Stile	Rail	Stile	Rail	
1	21	23	30+	18.5	20	20.5	21	20	16.5	16.5	15	16.5	+
2	22	22.5	30+	17.5	22.5	19.5	20.5	20.5	19	24	18	30+	-
3	20	30	23.5	21.5	23	30+	23	29	18.5	22.5	21.5	23	-
4*	22	30+	30+	30+	18.5	21.5	30+	30+	18	19	18	18	+
5*	20.5	19.5	24	17	16.5	20	19	19	18	20	17	19	-
6	24	30+	18.5	19	16.5	23.5	22.5	19	21.5	17.5	21	23.5	-
7	19	21	30+	23	30+	16	18.5	22	16	16.5	17.5	18.5	-
8	17.5	16	13.5	14.5	15.5	18.5	18.5	24	17.5	17.5	17	15.5	-
9	18	21	30+	21	30+	17	20	30+	16	17	16.5	18	+
10*	26	30+	23	30+	19.5	19.5	30+	18.5	19.5	18	18.5	17.5	+
11*	15.5	17	30+	18.5	30+	16.5	18	30+	15.5	16	17	18.5	+
12	19	18.5	19	17	22	20	16.5	24	14.5	15.5	15	15.5	+
13*	15.5	20	24	22.5	23	15.5	19.5	30	14.5	16.5	13	16.5	-
14†	19	22	20	26	22	15	18.5	24	13	13	14.5	13.5	-
15†	19	22	30+	23	22	15.5	19	27	17	15	15.5	17.5	-

*Wobbly, ie joints no longer tight. †Fixed and opening light unit with large mullion
Note - see fig 2.15 for position of sampling points

TABLE 2.5 DISTRIBUTION OF SAPWOOD AND DECAY IN LARKHILL WINDOWS

Window No	Proportion of sapwood in each member								Decay
	Left stile		Sill		Right stile		Top rail		
	F	B	F	B	F	B	F	B	
1									Slight decay front of sill at left end; spreading up left stile on inner face
2									Apparently sound
3									Apparently sound
4									Decay along top of sill, interior side of glass
5									Apparently sound
6									Apparently sound
8									Apparently sound
9									Possibly slight decay between beading and sill
10									Decay both ends of sill at front edge. Along interior part of the top of the sill
12									Decay left end of sill at front edge. Possible soft rot on sill where paint has peeled
15									Apparently sound

F front ie outside face B back ie inside face

Records to frames 7, 11, 13 and 14 were forwarded with the windows for treatment and use by two Preservation Companies,

proportion of sapwood visible on face of members adjacent to the wall

For sampling pattern, see fig 2.15.

TABLE 2.6 SUMMARY OF DECAY IN WINDOW FRAMES, CAMBRIDGE

Elevation†	Severely decayed		Slightly decayed (in the absence of severe decay)		Sound	Type of decay		
	Both joints	One joint	Both joints	One joint		White rot	Brown rot	Unknown
N	2	3	0	3	2	7	3	5
E	4	4	0	2	0	9	5	4
S	5	2**	1	0	2*	11	7	1
W	3	5	0	0	2**	13	2	1
Total	14	14	1	5	6	40	17	11

†Lower joints of 10 frames examined on each elevation
*Both part of a French window unit
**One part of a French window unit

TABLE 2.7 DETAILS OF DECAY IN WINDOW FRAMES, CAMBRIDGE

Elevation	Sample No	Left joint					Right joint					Remarks
		Decayed	WR	BR	U	Spread along sill	Decayed	WR	BR	U	Spread along sill	
N	1	+	+	+			slight			+		
	2	slight	+				+	+				
	3	-				slight	slight			+		Decay in sill remote from joint.
	4	-					slight			+		
	5	-					slight			+		
	6	-					-					
	7	-					-					
	8	+	+			3"	slight	+				
	9	+			+	24" bad	+	+			12" bad	Sill decayed whole length on upper face
	10	+		+			+	+	+			D. deliquescens in left joint
	Total	4 + 1 slight	3	2	1		3 + 5 slight	4	1	4		
E	1	+	+			12"	+	+	+			Mastic used to seal window/wall joint
	2	+		+			+	+				Mastic used to seal window/wall joint
	3	slight			+		+		+			
	4	slight			+		+	+				
	5	+	+				+	+				
	6	+	+				slight			+		Left end sill replaced as a repair;
	7	+		+			+	+				centre of sill decayed at joint.
	8	slight			+		-					
	9	-					slight	+				
	10	-					+		+		5"	
	Total	5 + 3 slight	3	2	3		7 + 2 slight	6	3	1		
S	1	+		+		15" bad	+	+	+			WR along most of sill
	2*	+	+	+			-					D. deliquescens at left end sill
	3	+	+			4"	+	+			6"	
	4*	-					-				15" bad	
	5	+	+				+		+			D. deliquescens at right end sill
	6*	-					-					WR at left end sill
	7	slight	+				slight	+				
	8	slight			+		+		+			D. deliquescens at right end sill
	9	+	+	+		8"	+	+	+			Myxomycete fruit body at centre
	10	+	+			6"	+	+			2"	Polystictus? collected from left end sill
	Total	6 + 2 slight	6	3	1		6 + 1 slight	5	4	0		
W	1	slight	+				+	+				
	2	+	+			6"	+	+				
	3	+	+	+		8"	+			+	6"	
	4	-					+	+			2"	
	5*	-					-					
	6	+	+				+	+			8"	
	7	slight	+				+	+				
	8	-					-					
	9	slight	+				+		+		18"	
	10	slight	+				+	+				Mastic used to seal window/wall joint.
	Total	3 + 4 slight	7	1	0		8	6	1	1		

*Centre rail joint of French windows

TABLE 2.8 CONDITION OF FRENCH DOOR/WINDOW UNITS, CAMBRIDGE

Elevation	Sample No	Centre rail joints				Bottom rail joints				Hardwood cill	Remarks
		Door		Window		Door		Window			
		Left	Right	Left	Right	Left	Right	Left	Right		
S	2	Sound	Sound	White rot and D. deliquescens	Sound	Sound	Sound	White rot	White and brown rot	Sound	Brown rot in weather bar on door
S	4	-	-	Sound	Sound	-	-	Brown rot top, white rot bottom	White rot		No door in this unit. Brown rot along top & white rot along bottom of lower rail of window unit.
S	6	Sound	Slight white rot	Sound	Sound	Brown rot	Sound	Sound	Sound	Sound	Brown rot in weather-bar on door. Additional water run off strips on door and window.
W	5	Sound	Sound	Sound	Sound	Sound	Sound	White rot	White rot	Sound	White rot along length of bottom rail/sill joint, in the rail only.

TABLE 2.9 DECAY OF SOFTWOOD FRAMES TO METAL WINDOW UNITS, CAMBRIDGE

Sample No	Description	Decay type			Remarks
		WR	BR	U	
1*	Bottom left hand corner of a fixed light adjacent to door.	+	+	+	Decay in door frame, sill and rail (see left hand portion fig 2.18).
2*	Bottom right hand corner of a fixed light adjacent to the wall.	+	+	-	Decay in rail and stile (see right hand portion fig 2.18).
3	Bottom left hand joint; 500 mm of sill and 300 mm of stile.	+	+	-	Extensive brown rot along front of sill, calcareous fruitbody, basidiospores 4 x 1.5 μ.
4	Bottom right hand joint; 500 mm of sill and 270 mm of stile.	+	+	-	Extensive white rot and brown rot along front edge of sill.
5	Bottom right hand joint; 600 mm of sill and 400 mm of stile.	+	+	-	White rot and brown rot of sill particularly at front edge; D. deliquescens fruitbodies Carpenter bee.
6	Complete sill plus 400 mm each stile.	+	+	-	Front of sill decayed at right end; Carpenter bee.
7	Complete sill plus 300 mm each stile.	+	+	-	Sill decayed at both ends. D. deliquescens fruitbodies
8	Complete sill only.	+	+	-	Sill decayed at both ends
9	Left hand stile only.	+	-	+	Decayed at "sill" end.
10	Complete frame	+	+	+	Sill decayed at both ends and entire length at front edge; D. deliquescens fruitbodies Carpenter bee
11	Complete frame	-	+	-	Decayed confined to inside face of one bottom joint.

*Part of French window/door unit, with hardwood sill.

WR - white rot

BR - brown rot

U - untypified attack

TABLE 2.10 SUMMARY OF THE INCIDENCE OF DECAY TYPES

Site	Rot type			Sample type and size
	White rot	Brown rot	Untypified attack	
Hayes	15	6	2	20 frames
Gosport	8	8*	**	12 frames
Larkhill	Type of decay not recorded			
Cambridge				
- site survey	40	17	11	80 joints
- lab examination	10	10	3	11 mixed samples
Chatham	No detailed observations			
Total	73	41	16	123

*Does not include one instance of imported decay
**Not recorded

Table 2.11

SUCCESS RATE FOR THE ISOLATION OF BASIDIOMYCETES FROM JOINERY

Source of joinery	Number of attempts	Nimber of successes		Per cent success	
		Definite*	Including** probables	Definite*	Including** probables
Hayes	10	1	1	10.0	10.0
Gosport	23	12	17	52.2	73.9
Cambridge	60	14	20	23.2	33.3
Larkhill (Set pattern)	60	11	11	18.3	18.3
Larkhill (Choice)	39	14	16	35.9	41.0

*organisms proved to be Basidiomycetes.
**including organisms thought to be Basidiomycetes but lacking clamp connections.

TABLE 2.12

DETAILS OF CULTURES FROM HAYES WINDOWS

FPRL No	Isolation No	Description/Comments	Probable identity/ rot reaction on sawdust	Source
B696	E1	White, cottony, simple clamps	White rot	Window 6 - Pocket of brown rot in pine

TABLE 2.13

DETAILS OF CULTURES FROM GOSPORT WINDOWS

FPRL No	Isolation No	Description/Comments	Probable identity where known/ rot reaction on sawdust	Source
B733	9Eb	White, fast growing, strands in sawdust tube, crystals on hyphae, simple clamps	White rot	Window 9 Mixed white and brown rot at base of a hemlock stile
B738	$7D_1$	Simple clamps, crystals on hyphae	White rot	Window 7 Uncertain rot type, spreading into hemlock bottom rail from the joint
B739	$7D_2$	Beige, growth in concentric zones, no clamps	Dacryomyces deliquescens Brown rot	Window 7 Uncertain rot type, spreading into hemlock bottom rail from the joint
B740	6R	Simple clamps, very pale, fruits in culture producing basidiospores, terminal swellings and contorted hyphae	Brown rot	Window 6 Brown rot in hemlock at a joint
B781	7F	White, turns agar brown, very rapid growth, terminal spores sometimes in chains, no clamps seen	No rot reaction	Window 7 Mixed white and brown rot at base of hemlock stile
B782	10A	Septate mycelium, possibly with rare clamps. Occasional basidium-like structures; free spores 3 x 2μ (representative of 10B and 10C)	Brown rot	Window 10 Brown rot in pine lower section of bottom rail
B783	11O	Simple clamps, swollen structures on some hyphal tips, dark staining bodies about 6μ across	No rot reaction	Window 11 Mixed white and brown rot in hemlock vertical weather bar
B785	$11Nb_1$	Simple clamps, terminal swellings, white	White rot, delayed	Window 11 White rot with small pockets of brown rot in pine weather bar
B786	11Na	Possible rare clamps, white, appressed	No rot reaction	Window 11 as B785
B789	12J	Simple clamps, chlamydospores, white (representative of 12G, H, I and L)	Fomes pinicola Brown rot	Window 12 Small pocket of brown rot in Douglas fir bottom rail

TABLE 2.14

DETAILS OF CULTURES FROM CAMBRIDGE WINDOWS

FPRL No	Isolation No	Description/Comments	Probable identity where known/ rot reaction on sawdust	Source* (see Table 2.9)
B790	C2E	Beige, powdery, growth in concentric rings. (Representative of C1G, C1H, C1I, C2B)	Brown rot	Window 2
B791	C2C	Beige, simple clamps	No rot reaction	
B792	C2D	Simple clamps, chains bulbil type cells, a few immature basidia	Sistotrema brinkmannii No rot reaction	
B793	C3B	No clamps, beige, growth in concentric rings	Dacryomyces deliquescens Brown rot	Window 3
B794	C3E	White, birefringent crystals on hyphae, simple clamps, deeply stained, thick walled swollen hyphal tips. Bleaches agar	White rot (weak reaction)	
B795	C4D	As B794	White rot	Window 4
B796	C4E	No clamps, beige, growth in concentric rings.	D. deliquescens Brown rot	
B797	C6A	Simple clamps, birefringent crystals on hyphae, swellings on hyphae. White with brown tinges on the agar	White rot	Window 6
B798	C6D	White, simple clamps, birefringent crystals, swollen hyphal tips, strong mushroom odour.	White rot	
B799	C7Db	As B794	White rot (slow reaction)	Window 7
B800	C7F	No clamps, beige, slightly zoned	D. deliquescens Brown rot	
B801	C8A	No clamps, beige, growth in concentric zones	D. deliquescens Brown rot	Window 8
B802	C10C	No clamps, beige, slightly zoned	D. deliquescens Brown rot	Window 10
B803	C10D	Beige, no clamps seen	Brown rot	
B804	C10E	Simple clamps, profuse white growth tinged brown at top of tube (also C10F)	White rot	

*The location of the sample points within each window was not recorded.

TABLE 2.15

DETAILS OF CULTURES FROM LARKHILL WINDOWS

FPRL No	Isolation No	Description/Comments	Probable identity where known/ rot reaction on sawdust	Source
B827	5B(D)1	White, fast growing, producing rings of mycelium beneath glass in sawdust tube, simple clamps	White rot	Window 5 Set pattern. Zone of discoloration in pine sapwood near joint
	5B(D)2	as 5B(D)1	White rot	Window 5 Set pattern. Zone of discolouration in pine sapwood near joint
	5B(D)12	as 5B(D)1 (also 5B(D)10)	White rot	Window 5 Choice. Zone of discoloration in Douglas fir beading near joint
	5D(B)5	as 5B(D)1 (representative of 5D(B)6, 7 and 8)	White rot	Window 5 Choice. Zone of discoloration in Douglas fir beading near joint
B828	5D(C)8	White, fast growing culture, no clamps	White rot	Window 5 Choice. Zone of discoloration in Douglas fir beading near joint
*	5C(D)3	White, fast growing culture, simple clamps, bulbil type cells, basidia	S. brinkmannii No rot reaction	Window 5 Choice. Zone of discoloration in pine beading near joint
	9B(D)1	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Set pattern. Apparently sound pine sapwood beading near joint
	9B(D)4	No clamps, bulbil type cells present	S. brinkmannii No not reaction	Window 9 Choice. Apparently sound Douglas fir beading near joint
	9D(B)1	as 5C(D)3	S. brinkmannii No not reaction	Window 9 Set pattern. Apparently sound pine sapwood near joint
	9D(B)2	as 5C(D)3	S. brinkmannii No not reaction	Window 9 Set pattern. Apparently sound pine sapwood near joint
	9D(B)3	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Set pattern. Apparently sound pine sapwood
	9C(D)2	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Choice. Zone of discoloration in pine heartwood beading near joint
	9C(D)5	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Choice. Apparently sound Douglas fir beading near joint
	9D(C)7	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Set pattern. Apparently sound pine sapwood near joint
	9D(C)8	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Set pattern. Apparently sound pine sapwood
	9D(C)9	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Set pattern. Apparently sound pine sapwood
B829	9D(C)11	Clamps, white fast growing culture. (Cultures rejected due to contamination.)		Window 9.Set pattern. Apparently sound pine sapwood beading
	9D(C)12	as 9D(C)11		Window 9 Set pattern. Apparently sound pine sapwood beading
*	9D(C)13	as 5C(D)3	S. brinkmannii No rot reaction	Window 9 Choice. Apparently sound Douglas fir beading near joint
	9D1	as 5C(D)3 (representative of 9D2, 3 and 4)	S. brinkmannii No rot reaction	Window 9 Choice. Brown rot decay pocket in centre of sill at the heart/sap boundary

*No FPRL Herbarium numbers were allocated since the cultures were rejected.

Table 2.16

TYPES OF BASIDIOMYCETE ISOLATES

Site	Rot type			
	White rot	Brown rot	Sistotrema	Inconclusive
Hayes	1	0	0	0
Gosport	3	5 + 5*	0	4
Cambridge	7	11	1	1
Larkhill	9	0	16	2
Total	20	16 + 5*	17	7

*organism present in timber when imported

Table 2.17

GROWTH RATE OF SOME GOSPORT CULTURES ON 5 PER CENT MALT AGAR AT 20°C

FPRL No	Culture* No	Replicate No	Incubation period						Growth rate**
			6 days			11 days			
			Two diameters (mm)		Mean	Two diameters (mm)		Mean	
B733	9Eb	1	23	25		45	46		
		2	29	24	25.3	47	50	47.0	2.175
B740	6Ra	1	6	7		11	11		
		2	10	9	8.0	15	15	13.0	0.500
	6Rb	1	7	9		15	13		
		2	10	10	9.0	16	15	14.8	0.575
B781	7Fa	1	dish covered						
		2	dish covered		>90				>7.5†
B783	110	1	33	34		66	64		
		2	32	31	32.5	65	65	65.0	3.250
B785	$11Nb_1$	1	35	30		76	78		
		2	32	37	33.5	73	77	76.0	4.250
B786	11Na	1	19	18		50	50		
		2	17	17	17.8	42	43	46.3	2.850
B789	12G	1	20	20		40	40		
		2	17	18	18.8	39	42	40.3	2.150
	12H	1	30	29		41	39		
		2	20	19	24.5	43	44	41.8	1.725
	12I	1	12	11		22	22		
		2	contaminated		11.5	contaminated		22.0	1.050
	12J	1	18	17		42	39		
		2	20	20	18.8	44	45	42.5	2.375
	12L	1	20	22		40	41		
		2	21	22	21.3	41	40	40.5	1.925

>Greater than

*The experiment was carried out during the isolation process prior to the matching of cultures and the allocation of FPRL Herbarium numbers.

**Increase in radius, in mm per day, between 6 and 11 days incubation.

†Growth rate calculated over the initial 6 days of growth.

Table 2.18

GROWTH OF SOME GOSPORT CULTURES ON GALLIC ACID AGAR

FPRL No	Culture No*	Sawdust reaction**	Incubation period		Remarks
			2 days	7 days	
B733	9Eb	W	No growth, inoculum dark brown, 33 mm diam. stain	No growth, inoculum dark brown, 50 mm diam. dark stain	Positive white rot reaction
B739	7D2	B	No growth, slight stain in agar	Slight growth, faint stain in agar 40 mm diam.	
B740	6Ra	B	Inoculum slightly brown, slight growth on inoculum	Slight growth, faint stain in agar 30 mm diam.	
	6Rb	B	Slight growth on inoculum	10 mm diam. growth, faint stain in agar 35 mm diam.	
B781	7Fa	N	19 mm diam. growth, inoculum slightly brown	Plate covered, no stain in agar, inoculum slightly brown	
	7Fb	N	23 mm diam. growth, inoculum rather brown	Plate covered, no stain in agar, inoculum slightly brown	
B782	10A	B	No reaction	Slight growth, no stain in agar	
	10B	B	Inoculum slightly brown, slight growth on inoculum	Slight growth, no stain in agar	
B783	11O	N	Slight growth on inoculum	15 mm diam. growth. No stain in agar	
B785	$11Nb_1$	W	Inoculum brown, 18 mm diam. stain in agar	Slight growth, 30 mm diam. moderate stain in agar	Probable white rot
B786	11Na	N	Slight growth on inoculum	Slight growth, no stain in agar	
B789	12G	B	Slight growth on inoculum	18 mm diam. growth, faint stain in agar	
	12H	B	Slight growth on inoculum	22 mm diam. growth, faint stain in agar	
	12I	B	Slight growth on inoculum	23 mm diam. growth, faint stain in agar	
	12J	B	Slight growth on inoculum	24 mm diam. growth, faint stain in agar	
	12L	B	Slight growth on inoculum	22 mm diam. growth, faint stain in agar	

* the experiment was carried out during the isolation process prior to the matching of cultures and the allocation of FPRL Herbarium numbers

**B brown rot W white rot N no rot reaction

Table 2.19

WEIGHT LOSS OF PINE SAPWOOD AFTER 6 WEEKS AT 22°C

FPRL No	Identity where known and rot reaction on sawdust	Weight loss of 6 replicates	
		Mean	Range
B733	white rot	1.7	1.2–2.7
B738	white rot	+ 0.3	+ 0.8–0.2
B739	*Dacryomyces deliquescens*	2.5	1.3–6.3
B740	brown rot	1.5	+ 0.8–7.7
B781	no rot	2.1	1.7–2.8
B783	no rot	1.6	0.9–1.9
B785	white rot	1.4	1.2–1.5
B786	no rot	1.4	0.9–2.0
B789	*Fomes pinicola*	1.0	0.6–1.5
B790	brown rot	0.9	0.5–1.4
B791	no rot	2.0	1.7–2.3
B792	*Sistotrema brinkmannii*	2.7	1.9–3.9
B793	*Dacryomyces deliquescens*	1.1	0.8–1.5
B794	white rot	1.3	1.0–1.6
B795	white rot	6.0	3.8–10.8
B796	*Dacryomyces deliquescens*	1.4	0.5–3.8
B797	white rot	1.3	1.0–1.5
B798	white rot	2.6	0.5–5.1
B799	white rot	+ 1.0	+ 2.2–0.5
B800	*Dacryomyces deliquescens*	0.9	0.3–2.6
B801	" "	1.4	0.6–2.1
B802	" "	1.4	0.3–2.2
B804	white rot	+ 0.7	+ 1.2–+0.3
B735	*Poria monticola*	43.5	38.1–47.0
B737	" "	44.5	41.0–49.4
B741	" "	33.2	32.0–34.5
B805	*Coniophora puteana*	16.2	14.0–19.0

Table 2.20

PRESERVATIVE TOLERANCE OF JOINERY ISOLATES

Rot reaction on sawdust medium	FPRL No	Toxic values as per cent w/w treating solution	
		TnBTO	PCP
white rot	B733	0.03–0.1	nt
	B738	erratic growth	<0.03
	B794	ng	ng
	B795	0.1–0.3	0.03–0.1
	B797	0.01–0.03	nt
	B798	0.1–0.3	0.03–0.1
	B799	<0.01	<0.03
	B804	>0.3	0.1–0.3
	28A	0.06–0.12*	0.3–1.0
brown rot	B739	nt	ng
	B782	nt	ng
	B789	ng	ng
	B790	nt	ng
	B793	nt	ng
	B796	nt	ng
	B800	nt	ng
	B801	nt	ng
	B802	nt	ng
	11E	0.06–0.12*	0.03–0.1
no reaction	B781	nt	<0.03
	B783	0.03–0.1	ng
	B786	ng	ng
	B791	0.03–0.1	<0.03
	B792	nt	ng

ng no growth on untreated filter paper
nt not tested
* extracted from PRL unpublished data

28A Coriolus versicolor
11E Coniophora puteana

TABLE 2.21 STAIN CATEGORIES, PERMEABILITY (AS % ABSORPTION OF DEKALIN) AND GERMINATION OF SPORES OF L. trabea ON BLOCKS FROM A 10 YEAR OLD WINDOW FRAME (SEE Fig 2.30)

No	A (E)			A (C)			B (A)			B (D)			C (A)			C (D)			D (B)			D (C)		
	S	% abs	G	S	% abs	G	S	% abs	G	S	% abs	G	S	% abs	G	S	% abs	G	S	% abs	G	S	% abs	G
1	O	15.73	-	O	15.86	-	L	18.10	-	H	70.81	+	L	20.61	-	L	19.80	-	H	99.41	+	O	13.41	-
2	O	15.29	-	O	15.94	-	L	17.67	-	H	55.90	+	L	19.00	-	V	16.20	-	H	91.29	+	O	13.39	-
3	O	15.62	-	O	16.12	-	L	18.72	-	H	43.08	-	L	19.01	-	V	16.04	-	H	69.90	-	O	13.83	-
4	O	15.29	-	O	15.71	-	L	18.53	-	M	27.96	-	L	18.91	-	O	15.90	-	M	61.66	-	O	14.49	-
5	O	15.22	-	O	15.57	-	L	18.37	-	M	22.85	-	L	17.81	-	O	16.30	-	M	57.02	-	O	16.52	-
6	O	15.51	-	O	16.03	-	L	17.51	-	M	23.02	-	L	15.08	-	O	16.55	-	M	54.60	-	O	12.08	-
7	O	15.76	-	O	15.31	-	L	17.37	-	M	22.36	-	L	18.21	-	O	16.61	-	L	53.57	-	O	15.26	-
8	O	15.61	-	O	16.02	-	L	16.62	-	M	22.24	-	V	16.13	-	O	16.76	-	L	50.00	-	O	13.30	-
9	O	15.45	-	O	16.20	-	L	18.18	-	M	21.69	-	V	16.53	-	O	15.69	-	L	45.91	-	O	16.16	-
10	O	15.77	-	O	16.84	-	L	19.34	-	M	22.32	-	V	15.76	-	D	15.63	-	L	41.62	-	O	16.01	-
11	O	17.70	-	O	15.02	-	L	18.99	-	M	20.42	-	V	16.68	-	O	15.28	-	L	36.41	-	O	15.53	-
12	O	16.20	-	O	15.56	-	L	18.00	-	M	21.50	-	O	15.43	-	O	15.48	-	L	29.85	-	O	15.39	-
13	O	15.39	-	O	15.59	-	L	18.00	-	M	22.61	-	O	15.87	-	O	15.36	-	L	22.02	-	V	16.20	-
14	O	16.02	-	O	16.34	-	L	18.43	-	M	21.71	-	O	15.85	-	O	14.96	-	O	15.79	-	V	16.01	-
15	O	15.32	-	O	15.93	-	L	19.46	-	M	22.31	-	O	14.94	-	O	14.79	-	O	13.73	-	V	16.86	-
16	O	15.39	-	O	15.50	-	L	19.16	-	M	22.48	-	O	16.30	-	O	15.69	-	O	14.04	-	V	16.86	-
17	O	15.89	-	O	14.79	-	L	19.55	-	M	22.51	-	O	16.10	-	O	14.96	-	O	13.14	-	V	16.97	-
18	O	15.49	-	O	16.05	-	L	18.68	-	M	21.39	-	O	16.01	-	O	14.57	-	O	12.03	-	V	15.70	-
19	O	16.05	-	O	16.26	-	L	19.97	-	M	20.65	-	O	17.56	-	O	14.91	-	O	11.64	-	V	16.83	-
20	O	15.25	-	O	15.69	-	L	19.04	-	M	21.05	-	O	15.63	-	O	15.71	-	O	11.94	-	V	17.15	-
21	O	16.31	-	O	16.49	-	L	19.53	-	M	20.27	-	O	14.31	-	O	14.16	-	O	11.68	-	V	17.61	-
22	O	15.97	-	O	15.50	-	L	18.63	-	M	19.25	-	O	8.66	-	O	14.91	-	D	6.62	-	V	17.11	-
23	O	16.49	-	O	15.51	-	L	19.90	-	M	15.78	-	O	14.96	-	O	8.82	-	O	11.76	-	V	16.14	-
24	O	18.87	-	O	17.94	-	L	20.38	-	M	21.52	-	O	15.35	-	O	15.31	-	O	12.63	-	V	17.32	-
25	O	15.93	-	O	14.28	-													O	14.02	-	V	16.09	-
26	O	15.08	-	O	15.20	-													O	14.20	-	V	15.88	-
27	O	15.51	-	O	15.30	-													O	14.52	-	V	16.13	-
28	O	14.86	-	O	15.93	-													V	16.42	-	V	16.54	-
29	O	15.19	-	O	15.93	-													V	23.00	-	V	18.17	-

S = stain category
O = no stain
V = very little stain
L = light stain
M = moderate stain
H = heavy stain
G = germination of spores of L. trabea
+ = germination
- = no germination

TABLE 2.22 MEAN PER CENT UPTAKE OF DEKALIN FOR EACH STAIN CATEGORY

Stain category	No of replicates	Mean % absorption
None	118	15.13
Very little	25	16.81
Light	39	22.46
Moderate	24	26.22
Heavy	6	71.73

TABLE 2.23 STATISTICAL ANALYSIS OF PERMEABILITY IN THE VARIOUS CATEGORIES OF STAIN INTENSITY

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio	
Between levels	20 229.57	4	5057.39	109.85	***
Residual	9 529.95	207	46.04		
Total	29 759.52	211			
	Detailed comparisons				
none v very little	58.65	1	58.65	1.274	nsd
very little v light	485.77	1	485.77	10.55	**
light v moderate	209.53	1	209.53	4.55	*
moderate v heavy	9 944.30	1	9 944.30	21.60	***

nsd = not significantly different
* = significantly different at 5 per cent level
** = significantly different at 1 per cent level
*** = significantly different at 0.1 per cent level

Fig 2.1 Hayes; general view of the site

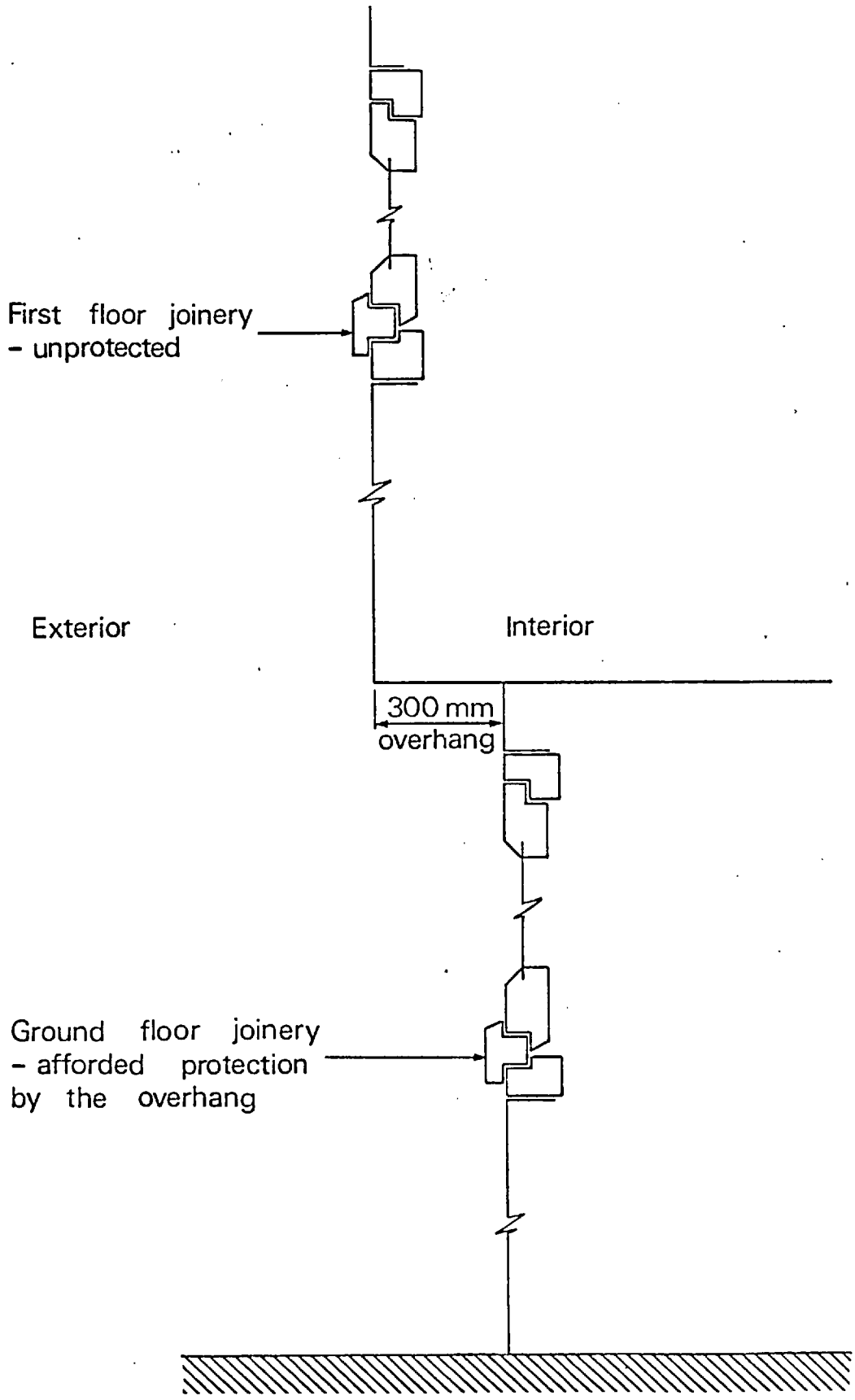
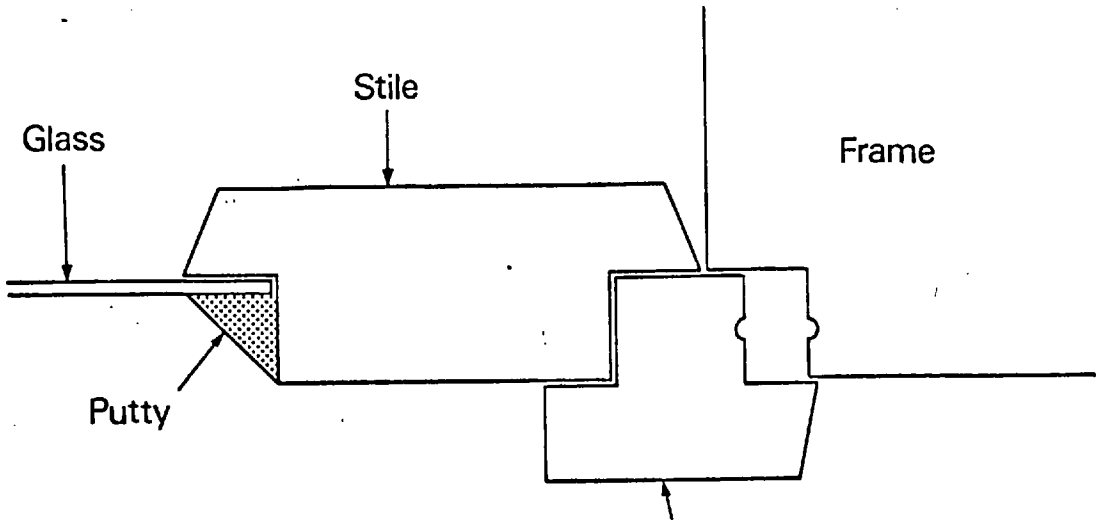
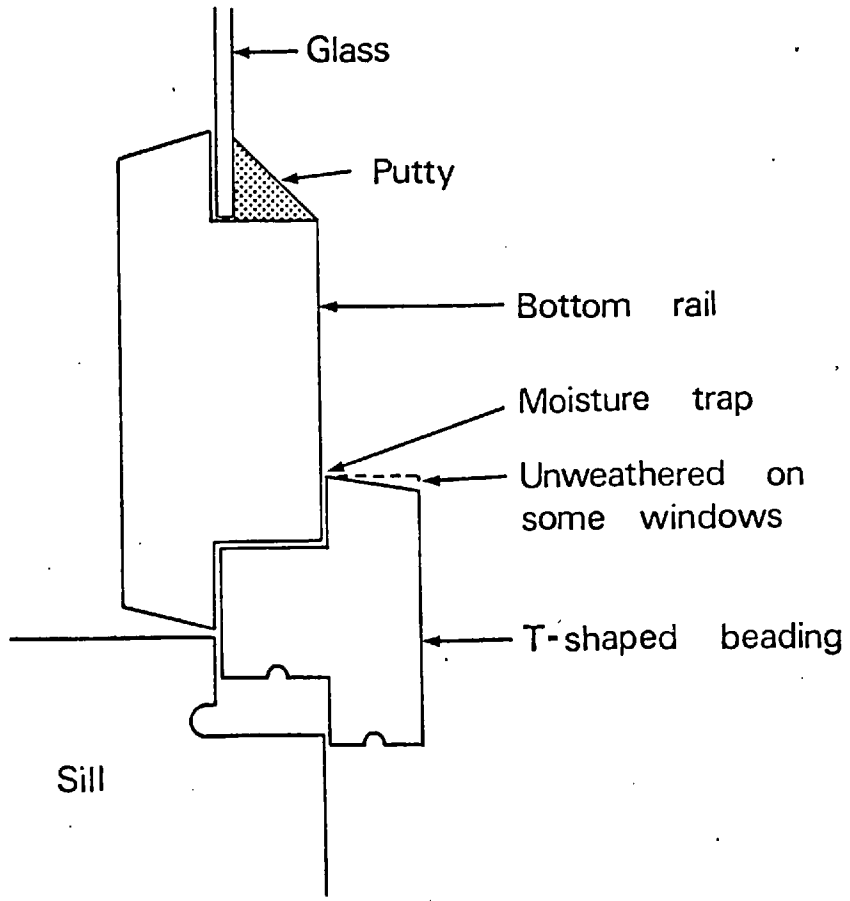


Fig. 2.2. Hayes; section through exterior wall of building



Section through stile below pivot



Section through bottom rail

Fig. 2.3. Hayes; section through opening lights

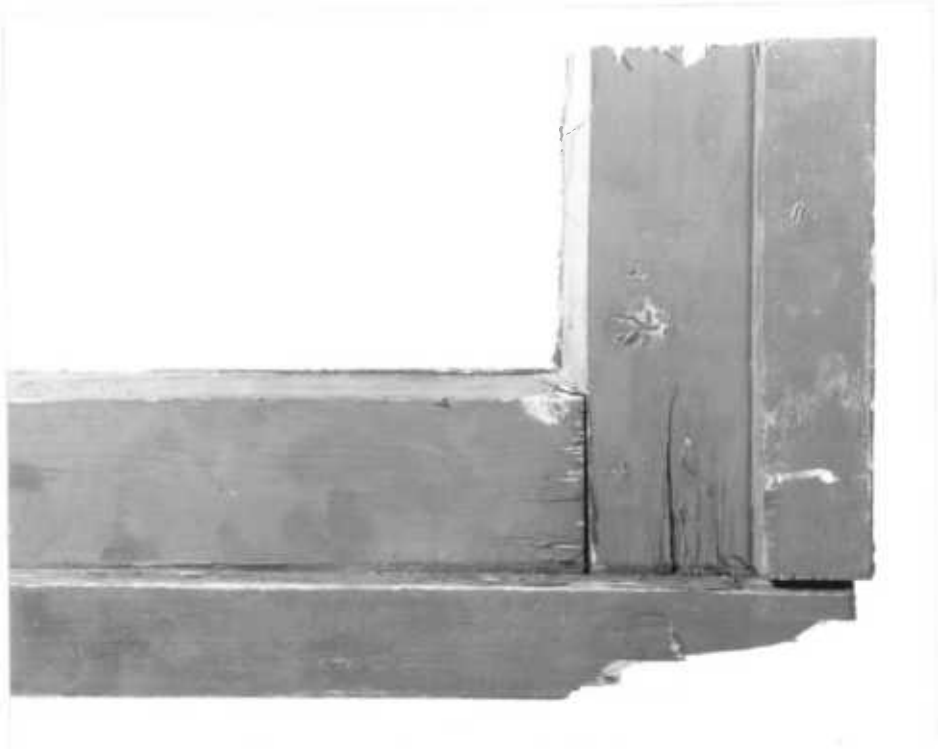


Fig 2.4 Hayes; decayed joint of centre pivot hung opening light.

(Note openness of joints in the main frame and between the frame and the T-shaped weatherbar; also splitting and decay of the frame and the weatherbar)

Fig 2.5 Gosport; general views of the site.

Fig 2.6 Gosport; decayed type A frame in situ

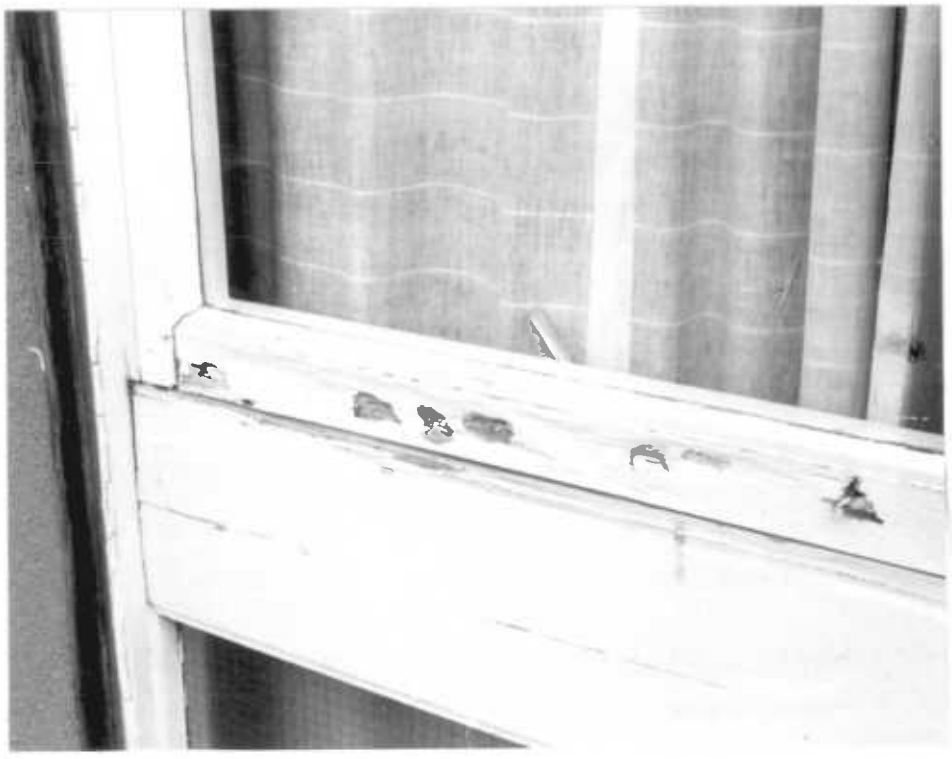


Fig 2.7 Gosport; decayed type B frame in situ

Window No. 3

Source Gosport.

Service

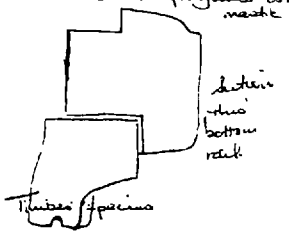
Exposure

Aspect

Position

Opening

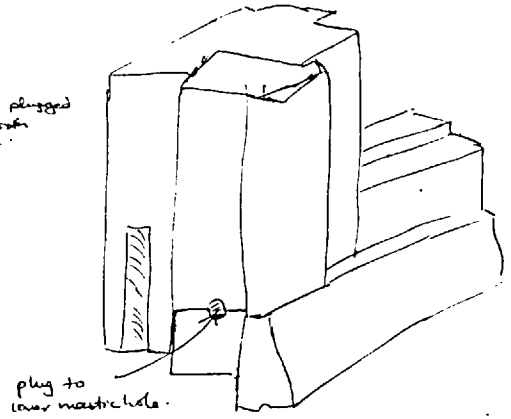
Machining Holes drilled + plugged to fill joints with mastic.



Glue

Date received 2/10/75

Reason for examination. Some decay.



Joints - glue failure LHJ both sides outer tenon, outside and bottom of inside of inner tenon.
RHJ almost throughout joint. Internal shoulder shrinkage

- water penetration Between 3 parts of bottom rail. Within mast & LHJ. - RHJ.
- decay None.
- type of rot none in LHJ. rail stained.

Beading - inside or out Out Red Meranti.

- priming of wooden Yes
- state of back fill putty Dirty at bottom of window
- sealing of beading Yes
- water penetration All around bottom of window

Information from sleep sensing

- Joint 1 } water penetration and glue failure on joints but no decay.
- " 2 }
- " 3
- " 4
- General. Bottom rail stained, slight blue stain

Further remarks.

Glue shedding on shoulders of joint on inside

Both styles + weather strips ~~all tops~~ tongue & lock

Top part of lower rail all tongue

Bottom " " " " ~~tongue~~ tongue & lock.

Name J Lacey
Date 25/11/75.

Fig 2.8 Record sheet for Gosport windows

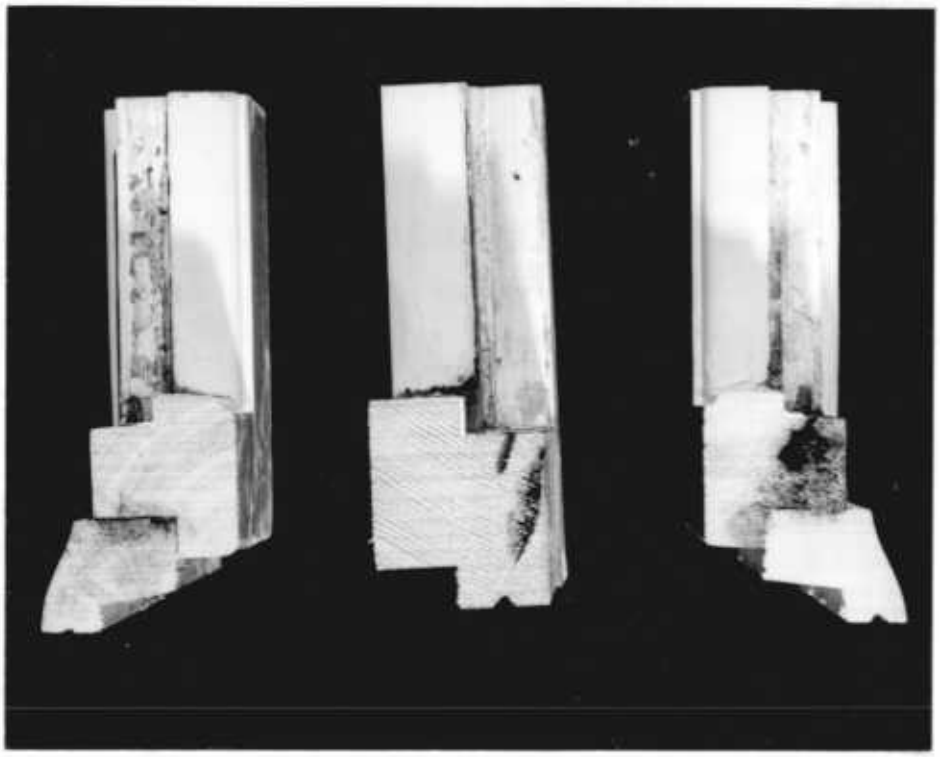


Fig 2.9 Gosport; sections through bottom rails to show different patterns of decay.

Left. Type A, pine and hemlock/fir mixture; decay associated with the horizontal joint.

Centre. Type B, Douglas fir; decay remote from normal paths of water entry.

Right. Type A, pine and hemlock/fir mixture; major decay associated with the glazing rebate.

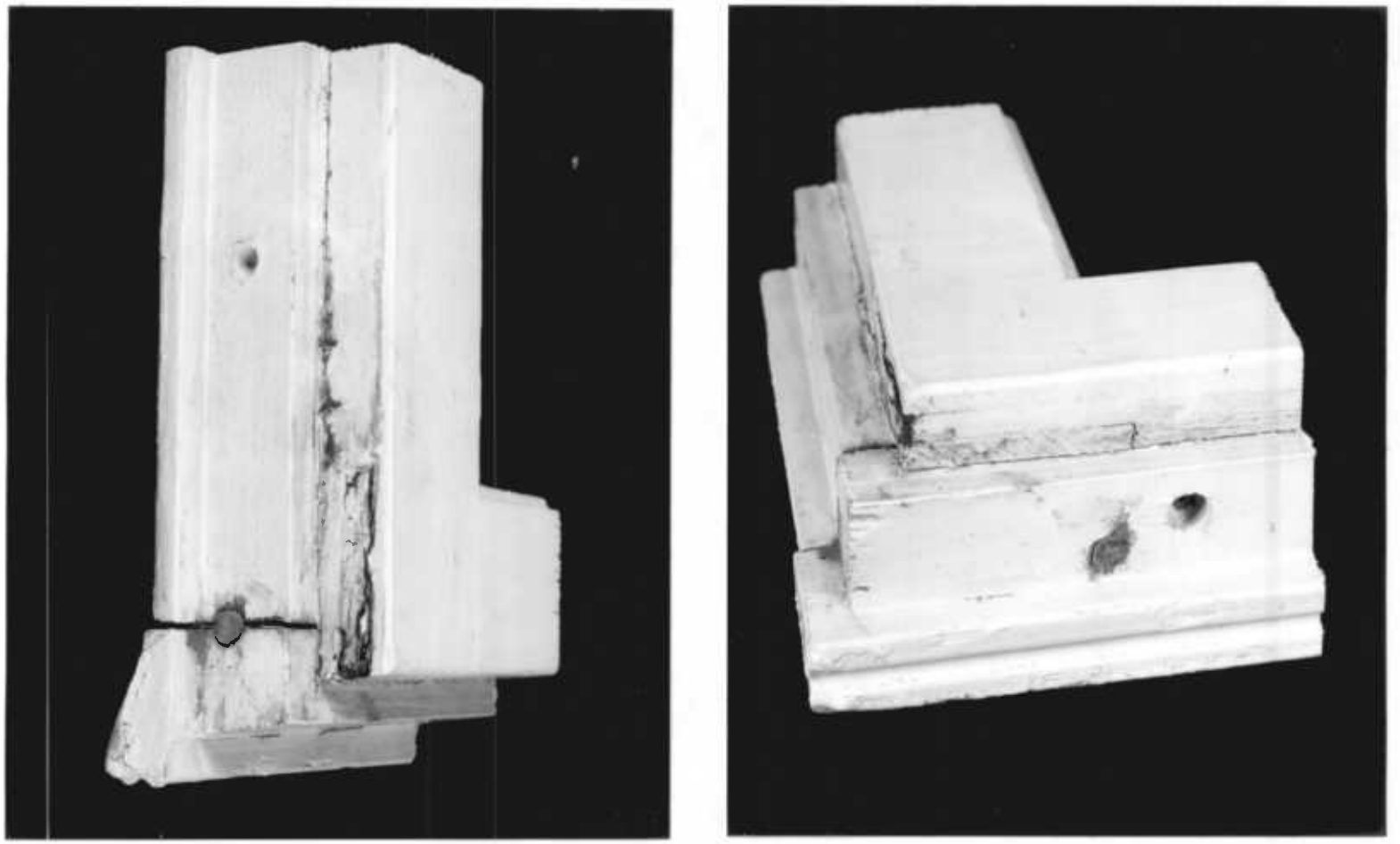


Fig 2.10 Gosport; use of mastic in an attempt to prevent moisture entry. (The horizontal join (left) and the vertical join (right) had been drilled out, filled with mastic and the hole closed with a timber plug.)

Fig 2.11 Larkhill; Noll Bingham road; a Scots pine door showing only slight decay after 45 years service.

(Note double mortice and tenon joint and long standing paint failure)

Fig 2.12 Larkhill; severe white rot at rail/stile joint

Fig 2.13 Larkhill; decay of a sill associated with the centrally positioned butt joint

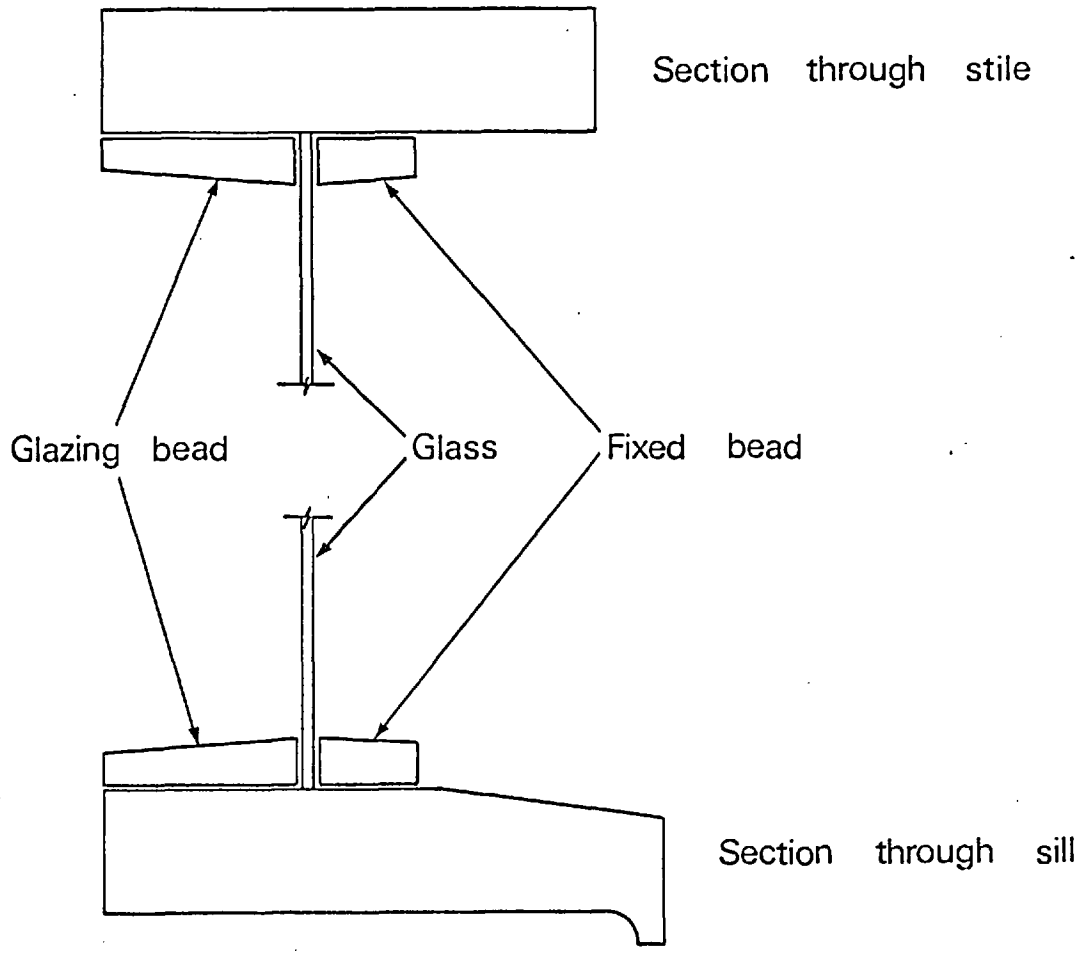


Fig. 2.14. Larkhill; section through window joinery

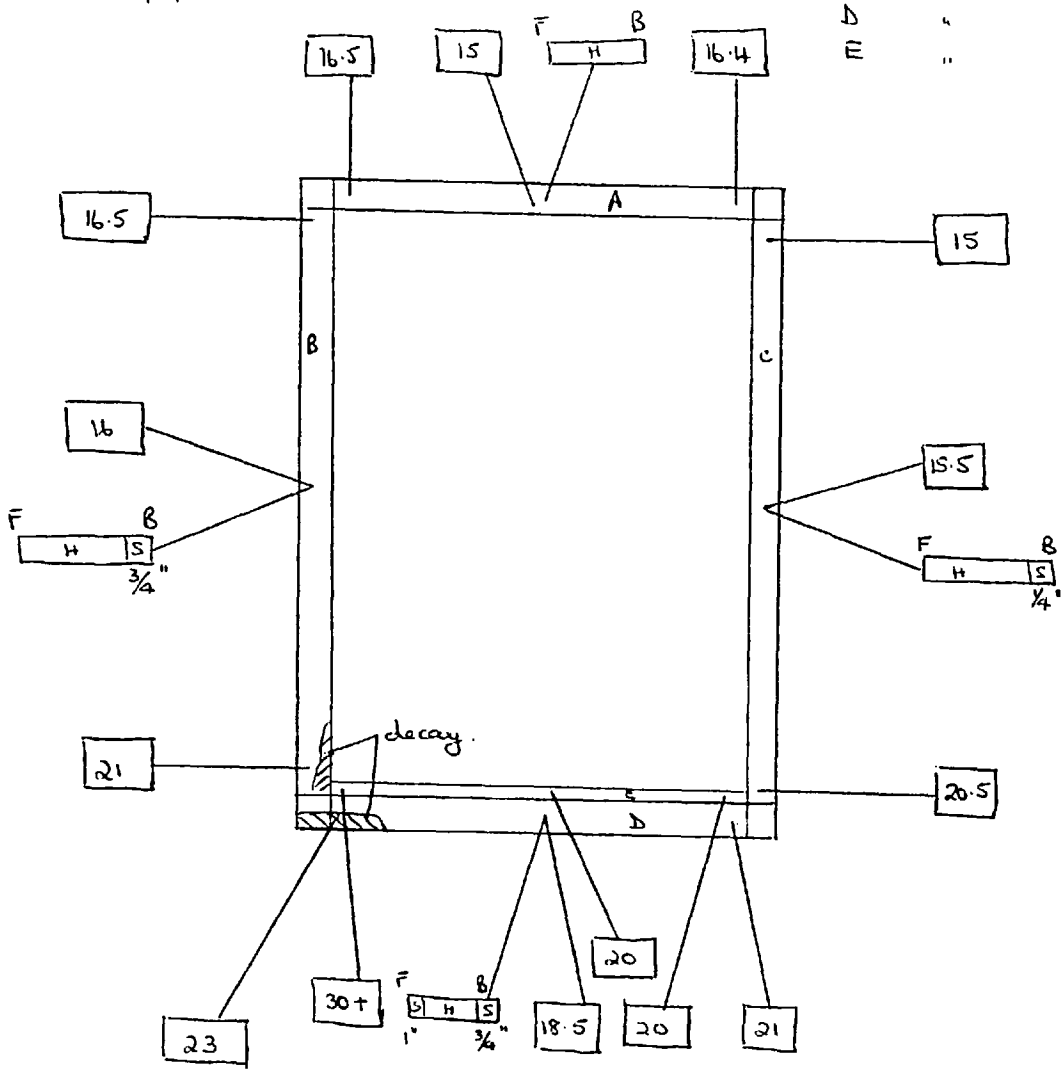
REMEDIAL TREATMENTS.

WINDOW No. 1 Frame to fixed light

SOURCE Larkhill

DATE 23/11/76

TIMBER SPECIES
A Scots pine
B "
C "
D "
E "



Notes. BD - slight decay at front of sill
Brown rot spreading up B from joint on interior face.

F front ie outside face B back ie inside face

H heartwood S sapwood

Per cent moisture content entered in the boxes

Fig 2.15 Record sheet for Larkhill windows

Fig 2.16 Cambridge; general view of the site

Fig 2.17 Cambridge; severe decay of sill associated with the water channel

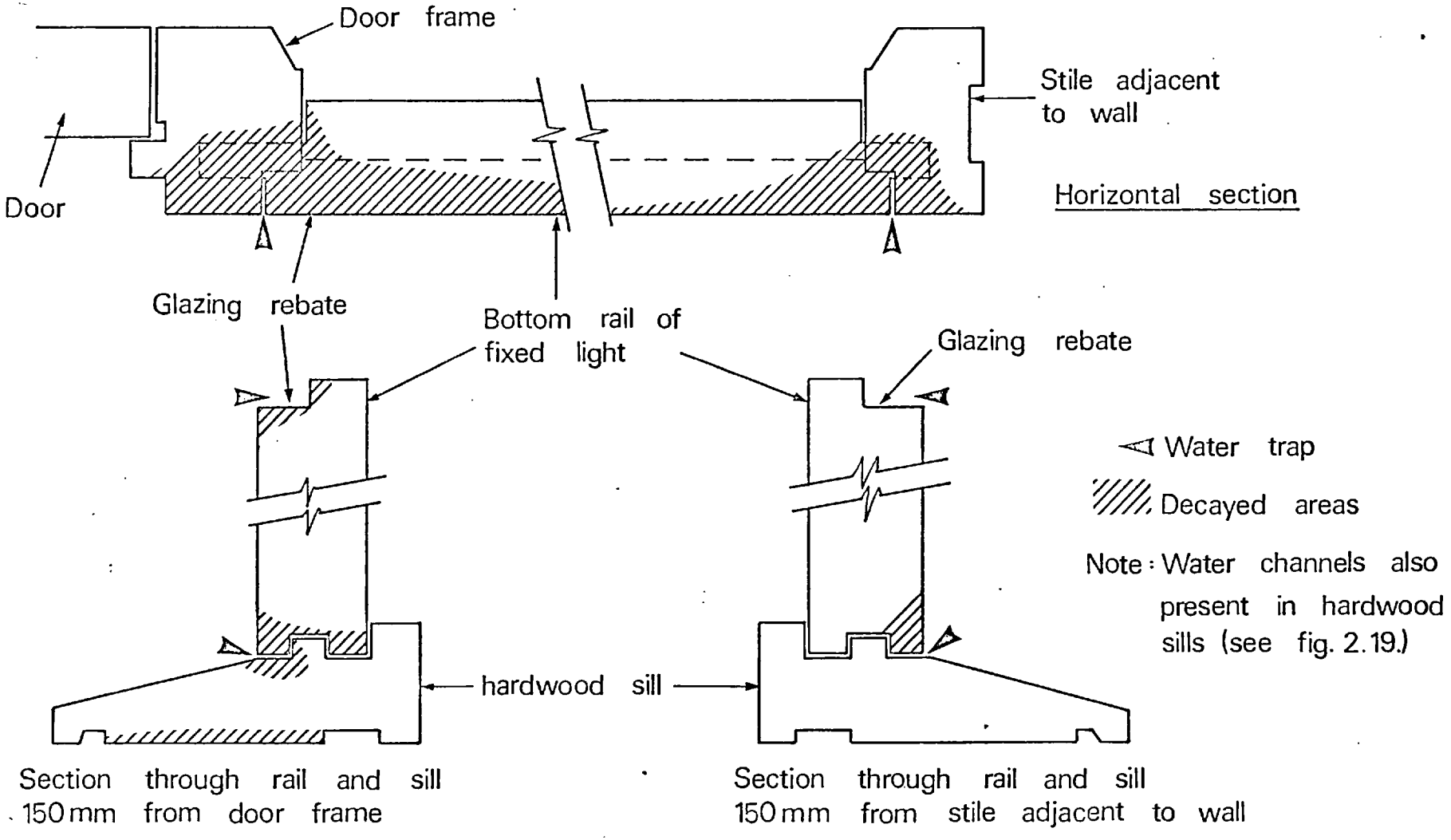


Fig. 2.18. Cambridge; construction and decay of lower portion of french door/window units

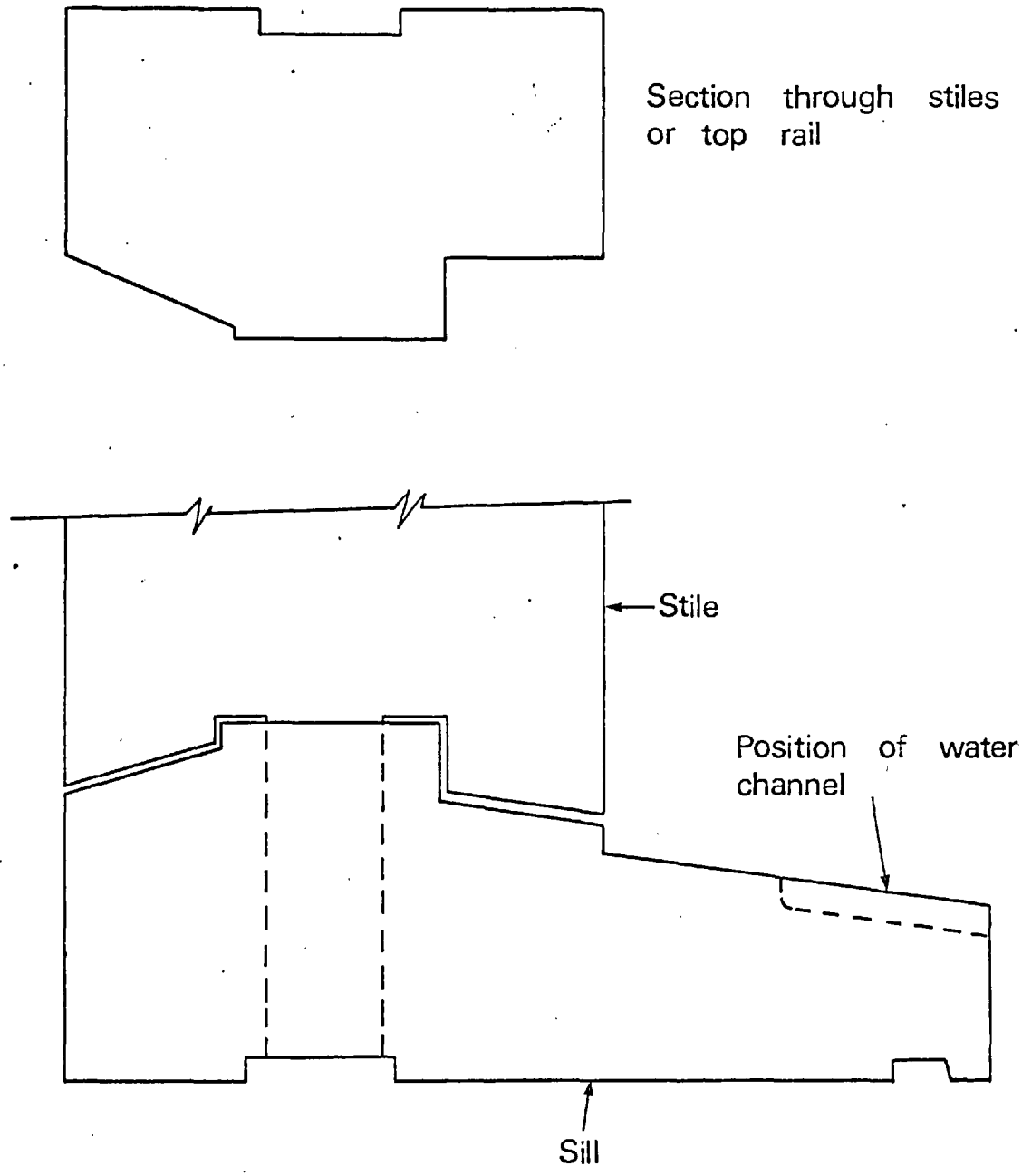


Fig. 2.19. Cambridge; construction of softwood frames to metal opening lights

Fig 2.20 Chatham; general views of the site

Fig 2.21 Chatham; decay in the centre of the bottom rail.

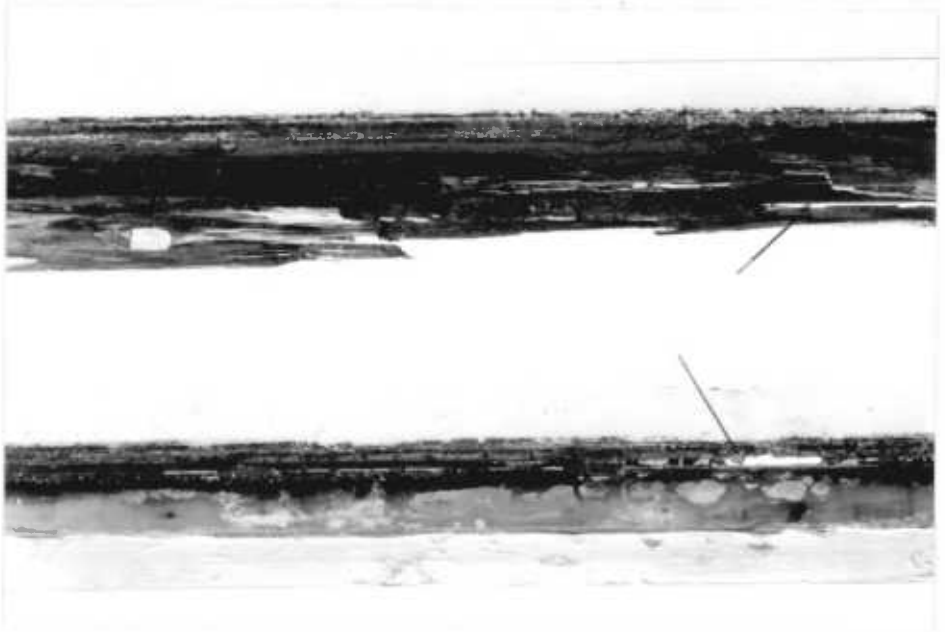


Fig 2.22 Chatham; closing mechanism rebate penetrating the glazing rebate in two sills (indicated by arrows).

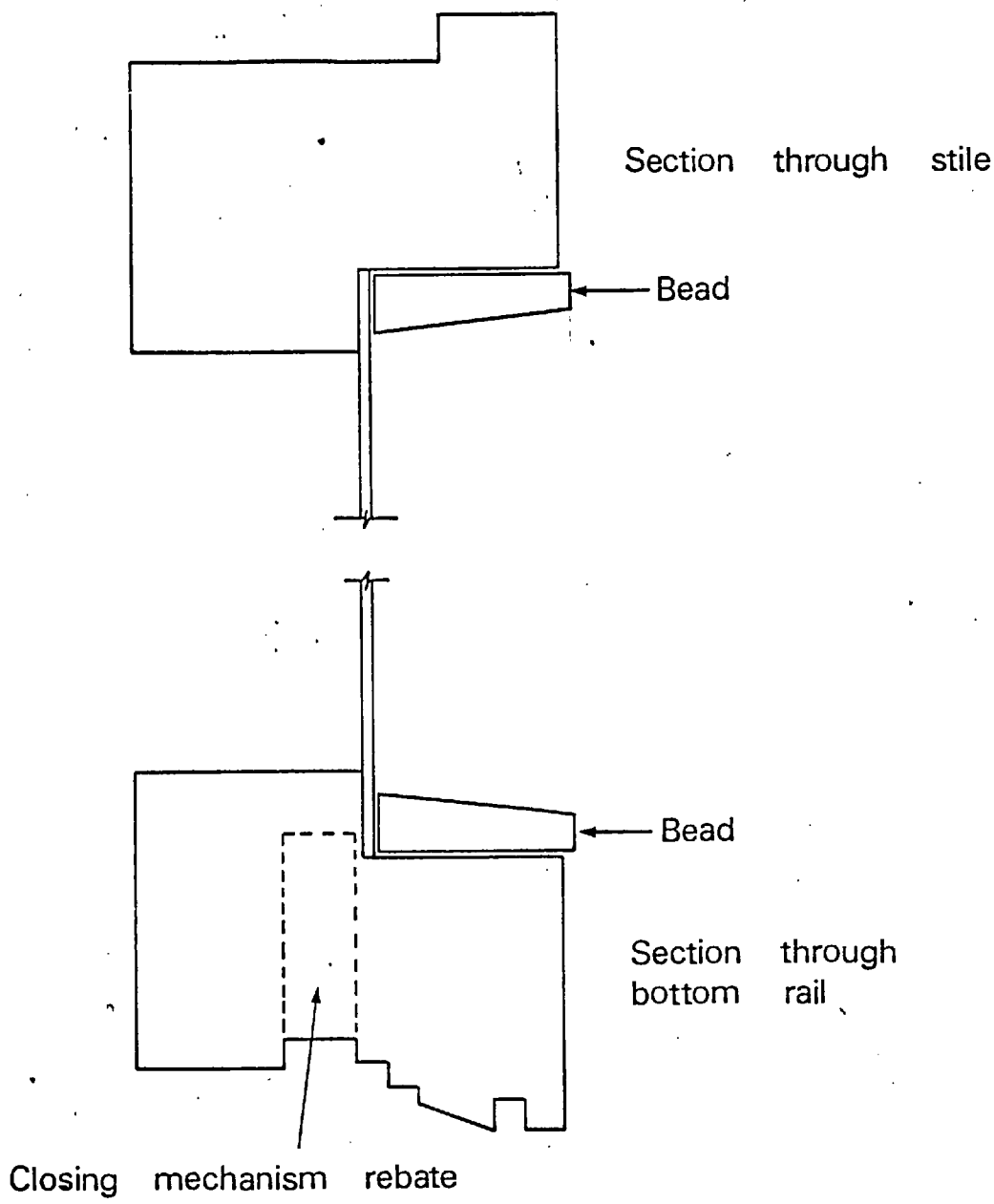
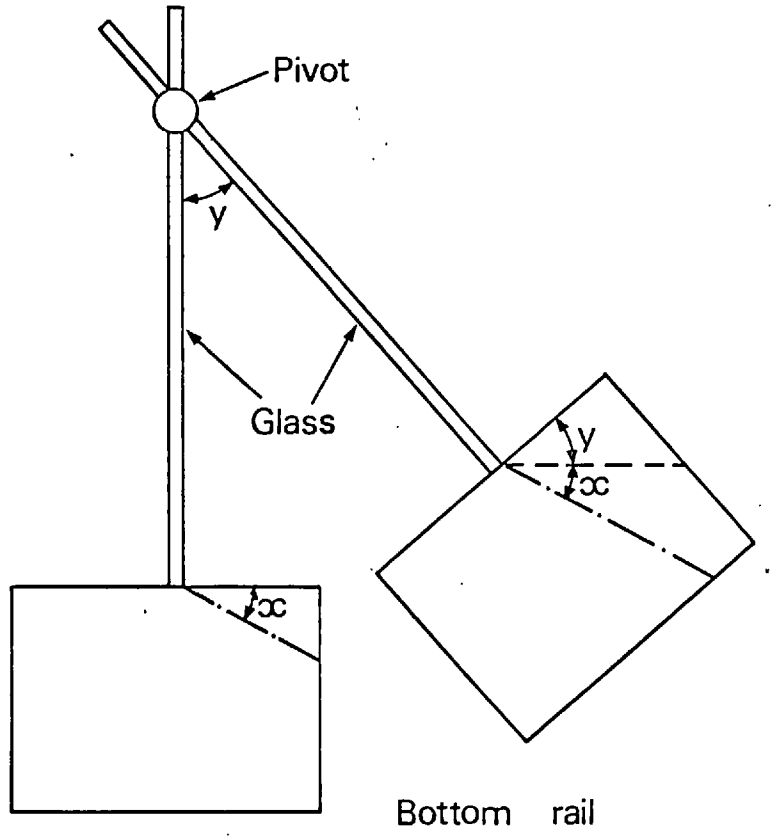


Fig. 2.23. Chatham; sections through window joinery

Fig 2.24 Sapwood in joinery grade redwood

Fig 2.25 Pith and juvenile heartwood in joinery grade redwood

Fig 2.26 Glue squeezed from around the dowels in a joint from a hemlock door. This was one of the best examples of spread of the glue although large areas are still not covered.

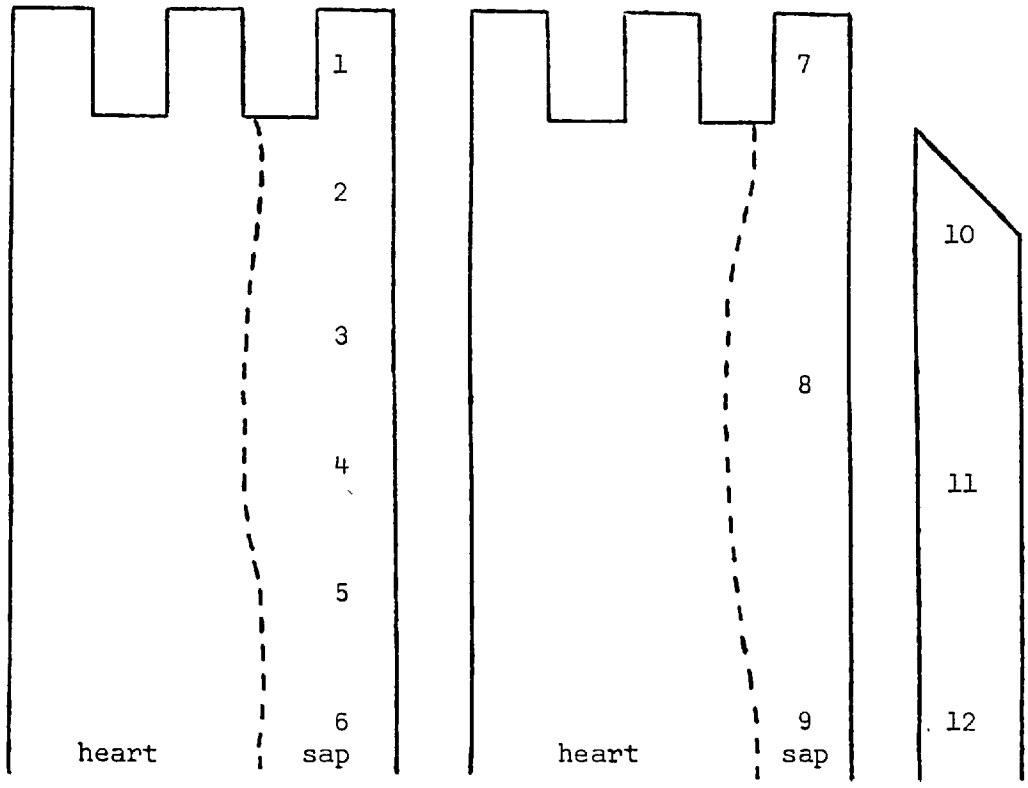


∝ = Angle necessary to prevent water accumulation when closed.

y = Angle of opening

∴ ∝ + y necessary to prevent water accumulation when opened to angle y

Fig. 2.27. Centre pivot hung window; theoretical considerations of preventing puddle formation



Section through centre of joint member

Upper exposed surface of joint member

Section through beading

Samples 1-6 taken from the mid-line of sapwood (when present) at 25 mm centres, on the face exposed by deep sawing.

Samples 7-9 taken from the mid-line of the sapwood on the surface exposed in service.

Samples 10-12 taken from the centre line of the beading on the surface exposed by deep sawing.

Fig 2.28 Larkhill windows; fixed plan of isolations

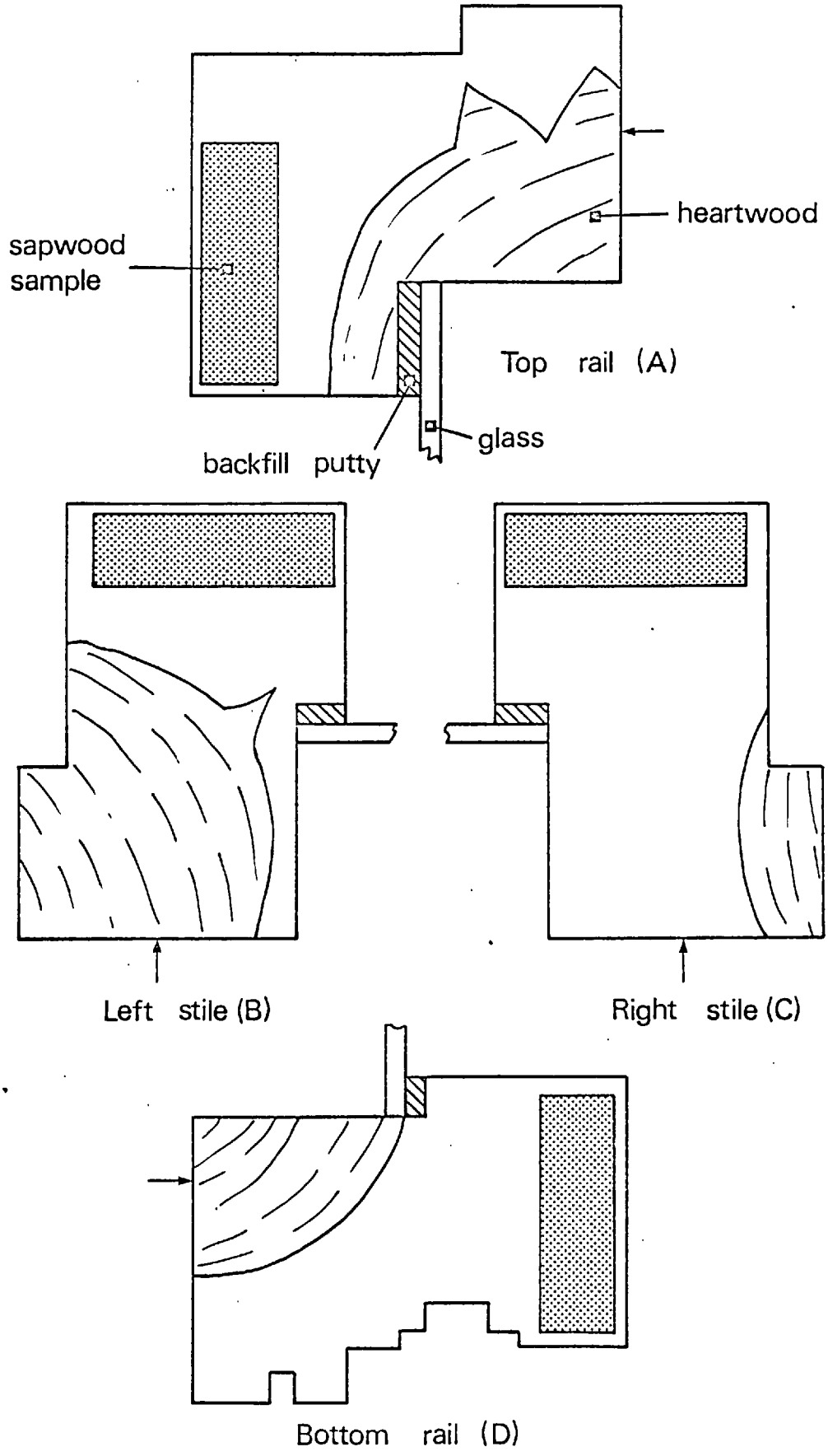


Fig. 2.29 Location of sample sticks in the cross - sections of the four members

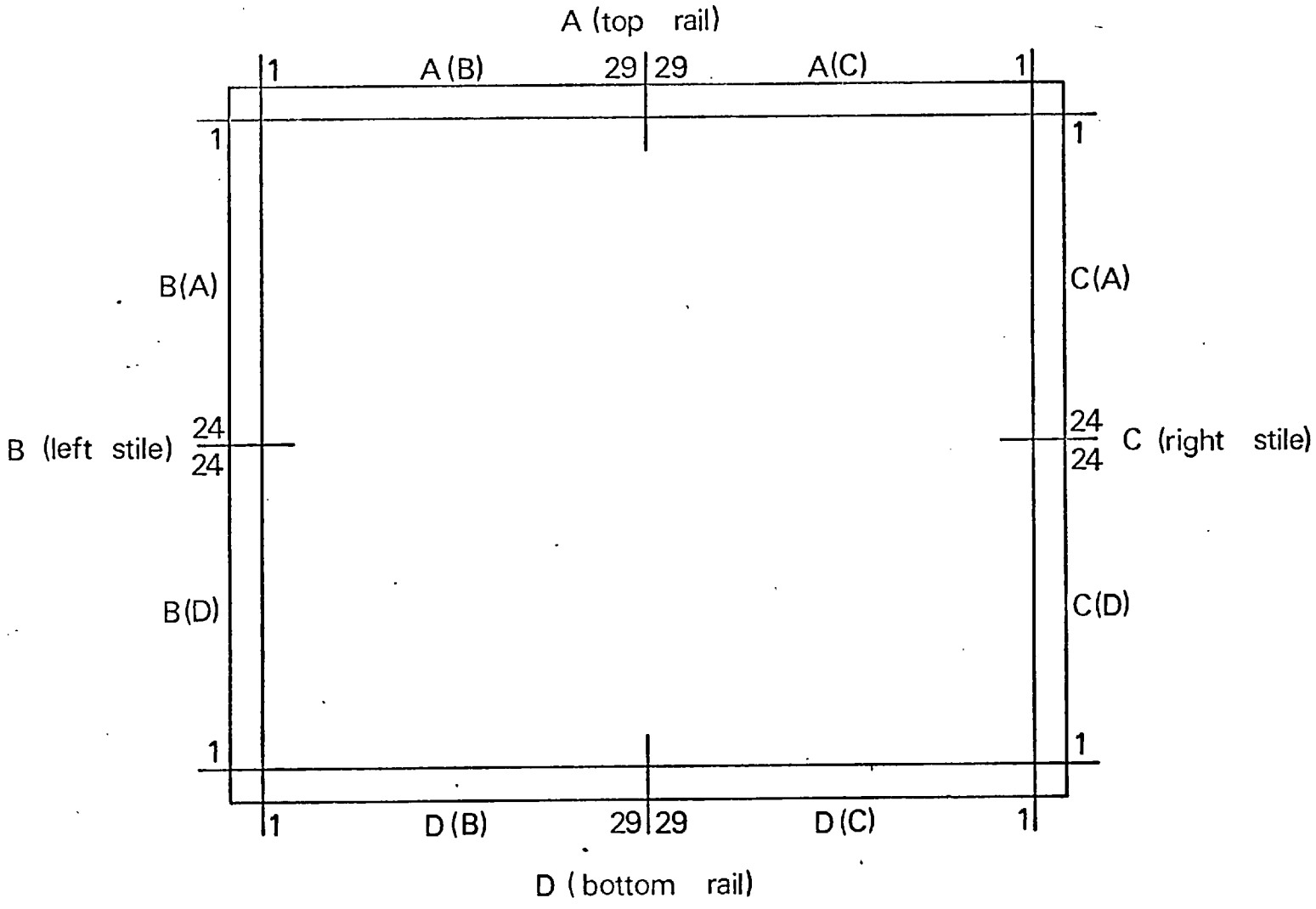


Fig. 2.30 Sampling of window frame (see section 2.4.1)

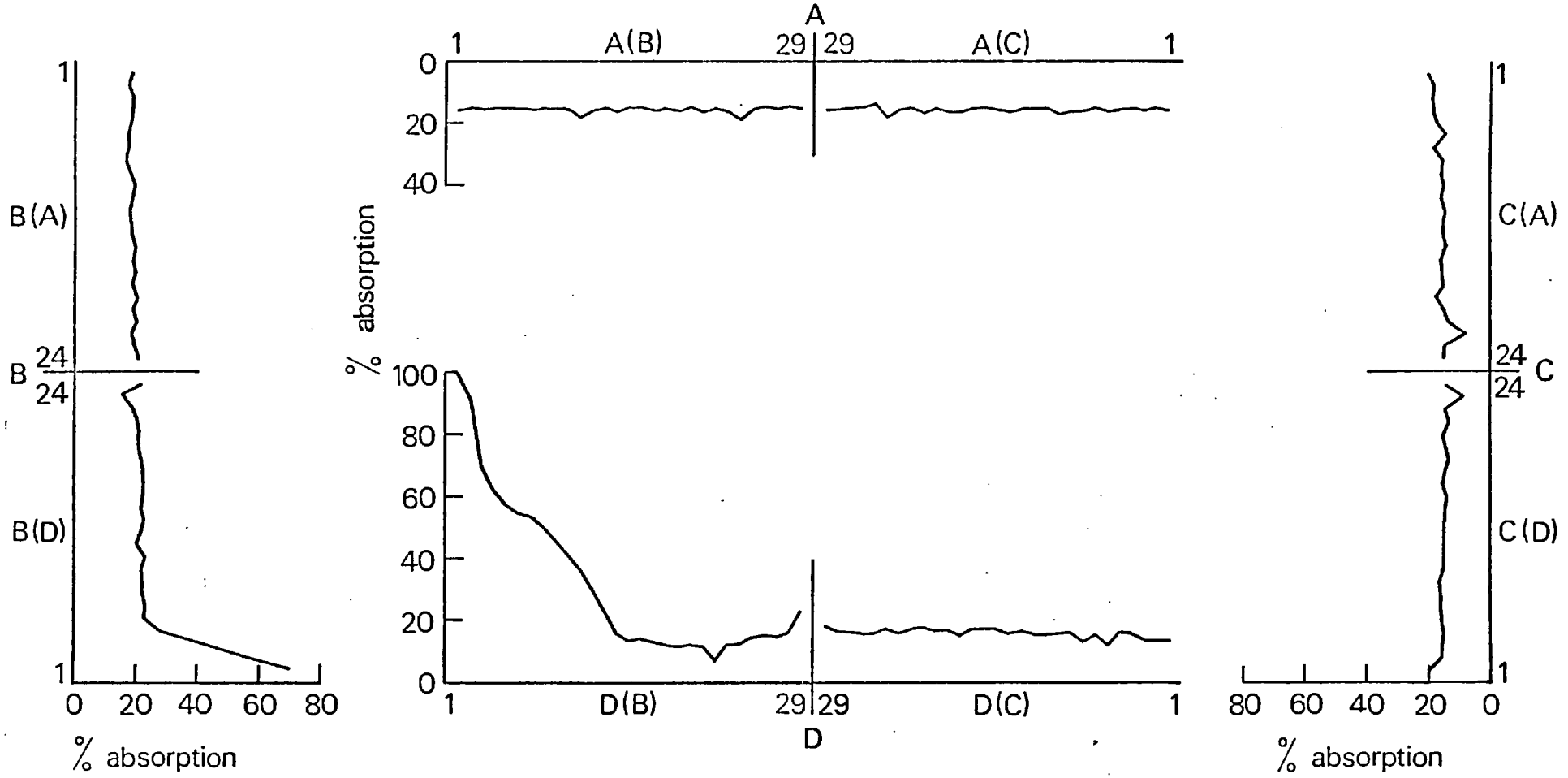


Fig. 2.31 Permeability (as per cent absorption of dekalin) of a window frame after 10 yrs. service (see fig. 2.30)

SECTION 3

OBSERVATIONS ON SIMULATED JOINERY UNITS; FIRST SERIES

3.1 INTRODUCTION

The use of exposure trials to evaluate preservative treatments designed for use in the less severe out of ground contact situation is well established. The level of interest can be gauged from the 33 replies received by Fougerousse (1976a) while conducting his survey of principles and methodology among members of the International Research Group on Wood Preservation. Numerous designs of exposure sample are under test, each reflecting a particular end use of preserved timber eg joinery, cladding, decking etc. In the joinery field, complete windows have been used (Purslow, 1975; Feist and Mraz, 1978) together with various units containing a single joint (Sedziak et al, 1970; Morgan, 1971; Shields and Krzyzewski, 1976; Feist and Mraz, 1978) representing a hazard situation. For example L-joint units simulate the lower joint of an opening light, which experience has suggested is one of the areas most susceptible to decay.

A series of L-joints, designed to test the effectiveness of water repellent systems, became available for destructive testing during 1974. The data obtained (Savory, Carey and Stribbling, 1977) indicated that such destructive testing could provide a valuable source of information on the deterioration of trial joints long before they failed due to decay. By the end of the exposure period ($4\frac{1}{2}$ years) visible decay had occurred even in those joints treated with 0.5 per cent tri-n-butyltin oxide (TnBTO); it was therefore concluded that observations should be made after shorter periods of exposure.

A pilot trial was therefore initiated using a novel design of L-joint and exposure method to compare the performance of untreated Scots pine sapwood with that treated by 3 minute immersion in one per cent TnBTO (the normal strength of solution used to treat window joinery). Additionally a small number of joints were prepared from imported western hemlock because of the then current interest in decay of hemlock exterior doors (Savory and Carey, 1975).

It was originally intended to study the uptake of moisture by the joints and the process of colonisation by bacteria (using a counting technique) and by fungi (using an isolation technique). In the light of subsequent information additional permeability monitoring was introduced in the later stages of exposure.

3.2 PREPARATION AND EXPOSURE OF TEST L-JOINTS

3.2.1 Materials

3.2.1.1 Sixty L-joints of Scots pine sapwood (Pinus sylvestris L) were prepared. The timber was British-grown and had been kiln dried immediately after conversion to 55 mm boards. Each L-joint consisted of two components measuring 203 x 38 x 38 mm (8 x $1\frac{1}{2}$ x $1\frac{1}{2}$ inches) with the grain parallel to the long axis and with random orientation of the annual rings.

The horizontal member provided the tenon for a bridle joint and was specially selected free from knots and heartwood, since this portion was to be used for sampling. The timber for the vertical member was not of such high quality since its rôle was solely to complete the joint. No glue or fixings were used.

3.2.1.2 Ten L-joints of western hemlock (Tsuga heterophylla (Rof) Sarg) were prepared. These joints were machined as above from joinery quality hemlock imported from western Canada. Although it was not checked, the sample was probably entirely heartwood since the timber is cut from large trees.

3.2.1.3 A one per cent by weight solution of TnBTO (Albright and Wilson Ltd) in white spirit was used to treat 20 of the pine L-joints.

3.2.1.4 Paint system:

1st coat ICI Dulux primer
2nd coat ICI Dulux undercoat, brilliant white
3rd coat ICI Dulux gloss finish, brilliant white

3.2.1.5 Hevikote (Thomas Ness Ltd) was used to seal the remote ends of both horizontal and vertical members. This is a two pack product combining the adhesion, chemical resistance and durability of epoxy resin with the

flexibility and water resistance of modified coal tar pitch; it dries partly by chemical action and partly by solvent evaporation.

3.2.1.6 The L-joints were marked with sequentially numbered aluminium labels using 12 mm anodised escutcheon pins for fixing.

3.2.1.7 Racks of Douglas fir plywood, canted back at 10 degrees to form a water trap in the joint area, facing south and approximately 900 mm above ground level were used to hold the test samples (fig 3.1).

3.2.2 Method

Twenty assembled Scots pine sapwood L-joints (3.2.1.1) were weighed, dipped for 3 minutes in the TnBTO solution (3.2.1.3) reweighed and allowed to dry for one day at room temperature. The three coat paint system (3.2.1.4) was applied to all L-joints after the end grain surfaces remote from the joint had been sealed using Hevikote (3.2.1.5). The L-joints were allowed to dry for two weeks, then were numbered (3.2.1.6) on the vertical member; the paint seal across each joint was then broken, by opening the joint and reassembling as tightly as possible. The L-joints were placed on the racks, on 5 March 1975, separated by a distance of 35 mm, maintained by spacer blocks attached to the rack near the top of the vertical member (fig 3.1). Those L-joints treated with TnBTO were exposed on separate racks from untreated L-joints to prevent any possible carry over of preservative.

The L-joints initially wetted very rapidly. During periods of heavy rain a puddle which persisted for several hours developed in each rack. It had not been anticipated that the rack design would trap water to this extent. To reduce the effect of the puddle, after 10 days exposure the L-joints were each raised on two 5 mm square stickers of Scots pine sapwood, both positioned away from the joint area (fig 3.2).

3.3 SAMPLING

3.3.1 Materials

3.3.1.1 Microwave moisture meter; the quantity of water in a sample is estimated by measuring attenuation of the microwave as it passes through the sample between the transmitter, adjacent to one face, and the receiver, placed directly opposite. This value, recorded in decibels, is converted to a moisture content value by use of the following formula:

$$M\text{-}5 = \frac{1.25 \times \text{decibels}}{T \times D}$$

M = per cent moisture content

T = thickness in cm

D = density in g/cc

The instrument cannot be used where the beam passes through layers of timber orientated in opposing directions, as encountered in joints. The distribution of measurements is also determined by the width of the measuring head (approx 35 mm). The maximum moisture content which can be determined, for the present test conditions, is just below 40 per cent.

3.3.1.2 Nutrient agar; Oxoid nutrient agar (Code CM3) prepared by suspending 28 g in 1 litre of deionised water. After autoclaving for 15 minutes at 121°C, either 9 ml aliquots were dispensed into test tubes, which were plugged and resterilised, or 20 ml aliquots were dispensed aseptically into 90 mm diameter disposable petri dishes.

3.3.1.3 Starch casein nitrate agar (Ottow, 1972)

soluble starch	10 g
casein	0.3 g
KNO_3	2 g
NaCl	2 g
K_2HPO_4	2 g
$MgSO_4.7H_2O$	0.05 g
$CaCO_3$	0.03 g
$FeSO_4.7H_2O$	0.01 g
agar	10 g
deionised water	1 l
rose bengal	0.350 g

The ingredients were mixed, autoclave sterilised for 20 minutes at 121°C then dispensed in 20 ml aliquots, under aseptic conditions into 90 mm disposable petri dishes.

3.3.1.4 Dekalin; decahydronaphthalene, the solvent currently used for testing penetration (BS 5707:Part I:1979).

3.3.2 Methods

3.3.2.1 Moisture content

The moisture content of the horizontal member of each L-joint was established at four positions (fig 3.2) using a microwave moisture meter (3.3.1.1) before exposure. This was repeated on a limited number of the replicates (see table 3.3) at various times during the first 101 days of exposure and again after 24 months.

After 24 months exposure the moisture content of three hemlock L-joints was determined by converting a 12 mm strip, from one side of the horizontal and vertical members, to 10 mm long blocks (fig 3.3). Each block was weighed and, after assessment for permeability (3.3.2.2), oven dried for 18 hours at 103°C then reweighed and the moisture content calculated. After 35 months exposure the moisture content was determined in this way for the horizontal members only of three replicates each of untreated pine, TnBTO treated pine and hemlock.

3.3.2.2 Permeability

A 12 mm strip was cut from one side of the horizontal member of L-joints exposed for varying lengths of time and subsequently air dried. Several unexposed check blocks were also sampled. The end-grain surface originally exposed within the joint was removed and the remainder cut into twelve 10 mm lengths which were carefully labelled to retain the L-joint number (indicating exposure period) and sequential position along the member (fig 3.3). The blocks were oven-dried to constant weight at 50°C, then weighed. After immersion for 10 seconds in dekalin, they were blotted, to remove excess solvent, reweighed and the uptake of dekalin calculated.

Joints exposed for 24* and 35 months were converted to the test blocks prior to air drying, utilising the system devised for the second series of tests (see section 4) which enables moisture contents above 40 per cent (the maximum possible with the microwave meter) to be determined.

Details of the experimentation carried out in the development of this method are reported in Appendix A.

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*This assessment was carried out on both the horizontal and the vertical members of the 3 hemlock L-joints sampled.

3.3.2.3 Bacterial counts (untreated pine L-joints only)
Using a flame sterilised handsaw and an alcohol sterilised vice, the horizontal member of each L-joint was cut to provide 10 mm long samples from the shoulders of the joint, the centre and the remote end (fig 3.4). A flame sterilised chisel was used to split each sample so as to prepare 6 test blocks, each approximately a 10 mm cube, from each sample. These blocks were placed in a sterile beaker, weighted down and covered with sterile deionised water. A vacuum of approximately 20 torr was drawn for 10 minutes, then released, thus saturating the blocks. Each replicate block was placed, in turn, within the mouth of a small sterile polythene bag and squeezed in a small vice. From 1 ml of the exudate thus collected in the bottom of the bag, a 1 in 10 dilution series in water was prepared; 1 ml of each dilution was mixed thoroughly with 9 ml of molten nutrient agar (3.3.1.2), maintained at 40°C in a water bath, then poured into a sterile plastic petri dish. The dishes were incubated at 22°C for 3 days, when the number of colonies was counted and the count per ml of exudate calculated.

3.3.2.4 Isolations
3.3.2.4.1 During the initial period of exposure (up to 93 days) small chips of wood were taken using the two chisel technique (Carey, 1975) from close to the joint, at the centre and at the remote end of the horizontal member of each L-joint being sampled. They were planted on dishes of 5 per cent malt agar (2.3.1.2), incubated at 22°C and observed for growth at frequent intervals. Preparations were observed microscopically to identify the organisms.

3.3.2.4.2 After one year's exposure a more comprehensive study of the microbial flora was carried out. One untreated (No 28) and one TnBTO treated (No 48) pine L-joint were sawn as shown in Fig 3.5. Each saw cut was made progressively from the remote end to the joint to ensure organisms could not be distributed further along the member than they had colonised. The samples were reassembled immediately after sawing and secured with elastic bands to minimise contamination.

Four small chips were cut from each sampling point using a 6 mm wide U-shaped gouge, and one planted on each of the following media:

nutrient agar (NA) (3.3.1.2)
two per cent malt agar (MA) (2.3.1.2)
benomyl/malt agar (Ben 2) (2.3.1.1 but using only 10 ml benlate suspension)
starch casein nitrate agar (SCN) (3.3.1.3).

Two L-joints prepared with the others, but never exposed, were sampled at position 3 only (fig 3.5), to act as checks on the sampling system.

In addition four untreated and four 1% TnBTO treated pine L-joints were sampled at position 3 using solely the Ben 2 agar, to investigate the Basidiomycete flora in more detail.

All dishes were incubated at 22°C and observed at frequent intervals. As each type of growth appeared, subcultures were made on to nutrient agar for the bacteria and 2 per cent malt agar (2.3.1.2) for the fungi. The fungi were subcultured until pure cultures were obtained.

Because of the diverse nature of the organisms obtained many were not observed in detail. They were divided into groups based on gross morphology so that patterns of colonisation could be established. The identity of certain groups has become apparent with the greater amount of work on identification carried out on cultures from the second series of L-joints reported in section 4.

Cultures showing Basidiomycete characteristics (eg no dark pigmentation, no sporing, silky appearance) were generally examined microscopically for the presence of clamp connections and other features to aid identification. Some of the confirmed Basidiomycete cultures were grown on sawdust medium (2.3.1.3) to provide typification as white or brown rots.

3.3.2.4.3 After 18 months exposure three untreated and three 1 per cent TnBTO treated pine L-joints were sampled; a similar set, plus three hemlock L-joints were sampled after 35 months exposure. Inocula were taken from position 3 and planted on Ben 10 agar (2.3.1.1). Subcultures of Basidiomycete-like growths were made on 2 per cent malt agar. Pure cultures were examined microscopically for the presence of clamp connections and other significant features.

3.3.2.5 Visual observation

After 15 months exposure, one untreated pine L-joint was converted as shown in Fig 3.6. Sections approximately 20 µm thick were cut, using a sledge microtome, from each numbered block. The sections were stained in safranin and picroaniline blue by the method described in TIL 52 (Anon,1974). Detailed observations were made on sections from radial, tangential and transverse faces for the presence and distribution of organisms and degrade of the timber structure. As a result of finding soft rot in these sections further sections were cut from the small blocks used to assess the changes in permeability (3.3.2.2) nearest to the joint (1) and from the remote end (12) of untreated and 1 per cent TnBTO treated joints exposed for one year.

3.4 RESULTS

A summary of the types of data collected after various exposure periods is given in Table 3.1.

The absorption, by each L-joint, of the 1 per cent TnBTO solution is given in Table 3.2.

The uptake of moisture by the L-joints recorded by the microwave moisture meter is presented in Table 3.3 and Fig 3.7. Moisture contents determined by oven drying after 24 months' exposure are presented in Table 3.4 and after 35 months' exposure in Table 3.5 together with mean values at each sample position for each set of replicates; values equivalent to the four moisture meter reading zones have been calculated and used in Fig 3.7 where appropriate.

Permeability values for each sample block and the mean at each sample position for each set of replicates are presented in Tables 3.6 to 3.8. Values for the hemlock samples are presented in Fig 3.8 together with equivalent moisture content readings; values for the pine samples are presented in Fig 3.9. Mean values for all sample positions for each set of pine replicates are presented in Table 3.9 and Fig 3.10.

The viable counts of bacteria per ml of exudate from each replicate sampled are presented in Table 3.10 together with the results from the fungal isolations over the same period.

The lists and brief descriptions of cultures isolated in the comprehensive study of L-joints 28 and 48 after 12 months' exposure are presented as an appendix to this section. The culture types have been mapped and are presented in Fig 3.11 and 3.12, for the untreated L-joint No 28 and in Fig 3.13 for the TnBTO treated L-joint No 48. The brief list of isolates from the two check blocks are also incorporated in the appendix.

The distribution of Basidiomycete cultures isolated after one year's exposure are presented in Fig 3.14 for untreated pine and in Fig 3.15 for 1 per cent TnBTO treated pine. Results after 18 and 35 months are presented in Figs 3.16 and 3.17 respectively. No Basidiomycetes were isolated from the hemlock joints after 35 months exposure. Data for the comparative performance of Scots pine sapwood and hemlock heartwood in laboratory tests and field trials are presented in Tables 3.11 and 3.12 respectively.

Microscopic observations on sections cut from one untreated L-joint after 15 months' exposure are presented in Table 3.13. Observations on untreated and 1 per cent TnBTO treated material after one year's exposure are presented in Tables 3.14 and 3.15 respectively. Photomicrographs of parts of the sections are presented in Figs 3.18-3.24.

3.5 DISCUSSION

3.5.1 Sample selection and exposure method

The L-joint design used for this section of work was chosen following the successful observations on larger specimens exposed for $4\frac{1}{2}$ years (Savory, Carey and Stribbling, 1977). For economy of materials and space a reduced specimen size was introduced. The cross-section was reduced to 38 mm square, the minimum width for measurements using the porosity meter developed by the Paint Research Association although in the event this apparatus was not employed. The length of each arm was reduced to 203 mm but the end-grain remote from the joint was sealed. By eliminating the influence of the end grain the length effectively becomes infinite, which overcomes the major criticism of the test method employed for the L-joints previously examined. The bridle joint was retained; although such a simple structure is rare in practice its use eliminates any influence a more complex design may exert on the rates of moisture entry

or colonisation by micro-organisms. Glue and often pins are normally employed in joinery; pins were not used because they would prevent the joint being taken apart and the joint surfaces examined during exposure. Glue would also have this effect during the early stages of exposure and should serve to seal joint surfaces against moisture entry. However, in practice it has been found that the UF type glues used in most low cost joinery are not resistant to water over prolonged periods. Glue was therefore omitted to reduce variation in the time taken for the glue to fail and allow entry of moisture and furthermore to reduce the overall exposure period. The paint film over the joint was broken before exposure so as to reduce variation in the time to failure and the overall exposure period. The joints were, therefore, all liable to wetting from the start although in practice this situation would occur at variable times depending on design, finishing, handling, exposure etc.

The exposure racks were canted back at an angle of 10 degrees to provide a moisture trap in the joint area of the samples. The heavy rainfall and snow experienced during the early days of exposure resulted in puddle formation in the rack and rapid wetting of the pine L-joints. Stickers were therefore placed under all L-joints after approximately 10 days exposure. Gross exposure to water, of comparable severity, can occur in practice eg the puddles which collect at the bottom rail/glass joint of partially open centre pivot hung lights.

TnBTO was selected as the preservative because it is the one used most widely for pretreatment of joinery in the UK. In practice, it is applied in a range of formulations, many containing water repellent additives. These are likely to influence the performance of any preservative. The effect may be beneficial for example by reducing water uptake by the timber but can be detrimental for example by reducing the toxicity of the preservative (Stalker, 1972). To avoid the complexity of such effects, the TnBTO was used in a simple solvent system, although it was realised that it did not represent the true practical situation.

The range of uptake of TnBTO solution (table 3.2) was within the normal variation for Scots pine sapwood treated by 3 minute immersion (Purslow, 1978).

3.5.2 Moisture content and permeability

The significance of moisture content in the decay of timber has long been established (Cartwright and Findlay, 1958) with levels of below 20-25 per cent being regarded as safe from attack. It has been shown in laboratory experiments (Duncan, 1953; Ammer, 1964) that within certain limits decay increases with increasing moisture content although at high moisture contents decay is often reduced due to lack of oxygen. There is some evidence which suggests this relationship is maintained under exposure trial conditions (Savory et al, 1977). Among the non-preservative treated L-joints examined, those treated with the higher levels of water repellent showed less decay. Although these joints were at risk of decay ie above 22 per cent for similar periods to those treated with lower levels of water repellent, the maximum moisture content achieved during exposure was lower (PRL, unpublished data).

The permeability of timber determines the rate of moisture absorption and thus influences moisture content. Over a century ago Britton (1875) noted the superior performance of red deals over yellow and white deals because they absorbed less water. More recently this argument has been used in favour of the use of spruce (white deal) as a joinery timber by Morgan (1975) who referred to data from T-joint exposure trials in which spruce showed lower moisture contents and less severe attack than pine sapwood after 5 years' exposure. It is believed by various PRL staff that some of the anomalies between the expected life based on laboratory natural durability tests and the actual life in service are a result of the inherent permeability of the timber.

Since the permeability of the timber affects the moisture content achieved under exposure conditions, these two properties are discussed together.

The microwave moisture meter used to monitor changes in moisture content has the disadvantage of a restricted range; the maximum moisture content that could be recorded under the prevailing test conditions was 36-38 per cent. The pine sapwood L-joints showed a very rapid initial uptake of moisture (table 3.3) at the joint end and transmission along the horizontal member so that after 10 days exposure the end remote from the joint was at a moisture content above the maximum recordable in some replicates. This situation continued for the remainder of the exposure period and so no true moisture contents were established until 35 months exposure when a determination was carried out by oven drying. The moisture contents

recorded varied from 67 to 175 per cent. The highest values were recorded close to the joint; values along the remainder of the member were consistent (table 3.5).

In contrast to the pine sapwood, the hemlock showed a much slower increase in moisture content (tables 3.3 to 3.5; fig 3.7). Adjacent to the joint, the moisture content did not exceed 20 per cent during the first 31 days of exposure; transmission along the member was very slow so that after 66 days the moisture content at the remote end had risen only to 16.2 per cent. During the period 66 to 101 days considerable drying took place at the joint end; this effect was not transmitted to the other sampling positions which all showed small increases over the same period. This period was characterised by above average temperatures corresponding to those normally reached during the summer period (local Meteorological Office records).

The moisture contents were not monitored again until 24 months' exposure, mainly due to a fault in the moisture meter. The moisture contents close to the joint were much higher than previously recorded; the horizontal member of L-joint No H9 which was more permeable than the other two (table 3.8) showed higher moisture contents and a greater transmission of moisture along the horizontal member (table 3.4). After 35 months exposure the moisture contents were generally lower than after 24 months. Both measurements were taken towards the end of the winter period (March 1977 and February 1978 respectively) and therefore probably represent the maximum moisture contents achieved during each year's exposure. The difference between the figures correlates with the weather conditions which were much wetter than average in the winter of 1976/77 and about average in the winter of 1977/78 (local Meteorological Office records).

The differences in the rate of moisture uptake and the moisture contents achieved in the pine sapwood and the hemlock can be accounted for by the inherent differences in permeability of the two timbers (Anon, 1977). The patterns of moisture uptake is, however, essentially the same since this is determined by the L-joint system. The end grain remote from the joint is effectively sealed with Hevikote; the paint system, while remaining intact, will only allow slow changes in moisture content (Anon, 1969). Gross uptake of moisture can therefore only be effected via the surfaces exposed within the joint.

The moisture content data suggest that hemlock should perform better than pine sapwood under equivalent conditions of exposure. In addition, there is some evidence which suggests the end grain of the hemlock is easily sealed to prevent moisture uptake. Moisture is not usually transmitted as far up the vertical member as it is along the horizontal member of an L-joint (Purslow and Williams, 1978) due to the effects of gravity. This effect was exaggerated in the hemlock L-joints examined after 24 months exposure (table 3.4, fig 3.8). Even within the joint the moisture content in the vertical members barely exceeded 30 per cent while the horizontal members were generally above 30 per cent for half their length and several individual readings near the joint exceeded 100 per cent. Apart from orientation during exposure, the end grain in line with the "horizontal" samples is exposed directly to moisture within the joint but the "vertical" samples are in line with end grain sealed with a paint film (fig 3.3). If end grain sealing can prevent moisture uptake to this extent, the use of sufficient quantities of a moisture resistant glue, or a water repellent should give similar protection and thus improve performance.

Furthermore, in contrast to pine sapwood (see later) the permeability of hemlock is not modified to any extent during exposure; Table 3.8 and Fig 3.8 show that the permeability at the joint end, where wetting has occurred, is very similar to the permeability at the remote end, where moisture contents have not exceeded 20 per cent. The heartwood of other timbers such as pine and spruce has also shown no increase in permeability in a ponding situation. (Dunleavy and McQuire, 1970; Boutelje et al, 1977).

Despite all these points in its favour, hemlock can decay rapidly in service. Savory and Carey (1975) and Soane (1978) record failures of hemlock external panelled doors, due to decay of the hemlock, after as little as five years service (replacements after 2-3 years were found to be due to the use of infected timber or decay of perishable hardwood dowels). Doors containing pine sapwood have been found to decay after similar periods. This rapid failure of hemlock could be a result of the distribution of the moisture that is absorbed. At the joint surface the moisture content exceeded 20 per cent within 40 days of exposure and therefore became susceptible to decay. Although the pine sapwood

exceeded 20 per cent within 3 days this difference in time would not be significant during an exposure period of, for example, 5 years. Data presented later (section 3.5.4) suggest the colonisation of the hemlock may be affected by factors in addition to moisture content and therefore the explanation may be more complex.

The permeability of the Scots pine sapwood was, in contrast to the hemlock, markedly modified during exposure (tables 3.6, 3.7 and fig 3.9). After one month's exposure two of the three replicate blocks at sample position 1 (ie closest to the joint) had uptakes of dekalin above the range of values established for unexposed sample blocks. This may indicate the beginning of increases in permeability. At 6 weeks' exposure there was wide variation between the two replicates which reflects the rapidity of reaction, during the initial period, once changes have been initiated. At later stages, variation was much reduced.

There is little variation in permeability with sample position along the length of the members; mean values for all samples have therefore been calculated (table 3.9) and plotted against time (fig 3.10). This shows the initial fast rate of reaction, after a short lag, followed by a steady rate of increase over the remainder of the exposure period for both untreated and 1 per cent TnBTO treated replicates. This pattern of reaction will be further discussed in conjunction with the results of the bacterial counts.

3.5.3 Bacterial counts

Counting the numbers of bacteria in a piece of wood is extremely difficult. Direct observation by light or electron microscopy is fraught with difficulty due to variable losses during preparation of specimens for examination. Comminution of the sample, followed by plating out dilutions of the washings can be plagued by high background counts (Savory, 1975). The alternative system of squeezing liquid from waterlogged blocks (Dunleavy et al, 1973) and enumerating the bacteria using a viable count was therefore attempted. Although one cannot remove all bacteria in this way it is hoped the numbers released will reflect the total number present and give indications of proliferation.

The counts were variable and therefore results from each L-joint are presented individually in Table 3.10. No reason for the complete failure of counts after 93 days exposure was established. The results show a sharp rise in numbers between 23 and 37 days exposure for the entire length of the L-joint member. This suggests that in a sample of this length the phase of initial colonisation (ie movement along the sample) is complete before proliferation takes place. The increase in bacterial numbers between 23 and 37 days correlates with the increase in permeability recorded between one month and 6 weeks' exposure. In the almost total absence of a fungal flora at this stage in exposure (table 3.10) it is postulated that the bacteria are responsible for the increase in permeability. It is well established (Suolahti and Wallén, 1958; Ellwood and Ecklund, 1959; Greaves, 1966) that bacteria are responsible for increases in the permeability of green timber stored commercially for protection under water in log ponds and in laboratory experiments. The present data extend the range of environments, in which such changes take place, to include processed pine sapwood in which a ponding situation is established by water being taken up via the joint and trapped within the timber by the impermeable paint film.

3.5.4 Isolations

Few attempts to isolate fungi were made during the early stages of exposure because of the concentration of effort on the bacterial counts. No fungi were isolated from unexposed material or after 9 days exposure. By 36 days an Aureobasidium type was present near the shoulders of the joint and by 93 days an Aspergillus sp was also present.

After one year's exposure a more comprehensive sampling system was introduced. Earlier observations had monitored numerical changes in the bacterial flora and the entry of staining fungi by 37 days exposure. By sampling to the pattern used (fig 3.5) it was hoped to be able to follow colonisation, as it progressed, and to detect the spatial arrangement of the different species present. Although there have been numerous ecological studies of timber in various environments many have initiated sampling only after active or incipient infection was present (Käärik, 1967 and 1968; Butcher, 1968; Banerjee and Levy, 1971;

Sedziak et al, 1970; Shields and Krzyzewski, 1976) or have relied on those fungi induced to fruit on the surface of bait blocks (Eaton, 1969; Eaton and Jones, 1971). The L-joints sawn after one year's exposure showed some bluestain but otherwise appeared sound and yet produced a complete spectrum of organisms including bacteria, moulds, stainers, soft rotters and Basidiomycetes (figs 3.11 to 3.13). This result emphasises the importance of a comprehensive sampling system to be used throughout exposure and not only when decay has been established.

The four media used are selective for different types of organisms and in this way it was hoped to establish the spectrum of species present. The nutrient agar provides a suitable environment for many bacteria and certain types of fungi, while malt agar provides a non-selective environment for many fungi. The benomyl/malt agar is selective for Basidiomycetes while the starch casein nitrate agar (plus rose bengal) promotes the growth of many soil inhabiting fungi (Ottow, 1972) and has the added advantage that Actinomycetes produce pink colonies by incorporating the rose bengal. It can be seen from the lists of culture numbers (see Appendix) that each medium contributed to the spectrum of organisms although a proportion were isolated on several of the media.

Several species of fungi grew from a single sample chip on many occasions. Although care was taken to separate as many pure cultures as possible it is inevitable that some of the species present at a particular point were not separated either because of a slower growth rate than co-habitants on the media provided or perhaps due to inhibition by other organisms. It can be seen (figs 3.11 and 3.12) that few organisms were isolated in the presence of Trichoderma viride (sensu Bisby, 1939) which has both a fast rate of growth and known antagonistic properties (Grosclaude et al, 1974; Toole, 1971). Various observations on the distribution of organisms are, however, pertinent.

No bacteria and few fungi were isolated from the check blocks sampled with L-joints exposed for one year. These blocks had been stored in a warm, dry atmosphere during the exposure period and do not, therefore necessarily reflect accurately the microbial population at the time of exposure. However the result shows that contamination by bluestain fungi did not occur during sawing and the isolation process.

Aspergillus/Penicillium types were not common on exposed material and therefore were probably true inhabitants of the check material.

Bacteria were present throughout both exposed L-joints; only a few "a" sites (ie the upper surface) near the remote end of the untreated L-joint failed to produce growth. Trichoderma viride was restricted to the joint area of the untreated L-joint, and was not present in the treated L-joint. This localised distribution is surprising in view of its common occurrence in isolations from timber in ground contact (Käärik, 1967 and 1968; Henningsson and Nilsson, 1976) or from stumps (Käärik and Rennerfelt, 1957) and as a contaminant of isolations from decayed joinery in service.

Bluestain organisms were widely distributed in both L-joints. The distribution of the various morphological types from the untreated L-joint (fig 3.9) shows some interesting patterns. BS 1 (typical Aureobasidium pullulans with hyaline conidia) was largely restricted to the outer surfaces of the block and was not isolated from the same position as BS 3 (Aureobasidium pullulans with lilac coloured conidia) which was restricted to a small area at positions 3 and 4 along the length of the sample. BS 6 and BS 7 were only isolated near the upper surface and BS 4 and BS 9 only close to the joint.

Yellow cultures (subsequently identified as the Phialophora imperfect stage of Coniochaeta ligniaria) were isolated only from the treated L-joint. Various Phialophora species have been shown to predominate in timber treated with various water-borne preservatives and exposed in the ground contact situation (Henningsson and Nilsson, 1976; Fougerousse, 1976b). In the out of ground contact situation Phialophora species have been isolated from naturally durable western red cedar shakes (Smith and Swann, 1976) and from preservative treated (copper naphthenate, PCP) trial joints (Sedziak et al, 1970; Shields and Krzyzewski, 1976). The present record of Phialophora from another preservative (TnBTO) treated substrate adds further evidence of the importance of this genus in the attack of treated timber.

The Basidiomycete flora of the pine L-joints varied little between exposure periods or between treated and untreated material (figs 3.11 and 3.13 to 3.17). The dominant organism was Sistotrema brinkmannii; other isolates have not been identified.

The rôle of Sistotrema in the process of colonisation and decay of timber is an enigma. It has been shown to be an early coloniser of stumps (Käärik and Rennerfelt, 1957; Hallaksela, 1977; Rayner, 1977b) and of timber in ground contact (Merrill and French, 1966; Käärik, 1967 and 1968). It has been isolated from shakes on roofs (Smith and Swann, 1976) in addition to joinery in service (see section 2.3) where it is often associated with an apparently slow acting brown rot. In the laboratory it readily colonises and produces an increase in the permeability of Scots pine sapwood (section 6.2) and shows cellulase activity by clearing cellulose agar (Suhirman, 1978). However it has not produced significant weight loss in decay capability trials (section 2.3; PRL unpublished data) although Käärik and Rennerfelt (1957) recorded 6.9 per cent weight loss of pine sapwood after 4 months exposure using a soil burial method. It has been shown (von Aufsess, 1976a and 1976b) that Sistotrema is antagonistic to certain other Basidiomycetes and has been classified by her as a "rot accompanist" although the antagonistic effects could be interpreted as a mechanism to facilitate its entry into the natural succession of fungi. The presence of Sistotrema does not affect the rate of decay of Scots pine sapwood blocks by either Coniophora puteana or Coriolus versicolor (section 6.4). On balance the rôle of the organism would appear to be that of a slow acting brown rot.

The significance of the patterns of distribution of isolates in relation to the process of colonisation and subsequent decay is difficult to assess. The bacteria, as previously recorded, are present in high numbers by 37 days exposure and are still present throughout the L-joints after one year. Fungi colonise more slowly but after one year many species are present. Evidence from the untreated L-joint sampled after one year's exposure suggests certain types are spatially separated from other types. However, since it is doubtful if all species present in each sample chip were successfully isolated, and due to the lack of proper observation of individual members of the morphological type groups, this conclusion is only tentative. Evidence from observations on patterns of colonisation in hardwood logs and stumps (Rayner, 1977a) shows that similar spatial arrangements occur, and therefore the patterns observed may be a real effect. It is evident

that further work on the early stages of colonisation should be undertaken; the isolation of Basidiomycetes from both treated and untreated material after one year's exposure shows any succession of organisms to be approaching completion by this time, since the observations made on joinery rejected after failure in service (section 2) indicate that ultimate failure is primarily the result of attack by the wood-rotting Basidiomycetes. None of the isolates (apart from Sistotrema) has been characterised as a brown rot; examination of decayed joinery has suggested that in many cases brown rots follow white rots, and so the succession may be lacking this final component. It is important to elucidate the stages involved in the colonisation process since it has been shown that basidiospores of one Basidiomycete, Lenzites trabea, are unable to germinate (in fact they lyse) on the sapwood of commercially dried Scots pine that has not been preconditioned for example by the growth of bacteria or staining fungi (Savory and Carey, unpublished data).

Isolations from three hemlock L-joints exposed for 35 months failed to yield any Basidiomycete cultures although in the joint area at least these samples had been "at risk" of decay (ie above 22 per cent moisture content) for virtually the entire exposure period. Basidiomycetes were present in pine sapwood joints within a year of exposure. This difference is difficult to attribute to a difference in natural durability since the information available is contradictory. Although the two timbers show different weight losses in laboratory tests (table 3.11) both are classed as "non-durable" and show almost identical life in stake tests (table 3.12).

An alternative explanation is, however, possible. In tests of the germination of basidiospores of Lenzites trabea and Poria monticola on a range of timber species (Savory and Carey, unpublished data) both fungi failed to germinate on western hemlock; spores of L. trabea were found to lyse. As previously stated, a similar phenomenon was observed with commercially dried Scots pine sapwood; germination was, however, possible after this timber had been "detoxified" by the growth of a variety of staining fungi, Trichoderma viride and bacteria from log pond water. It has been shown that this process does take place under service conditions (section 2.4). Hemlock heartwood is likely to be difficult to "detoxify" in this way, since in general, staining fungi and bacteria do not grow in heartwood. In the out of ground contact situation,

decay must occur largely as a result of spore-borne infection and if the spores cannot germinate infection cannot occur. In many instances of the failure of hemlock in joinery (Savory and Carey, 1975; section 2.2.2) the hemlock was used in association with other non-durable or perishable species which could have acted as the substrate for spore germination. Hemlock alone may have given improved service. Many of the early colonisers of hemlock are white rot fungi; these may be less affected by the hemlock "toxin" than the two brown rots used in the experiments, although no data are available to support this theory. In time, however, sufficient detritus is likely to build up in open joints or other niches to allow germination to take place; the hemlock would then be colonised from a mycelial source rather than directly from the spores.

The difference in the performance of hemlock as L-joints and as joinery does not appear to be due directly to a difference in the severity of exposure. The poor performance as joinery is more likely to be due to in-built water traps and association with easily colonised timbers. This observation serves to emphasise the need for careful design, selection of materials and execution of the design of timber structures for outdoor exposure situations.

3.5.5 Visual observations

The observations on sections cut from the L-joints are entirely subjective. Although the benefit of a more objective method has been shown by Dwyer and Levy (1976) and is currently being developed further by Clubbe (1978), it was felt the observations were not of sufficient importance to the current study to warrant the time necessary to adapt the system. The observations developed into scoring the presence or absence of the range of features used in Tables 3.13 to 3.15 and an estimate of the relative numbers of soft rot cavities observed.

The single joint sectioned after 15 months' exposure (table 3.13) showed, throughout, pigmented hyphae and/or spores (fig 3.18) very similar to those of Aureobasidium pullulans illustrated by Schmidt and French (1976). Hyaline hyphae bearing clamp connections (fig 3.19), indicating the presence of Basidiomycetes, were also present throughout, at times giving rise to chains of "spores" (fig 3.20) although the "spores"

were often observed without hyphae. Since the predominant Basidiomycete isolated from the L-joints has proved to be Sistotrema brinkmannii these "spores" are attributed to this organism and may prove to be a form of the "bulbil type cells" produced in culture (Baniecki and Bloss, 1969; Siepmann, 1969).

Soft rot cavities (fig 3.21) were present at all the locations observed although more abundant in the tenon. This observation was surprising in view of the normal pattern of attack by soft rot, ie a gradual advance from the surface. Superficial soft rot of joinery had been reported previously by Duncan (1963). At the time, it was thought the deep penetration could be due to the L-joints becoming very wet at an early stage in exposure; attack could also have been assisted by additional nitrogenous nutrients from the excreta of birds which use the exposure racks as perches. Baines (1976) also detected the presence of nitrogen fixing ability in an L-joint removed shortly afterwards, which provides an alternative supply of additional fixed nitrogen. It has been shown (Savory and Bravery, 1971) that in a short-term closed system the amount of soft rot attack varies with available nitrogen although over longer periods, the evidence of Bletchly (1959) points to some recycling of nitrogen taking place. A subsequent search of the literature has shown that Harmsen (cited by Henningsson, 1977) has found soft rot in window joinery in Denmark and latterly (Suhirman, 1978) in joinery from the site at Chatham (see section 2.2.5). However, no soft rot was observed in sections from Canadian experimental material (Shields and Krzyzewski, 1976) perhaps due to the less severe exposure conditions at this site.

As a consequence of finding soft rot in this untreated L-joint after 15 months exposure, sections were cut from four untreated and four one per cent TnBTO treated L-joints exposed for one year. Sections cut from untreated material adjacent to the joint (block No 1) showed as much or more soft rot attack than in the tenon of the previous sample, but three of the four samples from the remote end (block No 12) showed no cavities (table 3.14).

The amount of colonisation of the treated material was generally much lower than in the untreated material. A few soft rot cavities were found in two "joint" samples and one "remote" sample (table 3.15). The

treatment has therefore not provided complete protection. In addition to normal cavities (fig 3.21) an area of atypical attack was found in block 47/1. The attack did not produce the normal extinguishing effect under polarised light, did not follow the angle of the microfibrils in the S_2 layer (fig 3.22) and sometimes appeared branched (fig 3.23 and 3.24). The attack could be either cavities in the S_1 layer, as described by Bailey and Vestal (1937) or erosion of the S_3 layer (the type 2 attack of Corbett, 1965). Unfortunately transverse sections cut to pass through the decayed area did not show degrade analogous to that observed in longitudinal section probably as a result of the complete area having been removed during cutting of the longitudinal sections. In pure culture studies with Chaetomium globosum by Bravery (1971) two of his photographs (fig 17 and 18) could be interpreted as erosion of the S_3 layer parallel to the direction of the microfibrils, although the author did not interpret them in this way. Branched forms of attack have not been figured in published work although Nilsson (1976) reported seeing similar "abnormalities" in preservative treated poles. They may therefore be a result of attack of preservative treated wood by one of the many fungi shown to be present in these situations.

3.5.6 General discussion

The original design of L-joint used in exposure trials at PRL has shown decay of untreated, painted material, sufficiently severe to require replacement of frames in a practical situation, after some 7-10 years exposure (Purslow and Williams, 1978). They therefore represent an exposure of comparable severity to the worst experienced in practice (see section 2). Changing the orientation of the specimens on the rack, raising them on stickers and sealing the end grain remote from the joints should have made the test more realistic. The watertrap situation between the sample and the backing board has been removed and rapid uptake of moisture through end-grain surfaces has been restricted to those within the joint, as in most practical situations. The severity of exposure has probably therefore been reduced but one can only speculate as to the extent since the trial joints have not yet been exposed to failure.

Assuming that the L-joints are duplicating certain practical situations, the information derived from them, together with observations made in service (section 2) can be used as the basis for an hypothesis on the

process of infection and decay of exterior joinery. It should be remembered that the exposure periods of the L-joints relate to the time from which wetting began. In practice wetting does not begin until the paint film across the joint has failed; the time for this to occur will vary and could be a period of years.

Soon after wetting, the timber is invaded by bacteria. These proliferate and cause an increase in the permeability of the timber thus making it more susceptible to wetting. Nitrogen fixing ability among the bacteria could predispose the timber to soft rot attack. The timber is subsequently invaded by a wide range of fungi; moulds, bluestains, soft rots and Basidiomycetes are all present after one year's exposure. On the basis of the information obtained from this experiment it is not known if any succession operates among them. The fungi are responsible for the slower rate of increase in permeability recorded over the later stages of the exposure period with the change in emphasis from bacterial to fungal attack occurring gradually as fungal colonisation progresses. All four groups of fungi are implicated in the permeability increases. Within the Basidiomycetes, Sistotrema brinkmannii and various white rot organisms, are the first colonisers; the brown rots, present in a significant proportion of failures in service, colonise at a later stage.

Clearly further information is required to verify this hypothesis. At present, data on the process of colonisation by fungi over the first year of exposure are generally lacking for both untreated and 1 per cent TnBTO treated material. Similarly there are large gaps in the permeability and moisture content data. Further experimentation, concentrating on collection of data over the first year of exposure, seems essential.

The use of a three minute immersion treatment with one per cent TnBTO did not qualitatively affect the pattern of fungal colonisation for exposure periods of one year and over. No comment can be made on the period up to one year due to the paucity of observations. Microscopic examination indicates fungal colonisation was quantitatively less in treated material. Permeability increases after 12 months of exposure were equivalent to those of untreated material which could indicate bacterial colonisation was little affected.

The practical significance of these contra-indications to the effectiveness of the TnBTO treatment needs careful consideration. The increases in permeability will allow the timber to wet up and dry out more rapidly and therefore will increase preservative losses due to leaching, as well as providing easy access for the moisture required for decay to take place. Aureobasidium pullulans has been shown to reduce the toxicity of TnBTO in agar to basidiospores of Lenzites trabea (Section 6.3); its presence in TnBTO treated material therefore indicates a possible biological method of reducing toxicity. Sistotrema brinkmannii, although unlikely to be a direct cause of failure, could also induce degrade of TnBTO; it is more tolerant to TnBTO than Coniophora puteana (PRL unpublished data) which together with Coriolus versicolor has been shown, in wood, to increase the rate of degrade to the less toxic di- and mono-butyl tin derivatives (Henshaw et al, 1978). Soft rot was present in TnBTO treated material after one year's exposure. Though unlikely to cause direct failure of joinery, the group of organisms responsible for this type of attack are generally more tolerant of preservatives than Basidiomycetes and could again lead to detoxification and subsequent colonisation by the latter.

With the limited observations currently available, the practical significance of the apparent failure of TnBTO is difficult to assess but in view of the non-biological degrade shown by Henshaw et al (1978) the long term effectiveness of TnBTO must be in question. Clearly further experimentation is required. It is possible that the L-joint does not parallel the practical situation. To date, there is no proof of failure of treated joinery in service. However, as previously stated (section 2.2.6) exposure periods of treated joinery do not greatly exceed the most common age of failure of untreated joinery (7-9 years). Performance under similar exposure conditions of a preservative of proven long term effectiveness, such as pentachlorophenol (PCP) which has been used successfully as a joinery treatment in the USA for many years (Lance, 1958), could be used as a standard by which to judge the performance of TnBTO. Additionally the effect of application in a formulation containing water repellents needs to be investigated.

The final trial of any preservative is its performance in practice; an assessment of the performance of joinery treated with TnBTO is therefore essential, in the long term.

Table 3.1 SUMMARY OF THE TYPE OF DATA COLLECTED

Exposure period	Moisture content	Permeability	Bacterial counts	Isolations	Microscopic observation
Unexposed	U T H	U	U	U	
3 days	U T H				
9 days			U	U	
10 days	U T H				
17 days	U T H				
23 days			U		
31 days	U H	U			
37 days			U	U	
42 days		U			
45 days	U H				
58 days			U		
66 days	U H				
93 days			U	U	
101 days	U H				
12 months		U T		U T	U T
15 months					U
18 months		U T		U T	U T
24 months	U T H	H			
35 months	U T H	U T H		U T H	

U - untreated pine sapwood

T - 1% TnBTO treated pine sapwood

H - western hemlock

Table 3.2 ABSORPTION OF 1 PER CENT TnBTO IN WHITE SPIRIT BY THE L-JOINTS

L-joint No	Absorption (g)	L-joint No	Absorption (g)
41	18	51	25
42	13	52	28
43	17	53	13
44	16	54	21
45	15	55	21
46	16	56	18
47	19	57	23
48	20	58	19
49	24	59	19
50	16	60	22

Table 3.3 UPTAKE OF MOISTURE BY L-JOINTS MEASURED BY THE MICROWAVE MOISTURE METER (MEAN PER CENT MOISTURE CONTENT OF THOSE REPLICATES SAMPLED)

Test timber	Exposure period (days)	Sample position**				No of replicates
		1	2	3	4	
Scots pine sapwood	0	11.1	11.4	11.3	10.6	60†
	3	>35.8	>27.5	>18.0	12.9	33†
	10	>35.8	>35.6	>34.6	>30.6	37†
	17	>36.0	>35.7	>34.9	>31.4	37†
	31	>36.0	>36.0	>36.0	>36.0	5
	45	>36.0	>36.0	>36.0	>36.0	5
	66	>36.0	>36.0	>35.9	>34.6	5
	101	>27.8	>31.0	>31.3	>29.5	8
	24 months	>38.6	>38.6	>38.6	>38.6	24†
	*35 months	107.3	96.1	96.1	100.3	6†
Hemlock	0	10.4	10.7	10.8	10.3	10
	3	14.6	10.7	10.4	10.2	5
	10	16.4	11.2	10.6	10.2	10
	17	17.0	11.6	10.8	9.8	10
	31	17.8	11.8	11.1	10.0	6
	45	26.4	14.5	13.5	12.4	6
	66	31.0	16.2	14.4	12.9	3
	101	18.1	17.1	16.0	14.4	4
	24 months	>37.0	>29.5	18.7	16.2	10
	*35 months	45.3	20.2	19.1	18.2	3

> Shows the moisture content of a number of the replicates was above the maximum recordable by the meter under the conditions of test.

* Moisture contents established by oven drying.
** See fig 3.2
† Both untreated and treated joints included.

Table 3.4 MOISTURE CONTENT OF L-JOINTS DETERMINED BY OVEN DRYING; 24 MONTHS' EXPOSURE

Timber species	Orientation during exposure	L-Joint No	Sample position†														
			1	2	3	4	5	6	7	8	9	10	11	12	13*	14*	15*
Hemlock	Horizontal	H5	101.2	67.5	39.1	32.3	27.5	24.1	20.7	19.3	18.6	18.1	18.2	17.5			
		H8	98.6	84.3	55.4	51.1	42.6	34.9	25.0	20.4	19.9	19.2	18.6	18.1			
		H9	153.7	149.1	121.0	73.9	63.4	52.4	36.9	27.0	21.6	20.0	19.4	18.5			
		Mean	117.8	100.3	71.8	52.4	44.5	37.1	27.5	22.2	20.0	19.1	18.7	18.0			
		Moisture meter equivalent	96.6			44.7			23.2			18.6					
	Vertical	H5	28.6	29.2	28.3	25.3	24.2	23.6	22.7	22.3	21.2	19.8	18.9	17.8	17.1	16.9	16.6
		H8	24.8	26.1	24.4	23.2	21.8	21.8	20.8	19.7	19.2	18.5	18.1	18.1	17.2	18.2	17.4
		H9	30.4	31.5	30.8	29.0	26.7	25.3	24.1	22.9	22.0	21.5	20.5	20.8	21.0	20.7	20.4
		Mean	27.9	28.9	27.8	25.8	24.2	23.6	22.5	21.6	20.8	19.9	19.2	18.9	18.4	18.6	18.1

†See fig 3.3

*Not produced by horizontal members

Table 3.5 MOISTURE CONTENTS OF L-JOINTS DETERMINED BY OVEN DRYING; 35 MONTHS' EXPOSURE

Timber species	Orientation during exposure	L-Joint No	Sample position†											
			1	2	3	4	5	6	7	8	9	10	11	12
Hemlock	Horizontal	H1	64.0	29.4	21.7	20.3	20.2	19.2	19.4	19.2	19.0	18.9	18.8	18.4
		H4	84.9	32.5	23.0	21.0	20.1	19.7	18.9	18.7	18.4	18.1	17.9	17.2
		H7	96.2	33.3	23.0	21.6	20.4	19.0	18.8	18.9	19.0	18.6	18.1	17.7
		Mean	81.7	31.7	22.6	21.0	20.2	19.3	19.0	18.9	18.8	18.5	18.3	17.8
		Moisture meter equivalent	45.3			20.2			19.1			18.2		
Scots pine sapwood	Horizontal	10	91.4	83.4	81.2	81.6	81.5	85.0	86.5	88.2	90.3	91.9	92.4	112.0
		13	175.2	140.5	130.6	127.8	126.5	124.7	126.0	126.9	128.8	129.3	130.3	136.0
		40	125.0	106.0	102.3	95.5	91.2	86.8	85.9	88.1	88.1	93.3	93.2	95.7
		Mean	130.5	110.0	104.7	101.6	99.7	98.8	99.5	101.1	102.4	104.8	105.3	114.6
		51*	93.0	83.7	81.2	76.5	73.8	72.3	72.3	70.4	69.1	68.7	67.6	73.4
		57*	116.4	99.3	100.4	101.0	100.0	98.1	97.1	96.4	94.9	95.6	100.3	100.8
		60*	114.4	105.3	101.6	101.2	102.1	105.0	107.6	106.5	106.8	106.1	109.1	109.2
		Mean*	107.9	96.1	94.4	92.9	92.0	91.8	92.3	91.1	90.3	90.1	92.3	94.5
		Moisture** meter equivalent	107.3			96.1			96.1			100.3		

†See fig 3.3
*Treated with 1% TnBTO
**Including both treated and untreated L-joints

Table 3.6 UPTAKE OF DEKALIN BY UNTREATED PINE SAPWOOD

Exposure period	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
Unexposed*	Check 1	.371	.368	.390	.450	.417	.411	.408	.413	.408	.441	.458	.474
	Check 2	.342	.305	.314	.340	.342	.342	.353	.352	.377	.400	.394	.381
	Check 3	.472	.464	.470	.441	.503	.470	.434	.425	.448	.495	.499	.487
	Mean	.395	.379	.391	.410	.421	.408	.398	.397	.411	.445	.450	.447
One month	3	.510	.435	.434	.438	.445	.400	.413	.431	.428	.468	.443	.471
	7	.463	.430	.398	.387	.380	.373	.366	.363	.360	.349	.335	.326
	24	.507	.468	.450	.479	.467	.432	.433	.424	.401	.415	.418	.428
	Mean	.493	.444	.427	.435	.431	.402	.404	.406	.396	.411	.399	.408
6 weeks	37	.915	.911	.876	.860	.850	.878	.893	.910	.937	.942	.981	1.036
	38	.556	.522	.490	.468	.456	.468	.449	.451	.455	.460	.461	.465
	Mean	.736	.717	.683	.664	.658	.673	.671	.681	.696	.701	.721	.751
12 months	22	1.345	1.285	1.426	1.383	1.455	1.424	1.363	1.343	1.262	1.310	1.252	1.215
	23	1.378	1.348	1.396	1.380	1.244	1.254	1.221	1.116	1.028	.782	.670	.693
	26	1.330	1.340	1.292	1.300	1.319	1.238	1.178	1.148	1.046	.932	.891	.942
	31	1.336	1.256	1.237	1.160	1.123	1.190	1.190	1.231	1.260	1.279	1.226	1.225
	Mean	1.347	1.307	1.338	1.306	1.285	1.277	1.238	1.210	1.149	1.076	1.010	1.019
18 months	32	1.329	1.271	1.182	1.099	1.055	1.020	1.073	1.110	1.098	1.120	1.087	1.060
	33	1.683	1.658	1.622	1.634	1.621	1.584	1.544	1.544	1.552	1.582	1.577	1.575
	36	1.322	1.166	1.152	1.119	1.108	1.113	1.142	1.120	1.085	1.078	1.116	.991
	Mean	1.445	1.365	1.319	1.284	1.261	1.239	1.253	1.258	1.245	1.260	1.260	1.209
35 months	10	1.838	1.889	1.873	1.871	1.856	1.844	1.790	1.788	1.787	1.791	1.750	1.800
	13	1.944	2.064	2.002	1.973	2.003	2.056	2.020	2.013	2.011	2.013	2.064	2.022
	40	1.884	1.817	1.886	1.897	1.816	1.746	1.836	1.779	1.838	1.892	1.884	1.923
	Mean	1.889	1.923	1.920	1.914	1.892	1.882	1.882	1.860	1.879	1.899	1.899	1.915

*Unexposed samples were not painted
**See fig 3.3

Table 3.7 UPTAKE OF DEKALIN BY PINE SAPWOOD TREATED WITH 1% TnBTC

Exposure period	L-joint No	Sample position*											
		1	2	3	4	5	6	7	8	9	10	11	12
12 months	43	1.020	1.146	1.110	1.171	1.228	1.248	1.238	1.209	1.225	1.188	1.158	1.193
	47	1.238	1.304	1.397	1.307	1.260	1.273	1.178	1.110	1.021	.930	.869	.920
	53	.880	.812	.789	.833	.762	.775	.808	.811	.837	.832	.790	.763
	58	1.430	1.470	1.565	1.551	1.550	1.441	1.347	1.250	1.295	1.226	1.238	1.189
	Mean	1.142	1.183	1.215	1.216	1.200	1.184	1.143	1.095	1.095	1.044	1.014	1.016
18 months	41	1.459	1.421	1.386	1.415	1.382	1.410	1.382	1.372	1.385	1.339	1.378	1.337
	50	1.529	1.391	1.380	1.331	1.322	1.328	1.227	1.209	1.139	1.110	1.115	1.116
	54	1.486	1.471	1.514	1.495	1.416	1.405	1.364	1.318	1.279	1.205	1.123	1.149
	Mean	1.491	1.428	1.427	1.414	1.373	1.381	1.324	1.300	1.268	1.218	1.205	1.201
35 months	51	1.994	2.069	2.069	2.045	2.006	1.926	1.973	1.960	1.862	1.856	1.787	1.858
	57	1.676	1.727	1.703	1.706	1.653	1.699	1.709	1.758	1.750	1.687	1.737	1.742
	60	1.882	1.884	1.854	1.878	1.859	1.854	1.844	1.882	1.877	1.852	1.868	1.821
	Mean	1.851	1.893	1.875	1.876	1.839	1.826	1.842	1.867	1.830	1.798	1.797	1.807

*See fig 3.3.

Table 3.8 UPTAKE OF DEKALIN BY HEMLOCK

Exposure period	Orientation during exposure	L-joint No	Sample position*														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
24 months	Horizontal	H5	.267	.262	.246	.246	.247	.237	.247	.246	.229	.236	.229	.229			
		H8	.324	.315	.310	.310	.298	.287	.284	.269	.278	.268	.251	.232			
		H9	.483	.581	.681	.698	.672	.650	.627	.623	.566	.579	.577	.531			
		Mean	.358	.386	.412	.418	.406	.391	.386	.379	.358	.361	.352	.331			
	Vertical	H5	.490	.610	.588	.553	.573	.602	.591	.601	.604	.567	.572	.597	.588	.584	.572
		H8	.403	.421	.431	.403	.364	.354	.364	.372	.373	.367	.374	.369	.377	.377	.351
		H9	.560	.548	.541	.491	.462	.462	.470	.462	.436	.425	.419	.407	.412	.407	.413
		Mean	.484	.526	.520	.482	.466	.473	.475	.478	.471	.453	.455	.458	.459	.456	.445
35 months	Horizontal	H1	.326	.310	.300	.293	.282	.262	.277	.284	.304	.306	.306	.276			
		H4	.360	.359	.310	.292	.296	.296	.300	.291	.307	.309	.299	.305			
		H7	.355	.394	.364	.345	.338	.314	.342	.336	.303	.303	.312	.312			
		Mean	.347	.354	.325	.310	.305	.291	.306	.304	.305	.306	.306	.298			

*See fig 3.3

Table 3.9 UPTAKE OF DEKALIN; MEAN OF ALL SAMPLE POSITIONS

Exposure period	Mean uptake of dekalin (g)	
	Untreated	1% TnBTO
Unexposed	0.413	nt
1 month	0.421	nt
6 weeks	0.696	nt
12 months	1.214	1.129
18 months	1.283	1.336
35 months	1.896	1.842

nt - not tested

Table 3.10 BACTERIAL COUNTS AND EARLY FUNGAL ISOLATIONS FROM PINE SAPWOOD

Exposure period (days)	Bacterial count per ml exudate			Fungal isolations
	Shoulder	Centre	Remote end	
0	8.00×10^{1}	9.00×10^{1}	0	No growth
9	5.37×10^{4}	5.28×10^{4}	*	No growth
23	1.90×10^{2} 2.26×10^{3} 1.82×10^{3}	4.70×10^{2} 2.30×10^{2} 1.30×10^{2}	3.50×10^{2} 5.00×10^{1} 6.00×10^{1}	nt
37	5.92×10^{8} 1.71×10^{7} 3.50×10^{7}	1.53×10^{8} 1.73×10^{7} 1.80×10^{7}	3.10×10^{7} 3.00×10^{7} 1.36×10^{7}	Aureobasidium**
58	1.96×10^{6} 1.06×10^{9} 2.81×10^{5}	* 1.73×10^{8} 3.04×10^{4}	9.10×10^{5} 2.03×10^{6} 3.94×10^{4}	nt
93	0	0	0	Aureobasidium Aspergillus

*No result obtained

**At shoulders of joint only

nt - not tested

Table 3.11 LABORATORY* WEIGHT LOSS TESTS (UNPUBLISHED PRL REPORT)

Test timber	Per cent weight loss**		
	Test fungi		Mean
	Coniophora puteana	Serpula lacrymans	
Scots pine sapwood	41.2	32.1	36.7
hemlock heartwood	26.6	22.0	24.3

*Blocks measuring 50 x 25 x 15 mm were exposed in an agar/block test system for 12 weeks at 22°C.

**Mean of four blocks per timber per fungus

Table 3.12 STAKE TESTS (PURSLOW, 1976)

Test timber	Average life*		
	Test site		Mean
	Risborough	Thetford	
Scots pine sapwood	7 7	6 4	6.0
hemlock heartwood	6 5 9 5	7 3 7 7	6.1

*Mean life, in years, of 10 samples per site per test.

Table 3.13 MICROSCOPIC OBSERVATIONS ON ONE UNTREATED L-JOINT EXPOSED FOR 15 MONTHS

Sample position	Section type	Pigmented hyphae	Pigmented spores	Blue stain penetrations	Unpigmented hyphae	Clamps	*Unpigmented spores sometimes in chains	Soft rot cavities	Disrupted rays	Attacked pits	Bacteria	Remarks
1	RLS	+	+		+	+	+	++	+			
	TLS	+	+	+	+	+	+	++	+			
	TS	+	+		+		+	++	+			
2	RLS	+			+	+	+		+			
	TLS	+	+	+	+	+	+	+	+			
	TS											No TS observations
3	RLS			+	+	+	+					
	TLS	+		+	+	+	+	+	+		+	
	TS				+		+	+				
4	RLS	+	+	+	+	+	+	+	+	+?	+	
	TLS	+		+	+	+	+	+	+		+	
	TS											No TS observations
5	RLS				+	+	+		+		+	Hyphae with clamps producing short chains of spores.
	TLS	+		+	+	+	+	+	+		+	
	TS						+	+				
6	RLS			+	+	+	+	+	+	+?	+	
	TLS	+	+	+	+	+	+	+	+		+	
	TS											No TS observations
7	RLS	+	+	+	+	+	+	+	+		+	
	TLS	+	+	+	+	+	+	+	+		+	
	TS	+	+		+		+	+			+	

*Believed to be produced by Sistotrema brinkmannii

Table 3.14 MICROSCOPIC OBSERVATIONS ON UNTREATED L-JOINTS AFTER ONE YEAR'S EXPOSURE

Block No	Section type	Pigmented hyphae	Pigmented spores	Blue stain penetrations	Unpigmented hyphae	Clamps	*Unpigmented spores sometimes in chains	Soft rot cavities	Disrupted rays	Attacked pits	Wall erosion	Remarks
Check 2/1	RLS	+			+							
	TLS				+				+			
	TS				+				+			
22/1	RLS	+						++				
	TLS	+		+	+	+			+			
	TS	+	+					++				
22/12	RLS	+	+	+	+	+						
	TLS	+	+		+				+			
	TS	+	+									
23/1	RLS	+	+		+			++			+?	
	TLS	+	+	+	+			++	+			
	TS	+						++				Soft rot most common near rays
23/12	RLS	+	+		+					+		
26/1	RLS	+	+	+	+	+	+	+++		+		Soft rot most intense at joint surface
26/12	RLS	+	+	+	+		+			+		
31/1	RLS	+	+	+	+		+	++				
	TLS	+	+	+				+	+			
	TS	+	+					+++				
31/12	RLS	+	+		+		+					
	TLS	+	+		+		+		+			
	TS	+	+		+			+				

*Believed to be produced by Sistotrema brinkmannii

Table 3.15 MICROSCOPIC OBSERVATIONS ON 1% TnBTO TREATED L-JOINTS AFTER ONE YEAR'S EXPOSURE

Block No	Section type	Pigmented hyphae	Pigmented spores	Blue stain penetrations	Unpigmented hyphae	Clamps	*Unpigmented spores sometimes in chains	Soft rot cavities	Disrupted rays	Attacked pits	Wall erosion	Remarks
43/1	RLS	+	+	+	+			+				Generally low level of fungal activity
	TLS	+	+	+	+	+	+	+	+			
	TS		+									
43/12	RLS	+	+									No fungal activity near surface
	TLS	+	+		+				+			
	TS	+	+									
47/1	RLS	+	+		+		+					Some cavities apparently in S_1 (Photographs)
	TLS				+	+	+	+	+			
	TS		+		+			+			+	
47/12	RLS	+		+	+	+	+					
	TLS	+	+	+	+				+			
	TS	+	+	+	+		+		+			
53/1	RLS		+		+		+					
	TLS			+	+	+	+		+			
	TS	+			+							
53/12	RLS	+		+	+				+			
	TLS	+			+				+			
	TS	+	+		+							
58/1	RLS	+	+		+							Little fungal activity near surface ie in treated zone
	TLS	+		+	+		+		+			
	TS	+	+									
58/12	RLS	+			+			+				Only 3 soft rot cavities seen little fungal activity
	TLS								+			
	TS	+			+							

*Believed to be produced by Sistotrema brinkmannii

Fig 3.1 L-joints on exposure racks

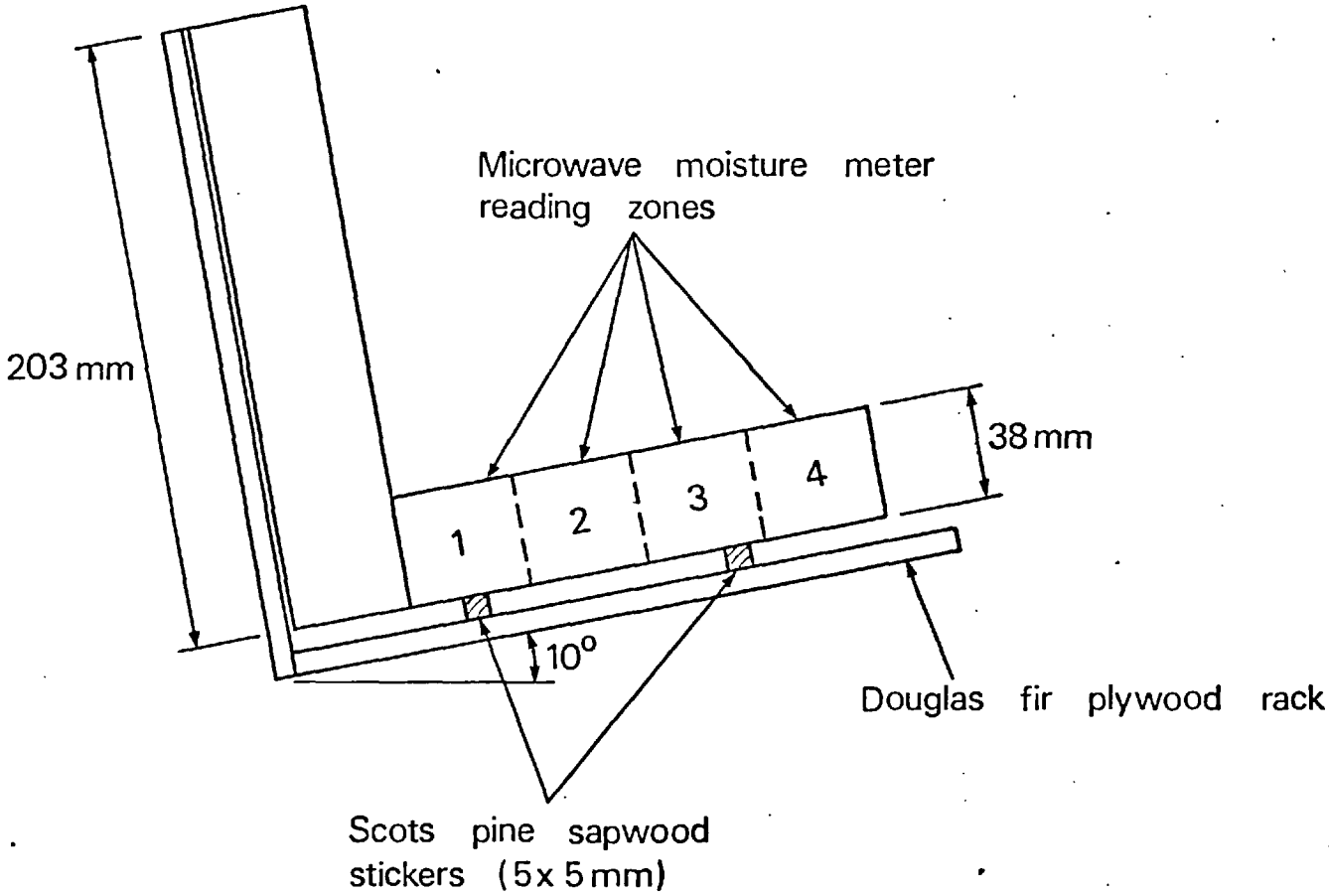


Fig. 3.2. L-joint during exposure

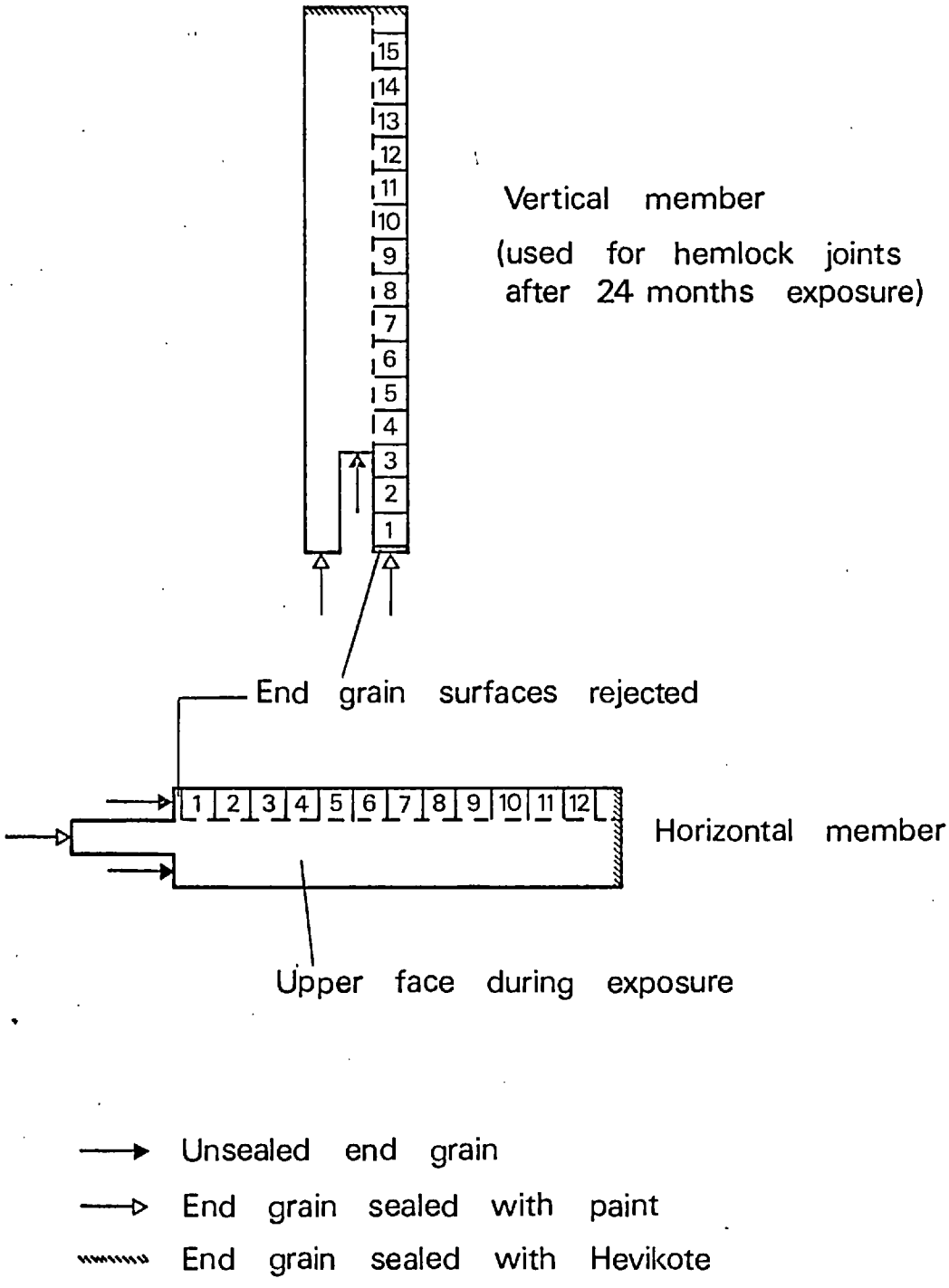


Fig. 3.3. Conversion for moisture content and permeability determination

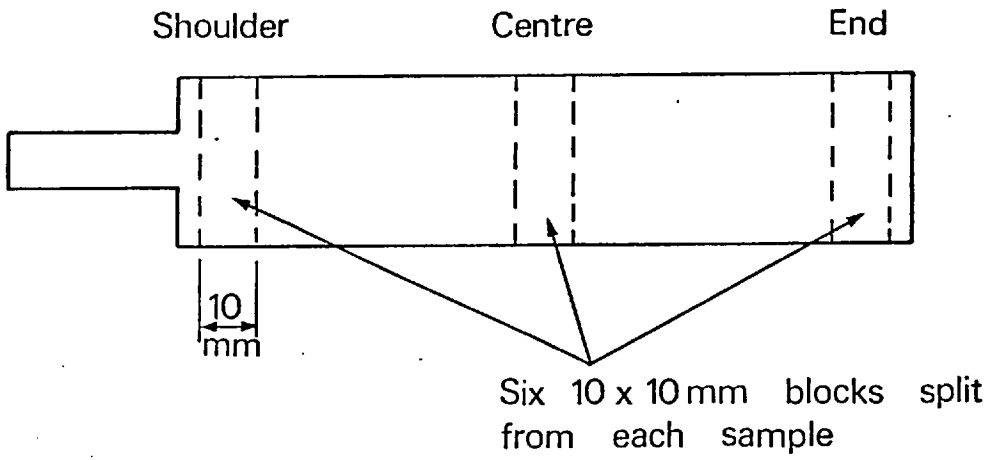
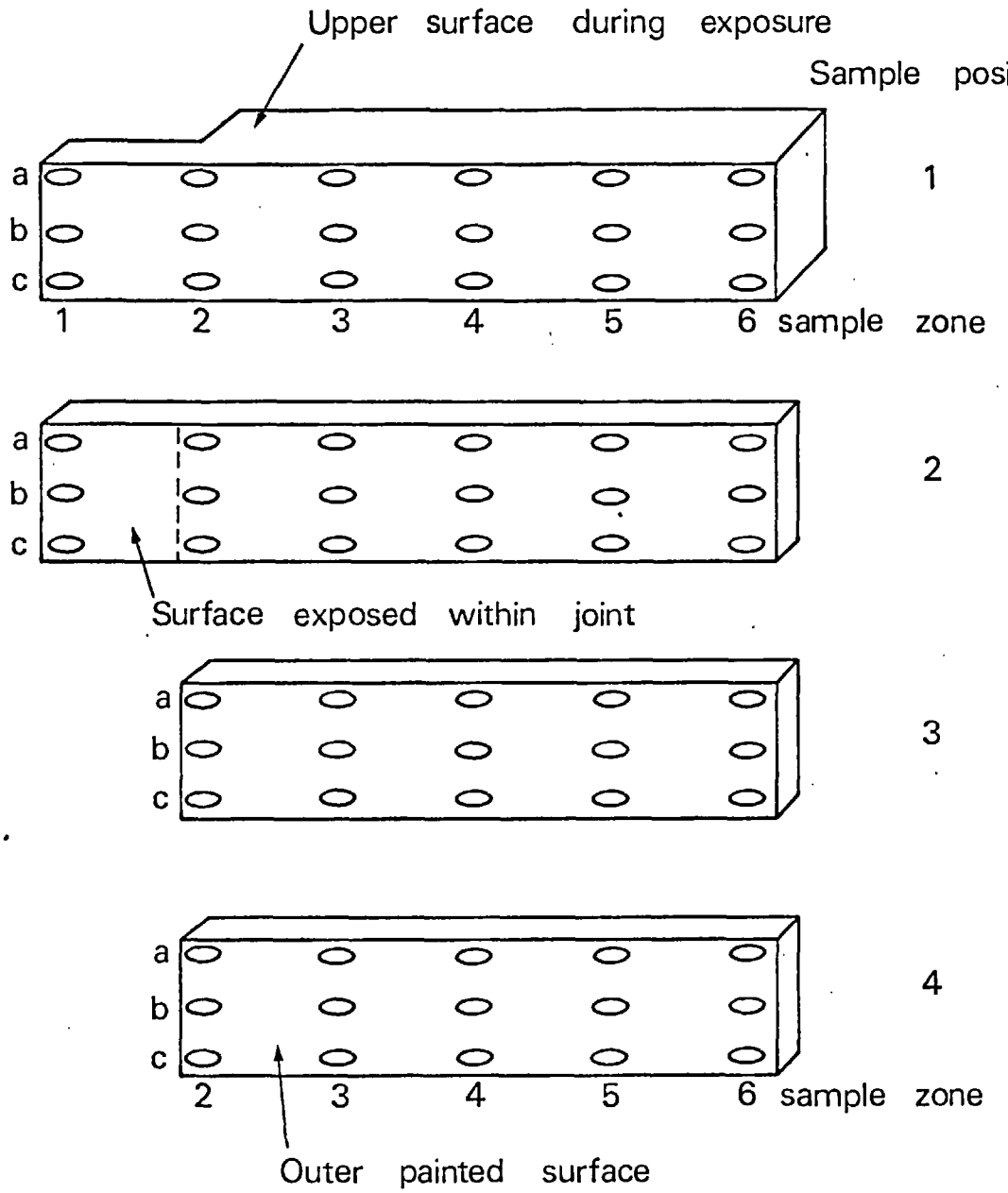


Fig. 3.4. Sampling for bacterial counts



○ Sampling point

Fungal culture numbering system :- 1 6 MA a 2

1 Sample position

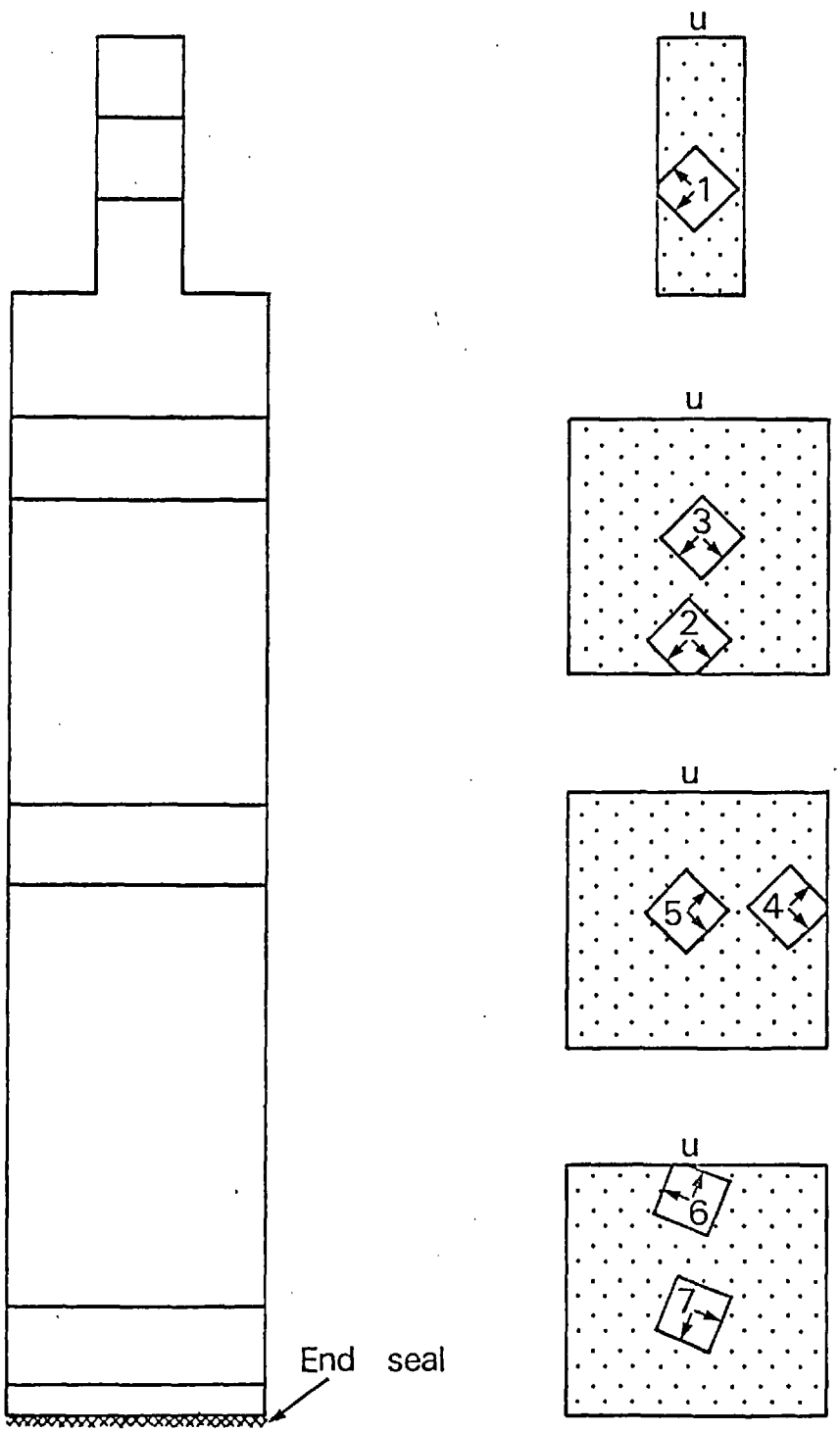
6 Sample zone

MA Isolation medium (see section 3,3,2,4,2)

a Within-zone position (near upper surface, at centre line or near lower surface during exposure.)

2 Number of the organism with all the above factors identical (no number indicates the culture was the sole fungus isolated.)

Fig. 3.5 Sampling method for isolations after 1yr exposure



→ Faces sectioned (in addition transverse sections were prepared from blocks 1, 3, 5 and 7)

u Upper face during exposure.

Fig. 3.6 Sampling of untreated L-joint after 15 months exposure.

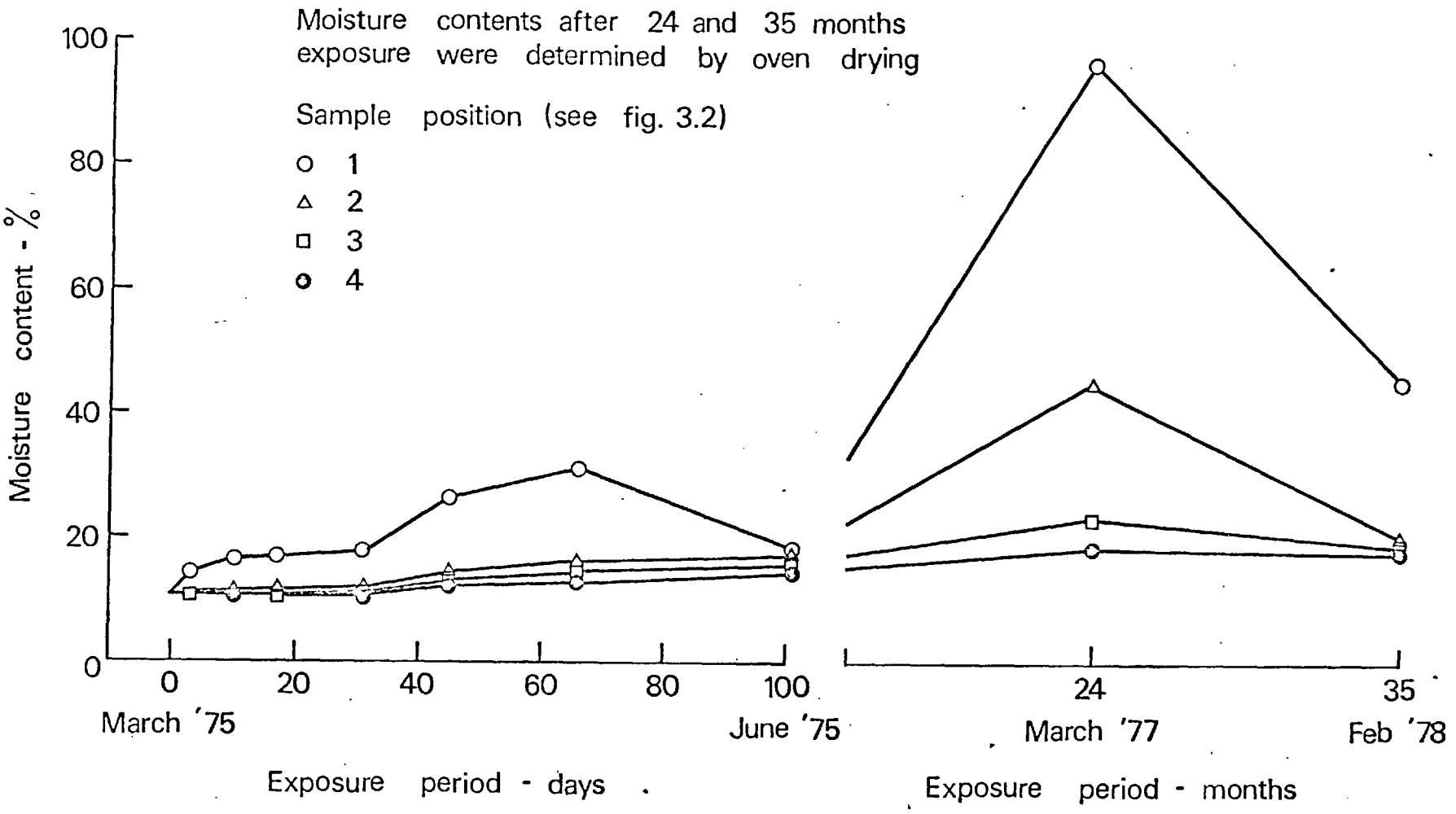


Fig. 3.7 Moisture content of hemlock L- joints

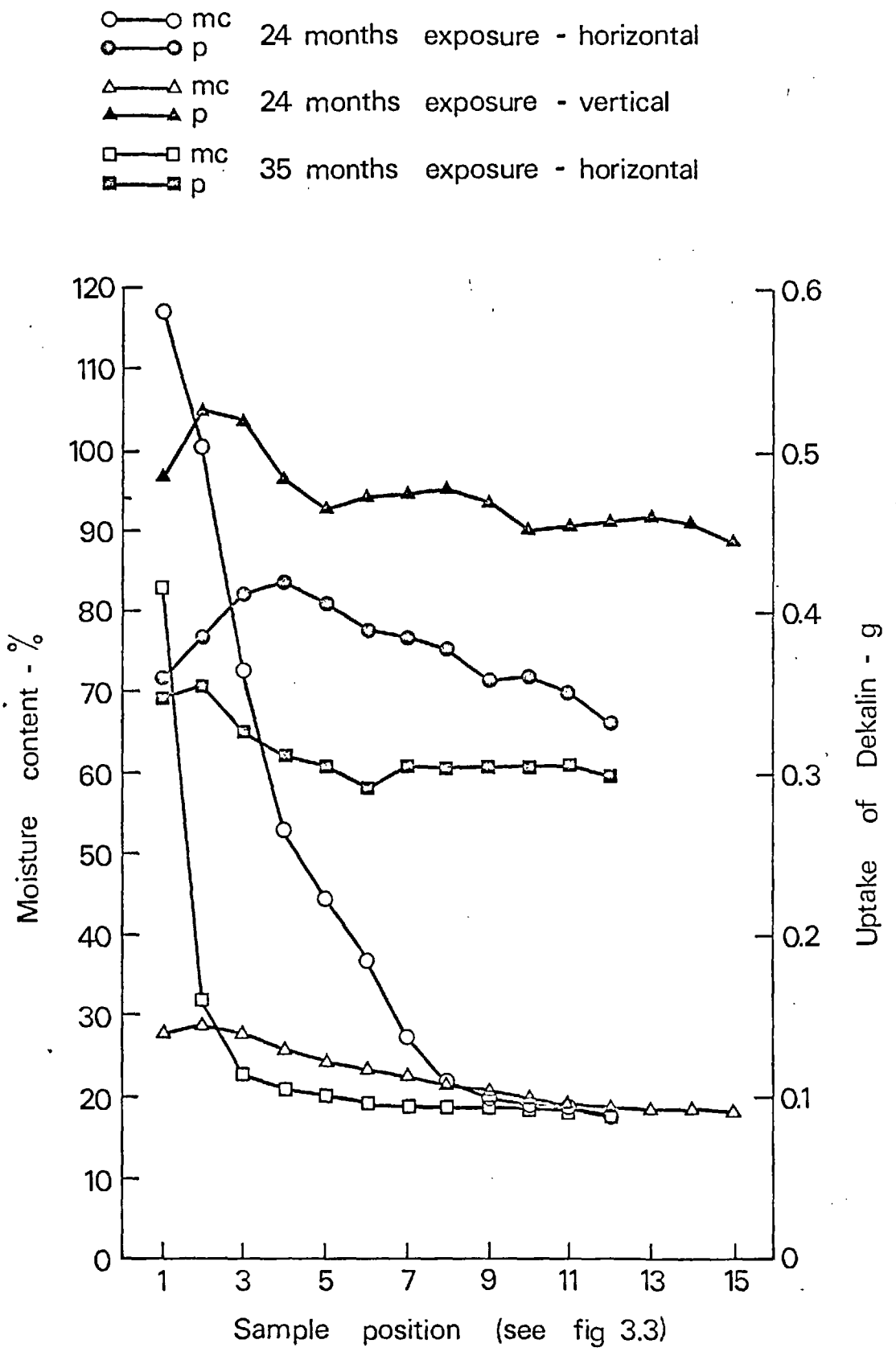


Fig. 3.8 Hemlock moisture content (mc) and permeability (p)

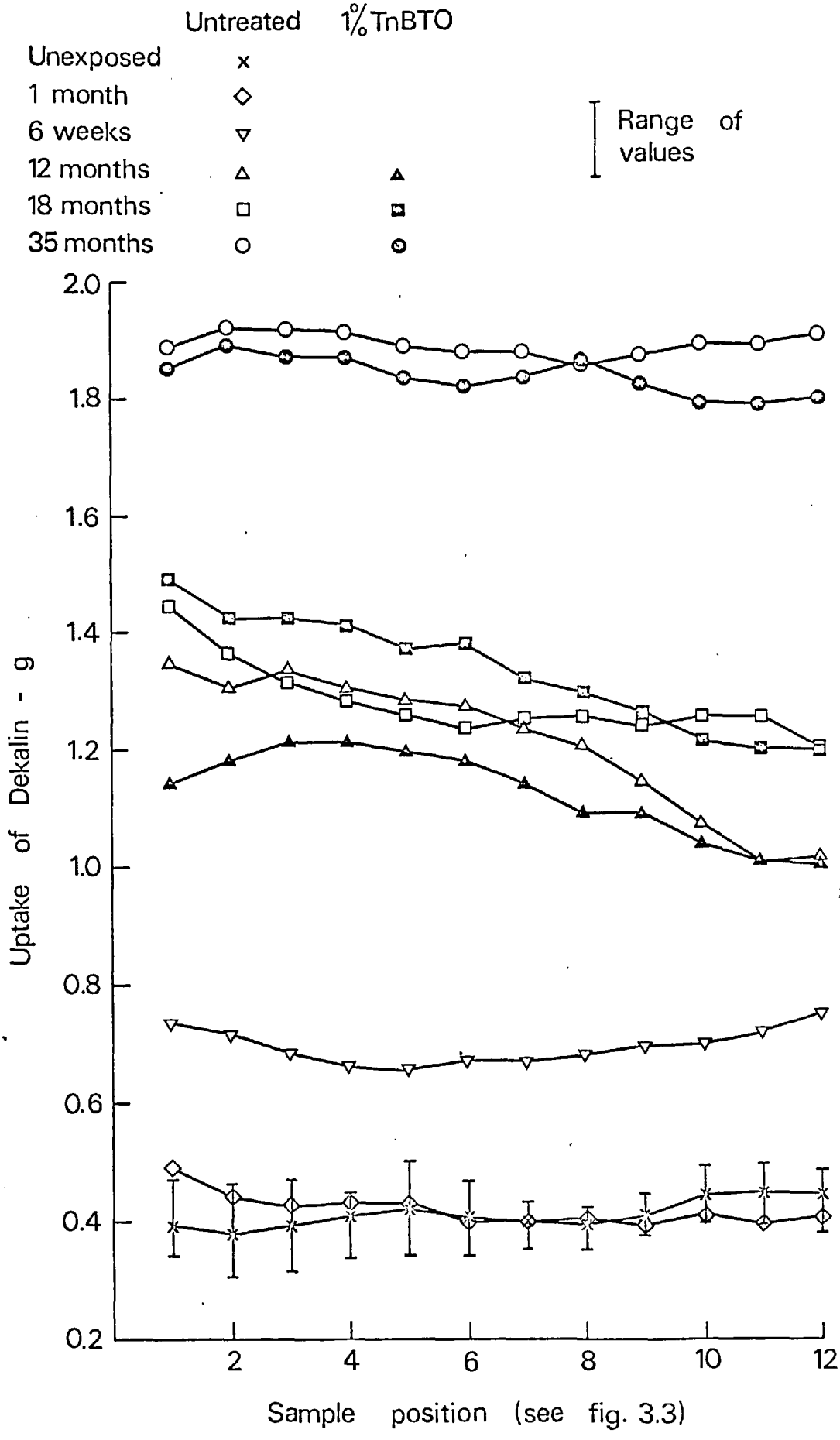


Fig. 3.9 Permeability of pine sapwood

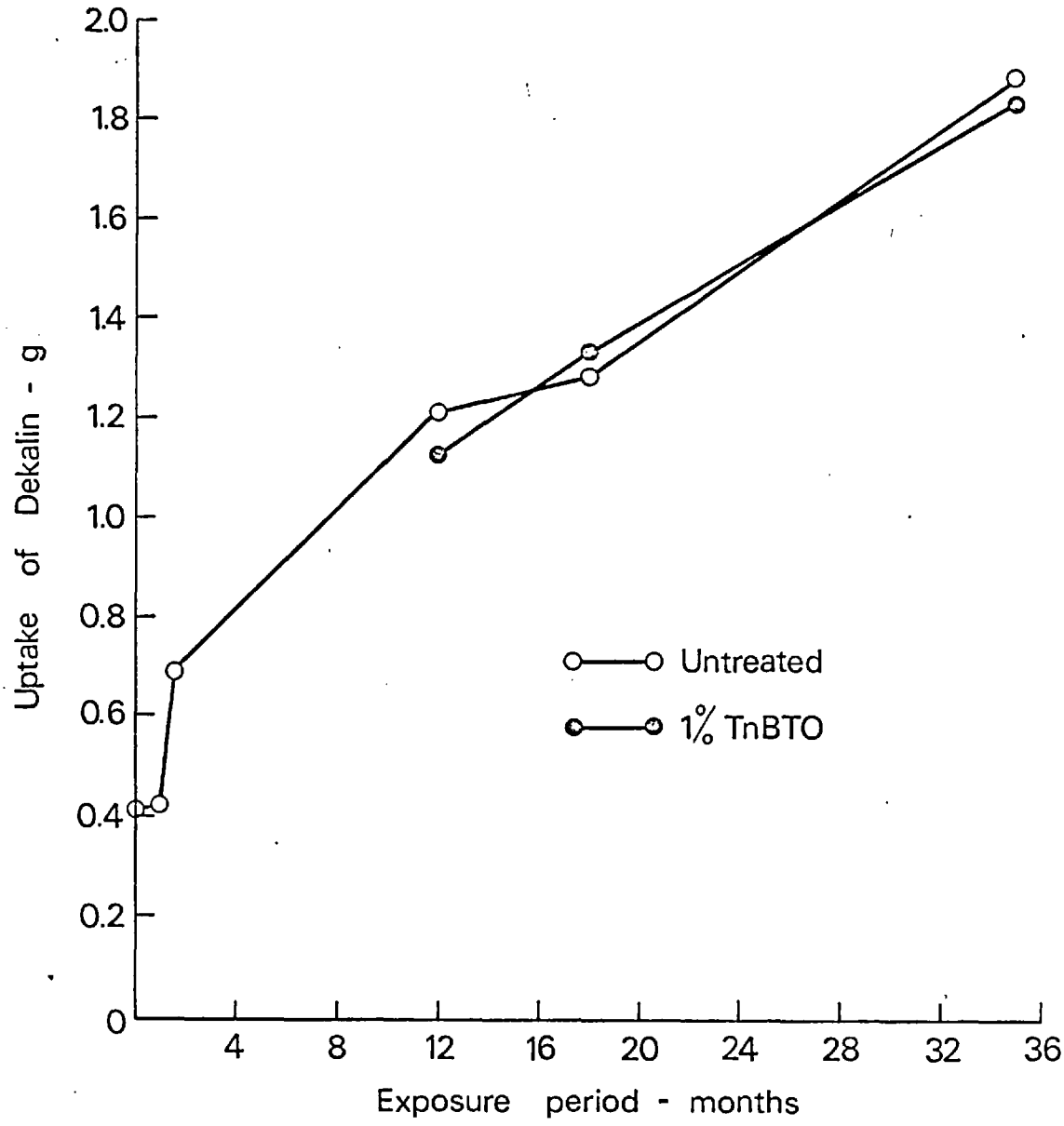


Fig 3.10 Increase in permeability of Scots pine sapwood (Mean of all test blocks)

KEY TO FIGURES 3.11 and 3.13-3.17

B bacteria

Bs bluestains

Tv *Trichoderma viride*

A *Aspergillus*/*Penicillium*

Y Yellow Phialophora

Sb *Sistotrema brinkmannii*

W White cultures

M Mucor type

O Other Basidiomycetes

U Untypified cultures

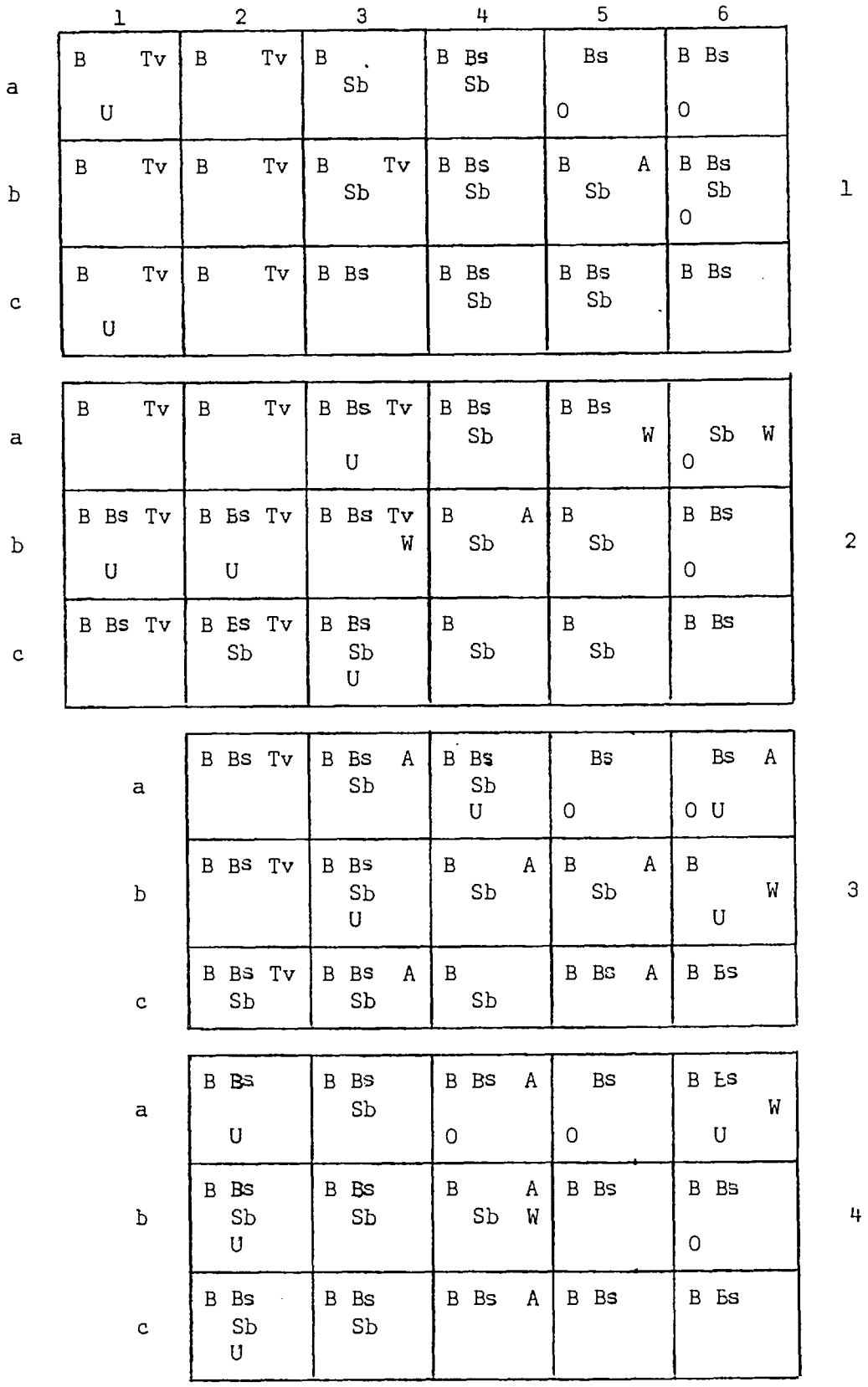
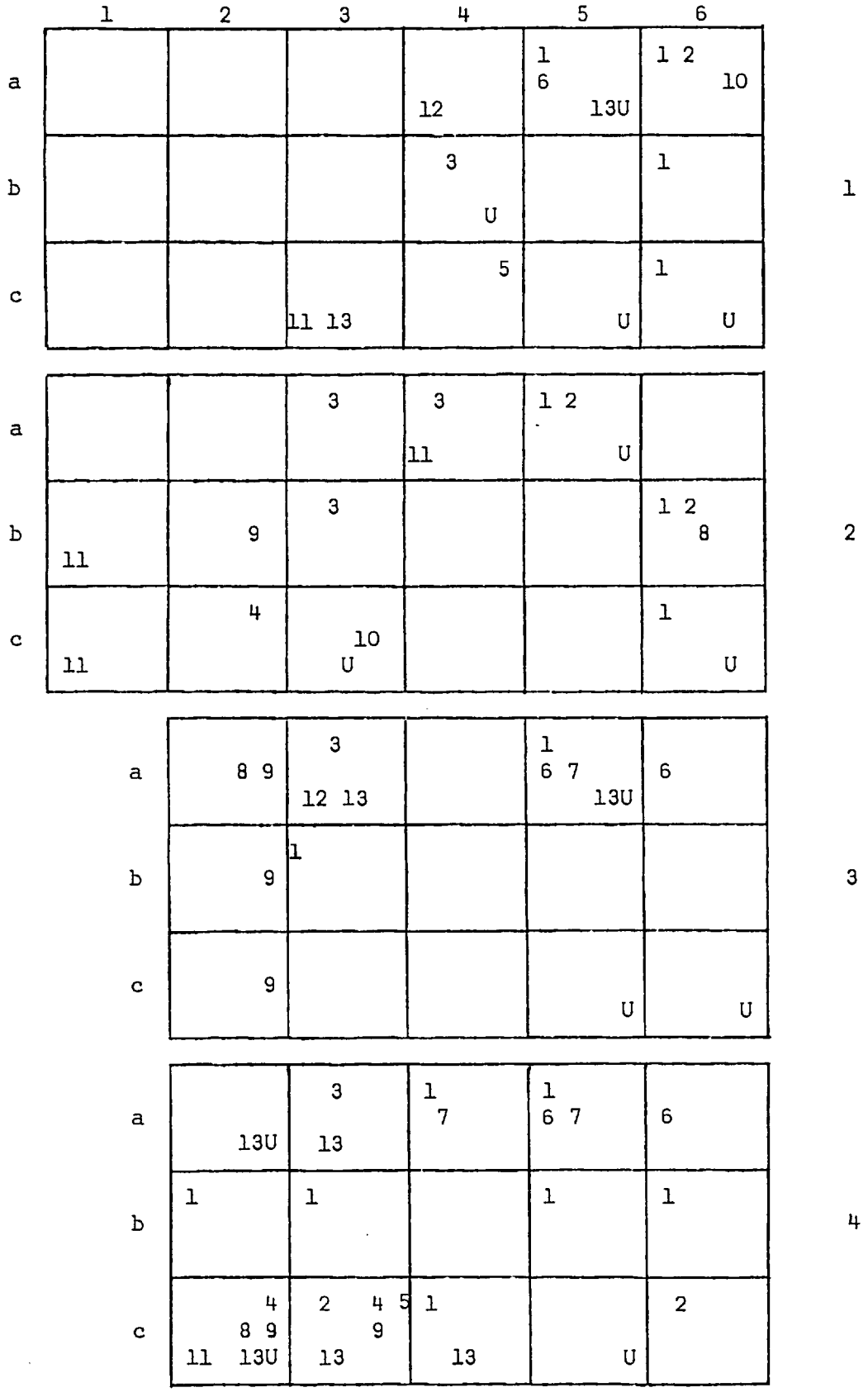


Fig 3.11 Distribution of organism types isolated from untreated L-joint No 28 after one year's exposure (see Fig 3.5)



U - untypified bluestains

1-13 - see appendix for information on these types

Fig 3.12 Distribution of bluestain types isolated from untreated L-joint No 28 after one year's exposure (see Fig 3.5)

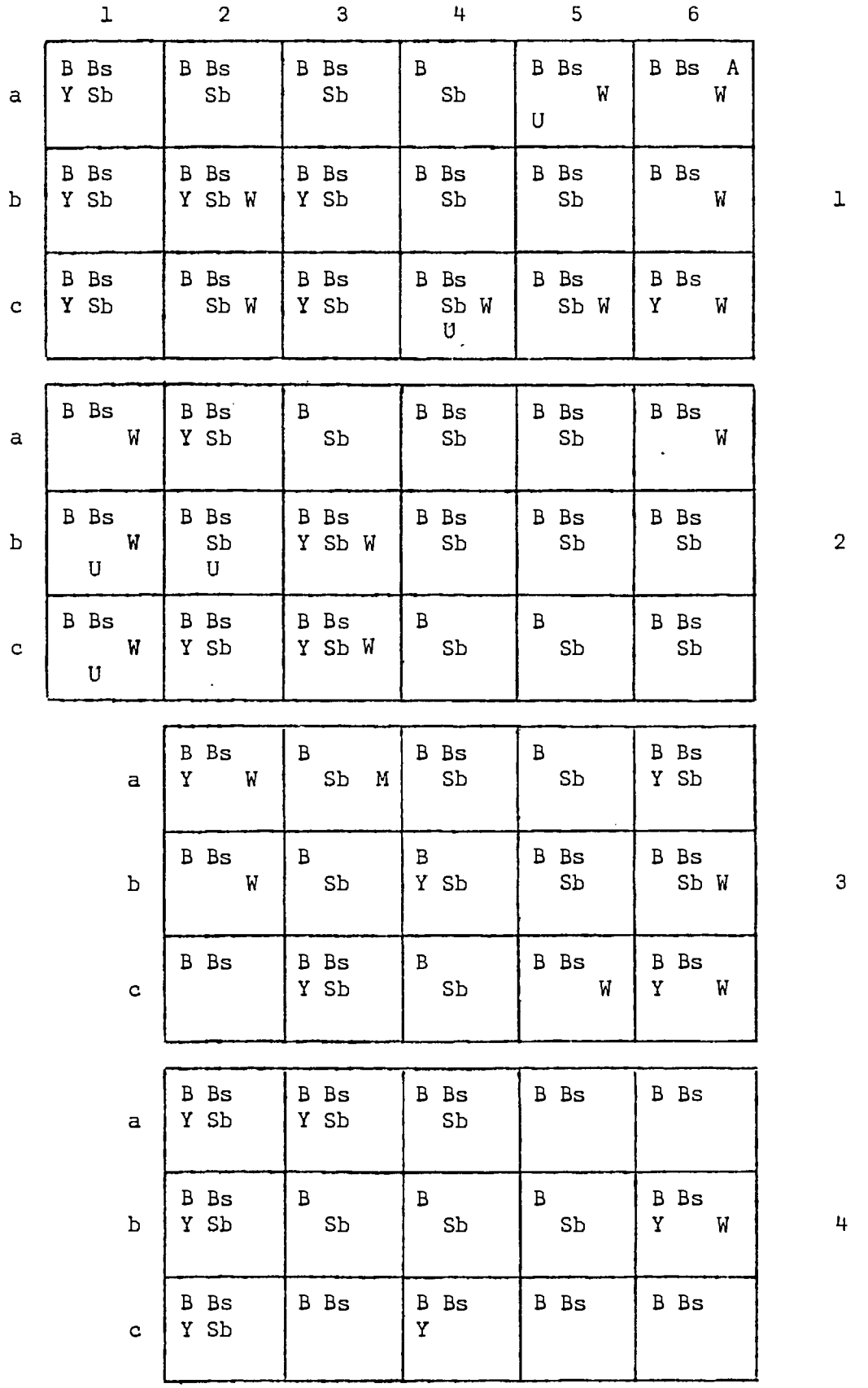


Fig 3.13 Distribution of organism types isolated from 1 per cent TnBTO treated L-joint No 48 after one year's exposure (see Fig 3.5)

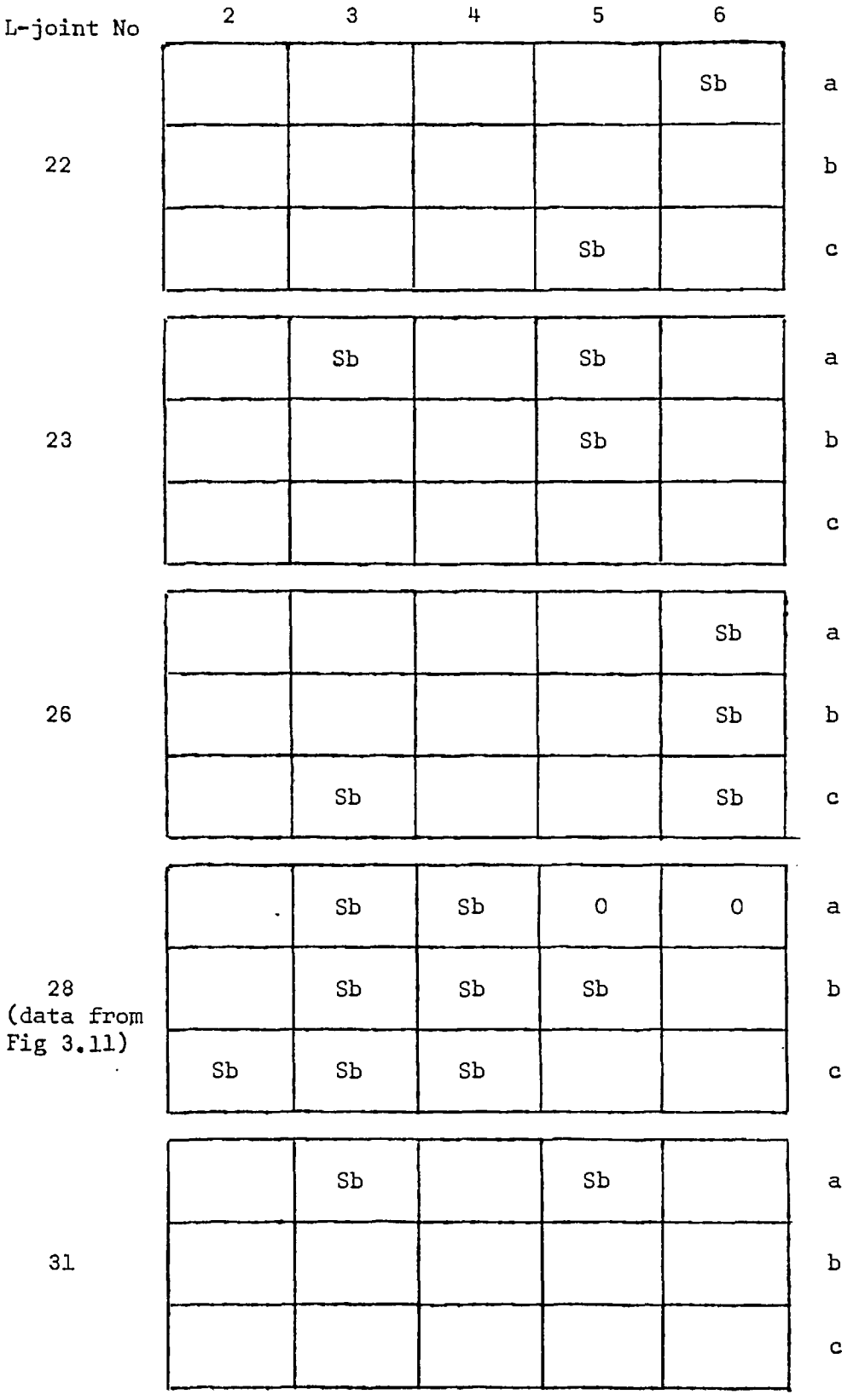


Fig 3.14 Distribution of Basidiomycete cultures in untreated L-joints after one year's exposure (sample position 3 only - see Fig 3.5)

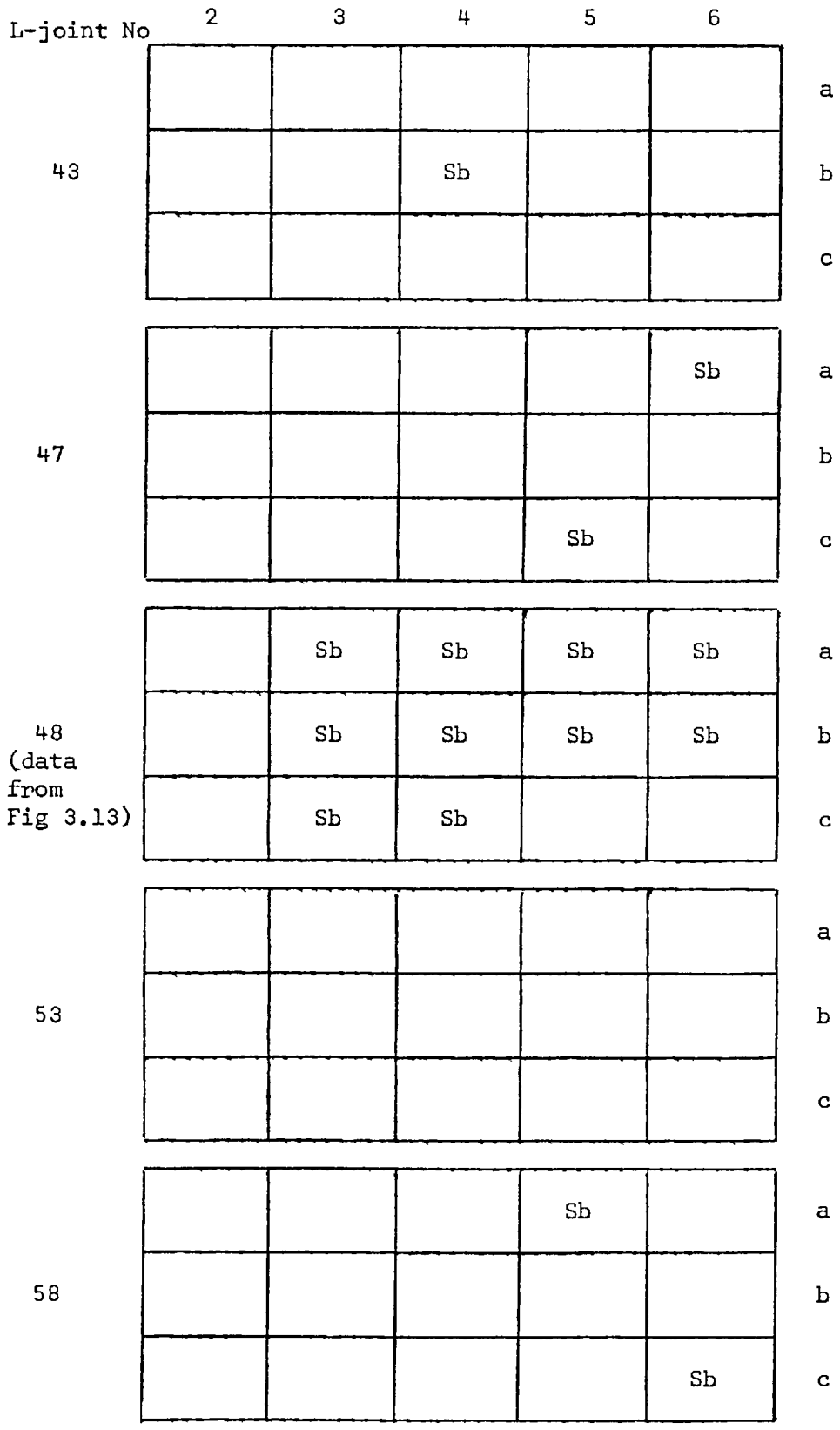


Fig 3.15 Distribution of Basidiomycete cultures in 1 per cent TnBTO treated L-joints after one year's exposure (sample position 3 only - see Fig 3.5)

L-joint No		2	3	4	5	6	
Untreated		Sb	Sb	Sb	Sb	Sb	a
	32	Sb	Sb	Sb	Sb	Sb	b
		Sb	Sb	Sb	Sb	Sb	c
			Sb	Sb	Sb	Sb	a
	33		Sb	Sb	Sb	Sb	b
			Sb	Sb	Sb	Sb	c
			Sb	Sb	Sb	Sb	a
	36	Sb	Sb	Sb	Sb	Sb	b
		Sb	Sb	Sb	Sb	Sb	c
1% TnBTO Treated		Sb	Sb	Sb	Sb		a
	41	Sb	Sb	Sb	Sb		b
		Sb	Sb	Sb	Sb		c
		Sb	Sb	Sb	Sb		a
	50	Sb		Sb	Sb		b
		Sb		Sb	Sb		c
							a
	54					Sb	b
		Sb			Sb	Sb	c

Fig 3.16 Distribution of Basidiomycete cultures in untreated and 1 per cent TnBTO treated L-joints after 18 months exposure (sample position 3 only - see Fig 3.5)

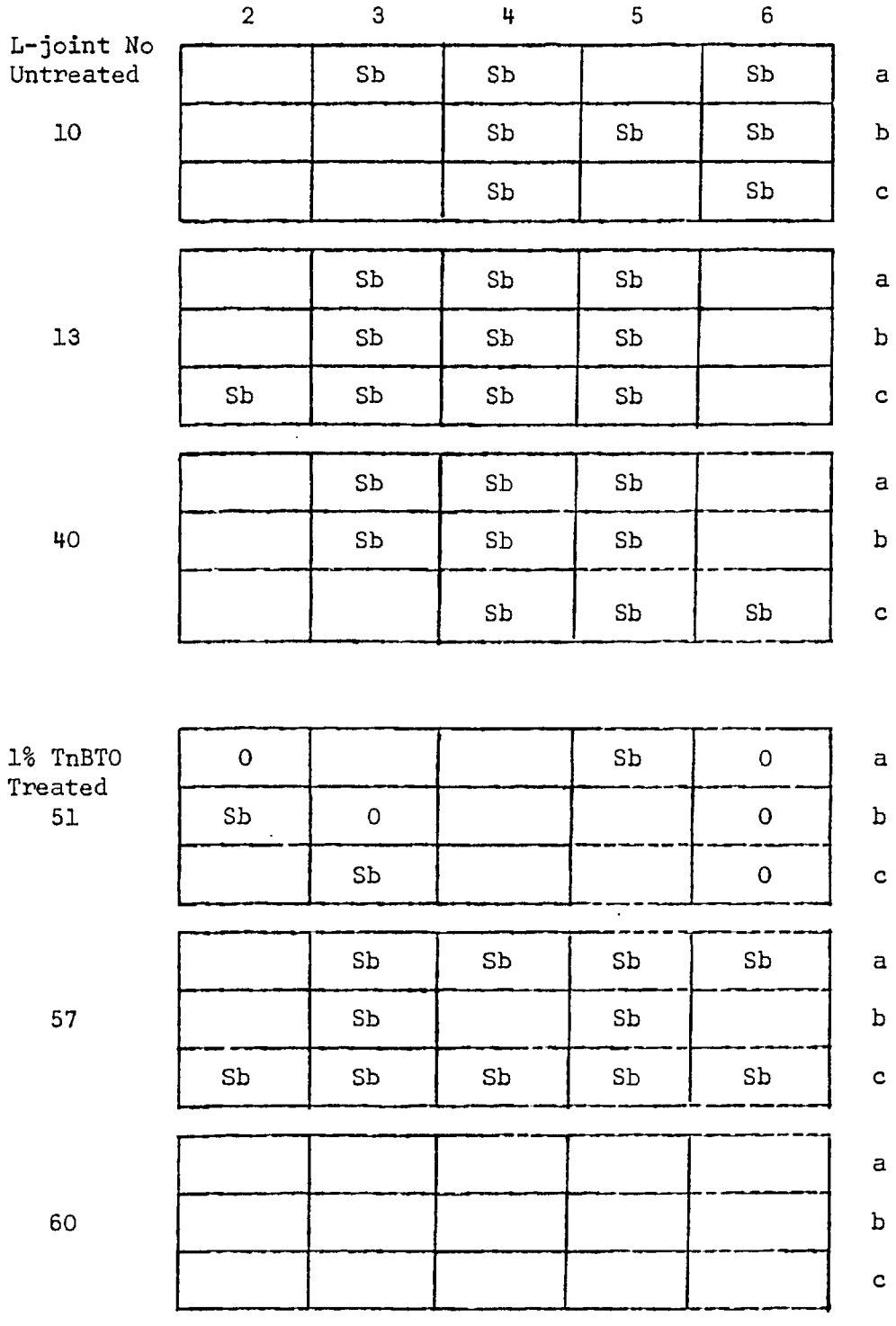


Fig 3.17 Distribution of Basidiomycete cultures in untreated and 1 per cent TnBTO treated L-joints after 35 months exposure (sample position 3 only - see Fig 3.5)

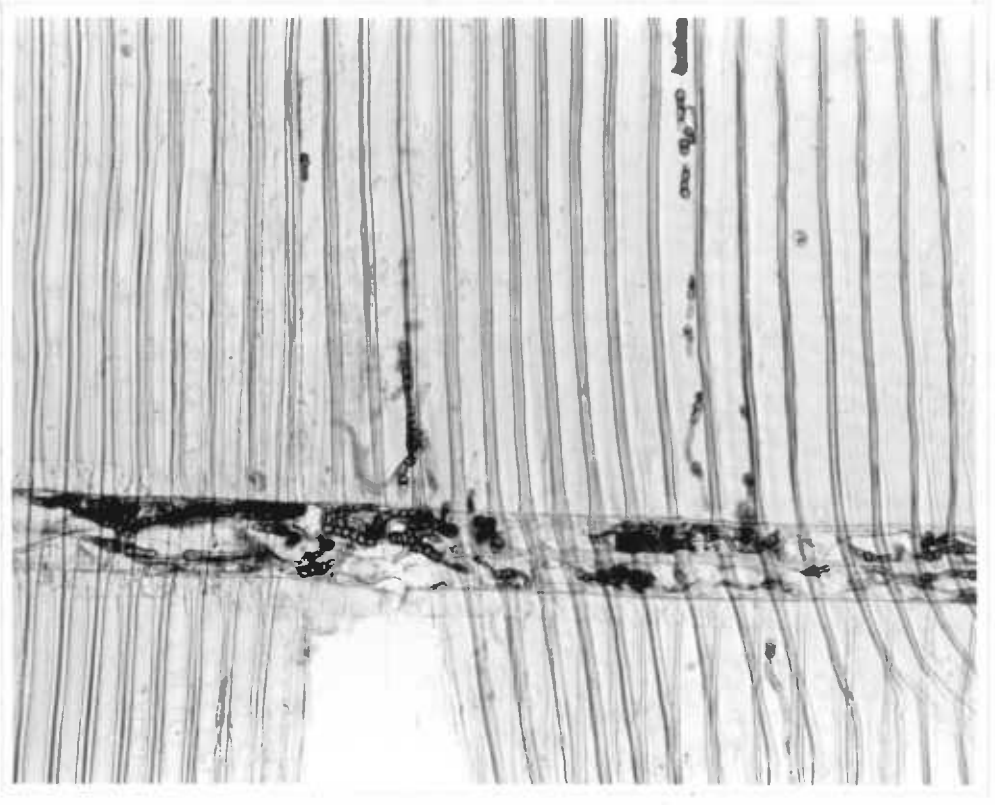


Fig 3.18 Pigmented hyphae and spores (x 175);

Fig 3.19 Hypha bearing a clamp connection (arrowed) (x 525)

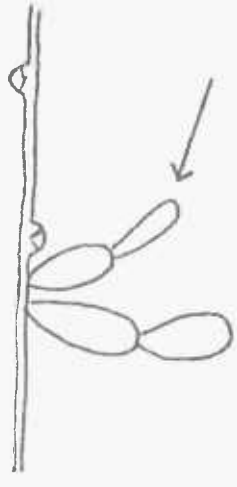


Fig 3.20 "Spores" attributed to Sistotrema brinkmannii
Above, photomicrograph (X700)
Below, freehand interpretation
Common feature arrowed.

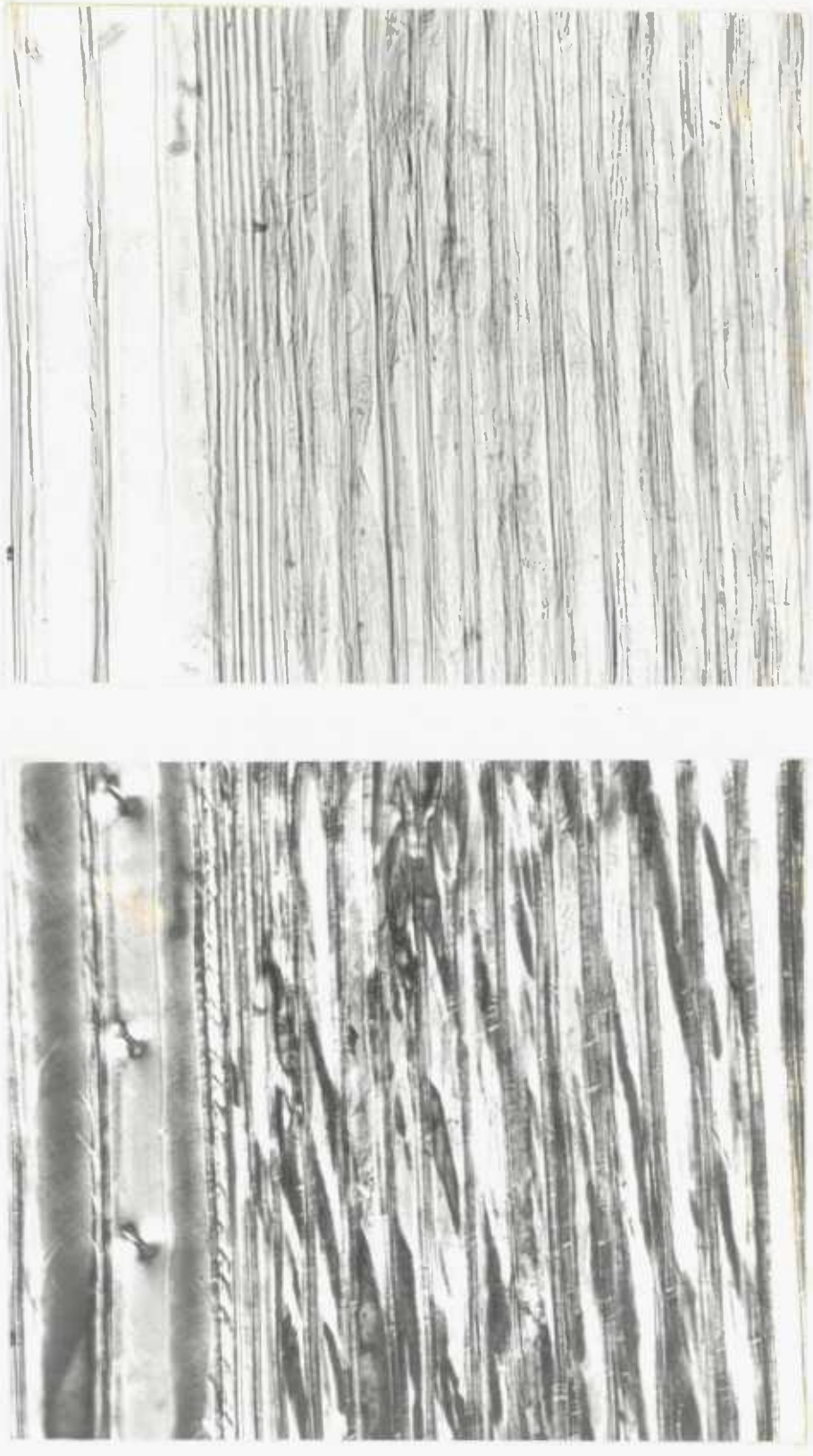


Fig 3.21 Typical soft rot cavities (x 275)
Above, normal light
Below, polarised light

Fig 3.22 Atypical attack (x 275)
Above, normal light
Below, polarised light

Fig 3.23 Branched cavities
Above, normal light (x 825)
Below, polarised light (x 275). Common feature arrowed

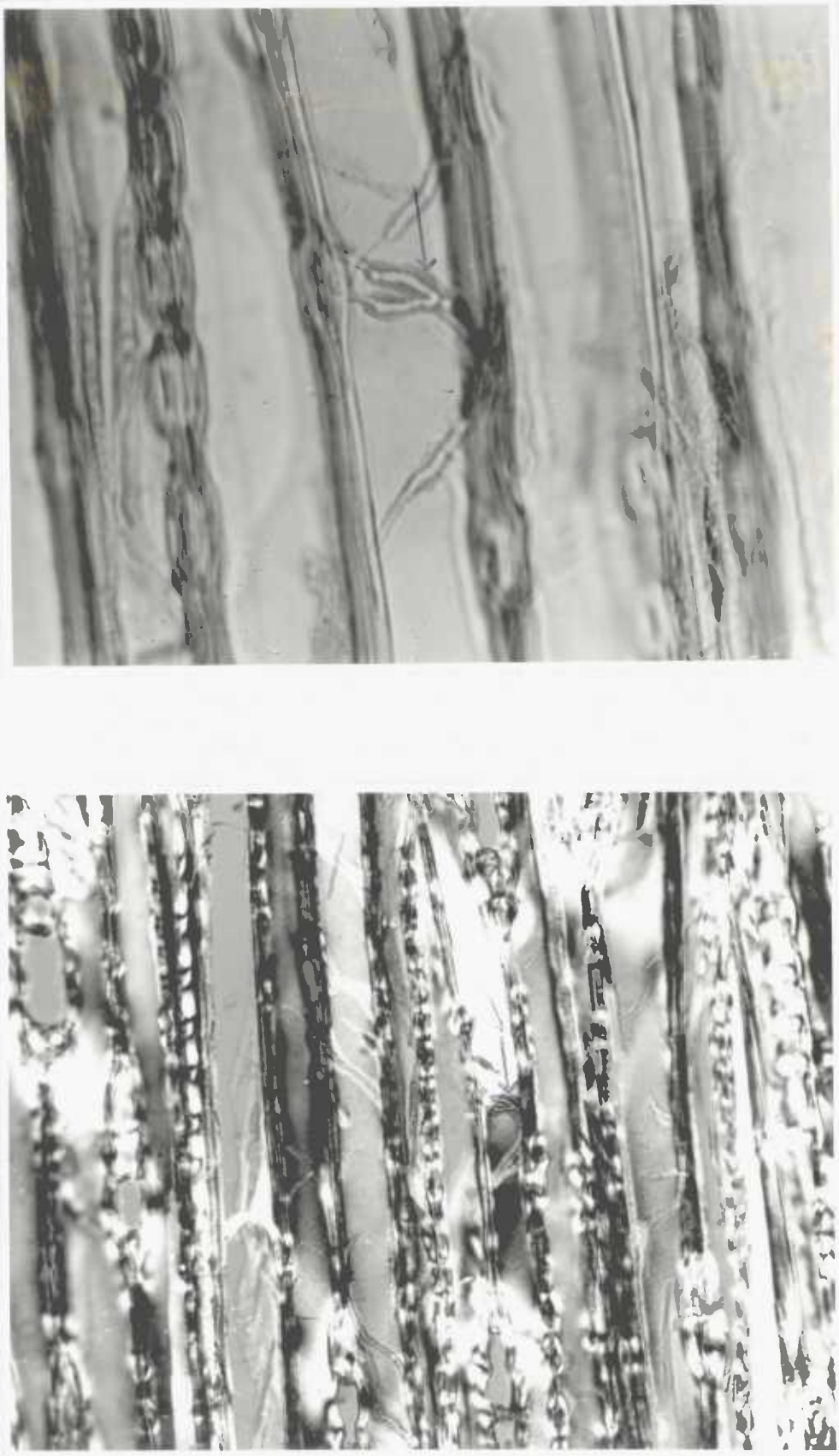


Fig 3.24 Branched cavities
Above, normal light (x 825)
Below, polarised light (x 275). Common feature arrowed

3.6 Appendix

3.6.1 Brief descriptions of bluestain (BS) types isolated from untreated L-joint No 28 after one year's exposure.

BS1 No aerial mycelium, masses hyaline conidia; typical Aureobasidium pullulans.

BS2 Rather brown, with aerial mycelium.

BS3 Little aerial mycelium, conidia lilac coloured in mass; an atypical Aureobasidium pullulans.

BS4 Hyphae only becoming pigmented with age.

BS5 Aerial mycelium.

BS6 Moisture droplets on the aerial mycelium.

BS7 Light coloured floccose aerial mycelium, dark reverse.

BS8 Pale brown, darker reverse.

BS9 Dark aerial mycelium.

BS10 Dark aerial hyphae, aggregating to form tufts.

BS11 Large sporing structure on the test-tube wall.

BS12 Stippled with spore heads.

BS13 Aerial mycelium aggregating, darker reverse.

3.6.2 List of cultures* isolated from untreated pine L-joint No 28 after one year's exposure.

BS type 1

15 MAa_1
16 $MAa=b=c_1=c_2$
25 MAa
26 $MAb=c_2$
33 MAb
35 MAa_1
42 MAb_2
43 MAb=SCNb
44 $MAc_2=SCNa_2$
45 $MAb=NAa_1=a_2$
46 MAb

BS type 2

16 $NAa=SCNa_1$
25 Bena
26 $SCNb_2$
43 $SCNc_1$
46 MAc

BS type 3

14 MAb_1
23 $MAa_1=a_2=b$
24 MAa_2
33 $MAa_1=a_2$
43 $MAa_1=a_2$

BS type 4

22 NAc=SCNc
42 $SCNc_2$
43 NAc_3

BS type 5

14 MAc
43 MAc_1

BS type 6

15 NAa_2
35 NAa_2
36 $MAa_1=NAa_1$
45 NAa_2
46 NAa

BS type 7

35 $NAa_1=SCNa_1$
44 $SCNa_1$
45 $MAa_1=a_3=SCNa$

BS type 8

26 $SCNb_1$
32 $Bena_2$
42 NAc

BS type 9

22 $Benb_1$
32 $Bena_1=b=NAc$
42 $SCNc_1$
43 $SCNc_3$

BS type 10

16 $SCNa_2$
23 MAc_1

BS type 11

13 $Benc_1$
21 NAb=c
24 NAa
42 $Benc_2$

BS type 12

14 MAa=Bena=NAa
33 NAa_2

BS type 13

13 $Benc_2$
15 $NAa_1=SCNa_1$
33 $Benb=c=NAa_1=SCNb_1=c_1$
35 $SCNa_2$
42 $MAc_1=SCNa_1$
43 $MAc_2=Benc=NAa=c_1SCNc_2$
44 Benc=SCNc

Untypified BS

14 $SCNb_2$
15 MAc
15 $SCNa_2$
16 $SCNc_1$
16 $SCNc_2$
23 MAc_2a
23 NAc_1
23 NAc_2
25 NAa
25 $SCNa_2$
26 MAc_1
26 SCNc
35 MAc
35 $SCNa_3$
35 $SCNa_4$
35 $SCNa_5$
36 SCNc
42 $MAa_2=SCNc_3$
42 MAc_2
42 $SCNa_2$
45 $SCNc_1$
45 $SCNc_2$

*The numbering system is explained in Fig 3.5.

Trichoderma viride

11 MAa=b=c=Bena=b=c

12 MAa=b=c=Bena=b=c=SCNa=b=c

13 MAb

21 MAa=b=c=SCNa=b=c

22 MAa=b=c=Bena=SCNa=b

23 Bena=SCNb

32 MAa=b=c=SCNa=b

Aspergillus/Penicillium

15 MAb_1

16 NAc

24 $SCNb_1$

33 $SCNa_1$

33 $SCNc_2$

34 MAb

35 MAb

35 SCNc

36 NAa_2

44 MAa=NAa

44 MAc_1

44 SCNb

Sistotrema brinkmannii

13 $Bena_1=a_2=b$

14 $MAb_2=b_3=Benb=c_1=c_2=SCNa=b_1=b_2=c_1=c_2$

15 MAb_2=Benb=c=SCNb=c

16 SCNb

22 $Benc_1$

23 Benc=SCNc

24 MAa_1=b=c=Bena=b=c=SCNa=$b_2=c_1=c_2$

25 MAb=Benb=c=SCNc

26 $SCNa_1$

32 Benc

33 $MAc_1=c_2$=Bena=$SCNa_2=b_2$

34 MAc=$Bena_1$=b=c=$SCNa_2=a_3$=b=c_1

35 SCNb

42 Benb=$SCNb_1=c_4$

43 MAc_3=Bena=b=SCNa

44 Benb

Other Basidomycetes*

15 Bena

16 Bena=b

26 Bena=b

35 $MAa_2=Bena_1=a_2$

36 MAa_2=Bena

44 Bena

45 $MAa_2=Bena_1=a_2$

46 Benb=SCNb

White cultures

23 Benb

25 $SCNa_1$

26 $MAa_1=NAa_1$

26 $MAa_2=NAa_2$

26 MAa_3

26 $SCNa_2$

36 SCNb

44 MAb

46 SCNa

*All identical; given the herbarium number B742

Other fungal cultures

22 $Benb_2$)
33 $SCNb_3$) pink/mauve, darker reverse
42 $SCNa_3$)

34 $MAa=SCNa_1$)
36 SCNa)
42 $Bena_2=c_1=NAa_2$) olivaceous, burned orange tints
46 $MAa=Bena_1=a_3$)

11 NAc_1 pale pink, dark inoculum
11 SCNa pale pink, floccose
21 Benb white with black spores
23 MAc2b pale ochre
23 SCNa
34 $Bena_2$
36 SCNb
42 $MAa_1=Bena_1=NAa_1$
42 MAb_1

Bacteria

11a	11b	11c
12a	12b	12c
13a	13b	13c
14a	14b	14c
	15b	15c
16a	16b	16c
21a	21b	21c
22a	22b	22c
23a	23b	23c
24a	24b	24c
25a	25b	25c
	26b	26c
32a	32b	32c
33a	33b	33c
34a	34b	34c
	35b	35c
	36b	36c
42a	42b	42c
43a	43b	43c
44a	44b	44c
	45b	45c
46a	46b	46c

3.6.3 List of cultures isolated from 1% TnBTO treated pine L-joint No 48 after one year's exposure

Bluestains

11 MAa_2
11 MAb_2
11 MAc_2
12 MAa_2
12 MAb_2
12 MAc_2
12 $Bena_2$
12 $Benb_2$
12 $Benc_2$
13 MAa_2
13 MAb_2
13 MAc_2
13 $Benb_2$
14 MAb_2
14 MAc_2
15 MAa_1
15 MAa_2
15 MAb_2
15 MAc_2
16 MAa_3
16 MAb_2
16 MAc_2
16 $Benb_2$
21 MAa_2
21 MAb_2
21 MAc_2
22 MAa_2
22 MAb_2
22 MAc_2
22 $Benb_2$
23 MAb_2
23 MAc_2
23 $Benb_2$
24 MAa_2
24 MAb_2
24 $Benb_2$

Bluestains cont

25 MAa_1
25 MAa_2
25 MAb_2
26 $MAa_2=b=c_1$
32 MAa_2
32 MAb_2
32 MAc_1
33 MAc_2
34 MAa_2
35 MAb_2
35 MAc_2
36 MAa_2
36 MAb_2
36 MAc_2
42 MAa_2
42 MAb_2
42 MAc_3
42 $Benc_1$
53 $Benc_2$
43 MAa_2
43 MAc
44 MAa_2
44 MAc_1
45 MAa_1
45 MAa_2
45 MAc_1
46 MAa_1
46 MAb_2
46 MAc_1
46 MAc_2
46 $Bena_1$
46 $Bena_2$

Aspergillus/Penicillium

16 MAa_2

Yellow Phialophora

11 $MAa_3=b_3=c_3$
12 MAb_3
13 $MAb_3=c_3$
16 MAc_3
22 $MAa_3=c_3$
23 $MAb_3=c_3$
32 MAa_3
33 MAc_3
34 MAb_2
36 $MAa_3=c_3$
42 $MAa_3=b_3=c_2$
43 MAa_3
44 MAc_2
46 MAb_1

S. brinkmannii

11 $Bena=b=c=MAa_1=b_1=c_1$
12 $Bena_1=b_1=c_1=MAa_1$
13 $Bena_1=b_1=c_1=MAa_1=b_1=c_1$
14 $Bena=b=c=MAa_1=b_1$
15 $Benb=c=MAb_1=c_1$
22 $Bena_1=b_1=c_1=MAa_1=b_1=c_1$
23 $Bena_1=b_1=c_1=MAa_1$
24 $Bena=b_1=c=MAa_1=b_1=c_1$
25 $Bena=b=c=MAb_1=c_1$
26 $Benb=c=MAc_2$
33 $Benb=c=MAa_1=b=c_1$
34 $Bena=b=MAa_1=b_1=c_1$
35 $Bena_1=b_1=MAa_1=b_1$
36 $MAa_1=b_1$
42 $Bena=b=MAa_1=b_1=c_1$
43 $Bena=b=MAa_1=b$
44 $Bena_1=b_1=MAa_1=b_1$
45 $Benb_1=MAb_1$

White cultures

12 MAb_1
12 MAc_1
14 MAc_1
15 MAa_3
15 MAc_3
16 MAa_1
16 MAb_1
16 MAc_1
16 $Benb_1$
16 $Benc_1$
21 MAa_1
21 MAb_1
21 MAc_1
21 $Bena_1$
21 $Benc_1$
23 MAb_1
23 MAc_1
26 MAa_1
32 MAa_1

32 MAb_1
32 Bena=b
35 MAc_1
36 MAc_1
36 Benb
46 MAb_3

Mucor type

33 Bena

Other fungal cultures

14	MAc_3	pink
14	MAc_4	brown
15	MAa_4	pink
21	$Benb_1$	amber
21	$Benb_2$	apricot
21	$Benc_2$	apricot
22	$Benb_3$	amber/pink

Bacteria

Isolated from all sample positions

3.6.4 List of cultures isolated from untreated, unexposed L-joints (checks)

Aspergillus*

check 1 MAa=NAa=c

check 2 MAc=Benc=NAa=c=SCNa

Aspergillus/Penicillium

check 1 MAc

check 1 Benb

check 1 SCNa

check 1 SCNb

check 2 MAb

*Produces a yellow stain in the agar.

SECTION 4

OBSERVATIONS ON SIMULATED JOINERY UNITS; SECOND SERIES

4.1 INTRODUCTION.

The first series of experiments on simulated joinery units provided a wealth of information on the process of infection of timber in the out of ground contact situation and associated changes in the wood. However there were a number of areas in which additional information was desirable such as the pattern of fungal colonisation during the first year of exposure or where changes in technique or the introduction and development of new techniques would provide more data from the same samples.

One per cent TnBTO failed to prevent colonisation and attack of the first series L-joints. A five per cent PCP treatment has therefore been added since this has long been regarded as a standard against which other exterior joinery preservative treatments may be judged (Anon, 1967) and has been widely used for joinery preservation in the USA (Lance, 1958).

Major changes were made in the methods of monitoring the process of colonisation.

4.2 PREPARATION AND EXPOSURE OF TEST JOINTS

4.2.1 Materials

4.2.1.1 One hundred and thirty five L-joints of Scots pine sapwood (Pinus sylvestris L) were machined from British grown, air dry stock. The overall dimensions and specification were as detailed for the first series L-joints (3.2.1.1) but the corners of the horizontal members (tenon) were rounded; this was not carried out on the vertical members since it would result in a groove at the joint, if machined for their entire length, and in any case, the vertical members are less important as they are only used to provide a joint and are not sampled in the same way as the horizontal members. After machining, the assembled L-joints were stored at room temperature; drying resulted in small splits from the corners of the mortice in some joints. These were ignored since they all occurred in a vertical member.

4.2.1.2 A one per cent by weight solution of bis tri n-butyltin oxide in Shellsol E was used to treat 45 L-joints.

4.2.1.3 A five per cent by weight solution of pentachlorophenol in Shellsol E, containing ten per cent by weight of dibutyl phthalate as co-solvent, was used to treat 45 L-joints.

4.2.1.4 The paint system employed was as follows:
1st coat: white wood primer manufactured by W & J Leigh and Co, Bolton to BS 5358 (1976)
2nd coat: ICI Dulux white undercoat
3rd coat: ICI Dulux brilliant white gloss finish top coat.

4.2.1.5 The vertical member of each L-joint was marked with a numbered aluminium label using anodised escutcheon pins for fixing.

4.2.1.6 Two coats of Hevikote (3.2.1.5) were used to seal the ends remote from the joint of both members after the paint system had been completed.

4.2.1.7 South facing racks of Douglas fir plywood, approximately 900 mm above the ground and canted back at 10° to provide a water trap in the joint area were used to support the test specimens. The upright position of the L-joints was maintained by spacing blocks fixed to the rack between the vertical members (fig 4.1).

4.2.2 Method
The L-joints were divided into three groups of 45 (fig 4.2). The first group, which were to remain untreated, were numbered from 1 to 45, the number being written in pencil on both exposed end grain faces. The second group, which were to receive treatment with 1 per cent TnBTO were numbered 51 to 95 and the third group, to be treated with 5 per cent PCP, were numbered 101-145.

Before any treatment was carried out, the L-joints were sampled to determine their permeability, to establish a base line for assessing any changes that might occur during exposure. Twelve L-joints, in which the vertical members complied with the timber quality

specification for the horizontal members, were selected from each of the treatment groups. A 10 mm length was cut from the end of each vertical and a 12 mm strip was cut from one side to provide a block of the same dimensions as that used in assessing permeability changes during exposure (4.3.4); their permeability was also assessed by the same method.

The L-joints in the two groups to be treated, were taken apart; each member was weighed, immersed for 3 minutes in the relevant preservative solution (4.2.1.2 and 4.2.1.3) then stood, on its end remote from the joint, on paper towelling to drain for a few minutes, and re-weighed. The members were then stood on fresh paper towelling in a fume cupboard and allowed to dry for about one week. The L-joints were reassembled and transferred, together with the untreated group, to a paint room at 20°C and 60 per cent rh. After light sanding, a three coat paint system was applied (4.2.1.4) with at least 24 hours between coats and ensuring all splits and gaps at joints were filled by each coat. During drying the L-joints were stood on their remote ends (fig 4.3). The L-joints were then labelled (4.2.1.5) near the top of the vertical member. A layer of Hevikote (4.2.1.6) was applied to each remote end and continued over the paint system for 3-4 mm. A second layer was applied when the first had dried, the L-joints being stood as shown in Figure 4.4 during drying.

The paint seal across each joint was broken by opening the joint which was then reassembled as tightly as possible.

The L-joints (with the exception of the three replicates from each treatment to be sampled prior to exposure) were then placed on the exposure racks (4.2.1.7) in an order derived from random number tables. Treatments were not mixed, to ensure no carry over of preservative between replicates, and two racks (each 6 feet in length) were necessary to hold each treatment. The L-joints were initially placed in direct contact with the rack but this resulted in greater wetting along the lower half of the horizontal member (fig 4.5) as a result of puddle formation in the rack during rain (see section 3.5.1). The L-joints were placed on two 5 mm square stickers of Scots pine sapwood, both situated away from the joint area (fig 4.1), 12 days after they first began to wet up.

4.3 SAMPLING

4.3.1 Conversion

Only the horizontal members of the L-joints were sampled. The system used for individual L-joints (fig 4.6) was devised to provide the maximum amount of data from each replicate, with several parameters (moisture content, CO_2 production, permeability) being determined on the same sample block (fig 4.7).

Where the end seal overlapped the painted faces, it was removed with a chisel to facilitate accurate sawing. A red line was drawn along the underside of the portion to be removed by cut A, to enable the exact orientation of each block, during exposure, to be distinguished. Cuts A (12 mm from outerface) and B (5 mm from outerface) were then made (fig 4.7), starting from the remote end, to prevent organisms being transmitted on the saw to a greater distance from the joint than they had actually reached. The "B" piece was immediately placed against the central portion and secured with an elastic band to minimise aerial contamination. After the end grain exposed within the joint had been removed, the "A" piece was converted into 12 blocks, each 10 mm in length, which were numbered sequentially from the joint end. All pieces were transferred to a polythene bag directly after cutting to reduce moisture loss.

4.3.2 Moisture content

The twelve blocks from each replicate were trimmed to prevent fragments subsequently being lost. Each block was then weighed and, after CO_2 production (4.3.3) and permeability (4.3.4) determinations were complete, was oven dried for 18 hours at 103°C, reweighed and the moisture content calculated.

4.3.3 CO_2 production

After the initial weighing for moisture content determination (4.3.2), each block was secured by a noose of nylon fishing line and suspended over 5 ml of freshly prepared 0.05 N sodium hydroxide solution in a 100 ml conical flask closed with a rubber bung (fig 4.8). Evolution of CO_2 during microbial respiration within the blocks was measured at the end of a 24 hour incubation period at room temperature, the CO_2 dissolving in the sodium hydroxide producing sodium carbonate. The

sodium carbonate produced by each block was precipitated as insoluble barium carbonate by the addition of 0.15 ml saturated barium chloride solution. The residual sodium hydroxide in each flask was estimated by titration with 0.05 N hydrochloric acid using phenolphthalein (one per cent in ethanol) as indicator; the solution change being from a magenta colour to colourless at the end point. Control flasks containing dry blocks (ie without microbial activity) or no blocks were included with each set of replicates.

4.3.4 Permeability

After completion of the CO_2 production determination (4.3.3) the nylon nooses were removed and the blocks allowed to air dry. The blocks were then oven dried to constant weight at 50°C and their weight recorded. After immersion for 10 seconds in dekalin (3.3.1.4) they were blotted, to remove excess solvent, reweighed and the uptake of dekalin calculated. The blocks were then placed in a fume cupboard for the solvent to evaporate prior to the final oven drying for moisture content determinations (4.3.2).

4.3.5 Bacterial counts

10 mm long blocks were cut from the shoulder, centre and remote end of the central portion (fig 4.7) of one of the three replicates of each treatment. These blocks, usually with unexposed control blocks, were immersed in freshly drawn deionised water in a beaker and weighed down. A vacuum was drawn for 10 minutes and then released to saturate the blocks. Each block was placed within the mouth of a small sterile polythene bag and squeezed in a small vice (fig 4.9). From 0.1 ml of the exudate thus collected in the bottom of the bag, a one in one hundred dilution series in water was prepared; 1 ml of each dilution was mixed thoroughly with 9 ml of molten nutrient agar (3.3.1.2), maintained at 40°C in a water bath, then poured into a sterile plastic petri dish. The dishes were incubated at 22°C for 3 days, when the number of colonies was counted and the count per ml of exudate calculated.

After 22 days exposure, a high count was obtained in the unexposed control blocks which was thought to be due to contamination by bacteria from exposed blocks; similar contamination could also have occurred between exposed blocks. For the subsequent counts, blocks

were impregnated individually, weighted down with glass beads in a test tube containing deionised water.

4.3.6 Bacterial growth estimation

The "B" piece from each replicate (fig 4.7) was placed in a modified bench hook (fig 4.10) and a sample approximately 12 mm long was cut from each of the 15 sampling points (fig 4.11) using a sterile 6 mm wide U-shaped gouge. Samples from the top (a), centre (b) and bottom (c) points of each position relative to the joint were streaked across and planted (fig 4.12) on a single petri dish of nutrient agar (3.3.1.2) previously dried for a few hours at 45°C to remove surface moisture. All operations were carried out on a laminar flow work bench using instruments sterilised by flaming with alcohol (fig 4.13). The dishes were incubated for 4 days at 22°C when both written and photographic records were made.

4.3.7 Fungal isolations

Three additional samples were cut from each of the positions used for estimation of bacterial growth (4.3.6) and planted, partly submerged, in each of the following media:

two per cent malt agar (MA) (2.3.1.2)
benomyl/malt agar (Ben 10) (2.3.1.1)
starch casein nitrate agar (SCN) (3.3.1.3)

Samples from the top (a), centre (b) and bottom (c) points of each position relative to the joint (fig 4.11) were placed equidistant and around the periphery of a single petri dish of each medium (fig 4.14). All dishes were incubated at 22°C for a minimum of 6 weeks. The dishes were observed during this time and records kept of both fungal and bacterial growth (fig 4.15). Subcultures of each type of fungal growth were transferred to dishes of MA or Ben 10 as appropriate; pure cultures obtained were transferred to test tube culture for maintenance. During this process cultures were numbered using a system which, although resulting in complex numbers, allowed immediate recognition of the exact location from which the organism had been isolated (fig 4.11). Each pure culture was provided with a reference card on which details of macroscopic appearance were recorded. Details of microscopic observations or any other

significant features were added as they became available. Various groups of similar organisms were further identified by punching the edges of the card (fig 4.16).

After 71 days exposure additional isolations were carried out on one untreated replicate (No 31). One additional sample was cut from each of the 15 isolation positions; 15 samples were cut, on the same pattern, from the outer painted surface after first surface sterilising by wiping over with 70 per cent ethanol. All 30 samples were planted on dishes of MA and incubated at 45°C. Subcultures were initially held at 45°C but later incubated at 22°C with the other isolates.

4.3.8 Visual observations

Radial longitudinal sections were cut, using a sledge microtome from sample block 1 (fig 4.7) of a number of the L-joints (table 4.1). The sections were stained with safranin and picro-aniline blue as described in TIL 52 (Anon, 1974) and mounted in canada balsam. The sections were examined to note the presence and distribution of soft rot attack, together with any other interesting features.

4.3.9 Paint film failures

After exposure, the painted surfaces of the L-joints were examined and any defects in the paint film noted.

4.4 RESULTS

Various features associated with preparation, exposure, sampling and recording of results are illustrated in Figs 4.1 to 4.16. A summary of the types of data collected after the various exposure periods is given in Table 4.1.

The absorption of dekalin by the 36 sample blocks cut from vertical members prior to treatment or painting is presented in Table 4.2 and Fig 4.17. The absorption of preservative solution by each member of the L-joints is presented in Table 4.3 for TnBTO, Table 4.4 for PCP and in Fig 4.18. The relationship between permeability and preservative uptake is shown in Fig 4.19.

Daily rainfall during the early stages of exposure and mean monthly rainfall over the whole exposure period are given in Fig 4.20.

The original system for the display of information is shown in Fig 4.21.

The moisture contents of the twelve blocks from each L-joint together with the mean value for each sample position for each set of three replicates are presented in Tables 4.5, 4.6 and 4.7 for untreated, 1 per cent TnBTO treated and 5 per cent PCP treated L-joints, respectively. The mean values are presented pictorially in Fig 4.22. The relationship between moisture content and permeability after 221 days exposure is shown in Fig 4.23 and the relationship between loss of moisture and permeability at sample position 1, in Fig 4.24.

CO_2 production by the twelve sample blocks from each L-joint together with mean values are presented in Tables 4.8, 4.9 and 4.10 for untreated, 1 per cent TnBTO treated and 5 per cent PCP treated L-joints respectively; mean values are shown in Fig 4.25. Equivalent data for permeability are presented in Tables 4.11, 4.12 and 4.13 and in Fig 4.26. The increase in permeability with time at sample block position 1 is compared with data from the first series L-joints in Fig 4.27.

The bacterial counts made during the initial stages of exposure are given in Table 4.14. Details of bacterial colonisation as shown by the streaking technique are presented in Table 4.15; representative petri dishes are shown in Figs 4.28 and 4.29. The results are summarised in Tables 4.16, 4.17 and Fig 4.30.

The numbers of fungal isolates obtained are recorded in Table 4.18 and summarised in Tables 4.19, 4.20 and Fig 4.31. The identity of the isolates and their frequency of isolation, after each exposure period, are given in Table 4.21. The progress of colonisation by these isolates, classified according to their role in deterioration, is shown in Tables 4.22, 4.23 and 4.24 from untreated, 1 per cent TnBTO treated and 5 per cent PCP treated L-joints respectively and in Fig 4.32.

Results of observations under the microscope are summarised in Table 4.25. Details of the defects observed in the paint film are given in Table 4.26 and illustrated by Figs 4.33 and 4.34.

The conversion system used to sample the untreated core of the L-joints exposed for 375 days is shown in Fig 4.35; the data collected are presented in Table 4.27 and compared with the normal permeability samples in Table 4.28.

4.5 DISCUSSION

4.5.1 Test method

The similarity between the L-joint simulated joinery units and the hazardous situation faced by joinery in service has been discussed in relation to the first series. This second series was set up to substantiate and increase the information obtained from the first series (section 3) and there were only minor modifications to the exposure technique. The corners of horizontal members were rounded to reduce the paint loss encountered in the first series, which often originated at the sharp corners, and an additional preservative treatment (5 per cent PCP applied by 3 minute immersion) was introduced to act as a standard.

As described in section 4.2.2, prior to preservative treatment, sample blocks were cut from the end remote from the joint of the vertical member of 36 L-joints. The permeability of these blocks was established by dipping in dekalin. The results (table 4.2; fig 4.17) show a variation in uptake from 0.258 g to 0.537 g per block; values between 0.30 g and 0.35 g were recorded most frequently. The range of values represents 77.5 per cent of the mean value (0.360 g) and is a little higher than the 64.8 per cent established for the sample of Scots pine sapwood dipped in white spirit used during development of the permeability test method (Appendix A). This emphasises the variable nature of this timber and it follows that care must be taken when interpreting the increases in permeability which occur during exposure (4.5.4).

Each component was treated separately with preservative, rather than as an assembled L-joint as in the first series; the modified method parallels the more common method used in practice and allows the preservative solution absorbed by each component to be determined. This has proved important. Three horizontal members (No 90, table 4.3; No 138 and 144, table 4.4) show abnormally high absorptions indicating a high initial permeability. The horizontal components of No 90 and 138 sampled to date, show a higher final permeability than the other replicates (tables 4.12 and 4.13) and a high moisture content (tables 4.6 and 4.7). With hind sight, these replicates should have been rejected prior to exposure. Their higher permeability should not be allowed to influence the results obtained from the other replicates; the results for both moisture content and permeability of these samples have, therefore, been omitted from the mean values.

The uptake of preservative solution by the L-joint members (fig 4.18) shows a similar pattern to that of dekalin by the sample blocks, both having a positive skew; this distribution has been recorded by other workers (Purslow, 1974). In general, the vertical members absorbed more than the horizontal members. Those vertical members which were not sampled for permeability have a greater volume than the horizontal members and therefore should absorb more solution; the timber quality of the vertical members is more variable and this too may have exerted an influence. The components treated with PCP solution absorbed more, on a weight basis, than those treated with TnBTO solution (SG 0.856) due in part to the higher specific gravity of the PCP solution (SG 0.893). When converted to the volume absorbed, the uptake of preservative solution broadly correlates with the weight of dekalin absorbed by the sample blocks cut prior to treatment (fig 4.19) although unfortunately, none of the very permeable material was sampled. In future trials this may provide a means to reject over absorptive timber prior to treatment.

The L-joints were exposed on Friday 13 May 1977. The first replicate sets of L-joints were examined after 3 and 5 days exposure. Despite a few showers (fig 4.20) during this period the L-joints showed only a minor increase in moisture content to a maximum of 16.3 per cent near the joint. Not surprisingly, there was no increase in the

bacterial flora detectable by either the streaking or the counting method (isolations of fungi were nct attempted).

No further sampling was carried out until wetting had been detected by using a microwave moisture meter (3.3.1.1); this occurred following rain on 5 June and exposure times have been calculated from this base date, disregarding the initial dry period.

In the CO_2 production tests, the 24 hour exposure period had been used in the trials and calculations indicated that the NaOH would be neutralised by CO_2 before lack of oxygen would become a limiting factor (Appendix B). During the initial dry period, the 5 ml aliquots of NaOH were measured using a graduated 10 ml pipette; the difference between the first and second aliquots was detectable and therefore a 5 ml pipette was used for all later series. The ability to detect such a small difference indicates the sensitivity of the test method.

During the early stages of the exposure period, data were accumulated very quickly and it became essential to display the information in a form which could be easily understood. For this purpose the scheme shown in Fig 4.21 was used; data on moisture content, permeability and colonisation by bacteria and fungi were recorded separately for each replicate*. As the volume of data increased this system became inadequate since its two-dimensional form was not suited to the three-dimensional nature of the results (position relative to the joint v time v value for the parameter being measured). A computer programme, already in existence (Baines, 1978), was therefore modified and used to present the data in three dimensions, using the mean value for the three replicates sampled for each treatment; the form of presentation is shown in Figs 4.22, 4.25 and 4.26. The time scale is not exact, due to limitations imposed by the computer, but is sufficiently accurate not to misrepresent the results.

The importance of the three isolation media for fungi became apparent, particularly during the later stages of exposure when many organisms were present. The malt agar medium (MA) is non-selective and

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*CO_2 production was not included at this stage because associated experiments were incomplete.

therefore, to a degree, the rate of growth of the organisms appears to determine whether they are isolated successfully or swamped by the fast growers. In the early stages growth was dominated by the bluestain organisms while later, the Basidiomycetes became important. Phialophora type A, though slow growing, was also isolated frequently on the MA. This is probably a result of the antagonism it shows towards other organisms, particularly bluestains (see section 5.3.19). The benomyl/malt agar (Ben 10) is selective for Basidiomycetes although occasionally other organisms such as Trichoderma viride were able to develop. Interestingly, different Basidiomycetes developed, on occasions, from the replicate chips placed on MA and Ben 10. This may reflect a different ability of the organisms to grow on the two media but equally well could show the chips contained different organisms growing in close proximity to one another. The starch casein nitrate agar (SCN) inhibits the growth of most Basidiomycetes and bluestains of the Aureobasidium type but allows the growth of various slow growing organisms such as Phialophora, Phoma, Coniothyrium, various unidentified isolates and some moulds. Consistently, these organisms have not been isolated on the other media which indicates the importance of the SCN in helping towards determining the total microflora. The cellulose containing media used by various workers (Greaves and Savory, 1965; Butcher, 1968 and 1971; Clubbe, 1978) probably fulfill a similar role. The SCN has the added advantage that Actinomycetes produce pink colonies by incorporating the rose bengal; in this particular study no Actinomycetes were isolated but in other situations eg ground contact or the marine environment this facility could prove important. Undoubtedly, if additional media had been used, additional fungi would have been isolated. However, the extra work resulting from expanding the programme of isolations in this way must be weighed against the additional information derived, and both must be reconciled with the time available.

4.5.2 Moisture content

Although there was variation between replicates (tables 4.5-4.7) the pattern of the results was essentially the same, with wetting only taking place from the joint end. The mean values have therefore been used in the presentation of the data (fig 4.22).

Following rain (fig 4.20) the uptake of moisture was rapid with a moisture content of over 70 per cent at the joint end in untreated material achieved after 11 days. TnBTO treated material showed similar uptake but PCP treatment reduced the rate so that the moisture content at the joint end after 11 days was only 50 per cent. This is still adequate to support the growth of wood decay fungi and so its practical significance may be doubtful. Nevertheless, the number of isolates from the PCP treated L-joints was much smaller than from the others (fig 4.31), which may be an indication of a contributory factor.

The results show that water taken in at the joint moved along the horizontal members so that increases could be detected at the remote end (up to 165 mm from the joint) after 8 days; mean moisture contents sufficient to support decay were not recorded in this position until 130 days of exposure although individual replicates did exceed 20 per cent after shorter periods. There were wide fluctuations in the moisture contents recorded close to the joint depending on both the rainfall (fig 4.20) and the drying which occurred in the intervening periods. The intensity of the fluctuations diminished as distance from the joint increased. Over certain periods the change in moisture content at the remote end was in the opposite direction to that recorded at the joint end. This suggests that the prevailing influence at the joint end takes time to influence the remote end; during the initial period of wetting this was between 4 and 8 days. Once wet, the L-joints did not dry to a "safe" moisture content of less than 20 per cent; this result agrees with earlier findings (section 3; Anon, 1969).

After 221 days exposure, the moisture content of individual blocks was, in general, correlated to their permeability (fig 4.23); this effect was investigated further (section 6.5) by a laboratory experiment. The results showed that when blocks of different permeabilities were exposed to unlimited water the moisture content achieved varied with their permeability for most of the 8 day period of the experiment. In the 13 days prior to the sampling of L-joints after 221 days exposure, a total of 24.5 mm of rain was recorded, which must have approximated to the unlimited water situation used in the laboratory

tests. This emphasises the importance of permeability in the performance of timber in service since in general the higher the moisture content, the greater the amount of decay (Duncan, 1953; Ammer, 1964).

Between 221 and 375 days exposure, the moisture contents of the L-joints fell. Rainfall was heavy at the beginning of the period (fig 4.20) then gradually declined suggesting the moisture contents may initially have risen and then fallen to the values recorded. The effect was greatest in the untreated L-joints, and the amount of drying was related to the permeability of the replicates (fig 4.24).

4.5.3 CO_2 production

Results during the early stages of exposure were variable although preliminary experiments (Appendix B) had shown that the test method could give reproducible results. Numerous assays showed a negative production of CO_2 as compared with the dry control blocks (shown as + values in tables 4.8-4.10). A pattern of results began to develop in which more negative results were recorded at the joint end than at the remote end. This lead to the hypothesis that the higher moisture contents recorded at the joint end were having an effect possibly by a proportion of any CO_2 produced dissolving in the water within the test blocks rather than in the NaOH solution. This was tested by blocks being wetted to varying moisture contents, then sealed in polythene bags and sterilised by exposure to ethylene oxide using an Amsco gas steriliser. After an extended period of ventilation, to allow gases to diffuse back into the blocks following the period of evacuation at the end of the sterilisation cycle, each block was removed from its bag, suspended in a fishing line "noose" and CO_2 production assessed by the method used for exposed blocks (section 4.3.3). No correlation was found between moisture content and CO_2 production. This may have been the result of an inadequacy in the test method but since, by the time the results were available, the CO_2 production tests were showing more meaningful results, an alternative system was not tested.

The computer diagrams (fig 4.25) have been prepared with negative production of CO_2 recorded as zero; the apparently greater production of CO_2 towards the remote end (see above) can be seen most clearly with the data from PCP treated samples.

Despite the variation experienced, two trends are apparent. First, CO_2 production increased with exposure, except for the last sampling time where reduced moisture contents may have influenced the results. Secondly, CO_2 production by untreated samples was greater than for TnBTO treated samples which in turn produced more CO_2 than PCP treated samples. These results parallel the gross patterns of colonisation by micro-organisms, particularly fungi (sections 4.5.6 and 4.5.7) but due to their variable nature, do not contribute significantly to the overall results.

CO_2 production monitors activity only under the conditions prevailing at the time of sampling rather than the potential activity. This is exemplified by the fall in activity between 221 and 375 days exposure although no fall in the numbers of bacteria or fungi present was recorded except for a small reduction of the numbers of bacteria in untreated samples. The inclusion, in future trials, of any method of monitoring CO_2 production should be considered carefully in light of the success of the various isolation techniques (sections 4.5.6 and 4.5.7); the present method does not appear suitable for this application.

4.5.4 Permeability

The permeability of the samples prior to exposure was consistent along the length of the untreated and TnBTO treated L-joint members (tables 4.11 and 4.12); there was more variation among PCP treated samples (table 4.13) and those blocks close to end grain exposed during treatment were of lower permeability, as predicted by the preliminary trials (Appendix A). During the initial period of exposure, permeability values towards the joint end were generally higher than those towards the remote end for all three treatments (fig 4.26); this result, which parallels the distribution of moisture (fig 4.22), suggested a relationship between the two factors. In a specific test,

blocks vacuum impregnated with deionised water and then dried to constant weight at 50°C took up 0.460 g dekalin during a 10 second immersion compared with only 0.300 g by a replicate series of blocks which had not been wetted. In this test part of the increase may have been due to the vacuum treatment. An increase in permeability following wetting does not appear to have been recorded previously. The increase is small in terms of the increases achieved by microbial action (see later) and is probably explained in terms of the de-aspiration of a small number of bordered pits. Because of this increase due to wetting alone, the values for permeability before exposure recorded in Table 4.2 cannot be used directly as a base line by which to judge the effects due to microbiological attack. A correction factor can be calculated from the test L-joints during the early stages of exposure prior to microbiological attack. Between 4 and 22 days exposure the absorption of dekalin by sample block 1 (nearest to the joint) was higher than that by sample block 12 (furthest from the joint) by an average of 24.5 per cent in untreated replicates. When this factor is applied to the Table 4.2 values, the mean becomes 0.448 g and the range 0.321 g to 0.669 g; only values in excess of 0.669 g can, therefore, be considered as indicative of microbiological attack. In 1 per cent TnBTO treated replicates the correction factor, calculated as above, was 14.8 per cent. This lower figure may be a result of the TnBTO, a non water-soluble compound, slowing down the rate of the effect rather than actually reducing it; the 'untreated' correction factor should therefore be applied to the TnBTO treated L-joints to ensure the proper safety margin. With PCP treated samples the same reasoning has been applied; in addition the lower permeability associated with the PCP treatment must be taken into account (Appendix A). When both correction factors are applied to the Table 4.2 values, the mean becomes 0.388 g and the range 0.278 g to 0.578 g.

Among the untreated replicates, sample block 1 from one L-joint exposed for 22 days (No 23, table 4.11) absorbed 0.677 g dekalin thus marginally exceeding the 0.669 g limit. However since all the other sample blocks showed above average permeability the effect is unlikely to be due to microbiological attack. Again, after 32 days exposure,

sample block 1 from one replicate (No 25) exceeded the 0.669 g limit. In this instance the remaining sample blocks showed only average absorptions; the effect is therefore believed to be the earliest evidence of microbiological attack. At the next sampling (after 43 days exposure) no similar increase was observed. After longer periods of exposure, all replicates showed significant increases which were generally more pronounced towards the joint end (fig 4.26).

In TnBTO treated L-joints the first significant increase in permeability occurred in one replicate after 71 days exposure (No 83; table 4.12); as with untreated L-joint No 23 this appears to be a generally more permeable replicate and the effect may not be due to microbiological attack. After 130 days exposure two replicates showed increases although, as previously discussed (4.5.1) the values recorded for L-joint No 90 have not been included in the mean due to its very high initial permeability.

In PCP treated L-joints, the permeability showed the same initial increase in response to wetting given by untreated and TnBTO treated L-joints but in contrast did not show any increases which could be attributed unequivocally to microbiological attack. Several L-joints gave values above the 0.578 g limit; of these No 138 had previously been rejected (4.5.1) due to its very high initial permeability. Three others (No 124, 128 and 135) had also initially taken up above average amounts of preservative (table 4.4) indicating a higher initial permeability. A further three (No 131, 134 and 142) exceeded the limit by only small amounts which could be due to natural variation in permeability.

The overall picture of increases in permeability can be seen in Fig 4.26. The TnBTO treatment delays the onset of increases due to microbiological activity but apparently does not reduce the rate at which the effect occurs (fig 4.27). Data from the first series of L-joint tests (section 3) are included to show the similarity in the results from the two series. The PCP treatment has prevented major permeability changes due to microbiological activity during the first year of exposure.

4.5.5 Bacterial counts

The process of obtaining viable counts of bacteria is time consuming and does not result in a direct count of the number of bacteria in a piece of wood but relies on the relationship between the number released and the total number present remaining constant. Because of these problems, an alternative system, the 'streak' method, has been devised (Appendix B); the counting method has been included as a back up for the new method (4.5.6) which has not previously been used under field conditions.

After 22 days exposure, check blocks showed high counts probably as a result of transfer of bacteria from infected blocks during the impregnation process. Similar transfer could have occurred between test blocks and so the counts of 10^5 per ml may not indicate proliferation of bacteria. After the longer periods of exposure the test blocks were impregnated individually to avoid cross-contamination; these results confirm that no proliferation of bacteria had occurred at 'centre' and 'end' positions after 22 days.

Comparison of the results obtained using the counting technique (table 4.14) with those obtained by the 'streak' method show certain differences. For example, the first indication of any proliferation of bacteria in the central portion was in the PCP treated L-joint exposed for 15 days, whereas the 'streak' method had detected a significant increase in numbers close to the joint in untreated and TnBTO treated material after only 8 days exposure (table 4.15). These differences may be the result of two factors. First, the counting technique provides an average for the whole sample block and cannot distinguish between a heavy concentration in a small pocket and an overall low concentration. The smaller sample in the 'streak' method should give a more accurate assessment of that small sample but the results from a large number of samples should be considered to gain an overall picture.

Secondly, the sample blocks used in the counting technique are taken from the central portion of the L-joint member (fig 4.7) and therefore are not in line with the end grain exposed within the joint.

Colonisation of this central portion is therefore a result of lateral as well as longitudinal movement. In contrast, the chips used in the 'streak' method are taken directly in line with the end grain and colonisation is a result of longitudinal movement alone. The delay before proliferation of bacteria is detected in the central portion could be the result of a slower rate of lateral movement. Alternatively, the moisture content in the central portion could be inadequate to support bacterial growth since water moves more slowly laterally than longitudinally.

After 43 days exposure, proliferation of bacteria was detected in the 'centre' position in the untreated replicate; this was also detected at the equivalent position (3), by the 'streak' technique for this particular replicate but was not apparent in the other replicates (table 4.15).

The two methods appear to be providing similar information. However, the 'streak' technique is providing information for all three replicates, instead of one, at five positions relative to the joint, instead of three, and in a direct line with the end grain exposed within the joint. After the results from replicates exposed for 43 days it was felt the counting technique had validated use of the 'streak' method under field conditions but was no longer providing the quantity of information comparable to the effort required. No further counts were, therefore, undertaken.

4.5.6 Bacterial colonisation

The method of following bacterial colonisation, the 'streak' technique, was devised for the present work; details of the initial experimentation are given in Appendix B. The use of the method has been further validated by comparison of results with those from a viable count technique (4.5.5).

Sample chips were taken at three positions through the depth of each replicate and at five positions relative to the joint; by using this pattern it was hoped to be able to follow the process of colonisation. Detailed results are presented in Table 4.15; representative photographs are presented as Figs 4.28 and 4.29. Each level on the subjective scale of assessment has been given a numerical value to

allow an adding up process. However, it must be remembered that a 10 fold increase is required in the bacterial numbers present in the exudate before the next level on the subjective scale is reached (Appendix B); a small increase in the numerical scale, therefore, represents a considerable increase in bacterial numbers. A few colonies along the streak, in the absence of growth around the chip, are attributed to contaminants; they are recorded as * in Table 4.15 and are not included in totals.

The data from Table 4.15 have been analysed in two ways. First the numerical ratings have been totalled for each position through the depth of the L-joint member and a grand total calculated for each exposure period (table 4.16). This analysis shows no consistent effect with the depth of the sample except perhaps for less bacteria being present along the bottom of PCP treated samples. The grand totals show an initial increase (fig 4.30) and then no further increase from 22 to 43 days. This was a period of low rainfall (fig 4.20) during which the moisture contents of the L-joints fell (fig 4.22), in many cases to below 40 per cent (tables 4.5, 4.6, 4.7). This obviously had an effect on bacterial growth. Following rewetting, growth was resumed. After 375 days exposure, untreated replicates showed reduced growth, again probably due to lower moisture contents as a result of low rainfall in the period prior to sampling (fig 4.20).

Secondly the numerical ratings have been totalled for each position relative to the joint (table 4.17; fig 4.32). This analysis shows initial colonisation close to the joint of both untreated and 1 per cent TnBTO treated replicates after 8 days exposure; colonisation of 5 per cent PCP treated replicates did not occur until 15 days exposure. Colonisation beyond position 2 appears to have been delayed by the dry period previously mentioned although some growth occurred near the remote end of untreated and 1 per cent TnBTO treated replicates after 15 days exposure. On rewetting, colonisation progressed and bacteria first reached the remote end of an untreated L-joint after 71 days and were present in large numbers at 130 days; by this time the moisture content at the remote end had risen to above 30 per cent. No colonisation of the remote end of treated replicates

was recorded until 221 days exposure although moisture contents of above 30 per cent were recorded in some replicates after 130 days.

The preservative treatments have not had any marked effect on the colonisation of the L-joints by bacteria; PCP treatment delayed initial colonisation by a few days and both delayed progress along the length of the samples. However, after 221 days, bacteria were recovered from the remote end of the three replicates treated with TnBTO and two of the three replicates treated with PCP. In the first series of L-joint tests (section 3) it was postulated that the bacteria were responsible for initial increases in permeability. In light of the above result in conjunction with no significant increase in the permeability of PCP treated samples, this hypothesis may have to be modified (see section 4.5.10).

4.5.7 Fungal colonisation

The system of sampling used in an attempt to follow the progress of fungal colonisation was developed from that used successfully to sample two first series replicates after one year's exposure (3.5.4). In order to be able to sample from nine replicates (three from each treatment) after each exposure period, samples were taken in one plain only, along the length of the sample. This was the mid-line of the end grain exposed within the joint (fig 4.7) and it corresponds to position 3 as used in the first series; this position had been selected previously when investigating the Basidiomycete flora after the longer exposure periods in the first series.

Because of the large numbers of fungi isolated, many of the details regarding identification and other features have been recorded separately (section 5). This discussion of fungal colonisation is therefore couched in rather general terms so as not to camouflage L-joint testing in mycological minutae.

The isolations incubated at 45°C were suggested by the work of Morton (1975) and Morton and Eggins (1976a, 1977). The two fungi isolated, Aspergillus fumigatus and Paecilomyces varioti, were also isolated from the samples incubated at 22°C and therefore did not

provide additional information on the species present. Although such organisms can continue to grow at the elevated temperatures recorded in insolated wood (Morton, 1975) they are not the ultimate cause of failure of untreated joinery or the few recorded failures of preservative treated joinery; this is the role of the Basidiomycetes (section 2.2; PRL,unpublished data). By carrying out isolations at lower temperatures, an appreciation of the range of viable fungi is obtained. Each organism will be able to fulfill its particular rôle in the deterioration process only when temperature, moisture and nutritional conditions are suitable for its growth. The ability to survive elevated temperatures would therefore appear more important than the ability to grow at elevated temperatures; no further isolations at 45°C were carried out and those results obtained are not included in the following discussion.

For simplicity untreated and preservative treated data will initially be considered separately. In untreated material, the number of fungal types isolated at each sampling point (table 4.18) inevitably show variation between replicates. To obtain a clearer picture, the data have been processed variously. By summation of the number of isolates at each depth it can be seen (table 4.19) that, as with the bacteria (table 4.16), there is no consistent effect. In future L-joint trials, therefore, it should be possible to reduce the number of sampling points to a single line along the length of each replicate. By reducing the effort per L-joint, a larger number could be sampled simultaneously.

A few fungi were isolated from unexposed material; the same species were isolated after various periods of exposure and tend to confuse the results. For example, of the 49 isolates after 11 days exposure, 18 were identified as organisms known to occur in unexposed material including 13 isolates of Aspergillus fumigatus from a single L-joint. If these are discounted, the number of isolates after 11, 22 and 32 days become similar (table 4.19). Over this period, the L-joints were drying out (table 4.5) which apparently discouraged invasion as has been noted previously for the bacteria (see section 4.5.6). The number of isolates increased with longer periods of exposure

(fig 4.31), the increases representing proliferation of the species present at individual sampling points (table 4.18) as well as colonisation along the samples (table 4.20). Rapid colonisation occurred; after 11 days one invading organism was isolated from position 3 which lies 75-90 mm from the joint surface. The rate of colonisation may have been influenced by the initial period of exposure prior to wetting (4.5.1); some localised areas may have achieved a moisture content adequate to support growth and thus build up the inoculum potential. On wetting colonisation could be more rapid than from de novo infection; additionally the conidia produced during growth of Aureobasidium type cultures in wood (see section 5.2) may be capable of being carried through the wood by liquid water. Between 11 and 43 days exposure, colonisation did not proceed further along the length of the samples. This period corresponds to a period of drying. At 43 days the total number of isolates showed an increase (fig 4.31) but obviously there is a lag phase during which water is transmitted along the samples and further colonisation can occur. After 71 days, isolates were recovered from position 5 indicating complete colonisation of the L-joint member; the numbers of isolates increased after longer exposure periods. In contrast to the bacterial isolations, there was no decrease in the numbers of fungal isolates between 221 and 375 days indicating the fungi possess a greater resistance to desiccation.

Comparison with the data for bacterial colonisation (table 4.17; fig 4.32) shows that, in general, the bacteria initially colonised at a slower rate than the fungi, reaching position 3 after 43 days in comparison with 11 days for the fungi. However, both types of organisms were isolated from position 5 after 71 days. This suggests that the bacteria were not playing a 'pathfinding' rôle, prior to fungal invasion.

Considering colonisation of preservative treated material, both treatments reduced the number of fungal isolates obtained (fig 4.31). In one per cent TnBTO treated L-joints, fewer fungi were present over the initial stages of exposure than in untreated material; during the later stages, the numbers increased, but at a slower rate. In five per cent PCP treated L-joints, there were few isolates throughout the exposure period and there was no tendency towards

higher numbers after the longer periods of exposure. Both preservative treatments, therefore, were reducing fungal colonisation with 5 per cent PCP being the more effective; this is in marked contrast to their ineffectiveness in the control of bacterial colonisation (compare figs 4.30 and 4.31). Despite the lower numbers of fungi present in the treated replicates, the rate of colonisation is remarkably similar to that of untreated material (table 4.20) apart from an initial delay caused by the PCP. The preservatives therefore appear to be selecting tolerant fungi rather than causing a general reduction in growth rate. This is borne out by the species present (see later). It also correlates with observations made on the colonisation of copper-chrome-arsenic (CCA) treated pine sapwood and birch stakes exposed in ground contact (Clubbe, 1978, 1980a, 1980b) and the increased incidence of copper-tolerant fungi in soil adjacent to CCA treated posts (Murphy, 1979).

A considerable amount of effort has been expended on the identification or characterisation of the fungal isolates. The details are recorded in Section 5 but a summary is presented in Table 4.21. The fungi are listed basically in the order in which they appear but the various unnamed bluestain and Basidiomycete types are listed together. Aspergillus fumigatus has been included with the fungi occurring in unexposed material although, in this series, it was not isolated. In the first L-joint trial (section 3) an Aspergillus sp producing a yellow stain in the agar was isolated from unexposed blocks; this is now believed to be A. fumigatus. In addition all 13 isolates from untreated material exposed for 11 days were from one of the three replicates; the fungus was not isolated from two positions close to the joint where bluestain fungi had become established. This pattern of isolation strongly suggests prior infection by A. fumigatus.

The preservative treated material appears to provide a selective environment. The bluestain types most common on the untreated material (A,B,C and K) are less common on preservative treated material or appear later in the exposure period. Bluestain (BS) type D grew equally well on untreated material or 1 per cent TnBTO treated material but BS type F was only isolated in the presence of TnBTO

and BS types E, G and I only in the presence of PCP. Similar patterns are shown by other organisms for example Botrytis cinerea was first isolated from untreated material after 11 days exposure, from TnBTO treated material after 43 days exposure but was not isolated from PCP treated material.

The organisms have been classified into various groups; the divisions are based on the experiments carried out on the cultures (section 5), information gleaned from relevant literature (eg Seehann et al, 1975) or a personal knowledge of the organism concerned.

For untreated L-joints, the numbers of isolates in each group, at each position relative to thejoint, are presented in Table 4.22. Those fungi known to occur in the material prior to exposure have been omitted to clarify the process of colonisation. The data are also presented in Fig 4.32, omitting the unclassified organisms and amalgamating Sistotrema brinkmannii with the white rotting Basidiomycetes. Data on bacterial colonisation (taken from table 4.17) have been included for comparison.

The small group of unclassified organisms were generally isolated close to the joint; most isolates showed no characteristic features although two were yeasts. The rôle of the organisms in this group is uncertain and therefore they have not been considered further.

The moulds appear relatively unimportant, occurring only sporadically apart from a large number of isolates of Trichoderma viride after 71 days exposure. Bluestains and soft rots were both present after 11 days exposure. The bluestains were most numerous adjacent to the joint but had penetrated as far as position 3 while the soft rots were only present adjacent to the joint; this suggests the soft rots colonised at a later time than the bluestains. During the dry period up to 43 days exposure (fig 4.20) neither group of organisms penetrated further along the samples although more soft rots were present after 43 days. During this period the first Basidiomycetes were isolated; the white rot isolated after 32 days did not possess clamp

connections and therefore could have been the result of the germination of a single spore. Between 43 and 71 days colonisation was able to progress as a result of wetting following rain. Bluestains and Basidiomycetes were both isolated from position 5, ie furthest from the joint, while the soft rots were not isolated beyond position 3. This may indicate a slower rate of colonisation by the soft rots; alternatively the presence of T. viride may have adversely affected their isolation.

After 130 days exposure, a large number of bluestains were isolated, and the numbers remained relatively constant over the remainder of the exposure period (table 4.22). However, their distribution varied (fig 4.32) with most isolates being obtained close to the joint (position 1) after 221 and 375 days. This could indicate a general decline in the population except at the joint, where reinfection can occur.

The number of soft rots increased rapidly between 130 and 221 days; there were less isolates after 375 days. This may represent a true peak followed by a decline although, due to the small number of replicates, the reduction in numbers could be due to experimental error. Results after longer periods of exposure would be necessary to determine the correct interpretation.

The number of Basidiomycete isolates increased over the later stages of exposure, and their distribution is very even along the length of the samples (fig 4.32).

The isolations from untreated L-joints suggest that the fungi invade in the order bluestains, then soft rots, then Basidiomycetes although all three groups are present with 32 days exposure. The three groups reach their maximum frequency in the same order as their initial colonisation but there is little evidence to support a subsequent decline in their importance.

Isolations from preservative treated L-joints have been presented in the same way as those from the untreated L-joints. The numbers of isolates in each group, at each position relative to the joint are

presented in Table 4.23, for 1 per cent TnBTO treated L-joints, and in Table 4.24, for 5 per cent PCP treated L-joints; these data are also presented in Fig 4.32.

As in untreated material, few moulds were isolated from the preservative treated L-joints with the exception of T. viride from PCP treated material, after 71 days exposure. This organism is capable of degrading PCP (Cserjesi, 1972) which may account for its presence.

In TnBTO treated material, bluestains were isolated after 11 days exposure, but they did not penetrate the full length of the L-joint members until 130 days. Few bluestains were isolated after 221 days when they may have been adversely affected by Sistotrema brinkmannii which outgrows most other organisms on malt agar, the growth medium on which most bluestains have been isolated. Soft rots were first isolated after 43 days but did not penetrate the full length of the L-joint members until 375 days. The first Basidiomycete, S. brinkmannii, was isolated after 221 days, followed by a white rot after 375 days, thus reversing the order of colonisation, by the two Basidiomycete types, established in the untreated L-joints. S. brinkmannii has been shown to be considerably more tolerant of TnBTO than the brown rot Coniophora puteana (PRL, unpublished report) but it has not been compared with the white rot Coriolus versicolor which is more tolerant of TnBTO in pine sapwood than C. puteana (Savory and Carey, 1976). The present result suggests it is more tolerant than the white rots and hence is able to colonise at an earlier stage.

In PCP treated material, bluestains were first isolated after 32 days exposure and one was recovered from the remote end of a sample (position 5) after 130 days; they were only recovered in low numbers and there was no indication that they were becoming more successful. Soft rots, all Phialophora type C, were only isolated after 375 days; no Basidiomycetes were isolated, with the exception of one isolate after 32 days exposure which possessed the characteristics of an Aureobasidium type culture under normal conditions but which produced clamp connections when grown under stress in a coverslip culture (section 5.2, BS type I).

As previously discussed, the preservative treatments reduced the number of fungal isolates. In addition, they lengthened the period over which the various groups of organisms became established. In TnBTO treated L-joints, bluestains were present after 11 days but soft rots were not isolated until 43 days, and Basidiomycetes not until 221 days. In PCP treated L-joints bluestains were first isolated after 32 days and soft rots after 375 days; no typical Basidiomycetes were isolated.

This investigation of the colonisation of painted wood exposed not in contact with the ground is the first of its kind. A few earlier studies including isolation of organisms have been carried out on similar material but not until after several years of exposure (Sedziak et al, 1970; Shields and Krzyzewski, 1975 and 1976; Savory et al, 1977) while another only occasionally used painted wood and was concerned largely with thermophilous fungi (Morton, 1975; Morton and Eggins, 1976a and 1977). It is not, therefore, possible to make comparisons with previous work. However, a considerable amount of work has been carried out on the initial colonisation of softwood stakes in ground contact, and it is interesting to compare the results.

Summarising the ecological studies carried out at the ground-line of untreated stakes, a true succession appears to occur in the order bacteria to primary moulds to bluestains to softrots to secondary moulds and Basidiomycetes with each group being dominant in turn (Corbett and Levy, 1963; Butcher, 1968; Banerjee, 1969; Clubbe, 1978, 1980a, 1980b); the importance of the bluestains in the succession seems to vary more than for the other components. As previously stated, the situation is different in the painted L-joint. Moulds were isolated only sporadically but bluestains were important at an early stage being quickly followed by soft rots and Basidiomycetes, all three groups being present in approximately equal numbers after 375 days. The lack of moulds and importance of bluestains may be related to the difference in exposure conditions. All organisms infecting the L-joints must arrive as air-borne spores whereas at the groundline of stakes most organisms probably originate in the surrounding soil.

Butcher (1968) showed bluestains were a more significant part of the flora in the upper part of stakes, where aerial colonisation is more important, than at the ground line. Many bluestain organisms occur naturally on the aerial parts of trees and their reproduction is suited to air-borne dispersal of spores.

The first Basidiomycete was isolated from the L-joints after only 32 days exposure; numbers then rose significantly. At the ground-line of stakes, the Basidiomycete flora has been present in only low numbers (Merrill and French, 1966; Butcher, 1968) or has arrived rather later in exposure (Clubbe, 1978, 1980a, 1980b). In both cases the predominant organisms have been white rots and Sistotrema brinkmannii. The incidence of white rots in joinery has been discussed previously (section 2) in comparison with the interior of buildings where decay of softwood is almost exclusively caused by brown rots. The above data, together with information derived from colonisation studies of posts and stumps (Käärik, 1967 and 1968; Hallaksela, 1977) suggest that in all these situations a succession from white rot to brown rot occurs and therefore, that the situation inside buildings is unique, with brown rot occurring alone. One possible reason for this difference is the less fluctuating and generally higher temperature within buildings which could prove either of advantage to the brown rots or of disadvantage to the white rots.

Studies with preservative treated material (Butcher, 1971; Clubbe, 1978, 1980a, 1980b) suggest the preservative (copper-chrome-arsenic) has the effect of elongating the period over which the various parts of the fungal succession occur. The effects of this have been monitored in laboratory tests by Smith (1971) who showed the period before strength loss could be detected, in thin strips of wood exposed to natural soil, increased with increasing preservative concentration. Within a finite period of observation, certain parts of the succession may not be observed eg the Basidiomycetes (Clubbe, 1978). In practice, the Basidiomycetes may be excluded for so long, that failure due to soft rot attack occurs, as in transmission poles in Sweden (Henningsson et al, 1975). In the L-joints, preservative treatment appears to be having a similar effect. The 1 per cent

TnBTO treatment has elongated the period over which the various groups of fungi colonise the material from 32 days to 221 days (time at which the first Basidiomycete was isolated). The 5 per cent PCP treatment has only reached the soft rot stage after one year's exposure thus showing considerable superiority over the 1 per cent TnBTO treatment.

4.5.8 Visual observations

Visual observations were carried out on a limited scale to determine the time at which soft rot cavities could first be detected. Observations on first series L-joints had shown cavities to be present in both untreated and 1 per cent TnBTO treated L-joints after one year's exposure and, after 15 months' exposure, to be present throughout one untreated replicate (section 3.5.5).

Radial longitudinal sections were cut from the small sample blocks closest to the joint (position 1) previously used for moisture content etc determinations (fig 4.7). These blocks were originally sited between 3 and 13 mm from the joint. After one year's exposure cavities were found in sections cut from untreated material but were only present at the end originally closest to the joint. The other end showed no cavities. In one replicate, sections were cut subsequently from the sample block No 2; no cavities were observed. This restriction of soft rot attack to the area within 10 mm of the joint surface is very different from that observed in the first series tests where, although attack was heaviest close to the joint, cavities were widely distributed. It was proposed (section 3.5.5) that the distribution of cavities could be associated with nitrogen fixation by bacteria in waterlogged conditions. The moisture contents in the second series have been generally lower and therefore it is less likely that conditions suitable for nitrogen fixing activity have occurred. The occurrence of soft rot close to the joint, where additional nitrogenous nutrients may be available from external sources eg bird droppings, would appear to be the more normal situation; this was the case in joinery from the Chatham site examined by Suhirman (1978).

Soft rot cavities were found in untreated material exposed for 130 days but not after 71 days (table 4.25) although pigmented hyphae characteristic of bluestain organisms were observed. Organisms capable of producing soft rot had been present in the material since before exposure (table 4.21) but, for at least 71 days, had produced no detectable cavities. It is possible this delay may be explained in terms of nutrition. Many organisms will grow through wood producing a bluestain type growth but are also capable of entering the S_2 layer of the cell wall and producing cavities by utilising the cellulose (Krapivina, 1960). In laboratory experiments, an external supply of sugar will discourage cavity formation (Banerjee and Levy, 1970; Kaune, 1970). It is probable, therefore, that cavities are not formed until alternative, more easily accessible, sources of nutrient have been exhausted.

In TnBTO treated material, cavities were present after 375 days but not after 221 days despite the presence of soft rot organisms throughout the exposure period. Although nutritional considerations account for some delay in the onset of cavity formation it seems likely that the TnBTO treatment has caused a further delay, perhaps by retarding penetration of the cell wall. Evidence from transmission electron microscopy (Bravery et al, 1975) shows that an electron dense material, regarded by the authors as TnBTO, is deposited around the lumen during treatment. This layer would need to be breached for the fungi to enter the S_2 layer from the lumen.

In PCP treated material, no cavities were observed after one year's exposure although pigmented hyphae characteristic of bluestain fungi were observed. Soft rot organisms were not isolated in significant numbers from PCP treated material until one year's exposure (table 4.24); the lack of cavities is therefore not unexpected in view of the delay between colonisation and cavity formation which occurred in TnBTO treated material.

4.5.9 Paint film failures

Minor defects in the paint films were noted early in exposure (table 4.26); these were close to the joint and appeared to be a result of swelling of the underlying timber as moisture was taken up. Defects due to fungal activity were observed at different times depending on the preservative treatment. In all cases these were pustules apparently produced by the fungus growing on the underlying wood pushing its way through the paint film. The process appears to have been largely mechanical as shown by the distention of the paint film prior to failure and the torn edges surrounding the holes (fig 4.33); hyphal growth from the pustules across the surface of the paint film occurred at a later stage (fig 4.34).

The first pustules were observed on untreated material after 32 days, on TnBTO treated material after 130 days but not until 375 days on PCP treated material (table 4.26). Both preservative treatments delayed the onset of pustule formation, with the PCP treatment being more effective.

After 71 days exposure, a limited study was undertaken of the organisms responsible for pustule formation on untreated material; this is reported in section 5.5. Unfortunately time was not available for further studies after longer periods of exposure and of preservative treated material. Since the range of organisms occurring within the wood varies between the three treatments (table 4.21) it would seem likely that different organisms are responsible for the paint film failures.

4.5.10 General discussion

Observations on the first series of L-joints reported in section 3 clearly demonstrated the suitability of the L-joint system for studying the process of colonisation of timber exposed out of contact with the ground. The results obtained showed that during the first year many types of organisms had colonised both untreated and 1 per cent TnBTO treated material and that associated physical changes had occurred. The information suggested that the TnBTO treatment was not performing well under the conditions of exposure. This second trial

was therefore undertaken to study, in greater detail, the first year of exposure and to judge the performance of TnBTO by comparison with PCP which has been used in the successful treatment of joinery for many years (Lance, 1958). It has yielded a wealth of information concerning the way in which untreated timber wets up, is rapidly colonised by a wide variety of organisms and undergoes initiation of attack as shown by permeability changes and soft rot cavity formation. The process is modified by both preservative treatments but the data indicate the 1 per cent TnBTO treatment is providing a lower standard of protection than the 5 per cent PCP treatment.

Each organism colonising the L-joints probably plays a significant rôle in the process ultimately leading to failure as a result of decay by Basidiomycetes. After the first series of tests (section 3) it was postulated that bacteria were responsible for the initial increases in permeability and that later in exposure, the fungi could play a more important rôle. If only the parallel data is examined, this second series of tests suggests the same conclusion. The bacteria became established in both untreated and TnBTO treated L-joints early in exposure (fig 4.32) and were present in high numbers, towards the joint end, at the time the first permeability increases were detected (32 days in untreated L-joints, 71 days in TnBTO treated L-joints). However, this reasoning must be questioned in light of the data from the PCP treated L-joints. Bacterial colonisation was only slightly delayed and yet, over the first year of exposure, no increases in permeability, due to microbiological activity, were detected. Ellwood and Ecklund (1959) showed that bacterial action requires a high moisture content, since only the submerged parts of logs became more permeable. This effect was not due to a difference in oxygen tension between the aerial and submerged parts of the logs since Banks and Dearling (1973) showed that permeability changes, due to bacterial action, occurred more rapidly when the test system was aerated. The PCP treatment initially retarded water uptake but over the later stages of exposure the moisture contents were higher and sustained over a longer period than those recorded in untreated and TnBTO treated L-joints during the period when the first permeability increases took place. Therefore, the moisture content should not have limited bacterial activity.

There are various alternative explanations of the results:

a the PCP treatment allows colonisation but prevents attack leading to permeability increases;

b the PCP treatment selects against those bacteria responsible for the permeability increases;

c the fungi, and not the bacteria, are responsible for the permeability increases.

Hypothesis (a) can be examined simply. Preservative applied to Scots pine sapwood by 3 minute immersion has a limited penetration; the central portion of the L-joint members therefore remains untreated. If the PCP treatment does not prevent colonisation, but only prevents attack, then this central portion should show increased permeability. The bacterial counts (section 4.5.5) show that the central portions close to the joint had been colonised by bacteria within the first 43 days exposure. The central portion was sawn out of the remnants of those L-joints exposed for 375 days (fig 4.35), cross-cut into 10 mm lengths which were tested for permeability using the method previously described (section 4.3.4). Since the cross-section of these blocks was smaller than that of the normal blocks used for testing permeability, the results (table 4.27) cannot be compared directly. The mean value for both types of blocks from each treatment has been used to calculate a ratio of treated blocks to untreated blocks (table 4.28). These values are similar for the two block types and indicate the central portion, containing no preservative is protected to the same extent as the normal test blocks which contain preservative; the hypothesis therefore falls.

For confirmation of (b) a comprehensive study of the bacterial species present in the different treatments would be required, together with an investigation of their ability to produce permeability changes in pure culture. Time has not permitted this study.

The third explanation (c), that fungi are responsible for the increase in permeability, is supported by the relative numbers of fungi isolated (table 4.19). Various fungi have been shown to increase permeability including Trichoderma viride (Lindgren, 1952; Unligil, 1969), bluestains (Lindgren and Sheffer, 1939) and Sistotrema brinkmannii (section 6.2), all of which have been isolated from the L-joints.

On the basis of the available information, neither of the two latter alternatives can be discounted although the theory of the selection of non active bacterial types appears unlikely.

Blanchette and Shaw (1978) have shown that bacteria and yeasts will increase the rate of decay by Basidiomycetes in laboratory tests and that in naturally infected material, the organisms are closely associated with one another. However, Jacquiot (1968) has demonstrated antagonistic action by bacteria in some instances. These observations offer alternative rôles for the bacteria.

Bluestain, soft rot and Basidiomycete fungi colonised untreated material in rapid succession; the rate and extent were reduced by preservative treatment. The bluestains, apart from causing failures of the paint film (sections 4.5.9 and 5.5) may be implicated in permeability increases and in detoxification of preservatives (section 6.3). Attack by soft rot fungi is unlikely to directly lead to failure (Savory and Carey, 1979); however, these organisms are generally tolerant to preservatives thus again, detoxification is possible. The Basidiomycetes are responsible for the final destruction of untreated joinery (section 2.2) and the rare cases of failure of preservative treated joinery (PRL, unpublished data). The rate of decay is slow; although a white rot was isolated from an untreated L-joint after only 32 days exposure, there was no obvious decay in L-joints exposed for one year. The entry of Basidiomycetes was delayed by TnBTO and, over the first year, prevented by PCP.

The deterioration process is, therefore, a complex in which a wide range of organisms interact with one another and the substrate, including any preservative present.

TABLE 4.1

Summary of the types of data collected

Exposure period (days)*	Moisture content	CO_2 production	Permeability	Parameter Bacterial** counts	Bacterial streaks	Fungal isolations	Microscopic observations
Unexposed	+	+	+	+	+	+	-
4	+	-	+	+	+	-	-
8	+	+	+	+	+	-	-
11	+	+	+	+	+	+	-
15	+	+	+	+	+	-	-
22	+	+	+	+	+	+	-
32	+	+	+	+	+	+	-
43	+	+	+	+	+	+	-
71	+	+	+	-	+	+	U
130	+	+	+	-	+	+	U
221	+	+	+	-	+	+	UT
375	+	+	+	-	+	+	+

*Calculated from first day of wetting
**One replicate only per treatment
+Three replicates each untreated, 1% TnBTO and 5% PCP treated sampled

U Untreated replicates sampled
T 1 per cent TnBTO treated replicates sampled
- Not sampled

TABLE 4.2

Absorption of dekalin by sample blocks cut from vertical members prior to treatment or painting

L-joint No	Absorption (g)	L-joint No	Absorption (g)	L-joint No	Absorption (g)
11	0.264	61	0.398	110	0.325
12	0.415	62	0.313	111	0.274
13	0.434	63	0.457	112	0.429
14	0.317	64	0.501	113	0.438
15	0.290	65	0.472	114	0.298
16	0.451	66	0.316	115	0.369
17	0.335	67	0.258	116	0.307
18	0.318	68	0.330	117	0.308
19	0.360	70	0.365	118	0.330
20	0.343	71	0.422	119	0.373
21	0.271	72	0.295	120	0.537
22	0.454	88	0.315	121	0.273

Mean 0.360 g Range 0.258-0.537 g

TABLE 4.3

Absorption of 1 per cent TnBTO solution by the L-joints

L-joint No	Absorption of preservative solution (g)		L-joint No	Absorption of preservative solution (g)		L-joint No	Absorption of preservative solution (g)	
	Horizontal	Vertical		Horizontal	Vertical		Horizontal	Vertical
51	7.277	8.158	66	8.469	6.943*	81	7.890	8.624
52	8.664	6.900	67	7.812	7.511*	82	6.277	10.498
53	9.051	8.581	68	8.253	7.185*	83	8.791	8.474
54	7.000	9.221	69	6.642	6.897	84	9.328	10.509
55	7.064	6.764	70	8.469	8.132*	85	6.663	8.882
56	6.687	8.145	71	7.708	9.562*	86	8.343	8.600
57	6.375	7.566	72	8.471	7.144*	87	8.583	7.594
58	6.399	7.005	73	6.920	8.615	88	7.214	6.955*
59	6.981	6.939	74	7.604	8.332	89	8.889	8.360
60	7.848	7.429	75	7.293	9.940	90	18.452	11.239
61	7.272	7.567*	76	6.169	9.441	91	6.418	9.461
62	6.772	7.226*	77	6.666	7.031	92	6.721	11.687
63	7.171	8.259*	78	7.086	7.720	93	6.836	7.231
64	8.140	8.270*	79	8.255	8.095	94	7.809	7.583
65	7.293	7.546*	80	7.011	10.261	95	10.799	7.432

*Sample removed prior to treatment to assess permeability

TABLE 4.4

Absorption of 5 per cent pentachlorophenol solution by the L-joints

L-joint No	Absorption of preservative solution (g)		L-joint No	Absorption of preservative solution (g)		L-joint No	Absorption of preservative solution (g)	
	Horizontal	Vertical		Horizontal	Vertical		Horizontal	Vertical
101	7.331	6.779	116	9.222	8.055*	131	8.848	7.026
102	7.684	9.775	117	8.410	8.663*	132	7.618	10.247
103	7.643	8.656	118	7.442	6.684*	133	7.787	8.650
104	7.973	7.118	119	8.399	7.386*	134	7.137	8.908
105	6.798	6.647	120	7.659	9.616*	135	9.366	8.869
106	7.977	8.769	121	6.996	7.499*	136	8.726	7.865
107	8.000	8.145	122	7.833	8.277	137	8.282	8.597
108	6.638	8.119	123	8.035	8.528	138	19.561	7.547
109	8.418	9.377	124	10.288	9.064	139	8.344	8.916
110	8.927	9.289*	125	8.302	9.814	140	9.254	11.553
111	9.119	8.806*	126	7.722	7.543	141	7.657	7.556
112	6.675	7.593*	127	7.552	9.447	142	6.326	7.318
113	9.040	9.950*	128	9.013	9.553	143	6.259	7.490
114	6.153	7.048*	129	6.978	8.859	144	13.311	15.465
115	7.996	9.300*	130	8.232	8.767	145	6.894	7.523

*Sample removed prior to treatment to assess permeability

TABLE 4.5

Moisture content* of untreated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	1	9.2	9.2	9.2	9.4	9.4	9.5	9.6	9.7	9.8	9.9	9.9	10.0
	2	9.2	9.4	9.5	9.5	9.5	9.6	9.7	9.6	9.8	9.7	9.8	9.9
	3	9.0	9.3	9.3	9.4	9.5	9.5	9.6	9.9	9.9	9.9	10.2	9.7
	Mean	9.1	9.3	9.3	9.4	9.5	9.5	9.6	9.7	9.8	9.8	10.0	9.9
4	10	40.8	39.0	35.7	27.2	19.2	14.8	12.4	11.4	10.9	11.0	10.9	10.4
	11	40.3	38.4	30.8	21.8	15.6	12.9	12.1	11.6	11.6	11.3	11.0	10.5
	12	31.9	24.8	18.9	15.1	12.9	12.1	11.6	11.5	11.4	11.2	11.0	10.4
	Mean	37.7	33.4	28.5	21.4	15.9	13.3	12.0	11.5	11.3	11.2	11.0	10.4
8	13	58.3	47.4	39.3	24.1	15.6	13.4	12.3	11.9	11.8	12.0	11.7	11.9
	14	70.2	62.1	58.4	56.6	46.0	27.5	17.6	15.5	13.3	12.9	13.3	12.7
	15	52.6	43.1	31.1	18.1	14.4	13.7	12.6	11.9	11.8	11.5	11.5	11.2
	Mean	60.4	50.9	42.9	32.9	25.3	18.2	14.2	13.1	12.3	12.1	12.2	11.9
11	16	80.5	56.8	47.7	48.4	39.5	24.8	17.9	15.6	15.8	15.0	13.8	14.4
	17	71.3	55.7	47.7	36.8	26.4	18.5	15.7	13.5	13.8	12.9	12.8	12.5
	18	63.1	61.3	63.7	63.8	60.7	55.5	42.3	30.2	19.7	15.3	14.5	15.6
	Mean	71.6	57.9	53.0	49.7	42.2	32.9	25.3	19.8	16.4	14.4	13.7	14.2
15	19	41.6	38.5	34.3	30.2	25.9	21.8	19.7	17.2	14.3	12.1	11.8	12.3
	20	55.7	46.6	40.9	31.2	21.5	17.9	15.9	17.5	13.9	13.3	13.1	12.8
	21	54.9	52.6	48.3	41.1	27.8	19.3	17.3	14.9	13.5	12.3	12.6	12.1
	Mean	50.7	45.9	41.2	34.2	25.1	19.7	17.6	16.5	13.9	12.6	12.5	12.4
22	22	51.9	55.2	51.9	45.3	41.8	39.8	37.8	30.8	22.9	18.6	17.2	15.4
	23	51.2	53.4	55.8	54.3	52.0	48.4	42.1	31.7	24.0	19.0	16.5	14.6
	24	37.2	34.5	32.6	30.0	27.5	24.6	21.9	18.7	16.3	15.1	13.8	12.3
	Mean	46.8	47.7	46.8	43.2	40.4	37.6	33.9	27.1	21.1	17.6	15.8	14.1
32	25	42.3	41.5	40.1	39.2	38.2	37.1	34.0	29.1	24.2	20.7	18.9	16.4
	26	32.5	32.4	31.5	28.2	24.6	21.0	18.8	17.7	16.7	16.2	15.3	13.7
	27	32.6	32.5	31.3	29.7	26.1	23.1	19.7	17.6	16.6	15.4	14.4	12.6
	Mean	35.8	35.5	34.3	32.4	29.6	27.1	24.2	21.5	19.2	17.4	16.2	14.2
43	28	44.9	33.9	31.6	29.5	26.8	23.0	20.1	18.8	17.6	16.8	15.8	14.6
	29	32.7	31.2	30.4	29.6	28.8	27.1	25.4	23.8	21.3	19.4	17.9	16.2
	30	30.8	29.6	28.8	27.9	26.7	25.4	23.7	22.0	20.0	18.3	17.1	15.9
	Mean	36.1	31.6	30.3	29.0	27.4	25.2	23.1	21.5	19.6	18.2	16.9	15.6
71	31	63.0	56.3	47.9	40.4	35.0	31.7	30.5	29.3	27.8	26.0	24.2	21.9
	32	72.6	62.3	52.3	40.0	31.7	26.6	24.2	21.6	20.0	19.2	18.3	16.8
	33	64.3	54.9	45.3	35.8	30.3	27.7	25.1	22.8	21.2	20.2	19.0	17.3
	Mean	66.6	57.8	48.5	38.7	32.3	28.7	26.6	24.6	23.0	21.8	20.5	18.7
130	34	60.9	57.2	54.9	53.2	51.3	50.3	49.6	46.7	44.6	42.3	39.4	34.0
	35	86.7	65.7	55.2	46.9	40.2	34.0	29.4	26.9	26.2	26.2	25.7	23.7
	36	75.8	59.2	55.1	52.3	50.9	50.2	49.3	47.4	43.5	39.5	37.4	37.3
	Mean	74.5	60.7	55.1	50.8	47.5	44.8	42.8	40.3	38.1	36.0	34.2	31.7
221	37	174.0	157.2	127.3	112.7	107.5	101.2	96.4	91.3	80.8	76.5	71.0	65.8
	38	87.3	84.5	80.5	77.1	74.2	71.4	67.6	63.0	57.7	54.3	49.3	44.2
	39	189.2	168.5	148.7	132.9	126.8	122.1	118.8	113.1	114.9	114.4	115.6	117.7
	Mean	150.2	136.7	108.0	107.6	102.8	98.2	94.3	89.1	84.5	81.7	78.6	75.9
375	40	54.0	62.1	54.9	51.9	51.3	51.6	54.4	52.8	48.5	48.5	49.5	45.1
	41	46.2	49.3	46.5	50.1	46.3	41.6	38.4	36.3	35.1	32.3	29.3	26.9
	42	56.1	55.2	56.3	56.3	48.9	44.4	42.3	40.6	36.5	32.8	29.3	27.4
	Mean	52.1	55.5	52.6	52.8	48.8	45.9	45.0	43.2	40.0	37.9	36.0	33.1

*Expressed as a percentage of the final dry weight
**See Fig 4.7

TABLE 4.6

Moisture content* of 1 per cent TnBTO treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	51	9.5	9.9	10.0	10.2	10.1	10.1	10.2	10.1	10.4	10.3	10.4	10.2
	52	9.1	9.5	9.5	9.5	9.5	9.6	9.8	9.9	10.0	10.2	10.5	10.5
	53	9.7	9.7	9.8	9.8	9.7	9.8	9.7	9.9	10.0	10.0	10.2	10.2
	Mean	9.4	9.7	9.8	9.8	9.8	9.8	9.9	10.0	10.1	10.2	10.4	10.3
4	60	29.7	31.5	27.8	20.6	15.0	12.3	11.5	11.1	10.8	10.9	10.9	9.8
	61	22.7	18.8	14.5	12.1	11.0	10.9	10.6	10.8	10.9	11.0	11.0	10.5
	62	21.8	19.1	15.8	13.4	11.4	10.3	9.8	9.8	9.8	9.8	9.7	9.3
	Mean	24.7	23.1	19.4	15.4	12.5	11.2	10.6	10.6	10.5	10.6	10.5	9.9
8	63	56.6	53.6	50.3	47.3	43.2	36.5	26.7	17.7	13.8	11.8	11.6	11.2
	64	60.3	54.9	52.8	46.8	39.7	35.1	29.6	22.0	15.2	13.0	13.1	12.3
	65	71.1	63.5	57.1	51.7	43.4	31.8	23.8	18.8	14.9	14.5	13.7	13.0
	Mean	62.7	57.3	53.4	48.6	42.1	34.5	26.7	19.5	14.6	13.1	12.8	12.2
11	66	62.0	55.6	57.3	54.7	54.0	50.3	44.7	37.9	24.8	18.6	16.4	14.9
	67	68.1	59.0	58.0	55.6	54.2	51.8	50.1	48.2	45.7	41.2	40.6	37.6
	68	59.9	54.0	49.3	47.8	42.0	35.4	30.6	21.6	17.9	17.1	15.6	14.7
	Mean	63.3	56.2	54.9	52.7	50.1	45.8	41.8	35.9	29.5	25.6	24.2	22.4
15	69	54.2	50.1	46.3	44.4	41.2	33.1	22.3	17.9	15.6	14.2	14.0	13.8
	70	30.5	29.4	25.8	19.5	17.2	14.9	13.7	12.3	11.9	12.1	11.2	11.0
	71	57.2	56.0	53.3	51.0	49.1	47.0	45.6	42.8	37.8	35.4	32.5	23.4
	Mean	47.3	45.2	41.8	38.3	35.8	31.7	27.2	24.3	21.8	20.6	19.2	16.1
22	72	43.9	41.7	39.0	37.0	34.3	25.8	20.1	16.9	14.9	14.3	14.0	12.4
	73	43.3	42.4	38.7	37.1	33.6	29.6	24.4	19.4	16.5	15.9	14.7	13.6
	74	42.7	40.6	37.7	34.0	28.3	25.1	21.3	19.0	17.3	16.5	15.3	13.7
	Mean	43.3	41.6	38.5	36.0	32.1	26.8	21.9	18.4	16.2	15.6	14.7	13.2
32	75	26.9	30.6	33.8	34.1	35.0	34.9	34.4	34.6	32.5	29.6	25.6	21.1
	76	32.8	33.4	33.0	32.4	30.8	26.7	21.5	20.2	18.0	16.7	14.7	13.3
	77	29.5	30.2	30.7	30.0	28.9	27.8	26.0	24.7	23.3	21.5	19.6	17.0
	Mean	29.7	31.4	32.5	32.2	31.6	29.8	27.3	26.5	24.6	22.6	20.0	17.1
43	78	42.3	39.2	38.2	37.1	34.6	31.9	31.6	31.2	30.2	28.9	26.8	22.9
	79	52.5	45.6	37.3	32.8	31.3	28.9	26.8	24.6	22.6	20.6	18.4	16.6
	80	36.1	35.8	34.9	34.2	32.6	30.6	28.8	26.6	24.1	21.6	20.0	18.1
	Mean	43.6	40.2	36.8	34.7	32.8	30.5	29.1	27.5	25.6	23.7	21.7	19.2
71	81	58.1	48.1	40.7	33.7	27.4	23.8	21.3	21.0	20.2	19.8	18.9	17.5
	82	56.5	45.6	39.8	32.7	29.1	26.4	24.4	22.9	22.0	20.7	20.1	18.1
	83	56.2	47.2	43.1	38.6	33.3	29.1	25.5	24.1	23.0	21.7	20.8	18.9
	Mean	56.9	47.0	41.2	35.0	29.9	26.4	23.7	22.7	21.7	20.7	19.9	18.2
130	84	75.9	73.7	70.4	63.6	56.8	52.3	47.7	42.6	38.9	36.0	34.3	31.5
	85	64.6	54.2	45.6	42.1	40.0	37.5	34.8	30.8	27.0	24.5	22.4	20.0
	86	114.8	118.4	107.1	92.4	83.7	75.7	70.5	64.9	59.3	54.6	47.9	45.9
	Mean	85.1	82.1	74.4	66.0	60.2	55.2	51.0	46.1	41.7	38.4	35.5	32.5
221	87	154.0	140.2	133.9	121.1	106.2	98.3	90.7	85.0	78.0	74.7	71.2	72.3
	88	108.0	113.8	112.5	100.5	91.0	81.0	75.8	72.1	69.4	65.5	62.0	60.0
	89	127.7	111.1	94.6	91.0	84.0	80.0	76.9	75.5	75.2	74.3	74.3	72.7
	Mean	129.9	121.7	113.7	104.2	93.7	84.9	81.1	77.5	74.2	71.5	69.2	68.3
375	90†	131.4	119.5	101.9	92.3	87.1	84.2	83.8	83.1	82.0	82.4	84.2	87.7
	91	100.1	114.5	111.6	107.5	109.7	105.3	97.5	91.6	88.2	85.8	82.5	77.3
	92	39.4	75.2	81.6	71.9	68.2	65.2	60.6	56.9	53.4	49.6	47.3	45.6
	Mean	69.8	94.9	86.6	89.7	89.0	85.3	79.1	74.3	70.8	67.7	64.9	61.5

*Expressed as a percentage of the final dry weight

**See Fig 4.7

†Values not included in mean (see text)

TABLE 4.7

Moisture contents* of 5 per cent PCP treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	101	10.4	9.7	9.8	9.8	9.9	9.9	10.0	10.1	10.2	10.4	10.6	10.6
	102	10.5	10.0	10.0	10.0	10.1	10.2	10.3	10.5	10.7	10.8	10.9	10.9
	103	10.0	9.9	9.8	10.0	10.1	10.3	10.5	10.7	10.8	10.9	11.0	11.2
	Mean	10.3	9.9	9.9	9.9	10.0	10.1	10.3	10.4	10.6	10.7	10.8	10.9
4	110	35.4	35.8	32.4	23.1	16.0	13.1	12.3	11.6	12.1	11.5	11.2	10.8
	111	27.2	26.7	20.6	15.4	11.8	11.7	12.0	11.7	11.4	11.2	11.1	10.6
	112	23.8	18.9	14.9	12.9	12.2	12.0	11.4	11.6	11.7	11.5	11.3	10.6
	Mean	28.8	27.1	22.6	17.1	13.3	12.3	11.9	11.6	11.7	11.4	11.2	10.7
8	113	50.2	43.3	35.6	28.4	18.2	14.7	13.8	15.1	15.6	13.4	13.7	13.1
	114	32.9	27.4	22.5	18.5	14.6	12.9	12.3	12.0	12.0	12.3	11.8	13.0
	115	48.2	43.5	38.3	27.1	17.6	15.2	13.7	13.3	12.8	12.9	13.1	12.8
	Mean	43.8	38.1	32.1	24.7	16.8	14.3	13.3	13.5	13.5	12.9	12.9	13.0
11	116	56.9	46.3	35.3	21.7	17.9	15.9	15.8	14.3	14.0	13.7	14.7	13.9
	117	51.1	40.0	31.2	25.6	21.8	17.7	15.8	16.6	14.8	15.5	14.9	14.2
	118	42.9	36.0	29.7	22.3	17.4	15.0	14.1	14.2	13.2	13.4	13.7	13.6
	Mean	50.3	40.8	32.1	23.2	19.0	16.2	15.2	15.0	14.0	14.2	14.4	13.9
15	119	36.9	36.0	32.3	24.8	20.9	17.7	15.2	13.7	13.3	12.9	12.3	11.8
	120	49.8	47.6	47.7	46.2	42.0	36.0	30.3	25.9	24.0	21.1	17.1	15.1
	121	33.1	22.1	17.5	15.4	13.5	12.7	12.2	12.0	11.8	11.8	12.0	12.0
	Mean	39.9	35.2	32.5	28.8	25.5	22.1	19.2	17.2	16.4	15.3	13.8	13.0
22	122	35.2	33.7	31.2	27.6	23.0	19.7	17.6	15.9	14.7	14.2	14.3	13.3
	123	32.9	31.9	28.4	24.4	20.7	18.2	16.1	15.2	14.5	14.3	13.4	13.0
	124	46.7	46.6	44.4	41.5	39.2	36.9	33.9	28.6	23.6	19.9	18.3	16.7
	Mean	38.3	37.4	34.7	31.2	27.6	24.9	22.5	19.9	17.6	16.1	15.3	14.3
32	125	29.6	31.1	32.5	32.1	30.7	28.5	25.3	21.6	19.7	18.5	17.1	15.5
	126	23.0	23.7	23.0	21.1	18.7	17.7	16.4	15.9	15.5	15.1	14.9	13.7
	127	26.8	27.8	28.2	26.9	24.3	21.4	19.0	17.8	17.1	16.4	15.9	14.9
	Mean	26.5	27.5	27.9	26.7	24.6	22.5	20.2	18.4	17.4	16.7	16.0	14.7
43	128	33.9	31.1	29.1	28.6	28.3	27.2	26.1	24.2	22.1	20.1	18.6	17.1
	129	43.4	40.5	38.5	35.0	30.0	25.5	23.7	21.6	19.3	18.0	17.2	16.3
	130	43.7	41.0	36.4	31.3	27.6	24.8	23.3	20.9	19.3	17.7	17.7	16.4
	Mean	40.3	37.5	34.7	31.6	28.6	25.8	24.4	22.2	20.2	18.6	17.8	16.6
71	131	51.6	42.4	35.9	32.2	29.2	26.7	23.4	21.4	20.3	19.7	19.0	17.8
	132	58.0	50.2	44.8	39.1	31.6	27.2	25.9	24.5	23.2	22.0	20.7	18.6
	133	53.1	36.4	29.0	25.4	22.9	20.2	18.7	19.0	18.1	17.9	16.9	16.0
	Mean	54.2	43.0	36.6	32.2	27.9	24.7	22.7	21.6	20.5	19.9	18.9	17.5
130	134	76.8	67.0	63.5	60.7	56.6	51.4	43.6	35.6	28.0	22.7	20.8	19.5
	135	58.6	53.8	50.9	46.5	43.2	41.4	40.0	39.0	37.8	36.0	34.9	33.9
	136	55.4	45.5	39.3	37.1	37.5	32.9	24.9	23.2	22.0	19.9	19.4	17.9
	Mean	63.6	55.4	51.2	48.1	45.8	41.9	36.2	32.6	29.3	26.2	25.0	23.8
221	137	95.5	100.2	106.0	108.9	107.6	105.8	103.8	93.3	81.3	66.7	46.3	32.0
	138†	149.9	151.3	154.1	151.9	146.6	141.4	138.8	132.4	130.2	128.1	130.1	130.5
	139	106.1	95.4	93.0	89.5	91.4	93.0	79.6	68.7	56.8	45.9	35.9	27.9
	Mean	100.8	97.8	99.5	99.2	99.5	99.4	91.7	81.0	69.1	56.3	41.1	30.0
375	140	115.5	103.1	89.6	82.7	83.3	81.0	75.7	69.7	63.4	56.4	50.9	47.2
	141	34.1	62.5	59.0	53.4	53.0	50.6	48.2	45.4	44.3	44.6	42.8	37.3
	142	63.9	108.6	108.1	93.4	94.9	93.3	87.4	78.7	70.8	63.8	55.5	51.1
	Mean	71.2	91.4	85.6	76.5	77.1	75.0	70.4	64.6	59.5	54.9	49.7	45.2

*Expressed as a percentage of the final dry weight

**See Fig 4.7

†Values not included in mean (see text)

TABLE 4.8

CO_2 production* by untreated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0†	1	3.33	3.36	3.31	3.18	3.22	3.24	3.30	3.19	3.23	3.16	3.18	3.09
	2	3.25	3.20	3.15	3.16	3.17	3.14	3.16	3.04	2.99	3.09	3.14	2.88
	3	3.18	3.15	3.22	3.14	3.15	3.12	3.12	3.18	3.12	3.16	3.13	3.23
	Mean	3.25	3.24	3.23	3.17	3.18	3.17	3.19	3.14	3.11	3.14	3.15	3.07
8	13	+0.72	+0.72	+0.36	+0.38	+0.10	+0.14	+0.25	+0.14	0.33	0.59	0.63	0.53
	14	0.98	0.75	0.61	0.64	0.57	1.00	0.80	0.86	1.06	0.76	1.07	1.09
	15	+0.25	+0.35	+0.37	+0.37	+0.32	+0.29	+0.45	+0.23	+0.13	+0.23	+0.15	+0.07
	Mean	0.00	+0.11	+0.04	+0.04	0.05	0.19	0.03	0.16	0.42	0.37	0.52	0.52
11	16	1.10	0.05	+0.08	+0.02	0.10	0.05	0.15	0.11	0.11	0.09	0.08	-
	17	0.42	0.10	0.20	0.25	0.21	0.20	0.12	0.17	0.22	0.18	0.29	0.15
	18	-	+0.09	+0.19	+0.11	+0.09	+0.10	+0.06	+0.01	+0.03	+0.10	+0.08	+0.02
	Mean	0.76	0.02	+0.02	0.04	0.07	0.05	0.07	0.09	0.10	0.06	0.10	0.07
15	19	+0.04	+0.20	+0.16	+0.18	+0.07	+0.09	+0.06	+0.06	+0.03	+0.10	+0.01	+0.06
	20	0.50	+0.07	+0.12	0.03	0.11	0.06	0.12	0.08	0.16	0.16	-	0.09
	21	0.30	+0.19	+0.03	+0.15	-	+0.07	0.10	0.04	0.10	0.05	0.07	0.00
	Mean	0.25	+0.15	+0.10	+0.10	0.02	+0.03	0.05	0.02	0.08	0.04	0.03	0.01
22	22	0.75	0.75	0.75	0.35	0.25	0.37	0.68	0.66	0.62	0.32	0.04	0.04
	23	0.67	0.65	0.72	0.29	0.39	0.30	0.35	0.34	0.33	0.38	0.45	0.35
	24	0.22	0.08	0.00	0.04	0.02	0.08	0.10	0.24	0.14	0.18	0.50	0.15
	Mean	0.55	0.49	0.49	0.23	0.22	0.25	0.38	0.41	0.36	0.29	0.33	0.18
32	25	1.44	0.84	0.63	0.09	+0.10	+0.11	+0.01	+0.03	0.05	+0.03	0.00	+0.06
	26	0.14	0.06	0.09	0.05	0.05	0.13	0.15	0.13	0.14	0.16	0.23	0.19
	27	0.24	0.15	0.22	0.31	0.13	+0.03	0.02	0.08	0.08	0.00	+0.01	0.04
	Mean	0.61	0.35	0.31	0.15	0.03	0.00	0.05	0.06	0.09	0.04	0.07	0.06
43	28	0.12	0.00	+0.08	0.01	0.25	+0.07	0.00	+0.08	+0.08	0.10	0.04	0.03
	29	0.32	+0.20	0.83	0.10	0.28	0.36	0.22	0.41	0.50	0.46	+0.14	0.97
	30	0.22	0.11	0.14	0.12	0.46	0.57	0.21	0.13	0.25	0.28	0.24	0.10
	Mean	0.22	+0.03	0.30	0.08	0.33	0.29	0.14	0.15	0.22	0.28	0.05	0.37
71	31	0.45	0.20	0.41	0.48	0.36	0.10	0.08	0.08	0.21	0.05	0.16	0.34
	32	0.73	0.45	0.27	0.28	0.40	0.36	0.56	0.32	0.16	0.16	0.19	0.17
	33	0.49	0.40	0.47	0.49	0.44	0.55	0.61	0.58	0.31	0.04	0.13	-
	Mean	0.56	0.35	0.38	0.42	0.40	0.34	0.42	0.33	0.23	0.08	0.16	0.26
130	34	0.52	0.85	0.85	0.97	1.09	1.09	1.39	1.49	1.40	1.49	1.54	1.59
	35	0.73	0.53	0.60	0.78	0.76	0.84	0.69	0.53	0.43	0.48	0.53	0.83
	36	0.50	0.13	0.12	0.11	0.10	0.14	0.17	0.21	0.21	0.27	0.39	0.49
	Mean	0.58	0.50	0.52	0.62	0.65	0.69	0.75	0.74	0.68	0.75	0.82	0.97
221	37	1.02	0.51	0.42	0.51	0.56	0.62	0.70	0.69	0.67	0.77	0.82	0.77
	38	0.77	0.87	0.95	1.23	1.38	1.41	1.57	1.44	1.38	1.52	1.49	1.17
	39	0.97	0.58	0.46	0.48	0.50	0.73	0.73	0.62	0.82	0.76	0.81	0.67
	Mean	0.92	0.65	0.61	0.74	0.81	0.92	1.00	0.92	0.96	1.02	1.04	0.87
375	40	0.02	0.01	+0.05	+0.03	+0.10	+0.04	0.14	0.08	0.10	0.05	0.13	0.17
	41	0.15	0.09	+0.01	0.02	0.07	0.07	0.11	0.10	0.19	0.30	0.37	0.34
	42	0.47	+0.01	0.10	+0.06	0.10	0.09	0.12	0.05	0.09	0.19	0.24	0.22
	Mean	0.21	0.03	0.01	0.02	0.02	0.04	0.12	0.08	0.13	0.18	0.25	0.24

*CO_2 produced is expressed as ml 0.05 N HCl (the difference between test values and controls containing dry blocks)

**See Fig 4.7

†ml 0.05 N HCl required to neutralise the residual NaOH (not corrected using controls)

+Negative production of CO_2

-No result

TABLE 4.9

CO_2 production* by 1 per cent TnBTO treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0†	51	3.20	3.10	3.13	3.21	3.05	3.13	3.09	3.22	3.11	3.07	3.02	2.92
	52	3.37	3.26	3.29	3.20	-	3.25	3.25	3.20	3.27	3.15	3.10	3.18
	53	3.12	3.18	3.20	3.12	3.20	3.10	3.01	3.09	3.05	3.05	3.09	3.01
	Mean	3.23	3.18	3.21	3.18	3.13	3.16	3.12	3.17	3.14	3.09	3.07	3.04
8	63	+0.13	+0.01	+0.12	+0.06	+0.05	0.08	0.22	0.00	0.23	0.34	0.28	0.31
	64	+0.67	+0.62	+0.59	+0.59	+0.45	+0.51	+0.51	+0.42	+0.29	+0.41	+0.44	+0.36
	65	+0.32	+0.27	+0.28	0.43	+0.01	+0.08	0.14	+0.03	0.23	0.25	0.25	0.34
	Mean	+0.37	+0.30	+0.31	+0.07	+0.17	+0.17	+0.05	+0.15	0.06	0.06	0.03	0.10
11	66	+0.02	+0.06	+0.05	+0.06	+0.06	0.00	+0.03	0.00	+0.05	0.05	0.09	0.03
	67	0.38	0.00	+0.01	+0.09	+0.06	+0.10	+0.04	+0.04	+0.07	+0.05	+0.07	+0.10
	68	0.01	+0.03	+0.05	0.01	0.08	0.11	0.05	0.15	0.17	0.15	0.16	0.04
	Mean	0.12	+0.03	+0.04	+0.05	+0.01	0.00	+0.01	0.04	0.02	0.05	0.06	+0.01
15	69	0.29	0.16	0.23	0.07	0.28	0.27	0.24	0.28	0.47	0.50	0.33	0.36
	70	+0.10	+0.13	+0.13	+0.13	+0.06	+0.06	+0.11	+0.09	0.02	+0.05	+0.09	+0.08
	71	0.48	0.13	0.04	+0.04	+0.05	+0.03	+0.03	+0.06	0.29	0.11	0.18	0.10
	Mean	0.22	0.05	0.05	+0.03	0.06	0.06	0.03	0.04	0.26	0.19	0.14	0.13
22	72	0.35	0.29	0.12	0.04	0.11	0.14	0.13	0.16	0.21	0.18	0.23	0.18
	73	0.19	0.10	+0.02	+0.05	+0.02	0.04	0.06	0.10	0.03	0.08	0.08	0.09
	74	0.51	0.10	0.04	0.11	0.10	0.14	0.16	0.19	0.14	0.21	0.16	0.25
	Mean	0.35	0.16	0.05	0.03	0.06	0.11	0.12	0.15	0.13	0.16	0.16	0.17
32	75	+0.07	+0.14	+0.07	+0.04	+0.06	+0.02	+0.01	+0.04	0.05	0.06	0.02	0.01
	76	0.09	+0.10	+0.05	0.02	0.09	0.08	0.05	0.37	0.14	0.11	0.20	0.18
	77	0.04	0.04	0.03	+0.14	0.11	0.04	0.07	0.08	0.08	0.19	0.13	0.13
	Mean	0.02	+0.07	+0.03	+0.05	0.05	0.03	0.04	0.14	0.09	0.12	0.12	0.11
43	78	0.44	0.12	0.19	0.20	0.22	0.26	0.58	0.19	0.28	0.28	0.33	0.34
	79	0.15	0.07	+0.06	+0.03	+0.11	+0.10	+0.01	+0.10	+0.08	+0.15	+0.04	+0.03
	80	+0.02	+0.03	+0.03	+0.08	0.00	+0.11	+0.02	+0.02	0.00	0.04	0.05	0.07
	Mean	0.19	0.05	0.03	0.03	0.04	0.02	0.18	0.02	0.07	0.06	0.11	0.13
71	81	0.32	0.30	0.46	0.94	0.88	0.40	0.13	0.14	0.09	0.16	0.18	0.10
	82	0.25	0.08	0.19	0.36	0.29	0.19	+0.01	+0.02	0.00	+0.06	+0.01	0.02
	83	0.29	0.35	0.30	0.38	0.01	0.08	0.06	0.09	0.05	0.14	0.12	0.20
	Mean	0.29	0.24	0.32	0.56	0.39	0.22	0.06	0.07	0.05	0.08	0.10	0.11
130	84	0.59	0.49	0.47	0.39	0.44	0.46	0.47	0.64	0.59	0.36	0.38	0.19
	85	0.14	0.00	0.11	+0.10	0.04	0.15	0.19	0.25	0.35	0.28	-	+0.06
	86	0.67	0.40	0.20	0.04	0.09	0.27	0.19	0.34	0.47	0.50	0.65	0.27
	Mean	0.47	0.30	0.26	0.11	0.19	0.29	0.28	0.41	0.47	0.38	0.52	0.13
221	87	0.67	0.52	0.52	0.51	0.35	0.45	0.52	0.71	0.59	0.75	0.77	0.55
	88	0.71	0.74	0.81	0.83	0.76	0.91	1.02	1.10	-	0.97	1.09	0.65
	89	1.18	0.76	0.79	0.91	1.08	1.01	1.45	1.37	1.42	1.50	1.34	0.81
	Mean	0.85	0.67	0.71	0.75	0.73	0.79	1.00	1.06	1.01	1.07	1.07	0.67
375	90	0.24	0.10	0.14	0.12	0.12	0.15	0.12	0.06	0.18	0.22	0.22	0.27
	91	0.20	+0.04	+0.01	+0.07	0.07	0.07	0.10	+0.01	+0.07	0.01	0.07	0.12
	92	0.14	0.20	0.12	0.12	0.30	0.26	0.25	0.27	0.27	0.22	0.27	0.21
	Mean	0.19	0.09	0.08	0.06	0.16	0.16	0.16	0.11	0.13	0.15	0.19	0.20

*CO_2 produced is expressed as ml 0.05 N HCl (the difference between test values and controls containing dry blocks)

**See Fig 4.7

†ml 0.05 N HCl required to neutralise the residual NaOH (not corrected using controls)

\+ Negative production of CO_2

\- No result

TABLE 4.10

CO_2 production* by 5 per cent PCP treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0†	101	3.35	3.21	3.37	3.22	3.40	3.08	3.29	3.21	3.30	3.16	3.24	3.20
	102	3.25	3.15	3.21	3.19	3.23	3.18	3.20	3.14	3.10	3.16	3.10	3.00
	103	3.20	3.16	-	3.25	3.26	3.22	3.20	3.38	3.37	3.09	3.14	3.02
	Mean	3.30	3.17	3.29	3.22	3.30	3.16	3.23	3.24	3.22	3.14	3.16	3.07
8	113	+0.67	+0.72	+0.63	+0.66	+0.67	+0.57	+0.52	+0.45	+0.52	+0.40	+0.30	+0.15
	114	+0.31	+0.06	+0.10	+0.11	+0.07	+0.09	0.17	0.19	0.42	0.34	0.32	0.50
	115	+0.83	+0.85	+0.72	+0.54	+0.59	+0.61	+0.63	+0.47	+0.48	+0.42	+0.52	+0.43
	Mean	+0.60	+0.55	+0.48	+0.44	+0.40	+0.36	+0.33	+0.24	+0.19	+0.16	+0.17	+0.03
11	116	+0.15	+0.15	+0.03	0.03	0.04	+0.05	+0.03	+0.01	0.05	+0.20	0.04	0.05
	117	+0.05	0.19	0.01	0.00	0.05	0.13	0.12	0.04	0.16	0.17	0.21	0.11
	118	+0.09	+0.04	0.03	0.01	+0.02	+0.01	0.01	+0.02	0.00	+0.03	0.10	0.04
	Mean	+0.10	0.00	0.00	0.01	0.02	0.02	0.03	0.00	0.07	+0.02	0.12	0.07
15	119	+0.08	+0.09	+0.07	0.00	+0.02	0.07	0.01	0.07	0.02	0.09	+0.03	0.03
	120	0.03	+0.02	+0.09	+0.01	0.13	0.13	0.07	0.13	0.19	0.28	0.18	0.52
	121	-	+0.02	+0.17	+0.05	+0.10	+0.08	+0.09	+0.06	0.01	0.03	0.03	0.03
	Mean	+0.02	+0.04	+0.11	+0.02	0.00	0.04	0.00	0.05	0.07	0.13	0.06	0.19
22	122	+0.06	+0.13	+0.06	0.00	+0.03	+0.08	0.01	0.01	+0.01	+0.03	0.03	0.05
	123	+0.05	+0.05	0.00	0.07	0.01	0.03	0.04	0.04	+0.04	0.06	0.09	0.11
	124	0.00	+0.04	+0.05	+0.03	0.02	0.02	+0.01	0.06	0.05	0.05	0.05	0.15
	Mean	+0.04	+0.07	+0.04	0.01	0.00	+0.01	0.01	0.04	0.00	0.03	0.06	0.10
32	125	0.03	+0.05	+0.04	0.06	0.05	0.04	0.20	0.18	0.14	0.05	0.32	0.17
	126	0.12	0.14	0.11	0.15	0.24	0.24	0.24	0.29	0.20	0.30	0.37	0.35
	127	+0.06	+0.06	0.00	+0.02	0.00	0.06	0.08	0.05	+0.02	0.05	0.09	0.14
	Mean	0.03	0.01	0.02	0.06	0.10	0.11	0.17	0.17	0.14	0.13	0.26	0.22
43	128	+0.23	+1.38	+0.33	+0.28	+0.07	0.00	+0.07	+0.03	+0.23	0.00	+0.01	+0.03
	129	+0.15	0.09	0.11	0.42	0.21	0.13	0.09	0.05	0.20	0.67	+0.51	0.18
	130	0.01	+0.03	+0.03	+0.14	+0.81	0.51	0.06	+0.03	0.02	0.02	0.17	0.32
	Mean	+0.12	+0.44	+0.08	0.00	+0.22	0.21	0.03	0.00	0.00	0.23	+0.12	0.16
71	131	0.20	0.09	+0.01	0.02	0.00	+0.10	+0.05	+0.04	+0.06	+0.02	0.02	0.29
	132	0.41	0.36	0.40	0.35	0.27	0.04	0.08	0.08	-	0.08	0.24	0.20
	133	0.13	0.07	+0.03	+0.10	0.00	+0.07	0.03	+0.02	0.05	+0.07	+0.04	0.23
	Mean	0.25	0.17	0.12	0.09	0.09	+0.04	0.02	0.01	+0.01	0.00	0.07	0.24
130	134	0.16	0.11	0.08	0.25	0.20	0.30	0.36	0.14	+0.06	+0.04	+0.02	+0.02
	135	0.34	0.16	0.23	0.10	0.22	0.21	0.20	0.33	0.41	0.14	0.29	0.41
	136	0.09	+0.03	0.00	0.04	0.17	0.17	+0.06	+0.09	0.00	+0.01	+0.04	0.00
	Mean	0.20	0.08	0.10	0.13	0.20	0.23	0.17	0.13	0.12	0.03	0.08	0.13
221	137	0.16	0.11	0.18	0.30	0.32	0.42	0.68	0.81	0.76	0.69	0.70	0.34
	138	0.45	0.41	0.33	0.37	0.39	0.35	0.32	0.42	0.37	0.32	0.37	0.44
	139	0.27	0.15	0.21	0.33	0.46	0.66	0.96	0.77	0.79	0.85	0.60	+0.01
	Mean	0.29	0.22	0.24	0.33	0.39	0.48	0.65	0.67	0.64	0.62	0.56	0.26
375	140	0.23	0.07	0.22	0.27	0.37	0.41	0.58	0.42	0.67	0.84	1.07	1.00
	141	+0.03	0.10	0.01	0.10	0.12	0.18	0.21	0.13	0.26	0.18	0.12	0.17
	142	0.15	0.33	0.11	0.10	0.12	0.00	0.42	0.32	0.28	0.25	0.28	0.33
	Mean	0.12	0.17	0.11	0.16	0.20	0.20	0.40	0.29	0.40	0.42	0.49	0.50

*CO_2 produced is expressed as ml 0.05 N HCl (the difference between test values and controls containing dry blocks)

**See Fig 4.7

†ml 0.05 N HCl required to neutralise the residual NaOH (not corrected using controls)

+ Negative production of CO_2

- No result

TABLE 4.11

Permeability* of untreated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	1	0.344	0.341	0.349	0.350	0.356	0.341	0.355	0.347	0.338	0.332	0.330	0.318
	2	0.330	0.295	0.317	0.326	0.315	0.335	0.332	0.325	0.335	0.343	0.340	0.351
	3	0.318	0.325	0.322	0.305	0.318	0.329	0.316	0.324	0.317	0.311	0.325	0.304
	Mean	0.331	0.320	0.329	0.327	0.330	0.335	0.334	0.332	0.330	0.329	0.332	0.324
4	10	0.405	0.402	0.409	0.352	0.312	0.289	0.294	0.282	0.289	0.277	0.298	0.292
	11	0.463	0.455	0.433	0.368	0.330	0.296	0.298	0.294	0.300	0.262	0.283	0.286
	12	0.253	0.256	0.255	0.236	0.239	0.233	0.226	0.244	0.227	0.239	0.229	0.247
	Mean	0.374	0.371	0.366	0.319	0.294	0.273	0.273	0.273	0.272	0.259	0.270	0.275
8	13	0.419	0.403	0.395	0.345	0.315	0.311	0.325	0.331	0.328	0.329	0.331	0.327
	14	0.493	0.534	0.540	0.542	0.504	0.458	0.388	0.383	0.391	0.417	0.419	0.443
	15	0.394	0.385	0.394	0.330	0.311	0.330	0.337	0.346	0.354	0.336	0.345	0.376
	Mean	0.435	0.441	0.443	0.406	0.377	0.366	0.350	0.353	0.358	0.361	0.365	0.382
11	16	0.501	0.469	0.470	0.455	0.435	0.368	0.322	0.332	0.342	0.303	0.323	0.338
	17	0.427	0.417	0.410	0.413	0.367	0.353	0.319	0.322	0.311	0.296	0.300	0.295
	18	0.534	0.572	0.568	0.555	0.527	0.529	0.549	0.519	0.451	0.482	0.497	0.499
	Mean	0.487	0.486	0.483	0.474	0.443	0.417	0.397	0.391	0.368	0.360	0.373	0.377
15	19	0.407	0.406	0.400	0.390	0.385	0.363	0.353	0.363	0.333	0.343	0.334	0.303
	20	0.478	0.462	0.451	0.438	0.377	0.355	0.361	0.377	0.379	0.396	0.448	0.396
	21	0.442	0.450	0.463	0.440	0.401	0.355	0.357	0.363	0.362	0.357	0.362	0.359
	Mean	0.442	0.439	0.438	0.423	0.388	0.358	0.357	0.368	0.359	0.365	0.381	0.353
22	22	0.541	0.552	0.546	0.552	0.548	0.534	0.537	0.508	0.464	0.430	0.428	0.432
	23	0.677	0.646	0.642	0.631	0.641	0.654	0.661	0.612	0.570	0.538	0.552	0.538
	24	0.430	0.407	0.507	0.467	0.461	0.433	0.415	0.404	0.416	0.400	0.392	0.383
	Mean	0.549	0.535	0.565	0.550	0.550	0.540	0.538	0.508	0.498	0.456	0.457	0.451
32	25	0.730	0.561	0.505	0.494	0.481	0.468	0.470	0.455	0.429	0.431	0.419	0.433
	26	0.556	0.529	0.527	0.530	0.506	0.503	0.489	0.497	0.500	0.496	0.500	0.493
	27	0.563	0.577	0.571	0.560	0.520	0.523	0.530	0.516	0.523	0.510	0.525	0.551
	Mean	0.616	0.556	0.534	0.528	0.502	0.498	0.496	0.489	0.484	0.479	0.481	0.492
43	28	0.574	0.611	0.603	0.602	0.596	0.592	0.582	0.549	0.532	0.572	0.569	0.565
	29	0.418	0.448	0.453	0.452	0.439	0.446	0.447	0.440	0.412	0.421	0.409	0.395
	30	0.473	0.481	0.484	0.462	0.439	0.440	0.437	0.422	0.410	0.396	0.390	0.396
	Mean	0.488	0.513	0.513	0.505	0.491	0.493	0.489	0.470	0.451	0.463	0.456	0.452
71	31	0.962	1.005	1.065	0.976	0.894	0.732	0.651	0.591	0.566	0.546	0.584	0.649
	32	0.717	0.566	0.509	0.473	0.420	0.419	0.413	0.409	0.388	0.388	0.416	0.419
	33	0.785	0.792	0.774	0.681	0.606	0.555	0.492	0.487	0.492	0.510	0.543	0.585
	Mean	0.821	0.788	0.783	0.710	0.640	0.569	0.519	0.496	0.482	0.481	0.514	0.551
130	34	1.258	1.261	1.291	1.297	1.272	1.239	1.192	1.168	1.082	1.021	0.930	0.768
	35	0.873	0.739	0.755	0.797	0.721	0.644	0.549	0.465	0.426	0.413	0.391	0.359
	36	1.224	1.279	1.358	1.282	1.279	1.273	1.272	1.257	1.288	1.301	1.239	1.145
	Mean	1.118	1.093	1.135	1.125	1.091	1.052	1.004	0.963	0.932	0.912	0.853	0.757
221	37	1.656	1.591	1.561	1.620	1.563	1.504	1.516	1.502	1.526	1.424	1.393	1.361
	38	0.769	0.768	0.835	0.905	0.880	0.866	0.816	0.763	0.648	0.564	0.522	0.505
	39	1.575	1.436	1.460	1.528	1.578	1.568	1.615	1.652	1.591	1.623	1.645	1.611
	Mean	1.333	1.265	1.285	1.351	1.340	1.313	1.316	1.306	1.255	1.204	1.187	1.159
375	40	1.449	1.186	1.071	1.059	0.906	0.811	0.737	0.691	0.700	0.645	0.688	0.660
	41	1.009	0.846	0.825	0.810	0.795	0.804	0.800	0.730	0.677	0.707	0.710	0.718
	42	1.532	1.536	1.553	1.386	1.433	1.335	1.294	1.360	1.370	1.220	1.157	1.113
	Mean	1.330	1.189	1.150	1.085	1.045	0.983	0.944	0.927	0.916	0.857	0.852	0.830

*Expressed as uptake of dekalin (g)

**See Fig 4.7

TABLE 4.12

Permeability* of 1 per cent TnBTO treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	51	0.344	0.340	0.341	0.346	0.336	0.338	0.325	0.333	0.325	0.344	0.331	0.337
	52	0.269	0.308	0.322	0.317	0.315	0.297	0.329	0.340	0.319	0.334	0.325	0.333
	53	0.286	0.267	0.265	0.278	0.283	0.279	0.287	0.288	0.300	0.309	0.324	0.318
	Mean	0.300	0.305	0.309	0.314	0.311	0.305	0.314	0.320	0.315	0.329	0.327	0.329
4	60	0.456	0.425	0.407	0.342	0.328	0.308	0.323	0.317	0.305	0.326	0.319	0.318
	61	0.416	0.345	0.270	0.238	0.224	0.215	0.215	0.225	0.241	0.239	0.245	0.243
	62	0.295	0.280	0.260	0.221	0.222	0.212	0.224	0.233	0.252	0.245	0.269	0.261
	Mean	0.389	0.350	0.312	0.267	0.258	0.245	0.254	0.258	0.266	0.270	0.278	0.274
8	63	0.468	0.496	0.500	0.507	0.481	0.475	0.469	0.442	0.420	0.431	0.428	0.420
	64	0.565	0.600	0.597	0.585	0.576	0.550	0.521	0.506	0.472	0.467	0.471	0.483
	65	0.385	0.408	0.414	0.413	0.416	0.384	0.380	0.369	0.371	0.365	0.346	0.360
	Mean	0.473	0.501	0.504	0.502	0.491	0.470	0.457	0.439	0.421	0.421	0.415	0.421
11	66	0.621	0.663	0.658	0.689	0.695	0.692	0.661	0.681	0.601	0.608	0.634	0.620
	67	0.568	0.589	0.578	0.585	0.570	0.580	0.576	0.585	0.608	0.604	0.602	0.598
	68	0.535	0.537	0.531	0.537	0.529	0.525	0.505	0.472	0.459	0.447	0.443	0.426
	Mean	0.575	0.596	0.589	0.604	0.598	0.599	0.581	0.579	0.556	0.553	0.560	0.548
15	69	0.390	0.389	0.402	0.380	0.368	0.363	0.334	0.311	0.318	0.313	0.322	0.325
	70	0.430	0.471	0.464	0.454	0.414	0.410	0.412	0.413	0.409	0.415	0.430	0.424
	71	0.553	0.587	0.571	0.563	0.560	0.550	0.549	0.551	0.550	0.536	0.521	0.452
	Mean	0.458	0.482	0.479	0.466	0.447	0.441	0.432	0.425	0.426	0.421	0.424	0.400
22	72	0.578	0.616	0.607	0.606	0.585	0.539	0.520	0.492	0.464	0.461	0.505	0.506
	73	0.424	0.449	0.457	0.454	0.447	0.433	0.418	0.412	0.382	0.371	0.380	0.370
	74	0.441	0.445	0.433	0.428	0.406	0.396	0.385	0.382	0.390	0.392	0.399	0.403
	Mean	0.481	0.503	0.499	0.496	0.479	0.456	0.441	0.429	0.412	0.408	0.428	0.426
32	75	0.482	0.463	0.461	0.469	0.462	0.451	0.448	0.441	0.439	0.430	0.427	0.431
	76	0.417	0.430	0.420	0.408	0.424	0.393	0.386	0.380	0.374	0.350	0.344	0.341
	77	0.527	0.522	0.515	0.479	0.505	0.495	0.444	0.449	0.437	0.422	0.436	0.419
	Mean	0.475	0.472	0.465	0.452	0.464	0.446	0.426	0.423	0.417	0.401	0.402	0.397
43	78	0.457	0.453	0.457	0.463	0.436	0.412	0.410	0.414	0.409	0.403	0.379	0.344
	79	0.544	0.535	0.524	0.500	0.497	0.510	0.508	0.507	0.501	0.502	0.482	0.483
	80	0.458	0.480	0.494	0.508	0.511	0.509	0.505	0.503	0.495	0.482	0.463	0.442
	Mean	0.486	0.489	0.492	0.490	0.481	0.477	0.474	0.475	0.468	0.462	0.441	0.423
71	81	0.541	0.544	0.527	0.502	0.460	0.407	0.401	0.407	0.399	0.430	0.430	0.422
	82	0.408	0.384	0.377	0.352	0.329	0.332	0.317	0.314	0.317	0.327	0.319	0.310
	83	0.712	0.698	0.746	0.678	0.632	0.608	0.578	0.566	0.573	0.578	0.559	0.559
	Mean	0.554	0.542	0.550	0.511	0.474	0.449	0.432	0.429	0.430	0.445	0.436	0.430
130	84	0.747	0.664	0.603	0.563	0.555	0.537	0.534	0.534	0.518	0.515	0.502	0.458
	85	0.452	0.429	0.420	0.424	0.412	0.404	0.410	0.394	0.348	0.319	0.303	0.320
	86	0.926	0.688	0.583	0.562	0.577	0.578	0.581	0.580	0.577	0.584	0.560	0.540
	Mean	0.708	0.594	0.535	0.516	0.515	0.506	0.508	0.503	0.481	0.473	0.455	0.439
221	87	1.018	1.082	0.897	0.703	0.641	0.623	0.640	0.614	0.560	0.595	0.528	0.539
	88	1.077	1.131	1.010	0.875	0.827	0.797	0.719	0.669	0.667	0.641	0.623	0.606
	89	1.071	0.889	0.702	0.710	0.659	0.661	0.682	0.705	0.670	0.664	0.693	0.673
	Mean	1.055	1.034	0.870	0.763	0.709	0.694	0.680	0.663	0.632	0.633	0.615	0.606
375	90†	1.630	1.614	1.621	1.602	1.551	1.600	1.523	1.513	1.546	1.573	1.592	1.690
	91	0.943	0.721	0.603	0.623	0.677	0.626	0.590	0.530	0.511	0.491	0.478	0.465
	92	1.172	0.890	0.738	0.732	0.699	0.678	0.670	0.680	0.654	0.642	0.604	0.601
	Mean	1.058	0.806	0.671	0.678	0.688	0.652	0.630	0.605	0.583	0.567	0.541	0.533

*Expressed as uptake of dekalin (g)

**See Fig 4.7

†Values not included in mean (see text)

TABLE 4.13

Permeability* of 5 per cent PCP treated L-joints

Exposure period (days)	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
0	101	0.259	0.301	0.299	0.319	0.317	0.326	0.334	0.330	0.351	0.338	0.346	0.332
	102	0.335	0.351	0.354	0.347	0.366	0.381	0.379	0.378	0.416	0.382	0.388	0.359
	103	0.269	0.273	0.368	0.318	0.331	0.326	0.340	0.353	0.345	0.329	0.337	0.305
	Mean	0.288	0.308	0.340	0.328	0.338	0.344	0.351	0.354	0.371	0.350	0.357	0.332
4	110	0.469	0.540	0.503	0.448	0.420	0.392	0.405	0.395	0.405	0.406	0.415	0.427
	111	0.481	0.443	0.390	0.341	0.303	0.305	0.342	0.354	0.323	0.323	0.331	0.332
	112	0.237	0.228	0.212	0.211	0.204	0.213	0.207	0.218	0.215	0.199	0.213	0.210
	Mean	0.396	0.404	0.368	0.333	0.309	0.303	0.318	0.322	0.314	0.309	0.320	0.323
8	113	0.492	0.559	0.551	0.536	0.502	0.506	0.480	0.481	0.490	0.466	0.503	0.481
	114	0.242	0.315	0.315	0.306	0.306	0.313	0.317	0.325	0.326	0.301	0.312	0.309
	115	0.432	0.497	0.493	0.467	0.427	0.409	0.411	0.416	0.436	0.433	0.404	0.377
	Mean	0.389	0.457	0.453	0.436	0.412	0.409	0.403	0.407	0.417	0.400	0.406	0.389
11	116	0.422	0.463	0.417	0.385	0.377	0.367	0.364	0.369	0.379	0.369	0.355	0.312
	117	0.438	0.440	0.462	0.458	0.446	0.434	0.437	0.429	0.452	0.452	0.439	0.427
	118	0.376	0.391	0.357	0.319	0.310	0.310	0.309	0.318	0.314	0.322	0.333	0.329
	Mean	0.412	0.431	0.412	0.387	0.378	0.370	0.370	0.372	0.382	0.381	0.376	0.356
15	119	0.380	0.436	0.437	0.417	0.405	0.378	0.362	0.372	0.364	0.362	0.351	0.346
	120	0.479	0.509	0.519	0.533	0.512	0.509	0.479	0.471	0.458	0.424	0.415	0.406
	121	0.287	0.311	0.313	0.323	0.324	0.325	0.318	0.312	0.318	0.311	0.329	0.342
	Mean	0.382	0.419	0.423	0.424	0.414	0.404	0.386	0.385	0.380	0.366	0.365	0.365
22	122	0.424	0.454	0.452	0.449	0.411	0.404	0.400	0.399	0.400	0.399	0.399	0.393
	123	0.466	0.509	0.494	0.460	0.429	0.444	0.437	0.440	0.435	0.434	0.427	0.402
	124	0.586	0.669	0.648	0.656	0.651	0.683	0.722	0.693	0.605	0.571	0.578	0.555
	Mean	0.492	0.544	0.531	0.522	0.497	0.510	0.520	0.511	0.480	0.468	0.468	0.450
32	125	0.448	0.501	0.507	0.515	0.516	0.506	0.501	0.491	0.474	0.449	0.446	0.455
	126	0.373	0.451	0.448	0.462	0.439	0.435	0.422	0.433	0.427	0.439	0.423	0.410
	127	0.423	0.473	0.484	0.491	0.484	0.486	0.475	0.491	0.488	0.478	0.431	0.455
	Mean	0.415	0.475	0.480	0.489	0.480	0.476	0.466	0.472	0.463	0.455	0.433	0.440
43	128	0.610	0.638	0.632	0.586	0.560	0.560	0.596	0.595	0.517	0.491	0.517	0.495
	129	0.380	0.353	0.364	0.352	0.349	0.338	0.335	0.334	0.323	0.325	0.331	0.324
	130	0.474	0.508	0.477	0.475	0.462	0.419	0.446	0.439	0.431	0.436	0.397	0.422
	Mean	0.488	0.500	0.491	0.471	0.457	0.439	0.459	0.456	0.424	0.417	0.415	0.414
71	131	0.591	0.611	0.597	0.588	0.576	0.539	0.530	0.522	0.538	0.536	0.529	0.508
	132	0.502	0.557	0.528	0.518	0.493	0.480	0.469	0.464	0.469	0.460	0.451	0.416
	133	0.395	0.425	0.380	0.388	0.379	0.352	0.335	0.344	0.367	0.338	0.351	0.390
	Mean	0.496	0.531	0.502	0.498	0.483	0.457	0.445	0.443	0.458	0.445	0.444	0.438
130	134	0.581	0.606	0.636	0.647	0.626	0.575	0.519	0.485	0.460	0.438	0.447	0.449
	135	0.706	0.799	0.786	0.765	0.750	0.749	0.808	0.743	0.778	0.739	0.702	0.674
	136	0.415	0.419	0.413	0.406	0.398	0.395	0.408	0.393	0.373	0.367	0.347	0.324
	Mean	0.567	0.608	0.612	0.606	0.591	0.573	0.578	0.540	0.537	0.515	0.499	0.482
221	137	0.478	0.517	0.519	0.508	0.513	0.521	0.540	0.522	0.511	0.477	0.467	0.405
	138†	1.132	1.245	1.242	1.317	1.299	1.314	1.303	1.304	1.457	1.483	1.412	1.391
	139	0.501	0.456	0.434	0.462	0.438	0.438	0.439	0.420	0.382	0.343	0.360	0.308
	Mean	0.490	0.487	0.477	0.485	0.476	0.480	0.490	0.471	0.447	0.410	0.414	0.357
375	140	0.527	0.573	0.578	0.565	0.541	0.484	0.458	0.436	0.436	0.435	0.420	0.415
	141	0.434	0.450	0.439	0.472	0.475	0.446	0.450	0.469	0.468	0.475	0.495	0.513
	142	0.466	0.615	0.581	0.579	0.622	0.578	0.585	0.538	0.536	0.497	0.457	0.453
	Mean	0.476	0.546	0.533	0.539	0.546	0.503	0.498	0.481	0.480	0.469	0.457	0.460

*Expressed as uptake of dekalin (g)

**See Fig 4.7

†Values not included in mean (see text)

TABLE 4.14

Bacterial counts*

Expsoure period (days)	Sample position	Untreated	1% TnBTO	5% PCP
0	S	1.55×10^2	10	1.12×10^3
	C	2.20×10^3	2.50×10^2	1.11×10^3
	E	1.01×10^3	4.00×10^2	1.81×10^3
3 (without wetting)	S	4.50×10^2	7.35×10^2	8.35×10^2
	C	1.69×10^3	ng	2.00×10^1
	E	8.20×10^2	10	2.95×10^3
4	S	ng	ng	2.00×10^1
	C	ng	ng	1.00×10^2
	E	ng	ng	ng
8	S	3.58×10^3	1.31×10^3	1.30×10^3
	C	1.91×10^2	1.14×10^2	5.08×10^2
	E	9.32×10^2	9.30×10^2	1.87×10^2
11	S	1.46×10^3	1.40×10^3	ng
	C	3.41×10^2	6.12×10^2	6
	E	1.15×10^3	5.34×10^2	ng
15	S	1.34×10^3	1.01×10^3	1.43×10^5
	C	1.34×10^3	3.80×10^2	3.76×10^2
	E	2.56×10^3	1.17×10^3	8.26×10^2
22**	S	4.80×10^5	2.40×10^7	uc ($> 2.40 \times 10^7$)
	C	3.00×10^5	1.85×10^5	9.40×10^5
	E	8.00×10^5	4.40×10^5	4.80×10^5
32	S	4.23×10^7	1.56×10^6	ng
	C	ng	ng	ng
	E	ng	3.00×10^4	ng
43	S	1.31×10^7	1.02×10^5	4.79×10^7
	C	uc ($> 4.79 \times 10^7$)	ng	ng
	E	nd	1.80×10^3	ng

*Expressed as bacteria per ml of exudate

**High counts in check blocks suggest cross contamination may have occurred between test blocks

ng - insufficient numbers of colonies for estimation .'. less than other values

uc - too many colonies to count .'. greater than other values

nd - not determined

S - shoulder

C - centre

E - end (see Fig 4.7)

TABLE 4.15

Bacterial colonisation*

Exposure period (days)	Position** through depth of sample	Position relative to the joint**														
		Untreated					1% TnBTO					5% PCP				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	a	000	000	000	000	000	+00	000	+00	000	000	000	000	00+	000	000
	b	000	000	+00	000	000	000	000	000	000	000	000	000	000	000	000
	c	000	000	000	00+	000	000	000	000	000	+0+	+00	+00	000	0+0	000
4	a	000	000	000	000	000	000	000	000	000	000	000	000	000	000	000
	b	000	000	0+0	000	000	000	000	000	000	00+	000	000	000	000	000
	c	000	000	+00	00+	000	000	000	000	000	000	000	0+0	000	000	000
8	a	020	000	000	000	000	101	000	000	000	000	000	000	000	0+0	000
	b	220	000	000	000	000	201	000	000	000	000	000	000	000	000	000
	c	12+	000	000	000	000	101	000	000	000	000	00+	000	0+0	000	000
11	a	223	020	000	000	000	001	000	+00	000	000	+00	000	000	000	000
	b	213	010	000	000	000	002	100	000	000	000	000	000	000	000	000
	c	213	000	000	000	000	022	000	000	000	000	000	000	000	000	000
15	a	322	+++	++0	220	2+0	300	+00	+++	000	++0	020	+2+	000	+00	0++
	b	332	1++	++0	++0	++0	330	000	++0	+00	030	220	+00	+00	000	++0
	c	433	213	+++	2+0	2+0	433	+0+	000	200	+00	333	++0	+0+	00+	++0
22	a	424	0+0	+++	00+	00+	143	002	+00	000	0+0	304	000	++0	000	000
	b	434	+1+	0++	+++	+0+	244	024	0+0	000	000	424	2+4	200	000	+00
	c	424	+0+	++0	00+	00+	444	004	002	0+0	00+	444	302	00+	000	0+0
32	a	432	4+0	000	000	000	140	220	000	000	000	200	001	000	000	000
	b	433	000	000	000	000	030	010	000	000	0+0	000	000	001	000	000
	c	223	100	+00	000	000	232	000	000	0+0	0+0	0+0	+00	000	000	000
43	a	323	100	100	000	000	244	024	002	000	000	000	000	000	000	000
	b	343	100	100	000	000	033	002	000	000	000	300	000	010	002	000
	c	233	022	000	000	000	032	022	000	000	000	200	000	000	000	000
71	a	344	403	330	022	002	444	444	230	000	000	444	444	203	000	000
	b	344	223	222	030	000	444	444	203	000	000	432	432	000	000	000
	c	344	333	+32	022	000	444	344	000	+00	000	433	110	000	000	000
130	a	442	400	440	442	420	244	442	442	000	000	432	443	042	000	000
	b	333	422	442	440	42+	444	443	442	000	00+	441	443	244	000	000
	c	443	430	440	440	430	444	444	434	000	000	411	422	430	0+0	000
221	a	424	424	414	414	404	444	444	443	442	432	422	322	434	444	043
	b	344	444	414	414	404	444	433	432	422	322	423	334	444	334	041
	c	423	413	414	414	404	444	443	433	421	322	432	424	234	142	040
375	a	322	222	222	322	222	223	434	444	443	443	323	444	432	343	223
	b	222	232	222	223	112	333	444	444	443	443	324	444	343	144	023
	c	222	223	323	212	112	432	444	444	433	443	322	434	443	334	022

*Data for the three replicates are presented from left to right across each column

**See Fig 4.11

0 No growth

\+ A few colonies along the streak but no growth around the chip

1 Growth only around the chip

2 "Few" colonies along the streak

3 "Some" colonies along the streak

4 "Many" colonies along the streak

TABLE 4.16

Bacterial colonisation; variation with the position through the depth of the sample (totals from table 4.15)

Exposure period (days)	Position* through depth of sample	Untreated		1% TnBTO		5% PCP	
		Total at each depth position	Grand total	Total at each depth position	Grand total	Total at each depth position	Grand total
0	a	0	0	0	0	0	0
	b	0		0		0	
	c	0		0		0	
4	a	0	0	0	0	0	0
	b	0		0		0	
	c	0		0		0	
8	a	2	9	2	7	0	0
	b	4		3		0	
	c	3		2		0	
11	a	9	22	1	8	0	0
	b	7		3		0	
	c	6		4		0	
15	a	13	42	3	24	4	17
	b	9		9		4	
	c	20		12		9	
22	a	10	32	10	44	7	42
	b	12		16		18	
	c	10		18		17	
32	a	13	31	9	20	3	4
	b	10		4		1	
	c	8		7		0	
43	a	10	34	18	35	0	8
	b	12		8		6	
	c	12		9		2	
71	a	30	86	29	81	29	59
	b	27		29		18	
	c	29		23		12	
130	a	38	120	30	98	26	77
	b	41		33		30	
	c	41		35		21	
221	a	46	138	54	147	45	130
	b	49		46		46	
	c	43		47		39	
375	a	32	92	52	161	46	134
	b	30		55		45	
	c	30		54		43	
Total	a	192	575	205	610	160	471
	b	190		200		168	
	c	193		205		143	

*See Fig 4.11

TABLE 4.17

Bacterial colonisation; variation with the position relative to the joint (totals from table 4.15)

Exposure period (days)	Position relative to the joint*														
	Untreated					1% TnBTO					5% PCP				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	9	0	0	0	0	7	0	0	0	0	0	0	0	0	0
11	19	3	0	0	0	7	1	0	0	0	0	0	0	0	0
15	25	7	0	6	4	19	0	0	2	3	15	2	0	0	0
22	31	1	0	0	0	30	12	2	0	0	29	11	2	0	0
32	26	5	0	0	0	15	5	0	0	0	2	1	1	0	0
43	26	6	2	0	0	21	12	2	0	0	5	0	1	2	0
71	33	23	17	11	2	36	35	10	0	0	31	23	5	0	0
130	30	19	26	26	19	34	33	31	0	0	24	30	23	0	0
221	30	30	27	27	24	36	33	30	25	23	26	27	32	29	16
375	19	20	20	19	14	25	35	36	32	33	24	35	30	29	16

*See Fig 4.11

TABLE 4.18

Fungal colonisation; number of fungi isolated at each sample position*

Exposure period (days)	Position** through depth of sample	Position relative to the joint**														
		Untreated					1% TnBTO					5% PCP				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	a	101	000	000	000	100	001	001	000	100	000	000	000	000	000	000
	b	000	000	000	000	000	000	000	000	000	100	000	100	000	000	100
	c	001	020	000	000	000	000	000	000	010	000	000	000	000	000	100
11	a	513	001	001	002	102	100	000	000	000	000	000	000	000	000	000
	b	313	302	002	001	001	000	100	000	000	000	000	000	000	100	000
	c	322	122	011	001	101	110	000	000	000	000	000	000	000	000	000
22	a	224	000	001	000	000	020	000	000	000	000	000	000	000	000	000
	b	113	000	000	000	000	010	000	100	000	000	000	000	000	000	000
	c	132	010	030	100	000	010	000	101	000	100	000	000	000	000	000
32	a	333	000	000	000	000	200	010	000	000	000	020	100	000	000	000
	b	132	000	000	000	000	200	000	000	000	000	000	100	001	000	000
	c	214	101	300	000	000	023	000	000	000	000	000	020	000	000	000
43	a	255	211	100	000	000	112	011	000	000	000	000	000	000	100	000
	b	323	200	100	100	000	201	000	000	000	000	000	000	000	000	000
	c	543	200	000	000	000	002	000	000	000	000	001	000	000	200	000
71	a	124	212	113	201	200	232	001	000	010	000	110	010	000	010	000
	b	123	212	311	100	200	120	111	000	002	000	111	110	000	030	000
	c	122	210	321	211	210	120	100	000	000	000	010	110	010	010	000
130	a	552	322	221	111	022	112	131	101	000	000	210	010	000	000	000
	b	432	425	213	111	012	221	121	122	112	000	110	030	000	000	000
	c	642	134	113	121	000	312	322	102	101	001	101	010	010	000	010
221	a	536	313	413	312	302	222	310	010	010	000	100	000	000	001	000
	b	536	334	312	322	302	212	220	000	000	000	100	100	000	001	000
	c	336	414	313	422	321	222	110	010	121	010	100	100	000	000	000
375	a	853	313	203	232	033	122	021	131	111	000	001	001	001	000	000
	b	453	132	243	023	222	132	121	121	112	100	001	000	000	000	000
	c	655	144	213	232	221	121	121	141	220	120	001	001	001	000	100

*Data for the three replicates are presented from left to right across each column
**See Fig 4.11

TABLE 4.19

Fungal colonisation; variation in the number of fungi with the position through the depth of the sample (totals from table 4.18)

Exposure period (days)	Position* through depth of sample	Untreated		1% TnBTO		5% PCP	
		Total at each depth position	Grand total	Total at each depth position	Grand total	Total at each depth position	Grand total
0	a	3	6	3	5	0	3
	b	0		1		2	
	c	3		1		1	
11	a	16	49	1	4	0	1
	b	16		1		1	
	c	17		2		0	
22	a	9	25	2	8	0	0
	b	5		2		0	
	c	11		4		0	
32	a	9	27	3	10	3	7
	b	6		2		2	
	c	12		5		2	
43	a	17	43	6	11	1	4
	b	12		3		0	
	c	14		2		3	
71	a	21	61	9	21	4	17
	b	19		8		8	
	c	21		4		5	
130	a	31	92	11	48	4	14
	b	32		18		5	
	c	29		19		5	
221	a	40	124	12	35	2	7
	b	42		9		3	
	c	42		14		2	
375	a	41	122	16	56	3	8
	b	38		19		1	
	c	43		21		4	
Total	a	187	549	63	198	17	60
	b	170		63		22	
	c	192		72		21	

*See Fig 4.11

TABLE 4.20

Fungal colonisation; variation in the number of fungi with the position relative to the joint (totals from table 4.18)*

Exposure period (days)	Position relative to the joint**														
	Untreated					1% TnBTO					5% PCP				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	(3)	(2)	0	0	(1)	(1)	(1)	0	(2)	(1)	0	(1)	0	0	(2)
11	22 (1)	8 (3)	1 (4)	(4)	(6)	3	1	0	0	0	0	0	0	1	0
22	19	1	4	1	0	4	0	2 (1)	0	(1)	0	0	0	0	0
32	22	2	3	0	0	9	1	0	0	0	2	3 (1)	1	0	0
43	32	6 (2)	2	(1)	0	9	2	0	0	0	1	0	0	3	0
71	18	12 (1)	16	8	7	13	3 (2)	0	3	0	6	5	1	5	0
130	33	25 (1)	16	10	7	15	16	10	6	1	7	5	1	0	1
221	38 (2)	21 (5)	16 (5)	20 (1)	15 (1)	17	10	2	5	1	3	2	0	2	0
375	44	22	20	19 (2)	17	15	11	15	11	4	3	2	2	0	1

*Numbers in parentheses indicate additional isolates known to occur in unexposed material

**See Fig 4.11

TABLE 4.21

Fungal isolates; frequency of isolation from 15 attempts on each of three replicates

Organism	Classification	Untreated								
		Exposure period (days)								
		0	11	22	32	43	71	130	221	375
Untypified/others **	U	2	2	2	2	1	3	3	5	2
Aspergillus fumigatus *	SR		13			3		1	14	
Chaetomium globosum *	SR	2	2							
Mucor type A *	M		3							
Penicillium type A *	M								1	
Penicillium **	M	2	1	1				2	3	2
Bluestain type A	BS		5	6	3	1		1	3	
" " B	BS		6	5	1	5	1	6	3	7
" " C	BS		4	2	2			3	12	3
" " D	BS		2	1		1			2	2
" " E	BS									
" " F	BS									
" " G	BS									
" " H	BS		1							
" " I	BS									
" " J	BS				2			2		
" " K	BS		1		3	2		5		2
" (no type given)	BS		3	5	4	6		4		
" (not type-tested)	BS		2	1	4	10	4	18	11	25
Aspergillus	M		1			1			1	1
Botrytis cinerea	SR		1	1	1	1	2			
Phialophora type A	SR		1	1	1	7	2	10	32	26
Pleospora	SR		1							
Trichoderma viride	M						27	4		
Diplodia gossypina	BS				1	1	4	2	1	1
Pestalotia	SR				1					
Ulocladium	SR				1			1		
Basidiomycete type A	WR							3		9
" " B	WR						6		4	2
" " C	WR						3			
" " D	WR							1		
" " E	WR									
" " F	WR							7		
" " G	WR				1					4
" " H	WR							2		
" " I	WR									10
" " J	WR								4	
" " K	WR									2
" " L	WR								4	
" " M	WR							3		
" (no type given)	WR					1	3	1		7
Sistotrema brinkmannii	Sb					1	3	4	15	12
Alternaria	SR					1	1		1	
Epicoccum purpurascens	SR					1		1		
Paecilomyces varioti	M						2	6		
Ascomycete type A	BS								1	
Phialophora type B	SR								1	
Phoma	SR							1	2	1
Rhinocladiella	BS							1		
Fusarium	SR								3	
Mucor type B	M								1	
Coniothyrium	SR									4
Phialophora type C	SR									

*Organisms present prior to exposure
**Most isolates unlikely to have been present prior to exposure

M Mould
BS Bluestain
SR Soft rot
WR White rot
Sb S. brinkmannii
U Unclassified

TABLE 4.21 Cont

Fungal isolates; frequency of isolation from 15 attempts on each of three replicates

1% TnBTO									5% PCP									Total
Exposure period (days)									Exposure period (days)									
0	11	22	32	43	71	130	221	375	0	11	22	32	43	71	130	221	375	
				3	1	1	5	2										34
		1			2													34
2												1						7
2		1																6
1									3					1		1		7
	2	1			1	2	1			1			1	3	1	1		25
						2												21
			1			1												36
	1					8	1											36
	1	3	5			1	1											19
												2	1		2			5
		1	3															4
												2						2
												1			3	1		6
												1						1
			1				1								3	1	1	11
						4	1									2		20
				1	4	7												34
				2		1	1	7							3	1		90
													2					6
				1	1													8
				4	6	15	14	16										135
		1			3									13				1
																		48
																		10
																		1
																		2
																		12
																		12
																		3
																		1
								1										1
																		7
																		5
																		2
																		10
																		4
																		2
																		4
																		3
							10											12
																		45
																		3
																		2
																		8
					3	3		16										23
						3		14										18
																		4
															2			3
																		3
																		1
																		4
																	7	7

TABLE 4.22

Colonisation of untreated L-joints by the various groups of fungi (totals from table 4.21)*

Exposure period (days)	Group of† fungi	Position relative to the joint**					
		1	2	3	4	5	Total
11	M		2				2
	BS	17	6	1			24
	SR	3					3
	WR						0
	Sb						0
	U	2					2
22	M		1				1
	BS	16		4			20
	SR	2					2
	WR						0
	Sb						0
	U	1			1		2
32	M						0
	BS	15	2	3			20
	SR	4					4
	WR	1					1
	Sb						0
	U	2					2
43	M		1				1
	BS	19	5	2			26
	SR	10					10
	WR	1					1
	Sb	1					1
	U	1					1
71	M	9	6	9	4	1	29
	BS	2		4		3	9
	SR	2	2	1			5
	WR	2	3	2	2	3	12
	Sb	3					3
	U		1		2		3
130	M	4	2	4	1	1	12
	BS	11	10	9	8	4	42
	SR	9	3			1	13
	WR	5	7	3	1	1	17
	Sb	3	1				4
	U	1	2				3
221	M	3	1	1	1		6
	BS	15	3	2	7	6	33
	SR	12	9	6	6	6	39
	WR	3	3	3	3		12
	Sb	3	3	3	3	3	15
	U	2	2	1			5
375	M	1	1		1		3
	BS	21	5	4	2	8	40
	SR	10	7	6	6	2	31
	WR	4	7	8	8	7	34
	Sb	6	2	2	2		12
	U	2					2

*Fungi known to occur in unexposed material have been omitted

**See Fig 4.11

†See Table 4.21

TABLE 4.23

Colonisation of 1 per cent TnBTO treated L-joints by the various groups of fungi (totals from table 4.21)*

Exposure period (days)	Group of† fungi	Position relative to the joint**					
		1	2	3	4	5	Total
11	M	2					2
	BS	1	1				2
	SR						0
	WR						0
	Sb						0
	U						0
22	M			2			2
	BS	4					4
	SR						0
	WR						0
	Sb						0
	U						0
32	M						0
	BS	9	1				10
	SR						0
	WR						0
	Sb						0
	U						0
43	M						0
	BS	2	1				3
	SR	4	1				5
	WR						0
	Sb						0
	U	3					3
71	M	1			3		4
	BS	6	1				7
	SR	5	2				7
	WR						0
	Sb						0
	U	1					1
130	M		1	1			2
	BS	5	9	7	5	1	27
	SR	10	5	2	1		18
	WR						0
	Sb						0
	U		1				1
221	M	1					1
	BS		2		3		5
	SR	9	5				14
	WR						0
	Sb	7	3				10
	U			2	2	1	5
375	M						0
	BS	6	6	6	5		23
	SR	9	5	7	5	4	30
	WR			1			1
	Sb						0
	U			1	1		2

*Fungi known to occur in unexposed material have been omitted

**See Fig 4.11

†See Table 4.21

TABLE 4.24

Colonisation of 5 per cent PCP treated L-joints by the various groups of fungi (totals from table 4.21)*

Exposure period (days)	Group of† fungi	Position relative to the joint**					
		1	2	3	4	5	Total
11	M				1		1
	BS						0
	SR						0
	WR						0
	Sb						0
	U						0
22	M						0
	BS						0
	SR						0
	WR						0
	Sb						0
	U						0
32	M						0
	BS	2	3	1			6
	SR						0
	WR						0
	Sb						0
	U						0
43	M				3		3
	BS	1					1
	SR						0
	WR						0
	Sb						0
	U						0
71	M	6	5	1	5		17
	BS						0
	SR						0
	WR						0
	Sb						0
	U						0
130	M		1				1
	BS	7	4	1		1	13
	SR						0
	WR						0
	Sb						0
	U						0
221	M		2				2
	BS	3			2		5
	SR						0
	WR						0
	Sb						0
	U						0
375	M						0
	BS					1	1
	SR	3	2	2			7
	WR						0
	Sb						0
	U						0

*Fungi known to occur in unexposed material have been omitted

**See Fig 4.11

†See Table 4.21

TABLE 4.25

Summary of microscopic observations

Exposure period (days)	Untreated		1% TnBTO		5% PCP	
	Soft rot	Bluestain	Soft rot	Bluestain	Soft rot	Bluestain
375	+	+	+	+	-	+
221	+	+	-	+	ne	ne
130	+	+	ne	ne	ne	ne
71	-	+	ne	ne	ne	ne

\+ feature present in one or more replicates
\- feature not observed
ne not examined

TABLE 4.26

Paint film failures

Exposure period (days)	Untreated		1% TnBTO		5% PCP	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
0	None	None	None	None	None	None
4	None	Slight splits from mortices	None	Slight split from mortice on 1 replicate	None	Slight splits from mortice on 1 replicate
8	None	Slight splits from mortices	Slight cracks on 1 replicate	Slight splits from mortices	None	Slight splits from mortices on 2 replicates
11	Slight cracks on 1 replicate	Slight splits from mortices	None	Slight splits from mortices on 2 replicates	None	Slight splits from mortice on 1 replicate
15	Slight cracks on 1 replicate	Slight splits from mortice on 1 replicate. Slight paint loss on 1 replicate	Slight split on 1 replicate	Slight splits from mortices on 2 replicates	Slight split from joint on 1 replicate	Slight splits from mortice on 1 replicate Slight cracks on 1 replicate
22	Slight cracks on 1 replicate	Slight splits from mortice on 1 replicate. Slight cracks on 2 replicates	Slight crack on 1 replicate	Slight splits from mortices on 2 replicates	None	Slight split from mortice on 1 replicate
32	Slight cracks on 1 replicate. Pustules through the paint on 1 replicate, not extensive	Pustules through the paint on 2 replicates; not extensive	Crack on 1 replicate	Slight split from mortice on 1 replicate	None	None
43	Pustules through the paint on 2 replicates, not extensive	Pustules through the paint on 3 replicates, quite extensive on 2	Crack on 1 replicate	Slight splits from mortice on 1 replicate. Slight cracks on 1 replicate	None	Slight split from mortice on 1 replicate
71	Pustules through the paint on 3 replicates; quite extensive	Slight splits from mortices on 3 replicates; Pustules through the paint on 3 replicates; quite extensive	Slight crack on 1 replicate. Slight paint loss on 1 replicate	Slight splits from mortices on 2 replicates	None	Slight splits from mortices on 3 replicates
130	Pustules through the paint on 3 replicates; extensive. Coniochaeta fruitbodies in joint on 1 replicate	Small splits from mortices on 3 replicates. Pustules through the paint on 3 replicates; extensive	Small splits on 1 replicate. Pustules through the paint on 3 replicates; not extensive	Slight paint loss on 1 replicate. Pustules through the paint on 2 replicates; not extensive	None	None

TABLE 4.26 Cont

Exposure period (days)	Untreated		1% TnBTO		5% PCP	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
221	Pustules through the paint on 3 replicates; extensive and intense in patches	Pustules through the paint on 3 replicates; extensive and intense in patches. Coniochaeta fruitbodies in joint on 1 replicate	Paint lifting on 1 replicate. Pustules through the paint on 3 replicates; not extensive	Slight paint loss and cracks on 3 replicates. Pustules through the paint on 3 replicates; not extensive	Paint lifting on 1 replicate	Slight paint loss on 1 replicate
375	Pustules through the paint on 3 replicates; extensive and intense in patches	Pustules through the paint on 3 replicates; extensive and intense in patches	Small splits and paint lifting on 1 replicate. Pustules through the paint on 2 replicates; not extensive	Paint lifting and slight paint loss on 3 replicates. Pustules through the paint on 3 replicates; not extensive	Pustules through the paint on 3 replicates; not extensive	Slight splits from mortice on 2 replicates. Slight paint loss on 2 replicates.

TABLE 4.27

Permeability* of central portion of L-joints exposed for 375 days

Preservative treatment	L-joint No	Sample position**											
		1	2	3	4	5	6	7	8	9	10	11	12
Untreated	40	0.405	0.317	0.302	0.326	0.356	0.363	0.369	0.329	0.337	0.341	0.342	0.342
	41	0.349	0.300	0.273	0.322	0.391	0.434	0.463	0.468	0.458	0.439	0.434	0.425
	42	0.698	0.761	0.669	0.736	0.818	0.712	0.621	0.546	0.577	0.489	0.449	0.409
	Mean	0.484	0.459	0.415	0.461	0.522	0.503	0.484	0.448	0.457	0.423	0.408	0.392
1% TnBTO	90†	0.914	0.881	0.898	0.919	0.917	0.913	0.890	0.880	0.893	0.926	0.940	0.949
	91	0.493	0.362	0.352	0.374	0.374	0.375	0.355	0.331	0.310	0.265	0.270	0.281
	92	0.491	0.363	0.356	0.335	0.305	0.317	0.317	0.308	0.303	0.308	0.306	0.286
	Mean	0.492	0.363	0.354	0.355	0.340	0.346	0.336	0.320	0.307	0.287	0.288	0.284
5% PCP	140	0.234	0.216	0.224	0.253	0.244	0.238	0.235	0.243	0.228	0.214	0.226	0.247
	141	0.264	0.254	0.272	0.274	0.303	0.286	0.263	0.236	0.245	0.248	0.261	0.246
	142	0.276	0.265	0.261	0.227	0.213	0.193	0.204	0.204	0.194	0.191	0.178	0.181
	Mean	0.258	0.245	0.252	0.251	0.253	0.239	0.234	0.228	0.222	0.218	0.222	0.225

*Expressed as uptake of dekalin (g)
**See Fig 4.7
† Not included in the mean (see text)

TABLE 4.28

Comparison of the permeability of the normal test blocks with those taken from the central portion of L-joints

Treatment	Normal test blocks		Central portion blocks	
	Mean * permeability	Ratio to Untreated	Mean * permeability	Ratio to Untreated
Untreated	1.009	1	0.455	1
1% TnBTO	0.668	0.66	0.339	0.75
5% PCP	0.499	0.49	0.237	0.52

*Expressed as uptake of dekalin (g)

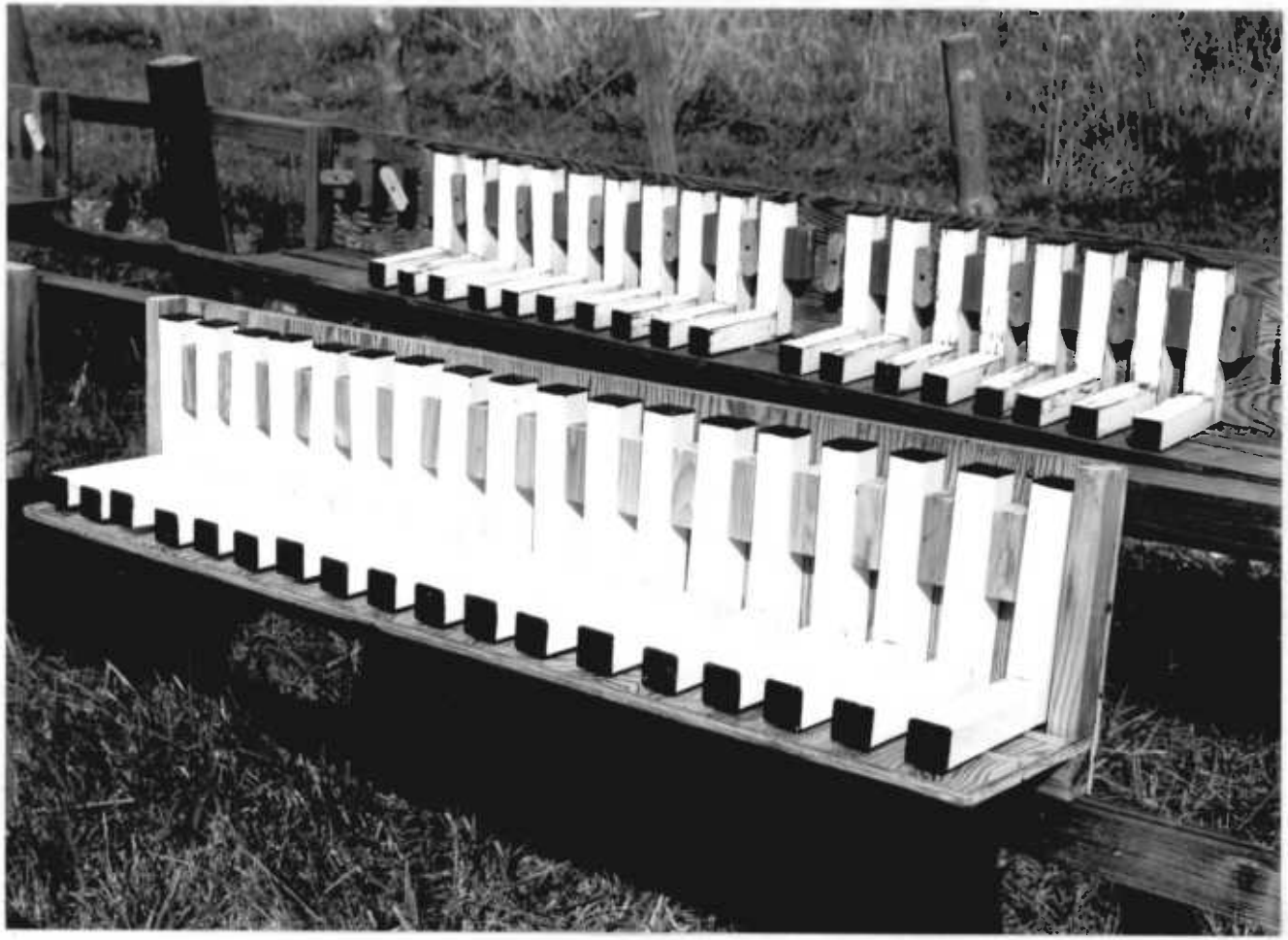


Fig 4.1 L-joints during exposure.

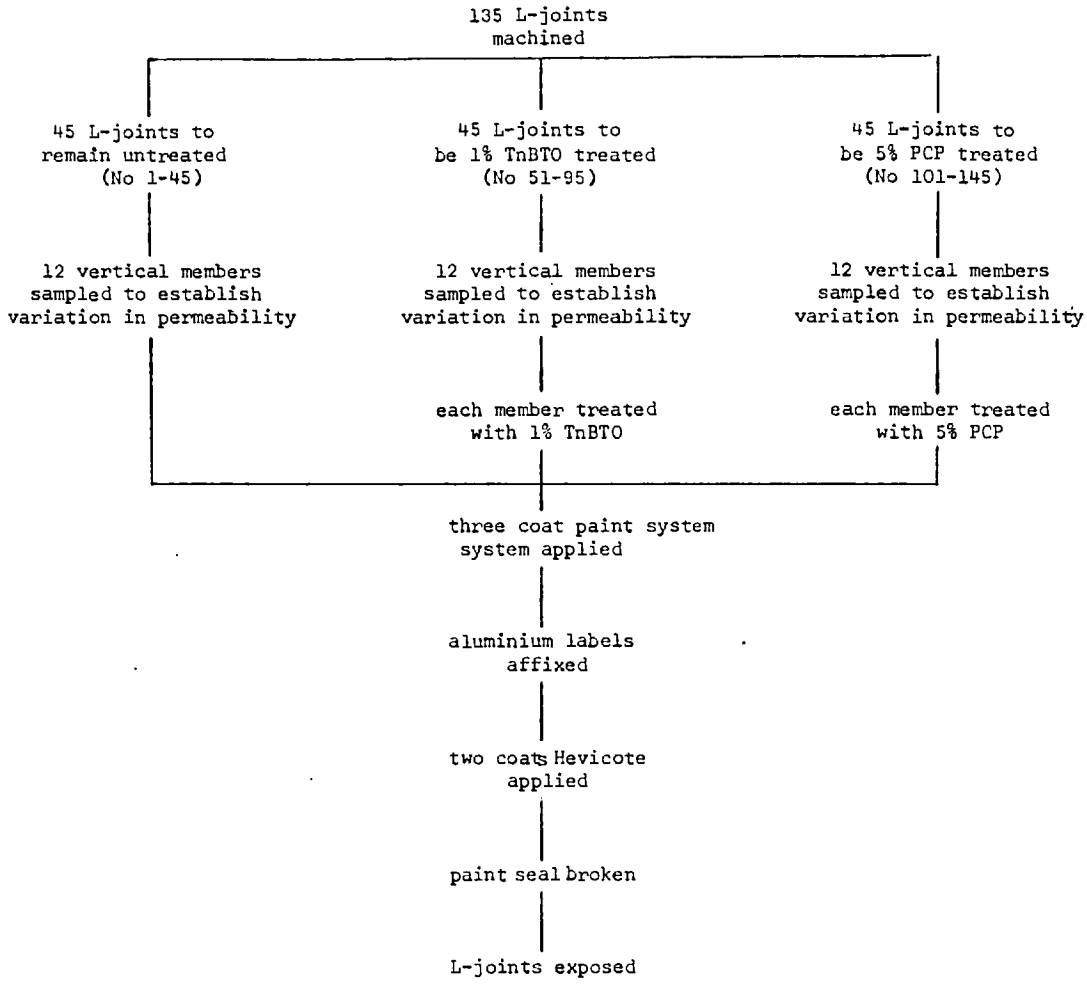


Fig 4.2 Preparation of L-joints

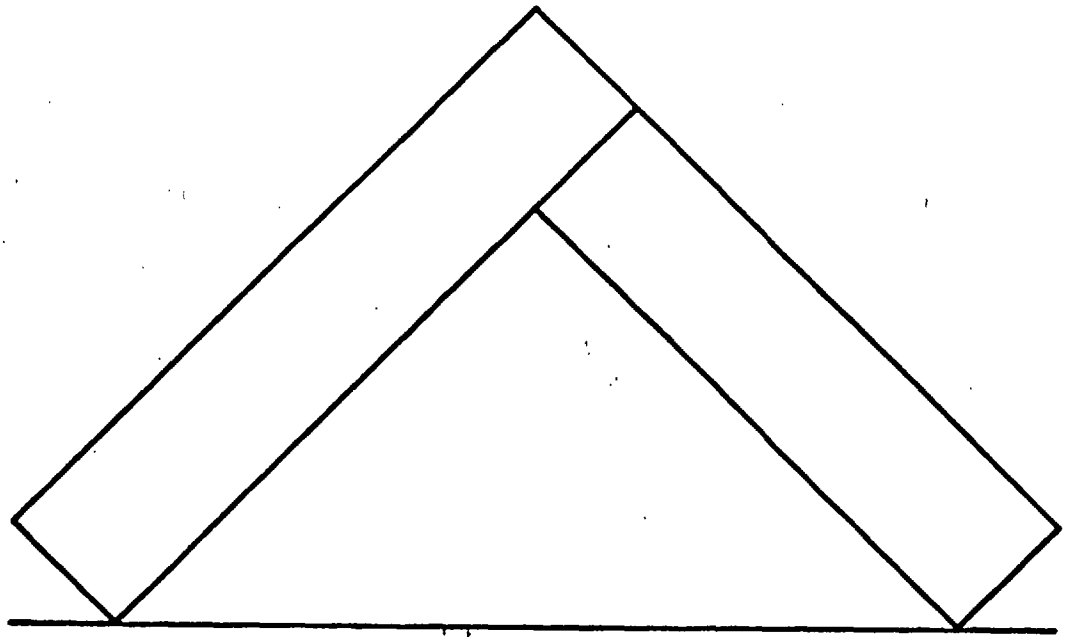


Fig. 4.3 Position of L - joints during painting

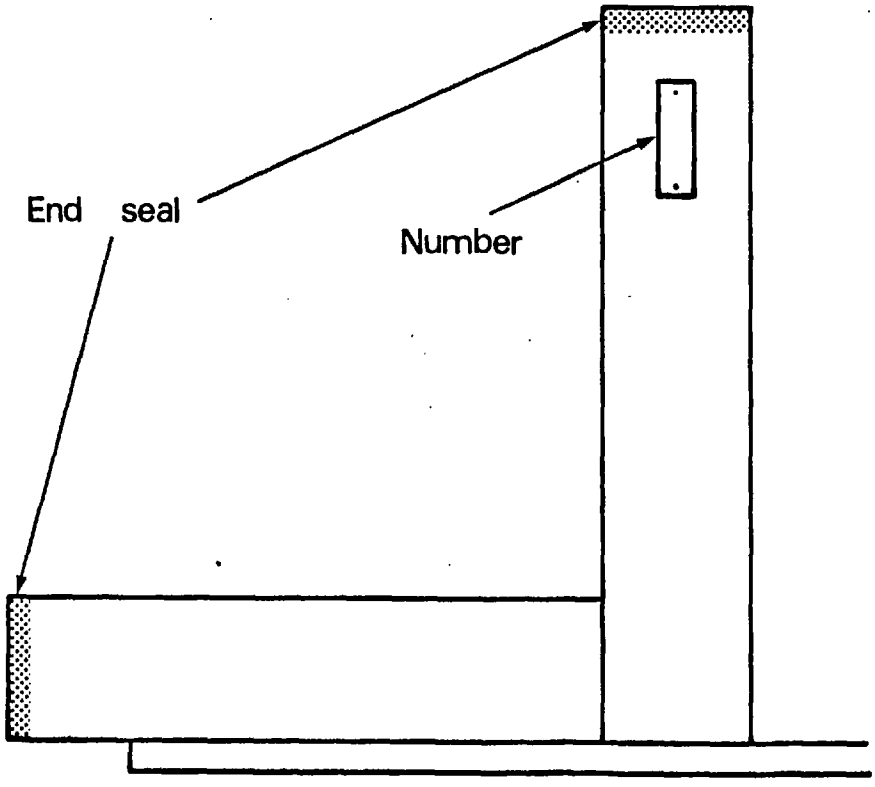


Fig. 4.4 Position of L - joints during end sealing

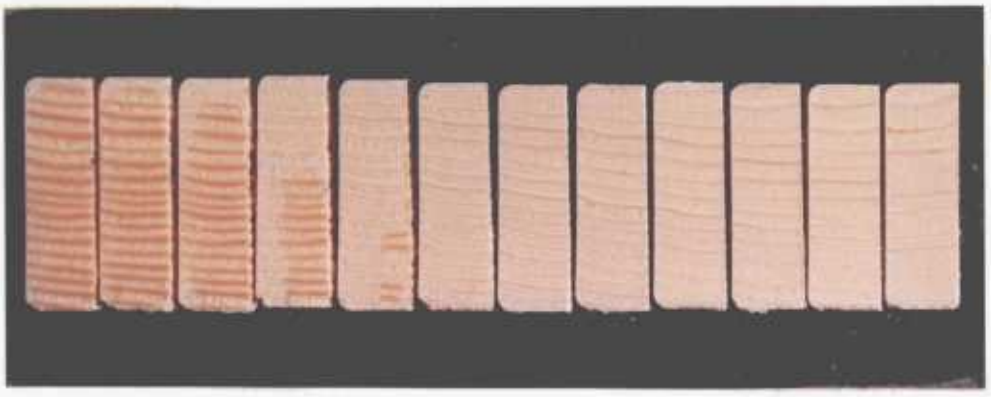


Fig 4.5 Moisture distribution after 11 days exposure.
Left - joint end. Right - remote end.
Note greater moisture penetration along the bottom.

Fig 4.6 Conversion of an L-joint during sampling.
(See Fig 4.7)

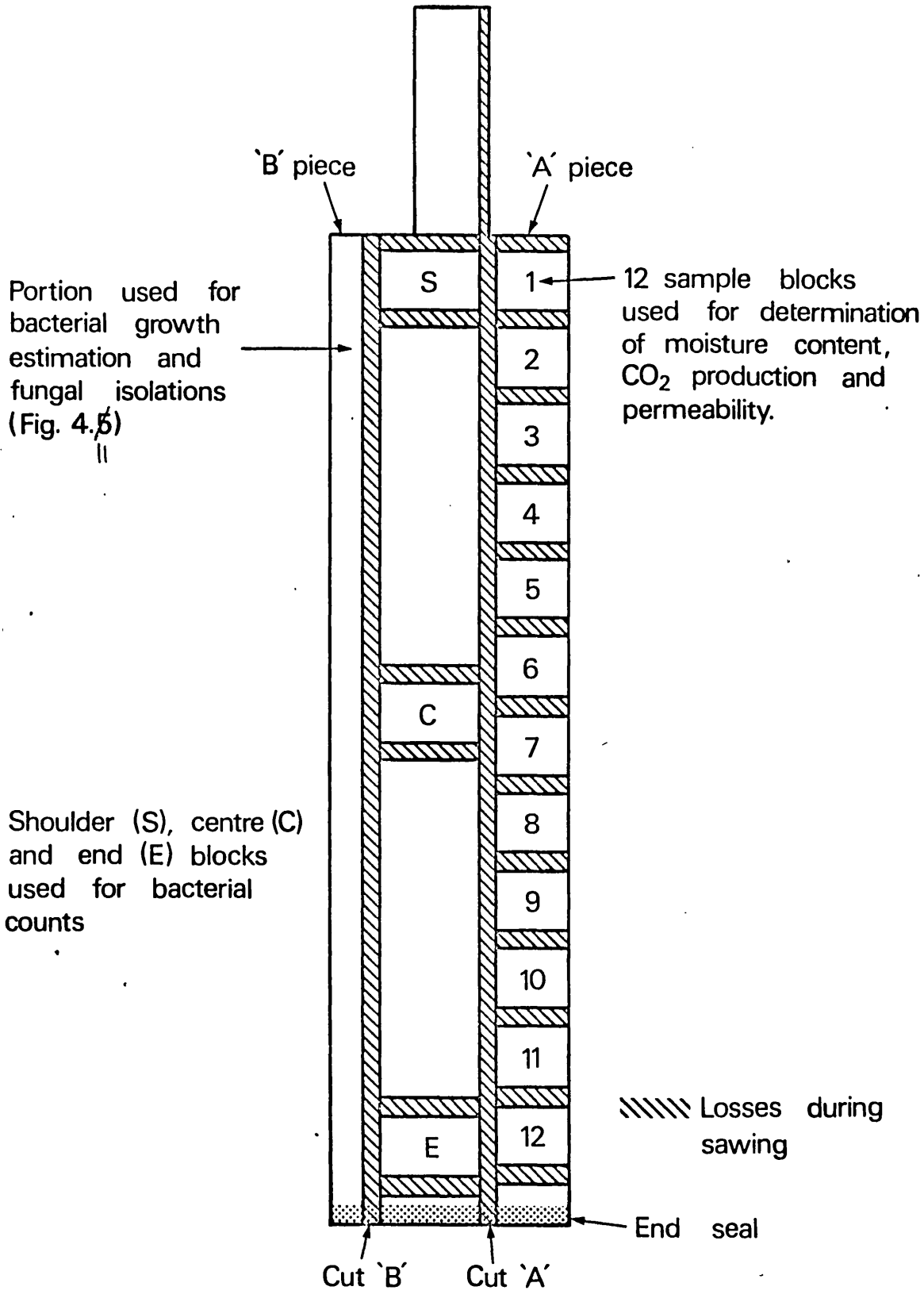


Fig. 4.7 Plan of the conversion of each L - joint

Fig 4.8 Titrating for CO_2 production

Fig 4.9 [illegible]

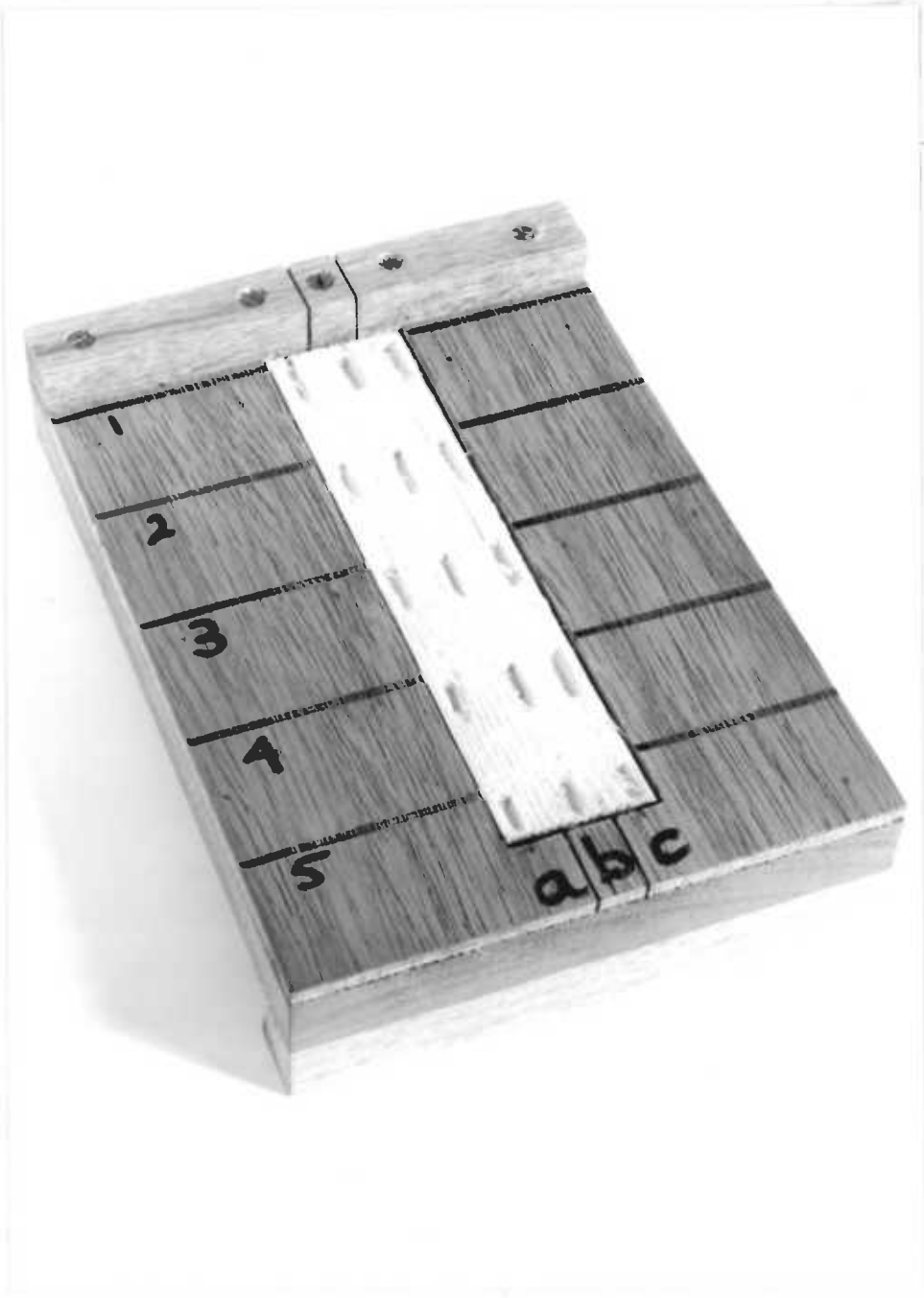
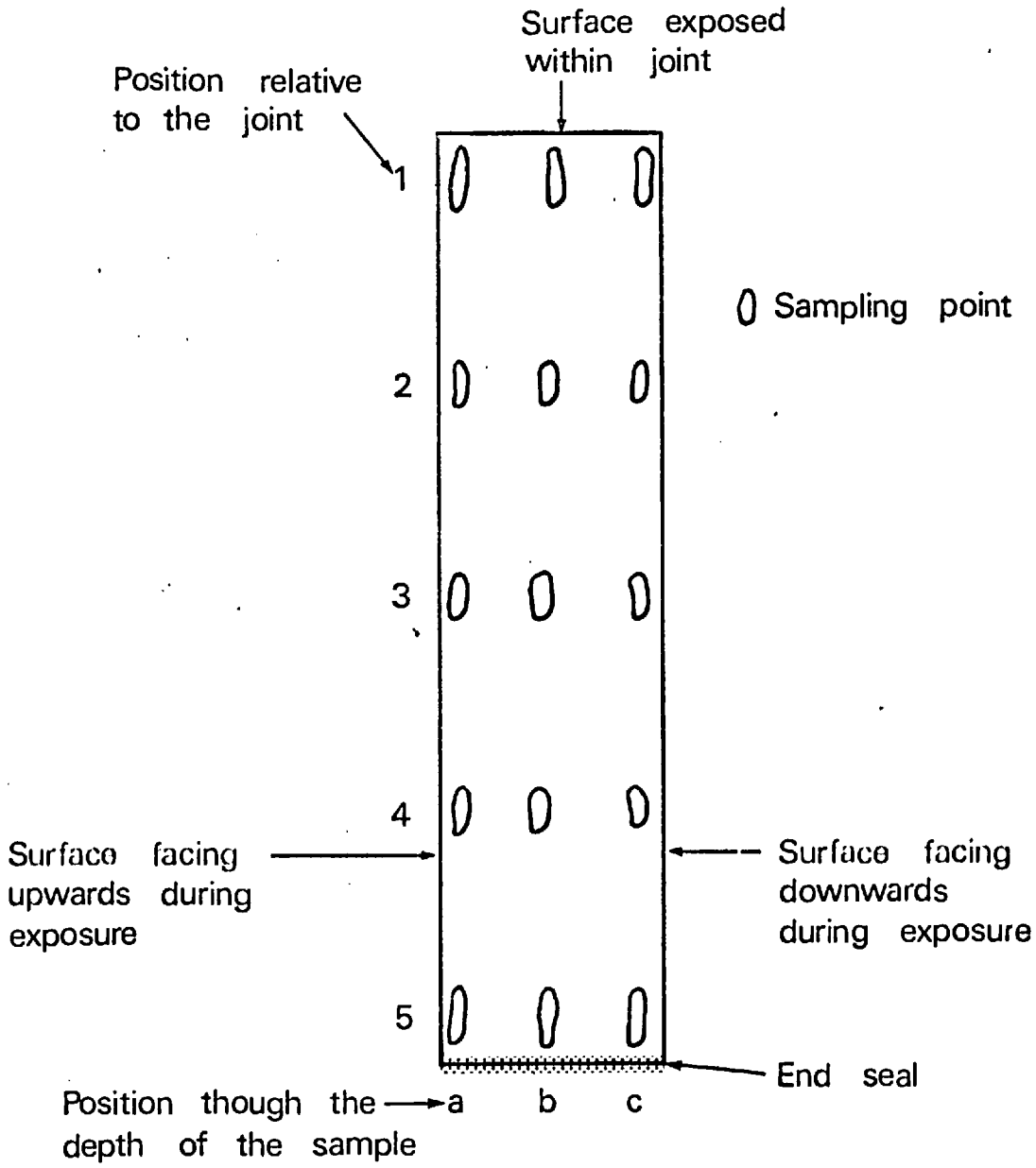


Fig 4.10 "B" piece in the modified bench hook (after sampling).



Culture numbering system :- 26/4 $SCNc_3$

26	L-joint number
4	Position relative to the joint
SCN	Isolation medium
C	Position through the depth of the sample
3	Number of the isolate (all other factors equal)

Fig. 4.11 Sampling system for bacterial growth estimation and fungal isolations

Fig 4.12 Bacterial growth test system.

Fig 4.13 Isolation procedure at the laminar flow work bench.

Fig 4.14 Fungal isolations; growth from samples
Top two per cent malt agar
Left benomyl/malt agar
Right starch casein nitrate agar

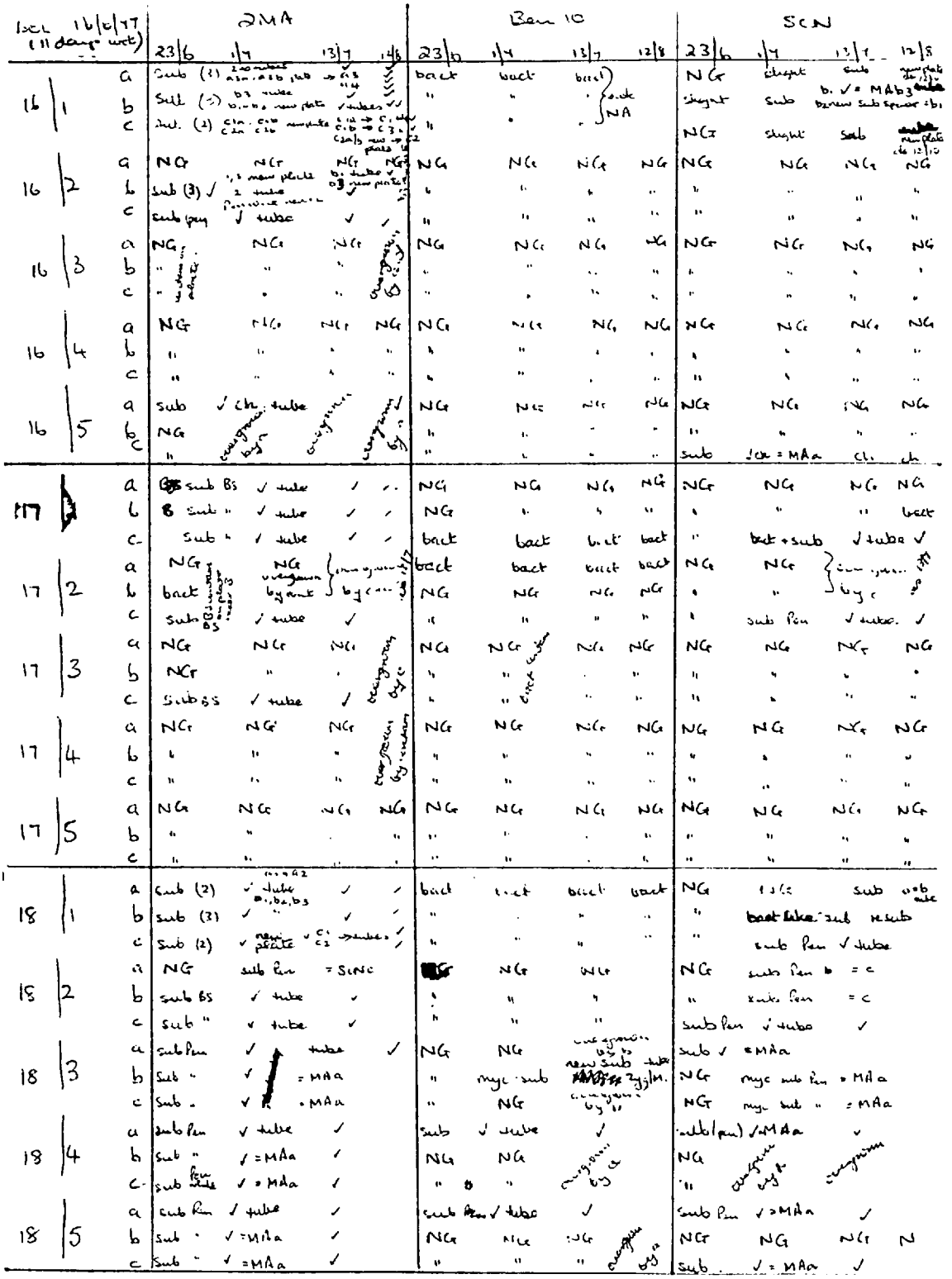


Fig 4.15 Isolation record sheet

34/4 MAb.

4/2/78 Bluestain, slow growing, dense growth. no hyaline margin. Aerial mycelium compact giving a velvety appearance, dark grey/green in colour.

4/2/78. Pigmented hyphae, very spiky appearance due to short side branches at 90° to main hypha. Many free conidia others attached to and portion of side branches. Scars visible where conidia detached Spores. ± 5 × 1·5 – 2·0 μ.

Rhinocladiella

9/3/78 Photomicrographs of coverslip cultures. Neg nos 780207 | A2 - A7 and C13, C14.

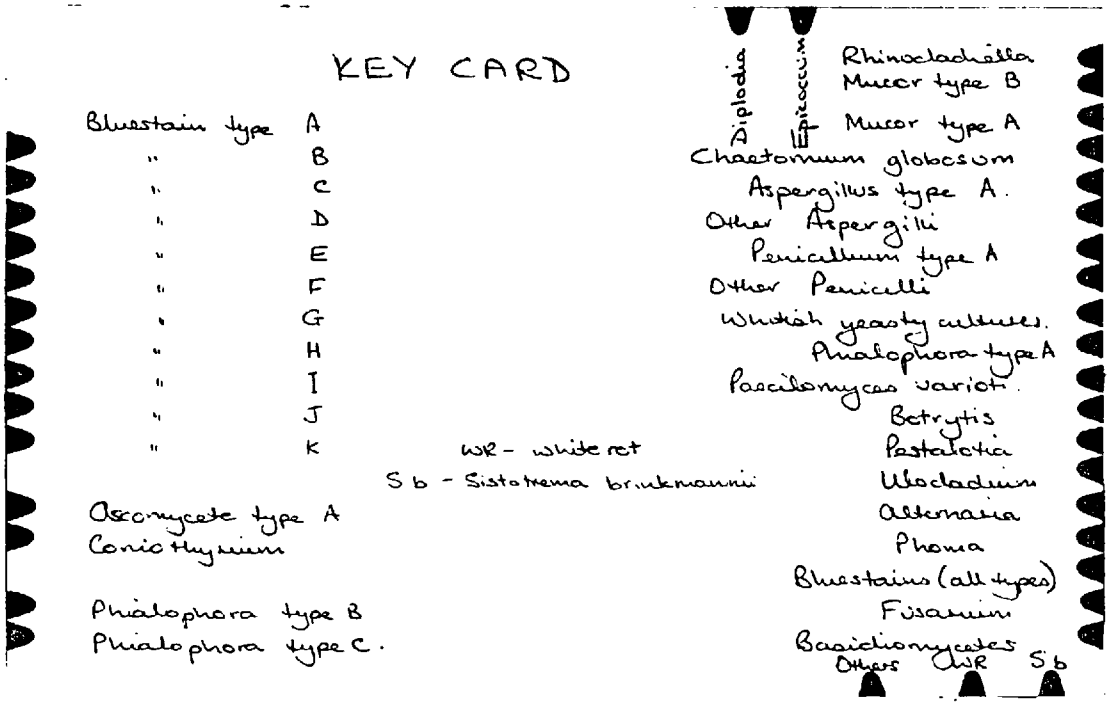


Fig 4.16 Fungal record card (top) and key card (bottom)

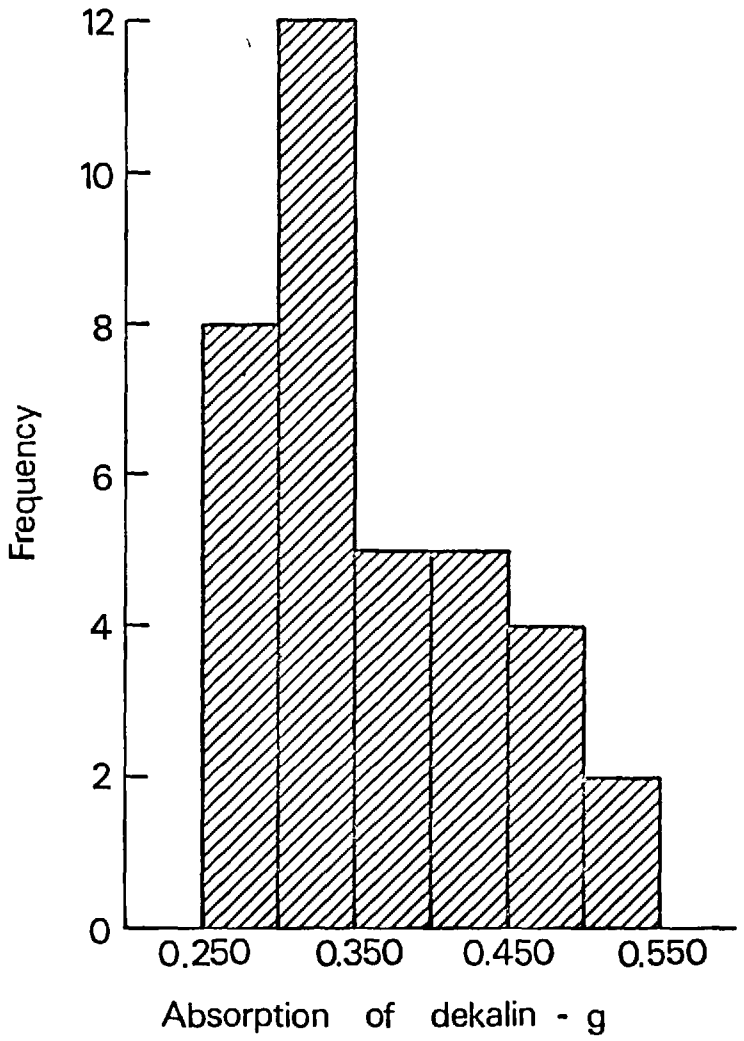


Fig. 4.17 Absorption of dekalin by sample blocks cut from vertical members prior to treatment or painting

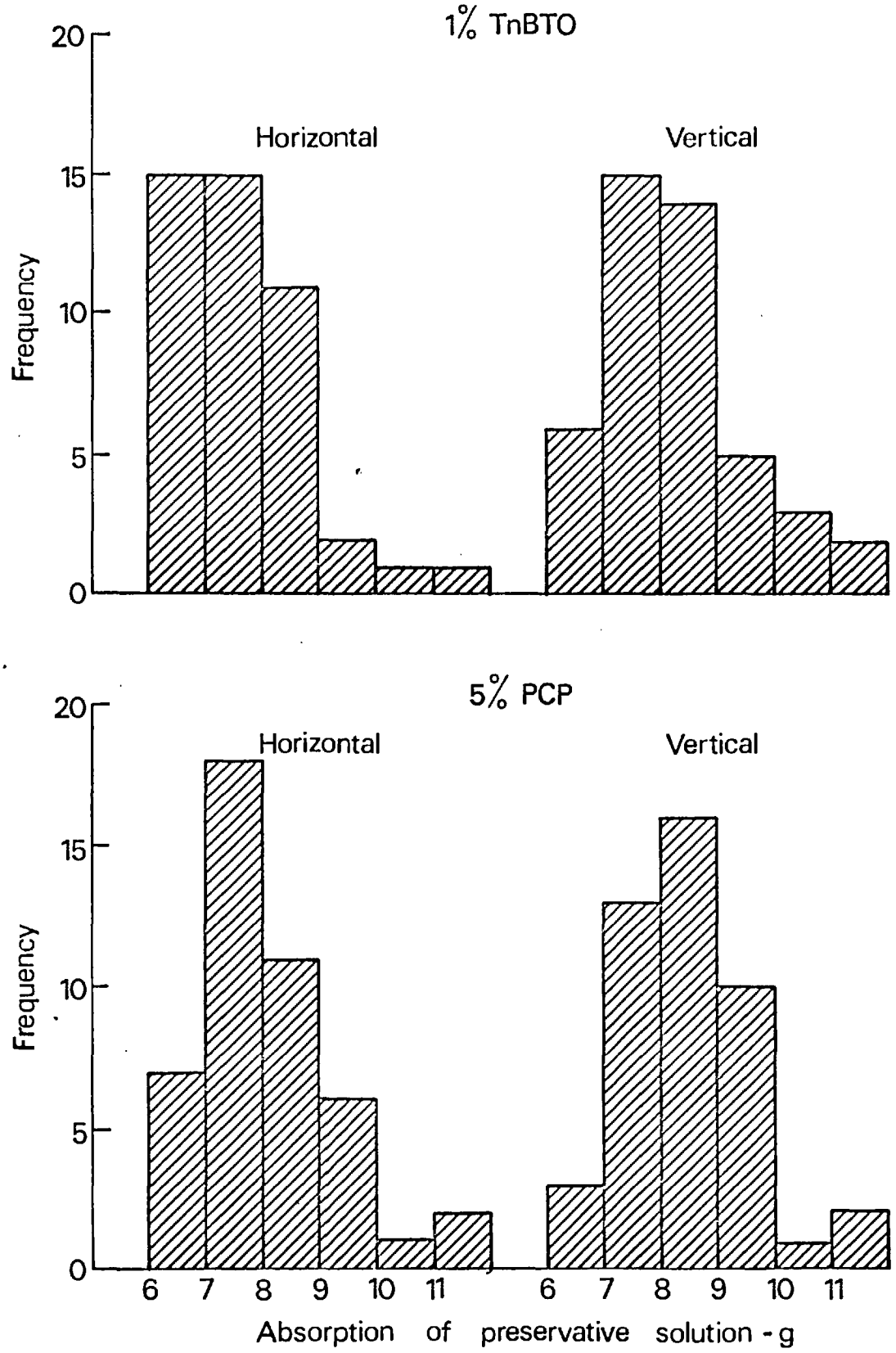


Fig. 4.18 Absorption of preservative solution by L-joint members

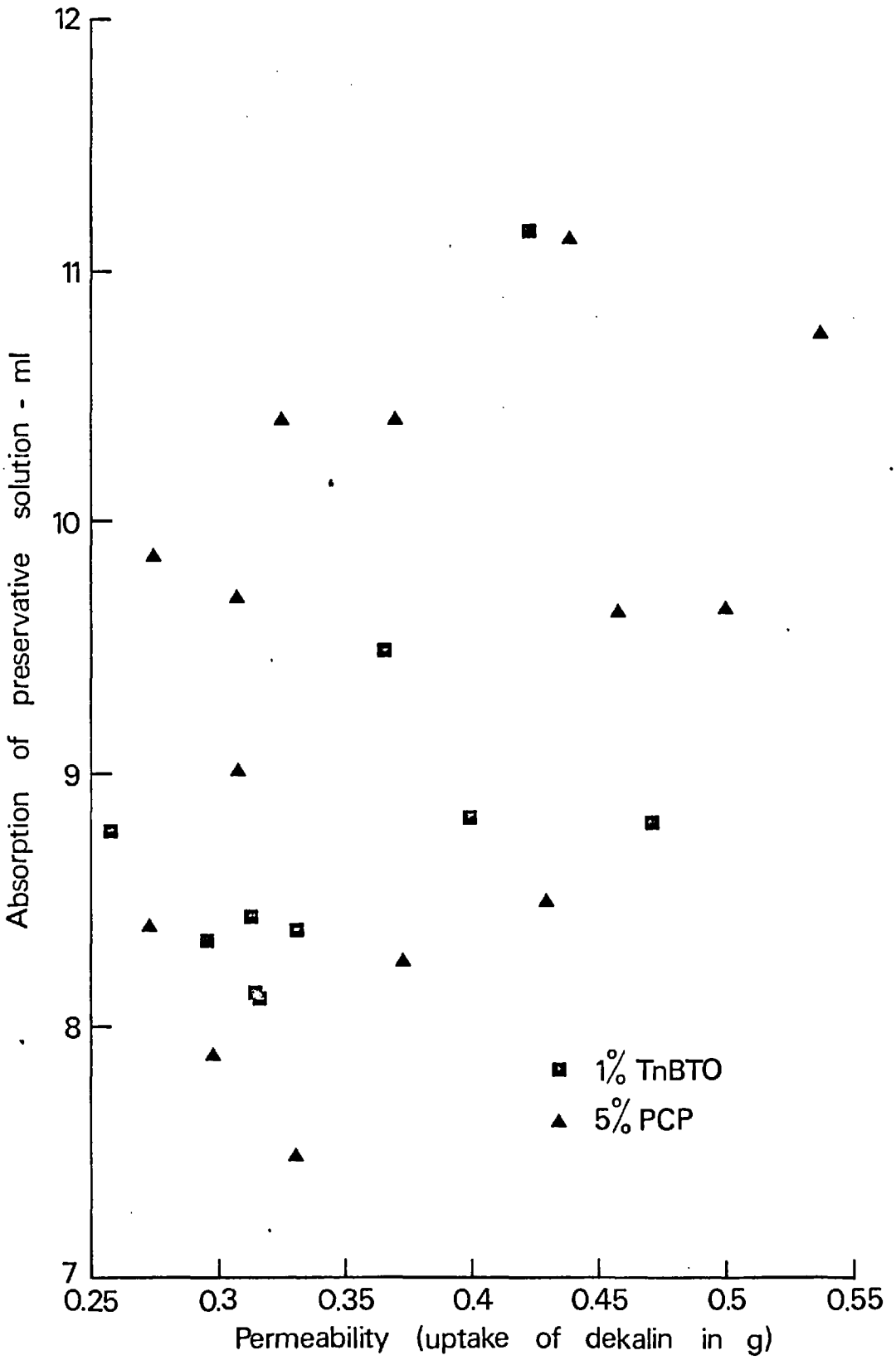


Fig. 4.19 Relationship between permeability and preservative absorption.

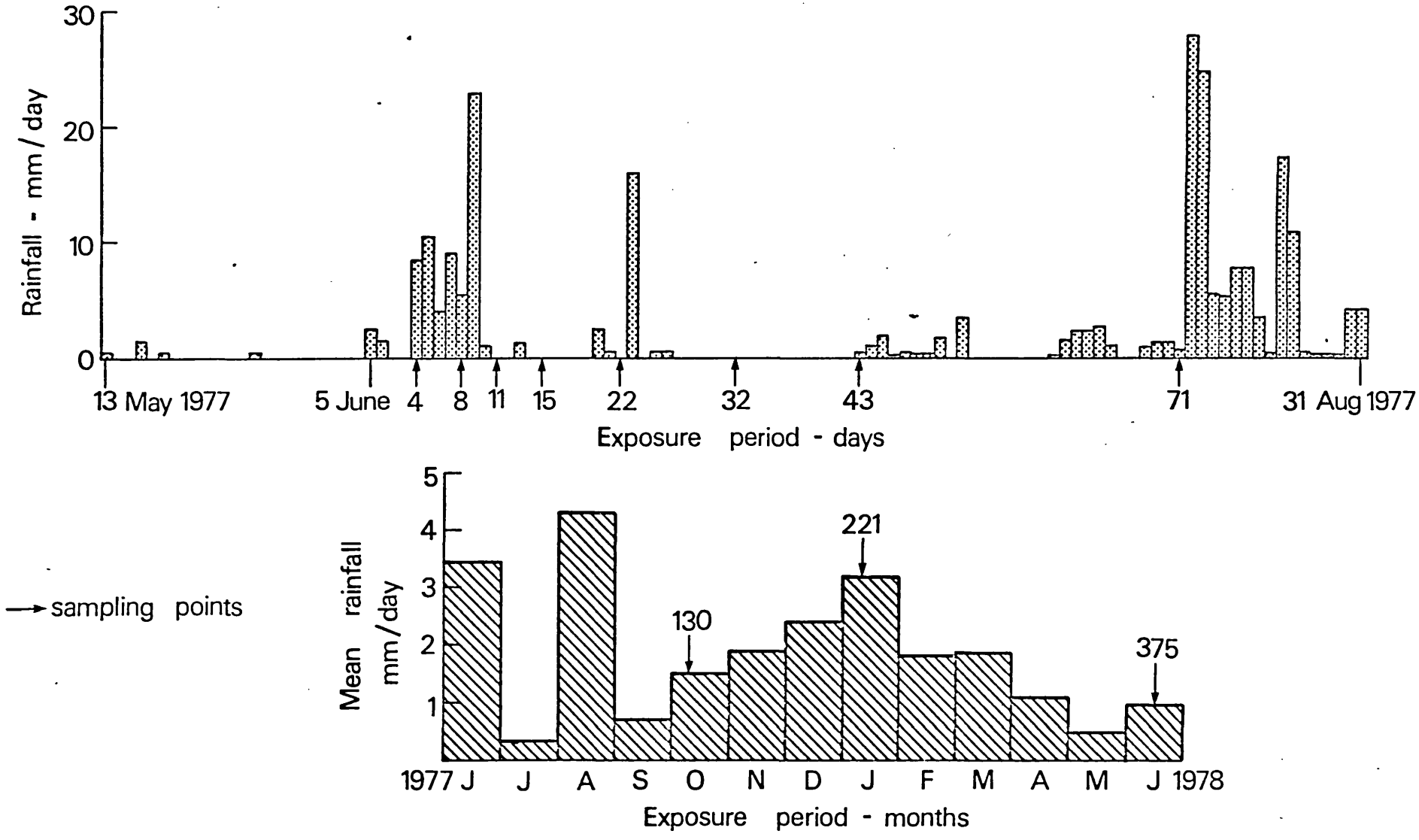
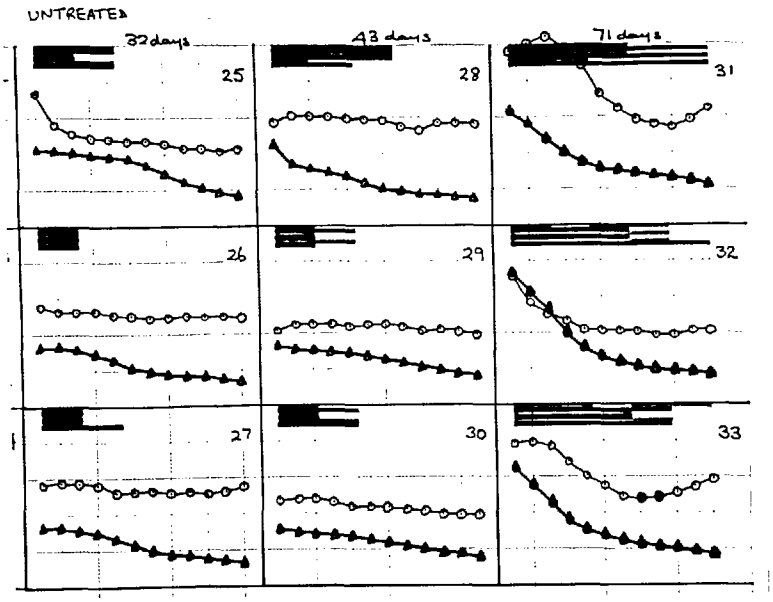


Fig. 4.20 Rainfall during the exposure period



Each rectangle records data for the L-joint identified by the number given top right.

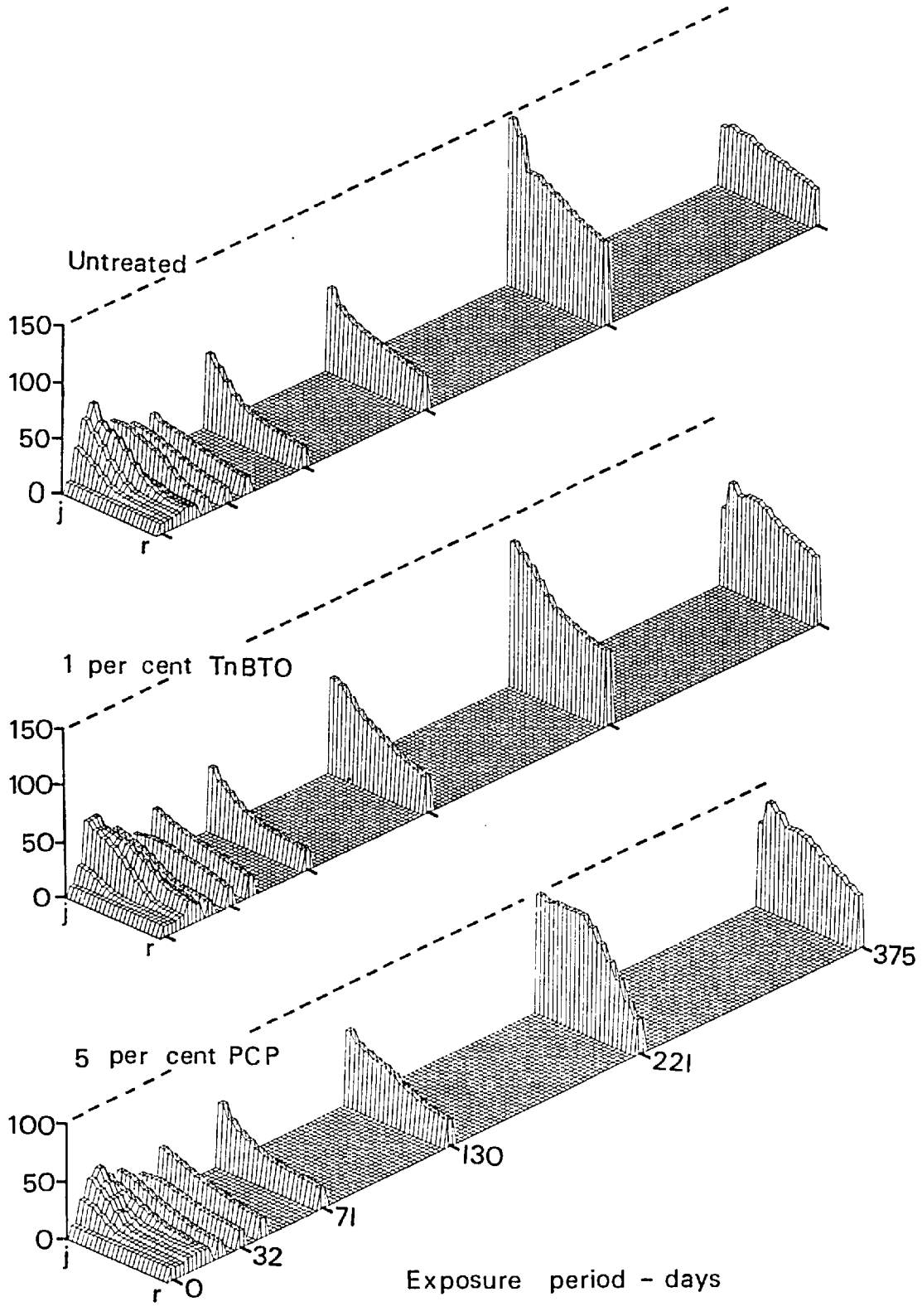
The left side of each rectangle represents the joint end, and the right side the end remote from the joint.

The vertical scale represents:
for permeability, a range of uptake of dekalin from 0-1.0 g
for moisture content, a range from 0-100 per cent.

Yellow bars - colonisation by bacteria
Brown bars - colonisation by fungi
The bar length represents the five sampling positions relative to the joint and the three pairs of bars, the positions through the depth of the sample - upper a, centre b, and lower c (see fig 4.11).

Red circles - permeability values
Blue triangles - moisture content values
Each point records the value for an individual sample block (see fig 4.7).

Fig 4.21 Initial data display system (untreated L-joints exposed for 32 to 71 days)



j Joint end (position 1)

r Remote end (position 12)

Fig 4.22 Moisture content of L-joints as a percentage of the final dry weight

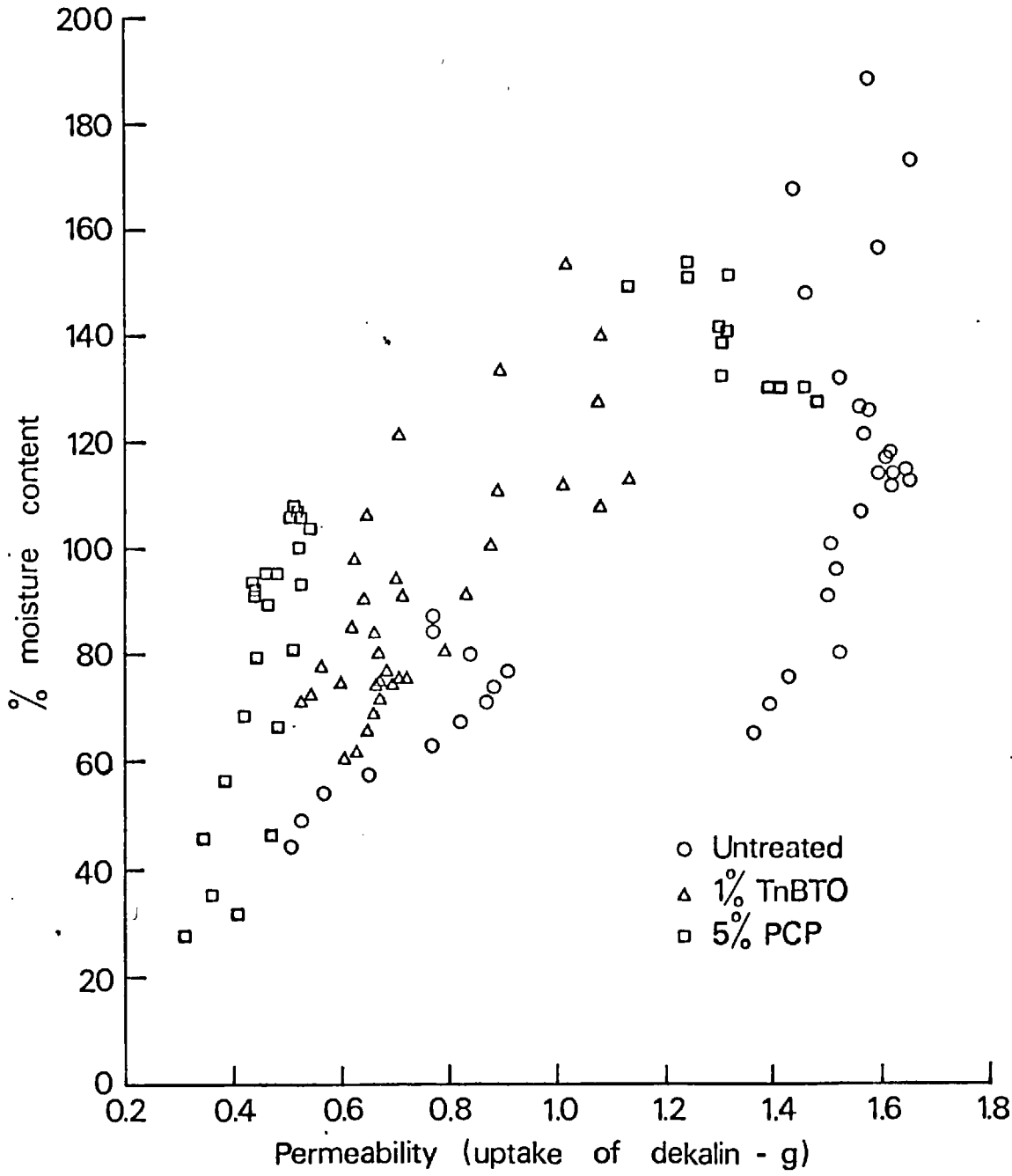


Fig. 4.23 Relationship between moisture content and permeability, after 221 days exposure

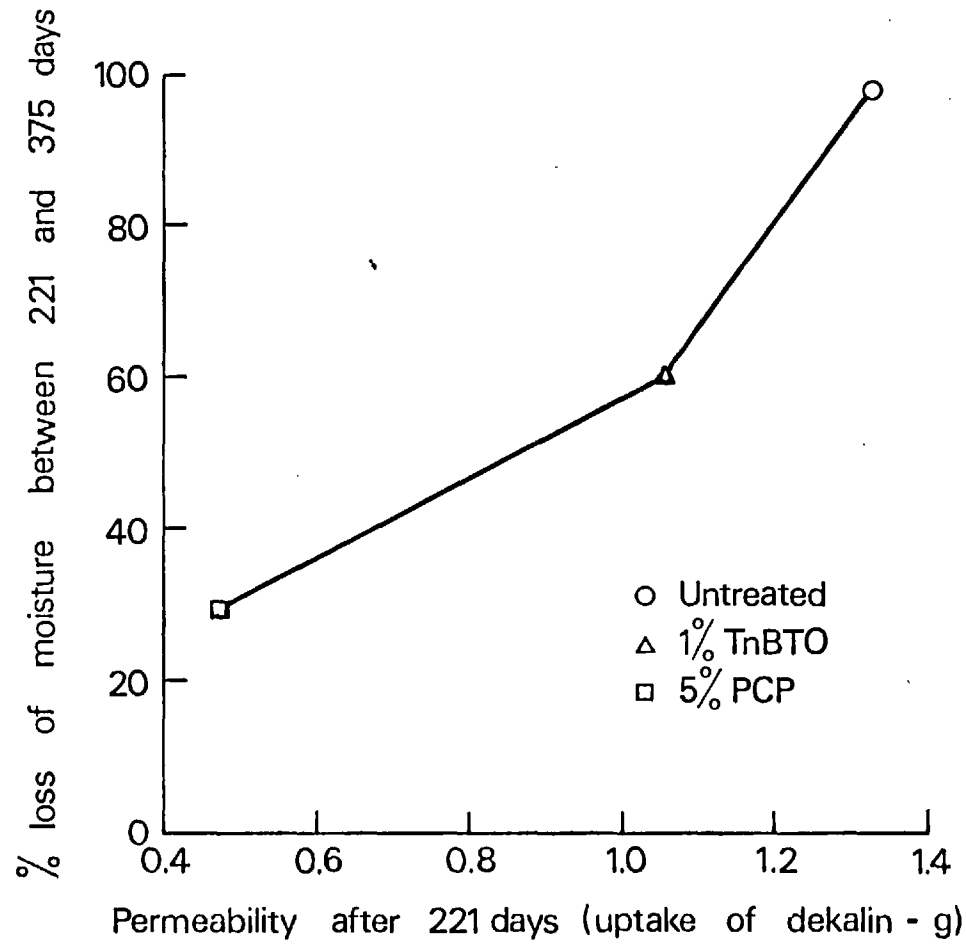
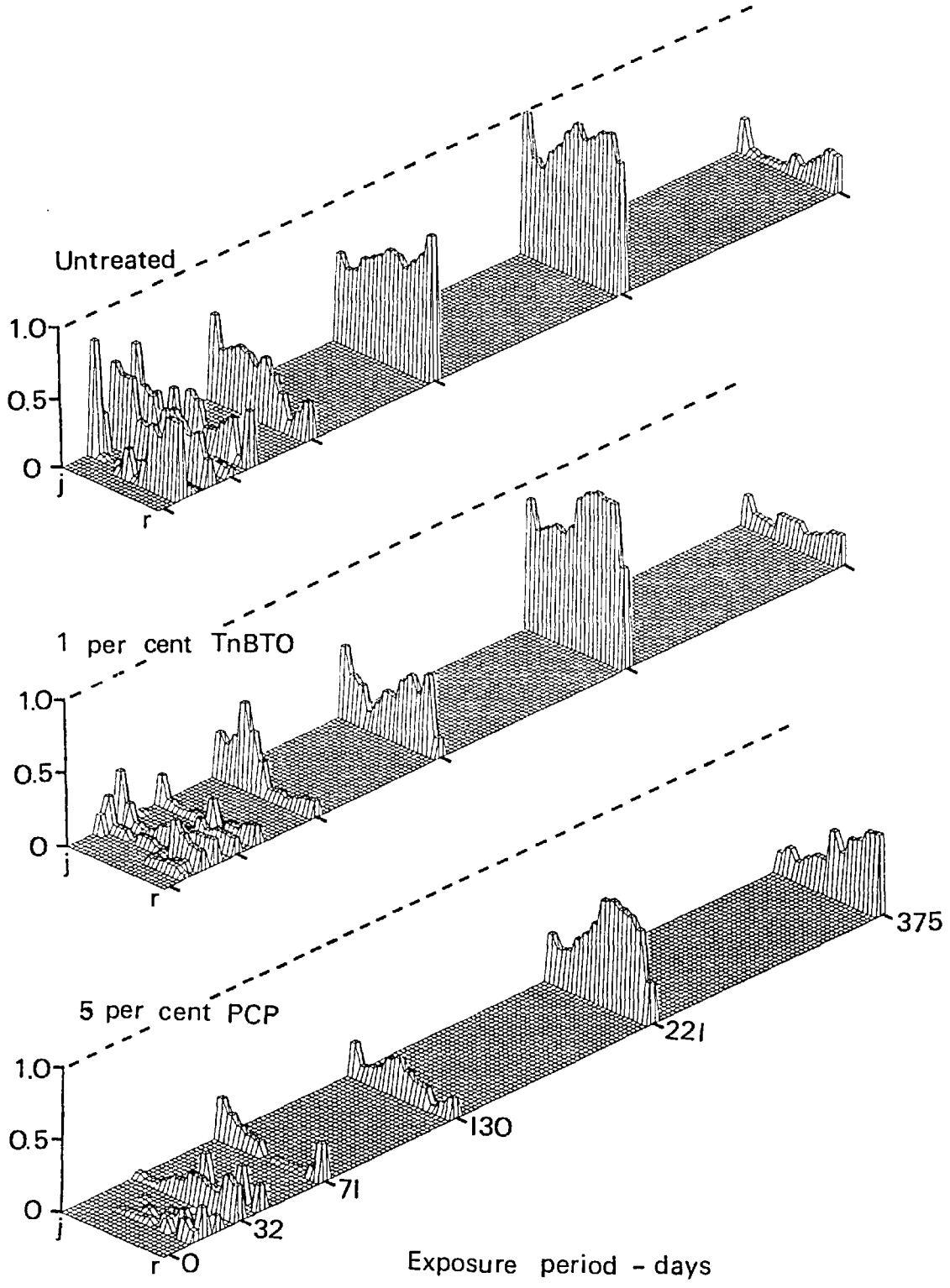


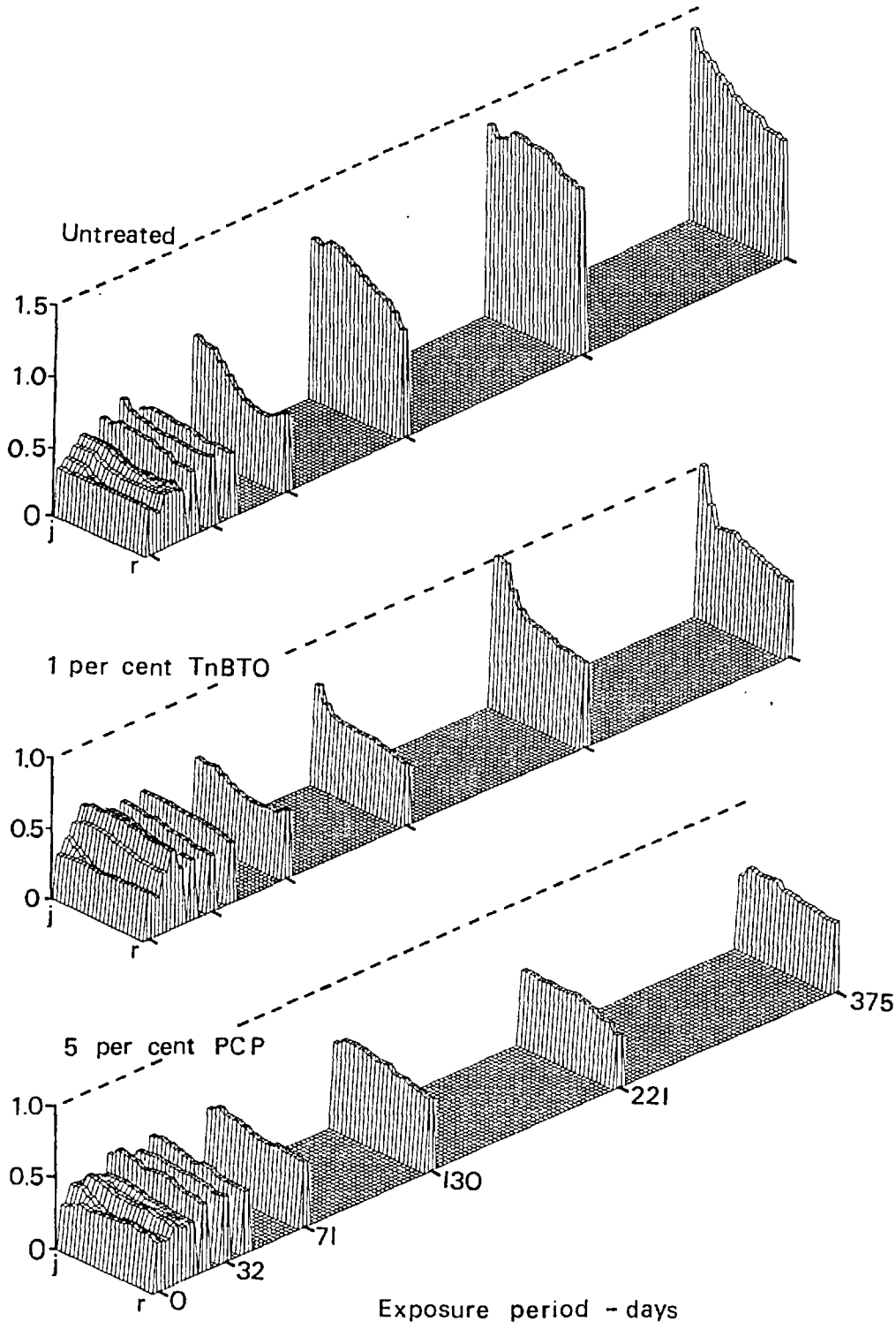
Fig. 4.24 Relationship between permeability and loss of moisture at sample position 1 (see fig. 4.7)



j Joint end (position 1)

r Remote end (position 12)

Fig 4.25 Carbon dioxide production as ml 0.05 N HCl



j Joint end (position 1)

r Remote end (position 12)

Fig 4.26 Permeability of L-joints expressed as uptake of dekalin in g

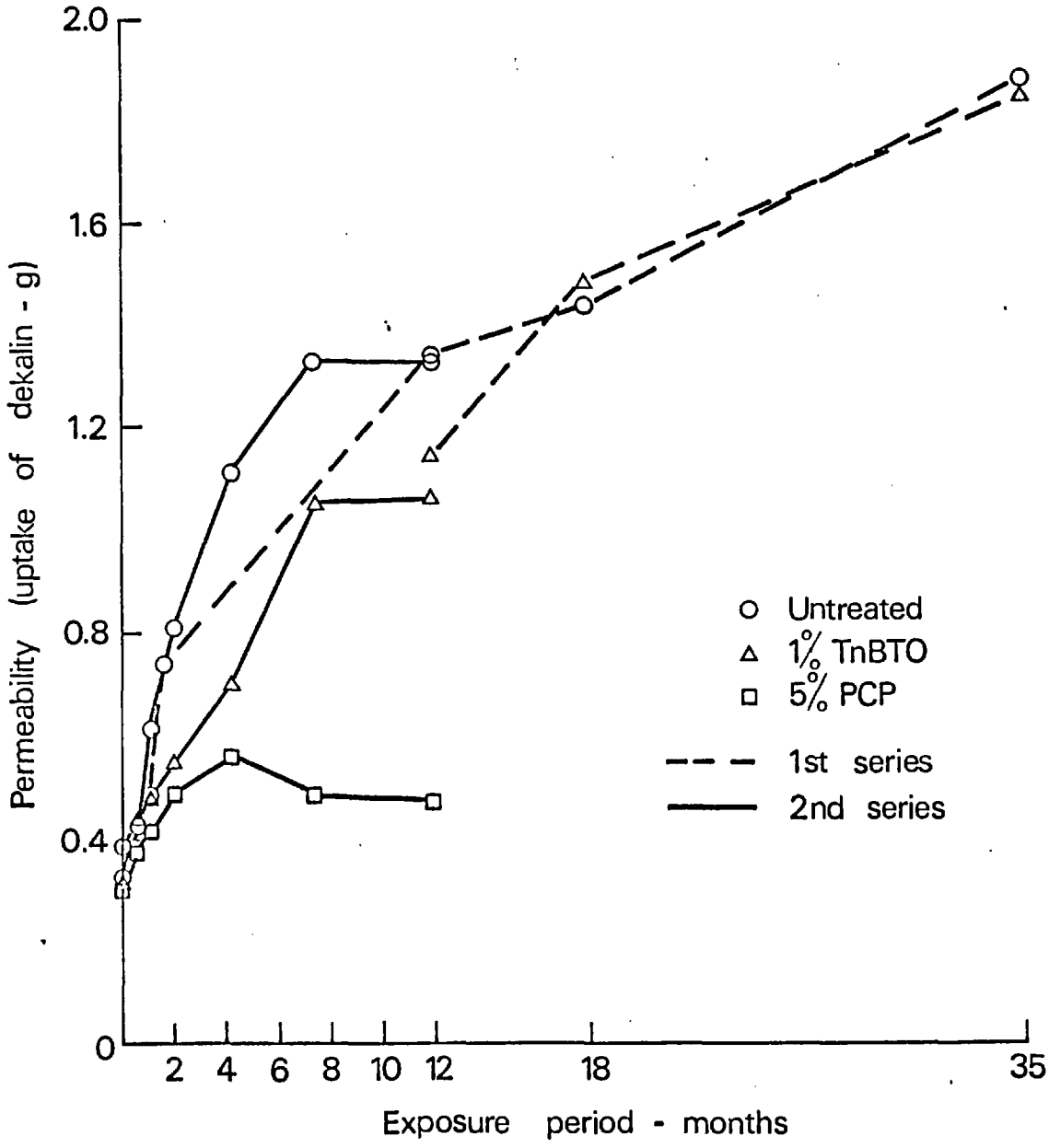


Fig. 4.27 Increase in permeability at the sample block 1 position (see fig. 4.7)

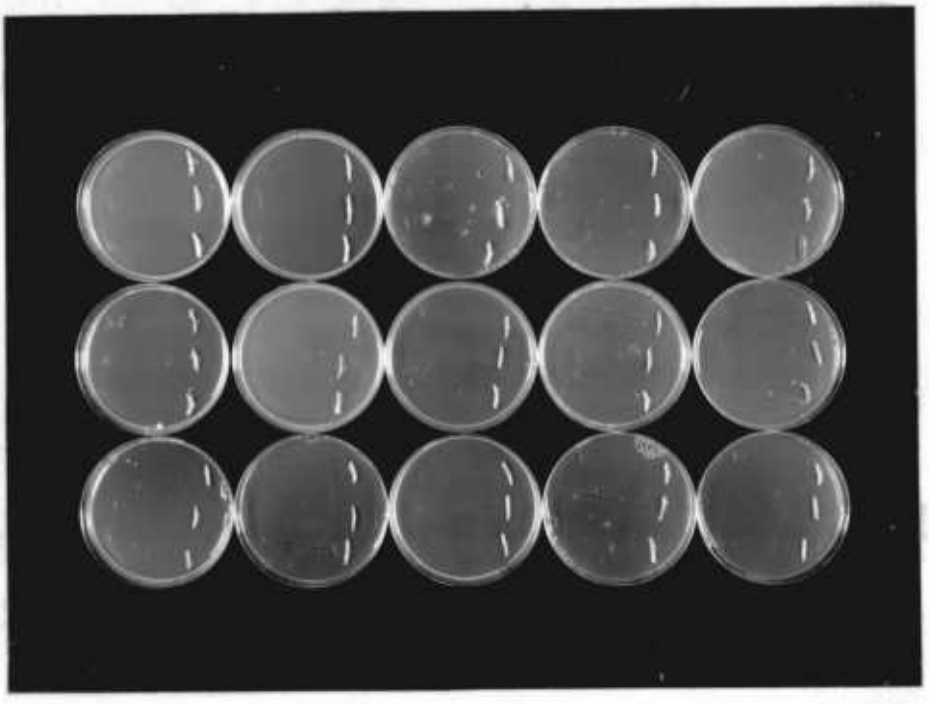


Fig 4.28 No bacterial growth from untreated, unexposed replicates

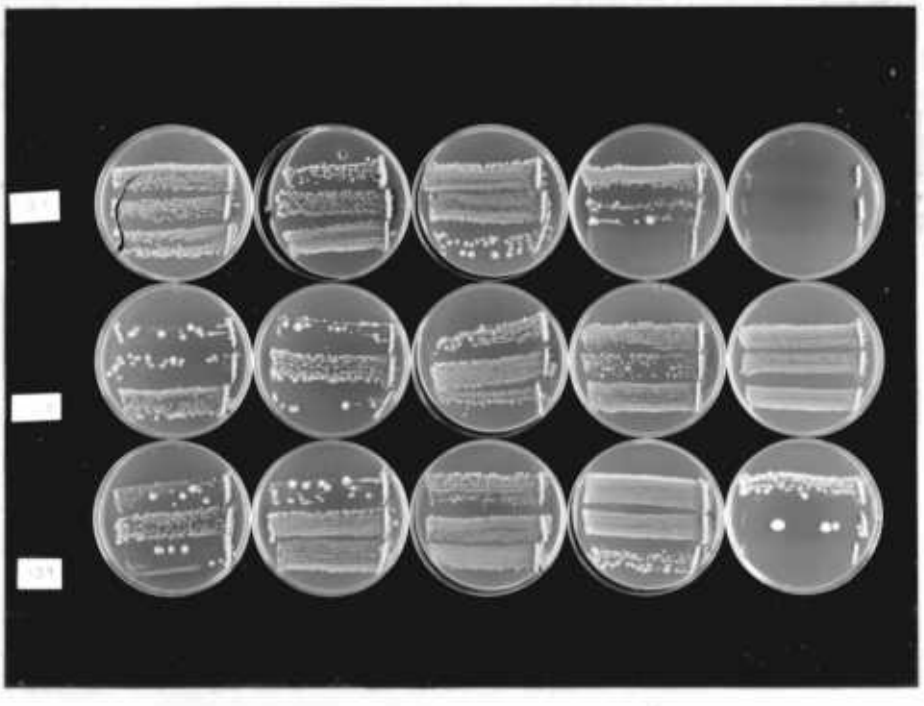


Fig 4.29 Bacterial growth from PCP treated replicates exposed for 221 days. Left-joint end, right-remote end. Chips from top, centre and bottom positions (see Fig 4.11)

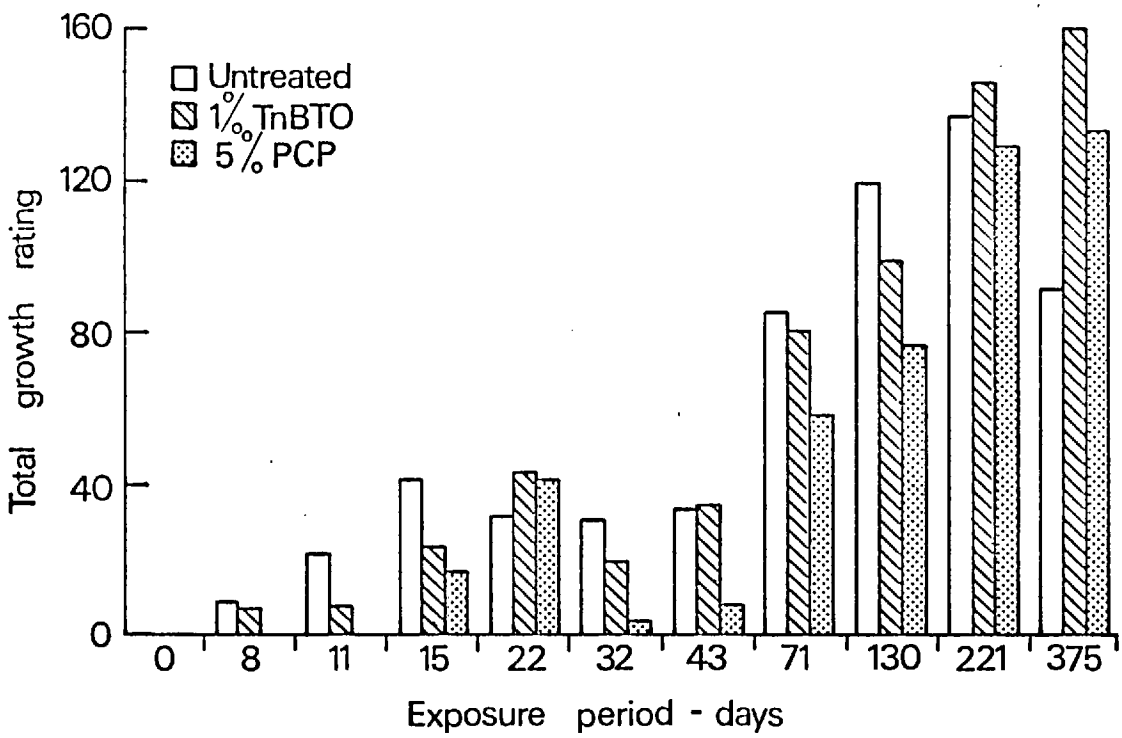
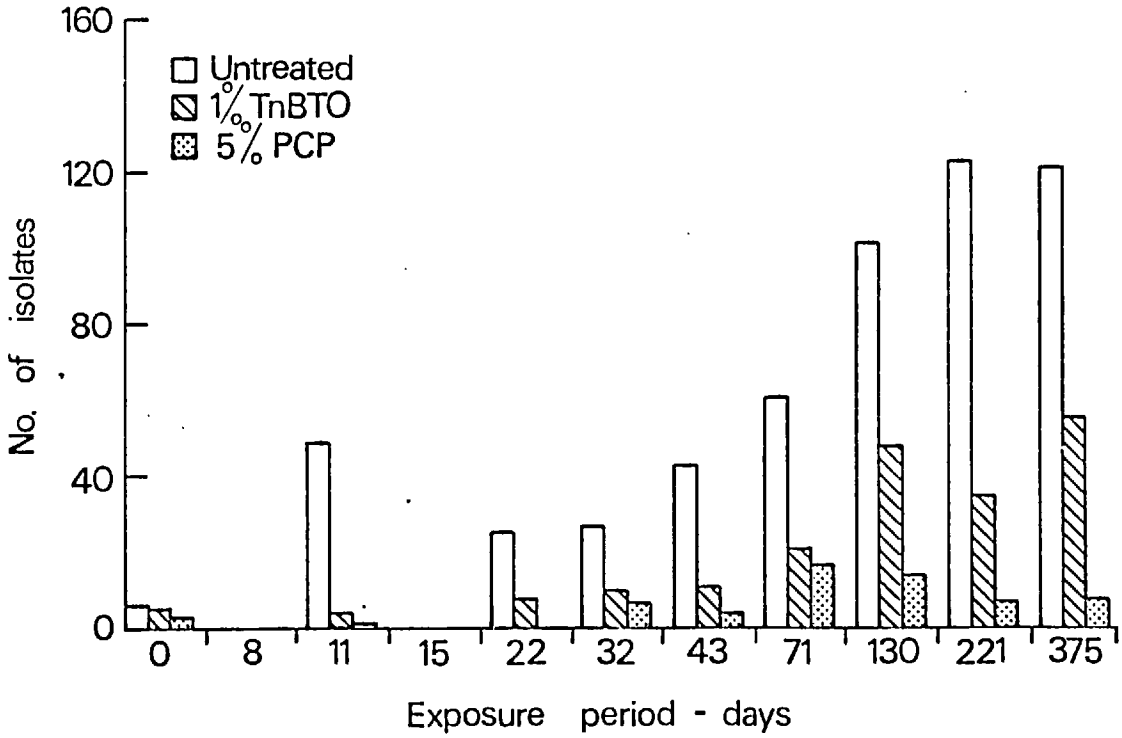
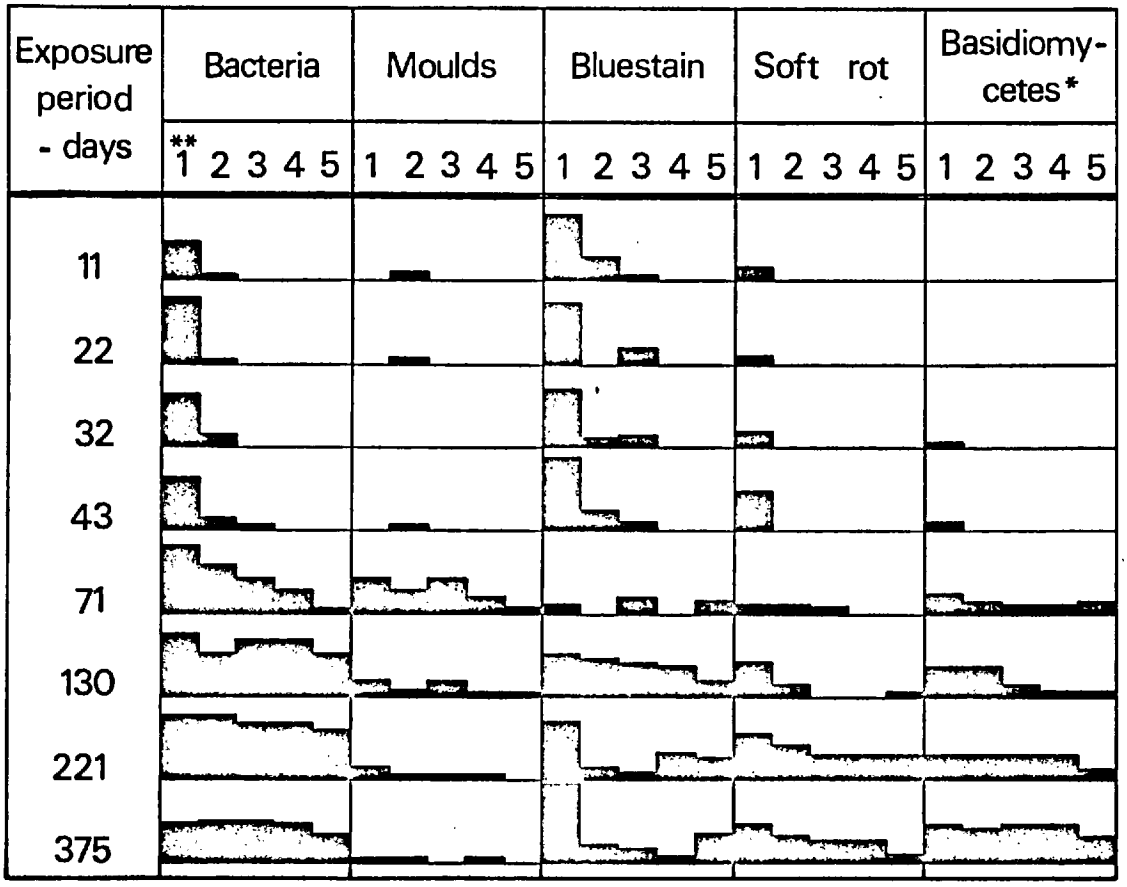


Fig. 4.30 Total bacterial growth rating



Note : No isolations were attempted after 8 and 15 days

Fig. 4.31 Number of fungal isolates



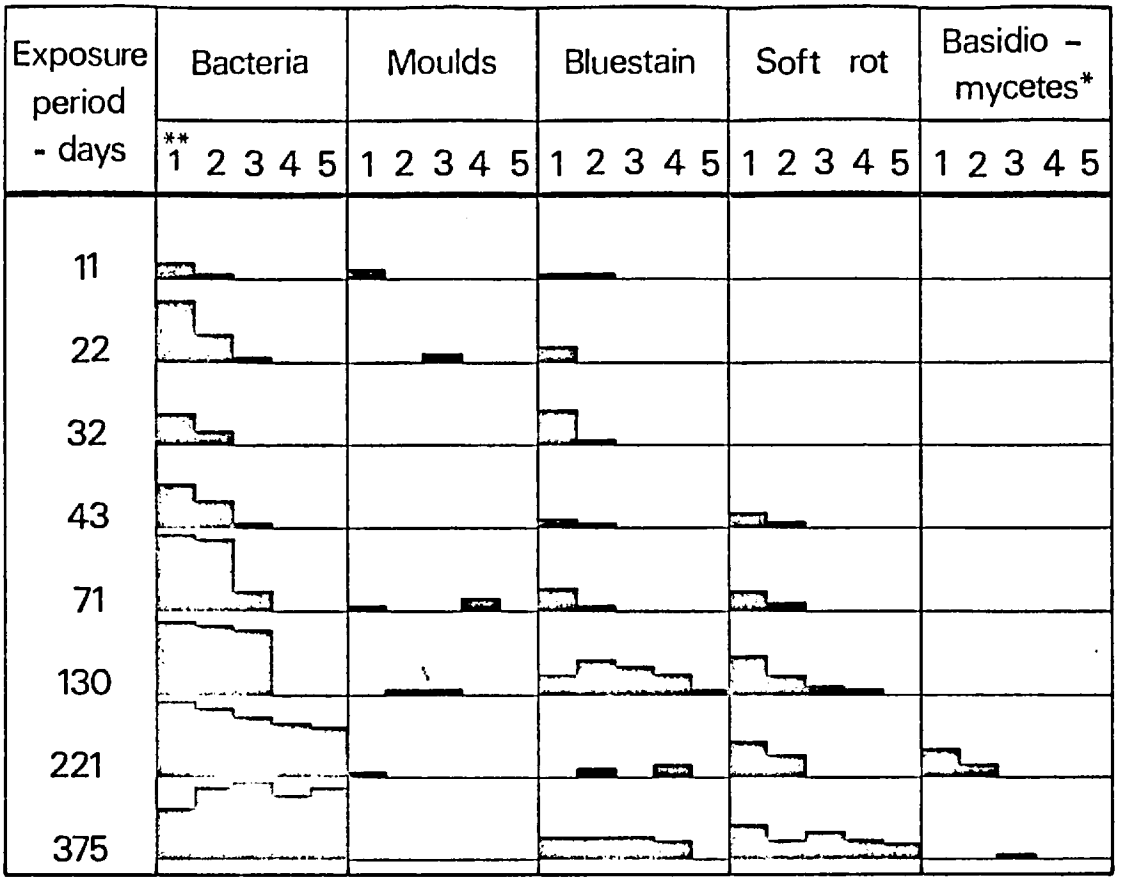
Untreated

* White rots plus Sistotrema brinkmannii

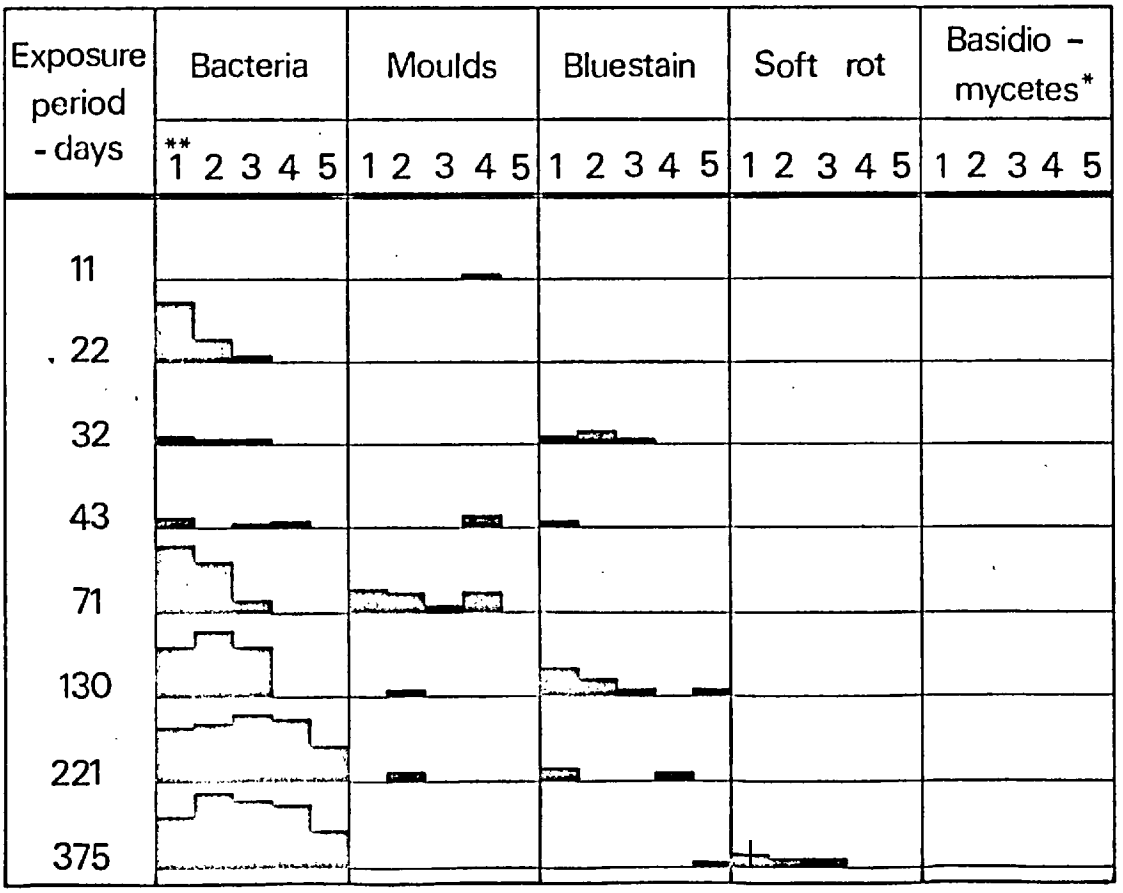
** Position relative to the joint (see fig. 4.11)

Note : fungi known to occur in unexposed wood have been omitted.

Fig. 4.32. Colonisation of untreated and preservative treated L-joints by bacteria and fungi.



One per cent TnBTO treated



Five per cent PCP treated

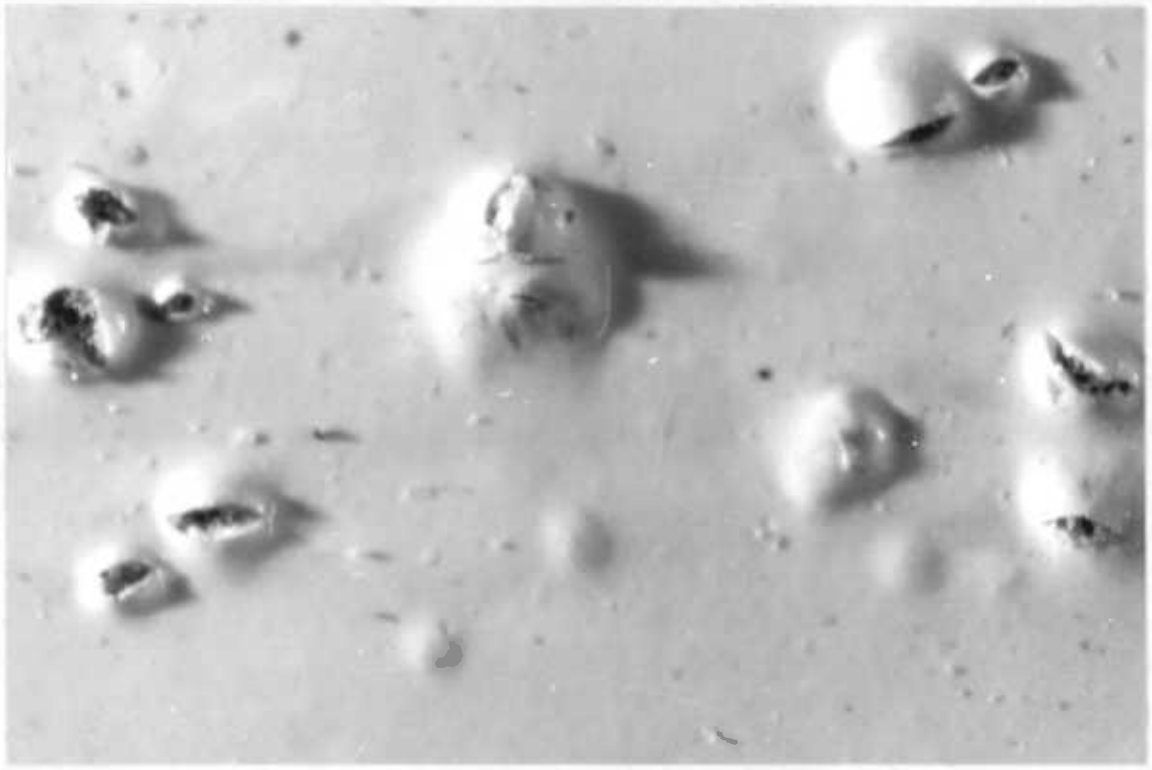


Fig 4.33 Pustules erupting through the paint film after 32 days exposure.

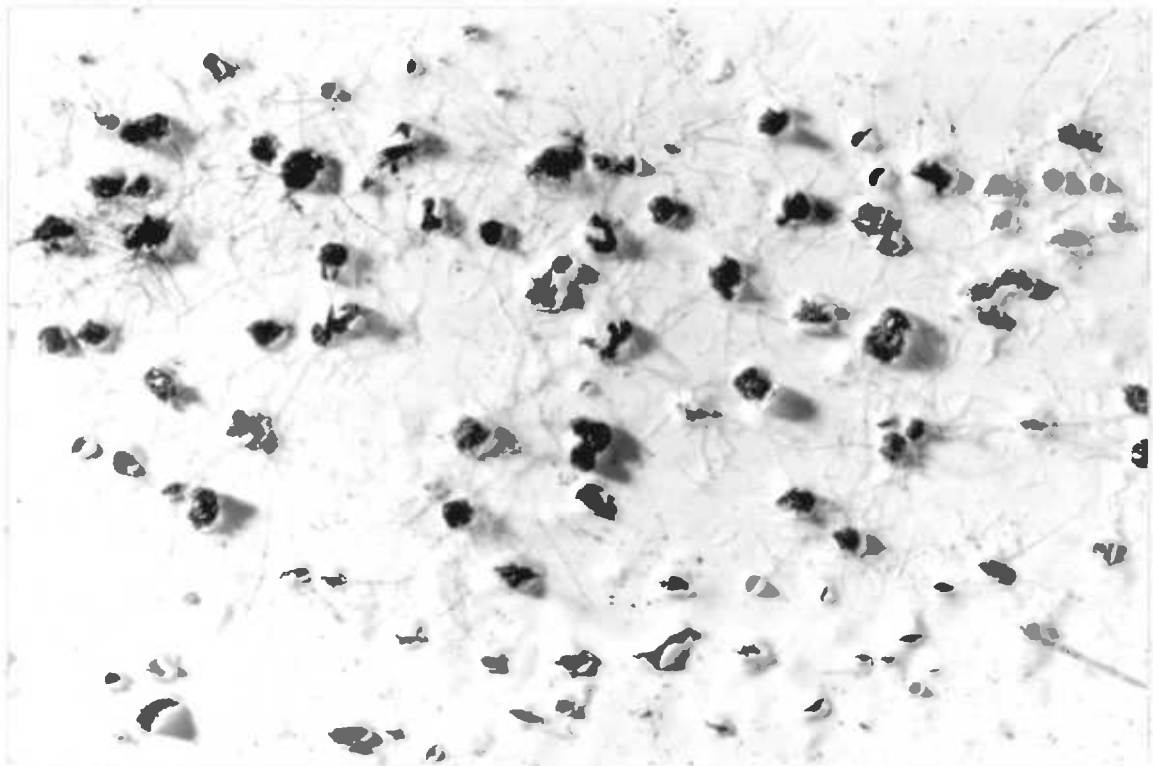


Fig 4.34 More mature pustules and superficial hyphal growth.

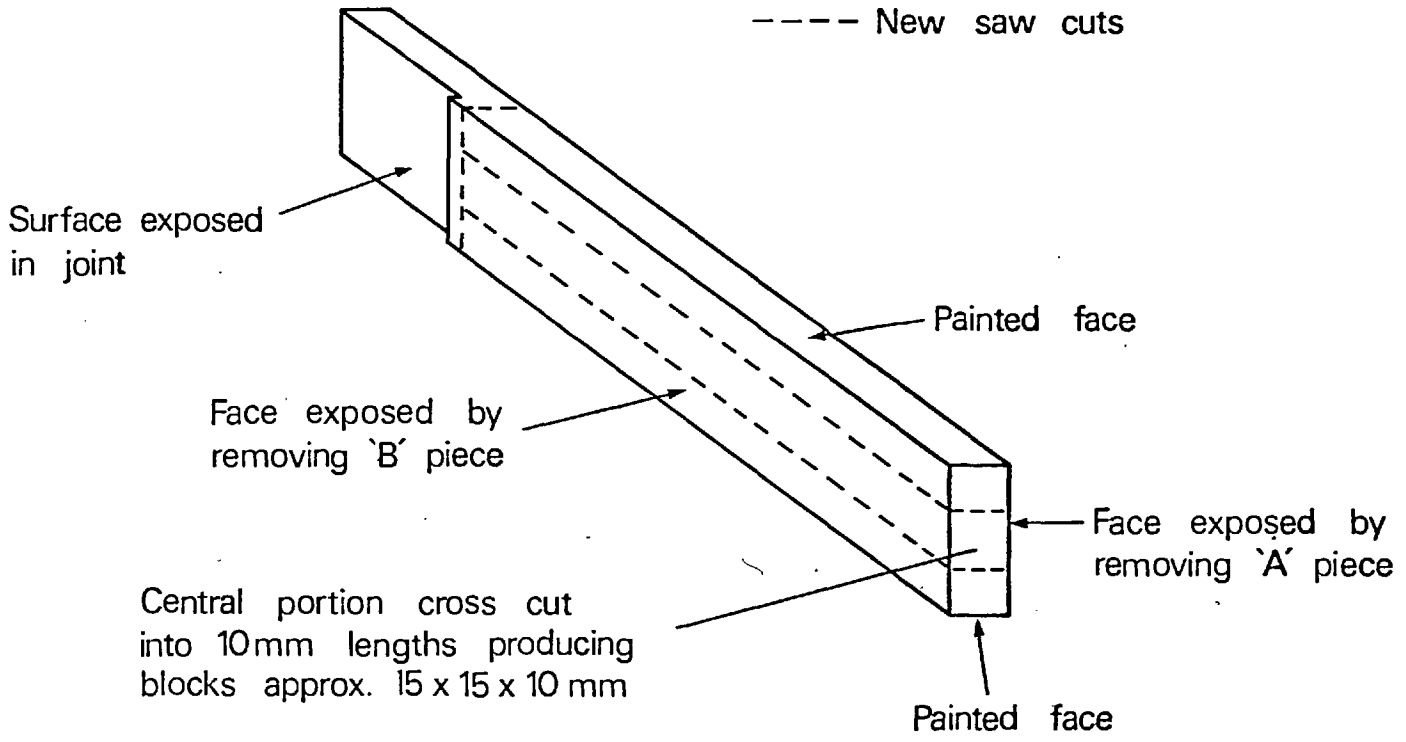


Fig. 4.35 Conversion for sampling the untreated core of L-joints

SECTION 5

FUNGI ISOLATED FROM SECOND SERIES L-JOINTS

5.1 INTRODUCTION

A total of 807 fungal isolates were separated during the isolation procedures in the second series L-joint tests (section 4). These were observed and identified where possible but the numbers involved together with work generated by the other facets of the investigation have prevented the process from being completed. Much of the time available has been devoted to the study of the Aureobasidium-type cultures which constituted a significant proportion of the total number of isolates (table 4.21). Additional experiments were undertaken with these organisms in an attempt to clarify the classification of this complex; these are reported in section 5.2. The remaining microfungi are documented, as far as possible, in section 5.3 and their possible rôle in the deterioration process discussed. The organisms have been classified as soft rots when thought to be capable of this type of attack. In the absence of sufficient evidence, the organisms have been classified as either bluestains or moulds depending on the pigmentation of their hyphae.

The Basidiomycetes, which, relatively, have been neglected, are documented in section 5.4. A small scale study of the organisms causing failure of the paint film on the L-joints after 71 days exposure is reported in section 5.5.

5.2 BLUESTAIN CULTURES OF THE *Aureobasidium pullulans* TYPE

5.2.1 Introduction

The true identity of *Aureobasidium pullulans* (de Bary) Arn, formally known as *Pullularia pullulans* (de Bary) Barkh, has been a source of considerable confusion over the years. Butin (1963) clarified the situation considerably by providing the basis for differentiating cultures of *P. pullulans* from those of *Sclerophoma pithyophila* (Corda) using pycnidium production, the shape of conidiogenous cells, hyphal characteristics and the maximum temperature for growth. Following Robak (1952), he considered *Hormonema dematioides* Lagerberg and Melin to be a synonym of *Sclerophoma pithyophila* but not of *Aureobasidium pullulans* although synonymy with *A. pullulans* had been accepted by both Ciferri et al (1956) and Cooke (1962). Furthermore, Butin showed various instances of work carried out on cultures originally identified as *A. pullulans* but subsequently shown to be *S. pithyophila* eg Schulz (1951), Tarocinski (1961). More recently, De Hoog and Hermanides-Nijhof (1977) have differentiated the organisms using the method of conidiogenesis. *Aureobasidium* is characterised by conidia produced synchronously, often from the tips of special structures protruding from the conidiogenous cells. *Sclerophoma pithyophila*, which they refer to by the Lagerberg and Melin name of *Hormonema dematioides*, produces conidia in basipetal succession from one or two points on each conidiogenous cell. This difference in conidiogenesis can be seen quite clearly in the drawings of Butin (1963) although he did not use it as one of his criteria.

With this rather confused background the task of identifying the numerous cultures, isolated from the second series L-joints (section 4), believed to belong to this complex, seemed daunting. During isolation, many of the cultures showed distinct morphological characteristics and a method of typifying them was sought. Each culture was grown on three different media, thus using the type of biochemical approach common in bacterial classification and gaining ground in certain areas of fungal taxonomy. This provided the basis for grouping various isolates; representatives from these groups have been used in further experiments.

5.2.2 Materials

5.2.2.1 Corn meal agar (Oxoid, code CM103)

The dehydrated medium contains:

corn meal extract (from 50 g whole maize) 2 g

agar 15 g

17 g was soaked in 1 litre of deionised water for 15 minutes then autoclave sterilised for 15 minutes at 121°C. 20 ml aliquots were dispensed, under aseptic conditions, into 90 mm diameter plastic disposable petri dishes.

5.2.2.2 Czapek dox agar (modified) (Oxoid, Code CM97)

The dehydrated medium contains:

	g per litre
sodium nitrate	2.0
potassium chloride	0.5
magnesium glycerophosphate	0.5
ferrous sulphate	0.01
potassium sulphate	0.35
sucrose	30.0
Oxoid agar No 3	12.0

45.4 g was soaked in 1 litre of deionised water for 15 minutes then autoclave sterilised for 20 minutes at 115°C. 20 ml aliquots were dispensed, under aseptic conditions, into 90 mm diameter plastic disposable petri dishes.

5.2.2.3 Abrams' agar (Abrams, 1948)

The medium contains:

	g per litre
ammonium nitrate ($NH_4 NO_3$)	3.0
di-potassium hydrogen phosphate ($K_2H PO_4$)	2.0
potassium di-hydrogen phosphate ($KH_2 PO_4$)	2.5
magnesium sulphate ($MgSO_4.7H_2O$)	2.0
agar	20.0

The ingredients were weighed out. The magnesium sulphate was added to a small quantity of water and the other ingredients to the remaining water. After autoclave sterilisation for 20 minutes at 121°C the two were mixed, under aseptic conditions, and 20 ml aliquots dispensed into 90 mm plastic disposable petri dishes. The magnesium sulphate is prepared separately to prevent precipitation of the other salts.

5.2.3 Methods

5.2.3.1 Growth on three media

Following isolation, cultures of the Aureobasidium type were inoculated in the centre of a plate of each of the following media, two per cent malt agar (2.3.1.2), corn meal agar (5.2.2.1) and czapek dox agar (5.2.2.2). The plates were incubated, in the dark, at 22°C. After 7 to 10 days, observations of gross colonial morphology were made and representative plates were photographed. The micromorphology on malt agar was studied by cutting 10 mm square blocks of agar from the advancing edge and more mature parts of the colony; one drop of aniline blue in lactophenol was placed on each agar block and a coverslip added before observation using a high power microscope and magnifications up to x600.

5.2.3.2 Coverslip cultures

After observations had been completed on the culture plates (5.2.3.1), samples were cut from representative plates using a 6 mm diameter cork borer. These were transferred, mycelium side uppermost, to a sterile microscope slide placed on top of a layer of 2 per cent sodium fluoride 2 per cent agar in a petri dish; each plug was covered with a sterile No 0 coverslip (fig 5.1). These assemblies were incubated at 22°C until the mycelial front was approximately half way between the plug and the edge of the coverslip. Each coverslip was carefully removed, washed thoroughly in 70 per cent ethanol and allowed to air dry. The washing served the dual purpose of removing excess conidia and fixing the remaining conidia and hyphae. When dry each coverslip was gently lowered on to a minimal quantity of aniline blue in lactophenol, gently warmed and observed. Representative areas were photographed.

5.2.3.3 Growth in Scots pine sapwood

10 mm cubes of Scots pine sapwood, with the faces orientated in the radial, tangential and transverse planes, were numbered then autoclave sterilised at 121°C for 20 minutes. Sterile 50 mm square pieces of filter paper were placed on top of 20 ml aliquots of normal Abrams (1948) agar (5.2.2.3) previously dispensed into 90 mm diameter petri dishes. Each corner of a filter paper was inoculated with one of the test fungi; a Scots pine block was then placed on top of each inoculation point, with a tangential face in contact with the filter paper. Under aseptic conditions, one block was removed from each dish after 2, 4, 6 and 8 weeks incubation at 22°C. The blocks were initially placed in formalin-acetic acid-alcohol fixative; later thin sections were cut in the radial longitudinal plane, using a sledge microtome. These were stained and mounted as described in TIL 52 (Anon, 1974) then observed to follow the progress of colonisation by the organisms. Representative areas were photographed.

5.2.3.4 Growth rate and temperature response

7.5 mm plugs were cut, using a sterile cork borer, from young plates of each test organism. One plug was placed in the centre of a plate of two per cent malt agar (2.3.1.2) with the mycelium in contact with the fresh substrate. Three replicates were incubated at each temperature. The diameter of each colony was measured on each working day along two predetermined axes, marked on the bottom of each dish, until the faster growing cultures had covered the plates. Plates incubated at the two lowest temperatures were observed over longer periods. Representative plates were photographed.

5.2.4 Results

The method used to produce coverslip cultures is shown in Fig 5.1.

The growth characteristics, on the three agar media, of the various bluestain groups are recorded in Table 5.1 and illustrated in Figs 5.2 to 5.11; the "not dense" growth of groups B and K is shown in Fig 5.12. Details of the micromorphology of each group are also given in Table 5.1 and various features illustrated in Figs 5.13 to 5.20.

The progressive colonisation of Scots pine sapwood blocks by a representative of the five major groups (A, B, C, D and K) is summarised in Table 5.2 and various features illustrated in Figs 5.21 to 5.23.

The problems associated with determining the growth rates of the fungi at a range of temperatures are illustrated by Table 5.3 and Figs 5.24 and 5.25. The growth rate of each organism at each temperature is recorded in Table 5.4 and the temperature response curves, constructed from these data, are presented as Fig 5.26. The division of the organisms based on these curves is shown in Fig 5.27. Representative plates at a range of temperatures are shown in Figs 5.28 and 5.29 for a type K and a type B organism respectively.

Classification of the bluestain groups according to Butin (1963) is shown in Table 5.5 and according to de Hoog et al (1977) in Tables 5.6 to 5.8.

5.2.5 Discussion

5.2.5.1 Growth on three media

The three media used for the growth of the organisms provide very different environments. The malt agar contains an easily accessible supply of sugars whilst the corn meal agar is a nutritionally impoverished medium recommended for the maintenance of stock cultures of fungi, especially the black-pigmented varieties. The czapek dox agar is a semi-synthetic medium containing sodium nitrate as the sole source of nitrogen.

All organisms grew well on the malt agar producing intensely pigmented hyphae (except group A) apart from the colony margin, and large numbers of conidia, but, as Figs 5.2 to 5.11 and Table 5.1 show, had a variable morphology. On corn meal agar most organisms grew more slowly than on malt agar; again a variety of morphological types were produced. On czapek dox agar a proportion of the cultures failed to produce significant growth; others produced growth, often very yeast-like, but without dark pigmentation.

On the basis of the gross morphology, the organisms were originally divided into groups A to J; the micromorphology within these groups appeared similar (see 5.2.5.2). Subsequently group F organisms were discarded as not belonging to the Aureobasidium type organisms and an additional group, K, was introduced. Finally, group E was considered to be a slight variant of group H. Some organisms tested did not give reactions allowing them to be placed in these groupings; in Table 4.23 these are recorded as "Bluestain (no type given)". This result suggests additional groups may exist although some of these organisms are no longer considered to fall within the Aureobasidium type group. More commonly, time was not available to process all the cultures; these are recorded as "Bluestain (not type tested)". At a later date (approximately one year after isolation), when an attempt was made to process some of these organisms, it was found that a larger proportion, than when freshly isolated organisms are tested, did not fit completely into the existing groupings. It is believed this is due to prolonged maintenance on sugary media and the resultant changes in physiology; in the case of the Basidiomycetes it has been shown that some lose the ability to decay wood under these conditions (PRL, unpublished data). These changes may prevent this system of grouping being extended to existing isolates although two long-standing cultures originally proposed by Butin as test fungi, now accepted in a draft European Standard for standardised test use (P. pullulans FPRL No S9E and S. pithyophila FPRL No S149A), were placed in groups J and K respectively.

5.2.5.2 Micromorphology

The micromorphology of the cultures was observed, initially, on malt agar by removing 10 mm squares of agar from the plates, adding stain and a coverslip, then observing using magnifications up to x600. These observations showed similar micromorphological features within each of the groups separated by growth on the three different media. In most cultures detailed observations were made difficult by the numerous free conidia, the density of growth and the intensity of pigmentation. In an attempt to observe the differences in micromorphology under better conditions, a representative from each group was grown in coverslip culture (5.2.3.2).

Most mycological textbooks describe one or more of the methods available for producing coverslip cultures and so the method chosen is a matter of personal preference. Providing a moist environment in which the cultures can grow across the coverslip without being troubled by contamination has been overcome in various ways. The use of sodium fluoride in agar in the bottom of a petri dish provides a simple self-sterilising humidity chamber which can be reused; this system does not appear to have been used before. The slides which resulted from the use of this technique were easy to observe and photograph.

The culture of type A only produced hyaline hyphae and was not photographed. Cultures of types B, C, D, E, G, H and J all produced conidia together with various other morphological features. These are recorded in Table 5.1; representative photographs are presented as Figs 5.13 to 5.16. In the absence of clear evidence of synchronous production of conidia of the type described for Aureobasidium by de Hoog et al (1977) it was thought all these groups belonged to the Hormonema genus. In contrast, conidiogenesis in group A, which was subsequently observed on corn meal agar (Fig 5.17), was characterised by the conidia being produced synchronously, often at the tips of specialised structures, as described for Aureobasidium. The isolation of group A from the rest, based on no conidiogenesis in coverslip culture and synchronous production of conidia, seemed logical. However, because of the importance of the method of conidiogenesis in identifying the cultures using the de Hoog et al (1977) system, members from each group were observed at a later time, growing on corn meal agar. By observing submerged hyphae, which was only possible due to the diffuse superficial growth on this medium, a very different picture emerged. With the exception of type B, all groups including I and K, exhibited conidiogenesis characteristic of Aureobasidium, with the groups of synchronously produced conidia held in place by the agar. This condition could be seen in the superficial mycelium as well but was confused by the presence of free conidia in many areas. In retrospect, indications of synchronous production of conidia can be seen in some of the coverslip culture photographs (figs 5.14 and 5.16).

In contrast to the other groups, conidiogenesis in the type B cultures was only associated with concentrations of thick-walled, darkly pigmented, very septate hyphae, perhaps constituting a rudimentary

pycnidium (fig 5.18, top). These hyphae also developed longitudinal septa, most easily seen in non-conidiogenous areas (fig 5.18, centre). Although two or three conidia were sometimes associated with the same cell, none of the large clumps of conidia (as seen in all other groups - see fig 5.17) were observed (fig 5.18, bottom). It is considered, therefore, that conidia in type B cultures are produced in basipetal succession usually from one, rarely from two or three points per cell; a characteristic of Hormonema species according to de Hoog et al (1977).

The difficulties encountered while observing conidiogenesis emphasise the importance of good descriptions of methodology in published work; where details are similar, line drawings, which incorporate the interpretation given by their originator, could be supplemented with photographs portraying that which the newcomer may expect to observe. The idea of observing submerged hyphae, where the conidia are held in place by the agar, is an obvious solution to the problem of free conidia obscuring detail, but is possibly only on a medium which permits diffuse growth.

Other interesting morphological features have been observed in some cultures. Group E/H organisms produced conidia at the tips of hyphae more often than other groups (fig 5.19, top). In addition they produced pigmented conidia (fig 5.19, centre) of similar length, but much wider than the hyaline conidia, apparently by the same method of conidiogenesis. Pigmented blastoconidia are not feature in previous descriptions although pigmented arthroconidia have been described (de Hoog et al, 1977). These cultures also produce a peculiar filamentous growth on corn meal agar when freshly isolated (fig 5.19, bottom). The group I organism shows a similar type of growth. Again this does not appear to have been described previously for Aureobasidium although a similar feature is described by de Hoog et al (1977) for Hormonema prunorum (Dennis and Buhagiar) Hermanides-Nijhof.

The type I organism, when grown in coverslip culture, produced only hyaline hyphae, away from the inoculum, bearing clamp connections. In various places the transition, along a single hypha, from pigmented without clamps to hyaline with clamps, was observed (fig 5.20) thus ruling out the possibility of a contaminating organism being responsible.

When repeated, over a year later, pigmented hyphae bearing conidia were produced. This suggests the ability to produce clamps had been lost during the period in culture. The possibility that an Aureobasidium type culture can be produced by a Basidiomycete does not appear to have been suggested previously but other bluestains such as Burgoa are linked with the Basidiomycetes (Goidànich et al, 1938; Weresub and LeClair, 1971). Alternatively, the Basidiomycete may have been growing as a parasite on the Aureobasidium.

5.2.5.3 Growth in Scots pine sapwood

A representative from each of the five most commonly occurring groups (A, B, C, D and K) was grown back on to Scots pine sapwood and the progress of colonisation observed after 2, 4, 6 and 8 weeks. The results, summarised in Table 5.2, show differences between the organisms particularly in the pigmentation of the hyphae and the time of production of conidia. In addition there were slight differences between the morphology of mature pigmented hyphae. However, because of the lack of replication, it is not known if these features would remain consistent in replicate experiments with the same isolate or with other members of each bluestain group and therefore only general observations are pertinent. All cultures produced conidia within the wood (fig 5.21). It is possible these conidia may be able to increase the rate of colonisation if they can be carried through the wood by liquid water. The initial rate of colonisation in the L-joints (see section 4.5.7) was very rapid and perhaps can be accounted for by this process. Widespread colonisation by pigmented hyphae (fig 5.22) occurred during the 8 week incubation period confirming all the cultures are capable of producing bluestain, but there was no direct evidence of them penetrating tracheid walls other than via the bordered pits. One organism (type C) produced large aggregations of pigmented hyphae (fig 5.23). Since the sections were cut from the superficial layers of the wood cubes, these may be associated with pycnidium development.

5.2.5.4 Growth rate and temperature response

In the literature three types of measurement of growth rate on a solid nutrient substrate are recorded. First, as described previously (section 2.3.4.2), the growth is divided into a lag phase, a mid phase and a senescent phase. The mid phase, characterised by a steady rate of increase in diameter, is regarded as the true growth rate. Growth

may also be recorded as the diameter after a given period of time or as the time necessary to reach a given diameter. These latter methods are more simple but can give a significantly different result from the first method as shown by the following examples.

The growth curves for two organisms are presented in Figs 5.24 and 5.25 together with the temperature response curves produced by calculating the growth rate over various parts of the growth curve (table 5.3). Fig 5.24 shows the effect of incorporating part of the senescent phase of growth. Cultures incubated at 17, 21 and 24°C produced less growth each day over the period 5-8 days. Consequently the temperature response curve calculated over this period shows lower rates of growth at these temperatures and as a result the apparent optimum growth temperature is raised to above 27°C whereas the other three curves give an optimum temperature of 23-24°C. In the initial part of the senescent phase at 21 and 24°C (5-6 days growth) the temperature response curve has not changed shape but the growth rate is lower than that recorded over days 1-5. At lower temperatures (6 and 11°C) the lag phase appears to last longer so that the curves calculated using data recorded in the initial stages of growth (0-7 days and 1-5 days) show lower growth rates. This effect can be seen more clearly in Fig 5.25 where the effect is most pronounced at 17°C. The two more simple methods both incorporate lag phase growth and thus are subject to similar inaccuracies.

Whilst aware of these problems, the temperature response curves have been derived for cultures placed within each group based on their growth characteristics on the three media. Growth of each organism at each temperature has been considered individually and that part of the growth curve deemed to represent the mid phase used to calculate the daily growth rate (table 5.4). Colony diameters after 7 days are also available for comparison where necessary with data presented by de Hoog and Hermanides-Nijhof (1977).

The temperature response curves (fig 5.26) generally show good reproducibility within groups particularly A, C, G and K; groups B and E/H show greater variation than the others. The shapes of the curves vary. Some show the normal pattern of response to temperature with the growth rate falling rapidly above the optimum eg groups C and

K; in others this effect is less pronounced and at times the curves are almost symmetrical eg group D. The curves are sufficiently characteristic to be used to separate various groups of organisms (fig 5.27). There is a major division into those organisms able to grow at 35°C and those which cannot. Among the former group, there is variation in optimum growth rate but otherwise the curves are similar and cannot, therefore, be subdivided; this group includes bluestain groups E/H, G, I and J. The organisms unable to grow at 35°C can be subdivided into 4 groups based on differences between the growth curves. Bluestain group C is characterised by an optimum above 27°C and bluestain group D by an optimum below 21°C. The remaining organisms have optima in the range 23-25°C but can be divided on the growth rate. Bluestain group A organisms have a maximum of less than 7 mm/day while the others, which includes bluestain groups B and K, have maxima of greater than 8 mm/day. Bluestain groups B and K can, however, be distinguished since group K organisms produce almost non-pigmented growth at 27°C (fig 5.28) while group B organisms produce more normal growth (fig 5.29) although the hyaline margin is considerably wider than at lower temperatures; group B organisms tend to grow faster than group K organisms at 27°C.

Although the temperature response curves do not enable any subdivision of those organisms capable of growth at 35°C, the ability to distinguish, individually, the five bluestain groups unable to grow at 35°C gives considerable support to the groupings derived by growth on the three media.

More generally, the temperature response curves are very interesting. This group of morphologically similar organisms has a wide range of optima from below 21°C (group D) to above 27°C (group C) and all organisms produced some growth over the range 5/6 to 31°C (up to 1.9 mm/day at 6°C; up to 4.4 mm/day at 31°C). Of those unable to grow at 35°C, only certain replicates belonging to groups A and B failed to produce growth when incubated at their optimum temperature after 8 days at 35°C. This indicates considerable "thermodurance" by the majority of these organisms. This, together with growth at low temperatures, would make them well adapted to the joinery situation

where wide fluctuations in temperature have been recorded (Morton, 1975) and could help to account for the preponderance of Aureobasidium type fungi isolated from the L-joints during the early stages of exposure.

5.2.5.5 General Discussion

The Aureobasidium type fungi were originally divided on the basis of their growth characteristics on three different media (section 5.2.5.1). These divisions have subsequently been supported by micromorphological details and temperature response curves; the growth, of a representative from each of five groups, on Scots pine sapwood does not contradict the groupings. The groups are, therefore, based on widely differing criteria.

As a natural extension of the studies an attempt has been made to identify each group using previously published methods (Butin, 1963; de Hoog and Hermanides-Nijhof, 1977). The two schemes use different features to differentiate Aureobasidium (Pullularia) from Hormonema (Sclerophoma) (tables 5.5 and 5.6) but both give culture descriptions; these differ on two points. First, Butin states that chlamydospores in Aureobasidium are terminal but de Hoog et al describe them in chains. Secondly, Butin states that conidiogenous cells in Aureobasidium are usually terminal but when conidia are formed within the mass of mycelium, then the cells are at least twice as long as wide; de Hoog et al describe conidiogenous cells as often wider than long. In addition, pycnidium production, which Butin states belongs exclusively to Sclerophoma, has been shown to occur in Aureobasidium by Xenopoulos and Millar (1977). Because of the confusion, the two schemes have been applied separately to the bluestain groups.

Using Butin's (1963) criteria (table 5.5) four groups (B, C, D and K) possess only Sclerophoma characteristics, one group (G) possesses only Pullularia characteristics and the remainder a mixture, in varying proportions. Two groups (C and D) possess the same characteristics using this system although in terms of gross morphology, they are very different (see figs 5.4 and 5.5). It is therefore not possible to assign the majority of groups to either Pullularia or Sclerophoma using this system which supports a division into a greater number of groups.

Using the de Hoog et al (1977) system (table 5.6) all groups produce conidia by the method characteristic of Aureobasidium except group B which is characteristic of Hormonema and would be classified as H. dematioides (table 5.7). Among the Aureobasidium groups (table 5.8) group A would be classified as A. pullulans var pullulans while the remainder (7 groups), apart from minor deviations from the conidium size and the additional features noted previously (5.2.5.2), fit the description of A. pullulans var melanigenum.

In general the method of conidiogenesis has become the basis for the classification of microfungi (eg Ellis, 1971 and 1976) and therefore the system of differentiating Aureobasidium from Hormonema used by de Hoog et al (1977) should take precedence over Butin's earlier system using a wider range of morphological features. Among morphologically similar yeasts, a more biochemical approach has been adopted (Barnett et al, 1979) and it cannot be denied that, under certain cultural conditions, Aureobasidium type cultures be me yeast-like and indeed they are often referred to as the "black yeasts" (Cooke, 1962; Cooke and Matsuura, 1963; de Hoog et al, 1977). This type of approach, using gross morphology on three different growth media has divided cultures meeting the description of A. pullulans (de Bary) Arn var melanigenum Hermanides-Nijhof into seven groups. It is believed these groups may represent different perfect forms which have been shown to produce this type of culture eg Dothidea, Guinardia, Potebniamyces and Xenomeris are listed by de Hoog et al (1977). This could be confirmed by obtaining fresh cultures from authenticated specimens of these fungi and subjecting them to classification by the method developed. Fresh cultures appear necessary since there are indications the reactions of "old cultures" are less reliable. If these links could be established it would be a major advance in the taxonomy of this difficult group of organisms. At present the groups only represent a very useful way of classifying the organisms for the purpose of distribution studies in untreated and preservative treated L-joints which show further differences between the groups (section 5.3.5).

5.3 IDENTIFICATION, INCIDENCE AND RÔLE OF THE MICROFUNGI

5.3.1 *Alternaria* Nees ex Fr; Nees

5.3.1.1 Basis for identification

A personal knowledge of the organism.

5.3.1.2 Culture description

Light grey, fast growing culture producing lighter grey floccose aerial mycelium containing conidia (fig 5.30).

5.3.1.3 Special features

Species of *Alternaria* are determined by the size and shape of the conidia (Ellis, 1971). However Misaghi et al (1978) have shown, for *A. alternata*, that typical conidia are produced only under natural conditions although near-typical conidia can be produced by manipulating the culture conditions for example by using osmotic media or lowering the temperature. No allocation of specific names has, therefore, been attempted.

5.3.1.4 Incidence and importance in L-joints

Only three isolates, all from untreated L-joints, have been positively identified as *Alternaria* sp; a few further isolates of similar colonial morphology failed to produce conidia under normal culture conditions.

Alternaria did not appear until 43 days exposure, quite late in the colonisation sequence but, due to the low number of isolates, it is not possible to comment on the duration of its presence. *Alternaria* species have been confirmed as soft rot organisms (Seehann et al, 1975) but are unlikely to be very important in the L-joints due to the low numbers isolated from untreated material and their exclusion by both preservative treatments.

5.3.2 Ascomycete type A

5.3.2.1 Basis for identification

Although this culture possesses many characteristic features it has not been possible to relate it to a particular genus. Using the key in von Arx (1974) the organism would be placed in the genus *Trichosphaeria* except that this has hyaline ascospores. Using the

key in Dennis (1978) the organism would be placed in the Sordariaceae in the genus Coniochaeta except that no germ slits have been seen in the ascospores. However, if the iodine reaction of the apical apparatus of the asci is very faint and is being overlooked, as suggested by Hawksworth (personal communication), the organism could be placed in the genus Rosellinia (family Sphaeriaceae). In view of the importance of this organism (5.3.2.3) further efforts should be made to establish its identity.

5.3.2.2 Culture description

On malt agar, a dark-pigmented culture, with a green tinge. The wide indented margin is yellow when grown in the dark but turns pink in the light. Aerial mycelium gives the culture a felted appearance (fig 5.31). On corn meal agar, the sparce growth is a uniform green-brown; there is no significant growth on czapek dox agar. Hyaline conidia, 5 x 2 µm are produced in clumps at the tips of short conidiophores; the precise method of conidio-genesis is unclear (fig 5.32) but is probably phialidic. Fresh isolates readily produce small, black, globose perithicia. These are mainly hairy but a few bristles are also present. The aseptate pigmented ascospores (fig 5.33) are elliptical, 7.5 x 4.5-5 µm and do not show the presence of either a germ slit or a germ pore during germination.

5.3.2.3 Incidence and importance in L-joints

Only one isolate was obtained from an untreated L-joint and none from PCP treated L-joints. The organism was first isolated from TnBTO treated L-joints after 71 days and after 375 days was one of the most frequently isolated organisms. It would appear, therefore, that the organism is tolerant to TnBTO but not to PCP but cannot compete among the wide range of organisms present in the untreated L-joints. One isolate (culture No 92/1 MAa) was grown onto Scots pine sapwood blocks by the method described for the Aureobasidium type cultures (5.2.3.3). After 8 weeks incubation the blocks showed no significant weight loss and when sectioned, showed only lightly pigmented hyphae and conidia, and no attack of the wood. The rôle of this organism is probably that of a bluestain; its importance may lie in its tolerance to TnBTO which could be linked to a degradation process as shown for two

Basidiomycetes (Henshaw et al, 1978) or the unknown detoxification mechanism shown for Aureobasidium pullulans (section 6.3). An investigation of these possibilities is desirable.

5.3.3 Aspergillus fumigatus Fresenius

5.3.3.1 Basis for identification

The cultures produce typical Aspergillus conidiophores (fig 5.34); they were placed in the fumigatus group by reference to Raper and Fennell (1977).

5.3.3.2 Culture description

Green, sporing cultures, rather granular in appearance due to the conidiophores. The cultures produced a yellow pigment in the agar when grown at 22°C but not at 45°C.

5.3.3.3 Special features

A. fumigatus together with Paecilomyces varioti were the only organisms to grow on the additional isolation plates prepared after 71 days exposure, and incubated at 45°C.

5.3.3.4 Incidence and importance in L-joints

The appearance of A. fumigatus is very erratic (table 4.21). All 13 isolates, after 11 days exposure of untreated material, originated from a single L-joint. Only two isolation points close to the joint, both heavily colonised by bluestain fungi, failed to yield this organism. The moisture content at the two sampling zones furthest from the joint had not risen above 20 per cent (table 4.5). These data suggest the timber was infected prior to use, rather than the alternative explanation of rapid growth through dry timber. For this reason A. fumigatus has been grouped in Table 4.21 with those organisms isolated from unexposed material.

The organism appears to survive in the untreated L-joints since 14 isolates, distributed between two L-joints, were obtained after 221 days exposure although after this time, de novo infection cannot be discounted.

Few isolates were obtained from TnBTO treated L-joints, and none from those treated with PCP, suggesting the organism is susceptible to both chemicals.

A. fumigatus has been shown to be an active soft rotter of hardwoods (Sehann et al, 1975; Flannigan and Sagoo, 1977) and capable of producing soft rot of softwoods (Ofusu-Asiedu and Smith, 1973). This, together with its tolerance of the high temperatures recorded in joinery (Morton, 1975) could make A. fumigatus an important organism in those situations where it is present.

5.3.4 Aspergillus Micheli (Corda) (other species)

5.3.4.1 Basis for identification

The cultues produce typical Aspergillus conidiophores.

5.3.4.2 Culture description

Green, sporing cultures, rather granular in appearance due to the conidiophores. The various isolates show differences in colour and colonial morphology.

5.3.4.3 Incidence and importance in L-joints

Single isolates were obtained from untreated L-joints after four of the exposure periods and two isolates were obtained from PCP treated L-joints after 43 days exposure. Although some Aspergillus species have been shown to cause soft rot, the ability is by no means universal in this genus. The isolates have, therefore, been classified as moulds, in the absence of identification at the species level, or tests of the soft rot ability of the isolates. The low numbers isolated indicate they are not an important part of the flora.

5.3.5 Aureobasidium type cultures (Bluestain types A, B, C, D, E/H, G, I, J and K).

5.3.5.1 Basis for identification

See Section 5.2.

5.3.5.2 Incidence and importance in L-joints

A total of 157 isolates have been allocated to these groups; they therefore constitute a significant proportion of the microflora of the

L-joints. The distribution of the isolates between the bluestain groups and between untreated and preservative treated L-joints is interesting (table 4.21; table 5.9). The numbers of isolates not type-tested particularly from untreated material could influence the distribution figures and therefore the following observations must not be regarded as unequivocal. Groups A and B were isolated from untreated material in large numbers, from TnBTO treated material only rarely, and not at all from PCP treated material. Group C and D organisms were isolated with similar frequency on the TnBTO treated material, and together provided almost 70 per cent of the total number of isolates. Group C organisms were also common on untreated material but group D were much less frequent; neither group was isolated from the PCP treated material. In contrast, groups E/H, G and I were isolated solely from the PCP treated material, with the exception of a single isolate from one untreated L-joint. Group J and, to a lesser extent, group K were more evenly distributed but tended to appear later in the exposure period of the preservative. treated material than of the untreated material. The groups therefore exhibit different tolerances to the two preservatives with groups A, B, C and D being controlled by PCP, and groups E/H, G and I by TnBTO although these latter groups are shown to be less competitive than the others by their low frequency of isolation from the untreated material. This provides an alternative explanation for their absence in the TnBTO treated material.

The rôle of Aureobasidium type organisms in the colonisation and ultimate decay of L-joints appears to be complex. They rapidly colonise the wood and may lead to an increase in permeability as shown for organisms causing bluestain of logs (Lindgren and Scheffer, 1939; Bellmann and Francke-Grosmann, 1952) although no increase was detected in Scots pine sapwood exposed to A. pullulans FPRL No S 9E for 24 days (section 6.2). A. pullulans has also been shown to reduce the toxicity of TnBTO in agar to the spores of Lenzites trabea (section 6.3); if this occurs in the L-joints it will reduce the toxicity of the TnBTO and may allow colonisation by Basidiomycetes. Finally, Aureobasidium type organisms are involved in the disruption of the paint film (section 5.5) which is aesthetically unacceptable and provides additional paths of moisture entry.

Aureobasidium type cultures therefore play an important preliminary rôle in the decay process and it would be desirable to exclude them from joinery in service. Neither of the preservative treatments used has achieved this in the L-joint trials which indicates the need for a more effective treatment which could be achieved either by increasing the quantity of toxicant or by improving the method of treatment eg use of a double-vacuum method or by using a combination of chemicals, as frequently used by the medical profession. Alternatively, if moisture were excluded, the organisms could not grow and thus the use of water-repellents and joint-sealants could provide protection which would have the bonus of being environmentally more acceptable than the use of fungicides.

5.3.6 Bluestain type F

5.3.6.1 Culture description

On malt agar produces a rust coloured, appressed culture lacking aerial mycelium and with a paler margin. Grows slowly on corn meal agar producing sparse, pale growth while on czapek dox agar produces pink yeast-like growth. The culture appears to lack septa suggesting that it is a Phycomycete. The rust colouration is due to spores, 12 µm in diameter, with an irregularly thickened wall (fig 5.35) produced by the swelling of the portion of a lateral hyphal branch just behind the hyphal tip (fig 5.36).

5.3.6.2 Incidence and importance in L-joints

Four isolates were obtained from TnBTO treated material during the early stages of exposure. It would appear to be tolerant of TnBTO but due to the low number of isolates and short duration of its presence, it is unlikely to be of major importance in the decay process.

5.3.7 Botrytis cinerea Persoon

5.3.7.1 Basis of identification

The cultures were compared with isolates from another source (mastic sealing materials) which had been identified by the Commonwealth Mycological Institute (CMI), and were found to be identical.

5.3.7.2 Culture description

Light coloured, fast growing culture producing under certain conditions masses of pale grey aerial mycelium bearing conidiophores and conidia. Black sclerotia of irregular size, shape and distribution also produced on the surface of the agar growth medium (figs 5.37 and 5.38).

5.3.7.3 Special features

The pattern of sclerotium production appeared to be constant for a given isolate but to vary between isolates (figs 5.37 and 5.38). This has been shown previously by Humpherson-Jones and Cooke (1977) who further showed that special sub-culturing techniques were necessary to maintain sclerotium production.

5.3.7.4 Incidence and importance in L-joints

This organism was isolated on eight occasions; it occurred consistently from 11 days to 71 days exposure in untreated material and was also isolated from TnBTO treated material after 43 and 71 days. It is therefore an early coloniser of untreated material but its appearance is delayed by TnBTO and precluded by PCP. It also disappears at a relatively early stage; since it outgrows other organisms it is unlikely that it was still present within the timber after this time but not isolated, unless inhibited by newly arrived organisms.

Botrytis cinerea is well established as a pathogen of a variety of plants. It has been shown by Leightley (1977) to cause soft rot of wood with the unusual feature of producing a greater weight loss of softwood than hardwood. Hale (1978) has confirmed its soft rotting ability by demonstrating cavity formation, in birch (Betula sp) sections in liquid culture, by two L-joint isolates (33/2 MAa and 79/2 MAa). Fiusello et al (1974-75) have also demonstrated lignolytic activity. Soft rot cavities were first observed in untreated L-joints after 130 days and in TnBTO treated L-joints after 375 days (table 4.28); B. cinerea which was not isolated after 71 days exposure is, therefore, unlikely to have played a major rôle in this attack.

5.3.8 Chaetomium globosum Kunze

5.3.8.1 Basis for identification

Personal knowledge of the organism and reference to Seth (1970).

5.3.8.2 Culture description

On malt agar, a fast growing pale culture rapidly producing abundant black, globose perithecia covered with a loose mass of undulate hairs. Ascospores light brown to dark olive-brown, lemon-shaped, 8.5-10.5 x 8-9 µm.

5.3.8.3 Incidence and importance in L-joints

Chaetomium globosum was isolated from unexposed untreated and TnBTO treated L-joints and during the early stages of exposure from untreated and PCP treated L-joints. This pattern suggests the organism was present in the stock of timber but quickly died out probably due to invasion of the L-joints by antagonistic organisms. It is unlikely, therefore, to have played any major rôle in the decay process. Although C. globosum is a very active soft rot (Seehann et al, 1975) and is frequently used in laboratory tests, it has not previously been isolated in large numbers from wood in ground contact and appears to be no more important in the out of ground contact situation.

5.3.9 Coniothyrium Corda

5.3.9.1 Basis for identification

Use of the key in von Arx (1974).

5.3.9.2 Culture description

On malt agar, initially a pale culture with aerial mycelium, later becoming amber. Hyphae of variable width, with some more darkly pigmented and with numerous septa. On corn meal agar, a sparse, feathery pigmented growth. At the advancing edge, numerous side branches arise as chains of swollen cells (fig 5.39) which, when left in daylight, develop into darkly pigmented pycnidia (fig 5.40). The pynospores measure 3 x 2.5 µm and are pigmented brown when viewed in mass.

5.3.9.3 Incidence and importance in L-joints

Four isolates were obtained from untreated L-joints after 375 days exposure. These organisms, therefore, occur late in the succession. Their importance is difficult to assess because they were only isolated after the longest exposure period. However, a number of isolates of this genus have been shown to be active soft rot organisms in softwood (Seehann et al, 1975); these isolates are probably attacking the untreated L-joints in this way.

5.3.10 Diplodia gossypina Cooke

5.3.10.1 Basis for identification

Comparison with cultures produced from single, typical, Diplodia spores in pustules developing through the paint film after 71 days exposure (section 5.5). One culture (35/4 SCNa) subsequently produced pycnidia and spores, 19 x 10 µm, more or less waisted and with no visible striations, on a plate of two per cent malt agar after a period in the light.

5.3.10.2 Culture description

On malt agar, the culture is fast growing and produces masses of aerial mycelium which is initially almost hyaline but later dark grey and with a tendency towards strand formation. The aerial mycelium remains much lighter in colour on czapek dox agar while on corn meal agar, the growth is sparse, appressed and almost hyaline.

5.3.10.3 Special features

Diplodia gossypina is currently accepted to be synonymous with many previous species in the genera Botryodiplodia, Chaetodiplodia, Diplodia and Lasiodiplodia (Punithalingam, 1976; Jones, 1977). The species is very variable, for example Verrall (1942) noted spore sizes of 24.2-30.4 x 11.8-15.4 µm. Jones (1977) unlike Zambettakis (1950, 1951) considers only paraphyses and spore ornamentation to be constant and suggests a possible future division into Diplodia, with striate spores, and Botryodiplodia, with smooth spores.

5.3.10.4 Incidence and importance in L-joints

D. gossypina was only isolated from untreated L-joints; it first appeared after 32 days exposure and was isolated at all subsequent sampling times. The greatest number of isolates (4) were recovered after 71 days by which time the organism, by fruiting, was disrupting the paint film (section 5.5).

The organism is known to produce soft rot cavities in hardwoods (Umezurike, 1978) and this has been confirmed with one L-joint isolate (35/3 SNCa) by Hale (1978) although a second isolate grown from a single spore failed to produce cavities. No cavities were seen in Scots pine sapwood blocks colonised by D. gossypina (culture No 27/2 MAc) growing on filter paper over normal Abrams (1948) agar (using the method described in 5.3.2.3 for Aureobasidium type cultures). The blocks were rapidly colonised; hyphae were present in rays and both earlywood and latewood tracheids after one week and could be seen passing through bordered pits (fig 5.41) but not directly through the wall. Colonisation progressed over the eight week observation period with an increase in the number of thick-walled spores (fig 5.42) and the intensity of staining of the blocks.

The rôle of D. gossypina in the L-joints would appear to be as a staining organism (its rôle as a soft rot in softwoods is not proven) capable of disrupting the paint film after only two months exposure, leading to disfigurement and additional points for entry of moisture. As with many bluestain organisms, it could be responsible for increases in permeability. Both preservative treatments have successfully excluded this organism over the first year of exposure.

5.3.11 Epicoccum purpurascens Ehrenb ex Schlecht

5.3.11.1 Basis for identification

The cultures were compared with isolates from another source (mastic sealing materials), which had been identified by CMI, and were found to be identical.

5.3.11.2 Culture description

Amber coloured culture producing thick aerial mycelium containing groups of black conidia visible with the naked eye.

5.3.11.3 Incidence and importance in L-joints.

Only two isolates, both from untreated L-joints, have been positively identified as E. purpurascens; a few similar cultures failed to spore under normal light conditions. The organism is capable of producing soft rot in hardwoods (Seehann et al, 1975) but does not appear to have been tested on softwoods. The first isolate was obtained prior to observed soft rot attack but the second was obtained afterwards; they may have contributed in a minor way to this form of attack in untreated L-joints but were controlled by both preservative treatments.

5.3.12 Fusarium Link

5.3.12.1 Basis for identification

The isolates were allocated to this genus on the presence of the characteristic sickle-shaped, multi-septate macroconidia.

5.3.12.2 Culture description

Pale apricot to light red cultures with paler fairly sparse aerial mycelium containing the macroconidia.

5.3.12.3 Incidence and importance in L-joints

Three isolates were obtained from untreated L-joints after 221 days exposure; these organisms therefore appear late in the succession and are probably transient. A number of Fusarium species produce soft rot in hardwoods and the two species tested (F. aquaeductuum and F. solani) also produce soft rot in softwood (Seehann et al, 1975). The isolates from L-joints, therefore, probably contributed to this type of attack in untreated L-joints but this genus was excluded by both preservative treatments.

5.3.13 Mucor type A

5.3.13.1 Basis for identification

The presence sporangiophores typical of the Mucoraceae.

5.3.13.2 Culture description

A slow growing culture producing mauve/grey aerial mycelium containing the sporangiophores.

5.3.13.3 Incidence and importance in L-joints

This organism was isolated a total of 6 times from unexposed material treated with TnBTO and during the early stages of exposure of TnBTO treated and untreated material. It is an organism which is present in the timber prior to utilisation but which quickly dies out and, therefore, is unlikely to play a significant rôle in the deterioration process.

5.3.14 Mucor type B

5.3.14.1 Basis for identification

The presence of sporangiophores typical of the Mucoraceae.

5.3.14.2 Culture description

A grey, fast growing culture producing abundant aerial mycelium containing the sporangiophores.

5.3.14.3 Incidence and importance in L-joints

This organism was isolated only once, after 221 days exposure of untreated material. It is, therefore, unlikely to play a major rôle in the deterioration process and is probably acting as a secondary mould, as described by Clubbe (1980a).

5.3.15 Paecilomyces varioti Bainier

5.3.15.1 Basis for identification

A personal knowledge of the organism.

5.3.15.2 Culture description

Green-yellow culture appearing powdery due to the production of smooth, elliptical conidia 4.5-6 x 2.5-4 µm borne in chains on conidiophores.

5.3.15.3 Special features

This organism, together with A. fumigatus grew from the samples incubated at 45°C.

5.3.15.4 Incidence and importance in L-joints

Two isolates were obtained from untreated material after 71 days exposure (plus two further isolates from samples incubated at 45°C) and 6 isolates after 130 days exposure. This organism is not recorded as causing soft rot and one isolate (culture No 32/3 Ben a) did not cause

any attack of Scots pine sapwood blocks over an 8 week period (using the method described in 5.3.2.3 for Aureobasidium type cultures).

The rôle of this organism appears to be that of a mould capable of growing over a wide range of temperature.

5.3.16 Penicillium Link type A

5.3.16.1 Basis for identification

The production of typical monoverticillate penicilli (fig 5.43); type A has been separated from the others by its production of dark pigmented hyphae. This is a known feature of P. coryophilum Dierckx but the penicilli of this species are typically biverticillate and asymmetric but with monoverticillate structures being present.

5.3.16.2 Culture description

The culture surface is a dark blue-green colour and powdery due to prolific sporulation. The reverse is almost black due to the growth of pigmented hyphae in the agar.

5.3.16.3 Incidence and importance in L-joints

This organism was isolated from TnBTO and PCP treated material prior to exposure and occasionally from untreated and PCP treated material after exposure periods of up to 221 days. This pattern suggests the organism is present in a small proportion of the timber prior to utilisation, is not killed by either of the preservative treatments used and does not proliferate when the timber is rewetted.
P. coryophilum is not recorded as being capable of causing soft rot; its rôle therefore appears to be that of a mould which may cause some staining if the pigmented hyphae are produced within the timber.

5.3.17 Penicillium Link (other species)

5.3.17.1 Basis for identification

The production of typical Penicillium conidiophores.

5.3.17.2 Culture description

The cultures varied considerably indicating the presence of different species. The colour varied from a light apple green to a dark blue-green and all were more or less powdery due to the production of conidia. Some caused minor changes to the colour of the agar but none

produced the red colouration typical of certain species eg P. funiculosum Thom. Differences in the structure of the conidiophores were noted.

5.3.17.3 Incidence and importance in L-joints

Isolates were obtained before exposure from untreated material and throughout the exposure period from untreated and preservative treated material. This indicates infection prior to use may have been killed by the preservative treatments although this is not certain because of the low number of replicates. The incidence remained similar throughout the exposure period (table 4.21) suggesting little proliferation occurs.

Although certain Penicillium species have been shown to cause soft rot in hardwoods (Seehann et al, 1975) only one P. funiculosum is confirmed as causing soft rot in a softwood. These isolates are therefore considered to be acting as moulds in the L-joints but their tolerance to both of the preservatives may indicate a rôle in detoxification.

5.3.18 Pestalotia de Notaris

5.3.18.1 Basis for identification

The identity of characteristic multiseptate spores with dark pigmented cells and setulae was established by reference to Barnett and Hunter (1972) and Domsch and Gamms (1972).

5.3.18.2 Culture description

A white fast growing culture with aerial mycelium bearing black spore droplets.

5.3.18.3 Special features

The nomenclature of organisms of this type is confused. Dube and Bilgrami (1966) note that various authors have severely criticised Steyaert's classification of this group into three different genera viz, Truncatella (for four-celled conidia), Pestalotiopsis (for five-celled conidia) and Pestalotia (for six-celled conidia), instead of the single genus Pestalotia which was created by de Notaris in 1839. They conclude, on the basis of observations on fifty-seven fungi, that the species show pronounced variation in the configuration of conidia and number of setulae borne over the superior hyaline cell and that the single genus Pestalotia is more appropriate to accommodate them. A

similar conclusion was reached by Shoemaker and Müller (1963) based on consideration of the relationships between conidial states and their perfect forms.

5.3.18.4 Incidence and importance in L-joints

This organism was isolated only once, from an untreated L-joint after 32 days exposure. Various Pestalotia isolates have been shown to cause soft rot in softwood (Seehann et al, 1975) but since no soft rot was observed until after 130 days of exposure it is unlikely to have been causing active decay. Both preservative treatments appear to prevent infection.

5.3.19.1 Phialophora Medlar type A

5.3.19.1 Basis for identification

The identity of these cultues was uncertain until single ascospore cultures were prepared from Coniochaeta fruitbodies, probably C. ligniaria (Greville) Massee (fig 5.44 and 5.45) present on first series L-joints, and yielded similar cultures. According to von Arx (1974) the imperfect stages of Coniochaeta spp are Phialophora spp. With these cultures, the phialides taper towards the tip and lack distinct collarettes (fig 5.46 left) thus resembling Cephalosporium. However occasional phialides are more typical (fig 5.46, right). Subsequently various isolates produced mature perithecia to confirm the link. Use of the key of Schol-Schwarz (1970) yields the tentative identification Phialophora hoffmannii (van Beyma) Schol-Schwarz.

5.3.19.2 Culture description

On malt agar, the cultures are yellow, slow growing, with paler coloured aerial mycelium and often giving a yellow pigment in the agar. Ageing cultures often produce small perithecia which are round, black with bristles. Asci containing 8 pigmented ascospores, 14 x 6 µm are often produced (fig 5.47). Growth on czapek dox agar is very dense with much aerial mycelium.

5.3.19.3 Special features

In culture single ascospore isolates will produce perithecia which outwardly appear typical, but which never contain ascospores. This ability would appear wasteful, but it may never be used under natural conditions.

During the process of isolation, organisms of this type frequently appeared to be antagonistic, at a distance, towards other cultures. Fig 5.48 shows this effect with two bluestain organisms, both isolated in close proximity to the Phialophora. No effect was obvious with a range of Basidiomycetes.

5.3.19.4 Incidence and importance in L-joints

A single isolate of the organism was obtained from untreated L-joints after the first three exposure periods; the number of isolates then built-up to a peak after 221 days. After 375 days there were fewer isolates but it was still the most common organism. Isolates were not obtained from TnBTO treated material until 43 days of exposure; between 130 days and 375 days the numbers remained constant at half the maximum level reached in untreated material. No isolates were obtained from PCP treated material.

This organism is, quite clearly, an important member of the flora of the untreated and TnBTO treated L-joints. The antagonism exhibited by the Phialophora towards bluestain organisms probably contributes towards its ability to invade successfully. The lower numbers recorded in the untreated material after 375 days could have been affected by the increasing number of white rot organisms which are not apparently adversely affected.

Three isolates have produced soft rot cavities in birch sections in liquid culture (Hale, 1978); numerous isolates of P. hoffmannii have previously been shown to be active soft rot organisms (Seehann et al, 1975). A significant number of isolates have been obtained from both untreated and TnBTO treated L-joints over the period when soft rot cavities were first observed (see table 4.25) which suggests this organism plays a rôle in this form of attack. P hoffmannii together with many other species of Phialophora have been isolated previously from preservative treated and naturally durable wood exposed in a variety of situations (see section 3.5.4).

5.3.20 Phialophora Medlar type B

5.3.20.1 Basis for identification

The cultures produced conidia from the tips of phialides, with a distinct collarette, typical of Phialophora (Schol-Schwarz, 1970). This type is differentiated from type C on the basis of spore size and shape.

5.3.20.2 Culture description

Slow growing culture producing grey aerial mycelium containing strands. The phialides, with a distinct collarette not darker than the rest of the phialide, are produced singly or in small groups. The spores vary in size and shape but the majority are elongate, measuring 5 x 2 µm

5.3.20.3 Incidence and importance in L-joints

This organism was isolated once from an untreated L-joint exposed for 221 days and a total of seventeen times from L-joints treated with TnBTO and exposed for between 130 and 375 days. No isolates have been obtained from PCP treated L-joints. It is, clearly, an important part of the flora of TnBTO treated material but has not been able to establish itself among the wide variety of organisms in untreated material.

Soft rot ability is widely distributed among Phialophora species (Seehann et al, 1975; Nilsson and Henningsson, 1978) and so it has been assumed that Phialophora type B is capable of this form of attack. Most isolates were obtained from the TnBTO treated material at the time when soft rot cavities were first observed (table 4.25) suggesting a link although another soft rot (Phialophora type A) had been established over a longer period. Nilsson and Henningsson (1978) list twelve species of Phialophora isolated from preservative treatment material, usually treated with mixed inorganic salts and exposed in ground contact, indicating the importance of this genus in this situation. The present study, with a different preservative and a different exposure method, adds further emphasis.

5.3.21 Phialophora Medlar type C

5.3.21.1 Basis for identification

The cultures produced conidia from the tips of phialides with a distinct collarette, typical of Phialophora (Schol-Schwarz, 1970). This type is differentiated from type B on the basis of spore size and shape.

5.3.21.2 Culture description

Slow growing culture producing aerial mycelium of grey with brown tinges. The phialides, with a distinct collarette not darker than the rest of the phialide, are produced singly or in small groups. The spores vary in size and shape but the majority are elleptical, measuring 2.5 x 2 µm.

5.3.21.3 Incidence and importance in L-joints

Seven isolates were obtained from PCP treated L-joints exposed for the longest period (375 days). As with Phialophora type B, it is assumed this organism is capable of causing soft rot although no cavities were observed at the time of isolation. A delay between colonisation and attack has been noted by other authors (eg Clubbe, 1980a).

The importance of Phialophora species in the attack of preservative treated wood has been discussed previously (sections 3.5.4 and 5.3.20.3). It is interesting that only one species of Phialophora has been able to colonise the PCP treated material and then, only after the longest exposure period. This is indicative of better protection than that provided by the TnBTO treatment.

5.3.22 Phoma (Fries) Desmazieres

5.3.22.1 Basis for identification

The pycnidia produced in culture were used to identify these cultures using the key in von Arx (1974).

5.3.22.2 Culture description

Dark pigmented cultures producing some aerial mycelium and black, globose thin-walled pycnidia approximately 2 mm in diameter exuding pycnospores 5 x 2 µm mostly hyaline but appearing pink in mass in some cultures.

5.3.22.3 Incidence and importance in L-joints

Four isolates were obtained from untreated L-joints after 130 to 375 days exposure. Numerous isolates of Phoma have been shown to produce soft rot in hardwoods and some also in softwoods (Seehann et al, 1975). It is therefore considered the isolates of Phoma could have contributed to the soft rot attack of the untreated L-joints. Both preservative treatments prevented colonisation over the first year of exposure.

5.3.23 Pleospora Rabenhorst

5.3.23.1 Basis for identification

The perithecia were used to follow the key in von Arx (1974) and compared with the drawings of Tulasne and Tulasne (1863).

5.3.23.2 Culture description

Slow growing culture on malt agar, producing grey/brown aerial mycelium and a yellow/brown pigment in the agar. On czapek dox agar the growth is similar but with pinkish tones particularly noticeable on the reverse of the colony.

Flask-shaped perithecia, 0.25 mm in diameter and with hairs, were produced in culture (fig 5.49). The ascospores were pigmented, measured 24 x 12 µm and had both transverse and longitudinal septa, Most spores were constricted at the central transverse septum (fig 5.50).

5.3.23.3 Incidence and importance in L-joints

This organism was isolated only once from an untreated L-joint after 11 days exposure. According to Seehann et al (1975) various Stemphylium species, the imperfect stage of Pleospora, are capable of causing soft rot. Because of its low incidence, the organism is unlikely to play any major rôle in the process of colonisation and decay.

5.3.24 Rhinocladiella Nannfeldt

5.3.24.1 Basis for identification

A personal knowledge of the organism; reference to the key in De Hoog and Hermanides-Nijhof (1977) gives the specific name R. atrovirens Nannfeldt.

5.3.24.2 Culture description

Dark grey to black slow growing cultures with velvety aerial mycelium which is sometimes tinged with brown. Numerous hyaline conidia, 4-6 x 1-2 µm, are borne on pigmented rachides (fig 5.51), which show small, distinct scars when the conidia become detached.

5.3.24.3 Incidence and importance in L-joints

Three Rhinocladiella cultures were obtained from L-joints exposed for 130 days, one from an untreated replicate and two from a single PCP treated replicate. No soft rot ability has been demonstrated and therefore this organism has been classified among the bluestains. The occurrence on PCP treated material suggests tolerance to this preservative and, therefore, the possibility of a detoxifying ability.

5.3.25 Trichoderma viride sensu Bisby (1939)

5.3.25.1 Basis of identification

Personal knowledge of the organism.

5.3.25.2 Culture description

Light coloured, fast growing culture producing masses of dark green conidia on the aerial mycelium. The culture smells of coconut, which is particularly strong when grown on benomyl/malt agar; this is a feature of T. viride Pers ex S F Gray according to Rifai (1969).

5.3.25.3 Special features

During the process of isolation and purification a number of isolates were inoculated, individually or in groups, onto petri dishes of two per cent malt agar. After incubation under similar conditions these plates showed varied patterns of sporulation (fig 5.52); ring patterns of sporulation have been noted previously for T. viride Pers ex S F Gray by Rifai (1969). Further strain variation was shown by the multi-isolate dishes (fig 5.53). On dishes 31/1 Ben and 132/1 two isolates show similar growth rates and patterns of sporulation but are different from the third. In each case the 3 isolates originated from the 3 sampling points through the depth of an L-joint (a,b and c) at a single position relative to the joint (fig 4.11) and were, therefore, growing within 38 mm of one another. On dish 131/1 MA, the two isolates appear similar.

Unfortunately time did not allow investigation of the reproducibility of these interactions or any relationship to the Trichoderma species recognised by Rifai (1969).

5.3.25.4 Incidence and importance in L-joints

Of the total of 48 isolates, the majority (43) were obtained after 71 days exposure; only three of these were from TnBTO treated L-joints. This confirms an earlier observation (section 3.5.4) on the first series L-joints where T. viride was not isolated from the TnBTO treated material. In contrast 13 isolates were obtained from PCP treated L-joints suggesting the organism is more tolerant of this preservative. Cserjesi (1972) has shown that T. viride can degrade PCP; its presence may, therefore, be indicative of a possible mode of failure of the preservative treatment.

The presence of T. viride over such a short period and its relatively late appearance in the succession of organisms is surprising in view if its common occurrence in ground contact situations (KÄÄrik and Rennerfelt, 1957; KÄÄrik, 1967 and 1968; Henningsson and Nilsson, 1976) although it was found only rarely in cedar shingles (Smith and Swann, 1976). Its appearance at 71 days (mid August) does not coincide with the most common fruiting time ("autumn" according to KÄÄrik and Rennerfelt, 1957) although fruitbodies were present within the joint of some samples when dismantled. The distribution of isolates from untreated and PCP treated L-joints (fig 5.54) suggests infection had occurred via the joint and therefore that the material sampled at 71 days had not been infected prior to exposure even though the chance of such a coincidence would have been very small. T. viride is normally easily isolated due to its fast growth rate and known antagonistic properties to other fungi (Grosclaude et al, 1974; Toole, 1971) and therefore is unlikely to be missed. However, the lack of isolates from position 2 of L-joint No 33 suggests circumstances can arise in which T. viride fails to grow. Botrytis cinerea, Alternaria sp a fast growing white rotting Basidiomycete and an untypified fungus were isolated from this position but they have not been tested for possible antagonism. The most probable explanation for the relatively rare occurrence is that the L-joint does not provide ideal growth conditions for T. viride.

The rôle of Trichoderma in causing permeability increases in timber has been reviewed by Unligil (1969), but it seems unlikely the organism has contributed significantly to permeability increases in the L-joints. Increases had occurred in untreated L-joints by 71 days exposure when Trichoderma was isolated in profusion but no significant increases had occurred in PCP treated L-joints by 375 days exposure by which time Trichoderma had died out.

5.3.26 Ulocladium

5.3.26.1 Basis for identification

The cultures were identified using the keys of Barnett and Hunter (1972) and Ellis (1971).

5.3.26.2 Culture description

A dark pigmented culture of moderate growth rate, which rapidly becomes granular due to the production of numerous conidia (fig 5.55). These are dark brown multiseptate and with a roughened wall.

5.3.26.3 Incidence and importance in L-joints

Two isolates were obtained from untreated L-joints, after 32 and 130 days exposure. Isolates of Ulocladium have been shown to produce soft rot (Seehann et al, 1975) although neither of these isolates produced cavities in birch sections in liquid medium (Hale, 1978). The low incidence, and exclusion by both preservative treatments indicate only a minor rôle in the colonisation of L-joints.

5.4 BASIDIOMYCETES

5.4.1 Sistotrema brinkmannii

5.4.1.1 Basis for identification

The fruitbody was identified by use of the key provided by Christiansen (1960). Later, comparisons were made with the culture descriptions given by Baniecki and Bloss (1969), Siepmann (1969), Weresub and LeClair (1971) and Hallaksela (1977).

5.4.1.2 Culture description

A white fast growing culture, with numerous simple clamp connections. A range of culture types exist from those with aerial mycelium which quickly become covered with a loose, rather granular fruitbody (fig 5.56) containing urniform basidia with up to 8 basidiospores (fig 5.57) to those with appressed growth and large groups of monilioid hyphae (bulbil type cells) within the agar (fig 5.58).

5.4.1.3 Special features

Twenty one isolates from L-joints (see table 5.12) plus isolates from joinery in service have been tested, using a modification of the Rautela and Cowling (1966) technique for detection of cellulose activity- all isolates gave positive reactions. During testing, distinct zones appeared. First a superficial zone, with dense hyphal growth, then a sharp transition to a zone of cleared cellulose and finally a less sharp transition to the uncleared cellulose (fig 5.59). These zones were investigated further (fig 5.60). It was found that monilioid hyphae were present in large numbers close to the edge of the zone of dense growth. Few hyphae were present beyond this zone but some penetrated to the edge of the uncleared zone. The effect, therefore, is unlikely to be due solely to the cellulase diffusing through the medium from relatively superficial growth but is probably dependent, to some extent, on the action of the sparse "foraging hyphae". The relative importance of the two effects cannot be judged from this experiment.

5.4.1.4 Incidence and importance in L-joints

Sistotrema brinkmannii was first isolated from one untreated L-joint after 43 days exposure; numbers of isolates built up to a peak after 221 days exposure (table 4.21) although at each exposure period isolates were obtained from only one of the three replicates (table 5.10).

The other replicates were colonised by white rot organisms; this may indicate a mutual antagonism between the types of Basidiomycetes with the first coloniser preventing invasion by the other. If this situation exists initially, it is overcome by longer periods of exposure since, after 375 days all three replicates were colonised by both S. brinkmannii and white rots (table 5.10).

S. brinkmannii was the first Basidiomycete to colonise TnBTO treated L-joints and was isolated from all three replicates exposed for 221 days. However, it was not isolated after 375 days; examination over a longer time period would be necessary to determine if the organism has died out or was absent only from replicates 90 to 92.

It is interesting to note that in 8 out of the 10 L-joints yielding S. brinkmannii the organism was recovered only from the isolation position closest to the joint (1) or the next position (2) (table 5.12). In the two other L-joints, colonisation had proceded further. The importance of this observation is not understood.

The rôle played by S. brinkmannii in the colonisation and decay of joinery has been discussed previously (section 3.5.4).

5.4.2 Other Basidiomycetes

5.4.2.1 Basis for identification

These organisms have been identified as Basidiomycetes by the presence of clamp connections or the ability to produce a white rot reaction when grown on sawdust medium (2.3.1.3). The majority of the isolates have been grouped into 13 types on the basis of colonial morphology and microscopic features observed in cultures growing under identical conditions. 12 isolates did not fall exactly within the groups; these are referred to as "others" at present although many are probably minor variants of the types.

5.4.2.2 Culture descriptions

A description of each group is given in Table 5.11; individual descriptions of the "other" cultures are not included.

5.4.2.3 Special features

Cultures in three groups (E, G and H) did not possess clamp connections when isolated. Various pairs of cultures were grown together and observed for clamp production. The only successful pairings were within group G, between culture 26/1a and those from L-joint No 40, thus confirming that group G cultures are a single species. The lack of success between the cultures from L-joint No 40 and between the two group H cultures from L-joint 34 suggests that, in each case, the cultures are part of a single mycelium originating from one basidiospore although non-compatible mating strains may be involved.

During the isolation of cultures from L-joint No 38 it was observed that the cultures from the a, b and c positions through the depth of the sample, were antagonistic to one another (fig 5.61) and that the same organism was isolated from the same depth at each position relative to the joint. The "c" cultures were placed in group B, the "a" and "b" cultures were given their own groups of J and L respectively. Similar patterns of compartmentalisation and antagonism have been shown by Rayner (1977b, 1978) in his work on the colonisation of hardwood stumps.

5.4.2.4 Incidence and importance in L-joints

The first white rot organism was isolated from an untreated L-joint after 32 days exposure; subsequently the number of isolates and the range of types increased. A single isolate was obtained from a TnBTO treated L-joint after one year's exposure. Although the organisms are capable of decaying wood, there were no visible signs of damage to the L-joints, suggesting a slow rate of attack. This is confirmed by other untreated, painted, L-joints exposed on the same site which were rejected due to decay after approximately 7 years (Purslow and Williams, 1978); the decay capacity of isolates from window joinery in service (section 2.3) is also low.

The failure of the L-joints can be divided into two distinct phases. First, the phase prior to infection by Basidiomycetes which will be referred to as the "protected" phase although, of

course, invasion by other micro-organisms is taking place. Secondly, the phase during which Basidiomycetes are present and decay is occurring albeit at a slow rate; this will be referred to as the "deterioration" phase. Similar phases in deterioration have been shown previously for textiles (Kempton et al, 1963) and thin strips of wood (Smith, 1980) buried in soil. From the results of the isolations it is obvious that the "protected" phase varies in length depending upon the treatment of the L-joints. In untreated L-joints it is only 32 days but, with 1 per cent TnBTO treatment, it is extended to 375 days (although S. brinkmannii was isolated after 221 days its ability to cause decay is unproven). No Basidiomycetes were isolated from the 5 per cent PCP treated L-joints; soft rot organisms were isolated for the first time after 375 days suggesting invasion by Basidiomycetes may not take place for at least a further year.

Using the results from L-joints previously exposed on the same site (Purslow and Williams, 1978) the "deterioration" phase for untreated L-joints is approximately seven years. Work by Smith (1971) and Purslow and Williams (1979) shows that with copper-chrome-arsenic treated wood exposed by soil burial, the rate of attack during the "deterioration" phase is reduced by increasing the treating solution concentration. Furthermore, Smith (1980) has shown that there is, commonly, a linear relationship between the lengths of the "protected" phase (his "induction" phase) and the "deterioration" phase (his "decay" phase) as the concentration is increased. If a similar relationship exists between preservative treatments of varying efficacy, such as the 1 per cent TnBTO and 5 per cent PCP in the present study, it should be possible to use the length of the "protected" phase to calculate the length of the "deterioration" phase and thus predict the service life of a given treatment.

Accepting the "protected" and "deterioration" phases given in Table 5.13 for 5 per cent PCP, and assuming a linear relationship between the lengths of the two phases, it can be calculated (fig 5.62) that the 1 per cent TnBTO treatment will have a "deterioration" phase of approximately 14.8 years and therefore a total life of approximately 15.8 years (fig 5.63).

Obviously this is a simplistic approach. In reality the relationship is probably complex but this can only be discovered by further observations. A further complication is that these trials of L-joints have been carried out using simple preservative solutions whilst commercial preservatives contain additives including water repellents which, alone, have been shown to extend service life considerably (Feist and Mraz, 1978). The possibility of a synergistic effect between water repellents and preservatives also exists. In addition, no data are available on the effect of different finishes on performance of preservatives. It should be possible to accumulate data on the effect of these variables in a relatively short time by duplicating the treatments given to samples already exposed for long periods. In addition any new trials should include sufficient samples for destructive testing during the early stages of exposure.

5.5 ORGANISMS CAUSING PAINT FILM FAILURES

5.5.1 Method

The "B" slices from untreated L-joints No 31 and 33, exposed for 71 days, were damp chambered on top of moist paper towel in a plant propagator. The development of pustules was observed using a binocular microscope, and preparation made for observation at magnifications up to x600. Isolations were made by transferring small samples from developing pustules to petri dishes of 2 per cent malt agar. Single spore cultures were prepared by dispersing spores in sterile deionised water, then spreading over petri dishes of water agar. Single germinating spores were picked off under a binocular microscope, and transferred to petri dishes of 2 per cent malt agar.

5.5.2 Results

5.5.2.1 Observations on pustule formation

During the damp chambering period, repeated observation of the same area showed that slight "bumps" in the paint film developed into pustules, apparently as a result of pressure exerted by the development of the fungus. However, some form of chemical attack, to give localised weakening of the film, could also take place. Further "bumps" developed in previously unaffected areas; these undoubtedly would have developed into pustules if the observation period had been extended.

5.5.2.2 Pustule types

Only two basic types of pustule were observed. The first consisted of a pycnidium-type structure, sometimes with superficial hairs, but apparently containing only a mass of dark pigmented hyphae. Isolations yielded yeast-like growth characteristic of Aureobasidium type cultures; these were not grouped using the system described in section 5.2.

The second type of pustule exuded pigmented, uniseptate spores, more or less waisted and approximately 19 x 10 µm. Some spores were scattered on the surrounding paint film while others were retained within superficial hyphae. Single spore cultures produced growth typical of Diplodia gossypina (section 5.3.10).

5.5.3 Discussion

Many of the pustules were produced by Aureobasidium type organisms which have long been established as causing this type of failure. It would have been interesting to investigate if all the different types separated in section 5.2 are capable of producing pustules or if the ability is limited, but there was not time in the present study.

Diplodia gossypina has previously been recorded as causing paint film failures in joinery samples from Kenya (Savory, personal communication).

At later stages in the exposure period it is likely that other organisms could be responsible for paint film failures.

TABLE 5.1 CHARACTERISTICS OF GROWTH ON AGAR MEDIA

Blue-stain type	Growth on three media				Micromorphology	
	Description			Fig No	Description**	Fig No
	Two per cent malt agar	Corn meal agar	Czapek dox agar			
A	Growth varying in colour from pink to greenish brown, often sectoring; hyaline margin to the colony. No aerial mycelium. Yeasty.	Lightly pigmented growth, rather sparse. Slower growth rate than on malt agar	Pale pink, very yeasty growth. Masses of conidia but some hyphae present.	5.2	Conidia hyaline, 4-8-13 x 2.5-3-7 µ produced synchronously, often at the tips of specialised structures. Conidiogenous cells elongate. No chlamydospores observed. Some thick-walled brown hyphae in old cultures.	5.17
B	Black colony with a hyaline margin. Some pigmented aerial hyphae. Usually dense* growth with pycnidial initials. Yeasty surface	Lightly pigmented growth, rather sparse. Slower growth rate than on malt agar.	No significant growth.	5.3	Conidia hyaline, 4.5-7-12 x 2-3-4 µ produced in basipetal succession from one or two loci per cell. Conidiogenous cells often shorter than the hyphal width. Some chlamydospores, also swollen sections of hyphae with thickened walls and longitudinal septa.	5.13 5.18
C	Black colony with an indented hyaline margin. Some pigmented aerial mycelium. Conidia lilac coloured in mass. Slower growing than most other types.	Lightly pigmented growth, rather sparse, similar growth rate to malt agar.	No significant growth	5.4	Conidia 6-9-14.5 x 2.5-3.5-5 µ produced synchronously from conidiogenous cells which may be shorter than the hyphal width. Chains of chlamydospores. Old hyphae thick-walled.	5.14
D	Dark brown colony with a lighter coloured margin. Metallic sheen to surface. Some pigmented aerial mycelium.	Lightly pigmented growth, rather sparse. Broad hyaline margin. Slower growth rate than on malt agar.	No significant growth.	5.5	Conidia hyaline, 5-7-11 x 2-3-3.5 µ produced synchronously. Conidiogenous cells variable in length but some shorter than the hyphal width. Chains of chlamydospores. Old hyphae thick-walled.	
E/H	Black colony with a hyaline margin. Pigmented aerial hyphae. Yeasty surface in patches.	Black growth in thick filaments in fresh isolates (fig 5.19). Hyphal development in old isolates. Slower growth rate than on malt agar.	Dark yellow growth with some darker pigmentation. No aerial mycelium. Yeasty surface.	5.6 5.7	Conidia spindle-shaped, hyaline, 4-9-12 x 2-3-3.5 µ produced synchronously often at the tips of hyphae. Pigmented blastoconidia 5-8-10 x 3-4.5-6 µ also produced. Some conidiogenous cells shorter than the hyphal width, chains of chlamydospores. Old hyphae thick-walled.	5.15 5.19

*See fig 5.12

**Conidial measurements are the minimum, modal and maximum length and width observed in preparations taken from mature colonies, mounted in lactophenol and warmed before observation.

TABLE 5.1 CONT: CHARACTERISTICS OF GROWTH ON AGAR MEDIA

Blue-stain type	Growth on three media				Micromorphology	
	Description			Fig No	Description**	Fig No
	Two per cent malt agar	Corn meal agar	Czapek dox agar			
G	Black colony with a hyaline margin. Hyaline aerial hyphae. Yeasty surface in patches.	Black growth, not sparse. Slower growth rate than on malt agar.	Yellow growth with a paler margin. Yeasty surface.	5.8	Conidia spindle-shaped, hyaline, 3.5-6-7.5 x 1.5-2-2.5 µ produced synchronously by elongate conidiogenous cells. No chlamydospores observed. Old hyphae thick-walled.	
I	Black colony with a hyaline margin. Hyaline aerial hyphae sometimes produced Centre of colony adpressed and very yeasty.	Black growth, rather sparse forming fine filaments when freshly isolated. Slower growth rate than on malt agar.	Pink yeasty growth with brown pigmentation around the inoculum.	5.9	Conidia hyaline, 5-7.5-10 x 2-3-4 µ produced synchronously by elongate conidiogenous cells. Chains of chlamydospores. Old hyphae thick-walled. In cover-slip culture produced hyaline hyphae with clamp connections.	5.20
J	Black colony with a hyaline margin. Hyaline aerial hyphae. Yeasty surface in patches.	Lightly pigmented growth rather sparse. Slower growth rate than on malt agar.	Pink yeasty growth with yellow/brown pigment around the inoculum.	5.10	Conidia hyaline, 5-7-11 x 2.5-3-4.5 µ produced synchronously by elongate conidiogenous cells. Chains of chlamydospores. Old hyphae thick-walled.	5.16
K	Black colony with a hyaline margin. Pigmented aerial hyphae behind advancing edge. Growth is not dense*, often shows concentric growth rings and pycnidial initials which may mature.	Lightly pigmented growth, rather sparse. Similar growth rate to malt agar.	Sparse pink yeasty growth Similar growth rate to malt agar.	5.11 5.12	Conidia hyaline, 5-6-10 x 2.5-3-4 µ produced synchronously by conidiogenous cells which may be shorter than the hyphal width. Chains of chlamydospores. Old hyphae thick-walled.	

*See fig 5.12

**Conidial measurements are the minimum, modal and maximum length and width observed in preparations taken from mature colonies, mounted in lactophenol and warmed before observation.

TABLE 5.2 CHARACTERISTICS OF GROWTH IN SCOTS PINE SAPWOOD

Blue-stain type	Exposure period				Fig No
	Two weeks	Four weeks	Six weeks	Eight weeks	
A	Hyphae, mostly without pigmentation, largely confined to the rays but a few in earlywood tracheids. Numerous conidia associated with the hyphae in places, a few still attached.	More pigmented hyphae present. Conidia.	Greater colonisation of earlywood tracheids Conidia.	More pigmented hyphae present in tracheids. Conidia.	
B	Rays and earlywood tracheids colonised by pigmented hyphae. Numerous conidia associated with some hyphae.	Some hyphae with thickened walls. No conidia.	As per 4 weeks	Some colonisation of latewood tracheids No conidia.	
C	Rays and earlywood tracheids colonised by unpigmented hyphae and by pigmented hyphae with areas of thickened wall. Numerous conidia associated with some hyphae.	Greater colonisation. More pigmented hyphae present. Conidia numerous in places.	Pigmented hyphae becoming more septate. Conidia.	Several solid masses of growth. Some colonisation of latewood tracheids. No conidia.	5.21 5.23
D	Rays and both early- and latewood tracheids colonised by pigmented hyphae, with areas of thickened wall. No conidia.	Hyphae more pigmented and more septate. No conidia.	As per 4 weeks.	Greater colonisation. A few conidia.	
K	Rays and earlywood tracheids colonised by unpigmented hyphae. Conidia associated with some hyphae.	Hyphae mainly pigmented and with many septa. A few conidia.	Latewood tracheids colonised. Many hyphae with thickened walls. No conidia.	As per 6 weeks.	5.22

Table 5.3

GROWTH RATES* DETERMINED OVER DIFFERENT PERIODS

Bluestain type and culture number	Incubation temp °C	Growth period (days after inoculation)			
		1–5	5–6	0–7**	5–8
B 28/1 MAa	6	0.4	1.4	0.6	1.5
	11	1.9	2.9	1.9	2.6
	17	5.0	5.0	4.2	4.3
	21	8.9	7.4	7.2	6.5
	24	9.7	8.9	8.0	7.3
	27	7.7	7.7	6.8	7.7
	31	0.4	0.3	0.4	0.3
	35	0	0	0	0
D 30/2 SCNa	6	0.7	1.3	0.8	1.6
	11	2.9	4.3	3.0	4.6
	17	5.5	7.9	5.4	7.6
	21	7.4	8.2	6.8	8.0
	24	7.6	7.8	6.7	7.5
	27	4.4	3.8	3.8	3.9
	31	0.8	0	0.6	0.1
	35	0	0	0	0

* mean increase in diameter (mm/day)

** discounting inoculum

Table 5.4

GROWTH RATES (MEAN INCREASE IN DIAMETER, MM PER DAY)

Bluestain type	Culture No	Growth temperature °C									
		5	6	11	17	18	21	24	27	31	35
A	28/1 MAc_3		0.7	2.1	3.9		5.3	5.6	4.0	0.2	0
	36/2 $SCNc_1$		1.2	2.8	4.1		5.5	6.5	4.7	0	0
B	28/1 MAa		1.1	2.0	5.0		7.4	8.9	7.7	0.3	0
	34/1 MAc_2		1.4	3.2	6.6		8.6	8.8	5.4	0.3	0
	26/1 MAc^2		1.7	2.8	6.8		8.9	9.4	8.4	1.1	0
C	35/1 MAa_3		0.6	1.1	1.5		2.6	3.5	5.0	0.6	0
	40/1 MAb_1		0.6	1.0	1.2		2.5	3.4	5.6	0.9	0
D	30/2 SCNa		1.9	4.1	7.6		8.0	7.5	3.9	0.1	0
	39/5 MAa_2		1.4	3.9	6.4		7.5	6.9	4.9	0.6	0
E	126/1 $SCNa_1$	0.4		2.0		4.2	5.9	6.9	5.7	3.4	1.1
	135/2 MAc	0.4		2.0		3.7	4.6	5.1	5.0	2.9	1.0
G	126/1 $SCNa_2$	0.5		2.5		4.9	6.6	7.3	6.9	3.6	0.9
	126/2 MAc_2	0.3		2.7		5.1	6.6	7.3	7.1	4.0	1.0
H	135/2 MAa		0.3	1.7	3.0		4.5	4.7	4.5	2.1	0.2
	134/1 MAa_2		0.3	1.8	3.4		5.7	5.6	4.3	1.5	0.5
I	125/2 SCNb		0.3	2.1	4.8		6.5	7.5	6.5	1.8	0.6
J	88/4 MAc_2		0.4	2.5	5.2		6.4	6.9	6.0	2.3	0.4
	36/5 SCNa		0.4	2.4		5.1	6.5	7.1	6.8	4.4	1.1
	136/1 MAc		0.4	2.2		4.9	6.1	6.2	5.0	2.2	0.2
K	84/2 MAa		1.8	3.1	5.9		8.1	8.5	3.6	0.2	0
	27/1 MAb_1		1.4	2.9	5.6		7.9	8.8	3.1	0.3	0

Table 5.5

CLASSIFICATION ACCORDING TO BUTIN (1963)

Butin characteristics		Features possessed by JKC groups								
Organism type and associated features		A	B	C	D	E/H	G	I	J	K
P – aerial hyphae grey-white if present at all S – aerial hyphae pigmented		P	S	S	S	S	P	P	P	S
P – conidiogenous cells at least twice as long as wide S – some conidiogenous cells shorter than their width		P	S	S	S	S	P	P	P	S
P – chlamydospores terminal S – chlamydospores in chains		–	S	S	S	S	–	S	S	S
P – significant growth at 30°C S – little growth at 30°C		S	S	S	S	P	P	P	P	S
P – no pycnidia* S – pycnidia produced		–	S	–	–	–	–	–	–	S
Total number of characteristics of each organism type	P	2	0	0	0	1	3	3	3	0
	S	1	5	4	4	3	0	1	1	5

P characteristic of Pullularia
S characteristic of Sclerophoma
– neither feature observed

* this character should be observed by growth on varnish-coated wood; this has not been attempted. Organisms in groups B and K have produced pycnidia in culture and are therefore recorded as "S". The ability within other groups to produce pycnidia is unknown.

Table 5.6

CLASSIFICATION ACCORDING TO DE HOOG AND HERMANIDES-NIJHOF (1977)

de Hoog et al characteristics	Feature possessed by JKC Groups								
Organism type and associated feature	A	B	C	D	E/H	G	I	J	K
A - conidia produced synchronously H - conidia produced sequentially	A	H	A	A	A	A	A	A	A

A characteristic of Aureobasidium

H characteristic of Hormonema

Table 5.7

APPLICATION OF THE DE HOOG ET AL (1977)

HORMONEMA SPECIES KEY TO JKC GROUP B

Feature	JKC Group B
- conidia less than 6μ wide	+
- daily growth rate at 24°C over 6 mm	+
Conclusion: Hormonema dematioides	✓

+ feature present

✓ correct conclusion based on the observed features

Table 5.8

APPLICATION OF THE DE HOOG ET AL (1977) *AUREOBASIDIUM* SPECIES KEY TO THE RELEVANT JKC GROUPS

Feature	JKC Groups							
	A	C	D	E/H	G	I	J	K
- conidia less than 22μ long	+	+	+	+	+	+	+	+
- conidia straight	+	+	+	+	+	+	+	+
- stromata absent	+	+	+	+	+	+	+	+
- brown hyphae in old cultures thick-walled	+	+	+	+	+	+	+	+
- conidia 9-11μ long	<	+	<	+	<	<	<	<
- constrictions of chlamydospore chains conspicuous	-	+	+	+	-	+	+	+
- cultures remaining pink, light brown or yellow for 3 weeks	+	0	0	0	0	0	0	0
- cultures rapidly becoming dark	0	+	+	+	+	+	+	+
Conclusion:								
A. pullulans var *pullulans*	✓							
A. pullulans var *melanigenum*		✓	✓	✓	✓	✓	✓	✓

\+ Feature present

0 Feature absent

< Most conidia less than 9μ long

\- Not applicable since no chlamydospore chains observed

✓ Correct conclusion based on the observed features

TABLE 5.9

Frequency of Isolation of Aureobasidium Type Cultures

Bluestain type	Preservative treatment		
	Untreated	1% TnBTO	5% PCP
A	19	2	0
B	34	2	0
C	26	10	0
D	8	11	0
E/H	1	0	10
G	0	0	2
I	0	0	1
J	4	2	5
K	13	5	2
Total No of isolates	105	32	20
No of groups represented	7	6	5

TABLE 5.10

Incidence of Basidiomycete types in L-joints

Preservative treatment	Exposure period (days)	L-joint No	Basidiomycete types* present
Untreated	32	25 26 27	G
	43	28 29 30	S.b O
	71	31 32 33	B O S.b C
	130	34 35 36	H A D F M O S.b
	221	37 38 39	S.b B J L
	375	40 41 42	S.b A G K O S.b A B O S.b I O
1% TnBTO	221	87 88 89	S.b S.b S.b
	375	90 91 92	E

*S.b Sistotrema brinkmannii

A-M see Table 5.11

O Other Basidiomycetes

Table 5.11

CULTURE DESCRIPTIONS OF BASIDIOMYCETE TYPES

Type	Five per cent malt agar		Sawdust medium		Microscopic features		
	Description	Growth* rate	White rot reaction	Special features	Clamp type	Hyphal width (μ)	Special features
A	White with floccose aerial mycelium, bleaching agar. Aerial hyphae becoming powdery and brown around inoculum.	4-5	+	Powdery growth, sawdust turning brown in patches in old cultures.	Simple, at all septa.	3-5	Sinuous growth near the hyphal tips
B	Pale with brown tinged aerial mycelium	4-5	+	Powdery growth in old cultures	Simple, at all septa	3-5	Sinuous growth near the hyphal tips
C	White with very powdery aerial mycelium, bleaching agar	4.5-5.5	+		Mostly in pairs, not common	4-7-10	Hyphae bleaching up into oidia, some hyphae encrusted with crystals
D	Apricot with masses aerial mycelium, bleaching agar, strong mushroom smell when bruised	5	+		Some in whorls, not common	5-6	Stereum sp
E	White and often appressed	0.5-1.5	+		None	2.5-5	
F	Pale, with concentric rings of pigmented growth and dark grey aerial mycelium, pigmentation lost on subculturing	3.3	+		Simple at all septa (none on pigmented hyphae)	3-5	
G	White with dense aerial mycelium, bleaching agar	1.5-2.5	+		None on individual isolates, simple at all septa when grown together	2-3	Branching near the hyphal tips
H	White developing a salmon-pink cushion at toe of slope, bleaches agar	5.5	+		None	5-7	Branching near the hyphal tips, oidia developing from some side branches
I	White with slight brown tinges in the aerial mycelium, becoming powdery with age	4.3	+	Very powdery growth	Simple at all septa	2.5-4.5	Sinuous growth and branching near the hyphal tips
J	White with aerial mycelium, bleaching agar	4.8	+		Simple at all septa, humped	3-6	Sinuous growth and branching near the hyphal tips
K	White with aerial mycelium becoming powdery, brown stain in agar around inoculum with age	4	+		Simple at all septa	3-5	Branching near the hyphal tips
L	White with aerial mycelium particularly behind the hyphal front	4.3	+	Powdery growth in old cultures	Simple at all septa, humped	5	Sinuous growth near the hyphal tips
M	White with powdery aerial mycelium, bleaching agar	3.5-4	+		Simple at all septa	2.5-5	Sinuous growth near the hyphal tips, much-branched hyphae present

*Radial increment, mm/day at 22°C

Table 5.12

DISTRIBUTION OF BASIDIOMYCETE ISOLATES†

Sistotrema brinkmannii

29/1a*
32/1a
32/1b*
32/1c
36/1a
36/1b
36/1c
36/2b*

37/1a*
37/1b*
37/1c
37/2a*
37/2b
37/2c
37/3a*
37/3b
37/3c
37/4a*
37/4b
37/4c
37/5a*
37/5b
37/5c

40/1a
40/1b*

41/1b*

42/1a*
42/1b
42/1c
42/2a*
42/2c
42/3b
42/3c
42/4b*
42/4c*(2)

87/1a*
87/1b
87/1c
87/2a
87/2b*

88/1a*
88/2b*

89/1a*
89/1b
89/1c

Type A

35/2a
35/2b
35/2c
40/1a
41/1c
41/2b
41/2c
41/3b
41/3c
41/5a
41/5b
41/5c

Type B

31/2b
31/2c
31/3b
31/3c
31/4a
31/4b
38/1c
38/2c
38/3c
38/4c
41/4c
41/4b

Type C

33/1a
33/1b
33/2b

Type D

35/5a

Type E

91/3c

Type F

35/2a
35/2b
35/2c
35/3a
35/3b
35/3c
35/4c

Type G

26/1a
40/1c
40/2c
40/3c
40/4c

Type H

34/1a
34/1c

Type I

42/2a
42/2b
42/2c
42/3a
42/3b
42/3c
42/4a
42/5a
42/5b
42/5c

Type J

38/1a
38/2a
38/3a
38/4a

Type K

40/3a
40/4a

Type L

38/1b
38/2b
38/3b
38/4b

Type M

35/1a
35/1b
35/2c

Others

30/1a
31/5a
31/5b
31/5c
35/1a
40/2a
40/3c
41/1a
41/4a
41/4b
41/4c
42/4b

†For culture numbering system see fig 4.11

*Isolates tested and shown to clear cellulose agar

TABLE 5.13

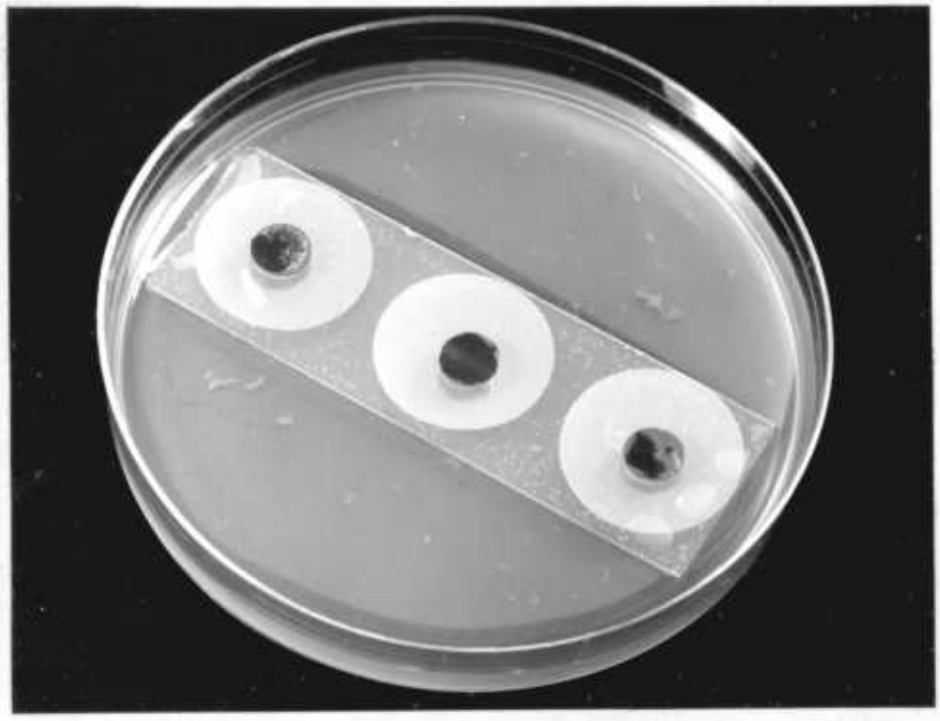
Data on "protected" and "deterioration" phases

Preservative treatment	Length of "protected" phase	Length of "deterioration" phase
Untreated	1 month	7 years†
1% TnBTO	1 year	?
5% PCP	2 years*	23.4 years**

† from Purslow and Williams (1978)

* estimated value based on isolation studies

** Verrall (1965) in tests of comparable severity to those of Purslow and Williams (1978) reported that all exposure samples, treated with 5 per cent PCP in kerosene, were sound after 13.9 years exposure. Assuming the first specimen will fail when the samples are next examined (15 years) and using the relationship given by Purslow (1976) the mean life can be estimated to be 25.4 years; accepting a "protected" phase of 2 years, the deterioration phase becomes 23.4 years.



Section through assembly

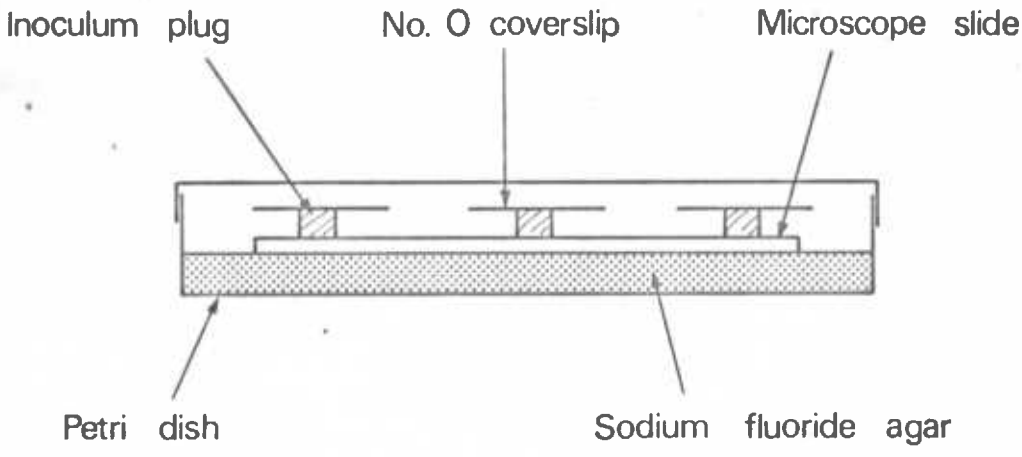
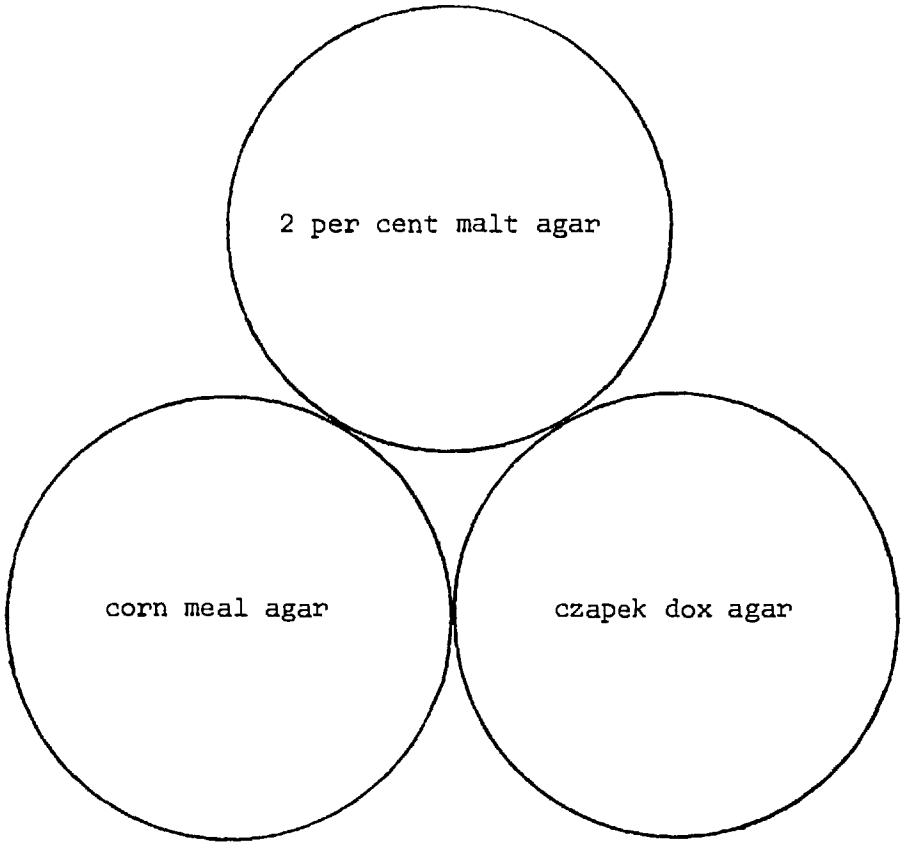


Fig. 5.1. Coverslip culture method



Key to Figs 5.2 to 5.11

top	growth on 2 per cent malt agar
left	growth on corn meal agar
right	growth on czapek dox agar

Fig 5.2 Bluestain type A. Culture No 18/1 MAb_3

Fig 5.3 Bluestain type B. Culture No 18/1 MAb_1
Key - see preceding page.

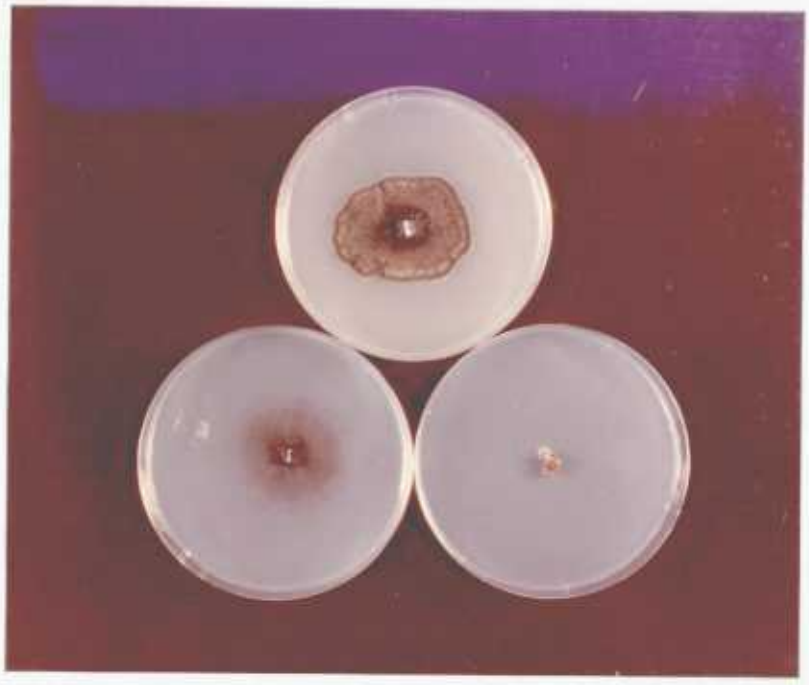


Fig 5.4 Bluestain type C. Culture No $18/1MAa_2$

Fig 5.5 Bluestain type D. Culture No $76/1MAc_2$

Key – see page preceding Fig 5.2

Fig 5.6 Bluestain type E. Culture No $126/1SCNa_1$

Fig 5.7 Bluestain type H. Culture No $126/2MAc_1$

Key - see page preceding Fig 5.2

Fig 5.8 Bluestain type G. Culture No 126/1SCNa$_2$

Fig 5.9 Bluestain type I. Culture No 125/2SCNb

Key - see page preceding Fig 5.2

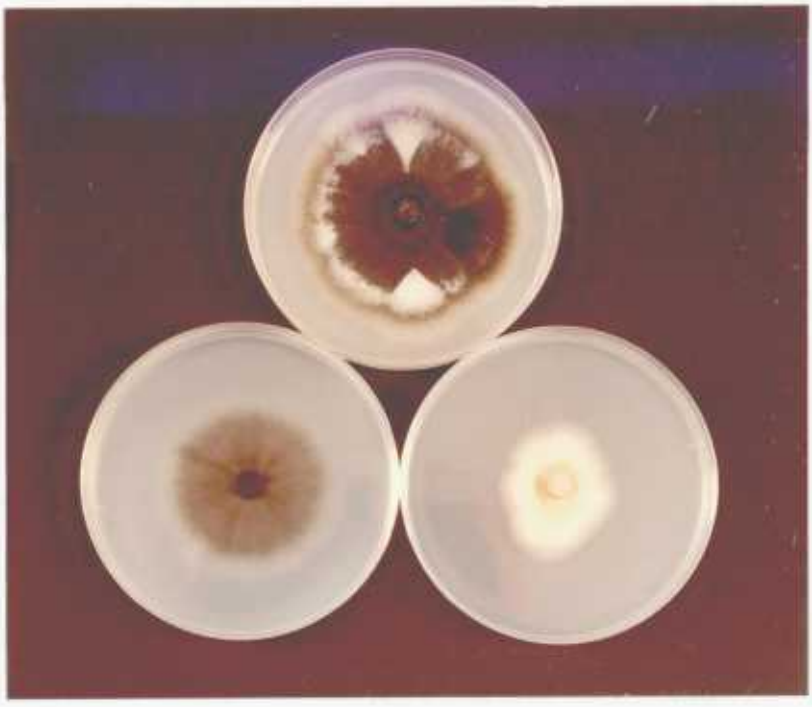


Fig 5.10 Bluestain type J. Culture No 88/4MAc$_2$

Fig 5.11 Bluestain type K. Culture No 84/2MAa

Key - see page preceding Fig 5.2

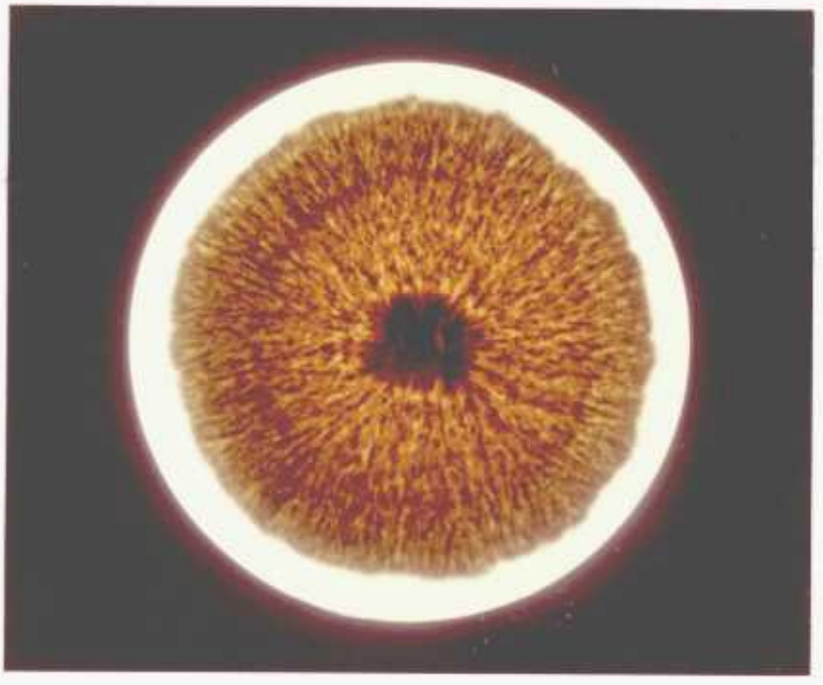


Fig 5.12 Bluestain type K. Culture No 84/2MAa "Not dense" growth. Reverse of colony showing transmission of light. The pattern of growth occurs with all type K cultures and some type B cultures.

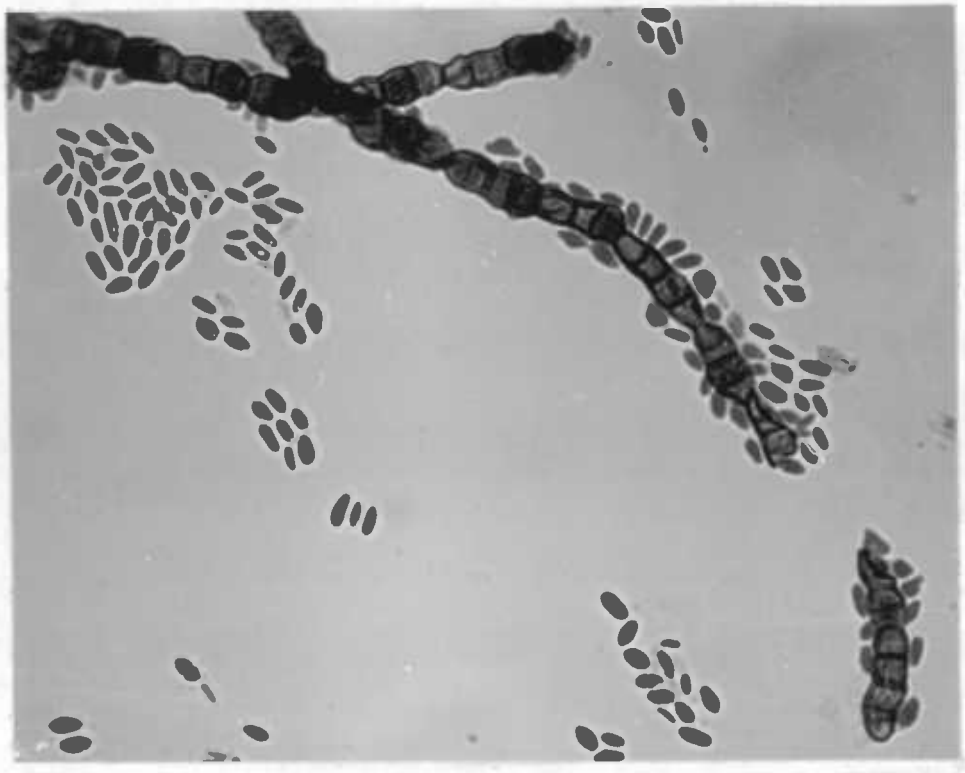


Fig 5.13 Bluestain type B. Culture No 18/1 MAb_1
Coverslip culture showing conidia (x 650)

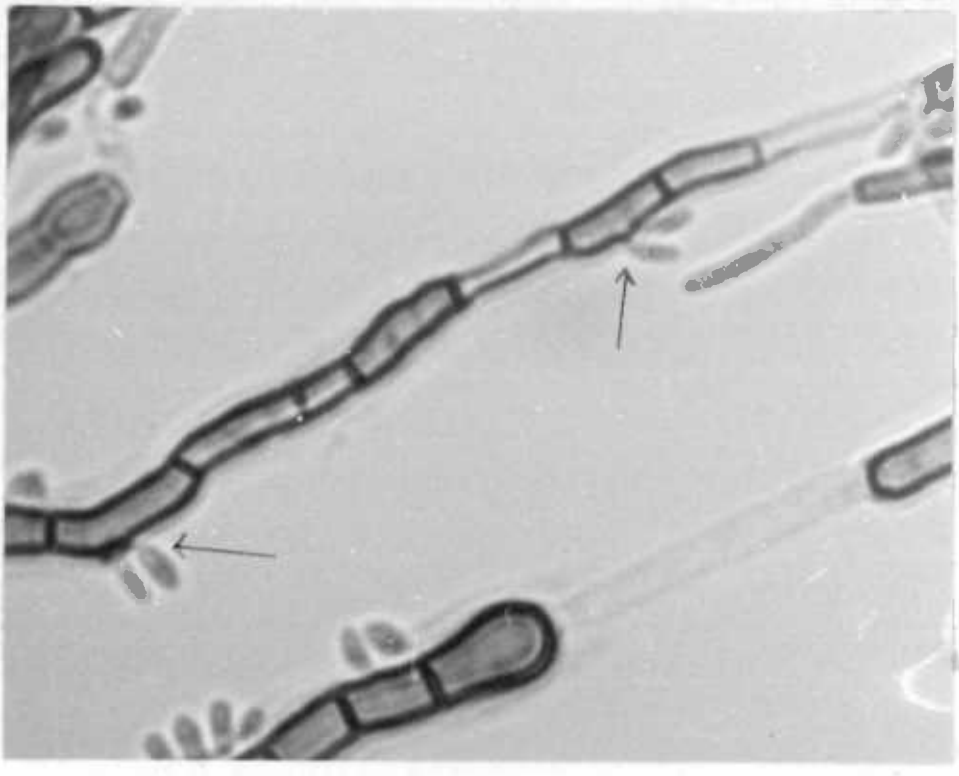


Fig 5.14 Bluestain type C. Culture No 18/1 MAa_2
Coverslip culture showing conidiogenesis (arrowed)
(x 1620)

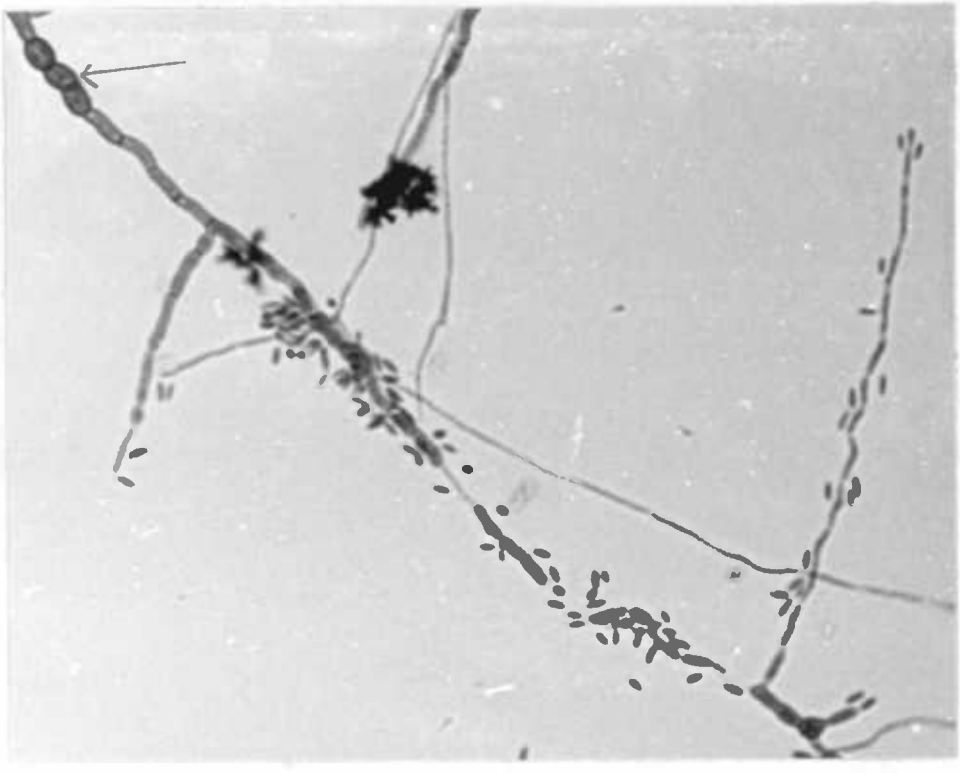


Fig 5.15 Bluestain type H. Culture No 126/2 MAc_1
Coverslip culture showing conidia and a short chain of chlamydospores (arrowed) (x 410)

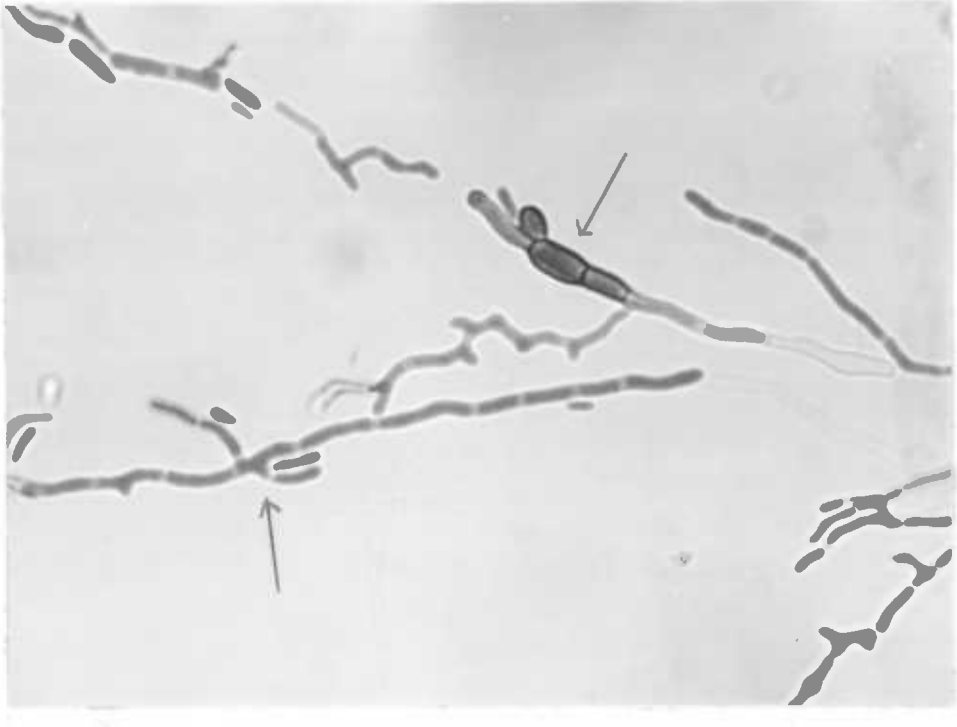


Fig 5.16 Bluestain type J. Culture No 88/4 MAc_2
Coverslip culture showing conidiogenesis and a short chain of chlamydospores (both arrowed) (x 580)

Fig 5.17 Bluestain type A. Culture No 28/1MAc$_3$
Conidiogenesis on corn meal agar
top (x 225) bottom (x 425)

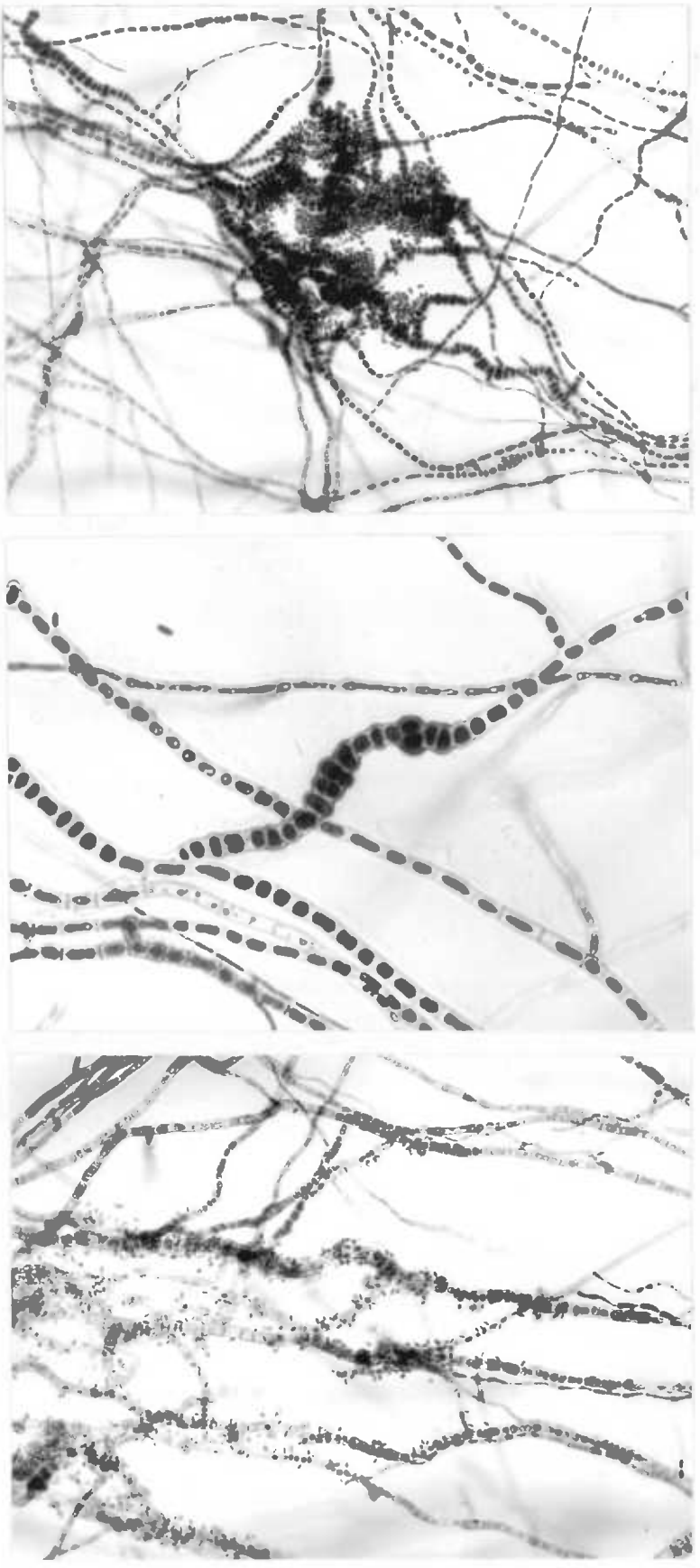


Fig 5.18 Bluestain type B. Culture No 28/1MAa
Conidiogenesis on corn meal agar
Top Rudimentary pycnidium (x 180)
Centre Longitudinal septa in thick-walled hyphae (x 430)
Bottom Conidia being produced in basipetal succession (x 160)

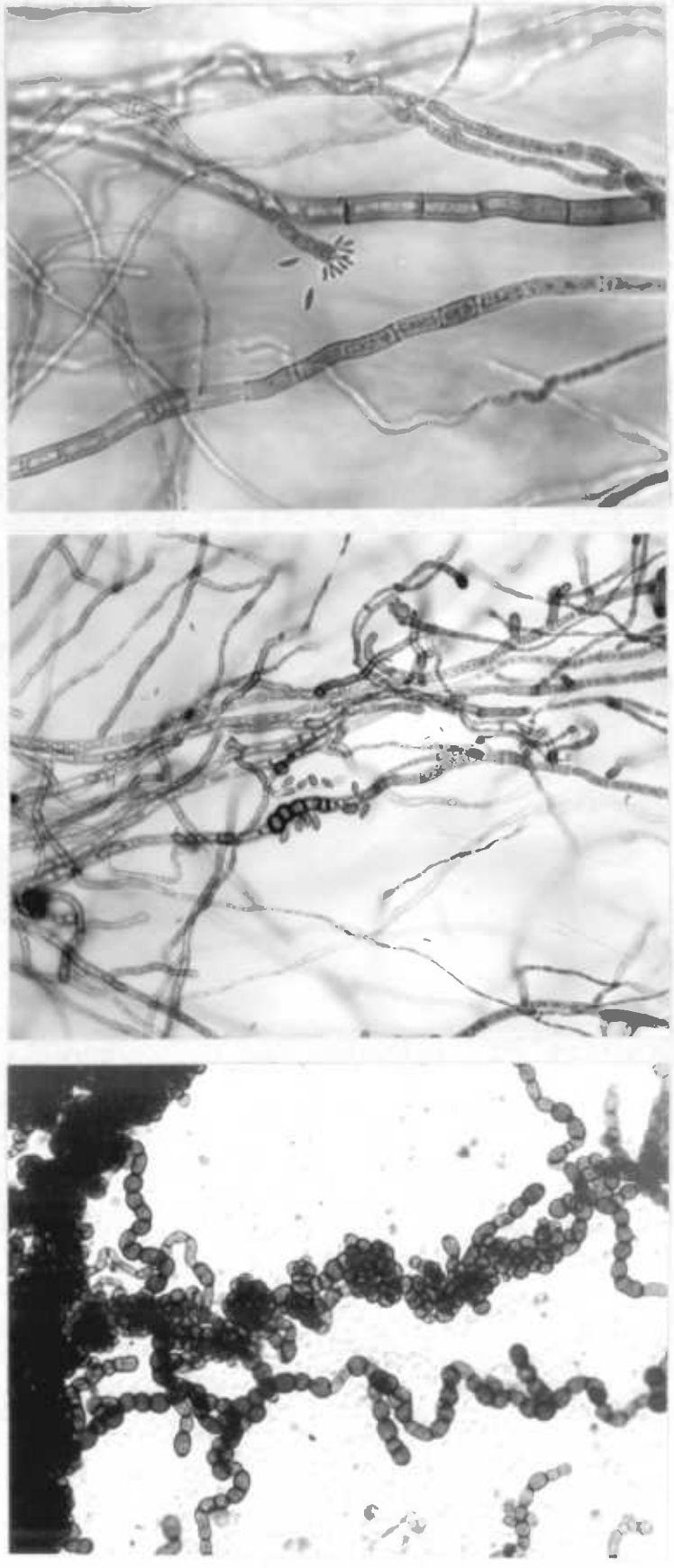


Fig 5.19 Bluestain type H.
Top Culture No 135/2MAa. Production of hyaline conidia (x 445)
Centre Culture No 135/2MAa. Production of pigmented conidia (x 445)
Bottom Culture No 126/2MAc$_1$. Filamentous growth on corn meal agar (x 185)

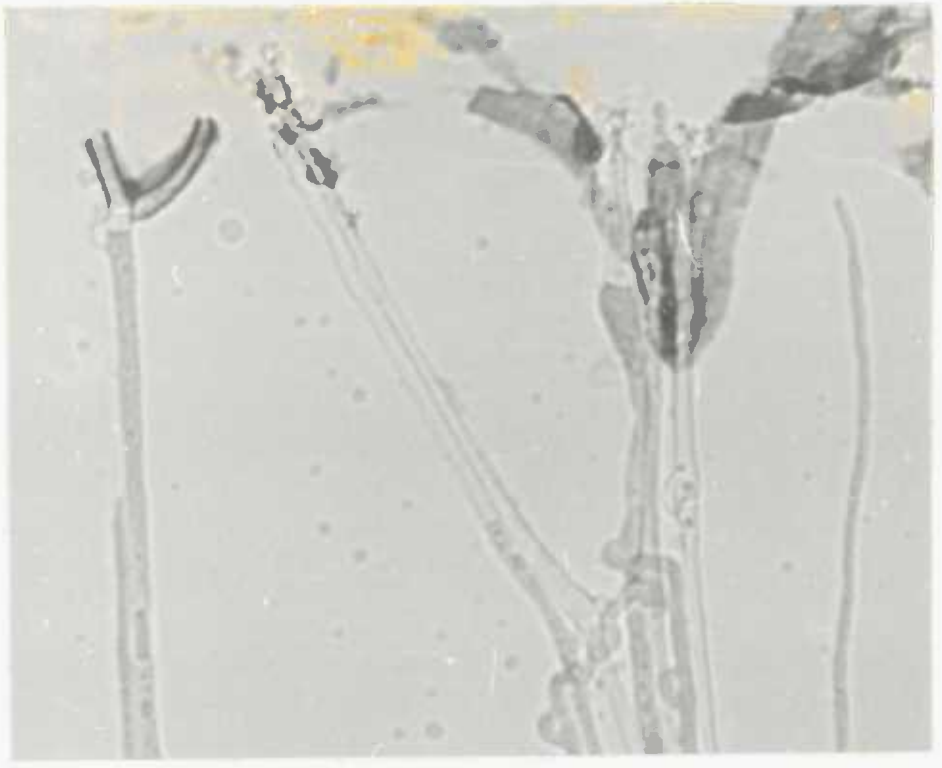


Fig 5.20 Bluestain type I. Culture No 125/2 SCNb
Coverslip culture showing clamp connections (x 950)

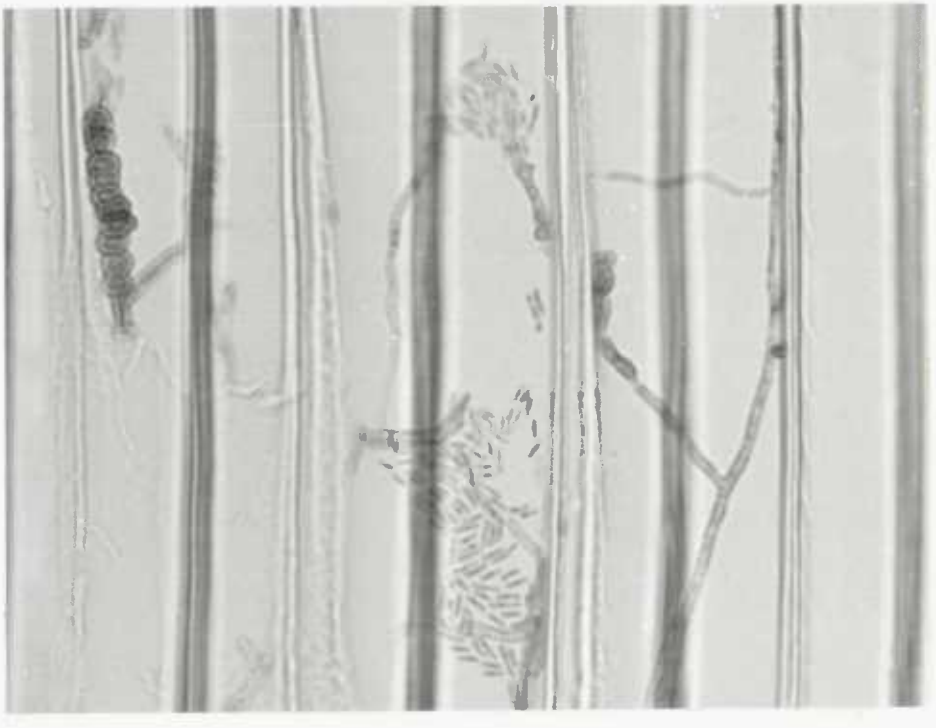


Fig 5.21 Bluestain type C. Culture No 35/1 MAa_3
Conidia present in Scots pine sapwood (RLS) after
4 weeks incubation (x 520)

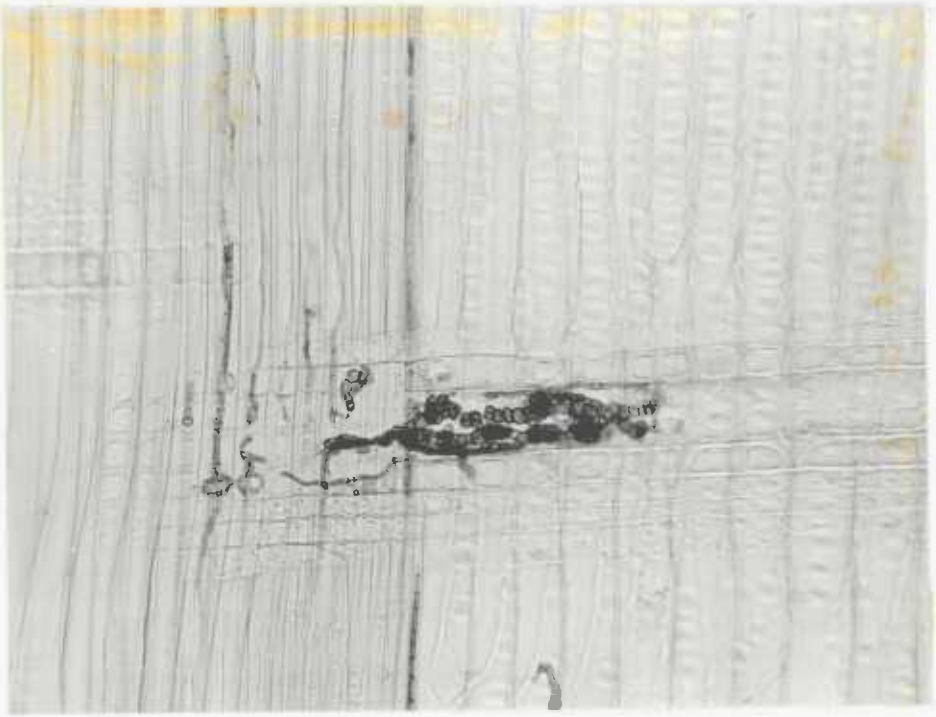


Fig 5.22 Bluestain type K. Culture No 84/2MAa
Colonisation of Scots pine sapwood (RLS) after 6 weeks incubation (x 135)

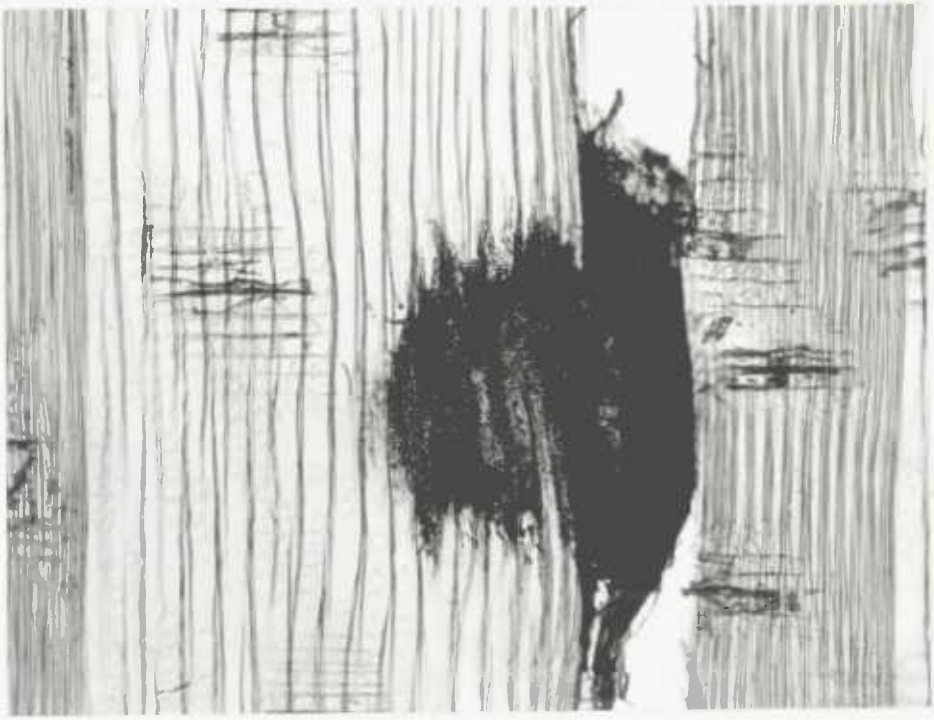


Fig 5.23 Bluestain type C. Culture No $35/1MAa_3$
Hyphal aggregation after 8 weeks incubation (x 85)

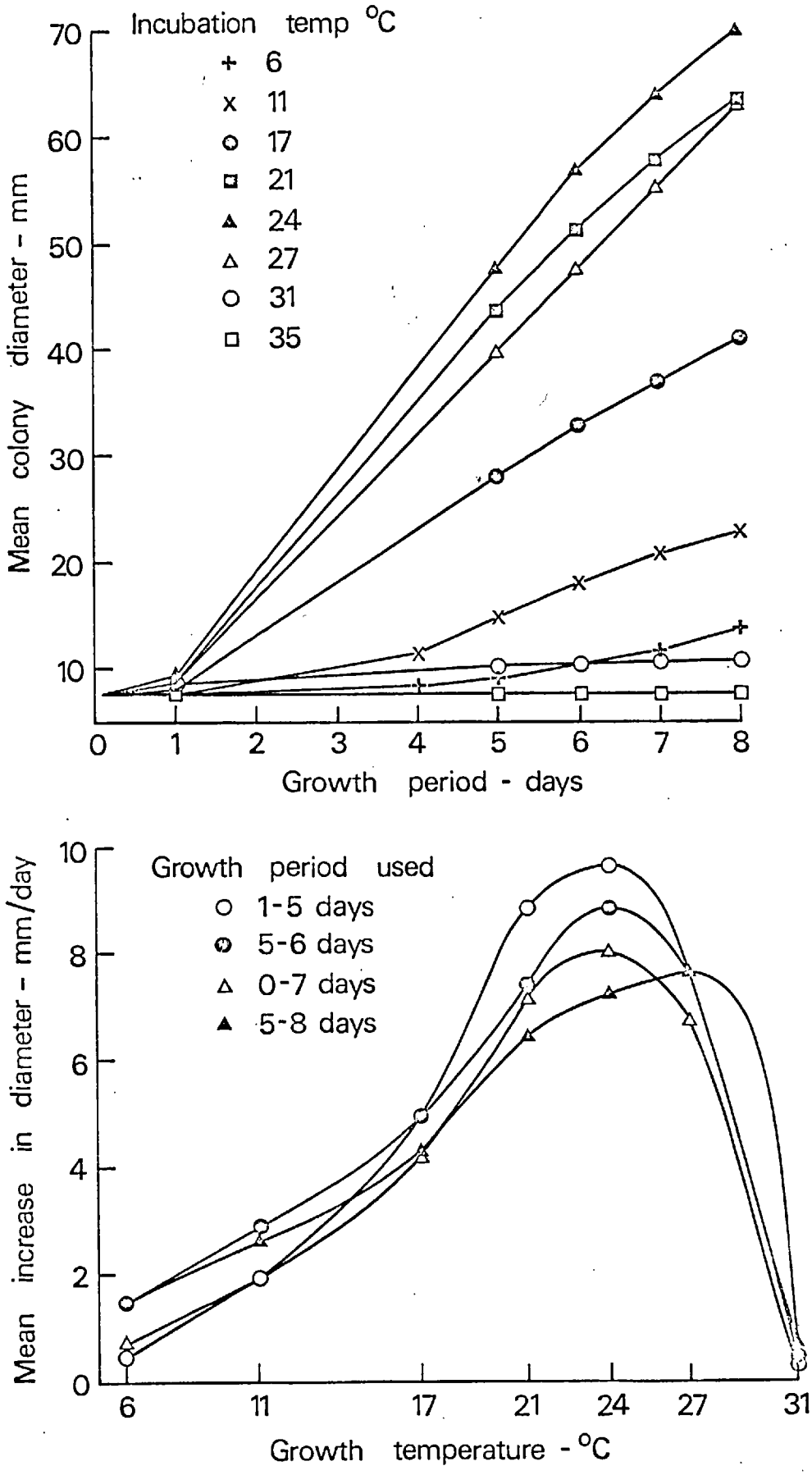


Fig. 5.24 Bluestain type 'B' (28/1 MAa)

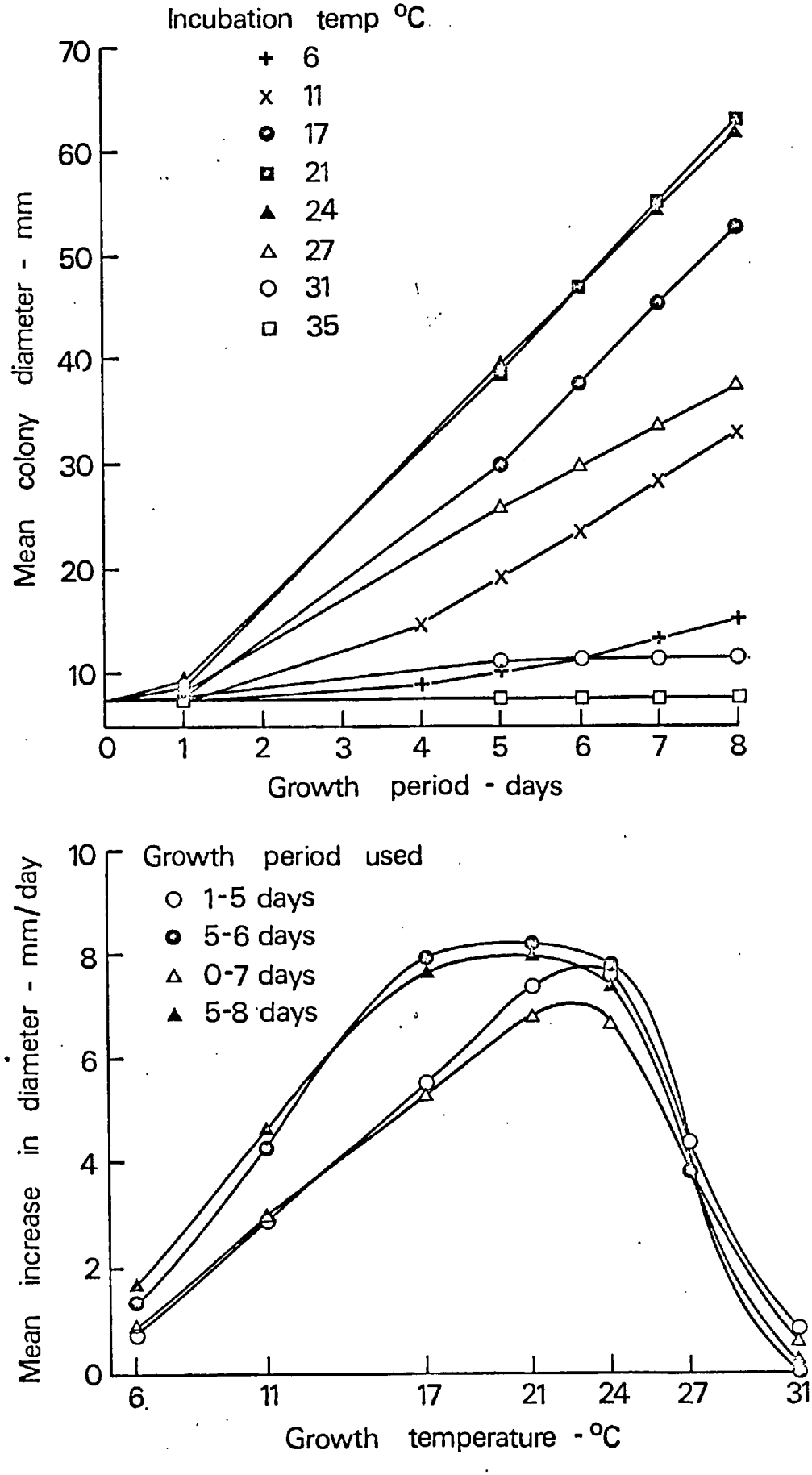


Fig. 5.25. Bluestain type 'D' (30/2 SCNa)

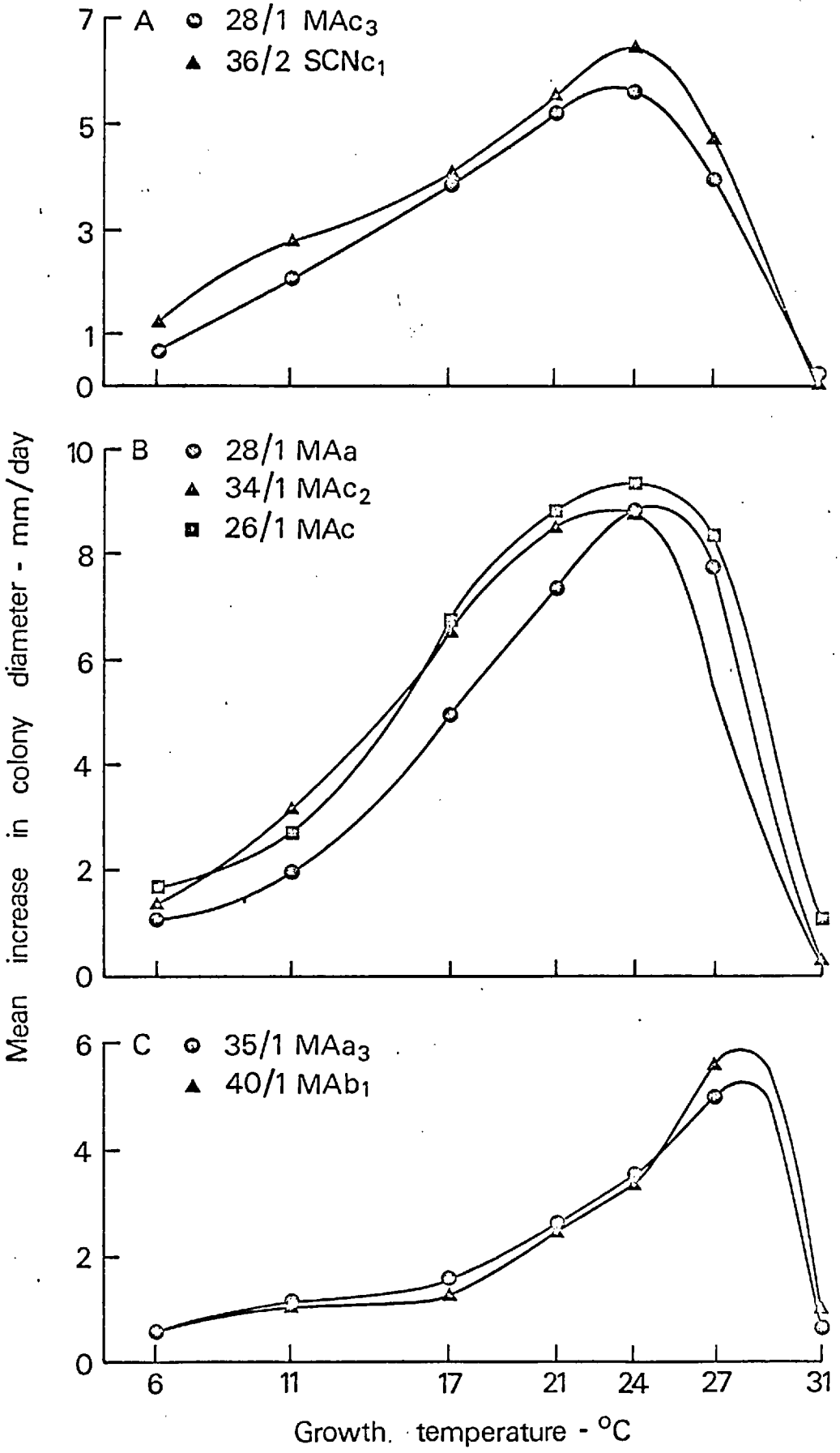


Fig 5.26 Temperature response curves

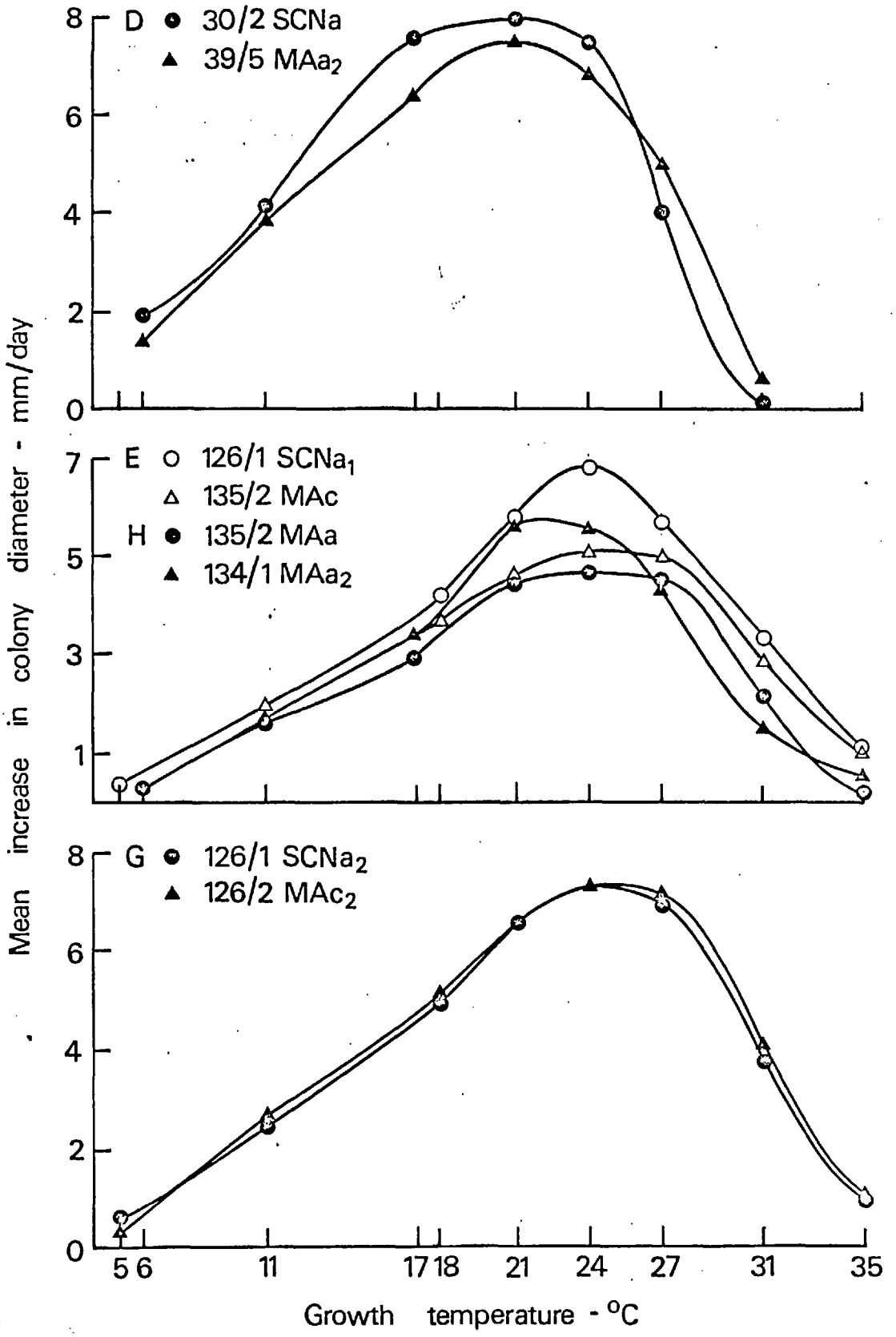


Fig. 5.26 cont. Temperature response curves

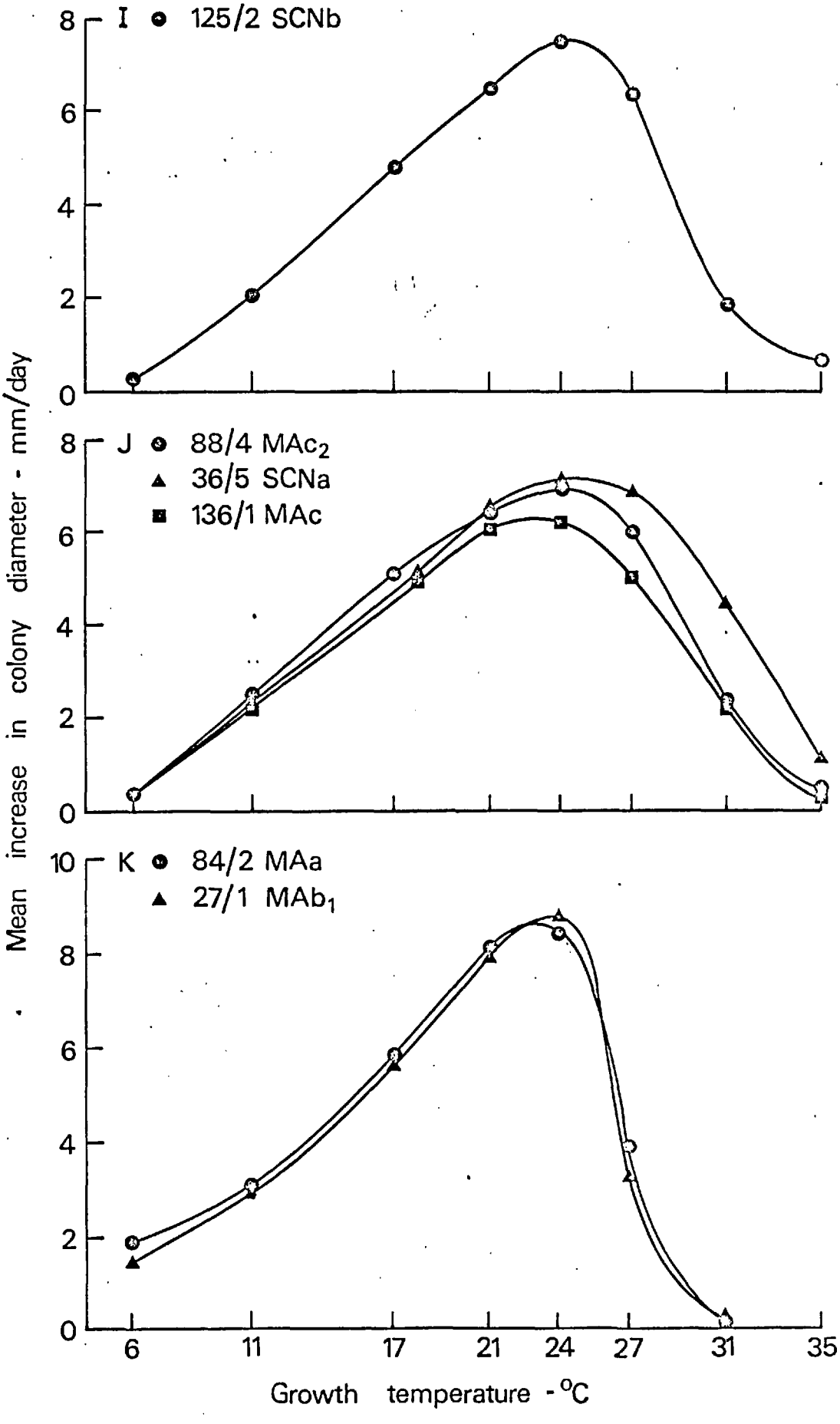


Fig. 5.26 cont. Temperature response curves

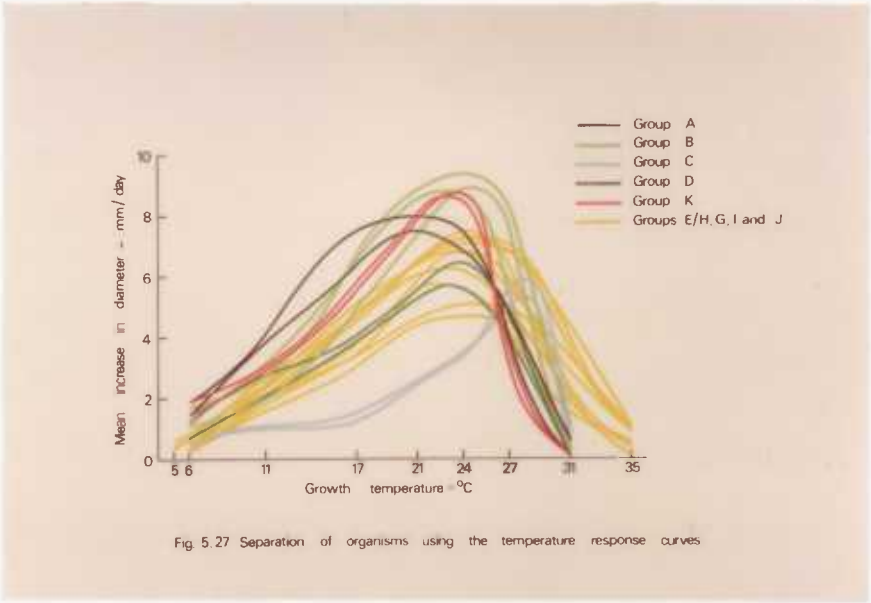


Fig 5.27 Separation of organisms using the temperature response curves

Fig 5.27 Separation of organisms using the temperature response curves.

Fig 5.28 Bluestain type K. Culture No 84/2MAa
Top 17°C 21°C 24°C
Bottom 27°C 31°C 35°C

Fig 5.29 Bluestain type B. Culture No 28/1MAa
Top 17°C 21°C 24°C
Bottom 27°C 31°C 35°C

Fig 5.30 Alternaria. Culture No 37/1 SCNb. Conidia in aerial mycelium (X 230)

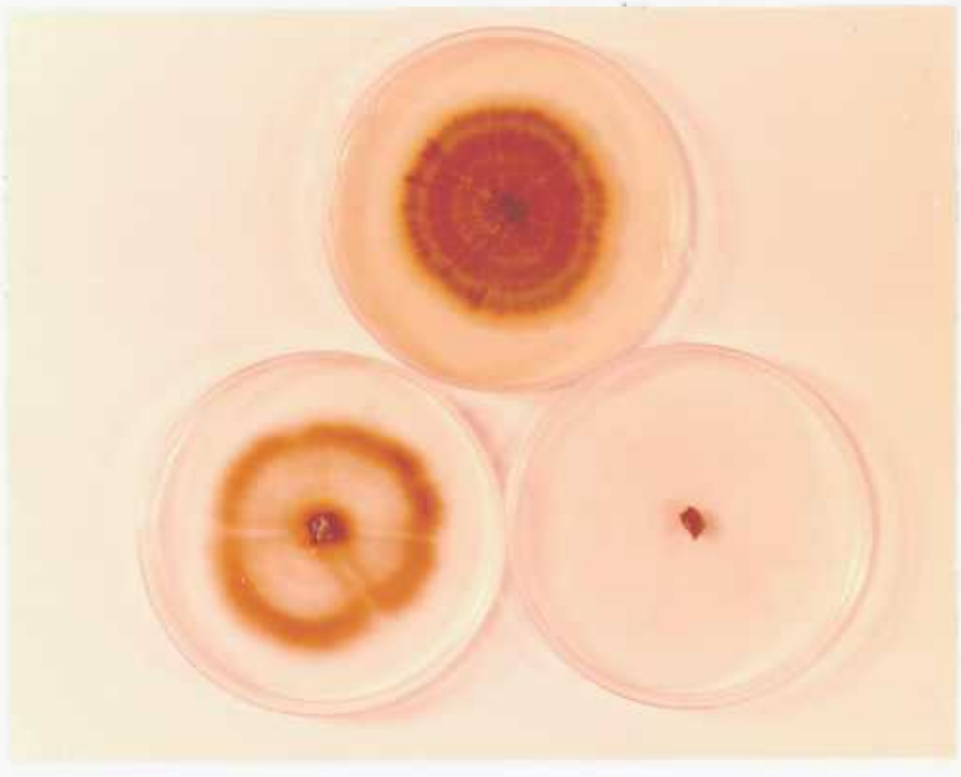


Fig 5.31 Ascomycete A. Culture No 86/2 MAc. Growth on three media. Top - 2 per cent malt agar, left - corn meal agar, right - czapek dox agar (X 0.5)

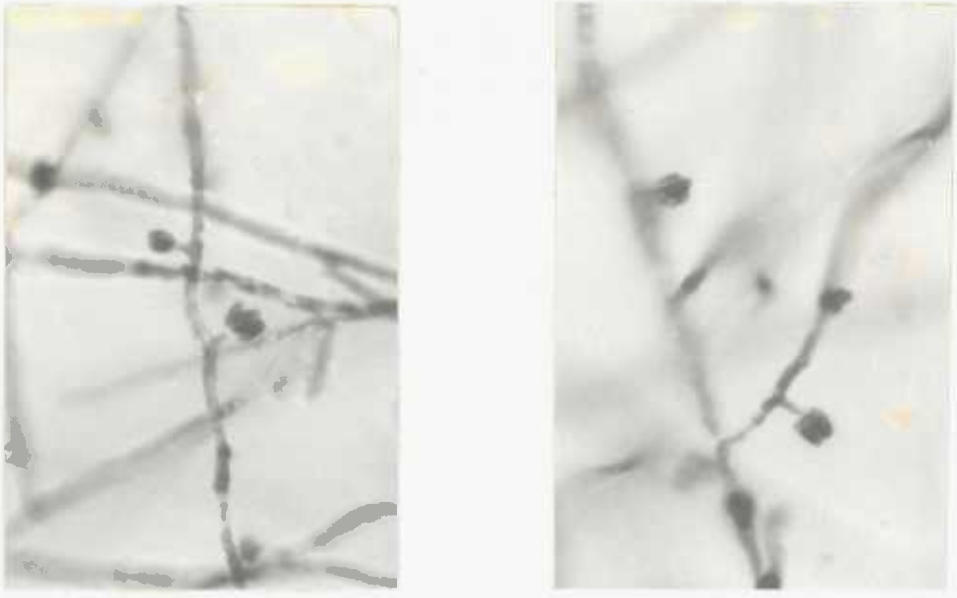


Fig 5.32. Ascomycete A. Culture No 94/4 MAa_2. Conidiogenesis (X 810)

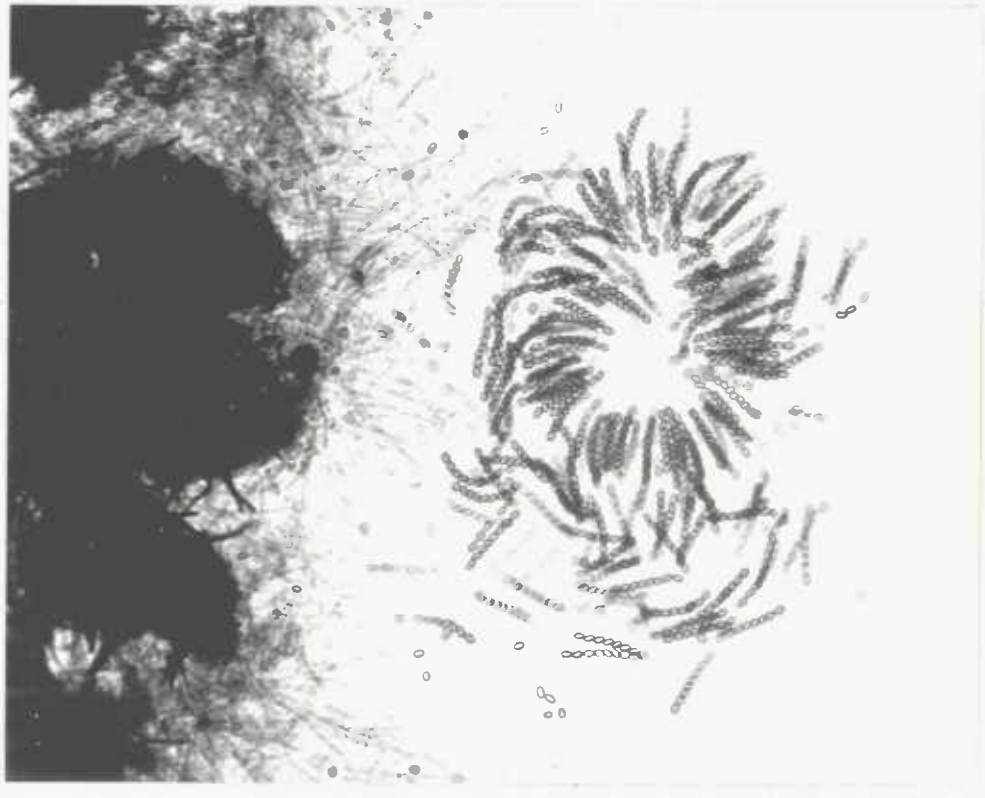


Fig 5.33 Ascomycete A. Culture No 92/2 MAa. Squash preparation of a perithecium (X 200)

Fig 5.34 Aspergillus fumigatus. Culture No 37/3 SCNa. Typical conidiophores (X 320)

Fig 5.35 Bluestain type F. Culture No 76/1 MAc_1. Mature spores showing irregularly thickened wall (X 650)

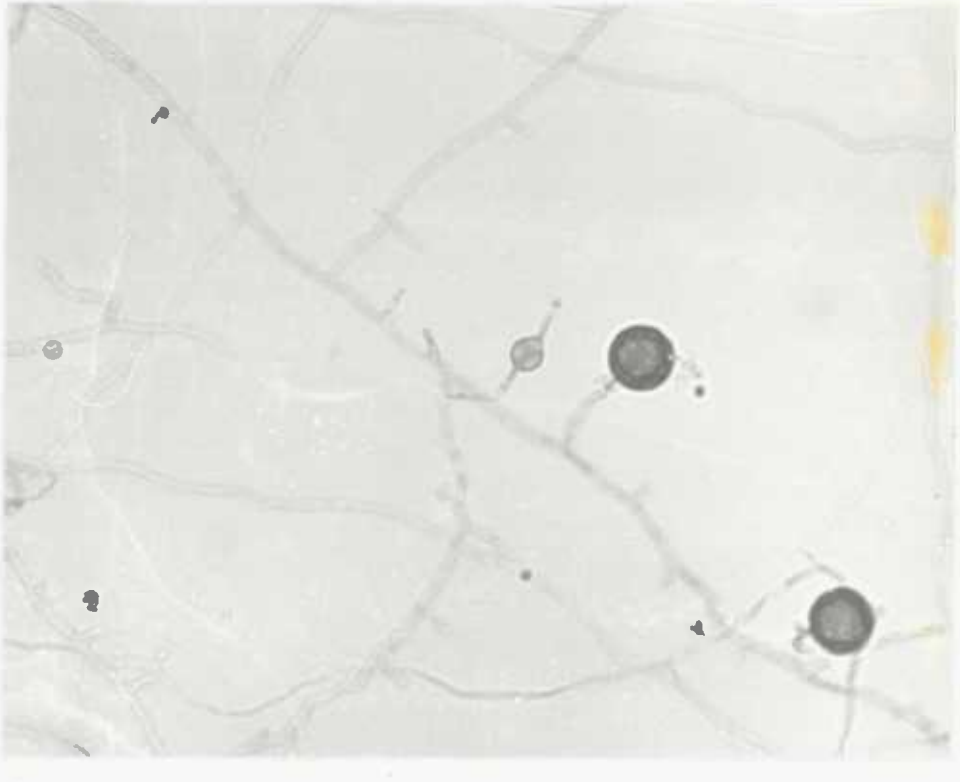


Fig 5.36 Bluestain type F. Culture No 76/1 MAc_1
Immature spore (X 650)

Fig 5.37 Botrytis cinerea. Culture No 16/1 SCNb.
Sclerotium production (X 0.9)

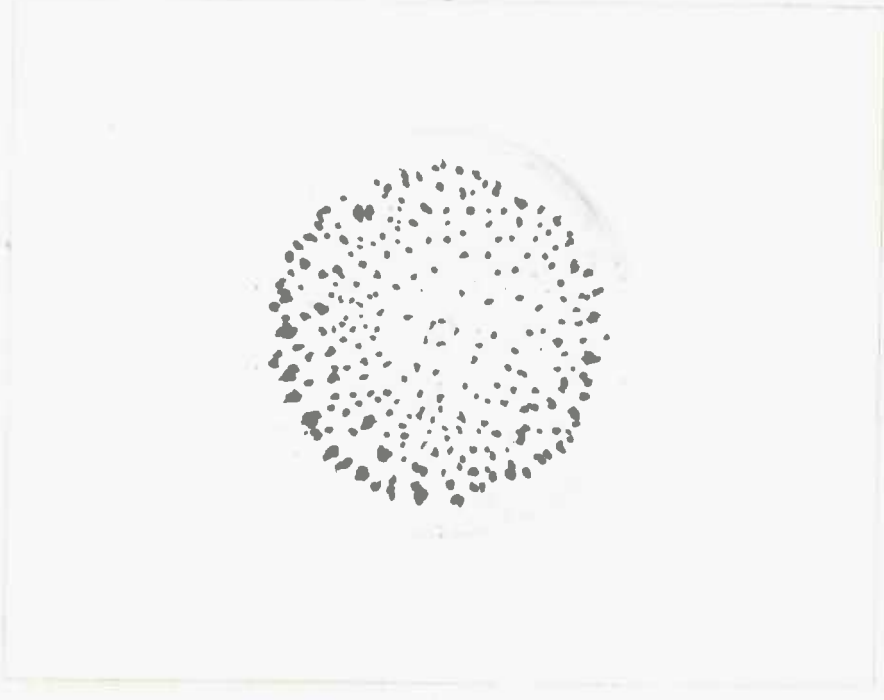


Fig 5.38 Botrytis cinerea. Culture No 26/1 MAa.
Sclerotium production (X 0.6)

Fig 5.39 Coniothyrium. Culture No 40/4 SCNa.
Pycnidium initials on corn meal agar (X 100)

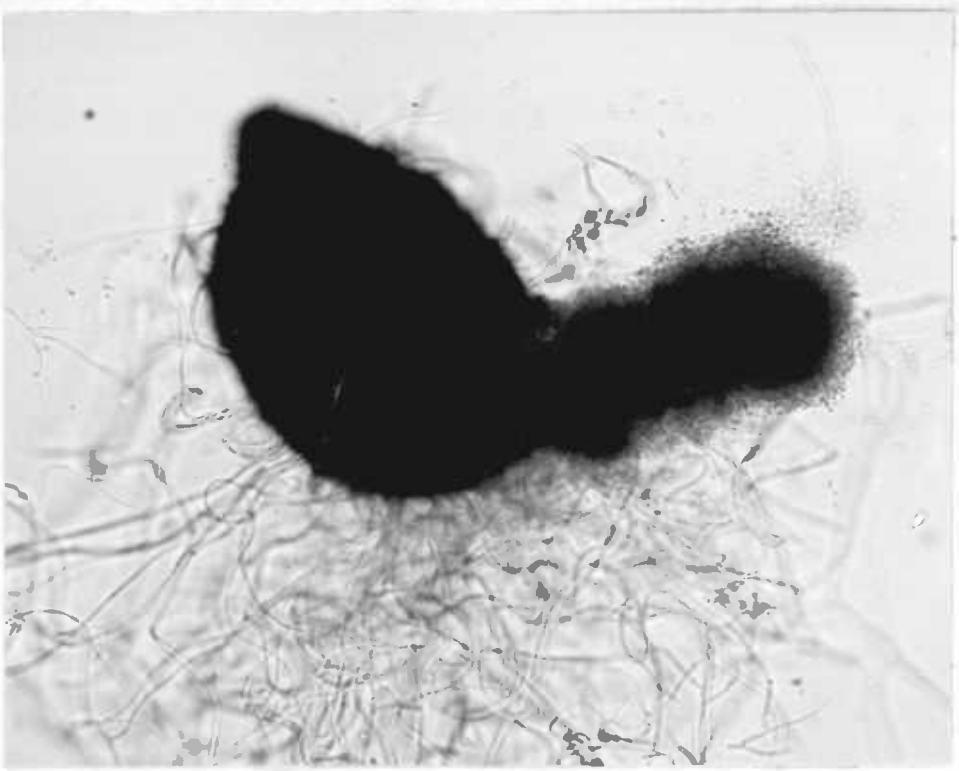


Fig 5.40 Coniothyrium. Culture No 42/1 MAb$_3$. Squashed pycnidium exuding spores, brown in mass (X 165)

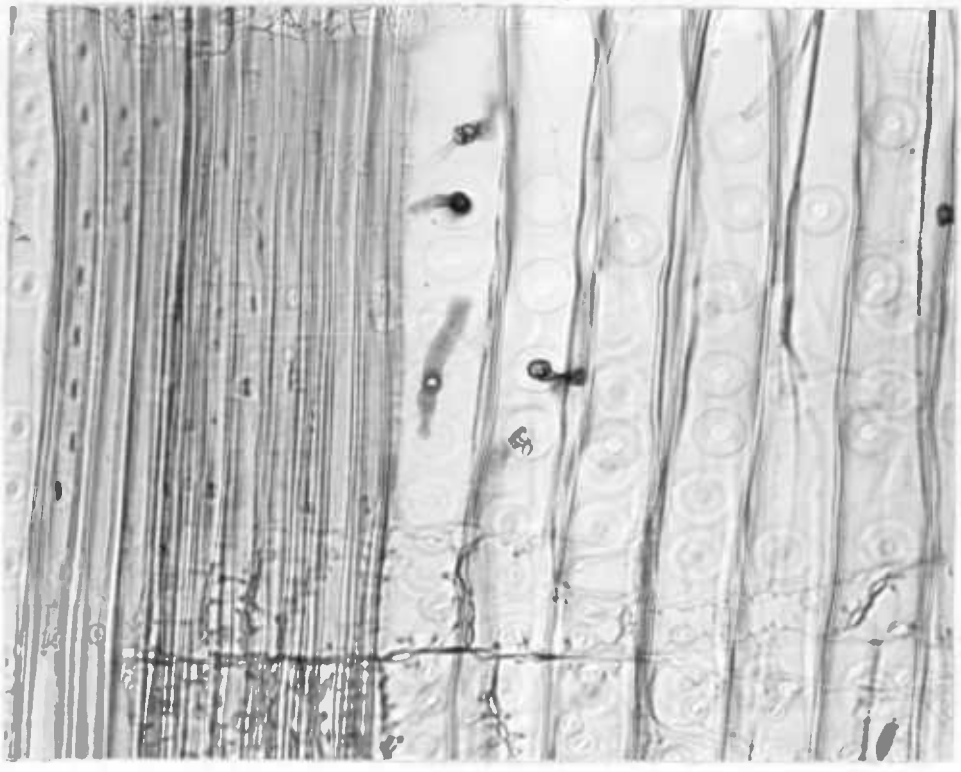


Fig 5.41 Diplodia gossypina. Culture No 27/2 MAc. Growth through bordered pits in Scots pine sapwood (X 260)

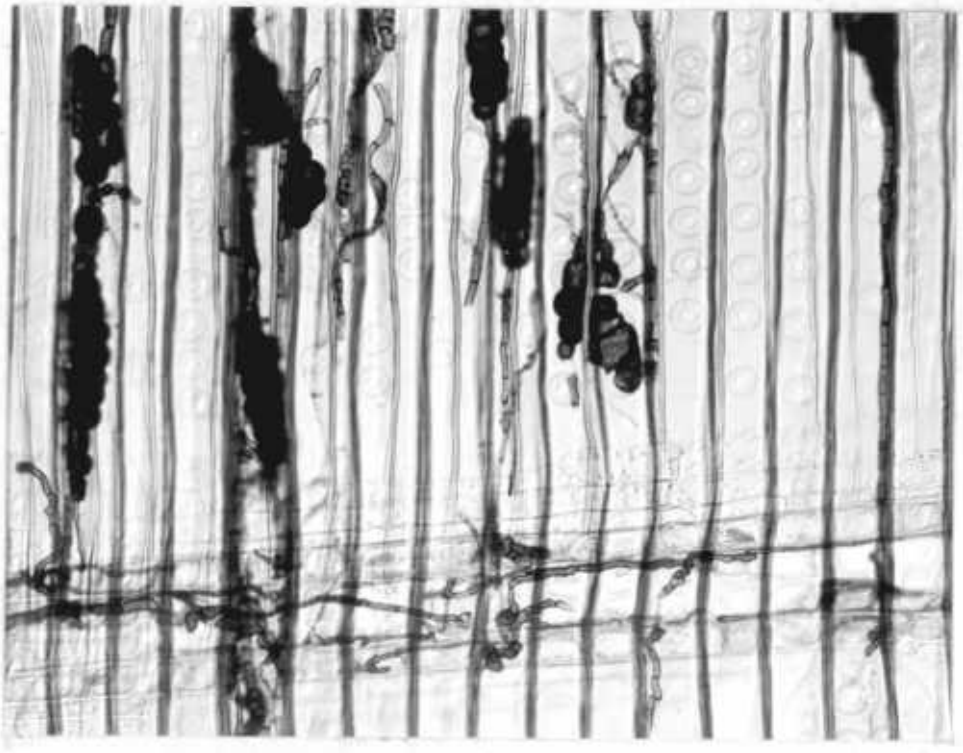


Fig 5.42 Diplodia gossypina. Culture No 27/2 MAc. Colonisation of Scots pine sapwood after eight weeks exposure (X 165).

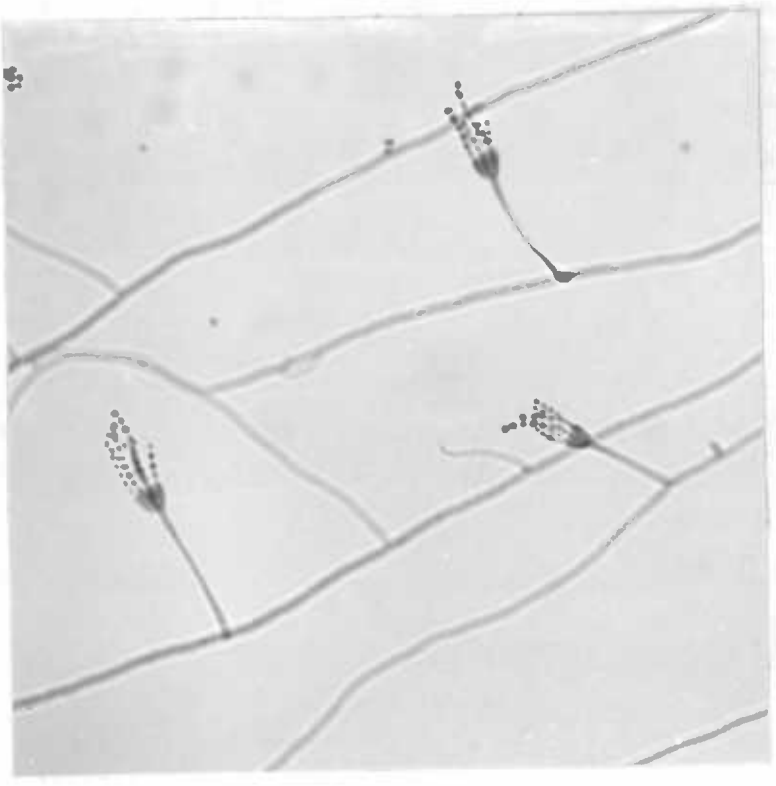


Fig 5.43 Penicillium type A. Culture No 137/2 MAb. Monoverticillate penicilli (X 350)

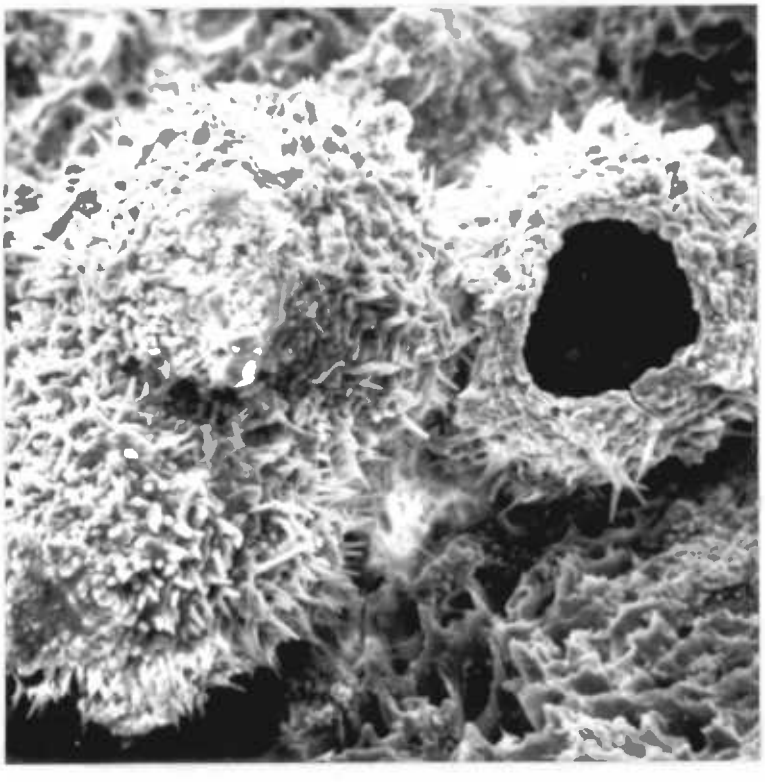


Fig 5.44 Coniochaeta perithecia viewed using an SEM. Note the change in form of the bristles around the ostiole and the similar changes in areas of the two other perithecia suggesting the position of the ostiole has been determined (X 220)

Fig 5.45 Coniochaeta perithecia attached to wood. Section showing the arrangement of the asci (X 140)

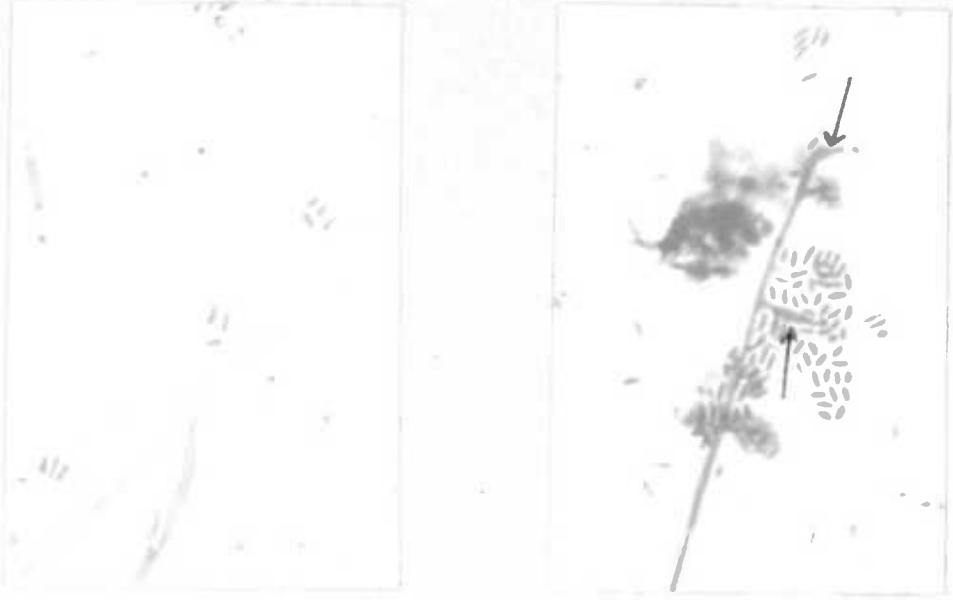
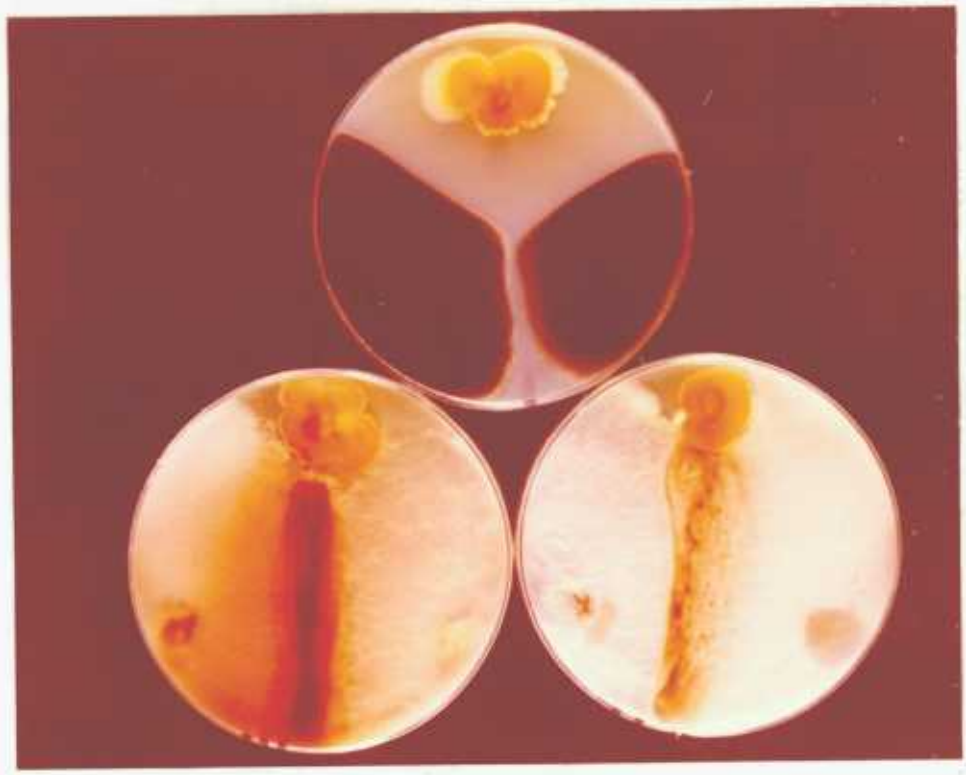


Fig 5.46 Phialophora type A. Culture No 36/5 SCNb.
Left - tapering phialides with newly released spores.
Right - more typically shaped phialides (arrowed) (X 665)

Fig 5.47 Phialophora type A. Culture No 34/1 SCNb. Squash preparation of a perithecium produced in culture showing ascospores contained within asci or free (X 160)



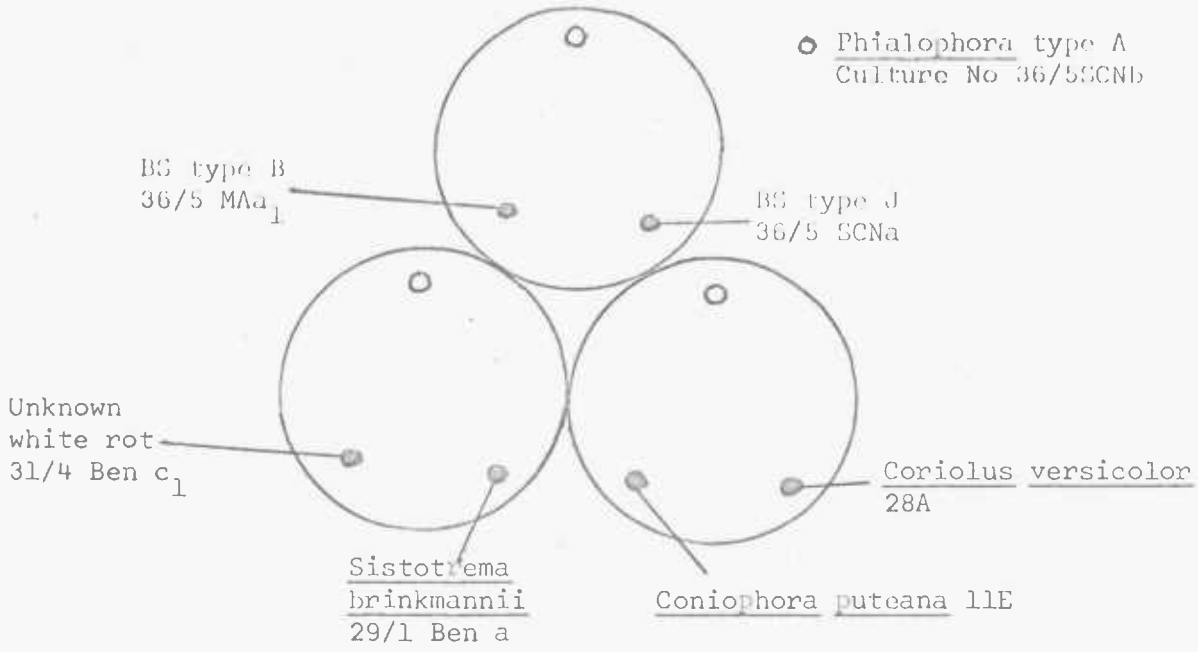


Fig 5.48 Antagonism between Phialophora type A and two bluestains but not with Basidiomycetes. Plates viewed from reverse side (x 0.6)

Fig 5.49 Pleospora. Culture No 17/1 SCNc. Squash preparation of a perithecium (X 100)

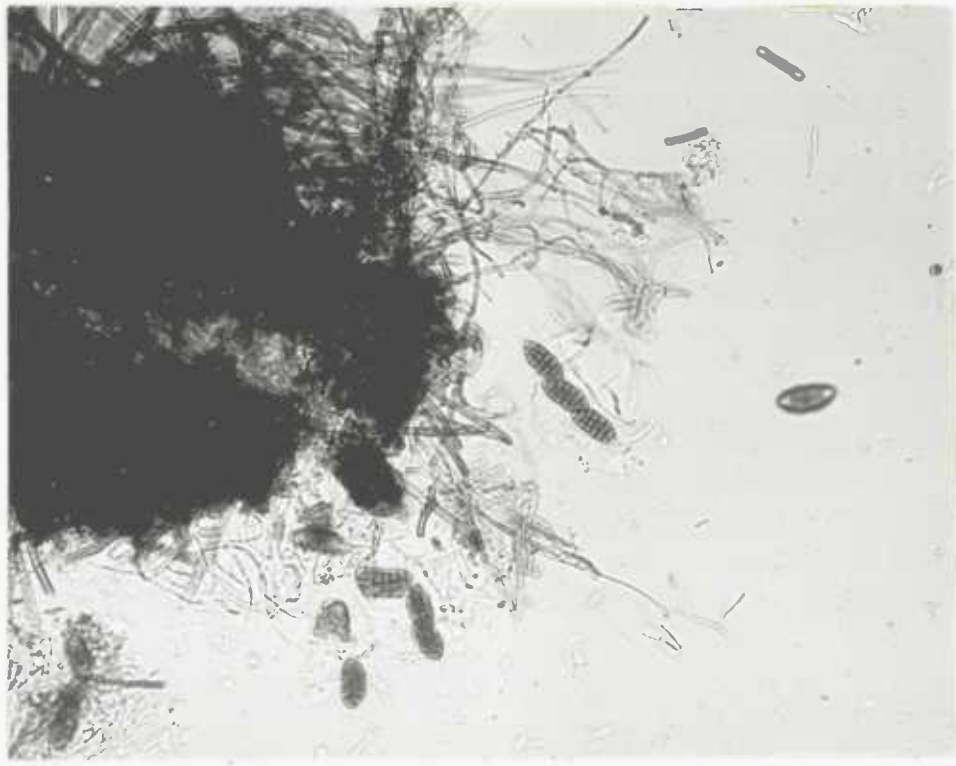


Fig 5.50 Pleospora. Culture No 17/1 SCNc. Ascospores, some retained in the remnants of asci, showing the constriction at the central transverse septum (X 260)

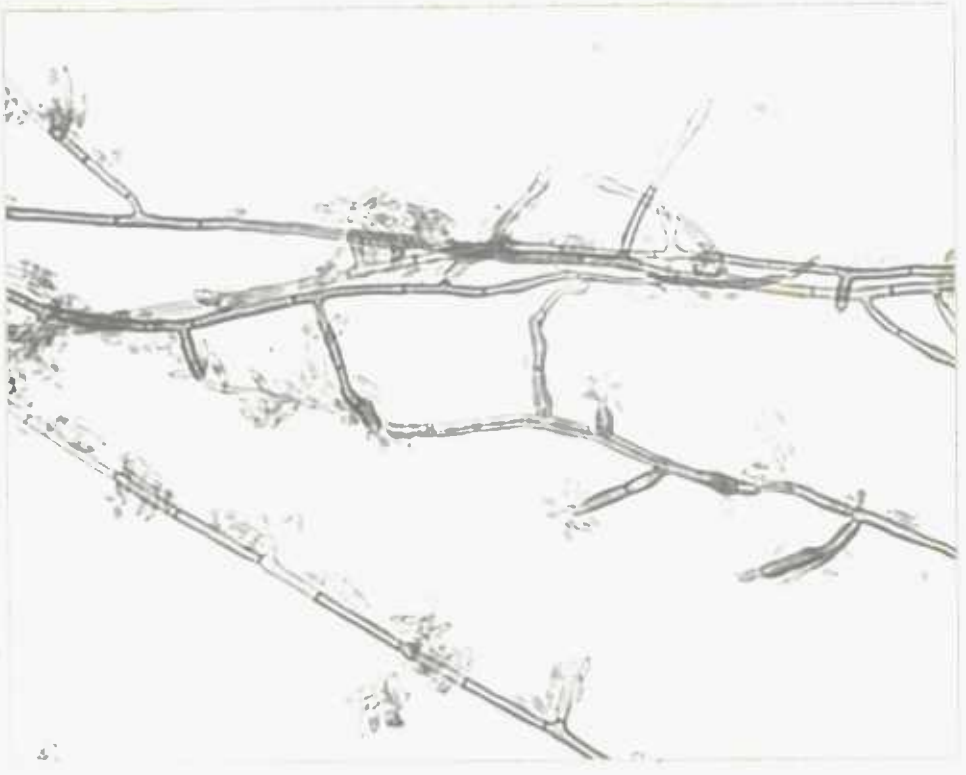
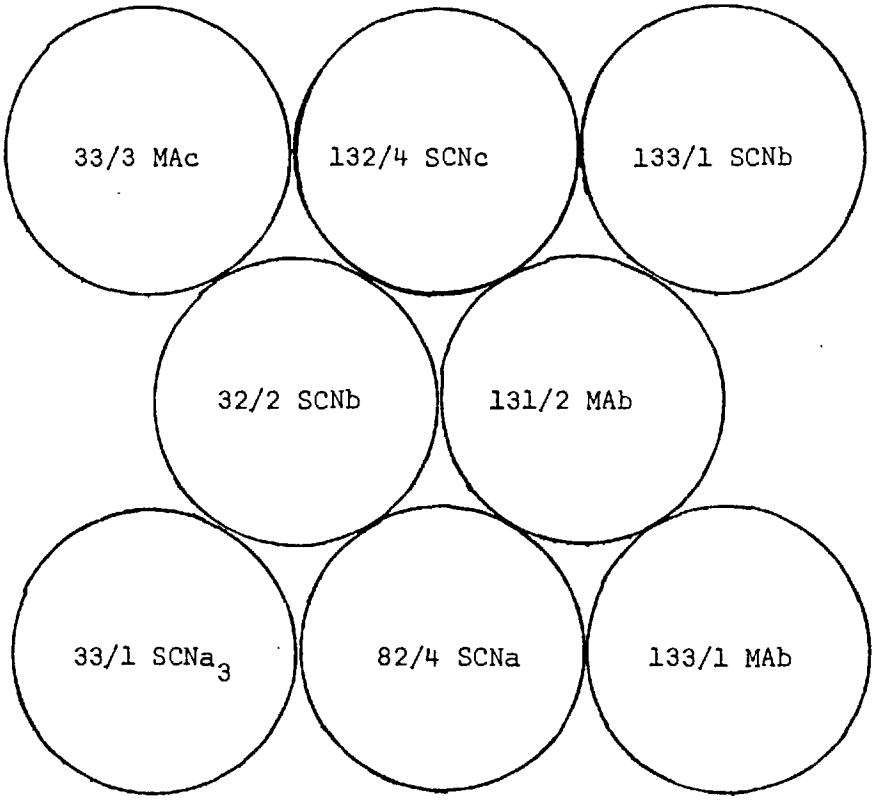
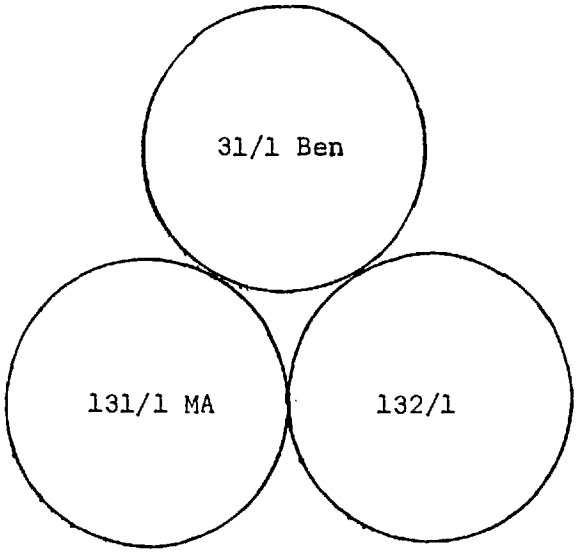


Fig 5.51 Rhinocladiella. Culture No 34/4 MAb.
Conidia borne on rachides (X 660)



Key to fig 5.52



Key to fig 5.53

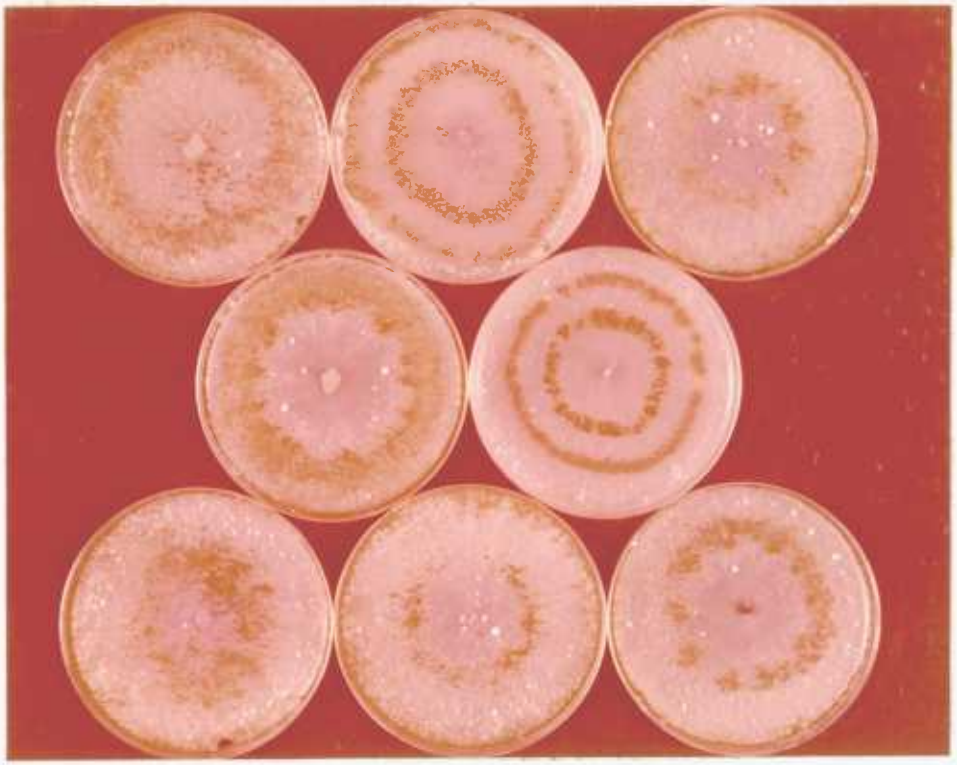


Fig 5.52 Trichoderma viride. Variation in sporulation between isolates.

Fig 5.53 Trichoderma viride. Interactions between isolates.

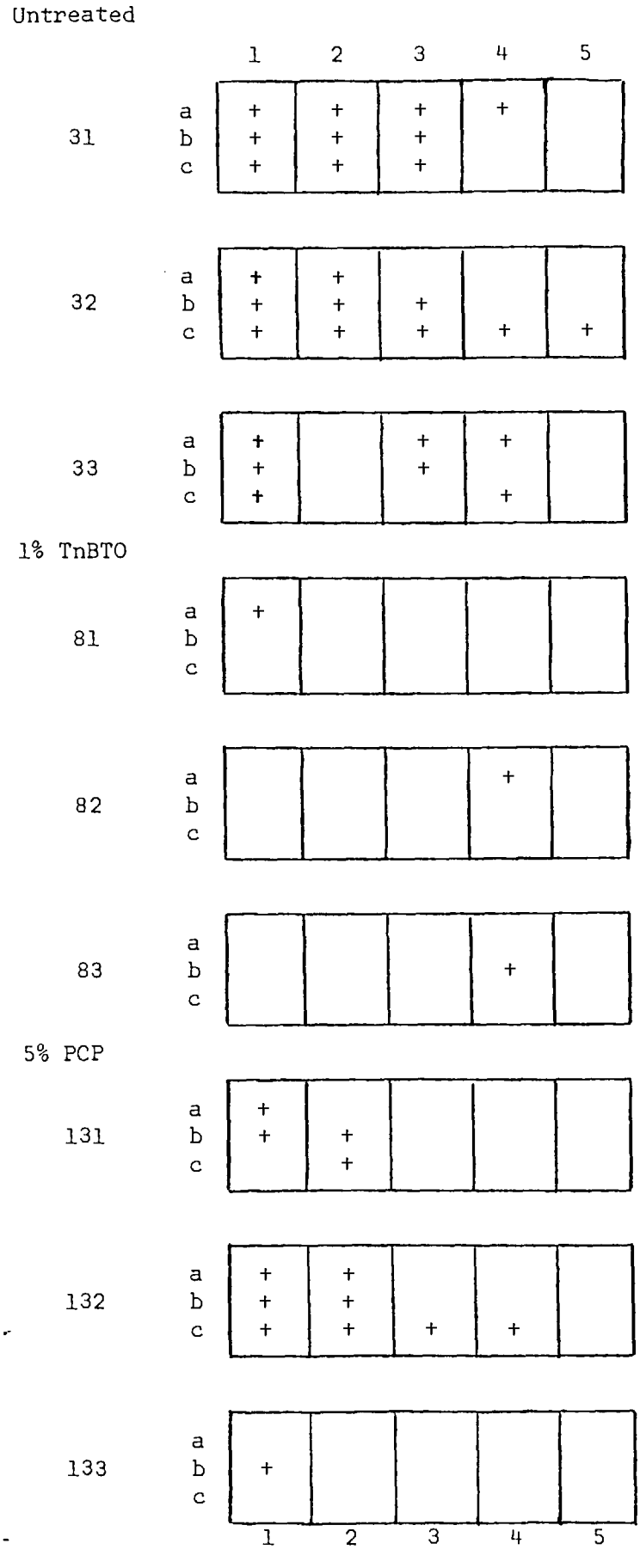


Figure 5.54 Distribution of T. viride isolates from L-joints after 71 days exposure

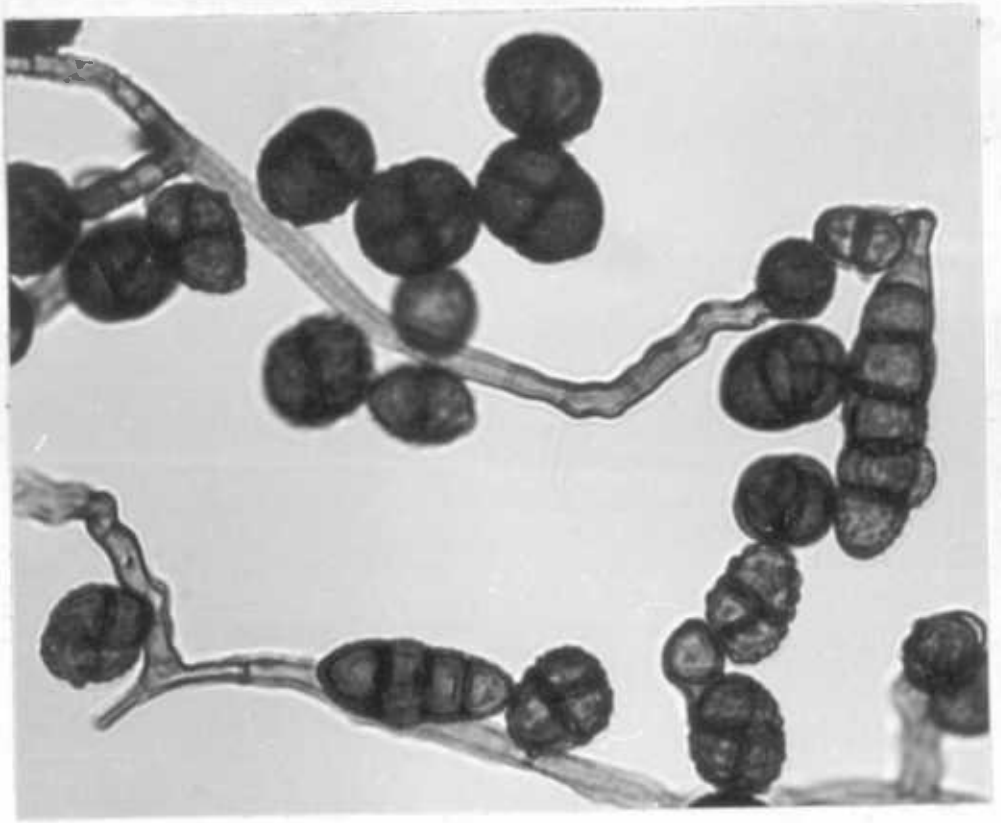


Fig 5.55 Ulocladium. Culture No 34/1 SCNa. Conidia produced in coverslip culture (X 810)

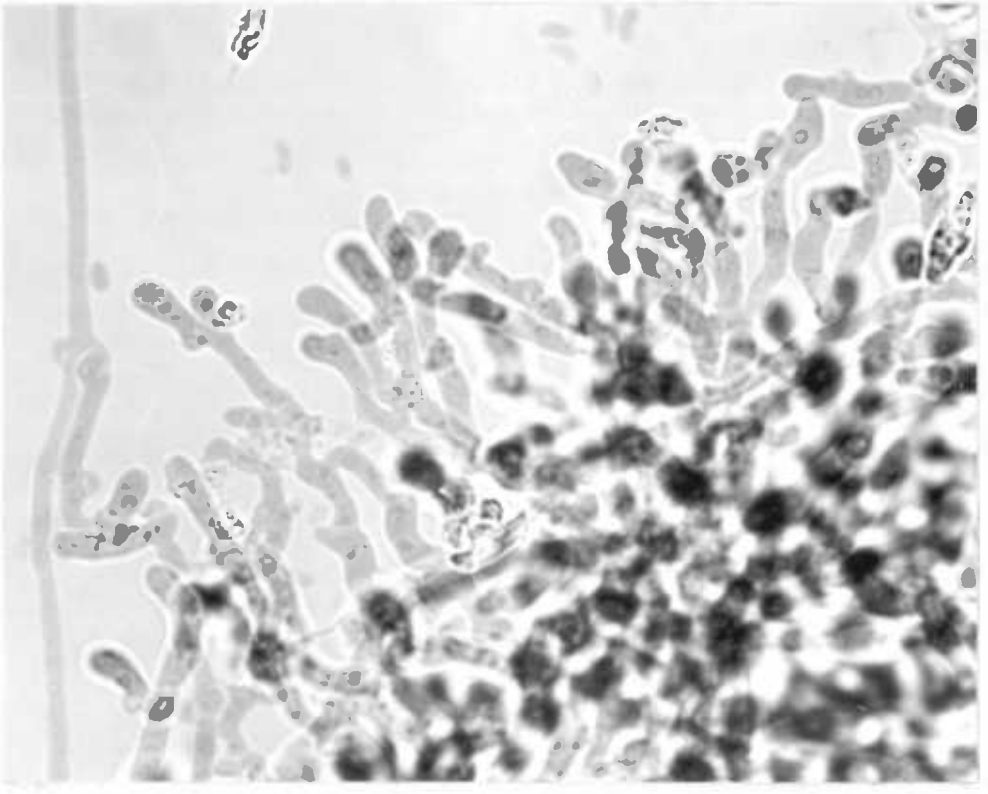


Fig 5.56 Sistotrema brinkmannii. Culture No 88/2 Ben b. Fruitbody showing its loose structure containing crystals, immature basidia with basal clamp connections, and free spores (X 830)

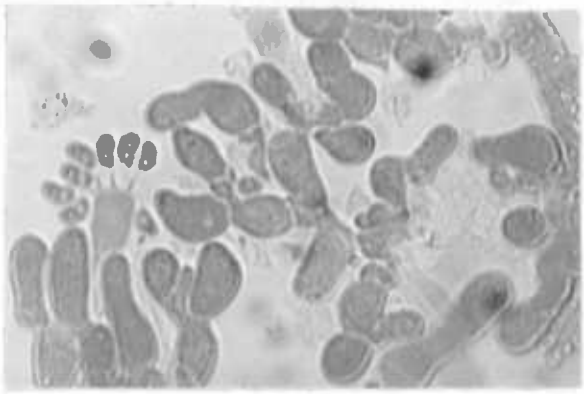


Fig 5.57 Sistotrema brinkmannii fruitbody showing eight basidiospores produced by one basidium (X 900)

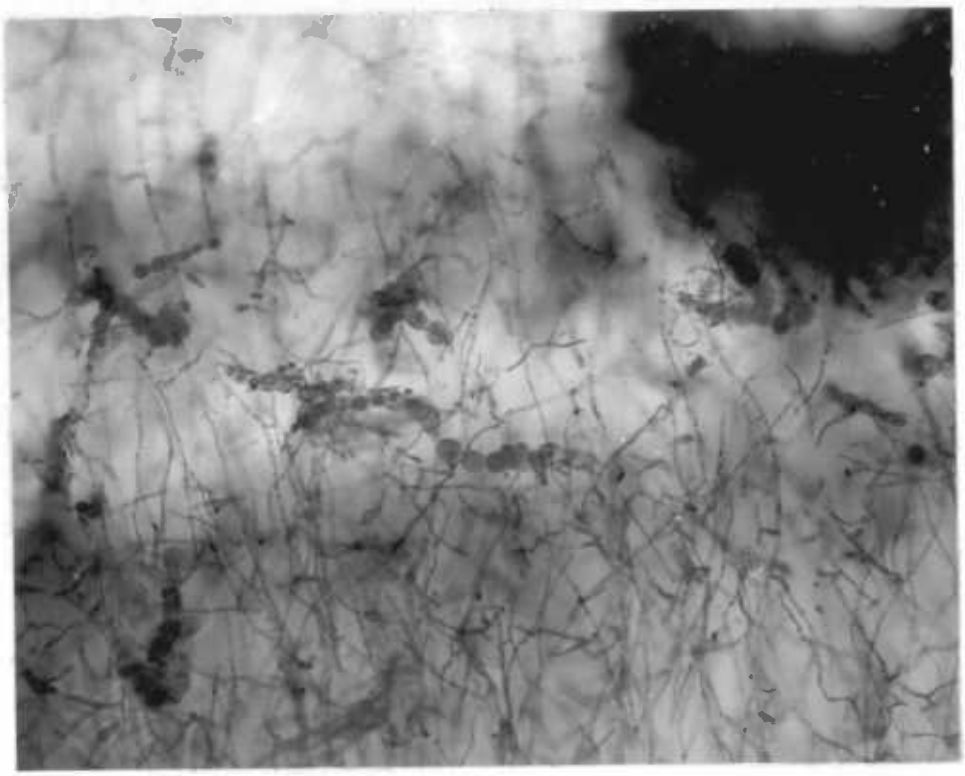


Fig 5.58 Sistotrema brinkmannii. Culture No 32/1 Ben b. Monilioid hyphae (large accumulation top right) (X 175)

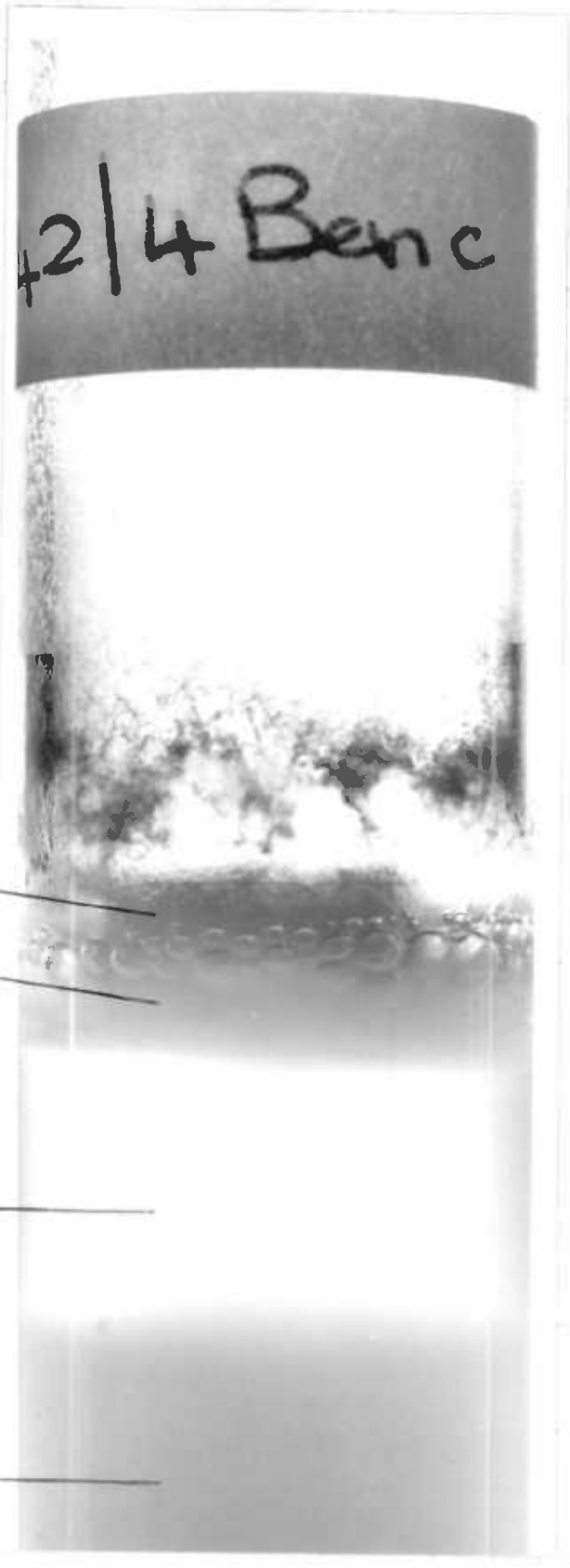
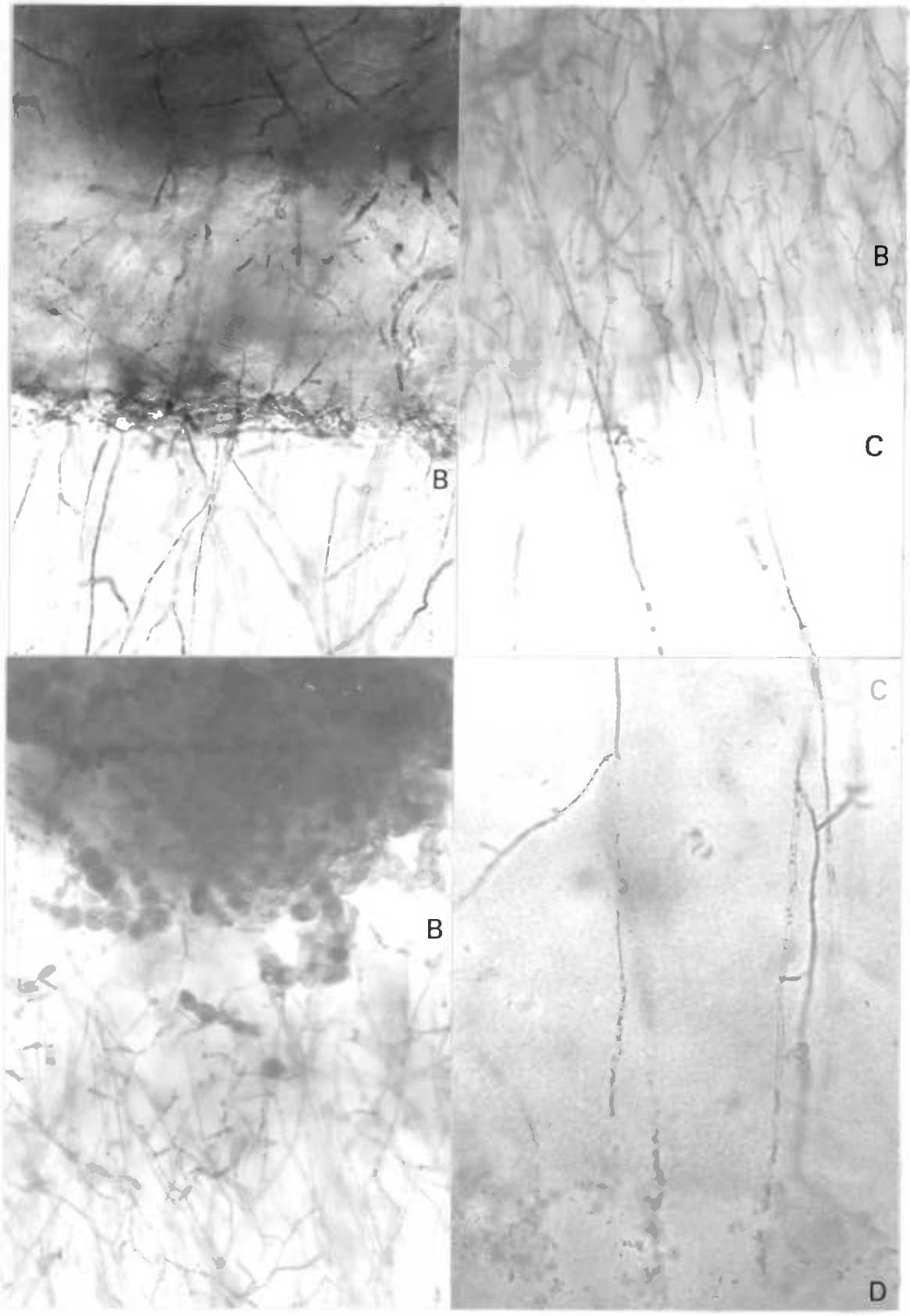


Fig 5.59 Sistotrema brinkmannii. Culture No 42/4 Ben c. Growth on cellulose agar (X 4)



A inoculum B zone of dense growth with monilioid hyphae

C zone of cleared cellulose D zone of uncleared cellulose

Fig 5.60 Sistotrema brinkmannii growth on cellulose agar (X 200)

Fig 5.61 Basidiomycete cultures from L-joint No 38.
Top b, left a, right c.

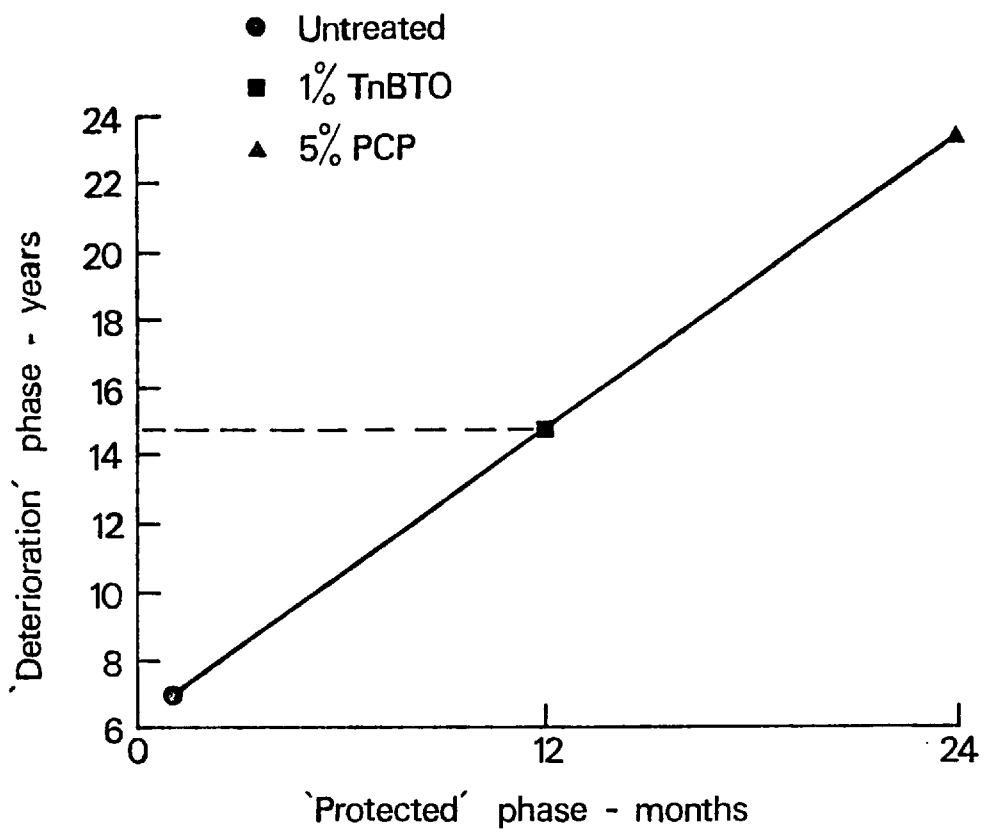


Fig. 5.62 Derivation of 'deterioration' phase for TnBTO

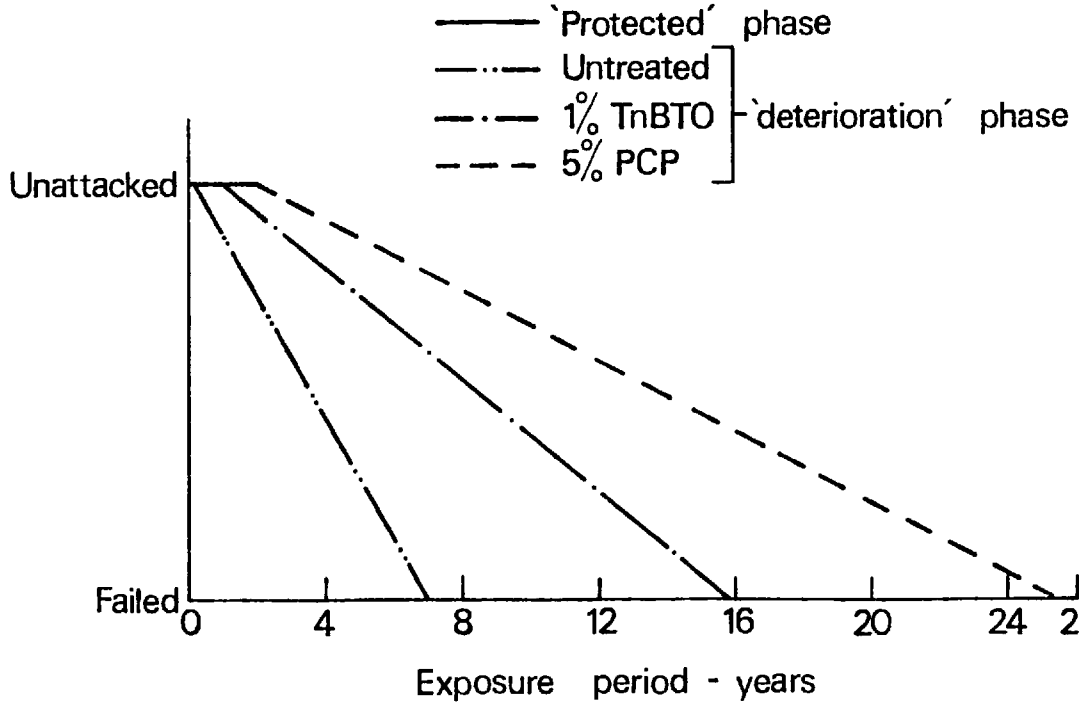


Fig. 5.63 The service life of L-joints

SECTION 6

MISCELLANY

6.1 INTRODUCTION

At various times during the course of the research work reported in sections 2 to 5 the results suggested additional areas of experimentation. These were undertaken as time permitted. They represent areas of associated research and have been referred to, where appropriate, in the text. They have been reported separately to avoid discontinuity in the previous sections. In several cases the experiments should have been repeated or further work undertaken but unfortunately time was not available.

6.2 THE EFFECT OF A RANGE OF ORGANISMS ON THE PERMEABILITY OF TEST BLOCKS OF SCOTS PINE SAPWOOD

6.2.1 Introduction

It is known that a variety of organisms including bacteria (Suolahti and Wallén, 1958; Ellwood and Ecklund, 1959) Trichoderma viride (Lindgren, 1952; Unligil, 1969) and bluestains (Lindgren and Scheffer, 1939) can be responsible for increasing the permeability of timber. Following the observation of increased permeability of the first series of L-joints (section 3) a variety of unknown bacteria and fungi from this environment were evaluated for their ability to increase the permeability of Scots pine sapwood blocks under laboratory conditions. In addition, four unknown bacteria isolated from the PRL log pond and various named fungi, known to occur in the joinery situation, were included.

6.2.2 Materials

6.2.2.1 Scots pine sapwood blocks measuring 38 x 24 x 10 mm with the annual rings parallel to the 24 mm dimension.

6.2.2.2 Modified Dubos medium of the following composition:

1 litre distilled water
0.5 g $NaNO_3$
1.0 g K_2HPO_4
0.5 g $MgSO_4\ 7H_2O$
0.5 g KCl
trace $Fe_2(SO_4)_3$

6.2.2.3 Cultures of the test fungi (table 6.1) growing on slopes of two per cent malt agar (2.3.1.2) in boiling tubes.

6.2.2.4 Cultures of the test bacteria (table 6.2) growing in flasks of nutrient broth (Oxoid code CMI).

6.2.2.5 Sterile glass bottles of 800 ml capacity (PRL jars) fitted with a ventilated screw cap and containing a piece of filter paper.

6.2.2.6 Sterile 500 ml erlenmeyer flasks containing 200 ml modified Dubos medium (6.2.2.2).

6.2.2.7 Dekalin; decahydronaphthalene

6.2.3 Methods

6.2.3.1 Fungi

Scots pine sapwood blocks (6.2.2.1) were soaked for four hours in distilled water, then wrapped in aluminium foil and autoclave sterilised at 121°C for 30 minutes. When cool 16 blocks were dipped in a mycelium macerate/spore suspension prepared from the culture of each fungus. An additional set of 16 blocks were kept as sterile controls. Sets of four replicate blocks were then placed on the filter paper in each replicate jar (6.2.2.5) and incubated at 25°C in polythene bags to prevent desiccation. The blocks were removed from one jar inoculated with each fungus and a control after 4, 8, 16 and 24 days growth, cleaned of adhering mycelium, then autoclave sterilised.

6.2.3.2 Bacteria

One ml of each bacterial culture (6.2.2.4) was added to each of six erlenmeyer flasks containing the sterile medium (6.2.2.6) which were incubated for 3 days at 25°C to establish growth. Six flasks were left uninoculated to provide sterile controls.

Three hundred and twelve wood blocks (6.2.2.1) were soaked in modified Dubos medium (6.2.2.2) for four hours then wrapped in aluminium foil and autoclave sterilised at 121°C for 15 minutes. Four blocks were added to the culture in each flask and incubated at 25°C, with daily agitation. The blocks were removed from one flask inoculated with each bacterium plus a control after 1, 2, 4, 8, 16 and 24 days growth, and autoclave sterilised.

6.2.3.3 Permeability testing

After exposure and sterilisation, all the blocks were dried to constant weight at 50°C, weighed, dipped for 10 seconds in dekalin (6.2.2.7), blotted to remove excess solvent and reweighed. The weight of dekalin absorbed was expressed as a percentage of the initial weight of each block.

6.2.4 Results

The mean uptake of dekalin was calculated for each set of four replicates. These values are presented in Table 6.1 for the fungi and Table 6.2 for the bacteria together with the number of replicates with absorptions above the range of the control values.

6.2.5 Discussion

Dipping in dekalin has been used as a simple method to detect changes in permeability; details of the development of this method are included in Appendix A. The method is reproducible and hence the variation in the control values reflects the variability of the timber which is so great that minor changes in permeability cannot be detected with certainty with so few replicates. Numerous series of replicates produced one block with a permeability above the range of control values (tables 6.1 and 6.2) but it is unlikely these results represent any real increase. Two of the fungi (BS-9 and S-6A) produced two blocks of higher permeability after the longest exposure period used (24 days); this could represent a real increase in permeability but the observation needs confirmation after a longer period of exposure. One fungus, Sistotrema brinkmannii, did produce significant increases in permeability after both 16 and 24 days exposure. This organism, though a Basidiomycete, has failed to produce significant weight loss of blocks in numerous laboratory tests (PRL unpublished reports; section 2.3) and yet possesses cellulase activity (Suhirman, 1978; section 5.4.1). It is possible the enzyme is not active against cellulose in the lignified cell wall but as with bacteria (Liese and Karnop, 1968) is able to attack the less lignified parenchyma cell wall and the cross field pits; destruction of the ray tissue in this way would result in increased permeability. Microscopic observations of blocks infected with S. brinkmannii show selective colonisation of the rays (PRL unpublished report); this, however, is probably associated with the sugars found in the ray cells rather than the cellulose; early colonisatión of the rays has been observed with various fungus/timber combinations by other workers (Bravery, 1972; Sorkhoh, 1976).

None of the bacterial isolates tested produced increased permeability. This may reflect deficiencies in the test method or too short an exposure period. However, although certain bacterial species have been shown to produce increases in permeability in pure culture (Ellwood and Ecklund, 1959; Greaves, 1966) tests with others, common in ponding

situations have been unsuccessful. Kurowski and Dunleavy (1976) proposed co-operation between species was a possible explanation in the field situation with a specific enzyme activity absent in one species being provided by another species.

6.3 DETOXIFICATION OF TnBTO BY ORGANISMS ISOLATED FROM JOINERY

6.3.1 Introduction

Isolation studies on the first series L-joints (section 3) showed a wide range of organisms to be present in TnBTO treated replicates after one year's exposure; visual observations confirmed the presence of soft rot attack. In an attempt to investigate the mechanism of tolerance to TnBTO, a range of organisms was grown on an agar medium containing various levels of TnBTO; their ability to detoxify the TnBTO was measured using germination of basidiospores of Lenzites trabea.

6.3.2 Materials

6.3.2.1 TnBTO, tributyltin oxide (Albright and Wilson Ltd).

6.3.2.2 Ten per cent gum arabic in aqueous solution, was sterilised by autoclaving for 15 minutes at 121°C.

6.3.2.3 Malt agar containing 2.5 per cent malt extract and 2.5 per cent agar was sterilised by autoclaving for 15 minutes at 121°C.

6.3.2.4 Cellophane, cut into 80 mm diameter circles, was boiled in distilled water for 30 minutes, to remove any surface dressing applied during manufacture, then sterilised by autoclaving for 15 minutes at 121°C

6.3.2.5 Cultures of the following fungi growing on plates of two per cent malt agar.

BS 12 - an unknown bluestain isolated from the first series L-joints (section 3)

S-6A - Phialophora fastigiata

S-9E - Aureobasidium pullulans

S-37E - Trichoderma viride

B730 - Sistotrema brinkmannii

6.3.2.6 Cultures of three unknown bacteria, isolated from joinery, growing in nutrient broth (Oxoid code CM1).

6.2.3.7 Basidiospores of Lenzites trabea (108E) were collected from fruitbodies produced in pure culture on agar containing 1 per cent cellulose and 0.5 per cent malt extract. Overnight, sterile slides were placed beneath the fruitbodies; the spores were washed off the slides using sterile distilled water and diluted to a concentration of 10^7 spores/ml.

6.3.3 Methods

0.016 g TnBTO (6.3.2.1) was incorporated in 50 ml gum arabic solution (6.3.2.2) and a one in two dilution series prepared. 5 ml of each concentration plus a control without TnBTO was mixed thoroughly with 195 ml molten malt agar (6.3.2.3); 20 ml aliquots were dispensed into 90 mm disposable petri dishes and allowed to solidify. The final concentration range in the agar was 0, 0.000025, 0.00005, 0.0001, 0.0002, 0.0004 and 0.0008 per cent w/v.

A cellophane disc (6.3.2.4) was placed on each of eight dishes at each concentration. One dish at each concentration was inoculated, centrally, with one of the test organisms using, for the fungi, a 6 mm diameter disc cut from the plate cultures (6.3.2.5) and for the bacteria, a single drop of the broth culture (6.3.2.6). The plates were incubated at 25°C for 12 days; the extent of the growth of each organism was marked on the bottom of the dish and its area calculated. The cellophane was then stripped off and the underlying agar streaked with basidiospores of L. trabea; three previously uninoculated plates at each concentration were also streaked with spores. All plates were incubated at 25°C for 24 hours then the agar surface was stained with 0.05 per cent aniline blue in lactophenol and observed using a binocular microscope.

6.3.4 Results

Growth of the test organisms on the malt agar plates containing various concentrations of TnBTO is recorded in Table 6.3 and germination of basidiospores of L. trabea in Table 6.4.

6.3.5 Discussion

The concentration range was selected to observe the effect of the growth of the range of organisms on subsequent germination of basidiospores of L. trabea. The growth of all the organisms on the highest concentration

of TnBTO does not, therefore, detract from the results. Comparison with toxic values established using a similar test method for three common decay test fungi - Coniophora puteana 0.0038-0.0075 per cent w/v, Coriolus versicolor 0.0019-0.0038 per cent w/v, Poria placenta 0.00031-0.00063 per cent w/v (PRL unpublished data) - shows the highest concentration is well below that required to inhibit the two more tolerant decay fungi. With the bacteria it was not possible to quantify the amount of growth but with the fungi, the area of growth was measured (Table 6.3). T. viride appeared most tolerant, its growth fully covering all the test plates; S. brinkmannii only showed reduced growth at the highest concentration. The unknown bluestain (BS 12) and A. pullulans showed a reduction in growth with each increase in concentration. P. fastigiata showed a stimulation of growth by the lowest concentration; this is a common effect with high dilutions of wood preservatives (Kaufert and Schmitz, 1937; Schulze-Dewitz, 1964). Growth was, however, reduced with further increases in concentration. Basidiospores of L. trabea germinated on concentrations of TnBTO up to 0.0001 per cent w/v (Table 6.4). Following growth by the test organisms, observation of basidiospore germination was often difficult due to the organisms having grown through the cellophane; no observations were possible with either P. fastigiata or S. brinkmannii. Among the other organisms, three types of reactions were observed. First, one bacterium (9D(B)5) inhibited basidiospore germination at both 0.00005 and 0.0001 per cent w/v; many examples of antagonism between organisms are found in the literature (Greaves, 1970; Dennis and Webster, 1971; Grosclaude et al, 1974) although in this case the bacterium could be acting indirectly by making the spores more sensitive to the TnBTO. Secondly, three organisms produced no effect on spore germination (unknown bluestain BS 12, unknown bacteria 9B(A)1 and 12C(D)1). Thirdly, growth of A. pullulans and probably T. viride, allowed spore germination to occur at higher concentrations of TnBTO. With A. pullulans the effect appeared to occur over most of the plate at 0.0002 and 0.0004 per cent but was limited to the area under the colony at 0.0008 per cent. The effect with T. viride was less definite and confined to 0.0002 per cent. The mechanism of the "detoxification" has not been investigated; two alternatives appear possible. First, the TnBTO has been "locked up" either inside the hyphae or perhaps in the extracellular pullulan

produced by A. pullulans. Second, the TnBTO has been degraded to the less toxic dibutyl- and monobutyl-derivatives. This occurs naturally during exposure of TnBTO treated wood and the rate can be accelerated by both C. puteana and C. versicolor (Henshaw et al, 1978). Whatever the mechanism, if such detoxification occurs within treated wood it could facilitate decay by Basidiomycetes which are, in general, less tolerant to preservatives than moulds, bluestains and soft rots (Butcher, 1971). Such associations have been noted previously between Fusarium oxysporum and Coprinus micaceus in the decay of posts treated with a fluor-chrome-arsenate-diphenol preservative (Madhosingh, 1961a, 1961b) and between Penicillium roquefortii and Stereum sanguinolentum in wood pulp treated with phenyl mercuric acetate (Russell, 1955) and quite clearly can be of practical significance in the failure of preservative treatments.

6.4 BASIDIOMYCETE INTERACTIONS

6.4.1 Introduction

During observations on joinery after various periods of exposure (section 2) it appeared that, within the decay fungi, a succession took place of first white rot and then brown rot. Occupiers of dwellings have often reported very rapid decay, by brown rots, although this is probably a result of the early stages of decay going unnoticed by the layman. Isolations from simulated joinery units (sections 3 and 4) have yielded white rots and Sistotrema brinkmannii but no brown rots. This experiment has therefore been designed to test the hypothesis that S. brinkmannii and a white rot (Coriolus versicolor) may have some effect on the subsequent rate of decay by other organisms using a method similar to that employed previously by Banerjee (1969) and von Aufsess (1976b).

6.4.2 Test fungi

Coniophora puteana FPRL No 11E
Coriolus versicolor FPRL No 28A
Sistotrema brinkmannii FPRL No B730

6.4.3 Methods

Test blocks of Scots pine sapwood, measuring 30 x 10 x 5 mm and within a narrow weight range were numbered, oven dried and weighed. The blocks were autoclave sterilised for 20 minutes at 121°C then planted, 3 per plate, on Netlon mesh over actively growing cultures of the first test fungus and incubated for 6 weeks at 22°C. Some blocks were removed from test at this time (options 7 and 9, table 6.5) while others were not disturbed (options 8 and 10). Most blocks (options 1 to 6) were transferred to a second fungus for a further period of 6 weeks; details of the intermediate handling procedures are given in Table 6.5. Additional blocks were also introduced (options 11 and 12). At the end of the final incubation period the blocks were cleaned, weighed, oven dried and reweighed.

6.4.4 Results

The combinations of fungi, methods of handling and incubation periods used are given in Table 6.5. Details of moisture content and per cent weight loss are presented in Table 6.6. Weight losses achieved by individual fungi (options 7-12) have been used to calculate a 'theoretical' weight

loss (table 6.7), assuming no interaction occurs between the fungi, when test blocks are exposed to two fungi in succession.

6.4.5 Discussion

At the end of the exposure periods the sets of replicates showed variable weight losses. Low weight losses were generally accompanied by high moisture contents indicating a waterlogged condition. This results in restricted access of oxygen and hence lower weight losses. Data for a number of the options have therefore been divided into two groups based on weight loss and moisture content (table 6.6); the division between the two groups was normally obvious, although in one set (option 2) the arbitrary line of 100 per cent moisture content was used. Groups with the lower moisture contents and therefore not affected by waterlogging (marked with a * in table 6.6) have been used as the basis of the comparisons made in Table 6.7.

The results showed a disconcerting variation between weight losses of the sets of replicates exposed to Coriolus versicolor during the first 6 week period (12.0 per cent) and that exposed during the second 6 week period (6.1 per cent). This variation must reduce the emphasis which can be placed on the results achieved using this organism.

Options 7 to 12 represent the various components of options 1 to 6. Using these data the theoretical weight loss for the various combinations of fungi used in options 1 to 6 have been calculated (Table 6.7); where blocks were not autoclaved between exposure to the two fungi the weight loss of the first fungus over a 12 week incubation period has been used.

In all four cases where S. brinkmannii was the first test fungus, the theoretical weight loss is very close to the actual weight loss which shows:

a S. brinkmannii does not predispose the timber to attack by either of the other test fungi.

b S. brinkmannii is not antagonistic to either of the other test fungi.

This second point is of interest since von Aufsess (1976a, 1976b) has shown antagonism does occur in laboratory tests between S. brinkmannii and Stereum sanguinolentum or Tyromyces stipticus but not with Armillariella mellea or Heterobasidion annosum.

Where Coniophora puteana followed Coriolus versicolor the actual weight loss was less than the theoretical weight loss independent of sterilisation between fungi. This suggests that timber previously attacked by a white rot organism is less available for subsequent attack by a brown rot; antagonism is not relevant since the effect is not altered by sterilisation of test blocks between exposure to the two fungi. This observation does not support the theory that brown rot may occur more rapidly on timber previously attacked by white rot. It does not, however, nullify the hypothesis of a succession from white rot to brown rot, where other factors, such as the ability of spores to germinate on the substrate (Savory and Carey, unpublished data) must be taken into consideration.

6.5 RELATIONSHIP BETWEEN PERMEABILITY AND MOISTURE CONTENT

6.5.1 Introduction

In the second series of L-joint exposure trials (section 4) it was noticed that, after 221 days exposure, the moisture of the sample blocks was related to their permeability (fig 4.23). Observation of results after shorter periods of exposure (71 and 130 days) showed a similar though less pronounced trend. Although, following discussion, it was thought the relationship was only a single point in a dynamic situation, a small scale experiment was undertaken to follow the absorption of water by blocks of varying permeability as measured by absorption of dekalin.

6.5.2 Methods

Six of the sample blocks exposed for 221 days, and covering the range of the permeability of the samples, were oven dried to constant weight at 50°C and weighed. Each block was held in forceps, with the transverse faces in the horizontal plain, and dipped in water for the relevant period of time, blotted to remove excess water, and reweighed. During longer periods of soaking, the blocks were orientated with the transverse faces vertical, to allow end grain penetration, and weighted down. After a total of 8 days soaking, the blocks were vacuum impregnated with water and the saturated weight obtained. The results were subsequently converted to moisture content, using the weights established previously after oven drying at 103°C for 18 hours. Finally the blocks were dried to constant weight at 50°C and their permeability reassessed.

6.5.3 Results

The permeability of the test blocks before and after the prolonged soaking in water are presented in Table 6.8. The uptake of water by the test blocks is recorded in Table 6.9 and Fig 6.1. The relationship between permeability and uptake of water is presented in Fig 6.2

6.5.4 Discussion

Between the two assessments of permeability (table 6.8) the blocks had been oven dried at 103°C and had been saturated with water by vacuum impregnation. Both these processes have been shown, previously,

to increase the permeability of blocks, as assessed by absorption of dekalin. The small increases recorded are, therefore, most likely to be due to these physical processes rather than to biological action occurring during the soaking period.

The initial rate of uptake of water by the test blocks was variable (fig 6.1) with the most permeable block (37/1) having the slowest uptake over the first minute. By 61 minutes soaking, the uptake by each block was related to its permeability (fig 6.2). This situation continued up to 5 days soaking although the gradient of the graph gradually decreased which indicates a reduction in the correlation between the two factors. After 8 days soaking, the more permeable blocks were approaching saturation which affected the relationship. If the uptake of moisture is expressed as the moisture content of the block the relationship is similar but due to the high initial weight of block 38/4 its moisture contents are relatively low. The relationship observed in L-joints after 221 days exposure (fig 4.23) therefore appears to be a real effect which develops when wood is exposed to unlimited water. A total of 24.5 mm of rain fell in the 13 days prior to sampling which probably allowed the L-joints to absorb the maximum amount of water. However, the situation is clearly complex since water must be transmitted along the member of the L-joint for those blocks at the end remote from the joint to have access to free water; these blocks are, in general, less permeable than those closer to the joint (fig 4.26). The lower moisture contents away from the joint are the result of a combination of these factors.

The relationship that has been established between permeability, measured by uptake of dekalin, and uptake of water is of practical significance. If the permeability of timber in service is increased by microbial action, it will absorb water more readily and thus attain a higher moisture content and therefore be more susceptible to decay (Duncan, 1953; Ammer, 1964). The relationship also adds weight to the use of this simple method for determining the permeability of small uniform sized samples of timber.

6.6 GERMINATION OF BASIDIOSPORES OF *Lenzites trabea* ON JOINERY FROM VARIOUS SOURCES

6.6.1 Introduction

It has been shown (Savory and Carey, unpublished data) that spores of *Lenzites trabea* will not germinate on commercially dried Scots pine sapwood unless preconditioned by, for example, the growth of bacteria, bluestain fungi or *Trichoderma viride*. In practice, pine joinery decays. It was, therefore, planned to carry out spore germination tests on joinery of various timbers, ages and exposure conditions to follow the process of detoxification. In the event, other parts of the programme took precedence, and apart from observations on a single window (section 2.4) only the following pilot tests were carried out.

6.6.2 Methods

Three blocks from each of the sources detailed in Table 6.10 were impregnated with deionised water, after the paint film had been planed off where necessary. The samples were placed on Netlon mesh over 40 g vermiculite plus 120 ml deionised water contained in PRL jars (6.2.2.5).

Paint films were separated from their substrate by chopping or using a scalpel where already partially detached. The film samples were placed exterior surface downwards on glass slides over moist filter paper in crystallising dishes.

Sterile sections of Scots pine sapwood, approximately 20 mμ thick were placed on each substrate, inoculated with 2 drops of a suspension of basidiospores of *L. trabea* (6.3.2.7) and incubated at 25°C for 3 days. The sections were then removed, stained with aniline blue in lactophenol and observed.

6.6.3 Results

A summary of the results is presented in Table 6.10. The germination rating is a subjective assessment based on both the number of germinated spores, and the extent of outgrowth.

6.6.4 Discussion

As expected, good germination and outgrowth occurred on those sections placed on non-antagonistic Scots pine sapwood; this procedure has been

used extensively in the evaluation of the toxicity of wood preservatives (Savory and Carey, 1976) and correlates well with the ability of spores, placed in direct contact with the substrate, to germinate and cause decay. The sample of commercial Scots pine sapwood caused the spores to lyse; this has been encountered previously (Savory and Carey, unpublished data). The effect also occurred on the back of the paint although this was probably a result of residual particles of wood. It proved impossible to detach paint alone due to the good adhesion between paint and wood, and the thinness of the single coat of primer. A few spores had started to germinate on the painted surface. The primer therefore helps to isolate the spores from the toxicity of the wood. However, it cannot provide a complete seal or is itself toxic since germination was reduced.

The 40 year old pine heartwood supported germination although fresh heartwood does not. This material must, therefore, have undergone a detoxification process which could be associated with decay by Phellinus or due to other agencies which led to the colonisation by Phellinus. The paint system, which was shown by analysis to contain lead, caused some reduction in germination and outgrowth by the spores but did not prevent growth. There has been much discussion on the possible role of lead based paint in the protection of joinery; under the conditions of exposure of this door the paint has not afforded complete protection, allowing attack by Phellinus, and does not show any great toxicity to spores of L. trabea. Clearly, many further samples should be tested to elucidate any toxic effect of the lead. However, on environmental grounds, a widespread return to the use of lead based paint would be unacceptable.

The 6-7 year old hemlock heartwood supported germination of L. trabea spores although a sample of fresh heartwood tested previously did not (Savory and Carey, unpublished data). Again, this material appears to have undergone a detoxification process, during its exposure period. The paint system used did not prevent germination but, on the outer surface, caused abnormal swelling of many of the spores and, therefore, was exerting some effect.

These few results point to a detoxification process occurring during the exposure of timber. An understanding of the process could lead to alternate ways of protecting the timber from decay organisms, by preventing the detoxification. L. trabea spore germination could be used to monitor natural detoxification in the same way as it has already been used in the laboratory to follow the detoxification of Scots pine sapwood by the action of various micro-organisms.

TABLE 6.1 EFFECT OF FUNGAL CULTURES ON THE PERMEABILITY* OF TEST BLOCKS OF SCOTS PINE SAPWOOD

Culture No	Identity	Exposure period (days)			
		4	8	16	24
BS-9	**	21.03	17.68	18.54	25.77 (2)
BS-12	**	20.15	20.08	19.06	23.57
BS-19	**	16.91	16.43	16.94	19.51
BS-21	**	23.61 (1)	25.35	22.89	21.10 (1)
BS-22	**	18.33	20.97	21.42	20.86
S-6A	Phialophora fastigiata	16.47	22.62	23.68	25.97 (2)
S-9E	Aureobasidium pullulans	16.02	20.58	21.95	21.06 (1)
S-37E	Trichoderma viride	19.37 (1)	16.66	19.70	21.89
S-149A	Sclerophoma pithyophila	16.98	20.77	18.28	23.10
B730	Sistotrema brinkmannii	16.09	17.19	44.36 (4)	84.81 (4)
Control	-	17.51	15.82	22.28	22.48

*The weight of dekalin absorbed during a 10 second dip expressed as a percentage of the initial weight of each block.

**unknown bluestain organism isolated from first series experimental L-joints after 1 year's exposure

Range of control values 12.11-28.73

Numbers in parentheses indicate the number of replicates, out of four, with an absorption above the range of control values

TABLE 6.2 EFFECT OF BACTERIAL CULTURES ON THE PERMEABILITY* OF TEST BLOCKS OF SCOTS PINE SAPWOOD

Culture** No	Exposure period (days)					
	1	2	4	8	16	24
PWB2	18.62	19.75	20.71 (1)	20.58 (1)	21.50 (1)	18.91
PWB3	14.63	18.23	17.68	18.54	17.69	17.32
PWB4	17.01	16.65	19.54	18.82	20.88	13.75
PWB5	20.05	17.64	18.65	15.80	19.30	18.74
1C(D)10	21.09 (1)	15.62	18.54	19.86	17.39	17.70
1D(B)13	17.04	17.64	20.16	16.58	20.39	20.03
1A(C) 9	17.35	17.70	14.31	15.47	16.35	17.98
9B(A) 1	17.14	15.44	17.18	18.60	18.92	21.63
9D(B) 5	18.68	15.81	19.11	18.07	17.99	21.57 (1)
12C(D)1	17.84	15.45	17.22	18.95	17.96	21.29
12D(B)4	17.61	16.66	15.83	24.55 (1)	17.51	21.05 (1)
12D(B)4	16.85	16.34	19.40	15.73	19.20 (1)	18.33
Control	19.03	15.31	15.39	22.09	15.82	16.23

*The weight of dekalin absorbed during a 10 second dip expressed as a percentage of the initial weight of each block.

**PWB cultures isolated from PRL log pond water; all other cultures isolated from joinery after 6-8 years service.

Range control values 11.08-25.92

Numbers in parentheses indicate the number of replicates, out of four, with an absorption above the range of the control values.

TABLE 6.3 AREA OF GROWTH OF THE TEST ORGANISMS (cm^2)

Test organism		Concentration of TnBTO in agar (% w/v)						
Type	Name and herbarium number	0	0.000025	0.00005	0.0001	0.0002	0.0004	0.0008
Fungus	Unknown bluestain BS 12	*	25.35	22.58	18.13	12.78	11.31	8.96
	Phialophora fastigiata S-6A	11.53	12.81	11.53	10.74	10.06	9.06	8.23
	Aureobasidium pullulans S-9E	16.40	14.83	13.10	10.27	8.28	7.39	6.03
	Trichoderma viride S-37E	*	*	*	*	*	*	*
	Sistotrema brinkmannii B730	*	*	*	*	*	*	20.90
Bacterium	Unknown bacterium 9B(A)1	+	+	+	+	+	+	+
	Unknown bacterium 9D(B)5	+	+	+	+	+	+	+
	Unknown bacterium 12C(D)1	+	+	+	+	+	+	+

*entire plate covered

+bacterial colonies present but size difficult to assess

TABLE 6.4 GERMINATION OF BASIDIOSPORES OF L. trabea AFTER GROWTH BY THE TEST ORGANISM

Test organism		Concentration of TnBTO in agar (% w/v)						
Type	Name and herbarium number	0	0.000025	0.00005	0.0001	0.0002	0.0004	0.0008
Fungus	Unknown bluestain BS 12	*	*	*+	*+	*-	*-	*-
	Phialophora fastigiata S-6A	*	*	*	*	*	*	*
	Aureobasidium pullulans S-9E	*+	*+	*+	*+	*+	*+	*(+)
	Trichoderma viride S-37E	*	*	*	*+	*+?	*-	*-
	Sistotrema brinkmannii B730	*	*	*	*	*	*	*
Bacterium	Unknown bacterium 9B(A)1	*+	*+	*+	*+	*-	*-	*-
	Unknown bacterium 9D(B)5	*+	*+	*-	*-	*-	*-	*-
	Unknown bacterium 12C(D)1	+	+	+	+	-	-	-
None	-	+	+	+	+	-	-	-

- no germination
+ germination
(+) germination limited to the site occupied by the test organism
* the test organism had grown through the cellophane and at times (no other rating given) could not be distinguished from growth of L. trabea

TABLE 6.5 TEST PLAN

Option No	First fungus	Intermediate handling	Second fungus
1	Sistotrema brinkmannii	A	Coriolus versicolor
2	Sistotrema brinkmannii	NA	Coriolus versicolor
3	Sistotrema brinkmannii	A	Coniophora puteana
4	Sistotrema brinkmannii	NA	Coniophora puteana
5	Coriolus versicolor	A	Coniophora puteana
6	Coriolus versicolor	NA	Coniophora puteana
7	Sistotrema brinkmannii	*	None
8	Sistotrema brinkmannii	not disturbed	Sistotrema brinkmannii
9	Coriolus versicolor	*	None
10	Coriolus versicolor	not disturbed	Coriolus versicolor
11	None	**	Coriolus versicolor
12	None	**	Coniophora puteana

* Cleaned, weighed, oven dried and weighed after 6 weeks

** Fresh blocks planted and incubated in parallel with the second part of the test

A Blocks cleaned, autoclaved for 20 minutes at 121°C and replanted on sterile mesh over the second organism

NA Blocks cleaned under aseptic conditions and replanted on sterile mesh over the second organism, without resterilisation.

TABLE 6.6 MOISTURE CONTENT AND WEIGHT LOSS OF SCOTS PINE BLOCKS EXPOSED TO VARIOUS COMBINATIONS OF FUNGI

Option No	Moisture content (per cent of final dry weight)		Weight loss per cent (per cent of initial dry weight)		No of replicates
	Range	Mean	Range	Mean	
1	57-201	121	6.2-11.4	8.0	9
2	72-96	85	6.1-9.1	6.9*	4
	102-194	136	1.7-6.4	4.3	5
3	70-119	85	17.5-28.3	21.6*	7
	160-200	180	3.4-6.2	4.8	2
4	78-196	107	18.9-27.1	23.0*	7
	162-168	165	0.7-7.6	4.1	2
5	72-213	129	18.5-39.6	25.6	9
6	59-130	83	23.6-40.9	31.5*	8
	161	161	12.9	12.9	1
7	40-71	51	0.8-2.0	1.3	9
8	52-189	76	1.0-2.2	1.7	9
9	53-80	60	8.7-17.5	12.0*	6
	69-81	73	0-2.4	0.9	3
10	48-116	81	8.6-23.7	17.4	9
11	44-72	55.3	4.9-7.1	6.1	9
12	46-115	90	11.2-24.2	20.0*	6
	152-190	169	0.4-14.2	6.6	3

*Figures used in comparisons made in Table 6.7

TABLE 6.7 THEORETICAL AND ACTUAL WEIGHT LOSSES OF SCOTS PINE SAPWOOD BLOCKS EXPOSED TO VARIOUS COMBINATIONS OF FUNGI

Option No	Incubation details			Theoretical weight loss per cent			Actual weight loss per cent
	First fungus	Intermediate handling	Second fungus	First fungus	Second fungus	Total	
1	Sistotrema brinkmannii	Autoclaved	Coriolus versicolor	1.3	6.1	7.4	8.0
2	Sistotrema brinkmannii	Not autoclaved	Coriolus versicolor	1.7*	6.1	7.8	6.9
3	Sistotrema brinkmannii	Autoclaved	Coniophora puteana	1.3	20.0	21.3	21.6
4	Sistotrema brinkmannii	Not autoclaved	Coniophora puteana	1.7*	20.0	21.7	23.0
5	Coriolus versicolor	Autoclaved	Coniophora puteana	12.0	20.0	32.0	25.6
6	Coriolus versicolor	Not autoclaved	Coniophora puteana	17.4*	20.0	37.4	31.5

*Results of 12 week incubation used since blocks were not sterilised on transfer to the second organism

TABLE 6.8 PERMEABILITY* OF THE TEST BLOCKS

Block No	Permeability before soaking	Permeability after soaking
37/1	1.656	1.665
37/8	1.502	1.681
138/3	1.242	1.338
38/4	0.905	1.031
87/8	0.614	0.679
139/12	0.308	0.460

*Uptake of dekalin (g) during a 10 sec dip

TABLE 6.9 UPTAKE OF WATER BY THE TEST BLOCKS (g)

Dipping time	Cumulative time	Block No (permeability)					
		37/1 (1.656)	37/8 (1.502)	138/3 (1.242)	38/4 (0.905)	87/8 (0.614)	139/12 (0.308)
5 sec	5 sec	0.078	0.168	0.333	0.252	0.310	0.258
5	10	0.157	0.366	0.682	0.453	0.550	0.390
10	20	0.243	0.566	0.968	0.625	0.731	0.480
10	30	0.354	0.742	1.210	0.763	0.902	0.553
10	40	0.469	0.894	1.385	0.911	1.049	0.621
10	50	0.572	1.023	1.560	0.998	1.160	0.691
10	60	0.672	1.142	1.631	1.078	1.228	0.750
20	80	0.809	1.320	1.687	1.168	1.341	0.841
20	100	0.910	1.398	1.739	1.231	1.360	0.896
20	120	1.022	1.466	1.770	1.277	1.389	0.946
20	140	1.089	1.548	1.795	1.314	1.387	1.008
20	160	1.162	1.580	1.827	1.363	1.429	1.028
20	3 min	1.228	1.632	1.853	1.381	1.414	1.048
1 min	4	1.389	1.709	1.898	1.428	1.426	1.068
1	5	1.480	1.766	1.920	1.458	1.431	1.088
1	6	1.544	1.816	1.943	1.482	1.443	1.105
5	11	1.673	1.936	1.994	1.538	1.454	1.144
5	16	1.824	1.999	2.020	1.563	1.472	1.162
5	21	1.912	2.061	2.051	1.584	1.490	1.182
10	31	2.005	2.094	2.056	1.616	1.498	1.197
30	61	2.254	2.150	2.095	1.669	-	1.236
30	91	2.374	-	2.087	1.725	1.518	1.248
30	121	2.414	2.217	2.123	1.744	1.530	1.242
59	3 hour	2.486	2.234	2.134	1.816	1.534	1.272
1 hour	4	2.492	2.274	2.136	1.821	1.534	1.270
1	5	2.506	2.308	2.156	1.851	1.564	1.276
19	1 day	2.639	2.494	2.249	2.002	1.691	1.439
1 day	2	2.834	2.718	2.333	2.252	1.868	1.759
3	5	3.017	2.968	2.640	2.544	2.392	2.151
3	8	3.060	3.076	2.778	2.713	2.692	2.372
After impregnation		3.055	3.107	2.994	2.824	3.030	2.700

TABLE 6.10 GERMINATION OF BASIDIOSPORES OF *Lenzites trabea* ON JOINERY FROM VARIOUS SOURCES

Source of sample	Substrate for spores	Observations	Germination rating*
Non-antagonistic Scots pine sapwood	Wood surface	Many spores showing extensive outgrowth and branching; a few ungerminated spores	++++
Scots pine sapwood, factory primed, taken from a building site	Painted surface	Occasional spores starting to germinate; ungerminated spores present	+
	Back of paint film	No spores seen	-
	Underlying wood	No spores seen	-
Scots pine heartwood, 40 years exposure, coated with a lead containing paint system. (Previously attack by *Phellinus contiguus*)	Painted surface	Germinated spores sparce and showing variable outgrowth; a few ungerminated spores.	++
	Back of paint film	A few spores showing extensive outgrowth; many spores ungerminated or just beginning to germinate	+++
	Underlying wood	Many spores showing extensive outgrowth; a few ungerminated spores	++++
	Unpainted wood from within the joint	A few spores showing extensive outgrowth; many spores ungerminated or with limited outgrowth	+++

*Germination rating ++++ > +++ > ++ > +

\- spores lysed

TABLE 6.10 CONTINUED

Source of sample	Substrate for spores	Observations	Germination rating*
Hemlock heartwood, 6-7 years exposure, coated with white gloss paint system	Painted surface	Many spores showing variable outgrowth and abnormal swelling during germination; numerous ungerminated spores	+++
	Back of paint film	Quite extensive mycelium in places; no ungerminated spores seen	++++
	Underlying wood	Sparce but extensive mycelium; a few spores with limited outgrowth	+++

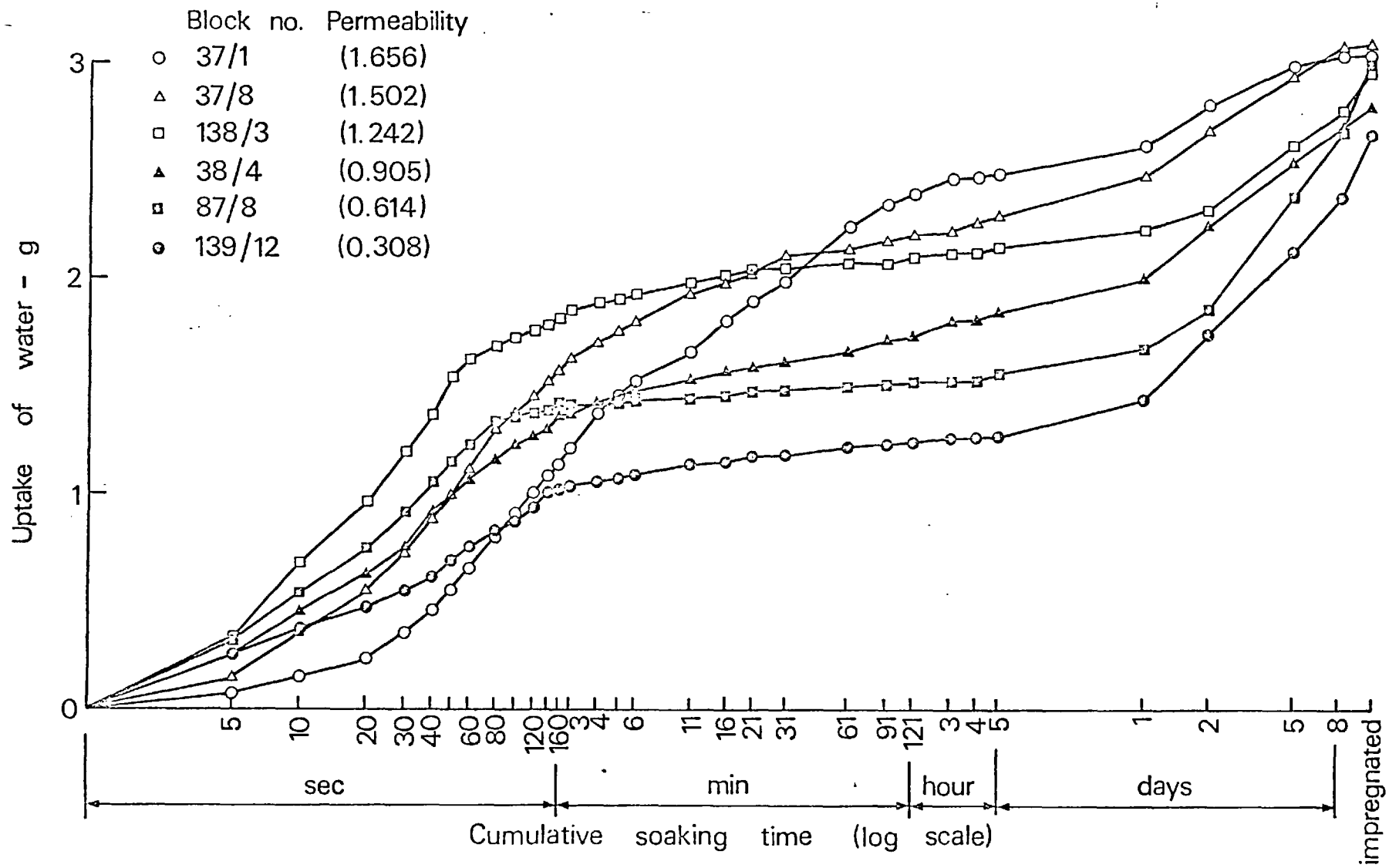


Fig. 6.1. Uptake of water by the test blocks

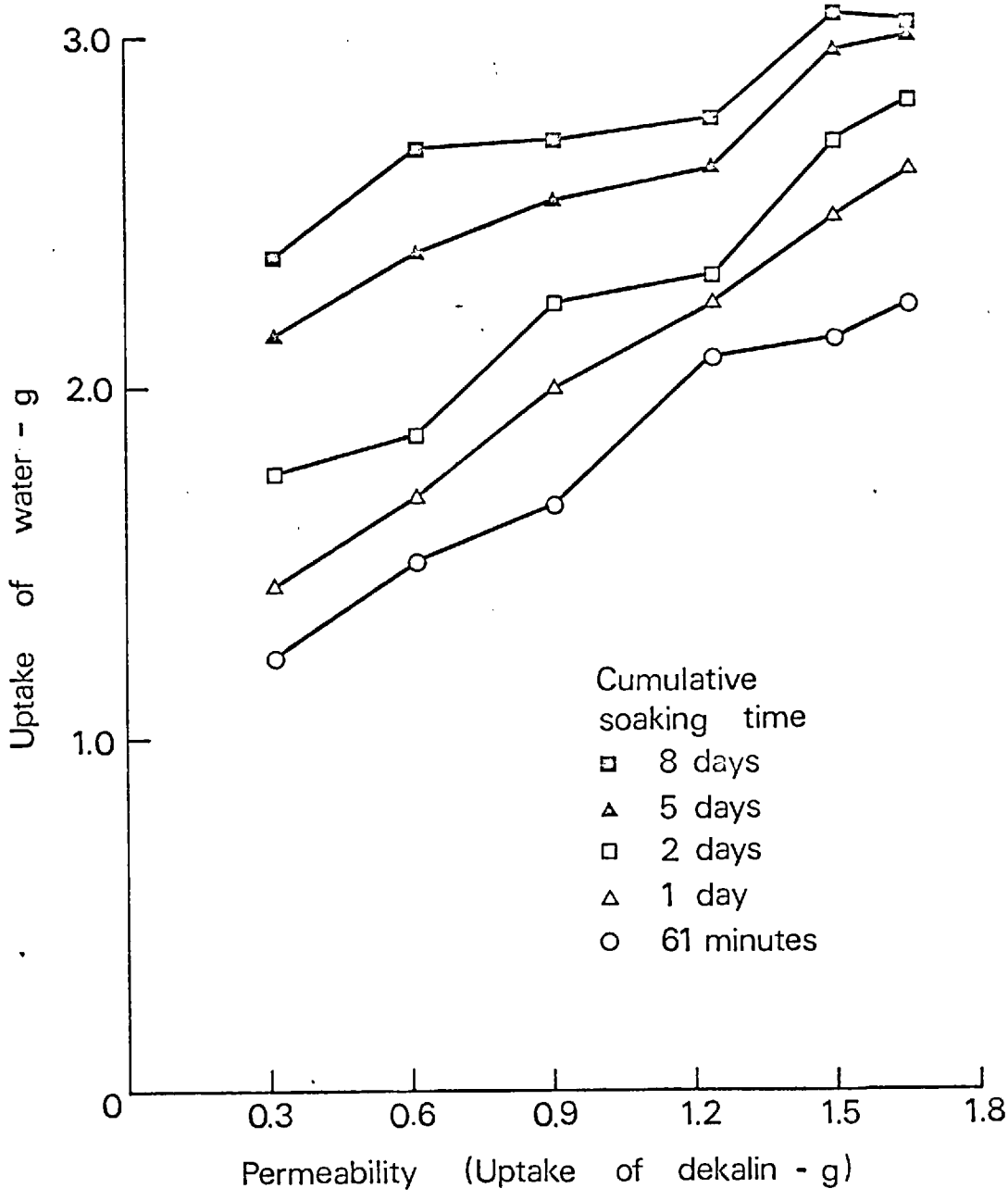


Fig. 6.2 Relationship between permeability and uptake of water

SECTION 7

GENERAL DISCUSSION

The results have been discussed in detail in individual sections, it remains for a final evaluation to be made in a more general way.

The survey of decayed window joinery in the United Kingdom has highlighted the rôle that bad design plays in accelerating the rate of failure. An indication of the severity of the hazard introduced is given by some failures which have occurred faster than in stakes of the same species exposed in ground contact. The preservative treatments currently advocated for joinery were not designed to afford protection under such severe conditions and therefore improvements in design must be made to ensure an adequate service life. This finding is regarded as being so important that it has already been published (Carey, 1980).

Although many of the Basidiomycetes isolated from decayed material have not been named, it can be concluded that they are unlike the fungi found elsewhere in buildings. Many isolates show a low decay capacity; this could reflect the inadequacy of the laboratory test method used but, more likely, reflects the true situation. Observations indicate that decay takes place over a period of years and over much of this time is not visible; Savory and Carey (1975) found internal decay in three out of four "sound" doors examined by deep sawing the frame members. Many of the isolates were white rots and showed tolerance to two widely used wood preservatives, TnBTO and PCP. Savory and Carey (1976) have shown one white rot (Coriolus versicolor) to be more tolerant of TnBTO in wood block decay tests than Coniophora puteana, the organism used to compare the toxicity of potential joinery preservatives to that of PCP (Anon, 1967). The present finding emphasises the need to consider the white rot/softwood combination when evaluating potential joinery preservatives using conventional laboratory decay tests.

A major part of the study has been devoted to elucidating the process of colonisation of simulated joinery components in the form of L-joints and the individual effect of two preservatives on the process. Although L-joint trials have been used before to evaluate water repellent and/or preservative treatments, destructive examination during the early stages of exposure has not been undertaken. It has been necessary, therefore, to devise and develop techniques for this purpose. A new method of following the progress of bacterial colonisation has been used (Carey, 1979). The concept of using changes in the permeability of wood as a simple yet sensitive indicator of attack has been developed. This has already proved of importance in the evaluation of preservative treatments applied to joinery in situ. The permeability increases occurring during service affect the subsequent penetration of the wood preservatives used in treatment necessitating the use of aged joinery for their assessment.

The approach of using several criteria to follow the progress of infection has proved of great value. Data from one assessment have provided cross-comparisons with those of other methods eg, the effect of moisture content on the progress of colonisation. Each method has contributed pieces to the puzzle so that, in the end, a more complete appreciation of the whole process has been achieved. The "objective" approach, as described by Pinzon-Picaseño (1976), to following the process of colonisation by making isolations from predetermined fixed points, has proved invaluable. It has enabled the progress of colonisation from the joint along the samples to be followed and the succession of the various types of organisms to be elucidated.

There was variation between the two L-joint exposure trials, particularly with respect to fungal colonisation and soft rot attack. It has been postulated that the differences were due to the extent of wetting of the samples. The first series were subjected to high precipitation, much as snow, during the early stages of exposure, which resulted in a very rapid uptake of water giving high moisture contents, while the second series wetted more slowly and the moisture contents were generally lower. The ecological niche created, favoured different organisms in each case. In the first series, the growth of fungi was retarded initially by the high moisture content but

ultimately widespread soft rot attack occurred, possibly as a result of the nitrifying activity of the bacteria under anaerobic conditions. In the second series, bacterial and fungal invasion occurred simultaneously.

The results, particularly from the second series, reveal that a multiplicity of organisms is involved in colonisation and that invasion by different types of organisms occurs in the order bacteria, bluestains, soft rots and Basidiomycetes; mould fungi are of low incidence. The concept of an ecological succession of this type is new to the area of timber in buildings although similar sequences have been established in timber in ground contact (eg Clubbe, 1980a). The preponderance of bluestain organisms in the early stages of exposure, particularly those of the Aureobasidium pullulans type led to a more detailed study. This showed that consistent differences existed between isolates and has allowed many of them to be assigned to nine subgroups. This study has contributed to our knowledge of this intransigent complex and, in time, may form the basis of a specific classification.

The effect of the preservatives TnBTO and PCP has been to select tolerant fungi and to extend the length of the colonisation process rather than to alter its form. This has important consequences in the development of the philosophy of testing potential preservatives for use in the joinery situation. Existing laboratory tests cover only discrete parts of the ecological system eg, toxicity to Basidiomycetes or the ability to prevent bluestains discolouring surface finishes. It is unlikely that a laboratory test could be devised to parallel the entire ecological system which is essential since, although only the Basidiomycetes are ultimately responsible for failure, the earlier colonisers may materially affect the final situation. Their activity modifies untreated Scots pine sapwood to allow germination of basidiospores of Lenzites trabea and is likely similarly to affect colonisation by other Basidiomycetes. Soft rot organisms and other microfungi, in general, have a much higher tolerance to preservatives than Basidiomycetes. Their activity is not directly responsible for joinery failure but if any of them are capable of detoxifying the preservative, as indicated by the results using A. pullulans, they may play a significant rôle in this way. This theory has been published (Savory and Carey, 1979).

It is evident that the approach of the destructive testing of L-joints during the early stages of exposure has provided an important advance in our knowledge of the process of infection and decay of timber exposed out of contact with the ground, such as joinery. In addition it presents intriguing possibilities in the prediction of preservative performance by comparing their effect on the pattern of initial colonisation. The presence of wood destroying Basidiomycetes has been used as a signal of the transition from the "protected" phase to the "deterioration" phase. More speculatively, it may be possible to use increases in permeability, indicative of microbial attack of the timber, in the same way. Both Basidiomycetes and permeability increases were detected in untreated L-joints after 32 days exposure; in TnBTO treated L-joints the first white rot organism was isolated after 375 days but permeability increases had been detected after 130 days. The earlier onset of permeability increase may permit more rapid prediction of comparative performance. In addition, permeability measurements require only simple equipment whereas microbiological facilities and expertise are required for the isolation of Basidiomycetes.

Accurate prediction of service life based on the results of destructive testing during the early stages of exposure is a real possibility although the problems, which have yet to be determined and then overcome, must not be underestimated. At present, using PCP as the standard by which to judge TnBTO, it can be seen that TnBTO is inferior in all aspects of performance except in the prevention of bacterial colonisation, where neither is effective. However, TnBTO treated material performed better than untreated material in all respects.

This study has contributed significantly to our knowledge of the process of joinery decay; the main achievements have been listed in Table 7.1 together with those items of technical significance originating from the work. The process of infection and decay of window joinery has been revealed as a complex in which a wide range of organisms interact with the substrate, one another and any preservative present.

TABLE 7.1 Main achievements of the study

Scientific

1 The Basidiomycetes causing decay of joinery are different from those elsewhere in buildings,

2 A new method of enumerating bacteria in wood has been developed.

3 The concept of using changes in permeability as a simple yet sensitive indication of attack of timber has been developed.

4 Joinery has been shown to be colonised by a succession of micro-organisms similar to that occurring in ground contact but apparently differing from the "pure culture" situation said to be found elsewhere in buildings.

5 Aureobasidium type cultures have been separated into physiological groups of ecological significance which may become the basis for a new classification.

Technical

1 The influence of bad design on the performance of joinery has been documented.

2 The importance has been shown of the white rot/softwood combination in laboratory tests of the toxicity of wood preservatives.

3 Increases in permeability of joinery in service have shown the need to use aged joinery in the evaluation of remedial preservative treatments.

4 Depletion of preservatives by the lower fungi which could facilitate decay by the Basidiomycetes has been demonstrated.

5 Destructive testing of L-joints has been shown to provide early comparative indicators of the performance of wood preserving chemicals which may serve as predictors of service life.

SECTION 8

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APPENDIX A

DEVELOPMENT OF A METHOD FOR MONITORING PERMEABILITY CHANGES

A.1 INTRODUCTION

Following the limited observation made by Research and Development staff of Wykamol Ltd, that injection of their in situ remedial treatment fluid was easier into windows in service than into new wood in the laboratory, it was evident that confirmation was needed, and if confirmed the cause should be investigated. It was known that areas of decay would be more permeable due to damage to the wood structure and that a variety of organisms including Trichoderma viride (Lindgren, 1952; Unligil, 1969) bluestains (Lindgren and Scheffer, 1939) and bacteria (Suolahti and Wallén, 1958; Ellwood and Ecklund, 1959) have been shown to increase permeability, particularly in the ground contact situation (Rossell cited by Levy, 1973). The wide range of organisms, which have been shown to be present in simulated joinery units (L-joints) after one year's exposure (section 3), may have, therefore, increased the permeability.

Various methods are available for testing the gas and liquid permeability of wood but the simple system of dipping small blocks, of uniform dimensions, for 10 seconds in a liquid was chosen for application to the current problem. Previous workers have used immersion in water (Lindgren and Scheffer, 1939), aqueous nigrosin (Greaves, 1966) and preservative solution (Lindgren, 1952; Ellwood and Ecklund, 1959); the system has been shown to be effective on a comparative basis when using white spirit by Newton (1976).

Two solvents were chosen for evaluation; white spirit as used by previous workers, and dekalin (decahydronaphthalene) the solvent then advocated for testing penetration of preservative solutions and subsequently included in BS 5707 (1979).

Because of the known variation in permeability of Scots pine sapwood and the unproven reproducibility of the test system in addition to possible effects due to preservative treatment, various preliminary experiments

were necessary before the method could be applied to either experimental L-joints, or to window joinery following a period of service.

A.2 INHERENT VARIABILITY OF PINE SAPWOOD AND REPRODUCIBILITY OF THE TEST SYSTEM

A.2.1 Method

Two unpainted unexposed L-joints remaining from the stock machined for the first exposure trial (section 3) were used. Each L-joint consisted of two components, both measuring 203 x 38 x 38 mm and machined to form a bridle joint (fig A.1). The two morticed components were labelled A and B and the two containing the tenon, C and D. Two 12 mm wide strips were then cut, one from each side of each component, and labelled 1 and 2. Each strip was cross-cut into 10 mm lengths which were numbered sequentially from the joint end. The small blocks, each measuring 38 x 12 x 10 mm, were lightly sanded to remove loose fragments and dried to constant weight at 50°C. Each block was then weighed and totally immersed for 10 seconds in white spirit; during immersion each block was orientated with the transverse faces in the vertical plain, as shown in Fig A.2. The blocks were then blotted on the four larger faces, using fresh paper towel for each block in order to standardise the process as much as possible, and reweighed.

The blocks were ventilated for several days to allow evaporation of the white spirit. They were again dried to constant weight at 50°C, weighed, then immersed for 10 seconds in the solvents and orientations given in Table A.3. The blocks were blotted, as previously described, and reweighed.

It was realised the first solvent treatment could affect the results of the second treatment. However, due to the variable nature of the timber, this process provided the simplest method to assess the reproducibility of the test system.

A.2.2 Results

The uptakes of solvent by the blocks are presented in Tables A.1 and A.2 and in Fig A.3. Mean values and variation for each sample are presented in Table A.3 together with the change in uptake between the two tests.

A.2.3 Discussion

The blocks were dried at 50°C to minimise the influence of the small splits known to develop during drying at the more usual temperature of 103°C. The decision was made on theoretical grounds that was later shown to be of practical significance when the permeability of a series of blocks was retested after drying at 103°C followed by a period of equilibration with room conditions. The uptake of dekalin was up to 10 per cent higher than in the first test; variation between tests without intermediate drying at 103°C is normally only 2 or 3 per cent.

The results have been presented as uptake of solvent rather than as a percentage of the initial weight as used by Newton (1976). When considering blocks of the same dimensions, variation in weight is due to variation in density. Since absorption tends to vary inversely with density expressing absorption as a percentage of the initial weight would exaggerate variation. The 'end' block of each sample was larger than the others; although the absorption values for these blocks are generally higher than for the uniform sized blocks, they are proportionally lower per unit volume. These blocks have therefore been omitted from the calculations but this result shows the need to use blocks of uniform dimensions.

The test blocks were initially all immersed in white spirit whilst orientated in the same way (with the transverse faces in the vertical plain) to establish the natural variation within the sample of pine sapwood. The results (table A.1; fig A.3) show considerable variation:

a between the four samples

b between the two sides of a single sample (particularly B)

c along the length of the samples

Overall variation in absorption (0.193-0.370 g) represents 64.8 per cent of the mean absorption (0.273 g). This figure appears very high, in view of the fact that the timber was from a single source and had been converted and kiln drying commenced within a week of felling. Because of the high

degree of variation experienced it was decided to retest some blocks to establish that the variation was due to the timber and not to some inadequacy in the test method. At the same time the effects of changing the orientation of the blocks during test and the test solvent were investigated.

Blocks from sample A were retested under identical conditions. The results (table A.2) show almost the same absorptions as previously recorded (table A.3; fig A.3); the slight reduction in absorption (on average 3.3 per cent lower) may be associated with the solvent treatment of the first test. The test system is, therefore, reproducible and variation in absorption must reflect variation in the permeability of timber.

Blocks from sample B were retested using white spirit but changing their orientation so that the transverse faces were in the horizontal plain during test. This modification was introduced because, in the first system used (fig A.2) the forceps covered part of the end grain, where the majority of penetration will take place. The absorptions were on average 4.4 per cent higher than before (table A.3) but showed almost identical variation along the length of the sample (fig A.3). This change in orientation would therefore seem desirable.

Blocks from samples C and D were retested in dekalin (table A.2); they show higher absorptions (table A.3) but again show almost identical variation along the length of the samples. The increase in the less permeable sample (D) can be accounted for by the higher specific gravity of the dekalin (0.884 g/cc) in relation to the white spirit (0.77 g/cc). The more permeable sample (C) has taken up a greater volume of dekalin; use of this solvent should result in a greater range of uptakes and therefore give a more sensitive test than white spirit.

Blocks from position 1 of samples C and D show rather low absorptions. This could be due to damage to the end grain or to collected detritus; removal of 2-3 mm prior to conversion to test blocks should solve this problem.

On balance the best test system appears to be dekalin used with the transverse faces of the test blocks horizontal during dipping.

Although the reproducibility of the test system has been shown to be very good, the inherent variability of the timber is such that detection of small changes in permeability would not be possible unless the initial permeability was known. It might be possible to remove samples from the remote ends of L-joints prior to exposure. The last uniform sized block (14 or 11) differs from the mean permeability of the sample by a maximum of 8.2 per cent (table A.1); information from such a block tested prior to exposure would therefore help to detect significant changes in the permeability of the remaining blocks after exposure. This system would not be applicable to joinery samples taken from service.

A.3 THE EFFECT OF ONE YEAR'S EXPOSURE ON PERMEABILITY

A.3.1 Method

A sample from one side of an untreated, painted, horizontal section of an L-joint (No 22) previously exposed in an out of ground contact situation for 12 months was cross-cut to 10 mm lengths rejecting the first few millimetres from the joint end. These blocks were dried at 50°C to constant weight, then immersed with the transverse faces horizontal, in dekalin for 10 seconds. The four larger faces were then blotted, and the blocks weighed.

A.3.2 Results

The uptake of solvent by each block is presented in Table A.4 and in Fig A.4 together with the most comparable set of data previously obtained (sample D retest) for unexposed controls.

A.3.3 Discussion

The exposed samples showed greatly increased absorption; the mean value is over four times that of the control material. The samples had been machined from different batches of Scots pine sapwood; although both batches were from a similar source and had been dried and stored in the same way, they may have had a different inherent absorption capacity. The exposed blocks were painted on both ends and one face which is likely to have slightly reduced absorption. However, since the difference between the exposed and unexposed samples is so great, none of these factors is likely to have significantly affected the result.

This observation confirms that of the Research and Development staff of Wykamol Ltd that timber exposed in an out of ground contact situation such as window joinery, becomes significantly more permeable.

A.4 EFFECT OF PRESERVATIVE TREATMENT ON PERMEABILITY

A.4.1 Method

Eighteen pieces of Scots pine sapwood measuring 165 x 38 x 38 mm were machined from the stock of timber used for preparation of the 2nd series of L-joints. After weighing, six samples were immersed for 3 minutes in a 1 per cent w/w solution of TnBTO in Shellsol E and six samples in a 5 per cent w/w solution of PCP in Shellsol E containing 10 per cent w/w dibutyl phthalate. The samples were allowed to drain on paper towel then reweighed to record uptake of preservative solution. Three samples were immersed in Shellsol E alone. All samples were laid flat and allowed to air dry. A 12 mm strip was cut from the side of each treated sample facing upwards during drying, and from one side of each of the three untreated samples. Five 10 mm long blocks were cut from each end of each strip and labelled a-e and v-z, a and z being the 'end' blocks (fig A.5). The blocks were sanded to remove loose fragments, oven dried to constant weight at 50°C then weighed, immersed in dekalin for 10 seconds with the transverse faces horizontal, blotted and reweighed.

A.4.2 Results

The absorption of preservative treating solution for each test sample is presented in Table A.5. The absorption of dekalin by each test block is given in Table A.6 together with mean values for the blocks from each sample. The mean absorption of dekalin at equivalent test block positions (eg a and z blocks are both 'ends') have been amalgamated in Table A.7. Details of statistical analyses are presented in Tables A.8, A.9 and A.10.

A.4.3 Discussion

The results presented in Table A.6 appeared to show variation between treatments; the data were therefore subjected to statistical analysis using the F test. Following advice, the mean value for the test blocks from each sample was used in the analysis since individual test blocks are not truly independent, having been cut from the same piece of wood. The analysis showed (table A.8) that there was no significant difference between untreated and solvent treated samples. The results from these groups were pooled and compared with the two preservative treatments.

There was no significant difference between the controls and the TnBTO treated samples but the PCP treated samples were significantly different from the controls at the 1 per cent level.

The data from equivalent test block positions along the length of each sample (table A.7) appeared to show that the 'end' blocks from the PCP treated samples were less permeable than the remainder. Separate analysis of the data from each treatment confirmed there was no significant difference along the length of untreated, solvent treated or 1 per cent TnBTO treated samples; the statistical table for the 1 per cent TnBTO treated material is presented as an example in Table A.9. The data for the 5 per cent PCP treated material (table A.10) showed the aborption of dekalin by the 'end' blocks (a/z) to be significantly lower at the 0.1 per cent level than the remaining blocks.

The hypothesis that the less absorbent 'end' blocks were responsible for the significantly lower absorption of dekalin by the PCP treatment as a whole was tested. New mean values for the PCP treated samples were calculated, omitting the 'end' blocks, and substituted for the original values in Table A.8. The PCP treated samples were still significantly different from the controls although the significance was reduced to the 5 per cent level.

If trial blocks (as suggested at the end of para A.2.3) were used, a separate set would be required for the PCP treated material; any block with end grain exposed during treatment would not be representative of the remaining length but the adjacent block could be used.

A.5 CONCLUSIONS

The method of immersing, for 10 seconds, small uniform blocks of Scots pine sapwood in dekalin and calculating the uptake provides a simple, convenient, and reproducible method for assessing permeability. The Scots pine sapwood tested was rather variable but an untreated painted L-joint exposed out of ground contact for one year showed a four-fold increase in the uptake of solvent. Permeability was reduced by 5 per cent PCP treatment, the effect being greatest in the 'end' blocks from each sample. These findings must be considered when further L-joint trials are undertaken.

TABLE A.1 ABSORPTION OF WHITE SPIRIT (g) BY TEST BLOCKS; 1st TEST

Test block position†	L-joint components							
	A		B		C		D	
	1	2	1	2	1	2	1	2
1	.272	.266	.368	.329	.245	.254	.195	.211
2	.265	.249	.343	.272	.304	.285	.220	.259
3	.252	.237	.350	.264	.326	.274	.234	.262
4	.269	.245	.360	.279	.315	.269	.231	.266
5	.245	.241	.370	.271	.325	.277	.234	.262
6	.228	.239	.359	.276	.331	.275	.231	.270
7	.232	.234	.354	.277	.326	.289	.232	.258
8	.236	.246	.344	.270	.323	.282	.213	.263
9	.230	.238	.360	.286	.312	.269	.205	.267
10	.239	.232	.354	.268	.335	.267	.193	.266
11	.233	.233	.335	.277	.343	.280	.208	.270
12	.228	.224	.338	.267				
13	.235	.239	.341	.273				
14	.233	.246	.335	.289				
end	.271	.273	.407	.395	.430	.411	.270	.359
mean*	.243	.241	.351	.278	.317	.275	.218	.259
range*	.228-.272	.224-.266	.335-.370	.264-.329	.245-.343	.254-.289	.193-.234	.211-.270
variation**	18.1	17.4	10.0	23.4	30.9	12.7	18.8	22.8

† see fig A.1
* excluding 'end' blocks
**the range expressed as a percentage cf the mean

TABLE A.2 ABSORPTION OF TEST SOLVENT (g) BY TEST BLOCKS; 2nd TEST

Test block position†	L-joint components							
	A		B		C		D	
	1	2	1	2	1	2	1	2
1	.245	.271	.380	.321	.312	.314	.245	.265
2	.235	.241	.373	.277	.408	.369	.273	.340
3	.231	.231	.377	.275	.413	.347	.297	.343
4	.234	.236	.375	.282	.415	.352	.293	.333
5	.220	.239	.397	.278	.425	.352	.298	.329
6	.221	.235	.385	.280	.427	.359	.294	.347
7	.234	.230	.381	.288	.436	.373	.294	.323
8	.225	.245	.380	.282	.427	.348	.278	.325
9	.221	.237	.365	.287	.421	.336	.258	.335
10	.230	.226	.384	.280	.454	.345	.255	.340
11	.222	.234	.351	.280	.460	.352	.257	.328
12	.228	.228	.356	.276				
13	.234	.237	.355	.270				
14	.231	.238	.347	.293				
end	.259	.281	.456	.401	.516	.518	.350	.422
mean*	.229	.238	.372	.284	.418	.324	.277	.310
range*	.220-.245	.226-.271	.351-.397	.270-.321	.312-.460	.314-.373	.245-.298	.265-.347
variation**	10.9	18.9	12.4	18.0	35.4	18.2	19.1	26.5

† see fig A.1
* excluding 'end' blocks
**the range expressed as a percentage of the mean

TABLE A.3 SUMMARY OF DATA FROM THE TWO TESTS

Test* joint	First test†		Second test		Per cent change in mean from first test to second test	Solvent and orientation during second test
	mean	variation**	mean	variation**		
A	0.242	19.8	0.234	21.8	- 3.3	White spirit, transverse faces vertical
B	0.315	33.7	0.328	38.7	+ 4.1	White spirit, transverse faces horizontal
C	0.296	33.1	0.371	39.9	+25.3	Dekalin, transverse faces vertical
D	0.266	28.9	0.294	34.7	+10.5	Dekalin, transverse faces horizontal

*results from the two sides amalgamated
**the range expressed as a percentage of the mean
†all blocks immersed in white spirit, with their transverse faces in the vertical plain

TABLE A.4 ABSORPTION AFTER ONE YEAR'S EXPOSURE

Sample position	Absorption (g per block)	
	One year's exposure	Unexposed*
1	1.345	0.255
2	1.285	0.307
3	1.426	0.320
4	1.383	0.313
5	1.455	0.314
6	1.424	0.321
7	1.363	0.309
8	1.343	0.302
9	1.262	0.297
10	1.310	0.298
11	1.252	0.293
12	1.215	-
mean	1.339	0.303

*Mean of values for two sides

TABLE A.5 UPTAKE OF PRESERVATIVE SOLUTION BY TEST SAMPLES

Preservative	Sample No	Absorption (g)
1% TnBTO	7	7.5
	8	7.1
	9	7.5
	10	7.3
	11	7.2
	12	8.0
	mean	7.4
5% PCP	13	7.8
	14	7.4
	15	7.5
	16	8.1
	17	7.5
	18	7.5
	mean	7.6

TABLE A.6 ABSORPTION OF DEKALIN (g) BY TEST BLOCKS

Treatment	Sample No	Test block position*										
		a	b	c	d	e	v	w	x	y	z	mean
Untreated	1	0.492	0.535	0.526	0.527	0.513	0.478	0.501	0.522	0.497	0.529	0.512
	2	0.480	0.446	0.463	0.432	0.429	0.428	0.434	0.462	0.470	0.439	0.448
	3	0.472	0.483	0.500	0.507	0.521	0.575	0.565	0.555	0.567	0.541	0.529
Solvent	4	0.464	0.462	0.460	0.448	0.450	0.454	0.444	0.423	0.427	0.464	0.450
	5	0.445	0.435	0.433	0.416	0.422	0.419	0.432	0.425	0.420	0.464	0.431
	6	0.405	0.369	0.380	0.386	0.396	0.347	0.384	0.375	0.382	0.367	0.379
1% TnBTO	7	0.429	0.430	0.405	0.422	0.444	0.471	0.474	0.466	0.483	0.474	0.450
	8	0.507	0.488	0.487	0.490	0.492	0.455	0.444	0.468	0.440	0.448	0.472
	9	0.450	0.397	0.408	0.432	0.439	0.531	0.532	0.497	0.509	0.487	0.468
	10	0.491	0.449	0.453	0.470	0.478	0.540	0.523	0.517	0.490	0.484	0.490
	11	0.410	0.370	0.359	0.357	0.355	0.456	0.452	0.432	0.428	0.399	0.402
	12	0.543	0.397	0.394	0.380	0.385	0.421	0.451	0.429	0.453	0.463	0.432
5% PCP	13	0.371	0.432	0.419	0.441	0.422	0.503	0.500	0.440	0.415	0.315	0.426
	14	0.317	0.391	0.401	0.378	0.369	0.380	0.362	0.364	0.370	0.263	0.360
	15	0.224	0.308	0.308	0.352	0.340	0.393	0.377	0.364	0.348	0.283	0.330
	16	0.295	0.372	0.364	0.382	0.387	0.451	0.421	0.464	0.496	0.336	0.397
	17	0.262	0.370	0.365	0.361	0.361	0.399	0.389	0.375	0.348	0.369	0.360
	18	0.310	0.400	0.407	0.429	0.431	0.403	0.401	0.426	0.413	0.286	0.391

*see fig A.5

TABLE A.7 MEAN ABSORPTION OF DEKALIN FOR EACH TEST BLOCK POSITION

Treatment	Test block position*				
	a/z	b/y	c/x	d/w	e/v
Untreated	0.492	0.500	0.505	0.494	0.491
Solvent	0.435	0.416	0.416	0.418	0.415
1% TnBTO	0.465	0.445	0.443	0.452	0.456
5% PCP	0.303	0.389	0.391	0.399	0.403

a/z blocks had end grain exposed during treatment

e/v blocks were furthest from the end during treatment

*see fig A.5

TABLE A.8 VARIATION BETWEEN TREATMENTS

	1 Untreated	2 Solvent	3 1% TnBTO	4 5% PCP	Total
n	3	3	6	6	18
T	1.489	1.260	2.714	2.264	7.727
$\bar{x}$	0.496	0.420	0.452	0.377	0.429
S	0.743	0.532	1.233	0.860	3.368
$\frac{T^2}{n}$	0.739	0.529	1.228	0.854	$\frac{T^2}{n}$ 3.350
					3.317

SS for random variation

0.004	0.003	0.005	0.006	0.018

Source of variation	SS	df	MS	F
between treatments	0.033	3	0.011	8.46 **
residual	0.018	14	0.0013	

Detailed comparisons:

	SS	df	MS	F
1 v 2	0.0087	1/4	0.0087	6.72 nsd
(1 + 2) v 3	0.0001	1/10	0.0001	0.078 nsd
(1 + 2) v 4	0.0196	1/10	0.0196	15.08 **

nsd no significant difference

** significantly different at the 1% level

TABLE A.9 NO VARIATION BETWEEN BLOCK POSITIONS* (1% TnBTO TREATED)

	a/z	b/y	c/x	d/w	e/v	Total
n	12	12	12	12	12	60
T	5.585	5.334	5.315	5.427	5.467	27.128
$\bar{x}$	0.465	0.445	0.443	0.452	0.456	0.452
s	2.618	2.391	2.378	2.484	2.522	12.393
$\frac{T^2}{n}$	2.599	2.371	2.354	2.454	2.491	12.269
					$\frac{T^2}{n}$	12.265
SS for random variation	0.019	0.020	0.024	0.030	0.031	0.124

Source of variation	SS	df	MS	F	
between positions	0.004	4	0.001	0.444	nsd
residual	0.124	55	0.00225		

nsd no significant difference
*see fig A.5

TABLE A.10 END BLOCKS SIGNIFICANTLY DIFFERENT FROM THE REMAINDER* (5% PCP TREATED)

	a/z	b/y	c/x	d/w	e/v	Total
n	12	12	12	12	12	60
T	3.631	4.663	4.697	4.793	4.839	22.623
$\bar{x}$	0.303	0.389	0.391	0.399	0.403	0.377
s	1.119	1.838	1.859	1.934	1.973	8.723
$\frac{T^2}{n}$	1.099	1.812	1.838	1.914	1.951	8.614
					$\frac{T^2}{n}$	8.530
SS for random variation	0.020	0.026	0.021	0.020	0.022	0.109

Source of variation	SS	df	MS	F	
between positions	0.084	4	0.021	10.61	***
residual	0.109	55	0.00198		
Detailed comparisons:					
a/z v b/y	0.0444	1/22	0.0444	22.41	***
b/y v e/x	0.000048	1/22	0.000048	0.024	nsd
a/z v rest	0.08318	1/58	0.08318	42.01	***

nsd no significant difference
*** significantly different at 0.1%
* see fig A.5.

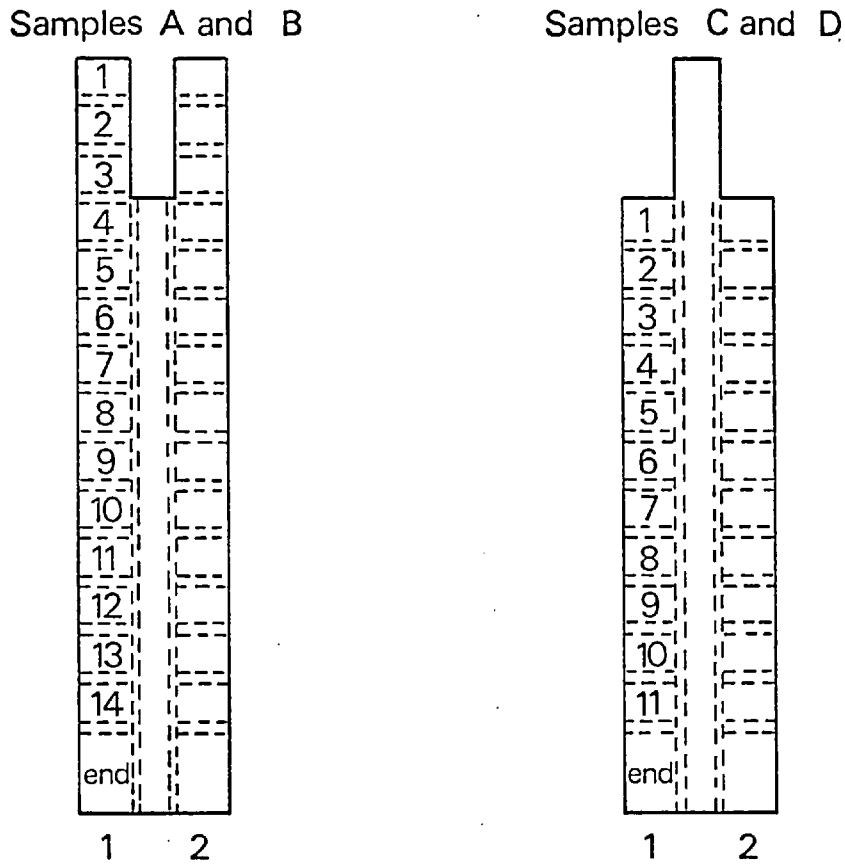

Fig. A1. Conversion of L-joints to test blocks

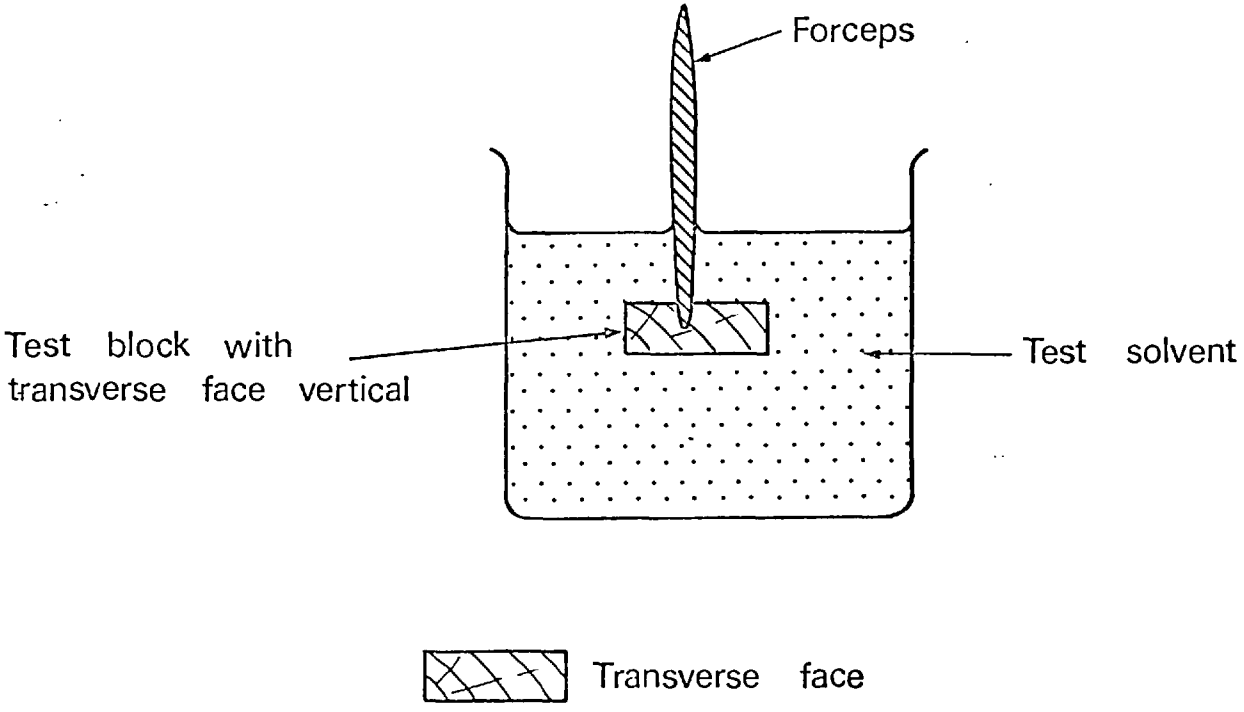

Fig. A2. Immersion of test blocks

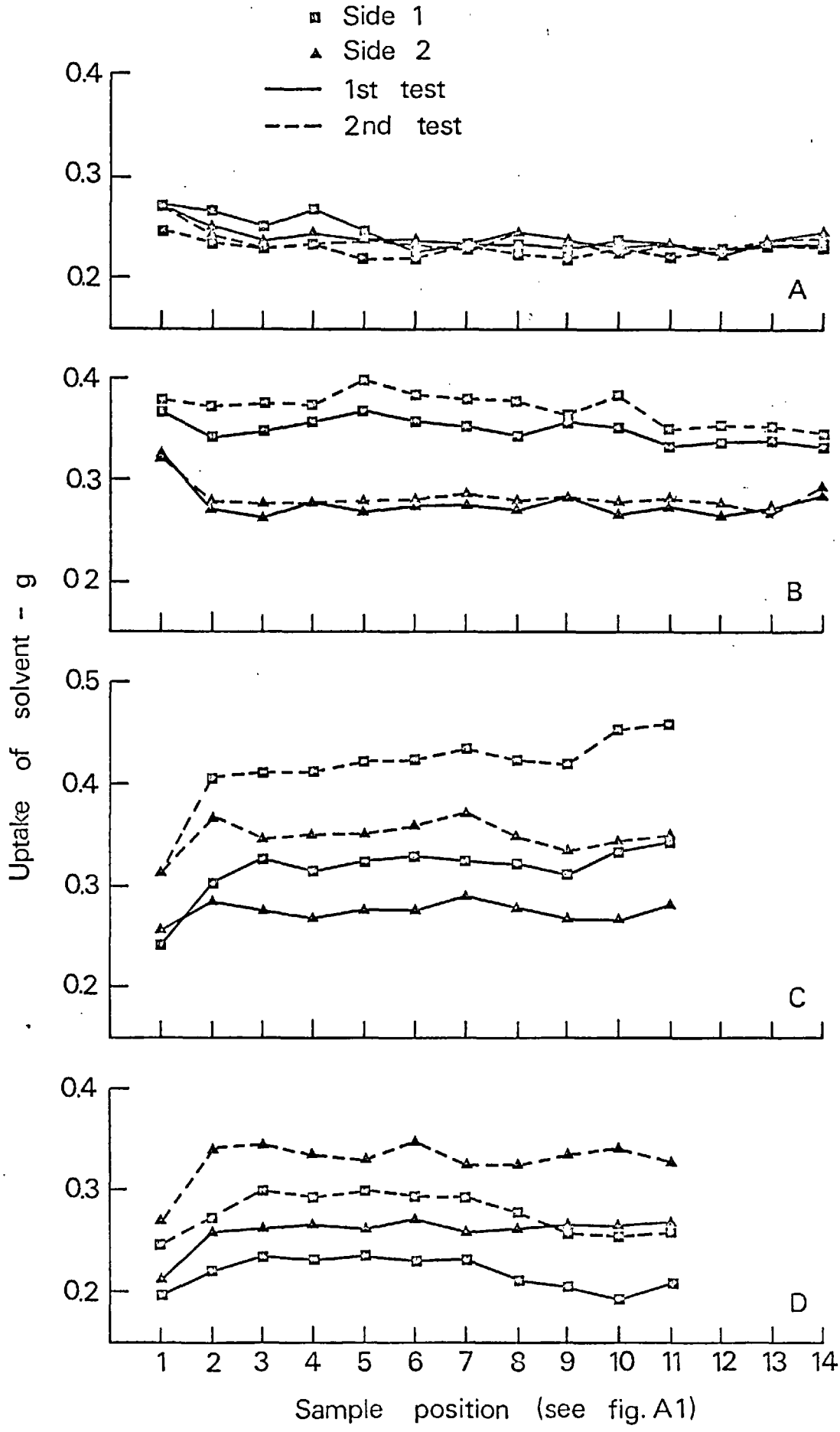

Fig. A 3. Uptake of solvent by test blocks

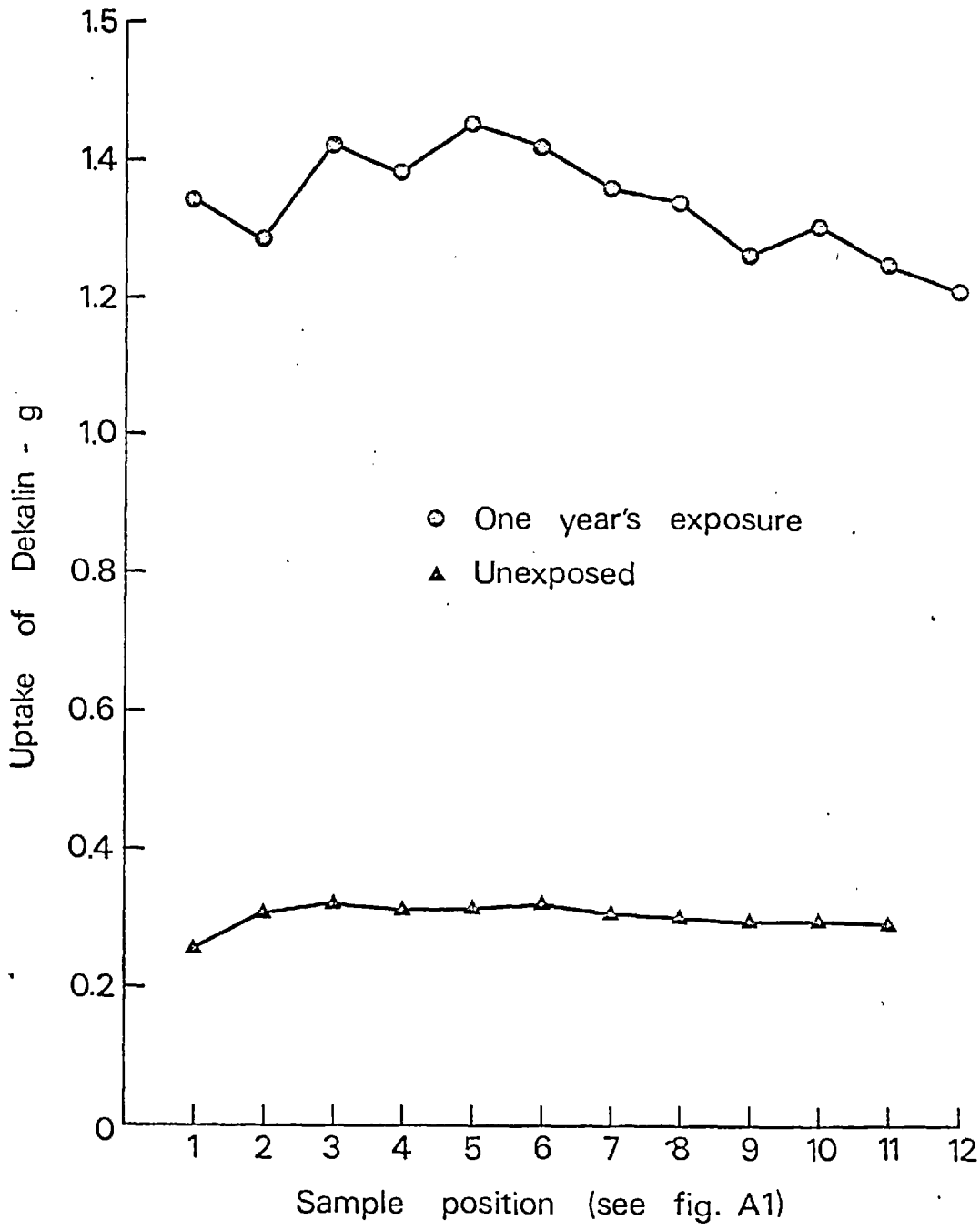

Fig. A4. Effect of one years exposure on absorption

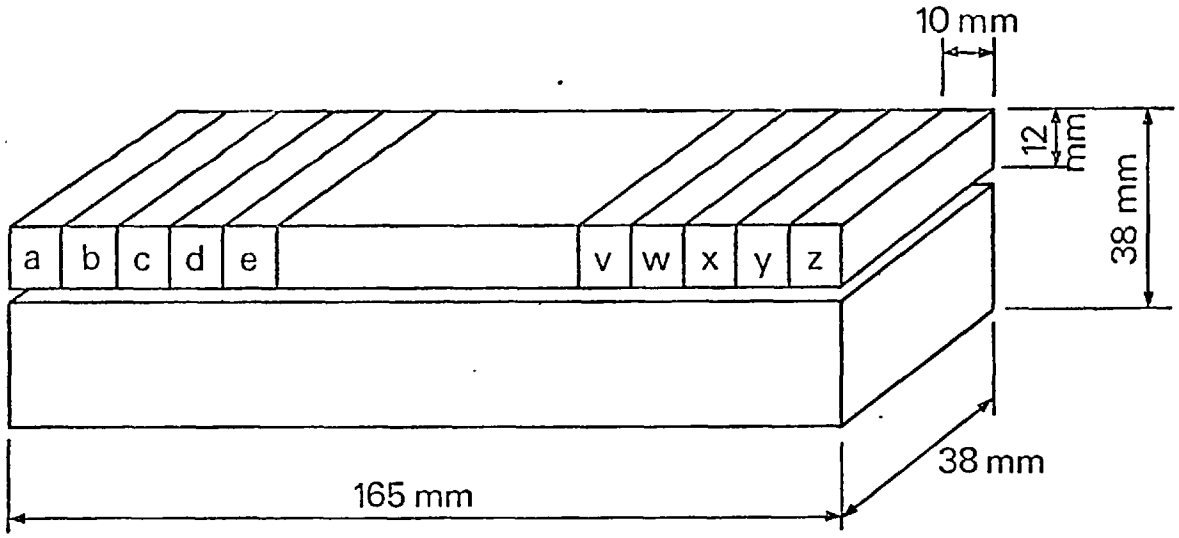

Fig. A 5. Conversion of blocks used for testing the effect of preservative treatment on permeability

APPENDIX B

DEVELOPMENT OF METHODS FOR ESTIMATING BACTERIAL NUMBERS IN SAMPLES

B.1 INTRODUCTION

In the second series of L-joint exposure trials (Section 4) it was proposed to follow gross microbial activity by measuring respiration in small blocks cut from the horizontal member of each joint. Various techniques using either carbon dioxide evolution or oxygen consumption have been used (Smith, 1975). Since it was not possible to obtain access to gas chromatographic or micromanometric apparatus over the time required the simple method using titration described by Klingström (1965) has therefore been investigated for application to the current problem. Carbon dioxide evolved during respiration was dissolved in sodium hydroxide solution. After a given period of exposure the sodium carbonate produced is precipitated as insoluble barium carbonate by adding barium chloride solution. The residual sodium hydroxide is estimated by titration with hydrochloric acid.

The bacterial counting technique used in the first series of L-joint exposure trials (Section 3) proved to be time consuming and gave variable results. A simple method of streaking sample chips across a nutrient medium has therefore been investigated as an alternative system.

B.2 METHOD

Blocks measuring 50 x 25 x 15 mm of Scots pine sapwood were cut from rapidly air dried stock. The blocks were placed in a beaker, weighted down and covered with water from the PRL log pond. A vacuum of approximately 20 torr was drawn for ten minutes, then released, thus saturating the blocks. Sets of three replicates were placed in 800 ml capacity glass jars and covered with pond water; the jars were closed with ventilated caps and incubated at 22°C.

After various periods of incubation the three blocks were removed from one jar; each block was cut into three parts and each part was sampled by one of the following methods.

B.2.1 Carbon dioxide evolution

A 10 mm length cut from one end of each block was suspended, using nylon fishing line, over 5 ml freshly prepared 0.05N sodium hydroxide in a 100 ml conical flask. Each flask was sealed with a rubber bung and incubated at room temperature for four hours. The bungs were then removed, 0.15 ml saturated barium chloride was added to each flask to precipitate the dissolved CO_2 as barium carbonate. The residual NaOH was titrated with 0.05N hydrochloric acid using one drop of phenolphthalein (1 per cent in ethanol) as indicator.

The effect of introducing dry blocks to act as controls, as recommended by Klingström (1965), was compared with the use of blocks freshly impregnated with sterile deionised water and therefore unlikely tc contain actively growing organisms. The effect of increasing the incubation period from 4 to 24 hours was investigated; control flasks without blocks were titrated immediately after being set up and after the same period of incubation as the test blocks.

B.2.2 Bacterial counts

Under sterile conditions, a 12 mm length cut from the other end of each block was placed near the top of a small, new polythene bag and squeezed in a small vice. A 0.1 ml sample of the exudate which collected in the bag was diluted in a 1 in 100 series using sterile deionised water. A 1 ml sample of each dilution was mixed with approx 9 ml molten nutrient agar, held at 40°C, and poured into a sterile plastic petri dish. The plates were normally incubated at 22°C for 3 days then counted. After certain incubation periods dry blocks were impregnated with freshly drawn deionised water and the bacterial count established as above. The bacterial numbers present in the pond water after 22 days incubation were estimated using a similar dilution plate technique.

B.2.3 Bacterial growth from sample chips

Under sterile conditions the remaining length of each block was split open and a small chip cut using a 6 mm wide U-shaped gouge. The chip was streaked across a plate of nutrient agar and partially embedded in the agar at the end of the streak (fig B.2). The plates were normally observed after incubation at 22°C for 3 days; deviations are noted in tabulated results. Chips were also taken from blocks impregnated with deionised water.

A second series of tests was carried out taking sample chips from replicate blocks impregnated with pond water on different days but sampled on the same day. The pond water was freshly drawn from the log pond on each occasion to avoid differences caused by incubation at room temperature. The sample chips (three from each of three replicates per incubation period) were streaked and planted on plates of nutrient agar that had previously been dried at 45°C for several hours, to remove all traces of condensation. The plates were incubated at 22°C for 3 days, then observed and photographed.

B.3 RESULTS

The quantities of HCl used in the titration of residual NaOH are given in Table B.1; double lines delimit sets of data obtained at the same time. Mean values and the difference between dry blocks and test blocks are presented in Table B.2. Bacterial counts on each dilution plate and the calculated number of bacteria per ml of exudate are presented in Table B.3. Observations on the growth of bacteria on the streak across the plate and around the sample chip in the first test are recorded in Table B.4. Data from these experiments are summarised in Table B.5 and Fig B.1.

Growth of bacteria from the streak and around the chip in the second test is shown in Fig B.2 and recorded in Table B.6. These data are correlated with counts per ml of exudate in Table B.7.

B.4 DISCUSSION

The first attempt at measuring CO_2 evolution (top set of data, table B.1) gave results which varied considerably between replicates. Some of the

variation can be attributed to the blocks being slightly too large to be withdrawn from the flasks at the end of the incubation period. During the titration, HCl came into contact with some test blocks and, although washed off with deionised water, may have affected the result. This does not explain the variation between replicates with dry blocks or without blocks. Subsequent series showed much better reproducibility and required consistently higher quantities of HCl to neutralise the NaOH, which may indicate the concentration of one of the solutions was inaccurate in the first set of tests. Klingström (1965) noted that dry blocks produced CO_2 and therefore used them as his control. Since, in these experiments, the test blocks were saturated with pond water, blocks saturated with deionised water were compared with dry blocks for use as controls (second set of data, table B.1). The results were almost identical, with mean values of 4.09 ml HCl for dry blocks and 4.08 ml HCl for saturated blocks; dry blocks alone were included in later tests to avoid the extra work involved in saturating them. The dry blocks usually produced CO_2 although the results after 16 days incubation are an exception and the system of using dry blocks as controls has therefore been adopted. Differences between the various sets of these controls corresponding to 7 to 22 days incubation (table B.2) can be accounted for by slight variation in the strength of the solutions used. The stock solution of NaOH must be freshly prepared for each test, to prevent reduction in strength due to uptake of atmospheric CO_2.

The titration values for flasks with dry blocks corresponding to the 0 and 3 day incubation periods differ from flasks without blocks by a much greater amount than subsequent sets, and as a consequence would give negative production of CO_2 if used to correct the test series. A "corrected" dry block value has therefore been calculated to correct the 0 and 3 day results (see table B.2). {The mean difference between flasks without blocks and flasks with dry blocks for the two subsequent sets of data (4.22 - 4.09 = 0.13 ml and 4.04 - 3.97 = 0.07 ml respectively; mean value 0.10 ml) has been used to correct the value for flasks without blocks from the first set of data (3.52 - 0.10 = 3.42)}.

The amount of CO_2 produced, measured as ml 0.05N HCl (ie the difference between "control" and "test" values) initially increased with the incubation period but subsequently declined (fig B.1). Bacterial counts (table B.3) showed quite good replication and a similar variation with time to CO_2 production (fig B.1) although on a logarithmic rather than linear scale. Such growth and decline follow the normal pattern for bacterial growth within a closed system (Stanier et al, 1963). The increase in bacterial numbers after 22 days incubation may indicate increased growth by a second group of organisms among the mixed flora known to be present in pond water (FPRL Unpublished report, 1963).

Calculations on the oxidation of $C_6H_{12}O_6$ units to CO_2 and water show that utilisation of 14 per cent of the atmospheric oxygen in the test flasks would saturate the NaOH with CO_2. Since this has not occurred, overall oxygen shortage within the sealed test flasks is not responsible for the low production but since the blocks were saturated with water, lack of oxygen within the blocks could have occurred. Alternatively the bacteria may not be utilising the substrate by a process resulting in free CO_2.

Increasing the incubation period for CO_2 production resulted in a higher value for NaOH used but not in proportion to the increase in time (table B.2). This again may have been a result of lack of oxygen within the blocks but should not preclude use of this longer period which is required by the logistics of the sampling planned for the second series of L-joint tests.

Observation of the growth of bacteria from chips streaked across the nutrient agar plates was impaired after 3 days incubation in pond water in the first tests by use of undried plates. This allowed the colonies to spread producing almost confluent growth. Subsequently, dried plates were used with much better results exemplified by the second tests shown in Fig B.2. Chips from blocks impregnated with deionised water did not give rise to growth either along the streak or around the chip although bacterial counts showed 8.19×10^2 bacteria per ml of exudate (table B.5). Blocks impregnated with pond water, with a count of 4.00×10^4 per ml of exudate only showed growth around

the chip. Blocks incubated for one day generally showed a few colonies along the streak; longer incubation periods produced more growth although there are indications that after 16 and 22 days incubation in pond water there was less growth, thus paralleling the data from the bacterial counts.

A subjective scale for recording visual observations was developed during the second test (table B.6; fig B.2). Providing bacterial numbers were similar in the two test series the scale can be loosely related to bacterial counts in the exudate (table B.7).

B.5 CONCLUSIONS

The data from these exploratory experiments have shown that:

a The simple system of measuring CO_2 production employed by Klingström (1965) can provide reproducible results.

b Bacterial growth from a chip streaked across a previously dried nutrient agar plate parallels bacterial counts in exudate from replicate blocks. By using the correlation established in these experiments, the streak method should provide information on relative bacterial numbers, with much reduced effort.

TABLE B.1 CO_2 EVOLUTION; ml HCl REQUIRED TO NEUTRALISE RESIDUAL NaOH AFTER 4 HRS INCUBATION

Blocks present	Previous conditioning of blocks		ml HCl	
	Impregnant	Incubation period (days)	Per replicate	Mean
-	-	-	3.35 3.77 3.45	3.52
+	-	-	2.45 3.42 3.25	3.04
+	pond water	0	3.70 3.51 3.41	3.54
+	pond water	3	3.61 2.81 3.75	3.39
-	-	-	4.25 4.15 4.25	4.22
+	-	-	4.15 4.12 4.00	4.09
+	deionised water	-	4.12 4.19 3.92	4.08
+	pond water	7	3.91 3.96 3.88	3.92
-*	-	-	4.13 4.04 3.93	4.03
-	-	-	4.02 4.08 4.01	4.04
+	-	-	3.98 3.94 3.99	3.97

*Titrated immediately flasks set up.
Note: Double lines separate sets of data obtained at different times.

TABLE B.1 CONTINUED

Blocks present	Previous conditioning of blocks		ml HCl	
	Impregnant	Incubation period (days)	Per replicate	Mean
+	pond water	9	3.74 3.74 3.74	3.74
-	-	-	3.83 3.89 3.91	3.88
+	-	-	3.97 3.96 3.91	3.95
+	pond water	16	3.70 3.66 3.70	3.69
+	-	-	3.96 3.98 4.06	4.00
+	pond water	22	3.70* 3.84 3.81	3.83
+**	-	-	3.77 3.76 3.90	3.81
+**	pond water	22	3.27 3.41 3.53	3.40

*Not included in mean since block touched NaOH
**Test flasks incubated for 24 hrs before titrated
Note: Double lines separate sets of data obtained at different times.

TABLE B.2 EVOLUTION OF CO_2 BY TEST BLOCKS IMPREGNATED WITH LOG POND WATER

Incubation period (days)	Mean titration of HCl		
	Dry blocks	Test blocks	†CO_2 production
0	3.04 (3.42)	3.54	-0.12**
3	3.04 (3.42)	3.39	0.03**
7	4.09	3.92	0.16
9	3.97	3.74	0.23
16	3.95	3.69	0.26
22	4.00	3.83	0.17
22*	3.81	3.40	0.41

†Measured as ml 0.05N HCl equivalent to the NaOH neutralised by the CO_2 produced, ie the difference between dry block controls and test blocks.

*Test flasks incubated for 24 hours.

**Calculated using the corrected value for dry blocks given in parentheses (see text).

-Negative production of CO_2 as compared with the dry block controls.

TABLE B.3 BACTERIAL COUNTS IN EXUDATE FROM TEST BLOCKS

Treatment of blocks prior to test. Impregnant; incubation period	Dilution				Count per ml exudate	
	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Diln used	Count
deionised water; not incubated	11	0	-	-	10^{-2}	1.10×10^{3}
	1	0	-	-	10^{-2}	1.00×10^{2}
	2	0	-	-	10^{-2}	2.00×10^{2}
						$\bar{x}$ 4.67×10^{2}
pond water; not incubated	252	0	0	-	10^{-2}	2.52×10^{4}
	928	4	0	-	10^{-2}	9.28×10^{3}
	20	0	0	-	10^{-2}	2.00×10^{4}
						$\bar{x}$ 4.00×10^{4}
pond water; 3 days	uncountable	416	6	-	10^{-4}	4.16×10^{6}
	uncountable	144	5	-	10^{-4}	1.44×10^{5}
	uncountable	32	1	-	10^{-4}	3.20×10^{6}
						$\bar{x}$ 1.97×10^{6}
deionised water; not incubated	15	0	-	-	10^{-2}	1.50×10^{3}
	13	0	-	-	10^{-2}	1.30×10^{3}
	7	0	-	-	10^{-2}	7.00×10^{2}
						$\bar{x}$ 1.17×10^{3}
pond water; 7 days	-	872	1	0	10^{-4}	8.72×10^{6}
	-	1768	18	2	10^{-4}	1.77×10^{7}
	-	1972	5	0	10^{-4}	1.97×10^{7}
						$\bar{x}$ 1.54×10^{7}
pond water; 16 days	uncountable	134	3	12*	10^{-4}	1.34×10^{6}
	uncountable	175	2	0	10^{-4}	1.75×10^{6}
	uncountable	315	3	0	10^{-4}	3.15×10^{6}
						$\bar{x}$ 2.08×10^{6}
pond water; 22 days	uncountable	215	1	-	10^{-4}	2.15×10^{6}
	uncountable	192	4	-	10^{-4}	1.92×10^{6}
	uncountable	374	6	-	10^{-4}	3.74×10^{6}
						$\bar{x}$ 2.60×10^{6}
pond water from around blocks after being incubated 22 days	uncountable	uncountable	48	-	10^{-6}	4.80×10^{7}

-Not tested

*Mostly adjacent to the side of the dish (suggesting dish was contaminated).

Note: Double lines separate sets of data obtained at different times.

TABLE B.4 BACTERIAL GROWTH FROM SAMPLE CHIPS (FIRST TEST)

Treatment of blocks prior to test. Impregnant; incubation period	Growth along streak	Growth around chip	Incubation period and temperature
deionised water; not incubated	- - -	- - -	3 days 22°C
pond water; not incubated	- - -	+ + +	3 days 22°C
pond water; 3 days	+ confluent + confluent + confluent	+ + +	3 days 22°C
deionised water* not incubated	- - -	- - -	3 days 22°C
pond water* 7 days	+ individual cols + individual cols + individual cols	+ + +	3 days 22°C
pond water* 9 days	+ almost confluent + almost confluent + almost confluent	+ + +	12 days 7°C
pond water* 16 days	+ few large cols + small ones + mostly separate cols + mostly separate cols	+ + +	5 days 22°C
pond water* 22 days	+ mostly sep cols (212) + mostly sep cols (376) + mostly sep cols (242)	+ + +	2 days 22°C 3 days 7°C

*Nutrient agar plates dried before use.
Note: Double lines separate sets of data obtained at different times.

TABLE B.5 SUMMARY OF RESULTS

Treatment of blocks prior to test. Impregnant; incubation period	CO_2† production	Bacterial count per ml exudate	Bacterial growth††	
			Along streak	Around chip
deionised water; not incubated	-	8.19×10^{2}*	none	-
pond water; not incubated	-0.12	4.00×10^{4}	none	+
pond water; 3 days	0.03	1.97×10^{6}	confluent**	+
pond water; 7 days	0.16	1.54×10^{7}	individual colonies	+
pond water; 9 days	0.23	-	almost confluent	+
pond water; 16 days	0.26	2.08×10^{6}	mostly individual colonies	+
pond water; 22 days	0.17	2.60×10^{6}	mostly individual colonies	+

†Measured as ml 0.05N HCl equivalent to the NaOH neutralised by the CO_2 produced.

††Data from first test only.

*Mean of two separate estimations.

**Growth affected by use of moist plates.

TABLE B.6 BACTERIAL GROWTH FROM SAMPLE CHIPS (SECOND TEST) cf FIG B.2

Replicate blocks	Within block replicates	Incubation period (days)				
		1	2	4	8	12
1	1	few +	few +	many +	some +	many +
	2	none +	few +	many +	some +	many +
	3	none +	few +	many +	many +	many +
2	1	2 +	few +	many +	many +	many +
	2	none +	some +	many +	few +	many +
	3	4 +	some +	some +	many +	many +
3	1	few +	some +	some +	some +	many +
	2	few +	some +	many +	many +	many +
	3	few +	some +	some +	many +	many +

Growth alone streak; many > some > few > actual numbers > none

Growth around chip; + growth - no growth

TABLE B.7 TENTATIVE CORRELATION OF GROWTH FROM SAMPLE CHIPS WITH ESTIMATED NUMBERS OF BACTERIA IN THE EXUDATE

Growth from sample chips		Bacterial count per ml exudate
Growth along streak	Growth around chip	
none	-	8.19×10^2
none	+	4.00×10^4
few	+	$<1.97 \times 10^6$
some	+	1.97×10^6
many	+	$>1.54 \times 10^7$

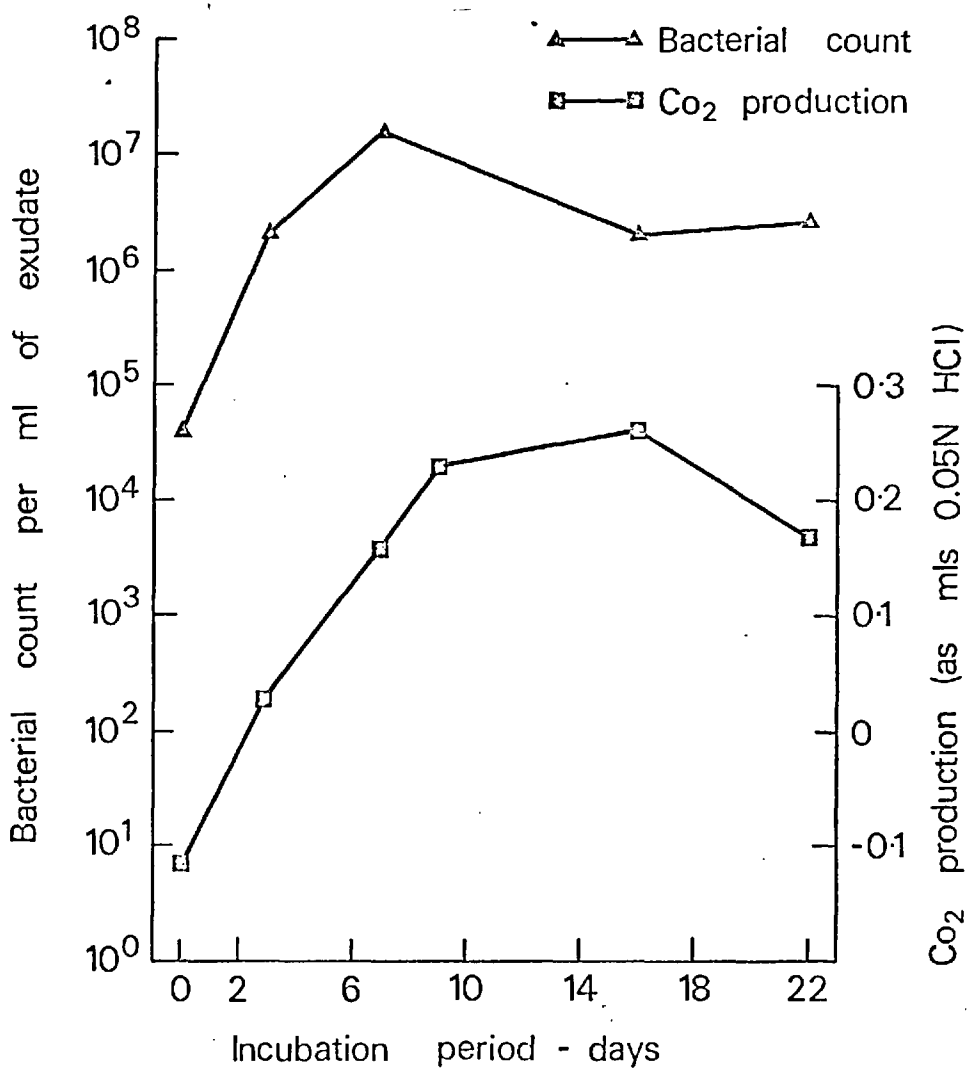

Fig. B.1. Bacterial counts in and CO_2 production from Scots pine sapwood blocks impregnated with pond water

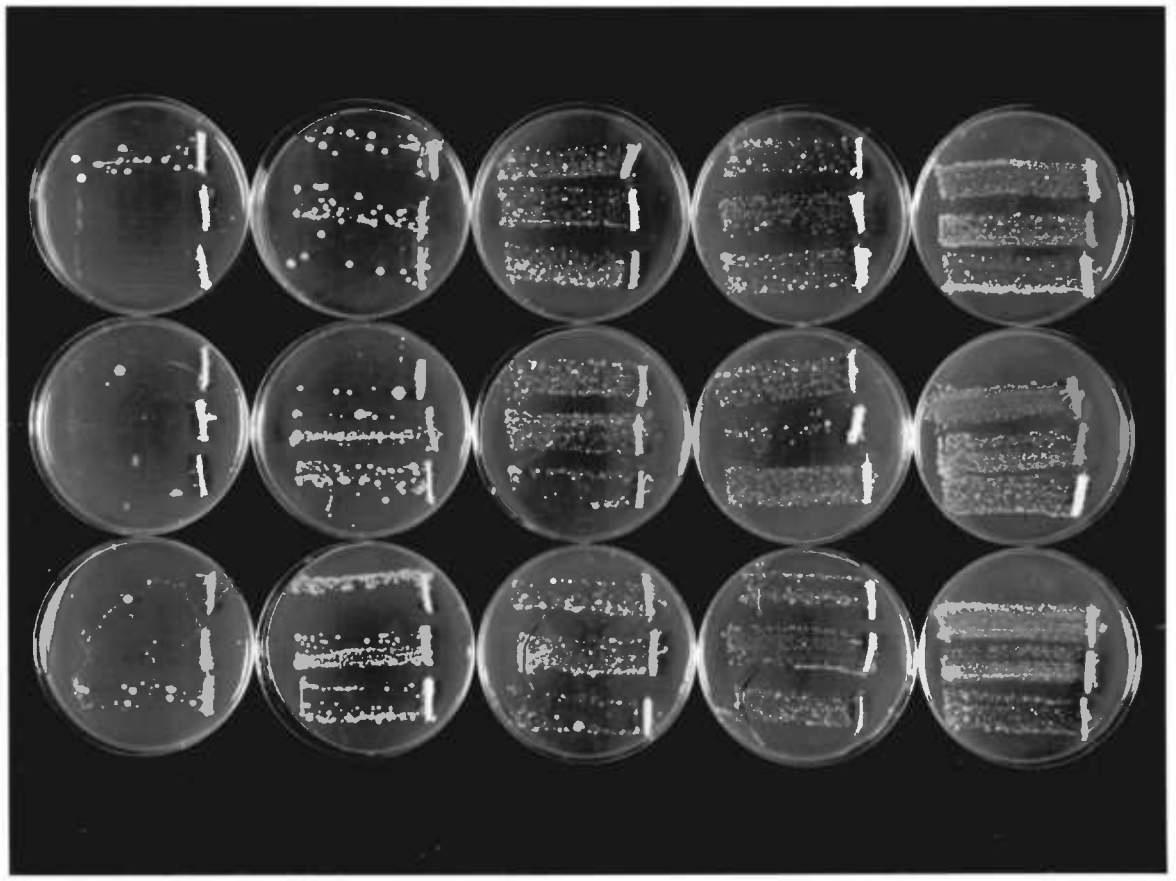

Fig B.2 Growth from 3 chips from each of 3 replicate Scots pine sapwood blocks impregnated with pond water and incubated at 22°C.
Columns from left to right incubated 1, 2, 4, 8 and 12 days. cf Table B.6.