## AN ABSTRACT OF THE THESIS OF

Sung-Mo Kang for the degree of Master of Science in Forest Products presented on December 1, 1998. Title: Fungal Colonization of Douglas-fir Sapwood.

Signature redacted for privacy.

Abstract approved: _____
Jeffrey J. Morrell

The fungi that cause mold and stain of freshly sawn lumber result in millions of dollars in losses to the forest products industry. Stain and mold are typically prevented by either kiln drying or by application of a topical fungicide. While these treatments are effective, kiln drying is costly and many mills object to use of chemicals. One alternative to conventional stain prevention is biological control. One problem with this approach on western wood species such as Douglas-fir is the lack of information on the target stain and mold fungi that colonize the sapwood of this species.

The fungi colonizing freshly sawn Douglas-fir sapwood were isolated and identified by culturing lumber obtained from 4 different locations in the Willamette Valley in western Oregon using regular, streptomycin amended or streptomycin/cycloheximide amended malt extract agar. The ability of each taxa to cause discoloration was evaluated in a laboratory discoloration test.

A total of 547 fungi were isolated from Douglas-fir sapwood, representing 24 genera and 45 species. *Graphium* species were the most frequently isolated fungi, representing 17.4 percent of all isolates. Most of these isolates were considered as anamorphs of *Ophiostoma piceae*. Other frequently isolated sapstaining fungi included *Cephaloascus fragrans*, *Aureobasidium pullulans*, *Rhinocladiella cellaris*, *Cladosporium cladosporioides* and *Scytalidium lignicola*. Molds, primary *Penicillium* and *Trichoderma* species, were frequently isolated, and may represent potential biological control organisms or competitors of biological control organisms because of their antagonistic characteristics. Many fungal species of unknown biological significance were also isolated from Douglas-fir sapwood, and their roles in the stain development merit further study.

Media and lumber source both affected the fungal flora isolated. Among stain fungi, *Graphium* species exclusively occurred on cycloheximide amended media. There was also close relationship between the degree of fungal colonization and the length of time after the lumber was cut.

The degree of discoloration exceeded 70 percent in all *Graphium* species. *Cephaloascus fragrans*, *Aureobasidium pullulans*, *Rhinocladiella cellaris*, *Cladosporium cladosporioides*, *Scytalidium lignicola* and *Thysanophora penicillioides* also produced extensive staining of Douglas-fir sapwood. Mold isolates also caused discoloration of Douglas-fir sapwood wafers, although their discoloration could be easily removed. All *Penicillium* species produced over 90 percent discoloration, while *Trichoderma* species produced 80 to 86 percent discoloration.

The results indicate that *Graphium* species are the primary stain fungi on Douglas-fir sapwood, however, other species were also capable of colonizing this material.

**Fungal Colonization of Douglas-fir Sapwood**

By

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Sung-Mo Kang

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## TABLE OF CONTENTS

**TABLE OF CONTENTS** (Continued)

**Page**

## TABLE OF CONTENTS (Continued)

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF TABLES (Continued)

**Fungal Colonization of Douglas-fir Sapwood**

## 1. INTRODUCTION

Because of its natural beauty, wood has been widely used for both construction and decorative purposes. Biological discoloration of unseasoned wood causes substantial degrade in wood quality by lowering the aesthetic value of wood. Discolored lumber is often more difficult to sell because a uniform color is preferred in the higher visual and structural grades. The growth of export markets for bright lumber has increased the importance of biological stains. For example, in 1975-76, depressed market conditions, transit and storage for long periods resulted in excessive stain, mold and decay on Hem-fir lumber, causing substantial economical loss (Nielson, 1978).

Although structural damage to timber by sapstain and mold fungi is minimal, the discoloration is objectionable to buyers and frequently results in the rejection of shipments. Financial losses due to sapstain in Spain can amount to as much as 40 percent of the products (Troya and Navarrete, 1989). The Canadian wood products industry spends millions of dollars to prevent stain annually (Seifert and Grylls, 1992). Fungal discolorations can be prevented by either drying below 20 percent moisture content or coating the wood surface with fungicides. Kiln drying is costly, while concerns about handling have led many mills to discontinue chemical treatments. One alternative for stain prevention is biological control, but this strategy has proven inconsistent in field applications.

One problem with biological control has been an inadequate knowledge of the target organism. Temperature, rainfall, and indigenous species vary widely and result in a diverse fungal flora on the wood surface. There are relatively few studies of the fungal flora of freshly sawn sapwood. Seifert and Grylls (1992) surveyed sapstain fungi occurring in Canadian lumber mills. Hutchison and Reid (1988a,b) reported on potential wood staining members of the *Ophiostomataceae*, *Pyrenomycetes*, *Coelomycetes* and *Hyphomycetes* in New Zealand.

# 2. LITERATURE REVIEW

## 2.1 Mold and stain

Numerous mold and sapstain fungi colonize wood, using the cell contents and certain components of cell walls. Molds colonize the sapwood and produce copious amounts of pigmented spores that cover the wood surface. These spores can be brushed from the wood. Stain fungi differ in that their hyphae produce dark pigments that deeply and permanently discolor the wood (Zabel and Morrell, 1992; Eaton and Hale, 1993). Mold control is not as critical; however, some customers consider the pigmented spores produced by these species objectionable (Seifert and Grylls, 1992).

## 2.2 Molds

Molds are asexual fungi. Many wood inhabiting molds are in the genera *Trichoderma*, *Penicillium* and *Aspergillus*. Species in these genera are also common inhabitants of soil and air, and they readily colonize wet lumber of nearly all of wood species (Seifert and Grylls, 1992). Molds discolor lumber primarily by their abundant production of spores, although some *Penicillium* species produce pigments that can diffuse into the wood (Zabel and Morrell, 1992; Seifert and Grylls, 1992; Eaton and Hale, 1993). Molds exhibit fast growth and may produce secondary metabolites that can include mycotoxins or antibiotics (Bruce and King, 1983; Bruce and Highley, 1991; Seifert and Grylls, 1992). Some molds have been considered as possible biocontrol agents because of their aggressive growth, but field tests have failed to produce complete protection (Henis, 1983; Seifert and Grylls, 1992; Kreber and Morrell, 1992; Liu and Morrell, 1996; Agrios, 1997).

## 2.3 Dematiaceous (stain) fungi

Sapstain fungi are Ascomycetes and Fungi Imperfecti that include truly pathogenic organisms that attack sapwood in living trees and saprobic fungi that only attack once the tree has been cut (Zabel and Morrell, 1992; Seifert, 1993). Sapstain caused by pathogenic fungi may be less important economically to the lumber industry because infected wood can be discarded before or during processing (Seifert, 1993). The sapwood of freshly sawn lumber is susceptible to colonization by a variety of saprobic fungi. Control of saprobic fungi is more difficult because colonization can occur at any time after the tree is felled if conditions are favorable for fungal growth.

Dematiaceous fungi are primary or initial wood colonizers that invade the ray parenchyma and wood cell lumens, where they assimilate the easily available carbon, carbohydrates, fats and proteins (Ballard et al., 1982). Dematiaceous fungi produce brownish pigments, but the wood appears blue due to light diffraction. Discoloration may also be green, purple, or brown, but it is most commonly grayish-blue, particularly in softwoods (Eaton and Hale, 1993).

Although many dematiaceous fungi cause stain in living or fallen trees or cut timber, the most important are species of *Ophiostomatales* including *Ophiostoma* and *Ceratocystis* (Hutchison and Reid 1988a; Seifert and Grylls, 1992).

### 2.3.1 *Ophiostomatales* (*Ophiostoma* and *Ceratocystis*)

The *Ophiostomatales* includes the genera *Ophiostoma* and *Ceratocystis* and their asexual stages which are classified in the genera *Graphium*, *Sporothrix*, *Leptographium* and *Hyalorhinocladiella*. (Hutchison and Reid, 1988a; Seifert and Grylls, 1992; Wingfield et al., 1994). These fungi produce dark brown to black sexual fruiting bodies (perithecia) with long necks and sticky masses of ascospores at the top (Hutchison and Reid, 1988a; Seifert and Grylls, 1992, Wingfield et al., 1995), and asexual stages with dry or wet conidia. Dry conidia may be transmitted through the air, while wet conidia may be dispersed in water films or on the bodies of beetles and other arthropods (Zabel and Morrell, 1992; Eaton and Hale, 1993).

Although they cause sapstain of lumber and forest products, many other species of *Ceratocystis* and *Ophiostoma* are of economic importance in agriculture. Some cause diseases of crop plants, such as sweet potato, coffee, rubber, mango, pineapple, sugarcane, and *Narcissus*. In the forest, *O. ulmi* and *C. fagacearum* cause Dutch-elm disease and oak wilt, respectively (Upadhyay, 1993).

2.3.1.1 Diseases caused by *Ophiostoma* and *Ceratocystis*

*Ophiostoma* species tend to colonize the woody xylem or phloem of temperate forest trees, whereas *Ceratocystis* species colonize a wide variety of herbaceous and woody plants in temperate and tropical temperature zones (Harrington, 1993; Kile, 1993).

*Ophiostoma* contains more species than *Ceratocystis*, but is a relatively homogeneous genus. Most *Ophiostoma* species are competitive saprophytes. For example, *Ophiostoma* species cause pole blight of western white pine, pine wilt disease, and oak tracheomycosis (Harrington, 1993). They are primarily transmitted by subcortical insects such as bark beetles (Hausner, 1992).

Most *Ceratocystis* species are necrotrophic plant pathogens that exhibit varying degrees of pathogenicity. Some species cause disease in roots, stems, shoots, leaves, tubers, fruits and seedpods. Important diseases caused by *Ceratocystis* species include myrtle wilt of beech, black root rot of tobacco and cotton, trunk scab of poplar, oak wilt and black rot of potato (Kile, 1993). The important vectors of *Ceratocystis* include flies and sapfeeding insects that disperse spores (Hausner et al., 1993).

2.3.1.2 Morphological characteristics of *Ophiostoma* and *Ceratocystis*

The genus *Ceratocystis* is a large heterogeneous group of Ascomycetes with evanescent asci and hyaline ascospores (Wingfield et al., 1987). They have *Chalara*-like anamorphs in which enteroblastic conidia develop through a ring wall building process (Harrington, 1981; Wingfield et al., 1987). Anamorphs of *Ophiostoma* include the

genera *Graphium, Sporothrix, Hyalorhinocladiella* and *Leptographium* in which conidia are produced by apical wall building (Wingfield et al., 1987; Wingfield et al., 1995).

The genus *Ceratocystis* was initially composed of *Ophiostoma* and *Ceratocystis*, and then, *Ophiostoma* species were segregated from *Ceratocystis* (Upadhyay, 1993). The subdivision of the genus *Ceratocystis sensu lato* has long been a source of taxonomic controversy (Hoog and Scheffer, 1984). The use of anamorph characters has been used for segregation of infrageneric groups; however, this approach can be confusing since teleomorphic characters are insufficiently concordant with those of the anamorph (Hoog and Scheffer, 1984).

Wingfield et al. (1995) described ascospore morphology in anamorphs of *Ceratocystis* and *Ophiostoma*. Species of *Ceratocystis* have short ascospores that are not falcate, with sheaths appearing half moon-shaped, hat shaped or cucullate. Ascospores in species of *Ophiostoma* can be surrounded by a hyaline sheath appearing ossiform, pillow, rectangular or dumbbell-shaped. Other species in *Ophiostoma*, however, can have ascospores that are sheathless, appearing lunate, cylindrical, ovate and orange section-shaped.

It is difficult to identify *Ophiostoma* and *Ceratocystis* using morphological features. Anamorph forms tend to be variable, and sometimes, more than two anamorph forms are present (Ellis, 1971; Upadhyay, 1993).

2.3.1.3 Other methods for distinguishing *Ophiostoma* and *Ceratocystis*

2.3.1.3.1 Cell wall structure and cycloheximide sensitivity

*Ophiostoma* and *Ceratocystis* can be distinguished from one another by differences in cell wall components. *Ceratocystis* species show more typical cell structures of Ascomycetes. While the cell walls of *Ophiostoma* contain cellulose, chitin and rhamnose, those of *Ceratocystis* are composed of only chitin and no cellulose or rhamnose (Wingfield et al., 1994; Hoog and Scheffer, 1984). Harrington (1981) studied cycloheximde sensitivity as a taxonomic character for separating *Ceratocystis* and

*Ophiostoma*. He proposed that the unique cell wall structure of *Ophiostoma* species made them more tolerant of cycloheximde. Since cycloheximide becomes bound to these cell walls, it cannot penetrate into the cell to exert its toxic effect. As a result, growth in cycloheximide amended media is one method for separating these genera (Harrington, 1981; Wingfield et al., 1987).

2.3.1.3.2 Ribosomal DNA or RNA sequence

The analysis of genetic materials is a new method for distinguishing *Ceratocystis* and *Ophiostoma*. The partial or complete rRNA and rDNA sequences of cultures can be used to assess relationships among a large variety of fungi for phylogenetic studies (Guadet et al., 1989). Hausner et al. (1993) subdivided *Ceratocystis* using analysis of partial rDNA sequencing. Their analysis did not support some subdivision groupings based on ascospore morphology. In addition, they asserted that some reassignment from *Ceratocystis* to *Ophiostoma* should be required for the species which lack *Chalara* anamorphs, are resistant to cycloheximide and have rhamnose in their cell walls.

2.3.2 Other dematiaceous fungi

Not all stain fungi belong to *Ophiostoma* and *Ceratocystis* species. A variety of other dematiaceous fungi have the potential to stain wood. Well-known wood staining fungi include members of the genera *Aureobasidium*, *Alternaria*, *Cladosporium*, *Stemphylium* and *Phialophora* (Zabel and Morrell, 1992; Eaton and Hale, 1993). These species generally produce dry masses of conidia that are dispersed by air currents. They cause stain either by producing masses of dark spores or pigmented hyphae within the wood cell lumens (Zabel and Morrell, 1992; Eaton and Hale, 1993; Seifert and Grylls, 1992).

## 2.4 Fungi of Douglas-fir

While colonization of wood by decay fungi has been extensively studied (Boyce, 1932; Smith et al., 1970; Morrell et al., 1987; Przybylowicz et al., 1987), the fungi colonizing sapwood have been studied to a limited extent. Boyce (1923) studied decay and discolorations in Douglas-fir wood used for airplane construction. *Ophiostoma* and *Ceratocystis* were the most important stain fungi, although *Cladosporium*, *Candida*, *Penicillium*, *Graphium* and *Fusarium* species also discolored wood. Kimmey and Furniss (1943) also reported that *Ophiostoma* and *Ceratocystis* were the primary cause of discoloration of Douglas-fir sapwood. *Graphium* and *Trichoderma* species were isolated from Douglas-fir pulp chips (Wright, 1954).

## 2.5 Life cycle of stain fungi and stain development

The spores of stain fungi are carried by air currents, water droplets or insects such as bark or ambrosia beetles or phoretic mites that live on bark beetles (Zabel and Morrell, 1992; Seifert and Grylls, 1992). Many stain fungi are found in bark beetle galleries, and are considered to have a symbiotic relationship with bark beetles (Whitney, 1982). After germination on the surface of wood, spores penetrate the wood through ruptured prosenchyma cells and exposed wood rays. Hyphae then grow into and consume nutrients stored within the parenchyma cells. Pigmentation is initiated five to six days after hyphal development. The rate of hyphal extension in the wood varies with wood species, temperature and moisture content. Hyphae can rapidly colonize wood cells under ideal conditions, and rates of growth as high as 5 mm/day longitudinally have been reported (Zabel and Morrell, 1992). These rapid rates of colonization give these fungi a distinct advantage in securing and utilizing sugars stored in the ray cells.

## 2.6. Importance of stain and mold fungi

### 2.6.1 Economic aspects

Sapstain is not permitted in the highest quality clear grades and top-grade industrial clears, but is allowed to a large degree under grading rules for lumber intended for structural purposes (WWPA, 1994). Estimates of the economic loss due to sapstain vary widely because of the fluctuating demand and supply that characterize the export lumber market (Roff et al., 1980). As demand becomes low, the economic loss increases as buyers become more selective (Roff et al., 1980). In the U.S., annual losses due to staining were estimated to be $10 million by Scheffer (1973). Hansen and Morrell (1997), however, claimed that this estimate was conservative, and actual losses should be much bigger. They found that the average loss per mill was $61,000 in western soft wood mills.

Long shipment times are often required when exporting lumber to other countries. The total period when lumber may remain in transit varies from four to twenty four months, depending on the market (Cserjesi, 1980). Conditions abroad ship can accelerate fungal development due to poor ventilation (Roff et al., 1980) and close-packing for easy handling (Cserjesi, 1980). The combination of wet lumber and favorable temperatures for fungal growth can result in rapid fungal growth and discoloration. Roff et al. (1980) reported losses can be $25,000 or more per shipment, and suggested that ventilation control should be required to reduce condensation during extended transport.

### 2.6.2 Effects of sapstains on wood properties

Discoloration decreases the aesthetic value of wood products, and the dark, melanistic pigments increase the consumption of bleaching chemicals during pulping (Zabel and Morrell, 1992). Although fungal stain in timber causes no appreciable loss

in most strength properties, there is some loss in toughness. Stained timber can be used for ordinary construction purposes except utility poles, piling, or glue-laminated timber or other single member critical applications (Scheffer, 1973).

The permeability of wood infested with sapstain fungi increases because of the removal of pit membranes (Eaton and Hale, 1993). Increased permeability has important effects on wood properties related to wood-water relationships such as finishing, preservative treatment, and dimensional stability (Eaton and Hale, 1993; Richardson, 1993; Zabel and Morrell, 1992). Sapstain does not change natural decay resistance significantly, but it can make wood more vulnerable to decay. Repeated wetting and drying resulting from increased permeability can lead to increased check development that provides points of entry for fungal spores (Eaton and Hale, 1993; Zabel and Morrell, 1992).

## 2.7. Stain controls

The best method for preventing sapstain is to reduce the moisture content below 20 percent. Ponding and continuous water spraying also inhibit the activity of stain and mold fungi by maintaining high moisture contents in the wood and limiting oxygen (Eaton and Hale, 1993). Surface treatments with fungicides are also employed for protecting wood that can not be immediately seasoned.

Although kiln drying is the most efficient method for preventing sapstain, it is also more costly. Kiln drying can cost $50-100/MBF, while the use of antistain chemicals costs only $4 – 8/MBF (Smith, 1992). Apart from the high cost, kiln drying cannot guarantee complete protection against biological discoloration because every piece of lumber cannot be adequately dried and remain so during transit and storage periods (Smith, 1992).

Chemicals are applied by either dipping or spraying on the surface of wood (Zabel and Morrell, 1992; Eaton and Hale, 1993). The fungicidal toxicity and the diffusibility of the active ingredients are important factors in efficacy. Generally

chemicals penetrate only slightly into wood. As a result, prompt treatment is critical for effective protection.

For decades, sodium pentachlorophenate or tetrachlorophenate were the most widely used fungicides. However, increased public concerns about environmental and toxic problems led to the use of alternatives such as oxine copper, triazoles, methyl thiobenzothiazole, methylene bisthiothiocyanate and several quarternary ammonium compounds (Zabel and Morrell, 1992; Eaton and Hale, 1993). However, none of these chemicals has been as effective and economical as penta (Zabel and Morrell, 1992; Richardson, 1993). Increased environmental concerns have also led to a search for non-chemical stain prevention methods. One possibility is to use biocontrol to prevent stain fungi from attacking wood materials.

Biological control is a well-known concept in agriculture and has been extensively applied to reduce agricultural dependence on chemical pesticides (Agrios, 1997). Historically, the basis for biological control can be explained with negative interactions such as amensalism, predation, and parasitism among microbial populations and between microbes and higher organisms (Atlas and Bartha, 1993). Biological control agents have been successfully used in a number of agricultural systems (Henis, 1983; Baker, 1987; Agrios, 1997).

Increased environmental regulation and a desire for decreased chemical reliance have encouraged the use of biocontrol in wood preservation. Some non-decay fungi may detoxify chemicals, and help other fungi to colonize the wood. These fungi may also inhibit re-colonization of other fungi by competition and amensalism (Giron et al. 1988). Biocontrol was first evaluated against decay fungi (Shields and Atwell, 1963; Ricard and Bollen, 1968; Ricard, 1976; Bruce and King, 1983; Dawson-Andoh and Morrell, 1991); however, the results were inconsistent (Seifert et al., 1988). Similar inconsistencies have been noted when biocontrol has been explored on fungal stains (Freitag et al., 1991). Although biocontrol agents such as *Trichoderma* species can prevent colonization by some brown rot fungi, they provided incomplete control against white rot fungi (Morrell and Sexton, 1990). Field tests also revealed that the effects of biological agent were too variable (Morris et al., 1984; Bruce and King, 1986 a and b); especially, when the wood was exposed to soil (Morrell and Sexton, 1990). From previous studies, Bruce and King

(1986a, b) concluded that no biocontrol agents provided complete and uniform protection against the broad spectrum of decay fungi that can degrade wood. These properties have limited the use of biological agents for controlling decay fungi in wood products

Biocontrol agents also show potential for preventing colonization of freshly cut logs during storage (Kallio, 1971; Hulme and Shields, 1972). A number of bacteria and fungi can prevent colonization by stain fungi (Benko, 1988; Seifert et al., 1988; Breuil et al., 1992; Kreber and Morrell, 1993). Organisms inhibiting stain fungi include *Gliocladium*, *Pseudomonas* and *Trichoderma* species. Many species in these genera produce antibiotics, although *in situ* anti-biotic production in wood remains to be demonstrated (Seifert et al., 1988; Breuil et al., 1992; Kreber and Morrell, 1993). *Trichoderma* species also produce chitinases that degrade chitin, a major structural component of the hyphae of target fungi (Henis, 1983; Ulhoa and Peberdy, 1991; Liu and Morrell, 1996; Agrios, 1997). *Trichoderma* species have also been applied to control soilborne pathogens on economically important crops (Henis, 1983; Agrios, 1997), and decay fungi (Ricard, 1976; Bruce and King, 1983; Bruce et al., 1984; Morris et al., 1984).

Biocontrol against stain fungi has showed limited potential because biocontrol agents appear to be differentially active against the many fungal species that colonize wood (Seifert et al., 1988; Breuil et al., 1992; Kreber and Morrell, 1993). One problem with this approach on western wood species such as Douglas-fir, is the lack of information on the target stain and mold fungi that colonize the sapwood of this species.

Improving our understanding of the organisms that colonize sapwood, how environmental conditions influence growth and how these organisms interact would provide valuable insights about the potential target organisms for a biological control agent and how environmental conditions can be manipulated to favor the control agent.

# 3. OBJECTIVE

1. To determine the identities of fungi present in Douglas-fir sapwood.
2. To assess the ability of isolates to cause discoloration in comparison with *Ophiostoma perfectum* and *Alternaria alternata*.

# 4. MATERIALS AND METHODS

## 4.1 Fugal flora in Douglas-fir sapwood

### 4.1.1 Collection of samples

The fungal flora of Douglas-fir (*Pseudotsuga menziesii*) sapwood was investigated by collecting boards from one lumber yard and three mills (Georgia Pacific Industries (GP), WTD Industries (WTD) and Frank Lumber Co). Sites were located in the Willamette Valley of western Oregon. These boards were freshly sawn at the mills or stored at the lumberyard. Samples were collected one to three times from the mills or lumberyard between July 1997 to June 1998 (Table 1). Freshly sawn boards were collected from the end-trim box of each mill, and stored boards were purchased from a local lumber yard. Every effort was made to select board sections with large percentages of sapwood. In June, 1998, samples were collected from the lumber yard, WTD and Frank Lumber at approximately the same time to compare fungal flora from different locations and lumber sources. It is obvious that the sampling used in this study could not account for the myriad of possible conditions under which Douglas-fir lumber is produced and stored. Instead, the objective was to provide a relative measure of fungal colonization under a variety of conditions.

Table1. The sampling dates and numbers of boards obtained from various mills or lumber Yards

| Location | Date of sampling |
|---|---|
| Georgia Pacific Industry | August 28, 1997 |
| Frank Lumber Co. | July 30, 1997 |
| | June 5, 1998 |
| Lumber yard | July17, 1997 |
| | October 9, 1997 |
| | June 1, 1998 |
| WTD | March 9, 1998 |
| | April 9, 1998 |
| | June 4, 1998 |

4.1.2 Culturing

A series of small chips were removed from each board as soon as possible after collection. The chips were briefly flamed then placed on the surface of three different media: 2 % malt extract agar (MEA), streptomycin (100 ppm) amended MEA and streptomycin (100 ppm)/cycloheximide (100 ppm) amended MEA (Appendix). Streptomycin was incorporated to inhibit bacterial growth, and cycloheximide was included to selectively isolate *Ophiostoma* species and their related asexual forms. Five chips were placed on each plate (Figure 1), which were incubated at room temperature, and observed daily for evidence of fungal growth. Any fungal growth from the chips was subcultured onto MEA for later identification.

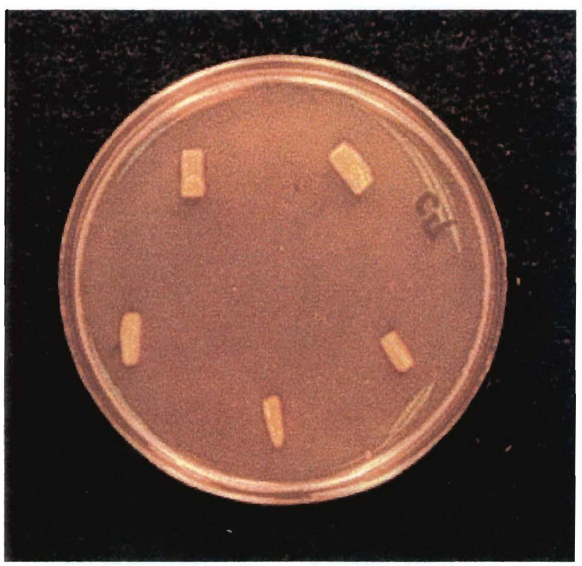


Figure 1. Pattern of placement of chips from Douglas-fir sapwood for isolating fungal inhabitants.

4.1.3 Identification of fungi

Fungal identification was based on cultural characteristics on suitable media in 90 mm plastic Petri plates. *Penicillium* species were incubated on Czapek's agar, Czapek's yeast agar and glucose amended malt extract agar (Appendix). The remaining isolates

were grown in MEA to determine their growth characteristics. All media were sterilized at 121 °C, 15 psi for 20 minutes. For *Penicillium* species, 14 days was chosen as the incubation time to measure growth rates, and observe textures and colors of the plates. Slide cultures were prepared according to Taschdjian's technique (1954) for better observation of the conidial structure. An alcohol-cleaned and sterile cover glass was dipped into molten 1% MEA, drained slightly, and placed on a 1.5 % water agar plate. Four cover glasses were placed on each plate. After solidification of the medium, the center of each cover glass was inoculated with a small amount of mycelia or conidia. One cover glass was removed 3, 7, 10 and 14 days after inoculation. The cover glass was covered with 70 % alcohol, and inverted over a drop of glycerol on a clean glass slide. Glycerol was used to prevent drying of mounts. The mounts were examined under Leitz and Microstar microscopes.

The isolates were identified using the appropriate literature (Gilman, 1957; Rifai, 1969; Ellis, 1971; Barnett and Hunter, 1987; Ramirez, 1982; Hutchison and Reid, 1988a, b; Wang and Zabel, 1990). After identification, the fungi were divided into four categories: dematiaceous fungi, *Graphium* species, molds and fungi of unknown significance.

### 4.1.4 Frequency of occurrence

Each isolate was identified with regard to mill/yard and board number. According to taxa, relative occurrence of the isolates was calculated as follows:

$$\% \text{ Frequency of Isolates} = \frac{\text{Number of Isolates}}{\text{Total Number of Isolates}} \times 100$$

## 4.2 Discoloration tests of isolates from Douglas-fir sapwood

The ability of isolates to cause discoloration on Douglas-fir sapwood was assessed in a laboratory test using *Ophiostoma perfectum and Alternaria alternata* for comparison.

### 4.2.1 Preparation of wood wafers

Douglas-fir sapwood wafers (5cm long and 5mm by 10mm) were cut from freshly sawn boards and sterilized by exposure to 2.5 Mrads of ionizing radiation from a Cobalt 60 source. The wafers were kept frozen before and after sterilization to minimize the risk of fungal attack.

### 4.2.2 Preparation of fungal inoculum

Isolates were grown on malt extract agar until the agar surface was covered by the fungus. The agar surface was flooded with sterile distilled water and rubbed to dislodge spores and hyphal fragments. The inoculum of *O. perfectum* and *A. alternata* were prepared by same methods. The number of isolates used from each species depended on frequency and their relative importance as sapstain fungi.

The resulting suspension of spores and hyphae was decanted into a small bottle and 15 sterile wafers were dipped for one minute into each suspension. Five wafers were placed on glass rods over moistened filter paper in a glass Petri dish. Bottles, glass rods, filter paper and glass Petri dishes were sterilized at 121 °C, 15 psi for 20 minutes. Each isolate was tested on 15 wafers in three dishes. The plates were sealed with para-film to retard moisture loss, and incubated for 4 weeks at room temperature.

4.2.3 Determination of discoloration

The degree of discoloration was visually assessed on a scale from 0 (no discoloration) to 100 % (complete discoloration) in 5 % increments, to estimate surface area covered by stain or pigmented spore/hyphae. The results were compared with those of similar trials using *Ophiostoma perfectum* and *Alternaria alternata*.

4.2.4 Analysis of results

The data were subjected to an ANOVA, and means were compared using Bonferroni's t-test at the 0.05 level of significance. Bonferroni's t-test was used because of the unequal sample sizes. The unequal sample sizes occurred due to loss of samples during the test due to contamination and the different numbers of isolates used for the discoloration test.

# 5. RESULTS AND DISCUSSION

## 5.1 Fungal flora of Douglas-fir sapwood

### 5.1.1 Fungi from Douglas-fir sapwood

A total of 547 fungal isolates were obtained from nine sample sets of Douglas-fir sapwood (Table 2). Over 93 percent of isolates were identified, representing 29 genera and 45 species. The fungal flora varied with sampling site, sampling time and media. *Graphium* species were the most frequently isolated fungi, occurring in 17.4 percent, followed by Zygomycetes, *Penicillium* and *Trichoderma* species representing 17.2, 16.6 and 16.1 percent of all isolations, respectively. Stain fungi were isolated more frequently from stored boards than freshly cut ones, suggesting that stain fungi were primary saprobic on Douglas-fir sapwood.

Table 2. Fungi isolated from 9 Douglas-fir sapwood sample sets from 4 sites in the Willamette Valley of western Oregon.

| Fungus | Frequency | |
|---|---|---|
| | % Isolates | % Sets |
| *Cephaloascus fragrans* | 5.9 | 22.2 |
| *Aureobasidium pullulans* | 1.8 | 44.4 |
| *Rhinocladiella cellaris* | 1.8 | 33.3 |
| *Cladosporium cladosporioides* | 1.7 | 44.4 |
| *Scytalidium lignicola* | 1.3 | 33.3 |
| *Epicoccum purpurascens* | 0.7 | 33.3 |
| *Mammaria echinobotryoides* | 0.6 | 11.1 |
| *Allescheriella crocea* | 0.4 | 11.1 |
| *Alternaria tenuissima* | 0.4 | 11.1 |
| *Alysidium resinae* | 0.4 | 11.1 |
| *Phoma fimeti* | 0.4 | 22.2 |
| *Aureobasidium* sp. | 0.2 | 11.1 |
| *Leptodontium elatius* | 0.2 | 11.1 |
| *Rhinocladiella atrovirens* | 0.2 | 11.1 |
| *Thysanophora penicillioides* | 0.2 | 11.1 |

Table 2. Continued.

| | | |
|---|---|---|
| *Trichocladium canadense* | 0.2 | 11.1 |
| Unidentified Dematiaceous fungi | 0.4 | 22.2 |
| *Graphium putredinis* str.1 | 5.7 | 44.4 |
| *Graphium putredinis* str.3 | 3.1 | 44.4 |
| *Graphium putredinis* str.2 | 2.7 | 33.3 |
| *Graphium penicillioides* str.1 | 1.3 | 44.4 |
| *Graphium penicillioides* str.2 | 0.9 | 33.3 |
| *Sporothrix* sp. A str.1 | 0.9 | 33.3 |
| *Sporothrix* sp. A str.2 | 0.4 | 22.2 |
| Unidentified *Graphium* sp. | 2.4 | 44.4 |
| *Trichoderma viride* | 8.2 | 55.6 |
| *Trichoderma harzianum* | 4.6 | 44.4 |
| *Penicillium frequentans* str.1 | 4.4 | 11.1 |
| *Trichoderma koningii* | 3.3 | 55.6 |
| *Penicillium purpurogenum* | 2.4 | 66.7 |
| *Penicillium frequentans* str.2 | 1.3 | 11.1 |
| *Penicillium frequentans* series | 1.1 | 11.1 |
| *Penicillium frequentans* str.3 | 0.9 | 11.1 |
| *Penicillium frequentans* str.4 | 0.9 | 22.2 |
| *Aspergillus fumigatus* | 0.6 | 33.3 |
| *Penicillium spinulosum* | 0.6 | 11.1 |
| *Penicillium diversum* | 0.4 | 11.1 |
| *Penicillium funiculosum* | 0.4 | 22.2 |
| *Penicillium odoratum* | 0.4 | 11.1 |
| *Penicillium expansum* | 0.2 | 11.1 |
| *Penicillium janthinellum* | 0.2 | 11.1 |
| *Penicillium para-herquei* | 0.2 | 11.1 |
| *Penicillium thomii* | 0.2 | 11.1 |
| *Penicillium vasconiae* | 0.2 | 11.1 |
| *Penicillium verrucosum* | 0.2 | 11.1 |
| Unidentified *Penicillium* sp. | 2.0 | 33.3 |
| Zygomycete | 17.2 | 88.9 |
| *Mucor* sp. | 5.3 | 77.8 |
| Mycelial yeast | 1.4 | 55.6 |
| *Hyalorhinocladiella* sp. | 0.6 | 11.1 |
| *Phialemonium dimorphosporum* | 0.4 | 22.2 |
| *Sporobolomyces salmonicolor* | 0.4 | 11.1 |
| *Sporothrix* sp. B | 0.4 | 11.1 |
| *Debaryozyme hansenii* | 0.3 | 11.1 |
| *Paecilomyces* | 0.3 | 11.1 |
| *Polyscytalum fecundissimum* | 0.3 | 11.1 |
| Unidentified fungi of unknown significance | 7.0 | 77.8 |

[a] Values represent percent frequency from 547 samples.

[b] Samples were obtained from GP, Frank, WTD and Lumberyard.

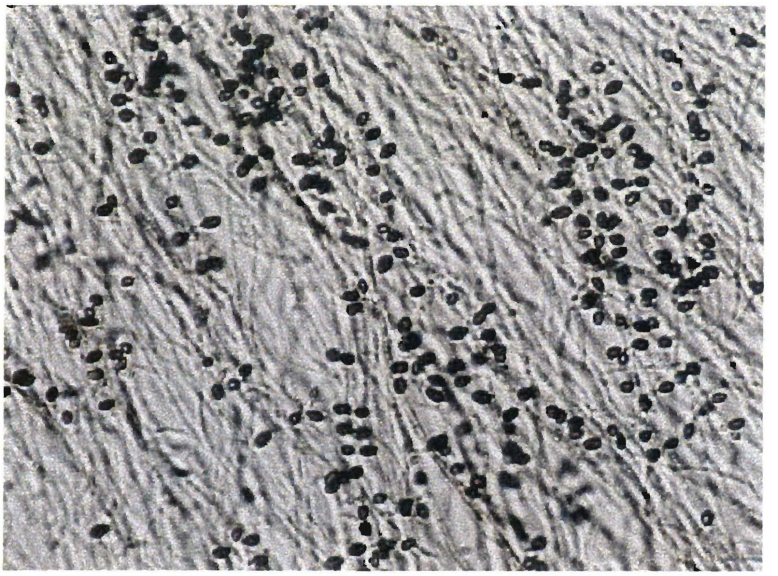
5.1.1.1 Dematiaceous fungi

Dematiaceous fungi represented 16.5 percent of total isolates, comprising 16 species and 2 isolates that could not be identified (Table 2, Figure 2). *Cephaloascus fragrans* was the most frequently isolated dematiaceous fungus (5.9 % of isolates) (Table 2); however, this fungus was only isolated from lumberyard samples. The boards from the lumberyard were produced by Seneca Saw Mill (Eugene, OR) and were believed to be treated with orthophenolphenate. *Cephaloascus fragrans* is resistant to phenol compounds (Roff, 1973), helping to explain its dominance in the sample. *Aureobasidium pullulans*, *Rhinocladiella cellaris*, *Cladosporium cladosporioides*, and *Scytalidium lignicola* were also frequently isolated, and were isolated from at least three mill/lumberyard samples (Table 2). *Cladosporium* species have been reported to discolor Douglas-fir (Boyce, 1923) and *Epicoccum* species have been isolated from Douglas-fir in Canada (Seifert and Grylls 1992). *Mammaria*, *Leptodontium* and *Phoma* species were infrequently isolated in our study (Table 2). Members of these genera have been classified as potential wood-staining fungi in New Zealand along with *Aureobasidium* and *Cladosporium* species (Hutchison and Reid, 1988b).

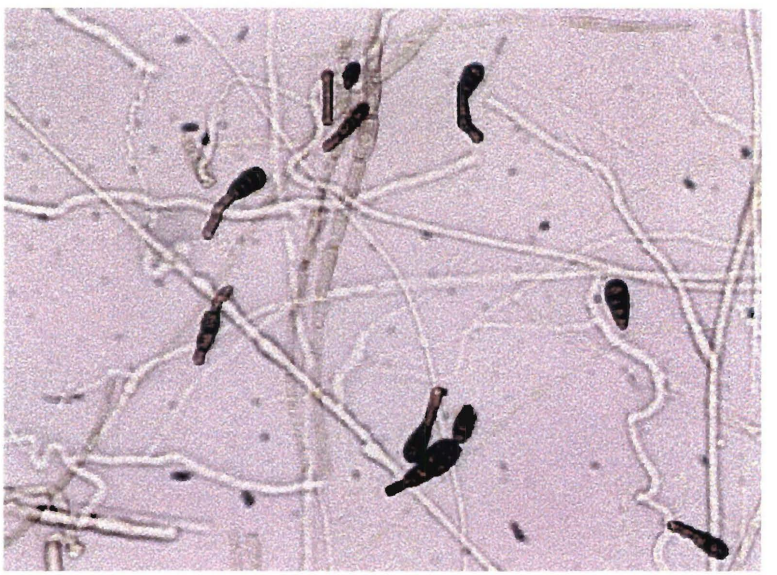
5.1.1.2 *Graphium* species

The anamorph genus *Graphium* was described by Corda (1837) and is the asexual form of *Ophiostoma* species (Wingfield et al., 1987; Wingfield et al., 1995). All *Graphium* species were sub-cultured on SCMEA to minimize other fungal contamination, and grew successfully on this media.

Three categories described by Ellis (1972) were used to group *Graphium* species as *G. putredinis*, *G. penicillioides* or *G. calicioides*. Only *G. putredinis* and *G. penicillioides* were isolated from the samples in our studies (Figure 3). Although Ellis (1972)'s classification is useful, it was insufficient to identify and describe many *Graphium* species. Therefore, we grouped *G. putredinis* isolates into three strains based upon amount of pigmentation on media. Isolates of *G. penicillioides* were also sub-grouped into two strains based upon pigmentation.



(A)

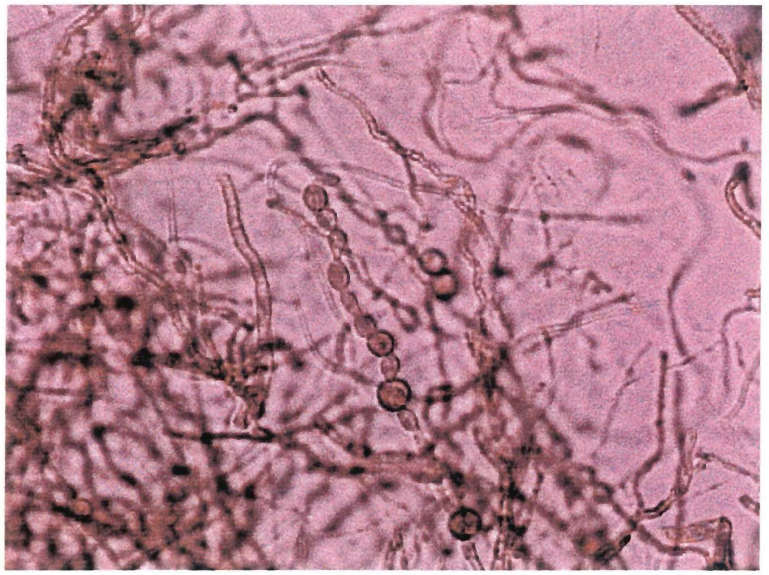


(B)

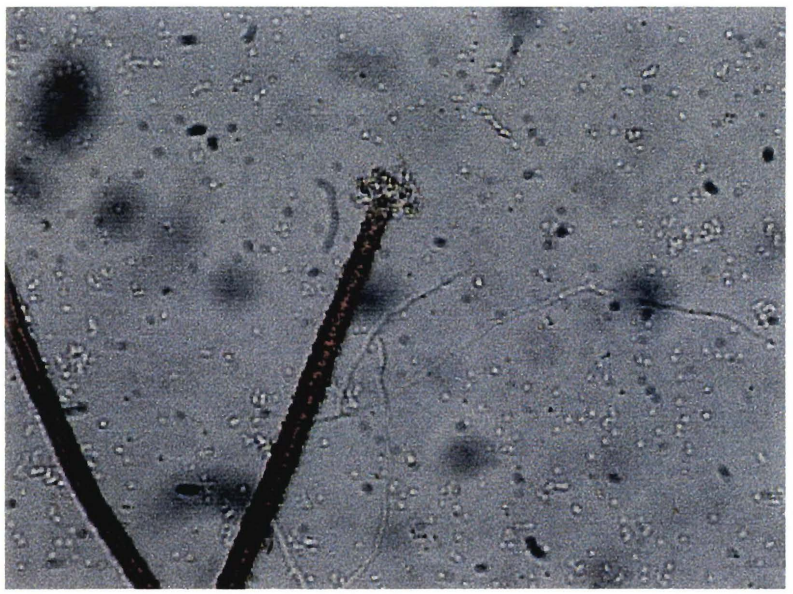
Figure 2. Microscopic characteristics of dematiaceous fungi isolated from Douglas-fir sapwood:

(A) *Allescheriella crocea* (400X)

(B) *Alternaria tenuisiima* (400X)



(c)

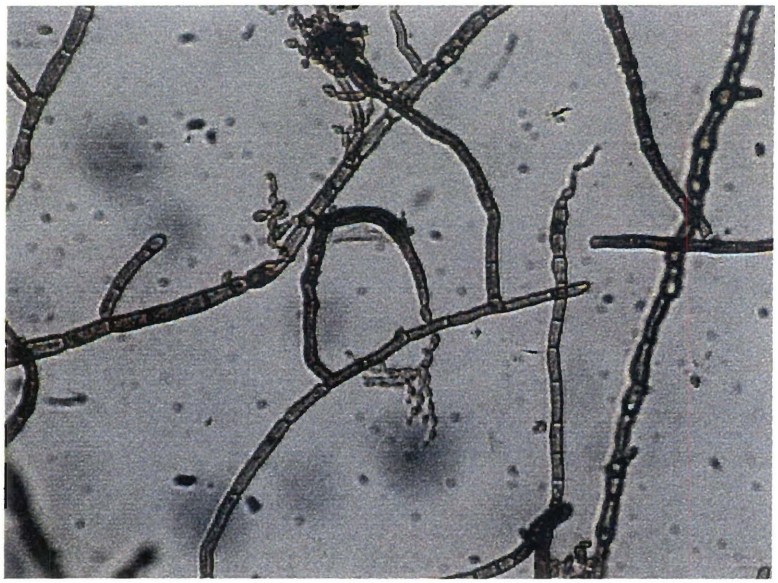


(D)

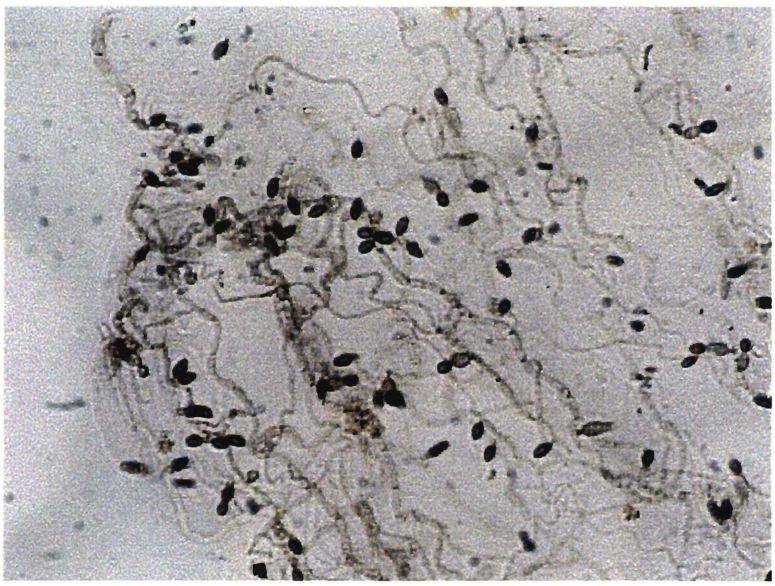
Figure 2. Continued.

(C) *Alysidium resinae* (400X)

(D) *Cephaloascus fragrans* (400X)



(E)



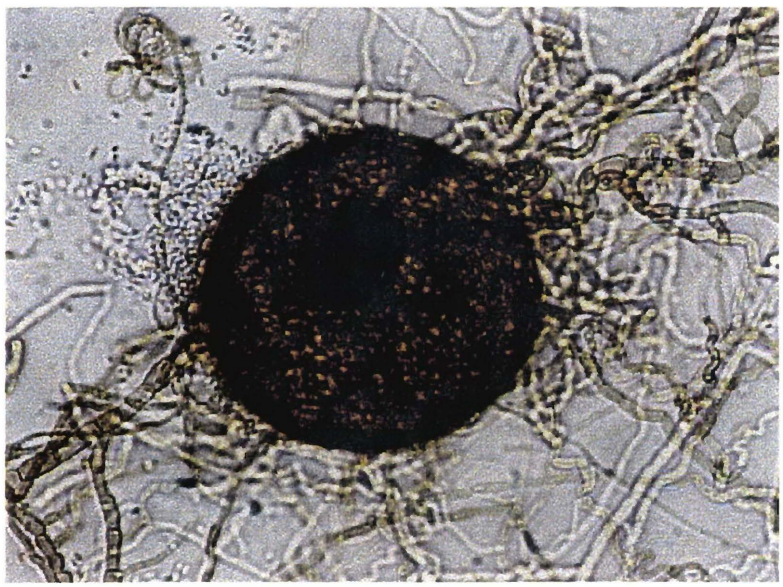
(F)

Figure 2. Continued.

(E) *Cladosporium cladosporioides* (400X)

(F) *Mammaria echinobotryoides* (400X)

(G)

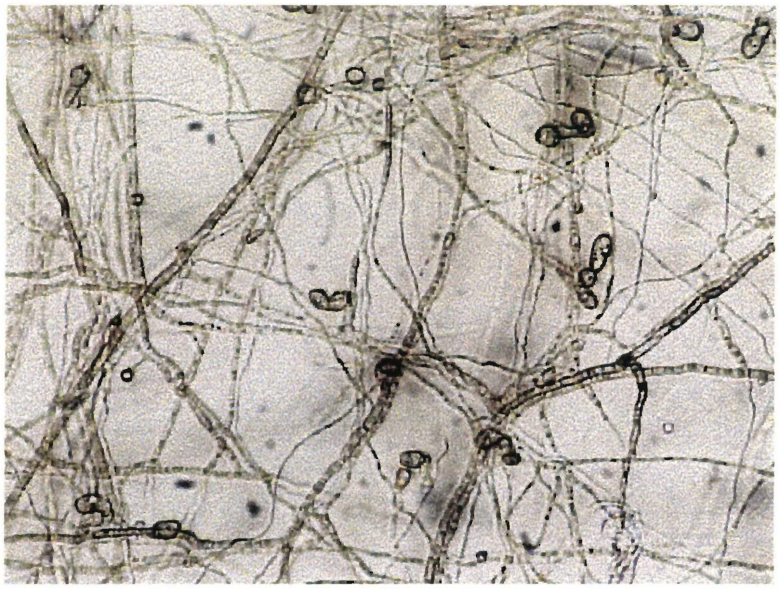


(H)

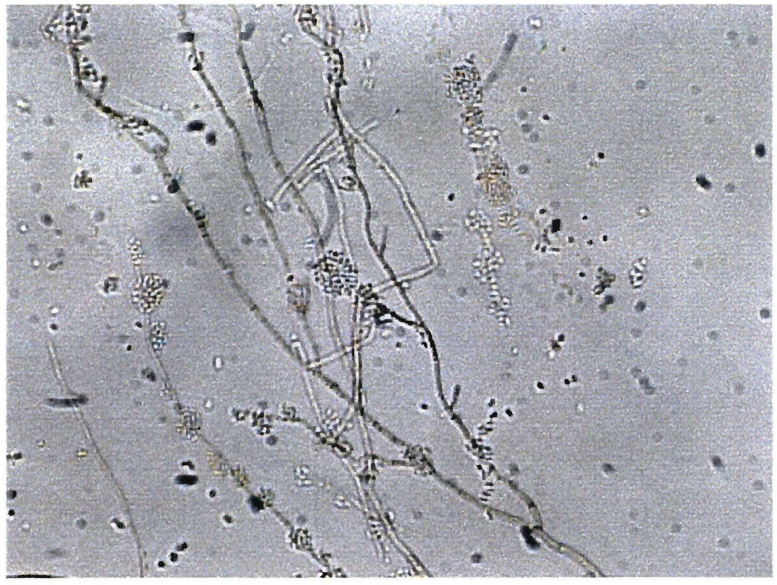
Figure 2. Continued.

(G) *Epicoccum purpurascens* (400X)

(H) *Phoma fimeti* (400X)



(I)

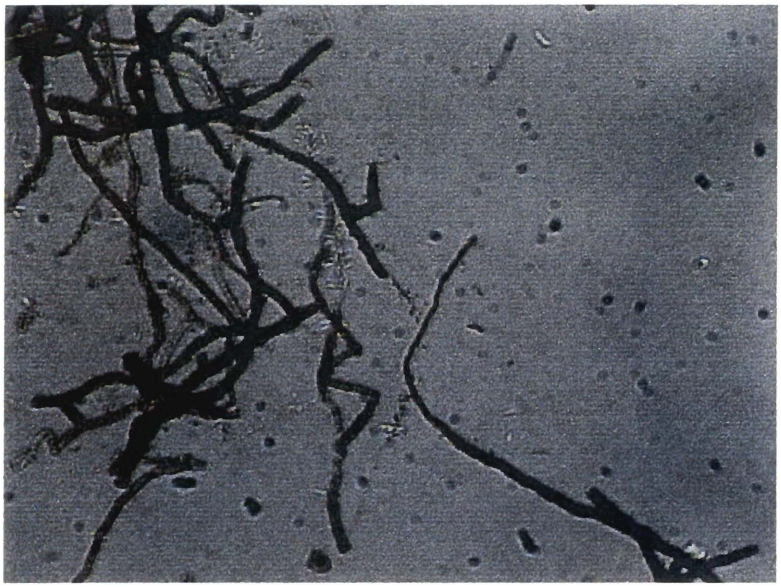


(J)

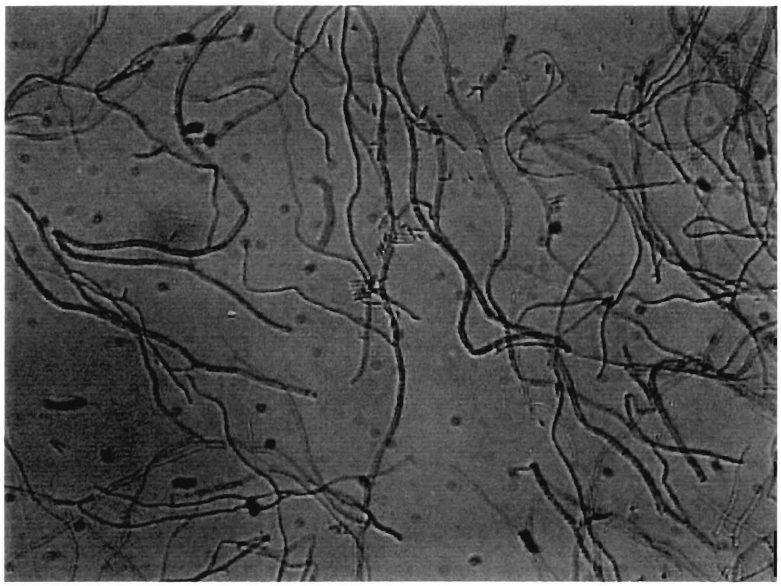
Figure 2. Continued.

(I) *Trichocladium canadense* (400X)

(J) *Leptodontium elatius* (400X)



(K)

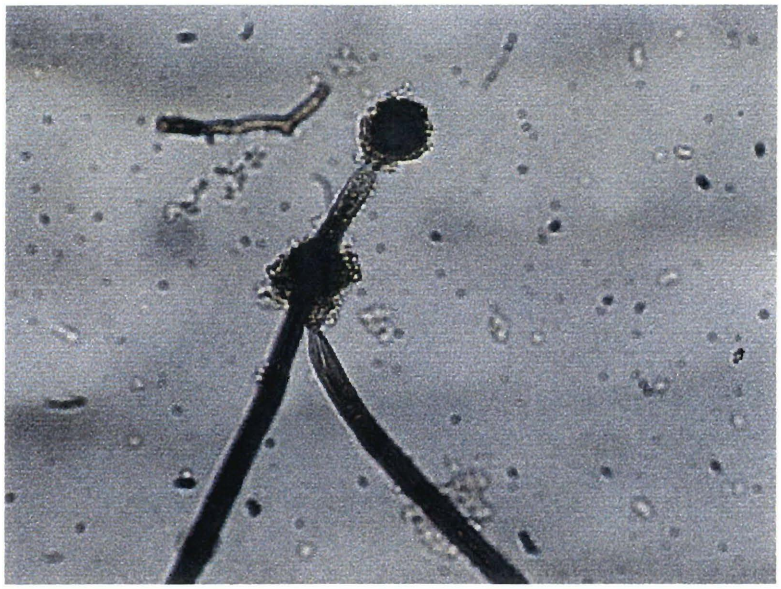


(L)

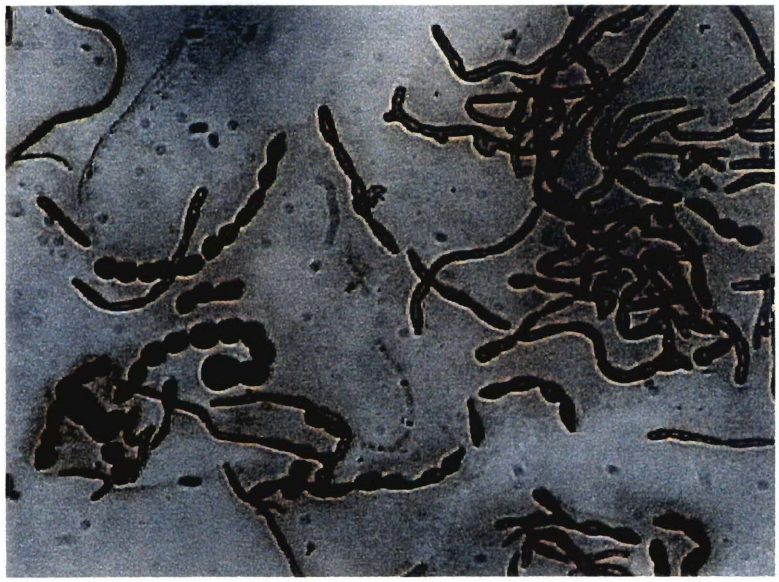
Figure 2. Continued.

(K) *Rhinocladiella cellaris* (400X)

(L) *Rhinocladiella atrovirens* (400X)



(M)

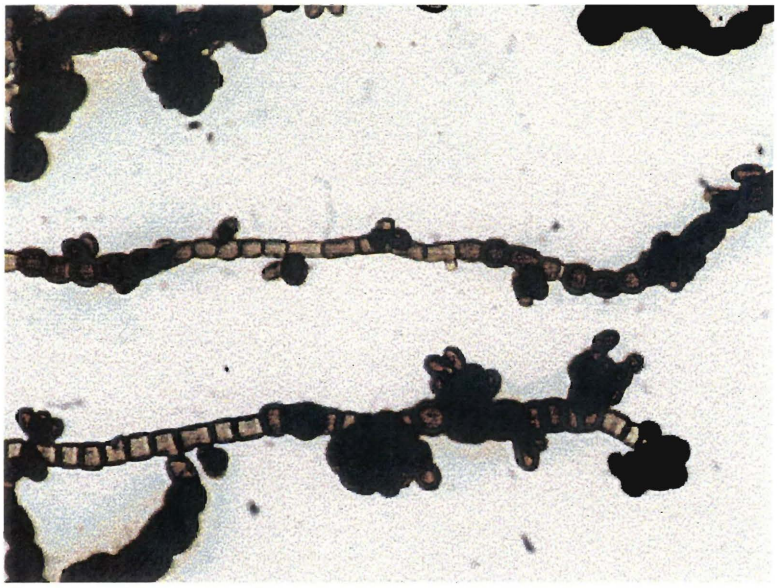


(N)

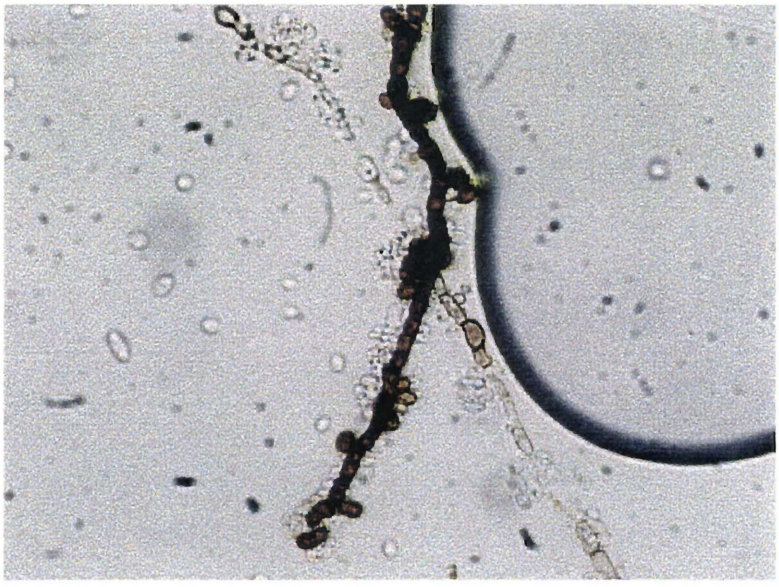
Figure 2. Continued.

(M) *Thysanophora penicillioides* (400X)

(N) *Scytalidium lignicola* (400X)



(O)



(P)

Figure 2. Continued.

(O) *Aureobasidium pullulans* (400X)

(P) *Aureobasidium* sp. (400X)

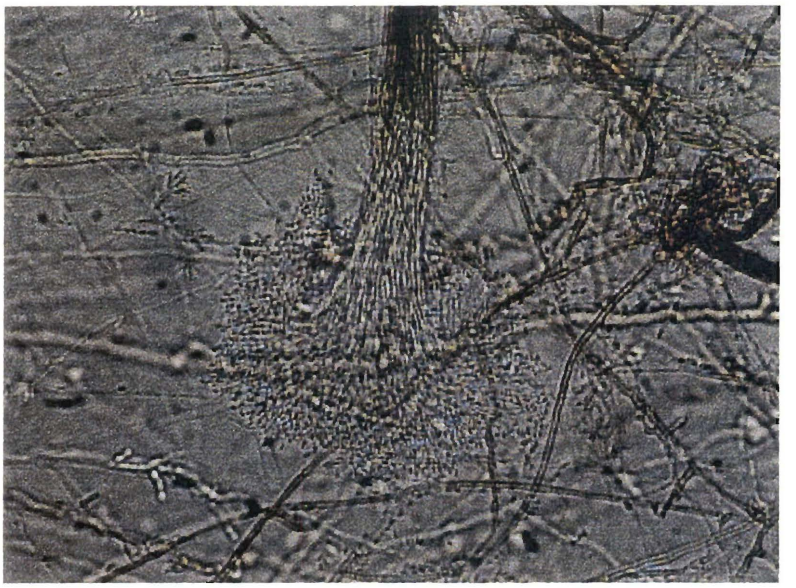
*Graphium putredinis* was isolated more frequently than *Graphium penicillioides*, representing 11.5 and 2.2 percent of isolates, respectively (Table 2). The frequency of strain #1 was higher than that of strain #2 in *G. penicillioides* although the significance of the difference is difficult to determine (Table 2).

Identification of *Graphium* species is difficult because the genus has never been comprehensively revised and critical comparisons of closely related species are lacking (Seifert and Okada, 1993). Some trials have been made to analyze this genus (Hughes, 1953; Uphadyay, 1981), but the presence of the teleomorph is necessary before the anamorph can be identified (Seifert and Okada, 1993).

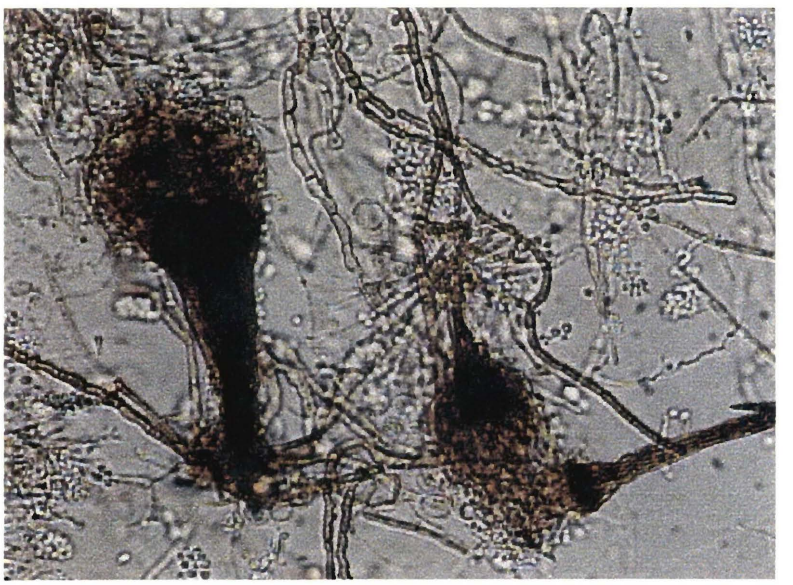
Many *Ophiostoma* species produce their ascomata with relative ease in culture. *O. piceae* mostly produces *Graphium* and *Sporothrix* anamorphs in abundance, but no perithecia (Hoog, 1993). In this study, the methods described by Hutchison and Reid (1988a) were used to induce perithecial production in fresh isolates. Although perithecia were not observed, we suggest that most *Graphium* species in our study were *O. piceae*. Most *Graphium* species isolated in this study produced both *Graphium* and *Sporothrix* stages. According to Hutchison and Reid (1988a), only *O. piceae* produced both *Graphium* and *Sporothrix* anamorphs. Seifert and Grylls (1992) reported that *O. piceae* was the most frequently isolated fungus from Douglas-fir in Canada.

*Sporothrix* species were incorporated into *Graphium* species because this genus is also considered to be an anamorph of *Ophiostoma* (Hutchison and Reid, 1988a; Seifert and Grylls, 1992; Wingfield et al., 1993). In addition, *Sporothrix* were observed along with *Graphium* species, especially *Graphium putredinis*. Hoog (1993) also reported that somewhat degenerate *Sporothrix* arose from some *Graphium* synnemata. *Leptographium* forms were also observed in some *Graphium* isolates. There are species of *Ophiostoma* that have anamorphs of both *Leptographium* and *Graphium* state (Wingfield, 1993). In this study, *Graphium* isolates with *Sporothrix* or *Leptographium* stages were grouped into *Graphium* species.

Isolates with only *Sporothrix* forms were listed as *Sporothrix* species. *Sporothrix* species represented 1.3 percent of all isolates (Table 2). These isolates were sub-grouped into two strains because further identification of this fungus using light microscopy was difficult. The main criteria for *Sporothrix* classification are the co-



(A)

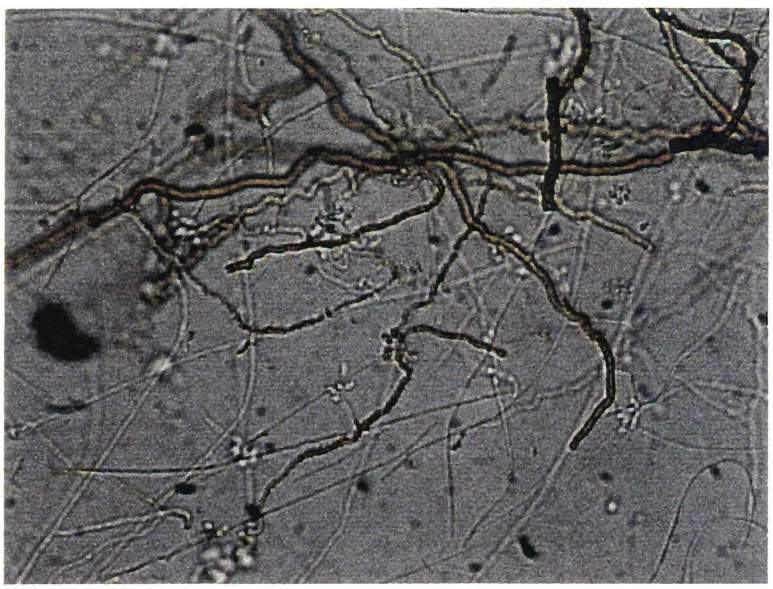


(B)

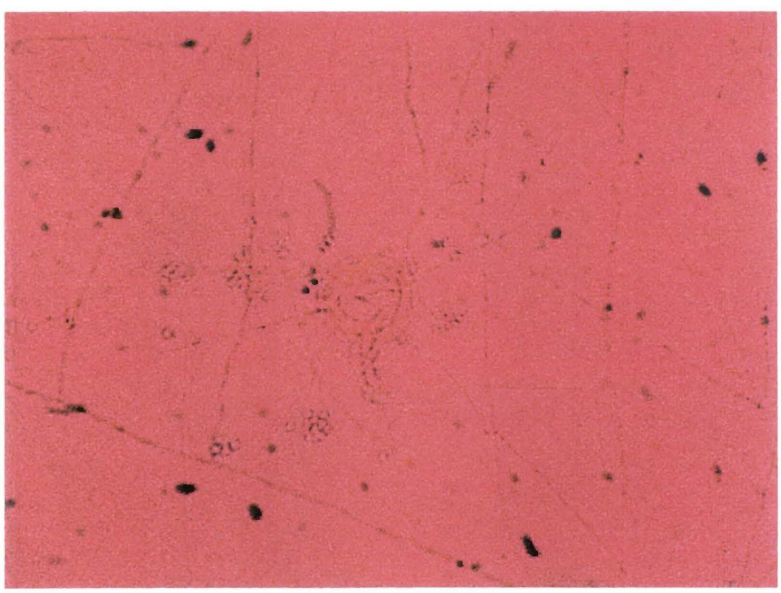
Figure 3. Microscopic characteristics of *Graphium* species isolated from Douglas-fir sapwood:

(A) *G. putredinis* (400X)

(B) *G. penicillioides* (400X)



(C)



(D)

Figure 3. Continued.

(C) *Sporothrix* sp. A str.#1 (400X)

(D) *Sporothrix* sp. A str.#2 (400X)

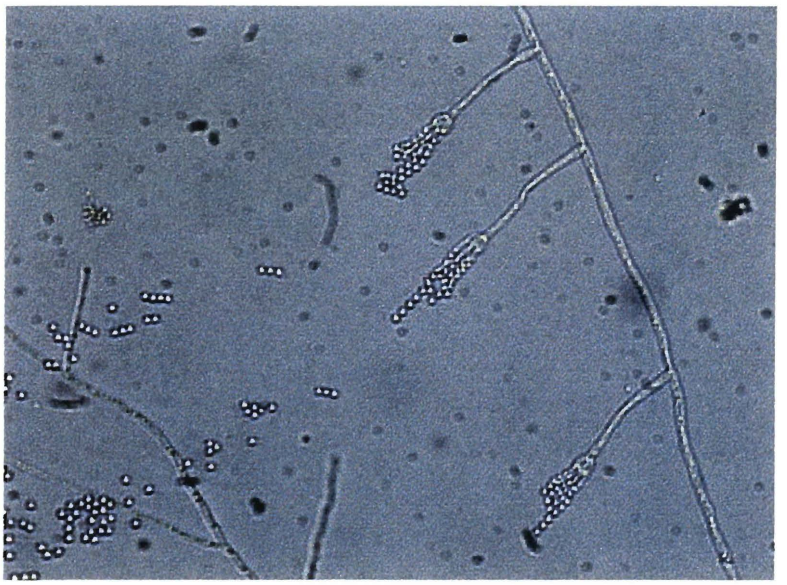
enzyme Q system and cell wall composition/structure. The genus *Sporothrix* can be classified into several species using these characters (Hoog, 1993). Strain #1 showed the typical structure of *Sporothrix*, while strain #2 produced both *Sporothrix* structures and hyphal loops that are commonly produced by *Phialophora* species (Figure 3).

5.1.1.3 Molds

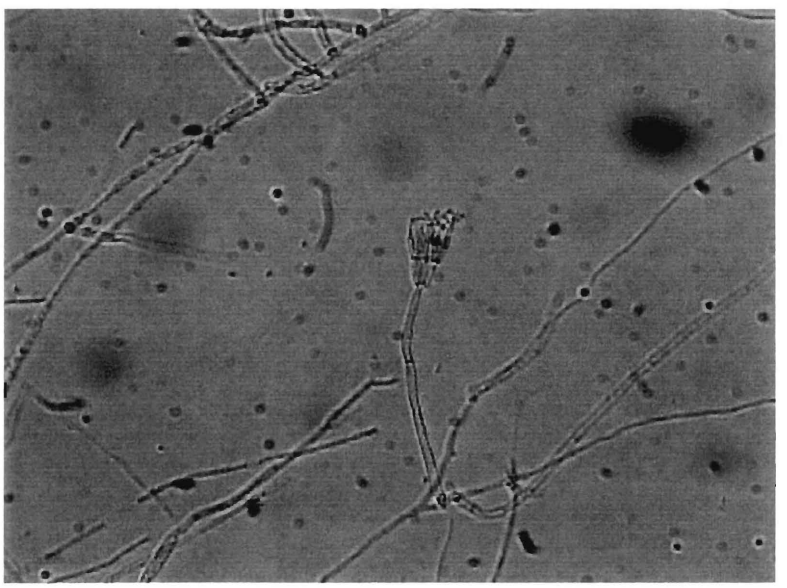
Isolates producing pigmented spores represented 32.4 percent of all isolates. Most isolates were *Penicillium* and *Trichoderma* species (Table 2 and Figure 4). Molds were more abundant in wood stored for longer periods. Molds are saprobic fungi that colonize wood after cutting (Agrios, 1997). Sawing increases cell damage, often spilling ruptured cell contents on the board surface where they can be readily utilized by mold fungi. *Penicillium* species were present on all sample sets, and represented 16.6 percent of total isolates (Table 2). *P. frequentans* was the most frequently isolated *Penicillium* species, representing 8.6 percent of the isolates (Table 2). *P. frequentans* were sub-grouped into four strains based upon growth characteristics on Czapek's agar, Czapek's yeast agar and glucose amended MEA,. Each strain showed slightly different colors and textures on the media, but their growth characteristics matched well with previously published descriptions of *Penicillium frequentans* (Ramirez, 1982). *Penicillium frequentans* strains are known to exhibit different growth characteristics (Ramirez, 1982).

Although *P. frequentans* occurred frequently, it was isolated exclusively from lumberyard samples suggesting that storage time may have enhanced colonization (Table 2). *P. purpurogenum* occurred in six of nine sampling sets, suggesting that this fungus was more broadly capable of attacking lumber shortly after sawing. (Table 2).

*Trichoderma* species were isolated from five of nine sample sets, comprising 16.1 percent of total isolates (Table 2). Three species of *Trichoderma* were isolated: *T. viride*, *T. harzianum* and *T. koningii*. Seifert and Grylls (1993) also reported that these three *Trichoderma* species were isolated from Douglas-fir lumber in Canada with *T. viride* being the most commonly isolated species. *Trichoderma* species are rarely reported on



(A)

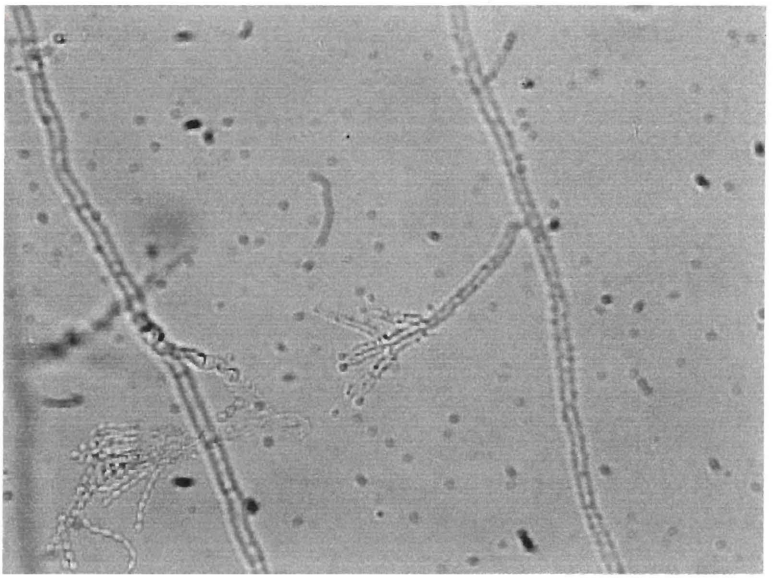


(B)

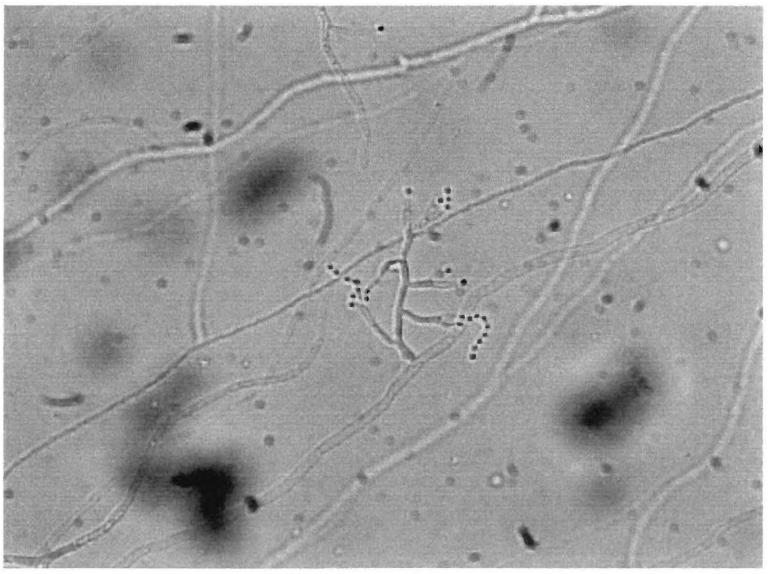
Figure 4. Microscopic characteristics of molds isolated from Douglas-fir sapwood:

(A) Simple penicillus as seen in *P. frequentans* (400X)

(B) Biverticillate-symmetric penicillus as seen in *P. purpurogenum* (400X)



(C)

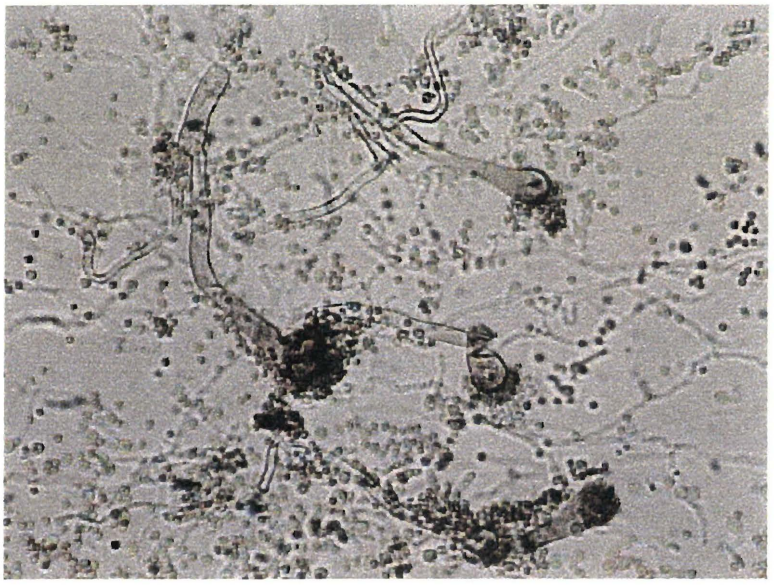


(D)

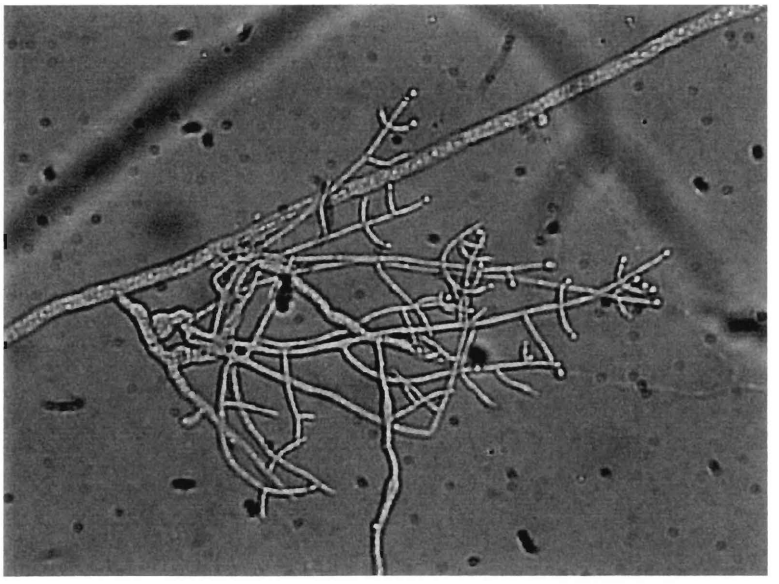
Figure 4. Continued.

(C) Biverticillate-asymmetric penicillus as seen in *P. expansum* (400X)

(D) Divariate penicillus as seen in *P. janthinellum* (400X)



(E)



(F)

Figure 4. Continued.

(E) *Aspergillus fumigatus* (400X)

(F) *Trichoderma viride* (400X)

living plants and are more common in soils (Samuels, 1996). *T. viride* is commonly present in Douglas-fir soil (Nelson,1982).

*Aspergillus fumigatus* was also isolated from a number of samples, and its growth characteristics were similar to those of *Penicillium* species (Figure 4).

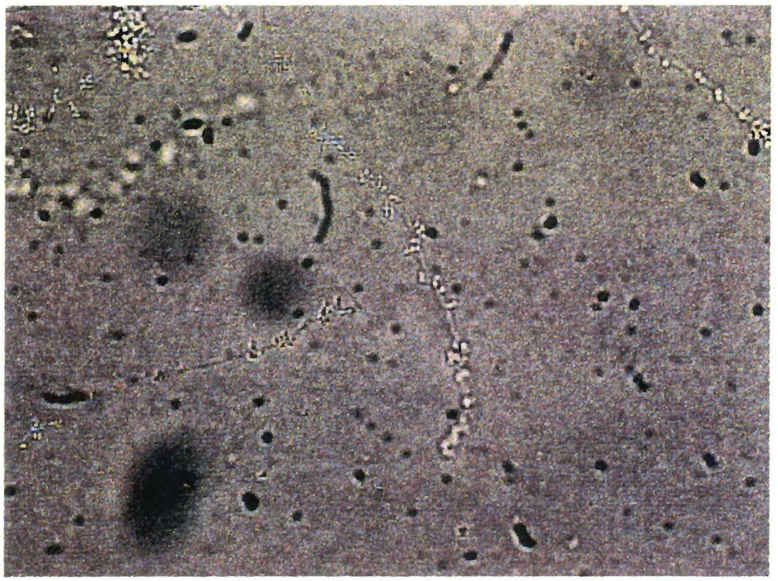
Molds are common on all kinds of lumber and soils (Seifert and Grylls, 1992). The role of these fungi in wood ecology merits further study.

5.1.1.4 Fungi of unknown significance

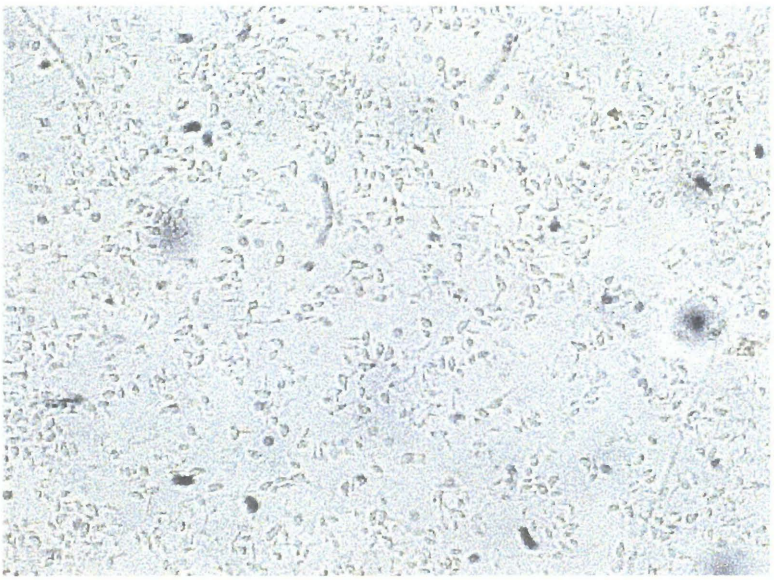
Fungi that lacked pigmented hyphae and spores were grouped into fungi of unknown significance. These fungi represented 33.6 percent of total isolates, comprising ten species (Table 2 and Figure 5). Zygomycetes represented 17.2 percent of total isolates, and they were isolated from eight of nine sample sets. *Mucor* species were also relatively abundant, and were isolated from all but two sample sets (Table 2).

*Hyalorhinocladiella* and *Sporothrix* sp. B were categorized in this group although some species in this genus are considered to be anamorphs of *Ophiostoma*. Since they did not produce any pigmentation, it was not possible to confirm whether these fungi were asexual forms of *Ophiostoma*.

Many fungi can colonize wood without producing any apparent decay or sapstain. These fungi may have synergistic or antagonistic roles in the growth of other fungi (Giron et al, 1988). Their biological activities and interactions with other wood-inhabiting organisms are poorly understood and merit further study (Seifert and Grylls, 1992).



(A)

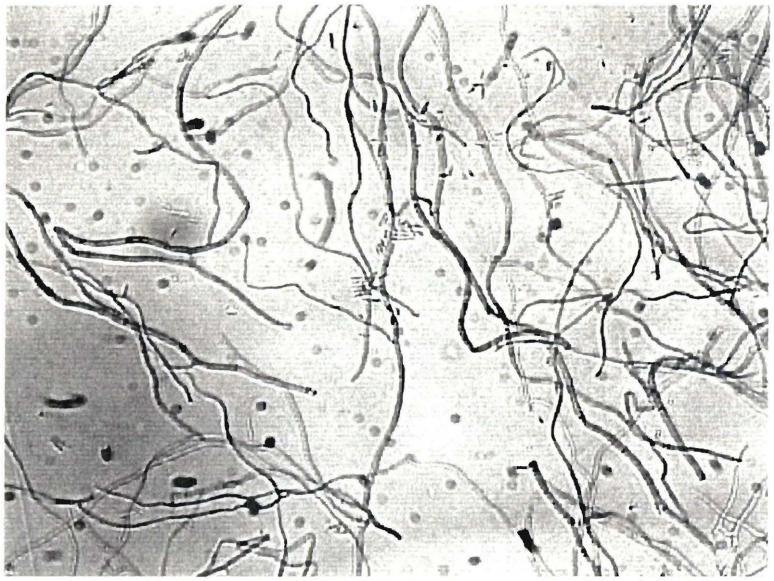


(B)

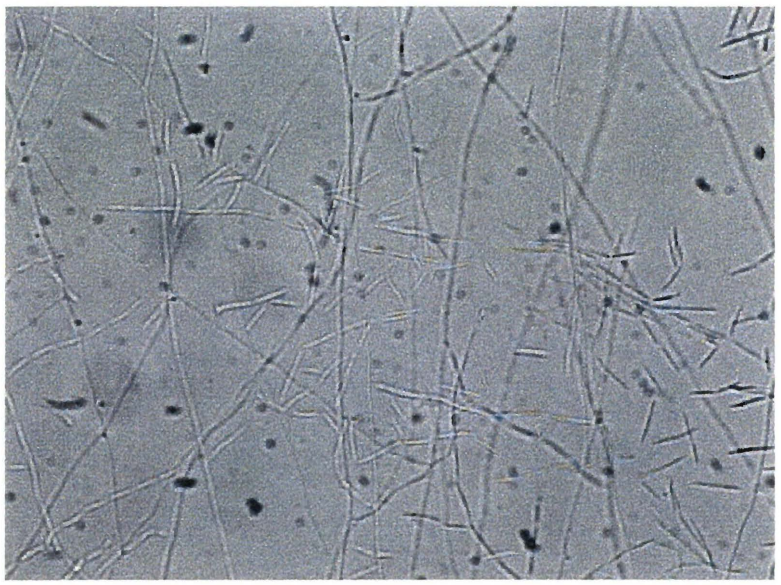
Figure 5. Microscopic characteristics of hyaline fungi isolated from Douglas-fir sapwood:

(A) *Debaryozyme hansenii* (400X)

(B) *Sporobolomyces salmonicolar* (400X)



(C)

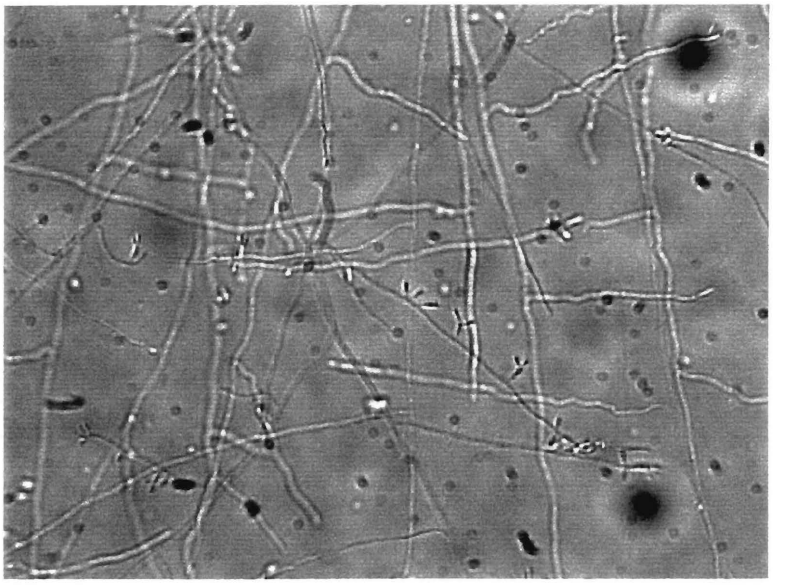


(D)

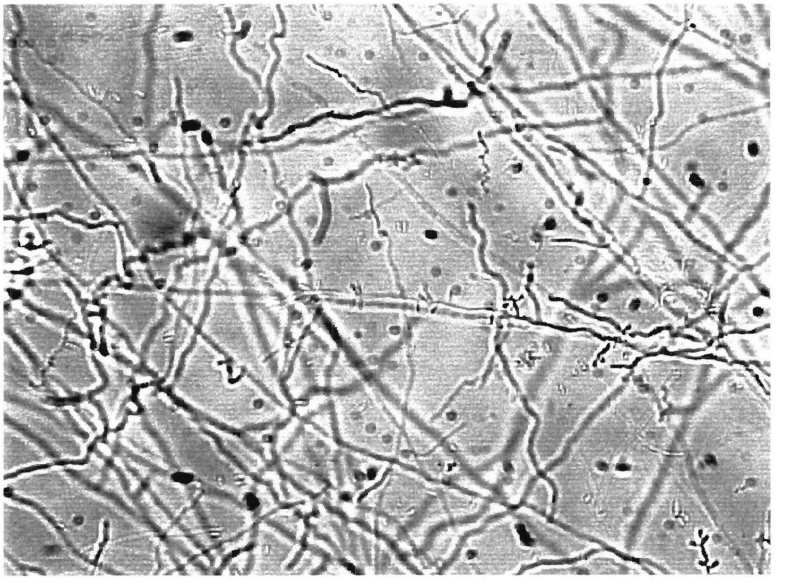
Figure 5. Continued.

(C) *Phialemonium dimorphosporum* (400X)

(D) *Polyscytalum fecundissimum* (400X)



(E)



(F)

Figure 5. Continued.

(E) *Sporothrix* sp. B (400X)

(F) *Hyalorhinocladiella* sp. (400X)

5.1.2 Effect of media on isolation frequency

Fungal flora and their frequencies can be affected by factors that include sampling site, sampling times and media used. More fungi were isolated from SMEA than MEA (Table 3). Streptomycin inhibited bacteria that could limit fungal growth. Bacteria colonies were abundant on MEA. A total of 55 isolates were obtained from the SMEA, compared with 42 isolates from MEA, although the fungal flora isolated were similar (Table 3).

Table 3. Frequency of fungi isolated from lumber obtained from Frank Lumber Co. using regular (MEA) and streptomycin amended malt extract agar (SMEA).

| | # of Isolates (% Frequency)[a] | |
|---|---|---|
| Fungus | MEA | SMEA |
| *Cladosporium cladosporioides* | 1 (2.4) | 2 (3.6) |
| *Leptodontium elatius* | - - | 1 (1.8) |
| *Rhinocladiella cellaris* | 3 (7.1) | 3 (5.5) |
| *Graphium putredinis* str.1 | 7 (16.7) | 6 (10.9) |
| *Graphium putredinis* str.2 | 2 (4.8) | 2 (3.6) |
| *Graphium putredinis* str.3 | 3 (7.1) | 2 (3.6) |
| *Sporothrix* sp. A str.1 | - - | 1 (1.8) |
| *Sporothrix* sp. A str.2 | - - | 1 (1.8) |
| Unidentified *Graphium* sp. | 2 (4.8) | 2 (3.6) |
| *Trichoderma harzianum* | 6 (14.3) | 5 (9.1) |
| *Trichoderma koningii* | 2 (4.8) | 4 (7.3) |
| *Trichoderma viride* | 4 (9.5) | 4 (7.3) |
| *Penicillium purpurogenum* | 1 (2.4) | - - |
| Unidentified *Penicillium* sp. | 1 (2.4) | 4 (7.3) |
| Zygomycete | 4 (9.5) | 7 (12.7) |
| Mycelial yeast | 1 (2.4) | 2 (3.6) |
| *Phialemonium dimorphosporum* | - - | 1 (1.8) |
| *Mucor* sp. | - - | 2 (3.6) |
| Unidentified Unknown fungi | 5 (11.9) | 6 (10.9) |
| Total Isolates | 42 | 55 |

[a] Fungi were isolated from 140 chips from 14 boards (10 chips from each board) collected on July 30, 1998.

The addition of cycloheximide had a dramatic impact on isolation frequency and composition of the flora (Table 4). Eleven species were isolated on SMEA, while only five species were isolated using SCMEA (Table 4). Cycloheximide is an antibiotic from *Streptomyces griseus* (Hawksworth et al., 1995) and is used as a fungicide in agar media for isolating bacteria (Pedersen, 1992). Cycloheximide is also used as selective media for distinguishing *Ophiostoma* from *Ceratocystis* species (Harrington, 1981; Wingfield, 1987). As expected, SCMEA selectively isolated *Graphium* species, asexual forms of *Ophiostoma* species. The frequency of *Graphium* species was 47.3 percent, while that on SMEA was 6.6 percent in lumberyard sample (Table 4). The cycloheximide effect was much more distinctive in samples from WTD where *Graphium* species represented 75 and 100 percent of total fungal isolations, respectively (Table 5 )

Table 4. Isolation frequencies of fungi from Douglas-fir lumber from the lumberyard Sample using streptomycin (SMEA) and streptomycin/cycloheximide amended malt extract agar (SCMEA).

| | # of Isolates (% Frequency)[a] | | | |
|---|---|---|---|---|
| Fungus | SMEA | | SCMEA | |
| *Cephaloascus fragrans* | 20 | (33.3) | - | - |
| *Scytalidium lignicola* | 2 | (3.3) | - | - |
| *Aureobasidium pullulans* | 1 | (1.7) | - | - |
| *Epicoccum purpurascens* | 1 | (1.7) | - | - |
| *Graphium putredinis str.1* | 2 | (3.3) | 7 | (19.4) |
| *Graphium putredinis* str.3 | - | - | 6 | (16.7) |
| *Graphium putredinis* str.2 | - | - | 1 | (2.8) |
| *Graphium penicillioides* str.1 | - | - | 1 | (2.8) |
| Unidentified *Graphium* sp. | 2 | (3.3) | 2 | (5.6) |
| *Penicillium frequentans* str.4 | 3 | (5.0) | 2 | (5.6) |
| *Trichoderma viride* | 3 | (5.0) | - | - |
| *Trichoderma koningii* | 2 | (3.3) | - | - |
| *Trichoderma harzianum* | 1 | (1.7) | - | - |
| Zygomycete | 13 | (21.7) | 10 | (27.8) |
| *Mucor* sp. | 4 | (6.7) | 3 | (8.3) |
| Unidentified Unknown | 6 | (10.0) | 4 | (11.1) |
| Total Isolates | 60 | | 36 | |

[a] Fungi were isolated from 100 chips from 10 boards (10 chips from each board) collected on October 9, 1998.

Table 5. Isolation frequencies of fungi from Douglas-fir lumber from WTD using streptomycin (SMEA) and streptomycin/cycloheximide amended malt extract agar (SCMEA).

| | # of Isolates (% Frequency)[a] | |
|---|---|---|
| Fungus | SMEA | SCMEA |
| Sample 1 | | |
| Unidentified Dematiaceous fungi | 1 (6.7) | - - |
| *Sporothrix* sp. A str.1 | - - | 2 (50.0) |
| *Sporothrix* sp. A str.2 | 1 (6.7) | - - |
| Unidentified *Graphium* sp. | - - | 1 (25.0) |
| *Aspergillus fumigatus* | 1 (6.7) | - - |
| *Penicillium purpurogenum* | 1 (6.7) | - - |
| *Penicillium verrucosum* | - - | 1 (25.0) |
| Unidentified *Penicillium* sp. | 1 (6.7) | - - |
| Zygomycete | 6 (40.0) | - - |
| *Polyscytalum fecundissimum* | 1 (6.7) | - - |
| Unidentified Unknown | 3 (20.0) | - - |
| Total Isolates | 15 | 4 |
| Sample 2 | | |
| *Graphium putredinis* str.2 | 1 (5.9) | 2 (66.7) |
| *Graphium penicillioides* str.2 | 1 (5.9) | 1 (33.3) |
| *Penicillium janthinellum* | 1 (5.9) | - - |
| *Aspergillus fumigatus* | 1 (5.9) | - - |
| Zygomycete | 8 (47.1) | - - |
| *Mucor* sp. | 3 (17.7) | - - |
| Unidentified Unknown | 2 (11.8) | - - |
| Total Isolates | 17 | 3 |

[a] Fungi were isolated from 150 chips from 10 boards (10 chips from each board) collected on March 9 and April 9, 1998.

SCMEA also prevented the growth of other dematiaceous fungi except *Ophiostoma*. For example, *Cephaloascus fragrans* was frequently isolated on SMEA (33.3 %), but was not observed on SCMEA (Table 4,5). The effect of cycloheximide on *Trichoderma*, *Penicillium* and *Cephaloascus* species isolated from SMEA was evaluated on SCMEA. All *C. fragrans* isolates were unable to grow on SCMEA, while *Trichoderma* species also grew poorly on this media (Table 6). As a result, the absence of *C. fragrans* and *Trichoderma* was not surprising although they represented 33.3 and 10.0 percent of total isolates from samples plated on SMEA (Table 4). Isolation of *Penicillium* species, however, did not appear to be affected by cycloheximide (Table 6).

Table 6. Effect of streptomycin amended malt extract agar (SMEA) and streptomycin/cycloheximide amended malt extract agar (SCMEA) on growth of selected fungi.

| | Colony diameter (mm)[a] | |
|---|---|---|
| Fungus | SMEA | SCMEA |
| *Cephaloascus fragrans* | 15.5 (0.8) | 0.0 (0.0) |
| *Trichoderma* sp. | 28.3 (0.9) | 3.9 (0.1) |
| *Penicillium* sp. | 32.9 (1.2) | 30.5 (0.8) |

[a] Values represent the means of 3 replicates/fungus for 7 days. Values in parentheses represent one standard deviation.

5.1.3 Effect of lumber source on isolation frequency

Fungal flora and frequencies varied widely with lumber source (Table 7). In total, 62, 37 and 6 isolates representing 15, 13 and 4 species were obtained from the lumberyard, WTD and Frank Lumber Co. samples, respectively. The time for which the boards were exposed prior to sampling varied with source. Boards from lumberyard were exposed for a relatively long time, boards from WTD were exposed for almost one month, and boards from Frank Lumber were less than 24 hours old. Fungi were present in 62, 37 and 6 percent of the chips, and 100, 90 and 50 percents of boards from lumberyard, WTD and Frank Lumber samples, respectively. Sapwood of freshly sawn lumber is susceptible to colonization by a variety of saprobic fungi (Seifert, 1993). The results indicated that fungal colonization on freshly sawn lumber was intimately related to the exposure time after cutting.

Differences in fungal flora among sites might reflect fungi that were dominant at the time of sampling. For example, temperature and rainfall can affect sporulation of specific fungi, while the presence of older, colonized lumber can provide a ready source of inoculum. High frequencies of *Trichoderma* species and low frequency of *Penicillium* species occurred in lumberyard samples, although both species are common saprobic fungi (Table 7). Dematiaceous fungi occurred frequently in WTD samples (37.8 %), while *Graphium* and *Trichoderma* species were abundant in samples from the lumberyard representing 22.6 and 30.6 percent of all isolates from these sites, respectively (Table 7). *A. pullulans* was present in 16.2 percent of isolations from WTD

samples, but was infrequent in lumberyard samples, and was absent in samples from Frank Lumber (Table 7).

Table 7. Incidence of fungi isolated from Douglas-fir lumber from the lumberyard, WTD and Frank Lumber Co.

| Fungus | # of Isolates (% Frequency of Isolates) | | |
|---|---|---|---|
| | Lumberyard | WTD | Frank |
| *Aureobasidium pullulans* | 1 (1.6) | 6 (16.2) | - - |
| *Aureobasidium* sp. | 1 (1.6) | - - | - - |
| *Allescheriella crocea* | - - | - - | 2 (33.3) |
| *Alternaria tenuissima* | - - | 1 (2.7) | - - |
| *Cladosporium cladosporioides* | | 2 (5.4) | - - |
| *Phoma fimeti* | 1 (1.6) | 1 (2.7) | - - |
| *Scytalidium lignicola* | - - | 3 (8.1) | - - |
| *Trichocladium canadense* | - - | 1 (2.7) | - - |
| *Graphium putredinis* str.3 | 4 (6.5) | - - | - - |
| *Graphium penicillioides* str.1 | 3 (4.8) | - - | - - |
| *Graphium putredinis* str.2 | 3 (4.8) | - - | - - |
| *Graphium penicillioides* str.2 | 2 (3.2) | - - | - - |
| *Graphium putredinis* str.1 | 2 (3.2) | - - | - - |
| *Trichoderma viride* | 12 (19.4) | - - | - - |
| *Trichoderma harzianum* | 5 (8.1) | - - | - - |
| *Trichoderma koningii* | 2 (3.2) | - - | - - |
| *Penicillium funiculosum* | 1 (1.6) | - - | - - |
| *Aspergillus fumigatus* | - - | - - | 1 (16.7) |
| *Penicillium purpurogenum* | - - | 1 (2.7) | 2 (33.3) |
| *Penicillium vasconiae* | - - | - - | 1 (16.7) |
| Zygomycete | 13 (21.0) | 12 (32.4) | - - |
| *Hyalorhinocladiella* sp. | 3 (4.8) | - - | - - |
| *Mucor* sp. | 3 (4.8) | 6 (16.2) | - - |
| *Sporothrix* sp. B | 2 (3.23) | - - | - - |
| Mycelial yeast | 1 (1.6) | 1 (2.7) | - - |
| *Sporobolomyces salmonicolor* | 1 (1.6) | 1 (2.7) | - - |
| *Debaryozyme hansenii* | - - | 1 (2.7) | - - |
| *Phialemonium dimorphosporum* | - - | 1 (2.7) | - - |
| Unidentified Unknown | 2 (3.2) | - - | - - |
| Total Isolates | 62 | 37 | 6 |

[a] Fungi were isolated from 100 chips from 10 boards per each lumber source (10 chips from each board) using streptomycin amended malt extract agar. All samples were collected on June, 1998.

Few fungi were isolated from freshly cut boards, but some fungi were isolated from end checks or beetle holes (Zabel and Morrell, 1992). *Allescheriella crocea* was isolated from beetle holes. This fungus might have symbiotic relationships with beetles.

## 5.2 Discoloration test of isolates from Douglas-fir sapwood

### 5.2.1 Selection of isolates

The number of isolates used from each species depended on frequency and their relative importance as sapstain fungi (Table 8). More *Graphium* isolates were tested because these fungi are considered to be important stain fungi. Although *Leptodontium elatius* and *Alysidium resinae* were isolated from Douglas-fir sapwood, they were excluded because pure cultures were not obtained.

### 5.2.2 Discoloration ability of isolate

The *O. perfectum* isolate used for comparison produced very heavy discoloration, while *A. alternata* failed to colonize Douglas-fir sapwood. Many *Graphium* species were isolated from Douglas-fir sapwood, but *A. alternata* was not isolated. Thus, the isolate evaluated may have lacked the ability to colonize this substrate.

Discoloration was more intense with time, although the increase varied depending on fungus. Some fast growing fungi produced up to 90 percent discoloration in 2 weeks, while slow growing isolates produced little discoloration after 2 weeks. Variations in degree of stain after 2 weeks was much bigger than that after 4 weeks with most fungi, suggesting that many fungi were not fully active after 2 weeks. This result indicates that wood discoloration tests require at least 4 weeks to allow slower growing fungi an opportunity to become fully functional on the substrate.

Table 8. Number of isolates of fungi isolated from Douglas-fir sapwood evaluated in a laboratory discoloration test.

| Fungus | No. of isolates tested |
|---|---|
| *Cephaloascus fragrans* | 3 |
| *Rhinocladiella cellaris* | 1 |
| *Cladosporium cladosporioides* | 1 |
| *Aureobasidium pullulans* | 1 |
| *Epicoccum purpurascens* | 1 |
| *Mammaria echinobotryoides* | 1 |
| *Scytalidium lignicola* | 1 |
| *Allescheriella crocea* | 1 |
| *Trichocladium canadense* | 1 |
| *Thysanophora penicillioides* | 1 |
| *Rhinocladiella atrovirens* | 1 |
| *Alternaria tenuissima* | 1 |
| *Aureobasidium* sp. | 1 |
| *Phoma fimeti* | 1 |
| *Graphium putredinis* str.1 | 10 |
| *Graphium putredinis* str.2 | 10 |
| *Graphium putredinis* str.3 | 10 |
| *Graphium penicillioides* str.1 | 2 |
| *Graphium penicillioides* str.2 | 2 |
| *Sporothrix* sp. A str.1 | 2 |
| *Sporothrix* sp. A str.2 | 2 |
| *Trichoderma viride* | 2 |
| *Trichoderma harzianum* | 2 |
| *Trichoderma koningii* | 2 |
| *Penicillium purpurogenum* | 2 |
| *Penicillium frequentans* str.1 | 2 |
| *Penicillium frequentans* str.2 | 1 |
| *Penicillium frequentans* str.3 | 1 |
| *Penicillium frequentans* str.4 | 1 |
| *Penicillium spinulosum* | 1 |
| *Aspergillus fumigatus* | 1 |
| *Penicillium funiculosum* | 1 |
| *Penicillium odoratum* | 1 |
| *Penicillium diversum* | 1 |
| *Penicillium thomii* | 1 |
| *Penicillium expansum* | 1 |
| *Penicillium janthinellum* | 1 |
| *Penicillium para-herquei* | 1 |
| *Penicillium vasconiae* | 1 |
| *Penicillium verrucosum* | 1 |

5.2.2.1 Dematiaceous fungi

Discoloration did not differ significantly among three isolates of *C. fragrans* ($\alpha$ = 0.05), suggesting that isolates of this fungus were uniformly capable of discoloration (Table 9).

Table 9. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of three isolates of *Cephaloascus fragrans*.

| Isolate | Degree of discoloration (%)[a] | |
|---|---|---|
| | 2 weeks | 4 weeks |
| 1 | 89 (2) A | 98 (3) A |
| 2 | 87 (7) A | 97 (4) A |
| 3 | 88 (5) A | 96 (6) A |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 15 specimens per each isolate. Values in parentheses represent a standard deviation. Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha$ = 0.05).

Degrees of discoloration after 4 weeks exceeded 90 percent for wafers exposed to *R. cellaris*, *C. fragrans*, *S. lignicola* or *C. cladosporioides*, and 85 percent for those exposed to *T. penicillioides* or *A. pullulans* (Table 10). These fungi represented from 1.28 to 5.85 percent of the total isolates and each occurred in more than 2 sample sets (Table 10). *T. penicillioides* produced 87 percent discoloration after 4 weeks, although it represented only 0.2 percent of the total isolates. Degrees of discoloration ranged from 23 to 52 percent for *A. tenuissima*, *T. canadense*, *A. crocea* and *P. fimeti* (Table 10). These fungi represent potential staining fungi for Douglas-fir sapwood. The remaining fungi produced little discoloration or did not grow on the wafers (Table 10).

Table 10. Degree of discoloration of Douglas-fir sapwood wafers exposed to selected dematiaceous fungi

| Fungus | Reps | Degree of discoloration (%)[a] 2 weeks | 4 weeks |
|---|---|---|---|
| *R. cellaris* | 15 | 75 (7) BC | 99 (2) A |
| *C. fragrans* | 45 | 88 (5) A | 97 (4) A |
| *S. lignicola* | 15 | 72 (11) C | 94 (6) AB |
| *C. cladosporioides* | 15 | 81 (6) AB | 94 (5) AB |
| *T. penicillioides* | 15 | 57 (42) D | 87 (14) B |
| *A. pullulans* | 15 | 1 (2) F | 86 (22) B |
| *P. fimeti* | 15 | 26 (16) E | 52 (13) C |
| *A. crocea* | 15 | 5 (2) F | 49 (29) C |
| *T. canadense* | 15 | 0 (0) F | 32 (13) D |
| *A. tenuissima* | 15 | 5 (4) F | 23 (9) E |
| *Aureobasidium* sp. | 15 | 3 (3) F | 5 (3) F |
| *R. atrovirens* | 15 | 0 (0) F | 0 (0) F |
| *E. purpurascens* | 15 | 0 (0) F | 0 (0) F |
| *M. echinobotryoides* | 15 | 0 (0) F | 0 (0) F |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values in parentheses represent a standard deviation. Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

5.2.2.2 *Graphium* species

Degrees of discoloration did not differ significantly between two isolates of *Sporothrix* sp. A or *G. penicillioides* except for *G. penicillioides* str. #1 (Table 11).

Table 11. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of two isolates of *Sporothrix* and *G. penicillioides*.

| Fungus | Degree of discoloration (%)[a] 2 weeks Isolates 1 | 2 | T-ratio | 4 weeks Isolates 1 | 2 | T-ratio |
|---|---|---|---|---|---|---|
| *Sporothrix* sp. A str.1 | 73 (4) | 75 (5) | -1.3653 NS | 85 (4) | 86 (4) | -0.2236 NS |
| *Sporothrix* sp. A str.2 | 67 (6) | 66 (5) | 0.3327 NS | 71 (6) | 72 (7) | -0.6904 NS |
| *G. penicillioides* str.1 | 70 (5) | 82 (3) | -7.8543 ** | 75 (4) | 86 (4) | -8.4887 ** |
| *G. penicillioides* str.2 | 65 (4) | 66 (4) | -0.7157 NS | 70 (4) | 71 (4) | -1.0000 NS |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 15 specimens per isolate. Values in parentheses represent a standard deviation. NS = not significant by a t-test ($\alpha = 0.05$). ** = significant by a t-test ($\alpha = 0.01$).

Ten isolates were evaluated for each *G. putredinis* strain. Discoloration differed significantly among isolates of all three strains ($\alpha = 0.05$) (Table 12). This variation suggests that classification by media pigmentation may be a poor method for segregating isolates of *G. putredinis*.

Table 12. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of 10 isolates of each *G. putredinis* strain.

| | Degree of Discoloration (%)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Strain #1 | | Strain #2 | | Strain #3 | |
| | 2 weeks | 4 weeks | 2 weeks | 4 weeks | 2 weeks | 4 weeks |
| 1 | 89 (3) A | 97 (2) A | 86 (4) A | 91 (4) A | 82 (7) A | 86 (6) A |
| 2 | 86 (5) AB | 93 (5) AB | 83 (7) AB | 88 (6) A | 78 (5) ABC | 85 (7) AB |
| 3 | 88 (4) A | 92 (4) ABC | 82 (4) ABC | 87 (3) A | 77 (5) BCD | 84 (6) AB |
| 4 | 82 (3) C | 90 (4) BC | 77 (8) ABCD | 83 (5) B | 76 (5) BCD | 84 (4) AB |
| 5 | 81 (9) C | 89 (12) BC | 70 (17) D | 82 (4) B | 79 (6) AB | 84 (6) AB |
| 6 | 82 (8) BC | 88 (8) C | 76 (10) BCD | 81 (7) BCD | 74 (5) CD | 81 (6) ABC |
| 7 | 73 (5) D | 79 (4) D | 46 (20) F | 80 (4) BCDE | 75 (9) BCD | 80 (9) BC |
| 8 | 68 (6) DE | 76 (5) D | 73 (4) CD | 78 (4) CDE | 73 (4) D | 77 (5) C |
| 9 | 69 (6) DE | 76 (6) D | 59 (16) E | 77 (6) DE | 65 (5) E | 71 (5) D |
| 10 | 67 (5) E | 71 (6) E | 71 (3) D | 77 (5) E | 54 (8) F | 60 (9) E |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 15 specimens per isolate. Values in parentheses represent a standard deviation. Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

*Sporothrix* sp. A and *G. penicillioides* showed significantly different discoloration ability with strain (Table 13). Degrees of discoloration by *G. penicillioides* strain #1 were 16.3 and 13.9 percent higher than for strain #2 after 2 and 4 weeks, respectively.

Table 13. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of two strains of *Sporothrix* sp. A or *G. penicillioides*.

| | Degree of discoloration (%)[a] | | | | | |
|---|---|---|---|---|---|---|
| | 2 weeks | | | 4 weeks | | |
| Fungus | Strain | | T-ratio | Strain | | T-ratio |
| | 1 | 2 | | 1 | 2 | |
| *Sporothrix* sp. A | 74 (5) | 66 (5) | 5.9646 ** | 86 (4) | 71 (5) | 11.8083 ** |
| *G. penicillioides* | 76 (7) | 66 (4) | 7.2147 ** | 81 (7) | 71 (4) | 6.8284 ** |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 30 specimens per strain. Values in parentheses represent a standard deviation. ** = significant by a t-test ($\alpha = 0.01$).

Degree of discoloration of wafers differed significantly among the three strains of *G. putredinis* after 4 weeks (Table 14). Strain #1 which produced more pigmentation on media, produced 85 percent discoloration of wafers, followed by strain #2 and strain #3 which produced average degrees of discoloration of 82 and 79 percent, respectively. While the trends followed the degree of pigmentation on media, the differences were relatively small and are probably of little biological or practical significance.

Table 14. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of 3 strains of *Graphium putredinis*.

| | Degree of Discoloration (%)[a] | |
|---|---|---|
| Strain | 2 weeks | 4 weeks |
| 1 | 79 (10) A | 85 (10) A |
| 2 | 72 (16) B | 82 (7) B |
| 3 | 73 (10) B | 79 (10) C |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 150 specimens per strain. Values in parentheses represent a standard deviation. Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

All *Graphium* species completely covered the wafers after 2 weeks, and produced numerous synnemata on the wood surface. The black necks of the synnemata increased the degree of discoloration slightly, but most of the discoloration was caused by hyphal pigmentation.

In discoloration produced by *Graphium* species (Table 15), *Sporothrix* sp. A strain #1 and *G. putredinis* strain #1 generally exceeded 85 percent. Degree of discoloration ranged from 79.1 to 82.3 percent for *G. putredinis* strain #2, *G. penicillioides* strain #1 and *G. putredinis* strain #2. *Sporothrix* sp. A strain #1 and *G. penicillioides* strain #2 produced over 70 percent discoloration. Isolates of *G. putredinis* generally caused more stain than *G. penicillioides*. All *Graphium* species produced more than 70 percent discoloration, illustrating the importance of these fungi in staining of Douglas-fir sapwood.

Table 15. Degree of discoloration of Douglas-fir sapwood wafers exposed to selected *Graphium* species.

| | | Degree of discoloration (%)[a] | |
|---|---|---|---|
| Fungus | Reps | 2 weeks | 4 weeks |
| *Sporothrix* sp. A str.1 | 30 | 74 (5) AB | 86 (4) A |
| *G. putredinis* str.1 | 150 | 78 (10) A | 85 (10) A |
| *G. putredinis* str.2 | 150 | 72 (16) B | 82 (7) AB |
| *G. penicillioides* str.1 | 30 | 76 (7) AB | 81 (7) B |
| *G. putredinis* str.3 | 150 | 73 (10) B | 79 (10) B |
| *Sporothrix* sp. A str.1 | 30 | 66 (5) C | 71 (5) C |
| *G. penicillioides* str.2 | 30 | 66 (4) C | 71 (4) C |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values in parentheses represent a standard deviation.
Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

5.2.2.3 Molds

Degree of discoloration did not differ significantly between the two isolates of *Penicillium* or *Trichoderma* species except for the 2 week readings of *Penicillium* species and *T. koningii* (Table 16). The differential discoloration after 2 weeks may represented variations in inoculum levels between isolates, since the differences disappeared after 4 weeks.

Table 16. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of two isolates of selected molds.

| | Amount of discoloration (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2 weeks | | | 4 weeks | | |
| Fungus | Isolates | | T-ratio | Isolates | | T-ratio |
| | 1 | 2 | | 1 | 2 | |
| *P. frequentans* str.1 | 77 (10) | 37 (26) | 5.5959 ** | 95 (2) | 94 (4) | 1.5378 NS |
| *P. purpurogenum* | 67 (29) | 34 (26) | 3.2988 ** | 97 (2) | 95 (3) | 1.3440 NS |
| *T. viride* | 78 (8) | 73 (8) | 1.4898 NS | 86 (1) | 86 (1) | 0.0000 NS |
| *T. harzianum* | 48 (35) | 62 (13) | -1.4120 NS | 82 (4) | 81 (6) | 0.7559 NS |
| *T. koningii* | 62 (7) | 74 (5) | -5.3090 ** | 79 (4) | 82 (4) | -2.0344 NS |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values represent means of 15 specimens per isolate. Values in parentheses represent a standard deviation. NS = not significant by a t-test ($\alpha = 0.05$). ** = significant by a t-test ($\alpha = 0.01$).

Discoloration ability did not differ among strains of *P. frequentans* after 4 weeks (Table 17), while significant differences were observed in 2 weeks. The difference after 2 weeks may reflect inoculum differences or a longer lag time before sporulation for some isolates.

Table 17. Degree of discoloration of Douglas-fir sapwood wafers exposed to one of 4 strains of *P. frequentans*.

| | | Amount of discoloration (%)[a] | |
|---|---|---|---|
| Strain | Reps | 2 weeks | 4 weeks |
| 1 | 30 | 57 (28) B | 95 (3) A |
| 4 | 15 | 82 (5) A | 94 (3) A |
| 3 | 15 | 76 (17) A | 94 (6) A |
| 2 | 15 | 53 (22) B | 92 (5) A |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values in parentheses represent a standard deviation.
Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

All *Penicillium* species produced over 90 percent discoloration, while degree of discoloration ranged from 80 to 86 percent for *Trichoderma* species after 4 weeks (Table 18).

*Penicillium* species produced spores over the entire wood surface, but *Trichoderma* species produced spores more sporadically. *Penicillium* and *Trichoderma* showed similar sporulation patterns in media: sporulation of *Penicillium* occurs evenly throughout the surface of media (Ramirez, 1982), while that of *Trichoderma* appears in concentric rings from the point of inoculation (Rifai, 1969; Wang and Zabel, 1990). The distribution of spores on the wafer surface was more even with *T. viride* than *T. harzianum* or *T. koningii*, and resulted in more discoloration with *T. viride* although growth rates of all three species on agar were similar.

*Aspergillus fumigatus* produced 93 percent discoloration on Douglas-fir sapwood wafers after 4 weeks, and produced a green color that was similar to that caused by *Penicillium* species.

Since molds produce masses of spores which can be easily removed, their control is not as critical issue. However, their presence is objectionable to some customers, and they can cause allergic reactions to people handling the wood (Seifert and Grylls, 1992; Zabel and Morrell, 1992). As a result, effective mold control must be considered when handling green lumber.

Table 18. Degree of discoloration of Douglas-fir sapwood wafers exposed to selected molds.

| | | Degree of discoloration (%)[a] | |
|---|---|---|---|
| Fungus | Reps | 2 weeks | 4 weeks |
| *P. thomii* | 15 | 86 (4) A | 96 (4) A |
| *P. odoratum* | 15 | 83 (6) A | 96 (4) AB |
| *P. purpurogenum* | 30 | 50 (31) EF | 96 (3) AB |
| *P. janthinellum* | 15 | 81 (4) AB | 95 (4) ABC |
| *P. para-herquei* | 15 | 85 (5) A | 95 (3) ABC |
| *P. vasconiae* | 15 | 80 (11) AB | 95 (4) ABC |
| *P. verrucosum* | 15 | 82 (3) A | 95 (3) ABC |
| *P. frequentans* | 75 | 65 (24) CD | 94 (4) ABCD |
| *P. funiculosum* | 15 | 63 (24) CDE | 93 (6) BCDE |
| *A. fumigatus* | 15 | 62 (9) CDE | 93 (4) CDE |
| *P. diversum* | 15 | 62 (21) CDE | 92 (6) DE |
| *P. expansum* | 15 | 38 (16) F | 92 (6) DE |
| *P. spinulosum* | 15 | 39 (17) F | 91 (4) E |
| *T. viride* | 30 | 76 (8) ABC | 86 (3) F |
| *T. harzianum* | 30 | 55 (27) DE | 82 (5) G |
| *T. koningii* | 30 | 68 (9) BCD | 80 (4) G |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values in parentheses represent a standard deviation.
Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

### 5.2.3 Comparison among three fungal categories

Although allowable discoloration is arbitrary and fluctuates depending on demand and supply in the market, a rating 20 percent or less was determined as the allowable degree of discoloration in this study. Degrees of discoloration of Douglas-fir sapwood exposed to most test fungi exceeded the allowable level except for some slow growing dematiaceous fungi (Figure 6 and Table 19). Discoloration caused by *A. crocea*, *T. canadense* and *A. tenuissima* was allowable after 2 weeks, but not 4 weeks.

The degree of discoloration with *O. perfectum* after 4 weeks did not significantly differ from that caused by *R. cellaris*, *C. fragrans* and most *Penicillium* species except *P. funiculosum*, *P. diversum* and *P. spinulosum* (Table 19). Seventeen of 37 fungi produced over 90 percent discoloration after 4 weeks. Discoloration ranged from 70 to 80 percent for 12 fungi, while four slow growing dematiaceous fungi produced 23 to 52 percent discoloration after 4 weeks. The remaining dematiaceous fungi fail to stain or colonize Douglas-fir sapwood. As a result, most fungi caused discoloration that would be unacceptable for many uses.

Table 19. Average degree of discoloration of Douglas-fir sapwood wafers exposed to selected fungi.

| | | Degree of discoloration (%)[a] | |
|---|---|---|---|
| Fungus | Reps | 2 weeks | 4 weeks |
| *O. perfectum* | 30 | 96 (3) A | 100 (0) A |
| *R. cellaris* | 15 | 75 (7) CDEFGH | 99 (2) AB |
| *C. fragrans* | 45 | 88 (5) AB | 97 (6) ABC |
| *P. thomii* | 15 | 86 (4) B | 96 (4) ABCD |
| *P. odoratum* | 15 | 83 (6) BCD | 96 (4) ABCD |
| *P. purpurogenum* | 30 | 50 (31) M | 96 (3) ABCD |
| *P. vasconiae* | 15 | 80 (11) BCDE | 95 (4) ABCD |
| *P. verrucosum* | 15 | 82 (3) BCDE | 95 (3) ABCD |
| *P. janthinellum* | 15 | 81 (4) BCDE | 95 (4) ABCD |
| *P. para-herquei* | 15 | 85 (5) BC | 95 (3) ABCD |
| *S. lignicola* | 15 | 72 (11) EFGHIJ | 94 (6) ABCD |
| *C. cladosporioides* | 15 | 81 (6) BCDE | 94 (5) ABCD |
| *P. frequentans* | 75 | 65 (24) HIJK | 94 (4) ABCD |
| *P. funiculosum* | 15 | 63 (24) IJKL | 93 (6) BCD |
| *A. fumigatus* | 15 | 62 (9) JKL | 93 (4) BCDE |
| *P. diversum* | 15 | 62 (21) JKL | 92 (6) CDE |
| *P. expansum* | 15 | 38 (16) N | 92 (6) CDE |
| *P. spinulosum* | 15 | 39 (17) N | 91 (4) DEF |
| *T. penicillioides* | 15 | 57 (42) KLM | 87 (14) EFG |
| *A. pullulans* | 15 | 1 (2) P | 86 (22) FGH |
| *T. viride* | 30 | 76 (8) CDEFG | 86 (3) FGHI |
| *Sporothrix* sp. A str.1 | 30 | 74 (5) DEFGH | 86 (4) FGHI |
| *G. putredinis* str.1 | 150 | 78 (10) BCDE | 85 (10) GHI |
| *G, putredinis* str.2 | 150 | 72 (16) EFGHI | 82 (7) GHIJ |
| *T. harzianum* | 30 | 55 (27) LM | 82 (5) GHIJ |
| *G. penicillioides* str.1 | 30 | 76 (7) CDEF | 81 (7) HIJ |
| *T. koningii* | 30 | 68 (9) FGHIJ | 80 (4) IJ |
| *G. putredinis* str.3 | 150 | 73 (10) DEFGH | 79 (10) J |
| *Sporothrix* sp. A str.2 | 30 | 66 (5) FGHIJK | 71 (5) K |
| *G. penicillioides* str.2 | 30 | 66 (4) HIJK | 71 (4) K |
| *P. fimeti* | 15 | 26 (16) O | 52 (13) L |
| *A. crocea* | 15 | 5 (2) P | 49 (29) L |
| *T. canadense* | 15 | 0 (0) P | 32 (13) M |
| *A. tenuissima* | 15 | 5 (4) P | 23 (9) N |
| *Aureobasidium* sp. | 15 | 3 (3) P | 5 (3) O |
| *M. echinobotryoides* | 15 | 0 (0) P | 0 (0) O |
| *E. purpurascens* | 15 | 0 (0) P | 0 (0) O |
| *R. atrovirens* | 15 | 0 (0) P | 0 (0) O |
| *A. alternata* | 30 | 0 (0) P | 0 (0) O |

[a] Degree of discoloration based on rating from 0 (no discoloration) to 100 (completely discolored). Values in parentheses represent a standard deviation.
Means in the same column that are followed by the same letter(s) do not differ significantly by Bonferroni's t-test ($\alpha = 0.05$).

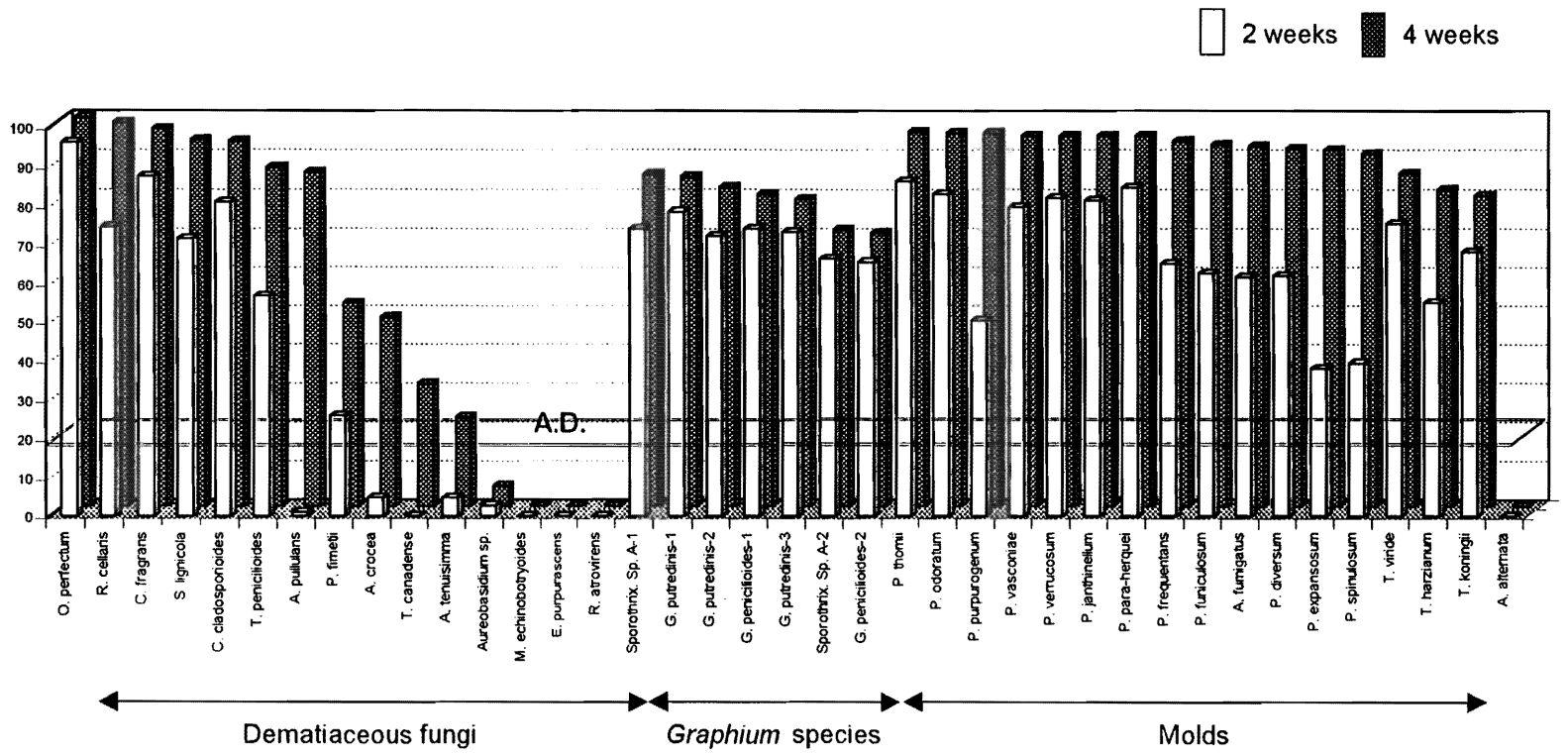



Figure 6. Degree of discoloration of Douglas-fir sapwood wafers exposed to fungi isolated from Douglas-fir sapwood.
A.D. stands for the level of discoloration that would be considered acceptable.

# 6. CONCLUSIONS

*Graphium* species were the most commonly isolated fungi from Douglas-fir sapwood, representing 17.37 percent of all isolates. Most *Graphium* species were considered as anamorphs of *O. piceae*. Other sapstaining fungi isolated with relatively high frequencies were *Cephaloascus fragrans*, *Aureobasidium pullulans*, *Rhinocladiella cellaris*, *Cladosporium cladosporioides* and *Scytalidium lignicola*.

Molds, primary *Penicillium* and *Trichoderma* species, were frequently isolated from Douglas-fir sapwood. These species may be antagonistic to other fungi and may be considered as potential biological control organisms or as competitors of biological control organisms added to lumber. A number of fungal species of unknown biological significance were frequently isolated from Douglas-fir sapwood. Their roles in the development of sapstain merit further study.

Fungal flora varied depending on media and lumber source. *Graphium* species were the only stain fungi isolated using cycloheximide amended media. Fungal colonization on freshly sawn lumber appeared to be related to exposure time after cutting.

All *Graphium* isolates caused more than 70 percent discoloration of Douglas-fir sapwood wafers. Mold isolates also discolored Douglas-fir sapwood wafers. All *Penicillium* species produced over 90 percent discoloration, while *Trichoderma* species produced 80 to 86 percent discoloration. These fungi, however, pose less of a concern since their blemishes are cosmetic and can be removed by sanding or planing the wood.

Most dematiaceous fungi were capable of staining Douglas-fir sapwood wafers, including *Cephaloascus fragrans*, *Aureobasidium pullulans*, *Rhinocladiella cellaris*, *Cladosporium cladosporioides*, *Scytalidium lignicola* and *Thysanophora penicillioides*.

The results indicate that, while *Graphium* species are the primary stain fungi on Douglas-fir sapwood, a variety of other species can opportunistically colonize this substrate.

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# APPENDIX

## Appendix. Culture media

Malt Extract Agar (MEA)

| | |
|---|---|
| Malt extract | 20 g |
| Agar | 20 g |
| Distilled water | 1000 ml |

Streptomycin amended Malt Extract Agar (SMEA)

| | |
|---|---|
| Malt extract | 20.0 g |
| Agar | 20.0 g |
| 10 % Lactic acid | 3.0 ml |
| Streptomycin | 100 ppm |
| Distilled water | 1000 ml |

Streptomycin/cycloheximide amended Malt Extract Agar (SCMEA)

| | |
|---|---|
| Malt extract | 20.0 g |
| Agar | 20.0 g |
| 10 % Lactic acid | 3.0 ml |
| Streptomycin | 100 ppm |
| Cycloheximide | 100 ppm |
| Distilled water | 1000 ml |

**Appendix. Continued.**

| Czapek's Agar | |
|---|---|
| $NaNO_3$ | 3.0 g |
| $K_2HPO_4$ | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g |
| KCL | 0.5 g |
| $FeSO_4 \cdot 7H_2O$ | 0.01 g |
| Sucrose | 30.0 g |
| Agar | 20.0 g |
| Distilled water | 1000 ml |

| Czapek's Yeast-Extract Agar | |
|---|---|
| $NaNO_3$ | 3.0 g |
| $K_2HPO_4$ | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g |
| KCL | 0.5 g |
| $FeSO_4 \cdot 7H_2O$ | 0.01 g |
| Sucrose | 30.0 g |
| Yeast Extract | 5.0 g |
| Agar | 20.0 g |
| Distilled water | 1000 ml |

| Glucose amended malt extract agar | |
|---|---|
| Malt extract | 20.0 g |
| Glucose | 20.0 g |
| Bacto-pepton | 1.0 g |
| Agar | 20.0 g |
| Distilled water | 1000 ml |