BLACK STAIN IN YELLOW CEDAR
CHAMAECYPARIS NOOTKATENSIS (D. DON) SPACH

by

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B.A., University of British Columbia, 1957

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

in the Department of
Biology and Botany

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
March, 1960

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ABSTRACT

From samples of stained yellow cedar wood, Chamaecyparis nootkatensis (D. Don) Spach, collected at three different localities on the British Columbia coast, isolation studies consistently yielded two members of the group Fungi Imperfecti. Reinoculation of these fungi into sound wood produced black stain similar to that found in nature.

In addition to these Deuteromycetes, three basidiomycetous fungi, Poria weirii Murr., Poria asiatica (Pilát) Overholts and Xeromphalina campanella (Batsch. ex Fr.) Kühner and Maire, were isolated. Of these the Poria species are believed to be first records for the host.

Studies on Petri plates revealed no marked antagonism between the two Deuteromycetes and P. weirii. The two deuteromycetous fungi gave positive reactions on gallic and tannic acid agars, demonstrating the production of the enzyme extracellular oxidase.

Two series of yellow cedar beams were respectively inoculated with macerated cultures of the Deuteromycetes by means of a special technique described. This technique produced exceptionally rapid and uniform fungal growth in comparison to that obtained under standard cultural conditions.

Inoculated beams were subjected to weight and strength loss tests, in comparison to control beams. Weight losses of 0.17 percent and 8.40 percent respectively were noted for the two fungi. No significant difference in impact modulus of rupture values was detected between test and control for either fungus.

Significance of experimental results is briefly discussed and recommendations for further study suggested.

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ACKNOWLEDGEMENT

To the Forest Products Laboratories Division, Forestry Branch, Department of Northern Affairs and National Resources, Canada, the author wishes to express her appreciation for initiation of the project and for the provision of work facilities and equipment.

Sincere thanks are extended to Dr. J. E. Bier of the Department of Biology and Botany, University of British Columbia, for his guidance, assistance and encouragement throughout the study and to Mr. R. W. Kennedy of the Faculty of Forestry, University of British Columbia, for assistance in the planning and execution of the strength tests described.

The help of all others who assisted in the various phases of this project is gratefully acknowledged.

INTRODUCTION

The possible significance of black stain in yellow cedar, Chamaecyparis nootkatensis (D. Don) Spach, was first recognized as early as 1938 when lumber concerns in the Vancouver area brought the problem to the attention of the Vancouver Laboratory of Forest Products Laboratories Division, Department of Northern Affairs and National Resources.

The main reason for concern on the part of commercial interests revolved around the effect of the stain on the strength properties of the lumber. Whether to grade stained wood as decayed or sound was a point of debate amongst lumber graders.

Unlike many stains, black stain in yellow cedar is present in the heartwood of living trees and is easily recognizable at the time of cutting.

Members of the Vancouver Forest Products Laboratory attempted to isolate the causal organism or organisms but were unable to obtain consistent results. Plantings of samples of stained wood on agar media were largely unsuccessful.

In May 1958 investigation of this problem was reopened, responsibility being delegated to the writer who, from May until September 1958, was employed as a Student Assistant at the Vancouver Laboratory. Under

the aegis of the University of British Columbia Department of Botany, and with the continued use of facilities at the Forest Products Laboratory, further work was carried out during the winter of 1958-59 with a view to summarizing the experimental results in thesis form.

The objectives of this study were: (1) To determine the cause of black stain in yellow cedar; (2) To ascertain the effect of this stain on the strength properties of the wood.

LITERATURE REVIEW

Information on stain-producing organisms in wood is not extensive and in the main is devoted to sapwood stains. In extensive work on stains in Sweden, Lagerburg et al (1927 : 244) described a blue heartwood stain in living Norway spruce (Picea excelsa Link.) from which the fungus Hormodendrum microsporum Lagerburg and Melin was isolated. Christensen and Kaufert (1941) reported a blue staining fungus inhabiting the heartwood of certain conifers in the Lake States. This organism, though unnamed, was compared with the Swedish fungus mentioned above. A heartwood blue stain of balsam fir (Abies balsamea (L.) Mill) and white spruce (Picea glauca (Moench) Voss) in Quebec was described by Crowell (1940). In the few samples available the heartwood was almost entirely stained. Though the staining organism was not identified it was stated that "It is probably not the same as that causing blue stain of spruce in Norway".

Hubert (1921) in a discussion of sap stain fungi, noted the occurrence of Ceratostomella spp. on northern white cedar (Thuja occidentalis L.). Illustrations accompanying his description show the typical penetration of cell walls by broad, dark-coloured hyphae. The general status of knowledge on heartwood stains was

summarized by Hubert (1931). He noted that blue stain organisms had been found in the heartwood of certain woods. Though tree species were not specified, it was stated that the woods involved were usually of a type showing little difference between heartwood and sapwood and in which the heartwood possessed a fairly high moisture content. More recently, Fritz (1951) observed that some heartwood discolourations are produced by staining fungi which are not known to cause rot in their final stages and which originate in the standing tree. In contrast to this type of stain, she mentioned sapwood stains which develop in felled timber.

With specific reference to yellow cedar, Perry (1954) has said, "Lumber from some areas shows frequent black unsound or loose knots with black stains spreading to adjacent areas." The last mentioned constituted the sole published reference on black stain in yellow cedar.

THE HOST

Yellow cedar (Chamaecyparis nootkatensis (D. Don) Spach) in Canada is confined to the west slope of the Coast Range of mountains in British Columbia and to the offshore islands from Alaska southward. In the south it is found at elevations of 2,000 to 5,000 feet. To the north it descends gradually until it reaches sea level at Knight Inlet. Usually it is associated with amabilis fir (Abies amabilis (Dougl.) Forb.) and western hemlock (Tsuga heterophylla (Raf.) Sarg.) occurring as scattered stems and patches. Only rarely is it found in pure stands (Canada, Department of Mines and Resources, 1951).

Yellow cedar wood has exceptionally desirable working qualities, being fine grained, fairly hard and strong, and possessing a very low shrinkage factor. It exhibits high durability in situations favouring decay and is considered valuable for use in conditions of exposure, notably for window frames, exterior doors and in boat construction (Jenkins et al, 1951).

The increased use of this tree species as sawn timber in the coastal region of British Columbia is evidenced by the fact that the total annual cut for that area has doubled in the period from 1951 to 1956 (Province of British Columbia, 1952, 1957).

PRESENTATION OF DATA

The Fungi

Deuteromycetes

In the course of isolation work, two fungi belonging to the class Fungi Imperfecti were consistently isolated. It was observed that these fungi grew directly out of plantings of stained wood. Cultures have been submitted to a specialist in the taxonomy of Fungi Imperfecti, but to date neither has been identified. For this reason, they have been arbitrarily designated as Fungus "A" (Figure 1) and Fungus "C" (Figure 2) and henceforth in this paper will be so called.

The two organisms demonstrate somewhat different cultural characteristics, permitting them to be easily differentiated. Both fungi appear very black in old cultures, while Fungus "C" is a slate grey colour in young cultures. Fungus "A" is jet black in colour throughout, even in young cultures.

PLATE I

Figure 1. Fungus "A" two months old culture on malt agar. X0.9

Figure 2. Fungus "C" two months old culture on malt agar. X0.9

PLATE I

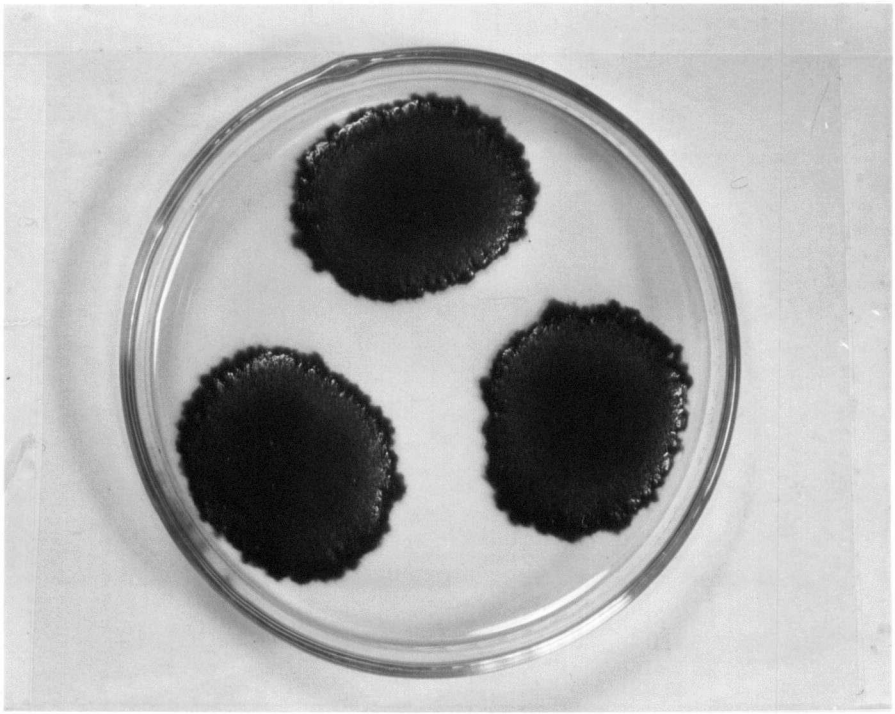

Figure 1

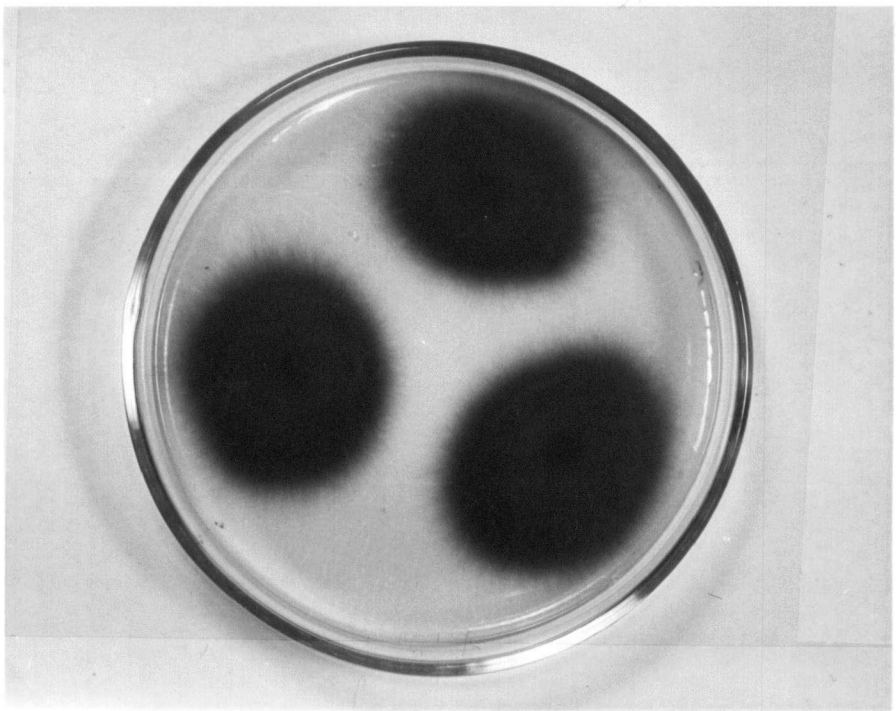

Figure 2

Cultural Characters of Fungus "A"

Growth characters

Mat jet black, opaque; aerial mycelium raised almost to the limit of growth, fine woolly, uniform. Submerged mycelium growing slightly beyond surface of colony. Reverse unchanged. Colonies on malt agar plates attaining a diameter of 4.5 centimeters in six weeks. On gallic acid agar,[1] reaction strong; trace of growth in one week. On tannic acid agar, reaction moderately strong with a trace of growth over the inoculum in one week.

Hyphal characters

Hyphae amber-brown coloured, simple septate, frequently branched at and between septa. Some suggestion of swellings or expansions on hyphae of advancing zone with some hyphal tips having a slightly clavate appearance. Hyphal width 2.4 to 3.2 microns. Aerial mycelium of older growth (three months old cultures) producing abundant pycnidia (Figures 3, 4 and 5) containing conidia (3.14 by 1.57 microns) which are cylindrical and hyaline.

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1 Gallic and Tannic Acid agars prepared by adding 0.5 percent of these acids, respectively, to malt agar in flasks immediately prior to pouring into Petri plates.

PLATE II

Figure 3. Fungus "A": camera lucida drawings showing various developmental stages of pycnidia.

PLATE II

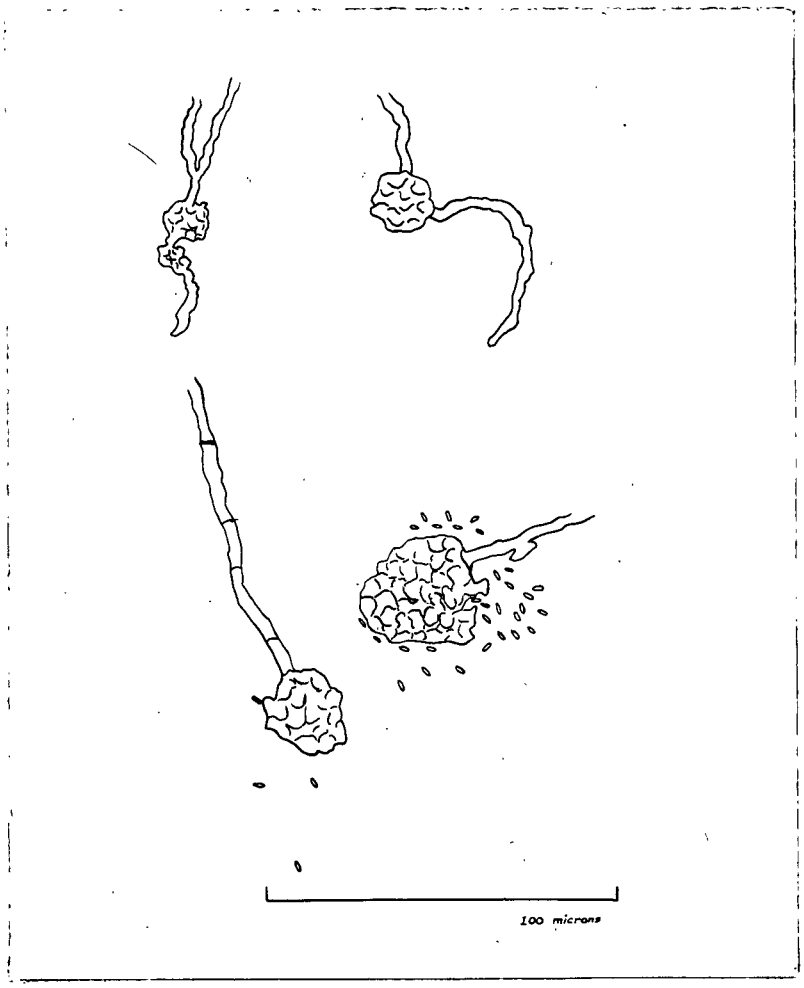

Figure 3

PLATE III

Figure 4. Fungus "A". Pycnidial formation. X500.

Figure 5. Fungus "A". Ruptured pycnidium releasing conidia. X775.

PLATE III

Figure 4

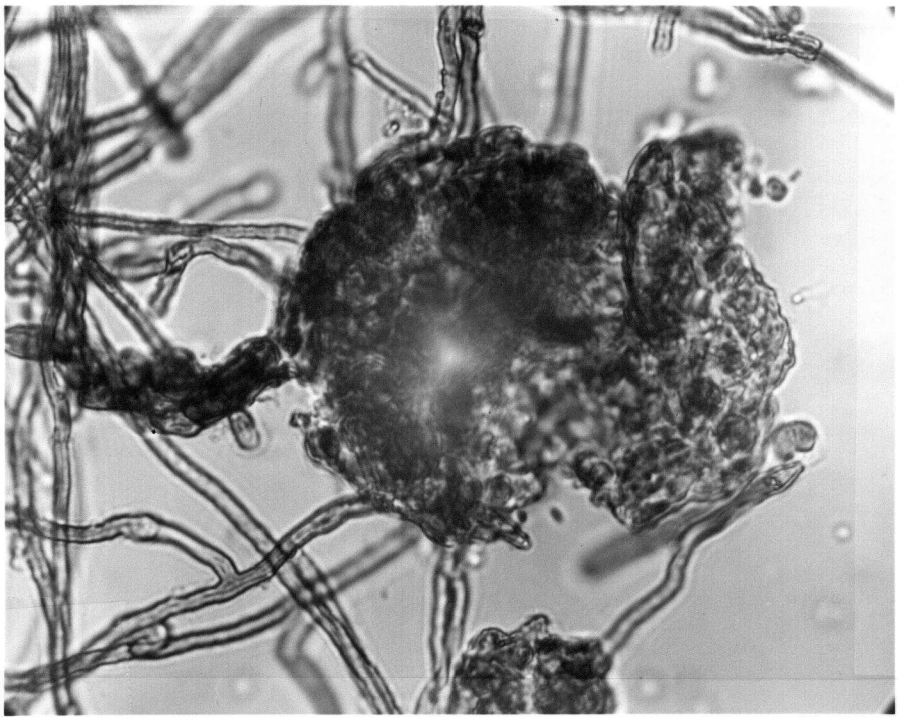

Figure 5

On the basis of its pycnidia, this fungus can be placed in the Form family Sphaeropsidaceae, Form order Sphaeropsidales, Form class Deuteromycetes.

Cultural Characters of Fungus "C"

Growth characters

Mat at first slate grey, opaque, becoming black in older cultures. Advancing zone even, aerial mycelium raised to the limit of growth; woolly to fine cottony, uniform. Reverse unchanged. Colonies on malt agar plates attaining a diameter of 4.0 cm. in six weeks. On gallic acid agar, reaction strong; no growth in one week. On tannic acid agar, reaction strong with a trace of growth over the inoculum in one week.

Hyphal characters

Hyphae amber-brown coloured, simple septate, infrequently branched (Figure 6). Hyphae 3.2 to 3.9 microns in width. Many dark, non staining, terminal, multiseptate conidial structures (Figure 7) up to 15.7 microns in diameter occurring singly, and occasional swollen terminal cells (up to 18.8 microns in diameter), the contents of which stain in phloxine, were observed in older cultures.

PLATE IV

Figure 6. Fungus "C". Hyphal characteristics. X290.

Figure 7. Fungus "C". Multiseptate conidial structure common in older cultures. X775.

PLATE IV

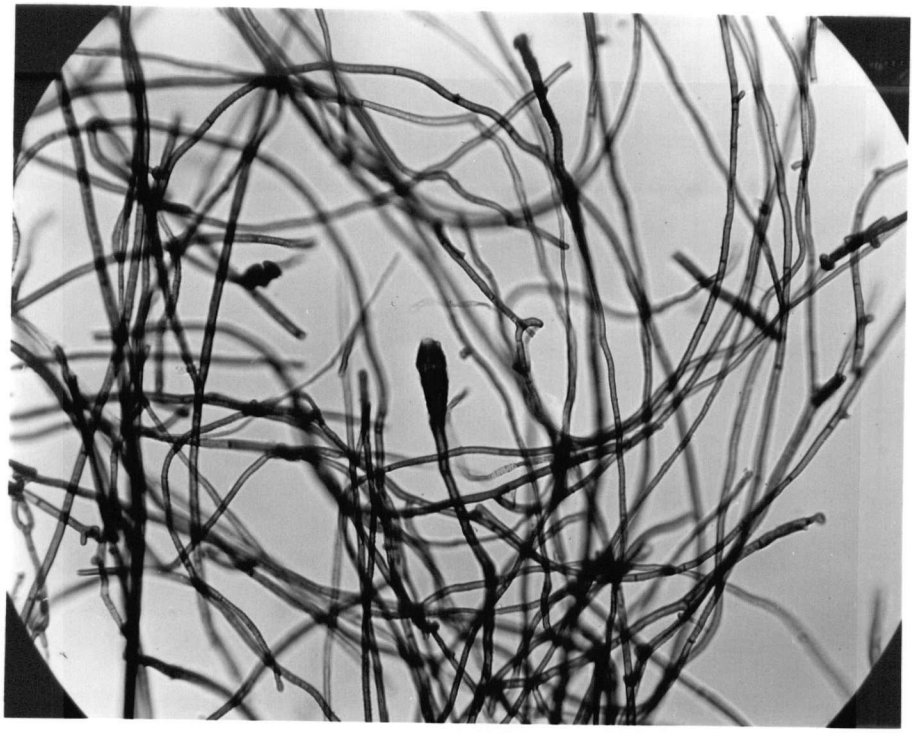

Figure 6

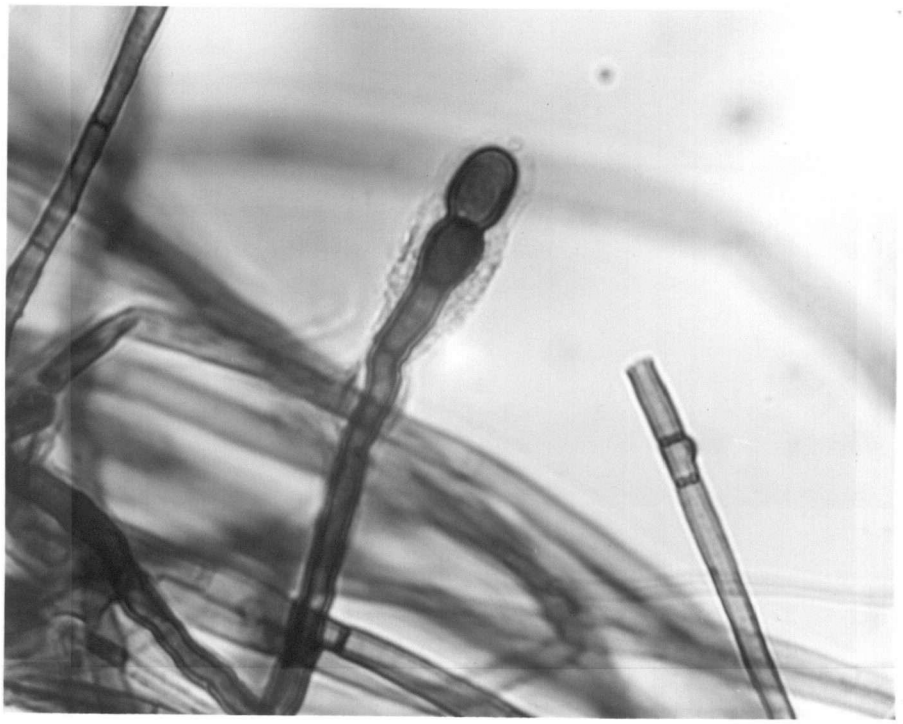

Figure 7

Basidiomycetes

Three basidiomycetous fungi were isolated in the course of this study: Poria weirii Murr., Poria asiatica (Pilát) Overholts and Xeromphalina campanella (Batsch. ex Fr.) Kühner and Maire.[2] P. weirii and P. asiatica are believed to be new records for this host. X. campanella was reported previously (Shaw, 1958 : 31).

Collection Localities

The material was collected from three areas on the British Columbia coast: Sechelt Peninsula; Harrison Lake; Buckley Bay, Vancouver Island.

Sechelt Peninsula

The stand where material was collected was a mixture of Douglas-fir (Pseudotsuga menziesii (Mirbel) Franco), western hemlock, western red cedar (Thuja plicata Donn) and yellow cedar growing in a swampy pocket on a hillside at about 3,500 feet above sea level. The sample trees were from 275 to 350 years of age and from 20 to 32 inches in diameter at breast height

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2 Nobles (1948) was the basis of the writer's identification of these fungi. Identity of the Poria species was confirmed by Dr. M. K. Nobles, Plant Research Institute, Department of Agriculture, Canada.

(4.5 feet above ground level). Most of them exhibited "dryside" about 8 to 10 feet above the ground, possibly as a result of fire scars. Several types of rot were evident in stumps; but black stain was only occasionally noted, either in association with advanced decay or in the outer heartwood of stumps in apparently sound wood. Stain was usually prominent in the top of the first log or beyond, from 40 to 60 feet above the ground.

Harrison Lake

The samples from this area were taken from timber that had been felled three weeks previous to collection as part of a logging operation of Canadian Forest Products Limited. The site was well irrigated and was located on a very steep east-north-east facing slope at about 3,500 feet above sea level. The stand was predominantly yellow cedar, amabilis fir and mountain hemlock (Tsuga mertensiana (Bong.) Carr.). Yellow cedar attained good growth on this site, some stumps being at least 4 feet in diameter. Butt rot was not prevalent, but there was a conspicuous yellow ring rot in the trunks of many of these trees. Black stain appeared to accompany this ring rot, but in many cases stain was observed in wood that showed no signs of decay.

Buckley Bay, Vancouver Island

This group of samples was brought into the laboratory by the British Columbia Forest Service. This site is similar to that of Sechelt Peninsula, the stand composition being mainly yellow cedar, western hemlock, western red cedar, mountain hemlock, western white pine (Pinus monticola Dougl.) and some Douglas-fir. Sites in this area are often flat and located in excess of 2,000 feet above sea level. Samples from seven trees were submitted, one of these being a plank that had been sawn from a butt log. This sample contained a brown cubical rot in addition to black heartwood stain. The log from which this plank had been recently sawn was from a tree felled approximately six months previously.

Description of Defects

Black stain appeared very frequently in the heartwood of the trees examined. Several forms were observed, the most common one being prominent longitudinal black streaks made up of short black lines, running in a radial direction in the wood (Figure 8). The location of the stain relative to the center of the tree was variable. Occasionally most of the heartwood

was stained a dull grey-black (Figure 9). Knots were frequently blackened, but no consistent relationship between knots and stained heartwood was apparent. In some instances, the stain was accompanied by obviously decayed wood; but in others it occurred in apparently sound wood (Figure 10).

Several types of rot were noted in the sample trees. These included the following.

1. Yellow laminated heartrot: associated with P. weirii
2. Brown cubical butt rot: associated with P. asiatica
3. Firm red-brown heartwood discolouration: associated with X. campanella

Isolation Studies

Methods

Fresh wood samples were used for the isolation work. Inoculations on three types of media were made from all of the samples in an effort to provide for a range of nutritional requirements. The media used were; standard Bacto-difco malt agar, standard Bacto-difco potato dextrose agar and acidified malt agar.

PLATE V

Figure 8. Yellow cedar showing longitudinal streak of black stain (center) and adjacent incipient decay.

Figure 9. Yellow cedar showing discolouration of almost entire heartwood area.

PLATE V

Figure 8

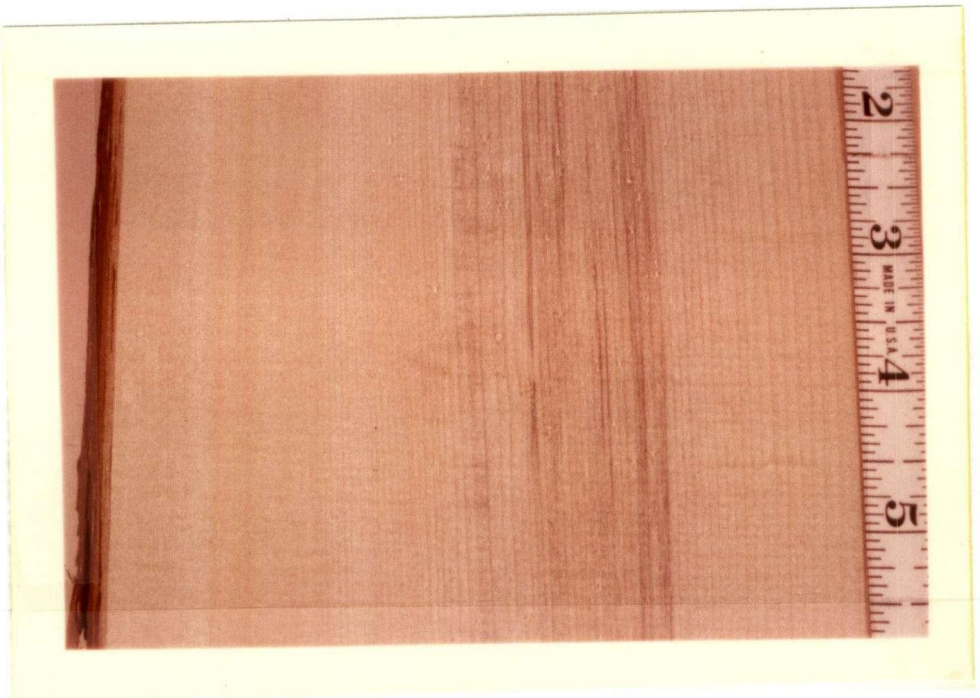

Figure 9

PLATE VI

Figure 10. Conspicuous black stain in apparently sound yellow cedar heartwood.

PLATE VI

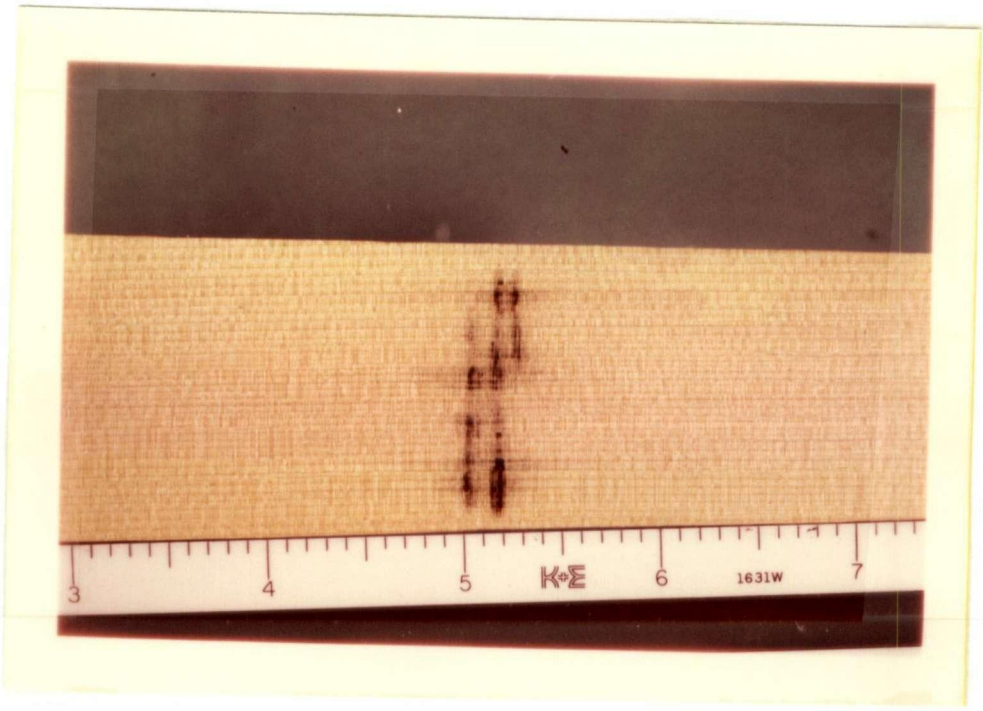

Figure 10

Because difficulty had been encountered in previous isolation work with yellow cedar, it was decided to explore the usefulness of malt agar acidified with lactic acid to pH of 4.5, in addition to the two previously noted media. All of these media were hardened at 2 percent agar concentration. The malt and potato dextrose agars were poured into culture tubes and slanted, and the acidified malt agar was poured into Petri dishes.

Inoculations were made in the conventional manner by aseptically placing small pieces of wood on the media. Plantings were taken from the different stages of stain and decay on the samples in an attempt to explore all the possibilities of fungal infection. The plantings were selected after splitting the bolts of wood to expose areas of stain and/or decay that would be free from surface contamination. Several plantings were made from each situation so exposed. Two hundred sixty four areas of stained and/or decayed wood were explored in this manner on the 24 samples, giving a total of 600 culture tubes and 150 Petri plates (three inocula per plate) of attempted cultures.

Results of Isolation Studies

The results of these isolation studies are summarized in Table I.

The three types of media used appeared equally suitable for isolation work, fungal growth being obtained in roughly the same proportion and variety on each.

Of the stained and/or decayed areas from which cultures were obtained, 42 percent yielded Fungus "C", 28 percent Fungus "A", 5 percent P. weirii, about 1.5 percent P. asiatica and about 1.5 percent X. campanella. About 3 percent of the stained and/or decayed areas yielded a mixture of Fungus "A" and another imperfect fungus. The remainder of growth was made up of a heterogeneous group of unidentified Fungi Imperfecti.

TABLE I

FUNGI ISOLATED IN A STUDY OF BLACK STAIN IN YELLOW CEDAR FROM THREE LOCALITIES

	Sechelt Peninsula	Harrison Lake	Buckley Bay, V.I.
Number of trees sampled*	7	8	8
Situations from which cultures were attempted	63	147	54
Situations producing fungal growth	56	104	42
Fungus "A"			
Number	17	31	11
Percent**	28	29	25
Fungus "C"			
Number	21	49	19
Percent	35	46	42
Fungus "A", Mixed Culture			
Number	7	--	--
Percent	12	--	--
Poria weirii			
Number	10	--	--
Percent	17	--	--
Xeromphalina campanella			
Number	--	--	3
Percent	--	--	7
Poria asiatica			
Number	--	--	3
Percent	--	--	7
Unidentified Fungi Imperfecti			
Number	5	26	9
Percent	8	25	19

*One bolt per tree.

**Percentage of the total "situation-fungus-growth types." Because of the isolation of more than one fungus from some situations, the total for Sechelt Peninsula, for example, is 60 and not 56: i.e., if a situation yielded Fungus "A" and P. weirii, it would be represented in both instances.

Cultural Interactions of Fungi

Exploration of the possibilities of inhibition or stimulation between cultures of Fungus "A", Fungus "C", Poria weirii and an undesignated imperfect fungus (this fungus was occasionally isolated during culture work) was carried out. Small squares of vigorously growing cultures of these fungi were inoculated on Petri dishes of malt agar. The inocula were placed at the periphery of the culture medium as far apart as the combinations permitted. Inoculations were made of the four fungi in all possible combinations and singly. The series was replicated four times, making a total of 60 plates. The cultures were grown in the dark at room temperature for six weeks, measurements and descriptions being recorded at weekly intervals. At the end of this period, measurements of the fungi plated singly were compared with those plated in combination.

No marked synergistic or antagonistic effects were observed, but the growth of P. weirii appeared to be slightly inhibited by both Fungus "C" and the undesignated imperfect fungus. Fungus "A" and Fungus "C" grew together with no effect of inhibition or

stimulation. These two fungi were subsequently replated (with inocula in closer proximity than in this series) to illustrate this fact (Figure 11).

Histological Studies

Naturally Stained Wood

Blocks of wood exhibiting typical black stain were prepared for microtome sectioning. Unstained sections were mounted in glycerin and examined under a microscope. Numerous amber-brown, broad, septate hyphae were observed (Figure 12). Oil droplets were frequent in the hyphal contents, and many of the hyphal walls were somewhat rough in appearance. In some cases structures resembling adpressoria (Lagerburg *et al*, 1928 : 172) were present.

These hyphae typically penetrated the tracheid walls and grew in a transverse direction (Figure 13). Marked constriction was noted when a hypha penetrated a tracheid wall with immediate widening on the other side of the wall. A single hypha could be traced passing through many tracheids in a straight line. Some similar hyphae were also found growing in a longitudinal direction within individual tracheids. The mycelium was occasionally found within the ray cells

PLATE VII

Figure 11. Fungus "A" and Fungus "C" growing together without antagonism in two months old culture on malt agar. X0.9

PLATE VII

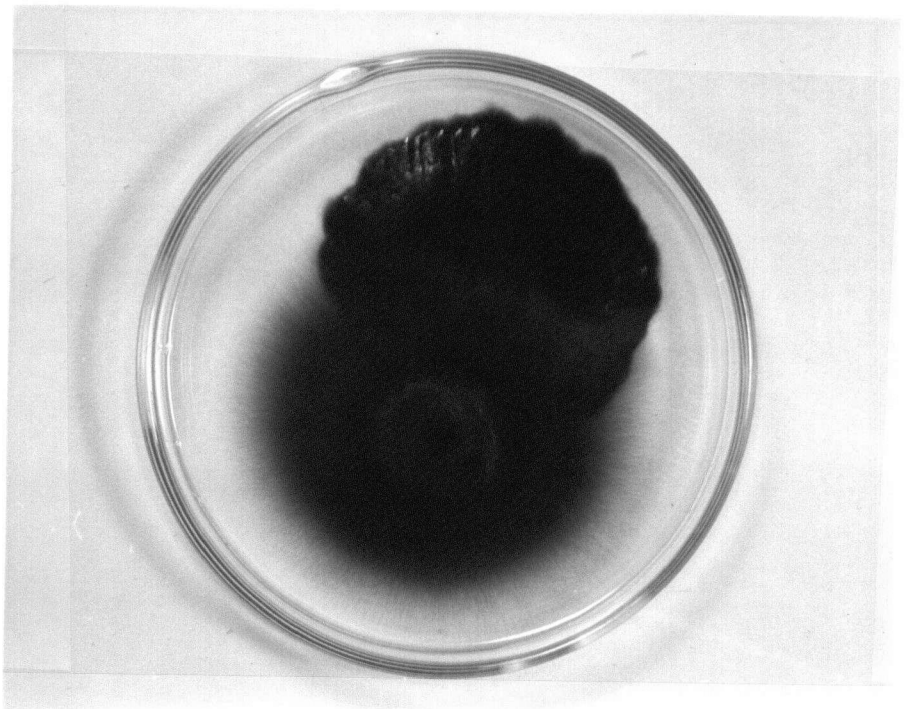

Figure 11

PLATE VIII

Figure 12. Tangential section of "stained" yellow cedar showing dark mycelium. X150

Figure 13. Radial section of "stained" yellow cedar showing dark mycelium passing through heartwood tracheids. X625

PLATE VIII

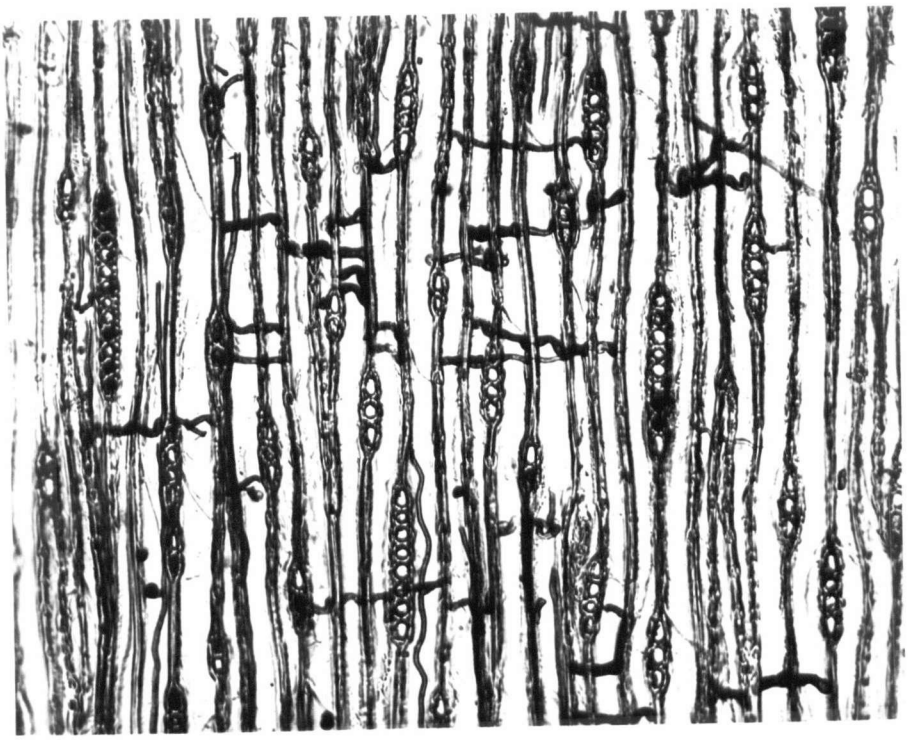

Figure 12

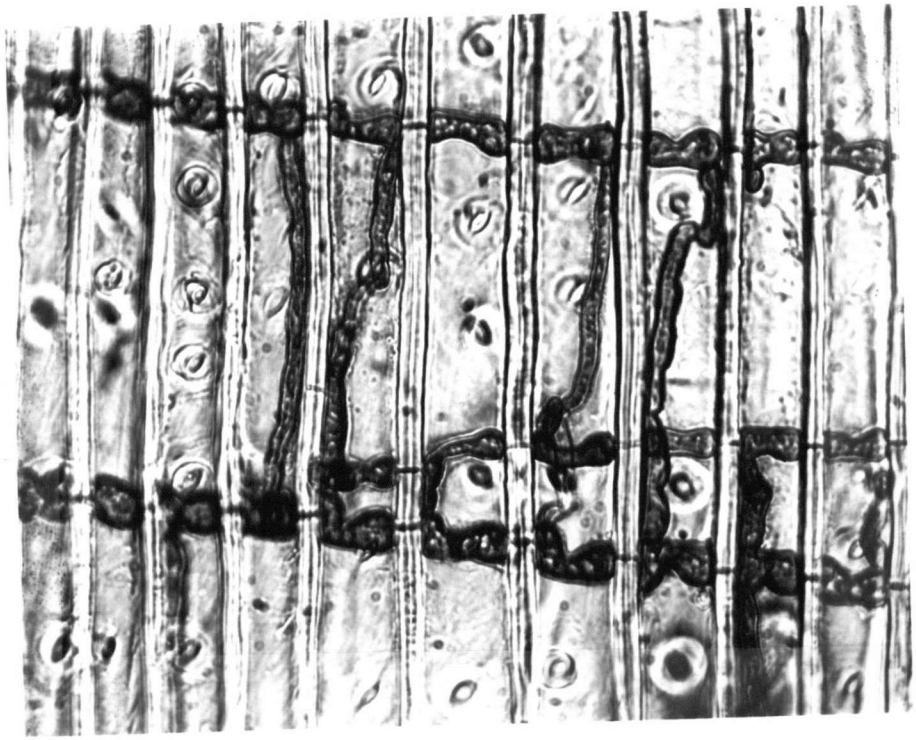

Figure 13

but did not generally appear to follow the rays, in contrast to most sapwood-staining organisms.

Narrow hyaline hyphae were also occasionally observed. These may have been of typical decay mycelium or the earlier stages of stain hyphae.

Inoculated Wood

Two series of yellow cedar beams were inoculated (see page 33) with Fungus "A" and Fungus "C" respectively. These beams were incubated for nine weeks at room temperature (about 22° C) after which they were removed from the culture bottles and placed in an "Airegulator"[3] to bring to a uniform moisture content before testing for possible losses of strength and weight. Subsequent to these tests, the beams were oven dried; and microtome sections were made from some of the beams of both series to observe the habit of the fungi when inoculated into wood under laboratory conditions.

In the case of the beams inoculated with Fungus "C", the stain hyphae could be found penetrating to the center of the beams in the characteristic manner (Figure 14). As in naturally stained wood, a single

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3 Custom made machine for maintaining constant conditions of temperature and humidity, located at Forest Products Laboratory, Vancouver.

hypha could be seen passing through many tracheids in a direct line (Figure 15). Macroscopically, the wood had a greyish color; and microscopically, hyphae were found in great abundance in the tracheids (Figures 16, 17).

Examination of beams from the series inoculated with Fungus "A" did not show as many hyphae in the tracheids as in the "C" series (Figure 18). Stain hyphae were found penetrating tracheids in the typical manner but usually in the outer part of the beams and rarely in the center (Figure 19).

PLATE IX

Figure 14. Fungus "C". Section from inoculated beam with typical hyphal penetration. X200

Figure 15. Fungus "C". Section from inoculated beam: hypha penetrating many tracheids in a direct line. Stain hyphae also obvious in ray cells. X200

PLATE IX

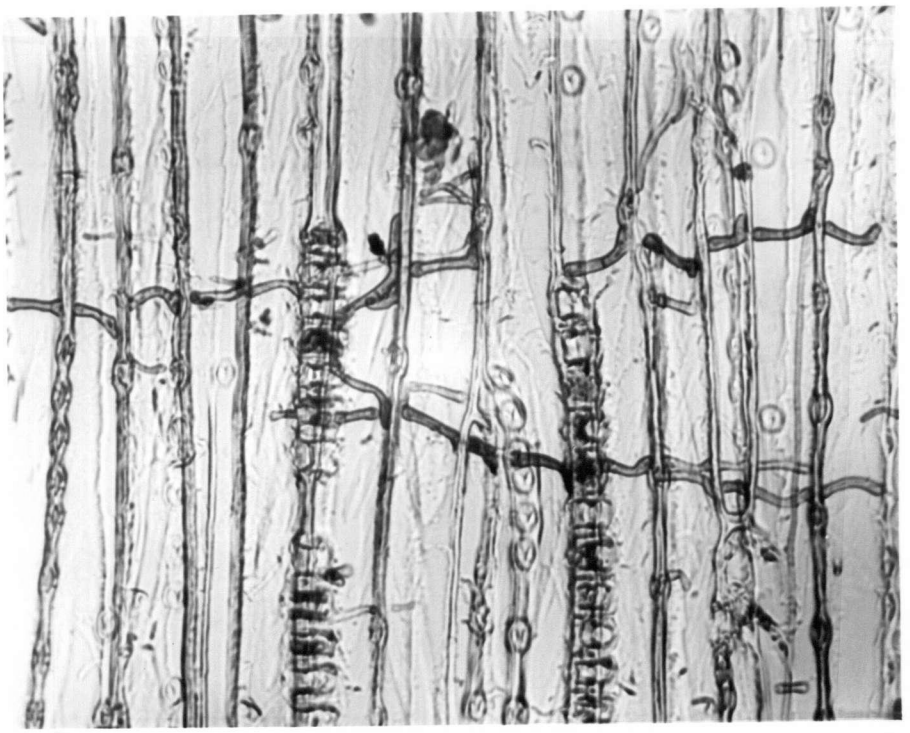

Figure 14

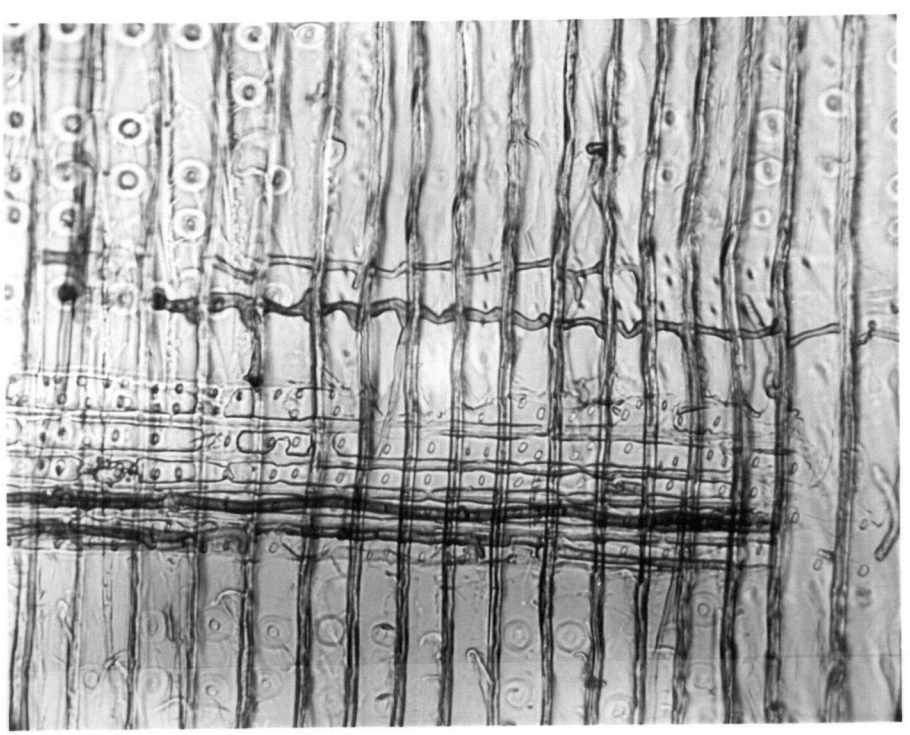

Figure 15

PLATE X

Figure 16. Fungus "C". Section from inoculated beam: penetration of yellow cedar ray tracheids by stain hyphae. X775

Figure 17. Fungus "C". Section from inoculated beam: penetration of tracheid walls by stain hyphae. Note adpressoria. X900

PLATE X

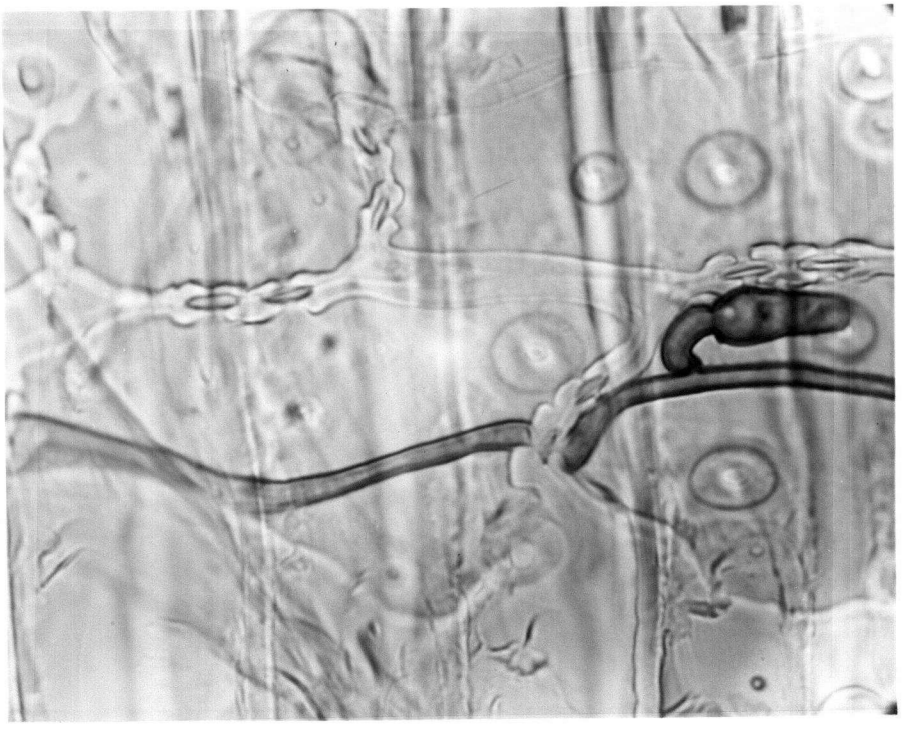

Figure 16

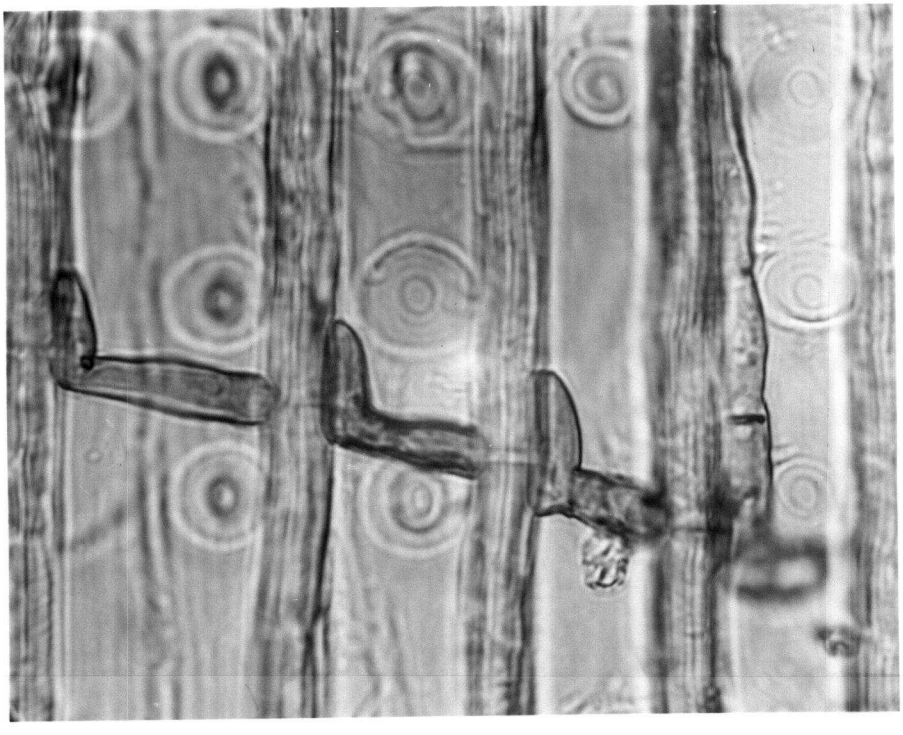

Figure 17

PLATE XI

Figure 18. Fungus "A". Section from inoculated beam: stain hyphae within the tracheids. X250

Figure 19. Fungus "A". Section fron inoculated beam: a few stain hyphae penetrating tracheids. X250

PLATE XI

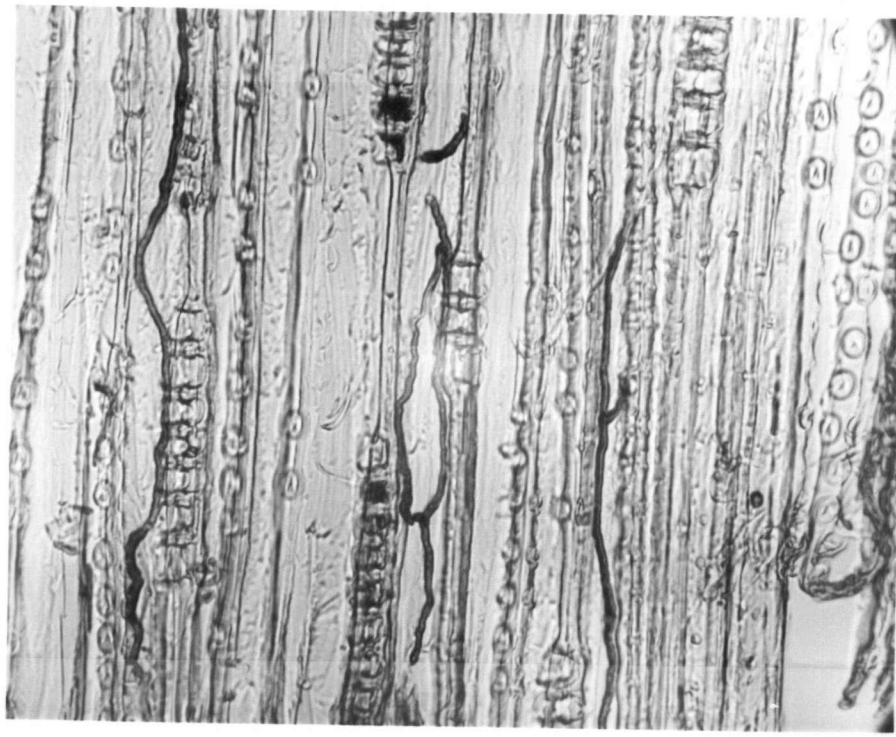

Figure 18

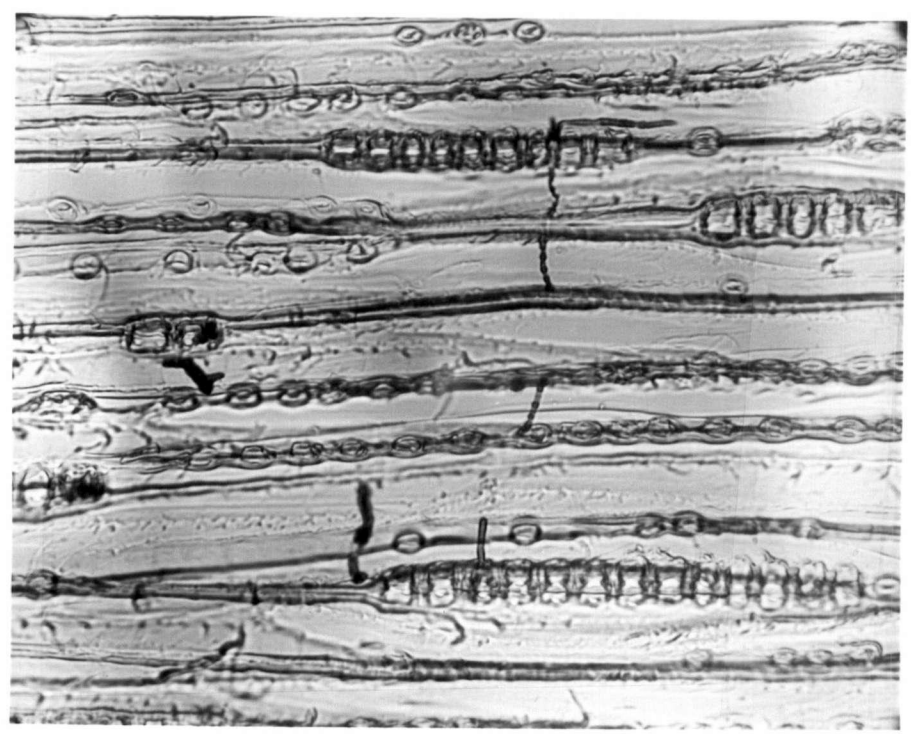

Figure 19

Strength and Weight Loss Tests

Method of Preparing Beams

A flat-sawn, clear, yellow cedar board approximately 8 inches by 3/4 inch by 5 feet was obtained from Kitsilano Lumber Company, Vancouver. This board was split into two pieces, each approximately 0.30 inches in thickness. One side of the board was used for each of the two series of inoculations and matching controls. For each series the halfboard was ripped into approximately 0.25-inch wide strips which were subsequently planed to a nearly uniform thickness of 0.20 inches. The strips were cut into four-inch beams and these were immediately numbered. Alternate beams within a single strip were chosen for "test" and "control" pieces (Figure 20) so that they would be end matched and a comparison could be made between beams from the same annual growth rings, thus avoiding some of the natural variation of wood properties within a single tree. Light sanding of the ends of the samples was done with a fine sandpaper to remove any loose splinters. Preparatory to weighing, the beams were placed in the "Airegulator" to bring them to a uniform moisture content. After two days and daily thereafter, dummy beams were taken out,

PLATE XII

Figure 20. Yellow cedar board. Diagram showing relative placement of test and control beams within strip. Not to scale.

PLATE XII

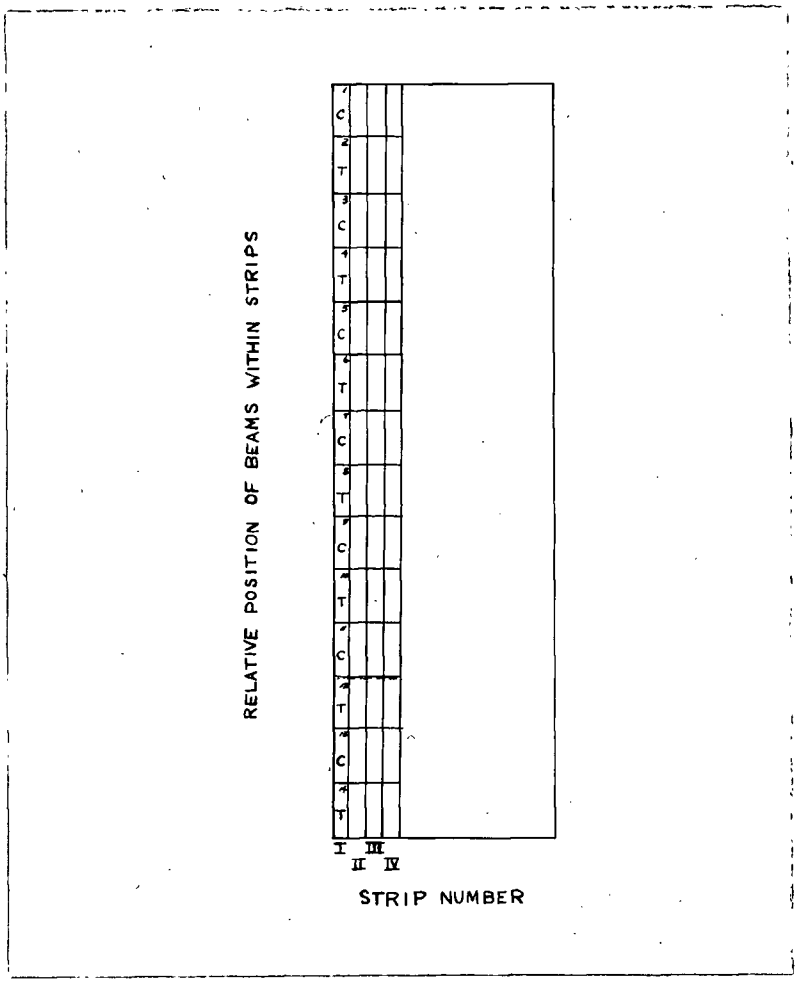

Figure 20

weighed, oven dried and reweighed to check moisture content. After one week, during which time the moisture content of the beams was found to have reached equilibrium, the beams were removed in small groups to polyethylene bags and quickly weighed. The test pieces were weighed individually, and the controls were weighed in the two groups in which they were to be used. Before placing in the inoculation chambers, all of the beams were placed in an autoclave under flowing steam (without pressure) for 25 minutes for the purpose of eliminating surface contamination.

Method of Preparing Culture Bottles

Pyrex "milk dilution bottles" of outside dimensions 5.5 by 1.75 by 1.75 inches were used as inoculation chambers for the beams. Thirty cubic centimeters of malt agar were placed in each bottle which was then closed with a cotton plug to allow aeration. The bottles were sterilized in an autoclave for 20 minutes at 15 pounds pressure per square inch. They were then placed in a horizontal position to provide the maximum culture medium surface upon cooling.

Because the two stain fungi have very slow growth rates, a special method of inoculation of the culture medium was devised that produced good growth

over the entire agar surface in a week. Two-month-old Petri plate cultures of Fungus "A" and Fungus "C", growing on malt agar, respectively, were macerated in a sterile Waring blendor with sterile distilled water. The mycelial colonies were scraped from the agar surface with a sterile scalpel, and care was taken to include as little of the substrate as possible. In each case, the blendor cylinder was approximately one-third to one-half filled; and blending was done for 1.5 minutes.

Two bent glass rods were placed aseptically on the agar in each bottle (Figure 21). The rods were heated slightly so that they would adhere to the surface of the medium. These were used to support the beams in contact with the mycelial mat, but not touching the agar surface.

Using a sterile pipette, 5 cubic centimeters of the appropriate macerated fungus suspension was placed in each upright bottle. The bottles were then tipped so that the suspension spread evenly over the agar surface. Vigorous uniform growth was obtained in all the bottles in about one week (Figure 22), illustrating the usefulness of this method for obtaining uniform growth of slow-growing fungi on an agar surface. Care in observing aseptic technique throughout the experiment completely obviated the

PLATE XIII

Figure 21. Culture bottle showing glass rods in place on medium. X 3/5

Figure 22. Culture bottle inoculated with Fungus "A". X 3/5

PLATE XIII

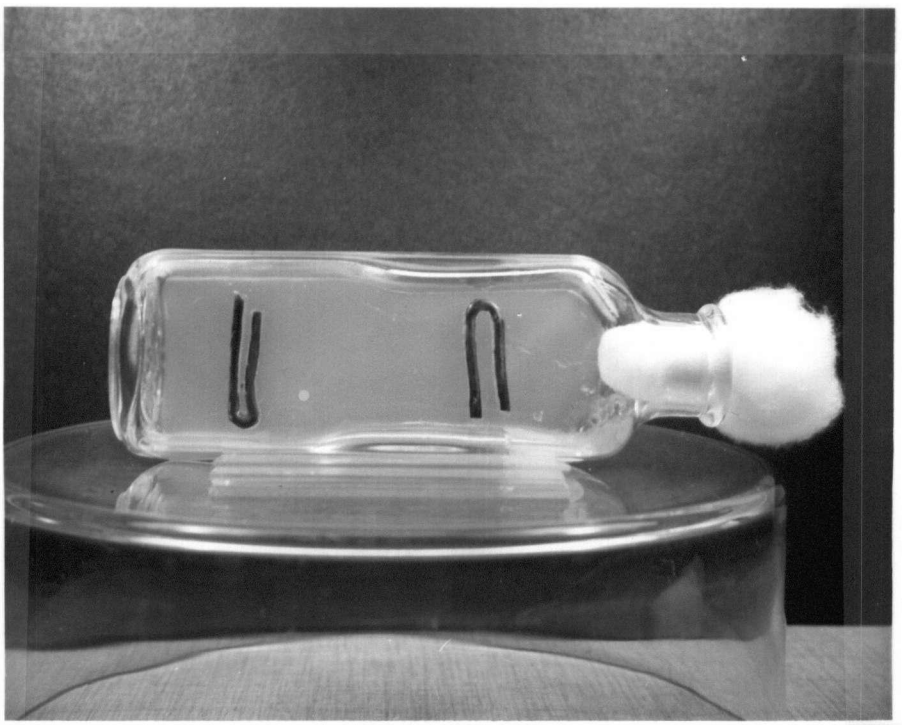

Figure 21

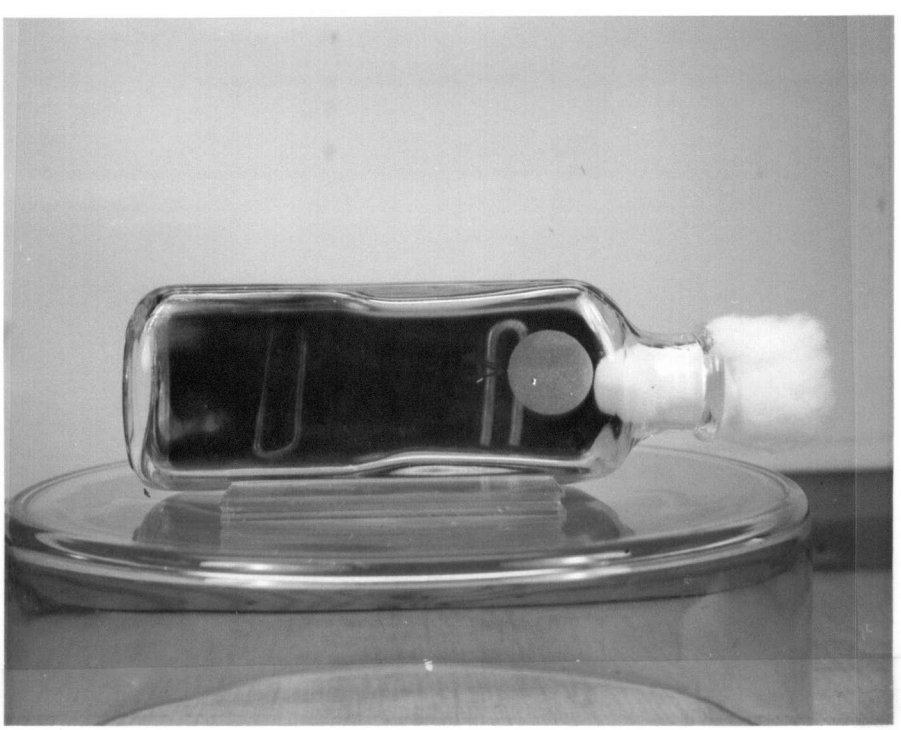

Figure 22

common problem of air-borne contaminants.

Before being introduced into its culture bottle, each beam was dipped in a macerated suspension of the appropriate fungus (Figure 23). This was done in an attempt to accelerate infection of the beams on all sides. Three beams were placed in each bottle (Figures 24, 25). The control beams for each series were treated in exactly the same manner as the test beams, except that the agar in the control bottles was not subjected to any fungus inoculation (Figure 26). Both series (Fungus "A" and Fungus "C") and their controls were incubated at room temperature in humidity chambers for nine weeks.

PLATE XIV

Figure 23. Method of dipping yellow cedar beams into macerated fungus suspension.

Figure 24. Introduction of beams into bottles. X½

PLATE XIV

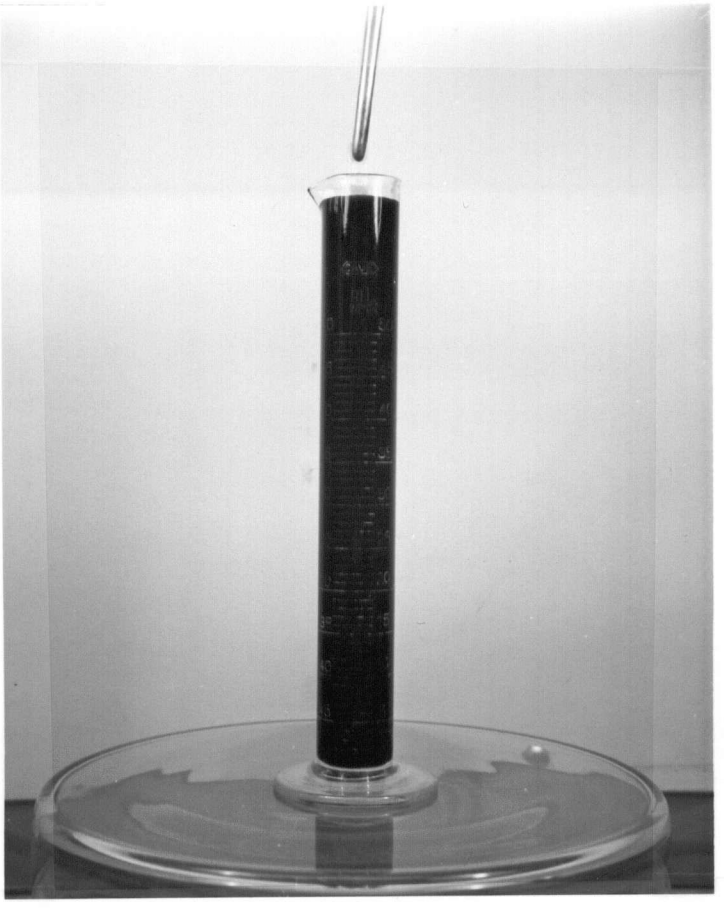

Figure 23

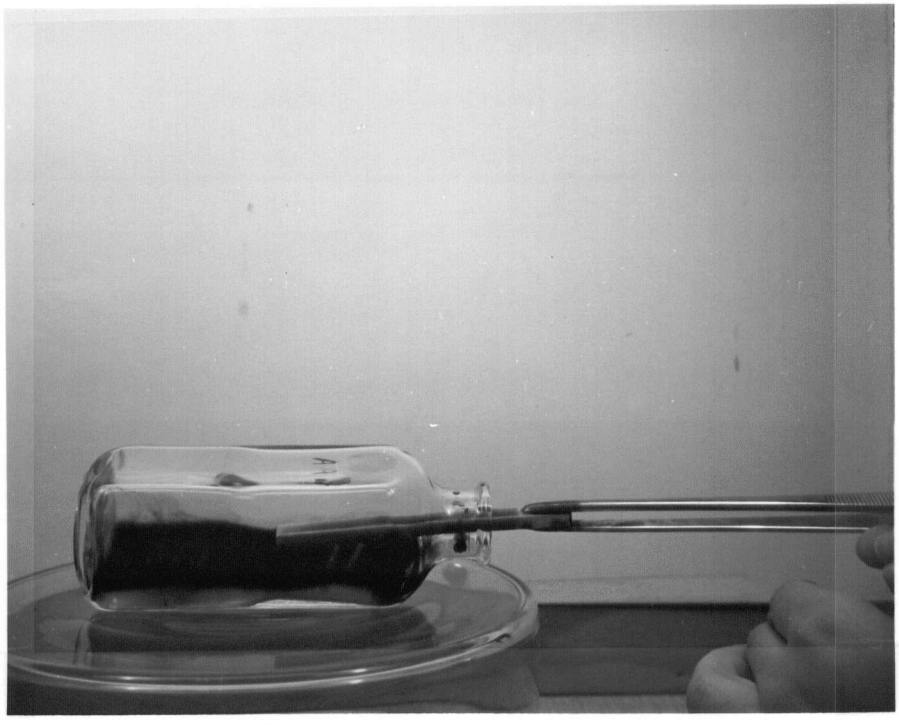

Figure 24

PLATE XV

Figure 25. Test bottle prepared for incubation. X 3/5

Figure 26. Control bottle prepared for incubation. X 3/5

PLATE XV

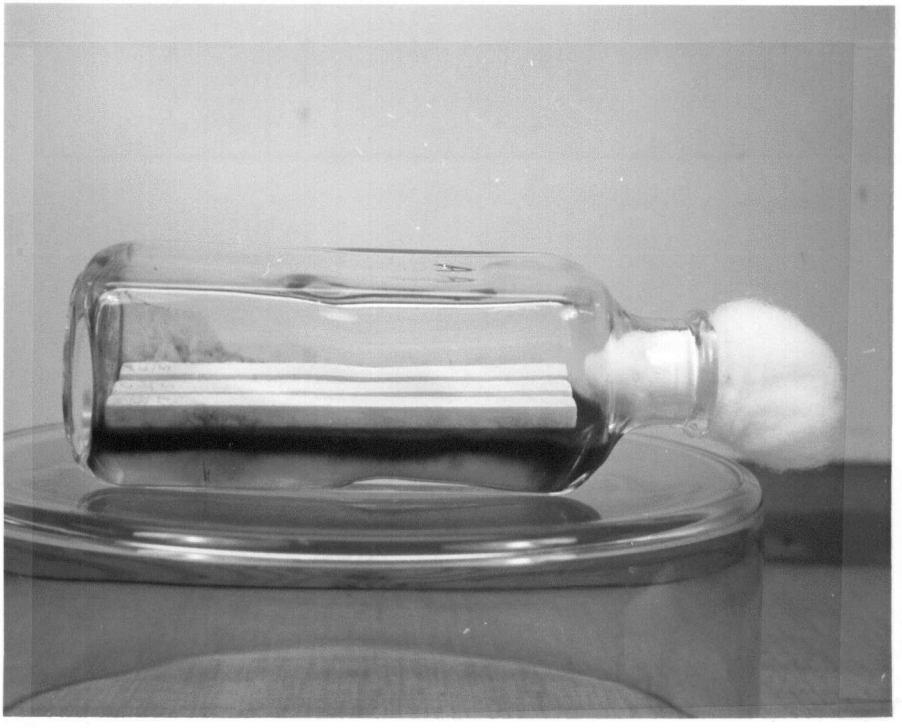

Figure 25

Figure 26

Test Procedure

At the end of the nine-week period, all of the beams were removed from the bottles; and the surface mycelium was gently removed from the test beams with a nylon brush. Both test series and controls were then placed in the "Airegulator" to bring them to a uniform moisture content (approximately 13 percent). When this was established, the beams were removed to polyethylene bags in preparation for immediate testing.

The width and the depth of each beam was individually measured by a micrometer to the nearest 0.001 inch. They were then subjected to a semi-impact bending test using a Tinius-Olsen Universal Testing Machine (Figure 27). This test was chosen because it is considered to be one of the most sensitive of accepted methods to changes brought about by decay-causing organisms. The machine had a capacity of 200 pounds and a load sensitivity of 0.2 pounds. Samples were center-loaded over a three-inch span and the load was applied at a head speed of 9.5 inches per minute. The time required for failure of the beams was approximately three seconds.

At the completion of the test, all beams were oven dried and weighed for the purpose of computing any weight losses.

PLATE XVI

Figure 27. Tinius-Olsen Universal Testing Machine. Yellow cedar beam in place prior to testing. X 1/5

PLATE XVI

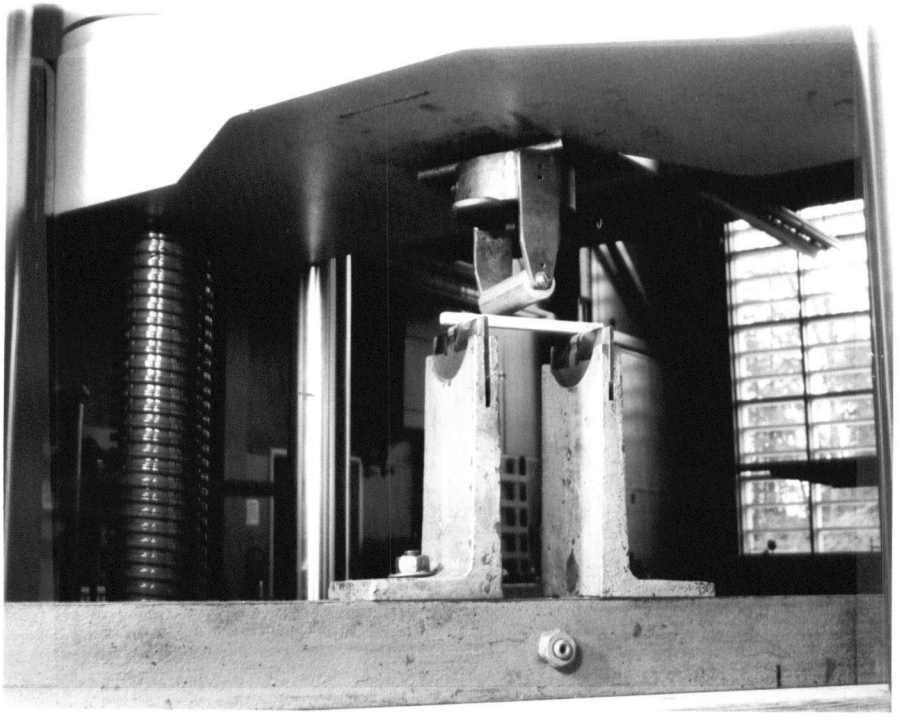

Figure 27

Results of Weight Tests

The weight test data are summarized in Table II. Each of the four groups (A test, A control, C test, C control) of beams was weighed, and the resultant value was compared with the computed original oven-dried weight of the groups. Weights of both sets of control beams remained relatively constant while the test beams suffered weight losses of 0.17 percent in the case of Fungus "A" and 8.40 percent in the case of Fungus "C".

TABLE II

WEIGHT LOSS OF YELLOW CEDAR HEARTWOOD BEAMS EXPOSED TO TWO STAIN FUNGI FOR NINE WEEKS

Series	Conditioned weight of beams prior to inoculation	Computed oven-dried weight of beams prior to inoculation*	Oven-dried weight subsequent to testing	Weight loss	Weight loss as percentage of column 3
	grams	grams	grams	grams	percent
A (test)	121.1907	106.6819	106.4992	0.1827	0.17
A (control)	131.4568	115.6579	115.6570	0.0009	0.0007
C (test)	128.3239	113.2602	103.7470	9.5132	8.40
C (control)	123.8695	109.3287	109.3170	0.0117	0.01

*Oven-dried weight prior to inoculation was computed using the following formula:

$$\text{O.d. wt.} = \frac{\text{wt. (as group)}}{1 + \text{moisture content}}$$

Moisture content was computed using the following formula:

$$\text{m.c.} = \frac{\text{conditioned wt.} - \text{oven-dried wt.}}{\text{oven-dried weight}}$$

(Brown et al, 1949: 60).

Results of Strength Tests

After conducting strength tests on the beams, the modulus of rupture (R) was calculated for each beam using the formula: (Brown et al, 1952 - Wakefield, 1957)

$$R = \frac{1.5 \times p \times l}{b \times h^2}$$

where l = length of span

p = maximum load (lbs) required for failure of the beam

b = width of beam

h = depth of beam

The experimental design was that of Randomized Complete Block (Cochran and Cox, 1957). Test values of R were compared with control values of R in an analysis of variance. The strips from which test and control beams were cut represented replicates, 11 strips in the case of "A" and 12 strips in the case of "C". "Test" and "control" constituted two treatments, of 55 and 60 beams each for "A" and "C" respectively.

Calculated F values were compared with tabled F values. No significant differences (at the 1 and 5 percent levels of confidence) were found between modulus of rupture values of test and control

beams in either series. A comparison was also made between the strips (blocks or replicates). A significant difference at the 5 percent level was found between the strips used in series "C". No difference was noted for series "A".

CONCLUSIONS

Two members of the group Fungi Imperfecti were consistently isolated from stained wood collected at three different localities.

Three basidiomycetous fungi were also isolated during the course of the study. These were Poria weirii, Poria asiatica and Xeromphalina campanella of which the two first named are believed to be new records for the host.

The results of a series of plate cultures indicated no marked antagonism between the two Deuteromycetes and Poria weirii. Similarly, no synergistic action was observed between any of these fungi.

Positive reactions of the two Deuteromycetes on gallic and tannic acid agars indicate the production of extracellular oxidase. This enzyme is generally considered to be a product of white-rotting fungi and to some extent is used as a taxonomic tool for separating the latter group from brown-rotting fungi (Nobles, 1958).

Two series of yellow cedar beams were respectively inoculated with macerated cultures of the two Deuteromycetes employing a special technique devised for this purpose. This technique was

successful in obtaining rapid and uniform growth of those fungi which had demonstrated a slow rate of growth under standard cultural conditions. As a result of the inoculations, stains similar to those observed in nature were produced in the beams.

Following inoculation, these beams were subjected to weight and strength loss tests, in comparison to uninoculated controls. Results of these tests showed weight losses of 0.17 percent and 8.40 percent respectively for series "A" and series "C" while analysis of variance detected no significant differences between modulus of rupture values for test and control samples of either series.

SIGNIFICANCE OF RESULTS

Two Fungi Imperfecti were consistently isolated from stained wood. These fungi produced typical stain in yellow cedar beams when inoculated under experimental conditions. Though it cannot be said that these fungi are the sole cause of black stain in yellow cedar, it would appear that they both may be of major importance. When grown together on artificial media, these fungi exhibit no antagonistic nor stimulatory effects. They can grow together in nature, both having been isolated from the same piece of stained wood in several instances.

That these fungi inhabit the heartwood of living trees and are capable of producing extracellular oxidase (under experimental conditions) indicate marked differences from the fungi generally associated with sapwood stains and certain parallels with wood-rotting fungi. Wood-rotting fungi are well represented as heartwood inhabiters whereas the fungi causing "blue stain" are usually restricted to sapwood. It is not known just how general the production of extracellular oxidase by wood-inhabiting fungi is, but this characteristic is considered to be a feature of white-rotting fungi and a means of separating this group from the brown-rotting fungi.

Penetration of tracheid walls of yellow cedar heartwood by the hyphae of black stain fungi is similar in appearance to the habit of decay fungi.

Under the conditions of this experiment, weight losses of 0.17 percent and 8.40 percent respectively were produced as a result of inoculation of test beams with stain fungi. Modulus of rupture values in impact were not significantly different in comparison with values for controls. Weight loss is generally considered to be a less sensitive test of decay proclivity of fungi than various strength tests including modulus of rupture (Kennedy, 1958). This appears to be particularly so in the case of brown-rot fungi, white-rot fungi having been found, in some cases, to produce significant weight losses accompanied by a relatively low effect on modulus of rupture values (Cartwright, Campbell and Armstrong, 1936).

Other than the preceding comparison, no attempt is made here to interpret the effect of this stain in terms of decay. Neither can it be interpreted in terms of sapstain though tests have similarly shown that the strength of sapwood in some species is not appreciably lessened by staining (Cartwright and Findlay, 1958). More work should

be carried out to add to the evidence provided by this experiment that black stain does not reduce the strength of yellow cedar heartwood.

RECOMMENDATIONS FOR FURTHER STUDY

Certain possibilities for further study of this problem become apparent:

1. Determination of mechanical properties of wood subjected to similar tests over a longer period of time.

2. Determination of the effect of stain fungi on the chemical wood components, notably lignin.

3. Comparison of decay resistance in stained and unstained wood.

4. Study of enzyme production of these fungi.

5. Field study of mode of infection of the fungi causing black stain of yellow cedar.

6. Identification and/or description as new species of the fungi herein designated as Fungus "A" and Fungus "C".

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APPENDIX I - SUMMARY TABLE OF ANALYSES OF VARIANCE

FUNGUS "A"

Source of Variation	d.f.	Sum of Squares	Mean Square	Calc. F	Tabled F at p = 0.05
Treatments	1	2,109,490	2,109,490	2.50	3.95
Blocks	10	12,975,679	1,297,568	1.54	1.93
T x B	10	12,949,961	1,295,996	1.54	1.93
Error	88	74,176,219	842,912		
Total	109	102,211,349			

	Mean	Standard Deviation
Control	11,155	± 976
Test	11,432	± 990

FUNGUS "C"

Source of Variation	d.f.	Sum of Squares	Mean Square	Calc. F	Tabled F at p = 0.05
Treatments	1	4,600	4,600.000	0.0043	3.94
Blocks	11	23,748,808	2,158,982.545	2.0052*	1.89
T x B	11	15,644,322	1,422,211.090	1.3209	1.89
Error	96	103,362,080	1,076,688.330		
Total	119	142,759,810			

	Mean	Standard Deviation
Control	12,050	± 1095
Test	12,038	± 1099

*Significant at the 5 percent level of confidence.