IN VITRO DECAY OF LODGEPOLE PINE BLOCKS BY SEVERAL SLASH-INHABITING HYMENOMYCETES

by

AUGUST ADRIAAN LOMAN

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1961

APPROVED:

Redacted for privacy

Professor of Botany

In Charge of Major

Redacted for privacy

Head of Department of Botany and Plant Pathology

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented MAY 2, 1961

Typed by Pearl Wagg

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. L. F. Roth for his critical reading of the manuscript.

Grateful acknowledgments are due to the Forest Biology Division, Department of Forestry, for permission to use data for thesis purposes.

Valuable assistance has been accorded by various members of the staff of the Forest Pathology Laboratory, Calgary. Thanks are extended in particular to Miss R. C. Robinson who identified the fungi encountered in this investigation, and to Mr. P. S. Debnam for the preparation of the photographs.

TABLE OF CONTENTS

TABLE OF CONTENTS Cont'd.

IN VITRO DECAY OF LODGEPOLE PINE BLOCKS BY SEVERAL SLASH-INHABITING HYMENOMYCETES

INTRODUCTION

Lodgepole pine (Pinus contorta Dougl.) has a very wide geographic range, and in Alberta it occupies nearly one third of the potential forest land (16, p. 3). Its main uses are pulp, poles, railway ties and lumber. Minimizing fire hazard due to accumulation of slash after logging is a vital problem to foresters involved in forest management. The effects of various means of slash disposal on fire hazard and on regeneration have received attention (10, p. 5-11; 21, p. 5-13).

The work reported here found its origin as a natural sequel to studies by the Calgary Forest Biology Laboratory, formerly Canada Department of Agriculture, involving the effect of cutting practices on the decay of lodgepole pine logging residues.

To date, in those studies, 85 species of fungi have been identified from the slash while an appreciable number of collections remain unidentified (14, p. 6-10). Of these 85 species, only seven have been consistently associated with decay.

In a volume and decay analysis conducted in the spring of 1959 on seven-year-old lodgepole pine slash, 966 cultural isolates were attempted of which 347 have been positively identified, yielding 11 fungi. All cultures were obtained from bits of wood which were taken aseptically from the upper, central and lower portions of the slash after splitting the slash perpendicularly to the soil surface. Approximately 80 per cent of all identified cultural isolates consisted of the four fungi: Lenzites saepiaria Wulf. ex Fr., Peniophora phlebioides Jacks. and Dearden, Stereum sanguinolentum Alb. and Schw. ex Fr. and Coniophora puteana (Schum. ex Fr.) Karst. Their relative frequency was in the order listed and ranged from 39.3 per cent for Lenzites saepiaria to 8.4 per cent for Coniophora puteana. Distribution of the fungi within the slash was of particular interest. Peniophora phlebioides appeared to predominate in the upper portions, Lenzites saepiaria in the central portions and Coniophora puteana and Stereum sanguinolentum in the lower portions of the slash.

Moisture and temperature are known to be important factors influencing growth and development of decay fungi and may be contributory to the above distribution. Extremes of both these factors occur

regularly between the upper exposed portions and the lower sheltered portions of individual pieces of slash, particularly in clearcut areas. Reid (15, p. 443) recorded temperatures as high as 47.5 degrees C. in subcortical areas of the upper and 22.0 degrees C. in subcortical areas of the lower portions of pieces of lodgepole pine slash at aerial temperatures between 23 and 25 degrees C.

This study was designed to provide information regarding the influence of temperature on the behaviour of Lenzites saepiaria Wulf. ex Fr., Peniophora phlebioides Jacks. and Dearden, Stereum sanguinolentum Alb. and Schw. ex Fr. and Coniophora puteana (Schum. ex Fr.) Karst. in lodgepole pine slash by: 1) determining growth of these four fungi on malt agar under controlled temperature; 2) determining the rate of decay by these four fungi of lodgepole pine test blocks under controlled temperature; and 3) by investigating possible antagonisms between fungi under various temperature conditions. In addition, an attempt was made to express decay intensity in terms of the number of hyphal penetrations per unit area of tracheid wall.

REVIEW OF THE LITERATURE

The effect of moisture on decay of wood in culture

Etheridge (3, p. 58) reported that at each initial moisture level test blocks of subalpine spruce inoculated with Coniophora puteana showed a gain in block moisture after a 12 week decay period. He concluded that Coniophora puteana may have the ability to regulate the moisture content of its substrate to a level suitable for its growth. Difficulties in maintaining constant moisture content in wood blocks during decay experiments were demonstrated by Cartwright and Findlay (2, p. 34) who concluded that considerable quantities of water are produced in the respiration process by certain wood rotting fungi when in active growth. They noted that once a rot was established, the fungus could continue to develop and spread independently of any external source of moisture, provided that the rate of evaporation of water from the wood did not greatly exceed its rate of production. These authors found that Coniophora cerebella was sensitive to drying, ceasing activity as soon as the supply of moisture was cut off.

Snell (17, p. 44-46) noted that maximum rates of wood decay occurred when moisture contents were at or slightly above fiber saturation point. Etheridge (4, p. 205) failed to obtain successful infection of subalpine spruce test blocks by Coniophora puteana at moisture contents lower than 8 per cent of saturation. Snell (18, p. 377-379) presented evidence that rate of decay remained constant at moisture content ranges from fiber saturation point upward, until a condition in the wood was reached where an air-moisture balance was unfavorable for fungus development. In his work on decay by Trametes subroseus and Fomes roseus on woods of various specific gravities and variable moisture contents, he showed that more than 20 per cent of the wood volume of the block must be available in air for fungus development (19, p. 543-546). Cartwright and Findlay (2, p. 38) concluded that optimum conditions for fungus growth on wood exist when the cell walls in addition to being fully imbibed are coated with a film of liquid water in which free diffusion of the enzymes and the products of their action can take place, but where some air remains in the cavities for diffusion of gases.

The effect of temperature on decay of wood in culture

Temperature relations of wood-destroying fungi have been studied primarily on culture media, probably because this method is the simplest and gives most promise in yielding immediate and consistent results. Cartwright and Findlay (2, p. 41) reported that the optimum temperature for growth of the majority of European species occurred between 25 and 30 degrees C. Humphrey and Siggers (9, p. 997-1008) tested the influence of temperature on rate of growth of 56 North American species of wood-rotting fungi. The results obtained indicated that most species had optimum temperatures for growth on agar between 24 and 30 degrees C. and that the majority of test fungi ceased growth within a range of 12 degrees C. above their optimum temperatures. They classified the fungi into three main groups as follows: 1) a low temperature group growing best at 24 degrees C. or below; 2) an intermediate temperature group growing best between 24 and 32 degrees C.; and 3) a high temperature group growing best above 32 degrees C. Cartwright and Findlay (2, p. 43) pointed out the ecological importance of temperature relations in determining not only the geographical range of a species, but the relative

dominance of any given species in certain situations. In this regard, Etheridge (3, p. 65) found that fungi causing trunk rots in subalpine spruce had a much higher temperature range than those causing butt rots.

Etheridge (3, p. 65) found the optimum temperature for growth of Coniophora puteana to be 20 degrees C. His strain of Stereum sanguinolentum had an optimum temperature of 25 degrees C., while Cartwright and Findlay (2, p. 88) reported that this species had optimum temperatures between 20 and 24 degrees C. Their strain of Coniophora cerebella had an optimum temperature of 23 degrees C. Humphrey and Siggers (9, p. 1004-1005) noted that the optimum temperature of Lenzites saepiaria on agar was 36 degrees C., while maximum growth of Coniophora cerebella occurred at 24 degrees C. Results of studies by Falck (5, p. 1-234) indicated that Lenzites saepiaria had a temperature range from 5 to 44 degrees C. with the optimum temperature at 35 degrees C. Lindgren (12, p. 76) reported that this fungus had a temperature range from 5 to 45 degrees C. with optimum temperatures between 32 and 35 degrees C. In comparing his results with those of other workers, he suggested that different strains of the same fungus might account for variations in cardinal temperatures.

Correlations between the rate of decay of wood and the rate of mycelial growth on culture media at different temperatures have been attempted by several investigators. Two possible reasons for the non-existence of such correlations were recognized from the start. The first concerned the chemical and physical differences between the agar and the wood. The second concerned the difference in the time factor involved. Although Lindgren (12, p. 79) confirmed the downward shifting of optimum temperatures of certain fungi over a period of time, he was of the opinion that for practical purposes the cardinal temperatures for the growth of a fungus on wood were approximately the same as those determined from measuring its growth on agar. Cartwright and Findlay (2, p. 43) supported this view. Gäumann (7, p. 59-69) compared the decay activity of Poria vaporaria and Schizophyllum commune on test blocks as determined by weight loss of oven dry substance, with growth rate of these fungi on malt agar, and he noted that the maximum intensity of the decay process occurred at 2 to 3 degrees C. lower than the optimum growth temperature on agar plates. Results of experiments by Lindgren (12, p. 81) showed that decay caused by Lenzites saepiaria and Polystictus versicolor progressed most rapidly at those temperatures most

favourable for their mycelial growth on culture media. However, decay caused by Lentinus tigrinus was most rapid at 27 degrees C., while the greatest mycelial development occurred at 35 and 32 degrees C.

Liese (11, p. 138-150) showed that extremely low temperatures did not kill the majority of his test fungi. Some fungi developed more vigorously after low temperature treatments than the same species not so treated. Lethal temperatures were determined for a number of fungi. Lenzites saepiaria was killed after 60 minutes of exposure to 60 degrees C. Coniophora puteana was killed after 15 minutes of exposure to 50 degrees C., and Stereum sanguinolentum was killed after 45 minutes of exposure to 45 degrees C. Cartwright and Findlay (1, p. 481-495) noted that high temperatures desiccated agar media rapidly, and increased chances of contamination due to slow growth of the test fungi.

The influence of sample size and other factors on decay of wood in culture

In studies of decay of wood in culture, Findlay (6, p. 160-162) found that vigor of the fungus and the amount of decay it can bring about is appreciably influenced by the size and shape of the test pieces, by the atmospheric condition within the test vessel and by

the amount of gaseous interchange between the outside air and the atmosphere within the vessel. Weight losses, expressed as percentages of initial ovendry weight were significantly different between small and large test blocks, the smaller blocks decaying more rapidly than the larger under similar conditions. He further reported that hyphal penetration throughout the larger test blocks was completed in a matter of days, and that decay as determined by loss in dry weight substance progressed more or less uniformly for a considerable time.

Etheridge (4, p. 202) in his study on subalpine spruce noted that ring frequency and specific gravity did not appreciably affect rate of decay.

Hyphal penetration of tracheid walls

Zycha and Brand (23, p. 411-419) investigated possible relationships between the degree of decay by various test fungi on pine and spruce test blocks, and the number of hyphal penetrations per unit area of tracheid wall of spring wood. The best relationship was found to exist for Fomes annosus on spruce test blocks. They found no hyphal penetrations through tracheid walls by Coniophora cerebella or Lenzites saepiaria.

THE INFLUENCE OF TEMPERATURE ON FUNGUS GROWTH IN MALT AGAR CULTURES

Materials

Mycelial growth studies were carried out on malt agar prepared according to the following formula:

Difco powdered malt extract broth	20 gm.
Difco Bacto agar	20 gm.
Distilled water	1000 cc.

Cultural isolates of the four test fungi originated from those portions of lodgepole pine slash where each was found to be most abundant. Cultures of Peniophora phlebioides Jacks. and Dearden originated from the upper portions of the slash, Lenzites saepiaria Wulf. ex Fr. cultures originated from central portions, and cultures of Coniophora puteana (Schum. ex Fr.) Karst. and Stereum sanguinolentum Alb. and Schw. ex Fr. originated from the lower portions of lodgepole pine slash.

Methods

Pure cultures of the four test fungi were plated on malt agar and allowed to grow at room temperature for about a week. Small, 4 mm. square pieces of mycelium from the vigorously growing advancing zone were centrally plated on 20 cc. of malt agar in 90 mm.

Petri dishes and placed in incubators. Five replicates were prepared of one isolate per organism. Measurements commenced when growth had just started from the inoculum. The last measurements were taken just before the colony covered the entire plate in the case of fast growing cultures. In the case of slow growing cultures, the last measurements were taken after one week of active growth. The difference between initial and final measurements was used to calculate the mean daily diameter growth of the cultures. Each growth measurement consisted of the average of two diameter measurements of the colony which were taken at right angles. Six incubators were used, ranging in temperature from 10 to 45 degrees C. at intervals of 7 degrees. A pilot study provided information regarding the choice of a suitable temperature range.

Results

Results are summarized in Table I and in Figures 4, 6, 8 and 10.

Peniophora phlebioides produced measurable growth over the entire temperature range. Between 17 and 38 degrees C. it grew rapidly, covering the plates with a very thin mat of hyaline hyphae, closely appressed to the substrate. Its optimum temperature for

growth on malt agar appeared to be approximately between 35 and 39 degrees C. The relatively large temperature intervals precluded a more accurate estimate of optimum temperatures. It is interesting to note that optimum temperatures for mycelial growth of Peniophora phlebioides are significantly higher than those of Lenzites saepiaria, which is a member of the high temperature group. This may explain in part the dominance of Peniophora phlebioides in the upper exposed portions of lodgepole pine slash, where temperatures as high as 47.5 degrees C. have been recorded (15, p. 443).

Lenzites saepiaria showed a much slower growth rate than Peniophora phlebioides in all temperatures. Its temperature range appeared to be more limited, with very little growth produced at 10 degrees C. and no growth at 45 degrees C. The optimum temperature for the strain of Lenzites saepiaria used in this test appeared to be between 30 and 32 degrees C., which is slightly lower than the optimum temperatures recorded for other North American and for European strains of this fungus.

Coniophora puteana produced measurable growth within a very limited temperature range. Very little growth was measured at 17 and 31 degrees C. The

optimum temperature for this fungus appeared to be between 23 and 25 degrees C. The apparent intolerance to high and low temperatures is indicated in its growth habits in forest stands. Coniophora puteana is limited to root and butt sections of standing trees, and to sheltered undersides of dead and down trees. Temperature fluctuations in these locations are known to be relatively small.

Stereum sanguinolentum showed a relatively slow growth rate with a range covering the lower temperatures. Negligible growth was recorded at 31 degrees C. while appreciable growth occurred at 10 degrees C. The optimum growth temperature of Stereum sanguinolentum was found to be between 20 and 24 degrees C., which is in agreement with results obtained by Cartwright and Findlay (2, p. 88).

TABLE I

AVERAGE DAILY DIAMETER GROWTH IN CM OF FOUR TEST FUNGI ON 2 PER CENT MALT AGAR AT VARIOUS TEMPERATURES

(Averages based on 5 replicates)

Organism	Temperature degrees C.					
	10	17	24	31	38	45
Peniophora phlebioides	.11	1.12	2.17	3.64	4.02	.55
Lenzites saepiaria	.08	.33	.78	1.18	.43	--
Coniophora puteana	--	.10	.90	.11	--	--
Stereum sanguinolentum	.25	.46	.55	.06	--	--

THE INFLUENCE OF TEMPERATURE ON DECAY INTENSITY

Source and preparation of test blocks

Since the upper limits of merchantability of lodgepole pine in Alberta are at a top diameter of four inches, most of the lodgepole pine logging slash consists of portions including the live crown. Three codominant trees were selected in the Strachan area and felled. Trunk sections were cut from the live crown with a maximum diameter of four inches and a minimum diameter of three inches. A total of 198 test blocks was cut from these sections, which consisted primarily of sapwood. Care was taken to exclude as much as possible from the central portions of the trunk in an attempt to eliminate heartwood. The test blocks measured 6 X 2 X 0.5 cm., with the long axis of each block parallel to the long axis of the tree elements. The width of all test blocks was oriented tangentially to the tree elements. The selection of blocks for inoculation with the four fungi and allocation to temperature treatment was random. The test blocks were oven dried for 24 hours at 104 degrees C. and weighed. Distilled and sterilized water was added to the blocks to the amount of 30 per cent of their oven dry weight by means

of a hypodermic syringe calibrated to 0.1 cc., in order to bring moisture content up to approximately fiber saturation point prior to inoculation. Twenty four hours at room temperature were generally sufficient for the blocks to absorb the water.

Preparation of decay vessels

French square bottles measuring 5.5 cm. X 13.5 cm. with metal screw tops were used (Figure 1). In order to keep the atmosphere inside the test vessel close to maximum saturation and to prevent rapid drying out of the agar substrate at the higher temperatures, a wad of cotton batten was tightly rolled in gauze and fastened with 21-gauge copper wire to the inside of the metal screw top. Two holes were punctured in the top through which the wire could be fastened. The pad was kept saturated with sterilized distilled water by means of a hypodermic syringe inserted through one of the holes in the screw top (Figure 2). Approximately 30 cc. of 2 per cent malt agar was used as a culture medium. The bottles containing the agar were steam sterilized for 20 minutes at 15 pounds pressure and laid on their sides to cool. Sterilized glass rods were placed inside the bottles to elevate the test blocks from the culture medium. The test fungi were introduced into the bottles and allowed

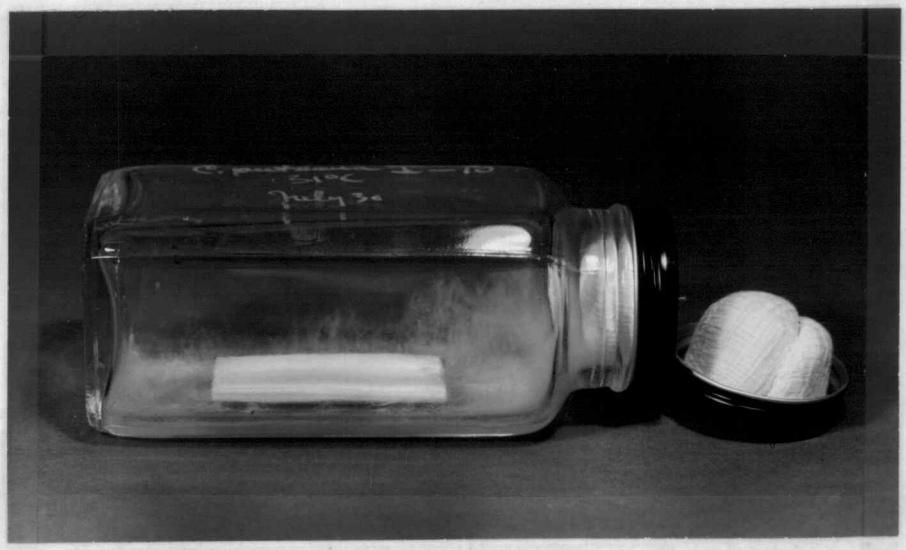

Figure 1. Decay bottle showing the cotton batten pad attached to the inside of the metal screw top. This pad was kept saturated with sterilized distilled water in an attempt to prevent rapid drying out of the medium at high temperatures.

Figure 2. Method of applying sterilized distilled water to cotton batten pad.

to grow for a period of 7 to 10 days at room temperature prior to admission of the test blocks.

Experimental design

Differences in mycelial growth rate on culture media are known to occur between different isolates of the same fungus. As far as could be determined from the literature, no comparisons have been made between decay capacities of different isolates of the same fungus. In order to achieve a maximum level of confidence in interpretation of results, it was decided to test four different isolates of each of the four fungi. This would permit comparison of weight losses between isolates of the same fungus prior to comparisons of weight losses between fungi. Conclusions regarding decay capacities of the four test fungi under various temperature treatments would be more meaningful when reached on this basis. The number of test blocks prepared per organism and temperature treatment is summarized in Table II.

Determination of decay rate

After an incubation period of 12 weeks, the test blocks were removed from their decay bottles. Surface mycelium was carefully removed with a camel hair brush

TABLE II

THE NUMBER OF LODGEPOLE PINE TEST BLOCKS INOCULATED WITH FOUR CULTURAL ISOLATES OF FOUR TEST FUNGI PRIOR TO INCUBATION AT VARIOUS TEMPERATURES

Organism, Isolate			Temperature treatment degrees C.					
			10	17	24	31	38	45
Peniophora	phlebioides	I	3	3	3	3	3	3
"	"	II	1	1	1	1	1	1
"	"	III	1	1	1	1	1	1
"	"	IV	1	1	1	1	1	1
Lenzites	saepiaria	I	3	3	3	3	3	3
"	"	II	1	1	1	1	1	1
"	"	III	1	1	1	1	1	1
"	"	IV	1	1	1	1	1	1
Coniophora	puteana	I	3	3	3	3	3	3
"	"	II	1	1	1	1	1	1
"	"	III	1	1	1	1	1	1
"	"	IV	1	1	1	1	1	1
Stereum	sanguinolentum	I	3	3	3	3	3	3
"	"	II	1	1	1	1	1	1
"	"	III	1	1	1	1	1	1
"	"	IV	1	1	1	1	1	1
CONTROL			3	3	3	3	3	3

and a scalpel before placing the blocks in aluminum containers for ovendrying at 104 degrees C. for 24 hours. Decay rate was expressed as a weight loss percentage of initial ovendry weight according to the following formula:

$$\text{Percentage of decay} = \frac{\text{initial o.d.wt.}^{*} - \text{decayed o.d.wt.}}{\text{initial o.d.wt.}} \times 100$$

Results

Weight losses experienced by the four cultural isolates of each test fungus were statistically compared. At the 5 per cent significance level, no significant differences were found between the decay activities of the four cultural isolates of each test fungus (Appendices A to D). Evidence that different isolates of the same fungus did not differ significantly in their decay capacities, allowed considering their weight losses as observations of one test fungus. In all further discussions no distinction is made between different isolates of one test fungus.

Hypotheses that neither temperature nor test fungus could influence decay rate, were rejected at the

*o.d.wt. = ovendry weight

5 per cent significance level. Similarly, the hypothesis that temperature did not affect decay rate of a test fungus was rejected at the 5 per cent significance level (Appendix E).

The relative significance of the test fungi as represented by average decay percentages for all temperature treatments was as follows: Lenzites saepiaria, 17.26 per cent; Coniophora puteana, 13.96 per cent; Peniophora phlebioides, 13.61 per cent; and Stereum sanguinolentum, 4.29 per cent. An average weight loss of .35 per cent was recorded for the control blocks.

Decay at 10 degrees C.

An appreciable amount of decay developed at this relatively low temperature. At the 5 per cent significance level, no significant difference was found between the decay capacities of Coniophora puteana, Lenzites saepiaria and Peniophora phlebioides which showed average weight losses of 8.68, 6.45 and 6.30 per cent respectively (Table III and Appendix K). An average weight loss of 3.40 per cent was recorded for Stereum sanguinolentum.

Decay at 17 degrees C.

At this temperature Lenzites saepiaria was the principal decayer, causing a weight loss of 22.83 per cent. No difference in decay rate was found at the 5 per cent significance level between Coniophora puteana and Peniophora phlebioides (Appendix J). These two fungi showed average weight losses of 16.39 and 15.50 per cent respectively. Stereum sanguinolentum was the least important decayer at 17 degrees C. causing an average weight loss of 8.16 per cent.

Decay at 24 degrees C.

Lenzites saepiaria was the most important decayer at 24 degrees C. causing a weight loss of 31.06 per cent of ovendry weight. Coniophora puteana and Peniophora phlebioides were second and third in importance, with average decay percentages of 20.43 and 16.93 per cent respectively. Stereum sanguinolentum was the least important decayer, with an average weight loss of 8.06 per cent.

Decay at 31 degrees C.

At the 5 per cent significance level, no significant difference was found between decay rates by

Lenzites saepiaria and Coniophora puteana, the principal decayers at this temperature, which showed weight losses of 38.46 and 37.79 per cent respectively (Appendix I). Peniophora phlebioides was third in importance showing an average weight loss of 26.54 per cent, and Stereum sanguinolentum was the least important decayer with an average weight loss of 5.01 per cent.

Decay at 38 degrees C.

Peniophora phlebioides was the only fungus to cause considerable decay at this high temperature. An average weight loss of 15.62 per cent was recorded for this fungus and 2.95 per cent for Lenzites saepiaria. Neither Coniophora puteana nor Stereum sanguinolentum caused any significant decay (Appendices G and H).

Decay at 45 degrees C.

No decay of significance took place at this temperature (Appendix F).

The optimum temperature for decay by Lenzites saepiaria was found to be 31 degrees C. At this temperature an average weight loss of 38.46 per cent was recorded. Optimum temperatures were the same for short term growth studies on malt agar as for long term decay studies on test blocks (Figures 6 and 7). This agreed

TABLE III

AVERAGE DECAY PERCENTAGES[1] OF LODGEPOLE PINE TEST BLOCKS BY FOUR SLASH-DECAYING HYMENOMYCETES AFTER A TWELVE WEEK INCUBATION PERIOD AT VARIOUS TEMPERATURES

(Averages based on 6 replicates)

Organism	Temperature treatment degrees C. 10	17	24	31	38	45
Peniophora phlebioides	6.30	15.50	16.93	26.54	15.62	.76
Lenzites saepiaria	6.45	22.83	31.06	38.46	2.95	1.81
Coniphora puteana	8.68	16.39	20.43	37.79	.33	.14
Stereum sanguinolentum	3.40	8.16[2]	8.06	5.01	.82[2]	.29[2]
CONTROL[3]	.54	.18[4]	.30	.19	.39	.41

[1] Percentages represent weight loss expressed as a percentage of initial ovendry weight.

[2] Averages based on 5 replicates.

[3] Averages based on 3 replicates.

[4] Average based on 2 replicates.

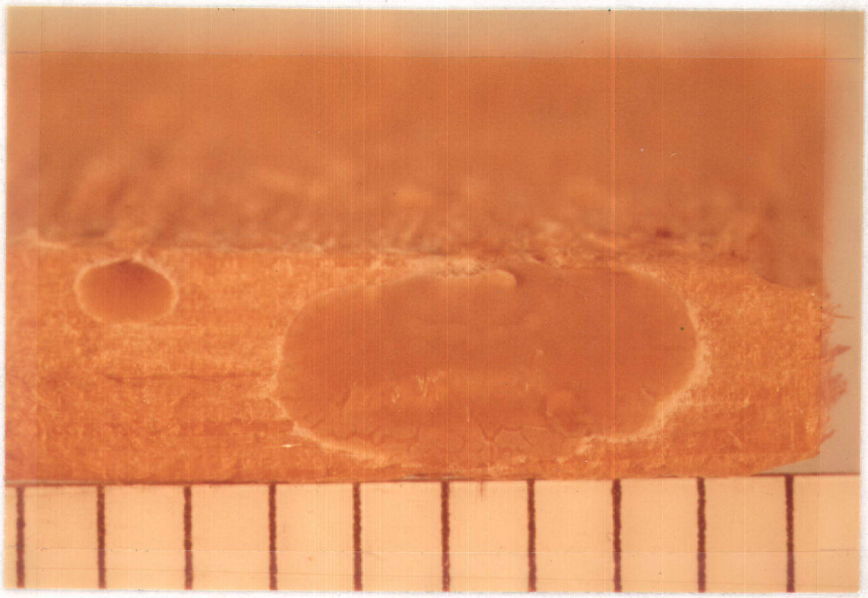

Figure 3. **Sporophore of *Peniophora phlebioides*** Jackson and Dearden developed *in vitro*. X7.

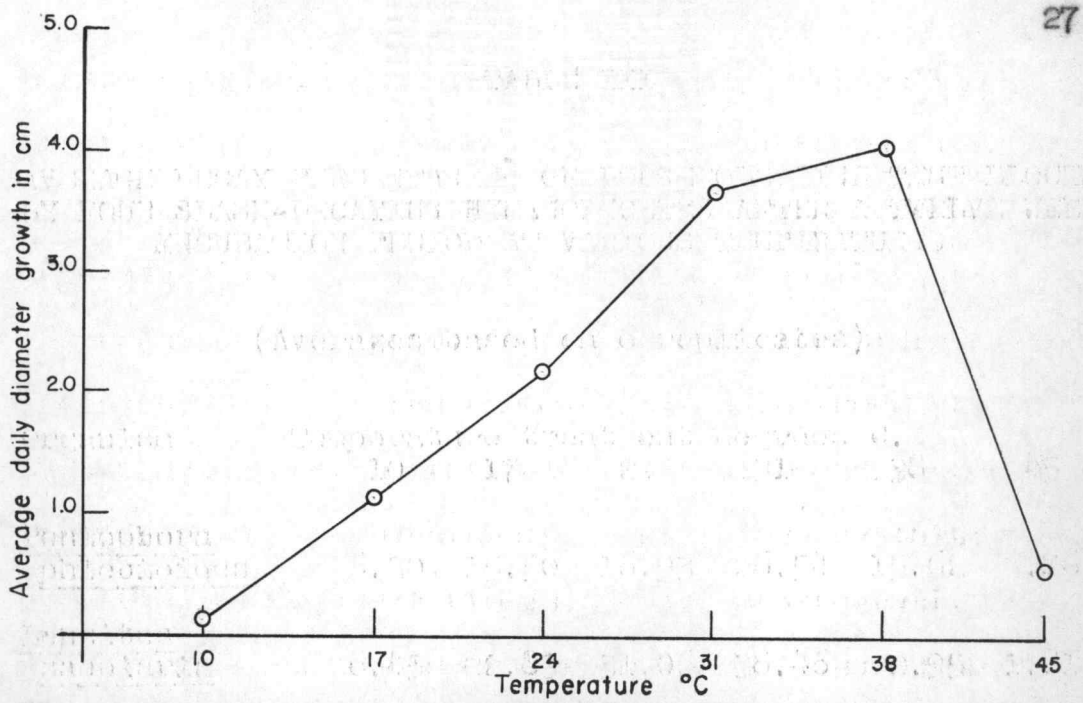

Figure 4. Average daily diameter growth of cultures of Peniophora phlebioides on 2 per cent malt agar, at various temperatures.

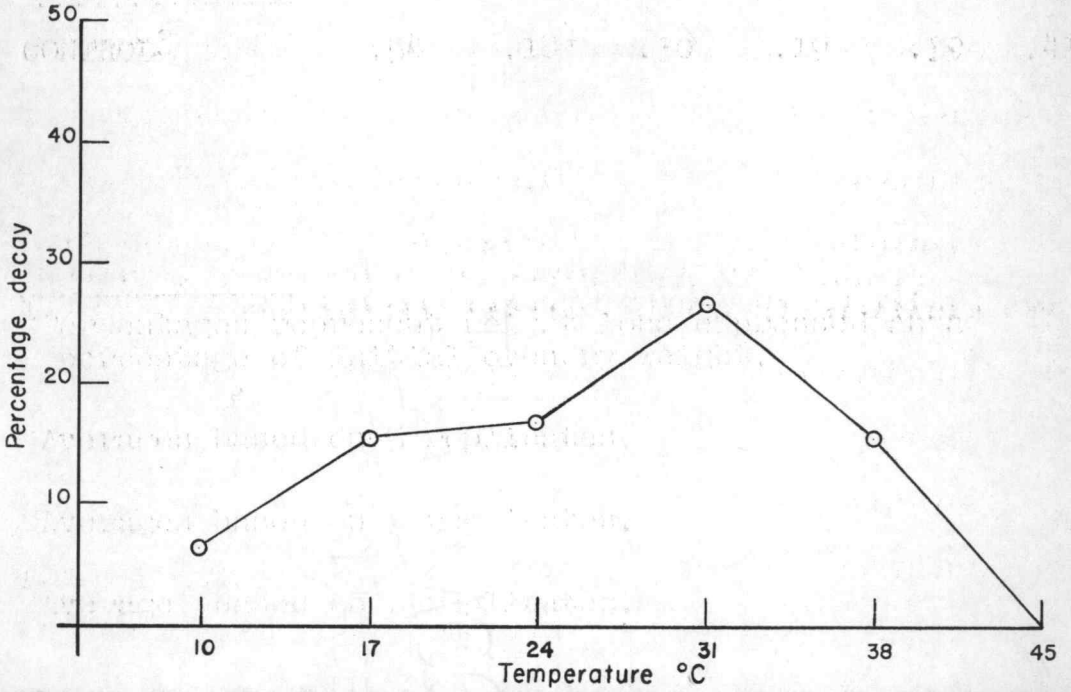

Figure 5. The loss in weight due to decay by Peniophora phlebioides of test blocks of lodgepole pine at various temperatures at the end of 12 weeks.

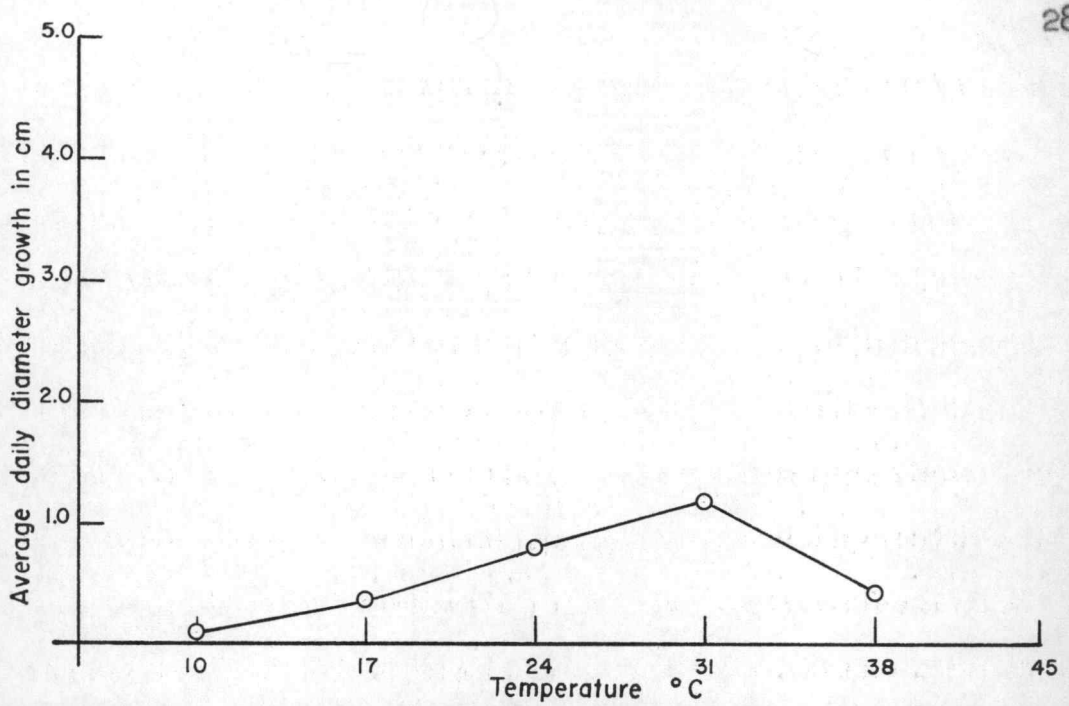

Figure 6. Average daily diameter growth of cultures of Lenzites saepiaria on 2 per cent malt agar, at various temperatures.

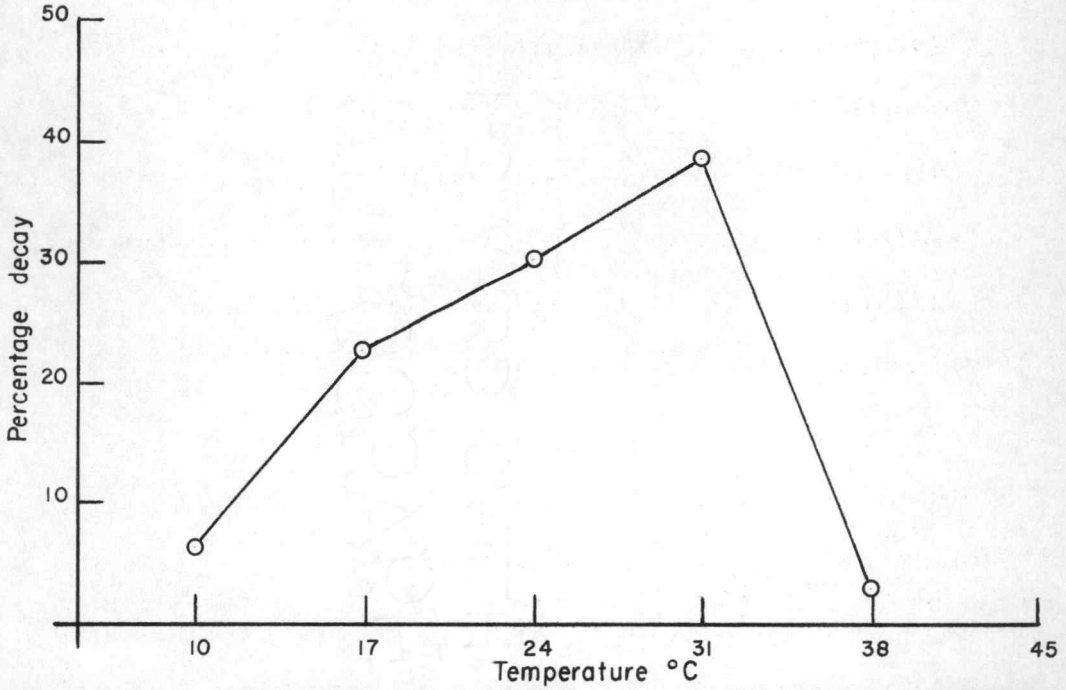

Figure 7. The loss in weight due to decay by Lenzites saepiaria of test blocks of lodgepole pine at various temperatures at the end of 12 weeks.

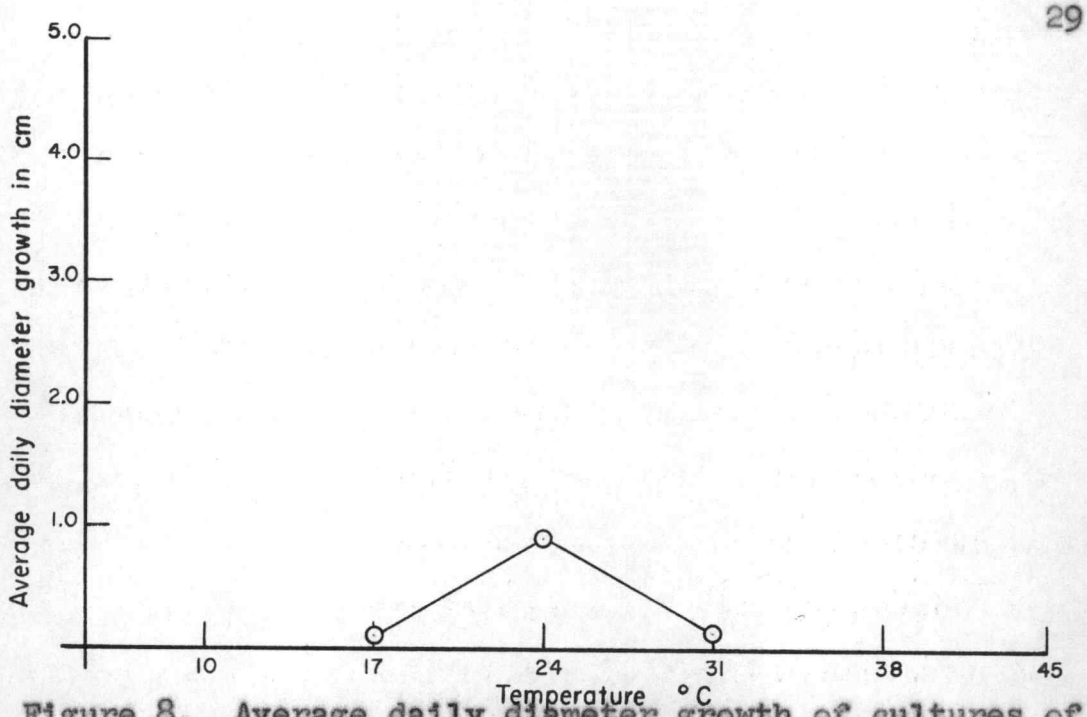

Figure 8. Average daily diameter growth of cultures of Coniophora puteana on 2 per cent malt agar, at various temperatures.

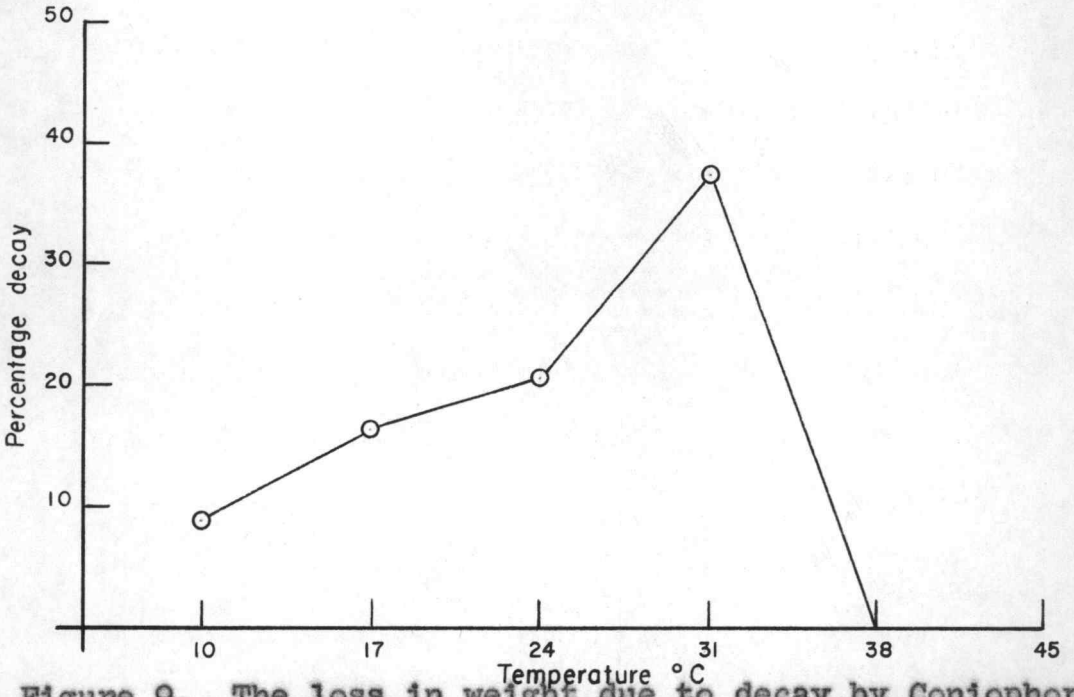

Figure 9. The loss in weight due to decay by Coniophora puteana of test blocks of lodgepole pine at various temperatures at the end of 12 weeks.

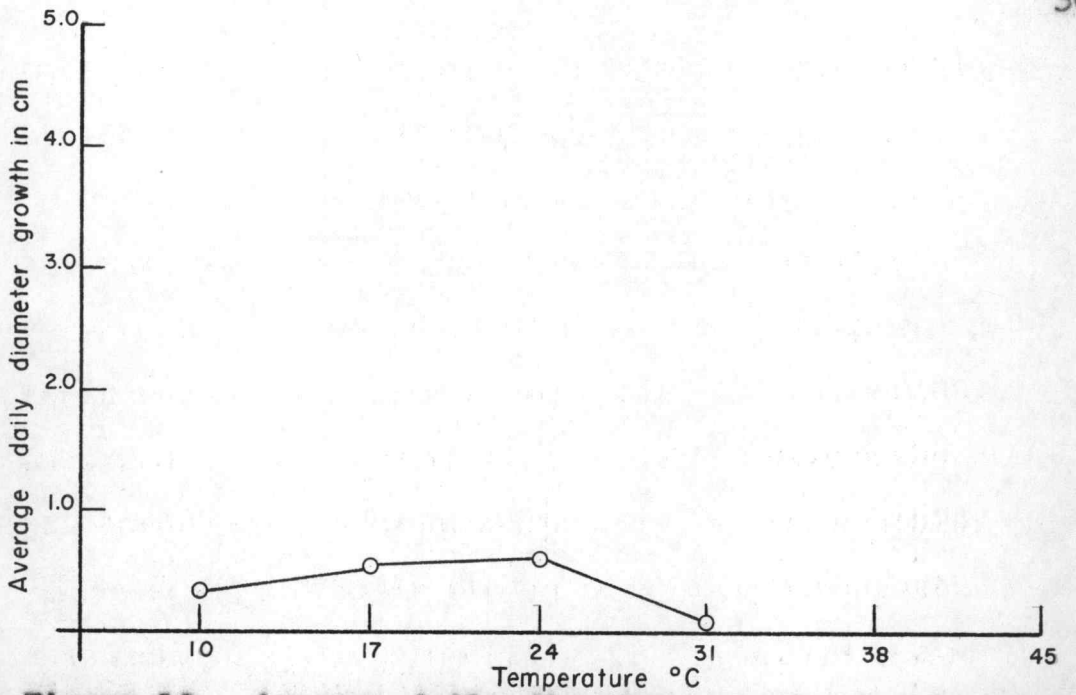

Figure 10. Average daily diameter growth of cultures of Stereum sanguinolentum on 2 per cent malt agar, at various temperatures.

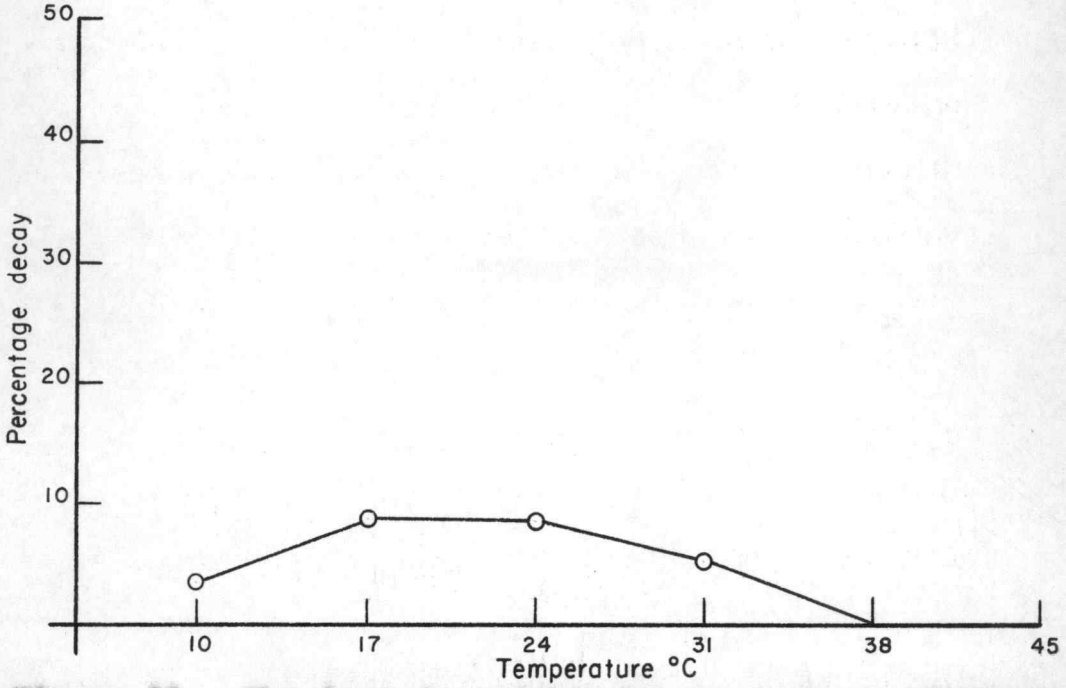

Figure 11. The loss in weight due to decay by Stereum sanguinolentum of test blocks of lodgepole pine at various temperatures at the end of 12 weeks.

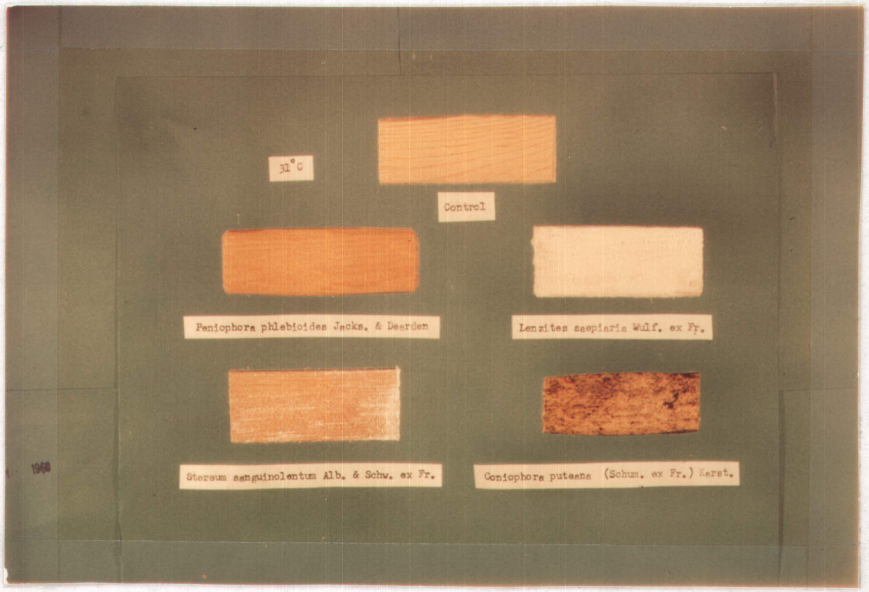

Figure 12. Lodgepole pine test blocks showing the effects of the activity of four slash-decaying hymenomycetes after 12 weeks of incubation at 31 degrees C.

with Lindberg's (11, p. 81) observations.

In this study, Coniophora puteana had an optimum temperature for decay of 31 degrees C., approximately 7 degrees C. higher than the optimum temperature for short term growth on malt agar (Figures 8 and 9). The optimum temperature for decay by Peniophora phlebioides was 31 degrees C., between 4 and 7 degrees C. lower than the optimum temperature for short term growth on malt agar (Figures 4 and 5). It is interesting to note that at 38 degrees C. Peniophora phlebioides was the only organism capable of causing appreciable decay. The optimum temperature for decay by Stereum sanguinolentum appeared to be between 17 and 23 degrees C., which was slightly lower than the optimum temperature for short term growth on malt agar (Table III, Figures 10 and 11).

THE INFLUENCE OF TEMPERATURE ON ANTAGONISM BETWEEN FUNGI

Antagonism is the inhibition of the growth of a particular organism by the presence of another. Most antagonistic effects are due to the production of specific metabolic products which are toxic to certain other organisms. These types of antagonism are expressed by a zone of clear medium between two colonies in Petri dish cultures. Part of the inhibitory effect on growth is due to competition for space and nutrients. This condition is generally expressed by a ceasing of growth along the line of contact of two colonies (8, p. 273).

The possibility of antagonism as a factor contributing to fungus distribution in lodgepole pine slash was investigated on 2 per cent malt agar and on lodgepole pine test blocks.

On 2 per cent malt agar

Methods. The possible combinations occurring between the four test fungi may be summarized as follows:

Peniophora phlebioides	X	*Lenzites saepiaria*
Peniophora phlebioides	X	*Coniophora puteana*
Peniophora phlebioides	X	*Stereum sanguinolentum*
Lenzites saepiaria	X	*Coniophora puteana*
Lenzites saepiaria	X	*Stereum sanguinolentum*
Coniophora puteana	X	*Stereum sanguinolentum*

Isolates number I of the four test fungi were plated and allowed to grow for about one week at room temperature to provide vigorously growing mycelium. The above combinations were then plated opposite one another near the margin of the substrate. Five replications per combination were prepared and incubated at 10, 17, 24, 31, 38 and 45 degrees C.

Results. Antagonism as expressed by a zone of clear medium between colonies was observed at 17 degrees C. between *Lenzites saepiaria* and *Stereum sanguinolentum* for each of the five replicates (Figure 13). Growth ceased along the line of contact of these two organisms at 24 and 31 degrees C. None of the other combinations exhibited antagonism as expressed by a zone of clear medium between colonies at any of the temperature treatments, but in all cases growth ceased along their line of contact. Aerial mycelium of *Coniophora puteana* frequently overran the other colony of the pair.

On lodgepole pine test blocks

Methods. Decay vessels and test blocks were prepared as previously described. Six combinations of fungi were introduced into the decay bottles and allowed to grow at room temperature for about a week. The test blocks were then placed in the decay bottles. Three replications were prepared per combination, and incubated at 24 and 31 degrees C.

Results. In all combinations involving Peniophora phlebioides, the other fungus of the pair failed to colonize the test blocks successfully. Peniophora phlebioides however, rapidly invaded the entire block (Figure 14). Similarly, in the combinations involving Lenzites saepiaria and Stereum sanguinolentum, the latter fungus failed to get established. Stereum sanguinolentum also failed to get established in combination with Coniophora puteana. Successful colonization of both Lenzites saepiaria and Coniophora puteana occurred at 31 degrees C. (Figures 14 and 15).

Decay percentages by fungus combinations corresponded closely with decay percentages by the pure cultures of the predominating fungus at 24 and 31 degrees C. Decay caused by the combination of Lenzites saepiaria and Coniophora puteana was appreciably less

than decay by the pure cultures of either of these two fungi at 24 and 31 degrees C. (Tables III and IV).

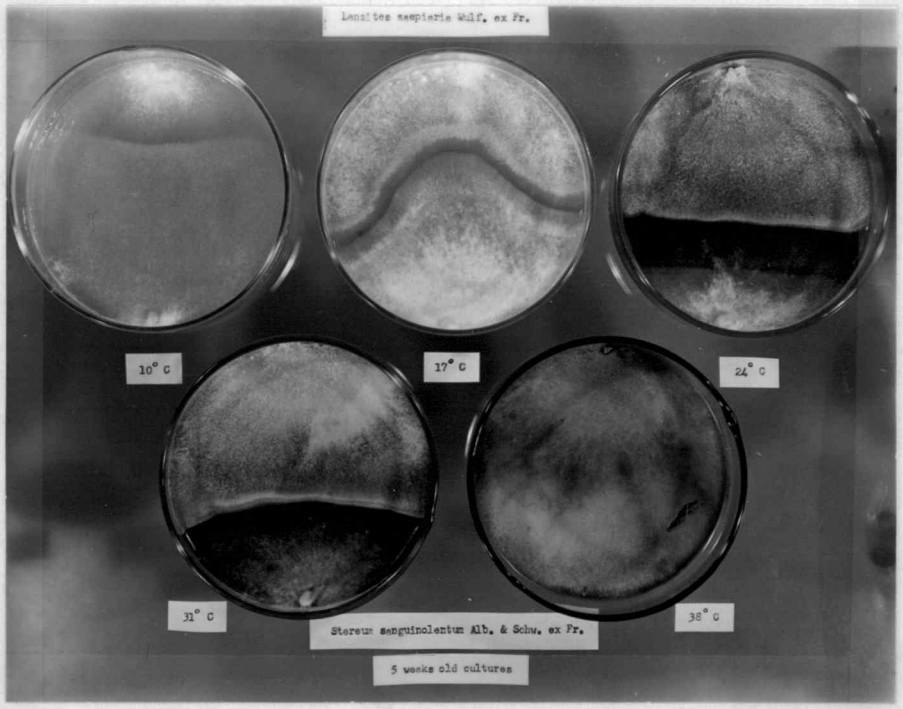

Figure 13. The effect of temperature on antagonism between *Lenzites saepiaria* and *Stereum sanguinolentum* on 2 per cent malt agar.

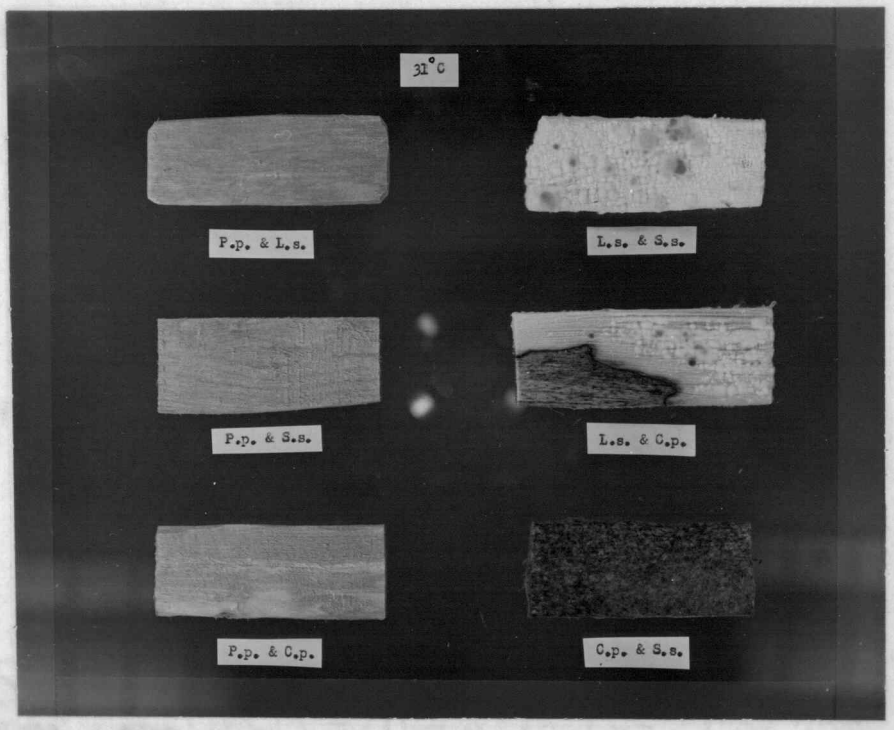

Figure 14. Lodgepole pine test blocks showing the effects of the activity of paired combinations of four slash-inhabiting hymenomycetes after 12 weeks of incubation at 31 degrees C.

Peniophora phlebioides	P.p.
Lenzites saepiaria	L.s.
Coniophora puteana	C.p.
Stereum sanguinolentum	S.s.

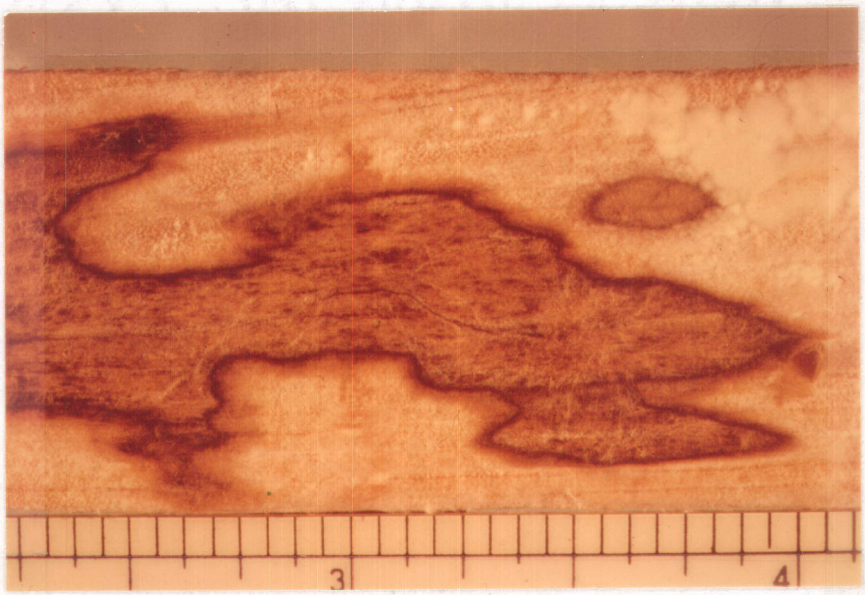

Figure 15. Lodgepole pine test block showing a contrast in activity of Coniophora puteana (brown) and Lenzites saepiaria (white) after 12 weeks of incubation at 31 degrees C. X2.3

TABLE IV

AVERAGE DECAY PERCENTAGES OF LODGEPOLE PINE TEST BLOCKS BY PAIRED COMBINATIONS OF FOUR SLASH-DECAYING HYMENOMYCETES AFTER A TWELVE WEEK INCUBATION PERIOD AT 24 AND 31 DEGREES C.

(Averages based on 3 replicates)

Combinations of fungi	Temperature treatment degrees C. 24	31
P. phlebioides X *C. puteana*	16.28	23.31
P. phlebioides X *L. saepiaria*	15.35	31.69
C. puteana X. *S. sanguinolentum*	17.17	32.57
L. saepiaria X *C. puteana*	17.20	30.63
P. phlebioides X *S. sanguinolentum*	17.28	29.27
L. saepiaria X *S. sanguinolentum*	31.00	40.44

HISTOLOGICAL STUDIES

In a study to determine relationships between frequency of hyphal penetration per unit area of tracheid wall and degree of decay of spruce and pine wood by selected fungi, Zycha and Brand (23, p. 411-419) found no hyphal penetrations by Coniophora puteana and Lenzites saepiaria. The possibility of a relationship between decay intensity and tracheid wall penetration by hyphae of Peniophora phlebioides, Stereum sanguinolentum, Lenzites saepiaria and Coniophora puteana was investigated.

Methods

The test blocks were separated into their individual cellular components by maceration, using Franklin's method as outlined by Wilson (22, p. 22). The blocks were split into match size pieces which were then placed in a solution consisting of equal parts of glacial acetic acid and 6 per cent hydrogen peroxide. The solution containing the pieces was heated to 60 degrees C. for 48 hours. The pieces were removed from the solution and washed overnight in running water. They were then carefully teased apart and placed in a strong Safranin O solution for a week. The fibers were

washed and mounted in Karo syrup. The cover slides were sealed with nail polish and the slides were examined under the microscope.

Results

Very few hyphae were found penetrating tracheid walls, even in test blocks which had lost an average of 38.46 per cent of their oven dry substance by decay. The abundance of pits in the radial walls of spring wood tracheids of lodgepole pine provides ample access to adjacent tracheids for fungus hyphae (Figure 16). This may be a contributing factor to the lack of hyphal penetrations of tracheid walls. Large accumulations of hyphae of Lenzites saepiaria were found in only a small number of tracheids and tracheid fibers (Figure 17). Generally, only a few hyphal strands were visible in the tracheids decayed by the four test fungi. Results of this experiment confirmed observations by Zycha and Brand (23, p. 411-419) that hyphal penetrations of tracheid walls by Coniophora puteana and Lenzites saepiaria are uncommon. Results further suggest that hyphae of Peniophora phlebioides and Stereum sanguinolentum do not as a rule penetrate tracheid walls, at least within the limits of a 12 week incubation period.

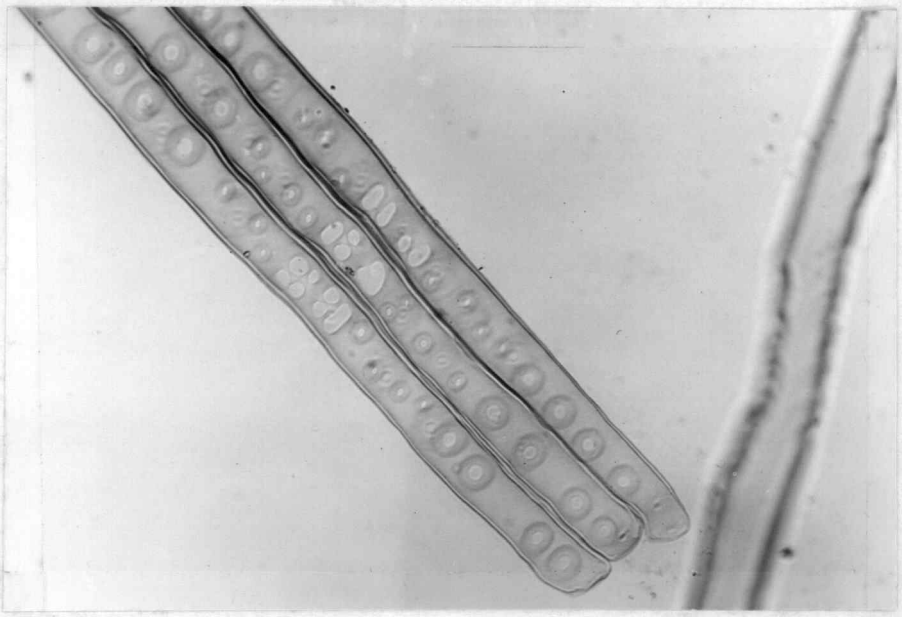

Figure 16. Radial view of springwood tracheids of lodgepole pine. X175.

Figure 17. Hyphae of Lenzites saepiaria in lodgepole pine tracheid. X225.

CONCLUSIONS

Optimum temperatures were approximately the same for growth rate on agar as for rate of decay of lodgepole pine test blocks for Lenzites saepiaria and Stereum sanguinolentum. However, appreciable differences were found between cardinal temperatures of growth rate on agar and decay intensity of wood for Peniophora phlebioides and Coniophora puteana. The optimum temperature of Peniophora phlebioides was between 4 and 7 degrees C. lower for decay than for growth rate on agar. On the other hand, the optimum temperature of Coniophora puteana was approximately 7 degrees C. higher for decay than for growth rate on agar.

Lenzites saepiaria was the most frequently isolated fungus from seven-year-old lodgepole pine slash, and it was the most important decayer of lodgepole pine test blocks in vitro.

Peniophora phlebioides (Figure 3), only recently described and relatively unknown (13, p. 207-208), was equally as important as Coniophora puteana as a decayer of lodgepole pine test blocks. It is the only fungus known to be capable of causing appreciable decay of lodgepole pine wood at temperatures as high as 38 degrees C.

Although Stereum sanguinolentum was frequently isolated from seven-year-old lodgepole pine slash, its decay capacity in vitro was appreciably below that of the other three test fungi.

Results of this study present strong evidence that temperature is indeed an important factor influencing the distribution of fungi in lodgepole pine slash.

Tests on malt agar showed that temperature may influence antagonism between two fungi. It is unlikely that antagonism between Lenzites saepiaria and Stereum sanguinolentum at 17 degrees C. contributes to the fungus distribution in lodgepole pine slash.

Antagonism tests on test blocks were carried out at 24 and 31 degrees C. The three fungi that were paired with Peniophora phlebioides failed to get successfully established in the test blocks. This may undoubtedly be contributed to the extremely rapid growth of Peniophora phlebioides at these temperatures.

The test fungi paired with Stereum sanguinolentum prevented this slow growing fungus to colonize the test blocks (Figure 14).

Decay by the combination of Lenzites saepiaria and Coniophora puteana at 31 degrees C. was considerably less than decay caused by pure cultures of either Lenzites saepiaria or Coniophora puteana at this

temperature.

Test blocks inoculated with combinations of Lenzites saepiaria and Coniophora puteana showed an interesting contrast in appearance at the end of the 12 week incubation period (Figures 14 and 15). Although both fungi produce brown cubical rots, the earlier stages of decay appear quite unlike each other.

Attempts to estimate decay intensity by microscopic examination of the tracheids were unsuccessful. No hyphal penetrations of tracheid walls could be found for any of the test fungi.

BIBLIOGRAPHY

1. Cartwright, K. S. G. and W. P. K. Findlay. Studies in the physiology of wood-destroying fungi. II. Temperature and rate of growth. Annals of Botany 48:481-495. 1934.

2. _____. Decay of timber and its prevention. 2d ed., Princes Risborough, England, Forest Products Research Laboratory, 1958. 322 p.

3. Etheridge, D. E. The influence of moisture and other factors on the activity of heartwood fungi in subalpine spruce. Ottawa, Canada, Dept. of Agriculture, Forest Biology Division, 1957. 114 p. (Mimeographed)

4. _____. The effect on variations in decay of moisture content and rate of growth in subalpine spruce. Canadian Journal of Botany 36: 187-206. 1958.

5. Falck, R. Die Lenzitesfaule des Coniferenholzes. In: A. Moller's Hausschwamm-forschungen, Vol. III, 1909. p. 1-234.

6. Findlay, W. P. K. Influences of sample size on decay rate of wood in culture. Timber technology and machine woodworking. 61(2166):160-162. 1953.

7. Gäumann, E. Uber die Wachstums und zerstörungs intensität von Polyporus vaporarius und von Schizophyllum commune bei verschiedenen Temperaturen. Angewandte Botanik 21:59-69. 1939.

8. Hawker, Lilian E. Physiology of fungi. London, England, University of London Press. 1950. 360 p.

9. Humphrey, C. J. and P. V. Siggers. Temperature relations of wood destroying fungi. Journal of Agricultural Research 47:997-1008. 1933.

10. Johnson, H. J. The effect of various slash disposal methods on the regeneration of cutover jack pine stands. Ottawa, 1955. 11 p. (Canada. Forestry Branch. Forestry Research Division. Technical Note No. 23)

11. Liese, J. Beobachtungen über die Biologie holzzerstörender Pilze II. Verhalten holzzerstörender Pilze gegenüber extremen Temperatureinwirkungen. Angewandte Botanik 13:2, 138-150. 1931.

12. Lindgren, R. M. Decay of wood and growth of some hymenomycetes as affected by temperature. Phytopathology 23:73-81. 1933.

13. Linzon, S. N. Peniophora phlebioides Jacks. and Dearden. A sapwood deteriorating fungus on eastern white pine. Canadian Journal of Botany 36:207-208. 1958.

14. Loman, A. A. Deterioration by decay of lodgepole pine logging slash near Strachan, Alberta. Ottawa, Canada, Dept. of Agriculture, Forest Biology Division, 1959. 22 p. (Mimeographed)

15. Reid, R. W. The bark beetle complex associated with lodgepole pine slash in Alberta. IV. Distribution, population densities and effects of several environmental factors. The Canadian Entomologist 89(10):437-447. 1957.

16. Smithers, L. A. Thinning in lodgepole pine stands in Alberta. Ottawa, 1957. 26 p. (Canada. Forestry Branch. Forestry Research Division. Technical Note No. 52)

17. Snell, W. H. Relation of moisture contents of wood to its decay. Paper Trade Journal 71(28):44-46. 1921.

18. _____. The relation of moisture contents of wood to its decay. II. Science n.s. 62:377-379. 1925.

19. _____. The relation of moisture contents of wood to its decay. III. American Journal of Botany 16:543-546. 1929.

20. Wagener, Willis W. and Ross W. Davidson. Heart rots in living trees. The Botanical Review 20(2):61-134. 1954.

21. Williams, D. E. Fire hazard resulting from jack pine slash. Ottawa, 1955. 16 p. (Canada. Forestry Branch. Forestry Research Division. Technical Note No. 22)

22. Wilson, J. W. An outline of microtechnique methods for the wood technologist. Vancouver, University of B. C., Dept. of Forestry, 1951. 27 p. (Mimeographed)

23. Zycha, H. and W. Brand. Eine Methode zur Bestimmung des Grades der Zerstörung von Fichtenholz durch Fomes annosus mit Hilfe des Mikroskopes. Phytopathologische Zeitschrift 35:411-419. 1959.

APPENDIX A

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY FOUR CULTURAL ISOLATES OF PENIOPHORA PHLEBIOIDES AFTER 12 WEEKS OF INCUBATION AT VARIOUS TEMPERATURES

Temperature	Isolate I	II	III	IV
10	8.39	7.80	5.66	6.33
	6.55			
	3.10			
17	20.06	12.97	12.72	13.02
	19.09			
	15.12			
24	17.39	16.90	14.09	15.01
	17.09			
	21.11			
31	29.81	23.63	26.94	26.17
	26.92			
	25.74			
38	16.64	14.91	9.04	21.06
	21.06			
	10.99			
45	.62	.52	1.42	.90
	.72			
	.39			

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among isolates	41.2193	3	13.7398	.1671
Within isolates	2630.7658	32	82.2114	
Total	2671.9851			

Hypothesis: The population means of Isolates I, II, III, and IV of Peniophora phlebioides are the same.
Significance level: 5 per cent
Critical region: $F > 2.9055$ with 3 and 32 degrees of freedom.
Conclusion: Since the F value at .1671 with 3 and 32 degrees of freedom lies outside the critical region, the hypothesis is accepted.

APPENDIX B

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY FOUR CULTURAL ISOLATES OF *LENZITES SAEPIARIA* AFTER 12 WEEKS OF INCUBATION AT VARIOUS TEMPERATURES

Temperature	Isolate I	II	III	IV
10	5.02	5.14	6.99	6.79
	6.67			
	8.09			
17	25.99	31.33	15.49	24.89
	18.65			
	20.63			
24	29.97	37.40	22.66	27.76
	32.24			
	36.30			
31	44.38	41.48	19.28	38.55
	45.68			
	41.41			
38	.43	4.87	3.29	2.56
	2.10			
	4.45			
45	.15	4.67	.56	5.19
	.31			
	.00			

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among isolates	291.9067	3	97.3022	.3926
Within isolates	7931.7934	32	247.8685	
Total	8223.7001			

Hypothesis: The population means of Isolates I, II, III, and IV of *Lenzites saepiaria* are the same.
Significance level: 5 per cent
Critical region: $F > 2.9055$ with 3 and 32 degrees of freedom.
Conclusion: Since the F value at .3926 with 3 and 32 degrees of freedom lies outside the critical region, the hypothesis is accepted.

APPENDIX C

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY FOUR CULTURAL ISOLATES OF CONIOPHORA PUTEANA AFTER 12 WEEKS OF INCUBATION AT VARIOUS TEMPERATURES

Temperature	Isolate I	II	III	IV
10	6.34	8.06	8.25	7.21
	8.35			
	13.86			
17	13.58	19.66	15.08	18.16
	14.90			
	16.97			
24	16.62	27.78	16.08	27.05
	19.16			
	15.89			
31	38.09	45.61	32.50	43.94
	33.14			
	33.48			
38	.54	.57	.45	.01
	.37			
	.03			
45	.00	.00	.60	.08
	.13			
	.00			

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among isolates	121.6465	3	40.5488	.2032
Within isolates	6384.7405	32	199.5231	
Total	6506.3870			

Hypothesis: The population means of Isolates I, II, III, and IV of Coniophora puteana are the same.
Significance level: 5 per cent
Critical region: $F > 2.9055$ with 3 and 32 degrees of freedom.
Conclusion: Since the F value at .2032 with 3 and 32 degrees of freedom lies outside the critical region, the hypothesis is accepted.

APPENDIX D

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY FOUR CULTURAL ISOLATES OF STEREUM SANGUINOLENTUM AFTER 12 WEEKS OF INCUBATION AT VARIOUS TEMPERATURES

Temperature	Isolate I	II	III	IV
10	3.02	5.24	3.80	1.65
	3.92			
	2.80			
17	7.81	12.33	10.10	2.59
	7.96			
	--			
24	6.43	16.08	7.36	7.11
	6.09			
	5.28			
31	5.00	7.92	5.03	1.97
	5.20			
	4.93			
38	.83	1.01	1.42	--
	.79			
	.07			
45	.00	.48	.65	--
	.25			
	.05			

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among isolates	63.6261	3	21.2087	1.5272
Within isolates	402.7344	29	13.8874	
Total	466.3605			

Hypothesis: The population means of Isolates I, II, III, and IV of Stereum sanguinolentum are the same.

Significance level: 5 per cent

Critical region: $F > 2.9340$ with 3 and 29 degrees of freedom.

Conclusion: Since the F value at 1.5272 with 3 and 29 degrees of freedom lies outside the critical region, the hypothesis is accepted.

APPENDIX E

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO THE DECAY BY FOUR SLASH-INHABITING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT VARIOUS TEMPERATURES

Organism	Isolate	Temperature treatments degrees C. 10	17	24	31	38	45
P. phlebioides	I	8.39	20.06	17.39	29.81	16.64	.62
	I	6.55	19.09	17.09	26.92	21.06	.72
	I	3.10	15.12	21.11	25.74	10.99	.39
	II	7.80	12.97	16.90	23.63	14.91	.52
	III	5.66	12.72	14.09	26.94	9.04	1.42
	IV	6.33	13.02	15.01	26.17	21.06	.90
L. saepiaria	I	5.02	25.99	29.97	44.38	.43	.15
	I	6.67	18.65	32.24	45.68	2.10	.31
	I	8.09	20.63	36.30	41.41	4.45	.00
	II	5.14	31.33	37.40	41.48	4.87	4.67
	III	6.99	15.49	22.66	19.28	3.29	.56
	IV	6.79	24.89	27.76	38.55	2.56	5.19
C. puteana	I	6.34	13.58	16.62	38.09	.54	.00
	I	8.35	14.90	19.16	33.14	.37	.13
	I	13.86	16.97	15.89	33.48	.03	.00
	II	8.06	19.66	27.78	45.61	.57	.00
	III	8.25	15.08	16.08	32.50	.45	.60
	IV	7.21	18.16	27.05	43.94	.01	.08
S. sanguinolentum	I	3.02	--	6.43	5.00	.83	.00
	I	3.92	7.81	6.09	5.20	.79	.25
	I	2.80	7.96	5.28	4.93	.07	.05
	II	5.24	12.33	16.08	7.92	1.01	.48
	III	3.80	10.10	7.36	5.03	1.42	.65
	IV	1.65	2.59	7.11	1.97	--	--
CONTROL		.04	--	.01	.47	.61	.57
		1.43	.22	.41	.01	.18	.34
		.14	.15	.49	.08	.39	.33

APPENDIX E cont'd

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Temperature	10494.8435	5	2098.9687	165.1457
Organism	5330.3080	4	1332.5770	104.8464
Interaction	5748.4654	20	287.4233	22.6143
Error	1626.8493	128	12.7098	58.5310
Total	44774.0831	157	285.1852	

i) Hypothesis: The population means of the six temperature levels are the same.
Significance level: 5 per cent
Critical region: $F > 2.2900$ with 5 and 128 degrees of freedom.
Conclusion: Since the F value, at 165.1457 with 5 and 128 degrees of freedom lies inside the critical region, the hypothesis is rejected.

ii) Hypothesis: The population means of the five organisms and control levels are the same.
Significance level: 5 per cent
Critical region: $F > 2.4472$ with 4 and 128 degrees of freedom.
Conclusion: Since the F value at 104.8464 with 4 and 128 degrees of freedom lies within the critical region, the hypothesis is rejected.

iii) Hypothesis: There exists no interaction between the two factors.
Significance level: 5 per cent
Critical region: $F > 1.6587$ with 20 and 128 degrees of freedom.
Conclusion: Since the F value at 22.6143 with 20 and 128 degrees of freedom lies inside the critical region, the hypothesis is rejected.

APPENDIX F

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY FOUR SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 45 DEGREES CENTIGRADE

	Organism				
	Peniophora phlebioides	Lenzites saepiaria	Coniophora puteana	Stereum sanguino-lentum	Con-trol
Observa-tions	.62	.15	.00	.00	.57
	.72	.31	.13	.25	.34
	.39	.00	.00	.05	.33
	.52	4.67	.00	.48	
	1.42	.56	.60	.65	
	.90	5.19	.08		

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among sample	10.4683	4	2.6171	1.7879
Within sample	30.7388	21	1.4638	
Total	41.2071			

Hypothesis: The population means of the four organisms and the control are the same.
Significance level: 5 per cent
Critical region: $F > 2.8401$ with 4 and 21 degrees of freedom.
Conclusion: Since the F value at 1.7879 with 4 and 21 degrees of freedom lies outside the critical region, the hypothesis is accepted.

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY THREE SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 38 DEGREES CENTIGRADE

	Organism			
	Lenzites saepiaria	Coniophora puteana	Stereum sanguinolentum	Control
Observations	.43	.54	.83	.61
	2.10	.37	.79	.18
	4.45	.03	.07	.39
	4.87	.57	1.01	
	3.29	.45	1.42	
	2.56	.01		

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among sample	25.5458	3	8.5153	9.3074
Within sample	14.6391	16	.9149	
Total	40.1849			

Hypothesis: The population means of the three organisms and the control are the same.

Significance level: 5 per cent

Critical region: $F > 3.2389$ with 3 and 16 degrees of freedom.

Conclusion: Since the F value at 9.3074 with 3 and 16 degrees of freedom lies within the critical region, the hypothesis is rejected.

APPENDIX H

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY TWO SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 38 DEGREES CENTIGRADE

	Organism		
	Coniophora puteana	Stereum sanguinolentum	Control
Observations	.54	.83	.61
	.37	.79	.18
	.03	.07	.39
	.57	1.01	
	.45	1.42	
	.01		

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among sample	.7307	2	.3654	2.9515
Within sample	1.3621	11	.1238	
Total	2.0928			

Hypothesis: The population means of the two organisms and the control are the same.

Significance level: 5 per cent

Critical region: $F > 3.9823$ with 2 and 11 degrees of freedom.

Conclusion: Since the F value at 2.9515 with 2 and 11 degrees of freedom lies outside the critical region, the hypothesis is accepted.

APPENDIX I

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY TWO SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 31 DEGREES CENTIGRADE

	Organism	
	Lenzites saepiaria	Coniophora puteana
Observations	44.38	45.61
	45.68	32.50
	41.41	43.94
	41.48	38.09
	19.28	33.14
	38.55	33.48

LEAST SIGNIFICANT DIFFERENCE

$$t = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{Sp^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Hypothesis: The population means of the two organisms are the same.

Significance level: 5 per cent

Critical regions: $t < -2.228$
$t > +2.228$ with 10 degrees of freedom.

Conclusion: Since the t value at .1429 with 10 degrees of freedom lies outside the critical regions, the hypothesis is accepted.

APPENDIX J

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY TWO SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 17 DEGREES CENTIGRADE

	Organism	
	Peniophora phlebioides	Coniophora puteana
Observations	20.06	13.58
	19.09	14.90
	15.12	16.97
	12.97	19.66
	12.72	15.08
	13.02	18.16

LEAST SIGNIFICANT DIFFERENCE

$$t = \frac{Y_1 - Y_2}{\sqrt{Sp^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Hypothesis: The population means of the two organisms are the same.

Significance level: 5 per cent

Critical regions: $t < -2.228$
$t > +2.228$ with 10 degrees of freedom.

Conclusion: Since the t value at - .5422 with 10 degrees of freedom lies outside the critical regions, the hypothesis is accepted.

APPENDIX K

WEIGHT LOSSES EXPRESSED AS PERCENTAGES OF INITIAL OVENDRY WEIGHT OF LODGEPOLE PINE TEST BLOCKS DUE TO DECAY BY THREE SLASH-DECAYING HYMENOMYCETES AFTER 12 WEEKS OF INCUBATION AT 10 DEGREES CENTIGRADE

	Organism		
	Peniophora phlebioides	Lenzites saepiaria	Coniophora puteana
Observations	8.39	5.02	6.34
	6.55	6.67	8.35
	3.10	8.09	13.86
	7.80	5.14	8.06
	5.66	6.99	8.25
	6.33	6.79	7.21

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Among sample	21.2384	2	10.6192	2.6823
Within sample	59.3842	15	3.9589	
Total	80.6226			

Hypothesis: The population means of the three organisms are the same.

Significance level: 5 per cent

Critical region: $F > 3.6823$ with 2 and 15 degrees of freedom.

Conclusion: Since the F value at 2.6823 with 2 and 15 degrees of freedom lies outside the critical region, the hypothesis is accepted.