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STUDIES OF VARIATION IN WOOD-DECAY ABILITY

AMONG ISOLATES OF SERPULA LACRIMANS

(WULF EX FRIES) SCHRÖT

A Thesis submitted to the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

by

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SUMMARY

1. (a) Great variations in wood-decaying ability were observed among the tested strains; some of them were significantly more active than the standard test strain, while others were significantly less active. There is close correlation between measurement of loss in dry weight and measurement of loss in breaking strength.

(b) The activity of the strains so far collected from the Clyde Valley area is generally lower than that of most of the others. The survey is being continued with a long term objective of looking for patterns in the geographical distribution of differences in wood-decaying ability.

2. (a) No correlation has been found between the differences of wooddecaying ability of a variety of dikaryotic and of monokaryotic cultures and their rate of increase of colony diameter on malt agar at 21°C.

(b) Monokaryotic cultures, all of which were isolated from basidiospores of a single fruit body showed much less variation in their rate of increase in colony diameter than that found in the dikaryotic cultures.

(c) The greater number of dikaryons showed a relatively faster growth than that demonstrated by monokaryons.

3. A close and consistent correlation has been found between wood decaying ability and the enzyme activity of 6 strains, as measured by rate of hydrolysis of sodium carboxymethyle cellulose by cell-free medium from liquid cultures. Monokaryotic cultures generally had greater enzyme activity and wood decay ability than the dikaryotic

- 4. The fungus has been shown to have a tetrapolar mating system by multiple pairing of two separate populations of monokaryons. The decay ability of the two populations has been found to range within similar limits to those of the dikaryons. The greater number of monokaryons had a higher decay ability than a dikaryotic culture isolated from tissues of the parent sporophore. The decay ability of fifty-seven dikaryons formed from monokaryons of different decay ability is consistently less than that of the most active partner.
- 5. A long term experiment carried out to examine any differences in wood-decaying ability resulting from storing the cultures on 2% malt agar and on wood pieces at each of three temperatures, has shown that at 20°C wood-decaying ability is significantly greater in cultures which have been held on wood pieces than those held on agar. The effects of storage at 15°C and at 24°C are generally similar, but the differences are not statistically significant.
- 6. We have confirmed earlier results obtained by Findlay (1932) and other workers that basidiospores germinated within 7-10 days after they were planted on 2% malt agar + 1% phosphoric acid at 21°C. The chances of germination were, however, increased by picking up the spores directly from the hymenium of fresh sporophore by use of sterile needle and spreading them on the surface of 2% malt agar medium which was sprayed by 8% phosphoric acid.
- 7. The mycelium of colonies of Strain 8 on 2% malt agar was killed by exposure to 40°C for 20 minutes. Slow growing and fast growing

sectors developed on colonies held at 40° for 15 minutes. The permanence of these changes of habit are being investigated.

Other strains reacted differently; some were killed off more quickly and some less quickly than strain 8.

- 8. In tests using five common building timbers, strains showing differences in decay ability on Scots Pine show similar differences on other common building timbers.
- 9. Experimental observations with different lengths of hyphal tips inoculae revealed that pieces which are less than 100 μ long do not survive. A fluorescent technique was used to determine the nuclear distribution within each of these apical cells. This has shown that nuclei tended to lie in the centres of the cells.

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GENERAL INTRODUCTION

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GENERAL INTRODUCTION

Serpula lacrimans (Wulf ex Fries) Schröt* is common and widely distributed throughout Britain and North of Europe, and it has also been reported as a cause of extensive damage to house timber in Russia (Rodigin, 1946; Bondartzev, 1948; and Demikhovs'ka, 1959).

The fungus generally occurs in buildings where the timbers have been allowed to remain in damp conditions. It has been found on external walls of infected houses in Britain, but only when local environment leads to high relative humidity and low temperature range (Hutchinson, personal communication).

There is also a single report of sporophores being formed on the external wall of an infected house in Norway (Danielsen, 1959).

In Britain, the fungus has not been reported in the field (Findlay, 1967), but very rare cases of its occurrence in the field have been reported in some other countries. Bagchee (1955) has reported the occurrence of the fungus on spruce logs in the temperate regions of the inner Himalayan forests, and Guzman (1963) found the fruiting bodies of the fungus together with some other wood-rotting fungi attacking the dead native trees in Mexico.

<u>S. lacrimans</u>, as far as is known, has never been reported in any tropical countries, and it is less common in U.S.A. and Mediterranean climate countries. It seems likely that this can be explained by the higher summer temperatures and perhaps also by more heating in the buildings during winter (Burt, 1917; Richards, 1933; Silverborg, 1953; and Verral, 1954).

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* In view of the extensive nomenclature of the fungus, the synopsis of information prepared by Segmüller and Walchli which appeared in the International Research Group on Wood Preservation, Document IRG/WP/108, 12 October, 1972, was discussed with Dr R. Watling, Mycologist, The Royal Botanic Garden, Edinburgh. We accepted his advice that the name shown above is the correct name of the fungus. Cartwright and Findlay (1934) were of the opinion that the sensitivity of <u>S</u>. <u>lacrimans</u> towards temperatures higher than 25°C is probably one of the reasons why the fungus is rarely found on felled timber in the field; under normal temperate summer conditions the timber may become heated by the sunlight to beyond this tolerable limit.

Occurrence of the fungus, however, fits the hypothesis that it is closely correlated with the need for abundant water, high relative humidity, and optimal growth at cool temperature range (20-22°C) (Theden, 1941; Cartwright and Findlay, 1934; and Brown <u>et al.</u>, 1968).

The economic and social importance of the decay has resulted in research being strongly concentrated towards direct field control measures. There has been little general investigation of either the differences in the degree to which separate strains of the fungus can decay wood in standard conditions or of the characters which lead to any such differences. In this thesis this property will be referred to as "decay potential". It has obvious similarity to that of virulence in pathological interactions.

Decay of wood was studied first primarily by forest pathologists interested mainly in the diseases of growing trees. The association between fungi and decay of wood was not established until the middle of the last century when Robert Hartig (1874, 1878) published the results of his research. His investigations are classic today, for he was the first investigator to diagnose properly the relationship between fungal hyphae and the decay of wood. His work covered such a wide field and was so well done that it has influenced workers in this subject to the present time (Cartwright and Findlay, 1958).

Amongst the early studies on the fungi which cause dry rot in

buildings is that of Falck (1909, 1912) who studied in some detail the various varieties of <u>Serpula lacrimans</u> and other related species. He found that <u>Merulius himantioides</u> Fr. (Syn: <u>M. Silvester</u>), which is sometimes found growing on coniferous trees and closely resembling <u>S. lacrimans</u>, could be readily distinguished from <u>S. lacrimans</u> by its ability to grow at temperatures above 30°C. His results were confirmed later by Cartwright and Findlay (1934), Cooke (1957) and Harmsen <u>et al</u>. (1958). Falck also carried out a number of physiological experiments and studied in some detail the germination of the spores of S. lacrimans.

There are several recent reviews of literature dealing with the variation in wood-decaying ability among various isolates of the same species of the most important wood rotting fungi. These reviews have indicated that considerable variation could exist within each of several species of these fungi.

Schmitz (1924) found differences in wood-decaying ability of different isolates of <u>Fomes pinicola</u> (Schw.) Karst. and concluded that there may be some physiological variation within the species, but he was not certain whether this variation was the result of host influence.

Mounce (1929) worked with the same species and concluded that the difference was the result of variation of the fungus rather than host influence. Owens (1936), Childs (1937), Hilborn (1942) and Aoshima (1954) arrived at a similar conclusion for various species of <u>Fomes</u> and <u>Polyporous</u>. Their conclusions were, however, not supported by any statistical analysis.

Relatively little rigorous work has been reported on the variation in decay abilities among various strains of <u>Serpula lacrimans</u>. Theden and Schulze (1942) showed that different strains of <u>S. lacrimans may</u> differ substantially in the rate at which they were able to decay pine sapwood. Gersonde (1958) found differences in the decay potential of

four strains of this fungus; the range of weight loss of <u>Pinus</u> sapwood in these strains varied from 20-40% over a period of sixteen . weeks.

Harmsen (1960) reported that he had found differences between the rates at which a number of different isolates of <u>S</u>. <u>lacrimans</u> and of a related species, could decay wood.

Suvorov (1970) showed that the decay potential caused by <u>S</u>. <u>lacrimans</u> was similar to that caused by <u>Coniophora cerebella</u> on different building timbers, although the growth rate of <u>C</u>. <u>cerebella</u> was considerably faster than that of <u>S</u>. <u>lacrimans</u> on malt agar medium. Similar results have also been obtained by Wazny (1963) and Rennerfelt (1963).

It is, however, important to note that the above mentioned studies include few details of the experimental design used, and no statistical analysis was done to determine the degree of significance of their results. Further, these studies do not include any analysis of the factors contributing to differences in the decay ability. No geographical survey of the distribution of different strains was performed.

Other studies related to the methods of measurement of decay, sexuality, and the relationship between enzyme activity, growth rates, and decay ability of the fungus etc. will be reviewed later on under the appropriate sections.

The present work has, therefore, been carried out on the following lines:

 Isolation and measurement of "decay potential" of different strains. The short term objective has been to obtain examples of strains with different degrees of potential for further examination. The longer term objective is to look for any

possible patterns in the geographical distribution of any differences in "potential" which may be found.

- (2) Identification of the physiological factors which contribute to any differences which are found. This comprises examination of response and tolerance to obvious physical and chemical factors in the environment, of variability of each strain in standard conditions, and of the relationship between "decay potential" and production of extra-cellular wood attacking metabolites.
- (3) Analysis of the genetic factors affecting wood decay ability and of the relationship of this to inheritance in culture and field conditions.
- (4) Some other investigations which developed from observations during this programme are also reported.

PART 1

GENERAL METHODS

GENERAL METHODS

This section of the thesis describes the methods used generally in many parts of the work. Those used specifically for particular investigations are described separately in the relevant parts.

(1) Source of Cultures

The sources of the 36 strains examined so far are given in Appendix 1. Most of them have been isolated by the author from infected houses in the Clyde Valley. Some strains from culture collections have been included for reference and comparison.

(2) Techniques used for Isolation of Strains in Culture

All isolates of the fungus made by the author were obtained from three different sources, pieces of sporophores, pieces of infected wood, and basidiospores.

(a) Pieces of sporophores

Pieces of mycelium were cut from fruit bodies under sterile conditions, and incubated in large dishes at 21°C under high relative humidity. A mass of new mycelium normally appeared on the surface within 7 to 10 days of the start of the incubations period; pieces of this mycelium were transferred to agar slants and incubated under the same conditions.

(b) Pieces of infected wood

Mycelium was obtained from wood by scraping infected samples to remove gross surface contamination, immersing the scraped samples for 30 seconds in 0.1% aqueous mercuric chloride solution, washing them several times with sterilized distilled water, and incubating them as above under high

relative humidity. In the course of one week, white mycelium bearing clamp connections grew out and pieces of this mycelium were transferred by normal sterile technique as indicated above.

(c) Basidiospore isolates

Germination and isolation of single basidiospore cultures is discussed in a separate section below.

Throughout this work, cultures obtained from sporophores or infected wood and containing clamp connections are termed "dikaryons". Cultures obtained from single basidiospores are termed "monokaryons".

(3) Culture Media, and Maintenance of Cultures

Unless otherwise stated, all cultures used for this work were grown on 2% malt extract agar. [Oxoid malt extract 20 g, Oxoid Agar 20 g, Deionized water 1000 ml. Autoclaved at 15 lb steam pressure for 20 minutes.] Stock cultures were maintained on this medium in test tube slopes. They were subcultured every three months; subcultures were incubated for ten days at 21°C, until vigorous growth was established. They were then stored at 5-10°C in a refrigerator. Inocula for experiments were prepared by subculturing stock cultures into 9 cm glass petri dishes. The dishes were incubated at 21°C for 14 days and the mycelium produced was used as inoculum for the experimental vessels.

(4) Design of method of measurement

(i) Previous work

Various methods of measuring the wood-decaying ability of fungi have been described and discussed by different investigators.

The earliest test to determine loss in mechanical properties due to decay appears to be that of Von Schrenk (1899) who measured the loss in strength of a few pieces of pecky cypress wood (<u>Taxodium distichum</u>) due to "peckiness". His tests were rather crude and the number of tests made was too small to determine whether any relation exists between weight losses and the breaking strength.

A more extensive but also rather crude investigation was reported in 1926 by Longyear, on the breaking strength of a number of woods representing different degrees of durability, after burial of the specimens for varying periods in unsterilized sand or soil in the laboratory. No clue was given as to the type of decay or the fungi that were responsible for it. His results showed that considerable loss in strength occurred before any significant loss in weight became apparent.

In other early studies, workers attempted to correlate the loss in strength with changes in the chemical and/or physical properties as decay proceeds. Cartwright <u>et al</u>. (1931) using the brown rot fungus <u>Poria monticola</u> (syn: <u>Trametes serialis</u>) to decay Sitka spruce, reported a reduction of over 15% in mechanical strength before any weight loss became apparent. They concluded that loss in strength would appear to be due to a chemical action on the cell wall substance rather than to a physical breaking down of the walls by hyphal penetration.

Liese and Stamer (1934) studied the influence of <u>S</u>. <u>lacrimans</u>, <u>Coniophora cerebella</u>, and other related fungi on the compressive strength of <u>Pinus sylvestris</u> sapwood (0.5 x 0.5 x 5 cm). From their table and graphs it is seen that <u>S</u>. <u>lacrimans</u> at all incubation periods from 1 to 6 months, caused roughly four times as much rot as calculated by both the weight and strength criteria, as its forest

relative, <u>Merulius sylvester</u>. <u>Poria vaporaria Fr. and C. cerebella</u> were intermediate in their effects. Percent of strength loss through the first three months was between 3 and 5 times the percent of weight loss. In computations based on Liese's and Stamer's table, differences in loss between duplicates expressed as percentages of the mean loss averaged 1.6 times as great for strength as for weight; this indicated that weight loss, though much less in value, had been definitely the more sensitive measure of decay in this investigation.

Armstrong (1935) investigated the effect of <u>Poria monticola</u> Murr. on the compressive and bending strength of samples of Sitka spruce. He pointed out that the reduction in impact bending strength (toughness) proceeds more rapidly than does the reduction in compressive strength.

Wazny (1958, 1959) carried out crushing and breaking tests on various timbers such as Pine (sap and hardwood), Spruce, Beech and Oak after six months exposure to <u>S. lacrimans</u> and <u>Coniophora</u> <u>cerebella</u>. He showed that impact bending strength decreased most of all, followed by bending strength, crushing strength and hardness.

Pechman and Shaile (1951) studied the influence of several brown rot fungi, including <u>S</u>. <u>lacrimans</u>, and the white rot fungus, <u>Polyporus</u> <u>versicolor</u>, on the impact bending strength of wood. They found that brown rots generally caused a greater reduction in strength of wood than did the white rots. They also concluded that the decrease in impact bending strength caused by both types of organisms was generally much more rapid than the loss in dry weight.

Similar results have also been obtained by Hinningsson (1967) who worked with various brown and white rot fungi.

Kennedy (1958) comparing the effects of brown and white rot fungi on the strength of a number of wood species, found that decay caused

by the brown rot organism, <u>Poria monticola</u> Murr., generally induced higher levels of strength loss than that caused by the white rot fungus <u>Polyporus versicolor</u>. Richards (1954), however, found no consistent difference in the ability of brown and white rotting fungi to reduce toughness at the same weight loss levels.

After considering this information and doing a little preliminary work, it was decided to start on the survey by recording loss of dry weight as one measure of chemical change, and a direct measure of change in a strength property.

(ii) Choice of the property to be measured

This was discussed in detail with the Department of Mechanical Engineering of Glasgow University. After careful reflection and consideration of the apparatus available we decided to measure the ability to cause loss of breaking strength across the grain of standard pieces of sapwood of <u>Pinus sylvestris</u>. The possibility of measuring loss of compression or tension strength was rejected as it was thought that would be more likely to be affected by minor differences in fibre arrangement etc. in the wood samples.

(iii) Choice of testing apparatus

A Hounsfield tensometer (Plate 1) was used as it was available in the Department of Mechanical Engineering. It was found to be convenient in preliminary tests, and a new one was subsequently purchased for use in the mycology laboratory.

(iv) Choice and sorting of wood samples

Pinus sylvestris sapwood was chosen as a common and conveniently



A Hounsfield Tensometer machine used for determining the breaking strength of Plate 1.

the test wood samples.

available building timber which would be likely to decay reasonably rapidly. It was appreciated that differences in the structure of the wood in the different samples would probably be responsible for a great part of the experimental errors which were to be expected. Separate preliminary investigations, reported in Appendices 2 and 3, were set up to determine the degree to which certain possible sorting by grain angle, and control of water content during the strength test, could lead to useful reduction in this error.

(v) Preparation of wood samples

Test pieces 60 mm x 10 mm x 5 mm were cut from a single batch of <u>Pinus sylvestris</u> sapwood kindly given to the Department by Robinson, Dunn & Co. in 1971. It was found that this particular size of test piece was convenient for use in the tensometer, and was a suitable size to permit five replicate pieces to be placed in each experimental bottle. Before cutting, planks were tested chemically with Benzidene to eliminate heartwood (Lominski & Hutchinson, 1949; and Phillips & Savory, 1958). This test requires two reagents, which may be prepared as follows:

(A) Benzidene 0.5 g
Conc. HCl 5.0 ml
Distilled 100 ml
H₂O

(B) Sodium Nitrite (NaNO₂) l0 g
Distilled H₂O l00 ml

Equal volumes of the two reagents are mixed just prior to testing. The mixed solution is brushed on to the end of each plank. After 3-5 min. heartwood stained with a bright red colour, while sapwood stained with yellow or

yellowish-brown colour.

Conversion was arranged so that the annual rings were approximately (± 45°) parallel to the narrow longitudinal axis; more rigorous sorting was not carried out in view of the findings of the preliminary investigation (Appendix 2).

(vi) Preparation of decay bottles

All decay experiments have been done using Roux bottles (one litre) fitted with cotton wool plugs as shown in Plate 2. Approximately 200 ml of 2% malt extract agar medium were poured into each bottle. They were autoclaved at 15 lb pressure for thirty minutes. The bottles were then removed and the medium allowed to solidify in a horizontal position. Each bottle was inoculated aseptically with discs of inoculum (approx. 10 mm in diameter) in six places with one of the isolates used in the test.

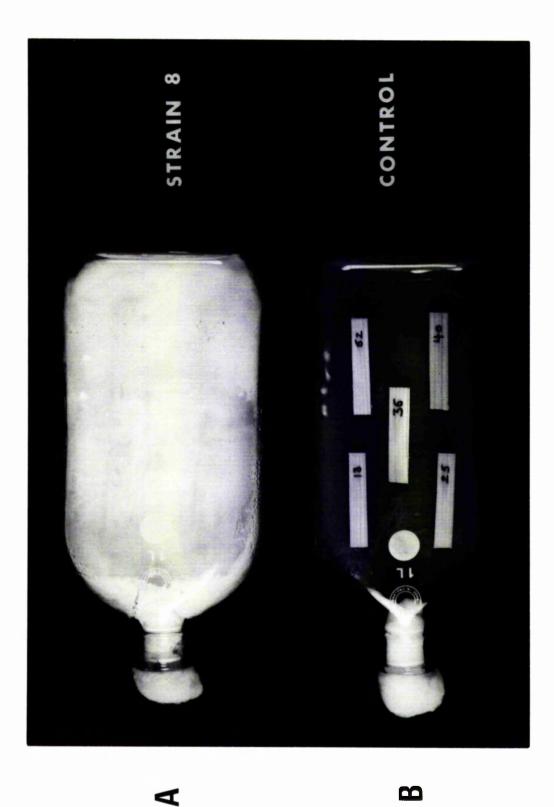
All cultures were incubated in the dark at 21°C in a thermostatically controlled chamber for two weeks, or until the mycelium had grown well over the surface of the medium.

This survey forms part of a longer term survey to be carried out in these laboratories. The various tests are set up in batches as cultures become available. To facilitate comparison of strains examined in different batches a "standard strain" (isolate 5) is included in each batch of tests.

PLATE 2

Roux bottles used as decay chambers.

- (A) Standard culture bottle at the end of incubation period.
- (B) Control bottle with five standard test pieces of wood placed in standard pattern.



PART 2

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SURVEY OF OCCURRENCE OF DIFFERENCES OF WOOD DECAY ABILITY

Materials and Methods

Appendix 4 describes a preliminary investigation to determine the amount of replication etc. which would be appropriate. From this investigation the following experimental design was adopted. Test pieces of <u>Pinus sylvestris</u> sapwood were taken at random from sorted stock and dried in an oven at 105°C for 48 hours to constant dry weights. They were then placed in desiccators at room temperature before their oven-dry weights were determined. Weights were recorded up to 0.001 gm.

Pieces were then soaked with distilled water for 15 minutes by which time their moisture contents were of the order of 25-40% of their dry weights. This was not recorded more precisely at this stage, as it was appreciated that it would change rapidly while in contact with the agar cultures during the experiment.

All pieces of wood were autoclaved at 15 lb. steam pressure for 30 minutes.

For each experiment five cultures of each test strain were set up on 2% malt extract agar, and five sterile test pieces were placed in standard pattern on the surface of each culture (see Plate 2).

After ten weeks incubation, all pieces were removed from each culture, the surface mycelium was lightly scraped off, and the pieces of wood were brought to constant oven dry weight at 105°C. Their dry weight was recorded, and their breaking strength (Newtons) was measured using a Hounsfield Tensometer. The results were compared with those of control pieces incubated in similar assemblies on uninoculated 2% malt agar.

Results and Discussion

The results of the three batches of tests are given in Appendix 5, Tables 14a, 14b and summarised in text figure 1.

The differences between the effects of the standard test strain 5 in the three batches are slight. For the purposes of this general survey this justifies the direct comparison of the activity of the other strains in the three batches without further calculation.

The results have therefore been consolidated in three sections.

(1) The general range of activity of the isolates

Figures 1 and 2 show that there is a great variation in the activity of the tested strains; 7 strains were significantly more active than the standard test strain (P = 0.05) and 4 were less active.

(2) Comparison of informativeness of measurements by loss of dry weight and by loss of breaking strength

Figure 2 shows that there is a close correlation between the two methods of measurements (rbatch 1 = -0.315; batch 2 = -0.283; batch 3 = -0.396 (P = 0.05)).

The overall pattern shows that the measurement by loss of breaking strength is less sensitive than that by loss of dry weight. Figure 2 shows that there is little appreciable difference between the results from Strain 16 to Strain 5 inclusive.

(3) Geographical distribution

The strains are grouped by origins in text figure 3. The number of strains tested is too small to justify extensive analysis. Y_{et} , the following possible patterns appear, which may be worth further

exploration as more results become available.

(a) The activity of the strains so far collected from the Clyde Valley area is generally lower than that of most of the others.

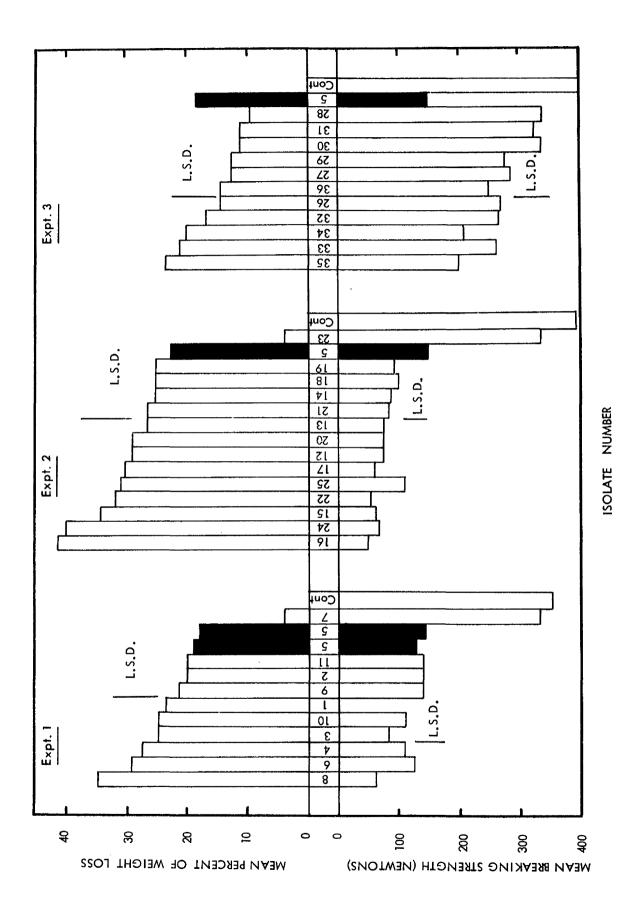
(b) The cultures obtained from the Netherlands (received from the C.B.S.), were more active than those from the Clyde and they included one of the most active strains (Strain 16).

(c) The strains which are known to have been in culture for more than 10 years are intermediate in their decay ability.

The data concerning the other strains shown in Figure 3 are insufficient to warrant further discussion.

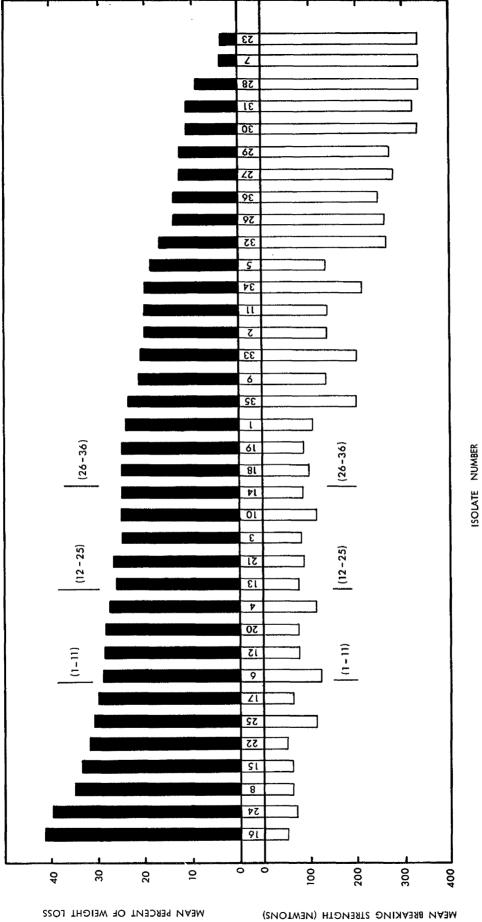
FIGURE 1

Histograms showing effects of different strains of <u>S. lacrimans</u> on wood pieces in three experiments. (Each column in these histograms represents the means of the measurements of five test pieces in each of five culture bottles.)



Histogram showing the mean percent of weight losses and reduction in breaking strength of test pieces of <u>P. sylvestris</u> sapwood decayed by 36 dikaryotic isolates of <u>S. lacrimans</u> at 10 weeks incubation. (Each column in this figure represents the mean of the measurements of five test pieces in each of five culture bottles.)

(This Figure is a summary of consolidation of results recorded in Appendix Table 14.)



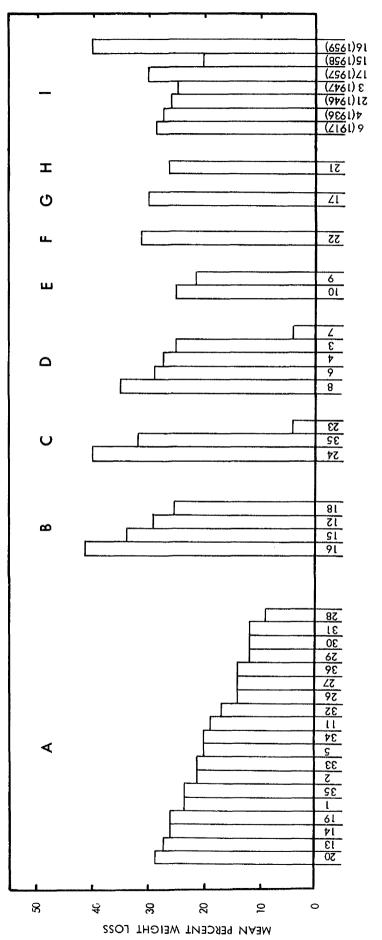
Dikaryotic cultures of <u>S</u>. <u>lacrimans</u> arranged in groups according to location from which they were isolated.

Group A = Strains collected from Clyde Valley area in Glasgow.

11	B =	11	obtained	11	Netherlands.				
11 ,	C =	11	11	11	Sweden.				
11	D =	11		. 11	F.P.R.L. (England).				
11	E =	u	n	11	Liverpool.				
п	F =	11	n	"	Cambridge.				
11	G =	Ħ	11	"	Canada.				
u	Н =	11	11	11	U.S.A.				

" I = Strains from culture collections isolated in the

year indicated in the figure.



ISOLATE NUMBER

PART 3

ANALYSIS OF THE FACTORS CONTRIBUTING TO THE DIFFERENCES IN

DECAY ABILITY

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SECTION 1

EXTRACELLULAR ENZYME ACTIVITY

Introduction and Previous Work

In the early years of the present century two types of wooddecaying fungi were distinguished, brown rots and white rots. Another type of decay caused by microfungi of the classes of Ascomycetes and Fungi Imperfecti is generally termed "soft rot". Decay of this nature usually occurs in timber immersed in sea water (Barghoorn and Linder, 1944) or in contact with soil (Savory, 1954).

The classification of fungi and the type of decay caused has been reviewed by Cartwright and Findlay (1958).

The white rot fungi, e.g. <u>Armillaria mellea</u> and <u>Fomes annosus</u>, are able to attack both cellulose and lignin, whereas the brown rot fungi, e.g. <u>Serpula lacrimans</u> and <u>Coniophora cerebella</u>, attack only cellulose leaving the lignin unattacked. Soft rot fungi are similar to brown rot fungi in that they preferentially attack the cellulose in the cell wall. Wood attacked by soft rot fungi does not undergo a rapid increase in alkaline solubility which is characteristic of brown rot fungi, and in this respect is similar to white rots (Savory and Pinion, 1958; Levy, 1964; Henningsson, 1967).

A classical test to distinguish white rot and brown rot fungi the Bavendamm reaction (1928) is based on the presence or absence of phenol oxidases. Many authors (Macdonald, 1937; Campbell, 1932; Davidson <u>et al.</u>, 1938; Law, 1950 and Nobles, 1958) have shown that the white rot fungi, when cultivated on 2% agar medium containing 0.2% tannic acid, produce a dark coloured ring around the culture, whereas brown rot fungi do not form this coloured ring.

The enzymes of wood-decaying fungi have received relatively little systematic investigation in comparison with those of many

other classes of organisms, although these fungi are principally responsible for attacking living trees and building timbers. Most of the information about the enzymes of these fungi is of a rather general nature. Relatively little is known about their chemistry or their precise mode of activity. Work in connection with their presence in wood-decaying fungi started in 1895 when Bourquelot and Herissey investigated the enzymes present in the juice of young sporophore of <u>Polyporus sulphureus</u> which attacks a number of broadleaved trees and conifers. They found evidence for the presence of the following enzymes: Emulsin, Cellulase, Maltase and Tanninase, but did not themselves consider this list to be exhaustive.

Later, other workers, such as Kohnstamm (1901) made some attempts to determine the enzymes present in <u>Serpula lacrimans</u>, <u>Armillaria mellea</u>, and <u>Polyporus squamosus</u> at different stages of growth. Kohnstamm's results were inconclusive as the technique of enzyme chemistry was not then fully developed.

In the recent years, the cellulolytic enzymes produced by certain basidiomycetes have been characterised by various workers (Sison <u>et al.</u>, 1958; Ahlgren & Eriksson, 1967; and Eriksson & Rzedowski, 1969).

Differences between cellulase activities produced by different strains of wood-decaying fungi have been studied by some other workers. Aschan and Norkrans (1953), working with various isolates of the white rot fungus <u>Collybia velutipes</u> (Curt) Fr., found that synthesized dikaryons gave a higher cellulolytic activity than either of their component monokaryons.

Cowling and Kelman (1963) stated that dikaryotic isolates of <u>Fomes annosus</u> showed relatively higher cellulase activity than the monokaryotic isolates. Bell and Burnett (1966) investigated the cellulase activity of different strains of Polyporus betulinus.

They found that the cellulase activity of these strains is not correlated with their growth rates in the presence or absence of .

The object of this study was to investigate the cellulase activity of some strains of <u>S</u>. <u>lacrimans</u> and to relate this activity to the decay ability and growth rate produced by these strains.

Materials and Methods

Four dikaryons (8, 4, 2, 7) and three monokaryons (AS9, AS15, AS7) were chosen to study the extracellular enzyme activity. These strains gave a comprehensive range of variety of decay ability.

The strains were grown in a chemically defined liquid medium; this medium was similar to that described by Pattersson <u>et al.(1963)</u>, except that 10 g/litre cellulose (Whatman cellulose powder) acted as carbon source. It contained 10 g Whatman powdered cellulose, 2.0 g $NH_4H_2PO_4$, 0.6 g KH_2PO_4 , 0.4 g K_2HPO_4 , 0.5 g $MgSO_47H_2O$, 10 mg FeCl₃, 4.4 mg $ZnSO_4^{7H}_2O$, 5 mg $MnSO_44H_2O$, 55 mg $CaCl_2$, 1.0 mg $CoCl_2.6H_2O$, 100 µg thiamine hydrochloride and distilled water to make one litre. The initial pH after autoclaving was 5.2.

Narrow mouth, 250 ml, Erlenmeyer flasks, containing 25 ml of liquid medium were used in these tests. All flasks, with their media, were autoclaved for 20 minutes at 15 lbs, steam pressure. Thiamine hydrochloride was then added to each flask. Each flask was then inoculated with three week old mycelial discs, (10 mm in diameter). Flasks were then incubated at 21°C for 30 days.

Extract of cellulase from cultures:

After the inoculated cultures had been incubated at 21°C for 30 days, the contents of the culture vessels were filtered through a filter paper. The residual cellulose powder and mycelium were washed

with a few mls of distilled water. The washings were added to the original filtrate and the whole made up to 30 ml in standard centrifuge tubes.

The culture filtrate was then centrifuged at 3000 r.p.m. for ten minutes, the supernatant decanted and its enzyme activity measured.

Before filtering, the filter papers had been oven-dried at 80°C until they reached their constant dry weight. After obtaining the mycelial mat on the filter paper, they were dried to constant weight and the dry weight of the mycelium produced by each isolate was calculated.

Enzyme assays:

Gascoigne and Gascoigne (1960) and Eriksson (1969) have reviewed the different methods used for measuring the cellulolytic activity of cellulase preparations. These assay methods have been based on the following procedures:

(a) Increase in reducing sugar value.

(b) Change in turbidity of cellulose suspensions.

(c) Decrease in viscosity of cellulose derivatives.

(d) Loss in weight of insoluble substrates.

(e) Decrease in mechanical properties of fibre and films.

(f) Measurements of clearance zones in cellulose agar.

(g) Colorimetric determination of dissolved decomposed products of cellulose.

In this work the method (a) has been used.

Measurement of increase in reducing sugars:

Cellulase activity was determined by the amount of soluble reducing sugars formed when the enzyme was incubated with carboxymethyl cellulose (CMC).

A 1 ml portion of the enzyme filtrate and 9 ml of a 1% (w/v)

aqueous solution of carboxymethyl cellulose of degree of substitution 0.52 (Gascoigne & Gascoigne, 1960, p. 108) were added to 2 ml of McLlvaine buffer (citric acid - sodium phosphate) solution at pH 5.0; the mixture was incubated in a water bath at 40°C for one hour. After incubation, a 1 ml aliquot of the assay mixture was withdrawn, 1 ml of distilled water added to it and then the solution was added to 1 ml of dinitrosalicylic acid reagent (DNS). The samples were incubated in a boiling water bath for 10 minutes followed by rapid cooling to room temperature.

A zero incubation time controls were made for every sample by adding 1 ml of reagent to 1 ml of substrate before the 1 ml of enzyme solution. The controls were immediately boiled and treated as the test samples.

The reducing sugars present in 1 ml of the enzyme incubation mixture were determined spectrophotometrically by measuring the absorbancy of the final solution at 540 mµ against a control prepared at zero time. This difference in reducing power (expressed as mg/ml glucose) at zero time and at one hour incubation gave a relative estimate of cellulase activity.

A calibration curve determined with an aqueous solution of glucose (0.05 to 1 mg per 1 ml of water) is used to convert the optical density readings into milligrams of glucose.

Results

The results are summarised in text Table 1 and shown in Figure 4. Cellulase activity and decay ability

Figure 4 shows that monokaryotic cultures with high decay ability had a relatively high enzyme activity whereas cultures with low decay ability had a relatively low enzyme activity.

A similar pattern of differences in cellulase activity was also

observed by the dikaryotic isolates.

Strain 2, which was isolated from sporophore from which the above monokaryons were isolated, showed a lower enzyme activity and a lower decay ability than any of these monokaryons.

On the basis of these results, it was suggested that monokaryotic isolates of <u>S</u>. <u>lacrimans</u> generally had a greater enzyme activity and a higher decay ability than the dikaryotic isolates.

Cellulase activity and growth rate

Growth rate as determined by (a) dry weight of mycelium after 30 days in defined liquid medium, and (b) growth represented by increase in colony diameter (mm) on 2% malt agar in petri dishes was compared with cellulase activity. No direct correlation between growth rate and cellulase activity was found, whether growth rate was measured in liquid culture during cellulase production or on malt agar.

Conclusion

From the foregoing results, it may be concluded that there is a close correlation between enzyme activity of these isolates and their decay abilities. However, cellulase activity and decay ability seemed to be independent of growth rate, both when cellulose is present in the medium or when it is absent.

There is no evidence on which to judge which difference in activity of the culture filtrates is due to differences in the amount of enzyme produced, or to differences in the activity of the enzymes due to culture conditions etc.

No. of	Incubation	Dry weight**	CX activity*	Decaj	Decay ability
isolates	· period (days)	of mycelium (mg)	mg/m1/hr.	Mean weight ~loss %	Mean breaking strength (Newtons)
AS9	30	29	0.24	42	50
AS15	11	21	0.20	34	70
AS7	II	28	0.13	17	130
ω	11	39	0.12	35	
4	II	22	0.05	27	105
7	11	36	0.04	20	140
7	1)	Т7	0.03	ю	330

Relationship between cellulase activity, dry weight of mycelium and wood decaying

Table 1.

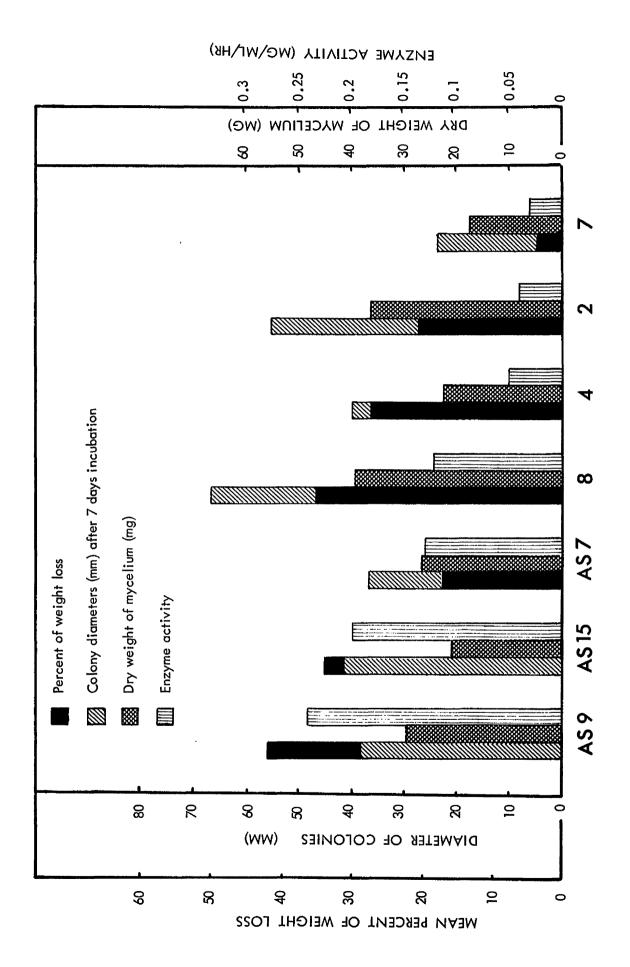
* Results expressed as reducing sugar in terms of glucose in mg/ml of mixture/hour. figure is a mean of readings of ten replicates.) .-

** Each figure is a mean of measurements of ten replicates.

For illustration see text figure 4.

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Comparison of wood decay ability with enzyme activity and increase in colony diameters (mm) of four dikaryotic (8, 4, 2, 7) and three monokaryotic (AS9, AS15, AS7) isolates of <u>S</u>. <u>lacrimans</u>.



SECTION 2

GROWTH RATE STUDIES

Introduction and Previous Work

There has been much previous work on saprophytic growth in a variety of cultural conditions. This has shown that the optimum temperature for vegetative growth of various strains of <u>S. lacrimans</u> is generally about 20-22°C. Maximum temperature for growth seemed to be about 26-28°C (Humphrey & Siggers, 1933; Jahn, 1941; Harmsen, 1960 and Langvad & Goksøyer, 1967).

Cartwright and Findlay (1934), in an extensive investigation of the effects of different temperatures on common wood-decaying fungi, found that the difference between maximum and optimum temperature was much smaller in <u>S</u>. <u>lacrimans</u> than in any other species tested. They agreed with Falck's (1909) earlier suggestion that this low temperature range of growth may distinguish <u>S</u>. <u>lacrimans</u> from the other species which they tested.

Davidson and Lombard (1953) have also pointed out that this fungus could be easily distinguished from cultures of other wood-decaying fungi by differences in growth rate and mycelial mat characteristics when grown on 2% malt agar medium.

Harmsen (1960) carried out some cultural and taxonomic studies of several species of the genus <u>Merulius</u>. He pointed out the importance of various cultural criteria (e.g. growth characters and mat colour) for separating closely related species.

It has been demonstrated by several investigators (Mounce, 1929; Herrick, 1939; Hilborn, 1942 and Hwang, 1955) that various strains of the same species of the most important wood-decaying fungi, and some other fungi, may, and often do, vary somewhat in their growth and cultural characteristics.

Variation in growth among both monokaryotic and dikaryotic isolates of these fungi have also been reported by many other investigators, such as Kaufert (1936) who reported that monokaryotic isolates of <u>Pleurotus corticatus</u> fr. grew only about half as fast as dikaryotic isolates. Verral (1936) found that dikaryotic isolates of <u>Fomes igniarius</u> (L.) Gill mostly grew faster than their monokaryotic components. Similar results have also been obtained by Hwang (1955) who worked with various strains of <u>Merulius americanus</u>.

Lindgren (1933) compared the rate of growth on agar at various temperatures of several species with the rate of decay of wood by these fungi at the same temperatures and concluded that in some fungi these might differ, but his data were not statistically analysed.

The only previous comparison of culture growth rates of different strains of <u>S</u>. <u>lacrimans</u> and decay ability seems to be the brief report by Harmsen (1960). He found that dikaryons mostly grew faster than monokaryons on malt agar, but that these growth rate differences were not correlated with differences in decay ability. He unfortunately gives little detail of this work and he does not support his deductions by statistical analysis.

Similar observations have also been found by Amburgey (1967, 1970) who worked with different isolates of <u>Lenzites trabea</u>.

This work, therefore, was carried out in order to determine whether or not growth (represented by the increase in diameter of colonies in mm) on 2% malt agar at the optimum temperature can be correlated with the "decay potential" of each isolate of <u>S. lacrimans</u>.

Materials and Methods

10 discs (10 mm in diameter) were cut from the edge of a 14 day old culture of each strain growing on 2% malt agar. One of these

discs was placed centrally on the surface of 20 ml aliquots of 2% malt agar in each of ten Petri dishes.

All cultures were incubated in the dark for seven days in a constant temperature incubator maintained at 21°C.

Two previously marked arbitrary diameters, at right angles to each other, were measured at the end of incubation period to the nearest millimetre.

The mean increase in diameter of colonies at the end of incubation period was based on the average of the ten measurements taken for each strain.

Results and Conclusion

The results are given in Appendix 5, Tables 15, 20a, and summarised in text Figure 5.

The variances between rates of increase in colony diameter were analysed and they are given in Appendix 5, Tables 15, 16 and 17. Plates 3, 4 and 5 illustrate the growth of some of these isolates on 2% malt agar medium.

Figure 5 shows clearly that the strains varied widely in their rate of growth on malt agar, as well as in their decay potential. There is no consistent relationship between the two; e.g.

Strains 8, 15 and 22 were fast growing with high decay potential. Strains 31, 28 and 23 were fast growing with low decay potential. Strains 16, and 24 were slow growing with high decay potential. Strains 21, 14 and 4 had moderate growth rate and decay potential.

Further confirmation was made by using monokaryotic isolates as shown in text figures 6 and 7. Monokaryotic isolates, collected from

each of two separate sporophores, showed a pattern of differences in growth rates and decay ability* similar to that shown by dikaryotic isolates discussed above. However, the monokaryotic isolates of each of the two sporophores showed generally less variation in the rate of increase in colony diameters than that shown by the dikaryotic isolates.

These results agreed fairly well with those obtained by Harmsen (1960) who worked with various strains of the same fungus, and Amburgey (1970) who worked with different strains of Lenzites trabea.

From the results discussed above, the following conclusions may therefore be stated:

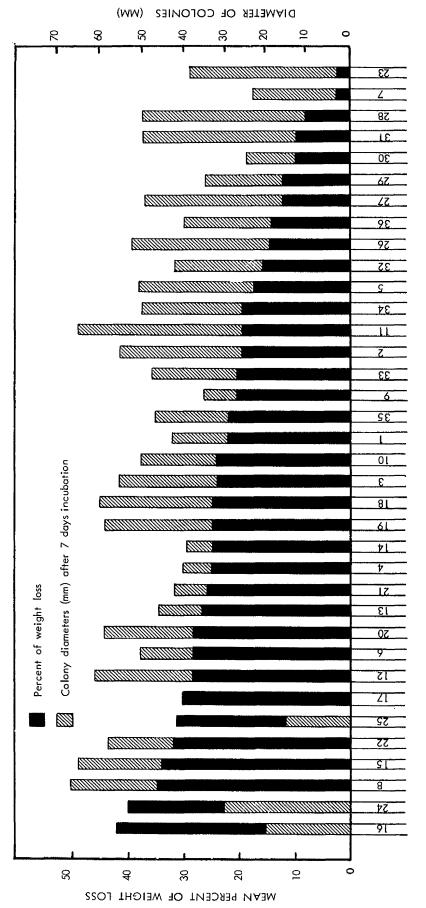
- No consistent correlation could be demonstrated between rate of increase in colony diameter on malt agar of a variety of dikaryotic and monokaryotic isolates, and their decay potential.
- (2) Monokaryotic cultures, all of which were isolated from basidiospores of a single fruit body, showed much less variation in their rate of increase of colony diameter than the dikaryotic cultures isolated either from sporophores or from decayed wood.
- (3) The growth rate of dikaryotic isolates tends to be faster than that of monokaryotic isolates.

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* Wood-decaying ability of monokaryotic cultures isolated from sporophores A and B is discussed in detail in Part 4.

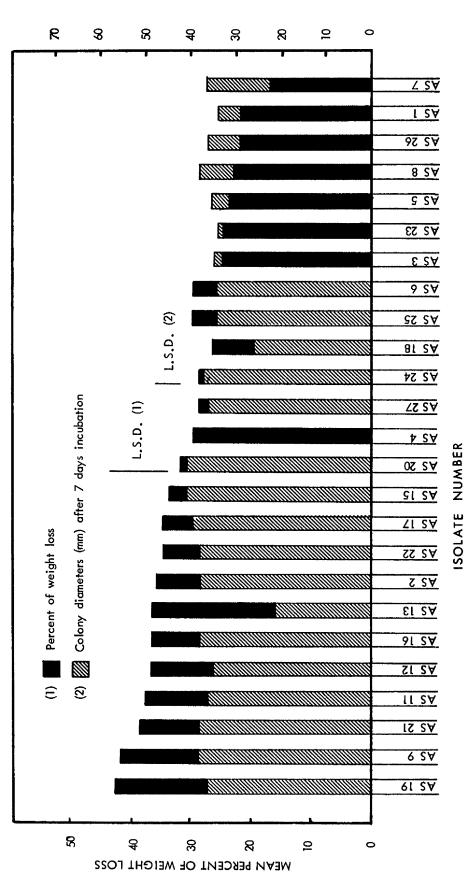
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Comparison of wood-decay ability of 36 strains with their rate of increase of colony diameters on malt agar at 21°C. Each column in this figure represents the mean of the measurements of five test pieces in each of five culture bottles, or the mean of seven days measurements of colony diameters in ten replicates taken for each strain.



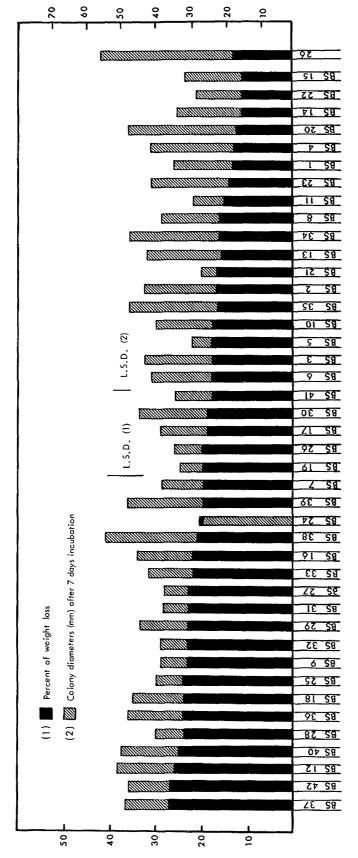


Comparison of wood-decay ability of 25 monokaryotic cultures (isolated from sporophore A) with their rate of increase of colony diameter on malt agar at 21°C. Each column in this figure represents the mean of the measurements of five test pieces in each of five culture bottles, or the mean of seven days measurements of colony diameter in ten replicates taken for each isolate.



DIAMETER OF COLONIES (MM)

Comparison of wood-decay ability of 42 monokaryotic cultures (isolated from sporophore B) with their rate of increase of colony diameter on malt agar at 21°C. Each column in this figure represents the mean of the measurements of five test pieces in each of five culture bottles, or the mean of seven days measurements of colony diameter in ten replicates taken for each isolate.





WEAN PERCENT OF WEIGHT LOSS

PLATE 3

One week old cultures of different growing isolates of S. lacrimans with different degrees of saprophytic ability. Strain 8 High in its decay ability with fast growth rate. n 11 tt 81 u n 11 ... Strain 2 Low Strain 16 High 11 11 Ħ slow Ħ 11 ... 17 'n 11 R 11 11 11 11 11 Strain 7 Low Strain 4 Moderate in its decay ability with moderate growth rate. 11 H , 17 n n 11 Strain 21 11 ĸ

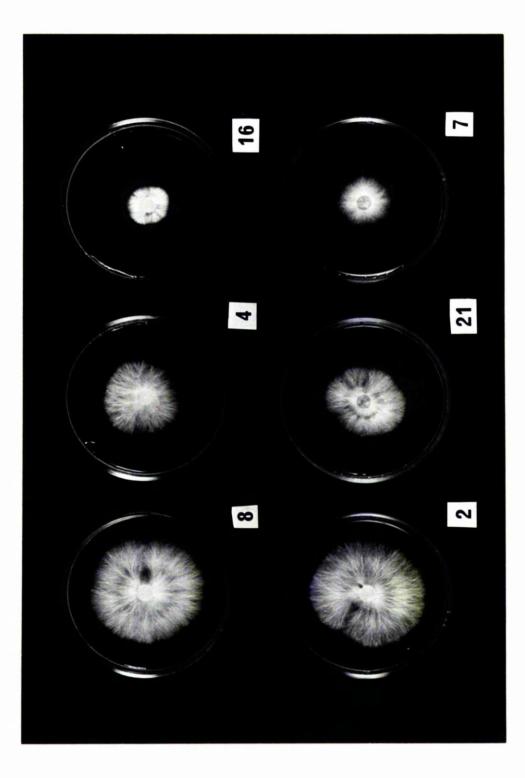


PLATE 4

One week old monosporous cultures (isolated from sporophore A) of <u>S</u>. <u>lacrimans</u> with different degrees of saprophytic ability.

AS9 High in its decay ability with fast growth rate. u ú n ... 11 AS8 Low II п AS13 High u 11 tı slow " п 11 11 11 u 11 11 IT AS7 Low 11 AS15 Moderate in its decay ability with moderate growth rate. ti 11 n n 11 11 н Ħ AS20 ŧ,

. .

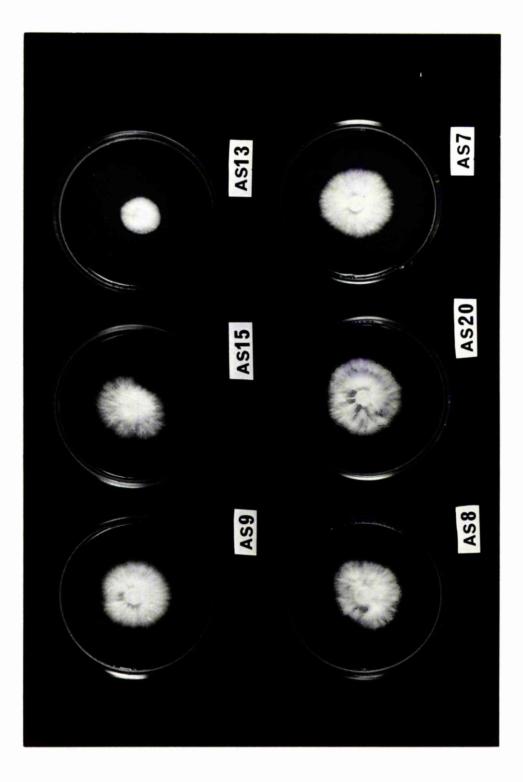


PLATE 5

One week old monosporous cultures (isolated from sporophore B)										
of <u>S. lacrimans</u> with different degrees of saprophytic ability.										
BS12	High	in	its	decay	abili	ty with	fast	growth	rate.	
						"			H .	
BS25	High	u	11	11	11	n	slow	u.		
BS15	Low	11	11	11	· 11	11	11	n	17	
BS20	Moder	cate	e in	its d	ecay al	bility	with	moderate	growth	rate.
BS40	IJ		11	11	11	n	11	tt	Ħ	

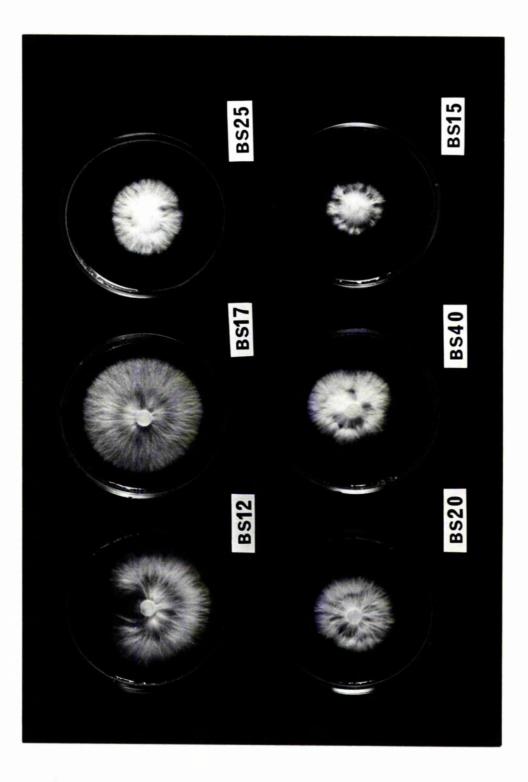
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PART 4

Genetics and inheritance of factors

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affecting wood-decay ability

GENETICS

Introduction

It has been well established by several investigators that there is a variation in the ability to cause decay of wood among various monokaryotic and dikaryotic isolates of some important wood-decaying fungi. Among these, Verral (1936) working with isolates of <u>Fomes</u> <u>igniarus</u> (L.) Gill and Kaufert (1936) working with <u>Pleurotus corticatus</u> Fr. both reported that monokaryotic isolates of these organisms decayed wood less rapidly than either of the dikaryotic cultures from which they were derived or the synthetic dikaryons formed by pairing them.

Da Costa and Kerruish (1965), indicated that monokaryotic cultures of <u>Poria vaillentii</u> (DC ex Fr.) Cke. were generally more destructive than related dikaryons, whereas dikaryotic cultures of <u>Lenzites trabea</u> (Pers.) Fr. tended to be slightly more destructive than related monokaryons.

Aoshima (1954) on the wood-decaying abilities of monokaryotic isolates of <u>Elfvingia applanata</u> (Pers.) Karst., and of the dikaryons formed by pairing them, indicate that more decay was caused by the monokaryons. He tentatively suggested that the results might be due to the denser aerial mycelium formed by the dikaryons on the wood pieces leading to a relatively poorer supply of oxygen within the pieces and hence less decay.

Similar results have also been obtained by Amburgey (1967, 1970) who worked with isolates of Lenzites trabea.

The only previous work on monokaryotic cultures of <u>S</u>. <u>lacrimans</u> which has been traced is the brief report made by Harmsen (1960). He reported that single spore cultures obtained from a single fruit body of <u>S</u>. <u>lacrimans</u> were found to have a somewhat greater decay ability and a somewhat lower growth rate than that in dikaryotic cultures isolated

from tissues of the same fruit body. His work was, however, inconclusive as he gives few details of the experimental design used, and he also gives no estimate of the degree of statistical significance of any differences in his results.

This part of the thesis is, therefore, concerned with examining the variability in wood decaying ability among different monokaryotic cultures derived from single spores from each of two sporophores, and a comparison of their ability with that of the dikaryotic cultures which were isolated from the tissues of the parent sporophores. It also reports on an examination of the mating type system, and of the relationship in decaying ability between compatible monokaryons and their synthesized dikaryons.

(1) Measurement of range of variation in decay ability

among monokaryotic isolates.

Materials and Methods

a. Isolation of single spores.

In early work, spore deposits were collected by holding each fresh sporophore, pore surface downwards, above sterile coverslips in a humid container at about 21°C for 24 hours. The whole assembly was sterilised before the sporophore was introduced.

Deposited spores were suspended in sterile 1% phosphoric acid (Findlay 1932) and 5 ml samples of the suspension were then spread on the surface of 2% malt agar medium. About 30% to 50% germination occurred during 7-10 days subsequent incubation.

In later work, spores were removed directly from the hymenium of fresh sporophores by use of a sterile needle. These spores were spread into 2% malt agar on which a few drops of sterile 8% phosphoric acid had been sprayed after autoclaving and cooling. About 50% to 70%

germination was recorded after 7-10 days full incubation.

Pieces of agar each containing single germinating basidiospores were transferred to agar slopes in test tubes and incubated in the standard conditions.

[Note: The better germination in acidic conditions is well known (Falck 1912; Findlay 1932; Girzitska 1933; Czaja and Pommer 1959). The technique by spraying the surface of agar plates permits a change of pH without affecting the setting of the agar. The possibilities that the better germination in these conditions is due to the high local acidity or to the difference in the treatment of the spores, has not been examined.]

b.Measurement of decay

A total of sixty-seven monokaryotic cultures have been obtained from two sporophores which grew in different localities in Clyde Valley.

25 monokaryotic cultures were isolated from sporophore A (from which dikaryotic strain No. 2 was isolated) and 42 monokaryotic cultures were isolated from sporophore B (from which dikaryotic strain No. 26 was isolated).

The method of determining the decay ability of these cultures and the period of incubation of this experiment were the same as described in the preceding experiments.

Results

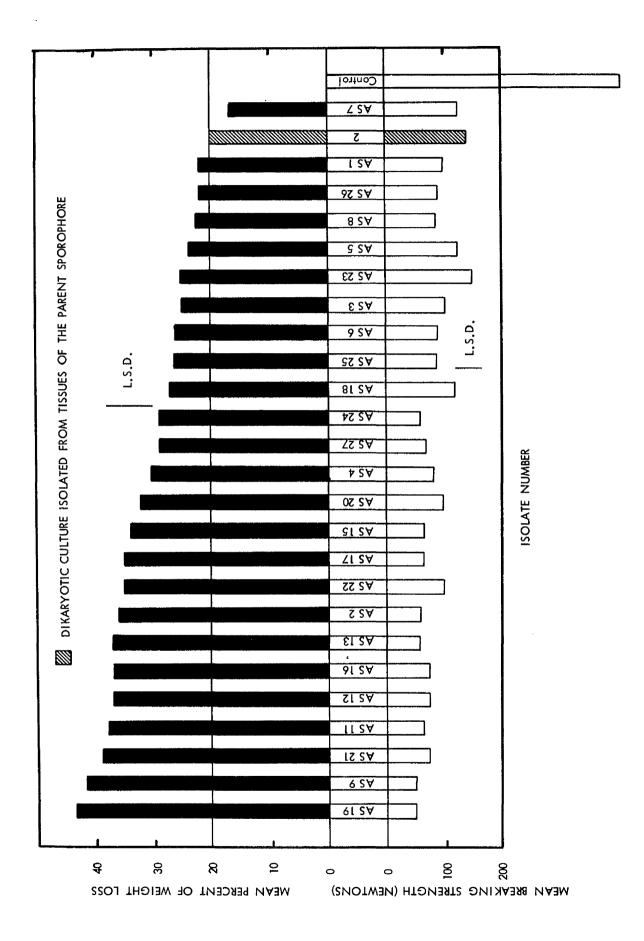
The results are recorded in Appendix Tables 18 and 19 and summarized in text figures 8 and 9.

Conclusion

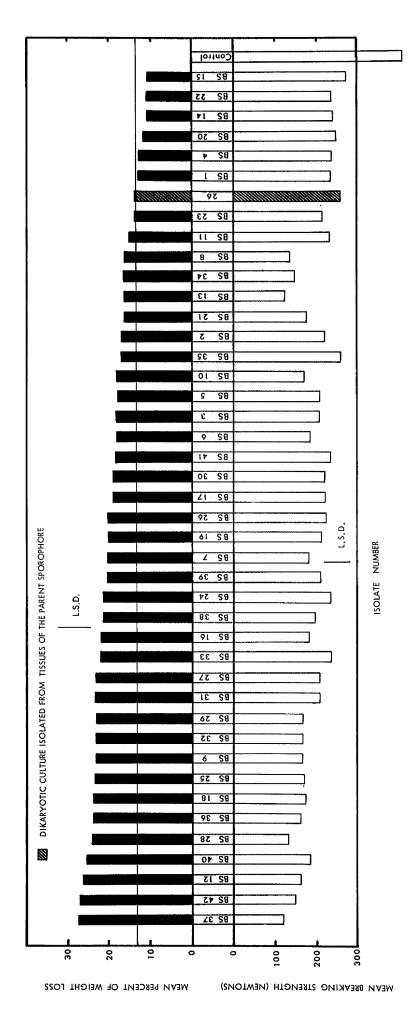
The recorded amount of decay caused by the monokaryotic cultures is regularly higher than that caused by the parent dikaryons. The differences were statistically significant (P = 0.05) for 15 of the

Histograms showing the mean percent of weight losses and reduction in breaking strength of test pieces of <u>P. sylvestris</u> sapwood decayed by 25 monokaryotic cultures (isolated from sporophore A) at 10 weeks incubation. (Each column in this Figure represents the means of the measurements of five test pieces in each of five culture bottles.)

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Histogram showing the mean percent of weight losses and reduction in breaking strength of test pieces of <u>P</u>. <u>sylvestris</u> sapwood decayed by 42 monokaryotic cultures (isolated from sporophore B) at 10 weeks incubation.



monokaryons from sporophore A, and for 15 of those from sporophore B. Only 8 monokaryotic cultures gave lower figures than the dikaryons.

The recorded amount of decay by all cultures from sporophore B was consistently less than that recorded for cultures from sporophore A. This conforms with the differences between those strains found in the general survey.

(2) Investigation of mating types

Methods

In order to determine the mating type system of isolates, monokaryotic mycelia of each sporophore were crossed in all possible combinations.

The crossings were usually made on malt agar slants in test tubes. When necessary, pairings on malt agar in petri dishes were also done. Small discs of inoculum (6 mm in diameter) taken from 2 weeks old malt agar cultures of each of two monokaryon mycelia (from the same sporophore) were placed on opposite sides of malt agar slants in a 15 cm test tube. The cultures were allowed to grow for at least two weeks, the paired and unpaired mycelia were then examined microscopically for the presence or absence of clamp connections. Those showing the presence of clamps or the compatible matings, were marked + and assumed to be dikaryons, and those showing only simple septa were marked with - and assumed to be monokaryons or incompatible matings.

Results

The results are shown in Figures 10 and 11.

Conclusion

The results of crosses of monokaryons from the two sporophores indicate clearly that all are heterothallic and possess the tetrapolar type of sexuality. This confirms earlier reports concerning the

Tetrapolar pattern of mating type obtained by rearrangement into groups of 23 monokaryotic cultures of <u>S</u>, <u>lacrimans</u> obtained from sporophore A.

11

The sign (+) indicates the presence of clamp connections.

" " (~) " " absence " "

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Tetrapolar pattern of mating type obtained by rearrangement into groups of 41 monokaryotic cultures of <u>S. lacrimans</u> obtained from sporophore B.

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The sign (+) indicates the presence of clamp connections.

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tetrapolarity of S. lacrimans (Harmsen et al. 1958 and Harmsen 1960).

(3) <u>Comparison of monokaryons and the dikaryons synthesized from them</u> Methods

In this section, a comparison was made of the wood-decaying ability of selected monokaryons from sporophores A and B and the dikaryons synthesized from them.

For each sporophore, the incompatibility reactions of its progeny were determined by mating each monokaryon to select representatives of each of the four incompatibility groups or mating types.

Five cultures of one mating type were chosen from the monokaryons from sporophore A, and mated in all possible combinations with five other cultures of compatible mating type from the same sporophore. A similar set of 6 x 6 matings were made with the monokaryons from sporophore B.

The decay abilities of the dikaryons have been tested and compared with the decay ability of their component monokaryons.

Results

Results are recorded in Appendix Tables 20, 21 and illustrated in text figures 12 and 13.

Discussion

Figure 12 shows that most of the dikaryotic cultures, obtained by pairing of compatible monokaryons from sporophore A, had a lower decay ability than either of their component monokaryons. Of the 25 dikaryotic cultures, only (AS5 x 7) and AS13 x 7) had a slightly higher amount of decay than either of their component monokaryons.

Text figure 13 summarizes the results of variation in wood-decay

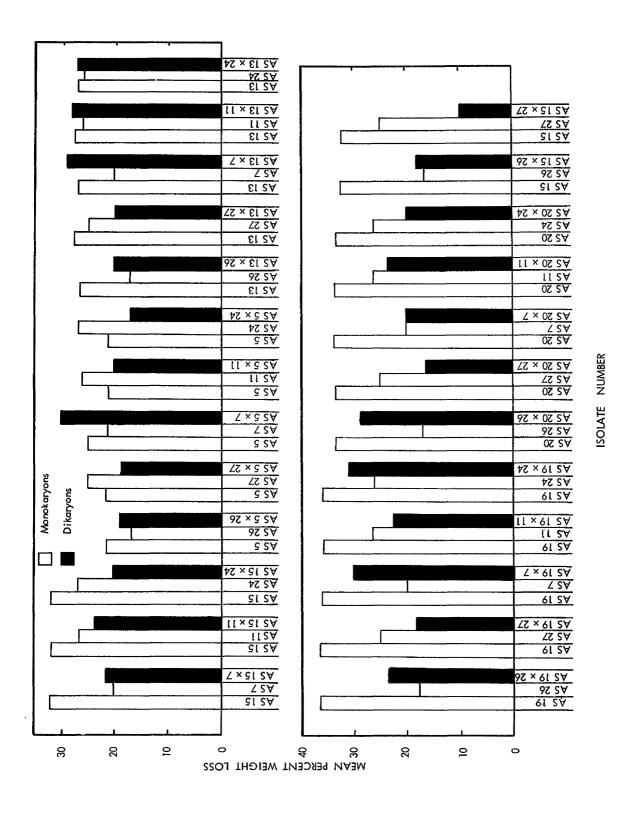
ability of dikaryotic cultures obtained by pairing of compatible monokaryons from basidiospores of sporophore B. In this experiment, similar differences in decay ability were observed. From figure 13, it can be seen that most of the dikaryons had a lower decay ability than either of their component monokaryons. In most cases monokaryons with low decay abilities produced a dikaryon with low decay ability. If a synthesized dikaryon is formed by mating a monokaryon with a low decay ability and one with an intermediate or high decay ability, the decay ability of the produced dikaryon will usually be equal to or lower than that of the monokaryon with the higher decay ability. Only two of the 36 dikaryotic cultures (BS34 x 42 and BS35 x 20), produced a slightly higher amount of decay than either of their component monokaryons.

Aoshima (1954) pointed out that dikaryotic isolates of <u>Fomes</u> <u>applanatus</u> (Pers.) Karst obtained from two monokaryotic mycelia both with high decay ability, do not always have a great ability to cause decay of wood. Similarly, dikaryotic mycelia synthesized from the monokaryotic ones, both with weak decaying ability do not always have weak ability to cause decay of wood. He concluded that the differences in ability to cause decay of wood existing among the dikaryotic mycelia cannot be explained on the basis of the decay ability of the monokaryons from which they were derived.

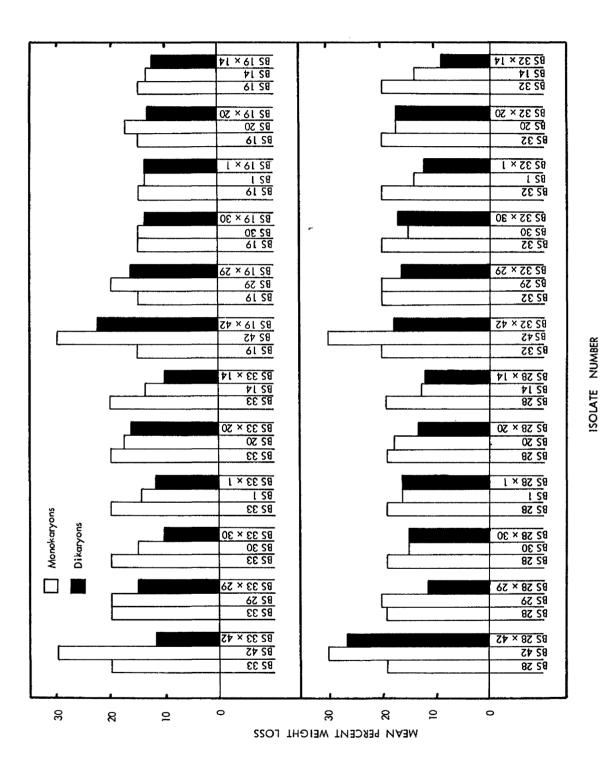
Amburgey (1967) indicated that dikaryotic isolates of <u>Lenzites</u> <u>trabea</u> (Pers) Fr. usually have a lower ability to cause decay of wood than their component monokaryons.

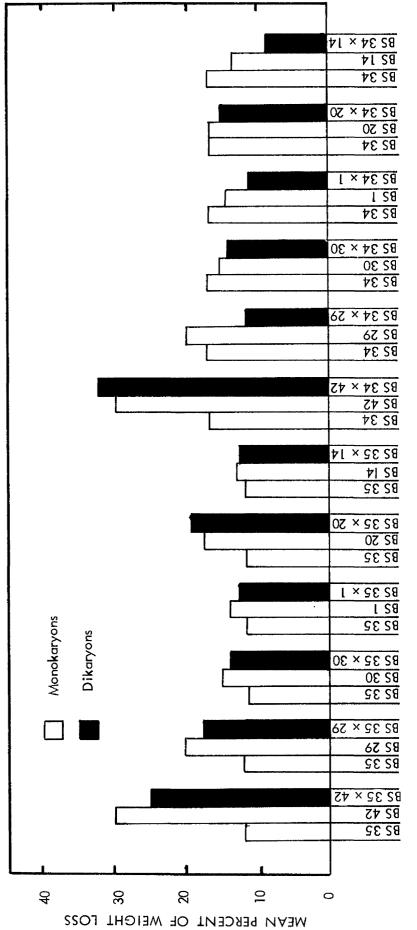
The results indicated by Amburgey are rather similar to that obtained by the present investigations in which it is indicated that the wood-decaying ability of dikaryotic isolates of <u>S</u>. <u>lacrimans</u> formed from monokaryons of different activities is consistently less than that of the most active partner, and in some cases less than the

Comparison of wood-decay ability of monokaryons (isolated from sporophore A) and their synthesized dikaryons formed by mating the monokaryons in all possible combinations.



Comparison of wood-decay ability of monokaryons (isolated from sporophore B) and their synthesized dikaryons formed by mating the monokaryons in all possible combinations.





ISOLATE NUMBER

ones with low decay ability.

(4) Genetical analysis

The design of the above experiment with monokaryons and the dikaryons synthesized therefrom was suggested by Dr C.G. Elliott with the aim of performing a genetical analysis of wood-decaying ability, using the techniques of biometrical genetics described by Mather and Jinks (1971) and Simchen and Jinks (1964).

The results of Dr Elliott's analysis may be summarized as follows:-

(a) The heritability of the character (percentage loss in weight of wood pieces) is low; only about 8% of the variation among dikaryons synthesized from sporophore A monokaryons and 14% from sporophore B is due to genetic factors, the remainder being experimental variation.

(b) In the sporophore A dikaryons, there is no evidence of dominance of one allele over the other for any of the genes segregating, or of any interaction between different genes. All gene action is additive; that is, the genetic component of the character measured is the sum of the contributions of the several genes, and the contribution of each gene is the sum of the contributions of its two alleles present.

(c) In sporophore B, there is evidence of dominance; alleles contributed by monokaryon BS42 are recessive to those contributed by the other monokaryons. Gene action in all cases excluding BS42 appears to be purely additive. It will be noted that monokaryon BS42 has a much greater decay ability than the other eleven monokaryons used. This is in line with the observation that the dikaryons generally have lower decay abilities than the average of their monokaryon 'parents' (Table 3). However, the biometrical analysis tells us about genes active in the dikaryons, and as there appears to be no evidence of dominance apart from BS42, one ought not to conclude that the lower decay ability of

Table 2.

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Upper figures: mean percent weight loss of dikaryons synthesized from monokaryons indicated in margins.

· · ·						
Monokaryons	AS5 21.92	AS13 27.48	AS15 32.72	AS20 34.00	AS19 36.12	Means
AS26	19.48*	20.12	17.64	18.84	23.76	19.97
16.92	19.42	22.20	24.82	25.46	26.52	
AS7	29.88*	29.16*	20.80	19.84	· 29.44*	25.82
20.16	21.04	23.82	26.44	27.08	28.14	
AS27	18.12	20.48	10.00	16.56	17.48	16.53
24.00	22.96	25.74	28.36	29.00	30.06	
AS11	19.76	28.36*	23.20	23.68	21.92	23.38
. 25.92	23.92	26.70	29.32	29.96	31.02	
AS24	16.92	27.20*	20.00	20.40	30.32	22.97
26.24	24.08	26.82	29.48	30.12	31.18	
Means	20.83	25.06	18.33	19.86	24.58	

Lower figureS: mean percent weight loss of the two component monokaryons (isolated from sporophore A).

* Dikaryons showed higher values than the average of their component monokaryons.

Table 3.

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Upper figureS: mean percent weight loss of dikaryons synthesized from monokaryons indicated in margins.

Lower figures: mean percent weight loss of the two component monokaryons (isolated from sporophore B).

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Monokaryons	BS14 12.64	BS1- 14.48	BS30 14.96	BS20 16.52	BS29 19.80	BS42 29.56	Means
BS35	9.16	12.28	14.04*	18.60*	18.08*	25.24*	16.23
12.28	12.46	13.38	13.62	14.40	16.04	20.92	
BS19	11.64	14.44	13.96	13.32	15.68	19.68	14.79
14.68	13.66	14.58	14.82	15.60	17.24	22.12	
BS34	7.08	11.36	14.12	15.16	10.56	31.72*	15.00
17.52	15.08		. 16.24	17.02	18.66	23.54	10000
BS32	9.12	11.40	15.88	18.48	16.72	17.52	14.85
19.32	15.98	16.90	17.14	17.92	19.56	24.44	14.03
DC 00	11 20	16 22	14 04	12.04	11 04	25.40*	15.33
BS28 19.64	11.32 16.14	16.32 17.06	14.84 17.30	13.04 18.28	11.04 19.72	25.40 [*] 24.60	12.33
							10 10
BS33 20.44	13.08 16.54	12.48 17.46	10.12 17.70	10.12 18.48	15.20 20.12	12.08 25.00	12.18
				,			
Means	10.23	13.05	13.83	14.79	14.55	21.94	

* Dikaryons showed higher values than the average of their component monokaryons.

the dikaryons is due to a recessive condition of the high decaypromoting alleles manifest in the monokaryons.

SECTION 2

. THE EFFECT OF STORAGE CONDITIONS ON WOOD

DECAYING ABILITY OF SERPULA LACRIMANS

Materials and Methods

A long term investigation has been started to investigate comparative changes in wood-decay ability of cultures stored on wood blocks and on 2% malt agar at each of three temperatures (15, 20 and 24°C).

Four dikaryotic (4, 5, 7, 8) and three monokaryotic (AS9, AS15, AS7) cultures with different degrees of saprophytic ability were chosen for this purpose.

Agar cultures were set up normally in Roux bottles. Wood block cultures were set up by inoculating the cultures in small quantities of malt agar in test tubes (20 centimetres long by 3.5 centimetres in diameter at the mouth) and placing a sterile standard piece of Scots Pine sapwood on the surface of each colony after two weeks incubation at 20°C.

One culture was set up for each of the seven strains. It was thought that replication by using of 7 strains would in these conditions be more informative than replication using one strain only.

The saprophytic ability of the cultures was tested by the standard methods after 6 and 12 months storage. At the time of testing, each malt agar culture was sub-cultured into fresh medium in similar Roux bottles. The wooden block cultures were also sub-cultured into small quantities of fresh malt agar in test tubes containing new pieces of wood.

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Results

The results after 6 months storage are given in Appendix Tables 22a, 22b and summarized in text figures 14, 15 and 16.

The results after one year storage are given in Appendix Tables 23a, 23b and summarized in text figures 14, 15 and 16.

Conclusion

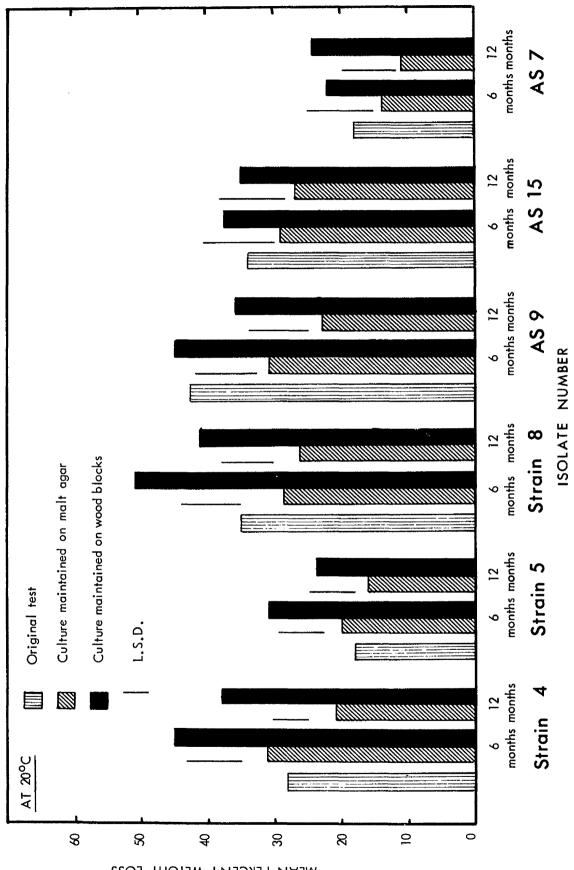
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The most interesting result from the experiment so far is that while saprophytic ability declined during storage on agar at 20°C, it increased during storage on wood. These results are consistent and statistically significant (P = 0.05).

The effects of storage at 15°C and at 24°C are generally similar, but the differences are not statistically significant.

Comparison of loss in dry weight in standard tests of cultures after storage on wood pieces and on malt agar for periods of six and twelve months. All results are means of measurements of five wooden test pieces in each of five fungal cultures.

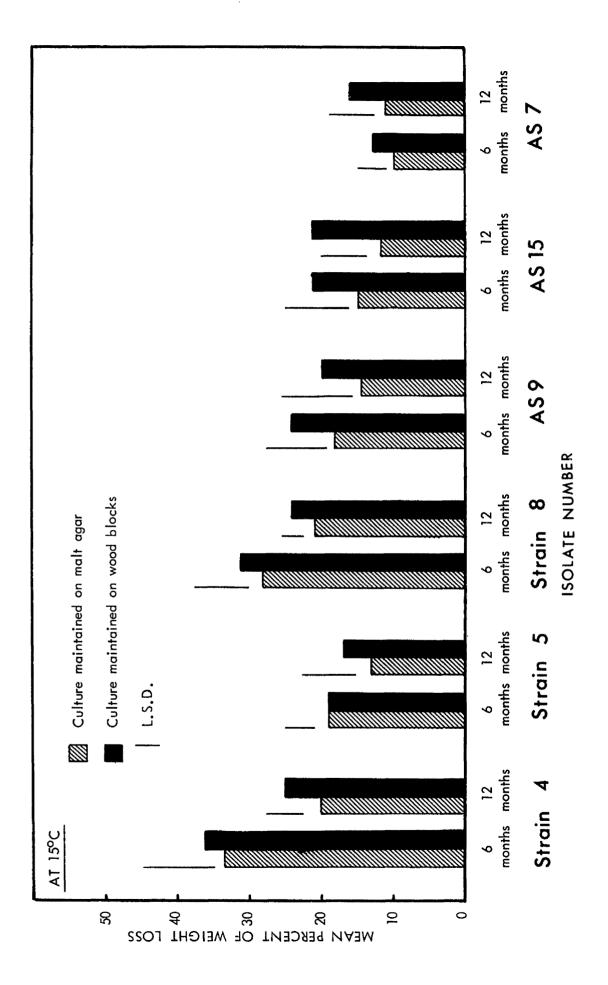
(Storage at 20°C.)



WEAN PERCENT WEIGHT LOSS

Comparison of loss in dry weight in standard tests of cultures after storage on wood pieces and on malt agar for periods of six and twelve months. All results are means of measurements of five wooden test pieces in each of five fungal cultures.

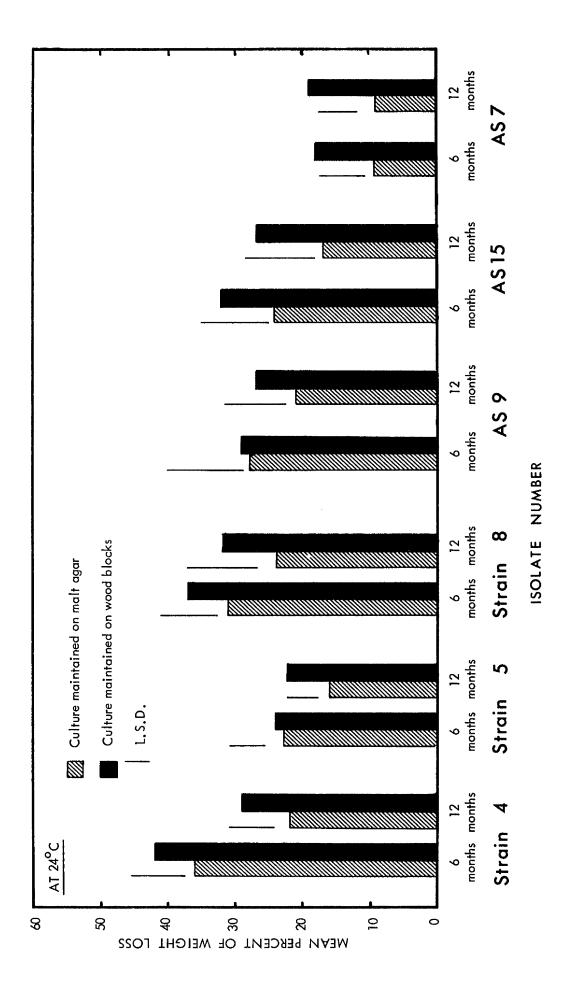
(Storage at 15°C.)



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Comparison of loss in dry weight in standard tests of cultures after storage on wood pieces and on malt agar for periods of six and twelve months. All results are means of measurements of five wooden test pieces in each of five fungal cultures.

(Storage at 24°C.)



PART 5

Effects of heat on mycelial development

and decay ability

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Introduction and Literature Review

In some field conditions it has been found necessary to attempt to eradicate <u>Serpula</u> infection in material by heat treatment <u>in situ</u>. It has been found difficult to apply this in completely rigorous conditions, and the Building Research Establishment (B.R.E.) has expressed interest in the possibility that sub-lethal exposure in parts of a site may have a significant effect on the saprophytic ability of surviving mycelia. The penetration of heat will presumably be affected by the size of the wood involved, the water content of wood, and its density (which will have been affected by the amount of decay which has gone on before treatment).

It is also common knowledge that the time required at various temperatures to kill fungi varies considerably according to the physical conditions of the medium in which the fungus is growing; e.g. the drier the medium the longer the time required. In most of the tests which have been carried out the fungi have been grown either in agar medium or in small pieces of wood containing a high moisture content and only very little information is yet available about the resistance to heat of fungal mycelium in air-dry wood. Snell (1923) subjected small test blocks of Sitka Spruce which had been decayed by five wood destroying fungi to various times and temperatures of moist and dry heat; he did not, however, include any species of Merulius in his tests. His results confirm that moist heat is much more effective in killing the fungi than dry heat, e.g., in moist heat, the most resistant fungus, Lenzites trabea, was killed in 12 hours at 55°C, but in dry heat, this species was not killed by 3 days at 70°C, although it succumbed in 12 hours at 105°C dry heat.

Liese (1931), using test tube cultures of 18 wood decaying fungi on agar, found the most sensitive was S. lacrimans which was killed by

15 min. exposure to a temperature of 40°C, while the most resistant were Lentinus squamosus, Lenzites abietina, L. sepiaria and schizophyllum, which were killed only after 30-60 min. exposure to 60°C. Montgomery (1936) reported that <u>S. lacrimans</u> growing on 2% malt agar or in small blocks of pine wood was killed after only 15 minutes exposure to a temperature of 40°C. He also noticed that inocula from the upper end of a sloped tube, where drier conditions existed, were viable after longer periods of exposure to heat than inocula from the middle of the slope where more moisture was present.

Langvad and Goksøyr (1967) found that the optimum temperature for rate of increase of colony diameters of S. lacrimans on 2% malt agar was 22°C, and that at 27°C there was slight aerial growth above the inoculum but no increasable change in diameter. At 28°C there was no visible They also found that colonies incubated for 4 hours or more growth. at 37.5° did not increase in size on subsequent incubation at 22°C; after more than 250 hours incubation at 28°C colonies increased in size on return to incubation at 22°C. They use the term "thermal death time" to refer to this temperature/time relationship needed to stop further increase in colony size. Their usage is confusing, however, as they pointed out that the respiration rate of the cells exposed to 37.5°C for 4 hours was "... not directly affected, as an appreciable respiration occurred even after 6 hours". They concluded that the "... detrimental effects of supraoptimal temperatures on this fungus include a degradation of nucleic acids and a subsequent leakage of the nucleotides out of the cells".

Subsequently, Langvad (1972) studied the effect of these treatments on the fine structures of the cells of <u>S. lacrimans</u>. His work was supported by electron micrographs. He found the following successive effects of the heat on the cells of the fungus. The first effect of heat was

observed on mitochondria, nuclei and the cytoplasm after 20 min. exposure to 37.5°C. Mitochondria seemed to be very sensitive to heat. After 20 min. exposure at 37.5°C the cristae started to break down and soon disappeared leaving double membrane vesicles. After 1 hour normal mitochondria could no longer be observed.

An early effect of high temperature was also observed on the nuclei; the nuclear membrane broke up, and the nucleoli leaked out of the cells. After 40 min. exposure, only a few compartments showed this damage but after 1 hour exposure normal nuclei could no longer be observed.

The cytoplasm assumed a granular appearance when exposed to high temperatures. Prolonged exposure at 37.5°C caused severe cytological damage. After 4 hours the hyphae were completely disorganized. All organelles and membrane systems, including the plasma membrane, were disrupted.

Langvad also noticed that when the fungus is exposed to high temperatures, some material, most probably nucleic acid degradation products, is excreted into the medium.

Langvad's work has not, however, examined the possibility that sub-lethal exposure to heat may reduce the growth rate of the surviving cells to an economically useful degree.

The Building Research Establishment (Savory, personal communication) has expressed interest in this question. It was therefore decided to investigate the following problems:-

(1) To determine the time of exposure to a range of high temperatures necessary to stop the increase of size of colonies of a representative isolate growing on 2% malt agar.

(2) To examine the nature of the effects of sub-lethal high temperature on the morphology and growth rates of the fungus, and to correlate the growth rates and decay ability of the surviving colonies with the growth

rate and decay ability of the parent culture.

(3) Continuation of the work, in a long term investigation, of determining the permanency of any effects found.

Materials and Methods

The fungus (Strain 8) was grown on 2% malt agar using 100 ml Erlenmeyer conical flasks; the flasks were first incubated at 21°C for four days after inoculation, and two marked diameters of each colony were measured.

The treatment was then applied by immersing a number of these conical cultures in thermostatically controlled water baths maintained at constant temperatures (30, 35, 38 and 40°C) for the following intervals - 10, 15, 20, 25, 30 and 35 minutes.

At each time interval, five conical flask cultures were removed, allowed to cool, and incubated further at 21°C for at least three weeks. The amount of growth was then determined by re-measuring the previously marked diameters.

The results of this test are recorded in text table 4 and illustrated in Plates 6 and 7. These results showed that the colonies did not increase in size after 20 minutes exposure to 40°C; however, 15 minutes of exposure to 40°C, or 25 minutes exposure to 38°C has had different effects on different parts of the young colonies. The mycelium at these stages was much slower in growth than the parent cultures, and it started to grow vertically from the centre of inoculum and then it spread over the surface of the medium. This change in the growth form induced by heat treatment has been studied in some detail.

The objective of this study is to find out whether or not the change in the growth form resulting from the heat treatment will be:-

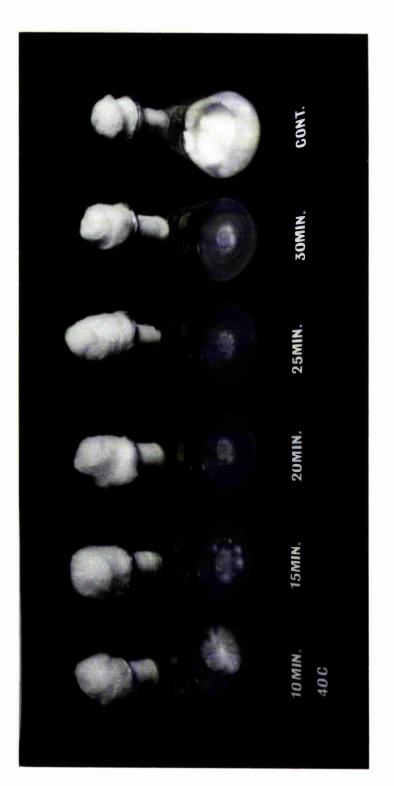
Temp. of	No. of	1	lime of	incubat	zi.on (mi	nutes)	
incubation (°C)	Flask	10	15	20	25	30	3 5 '
	1	+	+	+	· +	+	+
	2	" +	+	+	+	+	+
30	3	+	+	+	+	+	+
	4	+	+	+	+	+	+
	5	÷	+	+	÷	+	+
	1	+	+	÷	+	+	+
	2	+	÷	÷	+	+	÷
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Table 4 The effect of short period of exposures to various high temperatures upon the mycelium of <u>Serpula lacrimans</u> (Strain 8) growing on 2% malt extract agar.

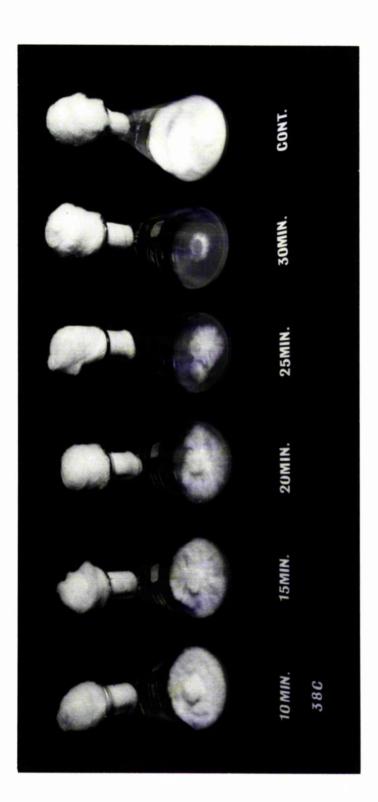
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Note. Each (+) or (-) indicates the presence or absence of fungal growth from a single flask culture.

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For more details see Appendix tables 24, 25, 26 and text figures 17 and 18. Photograph showing the effect of short period of exposures to high temperature (40°C) upon the mycelium of S. lacrimans (Strain 8) growing on 2% malt agar. Plate 6.



Photograph showing the effect of short period of exposures to high temperature (38°C) For more details see Appendix tables 24, 25, 26 and text figures 17 and 18. upon the mycelium of S. lacrimans (Strain 8) growing on 2% malt agar medium. Plate 7.

(i) Retained through successive subculturing.

(ii) Correlated with any change in decay ability.

The maintenance of the differences in growth form and decay ability after successive sub-culturing was measured by the following techniques:-

Pieces of mycelia from fast and slow growing sectors of the cultures which had been exposed to 40°C for 15 minutes, or to 38°C for 25 minutes, were transferred to 2% malt agar slopes in test tubes.

After two weeks incubation at 21°C, ten petri dishes were inoculated from each of these cultures, and incubated for seven days at the same temperature. The growth of these cultures was compared with the growth of ten controls set up from untreated culture.

Wood decaying ability of these different growing sectors and the parent culture has also been measured by the technique already described in the "general methods".

The cultures which had been subjected to high temperatures had been incubated further on malt agar at 21°C, and subcultures have been made at convenient intervals. Their increase in colony diameters and of decay ability have been tested once again after a period of six months storage.

Results and Discussion

The results of tests of cultures made within 30 days of heat treatment is shown in Appendix tables 24a, 24b, 26 and illustrated in text figure 17.

It can be seen from the results that the first transfers made from the most vigorously growing sectors of the treated culture tended to grow better than transfers of the slow growing sector. However, both of these differently affected parts were significantly slower in their

growth than the parent culture (P = 0.05).

No differences in the decay ability were found between the treated cultures and the parent control (P = 0.05).

The results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment are represented in Appendix tables 25a, 25b, 27 and illustrated in text figure 18.

In this test, the rate of increase of colony diameters of the cultures isolated from the fast growing sectors was not significantly different from that of the parent control. However, cultures isolated from slow growing sectors were just significantly lower than the parent control (P = 0.05).

No significant (P = 0.05) differences were found between the wood-decaying ability of the two growth forms (Table 5).

It must be mentioned that the results are of work on one strain only (Strain 8).

A further investigation has been started in this Department in collaboration with Mrs D. Leake. In the first experiment, the period at 40°C needed to stop all further growth of a colony has varied from 10 minutes to 20 minutes with three different strains. This work is being continued.

Comparison of variances of wood decaying ability of cultures of <u>S</u>. <u>lacrimans</u> isolated from different parts of colonies which had been subjected to high temperatures for different periods of time. **ب** Table

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Mean34.8%28.4%137 Newtons188 NewtonsRange-29.8 +30.2-21.4 +30.6-137 +263-188 +252Range-29.8 +30.2-21.4 +30.6-137 +263-188 +252Variance346.0273.618086.116612.8Variance346.0273.618086.116612.8Standard18.616.5134.5128.9Standard18.616.5134.5128.9Standard18.616.5134.5128.9Standard18.616.5134.5128.9Standard18.616.5134.5128.9Standard18.616.5134.5128.9Standard18.615.9223.822.8Standard12.412.6* $r^{12.4}$ 1.09*Stad12.412.412.41.26*		Percent of weight loss Test (1) Test (3	<i>w</i> eight loss Test (2)	Breaking Strength Test (1) Test	trength Test (2)
-29.8 +30.2 -21.4 +30.6 -137 +263 -1 ce 346.0 273.6 18086.1 rd 18.6 16.5 134.5 ion 18.6 16.5 134.5 ducial $\pm 3.29 \pm 2.92 \pm 2.92$ ± 23.8 ean $F^{124} = 1.26^*$ $F^{124} = 1.26^*$	Mean	34.8%	28.48	137 Newtons	188 Newtons
n 346.0 273.6 18086.1 n 18.6 16.5 134.5 cial ± 3.29 ± 2.92 ± 2.92 ± 23.8 n $r^{124} = 1.26^{*}$ $r^{124} = 1.0$	Range	-29.8 +30.2	-21.4 +30.6	-137 +263	-188 +252
$n = \frac{346.0}{18.6} = \frac{273.6}{16.5} = \frac{18086.1}{134.5}$ $r = \frac{134.5}{134.5} = \frac{134.5}{124} = \frac{134.5}{126*} = \frac{124}{126*} = \frac{124}{124} = \frac{1.26}{124} = \frac{1.0}{124}$				·	
18.6 16.5 134.5 ± 3.29 ± 2.92 ± 23.8 $F^{124} = 1.26^*$ $F^{124} = 1.09^{\pm}$ 124	Variance	346.0	273.6	18086.1	16612.8
± 3.29 ± 2.92 ± 23.8 F ¹²⁴ = 1.26* ± 1.09 * 124 = 1.09* 124	Standard deviation	. 18.6	16 • 5	134 . 5	128.9
$F^{124} = 1.26^{\circ}$ T^{24} T^{24} T^{24}	95% Fiducial limit of	±3.29	±2 . 92	±23.8	±22 . 8
- 1.40°	true mean		* 700 5	"124	*00
		r 124		r 124	

* Not significant at 0.05 level.

(1) Results of tests of cultures made within 30 days of heat treatment.

(2) Results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment.

(For detailed results see Appendix tables 24a, 24b, 25a, and 25b.)

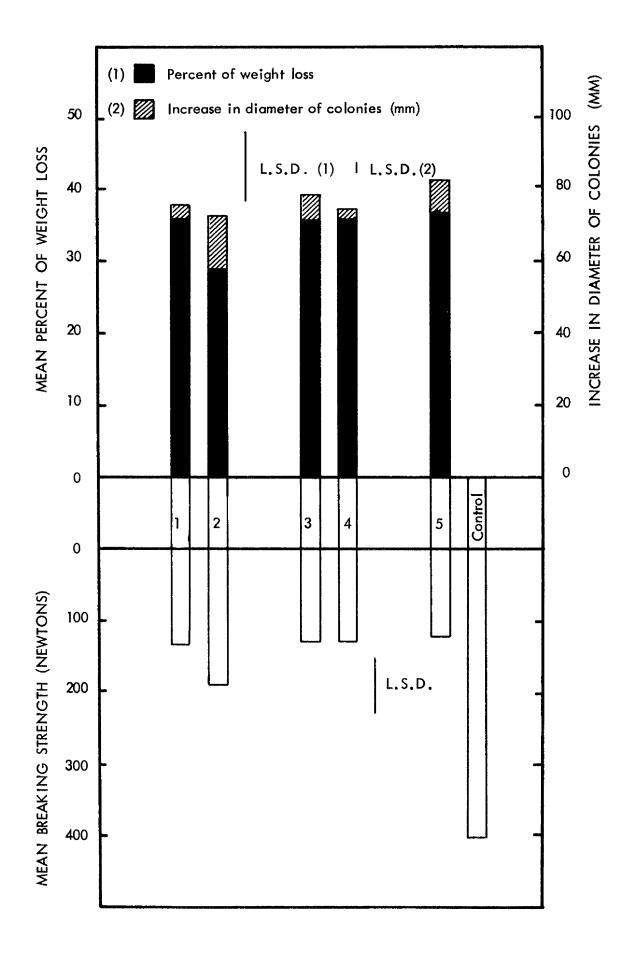
FIGURE 17

Comparison of saprophytic ability of cultures of <u>S</u>. <u>lacrimans</u> isolated from different parts of colonies which had been subjected to high temperature for different periods of time.

(1) Results of tests of cultures made within 30 days of heat treatment.

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.

Culture 5 Parent culture (Strain 8).



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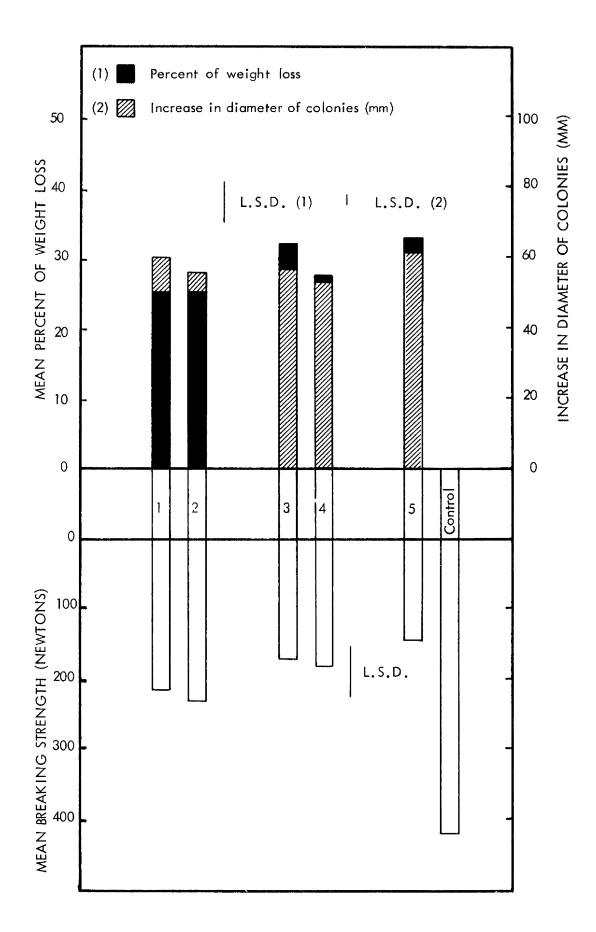
FIGURE 18

Comparison of saprophytic ability of cultures of <u>S</u>. <u>lacrimans</u> isolated from different parts of colonies which had been subjected to high temperatures for different periods of time.

(2) Results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment.

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.

Culture 5 Parent culture (Strain 8).



PART 6

Comparison of wood-decay ability of strains of

S. lacrimans on common building timbers

Introduction

It would be inappropriate here to attempt to review the whole of the work on relative durability of wood from different species of tree when exposed to fungal attack. It is common knowledge from this that woods can be graded into many rough categories of durability, e.g. softwoods are generally likely to decay faster than most hardwoods, teak and oak are generally durable, beech and birch are liable to decay quickly.

This survey of activity on wood of <u>Pinus sylvestris</u> has therefore been extended in a minor way by the examination of the effects of a few representative strains on four other timbers, e.g. Norway Spruce (<u>Picea</u> <u>excelsa</u> (Lam) Link), Sitka Spruce (<u>Picea sitchensis</u> Carr.), Beech (<u>Fagus sylvatica</u> L.) and Oak (<u>Quercus spp.</u>). These were chosen arbitrarily as a range which were conveniently available locally.

Methods

All tests were carried out with the standard methods described in Part 1. Five replicate Roux bottles each containing 5 pieces of wood were used for each treatment.

The wood used in the experiment were kindly supplied by Robinson Dunn & Co. in Glasgow.

Results

The results are recorded in Appendix 5, Tables 28a, 28b and illustrated in text Figure 19.

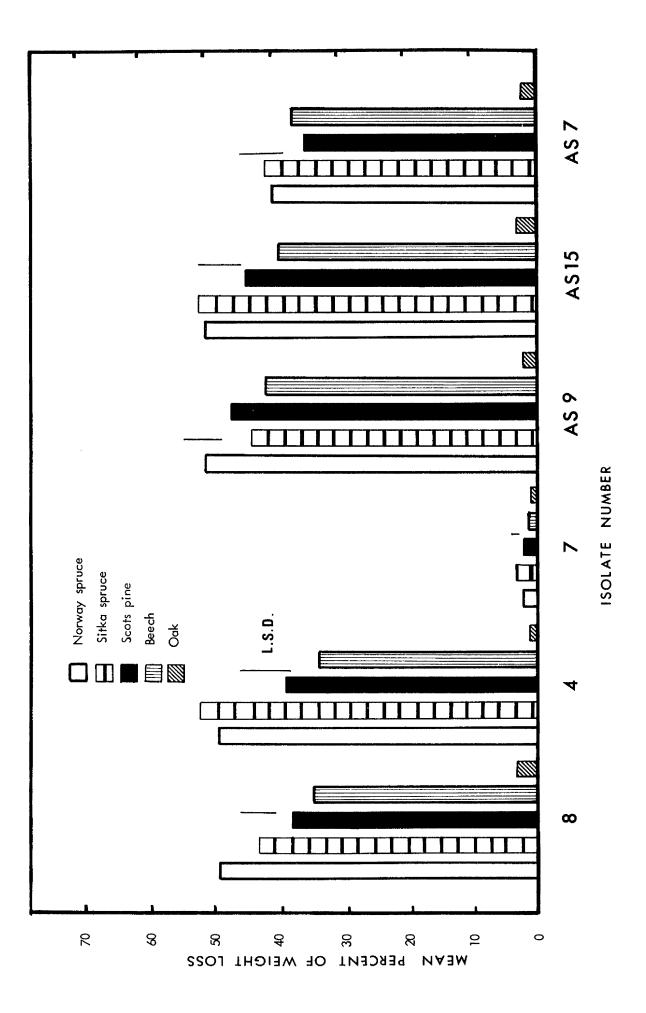
Conclusion

The patterns followed that of the survey with <u>P. sylvestris</u>. They suggest that Sitka Spruce wood might be a more sensitive "indicator" than that of <u>Pinus sylvestris</u>, but no useful, new information is likely to be obtained by using a greater range of test woods in the routine survey.

It may be that differences in relative saprophytic ability on more resistant woods might be shown if measurements were made after a much longer investigation period.

FIGURE 19

Histogram showing the comparison of wood decaying abilities of three dikaryotic (8, 4, 7) and three monokaryotic (AS9, AS15, AS7) cultures of <u>S. lacrimans</u> on five common building timbers.



PART 7

Extension to other biological studies; the effect of

mechanical damage to mycelium of the fungus

Introduction

Irregularities in the growth from different inoculae of <u>S</u>. <u>lacrimans</u> used during isolation work drew our attention to the possible relationships of inoculum size to survival of the transferred mycelium.

This work was intended as a preliminary investigation, and the main object of the work was to study the possibility of the survival of different lengths of hyphal tip inoculae after their severance from the parent hypha, and to see whether any of these fragments continued growth to form colonies.

The work has also continued to some extent to identify the nuclear arrangement within each of these hyphal apical cells. The correlation of these factors to inoculum size has been subject to further studies. Very little is known concerning these factors in S. lacrimans.

Materials and Methods

A thin layer of 2% malt agar medium was poured into a 9 cm petri dish and inoculated at the centre with a small inoculum (approx. 7 mm in diameter). All inoculum discs were cut from the margins of actively growing culture (Strain 8).

The cultures were incubated for one week at 21°C. The plates were then placed under a binocular microscope, and selected hyphal tips were cut off from the colonies by use of a glass needle on a micromanipulator. Cuts were made at different distances from the edge of the colony. Pieces of agar with the hyphal tips were then transferred to another sterilized petri dish containing a wet filter paper and incubated further at the same temperature.

Linear growth of the isolated hyphal tips was measured with a micrometer eyepiece. Measurements were taken at intervals of six and twelve hours for four days beginning at least two hours after the discs of the agar were cut out from the edge of the colony.

Cytological study

A fluorescent technique has been used to determine the nuclear distribution within each of these apical cells. This method has been used and developed by Milne (1967). The procedure employed was as follows:-

Sterilized cover slips were placed on top of the agar surface close to the edge of 5 days old colonies. The fungus was allowed to grow over the cover slip until the latter was half covered (usually takes 2-3 days). Coverslips were then removed and stained by the following technique:-

- Fix in Carnoys solution [30 ml Absolute alcohol; 5 ml Glacial acetic acid; 15 ml Chloroform] by adding a few drops onto the surface of the coverslip and allowing it to evaporate off.
- (2) Stain with acridine orange [25 mg acridine orange, N Michrom NO87, kindly supplied by Dr D.D. Clarke, per one litre of phosphate buffer, pH 7.0] for ¹₂-1 minute, the exact time varied and was determined by experiment.
- (3) Rinse in phosphate buffer for 30 seconds, mount in fresh phosphate buffer and examine immediately under blue fluorescent light. The nuclei of stained hyphal tips fluoresced yellow/green.

Results and Conclusion

The results are recorded in text tables 6, 7 and illustrated in Plate 8.

Experimental observations with different lengths of hyphal tip inoculae have shown that pieces which are more than 100 μ may be viable inoculae, but those which are less than 100 μ long do not survive.

Pieces, however, between 100 μ and 1000 μ long have shown much variation in growth rate after severance from their parent hypha.

The results also have shown that apical cells are generally 100 μ to 200 μ long, and nuclei tend to lie in the centres of the cells. These results support the hypothesis that the minimum length of hypha which is a viable inoculum is one which contains at least one complete "cell". e 6. Measurements of length (μ) of hyphae growing from hyphal tip inoculae of different lengths.

,		Length after	further	incubation	at 21°C	-
No. of hyphae	Original lengths of inoculum (µ)	Length after 24 hrs	Length after 48 hrs	Length after 60 hrs	Length after 70 hrs	Length after 84 hrs
l	1000	1300	1450	1580	1650	1820
2	840	900	1220	1440	1500	1620
3	720	950	1300	14 2 0	1440	1480
4	550	590	610	620	640	660
5	490	530	550	590	590	600
6	400	480	500	540	540	540
. 7	380	430	430	470	470	490
8	210	300	350	350	410	430
9	140	270	330	365	410	420
10	350	360	380	400	400	400
11	320	350	350	360	360	360
12	200	240	260	290	320	350
13	130	180	180	210	230	2 60
.14	240	240	260	260	260	260
15	180	180	180	190	190	190
16	60	80	90	90	90	90
17	50	70	70	90	90	90
18	80	80	80	80	80	80
19	70	70	70	70	70	70
20	60	60	60	60	60	60

<u>Table 6</u>.

•

No. of apical cell	Length of the apical cell (µ)	Distance between first nucleus and tip of the apical cell (µ)	Distance between second nucleus and tip of the apical cell (µ)
1	97	50	67
2	111	45	62
3	149	60	82
4	159	57	97
5	108	48	68
. 6	130	50	70
7	165	97	108
8	149	69	100
9	124	52	76
10	100	47	57 [′]
11	119	41	65
12	119	63	81
13	116	49	68
14	154	. 87	105
15	113	54	65
16	108	64	84
17	100	55	78
18	135	76	92
19	95	35	51
20	97	54	70

Table 7. Measurements of the length of apical cells and relative position of nuclei of different hyphal tips.

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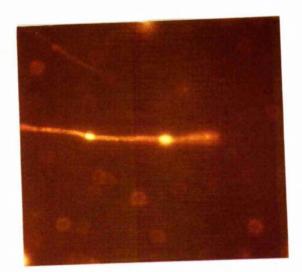
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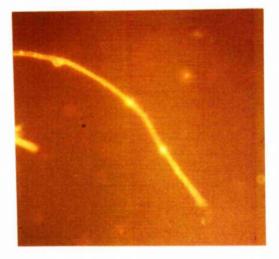
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"Studies of variation in wood-decay ability among isolates of Serpula Lecninans (Wulf ex Fries) Schr**o**t." Lacrumans

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UNIVERSITY OF GLASGOW





10µ

Plate 8. Photographs showing the nuclei of different hyphal tips stained with acridine orange and viewed in blue fluorescent light.

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PART 8

CONCLUSIONS

The aim of the work described in this thesis was to investigate the variation in wood-decaying ability of isolates of <u>S</u>. <u>lacrimans</u>, and to study the factors contributing to any differences found. The following main conclusions may be stated:

- (1) There is a substantial difference in saprophytic ability among various strains of the fungus. This may be an important factor which could influence the degree of risk which might be taken in remedial work.
- (2) No consistent relationship could be demonstrated between the rate of increase of colony diameter of monokaryotic and dikaryotic isolates and their ability to cause decay of wood. A close and consistent relationship was, however, observed between saprophytic ability and enzyme activity of 6 strains of the fungus.
- (3) It is interesting to note that the monokaryotic cultures generally had a greater ability to cause decay than the dikaryotic cultures.

This was so for dikaryotic cultures taken from sporophores tissue compared with monokaryons made from single spores isolated from these sporophores, and to dikaryons established artificially by pairing monokaryons of known activity.

Formal genetical analysis showed that this may be controlled by different systems in different populations.

These findings may provide useful information regarding fungus control in the field. They have also proved interesting to geneticists

who are developing them further.

It is possible to expand this work so as to serve the longterm objective of looking for patterns of the geographical distribution of differences in wood-decaying ability. It can also be extended to cover the study of variation in other characteristics affecting field decay, e.g., possible differences in ability to transfer water along hyphae in relation to different external humidity gradients. .

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BIBLIOGRAPHY

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APPENDICES

APPENDIX 1

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Cultural data and source of isolates of Serpula lacrimans used

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in the present investigation.

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Isolate No.	Collection Source	Locality	Date of Collection
1	Isolated from diseased wood from Bute Hydro Hotel, Rothesay. (Glasgow University Collection)	Rothesay	Established 17.4.71.
2	Isolated from Sporophore from Lily- bank Gardens, Glasgow W2.	Glasgow	Established 19.10.71.
3	Isolated from Sporophore from house at Hampton Hill; received from Forest Products Research Laboratory, England. Culture No. 12C.	Middlesex	Established 1947 and received 25.10.71.
4	BAM (Ebw) No. 315. Received from Eberswalde 1936 as stamn Urdingen Ebw 315. Cen test stain 1971 received from FPRL, England. Culture No. 12D.	Eberswalde	Established 1936 and received 25.10.71.
· 5	Isolated from Sporophore collected from house at Dowanside Road, Glasgow W2.	Glasgow	Established 29.9.71.
6	Origin not known, but believed to have been isolated circa 1917. Received from FPRL, England. Culture No. 12.		Established 1917 and received 25.9.71.
7	From Uerdingen as <u>M</u> . <u>domesticus</u> . Received from FPRL, England. No. 12B.	Uerdingen	Received 25.9.71.
8	Received from Forest Products Research Laboratories, England. No. 169A.		Received 25,9,71
. 9	Isolated from aerial mycelium coll- ected from the cellar of a house in Mill Lane, Liverpool. University Collection. LU6.	Liverpool	Established Dec. 1971 and received 4.2.72.
10	Germinated from basidiospores coll- ected from a sporophore produced on acidity malt agar, Liverpool Univ- sity Collection. LU7.	Liverpool	Established April 1971 and received 4.2.72.
11	Germinated from basidiospores coll- ected from a sporophore from Lily- bank Gardens, Glasgow W2.	Glasgow	Established 10.1.72.

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Isolate No.	Collection Source	Locality	Date of Collection
12	Obtained from Centraal Bureau Voor Schimmelcultures, Baarn, Nether- lands. Culture No. 217.29.	Netherlands	Received April 1972.
, 13	Isolated from Sporophore collected from a house at Deanston Drive, Glasgow.	Glasgow	Established 28.4.72.
14	Isolated from infected wood from a house at West Princes Street, Glasgow, W2.	Glasgow	Established 9.5.72.
15	Polysporous culture isolated from building timber in a house in the Hague. Received from Plant Res- earch Institute, Dept. of Agric- ulture, Mycology Section, Canada. Schure No. 11.	Hague Netherlands	Established Nov., 1958. Received 6.6.72.
16	Culture from Sporophore collected near Leiden in Oegstgeest, Neth- erlands, and received from Plant Research Institute, Canada. Schure No. 15.	Oegstgeast Netherlands	Established 29.4.59 and received 6.6.72.
17	Tissue culture from Sporophore on <u>Picea glauca</u> board covering a wall in a basement of house in Ottawa. Obtained from Plant Research Inst- itute, Canada. Culture No. 5435.	Ottawa	Established 14.5.57. Received 6.6.72.
18	Obtained from Centraalbureau, Baarn, Netherlands. Culture No. CO5-235-33.	Netherlands	Received 6.6.72.
19	Isolated from Sporophore collected from house at Newton Place, Glasgow	-	Established 10.8.72.
20	Germinated from basidiospores collected from a sporophore from a house at Hillhead St., Glasgow W2	Glasgow	Established 10.10.72.
21	Isolated from rot in a board of "buckeye" in a garage with assoc- iated sporophore. Received from Forest Products Laboratory, Madison, Wisconsin, U.S.A.	North Carolina U.S.A.	Established Oct., 1946. Received 22.12.72.
22	Received from Department of Botany, University of Cambridge.	Cambridge	Received 25.11.72.
23) 24) 25)	Received from Department of Forest Products, Royal College of Forestry Stockholm, Sweden. Culture Nos. AlO2, Alo9 and A332BAM respectively	,	Received 11.12.72.
26	Isolated from Sporophore collected from Garscube, Glasgow N.W.	Glasgow	Established 20.7.73.

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Isolate No.	Collection Source	Locality	Date of Collection
27	Isolated from sporophore collected from a church at Bromm Hill, Glasgow Gll.	Glasgow	Established 14.8.73.
28	Isolated from decayed wood coll- ected from old building at Park Circus, Glasgow C3.	Glasgow	Established 6.12.73.
29	Isolated from sporophore collected from Garscube, Glasgow, NW.	Glasgow	Established 13.12.73.
30	Isolated from basidiospores coll- ected from sporophore from old building at Park Circus, Glasgow, C3.	Glasgow	Established 10.1.74.
31	Isolated from infected wood from a house at Dumbarton Road, Glasgow, W4.	Glasgow	Established 12.1.74.
32	Isolated from sporophore collected from an infected house at Dumbarton Road, Old Kilpatrick, Glasgow.	Glasgow	Established 7.3.74.
33	Isolated from sporophore collected from a house at University Gardens, Glasgow, W2.	Glasgow	Established 28.3.74.
34	Isolated from sporophore collected from an infected house at Park Terrace, Glasgow, C3.	Glasgow	Established 1.4.74.
35	Isolated from decayed wood collected from Shawlands area, Glasgow, W.	Glasgow	Established 2.4.74.
36	Isolated from decayed wood collected from an infected house in Southpark Avenue, Glasgow, C3.	Glasgow	Established 10.4.74.

APPENDIX 2

The Relationship between Breaking Strength of Pinus sylvestris Sap Wood and the Orientation of Grain and Breaking Force.

The work in this Appendix has been done under the supervision of Dr S.A. Hutchinson; Mrs D. L. Leake assisted in the technical aspects of the project.

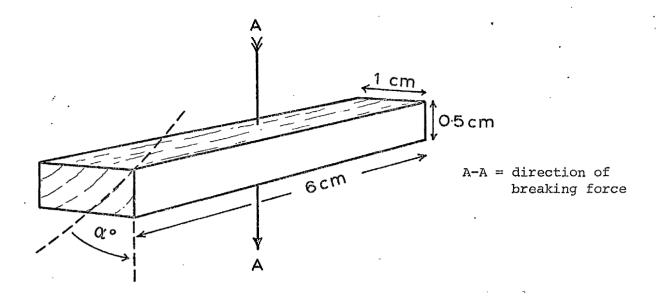
Introduction

Selection of timber to fine limits of uniformity is expensive and time consuming. In particular, selection for uniformity of grain orientation involves wasteful sawing angles which can only be set up on elaborate saw benches. This investigation examines the value of such a selection in relation to a current survey of decay potential of Serpula lacrimans; its findings may be useful in other work.

Materials and Methods

A plank of sap wood of <u>Pinus sylvestris</u> was sawn to yield strips 90 cm x 1 cm x 0.5 cm, each with longitudinal grain in which the annual rings lay at an angle α between 0° and 45° to the narrow long edge. Three of these strips were chosen in which angle $\alpha = 0^{\circ}$, and three were chosen in which it lay between 10° and 45°. Each of the six strips was then sawn transversely to yield fourteen 6 cm x 1 cm x 0.5 cm pieces. The pieces were dried for 12 hrs at 100°C; they were then weighed, angle α for each block was measured, and their breaking strength across the narrow long axis (A-A Fig. 1) was measured with a Hounsfield Tensometer.

In a confirmatory test six similar strips were cut from a plank of sap wood of <u>Pinus sylvestris</u> from a different source, three with angle $\alpha = 0^{\circ}$, three with angle α between 10° and 45°. Two similar pieces were cut from each end and two were cut from the middle of each strip. They were dried for 12 hrs at 100°C, and one of each pair of pieces was then immersed in deionised water for ten minutes. Angle α was measured as above.





Dimensions and orientation of test pieces

Results

The results are recorded in Figures 2 and 3, and summarised and analysed in Tables 1 and 2.

Discussions and Conclusions

In the principal experiment the mean breaking strength of population ABC (α between 10° and 45°) is significantly lower than that of population DEF ($\alpha = 0^{\circ}$) [t = 6.5], but the difference between the variances of the two is not significant [$F_{41}^{41} = 1.2$]. The varienace of the total population ABCDEF (α between 0° and 45°) is just significantly bigger than that of the selected population DEF [$F_{41}^{83} = 1.8$]. This is obviously the result of combining two populations with significantly different means, though the variance of each is similar.

Empirical judgement of Table 1 and Figure 3 shows that there is no significant difference between the variances of the total population ABCDEF and that in which α lies between 0° and 34° (ACDEF). There is a significant difference between the variance of the total population ABCDEF and that in which α lies between 0° and 26° (CDEF) [F⁸³₅₅ = 1.65]. The further sorting of the population for $\alpha = 0°$ (DEF) obviously has no significant additional effect on variance. The results of the confirmatory

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experiment (Table 2) agree generally with these, and they give no evidence to suggest that variance in wet samples is less than that in dry ones.

The relevance of these results to the design of decay tests for wood rotting fungi depends on the possibility of grain angle having other unmeasured effects on decay rates, and on the fineness of the measurements The first possibility seems unlikely in these conditions. required. The sorting of the pieces for a population with α between 0° and 26° recorded above resulted in a reduction of the 95% Fiducial Limit from ± In current tests with Serpula lacrimans 123 Newtons to ± 96 Newtons. differences of the order of 30 to 300 Newtons have been recorded; if the possibility of other effects can be discounted this improvement in sensitivity by ± 30 Newtons does not appear to justify the waste of material and cost of labour involved in this selection. Some useful ad hoc selection might be done if finer judgement of virulence is required, but the relationship of breaking strength to density suggested by Figure 2 indicates that piece weight might be a more useful first criterion. Density is also more likely to have a direct effect on rate of penetration by fungal hyphae, which might be a significant variable in tests.

The variance between strips is significantly greater than the variance between pieces in these tests. This is more likely to be a reflection of the large number of replicate pieces and the small number of strips than a measure of a real pattern of difference in the plank. Analysis of Breaking Strength Measurements of a Population of Pieces Table 1.

958 Fiducial Limits	±	+ 93	± 123	+ 96	± 128	
d Newtons	44	47	63	49	65	
Mean Breaking Strength	350	440	390	430	410	
Range of angle α	10° to 45°	0°	0° to 45°	0° to 26°	0° to 34°	
No. of pieces in samples	42	42	84	56	70	
Groups of strips from which pieces were cut	ABC	DEF	ABCDEF	CDEF	ACDEF	

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95% Fiducial Limit	1+ 1+	1 7 ±	± 73
d Newtons	32	40	37
Mean Breaking Strength	360	390	380
No. of pieces in sample	18	18	36
95% Fiducial Limit	+ 51	± 101	+ 82
α Newtons	32	51	42
Mean Breaking Strength	270	290	280
No. of pieces in sample	18	18	36
Range of angle a	10° to 45°	°O	0° to 45°
Groups of strips from which pieces were cut	GHI	JKL	GHIJKL
	Range of angle aNo. of piecesMean Breaking00f FiducialMean pieces0angle apiecesBreakingFiducialpiecesBreaking in0inStrengthNewtonsLimitinStrengthNewtonssamplesamplesamplesamplesamplesample	Range of angle aNo. of piecesMean BreakingO. of FiducialMo. of piecesMean Breaking in StrengthNo. of mean in StrengthMean Mean and in StrengthMean Mean and10° to 45°1827032± 511836032	Range of angle aNo. of piecesMean Breaking BreakingNo. of FiducialMean piecesOangle apiecesBreaking in StrengthNo. of in in StrengthMean in StrengthO10° to 45°1827032± 5118360320°1829051± 1011839040

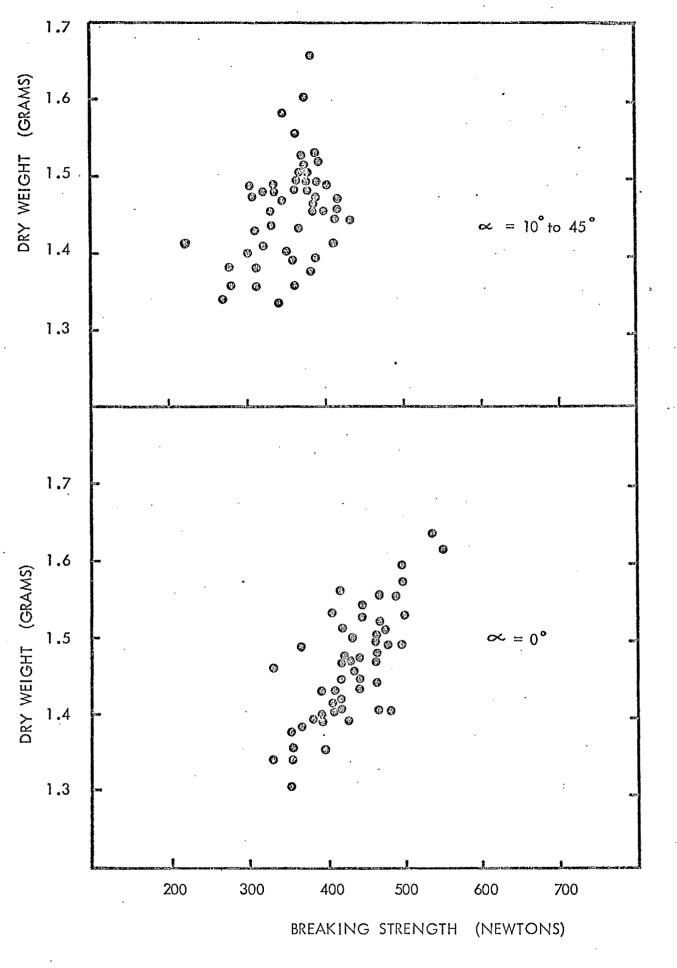
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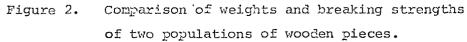
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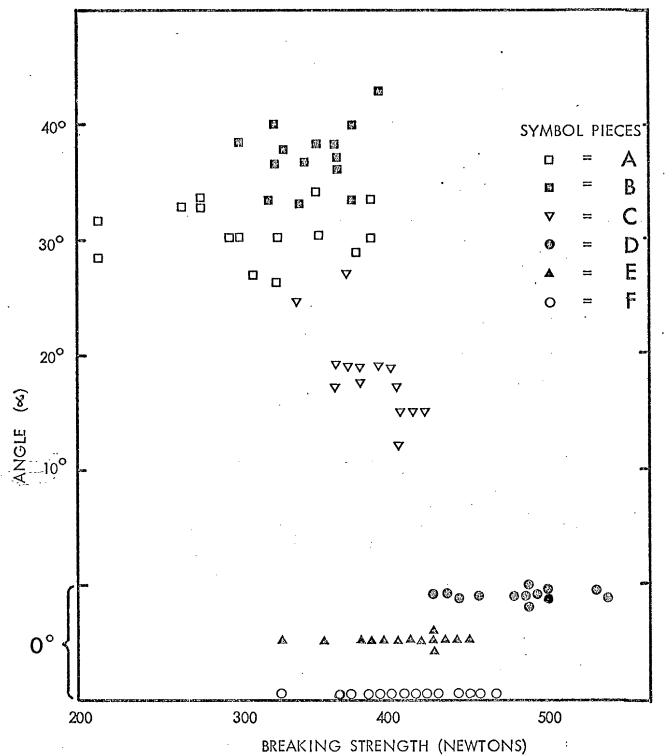
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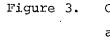
Analysis of Breaking Strength Measurements and Comparison of Variability of Measurements of Populations of Wet and of Dry Pieces

Table 2.









Comparison of breaking strength of wooden pieces and orientation of annual rings to the direction of breaking force.

APPENDIX 3

The effect of drying a sample of sapwood of Pinus sylvestris on the consistency of measurements of its cross-grain breaking strength

100 test pieces each 60 mm x 10 mm x 5 mm, cut from a single plank of even grained <u>P. sylvestris</u> showing approximately 4 rings per cm. The cuts were made so that the rings were approximately parallel to the narrow longitudinal axis. The pieces were dried at 110°C to constant weight. Half the pieces, chosen at random, were then immersed in tap water in a sealed container. The atmospheric pressure in the container was reduced to a few mm of Hg for 10 minutes, then allowed to return to normal. This process was repeated once. The pieces were then removed from the water and superficial water was shaken off. The breaking strength across the grain radial to the tree was then measured by a three point bending attachment on a Hounsfield Tensometer (of. Fig. 4).

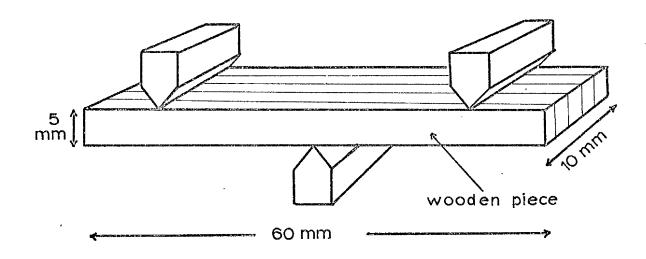


Fig. 4. Diagram of standard wooden pieces in 3-point bending machine.

The tests were carried out in a continuous series at uncontrolled laboratory temperature and humidity, and they took two hours to complete. The results are shown in Fig. 5 and Tables 3 and 4.

Table 3.	Comparison of	range of measureme	nts of breaking strength
	of wet pieces	and dry pieces of	P. sylvestris sapwood
•			
		Wet Pieces	Dry Pieces
Mean break	ing strength	223 Newtons	393 Newtons
Range		-93 +127 Newtons	-103 +157 Newtons
Variance		2121	3055
Standard d	eviation	46.1	55.2
95% Fiduci of true		± 13.2 Newtons	± 15.8 Newtons
	$F_{49}^{49} = 1.4$	< 0.05 significar	ace

Discussion

The mean breaking strength is clearly significantly different in the two populations without mathematic analyses, but the difference in the variance of the two populations is not significant in this test. The shape of Figure 5 suggests that greater consistency might well be found in the "wet" groups if more individuals were measured. The additional information is unlikely to justify the effort involved in many investigations, however; in the current survey useful information has been obtained from comparison of 25 individuals per treatment, in which the effect of wetness or dryness at testing would be less likely to be significant. It is therefore concluded that for this work the choice may be determined by convenience of other factors; e.g. it seems likely that errors due to crushing at the three suspension points in the bending test will be increased with wood softened by fungal attack, and that this would be minimised by measuring dry wood.

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pieces of P.	sylvestris		pwood.		sapwood.						
	DRY PJ	PIECES					M	WET PIECES	CES		
Group Replicate	1	2	ю	Ţ	ъ	Group Replicate	л Дта	5	ε	4	ы
н	295	420	350	365	335	н	290	170	245	135	190
II	450	460	380	410	375	II	195	270	210	230	190
TTT	355	550	390	380	350	TII	190	225	150	220	175
IV	340	370	500	385	410	IV	255	220	200	220	250
Λ	355	390	410	470	430	Λ	190	235	230	245	350
ΛI	440	380	410	290	340	ΥŢ	300	250	200	260	205
ΔII	385	370	420	420	420	ΛII	275	230	230	210	130
VIII	345	375	430	440	350	NIII	290	210	215	250	190
IX	350	320	410	550	470	XI	230	210	185	230	260
Х	380	380	350	320	385	X	185	210	340	220	130
Mean	370	402	405	403	387	Mean	240	223	221	222	207
Ane	Analysis (of var:	variance				Analysis		of variance		
Source of var	variance	S. S.	년 o	М. S.	V.R.	Source of	E variance	S.S.	년 0	M.S.	V.R.
Between groups	SC	9108	ず	2277	0.73*	Between groups	. sdnoxf	. 5510	4	1378	0.62*
Within groups (error)		140586	45	3124		Within groups (error)	roups or)	98402	45	2187	
Total		149694	49	3054.5		Total		103912	49	2120.	7
				'N *	ot signific	* Not significant at 0.05 level	e1				
L.S.D.	= 20	50.5 Newtons	tons				L.S.D.	"	42.2 Ne	Newtons	
		+									

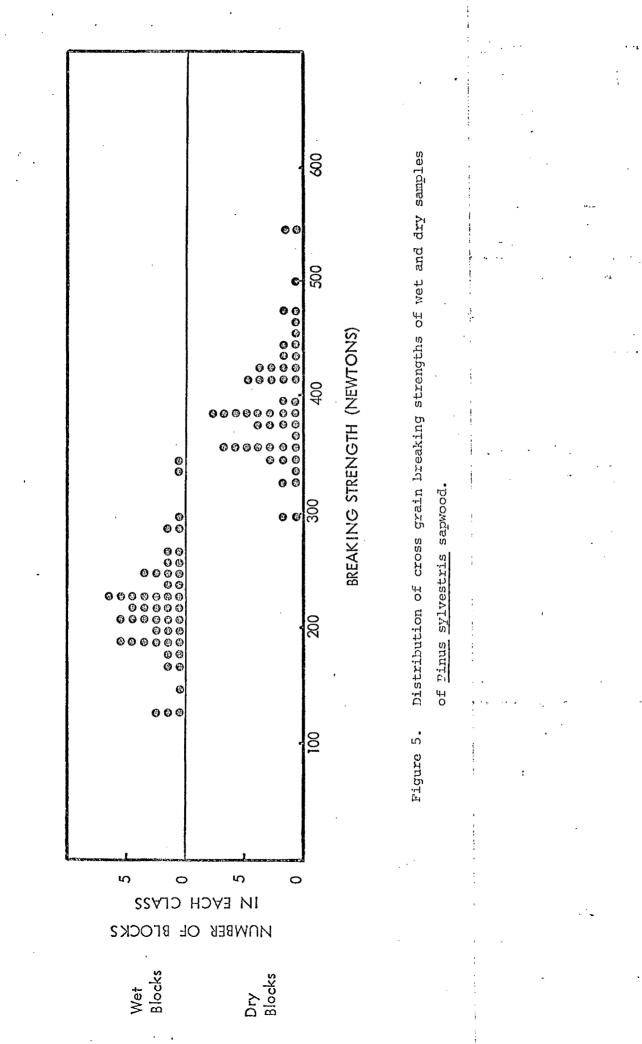
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APPENDIX 4

Initial Work made to Determine the Convenient Design for the Decay Experiments

This preliminary investigation was carried out to determine the period of incubation which would be suitable for our decay studies, and the amount of replication of cultures which would be required.

Methods

Five replicate cultures of each test strain were set up on 2% malt agar in Roux bottles and ten sterile test pieces were placed in standard pattern on the surface of each culture. One piece of wood was removed from each culture at fortnightly intervals (from 2-18 weeks), for drying, weighing and measurement of breaking strength. The results were compared with those of control pieces incubated in similar assemblies on uninoculated 2% malt agar.

Results

Tables of results are recorded in Appendix Tables 5-13 and summarised in Appendix Figure 6.

Discussion

From experimental observation, it was decided to examine the . results after 4 weeks and after 10 weeks in detail.

Tables 6 and 9 show that range of difference in breaking strength between most individuals in each strain is generally small, except for the occurrence of a small number which give grossly lower readings than the others. At 4 weeks there were 6 of these (1 strain 2, 2 strain 4, 1 strain 9, 1 strain 10, 1 control) in 60 individuals, at 10 weeks there were 4 (2 strain 4, 2 strain 8) in 60 individuals.

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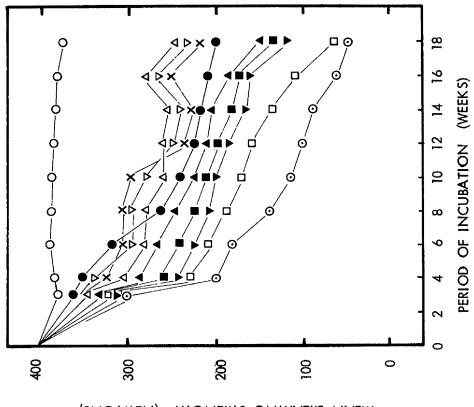
The consistency of general pattern of the results, and the close correlation between breaking strength and loss of dry weight (at 4 weeks = -0.133, at 10 weeks = -0.438) suggests that these erratic results were not due to experimental error in the use of the tensometer; it seems more likely that they are the result of differences between the wood of the test pieces. Their effects can therefore be reduced by increasing the number of pieces used for each experiment.

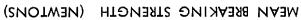
Hence, in a subsequent experiment the number of pieces tested was increased to 25, and the measurements were made at 4 weeks and 10 weeks only. The results (Figure 7) showed that 4 week measurements yielded little useful additional information to those from the 10 week measurement. Since the differences at 10 weeks were consistently bigger and easier to read, this period was chosen as a basis for the design of the main survey. This has produced a useful saving in apparatus, time, and labour involved in the measurements.

FIGURE 6

Graphs showing the mean percent of weight losses and reduction in breaking strength of test pieces of <u>P. sylvestris</u> decayed by 10 strains of <u>S. lacrimans</u> at various periods of incubation.

(Each point in these graphs is the mean of the measurements of five test pieces each taken from a separate culture.)





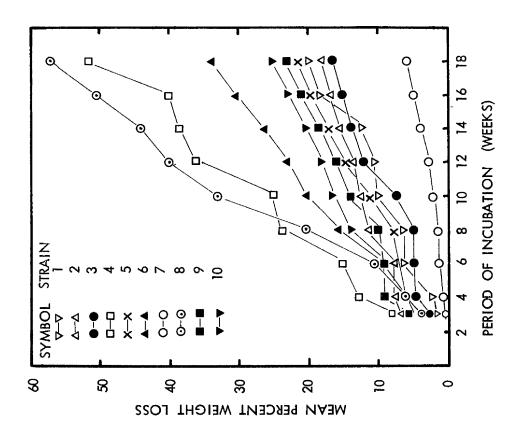
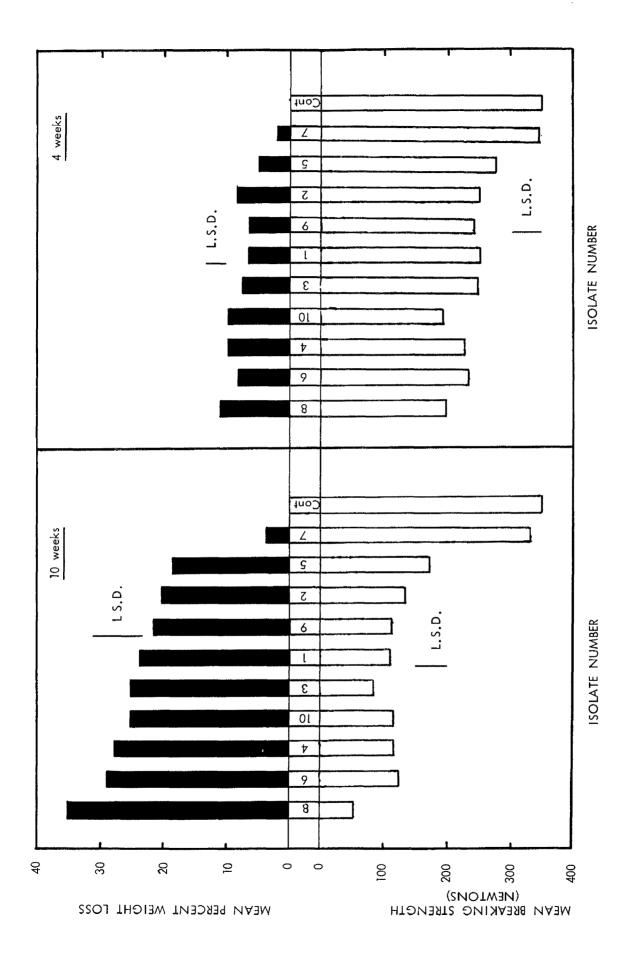


FIGURE 7

Histograms showing the mean percent of weight losses and reduction in breaking strength of test pieces of <u>P. sylvestris</u> sapwood decayed by 10 strains of the fungus at 4 and at 10 weeks incubations.

(Each column in these histograms represents the mean of the measurements of five test pieces in each of five culture bottles.)



Changes in measurement of decay of pieces of Pinus sylvestris sapwood

TABLE 5.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 3 weeks incubation.

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Strain No.			ŀ							
Piece No.	н	N	m	4	ъ	9	7	ω	0	10
Ч	7	m	7	11	4	თ	-1	7	0	ት
2	2	ი	N	с	IJ	m	щ	ო	ო	4
۳	Ч	ო	ъ	IJ	m	0	0	Ŋ	2	ი
4	7	12	0	12	4	ß	0	IO	-1	Ŋ
۲Û	5	7	ம	5	ヤ	ო	ы	9	r1	2
Mean	1.8	5.8	3.8	6.6	4.0	4.0	1.0	6.2	1.4	4.8
•										

Breaking Strength (Newtons) of wood pieces after 3 weeks incubation. ц М

Strain No.	-	c	· · ·		Ŀ		Г	c	c	¢	
Piece No.	-1	N	'n	ť	n	D	-	α	ת	D-T-	
r-ł	345	350	300	355	335	290	325	270	320	375	300
∾.	350	290	410	325	360	340	420	305	325	335	460
ε	350	340	350	320	370	310	400	315	380	170	390
4	330	300	390	310	320	390	395	290	300	375	385
S	325	360	350	330	400	350	380	270	360	335	380
Mean	340	328	360	328	357	336	384	290	337	318	383

Changes in measurement of decay of pieces of Pinus sylvestris sapwood TABLE 6.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 4 weeks incubation.

Strain No.										
Piece No.	н	7	ε	4	ហ	9	7	ω	ი	10
Т	2	18	ю	20	ю	£	۶H	9	10	12
7	-1	Q	Ø	9	ហ	14	7	თ	ヤ	ω
т	с	Ч	7	20	12	7	0	ؘۅ	11	12
ヤ	m	ε	Ţ	11	7	11	י ר	14	13	10
ы	4	ы	e	ъ	7	7	7	ω	10	5
Mean	2.6	6.6	4.0	12.4	2°8	8.4	1.2	8.6	9 . 6	8°8
B. Breaking Strength o	trength (of wood pieces	dieces af	ter 4 W	after 4 weeks incubation.	mation.				
Strain No.	,	(1		(1	
Piece	{	7	Υ	4	۵ م		œ	ת	P	Control
NO.										

Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 7.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 6 weeks incubation.

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B. Breaking strength of wood pieces after 6 weeks incubation.

Strain No.						,					
Piece No.	1	7	m	4.	Ω	S	2	ω	b	OT	Control
Ч	285	295	335	295	300	365	420	260	210	300	465
7	355	70.	275	300	280	240	,275	215	330	260	425
£	300	280	285	240	260	345	420	150	200	130	340
ъ	275	360	310	25	365	185	435	115	190	210	415
IJ	285	400	360	210	265	200	420	240	220	190	530
Mean	300	281	313	214	294	267	394	196	230	218	435

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Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 8.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 8 weeks incubation.

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Strain No.	,	ſ			1	ų	1		ſ	
Piece No.	-1	7	m	4	ъ	٥	7	ω	σ	IO
Т	10	16	4	15	13	ω	н	41	თ	14
7	9	ດ	7	29	7	12	7	11	19	22
m	ო	20	ъ	IO	თ	16	7	11	13	15
4	9	ŝ	7	57	4	თ	7	35	10	TT
ŝ	7	Ŋ	Ŋ	თ	7	26	2	7	ω	9
Mean	6.4	11.0	5.4	24.0	0 . 8	14.2	1.8	21.0	11.8	13.6

B. Breaking strength of wood pieces after 8 weeks incubation.

Strain No.											
Piece No.	r=l	5	m	4	ы	9	7	ω.	თ	10	Control
г	330	8	350	290	310	335	400	30	220	200	480
۲ ٦	300	290	235	20	260	255	405	200	150	160	500
ε	285	2 30	350	345	270	235	420	265	170	180	455
ተ	350	355	210	IO	355	350	385	25	250	220	425
ы	290	390	155	270	275	150	395	275	320	305	420
Mean	311	269	260	187	294	265	401	159	222	213	456

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Changes in measurement of decay of pieces of P. sylvestris sapwood

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 10 weeks incubation.

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1		ı	ı	•					•	
Strain No. Piece No.	-1	N	ĸ	4	ы	Q	7	ω	6	10
1	17	ი	9	23	თ	29	m	41	20	22
•0	7	18	თ	ТТ	11	12	0	28	Q	12
с	ŋ	14	ω	17	ω	18	г	25	11	lo
Ţ	0	11	ი	14	J 6	29	7	35	15	19
ъ	8	7	10	60	ω	10	2	18	18	18
Mean	9.2	11.8	8.4	25.0	25 . 0 · 10.4	19.6	2.0	29.4	14.6	16.2

Breaking strength of wood pieces after 10 weeks incubation. . ш

Strain No.											
Piece No.	-	5	т	4	١Ĵ	Q	7	ω	6	IO	Control
г	200	300	220	150	280	190	375	30	165	140	390
N	290	220	270	250	220	230	380	160	235	180	410
ო	310	230	200	190	290	245	420	185	210	210	470
4	300	290	310	230	310	200	400	25	230	240	460
S	320	240	260	IO	300	245	405	205	200	260	450
Mean	284	256	252	166	280	222	396	121	208	206	436

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TABLE 9.

Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 10.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 12 weeks incubation.

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Strain No.										
Piece No.	r1	7	m	4	ம	و	7	ω	თ	10
гщ	თ	10	9	22	J6	28	ы	35	30	ŢŢ
7	10	ΤΊ	17	17	13	1 8	Þ	51	ω	12
m	6	13	12	62	13	14	т	34	22	11
4	14	24	15	56	16	33	2	20	12	19
IJ	11	7	74	24	lo	17	m	59	01	26
Mean	10.6	13.0	12.8	36.2	13.6	22.0	2.6	39.8	16.4	18.4

B. Breaking strength of wood pieces after 12 weeks incubation.

Strain No. Piece No.	Г	5	£	7	ъ	9	7	ω	б	IO	Control
г	280	200	215	250	280	, 200	410	130	120	175	455
Ν	290	220	200	270	285	250	350	20	210	215	485
m	305	280	210	0	220	275	380	150	180	230	455
4	175	210	225	ហ	200	IIO	405	190	260	190	295
Ŋ	200	340	220	285	265	240	370	0	280	120	395
Mean	250	250	214	162	. 250	215	383	98	210	186	417

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Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 11.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 14 weeks incubation.

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	 		: 7 							
Strain No.										
Piece No.	r-I	7	m	4	ы	o	7	ω	ი	of I
. H	13	25	11	33	29	37	Ţ	46	28	16
7	12	15	17	66	ΤT	18	IJ	59	IO	35
ю	11	13	11	30	8	12	7	33	17	11
4	14	14 14	თ	40	16	30	9	52	32	28
IJ	13	6	15	24	19	33	m	30	11	12
Mean	12.6	15.2	12.6	38.6	16.6	26.0	4.0	44.0	19.5	20.4
•										

B. Breaking strength of wood pieces after 14 weeks incubation.

Strain No.				.							
Piece No.	-1	7	m	4	υ	o	7	ω	ס	10	Control
щ	310	220	200	160	225	160	390	90	155	240	370
7	200	250	185	0	260	240	370	35	225	70	375
т	245	2⊈0	195	185	265	260	420	135	200	200	420
4	195	230	280	25	220	220	350	70	100	160 [.]	.400
Ŋ	250	320	240	235	180	180	385	140	260	190	445
Mean	240	252	220	121	230	212	383	64	188	172	402

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Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 12.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 16 weeks incubation.

Strain No.										
Piece No.	г	2	m	ъ	ъ	9	7	ω	6	10
Ч	10	38	13	30	13	24	4	61	14	6
N	15	15	14	59	11	17	2	48	23	38
£	32	11	12	50	18	40	9	32	29	15
4	13	m	32	48	23	35	ω	57	18	42
ŝ	17	16	თ	25	28	33	m	51	26	TT
Mean	17.4	16.6	16.0	42.4	18.6	29.8	4.6	49.8	22.0	23.0

B. Breaking strength of wood pieces after 16 weeks incubation.

Strain No. Piece No.	г	7	m	4	ഹ	9	7	ω	თ	10	Contro l
щ	260	85	205	125	280	220	380	0	210	265	390
7	295	250	220	0	295	280	410	60	180	70	430
ť	100	310	260	0	250	70	370	90	160	210	470
4	355	ِ 350	150	260	200	110	365	20	200	45	450
ហ	265	260	200	190	170	135	400	35	120	220	450
Mean	255	251	207	115	239	163	385	41	174	162	438

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Changes in measurement of decay of pieces of P. sylvestris sapwood TABLE 13.

decayed by 10 strains of S. lacrimans.

A. Weight loss as % of original dry weight after 18 weeks incubation.

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Strain No.			1		1					
Piece No.		7	m	4	ы	o	7	ω	თ	P
Ч	28	IO	13	65	24	45	IJ	62	16	46
2	J 6	25	17	34	20	35	ቅ	57	21	18
m	თ	16	17	28	14	29	7	48	37	29
ሻ	26	TT	22	64	15	1 9	7	54	14	20
ſ	20	32	18 1	65	28	39	Ø	59	35	Ъ.
Mean	19 . 8	18.8	17.4	51.2	20.2	33.4	5.4	56.0	24.6	25.4

B. Breaking strength of wood pieces after 18 weeks incubation.

Strain No.											
Piece No.	r -1	5	ε	ず	ഹ	9	7	ω	თ	10	Control
H	160	300	220	0	250	35	390	0	200	20	350
CI	250	180	195	120	240	130.	395	55	170	190	375
т	320	240	200	190	245	200	410	65	60	100	460
ሻ	230	, 270	180	0	230	260	380	40	210	130	430
ы	190	150	205	10	150	80	355	35	70	200	440
Mean	230	228	200	64	223	141	386	39	142	128	411

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APPENDIX 5

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-21	 Neight loss as 5 of Gry weight after incuation period. 	t loss	as 5 2		reigne	arter	Include	1		1																														
Bottle Floce No. No.		п	m	4	5	و	2	8	91	1		2	12	13	14	15	16	17	18 1	19 2	50	21 2	52	23	24	25	5	26	27	58	29	8	R	33	ñ	ž	35	36	G	i
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1.5.D. = 7.6% (P = 0.05)	= d) 19°.	: 0.05)											L.S.D	L.S.D. = 7.18	-118 (P	= 0.05)	6											г.\$	= · · · ·	L.S.D. = 6.44 (P = 0.05)	(P ≝ O.	(50)								
Anilysis of variance for percent weight losses	lance fo.	r perce	ant vei	ght lo	SSes																																			
Strain 1-11 inclusive, the standard strain	l inclus.	ive, th	le star	idard s	train			1					Strai	ns 12-	25 inc	lusive	, the	standa	Strains 12-25 inclusive, the standard strain	ais								Str	ains 2	Strains 26-36 inclusive, the standard strain	inclusi	lve, th	ie star	ıdard s	train					
Cource of variation	variatio		SS	4.	SH	teV 62	Variance ratio																					Sol	urce o	Source of variation	ation	SS		۰. ۱	SM	Var	Variance ratio	1		
Between strains	trains	166	16696.2	11	1517.8		8.0.*	l					Sauce		Source of Variatio	e	2	*	2		ratio	8						Be	tween	Between strains	2	18195.6	5.6	Ħ	1654.1		12.29 •	1		
Setween bottles	ottles	68	8066.2	47 00	168.0		••63*0						Betve	Between strains	ains	32	32619-4	14	2330	0	14.0 *							Bei	tveen	Between bottles	ş	594	5940.2	48	123.8		0.92**			
Within bottles (error) 45468.2	ttles (er:	ror) 454	168.2	240	189-5	2		1					Betree	Between bottles	tles	σ.	9737.4	99		2-3	•-96-0	:						TM	(error)	Within bottles (error)		32303.2		240	134.6					
TATOT	-	202	70230.6	662				J						(error)			0.950	3	r.001			ļ							TOTAL	-		564 39.0		299				1		

56439.0 299

92252.8 374

TOTAL

For illustration see text figure 2.

Significant at 0.01 level.
 Not significant at 0.05 level

Toble 141. Charter in measurement of decay of pieces of Pinus sylvestris sapwood incubated for 10 weeks with 36 strains of S. lacrimans.

A. Height loss as 9 of dry weight after incubation period.

Tuble 14b. Changes in measurement of decay of pieces of Finus sylvestrie , sagwood incubated for 10 weeks with 36 strains of S. leotimans. E. Presting strength (Newtons) of wood pieces after incubation period.

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Proce	-1	10	6	-7	5	9	~	ω	6	3	1	s	Cont.	12	13	14	15	16	17 18	61 61	9 20	21	22	23	24	25	2	cont.	26	27	26 '	29	м Я	31 32	2 33	3	35	36	n	Cont.
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Mean	110	136	78	103	133	126	331	56	142	117	156	160 3	358	17	56	63	56	34 6	69 82	12 69	18 6	63	53	329	69	109 1	15	397	270	304	343	275 3	323 32	321 268	8 231	900 1	81	252	150	8
4 C-S-1	= 45.2 Newtons		(2 = 0.05)	05)											L.S.D.		= 39.4 Newtons		0 = 2)	0.5)										L.S.D.		= 82.1 Newtons		(P= 0.05)	05)					

Analysis of variance for breaking strength (exclusive controls)

Strains 1-11 inclusive the standard strain

Variance ratio	15.0 *	1.43***		
SW	115945.5	11050-1	2711-8	
6. •	11	с; т	240	299
SS	1275400.0	530432.0	1850840.0	3656672.0
Source of variation	Between strains	Between bottles	Within bottles (error)	TYLAL

Significant at 0.01 level
 Significant at 0.05 level
 Not significant at 0.05 level

For illustration see text figure 2.

Strains 12-25 inclusive the standard strain

	MS Variance ratio	127356.2 24.75 *	6925.l l.35 ***	5145.2	
	ы o	14 127	60 6	300	374
i	SS	1782967.3	415506.0	1543550.0	3742043.3 374
	Source of variation	Between strains	Between bottles	Within bottles (error)	TOTAL

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Strains 26-36 inclusive the standard strain

	SS	0.93538 11 0.9224.0	0.202951	5369940.0	7503e91.0 249
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Table 15. Mensurements of mate of increase of colony diareter (in mul for 36 strains after 7 days incubation on 24 main agar at 21°C.

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55 50	1 0	45	51	65	1 22	61	38	67	69	53	3	3	57	61	45	3	59	64	46	63	47	37	18	3	3	2	35	26	51	4	47	53	9	38
53 57	~	39	53	53	27	33	37	52	63	63	6 7	51	58	23	36	61	58	56	Q	64	43	38	14	59	3	47	ß	26	57	36	43	46	43	36
53	82	33	ያ	. 42	42	. 64	54	48	33	64	47	\$	ç	16	38	60	57	59	30	57	47	35	13	57	58	65	36	26	55	8	48	8	15	Ţ
56	54	28	48	35	41	19	4 2	53	4	59	ያ	38	63	14	ጽ	53	55	61	40	63	34	34	15	65	57	5 3	5	24	23	ň	t 3	48	47	Ě
53	59	46	ŝ	. 5	41	66	Q	43	65	63	48	43	3	26	46	63	58	57	43	56	32	37	14	32	4 5	ŝ	E	22	Ģ	ŝ	21	53	53	St.
53	53	88	. 52	53	38	R	38	53	62	19	47	36	58	26	ğ	57	56	59	39	55	27	42	12	5 5	42	56	32	22	8	58	5	ŝ	3	35
	52	55	55	58	27	72	22	47	54	63	42	Ξ	66	22	ß	63	58	61	42	53	48	34	13	ጽ	49	5	33	24	49	56	67	5	Я	ů
	55	51	54	5	23	11	21	53	68	63	47	39	63	22	53	61	63	60	4S	63	47	43	15	ጽ	46	52	39	27	45	\$	8	55	5 3	3 9
	56	35	45	.	8	Ľ.	عد	46	66	57	42 [.]	29	, 7 6	19	34	63	62	58	6£	49	28	38	20	44	43	46	15	5	47	6F	45	47	Ş	\$\$
	57	8	52	47	3	66	35	56	35	53	46	33	73	16.	ጸ	59	58	59	42	48	23	34	15	43	47	53	37	25	41	ន្	48	49	53	33
55.4	55.1	56	-9 SI-4	4 50.1	1 32.7	7 66.7	7 35.2	50.0	65.0	61-3	3 46.2	2 39.0	64.9	20.3	3 40.0	60.09	58.4	4 59.4	4 40.6	5 57.7	7 37.6	5 37.2	2 14.9	3	.3 49.0	50.0	0 35.0	0 25.2	2 49.5	5 42.2	\$ 47.2	5.0	47.0	0.04

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Analysis of variance for growth

Variance ratio	\$0.7 •	
SH	1489.1 29.4	
4 o	35 324	359
SS	52119.7 9508.6	61628.3
Source of variance	Between strains' Mithin strains (errof)	, THEOL

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Significant at 0.001 level

For illustration see text figure 5.

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Measurements of rate of increase of colony diameter (in mm) for 25 monderryotic cultures (isolated from sporophore A) after seven days incubation on 2% malt agar at 21°C. Table 16.

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Strain	10 A	2C)	253	224	255	a ch	724	82 Q	200	1124	AS12	AS13	ASI5	AS16	AS18	1 6124	AS20 /	AS21 7	AS22 1	AS23 A	AS24 A	AS25 AS	AS26 AS27
															·								
	34	37	\$	41	Q	40	37	45	37	37	34	53	41	41	23	37	40	40 40	64	24 4	40 41	1 38	36
	38	42	38	\$	36	43	38	45	38	34	36	22	42	\$	20	38	42	68	34	25 3	38 40	0 37	1
•	35	37	ጽ	41	37	40	36	38	36	32	65	23	39	41	35	33	41	37 4	42	22 4	40 42	32	38
	33	41	32	37	36	41	37	42	37	28	32	22	40	8	31	38	40	38	58	24 3	37 39	38	36
	34	34	38	42	40	40	38	35	Ş	40	34	21	41	36	26	36	42 3	39	38	26 3	39 41	1 39	35
	35	6	35	41	34	38	6 £	53	39	38	g	23	42	41	31	31	40	40	41	25 3	38 42	2 35	33
	36	41	31	ę	35	40	37	37	37	39	34	20	40	39	R	34	41	37 3	37 2	24 3	37 39	9 37	34
	38	39	36	37	34	38	36	35	C.\$	40	32	દા	45	31	26		65	38	្ន	23 3	35 41	1 33	36
••	34	\$	8	42	40	40	32	32	37	38	37	21	41	33	22	ş	42 4	40	41	26 3	37 40	0 32	34
	33	39	32	38	35	65	37	25	37	64	36	20	40	41	ß	34	41 4	6	đ	24 3	38 35	5 37	36
	35.0 39.0	1	34.2	39.9	36.9	39.9	36.7	37.7	38.1	36.6	34.4	21.4	41.1	38.6	27.4	35.7	40.8	38.8	39.2	24.2 3	37.9 4(40.0 36	36.3 35.8

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L.S.D. (P = 0.05) between the means of colony diameters = 2.40 mm

Analysis of variance for growth

Variance ratio	04 33 . 6 *	4	
SM	249.04	7.41	30.66
Бц о	23	216	239
SS	5727.9	1599.9	7327.8
Source of variance	Between strains	Within strains (error)	TOTAL

* Significant at 0.001 level.

For illustration see text figure 6.

Table 17. Mainterne of rate of interests of colony diameter (in ma) for 42 mm/arrolifectitures (isolated from sporohare 3) after seven days interaction on 34 milt agar at 21°C.

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1 8542	8	17	52	8	5	ţ;	3	1 6	2	3	1107
3541	B	% 1	Ş	E.	8	Сł	а	3	3	5	6.00
Ct SE	55	9	51	9	5	33	en V	5	а; 17	22	49.6
8839	3	3	е;	2	22	\$	1	8	a ;	5	5.14
8538	23	53	55	15	53	35	55	3	92	53	0.42
1ESE	3	2	47	6	4	51	2	67	5	51	49.0
3S]6	ß	51 17	47 00	8	47	55	9 7	47	Я	\$	
BS 35	3	46	47	48	2	44	47	87	\$	47	57.7
BS34	я	47	48	45	47	46	42	45	47	48	÷6.5
EESE	ą	42	6	41	42	ş	65	49	Ş	\$	41.3
BS 32	38	\$	35	ж	37	Ş	4	36	45	65	18.7
TE SE	х	£	ж	37	36	32	88	32	35	35	9.45
of se	44	15	ş	38	63	42	я	46	51	44	44.3
as29	47	44	42	45	47	\$	44	48	47	40	44.4
a\$28	37	44	41	65	44	40	38	6	39	62	40.1
BS27	32	8	32	31	29	26	32	29	34	29	2
BS26 F	33	16	38	35	36 2	34 2	36 3	32 2	e R	5 2	5 4.45
BS 25 B	E E	ĕ	27 3	31 3	29 29	32 3	33 3	36 3			г. 8 (г.
BS24 B	28	27 3	8	26 3	2	27 3	24 3	22 3	27 35	21 31	5 6 76
BS23 B	34	41 2	42	44 2	45 3						<pre></pre>
BS22 B						41	6 4	1 45	41	7 38	4 0 LE
ES21 B	3 42	5 41	34	38	36	65	33	34	\$	7E 37	26.4
R OCSE	5 23	5 25	0 26	5 25	23	31	0 25	õ	36	25	u
8519 35	46	45	8	45	IS .	52	8	49	48	ያ	37 0 82
BS18 B5	7 33	R	37	32	37	31	35	R	35	8	EE E 97
BS17 BS	47	43	45	48	45	46	47	48	46	48	ų
ιD.	32	55	35	33	35	41	36	51	\$	77	ά ά
B\$15 BS1	47	45	48	46	45	5	41	46	42	45	0 7 7
BS14 BS	28	TE	34	29	ŝ	ЗI	28	29	Ĩ	33	21 2 2
	37	33	32	E	ß	32	31	35	32	EE .	5 22 2
BSI2 BSI3	38	46	47	43	66	47	44	38	42	4	9 77 E
	53	3	53	52	•94	49	50	54	53	3	9.05
IISE	ΤE	27	25	33	õ	Ĩ	32	27	33	31	0.05
BSIO	\$	36	39	37	40	42	43	33	42	Ş	8. 0°.
9S9	\$	42	36	6	44	66	40	38	33	32	38.4
BSB	31	33	37	\$	41	42	37	38	37	36	37.4
BS7	37	41	38	37	54	36	34	ą	35	99	1.75
9S6	47	43 ,	46	39	36	41	39	45	41	36	517
BS5	23	38	29	27	26	23	24	ß	35	42	7.95
BS4	11	43	66	38	55	0	<u>6</u>	4	10	43	21.5
5%3	2	ц.	\$	4	5	\$	52	66	÷	55	1.02
C SS	ų	8 6	ő	35	9	9	<u>.</u>	9	3	17	4
195	37	35	2	Ľ.	8	М	3	5	2	8	14.4
	14			8		5	ij		H H	я	
Strain Neglicate						~	1	1111			12.07

L.S.L. (F = 0.05) between the means of colony diameters = 2.8 mm

Arilysus of versance for growth

Variance ratio	49.3 *		
SM	501.1	10.2	
ţد. ٥	41	378	419
SS	20543.6	3640.2	24383.8
Source of vertence	Setreen strains	Nitrin Strains Herrori	. 100M

* Support at 0.001 level.

Fir illustration see text figure 7.

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Bottle No.	Piece No.	13A	AS2	AS3	AS4	AS5	AS6	AS7	ASB	92A	AS11	AS12	AS13	AS15	- A5 16	AS17	AS18	AS19	AS20	AS21	AS22	AS23	AS 24	AS25	AS26	AS27
н		38 28 28 28	13 51 22 22	5 ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° °	43 15 67 67	200 000 000 000 000 00 00 00 00 00 00 00	33 4 11 33 33 4 11 33 33 8	15 41 41 11	20 4 50 20	14 14 14 25 25	30 34 60 60 1	665 1 3 3 0 6 6 1 3 3 0	38 13 61 61	54 54 10	50 50 50	56 8 3 5 4 5 6 8 3 6 4	13 8 8 9 0 13 8 8 9 0 13 8 8 9 0	18 23 60 51	61 13 12 44	36 22 27 11	40 60 112 60 70 70 80	51984 361984	221 14 26 26 26	47 15 60 14	711179 71117	35 35 35 35 35 35 35 35 35 35 35 35 35 3
II	1000 1000 1000	11 13 35 9	40 7 40 7 7 7	30 3 8 8 N	10 21 46 13	32 20 19 32 80 19	13 14 13 13 13 13 13 13 13 13 13 13 13 13 13	161 16 16	47 11 5 5	8 8 8 4 9 8	61 55 61 61	11 11 10 11 10 10	4 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	50 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	54742 54742	а 55 55 55 55 55 55 55 55 55 55 55 55 55	430 430 430 430 430 430 430 430 430 430	63 65 16 57	9 12 36 62 62	11 11 11 11 11 11 11 11 11 11 11 11 11	1 2 2 3 3 4 1 2 2 4 1 2 4 1 3 2 4 4	11 11 15 19 19	16 25 26 28	45 19 28 28 28 28 28	33 31 32 25	32 36 36 39 39
III	12212	315 48 8 m	57 49 46	36 51 15 15	19 55 22 22	36 110 36 12	7 II 8 9 7	57046 670	13 13 22 46	17 27 9 27 52 27 9	54 52 58 5 54 52 58 5	8 0 0 m 0	27 48 18 60	67 16 15	23 12 56 65	22 36 51 20 20	41 35 30 80	65 59 27 23 23	20 13 6 9 65	19 64 55 26	45 655 24 24 24 24 24 3	9870 10	19 16 16 30	30 4 5 30 4 5 30 5 4 5	3966833 396683	28 33 42 42
IV	16 13 19 20	404 81 14 14	4 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	35 12 7	4 6 7 6 F	0, 4, 9, 4 0, 9, 9, 4 0, 9, 4 1, 9, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,	40 40 40 40 40 40 40 40 40 40 40 40 40 4	1 36 2 8 1 36 2 8	4 50 6 430 6	579 539 63	58 13 25 63	22424 224314	5 4 4 5 6 6 6 7 4 5 6 1 6 7 1 7 1	22 52 48 26	1447 1447 1897	12948 13948 13948	45 45 45	26 55 32 32	19 32 41 60	41 11 55 55	50 744 185 185 185 185 185 185 185 185 185 185	25 25 25 25	35 42 21 36	34 17 24 22	с т к к к С 4 П 4 4	138 138 138 138 138 138 138 138 138 138
4,	22 23 25 25 25 25 25 25 25	23 15 27	17 61 7 7 7 7 7 7 7 7	23 74 73 73 73 73 73 73 73 73 73 73 73 73 73	181 180 180 180	517 3 8 a	31 50 24 80 23	or i oo	8 9 7 7 8 8 8 9 7 7 8 8	42 59 61 60	22 32 61 17	61 4 4 1 2 6 4 6 1 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	3666 3666 3666	33 75 75 75	5 2 9 9 9 5 0 9 9 0 7 0 9 9 0	22 28 18 50	16 25 21 13	4 0 0 4 0 8 8 0	36 14 59 59	38 43 58 52	21 48 63 15	57 28 39 39	27 39 33 33	29 29 29 29 29 29	36 11 28 28	39 28 37
	Mean	21.6	36.0	1 25.2	29.4	23.6	25.6	16.6	22.9	41.8	38.0	37.3	37.2	34.2	37.3	35.1	26.8	43.0	32.4	39.2	35.2	24.9	29.1	27.0	0.00	29.2

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f.S.D. (P = 0.05) between means of percent weight loss = 9.3%

Analysis of variance

Source of variation	SS	Ц. Ц.	SM	Variance ratio
Between isolates	30005-0	24	24 1259.2	4.26*
Between bottles	21763.4	100	217.6	0.74**
Within bottles (error)	146719.6	500	293.4	
TOTAL	198488.0 624	624		

* Significant at 0.01 level.

** Not significant at 0.05 level.

For illustration see text figure 8.

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Table 18b. Changes in measurement of decay of pieces of P. sylvestris sapwood incubated for 10 weeks with 25 monokaryoticcultures of S. Lacrimans.

B. Breaking strength (Newtons) of wood pieces after incubation period.

L.S.D. (P = 0.05) between means of breaking strength = 45.2 Newtons.

Analysis of variance for breaking strength (exclusive controls)

Variance ratio	2.4 *	1.08 **		
SW	16455.9	7332.6	6791.2	
а, °	24	8	50 02	624
SS	394942.6	733264.0	3395590.0	4523796.6
Source of variation	Between isolates	Between bottles	Within bottles (error)	TOTAL

* Significant at 0.05 level ** Not significant at 0.05 level

For illustration see text figure g.

period.
incubation
after
weight
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55
1053
Weight
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Notion Notin Notin Notin <th> </th> <th>B.4.</th> <th>2 4 3 4 5</th> <th>58888</th> <th>r 35 v 3</th> <th>raat.</th> <th>10 8 8 7 7 7 1 8 8 8 7 7 1 8 8 8 7 7 7 7 7</th> <th>26.6</th>		B.4.	2 4 3 4 5	58888	r 35 v 3	raat.	10 8 8 7 7 7 1 8 8 8 7 7 1 8 8 8 7 7 7 7 7	26.6
Nor 101 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>4</td>								4
Note 101 103 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>w l</td>								w l
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Note BI B								4
View 11 15 1								-
Note U1 U2 U								
Note 101 102 101 <td></td> <td></td> <td>¢</td> <td></td> <td></td> <td></td> <td></td> <td>1</td>			¢					1
Nice Bit Bit <td></td> <td>- 1</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		- 1						
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Note Not Not <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>æ</td>								æ
Note Hole Hole <th< td=""><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td>-</td></th<>		-						-
New Net Net <td></td> <td>- 1</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>-</td>		- 1						-
New Not Not <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>								
Nice Nice <th< td=""><td>1</td><td></td><td>8 C 8 4 6</td><td></td><td>9 4 7 9</td><td>. d</td><td>312268</td><td>1</td></th<>	1		8 C 8 4 6		9 4 7 9	. d	312268	1
Nice Nice <th< td=""><td></td><td></td><td>2 6 % 9 6</td><td>24198</td><td>63468</td><td>\$ n 4 9 7</td><td>21 20 21 20</td><td>22.</td></th<>			2 6 % 9 6	24198	63468	\$ n 4 9 7	21 20 21 20	22.
Piece Bol Bol </td <td></td> <td>4 1</td> <td>32228</td> <td>28 30 30 30 30 30</td> <td>9 n n n 9 N N N 1 N N 1</td> <td>យ័4 4 6 4</td> <td>តដីដីដី<i>ខ</i></td> <td>61</td>		4 1	32228	28 30 30 30 30 30	9 n n n 9 N N N 1 N N 1	យ័ 4 4 6 4	តដីដីដី <i>ខ</i>	61
Piece Bit Bit </td <td></td> <td></td> <td>3°° à 35°</td> <td>33 16 25 *</td> <td>899 H 8</td> <td>23 29 23</td> <td>1997 1997 1997 1997 1997 1997 1997 1997</td> <td>8</td>			3°° à 35°	33 16 25 *	899 H 8	23 29 23	1997 1997 1997 1997 1997 1997 1997 1997	8
Piece Bit Bit </td <td></td> <td></td> <td>1 8 10 23 1 8 10 23</td> <td>82311</td> <td>86 01 0 1 2 1</td> <td>.63 14 12 12</td> <td>22825</td> <td></td>			1 8 10 23 1 8 10 23	82311	86 01 0 1 2 1	.63 14 12 12	22825	
Piece Bit Bit </td <td></td> <td></td> <td>6 59 - 6 8 - 8</td> <td>25 9 11 9 9</td> <td>21 8 8 6 G</td> <td>39340</td> <td>33333</td> <td>4</td>			6 59 - 6 8 - 8	25 9 11 9 9	21 8 8 6 G	39340	33333	4
Piece B21 B21 </td <td></td> <td></td> <td>ដំពូន ខ្លះរ</td> <td>۲2°4%</td> <td>9 r 6 6 6</td> <td>rr 8 0 6</td> <td>00010</td> <td>1 1</td>			ដំពូន ខ្លះរ	۲2°4%	9 r 6 6 6	rr 8 0 6	00010	1 1
Piece Bit Bit </td <td></td> <td></td> <td>2 ~ % 3 K</td> <td>5ª4-1</td> <td>3 7 8 7 6 3</td> <td>122221</td> <td>1°1°,</td> <td></td>			2 ~ % 3 K	5ª4-1	3 7 8 7 6 3	122221	1°1°,	
Piece B21 B21 B21 B31 B31 </td <td></td> <td></td> <td>482°3</td> <td>0.ḋ0.ḋ.¥</td> <td>01 6 1 6 7</td> <td>111°5</td> <td>10 110 110 10</td> <td>1 1</td>			482°3	0.ḋ0.ḋ.¥	01 6 1 6 7	111°5	10 110 110 10	1 1
Piece Bit Bit </td <td></td> <td></td> <td>ដ្ខខត្ត</td> <td>5123</td> <td>1961,</td> <td>88180</td> <td>စ္ကစ္ခရစ</td> <td>1</td>			ដ្ខខត្ត	5123	1961,	88180	စ္ကစ္ခရစ	1
Piece BS1 BS1 </td <td></td> <td>BC18</td> <td>43983</td> <td>81685</td> <td>88448</td> <td>37 8 11 8 8</td> <td>22 51 51 51 51 51 51 51 51</td> <td></td>		BC18	43983	81685	88448	37 8 11 8 8	22 51 51 51 51 51 51 51 51	
Piece Bit Bit </td <td></td> <td>BS17</td> <td>8 a r 2 1</td> <td>ជង្ហាដ។</td> <td>18853</td> <td>39568</td> <td>24424</td> <td>1.61</td>		BS17	8 a r 2 1	ជង្ហាដ។	18853	39 5 68	24424	1.61
Piece B21 B23 B34 B55 B56 B57 B56 B51 B512 B513 B513 </td <td></td> <td>BSI6</td> <td>108537</td> <td>11 ° 20 23</td> <td>14 7 28 3 7 4</td> <td>8 5 9 9 9 8 5 9 9</td> <td>10 8 8 8 0 10 8 9 8 0</td> <td>22.1</td>		BSI6	108537	11 ° 20 23	14 7 28 3 7 4	8 5 9 9 9 8 5 9 9	10 8 8 8 0 10 8 9 8 0	22.1
No. Bit I State Bit I State Bit I B		BS15	8 2 4 3 3	8 11 8 19 6	4 6 4 8 I	0 4 C 7 0	9 7 7 8 7 7 8 7 7 8 7 7 8 7 7 8 7 8 7 8 7	10.9
Piece B21 B21 B31 B31 </td <td></td> <td>BS14</td> <td>ដទទទ</td> <td>8.9112</td> <td>88833</td> <td>e e r 11 51</td> <td>191-1</td> <td></td>		BS14	ដទទទ	8.9112	88833	e e r 11 51	191-1	
Piece B1 B2 B3 B54 B55 B56 B57 B58 B59 B510 B511 B51 1 8 12 6 7 14 6 8 7 <td< td=""><td></td><td>BS13</td><td>3110</td><td>82 8 28 F1</td><td>1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1</td><td>22238</td><td>* > * 19</td><td>16.0</td></td<>		BS13	3110	82 8 28 F1	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1	22238	* > * 19	16.0
Piece B31 B54 B55 B56 B57 B58 B59 B510 No. B2 2 7 14 6 8 9 8510 1 8 12 6 7 14 6 8 7 22 2 10 17 27 7 13 6 8 7 22 4 11 23 11 7 23 10 7 24 10 7 23 10 11 7 23 10 24 10 8 4 17 23 10 13 11 10 24 10 8 4 11 16 11 12 24 10 11 7 23 10 13 12 24 10 11 7 33 56 47 6 36 11 11 12		BS12	55 25 49 27	4 II 1 r 0	4 0 0 0 1	262548	충쳢₄빆ь	12
Place Bit Bit </td <td></td> <td>BSII</td> <td>13877</td> <td>8 6 6 8 I</td> <td>5 % ° 6 %</td> <td>0303I</td> <td>32300</td> <td></td>		BSII	13877	8 6 6 8 I	5 % ° 6 %	0303I	32300	
Piece BS1 BS2 BS3 BS4 BS5 BS6 BS7 BS8 10		BS10	- on d on 4 f2	5 x 1 5 v	ជនួង ្ខ ដ	2015 2015 2016 2016	1 v n 8 2	
Piece BS1 BS3 BS4 BS5 BS6 BS7 YO. 1 8 12 6 7 14 6 8 YO. 1 8 12 6 7 14 6 8 1 1 1 25 34 11 47 41 5 1 1 25 34 11 47 41 6 8 26 9 10 11 7 25 6 8 26 9 10 11 47 41 7 2 23 10 9 12 14 11 10 1 24 17 45 17 47 41 11 7 3 34 11 17 25 18 17 12 4 14 15 16 13 14 14 11 8		BS9	812518	26 I 65 I	8 1 S S S S	411112	138 38 4	1 1
Piece BSI BSI </td <td></td> <td>BS8</td> <td>8 7 7 6</td> <td>34233</td> <td>U - 4 6 6</td> <td>1 0 0 9 9 V</td> <td>rar dit</td> <td>13.2</td>		BS8	8 7 7 6	34233	U - 4 6 6	1 0 0 9 9 V	rar dit	13.2
Piece B:1 B:3 B:3 B:4 B:5 No. 1 8 12 6 7 14 1 8 12 6 7 13 2 10 10 7 7 71 13 4 14 11 25 34 111 7 2 21 10 9 12 6 8 26 9 10 11 7 2 22 13 34 11 7 2 22 10 9 12 13 8 4 17 45 17 9 12 14 10 1 2 13 26 9 12 14 11 16 11 7 3 3 4 11 16 11 16 11 16 12 12 12 12 12 12 <		BS7	8 5 8 1 1 1	22 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1	16 10 16 18	28331	31111	20.0
Pilecen BSI		BS6	80.85 m	7 6 0 8 1 3 8 7 4 4	8 1 8 8 8 7 8 8		25 9 36 336 37	18.2
Pilecen BSI		BSS	1 20048	33388	65550	, 91 65 18 81 65 18	*****	17.9
Piece B:1 B:3 B:3 No. B:1 B:3 B:3 No. B:1 B:3 B:3 1 0 10 10 7 5 1 0 27 5 6 8 26 9 6 8 26 9 7 5 221 10 10 1 4 24 14 11 1 5 22 11 11 1 4 24 14 11 1 4 4 14 11 1 4 4 14 11 1 4 4 14 11 1 4 4 14 11 1 4 1 3 11 1 4 9 3 4 11 1 1 1 3 3 3 <		-	L LI L 26	01 0 0 0 0 0 1 0 0	. 50 r su o r	. 44~°,	3"53"	
Piece BS1 10 1 1 8 1 8 2 1 4 14 6 8 7 5 8 7 9 4 10 14 11 7 12 3 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 7 11 1 12 1 13 1 14 1 15 1 16 1 17 1		BS3			1 R81~¥	4 1 4 1 8 8 8) Ц°Ц°°	17.8
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		Bott	2	н	II	н		

L.S.D. (P = 0.05) between means of percent weight loss = 8.3%.

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Analysis of variance

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Source of variation	ss	4	SH	Variance ratio
Between isolates	21119.5	41	515.0	2.25 *
Between bottles	45896.5	168	273.2	1.19**
Hithin bottles (error)	192386.5	840	229.0	
TOTAL	259402.5 1049	1049		

\$1971ficant at 0.05 level
Not significant at 0.05 level

For illustration see text figure 9.

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Breaking strength (Newtons) of wood pieces after incubation period.

Buttle Piece No. No.	н	ага е с 11	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	> 222222	Rean
ce BSI	150 22 150 250 150 250					
BS2	210 210 270 330 330					
BS3 1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	140 360 315 315				1
BS4 B	5 8 9 9 9 9 8 9 9 9 9 8 9 9 9 9 8 9 9 9 9					
BS5 BS6	280 160 280 160 220 100 120 100 130 100	170 140 310 170 300 270 240 85			190 210 170 200 140 150 200 110 250 50	205 185
6 BS7	0 10 10 10 10 10 10 10 10 10 10 10 10 10 1	5 190 120 5 190 120 5 190 120 5 190 120				5 179
BSB .	240 240 380 380 380 380	250 250 250 250 250 250 250 250 250		260 330 150 150	130 350 1 130 350 1 100 100	230
658	8 8 9 9 8 9	20 ° 12 22 22 50 ° 12 22	330 220 170 150	15 0 19 50 20 15 0 19 50 20	190 220 15 150	154
BSIO	190 250 120 120	210 270 230 230	120 120 120 120 120 120 120	23 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	150 170 210 210	174
BSII	410 360 180 180	210 270 380 370	140 310 110 410	130 380 250 250	170 220 220 220	234
BS12	160 280 35 280 233	8 1 x 1 8	120 230 390 120 20 390	315 20 275 10	8 3 3 8 3	153
BS13	210 270 160 200	170 380 380 270 270	50 210 290 100	215 320 110 380	180 190 180 180 180	525
3 BS14	220 370 270 280	190 200 230 230 110	210 22 25 26 20 25 20 20 20 20 20 20 20 20 20 20 20 20 20	899 999 899 599 999 999 999 999 999 999 999 999 9	210 120 390 180	240
a BS15	250 220 330 330 330	250 260 260 260 260 260 260 260 260 260 26	260 320 170 30 30	320 450 450 450 450 450 450 450 450 450 45	400 180 320 320	274
5 BS16	82888	240 280 310 180 180	170 180 300 140	90 ° 80 90	160 360 60 270 160	179
BS17	ឌ៩ខ្លួន	230 366 190 190	190 190 180 180		250 220 310 320	204
1 BS18	360 150 0 180 180	370 370 370 370 370 370 370 370 370 370	130 150 320 140	180 150 120 310	120 150 220 185	159
BS19	220 200 210 150	150 180 140 140	20 20 20 20 20 20 20 20 20 20 20 20 20 2	892282 8928 8928 8928 8938 8938 8938 893	150 150 150 150 150 150 150 150	207
9 BS20	340 270 390 390 390	320 210 350 350	8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	250 270 385	2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	248
BS21	85853	180 330 170 180	55 51 52 93 99 91 92 93 99 93 93 93 93	160 300 90 225	340 185 150 150 170	173
1 BS22	8 8 9 9 8 8 8 9 9 9 9 9 9 9 9 9 9 9 9 9	20 20 20 20 20 20 20 20 20 20 20 20 20 2	165 160 160 160 210	210 285 320 320 290	8 2 2 3 1 8 1 2 2 3 3 1	236
BS23	350 200 210 250	140 340 170 180	215 150 240 330 5	240 250 150 140	220 220 310 290 290 290	213
3 BS24	160 270 330 330 200	270 270 28 29 29 20 20 20 20 20 20 20 20 20 20 20 20 20	40 140 215 300 210	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	370 1160 215 215 280 215	515
BS25	88888	460 310 40 70 70	282282 2822	330 160 170	50 20 50 51 20 30 51 20	164
BS26	120 120 28 28 28 28 28 28 28	170 20 350 20 20	290 260 340 340	110 410 380 380 480	280 320 270 310 310	221
1258 0	20 360 20 360 20 360	320 370 370 40	50 10 20 250 30 20 250 30 20	20 160 20 20 20 20	360 376 376 376 376 376 376 376 376 376 376	208
BSZB	3 2 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	8 9 9 9 8 8 9 9 9 8	88888	410 240 130 130 10	\$ 8 8 8 8 9 \$ 8 8 8 9 \$ 8 8 9	133
BZ28	60 370 250 250	36 2 20 2 20 36 2 20 2	180 15 20 30 20	8888888	8 8 8 9 8 I	170
	5 5 5 5 5 5 5 5 5 5 5 6 1 5 6 5 1 5 5 6 1 5 5 5 5	20 130 220 220	140 250 210 120	250 450 120 120	360 360 390 390	222
~	220 310 310 310 310 310 310 310 310 310 31	190 330 330 330 330 330 330 330 330 330 3	400 280 140 140	15 140 320 310	8 9 9 9 9 9	505
<u> </u>	8888888	420 360 170 5	8 8 8 8 8	100 120 120 120 120 120 120	5 2 2 3 8 5	197
1	220 190 270 320	460 390 360 360 460	8 8 8 8 8 8 8 8 8 8 8	5 280 210 320	5555 S	237
~ I	25 210 240 180	330 320 180	180 395 395 395 395 395 395 395 395 395 395	130 240 370 410	280 190 190 190 190 190 190 190 190 190 19	251
<u> </u>	410 220 250 350 350	270 240 170	30 6 1 90 32 9 90 32 90	5 170 190 240	430 270 270 270 270	262
"	8 2 8 2 8	15 160 160 160 160	26 10 26 20 26 20 20 20 20 20 20 20 20 20 20 20 20 20	120 15 30 15 120	ខ្លីខន្ល	159
ina l	2 0 0 0 0 3	8 N N N S	35 6 1 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	260 100 150 20 25	85538	116
"	130 29 29 29 29 29 29	100 360 330 330	350 210 20 270 270	460 140 130 130	220 52 50 50 40	199
	36 55 6 0	15 350 290 190	80 390 80 25 25 25	5 1150 320 170	250 350 350 350 350 350 350 350 350 350 3	517
	350 350 290 290	12 250 13 4 30 19 50 19 50 10 50 10 10 10 50 10 10 10 10 10 50 10 10 10 10 10 10 10 10 10 10 10 10 10	390 290 350 350	868338	2 2 2 2 2 2	187
	270 310 120 150	400 370 370 370 370 370 370 370 370 370	340 190 270 270 270	250 250 250 250 250	128 139 130 128 139 139	8
. 1	8 9 9 9 <u>8</u>	8 1 2 0 3 0 1 8 0	25 25 10 10	2 2 2 3 <u>3</u>	20 20 20 20 20 20 20 20 20 20 20 20 20 2	8
	8 8 8 <u>8</u> 8	40 20 20 50 50 50 50 50 50 50 50 50 50 50 50 50	380 420 320 440	84 79 79 79 79 79 79 79 79 79 79 79 79 79	69 8 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	413

Analysis of variance (exclusive control)

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Source of variation	SS	4	R	Variance ratio
Between isolates	1338936.3	41	32657.0	2.57 •
B¢tween bottles	2284356.0	168	13597.4	1.07
Within bottles	1065678.0	840	12686.6	
(error)				

Significant at 0.05 level
 Not significant at 0.05 level

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for illustration see text figure 9.

LXESV	25 4 4 29 25 4 4 29	16 64 64 84 84 84 84 84 84 84 84 84 84 84 84 84	19 23 518 518	19 14 51 59	55 58 111 15	29.9
92×554	2227ø	22623	408669 408669	22222	22222	19.5
LZ×SSV				299229	25 26 38 38 38	18.1
ØZ×SSV	112 112 112 113 113 113 113 113 113 113	356114 356	2 2 2 3 2 2 2 3 2 2 2 3	6 2 2 8 0	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	16.91
TIXSEV						1 8.61
1	2004 12 2004 14 2014 14			126571		œ
7×052A	8524®	10128°23	2 7 9 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7	0.8101	μ 6 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 0 1 1 0 1 1 0 1 1 0 1 0 1 1 0	9.8 19.
92×05 24	9 10 10 10 10 17	10 17 10 17 10 17 10 17	1212 6 B	21191	66.0.28 26.0.28	.6 18.
72x022A	ч ч 0 г 4 4 и	52 122	1,0027	0 6 1 6 9 F H 16 9	512 22 11 22 12 22	.4 16.
\$2×0254	9 7 8 7 7 9 7 8 7 7	1 % % 1 °	3-46%	1991 1991 1997 1997 1997 1997 1997 1997	₽ 8 6 F M	.7 20.
TTXOZSV	16 54 18 18	27 64 17 19	42 11 12 11	16126	112232	23.
L×SISV	55 51 61 61	556 8 5	ដាដូន	31112	31 30 20 20 20	21.8
9Z×StSV	2 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 2 2 4 0 7 7 7 7 7 7 9 7 7 7 7 7 7 7 7 7 7 7 7 7	221 121 251 251 251 251 251 251 251 251	31,515	89999	17.6
LZXSTSV	9 0 0 1 T 4	12 20 20 20 20	404L4		დოდდო	10.0
\$\$X\$124	ט אימטיס אימטיר	2415	4 13 4	32 15 36	32 32 42 42 42	20.0
II*SISV	15 15 15 15	19211	6 2 3 6 7 9 7 9	2 11 11 12 5 26 11 12 12 5	ဇက္ခိ က အို အ	23.2
2×612A	56 8 2 4	11 26 8 17	6 24 25 25 25	11585	252 2 4 7 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	29.2
9Z×ETSV	11 8 17 17	52 8 6 E	50 4 50 50 4 50	6 10 10 10 10 10 10 10	8 6 21 25 55	20.1
LZXETSV	11 o the E	6 4 9 7 6 9 1 9 7 6	8 C 11 2 12 2 1	531 3 4 4 231 3 4 4	25 27 26	20.5
\$2×£134	22 51 23 12 24	31 16 24 24	9 7 6 8 7 e	37 23 10 1	44 35 35 36	27.2
TTXETSV	• 65 9 1 • 6 2 2 1 8 1 • 1 8 1	218 218 208 208 208 208 208 208 208 208 208 20	60 58 58	9 16 16	63 35 97 ° 11	28.4
L×GISV	51 1 9 53 51 51 1 9 53 51 51 1 9 53 51	55 55 51 55 55 55 55 55 55 55 55 55 55 5	5 5 3 3 2	9 7 1 3 4 3 4 5 3 4 0 3 4 5 4 5 4 0	36 36 16	29.4
92×615V	မစစ္ကအဖ	1 8 4 5 4 4 0 8 4 4 9 6	0.42 0.42 0.42 0.42 0.42 0.42 0.42 0.42	6 6 5 5 5 6 1 4 5 5 3 6	13 51 42 42	23.8
LEXGISV	4 × 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	12826	14 90 100 14	14 9 13 8	19 12 13 13	17.5
¢z×Gtsv	38258	12 12 13 18 15 1	44 172 20	120 130 150 150 150	214123 25 25	30.3
11×615V	9 14 14	13 14 15 15 15	54 34 210 210 210	24 19 19	12 11 2 14	21.9
	1		• 1			1
LSV	8 - 3 - 3	80 v 50 60	12 10 13 13 10 13	16 15 15	11 13 13	20.2
9254	22232	13 13 13 13 13 13 13 13 13 13 13 13 13 1	10 19 12 12	12 23 12 23	219 4 5 219 4 5	16.9
125V	0 2 9 6 9 6 7 9 6 9	E 6 6 7 1 1	12 F 2 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2	15 27 29 14	51185	24.0
\$254	33 22 34 33 32 32 34	16 28 28 28	23 35 35 22	8 5 5 5 1	19 25 22 16	26.2
Ttsv	14 11 11 11 12 14	21 21 22 21 21 21 21	23 2 3 8 6 7 5 7 9 8 6 7 5 7 9 8 6 7	22222	4 N N O N	25.9
SSA	58 10 11 24	45 66 11 113	22122	12 15 15 15 15 15 15 15 15 15 15 15 15 15	70 113 2	21.9
V550	85 1 5 8 1 5 8 1 5 8	135 11 135 135 131 135	22 24 22 22	10 12 12 12 12 12 12 12 12 12 12 12 12 12	889986	34.0
SIEV	64 65 65 65 7 65	3 4 6 8 9 3 4 6 8 9	9 EI 2 63 7 6	212 12 12 12 12 12 12 12 12 12 12 12 12		32.7
ETSV	26 20 18 18	335 52 52 53 53 53 53 53 53 53 53 53 53 53 53 53		251223		27.5
6154				44548		36.1 2
Plece No.		60 0 0 0 0 1	112645	116 118 202	22222	Hean
Bottle No.			III	N	>	

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Table 20a. Changes in measurement of decay of pieces of P. sylvestris sapwood incubated for 10 weeks with 10 monokaryons of S. lacrimans (isolated from sporophore A) and 25 dikaryons formed by maining the monokaryons in all possible comb inditions.

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Weight loss as 1 of dry weight after incubation period. ¥

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(ourroj	400 410 370 370 370	900 94 94 12 12 12 12 12 12 12 12 12 12 12 12 12	648844 648844 000000			3
7×68A	300 310 310 310 310 310 310 310 310 310	19 00 00 00 00 00 00 00 00 00 00 00 00 00	255 252 252 252 252 252 252 252 252 252		9 2 9 6 6 8 8 9 8 9 8 8 8 8 8 8 8 8 8 8 8 8	163
92×5:54	260 260 260 260 260 260 260 260	240 240 240 240 240 240 240 240 240 240	320 320 220 160	1) 1) 0) 1) 1) 10 0) 10 0) 10 10 11 10 0) 10 10 11 10 0) 10 11 10 0) 10 11 10 0) 10 1	270 230 350 350	523
LENGHV	170 250 250 250 250	280 280 280 280 280 280	75 260 260 250 250 250	250 250 250 250 250 250 250	47 0 0 0 1 1 0 0 0 1 1 0 0 0 1	2
vz×ssv	340 270 270 270	250 250 270 270 270	200 320 320 290 70	260 120 300 300	260 200 200 215	219
t × 58V	500 140 200 140 200 200 200 200	300 240 210 220	250 250 210 220 220	210 320 130 130	220 220 220 220 220 220 220 220 220 220	217
LXOZHV	230 330 310 260 240	140 300 10 155 190	290 240 260 260 260 260	290 340 300 275	100 75 230 235 270	222
92×025V	270 350 250 270 270	250 90 350 70	270 230 230 260	110 310 220 310	200 200 115	196
LZX025V	280 350 375 375	260 265 265 265 265 265 260	280 300 183 183 265	320 190 140 330	375 10 170 230 230	241
11×025V	190 50 180 220 170	200 5 220 220 170	60 240 233 333	200 230 190 160 290	220 225 300 300	192
L¥STBV	80 100 260 250 250	250 190 160 160 160	280 260 270 270 200	130 340 220 220 100	83 310 170 200	200
Va12*50	5 170 210 250 270	10 250 250 230 330	100 160 270 330 20	290 230 330 120	140 210 2200 320	215
LEXSISV	420 240 250 250 250	255 250 250 310	330 340 270 290	290 340 340 5	320 360 360 320 240	295
45×318V	30 265 273 32)	350 290 120 250	100 250 250 250 250	173 265 265 265 265	8657 667 69	207
ττ×ςτυν	105 25 275 275 220	260 250 250 250 250 320	180 10 315 240 0	240 240 320 260 160	300 3 4 0 3 0 0	208
LXETSV	230 55 280 280 280	250 230 230 230 230 230 230	320 70 160 160	337 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	360 360 360	156
92×618V	200 220 150 130 270	320 330 330 330	10 260 210 220 310	333 193 260 260 260	240 310 120 120	201
42×6 184	330 350 290 70	230	365 365 215 215	230 100 100 100 100 10	240 280 310 280 280 280 280	225
42×E 184	130 110 151 115	668888	230 300 300	60 10 10 10 10 10 10 10 10 10 10 10 10 10	20 50 135 30	95
ττ×ειαν	250 250 250 250	252 252 252 252 252 252	125325	0 250 80 170 270	320 260 170 170	154
2×618V	0 180 265 270 30	310 245 295 295 295 295 295 295 295 295 295 29	ខ្លុន្តទុក្ខ	330 260 260 260 260 260 260	270 100 290 240 300	ß
92×615V	340 250 230 230 230 230 230 230 230 230 230 23	22 23 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2			283 271 252 252	A
22°-515V	40 280 290 290 290 230 230	25 25 25 25 25 25 25 25 25 25 25 25 25 2		NAAAA	ผลสถุญ	162
V810*54	320 252 253 250 170	180 C 25 C 25 C 25 C 25 C 25 C 25 C 25 C 25	ននុទ្ធព្រ	N N K N	25 25 25 25 25 25 25 25 25 25 25 25 25 2	151
II×618V	CIE 571 511 512 512 512 512 512 512 512 512 51	25 55 55 55 56 55 55 55 57 55	a 8 8 8 8 7	****	20 30 20 30 20 20 20 20 20	197
LIN	100 310 220 2	320 320 270 170	305 220 225 230 230	265 210 250 250 250 250	300 230 230 220	206
928V	240 233 233 265 153 153	250 270 270 270 290	202 205 205 205 205 205 205 205 205 205	210 270 190 40 230	330 330 200 200 200	230
425V	22 23 21 23 23 21 23 23	333 323 323 165	240 350 380 260 260	210 210 235 235 235	330 335 243 243 293 190	503
428V	22 22 190 10 10 10	260 270 220 250 250	240 220 120 80 310	ខ ភ្លឺ ខ្លី ម្ពី អ	330 260 150 250 270	193
TISV	50 173 223 83	70 210 220 245	230 15 150 190	250 260 270 290 290	8 29 25 25 25 25 25 25 25 25 25 25 25 25 25	Ĕ
SUV	263 263 223 93	40 55 170 170	200 275 275 275 275 275 275 275 275 275 275	170 250 250 250 250	250 265 265 265 585 585	51
Vitso	20200	18 2 5 R 8	200 222 222 165	255 210 23 35	2 C C C	6
STIN	00000	8 8 8 4 3	330 212 330 330	355 345 345 255 255 255	190 200 120 120 0	51
E L SV	260 150 160 290 290	290 260 260 160	250 250 250 250 250 250 250 250 250 250	310 270 280 280 280 280 280	250 200 275 205	207
etsa	235 60 125 135	តកម្ពុជា	ំង អភិព ខ្ល	800000	នត្តដូច	чр Цэр
51.ock No.	rd 64 (M 48 66)	ቆ ሶ ጣ መ ሱ ጣ	ដងជា	យុ សេយុស្ស ក្រុមក្រុមស្	ជនសន	Nean
Nortle No.	14	::	11	ł;	5	

Changes in responsent of decey of picees of P. sylvestris supword inning firm 10 weeks with 11 rendkaryons of S. lacrimans (isolated from sporophore A) and 25 diversion formations in all possible combinated.

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Brestand strendth (Newtons) of pieces after incubation period.

Table 205.

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¢T×¢€Si	80 8 y 4	N 6 8 9 4	9 F 10 19 19	4 10 M 10 M	N 4 4 4 4
0Z×Þ€SU	8 H 6 5 H	5°5'	215 5 6 11 3	6 0 1 7 7 7 6 6	a a 1 a r
T×Þ€SE	23400	138 38 8 6 4 9 8 8	0 0 7 m 0	M W W W M	ង មួយ ទី មិន ទី
0Ex\$£28	81118	11 11 11 11 11 11 11	41201	199112	21 ⁰ 1 2
67×1/2 54	37233	L6 L 6 5 J	4 8 9 1 1	4 01 8 1 8 8	8 9 8 6 9 1 1
29×9650	5 6 8 9 S	22.00	16 54 54 54	22325	63 64 8
¢T×S€SQ	, , , , , , , , , , , , , , , , , , ,	1 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	8252	6 9 7 6 8 6 1 1	N 48 40 10 10 N 48 40 10 N 48 40 N
02×5680	14 2 2 8 18	10 8 7 8 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	8 9 45 9 41 9	5435 °	19 13 19 13 19 13
T×SESQ	1920	5 9 2 9 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	4 10 10 10	5° 5 5 5 5	11 35
0E×5E58	9 1 1 9 0	0 8 1 1 1 1 0 8 1 1 1 1	កដ _ិ ដដូរ	38272	28 61 8 22 22 6 5 2
62×5688	e a 5 5 6	15 10 15 8 10	C C C C 4	26 I B F	000
2\$×SESH	11 2 9 9 5 1 4	50 22 22 19	11 48 33 11 48	18 14 14	1216
\$T×6TSE	14044 1	പ്രഹംഗമ	12 48 5 m	19 12 4 19	8,48 m 9
02×6158	88121	19888		30111	നയനയൾ പ
1×6150		- 15 5 6 6	0.48 4 4	1198°	00000 00000
0E×6150	10004	6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	000000	8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	190580
62×6158	22222	85855 4	2 4 0 4 8 2 9 7 9 6	0 1 1 0 0 1 0 0 1 0 0 0 1 0 0 0 0 0 0 0	8 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8
PTAEESH	9 6 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7	40 10 26 14 14 8 56 56 56 56 14 8 56 56 14 8 56 56 14 8 56 56 56 56 56 56 56 56 56 56 56 56 56	8 14 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	26.9.7.11.7	14 6 7 5 15 7 5 15 15 10 10 10 10 10 10 10 10 10 10 10 10 10
OZ×EESE	90 0 0 0 4	0.000 9.909 9.9000 9.90000 9.90000 9.90000 9.900000000	1979 1979 1979 1979 1979 1979 1979 1979	16 8 9 1 1 8 2 8 2 8 2 8 2 8 2 8 2 8 2 8 2 8 2	na an
TXEESE	25 25 2 112 8 2 1 112 8 2 1	458°°	117 117 127 127 127 127 127 127 127 127	8 ° 7 5 5	2113 68 7 6 1 1 1
OEXEESU	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	**************************************	14 1 14 1 278 1	2014 a 2010	4 El 0, 2 El 2 E
67×EESB	52221 52221	181835	ຈຊູຄູອະ		* 51 0 r 0
z\$×ccsa	04000	9 L 2 8 H	18330	3.4 11 2.6 2.4	1 2222
ÞT×ZES8	18312		44224	4303r	
0Z×ZE SA	28713	0 7 4 5 9	r 12 8 8 9	• ដ ឆ ង ជ	222222
t×2€59	4 9 8 4 E	~ % 0 ~ ~	44051	2224	44400 N
0E×2E 58	82 r 11 8 6	ដក៥៦១	1 % X % 8	7 N N N N N N N N N N N N N N N N N N N	r ≉ s 15
0235×36	8 F 3 E 9	24 48 26 26	4 515 50 51 13 50	6 a J J &	12 0 8 7 0
ZÞ×ZESU	8 E E E E E E E	го 5 36 - 5	-8938	10222	22232
pt×925q	ちろうてて	1 ° % 5 ñ	40440	188 799 789	~ 6 ~ ~ ~
07×82 58	21862	r 88.62 93.88 4	15 28 10 8 8	ខ អ្កី ខ ស្ពី ខ	5 e a e a
t ×oz sa	48824	45,433	18 25 52 52	8 1 4 1 8 49 1 4 8	15 126 126 126
0E×9Z 58	12 e e 1 1	ミアアアタ	48843	រ ដែលសីស	35 27 27
67×82 58	ដូន្ <u>ន «</u> ដ	ស ល ល ល ។ ស	39 19 5 39 19 5	22247	21 Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
26×8259	23636	12230	• 16 1 1	6988	556514
¢tsu	๛๛๚๛๛	60 H 4 6	46 2 6 1 9 5 2 3 2 9 2 9 2 9 2 9 2 9 2 9 2 9 2 9 2 9	r @ 4 4 0	19 19 19 19 19 19 19 19 19 19 19 19 19 1
oz se	0 6 7 9 7 9 6 7 9	51483	202222	8 6 7 7 8 7 7 1 8	17 19 19 29 29 79 19 29 19 29 29 29 29 29 29 29 29 29 29 29 29 29
150	6 2 8 8 7 7	84848	****	2 2 2 4 4 2	99820
oesa	042ni	° ជីដីដីដី	28123	26r88	3 1 1 2 3
6Z 5 G	∾~≋11	585°85	28438	9 8 8 8 9 1 1 4 1	51 e & X a
29 S B	69 14 14 14	68484	12 61 921	112821	8•848
ÞESG	0 21 21 0 21 21 20	68 113 16	21 F 6 8 17	11 81 81 61 6	19145
scsa	02 6 8 15 6	23°83		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	6 n n 6 *
6150	8 7 4 7 1 41	8 11 18 8 1 1 1 8	8 6 7 F 8	88597	96769
EESU	13 7 7 7 9 13 8 7 7 8	4 0 m - 4	44 64 64 65 65 65 65 65 65 65 65 65 65 65 65 65	22201	0060J
7 E 5 H	32 32 15	16 52 23 13	10 14 14 14	16 2 2 6 6 1 1 0 1 2 2 2 6 6 1 1 0	ជន៍ជនរ
97 S E	14 17 17 17 17 17 17 17 17 17 17 17 17 17	01011	96 7 7 8 9 36 8 7 7 7 8	558315	45 25 15 25
8°.					
a Piece No.	1010040	97090	111111	11 11 19 19	88885
Bottle Xo.	н	Ħ	н	AT .	>

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Table 21a. Changes in melaureant of decay of pieces of P. sylvestris survood incubated for 10 weeks with 12 sonderyons of S. licrimans (isolated for sporphone 3) of 36 disaryons (oraged by machine the sonderyons in all possible combineds)

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A. Meight loss as a of dry weight after incubation period.

1613002	150 150 150 150 150 150 150 150 150 150	450 450 450 360 360 360	480 476 500 360 410	380 165 165 165 165 165 165	360 360 365 500	435
¢t×¢csa	340 250 260 120 390	420 310 130 370	350 330 370 350	400 270 270 430 320	280 360 390 330	322
oz×6e su	200 270 290 380 215	200 270 310 5	200 150 170 170	170 112 370 380 310	260 210 210 370 370	220
t×¢c su	280 320 370 370	210 250 320 210 210	400 100 340 320 380	330 325 360 360 360	300 380 160 140	276
0E×\$E58	190- 340 170 180	370 280 300 220	360 240 360 360	160 210 330 340	270 220 180 120 210	240
GZXPESU	270 270 120 260 270	340 340 290 210 210	230 230 230 230 230	260 280 120 280 280	310 300 310 310	258
Z\$×\$CSU	200 280	270 0 370 360	350 340 270 270	240 0 30 80 80	150 320 320	149
DT×SCSU	1350 1320	000000000000000000000000000000000000000	330 330	230 310 320	160 160 160 160	286
OZXSESE	220022	0.0000	2000	270	240 240 240 240	202
T×SESØ	280 340 280	160 260 300	350 270 270	160 310 270 10	300 300 300 300 300 300 300 300 300 300	250
0E×5E58	75 7 300 3 325 325 325	335 336 330 340	375 290 230 210	260 240 230 230	370 370 230 150	256
67×56 58	2500 3 2500 3 1400 3 1400 3	280 22	360 2 2 330 360 2 2 330 360 2 2 330	200 2 200 2 200 2 200 2	110 1 20 2 325 2 230 1	224 2
29×5€ 58	330 3 5 2 330 2 330 2 275 1	280 2 260 3 270 2 270 2 270 3	1150 3 70 3 230 1 230 3 230 3	280 140 3 215 3 230 2 230 2	190 1 200 2 190 2 210 2 210 2	193 2
\$T×GIS8	20000 20000 20000	380 21 380 21 380 22		2200 2300	270 2 1 2 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2	272 L
02×6128	0 250 360 320 320	0 200 200 190 210	0 330 0 385 0 250 0 250 0 310	0 290 0 290 0 250 0 280	0 390 0 290 0 320 0 290	0 275
T×6158	260 260 250 250 230 230 230 230 230 230 230 230 230 23		210 280	240 240 30 30 30 30 30 30 30 30 30 30 30 30 30	2200 2200 270 350	1 230
0E×6TS9	0 300 300 360 360	250 250 250 250 250 250 250 250 250	2300 2300 2300 2300 2300 2300 2300 2300	20 270 190 350 350	400 400 400	254
62×6158	0 230 0 280 0 280 0 280	0 270 0 240 0 250 0 310 0 200	0 320 90 320 0 320 160	0 200 0 350 0 250 0 270 0 270	0 110 5 170 5 240 0 240	240
29×6158	110	100 100 100 100 100 100 100 100 100 100	0 120 5 5 0 218 1 280	280 330 340	330 200 200	190
\$T×CC58	270 270 320 320	110 420 270 400	2600	190 320 170 160	240 420 220 220	297
OZXEESB	250 250 310 250	230 175 400 230	320 370 320 230	290 290 290 290 290 290	240 210 260 240 310	265
TXEESE	260 280 280 280 280 280 280 280 280 280 28	280 280	240 270 240 325	150 110 210 350 250	300 320 320 370	254
06 ×66 88	350 280 320 320 320	280 380 280 280 280	350 220 150 160	260 340 351 351	300 350 240 240 240	F/2
62×Ef su	230 250 310 320	110 160 100 270	360 200 200 200	430 260 335 310	300 280 240 370	244
St-XCE SU	310 290 340 340	240 310 290 290 200	240 150 340 340	340 210 320 360 170	370 290 210 300	274
\$T×2620	120 170 250 260 410	110 360 310 310 450	300 280 220 220	290 260 300 340	340 260 350 150 150	, g
02×2¢ su	170 265 280 280 280	275 100 30 280 60	320 190 300 325	290 300 70 225	330 240 270 130 240	230
t×zេទម	330 360 250 280	430 370 380 290	230 230 230 280 280	280 280 350 380	330 380 340 165	779
OC×ZCS8	1135 250 250 350 300	270 330 140 260	320 20 170 190	150 40 350 350 350	330 350 270 240 50	910
62×2658	320 215 230 320	160 320 250 140	210 270 210 300	10 270 250 270	260 315 215 215 270	727
29×2558	50 340 320 320 320 320	370 310 335 260 190	360 260 260 180	200 200 200 200 200	230 230 230 230 230	240
\$T×8259	320 350 300 300	280 290 340 110	330 270 350 210	330 240 310 290 160	310 370 380 250	286
0Z×8ZSA	170 270 300 310	240 340 280 290	270 240 75 75 280	400 160 300 290 270	290 260 260 260 250	259
t×ezse	330 265 30 30	180 170 210 230	205 200 300 10	370 270 215 15	250 280 280 315	226
0E×8258	290 230 210 220 220	240 240 240 230	200 2300 200	250 260 350 280	20 20 8 20	213
62×6258	200 250 320 270	330 260	370 3260 30 30	260 250 340 175	115 360 350 1 260 1	268 3
29×82 58	220 220 220 220 0	250 250 250	270 280 250 250 250	150 150 150 150 150 150 150	270 250 250 250 250 250 250 250 250 250 25	170
† T58	130 400 90 175	250 380 320 260 10	10 270 200 200 10	300 150 270 330 260	300 360 320 310	246
02.28	250 1 320 4 400 4 360 1	360 2 200 2 200 2 200 2	120 2 150 2 160 2 160 2	280 280 190 190 190 190 190 190 190 190 190 19	2200 3270 2300 3270 2300 3370	260 24
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Table 21b. Changes in measurement of decay of pieces of P. sylvestris suppood incubated for 10 weeks with 12 monokaryons of S. lacrimans (isolated from sporcyhore B) and 36 dikaryons forred by mating the monokaryons in all possible comp. instions.

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Breaking strength (Newtons) of pieces after incubation period.

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Taile 22a. Changes in messurement of decay of pieces of P. sylvestris sapwood decayed by three diffaryotic (4, 5, 8) and three monokaryotic(AS9, AS15, AS7) isolates of S. lactimans after they had been stored on mait agar or on wood blocks at three temperatures for a period of six months.

Weight loss as § of original dry weight after 10 weeks incubation.

Table 214. Changes in Forstantion of docay of Filestris saywood decayed by three districtio (1, 5, 8) and three consignees (859, A515, A57) isolates of 3. Jactions after thay had been scored on rait agai or on wood blocks at three temperatures for a period of six montums.

5. Breaking strength (Vewtons' of pieces after 10 weeks incubation.

Gentrel	ប្រុក្ខុក្ ល្លាក្ខណៈភ្ ម្រុក។ខេទា	000000 00010 01000	9 10 9 10 9 9 10 9 10 9 10 10 9 9		014 014	392
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C Dock	8 8 8 8 8 8 8 8 8 8 8 8 8 8		22 22 32 3 22 2 23 3 22 2 3 22 3 2 3 2 3		2 12 0 15 0 14 16 0 15 14 16 16 16 14 16 16 16 14 16 1	57 23
50ar 8	150 250 75	na -				67
Kood A	280 51 280 51 280 51					200
10 10 10 10 10 10 10 10 10 10 10 10 10 1	135 3 2				1200 m 1200 m 1200 m	125 2
Kood 3	1 10 1 1 20 1 1 20 1				10 1 45 2 40 1	66
97 Lally C			115 60 270			SS
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Actie No.		2-4 2-4		A	:-	

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AST	Wood		896011	753 4 5	92 22 20 20 00 00 00 00 00 00 00 00 00 00	19185	3 - 1 3 8
A	Agar		80 F 80 10 0	60 mg 49 49 mg	94240	16 7 10 15 7 10	0 7 7 7 5 0 9 7 8 7 6 9
AS 15	Mood		12 7 12 33	a a si si 4	19 19 19 19 19 19 19 19 19 19 19 19 19 1	01 6 1 7 2 2 1 2 2 1 2 1 2 1 2 1 2 1 2 1 2 1	14 15 1 B
A	Aga		4-3 ₂₈	14 18 10 10 10 10 10 10 10 10 10 10 10 10 10	80 10 10 17 N	01 0 m 61 r	20345
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	Acar		18 24 21 22 22	23 21 25 25	11 11 12 20	22 52 FI	10 57 57 57 57 57 57 57 57 57 57 57 57 57
	AS7		12 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	2 4 0 4 N	15 29 41	39 29 4	13 26 26
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11	A	Adar	5300 g	26 39 10 10 10 10 10 10 10 10 10 10 10 10 10	16 31 56 31 56	4 6 4 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	2 6 6 6 6
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		Agar	25 25 26 21 21	32 6 8 8 7 3 7 6 8 8 7 3 7 6 8 8 7	28 37 30 30 30 30 30 30 30 30 30 30 30 30 30	26 27 27 26	21 23 23
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		Agar	14 14 14 14 14 14 14 14 14 14 14 14 14 1	45 10 10 10 10	12 14 16	м м Ö л Ö	22 16 8 22 9
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	Å:	Agar	10 10 8 %	ឲដីឲដ <u>ី</u> 4	10 12 18	500 A 6 10	r r 80 61
	AS15	r Wood	67 6 19 19	დი. 14 დი ი	20 12 12	23 24 26 23 22 22 22 22	23 23 23
		d Agar	12 13 13 15 15 15 15 15 15 15 15 15 15 15 15 15	63 29 11 11	15 23 16	22 LL 7 68 10	ගගගට
	AS9	r Wood	55 10 10 10 10 10 10 10 10 10 10 10 10 10	10 10 10 10 10 10 10	5 1 1 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1	52 F1	14 14 18 19 19 19 19 19 19 19 19 19 19 19 19 19
0		d Agar	1 ⁸ 7 32	16 14 25 25	44 35 17 17	5572S	23 16 18
24°C	ω	r Wood	52 37 37 37 37 37 37 37 37 37 37 37 37 37	55 51 57 9	60 55 48 55 70 70 70 70 70 70 70 70 70 70 70 70 70	15 57 16	45 10 [.] 19
		B Agar	55 22 3	40 60 70 600	13 17 17 17 17 17 17 17 17 17 17 17 17 17	90190n	53 33 25
	5	r Wood	8 02 6 % []	278 27 29 29 21 21 29 21	53 51 58 5 8 53 51 58 58	18 18 18 18	20 25 16
		1 Agar	5 E E E E E E E E E E E E E E E E E E E	6 19 19 10 10 10 10 10 10 10 10 10 10 10 10 10	21117 88	17 18 17 17	4821
	4	r Wood	30 14 14 14	53 30 F F F	1 8 7 4 6 5 9 4 6 5 9 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6	12 12 59 18	47 14 13
		Agar	13844	11 25 19 17 25 19	31 5 33 5 5 ·	34 34 37 28	18 16 20
	Piece	No.	-1 N M 4 M	9 9 F 8 6 0	11111	16 19 19 20	2 2 2 2 5
	Bottle		н	H	III	2	~

24.0 27.0 41.0 23.6 36.0 27.0 35.1 10.5 24.2 20.2 25.2 12.8 17.1 21.4 23.9 14.6 20.0 11.9 21.2 11.5 15.8

8.2 19.2 21.4 37.5 16

21.5 27.3 17.4 26.8

21.6 29.1 15.9 22.2 23.6 32

Mean

Table 23a. Changes in pessurement of decay of pieces of P. sylvestris sapwood becayed by three dikaryotic (4, 5, 8) and three mondwrmatic(AS9, AS15, AS7) isolates of 5. lactimans after they had been stored on malt agar or on wood blocks at three temperatures for a period of one year.

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Weight loss as 1 of original dry weight after 10 weeks incubation.

Control	325 480 485 460	500 240 270 490	440 500 390 390	480 460 420 375	480 415 465 415 415	435
	270 170 350 120				240 265 270 230 290	228
od Aga	250 250 250 250 250 250 250 250 250 250			0 350 0 260 0 270 0 220 0 230	0 760 0 150 0 310 0 310	8 253
AS15 Agar Wood	0 120 0 330 0 140 0 210 5 280			0 220 0 320 0 230 0 250	120 120 120	258 188
No.	115 300 250 280 200 320 220 280 370 175		220 290 200 290 10 130 370 310 10 300	240 260 230 360 10 300 90 340 190 80	410 260 220 240 210 250 210 250 230 220 230 220	188 25
Agar Wc	170 1170 1170 1130 2310 2310 2310 2310 2310 2310 231		220 22 20 22	220 380 270 350 22 1160 22	4221230 4221230 4221232	234 1
g	220 1 120 2 190 1 150 3	220 4 140 2 140 1 240 1 165 1	100 2 170 3 170 3 150 2 150 2	120 3 120 3	2210 30 2 2210 1 190 2 190 2	161 2
Agar Wo	150 160 160 160	150 180 250 250	150 150 140	120 180 220 190	190 170 130 200	166
Kood	240 280 290 130 200	230 210 270 270	10 250 150 250 250	200 300 310 120	290 210 200 190 170	200
4 Agar Wood Agar	250 160 190 210	230 270 210 210 30	70 200 40 150 270	80 390 160 240	200 200 320 360 360	195
4 Wood	180 170 190 110 270	170 160 120 110 30	165 250 310 325	310 120 120 195	270 295 300 70	178
Agar	290 130 120 120	195 220 145 150	135 160 260 .175 .175	220 250 250 160 180	210 235 235 240 190	161
Control	390 450 510 310	400 370 425 530	260 420 440 390	510 360 450 490	390 450 380 450	420
R	360 200 370 15	140 360 15 260 20	250 160 110 110	130 160 380 300	150 250 20 20 20	159
AGar Wo	330 190 120 250		350 170 190 320	320 400 120 340	200 280 80 120	264
15 Wood	120 250 70	90 290 330 100	70 5 390 80 0	210 120 290 355	310 290 200 200	145
AS15 Agar We	270 190 200 260	0 320 250	280 350 250 250 270	80 310 290 290	190 250 10 170	177
AS9 r Wood	200 180 200 85		60 360 170 310	50 10 10	0 15 10 10	85
Aga	30 290 170 170		200 230 100 40	460 20 30 20	70 150 150 310	164
20°C B ar Wood	210 210 210 210		19999 1999 1999	0 120 120 140	0 160 140 20 140	80
Wood Agar	0 170 5 195 0 230 0 230		135 130 125 165 120 110 140 70 90 120	80 140 140 150 135 170 155 90 120 75	5 90 5 135 0 115 0 70	4 130
5 Agar Wo	200 200 20 140 20 125 20 125 210 150		280 13 270 12 260 12 350 14	320 14 320 14 250 13 210 13	80 160 250 125 150 145 300 170 50 90	198 134
Wood Ac	998899		6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	130 130 140 140 140 140 140 140 140 140 140 14	102 1
Agar W	210 1 210 1 45 1 130 1		40 220 1 270 1 170	150 1 320 160 230 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	171
Control	450 360 440	465 510 440 410 410	430 500 280 430 460 ·	420 500 520 410	500 440 480 480	443
Rood	310 200 250	290 280 300 60	250 250 220 220	280 200 260 180	230 230 250 250	202
Agar	350 380 410	380 330 330 300 250	360 300 310 260	330 330 340 370	330 310 290 310 350	326
AS15 ar Wood	180 180 180 180	2/0 90 10 310 310	190 340 290 290 70	210 240 190 190	310 220 200 200	188
A 1			270 220 220 220	260 170 250 350	400 340 280 290 190	239
AS9 AS9	0 160 150 35		120 120 120 120	200 340 290 290 290	0 30 315 150 150	153
C As	0 100 0 360 0		0 100 240 210 230 230			8 221
24°C B Anar Wood	10 30 250 10 10 180 130 190		280 130 220 130 220 80 230 80	280 220 290 15 90 10 220 250		6 128
			120 280 130 260 125 220 145 290 150 5			164 176
Post N						230 16
						147 2
3	250	310 310 280 280 280	170 180 100 100			205
ar Bood						1
Piece 4		N 0 N 0 N 0	12222	1111 111 111 111 111 111 111 111 111 1	22 22 25 25 25 25	Mean

Table 23b. Changes in measurement of decay of pieces of P. sylvestris sapwood decayed by three dikarrotic (4, 5, 8) and three monokaryotic (AS9, AS15, 357) isolates of 5. lacrimans after they had been stored on mait agar or on wood blocks at three temperatures for a period of one year.

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B. Breaking strength (Newtons) of pieces after 10 weeks incubation.

Table 24a. Comparison of saprophytic ability of cultures of S. lacrimans isolated from different parts of colonies which had been subjected to high temperatures for different periods of time.

(1) Results of tests of cultures made within 30 days of heat treatment.

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(A) Weight loss as % of original dry weight after 10 weeks incubation.

Bottle	Culture No.					Parent
No.	Piece No.	1	2	3	4	Culture
	1	19	60	40	19	49
	2	63	20	18	17	19
I	3	51	24	20	14	57
	4	12	26	21	6	13
	5	44	12	39	30	. 53
	6	1.3	41	63	64	37
	. 7	17	6	15	29	12
II	8	60	62	39	64 ·	19
	9	56	21	43	12	14
	10	. 22	37	34	18	64
	11	53	11	42	15	56
	12	56	20	56	21	13
. III	13	53	59	53	58	55
	14	28	47	45	61	31
	15	13	32	53	14	22
	16	29	11	9	35	51
	17	13	5	21	32	14
IV	18	12	13	48	32	48
	19	50	6	57	44	54
	20	17	65	37	63	53
	21	55	9	12	54	20
	22	37	52	21	56	56
v	23	44	18	23	46	19
	24	16	33	26	50	59
	25	36	33	62	55	19
	Mean	34.8	28.9	35.9	36.4	36.3

L.S.D. (P =0.05) between means of percent weight losses = 10.13%.

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.

For illustration see text figure 17.

Table 24b. Comparison of saprophytic ability of cultures of \underline{S} . Lacrimans isolated from different parts of colonies which had been subjected to high temperatures for different periods of time.

(1) Results of tests of cultures made within 30 days of heat treatment.

(B) Breaking strength of wood pieces after 10 weeks incubation.

Bottle	Culture No.						
No.		1	2	3	4	Parent	Control
	Piece No.		•			Culture	
	1	250	0	110	250	40	350
	2	0	270	320	290	280	430
I	3	10	290	260	310	0	380
	4	10	250	180	320	280	450
	5	60	280	50	190	10	320
	<u>,</u>	240	40	0	0		250
	6 7	340	40	0	0 110	130	350
II	8	320 5	340 0	310 30	0	290 180	330 410
Т.Т.	9	5 10	270	30 45	310	150	410
	10	290	270	40	290	5	450
	IO	290	90	40	290	5	450
	11	· 15	320	30	270	10	340
	12	0	350	10	290	320	400
III	13	10	5	10	0	10	330
	14	120	15	10	0	120	380
•	15	310	70	10	240	200	390
	16	100	320	340	50	10	380
	17	300	360	260	80	300	400
IV	18	260	320	5	45	40	350
	19	10	260	10	10	5	460
	20	310	0	65	0	10	390
	21	10	400	320	5	200	400
	21 22	100	400 10	320 290	5	200 5	390
v	22	40	240	290	10	290	390 420
v	23	300	100	150	10 5	290 0	420 390
	24 25	300	50	150	0	270 ·	460
6	Mean	128	185	122	125	125	393

L.S.D. (P = 0.05) between means of breaking strength = 70.5 Newtons

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.

Table 24c. Analysis of variance of data in Appendix Tables 24a and 24b.

A. Percent loss of dry weight

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Source of variation	SS	°F	MS	Variance ratio
Between Isolates	1069.0	4	267.3	0.80 *
Between Bottles	8455.0	20	422.8	1.27 *
Within Bottles (error)	33385.0	100	333.9	
TOTAL	42909	124		

B. Breaking strength [exclusive control]

Source of variation	SS	°F	MS	Variance ratio
Between Isolates	75163.0	4	18791.0	1.16 *
Between Bottles	552247.0	20	27612.0	1.71 *
Within Bottles (Error)	1615270.0	100	16152.7	
TOTAL	2242680.0	. 124	*****	

* Not significant at 0.05 level.

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Table25aComparison of saprophytic ability of cultures ofS. lacrimansisolated from different parts of colonies which had beensubjected to high temperatures for different periods of time.

(2) Results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment.

Bottle	Culture No.	1	2	3	4	Parent
No.	Piece No.	.بلد مدر بین کار میں کار میں کار میں کار	ے بینی بینی بین		1- 	culture
	1	11	15	46	48	58
	2	12	57	23	14	12
I	3	63	38	10	50	40
	4	10	30	40	10	58
	5	32	11	42	7	31
·	6	34	33	58	9	10
	7	17	20	18	46	12
II	8	12	23	16	49	54
	9	27	20	16	16	56
	10	33	21	46	52	45
	11	44	11	58	10	39
	12	11	55	28	49	21
III	13	59	11	18	10	38
	14	47	12	31	47	17
	15	34	15	23	7	24
	16	16	14	26	20	56
	17	3 5	14	26	15	58
IV	18	18	10	57	8	20
	19	15	25	41	23	31
	20	15	48	51	44	11 '
	21	17	12	13	12	42
	22	15	58	15	28	13
v	23	18	11	40	50 .	16
	24	17	10	25	11	36
	25	20	50	30	50	15
	Mean	25.3	25.0	31.9	27.4	32.5

(A) Weight loss as % of original dry weight after 10 weeks incubation.

L.S.D. (P = 0.05) between means of percent weight losses = 9.5%.

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had b been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.

For illustration see text figure 18.

Table 25b. Comparison of saprophytic ability of cultures of S. lacrimans isolated from different parts of colonies which had been subjected to high temperatures for different periods of time.

(2) Results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment.

Bottle No.	Culture No. Piece No.	1	2	3	4	Parent Culture	Control
	FIECe NO.					·····	
	1	340	340	50	10	10	360
	2	280	10	270	200	290	450
I	3	0	170	440	5	140	500
	4	270	310	90	300	20	470
	5	230	340	60	300	165	310
	6	190	90	5	290	250	410
	. 7	265	270	270	20	250	490
II	8	280	270	340	0	30	470
	· 9	270	290	300	230	10	395
	10	30	360	30	5	60	500
	11	40	340	10	390	50	500
	12	300	20	150	10	250	300
III	13	5	290	260	300	70	460
	14	5	300	100	10	200	500
	15	150	350	200	360	5	380
	16	370	280	265	300	230	440
	17	50	310	70	310	10	440
IV	18	280	290	10	310	210	400
	19	370	180	260	270	220	380
	20	50	10	20	20	250	4 90
	21	3 20	310	270	310	50	450
	22	320	5	320	180	280	460
v	23	370	330	140	30	250	400
	24	240	260	250	320	95	215
	25	340	5	120	10	235	410
	Mean	215	230	172	180	145	423

(B) Breaking strength of wood pieces after 10 weeks incubation.

L.S.D. (P = 0.05) between means of breaking strength = 72.3 Newtons.

- Culture Isolated from a fast growing sector of a colony which had been 1 subjected to 40°C for 15 minutes.
- Culture Isolated from a slow growing sector of a colony which had been 2 subjected to 40°C for 15 minutes.
- Culture Isolated from a fast growing sector of a colony which had been 3 subjected to 38°C for 25 minutes.
- Culture Isolated from a slow growing sector of a colony which had been 4 subjected to 38°C for 25 minutes.

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A. Percent loss of dry weight

Source of variation	SS	°F	MS	Variance ratio
Between Isolates	1291.2	4	322.8	1.11 **
Between Bottles	3614.6	20	180.7	0.62 **
Within Bottles (error)	29026.4	100	290.3	
TOTAL	33932.2	124		······································

B. Breaking Strength [exclusive control]

Source of variation	SS	°F	MS	Variance ratio
Between Isolates	144083.2	4	3 6020.8	3.34 *
Between Bottles	215690.0	20	10784.5	0.63 **
Within Bottles (error)	1700210.0	100	17002.1	
TOTAL	2059983 .2	124		

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* Significant at 0.05 level

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****** Not significant at 0.05 level

<u>Table 26</u>. Measurement of colony diameter (mm 7 days at 21°C) of cultures of <u>S</u>. <u>lacrimans</u> isolated from different parts of colonies which had been subjected to high temperature for different periods of time.

(1)	Results	of	tests	of	cultures	made	within	30	days	of	heat	treatment.
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Culture Replicate	1	2	3	4	Parent* Culture
1	77	71	75	74	80
2	78	75	77	77	83
3	75	72	78	75	84
4	74	74	74	78	85
5	76	73	73	74	83
6	78	76	76	72	79 .
7	73	70	75	76	86
8	77	73	73	73	82
9	79	75	70	79	84
10	75	74	77	72	87
Mean	76.2	73.3	74.8	75.0	83.3

L.S.D. between means of colony diameters = 2.0 mm (P = 0.05)

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.
 - * Untreated culture (Strain 8).

Source of variation	SS	°F	MS	Variance ratio
Between cultures	617.1	4	154.3	30.25**
Within cultures	227.4	45	5.1	
TOTAL	844.5	49	17.2	

Analysis of Variance

** Significant at 0.001 level.

For illustration see text figure 17.

Table 27. Measurements of colony diameters (mm 7 days at 21° C) of cultures of <u>S</u>. <u>lacrimans</u> isolated from different parts of colonies which had been subjected to high temperature for different periods of time.

Culture				a	Parent*
Replicate	1	2	3	4	culture
1	63	59	62	58	66
2	60	55	55	59	67
3	58	57	58	44	66
4	55	55	63	61	61
5	60	52	59	57	65
6	58	58	60	41	60
7	60	57	58	56	66
8	61	54	56	57	61
9	57	59	59	56	66
10	57	54	58	57	59
Mean	58.9	56.0	58.8	54.6	63.7

(2) Results of tests made of cultures which had been stored on malt agar for 6 months after the heat treatment.

L.S.D. between means of colony diameters = 3.4 mm (P = 0.05).

- Culture 1 Isolated from a fast growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 2 Isolated from a slow growing sector of a colony which had been subjected to 40°C for 15 minutes.
- Culture 3 Isolated from a fast growing sector of a colony which had been subjected to 38°C for 25 minutes.
- Culture 4 Isolated from a slow growing sector of a colony which had been subjected to 38°C for 25 minutes.
 - * Untreated culture (Strain 8).

Analysis of Variance

Source of variation	SS	°F	MS	Variance ratio
Between cultures	487.0	4	12.8	8.76**
Within cultures	627.0	45	13.9	
TOTÁL	1114.0	49	22.7	

**Significant at 0.001 level .

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For illustration see text figure 18.

Table 28a. The comparison of wood decaying ability of three multisporous (8,4,7) and three monosporous (AS9, AS15, AS7) cultures of S. lacrimans incubated for 10 weeks with five different building timbers.

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Weight loss as 1 of original dry weight after incubation period. Å.

No. No. <th>Bottle</th> <th>Piece</th> <th></th> <th>8</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>4</th> <th></th> <th></th> <th></th> <th></th> <th>7</th> <th></th> <th></th> <th></th> <th>AS9</th> <th></th> <th></th> <th></th> <th></th> <th>ASI5</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>AS7</th> <th></th> <th></th> <th>ļI</th>	Bottle	Piece		8						4					7				AS9					ASI5						AS7			ļI
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porous (AS9, AS15, AS7) cultures of S. lacrimans incubated	
of three multisporous (8,4, 7) and three monosi	ng timbers.
. The comparison of wood decaving ability	for 10 weeks with five different buildi
Cable 285	

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B. Breaking strength (Newtons) of pieces after incubation period.

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Source of variance	SS	4.	SK.	Variance ratio
Between strains	4589662.4	S	917932.5	7.31*
Between woods	24364865.5	4	6091216.4	48.50**
Interaction	2511704.9	20	125585.3	26.03**
Between bottles	659668.0	120	5497.2	1.14***
Within bottles (error)	2894705.0	600	4824.5	
TOTAL	35020605.8 749	749		

Significant at 0.01 level
Significant at 0.001 level
Not significant at 0.05 level

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