# THE DISTRIBUTION OF THERMOPHILIC AND THERMOTOLERANT FUNGI IN A SPRUCE-PINE CHIP PILE AND THEIR EFFECTS ON SOME CONIFEROUS WOODS

by

ALBERT OFOSU-ASIEDU
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M.Sc., Hebrew University, 1963
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## ABSTRACT

A study into the distribution of thermophilic and thermotolerant fungi in a spruce-pine wood chip pile in Prince George was carried out. Five treatments consisting of pine, spruce, incorporation of wood fines into spruce, sterilized spruce and sterilized spruce inoculated with a Ptychogaster sp. were examined. Samples of wood chips buried at six different locations in the chip pile were examined after 3, 6 and 12 months storage periods. From 100 randomly selected chips from each sample the fungi were isolated on 2% malt, 0.5% malic acid and 2% agar at 25° and 45°C. Data on temperature during storage and acidity of wood chips, moisture content and weight loss at the time of sampling were recorded for the six positions in the wood chip pile.

Thermophilic fungi colonized the inner regions while thermotolerant fungi inhabited the outer regions of the wood chip pile. Among the thermophilic fungi, listed according to frequency of isolation were Byssochlamys emersonii Stolk-Apinis, Allescheria terrestris Apinis, Sporotrichum thermophile Apinis, Thermoascus aurantiacus Miehe and Humicola lanuginosa (Griffonand Maublanc) Bunce. The most common thermotolerant fungi were Aspergillus fumigatus. Fresenius and Chrysosporium pruinosum (Gilman and Abbot) Comb. Nov.

Fungal distribution was generally related to position in the wood chip pile. Of the associated factors temperature (17°-45°C) was most strongly related to fungal distribution, whereas acidity of wood chips and moisture content did not vary greatly between positions.

Incubation of wood samples on cultures demonstrated the ability of all the common thermophilic and thermotolerant fungi to cause weight loss of lodgepole pine Pinus contorta Dougl. varlatifolia, ponderosa pine Pinus ponderosa Laws. and spruce Picea glanca (Moench) Voss sap wood samples. These weight losses varied from 0.65% to 25% after six weeks incubation. Temperature, medium and type of wood affected the ability of the fungi to cause weight loss. No synergistic or antagonistic effects existed between the thermophilic fungi.

Chemical analysis of degraded wood indicated that the thermophilic fungi utilized the arabinose fraction of the hemicellulose preferentially.

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Department of Forestry
The University of British Columbia
Vancouver 8, B.C., Canada

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# I INTRODUCTION

The storage of wood in the form of wood chip piles is a practice widely adopted by pulp mills around the world. The method has many advantages over roundwood storage such as reduced handling cost, reduced storage area, the ability to mix various types of chips to any proportion easily, and the ability to use sawmill residues for pulp economically. Storage of wood in chip piles also has certain disadvantages such as the high temperatures commonly generated in the pile with the attendant risk of fire and the degradation of the wood chips. It has been estimated, for instance, that in the interior of British Columbia, annual losses of $5,000,000 to $30,000,000 will occur by 1987 due to these factors if corrective action is not taken (Hatton, Smith and Rogers, 1968).

In the interior the main species used are lodgepole pine (Pinus contorta Dougl. var latifolia) and spruce. For the purpose of this thesis spruce refers to trees belonging to the complex consisting of Picea glauca (Moench) Voss, Picea engelmanii Parry and the various hybrids formed between these two species as it is found around Prince George B.C. No attempt was made to distinguish between the various forms.

The wood is either chipped at the pulp mill from freshly cut logs or else it arrives at the mill as chips produced from sawmill residue. In

contrast to the losses sustained by pulp mills in the interior, coastal pulp mills in British Columbia and the Pacific North West, using different species of wood, report little degradation of wood chips even after a year of storage (Wright, 1954). It follows that the experience gained on the coast cannot be applied directly to the new situation in the interior. Hence a large scale study of the problems associated with the storage of wood in chip piles in the interior was initiated by the Forest Products Laboratory.

The phenomenon of heat generation and degradation is not restricted to piled wood chips, but it occurs generally in piled organic matter such as hay, straw, manure, peat and grain (Miche, 1907; Isachenko and Mal'chevskaya, 1936; Waksman, Cordon and Hulpoi, 1939). In general two conditions must be met. Firstly, there must be an adequate supply of water and secondly, the pile must be large enough so that heat dissipation does not exceed heat generation until a high temperature is reached. The maximum temperature reached depends on these two factors plus the nature of the material being stored and the effects of various environmental conditions on the mechanisms of heat production.

Various mechanisms for the production of heat have been proposed. These are respiration of the plant material, respiration of organisms that utilize the stored material as a source of food, and auto-oxidation of organic substances in the stored materials. The origin of heat production in chip piles is not a subject of this thesis.

Isolations from such naturally heated piles of organic matter have yielded bacteria, fungi and actinomycetes, all with the peculiar ability to be metabolically active at high temperatures. All these classes of organisms have been isolated from wood chip piles. General experience with wood degradation has shown, however, that on wood fungi are much more important than either bacteria or actinomycetes. Since, it was impossible to sample all three groups adequately in the experimental chip pile used in the present study because of lack of time and space, it was decided to limit sampling to fungi.

Most fungi in contrast to bacteria are unable to grow at temperatures above 35°C, although spores and various other resting stages of these fungi such as sclerotia may occasionally survice exposure to higher temperatures. A relatively small number of fungi can grow at temperatures up to 60°C. These have been divided into two groups called thermophilic and thermotolerant fungi.

For the purpose of this thesis, thermophilic fungi are defined as those fungi able to grow on a 2% malt extract, 2% agar, and 0.5% malic acid medium at 45°C but not at 25°C. Thermotolerant fungi are those fungi which grow at both 25°C and 45°C on the above medium. Fungi which are able to grow at 25°C but not at 45°C are called mesophilic fungi.

In large commercial wood chip piles, the temperature attained in the interior region generally exceeds 45°C. It appears therefore that apart from the outer layers of such a chip pile, fungal activity is restricted to thermophilic and thermotolerant fungi. For this reason this study deals only with thermophilic and thermotolerant fungi and does not concern itself with

the activity of mesophilic fungi in the outer, cooler regions of the pile.

This thesis forms a part of the larger investigation mentioned above and deals with the role of those fungi which inhabit the hotter parts of a wood chip pile. The central hypothesis is that the fungi inhabiting the hotter parts of the chip pile are directly responsible for the loss of wood substance and that this loss leads to a decrease in pulp production. To support this hypothesis the following will be necessary. Firstly, it must be verified that fungi are present in the hotter parts of a chip pile and that weight losses are incurred in these positions. Secondly, it must be demonstrated that the variation in weight losses observed between various positions in the pile can be attributed to the distribution of fungi. Thirdly, it must be shown that environmental conditions in the pile due to such factors as temperature, moisture and acidity, if they can be correlated with weight losses, cannot by themselves result in the observed weight losses, and that such correlations can be explained by the effect of these environmental factors on the presence of fungi and their ability to degrade wood chips. Fourthly, it must be established that the fungi isolated from the chip pile are capable of causing weight losses of wood chips under carefully controlled environmental conditions. Lastly, it must be shown that at least part of the weight loss of wood incurred during storage is attributable to the loss of components of the wood which are utilized as pulp.

The thesis is divided into three parts. The first part deals with the study of fungi, environmental conditions, and weight losses in an experimental chip pile, the second is concerned with the demonstration of the ability of commonly isolated fungi to cause weight losses and the last part deals with the chemical analysis of degraded wood.

# II FIELD STUDY OF FUNGAL DISTRIBUTION

## A INTRODUCTION

This section of the thesis is concerned with the demonstration that thermophilic and thermotolerant fungi are present and active in the hot regions of a chip pile, that weight losses of wood occur in these regions, and that such weight losses are related to the distribution of fungi. Furthermore, it deals with the relationships between fungal distribution, weight loss, and various environmental factors. Lastly, an attempt is made to show how fungal distribution, weight losses and environmental conditions develop in time.

The experimental chip pile utilized for this study was designed by the Forest Products Laboratory. It was not planned solely for the purpose of this study. For instance samples inserted into the pile had to be large enough to allow experimental pulping studies of the stored chips. This made the accurate determination of weight losses rather difficult.

The pile consisted of four sections, to be broken down after 3, 6, 12 and 24 months. This study contains the results of the first three periods only. It was assumed that similar conditions prevailed at similar position throughout the whole chip pile.

Several treatments were incorporated into the chip pile. The first two consisted of bags filled with pure spruce and pure pine chips in order to determine the effect of these species on the various factors studied. A third treatment consisted of the incorporation of "fines" into the sample bag. Lastly two treatments consisted of the incorporation of a "core" bag containing spruce chips inoculated with a species of Phytogaster, a thermotolerant basidiomycete isolated from chip piles in eastern Canada into the sample bag, and a control consisting of sterile spruce chips in a core bag.

## B. LITERATURE REVIEW

### 1. Advantages and disadvantages of outside wood chip storage

Compared to roundwood storage, the advantages of outside chip storage are many (Anon, 1961; Holekamp, 1962; Westaway, 1968). One of these advantages is the lower cost in handling and storage of the material. It is estimated that a medium sized mill can save up to $50,000 annually using outside wood chip storage instead of roundwood storage (Clark, 1963). Operational advantages (Holekamp, 1962; Clark, 1963) have been reported as follows:

1. Reduced fiber loss.
2. Decreased handling cost.
3. Large manpower saving.
4. Twenty to twenty-five percent increase in production from wood yard to wood room.
5. Reduced maintenance costs.
6. Elimination of production loss because of wood room-wood yard breakdowns.
7. Increased bark yield and reduced fuel costs.
8. Improved uniformity in the digester furnish.
9. Greater storage per acre.
10. Less housekeeping.
11. Smooth wood yard and wood room operation.
12. Better and more accurate species handling.

This method, however, has its disadvantages which are as follows (Allen, 1968; Hatton, Smith and Rogers, 1968):

1. Risk of fire resulting from the tendency of piles to heat up.
2. Losses in pulp yield due to deterioration of chips during storage.
3. Reduction in strength properties of the chemical and refiner-groundwood pulps.
4. Losses in refiner-groundwood pulp brightness.
5. Increased requirements for cooking and bleaching chemicals.

6. Corrosion problems with chip reclaiming systems due to the low pH of severely deteriorated chips.
7. Production of off-grade chips due to contamination of chip piles by air borne particles, such as fly ash and sand.

Despite these disadvantages, the fact that a large number of mills are using or contemplating using this method might indicate that the advantages outweigh the disadvantages.

## 2. Types of wood chip piles

There are as many pile forms as there are mills. No two piles are the same in size, location or volume. However, two main shapes are in common use, the conical piles (Björkman and Haeger, 1963) and the rectangular piles (Björkman and Haeger, 1963; Shields and Unligil, 1968). There is also no standard design for a system of outside chip storage (Hajny, 1966). Recently, construction of piles in a ring form with a section of the ring pile removed so that the two resulting surfaces constitute the beginning and end of the pile has been advocated (Croon, 1966).

Chips are transported to the mills by trucks, trailers, rail, barge and ship (Bergman and Nilsson, 1966; Shields, 1967). In some instances round wood is transported to the mill to be chipped there (Shields and Unligil, 1968). There is little agreement as to what material to use for the base of the pile (Blackerby, 1958; Holekamp, 1959; Annergren, Dillner, Häglund and Jagerud, 1965). The earliest piles were built directly on the ground, and packed clay and sand were used as the base. Under these conditions 2 to 3 feet of chips have been left as the base of

a new pile. In some cases hogged fuel or bark have been used in place of this layer of chips.

A hard surfaced base such as black top or concrete is generally considered to be the most satisfactory, although the most expensive. An experimental pile was constructed on a polyethylene sheeting (Butcher and Howard, 1968).

Chips are blown on to the area by pneumatic chip blowers and are then spread out by crawler type tractors which compact them at the same time. Not all the piles are mechanically compacted and in such cases the chips are allowed to compact themselves.

The volume and the final height of the chip pile are two important factors determining the efficiency of the processes which take place in the pile. Experimental piles have generally been small and low (Butcher and Howard, 1968) varying from 9 feet to 145 feet. The experimental piles in Sweden were usually large and tall (Annergren, Dillén and Vardheim, 1964; Annergren, Dillner, Häglund and Jagerud, 1965). The volumes of piles in Canada have averaged from 21,000 cords to a huge 100,000 cords (Robinson, 1968; Shields and Unligil, 1968). These varying physical characteristics of the piles must account in part for the tremendous variability of the results from studies on chip piles.

The major species used in chip piles on the west coast of North America are Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco], western hemlock [Tsuga heterophylla (Ref.) Sarg] white fir (Abies concolor Gord.

and Glend), western red cedar (Thuja plicata Don), pines (Pinus sp.), red alder (Alnus rubra Bong.) and cottonwood (Populus trichocarpa Torr. and Gray) (Wright, 1954; Blackerby, 1958; Hensel, 1958). In the southern U.S.A. many piles are built of pine species.

In eastern Canada, balsam fir (Abies balsamea (L.) Mill), spruce (Picea sp.), pine (Pinus sp.) and eastern hemlock (Tsuga canadensis (L.) Carr) have been stored as chips (Blackerby, 1958; Shields and Unligil, 1968). Pulp mills in Scandinavian countries have experimented with birch (Betula sp.) spruce and pine species (Annergren, Dillén and Vardheim, 1964; Annergren, Dillner, Häglund and Jagerud, 1965; Björkman and Haeger, 1963; Bergman and Nilsson, 1966). Butcher and Howard (1968) have studied the behaviour of Pinus radiata D. Don. in small piles during winter and summer storage in New Zealand.

Conflicting accounts of the behaviour of wood in outside storage are common, possibly because of the variation in resistance to deterioration of the wood of different tree species. Piles of Douglas-fir on the west coast of North America do not seem to suffer even after three years of outside storage while alder is badly deteriorated after four months of storage. A mixture of alder and Douglas-fir could stand longer outside storage than alder alone (Wright, 1954).

The length of time that chips can be kept in storage varies with species, climate and pulping process (Hajny, 1966; Shields, 1967). In the Pacific Northwest chips have been stored for 2 to 3 years or more with little evidence of deterioration (Blackerby, 1958; Burke, 1962).

It is concluded that although many of the west coast species can be stored for lengthy periods without deterioration, such a condition might not prevail with other commercial species and that testing each species separately might be necessary.

In the southern United States chips of southern pines deteriorate much more rapidly than in the Pacific Northwest. This condition is reflected in the storage time recommended by workers in the southern United States. Holekamp (1959) recommends summer storage of three months, whilst Somsen (1962) gives 16 weeks as the maximum storage time. Rothrock, Smith and Lindgren (1961) stored slash pine chips for 5 months in the southern U.S.A. and showed that outside summer storage of pine wood is feasible within this limit. In Nova Scotia, Robinson (1963) recommends two months storage.

Deterioration of chips was found to be severe in the lower third of a spruce and balsam fir chip pile at approximately nine months, indicating that complete utilization of a pile should occur before eight months at least in order to minimize reductions in chip quality that could affect the pulp adversely (Shields and Unligil, 1968). Storage of softwood chips for 15 or more months involves risks of deterioration to spruce and pine chips (Björkman and Haeger, 1963) and Forssblad (1965) suggests that chip piles be mixed completely every few months to minimize this risk.

Experiments in Georgia (Saucier and Miller, 1961) indicate that deterioration of pine chips stored in the winter is one-third to one-half less than that of summer stored chips. Many of the pine chips stored in Sweden (Bergman and Nilsson, 1966) from the end of October to the following

May were found to be frozen during most of this period at the bottom level of the pile. Where average storage temperature is close to freezing, it is found that specific gravity losses are very low in comparison to losses in samples stored in the warmer areas of the pile.

### 3. Environmental conditions in wood chip piles

A spontaneous rise in temperature in chip piles occurs as a result of processes which go on in the pile. This heat generation affects water vapour movement within the pile, causes changes in wood extractives, and creates conditions for a different microflora not normally associated with wood deterioration.

Several small experimental chip piles have been built in the southern United States from southern pine chips (Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961; Somsen, 1962; Davis, 1963). The sloping sides of these piles were not compacted compared to the main body of the piles, however, temperatures in these piles showed a degree of uniformity. In the interior compacted portions of the piles, a rapid initial rise of temperature occurred. No mention was made of the ambient temperatures. Within the first two weeks the temperature rose to between 54° and 63°C. During the next two to four weeks the temperature dropped sharply to about 49°C followed by a gradual decline to about 38°C after about five months storage. In the uncompacted portions of the piles temperatures rose rapidly at first, but only to 38-49°C, and soon thereafter fell to ambient temperature.

In Sweden, Björkman and Haeger (1963) built a softwood pile in the shape of a cone with a base diameter of 15 meters and a height of 11 meters. In the deep interior of the pile the temperature remained above ambient for 15 months, the maximum difference being 20.6°C in January. The temperature near the periphery followed the air temperature.

In two studies on large piles of 7400-8800 cubic meters, Annergren, Dillén and Vardheim (1964) and Annergren, Dillner, Häglund and Jagerud (1965) made observations on temperature changes. Three of the piles were of spruce and one of birch. Temperatures in the spruce piles rose to about 55°C and remained at this level for 3 to 4 months followed by a decline. In the birch pile the temperature rose to 65°C in the first two weeks and remained at this level for 4 1/2 months at which time the pile was dismantled. Annergren, Dillén and Vardheim (1964) and Annergren, Dillner, Häglund and Jagerud (1965) also confirmed the finding that temperatures near the sides of the pile are lower than those near the centre of the pile.

Ljungqvist (1965) made an extensive study of temperature variation in several commercial piles in Sweden. Piles of birch, pine and spruce (exact species not identified in paper) built in summer reached maximum temperature of 69°C, 63°C and 58°C respectively.

Although temperatures in the interior of eastern Canadian chip piles do rise very sharply after the first weeks of construction, with longer storage the temperatures drop to between 30°C and 43°C (Shields and Unligil, 1968). The drop sometimes occurs very abruptly (Rothrock,

Smith and Lindgren,1961; Saucier and Miller, 1961) but this decline has also been shown to occur more gradually in the larger, more compacted piles in Sweden (Annergren, Dillén and Vardheim, 1964).

This difference in the internal temperatures has been attributed to the larger volume of chips in the Swedish piles which serves to insulate the inside of the pile from environmental changes. The degree of compaction is believed to be one of the factors responsible for the internal temperature fluctuations (Annergren, Dillén and Vardheim, 1964). Greater compaction results in higher average temperatures.

Studies in New Zealand on very small piles clearly illustrate the effect of volume of chips and compaction on the temperature devleopment in the pile. Butcher and Howard (1968) have shown that there are differences in the temperature of winter piles and summer piles. The temperatures recorded in a winter pile 9 feet on a 30 by 50 foot base, with self-compacted chips, were very erratic over the first seven weeks of storage. Reheating occurred, followed by several major fluctuations in temperature. The summer pile, which was 10 feet high on a 70 by 35 foot base and in which the chips were compacted by crawler tractor, had temperatures which were not influenced by ambient conditions. The rise in internal temperature was around 37°C to 38 °C for most of the nine months of storage.

It has been suggested that the rise in temperature which occurs in the outside stored chip piles is in part due to the condensation of water vapour on the chips, the presence of fines, the presence of metals and the

microbial activity within the pile (Shields, 1967; Allen, 1968; Chalk, 1968). No experiments have been done on any of these factors. In large chip piles a transfer of warm moist air from the lower regions of the pile to the upper cooler chips is believed to occur. This transfer creates a chimney effect whereby the evaporation of moisture from the lower chips results in cooler temperatures in these regions while condensation of water vapour on the chips in the uppor portion of the piles causes an increase in temperature (Rothrock, Smith and Lindgren, 1961; Annergren, Dillén and Vardheim, 1964; Ljungqvist, 1965).

Moisture distribution in chip piles is irregular and a tremedous variation in the moisture of the wood occurs during the storage period. Björkman and Haeger (1963) indicated that the relative humidity measured in a pile stored for 15 months averaged 98 to 100%.

It is generally accepted that the interior of a pile is less moist than the outer regions owing to the high temperatures that are associated with the core of the pile (Rothrock, Smith and Lindgren, 1961; Annergren, Dillén and Vardheim, 1964; Bergman and Nilsson, 1966).

Fungal activity may lead to an increase in the moisture content of the chips to some extent. Increased fungal metabolism results in the production of carbon dioxide and water (Lindgren and Eslyn, 1961) as end products of degradation of carbohydrates in wood.

Precipitation is an important source of moisture which can drastically affect the behaviour of the pile. Small piles react to changes in the rainfall (Butcher and Howard, 1968). The amount of moisture in small

piles in the west coast of the U.S. was not found to be directly related to the precipitation because the moisture content remained uniformly high during storage (Wright, 1954).

The initial moisture content of stored chips is generally within the range of 45 to 50 percent, green weight basis. With longer storage periods the moisture content becomes more or less uniform throughout the pile (Wright, 1954; Saucier and Miller, 1961). Additional moisture in the surface of the pile is contributed by rainfall or snow (Holekamp, 1958; Rothrock, Smith and Lindgren, 1961; Zak and Krauthauf, 1964).

High temperatures and moisture are factors which contribute to brown chemical staining and are reported (Annergren, Dillén and Vardheim, 1964) to result in the deacetylation of hemicelluloses in the wood.

The pH of wood chips gradually decreases during storage. Acetic acid odour is a very common phenomenom. This product is found to result from the deacetylation of the hemicelluloses in the wood. Shields (1970) has shown that the pH of the wood dropped from 5.92 in the fresh chips to 2.87 in chips stored for 259 days. This pH affected the growth and type of microorganisms appearing in the pile and the bacterial isolations dropped suddenly at pH 3.50 while the frequency of fungi isolations was highest at this pH.

Annergren, Dillén and Vardheim (1964) attribute the brown discolouration of chips to the pH and the high temperatures. It is believed that acetic acid itself is not directly responsible for the brown dis-

colouration but that other chemical or enzymatic reactions are probably involved (Shields, 1970).

4. Fungi isolated from wood chip piles

A wide variety of fungi, belonging to all classes, has been isolated from chips. Some of these organisms cause wood decay while others stain the wood. A large group of organisms has been isolated whose behaviour on wood is as yet unknown. The number of different fungi is generally much larger in chip storage than in round wood storage per wood unit (Nilsson, 1965) and probably influences the rate and type of deterioration. Because of the high temperatures developed in a chip pile a unique group of fungi is found which is able to survive at high temperatures. These fungi are called thermophilic fungi.

The largest group of fungi isolated belongs to the Fungi Imperfecti class and includes Trichoderma sp., Paecilomyces sp., Graphium sp., Phialophora sp., Gliocladium sp., Penicillium sp., and Aspergillus sp. (Lindgren and Eslyn, 1961; Björkman and Haeger, 1963; Nilsson, 1965; Bergman and Nilsson, 1966; Shields and Unligil, 1968; Shields, 1970). Members of the genera Chrysosporium found in chips during storage are among the most destructive fungi. Chrysosporium lignorum, a thermotolerant fungus, was reported to cause 33 percent weight loss in laboratory tests on pine sapwood after four months incubation at 25°C (Nilsson, 1965; Bergman and Nilsson, 1966). Another thermotolerant Fungus Imperfectus commonly isolated is

Aspergillus fumigatus. Unlike Chrysosporium lignorum it is not highly destructive.

The second commonest class of fungi found in chip piles is the Ascomycetes, which includes Ceratocystis sp. causing staining of wood, Chaetomium sp., Allescheria sp., Thermoascus sp. and Byssochlamys sp. (Nilsson, 1965; Bergman and Nilsson, 1966).

Basidiomycetes are not very common in outside chip storage except in small piles where the ambient conditions affect the behaviour of the pile, and in piles stored over long periods (Nilsson, 1965; Bergman and Nilsson, 1966). From two spruce piles stored for 6 and 7 months respectively, no Basidiomycetes were isolated. From the piles stored over a long period, 13 months, a number of Basidiomycetes were isolated. Two of these were Fomes annosus and Odontia bicolor (Nilsson, 1965). When the Basidiomycetes were present their frequency of isolation was very low. A heat tolerant Basidiomycete, Ptychogaster species has been commonly isolated from eastern Canadian chip piles (Shields and Unligil, 1968). It caused considerably more decay of pine sapwood when incubated at 37°C than at 27°C.

The majority of the wood-decaying Basidiomycetes from wood chip piles caused white rot in laboratory tests (Nilsson, 1965). Peniophora gigantea and Polyporus species were the most commonly identified organisms in this group although other species such as Ptychogaster do cause white rot.

Several of the Basidiomycetes have caused wood losses between 20-40% over 3 months (Nilsson, 1965; Bergman and Nilsson, 1966). Especially dangerous in chip storage are those rot fungi which can tolerate high temperatures. Among these are Polyporus species and an unknown Basidiomycete both isolated in Sweden (Nilsson, 1965).

Few Phycomycetes have been isolated and the commonest from coniferous piles is Mucor (Björkman and Haeger, 1963; Nilsson, 1965; Bergman and Nilsson, 1966). The damage caused by the Phycomycetes is as yet unknown. Rhizopus has been isolated from a Pinus radiata pile (Butcher and Howard, 1968).

A large number of the thermophilic fungi are Ascomycetes but a few are Fungi Imperfecti. The Fungi Imperfecti class includes: Sporotrichum thermophile Apinis and Humicola lanuginosa (Griffon and Maublanc) Bunce (Nilsson, 1965). Among the thermophilic Ascomycetes are Allescheria terrestris Apinis, Byssochlamys emersonii, Chaetomium thermophile La Touche and Thermoascus aurantiacus Miehe. Most of the isolates of these fungi have come from softwood chip piles (Nilsson, 1965; Bergman and Nilsson, 1966; Shields and Unligil, 1968).

Little is known about the nature of the thermophilic fungi's attack on wood. It has been demonstrated that A. terrestris caused a soft rot of hardwoods (Bergman and Nilsson 1967). However, this may not be the case in softwoods. The nature of attack is very important in elucidating the function of thermophilic fungi in chip piles.

Sporotrichum thermophile, A. terrestris and Humicola sp. can utilize callulose as a sole source of carbon whilst T. aurantiacus cannot (Fergus, 1969). No such study has been done for varieties of Byssochlamys sp. isolated from chip piles.

It is believed that these thermophilic fungi are partly responsible for the high temperatures usually recorded in chip piles. No results of heat production by fungi on wood are available. Results from heat generation experiments in other organic substrates like hay, straw, compost and grain indicated that thermophilic fungi are mainly responsible for the rise in temperature. Carlyle and Norman (1941) infected sterilized straw with Aspergillus fumigatus, a very common fungus in chip piles, and demonstrated that the temperature could rise from 25°C to 55°C during 38 hours. Fenstenstein, Lacey, Skinner, Jenkins and Pepys (1965) have shown that a large number of thermophilic fungi can raise the temperature of straw to about 60°C in a very short time.

## 5. Damage in wood chip piles

Chips in storage are damaged by the activity of micro-organisms, such as fungi, which either stain or degrade the wood and also by conditions which develop in the pile such as high temperatures. Damage by micro-organisms leads to a reduction of quality and/or quantity of the wood.

A common damage to wood chips is staining. Little deterioration of this type occurs in chips stored on the west coast of North America (Blackerby, 1958; Hensel, 1958; Burke, 1962). Blackerby (1958) reported discolouration and rot in softwood chips stored for one to three years at mills in B.C. and Oregon. Staining occurred early and was confined to the outer uncompacted parts of the pile. Studies in Georgia (Holekamp, 1958) indicated that staining to a depth of about one foot from the surface of the pile occurred after four months storage. Deterioration due to staining may be very minor in pine chips after 4 months storage (Blackerby, 1963). Most of the blue gray fungal discolourations in the pine chips were situated in the outer one to two feet of uncompacted sides of one pile after one month's storage (Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961). A softwood pile became dark in colour after 9 to 12 months storage in Nova Scotia (Robinson, 1963) and did not regain its colour after drying. Shields and Unligil (1968) found insignificant discolouration in spruce and balsam fir chips stored in New Brunswick after 4 to 8 months storage.

A non-fungal yellowish brown or orange discolouration was found to occur in a pine chip pile in the southeastern U.S.A. (Lindgren and Eslyn, 1961; Rothrock, Smith and Lindgren,1961; Saucier and Miller, 1961) within one month of storage. The discolouration was located primarily around resin ducts in the heartwood areas of pine chips.

Brownish discolourations have been noticed in the interior of several softwood piles and have been attributed to chemical changes in the pile (Saucier and Miller 1961; Shields and Unligil 1968). The development

of the brown stain is progressive with increased storage and appears to be more widespread near the bottom than near the top of one pile (Young, 1961).

After 4 months of summer storage and 5 months of winter storage, heavily stained pine chips were found to be soft and brashy (Lindgren and Eslyn, 1961; Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961). This condition has been attributed to the activity of soft rot fungi. In one study it was found that the number of decay fungi isolated from softwood chips in the bottom half of a large pile was greater than the number isolated from other parts of the pile (Shields and Unligil, 1968).

Losses in wood substance vary from one pile to another as work in Sweden and southern U.S.A. indicates. Minor reductions in specific gravity have been noticed in pine chips stored for up to two months (Anon, 1961). The reduction in the specific gravity corresponded to the lowering of temperature, to increase in moisture content and perhaps to depletion of oxygen.

After five month's storage (Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961) there was an approximate average loss of 7% of wood. Losses in the compacted centres of the piles were low (about 4%). Even after 12 months storage, greater reductions (about 9%) occurred in the outer three feet of the sloping sides of the pile where there was the least compaction and where visible chip deterioration resulting from growth of stain and decay fungi was evident (Lindgren and Eslyn, 1961; Rothrock, Smith and Lindgren, 1961).

Losses in Swedish spruce chip piles (Annergren, Dillén and Vardheim, 1964; Nordin and Selleby, 1965) were reported to vary from two to five percent for storage periods of four to five months. At temperatures of 20°C to 30°C, losses of 5 to 10 percent occurred in pine chips (Bergman and Nilsson, 1966) stored for seven months in Sweden while little or no loss occurred in frozen chips during this time.

A loss of approximately 11 percent in the specific gravity of brown and deteriorated balsam fir and spruce chips was determined from samples which had been sotred for just over nine months near the bottom of a pile in New Brunswick (Shields and Unligil, 1968). Further decreases in the specific gravity of pine (Burks, 1962; Somsen, 1962; Selleby, 1965) spruce (Annergren, Dillén and Vardheim, 1964; Selleby, 1965) and birch (Selleby, 1965) chips were slight beyond five months.

## C MATERIALS AND METHODS

### 1. The experimental wood chip pile

The experimental chip pile, used in the study of the distribution of thermophilic and thermotolerant fungi, was located on the grounds of the Intercontinental Pulp Company Ltd., Prince George, British Columbia. It was constructed on a level site and oriented longitudinally east-west.

The chip pile matrix consisted of white spruce and lodgepole pine into which the chip samples were inserted. The chips were from sawmill residues supplied by 15 sawmills. The pile contained 2,900 units of chips, each unit being 1,080 kg. of wood chips. The ratio of spruce to pine was two to one. The building of the pile took three weeks and data collection started on the second week of June, 1968.

The south face of the pile was uniformly exposed to the weather throughout these studies so that the effects of the prevailing climatological conditions on the entire front face would be constant. The chips were pneumatically delivered to the pile site, then a caterpillar tractor was used to level the pile. This levelling made it easy for the insertion of the samples and the compaction of the pile.

The pile was 75ft (23.2m.) wide at bottom 28ft. (8.6m.) wide at the top and 25ft (7.7m.) high. The length of the pile was 400ft (124.m). Figures 1 and 2 give the dimensions of the pile in the east and west and the south views.

Samples of chips in "flexmesh" plastic bags were incorporated into the pile during construction. For each treatment, sample bags were placed in six different positions within the pile as shown in the east-west view of Figure 1.

The spacing between the centres of any two sample bags was 8ft. (2.4m.). The pile was constructed to comprise four individual sections, each section containing all the treatments required for a study of one time period.

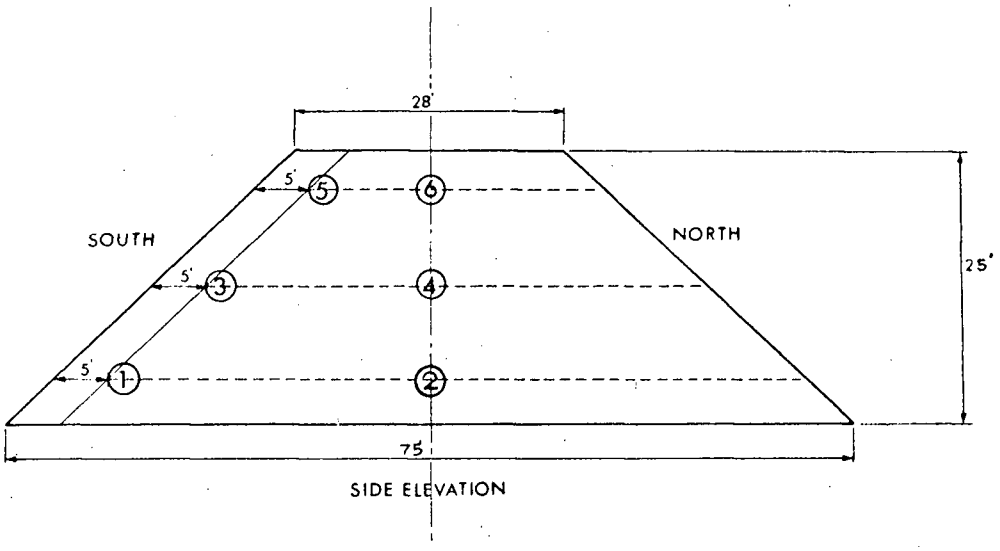



Fig. 1. East and West view of the chip pile showing location of chip samples 1 to 6.

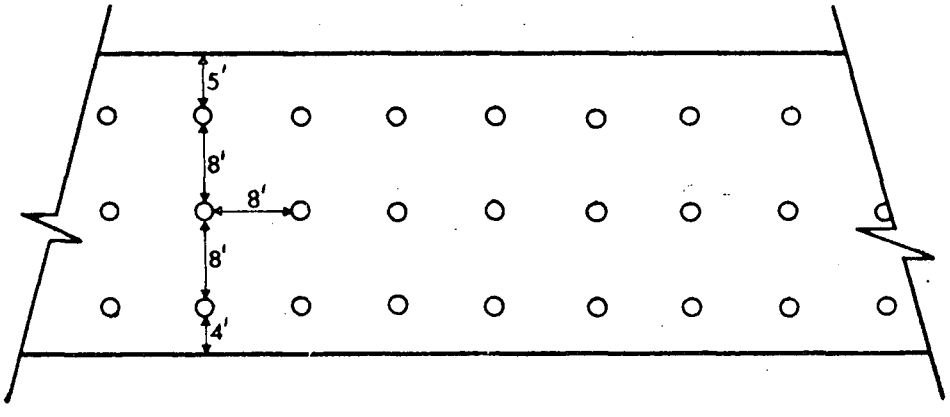



Fig. 2. South view section of the chip pile.

The four time periods (Fig. 3) were 3, 6, 12 and 24 months but only the first three were investigated in this study.

The sample bags were retrieved by carefully breaking up each section of the pile at the required time interval (Fig. 4). To reduce the possibility of environmental changes to the remaining sections, the samples in each section were separated by 20ft. (6.2m.) long sections of matrix chips. When a section was removed from the pile, the position and slope of the new face was adjusted to conform to those of the old.

Samples removed from the pile were placed in plastic bags and transported under refrigeration to the laboratory in Vancouver where they were stored at 1°±0.1°C until used.

2. Sampling positions

Each of the four sections of the wood chip pile (Fig. 3) had all the treatments placed in six different positions which were inner top, inner middle, inner bottom, outer top, outer middle and outer bottom (Fig. 1). The outer positions were on the south face of the pile.

3. Treatments

The treatments were as follows:

1. Sample bags of pine chips
2. Sample bags of spruce chips
3. Sample bags of spruce chips containing a core bag of fines

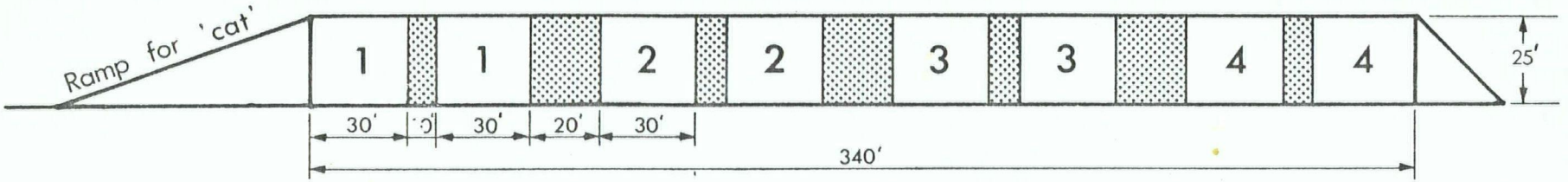



Fig. 3. The four sections of the chip pile which were broken down after 3, 6, 12 and 24 months.

Fig. 4. Retrieval of chip samples.

4. Sample bags of spruce chips containing a core bag of sterilized spruce chips
5. Sample bags of spruce chips containing a core bag of spruce chips inoculated with Ptychogaster sp.

Each treatment was replicated twice.

## 4. Preparation of wood chip samples

White spruce and lodgepole pine were used in preparing the samples. Sufficient sound wood for the entire experiment was obtained from several logs in the Prince George area. The wood was then chipped in the mill.

Individual species were well mixed to give homogeneous samples using the mixing facilities of Rayonier Inc. in Shelton, Washington. These samples were then transported back to Prince George in a refrigerated truck. The individual samples were prepared on the site by weighing 58 lb. (26.1 kg.) of chips into each flexmesh bag. Samples were taken from each bag, put in a screw-capped bottle, and weighed. These small samples were brought into the laboratory to determine the moisture content.

A "core sample bag" technique proposed by J.V.Hatton (1970) was used to prepare the majority of the samples. The technique consists of placing a smaller "flexmesh" plastic bag containing a test sample into a bigger bag containing chips so that the small bag essentially forms the central core. Any influence due to the core must then pass outward and affect the sample chips.

The main sample bags weighed between 58 lb. (26.1kg) and 60 lb. (27.kg.) green weight of chips while the core samples weighed between 8 lb. (3.6kg.) and 10 lb. (4.5kg.).

The core bags for treatments 4 and 5 were prepared by autoclaving 10 lb. (4.5 kg.) spruce chips in a wooden container at 15 psi for 90 minutes. These were put aseptically into "flexmesh" plastic bags which had been sterilized by dippling them in 5% phenol for a number of hours, followed by a rinse in sterile water. The core bags in treatment five were inoculated with cultures of a Ptychogaster sp., growing on 2% malt agar, by carefully removing the colony from the petri dish with a sterile spatula and putting it into the plastic bag containing the sterile chips. The flexmesh plastic bags were placed in sterile polyethylene bags and incubated for two and a half months at room temperature. They were then put in another thick plastic bag after the incubation period and transported in a refrigerated truck to the site of the pile.

## 5. Measurement of environmental conditions

The environmental factors measured were temperature in the wood chip pile, moisture content of wood chips and acidity of wood chips.

### a. Temperature in the wood chip pile

Thermocouples were inserted into the 2 year section of the pile to measure the temperatures in every sample bag (60 bags). The thermal gradient across the pile face to the depth of 96 in. (2.4m) was also measured

on the south and north faces by placing thermocouples at the surface and at depths of 3, 6, 12, 24, 48 and 96 in (0.08, 0.15, 0.31, 0.61, 1.22 and 2.43m). In all, 74 thermocouples were used.

Temperature readings were taken at daily intervals for three months after which time twice weekly recordings were made. All 74 thermocouples were connected to a master control panel situated within an insulated hut near the 2 year section of the pile.

High temperatures are more favourable for the growth and reproduction of thermophilic fungi (Cooney and Emerson, 1964). Therefore the total number of fungi isolated from a sampling bag after a given sampling period could, infact, be a reflection of the temperature regime the bag experienced during storage.

In determining a measure of the temperature regime corresponding to each bag, temperature values measured at all the bag positions in the 24 month section of the chip pile were considered applicable to the corresponding positions in the 3, 6 and 12 month sections of the chip pile; also weekly average temperature values for each bag position were summated for the period during which the respective bags were in the pile and these summations called the "total temperatures". These total temperatures were then correlated with the total counts of fungi for each bag.

b. Moisture content of chips

The change in moisture content was determined by comparing the moisture content determination at the time t = 0 with those determined after 3, 6 and 12 months storage. To determine the moisture content the sample chips were first weighed and then oven-dried to constant weight. The difference in weight represents the actual weight of water in the chips. Using the formula M.C. % =

$$\frac{\text{Weight of water in chips}}{\text{Oven dry weight of chips}} 100$$

the percentage moisture content was calculated.

c. Acidity of wood chips

Five to 10g of chips were ground in a Wiley Mill (Model ED-5) to pass a 1-mm screen. One gram of wood meal (oven-dry basis) was stirred initially for 15 seconds with 25-ml distilled water (of pH 6.0 - 6.5) and was determined with a Corning pH meter (Model 7).

6. Isolation of fungi

a. Sampling procedure

A random sample of 100 chips was taken from each 60 lb. sample bag. These were surface sterilized individually by quickly passing them through a flame. A piece was removed from the centre of the chips using sterile bone forceps. This piece was split into two halves, and then surface sterilized again and plated on acid malt agar composed of 2% malt, 2% agar

and 0.5% malic acid. One piece on one plate was incubated at 45°C and the other piece on another plate at 25°C. Each petri dish contained ten pieces of chips (Fig. 6).

The plates were examined daily and colonies growing on them were sub-cultured onto 2% malt 2%agar slants. The part of the colony that remained was removed and discarded. This process was repeated until drying of the agar prevented further isolation.

b. Isolation and grouping of fungi

At the end of the sampling, all the slant cultures were examined under the binocular microscope and were grouped into broad categories, mainly generic. The slants were then counted and recorded.

After a thorough examination of a selected number of cultures from each group the remainder of the tubes were discarded. These representative cultures were then examined using a phase contrast microscope and identified wherever possible. The following categories were used.

Group 1. This group was comprised of aspergillus type fungi which had no perfect stage. The culture was light green to green. The majority of the isolates in this group were Aspergillus fumigatus.

Group II. This group was made up of all types of fungi which had penicillate conidiophores. Some produced ascocarps while others did not. The most common fungus was Byssochlamys emersonii.

Figure 5. The chip pile.

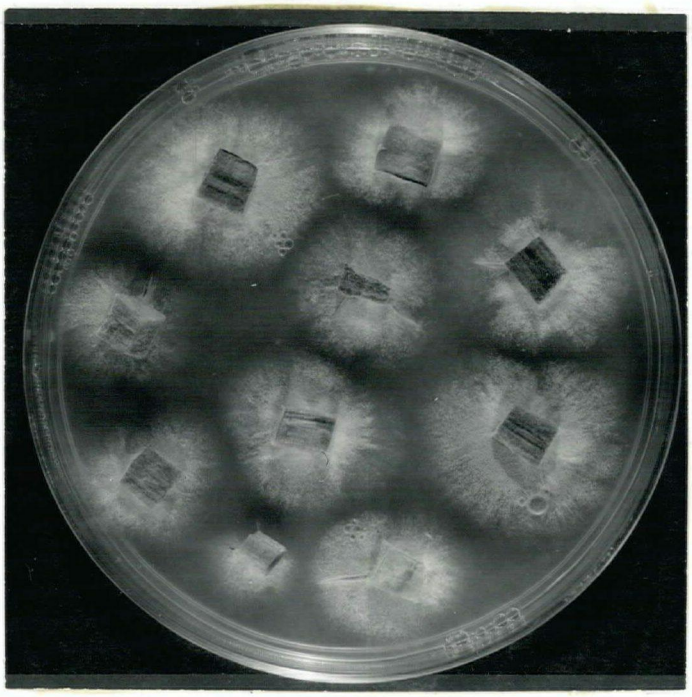


Figure 6. Byssochlamys emersonii growing out of chips on 2% malt and 0.5% malic acid agar.

Group III. This group consisted of a species with white thread-like mycelium. It had no asexual stage, grew slowly in culture and had light brown ascocarps. The fungus is still unidentified but resembles Byssochlamys.

Group IV. This group was made up of a single fast growing species which was identified as Chrysosporium pruinosum. The culture was cream in colour and powdery on top.

Group V. The hyphae of the fungi in this group were hyaline and had no conidiophores. The cleistothecia which were produced in large numbers were reddish brown and somewhat angular. This fungus grew very fast in culture.

Group VI. This group was made up of fungi which had white to cream mycelium. They produced hyaline conidia which were pyriform to oval. Some produced black cleistothecia. It contained both Allescheria and Sporotrichum.

Group VII. This group contained the yeast like organisms. They were infrequently isolated and were present mainly in the samples stored for three months.

7. Determination of wood chip weight loss

The weight losses were determined by comparing the calculated oven-dry weight of chip samples at storage time t = 0 with those measured after 3, 6 and 12 months storage. The weight losses were expressed as percent of original oven-dry weight of wood using the formula percent weight loss =

$$100 \times \frac{\text{Original ovendry weight of wood - ovendry weight of wood after test}}{\text{Original ovendry weight of wood.}}$$

# D RESULTS

## 1. List of fungi isolated

The following most commonly occurring fungi were identified:

*Aspergillus fumigatus* Fresenius

*Allescheria terrestris* Apinis *Cephaslosporium terrestre*

Asexual state of *A. terrestris*.

*Byssochlamys emersonii* Stolk-Apinis.

*Chrysosporium pruinosum* (Gilman and Abbot) Comb. Nov.

*Humicola lanuginosa* (Griffon and Maublanc) Bunce.

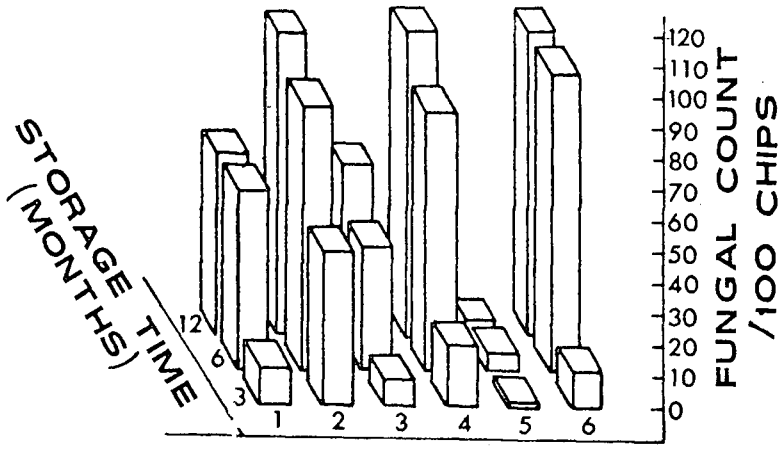
*Sporotrichum thermophile* Apinis.

*Thermoascus aurantiacus* Miehe.

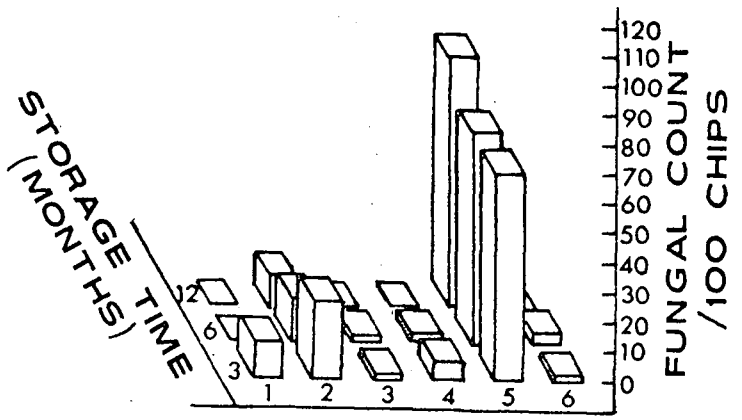
In addition to the fungi listed above, 21 distinguishable fungi isolated from the pile were not identified.

## 2. Distribution of common thermophilic and thermotolerant fungi

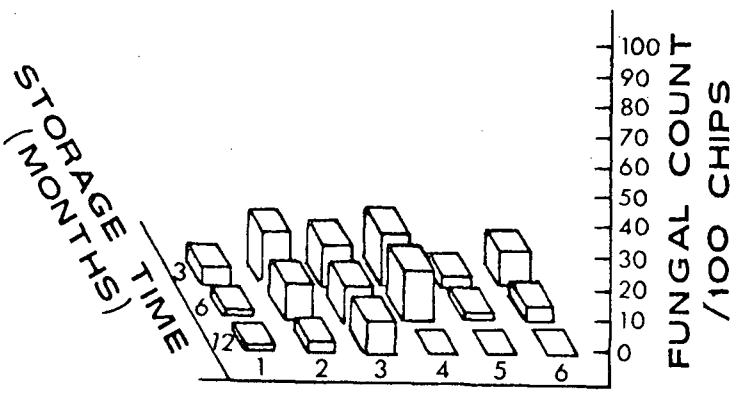
The most common thermophilic fungus occurring in the pile was *B. emersonii* (Fig. 7), which occurred mainly in the interior region of the pile where temperatures were high. The numbers of isolates of this fungus increased with time of storage.




*Byssochlamys emersonii*




*Aspergillus fumigatus*




*Allescheria terrestris and Sporotrichum thermophile*

POSITION IN PILE

Fig. 7. Fungal count.

A. terrestris and S. thermophile (Fig. 7) were commonly isolated from the inner region of the pile and were most common in the samples stored for three months. Thereafter, their numbers decreased with time of storage.

T. aurantiacus (Appendix 1) did not occur as frequently as the other fungi but would grow fast out of the wood if it were present. It was erratically isolated from both inner and outer regions of the pile.

A. fumigatus (Fig. 7) was confined to the outer region of the pile and appeared in large numbers. It frequently occurred in the outer top position. Its numbers remained stable throughout the period of storage of the chips.

C. pruinosum (Appendix 1) was more commonly isolated from spruce than from pine chips. The highest number of isolations came from outer top and outer middle positions of the pile. It was occasionally isolated from the outer bottom position. The numbers of isolates of C. pruinosum remained stable throughout the duration of storage of the chips.

### 3. Environmental factors

#### a. Temperature in the wood chip pile

Changes in temperature in the six positions in the pile for 34 days and 80 weeks are shown in Figures 8 and 9 respectively.

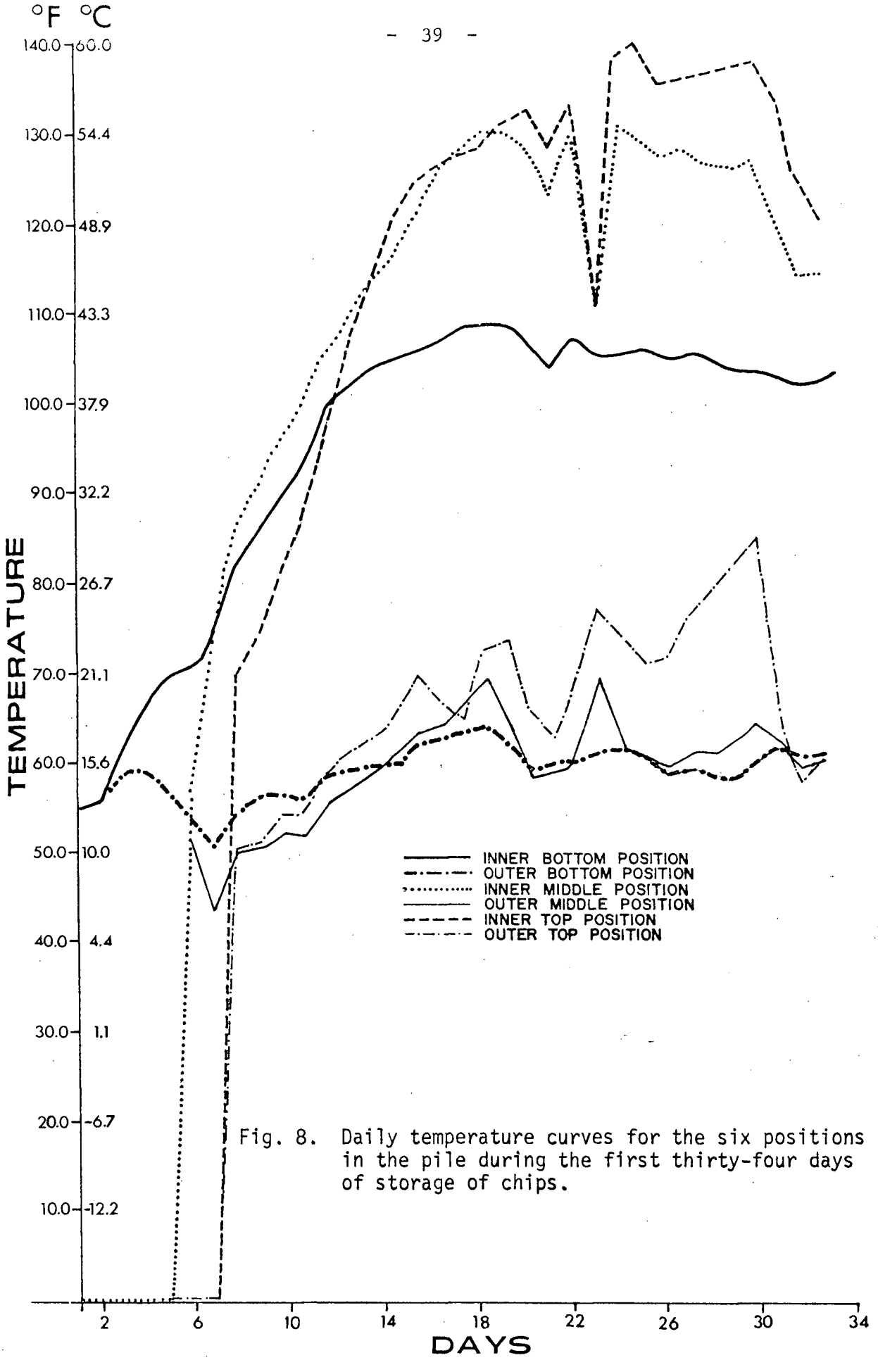



Fig. 8. Daily temperature curves for the six positions in the pile during the first thirty-four days of storage of chips.

The inner region of the pile had higher temperatures than the outer region. In the inner region, the highest temperatures were at the top followed by the middle and the bottom. The rise in temperature at the top was very rapid and attained the maximum temperature of 60.6°C in 19 days. Two weeks later the temperature had dropped to 49.4°C.

The temperature trend was the same for the inner middle position as the inner top position but temperatures were never as high. The maximum temperature was 54.4°C and was attained thirteen days after the burial of the bags. The drop in temperature was gradual, going from 54.4°C to 46.1°C in two weeks.

Low temperatures were recorded for the inner bottom position of the pile compared to the other interior regions. A longer time was required to attain a maximum temperature of 42.5°C. Temperatures were stable in this part of the pile.

In the outer regions of the pile temperatures fluctuated a great deal, and except for the outer top positions, temperatures never rose above the initial temperatures of the pile. The highest temperature recorded in the outer region was 29.4°C at the outer top position.

Temperatures were relatively high for the first three months after which there was a rapid drop. Temperatures were low during the late fall, winter and early spring but started to rise again in the summer months, though not attaining the values of the previous summer. In the winter months ambient temperature affected the inner top position more than the other

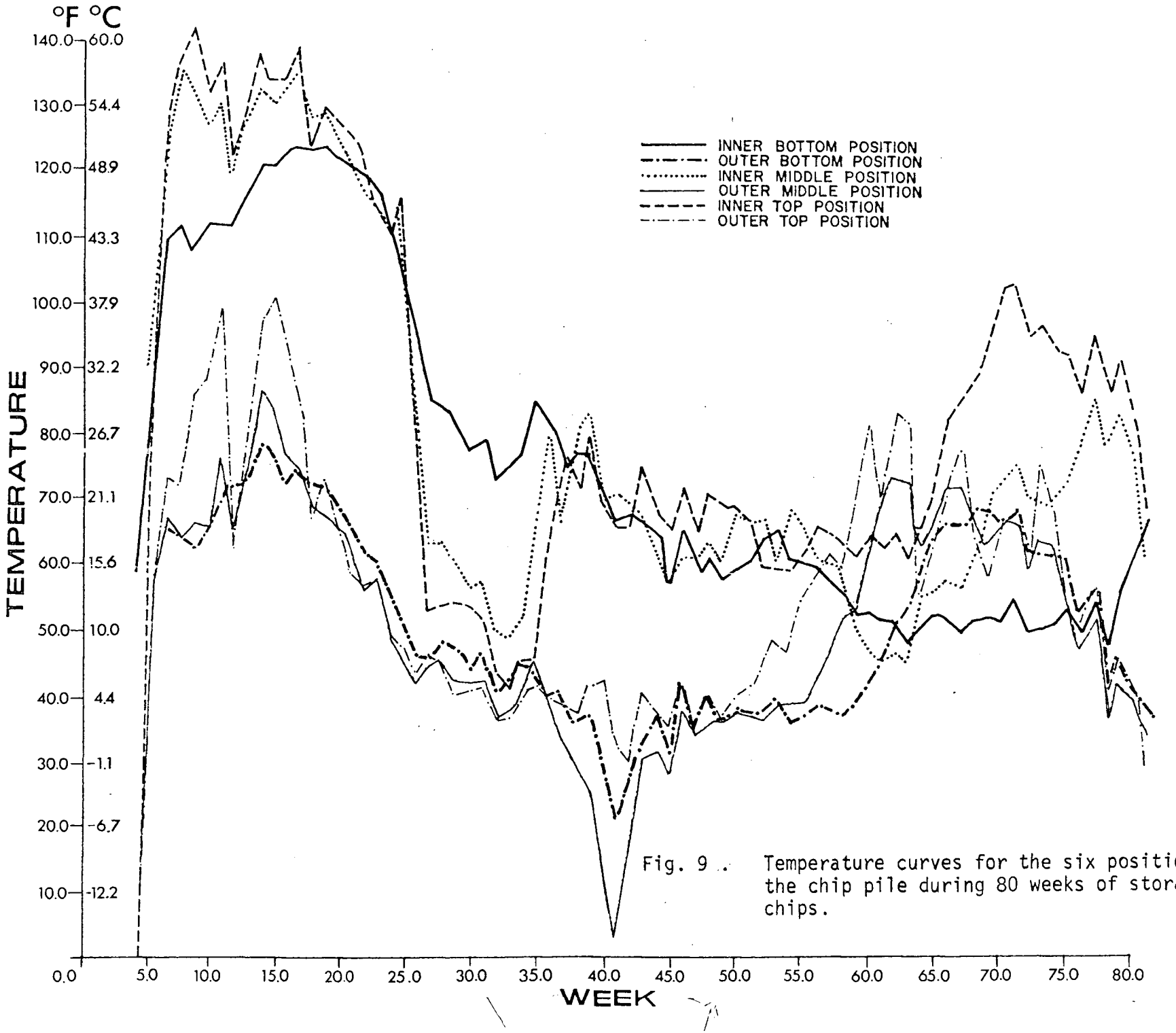



Fig. 9 . Temperature curves for the six positions in the chip pile during 80 weeks of storage of chips.

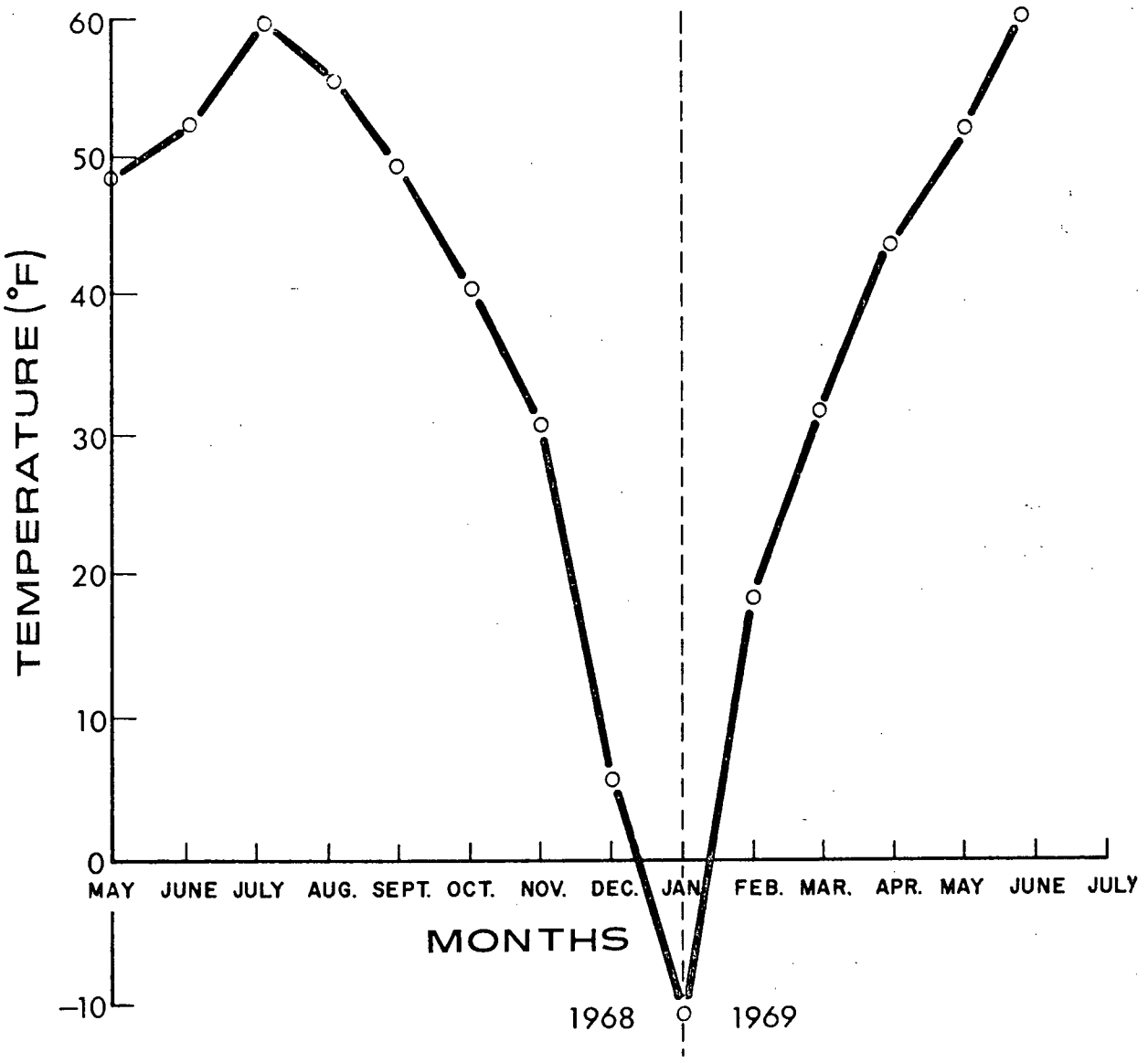



Fig. 10. Average monthly ambient temperature in Prince George.

position and the inner middle temperatures were sometimes higher than those of the inner top position.

Temperatures in the outer regio followed the ambient temperature for most of the time (Fig. 9, 10). Freezing temperatures were common. Temperature rise was noticed in the summer but was only marked in the outer top position of the pile (Fig. 9).

b. Moisture content of wood chips

No definite pattern of moisture content existed for all the treatments or for the pile as a whole. The moisture content of the samples was highly variable (Table 1).

Spruce always had a higher moisture content than pine. The chips in the outer positions had higher moisture content than the chips in the inner position until the "wet lens" effect developed at the inner top position, thus biasing the moisture content values of the chips in the inner positions in the later time periods. Moisture, generally, must have been adequate for fungal development.

Table 1. Final moisture content of wood chips.

| Treatment | 3 months % M. C. | 6 months % M. C. | 12 months % M. C. |
|---|---|---|---|
| Pine (1) | | | |
| Outer samples, 1, 3, 5 | 42.6 | 39.2 | 46.7 |
| Inner samples 2, 4, 6 | 33.6 | 38.1 | 39.7 |
| Spruce (2) | | | |
| Outer samples 1, 3, 5 | 64.7 | 66.2 | 65.6 |
| Inner samples 2, 4, 6 | 58.6 | 56.4 | 72.0 |
| Sterilized spruce (4) | | | |
| Outer samples 1, 3, 5 | 70.8 | 63.2 | 63.9 |
| Inner samples 2, 4, 6 | 65.6 | 67.5 | 72.9 |
| Inoculated spruce (5) | | | |
| Outer samples 1, 3, 5 | 64.9 | 61.6 | 66.4 |
| Inner samples 2, 4, 6 | 61.3 | 76.7 | 74.4 |

c. Acidity of wood chips

Measurements of acidity of wood chips were taken only for the spruce and pine treatments except for samples in the outer psotions of the first sampling period (Table 2).

Table 2. Final acidity of wood chips

| Storage periods | Pine | | Spruce | |
|---|---|---|---|---|
| Months | Outer 1, 3, 5 Position | Inner 2, 4, 6 Position | Outer 1, 3, 5 Position | Inner 2, 4, 6 Position |
| 0 | 4.80 | 4.80 | 5.40 | 5.40 |
| 3 | - | 4.60 | - | 5.00 |
| 6 | 4.96 | 4.75 | 5.37 | 4.93 |
| 12 | 5.01 | 4.81 | 5.57 | 5.30 |
| Mean | 4.92 | 4.74 | 5.45 | 5.16 |

Changes in pH were not very marked but generally followed the initial pH of spruce and pine which indicated that the pH of spruce was higher than pine. The pH of the inner samples was lower than that of the outer samples.

The change in pH with duration of storage was erratic. pH generally went down after storing the wood for three months but rose again after six months and continued to increase. The behaviour of the inner samples of

spruce did not follow the general pattern. pH decreased up to the six months' storage and started to increase only at twelve months.

4. Weight losses of wood chips

The weight losses of wood chips are summarized in Table 3. Weight losses were lower in the outer region of the pile than in the inner region. This difference was significant at 5%. There was no significant difference between treatments. Weight losses increased from three to six months, the difference being significant at 5%. Although weight losses at twelve months were lower than weight losses at six months, the difference was not statistically significant.

Table 3. Final weight loss of samples in percent

| Position | Percent weight loss | | |
|---|---|---|---|
| | 3 months | 6 months | 12 months |
| Outer: 1, 3, 5 | 1.1 | 2.0 | 1.9 |
| Inner: 2, 4, 5 | 2.4 | 4.0 | 3.2 |

5. Evaluation of treatments

The treatments were analyzed on the total count of thermophilic and thermotolerant fungi isolated at 45°C following incubation. The total count of fungi is the number of fungi isolated from one hundred chips.

The treatments (Tables 4, 7) generally had no effect on the distribution of fungi in the pile. It was only at the six months sampling period that differences existed between treatments. Pre-inoculating spruce with Ptychogaster sp had no effect on the distribution of the fungi. There were also no differences between pine and spruce.

The length of storage of chips (Tables 6,7) had a significant effect on the distribution of the fungi. Fungal population increased from the three months period to the six months period and remained constant between the six months and the twelve months periods.

The position of the samples in the pile (Table 5) played a dominant role in the changes in numbers of the fungal population. Most of the fungi were isolated from the inner bottom and outer top positions of the pile in the first three months. Examination of the chips after six months storage showed a large increase in the total fungal population in the inner upper and the inner middle positions although increases occurred at all positions. Changes in total fungal population did not occur after twelve months storage.

The evaluation of the treatments was carried out in two steps using the analysis of variance. The analysis of the three months samples was done separately because a treatment which was not examined in the subsequent time periods was included in this analysis. The second analysis was for the four treatments at the three time periods.

Table 4. Mean fungal count for different treatments after storing chips for three months.

| Treatments | Fungal count |
|---|---|
| Pine | 44 |
| Spruce | 55 |
| Fines incorporated into spruce | 65 |
| Sterilized spruce | 53 |
| Inoculated spruce | 43 |

In the three months storage (Table 4) differences between treatments were not significant but differences between positions (Table 5) were significant at 5%. No significant interaction existed between treatments and positions.

A Duncan's multiple range test (Table 5) carried out on the mean fungal counts for positions showed significant differences at 5% between fungal counts from inner bottom, outer top positions and all the other

positions. The highest count of fungi was from inner bottom position followed by the outer top position.

Table 5. Average number of fungi isolated per position after three months storage. *

| Position | Mean Fungal Count |
|---|---|
| Inner top | 24 |
| Outer bottom | 28 |
| Inner Middle | 39 |
| Outer middle | 45 |
| Outer top | 79 |
| Inner bottom | 99 |

* Any two positions means sidescored by the same line are not significantly different at 5% level.

In the second analysis significant difference at 5% existed between positions and time but not between treatments. There were significant interactions between all combinations of these factors except time and treatments (Table 6).

Table 6. Fungal counts at different storage times at the different positions.

| Positions | Storage period in months | | |
|---|---|---|---|
| | 3 | 6 | 12 |
| Outer bottom | 32 | 67 | 73 |
| Inner bottom | 99 | 119 | 108 |
| Outer middle | 48 | 68 | 73 |
| Inner middle | 41 | 112 | 100 |
| Outer top | 79 | 113 | 105 |
| Inner top | 25 | 118 | 100 |

The population of fungi changed with time of storage but not with the treatments. The significant differences existed between the treatments stored for 6 months but not for 3 or 12 months (Table 7).

Table 7. Fungal counts at different storage times with different treatments. These are average for 6 positions.

| Treatments | Storage period in months | | |
|---|---|---|---|
| | 3 | 6 | 12 |
| Pine | 44 | 82 | 92 |
| Spruce | 55 | 107 | 93 |
| Sterilized spruce | 65 | 116 | 96 |
| Inoculated spruce | 53 | 94 | 92 |

## 6. Relationships between variables measured

Figure 11 shows the relationship between total fungal count and moisture content. A simple regression analysis indicated that there was no significant correlation between these variables ($r^2 = 0.014$).

Figure 12 shows the relationship between total fungal count and total temperature. Figure 13 shows the relationship between total fungal count and weight loss. A multiple regression analysis on the data showed significant correlation ($R^2 = 0.34$) between fungal count, total temperature and weight loss (Table 8).

Table 8. Summary of regression analysis of total temperature and weight loss on total fungal count

| variable | $R^2$ | $F_{0.5}$ | |
|---|---|---|---|
| temperature and weight loss | 0.34 | 17.8 | * |
| temperature | 0.29 | 28.9 | * |

The final pH of the wood chips (Table 2) was not related to any of the other factors measured. pH varied from 4.74 to 5.57.

It is concluded that temperature, weight loss and fungal count are related. It is not clear which of these factors is the independent variable.

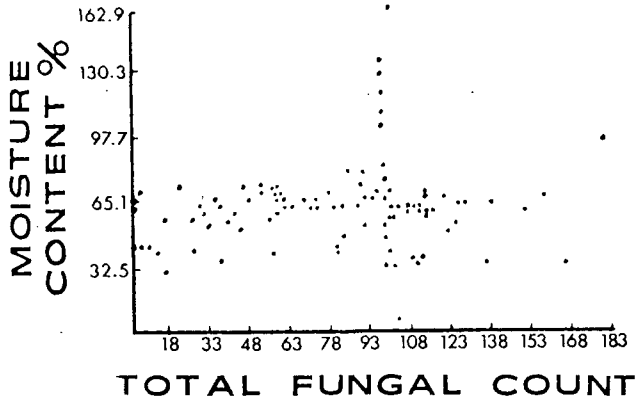



Fig. 11. The relationship between total fungal count and moisture content of chips.

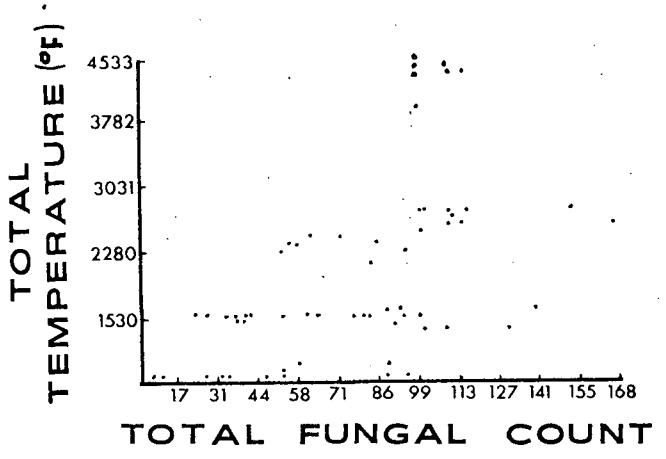



Fig. 12. The relatinship between total fungal count and total temperature.

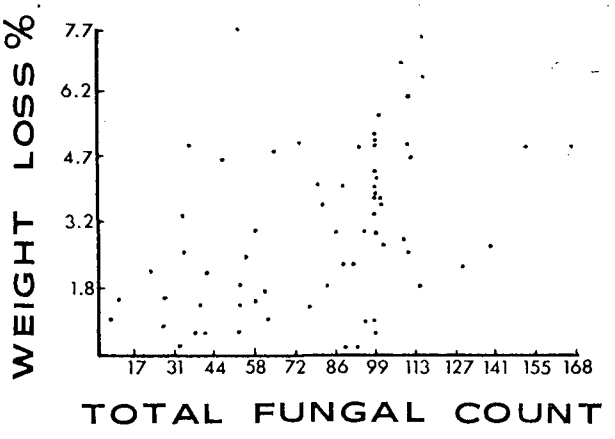



Fig. 13. The relationship between total fungal count and wood weight loss.

# E DISCUSSION

## 1. Fungi in the chip wood pile

This part of the study indicates that thermophilic and thermotolerant fungi occupy different regions of the chip pile (Fig. 7). The thermophilic fungi mainly inhabit the inner regions whilst the thermotolerant fungi are found in the outer regions of the pile. Any distributional and successional changes of fungi in the pile result from tenperature "preferences" of the different fungi.

Bergman and Nilsson (1967, 1968) showed a significant correlation between fungi and the temperatures in the pile. This was not a specific correlation based on any fungal group. In the present study a correlation (0.29) is shown between fungal isolations at 45°C and temperatures in the chip pile (Table 8).

The total number of fungi does not decrease with length of storage of chips even after storage of the chips for twelve months. The low unfavourable temperatures from the six months' storage period to the twelve months period did not affect the population of the fungi. The reduction in the numbers of fungi from the six months' sampling period to the twelve months sampling period was not significant. This finding is contrary to that of Shields (1970), who showed that the numbers of fungi decreased sharply

with storage time and even fell to negligible values after seven and one-half months' storage of the chips. No explanation is offered for this sharp decline, but it might imply that the chips were sterilized after being at the high temperatures for that length of time. Alternatively, it could signify that the low isolation temperatures which Shields used were responsible for his failure to isolate the thermophilic fungi which would have been present in the chips even after that period of storage.

The population of fungi in the hotter region of the pile is initially low and comprised of A. terrestris, S. thermophile and B. emersonii. With storage beyond three months, the population of fungi increases. The entire increase is accounted for by increase in B. emersonii population. The population of A. terrestris and S. thermophile decreases, and probably they are replaced by B. emersonii. In succession, therefore, B. emersonii follows A. terrestris and S. thermophile. The high frequency of occurrence of the three fungi is confirmed by Bergman and Nilsson (1966) but no mention is made of their succession.

A. fumigatus is an early colonizer of the outer regions of the pile and it may not be replaced by other fungi. In this study the high initial population of this fungus remained at the same level throughout the three sampling periods. No successional pattern is shown for C. pruinosum except that it occurs in the outer top and middle positions. Although in comparison to A. fumigatus its numbers were low, it is stable in those areas where it appeared. The chips in these areas, however, were not heavily degraded, and unlike the findings of Bergman and Nilsson (1966), the decaying activity of this organism must have been low in the spruce-pine

chip pile at Prince George. The cause of this low decaying activity is not clear but may be due to competition from fungi like A. fumigatus.

The high wood substance loss in the inner region of the chip pile is attributed partly to the activity of the thermophilic fungi in the pile. Indeed a significant correlation was found between fungal counts and weight losses of wood (Fig. 13 Table 8) although this correlation was not as strong as the correlation between fungal counts and temperature (Fig. 12 Table 8) in the pile.

The thermophilic and thermotolerant fungi through their metabolic activity must be partly responsible for thermogenesis in the chip piles. Thermogenesis through fungal activity has not been shown in wood, but thermogenetic capabilities have been shown for A. fumigatus in other plant materials (Carlyle and Norman, 1941).

The inner bottom region probably had the highest fungal counts because the temperature was mild and stable. Because of the mildness and stability this area of the pile can harbour both thermophilic and thermotolerant fungi. A similar observation was made by Shields and Unligil (1968) but they thought that the foundation of old chips contributed to the greater numbers of fungi in that region.

Ptychogaster sp. does not spread out of the inoculum bag into the chips. It was not isolated from the chip samples. Shields and Unligil (1968) isolated Ptychogaster sp. from balsam fir and spruce chip samples in the upper and middle layers of the pile. This fungus has not been isolated from other piles (Shields, 1967). It is possible that Ptychogaster

sp. is not able to compete with the thermophilic and thermotolerant species of fungi. Its inability to compete is inferred by Shields and Unligil (1968) who wrote that the fungus cannot stand temperatures above 50°C.

2. Temperature in the wood chip pile

A sharp rise in temperature occurs shortly after the construction of most chip piles. This generally takes two weeks to attain maximum temperature, and, depending upon the species of wood which is piled, the volume of the pile and the extent of compaction, the temperature will vary from 37°C to 69°C (Björkman and Haeger, 1963; Annergren, Dillén and Vardheim, 1964; Ljungqvist, 1965; Shields and Unligil, 1968; Butcher and Howard, 1968). A similar sharp rise occurred in the spruce-pine chip pile in Prince George. In this study, however, it was noticed that the temperature rise was not the same for all the positions in the inner regions of the pile, nor did it take the same time for all the positions to attain maximum temperatures. The time taken for these positions to attain maximum temperature varied from two to three weeks. The pattern of the decline in temperature is the same as given by other workers (Björkman and Haeger, 1963; Annergren, Dillén and Vardheim, 1964; Shields and Unligil, 1968).

Although differences in temperature occur between piles composed of different wood species, no differences were noticed between samples of spruce and pine. Ljungqvist (1965) found that as much as 5°C difference can

exist between pine and spruce piles. There are, however, species such as Douglas-fir (Hensel, 1958) where no rise in temperature occurs during storage. This may be due to high extractive content of the wood which inhibits the growth of fungi.

Temperatures in the outer region of the Prince George wood chip pile were much lower than those reported for southern pine species (Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961; Davies, 1963) although the compaction in all these cases must have been the same. Such a difference can be accounted for by the differences in the ambient temperatures of the different locations. Comparison of the ambient climatic conditions (Fig. 10) and the temperature changes (Fig. 9) in the wood chip pile studied shows that the ambient conditions had a strong effect on the changes in temperature.

Seasonal changes affected the pile in Prince George more than the other studies indicate. Generally, the outer regions freeze during the winter months, but even when the inner temperatures drop they do not approach ambient (Rothrock, Smith and Lindgren, 1961; Saucier and Miller, 1961; Björkman and Haeger, 1963; Shields and Unligil, 1968). In the cold weather of Prince George, the inner regions of the piles had temperatures near freezing.

The second rise in temperature (Fig. 9) after the decline from the maximum is not common with most piles. This happens in smaller piles in summer months (Butcher and Howard, 1968). This second rise in temperature

is probably due initially to increasing external temperature. This perhaps will, in time, bring temperatures to levels which will allow the resumption of thermophilic fungal activity. This is shown by Figure 9 where the second rise in temperature began with the warm whether.

3. Acidity of wood chips

The results on the pH of wood chips are at variance with findings of several authors who reported a rectilinear decrease of pH during storage (Annergren, Dillén and Vardheim, 1964; Shields, 1970). No attempt at correlating pH with time of storage was possible in this study since no set pattern existed for changes in pH.

The drop in pH is attributed to the production of acetic acid as a result of deacetylation of the hemicelluloses in the wood (Annergren, Dillén and Vardheim, 1964; Shields, 1970). Although some deacetylation of hemicelluloses must have occurred in the pile under study, the low wood substance loss could not have led to large acetic acid production. Changes in pH in this pile will not affect the activity and succession of the thermophilic and thermotolerant fungi.

4. Moisture in the wood chip pile

The extreme variation in moisture of chips during storage is said to be responsible for the distribution of fungi, and the rise in temperature in the inner top position of the chip pile (Rothrock, Smith

and Lindgren, 1961; Saucier and Miller, 1961; Björkman and Haeger, 1963; Annergren, Dillén and Vardheim, 1964; Nilsson, 1965; Bergman and Nilsson, 1966). Although the moisture content of the chips in this study was highly variable, especially between inner and outer samples, it had a minimal effect on the distribution of fungi. Moisture in the wood must have been adequate for the support of fungal activity.

The average moisture content of the chips was above 50% in all samples. However, moisture content above 100% was common in the samples from the inner top region, when they were stored for 12 months. This is similar to the findings of Björkman and Haeger (1963).

The high moisture content of the wood in the inner top of the pile is due to the melting of the ice and snow after the winter and to rainfall. Changes in rainfall have affected the moisture content of chips in small piles (Butcher and Howard, 1968). The edges of the pile dry out quicker than the top, consequently the inner top is wetter than the outer sides.

5. Damage

No serious damage as diagnosed by visual inspection was done to the chips during the outside storage for a period of 12 months at Prince George. Staining of the chips was minimal and weight losses were low. Moderate staining of the chips occurred in the outer chips stored for twelve months. These chips were mouldy, with very little blue staining present.

Staining of balsam fir and spruce has been reported after six to twelve months' storage, with little blue staining occurring (Shields and Unligil, 1968). The two findings are in agreement.

Although chips from the inner regions of the pile were light brown, they were not discoloured to any extent. In this respect the spruce-pine pile at Prince George behaved differently from piles in Sweden and eastern Canada (Annergren, Dillen and Vardheim, 1964; Shields, 1970) where it was found that owing to the low pH of the chips, they were brown and discoloured.

The weight losses of pine and spruce samples were low and did not increase from the six to twelve months' sampling period. The weight losses may not have increased from the second sampling period to the third sampling period because of the inclement weather conditions which could have affected fungal activity. Bergman and Nilsson (1966) showed that pine chips stored in the warmer parts of the pile lost 1% per month during storage.

High weight losses occur in the inner, hotter regions of the pile. Saucier and Miller (1961) found lower wood losses in the centre of southern pine pile than in the sides during both summer and winter storage. Bergman and Nilsson (1966) found lower wood losses in the centre of a summer stored chip pile while in winter storage the inner samples lost more. The spruce-pine pile in Prince George behaved like the winter pile which Bergman and Nilsson (1966) studied.

The conclusion from these findings is that the weight losses are affected by external conditions. This is possible in a small pile where the inside is affected by external conditions (Butcher and Howard, 1968). However, in a big pile the inside is well insulated and is not affected by external conditions (Björkman and Haeger, 1963). Of the factors tested only temperature and distribution of micro organisms are related and therefore could be responsible for weight losses.

# III LABORATORY INVESTIGATION OF WOOD DEGRADATION CAUSED BY THERMOPHILIC AND THERMOTOLERANT FUNGI

## A INTRODUCTION

Recent accounts (Bergman and Nilsson, 1966) of fungi isolated from wood chips during storage have indicated that the thermophilic and thermotolerant fungi might play a role in wood chip deterioration, especially in areas of the chip pile where high temperatures exist. In the first part of this study it was shown that the thermophilic and thermotolerant fungi were common in the inner region of the spruce-pine chip pile, where the highest weight losses of wood also occurred. There was a relationship between the wood substance loss and the number of these fungi. Relatively few laboratory studies have been made into the ability of the thermophilic and thermotolerant fungi to degrade wood and the factors affecting this phenomenon. These fungi caused low weight losses and the results of the experiments performed using these fungi were also variable (Nilsson, 1965; Bergman and Nilsson, 1966; Shields and Unligil, 1968).

The reason for the variation in the results of the previous studies could be that the basic factors governing the ability of these fungi to degrade wood have not been thoroughly investigated. The methods that have been used in the earlier work were borrowed from those methods designed for the investigation of wood decay caused by Basidiomycetes and soft rot of wood caused by Ascomycetes and Fungi Imperfecti.

This section is concerned with the demonstration that the common thermophilic and thermotolerant fungi isolated from the wood chip pile are capable of causing weight losses of wood. In order to accomplish this it was first necessary to develop a method to test for this ability. This section of the thesis is therefore subdivided into two parts. The first deals with the development of a method of testing, the second deals with the demonstration of the ability of the common isolates to cause weight losses.

The factors which were considered in the development of a method for testing the ability of thermophilic and thermotolerant fungi to cause weight losses of wood were the following:

1. The effect of the culture medium
2. The effect of wood sample size
3. The effect of method of inoculation
4. The effect of duration of incubation
5. The effect of temperature

## B LITERATURE REVIEW

The effect of medium on weight losses of wood caused by thermophilic and thermotolerant fungi has been little studied. In Sweden, Nilsson (1965) and Bergman and Nilsson (1966) used malt solution in

vermiculite, while in Canada, Shields and Unligil (1968) used the soil block burial test to study the activity of thermophilic fungi on wood. Cooney and Emerson (1964) have pointed out the difficulty of establishing thermophilic fungi on laboratory media. Of the media tested they found these four to be useful:

1. Yeast starch agar
2. Yeast glucose agar
3. Oatmeal agar
4. Czapek agar

Yeast starch agar and yeast glucose agar were particularly favourable for the growth of thermophilic fungi.

It has been pointed out that large numbers of microfungi are isolated from chips because of the chip size (Bergman and Nilsson, 1966). The small wood chips have large surface area to volume ratio and this may be critical in affecting the rate at which they are deteriorated. Findlay (1953), in laboratory studies of natural durability of wood, found that the amount of decay increased as the volume of the test species was reduced.

In the previous studies of thermophilic fungi on wood, incubation periods ranged from two months to three months (Bergman and Nilsson, 1966; Shields and Unligil, 1968). Bergman and Nilsson (1967) incubated C. lignorum on pine sapwood at 40°C and showed that the weight losses starting from the first month of incubation continued to increase every month till the fifth month when the experiment was terminated. In studies of weight losses caused by soft rot fungi the usual incubation period is six weeks (Duncan, 1953;

Savory, 1954).

Bergman and Nilsson (1966, 1968) and Shields and Unligil (1968) have tested some thermophilic and thermotolerant fungi and have shown that they could attack and cause weight losses in wood samples in the laboratory. The weight losses of coniferous species were lower than the weight losses of hardwood species and most of the fungi would not attack spruce. The thermophilic fungi caused considerably higher weight loss in aspen and birch samples.

Work on soft rot of wood has shown that soft rot fungi extensively attack hardwoods, whilst causing little or no rot in softwoods. Chaetomium globosum caused extensive damage to hardwoods but did not cause a weight loss in softwoods (Savory, 1954). The ability of the soft rot fungi to cause weight losses in wood is affected by the availability of nutrients, especially nitrogen, in the wood. It was shown that impregnation of the test wood with Abram's solution increased the weight losses substantially (Savory, 1954; Eslyn, 1969). Addition of nutrient salts increased the rate of decay by C. globosum, and the extent of decay appeared to be proportional to the amount of nutrients available to the fungus.

Temperature is a very important ecological factor affecting the distribution of many fungi. For a thermophilic fungus, temperature may be the most critical factor. Increasing the temperature up to the optimum generally increased the rate of growth and decay of wood by fungi. Bergman and Nilsson (1967) showed that the weight losses in birch, aspen, spruce

and pine wood caused by C. lignorum increased rapidly with increasing temperature and were highest at the optimum temperature for growth, which was 40°C. A. terrestris behaved similarly. Nilsson (1965) demonstrated that S. thermophile at its optimum temperature of 45°C caused up to 7% weight loss to wood during two months incubation.

Unlike the thermophilic fungi most Basidiomycetes are active at lower temperatures. Henningsson (1967) found that only three Basidiomycetes of the many isolated from birch and aspen pulpwood had optimal growth and decay activity above 30°C. He also showed that low temperature fungi have a comparatively low decay activity and that many fungi with higher temperature optima were more active at low temperatures than the low temperature fungi.

## C GENERAL METHODS

Test wood pieces (1/16 x 3/4 x 1 3/4 in.) 0.2 x 1.9 x 4.4 cm. were cut from ponderosa pine sapwood so that the long axis was parallel to the grain of the wood. The pieces were quickly impregnated with water and then conditioned at a constant temperature of 22.2°C and relative humidity of 50 ± 2% for seven days. The initial conditioned weights then were measured.

The test pieces were either sterilized in ethylene oxide or autoclaved. They were then placed on "S" shaped glass rods on the fungal cultures growing in petri dishes on a medium. The wood had no direct contact with the medium. Three pieces were put in each 9 cm. petri dish.

The samples were incubated at 45°C for six weeks. To prevent the cultures from dessicating the relative humidity in the incubator was kept high.

After incubation the surfaces of the wood samples were cleaned of all mycelium and were weighed while wet. The treated wood was again conditioned at the same temperature and relative humidity for seven days when final weights were taken.

Except for one experiment where different media were studied, Abrams cellulose medium was used in all studies. Media were autoclaved for thirty minutes at 121°C. Sixty-ml portions were dispensed into petri dishes and after cooling were inoculated with disks of inocula. The plates were incubated at 45°C for about four days to establish the fungus. The general design was the randomized complete design. The analysis of variance was used to analyse the results.

The fungi used in these experiments had previously been isolated from the spruce-pine chips from the Prince George experimental pile. Much attention was paid to the following because of their abundance in the pile:

1. B. emersonii
2. A. fumigatus
3. A. terrestris
4. S. thermophile
5. C. pruinosum

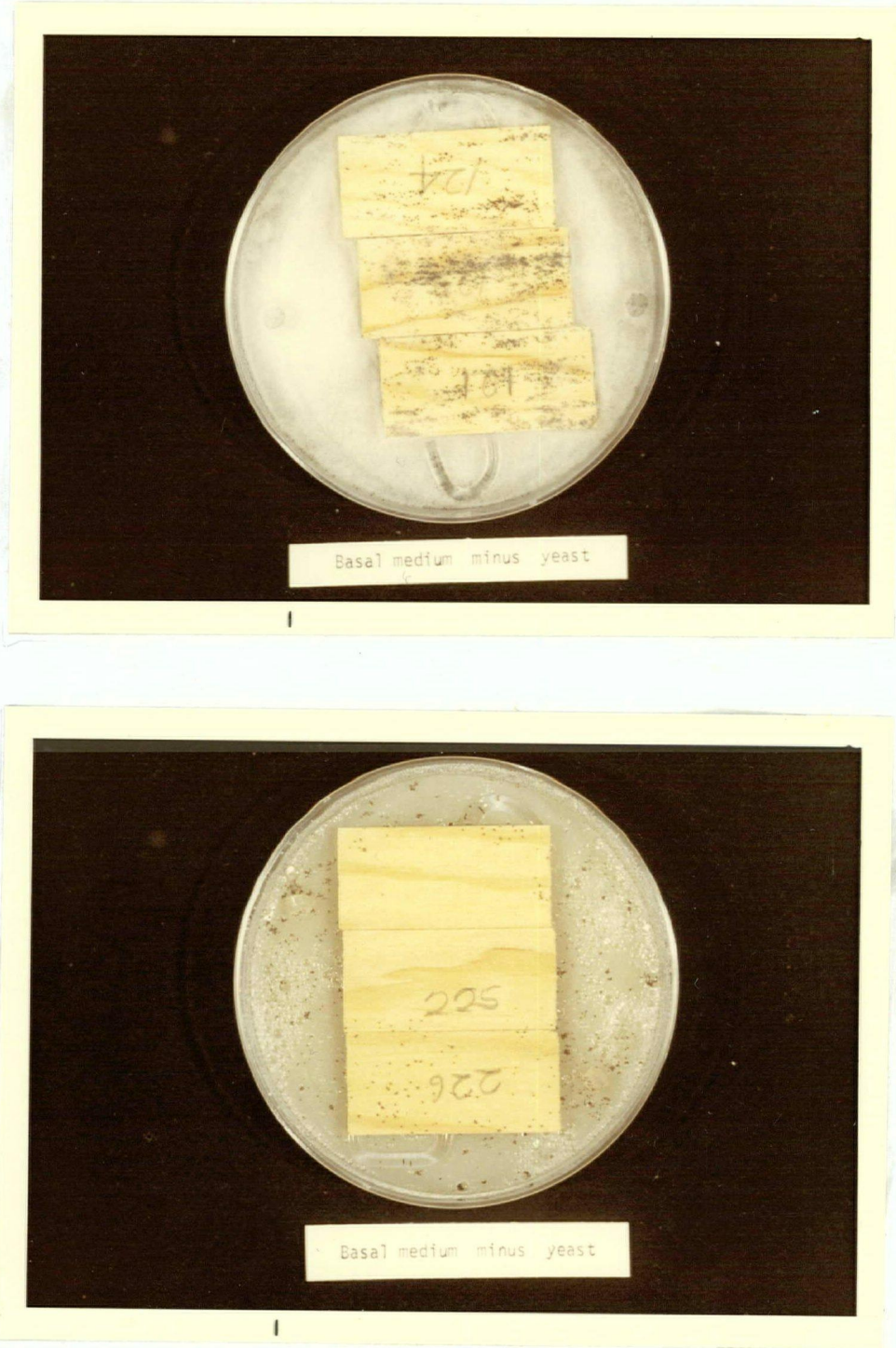



Figure 14. Allescheria terrestris and Thermoascus aurantiacus growing on Abrams cellulose medium and ponderosa pine. Top, A. terrestris; bottom, T. aurantiacus.

## D DEVELOPMENT OF A METHOD FOR THE STUDY OF THE ABILITY OF THE FUNGI TO CAUSE WEIGHT LOSSES OF WOOD

### 1. Effect of media

Standard methods are available for testing the ability of fungi, especially the Basidiomycetes, to degrade wood (BS 838 test, 1961 and ASTM D1413-61, 1961). These tests have been based on the careful study of media which serve as the substrate for the growth of the fungi.

No study of this nature is available for the thermophilic fungi. This study is intended to evaluate the effect of growth media on the ability of thermophilic fungi to cause weight losses of wood. The following media, the composition of which appear in Appendix 2, were used:

1. Abrams medium
2. 2% malt agar
3. Yeast-starch medium $Y_pSs$
4. Abrams-cellulose medium
5. 2% malt-cellulose medium
6. Yeast-cellulose medium $Y_pSc$

Table 9. Percentage weight losses caused to ponderosa pine sapwood on different media.

| Medium | Abrams | | Abrams cellulose | | 2% Malt | | 2% Malt 1% cellulose | | $Y_pSs$ | | $Y_pSc.$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fungus | % Wt. loss | S.D. | % Wt. loss | S.D. | % Wt. loss | S.D. | % Wt. loss | S.D. | % Wt. loss | S.D. | % Wt. loss | S.D. |
| Control | 1.42 | 0.24 | 0.93 | 0.24 | 1.15 | 0.15 | 0.85 | 0.52 | 0.50 | 0.35 | 0.97 | 0.52 |
| *Thermoascus aurantiacus* | 3.46 | 0.40 | 3.41 | 0.28 | 1.61 | 0.16 | 1.64 | 0.43 | 2.63 | 0.83 | 4.08 | 0.33 |
| *Humicola* sp. | 3.10 | 0.41 | 3.44 | 0.51 | 2.82 | 0.22 | 3.21 | 0.57 | 3.80 | 0.57 | 3.72 | 0.42 |
| *Sporotrichum thermophile* | 3.52 | 0.64 | 3.82 | 0.45 | 1.21 | 0.92 | 2.50 | 0.69 | 2.69 | 2.84 | 3.67 | 1.01 |
| *Allescheria terrestris* | 3.64 | 0.80 | 4.39 | 0.46 | 3.51 | 1.10 | 3.31 | 0.89 | 3.90 | 0.88 | 3.71 | 0.81 |

Table 10. Moisture content of samples on each medium

| | Abrams | | Abrams cellulose | | 2% malt | | 2% malt 1% cellulose | | YpSs | | YpSc. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % M.C. | S.D. | % M.C. | S.D. | % M.C. | S.D. | % M.C. | S.D. | % M.C. | S.D. | % M.C. | S.D. |
| Control | 22.1 | 2.2 | 62.5 | 42.9 | 48.5 | 23.2 | 50.3 | 7.1 | 42.0 | 19.0 | 34.8 | 14.9 |
| Thermoascus aurantiacus | 36.4 | 5.0 | 33.5 | 3.8 | 65.0 | 14.2 | 53.2 | 13.4 | 91.3 | 32.7 | 37.5 | 6.0 |
| Humicola sp. | 30.3 | 1.8 | 33.3 | 3.2 | 39.1 | 6.4 | 44.0 | 9.0 | 44.2 | 9.8 | 43.0 | 6.8 |
| Sporotricum thermophile | 35.6 | 7.2 | 33.0 | 7.4 | 178.1 | 9.9 | 187.6 | 15.7 | 96.7 | 34.7 | 46.7 | 16.7 |
| Allescheria terrestris | 31.5 | 6.6 | 41.3 | 7.4 | 103.4 | 34.7 | 140.5 | 59.3 | 55.5 | 20.1 | 49.5 | 13.3 |

The first three media normally are used for the study of fungal decay and the other three were made by adding 1% cellulose to the first two media and replacing starch with cellulose in the third medium. The following fungi were used: A. terrestris, Humicola sp., S. thermophile and T. aurantiacus. Each treatment was replicated twelve times.

Media had an affect on the ability of the fungi to cause weight losses of ponderosa pine sapwood (Table 9). There were significant differences between media and between fungi. No single medium was the best for all the fungi and there was significant interaction between fungi and media.

The two media resulting in the greatest wood weight losses were Abrams-cellulose and yeast-cellulose media. The addition of cellulose generally improved the effectiveness of the fungi to cause weight losses to ponderosa pine sapwood. Almost all fungi gave low weight losses when grown on malt agar medium.

On some media the moisture content of the samples (Table 10) was high and an average moisture content above 100% was measured in some samples incubated on malt-cellulose medium. S. thermophile caused high weight losses at higher moisture contents than the other fungi.

In the test of the effect of media on the weight losses caused by thermophilic fungi. it was shown that fungi did not behave independently of medium. There were "preferences" for media and the most pronounced was the "preference" of A. terrestris for Abrams-cellulose medium and T. aurantiacus for yeast-cellulose medium.

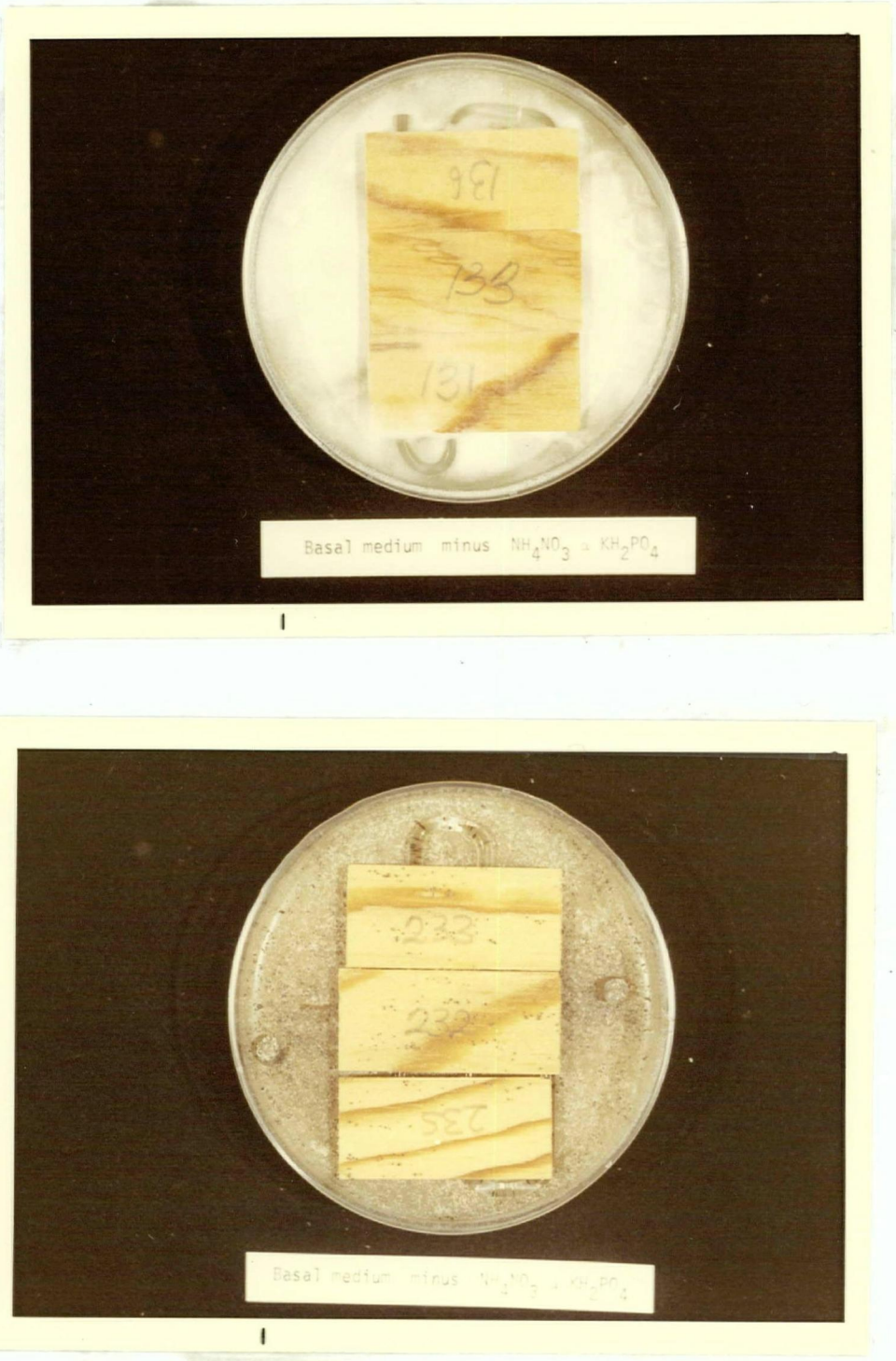



Figure 15. Growth of Allescheria terrestris and Thermoascus aurantiacus on yeast-cellulose medium and ponderosa pine. Top, A. terrestris; bottom, T. aurantiacus.

The differences between these two media were the absence of yeast from Abrams-cellulose and ammonium nitrate and potassium dibasic phosphate from yeast-cellulose medium. Since the search was for a medium which would be suitable for all the fungi, it was felt that a combination of these components might give the best medium. An experiment was set up to test the behaviour of the two fungi A. terrestris and T. aurantiacus to the three constituents and their combinations.

Eight media were tested. Each medium had a basal composition of magnesium sulphate, dipotassium monobasic phosphate, cellulose and agar. The composition of the media appear in the appendix 2.

The two fungi behaved differently to the different media (Table 11). Differences between fungi and between media were significant. There was significant interaction between fungi and medium.

Exclusion of yeast increased the weight losses caused by A. terrestris while decreasing that caused by T. aurantiacus. When potassium dibasic phosphate was removed the activity of A. terrestris was depressed while having no effect on the performance of T. aurantiacus. The reaction of the fungi to the exclusion of ammonium nitrate was similar to the exclusion of yeast. The removal of all three components from the medium had a more pronounced effect on A. terrestris than on T. aurantiacus.

Table 11. Percent weight losses obtained for A. terrestris and T. aurantiacus growing on medium prepared from cellulose, $MgSO_4 \cdot 7H_2O$ and $K_2HPO_4$ with the absence of yeast or $NH_4NO_3$ or $KH_2PO_4$.

| Composition | A. terrestris % Wt. loss | T. aurantiacus % Wt. loss |
|---|---|---|
| Complete medium | 3.89 | 4.46 |
| Without yeast | 4.42 | 3.85 |
| Without $KH_2PO_4$ | 3.25 | 4.31 |
| Without $NH_4NO_3$ | 4.32 | 4.03 |
| Without yeast & $KH_2PO_4$ | 3.78 | 3.94 |
| Without yeast & $NH_4NO_3$ | 2.16 | 3.71 |
| Without $KH_2PO_4$ & $NH_4NO_3$ | 3.91 | 4.17 |
| None | 1.92 | 3.60 |

T. aurantiacus was generally not as sensitive to changes in the nutrient composition of the medium as A. terrestris and benefited most from the incorporation of all three components into the medium.

After these experiments, Abrams-cellulose medium was selected for the rest of the study.

2. Effect of wood sample size

Two sample sizes selected for this study were similar in size to chips and the third size was chosen to be the same as that in the ASTM

D1413-61 test. The influence of sample size on weight losses of wood caused by thermophilic fungi was investigated.

The sizes of samples were 0.2 x 1.9 x 4.4 cm. (1/16 x 3/4 x 1 3/4 ins.), 0.4 x 2.5 x 5.1 cm. (1/8 x 1 x 2 in.) and 1.9 x 1.9 x 1.9 cm (3/4 x 3/4 x 3/4 in). and were cut so that the long axis was parallel to the grain of the wood. The sizes will be referred to as small, medium and large hereafter. They were all cut from ponderosa pine sapwood. The tangential surface was placed on the culture. The fungi used in this experiment were A. terrestris, B. emersonii and S. thermophile. Each treatment was replicated six times.

Sample size (Table 12) influenced the absolute weight loss of wood and the percentage weight loss of wood. There were significant differences between the percentage weight loss in the different sizes. The percent weight loss values for the small and medium sized pieces were greater than those for the large pieces,- except for S. thermophile where higher percent losses occurred in the medium sized samples than in the small or large samples. The reaction of the fungi was independent of the size of wood.

Sample size (Table 12) affected the absolute weight loss of wood in such a way that the small pieces lost less weight than the medium and large pieces. The difference in weight loss between different sizes of blocks was significant. The reaction to the different sizes of samples was dependent on the sample, since a significant interaction existed between fungi and size of sample. The results of this experiment did not lead to a change in the size of samples and in all subsequent experiments the small size pieces were still used.

Table 12. Percent weight loss and absolute weight loss of wood caused by thermophilic fungi growing on different sizes of ponderosa pine sapwood samples during six weeks incubation.

| | Percent weight loss | | | Absolute weight loss in grams | | |
|---|---|---|---|---|---|---|
| | (0.2 x 1.9 x 4.4) | (0.4 x 2.5 x 5.1) | (1.9 x 1.9 x 1.9) | (0.2 x 1.9 x 4.4) | (0.4 x 2.5 x 5.1) | (1.9 x 1.9 x 1.9) |
| | Small | Medium | Large | Small | Medium | Large |
| Control | 0.25 | 0.51 | 0.31 | 0.001 | 0.009 | 0.009 |
| *Allescheria terrestris* | 3.61 | 3.67 | 3.21 | 0.019 | 0.063 | 0.090 |
| *Byssochlamys emersonii* | 3.58 | 3.56 | 2.82 | 0.019 | 0.061 | 0.078 |
| *Sporotrichum thermophile* | 3.08 | 3.65 | 2.98 | 0.017 | 0.061 | 0.084 |

3. Effect of methods of inoculation

This study was designed to investigate the effect of different methods of providing the inoculum source on the weight losses of wood caused by thermophilic fungi. The usual method of providing inoculum in wood decay studies consists of growing the fungus on the basal medium for a period of time before the test blocks are planted. This method is unsuitable for soft rot studies (Duncan, 1953; Savory, 1954). In soft rot studies the wood, planted on Abrams medium, is directly inoculated with a spore suspension. Although these two methods have been extensively used in studies of microfungi from wood chips, no information is available on the effect of the different methods of providing inoculum source on the weight losses.

The following methods were tested:

1. Inoculation of wood samples.
2. Inoculation of medium, with wood samples subsequently planted on the medium on the same day.
3. Inoculation of medium, with wood samples subsequently planted on the culture after one week.

Two fungi, A. terrestris and B. emersonii were used and the method was the same as outlined under C-General methods. Each treatment was replicated six times. The results are shown in Table 13.

Table 13. Average percentage weight loss of wood samples caused by thermophilic fungi using different methods of providing the inoculum source.

| Methods of providing inoculum | A. terrestris % Wt. loss | B. emersonii % Wt. loss |
|---|---|---|
| Direct on wood | 2.87 | 2.91 |
| Inoculate medium-wood planted same day | 2.65 | 2.91 |
| Inoculate medium-wood planted after one week | 3.24 | 3.03 |

The method of providing the inoculum source had an effect on the weight losses caused to the wood. Differences between these methods were significant. The expression of these differences was linked to the fungus used in the test.

Both fungi caused higher weight losses if the medium was inoculated a week before the wood samples were planted. With both fungi there was no significant differences in weight loss if the wood was inoculated directly or if the medium was inoculated and the wood samples were planted on it on the same day. Inoculating the medium a week before planting the wood samples was considered the best method for inoculating the wood samples and was used in all subsequent experiments.

4. Effect of duration of incubation

Limited information is available on the effects of duration of incubation on the weight losses caused by thermophilic and thermotolerant fungi. The incubation periods for previous studies were arbitrarily chosen. This investigation was conducted to determine how duration of incubation affected weight losses caused by the thermophilic and thermotolerant fungi.

The fungi used in the study were A. terrestris, B. emersonii, C. pruinosum and S. thermophile. Six incubation periods were used varying from two to twelve weeks. Every two weeks, a whole set of treatments was taken out, conditioned and weighed. Each treatment was replicated six times. The rest of the method has been outlined before under C-General methods.

The weight losses were plotted against period of incubation and the results appear in Figure 16. All the graphs have been drawn from corrected data by deducting the weight losses in the control samples from the treated samples. Rapid weight losses were caused by all of the fungi during the first six weeks with the exception of C. pruinosum, which produced low weight losses in the first four weeks followed by a rapid increase from the fourth week to the sixth week.

Generally, no appreciable increases in weight losses occurred after the sixth week except for A. terrestris which attained a stable condition after the eighth week. Weight losses went up slightly for three of the fungi in the twelfth week, but this could be the result of a general drop in moisture content of the wood since the samples started to dry out.

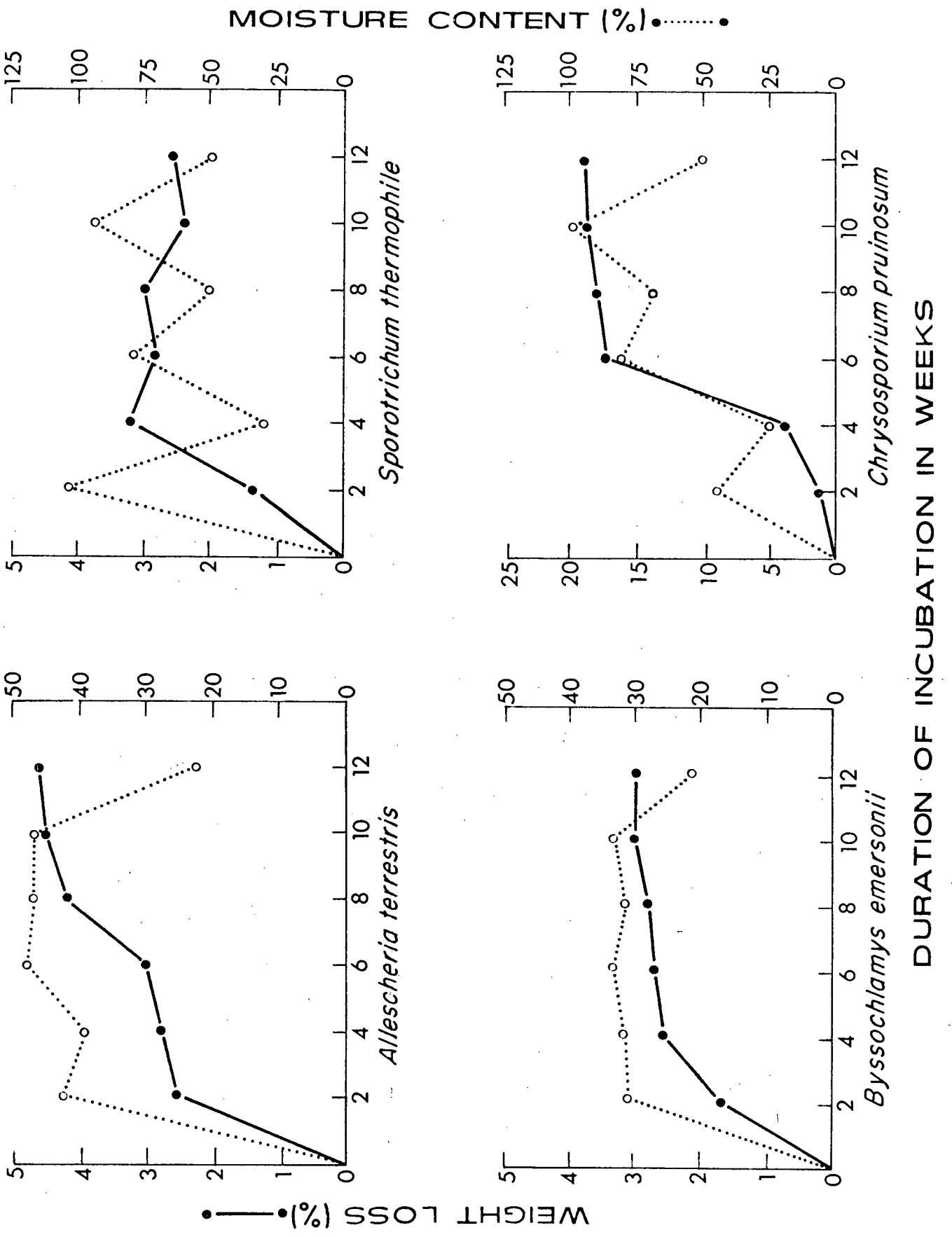



Fig. 16. Changes in weight losses with duration of incubation.

Fluctuations in weight losses were common for samples inoculated with S. thermophile. These fluctuations were opposite to changes in moisture content of the samples.

Since the activity of most of the fungi had stabilized after six weeks incubation, this time was chosen as the incubation period for the rest of the experiments.

5. Effect of temperature.

It was shown in the first part of this study that temperature affected the distribution of thermophilic and thermotolerant fungi in the chip pile. Wood substance loss was also higher in the inner region of the pile where temperatures were high. This experiment was conducted to investigate the effect of temperature on the weight losses caused by thermophilic and thermotolerant fungi.

Temperatures ranging from 25°C to 60°C were used for incubating the fungi. The temperature interval was 5°C. In a few cases 35°C incubation was not used. All cultures were incubated at 45°C before the start of the experiment. The incubation period was four weeks and six samples were used in each examination.

The following fungi were tested.

A. terrestris

A. fumigatus

B. emersonii

C. pruinosum

S. thermophile

T. aurantiacus

The results appear in Figure 17. All the graphs have been drawn from corrected data by deducting the weight losses in the control samples from the treated weight losses.

For all the fungi, increases in temperature led to increases in weight losses caused by them until the optimum temperature was reached and then weight losses decreased. Except for B. emersonii and T. aurantiacus, the highest weight losses occurred at 40°C. These two fungi caused their highest weight loss at 50°C.

All the fungi were active at 25°C except for B. emersonii and T. aurantiacus, which showed no activity at 25°C but started at 30°C. The temperature at which the activity of the organisms stopped, varied for different organisms. C. pruinosum activity ended at the maximum temperature 50°C, whilst that of B. emersonii and T. aurantiacus ended at or a little above 60°C. The other three fungi had the same limitation of activity maximum at a temperature of 55°C.

The increase in activity from the minimum to the optimum was rapid but the decline in activity from optimum to the maximum was gradual

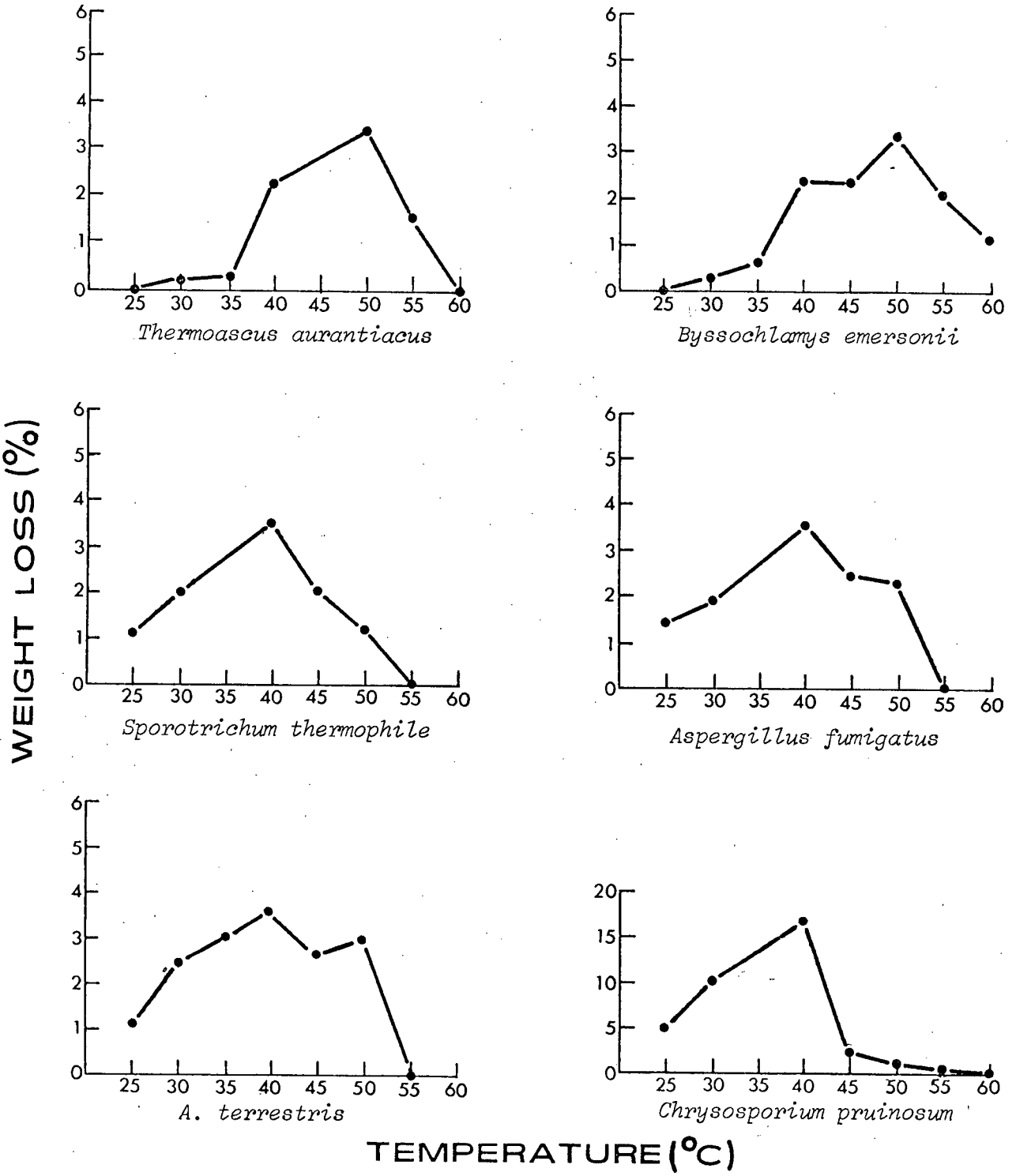



Fig. 17. Changes in weight losses with changes in temperature.

except for C. pruinosum and T. aurantiacus.

The production of ascocarps was greatly reduced at temperatures above 45°C and stopped completely at temperatures beyond 55°C. Mycelia production was sparse after 55°C except for B. emersonii and T. aurantiacus.

Samples, generally, showed a moisture deficit and charring after four weeks incubation at 60°C.

### 6. Summary

It appears from these results that the most suitable method for evaluating the ability of thermophilic and thermotolerant fungi to cause weight losses of wood will be by growing the test fungus on Abrams cellulose medium for one week at temperatures between 40° and 50°C, after which the wood samples (0.2 x 1.9 x 4.4 cm) are planted onto the culture. The test is then incubated for 6 weeks at the optimum temperature for the growth of the fungus. This may vary from 40° to 50°C.

## E WEIGHT LOSSES CAUSED BY THE THERMOPHILIC AND THERMOTOLERANT FUNGI

### 1. Evaluation of the common isolates

The following fungi, isolated at 45°C from wood chips, but not used in previous experiments, were examined for their ability to cause

weight losses of ponderosa pine sapwood. H. lanuginosa was not examined because it failed to grow on the Abrams cellulose medium which was used for this test. The method for the test was the same as outlined in C-General methods. Each treatment was replicated twelve times.

Table 14. Percent weight loss of ponderosa pine caused by some thermophilic and thermotolerant fungi at 45°C incubation.

| Fungi | % Wt. loss | S.D. |
|---|---|---|
| Control | 0.12 | 0.12 |
| Sporotrichum B. | 4.08 | 0.49 |
| Aspergillus fumigatus | 3.62 | 0.42 |
| Unknown ACIO | 3.52 | 0.14 |
| Unknown 61 | 3.40 | 0.24 |
| Unknown 6214 | 3.35 | 0.77 |
| Byssochlamys emersonii 11 | 3.30 | 0.23 |
| Byssochlamys species 201 | 3.26 | 0.36 |
| Byssochlamys emersonii 12 | 3.07 | 0.29 |
| Unknown 618 | 3.01 | 0.43 |
| Unknown 624 | 1.68 | 0.37 |
| Unknown 6310 | 0.71 | 0.40 |

All the fungi examined could cause weight losses of ponderosa pine sapwood (Table 14). Sporotrichum B. caused a high weight loss compared

to the other fungi. Two fungi Unknown 624 and Unknown 6310 caused very low weight losses, probably because of their poor growth on Abrams-cellulose agar.

2. Susceptibility of lodgepole pine and white spruce wood to attack by several fungal isolates.

In the earlier experiments ponderosa pine sapwood was used because of its availability in the laboratory and its extensive use in wood decay studies in North America. The chip pile from which the fungi used in this study have been isolated was made up of lodgepole pine and spruce. Therefore an investigation was made to study the action of some of the fungi on lodgepole pine and spruce. The experimental procedure was the same as outlined in C-General methods except for the different tree species used. Each treatment was replicated twelve times. The results are shown in Table 15.

There were remarkable visual differences in the attack of the fungi on pine and spruce. Most of the fungi completely covered pine after ten days of incubation, but not spruce. Not until halfway into the incubation period did all fungi completely cover spruce, even though staining of spruce was noticed earlier.

Table 15. Percentage weight loss of lodgepole pine and spruce sapwood caused by some thermophilic and thermotolerant fungi at 45°C after six weeks incubation.

| Fungi | Spruce % Wt. loss | Lodgepole pine % Wt. loss |
|---|---|---|
| Control | 1.53 | 1.96 |
| *Allescheria terrestris* | 3.06 | 9.17 |
| *Sporotrichum thermophile* | 3.39 | 5.18 |
| *Sporotrichum* B. | 5.02 | 6.69 |
| *Chrysosporium pruinosum* | 2.03 | 3.74 |
| *Byssochlamys emersonii* 19 | 2.54 | 3.88 |
| *Byssochlamys emersonii* 12 | 2.65 | 3.63 |
| Sterile mycelium | 2.85 | 3.72 |
| Unknown 6214 | 3.60 | 5.65 |
| *Aspergillus fumigatus* | 2.51 | 3.91 |
| *Thermoascus aurantiacus* | 2.58 | 3.95 |

All the fungi examined caused weight losses of lodgepole pine and spruce. Higher weight losses occurred in lodgepole pine than in spruce as shown in Table 15.

At the end of the experiment, some of the spruce samples were stained throughout and some had a streak of stain in the middle. Staining was observed for samples inoculated with the following fungi:

A. terrestris

S. thermophile

Sporotrichum B.

Unknown 6214

When stained and unstained samples were compared, the unstained samples had lost more weight than the stained samples (Table 16).

Table 16. Percentage weight loss for stained and unstained spruce inoculated with some thermophilic fungi

| Fungus | Unstained % Wt. loss | Stained % Wt. loss | $t_{0.5}$ | $t_{0.5}$ |
|---|---|---|---|---|
| Unknown 6214 | 4.69 | 2.82 | 2.79* | 2.28 |
| Sporotrichum B. | 6.02 | 4.29 | 3.46* | |
| Sporotrichum thermophile | 3.77 | 3.01 | 2.62* | |

The differences between the unstained and stained spruce samples were significant for all the three fungi examined. A comparison was not carried out for A. terrestris, since there were not enough unstained samples.

3. Effect of mixed isolates on weight losses

From a small sterilized piece of wood it was possible to isolate different species of thermophilic fungi. In mixed cultures of these fungi on malt agar, zones of inhibition were not found. Since all the different species have different capacities for attacking wood, it was thought that some of the fungi might depend on others to obtain their nutrients from the wood. If this were so, then it might be possible to obtain higher losses if more than one fungus were used to inoculate the wood.

An experiment to study the effect of mixed cultures of thermophilic fungi on weight losses of ponderosa pine sapwood was performed. The following thermophilic fungi which could grow together in culture and were very common in the pile were examined:

A. terrestris

B. emersonii

S. thermophile

Two incubation temperatures of 45°C and 50°C were used because the maximum activity of the three fungi was found to occur at different temperatures. Plates were inoculated with more than one fungus by placing side by side 2mm disks from malt agar cultures. Each treatment was replicated 6 times.

The mixed cultures did not generally cause higher weight losses than the single cultures except for the culture of B. emersonii and S. thermophile incubated at 45°C and the mixed cultures of all three fungi incubated at 50°C (Table 17).

Table 17. Effect of interaction between A. terrestris, B. emersonii and S. thermophile on weight losses of ponderosa pine incubated at 45°C and 50°C.

| Fungus | 45°C % Wt. loss | 50°C % Wt. loss |
|---|---|---|
| Control | 0.49 | 0.42 |
| Allescheria terrestris | 2.99 | 3.39 |
| Byssochlamys emersonii | 3.22 | 3.72 |
| Sporotrichum thermophile | 3.50 | 0.93 |
| A. terrestris & B. emersonii | 3.32 | 3.81 |
| A. terrestris & S. thermophile | 3.24 | 2.71 |
| B. emersonii & S. thermophile | 4.14 | 3.59 |
| A. terrestris & B. emersonii & S. thermophile | 3.22 | 4.07 |

Dependence of the fungi on temperature was more significant than the interaction between the fungi. Although the activity of the three fungi was generally not complementary, no obvious inhibition of one fungus by the other occurred.

## F DISCUSSION

Medium, unlike temperature, has not been studied extensively in the investigation of the effects of thermophilic fungi on wood. The ability of thermophilic fungi to degrade wood depends on the medium on which it is grown. These differences have been shown in the present study, in which no single medium was found to be the best for the study of weight losses caused by the total spectrum of thermophilic fungi. In all the previous studies of this subject, only one medium has been used, and no attention has been paid to the fact that the use of a single medium can oversimplify the weight loss results (Nilsson, 1965; Bergman and Nilsson, 1966, 1967, 1968; Shields and Unligil, 1968). For this reason these authors failed to show that a large number of thermophilic fungi from wood chips can be destructive.

Some thermophilic fungi are highly sensitive to changes in the composition of the medium in which they grow, while others are not. Drastic changes in the medium affected the ability of A. terrestris to cause weight losses more than they affected T. aurantiacus. The presence of yeast in a medium, while depressing the weight losses caused by A. terrestris, increased those caused by T. aurantiacus. Savory (1954) showed that for a soft rot fungus, impregnation of the wood with ammonium sulphate increased the weight losses to the wood caused by C. globosum while the impregnation with magnesium sulphate led to negligible losses. The two examples indicate that fungi have different "preferences" for different composition of media.

Although impregnation of wood with salts and solution like Abrams has been found useful for the study of weight losses caused by microfungi (Savory, 1954; Eslyn, 1969) it may not be a reliable method, since it could lead to a hydrolysis of some chemical constituents of the wood which will give misleading results when fungi are grown on the wood. Autoclaving mono and oilgosaccharides with salts leads to a change in the original structure (Ball, 1953; Bretzloff, 1954). High weight losses might be obtained which might not be due to the activity of the fungus alone. The aim of this study was to obtain a medium that would allow the fungus to cause high weight losses of wood without previously altering the composition of the wood.

Some of the thermophilic fungi have a tendency to accumulate moisture in the wood samples, depending upon the substrate on which they grow. The high moisture content of the sample might reduce the activity of the fungi. The thermophilic fungi were highly variable and the same medium which was efficient at one time could give a poor result at another time. This is probably the reason for the statement by Shields and Unligil (1968) that it is difficult to assess the role of the thermophilic fungi in the deterioration of wood chips.

There is no single medium which is suitable for studies of the effect of thermophilic fungi in wood. Based on the present results Abrams-cellulose medium was chosen as giving reasonable weight losses for the fungi tested.

The size of the wood chips may contribute to the abundance of Fungi Imperfecti and Ascomycetes in the chip pile (Bergman and Nilsson, 1966). It is not probable that the chip size *per se* increases the weight of wood loss. How chip size affects weight losses depends on how the losses are calculated.

The absolute weight loss caused by thermophilic fungi is directly proportional to the size of the wood; the bigger the sample the greater the weight loss. The percentage weight loss is inversely proportional to the size of the sample (Findlay, 1953). This was not the case with the thermophilic fungi examined, since the percentage weight losses of the medium sized samples were greater than those of the small sized samples.

Two different classes of fungi were used in the two studies and this may have brought about the differences. The thermophilic fungi may be limited in the quantity of nutrients available to them in a given piece of wood if they do not attack cellulose or lignin. The Basidiomycetes in attacking cellulose or cellulose and lignin will undoubtedly have a larger proportion of the wood to attack. In this situation the attack of wood by Basidiomycetes might depend more upon the ratio of surface area to volume. The reduced sample will, on the other hand, limit what is available to the thermophilic fungus.

However, little is known about the nature of attack of wood by thermophilic fungi. It has been shown that *A. terrestris* causes soft rot of hardwoods and therefore will attack cellulose (Bergman and Nilsson, 1967). These authors also pointed out that the moulds isolated from chip piles do not attack lignin but attack carbohydrates.

If weight losses are to be calculated in percentages, then small sample pieces should be chosen for the study. However, if weight losses are calculated in absolute terms, larger sample pieces are much more appropriate.

Thermophilic fungi will attack wood so long as the moisture in the wood is adequate and the temperature is suitable. It was noticed that unless the conditioned wood had absorbed sufficient moisture it was not possible to get the fungi to attack the wood.

Inoculating wood with thermophilic fungi did not present any of the difficulties associated with soft rot fungi. Savory (1954) could not successfully inoculate wood with soft rot fungi until he planted unsterilized wood samples on Abrams medium and seeded them with a spore suspension of C. globosum which led to the inoculation of the wood.

Planting the samples on weak old cultures of thermophilic fungi might have given higher weight losses because the fungi would probably have had enough time to grow and produce adaptive enzymes which are necessary for attacking wood.

In many studies on the effects of thermophilic fungi on wood, samples have been incubated for two to three months (Nilsson, 1965; Bergman, and Nilsson, 1966; Shields and Unligil, 1968). It appears from the present study that incubation beyond six weeks does not serve any useful purpose. In this respect the thermophilic fungi may be similar to the soft rot fungi which are usually incubated for six weeks (Savory, 1954).

A short period of incubation has certain advantages considering the conditions in which these tests are carried out. Results are obtained earlier and the drying problem at high temperatures is considerably reduced.

Most of the wood degradation in the laboratory takes place in the first few weeks of incubation, and the rest of the incubation time does not contribute a great deal to the total losses. Why this happens is not clear, but the following hypothesis may explain the phenomenon. The rapid growth of the thermophilic fungi may lead to the rapid depletion of the nutrients on which the fungi depend, probably resulting in the accumulation of metabolic by-products which may be inhibitory.

The percentage weight loss of wood caused by a thermophilic fungus increases with temperature until optimum temperature is reached, at which point it starts to decrease until the maximum temperature is reached, where activity is terminated. Studies in which the weight losses caused by A. terrestris increased eleven fold from the minimum to the optimum temperature (Bergman and Nilsson, 1967) support this conclusion.

The rise in the activity of the fungus as the temperature increases to the optimum is slower than the decline in the activity as the temperature increases from optimum to the maximum.

The optimum temperature for the activity of a thermophilic fungus is affected by the substrate on which it grows. The optimum temperature for

A. terrestris for radial growth on malt agar is 45°C, but on birch the optimum temperature associated with the highest weight losses is 50°C (Bergman and Nilsson, 1967). In the present study the optimum temperature at which A. terrestris caused the highest weight losses in pine was 40°C. This finding agrees with the work of Henningsson's (1967). He found that for several fungi from birch and aspen the temperature for optimum radial growth is higher than that for optimal decay activity.

The ability to cause weight losses by some thermophilic fungi may extend beyond 60°C. It has been pointed out that temperatures above 60°C in the pile may completely sterilize the chips (Nilsson, 1965). B. emersonii was very active when the temperature of incubation was 60°C for four weeks. It is possible that above 60°C incubation B. emersonii may cause weight losses.

Unlike the optimal temperature, the substrate did not affect the minimum temperature at which the activity of the fungus began. The minimum temperature for radial growth of T. aurantiacus on malt is 30°C (Bergman and Nilsson, 1966) and 30°C was the temperature at which it began to cause weight losses in pine as observed in this study.

The capacity of thermophilic fungi to attack different species of coniferous wood is variable, although nearly all the fungi examined will degrade pine and spruce. The observed degradation of lodgepole pine is a little greater than spruce. Similar results were obtained by the Swedish

workers except that some of the fungi they tested could not attack spruce at all (Bergman and Nilsson, 1966). The extent of attack was much lower in the fungi which Bergman and Nilsson (1966) investigated. This might be expected because of differences in species of wood, media and variation in the fungi themselves.

The cause of staining in the spruce samples is unknown. Staining may be dependent upon the position in the tree from which the samples came, since some heartwood might have been inadvertently included in the samples due to difficulty in differentiating between heartwood and sapwood zones in spruce. However, the nature of the observed staining, which may sometimes form a streak in the middle of a sample, does not support this contention.

Stained spruce lost less weight than unstained spruce. Hossfeld, Oberg and French (1957), found also that discoloured aspen associated with knots, Nectria canker and wet wood was more resistant to decay than the sapwood controls. It was suggested by Hossfeld, Oberg and French (1957) that the discoloured aspen wood contain extractive components some of which were toxic towards wood decaying fungi.

The thermophilic fungi examined were neither antagonistic nor synergistic in their interaction. Each fungus probably occupies its own zone in the wood without depending upon the other fungi present. Any interdependence between fungi that may take place will rely to a large

extent on temperature. Trichoderma lignorum was found to inhibit the ability of C. lignorum to degrade wood but only at low temperatures (Bergman and Nilsson, 1967). As the temperature increased the effect of T. lignorum disappeared.

In summary, it has been shown that in controlled laboratory experiments, thermophilic and thermotolerant fungi can cause wood weight losses and that these losses are affected by medium, duration of incubation, temperature of incubation, the tree species used and method of inoculation. Although sample size and interaction of fungi were considered, they were found to have little influence on the eventual percentage weight losses of the wood samples.

In conclusion, these results suggest that the optimal conditions for evaluating weight loss of wood caused by these fungi would involve using Abram-cellulose medium, growing the fungi on this medium for one week, planting wood samples (0.2 x 1.9 x 4.4 cm.) on the one week old cultures and incubating the test for 6 weeks at 45°C for thermophilic fungi and 40°C for thermotolerant fungi. This method developed for the evaluation of weight loss of wood caused by these fungi is an efficient one and the results obtained show less variability than those of previous workers.

# IV CHEMICAL ANALYSIS OF DEGRADED WOOD

## A INTRODUCTION

Thermophilic fungi have been isolated from chips and have also been shown to degrade wood in laboratory studies (Bergman and Nilsson, 1966, 1968; Shields and Unligil, 1968). Relatively little information is available on the nature of their specific chemical activity in wood. The present study was undertaken to provide some information on the specific chemical activity of some thermophilic fungi in wood.

The method of analysis followed was the hydrolysis of both cellulose and hemicellulose to monosaccharides, the reduction of the monosaccharides to sugar alcohols and the acetylation of the sugar alcohols. These acetylated compounds were then injected into a gas chromatograph.

## B LITERATURE REVIEW

Bergman and Nilsson (1968) noted that a number of soft rot fungi, including thermophilic fungi, isolated from a birch chip pile did not cause any lignin losses and thus the soft rot fungi mainly degraded the carbohydrates of the wood. Chang (1967) found that both hemicellulose and

cellulose were quite susceptible to microbial attack during self heating of straw and in 60 days of composting about 70.7% of the cellulose was removed. Henssen (1957) and Chang (1967) demonstrated the ability of S. thermophile to decompose hemicellulose and pectin. Chang (1967) and Fergus (1969) have shown that S. thermophile would degrade cellulose.

## C MATERIALS AND METHODS

The method used for the preparation of the wood samples for this analysis is the same as outlined in Part III. The fungi used were A. terrestris, B. emersonii and S. thermophile. Incubation periods of 2, 6 and 12 weeks were employed. Six of the wood samples for each treatment were ground together to pass through a 60 mesh sieve. From this 1 gm. was taken for the analysis.

Lignin contents were determined by the standard Klason method (Tappi standard T 13 05 - 54, 1954) and were not corrected for acid-soluble lignin. Carbohydrate analyses were made on the filtrate from the Klason lignin determination.

One gram of ground wood to which 250 mg. of inositol was added as an internal standard, was hydrolysed with 15 ml. of 72% sumphuric acid. The mixture was diluted with distilled water and heated in an autoclave

at 212°F for four hours. The lignin was removed by filtration and estimated by the standard Klason method. To convert the sugars to alditols an aliquot of the Klason lignin filtrate was brought to a pH of 4 by addition of saturated barium hydroxide solution. The resulting precipitate was then removed by centrifugation. The centrifugate was treated with 50mg. sodium borohydride and left over night. The reducing solution was acidified with 0.1ml glacial acetic acid and taken to dryness under vacuum using a flash evaporator in a water bath at temperature of 35° to 40°C. The boric acid was removed as methyl borate (Wolfrom and Thompson, 1963) by four evaporations under vacuum with methanol. The residue was taken up in a small volume of methanol and transferred to a volumetric tube where the methanol was again removed by evaporation under vacuum. The additive mixture was acetylated by adding 1 1/2ml acetic anhydride and heating the mixture for three hours at 120°C.

The excess acetic anhydride was removed by evaporation and the residue taken up in 2ml methylene dichloride. This solution was then injected into a gas chromatograph for analysis.

The gas chromatograph used was Series 1520 Varian-Aerograph with flame ionization detector, initial oven temperature 140°C, heating rate 1/2°/minute for 25 minutes, injection temperature 250°C, detector temperature 225°C with nitrogen as carrier gas. The copper column (1/8" x 2.5') was packed with 3% ECNSS-M on Gas Chrom Q (Sawardeker, Sloneker and Jeanes, 1965; Oades, 1967). The peak areas were measured with a Model 476 Varian-Aerograph digital integrator with inositol as the internal standard.

D RESULTS

In this exploratory test, considering the experimental variation in the chemical method (approximately ± 2%, personal communication, Dr. K. Hunt) and also the experimental variation to be expected in the mycological method, any changes in the results for glucose, mannose, xylose and lignin appear to be inconclusive. However, the results for arabinose indicate a decrease of this branch residue with increasing time of attack by all of the fungi.

Table 18. Concentration of various chemical components of ponderosa pine sapwood after degradation by some thermophilic fungi. Percentages are based on degraded wood.*

| Fungi | Time in wks. | Carbohydrate % & Lignin % | | | | |
|---|---|---|---|---|---|---|
| | | Lignin | Glucose | Mannose | Xylose | Arabinose |
| Control | 2 | 24.7 | 40.0 | 8.0 | 5.0 | 1.5 |
| | 12 | 25.1 | 41.3 | 12.3 | 5.6 | 1.6 |
| Allescheria terrestris | 2 | 25.2 | 38.0 | 10.0 | 5.1 | 1.5 |
| | 6 | 25.2 | 46.0 | 9.5 | 5.6 | 0.0 |
| | 12 | 26.3 | 46.2 | 11.0 | 5.6 | 0.0 |
| Byssochlamys emersonii | 2 | 25.5 | 41.7 | 10.4 | 4.5 | 0.8 |
| | 6 | 25.1 | 46.0 | 11.0 | 5.0 | 0.2 |
| | 12 | 25.8 | 43.0 | 13.6 | 4.8 | 0.1 |
| Sporotrichum thermophile | 2 | 25.5 | 48.7 | 11.0 | 4.0 | 0.4 |
| | 6 | 26.1 | 41.6 | 10.1 | 5.3 | 0.2 |
| | 12 | 25.7 | 47.0 | 13.3 | 5.3 | 0.2 |

* Since previous tests showed that these 3 fungi would give no more than about 4% weight loss, calculation of chemical results could be based on either sound or degraded wood with very little difference in the resulting values. In this test degraded wood weights were used.

## E DISCUSSION

The thermophilic fungi investigated do not attack lignin and do not appear to attack any of the carbohydrate constituents except arabinose. Bergman and Nilsson (1968) have indicated that soft rot fungi from a birch chip pile, which included some thermophilic fungi, did not attack lignin but attacked carbohydrates. No specific component of the carbohydrate was indicated as being attacked. Henssen (1957) and Chang (1967) showed that S. thermophile would decompose hemicelluloses and pectin. From this limited study it is possible to hypothesize that some of the sugar constituents of the xylans in the wood, especially arabinose may be attacked by the thermophilic fungi.

# V GENERAL DISCUSSION AND CONCLUSIONS

A study of the distribution of fungi in a chip pile should include all types of fungi which might inhabit this ecological location. However, much of the earlier studies concentrated mainly on mesophilic fungi (Bergman and Nilsson, 1966, 1967, 1968; Eslyn, 1967; Shields and Unligil, 1968; Shields, 1970) which occur commonly at ordinary temperatures. The peculiar ecology of a chip pile called for an emphasis on fungi capable of living at much higher temperatures. At the beginning of this study, all fungi were isolated, using two isolation temperatures, 25°C and 45°C. It was soon evident that many more fungi were isolated at 45°C than at 25°C, especially from the areas of the chip pile where higher temperatures occurred. The wood chips stored in the areas of the pile which produced large numbers of isolates at 45°C lost more weight. Therefore it appeared that greater attention should be paid to the isolates from 45°C than previous workers had given these fungi. Actinomycetes and Bacteria will be common in the chip piles. Eslyn (1967) and Shields (1970) have isolated bacteria and Eslyn (1967) has isolated Actinomycetes from chip piles, but these organisms were not considered to be important in quantitative wood degradation and were not treated in the present study.

The succession of fungi in a chip pile will depend upon many environmental factors such as temperature, the ability of the fungi to use wood as a nutrient source, the availability of moisture, the pH of the wood and aeration in the chip pile. Aeration in the chip pile at Prince George was not investigated but it has been suggested that accumulation of carbon dioxide or depletion of oxygen does not occur in chip piles (Hajny, Jorgensen and Ferrigan, 1967; Bergman and Nilsson, 1968). Moisture and pH were not found to be limiting factors in the development of fungi in the Prince George pile while all the fungi examined could use wood as nutrient source. The major factor which determined the succession of fungi in the present study was temperature. Thus a successional pattern based on groups of fungi delimited according to their temperature tolerances is given for the Prince George chip pile. This pattern will vary from one area of the pile to the other.

The initial fungal colonizers of the chip pile were fungi capable of growing at ordinary temperatures, like various species of Trichoderma, Gliocladium, Ceratocystis and Penicillium, which were isolated but not included in this study. Together with these fungi were thermotolerant fungi (Cooney and Emerson, 1964) like A. fumigatus and C. pruinosum. With rising temperatures, the thermophilic fungi (Cooney and Emerson, 1964) began to grow in the pile especially when temperatures rose above 22°C. These included organisms like S. thermophile and A. terrestris. Above 30°C, B. emersonii and T. aurantiacus

started to grow while some of the mesophilic fungi started to disappear from the chip pile. Most of the mesophilic fungi in the pile might have died above 40°C when the activity of both the thermophilic and thermotolerant fungi was optimum. Some of the thermotolerant fungi probably died at temperatures above 50°C when B. emersonii and T. aurantiacus were most active.

With temperatures above 55°C few fungal species will remain active among which were B. emersonii and T. aurantiacus. This pattern is supported by the distribution of fungi with storage time and the behaviour of some of these organisms on wood with changing temperatures.

B. emersonii may be the most important fungus in chip storage in the Prince George area. Its development could result in loss of wood substance, increased temperature and eventual serious degradation of wood. In chip piles in Sweden (Nilsson, 1965; Bergman and Nilsson, 1966, 1967, 1968) C. lignorum has been found to be most destructive. In this study, however, Chrysosporium was found to be a rare colonizer of the chip pile and these results suggest that it should not pose a problem in chip piles in and around Prince George. It is suggested here that the findings on a chip pile from one part of the world may not be true for another part of the world, however similar conditions may be. No thermophilic or thermotolerant Basidiomycetes were isolated from the spruce-pine chip pile in Prince George.

Chips will come onto the pile heavily contaminated by fungi because of the present methods used in handling chips. When a new pile is near to an old pile, further inoculum will come from the old pile. In a new area where no

piles have been built before, where will the inoculum come from? It is postulated that sufficient inoculum will come from the soil and from air-borne spores to initiate chip pile degradation. It is, however, suggested here that new chips should not be put on a base of old chip piles which must act as a large reservoir of infection.

Weight losses of wood caused by thermophilic and thermotolerant fungi in the laboratory were higher than losses of wood substance obtained in the Prince George pile. The higher losses of wood in the laboratory are to be expected since weight losses are studied under optimal conditions which do not prevail in the chip pile. When the weight losses of wood caused by thermophilic and thermotolerant fungi are compared to decay losses of wood caused by Basidiomycetes, the losses by the thermophilic and thermotolerant fungi are low. In chip piles, the frequency of isolation of Basidiomycetes is low (Nilsson, 1965; Shields, 1970). These factors indicate that losses in the chip piles are generally caused by microfungi. In areas of the chip pile where high temperatures occur most of the damage will be done by the thermophilic fungi. This view is supported by the observation from this study that no further weight losses occurred from the six months' to the twelve months' sampling period when the low temperatures in the pile would probably have affected the metabolic activity of the thermophilic fungi. It has also been shown that no increases in thermophilic and thermotolerant fungi occurred from the six months' to the twelve months' sampling period. It was also shown in the laboratory studies that all the thermophilic and thermotolerant

fungi would cause high weight losses of wood at temperatures above 40°C. This study has provided a method for testing thermophilic fungi on wood.

A chip pile is obviously not built only of sapwood, but will also include some heartwood. However, the percentage of heartwood is low and may be less than 10% (Keays, 1970). A fungus' ability to attack sapwood as shown in these studies will reflect its ability to destroy chips during storage. Both lodgepole pine and spruce are non-durable species and the thermophilic fungi may attack spruce and lodgepole pine heartwood during storage although not as strongly as the sapwood is attacked. Bergman and Nilsson (1967) showed that C. lignorum would attack both the heartwood and sapwood of pine.

Studies into the nature of attack of wood chips, by thermophilic and thermotolerant fungi, especially the chemical nature of the attack, are necessary. It is suggested that control of chip deterioration in Prince George should involve the prevention of the spread of thermophilic and thermotolerant fungi, especially B. emersonii in these piles. It might be shown conclusively that B. emersonii does not attack cellulose in the wood, but this will not nullify the control of the fungus since it may be responsible for thermogenesis in the chip pile.

Bergman and Nilsson (1968) have suggested that smaller piles, where the inside of the pile is near to freezing, be built in the winter and larger piles, where the inside of the pile will be at or about 60°C be built in the summer. This suggestion is the result of larger losses in winter piles in

Sweden when the temperature was about 40°C in the interior of the pile and smaller losses in summer piles when the temperature was about 60°C in the interior. It must be pointed out here that the pile at Prince George behaved like the winter pile in Sweden and that if larger piles are built during the summer in Prince George, losses can only be prevented by keeping the inside temperature of the pile above 60°C for most of the time. This may be difficult since larger volumes of chips can start a chain reaction which may lead to spontaneous combustion of the chips.

In conclusion, it has been shown that the hotter parts of the pile are inhabited by fungi and that weight losses are incurred in these regions of the pile. Furthermore, weight loss was positively correlated with both fungi and temperature. Controlled laboratory experiments have shown that the fungi commonly isolated from the chip pile were capable of causing weight losses when compared to uninoculated controls maintained under the same experimental conditions. A chemical analysis of wood exposed to common thermophilic fungi indicated that some of the sugar constituents of the xylans especially arabinose may be destroyed. It is concluded that thermophilic and thermotolerant fungi are directly responsible for the weight losses incurred in the experimental wood chip pile.

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Appendix 1

Total count/100 chips of T. aurantiacus, Byssochlamys sp. and C. pruinosum after storing chips for 12 months

| Position in Pile | Thermoascus aurantiacus | | | Byssochlamys sp. | | | Chrysosporium pruinosum | | |
|---|---|---|---|---|---|---|---|---|---|
| | DURATION OF STORAGE OF CHIPS IN MONTHS | | | | | | | | |
| | 3 | 6 | 12 | 3 | 6 | 12 | 3 | 6 | 12 |
| Outer bottom | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 2 | 1 |
| Inner bottom | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Outer middle | 0 | 1 | 1 | 0 | 1 | 0 | 21 | 18 | 15 |
| Inner middle | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 2 |
| Outer top | 0 | 0 | 0 | 1 | 11 | 0 | 6 | 6 | 5 |
| Inner top | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

## Appendix 2

### Composition of media used

Abrams medium

| | |
|---|---|
| $NH_4NO_3$ | 3.0 g. |
| $K_2HPO_4$ | 2.0 g. |
| $KH_2PO_4$ | 2.5 g. |
| $MgSO_4 \cdot 7H_2O$ | 2.0 g. |
| Agar | 20.0g. |
| Distilled water | 1000.0 cc. |

Abrams-cellulose medium

Same as Abrams with 10.0 g. of crystalline cellulose added.

$Y_pSs$: Yeast-Starch agar (Emerson, 1941)

| | |
|---|---|
| Difco powdered yeast extract | 4.0 g. |
| $K_2HPO_4$ | 1.0 g. |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g. |
| Soluble starch | 15.0 g. |
| Agar | 20.0 g. |
| Water (1/4 tap, 3/4 distilled) | 1000.0 g. |

$Y_pCs$: Yeast-cellulose agar

| | |
|---|---|
| Difco powdered yeast extract | 4.0 g. |
| $K_2HPO_4$ | 2.0 g. |
| $MgSO_4 \cdot 7H_2O$ | 2.0 g. |
| Cellulose | 10. g. |
| Distilled water | 1000.0 cc. |

Malt agar

| | |
|---|---|
| Difco malt | 20.0 g. |
| Agar | 20.0 g. |
| Water (distilled) | 1000.0 cc. |

Malt-cellulose agar

| | |
|---|---|
| Difco malt | 20.0 g. |
| Cellulose | 10.0 g. |
| Agar | 20.0 g. |
| Distilled water | 1000.0 cc |

Combinations of $NaNO_3$, $KH_2PO_4$, Difco yeast extract

| Components | | Medium 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| $NH_4NO_3$ | 3.0 g. | - | + | + | - | - | - | + | + |
| $K_2HPO_4$ | 2.0 g. | + | + | + | + | + | + | + | + |
| $KH_2PO_4$ | 2.5 g. | - | + | - | + | - | + | - | + |
| $MgSO_4 \cdot 7H_2O$ | 2.0 g. | + | + | + | + | + | + | + | + |
| Difco yeast extract | 4.0 g. | - | - | + | + | + | - | + | + |
| Cellulose | 10.0 g. | + | + | + | + | + | + | + | + |
| Agar | 20.0 g. | + | + | + | + | + | + | + | + |
| Distilled water | 1000.0 cc. | + | + | + | + | + | + | + | + |

+ = Component is present

- = Component is absent

Medium for isolating fungi

| | |
|---|---|
| Difco malt extract | 20.0 g. |
| Difco agar | 20.0 g. |
| Malic acid | 5.0 g. |
| Distilled water | 1000.0 cc. |

Medium for growing fungi

| | |
|---|---|
| Difco malt extract | 20.0 g. |
| Difco agar | 20.0 g. |
| Distilled water | 1000.0 cc. |