SAPROPHYTES IN THE STEM OF LIVING, HEALTHY TSUGA HETEROPHYLLA (RAF.) SARG. AND THEIR ROLE IN DECAY RESISTANCE OF THE WOOD.

by

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Department of Forestry

The University of British Columbia,
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Date April 8, 1964.

ABSTRACT

It was demonstrated that the sound wood of two stems of living western hemlock, Tsuga heterophylla (Raf.) Sarg., was colonized by microorganisms, which were isolated by shaking samples of the wood in sterile distilled water. The microfloras inhibited the growth of Fomes annosus (Fr.) Cke., and Poria monticola Murr. when added to one per cent malt agar. The test fungi were also inhibited on untreated surface sterilized wood incubated at one hundred per cent relative humidity at room temperature. Significant variation was observed, in the rate of inhibition between the sapwood and heartwood and between the various sections of the two stems investigated.

A drier environment (higher agar concentration of malt agar media, and lower relative humidity with wood) resulted in a significant decrease in inhibition rate of F. annosus and P. monticola.

Autoclaving the experimental material led to the loss of inhibition with both malt agar media and wood. Removal of the microorganisms from the shake solutions by Millipore filtration also resulted in loss of the inhibiting factor.

The relatively drier environment did not effect the rate of growth of the test fungi on autoclaved wood and in malt agar containing the autoclaved portion of the shake solutions.

Results of the experiment provide evidence that the microfloras inhabiting the sound wood were responsible for inhibition of F. annosus and P. monticola. Furthermore, sufficient moisture level appeared necessary for maintaining the inhibiting power of these microorganisms.

ACKNOWLEDGEMENTS

I am indebted to Dr. John E. Bier, Professor of Forest Pathology, for his encouragement, prior to initiation of this work, and his quidance, valuable suggestions and generous help throughout the course of the study are gratefully acknowledged.

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INTRODUCTION

The importance of decay problems in forestry practice is recognized both in living trees and in wood in service. Accordingly a great deal of work has been done in these fields, including detailed descriptions of the effects of various wood decaying fungi on different hosts, substrata and host-organism relationships in successive stages of decay. It would appear that most decay studies undertaken to date were similar in that woody tissues in living trees were considered as entities, varying in structure, chemical composition and other properties.

The development of a concept in recent years (Bier 1958, 1959, 1960, 1961) has led to the establishment of a relationship between host resistance and its water content expressed a relative turgidity. Resistance was positively correlated with high relative turgidity. It was suggested, furthermore, that healthy, living host tissues may not be entities but biological communities comprised of host tissues and a saprophytic microflora. If this is true, these microorganisms may have a significant role in the degree of host resistance by inhibiting the development of pathogens under certain circumstances. The establishment of host resistance - high relative turgidity relationship and the possible role of a microflora resulted from work undertaken on a variety of tree species in connection with canker diseases caused by native facultative parasites (Bier 1961, 1962). It was of particular interest to determine whether this principle would hold in the case of other types of pathogens, e.g., wood

decaying fungi.

This study was undertaken to provide evidence in support of this hypothesis and possibly establish the distribution of microorganisms in healthy wood, and to determine their inhibitory effect on the development of wood decaying fungi.

Tsuga heterophylla (Raf.) Sarg. was chosen as host tree, since this species is of major economic importance and was not under investigation in the Pathological Laboratory in the University at the beginning of this study. It was considered of interest to use a representative of both types of decay organisms, namely a white rot and a brown rot fungus. Fomes annosus (Fr.) Cke. and Poria monticola Murr. were chosen for this purpose respectively. Both fungi are pathogens of international importance and grow rapidly, characteristics which lead to their selection for laboratory studies.

MATERIALS AND METHODS

The cultures of F. annosus and P. monticola were supplied by Dr. John E. Bier and designated as No. 13 and No. 124A respectively.

A vigorously-growing hemlock tree eighty years of age was felled in the University Campus Forest and bucked into ten-foot sections. The first commencing from the base of tree was termed the "butt section"; the second was discarded; the third was named the "middle section"; the fourth discarded, and the fifth was labelled as the "top section". This terminology - butt -, middle -, and top section - will be used in the text.

The three sections were taken immediately to the Vancouver Laboratory, Forest Products Research Branch, Federal Department of Forestry, where a one inch thick board including the pith was sawn from each section. The boards were edged, trimmed and planed to three-quarter inch thickness. The lower four-foot portion of each board was used for sampling. Two three-quarter inch wide beams were cut from both edges of the four-foot boards and were considered to represent sapwood. The next one-inch wide strip of the edges was discarded. The remainder of the boards was regarded as heartwood. Three-quarter inch cubes were cut from the sapwood and heartwood beams of the three sections. The cubes were placed in plastic bags and stored at 5° C.

Four cubes were chosen randomly from both sapwood and heartwood from each of the three sections. Every cube was surface strilized by flaming and placed individually in a shaking flask containing 75cc of sterile, distilled water. The flasks were shaken continuously on a low

speed shaker for five days at room temperature. The final solutions obtained were believed to contain a sample of the population of microorganisms present in the cubes. The solutions were used in this condition for the preparation of the culture media.

One, four and six per cent of agar in three per cent malt extract were prepared; - the shake solution represented ten per cent of the water required to prepare the malt agar. The solution was added by sterilized pipette to the malt agar after it had cooled down considerably (approximately 40-45 °C), in order to prevent the harmful effect of high temperature on the microorganisms. The solution obtained from each cube was used separately to prepare one, four and six per cent malt agar, and four Petri plates were poured from each concentration, two of which were inoculated with F. annosus and the other two plates with P. monticola. Control plates were prepared for each concentration of agar and inoculated with each pathogen.

The inoculated plates were incubated for a period of two weeks at room temperature. Mycelial growth was measured on each plate on the fourth, sixth, eighth, tenth, twelfth and fourteenth day after inoculation. Two diameter measurements at right angle to each other were taken on each plate and the arithmetic mean of these two measurements was recorded.

At the end of the incubation period several plates showing the strongest inhibition to the wood destroying fungi were selected for further study. Forty discs, five millimeters in diameter, were cut with a sterilized cork borer from the malt agar medium where the growth of the solution microorganisms was evident. Each of twenty discs was placed

individually on a sterile microscope slide, in a growth chamber, adjacent to a disc of the same size cut from a pure culture of F. annosus (Fig. 1). The distance between the two discs was about five millimeter. The growth chamber consisted of a sterile Petri plate containing glass rods for the support of the microscope slide. Twenty milliliters of sterile distilled water was added to ten of the plates, so that the relative humidity within the plates had reached the one hundred per cent prior to the introduction of malt agar discs. The slides with the discs always remained above water level. The other ten growth chambers contained 1.5 weight molar sucrose solution in order to decrease the relative humidity to a level of approximately ninety-seven per cent (Luther 1935). Similar series of growth chambers were tested with discs con-

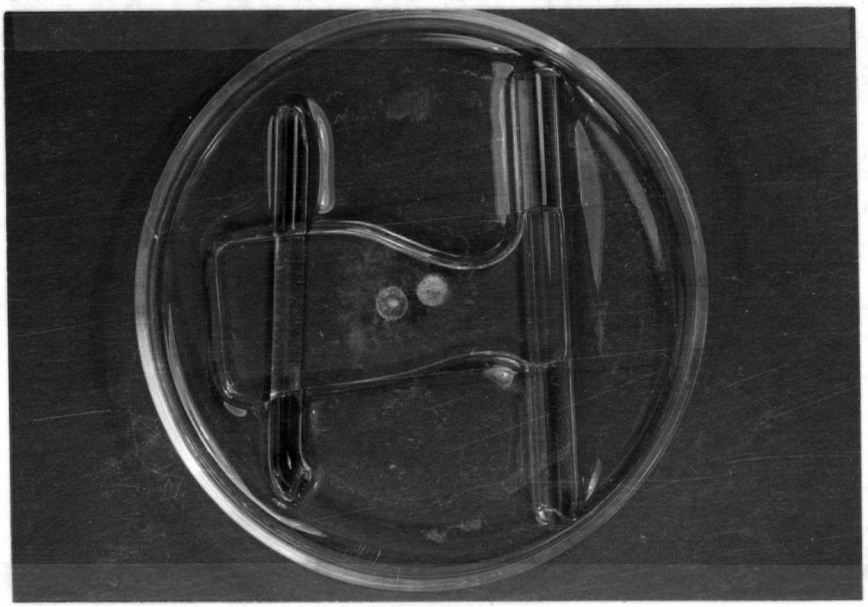


Figure 1. Growth chamber for studying effects of various relative humidity levels on mycelial spread.

taining mycelium of P. monticola. The growth chambers were incubated at room temperature for twenty-one days during which the mycelial growth of the wood destroying fungi was observed.

Another four blocks from both sapwood and heartwood of each of the three sections were chosen randomly. Shake solutions were prepared. One, four and six per cent malt agar containing ten per cent solution were prepared using the same techinque. However, the solutions prior to the addition to the malt agar were autoclaved for twenty-five minutes at fifteen pounds gauge pressure. Petri plates were poured for each pathogen as with the untreated, natural solution. The plates were inoculated and incubated for two weeks, and mycelial growth was measured on each plate on the fourth, sixth, eight, tenth, twelfth, and fourteenth day after inoculation.

Besides using various concentrations of malt agar, it was felt that it would be of importance to demonstrate the inhibitory effect of the saprophytes towards the wood decaying fungi on wood.

F. annosus and P. monticola were tested on three-quarter inch wood cubes in growth chambers which consisted of seventeen ounce massage jars with screw-on lids. A shell vial of fiteen by forty-five millimeters was placed at the center of each jar and kept in vertical position by a wire support (Fig. 2). Chrome-nickel resistance wire was used for the reason of preventing any rust formation in the jars which might interfere with the growth of fungi.

The growth chambers were autoclaved. The wood blocks were weighed to the nearest hundredth gram and seated individually on the top of the vials in the jars.

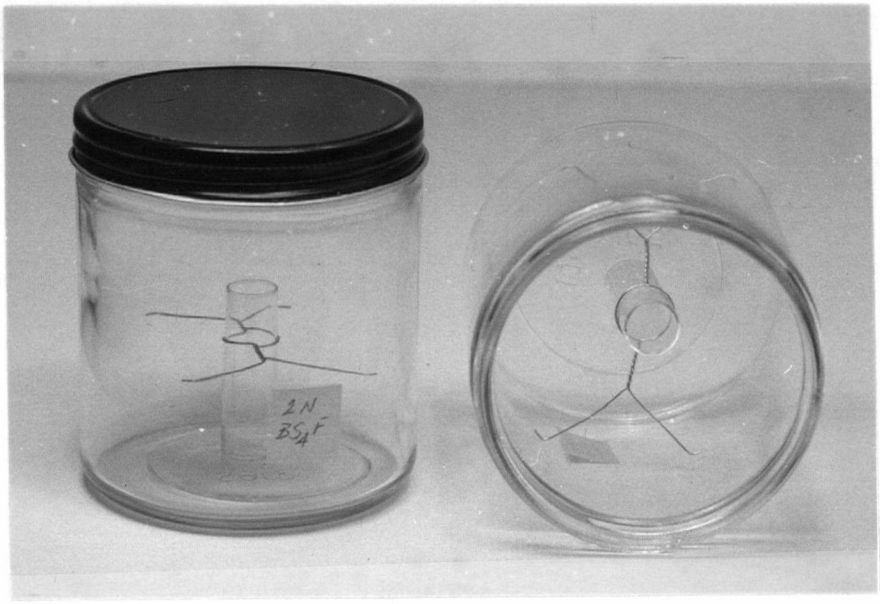


Figure 2. Growth chamber for decay test on wood.

Two sets of three-quarter inch cubes were drawn randomly from both sapwood and heartwood of the three sections of the trunk and each was treated prior to the inoculation as indicated in Table 1.

One set of four blocks from both sapwood and heartwood of the three sections for both pathogens was not subjected to any special treatment, except to surface sterilization by flaming in order to exclude contamination from the atmosphere. The second set of four blocks from both sapwood and heartwood were autoclaved at fifteen pounds gauge pressure for forty minutes.

Within each set of blocks a series of two blocks were kept at one hundred per cent relative humidity. One hundred milliliters of sterile distilled water was poured in the jar in order to keep its atmosphere saturated. The lid of the jar was not tight so as to provide sufficient aeration. In order to secure the one hundred per cent rela-

tive humidity the jars were placed on a shelf in a tray with tap water in it, and covered with a transparent plastic cover. Sufficient water supply was maintained in the tray during the three-month incubation period. The other series in each set was kept at ninety-seven per cent relative humidity. This humidity was set by using 1.5 weight molar sucrose solution. One hundred milliliters sucrose solution was in the growth chamber. Each block was inoculated with a disc five millimeters in diameter cut from pure cultures of the decay fungi and was incubated at room temperature for a period of three months.

Table 1.

Outline of the experiment carried out on three-quarter inch cubes of western hemlock sapwood and heartwood.

Wood		Sapwood				Heartwood			
Treatment		Natural		Autoclaved		Natural		Autoclaved	
Test fungus		Fomes	Poria	Fomes	Poria	Fomes	Poria	Fomes	Poria
R. humid. %		100 97	100 97	100 97	100 97	100 97	100 97	100 97	100 97
Butt sec'n.	1 cube 2								
Middle sec'n.	1 cube 2								
Top sec'n.	1 cube 2								

Simultaneously with the drawing of the blocks used in the experiment, four blocks from both sapwood and heartwood of the three sec-

tions were chosen randomly for the purpose of moisture content determination. The blocks were weighed when fresh, ovendried at 105 °C for 24 hours, reweighed and the moisture content was calculated on ovendry basis. The average of moisture content of four blocks of both sapwood and heartwood of the three levels were considered as an approximation to that of the blocks used in the experiment. On the basis of this moisture content and the fresh weight of the blocks used in the experiment their ovendry weight was calculated and termed as estimated ovendry weight. At the end of three months the superficial mycelial growth was removed from the surface of the blocks by a soft tooth-brush, exercizing care not to remove any wood particles. The blocks were weighed, then ovendried at 105 ° C and reweighed. Moisture content of the blocks at the end of the three-month period and weight loss, if any, were calculated.

Both experiments (test on malt agar - and test on wood) were repeated using the same technique, on material from another vigorously growing healthy hemlock tree of about the same age as used in the first experiments.

Statistical analyses were carried out on the data from experiments on both trees and the results are presented herein.

Although the final growth measurements were made on the fourteenth day after inoculation, the statistical analyses are based on the data recorded on the eighth day, since at this time some mycelial colonies had covered the diameter of the plates.

EXPERIMENTAL RESULTS

Cultural studies on malt agar media.

Large numbers of various organisms were obtained from both the sapwood and heartwood of each section of the healthy western hemlock stem. Every plate containing untreated shake solutions showed evidence of a microflora being present in the tissues. The organisms in the majority of the instances formed small colonies on the malt agar medium, and seemed to inhibit the growth of F. annosus and P. monticola (Fig. 3a, b, and 4a, b). Although no attempt was made to identify the organisms, it was noted that the population consisted of bacteria, yeasts, moulds and imperfect fungi. In a few cases the organisms produced no macroscopic, aerial growth but microscopic examination of the media revealed their presence (Fig. 5).

The summarized experimental data are given in Figures 6, 7 and 8. The two trees used in this study were not treated in the analysis as true replicates (Table 2) for the reason that significant tree to tree variation was expected. Variance was calculated for the trees and removed from the experimental error thus making the analysis more sensitive.

When a wet environment was provided - one per cent malt agar - the growth of both F. annosus and P. monticola was significantly inhibited in plates containing natural, untreated shake solutions. The test fungi grew better on media containing solutions from heartwood than on those with solutions from sapwood (Fig. 6a, b), and this growth pattern was consistent in the three sections of the stem. The strongest inhibition was exerted by the solution from sapwood of the butt section, followed in decreasing order by those from the top and middle sections.

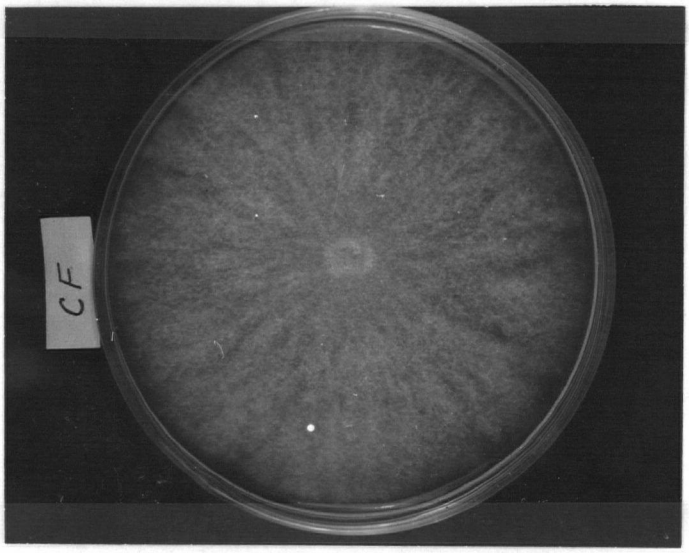


Figure 3 a. Fomes annosus on one per cent malt agar containing no shake solution (control).

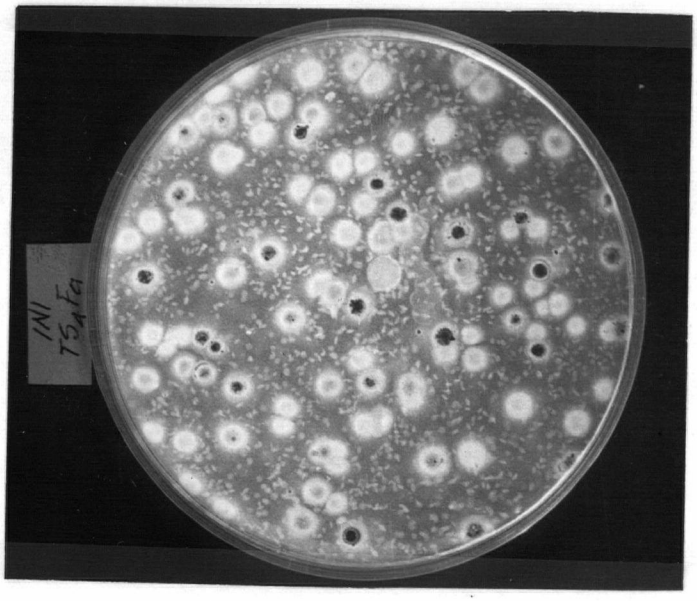


Figure 3 b. Fomes annosus on one per cent malt agar containing shake solution. Note lack of mycelial growth from inoculum plug at center.

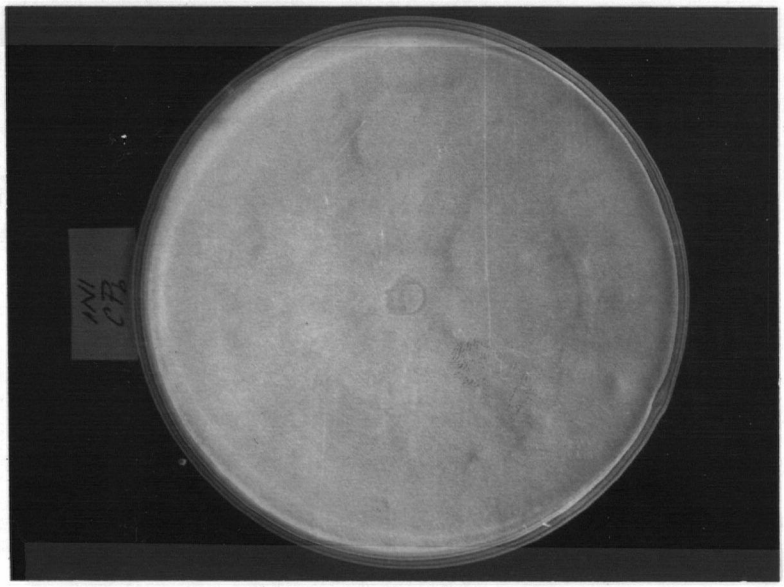


Figure 4 a. Poria monticola on one per cent malt agar containing no shake solution (control).

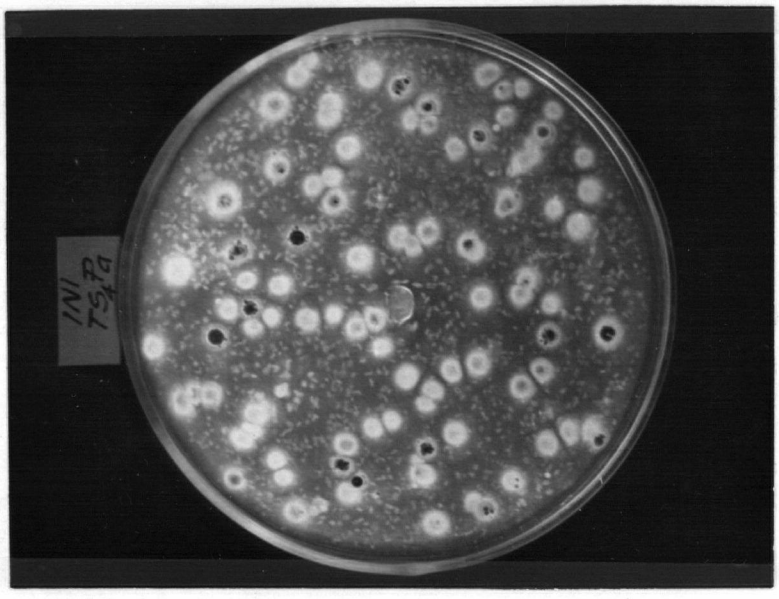


Figure 4 b. Poria monticola on one per cent malt agar containing shake solution. Note lack of mycelial growth from inoculum plug at center.

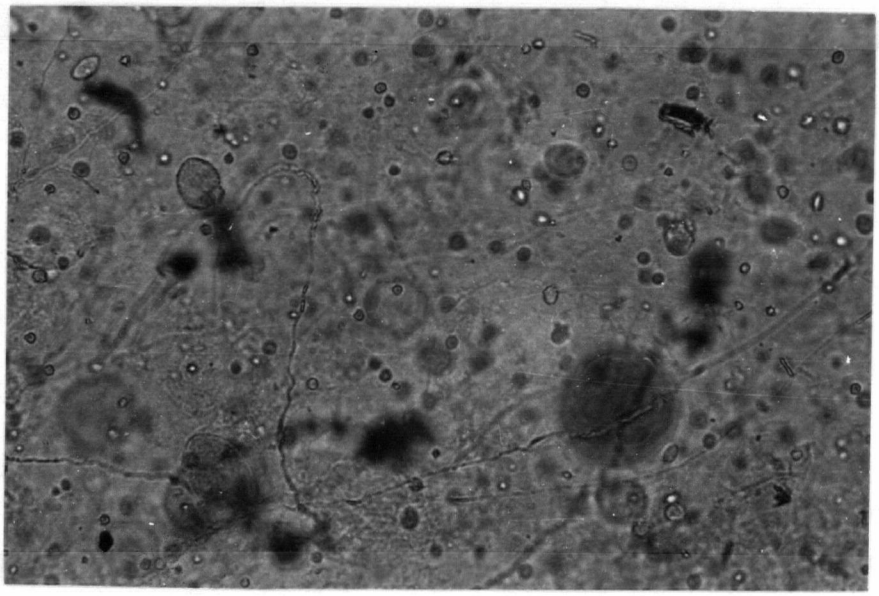


Figure 5. Photomicrograph showing the microorganisms in one per cent malt agar medium (approximately 600 x).

Figure 6 a. Upper left. Growth of F. annosus on one per cent malt agar containing the various untreated shake solutions.

Figure 6 b. Upper right. Growth of P. monticola on one per cent malt agar containing the various untreated shake solutions.

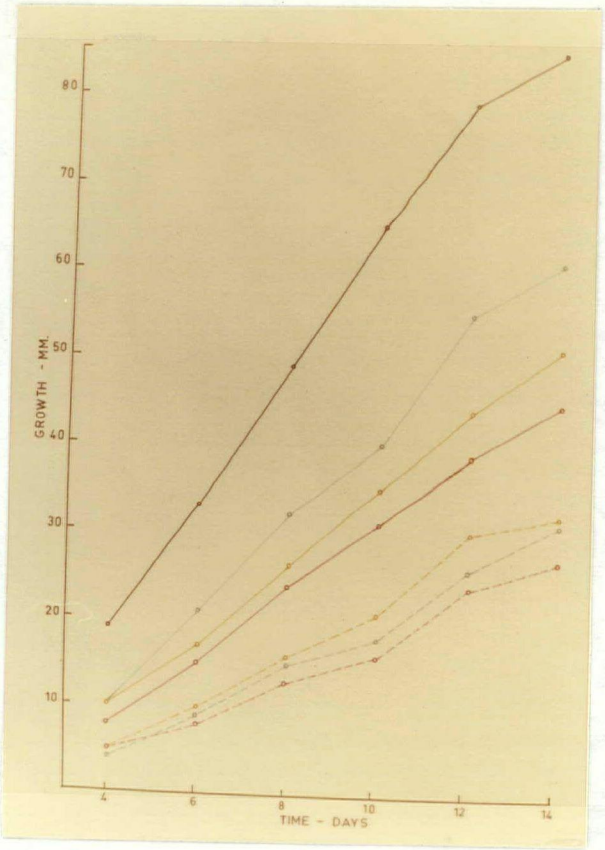
Figure 6 c. Lower left. Growth of F. annosus on one per cent malt agar containing the various autoclaved solutions.

Figure 6 d. Lower right. Growth of P. monticola on one per cent malt agar containing the various autoclaved solutions.

Legend:

Black solid line - no shake solution added (control)
Red dash line - butt section, sapwood solution
Red solid line - butt section, heartwood solution
Orange dash line - middle section, sapwood solution
Orange solid line - middle section, heartwood solution
Green dash line - top section, sapwood solution
Green solid line - top section, heartwood solution

Each plotted value represents the average of eight experimental values with each of the two trees.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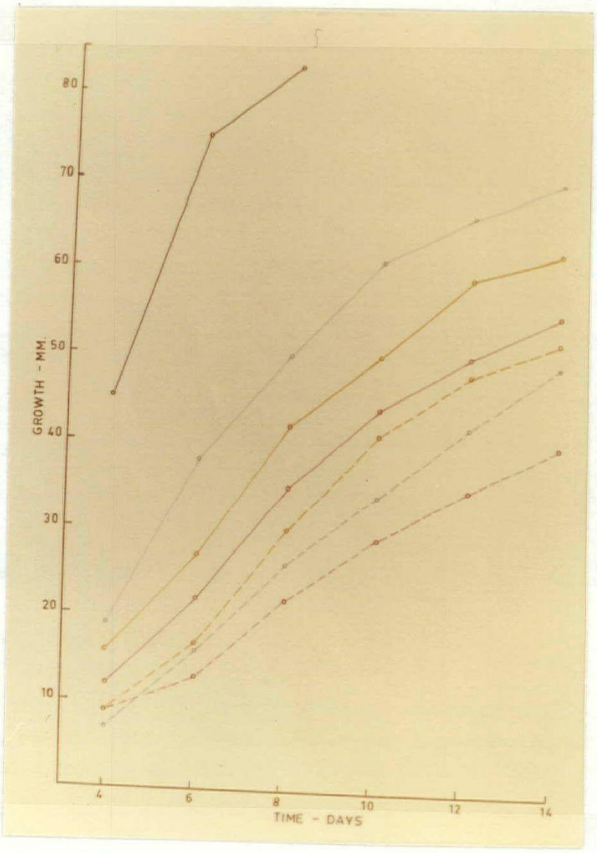
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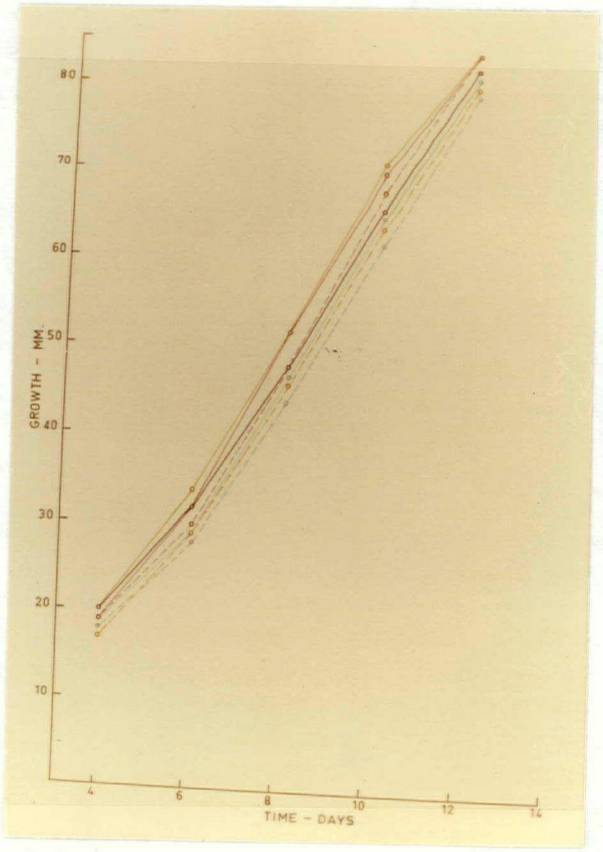
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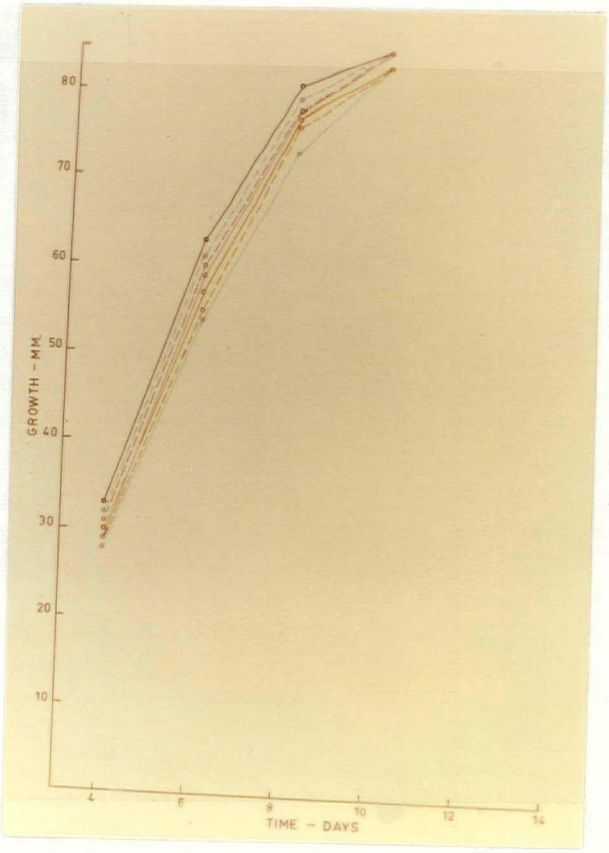

GROWTH - MM.
TIME - DAYS

Figure 7 a. Upper left. Growth of F. annosus on four per cent malt agar containing the various untreated shake solutions.

Figure 7 b. Upper right. Growth of P. monticola on four per cent malt agar containing the various untreated shake solutions.

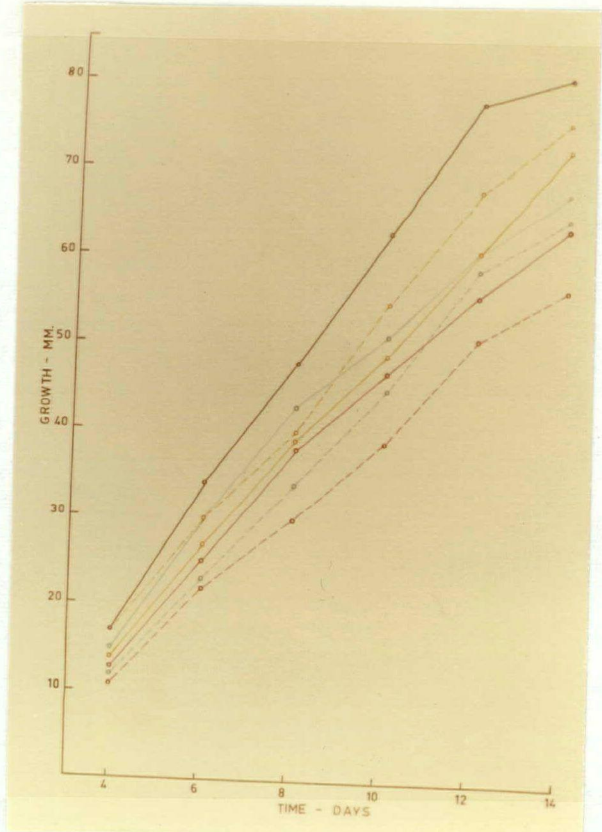
Figure 7 c. Lower left. Growth of F. annosus on four per cent malt agar containing the various autoclaved solutions.

Figure 7 d. Lower right. Growth of P. monticola on four per cent malt agar containing the various autoclaved solutions.

Legend:

Black solid line - no shake solution added (control)
Red dash line - butt section, sapwood solution
Red solid line - butt section, heartwood solution
Orange dash line - middle section, sapwood solution
Orange solid line - middle section, heartwood solution
Green dash line - top section, sapwood solution
Green solid line - top section, heartwood solution

Each plotted value represents the average of eight experimental values with each of the two trees.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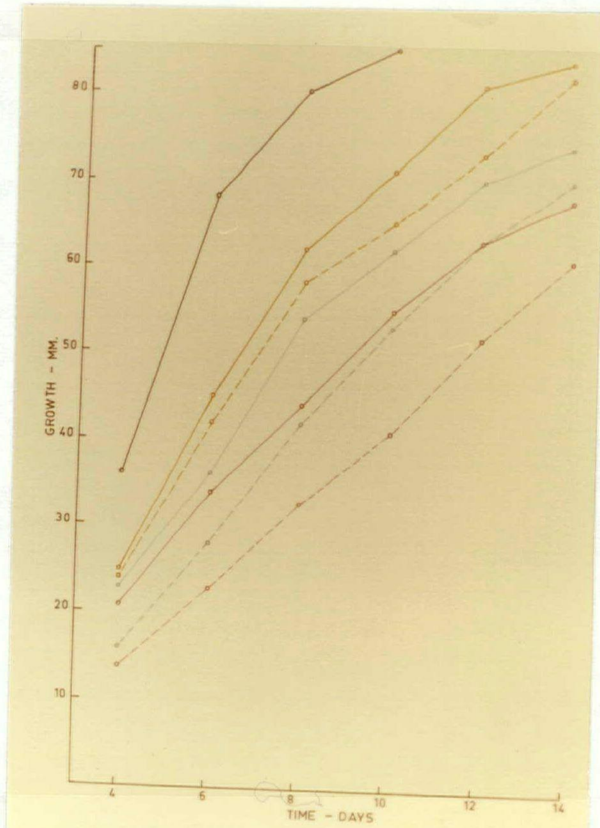
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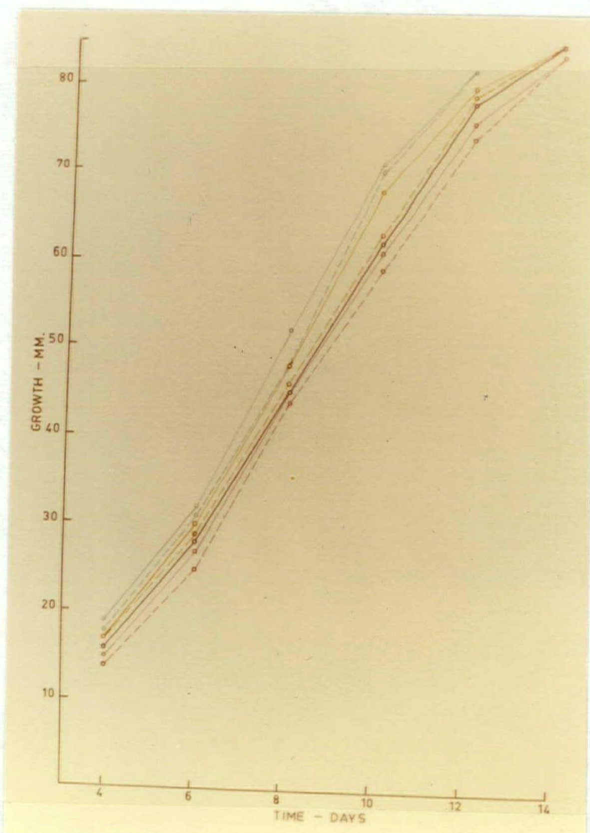
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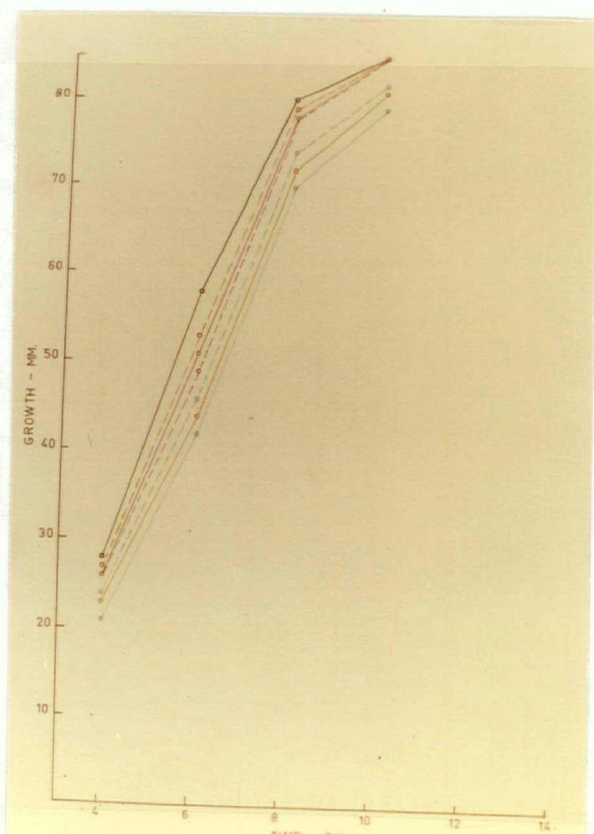
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TIME - DAYS
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Figure 8 a. Upper left. Growth of F. annosus on six per cent malt agar containing the various untreated shake solutions.

Figure 8 b. Upper right. Growth of P. monticola on six per cent malt agar containing the various untreated shake solutions.

Figure 8 c. Lower left. Growth of F. annosus on six per cent malt agar containing the various autoclaved solutions.

Figure 8 d. Lower right. Growth of P. monticola on six per cent malt agar containing the various autoclaved solutions.

Legend:

Black solid line - no shake solution added (control)
Red dash line - butt section, sapwood solution
Red solid line - butt section, heartwood solution
Orange dash line - middle section, sapwood solution
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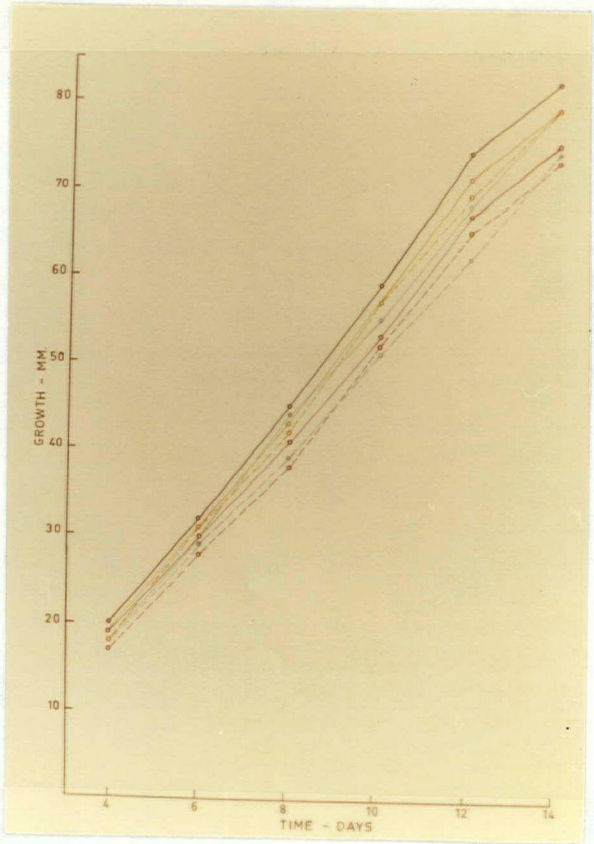
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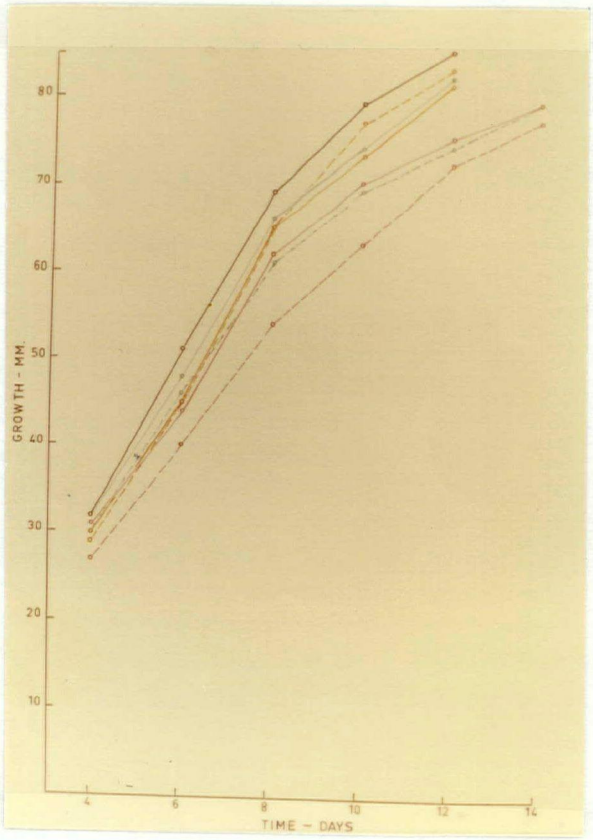
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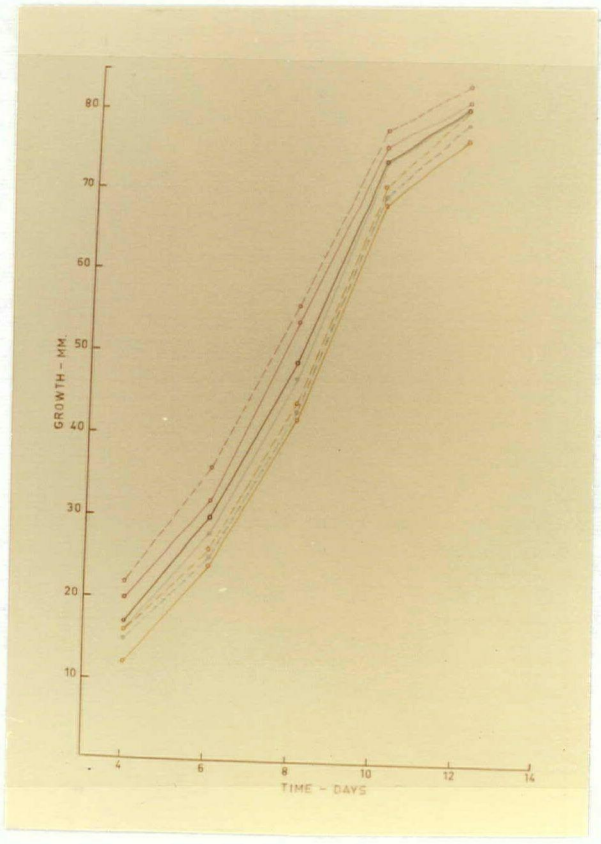
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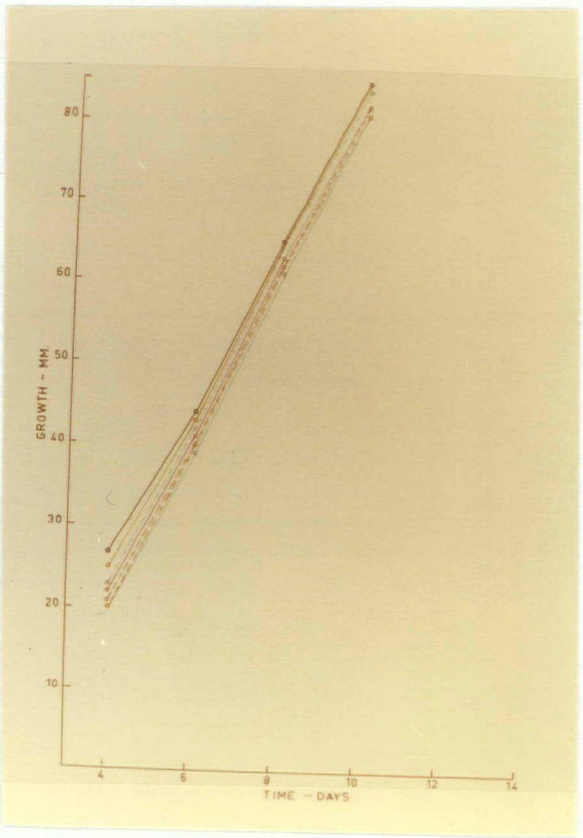
GROWTH - MM
TIME - DAYS

Table 2.

Analysis of variance based on data recorded in Figures 6, 7 and 8.

Source of variation	DF.	Sum square	Mean square	F.
A Tree	1	367.3600	367.3600	15.10 **
B Sapw.-Heartw.	1	625.0000	625.0000	25.70 **
C Nat.-Autocl.	1	12731.3000	12731.3000	523.60 **
D Fomes-Poria	1	13963.3000	13963.3000	574.27 **
E Agar % 1-4-6	2	2024.5400	1012.2700	41.63
F Section B-M-T.	2	150.4900	75.2450	3.09
AB	1	132.2300	132.2300	5.43 *
AC	1	21.7700	21.7700	0.89
AD	1	5.4400	5.4400	0.22
AE	2	101.0000	50.5000	2.07
AF	2	193.5400	96.7700	3.97
BC	1	616.6900	616.6900	25.36 **
BD	1	4.6900	4.6900	0.19
BE	2	266.6200	133.3100	5.48 **
BF	2	90.4900	45.2450	1.86
CD	1	624.9900	624.9900	25.70 **
CE	2	5676.5000	2838.2500	116.72 **
CF	2	713.5500	356.7750	14.67 **
DE	2	58.5800	29.2900	1.20
DF	2	155.0500	77.5250	3.18 *
EF	4	228.8300	57.2075	2.35
ABC	1	289.0200	289.0200	11.88 **
ABD	1	75.1200	75.1200	3.08
ABE	2	67.5600	33.7800	1.38
ABF	2	143.2000	71.6000	2.94
ACD	1	26.6900	26.6900	1.09
ACE	2	52.1100	26.0550	1.07
ACF	2	215.4100	107.7050	4.42 *
ADE	2	67.3700	33.6850	1.38
ADF	2	11.0800	5.5400	0.22
AEF	4	161.9600	40.4900	1.66
BCD	1	93.4400	93.4400	3.84
BCE	2	153.1000	76.5500	3.14
BCF	2	132.0600	66.0300	2.71
BDE	2	8.5200	4.2600	0.17
BDF	2	26.0700	13.0350	0.53
BEF	4	32.5000	8.1250	0.33
CDE	2	832.6500	416.3250	17.12 **
CDF	2	51.5200	25.7600	1.05
CEF	4	93.4600	23.3650	0.96
DEF	4	163.6200	40.9050	1.68
ERROR	65	1580.4600	24.3147	
TOTAL	143	43029.0000		

* Significant at 0.05 probability level.
** Significant at 0.01 probability level.

The inhibition on plates containing solutions from heartwood, in decreasing order were as follows: butt, middle and top sections, for both F. annosus and P. monticola. The best growth (least inhibition) of the two wood decay fungi occurred on plates with solutions from the heartwood of the top section, but this was still significantly less than the growth on control plates containing one per cent malt agar without the shake solution (Fig. 6 a, b).

Application of Duncan's new multiple-range test to these data showed that an increase of the agar concentration to four per cent (drier environment) brought about a highly significant change in the rate of growth of the test fungi. The inhibiting factor decreased with the increase in agar concentration. Both F. annosus and P. monticola produced better mycelial growth on four per cent malt agar than on one per cent malt agar regardless of the source of shake solutions (sapwood or heartwood, butt middle or top section) used in preparing the media (Fig. 7 a, b). The inhibition of F. annosus in plates containing various shake solutions was found to be as follows in decreasing order: butt sapwood, top sapwood, butt heartwood, middle heartwood and sapwood, and top heartwood (Fig. 7 a). P. monticola produced the poorest growth in plates containing the solution from the butt sapwood; followed by top sapwood, butt heartwood, top heartwood, middle sapwood and heartwood (Fig. 7 b). Furthermore, the distinctly different degrees of inhibition between the solutions from sapwood and heartwood on one per cent malt agar, lessened when the solutions were added to a drier substratum (four per cent malt agar).

Further increase in the agar concentration to six per cent

resulted in a more drastic change in the growth rates. Both F. annosus and P. monticola seemed to grow freely in the plates containing the shake solutions, producing colonies of almost the same size as those on control plates with no shake solutions (Fig. 8 a, b). Although growth of the microorganisms occurred in the dry medium, the inhibition factor was significantly reduced. Both F. annosus and P. monticola has overgrown the small colonies of the saprophytes on the dry medium - six per cent malt agar (Fig. 9 a, b).

The increase of the agar content of the media from one to four and six per cent was considered to provide a drier environment for the growth of test fungi. However, it was not possible to conclude that the decrease in the amount of available water was the sole factor responsible for the decline of inhibition.

This relationship was demonstrated in another experiment when malt agar discs of five millimeters in diameter, colonized by the saprophytes, were placed in growth chambers adjacent to a disc of the same size containing mycelium of the decay fungi. The growth of both F. annosus and P. monticola was inhibited at one hundred per cent relative humidity. On the other hand, a decrease of relative humidity in the growth chambers to approximately ninety-seven per cent resulted in free growth of the mycelium of the test fungi which covered the discs of saprophytes (Fig. 10 a, b, 11 a, b).

In another part of the experiment the shake solutions were sterilized by autoclaving prior to their being added to the malt agar. This treatment led to the loss of inhibition.

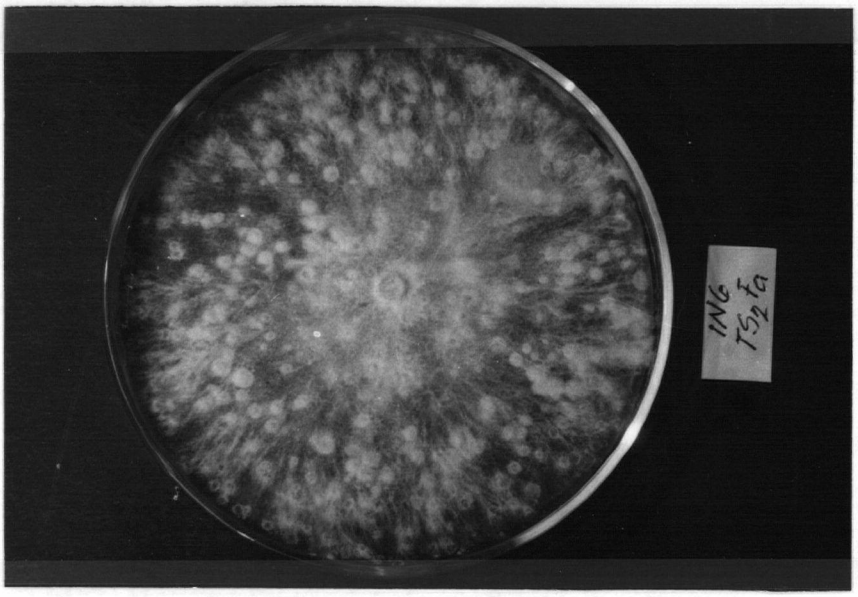

Figure 9 a. F. annosus on six per cent malt agar containing untreated shake solution.

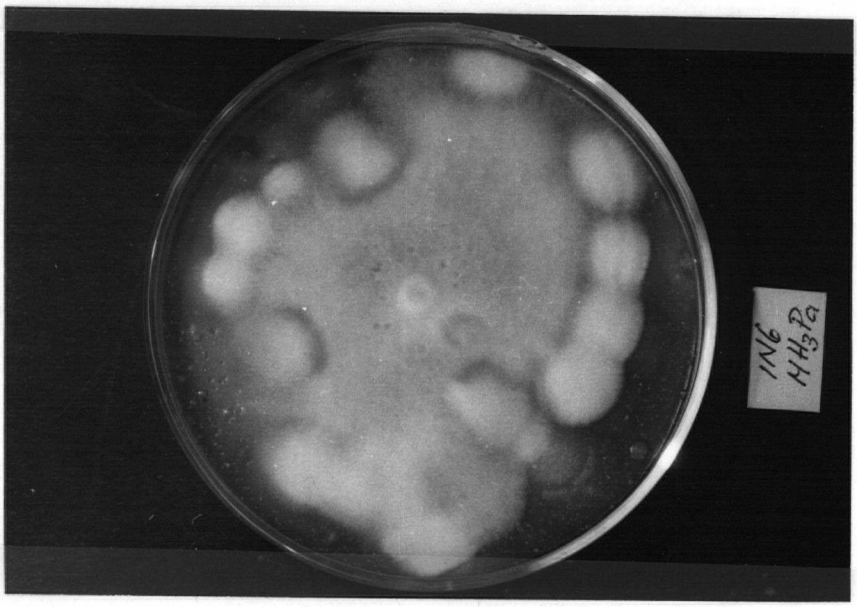

Figure 9 b. P. monticola on six per cent malt agar containing untreated shake solution.

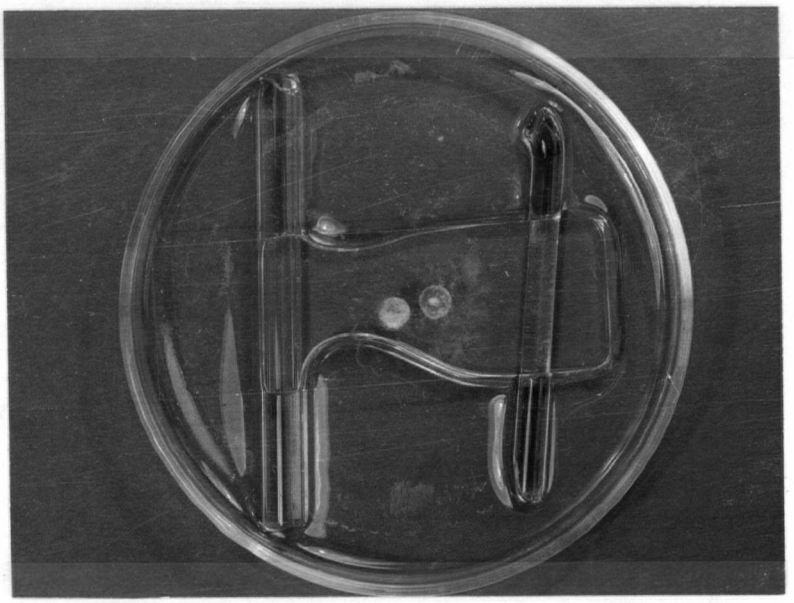

Figure 10 a. Mycelial spread of F. annosus (left disc) at one hundred per cent relative humidity.

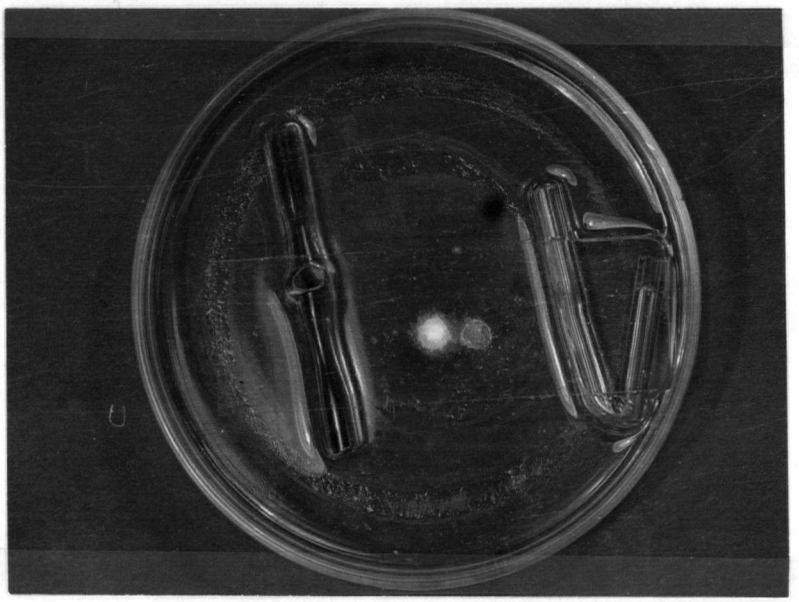

Figure 10 b. Mycelial spread of F. annosus (left disc) at ninety-seven per cent relative humidity.

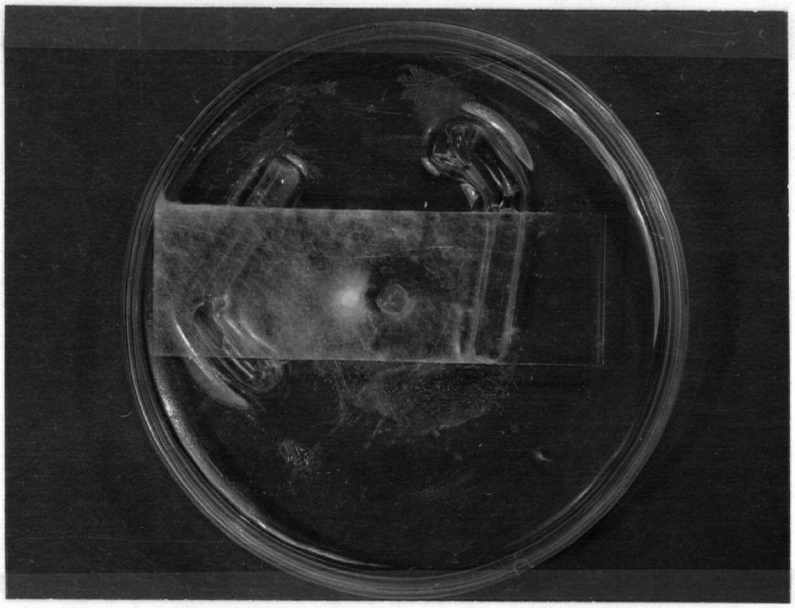

Figure 11 a. Mycelial spread of P. monticola (left disc) at one hundred per cent relative humidity.

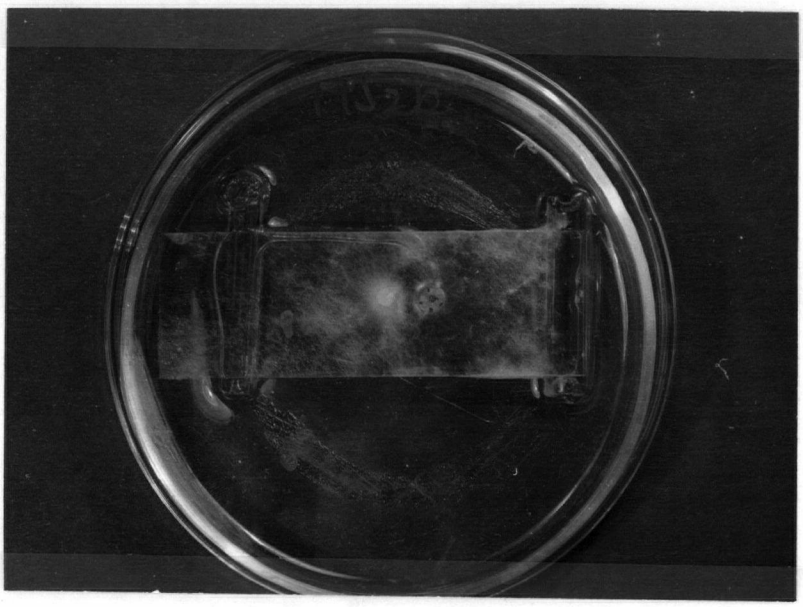

Figure 11 b. Mycelial spread of P. monticola (left disc) at ninety-seven per cent relative humidity.

On media containing the autoclaved solutions, both F. annosus and P. monticola grew approximately as well as on those without solutions, regardless of the source of solutions and the agar concentration of the media (Fig. 6 c, d, 7 c, d, 8 c, d). Stimulation occurred instead of inhibition in some cases. F. annosus produced better growth than the control in the following instances; on wet medium - one per cent malt agar - containing the solution of butt and middle heartwood (Fig. 6c); on drier medium - four per cent malt agar - with the solution from the middle heartwood and top sapwood and heartwood (Fig. 7 c); on driest medium - six per cent malt agar - containing the solution obtained from butt sapwood and heartwood (Fig. 8 c). The addition of the autoclaved solutions provided no stimulation in the growth of P. monticola.

Autoclaving is a common technique for sterilization. It was felt that the high temperature (250 °F) reached during the process may have altered the chemical composition of the solutions, thus destroying their inhibitory effect on the growth of the decay fungi. Therefore, it was of prime importance to express the inhibiting factor in a more explicit form.

The solutions containing the organisms which had shown the strongest inhibition were added to each other and divided into three equal portions. One part had no further treatment; the second was filtered through Millipore of type HA, 0.45 micron pore size; the third portion was autoclaved at fifteen pounds gauge pressure for twenty-five minutes. Each portion of the solution was then used in one per cent malt agar culture media as before and tested with the decay fungi. Mycelial growth was recorded on the fourth, sixth, eighth, tenth, twelfth, and

fourteenth day after inoculation.

The growth of both F. annosus and P. monticola was significantly inhibited on plates containing the untreated, natural shake solution. However, the growth of the decay fungi in plates with autoclaved solution and in plates with Millipore filtered solution were almost identical with the growth produced in the control plates having no solution (Fig. 12 a, b, c, d, and 13 a, b, c, d). Both treatments of sterilization, autoclaving and Millipore filtering resulted in loss of the inhibiting factor (Fig. 14 and 15).

Figure 12. F. annosus on one per cent malt agar,

a. containing no shake solution (control)

b. containing untreated solution; note lack of mycelial growth from inoculum plug at center

c. containing autoclaved solution

d. containing Millipore filtered solution

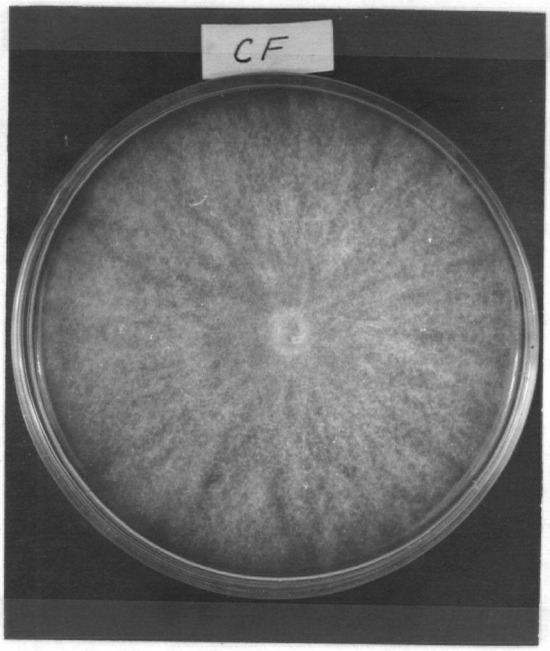

Figure 12 a.

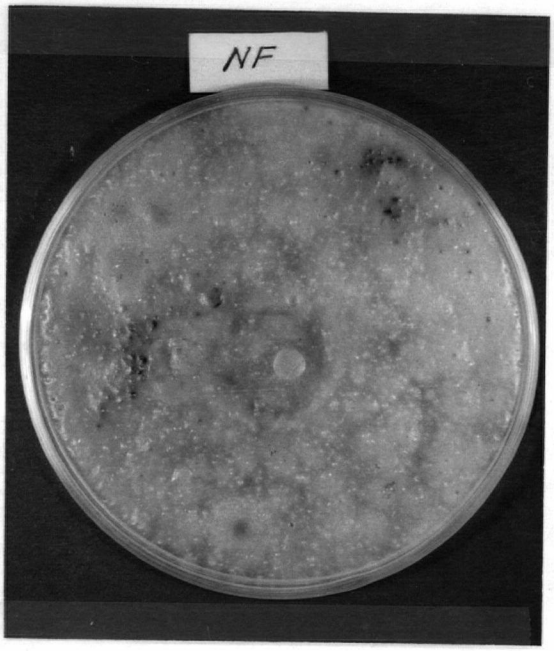

Figure 12 b.

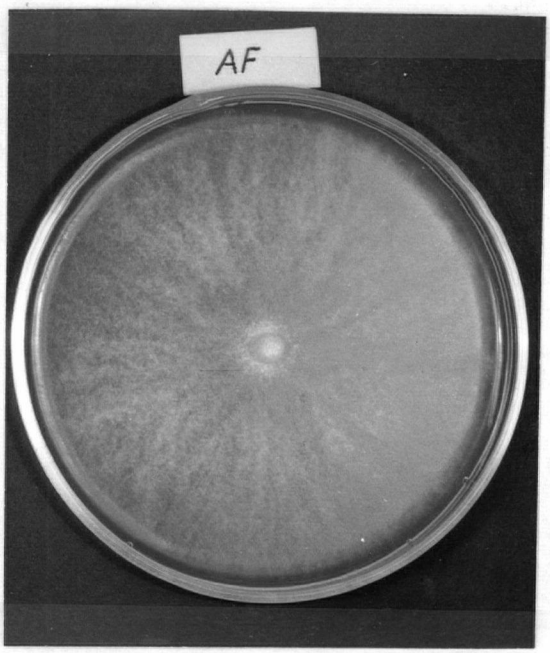

Figure 12 c.

Figure 12 d.

Figure 13. P. monticola on one per cent malt agar,

a. containing no shake solution (control)

b. containing untreated solution; note lack of mycelial growth from inoculum plug at center.

c. containing autoclaved solution

d. containing Millipore filtered solution

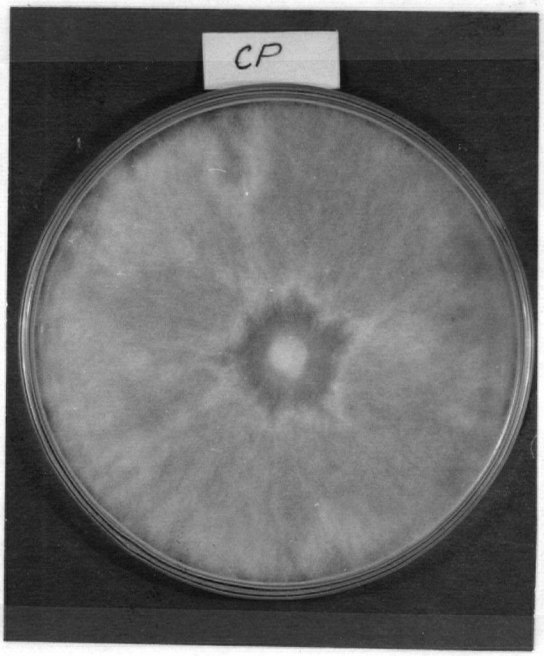

Figure 13 a.

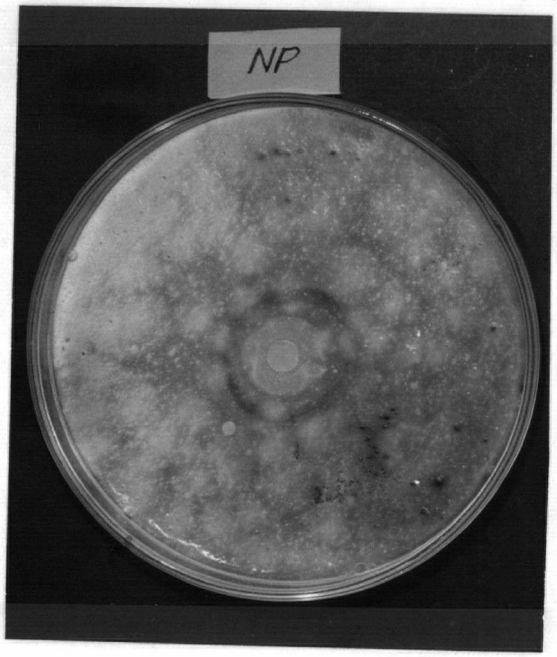

Figure 13 b.

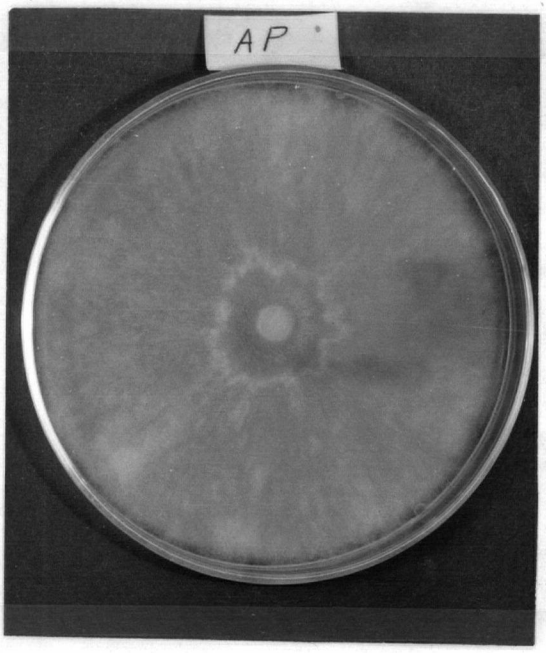

Figure 13 c.

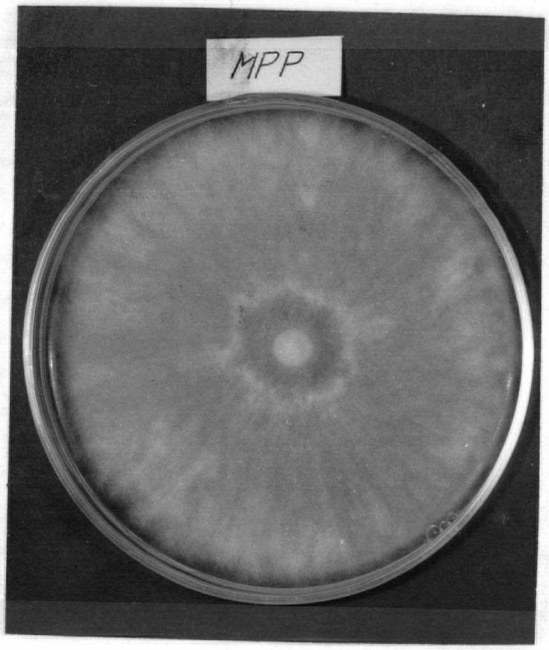

Figure 13 d.

Figure 14. Graph representing the growth rate of F. annosus on one per cent malt agar containing no shake solution, untreated solution, and solutions autoclaved and Millipore filtered.

Legend:

Solid line - no shake solution added (control)

Dash - two dots line - untreated solution

Dash line - autoclaved solution

Dotted line - Millipore filtered solution

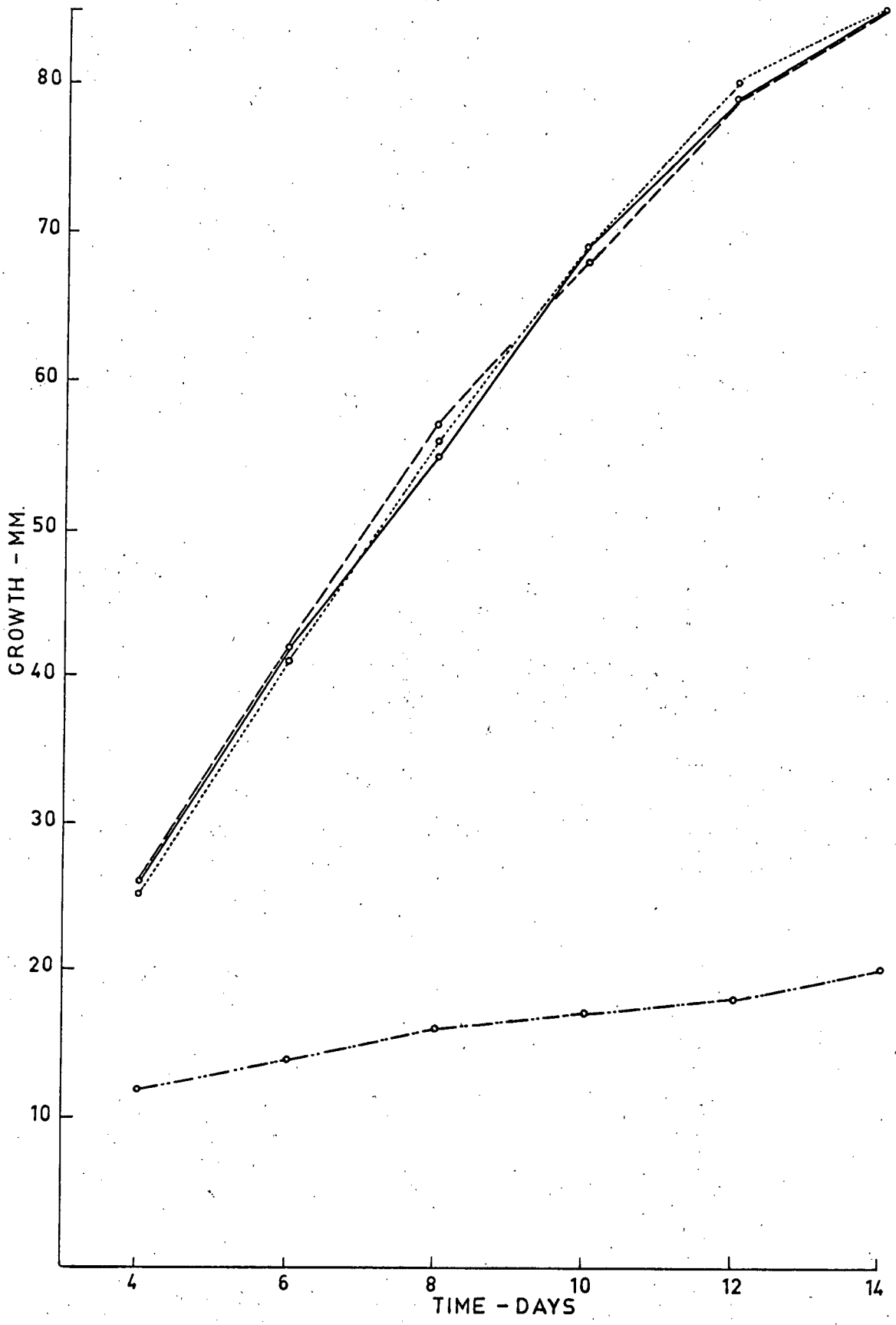

Figure 14.

Figure 15. Graph representing the growth rate of P. monticola on one per cent malt agar containing no shake solution, untreated solution, and solutions autoclaved and Millipore filtered.

Legend:

Solid line - no shake solution added (control)

Dash - two dots line - untreated solution

Dash line - autoclaved solution

Dotted line - Millipore filtered solution

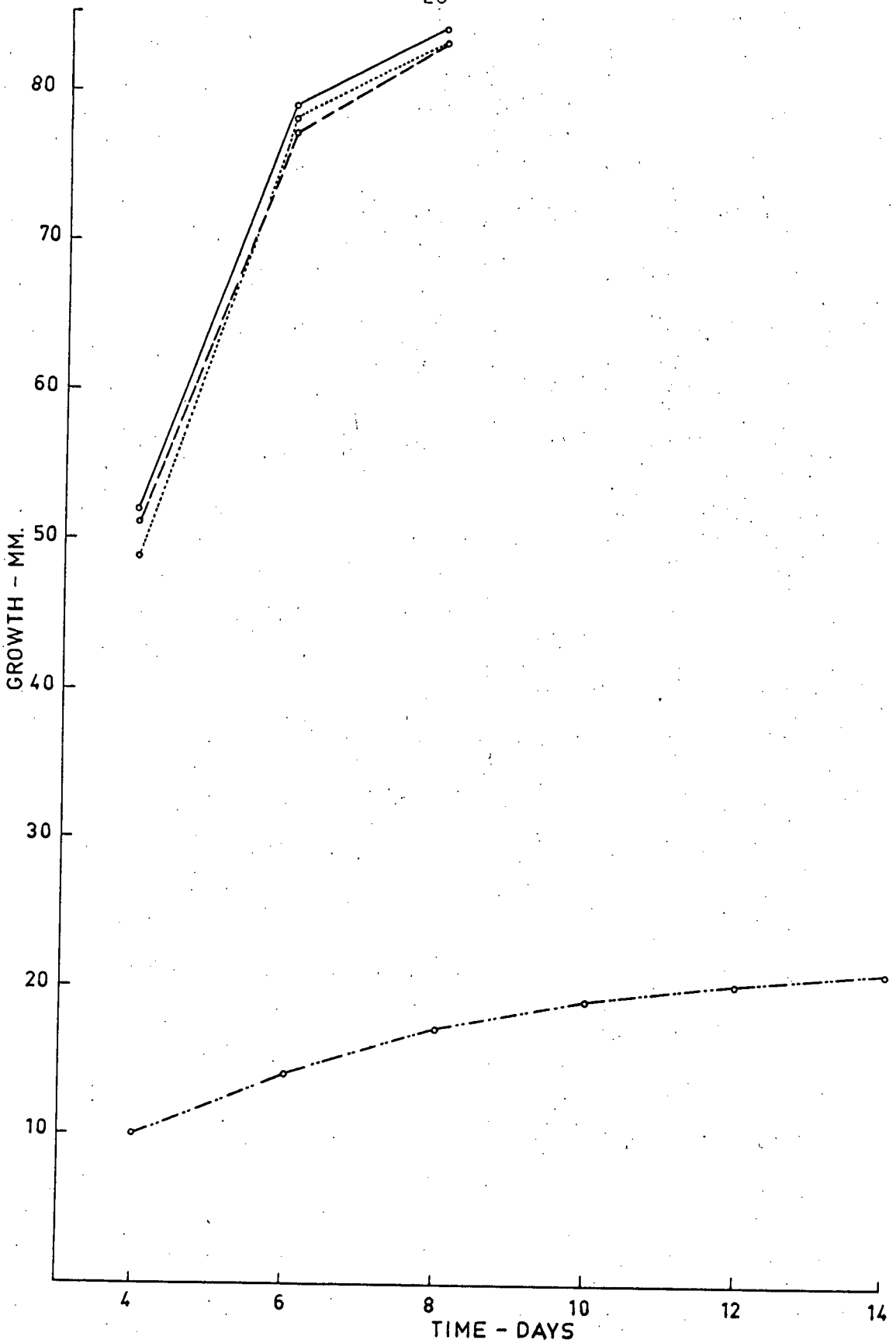

Figure 15.

Cultural studies on wood blocks.

The growth rate of F. annosus and P. monticola was studies on three -quarter inch wood cubes incubated in growth chambers. The growth of the decay fungi is expressed as a percentage of loss in weight of the cubes during the incubation period of three months. The percentage loss in weight was calculated as a difference between the initial estimated ovendry weight and the final ovendry weight over the final ovendry weight. (Final ovendry weight = ovendry weight of cube at the end of incubation period). The results are summarized in Table 3. Analysis of variance was made on the data and this is given in Table 4.

The growth of F. annosus and P. monticola was partially inhibited on natural, untreated wood (Fig. 16 and 17). It was possible to detect the microorganisms on natural, untreated cubes. In many instances they produced visible growth on the surface of the cubes invading the inoculum plug (Fig. 18 a, b). The weight loss of untreated sapwood and heartwood cubes of three sections of the stem was significantly smaller at 0.01 probability level than that of autoclaved blocks. Both test fungi produced better growth - more weight loss - on sapwood cubes than on cubes of heartwood (Fig. 19 and 20). This result is in contradiction with that of the studies on malt agar media.

The treatment of two levels of relative humidity yielded significantly different growth for F. annosus and P. monticola on untreated wood. The test fungi caused more weight loss in blocks at ninety-seven per cent relative humidity than in blocks kept at one hundred per cent relative humidity. The drier environment seemed to reduce consistently the inhibiting factor in untreated, natural sapwood and heartwood.

Table 3.

Per cent weight losses of three-quarter inch sapwood and heartwood cubes of western hemlock, caused by F. annosus and P. monticola. Underlined figures represent mean of values recorded on materials from the first and second trees.

Wood		Sapwood								Heartwood							
Treatment		Natural				Autoclaved				Natural				Autoclaved			
Test fungus		Fomes		Poria		Fomes		Poria		Fomes		Poria		Fomes		Poria	
R. humid. %		100	97	100	97	100	97	100	97	100	97	100	97	100	97	100	97
Butt	1	0.4*	1.3	13.2	10.8	6.1	5.5	23.3	16.5	0.3	1.5	15.3	14.8	3.8	4.4	20.6	14.2
tree		0.8	3.3	12.0	13.8	6.1	6.1	26.4	26.3	0.8	1.5	11.9	12.4	5.8	6.0	18.3	21.3
sec'n.	2	1.2	5.4	10.9	16.9	6.2	6.7	29.5	31.6	1.3	1.6	8.6	10.1	7.9	7.6	16.1	27.5
Middle	1	0.4	1.8	14.5	14.8	8.8	8.6	27.7	23.4	0.6	1.5	13.1	17.6	6.5	6.6	19.5	24.5
tree		1.0	1.6	14.9	15.6	8.9	8.3	23.5	21.7	0.8	1.7	14.1	15.6	7.3	6.2	18.6	20.9
sec'n.	2	1.6	1.3	15.4	16.4	9.0	8.0	19.3	20.0	1.0	1.9	15.1	14.6	8.1	5.9	17.8	17.4
Top	1	0.8	5.3	16.4	18.7	9.4	9.6	36.0	42.9	0.8	1.6	15.5	14.8	6.4	6.3	25.5	27.3
tree		0.8	3.0	18.9	20.4	7.9	8.0	39.5	41.7	1.1	1.7	16.9	14.6	7.8	7.3	25.3	25.7
sec'n.	2	0.7	0.8	21.5	22.1	6.5	6.4	43.2	40.5	1.4	1.7	18.4	14.5	9.2	8.3	25.1	24.2

* Every figure in each tree represents the mean of two experimental values.

Table 4.

Analysis of variance based on data shown in Table 3.

Source of variation	DF.	Sum square	Mean square	F.
A Tree	1	11.2620	11.2620	3.05
B Sapw.-Heartw.	1	98.1720	98.1720	26.59 **
C Nat.-Autocl.	1	1578.8500	1578.8500	427.72 **
D. Fomes-Poria	1	5757.9000	5757.9000	1559.85 **
E R.H.% 100-97	1	19.2320	19.2320	5.21 *
F Section B-M-T.	2	185.5820	92.7910	25.13 **
AB	1	0.8780	0.8780	0.23
AC	1	0.2580	0.2580	0.06
AD	1	0.8460	0.8460	0.22
AE	1	0.0000	0.0000	0.00
AF	2	40.3710	20.1855	5.46 **
BC	1	27.8440	27.8440	7.54 **
BD	1	36.5290	36.5290	9.89 **
BE	1	0.7950	0.7950	0.21
BF	2	20.4230	10.2115	2.76
CD	1	11.2180	11.2180	3.03
CE	1	15.7120	15.7120	4.25 *
CF	2	30.2700	15.1350	4.10 *
DE	1	3.0170	3.0170	0.81
DF	2	102.4120	51.2060	13.87 **
EF	2	2.3160	1.1580	0.31
ABC	1	5.1450	5.1450	1.39
ABD	1	36.0850	36.0850	9.77 **
ABE	1	1.4710	1.4710	0.39
ABF	2	20.0090	10.0045	2.71
ACD	1	0.7680	0.7680	0.20
ACE	1	4.7490	4.7490	1.28
ACF	2	35.7490	17.8745	4.84 *
ADE	1	11.7660	11.7660	3.18
ADF	2	19.8700	9.9350	2.69
AEF	2	51.2820	25.6410	6.94 **
BCD	1	20.5610	20.5610	5.57 *
BCE	1	4.7000	4.7000	1.27
BCF	2	3.5030	1.7515	0.47
BDE	1	1.9250	1.9250	0.52
BDF	2	33.0480	16.5240	4.47 *
BEF	2	7.8770	3.9385	1.06
CDE	1	14.8610	14.8610	4.02
CDF	2	47.6420	23.8210	6.45 **
CEF	2	1.3150	0.6575	0.17
DEF	2	1.8560	0.9280	0.25
ERROR	38	140.2700	3.6931	
TOTAL	95	8408.3300		

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

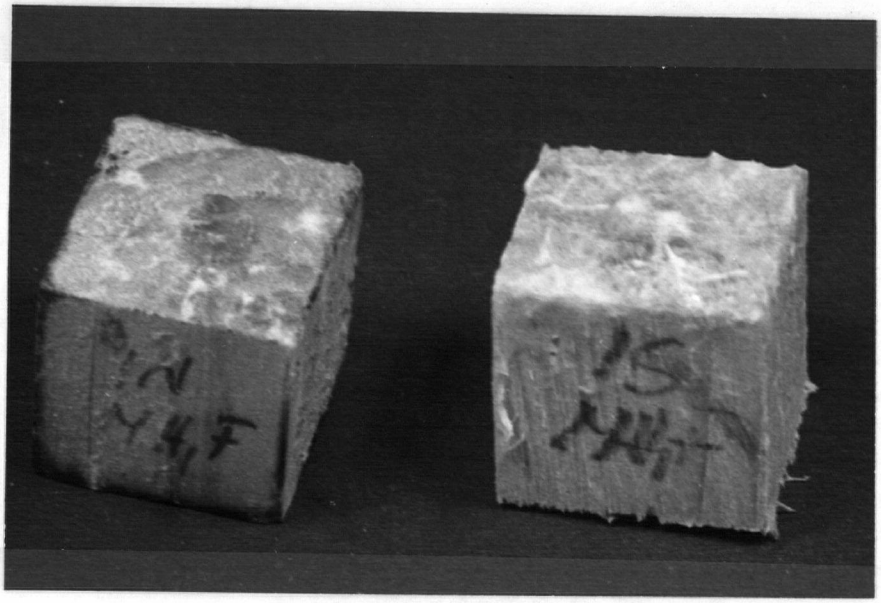

Figure 16. Growth of F. annosus on untreated (left) and autoclaved (right) cubes.

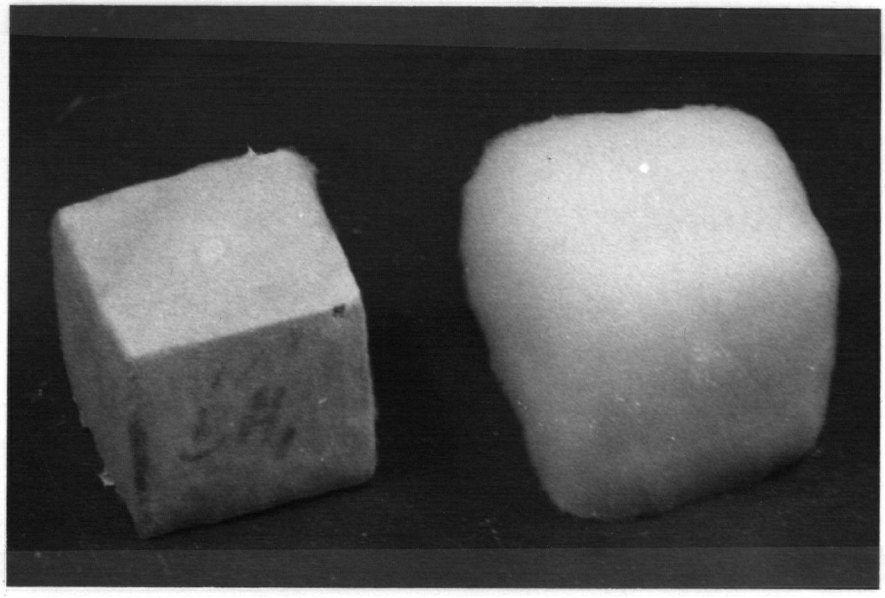

Figure 17. Growth of P. monticola on untreated (left) and autoclaved (right) cubes.

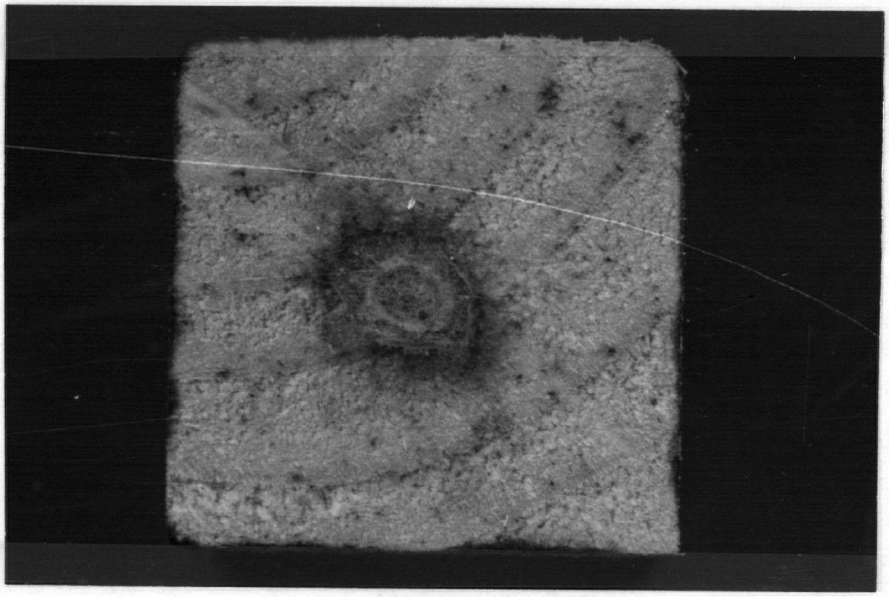

Figure 18 a. Untreated wood cube inoculated with F. annosus.

Figure 18 b. Showing the inoculum invaded by the microorganisms of Figure 18 a.

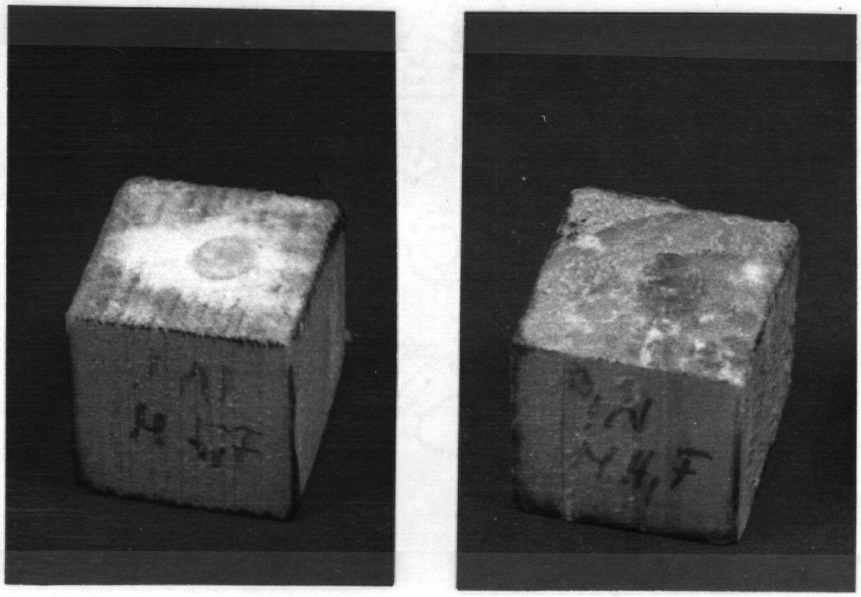

Figure 19. Growth of F. annosus on sapwood (left) and heartwood (right) cubes.

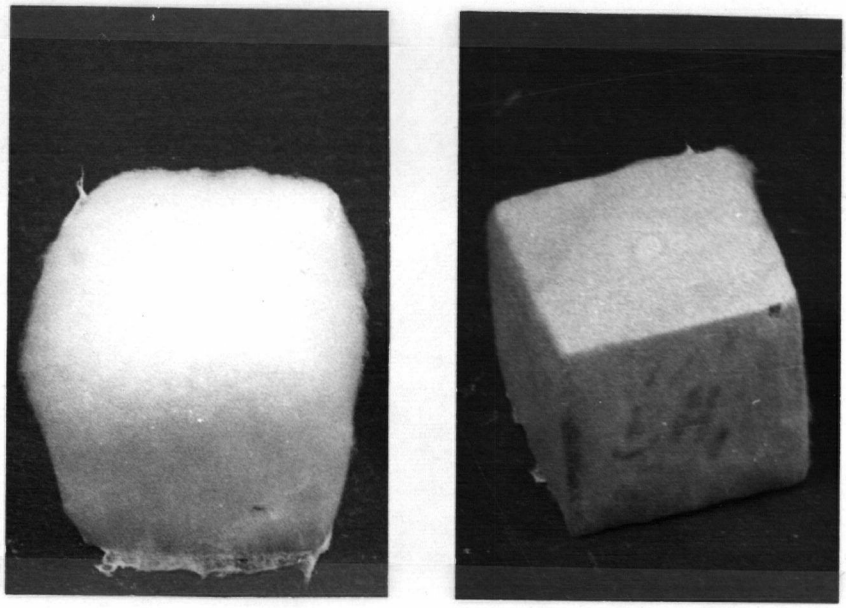

Figure 20. Growth of P. monticola on sapwood (left) and heartwood (right) cubes.

The difference in relative humidity, however, did not effect the growth of the test fungi on autoclaved wood. Both F. annosus and P. monticola caused approximately the same loss in weight of autoclaved cubes at one hundred per cent relative humidity as at ninety-seven per cent relative humidity.

Application of Duncan's new multiple range test to the data showed that the test fungi produced significantly the best growth - most weight loss - on cubes from the top section of the tree. Poorest growth occurred on cubes from the butt section, but there was no significant difference between the weight losses of cubes from the butt section and those of the cubes of the middle section.

The growth of P. monticola was significantly better at 0.01 probability level in all instances than that produced by F. annosus. This is in accordance with the expected since the former is known as a fungus of very rapid growth (Cartwright et al., 1958).

DISCUSSION

In this study samples were taken from sapwood and heartwood of the butt, middle and top sections of the stems of two healthy trees of western hemlock. Solutions were prepared by shaking three-quarter inch, surface sterilized wood cubes in seventy-five cubic centimetres of sterile distilled water. This technique was chosen to recover micro-organisms in wood, since it was considered to provide better coverage of the sample material than other methods of isolation, e.g. the trans-

fer of small portions of wood into plates of malt agar.

The individual solutions obtained from the samples of various parts of the stem were used in the preparation of malt agar, representing ten per cent of the water required to prepare the malt agar. Petri plates were poured and one series was inoculated with F. annosus and the other with P. monticola.

All untreated solutions yielded a variety of organisms capable of significantly inhibiting the growth of the test fungi when added to one per cent malt agar, (Fig. 6 a, b). The degree of inhibition of both test fungi on one per cent malt agar was greater in the plates with solutions from sapwood than in plates containing the solutions from heartwood. The malt agar provided uniform nutrition for the test fungi, and it was considered that the large numbers of microorganisms in the sapwood solutions were responsible for the greater degree of inhibition.

The results also indicated a significant variation in the rate of inhibition between the solutions from the three sections of the stem. The poorest growth on one per cent malt agar occurred in the plates with the solutions of the butt sections, and the best growth in those plates which contained the solutions of the top sections. The growth of test fungi in plates having the solutions of middle sections did not differ significantly from that in plates containing the solutions of the top sections. However, it was positively greater than the growth in plates containing the solutions of butt sections.

The results of the cultural studies on malt agar should not be related to those obtained on natural substrata. Therefore, both F. annosus and P. monticola were tested on three-quarter inch surface

sterilized wood cubes in growth chambers at one hundred per cent relative humidity. The resulting weight losses of the cubes were calculated as percentages of their initial estimated ovendry weight. The estimated ovendry weight in turn was calculated by using the moisture content values determined on the ovendry basis of a sample of four blocks drawn from the same population for this purpose. This method is known to be subject to error, because of variation in density of the samples. This factor was not overlooked, but it was not feasible to apply other techniques such as described by Etheridge, (1957) where the moisture content is expressed as a percentage of moisture present at saturation. The growth of both F. annosus and P. monticola was significantly inhibited on untreated, surface strilized wood cubes incubated at one hundred per cent relative humidity.

The degree of inhibition of both test fungi was significantly greater on heartwood than on sapwood samples. These results were the reverse of those obtained when the solutions of sapwood and heartwood samples were added to one per cent malt agar. It is possible that although the sapwood contained greater number of microorganisms capable of inhibiting the test fungi, better nutritional properties of the sapwood afforded more favourable conditions for the development of the wood destroying fungi.

The results of the cultural studies on wood also indicated a significant variation in the rate of inhibition between the three sections of the stems. These results were similar to those obtained on one per cent malt agar. The poorest growth and least weight losses occurred in the cubes of butt sections. Significantly better growth and

greater weight losses were observed in cubes of the top section. The growth and weight losses in the middle section did not differ significantly from those in the butt section, but they were positively below those recorded in the top section. These differences in the rate of growth and weight losses between the three sections of the stem seem to conform with the variations that exist in the age, structure, nutrient and water content (Brown et al., 1949), and populations of microorganisms of the sections. This study, however, was not designed to provide experimental evidence for further explanation of the variation on the basis of tree characteristics.

Environmental change brought about by increasing the agar concentration to four and six per cent, in malt agar, and by a drop in the level of relative humidity to ninety-seven per cent in the jars with the wood blocks, resulted in a significant change in the degree of inhibition.

Both F. annosus and P. monticola produced better growth on four per cent than on one per cent malt agar containing samples of all solutions. This environmental change, however, did not seem to affect the rate of growth in the plates with the various solutions to the same extent. It did become evident when the rank of the inhibiting powers changed, e.g. P. monticola on one per cent malt agar produced the best growth in the plates with the solution from top heartwood (Fig. 6 b); on four per cent malt agar the best growth of this fungus occurred in plates containing the solution of the middle heartwood (Fig. 7 b). The reason may have been that the organisms of the top heartwood were more tolerant to the change of environment while the organisms of the middle

heartwood were more sensitive to the change, which resulted in a greater loss of inhibiting power. The increase of agar concentration to four per cent also lessened the difference in the degrees of inhibition between the solutions from sapwood and heartwood. A further increase of the agar concentration to six per cent led to almost complete loss of inhibition.

The increase in the agar concentrations of the media was believed to result in a drier environment for the growth of microorganisms, but at the same time another variable was introduced represented by different nutrition. It was realized that the technique did not permit the separation of the linked variables, moisture content and nutrition in the media. Therefore, this technique was supported by another method when agar discs of the microorganisms were paired with those of the test fungi and kept in growth chambers at different relative humidities. The growth of F. annosus and P. monticola was inhibited at one hundred per cent relative humidity. A decrease in the relative humidity to approximately ninety-seven per cent led to the free growth of the test fungi, over-growing the discs containing microorganisms (Fig. 10 a, b, c, d). These results appear to indicate that the moisture content of the environment was an important factor in the occurrence and degree of inhibition.

A decrease in the level of relative humidity to approximately ninety seven per cent in the jars containing untreated wood brought about a significant loss in the degree of inhibition of F. annosus and P. monticola. Both test fungi produced better growth and caused greater weight losses in the cubes incubated at ninety-seven per cent relative

humidity, than in those in a saturated atmosphere. The increase in the weight losses at the lower level of relative humidity was consistent in the sapwood and heartwood of all sections. These results appeared to further support the hypothesis that the amount of available moisture in the environment may play an important role in the degree of inhibition.

F. annosus and P. monticola were also tested on sterilized media (one, four and six per cent malt agar with sterilized solutions, and sterilized wood). Autoclaving was used for the sterilization of the experimental material. The treatment led to the loss of inhibiting factor in both malt agar media and wood.

On malt agar there was no significant inhibition in the growth of the test fungi when the medium contained the autoclaved solutions. On the media containing the autoclaved solutions, both F. annosus and P. monticola grew approximately as well as on those without solutions, regardless of the source of solution and the agar concentration of the media. Rather, stimulation occurred instead of the inhibition in some instances in the growth of F. annosus e.g. on one per cent malt agar containing the solution from the middle heartwood. The rate of stimulation was not significant, and may have been induced by the nutritional properties of the solutions.

The possible harmful effect of the high temperature of autoclaving on the properties of the solutions was considered of importance. Consequently the results of the treatment were compared with those obtained from Millipore filtration, which served as another method of sterilization. The Millipore filtration served the purpose of removing microorganisms from the solutions, without serious alteration of the

chemical constituents of the filtrate. The Millipore filteres solution when added to one per cent malt agar had no inhibitory effect on the growth of the test fungi. Therefore, it was believed that the microfloras were responsible for the inhibition of the wood-decaying fungi.

Both F. annosus and P. monticola were tested on autoclaved wood and both fungi produced significantly better growth and greater weight loss in autoclaved than in untreated wood cubes. Both F. annosus and P. monticola caused more weight loss in the sapwood than in the heartwood, probably because the sapwood provided better nutrition for their growth.

It was informative that the relatively drier environments (higher agar concentration of the malt agar media and the wood blocks incubated in jars at a relative humidity of ninety-seven per cent) did not seem to affect the rate of growth of the test fungi on autoclaved material. There was no significant difference in the weight losses between the autoclaved cubes kept at one hundred per cent relative humidity and those incubated at ninety-seven per cent relative humidity. Therefore, the results suggested that the factors responsible for the inhibition were twofold, namely, the presence of microorganisms, and sufficient amount of moisture to maintain their activity.

The pathway, time of entrance of the microorganisms into the tree, their identification, the variation in their density and kind with the site and age of the tree are some questions which remain to be answered by future investigation.

CONCLUSIONS

The results of this study provide a basis for the following conclusions:

The healthy, normal wood of two living western hemlock trees sampled was colonized by microorganisms, consisting of bacteria, yeasts, moulds, and members of imperfect fungi.

It was demonstrated on malt agar media and wood, that the microorganisms had a significant role in the inhibition of F. annosus and P. monticola.

The moisture content of the environment seemed to be an important factor in the degree of inhibition. Drier environments significantly decreased the inhibiting power of the microorganisms.

Significant variation was found in the degrees of inhibition between the three sections of the stems. Greatest inhibition occurred in the butt sections, and the least in the top sections. On malt agar media the solutions of the sapwood samples provided greater inhibition than those from the heartwood. Using wood as substratum the inhibition was greater in heartwood than that in sapwood. It is believed that this may have been due to the superior nutritional properties of the sapwood. The difference in the behaviour of the test fungi on the two substrates emphasises the importance of using natural substrates in addition to artificial media to arrive at more reliable conclusions.

Autoclaving the experimental material resulted in the loss of inhibiting factor. The relatively drier environments did not affect significantly the rate of growth of F. annosus and P. monticola on

autoclaved material. Consequently, it was concluded that the occurrence of inhibition required the presence of the microorganisms and sufficient moisture to maintain their activity.

Millipore filtration of the shake solutions led to the loss of inhibiting power. This method of sterilization was considered to have little effect on the chemical constituents of the solutions, which provided further evidence in support of the hypothesis that the micro-organisms played a positive role in the inhibition of F. annosus and P. monticola.

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