THE INFLUENCE OF MOISTURE AND OTHER FACTORS ON THE ACTIVITY OF HEARTWOOD FUNGI IN SUBALPINE SPRUCE

by

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INTERIM REPORT

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INTRODUCTION

The importance of studying factors that contribute to variation in decay in forest trees has been emphasized by the need for reliable criteria upon which to classify forest stands for hidden defect. Differences in the incidence and amount of decay between stands, often on adjacent sites, make it difficult to apply to any one locality decay factors based on averages of different localities. Experience has shown that the response of trees to different site and environmental conditions, is in many cases, the major factor affecting the amount of decay. Consequently, various investigators have sought a characteristic of site which could be measured and which would be indicative of the trees to resist decay.

During a recent investigation of the decay in subalpine spruce on the Rocky Mountain Forest Reserve in Alberta, (Etheridge 1956), the writer was able to distinguish two types of sites which differed in the nature of the ground vegetation, the growth rate of the trees, and in the incidence of infection by heartrot fungi. This study revealed that there were about twice as many infected trees in the faster-growing stands on sites that were described as "moist" on the basis of the ground vegetation. Although the plants in this case provided a useful index to the incidence of decay, it was realized that the application of such a system to other regions with different forest types should be based upon a knowledge of how these local conditions affect the rate of decay in individual trees. Undoubtedly, site represents a complex of factors any one of which may be of major importance in determining the rate of decay in trees.

Davidson, Newell and Cochrane (1954), who investigated large regional variations in decay in balsam fir in the Maritime Provinces of Canada considered that a large number of site and stand factors influenced, either directly or indirectly, variations in the amount of decay, and concluded that the study of these factors be limited to a small number which could be adequately measured. They were of the opinion that much valuable information on the relation of such factors as stand composition, suppression, growth rate to decay might be obtained in field and laboratory experiments on individual trees. Such experiments should aim at determining whether trees are predisposed to fungal infection by virtue of their growth characteristics, or whether infection is favored by certain site influences which act primarily upon the pathogen. The former would include physical and chemical properties of the wood which might be expected to influence the progress of decay, whereas, the latter would include factors such as moisture and temperature conditions of the site and

fungal flora which might affect the occurrence of the more destructive types of decay fungi.

While it is possible that a number of conditions operate together to predispose trees to infection, the results of the author's recent studies in Alberta indicated that differences in the incidence of infection were closely related to the characteristics of the host, i.e., rate of growth and properties of the heartwood, but were not related to the relative abundance of the different species of heart-rot fungi.

The purpose of the present study is, therefore, to consider the relationship between site and the characteristics of the wood of subalpine spruce, and by laboratory experiments to examine the influence of these factors on the activity of the fungi responsible for decay in this species.

REVIEW OF LITERATURE

Decay and site

The relationships between site and decay have been much studied, but most investigators have dealt with the rating of decay losses by site quality classes, or have attempted to assess hidden defect by the use of certain characteristics of the site and stand. According to Wagner and Davidson (1954), who recently reviewed much of the work published on this subject, much less has been done "to establish the fundamental reasons for the observed relationships, or to separate the influence of environment from others that may have had a part in bringing about the decay status found in the particular stands under study." These authors feel that when the fundamental relationships have been sufficiently studied a better appreciation of the effects of site will be possible.

Decay and growth rate

The tree characteristic most closely related to site and one which probably has received most attention in relation to decay is the rate of growth. Many investigators have looked for a relationship between the rate of growth or site quality and the rate of decay in conifers but no consistent, clear cut and generally accepted conclusions have emerged. The literature on this matter is conflicting and possibly results from the absence of any satisfactory basis for analysing and comparing the data. Evidence that supports the probability that decay is more rapid in fast-growing trees, or in

trees on the better sites in Canada is given by McCallum (1928), Basham (1951), Davidson et al (1954), and Thomas and Thomas (1954). On the other hand, Bier, Salisbury, and Waldie (1948) found no consistent differences in the percentage of decay in fast- and slow-growing fir in the Upper Fraser Region of British Columbia, and White (1953) working in Ontario found a greater percentage of infected trees and larger volumes of decay in slow-growing white pine. These studies were based on the average condition of the residual trees in the different age classes, however, and make no provision for normal mortality in the stand which would result in the removal of infected trees at different ages. Thus, it is perhaps significant that the latter two studies were based on samples which contained almost twice as many trees in the slow-growing group. This could mean that mortality due to fungal infection in the different age classes, particularly in the older age classes, is much greater among the faster-growing trees. By the same token, conclusions based on the decay resistance of fast- and slow-growing stands determined by the use of site quality criteria, i.e., height growth at 50 or 100 years, should be interpreted with caution since it is generally recognized that good quality sites where growth is relatively rapid possess fewer trees in the older age classes. Such a condition is due primarily to the "dropping out" of badly infected trees at an earlier age than occurs on poor sites which are more often characterized by a top-heavy age structure.

In a few instances attempts have been made to correlate the progress of decay in trees with the width of the annual growth rings on the supposition that fungi penetrate more easily the less dense wood of the wide annual rings. Thus, in a study of pathological deterioration of insect-killed balsam fir in Ontario, Basham (1951) found that the greatest depth of radial penetration (centripetally) by decay fungi tended to occur in those trees with wide growth rings in the outer sapwood. The relationship was particularly so in trees killed during the winter months. Similarly, a correlation between the average width of the annual rings and fungal attack on pine was demonstrated by Passarge (1953) in the Magdeburg forest region in Germany. In this region the maximum incidence of Fomes pini attack on pine is attained on fertile soils with a high water table, and in trees with wide annual rings in the first 20 years of growth.

These observations generally agree with those of Schulman (1954) on the longevity of several species of conifers on marginal sites in semi-arid regions of the Western United States. Extremely slow-growing overage trees were in general relatively free of centre-rot compared with fast-growing favorably situated trees of the same age and species. The site characteristics usually associated with these overage trees included steep rock slopes or ridges, margins of the geographic range for the species, and open stands.

A relationship between adverse site conditions, narrow

annual rings, and a low incidence of disease appears to be general in conifers. This is demonstrated in unpublished data obtained by the present author during a study of site factors in relation to the incidence of root- and butt-infections in subalpine spruce in Alberta. The relationships obtained may be tabulated as follows:

Site:	Dry	Intermediate	Wet
Percentage of Infected trees:	10	22	52
Average width of the annual rings:			
Overstorey trees	0.039 ins.	0.047 ins.	0.053 ins.
Understorey trees	0.027 ins.	0.029 ins.	0.032 ins.

Although the incidence of root- and butt-infections in subalpine spruce were not determined separately for overstorey and understorey trees, there appeared to be a tendency for fewer infections to occur among the slower-growing trees of the understorey.

Decay and the physical properties of wood

Various attempts to discover the reasons for different rates of decay in slow-grown and fast-grown wood have been made by testing wood specimens in the laboratory under controlled conditions. The conclusions in most cases have been based on comparisons of losses of weight of test specimens of the different kinds of wood after infection by various wood-decay fungi for different periods of time. Among the earliest, and perhaps the most elaborate of these investigations were those of Zeller (1917) with three species of yellow pine, Pinus palustris, P. echinata, and P. Taeda. Special attention was given to the effect on decay of the physical properties of each wood used, data being obtained on resin content, specific gravity, percentage of summer-wood in the annual rings, sapwood and heartwood, and the distance of the sample from pith. Briefly his conclusions can be stated as follows: there is an inverse correlation between specific gravity and ring frequency, and weight loss in the heartwood, but no such correlation in the sapwood. The age or distance from the pith of the heartwood shows no relation to durability. An increase in the proportion of summer-wood results in an increase in specific gravity. Finally, he found that wood decays irrespectively of the resin content.

Southam and Ehrlich (1943) and others, questioned Zeller's results on the grounds that the data for all the wood specimens which were used to determine the relationship between specific gravity and

ring frequency and the rate of decay were considered together in these tests, whereas when the data for the different types are examined separately, no consistent relation was evident. Southam and Ehrlich used another species (Thuja plicata D. Don.) but failed to find any correlation between ring frequency or specific gravity and decay. Also, there was no correlation between ring frequency and specific gravity although this had been demonstrated by Zeller when the data for the three pines were considered together. These investigators attempted to explain the differential rates of decay that were often found in wood of different specific gravities on the basis of variation in the size of the intracellular spaces, which they suggested might affect the water-to-air ratio in the cells if the moisture content (based on the oven-dry weight of the wood) was not adjusted accordingly. Thus, presuming that there was a particular water-to-air ratio for the maximum development of decay-producing fungi, it followed that the optimum moisture content for decay would be different in wood of different densities. If this explanation is a correct one, many of the discrepancies among the data of the various workers might be explained by the lack of adequate control over moisture, particularly in comparative studies such as those undertaken by Zeller.

Moisture and decay

Most of the data on this subject have been derived from laboratory experiments on the influence of various moisture contents of air and wood on the rate of decay in test specimens.

In laboratory experiments, Snell (1929) demonstrated two cardinal values representing the upper moisture limit for maximum decay activity, and the limit at which there was no decay. These values, which were based on averages for six fungi, were seen to vary with the specific gravity of the wood, and this was attributed to different water-to-air ratios in the woods of different densities. Snell found that the water content of wood in which decay was inhibited corresponded to 80 per cent saturation.

Similarly, Bjorkman (1946) investigating the moisture requirements of storage-decay fungi, found that most species developed best at a moisture content equal to 20 to 50 per cent of saturation, whereas at 70 per cent of saturation fresh infection was generally prevented. At the other extreme, sound wood with a water content below the fibre saturation point (24 to 28 per cent of the oven-dry weight), was not infected. Bjorkman also obtained information which suggested that different wood-destroying fungi require different moisture ranges for optimum development. Thus, some fungi, including Stereum sanguinolentum, Corticium evolvens, and Polyporus abietinus, which he called "early rot" fungi owing to their early appearance in the succession

of fungi on wood-pulp piles, developed most rapidly at the lower values of the range (20 to 50 per cent of saturation), while most of the "late rot" fungi, for example, Poria vaporaria, Trametes serialis, T. trabea, Lenzites saepiaria, Lentinus lepideus, Paxillus panuoides developed best at high values. Some decay fungi were also able to regulate, to some extent, the water content of the wood according to their respective water requirements. In addition, the moisture range for initial infection of wood by various fungi was found to be considerably narrower and higher than that required for the subsequent decay. In this connection, Zeller (1920) has demonstrated that the germination of spores of L. saepiaria on wood require saturated air, or a relative humidity high enough to maintain fibre saturation of the wood.

The possibility that wood-destroying fungi might be classified on the basis of their air- and wood-moisture relations emerges from these studies. This suggestion is supported by the earlier work of Snell, Hutchinson, and Newton (1928) who demonstrated that two wood-destroying fungi, Fomes roseus and Trametes subroseus, species difficult to separate on morphological criteria, could be separated on the basis of their different moisture requirements for development.

While it has been shown that the moisture content of wood may confine the decay activity of fungi within a fairly narrow range, surprisingly little information is available on the similar relationships that might exist in the living tree. The only direct reference to a relationship between moisture and decay in living trees appears to be that by Henriksen and Jørgensen (1952) in a study of factors relating to the higher incidence of Fomes annosus attack generally found in heavily-thinned stands in Denmark. These investigators found differences in the moisture content, specific gravity, and the growth form of the trees and in the ground flora. From sample borings taken at 1.3 meters in height in June 1952, the moisture content of the trees in unthinned stands where the incidence of F. annosus was rated at one per cent, was found to be 23.9 per cent of the oven-dry weight, while the moisture content of the most heavily thinned stand where the incidence of F. annosus was 25 per cent, was 31.2 per cent. The specific gravity was about six per cent higher in the unthinned stands than in the heavily thinned stands, but on the basis of calculations made by the present author, a higher degree of saturation nevertheless occurred in the heavily thinned stands where the attack of F. annosus was greater.

Direct evidence that the moisture content of the stem of living trees is regulated to some extent by the moisture and growing conditions of the site has been recently obtained by Chalk and Bigg (1956) for Sitka spruce in England. Expressing the moisture content as the percentage of saturation, thus eliminating the differences in moisture content depending on density, these investigators were able to

compare fast- and slow-growing trees on a moist site with fast- and slow-growing trees on a dry site. For example, in July, the degree of saturation in the outer 2 cms. of the stem for a dominant tree and a suppressed tree on a wet site was found to be 94 per cent and 86 per cent respectively, while these values for two similar trees on a dry site were 77 per cent and 60 per cent. Using the conventional method of expressing moisture, i.e., percentage of oven-dry weight, other investigators, including Fielding (1952) and Nylinder (1953), have obtained similar results, indicating that moisture content tends to be higher in fast-growing stands on the better sites. Although these results, for the most part apply to moisture determination made in the sapwood, there is some evidence that parallel trends occur in the heartwood of the trees.

Temperature and decay

It is generally recognized diurnal, seasonal, and spatial fluctuations occur in the temperatures of the heartwood of trees (Gibbs 1950, Haarlöv and Peterson 1952, Reynolds 1939), and in one instance these have been correlated with site conditions (Saharov 1952). However, very little is known about the effect of heartwood temperature on the decaying activity of fungi apart from data derived from measurements in the laboratory of the growth of fungus colonies on agar plates at different temperatures. According to Wagner and Davidson (1954) there are no experiments on the effect of heartwood temperature on the activity of a decay fungus. However, there can be little doubt that temperature affects the rate of decay in the heartwood of trees, particularly, in extremes of latitude and altitude. In this connection, it is of some interest that the California Forest Experiment Station is now making continuous temperature measurements within trees to see how the temperatures of the heartwood of trees of different sizes and species vary under different conditions, and how these might influence the rate of decay. It is anticipated that the data obtained will indicate how fast decay may be expected to progress under different site conditions.

Decay and the chemical properties of wood

The occurrence of substances of the heartwood which are fungicidal, and which are responsible for decay resistance in certain species of trees, has been extensively reported in the literature. However, it is doubtful that such substances occur in spruce. Picea species often contain conidendrin (also known as lactone and tsugarsinol), which is a lignan related to pinorsinol from the gum resin derived from several species of spruce and pine. None of these substances, however, have been found to be fungicidal when tested against Pullularia pullulans (Rennerfelt and Nacht 1955).

On the other hand, the occurrence in the heartwood of trees of substances that act to promote the growth of wood-destroying fungi has received little attention. The effect on the decay of wood of nitrogen compounds has been studied, *in vitro*, by a number of investigators, but it is not known whether the concentrations or the forms of nitrogen that were used compares with those in the living trees.

In this connection, Findlay, (1934) has demonstrated that the addition of inorganic nitrogen (NH_4NO_3) in low concentrations (0.5 per cent solution) slightly increased the rate of decay of Sitka spruce, whereas wood treated with a one per cent solution of peptone increased the loss through decay from 25.8 to 40,8 per cent.

Schmitz and Kaufert (1936) using sawdust from Norway pine and paper birch heartwood, found that the addition of asparagine at approximately, .2, 1, and 2 per cent of the dry weight of pine sawdust increased significantly the rate of decay caused by *Lenzites trabea*. However, the addition of the same concentrations of asparagine to the paper birch sawdust caused statistically significant decreases in the rate of decay by *Polystictus versicolor*; the latter results were thought to be partly due to the presence of toxic substances in the heartwood of this species. The addition of similar amounts of NH_4NO_3 decreased the rate of decay in these woods.

Although, it appears that the rate of decay by wood-destroying fungi may be increased by adding certain organic nitrogen compounds to wood, it is difficult to assess nitrogen as a factor in decay variability until more information is available on the nature and concentration of such compounds in living trees. For example, the nitrogen content of wood has been reported of the order of 0.05 to 0.30 per cent of the dry weight (Findlay 1934), Schorger 1926), but whether nitrogen in these concentrations can be limiting depends on how efficiently it is used by wood-destroying fungi. It appears from studies made by Hungate (1940) on the nitrogen content of sound and decayed coniferous wood that the total nitrogen content of wood changes very little during decay. This was thought to indicate that decay fungi use nitrogen very efficiently in the decomposition of wood. However, Findlay (1934) found that about 40 times the amount of the original nitrogen in Sitka spruce (which was found to be 0.04 per cent) was accumulated in the mycelium of the fungus, and took the view that wood substance as a food material for fungi was very deficient in nitrogen. On the basis of these observations he postulated that small additions of nitrogen to wood might be expected to result in a greater rate of decay.

The general conclusions that may be drawn from the literature are as follows: While there have been many attempts in the laboratory to study factors associated with decay resistance of wood, with the result that much valuable information has been obtained

on the relation of physical and chemical properties of wood to decay, it is not known how these factors compare with conditions in the living tree. For example, there is abundant evidence from field investigations that fast-growing trees are more prone to infection and decay than slow-growing trees, but there are still differences of opinion on the nature of the decay-resisting properties of slow-grown wood in spite of much intensive work done in the laboratory.

MATERIALS AND GENERAL METHODS

Sample areas

The governing features in the choice of the areas from which the samples of spruce (Picea glauca (Moench)Voss) were collected were the moisture conditions of the site, the rate of growth of the trees, and the disease status of the stand. Two suitable areas, representing a wet site and a dry site located in the Bow River Forest in the Rocky Mountain Forest Reserve in Alberta were selected on the basis of data obtained by the author from surveys in the region during 1952 and 1953.

The dry site, with characteristic plants of Shepherdia canadensis (L.) Nutt. and Arctostaphylos uva-ursi (L.) Spreng., was located in a mixed stand of spruce and lodgepole pine (Pinus contorta var. latifolia), on the summit of a well-drained ridge, at an elevation of 4600 - 4800 feet. The wet site, with characteristic plants of Mertensia paniculata (Ait.) Don. and Smilicina amplexicaulis Nutt., was located about 4½ miles to the south east in a pure stand of spruce, in a valley, at an elevation of 4300 - 4500 feet. The estimated incidence of infected trees for the two stands was 0-5 per cent and 60 per cent respectively. The average gross volume for trees on the wet site was about twice that for trees of similar ages on the dry site.*

Three overstorey and three understorey healthy trees on each of these sites were felled in July 1954. The sites and the trees are briefly described in Table I.

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* The plant indicators that were used to distinguish the wet and dry sites, and the volume estimates for these sites are based on previous studies made by the author (Etheridge, 1956).

TABLE I

SITE AND TREE DATA

Description of the site	Tree No.	Class of tree	Total ht. (ft.)	D.b.h. o.b. (ins.)	Age at 1-ft. (yrs.)
Dry Site	1 DO	Over-storey*	72.2	16.2	184
Aspect: flat, ridge top	4 DO	"	67.7	12.2	197
Slope: zero	5 DO	"	71.7	12.9	140
Elevation: 4600-4800 ft.	2 DU	Under-storey	47.1	5.9	110
Soil **p^H: 5.5	3 DU	"	60.0	8.1	90
Soil tecture: heavy	6 DU	"	41.3	6.9	102
Soil nitrogen: 0.136% (% dry wt.)					
Wet Site	7 WO	Over-storey	79.8	14.6	117
Aspect: south, valley bottom	8 WO	"	81.0	13.0	117
Slope: 16%	9 WO	"	75.4	11.7	115
Elevation: 4300-4500 ft.	10 WU	Under-storey	55.0	6.7	108
Soil p^H: 6.1	11 WU	"	73.2	9.4	109
Soil nitrogen: 0.152% (% dry wt.)	12 WU	"	45.4	8.4	133

* Overstorey trees are defined as dominant trees having a diameter at breast height outside bark (d.b.h.o.b.) greater than 11 ins.

** Soil samples were taken from the "B" horizon: p^H was estimated electrometrically from samples mixed with distilled water (1:1); nitrogen represents the total nitrogen of the sample.

The tree designations, DO,DU,WO,WU, refer to dry overstorey, dry understorey, wet overstorey, and wet understorey respectively.

Collecting samples for moisture deterioration

Immediately after each tree was felled, discs about two inches thick were cut from the stem at the butt-level (at a height of from one to two feet) and at distances of 20 and 40 feet. Three samples of heartwood, each about two inches square, were cut in sequence from the butt-disc, the middle block of each group coming from the centre of the disc. One sample of heartwood was cut from each disc at the 20- and 40- foot level of the trees. After each sample block was obtained it was placed in an aluminium container and the lid quickly sealed with "cellulose tape" to prevent the loss of any water. In the laboratory, the containers together with the wood blocks were weighed, and then dried to constant weight at 105°C., and re-weighed. The difference between the initial weight and the final weight, represented the amount of water in each sample. The moisture content (known as the conventional moisture content, Chalk and Bigg (1956)) was expressed as a percentage of the oven-dry-weight (o.d.w.) of the sample.

Collecting and preparing the samples for study

The trees were also sampled by cutting sections, about one foot in length, from the stem at the butt-level and at intervals of 10 feet upwards until the tree was less than four inches in diameter. These samples, after being marked as to their position in the tree, were taken to the laboratory and furnished the basic material for the investigation.

Small pieces 1 x 1 x 6-7 inches, were cut from the sapwood, the heartwood, and the centre of each of the sections of the trees and were labelled so that their origin could be identified later. These pieces were maintained at room temperature and humidity until required for the various experiments.

For the decay experiments, nine blocks of wood measuring about 1 x 1 x $\frac{3}{4}$ inches, were sawn in sequence from an appropriate piece of wood, and except for an end-block which was selected for determining the moisture content of the sample, each block was machined on one side to furnish a cavity which was designed to take a disc or plug of wood inoculum (see Figs. 1 and 2). The nine blocks were then numbered for identification later and weighed. The oven-dry weight of each of the machined blocks was calculated, assuming that they contained the same moisture as the selected block which had been oven-dried at 105°C. for 24 hours.

The blocks used for the moisture determinations were also used to determine the specific gravity and the ring-frequency, i.e., number of annual rings per inch, of the group. The specific gravity was calculated from the oven-dry weight and the volume of the wood at

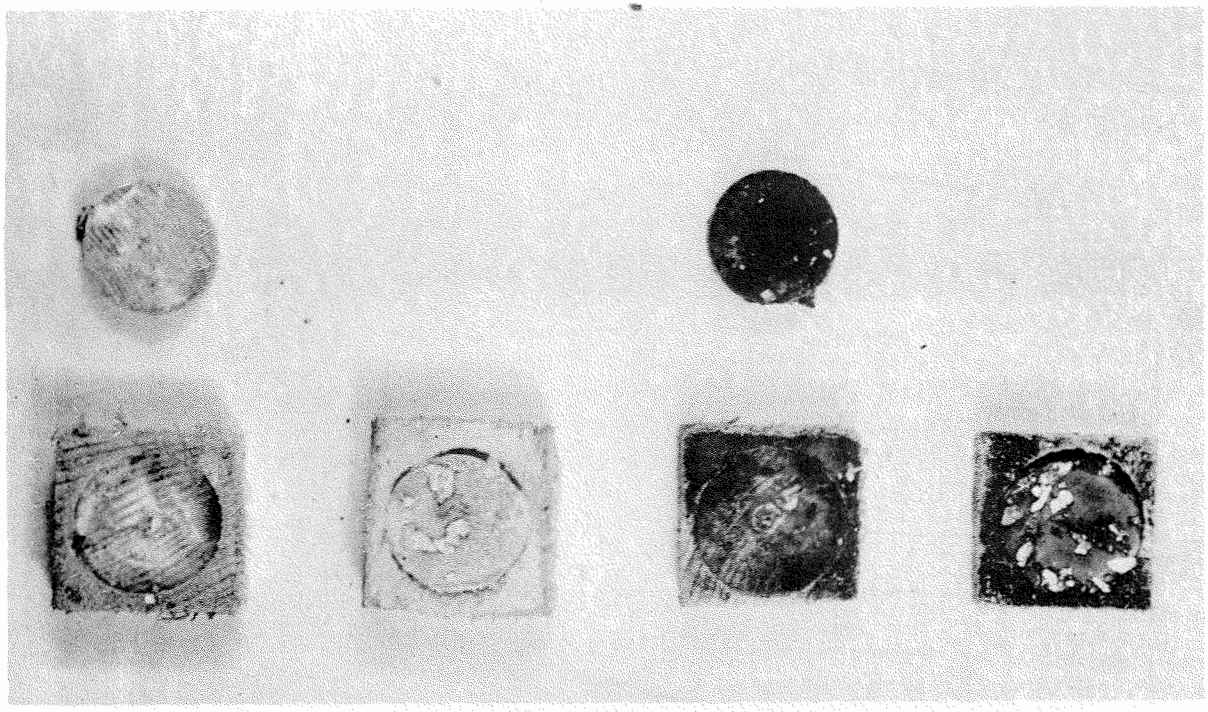

Fig. 1. Test blocks, cavity type (1 x 1 x $\frac{3}{4}$ ins.), infected with pure cultures of Stereum sanguinolentum (white mycelium) and Coniophora puteana (black mycelium) showing the method of inoculation.

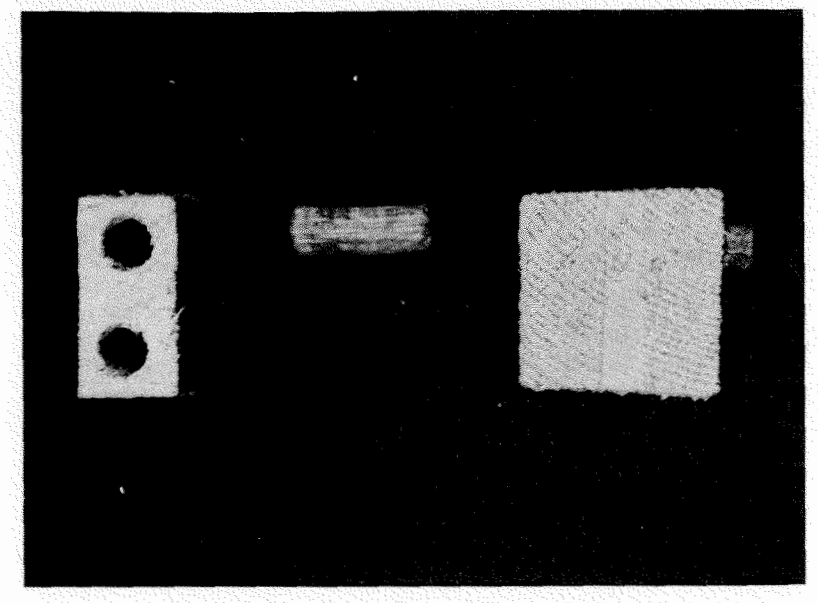

Fig. 2. Test blocks, socket type (1 x 1 x $\frac{3}{4}$ ins.), used in the moisture - decay experiments showing the method of inoculation.

a moisture content above the fibre saturation point, i.e., the green volume. The blocks were brought to the green volume by soaking in water for 24 hours, and then immersing them in mercury in a graduated cylinder; the volume of the block was estimated to the nearest ml.

To eliminate some of the differences in the moisture content due to differences in specific gravity, for comparative purposes, the moisture content was expressed as the percentage of the cell-cavity which contained water, i.e., percentage of saturation. These determinations were calculated according to a formula given by Brown, Panshin, and Forsaith, (p.34, 1952), which uses the specific gravity based on the volume of the wood when the moisture content is above the fibre saturation point:

$$\text{Percentage of saturation} = \frac{(M - 28)\,SG}{1 - 0.93\,SG}$$

where, M = conventional moisture content

SG = the specific gravity of the wood based on the green volume assuming the fibre saturation point to be at 28 per cent (o.d.w.), and the specific gravity of the dry wood substance to be 1.46

Inoculum

The inocula used in these experiments were either plugs from agar plate cultures or pieces of infected wood. The agar plugs, about 5 mm. in diameter were cut with a cork borer from plate cultures of the fungi grown on 2 per cent malt extract agar. The wood inoculum was prepared in 250 ml. flasks by placing discs (about 22 mm. in diameter) or plugs (about 6 mm. in diameter) (see Figs. 1 and 2) on a layer of spruce sawdust, about three grams, to which was added five per cent of an accelerator (Badcock, 1941), composed of:

Maize meal	-	50 parts by wt.
Bone meal	-	30 " " "
Potato starch	-	17 " " "
Sucrose	-	2 " " "
Wood ash	-	1 " " "

Water was added at the rate of 170 per cent of the oven-dry weight of the sawdust. After sterilization at 15 lb./sq. in. for 30 minutes, the flasks were inoculated with plugs of agar taken from a 2 to 3 week-old plate culture of the appropriate fungus. Incubation was, in most cases for three months; water was added to the cultures periodically to maintain the moisture content of the wood at about 70 to 80 per cent (o.d.w.).

Decay cultures

For the decay experiments wide-mouthed jars 5.5 cm. x 5.5 cm., with aluminium screw-top lids were used (Fig. 3). The glass jars were thoroughly washed, made slightly acid in a dilute solution of HCl, rinsed in distilled water, and then sterilized in an oven for two hours at 160°C.

The wood blocks were sterilized by steam or propylene oxide. When steam was used, the blocks of wood having an initial moisture content of about 10 per cent (o.d.w.) were placed in the jars with the lids loosely screwed down. The jars were then steamed from the cold for 1½ hours, one half hour of the time being at 100°C., and then allowed to cool slowly. This was repeated after 48 hours. The effect of the treatment on the initial moisture content of the blocks was determined by weighing control blocks after the steaming.

Propylene oxide was used in the way described by Hansen and Snyder (1947). The blocks were placed in a large desiccator on a perforated tray over propylene oxide which was introduced at the rate of 1 ml. per litre of the container. The blocks were left overnight and the gas was then removed with a suction pump, and the vessel flushed a number of times with filtered air. The treated blocks were transferred to sterile culture jars. This treatment was found to have a negligible effect on the moisture content of the blocks.

Adjusting the moisture content of the blocks

After sterilizing the blocks and before adding the inoculum, the required moisture content was obtained by adding the required amount of sterile distilled water to the cavity of each block from a hypodermic syringe calibrated to 0.1 ml. The amount of water added took into account the moisture content after sterilization, and for certain purposes, the specific gravity of the wood. After this initial adjustment of the moisture content, the blocks were inoculated with the appropriate fungus, the lids loosely screwed down, and the jars immediately placed in a saturated atmosphere in humidity chambers shown in Fig. 3.

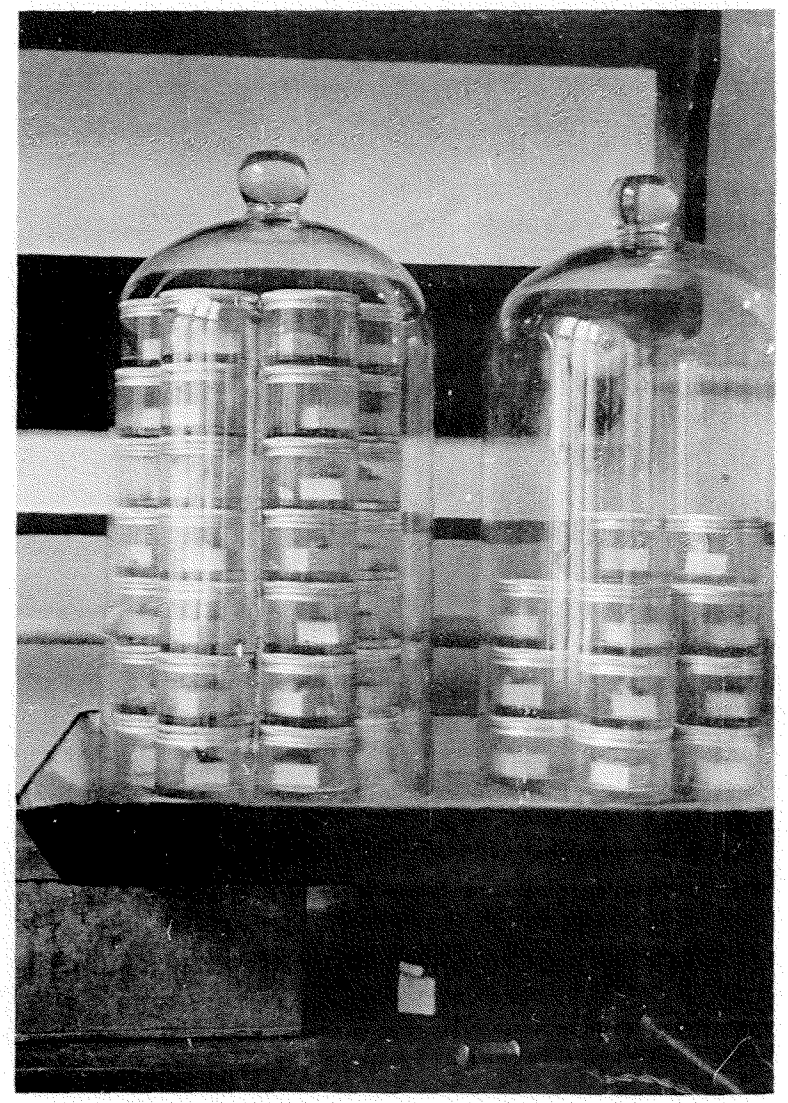

Fig. 3. Humidity and decay chambers showing how the test specimens of wood were indivually incubated in small screw-top type jare. Water was kept in the tray so as to maintain a saturated atmosphere in the chambers.

TABLE II

SOURCE OF CULTURES

Fungus	Culture No.	Host	Locality
Butt rots			
Coniophora puteana (Schum. ex. Fr.) Karst.	A263	*Picea glauca*	Nordegg, Alberta
	C4	*Picea mariana*	County Champlain, P.Q.[3]
	C5	*Picea glauca*	Wasagaming, Manitoba[2]
Flammula connissans Fr.	A300	" "	Nordegg, Alberta
	A259	" "	Nordegg, Alberta
	A86	" "	Fawcett Lake, Alberta[2]
	T510	*Picea engelmanni*	Bow River Forest, Alta.
Polyporus circinatus var. *dualis* Peck	A361	" "	Burmis, Alberta
	A79	*Picea glauca*	Eagle Creek, Alberta
	A20	" "	Fawcett Lake, Alberta[2]
	339	*Picea engelmanni*	Burmis, Alberta
Unknown C[1]	A212	*Picea glauca*	Fisher Creek, Alberta
	T173	" "	Bow River Forest, Alta.
Stereum sulcatum Burt	T491	*Abies lasiocarpa*	Clearwater Forest, Alta.
Trunk rots			
Fomes pini (Thore) Lloyd	T434	*Picea glauca*	Clearwater Forest, Alta.
	E25	" "	Waterton Lakes, Alberta
	E24	*Abies lasiocarpa*	" " "
Stereum sanguinolentum Alb. and Schw.	A94	*Picea glauca*	Otter Creek, Alberta
	T492	*Picea mariana*	Clearwater Forest, Alta.
	A107	*Picea glauca*	Otter Creek, Alberta
Peniophora septentrionalis Laur.	B356	*Picea engelmanni*	Burmis, Alberta
	A90	*Picea glauca*	Eagle Creek, Alberta
	A2	*Picea glauca*	Clearwater Forest, Alta.
Miscellaneous			
Coryne sarcoides (Jacq.) Tul.	T157	*Picea glauca*	Bow River Forest, Alta.
	L2C	" "	Lac la Biche, Alberta.

TABLE II (Concl'd)

Fungus	Culture No.		Locality
Miscellaneous (Cont'd)			
	A56	Picea glauca	Fawcett Lake, Alberta[2]
	A29	" "	" " " [2]
	A110	" "	Otter Creek, Alberta[2]
	C103	Pinus contorta	Strachan, Alberta
	C52	Pinus banksiana	? - Canada[3]
	BY1	Fagus sylvatica	Middlesex, England
	BG2	" "	" "
	BA3	" "	" "
	A11	? (fruit body)	Windsor Great Park, Eng. England
	FC1	Picea sitchensis	
Myrothecium verrucaria	144743	?	Commonwealth Mycological Institute, Kew, England

1 Designation used by Dr. M. K. Nobles, Senior Mycologist, Ottawa, Canada, pending the identification.

2 Collected by members of the Forest Biology Laboratory, Saskatoon, Saskatchewan.

3 Obtained from the Mycological Herbarium, Dept. Agr., Ottawa.

Stock cultures of the isolates were grown on a two per cent malt extract agar medium and kept in a refrigerator at approximately 4°C.

For reasons to be discussed later, it was found necessary for certain purposes to check the moisture level of the blocks during incubation. The blocks were removed from the jars in a room exposed to ultra-violet light from a germicidal lamp for one hour, and after removing the disc of inoculum, the blocks were weighed asceptically to the nearest .01 g. on a Joly spring-balance the pan of which was contained in an inoculating hood. The water content of the blocks was then calculated; if it were excessive, the blocks were allowed to lose water by keeping them on the laboratory bench for a few days, if it were too low, the necessary amount of water was added from a hypodermic syringe. Little contamination followed this procedure.

In the decay experiments, decay activity was measured as the loss of weight during incubation as a percentage of the original oven-dry weight. Thus, it was necessary to test the reliability of the method when the oven-dry weight was calculated and used. A control consisting of 10 uninoculated blocks were given the same treatment as the inoculated blocks and the differences between the final oven-dry weight and the initial calculated oven-dry weight was determined. On this basis, it was found that the calculated weights were, on the average, 0.5 per cent higher than the average weight obtained by drying the blocks in an oven at 105°C. for 24 hours. This source of error was taken into account in the interpretation of the results of the experiments.

Cultures and culture media

Thirty-five isolates, representing 10 genera of fungi were used in this investigation. Data about the cultures are given in Table II.

Many of the cultures were isolated by the author in the course of studies of the decay of subalpine spruce in Alberta, and for the most part represent genera of fungi that normally occur on this host. An isolate of *Myrothecium verrucaria* was obtained from the Commonwealth Mycological Institute, Kew, England, for comparison with the wood-destroying fungi.

In enzyme studies cell-free filtrates of the cultures grown on a semi-synthetic medium were used. The following *basal* medium, found by Jennison *et al* (1952) to support growth of a large number of species of wood-destroying fungi, and used by them for cellulase production, was adopted with certain modifications:

Casein hydrolysate (vitamin free)	120 mg./l.
KH_2PO_4	1.5 g./l.

$MgSO_4$	0.5 g./l.
Adenine	13 mg./l.
Guanine	13 mg./l.
Uracil	13 mg./l.
Thiamin HCl	1 mg./l.
Glucose	3 g./l.

Trace elements

B (as H_3BO_3)	0.10 mg./l.
Mn (as $MnCl_2 \cdot 4 H_2O$)	0.01 mg./l.
Zn (as $ZnSO_4 \cdot 7 H_2O$)	0.07 mg./l.
Cu (as $CuSO_4 \cdot 5 H_2O$)	0.01 mg./l.
Mo (as $(NH_4)6 Mo_7O24 \cdot 4 H_2O$)	0.01 mg./l.
Fe (as $FeSO_4 \cdot 7 H_2O$)	0.05 mg./l.

initial p^H - 5.0

Modification "A"

As in basal medium but with the following changes:

Yeast extract: 300 mg./l.

Carbohydrate: glucose reduced to - 400 mg./l.

cellulose in the form of filter paper (No. 1 Whatman) macerated in a Waring blender for 2 minutes, was added at the rate of - 5 g./l.

initial p^H - 5.7

Modification "B"

Yeast extract: 100 mg./l.

Carbohydrate: glucose increased to - 20 g./l.

initial p^H - 4.5

For comparative studies of enzyme production by the fungi in a liquid culture, 40 ml. of the medium (Modification "A") were sterilized for 15 minutes at 15 lb./sq. in. in 12-oz. medicine flats and then inoculated with 10 plugs taken from 4-week-old plate cultures of the isolates on two per cent malt extract agar. Except where stated otherwise, the flats were incubated for 17 days at 22.5°C., and then stored for one or two days at 4°C. The contents of two flats of each culture were strained through a single layer of muslin to remove the larger particles, then the remaining liquid was pressed from the mycelial matter in the muslin. The filtrate was centrifuged at 10,000 r.p.m. for 10 minutes to make it cell-free, and stored in 20-ml. bottles at -17°C.

Details of the method used for measuring the enzyme activity of the filtrates, and other special techniques will be given later.

EXPERIMENTAL

A. SITE AND TREE RELATIONSHIPS

(a) Growth and properties of the heartwood

The effect of site and dominance on growth and properties of the heartwood of subalpine spruce was investigated by sampling three overstorey and three understorey trees from each of two sites. The samples were those originally used to determine the moisture content of the trees and included three specimens of the heartwood from 1.5 feet, and one each from the 20- and 40-ft. level of the trees. The specific gravity and the ring-frequency were determined on specimens at the green volume. An estimate of the nitrogen content was based on a single 1 g. (o.d.w.) sample of wood-chips from the centre specimen collected at 1.5 feet from the ground. The nitrogen determinations

were made by micro-Kjeldahl methods described by Clark, (1943), and Hiller, Plazin and Van Slyke, (1948).

The influence of site and dominance class on specific gravity and ring-frequency was consistent for the four groups of trees, both the specific gravity and the number of annual rings per inch decreasing with increasing site moisture and with dominance. From Table III, it will be noted that the specific gravity is lowest (0.362) for overstorey trees on the wet site and reaches a maximum of 0.408 for the understorey trees on the dry site. Likewise, ring-frequency is seen to be lowest, viz., 12.5 annual rings per inch, for the overstorey trees on the wet site, and highest, viz., 25.5 rings per inch, for understorey trees on the dry site. It is noteworthy that the general trend holds for the four growth-rate classes, particularly for ring-frequencies, in spite of the much higher average age for the three overstorey trees on the dry site. A comparison of the means between the two sites revealed significant differences in specific gravity ($P = .02$), and highly significant differences in ring-frequency ($P = .001$). It was evident, therefore, that both specific gravity and ring-frequency were related to the rate of growth of the trees although the width of the annual rings appeared to be the more satisfactory measure of growth.

To find the best method for comparing the rate of growth of the trees, the mean annual growth increment, i.e., height index, was calculated for each tree by dividing the length of the stem (above a 10ft. stump) by the age of the tree at the 1ft. level. These indices are tabulated according to site and dominance classes in Table III. It will be seen that the rather low height index of 0.41 obtained for the dominant group of trees on the dry site, does not follow the expected trend but reflects the much greater age of the trees in this group. The figure for the average number of rings per inch based on the younger heartwood, which eliminates the slowing-down of terminal growth coincident with advancing age, was considered a better measure for making comparisons between trees of uneven age.

There was some indication that the total nitrogen content of the heartwood might be influenced by site and dominance (Table III). Although the sample was inadequate for statistical treatment of the results, it appears that nitrogen reached its highest concentration in the faster-growing dominants on the wet site. It also appears that the nitrogen content of the heartwood of subalpine spruce may be greater (0.22 to 1.45 per cent of dry wt.) than that previously reported for wood, viz., .05 to .30 per cent dry wt. (Findlay, (1934), Schorger, (1926), but it is not known whether the present results which are based on only 11 samples, can be regarded as comparable with those obtained by other workers.

TABLE III

EFFECT OF SITE AND DOMINANCE ON THE SPECIFIC GRAVITY, RING-FREQUENCY, AND THE TOTAL NITROGEN CONTENT OF THE HEARTWOOD OF 12 HEALTHY SUBALPINE SPRUCE

Site and Dominance	Tree No.	Origin of Sample Ht. in Ft.	Specific Gravity	Ring-frequency	Total N % dry wt.	Ht. index	Age at 1 ft. (yrs.)
Dry Site Overstorey	1 DO	1.5	0.412	24.5	0.608	0.38	184
		1.5	0.340	36.0			
		20.0	0.362	27.0			
		40.0	0.363	19.0			
		Average	0.369	26.6			
	4 DO	1.5	0.374	31.0	0.527	0.34	197
		1.5	0.409	31.5			
		40.0	0.378	19.0			
		Average	0.387	27.1			
	5 DO	1.5	0.365	25.0	0.446	0.50	140
		1.5	0.384	24.0			
		20.0	0.359	14.0			
		40.0	0.462 K2	14.0			
		Average	0.392	19.2			
	Class Average		0.382	24.1	0.527	0.41	173
Dry Site Understorey	2 DU	1.5	0.450 K	41.0	0.556	0.42	110
		1.5	0.442	42.0			
		20.0	0.407	27.0			
		Average	0.433	36.6			
	3 DU	1.5	0.404	27.0	0.219	0.65	90
		1.5	0.380	23.0			
		20.0	0.413 K	20.0			
		40.0	0.426	13.0			
		Average	0.406	20.7			
	6 DU	1.5	0.367	20.0	-	0.39	102
		1.5	0.433	39.0			
		20.0	0.360	18.5			
		Average	0.386	25.8			
	Class Average		0.408	27.0	0.387	0.49	101
	Site Average		0.359	25.5	0.471	0.45	137

TABLE III (Concl'd)

Site and Dominance	Tree No.	Origin of Sample Ht. in Ft.	Specific Gravity	Ring-frequency	Total N % dry wt.	Ht.[1] index	Age at 1 ft. (yrs.)
Wet Site Overstorey	7 WO	1.5	0.373	9.2	0.688	0.67	117
		1.5	0.346	12.8			
		20.0	0.397	11.0			
		Average	0.372	11.0			
	8 WO	1.5	0.358	10.5	1.450	0.68	117
		20.0	0.338	11.0			
		40.0	0.405[K]	9.5			
		Average	0.367	10.3			
	9 WO	1.5	0.332	14.7	0.700	0.64	115
		1.5	0.350	17.5			
		20.0	0.365	13.0			
		40.0	0.364	16.0			
		Average	0.353	15.3			
	Class	Average	0.362	12.5	0.946	0.66	116
Wet Site Understorey	10 WU	1.5	0.329	15.0	0.850	0.50	108
		1.5	0.361	14.0			
		20.0	0.357	15.5			
		Average	0.349	14.8			
	11 WU	1.5	0.430[K]	14.0	0.802	0.66	109
		1.5	0.386[K]	15.0			
		20.0	0.400[K]	10.5			
		Average	0.402	13.1			
	12 WU	1.5	0.373	16.0	0.876	0.33	133
		1.5	0.385	22.0			
		20.0	0.400[K]	16.0			
		Average	0.386	18.0			
	Class	Average	0.380	15.3	0.843	0.50	116
	Site	Average	0.369	18.8	0.894	0.58	116

1 Height Index = $\frac{\text{Height of tree (ft.)} - 1}{\text{Age of tree at 1 foot}}$

2 "K" indicates the presence of a knot

Significance of difference between means:

Wet and dry sites, specific gravity - P = .02

" " " " ring-frequency - P = .001

(b) Moisture content of the heartwood

The samples that were collected immediately after felling the trees were used to examine the influence of site and dominance class on the moisture content of the heartwood. The methods of sampling, and of measuring the moisture content of the trees were described earlier.

The moisture present in the samples is expressed as the percentage of the dry weight, and as a percentage of that present at saturation. Both methods are used to express the amount of moisture in wood but the latter method, which takes into account differences in the density of the sample, is the more suitable for comparing the moisture contents of fast- and slow-growing trees.

Examination of Table IV shows a marked influence of site and dominance class on the amount of moisture present in the heartwood of the trees by both methods of expressing the moisture content. The average moisture content of the four classes of trees appears to be related to the rate of growth; the moisture content is seen to decrease from a maximum of 48.4 per cent of dry wt. (10.0 per cent of saturation) in the faster-growing dominants on the wet site, to a minimum of 38.8 (7.0) per cent for the slow-growing understorey trees on the dry site; differences which are significant (% d.w., $P = .01$; % sat., $P = .02$). When the means for all samples of each site class were considered, the difference was significant only when the moisture content was expressed as percentage of dry weight ($P = .01$). Fewer samples, however, were available for the percentage saturation determinations.

Tables V and VI demonstrate the relationships between the moisture content, specific gravity, and the ring-frequency of samples taken at 1.5 feet from the ground. There is evidence of a relationship by both methods of expressing the moisture content although a better relationship is evident between ring-frequency and moisture content than between specific gravity and moisture content. Despite some inadequacies in sampling which occurred in the higher specific gravity and ring-frequency classes, there is a definite "falling-off" of the moisture content with increasing specific gravity, and with increasing number of rings per inch. For example, the moisture content expressed as the percentage of saturation in samples with 13.3, 22.8, 30.3, and 41.5 annual rings per inch was, respectively, 12.1, 10.6, 7.5, and 5.3.

From Table IV, it will be noted that the moisture contents of the samples taken at 1.5 feet are considerably higher than those from 20 feet or higher in the trees when the results are expressed by both methods. This condition is characteristic of the distribution of moisture in the stems of the 12 trees, it does not appear to be connected with differences in the specific gravity of the wood, and the following data show that it had an important influence on the

TABLE IV

THE EFFECT OF SITE, DOMINANCE, AND THE ORIGIN OF THE SAMPLE ON THE AMOUNT OF MOISTURE AND PERCENTAGE OF SATURATION IN THE HEARTWOOD OF 12 HEALTHY SUBALPINE SPRUCE

Site and Dominance	Tree No.	Sample[1]	Percentage Dry Wt. (ht. in ft.) 1.5	20	40	Avg.	Percentage Saturation (ht. in ft.) 1.5	20	40	Avg.
Dry Site Overstorey	1 DO	H1	44.7	38.2	36.0		11.1	5.6	4.4	
		H2	51.6				11.8			
		P	59.1							
	Average		51.8			45.9	11.4			8.2
	4 DO	H1	41.5	38.3	37.8		7.8	-	4.5	
		H2	33.7				3.7			
		P	42.8							
	Average		39.3			38.8	5.7			5.3
	5 DO	H1	37.1	40.0	36.4		5.0	6.5	6.8	
		H2	48.3				12.1			
		P	47.6							
	Average		44.3			41.9	8.5			7.6
Class Average			45.1	38.8	36.7	42.2	8.6	6.0	5.2	7.2
Dry Site Understorey	2 DU	H1	34.8	39.3	-		5.3	7.4	-	
		H2	35.2				5.4			
		P	34.6							
	Average		34.9			35.9	5.3			6.0
	3 DU	H1	51.4	36.8	35.5		15.2	5.9	5.3	
		H2	47.5				11.4			
		P	43.7							
	Average		47.5			42.9	13.3			7.5
	6 DU	H1	32.7	34.7	-		2.6	3.6	-	
		H2	39.3				8.2			
		P	39.3							
	Average		37.1			36.5	5.4			4.8
Class Average			39.8	36.9	35.5	38.8	8.0	5.6	5.3	7.0
Site Average			42.4	37.8	36.4	40.6	8.3	5.8	5.2	7.1

TABLE IV (Concl'd)

Site and Dominance	Tree No.	Sample[1]	Percentage Dry Wt. (Ht. in Ft.) 1.5	20	40	Avg.	Percentage Saturation (Ht. in Ft.) 1.5	20	40	Avg.
Wet Site Overstorey	7WO	H1	40.5	40.0	38.8		7.2	7.5	9.4	
		H2	62.4				16.8			
		P	40.0							
	Averages		47.6			44.3	12.0			10.2
	8WO	H1	62.2	43.2	35.9		18.8	7.5	5.1	
		H2	52.3				-			
		P	65.7							
	Averages		60.0			51.8	18.8			10.5
	9WO	H1	49.7	42.1	39.4		10.4	7.2	6.2	
		H2	55.0				14.1			
		P	59.3							
	Averages		54.6			49.1	12.2			9.5
Class Averages			54.1	41.8	38.0	48.4	13.4	7.4	6.9	10.0
Wet Site Understorey	10WU	H1	36.0	37.4	-		3.7	5.0	-	
		H2	41.1				7.1			
		P	43.9							
	Averages		40.3			39.6	5.4			5.3
	11WU	H1	47.7	40.0	-		19.5	7.2	-	
		H2	47.4				11.7			
		P	55.2							
	Averages		50.1			47.6	12.1			10.8
	12WU	H1	36.5	36.6	-		4.9	5.5	-	
		H2	53.1				15.1			
		P	54.6							
	Averages		48.1			45.2	10.0			8.5
Class Averages			46.1	38.0	-	44.1	9.3	5.9	-	8.2
Site Averages			50.1	39.9	38.0	46.5	11.2	7.1	6.9	9.2

1 H1 and H2 represent samples of wood taken from the heartwood. P represents samples taken from the centre of the tree and includes the pith

Significance of difference between means:
Wet and dry sites, (% dry wt.) - P = .01
" " " " (% sat.) - not significant
Wet overstorey (% dry wt.) - P = .01
and dry understorey (% sat.) - P = .02

TABLE V

THE RELATION OF SPECIFIC GRAVITY TO THE MOISTURE CONTENT OF THE HEARTWOOD[1] OF 12 HEALTHY SUBALPINE SPRUCE

Specific Gravity Class	No. of Trees Sampled	Average Specific Gravity	Average Moisture Content[2] % dry wt.	% saturation
0.341-0.360	4	0.348	49.6	12.1
0.361-0.380	3	0.376	45.2	9.9
0.381-0.400	3	0.394	41.0	8.1
0.401-0.420	1	0.408	47.5	12.1
0.421 Plus	1	0.446	35.0	5.3

TABLE VI

THE RELATION OF RING-FREQUENCY TO THE MOISTURE CONTENT OF THE HEARTWOOD[1] OF 12 HEALTHY SUBALPINE SPRUCE

Ring-Frequency Class	No. of Trees Sampled	Average Ring-Frequency	Average Moisture Content[2] % dry wt.	% saturation
10.1 - 18.0	5	13.3	49.2	12.1
18.1 - 26.0	3	22.8	45.6	10.6
26.1 - 34.0	3	30.3	40.6	7.5
34.1 - 42.0	1	41.5	35.0	5.3

1 Averages for each tree are based on two samples taken at 1.5 feet from the ground

2 At time of felling - July 1954

distribution of fungi in the trees; invariably fungi were confined to the wetter basal portion of the trees, none being isolated from samples higher than 20 feet fron the ground.

(c) The occurrence of fungi in the heartwood

The relation of site and characteristics of the heartwood to the occurrence of fungi in living subalpine spruce was investigated within a few days after felling the trees. Three hundred and fifty isolations were made from discs of wood about 6 in. thick cut from sections of the stem at 1.5 feet and at intervals of 10 feet up the tree until a minimum diameter of 4 in. was reached. Each disc was split through the centre to expose a clean surface, then five samples of wood were removed from underneath the freshly exposed surface with a flamed chisel, and placed aseptically on slopes of 2 per cent malt extract agar in test-tubes. To exclude the possibility that some of the isolates from the wood might represent organisms other than those comprising the heartwood flora, control samples of a similar size were taken from the exposed surface of the wood, or from the bark of each disc. A comparison of the culture types obtained by both methods was made.

Fourteen fungi, including six different species were isolated from the samples of wood (Table VII); no fungi were isolated from samples more than 20 feet from the ground. The most common fungus was Coryne sarcoides which was isolated five times. Fomes pini, a wood-destroying fungus, was obtained from samples in one tree at 20 feet but was not associated with decay. The remaining isolates as yet unidentified, were called Unknown No. 1 (1), Unknown No. 2 (2), and Unknown No. 11 (2); the frequency of occurrence of each is shown in brackets. The following organisms, most of them still not yet fully identified, were obtained from the control isolations: Penicillium spp. (15), Bacteria (2), Cladosporium sp. (1), Unknown No. 4 (1), Unknown No. 5 (2), Unknown No. 7 (1), Unknown No. 8 (1), and Unknown No. 9 (1). None of these species, however, was isolated from the unexposed wood. There is evidence, therefore, that the fungi listed in Table VII are species that commonly inhabit the wood of "healthy" subalpine spruce.

The data in T ble VII show the relationship between the incidence of fungi, site, and the dominance class of the trees. Nearly four times the number of infections occurred in trees from the wet site than in trees from the dry site. The percentage of samples that yielded fungi from fast and slow-growing trees on the wet and dry sites was, respectively, 13.3 11.1, 7.0, and 0.0. It is noteworthy that no fungi were isolated from the three understorey trees on the dry site. Some species only occurred on certain sites, e.g., Fomes pini and Unknown No. 1 were isolated only from dry site trees, whereas, Unknowns No. 3, No. 10, No. 11, and Coryne sarcoides were found on the wet site only.

TABLE VII

EFFECT OF SITE, DOMINANCE, AND ORIGIN OF THE SAMPLE ON THE OCCURRENCE OF FUNGI IN 12 HEALTHY SUBALPINE SPRUCE

Site and Dominance	Tree No.	Occurrence of Fungi[1]									No.[2] of Samples	No. and % of samples yielding Fungi	
		Pith			Heartwood			Sapwood					
		1.5 (ht.-ft.)	10	20	1.5 (ht.-ft.)	10	20	1.5 (ht.-ft.)	10	20			
Dry Site (OS)	1 DO	+1	-	-	-	-	-	-	-	+2	15	2	13.3
	4 DO	-	-	+FP	-	-	(+FP)	-	-	-	15	1	7.0
	5 DO	-	-	-	-	-	-	-	-	-	15	0	0.0
Total or Average		1	0	1	0	0	0	0	0	1	45	3	7.0
Dry Site (US)	2 DU	-	-	-	-	-	-	-	-	-	15	0	0.0
	3 DU	-	-	-	-	-	-	-	-	-	15	0	0.0
	6 DU	-	-	-	-	-	-	-	-	-	15	0	0.0
Total or Average		0	0	0	0	0	0	0	0	0	45	0	0.0
Site Totals		1	0	1	0	0	0	0	0	1	90	3	3.3
Wet Site (OS)	7 WO	+CS	-	+3	-	-	-	-	-	-	15	2	13.3
	8 WO	-	-	+3	-	-	-	-	-	-	15	1	7.0
	9 WO	-	+CS	-	+10	+11	-	-	-	-	15	3	20.0
Total or Average		1	1	2	1	1	0	0	0	0	45	6	13.3
Wet Site (US)	10 WU	-	-	-	-	-	-	-	-	-	15	0	0.0
	11 WU	+CS	+2	-	-	-	-	-	-	-	15	2	13.3
	12 WU	+CS	+CS	-	-	+11	-	-	-	-	15	3	20.0
Total or Average		2	2	0	0	1	0	0	0	0	45	5	11.1
Site Totals		3	3	2	1	2	0	0	0	0	90	11	12.2

1 The number above the plus sign represents the designation given to unknown fungi; FP and CS represent Fomes pini and Coryne sarcoides respectively.

2 The samples were distributed as follows: Pith - (1), Heartwood - (2), Sapwood -(2). One control isolation was made at each 10-ft. level up the tree (see text).

Unknown No. 2, which was isolated from the relatively wetter sapwood of tree No. 1 DO, occurred also in tree No. 11 WU, an understorey tree on the wet site for which the highest moisture content was recorded.

There was evidence that the incidence and occurrence of the fungi might depend upon the moisture content of the wood. This possibility was examined by arranging the samples in moisture content classes (percentage of saturation), and calculating the percentage of the samples which yielded fungi. No fungi were present in samples where the moisture content was less than 5 per cent saturation, whereas, 36.3 per cent of the samples falling in the 5 to 10 per cent saturation class, and 62.5 per cent of those in the 10 to 15 per cent class were infected (see Table VIII). Samples from the three height levels, i.e., 1.5, 10, and 20 feet from the ground, and of the two sites occurred indiscriminately among the three moisture content classes, thus, there is evidence that both the frequency of occurrence and the distribution of the fungi in the stems are determined by the amount of moisture present in the heartwood.

TABLE VIII

EFFECT OF MOISTURE CONTENT (PERCENTAGE OF SATURATION) ON THE NUMBER OF INFECTIONS BY HEARTWOOD FUNGI IN LIVING SUBALPINE SPRUCE

Saturation Class	No. of Samples	Average Percentage Saturation	No. and Percentage of Samples Yielding Fungi	
0 - 5.0	3	4.5	0	0.0
5.1 - 10.0	22	7.1	8	36.3
10.1 - 15.0	8	11.8	5	62.5

A. CONDITIONS INFLUENCING THE RATE OF DECAY IN TEST BLOCKS

To investigate effectively the influence of moisture and characteristics of the heartwood on the rate of decay of subalpine spruce, the chief immediate objective was the development of a relatively reliable method for adjusting and maintaining an uniform amount of moisture in the test blocks over the period required for a measurable amount of decay to take place. Preliminary trials, using cultures of Polyporus circinatus and Fomes pini as test fungi, had shown that the rate of decay by both fungi was about twice as high in wood with a specific gravity of 0.390 than in wood with a specific gravity of 0.335 despite relatively similar mean moisture contents of 42.6 and 45.1 per cent of oven dry weight recorded respectively for the two samples, a difference of only 2.5 per cent. Because of the higher dry weight in the dense samples, it was obvious that they held much more water than the difference in these values suggested. Therefore, using the method described on page 13 the moisture contents of the two samples were recalculated as a percentage of the water present at saturation. Values of 6.6 for the less dense and 10.4 per cent for the dense samples were obtained which indicated that nearly twice as much water had occupied the cells of the more rapidly decayed wood. Consequently, in comparative experiments with wood of different densities the amount of moisture was brought to a uniform level of saturation.

In preliminary experiments, the initial moisture content was maintained during incubation by making periodic adjustments to the moisture level of test blocks on the basis of changes taking place in "control" blocks which were sampled from time to time. This method, however, required a large number of additional blocks since the samples of different densities and of the different treatment series, gained or lost water at different rates during incubation. In view of the difficulty of keeping such a large number of blocks for controls, this method was abandoned in favor of the method described on page 18 which entailed weighing each block aseptically mid-way through the incubation, and then adjusting the moisture content.

One factor which undoubtedly contributed to the success of the latter procedure was the adoption of Coniophora putoana as the test fungus in subsequent experiments. It was found that this fungus very rapidly became established in wood, particularly in the inoculum; as a result, inocula and test blocks were rarely lost through the introduction of contaminants. On the other hand, the more slowly growing cultures of P. circinatus and F. pini, which were originally used as test fungi, were highly susceptible to contamination, and more often than not, inocula became contaminated before they could be used to infect the test blocks.

The effect of moisture, site, and dominance class on the decay of the heartwood of subalpine spruce was now investigated. One hundred and ninety-two specimens of heartwood from two positions in each of the 12 trees were prepared as already described (pp. 13 - 18). A statistical design employing the split-plot system of analysis was used. The principal object of the experiment was to discover whether site, dominance class, or the position in the tree, i.e., butt and trunk, from which the specimens of heartwood came had any effect on the rate of decay by Coniophora puteana, but three other variables could be conveniently introduced and this added considerably to the value of the experiment. These were, (a) moisture content (b) method of sterilization, i.e., steam and propylene oxide, and (c) different types of inocula. In addition, the general effect on decay of such variables as ring-frequency and specific gravity could be investigated since these might be evaluated on the basis of any differences between the decay rates of the classes of trees.

Eight test blocks were used from each of the two positions in the 12 trees, of these, one series of four were inoculated with pieces of infected wood, and another series with agar plugs. Two replicates of each series were sterilized in propylene oxide gas while the other two replicates were sterilized in steam as described on page 14, and one replicate series of each sterilization treatment was kept at different moisture levels. At the end of three months, the series inoculated with agar plugs, a total of 96 test blocks, had decayed very little compared with the series inoculated with discs of infected wood; the former, therefore, were left for another month, and the results were analysed separately. The results for the series inoculated with wood discs are set out in Table IX. An analysis of variance was made with this data; the scheme consisted of data for 12 trees and each tree furnished data from samples taken at the butt (1.5 ft.) and the trunk (20ft.). Each sample from each position consisted of four test blocks which included all combinations (2 x 2) of the sterilization and moisture series. There was, therefore, in the experiment three sections dealing with comparisons between the 12 trees, 24 positions, and 96 test blocks, entailing in the analysis of variance three separate estimates of error, each one applicable to its own particular treatment comparisons. Accordingly, the variances of the components in each of these three sections were tested against the particular error variance calculated for the section. The analysis of variance is set out in Table X. When the items were tested by the use of the variance ratio tables, it was found that only moisture had any significant effect on the rate of decay, having a probability of less than .05. The chief results of the experiment are given below under appropriate headings.

TABLE IX

PERCENTAGE LOSS IN WEIGHT OF BLOCKS OF WOOD OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA, AFTER 3 MONTHS

Tree No.	Position in Tree	Method of Sterilizing Blocks: Propylene Oxide M_1	Propylene Oxide M_2	Steam M_1	Steam M_2	Total M_1	Total M_2	GRAND TOTAL
Dry Site Overstorey								
1 DO	Butt	13.2	15.1	10.8	12.7	24.0	27.8	51.8
	Trunk	12.2	11.9	17.9	12.1	30.1	24.0	54.1
Total.....		25.4	27.0	28.7	24.8	54.1	51.8	105.9
4 DO	Butt	10.7	9.1	13.1	8.4	23.8	17.5	41.3
	Trunk	10.9	10.9	9.5	12.1	20.4	23.0	43.4
Total.....		21.6	20.0	22.6	20.5	44.2	40.5	84.7
5 DO	Butt	11.8	10.5	10.8	7.9	22.6	18.4	41.0
	Trunk	12.9	11.0	8.0	9.6	20.9	20.6	41.5
Total.....		24.7	21.5	18.8	17.5	43.5	39.0	82.5
Position Totals	Butt	35.7	34.7	34.7	29.0	70.4	63.7	134.1
	Trunk	36.0	33.8	35.4	33.8	71.4	67.6	139.0
Class Totals.....		71.7	68.5	70.1	62.8	141.8	131.3	273.1
Dry Site Understorey								
2 DU	Butt	14.7	11.8	14.1	13.4	28.8	25.2	54.0
	Trunk	5.7	8.1	15.0	14.2	20.0	22.3	42.3
Total.....		19.7	19.9	29.1	27.6	48.8	47.5	96.3
3 DU	Butt	9.4	10.0	9.3	7.1	18.7	17.1	35.8
	Trunk	12.0	13.2	18.3	14.1	30.3	27.3	57.6
Total.....		21.4	23.2	27.6	21.2	49.0	44.4	93.4
6 DU	Butt	12.1	10.5	9.5	8.7	21.6	19.2	40.8
	Trunk	9.9	12.3	10.4	9.8	20.3	22.1	42.4
Total.....		22.0	22.8	19.9	18.5	41.9	41.3	83.2
Position Totals	Butt	36.2	32.3	32.9	29.2	69.1	61.5	130.6
	Trunk	26.9	33.6	43.7	38.1	70.6	71.7	142.3
Class Totals.....		63.1	65.9	76.6	67.3	139.7	133.2	272.9
Total for site....		134.8	134.4	146.7	130.1	281.5	264.5	546.0

TABLE IX (Concl'd)

Tree No.	Position in Tree	Method of Sterilizing Blocks: Propylene Oxide M_1	Propylene Oxide M_2	Steam M_1	Steam M_2	Total M_1	Total M_2	GRAND TOTAL
Wet Site Overstorey								
7 WO	Butt	14.5	15.0	14.3	13.1	28.8	28.1	56.9
	Trunk	14.8	11.8	15.0	14.8	29.8	26.6	56.4
Total.....		29.3	26.8	29.3	27.9	58.6	54.7	113.3
8 WO	Butt	13.0	8.0	13.4	13.4	26.4	21.4	47.8
	Trunk	9.1	9.9	11.9	10.8	21.0	20.7	41.7
Total.....		22.1	17.9	25.3	24.2	47.4	42.1	89.5
9 WO	Butt	11.8	12.9	11.4	10.8	23.2	23.7	46.9
	Trunk	10.5	10.6	9.8	11.5	20.3	22.1	42.4
Total.....		22.3	23.5	21.2	22.3	43.5	45.8	89.3
Position Totals	Butt	39.3	35.9	39.1	37.3	78.4	73.2	151.6
	Trunk	34.4	32.3	36.7	37.1	71.1	69.4	140.5
Class Totals.....		73.7	68.2	75.8	74.4	149.5	142.6	292.1
Wet Site Understorey								
10 WU	Butt	10.8	10.4	11.4	12.2	22.2	22.6	44.8
	Trunk	21.2	12.5	13.6	11.4	34.8	23.9	58.7
Total.....		32.0	22.9	25.0	23.6	57.0	46.5	103.5
11 WU	Butt	13.8	13.0	15.5	14.4	29.3	27.4	56.7
	Trunk	10.4	10.0	10.6	9.0	21.0	19.0	40.0
Total.....		24.2	23.0	26.1	23.4	50.3	46.4	96.7
12 WU	Butt	11.6	7.2	6.4	7.8	18.0	15.0	33.0
	Trunk	12.1	8.8	10.3	11.3	22.4	20.1	42.5
Total.....		23.7	16.0	16.7	19.1	40.4	35.1	75.5
Position Totals	Butt	36.2	30.6	33.3	34.4	69.5	65.0	134.5
	Trunk	43.7	31.3	34.5	31.7	78.2	63.1	141.2
Class Totals.....		79.9	61.9	67.8	66.1	147.7	128.0	275.7
Total for Site....		153.6	130.1	143.6	140.5	297.2	270.6	567.8

*

(M_1) - Moisture content at 60 per cent of the dry weight

(M_2) - Moisture content at 14 per cent of saturation

TABLE X

ANALYSIS OF VARIANCE OF LOSS IN WEIGHT OF BLOCKS OF WOOD OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA, AFTER 3 MONTHS

Factor	Sum of Squares	Degrees of Freedom	Variance	Variance Ratio
Wet site vs. Dry site	4.95	1	4.95	3.90
Overstorey vs. Understorey	2.86	1	2.86	6.75
Site vs. Dominance Class Interaction	2.76	1	2.76	7.00
Error (i)	154.48	8	19.31	
Total (i).....	165.05	11		
Butt vs. Trunk	1.55	1	1.55	11.50
Butt vs. Trunk Interaction	12.14	3	4.05	4.40
Error (ii)	142.70	8	17.83	
Total (ii).....	321.44	23		
Treatments:				
Sterilization Propylene oxide vs. Steam	0.66	1	0.66	6.13
Moisture Content 60% dry wt. vs. 14% sat.	19.80	1	19.80*	4.80*
Sterilization vs. moisture Interaction	0.79	1	0.79	5.12
Remaining Interactions	94.51	21	4.50	1.11
Error (iii)	194.58	48	4.05	
Total (iii).....	631.78	95		

* Significant at 5 per cent point

(a) Moisture

It has already been pointed out that when moisture content is expressed as a percentage of the dry weight, an erroneous picture is given of the amount of water held by wood of different densities. Thus, in the following experiments for comparative purposes, the mean* moisture contents of the samples were expressed as percentages of those held at saturation.

To elucidate certain discrepancies that occurred in previous decay data through expressing the moisture content on the basis of dry weight, the two methods were compared by adding an amount of moisture to one series of blocks (called M_1 series) equal to 60 per cent of the dry weight, ignoring differences in density, while to another series (called M_2 series), an amount of water was added to give 14 per cent saturation. Since the moisture content at 14 per cent saturation was equivalent to a moisture content of 60 per cent of dry weight for wood with a specific gravity of .310, the moisture contents for test blocks with higher specific gravities could be obtained by substitution in the original formula given on page 13 thus,

Moisture content as a percentage of the dry wt. of the test block = $\frac{14 \; [\text{illegible}] + 14.98}{SG}$

where, 14 and 14.98 are constants, and SG is the specific gravity based on the green volume of the particular test block.

This procedure, gave two moisture levels for the experiment, the M_1 series, in effect had higher moisture contents (% sat.) than the M_2 series for test blocks of specific gravity greater than .310. Table XI, which sets out the mean moisture contents corresponding to the decay values given in Table IX, illustrates the results that were obtained. The mean moisture contents of the M_2 series were relatively uniform at 16.9 and 16.6 per cent of saturation respectively for samples from the two sites, despite very different values of .344 and .372 in specific gravity for the wet and dry site samples. In contrast, the mean moisture contents of the denser, dry site samples in the M_1 series reflect the effect of specific gravity, with mean moisture contents of 18.7 and 21.6 per cent respectively.

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* The mean percentages of saturation values were calculated from the initial and final moisture contents which were expressed originally as percentages of the dry weights of the test blocks. (See formula on p. 13).

TABLE XI

MEAN MOISTURE CONTENT (% SATURATION), DURING INCUBATION, OF BLOCKS OF WOOD OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA

Tree No.	Position in Tree	Moisture Treatment* M_1			M_2		
		Prop.	Oxide	Steam Avg.	Prop.	Oxide	Steam Avg.
Dry Site Overstorey							
1 DO	Butt	20.2	22.0	21.1	16.6	17.8	17.2
	Trunk	17.8	18.7	18.2	15.0	16.4	15.7
4 DO	Butt	21.1	20.5	20.8	15.8	13.8	14.8
	Trunk	18.8	15.8	17.3	16.2	14.3	15.2
5 DO	Butt	23.0	21.3	22.1	15.2	13.9	14.5
	Trunk	20.0	16.8	18.4	17.8	15.6	16.7
Class Total or Average		120.9	115.1	19.6	96.6	91.8	15.8
Dry Site Understorey							
2 DU	Butt	25.9	29.4	27.6	18.2	21.6	19.9
	Trunk	20.6	28.0	24.3	16.9	19.4	18.1
3 DU	Butt	25.8	21.8	23.8	17.3	16.2	16.7
	Trunk	22.2	21.4	21.8	17.2	16.6	16.9
6 DU	Butt	22.7	20.4	21.5	16.7	13.5	15.1
	Trunk	22.6	21.6	22.1	19.7	17.3	18.5
Class Total or Average		139.8	142.6	23.5	108.0	104.6	17.5
Dry Site Total or Average		260.7	257.7	21.6	204.6	196.4	16.6

TABLE XI (Concl'd)

MEAN MOISTURE CONTENT (% SATURATION), DURING INCUBATION, OF BLOCKS OF WOOD OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA

Tree No.	Position in tree	Moisture Treatment M_1			M_2		
		Prop.	Oxide	Steam Avg.	Prop.	Oxide	Steam Avg.
Wet Site Overstorey							
7WO	Butt	15.4	17.2	16.3	15.4	19.7	17.5
	Trunk	19.0	19.5	19.2	19.1	16.9	18.0
8WO	Butt	17.0	16.2	16.6	14.9	19.1	17.0
	Trunk	17.3	16.9	17.1	16.3	15.0	15.6
9WO	Butt	19.4	19.1	19.2	16.2	16.2	16.2
	Trunk	18.5	17.1	17.3	18.7	16.2	17.4
Class Total or Average		106.6	106.0	17.7	100.6	103.1	16.9
Wet Site Understorey							
10WU	Butt	19.5	20.0	19.7	17.3	18.9	18.1
	Trunk	23.0	18.5	20.7	16.5	17.8	17.1
11WU	Butt	22.0	20.2	21.1	18.8	18.5	18.6
	Trunk	20.7	19.6	20.1	15.9	15.2	15.5
12WU	Butt	19.1	18.3	18.7	19.1	16.1	17.6
	Trunk	19.1	18.7	18.9	15.8	14.3	15.0
Class Total or Average		123.4	115.3	19.9	103.4	100.8	17.0
Wet Site Total or Average		230.0	221.3	18.7	204.0	203.9	16.9

* (M_1) - Moisture content at 60 per cent of the dry weight

(M_2) - Moisture content at 14 per cent of saturation

The mean moisture content values for the two series were 20.2 per cent for M_1 and 16.8 per cent for M_2 representing a difference between the treatments of 3.4 per cent. Since the percentage loss in weight due to decay for series M_1 and M_2 was 12.05 and 11.30 respectively, a difference of only 3.4 per cent saturation in the moisture content resulted in a difference of 0.75 per cent in decay (Table XII). It is noteworthy that differences in the moisture content smaller than 3.4 per cent, viz., 1.2 per cent between the two sites, 2 per cent between the two dominance classes, and 0.7 per cent between the two positions did not result in significant differences in the rate of decay by C. puteana.

An analysis of the relation of moisture content to decay when the data were arranged in specific gravity and ring-frequency classes showed that, without exception, the amount of decay increased regularly with increasing moisture content of the samples (Tables XIII and XIV). Thus, the samples in each of the specific gravity classes show an increase in decay with increasing moisture content, a trend that was closely paralleled in the ring-frequency classes. A comparison of the mean moisture content values obtained from the averages of the two sets of data (ring-frequency values in brackets), viz., 14.9 (15.2), 17.2 (17.2), and 22.7 (22.2) with the corresponding decay values, viz., 10.3 (10.1), 11.7 (11.3), and 12.5 (12.2) shows a close relationship although neither one of these properties appear to influence the rate of decay by C. puteana.

(b) Wood Characteristics

(1) Specific gravity and ring-frequency

From the foregoing analysis, there was no consistent evidence of a relationship between ring-frequency and decay, or between specific gravity and decay, but there was a tendency in most of the classes for the high moisture values to be associated with high specific gravity and ring-frequency values, a condition which may have obscured the effect of any decay-resisting properties that were present in the dense wood. This possibility was investigated in the following way. Samples with moisture contents falling in the relatively narrow range of 17.5 to 19.5 per cent of saturation were selected, arranged in ring-frequency classes, and values calculated as before (Table XV), but there was no evidence of a relation between ring-frequency and decay with the moisture contents thus controlled.

TABLE XII

CHARACTERISTICS OF THE SPECIMENS OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA IN RELATION TO SITE, DOMINANCE CLASS, POSITION, AND TREATMENT FACTORS AS TESTED BY ANALYSIS OF VARIANCE

Factor Analysed	Average* Percentage Saturation	Loss-in-weight (% dry wt.)	Ring-frequency	Specific Gravity
Wet Site	17.8	11.9	18.0	0.344
Dry Site	19.0	11.3	28.2	0.372
Difference....	1.2	0.6	10.2	0.028
Overstorey	17.4	11.7	19.1	0.339
Understorey	19.4	11.5	27.1	0.377
Difference....	2.0	0.2	8.0	0.038
Butt	18.8	11.4	26.4	0.366
Trunk	18.1	11.7	19.8	0.350
Difference	0.7	0.3	6.6	0.016
Moisture-60% d.w.	20.2	12.05	samples from the same piece of wood	
Moisture-14% Sat.	16.8	11.30		
Difference....	3.4	0.75**	None	None

* Each value is based on the following number of observations:
Percentage of Saturation - 48
Loss-in-Weight - 48
Specific Gravity - 12
Ring-frequency - 12

** The difference is significant at the 5 per cent point

TABLE XIII

THE AVERAGE DECAY LOSS CAUSED BY CONIOPHORA PUTEANA IN SPECIMENS OF SUBALPINE SPRUCE IN RELATION TO MOISTURE CONTENT AND SPECIFIC GRAVITY

Specific Gravity Class	Moisture Content - Percentage of Saturation									Class Averages	
	<16			16 to 20			>20				
	No.*	Avg. % Sat.	Avg. Decay	No.	Avg. % Sat.	Avg. Decay	No.	Avg. % Sat.	Avg. Decay	% Sat.	Decay
.301-.320	5	14.7	11.9	7	17.5	14.0	0	-	-	16.3	13.2
.321-.340	3	15.0	10.1	13	17.8	12.0	0	-	-	17.4	11.6
.341-.360	3	15.1	9.0	11	18.9	11.2	2	20.8	11.9	17.9	10.9
.361-.380	4	14.9	9.9	18	17.0	11.3	11	21.7	12.9	18.3	11.6
.381-.400	1	15.2	7.8	2	18.2	11.1	2	25.5	12.9	25.1	13.3
.401-.420	0	-	-	2	16.7	8.5	2	23.8	9.3	19.8	8.9
.421-.440	0	-	-	1	18.2	13.4	3	25.6	13.5	23.8	13.5
Total or Average	16	14.9	10.3	54	17.2	11.7	20	22.7	12.5	90 samples	

* Number of samples

TABLE XIV

THE AVERAGE DECAY LOSS CAUSED BY CONIOPHORA PUTEANA IN SPECIMENS OF SUBALPINE SPRUCE IN RELATION TO THE MOISTURE CONTENT AND THE NUMBER OF RINGS PER INCH

Ring Frequency (No. rings per inch)	Moisture Content - Percentage of Saturation: 16			16 to 20			20			Class Averages		
	No.*	Avg. % Sat.	Avg. Decay	No.	Avg. % Sat.	Avg. Decay	No.	Avg. % Sat.	Avg. Decay	Avg. R.F.**	Avg. % Sat.	Avg. Decay
8 - 12	2	15.6	10.3	14	17.7	12.9	0	-	-	9.1	17.4	12.7
12 - 16	2	15.0	10.0	8	17.7	11.2	2	20.7	17.4	14.3	17.7	12.0
16 - 20	2	15.5	9.5	4	17.5	10.3	2	20.1	10.5	18.7	17.7	10.2
20 - 24	2	15.2	11.5	12	17.6	11.2	2	21.8	15.1	20.9	17.8	11.7
24 - 28	6	15.0	9.9	4	18.3	13.0	6	21.6	11.1	25.4	18.3	11.1
28 - 32	0	-	-	2	19.9	12.6	2	27.6	14.0	30.5	23.7	13.3
32 - 36	2	15.1	9.6	8	17.8	10.9	10	24.3	11.3	31.3	18.0	9.9
36 - 40	0	-	-	4	18.9	11.2	0	-	-	38.0	18.9	11.2
Total or Average	16	15.2 (Avg. R.F.-21.6)	10.1	56	17.2 (Avg. R.F. - 20.2)	11.3	24	22.2 (Avg. R.F. - 27.9)	12.2	Total of 96 samples		

* Number of samples

** R.F. - Ring Frequency

TABLE XV

RELATION BETWEEN RING-FREQUENCY AND LOSS-IN-WEIGHT IN SPECIMENS OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA, MOISTURE CONTENT APPROXIMATELY UNIFORM

Ring-Frequency Class	No. of Samples	Average Ring-Frequency	Average Moisture Content (% sat.)	Average Loss-in-Weight (% dry wt.)
8 - 12	3	10.8	18.2	14.1
12- 16	1	13.0	18.9	11.2
16 - 20	1	20.5	18.4	10.4
20 - 24	3	23.3	18.5	10.6
24 - 28	2	26.2	18.3	13.0
28 - 32	0	-	-	-
32 - 36	2	36.0	18.3	12.4
36.- 40	1	38.0	18.1	11.3

When the wood characteristics, ring-frequency and specific gravity, were compared for the site, dominance class, and position series it was seen that differences of 10.2, 8.0., and 6.6 in the number of rings per inch, and differences of 0.028, 0.038, and 0.016 in the specific gravity of the samples had no significant effect on decay. (see Table XII). It is of some interest, however, that in each of these comparisons there was less decay in the denser wood in spite of higher moisture contents. Whether this indicated a connection between decay resistance and the structure of the wood was not clear from the data, but the evidence suggests that wood structure is possibly less important than moisture and does not affect significantly the rate of decay.

(2) Knots

The possibility that knots might affect, either directly or indirectly, the progress of decay was investigated by noting the occurrence of knots in each of the test blocks used in the experiment. Knots were present in 25 per cent of the test blocks derived from dry site trees, and in 22.9 per cent of those from wet site trees. No attempt was made to classify the knots by size; their diameters were between 1 and 10 mm.

The occurrence of knots in relation to the four dominance classes was investigated more comprehensively with 236 samples (1 x 1 x 6-7 inches) derived from the 12 trees. The average number of knots counted in the samples from the wet overstorey, wet understorey, dry overstorey, and dry understorey trees was, respectively, 1.71, 1.73, 1.70 and 1.87. The mean number of knots per sample for the wet site was 1.72 and the mean for the dry site was 1.74. Thus, there was no connection in the samples between the occurrence of knots, the dominance classes, and resistance to decay.

Because the specimens obtained from the trees came originally from the relatively knot-free internodal portion of the stem, itwas interesting to see how the occurrence of knots in the samples compared with the occurrence in trees. A characteristic of conifer, particularly spruce, is the regular formation of branch whorls which are coincident with annual growth. Thus, the age of the tree divided by the height of the tree gave an estimate of the number of knots,* i.e. branch whorls per foot of the trees. The values obtained for the wet overstorey and understorey, and dry overstorey and understorey were, respectively, 1.49, 2.16, 2.50, and 2.16, with mean values of 1.82 for the wet site, and 2.33 for the dry site.

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* It is realized that a number of knots, possibly 5 or 6, might occur in a cluster at each node, but for convenience and simplicity each node has been assumed to contain one knot.

The dry site overstorey trees were much older so that the number of knots per linear foot in the three remaining classes of trees gave a better indication of a relation between site, dominance class, and the occurrence of knots in living trees. The theoretical values thus obtained show that knots were much more abundant in the wood of the slower-growing trees, but this fact was not demonstrated in the sample blocks. The question whether knots might affect the progress of decay in living trees was, therefore, unanswered by the laboratory experiments owing to the inadequacies of the sample. In other respects, however, the relationship observed between the frequency of branch whorls in the trees and site was an interesting one, particularly since it also suggested a possible connection between the occurrence of branches (or branch stubs) and the moisture content of the stems. This possibility has some significance in the light of studies recently made by Chalk and Bigg, (1956) who obtained evidence that the moisture content of the main stem of European larch was reduced upon the removal of dead branches.

(c) Origin of the sample

(1) Site and dominance class

Site and dominance class were found to have no significant effect on decay when the decay rates of 48 test-blocks in each of these categories were compared and tested by analysis of variance (Tables X and XII). There was a tendency, however, for decay to be greater in the samples from the wet site trees than in those from dry site trees. Similarly, decay was greater in the samples from the overstorey than in those from the understorey. Lower moisture values were recorded for the two categories where greater decay losses occurred.

Because of the close relationship which existed between the physical characteristics, i.e., ring-frequency and specific gravity, and the site and dominance classes, it was not possible to separate the effect on decay of these and other properties that might characterize the heartwood of the different trees. If, as the evidence already given suggests, the principal effect on decay of the wood structure is determined by the water relations in cells of different sites, it might be possible that the consistently lower decay rates obtained in specimens from understorey and dry site trees is a response to heartwood properties other than the amount of water that occupied the cells. For example, in Table XI it is seen that decay rates of 11.55 per cent and 11.00 per cent of the dry weight were obtained for overstorey and understorey trees respectively when mean values for the space occupied by water in the cells of the two classes of

trees differed by only 0.3 per cent. It is unlikely that a difference in the moisture content of this magnitude would have any measurable effect on the rate of decay.

(2) Position in the tree

A comparison of the rates of decay of 48 test blocks of heartwood from the butt (at 1.5 feet from the ground) with an equal number from the trunk (at 20 feet from the ground) revealed differences of only 0.3 per cent in the average loss in weight between the samples (Tables X and XII). The samples from the butt were characterised by higher mean values for ring-frequencies and specific gravities, and slightly lower moisture contents than the samples from the trunk position.

(d) Cultural conditions

(1) Method of sterilization

Since it was possible that some natural constituents of the heartwood, viz., resins, might be affected by steaming the test-blocks during the sterilization process it was necessary to investigate this factor. Consequently, one series of 48 test-blocks were sterilized by steaming and another series which served as controls, were sterilized over propylene oxide gas in a manner already described, and the two methods were compared and tested by analysis of variance (Tables IX and X). The average loss by decay of the steam sterilized blocks was 11.85 per cent of dry weight compared with 11.50 per cent obtained for the blocks sterilized by propylene oxide, but this difference was not statistically significant. The experiment demonstrated, therefore, that decay-resisting resins or other volatile substances which might be removed or reduced in quantity by steaming were unimportant in preventing decay by C. puteana. Where the blocks were inoculated with agar plugs, propylene oxide gas proved the more reliable sterilizing agent although in the above experiment where wood discs were used, both sterilizing agents were equally effective.

(2) Source of inoculum

As already stated, a total of 192 test-blocks were divided into two series, which were inoculated with infected discs of wood or agar plugs from plate cultures. The objective was to determine the effect of the source and size of inoculum on the rate of decay by C. puteana, but the differential effect that the inoculum might have on decay in the test-blocks from the two sites was also studied.

Because the rate of decay in the blocks inoculated with wood discs was obviously greater at the end of three months than in the blocks inoculated with agar plugs, the latter were left for another month. In the agar-inoculated blocks the average decay loss after four months was 5.9 per cent of the dry weight, whereas, the average decay loss was twice as high in the wood-inoculated blocks after only three months, infection was well established after three weeks in the latter blocks and after six weeks in the former. Twelve test-blocks representing two trees in the agar-series were contaminated at the end of the four month period. These blocks had been steam sterilized. It is noteworthy, however, that no other blocks were lost through contamination out of a total of 180 blocks. For these two reasons, an analysis of variance as originally intended was not undertaken on the combined data.

Because the two treatments differed significantly in their effect on decay, the results were examined to find the cause for the appreciably slower decay of the agar-inoculated blocks. The mean moisture values of 128 successfully infected test-blocks representing four trees and two sites from each of the two series were compared (see Table XVI). There were lower moisture values in the agar series in each of the four dominance classes but it has already been shown that these differences in moisture were not considered sufficiently great to account for the large differences in decay. For example, when the data for the two series were arranged in moisture content classes, and the average values for moisture were plotted against the average values for decay, two distinct curves were obtained (Fig. 5) which indicated that the difference in the rates of decay could not be attributed to differences in the moisture content. Differences in the decay rates, therefore, could be attributed either to the size or to the nutrients contained in the inoculum, i.e. the inoculum potential described by Garrett (1956). From the standpoint of determining the exact nature of the superior infection- and decay-promoting qualities of the wood-inocula, the different sizes of the wood discs, 22 mm., and agar plugs, 5 mm., were unfortunate since neither the size nor the supply of nutrients of the inocula could be properly assessed. When agar was used, two plugs of inocula were placed diametrically opposite at the base of the cavity; thus, compared with the wood discs which filled the cavity, the agar did not come in contact with as large an area of the cavity. It is unlikely that a disc of wood contained more of any growth factor than the two agar plugs, but probably contained more water. In the saturated atmosphere of the decay chambers, however, any effect of the water content of the inoculum was probably very little.

TABLE XVI

EFFECT OF TYPE OF INOCULUM ON LOSS-IN-WEIGHT IN SPECIMENS OF SUBALPINE SPRUCE DECAYED BY CONIOPHORA PUTEANA

Site and Dominance Class	Tree No.	Agar Inoculum Series*		Wood Inoculum Series**	
		Moisture % Sat.	Decay % d.w.	Moisture % Sat.	Decay % d.w.
Dry Site Overstorey	1 DO 4 DO	15.1	4.8	17.5	11.9
Dry Site Understorey	2 DU 3 DU	18.9	8.7	21.0	11.8
Dry Site Average.....		17.0	6.7	19.2	11.8
Wet Site Overstorey	7 WO 8 WO	14.6	5.0	17.2	12.7
Wet Site Understorey	10 WU 12 WU	16.0	5.3	16.9	11.2
Wet Site Average.....		15.3	5.1	17.0	11.9
AVERAGE FOR SERIES		16.1	5.9	18.1	11.9

NOTE: Each value is the average of 16 replidates representing two positions in each of two trees

* After four months.

** After three months

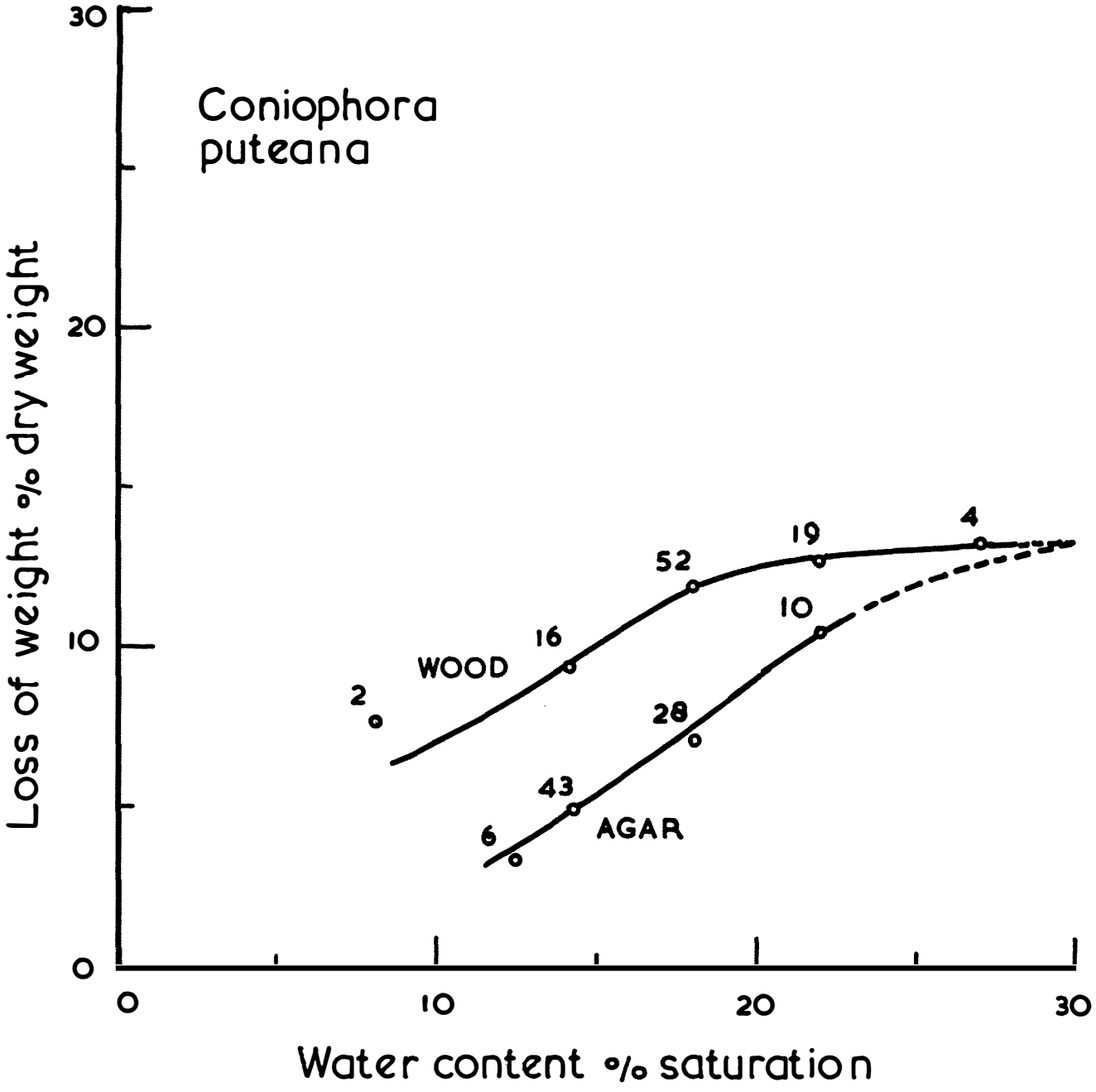

Fig. 5. Curves showing the relationship between the decaying activity of Coniophora puteana, the moisture content of the substratum, and the method of inoculating the test blocks. Wood-inoculum series at the end of three months, agar-inoculum series at the end of four months.

The evidence, although inconclusive, suggested that it was the larger mass of the wood-inoculum, and the larger area of contact, which was responsible for the increased rate of decay.

Although an interaction between inocula and site could not be satisfactorily tested by analysis of variance because 12 trees were needed and only eight were available, comparisons of decay losses similar to those between sites in the wood-inoculated series were obtained.

B. CONDITIONS INFLUENCE IMBIBITION OF WATER BY WOOD

It has been shown that marked differences occurred between the moisture content of the heartwood of subalpine spruce on wet and dry sites, and relationships have been demonstrated between the moisture contents of particular classes of trees and the incidence of heartwood fungi. There was also shown to be a connection between moisture content and the physical properties of wood, i.e., width of the annual rings and specific gravity, but it was not known whether this was due to the physical characteristics of the wood itself, i.e. its water-holding capacity, or, whether the amount of moisture in the heartwood was determined independently by the amount of water available to the particular class of trees. It seemed, therefore, that it would be of some importance to investigate the hygroscopic properties of wood from the different site and dominance classes to see if differences in wood structure alone would explain the differences between the moisture contents observed in the living trees.

(a) Origin of the sample

In the first experiment, the hygroscopic properties of samples of wood from the different site and dominance classes were investigated by comparing the relative amounts of water taken up by the different samples after equilibrium had been reached in the atmosphere of the room (about 65 per cent relative humidity), and in a saturated atmosphere. Tests were made with pieces of air-dried wood about 1 x 1 x $\frac{1}{4}$ inches which were taken from the 1.5 foot level of the trees. One sample from each of the trees was weighed, then oven-dried at 105° C. for 24 hours, reweighed, and the difference between the weighings was calculated as a percentage of the oven-dried weight of the sample. The samples were then suspended in test-tubes over water and the tubes sealed with aluminium foil. Increases in the weight of the samples caused by the absorption of water were determined at intervals of three days; after 15 days no further increases were observed, it was assumed

that the moisture in the wood was in equilibrium with the atmosphere and that the fibre saturation point had been reached. The results are summarized in Table XVII, in which the values quoted are for moisture contents after three months in the atmosphere of the room, and after 14 days in 100 per cent relative humidity. From these preliminary trials, the water-imbibing capacities of the samples from the wet site trees were slightly greater than those in samples from the dry site trees but the differences were such that the small sample which was used did not exclude the possibility of experimental error.

As this question was of some importance a further experiment was made with an adequately replicated sample. 48 samples of heartwood each about 1 x 1 x 6-7 inches, two from each of two positions (10ft. and 30ft. from the ground) of the 12 trees were selected; the samples were then sawn in half. The 96 samples were oven-dried, weighed, and placed in large desiccators over water and sealed. The blocks were left to absorb water until no further increase in weight could be observed (this took about two months), then each block was removed and immediately weighed. Data was also obtained on the amount of water taken up by the samples after they has been kept in the laboratory for six months. From the two weighings, the amount of moisture absorbed by the samples in 100 per cent and in 60-65 per cent relative humidity was calculated and expressed as a percentage of the oven-dried weight of the sample (Table XVIII).

TABLE XVII

THE EFFECT OF THE ORIGIN OF THE SAMPLE ON IMBIBITION OF WATER BY WOOD
(BASIS - 11 SAMPLES)

Site and Dominance Class	Average Equilibrium Moisture Content[1]	
	Relative Humidity (Room)	Relative Humidity (100%)
Wet Site Overstorey	9.65	28.9
Wet Site Understorey	9.49	28.5
Dry Site Overstorey	9.29	27.5
Dry Site[2] Understorey	9.29	27.9

[1] Values represent averages of three tree samples

[2] Values represent averages of two tree samples

TABLE XVIII

THE EFFECT OF THE ORIGIN OF THE SAMPLE ON IMBIBITION OF WATER BY WOOD

(BASIS - 96 SAMPLES)

Site and Dominance Class	Average Ring-Frequency	Average Equilibrium Moisture Content	
		Relative Humidity (Room)	Relative Humidity (100%)
Wet Site Overstorey	14.9	7.44	28.85
Wet Site Understorey	22.1	7.56	29.62
Average.....	18.5	7.50	29.23
Dry Site Overstorey	21.4	7.44	28.63
Dry Site Understorey	29.3	7.51	27.94
Average.....	25.3	7.47	28.28

The average moisture contents at which the fibres became saturated for samples from the wet and dry sites were, 29.15 and 28.45 respectively, but the difference between the two values was not significant statistically. The moisture absorbed at room humidity, i.e., a moisture content below the fibre saturation point, was about the same for samples from the two sites. There was, therefore, no difference in the water-imbibing properties of the wood fibres themselves. These experiments showed that differences due to the origin of the sample only slightly affected the amount of water absorbed at the fibre saturation point of the wood, thus, it was unlikely that variations in the moisture content of living trees could be explained on this basis.

(b) Width of the annual rings

To elucidate the apparent connection between the characteristics of growth of the trees and the water absorbing power of the wood, the relation between the ring-frequency and the hygroscopic properties of the samples was investigated. The number of annual rings per inch were counted for each of the samples; the samples then arranged in four ring-frequency classes and the average ring-frequency and moisture content values at the two humidity levels were calculated. The results are summarized in Table XIX.

TABLE XIX

THE EFFECT OF THE RING-FREQUENCY OF THE SAMPLES ON IMBIBITION OF WATER BY WOOD

Ring-Frequency Class (rings per inch)	No. of Samples	Average Ring-Frequency	Average Equilibrium Moisture Content	
			Room Relative Hum.	100% Relative Hum.
8 - 16	10	12.6	7.49	29.41
16 - 24	16	20.8	7.46	28.85
24 - 32	9	26.3	7.51	28.40
32 - 40	2	35.0	7.43	27.47

The relationship between the growth characteristics of the wood and the moisture content at which the fibres became saturated was more clearly seen when ring-frequencies were used as a measure of the growth rate. The average moisture content at fibre saturation for samples with ring-frequencies of 12.6, 20.8, 26.3 and 35.0 was, respectively, 29.41, 28.85, 28.40, and 27.47, that is, more water was taken up by the lighter wood having the larger cells. This analysis also confirmed that the moisture content of wood below the fibre saturation point was not affected by the size of the cells.

(c) Temperature

Variations in temperature are known to occur in the heartwood of forest trees, and in one instance these have been correlated with site moisture conditions (Saharov, 1952). It was possible, therefore, that variations in temperature might cause differences in the moisture content of trees by influencing the rate of moisture imbibition by wood.

In the following experiment, wood shavings were used instead of wood blocks, since Zeller, (1920) had found that the moisture-imbibing power of wood was not changed by shaving, but imbibition was more rapid than when blocks were used. Zeller also claimed that oven-drying affects the hygroscopic properties of wood by reducing the water absorbing capacity below that shown by air-dried samples, so, shavings were planed from the (radial) surface of samples which had not been oven-dried, and the oven-dry weights were determined at the end of the experiment. Ring-frequency and specific gravity determinations were made on the original sample and by the usual methods. Samples of shavings from wood of various ring-frequencies, each weighing about 1 g., were loosely rolled into tubes, suspended over water in flat-bottomed test-tubes (1 x 5 inches), and then sealed with a cork. The shavings were held in position largely by the natural resilience of the wood, but dents were made in the glass half way up the test tubes to keep the shavings at a standard distance from the water. The tubes containing the samples were kept at three different temperatures over a period of 10 weeks; the period at each temperature was determined when no increase in weight was observed in a control sample which was removed at weekly intervals from the tubes, placed immediately in weighing-bottles, and weighed. Two identical weighings for each sample were obtained before the series were changed to a different temperature. The shavings were equilibrated at room temperature (about 22.5°C.) for 28 days, at 33°C. for 29 days, and at 10°C. for 26 days; the moisture contents were recorded at the beginning and at the end of each successive period. Generally, the shavings responded fairly rapidly to any change in temperature (usually, equilibrium was attained after from one to two weeks) and increases were noted in the moisture content when

the temperature was raised or lowered. Characteristic curves showing the typical response in water imbibition by four samples, each of different ring-frequencies, at the three temperatures are given in Fig. 6.

The average maximum moisture content values for the ten samples, at the three temperatures were 30.98, 32.56, 33.43, respectively, representing a rather sharp response when the temperature was increased, and a less pronounced one when the temperature was lowered. The principal effect on moisture-imbibition in the experiment was seen to be caused by fluctuations in temperature rather than by the amount or the direction of the temperature change. It was also seen that abrupt changes in temperature caused an increase in the moisture content of the wood above the point at which the fibres were normally saturated, i.e., 28 to 30 per cent o.d.w.; there was evidence, therefore, that the amount of free-water held by wood was to some extent regulated by fluctuations in temperature.

There was no consistent relation between the final moisture content values and ring-frequency among the 10 samples used in this experiment, although the extremes in moisture content values generally coincided with extremes in ring-frequency values. Temperature was seen to have no differential effect on the general tendency for wood with wide annual rings to absorb more water when the fibres were saturated.

(iii) Fungi in the Heartwood

A. COMPARATIVE PHYSIOLOGICAL STUDIES

During the course of field studies in Alberta, the following wood-destroying fungi were found associated with nearly 90 per cent of the decay in a representative sample of subalpine spruce. Fomas pini, which occurred in the butt, top, and trunk of the trees, was associated with 34.4 per cent of the decay volume. Polyporus circinatus, which occurred only in the lower part of the trees, was associated with 16.6 per cent of the total decay volume. Stereum sanguinolentum and Peniophora septentrionalis, occurring in the upper part of the trees, were associated with 14.0 and 11.2 per cent, respectively, of the decay volume. Three others, Flammula connissans, Unknown C, and Coniophora puteana were confined to the root and butt regions, and were associated with considerably smaller volumes of decay. Polyporus circinatus and S. Sanguinolentum each caused more infections than F. pini, but together were responsible for less decay. It is thus seen that considerable individual variation occurred in the ability of the different fungi to cause decay,

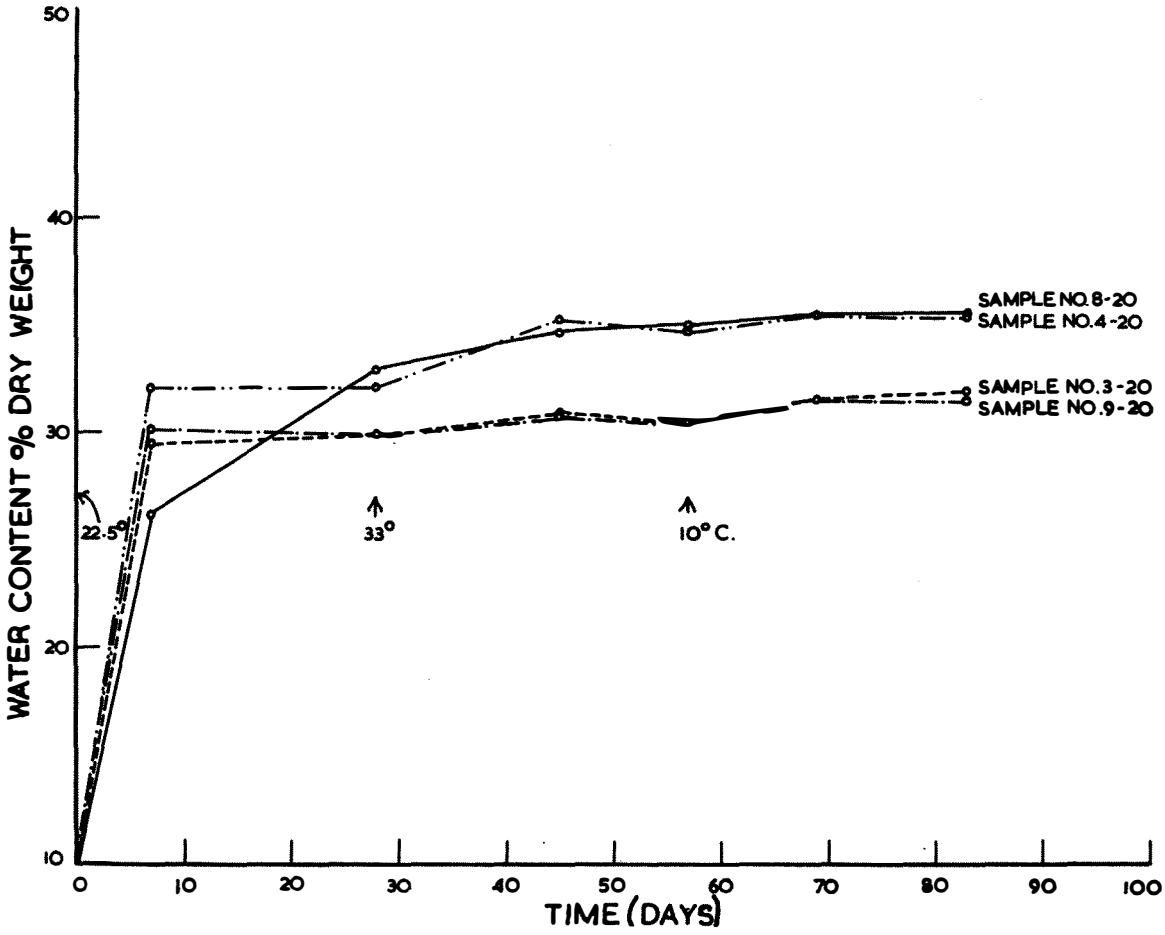

Fig. 6. Curves showing the rate of imbibition of water, at three temperatures, of spruce shavings representing different wood properties, freely suspended in a saturated atmosphere. A description of the samples may be found in the text.

and that preferences were shown by some of the fungi for specific parts of the trees. In view of the possible influence that different species of fungi might have on variations in decay between stands, it seemed of some importance to compare the rates of decay by these fungi in the laboratory, and to investigate some of the factors which might explain their specific behavior in nature.

(a) Moisture

Since it has been shown that the amount of moisture in the heartwood can determine the rate of decay, and can influence, to some extent, the distribution of fungi in the stems of living trees, the optimum moisture requirements for decay were determined for isolates of the above fungi.

The samples of heartwood used in this experiment came from a single section of wood of specific gravity about 0.34. The samples differed from the test-blocks used in the previous experiments by having two holes (about $\frac{1}{4}$ inch in diameter) in one side of the block to take separately the water amendment and the plug of inoculum as shown in Fig. 2; in other respects the procedure was similar to that which was carried out previously. Water was added to the blocks to give the following initial moisture contents (c.d.w.): 27,35,45,60, 100, 125, 150, and 175 per cent. However, in these tests the moisture contents were not corrected during incubation. Cultures of the following fungi were used to inoculate the blocks:

Culture No.	Fungus
A90	Peniophora septentrionalis
A94	Stereum sanguinolentum
T173	Unknown C
C5	Coniophora puteana
A300	Flammula connissans
E24	Fomes pini
A79	Polyporus circinatus var dualis

Tests were made in triplicate, except for the last two fungi which were used in duplicate. The decay cultures were kept in a saturated atmosphere in the manner shown in Fig. 3. Where it was necessary to maintain a uniform moisture content at 27 percent of the dry weight, the blocks were kept in a constant humidity of 98 per cent*. After three months, the mycelium adhering to the surface of the blocks was carefully removed with a brush, the plugs of inoculum removed, then the blocks were weighed, oven-dried at 105°C. for 24 hours, and re-weighed. The mean moisture contents were expressed as percentages of saturation, and the loss in weight of the blocks was expressed as percentages of their original oven-dry weights.

Table XX, records the initial and final moisture contents of the blocks for each of the seven fungi. Changes in the moisture content that took place in uninoculated controls are recorded for comparison.

It is apparent that, with the exception of the blocks inoculated with C. puteana, there was a considerable loss of water in the blocks at initial moisture contents greater than 45 per cent of the dry weight. This was noted especially in the two series infected with P. septentrionalis, and S. sanguinolentum, although losses of water also occurred at the higher moisture content values for the four remaining fungi, and for the controls. In contrast, the blocks inoculated with C. puteana showed a gain of water at each moisture level; this suggested that the moisture relations of this fungus, which caused a brown butt-rot, and the other six fungi which cause white-rots in wood, are fundamentally different. It has been reported by Cartwright and Findlay (1946) and Bjorkman (1946), that C. puteana requires a relatively high moisture content for growth in wood. These results might indicate, therefore, that C. puteana has the power to regulate the moisture content to a level more suitable for its growth. Whether this characteristic of C. puteana is associated with the ability to produce water by its respiratory activities, as shown for Merulius lacrymans (Wulf.) Fr. (the dry rot fungus), is not known, but C. puteana characteristically produces thick strands of mycelium which might have the power to absorb moisture from a saturated atmosphere and then conduct it to the wood in which it is growing.

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* A constant humidity of 98 per cent was maintained by using a saturated solution of $Pb(NO^3)_2$ instead of water.

TABLE XX

THE MOISTURE CONTENTS OF WOOD BLOCKS DECAYED BY SEVEN SPECIES OF WOOD-DESTROYING FUNGI, AT THE END OF THREE MONTHS

Test Fungus	Final Moisture Contents (% dry wt.) at Various Initial Moisture Levels							
	27	35	45	60	100	125	250	175
White Rots								
P. septentrionalis	27.8	37.1	55.3	40.3	70.4	77.5	110.6	129.3
S. Sanguinolentum	26.8	34.0	46.7	44.5	70.3	96.3	133.0	136.7
F. Pini	31.8	39.9	45.2	52.8	89.1	98.6	153.1	157.5
P. circinatus	27.0	34.3	46.3	52.9	94.6	112.4	142.6	126.6
Unknown C.	26.1	34.1	45.8	51.3	62.8	91.9	119.8	118.5
F. connissans	25.3	35.2	41.5	55.2	94.6	116.2	137.3	160.6
White Rots Average	27.4	35.7	46.8	49.5	80.3	98.8	132.7	138.2
Gain or Loss (+ -)	+ .04	+0.7	+1.8	-10.5	-19.7	-26.2	-17.3	-36.8
Brown Rots								
C. puteana	27.2	39.6	51.7	72.2	113.6	135.1	168.4	203.5
Gain or Loss (+ -)	+ 0.2	+4.6	+6.7	+12.2	+13.6	+10.1	+18.4	+28.5
Uninoculated Control	27.0	35.1	43.2	46.7	90.3	104.0	137.3	141.9
Gain or Loss (+ -)	0.0	+.1	-1.8	+1.7	-9.7	-21.0	-12.7	-33.1

NOTE: By substitution in the following formula, approximate moisture contents in terms of percentage of saturation may be obtained.

$$\frac{\%\text{ dry weight}}{\%\text{ saturation}} = \frac{\%\text{ sat. value}}{} = \frac{.35x - 9.80}{0.6745}$$

where, X is the moisture content expressed as per cent of dry weight

In addition to the irregularities in moisture contents that are noted above, irregularities frequently occurred in the moisture and decay values between replicates; thus, attempts to obtain a relationship between moisture and decay based on averages at each moisture level were unsuccessful. It was found more practicable to arrange the data into five or six moisture content classes (percentage of saturation), and then to calculate the average moisture and decay values for each class. These values were plotted on semi-logarithmic graph paper with decay values at logarithmic values on the ordinate axis,* and rough curves were drawn through the points. The curves were adjusted so as to obtain minimal deviations for the individual decay values and then redrawn as smooth curves to show the characteristic relationship between moisture and decay for each of the seven fungi. The curves obtained for the six fungi which cause white-rots in the wood are shown in Fig. 7. For comparison, the three curves for the fungi causing butt-rots are shown on the left of theplate and the curves for the fungi causing trunk-rots are on the right.

Most of the fungi showed a close similarity on their ability to cause decay in the samples. Peniophora septentrionalis was responsible for the greatest decay among the six white-rot fungi, and was responsible for a maximum loss in weight of nearly 4 per cent and this occurred in wood which was about 40 per cent saturated. Fomes pini caused the smallestloss in weight of the blocks, but tests with this fungus were unsatisfactory because only eight out of a total of 20 blocks were successfully infected. Although these results were not strictly comparable with the results for the other fungi, there was some evidence that maximum decaying activity for F. pini occurred at moisture contents of about 10 per cent saturation. The maximum decay values obtained for the remaining fungi correspond to a loss in weight of about 2 per cent, and this occurred in wood at moisture contents ranging from 29.5 per cent for S. sanguinolentum to 44 per cent for F. connissans.

The relationship between moisture and decay for the brown-rot fungus, C. puteana, is shown in Fig. 8. This fungus undoubtedly caused the greatest decay in these tests, and was responsible for a maximum loss in weight of about 12 per cent in samples with moisture contents of 35.2 per cent of saturation.

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* A logarithmic scale divided into two cycles was used to reduce the spread in values obtained for the different fungi, and to make comparisons easier.

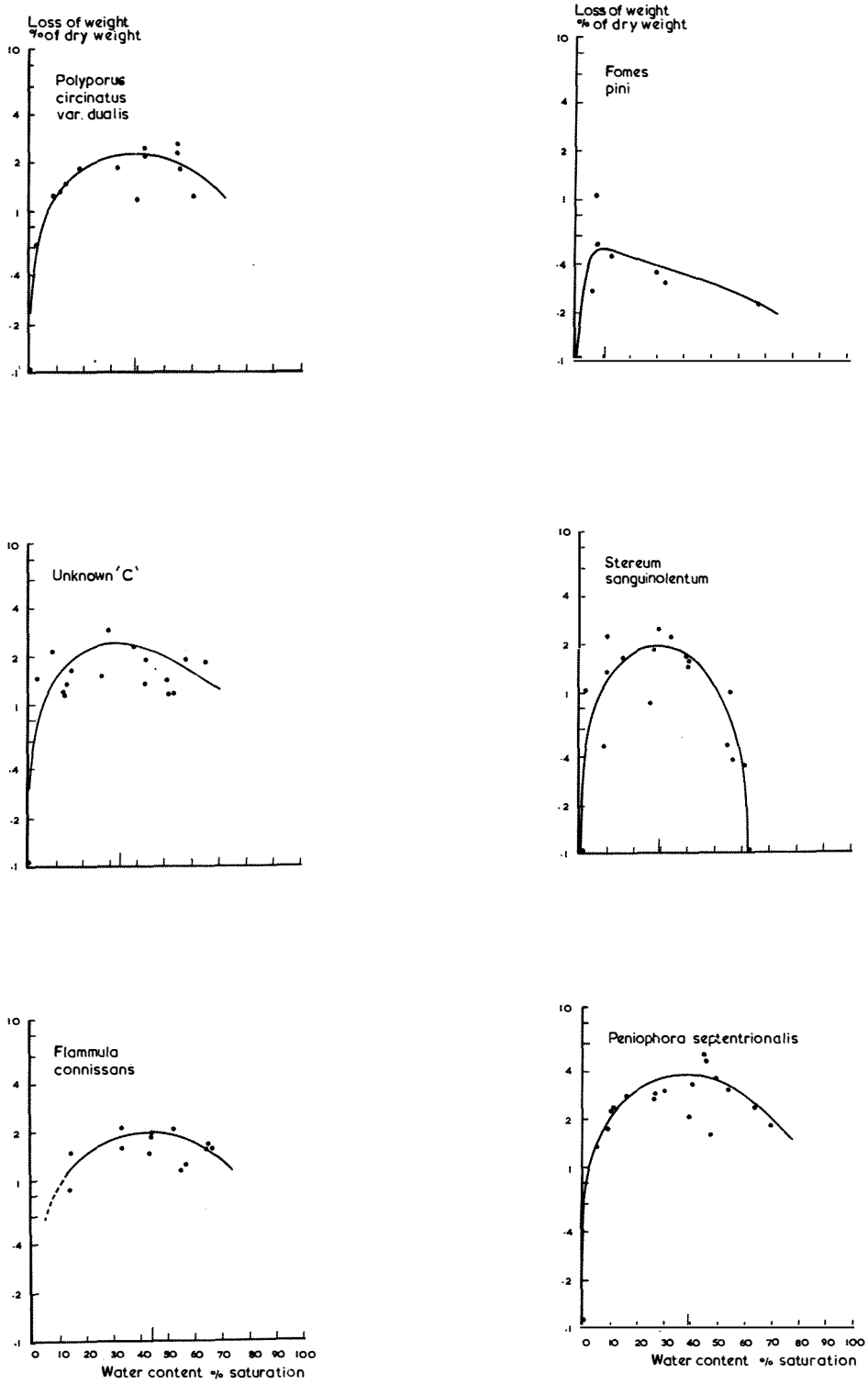

Fig. 7. Characteristic curves showing the relationship between the loss in weight due to decay by three butt-rotting (1st column) and three trunk-rotting (2nd column) fungi and the moisture content of test blocks of spruce heartwood at the end of three months.

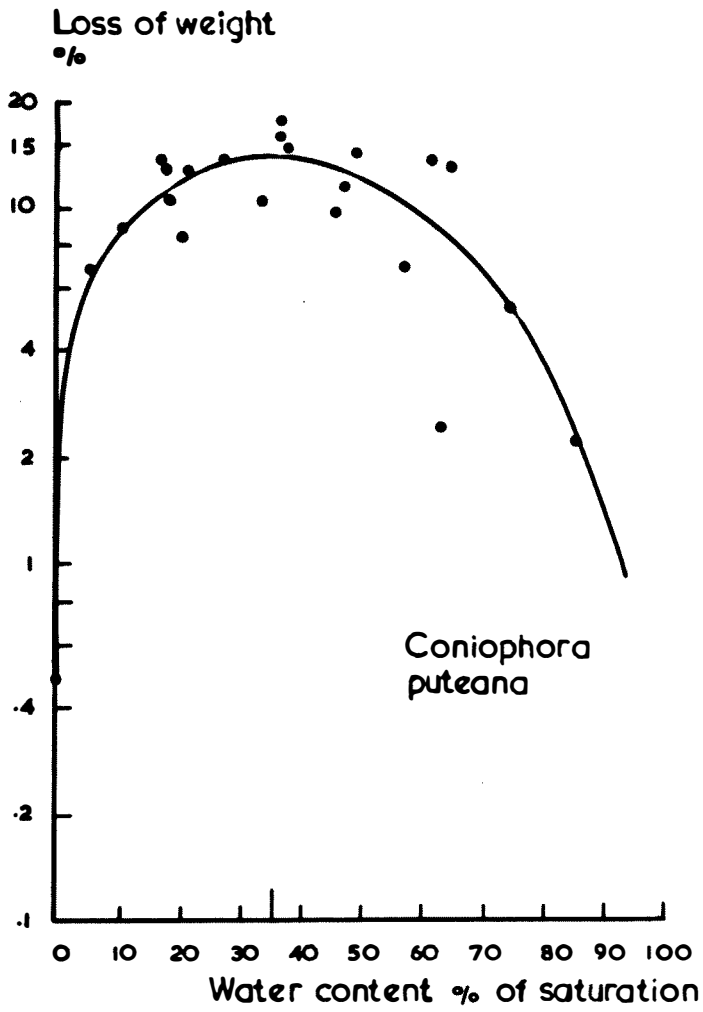

Fig. 8 Characteristic curve showing the relationship between the loss in weight due to decay by Coniophora puteana and the moisture content of test blocks of spruce heartwood at the end of three months.

From the curves it was seen that the optimum moisture contents for the seven fungi ranged from 10 to 44 per cent of saturation; the two trunk-rotting fungi, F. pini and S. sanguinolentum reached their maximum development at the lower end of this scale. To see whether the optimum moisture contents bore any relation to the specific behavior of the fungi these values were compared for the four butt-rotting and the three trunk-rotting fungi. The average moisture optimum for decay for the four butt-rotting fungi was found to be 37.9 per cent of saturation, or 104 per cent of the dry weight (assuming the specific gravity to be constant at 0.35), whereas, the average values for the three trunk-rotting fungi were 26.3 or 78.0 % respectively. Thus, the fungi which occurred in the root or butt regions of the trees appeared to need a higher moisture content for maximum development than that required by the fungi which occurred higher in the trees. Of the trunk-rotting fungi, P. septentrionalis, however, needed a much higher moisture content for maximum development than the other two so it would appear that the different moisture requirements for the two groups of fungi, although probably of some ecologic l importance, do not entirely explain their specific behavior in nature.

An interesting characteristic found associated with decay by P. circinatus was the formation of a pseudosclerotia on the surface of the blocks which had moisture contents ranging from 35 to 60 per cent of their dry weight. The formation of pseudosclerotia on the surface of blocks can be seen in Fig. 9. The mean moisture contents for the five blocks expressed as a percentage of saturation were 0, 3.5, 9.4, 18.2, and 40.0 respectively. Individual decay losses of 0, 0.62, 1.24, 1.81, and 1.17 per cent of the dry weights were obtained at these five moisture levels. Maximum development of sclerotia was seen on the surface of the blocks which had the most decay (1.81 per cent). About as much decay occurred in the block with the maximum moisture content as in the block with only 9.4 per cent of saturation, but sclerotia did not form under the wetter conditions. This suggested that the formation of pseudosclerotia depended upon suitable moisture and aeration conditions on the surface of the wood, but not on the progress of decay in the wood.

(b) Temperature

Information on temperature within the heartwood of trees in not extensive, but it is sufficiently definite to indicate that variations occur within the stems, and these differences may exert an important influence on the rate of progress of decay fungi in the heartwood. Consequently, it was of some importance to investigate the temperature relations of the fungi to see whether the optimum temperature requirements of butt - and trunk-decaying fungi were

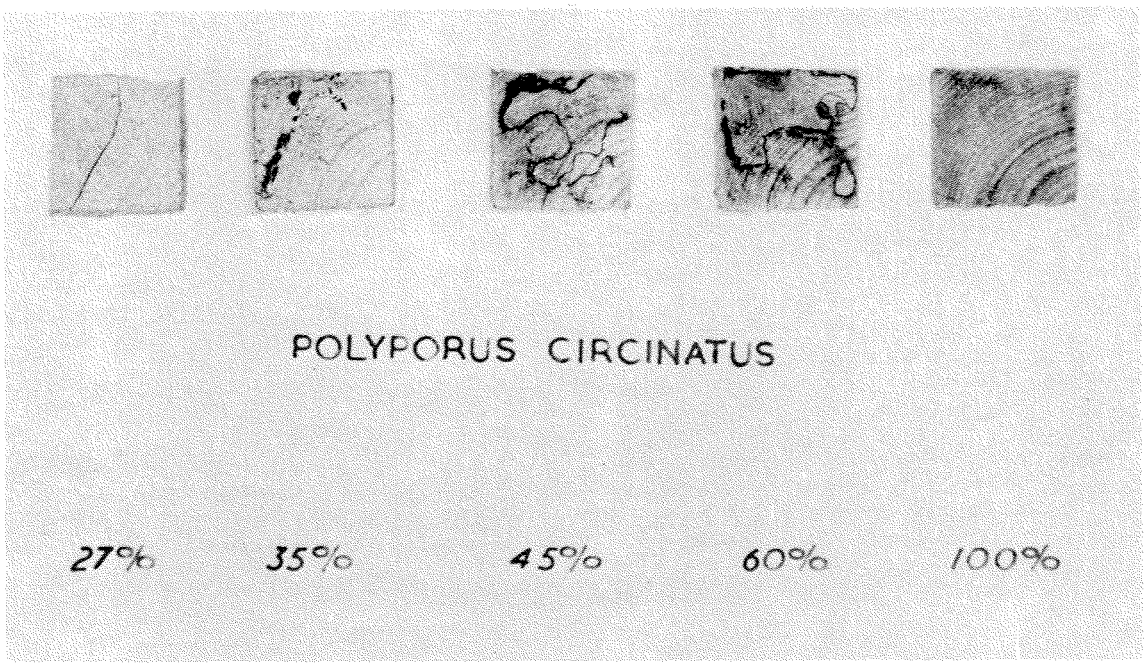

Fig. 9. The effect of the moisture content of the substratum on the formation of pseudorhizomorphs by Polyporus circinatus var. dualis (moisture content = percentage of dry weight).

sufficiently different to explain the relative dominance of these fungi in certain situations.

Cartwright and Findlay (1946) are of the opinion that for practical purposes the cardinal temperatures for the growth of a fungus on wood are approximately the same as those determined from measuring its growth on agar. Therefore, the effect of temperature on the growth of the fungi on plates containing two per cent malt agar was studied. Cultures of the seven species of wood-destroying fungi used previously in the moisture experiment (see page 57), and cultures C103, L2C, and T157 of Coryne sarcoides were used in these tests. The experiment was conducted in duplicate at temperatures of 4°, 10°, 20°, 25°, 30°, and 35°C. Two measurements at right angles of the diameters of the colonies on the two plates were made at the end of seven and fourteen days. The difference between the two measurements which represented the growth during the second week, were used to calculate the mean daily growth of the cultures. The temperature-growth curves developed from these data are shown in Figs. 10, 11, and 12.

First, considering the curves for the seven wood-destroying fungi shown in Figs. 10 and 12, it can be seen that the three trunk-rotting fungi (shown on the right of Fig. 10) have temperature optima of about 25°, whereas, maximum growth for the four butt-rotting fungi (including C. puteana. Fig. 12) occurred at lower temperatures, at about 20°C. It is noteworthy, that maxima for the four butt-rotting fungi occurred at 30°, while two of the trunk-rotting fungi, P. septentrionalis and F. pini had temperature maxima above 30°. The strain of S. sanguinolentum used in these tests showed an optimum at 25°, and a maximum at 30°. Cartwright and Findlay (1946), however, found that the optimum for a British strain of S. sanguinolentum was 20° and 24° and the maximum for growth was at 36°. These investigators also give data on a British strain of F. pini, recording a relatively high optimum of 24°, and a maximum at 30°. However, Percival (1933), presumably using a N. American strain of F. pini, found the optimum temperature for growth to be 25°, and the maximum to be between 30° and 35°. The first named authors found the optimum temperature for growth of C. puteana to be about 23°, and a maximum for growth at about 35°. There was no comparable data available for the four remaining species of fungi that were tested.

As far as evidence is available, the results indicated a much higher temperature range for fungi which caused trunk-rots than those which caused butt-rots, a characteristic which could be of considerable ecological importance in determining the relative dominance of these fungi in certain parts of the trees.

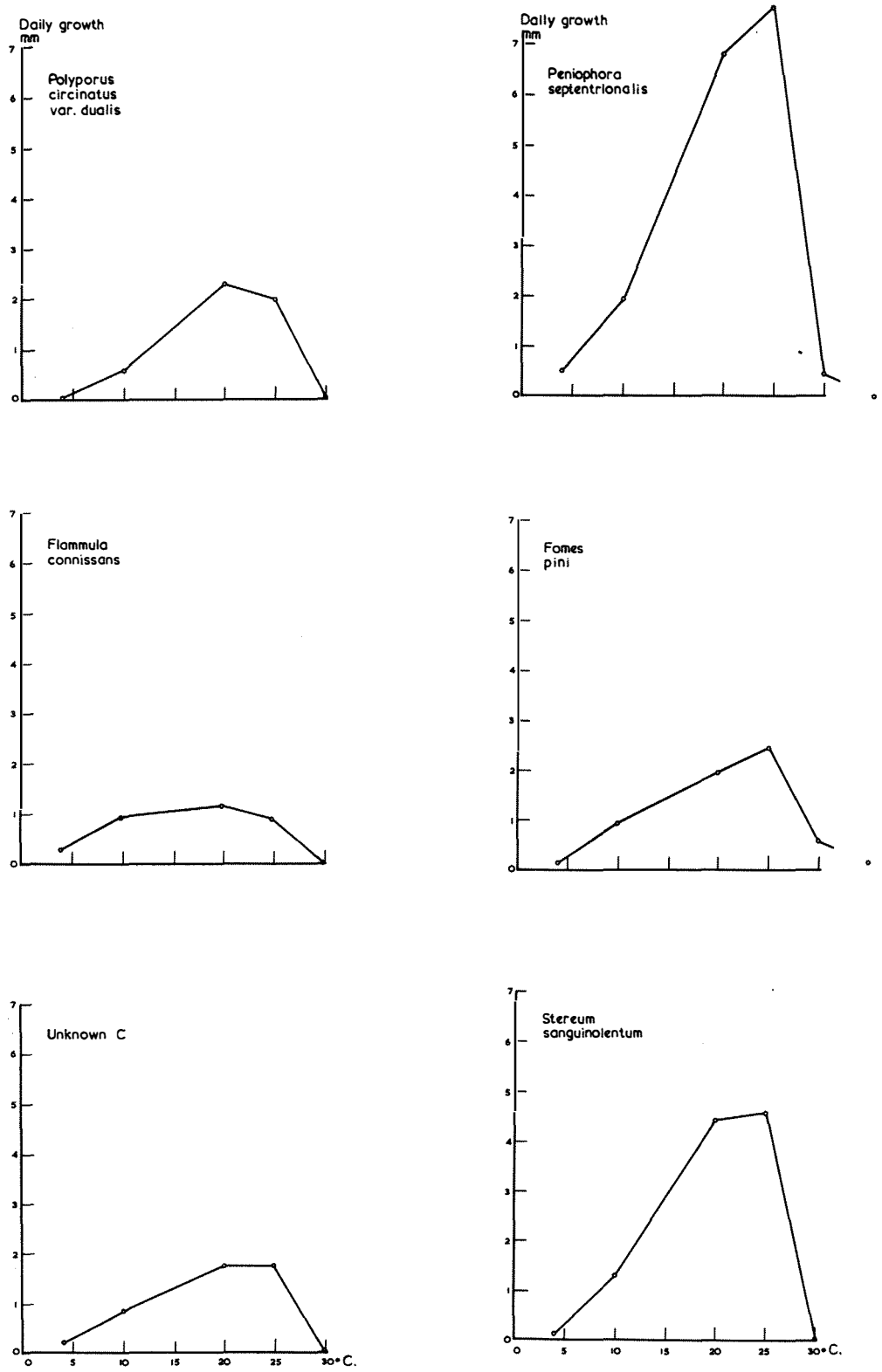

Fig. 10. Daily radial growth of representative cultures of butt-rotting (1st column) and trunk-rotting (2nd column) fungi on 2 % malt agar for 14 days, at various temperatures. Each point represents the average of two Petri-dish cultures.

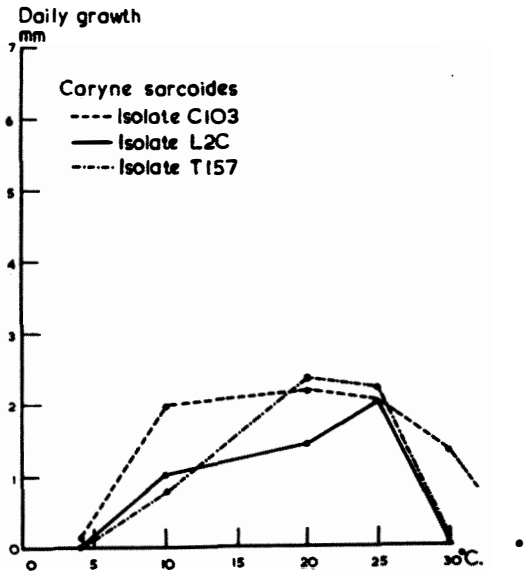

Fig. 11. Daily radial growth of cultures of Coryne sarcoides from Picea glauca (L-2-C,T157) and Pinus contorta (C-103), on 2 % malt agar for 14 days, at various temperatures.

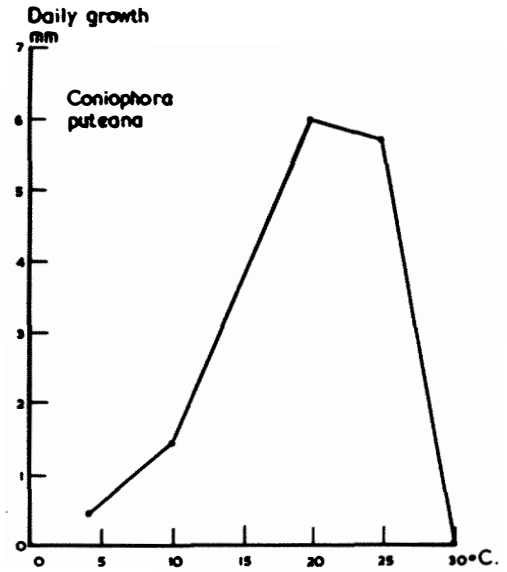

Fig. 12. Daily radial growth of Coniophora puteana on 2% malt agar for 14 days, at various temperatures.

There was evidence also of a relationship between the maximum growth of the fungi on agar and their maximum development in wood (Figs. 7, 8, 10, and 12). Of the three trunk-rotting fungi, P. septentrionalis showed the fastest growth rate on agar and also caused the most decay, while F. pini showed the slowest growth on agar and caused the smallest amount of decay. A similar trend was observed between growth on agar and decay for the butt-rotting fungi where growth on agar and in wood increased from a minimum for F. connissans to a maximum for C. puteana.

A comparison of the temperature ranges for growth for three Canadian strains of Coryne sarcoides (Fig. 11), showed an interesting relationship between the geographic and host origin of the cultures and their particular temperature requirements. A culture of C103 showed the widest temperature range with maximum growth occurring at 35°C. This isolate was collected on Pinus contorta var. latifolia, a host which commonly occurs on warm, dry sites. On the other hand, the cultures of T157 and L2C which were isolated from spruce, a host which is generally associated with moist, cool conditions, had appreciably narrower temperature ranges for growth. The culture T157 which showed a temperature optimum for growth at 20° came from an alpine location, and the culture of L2C which showed a higher temperature optimum was collected at a much lower, and more temperate, elevation.

The association of these fungi with certain hosts appears to be largely due to their ability to tolerate particular host-temperature conditions which are necessary for their development. In this respect, their behavior appears to correspond to the specific behavior shown by the wood-destroying fungi in relation to their temperature requirements and their occurrence in the trees.

(c) Ability of the Fungi to cause Decay

All the isolates listed in Table II were tested for their ability to cause decay. These were: 22 isolates of various wood-destroying fungi, one isolate of Stereum sulcatum, and five isolates of Coryne sarcoides which were obtained from decays in living subalpine spruce; two Canadian isolates of C. sarcoides shich were obtained from decays in Pinus spp. in Canada, three British isolates of this fungus which were obtained from sporophores, and one isolate of Myrothecium verrucaria. Stereum sulcatum and Coryne sarcoides are species that are found commonly associated with decay in living trees; their decaying ability has not been previously studied.

The procedure previously followed in the decay experiments was adopted, with certain modifications. Each fungus was tested on three blocks of spruce heartwood; these were placed, one on top of the other, in a screw-top jar to which had been added three grams of spruce sawdust enriched with Badcock's Accelerator (described on page 13). The lower-most block rested on glass rods which were placed on the sawdust. Water was added to the blocks to give a moisture content of approximately 70 per cent of their dry-weight. The jars were sterilized by steam in the usual manner, and then three plugs of agar from actively growing plate culture of the appropriate fungus were placed on top of the enriched sawdust. The cultures were kept in a saturated atmosphere as shown in Fig. 3, for three months. The loss-in-weight of wood substance, and the final moisture contents for the blocks were calculated in the usual way. The results are given in Table XXI which records the losses in weight and the final moisture contents for thirty-one series of blocks which had rested on sawdust in which the fungus had become established; four series of blocks were lost through contamination.

There was considerable difference in the decay caused by the different isolates of each of the seven species of wood-destroying fungi tested, but the mean values obtained for each of the species agreed fairly well with the results obtained in the first decay experiment (see Figs. 7 and 8). They differed, however, in that greater losses in weight occurred in this experiment in which enriched sawdust was used as an accelerator. The results of both experiments showed that the decay caused by C. puteana, the brown-rot fungus, was about three times as great than that caused by any of the white-rot fungi. The most active decay-producer of the white-rot group was P. septentrionalis. Of the three fungi causing white-butt rots, Unknown C. was responsible for the greatest loss in weight, and F. connissans for the least. In both experiments, F. pini caused the least decay of the three white trunk-rotting fungi, but the present results based on two isolates were more reliable and decay values which were obtained were of the same order as for the other fungi tested.

A comparison of the final moisture contents of the blocks shows that the fungi are capable of regulating the moisture to a level more suitable for their growth; the moisture contents of the blocks infected with the fungi causing butt-rots are seen to be much higher (69.4) than those for the fungi causing trunk-rots (57.4). This was also characteristic of the two types of fungi in the first experiment.

TABLE XXI

LOSSES IN WEIGHT (% DRY WT.) AND MOISTURE CONTENTS (% DRY WT.) OF WOOD BLOCKS INFECTED WITH VARIOUS FUNGI, AT THE END OF THREE MONTHS

Species	Isolate	Loss in weight (PerCent Dry Wt.) Replicate*				Moisture Content** (PerCent Dry Wt.) Replicate**			
		1	2	3	Avg.	1	2	3	Avg.
P olyporus	A361	2.30	3.18	-	2.74	55.4	69.3	39.4	62.4
circinatus	A79	4.56	3.29	3.26	3.70	76.5	51.2	31.8	53.2
var dualis	A20	3.67	4.90	0.93	3.16	70.8	66.8	40.8	59.5
Average					3.26				57.8
Flammula	A259	1.33	0.76	-	1.04	59.5	72.0	(39.2)	65.7
connissans	A86	3.95	1.50	-	2.72	86.5	58.4	(42.2)	72.4
	T510	3.31	1.64	1.84	2.26	124.2	81.8	52.3	86.1
	A300	-	1.87	-	1.87	(101.0)	96.1	(42.6)	96.1
Average					2.02				78.8
Unknown C	A212	3.88	1.78	2.31	2.66	54.0	50.7	48.0	50.9
	T173	6.97	3.60	1.25	3.94	109.0	72.5	40.2	73.9
Average					3.30				62.4
Coniophora	A263	27.6	21.9	14.0	21.20	168.0	69.0	52.2	96.4
puteana	C5	10.1	6.3	9.15	8.52	86.0	69.7	56.1	70.6
	C4	7.2	6.8	5.0	6.30	64.5	93.2	48.2	68.9
Average					12.00				78.6
Stereum sulcatum	T491	0.0	0.0	0.0	0.0	54.7	52.5	36.7	47.9
Myrothecium verrucaria	144743 (CMI)	0.75	0.55	-	0.65	104.0	66.3	(40.5)	85.1

TABLE XXI (Concl'd)

Species	Isolate	Loss in Weight (Per Cent Dry Wt.) Replicate				Moisture Content (Per Cent Dry Wt.) Replicate			
		1	2	3	Avg.	1	2	3	Avg.
Stereum sanguinolentum	A94	4.18	7.70	3.23	5.03	59.0	59.5	49.9	56.1
	A107	3.93	3.60	1.32	2.95	74.0	58.4	39.1	57.2
	T492	0.74	2.66	0.75	1.38	48.0	52.7	42.2	47.6
Average					3.12				53.6
Fomes pini	E24	2.86	2.33	-	2.59	57.6	49.2	(40.2)	53.4
Average					3.09				53.9
Peniophora septentrionalis	B356	6.20	6.41	6.03	6.21	58.1	59.9	59.4	59.9
	A2	3.94	3.38	1.93	3.75	64.3	60.2	43.8	56.1
	A90	1.16	0.87	1.19	1.07	84.6	89.0	59.7	77.7
Average					3.68				64.6
Coryne sarcoides	BA3	0.63	-	-	0.63	55.2	(46.7)	(47.4)	55.2
	BG2	1.70	-	-	1.71	48.8	(57.2)	(55.9)	48.8
	L2C	0.0	0.0	-	0.0	53.0	102.0	(40.0)	77.5
	C103	0.0	0.0	0.0	0.0	53.3	64.5	36.8	51.5
	A29	0.0	0.0	0.0	0.0	59.0	60.6	36.2	51.9
	A110	0.0	0.0	0.0	0.0	85.2	65.7	39.4	63.4
	C52	0.0	0.0	0.0	0.0	46.6	47.8	55.7	50.0
	T157	0.0	0.0	0.0	0.0	52.8	63.3	38.7	51.6
	A56	0.93	0.0	0.0	0.31	60.8	52.9	52.0	53.2

* The positions of the replicates in the jars were: (1) lowermost block, (2) middle block, (3) uppermost block

** The values in brackets are for blocks in which only a trace of decay occurred, (between .2 and .5 per cent of the dry wt.) such values do not figure in the average

It is interesting to note that, with few exceptions, decay was influenced by the position of the replicates in the jars; and that differences in the amount of decay between the blocks in any one jar corresponded to differences in their moisture contents. The uppermost block in each jar (replicate 3) was usually the driest and also contained the smallest amount of decay, while the bottom block (replicate 1) was usually the wettest and had the largest amount of decay. Decay was rarely seen in blocks with moisture contents of less than 40 per cent of their dry weight.

Turning to the results obtained for M. verrucaria, S. sulcatum, and C. sarcoides, it is seen that all these fungi did not cause any appreciable decay under the conditions of the experiment. It seems probable, however, that conditions were not suitable for M. verrucaria since losses in weight of about 4 per cent at moisture contents of 60 per cent were obtained in preliminary tests with this fungus when enriched sawdust was not used. Earlier tests with S. sulcatum and C. sarcoides, however, gave substantially the same results. For example, the decaying ability of isolate C52 of C. sarcoides was tested with 20 blocks over a wide moisture range and there was no decay in any although fruit-bodies (conidial stage) of the fungus frequently occurred on the surfaces of the blocks. The results with the last two species are of particular interest because both fungi may be associated with decay in living trees, but it was not known whether they were capable of destroying wood (Etheridge (1954, 1955, 1956)).

(d) Enzyme Studies

There are few comparative studies of the enzymes of wood-destroying fungi, and most of the information about them is of a general nature. For instance, little is known about the relationship between the production of enzymes that catalyse the degradation of cellulose and lignin and the ability of fungi to cause decay. It was, therefore, of interest to study the production of enzymes by the different fungi, and to compare the results with those obtained in the decay experiments.

(1) Cellulolytic Activity

The culture fluid of each of the isolates listed in Table II was tested for cellulolytic activity in the following way. The fungi were grown in cazein hydrolysate medium and cell-free filtrates were prepared as described on pages 19 and 20. The optimum pH for cellulose activity was determined as follows. Equal volumes (1.25ml.) of Sorensen's Sodium Citrate buffers at pH 3.2, 4.4, 5.1, 6.0, and 7.2, and a culture filtrate of C. puteana (A263) were put into a 25-ml bottle containing 2.5 ml. of a 2 per cent solution of CMC.*

* Sodium carboxy methyl cellulose, sample designated CMC with a degree of substitution of 0.71

The mixture was incubated at 55°C. for 18 hours as recommended by Jennison et al (1952). The mixture was then assayed for cellulose activity by determining the glucose in a sample of the mixture before and after incubation by the copper iodometric method described by Shaffer and Somogyi (1953) and modified by Somogyi (1937). By this method, quantities of reducing substances (expressed as glucose) as low as 0.002mg. per ml. can be determined titrometrically. The determinations were made in duplicate and the results are shown in Table XXII.

It was apparent that the highest activity was at pH 4.4. These results agreed closely with those obtained by Jennison et al (1952) and other investigators, Cellulose activity was therefore tested in mixtures buffered at pH4.4.

It was decided to use cellulose in the form of filter-paper as a substrate for the comparative studies. Tests were made to determine the best concentration for use in the reaction mixture. The cellulose was prepared as follows: filter -papers (Whatman No.40) were first macerated in a Waring type blendor cup with 500 ml. of distilled water for two minutes, the suspension was left to stand overnight at 4°C. to extract impurities; the supernatant liquid was poured off and the cellulose pressed between layers of muslin and dried. When dry, the cellulose was shredded with a course wood-rasp, and kept in a sealed container until needed. For the assay, 0.025, 0.050, and 0.100 g. (to give a 0.5, 1.0, and 2.0 per cent final concentration in the mixture) of shredded cellulose were weighed and then mixed with 1.25 ml. of distilled water in 25-ml. bottles until a homogenous suspension was obtained. 1.25 ml of buffer (pH 4.4) and 2.50 ml of filtrate were then added and the mixture again stirred. The bottles were sealed and incubated in a constant temperature bath at 27°C.; one series was assayed at the end of three hours and the other at the end of 17 hours. The mixture was agitated during incubation by a reciprocating shaking device. The mixture was then filtered through muslin, and the glucose in the mixture was determined as before. Curves showing the liberation of reducing groups, as glucose, in mixtures containing 0.5, 1.0, and 2.0 per cent concentrations of cellulose after different periods of incubation were plotted from the data (Fig. 13).

The highest glucose values were obtained with a 2 per cent concentration of cellulose after incubation for 17 hours. Under these conditions of assay, 0.274 mg./ml. of glucose were present in the filtrate compared with 0.095 mg./ml. when the incubation time was three hours, and 0.070 mg./ml. when the concentration of cellulose was 0.5 per cent. In further tests, therefore, activity was measured after 17 hours incubation of mixtures containing 2 per cent filter-paper. Table XXIII records the results obtained from two determinations for each of the 33 isolates. It also gives the mean values for the seven species of wood-destroying fungi, and for the Canadian and British isolates of Coryne sarcoides.

TABLE XXII

ACTIVITY OF REACTION MIXTURE BUFFERED AT VARIOUS pH VALUES

pH of Buffer	Replicate	Activity (as glucose) mg./ml.		
		Before*	After	Gain
3.2	1	0.042	0.260	0.218
	2	0.042	0.267	0.225
4.4	1	0.042	0.491	0.449
	2	0.042	0.491	0.449
5.08	1	0.042	0.060	0.118
	2	0.042	0.190	0.148
6.01	1	0.042	0.106	0.064
	2	0.042	0.092	0.050
7.16	1	0.042	0.088	0.046
	2	0.042	0.092	0.050
Control (no buffer)	1	0.042	0.090	0.048

* Glucose value based on three determinations

TABLE XXIII

CELLULOLYTIC ACTIVITY OF FILTRATES FROM CULTURES OF DIFFERENT FUNGI

Species	Isolate	Activity of Filtrate*
Polyporus circinatus	A361	.056
	A79	.041
	A339	.060
	A20	.048
Average		.051
Flammula connissans	A259	.052
	A86	.044
	T510	(.006)
Average		.048
Unknown C	A212	0.29
	T173	0.38
Average		.034
Coniophora puteana	A263	.029
	C5	.107
	C4	.083
Average		.073
Stereum sulcatum	T491	.009
Myrothecium verrucaria	144743	.041
Stereum sanguinolentum	A94	.064
	A107	.120
	T492	.047
Average		.077
Fomes pini	E24	.086
	E25	.096
	T434	.080
Average		.087

TA BLE XXIII (Concl'd)

Species	Isolate	Activity of Filtrate
Peniophora septentrionalis	B356	.088
	A90	.086
	A2	.082
Average.....		.085
Coryne sarcoides	BX1	.140
	BA3	.116
	BG2	.101
Average (British Isolates).....		.119
Coryne sarcoides	L2C	(.000)
	C103	.70
	A29	.065
	A110	.022
	C52	.061
	T157	.023
	A56	.028
Average (Canadian Isolates).....		.045

* Activity expressed in terms of reducing sugar as mg. glucose per ml. of reaction mixture, incubated 17 hours at 27°C.

Reaction mixture: Macerated filter-paper - 0.1 g.
Sodium citrate buffer, pH 4.4 - 1.25 ml.
Distilled water - 1.25 ml.
Cell-free filtrate - 2.50 ml.

Note: Values in brackets do not figure in the average

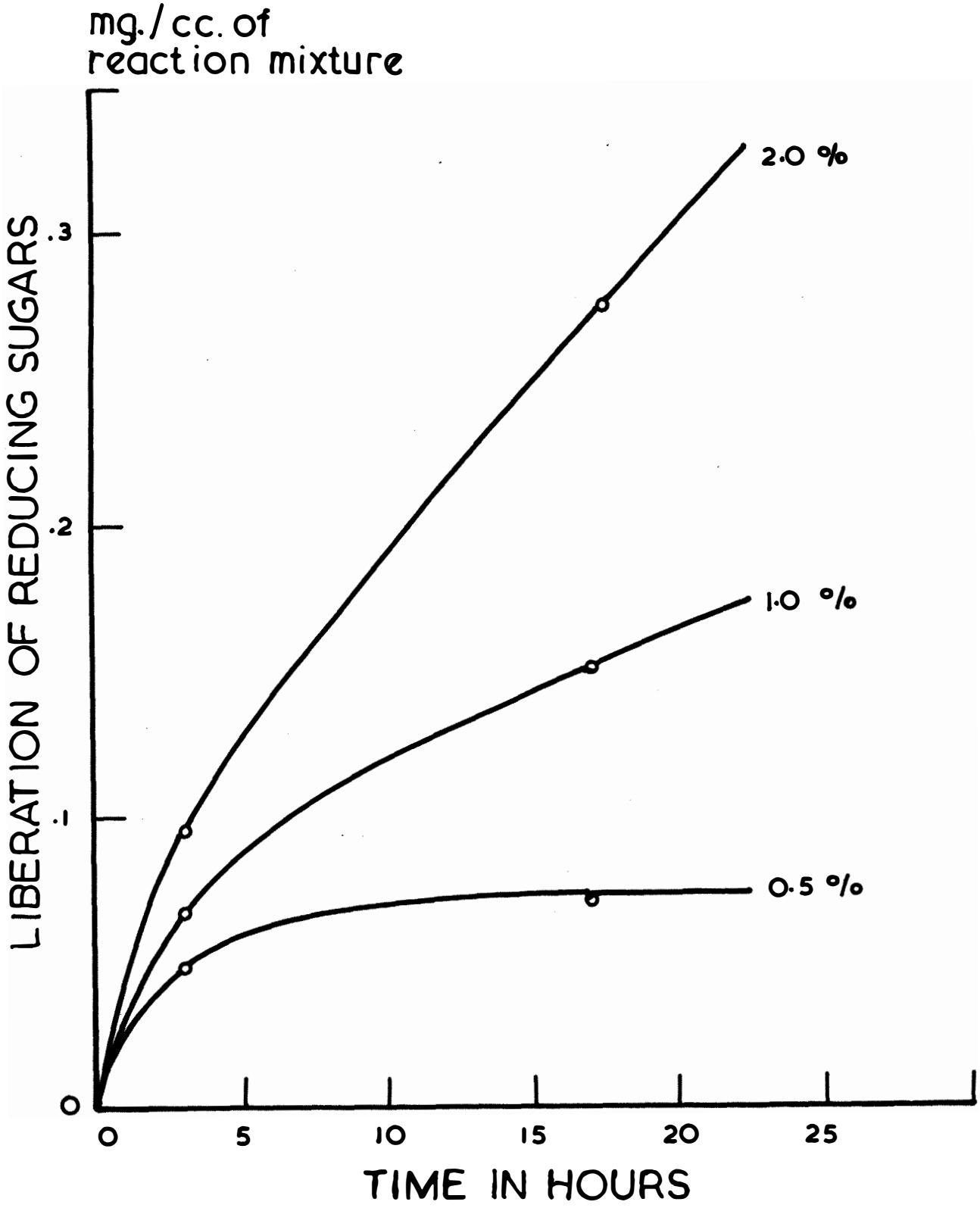

Fig. 13. The effect of the concentration of cellulose (macerated filter-paper) on the liberation of reducing sugars in a 1:3 reaction mixture, buffered at pH 4.5, after 3 and 17 hours, at 27° C.

Before any conclusions are drawn from these data it is important to point out that an effort was made to grow the cultures under uniform conditions, and the filtrates were prepared and tested in a standard way. Except during the final assays, each step in the procedure was completed with all the cultures in one day. Assuming therefore that the results are comparable, it is obvious that the culture filtrates differed considerably in their cellulolytic activity. The three fungi producing white trunk-rots (which attack both the cellulose and lignin in wood) showed about the same activity as did the brown-rot fungus, C. puteana, which attacks only the cellulose. Moreover, the culture filtrates of the trunk-rotting fungi were considerably more active than those of the butt-rotting fungi. It is of some interest that F. pini, which is responsible for the greatest decay losses in living subalpine spruce, also showed the highest cellulolytic activity among the wood-destroying fungi, although this fungus showed only a moderate ability to attack wood under the conditions of the laboratory experiments. Culture filtrates of C. sarcoides, on the other hand, hadsurprisingly high activities, particularly the British isolates, despite their apparent inability to cause decay in the laboratory tests. The three British isolates of C. sarcoides were the most active producers of cellulose, while the isolate of S. sulcatum, isolate T510 of F. connissans, and isolate L2C of C. sarcoides all showed extremely low, or negative activity in their filtrates.

(2) Polyphenol Oxidase Activity

As early as 1928 Bavendamm (1928) observed that fungi causing white-rots in wood, when grown on agar media containing tannin, caused an oxidative browning of this compound which appeared as a dark ring around the culture. This phenomenon was absent with fungi of the brown-rot type which are not capable of decomposing lignin. Bavendamm concluded that tannin oxidation was catalysed by enzymes of the phenolase type excreted by the fungus, and he suggested that these enzymes would also play a part in the degradation of lignin. The reaction was later examined by Davidson, Campbell, and Blaisdell (1938) by growing a large number of species of wood-rotting fungi on an agar medium containing tannic and gallic acids. They found that the two wood-rotting types generally gave the correct Bavendamm reaction, although, the intensity of the reaction differed with different fungi, and a few of the fungi associated with brown-rots did not give the expected results. A different method for the separation of brown - and white-rot fungi in culture was used by Preston and McLennan (1943). Their method was based on the decolorization by fungi of the white-rot type, of various dyes that were incorporated with the medium, one of the most satisfactory for this purpose being gentian violet. Although the nature of the reaction was not investigated, these workers concluded that the decolorization

of the dye was probably due to the production, by the white-rot fungi, of an extracellular oxidase system. Studies regarding the kind of enzymes concerned in the reaction, and their relation to the oxidation of lignin have been made by a number of workers, but no consistent and generally accepted conclusions have emerged. Cartwright and Findlay (1946) were of the opinion that there is some evidence for assuming that the rate of attack of lignin is directly related to the phenol oxidase activity of the fungus since fungi that cause an active white-rot generally give a very strong reaction with certain phenolic compounds. The relationship between exidase activity and decaying activity for the white-rot fungi, therefore, seemed to be an interesting one to study, particularly since it was possible to compare the oxidase activities of the different isolates with their activities in wood. In the experiments reported below, oxidase activity was estimated and compared semi-quantitatively by observing the reaction of the fungi on agar media containing tannic and gallic acids, and gentian violet according to procedures developed for these tests which were found in the literature.

Media composed of 15 g. malt extract, 20 g. agar, and 5 g. of tannic or gallic acid to one litre of distilled water was prepared according to the method described by Davidson, et al (1938). The acids were sterilized separately and added to the cooled agar before pouring the plates. The plates were inoculated in duplicate with agar plugs from 3 to 4 week old cultures of the appropriate fungus and kept at room-temperature for 7 days. The following system which was used by Davidson et al (1938) to record the reactions of the fungi was adopted:

- \- Negative, no brown decoloration of the agar under or about the mat.
- \+ Diffusion zone light to dark brown formed under inoculum at centre of mat and visible only from underside of dish. In case no growth takes place, a faint brown decoloration under the inoculum.
- ++ Diffusion zone light to dark brown formed under most of mat, but not extending to margin. Visible from underside only.
- +++ Diffusion zone light to dark brown extending a short distance beyond the margin of the mat, and visible from the upperside.
- ++++ Diffusion zone dark brown, opaque, extending considerably beyond margin of fungus mat.
- +++++ Diffusion zone very intense, dark brown, opaque, forming a wide corona about mat. Usually such intense reactions occur with species giving no growth on the medium, and are most common on gallic acid medium.

The gentian violet medium consisted of 12.6 g. malt extract, 17.5 g. agar, 70 ml. of a 0.07 per cent solution of gentian violet (to give a final concentration of 0.007 per cent) to 630 ml. of distilled water (Preston and McLennan, 1948). 5 ml. of the media were poured into tubes which were inoculated by placing an agar plug from an actively growing culture, about one inch from the top of the slope. The cultures were incubated at room-temperature for three weeks and notes were made of their appearance at 7-day intervals. The following system was used to record the reactions of the fungi:

- \- No decolorization, slight to heavy growth.

- \+ A slight decolorization of dye directly beneath the inoculum after three weeks, mat not visible from underside.

- ++ Slight decolorization of dye beneath the mat after three weeks, mat visible from underside.

- +++ Pronounced decolorization of dye after two weeks accompanied by good growth of the fungus.

Table XXIV records the reaction of the isolates on tannic, gallic acids, and gentian violet media. Generally, there was good agreement between the reactions on the three media but some important inconsistencies were observed. Positive reactions were obtained for all isolates of the white-rot type, except culture A107 of S. sanguinolentum which gave a negative reaction with gallic acid. The results obtained for the three isolates of C. puteana were surprising because brown-rot fungi would be expected to give a negative reaction, but these reactions were only shown by cultures A263 and 05 on gallic acid, and by culture C4 on tannic acid, Davidson et al (1938) obtained negative reactions for five different isolates of C. puteana on both these media. For diagnosis, therefore, none of these media were satisfactory, particularly gentian violet, which gave position reactions with the three isolates.

Some interesting results were obtained for the fungi that were found incapable of causing decay. S. sulcatum gave positive reactions on the three media, as did culture BX1 of C. sarcoides. Three ether isolates of C. sarcoides, BG2, A110, and T157 gave a positive reaction on on tannic acid, but the rest of the isolates including M. verrucaria, gave negative reactions on the acid media.

Two facts emerge from these results. First, there appears to be good evidence that different enzymes are concerned in these reactions, e. g., cultures T157 and A110 of C. sarcoides were only able to oxidize tannic acid, while culture C4 of C. puteana was able to oxidize gallic acid and gentian violet but not tannic acid.

TABLE XXIV

REACTION AND GROWTH OF VARIOUS FUNGI ON TANNIC ACID, GALLIC ACID, AND GENTIAN VIOLET AGARS

Species	Isolate	Activity*					
		Tannic Acid		Gallic Acid		Gentian Violet	
		Reaction	Growth	Reaction	Growth	Reaction	Growth
Polyporus circinatus	A361	+++	-	++++	tr.	++	+
	A79	++++	tr.	++++	tr.	++	+
	A339	++++	10	++++	tr.	+	-
	A20	++++	tr.	++++	-	++	-
Average.....		++++	tr.	++++	tr.	++	tr.
Flammula connissans	A259	+++	-	+++	-	++	+
	A86	+++	-	+++	-	++	+
	T510	+++	-	++	-	++	+
	A300	++++	-	+++	-	+++	+
Average.....		+++	-	+++	-	++	+
Unknown C	A212	++++	tr.	++++	-	+++	+
	T173	++++	tr.	++++	-	+++	+
Average.....		++++	tr.	++++	-	+++	+
Coniophora puteana	A263	+	tr.	-	27	+++	+
	C5	+	25	-	29	+	-
	C4	-	25	++	27	++	+
Average.....		+	tr. to 25	-	25 to 30	++	+
Stereum sulcatum	T491	++++	10	+++	tr.	+	+
Myrothecium verrucaria	144743	-	18	-	9	++	+

TABLE XXIV (Concl'd)

Species	Isolate	Activity*					
		Tannic Acid		Gallic Acic		Gentian Violet	
		Reaction	Growth	Reaction	Growth	Reaction	Growth
Stereum sanguinolentum	A94	++++	12	+	10	++	-
	A107	+++++	tr.	-	-	++	+
	T492	+++++	tr.	+	-	++	+
Average.....		+++++	tr.to 12	+	0 - 10	++	+
Fomes pini	E24	++++	tr.	+++	tr.	++	+
	E25	+++++	tr.	++++	9	++	-
	T434	+++++	12	++++	tr.	++	-
Average.....		+++++	tr.-12	++++	tr.-9	++	-
Peniophora septentrionalis	B356	++++	35	+++++	9	+++	+
	A2	+++++	25	+++++	14	+++	+
	A90	++++	25	+++++	10	+++	+
Average.....		++++	25-35	+++++	9-14	+++	+
Coryne sarcoides	BA3	-	25	-	10		
	BX1	++++	15	+++++	tr.	+	+
	BG2	++++	25	-	14	+	-
	L2C	-	tr.	-	?	-	-
	C103	-	-	-	9	-	-
	A29	-	-	-	11	-	+
	A110	+++	9	-	tr.	-	+
	C52	-	-	-	tr.	-	-
	T157	++++	8	-	tr.	-	+
	A56	-	10	-	12	-	-

* Growth on tannic and gallic acid media are given as colony diameters (mm.) of Petri-dish cultures at the end of seven days. The presence or absence of growth on gentian violet slants after three weeks is indicated by plus or minus signs. The basis for the reaction ratings is given in the text.

In contrast, M. verrucaria did not react on either tannic or gallic acid, yet this fungus was able to decolorize gentian violet. It was also evident that certain fungi might react on the Bavendamm's substrates and yet be incapable of causing decay, e.g., C. sarcoides and S. sulcatum. There was evidence, therefore, that two or more enzymes are concerned in Bavendamm's reaction, but it seems unlikely that they can play a part in catalysing the degradation of lignin.

(3) Observations on the Browning of Sawdust by White-rot Fungi

A characteristic browning was associated with fungi of the white-rot type in sawdust cultures containing Badcock's Accelerator; it also occurred in wood-block cultures which had not received additional nutrients. Since extracellular enzymes of the phenol oxidase type are characteristically produced by white-rot fungi, it seemed likely that the reaction was an oxidative browning of certain phenolic compounds in the wood, but it was also possible that the reaction was caused by lignin-oxidizing enzymes. If it could be demonstrated that the browning followed oxidation of phenolic substances, it was possible that information might be gained about identity of the particular substrates concerned in the reaction; otherwise it might be possible to show that the substrate was lignin. These possibilities were tested by seeing of the reaction would occur in sawdust after the phenolic substances had been removed by appropriate solvents.

The phenolic substances were extracted by the method described by Lindstedt (1949). Air dry samples of heartwood of subalpine spruce were cut into pieces 1 - 2 inches long, and about $\frac{1}{4}$ inch diameter, ground in a mill, and then passed through a 60-mesh seive. Thirty-three g. of sawdust were extracted with 300 ml. of ether in a glass percolator at room temperature for 24 hours. The extract was then evaporated to 33 ml. Lindstedt, (1949) found that most of the phenolic constituents in pine heartwood were in the ether extract, but some were also present in further acetone and alcohol extracts of the wood. Therefore, after the ether extraction, the sawdust was again extracted with 400 ml. of acetone for about 60 hours, and the extract evaporated to 33 ml. In a similar manner 13 g. of the ether and acetone extracted sawdust were extracted with 70 ml. of 90 per cent ethanol, and the extract was evaporated to 13 ml, The browning reaction with the following was now investigated: (1) sawdust extracted with ether and acetone, (2) sawdust extracted with ether, acetone, and alcohol and (3) untreated sawdust. In addition, tests were carried out on extracted and untreated sawdust to which had been added the extractives which had been removed with the solvents, e.g. 1 ml. of the ether extract containing the extractives from 1 g. of wood was thoroughly mixed with 1 g. of the extracted sawdust in a mortar, and then the ether was evaporated off. Each

sample of sawdust was treated similarly; in the controls, solvents alone were used. The tests were made with one gram of treated sawdust in 100 ml. flasks containing 5 ml. of casein hydrolysate medium (Modification B). These were autoclaved at 20 lbs. p.s.i. for 20 minutes. The flasks were inoculated by placing an agar plug from culture T173 of Unknown C. in the centre of the thin layer of sawdust in the bottom of each flask. The cultures were incubated at room temperature and the reaction was recorded at the end of 7, 11, and 14 days by measuring the diameter of the brown zone in the sawdust from the underside of each flask (see Fig. 14). Table XXV records the results that were obtained for the various treatments at the end of 14 days.

The browning reaction in the extracted sawdust culture was stronger than in the controls. This was especially so in solvent extracted sawdust. There was very little difference between the reactions when the extractives were returned to the sawdust. For example, reaction zones measuring 29 and 52 mm., respectively, were recorded for cultures on extracted sawdust and on sawdust that had been treated with the three solvents, whereas, values of 27.2 and 47.7 were recorded for the corresponding cultures in which the extractives had been replaced. It is noteworthy, however, that a maximum reaction of 55.0 mm. was obtained when the alcohol extract alone was added to extracted sawdust. This indicated that although the constituents removed by the ether and acetone were not responsible for the browning, their presence in the wood might have reduced the intensity of the reaction. That this did not occur when the alcohol extract was returned strongly suggested that a relatively stable but partly alcohol-soluble component of wood, possibly lignin, gave rise to the browning, and that the reaction might be intensified when the lignin became more accessible. If the intensity of the reaction was determined by the amount of substrate made available by the treatment, it seemed likely that relatively stronger reactions could be obtained in wood that had a greater initial concentration of lignin.

Since highly lignified tissues occur in knots, an experiment was set up to compare the browning reactions in sawdust obtained from knots and knot-free wood. The sawdust for these tests were prepared in a similar manner to that of the previous experiment. Half of each kind of sawdust was left untreated (this was exposed to the solvents as before but not extracted), the other half wasextracted with ether and acetone and the extracts evaporated to the appropriate volumes as before. There appeared to be a larger quantity of extractives in the extract from the knot-free sawdust than in that obtained from the knot sawdust: the extract from the former was deep wine-red in colour, while the extract from the knot sawdust was pale yellow. In addition, a sample of sawdust was prepared from wood adjacent to the knots, but this was not extracted.

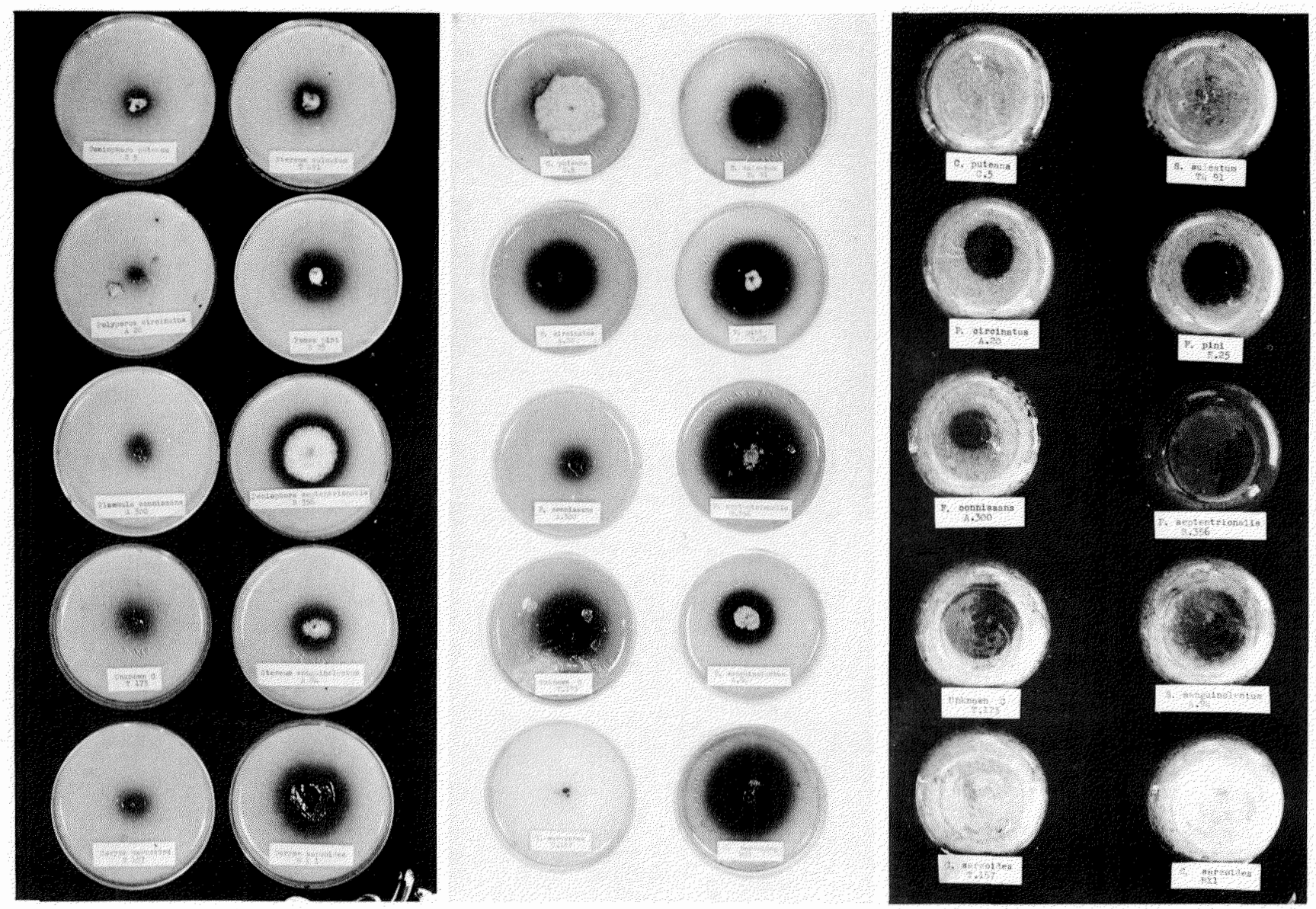

Fig. 14. A comparison of the reaction and growth of representative heartwood fungi on tannic and gallic acid media with the reaction obtained by growing the same cultures on extracted spruce sawdust. From left to right, tannic and gallic acid series at the end of seven days, sawdust series at the end of 10 days.

TABLE XXV

BROWNING REACTION ON SPRUCE SAWDUST EXTRACTED WITH VARIOUS SOLVENTS

Treatment	Replicate	Diameter of Brown Oxidation Zone on Sawdust (mm.)
Untreated (I) sawdust from tree No.500	1	26.5
	2	31.5
	Mean	29.0
Extracted with ether and acetone (II)	1	37.5
	2	49.5
	Mean	43.5
Extractedwith ether, acetone and alcohol (III)	1	49.0
	2	55.0
	Mean	52.0
Sawdust of Treatment II plus ether extract	1	-
	2	50.0
	Mean	50.0
Sawdust of Treatment II plus acetone extract	1	44.0
	2	44.5
	Mean	44.2
Sawdust of Treatment III plus alcohol extract	1	56.0
	2	54.0
	Mean	55.0
Sawdust of Treatment III plus ether, acetone, and alcohol extracts	1	49.5
	2	46.0
	Mean	47.7
Untreated sawdust (1) plus the extracts which were removed	1	27.5
	2	27.0
	Mean	27.2
Untreated (IV) sawdust from tree No.7W0	1	-
	2	30.0
	Mean	30.0

Note: Test fungus - Unknown C (T173)

Sawdust cultures were prepared in duplicate as before, and the intensity of the reaction was recorded after 7, 11, and 14 days. Table XXVI, records the results that were obtained after 14 days, for the seven different treatments.

Unextracted sawdust from both kinds of wood gave about the same reaction, the diameters of the zones being 29.1 and 27.2 mm., respectively, but the reaction of knot sawdust was brick-red in colour, while that of the knot-free sawdust was orange brown. The extracted knot sawdust gave a much stronger reaction (46.7 mm.) than the extracted knot-free sawdust (37.0) mm.); the colours were as before, The extract from the knot sawdust appeared to retard the browning reaction, while the extract from the knot-free sawdust slightly increased the intensity of the reaction, despite the fact that this extract seemed to contain more extractives. Apart from these inconsistencies the experiment confirmed the earlier results that when the sawdust was extracted the reaction was stronger. It also provided evidence that lignin gave rise to the reaction because a greater concentration of lignin was probably contained in the knots.

In view of the possible significance of lignin in this reaction the results will be given of an attempt to prepare a sample of "native" lignin for use in these studies. According to Brauns (1939), it is possible to obtain an unchangedfraction of about 3 to 4 per cent of the total lignin in wood by extracting finely divided wood-meal (100 to 150) mesh), first with cold water and ether, and then with alcohol at room-temperature until the alcohol which drains off is colourless (this usually takes about two weeks.) After the alcohol is removed under reduced pressure, a finely divided creamy precipitate separates out which is then washed with cold water and ether, repeatedly dissolved in dioxane and precipitated in distilled water, and finally precipitated into ether until the methoxyl content remains constant. The lignin so obtained is a light cream coloured powder. Brauns showed that this lignin behaves chemically in a manner similar in every respect to the total lignin in wood. Following this procedure an attempt was made to obtain a sample of lignin for tests but only a small amount of wood-meal was available and the amount of the final produce wasless than 0.1 g. No attempt, therefore, was made to purify this product, and it was not tested for methoxyl content. When tested for lignin with phoroglucinol and HCl, however, it gave a strong purple-red colour. This sample was used in the following way. The tests were carried out by soaking four filter-papers (Whatman No. 40, 5 cm.) in aconcentrated dioxane solution of the unpurified lignin product until all the solvent was taken up. The filter-papers were then dried at room-temperature, placed on glass beads on 100 ml. flasks containing 5 ml. of casein hydrolysate medium (Modification B) and autoclaved at 15 lbs. p.s.i. for 15 minutes. An agar plug from culture T173 of Unknown C was placed on the centre of each lignin-impregnated filter-paper and four untreated filter-

TABLE XXVI

COMPARISON OF THE BROWNING REACTION ON KNOT-SAWDUST AND KNOT-FREE SAWDUST AFTER EXTRACTION WITH ACETONE

Treatment		Replicate	Diameter of Brown Oxidation Zone Around Inoculum (mm.)
I	Untreated knot-sawdust (I)	1	35.2
		2	23.0
		Mean	29.1
II	Untreatedknot-free-sawdust (II)	1	23.0
		2	31.0
		Mean	27.2
III	Knot-sawdust (1) extracted with ether and acetone	1	44.5
		2	49.0
		Mean	46.7
IV	Knot-free-sawdust (II) extracted with ether and acetone	1	37.0
		2	37.0
		Mean	37.0
V	Knot-sawdust of Treatment III plus ether and acetone extract	1	34.5
		2	34.0
		Mean	34.2
VI	Knot-free sawdust of Treatment IV plus ether and acetone extract	1	38.5
		2	40.5
		Mean	39.5
VII	Untreated sawdust from wood adjacent to knots	1	36.5
		2	32.5
		Mean	34.5

Note: Test fungus - Unknown C (T173)

papers were inoculated in the same way as controls. At the end of two weeks, a faint, but distinct, brown reaction zone appeared around the inoculum on the filter-paper containing the lignin product, but not in the controls. Although it was not possible to repeat the experiment with a chemically pure sample of lignin, there was some evidence from the phloroglucinol test that the alcohol extracted product contained lignin and this was seen to give a reaction similar to that obtained on the sawdust.

(4) Oxidation of Lignin

Using sawdust cultures and the technique described above, the 34 isolates listed in Table II were screened for their ability to oxidize lignin. The sawdust for these tests was extracted with ether and acetone, and then placed in 100 ml. flasks containing 5 ml. of casein hydrolysate medium (B). Two flasks were inoculated with agar plugs from plate cultures of the fungi, and incubated at room temperature for 10 days. The reaction on the sawdust was measured as before. The final pH of the medium was also obtained by extracting the sawdust with 5 ml. of distilled water for 2 to 3 hours, filtering, then measuring the pH of the filtrate with a meter. The results are given in Table XXVII which records the averages obtained for two cultures of each fungus.

The values obtained for replicate cultures agreed very closely, and it can be seen from Table XXVII that there was also good agreement between isolates of the separate species. Positive reactions were obtained with each isolate of the white-rot species, while negative reactions were shown by the three isolates of C. puteana, the single isolates of M. verrucaria and S. sulcatum*, and by the 10 isolates of C. sarcoides. Thus, the experiment clearly demonstrated that only fungi of the white-rot type were able to produce the browning reaction.

Growth of none of the cultures was inhibited by the presence of lignin since all grew on the sawdust containing the nutrient solution and all produced changes in the pH. Nearly all the fungi lowered the pH; particularly the three isolates of C. puteana where the final pH values were about 3 compared with 5.1 for the uninoculated control. F. connissans grew well on the sawdust but did not change the pH appreciably.

(5) The Oxidation of Lignin in Relation to Bavendamm's Reaction

Although most fungi of the white- and brown-rot type may be differentiated by tests such as that of Bavendamm (which uses tannic and gallic acid substrates) certain inconsistencies were noted by

* Subsequent studies have cast some doubt upon the typicalness of the culture of S. sulcatum used in these experiments. There is evidence of degeneration in this isolate in the ability to oxidize lignin, in cellulolytic activity, as well as in spore production which might explain the negative results obtained in the decay tests.

TABLE XXVII

THE OXIDATION OF LIGNIN BY VARIOUS FUNGI AS DETERMINED BY THEIR REACTION ON EXTRACTED SPRUCE SAWDUST,* AT THE END OF 10 DAYS

Species	Isolate	Intensity of ** Reaction	Final pH of Sawdust
Polyporus circinatus	A361	18	4.40
	A79	18	4.85
	A339	22	4.45
	A20	13	4.42
Average.....		18	4.53
Flammula connissans	A259	9	5.36
	A86	6	5.28
	T510	6	5.28
	A300	7	5.00
Average.....		7	5.23
Unknown C	A212	15	4.46
	T173	20	4.51
Average.....		18	4.48
Coniophora puteana	A263	-	2.88
	C5	-	2.86
	C4	-	3.16
Average.....		-	2.96
Stereum sulcatum	T491	-	4.05
Myrothecium verrucaria	144743	-	5.18
		Uninoculated Control.....	5.07

TABLE XXVII (Concl'd)

Species	Isolate	Intensity[**] of Reaction	Final pH of Sawdust
Stereum sanguinolentum	A94	22	4.92
	A107	27	4.02
	T492	19	4.82
Average.....		23	4.59
Fomes pini	E24	20	4.02
	E25	20	4.07
	T434	22	4.21
Average.....		21	4.10
Peniophora septentrionalis	B356	52	4.36
	A2	42	4.28
	A90	51	4.30
Average.....		48	4.31
Corvne sarcoides	BX1	-	4.36
	BA3	-	4.76
	BG2	-	4.50
	L2C	-	4.65
	C103	-	4.51
	A29	-	4.66
	A110	-	5.00
	C52	-	4.54
	T157	-	4.69
	A56	-	4.68
Average.....		-	4.63
	Uninoculated Control.....		5.07

* Most of the tannins, resins, and phenolic compounds were removed from the sawdust by extracting with water, ether, and alcohol for 24 hours at room temperature.

** Intensity of reaction in terms of diameter (mm.) of "browning" zone in sawdust. Tests were run in duplicate.

Davidson et al (1938), and still others were demonstrated by the present work. The more consistent results obtained by the sawdust tests suggested that the browning reaction might be more useful diagnostically than the Bavendamm reaction, or that of Preston and McLennan (1948), which is based on the decolorisation of dyes in the medium. To demonstrate this, a comparative study was made with 10 cultures with the Bavendamm substrates and sawdust extracted by the methods already described. The reactions obtained on the three media are shown in Fig. 14: the photographs illustrate the reactions obtained on plates containing tannic and gallic acid after 7 days, and the sawdust after 10 days. It can be seen that the sawdust reaction was better for differentiating the fungi of the white-rot type from the brown-rot fungus C. puteana. It was also a better indicator of the decaying ability of the fungi. For example, although cultures T157 and BX1 of C. sarcoides, and culture T491 of S. sulcatum did not cause decay in laboratory tests, they gave a positive reaction on one or both of the Bavendamm substrates, but not on the sawdust. It is of some interest, however, that cultures which produced strong reaction zones on tannic and gallic acid media generally produced a strong reaction on the sawdust, e.g., P. septentrionalis and Unknown C.

(c) Relation of Enzyme Activity to Decaying Activity

The results of the enzyme and decay studies recorded in Tables XXI, XXIII, XXIV, and XXVII were compared to see if there was a relationship between the production of certain enzymes and the ability of the fungi to cause decay. These data are summarized in Table XXVIII which shows the mean values obtained for 10 species of fungi for cellulolytic activity, phenol oxidase activity on tannic and gallic acids, oxidation of lignin (browning reaction), and the decaying activity as determined by loss-in-weight of the wood blocks in the laboratory.

Considering first the relation between the cellulolytic activity of culture filtrates and decay, it is seen that filtrates from the British isolates of C. sarcoides were the most active, but these cultures caused negligible decay. C. puteana, on the other hand, produced most decay but only ranked fifth among the fungi in the activity of its culture filtrates. It was also clear from the values obtained for the other cultures that the cellulolytic activity of culture filtrates was no indication of the ability of the fungi to cause decay under laboratory conditions.

The reactions of the fungi on tannic and gallic acid were also not uniformly related to decay activity. S. sulcatum gave a moderately strong reaction on both media, but did not cause decay in laboratory tests. Similarly, certain cultures of C. sarcoides gave positive reactions on these substrates but did not cause decay.

TABLE XXVIII

COMPARISON OF CERTAIN ENZYMATIC ACTIVITIES AND THE DECAYING ABILITY OF 10 SPECIES OF FUNGI

Species	Cellulolytic Activity	Polyphenol oxidase Activity		Enzymic Oxidation of Lignin	Decaying Ability % loss in weight
		Tannic Acid	Gallic Acid		
Polyporus circinatus	.051	++++	++++	18	3.26
Flammula connissans	.048	+++	+++	7	2.02
Unknown C	.034	++++	++++	18	3.30
Coniophora puteana	.073	+	-	-	12.00
Stereum sulcatum	.009	++++	+++	-	0.0
Myrothecium verrucaria	.041	-	-	-	0.65
Stereum sanguinolentum	.077	+++++	+	23	3.12
Fomes pini	.087	+++++	++++	21	3.09
Peniophora septentrionalis	.085	++++	+++++	48	3.68
Coryne (British) sarcoides	.119	-to ++++	- to +++++	-	1.20*
Coryne (Canadian) sarcoides	.045	- to ++++	-	-	0.0

* Average of two test-blocks only.

Note: Except for S. sulcatum and M. verrucaria (one isolate of each), the determinations were based on three ormore isolates of each species.

In contrast, the ability to oxidize lignin shown by the browning reaction was fairly well related to decaying ability. The only inconsistencies were those of M. verrucaria and C. sarcoides (British strain) which gave no reaction on sawdust, although these fungi did cause some decay. Since both fungi produce cellulase (a) decay might have been caused by degradation of cellulose and not of the lignin. In this respect, these fungi behave like the brown-rot fungi, and thus might be compared with C. puteana.

The intensity of the browning reaction to some extent could be correlated with the decaying activity of the white-rot fungi. Thus the sizes of the brown zones and the percentages of decay for the three butt-rot fungi, Unknown C, P. cinctipus, and F. connissans were 18, 18, 7 and 3.30, 3.26, 2.02 respectively, while these values for the three trunk-rot fungi, P. septentrionalis, S. sanguinolentum, and F. pini, were 48 and 25, 21 and 3.68, 3.12 and 3.09 respectively.

B. ANTAGONISM BETWEEN HEARTWOOD FUNGI

Certain species of fungi, notably C. sarcoides, are frequently found associated with decay in living trees, but there is evidence from field and laboratory studies indicating that these fungi are incapable of initiating decay. Butfrom an ecological standpoint other aspects of the relationship between decay-producing and non-decay-producing fungi in the heartwood might be important. For example, although samples of both the white and brown types of decay taken from living trees have yielded cultures of C. sarcoides it was not always possible to obtain cultures of the causal wood-destroying fungus, (Etheridge, 1954). This suggested that, under certain conditions, C. sarcoides might be important, and when well established in the wood, might seriously affect the progress of decay. It has also been found from more recent studies (Etheridge, 1955), and from the present work, that this fungus is sometimes present in the heartwood of trees that have not been attacked by heart-rot fungi. It was important, therefore, to know how the presence of this fungus in wood might affect subsequent decay by wood-destroying fungi.

This was investigated in the following manner. Fifteen cultures each of isolates T157 and A29 of C. sarcoides were grown on 20 ml of2 per cent malt extract agar in screw-top jars for 5 to 6 weeks. Six additional jars were prepared in the same way but were not inoculated. Thirty-six test-blocks of the type shown in Fig. 1 were prepared as for the decay experiments. These were sterilized with steam and one block was placed in each of the culture jars. Water was added to the blocks to give an initial moisture content of 50 per cent of their dry-weight, and the cultures were

incubated at room-temperature for two months. The blocks were then examined and it was seen that the 30 inoculated blocks were stained either pink or purple, or bore fruit-bodies of the fungus; this was taken as evidence that C. sarcoides was well established on the wood. Microscopic examination of a section of wood taken from one of the blocks revealed that the fungus had also penetrated into the wood. Evidence of this is shown in the photomicrograph in Fig. 16 which shows a hypha penetrating a cell-wall. The blocks of wood were then removed aseptically from the agar cultures and placed in washed and sterilized jars without agar. The procedure was then according to the table:

Blocks infected
T157 of C. sarcoides

3 inoculated C. puteana (C5)
3 " P. circinatus (A20)
3 steamed and then inoculated C. puteana
3 " " " " P. circinatus
3 blocks no further treatment

Blocks infected
A29 of C. sarcoides

3 inoculated C. puteana (C5)
3 " P. circinatus (A20)
3 steamed and then inoculated C. puteana
3 " " " " P. circinatus
3 blocks no further treatment

Blocks not infected C. sarcoides

3 inoculated C. puteana
3 " P. circinatus

One ml. ofwater was then added to each block under the disc of inoculum and they were incubated at room-temperature for three months. The loss in weight and the final moisture content was determined and the results are recorded in Table XXIX.

Six of the cultures were lost through contamination, nevertheless, the experiment showed that isolate A29 of C. sarcoides was able to retard considerably decay by C. puteana and P. circinatus. WithC. puteana, the loss in weight was reduced by about 75 per cent of that obtained in the controls where C. sarcoides was absent, and of that in the blocks where the fungus had been killed by steam.

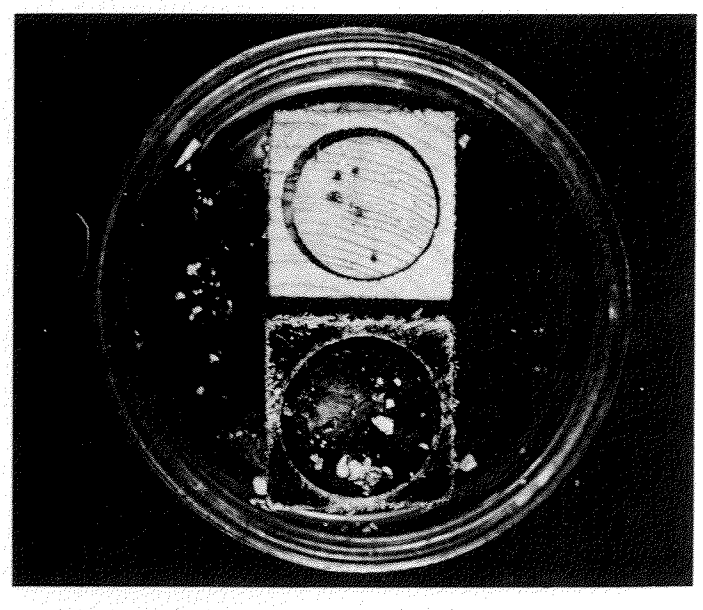

Fig. 15. Test-blocks of wood infected with pure cultures of isolate A 29 of Coryne sarcoides (upper) and isolate C5 of Coniophora puteana (lower) after being incubated together for three months. The presence of the antagonistic strain of C. sarcoides in the upper block has prevented infection by the wood-rotting fungus.

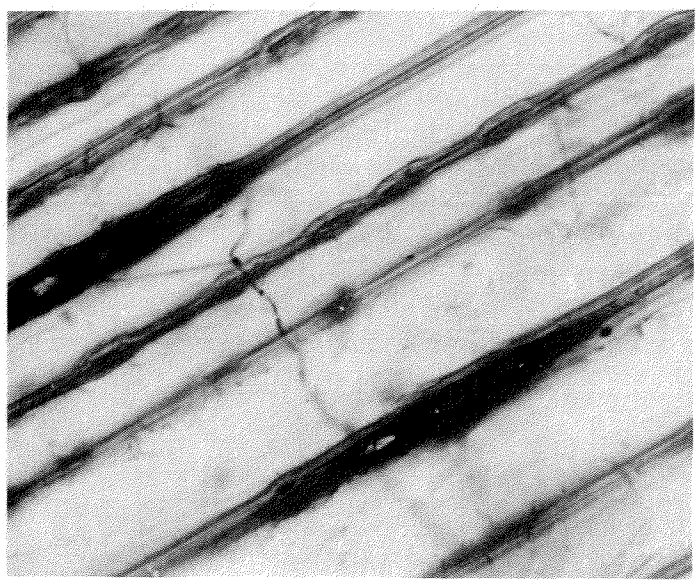

Fig. 16. Photomicrograph of a section of spruce heartwood infected with a pure culture of Coryne sarcoides, showing a hypha penetrating the wall of a tracheid. Approximately 250 X.

TABLE XXIX

THE EFFECT OF TWO ISOLATES OF CORYNE SARCOIDES ON THE DECAYING ACTIVITY OF CONIOPHORA PUTEANA AND POLYPORUS CIRCINATUS, AS DETERMINED BY THE LOSS IN WEIGHT OF SAMPLES OF SUBALPINE SPRUCE AFTER THREE MONTHS

Fungus	Coryne sarcoides present: Isolate T157 living	Isolate T157 dead	Isolate A29 living	Isolate A29 dead	Coryne sarcoides absent
		Decay			36.57
Coniophora (C5) puteana	contd.	19.44	0.0	20.38	28.86
	30.64	17.58	13.32	21.46	22.41
	28.04	25.98	5.75	29.26	18.12
Average.....	29.34	21.00	6.36	23.70	26.49
		Final Moisture Content			235.8
	-	125.0	223.7	123.4	174.6
	183.5	110.1	131.1	247.9	152.7
	215.9	159.2	198.1	218.2	215.6
Average.....	199.7	131.4	184.3	196.5	194.7
		Decay			
Polyporus (A20) circinatus	0.90	2.40	0.00	2.05	- unsucc.
	0.59	2.15	1.99	1.83	-
	1.41	0.90	0.39	2.43	1.80
Average.....	0.96	1.81	0.79	2.10	1.80
		Final Moisture Content			
	87.0	52.7	90.6	81.1	-
	59.6	42.5	105.7	96.7	-
	70.3	71.5	84.6	91.7	64.0
Average.....	72.3	55.6	93.6	89.8	64.0

	Decay	Moisture	Decay	Moisture
	0.0	-	0.0	124.8
	0.5	44.9	0.0	50.3
	0.0	144.0	0.0	-
Average.....	0.0	94.4	0.0	87.5

With P. circinatus, the loss in weight was reduced by about half of that obtained in the single block which was available as the control (two of the control blocks were lost through contamination), and almost by two thirds of that obtained in the three blocks which had been steamed. To some extent the living mycelia of the isolate T157 retarded decay by P. circinatus, but this strain promoted decay by C. puteana. Thus, there was evidence that only living cultures of C. sarcoides affected the rate of decay by the wood-destroying fungi. There was no loss in weight after five months in the blocks inoculated only with cultures of C. sarcoides.

Since some form of antagonism by C. sarcoides was indicated by the results obtained in the above experiment, 12 isolates of C. sarcoides were screened for their antagonism against isolate C5 of C. puteana. Five British and 7 Canadian isolates were tested as follows: An agar plug from an actively growing culture of C. puteana was placed at one edge of a plate containing 20 ml. of 2 per cent malt extract agar, and a spore suspension of C. sarcoides was streaked on the opposite side of the plate at a distance of about 6 cm. The width of the zone between the two cultures was measured at the end of 7, 10 and 14 days, and observations were made of the mutual effects of the growth of the two fungi. The results obtained at the end of 14 days are summarized in Table XXX.

Isolates BX1, BG2 (Br.), and A29 (Cdn.) of C. sarcoides prevented the growth of C. puteana at distances of 5.0, 6.0, and 3.0 cm., respectively, but the remaining nine isolates of C. sarcoides were overgrown to a greater or lesser extent by the decay fungus. The antagonism of the three active isolates of C. sarcoides is shown in Fig. 18 which also shows five inactive isolates that were overgrown and completely covered by C. puteana.

The tests with isolates A29 and T157 of C. sarcoides were of some value in explaining the results obtained from the decay experiment since isolate A29 which retarded decay by C. puteana in the wood blocks also prevented growth of this fungus on agar. Similarly, it is seen that T157 was inactive on agar, and did not affect the fungus on wood. The antagonism of isolate A29 against isolate C5 of C. puteana on wood and on agar cultures is shown in Figs. 15 and 17. Fig. 17 also shows T157 completely overgrown by C. puteana on agar.

From an ecological standpoint, it is of some interest that two, i.e., 40 per cent, of the British isolates of C. sarcoides were antagonistic in these tests, while only one representing 14 per cent of the Canadian isolates was active.

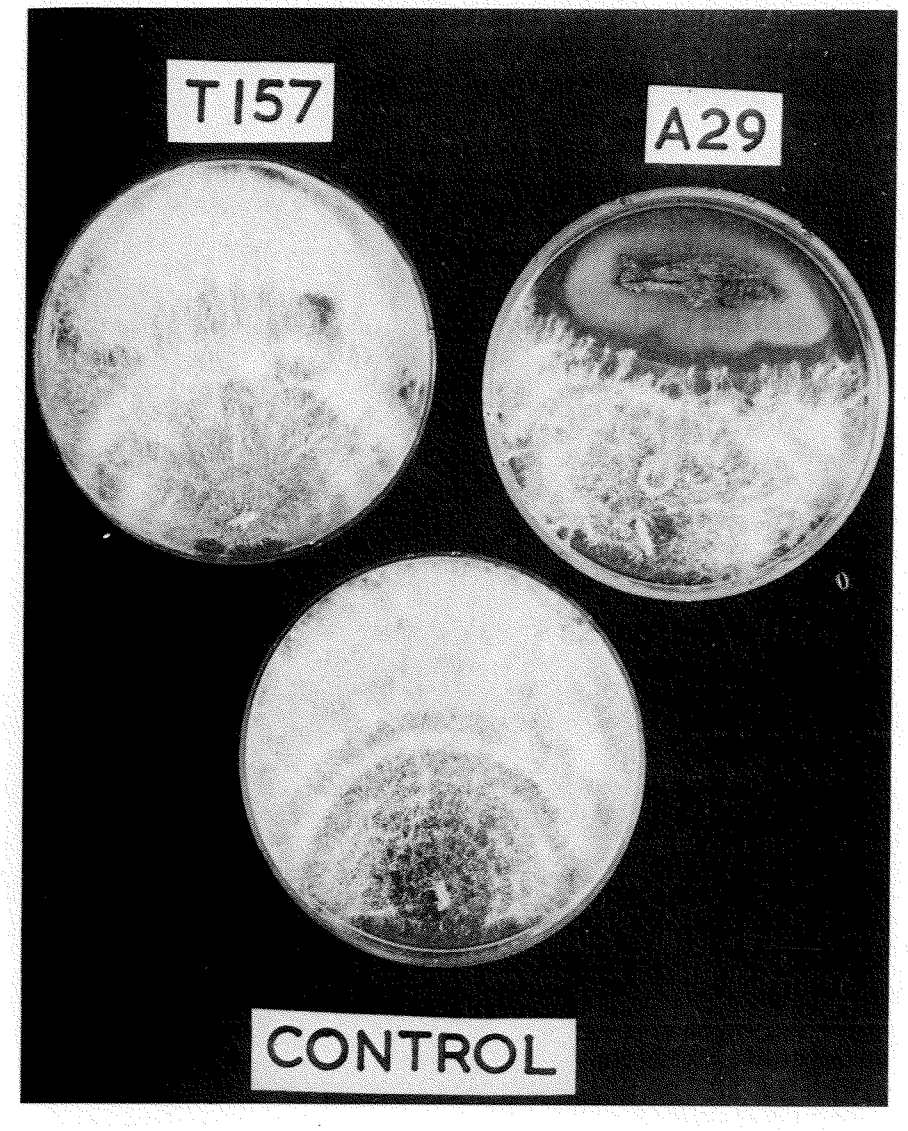

Fig. 17. Antagonistic action of Coryne sarcoides (upper colony) on Coniophora puteana (C-5), two weeks after inoculating the plates.

Culture No. T-157. Unactive isolate of Coryne sarcoides
Culture No. A-29. Active isolate of Coryne sarcoides

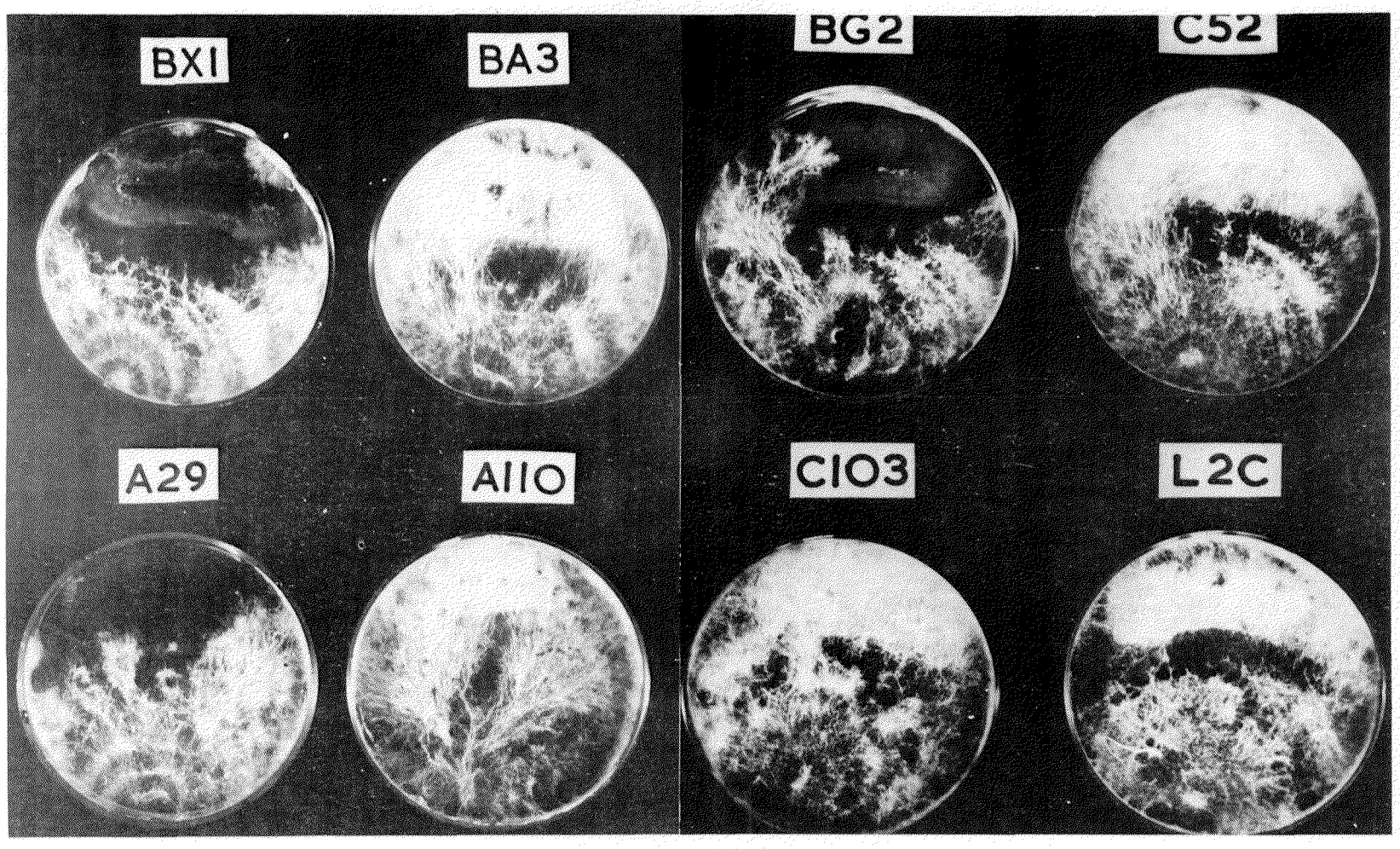

Fig. 18. Differential antagonistic action of eight isolates of Coryne sarcoides (upper colony) on Coniophora puteana (C-5), two weeks after inoculating the plates.

Cultures No. BX-1, BG-2, and A-29 are active isolates of Coryne sarcoides.

TABLE XXX

ANTAGONISTIC ACTIVITY OF CORYNE SARCOIDES AGAINST CONIOPHORA PUTEANA (C5), AS DETERMINED BY WIDTH OF INHIBITION ZONE BETWEEN COLONIES OF THE FUNGI ON MALT AGAR AT THE END OF 14 DAYS

Isolate of Coryne sarcoides	Width of Inhibition zone between the two colonies (cm.)	Remarks
BX1 (British)	5.0	Inhibition zone completely encircles C. sarcoides
BA3 "	-	C. sarcoides half overgrown by C. puteana
BG2 "	6.0	Aerial mycelium of C. puteana growing over zone in one place
A11 "	-	C. sarcoides completely overgrown by C. puteana
FC1 "	-	C. sarcoides two thirds overgrown by C. puteana
12C (Canadian)	-	C. sarcoides about half overgrown by C. puteana
C103 "	-	C. sarcoides almost completely overgrown by C. puteana
A29 "	3.0	Aerial mycelium of C. puteana growing over zone in one place
A110 "	-	C. sarcoides completely overgrown by C. puteana
C52 "	-	C. sarcoides two thirds overgrown by C. puteana
T157 "	-	C. sarcoides completely overgrown by C. puteana
A56 "	-	C. sarcoides about one third overgrown by C. puteana
CONTROL		
Coniophora puteana (C5) alone.....		Plate almost completely overgrown

DISCUSSION

One of the results of this investigation has been to demonstrate a connection between site moisture conditions, growth and vigor of the trees, and the moisture content in the heartwood of subalpine spruce. These results are similar to those obtained by Chalk and Bigg (6) for Sitka spruce in Britain who found that the moisture content in the sapwood tended to be highest in the more vigorous trees on the moist sites. Since the conclusions in both these investigations have been based on comparisons when the moisture content was expressed as a percentage of the moisture held by the wood at saturation, it is unlikely that the results can be explained by differences in the specific gravity of the samples. There is evidence that the distinctly wetter heartwood in trees on the moist site and in overstorey trees, is directly influenced by the amount of available water, not by the properties of wood associated with the faster rate of growth. For instance, tree "12 WU" on the moist site, and tree "5DO" on the dry site have similar densities and ring widths but differ only in their moisture contents; similarly, an overstorey tree, viz., "9 WO" on the moist site had a distinctly higher moisture content than an understorey tree, viz., "10 WU" growing on the same site in spite of otherwise similar characteristics of the heartwood. Moreover, there was a sharp drop in the moisture content in all the trees at the 20 ft. level of the stem and a further drop at the 40 ft. level which bore no apparent relation to the specific gravity of the wood.

It is shown here that whilst a difference in the moisture content of only 3% of saturation existed between the fast-growing dominants on the moist site and the slow-growing understorey trees on the dry site, this small difference was statistically significant and it had a marked effect on the occurrence of fungi in the trees. However, the point of interest is that the lower moisture level characterizing the heartwood of the dry site trees was sufficiently critical to prevent infection by the fungi. For example, 13.3% of the samples from the moist site trees having an average moisture content of 10% saturation yielded fungi, whereas, no fungi were isolated from the dry site samples which had moisture contents averaging 7%. A similar pattern in the distribution of moisture appears to explain the absence of fungi above the 20 ft. level of the trees since average moisture content values in the butt and upper portions of the stem were, respectively, 9.8 and 6.0%. It is, therefore probable that the threshold moisture content for infection in the trees lies somewhere around 7% of saturation. This is borne out by laboratory experiments which have indicated that infection fails to take place in test blocks with moisture contents lower than 8% saturation (Fig. 3).

A significant effect on decay induced by small changes in the moisture content of wood has been clearly demonstrated by the laboratory tests. Results of this study have shown that a difference in the moisture content as little as 3.4% of saturation has a critical effect on the rate of decay by C. puteana. It has also been seen that when moisture is controlled the differences in ring-frequency and specific gravity which represented differences between these properties in the living trees, did not result in significant differences in decay by this fungus. Neither is there any evidence that decay is influenced by the origin of the samples. This appears to exclude the possibility that variations in the distribution of decay-promoting or decay-retarding substances occur in the trees.

Although only one fungus was used in the tests, there is some evidence from collateral studies that other species of wood-destroying fungi occurring on subalpine spruce have similar moisture requirements. Tests with seven different species of wood-destroying fungi have shown that moisture contents of from 5 to 10% of saturation are needed before any appreciable decay can occur.

The differences in the moisture and temperature requirements for butt - and trunk-rot fungi seem to be related to their specific behavior in the trees, These studies show that the most favorable moisture range for decay by butt-rotting fungi lies between 34 and 44 per cent of saturation, while that for the trunk-rotting fungi is lower and lies between 11 and 39 per cent of saturation. Moreover, butt-rot fungi characteristically produced higher moisture contents as a result of their growth in the wood than those produced by the trunk-rot fungi. This might indicate adaptation by the butt-rot fungi to a wetter environment since moisture contents are generally higher in the lower part of the trees. Studies made with the fungi on agar plates at various temperatures show that the optima and maxima temperatures for the species of butt-rot fungi are about 5°C lower than those for the species of trunk-rot fungi. It is of interest that differences of the same order have been found to exist in the stem temperatures of conifers. Sarahov (1952), has noted that in Pinus sylvestrias, the stem temperature tends to be higher at 4.5 feet from the ground than at ground level, and in July on the south side of the trees he observed a difference of 5°C. It may well be that both the moisture and temperature conditions in the trees are of considerable ecological importance in determining the relative dominance of fungi in certain situations.

The relative ability of the fungi to cause decay under laboratory conditions was no indication of their relative importance as decay pathogens in nature. This is not surprising in view of the different standards for estimating the decaying activity of the fungi in field and laboratory studies. In laboratory tests, the criterion of comparison is the loss of weight in test specimens within a specified time, but in the living tree it is the relative volume of wood affected by the lineal progress of decay over a period of years. For example, F. pini is by far the most destructive decay pathogen in living conifers in the Northern Hemisphere, yet most of the other fungi used in the laboratory tests showed a greater ability to cause decay. In contrast, C. puteana caused the most decay in the laboratory, yet was responsible for only 2 per cent of the total decay volume in living subalpine spruce. The practice in laboratory tests of adding various food materials to the test-blocks is also a disadvantage when comparisons are made between field andlaboratory data. The present study shows that the rate of decay by fungi is substantially increased when the test-blocks are in contact with nutrients, i.e., malt extract or Badcock's Accelerator. When no additional food material was added to the wood, much smaller losses in weight resulted, but for some fungi these lower values approached more closely those obtained under natural conditions. For example, Bjorkman, (1946) gives data on the loss in weight caused by S. sanguinolentum in pulp logs over a period of two years; in three months the logs lost from 2 to 3 per cent of their weight. This compares favorably with losses of 2 per cent obtained for this fungus on blocks of untreated wood, although losses of 5 to 6 per cent were obtained for blocks of wood placed on enriched sawdust. With C. puteana, much greater differences in the loss of weight were obtained between untreated test-blocks and blocks which had been in contact with malt extract agar. Tests made by both methods over a period of three months show that about 70 per cent more decay occurred in the blocks which had been in contact with malt extract for two months prior to the test.

It is seen that all the wood-destroying fungi secrete cellulases into their culture filtrates which show varying activities when tested against filter-paper. The trunk-rot fungi show greater cellulolytic activity than the butt-rot fungi, and this is correlated to a limited extent with the ability to cause decay. When each species is considered separately, there is no relation between cellulase activity and decay under laboratory conditions, but the activities of the trunk-rotting fungi, F. pini, P. septentrionalis and S. sanguinolentum can be correlated with the relative importance of these fungi as decay pathogens in subalpine spruce. A similar relationship is seen between the butt-rotting fungi, F. circinatus, F. connissans and Unknown C. Tests with C. puteana showed only moderately strong cellulase activity, and

there was no suggestion of a connection with the ability to cause decay. Of the non-decay-producing fungi, M. verruceria gave a relatively week cellulase test, and S. sulcatum, showed no cellulolytic activity in the tests. In the case of C. sarcoides, considerable variation occurred in the activities between the British and Canadian isolates; the three British isolates have the strongest cellulase activity of all the fungi that were tested.

Tests with the fungi for polyphenol oxidase activity on the Bavendamm substrates (tannic and gallic acid) show varying activities for the different fungi. The trunk-rotting fungi gave stronger reactions than the butt-rotting fungi, but there was no consistent relation between the intensity of the reaction and the ability of the separate species to cause decay under laboratory or natural conditions. Two isolates of C. puteana, a brown-rot fungus, gave positive reactions on tannic acid, while another isolate of the fungus gave positive reaction on gallic acid. According to Law (1955), a positive reaction on gallic acid indicates the presence of lacease, a phenol oxidase which is generally associated with fungi of the white-rot type, but she found no avidence that this enzyme was produced by C. puteana. The same author found some evidence of weak tyrosinase activity for C. puteana, but the production of this enzyme was entirely intracellular.

A positive reaction on Bavendamm substrates has been associated with the ability to decompose lignin, but it is shown here that fungi of the brown-rot type, and also species incapable of causing decay, can produce a positive reaction on these substrates. These inconsistencies are not encountered when extracted sawdust (Phenol-free) is used as a substrate. Positive reactions shown by a browning of the sawdust around the culture were only obtained with white-rot fungi in these tests. The production by certain fungi of enzymes which oxidize tannic or gallic acid, but not lignin, may indicate the occurrence of three distinct enzymes. These studies show that the type of phenolase produced by isolates of C. puteana, C. sarcoides, and S. sulcatum can oxidize either tannic or gallic acid, but these enzymes are not able to oxidize lignin. Although, it it possible that the lignin-oxidizing enzyme is also a phenolase, it appears that further information in its specificity can only be gained by testing the phenolase activity of a purified preparation of the enzyme.

Some information on the role of C. sarcoides in the decay complex in living trees has been gained from these studies. On the basis of laboratory tests, it can be stated with reasonable certainty that this fungus does not cause decay, but its presence in the heartwood may affect the progress of decay by antagonism of wood-destroying fungi. Although, studies with this fungus show

that any of the isolates are quite ineffective, several were very active in preventing the growth of C. puteana on agar plates, and the antagonism of one isolate was shown to the extent that decay by C. puteana and P. circinatus was reduced by 50 to 75 per cent of that obtained in the controls. Further studies are needed to determine the action of C. sarcoides on the chemical and physical properties of wood.

SUMMARY

1. Factors that influence variations in decay in subalpine spruce have been studied in 12 trees which occurred on a wet and a dry site in Alberta.

2. Differences in the moisture content (expressed as a percentage of saturation and as apercentage of dry weight) were found to exist between overstorey and understorey trees, and between trees of the two sites. Trees of the site and dominance classes were characterized by differences in the rate of growth, the number of annual rings per inch, and the specific gravity.

3. The moisture content was highest in the overstorey trees on the wet site (10 per cent of saturation), and lowest in understorey trees on the dry site (7 per cent of saturation). An increase of three per cent in the moisture content of the heartwood resulted in an increase in the incidence of non-decay-producing fungi from 0.0 to 13.3 per cent. The threshold moisture content for infection appeared to be at 8.5 per cent of saturation.

4. In laboratory tests, an increase of 3.4 per cent in the moisture content in specimens of wood taken from the different trees resulted in a statistically significant difference in the rate of decay by C. puteana. Differences in the chemical or physical properties of fast- and slow-grown wood had no appreciable effect on decay by C. puteana.

5. Propylene oxide gas proved to be equally effective as steam for sterilizing the test-blocks, and neither of these agents produced any measurable effect on the natural decay-resisting or decay-promoting properties of the wood. Pieces of infected wood proved to be better than plugs of infected agar for initiating decay in the blocks of wood.

6. The structure of the wood does not appear to influence the rate of imbibition of water in a saturated atmosphere, although fluctuations in the temperature cause an increase in the amount of water held by the wood after thefibres were saturated, i.e., 27 to 28 per cent of dry weight.

7. The optimum moisture and temperature requirements for butt-rotting and trunk-rotting fungi appear to be fundamentally different. The moisture optima for decay tended to be higher for the butt-rotting fungi, C. puteana, F. circinatus, F. connissans, and Unknown C than for the trunk-rotting fungi, F. pini, S. sanguinolentum, and P. septentrionalis, and the two types of fungi showed some ability to regulate the moisture content to a level more suitable for their growth. The temperature optima and maxima for the butt-rotting fungi was about 5°C. lower than for the trunk-rotting fungi.

8. There was no consistent relation between the production of enzymes by 34 isolates, representing 10 different species of fungi, and their ability to cause decay. The relative cellulase activity of the white butt- and trunk-rot types of fungi was correlated with the relative importance of these fungi as decay pathogens in nature. In contrast, the ability of these fungi to oxidize lignin (browning reaction) appears to be related to their ability to cause decay under laboratory conditions. Tests for phenolase activity on Bavendamm substrates, i.e., tannic and gallic acids, showed no consistent relation with the decaying activity of the fungi.

9. A method for differentiation of white- and brown-rot fungi based on the ability of white-rot fungi to produce a browning reaction in sawdust cultures is presented. This reaction appears to follow the oxidation of lignin since it was intensified when phenolic compounds were removed from the sawdust, and when sawdust containing a higher concentration of lignin, i.e., knot-sawdust, was used. This method appears to have none of the disadvantages of such tests as that of Bavendamm (1928), and Preston and McLennan (1948), since only fungi which are known to be capable of decomposing lignin produced the reaction.

10. Tests with the fungi on three different substrates indicate the occurrence of three distinct enzymes which show specificity for tannic, gallic acids, and lignin, respectively.

11. The occurrence of Coryne sarcoides (Jacq.) Tul. in the heartwood of sound subalpine spruce is demonstrated. Tests made with this fungus in the laboratory show that the fungus does not cause decay, but several isolates show antagonism to wood-destroying fungi. Wood inoculated with an active isolate of this fungus was comparatively resistant to decay by C. puteana and P. circinatus.

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