

CONTROL OF BLUE-STAIN ON RUBBER (*HEVEA BRASILIENSIS*, WOOD DURING THE BORON DIFFUSION TREATMENT

By

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SUMMARY

Stain development was caused by the discolouration and accumulation of dark deposits and mycelia in wood ray cells and xylem tracheids due to the growth of a *Penicillium* sp. and an *Aspergillus* sp. A Double Dip method of treatment where timber was first treated with aqueous sodium pentachlorophenate and subsequently with borax/boric acid solution, was developed and was found to be effective.

INTRODUCTION

Large quantities of rubber (*Hevea brasiliensis*) wood are available in Sri Lanka when uprooting the old stand prior to replanting. Untreated rubber wood is not durable as it is highly susceptible to decay and to attack by Bostrychid borers and stain fungi (Tisseverasinghe, 1969). Therefore, until recently it was not considered for use in the building and furniture industries.

Recent attempts to increase the utilization of rubber wood upgraded by the Boron Diffusion Treatment of Mason (1966), has focussed attention on treatment problems. Staining of wood is a common feature of the current boron treatment method (Tisseverasinghe, 1969; 1970), which envisage a diffusion period of 3-4 weeks (Mason, 1966) for boron salts to penetrate into the wood. During this period, treated timber is kept 'block-stacked' and covered to prevent drying. This allows the development of stain fungi and other moulds. Staining appears as bluish grey streaks or patches in the wood and precludes its use in situations where a natural finish is desired.

Among the fungicides that are widely used for the prevention of stain, chlorinated phenols. e.g. sodium pentachlorophenate, have proved to be effective (Roff & Cserjesi, 1965). Tisseverasinghe (1969, 1970) and Gunasekera & Sumanaweera (1973) have indicated that 1% sodium pentachlorophenate in borax /boric acid solution of 25% BAE (boric acid equivalent) is ineffective in controlling blue-stain on rubber wood.

Other methods of controlling blue-stain are known, but they are impracticable under local conditions. Therefore, the use of aqueous sodium pentachlorophenate was further investigated. This paper describes the nature of staining and a simple Double Dip method of treatment developed for the prevention of blue-stain on rubber wood.

MATERIALS AND METHODS

The internal distribution of the causal fungi was studied in 10-15 m μ thick, transverse, radial longitudinal and tangential longitudinal sections cut on a Rotary Microtome and stained with safranin-picroaniline blue (Cartwright, 1929). The causal fungi were isolated from infected planks and grown on 2% malt extract agar (MEA) at 28°C. They were tested in the laboratory to establish their staining capabilities by the experimental procedure of Cserjesi & Roff (1970), using test pieces of rubber wood (0.7 x 1.5 x 7.0 cm), sawn in the same manner as a normal plank.

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Penicillium sp. and *Aspergillus* sp., either individually or in combination were used in artificial inoculations. Spore suspensions were prepared from 7 day old cultures. Tween 80 (0.05%) was added to disperse the spores which were clumped. Spore suspensions were filtered through a nylon cloth to remove the mycelial fragments. Inoculations were done, using a 1ml micropipette, at a spore concentration of about 10×10^4 per ml.

Stain development was assumed to be equivalent to the superficial fungal growth, which was graded as follows:

- 0 = no fungal growth
- 1 = few scattered fungal colonies
- 2 = fungal colonies covering about 25% of the surface area
- 3 = fungal colonies covering 25-50% of the surface area
- 4 = fungal colonies covering 50-100% of the surface area.

A colonisation index was computed giving the sum of grades as a percentage of the maximum score:

$$\text{Colonisation index} = \frac{100 \times \text{sum of grades}}{4 \times \text{total number of test pieces}}$$

Colonisation was also estimated as a percentage of the total number of test pieces used in a particular treatment. Boron uptake and penetration was detected using the turmeric test (Mason, 1966).

RESULTS

Nature of infection and development of stain

Colonisation of treated planks by stain fungi can be detected by the appearance of small white colonies on the surface of the planks 3-4 days after 'block-stacking' for diffusion. Later these colonies either turn blue or yellow brown and at the same time they coalesce so that the planks are covered to varying extents with a thick mat of mycelium (Plate I, Fig. 1). Planing or sawing through the colonised planks reveals stained areas (Plate I, Fig. 2) which are either superficial or penetrate deep into the planks (Tisseverasinghe, 1969; 1970).

Unstained areas of boron treated wood were free of fungal hyphae when examined under the microscope (Plate II, Fig. 1). Stained areas, however showed, discoloured cell contents (Plate II, Fig. 2 & Plate III, Figs. 1 & 2) and fungal hyphae (Plate III, Figs. 1 & 2). Fungal hyphae and discolourations occur mainly in the xylem and ray parenchyma cells and to a lesser extent in xylem tracheids and vessels. The fungus is seen to pass from cell to cell in ray parenchyma cells (Plate III, Fig. 1) and from ray parenchyma cells to xylem tracheids (Plate III, Fig. 2). No sporulation was observed inside the cells.

Causal fungi

Two main types of fungi were isolated from the treated planks: 1. A single *Penicillium* sp. isolated from bluish areas, and producing a bluish pigment at the time of sporulation, 3-4 days at 28 °C on MEA. 2. A single *Aspergillus* sp. isolated from yellowish brown areas, and producing a yellow brown pigment at the time of sporulation, 4-5 days at 28 °C on MEA.

These two fungi produced a prominent bluish stain and a less conspicuous brown stain, respectively, in test pieces of rubber wood. Both fungi were successfully reisolated from the test pieces at the conclusion of the experiment.

Effect of sodium pentachlorophenate

The influence of 0, 12.5, 25, 50, 100 ppm of sodium pentachlorophenate on the growth of *Penicillium* sp. *in vitro* was next examined. Dilutions of the fungicide were prepared in sterile distilled water so that 1 ml portions of these when added to 14 ml of sterile MEA give the desired concentration in the final medium. Each plate was inoculated centrally with a 3 mm disk, cut from the edge of a 3-day old culture of the fungus on MEA, placed with the mycelium on agar. Four such plates for each concentration was incubated at 28°C, and growth estimated daily by measuring the colony radius along two diameters. Results given in Fig. 1 show that 50 ppm of sodium pentachlorophenate, when incorporated into agar, completely checked the mycelial growth of *Penicillium* sp. A noteworthy feature was the growth of the mycelium 8 days after inoculation at a concentration of 25 ppm.

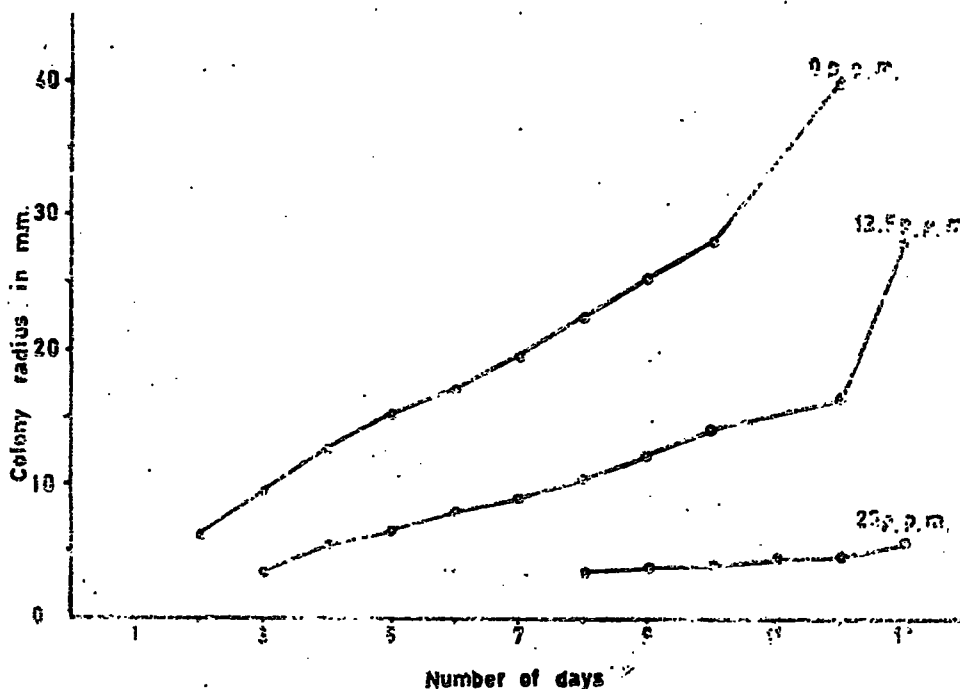


Fig. 1. Effect of sodium pentachlorophenate (p.p.m.) on mycelial growth of *Penicillium* sp. on 2% malt extract agar at 28°C (No growth at concentrations greater than 25 p.p.m.).

The colonisation levels of stain fungi on rubber planks, not exposed to treatment with borax/boric acid solution, was measured using the test technique of Cserjesi & Roff (1970). The fungal growth on rubber wood was completely suppressed by aqueous sodium pentachlorophenate at concentrations $> 1\%$ (Table 1).

TABLE 1
COLONISATION BY *PENICILLIUM* SP. & *ASPERGILLUS* SP. OF TEST PIECES TREATED WITH SODIUM PENTACHLOROPHENATE

sodium pentachlorophenate (%)	<i>Penicillium</i> sp.	<i>Aspergillus</i> sp.	<i>Penicillium</i> + <i>Aspergillus</i> sp.
0	+++	+++	+++
0.5	+++	++	++
0.75	++	++	++
1.0	+	+	+
1.5	—	—	—
2.0	—	—	—
2.5	—	—	—

— = unaffected

+ = slight
++ = moderate
+++ = severe } colonisation

Solubility of sodium pentachlorophenate was as follows :—

TABLE 2
SOLUBILITY OF SODIUM PENTACHLOROPHENATE

	solvent		
	water	borax/boric acid of 25% BAE	borax/boric acid <25% BAE
maximum solubility of sodium pentachlorophenate at 45°C (%)	4.0	0.5	1.2
pH	9.0	7.4	9.2

Sodium pentachlorophenate (0.5%) is not very soluble in borax/boric acid mixture of 25% BAE; when 1% by weight is added, only half of it goes into solution and the balance appears as a scum. Table 1 shows that sodium pentachlorophenate is ineffective in controlling stain fungi at a concentration of 0.5%.

Double Dip method of treatment

The limited solubility of sodium pentachlorophenate in borax/boric acid mixture, and the inability to modify the BAE of the treatment mixture led to the development of a Double Dip method of treatment, using an aqueous solution of this fungicide and the standard boron mixture separately. In this method of treatment test pieces were first bulk treated with 2% aqueous sodium pentachlorophenate, kept for 24h under humid conditions, and then treated with 25% BAE solution in the usual manner. Higher concentrations of the fungicide and a 24h storage

period were introduced to allow the fungicide to react with the surface of the wood and get deposited as insoluble pentachlorophenol and also to minimise any leaching (Cserjesi & Roff, 1964), that may take place during the second dip.

The double dip method was compared with Mason's single dip method (1966) and a modified single dip method incorporating 1.0% sodium pentachlorophenate regardless of BAE recommendation. Twelve test pieces were used for each of the fungal combinations amounting to a total of 36 test pieces per treatment method and a grand total of 108 test pieces. Inoculations were done in the usual manner. Visual observations made after 4-weeks of diffusion in petri dish chambers (Cserjesi & Roff, 1970) are summarised in Table 3.

TABLE 3

COLONISATION LEVELS AND QUALITY OF TEST PIECES TREATED BY MASON'S SINGLE DIP (1966), MODIFIED SINGLE DIP AND THE DOUBLE DIP METHOD OF TREATMENT

Method	borax/boric acid in g/500 ml	aqueous sodium pentachlorophenate in g/500 ml	Fungal combination	Colonisation level	Quality of test pieces
Mason's single dip	98.0/62.5	0.25**	{ a b c }	{ +++ +++ +++ }	good, but heavily stained
Modified single dip	135.0/25.0	5.00**	{ a b c }	{ ++ ++ ++ }	poor, but with less stain
Double dip (I II*)	— 98.0/62.5	10.00 —	{ a b c }	{ — — — }	good stain rare

* step II done
24 h after step I

a= *Penicillium* sp.
b= *Aspergillus* sp.
c= *Penicillium* +
Aspergillus sp.

— = not colonised
+ = slight
++ = moderate colonisation
+++ = severe

** maximum solubilities

Comparison of Mason's single dip and modified single dip treatments show that both methods do not yield stain-free good quality wood; Mason's method producing heavily stained wood and the modified method producing less stained but poor quality wood. Less stain in the latter method is probably due to the high concentration of sodium pentachlorophenate (1.0%) in solution. With the double dip method staining was observed to be rare and shows promise of producing unstained good quality wood.

Further evaluations of the Double Dip method

The double dip method was further tested for its efficacy using test pieces of rubber. In these evaluations fungal colonisation was estimated as a percentage of the total number of planks colonised and in the form of a colonisation index.

TABLE 4

PERCENTAGE COLONISATION AND COLONISATION INDICES OF DOUBLE DIP TREATED TEST PIECES INOCULATED WITH STAIN FUNGI

Fungal combination	Percentage colonisation	Colonisation index
<i>Penicillium</i> sp.	33.0	17.0
<i>Aspergillus</i> sp.	13.0	3.2
<i>Penicillium</i> + <i>Aspergillus</i> sp.)	25.0	8.4

Though a maximum of 33% of the test pieces show colonisation (Table 4), the degree of staining was found to be negligible and superficial. Most of the small colonies disappeared during air drying and the surfaces appeared clean.

The pattern of distribution of fungal colonies showed that their development occurs in areas where planks are in contact during bulk treatment. This is apparently due to the fact that the exposure of the wood to the fungicide is reduced at such regions of contact and this lessens the amount of fungicide and boron solutions reaching the wood.

The results of an experiment using (2.5 x 6.5 x 25.5 cm) test pieces individually treated and inoculated with a spore suspension (10×10^4 per ml) of *Penicillium* sp. appear in Table 5. Both double dip treatments were found to be superior to the presently used Mason's single dip treatment method in controlling the colonisation by *Penicillium* sp. The percentage colonisation and colonisation indices for the double dip treatments are 30% and 10%, respectively, whereas those for Mason's single dip treatment are 100% and 90%, respectively.

TABLE 5

SOLUTION UPTAKE FOR 6 TREATMENTS, USING INDIVIDUALLY TREATED TEST PIECES (2.5 X 6.5 X 25.5 CM), % COLONISATION AND COLONISATION INDICES AFTER 4 WEEKS OF DIFFUSION SINCE SPRAY INOCULATION WITH *PENICILLIUM* SP.

Treatment	Temp. °C	Solution uptake		BAE uptake g/M ³	% with colonies	Colonisation index
		g/test piece	l/M ²			
Water	45	—	—	—	100	100
25% BAE	45	7.4	0.13*	3.30	100	100
25% BAE + penta-chlorophenate	45	9.1	0.15*	4.06	100	90
2% pentachlorophenate	28	6.5	0.11	—	25	7.5
2% pentachlorophenate	45	6.3	0.11	—	35	12.5
I. 2% pentachlorophenate	28	5.2	0.09	1.53	30	10
II. 25% BAE	45	4.6	0.08**			
I. 2% pentachlorophenate	45	6.5	0.11	2.54	30	10
II. 25% BAE	45	5.5	0.09**			

* average 0.11 (Tisseverasinghe, 1970)

** well within extremes 0.07—0.13 (Tisseverasinghe, 1970)

Standards for BAE uptake = 3.81 g/M³ (Mason, 1966), for solution uptake = 0.32 l/M² (Mason, 1966)

DISCUSSION

Present study shows that stain is caused by a *Penicillium* sp. and an *Aspergillus* sp. which grow on rubber wood consuming starch and sugars present in it as food. Hence, stain development does not cause any decay of the wood. However, the conditions that favour staining may subsequently predispose them to be attacked by wood decaying fungi.

Studies under the microscope have shown that stain in rubber wood results from the accumulation of pigmented hyphae as observed by Butcher (1968a) in sapwood of *Pinus radiata* and by the discolouration of cell contents and walls of medullary ray and xylem parenchyma cells as found in Red Beach (Butcher, 1968b). The xylem tracheids of rubber wood also show accumulation of dark deposits in their lumina and discolouration of their walls (Plate II, Fig. 2 & Plate III, Fig. 2), whereas xylem vessels only show a discolouration of their walls (Plate II, Fig. 2). Discolouration of cell contents and walls may be caused either by the secretion of coloured pigments by the causal fungi (Cartwright & Findlay, 1958) or by a reaction of the host cells to products of fungal metabolism. Both processes may be operative at the same time since both fungi were found to produce pigments *in vitro*.

To control the stain, growth of the causal fungi must be arrested throughout the diffusion period and the early part of the air-drying period. But moist conditions are required for boron salts to diffuse into the wood. Thus, quick drying, such as kiln drying cannot be coupled with the present method of treatment for boron diffusion. Therefore the wood is made unsuitable for the establishment of stain fungi by the incorporation of fungicides e.g. sodium pentachlorophenate into the treatment solution of borax/boric acid. Aqueous sodium pentachlorophenate at concentrations $> 1\%$ was effective in controlling stain fungi. However, the use of 1% sodium pentachlorophenate in borax/ boric acid was ineffective under field conditions, confirming the observations of Tisseverasinghe (1969, 1970), and also under laboratory conditions.

Several reasons can be attributed to the failure of sodium pentachlorophenate to control stain fungi. (i) the fungicide may be detoxified or biologically degraded to an inactive form by the causal fungi, as suggested by the growth of the mycelium 8 days after inoculation on 2% MEA with 25 ppm of sodium pentachlorophenate (Fig. 1). Such detoxification by fungi was observed (Duncan & Deverall, 1964; Cserjesi, 1967 & Unligil, 1968) and implications of such phenomena in causing stain and decay has been discussed by Butcher (1968a). (ii) 1% sodium pentachlorophenate was less than the minimum dose required for control. (iii) sodium pentachlorophenate; a surface protective fungicide (Cserjesi, Quon & Kozak, 1967), is effective in the form of insoluble pentachlorophenol (Cserjesi & Roff, 1964). The scum formation observed in this study and by Tisseverasinghe (1969) on dissolving sodium pentachlorophenate in borax/boric acid solution of 25% BAE at 45°C , may be due to the formation of insoluble pentachlorophenol.

Sodium pentachlorophenate should remain in soluble form till it comes into contact with the surface of wood; it then gets converted to insoluble pentachlorophenol and gets deposited on the surface of wood as a protective layer (Cserjesi & Roff, 1964). Limited solubility of sodium pentachlorophenate in borax/boric acid solution of 25% BAE as seen by scum formation at concentrations $> 0.5\%$ at 45°C , accounts for the failure of the process.

Rigorous leaching, by immersing in running water for 24h employed by Cserjesi & Roff (1964) on western hemlock samples, immediately after treatment with sodium pentachlorophenate resulted in a complete loss of the fungicide, whereas 24 to 48h delay in leaching resulted only in a 50% and negligible loss of the fungicide. To minimise any leaching the second dip treatment in this study was done 24h after the treatment with 2% sodium pentachlorophenate. With a 2% solution of the fungicide (slightly higher than the estimated requirement) sufficient retention was obtained after the second treatment with borax/boric acid solution to prevent the development of stain fungi.

Though the uptake of BAE was less in the double dip treatments than in the single dip treatment and the standard of 3.81g/M³, boron retention was found to be adequate according to turmeric test standards of Mason (1966). The range of solution uptake for borax/boric acid in the double dip treatment, though less than the standards of Mason (1966), is well within the extremes found to be effective for timber under field conditions by Tisseverasinghe (1970). The variations in solution uptake recorded here and by Tisseverasinghe (1970), can be attributed to the differences in physical properties and moisture content of the timber used on these occasions or to the differences of the timber surfaces caused by saw blades (Cserjesi, Quon & Kozak, 1967).

Further work under field conditions may lead to a reduction in the required sodium pentachlorophenate concentration coupled with a longer period of storage prior to treatment with borax/boric acid solution.

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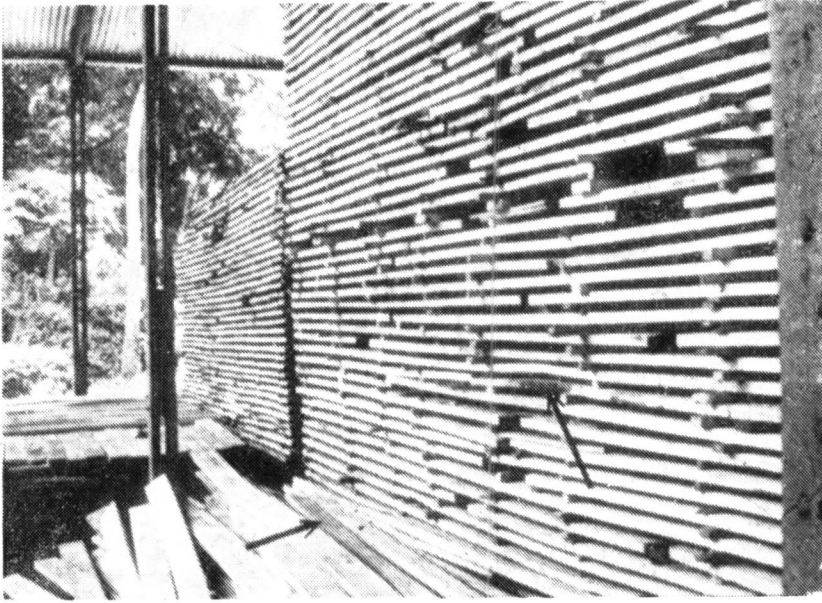


Fig. 1. Treated planks showing fungal colonies (arrowed). Note the planks stacked for air seasoning at the right. (Site: Kuda Uduwa Mill, Horana).

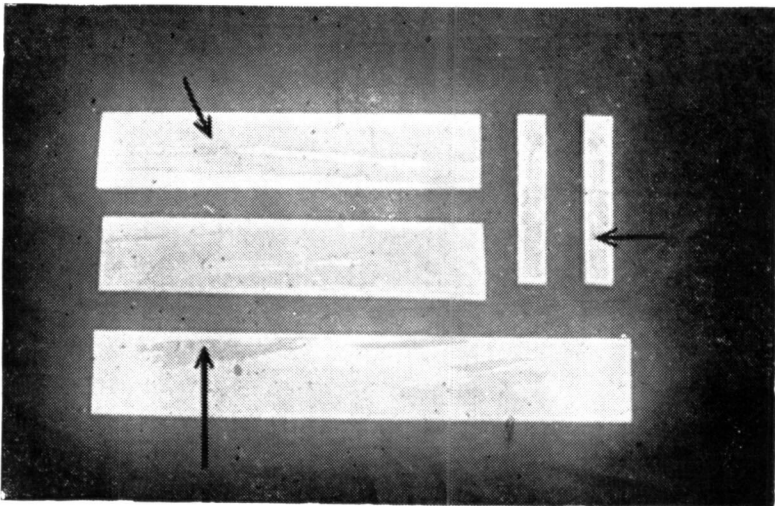


Fig. 2. Planks sawn through revealing stained areas (arrowed). Note deep penetration of the stain in the two edge views on the top right (arrowed). Dimensions of the bottom plank 60 x 15 x 2.5 cm.

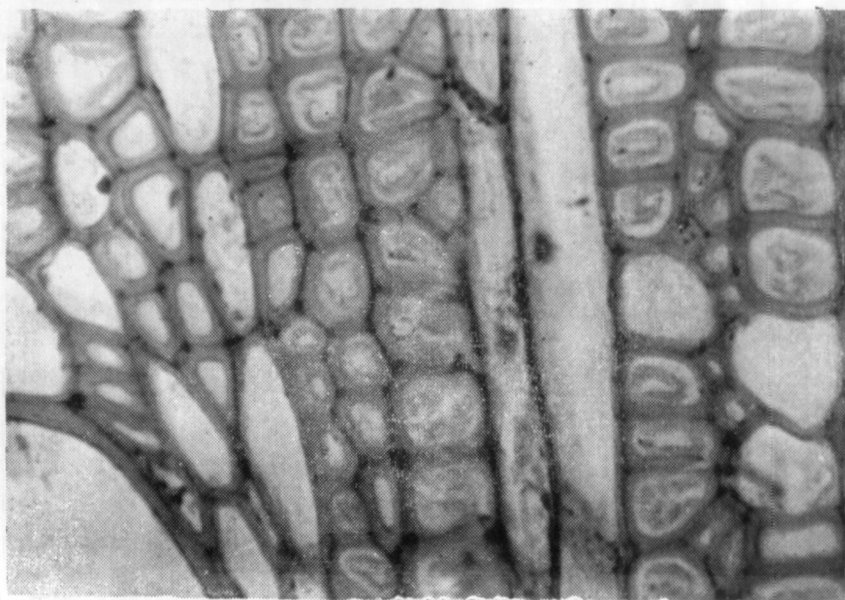


Fig. 1. Transverse section through an unstained area of a plank, x400.

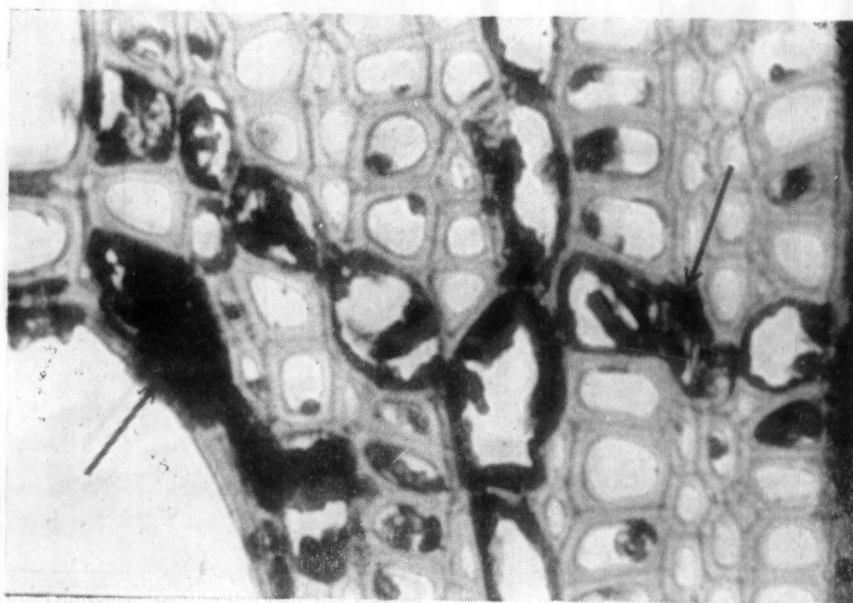


Fig. 2. Transverse section through the stained area of a plank showing discoloration and dark contents (arrowed) in the xylem and ray parenchyma, tracheid and a vessel, x400.

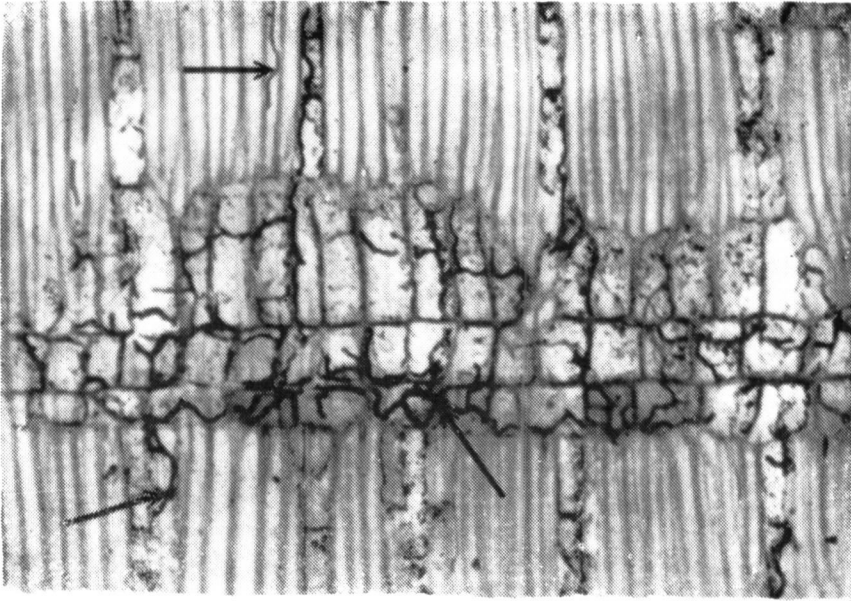


Fig. 1. Radial longitudinal section through the stained area of a plank showing the distribution of fungal hyphae (arrowed) in the xylem and ray parenchyma cells and tracheids. x100.

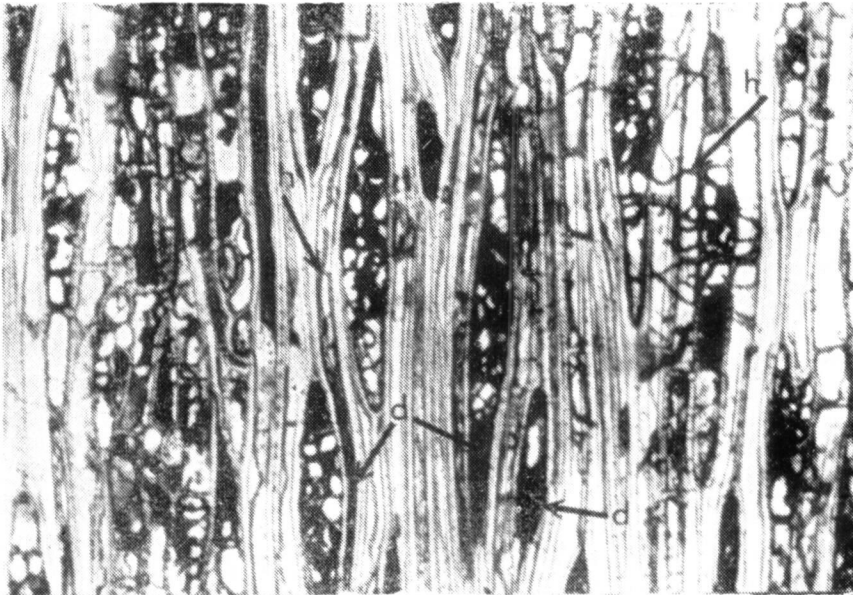


Fig. 2. Tangential longitudinal section through the stained area of a plank showing the distribution of fungal hyphae (h) and dark deposits (d) in xylem and ray parenchyma cells and tracheids. x100.