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The occurrence, identity and physiology of microfungi colonizing untreated, or, Vapam (sodium N-methyldithiocarbamate), chloropicrin (trichloronitromethane), or Vorlex^{R} (20% methylisothicyanate, 80% chlorinated C_3 hydrocarbons) treated Douglas-fir poles were evaluated by culturing increment cores from poles remedially treated 5 and 15 years prior to sampling. A total of 14 species of microfungi were isolated from the fumigant treated poles while 16 species were isolated from the untreated controls. As expected, the fungal population was higher in the untreated than in the the treated poles.

Two species of Scytalidium and Penicillium were the most commonly isolated microfungi from the older treated poles as well as the untreated controls. Vorlex appeared to have the most varied microflora, while there were minor differences between the microflora found in the Vapam and chloropicrin treated poles.

Among the 18 fungi tested, none caused severe weight losses on pine blocks after 12 weeks of exposure in a vermiculite burial test. Only the two Trichoderma viride strains produced cell wall erosion (Type 2 soft rot attack) and Oidiodendron tenuissimum caused scattered

cavity formation (Type 1 soft rot attack).

Responses of microfungi to pentachlorophenol and creosote varied. The tests illustrated the relationship between isolation frequency and tolerance to the preservatives. The ability of the microfungi to survive exposure to methylisothiocyanate (MIT) or chloropicrin also varied. chloropicrin appeared to require a longer exposure period to inhibit fungal growth.

Agar and soil block tests showed that exposure to five selected microfungi reduced the decay capacity of Poria carbonica and P. placenta particularly when blocks were exposed to the microfungi first.

The results indicate that fumigant treated Douglas-fir poles were colonized by a limited microflora, even 15 years after treatment. Some of the microflora exhibited preservative and fumigant tolerance which may, in part, explain their presence in the treated wood. Furthermore, several of these fungi exhibit antagonism to decay fungi which commonly attack Douglas-fir wood in ground contact. The presence of these fungi may help to explain the long term performance of wood fumigants.

MICROFUNGI OF PRESERVATIVE TREATED DOUGLAS-FIR POLES BEFORE AND AFTER FUMIGANT TREATMENT

by

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MICROFUNGI OF PRESERVATIVE TREATED DOUGLAS-FIR POLES BEFORE AND AFTER FUMIGANT TREATMENT

INTRODUCTION

When properly handled, wood is a durable material that can last for centuries. Unfortunately, wood is often used in environments conducive to decay. To protect wood under these conditions, chemical preservatives are applied under pressure, extending its service life to 40 or more years.

Some wood species are difficult to treat with chemicals because of their relatively thin sapwood. These species are susceptible to internal decay which shortens wood service life. The characteristics which render the internal heartwood core resistant to preservative penetration also make it difficult to deliver internal treatments which can arrest decay.

By 1938, externally applied pastes were extensively used to control wood decay in poles in service. Since these pastes lack the ability to penetrate beyond the preservative treated shell, effective and long term protection against fungal decay could not be fully achieved.

More recently, volatile agricultural fumigants were shown to eliminate decay fungi from wood and to prevent fungal reinvasion for up to 17 years. These chemicals are often applied as liquids but the active ingredient diffuses through the wood as a gas. The performance of these chemicals has led to their use in 85 percent of the utility maintenance programs in the United States (Goodell and Graham, 1983).

While fumigants have performed well, the nature of long term fumigant protection is poorly understood. Several fumigants have been

shown to remain in the wood for up to 17 years, while the most commonly used fumigant, Vapam, cannot be detected after 2 to 3 years. A retreatment cycle of 6 to 7 years for Vapam and 10 years for Vorlex and chloropicrin was recommended to prolong the service life of fumigant treated poles (Graham et al, 1975).

Previous studies have shown that fumigant treated wood is rapidly colonized by fungi when large quantities of fungal inoculum are used. However, the critical density of inoculum and the effects of individual spores on the colonization process are not fully elucidated.

The low rate of fungal invasion by decay fungi in fumigant treated wood may be due to a natural slow colonization process, the presence of non-volatile Vapam residues or decomposition products, or the colonization of the fumigant treated wood by fungi capable of inhibiting the growth of decay fungi. Conversely, the surviving fungal flora in the treated wood might alter the fumigant more rapidly, thereby permitting more rapid recolonization of the wood by decay fungi. Understanding the nature of wood/chemical/microflora interactions would help in the development of more effective remedial decay control strategies.

At present, there is limited information on the microflora of fumigant treated wood. This research evaluates the fungal flora of Douglas-fir poles treated with Vapam, Vorlex, methylisothiocyanate, or chloropicrin, 5 to 15 years after fumigant treatment.

REVIEW OF LITERATURE

A. Ecology of fungi infecting untreated and treated wood.

Many members of the Fungi Imperfecti and Ascomycetes are known to colonize living trees, freshly cut stumps, logs or timber in service (Bourchier, 1961; Lindgren & Eslyn, 1961; Shigo, 1962; Merrill & French, 1966; Greaves & Levy, 1968; Levy, 1968; Maloy & Robinson, 1968; Hansen & Hansen, 1973; Shortle & Cowling, 1975:). A wide variety of fungi also colonize untreated and treated wood in ground contact (Savory, 1954a, 1954b; Cowling, 1957; Duncan, 1960; Corbett & Levy, 1963; Greaves & Savory, 1965; Merrill & French, 1966; Kaarik, 1967; Butcher 1968, 1971; Cavalcante, 1980).

Butcher (1968) made one of the first detailed studies on relative fungal frequency and distribution in untreated and chromated copper arsenate (CCA)-treated Pinus radiata sapwood stakes. The fungal flora in untreated stakes varied with degree of ground contact and these differences were attributed to changes in wood moisture content and source of infection. Fungal succession aboveground proceeded from primary molds to blue stain fungi. At groundline, succession shifted from primary molds to soft rot fungi to secondary molds and primary Basidiomycetes (white rot fungi). In preservative treated stakes, fewer fungal species were isolated but the succession profile was similar to untreated stakes. Members of the Moniliaceae occurred with the highest frequency and were found to be most active, particularly Penicillium sp. and Cephalosporium sp. which were the most common fungi isolated above-ground. Trichoderma viride was a common member of the

flora below-ground and was encountered sporadically above and at groundline. The soft rot fungi, Chaetomium globosum, Cephalosporium sp. and Coniothryrium sp. were identified from the untreated stakes and caused severe weight losses in decay tests.

On CCA-treated Eucalyptus regnans and P. radiata sapwood stakes, T. viride, Cladosporium sp. and Paecilomyces roseus were isolated after two months of exposure while C. globosum and Humicola grisea were observed after four months (Greaves, 1972). Penicillium sp. once again was found to be the most dominant fungal group isolated.

A total of 56 cellulolytic fungal species were isolated from preservative treated Scots pine transmission poles and beech sapwood stakes that were in service for 28-29 years (Greaves and Savory, 1965). Two fungal species, Cladosporium resinae and Paecilomyces varioti, were only isolated from creosoted material. The results of the study indicated that these species may play an important role in the depletion of creosote in the wood. The depletion may facilitate the establishment of less creosote-tolerant microorganisms (Greaves & Savory, 1965; Marsden, 1954).

Cladosporium resinae is commonly isolated from creosote treated wood and can utilize creosote as the sole carbon source (Christensen, et al, 1942; Marsden 1954). Like Penicillium stecki, C. resinae is believed to be capable of modifying the phenolic components of creosote (Kerner-Gang, 1975).

The occurrence of non-basidiomycetous fungi in Douglas-fir poles treated with Vorlex, Vapam and Chloropicrin has been previously noted (Graham, 1972). All the fumigated poles experienced a steady increase

in the population of microfungi between the first and second year after treatment, the effect being most pronounced with Vapam treated poles (Graham & Helsing, 1973). The incidence of microfungi continued to increase with time.

The effects of microfungi on fumigant performance is poorly understood. Graham (1975) suggested that the microfungi could enhance reinvasion by decay fungi by detoxifying the residual fumigant. Conversely, these fungi may also act as a biological buffer to delay reinvasion by decay fungi.

Vapam and Chloropicrin have controlled decay fungi in the interior of waterfront timbers but have not limited the occurrence of molds (Eslyn, 1970). While the Basidiomycetes colonizing Douglas-fir in service have been characterized (Eslyn, 1970; Zabel et al, 1980), there is limited data on the Ascomycetes and Fungi Imperfecti colonizing this wood species. These fungi may play significant roles in preservative performance and colonization by more aggressive decay fungi.

B. Effects of non-decay fungi on the preservative treated wood.

Although the microfungi are often overlooked, their effects on conventional decay fungi may be important factors in preservatives performance. The inactivation of arsenic, copper, mercury and phenolic compounds by fungi had been demonstrated by various workers. Madhosingh (1961) observed that Fusarium oxysporum was frequently associated with Coprinus micaceus and suggested that the former reduced the toxicity of the preservative (Composition: sodium fluoride 34%;

potassium dichromate 34%; sodium arsenate 25% and 2,4 dinitrophenol 7%), thus facilitating the entry and spread of the decay fungus. Other laboratory tests also indicate that some Phoma, Orbicula, Chaetomium and Graphium species are capable of degrading or inactivating arsenic, copper, chromium and fluoride salts (Duncan & Devarall, 1964).

Cultures of certain Aspergillus, Penicillium and Scytalidium species were also capable of growth on media saturated or nearly saturated with copper sulphamate (Starkey, 1973). Resistance increased markedly with increase nitrogen content of the substrate. Copper tolerance has been demonstrated for two Phialophora species. Members of this genus are among the species most commonly isolated from preservative treated wood in ground contact (Henningson & Nilsson, 1975)

Pentachlorophenol (PCP), a widely used, broad- spectrum preservative has also been reported to be degraded by various fungi. Unligil (1968) found that T. viride, Cephalosporium, and Pullularia pullulans were the most pentachlorophenol-tolerant of the twenty seven fungi tested. The PCP loss was attributed to the fungal activity of T. viride, which colonized the wood without causing weight loss. Trichoderma viride was found to deplete 6.0 percent of the PCP from the samples containing 5.8 kg PCP/m3. Other studies have shown that T. viride, Fusarium sp. and Scytalidium sp. isolated from PCP-treated Douglas-fir poles can tolerate high retentions of this chemical in the outer 0.6 cm zone (Lew & Wilcox, 1981). Dinitrophenol, is more easily degraded by Fusarium oxysporum, probably via a nitro-reductase system similar or comparable to that occurring in mammalian tissues

(Madhosingh, 1961).

Some fungi are naturally tolerant to specific chemicals, although some species such as Cephaloascus fragrans can be adapted to high concentrations of preservatives (Cserjesi, 1967). The ability of microfungi to degrade preservatives is well documented.

While much is known about the tolerance of fungi isolated from preservative treated wood, information on the tolerance of microfungi isolated from fumigant treated wood is not available. Similarly, very little is known on the effects of colonization by preservative-degrading microfungi on subsequent invasion by decay fungi.

C. Associations between microfungi and decay fungi.

The colonization of wood by microorganisms and the interactions which occur between species in a particular piece of wood depend upon the microorganisms adjacent to the wood, the intrinsic characteristics of the wood species and the environmental conditions over the course of decay. The organisms which occupy a particular niche will depend on temperature, moisture and the availability of nutrients.

As microorganisms invade the wood they can destroy the pit membranes, degrade the wood, compete for nutrients, enhance the growth of other microorganisms, and inhibit other organisms (Levy, 1975). There are numerous studies on associations between wood inhabiting microorganisms in wood (Shields & Atwell, 1963; Shigo, 1965; Ricard & Bollen, 1968; Ricard, Wilson & Bollen, 1969; Sharp, 1975; Tanaka, 1983; Morris et al, 1984; Benko, 1986; Bruce & King, 1986).

Among the first wood colonizers are the bacteria, which may

exhibit either synergistic or antagonistic properties. Studies indicate that pioneering bacteria found in sapwood may alter the rate of cellulase activity of decay fungi and interact with wood parenchyma to alter the rate of fungal growth (Shortle, Menze & Cowling, 1978; Levy, 1975).

Groups of bacteria which have the ability to fix nitrogen play an important role in the colonization process. Nitrogen fixation by bacteria has been detected in living white fir (Seidler et al, 1973) and deteriorating wood (Sharp & Millbank, 1973). This process may make nitrogen available for the development and growth of secondary invaders which could ultimately lead to decay of the wood.

Interactions between bacteria and decay fungi isolated from unbleached birch pulpwood showed that fungal growth was usually reduced in the presence of bacteria (Henningson, 1967). The bacteria were believed to produce diffusible substances which inhibited growth of the decay fungi at a considerable distance from the bacterial colony.

Studies on the antagonistic interactions between Actinomycetes and decay fungi have also been reported. As a case in point, Streptomyces sp. produces an antibiotic that inhibits growth of Polystictus sanguineus and Sistotrema brinkmanii (Cavalcante, 1981; Cavalcante & Eaton, 1981).

In addition to the effects of bacteria and Actinomycetes, many microfungi have shown antagonistic properties under laboratory and field conditions. Trichoderma viride, Pestalotia sp., Gliomastix chartarum, Cephalosporium acremonium and a mutant strain of Penicillium humuli isolated from forest soil were consistently antagonistic to

Poria weirii, a destructive root disease of Douglas-fir (Nelson, 1969).

The inoculation of freshly cut pine stumps with a weak pathogen, Phlebia gigantea, has prevented the invasion of Heterobasidion annosum (Risbeth, 1963). This method is now used in the management of pine forests in England (Cook & Baker, 1983). The role of Scytalidium sp. and Trichoderma sp. on the control of internal decay has also been extensively studied. The former produces a substance known as sytalidin, a non-soluble, chemically stable antibiotic that inhibits the attack of wood decay fungi. The antagonistic properties of scytalidin have been proven effective against Poria carbonica and Poria placenta in culture (Ricard & Bollen 1969: Stillwell, Wall & Strunz,1973; Klingstrom & Johansson 1973: Morris, 1980 ; Morris & Dickinson 1981). These two immunizing commensals can also parasitize and control Lentinus lepideus (Ricard, 1973).

Trichoderma sp. releases volatile substances which produce fungistatic effects on Heterobasidion annosum, and both fungistatic and fungicidal effects on two strains of L. lepideus (Bruce et al, 1984). Acetaldehyde was identified as one inhibitory volatile metabolite, while chloroform-soluble antibiotics, trichodermin and peptide antibiotics have also been detected (Dennis & Webster, 1971). Trichoderma was not only found to produce volatile metabolites and antibiotics, but also parasitizes some decay fungi, e.g., Armillaria mellea, Polyporus schweinitzii and Lentinus edodes (Dennis and Webster, 1971). Parasite coiling by the antagonist results in the bursting of some fungal cells, releasing the cytoplasm that eventually serves as a nutrient source for the Trichoderma. This hyphal

interaction, in conjunction with the action of antibiotics and activity of the enzyme systems, enhances the effectiveness of this fungus against decay fungi. Mycoparasitism by Trichoderma has also been demonstrated on some soil fungi (Weindling, 1932).

Prior infection of southern pine sapwood by the mold fungus, T. viride or P. placenta indicated that these organisms were mutually antagonistic (Toole, 1972). Blocks infected with the actively growing mold fungus were not decayed when placed on cultures of P. placenta. When the mold was killed before P. placenta exposure, weight losses were significantly greater than those found when blocks were exposed to the decay fungus alone. These results indicate a definite reduction in decay capability caused by T. viride. In a separate laboratory study, Gliocladium deliquescens inhibited the decay capacity and destroyed the mycelium of Merulius lacrymans, P. gigantea and Stereum sanguinolentum (Kallio & Salonen, 1972).

The early depletion of more accessible nutrients by the primary saprophytes, S. lignicola, Paecilomyces varioti, T. viride, Gliocladium viride, a Penicillium sp. and Mycelia sterilia was shown to hinder the normally rapid process of colonization by Coriolus versicolor, P. hirsutus, P. adustus and two Peniophora spp. (Shields & Hulme, 1972). Nutrient competition may thus represent an important aspect of colonization by secondary organisms.

The above studies indicate that a variety of interactions could occur under particular environmental conditions. The same fungal associations may also occur among fungi that colonize preservative or fumigant-treated wood.

CHAPTER I
Occurrence, Frequency, Morphology and Physiology of Microfungi Isolated from Fumigant Treated Poles

ABSTRACT

The occurrence, frequency, morphology and preservative tolerance of microfungi colonizing untreated or Vapam, chloropicrin, or Vorlex treated Douglas-fir poles were evaluated by culturing increment cores from poles remedially treated 5 and 15 years prior to sampling. The microfungi were generally isolated from the inner segments of the cores removed 15 cm below and at groundline. Scytalidium lignicola and Scytalidum aurantiacum were identified as the most common of the 14 species isolated from poles that were in service 15 years after fumigant treatment. Scytalidium lignicola and Penicillium sp. 1. were the only species isolated from poles treated 5 years before sampling.

Two strains of Trichoderma viride produced cell wall erosion while Oidiodendron tenuissimum produced scattered soft-rot cavities on pine test blocks. None of the test fungi caused severe weight losses on pine test blocks after exposure for 12 weeks.

Cladosporium cladosporioides was the most tolerant fungus to creosote treated media while Scytalidium lignicola and S. aurantiacum were the most tolerant fungi to pentachlorophenol in the media. Survival of the microfungi following exposure to methylisothiocyanate (MIT) and chloropicrin varied. Penicillium sp. #3 was the most tolerant microfungus to MIT while Scytalidium lignicola, two strains of Trichodrma viride, T. polysporum and Oidiodendron tenuissimum were the microfungi most tolerant to chloropicrin.

INTRODUCTION

Internal decay causes substantial damage in preservative treated Douglas-fir transmission poles. This decay, however, can be effectively controlled by the application of volatile chemicals or fumigants (Helsing et al, 1984; Morrell and Corden,1986). Previous studies indicate that the application of Vapam (sodium N-methyldithiocarbamate), Vorlex (20% methylisothiocyanate, 80% chlorinated C3 hydrocarbons) or chloropicrin (trichloronitromethane) can eliminate established decay fungi and protect preservative treated poles from the re-entry of the decay fungi. As a result, service life of remedially treated poles can be extended significantly.

Fumigant treated poles, however, can be colonized by a non-basidiomycetous fungal flora. Microfungi have been consistently isolated from cores taken from the groundline and 30 cm below ground, but these fungi have not been identified (Helsing et al, 1984).

There is limited information concerning the microfungi that colonize fumigant treated wood and their effects on fumigant performance. The declining fumigant levels in previously treated Douglas-fir poles and the impending need to augment previous treatments suggest a need to identify these microfungi and to understand their role in the treated wood.

This study establishes the occurrence, frequency and characteristics of several microfungi in fumigant treated wood. Inherent microfungal ability to degrade wood is evaluated. Tolerance of microfungal isolates to the preservatives creosote and pentachlorophenol, and the fumigants methylisothiocyanate and chloropicrin is also determined.

MATERIALS AND METHODS

A. Frequency and distribution of microfungi in fumigant treated and untreated Douglas-fir poles.

1. Collection of samples - The frequency of Fungi Imperfecti and Ascomycetes in fumigant treated Douglas-fir (Pseudotsuga menziesii (Mirb) Franco) poles was assessed by sampling poles fumigated 5 and 15 years prior to collection of cores. Samples were collected from 18 Bonneville Power Administration transmission poles at the Santiam-Toledo Lines. These poles were initially treated with creosote or pentachlorophenol and remedially treated in 1969 with either Vapam, Vorlex or chloropicrin. One group of three poles had been treated with Vorlex while two groups of four poles were treated with Vapam or chloropicrin and wrapped to retain the chemical. Samples were also collected from four poles which were treated with Vapam (unwrapped). The fungal flora of these poles was compared with that found in three non-fumigant-reated poles.

Samples were also taken from Portland General Electric poles located near Salem, Oregon. These poles were remedially treated in 1978 with Vorlex, 20% MIT in diesel oil and 100% MIT. One group of cores were taken from four poles that were treated with Vorlex. A second group of core samples were extracted from two poles treated with 20% MIT in diesel oil, a third set from two poles treated with 100% MIT and a fourth set were taken from four non-fumigant treated poles.

The poles were sampled by removing 15-cm-long increment cores from four equidistant locations around the pole; at the groundline, 16 cm below ground, 90 cm and 182 cm above-ground. The cores were

individuallly placed in plastic drinking straws, which were closed at one end with masking tape and stapled at the other end. Cores were plated within 24 hours after collection.

2. Preparation and plating of core sections - The outer preservative treated part of the cores was cut off and discarded, the remaining portion was divided into outer, middle and inner zones. Eight 1-2 mm segments were cut from each zone and planted on 2.5 percent malt agar in petri plates. The plates were incubated at room temperature and were observed daily. Fungal mycelium growing out of the wood pieces was subcultured onto fresh media for subsequent identification purposes. There was little difference between isolates from the middle and inner zones and these zones were combined in later analyses.

3. Computation of the relative occurrence of the microfungi - The relative occurrence of the microfungi was computed as follows:

$$\text{Rel. occurrence} = \frac{\text{Number of isolates}}{\text{Total attempted isolations}} \times 100$$

B. Cultural characteristics and identification of the microfungi isolated from the fumigant-treated poles.

1. Cultural characterization - Penicillium species were grown on Czapeck's agar, Czapeck's yeast agar and malt extract agar (Appendix Table I.1), while the rest of the microfungi were grown in malt agar to determine their growth characteristics.

2. Staining procedure - Hyphal characteristics and fruiting structures of the isolates were examined on cultures grown on malt agar and potato dextrose agar.

Fungal morphology was examined by staining mycelia and spores

with lactophenol in cotton blue. Slide cultures were prepared and these cultures were also stained with lactophenol in cotton blue. The nature and arrangement of the spores or conidia were easily observed using this technique. The sections were examined under Leitz and Microstar microscopes.

3. Identification - The isolates were identified using the appropriate literature (Bisby, 1939; Raper & Thom, 1949; Ainsworth, 1961; Barron, 1962; Klingstrom & Beyer 1965; Wang, 1965; Rifai, 1969; Ellis, 1971; 1976; Barnett, 1972; Cole & Kendrick, 1973; Ramirez, 1982).

The fungi isolated from the fumigant treated poles were then grouped into taxa and were characterized for their decay capability, preservative tolerance and fumigant resistance.

C. Ability of the microfungi to cause weight loss in wood.

The presence of microfungi in Douglas-fir heartwood may reflect their ability to degrade the wood. For this reason, the microfungi isolated from the fumigant treated wood were evaluated for their ability to degrade pine blocks in a vermiculite burial test.

1. Preparation of test blocks and decay chamber - One hundred ninety ponderosa pine blocks (Pinus ponderosa) (1.0 x 0.5 x 2.0 cm) were labeled, conditioned and weighed to the nearest 0.001 g. The test blocks were then vacuum impregnated with distilled water. Five g of vermiculite was placed in a 227 ml glass jar and a 1.0 cm square of filter paper was laid on the vermiculite. Two test blocks were then placed on the filter paper and a second filter paper square was put on top of the test blocks. Vermiculite (5 g) was then added to completely

cover the test blocks. A third piece of filter paper was placed on top of the vermiculite and about 35 ml of a nutrient solution was poured into the chambers, which were capped and autoclaved for 20 minutes at 121°C. The nutrient solution contained: NH_4NO_3, 6.0 g; K_2HPO_4, 4.0 g; KH_2PO_4, 5.0 g; $MgSO_4.7H_2O$, 4.0 g; glucose, 2.5 g; 0.001 g of thiamine hydrochloride and distilled water, 1000 ml (Nilsson, 1973).

After cooling, the chambers were inoculated by placing plugs cut from the edges of 7-10 day old cultures of the test fungi on the filter paper. The chambers were incubated for 12 weeks at 28°C. Groups of ten blocks were exposed to each of the 18 microfungi isolated from the fumigant treated poles.

2. Determination of weight loss - After 12 weeks, the blocks were removed and any adhering mycelia was scraped away. Five test blocks were conditioned to constant weight to determine weight loss due to fungal attack, while the rest were fixed in a formalin-aceto-alcohol (FAA) solution (95% ethyl alcohol, 50 ml; acetic acid, 10 ml; formaldehyde, 5 ml; distilled water, 35 ml) for anatomical study.

3. Microscopic examination of wood sections - A series of 20-25 um thick transverse and radial sections were cut from the FAA fixed blocks using a sliding microtome. The sections were stained with 1% aqueous safranin followed by steaming with picro-aniline blue (25 ml saturated aqueous aniline blue in 100 ml saturated picric acid). The sections were then examined for the presence of soft rot cavities or cell wall erosion.

D. Tolerance of the microfungi to toxicants.

The ability of the fungi isolated from the fumigant treated wood

to tolerate preservatives or wood fumigants may help explain their position within the wood. To evaluate this characteristic, the isolates were grown on a 1.25 percent malt agar media amended with various concentrations of creosote or pentachlorophenol.

1. Preparation of creosote or pentachlorophenol/malt agar media - Creosote (marine grade) amended media was prepared at concentrations of 2, 10, 15, 20, 25 and 50 ul/ml of malt agar media, while pentachlorophenol amended media was prepared at 0.001, 0.002, 0.006, 0.0012, 0.031 and 0.062 ug/ml of malt agar medium. The desired amount of pentachlorophenol or creosote was dissolved in 2.5 ml dimethylsulfoxide and added to the malt agar to make 1.0 of liter media. For creosote, the quantities of creosote necessary to produce the desired concentrations were placed in small test tubes, while the pentachlorophenol concentrates were prepared in 25 ml Erlenmeyer flasks. The chemicals were steamed at 100^{o}C for 10 minutes, then mixed together with the malt agar to produce the desired preservative concentration and poured in petri dishes. These plates were cured for 2-3 days to allow vapors to diffuse from the medium.

2. Inoculation procedures - Three 0.5 cm agar plugs cut from the edge of actively growing cultures of the test fungi were planted equidistantly on the creosote or pentachlorophenol media. Each fungus was replicated on three plates per chemical concentration. The plates were incubated at room temperature and radial growth was measured 7 and 14 days after inoculation. The results were compared with the growth of the same fungus on unadulterated malt extract agar.

3. Preparation of test blocks colonized by the microfungi for exposure to fumigant - The effects of methylisothiocyanate or

chloropicrin exposure on the survival of the isolates was determined by exposing steam-sterilized (10 minutes at 100°C) Douglas-fir heartwood test blocks (1.0 x 1.0 x 0.5 cm) to the selected test fungi. The blocks were aseptically placed on agar inoculated with the test fungus and were incubated until they were thoroughly colonized. The blocks were then removed from the plates and exposed to 2 ug of either methylisothiocyanate or chloropicrin per ml of air in a closed desiccator system (Fig. I.1).

The blocks were removed from the dessicator at periods ranging from 2 to 48 hours. Blocks were placed on fresh malt agar plates at the end of each exposure period and were incubated for 2-4 weeks to determine whether the test fungus survived fumigant exposure.

RESULTS AND DISCUSSION

A. Frequency and distribution of microfungi in fumigant treated and untreated Douglas-fir poles.

A total of 14 species of microfungi were isolated from the fumigant-treated poles, while 16 taxa were isolated from the untreated controls (Table I.1). As expected, the untreated poles contained much higher fungal populations than the comparable fumigant-treated controls. The majority of the fungi were isolated from the poles which had been fumigated 15 years earlier, while the more recently treated poles contained only two species (Table I.2). Some cores from the poles treated with fumigants 15 years ago showed some evidence of advanced decay; however, it was not possible to determine when this damage had occurred. There were minor differences in the number and identity of the microflora found in Vapam (unwrapped) and Chloropicrin

Fig. I.1. Set-up of a closed dessicator system used for fumigant exposure.

Table I.1. Occurrence and distribution of microfungi in untreated and Vapam, chloropicrin or Vorlex treated poles as measured by culturing increment cores removed from sites above and below ground 15 years after treatment.

Chemical Treatment	Position	Zone[a]	Frequency by Fungal Species																		
			S. lignicola	S. thermophilum	S. aurantiacum	I. viride str.1	I. viride str.2	I. polysporum	Penicillium sp.1	Penicillium sp.2	Penicillium sp.3	Q. tenuissimum	A. pullulans	P. richardsiae	R. atrovirens	Rhinocladiella sp.2	C. cladosporioides	Cladosporium sp.2	G. phycomyces	Mycelia sterila	Total Isolates
Vapam (unwrapped)	+1.8	0	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
		I	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	4
	+0.9	0	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12
		I	2	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	0	0	17	-	8	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	29
		I	22	-	9	-	-	-	7	2	-	-	-	-	-	-	-	-	-	-	40
	-0.15	0	4	-	4	-	-	-	4	8	-	-	-	-	-	-	-	-	-	-	20
		I	32	-	20	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	54
Vapam (Wrapped)	+1.8	0	2	2	-	-	-	-	4	8	-	-	-	-	-	-	-	-	-	-	16
		I	16	-	9	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	27
	+0.9	0	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	6
		I	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	6
	0	0	29	-	-	6	-	-	-	2	-	-	-	-	-	-	-	-	-	-	39
		I	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31
	-0.15	0	26	2	21	3	4	-	-	3	-	-	-	-	-	-	-	-	-	-	59
		I	15	1	29	12	8	-	-	-	-	-	-	-	-	-	-	-	-	-	65
Chloropicrin (Wrapped)	+1.8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		I	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	+0.9	0	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
		I	-	-	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	8
	0	0	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4
		I	17	-	12	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	33
	-0.15	0	8	-	24	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	35
		I	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Vorlex (wrapped)	+1.8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		I	16	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
	+0.9	0	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
		I	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
	0	0	4	-	4	-	-	-	2	5	10	-	2	-	-	-	-	-	-	-	27
		I	8	4	44	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	60
	-0.15	0	-	-	12	-	-	-	-	-	-	1	4	-	1	-	8	2	8	-	36
		I	5	-	20	-	-	-	-	-	-	12	4	-	8	-	-	7	2	-	58
Untreated Control	+1.8	0	-	-	-	-	-	-	-	4	8	-	-	-	-	-	-	-	-	-	12
		I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
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		I	7	4	17	3	5	8	-	-	4	4	-	19	-	-	8	2	-	8	89
	-0.15	0	31	-	6	4	-	3	-	-	6	-	-	8	-	-	-	2	-	-	60
		I	18	-	12	3	1	-	-	-	8	-	12	-	-	-	8	4	-	-	66
Total Isolations per site	-----	0	130	4	114	16	8	3	17	40	28	5	6	8	1	4	8	6	8	8	414
		I	236	16	184	18	18	8	20	6	12	16	18	19	8	0	16	15	2	0	612
Total Isolations all sites			366	20	298	34	26	11	37	46	40	21	24	27	9	4	24	21	10	8	1026

a-Cores were divided into outer (0-4.0 cm) and inner zones (4.0-12.5 cm).

Table I.2. Occurrence and distribution of microfungi in untreated and Vorlex, 100% and 20% MIT treated Douglas-fir poles 5 years after fumigant treatment.

Chemical Treatment	Position	Zone[a]	Frequency by Fungal Species				
			S. lignicola	*S. aurantiacum*	*Penicillium* sp.#1	*T. viride* str.#1	Total Isolates
Vorlex	+1.8	O	-	-	-	-	0
		I	-	-	-	-	0
	+0.9	O	-	-	-	-	0
		I	-	-	-	-	0
	+ 0	O	-	-	-	-	0
		I	-	-	-	-	0
	-.15	O	3	-	-	-	3
		I	5	-	-	-	5
MIT(100%)	+1.8	O	-	-	-	-	0
		I	-	-	-	-	0
	+0.9	O	-	-	-	-	0
		I	-	-	-	-	0
	+ 0	O	-	-	-	-	0
		I	-	-	-	-	0
	-.15	O	-	-	-	-	0
MIT(20%)	+1.8	O	-	-	-	-	0
		I	-	-	-	-	0
	+0.9	O	-	-	-	-	0
		I	-	-	-	-	0
	+ 0	O	-	-	-	-	0
		I	-	-	-	-	0
	-.15	O	-	-	3	-	3
		I	-	-	-	-	0
Untreated	+1.8	O	-	-	-	-	0
		I	-	-	-	-	0
	+0.9	O	-	-	-	-	0
		I	-	-	-	-	0
	+ 0	O	5	6	26	5	42
		I	10	9	25	7	51
	-.15	O	11	7	29	0	47
		I	17	9	25	0	51
Total		O	19	13	58	5	95
		I	32	18	50	7	107
Grand Total			51	31	108	12	202

a-Cores were divided into outer (0-4.0 cm) and inner zones (4.0-12.5 cm).

treated poles. Except for Penicillium sp. #2, which was only isolated from the former, S. lignicola, S. aurantiacum and Penicillium sp. #1 were isolated from poles treated with Vapam or chloropicrin.

The isolation frequencies from the Vorlex and Vapam (unwrapped) treated poles were 84 to 90 percent, respectively, of those found in the untreated controls (Table I.3). Conversely, poles treated with chloropicrin had only 35 percent of the fungal population found in the untreated controls.

Of the species colonizing the fumigant treated wood, S. lignicola and S. aurantiacum comprised the majority of isolates from pole sections treated 15 years ago. These species were also the common inhabitants of the untreated control poles, suggesting that the treated poles were being recolonized by the naturally occurring microflora. In general, the Vorlex poles appeared to have the most varied microflora, including a Graphium sp. that was not found in the untreated controls.

In addition to the differences in microflora between treated and untreated controls, there were more subtle differences in fungal distribution within individual poles. Penicillium sp. #3 was most frequently isolated from the outermost position of the cores removed 15 cm below ground, while S. lignicola was isolated from the inner portion of the same cores removed from poles treated with Vapam (wrapped). The latter fungus is a common inhabitant of untreated Douglas-fir heartwood, and its presence is not unexpected.

In the older chloropicrin treated poles, microfungi were most frequently isolated from the outer zone below the groundline. This could be explained by the fact that chloropicrin is strongly bound to Douglas-fir heartwood (Goodell et al., 1985), but appears to provide

Table I.3. Summary of the frequency and distribution of microfungi isolated from untreated and Vapam, chloropicrin or Vorlex treated poles 15 years after fumigant treatment.

Chemical Treatment	Attempted Isolations	Number of Isolates	% Relative Occurrence[a]	% Isolation Frequency[b]
Vapam (unwrapped)	495	169	34	61
Vapam (wrapped)	576	249	43	90
Vorlex	576	234	40	84
Chloropicrin	576	98	17	35
Untreated	437	276	63	-
Grand Total	2660	1026	-	-

a-Percentage based on the number of attempted isolations.
b-Percentage based on the number of isolates from the untreated poles.

B. Cultural characteristics and identification of the non-decay fungi isolated from the fumigant treated poles.

Sexual reproductive structures of the microfungi were not observed on the core samples nor were they produced in culture. This could be due to the absence of conditions favorable to the development of sexual spores. Microscopic examination showed the presence of fungal hyphae in the lumen of the wood cells, whose abundance varied with chemical treatment. As expected, sections taken from chloropicrin treated poles contained fewer hyphal cells.

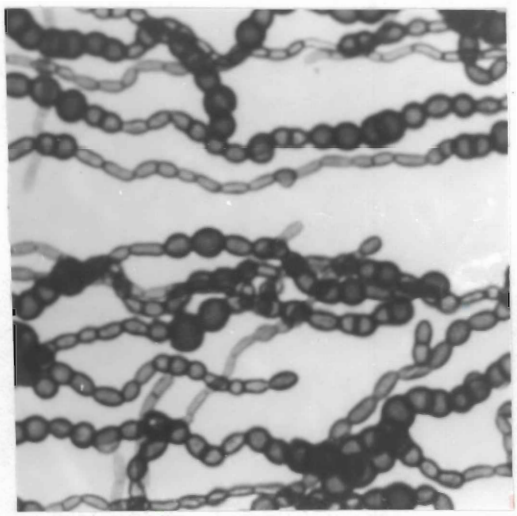
The following are descriptions of the microfungi that were isolated from the fumigant and non-fumigant treated poles.

Scytalidium lignicola Pesante: On malt agar, the immersed mycelium was initially hyaline, then turned gray to black with age. Older cultures developed whitish to yellowish short aerial mycelium, but culture media rarely turned yellow.

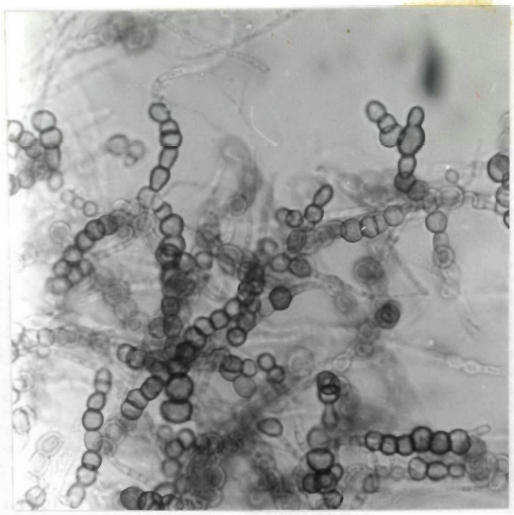
The hyphae were smooth, narrow, cylindrical pale brown to brown, with slightly swollen cells that were separated by thick brown septa (Fig. I.2a). The hyphae were parallel to one another or were closely appressed to form bundles. Younger cells were hyaline, even after 7 days of incubation. The brown conidiophores were branched or unbranched and smooth. The hyphae were 1.48 to 5.8 um wide, while the thick swollen cells were up to 8.8 um thick.

Conidiogenous cells fragmented to form arthroconidia which were mostly determinate, but were commonly intercalary. Conidia were simple, 0-1 septate, thick-walled, hyaline when young, turning brown at maturity and measured 5-6 x 5-10 um.

This isolate conforms with the features characteristic of



(a)



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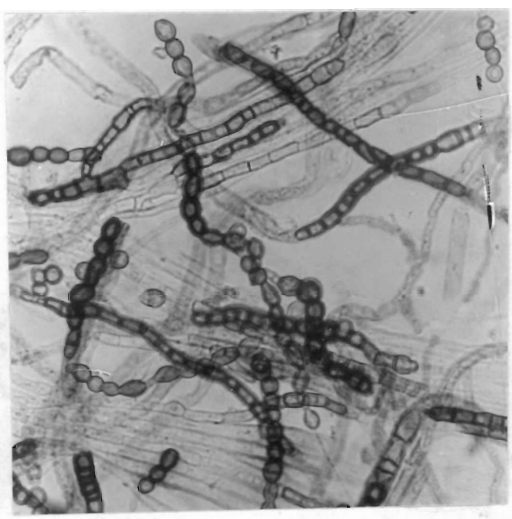
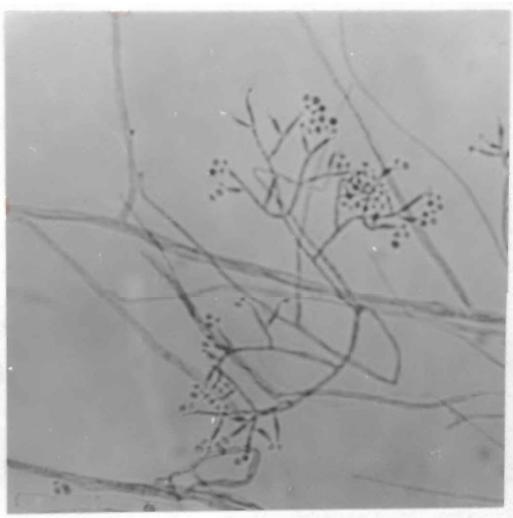


(c)



(d)

Fig. I.2. Microscopic characteristics of: (a). S. lignicola (250X), (b). S. thermophilum (300X), (c). S. aurantiacum (200X), and, (d). T. viride str. #1 (500 X).

S. lignicola as described by Pesante (Ellis, 1971; Klingstorm, 1979).

Perfect state: Unknown

Scytalidium thermophilum (Cooney & Emerson) Austwick: The colonies were at first white turning grey to black with age. The smooth hyphae were hyaline but became black as thick-walled, brown arthrospores developed (Fig. I.2b). Arthroconidia were intercalary or determinate and slightly lighter in color than those produced by S. lignicola.

The hyphae were 2.5-5.2 um in diameter and were slightly thinner when young. Conidia were spherical, smooth, dark brown and 8.8 -13.7 um in diameter. Some conidia were oblong to ellipsoidal.

The isolate fits the description of S. thermophilum as described by Ellis (1976) and Cooney & Emerson 1954).

Perfect State: Unknown

Scytalidium aurantiacum Klingstorm & Beyer: Colonies were flat and bristly. The fungus produced a yellow diffusible pigment on malt agar. With age, the culture turned black as conidia were formed.

The fungus was microscopically similar to S. lignicola (Fig. I.2c). The hyaline hyphae were thin-walled, but later turned black. The yellow pigment still showed through the underside of the darkened plate. Conidia were ellipsoidal, 5.0 -9.0 x 1.6 -2.5 um, while the hyphae were 2.0- 5.0 um in diameter.

Perfect state: Unknown

Trichoderma viride str. #1. Pers. ex S. F. Gray: Colonies grew rapidly on malt agar, covering the petri plates in four days. Colonies were at first translucent white, but turned green as the spores developed and finally became dark green color on the agar surface.

The mycelium consisted of hyaline, smooth, septate and much branched hyphae. Conidiophores arose in compact to loose tufts with the main branch forming two to three or several branches. All the side branches stood at wide angles to the main branch, with their apices terminated by phialides (Fig. I.2d). Phialides arose singly or in opposite pairs along the strand.

The hyaline phialospores were globose, 3.6-5.3 um in diameter with indistinctive minute roughenings. The conidia accumulated at the tip of each phialide to form a globose conidial head.

This isolate conforms with I. viride described by Rifai (1969).

Perfect state: Hypocrea rufa

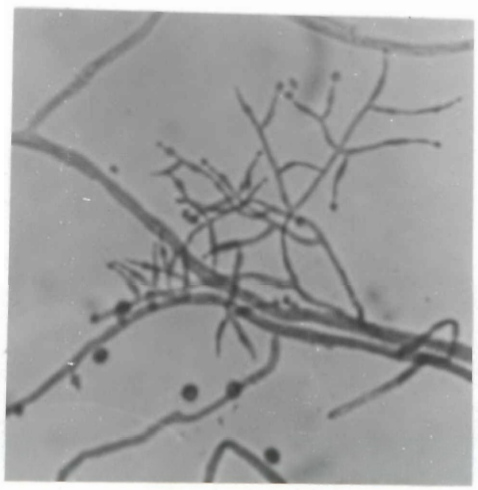
Trichoderma viride str. #2. Pers. ex. S. F. Gray: Fungal growth of this isolate in culture and microscopic characteristics were similar to the above-mentioned strain (Fig. I.3a); however, aggregates of conidia on the surface of the malt agar were much larger.

Perfect state: Hypocrea rufa

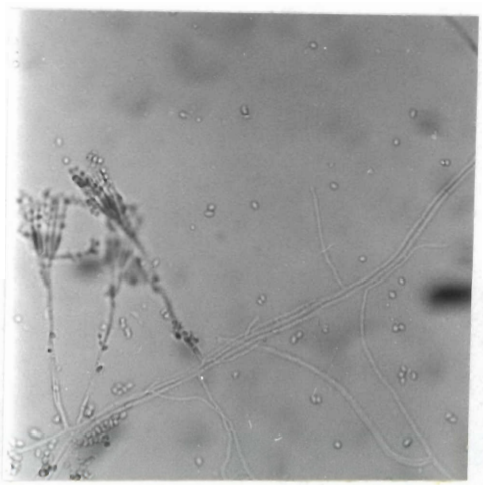
Trichoderma polysporum (Link ex. Pers.) Rifai: Growth of the fungus on malt agar was thin or transparent, with white mycelial strands and white conidial areas.

Hyphae were hyaline, septate, smooth and 1.8-10 um in diameter. Conidiophores arose irregularly and close together. Hyphal elongations were curved and flexuous, with 1-2 sterile branches.

The phialides were 4.0-6.5 x 3-3.5 um, short and almost pear-shaped, being slightly wider above the middle than the base. Phialospores were produced singly and successively from the tips of the phialides to form a slimy conidial head.

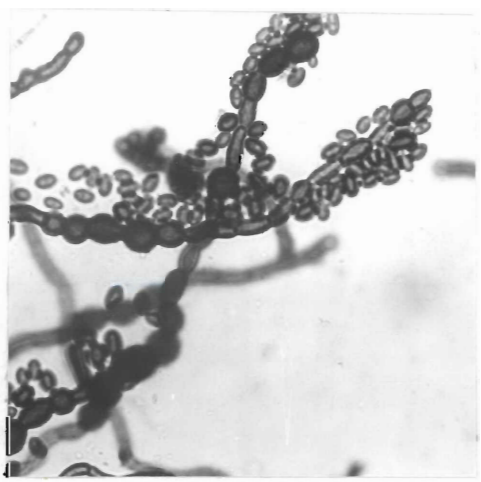


(a)



(b)

(c)



(d)

Fig. I.3. Microscopic characteristics of: (a). I. viride str. #2, (500X), (b). Penicillium sp. #1 (200X), (c). O. tenuissimum (300X), and, (d). A. pullulans (300X).

The spores were colorless, ellipsoidal, smooth-walled and 2.8-3.7 x 1.8-2.0 um.

Perfect state: Hypocrea pilufera Webster & Rifai est.

Penicillium sp. #1: On Czapeck's agar, the slow growing colonies attained a diameter of 14 mm in 5 days. The colonies were composed of loosely textured basal felt with an appressed, white mycelial margin extending 1-2 mm beyond the aerial growth.

Penicilli were biverticillate with three metulae measuring 17.48 um long, producing smooth-walled conidiophores (Fig. I.3b). Phialides were slender, cylindrical and 11.64-15.54 um long. The hyaline conidia were ellipsoidal to sub-globose, measuring about 1.94-3.88 um in diameter, with thin, smooth walls.

The fungus produced abundant blue-green colored spores and a characteristic fragrant odor, but no exudate was detected. An amber-colored pigment diffused in the medium which became purple with age.

Perfect state: Many species of the genus belong to the Eurotiales.

Oidiodendron tenuissimum (Peck) Hughes: Colonies on malt agar produced black mycelial growth with a whitish margin that was evident after five days of incubation. Mycelium was partly immersed, with short black aerial mycelium arising from the center of the colony.

Hyphae were hyaline when young and became gray to light brown at maturity, due to abundant sporulation. Colonies were relatively fast growing with simple, septate vegetative hyphae and conidiophores produced singly along the hyphae. Conidiophores were slender, smooth

and branched into two or more trunks, which were 3.8 x 22.8 um long (Fig. I.3c).

The one-celled hyaline conidia were smooth, globose to ovoid to ellipsoidal, measuring 3.88 x 6.0 um.

It has been reported that the perfect state of the fungus were some unknown basidiomycetes.

Aureobasidium pullulans (de Bary) Arnand: Colonies on malt agar were black with distinct mycelium that radiated from the center of the slimy colony. The hyphae were at first hyaline, but became brown with age and were 2.0-10.0 um thick, reaching 15.0 um in diameter.

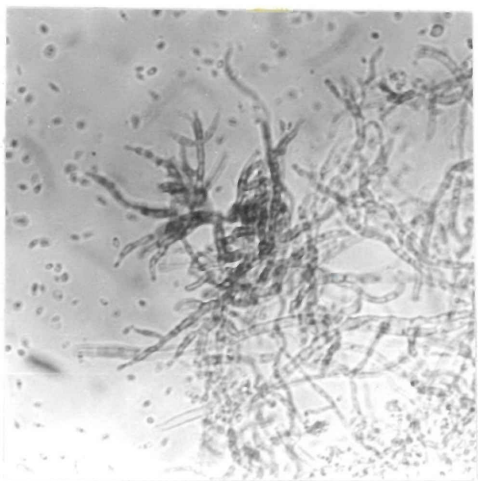
Mycelium was immersed and varied in thickness. Conidiophores were branched, turning brown and thick-walled (Fig. I.3d). The simple, ovoid to ellipsoidal conidia were integrated in a slimy mass of colorless, smooth and non-septate spores measuring 2.0-3.0 x 4.0-6.0 um. Each spore was completely encased in a slimy coat. Intercalary arthrospores measuring 6.0 x 10 um were also produced.

Perfect state: Dothidea acerva Barr.

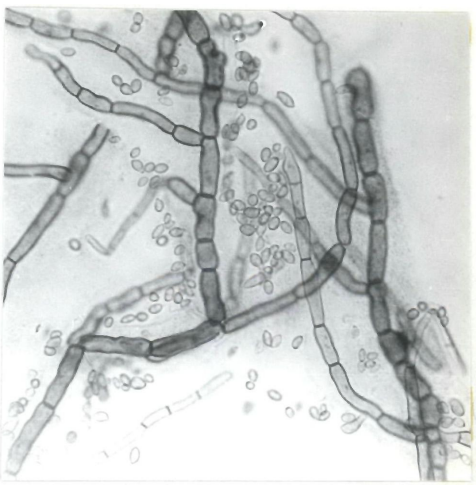
Muellerites Holm.

Phialophora richardsiae (Nannf) Conant: Colonies were creamy white, turning gray to light brown with age. The white mycelium was generally immersed or superficial, but some aerial, white mycelium was observed near the center of the colonies.

The conidiophores were branched, smooth, and hyaline to light brown in color. Phialides (6.0 - 12.0 um long) were produced singly, terminally or along the length of the conidiophore (Fig. I.4a). Collarettes were saucer-shaped and flaring, and a few produced percurrent collarettes.

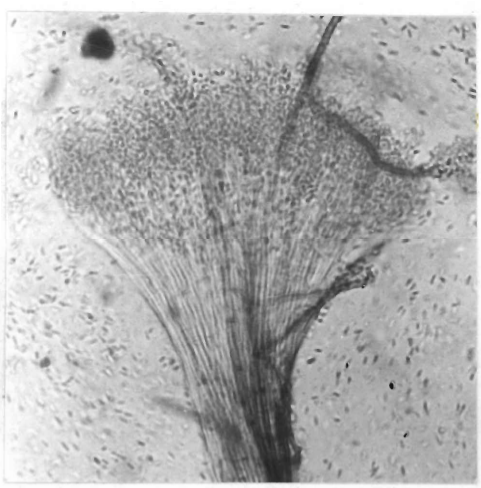


(a)



(b)

(c)



(d)

Fig. I.4. Microscopic characteristics of: (a). P. richardsiae (400X), (b). C. cladosporioides (200X), (c). R. atrovirens (200X), and, (d). G. phycomyces (250X).

The single celled phialospores were hyaline, simple, and hyaline to light brown in color, ellipsoidal or oblong, and were 3.0-6.0 um long.

The isolate conforms with the range of spore sizes and features of P. richardsiae described by Wang (1965).

Perfect state: No sexual stage has been reported.

Cladosporium cladosporioides (Fresen.) de Vries: Colonies on malt agar were olivaceous, hairy at the center and very slow growing. Conidiophores were short, 1.8-5.6 um thick, pale to light brown and smooth (Fig. I.4c). The non-septate conidia were smooth, in chains, ellipsoidal to limoniform and measured 2.5-10.0 x 2.0-4.8 um.

Perfect state: Didymella paecilospora. Other species of the genus has been reported to have some connections with Leptosphaeria, Mycosphaerella, and Venturia.

Cladosporium sp. #2: Colonies on malt agar were slow growing, velvety, hairy and blackish brown in color with short, black aerial and immersed mycelium. Hyphae were brown, with 1.5-3.0 um thick terminal conidiophores. The hyphae had numerous scars. The conidia were ellipsoidal, light brown, smooth, non-septate, and measured 4-7 x 9.0-25 um.

Perfect state: The genus has some connections with Leptosphaeria, Mycosphaerella, Venturia and Dothidea.

Rhinocladiella atrovirens (Nannf): The compact, slow growing colonies were velvety, cushion-like and black, with mycelium that was partly immersed. Short aerial grayish mycelia was also produced.

Conidiophores were flask-shaped to cylindrical with numerous denticles bearing conidia (Fig. I.4c) that were colorless to pale

brown, 1-celled, elliptical to obovate and measured 3.8 -5.6 x 1.2-1.8 um. Conidiophores were pale to mid-olivaceous brown and 2.0-3.0 um in diameter.

Perfect state: Dictyotrichiella mansonii. The genus has some connections with Dictyotrichella.

Rhinocladiella sp #2: The slow-growing colonies were blackish green with short aerial mycelium and a tough, immersed mycelium. Conidiophores were brown in color and scars were present at the end of the brown, 1.6-3.0 um thick hyphae.

The conidia were colorless, ellipsoidal, smooth and rarely 1-2 septate, measured 4.5 -7.0 x 1.6 -2.5 um, with some spores up to 12.0 um long.

Graphium phycomyces (Phialocephala phycomyces) Kendrick: On malt agar, the mycelium of the fungus was white, turning brown with age and accompanied by the production of numerous, erect, short, dark conidiophores, each with a glistening white or buff head of conidia.

The conidiophores were 80-90 um long, composed of unbranched, septate, darkly pigmented cells. The hyaline, unicellular conidia developed at the tip to form a capitate globose head (Fig. I.4d). The conidia were ellipsoidal, hyaline, smooth and measured 0.5-2.5 x 3.8-5.8 um.

Perfect state: The genus has connections with Ceratocystis.

Mycelia Sterilia: The fungus was a very slow grower, producing black, radiating mycelium from the center of the colony. A tuft of short aerial mycelium was also produced at the center of the colony. The smooth, thick-walled hyphae were brown, very long, and 2.0-4.0 um thick (Fig. 4a).

Penicillium sp. #2: On malt agar, the colonies were blue green in color, with a zonate margin and submerged mycelium extending 3 mm beyond the aerial region that was loosely textured with abundant blue green spores. The medium remained unchanged in color. On Czapeck's yeast agar, a dark amber pigment diffused through the medium.

On Czapeck's agar, the colony was radiately wrinkled with a white margin. The reverse was yellow-orange.

The hyphae had smooth walls, enlarged at the tip, with simple penicilli and phialides in clusters of 8-12 with chains of conidia that were globose to subglobose. The conidia were smooth and measured 2.8-3.5 um in diameter.

Perfect state: The genus has been reported to have some connections with Eurotium.

Penicillium sp. #3: Colonies on malt agar were velvety, with abundant yellow spores. On malt extract agar (Ramirez, 1982), colonies were green and the underside of the medium was amber colored.

Conidiophores were smooth-walled with verticills measuring 8-10 x 2-3 um. Hyaline spores were globose to subglobose (2.5-3 um), smooth-walled, and in chains.

Perfect state: The genus has some connections with the genus Eurotium and others.

C. Ability of the microfungi to cause wood weight loss.

Since the microfungi were isolated from the untreated heartwood, it was important to determine if the microfungi isolated from the fumigant-treated poles were capable of causing wood degradation. The results indicated that none of these microfungi caused severe weight

results indicated that none of these microfungi caused severe weight losses on pine test blocks after 12 weeks exposure (Table I.4).

Aureobasidium pullulans and C. cladosporioides did not cause any weight loss, while S. lignicola, S. aurantiacum, Penicillium sp. 1, T. viride st. #1, T. viride st. #2, T. polysporum and Q. tenuissimum caused weight losses ranging from 2.25 to 3.86 percent. Similar results have been obtained in previous tests conducted using Pinus sylvestris (Nilsson, 1973). Weight losses for all of the decay tests ranged from 0.5-7.9 percent, with Phialophora sp. causing the largest weight loss (Nilsson, 1973).

Among the 18 microfungi tested, only the two T. viride strains exhibited erosion patterns (Type 2 soft rot) on the pine test blocks, while Q. tenuissimum produced scattered Type 1 soft rot cavities. The latter species was only present in Vorlex treated poles and its limited presence appears to be of little concern. Trichoderma viride st. #1 was the seventh most common isolate, but was only found in Vapam treated poles, where it colonized the inner zones of the wood.

The tests indicate that most of the microfungi lacked the ability to cause soft rot damage; however, the use of more susceptible wood species such as beech or birch might alter these results (Nilsson, 1973).

D. Tolerance of microfungi to chemical toxicants.

Cladosporium cladosporioides was the most tolerant fungus to creosote, growing at 80 percent of the control at concentrations of 2.0 ul/ml media (Table I.5). The growth rate of C. cladosporioides was quite low after 7 days, but increased after 14 days (Appendix Table

Table I.4. Weight losses produced on Ponderosa pine blocks by microfungi isolated from Douglas-fir transmission poles after 12 weeks of exposure in a vermiculite burial test at 28°C.

Microfungi	Wood Weight Loss[a] (%)	Soft-Rot Attack Pattern[b]
Scytalidium lignicola	3.42	-
Scytalidium thermophilum	3.11	-
Scytalidium aurantiacum	2.59	-
Trichoderma viride str. #1	2.70	2
Trichoderma viride str. #2	2.66	2
Trichoderma polysporum	2.25	-
Oidiodendron tenuissimum	2.58	1
Aureobasdium pullulans	0	-
Phialophora richardsiae	2.19	-
Rhinocladiella atrovirens	1.86	-
Rhinocladiella sp. #2	0.81	-
Cladosporium cladosporioides	0	-
Cladosporium sp. #2	1.23	-
Penicillium sp. #1	3.86	-
Penicillium sp. #2	0.76	-
Penicillium sp. #3	0.51	-
Graphium phycomyces	1.45	-
Mycelia Sterilia	0.30	-

a-Each value represents the average of 5 test blocks.
b-Soft-rot attack can be classified as Type 1 for for formation of cavities in the S-2 cell wall layer or Type 2 for the occurrence of erosion in the wood cell wall.

Table I.5. Ability of microfungi isolated from fumigant treated or untreated Douglas-fir heartwood to grow on malt agar amended with creosote or pentachlorophenol[a].

Microfungus Tested	Fungal Growth (%)[b]											
	Creosote Concentration (ug/ml)						Pentachlorophenol Concentration (ug/ml)					
	2	5	7	10	12	25	0.001	0.002	0.006	0.012	0.031	0.062
S. lignicola	56	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	88	88	88	48	4	c
S. thermophilum	22	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	51	50	26	8	0 (0)	0 (0)
S. aurantiacum	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	100	100	85	82	28	1
I. viride st.1	57	0 (100)	0 (100)	0 (80)	0 (0)	0 (0)	79	72	39	16	0 (0)	0 (0)
I. viride st.2	7	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	100	100	100	51	4	0 (0)
I. polysporum	(100)	41	0 (40)	0 (20)	0 (0)	0 (0)	73	68	39	18	0 (0)	0 (0)
Q. tenuissimum	0 (100)	0 (100)	0 (100)	0 (100)	0 (80)	0 (0)	33	27	13	8	c	0 (0)
A. pullulans	0 (100)	0 (100)	0 (100)	0 (100)	0 (60)	0 (0)	100	100	86	68	0 (0)	0 (0)
P. richardsiae	c	0 (100)	0 (100)	0 (80)	0 (0)	0 (0)	92	88	74	67	15	0 (0)
R. atrovirens	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (20)	94	91	67	18	c	0 (0)
Rhinocladiella sp.2	83	c	c	0 (80)	0 (0)	0 (0)	100	90	67	50	20	0 (0)
C. cladosporioides	80	75	76	68	68	64	50	36	0 (100)	0 (100)	0 (100)	0 (100)
Cladosporium sp.2	0 (100)	0 (100)	0 (40)	0 (0)	0 (0)	0 (0)	92	51	22	16	c	0 (100)
Penicillium sp.1	0 (100)	0 (100)	0 (40)	0 (0)	0 (0)	0 (0)	100	68	24	15	7	0 (0)
Penicillium sp.2	7	0 (100)	0 (100)	0 (100)	0 (40)	0 (0)	85	35	27	11	c	0 (0)
Penicillium sp.3	29	13	11	8	7	0 (0)	82	36	26	13	c	0 (0)
G. phycomyces	21	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	56	47	30	11	0 (0)	0 (0)
Mycelia sterila	25	0 (100)	0 (100)	0 (100)	0 (100)	0 (0)	78	45	30	17	2	0 (0)

a-As compared with growth of the same fungus on malt agar without the toxicant.
b-As a percent of growth on non-amended media. Figures represent average of nine replicates, while values in parenthesis represent percentage of original inoculum plugs which grew when replated.
c-Aerial mycelium grew from inoculum plug, but did not touch the agar surface.

the degradation of creosote by C. resinae, a related species (Marsden, 1954).

Creosote at the 2.0 ul/ml level completely inhibited the growth of S. aurantiacum, A. pullulans, O. tenuissimum, Cladosporium sp. #2, R. atrovirens and Penicillium sp. #1 (Table I.5). All the remaining fungi were able to tolerate creosote at the 2.0 ul/ml level, but were inhibited at higher concentrations. Growth of S. lignicola, S. thermophilum, I. viride. st. #1 and G. phycomyces were 56.0, 22.0, 57.0 and 21.0 percent of the control, respectively at 2.0 ul creosote/ml of media. The rate of growth of S. lignicola was slightly higher after 14 days of incubation. Conversely, the growth rate of I. viride str. 1 was lower on the 2nd than on the 1st week of incubation. Scytalidium thermophilum and G. phycomyces grew after 14 days, but the growth rate was considerably lower than the controls.

The increased growth rate of some microfungi following an additional 7 days of incubation could be due to their ability to detoxify the preservative. Some isolates may produce enzymes which detoxify the toxicant after prolonged exposure to the preservative.

Subsequent transfers of the original inoculum plugs of S. lignicola and S. thermophilum from the creosote-malt agar media to fresh malt agar plates resulted in a 100 percent regrowth of the test fungus indicating that creosote only inhibited these two fungi. Replated I. polysporum inoculum plugs from the plates with 12.5 ug creosote/ml of media failed to grow suggesting that this concentration of creosote was toxic to the fungus. Creosote at 50.0 ug/ml of media was lethal to most of the remaining microfungi, which failed to grow when transferred to fresh malt agar.

Tests on pentachlorophenol toxicity indicated that all the microfungi tolerated this chemical at concentrations ranging from 0.001 to 0.031 ug/ml of culture media. The exception was C. cladosporioides, which was inhibited at 0.006 ug/ml (Table I.5). Subsequent transfer of this fungus to fresh malt agar indicated that pentachlorophenol was only inhibitory at this level.

Pentachlorophenol at 0.031 ug/ml of media was fungistatic to most of the test fungi, preventing the growth of S. thermophilum, T. viride st. #1, T. polysporum, A. pullulans and P. phycomyces. S. lignicola and S. aurantiacum were the most pentachlorophenol-tolerant species, growing at 0.062 ug/ml of media; however, their growth was restricted to the edge of the fungal inoculum. Rhinocladiella sp. #2, was able to tolerate PCP at concentrations up to 0.031 ug/ml of media.

In general, 0.062 ug pentachlorophenol/ml of media inhibited and killed all the test fungi except S. lignicola and S. aurantiacum (Table I.5), which were able to grow back when transferred to fresh malt agar. Cladosporium cladosporioides was inhibited at 0.006 ug pentrachlorophenol /ml of media but subsequent transfers of the original inoculum to fresh malt agar resulted in the re-growth of this fungus.

The tests showed that the microfungi exhibited wide variations in their responses to the presence of creosote and pentachlorophenol. The results also illustrate the relationship between isolation frequency and tolerance to the preservatives. Scytalidium lignicola and S. aurantiacum, two of the most frequently isolated microfungi, showed tolerance to pentachlorophenol, while creosote was inhibitory to both fungi.

Many of the fungi had higher growth rates after 7 days of incubation (Appendix Table I.3). The results showed that 10 of the 18 test fungi had higher growth rates after 14 days than after 7 days of incubation. The growth rate of Oidiodendron tenuissimum was slightly higher on the 14th than on the 7th day when exposed to 0.001 ug pentachlorophenol/ml of media.

The ability of the microfungi to survive exposure to MIT or chloropicrin varied widely (Table I.6). Most of the microfungi were killed after an 8 hour exposure to MIT.

Oidiodendron tenuissimum was very sensitive to MIT, failing to survive 2 hours of exposure to 2.0 ug MIT/ml of air, while Penicillium sp. #3 was the most tolerant, surviving a 36 hour exposure. Scytalidium lignicola, S. aurantiacum and T. polysporum were less tolerant, failing to survive after 24 hours of exposure.

While MIT rapidly controlled most of the microfungi, chloropicrin appeared to require longer exposure periods to inhibit fungal growth. Scytalidium lignicola, both T. viride strains, T. polysporum and O. tenuissimum were the most tolerant, while G. phycomyces was the most sensitive, failing to survive a 2-hour exposure period. These results were similar to previous reports on the tolerance of S. lignicola to fumigants (Zabel et al, 1982: Graham and Corden, 1980). Fungi that are tolerant to fumigants might be capable of colonizing wood where the fumigant levels are declining.

CONCLUSIONS

The majority of the isolates were isolated from the poles which were fumigant treated 15 years earlier, while the more recently treated

Table I.6. Ability of microfungi isolated from fumigant treated or untreated Douglas-fir heartwood to survive exposure to 2 ug methylisothiocyanate or chloropicrin per ml of air at varying time periods.

Microfungus Tested	Fungal Survival (%) by Exposure Time (Hr)																			
	Methylisothiocyanate									Chloropicrin										
	0	2	4	6	8	10	12	24	36	0	2	4	6	8	10	12	24	36	48
S. lignicola	100	100	100	100	100	80	80	0	0	100	100	100	100	100	100	100	100	100	100
S. thermophilum	100	80	20	0	0	0	0	0	0	100	100	100	100	100	100	20	0	0	0
S. aurantiacum	100	100	100	100	100	100	100	0	0	100	100	100	100	100	100	100	0	0	0
T. viride st.1	100	100	80	80	80	0	0	0	0	100	100	100	100	100	100	100	100	0	0
T. viride st.2	100	100	80	80	80	60	0	0	0	100	100	100	100	100	100	100	100	0	0
T. polysporum	100	100	100	100	80	80	60	0	0	100	100	100	100	100	100	100	100	0	0
Q. tenuissimum	100	0	0	0	0	0	0	0	0	100	100	100	100	100	100	100	100	0	0
A. pullulans	100	100	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0
P. richardsiae	100	100	80	0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0
R. atrovirens	100	100	80	0	0	0	0	0	0	100	100	100	40	40	40	0	0	0	0
Rhinocladiella sp.2	100	100	80	40	40	40	0	0	0	100	100	100	100	100	100	20	0	0	0
C. cladosporioides	100	100	100	100	100	100	100	40	0	100	100	100	100	100	100	0	0	0	0
Cladosporium sp.2	100	100	40	20	0	0	0	0	0	100	100	100	100	100	100	20	0	0	0
Penicillium sp.1	100	100	100	100	100	0	0	0	0	100	100	100	100	100	0	0	0	0	0
Penicillium sp.2	100	100	100	100	0	0	0	0	0	100	100	100	40	40	40	0	0	0	0
Penicillium sp.3	100	100	100	100	100	100	100	100	100	100	100	100	100	40	0	0	0	0	0
G. phycomyces	100	100	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
Mycelia sterila	100	100	100	0	0	0	0	0	0	100	100	100	100	100	100	100	0	0	0
L. lepideus[a]	100	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0

a-Values represent percent survival from 5 replicates.
b-Basidiomycete included for comparative purposes.

poles contained only two taxa. The results indicate that the fumigant-treated poles are gradually being recolonized by microfungi.

Of the species colonizing the fumigant-treated wood, S. lignicola and S. aurantiacum comprised the majority of isolations from pole sections treated 15 years before sampling and were also the common inhabitants of the untreated controls poles. Penicillium sp. #3 was the most frequently isolated microfungus from the outermost position of cores removed below ground. Scytalidum lignicola was generally isolated from the inner portions of the cores at groundline and 15 cm below ground.

Among the 18 microfungi that were isolated, none caused severe weight losses on pine test blocks after 12 weeks of exposure. The results suggest that most the microfungi lacked the ability to cause soft rot damage; however, the use of a more susceptible species might alter these results.

Results of tests on the effect of toxicants to the microfungi indicated that there was a direct relationship between isolation frequency and tolerance to preservatives. Tolerant species were generally more frequently isolated than the less tolerant ones.

The survival of microfungi upon exposure to MIT or chloropicrin varied greatly, but longer exposures to choloropicrin were required to inhibit fungal growth of some of the microfungi. The results indicate that fumigant treated poles are recolonized by a microflora which generally do not damage the wood, exhibit some preservative tolerance, and can tolerate some exposure to fumigants. These traits help to explain the position of these fungi in the wood and may provide clues concerning the long term performance of fumigant treated wood.

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CHAPTER II
Interactions of Microfungi Isolated from Fumigant Treated and Untreated Douglas-fir Heartwood

ABSTRACT

The effects of exposure to microfungi on weight loss of fumigant treated or untreated pine blocks following subsequent exposure to Poria carbonica Overnh. or Poria placenta Fr. was evaluated using agar and soil block tests.

Blocks that were inoculated with the microfungi first had lower weight losses than those that were exposed to the decay fungus alone. Weight losses of the fumigant-treated blocks were generally lower than those for untreated blocks. This effect was least noticeable with the lowest Vapam treatment and largest in the chloropicrin treatment.

Results of agar block tests showed that weight losses were significantly reduced in test blocks treated with 0.015 ug Vapam/cc of wood and exposed to Scytalidium lignicola Pes. prior to exposure to Poria placenta. Similar results were obtained with the two strains of Trichoderma viride. Conversely, significant reductions were not obtained in inoculation experiments with Poria carbonica.

In soil block tests, prior exposure of fumigant treated blocks to S. lignicola Pesante, Scytalidium aurantiacum Klinstrom & Beyer, T. viride str. 1 Pers. ex. S. F. Gray, T. viride st. 2 or Penicillium sp. #1 caused significant reductions in subsequent weight losses caused by P. placenta. As in the agar block tests, experiments with these same fungi failed to produce significant reductions in weight loss caused by P. carbonica. Further tests with this fungus using a more aggressive strain may produce more significant interactions.

INTRODUCTION

Wood in ground contact is colonized by a variety of microorganisms, including bacteria and fungi. These organisms alter the properties of the wood, allowing other organisms to invade the substrate and eventually returning nutrients to the surrounding soil. As they invade, these organisms compete, enhance other organisms, or affect each other.

Microbial competition has been exploited in other fields for biological control strategies (Baker and Cook, 1974), but this approach has seen limited application in the protection of wood. Ricard et al. (1967) reported on the ability of Scytalidium strain FY to control Poria carbonica in Douglas-fir heartwood; however, subsequent tests indicated that this fungus was unable to effect complete control (Graham, 1973).

More recently, a commercial formulation containing Trichoderma polysporum and Trichoderma harzianum has been applied to standing Scots pine (Pinus sylvestris) utility poles in Europe. This formulation has produced mixed results, mostly due to the inability of the biocontrol fungi to thoroughly colonize the wood and overcome existing Basidiomycete colonization (Morris et al, 1984).

One method for overcoming the lack of effective control of established fungal infestations is the use of fumigants before the addition of the biological control agents. Wood fumigants represent the most useful chemicals since they have the ability to migrate through normally impermeable wood for distances up to 3 meters from the point of application (Helsing, et al, 1984). A drawback to this approach is the long residual time of many currently registered wood

fumigants in the wood. However, this problem can be overcome by applying the biological agent several years after sterilization and by selecting control agents with high fumigant tolerance.

This report describes the ability of fungi isolated from fumigant treated Douglas-fir heartwood to control P. carbonica and P. placenta in agar and soil block decay tests.

MATERIALS AND METHODS

A. Block preparation.

Douglas-fir (Pseudotsuga menziesii (Mirb) Franco) heartwood blocks (2.0 by 0.3 by 1.0 cm) with the long axis parallel to the grain of the wood were labeled, oven-dried, and weighed (0.001 g). Blocks were then conditioned to 20 to 25 percent moisture content, by placing the blocks on a nylon screen held over water in a closed chamber.

B. Fumigant treatment.

Groups of 90 blocks were selected for treatment with 0.015 and 0.075 ug Vapam/cc of wood or 0.001 and 0.015 ug chloropicrin/cc wood. One ml of each fumigant was placed in a small vial which was introduced into a previously prepared dessicator containing the conditioned test blocks on a nylon screen. Water was placed at the bottom of the dessicator to maintain moisture in the test blocks, which were exposed to the fumigant for 5-7 days. After fumigant exposure, blocks which contained high levels of chloropicrin or methylisothiocyanate (MIT from Vapam) were aerated to reduce chemical levels to the point where fungal colonization could occur. At each aeration time point, selected blocks were removed, placed in 5 ml of the appropriate solvent (ethyl acetate

for Vapam and hexane for chloropicrin), and extracted for 24 hours. The extract was then analyzed for chloropicrin or MIT content determination using a Varian 3700 gas chromatograph equipped with an electron capture detector and a flame photometric detector as previously described (Zahora and Morrell, 1988; Morrell and Scheffer, 1985).

Once the blocks had been aerated to the desired concentrations, they were removed from the dessicator and stored in teflon^{R} sealed jars. The storage period served the dual purpose of insuring that the chemical remained in the wood and allowing equilibration of blocks in the same jar to uniform chemical contents. Additional sets of blocks were left untreated to serve as controls.

The blocks were then used to evaluate the ability of Scytalidium lignicola, Trichoderma viride str. 1, T. viride str. 2, Scytalidium aurantiacum, and Penicillium sp. 1 to prevent weight loss by P. carbonica or P. placenta in agar and soil block tests. These microfungi were isolated from Douglas-fir poles which had been treated with Vapam, Vorlex (20 percent methylisothiocyanate, 80 percent chlorinated C_3 hydrocarbons), or chloropicrin.

The test blocks were exposed to the microfungi in the following sequences using agar and soil blocks tests: (a). Exposure to the microfungus for 4 weeks, followed by exposure to the decay fungus for 4 weeks (microfungus/decay fungus combination); (b). Exposure to the decay fungus for 4 weeks followed by exposure to the microfungus for 4 weeks (decay fungus/microfungus combination); (c). Exposure to the microfungus alone for 8 weeks; and, (d). Exposure to the decay fungus alone for 8 weeks.

C. Agar block test.

1. Exposure "a" - Three petri plates each containing 20 ml of 2.5 percent malt agar were inoculated with the microfungus (first fungus) and incubated until the surface was covered by the test fungus. At that point, glass rods were placed on the agar surface of the petri dishes and seven test blocks were aseptically placed on the rods. The plates were sealed with parafilm to retain moisture and incubated for 4 weeks at 25 C.

At the end of the incubation period, the blocks were removed from the petri plates and scraped clean of adhering mycelium. Five test blocks were weighed after conditioning in an oven at 50°C to determine weight lost due to fungal exposure after the 4 week incubation period. Two test blocks were placed in the appropriate solvent, extracted and analyzed using the previously described gas chromatographic procedures to determine residual fumigant content of the blocks.

Fourteen of the remaining test blocks were surfaced sterilized by briefly passing them over a flame prior to exposure to the decay fungus (second fungus). One half of the blocks were introduced into a plate previously inoculated with P. carbonica and the other one half to plates with P. placenta. These plates were incubated and weight loss was determined at the end of another 4 week incubation period.

2. Exposure "b" - The procedures followed were similar to those used for exposure "a", except that the blocks were first exposed to the decay fungus prior to introduction of the microfungus.

3. Exposures "c" and "d" - Two petri dishes with 20 ml of 2.5 percent malt agar media were inoculated with the microfungus or decay

fungus in the same manner as in exposures "a" and "b". These plates with each set of blocks were incubated at 25°C and at the end of a 4 week incubation period, five test blocks were removed from one of the petri dishes and wood weight loss was determined. The test blocks in the other petri dish were incubated for an additional 4 weeks and weight loss was determined at the end of that incubation period.

D. Soil block test.

Decay chambers were prepared by adding 10 grams of soil to a 112 ml glass jar, then placing a western hemlock (Tsuga heterophylla (Raf.) Sarg.) feeder strip (2.3 x 2.3 x 0.3 cm) on the soil surface, and adding 3-5 ml of water. The chambers were capped and autoclaved for 45 minutes at 121°C. After cooling overnight, the chambers were re-autoclaved for 15 minutes at 121°C.

After cooling, the chambers were inoculated with a 5 mm diameter plug of agar cut from the edge of an actively growing colony of the test fungus. Once the test fungus had covered the feeder strip, the test blocks were individually placed onto the feeder strip and the chambers were incubated for 4 weeks. The sequence of inoculation, weighing, conditioning and extraction of MIT or chloropicrin were similar to the procedures use for the agar plate test.

E. Analysis of results.

The data were subjected to an Analysis of Variance. Mean separation procedures were performed using Scheffe's mean separation procedure at the 0.10 level of significance. A comparison of all treatment groups was also performed using Duncan's Multiple Range Test

(DMRT) at the 0.05 level of significance. The latter was not used in the discussion because the test could only compare 20 treatment means.

RESULTS AND DISCUSSION

A. Agar block test.

As expected, the presence of the fumigants caused reductions in the fungal associated weight losses. All of the test fungi were capable of growth on the blocks, suggesting that the fumigant levels chosen were appropriate for this study (Tables II.1 and II.2). In addition, the fumigants appeared to dissipate over the course of exposure and were not detectable at the end of the test period.

Weight losses caused by P. carbonica and P. placenta were reduced when the test blocks were first exposed to any of the five microfungi. The trend was observed in all the microfungi/decay fungi and decay fungi/microfungi combinations.

Weight losses caused by the microfungi alone ranged from 1.3 to 4.1 percent on the untreated blocks, with lower weight losses in the fumigant treated blocks. These weight losses were significantly lower than those caused by the decay fungi used in the test, which ranged from 12.0 to 14.0 percent for P. placenta and 7.0 to 10.0 percent for P. carbonica. These weight losses were somewhat lower than those normally found in soil block tests, but the exposure period was much shorter.

Weight losses from the agar block tests indicated that Scytalidium lignicola was associated with reductions in the resulting basidiomycete weight losses (Table II.1). Blocks exposed to this microfungus prior to basidiomycete exposure were associated with 50 to

Table II.1. Average percent weight losses of Douglas-fir heartwood test blocks treated with Vapam or chloropicrin prior to exposure to selected microfungi and basidiomycetes in various combinations using an agar plate technique.

Fungal Comb[a]	Weight Loss (%)[b] Vapam[c] 0.015	Vapam[c] 0.075	Chloropicrin[c] 0.001	Chloropicrin[c] 0.015	Control
SL+PC	5.63^{abcdef}	2.09^{abcd}	1.28^{abcd}	1.12^{abc}	6.05^{abcdef}
PC+SL	4.57^{abcdef}	2.74^{abcdef}	1.51^{abcd}	1.24^{abc}	6.63^{bcdefg}
PC	6.22^{abcdef}	4.94^{abcdef}	2.04^{abcd}	1.61^{abcd}	7.02^{cdefgh}
SL+PP	6.20^{abcdef}	4.15^{abcdef}	1.57^{abcd}	1.39^{abcd}	6.94^{bcdefgh}
PP+SL	7.61^{defgh}	6.09^{abcdef}	2.34^{abcde}	2.15^{abcde}	8.61^{fgh}
PP	2.36^{gh}	8.11^{efgh}	8.54^{abcdef}	2.73^{abcdef}	12.92^{h}
SL	4.10^{abcdef}	2.57^{abcde}	0.87^{ab}	0.50^{a}	4.11^{abcdef}
TV1+PC	2.82^{abcd}	2.11^{abc}	1.58^{abc}	1.19^{ab}	6.10^{cdef}
PC+TV1	4.30^{abcde}	3.50^{abcd}	2.46^{abcd}	1.67^{abc}	7.04^{defg}
PC	5.42^{bcde}	3.77^{abcd}	2.59^{abcd}	2.13^{abc}	10.73^{fg}
TV1+PP	5.21^{bcde}	5.21^{abcd}	2.38^{abc}	2.17^{abc}	6.25^{cdef}
PP+TV1	6.00^{cdef}	4.90^{abcde}	3.21^{abcd}	1.80^{abc}	8.92^{efg}
PP	8.89^{efg}	4.92^{abcde}	5.60^{bcde}	2.19^{abc}	11.36^{g}
TV1	2.12^{abc}	1.84^{abc}	2.20^{abc}	0.31^{a}	2.46^{abcd}
SA+PC	1.53^{ab}	1.06^{ab}	0.78^{ab}	0.31^{a}	5.32^{abcde}
PC+SA	1.15^{ab}	1.24^{ab}	1.16^{ab}	0.77^{ab}	6.26^{bcde}
PC	3.21^{abcd}	2.13^{ab}	2.30^{abc}	1.45^{ab}	7.71^{cdef}
SA+PP	1.49^{ab}	1.27^{ab}	1.41^{ab}	0.69^{ab}	8.52^{def}
PP+SA	2.86^{abcd}	2.37^{abc}	1.98^{abc}	1.18^{ab}	9.38^{ef}
PP	5.64^{abcde}	4.05^{abcde}	4.96^{abcd}	1.95^{abc}	12.23^{f}
SA	1.21^{ab}	1.07^{ab}	1.04^{ab}	0.73^{ab}	1.36^{ab}
TV2+PC	2.23^{ab}	1.99^{ab}	2.15^{ab}	1.53^{a}	3.64^{abcd}
PC+TV2	3.29^{abc}	2.82^{abc}	2.29^{ab}	2.21^{ab}	4.71^{abc}
PC	4.12^{abc}	3.42^{abc}	3.18^{abc}	2.42^{ab}	7.04^{abcd}
TV2+PP	2.62^{abc}	2.49^{ab}	3.38^{abc}	2.28^{ab}	5.79^{abc}
PP+TV2	3.36^{abc}	2.28^{ab}	5.88^{abc}	2.65^{abc}	8.25^{bcd}
PP	7.16^{abcd}	4.88^{abc}	8.93^{cd}	2.53^{ab}	13.38^{d}
TV2	2.07^{ab}	1.64^{a}	1.47^{a}	0.90^{a}	3.12^{abc}
PE+PC	2.91^{abcd}	1.07^{a}	1.26^{ab}	0.94^{a}	3.36^{abcd}
PC+PE	2.97^{abcd}	2.46^{abcd}	2.51^{abcd}	2.06^{abcd}	5.62^{abcd}
PC	4.72^{abcd}	3.3^{abcd}	3.10^{abcd}	2.43^{abcd}	8.18^{abcde}
PE+PP	7.25^{abcde}	5.57^{abcd}	3.88^{abcd}	2.84^{abcd}	7.11^{abcde}
PP+PE	7.78^{abcde}	7.22^{abcde}	5.58^{abcd}	3.14^{abcde}	9.25^{bde}
PP	8.73^{bcde}	5.23^{abcd}	7.17^{abcde}	3.27^{abcd}	14.77^{e}
PE	2·26^{abcd}	1.85a^{bcd}	1.62^{abcd}	1.30^{abc}	2.34^{abcd}

a-Abbreviations. SL=S.lignicola; TV=T.viride (st.1 &)2;SA=S. aurantiacum; PE=Penicillium sp.1; PC=P.carbonica; P.placenta.
b-Means followed by the same letter are not significantly different (P=0.10) based upon Scheffe's test.
c-Chemical dosages reported in ug of chemical per cc of wood.

Table II.2. Average percent weight losses of Douglas-fir heartwood test blocks treated with Vapam or chloropicrin prior to exposure to selected microfungi and basidiomycetes in various combinations using a soil block technique.

Fungal Comb[a]	Wood Weight Loss (%)[b] Vapam[c] 0.015	Vapam[c] 0 .075	Chloropicrin[c] 0 .001	Chloropicrin[c] 0 .015	Control
SL+PC	13.12^{abcdef}	6.67^{abcde}	7.97^{abcde}	5.39^{abcd}	13.24^{abcdef}
PC+SL	12.49^{abcde}	10.45^{abcdef}	9.63^{abcde}	7.24^{abcde}	14.81^{abcdef}
PC	14.61^{abcdef}	11.50^{abcde}	11.40^{abcde}	8.98^{abcde}	21.08^{bcdef}
SL+PP	14.84^{abcdef}	11.08^{abcde}	8.20^{abcde}	6.61^{abcde}	16.03^{abcdef}
PP+SL	25.96^{efg}	24.80^{defg}	25.57^{efg}	21.50c^{def}	47.50^{h}
PP	43.86^{gh}	32.46^{fgh}	41.84^{gh}	12.48^{abcde}	49.63^{h}
SL	5.38^{abcd}	2.28^{abc}	1.93^{ab}	0.88^{a}	5.21^{abc}
TV1+PC	5.27^{ab}	2.49^{a}	2.65^{a}	2.15^{a}	6.41^{ab}
PC+TV1	5.60^{ab}	3.20^{a}	3.12^{a}	2.58^{a}	7.78^{ab}
PC	8.68^{abc}	5.26^{ab}	4.22^{a}	3.01^{a}	17.99^{abcd}
TV1+PP	12.54^{abc}	4.25^{a}	3.80^{a}	2.50^{a}	15.93^{abcd}
PP+TV1	34.28^{def}	23.15^{bcde}	12.74^{abc}	8.50^{ab}	34.94^{def}
PP	38.98^{ef}	33.64^{def}	26.97^{cde}	12.90^{abc}	47.90^{f}
TV1	2.31^{a}	1.23^{a}	0.77^{a}	0.60^{a}	3.09^{a}
SA+PC	3.71^{a}	3.01^{a}	3.38^{a}	2.56^{a}	6.08^{ab}
PC+SA	5.27^{ab}	3.82^{a}	4.24^{ab}	3.32^{a}	7.55^{ab}
PC	15.41^{abcde}	5.24^{ab}	8.92^{ab}	5.94^{ab}	35.06^{g}
SA+PP	9.33^{ab}	2.70^{a}	5.54^{ab}	4.97^{ab}	10.73^{abc}
PP+SA	27.74^{efg}	25.48^{cdefg}	19.69^{bcdef}	11.73^{abcd}	34.67^{fg}
PP	36.63^{g}	26.14^{defg}	29.69^{efg}	10.53^{abc}	56.58^{h}
SA	2.62^{a}	2.24^{a}	2.57^{a}	2.03^{a}	2.53^{a}
TV2+PC	5.81^{a}	5.20^{a}	4.60^{a}	3.65^{a}	6.32^{ab}
PC+TV2	6.78^{ab}	5.82^{a}	5.35^{a}	4.79^{a}	9.58^{ab}
PC	9.16^{ab}	7.06^{ab}	6.22^{ab}	5.43^{a}	20.88^{bcde}
TV2+PP	8.53^{ab}	5.94^{a}	5.47^{a}	4.91^{a}	13.61^{abc}
PP+TV2	27.22^{cdef}	25.27^{cdef}	16.02^{bcd}	9.75^{ab}	30.30^{def}
PP	35.69^{f}	27.37^{cdef}	29.51^{def}	33.44^{ef}	61.14^{g}
TV2	4.86^{a}	4.32^{a}	3.50^{a}	2.65^{a}	5.73^{a}
PE+PC	7.88^{ab}	6.07^{a}	6.73^{a}	5.11^{a}	8.97^{abc}
PC+PE	10.13^{abc}	8.92^{ab}	7.32^{a}	6.47^{a}	12.43^{abc}
PC	12.19^{abc}	10.61^{abc}	9.67^{ab}	6.74^{a}	26.61^{bcdef}
PE+PP	37.30^{defghi}	21.06^{abcde}	17.63^{abcd}	7.82^{ab}	42.52^{fghi}
PP+PE	44.84^{fghi}	37.86^{efghi}	33.39^{defgh}	29.02^{cdefg}	45.70^{ghi}
PP	52.23^{hi}	45.98^{ghi}	41.72^{fghi}	33.64^{defgh}	54.60^{i}
PE	4.95^{a}	4.01^{a}	3.94^{a}	2.62^{a}	5.66^{a}

a-Abbreviations. SL=S.lignicola; TV=T.viride (st.1 & 2); SA=S. aurantiacum; PE=Penicillium sp.1; PC=P.carbonica; PP=P.placenta.
b-Means followed by the same letter are not significantly different (P=0.10) based upon Scheffe's test.
c-Chemical dosage reported in ug of chemcial per cc of wood.

75 percent decreases in weight loss by P. placenta and a 10 to 50 percent reduction in weight loss by P. carbonica. As expected, these effects were somewhat diminished when the blocks were exposed to the basidiomycete first. The remaining four test fungi were also associated with reduced weight losses, particularly when the blocks were pre-exposed to the microfungus.

The high variations within individual treatments limited the usefulness of mean separation procedures; however, a significant difference was found in the 0.015 ul Vapam treated test blocks that were exposed to P. placenta alone and those that were exposed to S. lignicola prior to P. placenta inoculation. The two strains of T. viride also caused significant reductions in weight loss in the control blocks, but the differences were not significant in the fumigant treated blocks based upon Scheffe's test for comparison of means. These results suggest that the fumigant has an effect on the interactions occurring between the decay fungi and the microfungi.

None of the treated test blocks contained detectable amounts of MIT or chloropicrin at the end of each exposure period; however, fumigant treated test blocks had lower weight losses than the untreated controls. These results suggest that very minute amounts of the chemical were still present in the wood. Chloropicrin is strongly bound to the wood (Goodell et al., 1985) and remains in the wood for long periods of time (Helsing et al., 1984). Conversely, Vapam does not appear to remain in the wood for extended periods (Helsing et al., 1984) and its decreased inhibition of fungal growth in these tests is not surprising.

All of the microfungi tested are important components of the

microflora present in the fumigant treated Douglas-fir poles. This environment is characterized by a diminished microflora and by the presence of residual volatile and non-volatile fungitoxic compounds. The fungi present in this system appear to have slightly higher fumigant tolerance, which allows them to reinvade the relatively sterile wood prior to other, more common soil fungi.

B. Soil block test.

In general, the soil block test produced higher weight losses than those found with the agar block test (Table II.2). Weight losses ranged from 2.5 to 5.75 percent for the microfungi on untreated wood, while weight losses ranged from 47 to 61 and 17 to 35 percent for P. placenta and P. carbonica, respectively. The latter fungus generally had difficulty colonizing fumigant treated blocks. Poria carbonica is considered to be more of a problem in wood in service, but is much less aggressive than P. placenta in culture. Poria carbonica grew very slowly and was observed to attack just the lower surface of blocks that were in contact with the feeder strip. Weight losses in this study apparently reflect this difference.

As observed in the agar block test, prior exposure to a microfungus resulted in lower basidiomycete weight losses in the fumigant treated blocks. This effect was only significant when P. placenta was challenged by the microfungi and varied with fumigant concentration. Weight losses produced by P. placenta were reduced by 60 to 80 percent by Scytalidium lignicola and 40 to 80 percent by Penicillium sp. Weight loss reductions ranging from 20 to 60 percent were noted for P. carbonica following similar exposures to these

microfungi.

There were significant reductions in weight losses of fumigant treated test blocks exposed to I. viride str. 2 and subsequently exposed to P. placenta. Weight losses were also significantly lower in test blocks treated with 0.015 ul chloropicrin/cc of wood prior to exposure to I.viride str.2/P. placenta than in similar blocks exposed to P. placenta/I. viride str.2 or blocks that were exposed to the decay fungi alone. These reductions were not significant in the other fumigant treated test blocks. The results indicate that the fumigant in the blocks did not adversely affect the activity of the microfungi, which were subsequently able to reduce attack by the decay fungi.

Test blocks treated with both levels of Vapam or 0.001 ul chloropicrin and exposed to either S. lignicola, S. aurantiacum and I. viride str. 1, then exposed to P. placenta had significantly lower weight losses than similar test blocks exposed to P. placenta alone. The results indicate that the inhibitory action of the microfungi may be masked by the presence of the fumigant, indicating that wood/chemical/microflora interactions may help explain long term fumigant performance.

The effect of colonization by the above mentioned microfungi was also evident in the non-fumigant treated control blocks, as shown by the significant reductions in weight loss. However, weight losses were still lower in the treated than in the untreated blocks.

While most of the isolates inhibited the basidiomycetes, Penicillium sp. 1 did not significantly reduce weight losses by P. placenta in the 0.015 ul Vapam/cc treated or untreated blocks. Weight losses were significantly reduced in the other chemical treatments,

suggesting that the fungus was inhibitory to P. placenta, even at higher fumigant concentrations.

The results indicate that prior exposure to the microfungi would minimize, if not completely inhibit, the growth of decay fungi. These results also support the suggestion that antagonistic microfungi may be the first organisms to reinvade fumigant-treated wood. As such, these organisms may help explain the remarkable performance of fumigant treated wood in soil contact. However, these fungi do not appear to be effective in the absence of a conventional preservative barrier. Previous studies have shown that otherwise untreated, Douglas-fir poles treated with fumigants experience extensive surface decay and insect attack (Morrell et al, 1986). The conventional preservative barrier may provide a selective microbial screen that prevents competing microfungi from entering the wood, permitting the antagonist to monopolize the substrate.

The soil provides a ready source of inoculum for reinvasion. Since many decay fungi can tolerate relatively high levels of residual fumigant, it would appear that other factors are functioning to restrict recolonization of fumigant treated wood. Antagonistic microfungi may represent one possible explanation for this retarded basidiomycete recolonization.

The nature of the weight loss reductions associated with the five microfungi can not be deduced from the results of these tests. However, previous studies indicate that fungal inhibition occurs by several mechanisms including; competitive inhibition, production of water soluble toxins, production of volatile inhibitors, or by mycoparasitism (Bruce & King, 1983). The isolates in this study have not been

previously tested. Nevertheless, earlier studies of similar species may shed some light on the possible mechanisms effecting reductions in weight losses by decay fungi.

Both species of Scytalidium that were used in the trials inhibited the activity of both decay fungi. Some Scytalidium species have been reported to produce scytalidin (Ricard et al, 1969: Klingstrom & Beyer, 1965; Ricard & Bollen, 1968; Hulme & Shields, 1972) which has an antibiotic effect over a period of several months. This substance was only released when a Scytalidium sp. strain FY was growing in competition with certain wood decay fungi such as P. carbonica (Ricard, 1969).

Trichoderma viride has been shown to inhibit decay caused by Coriolus versicolor, Bjerkandra adusta, Polyporus hirsutus and two Peniophora spp. This inhibition appeared to be related to an early depletion of more accessible nutrients by the microfungus, hindering the normally rapid process of colonization by the basidiomycete (Hulme & Shields, 1972).

Toole (1969) noted that prior infection of southern pine by T. viride inhibited the development of P. placenta. Conversely, prior infection by P. placenta inhibited the development of T. viride. In the present study, the presence of T. viride inhibited the decay fungi as shown by the significantly lower weight losses of test blocks; however, the microfungus did not cause significant weight losses in the exposed blocks and did not appear to be utilizing large portions of the available substrate.

The antagonistic properties of the Trichoderma species have been attributed to the production of non-volatile antibiotics such as

trichodermin and peptide antibiotics (Dennis & Webster, 1971). Acetaldehyde has also been identified as an inhibitory metabolite. In addition to the soluble antibiotics, Trichoderma coils around the hyphae of some fungi (e.g., Rhizoctonia solani), but does not penetrate the hyphae. Vacuolation, coagulation of the cytoplasm and bursting of the hyphae have been reported on Heterobasidion annosum and Rhizoctonia solani exposed to antibiotic producing Trichoderma strains (Dennis & Webster, 1971).

Residues from Trichoderma have been found to control wood decay by Lentinus lepideus (Bruce & King, 1983). These residues may be able to protect poles, even after active growth and colonization has ceased.

CONCLUSIONS

The results of the tests showed that prior exposure to S. lignicola, S. aurantiacum, T. viride strains #1 and #2 and Penicillium sp. #1 caused noticeable reductions in weight losses by P. carbonica or P. placenta. The effects were significant in the microfungi/P.placenta inoculation experiments using the soil block test.

Both basidiomycetes are important decay fungi in Douglas-fir heartwood, but are very slow to colonize fumigant treated wood. The presence of an antagonistic microflora in the wood may help explain the slow rate of Basidiomycete recolonization.

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GENERAL CONCLUSIONS

A variety of microfungi colonized Douglas-fir poles that were treated with Vapam, chloropicrin or Volrex 15 years prior to sampling. Many of these fungi were also present in untreated wood. Poles which were fumigated 5 years before sampling contained only two species of microfungi.

Only three of the microfungi isolated from the poles were capable of producing soft-rot damage in pine blocks, and none of the fungi produced severe weight losses. These results indicate that the fungi present in the poles are subsisting on non-wood cell nutrients.

Some of the microfungi exhibited tolerance to wood preservatives. Several of the isolates which were tolerant to pentachlorophenol or creosote were frequently isolated near the treated shell, suggesting that preservative tolerance had allowed them to penetrate beyond the treated zone. The fumigant tolerance of the microfungi may, in part, explain their presence in the fumigant treated heartwood.

The ability of several microfungi to inhibit decay by P. carbonica and P. placenta suggests that these fungi may play an important role in long term fumigant performance. These fungi may be particularly important in Vapam treated poles, since this chemical has a relatively short residual time in the wood.

While the nature of the interactions between the microfungi and the Basidiomycetes was not investigated, the results suggest that further studies be undertaken to confirm the results. Ultimately, microfungi could be inoculated in fumigant-treated poles to provide longer, non-chemical protection to the wood.

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APPENDIX

Appendix I.1. Culture media (Ramirez 1982).

Czapeck's Agar	
$NaNO^3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCL	0.5 g
$FeSO_4.7H_2O$	0.01 g
Sucrose	30.0 g
Agar	20.0 g
Distilled water	up to 1000 ml

Malt Extract Agar	
Malt extract	20.0 g
Glucose	20.0 g
Bacto-peptone	1.0 g
Agar	20.0 g
Distilled water	1000 ml

Czapeck's Yeast-Extract Agar	
K_2HPO_4	1.0 g
Czapeck's concentrate	10.0 ml
Yeast extract	5.0 g
Sucrose	30.0 g
Agar	20.0 g
Distilled water	up to 1000 ml

Malt Agar	
Malt extract	25 g
Agar	10 g
Distilled water	1000 ml

Czapeck's Concentrate	
$NaNO_3$	300.0 g
$MgSO_4$	50.0 g
KCL	50.0 g
$FeSO_4$	1.0 g
Distilled water	1000 ml

Appendix Table I.1. Non-decay fungi frequently isolated from Douglas-fir poles in Oregon (Graham & Corden 1980).

Alternaria alternata (Fr.)Keissler

Alternaria sp.

Arthrinium sp.

Aspergillus spp.

Bispora betulina (Corda)Hughes

Cladosporium herbarum Link (Fresen)de Vries

Cladosporium cladosporiodes (Fresen)de Vries

Cladosporium sp.

Epicoccum sp.

Fusarium oxysporum Schlecht. emend. Snyder & Hansen

Geotrichum sp.

Helicosporae sp.

Hyalodendron lignicola Diddens

Hyalostachybotrys sp.

Mycotypha sp.

Pachnocybe feruginea (Sow. ex. Fr.)Berk.

Paeciliomyces varioti Bainier

Penicillium spp.

Periconiella sp.

Pestalotia sp.

Phialophora fastigiata (Lagerb. & Melin.) Conant

Phialocephala dimorphospora Kendrick

Phialophora sp.

Rhinocladiella mansonii (Schol-Schwarz)

Scytalidium lignicola Pesante

Stemphyllum sp.

Trichoderma viride Pers. ex. S. F. Gray

Ulocladium atrum Preuss

Ulocladium sp.

Appendix Table I.2. Growth rate of microfungi exposed to various concentrations of creosote in 1.25% malt agar after 7 and 14 days of incubation at 28°C.

Microfungi	Test Period (Days)	Growth Rate (mm/day)[a] Concentration of Creosote[b] 0	2	10	15	20	25	50
S. lignicola	7	2.7	1.0	0[c]	0	0	0	0
	14	1.9	1.3	0	0	0	0	0
S. thermophilum	7	1.4	0	0	0	0	0	0
	14	0.7	0.5	0	0	0	0	0
S. aurantiacum	7	2.7	0	0	0	0	0	0
	14	2.0	0	0	0	0	0	0
T. viride str. #1	7	2.7	1.5	0	0	0	0	0
	14	d	0.9	0	0	0	0	0
T. viride str. #2	7	2.7	0	0	0	0	0	0
	14	-[d]	0.3	0	0	0	0	0
T. polysporum	7	2.7	2.4	0.8	0	0	0	0
	14	-	1.8	0.8	0	0	0	0
O. tenuissimum	7	1.7	0	0	0	0	0	0
	14	0.8	0	0	0	0	0	0
A. pullulans	7	2.7	0	0	0	0	0	0
	14	1.5	0	0	0	0	0	0
P. richardsiae	7	0.7	+[e]	+	0	0	0	0
	14	1.2	+	+	0	0	0	0
R. atrovirens	7	0.7	0	0	0	0	0	0
	14	0.8	0	0	0	0	0	0
Rhinocladiella	7	0.7	0.5	+	+	0	0	0
sp. #2	14	0.7	0.6	+	+	0	0	0
C. cladosporioides	7	2.1	0.8	0.7	0.7	0.6	0.6	0.5
	14	2.0	2.0	1.9	1.9	1.7	1.7	1.7
Cladosporium	7	1.4	0	0	0	0	0	0
sp. #2	14	1.0	0	0	0	0	0	0
Penicillium	7	1.8	0	0	0	0	0	0
sp. #1	14	0.4	0	0	0	0	0	0
Penicillium	7	1.0	0	0	0	0	0	0
sp. #2	14	0.8	0.1	0	0	0	0	0
Penicillium	7	1.1	0.3	0.2	0.1	0	0	0
sp. #3	14	0.1	0.2	0.1	0.1	0.1	0.1	0
G. phycomyces	7	0.8	0	0	0	0	0	0
	14	1.2	0.4	0	0	0	0	0
Mycelia Sterilia	7	0.7	0	0	0	0	0	0
	14	0.1	0.4	0	0	0	0	0

a-Averge of 9 inoculum plugs.
b-Amount of creosote in ul/ml media.
c-No growth. Creosote was either fungicidal or fungistatic.
d-Plate completely covered with mycelial growth.
e-Mycelium grew only at the edge of the inoculum plug.

Appendix Table I.3. Growth rate of microfungi exposed to various concentrations of pentachlorophenol in 1.25% malt agar after 7 and 14 days of incubation at 28°C.

Microfungi	Test Period (Days)	Growth Rate (mm/day)[a] Concentration of PCP[b] 0.0	.001	.002	.006	.012	.031	.062
S. lignicola	7	2.6	0.6	0.6	0[c]	0	0	+[e]
	14	1.9	1.8	0.9	0.9	0.5	0.2	+
S. thermophilum	7	1.4	0.5	0.5	0.2	0	0	0
	14	0.7	0.6	0.5	0.3	1.8	0	0
S. aurantiacum	7	2.7	1.8	1.7	1.7	1.0	0	0
	14	1.9	2.2	2.3	2.0	2.4	1.1	0.5
T. viride str. #1	7	2.7	1.1	1.1	0.8	0.4	0	0
	14	-[d]	2.6	2.1	1.2	1.0	0	0
T. viride str. #2	7	2.7	2.7	2.7	2.7	0.6	0	0
	14	-	-	-	-	1.7	1.0	0
T. polysporum	7	2.7	2.1	2.0	0.7	0	0	0
	14	d	0.9	0.9	0.9	0.7	0	0
O. tenuissimum	7	1.7	0.3	0.3	0.1	+	+	0
	14	0.8	0.4	0.3	0.1	0.2	+	0
A. pullulans	7	2.7	2.7	2.5	2.2	2.4	0	0
	14	1.5	1.5	0.7	0.6	0.1	0	0
P. richardsiae	7	1.4	1.4	1.5	1.2	1.1	0	0
	14	1.0	0.4	0.2	0.2	0.3	+	0
R. atrovirens	7	0.7	0.3	0.3	0.1	+	+	0
	14	0.8	0.4	0.4	0.4	0.1	+	0
Rhinocladiella sp. #2	7	0.7	0.4	0.3	0.2	0.2	0	0
	14	0.7	0.4	0.4	0.3	0.2	0.1	0
C. cladosporioides	7	2.0	1.1	0.7	0	0	0	0
	14	2.1	0.6	0.5	0	0	0	0
Cladosporium sp. #2	7	1.4	1.1	0.6	0.2	0	0	0
	14	1.0	1.2	0.7	0.3	0.4	+	0
Penicillium sp. #1	7	0.8	0.8	0.5	0.1	0	0	0
	14	0.8	1.0	0.7	0.2	0.2	0.1	0
Penicillium sp. #2	7	1.0	1.0	0.7	0.5	0	0	0
	14	0.8	1.2	0.2	0.1	0.1	+	0
Penicillium sp. #3	7	1.1	1.1	0.5	0.4	0	0	0
	14	1.0	0.8	0.3	0.2	0.2	+	0
G. phycomyces	7	1.2	0.4	0.2	0.1	0	0	0
	14	0.8	1.2	1.1	0.7	0.3	0	0
Mycelia Sterilia	7	0.7	0.8	0.5	0.2	0	0	0
	14	0.1	0.8	0.4	0.3	0.3	0.2	0

a-Average of 9 inoculum plugs.
b-Amount of pentachlorophenol in ug/ml media.
c-No growth. Pentachlorophenol was either fungicidal or fungistatic.
d-Plate completely covered with mycelial growth.

e-Mycelium grew out from the edge of the inoculum plugs.