ROLE OF NON-DECAY FUNGI ON THE WEATHERING OF WOOD

by

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Abstract

In this thesis I hypothesized that the graying of wood exposed outdoors is due to the presence of melanized fungi that are relatively resistant to UV-light. To test this hypothesis I examined the color and chemical changes at wood surfaces exposed to the weather and filtered solar radiation, isolated and identified fungi colonizing wood samples by DNA analysis and microscopy and examined the survival, growth and melanin production of staining fungi under UV, visible or no light. The ability of isolated fungi to decay wood was also tested by evaluating changes in the microstructure, mechanical, viscoelastic and chemical properties of spruce and lime wood incubated with fungi. Finally, I tested a novel non-biocidal approach to reduce the staining of wood by fungi, which employed melanin biosynthesis inhibitors (MBIs). My results support the general hypothesis (above) and reveal that weathered wood surfaces are grayed by the interactive effects of solar radiation and fungal colonization. UV-radiation increased the production of melanin by the fungus most frequently isolated from weathered wood (*Aureobasidium pullulans*), which leads to darker weathered wood surfaces. Decay tests showed that species of *Cladosporium*, *Coniochaeta*, *Epicoccum*, *Lewia*, *Mollisia* and *Phialocephala*, were able to degrade wood tissues. In artificial media, MBIs in combination with UV-radiation blocked the growth of staining fungi, but at wood surfaces MBIs reduced fungal staining irrespective of the type of light that samples were exposed to. I conclude that UV-radiation and melanized fungi interact to influence the color of weathered wood surfaces. Degradation of wood by surface fungi is possible, but the extent of damage probably depends on the presence of conditions that favor microbial decay. Finally, the use of MBIs is a promising approach to control graying of weathered wood surfaces, but further research is required to optimize the treatments and test them outdoors.

Preface

Elements of Chapter 5 were presented at the IRG-Americas Regional Meeting; Guanacaste, Costa Rica; 2008, under the title: “The effects of solar radiation on the fungal colonization and color of weathered wood”. I conducted the experimental research, wrote the manuscript and presented the results at the conference. Co-authors and academic supervisors Dr Philip Evans and Dr Colette Breuil, helped with the experimental design, statistical analyses and edited the final manuscript. The citation for the paper is:

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Dedication

To William "Bill" New

Thanks for sharing your knowledge, wisdom

and your sincere friendship with me

Chapter 1: General introduction

1.1. Introduction

Wood has historically been an important material for construction. Since ancient times it has been favored over other construction materials due to its widespread availability and low cost (Duncan 1963). Even today, with remarkable technological advances in material sciences, wood's aesthetic properties confer advantages which add extra value to its other well known structural and environmentally-friendly credentials. Unfortunately, the aesthetic properties of wood are rapidly lost when it is exposed outdoors. Wood exposed outdoors rapidly interacts with the environment and it is particularly susceptible to surface degradation called 'weathering' (Feist, 1983). Weathering can be defined as 'surface degradation resulting from environmental factors that can permanently change the natural appearance of wood surfaces, decreasing their aesthetic value by producing discoloration, checks and cracks, which are often accompanied by various forms of distortion (cup, twist, etc)' (Feist, 1990; Evans, 2008). The environmental factors responsible for the weathering of wood are: (1) solar radiation, (2) moisture (water in its different states), (3) molecular oxygen, (4) heat, (5) pollutants and (6) microorganisms and insects (Evans 2008). Of the above mentioned factors, solar radiation is the most important factor responsible for chemical changes at weathered wood surfaces. Elevated levels of solar radiation occur at wood surfaces exposed outdoors. For example, on a clear day the amount of solar radiation reaching the earth is approximately 1000 W/m^2. This is composed of 5% (UV radiation), 45% (visible light) and 50% (Infra-red light) (Evans et al. 2005). UV radiation and visible light from

solar radiation are responsible for the depolymerization of lignin, which causes the color of wood to change (yellowing and browning), because unsaturated lignin breakdown products accumulate at the surface of wood (Gellerstendt and Gierer, 1975; Feist and Hon, 1984). Also, photo-depolymerization of lignin affects the integrity of the middle lamella which results in the separation of wood cells and causes micro-checking. Over time micro-checks can develop into macro-checks (Miniutti, 1974; Evans, 2008). Furthermore, UV radiation also depolymerizes cellulose and hemicelluloses creating low molecular weight carbohydrates at wood surfaces (Bourbonnais and Paice 1987; Schoeman and Dickinson 1997; Evans 2008). Hence, UV radiation creates a nutrient rich surface layer in wood exposed outdoors. Such layer is an important food source for a number of microorganisms, especially fungi. Many fungi have been found colonizing weathered wood and metabolizing simple sugars and phenolic photodegradation products (Seifert, 1964; Sell and Wälchli, 1969; Bourbonnais and Paice, 1987; Schoeman and Dickinson, 1997). An important proportion of the fungi colonizing weathered wood are black ascomycetes, which cause the staining of wood surfaces due to the dark pigment (melanin) in their hyphae and spores (Brisson et al. 1996; Chedgy, 2006). The graying of wood by these fungi is one of the defining features of weathered wood (Feist 1990; Evans 2008). Fungi responsible for the staining of weathered wood are often accompanied by other fungi which do not seem to contribute to staining. The role played by these organisms is not clear, but there is some evidence that they may be involved in the decay of wood (Schmidt and French 1976).

The effects of such microorganisms and those of other factors involved in the weathering of wood can be blocked by various treatments. For example, UV absorbers and hindered

amine light stabilizers are commonly added to finishes, such as varnishes, stains and water-repellents (Evans 2008). Fungicides and wood preservatives have long been used to protect wood from fungi and other microorganisms. However, some fungi can grow underneath finishes, and others have shown tolerance to wood preservatives (Savory 1973; Kim et al. 2007). The number of biocides that can be used as wood preservatives has been restricted, and there is a need to develop new ways of controlling the decay and discoloration of wood by fungi (Evans, 2003). Blocking the production of pigments (melanin) inside fungal hyphae might be one way of controlling fungal stains in weathered wood. In addition, blocking of melanin biosynthesis would make fungi more susceptible to the damaging effects of UV radiation, which might eventually kill them.

This research examines the colonization of wood surfaces exposed outdoors by fungi. I seek to understand the effects of fungi on the wood and examine the complex interaction between solar radiation and fungal colonization. I also aim to generate new approaches to eliminate or decrease fungal stains based on the combined effects of UV radiation and inhibition of fungal melanin biosynthetic pathways.

1.2. General Hypothesis

UV radiation is very energetic and harmful to most living organisms (Diffey, 1991; Ranby and Rabek, 1975). Living organisms, including fungi, synthesize melanin to protect themselves from solar radiation and other stressing factors, such as high temperatures and desiccation (Fogarty and Tobin 1996; Butler and Day 1998; Henson et al. 1999). These factors can all be

found at wood surfaces exposed outdoors. Studies have indicated that, at wood surfaces exposed outdoors, the predominant fungal flora is dominated by black moulds (Duncan, 1963; Seifert, 1964; Sell and Wälchli, 1969). These fungi apparently can use the melanin in their hyphae to provide a competitive advantage and prevail at wood surfaces. However, as a result the same fungi cause the staining of wood surfaces exposed outdoors as the melanin in their hyphae and spores stains the first few layer of cells at exposed wood surfaces (Brisson et al. 1996). Such staining decreases the aesthetic and economic value of wood and wood product exposed outdoors, as mentioned above.

Base on this information, the general hypothesis for this Thesis is:

"The graying of wood exposed outdoors is due to the presence of melanized fungi with relatively high resistance to UV light".

The treatments used to block the staining of wood generally focus on killing the staining fungi using biocides, but there has been no research that examines the possibility of reducing staining by blocking the biosynthetic pathway of fungal melanins. Melanin biosynthesis inhibitors (MBIs) are chemical substances produced to interrupt the enzymatic pathway involved in the biosynthesis of fungal melanins (Kurahashi 2001). They are commonly used in agriculture as a foliar treatment to prevent blast rice disease produced by the fungus *Magnoporthe grisea* (Kurahashi 2001). This ascomycete synthesizes dehydroxynaphtalene (DHN) melanin similar to many of the fungi colonizing weathered wood (Bell and Wheeler 1986).

If the general hypothesis of this thesis is correct we should be able to use melanin synthesis blockers as a preservative treatment since blocking melanin production may decrease fungal resistance against UV light, possibly leading to the destruction of staining fungi. One problem with this approach is that the biosynthesis of melanin is complex and can vary from one fungal species to another, and some of the different MBIs have different modes of action (Butler and Day, 1998; Kurahashi, 2001). Hence, individual MBIs may not be effective in blocking melanin biosynthesis in all species.

1.3. Scope and importance

The scope of this thesis is to study the relationship between fungi colonizing and staining weathered wood, and UV radiation within the solar spectrum. I seek to obtain fundamental information on the fungi involved in the weathering of wood and their interactive response to exposure to UV radiation under controlled conditions. Also, I seek to generate a new approach to control the graying of weathered wood based on the use of fungal melanin biosynthesis inhibitors and the sterilizing effects of UV radiation. I also perform fundamental research to isolate and characterize fungi colonizing weathered wood and examine their ability to degrade wood.

The aesthetic disfiguration of wood exposed outdoors significantly decreases the value of wood and wood products. This problem is economically important as illustrated by the problem that the weathering of wood causes for the use of wood for decking. This market is forecasted to reach $6.2 billion per annum by 2014 in USA (Freedonia Group, 2011).

However, statistics show that one third of the decks installed in the USA are replaced after only a few years of service due to weathering of exposed wood surfaces (Amburgey and Ragon, 2008). The cost of replacing such decks could be in excess of US$ 1.5 billion. This generates a negative impression of wood as a building material for outdoors uses, which has led to its substitution by other materials such as wood plastic composites.

My research focuses on fungi colonizing wood surfaces in Vancouver BC, Canada, but the results might be reasonably extrapolated to different regions of earth with similar climate and flora. It is important to note that the research does not encompass other organisms which colonize weathered wood, such as, algae, bacteria and mosses because these organisms do not appear to be involved in the graying of weathered wood.

1.4. Study outline

In this chapter (Chapter 1) the general introduction and rationale for the thesis are presented. Chapter 2 reviews the literature on: (1) the weathering of wood; (2) deleterious effects of UV radiation on wood; (3), biological organisms colonizing weathered wood; (4), fungi colonizing weathered surfaces and their possible effects on wood; (5) effect of UV radiation on living cells; (6), fungal melanins and MBIs. In Chapter 3, the fungi colonizing weathered wood exposed outdoors in Vancouver, Canada, are isolated, identified and characterized. Emphasis is given to the use of molecular techniques (DNA analysis) to efficiently identify fungi. In the following chapter (Chapter 4), the ability of fungi isolated from weathered wood to degrade wood surfaces is studied using several techniques

including examination of the effects of fungi on the mechanical and viscoelastic properties of wood (peak tensile stress, modulus of elasticity, peak stiffness, peak toughness and storage modulus). Chapter 5 examines the effect of UV radiation within the solar spectrum on the staining of weathered wood. Insights into the effect of UV radiation on the color of weathered wood are provided by the results presented in this chapter. Chapter 6 complements the previous chapter because it examines the effect of UV radiation on staining fungi growing on artificial culture media. This chapter also examines how UV radiation influences the production of melanin by staining fungi. The last experimental chapter (Chapter 7) seeks to demonstrate the potential use of melanin inhibitors and UV light as a novel treatment to block the fungal staining of wood surfaces. Promising results of *in-vitro* tests are presented in this chapter. In the final chapter (Chapter 8), I discuss the results of all of the experimental chapters and relate them to the general hypothesis and aims of the thesis. I make conclusions and suggest future research that should be performed to strengthen my findings and conclusions.

Chapter 2: Literature review

This chapter describes the literature on the weathering of wood and the colonization of wood surfaces by fungi that cause the graying of weathered wood. The review focuses on the key literature that is relevant to my thesis.

2.1. Weathering of wood

Weathering of wood is caused by damaging reactions that occur at wood surfaces when they are exposed outdoors. These reactions, which are caused by various environmental factors (mentioned in Chapter 1), permanently change the appearance of wood and decrease its appeal (Figure 2.1).

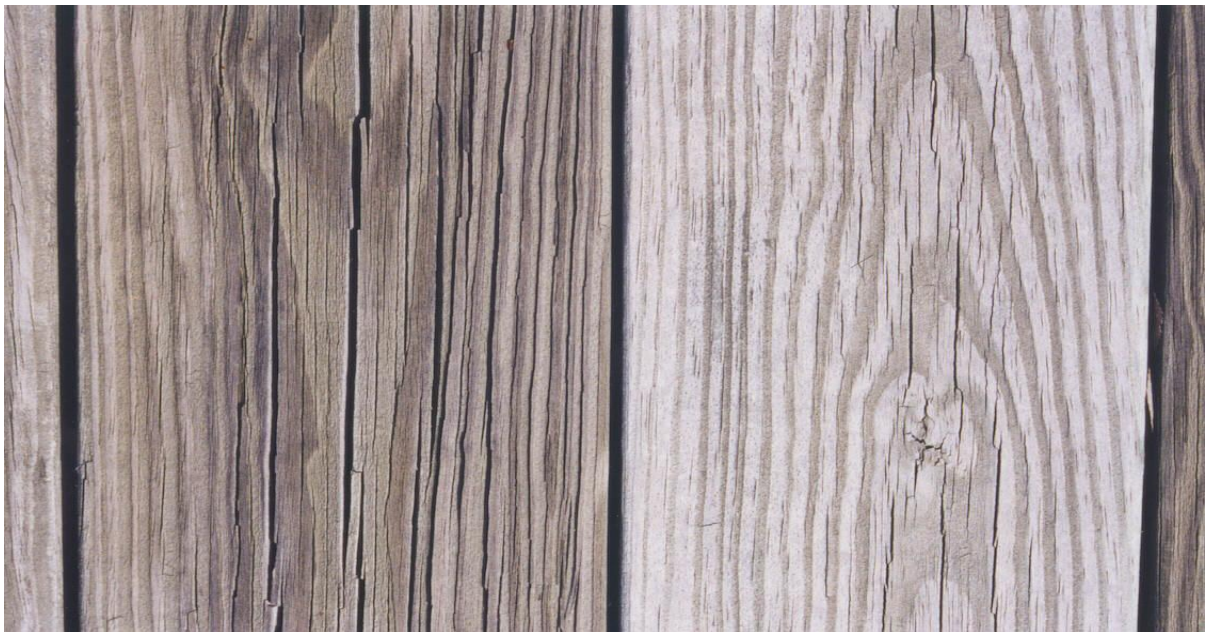


Figure 2.1: Appearance of weathered Southern pine (*Pinus* sp.) wood. Note the graying and surface checking of the wood

Feist (1990) described the changes that occur when wood is weathered as follows: *"During weathering the original surfaces become rough as the grain raises, the wood checks, and the checks grow into large cracks. Boards cup, warp, and pull away from fasteners. Surface color changes, the wood gathers dirt and mildew and becomes unsightly"*. The environmental factors responsible for the weathering of wood are solar radiation, water, molecular oxygen, heat, pollutants, microorganisms and insects (Evans 2008). Solar radiation is the most important factor responsible for the weathering of wood. Solar radiation can be absorbed by all of wood's main structural polymers (cellulose, hemicelluloses and lignin), depending on the wavelength of the incident light (Kalnins, 1966). Wood exposed outdoors also gains and loses moisture, which causes dimensional changes that generate surface and internal stresses, leading to checking and warping (Feist 1990). The swelling of wood by water may also open up inaccessible regions of the cell wall making them accessible to other environmental factors that may increase the depth of weathering, according to Feist and Hon (1984). Water in the form of rain can also wash and leach photodegraded wood products from wood surfaces (Derbyshire and Miller 1981). Molecular oxygen contributes to the weathering of wood as most of the processes related to wood photodegradation are oxidative. Molecular oxygen plays a fundamental role in the formation of peroxy radicals, which is a key step in the photodegradation of lignin and holocellulose (Feist and Hon, 1984). Photochemical reactions are accelerated by heat from solar radiation. Many chemical reactions involved in weathering are increased as temperature increases (Maddock, 1920). Wood surfaces exposed outdoors are also contaminated by dust, smoke particles and volatile pollutants, for example, sulfur compounds (Spedding, 1970; Williams,

1987). Atmospheric sulfur dioxide, in the form of acid rain, may reduce the mechanical properties of wood surfaces exposed in polluted environments (Raczkowski 1980).

A diverse range of fungi, algae, lichens and insects are able to colonize and attack weathered wood surfaces. In most cases the damage is superficial. Nevertheless, most modern studies on the weathering of wood point out that these microorganisms are responsible for the graying and staining of weathered wood (Duncan, 1963; Feist, 1990). However, the precise nature of the damage caused by micro-organisms colonizing weathered wood surfaces has not been fully elucidated. This topic will be examined in greater depth in this literature review. The damage to wood surfaces caused by insects is not described in the literature except for the superficial erosion caused by wasps and hornets that use fragments of weathered wood to make their paper-like nests (Schmolz et al. 2000).

2.1.1. Degradation of wood polymers by solar radiation

Solar radiation degrades wood because it is absorbed by wood's molecular components. The extent of degradation depends on the wavelength of the incident radiation. The critical wavelengths to dissociate the most important bonds in wood are 346, 334 and 289 nm, corresponding to carbon-carbon, carbon-oxygen, and carbon-hydrogen bonds, respectively (Evans 2008). These wavelengths are found in the UV components of solar radiation (Diffey 1991). Thus, UV radiation is the most damaging portion of the solar spectrum. In addition, the violet light component of visible light has sufficient energy to photodegrade lignin, and

it ‘extends photodegradation into wood beyond the zones affected by UV radiation’ (Kataoka et al. 2007).

Lignin is the most sensitive of wood’s polymers to photodegradation (Derbyshire and Miller 1981), but the complex mechanisms involved in the photodegradation of lignin have not been completely clarified. However, the process can be summarized as follows: ‘Lignin, which is an amorphous phenolic polymer, is rich in chromophoric groups that strongly absorb UV light’ (Hon 1979). ‘These groups, including phenolic, double bonds, carbonyls, quinones, quinomethides and biphenyls (Hon 1979), readily interact with UV light to form free radicals’. ‘These radicals react with molecular oxygen to form new radicals such as peroxides, hydroperoxides, peroxyl and alkoxyl radicals’ (Kalnins, 1966). George et al. (2005) noted that the main free radicals resulting from the photodegradation of lignin are phenoxy radicals (Figure 2.2).

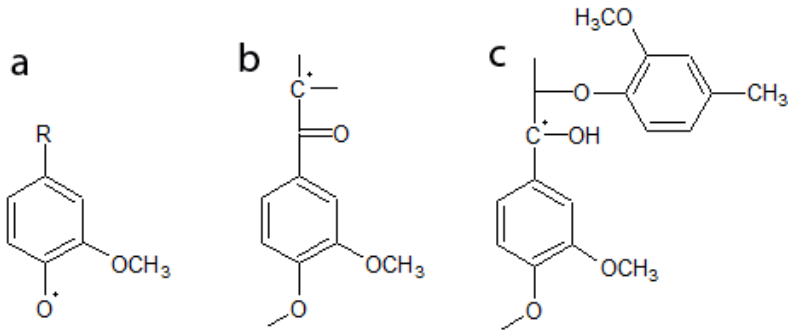


Figure 2.2: Phenoxy radicals produced during photodegradation of lignin. (a) Guaiacoxyl radical; (b) Phenacyl radical; and (c) Cetyl radical

According to their review ‘these free radicals are transformed into quinoid structures which accumulate at wood surfaces causing the first color changes during weathering’ (George et al., 2005). Cellulose seems to be more resistant to weathering than lignin as it is only

sensitive to wavelengths shorter than 280 nm, and the ozone layer prevents such radiation from reaching the earth's surface. However, cellulose is rapidly depolymerized during natural weathering (Derbyshire and Miller 1981; Evans et al. 1996). The depolymerization of cellulose is linked to the formation of aromatic radicals and/or presence of metal ions. In the presence of promoters, such as metal ions and certain dyes, free radicals can be formed even when cellulose is exposed to wavelengths longer than 340 nm (Hon 1975; Feist and Hon 1984). When cellulose in wood is subjected to sunlight, its glycosidic linkages are cleaved causing a loss of strength and degree of polymerization (Derbyshire and Miller, 1981). Hon and Chang (1984) suggested that UV light absorbed by lignin can help to degrade cellulose by energy transfer mechanisms. Nevertheless, cellulose rich surfaces are produced by the photodegradation of lignin at wood surfaces exposed to natural weathering (Feist, 1990)

Hemicelluloses seem to be affected by solar radiation in the much same way as cellulose (Feist and Hon 1984). Hemicelluloses, particularly those containing xylose and arabinose, are depolymerized during weathering and leached from wood surfaces (Evans et al. 1992). Leachates from weathered wood surfaces contain a high proportion of mannose and xylose, suggesting the degradation of galactoglucomannan and arabinoglucoronoxylan, respectively (Evans et al. 1992).

2.1.2. Macro and microscopic effect of weathering

The first visible effects of weathering at wood surfaces are color changes (Feist, 1990; George et al., 2005; Evans, 2008). Color changes at weathered wood surfaces are initially

due to the accumulation of photodegraded lignin fragments in the wood which turns the wood yellow or brown (Gellerstendt and Gierer, 1975; Feist and Hon, 1984). Later, wood starts to turn gray; becoming darker after a few months of outdoor exposure. As mentioned above, the graying and darkening of weathered wood surfaces is attributed to colonization of the wood by staining fungi (Duncan 1963). However, the accumulation of dust and pollutants at wood surfaces also contributes to the graying of weathered wood. Other obvious physical effects of weathering at wood surfaces are the formation of macro-checks and cracks. Checks and cracks are caused by the separation of fibers due to surface and internal stresses resulting from moisture gradients and shrinkage and swelling of inner and outer wood layers (Panshin and De Zeeuw 1980). The photodegradation of lignin also increases the susceptibility of surface layers of wood to check because lignin plays an important role in bonding wood cells together (Evans et al. 2008). Cells at exposed wood surfaces are eroded, but the erosion of weathered wood surfaces is highly dependent on the density of wood (Evans et al. 2005). Thus, the rate of erosion of lower density earlywood is higher than that of latewood. Feist (1983) noted that wood erodes at a rate of 6 to 3 mm per century, for softwoods and hardwoods, respectively.

At the microscopic level the effects of weathering are most noticeable in the middle lamella. The high concentration of lignin in this layer makes it very susceptible to UV radiation (Feist 1990). Damage to the middle lamella can be seen in both transverse and longitudinal sections (Feist 1990). Bordered and half bordered pits are also very susceptible to weathering; and small checks originating from pit apertures have been observed in many weathered softwoods (Miniutti, 1974; Chang et al. 1982; Evans, 1989). Checks in tracheid

walls follow the microfibril angle of the S_2 layer of the secondary wall (Feist 1990). Separation of tracheids and fibers occurs due to erosion of the middle lamella and this, plus the presence of microchecks, causes small sections of cell wall to detach, which produces a progressive loss of integrity of exposed surfaces (Evans, 2008). Thinning and delamination of different cell wall layers can be observed in weathered wood. Thin walled cells, for example epithelial cells in resin canals are more susceptible to weathering than thicker walled cells (Evans, 1989).

2.1.3. Depth of weathering

The depth to which weathering extends into wood is related to how deep light penetrates wood. The depth of color changes in wood exposed to weathering acts as a guide to the depth of penetration of wood by light. Browne and Simonson (1957) described two layers in weathered wood: (1) a gray layer, 125 µm in thickness; and (2) a brown layer ranging from 0.51 mm to 2.54 mm in thickness. UV and visible light are not able to penetrate wood to a depth of 2.54 mm. Hence, Browne and Simonson (1957) explained their observation that weathered wood contained a brown layer up to 2.54 mm deep by stating that free radicals formed in outer layers may migrate deeper into the wood and react with the wood producing color changes. Kataoka et al. (2004) found photo-induced changes in Japanese cedar earlywood exposed to artificial solar radiation to a depth of up to 75 µm. They also found an exponential decrease in light penetration with wood depth, but sufficient photochemically active light was present which could degrade wood at a depth of 700 µm.

2.2. Biological organisms colonizing weathered wood surfaces

A wide range of organisms are able to colonize wood surfaces exposed outdoors. These organisms create a 'biofilm' at wood surfaces which can include, fungi, bacteria and algae (Gaylarde and Morton 1999; Sailer et al. 2010). Algae are a very diverse photosynthetic group of plants lacking roots, leafy shoots and vascular tissues (Hoek et al. 1995). They often disfigure the surface of buildings located in shaded areas with high humidity. Algae growing on surfaces require little nutrients, because they can photosynthesize (Gaylarde and Morton 1999). Coccoid green algae that reproduce by autosporulation are suited to environments found at wood surfaces. For example, the coccoid green alga *Hylodesmus singaporensis* gen. et sp. nov. grows at decayed wood surfaces (Elias et al. 2010). Other algal species found on wood in shaded areas are *Protococcus viridis*, *Chlorococcum* sp., *Hormidium* sp. and *Cyanophyceae* sp. (Ohba et al. 2001). Algae such as *Chlorococcum* sp. and *Amphora* sp. are even able to grow beneath a coat of varnish (de Souza and Gaylarde, 2002). The moisture content at weathered wood surfaces is not always suitable for algae, but they can survive dry periods by developing a symbiotic relation with fungi to form lichens. 'A lichen is an association of a fungus and a photosynthetic symbiont resulting in a stable thallus of specific structure' (Hawksworth and Hill 1984). Around one in five of all known fungi can be 'lichenized', and across the spectrum of lichenizable fungi about 46% of them belong to the phylum ascomycota (Hawksworth and Hill 1984). Little information is available on the colonization of weathered wood surfaces by lichens, but Schmidt and French (1976) described the colonization of weathered shingles exposed in Portland, Oregon, by the lichen *Lecidea granulose* (Hoffm.) Ach. They also discuss whether the

lichenization of *Aureobasidium pullulans* (de Bary) G. Arnaud, one of the most common fungi isolated from weathered wood, might be involved in colonization of wood shingles by lichens. Bacteria can also colonize wood surfaces exposed outdoors.

Bacteria are unicellular prokaryotes, but some forms such as those found in the *Actinomycetes* can form chains of cells and have filamentous forms. Many bacteria are adapted for growth on surfaces and they can rapidly exploit a wide range of energy sources. Some of them are very resistant to environmental extremes (Zabel and Morrell, 1992). Bacteria can be present in sufficient numbers to exert adverse effects on apparently clean surfaces. They are notable for their ability to grow at low concentrations of oxygen. Hence, they can be very active in anoxic wet environments and beneath biofilms formed on surfaces exposed outdoors (Gaylarde and Morton 1999). Several bacterial species can damage wood. For example, *Clostridium xylanolyticum* is able to cause tunneling decay (Zabel and Morrell, 1992). This bacterium produces a xylanase enzyme, which seems to be very active even under anaerobic conditions (Rogers and Baecker 1991). Other members of the genera *Clostridium* can produce cellulase enzymes, which are even more effective at degrading wood (Boutelje and Bravery 1968; Greaves 1971). *Bacillus polymixa* can breakdown pectin in pits and consequently increase the permeability of wood (Knuth and McCoy 1961). Bacteria can attack wood even when it has been treated with preservatives (Singh et al. 1992; Eaton 1994).

Insects can also affect wood exposed outdoors. Insects live in wood or use it as a food source, but in both cases the wood is chewed into small fragments (Zabel and Morrell, 1992). Insects can benefit from the modified characteristic of weathered wood. For

example; termites and wasps frequently excavate weathered wood surfaces. Termites excavate wood by chewing on it, but the digestion of wood is due to the action of enzymes from symbiotic protozoa and bacteria that live in their gut (Breznak and Brune 1994). Termite colonization of wood depends mainly on its moisture content and natural durability (Zabel and Morrell, 1992).

Paper wasps, genera *Polistinae*, and other social wasps, such as yellow jackets and hornets (*Vespinae*), construct paper covers for their nests using weathered or decayed wood. The covers are made by removing and intensively chewing the weathered wood and using saliva as an adhesive (Schmolz et al., 2000). Weathered or rotten wood is preferred by the insects over sound wood. Other insects that attack weathered wood surfaces are carpenter bees and carpenter ants. Carpenter bees excavate galleries in wood to construct their nests. The galleries are used as a depot for eggs, nectar and pollen (Keasar, 2010). Carperter bees generally attack uncoated softwood, but Zabel and Morrell (1992) reported that after weathering almost all wood species were susceptible to attack by carpenter bees. Carpenter ants behave in similar way, excavating galleries in the wood (Hansen and Klotz 2005). In both cases wood is not used as a food source.

2.2.1. Fungi classification

Fungi are very successful at colonizing wood surfaces exposed outdoors, as mentioned above. Fungi are eukaryotic heterothophs belonging to the monophyletic group eumycota (Kendrick, 2000). The fungal body, known as *thallus*, is formed by multicellular filamentous structures called hyphae. Some fungi form a complex net from their hyphae called mycelia.

Other fungi may form yeast (yeast-like fungi) or may grow using both stages (dimorphic fungi) (Kendrick 2000). The hyphal system is adapted to penetrate, externally digest, absorb and metabolize a wide range of organic materials (Zabel and Morrell 1992). A wide range of fungi can colonize wood in trees or when it is used for timber products. Some fungi utilize simple products accumulated in cell lumens, resin canals and parenchyma cells of trees. Other fungi can directly attack the wood's structural polymers producing decay. The extension and type of damage depends on the type of fungi colonizing the wood.

Not all fungi are part of the eumycota kingdom. Certain slime moulds (Phyla: myxosteida, dictyostelida, labyrinthulida, plasmodiophorida) as well as certain chromistan organisms (Phyla: hyphochytriomycota, oomycota) do not belong to the eumycota, but they are still classified as fungi. The main streams of fungi in the eumycota kingdom are part of the phyla: Chytridiomycota, Zygomycota and Dikariomycota. The last phylum includes most of the wood-colonizing fungi in the subphyla ascomycotina and basidiomycotina (Kendrick 2000) (Figure 2.3).

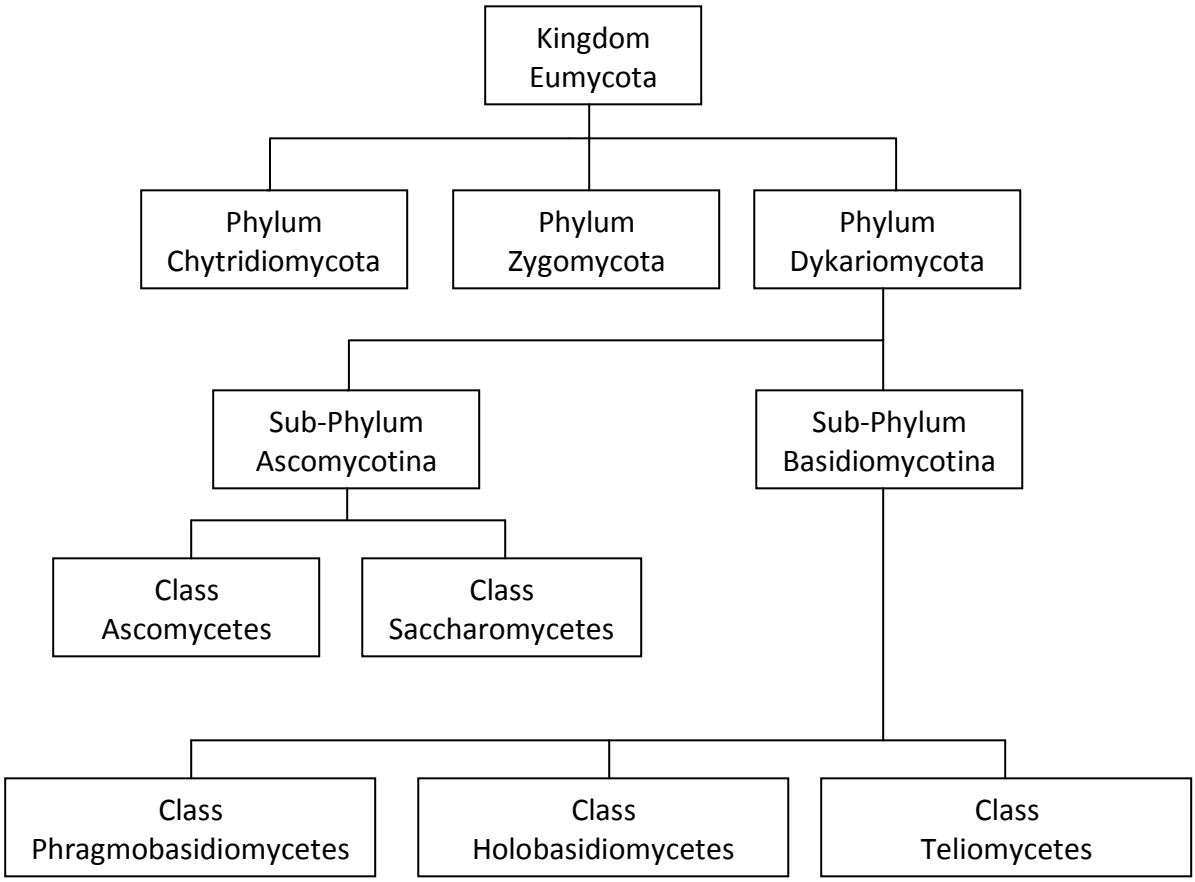


Figure 2.3: Biological classification of true fungi as described by Kendrick (2000)

Fungi can also be classified as decaying or staining fungi. Decay fungi fall into three sub-categories according to the mode of degradation of woody tissues: (1) brown-rot; (2) white-rot; and (3) soft-rot (Zabel and Morrell, 1992). Brown-rot breaks down cellulose and hemicelluloses, but decomposition of lignin is limited (Cartwright and Findlay, 1958; Green and Highley, 1997). Brown-rot rapidly degrades cellulose and the S2 layer of the wood cell wall, but highly lignified wall layers such as the middle lamella appear to be resistant to degradation (Eriksson et al. 1990). Brown-rotted wood is brittle, heavily cracked and powdery (Schwarze 2007). White-rot fungi can degrade lignin as well as cellulose and hemicelluloses.

White-rot fungi are classified into two types that cause simultaneous rot and selective delignification, respectively. In the former, lignin and carbohydrates are degraded simultaneously whereas selective delignification involves removal of lignin from cell walls before the holcellulose is degraded (Zabel and Morrell, 1992).

Soft-rot is different from white and brown rot mainly due to the different way it degrades cell walls layers. Soft-rot is chemically more similar to brown-rot than white-rot, as carbohydrates are decomposed while lignin is only slightly modified (Savory, 1954; Greaves and Levy, 1965; Schwarze, 2007). Soft-rot decay is sub-classified into Type 1 and 2. In Type 1 decay cavities are formed inside the S2 layer of the secondary wall, while in Type 2 discrete notches are eroded in the cell wall layer adjacent to lumens (Zabel and Morrell 1992; Schwarze 2007). Soft-rot fungi require less moisture than basidiomycete fungi (Duncan 1963).

Staining fungi belong predominantly to the sub-phylum ascomycotina, but they include a wide variety of pathogenic and non-pathogenic fungi, plus an important number of moulds. Two groups of staining fungi can be distinguished; (1) sap-staining fungi and; (2) surface staining fungi. Sap-staining fungi can be further classified into pathogenic or non-pathogenic fungi. In both cases fungi develop by metabolizing substances accumulated in the parenchyma cells of trees, logs or unseasoned wood. Fungal staining can extend throughout the sapwood (Zabel and Morrell, 1992; Krokene and Solheim, 1998). Surface staining fungi include a great number of moulds, which colonize wood surfaces creating black and dark stains that only extend few millimeters underneath the wood surface (Duncan 1963;

Dickinson 1971; Savory 1973). These fungi play a predominant role in changing the color of weathered wood to grey (as mentioned above).

2.2.2. Factors affecting fungal survival in wood

Fungal development in wood requires the presence of water, oxygen, moderate temperatures, nutrients, appropriate pH, nitrogen, vitamins and minerals. The moisture content of wood needs to be slightly greater than the fiber saturation point. Free water in cell lumens is a reactant in hydrolysis and a diffusion medium for enzymes. It also solubilizes substrate molecules, and acts as a solvent or wood-capillary swelling agent (Zabel and Morrell 1992). Most fungi are obligate aerobes or in other words they require free oxygen for metabolic reactions (Scheffer 1986). The metabolic activities of fungi, such as digestion, assimilation, respiration and translocation are affected by temperature (Cochrane 1958). Metabolites within the wood in trees are used by fungi to create a wide range of compounds needed for their growth and development, including chitin, glucan, nucleotides, enzymes, proteins and lipids (Zabel and Morrell, 1992). The pH of wood primarily affects substrate availability, rate of exoenzymatic reactions, exoenzyme stability, cell permeability, extracellular components and solubility of minerals and vitamins (Zabel and Morrell, 1992). Nitrogen is required by fungi to synthesize proteins and other cell constituents or products such as nucleoproteins, lipoproteins, enzymes and chitin in hyphal cell walls. Many fungi also require thiamine, as well as phosphorous, potassium, magnesium and sulfur, trace amounts of iron, zinc, copper manganese and molybdenum (Cochrane, 1958; Griffin, 1981; Zabel and Morrell, 1992).

2.2.3. Fungi colonizing weathered surfaces

2.2.3.1. Introduction

The presence of fungi in weathered wood was first noticed by Schacht (1863) and later by Möbius (1924). Both authors described the presence of fungi in wood, but only Möbius attributed the graying of wood surfaces to the presence of fungi. Before Möbius (1924) it was thought that weathered wood became gray due to the accumulation of dirt. Subsequent microscopic studies confirmed Möbius's observations that the graying of weathered wood is almost exclusively the result of growth of dark colored fungi at the wood surface (Duncan 1963; Dickinson 1971).

2.2.3.2. Organisms colonizing weathered wood

The fungi colonizing weathered wood surfaces are moulds, which can grow on most carbon-containing materials including wood, leather, plastic, food and paints. Wood-staining moulds have dark hyphae and spores, but their growth on weathered wood seems to be limited to periods of high humidity or intermittent rain (Kuhne et al. 1970; Hansen 2008). Nevertheless, the surface moulds that colonize weathered wood are capable of withstanding dry conditions and the relatively high temperatures at wood surfaces (Duncan 1963).

The growth of moulds occurs after their spores alight and germinate on wood surfaces. After germination, hyphae, ramify through the wood cells, by penetrating cell lumina,

bordered pits and rays. Hyphae of fungi colonizing softwoods are most prominent in rays and resin ducts. Here the fungi metabolize sugars, starches, resin acids and hemicelluloses for growth. The walls of the ray parenchyma and epithelial cells surrounding resin ducts are often destroyed, leaving elongated open channels that increase the permeability of the affected wood. This effect may contribute to pronounced fluctuations in the surface moisture content of wood (Duncan 1963).

Fungi colonizing weathered wood have been isolated and identified by several researchers. Sell and Wälchli (1969) isolated *A. pullulans, Macrosporium sp., Tetracoccosporium sp., Cladosporium sp.* and *Sclerophoma sp.* from weathered wood in the late 1960's. However, *A. pullulans* was isolated from weathered wood before this by Seifert (1964). Subsequently, Dickinson (1971) isolated a range of mould fungi from Scots pine (*Pinus sylvestris* L.) and Western red cedar (*Thuja plicata* Donn ex D.Don), in England and Sweden. The main species he isolated were *A. pullulans*, *Cladosporium sp*., *Alternaria sp*., *Stemphylium sp*. and *Torula sp*. Later, and based on more isolations, he pointed out that *A. pullulans* was the main fungus responsible for the graying of weathered wood. More recent studies have observed that *A. pullulans* also frequently colonizes painted wood surfaces (Amburgey, 1974; Schmidt and French, 1976; Bardage and Bjurman, 1998). The frequent isolation of *A. pullulans* from weathered and painted wood surfaces seems to be related to its ability to metabolize photodegraded lignin product from weathered wood surfaces and also its capacity to withstand desiccation and high temperatures (Park 1982; Schoeman and Dickinson 1996; 1997). These characteristics may give it an advantage over many other moulds that colonize wood surfaces. The ubiquitous colonization of wood by moulds is also clearly related to

their successful modes of propagation. According to Hansen (2008) airborne conidia are easily carried by the wind for long distances, even from one continent to another. Thus, spores are abundant everywhere in the world. Therefore the successful colonization of a newly exposed wood surface will largely depend on the substrate and its surface microclimate. Mould fungi are able to colonize wood surfaces even in an extreme climate like that in Antarctica. For example, four species of soft rot fungi, *Candophora sp*., *Cladosporium sp., Hormonena dematioides, sp., Lecythophora hoffmannii and Penicillium sp.* were isolated from a 40+ years old wood structure at New Harbor, Antarctica by Held et al. (2006). More recently fungal diversity on weathered western red cedar fences and decks exposed in Vancouver, Canada, was examined by Lim et al. (2005; 2007). They isolated a wide range of basidiomycetes and ascomycetes. The ascomycetes they isolated were *Oidiodendron griseum*, *Rhinocladiella atrovirens*, 2 species of *Sporothrix*, several species of *Phialophora*, *Acanthophysium lividocaeruleum*, *Coniophora puteana*, *Dacrymyces stillatus, Hyphoderma praetermissum, Pachnocybe ferruginea, Phellinus ferreus, A. pullulans, Exophiala heteromorpha, Phialocephala dimorphospora, Rhinocladiella atrovirens, and Umbelopsis autotrophica.* An earlier study isolated *A. pullulans*, *Cladosporium* spp., *Oidiodendron* spp., *Penicillium* spp., *Phialocephala* spp., *Raffaelea* sp., *Rhinocladiella* spp., *Sepsonema* sp., *Sporothrix* spp., *Trichoderma* spp., from weathered Western red cedar shingles and shakes (Smith and Swann, 1976).

A comprehensive review of fungi isolated from wood surfaces exposed outdoors (above the ground) around the world shows that the most frequent fungus isolated from weathered wood is *A. pullulans* (Table 2.1). This fungus is followed, in decreasing order of importance,

by species of *Cladosporium*, *Penicillium*, *Phialocephala, Alternaria, Curvularia, Fusarium, Nigrospora, Rhinocladiella, Sporothrix,* and *Trichoderma*. All these organisms have been isolated from virtually all continents (excepting Africa for which data are not available) from durable and non durable wood species and in some cases from preservative treated wood. However, the review also indicates that other fungi are able to colonize weathered wood. Such fungi have only been isolated once or twice but they are a highly diverse group of microorganisms distributed across at least 46 genera.

Table 2.1: Fungi isolated from wood surface exposed outdoors above the ground. The table also reports the author, substrate and country of isolation. Question mark (?) is featured when information was not available

Isolate	Author; substrate; country
A. pullulans	Sell and Walchli (1969); ?; ?
	Dickinson (1971); Scots pine, WRC; England
	Lim et al. (2005; 2007); WRC; Vancouver-Canada
	Kim et al. (2007); treated radiata pine; Korea
	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
	Amburgey (1974); asphalt shingles (wood based); USA
	Schmidt and French (1976); lauan, cedar and redwood; USA
	Hansen (2008); ?; USA, Thailand, Brazil
	Smith and Swann (1976); WRC; USA, Vancouver Canada
	Doi and Horisawa (2001); sugi; Japan
Acanthophysium lividocaeruleum	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Acremonium sp.	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Alternaria spp.	Dickinson (1971); Scots pine, WRC; England
	Amburgey (1974); asphalt shingles (wood based); USA
	Hansen (2008); ?; Germany, Malaysia, USA, Thailand, Brazil
	Doi and Horisawa (2001); sugi; Japan
Arthrinium sp.	Doi and Horisawa (2001); sugi; Japan
Aspergillus spp.	Amburgey (1974); asphalt shingles (wood based); USA
	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Brachysporiella sp.	Sudayani et al. (2002); Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Candophora sp.	Held et al. (2006); ?; Antarctica
Cladosporium spp.	Sell and Walchli (1969); ?; ?
	Dickinson (1971); Scots pine, WRC; England
	Held et al. (2006); ?; Antarctica
	Kim et al. (2007); treated radiata pine; Korea
	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
	Hansen (2008); ?; Germany, Malaysia, USA, Thailand, Brazil
	Smith and Swann (1976); WRC; USA, Vancouver Canada
Coniophora puteana	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Curvularia spp.	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
	Amburgey (1974); asphalt shingles (wood based); USA
	Doi and Horisawa (2001); sugi; Japan
	Hansen (2008); ?; Brazil

Isolate	Author; substrate; country
Dacrymyces stillatus	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Epicoccum sp.	Doi and Horisawa (2001); sugi; Japan
Exophiala heteromorpha	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Fumago sp.	Amburgey (1974); asphalt shingles (wood based); USA
Fusarium spp.	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia Amburgey (1974); asphalt shingles (wood-base); USA Hansen (2008); ?; Brazil
Fusicladium sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Geotrichum sp.	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Gliomastix sp.	Doi and Horisawa (2001); sugi; Japan
Hormonema dematioides	Held et al. (2006); ?; Antarctica
Hyalodendron sp.	Kim et al. (2007); treated radiata pine; Korea
Hyphoderma praetermissum	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Lecythophora hoffmannii	Held et al. (2006); ?; Antarctica
Macrosporium sp.	Sell and Walchli (1969); ?; ?
Melasmia sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Monilia sp.	Sudayani et al. (2002); Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia Amburgey (1974); asphalt shingles (wood-base); USA
Monochaetia sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Mucor sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Nectria sp.	Doi and Horisawa (2001); sugi; Japan
Neurospora spp.	Doi and Horisawa (2001); sugi; Japan Sudayani et al. (2002); Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Nigrospora spp.	Doi and Horisawa (2001); sugi; Japan Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia Hansen (2008); ?; USA
Oidiodendron spp.	Smith and Swann (1976); WRC; USA, Vancouver Canada Lim et al. (2005; 2007); WRC; Vancouver-Canada
Pachnocybe ferruginea	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Paecilomynes sp.	Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia
Penicillium spp.	Held et al. (2006); ?; Antarctica Kim et al. (2007); treated radiata pine; Korea Sudiyani et al. 2002; Albizia, kapur, Mahoni, Nangka, Puspa; Indonesia Amburgey (1974); asphalt shingles (wood-base); USA Hansen (2008); ?; Germany Smith and Swann (1976); WRC; USA, Vancouver Canada

Isolate	Author; substrate; country
Pestalotia sp.	Doi and Horisawa (2001); sugi; Japan
Phellinus ferreus	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Phialocephala spp.	Lim et al. (2005; 2007); WRC; Vancouver-Canada
	Smith and Swann (1976); WRC; USA, Vancouver Canada
	Kim et al. (2007); treated radiata pine; Korea
	Lim et al. (2005; 2007); WRC; Vancouver-Canada
Phoma spp.	Hansen (2008); ?; Malaysia, Thailand, Brazil
	Kim et al. (2007); treated radiata pine; Korea
Pithomyces spp.	Amburgey (1974); asphalt shingles (wood-base); USA
Pithomyces spp.	Doi and Horisawa (2001); sugi; Japan
Raffaelea sp.	Smith and Swann (1976); WRC; USA, Vancouver Canada
Rhinocladiella spp.	Lim et al. (2005; 2007); WRC; Vancouver-Canada
	Smith and Swann (1976); WRC; USA, Vancouver Canada
Sclerophoma sp.	Sell and Walchli (1969); ?; ?
Scolecobasidium sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Sepsonema sp.	Smith and Swann (1976); WRC; USA, Vancouver Canada
Sordaria sp.	Doi and Horisawa (2001); sugi; Japan
Sphaeropsis sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Sporothrix spp.	Lim et al. (2005; 2007); WRC; Vancouver-Canada
	Smith and Swann (1976); WRC; USA, Vancouver Canada
Stemphylium sp.	Dickinson (1971); Scots pine, WRC; England
	Hansen (2008); ?; Germany
Tetracoccosporium sp.	Sell and Walchli (1969); ?; ?
Thielaviopsis sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Torula sp.	Dickinson (1971); Scots pine, WRC; England
	Amburgey (1974); asphalt shingles (wood-base); USA
Trematisphaeria sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Trichocladium sp.	Amburgey (1974); asphalt shingles (wood-base); USA
Trichoderma spp.	Amburgey (1974); asphalt shingles (wood-base); USA
	Smith and Swann (1976); WRC; USA, Vancouver Canada
	Kim et al. (2007); treated radiata pine; Korea
Umbelopsis autotrophica	Lim et al. (2005; 2007); WRC; Vancouver-Canada

2.2.3.3. Effects of surface fungi on wood

The growth of moulds at wood surfaces can produce a range of colors, including black, gray, green, purple and red. Heavy colonization of wood surfaces by mould can also produce characteristic mould-like odors, and their spores represent a potential cause of allergies (Zabel and Morrell, 1992). A number of moulds have the ability to attack pit membranes, and this effect of moulds on the structure of wood has been used to develop biological treatments to increase the permeability of difficult-to-treat wood species (Schulz, 1956). Others moulds are antagonist to decay fungi (Hulme and Shields, 1972) and others can detoxify wood preservatives (Brown, 1953). Some moulds isolated from weathered wood can also cause soft-rot decay. Such fungi include *Alternaria sp.*, *Phialophora sp., Lecythophora hoffmannii, Coniochaeta ligniaria, Phoma sp., Aspergillus sp., Penicillium sp., Trichoderma sp.* (Savory 1954; Rajderkar 1966; Bugos et al. 1988; Zabel and Morrell 1992; Lim et al. 2007). In fact this phenomenon was observed in weathered western red cedar shingles over 30 years ago by Smith and Swann (1976). *A. pullulans* is able to depolymerize carbohydrates and previous studies have shown that it can cause weight losses of 7% and 3-4% when grown on cellulose and hemicelluloses, respectively (Seifert, 1964). In addition, *A. pullulans* exhibits cellulase, polygalacturonase, pectinesterase and laccase activity suggesting that it is capable of attacking carbohydrates directly in lignified cell walls (Dickinson 1971). Indirect evidence of the ability of moulds to degrade wood is available from a study carried out by Merrill et al. (1965). They examined the effects of common moulds on fiberboards, and found that most of the moulds caused strength and weight losses. Chemical analyses showed that they were able to reduce the α-cellulose and

hemicellulose content of the fiberboards. In addition, *Alternaria* sp. and *Penicillium* sp. were able to reduce the lignin content of the fiberboards (Merrill et al. 1965). Today, it is known that hemicelluloses influence the mechanical properties of wood (Curling et al. 2002), and their degradation may account for the strength losses of fiberboards that Merrill observed (Merrill et al. 1965).

2.2.3.4. Staining of coated and modified wood

Wood is still susceptible to fungal attack by moulds even when it is covered by coatings. *Alternaria* sp.*, Phoma* sp.*, Cladosporium* sp.*, Stemphylum* sp. *and A. pullulans* have all been isolated from coated wood (Duncan 1963; Savory 1973). These fungi can sometimes grow within the finish without colonizing the wood, by using some of the chemical components of the coating as a food source, for example oil-based binders (Duncan 1963; Savory 1973). Evidence for this is that *A. pullulans* grows on paints applied to metals (Savory 1973). A number of theories have been proposed to explain the colonization of coated wood by mould fungi. The first postulates that spores land on wood prior to the application of coatings and germinate later using moisture from within the wood (Duncan 1963; Savory 1973). A second theory suggests that fungi grow directly on finishes and penetrate into the wood using imperfections in the coating, raised fibers, or via enzymatic mechanisms (Duncan 1963; Savory 1973). Once fungi colonize the wood surface under the coating, the growth of hyphae can generate mechanical stresses which cause the coating to blister, fracture and finally fail (Duncan 1963).

According to Dickinson (1971) the most effective treatment at preventing fungal colonization of finished wood is a pre-treatment containing a water repellent and fungicide. However, good control of fungi has also been obtained using a primer containing a mix of fungicides (propiconazole + 3-Iodo-2-propynyl butylcarbamate (IPBC), 0.5+0.2 %, respectively) (Hannu and Ahola 1998). Fungi colonizing weathered wood, however, exhibit some tolerance to preservative treatments. This behavior includes tolerance to preservatives such as chromated copper arsenate (CCA). For example, Kim et al. (2007) isolated 16 species from the genera *Phoma, Cladosporium, Penicillium, Aureobasidium, Phialophora, and Trichoderma* from CCA-treated radiata pine (*Pinus radiata* D.Don). They concluded that staining fungi are more tolerant to CCA salts than basidiomycete fungi (Kim et al. 2007). *Cladosporium* sp. and *Aspergillus* sp., are also tolerant of the fungicides found in some preservative formulations. According to Shirikawa et al. (2002) paint containing a mix of preservatives was able to prevent the growth of large numbers of microorganisms on wood. However, it could not inhibit the growth of *Cladosporium* sp. and *Aspergillus* sp.

The use of photocatalytic substances such as TiO_2 has been shown to be effective against microorganisms growing on concrete and other materials surfaces (Gumy et al., 2006), but this approach has not been tested on weathered wood.

Fungi also seem to be able to colonize modified wood surfaces. Wood surface fungi have been reported colonizing thermally and chemically modified wood. Raberg et al. (2006) reported colonization of thermally modified Norway spruce (*Picea abies* (L) H.Karst.) by *Mucor* sp. and *Hormonema dematioides*; and colonization of acetylated Scots pine by *Cladosporium* sp. and *Phoma* sp. Recently, a wide range of fungi were found colonizing

specimens of Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) modified with an amino-alkyl-functional oligomeric siloxane, sodium water glass or 1,3-dimethylol-4,5-dihydroxyethylene urea (DMDHEU) (Pfeffer et al. 2012). In such work *Trichoderma* sp. and *Epicoccum* sp. were the predominant fungi isolated from the modified woods, but DMDHEU modified wood was only colonized by *A. pullulans*.

2.3. Ultraviolet radiation and fungal melanins

2.3.1. Effect of ultraviolet radiation on living cells and fungi

The ultraviolet (UV) region of the electromagnetic spectrum has been subdivided into three regions: UVA (400-320 nm); UVB (320-290 nm); and UVC (290-200 nm). The division between UVB and UVC at 290 nm is chosen because ultraviolet radiation at wavelengths shorter than 290 nm is unlikely to be present in terrestrial sunlight, except at high altitudes (Henderson 1977). The quantity and quality of UV light reaching the earth's surface depends on the output from the sun and the properties of earth's atmosphere, but UVB is the most important part of the terrestrial UV spectrum in terms of its damaging effects on biological organisms and materials (Diffey 1991).

The biological effects of UV light start with its photochemical absorption by biological molecules. The biological molecules that are most susceptible to UV radiation are nucleic acids and proteins, and their nucleotides which act as chromophores (absorbers of light) (Harm 1980). In nucleic acids like deoxyribonucleic acid (DNA) the nucleotides are adenine, guanine, thymine and cytosine. DNA nucleotides absorb UV radiation at slightly different

wavelengths, between 260 – 265 nm. In contrast, proteins absorb less UV radiation than DNA, and at wavelengths closer to 280 nm (Diffey 1991). The products of UV absorption are mainly derivates of pyrimidine (pyridime dimers). In addition, DNA and proteins in cells cross-link when they are exposed to UV radiation (Patrick and Rahn 1976). Cells exposed to UV radiation can reach a state of inactivation, losing their ability to reproduce (Diffey 1991). The range of responses of DNA in biological organisms to UV radiation is summarized in Figure 2.4.

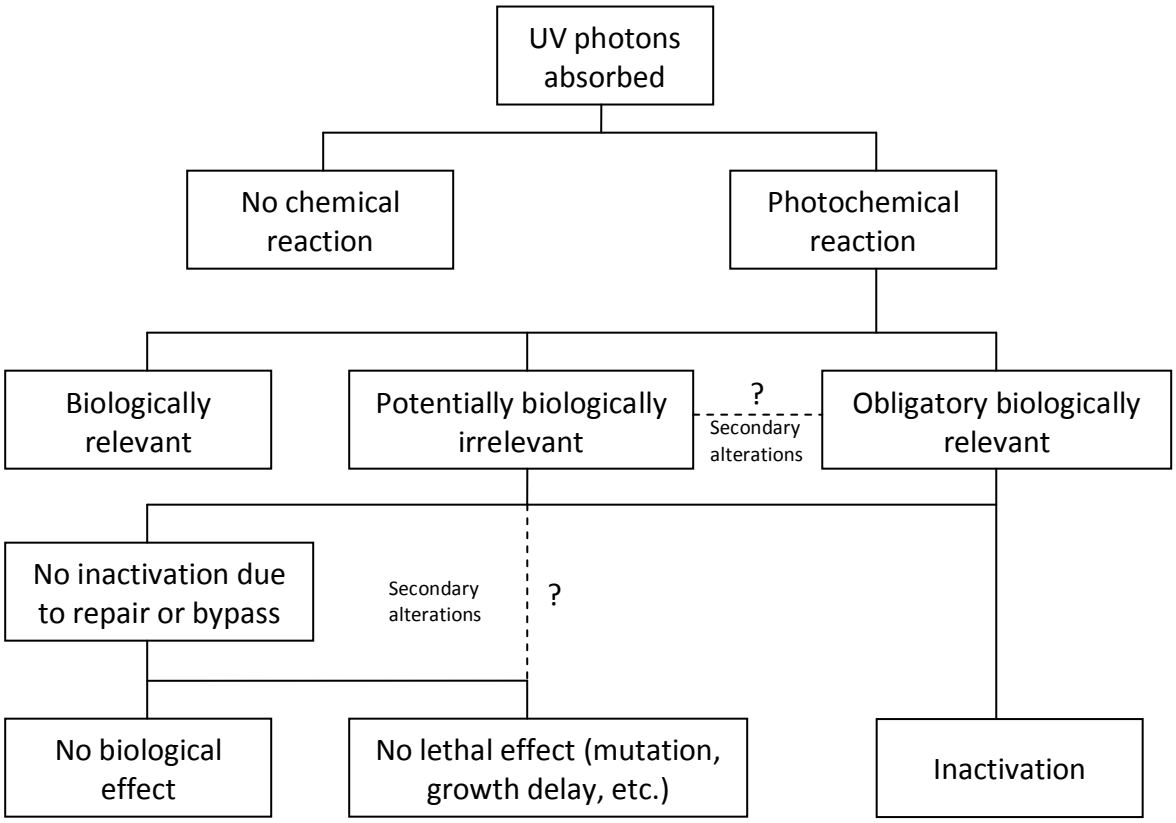


Figure 2.4: Possible effects of absorption of UV radiation by deoxyribonucleic acid (DNA) (Harm 1980)

Living cells have the ability to repair their DNA despite the damage caused by UV exposure. Repairing mechanisms have been identified and are described here according to Freifelder's (1987) terminology. (1) '*Photoreactivation repair*: this mechanism makes possible the repair

of DNA by the separation of a photoreactivating enzyme attached to the resultant pyrimidine dimers in the presence of radiation between 330 and 600 nm. The separation leaves a repaired section of DNA'. (2) '*Excision repair*: this repair process takes places in the dark. The defective zone of DNA is excised by enzymes and then replaced with normal nucleotides utilizing the complementary base pairing information in the interactive strand (in case the complementary strand is intact)'. (3) '*Post-replication repair*: UV damaged DNA can replicate in such a way that gaps are left in the daughter strand opposite the damaged sites. Subsequently the gaps are filled by DNA synthesis'. (4) '*SOS repair*: this mechanism is not fully understood, but it is thought to include a bypass system that allows the growth of the DNA chain across the damaged site'. 'This is achieved at the cost of fidelity of replication, and a great deal of evidence now indicates that SOS repair is the major cause of ultraviolet induced mutagenesis' (Freifelder 1987).

Living cells in fungal hyphae and spores are susceptible to solar radiation and especially to UV light. Exposure to solar radiation has been shown to be one of the most important factors affecting the survival of fungi (Rotem and Aust, 1991). The inactivation of microorganisms by light depends on the wavelength of the incident light, its intensity, and other physical and chemical parameters such as temperature, and substrate conditions (roughness and nutrients). The concentration of microorganisms at the exposed surface also plays an important role (Ozcelik, 2007; Schoenen and Kolch, 1992). The germicidal effect of UV light is well known and it is routinely used in air handling units (Levetin et al. 2001). Such units contain UV lamps that are able to reduce spore concentrations in air ducts. The effectiveness of such systems has been demonstrated against *Cladosporium* sp., and

Alternaria sp. spores (Levetin et al. 2001). Shorter wavelengths closer to 254 nm have greater fungicidal effects than longer ones such as 354 nm which, according to Ozcelik (2007) are unable to inactivate moulds even after 75 minutes of exposure. Nevertheless such exposure may decrease growth rates of fungi. Accordingly, Cagan and Svercel (2001) found that the radial growth of the fungus *Beauveria bassiana* decreased with an increase in time of exposure to UV light with an average wavelength of 253.7 nm. In contrast, other fungi exhibited different behavior to solar radiation or UV radiation (Rotem and Aust 1991). In some fungi their survival when exposed to UV radiation was proportional to the melanin content of their spores walls (Durrell 1964). For example, Wang and Casadevall (1994) found that non-melanized hyphae were more susceptible to UV radiation than melanized ones when exposed to different doses of UV light with a wavelength peak at a 254 nm. Kawamura et al. (1999) found that melanin conferred UV tolerance to *Alternaria alternata*. Frederick et al. (1999) found that exposure to UV light resulted in the melanization of hyaline hyphae of the fungus *G. graminis var. graminis*. As a result the hyphae became more tolerant to UV radiation compared to the hyphae of a non-melanized mutant. Melanin also confers UV tolerance to most spores and propagules (Henson et al. 1999). Another mechanism used by fungi to tolerate exposure to UV radiation involves the aggregation of spores and propagueles. For example, Rotem and Aust (1991) found a higher survival ratio for spores exposed to UV radiation when they formed aggregates.

2.3.2. Fungal melanins

2.3.2.1. Properties and role of melanins

Fungal melanins are high molecular weight, dark brown or black pigments formed by enzymatic or auto-oxidative polymerization of phenols and amino acid derivates or amino sugars, which are synthesized from carbohydrates by fungi during biosynthetic processes (Butler and Day, 1998; Paim et al. 1990). Melanin pigments are not essential for fungal growth. In fact, their synthesis is sometimes classified as 'secondary metabolism' and both pigmented and albino strains of the same fungi may exist (Henson et al. 1999). However, pigmented fungi may have comparative advantages when growing in certain environments (Butler and Day, 1998; Fogarty and Tobin, 1996). Hence, melanin can account for approximately 30 percent of the dry weight of a fungal cell. This quantity underscores its importance to fungi (Butler and Day 1998). Melanins can be found within or outside cell walls. The latter occurs via secretion of phenol compounds, which are subsequently oxidized, or through secretion of phenol oxidases enzymes to oxidize phenolics compounds in the medium external to the fungus. An example of this process occurs in *A. pullulans,* which releases extracellular granules of melanin (Butler and Day, 1998; Fogarty and Tobin, 1996). In general, melanins from different organisms share some common characteristics. They are often sparingly soluble in alkali and generally insoluble in water, aqueous acids, and common organic solvents, and they can interact with metals (Butler and Day, 1998; Caesar-TonThat et al. 1995; Fogarty and Tobin, 1996). For example, supernatant culture fluids from *Cladosporium resinae* and *A. pullulans*, containing extracellular melanin, can

bind Cu. Melanin from *A. pullulans* is also produced in response to Cu, Co, Pb, Hg, Cd, Fe, Mn, Ag, Al, and Ni, but not Mg, or Zn (Caesar-TonThat et al. 1995).

The dark color of melanins occurs because they do not re-radiate absorbed radiation as visible light (Butler and Day 1998). An impressive characteristic of fungal melanins is that they may exist as free radicals which are easily formed under various conditions such as incubation at increased temperature, irradiation with UV, γ-rays, or reaction with chemical reductants (Fogarty and Tobin 1996). In this sense, melanins are unique biopolymers because they contain stable free radicals that can act as proton receivers or donors; although they can be reduced by silver ions and oxidized by H_2O_2 (Fogarty and Tobin 1996; Henson et al. 1999). Several studies have shown that the presence of melanin enhances the survival of fungi exposed to environmental stress. The melanin present in fungal conidia reduces damage caused by UV light, solar radiation, γ-radiation, and X rays. The degree of protection against UV radiation is proportional to the concentration of melanin in conidial walls (Fogarty and Tobin 1996; Henson et al. 1999; Butler and Day 1998; 2001). Melanins may also provide fungi with increased resistance to desiccation and extreme temperatures. Melanins are synthesized in fungal pathogenesis by fungi to develop turgor in appressoria, and to increase virulence (Fogarty and Tobin 1996; Henson et al. 1999; Butler and Day 1998; 2001). Melanins provide protection against lysis in natural soils and protection against oxidizing agents (Butler and Day 1998). They also act as a physical boundary between the cell and its often hostile surroundings. Thus, melanin isolates the fungus from physical and biological stresses including poisons (Butler and Day 2001). Some melanins can bind drugs such as chlorpromazine and chloroquine. It is possible that some fungicides can be bound to

and inactivated by fungal melanins in a similar fashion (Butler and Day 1998; 2001). Melanins can also limit the leakage of useful compounds from fungal cells (Butler and Day 2001).

2.3.2.2. Synthesis of fungal melanins

Tyrosine, 3,4-dihydroxyphenylalanine (DOPA), γ-glutaminyl-4-hydroxybenzene (GHB), catechol, catecholamines, and 1,8-dihydroxynaphthalene (DHN) are the known precursors of fungal melanins (Fogarty and Tobin 1996). These precursors generate 4 different types of fungal melanins: DOPA, GHB, Chatechol and DHN (Figure 2.5).

DOPA melanins are heteropolymers made from a number of different compounds derived from tyrosine and DOPA (Butler and Day, 1998; Fogarty and Tobin, 1996). The biosynthetic pathway of DOPA melanins starts when tyrosine is hydroxylated to form DOPA followed by formation of DOPA-quinone by dehydrogenation of DOPA (Fogarty and Tobin 1996). DOPA melanins are able to switch incident visible, UV, and infrared energy into heat by converting the electronic energy of the radiation into vibrational and rotational activity in the molecular structure of the melanin. DOPA melanin is synthesized by basidiomycete fungi (Butler and Day 1998).

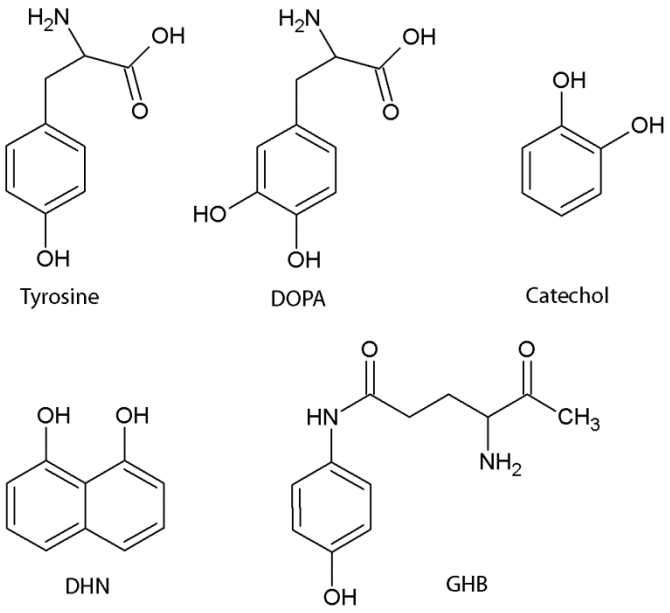


Figure 2.5: Precursors of fungal melanins

The biosynthesis of GHB melanins was described for *Agaricus bisporus* by Fogarty and Tobin (1996): “GHB melanin is generated from the precursor glutaminyl-4-hydroxybenzene, synthesized via the shikimic acid pathway. The shikimic acid is *o*-hydroxylated, followed by dehydrogenation of diphenol and polymerization of γ-glutaminyl-3,4-benzoquinone (GBQ) and quinoid products of GBQ. The γ-glutaminyl moiety of GHB may be removed prior to polymerization by a γ-glutaminytransferase present in the fruiting body. The γ-glutaminyl residue may thus be transferred to a receptor, liberating 4-aminiphenol (or 4-aminocathechol if the γ-glutaminylmoiety from GDHB is removed), which can be converted to very reactive oxidized intermediates, such as 2-hydroxy-4-iminoquinone. The intermediates can then polymerize to yield melanin”. As for DOPA melanin, it is well accepted that GHB melanin is produced by fruiting bodies of basidiomycetes.

Cathecol melanin contains percentages of carbon, hydrogen, nitrogen and carboxyl groups, but its biosynthesis is still unclear (Fogarty and Tobin 1996).

The starting molecule for the DHN melanin pathway is 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN), which is formed by the head-to-tail joining and cyclization of acetate molecules. After that an alternating pair of reduction and dehydration reactions results in the formation of an immediate precursor (the monomer) to the melanin polymer, which is DHN. In brief, 1,3,6,8-THN is reducted to scytalone, and a dehydration reaction then forms 1,3,8-trihydroxynaphthalene (THN). A second reduction reaction forms vermelone from 1,3,8-THN, which is converted to DHN by a second dehydration reaction, and DHN is finally polymerized in a final step to form DHN melanin (Figure 2.6) (Fogarty and Tobin 1996). DHN melanins are synthesized by a number of ascomycetous and imperfect fungi, mainly

filamentous fungi. Among them are: *Sporothrix shenckii*, *Alternaria alternata*, *A. pullulans*, *Cladosporium carrionii*, *Cladosporium bantianum* and *Cladosporium cladosporioides*, *G. graminis*, *Magnaporthe grisea*, *C. lagenarium*, *Cochliobolus heterostrophus*, and *Aspergillus* sp. (Caesar-TonThat et al. 1995; Kawamura et al. 1997; Henson et al. 1999; Romero-Martinez et al. 2000; Kogej et al. 2004). However, the complex factors involved in the biosynthesis of DHN melanin can generate slightly different polymers in different fungi. As a result, color differences can be found between melanins from different fungi. These differences are related to the amounts and wavelengths of light that melanins absorb, and with the polymer's structure, size, crosslinking, oxidation state, cellular location, and complexation with other cellular components (Henson et al. 1999). Comparison of melanins derived from DOPA, DHN, GHB, and catechol shows that they have similar (but not identical) chemical and physical properties. One explanation for this similarity, which is supported by Fourier transform infrared spectroscopy, is that they all contain identical functional groups (Fogarty and Tobin 1996).

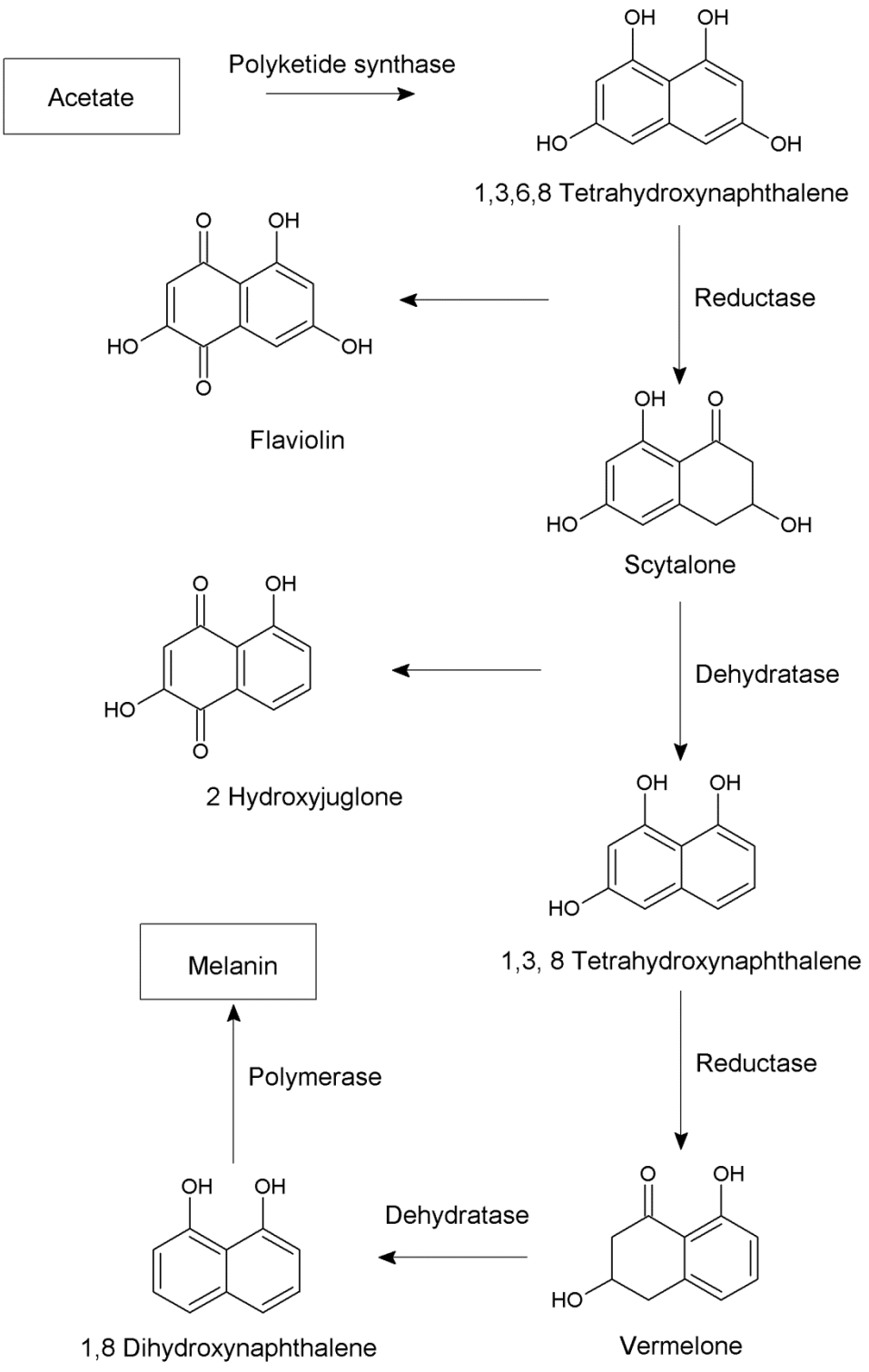


Figure 2.6: DHN melanin biosynthesis

2.4. Fungal melanin biosynthesis inhibitors

Fungal melanin biosynthesis inhibitors (MBIs) are chemical substances initially developed as systemic and multi-systemic fungicides against rice blast disease (Kurahashi 2001). Their mode of action is based on impeding the penetration of fungal hyphae inside plant's tissues by affecting the thickening mechanism of fungal appressoria (Kurahashi 2001). Appressoria need to attain a specific turgor to penetrate plant tissue, and this is achieved by the accumulation of melanin. Production of melanin can be blocked by MBIs which impedes the thickening of appressoria and consequently prevents the penetration of plant tissues by the rice blast fungus (Kubo 2005). The fungus responsible for blast rice disease (M. grisea) and fungi responsible for other infections in crops are normally filamentous ascomycetes which synthesize melanin via 1,8-DHN. The synthesis of DHN melanin can be interrupted by MBIs which target the different enzymes involved in the biosynthetic pathways of DHN-melanin (Kim et al. 1998). The target site where MBIs act vary according to the enzyme they target. In general, MBIs are able to block three different enzymatic pathways: (1) at the earliest stages of melanin biosynthesis (possibly before and on pentaketide formation or cyclization); (2) at the reductive stage (reductase enzyme inhibited); and (3) at the dehydrate stage (dehydratase enzyme inhibited) (Figure 2.6). Melanin biosynthesis inhibitors are also useful for providing insights into the different pathways involved in the synthesis of melanin. The inhibition of specific enzymatic activity hints at the biosynthetic process involved in melanin synthesis. This research involves analyzing the chemicals that accumulate due to the action of MBIs (Butler and Day, 1998).

2.4.1. MBIs targeting early stages of DHN melanin biosynthesis

The compound cerulenin [(2R,3S)-3-[(4E,7E)-nona-4,7-dienoyl]oxirane-2-carboxamide] (Figure 2.7a) is a strong inhibitor of melanin biosynthesis at the polyketide synthase step of DHN synthesis. Cerulenin also inhibits the enzyme fatty acid synthase, a physiologically critical enzyme. Therefore at low concentrations cerulenin is able to inhibit fungal growth *in-vitro* (Fleet and Breuil 2002).

The fungicide KC10017 [3-[4'-bromo-2',6'-dimethylphenoxy]methyl-4-[(3"-methylphenyl) aminocarbonyl]methyl-1,2,4-oxadiazol-5-one] (Figure 2.7b) also blocks DHN-melanin biosynthesis at the earliest stage of melanin biosynthesis. The target sites for this chemical are the reaction steps prior to 1,3,6,8-THN formation, namely pentaketide synthesis and /or pentaketide cyclization (Kim et al. 1998). According to Kim et al. (1998) the fungicide is very effective at blocking the biosynthesis of melanin by *M. grisea*, but when it was tested against other microorganisms like *A. alternata* and *C. lagenarium* it did not cause color changes in mycelia suggesting that it did not act as a melanin biosynthesis inhibitor. Kim et al. (1998) accounted for this discrepancy by suggesting that the biosynthetic pathway prior to 1,3,6,8-THN formation for *M. grisea* and *A. alternata* and *C. lagenarium* might be different, or alternatively that the structure of the enzyme blocked by KC10017 in *M. grisea* might be different from that in *A. alternata* and *C. lagenarium* (Kim et al. 1998).

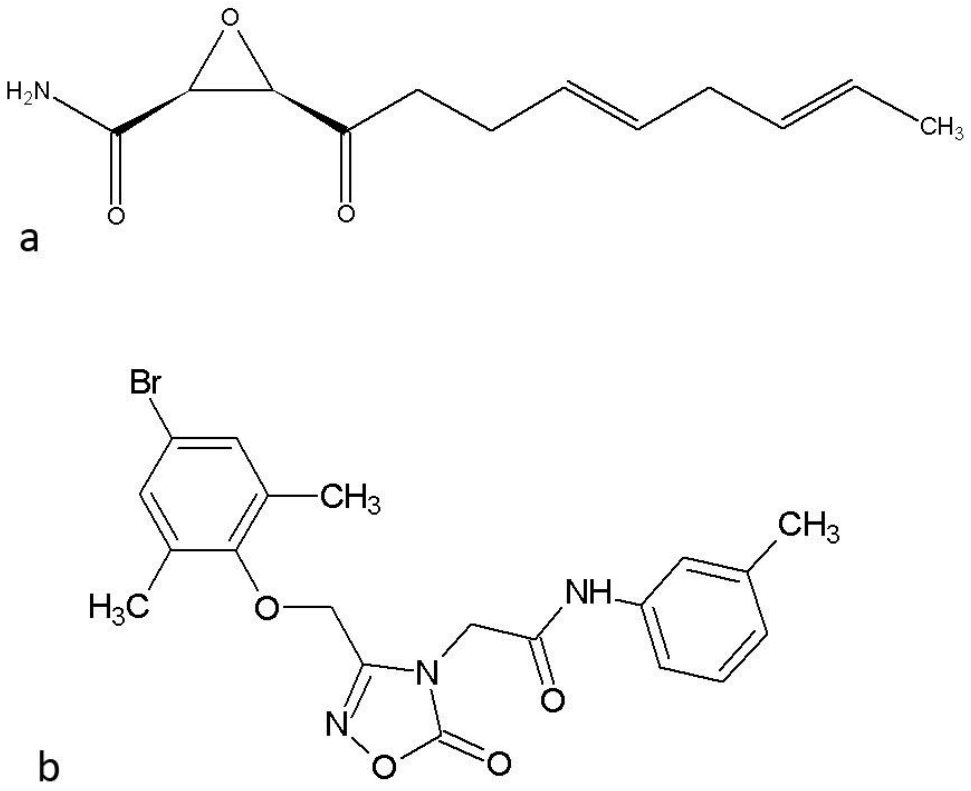
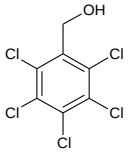
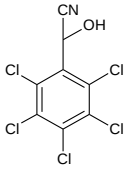
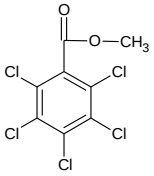
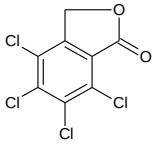
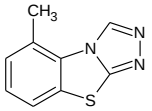
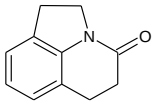
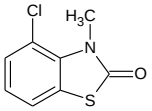
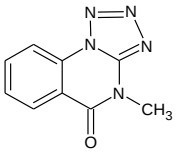
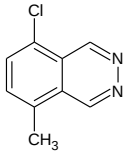


Figure 2.7: Melanin biosynthesis inhibitors acting on the early stages of the biosynthesis of melanin. (a) Structure of cerulenin (Fleet and Breuil 2002) and (b) [3-[4'-bromo-2',6'-dimethylphenoxy]methyl-4-[(3"-methylphenyl) aminocarbonyl]methyl-1,2,4-oxadiazol-5-one] (KC10017) (Kim et al. 1998)

2.4.2. MBIs targeting reductase enzymes

A second target site for MBIs is the enzymatic reduction of 1,3,6,8-THN to scytalone and 1,3,8-THN to vermelone. This can be achieved by blocking the enzyme polyhydroxynaphthaline reductase (Kurahashi and Pontzen 1998; Kim et al. 1998; Kubo et al. 1996; 2005). The list of MBIs that block the reductase enzyme system and are registered as fungicides in Japan are listed in Table 2.2 (Kurahashi, 2001).

Table 2.2: Melanin biosynthesis inhibitors of reductase registered in Japan in 2001

Chemical group	Name	Chemical structure
Poly chlorinated aromatic compounds	PCBA	
	PCMN	
	CPA	
	Fthalide	
Fused heterocyclic compounds	Tricyclazole	
	Pyroquilon	
	Chlobenthiazole	
	PP 389	
	Phthaladine	

Tricyclazole is the reductase inhibitor that has been most widely studied. Tricyclazole was first developed as a fungicide, but it has been widely used in studies of melanin biosynthesis

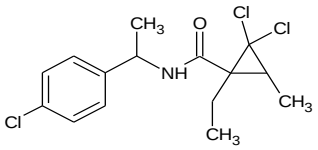
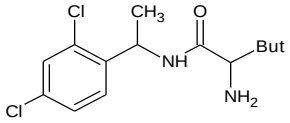
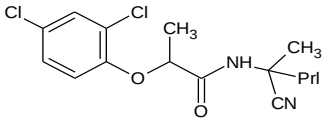
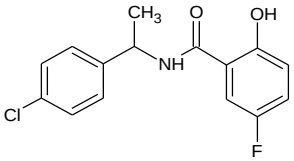
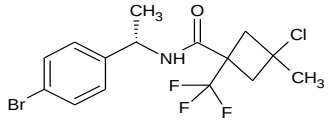
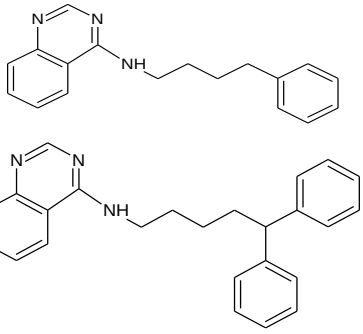
(Cooper and Gadd, 1984; Fleet and Breuil, 2002; Kogej et al., 2004; Romero-Martinez et al., 2000). The effect of tricyclazole on pigmented fungal strains *in-vitro* is to induce hyphae to become pink initially. The hyphae then darken to red and brown as the fungal colony ages (Cooper and Gadd, 1984). These color changes are due to the accumulation of 'shunt' products from the blocked pathway. Flaviolin and 2-hydroxyjuglone (2-HJ) (Figure 2.6) are auto-oxidative products of 1,3,6,8-THN and 1,3,8-THN, respectively, and they have been isolated from cultures treated with tricyclazole (Butler and Day, 1998; Kogej et al., 2004). Wheeler and Klich (1995) evaluated the inhibition of pigmentation in *Penicillium* and *Aspergillus* species using several MBIs. They showed that tricyclazole, chlobenthiazone and pyroquilon were the most successful treatments, followed by phthalide, PCBA, and others. They also noticed that the fungicide chlobenthiazone did not inhibit mycelial growth at a concentration of 8 µg/mL. According to Cooper and Gadd (1984), tricyclazole might affect other types of melanins because it was able to inhibit induced colorization by DOPA and indole, which are precursors of the tyrosine type melanin.

2.4.3. MBIs targeting dehydratase enzymes

A third target for fungal MBIs is the enzymatic dehydration of scytalone into 1,3,8-THN by elimination of water, and also a second dehydration reaction for the conversion of vermelone into 1,8-DHN (Kurahashi and Pontzen 1998; Kubo et al. 1996; 2005). The fungicides that target these reactions were developed later than reductase inhibitors; Kurahashi (2001) published a list of MBIs inhibitors of dehydratase that were registered in Japan in 2001 (Table 2.3).

The fungicide from this list that has been most commonly tested is carpropamid [(1R*,3S*)-2,2-dichloro-N-[1-(4-chlorophenyl)+ethyl]-1-ethyl-3-methylcyclopropanecarboxamide]. Carpropamid is used as a foliar fungicide (Kurahashi and Pontzen 1998; Kurahashi et al. 1999; 2001; Hewitt 2000; Rohilla et al. 2001). It has also been used in laboratory studies to confirm the presence of the DHN-melanin pathway in fungi (Fleet and Breuil, 2002).

Table 2.3: Melanin biosynthesis inhibitors of dehydratase registered in Japan by 2001 (Kurahashi, 2001)

Chemical group	Name	Chemical structure
Carboxamide derivatives	Carpropamid (CAR)	
	Dichlocymet (DCM)	
	Fenoxanil	
	BFS	
	Cyclobutane carboxamid	
4-aminoquinazolin dereviates		

2.4.4. Other inhibitors

Other MBIs are also mentioned in the literature. For example, Wheeler and Klich (1995) mention the ability of MQ (N-methyl-2-quinolone), TQ (s-triazolo-[4,3-a]quinoline) and coumarin (Figure 2.8 (a), (b) and (c), respectively) to inhibit the melanization of *P. oryzae*. However, there is no information on the metabolic targets for these molecules.

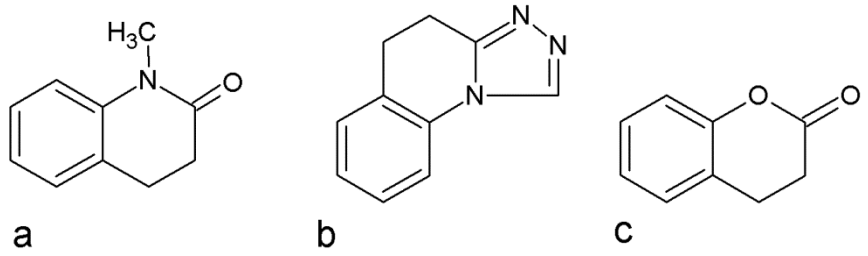


Figure 2.8: Compounds that inhibit DHN-melanin biosynthesis in *P. oryzae* and other brown and black fungi. (a) N-methyl-2-quinolone (MQ), (b) s-triazolo-[4,3-a]quinoline (TQ) and (c) Coumarin (Wheeler and Klich 1995)

2.5. Summary

This literature review provides background information on the weathering of wood, biological agents colonizing wood surfaces with emphasis on moulds colonizing wood surfaces, effect of UV radiation on microorganisms, fungal melanins and chemical inhibition of melanin biosynthesis. This information enables the reader of this thesis to understand the experimental chapters that follow.

This review shows that only a few studies have examined the effect of moulds on the structural properties of wood and its polymeric constituents. Such studies do not conclusively establish whether moulds can degrade wood's structural tissues. Similarly, the effect of UV radiation on the growth, survival and melanization of fungi have been studied in general, but the effect of UV radiation on the growth and melanization of moulds

colonizing weathered wood has not been examined. In addition, the control of surface fungi to prevent the graying of wood has been restricted to the use of fungicides. The possibility of using melanin biosynthesis inhibitors to reduce the staining and graying of weathered wood has not been examined. This thesis intends to fill these gaps and provide new information to enhance our understanding of the role of non-decay fungi on the weathering of wood, with emphasis on ability of wood surface moulds to decay wood, effects of UV radiation on melanization and growth of surface moulds and prevention of graying of weathered wood surfaces using chemicals that inhibit the biosynthetic pathways of fungal melanins.

Chapter 3: Fungi colonizing the surface of southern pine exposed to natural weathering

3.1. Introduction

Early observations of fungi causing the graying of wood exposed outdoors date back to the 19th and early 20th century, as mentioned in Chapter 2 (Möbius, 1924; Schacht, 1863), but the fungi colonizing weathered wood surfaces were not identified until the mid 20th century (Duncan 1963). A comprehensive list of fungi isolated from weathered wood surfaces around the world was tabulated in Chapter 2. Many of the organisms colonizing weathered wood have remarkable ability to grow in adverse environments (Duncan 1963), but their diversity normally depends on wood species (substrate), exposure conditions and climate (Hansen 2008). Most of the species isolated from weathered wood were identified using their morphological features (observed under the light microscope). This method of identification requires great skill and experience to produce accurate results (Gutzmer et al. 2004), because many fungal species share similar morphological features. On the other hand, identification of fungi using DNA analysis, can be more accurate (Ray et al. 2004; Balajee et al. 2007). In such analysis ribosomal genes are the most common targeted genes used for differentiating fungi at the genus and species levels. Genes are multiple copied, sequenced and blasted against genes from known (identified) organisms. The drawback of this technique is that the gene sequences of the target organisms must be available in data-bases for the identification to be accurate (Dismukes et al. 2003). Nevertheless, I hypothesize here that the combination of both molecular techniques and microscopy will

be highly effective at identifying the different fungi colonizing wood surfaces exposed outdoors.

The aim of the research in this chapter was to isolate, identify and characterize the fungi colonizing untreated wood surfaces exposed outdoors. Southern pine wood was the test substrate because it is a commercially important wood species and it is prone to fungal staining (Himelick, 1982). Southern pine is a generic name given to most pine species whose major range is in the United States south of the Mason-Dixon line (lat. 39° 43' N.). Southern pine comprises at least 10 species, all hard pines-diploxylon members of the genus *Pinus*, family Pinaceae, and order Coniferales, e.g. *P. palustris*, *P. elliottii*, *P. tadea*, *P. echinata*, *P. glabra*, and others (Koch, 1972). Fungi growing on wood samples exposed outdoors for 40 weeks were isolated and identified using both molecular techniques and microscopy. The growth rate and mycelia color of the fungi were then measured in solid culture media. The morphology, color and area of exposed wood surfaces affected by stain were also quantified. Chemical changes occurring at weathered wood surfaces were assessed using Fourier transform infra red spectroscopy (FTIR). Fungi isolated from weathered southern pine surfaces were used for subsequent experimentation in Chapters 4 and 5.

3.2. Materials and methods

3.2.1. Wood samples and exposure

Five flat-sawn southern pine boards measuring 381 mm x 1397 mm x 24000 mm, supplied by CSI (now Viance) in North Carolina, USA, were used in this experiment. The growth rate and wood density of sample boards is shown in Table 3.1 below. The boards were stored in a conditioning room at 20 ± 1 °C and 65 ± 5% relative humidity (r.h.) for 24 weeks (12% equilibrium moisture content), and were cross-cut to produce 5 samples (one per board), each 320 mm long. These samples were planed on their tangential faces with growth rings oriented convex to the face (bark-side up) using a Martin T54 thickness planer. Then, sixteen strips, 20 mm wide, were made on the exposed face of each sample by cutting transversally to the grain 15 grooves, 3 to 5 mm in depth, with a band saw (Meber, Model SR-500). The strips, intended to facilitate measurement of stained area, were isolated from each other by filling the grooves with a hot melt resin (commercial grade) applied with a heating gun. The end grain on samples was sealed with epoxy resin (Quick cure 5; System three resins, Inc. WA, USA), to minimize further drying and development of checks. Samples were exposed outdoors to the weather, at ≈ 400 mm above the ground for 40 weeks in Vancouver, Canada. The 40 weeks (August to May) included many sunny days and periods when samples were exposed to heavy rainfall. The superficial moisture content of the samples was measured during the most rainy months of the exposure trial (week 10 to 32) using a portable resistance-type moisture meter (Delmhorst RDM³, Delmhorst Instrument Company). Monthly weather conditions for the exposure period are shown in Table 3.2.

Table 3.1: Density and growth rate of southern pine samples

Board/Block	Growth [rings/cm]	Basic density [g/cm^3]
1	8.28	0.429
2	5.14	0.432
3	4.57	0.560
4	4.50	0.505
5	5.42	0.452

Table 3.2: Monthly weather conditions during the exposure period in Vancouver, Canada; reported by Canada's National Weather Archive

Year	Month	Mean max. temp. [°C]	Mean temp. [°C]	Mean min. temp. [°C]	Extrem. max. [°C]	Extrem. min. [°C]	Total rain [mm]	Total snow [mm]	Total precip. [mm]
2007	Aug	21.9	17.8	13.6	26.7	11.3	8.4	0.0	8.4
2007	Sept	17.6	14.2	10.8	22.4	6.2	73.6	0.0	73.6
2007	Oct	12.4	9.6	6.7	17.3	1.5	155.2	0.0	155.2
2007	Nov	8.9	5.9	2.8	12.8	-3.3	116.2	0.0	116.2
2007	Dec	5.8	3.2	0.6	12.9	-5.3	181.6	19.6	210.6
2008	Jan	5.5	2.8	0.1	10.3	-4.9	122.2	14.2	137.6
2008	Feb	8.6	5.5	2.4	14.1	-2.9	67.4	0.8	68.6
2008	Mar	9.1	5.9	2.7	11.6	-1	72.8	2.4	75.2
2008	Apr	11.3	7.6	3.8	18.8	-2.1	56.8	2.2	62.2
2008	May	16.6	12.8	8.9	29	3.3	43.2	0.0	43.2

3.2.2. Isolation, purification, identification and storage of fungi

The isolation of fungi from the surface of weathered southern pine samples used the method of Lim et al. (2005). A small fragment of wood, was excised from under the wood surfaces using a sharp scalpel and seeded directly onto 1% malt extract agar (MEA) Difco Different fungi growing on agar were separated by simple replication on to new plates, or by single spore isolation as described by Choi et al. (1999). Fungal isolations were performed on all samples after they were exposed to 40 weeks of natural weathering.

Isolated fungi were identified using both molecular techniques and microscopy, as mentioned above. Molecular techniques were used first to identify fungi and their identities were confirmed by examining their morphological features (Table 3.3). Some fungi had specific morphological characteristics that made it easier to identify them using light microscopy (Barnett and Hunter 1998). Identification using molecular techniques involved the extraction, amplification, purification and sequencing of fungal ribosomal DNA (rDNA). rDNA extraction was carried out using a modified version of the method developed by Lim et al. (2005). Modifications included the use of TES buffer as an extraction buffer and mechanical breakage of fungal cells by stirring the solution for 3 minutes at 600 rpm using a sterile stainless steel rod. The internal transcribed spacer (ITS) region of the rDNA was amplified using the universal primers ITS4 – ITS5 (Schmidt and Moreth 2002). Purification used the QIAquick PCR purification kit for enzymatic reactions (Quiagen Sciences Maryland, USA), and sequencing was performed at the DNA Synthesis and Sequencing Facility, at Macrogen (Seoul, Korea). The information obtained from the sequences was cross-referenced in the GeneBank data-base website (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). This data-base identifies similarities of the unknown fungus with those of known fungi in the data-base. Fungi were identified to the level of the genus or species depending on the information available.

Stocks of isolated and purified fungi were prepared by placing 4 to 6 agar plugs (5 mm in diameter) of isolated fungi into 2 mL screw-cap collection tubes filled with 900 µL nano pure water and 100 µL of glycerol. Stock tubes were stored at -80°C.

Table 3.3: Morphological features of common darks moulds colonizing weathered wood (Barnett and Hunter 1998)

Genera	Features
Aureobasidium	Mycelium not extensive, hyaline when young, becoming dark with age, black and shiny in old cultures, bearing abundant conidia laterally; conidia (blastospores) subhyaline to dark, 1-celled, ovoid, producing other conidia by budding; saprophytic or weakly parasitic; common in soil.
Alternaria	Conidiophores dark, mostly simple; determinate or sympodial, rather short or elongate; conidia (porospores) dark, typically with both cross and longitudinal septa; various shapes, obclavate to elliptical or ovoid, frequently borne acropetally in apical or branched appendages; parasitic or saprophytic on plant material.
Cladosporium	Conidiophores tall, dark, upright, branched variously near the apex, clustered or single; conidia (blastospores) dark, 1 or 2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon-shaped; often in simple or branched acropetalous chains; parasitic on higher plants or saprophytic.
Epicoccum	Sporodochia dark, more or less cushion-shaped, variable in size; conidiophores compact or loose, dark, rather short; conidia dark, several-celled (dicyosporous), globose; mostly saprophytic, or weakly parasitic.
Phoma	Pycnidia dark, ostiolate, lenticular to globose, immersed in host tissue, erumpent or with a short beak piercing the epidermis; conidiophores short; conidia small, 1 celled, hyaline, ovoid to elongate; parasitic, producing spots, principally on leaves.

3.2.3. Fungal diversity

The diversity of fungi colonizing weathered southern pine samples was assessed using two measures: (1) fungal richness and (2) reciprocal Simpson index. Fungal richness is simply the total number of species isolated per sample (Adams 2009) and the reciprocal Simpson index corresponds to the number of fungal species that in theory must be colonizing the wood after exposure (Peet 1974). The reciprocal Simpson index is calculated using the following formula (Maria and Sridhar 2002):

$$\text{Reciprocal Simpson index} = [1 / \Sigma (pi)^2]$$

Where, pi = proportion of individuals that species *i* contributes to the total per sample. Simpson index was calculated separately for each weathered southern pine sample.

3.2.4. Growth and color of fungi on solid culture media

Isolated fungi were grown on 1% MEA Difco. A 5 mm diameter agar plug, from the original fungal culture, was placed on agar in a 150 mm x 15 mm Petri dish. Under standard conditions of illumination a digital image of the hyphal mat from each plate (1:1 scale) was obtained after 7 days using a desktop scanner (Microtek Scan Maker i800). The diameter of the hyphal mat was digitally measured with the ruler tool of the software Adobe Photoshop CS3 Extended, version 10.0.1 (Adobe Systems Incorporated, USA), Figure 3.1a. The plates were re-scanned without their lids after 20 days and the images were used to digitally measure the color of the hyphal mats (using Photoshop, as above). Color measurement of hyphal mats involved the selection of a relevant portion of mycelia in the image and the evaluation of red-green-blue (RGB) color of the selection using the color histogram provided by the software. The average RGB color was registered and then entered in the picker color tool of the software, which provides equivalent colors in different color systems, including the CIELab system, Figure 3.1b. Color of fungal mats was recorded using the CIELab color coordinates, L (lightness on scale of 0, [black] to 100 [white]), a* (+60 [red] to -60 [green]) and b* (+60 [yellow] to -60 [blue]) (International Commission on Illumination 2007). Only lightness results are presented and discussed here.

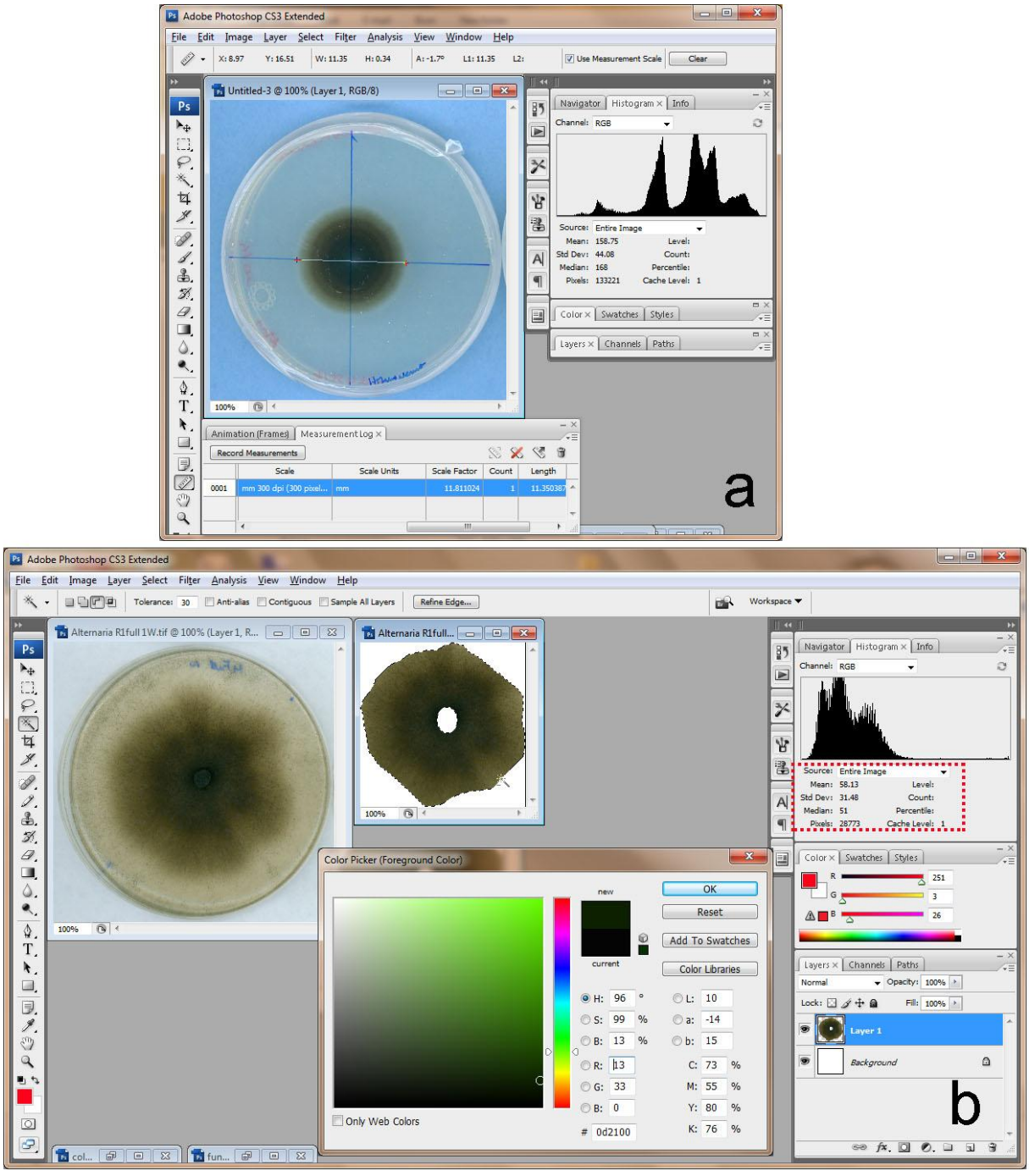


Figure 3.1: Growth rate and fungal mat color measurements. (a) Growth measurement in Photoshop of a fungal colony after 7 days of growth on malt extract agar (MEA) 1%; note the use of the ruler tool to estimate the diametrical growth of the fungal colony; (b) Fungal mat color measurement in Photoshop after 20 days of growth onto MEA 1%; note the original image of the colony, the selection of a relevant area for the measurement, information about the RGB color of the selected pixels (red square right side of the image) and color picker tool for transformation from RGB into CIELab color

3.2.5. Microstructure of wood colonized by fungi

The microstructure of wood colonized by fungi was examined using light microscopy. Pieces of wood measuring 10 mm x 10 mm were cut from the surface of exposed southern pine specimens and soaked in distilled water for 2 days. Each water-saturated block was clamped in a microtome and 20 µm sections were cut from the block using a disposable blade (Type S35, Feather Safety Razor Co., Japan) bolted to a microtome blade-holder. Sections were dehydrated in ethanol (industrial grade) for 2 days and then transferred to a saturated solution of safranin (BDH Chemical Ltd, England) in ethanol for 2 days. Each stained section was placed on a droplet of DPX (dibutyl phthalate xylene) mountant (Fluka Analytical, Germany) on a glass slides measuring 76 mm x 26 mm x 1 mm (Matsunami Glass Ind. Ltd. Japan), covered with a glass cover slip measuring 22 mm x 40 mm x 0.20 mm (Fisher Finest Premium Cover Glass, Fisher Scientific, Pittsburgh, USA), and dried at room temperature for 48 hours. The sections were examined using a light microscope (Carl Zeiss, Germany) at various magnifications. An Olympus DP71 digital camera attached to the microscope was used to take photographs of fungi colonizing the wood sections.

3.2.6. Color of weathered wood and area stained by fungi

The color of wood samples exposed to the weather was measured periodically. Samples were removed from the weathering racks and their color was measured: weekly during the first 4 weeks of exposure, every two weeks until week 20 and then at weeks 24, 32 and 40. Color expressed in CIELab color coordinates (as shown in section 3.2.4) was measured using

a portable spectrophotometer (Minolta CM-2600d). After color measurements, digital images of wood samples, scale 1:1; 96 dpi resolution, were taken with a desktop scanner (as above) to assess the area of wood stained by fungi. Digital images were examined using Photoshop (as above) at increased magnification (150 %) for fungal stains, and an additional transparent layer (same pixels size and resolution) was added to each picture. In this layer the area colonized by fungi was manually colored with Photoshop's brush tool. The number of dark colored pixels, measured with the automatic counting tool of the software, divided by the total number of pixels in the layer, multiplied by 100 was recorded as the stained area (Figure 3.2).

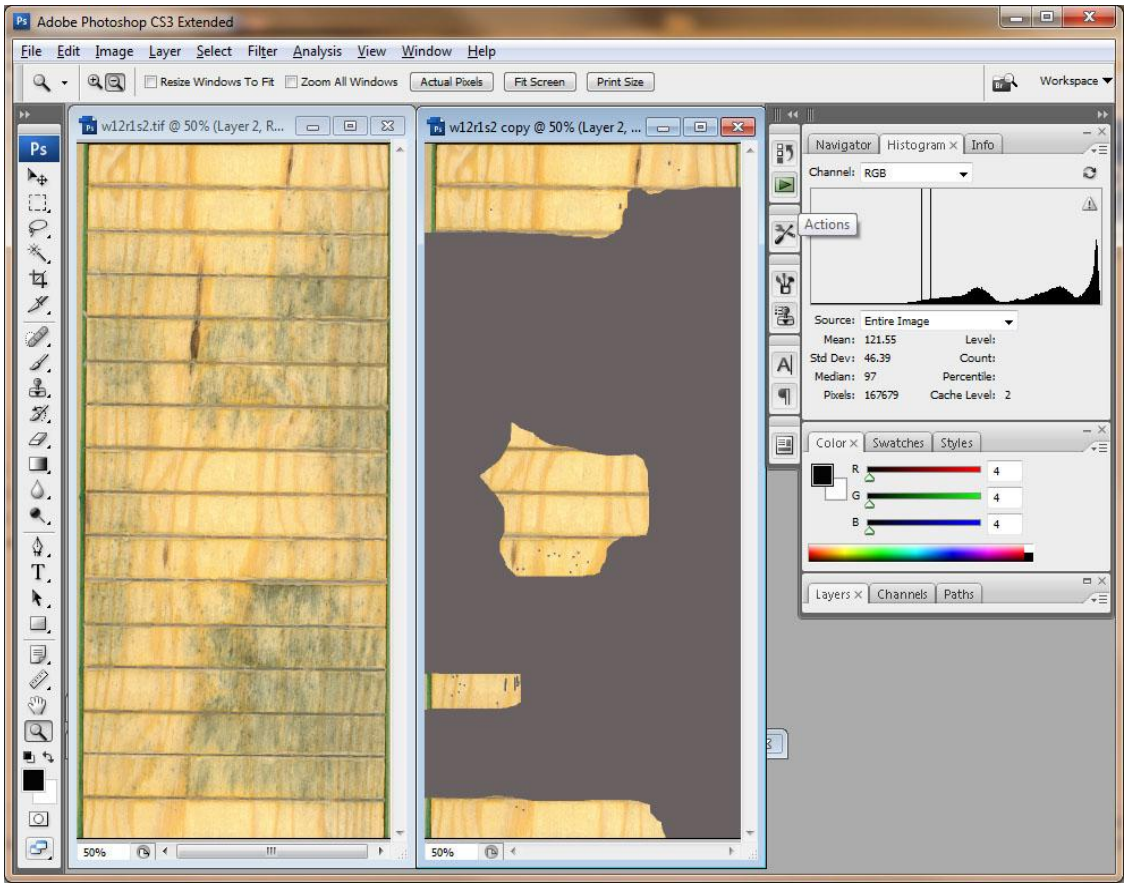


Figure 3.2: Measurement using Photoshop of the area of a wood sample stained by fungi. Original image (left) and colored pixels (centre) for quantification of stained area

3.2.7. Chemical changes at weathered wood surfaces

FTIR spectroscopy was used to examine chemical changes occurring at wood surfaces exposed outdoors. Pieces of wood measuring 20 mm (width) x 60 mm (length) x 8 mm (thickness) were sawn from each sample and stored for 5 days in a vacuum desiccator over silica gel. Direct reflectance (ATR-IR) FTIR spectra of weathered (gray) surfaces were obtained using a single bounce attenuated total reflectance accessory (PikeMiracle, PIKE technologies, WI, USA) attached to a spectrometer (Perkin Elmer Spectrum one, Waltham MA, USA). The penetration of infrared radiation into the wood sample was expected to be approximately 1.2 µm (Evans et al. 2008). Spectra of the fingerprint region 1800 to 800 (cm^{-1}) represented 16 accumulations at 8 cm^{-1} of resolution. Relevant peaks in the spectra were highlighted in the Spectrum software (v 5.3.1) on a PC attached to the spectrometer.

3.3. Results

3.3.1. Fungal diversity

A total of 26 isolates from 10 different genera, all in the phylum ascomycota, were isolated from the five replicate (boards) weathered southern pine samples. Of the 10 genera 4 were identified exclusively by DNA analysis, representing 15 % of the total isolates; 2 genera were identified exclusively by light microscopy, representing only 12 % of the total isolates; and 4 genera were identified using both techniques, representing 73 % of the total isolates (Table 3.4). The fungal richness on samples varied from 2 to 7, and the Simpson index from 2 to 5 (Table 3.5). Among the isolated fungi several were very well known colonizers of weathered wood including *Aureobasidium pullulans*, *Hormonema dematioides, Cladosporium sp., Alternaria sp.,* and *Phoma sp*. Other fungi isolated were *Truncatella angustata* (Pers.) S. Hughes*, Glonium pusillum* Zogg Zogg H.*, Mollisia minutella* (Sacc.) Rehm and a fungus from the genus *Lecythophora*. In addition, further characterization of isolated *A. pullulans* on solid media revealed that two varieties were present: a dark-type and a white-type. The latter white fungus melanized approximately one week after being seeded onto 1% MEA.

Table 3.4: Fungi isolated from southern lodgepole pine wood samples after 40 weeks of outdoor exposure in Vancouver, Canada

Fungi	Phylum	Source (Exposure/Rack)	Codification	Identification	Primer sequenced	Closest match in Blast (GeneBank)		Identity
Aureobasidium pullulans (black)	Ascomycota	Full / Sample 2	2	rDNA / Microscopy	ITS4	*Aureobasidium pullulans*	FJ216455	556/561 (99%)
Aureobasidium pullulans (black)	Ascomycota	Full / Sample 3	1_1	Microscopy*				
Aureobasidium pullulans (white)	Ascomycota	Full / Sample 3	3	rDNA / Microscopy	ITS4	*Aureobasidium pullulans*	AF455533	549/564 (97%)
Aureobasidium pullulans (black)	Ascomycota	Full / Sample 4	4	Microscopy*				
Hormonema dematioides	Ascomycota	Full / Sample 3	5_2	Microscopy*				
Hormonema dematioides	Ascomycota	Full / Sample 4	1	Microscopy*				
Hormonema dematioides	Ascomycota	Full / Sample 5	4	Microscopy*				
Hormonema dematioides	Ascomycota	Full / Sample 1	1S	rDNA / Microscopy	ITS4	*Hormonema dematioides*	AY253451	561/571 (98%)
Hormonema dematioides	Ascomycota	Full / Sample 1	6_1S	rDNA / Microscopy	ITS4	*Hormonema dematioides*	AY253451	566/573 (98%)
Alternaria sp.	Ascomycota	Full / Sample 1	3.2	Microscopy				
Alternaria sp.	Ascomycota	Full / Sample 1	1	Microscopy				
Cladosporium sp.	Ascomycota	Full / Sample 3	4_1	Microscopy				
Epicoccum nigrum	Ascomycota	Full / Sample 1	7W	rDNA / Microscopy	ITS4	*Epicoccum nigrum*	FJ904918	526/531 (99%)
Epicoccum sp.	Ascomycota	Full / Sample 3	2	Microscopy*				
Epicoccum sp.	Ascomycota	Full / Sample 3	6	Microscopy*				
Epicoccum sp.	Ascomycota	Full / Sample 4	5	Microscopy*				
Epicoccum sp.	Ascomycota	Full / Sample 5	2	Microscopy*				
Phoma herbarum	Ascomycota	Full / Sample 4	6	Microscopy*				
Phoma sp.	Ascomycota	Full / Sample 1	4S	rDNA / Microscopy	ITS4	*Phoma sp.*	AM901684	532/535 (99%)
Phoma sp.	Ascomycota	Full / Sample 2	1	rDNA / Microscopy	ITS4	*Phoma herbarum*	DQ132841	510/519 (98%)
Phoma sp.	Ascomycota	Full / Sample 4	2	rDNA / Microscopy	ITS4	*Phoma herbarum*	DQ132841	514/526 (97%)
Phoma sp.	Ascomycota	Full / Sample 4	3	rDNA / Microscopy	ITS4	*Phoma herbarum*	AY337712	463/471 (98%)
Truncatella angustata	Ascomycota	Full / Sample 2	4	rDNA	ITS4	*Truncatella angustata*	AF405306	557/558 (99%)
Glonium pusillum	Ascomycota	Full / Sample 2	1_1	rDNA	ITS4	*Glonium pusillum*	EU552134.1	507/509 (99%)
Lecythophora sp.	Ascomycota	Full / Sample 2	5_1	rDNA	ITS4	*Lecythophora sp.*	AY219880.1	528/539 (97%)
Mollisia minutella	Ascomycota	Full / Sample 3	4_2	rDNA	ITS4	*Mollisia minutella*	DQ008242.1	448/448 (93%)

*: Morphological features cross references against fungi identified by DNA analysis

Table 3.5: Fungal diversity in southern pine wood samples exposed to the weather for 40 weeks in Vancouver, Canada

Sample	Fungal richness	Simpson index
1	6	3.6
2	5	5
3	7	4.5
4	6	3
5	2	2
Average	5.2	3.6
Total	26	6.76

3.3.2. Growth and color of isolated fungi

The radial growth of fungi after 7 days is expressed as mm of growth per week (Table 3.6). *Epicoccum* sp., *T.* angustata and *Phoma* sp. grew the fastest, 17 and 24 mm per week, respectively. *A. pullulans* and *H. dematioides* grew at similar rates, of around 13 mm per week. Other fungi grew more slowly particularly *Mollisia minutella* (2 mm), *Lecythophora* sp. (3.5 mm) and *Cladosporium* sp. (5.6 mm).

Lightness of fungi after 20 days of growth expressed as the CIE L coordinate is shown in Table 3.7. *A. pullulans* (black)*, H. dematioides, Cladosporium* sp. and *Mollisia* sp. produced the darkest mycelia whereas *A. pullulans* (white variety), *Alternata* sp., *Epicoccum* sp., *T. angustata* and *G. pusillum* were lighter. Hyaline (white) growth was shown by *Phoma* sp. and *Lecythophora* sp. (Table 3.7). Scanned images of fungi growing on MEA show the variation in color of the different fungi that were isolated from weathered wood and these images accord with color measurements (Figure 3.3 and Figure 3.4).

Table 3.6: Growth of fungi cultured onto solid malt extract agar (1% Difco) after 7 days of growth

Fungi	Growth at day 7	
	Avg (mm)	[SD]
Truncatella angustata	24.1	[NA]
Epicoccum sp.	20.7	[5.2]
Phoma sp.	17.9	[1.0]
Alternaria sp. 5	15.1	[8.3]
Aureobasidium pullulans (white)	13.4	[0.6]
Hormonema dematioides	13.4	[2.4]
Aureobasidium pullulans (black)	12.9	[2.7]
Glonium pusillum	10.2	[NA]
Cladosporium sp.	5.7	[NA]
Lecythophora sp.	3.6	[NA]
Mollisia minutella	2.0	[NA]

Table 3.7: Lightness of fungi cultured onto solid media malt extract (agar 1% Difco) after 7 days of growth

Fungi	Lightness at day 20	
	Avg (L)	[SD]
Hormonema dematioides	16.6	[3.6]
Cladosporium sp.	24.0	[NA]
Alternaria sp. 5	27.5	[0.7]
Aureobasidium pullulans (black)	28.0	[NA]
Mollisia minutella	29.0	[NA]
Glonium pusillum	50.0	[NA]
Epicoccum sp.	58.5	[13.3]
Truncatella angustata	61.0	[NA]
Lecythophora sp.	69.0	[NA]
Aureobasidium pullulans (white)	73.0	[NA]
Phoma sp.	76.2	[8.5]

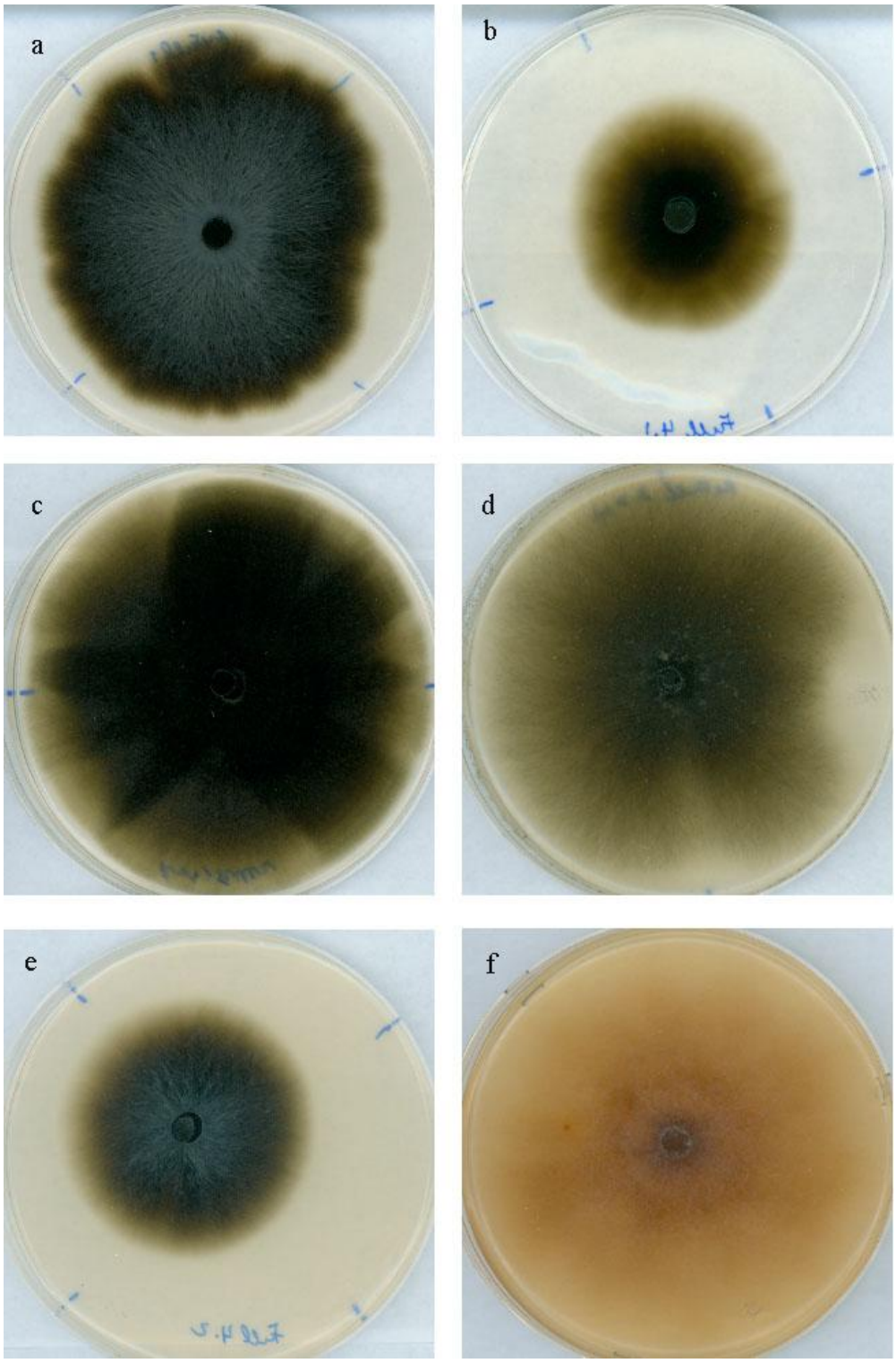


Figure 3.3: Dark fungi isolated from weathered wood, after 20 days of growth on malt extract agar (1% Difco): (a) *Hormonema dematioides*; (b) *Cladosporium* sp.; (c) *Aureobasidium pullulans*; (d) *Alternaria* sp.; (e) *Mollisia minutella;* and (f) *Glonium pusillum*

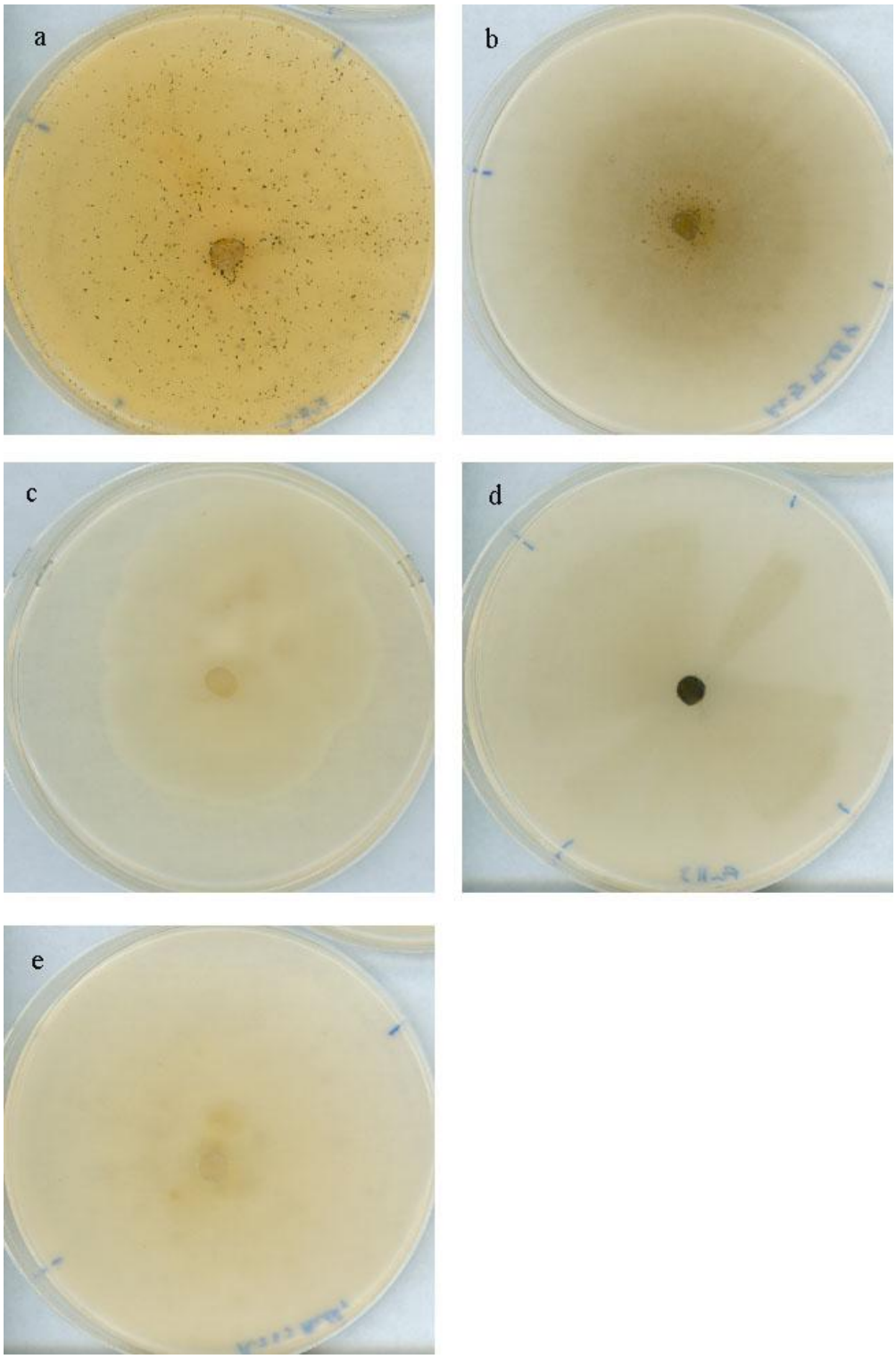


Figure 3.4: Light fungi isolated from weathered wood, after 20 days of growth on malt extract agar (1% Difco): (a) *Epicoccum nigrum*; (b) *Phoma* sp.; (c) *Lecythophora* sp.; (d) *Aureobasidium pullulans;* and (e) *Truncatella angustata*

3.3.3. Fungal colonization under light microscopy

Visual examination of end-grain of samples exposed to the weather for 40 weeks revealed that some of the samples were stained all the way through. Light microscopy revealed that fungi colonized and degraded parenchyma cells in the rays. Also, they were present in adjacent longitudinal tracheids. Hyphae penetrated the wood via ray parenchyma cells rather than via tracheids or ray tracheids. Hyphae grew longitudinally using the lumens of tracheids as a pathway (Figure 3.5).

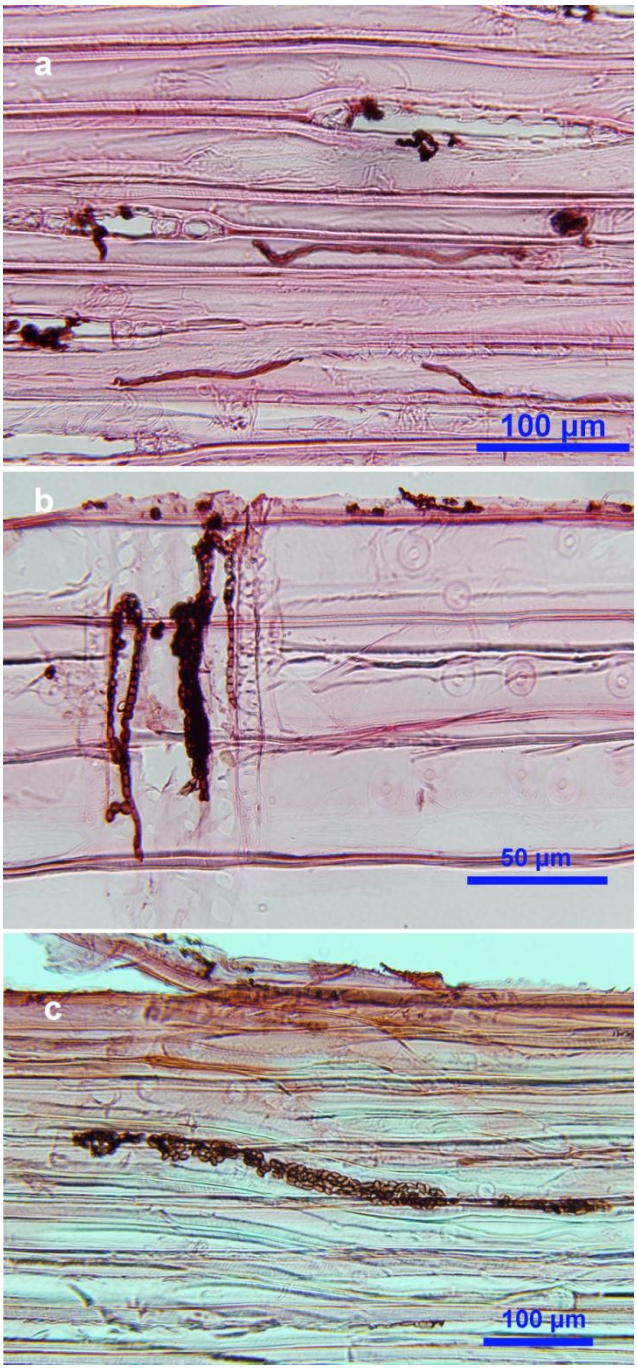


Figure 3.5: Light microscopy images of sections from southern pine wood samples exposed outdoors for 40 weeks. (a) Tangential longitudinal section showing dark hyphae in degraded rays and tracheids; (b) Radial longitudinal section showing dark hyphae colonizing ray parenchyma cells, but not ray tracheids in rays; (c) Radial section showing dark hyphae colonizing tracheids approximately 200 micrometers beneath the weathered wood surface

3.3.4. Color of weathered wood and area stained by fungi

Dark stains appeared on the surface of the southern pine samples 6 to 8 weeks after they were exposed outdoors. The increase in the percentage of the area of samples stained by fungi is shown in Figure 3.6. There was some evidence of fungal growth on wood surfaces as early as the second week of exposure. At this stage, small black fungal colonies were present, which increased in number over the next four weeks (week 6). After 8 weeks of exposure, the area colonized by fungi increased noticeably, covering approximately 50 % of the total area of samples. This increase coincided with an increase in the number of rainfall episodes. After 10 weeks exposure, the entire surface of the specimens was colonized by microorganisms. Subsequently there were only small changes in the color of the exposed surfaces. Evolution of wood graying is depicted in Figure 3.7.

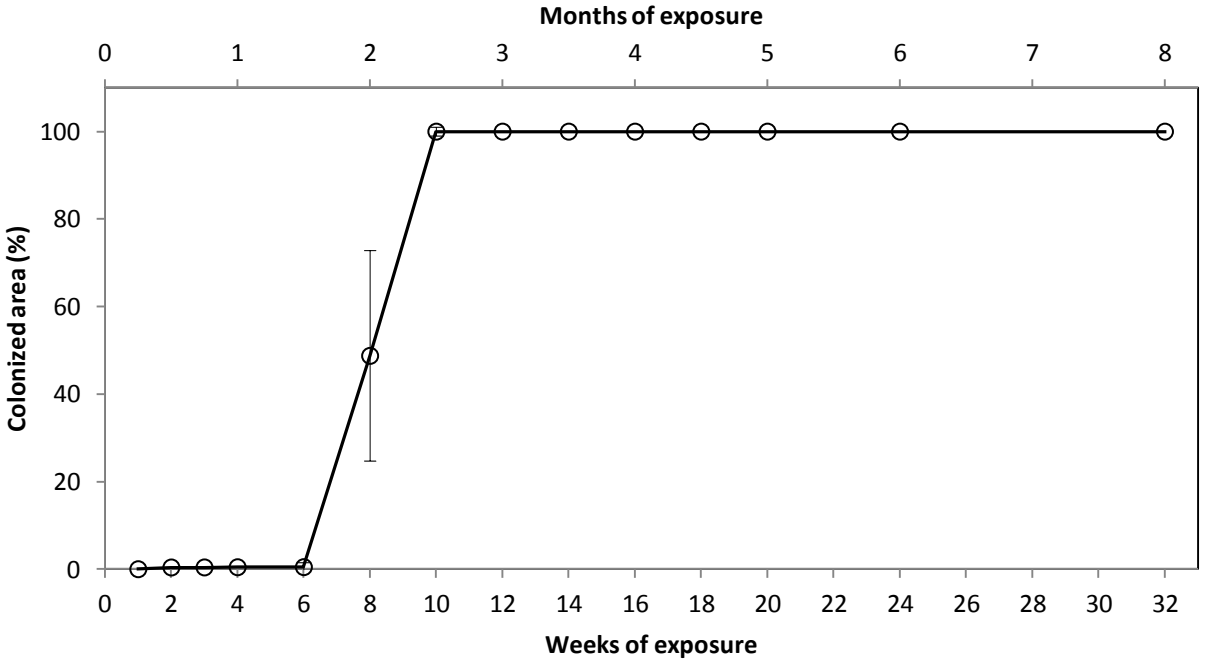


Figure 3.6: Area of southern pine wood samples colonized by fungi during 40 weeks of exposure outdoors. Error bars depict standard deviations

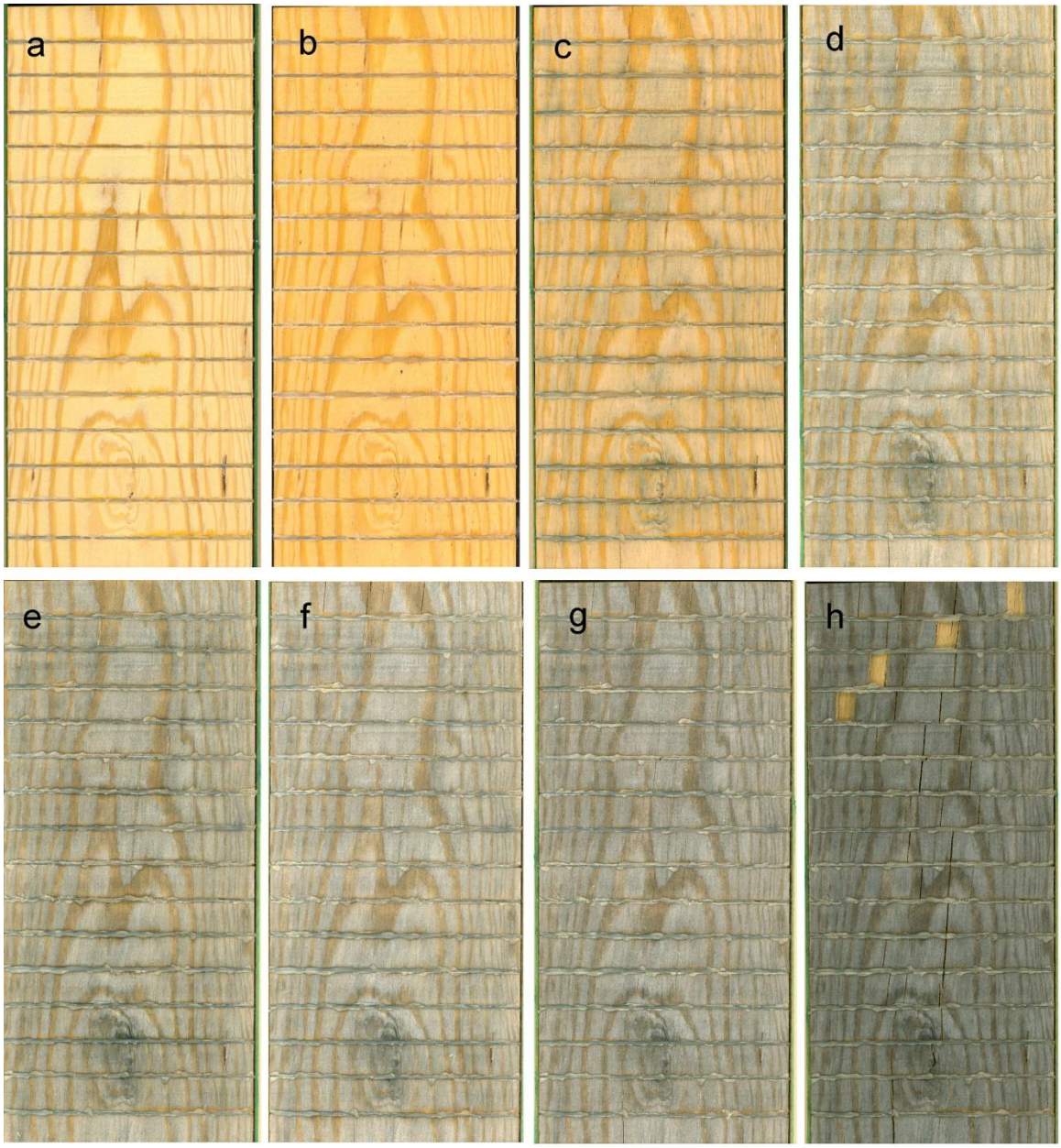


Figure 3.7: Changes in color and colonized area of southern pine wood samples exposed to weather for 40 weeks in Vancouver, Canada. (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

The color at the surface of southern pine specimens expressed as lightness, redness–greenness and yellowness–blueness using the CIELab color space system, was measured throughout the 40 week exposure trial. Color measurements were also made on samples that were kept in a dark conditioning room for the duration of the trial. Samples became darker even after one week of exposure, but then their color remained the same until week 8. Afterwards, there was further darkening which coincided with the increase in colonization of samples by fungi. Lightness plateaued after 14 weeks of exposure (Figure 3.8).

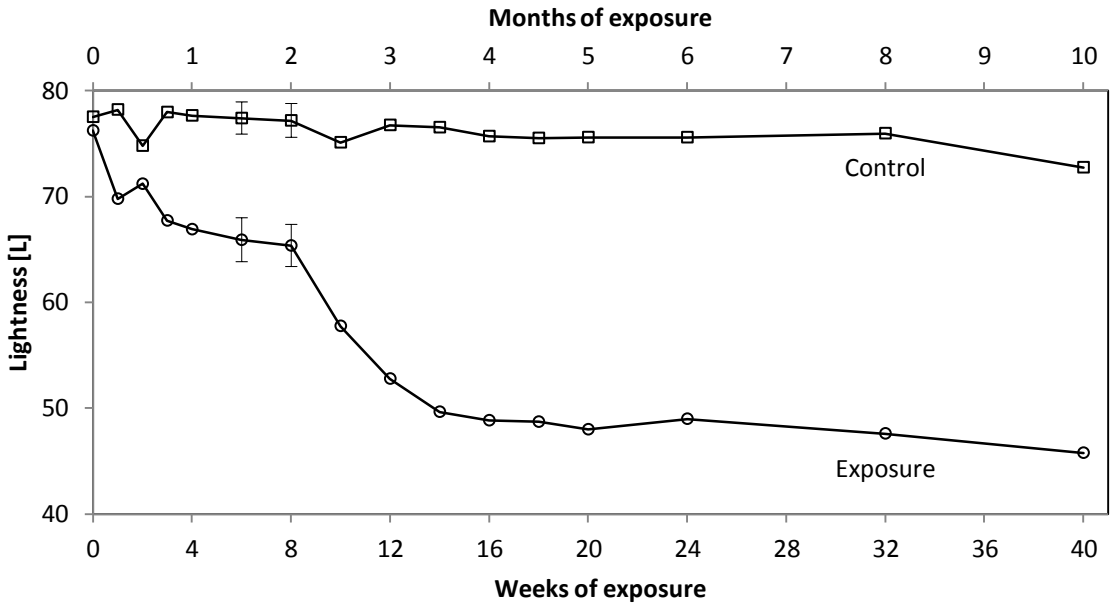


Figure 3.8: Changes in lightness of southern pine wood samples exposed to the weather in Vancouver for 40 weeks. Lightness is expressed using the CIELab system, L [100=white; 0=black]. Error bars depicting standard deviations

Redness–greenness of exposed samples is shown in Figure 3.9. Samples became redder over the first 6 weeks of the trial, but thereafter their redness decreased as they became greener. From week 14 to week 24 the redness/greenness of samples remained relatively

constant, until week 24, when they became greener ([a] decreased). As with lightness, redness–greenness values showed an inflection point close to week 6 corresponding to pronounced staining of wood by fungi.

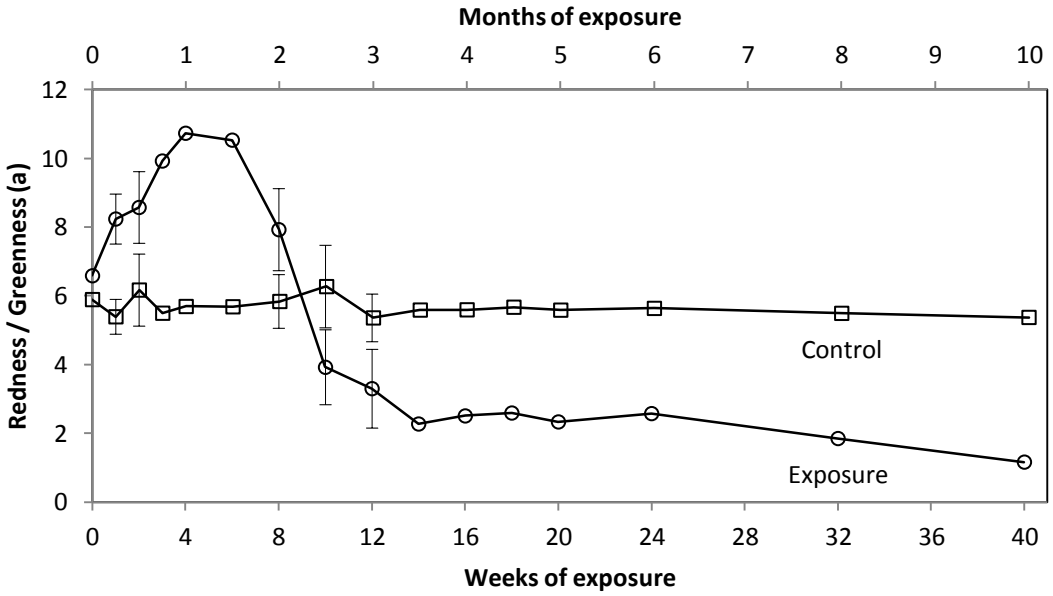


Figure 3.9: Changes in redness/greenness of southern pine wood samples exposed to the weather in Vancouver for 40 weeks. Redness/greenness is expressed using the CIELab system, a [+60=red; -60=green]. Error bars depict standard deviations

Yellowness–blueness [b] values of samples during the exposure trial are depicted in Figure 3.10. Changes in [b] are similar to those of redness. Yellowness increased initially reaching a maximum at the end of the first week and then stayed approximately constant until week 4. Thereafter, yellowness of samples decreased until week 14, when it stayed approximately the same for the remainder of the exposure trial. As with the previous color components, [b] showed an inflection point after 6 weeks corresponding to extensive colonization of samples by fungi.

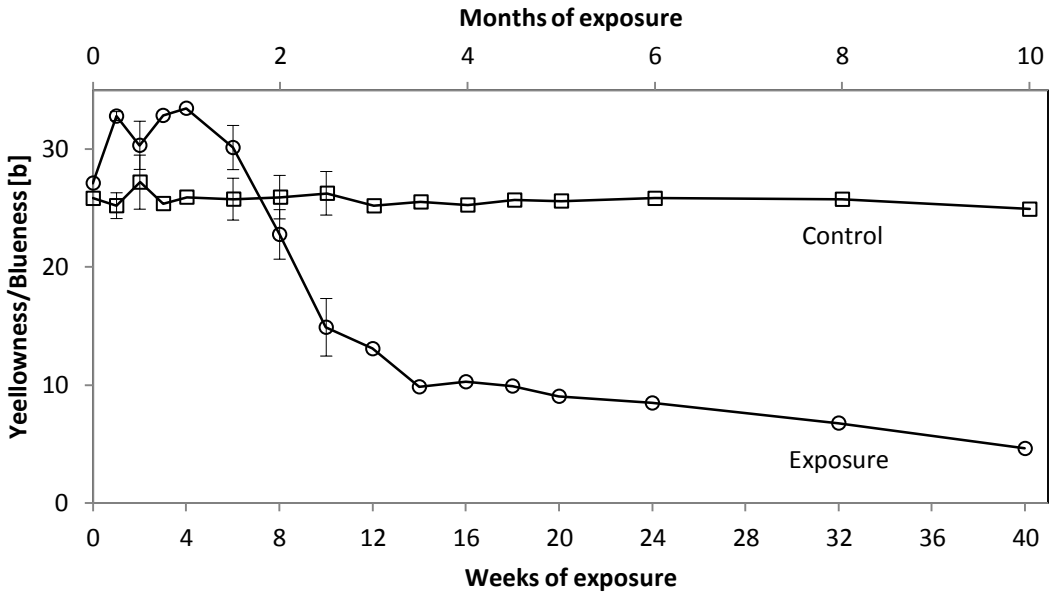


Figure 3.10: Changes in yellowness/blueness of southern pine wood samples exposed to the weather in Vancouver for 40 weeks. Yellowness/blueness is expressed using the CIELab system, b [+60=yellow; -60=blue]. Error bars depict standard deviations

3.3.5. Moisture content

The superficial moisture content of the southern pine wood samples was measured from weeks 10 to 32 of the exposure trial. The moisture content of samples was always below the fiber saturation point (≈ 30% moisture content) and appeared to vary depending on the number and severity of rainfall events (Figure 3.11).

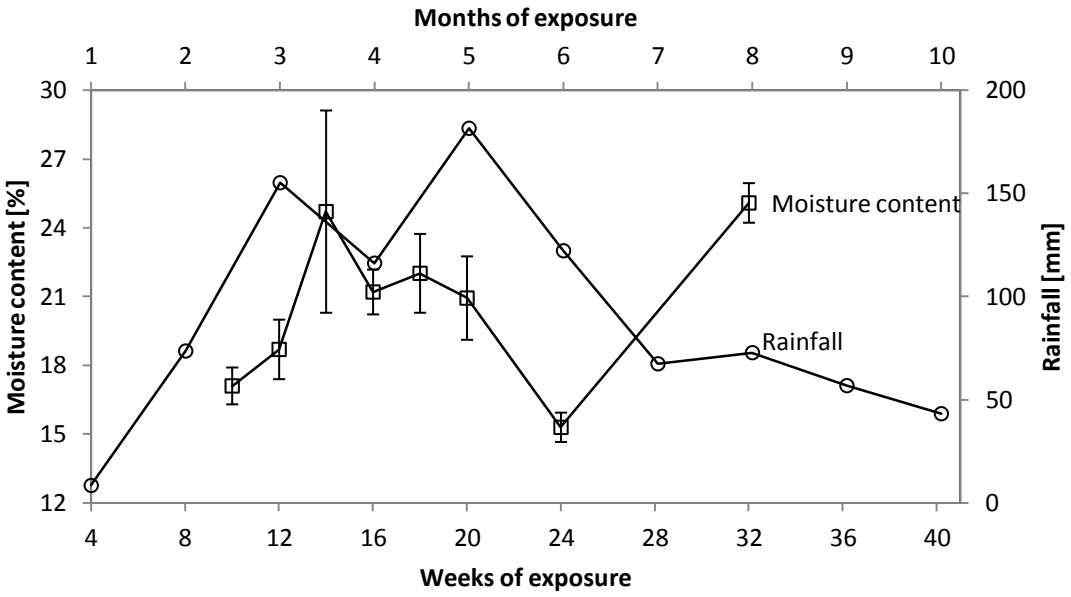


Figure 3.11: Changes in moisture content of southern pine wood samples exposed outdoors for 40 weeks in Vancouver Canada (data available for week 10 to 32). The figure includes the rain that fell (mm) during the exposure trial. Error bars depict standard deviations

3.3.6. FTIR spectra of samples exposed outdoors

FTIR spectra of samples exposed to the weather for 40 weeks and unexposed controls are shown in Figure 3.12.

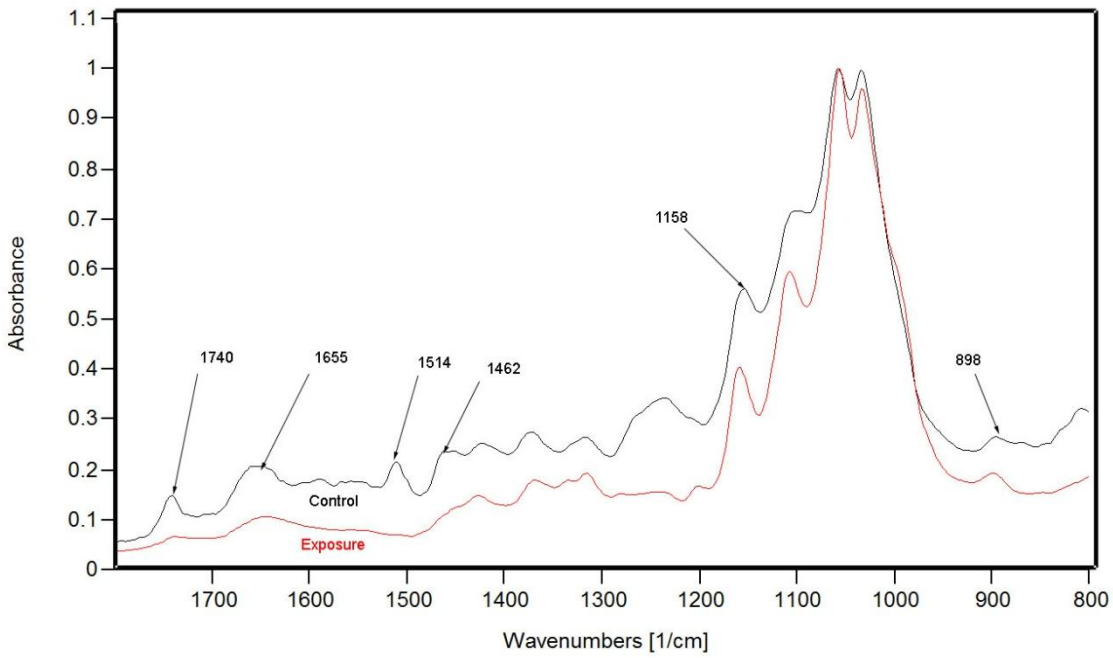


Figure 3.12: FTIR absorbance spectra of southern pine wood surfaces exposed to the weather for 40 weeks and unexposed control. Exposed sample showing decrease of peaks at 1740, 1655, 1514 and 1462 cm^{-1} related to lignin and little change in peaks at 1158 and 898 cm^{-1} related to carbohydrates

After exposure, peaks at 1514 and 1462 cm^{-1} decreased in size in comparison to those in the spectrum of the control. These peaks correspond to stretching vibration of carbonyl groups in lignin benzene rings and C-H deformations in lignin, respectively (Anderson et al. 1991; Pandey and Pitman, 2003). Peaks at 1740 and 1655 cm^{-1} also decreased during weathering. These peaks correspond to conjugated C-O absorptions which typically increase at early stages of weathering and then decrease after extended exposure (Anderson et al., 1991; Pandey and Pitman, 2003; Williams, 2005). These changes indicate a decrease in the lignin content of samples. Conversely, peaks at 898 and 1158 cm^{-1}, corresponding to C-H stretching and C-O-C stretching in pyranose rings in cellulose and hemicelluloses (Huang et al. 2008), showed little change.

3.4. Discussion

The isolation and identification of fungi conducted in this Chapter revealed that only fungi belonging to the ascomycota phylum were able to colonize southern pine wood surfaces exposed outdoors and above ground for 40 weeks in Vancouver, Canada. An average of 5 fungal isolates was recovered per sample (fungal richness), but the number of fungal species expected to be found in each sample was estimated at 4 (average reciprocal Simpson index per sample). Neither of these two parameters (fungal richness and reciprocal Simpson index) have been used before to quantify fungal diversity in wood surfaces exposed outdoors.

A. pullulans, *H. dematioides*, *Epicoccum nigrum* and *Phoma* sp. were the most frequently isolated fungi and they represented more than 70% of the fungal flora. Therefore, they were the main colonizers of weathered southern pine here and they are probably also responsible for the changes in color of wood during weathering. *A. pullulans* has been frequently isolated from weathered wood and coatings, as mentioned in Chapter 2 (Seifert 1964; Dickinson 1971; Amburgey 1974; Schmidt and French 1976; Bardage and Bjurman 1998). Physiological studies on *A. pullulans* have shown that it can metabolize simple sugars and phenolic compounds, which are chemically similar to the photodegradation products of hemicelluloses and lignin, respectively (Bourbonnais and Paice 1987; Schoeman and Dickinson 1996; 1997). Furthermore, *A. pullulans* is able to synthesize a polysaccharide (pullulan) that allows its blastospores to adhere to wood and enhances its asexual reproduction (Bardage and Bjurman 1998). Also, *A. pullulans* produces highly melanized mycelia which is a desirable attribute for a microorganism exposed to UV radiation,

fluctuating temperatures and high intermitant availability of water at weathered wood surfaces (Fogarty and Tobin 1996; Butler and Day 1998; 2001; Henson, Butler, and Day 1999). Microorganisms with these characteristics would be well adapted to weathered wood surfaces. I isolated two varieties of *A. pullulans.* One was darkly pigmented while the other was much lighter. Physiological differences between strains of *A. pullulans* have been reported by Schoeman and Dickinson (1997). They attributed these differences to biological adaptations related to the environments that the strains inhabited. Another fungus, *H. dematioides*, which is similar morphologically to *A. pullulans,* has also been isolated from weathered wood surfaces (Held et al., 2006). It is possible that the two species are physiologically similar, which would explain my observation of the frequent isolation of *H. dematioides* from weathered southern pine samples. *Epicoccum nigrum* and *Phoma* sp. have also been found colonizing weathered wood surfaces (Doi and Horisawa, 2001; Hansen, 2008). These fungi have colorless rather than melanized hyphae. Therefore, they must use a different mechanism to that used by *A. pullulans* and *H. dematioides* to survive at the surface of weathered wood. According to the literature, *E. nigrum* is able to produce black sporodochia (spore aggregations). This structure increases the survival of spores exposed to UV radiation (Barnett and Hunter, 1998; Rotem and Aust, 1991). On the other hand, *Phoma* sp. produces dark structures known as pycnidium. Inside the pycnidium spores are kept safe until released (Barnett and Hunter 1998). Another survival strategy that hyaline fungi might use when colonizing wood exposed outdoors is to grow underneath darker fungi. In weathered wood the dark layer extends to a depth of a few millimeters (Duncan 1963). This dark color is due to the presence of melanized fungi (Dickinson 1971).

The melanin concentrated in this layer may absorb part of the harmful UV radiation that reaches the wood surface. Organisms and also the wood itself, below this layer, may be shielded from UV light and hence sub-surface fungi may not need to be highly melanized to survive. Other fungi isolated during this trial were *Lecythophora* sp., *Truncatella angustata*, *Glonium pusillum* and *Mollisia minutella*. Each of these fungal species was isolated only once. Most of them are recognized pathogens of trees, plants and fruits, and are normally found on wood debris and soil (Sherwood 1973; Crawford et al. 1987; Allmer et al. 2006; Held et al. 2006). *Lecythophora* sp. has also been reported degrading resin acids from lodgepole pine chips, which may help it to colonize wood surfaces (Wang et al. 1995).

Identification of fungi using DNA analysis was particularly valuable because there was little information on some of the fungal species growing in exposed wood surfaces, and also for the identification and separation of *H. dematioides* sp. and *A. pullulans* sp. These two species are very difficult to identify and separate using their morphological features (Ray et al. 2004). Some other fungi had distinctive morphological characteristics and were easily recognized under the microscope. Hence, it was not necessary to use DNA analysis to identify them. Identification of fungal species isolated only once was difficult. In such cases, DNA sequencing was essential. Identification of organisms using more complex molecular techniques, for example, sequencing of specific genes, can be very accurate (Tsui et al. 2010), and makes the use of microscopy redundant. Nevertheless, such techniques are costly and consequently they are normally limited to very specific situations. In contrast, in this chapter, DNA identifications were achieved by sequencing only the DNA strand amplified by the primer ITS4. This approach did not decrease the efficiency of the

technique, but made it less expensive, since overall costs for sequencing were reduced by fifty percent. Therefore, the use of basic DNA identifications complemented those achieved using microscopy and this combined approach proved to be a suitable and affordable way to identify microorganisms colonizing weathered southern pine wood.

Previous research on fungal flora colonizing wood surfaces exposed outdoors in Vancouver has focused on fungi that colonize western red cedar. This wood is widely used for outdoor applications due to his natural durability (Wethern, 1959). Comparison of fungal species isolated here in southern pine with those isolated from western red cedar revealed that certain fungi colonize both wood species. For example, Smith and Swann (1976) isolated and identified fungi colonizing western red cedar shingles exposed for 5 to 28 years outdoors. From a total of 708 isolates approximately 14 different genera were isolated. *Philophora* and *Rhinocladiella* were the most frequently isolated genera, but also *A. pullulans*, and *Cladosporium* spp., as well as species of basidiomycetes, actinomycetes and bacteria were also frequently isolated. In two other studies Lim et al. (2005; 2007) found a wide range of basidiomycetes and ascomycetes growing on western red cedar decks and fences. They frequently isolated *A. pullulans* from western red cedar.

Studies performed outside Vancouver support the ability of the fungi I isolated to colonize weathered wood and also tolerate diverse climatic conditions. For example, Cronin et al. (2000) isolated and identified fungi responsible for the graying of white cedar (*Thuja occidentalis* L.) shingles in maritime climates. They isolated fungi directly from wood pieces and identified by microscopy *A. pullulans*, *Alternaria* sp., *Penicillium* sp. and *Cladosporium* sp. Sudiyani et al. (2002) exposed several tropical wood species outdoors in Indonesia and

isolated the moulds colonizing the woods. Identification of organisms was performed by light microscopy. Fourteen different fungal genera were identified in the subphylum ascomycotina, 3 were basidiomycetes and 1 was an actinomycete, but also several organisms were unidentified. Like here, *Aureobasidium* and *Cladosporium* were frequently isolated. In the extreme conditions of Antarctica, Held et al. (2006) was able to isolate fungi from 5 different genera. Through microscopy and DNA analysis they were able to identify *Cadophora*, *Cladosporium*, *Hormonema*, *Lecythophora* and *Penicillium* species (Held et al., 2006). Three species from these 5 genera were isolated here in southern pine, indicating the ability of these species to withstand adverse climatic conditions.

Hence, my results are in partial agreement with those of other studies because the fungi isolated from southern pine wood samples here have been found colonizing a variety of wood substrates exposed outdoors, not only in Vancouver, but also in diverse locations and climates around the world. Differences between my results and those of other studies, e.g. number of genera and species isolated and absence of basidiomycetes, may be attributed to differences in substrates, climate, size and methods for sampling and length of time that samples were exposed to the weather. Sampling method in particular may have influenced the results obtained by several authors in the past. The method of sampling used here was selected according to the target organisms that I was seeking to isolate. For example, in my samples, fungi with the ability to grow in the thin layer of weathered wood were of interest. Therefore an appropriate method of sampling this layer was chosen. Other sampling methods, for example scratching or swabbing the surface may have inflated the number of fungi isolated by other studies because these techniques can isolate fungi (via mycelia and

spores) that are opportunistically present at the wood surface, but do not colonize weathered wood.

Changes in the color of southern pine wood exposed outdoors appear to be due initially to photodegradation of wood and thereafter to colonization of the surface by fungi. Photodegraded wood surfaces turned red and yellow initially probably because of photo-oxidation of lignin and the accumulation of unsaturated aromatic compounds in the wood (Feist and Hon, 1984; Gellerstendt and Gierer, 1975). Accordingly, FTIR spectroscopy showed a decrease in the functional groups assigned to lignin (1514 cm^{-1} stretching vibration of carbonyl groups in benzene rings and 1462 cm^{-1} C-H deformations in lignin, Anderson et al. 1991; Pandey and Pitman 2003) and a relative increase in the groups assigned to cellulose (898 cm^{-1} C-H stretching and 1158 cm^{-1} C-O-C stretching in pyranose rings in cellulose and hemicelluloses, Huang et al. 2008). After 8 weeks of exposure wood surfaces became darker (L decreased). This color change coincided with significant colonization of the weathered surface by fungi. Later, after 14 weeks of exposure, the darkening of the wood surface tended to stabilize, coinciding with the complete staining of the wood surface by fungi. The two main fungi isolated from weathered wood were black, supporting previous suggestions in the literature that the graying of wood exposed outdoors is due to colonization of weathered wood surfaces by fungi.

The diversity and types of fungi colonizing wood exposed outdoors must be taken into account when developing treatments to prevent the unwanted graying of wood exposed outdoors. The organisms isolated most frequently here (and by other related studies) should be used in bioassays to test the effectiveness of biocides at preventing the fungal

staining of weathered wood. Complementary experiments should be performed to increase our understanding of the effects that ascomycetes fungi have on the properties of wood surfaces. For example, some ascomycetes isolated from wood surfaces are regarded as soft-rot fungi (Savory 1954; Rajderkar 1966; Bugos et al. 1988; Zabel and Morrell 1992; Lim et al. 2005; Lopez et al. 2007). There have been no studies that have examined in detail whether fungi colonizing weathered wood can cause significant degradation of the wood. Hence, the next chapter (Chapter 4) examines whether the fungi isolated from weathered southern pine wood here are able to cause significant degradation of wood.

3.5. Conclusions

The combination of molecular techniques and microcopy can complement each other making identification of fungi isolated from weathered wood surfaces faster, more affordable and accurate. Furthermore, identification of fungi (to the level of genus) is possible using these methods without the need for highly trained personnel.

Ascomycete fungi dominated the fungal flora isolated from southern pine wood exposed outdoors for 40 weeks in Vancouver, Canada. *A. pullulans*, *H. dematioides*, *Epicoccum nigrum* and *Phoma* sp. were the fungi most frequently isolated from weathered southern pine wood. It is likely that these microorganisms posses adaptations that enable them to survive at weathered wood surfaces. These adaptations may include high level of melanization, abilities to metabolize wood extractives, sugars and photodegradation product, and appropriate reproductive strategies.

Ascomycete fungi colonizing wood surfaces exposed outdoors are responsible for the graying of weathered wood (as other have noted), but color changes at wood surfaces, during the first weeks of outdoor exposure (0 to 8 weeks) involve yellowing and reddening, which is probably due to photodegradation of lignin. Color changes related to fungal colonization became more pronounced after approximately 8 weeks of outdoors exposure outdoor, and complete graying of the surface occurred after 14 weeks exposure. The fungi responsible for such graying are the black fungi that were frequently isolated here, *A. pullulans* and *H. dematioides*.

Chapter 4: Decaying abilities of fungi isolated from weathered wood

4.1. Introduction

Fungi colonizing weathered wood surfaces include a broad spectrum of micro-organisms, but wood decaying basidiomycetes do not seem to predominate (Duncan, 1963; Seifert, 1964; Sell and Wälchli, 1969; Dickinson, 1971; Feist, 1990). The fungi colonizing weathered wood disfigure the wood to a depth of a few millimeters (Duncan 1963; Dickinson 1971; Savory 1973), but there is a body of opinion that suggests that they are unable to degrade the wood (Feist 1983). This opinion is underpinned by studies which have failed to detect soft-rot cavities in the walls of tracheids at weathered wood surfaces (Evans, 1989; Paajanen, 1994) and the fact that environmental conditions at weathered wood surfaces are generally unfavorable for microbial degradation (Evans 2008). However, Smith and Swann (1976) have a different opinion. Their histological studies on weathered western red cedar shingles found evidence of soft-rot cavities and enzymatic erosion of wood cell walls. Furthermore, cellulolytic and lignolytic fungi that have the ability to produce soft-rot decay are frequent colonizers of weathered wood (Savory 1954; Rajderkar 1966; Bugos et al. 1988; Zabel and Morrell 1992; Lim et al. 2005). Therefore, it seems reasonable to assume that under certain circumstances, fungal degradation of wood surfaces (particularly the occurrence of soft-rot decay) may occur when wood weathers. In addition, such degradation might be enhanced by the photo-induced delignification of wood surfaces as suggested by Evans and Banks (1986).

The techniques used to assess soft-rot decay such as microscopy and measurement of weight loss are not very good at detecting the early stages of soft-rot. In contrast, measurement of wood strength losses is far more sensitive to early decay (Wilcox, 1978; Morrell and Zabel, 1985; Sexton et al., 1993; Nicholas and Jin, 1996). In this chapter, I hypothesize that some of the fungi isolated from weathered wood will be able to degrade wood tissues and such degradation will lead to losses in the mechanical properties of wood. To test this hypothesis a range of fungi isolated from weathered wood surfaces (in Chapter 3) were screened for their ability to produce cellulolytic and lignolytic enzymes. Then, they were used in a bioassay, which measured changes in mechanical properties of wood exposed to the different fungi. In addition other techniques including dynamic mechanical analysis, FTIR spectroscopy and light and scanning electron microscopy were used to examine whether fungi were able to break down the wood, and identify the type of degradation caused by the fungi (if any).

4.2. Materials and methods

4.2.1. Fungal screening

Fungi isolated from weathered wood in Chapter 3 were screened for their ability to synthesize lignolytic and cellulolytic enzymes *in-vitro* (Table 4.1). Laccase producing organisms were indentified by their ability to breakdown the aromatic compound guaiacol, a widely used lignin model (Kiiskinen et al. 2004). When fungi are inoculated on solid media containing guaiacol, fungi able to produce laccase form reddish-brown halos around their mycelia, as their lignollytic enzymes breakdown the guiacol (Figure 4.1a) (Kiiskinen et al. 2004). Five mm (diameter) agar plugs from different cultures of surface fungi were transferred onto 150 mm x 15 mm Petri dishes with solid media containing: peptone (3 g/l), glucose (10 g/l), KH_2PO_4 (0.6 g/l), $ZnSO_4$ (0.001 g/l), K_2HPO_4 (0.4 g/l), $FeSO_4$ (0.0005 g/l), $MnSO_4$ (0.05 g/l), $MgSO_4$ (0.5 g/l), agar (20 g/l) and guaicol (0.2 g/l) (Viswanath et al. 2008). The enzymatic activity after one week of growth was ranked visually according to the intensity and extension of the reddish-brown halos as follows: (1) negative (-); (2) low (+); (3) medium (++); and (4) high (+++). On the other hand, the ability of surface fungi to produce cellulolytic enzymes was tested using a carboxymethyl cellulose (CMC) assay (Peciulyte 2007). In this CMC assay, fungi are grown on solid media containing CMC as a sole source of carbon. During this assay cellulolytic enzymes break down the CMC. The enzymatic reaction can be visualized by adding Congo red dye to the growth medium. Congo red strongly bonds to contiguous β-(1-4)-bound-D-glucopyranosyl units (Sazci et al. 1986). At the end of the bioassay Congo red is removed from the medium using a solution

of 1M NaCl, but yellower halos remain in areas where cellulolytic enzymes were active. Enzymatic activity is quantified using an index for enzyme activity for CMC (Icmc), as follows: Icmc = Clear or yellower halo diameter/Fungi colony diameter (Peciulyte 2007). Specifically in my experiment 5 mm (diameter) agar plugs from the original cultures were transferred onto 150 mm x 15 mm Petri dish with solid media containing: NH_4NO_3 (1.6g/L), Na_2HPO_4 (0.5g/L), K_2HPO_4 (0.65 g/L), $MgSO_4.7H_2O$ (3 g/L), $CaCl_2.2H_2O$ (0.4 g/L), yeast extract (0.3 g/L), Triton X100 (0.1 g/L), agar (15 g/L) and CMC (10 g/L). After a period of incubation for 14 days, cultures were flooded with Congo red dye (1% aqueous solution) and 1M NaCl for 15 and 20 minutes, respectively. Diameter of fungi colonies and clear halos were calculated using image analysis of digital pictures. Digital images of the fungal colonies on each plate, 1:1 scale; under standard conditions of illumination were obtained using a desktop scanner (Microtek Scan Maker i800). The diameter of each hyphal mat and clear halos were digitally measured with the ruler tool of the software Adobe Photoshop CS3 Extended, version 10.0.1 (Adobe System Incorporated, USA), Figure 4.1b.

Fungi showing strong enzymatic activity and those most frequently isolated from weathered wood were selected for subsequent experimentation. White-rot and brown-rot decay fungi, and a known soft-rot fungus were used as controls.

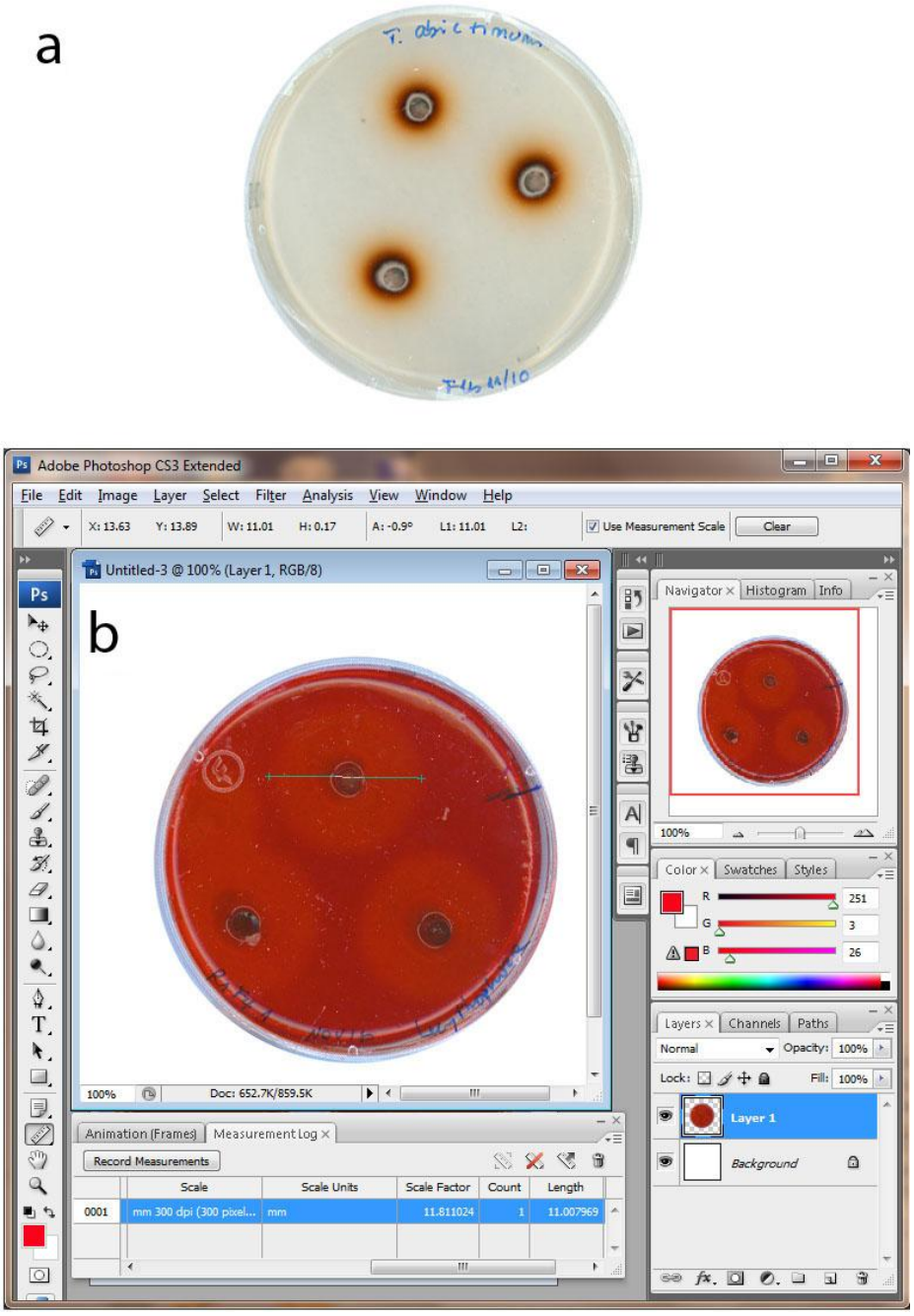


Figure 4.1: Fungal screening: (a) *Trichaptum abietinum* after seven days of growth on media containing guiacol (0.2 g/L), the enzymatic activity of the fungus was ranked as high (+++); (b) carboxymethyl cellulose (CMC) assay; measurement of halo diameter using the ruler tool of Photoshop. The fungus in the image is *Lecythophora* sp. after 14 days of growth in media containing CMC 10 (g/L) stained with Congo red

Table 4.1: Fungi tested for their ability to synthesize lignolytic and cellulolytic enzymes

No	Fungi	Strain(s) tested
1	*Allantophomopsis lycopodina* (Höhn.) Carris	1
2	*Alternaria* sp.	5
3	*Aureobasidium pullulans* (de Bary) G. Arnaud (black)	6
4	*Aureobasidium pullulans* (de Bary) G. Arnaud (white)	6
5	*Botryosphaeria stevensii* Shoemaker	1
6	*Botryotinia fuckeliana* (de Bary) Whetzel	4
7	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries	5
8	*Cladosporium* sp.	1
9	*Coniochaeta ligniaria* (Grev.) Massee	2
10	*Epicoccum nigrum* Link	5
11	*Epicoccum* sp.	1
12	*Glonium pusillum* H. Zogg	1
13	*Hormonema dematioides (Lagerb. & Melin)*	7
14	*Lecythophora* sp.	2
15	*Leptosphaerulina chartarum* Cec. Roux	1
16	*Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons	2
17	*Mollisia minutella* (Sacc.) Rehm	1
18	*Penicillium expansum* Link ex. Thom	1
19	*Peniophora aurantiaca* (Bresadola) von Höhnel & Litschaue	1
20	*Phialocephala* sp.	1
21	*Phialophora* sp.	2
22	*Phoma* sp.	5
23	*Rhizopogon* sp.	1
24	*Trichoderma viride* Pers.	1
25	*Truncatella angustata* (Pers.) S. Hughes	1
26	*Valsa ambiens* (Pers.) Fr.	1
27	*Trichaptum abietinum* (Pers.) Ryvarden (white-rot control)	1
28	*Coniophora puteana* (Schum. ex Fries) Karst. (brown-rot control)	1

4.2.2. Decay test

4.2.2.1. Experimental design

An experiment was designed to test the effect of fungi isolated from weathered wood on the tensile properties of two wood species. Twelve 'blocks' provided replication at the higher level. Each block included 18 treatments (17 fungi plus a control), which were randomly assigned to 18 Petri dishes. The internal area of each Petri dish was subdivided into two; a hardwood (lime, *Tilia vulgaris* Hayne) and a softwood (White spruce, *Picea*

glauca, Moench (Voss)) were randomly assigned to the two sectors within each dish. The resulting split-plot design accounted for random variation in fungal growth and wood properties. Analysis of variance (ANOVA) was used to examine the effect of fungal species and wood species and the interactions of these factors on the mechanical properties of thin wood veneers (see below). The analysis of data was performed using the software Genstat v. 12 (VSN International 2009). The assumptions of ANOVA were tested prior to the final analysis (normality of residuals and homogeneity of variances). After ANOVA ($p<0.05$), significant differences were estimated using Fisher's least significant test (l.s.d.). Results are presented in graphs featuring means and either standard error of the differences (s.e.d.) or l.s.d bars for the different tested parameters. The detailed output of the statistical analyses in this chapter is appended to this thesis (Appendix 1). A summary of the experimental design is presented in Table 4.2.

Table 4.2: Summary of the experimental design used for the decay test

Blocks	Fungal species	Wood species	Petri dishes
1	17 + control	2	18
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
12	17 + control	2	18

4.2.2.2. Wood samples

Two non-durable wood species were used as test substrates for the bioassay. White spruce was selected because of its susceptibility to fungal degradation and homogeneous properties (Forest Products Laboratory 1999). Lime wood was selected because previous

work demonstrated that thin wood veneers from this wood species can be successfully used to detect degradation of fungi when tested in tension (Evans and Banks 1986). Wood veneers were cut from white spruce and lime using the method described by Evans (1988). Blocks measuring 18 mm (radial) x 25 mm (tangential) x 85 mm (longitudinal) were cut from five different lime and white spruce boards. These blocks were soaked in distilled water for 5 days. Individual blocks were firmly clamped in a custom-made sample holder attached to a sliding microtome (Spencer Lens Co. Buffalo, USA; Figure 4.3a) with the radial face uppermost. Eighty micrometers (80 µm) veneers were cut from each block using a disposable stainless steel blade (Type S35, Feather Safety Razor Co., Japan) mounted in a blade holder. Veneers were placed on glass plates and clamped at their ends using strips of Perspex and butterfly clips. Restrained veneers were air dried in a conditioning room at 20°C ± 1°C at 65% ± 5% r.h. for seven days. Each veneer was labeled using a pencil and their thickness and weights were measured with a digital micrometer (Lorentz & Wettre HWS 5781) and an analytical balance A & D (Model GR-200 from B.C. Scale Co. Ltd; 210 g x 0.0001 g), respectively. Veneers were then oven dried (100 ± 5°C) for 24 hours to a constant weight (as above) and sterilized in autoclave at 121°C and 103.4 kPa for 20 min. Veneers were re-hydrated by soaking them in nano-pure sterile water under sterile conditions.

The effect of fungi on the microstructure of wood used small lime and white spruce samples. These samples measured 35 mm (longitudinal) x 12 mm (radial) x 2.5 mm (tangential), and were cut and planed from parent boards and then labeled with pencil. They were then conditioned for 14 days, oven dried until they reached constant weight,

sterilized in an autoclave, and re-hydrated with nano-pure water under sterile conditions (as above).

4.2.2.3. Fungal inoculation and incubation

Black colored and control fungi were tested for their ability to breakdown wood veneers and solid wood samples. Three or two or sometimes one isolate were used per treatment. Not all of the test fungi were able to produce spores on solid media. Therefore, fungi were inoculated from aqueous solutions containing a known and standard concentration of fungal mycelia. To obtain such solutions 1% w/v malt extract agar (MEA) – Difco Petri dishes, overlaid with a layer of cellophane were inoculated with five agar plugs (5 mm in diameter) from original fungal cultures. After two weeks when fungi had completely covered the cellophane layer the fungal mycelia was collected in 1.5 mL screw-cap tubes using a sterile scalpel. Then, 500 µL of nano-pure water was added to the tube and mycelia were crushed using a sterile stainless steel rod and the solution was stirred for 3 minutes at 100 rpm. Crushed mycelia was then transferred to 50 mL falcon tubes and diluted with nano-pure water until a total volume of 40 mL was obtained. The dry weight of fungal mycelia in 3 mL of solution was used to estimate fungal biomass per mL. Later, fungal biomass concentrations were adjusted to 2.13×10^{-4} g/mL. Petri dishes (150 mm x 15 mm) with 1% MEA and cellophane were then inoculated with 1000 µL of fungal solutions. The inoculum was evenly spread over the cellophane using a glass rod. Inoculated cultures were left for approximately 48 hours until clear signs of new mycelial growth was noted. Then the cellophane sheets were transferred onto new plates containing the following mineral media

designed to encourage soft-rot fungal decay: NH_4NH_3 (6 g/L), K_2HPO_4 (4 g/L), KH_2PO_4 (5 g/L), $MgSO_4$.7H2O (4 g/L), thiamine HCl (0.02 g/L) and agar (15 g/L) (Leightley 1980). Wood veneers and solid wood samples were allocated to segments inside the Petri dishes, as mentioned above. The dishes were then sealed using plastic foil (The Glad Company, USA) and incubated for 12 weeks under sterile conditions at 20°C in dark room, Figure 4.2.

4.2.2.4. Mechanical property losses of veneers

All veneers exposed to fungi for 12 weeks were conditioned (as above) for 14 days. Tensile strength (ability to resist an applied stress in tension) tests were carried out using an Instron Universal Tension Tester (model 5565, Figure 4.3b) using 20 mm/min cross-head speed and 38.1 mm span-length. Data collected from each test were used to plot stress-strains curves for each veneer (see Appendix 2). Stress (amount of force for a given area unit) and strain (deformation per unit of the original length) were calculated as follows (Bodig, 1982):

Stress = force applied / Area tested

Strain = displacement / original length

Stress-strains curves on graphs were used to determine: (1) peak tensile stress (PTS, maximum tensile stress value) and (2) modulus of elasticity (MOE, slope of the curve). PTS and MOE were used to calculate the peak work done, which is equivalent to the maximum toughness (ability of the material to absorb and distribute energy within itself) of the

samples (PWD, peak toughness), and peak stiffness (PS, maximum stiffness), as follows (Bodig, 1982):

$$PWD = PTS^2 / (2 \times MOE)$$

$$PS = \text{Peak force applied / peak displacement}$$

Mechanical property losses results of veneers are expressed as the ratio of matched controls.

4.2.2.5. Fourier transform infra-red spectroscopy

Fourier transform infra-red spectroscopy was used to examine chemical changes at the surface of wood veneers exposed to fungi. A small piece of veneer measuring 10 mm (tangential) x 10 mm (longitudinal) was cut from the parent veneer using scissors. Pieces of veneers were stored for 5 days in a vacuum desiccator over silica gel. Direct reflectance (ATR-IR) FTIR spectra of veneers surfaces were obtained using a single bounce attenuated total reflectance accessory, as described in Chapter 3 (section 3.2.7).

4.2.2.6. Viscoelastic properties

The viscoelastic properties of solid wood samples were quantified because of their sensitivity to small polymeric changes such as those produced by enzymatic fungal degradation. The dynamic elastic response or storage modulus (SM) of solid wood samples

exposed to fungi that caused the greatest losses in tensile strength was measured. Solid wood samples measuring 35 mm (longitudinal) x 12 mm (radial) x 2.5 mm (tangential) were reduced in size to 1 (tangential) x 3 (radial) x 25 (longitudinal) mm and tested in a dynamic mechanical analyser (DMA, Perkin Elmer model DMA 7e, Figure 4.3c). The test was performed as follows: (1) double cantilever bending geometry; (2) 20 mm span-length; (3) temperature range of 25 to 200°C with a heating rate of 5°C/min; (4) frequency 1Hz; and (5) ratio static/dynamic charge 550/500 mN.

4.2.2.7. Microscopy

The microstructure of solid wood samples exposed to fungi was examined using light microscopy. Pieces of wood measuring 10 mm (radial) x 2.5 mm (tangential) x 10 mm (longitudinal) mm were cut from the surface of exposed lime and spruce specimens and soaked in distilled water for 2 days. Each water-saturated block was clamped in a microtome (as above) and 20 µm sections were cut from the block using a disposable stainless steel blade (Type S35, Feather Safety Razor Co., Japan) bolted to a microtome blade-holder. Sections were dehydrated in ethanol (industrial grade) for 2 days and then soaked in a saturated solution of safranin (BDH Chemical Ltd, England) in ethanol for 2 days. Each stained section was placed on a droplet of DPX (dibutyl phthalate xylene) mountant (Fluka Analytical, Germany) on a glass slide measuring 76 mm x 26 mm x 1 mm (Matsunami Glass Ind. Ltd. Japan), covered with a glass cover slip, 22 mm x 40 mm x 0.20 mm in size (Fisher Finest Premium Cover Glass, Fisher Scientific, Pittsburgh, USA). Slides were dried at room temperature for 48 hours. The sections were examined using a light microscope (Carl Zeiss,

Germany) at various magnifications. An Olympus DP71 digital camera attached to the microscope was used to take photographs of fungi colonizing wood.

Scanning electron microscopy (SEM) was used to examine structural changes in veneers exposed to fungi. A small piece of veneer measuring 5 (radial) mm x 5 (longitudinal) mm, was cut from the parent veneer using scissors and glued to aluminum stubs using nylon nail polish as an adhesive. The stubs containing the veneers were stored for 5 days in a vacuum desiccator over silica gel. The stubs were coated with a 10 nm layer of gold using a sputter coater (Nanotech SEMPrep II) and then examined using a Zeiss Ultraplus field emission scanning electron microscope at an accelerating voltage of 5 kV. Secondary electron images of veneers were obtained and saved as TIFF files.

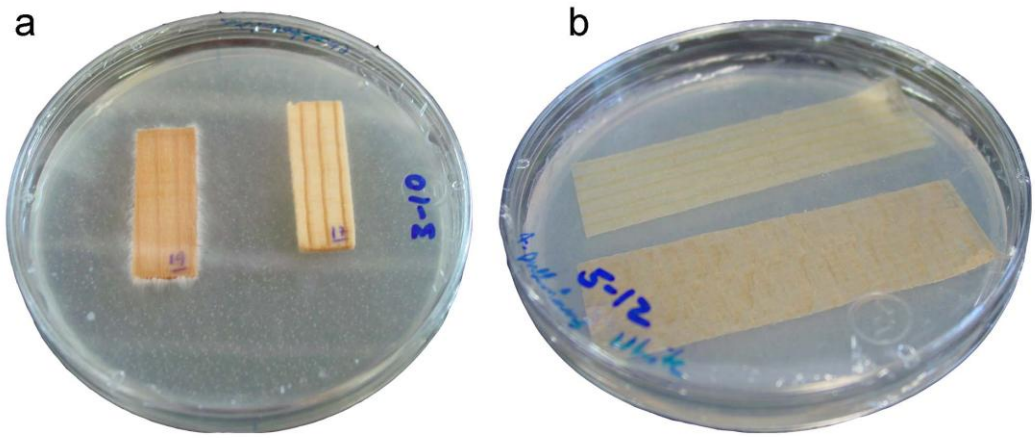


Figure 4.2: Wood samples after 1 week of exposure to fungi: (a) solid wood samples; (b) wood veneers

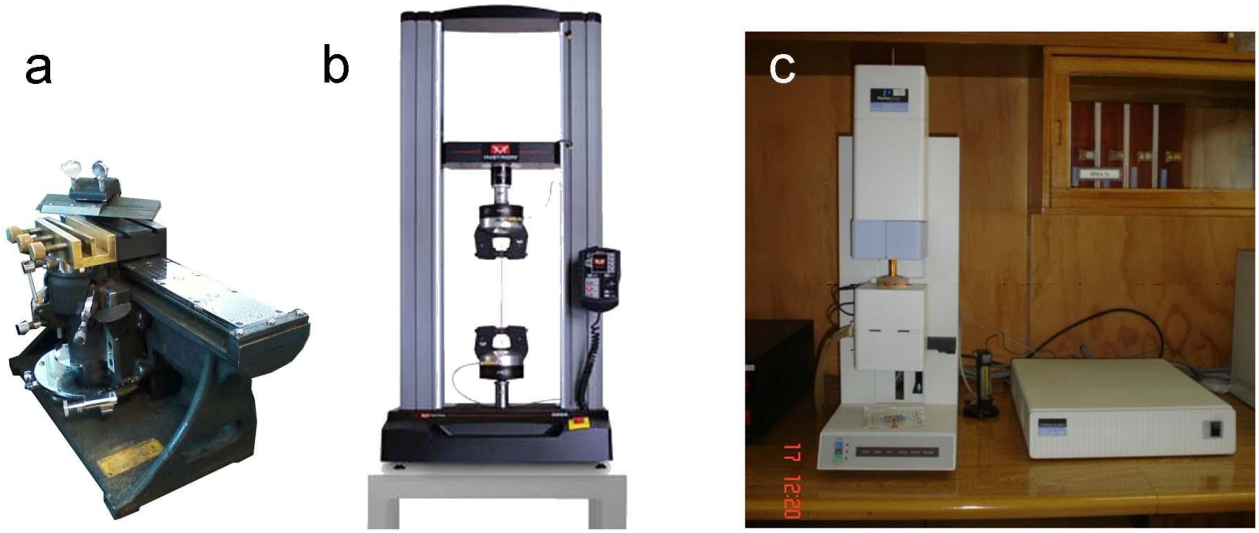


Figure 4.3: Equipment for sample preparation and testing; (a) sliding microtome with blade holder and clamping device for wood samples; (b) Instron Universal tensile tester (model 5565) and; (c) Dynamic mechanical analyzer (Perkin Elmer model DMA 7e)

4.3. Results

4.3.1. Fungal screening

The results for lacasse activity and index for enzyme activity of fungi on CMC are shown in Table 4.3. Five fungi showed lignolytic activity, while 24 out of 28 exhibited cellulolytic activity on CMC. The enzymatic activity of the fungi and their frequency of isolation on weathered wood (Chapter 3) and in other studies were used as criteria to select fungi for the decay test described below. Selected organisms are shown in Table 4.4.

Table 4.3: Laccase activity and index for enzymatic activity for carboxymethyl cellulose (CMC)

No	Fungi	Strains tested	Laccase activity† after 12 days	Icmc* after 7 days
1	*Mollisia minutella*	1	+++	5.00
2	*Rhizopogon* sp.	1	-	5.00
3	*Coniophora puteana* (brown-rot control)	1	-	5.00
4	*Phialophora* sp.	2	-	2.43-2.59
5	*Coniochaeta ligniaria*	2	-	2.23-2.55
6	*Lecythophora* sp.	2	+++	2.15-2.22
7	*Penicillium expansum*	1	-	2.04
8	*Valsa ambiens*	1	-	1.96
9	*Botryosphaeria stevensii*	1	-	1.82
10	*Aureobasidium pullulans* (white)	6	-	1.62-2.84
11	*Aureobasidium pullulans* (black)	6	-	1.56-1.94
12	*Cladosporium cladosporioides*	5	-	1.51
13	*Botryotinia fuckeliana*	4	+++	1.5-1.94
14	*Phoma* sp.	5	-	1.44-1.99
15	*Lewia infectoria*	2	-	1.38-1.48
16	*Glonium pusillum*	1	-	1.33
17	*Peniophora aurantiaca*	1	-	1.29
18	*Cladosporium* sp.	1	-	1.31-1.63
19	*Epicoccum nigrum*	5	-	1.26-1.3
20	*Epicoccum* sp.	1	-	1.21
21	*Leptosphaerulina chartarum*	1	-	1.21
22	*Alternaria* sp.	5	-	1.17-1.25
23	*Truncatella angustata*	1	-	1.15
24	*Trichoderma viride*	1	-	1.01
25	*Allantophomopsis lycopodina*	1	-	0.00
26	*Hormonema dematioides*	7	-	0.00
27	*Phialocephala* sp.	1	+++	0.00
28	*Trichaptum abietinum* (white-rot control)	1	+++	0.00

†Rank of enzymatic activity: negative (-); low (+); medium (++); high (+++)

*Icmc: index for range of enzyme activity on carboxymethyl cellulose

Table 4.4: Fungi isolated from weathered wood and tested for their ability to breakdown wood

Treatment	Fungi	Code Name	Strains tested
1	*Alternaria* sp.	Alt.	3
2	*A. pullulans* (black)	Aur. (black)	3
3	*A. pullulans* (white)	Aur. (white)	3
4	*B. fuckeliana*	Botr.	3
5	*Cladosporium* sp.	Clad.	3
6	*C. ligniaria*	Conio.	2
7	*E. nigrum*	Epic.	3
8	*H. dematioides*	Horm.	3
9	*Lecythophora* sp.	Lecyth.	1
10	*L. infectoria*	Lew.	3
11	*M. minutella*	Moll.	1
12	*Phialocephala* sp.	Phialoc.	1
13	*Phialophora* sp.	Phialop.	2
14	*Phoma* sp.	Phom.	3
15	*T. abietinum* (white-rot control)	Trich.	1
16	*C. puteana* (brown-rot control)	Coniop.	1
17	*Chaetomium globosum* Kunze ex Fr. (soft-rot control)	Chaet.	1

4.3.2. Decay test

4.3.2.1. Mechanical property losses of veneers

Analysis of variance showed a significant effect (P-value < 0.001) of fungal species (F), wood species (W) and interaction of FxW, on the different mechanical properties of spruce and lime veneers tested in tension (peak tensile stress ratio, modulus of elasticity (MOE) ratio, peak stiffness ratio, and toughness ratio). Table 4.5 shows the statistical significance (P-values) of experimental variables (fungi, wood species) and interaction of fungi with wood species on the different response variables. Since in some cases interactions were produced by unusual variations in one or two treatment, the main effects were also included in the results in order to facilitate interpretation of results.

Table 4.5: Significant effects of, and interactions between fungal species and wood species, on mechanical properties of veneers exposed to test fungi

	P-value			
Source of variation	Peak tensile stress	MOE	Peak stiffness	Toughness
Fungi	<0.001	<0.001	<0.001	<0.001
Wood sp.	<0.001	<0.001	<0.001	<0.001
Fungi x Wood sp.	<0.001	<0.001	<0.001	<0.001

4.3.2.1.1. Peak tensile stress ratio

Fungal species had a significant effect (P-value < 0.001) on the peak tensile stress ratios (maximum tensile stress as a ratio to that of the sound wood control) of wood veneers. *Cladosporium* sp. and *C. ligniaria* produced the greatest losses in peak tensile stress, followed by the control fungi, *C. globosum* and *T. abietinum*. Less pronounced losses in strengths were produced by *Phialocephala* sp., *M. minutella*, *L. infectoria* and *E. nigrum*. The rest of the fungi produced tensile stress ratios for treated veneers that were close to one, indicating that peak tensile stress of veneers was similar to that of the sound wood controls, Figure 4.4.

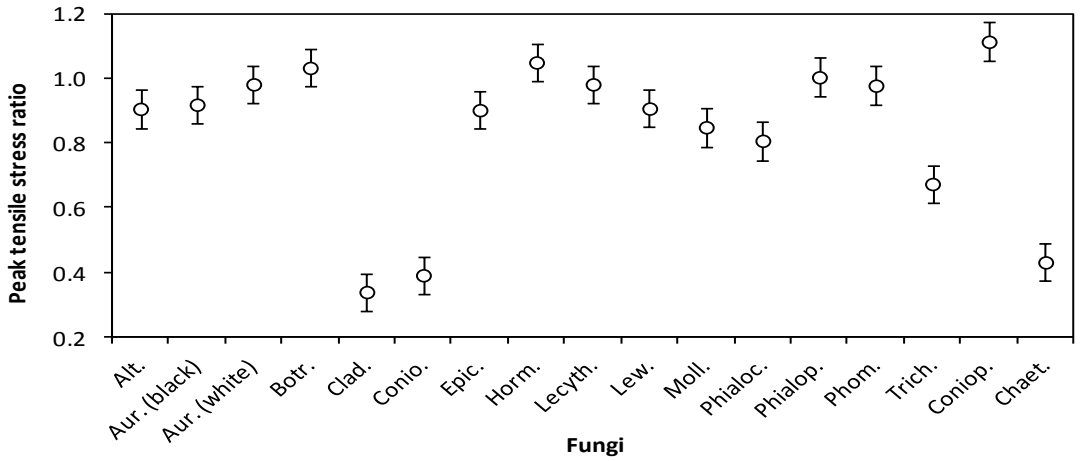


Figure 4.4: Peak tensile stress ratio (peak tensile stress of bioassayed veneer/peak tensile stress sound wood) of wood veneers exposed to fungi isolated from weathered wood. *Cladosporium* sp. and *C. ligniaria* produced the highest losses in peak tensile stress followed by the control fungi *C. globosum* and *T. abietinum*. Peak tensile stress ratio close to one indicates that tensile stress was similar to that of sound wood. Error bars correspond to ±SED

Wood species also had a significant effect (P-value < 0.001) on the peak tensile stress ratio of tested veneers. Lime veneers exposed to fungi isolated from weathered wood showed a significantly lower peak tensile ratio than spruce veneers, Figure 4.5.

Figure 4.5: Peak tensile stress ratio (peak tensile stress of bioassayed veneer/peak tensile stress sound wood) of lime and spruce veneers. Lime veneers treated with fungi isolated from weathered wood showed a significantly lower peak tensile ratio than spruce veneers. Peak tensile stress ratio close to one indicates that tensile stress was similar to that of sound wood. Error bars correspond to ±SED

The interaction of fungal species x wood species also had a significant effect (P-value < 0.001) on the peak tensile stress ratio of tested veneers. The interaction was caused by an inconsistent variation in peak tensile stress ratio of wood veneers exposed to *Phialophora* sp. The peak tensile stress ratio of spruce veneers was generally significantly higher than that of lime wood veneers (Figure 4.5), but in veneers incubated with *Phialophora* sp., the opposite was the case (circled in Figure 4.6).

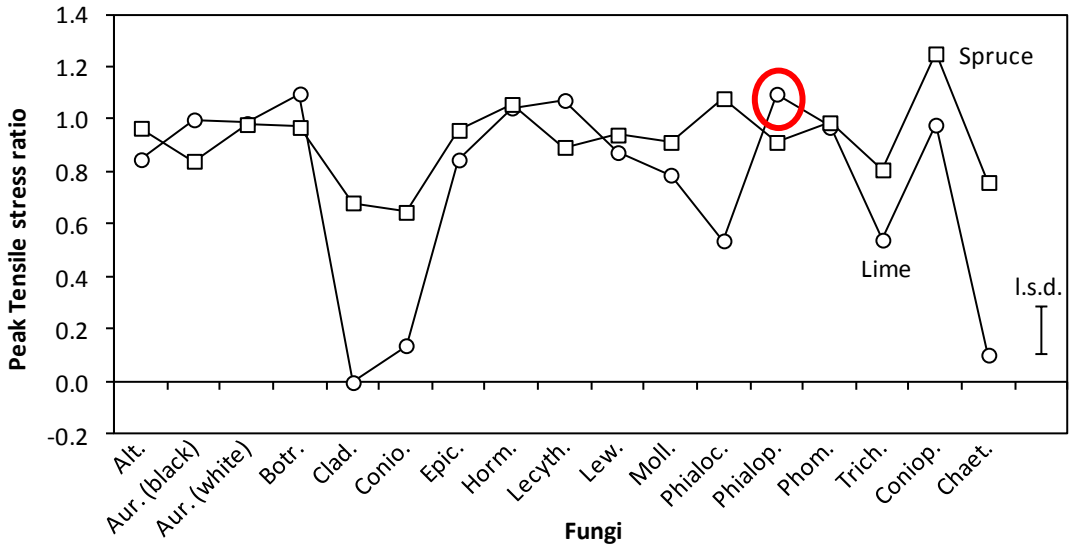


Figure 4.6: Peak tensile stress ratio (peak tensile stress of bioassayed veneer/peak tensile stress of sound wood) of lime and wood veneers inoculated with fungi isolated from weathered wood. Statistical interaction of fungi x wood (encircled in red) occurred due to the behavior of lime and spruce veneers incubated with *Phialophora* sp. Peak tensile stress ratio close to one indicates that tensile stress was similar to that of sound wood.

4.3.2.1.2. Modulus of elasticity (MOE) ratio

Fungal species had a significant effect (P-value < 0.001) on the MOE ratio of wood veneers. *Cladosporium* sp., *C. ligniaria* and *C. globosum* produced the highest losses in MOE. Less pronounced losses were caused by *Phialocephala* sp. and *T. abietinum*. The rest of the tested fungi produced MOE ratios in veneers that were close to one, indicating that the MOE of the veneers was similar to that of the sound wood controls, Figure 4.7.

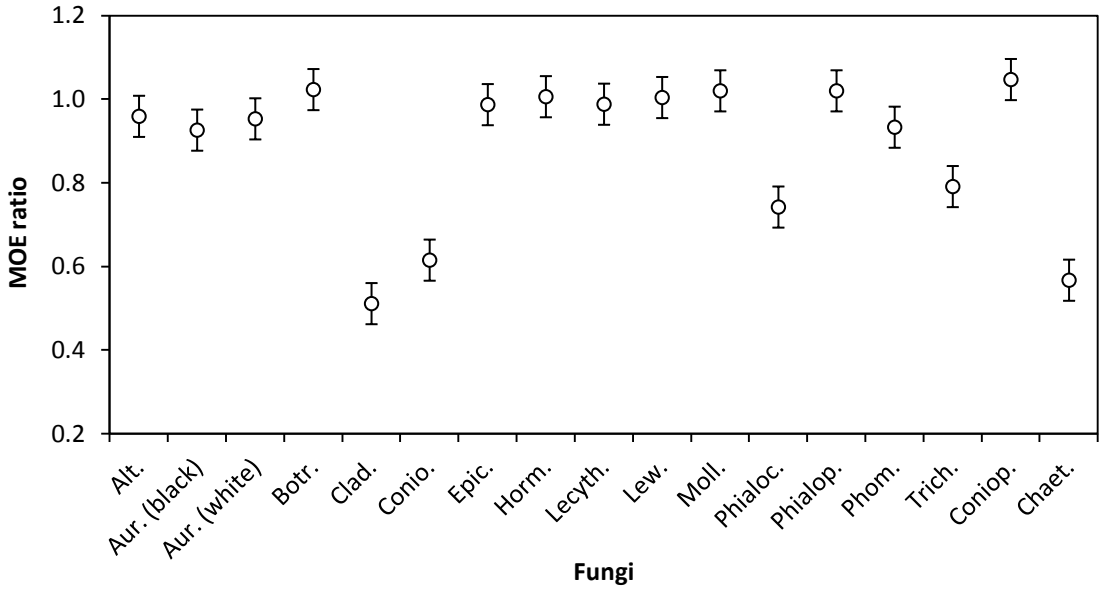


Figure 4.7: Modulus of elasticity (MOE) ratio (MOE bioassayed veneer/MOE sound wood) of wood veneers exposed to fungi isolated from weathered wood. *Cladosporium* sp. and *C. ligniaria* produced the highest losses in MOE followed by *C. globosum*, *Phialocephala* sp. and *T. abietinum*. MOE ratio close to one indicates that MOE was similar to that of sound wood. Error bars correspond to ±SED

Wood species also had a significant effect (P-value < 0.001) on MOE ratio. Lime veneers exposed to fungi isolated from weathered wood showed significantly lower MOE ratio than spruce veneers, Figure 4.8.

Figure 4.8: Modulus of elasticity (MOE) ratio (MOE bioassayed veneer/MOE sound wood) lime and spruce veneers. Lime veneers incubated with fungi isolated from weathered wood showed a significantly lower MOE ratio than spruce veneers. MOE ratio close to one indicated that MOE was similar to that of sound wood. Error bars correspond to ±SED

The interaction of fungal species x wood species also had a significant effect (P-value < 0.001) on the MOE ratio of tested veneers. Inconsistent variation in MOE ratio of spruce veneers exposed to *Cladosporium* sp. accounts in part for this interaction. Lime veneers exposed to *Cladosporium* sp. had the lowest MOE ratio of all veneers (Figure 4.7). However, spruce veneers exposed to *Cladosporium* sp. showed no losses in MOE, whereas other fungi that caused losses in MOE of lime veneers also caused losses in MOE of spruce veneers (Figure 4.9).

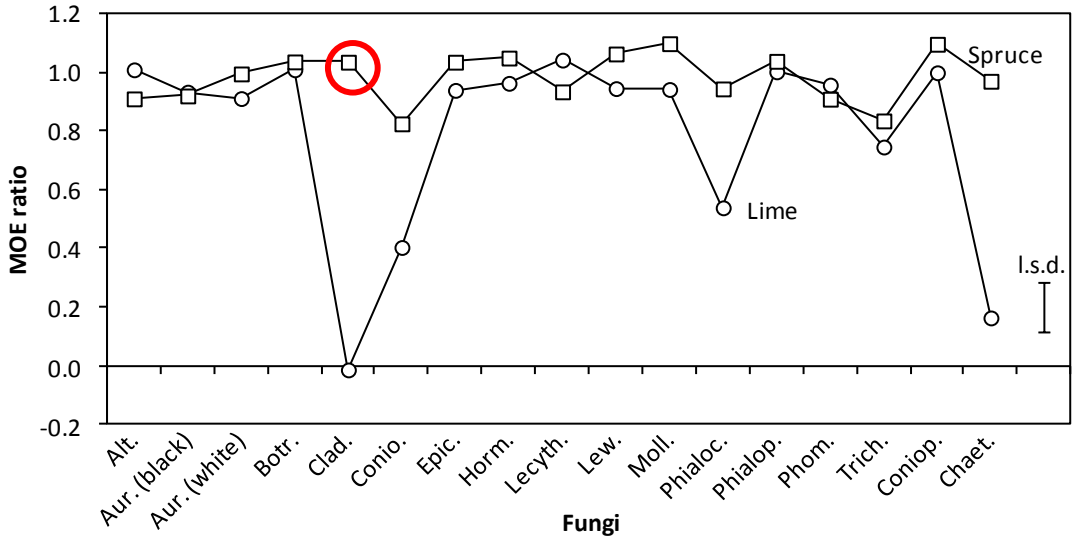


Figure 4.9: Modulus of elasticity (MOE) ratio (MOE bioassayed veneer/MOE sound wood) of lime and wood veneers incubated with fungi isolated from weathered wood. MOE ratio close to one indicates that MOE was similar to that of sound wood

4.3.2.1.3. Peak stiffness ratio

Fungal species had a significant effect (P-value < 0.001) on the peak stiffness ratio of wood veneers. *Cladosporium* sp., *C. ligniaria* and *C. globosum* produced the highest losses in peak tensile stress. Less severe losses in stiffness were caused by *T. abietinum*. All other tested

fungi produced peak stiffness ratios in tested veneers that were close to one, indicating that the peak stiffness of veneers was similar to that of the sound wood controls, Figure 4.10.

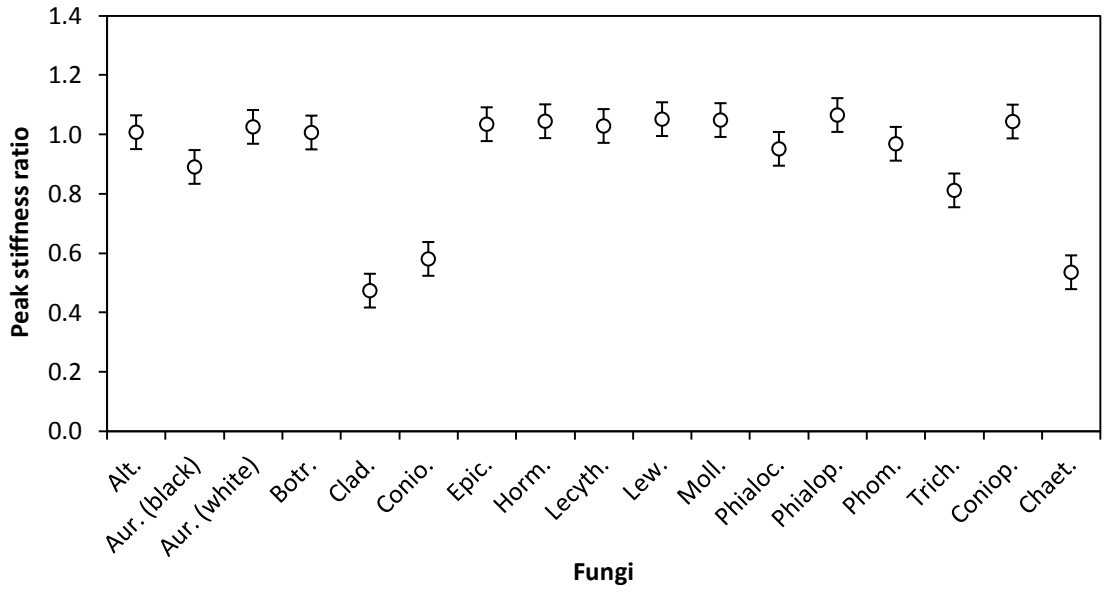


Figure 4.10: Peak stiffness ratio (peak stiffness bioassayed veneer/peak stiffness sound wood) of wood veneers exposed to fungi isolated from weathered wood. *Cladosporium* sp., *C. ligniaria* and *C. globosum* produced the highest losses in peak tensile stress. Peak stiffness ratio close to one indicates that peak stiffness was similar to that of sound wood. Error bars correspond to ±SED

Wood species also had a significant effect (P-value < 0.001) on peak stiffness ratio. Lime veneers incubated with fungi isolated from weathered wood showed a significantly lower peak stiffness ratio than spruce veneers, Figure 4.11).

Figure 4.11: Peak stiffness ratio (peak stiffness bioassayed veneer/peak stiffness sound wood) of lime and spruce veneers. Lime veneers incubated with fungi isolated from weathered wood showed a significantly lower peak stiffness ratio than spruce veneers. Peak stiffness ratio close to one indicated that peak stiffness was similar to that of sound wood. Error bars correspond to ±SED

The interaction of fungal species x wood species also had a significant effect (P-value < 0.001) on peak stiffness ratio. The interaction occurred for the same reason as the interaction detected for losses in MOE (as expected), Figure 4.12.

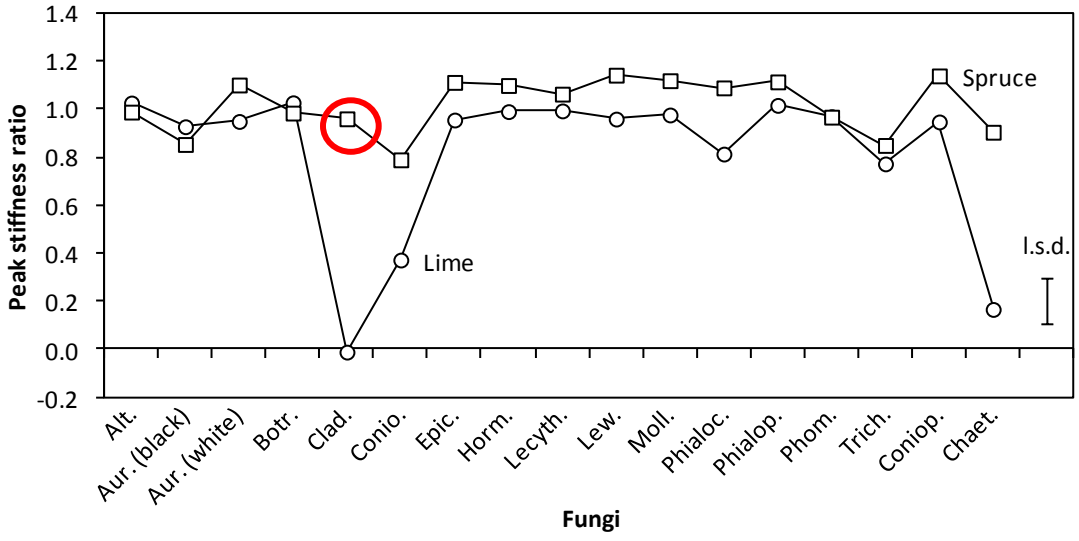


Figure 4.12: Peak stiffness ratio (peak stiffness bioassayed veneer/peak stiffness sound wood) of lime and wood veneers incubated with fungi isolated from weathered wood. Peak stiffness ratio close to one indicates that peak stiffness was similar to that of sound wood control

4.3.2.1.4. Peak toughness ratio

Fungal species had a significant effect (P-value < 0.001) on the peak toughness ratio (maximum amount of energy absorbed by the wood as a ratio to that of the sound wood control) of wood veneers. *Cladosporium* sp., *C. ligniaria* and *C. globosum* produced the greatest losses in peak toughness ratio followed by *T. abietinum*. Less pronounced losses in toughness were caused by *E. nigrum*, *L. infectoria*, *M. minutella* and *Phialocephala* sp. A slight increase in peak toughness ratio was caused by *C. puteana*. All other tested fungi produced peak toughness ratios for tested veneers that were close to one, indicating that the toughness of veneers was similar to that of sound wood, Figure 4.13.

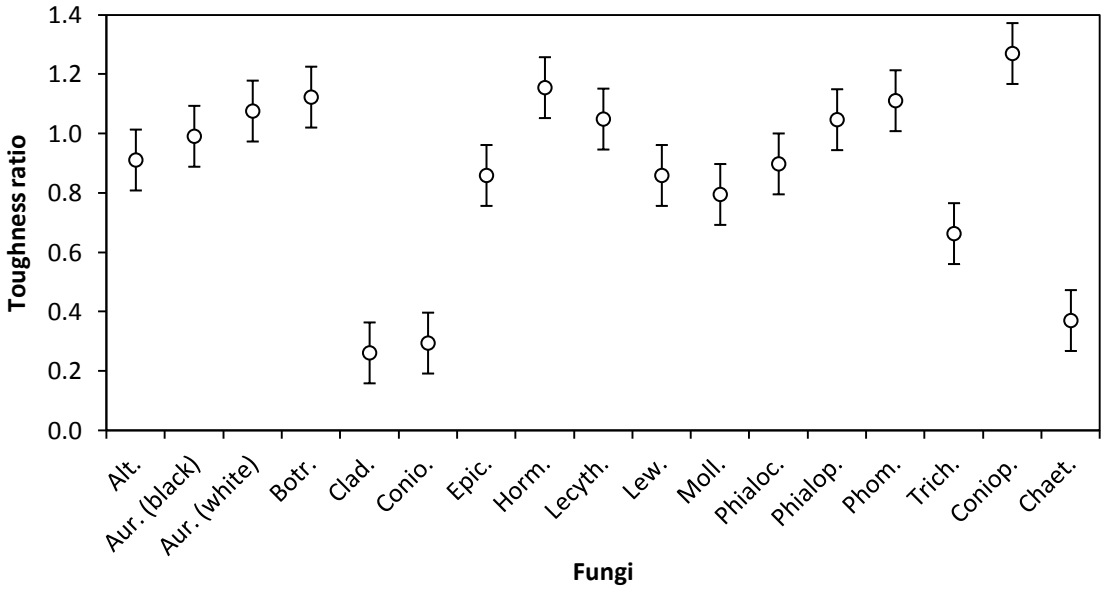


Figure 4.13: Peak toughness ratio (peak toughness bioassayed veneer/peak toughness sound wood) of wood veneers incubated with fungi isolated from weathered wood. *Cladosporium* sp., *C. ligniaria* and *C. globosum* produced the highest losses in peak tensile stress followed by *T. abietinum*. Peak toughness ratio close to one indicates that peak toughness was similar to that of sound wood. Error bars correspond to ±SED

Wood species also had a significant effect (P-value < 0.001) on toughness ratio. Lime veneers incubated with fungi isolated from weathered wood showed a significantly lower toughness ratio than spruce veneers, Figure 4.14.

Figure 4.14: Peak toughness ratio (peak toughness treated veneer/peak toughness sound wood) of lime and spruce veneers. Lime veneers treated with fungi isolated from weathered wood showed significantly lower peak stiffness ratio than spruce veneers. Peak toughness ratio close to one indicates that peak toughness was similar to that of sound wood control. Error bars correspond to ±SED

The interaction of fungal species x wood species also had a significant effect (P-value < 0.001) on peak toughness ratio (Figure 4.15). This interaction occurred for the same reason that there was a significant (P-value < 0.001) interaction of fungal species x wood species on peak tensile strength ratio (see Figure 4.6).

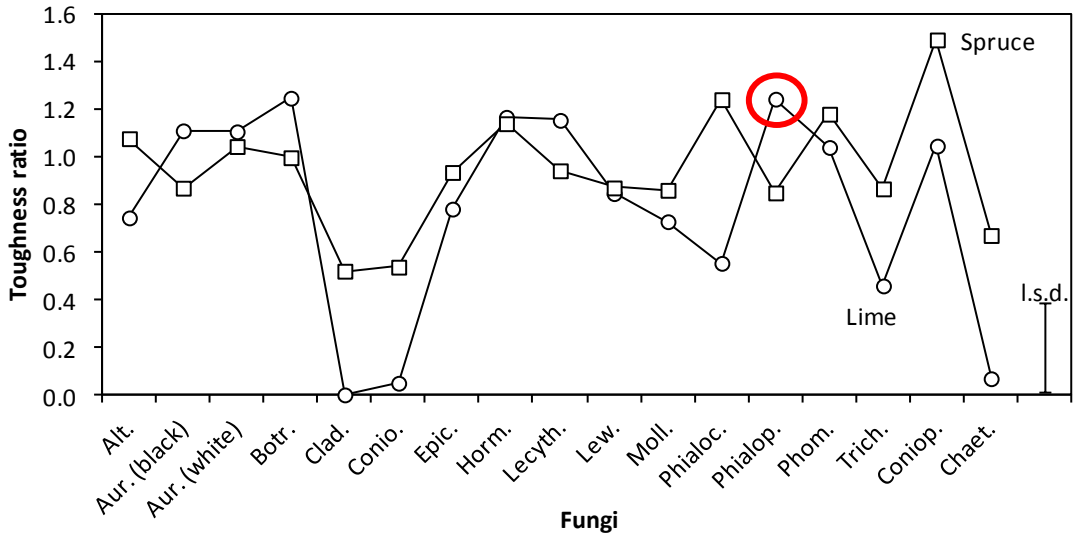


Figure 4.15: Peak toughness ratio (peak toughness bioassayed veneer/peak toughness sound wood) of lime and wood veneers incubated with fungi isolated from weathered wood. Peak toughness ratio close to one indicated that peak toughness was similar to that of sound wood control

4.3.2.2. Viscoelastic properties

The storage modulus (SM) of lime and spruce samples exposed to different fungi are depicted in Figure 4.16 and Figure 4.17, respectively. SM decreased with temperature for both wood species showing transitions at about 75 °C and 150 °C. The SM of lime wood incubated with *Alternaria* sp., *Cladosporium* sp., *C. ligniaria*, *Phialocephala* sp., *C. globosum* and *T. abietinum* showed lower values than that of untreated wood, and there was no second transition point at 150 °C. In contrast the SM of lime samples incubated with *M. minutella*, *E. nigrum* and *Lewia* sp. was slightly higher than that of the untreated control. Spruce samples did not show an inflection point at about 150 °C and their SM remained lower than that of the sound wood control. The SM of spruce wood incubated with *Phialophora* sp. had the highest SM of all spruce wood samples exposed to the different fungi.

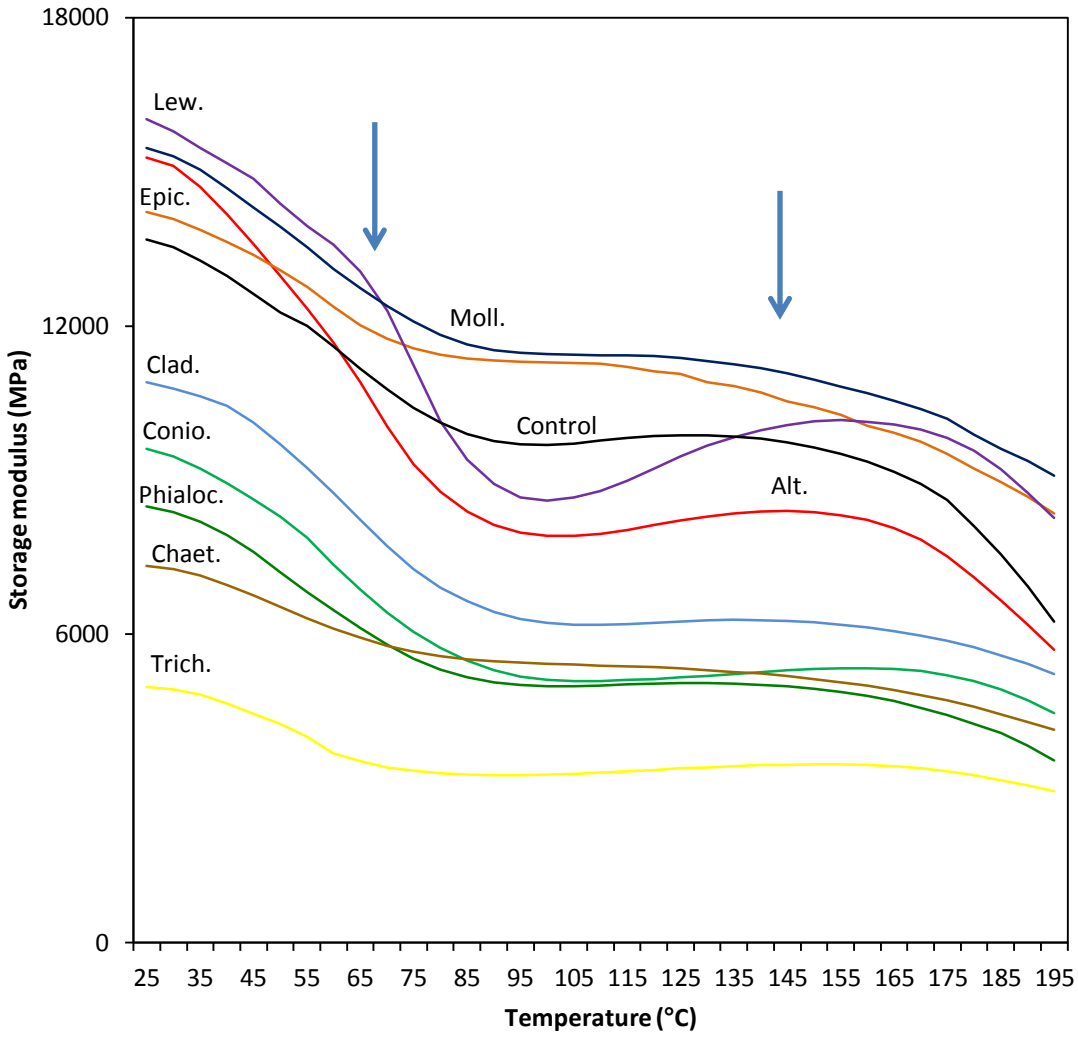


Figure 4.16: Storage modulus of lime wood samples after 12 weeks of incubation with fungi isolated from weathered wood, blue arrows indicate zones of viscoelastic transition

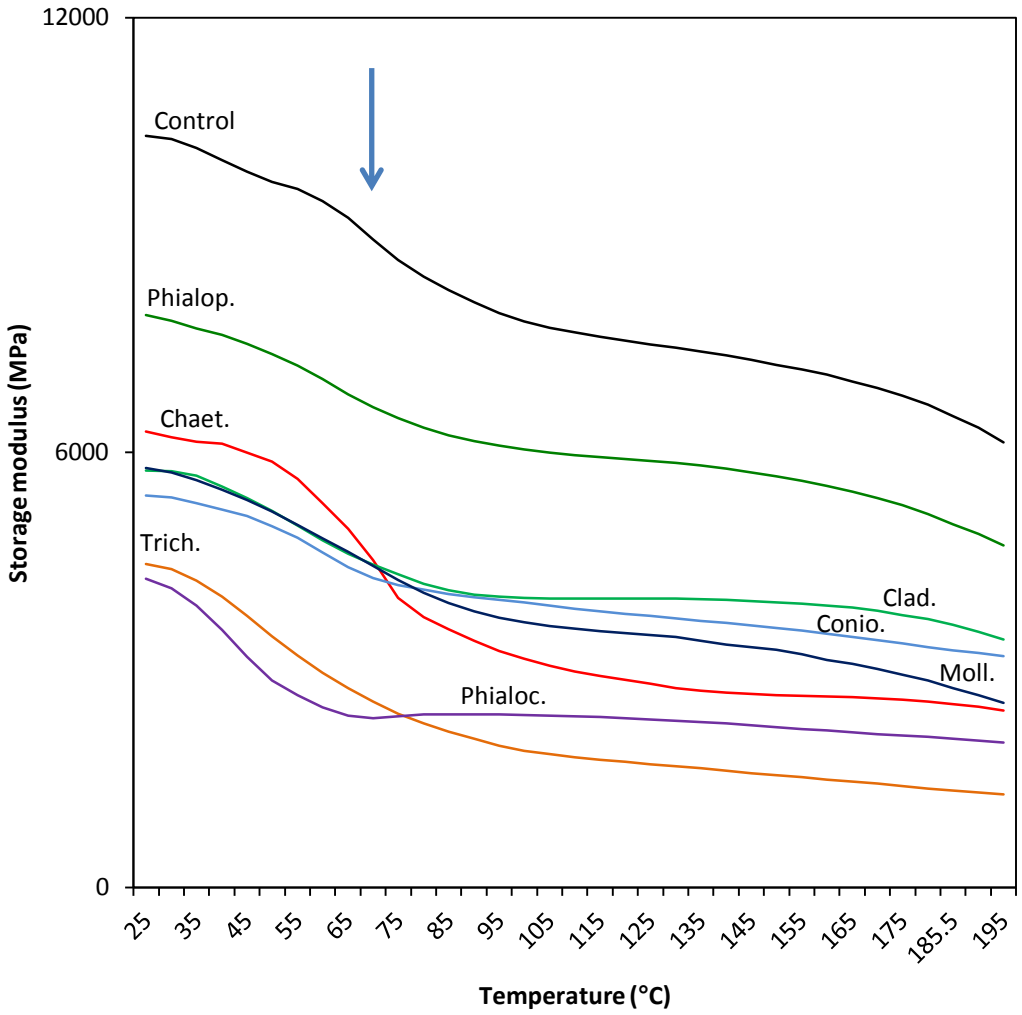


Figure 4.17:Storage modulus of spruce wood samples after 12 weeks of incubation with fungi isolated from weathered wood, blue arrow indicate a zone of viscoelastic transition

4.3.2.3. Fourier transform infra-red spectroscopy

FTIR spectra for lime and spruce wood samples exposed to the different fungi are shown in Figure 4.18 to Figure 4.23 and Figure 4.24 to Figure 4.29, respectively. In lime wood incubated with *Alternaria* sp., *A. pullulans* (black) and *A. pullulans* (white), *Cladosporium* sp., *Lewia* sp., *Phialocephala* sp., *Phoma* sp., *C. globosum* and *C. puteana* there were significant decreases, or in some cases elimination of peaks related to cellulose and hemicelluloses.

Among the peaks affected were 1059 cm^{-1}, C-O stretching in cellulose (Faix and Böttcher 1992; Pandey and Theagarajan 1997; Popescu et al. 2010), 1108 cm^{-1}, C-O-H deformation in hemicelluloses and cellulose; (Faix and Böttcher 1992; Popescu et al. 2010), 1165 cm^{-1}, C-O-C stretching in hemicelluloses and cellulose (Faix and Böttcher 1992; Pandey and Theagarajan 1997; Popescu et al. 2010), 1244 cm^{-1}, guaiacyl ring and C-O stretching in xylan and lignin (Faix and Böttcher 1992; Popescu et al. 2010), 1376 cm^{-1}, C-H Deformation, CH3 symmetric deformation in hemicelluloses and cellulose (Faix and Böttcher 1992; Pandey and Theagarajan 1997; Popescu et al. 2010), 1737 cm^{-1}, C=O stretching of carbonyl and acetyl groups in hemicelluloses (Faix and Böttcher 1992; Popescu et al. 2010). Conversely, samples inoculated with *Lecythophora* sp. and *T. abietinum* showed an increase in the peak at 1108 cm^{-1}. Peaks related to lignin decreased in veneers inoculated with *T. abietinum* or *Phialophora* sp. Peaks affected were 1510 cm^{-1}, C=C stretching of substituted aromatic rings in lignin (Harrington et al. 1964; Faix and Böttcher 1992), 1598 cm^{-1}, C=C stretching of substituted aromatic rings in lignin (Pandey and Theagarajan 1997; Popescu et al. 2010). Decreases in both, cellulose and lignin, peaks were observed in lime wood incubated with *C. ligniaria, H. dematioides* or *Mollisia* sp. The peaks most affected were 1059 cm^{-1}, 1244 cm^{-1}, 1376 cm^{-1}, 1510 cm^{-1}, 1598 cm^{-1} and 1737 cm^{-1}. In addition, all fungi but *T. abietinum* and *B. fuckeliana* produced increases in the peak at 1655 cm^{-1}, C=O conjugated stretching of phenolic groups in lignin. Such an increase has been attributed to the increase of carbonyl moieties as decay occurs (Popescu et al. 2010). The spectrum of lime wood incubated with *B. fuckeliana* was similar to that of the sound wood control.

In spruce wood inoculated with *Alternaria* sp., *A. pullulans* (white), *E. nigrum*, *Lewia* sp. and *C. globosum,* there was also a significant decrease in peaks related to cellulose and hemicelluloses. Peaks affected were 1462 cm^{-1}, C-H deformation in lignin and carbohydrates (Pandey and Theagarajan 1997) and 1737 cm^{-1}, C=O stretching of carbonyl and acetyl groups in hemicelluloses (Faix and Böttcher 1992; Pandey and Pitman 2003). A decrease in peaks related to lignin was produced by *A. pullulans* (black), *B. fuckeliana, C. ligniaria,* and *T. abietinum*. Peaks affected were 1268 cm^{-1}, C-O guaiacyl ring breathing, C-O stretching, C-O linkage in guaiacyl aromatic methoxyl groups in lignin (Pandey and Theagarajan 1997) and 1505 cm^{-1}, C=C stretching of substituted aromatic rings in lignin (Harrington et al. 1964). Conversely, samples inoculated with *Phialocephala* sp. showed an increase in the peak at 1268 cm^{-1}. Decreases of both, cellulose and lignin, peaks was observed in spruce wood inoculated with *H. dematioides, Lecythophora* sp., *Mollisia* sp. and *C. puteana*. The peaks most affected were 1268 cm^{-1}, 1462 cm^{-1}, 1505 cm^{-1} and 1737 cm^{-1}. All fungi but *Phoma* sp. and *C. puteana* increased the peak at 1655 cm^{-1} (as above). The FTIR spectrum of spruce wood inoculated with *Phoma* sp. appeared to be unaffected by fungal exposure.

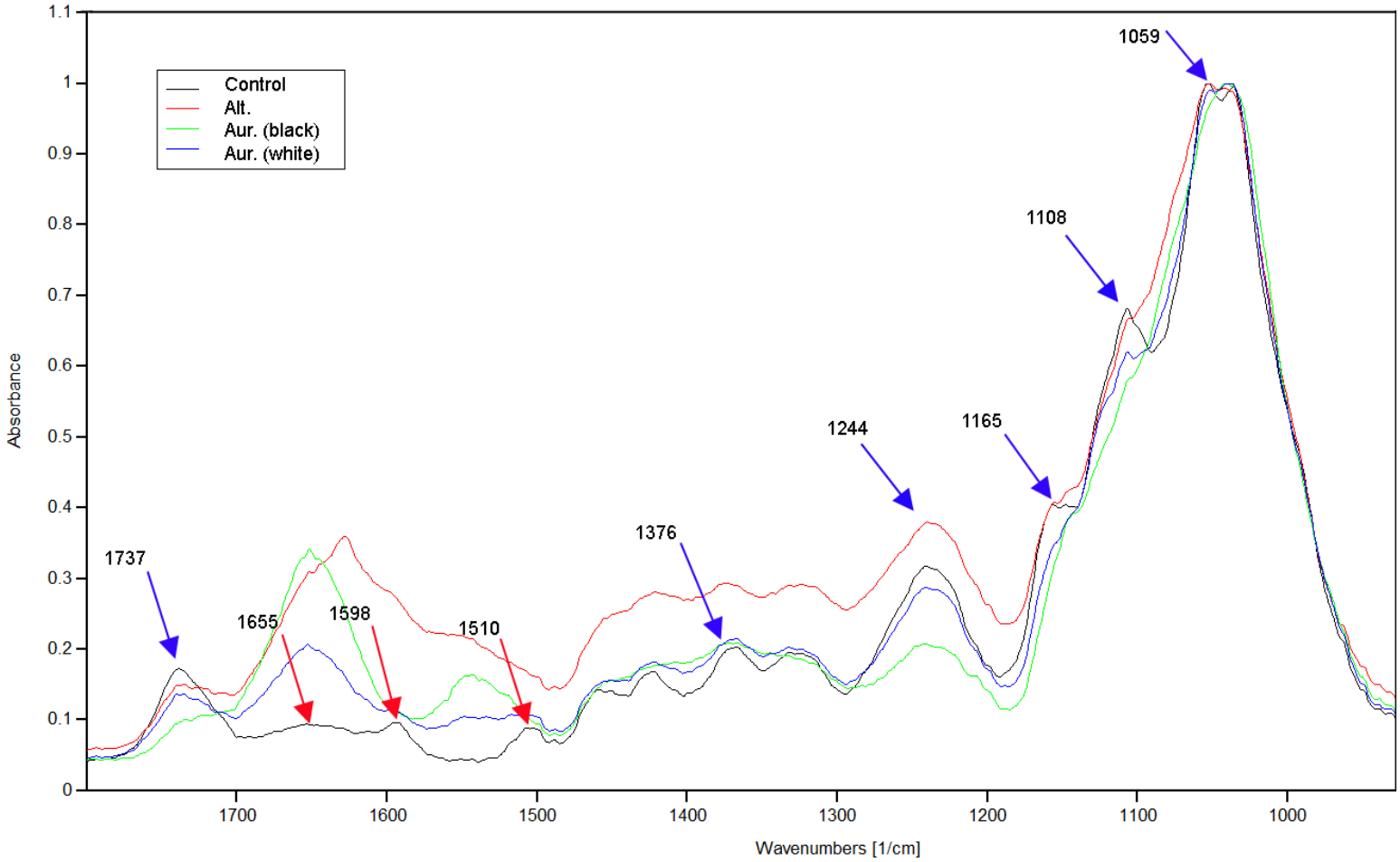


Figure 4.18: Normalized FTIR spectra of lime wood exposed to *Alternaria* sp., *A. pullulans* (black) and *A. pullulans* (white). Peaks related to cellulose and hemicelluloses at 1108 and 1737 cm^{-1} were reduced in size by *Alternaria*. *A. pullulans* (black) reduced the sizes of the peaks at 1059, 1108, 1165 and 1737 cm^{-1} and *A. pullulans* (white) reduced the sizes of the peaks at 1165 and 1737 cm^{-1}. All fungi increased the peak at 1655 cm^{-1}. The spectrum for the sound wood control is shown for comparison

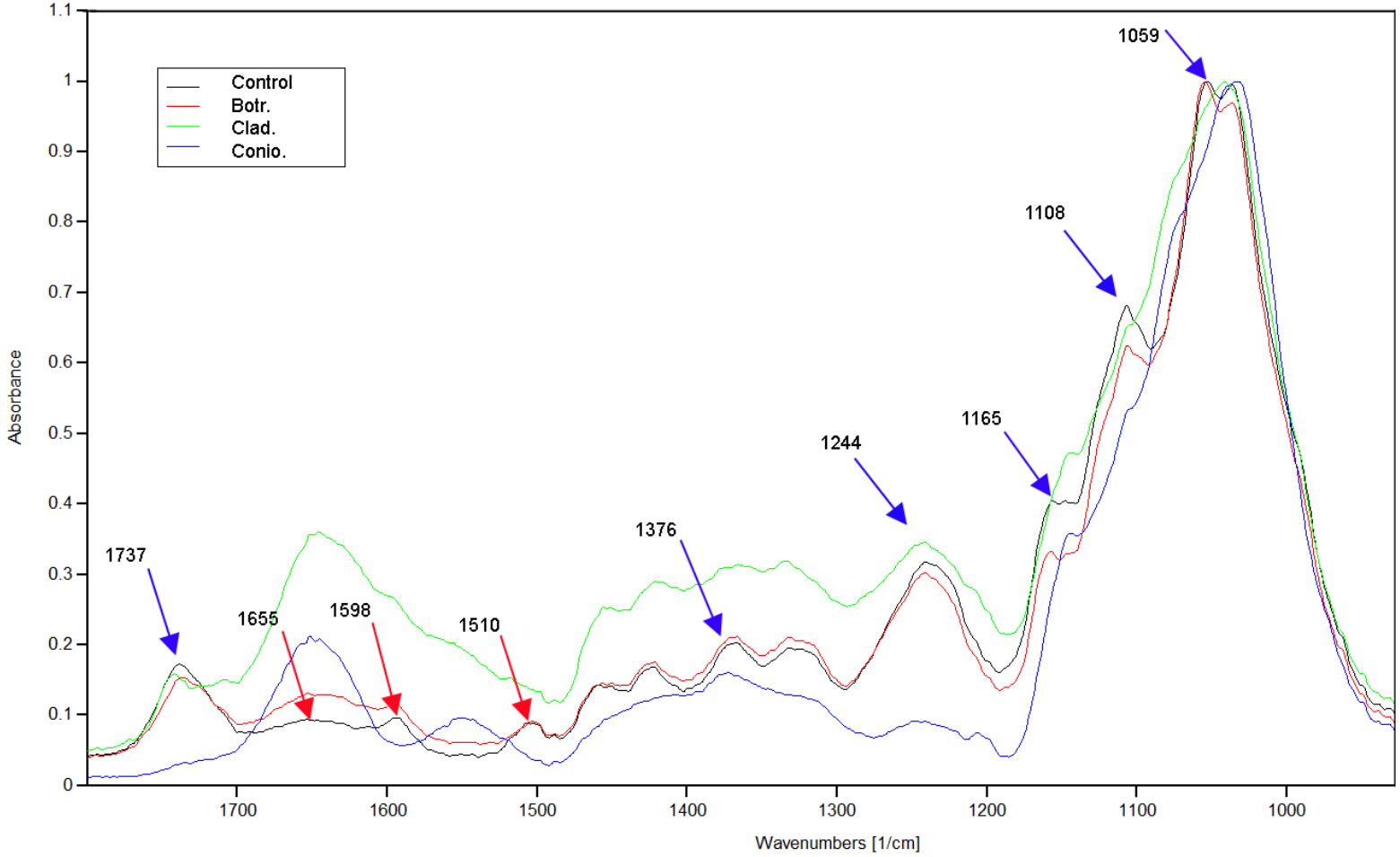


Figure 4.19: Normalized FTIR spectra of lime wood exposed to *B. fuckeliana, Cladosporium* sp., and *C. puteana. Cladosporium* sp. decreased the peak related to cellulose and hemicelluloses 1059 cm^{-1}. *C. ligniaria* decreased peaks related to cellulose, hemicelluloses and lignin at 1059, 1244, 1376, 1510, 1598 and 1737 cm^{-1}. Both of the latter fungal species increased the peak at 1655 cm^{-1}. No changes in the spectrum of lime wood were produced by *B. fuckeliana*. The spectrum for the sound wood control is shown for comparison

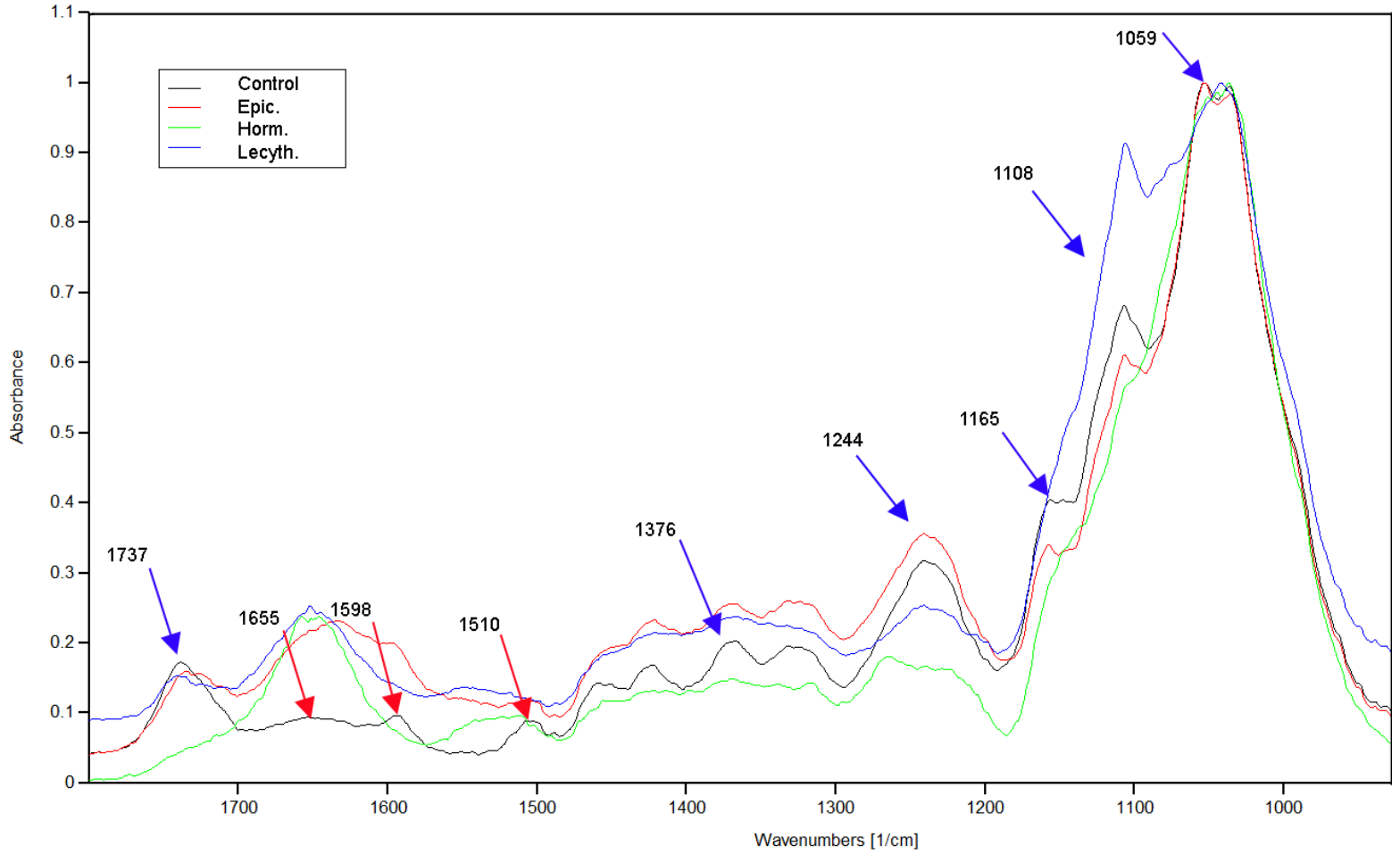


Figure 4.20: Normalized FTIR spectra of lime wood exposed to *E. nigrum, H. dematioides* and *Lecythophora* sp. *H. dematioides* decreased peaks related to cellulose and lignin at 1244, 1376, 1598, 1737 cm^{-1}. *Lecythophora* sp. increased the peak at 1108 (cellulose and hemicelluloses). All fungi increased the peak at 1655 cm^{-1}, but *E. nigrum* did not produce any other changes. The spectrum for the sound wood control is shown for comparison

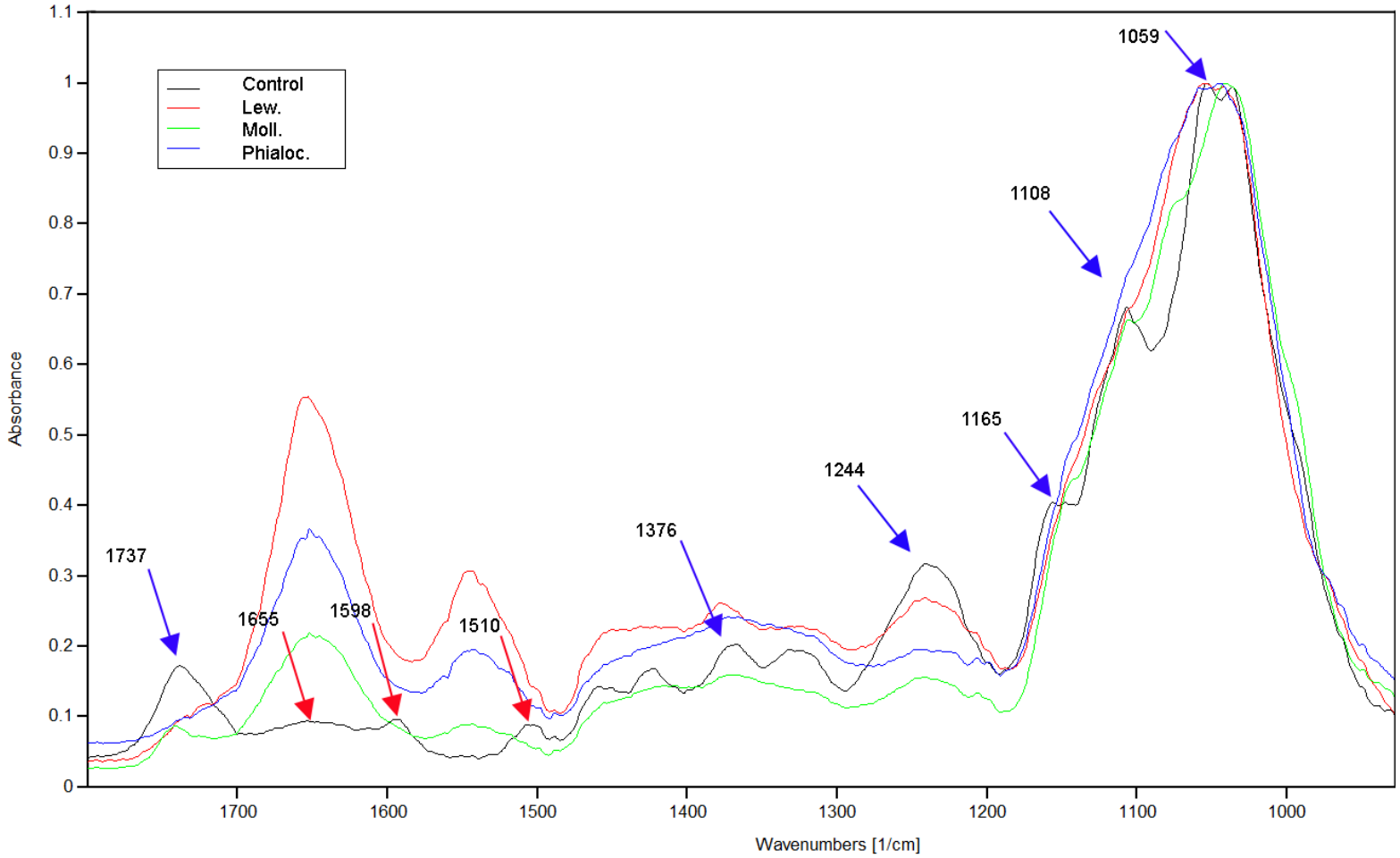


Figure 4.21: Normalized FTIR spectra of lime wood exposed to *L. infectoria*, *M. minutella* and *Phialocephala* sp. *L. infectoria* decreased the peak related to cellulose and hemicelluloses at 1737 cm^{-1}. *Phialocephala* sp. decreased peaks related to cellulose and hemicelluloses at 1244, 1376 and 1737 cm^{-1}. *M. minutella* decreased peaks related cellulose, hemicelluloses and lignin at 1244, 1376 1510 and 1737 cm^{-1}. All fungi increased the peak at 1655 cm^{-1}. The spectrum of the sound wood control is shown for comparison

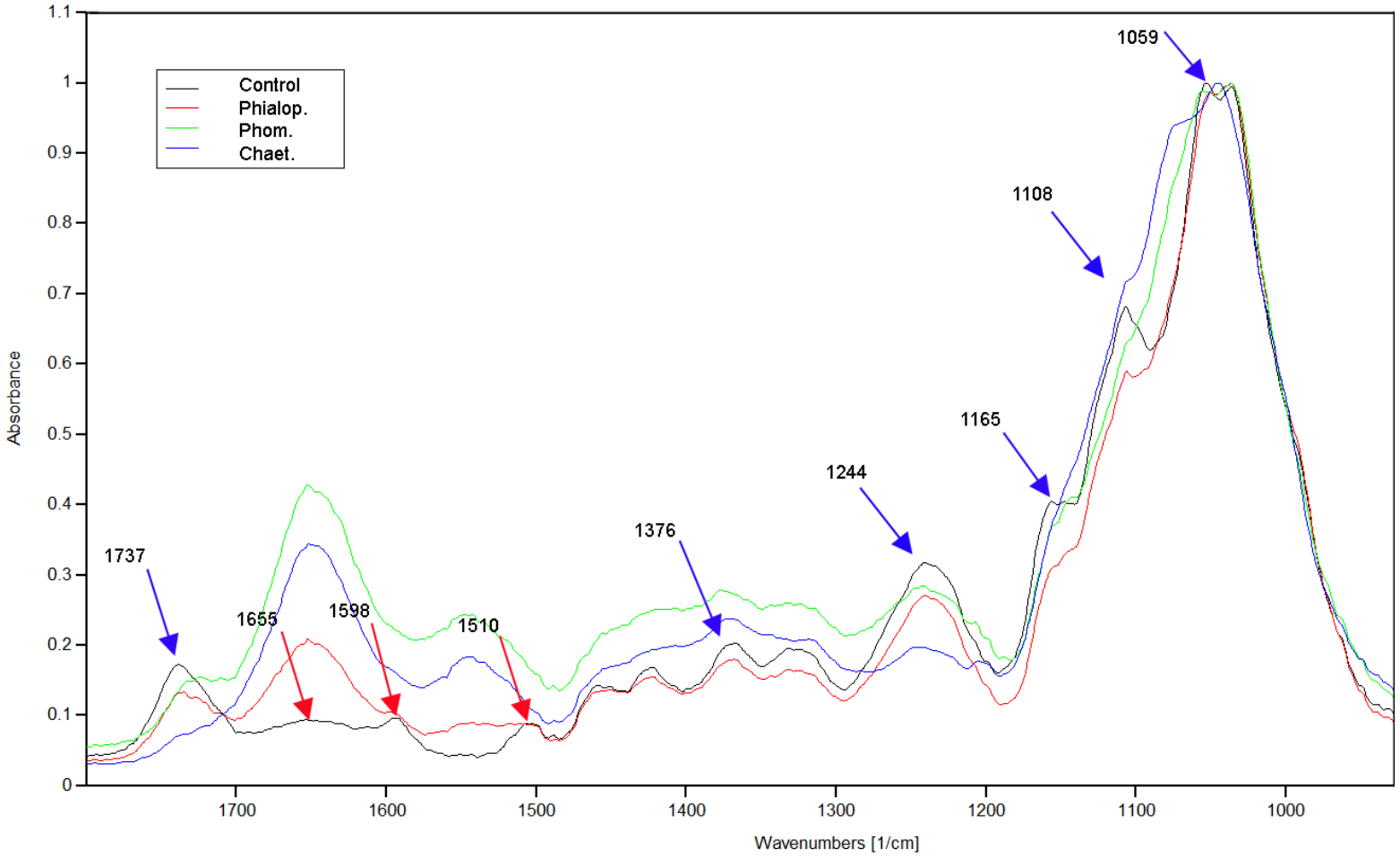


Figure 4.22: Normalized FTIR spectra of lime wood exposed to *Phialophora* sp., *Phoma* sp. and *C. globosum*. *Phialophora* sp. decreased the peak related to lignin at 1510 cm^{-1}, *Phoma* sp. decreased the peak related cellulose and hemicelluloses at 1737 cm^{-1}. *C. globosum* decreased the peaks at 1244 and 1737 cm^{-1} related to cellulose and lignin. All fungi increased the peak at 1655 cm^{-1}. The spectrum for the sound wood control is shown for comparison

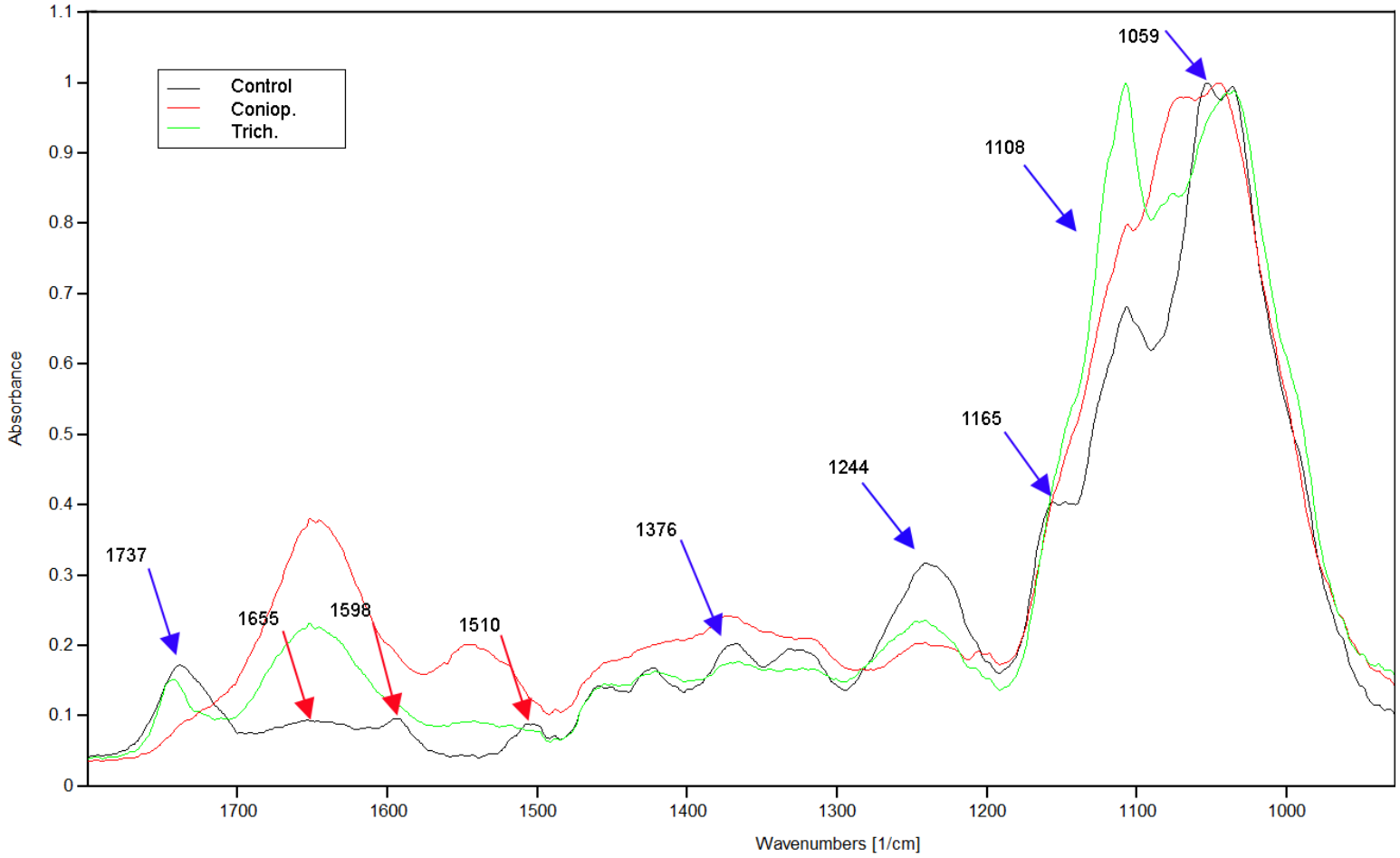


Figure 4.23: Normalized FTIR spectra of lime wood exposed to *C. puteana* and *T. abietinum*. *C. puteana* decreased the peaks at 1244 and 1737 cm^{-1} related to cellulose and lignin. *T. abietinum* increased the peak at 1165 cm^{-1} related to cellulose and hemicelluloses and decreased the peaks at 1510 and 1598 cm^{-1} related to lignin. Both fungal species increased the peak at 1655 cm^{-1}. The spectrum for the sound wood control is shown for comparison

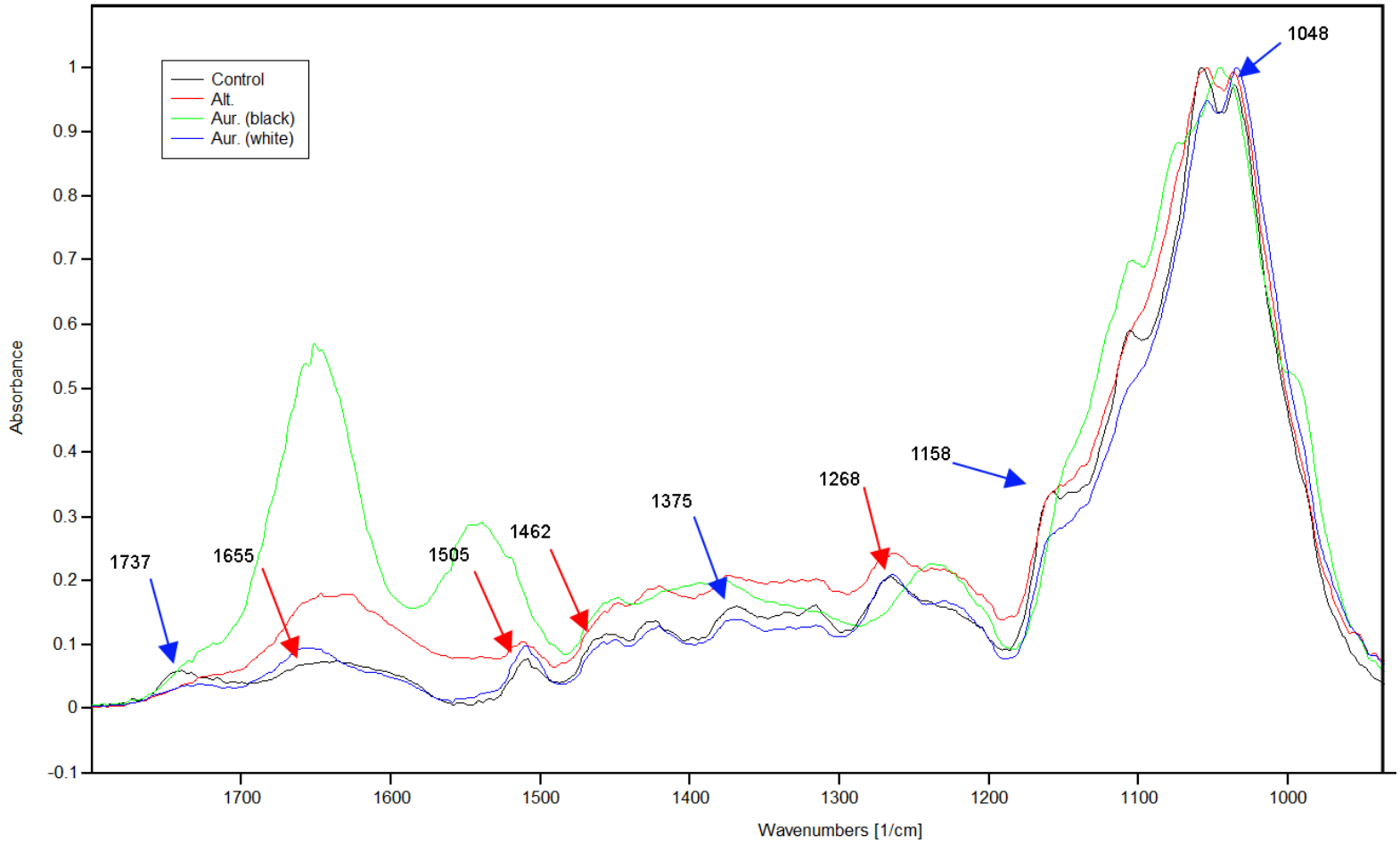


Figure 4.24: Normalized FTIR spectra of spruce wood exposed to *Alternaria* sp., *A. pullulans* (black) and *A. pullulans* (white). The peak related to cellulose and hemicelluloses 1737 cm^{-1} was decreased by *Alternaria*. *A. pullulans* (black) decreased the peak at 1268 cm^{-1} (lignin). *A. pullulans* (white) decreased the peak at 1731 cm^{-1} (cellulose and hemicelluloses). All fungi increased the peak at 1655 cm^{-1}. The spectrum of the sound wood control is shown for comparison

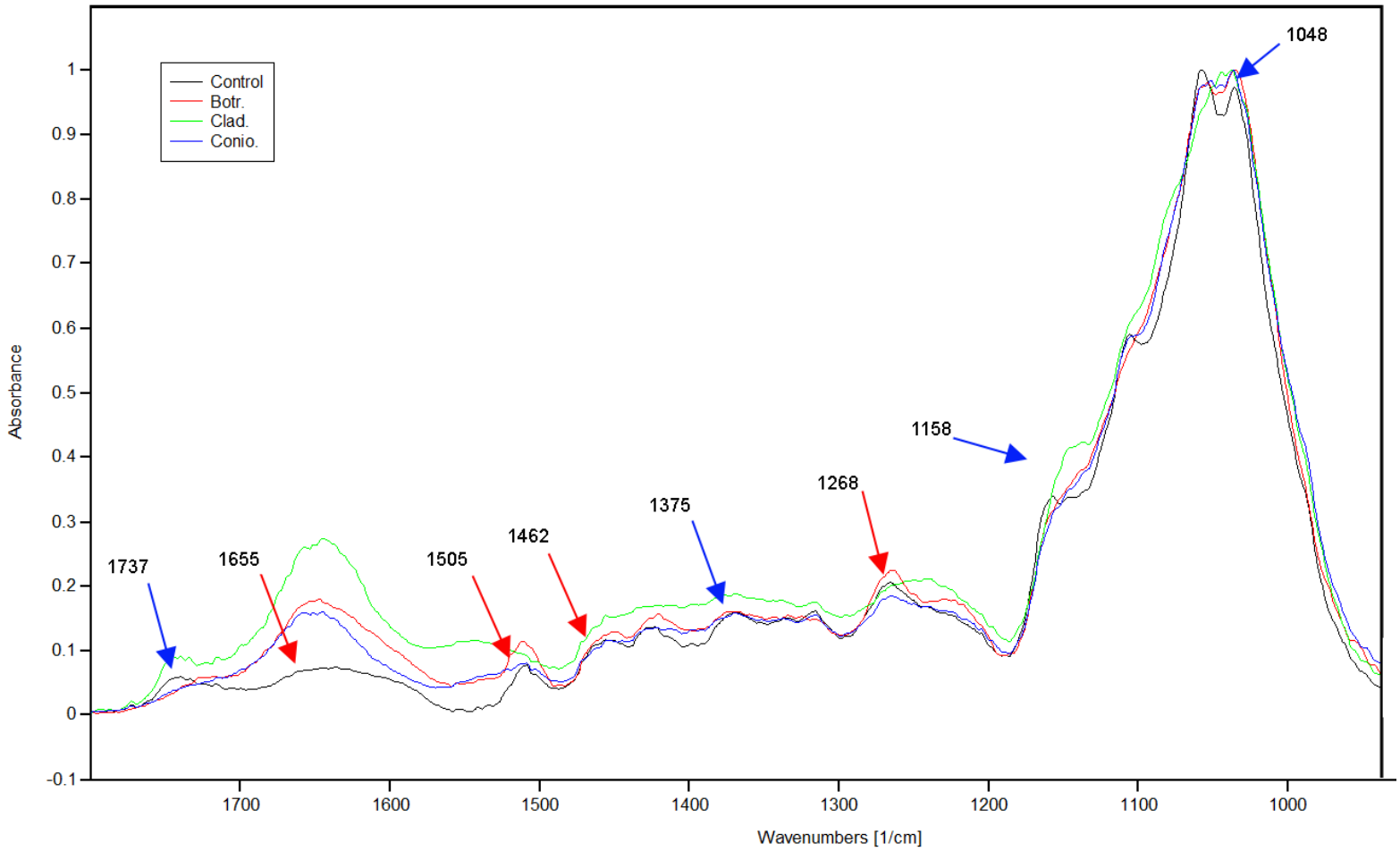


Figure 4.25: Normalized FTIR spectra of spruce wood exposed to *B. fuckeliana, Cladosporium* sp., and *C. puteana*. *B. fuckeliana* and *C. ligniaria* decreased the peak at 1505 cm^{-1} related to lignin. All fungal species increased the peak at 1655 cm^{-1}. *Cladosporium* sp. did not produce any further changes. The spectrum of the sound wood control is shown for comparison

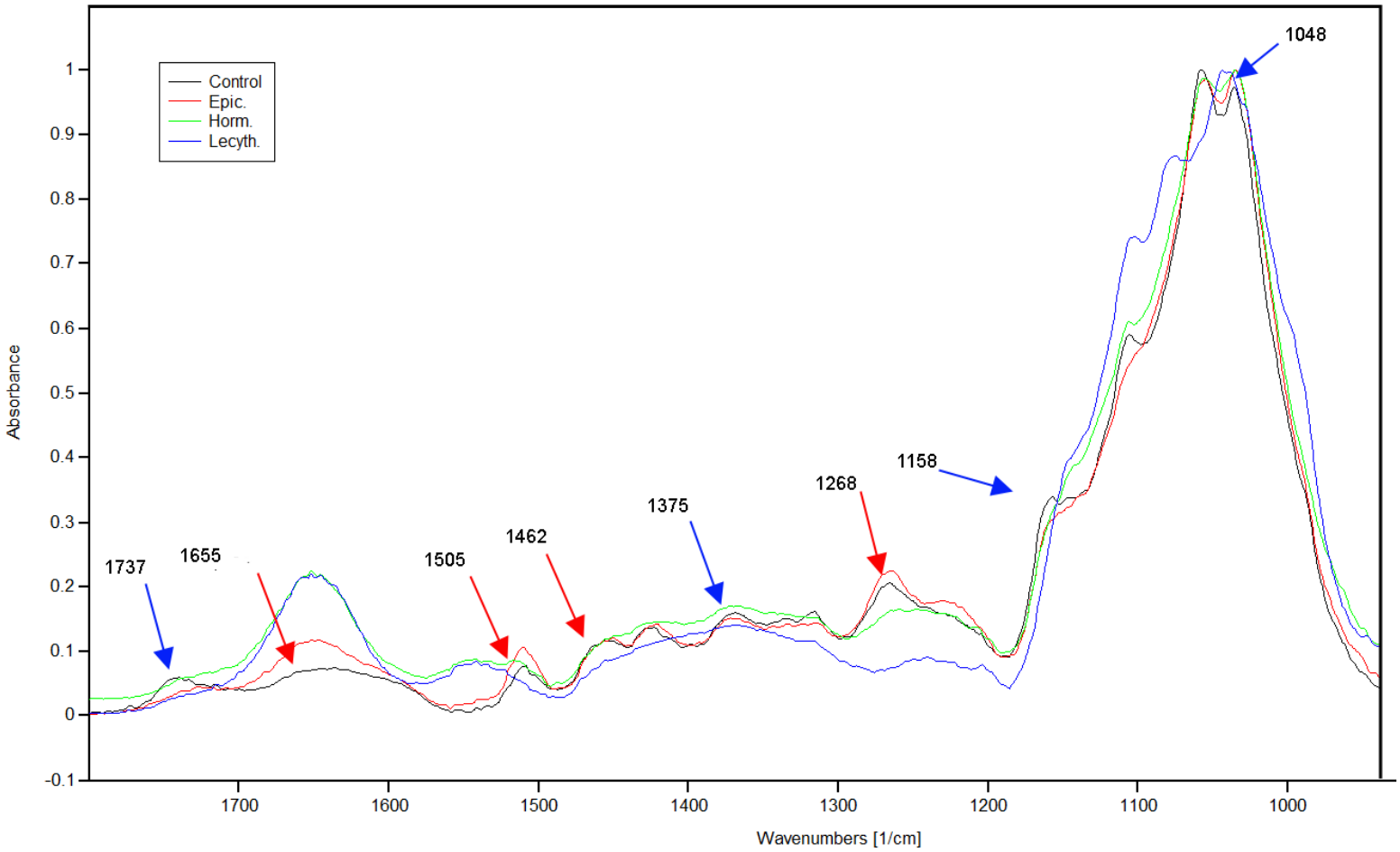


Figure 4.26: Normalized FTIR spectra of spruce wood exposed to *E. nigrum, H. dematioides* and *Lecythophora* sp. *H. dematioides* decreased peaks related to cellulose and lignin at 1268, 1505 and 1737 cm^{-1}. *Lecythophora* sp. decreased peaks at 1737 (cellulose and hemicelluloses) and 1268, 1462 and 1505 cm^{-1} (lignin). *E. nigrum* decreased the peak related to cellulose and hemicelluloses at 1737 cm^{-1}. All fungi increased the peak at 1655 cm^{-1}. The spectrum of the sound wood control is shown for comparison

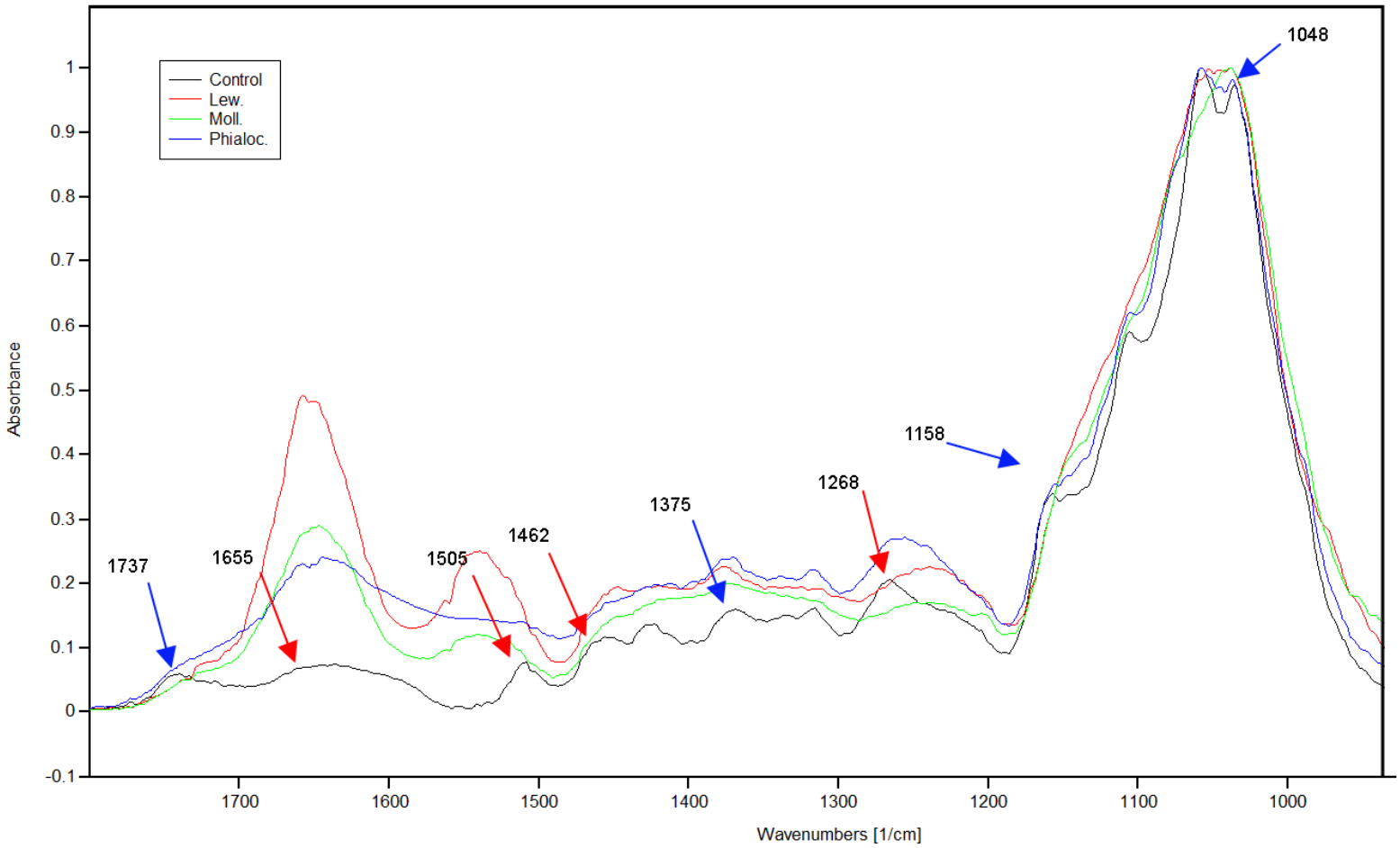


Figure 4.27: Normalized FTIR spectra of spruce wood exposed to *L. infectoria*, *M. minutella* and *Phialocephala* sp. *L. infectoria* decreased the peak related to cellulose and hemicelluloses at 1737 cm^{-1} and 1462 cm^{-1} related to lignin. *Phialocephala* sp. increased the peak at 1268 cm^{-1} (lignin), *M. minutella* decreased peaks related to cellulose, hemicelluloses, and lignin at 1268 and 1737 cm^{-1}. All fungi increased the peak at 1655 cm^{-1}. The spectrum of the sound control is shown for comparison

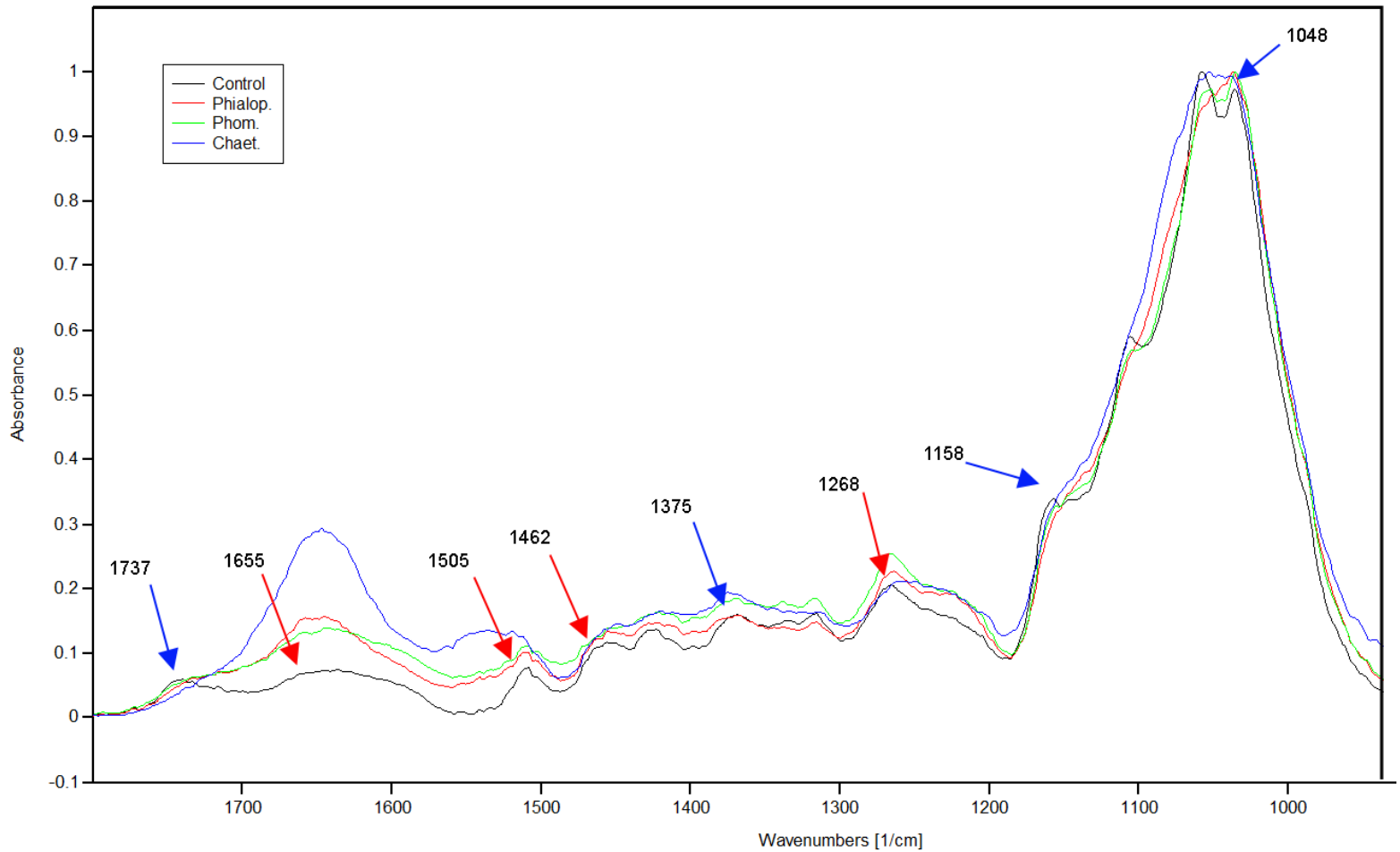


Figure 4.28: Normalized FTIR spectra of spruce wood exposed to *Phialophora* sp., *Phoma* sp. and *C. globosum*. *C. globosum* decreased the peak at 1737 cm^{-1} related to cellulose and hemicelluloses. *Phialophora* and *C. globosum* increased the peak at 1655 cm^{-1}. No changes were produced in wood exposed to *Phoma* sp. All fungi increased the peak at 1655 cm^{-1}. The spectrum of the sound wood control is shown for comparison

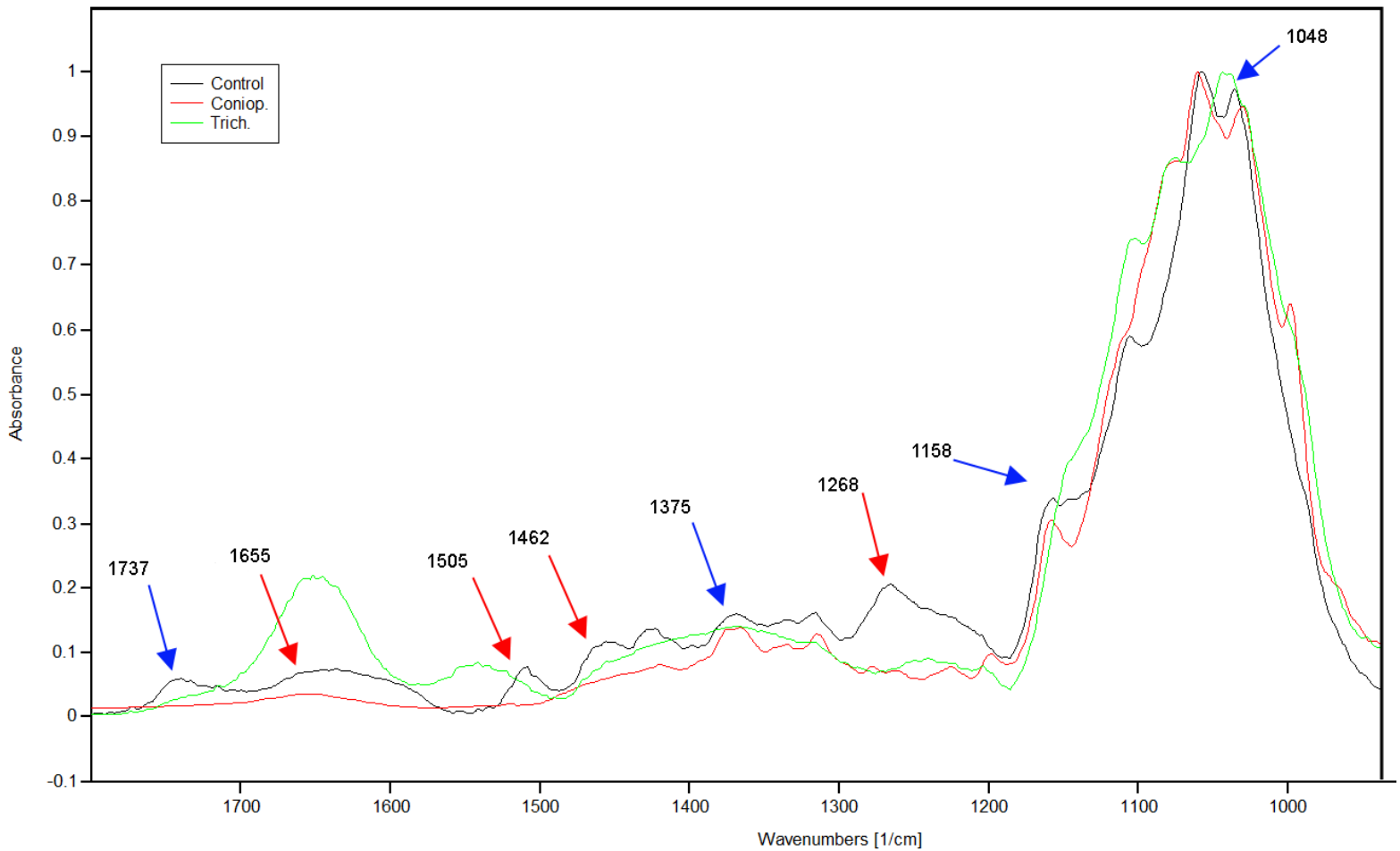


Figure 4.29: Normalized FTIR spectra of spruce wood exposed to *C. puteana* and *T. abietinum*. *C. puteana* decreased the peaks at 1268, 1462, 1505 and 1737 cm^{-1} related to cellulose, hemicelluloses and lignin. *T. abietinum* decreased the peaks at 1268 cm^{-1} related to lignin and increased the peak at 1165 cm^{-1}. The spectrum of the sound wood control is shown for comparison

4.3.2.4. Light microscopy

Light microscopy revealed degradation of lime wood by *Alternaria* sp., *C. ligniaria*, *Cladosporium* sp., *Phialocephala* sp., *Phialophora* sp. and *C.* puteana. Wood colonized by *C. ligniaria*, *Phialocephala* sp., *Phialophora* sp. and *C. puteana,* showed a decay pattern resembling that caused by soft-rot fungi: a mixture of soft-rot decay cavities and erosion of fibers and vessels walls. The decay pattern caused by *Cladosporium* sp. consisted mainly of erosion of wood cell walls, while *Alternaria* sp. produced a general disruption of colonized wood tissues, but no clear signs of cell wall degradation. Some damage of wood tissue was found in samples colonized by *B. fuckeliana, Lecythophora* sp., *L. infectoria, M. minutella* and *T. abietinum.* In these samples rupture of vessels cell walls was observed, which may have been due to the enzymatic action of fungi. No degradation was observed in samples colonized by *E. nigrum, H. dematioides, A. pullulans* (black and white) or *Phoma* sp. Irrespective of the different patterns of degradation caused by the fungi, most of them colonized parenchyma cells (ray and axial parenchyma) and the lumens of vessels where spores and hyphae accumulated.

In samples colonized by *Alternaria* sp. a general disruption of wood tissues was observed, but no clear pattern of wood cell wall degradation was seen. Samples colonized by *A. pullulans* (black) and *A. pullulans* (white) showed no signs of fungal degradation. Samples colonized by *B. fuckeliana* showed erosion of wood tissues in cells adjacent to vessels. Samples colonized by *C. globosum* showed clear signs of soft rot decay-presence of soft-rot cavities and erosion of wood cell walls. Samples colonized by *Cladosporium* sp. showed erosion-type decay of fibers and vessels, but rays appeared to be resistant to degradation

(Figure 4.30). Samples colonized by *C. ligniaria* showed erosion of wood cell walls and soft-rot cavities. Samples colonized by *C. puteana* showed clear degradation of wood cell walls (erosion) and presence of soft rot-like cavities. Samples colonized by *H. dematioides* showed the presence of spore aggregations in vessel lumina. In samples colonized by *Lecythophora* sp. and *L. infectoria* vessels walls were degraded (Figure 4.31). Samples incubated with *M. minutella* showed rupture of cell walls. Samples colonized by *Phialocephala* sp. showed erosion of wood cell walls. Samples colonized by *Phialophora* sp. showed erosion and rupture of wood cell walls in tissue close to the surface of the sample. Samples incubated with *T. abietinum* showed degradation of vessels. No signs of degradation were seen in samples incubated with *Phoma* sp. (Figure 4.32).

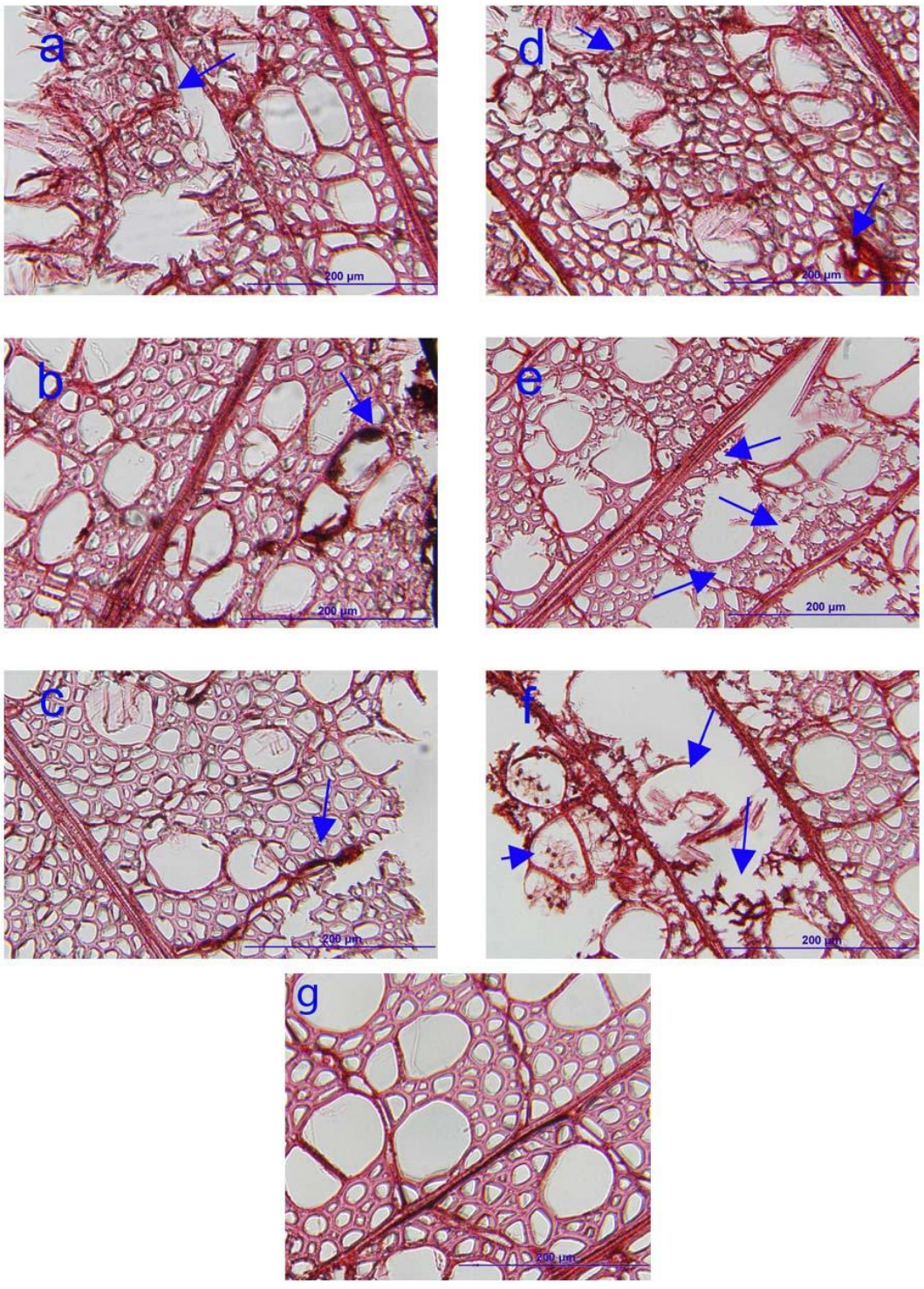


Figure 4.30: Light microscopy images of lime wood colonized by (a) *Alternaria* sp.; (b) *A. pullulans* (black); (c) *A. pullulans* (white); (d) *B. fuckeliana*; (e) *C. globosum;* (f) *Cladosporium* sp.; (g) control

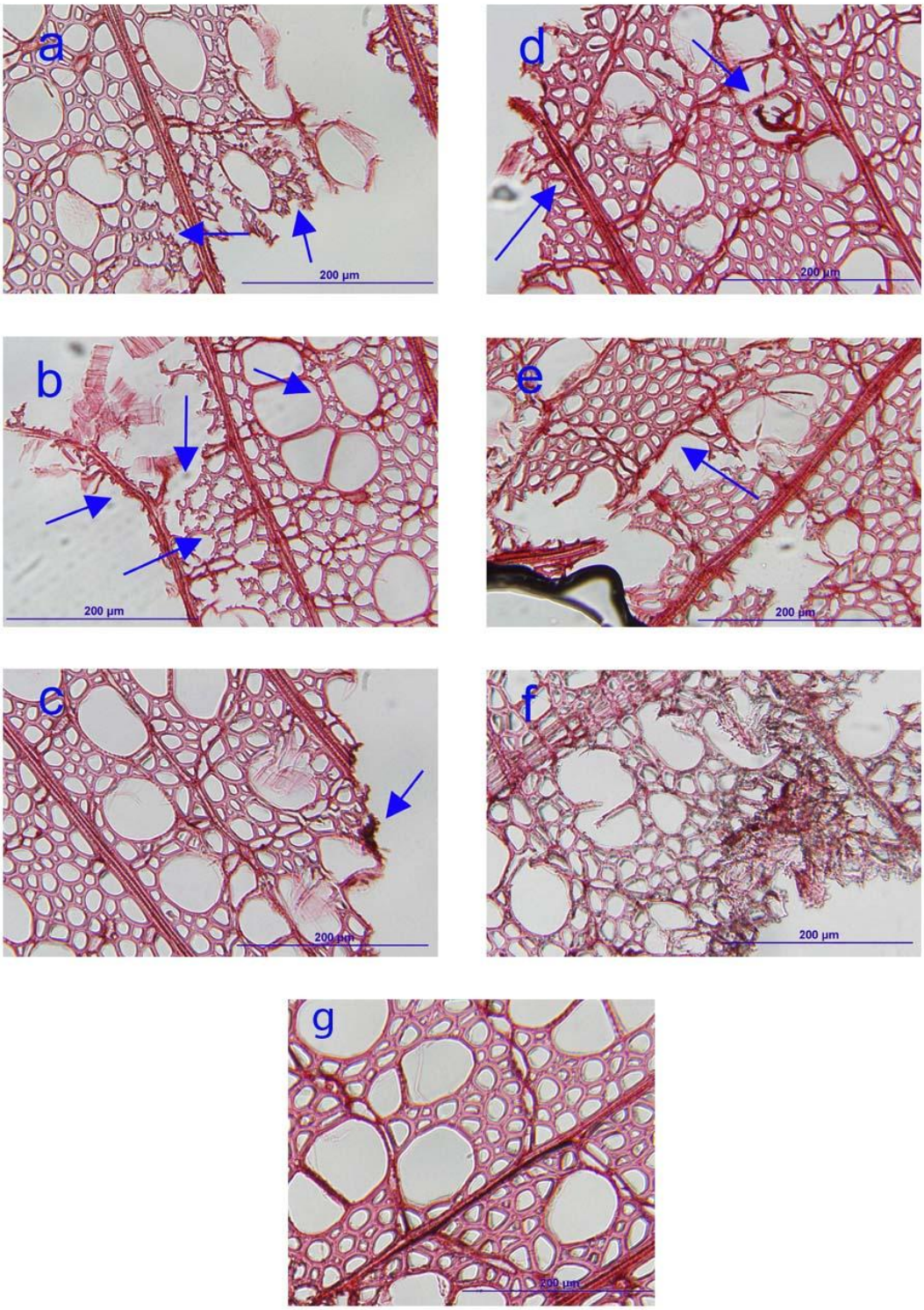


Figure 4.31: Light microscopy images of lime wood colonized by (a) *C. ligniaria*; (b) *C. puteana*; (c) *E. nigrum*; (d) *H. dematioides;* (e) *Lecythophora* sp.*;* (f) *L. infectoria*; and (g) control

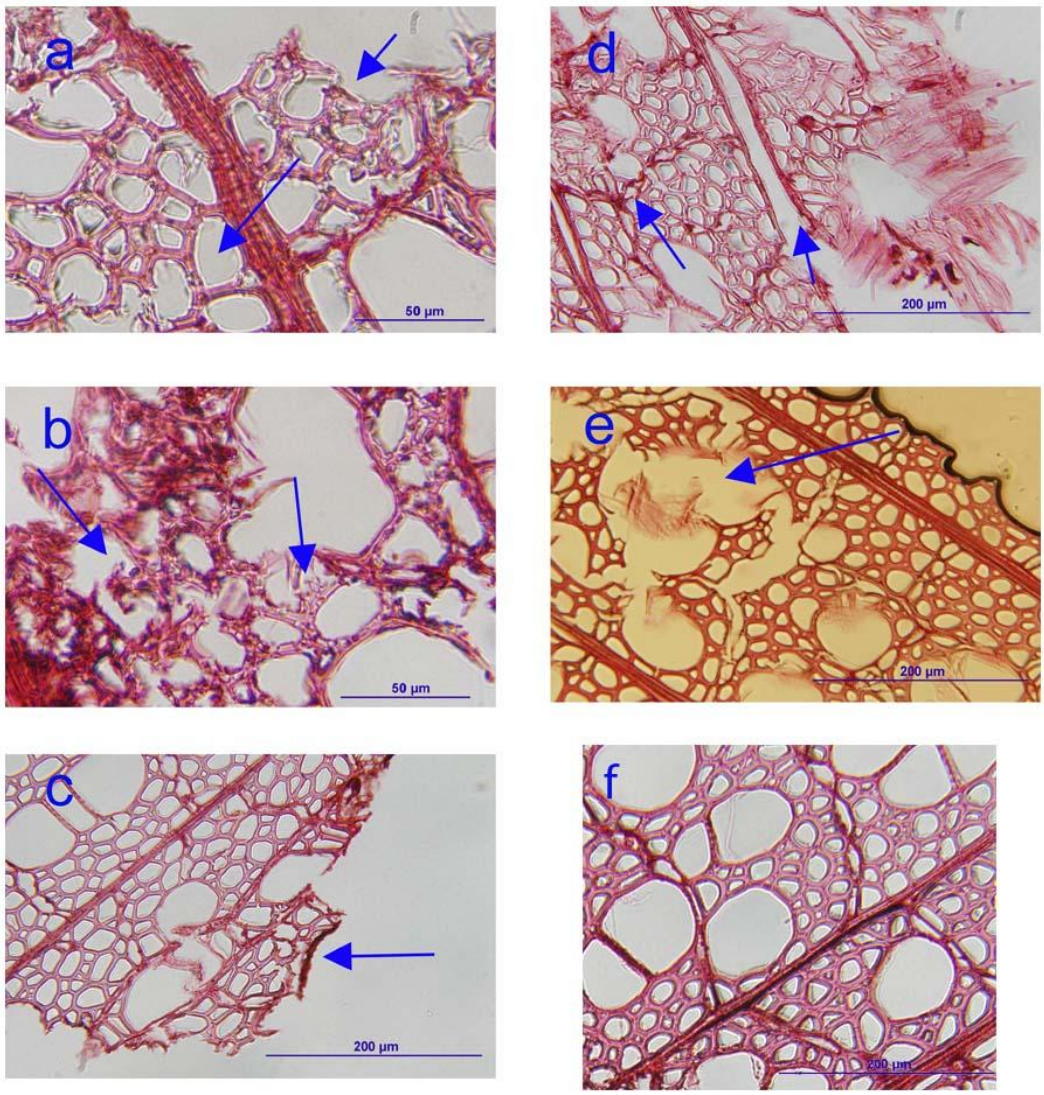


Figure 4.32: Light microscopy images of lime wood colonized by (a) *M. minutella*; (b) *Phialocephala* sp.; (c) *Phialophora* sp.; (d) *Phoma* sp.; (e) *T. abietinum;* and (f) sound wood control

Light microscopy showed that spruce wood was much less susceptible to degradation by test fungi than lime. Despite colonization of spruce by fungi little decay was observed.

In samples colonized by *Alternaria* sp., *C. globosum* and *Cladosporium* sp. disruption of wood tissues was observed close to the surface of the samples- it seems that cell walls were weakened by the presence of the fungus. Samples colonized by *A. pullulans* (black) and *A.*

pullulans (white) showed colonization of parenchyma rays and tracheids. Samples colonized by *B. fuckeliana* showed disruption of wood tissues close to the surface of the sample and detachment of wood cell walls colonized by fungi (Figure 4.33). In samples colonized by *C. ligniaria, C. puteana, Lecythophora* sp. disruption of wood tissues was observed close to the surface of the samples. In samples colonized by *E. nigrum* and *L. infectoria* colonization of parenchyma ray cells and resin canal was observed, but there were no signs of decay. The sample colonized by *H. dematioides* showed colonization of rays close to the surface and deterioration and staining of the first rows of tracheids (Figure 4.34). Samples incubated with *M. minutella* and *Phialocephala* sp. showed disruption of wood tissues close to the surface of the sample and detachment of wood cell walls in cells colonized by fungi. Samples colonized by *Phialophora* sp. *T. abietinum* and *Phoma* sp. showed a general disruption of wood tissues close to the surface of the samples (Figure 4.35).

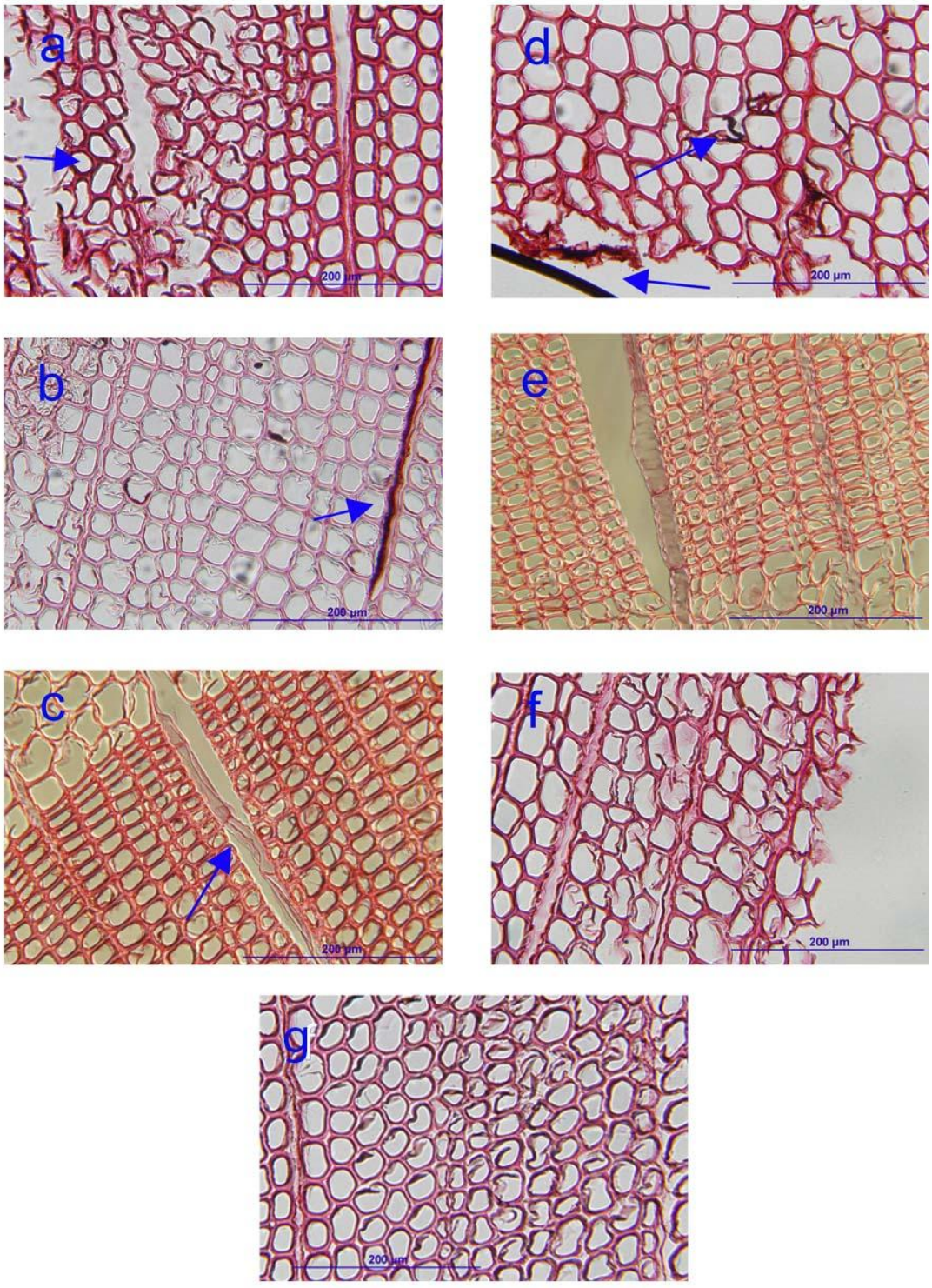


Figure 4.33: Light microscopy images of spruce wood colonized by (a) *Alternaria* sp.; (b) *A. pullulans* (black); (c) *A. pullulans* (white); (d) *B. fuckeliana;* (e) *C. globosum;* (f) *Cladosporium* sp.; and (g) control

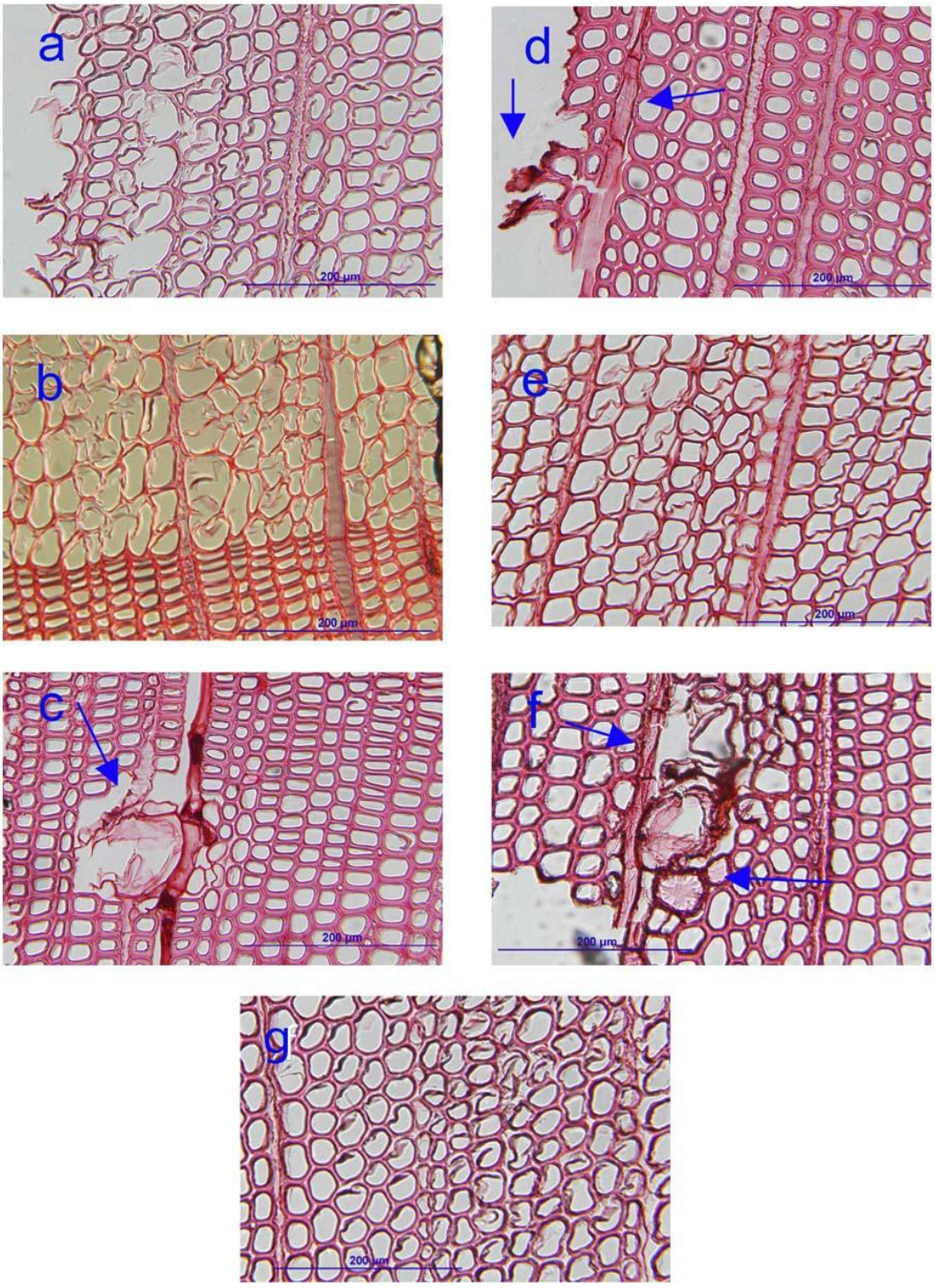


Figure 4.34: Light microscopy images of spruce wood colonized by (a) *C. ligniaria*; (b) *C. puteana*; (c) *E. nigrum*; (d) *H. dematioides;* (e) *Lecythophora* sp.*;* (d) *L. infectoria*; and (g) Control

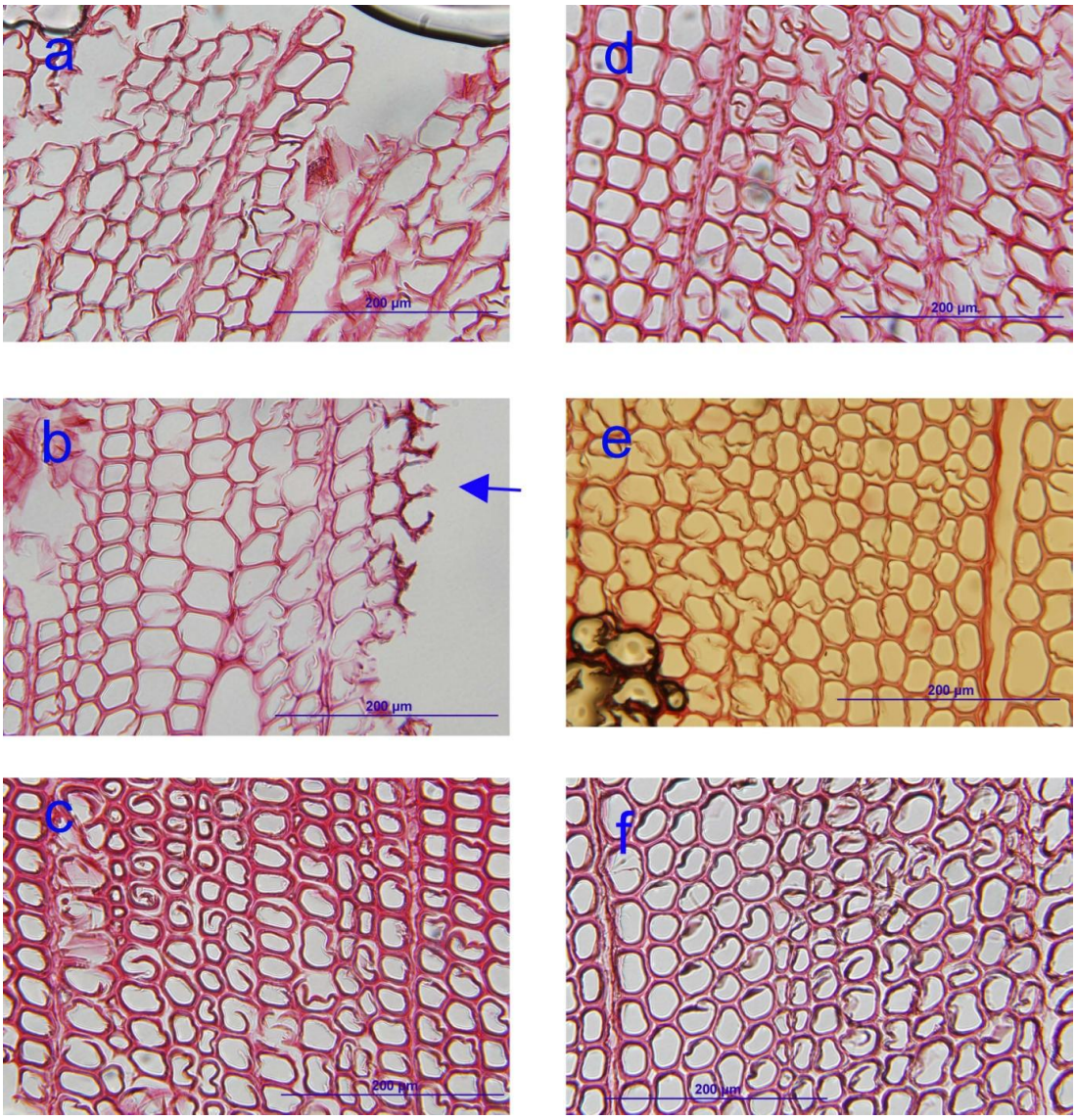


Figure 4.35: Light microscopy images of spruce wood colonized by (a) *M. minutella*; (b) *Phialocephala* sp.; (c) *Phialophora* sp.; (d) *Phoma* sp.; (e) *T. abietinum;* and (f) sound wood control

4.3.2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) was carried out on lime and spruce wood samples colonized by *Cladosporium* sp., and lime wood samples colonized by *A. pullulans* (Figure 4.36 and Figure 4.37). *Cladosporium* sp. was able to form a compact mycelial mat that evenly covered the surface of lime. The lime wood was very heavily and evenly degraded. Wood fibers appeared to be degraded to more basic sub-units. SEM images suggest that degradation of lime occurred from the direct effects of enzymes diffusing from the hyphal mat towards the wood surface. No cavities or bore holes were observed. In contrast, *Cladosporium* sp. was not effective at degrading spruce, and the hyphal mat formed by the fungus on spruce was much less compact than that on lime. Similarly, *A. pullulans* did not produce any changes to the microstructure of spruce or lime wood.

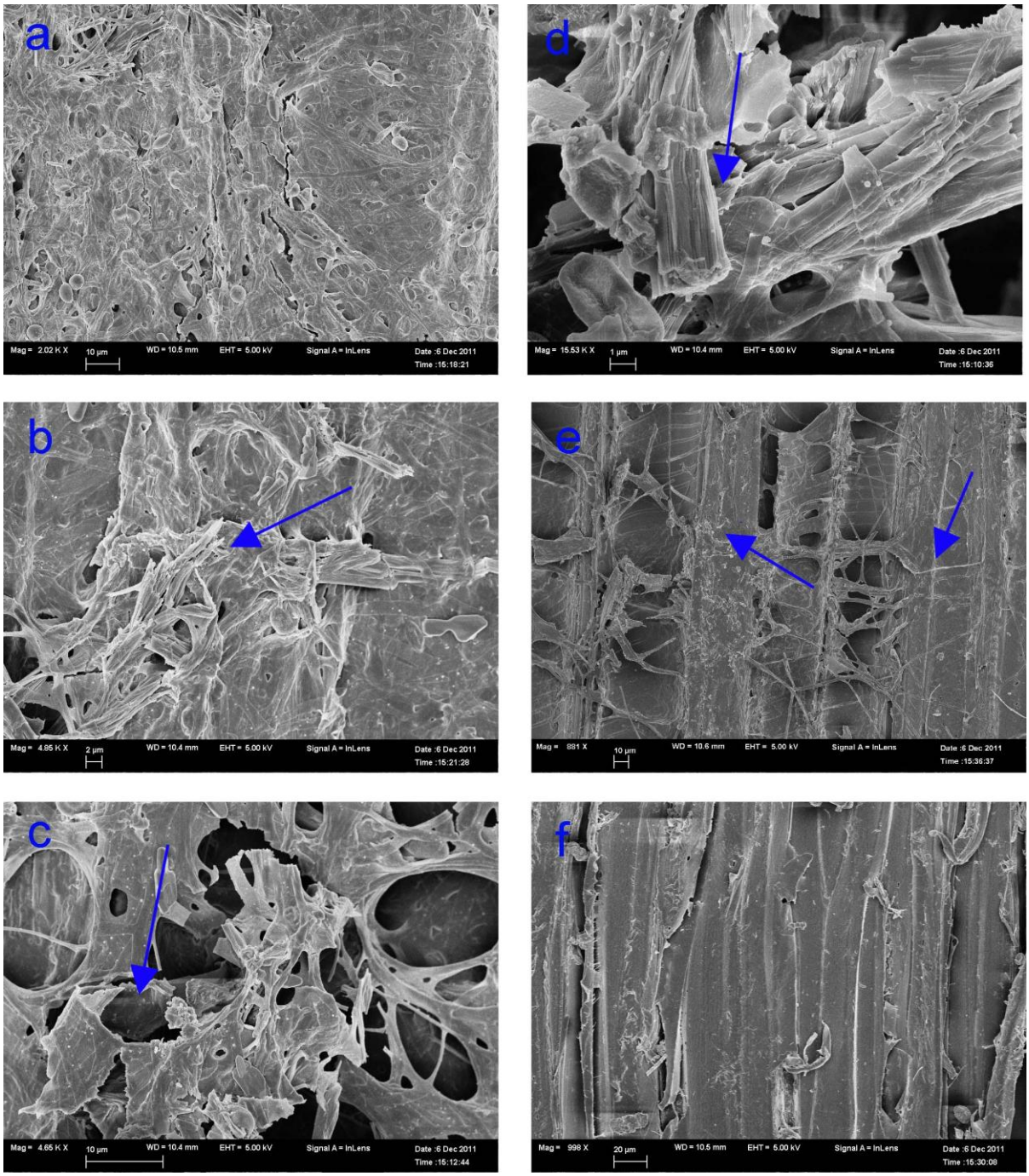


Figure 4.36: SEM images of lime wood colonized by *Cladosporium* sp. and *A. pullulans* (black). a) *Cladosporium* sp. formed a complex and packed net of hyphae on the surface the veneer; b) and c) *Cladosporium* sp. eroded the wood and the whole surface was affected; d) higher magnification image of a veneer degraded by *Cladosporium* sp. revealed that in some cases the wood cells were degraded to more basic sub-units; e) lime wood veneers colonized by *A. pullulans* showed no sign of decay at the surface despite colonization by hyphae; f) sound wood control

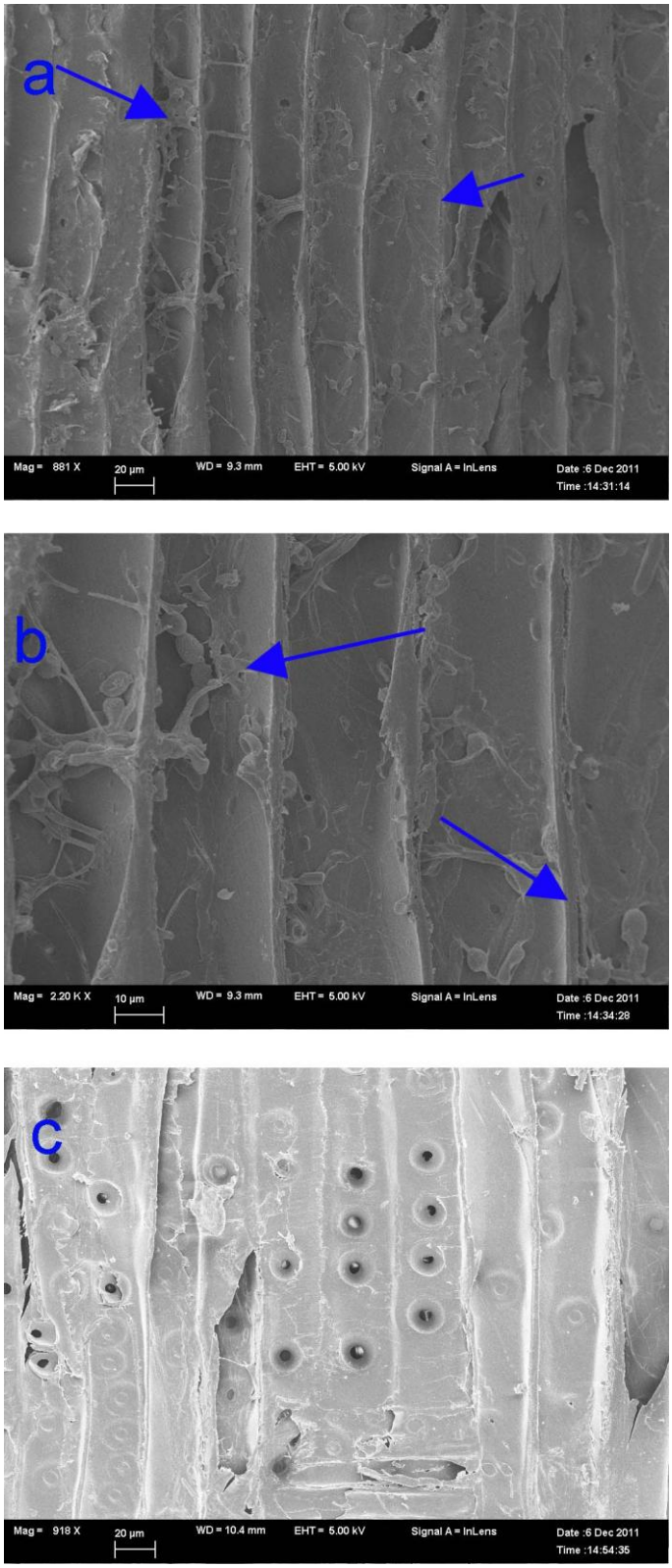


Figure 4.37: SEM images of spruce wood colonized by *Cladosporium* sp. a) presence of hyphae covering the wood surface; b) higher magnification imagine showing the presence of a complex network of hyphae and spores on the veneer, but no signs of degradation were observed; c) sound wood control

4.4. Discussion

In the introduction to this Chapter, I hypothesized that some of the fungi isolated from weathered wood would be able to degrade wood. Some of my findings support this hypothesis as *Cladosporium* sp., *C. ligniaria*, *E. nigrum*, *L. infectoria*, *M. minutella* and *Phialocephala* sp. were all able to significantly decrease some of the mechanical properties of lime and spruce wood veneers. Peak tensile stress ratio and toughness ratio were the parameters most affected by these fungi. I chose to examine the changes in mechanical properties of lime and spruce veneers caused by fungi, because mechanical properties depend on the integrity of cellulose, hemicelluloses and lignin. Cellulose is responsible for strength in wood fibers mainly due to its high degree of polymerization and linear orientation, while hemicelluloses also contribute to tensile properties as they act as coupling agents between cellulose and lignin (Ifju, 1964; Spiegelberg, 1966). Lignin helps to bind carbohydrates molecules together within the cell wall of wood fibers (Jeffries, 1994). In support of these results, the dynamic stiffness (storage modulus) of samples incubated with these organisms also exhibited lower values than sound wood. Storage modulus (SM) represents the elastic component of a viscoelastic material or the ability of wood to recover from elastic deformation (Menard 1999). The SM of wood is negatively correlated with temperature as the hemicelluloses and lignin become ductile when temperature increases and produce movements in their polymeric side chains (Birkinshaw et al. 1999). A lower dynamic stiffness as was observed for wood samples exposed to some fungi provides evidence of changes to the structure of hemicelluloses and lignin. Changes to the chemical composition of hemicelluloses, lignin and cellulose were confirmed in both spruce and lime

by FTIR spectroscopy. FTIR spectroscopy detected changes in functional groups associated with all three of wood's polymeric constituents in wood exposed to fungi. Early decreases in the mechanical properties of wood as a result of exposure to fungi have been related to degradation of hemicelluloses (Winandy and Morrell 1993). For example, observation of *Pinus* spp. colonized by brown-rot fungi showed that early losses in bending strength (> 40%) were related to degradation of hemicelluloses. In contrast, later more pronounced strength losses (> 75%) were caused by degradation of cellulose (Curling et al. 2002).

Changes in the microstructure of wood colonized by the fungi also provide evidence of the ability of some of the fungi to degrade wood. The decay pattern observed by light microscopy of transverse sections of solid wood samples exposed to fungi consisted of cavities and erosion of cell walls. Such a pattern is typical of soft-rot decay caused by ascomycete fungi (Savory 1954). However, SEM revealed that the surface of highly degraded veneers colonized by *Cladosporium* sp. was different to that of the surface of solid wood samples. Wood fibers at veneer surfaces appeared to be very heavily degraded by direct enzymatic leakage from fungal hyphae established on the veneer surface. SEM images showed fiber cell walls breaking down to more elemental sub-units.

Amongst the different fungi that were tested, results showed that *Cladosporium* sp. and *C. ligniaria* caused the greatest losses in peak tensile stress ratio, MOE ratio, peak stiffness ratio and toughness ratio of lime and spruce wood. Changes in FTIR bands for carbohydrates and lignin in spruce and lime provided evidence of the ability of these fungi to modify wood's polymers. *Cladosporium* spp. have been reported to possess cellulase, xylanase, mananase, amylase and cellobiose dehydrogenase enzymes (Ghahfarokhi et al. 2004;

Nilsson, 1974). *C. ligniaria* produces cellulase, xylanase, manganese peroxidase and lignin peroxidase (Lopez et al., 2007). The action of these enzymes may account for why both fungi were able to degrade lime and to a lesser extent spruce veneers. Furthermore, Zyani et al. (2009) observed that *Cladosporium cladosporioides* was able to decay wood in *in-vitro* tests, although they did not specify the wood species that was tested. Therefore, my findings are consistent with their observations. *Cladosporium* sp. is a highly melanized fungus which is adapted to the conditions found at wood surfaces exposed outdoors (Park 1982). Moreover, it is frequently isolated from weathered wood (Hansen, 2008). *Cladosporium*'s cellulolytic abilities may represent another beneficial adaptation for survival at weathered wood surfaces. Likewise, *L. infectoria*, *M. minutella*, *Phialophora* sp and *Phialocephala* sp. also significantly reduced the tensile properties of wood; and with the exception of *M. minutella* and *L. infectoria* in lime wood, all the other fungi I tested were able to modify the storage modulus of wood. The latter observation suggests that wood exposed to fungi was less rigid than the sound wood controls. Fungal species from these genera have been reported to produce soft-rot decay of solid wood and also forest debris (Morrell and Zabel, 1985; Hale and Eaton, 1985; Allmer et al. 2006). *Phialophora* and *Phialocephala* fungi also cause soft-rot cavities and erosion-type decay in pine and beech wood (Morrell and Zabel 1985). Light microscopy images here showed that presence of soft-rot cavities in samples of lime wood exposed to some of the fungi, but erosion of cell walls was more frequently observed. Enzymatic erosion is the simplest type of soft-rot decay since it only requires the presence of diffusible enzymes inside cell lumens, whereas soft-rot cavity formation is more complex as it requires fungal hyphae to penetrate into the cell

wall, align in the direction of the microfibrils and produce enzymes that dissolve the wall around the hyphae (Nilsson, 1973).

Alternaria sp. and *E. nigrum* reduced the peak tensile stress of lime veneers, but not that of spruce. Both fungi commonly colonize weathered wood (Morrell and Zabel, 1985; Pfeffer et al., 2012), but only *Alternaria* species have been reported as being able to produce soft-rot decay (Rajderkar 1966; Morrell and Zabel 1985). *A. pullulans* is probably the most ubiquitous colonizer of weathered wood surfaces (Dickinson 1971). It did not significantly reduce the tensile properties of lime or spruce. However, its effect on the toughness approached statistical significance. *A. pullulans* together with *Alternaria* sp. and *Cladosporium* sp., are well adapted to survive at weathered wood surfaces, as mentioned in Chapter 2. Tests have demonstrated that they can withstand long periods of dryness and high temperatures (Park 1982). Several studies have tried to elucidate whether *A. pullulans* can degrade wood and model wood compounds. For example, Sharpe and Dickinson (1992) carried out an *in-vitro* test on the ability of *A. pullulans* to use cellulose, different hemicellulose monomers and dimers, and lignin model compounds, as sole carbon sources. Their findings suggested that *A. pullulans* is not able to degrade cellulose, but it can use simple sugars. Accordingly, the authors concluded that wood cell wall carbohydrates need to be broken down possibly to mono or disaccharides before *A. pullulans* can utilize them (Sharpe and Dickinson 1992). Sharpe and Dickinson (1992), also found that *A. pullulans* was able to utilize phenolic compounds more readily than oligosaccharides. In support of their findings I observed that the FTIR band at 1268 cm^{-1} (C-O guaiacyl ring breathing, C-O stretching, C-O linkage in guaiacyl aromatic methoxyl groups lignin; Pandey and Theagarajan

1997) appeared to decrease in spruce wood incubated with *A. pullulans*. According to Bourbonnais and Paice (1987) *A. pullulans* is able to cleave β-O-4 linkages in model lignin compounds, but it does not have the ability to degrade non-phenolic dimers. This observation indicates that the capacity of *A. pullulans* to degrade lignin is limited. Nevertheless, fungi with limited ability to degrade wood tissues may increase the overall rate of decay because their enzymes may eventually act on wood that has been degraded by other fungi. For instance, *L. hoffmanni* can metabolize phenolic compounds, and fungi from the genus *Phoma* have been isolated from soft-rotted wood (Savory 1954; Bugos et al. 1988). Both of these fungi may contribute to the degradation of wood during the later stages of decay, even though they are unable to degrade sound wood.

Lime wood was more susceptible to fungal degradation than spruce. The greater susceptibility of lime to degradation by ascomycetes isolated from weathered wood (here) accords with the observations of Nilsson and Daniel (1989) and Encinas et al. (1998) who reported weight and toughness losses in birch and pine spp. colonized by staining fungi. Faix et al. (1985); Nilsson and Daniel (1989) and Blanchette (1991) all suggested that differences in the lignin content of hardwood (lime) and softwood (spruce) may account for the greater susceptibility of the former to degradation by ascomycetes. Lignin is a significant barrier to hyphae and enzymes since it encrusts cell walls preventing enzymes from hydrolyzing carbohydrates (Winandy and Rowell 2005). Softwoods with a high concentration of guaiacyl lignin units are particularly resistant to fungi that cause soft-rot, whereas wood consisting predominantly of syringyl lignin is more susceptible to soft-rot fungi (Faix et al. 1985; Nilsson and Daniel 1989; Blanchette 1991).

The toughness of wood veneers was severely affected by fungi, as mentioned above Toughness is the most sensitive indicator of fungal decay (Wilcox 1978). Some studies have shown that staining fungi cannot alter the toughness of wood (Schirp et al. 2000), whereas other have shown the opposite. For example, Encinas et al. (1998) reported that the blue stain fungus *Lasiodiplodia theobromae* was able to produce significant losses in toughness in birch and Pine species. Such losses were well correlated with weight losses in the tested samples. Discrepancies on the effect of staining fungi on the mechanical properties of wood, and specifically on toughness properties could be related to the fact that degradation of wood varies with both fungal and wood species (Zabel and Morrell 1992). Nevertheless, here I showed that surface fungi isolated from weathered wood are able to affect the toughness of thin wood veneers. Furthermore, microscopy showed that fungi reducing the toughness of veneers also eroded and produced cavities in wood cell walls. The erosion of wood cell walls during the weathering has been attributed to the action of UV radiation and water (Evans 2008). It is not clear whether fungi also contribute to the erosion of wood cell walls during weathering.

As stated in the introduction to this thesis, there is a body of opinion that indicates that fungi colonizing weathered wood are unable to degrade woody tissues (Feist 1983). Thus, references to their effect on wood mainly describe how they discolor and affect the appearance of wood. However, Duncan (1963) described early research suggesting that fungi colonizing weathered wood might degrade wood tissues. My findings for some of the fungi isolated from weathered wood support such suggestions, although the contribution of these organisms to the degradation of wood surfaces *in-vivo* would depend on conditions at

weathered wood surfaces being favorable to decay. Soft-rot decay in weathered western red cedar shingles was documented by Smith and Swann (1976). Their samples were actively colonized by a number of moulds, which produced soft-rot cavities and erosion of wood cell walls. In addition, Seifert (1964) reported that *A. pullulans*, possibly the most successful colonizer of weathered wood surfaces (Dickinson 1971), was able to produce weight losses and reduce cellulose and pentosans in Scots pine wood. Results here showed that *A. pullulans* was able to affect the strength properties and chemical composition of wood veneers, but not as much as *Cladosporium* sp. or *C. ligniaria* did. Soft-rot can occur slowly at low moisture levels according to Blanchette et al. (1994). Whereas, Worrall et al. (1991) suggested that soft-rot fungi may not differ from basidiomycetes in their preference for moderate moisture conditions. Moisture conditions favoring microbial degradation at wood surfaces are probably met sporadically year round but more commonly during winter and autumn as evaporation rates are slower due to lower temperatures (Denig et al. 2000). Microbial degradation of weathered wood surfaces may be more pronounced in wet tropical climates, which produce conditions at wood surfaces that are more favorable for decay. Furthermore, weathered wood is a modified substrate that is more susceptible to microbial degradation because solar radiation degrades carbohydrates and lignin. Such degradation may facilitate the enzymatic degradation of the remaining wood tissues as suggested by Evans and Banks (1986).

The ability of some of the fungi colonizing weathered wood to break down wood tissues *in-vitro* has been shown in this Chapter. However, further research is needed to elucidate whether the conditions for microbial degradation are met at wood surfaces either

sporadically or seasonally in different climates. If such conditions occur it would be pertinent to clarify how much of the erosion at weathered surfaces is due to the action of surface fungi.

4.5. Conclusions

My observations support the hypothesis that fungi isolated from weathered wood can degrade wood tissues because *Cladosporium* sp., *C. ligniaria*, *E. nigrum*, *L. infectoria*, *M. minutella* and *Phialocephala* sp. were able to significantly reduce the mechanical properties of lime and spruce wood. Tensile stress and toughness were the parameters most affected by fungi. *Cladosporium* sp., *C. ligniaria* produced the most dramatic changes in these mechanical properties. As a result veneers colonized by these fungi became very brittle. These fungi caused erosion of cell walls (soft-rot decay type II) and to a lesser extent soft-rot cavities (soft-rot decay type I). *A. pullulans,* one of the most successful organisms colonizing weathered wood worldwide, produced no significant changes in the tensile properties of incubated wood. However, its effect on the toughness of spruce was nearly significant. The dynamic stiffness of samples exposed to some of the fungi was lower than that of sound wood. Furthermore, some of the fungi were able to degrade wood's chemical components. Therefore, I conclude that some the fungi colonizing weathered wood surfaces are capable of causing significant degradation of wood particularly hardwood.

Further research is necessary to elucidate whether the conditions for microbial degradation are met at wood surfaces either sporadically or seasonally in different climates and how much of the erosion of wood during weathering is caused by the action of surface fungi.

Chapter 5: Effects of solar radiation on the colonization of weathered wood by fungi

5.1 Introduction

Wood exposed outdoors rapidly acquires a rough, gray color, which adversely affects its appearance (Feist 1990). The graying of wood surfaces is caused by the colonization of wood by melanized fungi, which have the ability to metabolize photodegraded wood polymers (Duncan 1963; Dickinson 1971). Melanin in these fungi is apparently synthesized as a protective response against solar UV radiation, but this response may darken the wood (Brisson et al. 1996; Fogarty and Tobin 1996; Butler and Day 1998; Henson et al. 1999). Information in the literature supports the concept that UV radiation increases the fungal staining of wood surfaces, as it has been shown that UV radiation increases melanin production in several fungi (Frederick et al. 1999). In addition, UV radiation may restrict fungal diversity at wood surfaces to those organisms able to survive exposure to energetic radiation, e.g. *Aureobasidium pullulans* (de Bary) G. Arnaud and *Hormonema dematioides Lagerb. & Melin* (Ray et al. 2004).

In this Chapter I hypothesize that blocking UV radiation from reaching wood surfaces will influence the diversity of fungi colonizing the wood surfaces. In the absence of UV radiation the adaptations of melanized fungi that commonly colonize weathered wood surfaces may not provide a competitive advantage and other fungi might successfully out compete them. In such conditions fungal staining of wood may be less severe than that of wood exposed to the full solar spectrum. This hypothesis was tested by exposing 25 southern pine boards for 40 weeks under polymethylmethacrylate filters which blocked different wavelengths of

solar radiation from reaching wood surfaces. Fungi colonizing the samples exposed under different filters were isolated, identified and characterized. Changes in fungal diversity were recorded and the color of wood surfaces exposed under the different filters was measured and related to the ecology of fungi colonizing the samples. The final appearance of exposed wood surfaces was evaluated by measuring the area colonized by fungi and color of wood surfaces. Chemical changes at wood surfaces under different filters were evaluated using FTIR internal reflectance spectroscopy. In summary, in this Chapter I seek to better understand the importance of melanin for fungi colonizing wood surfaces exposed to solar UV radiation and record the frequency of highly melanized fungal hyphae colonizing wood surfaces exposed to the weather.

5.2 Materials and methods

5.2.1 Experimental design and statistical analyses

The experiment was initially designed to assess the effect of different chemical treatments and wavelengths of solar radiation on the color of wood surfaces exposed outdoors. However, later on as results became available it was realized that the experiment could also provide important information on the effect of solar radiation on the ecology of fungi colonizing exposed samples. Therefore, initially a split-split-plot design was used to examine the effect of different components of the solar spectrum and four chemical treatments at four different concentrations (chemical loads) on the color of wood surfaces. The design included five decking boards cut from five different trees (blocks), which provided replication at the higher level. Each sample (whole-plot) cut from these decking boards was

sub-divided into 4 areas, which were assigned to three treatments plus a control (water) (sub-plots). Such areas were then sub-divided into four strips (sub-sub-plots), which were randomly assigned to the four chemical loads. The samples were exposed in racks under one of five different filters that blocked selected regions of the solar spectrum (Evans et al., 2008). The resulting experimental design accounted for random variation in wood properties of decking samples, that due to exposure of samples under various filters in different testing racks (spatial effects of location of samples between and within racks) and that due to the spatial effect of location of different chemical treatments and chemical loads. Separate analyses of variance (ANOVA) were performed after the first 4 weeks of exposure, every two weeks until week 20 and then at weeks 24, 32 and 40. Data for fungi colonizing and staining the wood were only acquired from untreated areas of the samples. Therefore, such data were analyzed as a factorial experiment with random blocks. ANOVA was performed to examine the effect of filter type (F), fungal species (S) and F x S on the frequency of isolation of fungi and Simpson index for fungal diversity, and filter type on the area colonized by fungi (stained area). Analysis of variance (ANOVA) was performed using the Software Genstat v. 12 (VSN International 2009). The assumptions of ANOVA were tested prior to the final analysis (normality of residuals and homogeneity of variances), and as a result of such tests the data for fungal diversity were transformed into natural logarithms and analyzed as logarithms. After ANOVA ($p<0.05$), significant differences were estimated using Fisher's least significant test (l.s.d.). Results are presented in graphs as means and either standard error of the differences (s.e.d.) or l.s.d. bars can be used to

compare means. The output from the statistical analyses of data in this Chapter is appended to this thesis (Appendix 3). A summary of the experimental design is presented in Table 5.1.

Table 5.1: Summary of experimental design used to test the effect of solar radiation on wood surfaces and fungal colonization

Blocks	Exposure type	Wood samples	Chemical treatments	Chemical loads	Strips per sample
1	5 + control	6	3 + water	4	16
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
5	5 + control	6	3 + water	4	16

5.2.2 Wood samples

The same five flat-sawn southern pine boards used for the experiment described in Chapter 3 were used in this experiment. The preparation of samples was the same as that of the samples prepared for the experiment described in Chapter 3. Then, sixteen strips, 20 mm wide, were cut into the upper face of each sample by cutting 15 grooves, 3 to 5 mm deep (transverse to the grain), with a band saw Meber (Model SR-500). Strips were isolated from each other by filling the grooves with a hot melt resin (commercial grade) applied with a heat gun. The end grain of samples was sealed with epoxy resin (Quick cure 5; System three resins, Inc. WA, USA) to restrict rate of drying and the development of end checks.

5.2.3 Chemical treatments

Three chemicals plus a control were applied to the surface of the wooden samples to test their ability to decrease color changes of wood during exposure to different wavelengths of the solar spectrum. Treatments included: (1) carpropamid, an inhibitor of dihydroxynaphthalene (DHN) fungal melanins (Bayer Crop Science, Germany); (2) acetic acid, a by-product in wood after acetylation (Glacial acetic acid, Fisher Scientific, Nepean-Ontario, Canada); (3) tinuvin 384, a benzotriazole UV absorber (Ciba Specialty Chemical Corporation, Tarrytown - New York, USA); and (4) distilled water (control). Each chemical treatment was brushed onto one of the four pre-designated areas on the wood surfaces. Each area consisted of 4 strips, which randomly received one of the 4 chemical loads, determined in agreement with the recommendations of the companies that manufacture the chemicals (Figure 5.1). Grooves at the wood surface and the chemical sealant (described at section 5.2.2) prevented the chemicals from diffusing from one strip to another. Details on the chemical treatments can be found in Table 5.2.

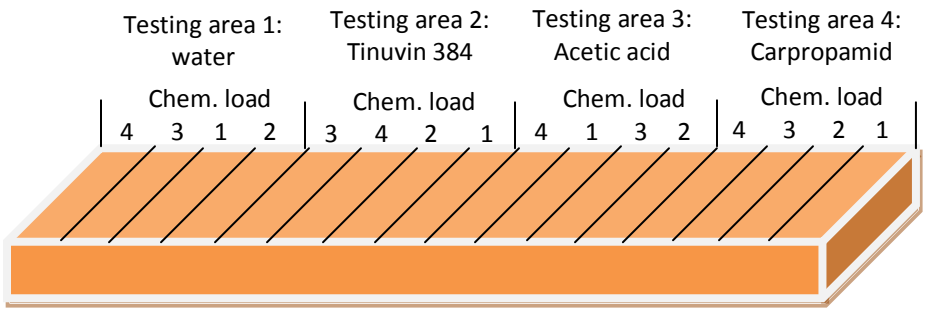


Figure 5.1: Distribution of chemical treatments, testing areas and chemical loads. The figure shows the treatments applied to sample 3 (block 1) exposed under a filter transmitting all wavelengths of solar spectrum (Filter 1)

Table 5.2: Chemical treatment applied to southern pine wood samples exposed outdoors for 40 weeks in Vancouver (Canada) and exposed to different wavelengths of the solar radiation

Chemical treatment	Solvent	Mother solution concentration (ppm)	Chemical load	Amount applied (mg)
Carpropamid	Acetone industrial grade	100	1	0.02
			2	0.03
			3	0.05
			4	0.06
Acetic acid	Water	100	1	0.01
			2	0.03
			3	0.04
			4	0.06
Tinuvin 384	Mineral spirit	10000	1	1.00
			2	2.00
			3	3.00
			4	5.00
Distilled Water	n/a	n/a	1	100.00
			2	200.00
			3	300.00
			4	400.00

5.2.4 Exposure

Samples were exposed in racks which contained five horizontal openings for different polymethylmethacrylate filters (CRYO Industries, Rockaway, USA). These filters transmitted selected regions of the solar spectrum (Table 5.3). Matching filters on the sides and ends of the openings prevented unfiltered light from reaching the samples. Five samples, cut from the same board, were randomly assigned to the five different areas in each rack. The samples were oriented parallel to the long axis of the racks on 40 mm wide spacer blocks. The construction of the racks is described by Urban (2005); and Evans et al. (2008). Angled aluminum sheet captured rain water and directed it on to the surface of samples (Figure 5.2). This sheeting and the wooden frame were painted dark brown to minimize reflection

of light on to the samples. Racks were inspected daily and dust accumulating on the filters was removed when necessary. Samples were exposed outdoors to the weather, ≈ 400 mm above ground for 40 weeks in Vancouver, Canada. The superficial moisture content of the wood samples was measured from week 10 to 32, using a portable resistance-type moisture meter (Delmhorst RDM³, Delmhorst Instrument Company). Meteorological conditions during the exposure trial are shown in Table 3.2 (Chapter 3). During the trial un-weathered southern pine controls samples were kept in the dark in a conditioning room.

Table 5.3: Filters used to block selected regions of the solar spectrum from reaching samples

Filter No	Filter type	Light type transmitted	Wavelengths blocked [nm]
1	OP-4	UVB+ UVA+Vis.light+IR	None
2	GP	UVA+Vis.light+IR	260-345
3	OP-2	Vis.light+IR	260-400
4	GP-Black 1146-0	IR	260-760
5	GP-Black 199-0	No light	All

IR: infra red; Vis: visible light; UVA: ultra violet light type A; UVB: ultra violet light type B

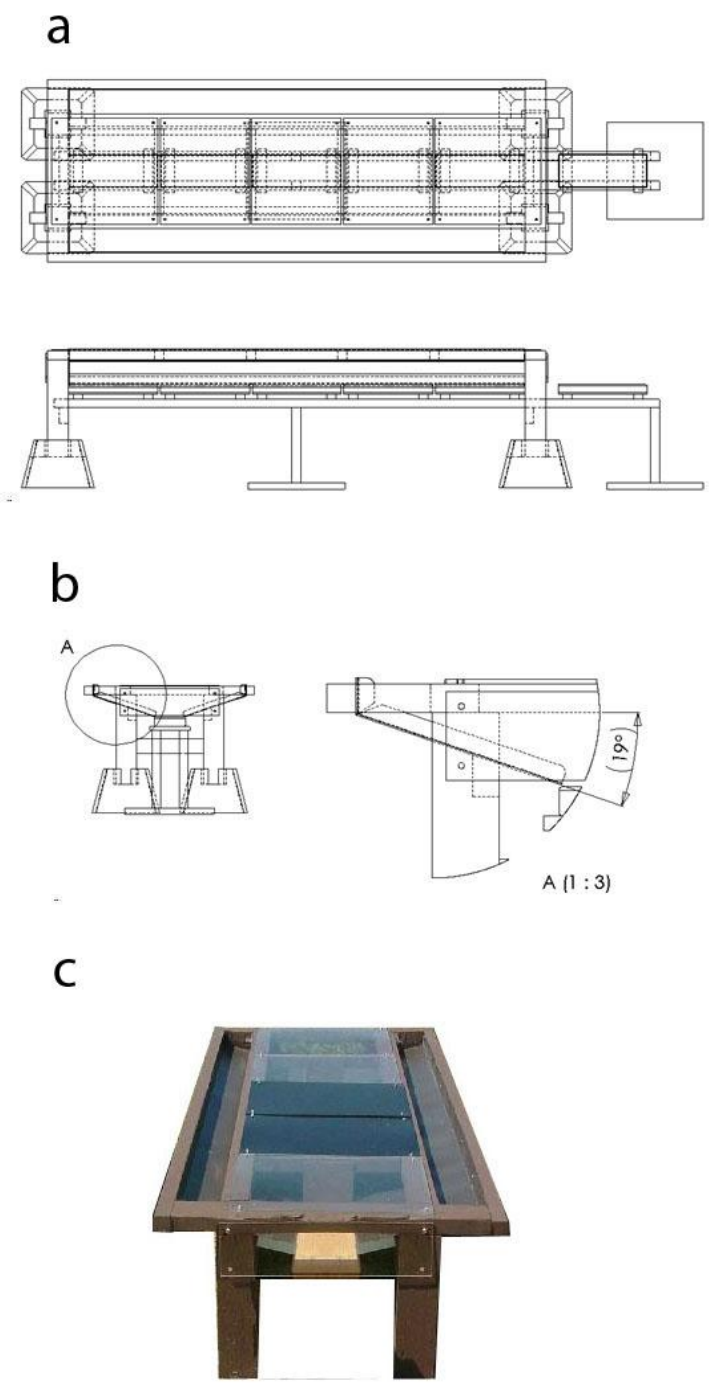


Figure 5.2: Rack used for exposure of wood to different wavelengths of the solar radiation. (a) and (b) engineering drawings of the rack featuring angled aluminum sheets; (c) actual view of the rack and the five different polymethylmethacrylate filters

5.2.5 Determination of wood color and area colonized by fungi

Every month during the 40 weeks that the wooden samples were exposed under the different filters their color was measured. Samples were removed from the racks every week during the first 4 weeks of exposure, every two weeks until week 20 and then at weeks 24, 32 and 40. The color of the wood was measured using a portable spectrophotometer and expressed using the CIELab color coordinates as described in Chapter 3 (section 3.2.6). Digital images of the wooden samples, scale 1:1; 96 dpi resolution, were obtained using a desktop scanner (Microtek Scan Maker i800) and the area of wood stained by fungi was quantified using Adobe Photoshop CS3 (Extended version 10.0.1, Adobe Systems Incorporated, USA) as described in Chapter 3 (section 3.2.6). Such quantification was performed only in the control areas (treated with water) of the samples.

5.2.6 Chemical changes at weathered wood surfaces and isolation and identification of fungi

FTIR spectroscopy was used to examine chemical changes occurring at untreated wood surfaces when samples were exposed under the filters for 40 weeks. Pieces of wood measuring 20 mm (width) x 60 mm (length) x 8 mm (thickness) were sawn from the control area of each sample and stored for 5 days in a vacuum desiccator over silica gel. Direct reflectance (ATR-IR) FTIR spectra of weathered (gray) surfaces were obtained as described in Chapter 3 (section 3.2.7).

The isolation of fungi from the surface of weathered southern pine samples used the method described in Chapter 3 (section 3.2.2). Four wood fragments obtained from the

control area of each sample were used for the fungal isolations from wooden samples exposed outdoors for 40 weeks under the different filters.

Isolated fungi were identified using both microscopy and DNA analysis. Molecular techniques were used first to identify fungi. Then, their identities were confirmed by examining their morphological features (Table 3.3, Chapter 3).

5.2.7 Fungal ecology and characterization of isolated fungi

The frequency of occurrence of fungi (FIF) colonizing each southern pine sample exposed under the different filters was calculated as follows:

FIF = number of fungi of the species *i* in the sample / total number of fungi in the sample

FIF results were grouped into seven categories. Six categories for the most frequently occurring fungi, plus one for "others", which comprised a diverse group of fungi isolated one or two times per sample. Results for the five categories isolated from wood exposed under the different filters are presented in graphs.

The diversity of fungi colonizing the southern pine samples exposed under the different filters was quantified using the reciprocal Simpson index, as described in Chapter 3 (section 3.2.3).

Isolated fungi were grown on 1% malt extract agar. A 5 mm diameter agar plug, from the original fungal culture, was placed on agar in a 150 mm x 15 mm round Petri dish. A digital image of the hyphal mat on each plate (1:1 scale) at standard conditions of illumination, was obtained after 7 days using a desktop scanner (as described in Chapter 3 section 3.2.4).

The diameter of the hyphal mat was measured using Photoshop as described in Chapter 3. After 20 days the plates were re-scanned without their lids and the images were used to calculate the lightness of the hyphal mats as described in Chapter 3.

5.3 Results

5.3.1 Color of wood after exposure

Independent analyses of variance were performed on color of wood for each exposure period from 1 to 40 weeks. There was no significant effect of the different chemical treatments, chemical loads and their interactions on the color of wood surfaces. However, filter type significantly affected (P-value > 0.001) the color of southern pine wood samples from week 1 to 40 (end of the exposure trial).

The color of southern pine specimens expressed using the CIELab color co-ordinates lightness (L), redness–greenness (a) and yellowness–blueness (b) were measured throughout the 40 week exposure trial. The color of samples that were kept in the dark in a conditioning room (20 ± 1 ˚C and 65 ± 5% r.h.) for the duration of the trial was also measured. During the first 20 weeks of exposure, lightness of samples exposed under filters 1,2, 4 and 5 decreased, although samples exposed to the most energetic radiation (filters 1 and 2) showed lower lightness values. Conversely, the lightness of samples exposed under filter 3 increased during the first 2 weeks of exposure, but thereafter their lightness decreased. After 20 weeks, less pronounced changes in lightness occurred in all samples. Samples exposed under filters that transmitted UV radiation were darker than those exposed under filters that blocked such radiation (Figure 5.3).

Significant differences in redness–greenness [a] of samples exposed under the different filters occurred after the first week of exposure (Figure 5.4). After the first week of exposure samples became greener. Samples exposed under filter 1 and 3 reddened until 8 to 10 weeks of exposure, and then they became greener ([a] value decreased). Greening continued until 20 weeks of exposure for samples that were shielded from UV radiation. Samples exposed under the filters that transmitted UV radiation continued to become greener until the end of the exposure trial. It was noticeable that samples exposed to UV radiation initially reddened significantly more than samples shielded from UV radiation, but the latter were generally greener than samples exposed to UV radiation.

Significant changes in yellowness–blueness also occurred in all samples except the ones exposed under filter 1, which became more blue (Figure 5.5). After two weeks exposure, samples exposed to UV and visible light (filters 1, 2, and 3) yellowed, but no major changes were observed in samples exposed under filters that blocked UV and visible light. After 4 to 10 weeks of exposure samples became bluer ([b] value decreased). This trend continued for approximately 20 weeks for all samples except the ones exposed under filter 1. Subsequently, the decrease in [b] was less pronounced except for samples exposed under filter 1, which continued to become bluer until the end of the exposure trial.

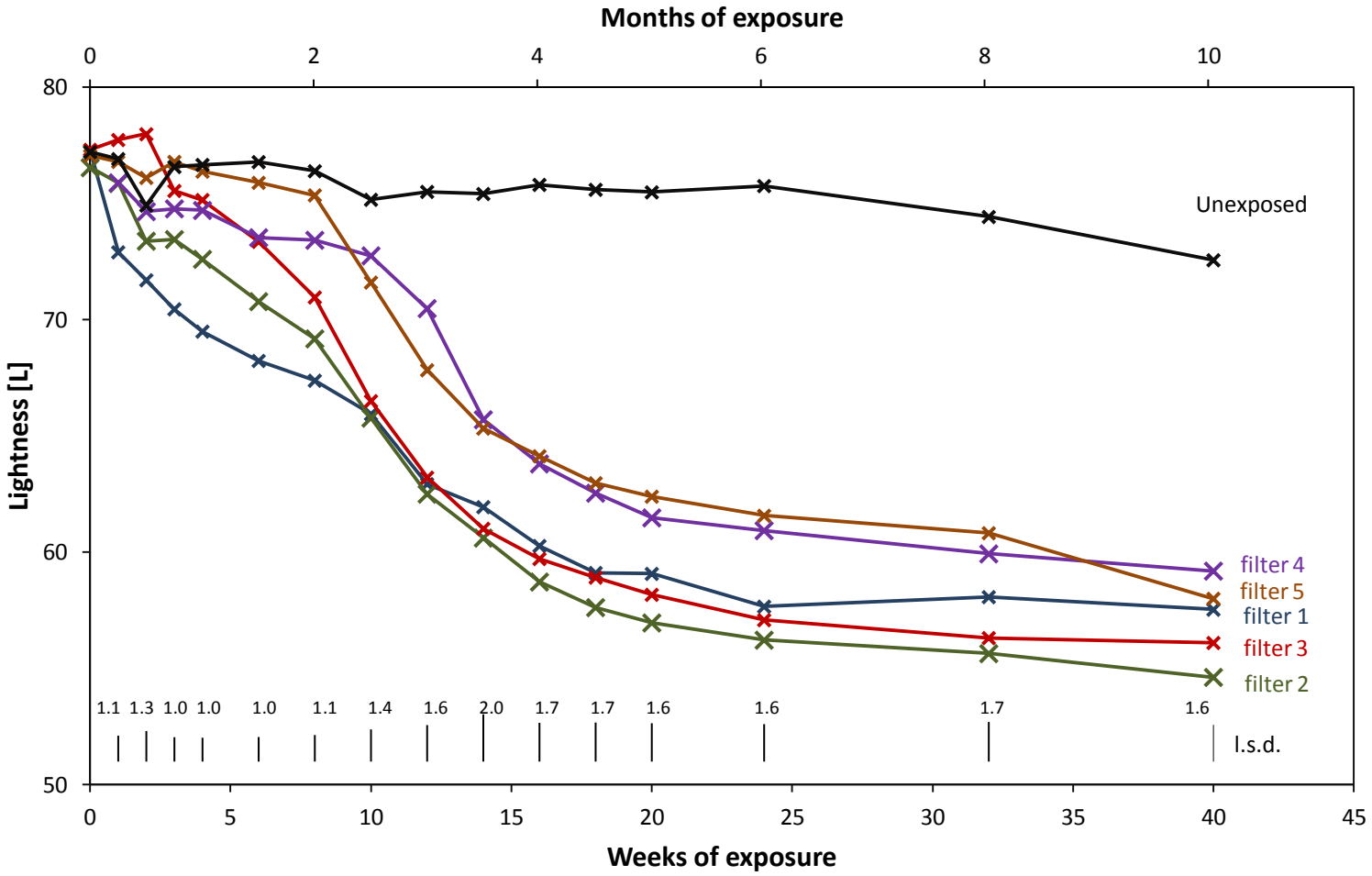


Figure 5.3: Lightness (L) of southern pine wood samples during 40 weeks of exposure under polymethylmethacrylate filters. Lightness is expressed using the CIELab parameter, L [100=white; 0=black]. Filter 1 transmitted UVB+UVA+Vis.light+IR; Filter 2 transmitted UVA+Vis.light+IR; Filter 3 transmitted Vis.light+IR; Filter 4 transmitted IR; and Filter 5 transmitted no radiation (L.s.d. bars for comparison of means only apply for the specific week in which they are located)

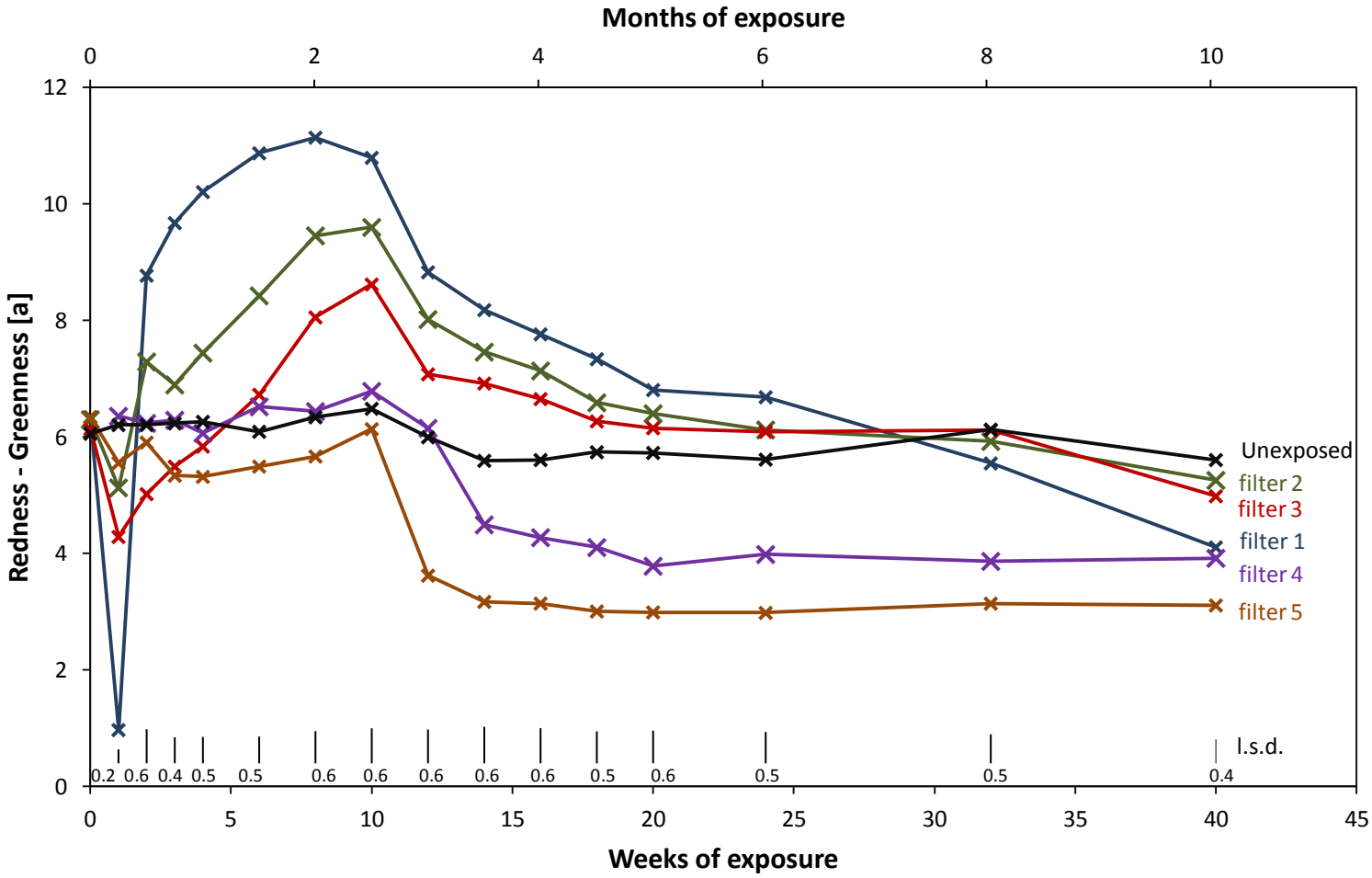


Figure 5.4: Redness-greenness (a) of southern pine wood samples during 40 weeks of exposure under polymethylmethacrylate filters. Redness/greenness is expressed using the CIELab parameter, a [+60=red; -60=green]. Filter 1 transmitted UVB+UVA+Vis.light+IR; Filter 2 transmitted UVA+Vis.light+IR; Filter 3 transmitted Vis.light+IR; Filter 4 transmitted IR; and Filter 5 transmitted no radiation (L.s.d. bars for comparison of means only apply for the specific week in which they are located)

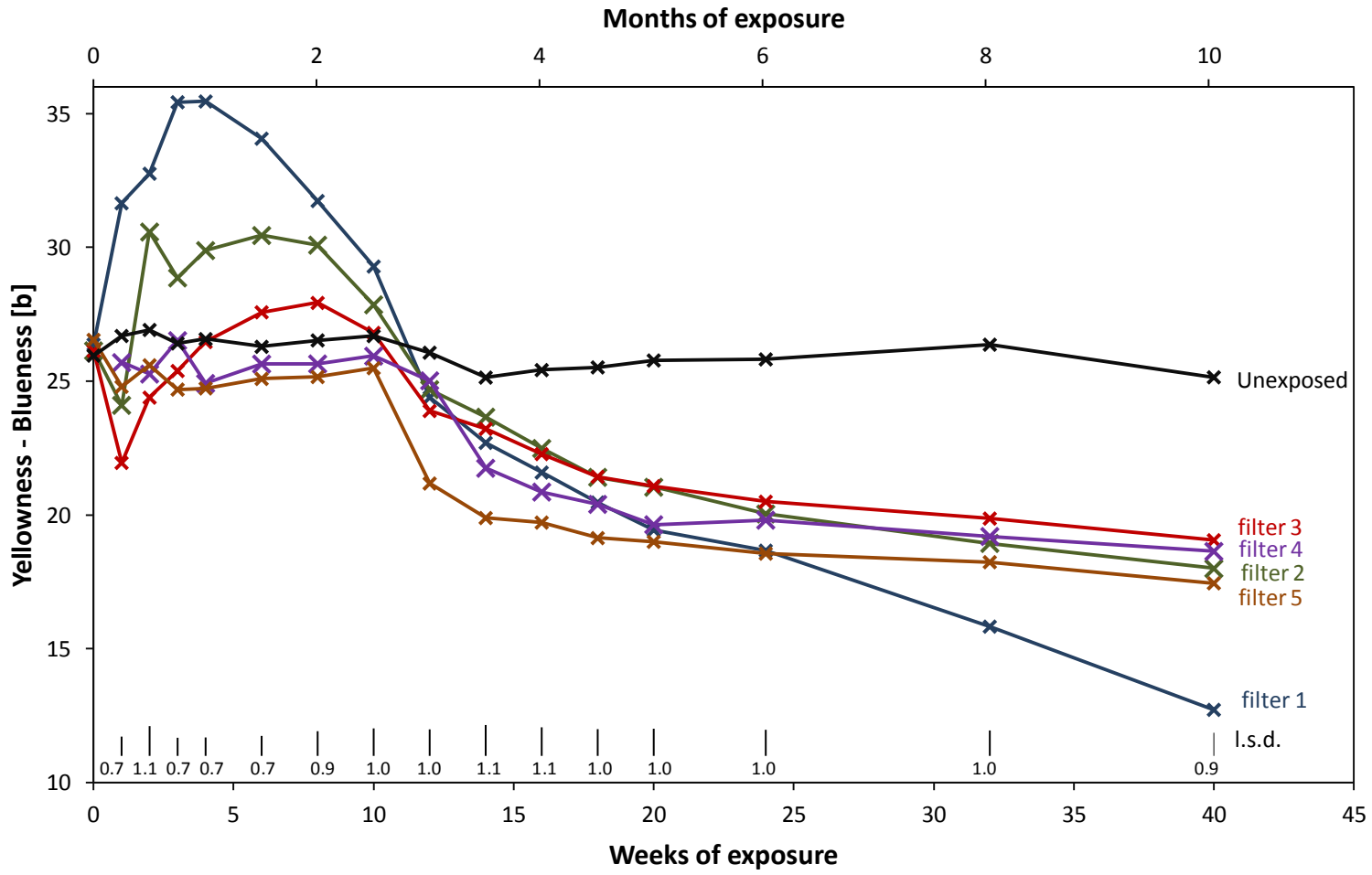


Figure 5.5: Yellowness-blueness (b) of southern pine wood samples during 40 weeks of exposure under polymethylmethacrylate filters. Yellowness/blueness is expressed using the CIELab parameter, b [+60=yellow; -60=blue]. Filter 1 transmitted UVB+UVA+Vis.light+IR; Filter 2 transmitted UVA+Vis.light+IR; Filter 3 transmitted Vis.light+IR; Filter 4 transmitted IR; and Filter 5 transmitted no radiation (L.s.d. bars for comparison of means only apply for the specific week in which they are located)

5.3.2 Area colonized by fungi

The area colonized (stained) by fungi on the control strip of samples (treated with water) was analyzed independently for each exposure period from 1 to 40 weeks. Analysis of variance indicated that after 2 weeks of exposure the area stained by fungi was significantly affected (P-value < 0.001) by filter type.

Dark stains started to appear 6 to 8 weeks after the southern pine samples were exposed outdoors under the different filters (Figure 5.6). However, small black fungal colonies appeared as early as the second week of exposure (Figure 5.7). These colonies increased in number over the next four to five weeks. After 12 weeks of exposure, the area colonized by fungi increased noticeably, covering approximately 40% to 90% of the total area of exposed specimens (Figure 5.8). After 20 weeks of exposure greater than 90 percent of the entire surface of all specimens was colonized by microorganisms. Samples exposed under the filter that blocked all solar radiation (filter 5) were colonized faster than the other samples. In contrast, samples exposed under the filter that transmitted the entire solar spectrum were less stained than samples exposed under the other filters (Figure 5.9 and Figure 5.10). The increase in area of samples stained by fungi is shown in Appendix 4.

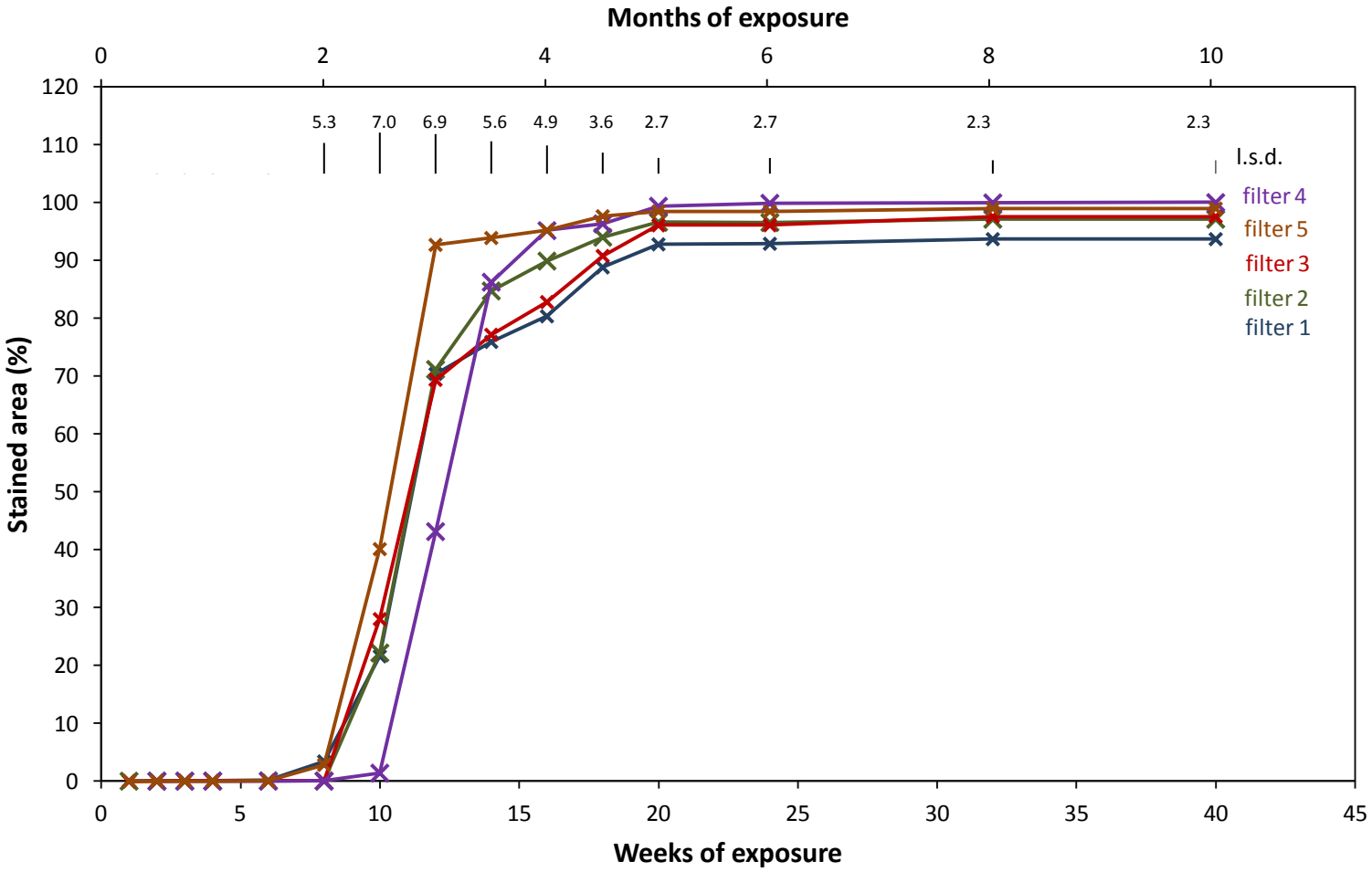


Figure 5.6: Area of southern pine wood samples colonized by fungi during 40 weeks of exposure under different polymethylmethacrylate filters. Filter 1 transmitted UVB+UVA+Vis.light+IR; Filter 2 transmitted UVA+Vis.light+IR; Filter 3 transmitted Vis.light+IR; Filter 4 transmitted IR; and Filter 5 transmitted no radiation. After 12 weeks of exposure the total area of specimens stained by fungi ranged from 40 % to 90 %. After 20 weeks exposure, greater than 90 percent of the area of specimens was stained. L.s.d. bars for comparison of means apply only for the specific week in which they are located

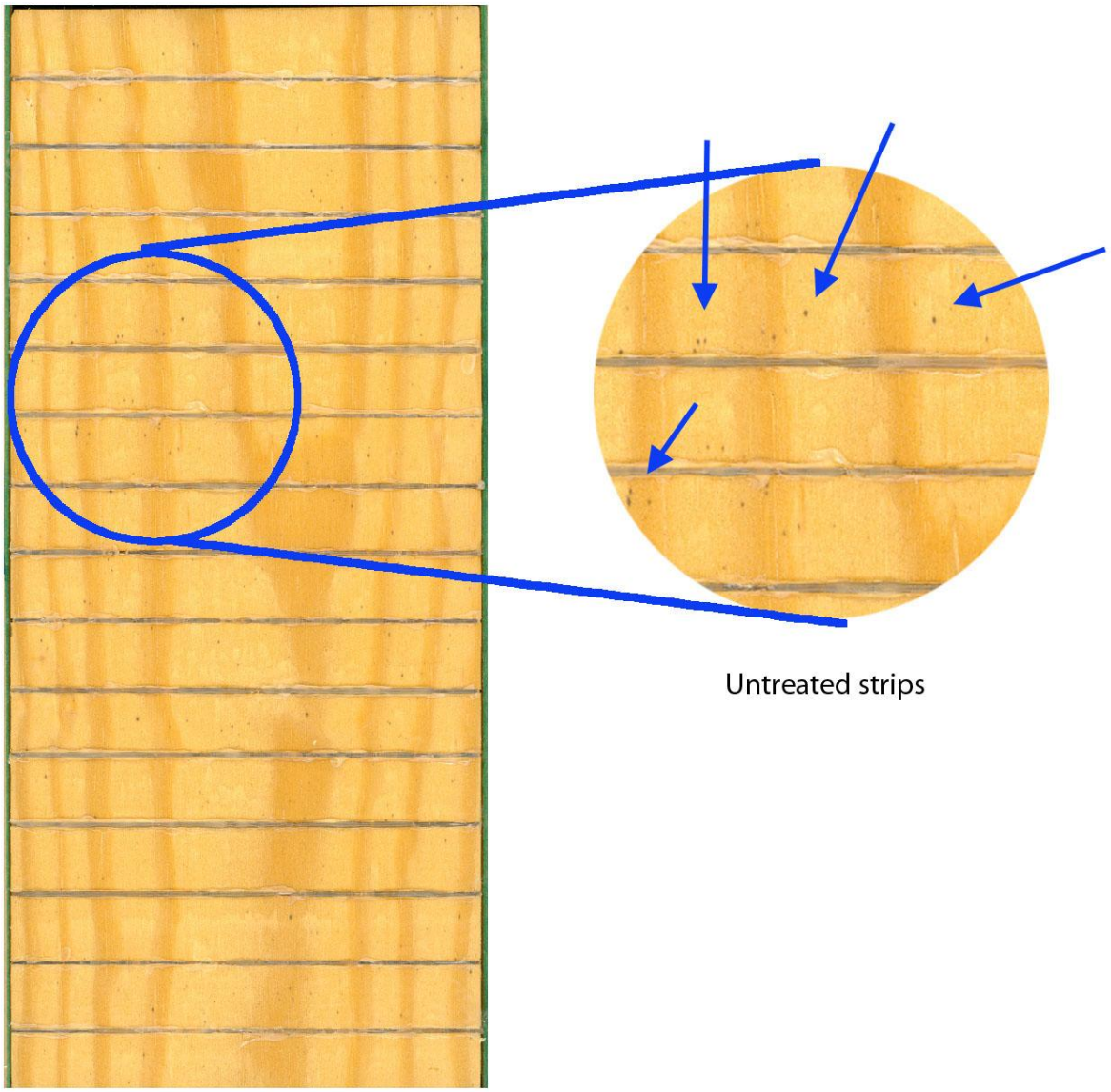


Figure 5.7: Appearance of southern pine wood samples exposed to the weather for 2 weeks in Vancouver, Canada, under a polymethylmethacrylate filter transmitting all wavelengths of solar radiation (Filter 1). Blue arrows show black dots attributable to early stages of fungal colonization

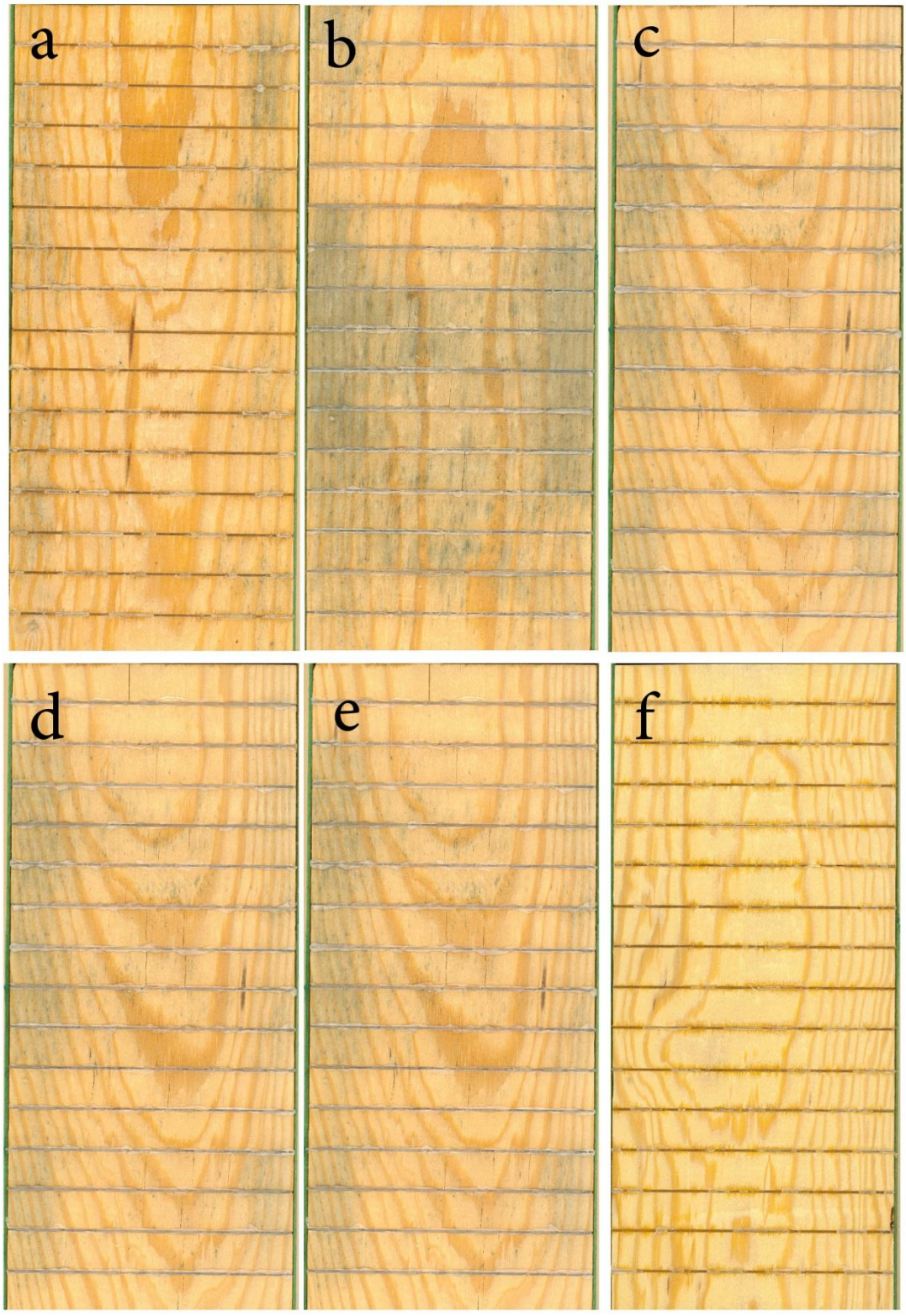


Figure 5.8: Appearance of southern pine wood samples exposed to the weather for 12 weeks in Vancouver, Canada, under filters 1 (a), 2 (b), 3 (c), 4 (d), 5 (e) and control sample stored in a conditioning room (f)

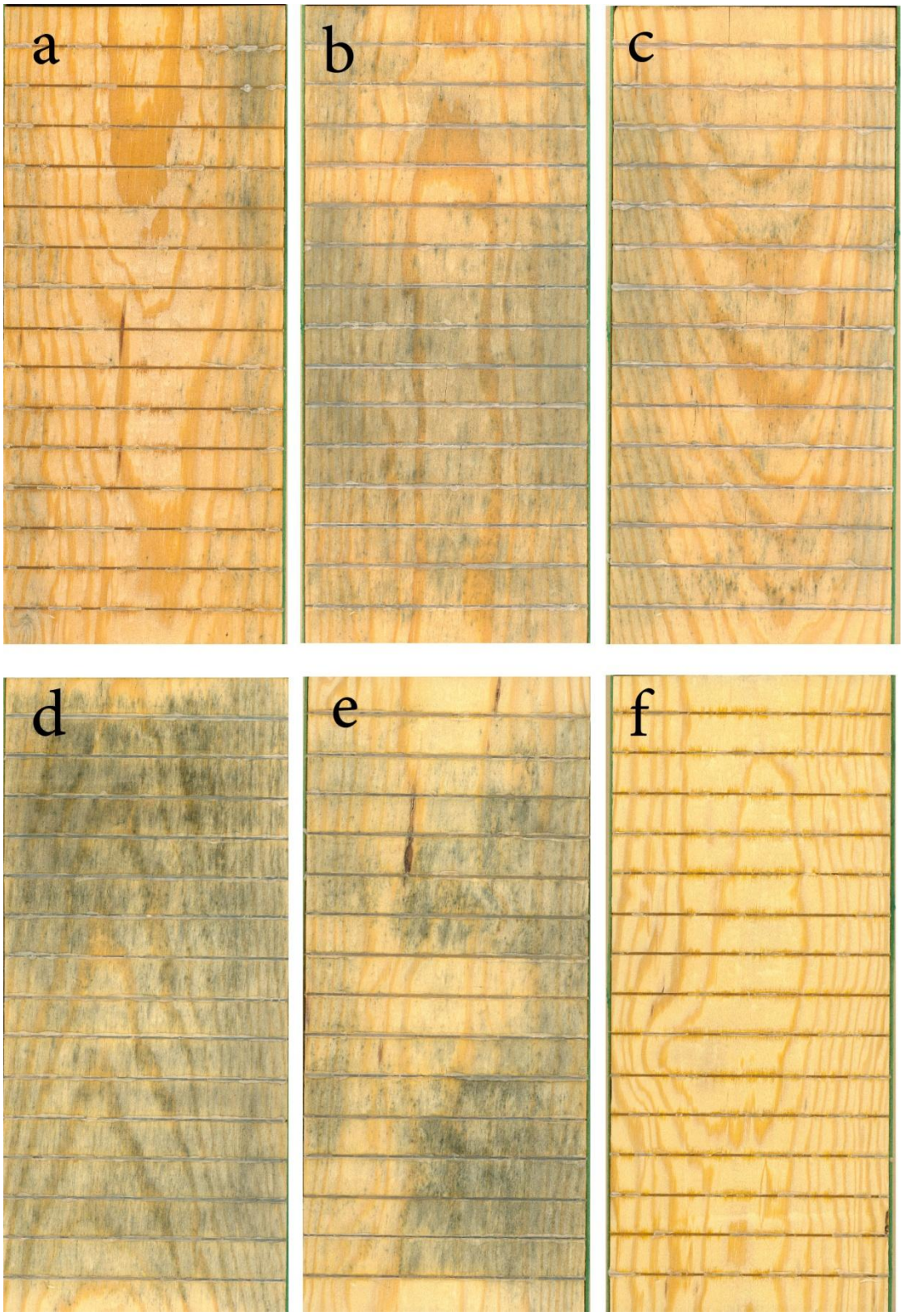


Figure 5.9: Appearance of southern pine wood samples exposed to the weather for 16 weeks in Vancouver, Canada, under filters 1 (a), 2 (b), 3 (c), 4 (d), 5 (e) and control sample stored in a conditioning room (f)

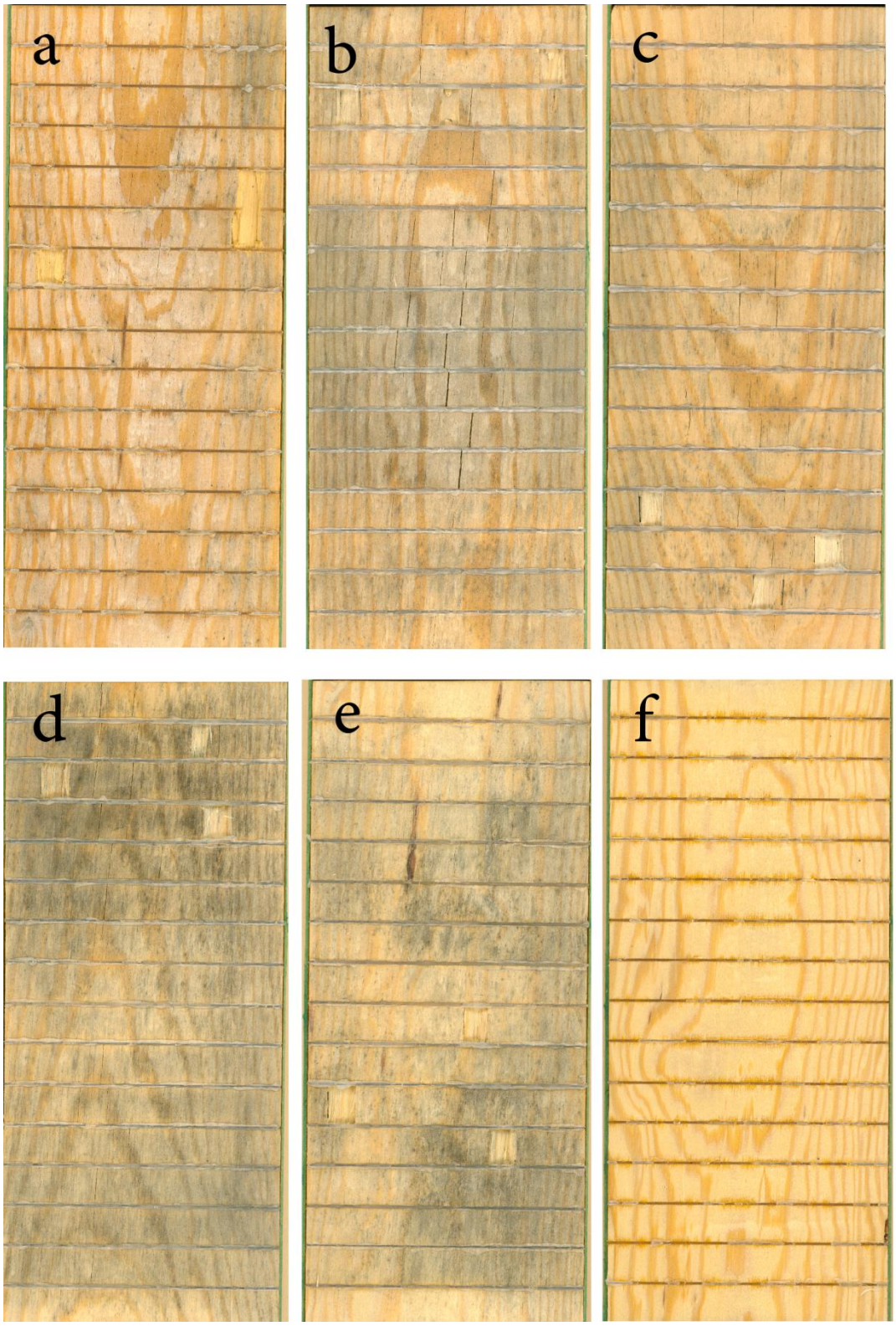


Figure 5.10: Appearance of southern pine wood samples exposed to the weather for 40 weeks in Vancouver, Canada, under filters 1 (a), 2 (b), 3 (c), 4 (d), 5 (e) and control sample stored in a conditioning room (f)

5.3.3 Moisture content

The superficial moisture content of the southern pine wood samples was measured every week from weeks 10 to 32 of the exposure trial. The moisture content of samples was always below the fiber saturation point and appeared to vary depending on the number and severity of rainfall events. Analysis of variance revealed no significant differences (P-value > 0.05) in the weekly moisture contents of samples exposed under the different filters (Figure 5.11).

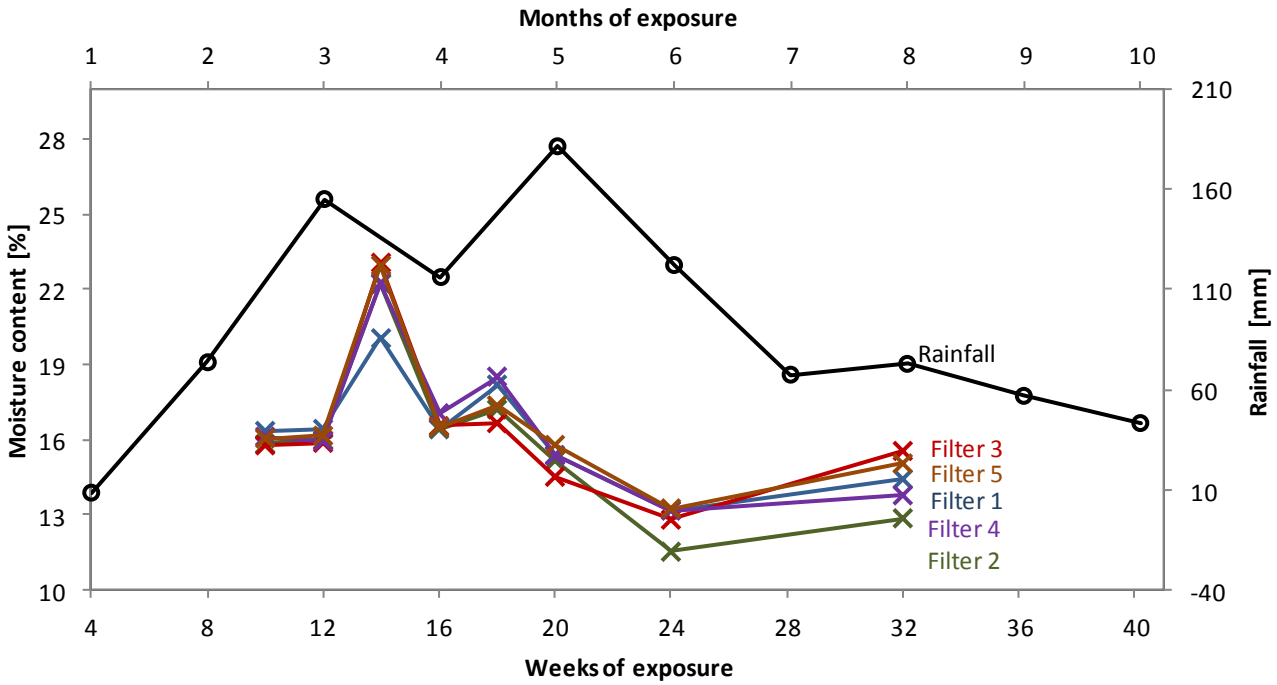


Figure 5.11: Changes in moisture content of southern pine wood samples during 40 weeks of exposure under polymethylmethacrylate filters in Vancouver, Canada (data available from week 10 to 32). The figure includes the monthly rainfall total during the exposure trial. Analysis of variance revealed no significant differences in the weekly moisture contents of samples exposed under the different filters

5.3.4 Chemical changes at weathered wood surfaces

FTIR spectra of samples exposed to the weather under the polymethylmethacrylate filters for 40 weeks and unexposed controls are shown in Figure 5.12. After exposure, wavenumbers of peaks at 1514 and 1462 cm^{-1} were smaller than those in the control, although the decreases in peak heights were more pronounced for samples exposed to the most energetic radiation (filter 1, 2 and 3). These peaks correspond to stretching vibration of carbonyl groups in lignin benzene rings and C-H deformations in lignin, respectively (Anderson et al., 1991; Pandey and Pitman, 2003). The peaks at a wavenumber of 1740 cm^{-1} decreased for all samples, and the peak at 1655 cm^{-1} decreased only for samples exposed under filter 1 and increased for samples exposed under filters 4 and 5. These peaks correspond to conjugated C-O absorptions which typically increase during the early stages of weathering, and then decrease with extended exposure to solar radiation (Anderson et al., 1991; Pandey and Pitman, 2003; Williams, 2005). On the other hand, the peak at a wavenumber of 1158 cm^{-1} (C-O-C stretching in pyranose rings in cellulose and hemicelluloses, Huang et al. 2008), decreased in comparison to the peak in the unexposed control. Again decreases in peak height were more pronounced for samples exposed under filters that transmitted more energetic radiation.

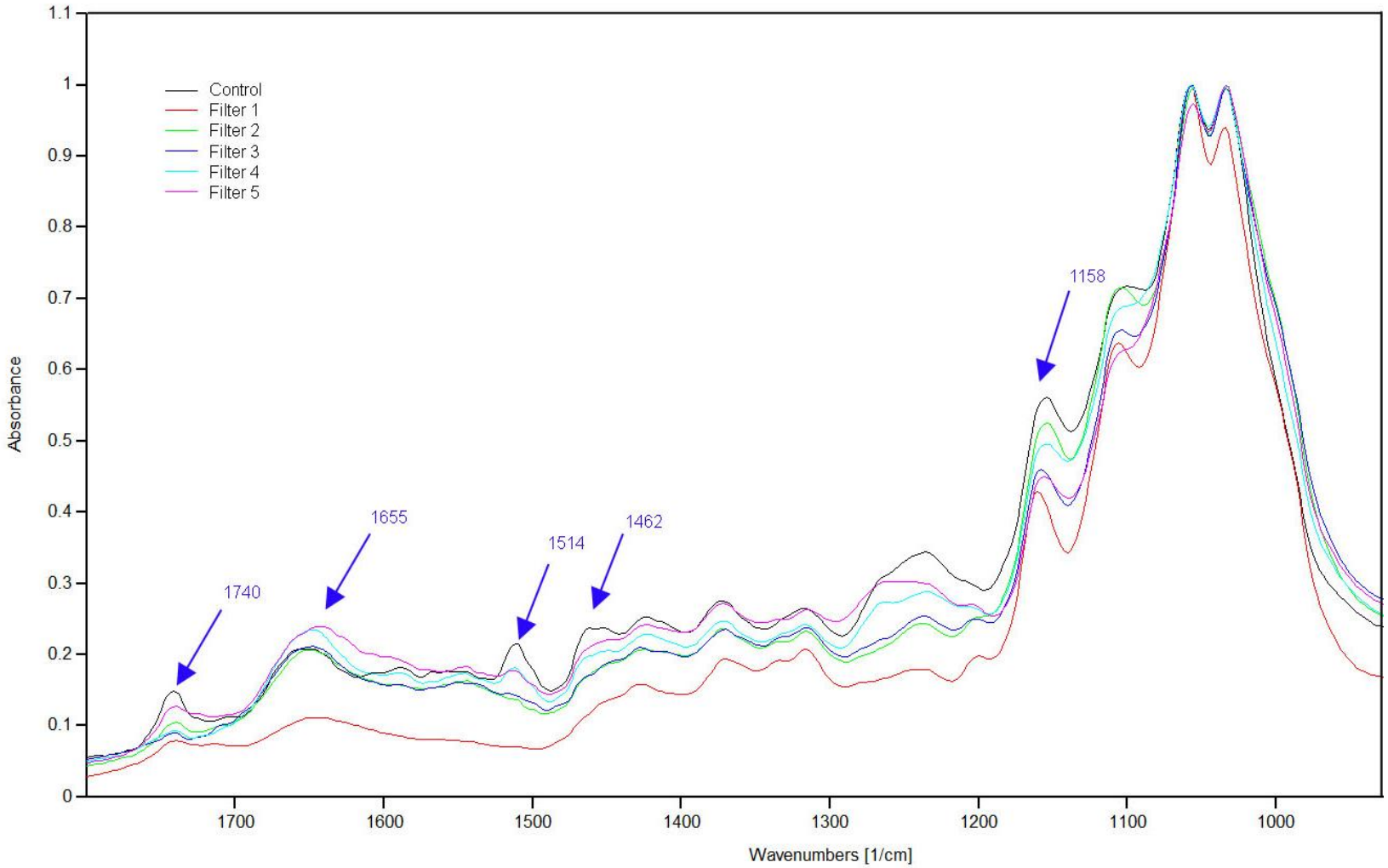


Figure 5.12: Normalized FTIR absorbance spectra of southern pine wood surfaces exposed to the weather for 40 weeks under polymethylmethacrylate filters and unexposed control. Filter 1 transmitted UVB+UVA+Vis.light+IR; Filter 2 transmitted UVA+Vis.light+IR; Filter 3 transmitted Vis.light+IR; Filter 4 transmitted IR; and Filter 5 transmitted no radiation. Exposed samples showed decreases in peaks at 1740, 1514 and 1462 cm^{-1} related to lignin and 1158 cm^{-1} related to carbohydrates. The spectrum of the unexposed control is included for comparison

5.3.5 Fungal ecology and characterization of isolated fungi

A total of 126 fungi from 21 different genera were isolated from the southern pine samples exposed under the different polymethylmethacrylate filters for 40 weeks. All of the fungi except for one belonged to the phylum ascomycota. The exception was a basidiomycete fungus from the genera *Rhizopogon*, which was isolated from a sample shielded from light (filter 5). Several very well known colonizers of weathered wood were isolated including *Aureobasidium* pullulans, *Hormonema dematioides, Cladosporium sp., Alternaria sp., Phoma sp,* and *Epicoccum nigrum* Link. Other fungi isolated were *Allantophomopsis lycopodina* (Höhn.) Carris, *Botryosphaeria stevensii* Shoemaker, *Botryotinia fuckeliana* (de Bary) Whetzel, *Coniochaeta ligniaria* (Grev.) Massee, *Lecythophora* sp., *Leptosphaerulina chartarum* Cec. Roux, *Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons, *Penicillium expansum* Link ex. Thom, *Peniophora aurantiaca* (Bresadola) von Höhnel & Litschaue, *Phialocephala* sp., *Phialophora* sp., *Rhizopogon* sp., *Trichoderma viride* Pers., *Valsa ambiens* (Pers.) Fr., and Exophiala sp. A list of fungi isolated from samples exposed under the different filters and the methods used to identify them are given in Table 5.4 to Table 5.8. In addition, as in Chapter 3, further characterization of isolated *A. pullulans* in solid media revealed that two types were present: a dark-type and a white-type. The latter white fungus melanized approximately one week after being inoculated onto 1% MEA.

Table 5.4: Fungi isolated from samples exposed to UVA+UVB+Vis.light+IR. Primer sequenced for rDNA identification ITS4

Fungi	Rack	Codification	Identification	Closest match in Blast	GenBank Acc No.	Identity
Aureobasidium pullulans	3	1_3	Microscopy			
Aureobasidium pullulans (white)	3	4	Microscopy			
Aureobasidium pullulans (white)	4	3	Microscopy			
Aureobasidium pullulans (black)	1	4W	rDNA	*Aureobasidium pullulans*	AY225167.1	550/554 (99%)
Aureobasidium pullulans (black)	2	5_3	Microscopy			
Aureobasidium pullulans (black)	5	3_1	Microscopy			
Aureobasidium sp. (black)	4	5	rDNA	*Aureobasidium* sp.	AM901687.1	555/557 (99%)
Epicoccum nigrum	1	3W	rDNA	*Epicoccum nigrum*	FJ904918.1	524/528 (99%)
Epicoccum nigrum	2	2	rDNA	*Epicoccum nigrum*	FJ424264.1	518/521 (99%)
Epicoccum sp.	3	2	Microscopy			
Epicoccum sp.	4	1	Microscopy			
Epicoccum sp.	4	2	Microscopy			
Epicoccum sp.	5	1	Microscopy			
Epicoccum sp.	5	2	Microscopy			
Hormonema dematioides	1	5W	rDNA	*Hormonema dematioides*	AY253451.1	557/565 (98%)
Hormonema dematioides	3	8	Microscopy			
Hormonema dematioides	4	4_1	Microscopy			
Hormonema dematioides	5	3_2	Microscopy			
Botryosphaeria stevensii	2	3_2	rDNA	*Botryosphaeria stevensii*	EU856766.1	532/535 (99%)
Botryotinia fuckeliana	3	7	rDNA	*Botryotinia fuckeliana*	EF207415.1	507/513 (98%)
Alternaria sp.	2	4	Microscopy			
Cladosporium sp.	1	2W	rDNA	*Cladosporium* sp.	GU214631.1	528/530 (99%)
Phoma sp.	3	6	rDNA	*Phoma herbarum*	AY337712.1	469/475 (98%)
Coniochaeta ligniaria	3	5_1	rDNA	*Coniochaeta ligniaria*	AY198390.1	521/525 (99%)
Penicillium sp.	3	1_1	rDNA	*Penicillium expansium*	FJ008997.1	554/556 (99%)
Phialophora sp.	3	3	rDNA	*Phialophora* sp.	AY618679.1	503/505 (99%)

Table 5.5: Fungi isolated from samples exposed to UVA+Vis.light+IR. Primer sequenced for rDNA identification ITS4

Fungi	Rack	Codification	Identification	Closest match in Blast	GenBank Acc No.	Identity
Aureobasidium pullulans (white)	1	2W	rDNA	*Aureobasidium pullulans*	GQ376094.1	551/556 (99%)
Aureobasidium pullulans (white)	2	6	Microscopy			
Aureobasidium pullulans (white)	3	2	Microscopy			
Aureobasidium pullulans (white)	4	3	Microscopy			
Aureobasidium pullulans (white)	5	3	Microscopy			
Aureobasidium pullulans (black)	2	5	rDNA	*Aureobasidium pullulans*	AM901687.1	543/547 (99%)
Aureobasidium pullulans (black)	3	5_1	Microscopy			
Aureobasidium pullulans (black)	4	5	Microscopy			
Hormonema dematioides	1	6_1W	rDNA	*Hormonema dematioides*	AY253451.1	557/567 (98%)
Hormonema dematioides	2	2	rDNA	*Hormonema dematioides*	AY253451.1	558/563 (99%)
Hormonema dematioides	3	3_1	rDNA	*Hormonema dematioides*	AY253451.1	545/551 (98%)
Hormonema dematioides	4	4_1	Microscopy			
Hormonema dematioides	5	1	Microscopy			
Cladosporium sp.	1	3	Microscopy			
Cladosporium sp.	4	1_2	Microscopy			
Cladosporium sp.	5	2	Microscopy			
Epicoccum nigrum	1	5W	rDNA	*Epicoccum nigrum*	AF455403.1	513/520 (98%)
Epicoccum sp.	4	2	Microscopy			
Botryotinia fuckeliana	5	4	rDNA	*Botryotinia fuckeliana*	EU128648.1	501/504 (98%)
Botryotinia sp.	2	4	Microscopy			
Alternaria sp.	1	1	Microscopy			
Phoma sp.	2	3	rDNA	*Phoma* sp.	FJ903335.1	507/509 (99%)
Leptosphaerulina sp.	3	4	rDNA	*Leptosphaerulina* sp.	AM901681.1	562/564 (99%)

Table 5.6: Fungi isolated from samples exposed to Vis.light+IR. Primer sequenced for rDNA identification ITS4

Fungi	Rack	Codification	Identification	Closest match in Blast	GenBank Acc No.	Identity
Aureobasidium pullulans (white)	2	1_1	Microscopy			
Aureobasidium pullulans (white)	3	5_3	Microscopy			
Aureobasidium pullulans (white)	4	1	Microscopy			
Aureobasidium pullulans (white)	5	1	Microscopy			
Aureobasidium pullulans (white)	1	5S	rDNA	*Aureobasidium pullulans*	AF121283.1	516/516 (100%)
Aureobasidium pullulans (black)	1	1_2S	rDNA	*Aureobasidium* sp.	AM901687.1	548/554 (98%)
Aureobasidium pullulans (black)	2	4	rDNA	*Aureobasidium pullulans*	DQ787427.1	527/531 (99%)
Aureobasidium pullulans (black)	3	3_2	rDNA	*Aureobasidium* sp.	AM901687.1	559/565 (98%)
Aureobasidium pullulans (black)	4	2	rDNA	*Aureobasidium* sp.	AM901687.1	556/556 (100%)
Aureobasidium pullulans (black)	5	3	rDNA	*Aureobasidium* sp.	AM901687.1	553/555 (99%)
Hormonema dematioides	2	2	rDNA	*Hormonema dematioides*	AY253451.1	565/569 (99%)
Hormonema dematioides	4	3	Microscopy			
Hormonema dematioides	5	9	Microscopy			
Phoma sp.	1	5_2	rDNA	*Phoma* sp.	AM901684.1	521/537 (97%)
Phoma sp.	4	5	rDNA	*Phoma herbarum*	AY337712.1	509/516 (98%)
Phoma sp.	5	8	rDNA	*Phoma herbarum*	AY337712.1	504/512 (98%)
Alternaria sp.	1	4	Microscopy			
Alternaria sp.	3	2_1	Microscopy			
Cladosporium sp.	2	3	rDNA	*Cladosporium cladosporioides*	GQ241276.1	501/505 (99%)
Cladosporium sp.	5	2	Microscopy			
Epicoccum sp.	2	5	Microscopy			
Epicoccum sp.	5	4	Microscopy			
Botryotinia sp.	5	5	rDNA	*Botryotinia fuckeliana*	EU128648.1	505/511 (98%)
Coniochaeta ligniaria	3	5_4	Microscopy			
Lewia sp.	5	11	rDNA	*Lewia infectoria*	GQ376103.1	561/568 (98%)
Phialophora sp.	3	4A	rDNA	*Phialophora* sp.	AY618679.1	503/505 (99%)

Table 5.7: Fungi isolated from samples exposed to IR. Primer sequenced for rDNA identification ITS4

Fungi	Rack	Codification	Identification	Closest match in Blast	GenBank Acc No.	Identity
Aureobasidium pullulans (white)	3	2_1	rDNA	*Aureobasidium pullulans*	GQ376094.1	551/554 (99%)
Aureobasidium pullulans (white)	2	7	Microscopy			
Aureobasidium pullulans (white)	3	5	Microscopy			
Aureobasidium pullulans (white)	4	4	Microscopy			
Aureobasidium pullulans (black)	1	7_1W	rDNA	*Aureobasidium pullulans*	DQ787427.1	528/531 (99%)
Aureobasidium pullulans (black)	5	6	Microscopy			
Cladosporium sp.	1	4	Microscopy			
Cladosporium sp.	2	5	Microscopy			
Cladosporium sp.	3	1	Microscopy			
Cladosporium sp.	4	3	Microscopy			
Epicoccum nigrum	5	8	rDNA	*Epicoccum nigrum*	FJ424264.1	511/514 (99%)
Epicoccum nigrum	2	1_1	rDNA	*Epicoccum nigrum*	AF455403.1	508/511 (99%)
Epicoccum nigrum	3	3	Microscopy			
Epicoccum nigrum	4	7	Microscopy			
Hormonema dematioides	1	1_2W	rDNA	*Hormonema dematioides*	AY253451.1	552/564 (97%)
Hormonema dematioides	4	2_1	rDNA	*Hormonema dematioides*	AY253451.1	541/552 (98%)
Phoma sp.	2	2	rDNA	*Phoma herbarum*	AY337712.1	498/507 (98%)
Phoma sp.	5	1	rDNA	*Phoma herbarum*	AY337712.1	501/508 (98%)
Lewia sp.	4	1_1	rDNA	*Lewia infectoria*	AF4555012.1	520/539 (96%)
Lewia sp.	5	2	rDNA	*Lewia infectoria*	EF104194.1	528/531 (99%)
Allantophomopsis lycopodina	4	5	rDNA	*Allantophomopsis lycopodina*	AB041243.1	498/498 (100%)
Botryotinia sp.	3	4	rDNA	*Botryotinia fuckeliana*	GU062311.1	470/471 (99%)
Exophiala sp.	1	2S	rDNA	*Exophiala xenobiotica*	DQ182589.1	531/534 (99%)
Lecythophora sp.	1	1	rDNA	*Lecythophora* sp.	AY219880.1	521/543 (95%)
Phialocephala sp.	5	4_1	rDNA	*Phialocephala* sp.	AY524844.1	778/836 (93%)
Trichoderma viride	4	6	rDNA	*Trichoderma viride*	FJ872073.1	548/548 (100%)

Table 5.8: Fungi isolated from samples exposed to No light. Primer sequenced for rDNA identification ITS4

Fungi	Rack	Codification	Identification	Closest match in Blast	GenBank Acc No.	Identity
Cladosporium sp.	1	1	Microscopy			
Cladosporium sp.	3	6	Microscopy			
Cladosporium sp.	4	3_1	Microscopy			
Cladosporium sp.	4	3_2	Microscopy			
Cladosporium sp.	5	3	Microscopy			
Cladosporium sp.	5	4	Microscopy			
Aureobasidium pullulans (black)	3	2_2	rDNA	*Aureobasidium pullulans*	GQ376094.1	556/561 (99%)
Aureobasidium pullulans (black)	2	5	Microscopy			
Aureobasidium pullulans (black)	5	1_1	Microscopy			
Aureobasidium pullulans (white)	3	2_1_1	rDNA	*Aureobasidium pullulans*	GQ376094.1	533/537 (99%)
Aureobasidium pullulans (white)	5	2	Microscopy			
Epicoccum nigrum	1	3	Microscopy			
Epicoccum nigrum	2	3	Microscopy			
Epicoccum nigrum	3	3	Microscopy			
Epicoccum nigrum	4	7	Microscopy			
Epicoccum nigrum	5	6	Microscopy			
Hormonema dematioides	2	1	Microscopy			
Hormonema dematioides	3	1_1	Microscopy			
Alternaria alternata	4	1_1	rDNA	*Alternaria alternata*	FN179367.1	499/500 (99%)
Alternaria tenuissima	4	2	rDNA	*Alternaria tenuissima*	FJ827038.1	499/501 (99%)
Alternaria sp.	1	2	Microscopy			
Leptosphaerulina sp.	1	4W	rDNA	*Leptosphaerulina chartarum*	DQ384571.1	465/470 (98%)
Peniophora sp.	4	4	rDNA	*Peniophora aurantiaca*	AF210825.1	586/607 (96%)
Rhizopogon sp.	4	3_3	rDNA	*Rhizopogon* sp.	AF377159.1	394/466 (84%)
Valsa ambiens	2	6	rDNA	*Valsa ambiens*	EF447369.2	530/531 (99%)

5.3.5.1 Frequency of isolation

Analysis of variance showed a significant effect of species (P-value < 0.001) and a significant interaction of filter type x fungal species (P-value = 0.018) on the parameter FIF (frequency of occurrence of fungi). There was no significant effect of filter type (P-value > 0.05) on FIF.

A. pullulans was more frequently isolated from the southern pine samples than any other fungal species. In contrast, the frequency of isolation of *Alternaria* sp. and *Phoma* sp. was significantly (P-value < 0.05) lower than that of other fungi (Figure 5.13).

The interaction of filter type x fungal species occurred because the frequency of occurrence of *A. pullulans* was significantly higher than that of all other fungi on samples exposed under filters 1, 2 and 3, whereas under filters 4 and 5 some other fungi were more frequently isolated. For example, under IR and in the absence of light (filter 4 and 5, respectively) *Cladosporium* sp., *Epicoccum* sp. and "others" were more frequently isolated. The occurrence of *H. dematioides* was considerably lower after UV radiation was blocked from reaching samples, Figure 5.14.

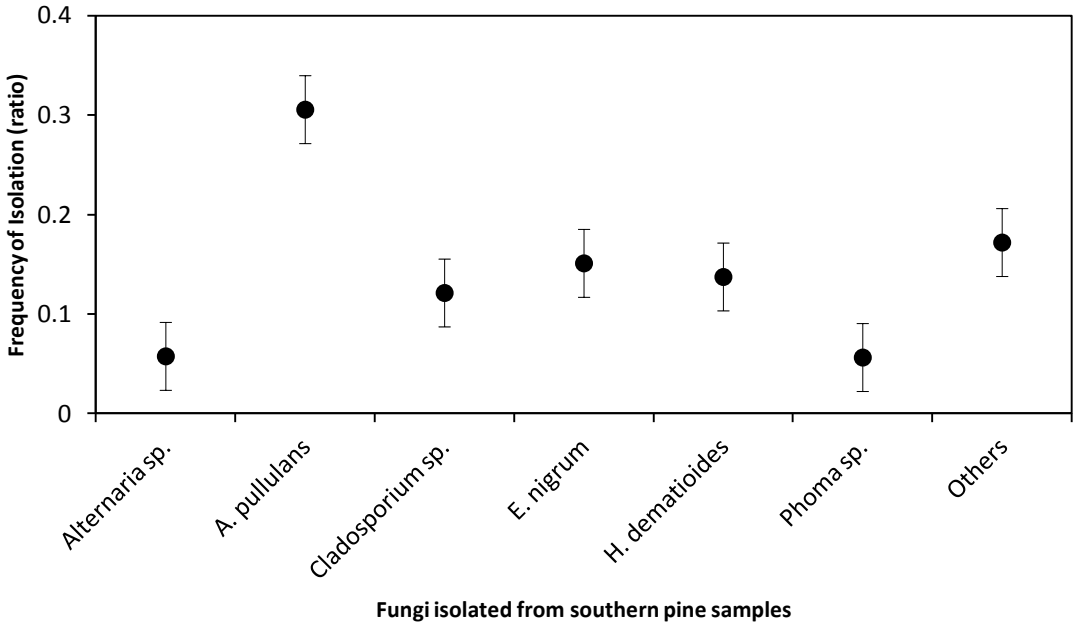


Figure 5.13: Frequency of isolation of fungi from southern pine samples exposed to different wavelengths of solar radiation under polymethylmethacrylate filters (results averaged across filter type and expressed as ratio of occurrence)

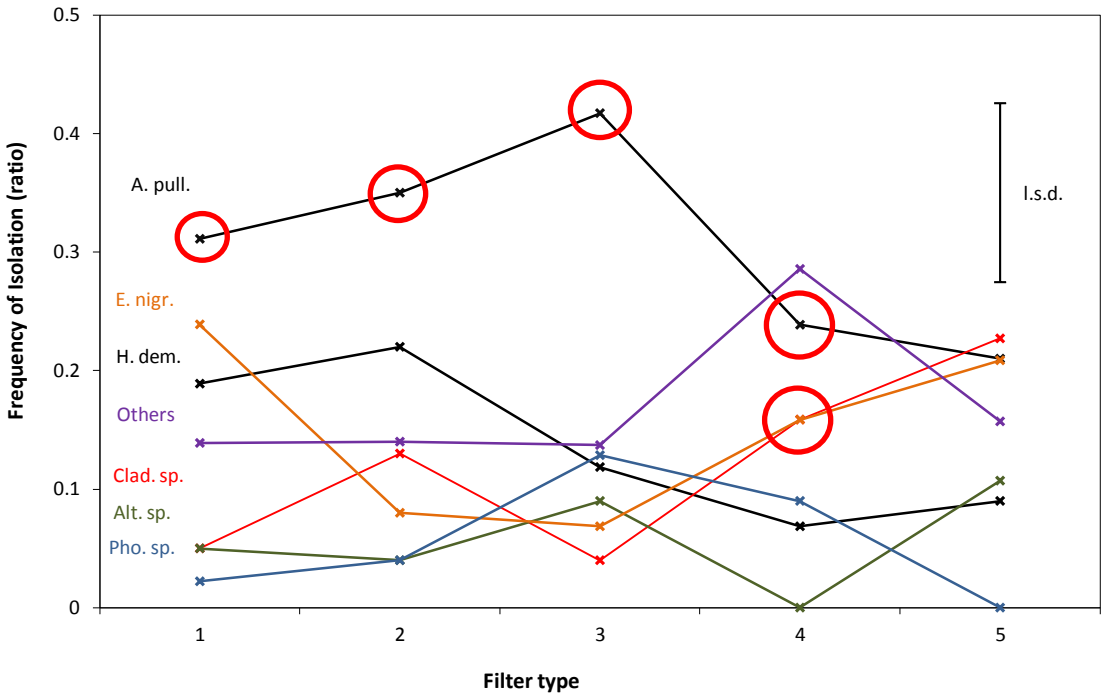


Figure 5.14: Frequency of isolation of fungi from southern pine samples exposed to different wavelengths of solar radiation under polymethylmethacrylate filters. Factor responsible for the interaction of filter type x fungal species (encircled in red). Results expressed as ratio of frequency of occurrence

5.3.5.2 Fungal diversity

Analysis of variance showed that there was no significant effect of filter type on the diversity of fungi isolated from the southern pine wood samples (P-value = 0.839), but the average diversity was lower in samples exposed under filter 1. Results for reciprocal Simpson index are appended to this thesis (Appendix 5).

5.3.5.3 Characterization of fungi on solid culture media

The lightness of fungal mycelia after 20 days of growth expressed using the CIELab coordinate (L) is shown in Table 5.9. *A. pullulans*, *H. dematioides*, *Cladosporium* sp., *A. lycopodina* and *Alternaria* sp. possessed the darkest mycelia whereas *Lecythophora* sp., *B. fuckeliana*, *Peniophora* sp., *Trichoderma viride* and *Rhizopogon* sp. were the lightest. Scanned images of fungi growing on malt extract agar arranged from the darkest to the lightest fungi are shown in Figure 5.15.

Table 5.9: Lightness of fungi grown on solid media malt extract agar (1% MEA)

Fungi	Lightness (L)	
	Ave	SD
Rhizopogon sp.	83.95	[NA]
T. viride	83.19	[NA]
Peniophora sp.	81.08	[NA]
B. fuckeliana	80.55	[4.19]
Lecythophora sp.	80.5	[NA]
C. ligniaria	76.92	[0.35]
V. ambiens	76.44	[NA]
Penicillium sp.	74.98	[NA]
Phoma sp.	74.32	[5.88]
A. pullulans (white)	70.49	[9.10]
Phialocephala sp.	54.73	[1.81]
Leptosphaerulina sp.	52.99	[4.60]
E. nigrum	52.19	[18.56]
B. stevensii	47.88	[NA]
Lewia sp.	41.04	[18.42]
Alternaria sp.	34.14	[10.01]
A. lycopodina	21.62	[NA]
Cladosporium sp.	18.81	[4.54]
H. dematioides	14.63	[6.75]
A. pullulans (black)	14.62	[9.73]

The radial growth of isolated fungi after 7 days is expressed as mm growth per week (Table 5.10.). The less melanized fungi, which were isolated more frequently from samples under the filter that shielded wood from UV radiation, grew faster than black fungi. For example, *A. lycopodina*, *B. stevensii*, *B. fuckeliana*, *T. viride* and *V. ambiens*, grew the fastest, at 30 to 35 mm per week. More pigmented fungi like *Alternaria* sp., *E. nigrum*, *Lewia* sp. and *Peniophora* sp. grew at a rate of 20 to 25 mm per week. The remaining fungi, including very dark fungi such as *A. pullulans, H. dematioides* and *Cladosporium* sp. grew even more slowly (6 to 17 mm per week). Scanned images of fungi growing on malt extract agar arranged from the fastest to the slowest growing fungi are shown in Figure 5.16.

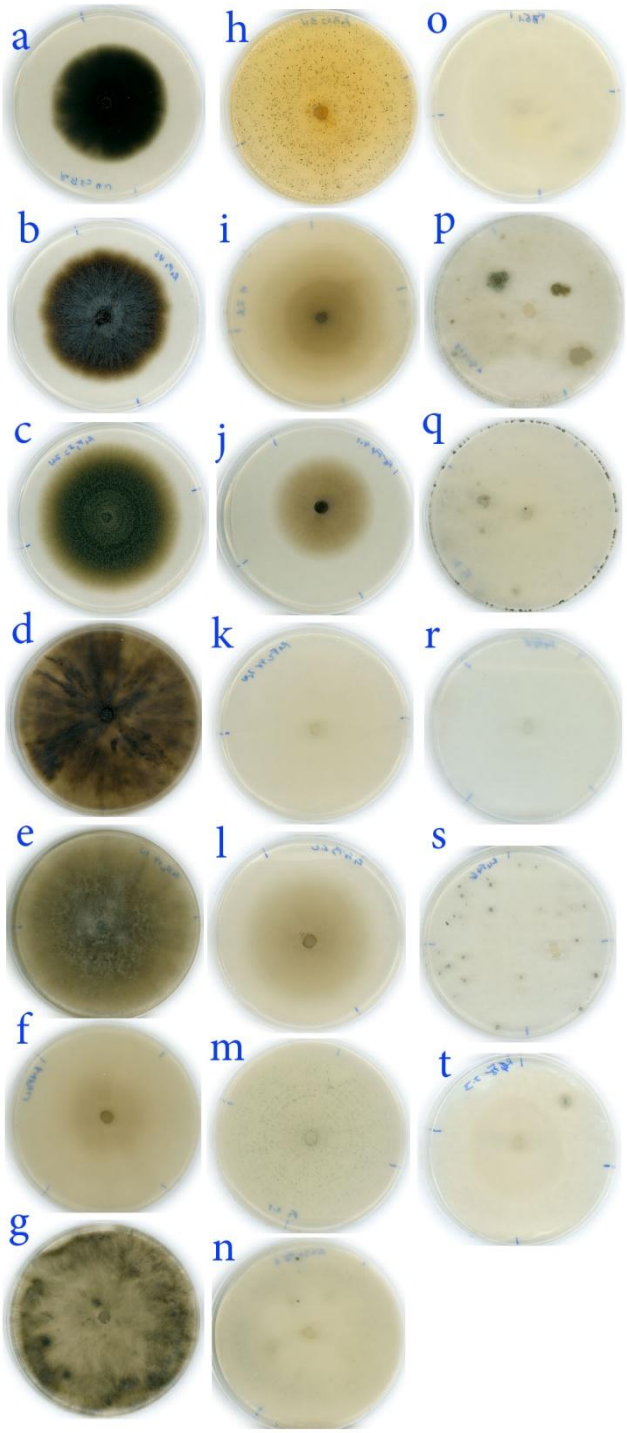


Figure 5.15: Fungi isolated from weathered wood after 20 days of growth on 1% malt extract agar arranged from the darkest to the lightest: (a) *A. pullulans* (black); (b) *H. dematioides;* (c) *Cladosporium* sp.; (d) *A. lycopodina*; (e) *Alternaria* sp.; (f) *Lewia* sp.; (g) *B. stevensii*; (h) *E. nigrum;* (i) *Leptosphaerulina* sp.; (j) *Phialocephala* sp.; (k) *A. pullulans* (white); (l) *Phoma* sp.; (m) *Penicillium* sp.; (n) *V. ambiens*; (o) *C. ligniaria*; (p) *Lecythophora* sp.; (q) *B. fuckeliana*; (r) *Peniophora* sp.; (s) *T. viride*; and (t) *Rhizopogon* sp.

Table 5.10: Growth of fungi grown on solid malt extract agar (1% MEA) after 7 days

Fungi	Radial growth	
	Ave	SD
T. Viride	35.45	[NA]
V. ambiens	34.28	[NA]
A. lycopodina	32.32	[NA]
B. stevensii	32.24	[NA]
B. fuckeliana	30.41	[11.34]
Lewia sp.	24.38	[5.10]
Peniophora sp.	23.72	[NA]
Alternaria sp.	23.59	[3.53]
E. nigrum	23.36	[6.06]
Leptosphaerulina sp.	17.45	[0.49]
Phoma sp.	16.63	[3.75]
Penicillium sp.	16.33	[NA]
H. dematioides	13.9	[4.87]
A. pullulans (black)	12.53	[3.09]
A. pullulans (white)	12.47	[2.59]
Phialocephala sp.	11.01	[3.47]
Cladosporium sp.	10.36	[2.25]
Lecythophora sp.	9.01	[NA]
C. ligniaria	7.61	[0.12]
Rhizopogon sp.	6.88	[NA]

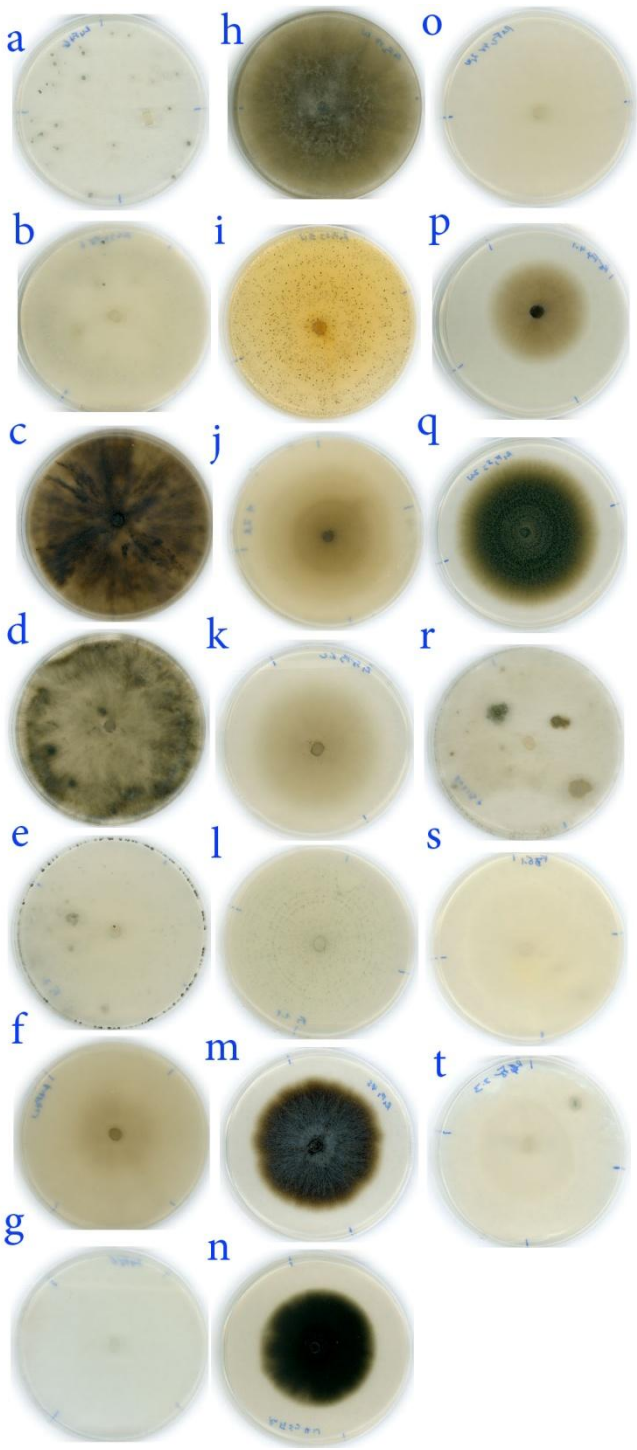


Figure 5.16: Fungi isolated from weathered wood after 20 days of growth on 1% malt extract agar arranged from the fastest to the slowest growing species: (a) *T. viride*; (b) *V. ambiens*; (c) *A. lycopodina*; (d) *B. stevensii*; (e) *B. fuckeliana*; (f) *Lewia* sp.; (g) *Peniophora* sp.; (h) *Alternaria* sp.; (i) *E. nigrum*; (j) *Leptosphaerulina* sp.; (k) *Phoma* sp.; (l) *Penicillium* sp.; (m) *H. dematioides*; (n) *A. pullulans* (black); (o) *A. pullulans* (white); (p) *Phialocephala* sp.; (q) *Cladosporium* sp.; (r) *Lecythophora* sp.; (s) *C. ligniaria*; and (t) *Rhizopogon* sp.

5.4 Discussion

Filter type did not have a statistically significant effect on the diversity of fungi isolated from southern pine samples exposed to the weather, but *A. pullulans* was more common in samples exposed to more energetic wavelengths (UVB, UVA and visible light). Furthermore, it was isolated less frequently from samples exposed under filters that blocked UV and visible light. Similarly, *H. dematioides* was also more prevalent in samples exposed to UV light. The fungi that were most frequently isolated from samples exposed to UV and visible light were often highly melanized. This finding accords with results for the color of wood samples exposed under the different filters. Samples exposed to UV and visible light (Filters 1 – 3) were darker than samples exposed under filters that blocked such radiation (Filters 4 – 5). The literature mentions that melanized fungi are better able to survive exposure to more energetic radiation (Wang and Casadevall 1994; Kawamura et al. 1999). In addition, *A. pullulans* has been reported to be able to metabolize simple sugars and phenolic compounds, which are generated at wood surfaces due to photodegradation of cellulose hemicelluloses and lignin (Bourbonnais and Paice, 1987; Sharpe and Dickinson, 1992). Furthermore, *A. pullulans* synthesizes 'Pullulan', a polysaccharide that enables its blastospores to adhere to weathered wood surfaces (Bardage and Bjurman 1998). These characteristics undoubtedly provide *A. pullulans* with competitive advantages when colonizing wood surfaces exposed outdoors. It is possible that other black fungi may share some of these features. For example, *A. pullulans* and *H. dematioides* are morphologically similar (Ray et al. 2004) and both colonize weathered wood surfaces. Like *A. pullulans*, *H.*

dematioides was frequently isolated from samples exposed to UV radiation, but was less frequently isolated from samples exposed under filters that blocked UV radiation.

In the absence of UV and visible light the diversity of fungi colonizing wood was slightly greater than that of samples exposed to more energetic radiation. Evidence in the literature supports the finding that the growth of less melanized fungi may be favored by the absence of UV radiation (Singaravelan et al. 2008). FTIR spectroscopy of weathered samples revealed a reduction of bands at 1514 and 1462 cm^{-1} (stretching vibration of carbonyl groups in lignin benzene rings and C-H deformations in lignin, respectively; Anderson et al. 1991; Pandey and Pitman 2003). Such changes were less pronounced in samples shielded from UV radiation indicating less photodegradation of lignin. For such samples the concentration of simple phenolic compounds that support fungi adapted to metabolize these substances may be limited (Bourbonnais and Paice, 1987; Feist, 1990; Schoeman and Dickinson, 1997).

The use of different amounts of carpropamid, acetic acid and tinuvin 384 did not have a significant effect on the color of wood surfaces exposed under the different filters. Tinuvin 384, a liquid UV absorber developed for coatings (Ciba, 1998), was applied directly onto the wood surface without the addition of a binder to prevent it from leaching it is likely that acetic acid and carpropamid were also leached from samples by rain.

Color changes in samples exposed under some of the filters resembled those of samples that were fully exposed to the weather (Chapter 3). Color changes during the first 8 weeks of exposure were probably due to photodegradation of wood. Thereafter, the wood's color was strongly influenced by the colonization of wood surfaces by fungi. The initial color changes in wood exposed outdoors are mainly due to photodegradation of lignin. Feist and

Hon (1984) mentioned that photodegradation of lignin causes wood to become red and yellow, which accords with my findings. However, after 8 weeks exposure, fungal staining began to influence the wood color, but samples were not fully stained by fungi until approximately 20 weeks of exposure. Wood surfaces exposed to more energetic radiation were darker than those shielded from such wavelengths. Such darkening appeared to be associated with the presence of a greater proportion of dark, melanized, fungi. Conversely, samples shielded from UV/visible radiation were colonized more frequently by less highly melanized fungi and tended to be greener. Melanin in hyphae and spores of fungi colonizing wood surfaces is responsible for the staining of wood (Brisson et al. 1996) and its biosynthesis can be increased by the presence of UV radiation (Frederick et al. 1999). Therefore, the presence of UV and visible light seems to increase the severity of fungal staining at weathered wood surfaces. My results suggest that UV radiation influences fungal ecology and the color of weathered wood surfaces exposed outdoors. Accordingly, in the absence of UV radiation the adaptations of certain fungi may not provide a competitive advantage and other fungi become more prevalent.

A total of 126 fungi from 21 different genera were isolated in this experiment. A number of these organisms are associated with the staining of wood, but the role played by many of the others species is not completely clear. My results suggest that the fungi most likely to be responsible for staining of southern pine samples were *A. pullulans*, *H. dematioides, Cladosporium sp.* and *Alternaria sp.* Other fungi like *Epicoccum nigrum* and *Phoma sp.* were also frequently isolated, but they do not possess highly melanized hyphae. Their contribution to staining may come from their spores and propagules (Barnett and Hunter

1998). In addition to staining, the literature and results in Chapter 4 suggests that some of the 'other' fungi isolated here might produce soft-rot decay of wood surfaces. For example *Cladosporium cladosporioides*, *Lewia infectoria*, *Phialophora* sp., *Phialocephala* sp., *Alternaria* sp. and *C. ligniaria* have all been found to produce soft-rot (Rajderkar, 1966; Hale and Eaton, 1985; Morrell and Zabel, 1985; Allmer et al. 2006; Zyani et al., 2009). In addition, *L. hoffmanni* can metabolize phenolic compounds, and *Phoma* spp. have been isolated from soft-rotted wood (Savory 1954; Bugos et al. 1988). The presence of these fungi at the surface of weathered wood suggests that soft-rot could occur if the conditions were favorable for fungal growth. Soft-rot fungi were poorly melanized when grown in solid media. Therefore, a valid question is how these fungi can withstand the unfavorable conditions present at wood surfaces exposed to weathering? Two mechanisms are proposed to account for this. The first one is the use of sporulative strategies, for example, propagules such as sporodochia and spore aggregations, which are resistant to UV radiation (Barnett and Hunter, 1998; Rotem and Aust, 1991); and the second is colonization and growth of soft-rot fungi under the surface of wood that is heavily colonized by staining fungi. The hyphae of staining fungi are rich in melanin, which strongly absorbs UV radiation between 250 to 700 nm (Suryanarayanan et al. 2004). The melanin concentrated in the fungi colonizing the weathered surface layer may absorb part of the UV radiation that is incident upon the surface, thereby reducing the amount that reaches sub-surface layers. As a result less highly melanized fungi might be able to grow in this sub-surface layer.

Differences in melanin production by the different fungi isolated here were not examined. Therefore, future work should focus on gaining a better understanding of the relationship

between exposure to UV light and the production of melanin by staining fungi. Understanding the colonization of weathered wood by fungi is also a key step in developing new protective treatments to maintain the color and appearance of wood exposed outdoors.

5.5 Conclusions

The experimental results in this Chapter show that changes in fungal ecology of wood surfaces occurred when UV and visible light were blocked from reaching the surface of wood. Under UV and visible light *A. pullulans* was the dominant fungus colonizing southern pine wood samples, but when such radiation was blocked other fungi became more common. Results also indicate that color changes at exposed wood surfaces during the first 8 weeks of exposure seem to be related to photodegradation of wood. Thereafter, changes appeared to be influenced to a greater extent by the staining of wood by fungi. Therefore, I conclude that solar radiation is an important factor affecting the fungal flora at wood surfaces, and also the color of weathered wood surfaces.

Due to their frequency of isolation and the fact that they possess dark mycelia, *A. pullulans*, *H. dematioides, Cladosporium sp.* and *Alternaria sp.* seem to be the fungi most responsible for the grey colorization of weathered wood surfaces. However, the role played by a number of other isolated fungi is unclear. It is possible they could cause soft-rot decay immediately below the weathered wood surface.

The results in this chapter enlarge our understanding of aesthetic disfiguration of wood surfaces exposed outdoors. However, the results may also be relevant to situations where wood surfaces are shielded from UV radiation for example by building components or beneath semi-transparent finishes.

Chapter 6: Effect of UV radiation on melanization and growth of fungi isolated from weathered wood surfaces

6.1. Introduction

A large number of black moulds colonize and stain weathered wood surfaces, but the stain only extends few millimeters into the wood (Duncan 1963; Dickinson 1971; Savory 1973). The black-blue stain caused by these fungi occurs because the fungal hyphae growing within wood's cell lumens, parenchyma cells and resin canals are heavily pigmented. These pigments, which absorb visible radiation and hence are dark brown, are referred to as melanins (Brisson et al., 1996; Butler and Day, 1998). Fungi synthesize melanins via enzymatic or auto-oxidative reactions of phenols, amino acid derivates or amino sugars (Paim et al. 1990; Butler and Day 1998). Melanin in fungal hyphae enhances the survival of fungi under environmental stresses (Henson et al. 1999; Butler and Day 2001). For example, melanin present in fungal conidia reduces damage caused by UV light, solar radiation, γ-radiation, and X-rays (Fogarty and Tobin 1996; Butler and Day 1998; Henson et al. 1999). Melanins may also play a role in fungal resistance to desiccation and extreme temperatures (Fogarty and Tobin 1996; Butler and Day 1998). The degree of protection provided by melanin against UV light is proportional to the concentration of melanin in fungal cell walls (Butler and Day, 1998; Durrell, 1964; Fogarty and Tobin, 1996). Accordingly, non-melanized hyphae are more susceptible to UV radiation than melanized ones when they are exposed to different doses of UV light at 254 nm (Wang and Casadevall, 1994). Similarly, Kawamura et al. (1999) found that melanin conferred UV tolerance to *Alternaria alternata*, and Frederick et al. (1999) found that hyaline hyphae of the fungus *G. graminis var. graminis*

melanized upon irradiation with UV light. As a result the melanized hyphae were more tolerant to UV radiation compared to non-melanized (mutant) hyphae of the same fungal species. Consequently, pigmented (melanized) fungi may have adaptive advantages in environments where they are exposed to UV radiation.

It is well documented that the most successful organisms colonizing weathered wood surfaces outdoors are black/dark moulds (Duncan, 1963; Sell, 1968; Dickinson, 1971; Sharpe and Dickinson, 1993). It has been suggested that these dark moulds occupy this niche because they are capable of withstanding long periods of dry conditions, relatively high temperatures and high levels of UV radiation at exposed wood surfaces (Duncan, 1963). It seems likely that these adaptations are due in part to their ability to synthesize melanin.

In this chapter I hypothesize that fungi isolated from weathered wood will respond to elevated levels of UV radiation by increasing their production of melanin and as a result will be able to survive such exposure better than fungi that lack the ability to respond in the same way. To test this hypothesis the melanin, biomass and spore production, radial growth and mycelial color of *Aureobasidium pullulans* (de Bary) G. Arnaud [strains R2F32.2 and R1F22W] and *Cladosporium cladosporioides* (*Fresen*.) de Vries [strain R2F33], isolated from weathered wood, were evaluated under three conditions, (1) exposure to artificial UV (340 nm); (2) exposure to visible light (450 nm); (3) and complete darkness. Two albino fungi: *A. pullulans* [strain ATCC 42371] and *Ophiostoma piliferum* (Fries) *Syd. & P. Syd* [strain Cartapip97]; and one pigmented *O. piliferum* [strain TAB28] were used as controls.

6.2. Materials and methods

6.2.1. Experimental design

A factorial experiment was designed to examine the effect of different light conditions on melanin, biomass and spore production, radial growth and mycelial color of wood surface fungi grown on artificial media. The design included 6 blocks, which provided replication at the higher level, three light conditions: UV light, visible light and darkness, and 6 different fungi. Analysis of variance (ANOVA) was used to examine the effect of fixed factors (light conditions and fungal species) on factors of interest. The analysis of data was performed using the Software Genstat v. 12 (VSN International 2009). The assumptions of ANOVA (ANOVA) were tested prior to the analysis (normality of residuals and homogeneity of variances), and as a result the spore concentration and radial growth data were transformed into natural logarithm (LN) and analyzed as logarithms. Significant differences between means were estimated using Fisher's least significant test (l.s.d.). Results are presented in graphs as means and these means can be compared using the relevant standard error of the differences (s.e.d.) or l.s.d. bars. Detailed statistic outputs of the analyses in this chapter are appended to this thesis (Appendix 6). A summary of the experimental design is presented in Table 6.1.

Table 6.1: Summary of experimental design used to test the effect of different light sources on fungal development and melanization

Blocks	Exposure (light sources)	Fungal species	Petri dishes
1	3	6	18
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
6	3	6	18

6.2.2. Fungi and culturing conditions

Six ascomycete fungi were selected including three isolates from weathered wood: *Aureobasidium pullulans* (de Bary) G. Arnaud [strains R2F32.2 and R1F22W] and *Cladosporium cladosporioides* (*Fresen*.) de Vries [strain R2F33], which were selected because they were frequently isolated from weathered wood and were deeply pigmented. Three control fungi were used: albino species of *A. pullulans* [strain ATCC 42371] and non pigmented *Ophiostoma piliferum* (Fries) *Syd. & P. Syd* [strain Cartapip97] and a pigmented *Ophiostoma* strain, *O. piliferum* [strain TAB28]. The albino *A. pullulans* was donated by Viance LLC. Albino and pigmented controls were included in the experiment to compare their melanin production under dark and light conditions with those of test fungi. All fungi were cultured in 100 mm x 15 mm Petri dishes with 1% MEA Difco media at room temperature, and sub-cultured in identical plates every two weeks to ensure the cultures were fresh. For the experiment 60 mm x 15 mm Petri dishes with 1% MEA Difco and a cellophane layer were used. These plates were inoculated with 7 mm (diameter) agar plugs containing fresh fungal mycelia. The plate's lids were replaced by UV transparent quartz

glass disks 63.5 (diameter) x 1.6 (thickness) mm (Technical Glass Products, Inc. Painesville, OH, USA) which transmit radiation between 245 to 780 nm (Figure 6.1). After inoculation the plates were sealed with parafilm, and the fungi were allowed to grow in the dark at room temperature for two days before they were exposed to light. Digital (TIFF) images of the plates were taken using a Microtek Scan Maker i800 scanner, as described above (Chapter 3, section 3.2.4).

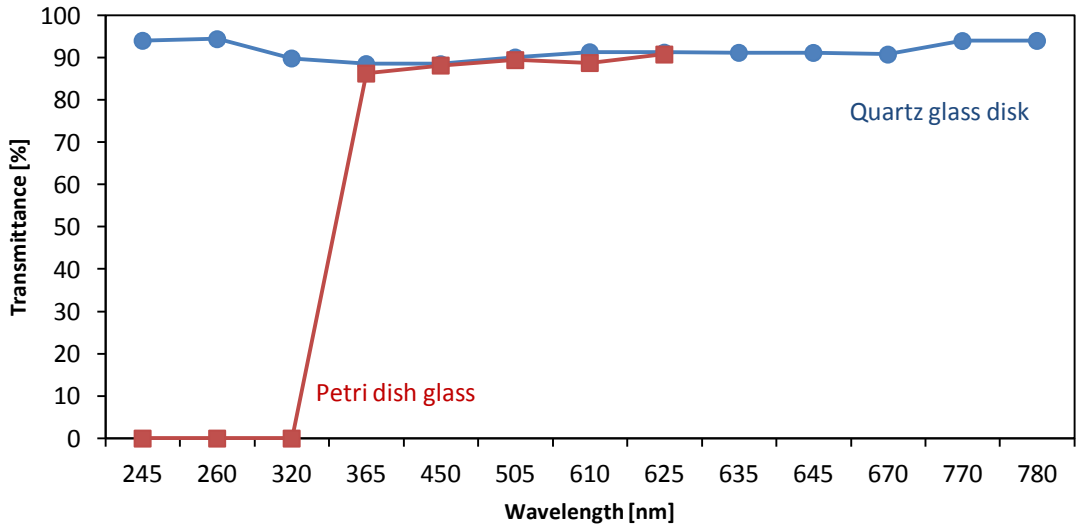


Figure 6.1: Transmittance of a quartz glass lid to UV (340 nm) and visible light (450 nm approx.), Petri dish glass is shown. Transmittance was measured using a UV-VIS spectrophotometer (Varian Model Cary 50 Bio)

6.2.3. Exposure

Plates containing test fungi and controls were exposed to different light conditions in separate exposure units. These units were rectangular boxes made of oriented strand boards. The boxes were painted on the inside with matt paint to reduce reflection, and a sheet of black cloth at the front of the box prevented external light from shining into the

box, but there was still circulation of fresh air into the interior of the box. The unit that exposed fungi to UV light contained 2 UV tubes 340 nm, 40 W (Q-Lab Corp.) (Figure 6.2a). The other two units contained 2 fluorescent tubes (F40L/AQ/ECO wide spectrum 40W, General electric) (Figure 6.2b), or no light source. Irradiance charts for both tubes types were kindly provided by the manufacturers (Figure 6.2 c and d). Fungi were exposed in these boxes to 1700 ($\mu W \times m^{-2}$) of UV radiation or 114 ($\mu mol \times s^{-1} \times m^{-2}$) of visible light for 7 days. The experiment was performed in a conditioning room at 20°C ± 1°C at 65% ± 5% r.h.

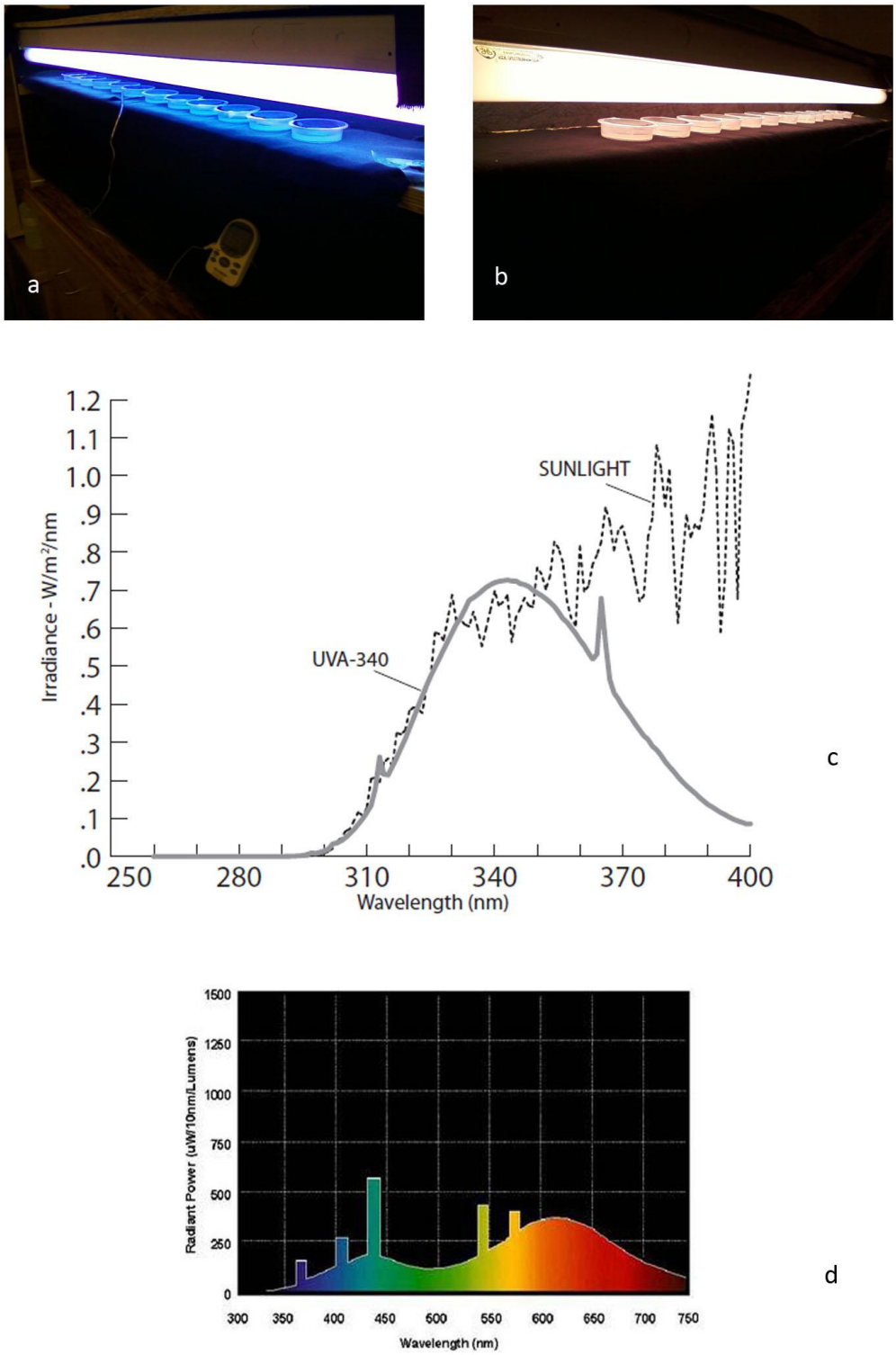


Figure 6.2: UV and visible light exposure units and irradiance charts. (a) UV exposure unit, the unit included 2 UV bulbs 340 nm, 40 W (Q-Lab Corp.); (b) visible light exposure unit, the unit included 2 fluorescent bulbs 450 nm approx. F40L/AQ/ECO wide spectrum, 40W (General electric); (c) irradiance chart for UV tubes; and (d) irradiance chart for visible light tubes. Irradiance charts were kindly provided by the manufacturers

6.2.4. Determination of radial growth, mycelial color, spores, biomass and melanin production

6.2.4.1. Measurement of radial growth of fungal colonies

Digital images of the plates were obtained at the start and the end of the experiment using a desktop scanner (as described in section 6.2.2). Images were analyzed using Adobe Photoshop CS3 Extended, version 10.0.1 (Adobe System Incorporated, USA). The ruler tool of the software was used to quantify the radial growth of the fungi during the 7 day exposure period as described in Chapter 3 (Figure 3.1a). For comparative purposes radial growth (mm/week) was expressed as a function of fungal biomass [(mm/week)/mg biomass].

6.2.4.2. Lightness of mycelia

Images of the plates without their quartz glass lid after exposure were acquired using a desktop scanner (as described in section 6.2.2). The lightness of the mycelial mats was estimated using these images as described in Chapter 3 (Figure 3.1b) (Papadakis et al. 2000). Images were loaded into Photoshop and the lightness of mycelia was measured and expressed using the CIE coordinate, L (lightness on scale of 0, [black] to 100 [white]) (International Commission on Illumination 2007).

6.2.4.3. Spore production

Three mililiters of nano pure water were placed on the surface of each exposed plate. Fungal spores in the moistened mycelial mat were loosened from the mat using a sterile glass rod. The supernatant from each plate was collected in separate 15 mL falcon tubes. The concentration of spores in each tube was determined with a hemocytometer (La Fontaine, Germany) in accord with manufacturer's guidelines (Figure 6.3) (Smith et al., 1988). The concentration of spores was expressed as colony forming units per mL per week, but it was also expressed as a function of the fungal biomass [((CFU/mL)/week)/fungal biomass] to be consistant with the expression of radial growth.

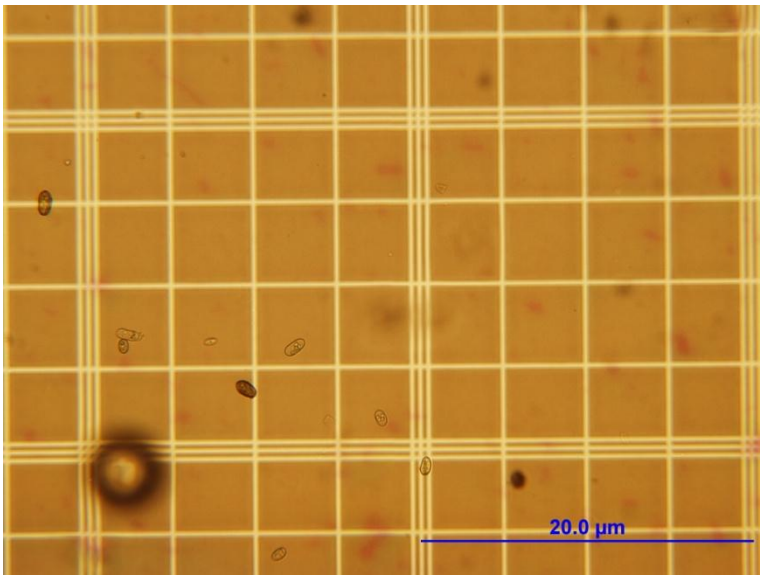


Figure 6.3: Determination of spore concentration by hemocytometer counting

6.2.4.4. Mycelial biomass

Mycelial mats growing on top of cellophane layers were scraped from the plates using a sterile scalpel and transferred onto pre-weighed and labeled glass plates. The plates were

placed in an oven and dried (100 ± 5 °C) for 24 h and then allowed to cool for 2 h in a glass dessicator over silica gel. The plates were re-weighed using an analytical balance (A & D; Model GR-200 from B.C. Scale Co. Ltd; 210 g x 0.0001 g). Biomass produced by fungi in each plate was expressed as mg/week.

6.2.4.5. Isolation of melanin

Dry fungal biomass was rehydrated with 2 mL of nano pure water and placed in separate 5 mL collection tubes containing ceramic beads. One mL of water was added to each tube. Tubes were vortex-mixed until fungal mycelia in the tubes disintegrated. Extraction of melanin from hyphae used hot NaOH according to the method of Gadd (1982). This method involved autoclaving the fungal suspensions in 50 mL glass vials containing 10 mL of 1M NaOH for 1.5 hours at 121°C. The supernatants were then collected and the vials were re-filled with 10 mL of 1M NaOH and the extraction procedure was repeated. Twenty mL of supernatant was collected from each tube and filtered using 40 µm sterile cell strainers Fisherbrand (Fisher Scientific, AB; Canada). Melanin in the solutions was precipitated by adding 5 mL of 7M HCl (final pH approx. 1) to each tube and leaving the tubes overnight at room temperature. Tubes were centrifuged (3700 rpm, 15 min.) and the raw melanin (pellet) residues were purified by acid hydrolysis using the method of Bell and Wheeler (1986). Purification used sealed glass vials containing 5 mL 7M HCl and stored at 100°C for 12 hours. The tubes were allowed to cool and centrifuged (as above). Pigments were dissolved in 5 mL 1M NaOH overnight, purified from solid by centrifugation and transferred into new 15 mL falcon tubes.

6.2.4.6. Melanin concentration

The concentration of melanin in material extracted from fungal mycelium (above) was calculated using absorbance of UV/Vis. light at 420 nm measured using a UV-VIS spectrophotometer (Varian Model Cary 50 Bio) (Singaravelan et al., 2008). Melanin in the solutions was precipitated with HCl (as above), washed with nano pure water, dried and weighed. Then, known amounts of dried melanin, were dissolved in 1M NaOH and the absorbance of light at 420 nm was measured for different solution concentrations (50, 20, 10, 5, 2, 1.42, 1 and 0.5 percent). Absorbance data were used to plot a calibration curve, for absorbance versus concentration of melanin (data available in Appendix 7). These curves were used to calculate the concentrations of the purified melanin in the original parent solutions. Finally the amount of melanin produced by the cultured fungi after seven days of growth in each plate was calculated as:

CM plate = CM extracted solution x SW solution / biomass

Where:

CM plate: concentration of melanin in each plate (mg melanin / mg biomass)/week

CM extracted solution: concentration of melanin in each extracted solution (calculated from calibration curve and expressed as mg melanin/g solution)

SW solution: standard weight of each extracted melanin solution (5.20 g)

biomass: fungal biomass per plate (mg)

6.3. Results

There were significant effects of exposure to light (E), fungal species (F) and interactions of these two factors (ExF) on melanin concentration, biomass, spore concentration, radial growth and lightness of fungal cultures.

Table 6.2: Significant effects of, and interaction between exposure to light and fungal species on melanin concentration, biomass, radial growth and lightness of fungal cultures

	P-value				
Source of variation	melanin concentration	biomass	LN (1+spore concentration)	LN (1+ radial growth)	lightness
Exposure	0.032	<.001	<.001	<.001	<.001
Fungi	<.001	<.001	<.001	<.001	<.001
Exposure x Fungi	0.007	<.001	<.001	<.001	<.001

6.3.1. Melanin concentration

Melanin production of fungi isolated from weathered wood and controls are depicted in Figure 6.4. The melanin concentration of *A. pullulans* increased with exposure to both visible and UV light. However, variation occurred between strains. *A. pullulans* [R2F32.2] showed no significant difference in melanin production when grown under UV or visible light. Melanin concentration of this strain when grown in the absence of light was, however, significantly lower than those of cultures grown under UV or visible light. *A. pullulans* [R1F22W] behaved differently. This strain produced significantly more melanin when grown under UV light than when it was grown under visible light or in the absence of light. Furthermore, there was no significant difference in the melanin production of cultures grown under visible light or in the dark. The production of melanin by *A. pullulans* [ATCC 42371], an albino control, showed the same trends as that of *A. pullulans* [R2F32.2]; although it produced higher concentrations of melanin than the other strains when grown

under UV or visible light. It should be noted that *A. pullulans* [ATCC 42371] is classified as an albino in the American Type Culture Collection, however, my results indicate that it can synthesize melanin. Therefore it should not be classified as an albino strain. *Cladosporium cladosporioides* [R2F33], showed no significant differences in melanin production when grown under UV or visible light or in the dark (although the difference in melanin production of cultures grown under UV light or in absence of light was almost significant). This fungus produces high amount of melanin in the dark unlike *A. pullulans* which showed decreased production of melanin in the absence of light. *A. pullulans* produced more melanin than *C. cladosporioides*, although the difference was not statistically significant. The control fungi *O. piliferum* [TAB28] and [Cartapip97] were unable to synthesize melanin under UV radiation. Under visible light both fungi produced small amounts of pigmentation. However, in the absence of light *O. piliferum* [TAB28] produced larger amount of pigments. *O. piliferum* [Cartapip97] produced only small amounts of melanin when grown under visible light or in the dark.

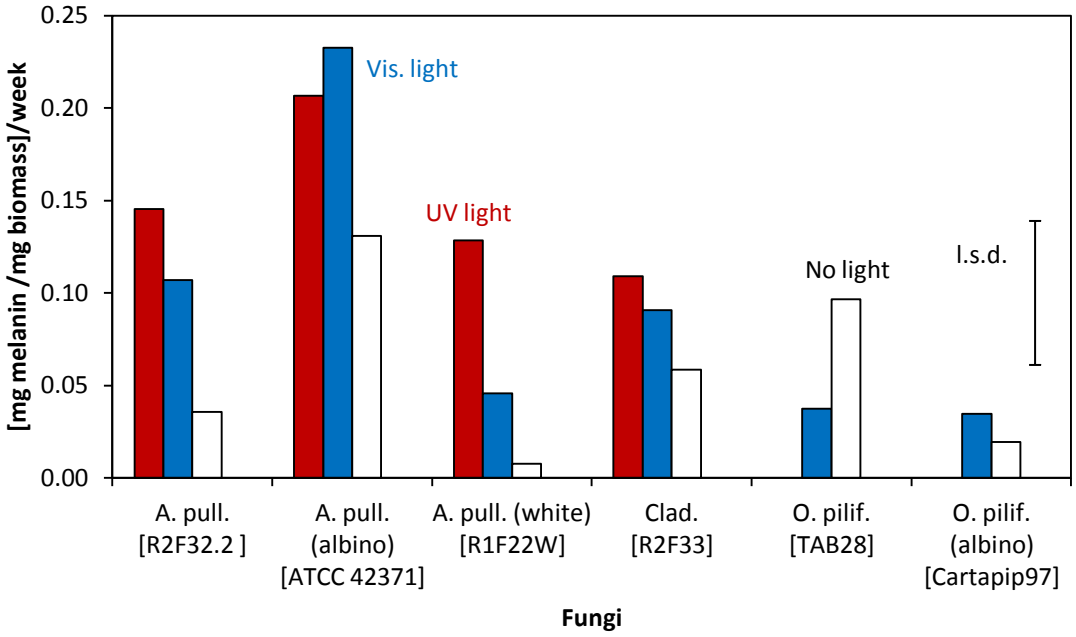


Figure 6.4: Melanin production of fungi isolated from weathered wood (including controls) after 7 days of growth under UV or visible light, or when grown in the dark. L.s.d. (least significant difference bar)

6.3.2. Fungal biomass

The biomass of fungi grown under different light conditions is shown in Figure 6.5. The two strains of *A. pullulans* [R2F32.2] and [R1F22W], again behaved differently. *A. pullulans* [R2F32.2] produced the highest amount of biomass when grown under visible light, but there was no difference in biomass production when it was grown in the dark or under UV light. The production of biomass by *A. pullulans* [R1F22W] increased significantly when it was exposed to less energetic radiation (visible or no light). The *A. pullulans* control behaved in the same way as *A. pullulans* [R2F32.2], although the amount of biomass produced by this fungus was significantly lower than that of the fungi isolated from weathered wood. *C. cladosporioides* [R2F33] behaved in the same way as *A. pullulans* [R1F22W] and [ATCC 42371]. Significant differences occurred in cultures grown under UV light or in the dark, although the amount of biomass produced by cultures grown under UV

light was not different from that of cultures of *A. pullulans* [R2F32.2]. The *O. piliferum* controls [TAB28] and [Cartapip97], did not grow under UV radiation and their production of biomass when grown under visible light or in the dark was not significantly different from that of *C. cladosporioides* and *A. pullulans* [R1F22W]. The biomass of *O. piliferum* [Cartapip97] cultures was significantly higher when they were grown in the dark than when they were grown under visible light (biomass was almost double that of cultures grown under visible light).

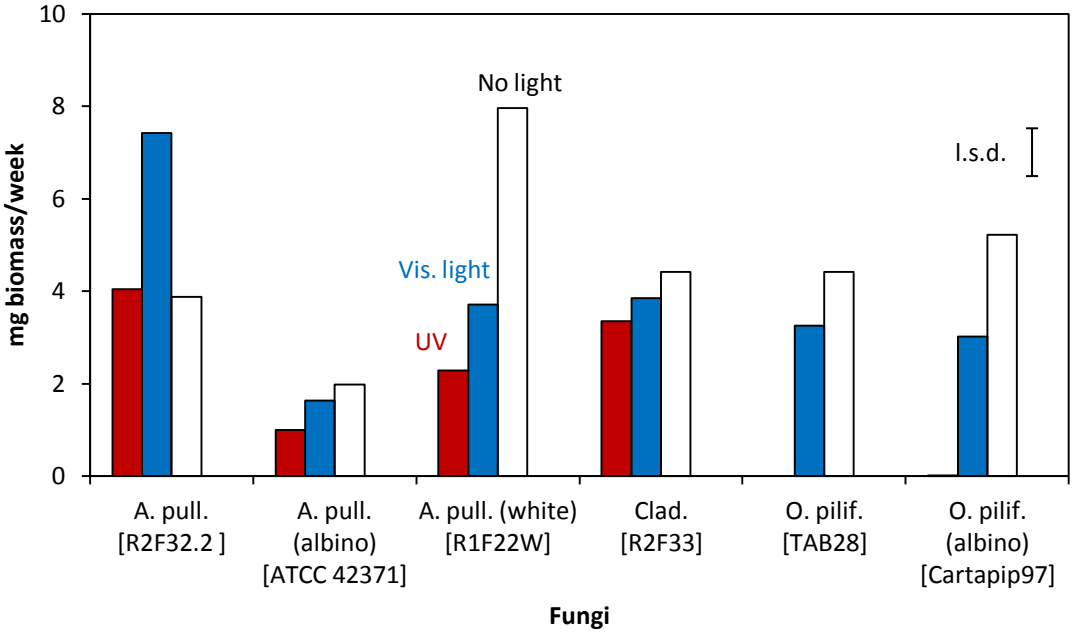


Figure 6.5: Production of biomass by fungi isolated from weathered wood (including controls) after 7 days of growth under UV or visible light, or when grown in the dark. L.s.d. (least significant difference bar)

6.3.3. Spore production

The production of spores by the fungi when they were grown under different conditions expressed as LN [1+ (unit forming colonies/mL)/mg biomass] is depicted in Figure 6.6. The production of spores by all of the tested fungi appeared to be affected by the presence of

radiation. In the case of *A. pullulans* [R2F32.2] the presence of UV and visible radiation decreased the production of spores in comparison to that of cultures grown in the dark. The production of spores by *A. pullulans* control [ATCC 42371] increased when the energy of the incident light decreased (visible light) or when the fungus was grown in the dark. On the other hand, spore production by isolates of *A. pullulans* [R1F22W] peaked when they were exposed to visible light. The behavior of *C. cladosporioides* was very similar to that of the *A. pullulans* control, as spore production of cultures grown under visible light and in the dark was similar. *O. piliferum* [TAB28] and [Cartapip97] behaved differently. These fungi were unable to produce spores in presence of UV radiation. Nevertheless, under visible light they produced significantly higher amounts of spores than when they were grown in the dark.

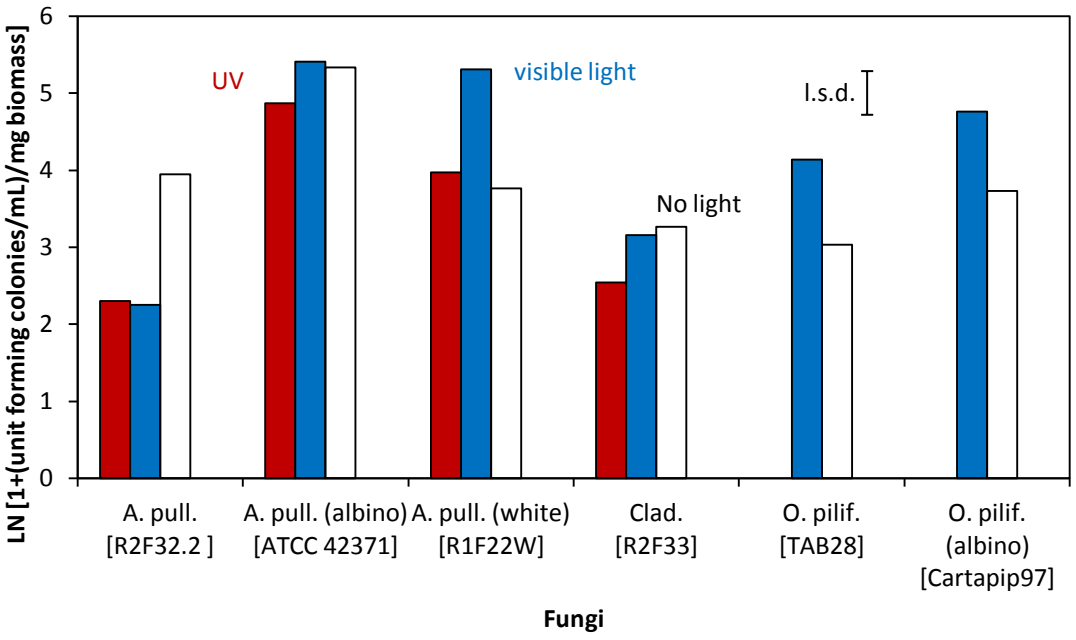


Figure 6.6: Production of spores by fungi isolated from weathered wood (including controls) after 7 days of growth under UV or visible light or when grown in the dark. L.s.d. (least significant difference bar)

6.3.4. Radial growth of fungal cultures

The growth of fungal cultures (measured as described in section 3.2.4) when they were exposed to different light conditions is depicted in Figure 6.7 (analyzed as LN [1+ (mm/week)/mg biomass]. The growth of all of the fungi isolated from weathered wood was affected by UV radiation. All fungi grew well in the dark and under visible light. *A. pullulans* [R2F32.2] grew significantly faster under visible light than when grown in the dark. However, the differences in growth of *A. pullulans* [R2F32.2] and [ATCC 42371] when grown under visible light or in the dark were not statistically significant. *C. cladosporioides* behaved in a similar way to *A. pullulans* [ATCC 42371]. The growth of *O. piliferum* controls [TAB28] and [Cartapip97] again was seriously affected by UV radiation, as no growth occurred when the fungi were exposed to UV radiation. However, both strains of *O. piliferum* grew well under visible light and in the absence of light.

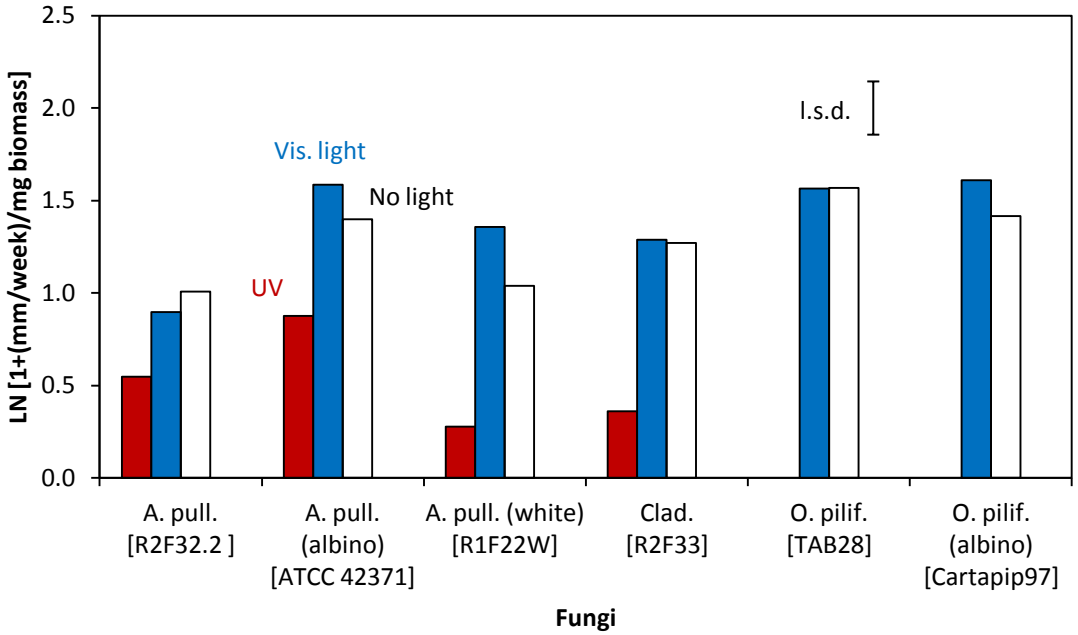


Figure 6.7: Radial growth (LN [1 + radial growth]) of fungi isolated from weathered wood (including controls) after 7 days growth under UV or visible light or when grown in the dark. L.s.d. (least significant difference bar)

6.3.5. Lightness of mycelia

The lightness of mycelium from fungi isolated from weathered wood and the controls are depicted in Figure 6.8. The results indicate that all strains of *A. pullulans* were dramatically affected by exposure to UV radiation. The lightness of *A. pullulans* [R2F32.2] under UV radiation and visible light was similar, but in the absence of light fungal mycelia was significantly lighter. *A. pull* [R1F22W] and [ATCC42371] behaved in the same way. They became lighter when grown under less energetic light. Similarly, mycelia of *C. cladosporioides* was lighter when it was grown under less energetic light, but significant differences in lightness were only found in cultures grown under UV radiation or in the absence of light. The control *O. piliferum* [TAB28] was lighter when grown under visible light

(significantly), but [Cartapip97] showed no significant variation in lightness, irrespective of the light it was exposed to.

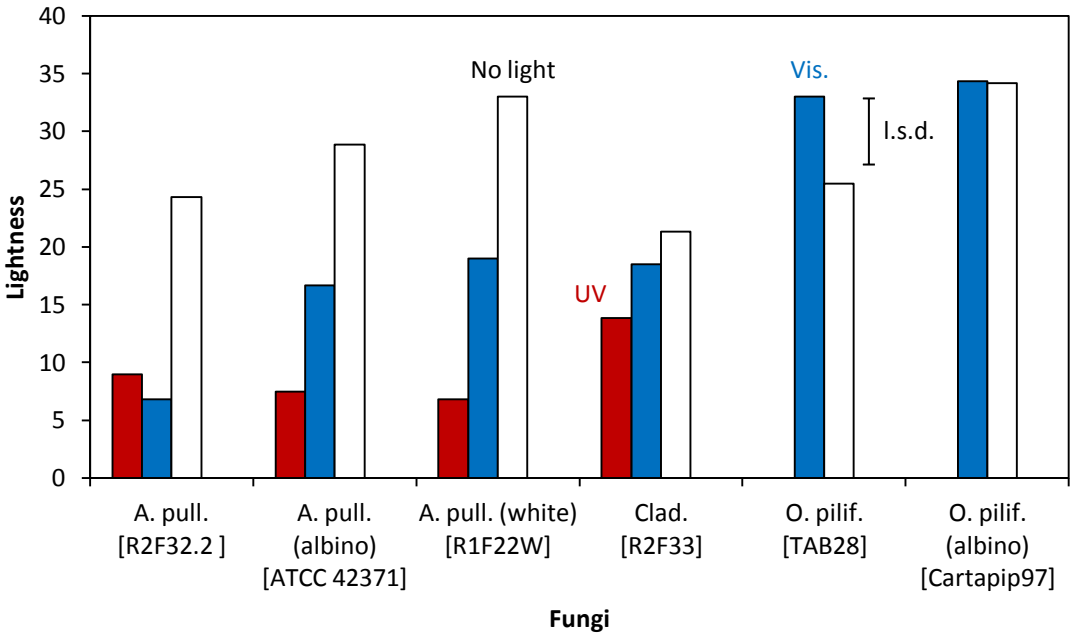


Figure 6.8: Lightness of mycelia from fungi isolated from weathered wood (including control) after 7 days of growth under UV or visible light or when grown in the dark. No measurements were performed for *Ophiostoma* fungi exposed under UV radiation. L.s.d. (least significant difference bar). Lightness is expressed using the CIE parameter L, 0: black – 100: white

6.4. Discussion

The radial growth, biomass and spore production of fungi isolated from weathered wood and grown on artificial media was affected by exposure to UV light. Under UV light only *A. pullulans* and *C. cladosporioides*, and *A. pullulans* strain [ATCC42371] grew, generated biomass and spores and synthesized melanin. *A. pullulans* strain [ATCC42371] was expected to act as an albino control, but contrary to expectations it produced more melanin when exposed to UV and visible light than the other microorganisms. Therefore, for the remainder of this discussion results for *A. pullulans* [ATCC42371] will be grouped along with those of the other strains of *A. pullulans*, but the stability of its albino condition will be discussed later.

The amount of melanin synthesized by *A. pullulans* and *C. cladosporioides* increased when they were exposed to UV radiation. The tendency of fungi to increase melanin production under UV radiation has been reported for the fungus *Gaeumannomyces graminis* (Frederick et al., 1999), but it has not been reported before for fungi isolated from weathered wood. Thus, my experimental data support the hypothesis that *A. pullulans* and *C. cladosporioides* are able to increase melanin production when exposed to UV radiation, an adaptation that would be beneficial to their survival at weathered wood surfaces. The ability of melanized moulds to tolerate elevated levels of UV radiation, high temperatures, water deficiency and chemical and radioactive pollution has been documented before (Fogarty and Tobin 1996; Henson et al. 1999; Butler and Day 1998; 2001; Dadachova et al. 2007). My findings also accord with those of Singaravelan et al. (2008), who described the ability of *Aspergillus niger* to increase melanin production as an adaptive response to elevated levels of solar

radiation. In this experiment two strains of *A. pullulans*, isolated from weathered wood, were tested. These strains were selected because they produced different patterns of melanization when grown on artificial media. *A. pullulans* [RF2F32.2] produced melanized mycelia almost simultaneously as it grew. In contrast, *A. pullulans* [R1F22W] produced colorless mycelia during the first two weeks of growth and then there was mild melanization of its mycelial mat. When exposed to UV light both strains were able to produce melanin. This finding suggest that melanin biosynthesis in both of these fungi is an adaptation that can be enhanced by exposure to UV radiation. Accordingly, both strains synthesized less melanin when they were grown under visible light or in the dark.

Exposure to visible light or the absence of light, favored the growth and spore production of tested fungi. According to Griffin (1996) "The effect of light on sporulation and growth can vary from inductive to inhibitive even at different stages of the same sporulative process". Accordingly, results here indicate that exposure to UV radiation influenced sporulation. Nevertheless, differences in the production of biomass by the different *A. pullulans* strains were observed. These differences might be the result of biological variations within species.

C. cladosporioides behaved differently, for example it showed no significant differences in the production of melanin when exposed to UV or visible light or when grown in the dark. Apparently this fungus produces melanin not only when exposed to UV radiation. Studies on *Cladosporium sp.* have pointed out that its spores are widely distributed all over the world and are an important component of the biological particles that are suspended in the atmosphere (Iannone et al. 2011). Melanization of spores may be advantageous when they are subjected to low temperatures and high levels of UV radiation in the upper atmosphere.

Similarly, *O. pilif.* [TAB28] produced an equal amount of melanin when grown under visible light or in the absence of light. This fungus is a wild-type strain able to synthesize melanin. Its natural habitat is in insect galleries under the bark of conifer trees where it is not exposed to UV radiation (Perry 1991). UV radiation has been reported to penetrate wood surfaces to depths of approximately 200 micrometers approximately, but it would be unable to penetrate the bark of conifer trees (Kataoka et al. 2004). The albino control *O. piliferum* [Cartapip97] used here was originally developed as a bio-control agent for removal of extractives from wood chips prior to pulping. According to Behrendt et al. (1995) it is not able to synthesize melanin. However, I found that it produced small amounts of spectroscopically active substances (absorbance at 420 nm), suggesting that it may produce melanin. However, it is important to point out that the method for melanin extraction used here has not been optimized to obtain pure melanins (Gadd 1982; Rosas et al. 2000). Therefore, the presence of small amounts of chromophores in extracts does not conclusively prove that melanin was present.

My finding that common fungi isolated from weathered wood produce more melanin when they are exposed to UV and visible radiation changes our understanding of the discoloration of wood surfaces exposed outdoors. For example, it becomes clear that UV radiation and fungi may interact to produce darker surfaces because in presence of UV radiation *A. pullulans* became darker and more heavily melanized. On the other hand, the presence of a layer extending to a depth of 100 micrometers at wood surfaces, which contains heavily melanized fungi may shield the underlying wood from UV radiation. Fungal melanin absorbs wavelengths between from 250 to 700 nm, but peak absorbance is at 250 nm

(Suryanarayanan et al. 2004). Hence, melanized fungi colonizing weathered wood surfaces may protect lignin in sub-surface wood tissues from photodegradation because it strongly absorbs UV radiation (Kalnins, 1966). The 'shielding effect' of melanized fungi might explain why the rate of erosion of wood surfaces exposed to natural weathering decreases over time whereas the rate of erosion of wood exposed to accelerated weathering in the absence of fungal colonization stays constant (Liu 2011). In accord with this suggestion Sailer et al. (2010) proposed a treatment to encourage the complete colonization of wood surfaces by *A. pullulans*. Sailer et al. reasoned that such a highly melanized biofilm could protect wood surfaces from photodegradation.

A. pullulans strain [ATCC42371] is reported to be an albino mutant by Gadd and De Rome (1988); and Gadd et al. (1990). Its definition as an albino implies total inability to synthesize melanin, and, at the genetic level, inhibition of the expression of the polyketide synthase gene (Fleet and Breuil 2002; Starr et al. 2010). My results show that *A. pullulans* strain [ATCC42371] was able to produce pigmentation when exposed to UV and visible light, although, under normal culturing condition in the laboratory (1% malt extract agar), the isolate was colorless. My results suggest that *A. pullulans* [ATCC42371] should be re-classified as a white strain similar to those that I isolated from weathered wood surfaces in Chapters 3 and 5, and others reported by previous studies (Schoeman and Dickinson, 1997).

The main hypothesis proposed at the start of this chapter can be accepted in part. Further research to better understand the complex interaction between exposure to UV radiation, fungal colonization and the weathering of wood would be desirable. In particular research should examine the occurrence of fungi at wood surfaces and their influence on the depth

and extent of photodegradation of underlying woody tissues. It would also be interesting to compare the diversity and ecology of organisms at wood surfaces with those found beneath the surface.

6.5. Conclusions

My results show that *A. pullulans*, one of the most successful fungi at colonizing weathered wood, has the ability to increase its production of melanin when exposed to UV radiation. This could be an adaptive response to the high levels of UV radiation found at wood surfaces exposed outdoors. Conversely, *C. cladosporioides* did not produce more melanin when it was exposed to UV radiation. Therefore, I conclude that not all fungi need such an adaptive response to survive exposure to UV radiation at wood surfaces outdoors, although it seems that fungi lacking this response need to be highly melanized to grow at weathered wood surfaces. Further research on other fungi isolated from weathered wood is needed to strengthen these conclusions. My results also indicate that our current understanding of the discoloration of weathered wood surfaces needs to be revised, as it is clear that darker staining is produced by the interaction of UV radiation and colonization of wood surfaces by fungi. More research on the influence of staining fungi at wood surfaces on the depth and extent of photodegradation of sub-surface woody tissues is needed to better understand the role that fungi play in the weathering of wood.

Results in this chapter have provided new insights into the adaptive response of surface fungi to the high levels of UV radiation that they are exposed to at weathered wood surfaces. The next chapter will study the response of two surface fungi to UV radiation when they are prevented from synthesizing the melanin biopolymer that protects them from UV radiation.

Chapter 7: UV light and melanin biosynthesis inhibitors as potential treatments against fungal staining

7.1. Introduction

Discoloration of wood surfaces exposed to weathering has been attributed to the colonization of wood by melanized fungi (Duncan, 1963; Dickinson, 1971). Melanin synthesized by these fungi protects their cells against the deleterious effects of solar UV radiation, extreme temperatures and desiccation (Fogarty and Tobin 1996; Butler and Day 1998; Henson et al. 1999). However, melanin does not re-radiate absorbed radiation as visible wavelengths (Butler and Day, 1998). Therefore, wood colonized by melanized fungi is a blue/dark color due to the photochemical properties of the melanin contained in the fungal cells (Brisson et al., 1996).

Melanin synthesis by fungi can be blocked by specific chemicals. These chemicals have been extensively used in agriculture as fungicides, for example to prevent blast rice disease (Kurahashi 2001). The chemicals target specific enzymes involved in the synthesis of dehydroxynaphthalene (DHN) melanins by ascomycetes. DHN melanins are synthesized by some of the fungi that colonize weathered wood surfaces (Kawamura et al., 1997; Kogej et al., 2004). Therefore in principle melanin biosynthesis inhibitors (MBIs) should be able to reduce the level of fungal melanization and staining of weathered wood surfaces. Furthermore, if surface staining fungi lack sufficient melanin, when exposed outdoors, their death might be hastened because many of them seem to tolerate solar UV radiation primary because of their ability to synthesize melanin (Durrell 1964; Wang and Casadevall 1994; Kawamura et al. 1999).

In this chapter I hypothesize that the interruption of melanin biosynthesis in fungi colonizing weathered wood can be achieved by using MBIs. If this occurs fungal staining at wood surfaces would decrease because of high mortality of melanized fungi following exposure to UV radiation. Two *in-vitro* experiments were carried out to test this hypothesis. In the first one, three different MBIs, cerulenin, tricyclazole and carpropamid, were added to artificial media (1% malt extract agar). Spores of two melanized fungi that were frequently isolated from weathered wood, *Aureobasidium pullulans* (de Bary) G. Arnaud and *Cladosporium cladosporioides* (*Fresen*.) de Vries, were inoculated onto the plates. The plates were exposed to artificial UV or visible light and the growth of the fungi on the plates was examined. For the second experiment spruce veneers were impregnated with carpropamid, which were then inoculated with spores of *A. pullulans.* Veneers were exposed to artificial UV or visible light. The effectiveness of the treatment was evaluated by measuring the staining and color of the treated veneers and untreated controls.

7.2. Materials and methods

7.2.1. In-vitro testing of the melanin biosynthesis inhibitors cerulenin, tricyclazole and carpropamid, and the fungicide quinoxyfen

7.2.1.1. Experimental design

A factorial experiment was designed to test the effects of different melanin biosynthesis inhibitors (MBIs) on the survival of fungal colonies grown on artificial media and exposed to either UV or visible light. The experiment included three MBIs, two fungal species, and two exposure conditions. Controls consisting of plates supplemented with a fungicide and the solvent used to prepare the MBIs (acetone) were also included. The design accounted for random variation in media preparation, fungal inoculation, exposure and spatial distribution of the plates under the different light sources. Analysis of variance (ANOVA) was used to examine the effect of fixed factors and their interactions on the number of fungal colonies that grew on plates. Analysis of data was performed using the software Genstat v. 12 (VSN International 2009). The assumptions of ANOVA were tested prior to the analysis (normality of residuals and homogeneity of variances). However, no transformation of data was required. Significant differences ($p < 0.05$) between means were tested using Fisher's least significant test (l.s.d.). Results are presented in graphs as means and these means can be compared using either standard error of the differences (s.e.d.) or l.s.d. bars. The detailed statistic output for the analysis of data is appended to this thesis (Appendix 8). A summary of the experimental design is presented in Table 7.1.

Table 7.1: Summary of experimental design used to test the effect of different melanin biosynthesis inhibitors and a fungicide on the survival of fungi

Blocks	Exposure (light sources)	Chemicals supplemented	Concentrations tested	Fungal species	Petri dishes
1	2	4 + control	1	2	20
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
5	2	4 + control	1	2	20

7.2.1.2. Chemicals and culture media

Three MBIs were selected based on their ability to interrupt the biosynthetic pathway for DHN melanins. MBIs were: (1) Cerulenin [(2R,3S)-3-[(4E,7E)-nona-4,7-dienoyl]oxirane-2-carboxamide], (2) tricyclazole [5-methyl-1,2,4-triazolo[3,4-b]benzothiazole], and (3) carpropamid [(1R*,3S*)-2,2-dichloro-N-[1-(4-chlorophenyl)+ethyl]-1-ethyl-3-methylcyclopropanecarboxamide] (Figure 7.1 a, b and c, respectively). In addition the fungicide quinoxyfen [5,7-dichloro-4-(4-fluorophenoxy)] quinoline was tested because of its efficacy against powdery mildews (Coghlan et al. 1991) (Figure 7.1d). Chemicals were purchased as powders from Sigma-Aldrich Co, St Louis, MO, USA. Stock solutions of these chemicals at 100 ppm were prepared in acetone (industrial grade) and added to autoclaved malt extract agar (MEA) (1% Difco), when still liquid (45°C approx.) under magnetic stirring, until the final desired concentration of 10 ppm was reached. Control plates were supplemented with acetone at a level similar to that used to dissolve the MBIs. Media supplemented with these chemicals and acetone was poured into 60 x 15 mm Petri dishes. Plates were stored at 4°C until they were inoculated with test fungi.

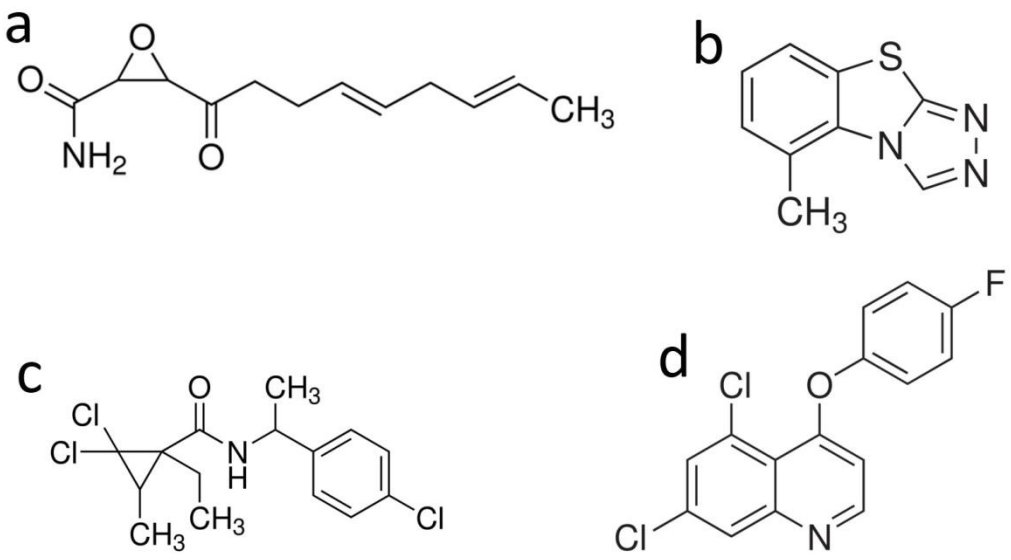


Figure 7.1: Chemical structures of three melanin biosynthesis inhibitors (MBIs) and a fungicide used to inhibit growth of *A. pullulans* and *C. cladosporioides.* (a) cerulenin, inhibitor of melanin biosynthesis at the polyketide synthase step; (b) tricyclazole, inhibitor of polyhydroxynaphthalene reductase in the enzymatic reduction of 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) to scytalone and 1,3,8-trihydroxynaphthalene (!,3,8-THN) to vermelone; (c) carpropamid, inhibitor of the dehydratase enzyme in the enzymatic dehydration of scytalone into 1,3,8-THN and dehydration for the conversion of vermelone into 1,8-dihydroxynaphthalene; and (d) quinoxyfen, disruptor of early cell signaling events in fungal cells

7.2.1.3. Inoculation of media with *A. pullulans* and *C. cladosporioides*

Aureobasidium pullulans (de Bary) G. Arnaud [strain R2F32.2] and *Cladosporium cladosporioides* (Fresen.) de Vries [strain R2F33] were used as the test fungi. These fungi were selected based on their frequency of isolation from weathered wood (Chapters 3 and 5) and their ability to synthesize melanin (Chapter 6). Fungi were cultured in 100 x 15 mm Petri dishes with 1% MEA Difco media at room temperature, and sub-cultured in similar plates every two weeks to ensure that fresh material was available for the duration of the experiment. Spores in the plates were harvested under sterile conditions after 1 week by flooding the plates with 3 mL of nano pure water. Spores produced by the fungi were

loosened from fungal mycelia using a sterile glass rod. The supernatants were collected in 15 mL falcon tubes and the concentration of spores in these solutions was determined using a hemocytometer (La Fontaine, Germany) in accord with manufacturer's guidelines. Then the supernatants were adjusted to one spore per µL (Figure 6.3). Plates containing MBIs were inoculated with 50 µL of spore solutions, which were evenly spread onto the media using a glass rod. The lids of the plates were replaced by quartz glass disks 63.5 (diameter) x 1.6 (thickness) mm (Technical Glass Products, Inc. Painesville, OH, USA) which transmitted radiation between 245 to 780 nm (Figure 6.1). The plates were sealed with parafilm and fungi were allowed to grow in a dark room at ambient temperature for 24 hours before they were exposed to UV radiation or visible light.

7.2.1.4. Exposure to UV and visible light and quantification of number of fungal colonies after exposure

Plates containing the tested fungi and control were exposed to UV or visible light in separate wooden boxes, painted inside with matt paint to reduce reflection of light, as described in Chapter 6 (section 6.2.3). A box with no light source acted as a control. Fungi were exposed in these boxes to 1700 (µW x m^{-2}) of UV radiation or 114 (µmol x s^{-1} x m^{-2}) of visible light for 7 days. The experiment was performed in a conditioning room at 20°C ± 1°C and 65% ± 5% r.h.

After 7 days of exposure to UV or visible light, digital images of the plates without their lids, at a resolution of 300 dpi, were obtained using a Microtek Scan Maker i800 desktop scanner. Digital images of the mycelial mats were loaded into Adobe Photoshop CS3

Extended, version 10.0.1 (Adobe System Incorporated, USA) and observed at a magnification of 150%. Individual fungal colonies within each plate were manually counted and labeled using the software's counting tool (Figure 7.2).

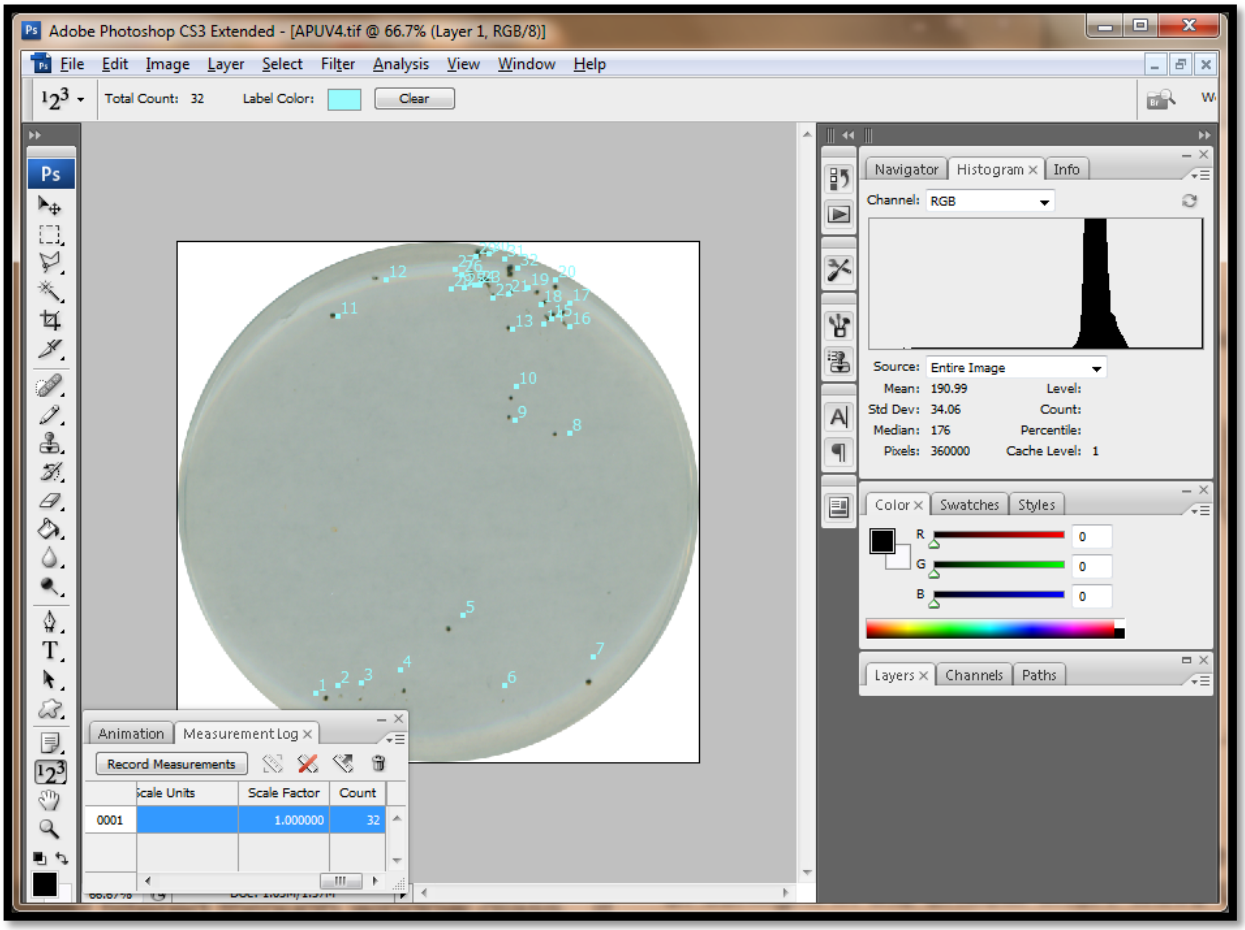


Figure 7.2: Screen-shot of the software used to count the number of fungal colonies in each plate

7.2.2. Effect of chemicals and UV radiation on fungal staining of wood

7.2.2.1. Experimental design

A factorial experiment was used to test the effect of the MBI carpropamid and the fungicide quinoxyfen on the staining and color of wood veneers inoculated with an aqueous solution of fungal spores or water and exposed to UV or visible light. The experimental used two exposure conditions (UV and visible light), two chemicals at two concentrations, and one fungal species. The control was veneers inoculated with sterile water. Five blocks provided replication at the higher level. The resulting design accounted for random variations in the media preparation, impregnation of chemical into the wood, inoculation with fungi, exposure and spatial distribution of plates in each light box. Analysis of variance (ANOVA) was used to determine the effects of the fixed factors (chemicals and light conditions) and random effects on the area of wood stained by fungi and color differences of samples. Analysis of data used Genstat v. 12 (VSN International 2009). The assumptions of ANOVA were tested prior to the analysis (as metioned in section 7.2.1.1) and as a result fungal staining (ratio of stained area of treated veneers divided by stained area of untreated veneers) was transformed into natural logarithms. Significant differences ($p<0.05$) were tested using Fisher's least significant test (l.s.d.). Results are presented in graphs as means, and either standard error of the differences (s.e.d.) or l.s.d. bars can be used to compare means. Statistical output for this section is appended to this thesis (Appendix 9). A summary of the experimental design is presented in Table 7.2.

Table 7.2: Summary of experimental design used to test the effect of a melanin biosynthesis inhibitor and UV radiation on fungal staining of wood

Blocks	Exposure (light sources)	Chemicals supplemented	Concentrations tested	Fungal species	Wood species	Petri dishes
1	2	2 + control	2	1	1	12
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
5	2	2 + control	2	1	1	16

7.2.2.2. Wood samples

White spruce (*Picea glauca,* Moench (Voss)) was selected for this experiment because it is susceptible to staining and its homogeneous properties make easy to cut thin veneers from it (Forest Products Laboratory, 1999; Harrington, 1988). Wood veneers measuring 18 mm (radial) x 25 mm (tangential) x 85 mm (longitudinal) were cut from blocks of white spruce as described in Chapter 4. Veneers were placed on glass plates and clamped at their ends as described in Chapter 4, and dried at room temperature for seven days. Each veneer was cut with a scissor to produce approximately 4 sections, each 20 mm in length. These sections were mixed together and randomly allocated to the different treatments. Pieces of veneer were oven dried (100 ± 5°C) for 24 hours and sterilized in an autoclave at 121°C and 103.4 kPa for 20 min. They were then placed in sterile Petri dishes and sealed until they were needed.

7.2.2.3. Preparation of solutions and impregnation of wood veneers

The melanin biosynthesis inhibitor (MBI) carpropamid and the fungicide quinoxyfen were used to treat wood sections. Fifty mL solutions of these chemicals in acetone (industrial grade) at concentrations of 3000 or 6000 ppm were prepared in 100 mL glass beakers. Five batches of 4 veneers sections were immersed in these solutions for 24 hours. Each batch represented a 'block'. Similarly, control veneers were impregnated with pure acetone (as above). Treated veneer sections were dried at room temperature for 3 days inside a laminar flux chamber that provided a clean environment and favored the evaporation of solvent. Sections were transferred onto 60 mm x 15 mm Petri dishes containing water-agar media (1 %). The water-agar medium was used to maintain moisture content and support fungal growth in the veneers when they were exposed to different light sources *in-vitro*. Veneer sections were placed on the center of the plates and allowed to re-hydrate for 3 hours before they were inoculated with fungi.

7.2.2.4. Inoculation of media with *A. pullulans* and exposure of treated wood sections to UV and visible light

A. pullulans [strain R2F32.2] was grown in 100 mm x 15 mm Petri dishes with 1% MEA (Difco) at room temperature, and sub-cultured in similar plates every two weeks to ensure that fresh material was available for the experiment. After a week of growth, spores in the plates were harvested, under sterile conditions, by flooding the plates with 3 mL of nano pure water. The spores produced by the fungi were loosened from mycelia with a sterile glass rod. The supernatants were collected in 15 mL falcon tubes and the concentration of

spores in these solutions was measured with a hemocytometer (as described in section 7.2.1.3) and adjusted to a concentration of 1 spore per µL. Veneer sections on the water-agar media were inoculated with 50 µL of spore solution (Figure 7.3). Solutions were evenly spread onto the sections with a glass rod. The plates were sealed using quartz glass disks as lids and parafilm (as described in section 7.2.1.3), and kept in a dark room for 24 hours.

Veneer sections were exposed to UV, visible or no light at 20°C ± 1°C at 65% ± 5% r.h. as described in section 7.2.1.4.

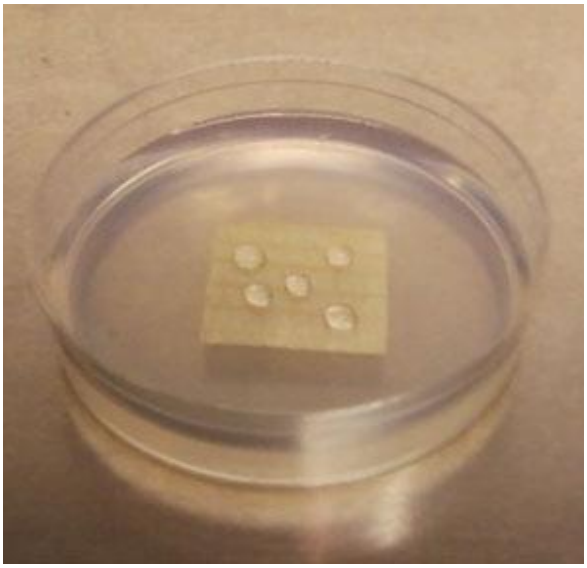


Figure 7.3: Inoculation of spruce veneers with 50 µL of spore solution (1 cell/µL)

7.2.2.5. Quantification of staining and color of treated and inoculated veneer sections exposed to UV or visible light

After 5 days of exposure, digital images of the plates without their lids, at a resolution of 300 dpi, were obtained using a desktop scanner (as described in section 7.2.1.4). Measurements of the staining of veneer sections (stained area) involved adjusting the tonal

range of images with Photoshop to help visualize fungal staining (Figure 7.4 a and c). Then, the pixels corresponding to fungal stain were visualized in black by adjusting the threshold level of images (Figure 7.4 b and d). Finally, the number of black pixels in the image's histogram was used to calculate the stained area of the veneer section as a percentage of the total number of pixels in the image.

The color of veneer sections was evaluated using the CIELab color system. Digital images (TIFF format) of the exposed samples (inoculated and without fungi) were loaded into Photoshop. Color measurements used the entire exposed surface contained in each image. Color was initially expressed using the RGB color system obtained using a color histogram (Figure 7.5a). The average RGB color was obtained and converted to the equivalent colors in the CIELab system in Photoshop (Figure 7.5b). Photoshop provides CIELab color using the standard scale of 0 to 100, for lightness, but redness-greenness and blueness-yellowness are expressed at 255 levels in scales ranging from -127 to 128. Therefore, redness-greenness and blueness-yellowness were transformed into the normal CIELab scale (-60 to +60) using the following equation (Papadakis et al., 2000):

$$a^* = [120 \times (a + 128) / 255] - 60$$

Where:

a^* = CIELab color from -60 to + 60

a = CIELab color provided by Photoshop

The CIElab parameters were used to calculate the color difference between treated veneers and controls. Color differences were calculated using following equation (ASTM, 1993):

$$\Delta E = [(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2]^{1/2}$$

Where:

ΔE = color difference

L_1, a_1 and b_1 = CIElab color components of control veneer

L_2, a_2 and b_2 = CIElab color components of treated veneer

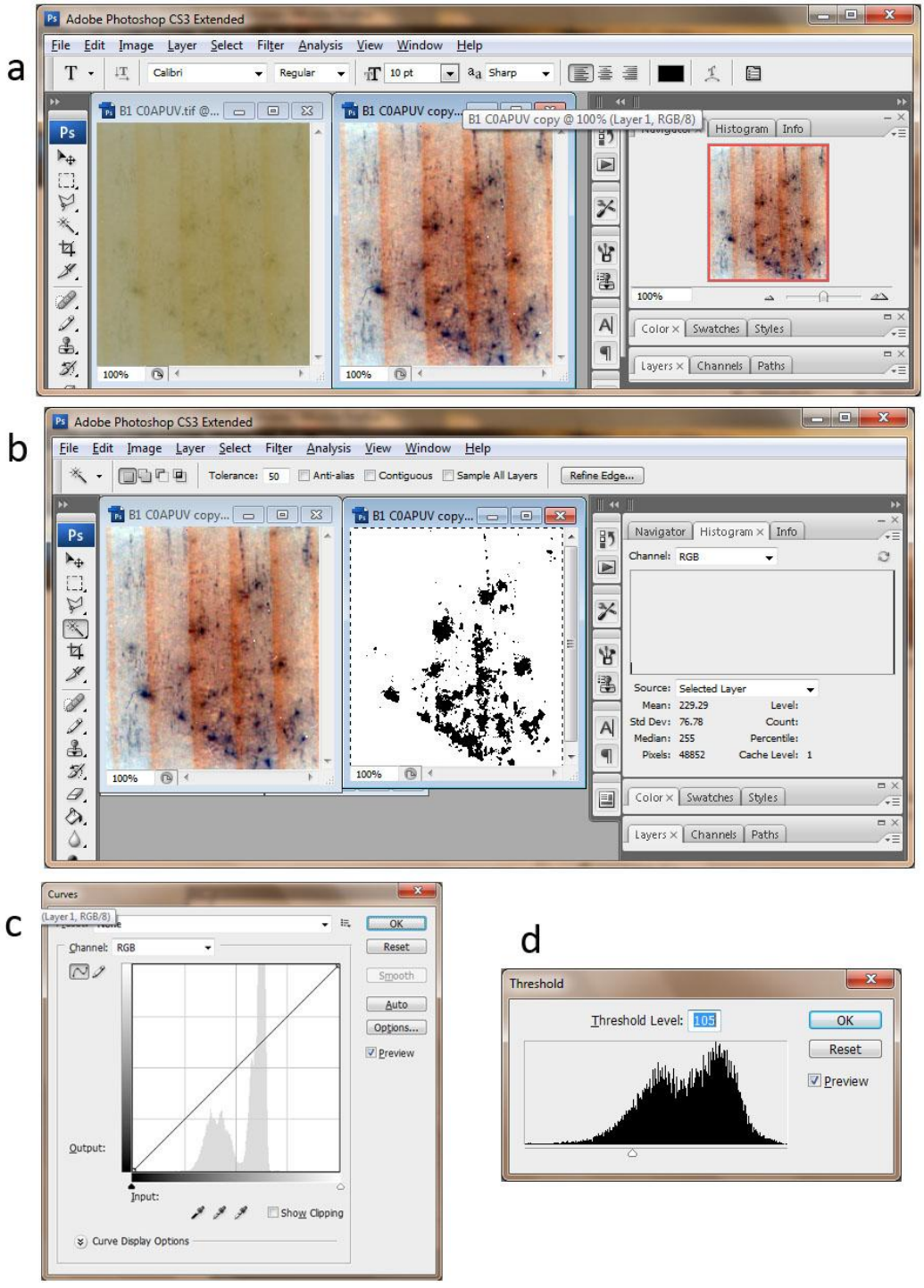


Figure 7.4: Color measurement of stained area on spruce veneer sections inoculated with *A. pullulans* and exposed for 5 days under UV or visible light: (a) adjustment of tonal range; (b) stained pixels selected using threshold adjustment; (c) 'curves' function of the software used to adjust the tonal range; and (d) threshold adjustment

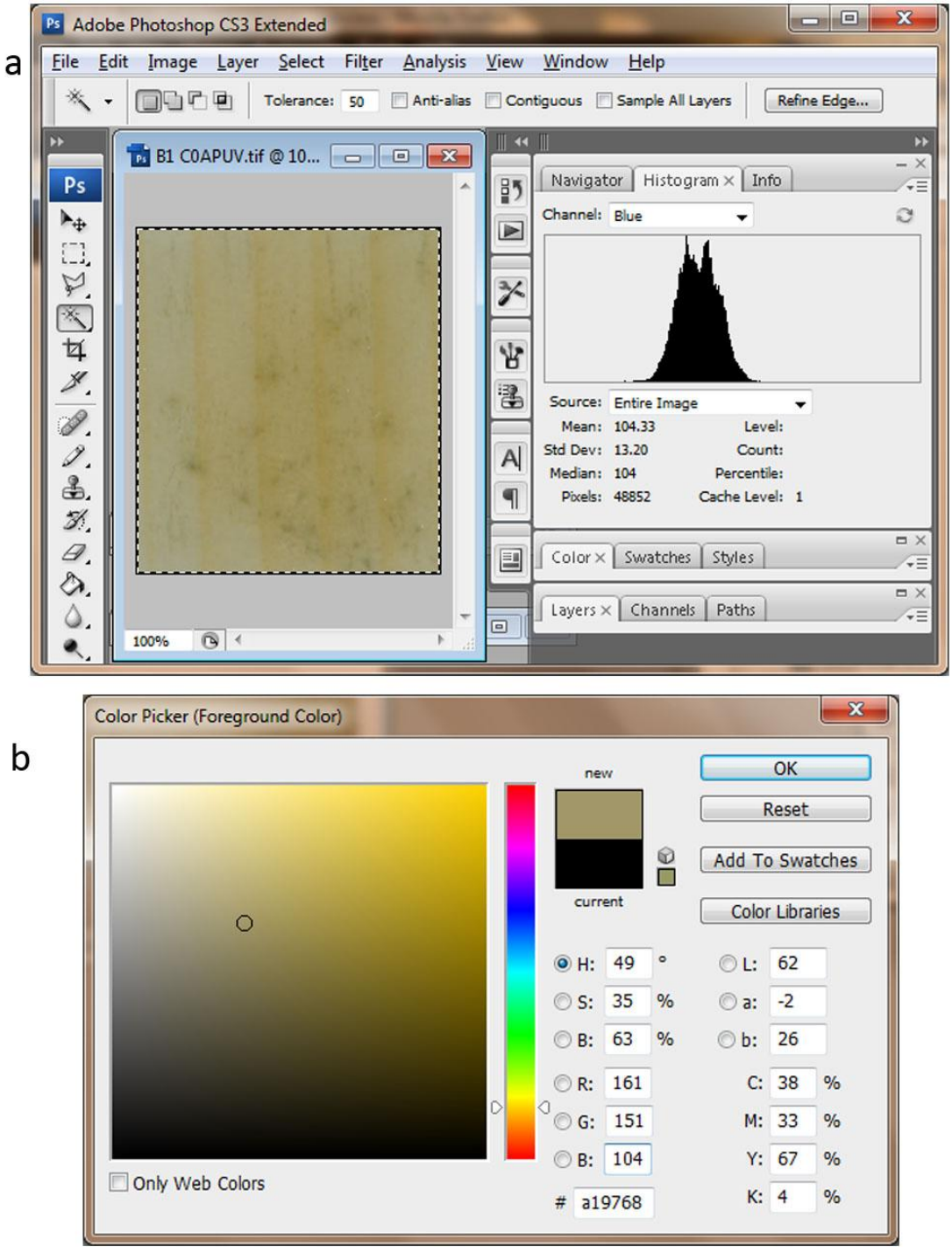


Figure 7.5: Color measurement of stained spruce veneers inoculated with *A. pullulans* and exposed for 5 days under UV or visible light: (a) Use of histogram in Photoshop to acquire information about the RGB color of the image; and (b) color picker tool for transformation of RGB into CIELab color

7.2.2.6. Microscopy

Growth of fungi on the surface of wood veneer sections was confirmed by observing the sections under a stereomicroscope (Wild Makroskop M420; Wild Leitz Canada, Willowdale, Ontario). Images of the wood surfaces were acquired using a digital camera (Nikon Coolpix S8100. Nikon Corp. Japan).

7.3. Results

7.3.1. MBIs tested in malt extract agar

The results of analysis of variance of the effect of different melanin biosynthesis inhibitors on number of fungal colonies that grew on agar plates are shown in Table 7.3. There were significant effects (p-value < 0.001) of exposure (E), chemicals (Ch) and fungi (F) on the number of colonies in plates. The interactions of E x Ch and E x F were also significant. Main effects were included in the results to facilitate the interpretation of the results.

Table 7.3: Significant effect of, and interactions between exposure to light, chemical, and fungal species on the number of colonies growing on agar plates

Colonies after exposure	
Source of variation	p-value
Exposure (E)	<.001
Chemical (Ch)	<.001
Fungi (F)	<.001
ExCh	<.001
ExF	<.001
ChxF	0.092
ExChxF	0.195

The number of fungal colonies growing in the plates was significantly lower when the plates were exposed to UV radiation compared to plates exposed to visible light (Figure 7.6).

Cerulenin, carpropamid and quinoxyfen also reduced the number of colonies growing on the media compared to the control, whereas tricyclazole had no such effect. However, there were no significant differences in colony numbers on plates containing quinoxyfen and carpropamid, or between plates containing carpropamid and cerulenin (Figure 7.7). The number of colonies of *C. cladosporioides* growing on plates was significantly higher than that of *A. pullulans* (Figure 7.8). A significant interaction between exposure and chemical occurred because the number of fungal colonies growing on plates containing MBIs was significantly lower when the plates were exposed under UV light compared to those on plates exposed to visible light, except for plates containing tricyclazole (Figure 7.9). The interaction between exposure and fungi occurred because the number of colonies of *A. pullulans* on plates exposed to either UV or visible light was similar whereas the number of colonies of *C. cladosporioides* was significantly lower on plates exposed to UV light compared to those exposed to visible light (Figure 7.10). Plates exposed to UV and visible light and inoculated with *A. pullulans* and *C. cladosporioides* are shown in Figure 7.11 and Figure 7.12, respectively.

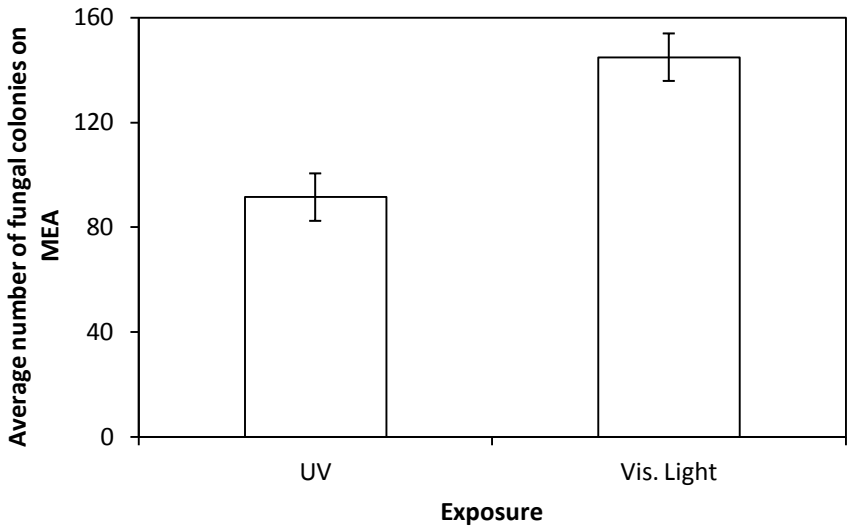


Figure 7.6: Average number of fungal colonies growing on malt extract agar in Petri dishes exposed to either UV or visible light. Results averaged across plates containing different MBIs (plus control) and inoculated with *A. pullulans* or *C.* cladosporioides. Error bars correspond to ±SED

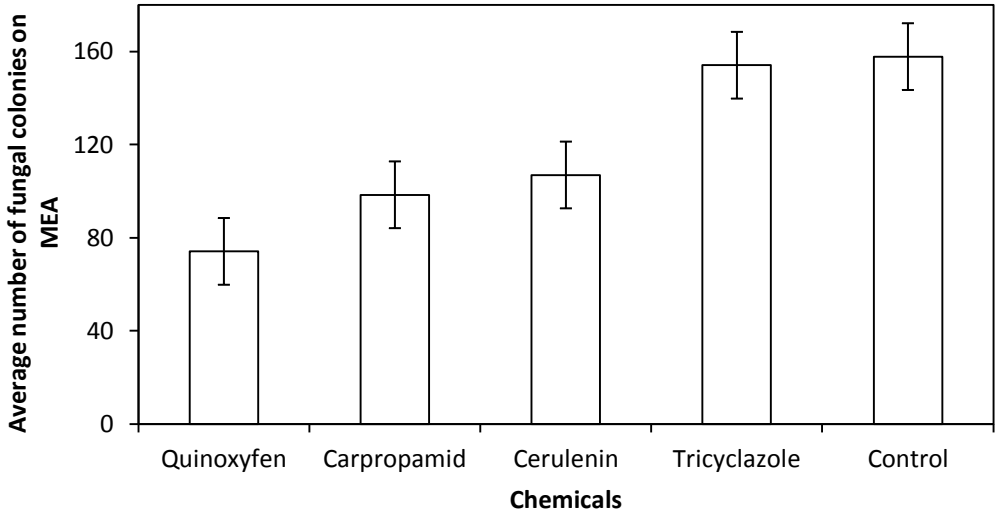


Figure 7.7: Average number of fungal colonies growing on malt extract agar in Petri dishes containing different MBIs, the fungicide quinoxyfen, or acetone (as control). Results averaged across plates exposed to UV and visible light and inoculated with *A. pullulans* or *C. cladosporioides*. Error bars correspond to ±SED

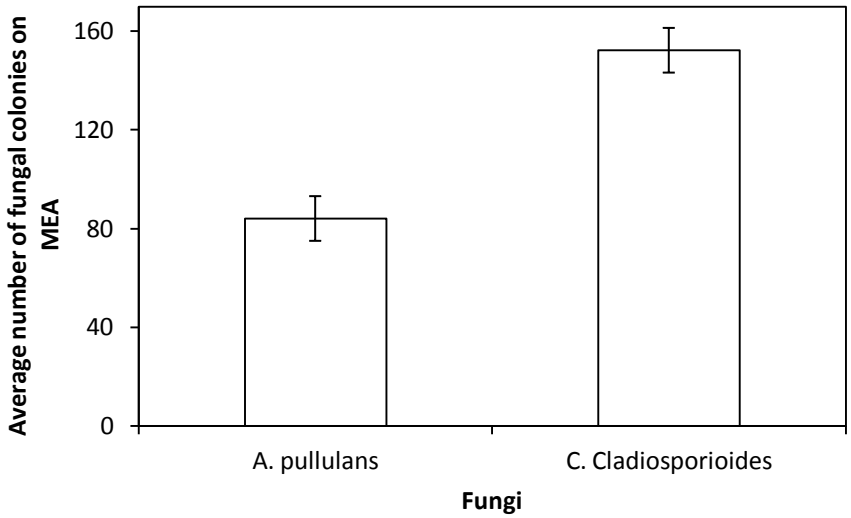


Figure 7.8: Average number of colonies of *A. pullulans* and *C. cladosporioides* growing on malt extract agar in Petri dishes. Results averaged across plates containing different chemicals and exposed to UV or visible light. Error bars correspond to ±SED

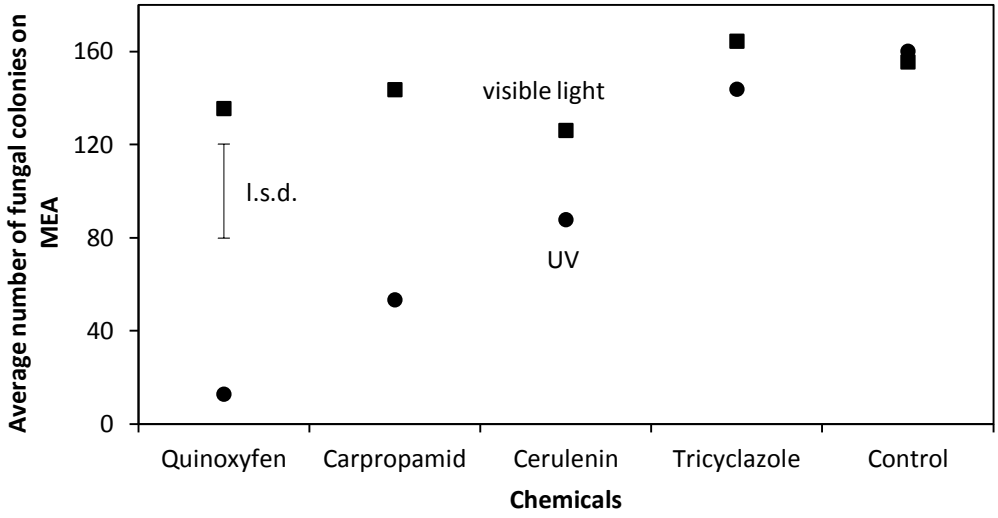


Figure 7.9: Average number of fungal colonies growing on malt extract agar in Petri dishes containing the MBIs carpropamid, cerulenin and tricyclazole, the fungicide quinoxyfen, and acetone (control plates); and exposed to UV or visible light. Results averaged across plates inoculated with *A. pullulans* or *C. cladosporioides.* L.s.d. bar for comparison of means

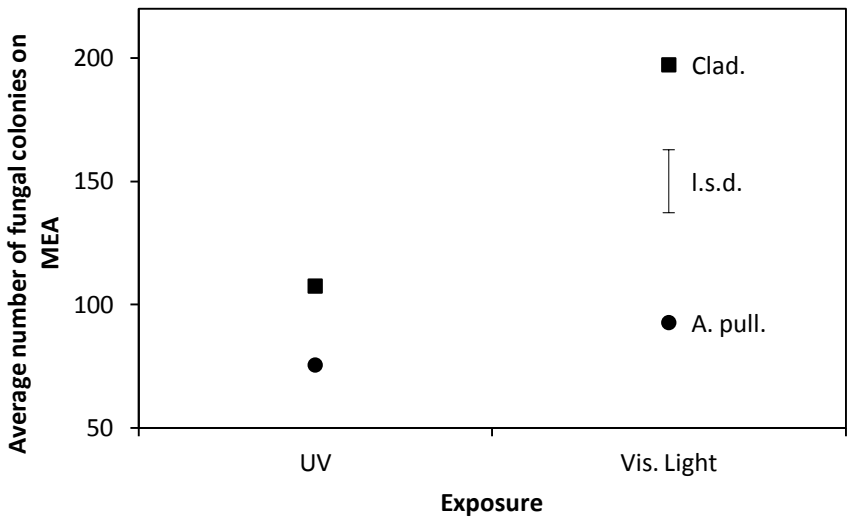


Figure 7.10: Average number of fungal colonies growing on malt extract agar in Petri dishes exposed to UV or visible light, and inoculated with either *A. pullulans* or *C. cladosporioides.* Results averaged across plates containing melanin biosynthesis inhibitors, quinoxyfen or acetone. L.s.d. bar for comparison of means

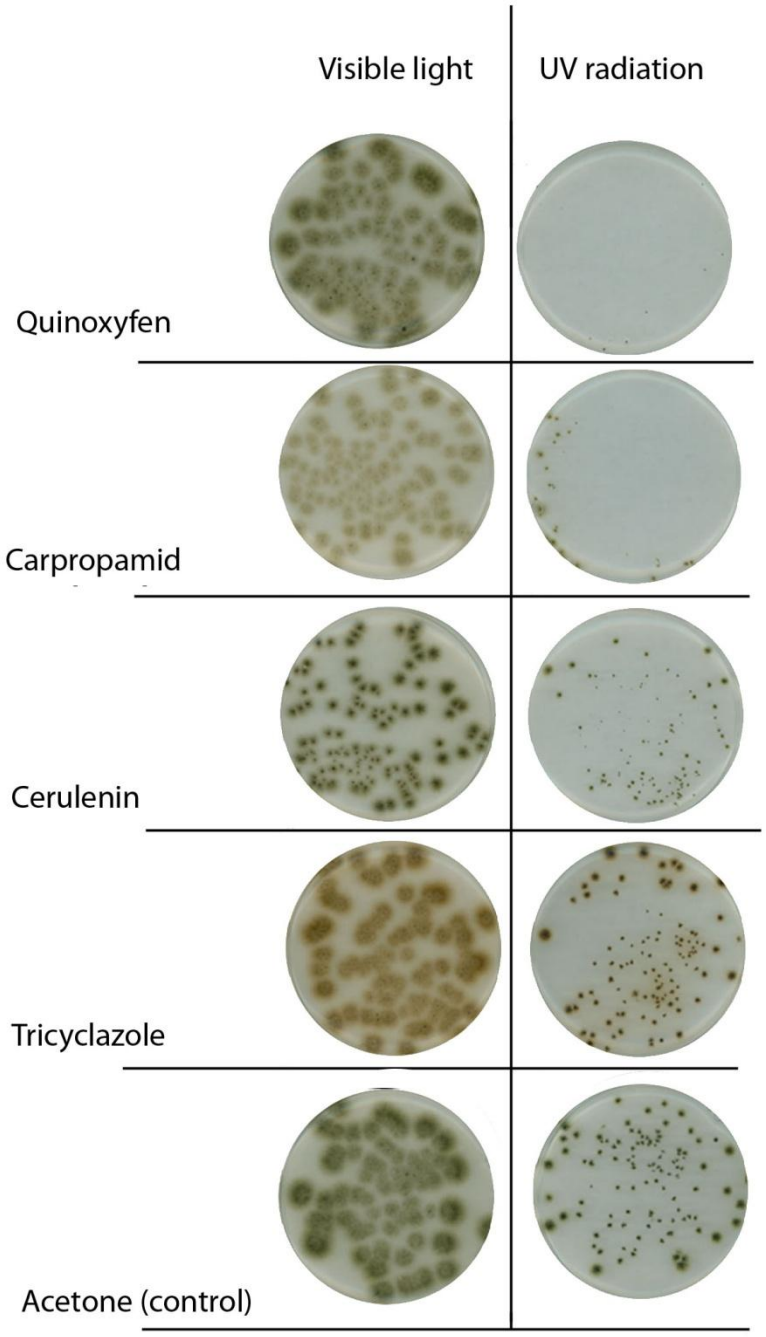


Figure 7.11: Effects of chemical types (MBIs, fungicide [quinoxyfen] or acetone [control]) and exposure to UV radiation or visible light on growth of *A. pullulans* on artificial media. Concentration of MBIs and quinoxyfen = 10 ppm; acetone in control plates was added at a level that was the same as that used to dissolve the MBIs

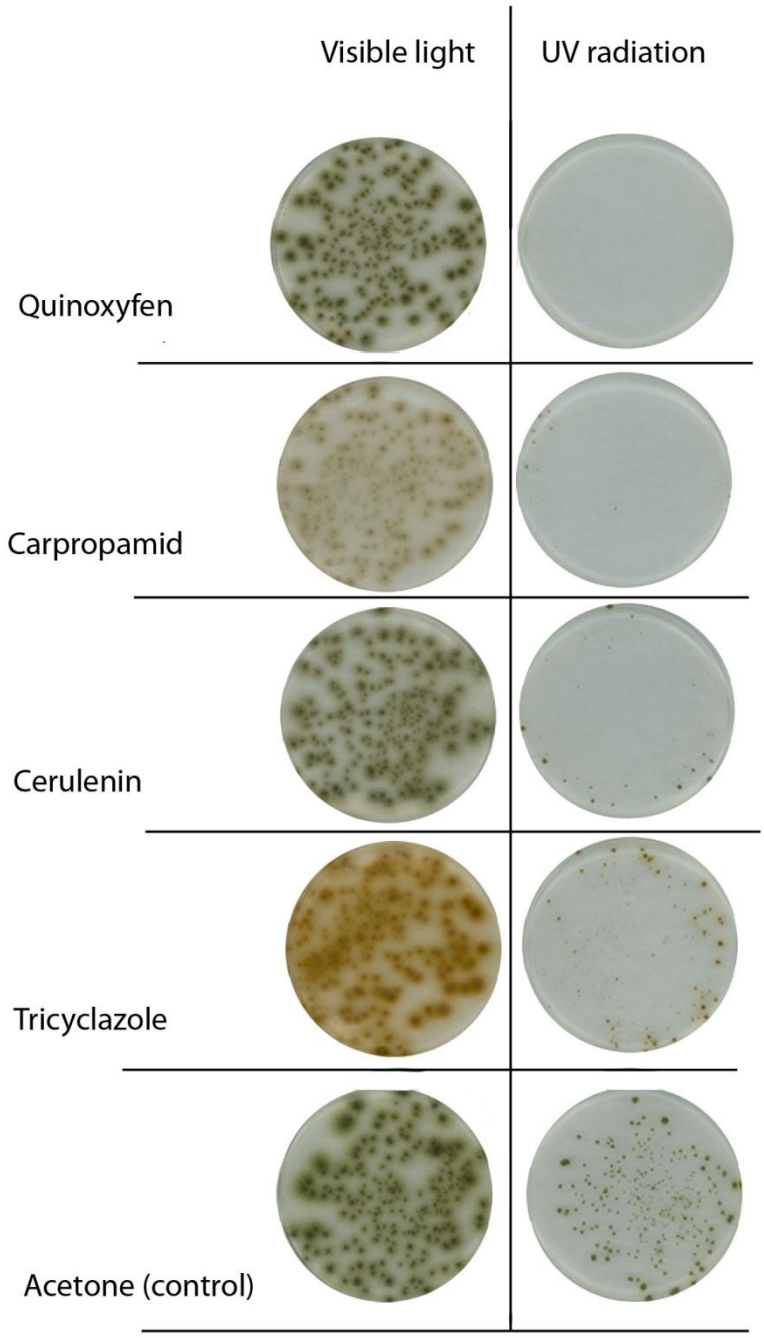


Figure 7.12: Effects of chemical types (MBIs, fungicide [quinoxyfen] or acetone [control]) and exposure to UV radiation or visible light on growth of *C. cladosporioides* on artificial media. Concentration of MBIs and quinoxyfen = 10 ppm; acetone in control plates was added at a level that was the same as that used to dissolve the MBIs

7.3.2. Effects of MBIs and UV radiation on fungal staining and color of wood

Table 7.4 summarizes the results of the analyses of variance of the effect of fixed factors (exposure to light, chemical treatment and concentration and their interactions) on fungal staining and color of veneer sections (both inoculated with fungi and not inoculated controls).

Table 7.4: Significant effect of, and interaction between exposure to light, chemical treatments and concentration on stained area and color change (ΔE) of fungal and water inoculated spruce veneers surfaces, after 5 days of exposure. Stained area of veneers was analyzed as ratio of stained area of impregnated veneers over control veneers. Natural logarithm (LN) transformation was used to fulfill assumptions of analysis of variance

P-value				
Source of variation	LN [1 + stained area ratio]	ΔE inoculated veneers	ΔE no fungi veneers	ΔE inoculated veneers v. no fungi veneers
Exposure (E)	0.398	0.16	0.583	0.259
Chemical (Ch)	<.001	<0.001	0.892	<0.001
Concentration (C)	0.492	0.268	0.598	<0.001
E x Ch	0.106	0.506	0.865	0.46
E x C	0.56	0.35	0.735	0.12
Ch x C	0.17	0.691	0.808	<0.001
E x Ch x C	0.666	0.913	0.92	0.57

7.3.2.1. Effect on fungal staining

Fungal stains in veneer sections exposed to either UV or visible light are shown in Figure 7.13 and Figure 7.14, respectively. Staining of veneer sections was significantly affected ($p < 0.001$) by the type of chemical impregnated into the sections, but not by the other factors. However, the interaction of chemical and exposure approached statistical significance ($p = 0.106$) (Table 7.4), although reduction of staining due to such an interaction was not apparent visually (Figure 7.13 and Figure 7.14). Veneer sections impregnated with carpropamid showed significantly less staining than those impregnated with quinoxyfen

(Figure 7.15). The fungi in the veneer sections had melanized hyphae, but in sections exposed to UV radiation hyphae were darker than those exposed to visible light. Light microscopy confirmed presence of *A. pullulans* hyphae in veneer sections and this observation discounts the presence of contamination due to other microorganisms (Figure 7.16 and Figure 7.17). The light contrast used to obtain the micrographs helped to see the presence of highly melanized hyphae in veneer sections exposed to UV radiation.

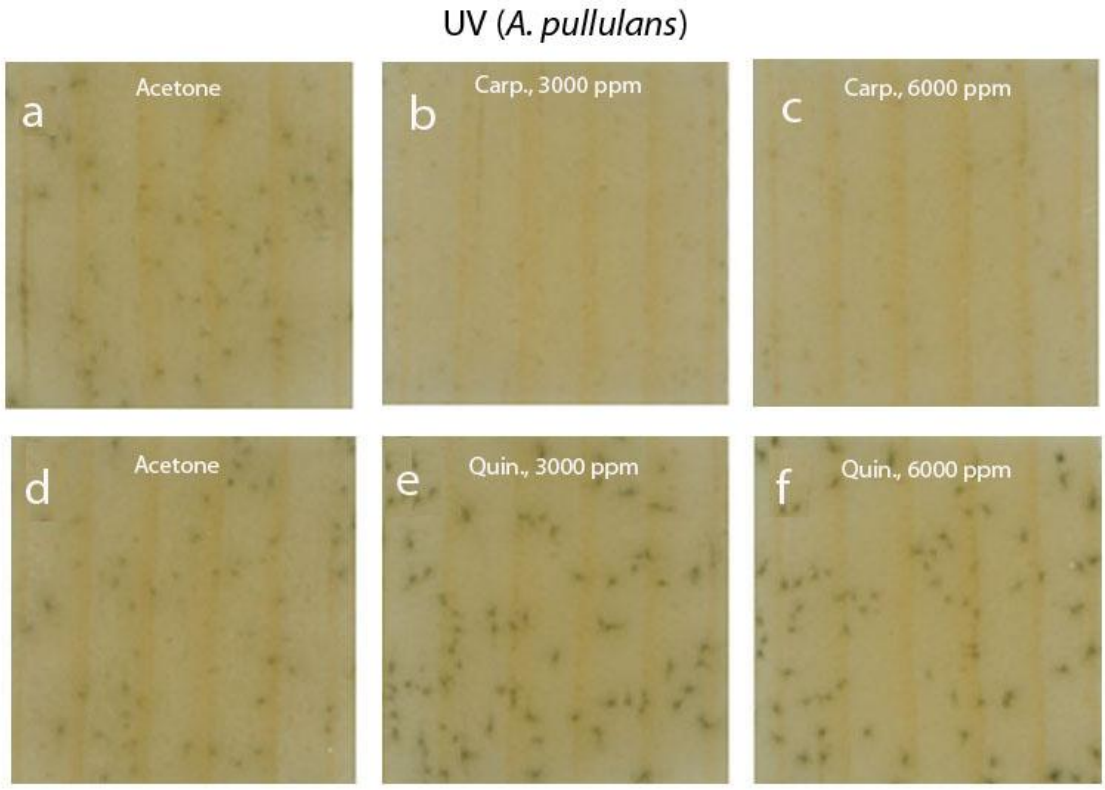


Figure 7.13: Appearance of spruce veneer sections impregnated with carpropamid or quinoxyfen, inoculated with spores of *A. pullulans* and exposed for 5 days to UV radiation: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm. Veneers impregnated with carpropamid stained significantly less than the control. In contrast, impregnation with quinoxyfen appeared to encourage fungal colonization

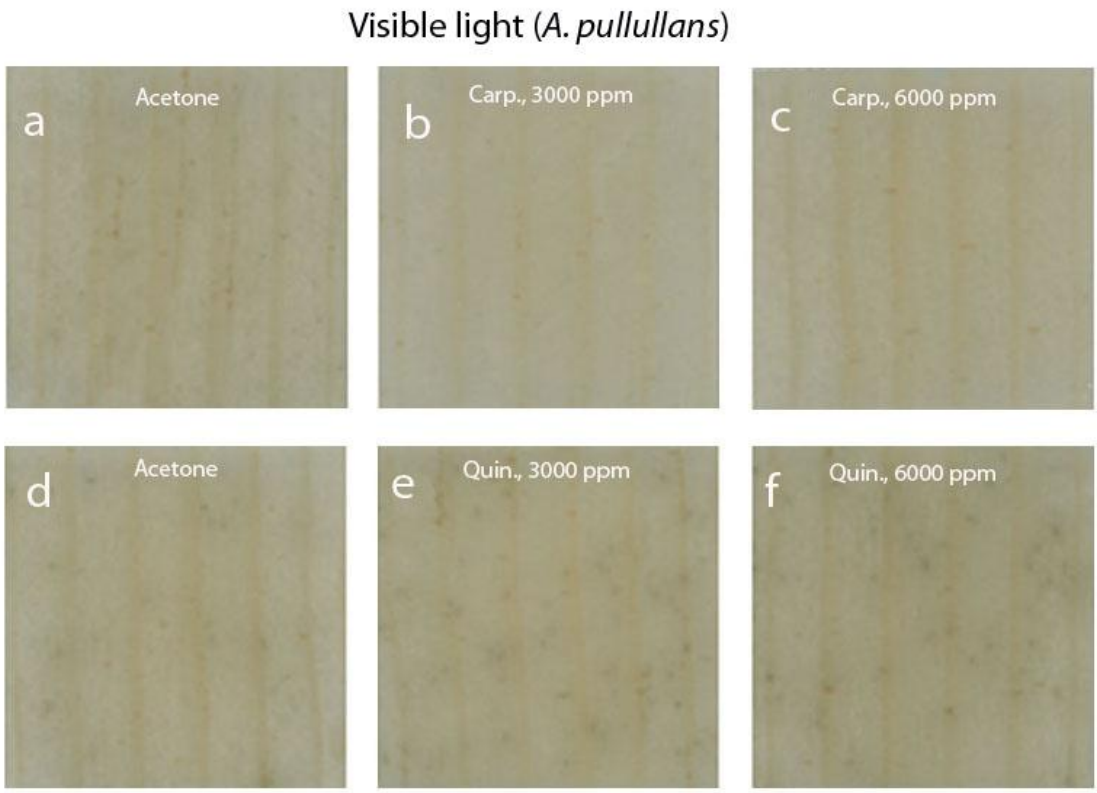


Figure 7.14: Appearance of spruce veneer sections impregnated with carpropamid or quinoxyfen, inoculated with spores of *A. pullulans* and exposed for 5 days to visible light: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm. Veneers impregnated with carpropamid stained less than the control. The presence of quinoxyfen appeared to encourage melanization of *A. pullulans*

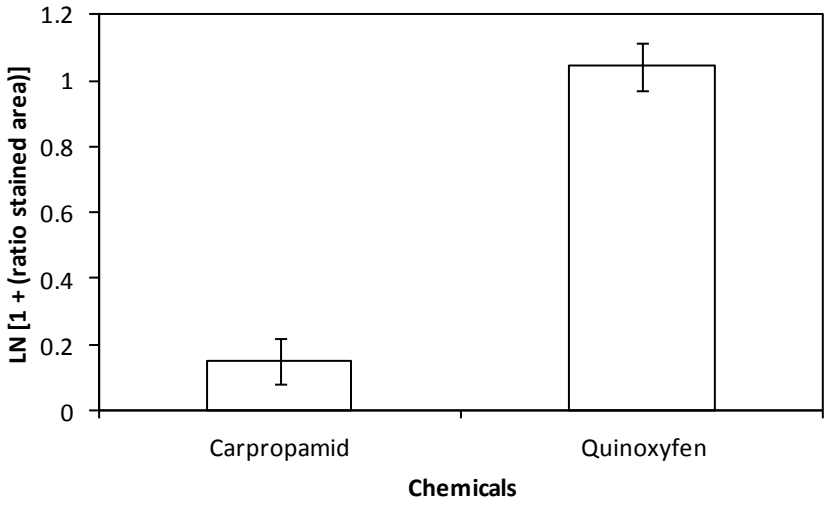


Figure 7.15: Effect of chemical treatment on staining (evaluated as LN (1 + Stained area ratio)) of spruce veneers. Results averaged across veneer sections treated with different concentrations of chemicals and exposed to UV or visible light. Error bars correspond to ±SED

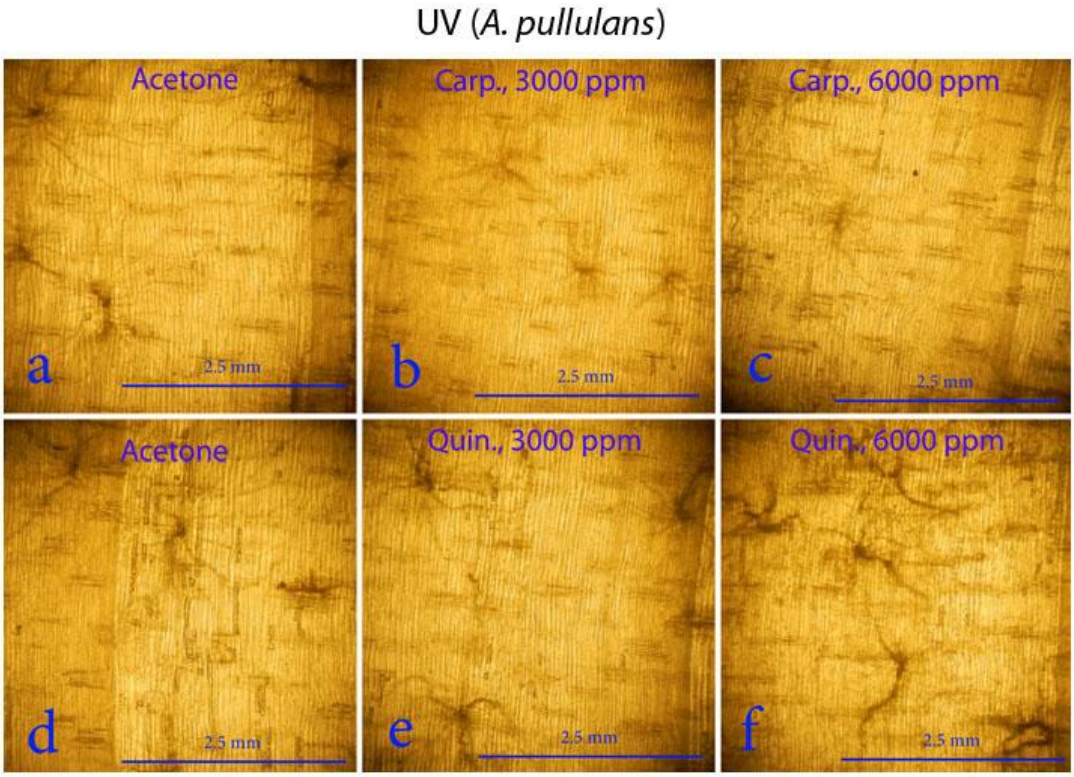


Figure 7.16: Magnified appearance of spruce veneer sections impregnated with carpropamid or quinoxyfen, inoculated with spores of *A. pullulans* and exposed for 5 days to UV radiation: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm. Greater staining of sections treated with quinoxyfen was observed

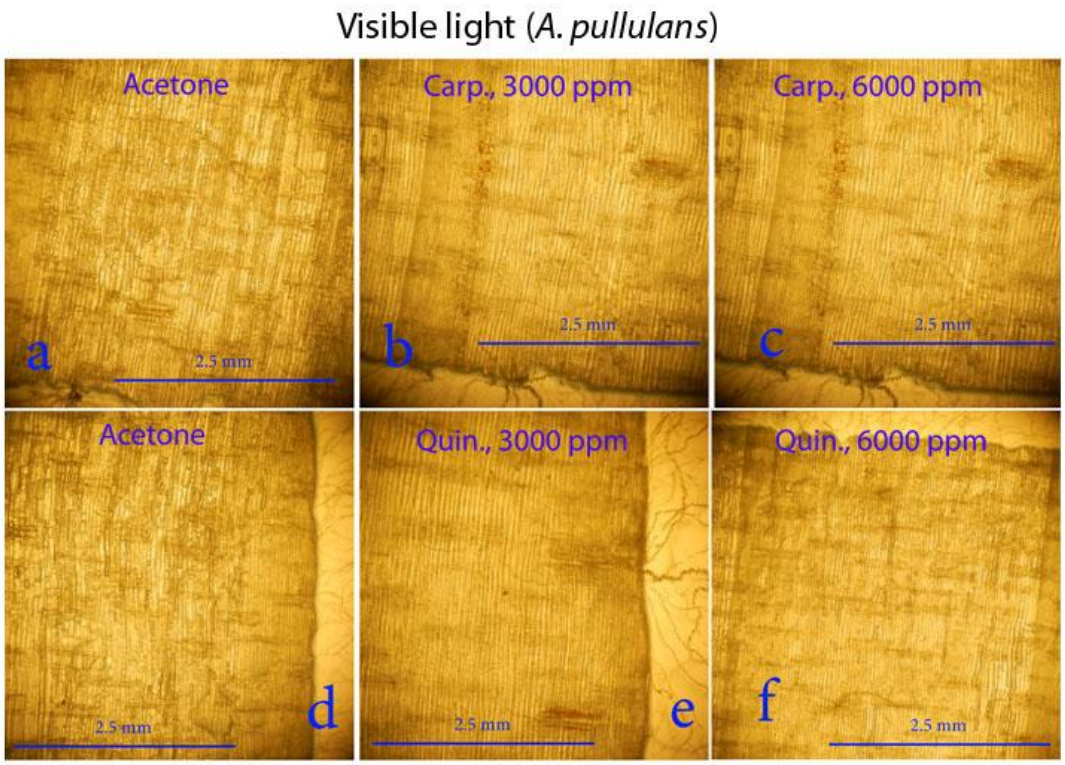


Figure 7.17: Magnified appearance of spruce veneer sections impregnated with carpropamid or quinoxyfen, inoculated with spores of *A. pullulans* and exposed for 5 days to visible light: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm. Less staining of wood samples was observed compared to sections exposed to UV radiation

7.3.2.2. Effect on color; comparison of stained wood surfaces

Determination of color difference (ΔE) between sections inoculated with fungi and untreated stained sections made it possible to compare how effective carpropamid and quinoxyfen were against fungal staining. The color difference of veneer sections impregnated with carpropamid and quinoxyfen (inoculated with spores of *A. pullulans*) against control veneers (impregnated with acetone and inoculated with spores of *A. pullulans*) was significantly affected ($p < 0.001$) by chemical treatment (Table 7.4). ΔE was significantly higher in veneers sections impregnated with carpropamid, indicating that the

color of such sections was different to that of the heavily stained control veneer sections (Figure 7.18).

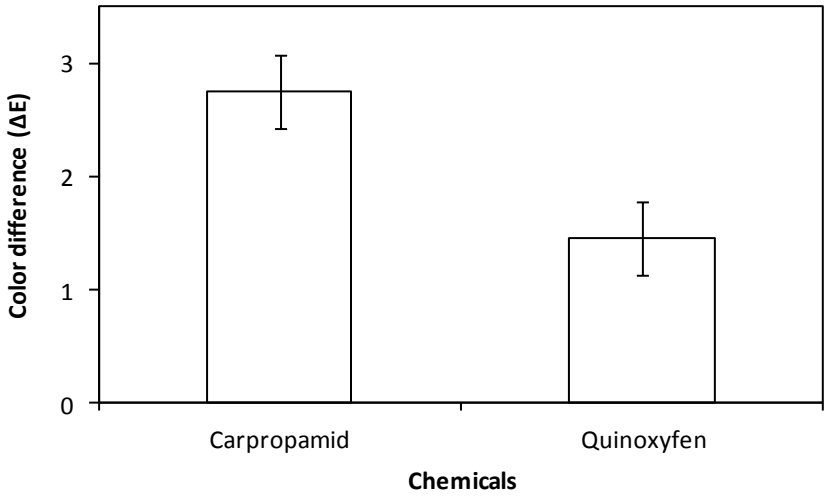


Figure 7.18: Effects of chemical treatment on color differences (ΔE) of spruce veneers treated with carpropamid or quinoxyfen; inoculated with spores of *A. pullulans* v. spruce control veneers (impregnated with acetone) inoculated with *A. pullulans*, after 5 days of exposure to UV and visible light. Results averaged across veneer sections treated with different concentrations of chemical and exposed to UV or visible light. Error bars correspond to ±SED

7.3.2.3. Effect on color of wood veneers in comparison to unstained wood surfaces

Determination of color differences (ΔE) between veneer sections inoculated with *A. pullulans* and sections that were not inoculated with fungus provides another measure of the effectiveness of carpropamid and quinoxyfen at reducing fungal staining. ΔE of inoculated veneer sections v. not inoculated sections was significantly affected ($p < 0.001$) by chemical treatment (Table 7.4). Sections treated with carpropamid had significantly lower ΔE than veneers impregnated with quinoxyfen (Figure 7.19), indicating that color of veneer sections treated with carpropamid was similar to that of sound veneers. ΔE was also affected by the concentration of chemicals ($p < 0.001$), as veneer sections treated with

carpropamid and quinoxyfen at 3000 and 6000 ppm had a significantly lower ΔE than control veneers impregnated with acetone (Figure 7.20). However, the interaction between chemicals and concentrations was also significant ($p < 0.001$). This interaction occurred because as the concentration of carpropamid increased the color differences of veneer sections decreased, whereas no such effect of concentration was seen in sections treated with quinoxyfen (Figure 7.21).

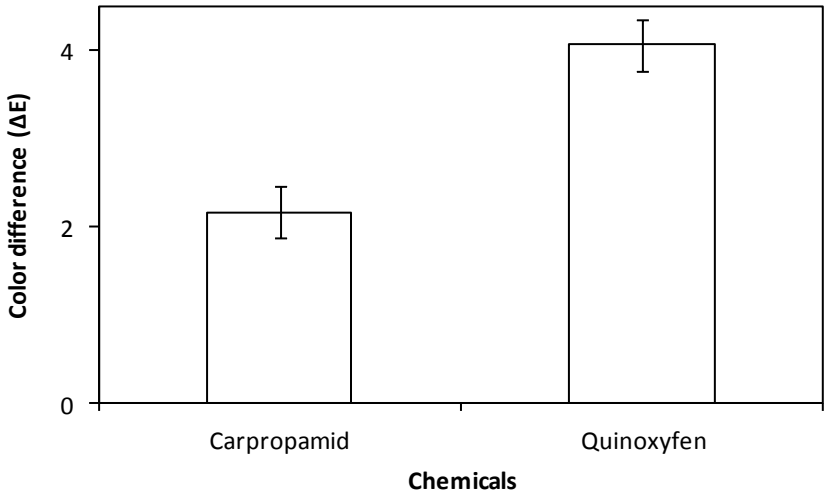


Figure 7.19: Effects of chemical treatment on color differences (ΔE) of spruce veneers impregnated with carpropamid or quinoxyfen inoculated with spores of *A. pullulans* v. spruce veneers impregnated with carpropamid or quinoxyfen and not inoculated with the fungus, after 5 days of exposure to either UV or visible light. Results averaged across veneer sections treated with different concentrations of chemicals and exposed to UV or visible light. Error bars correspond to ±SED

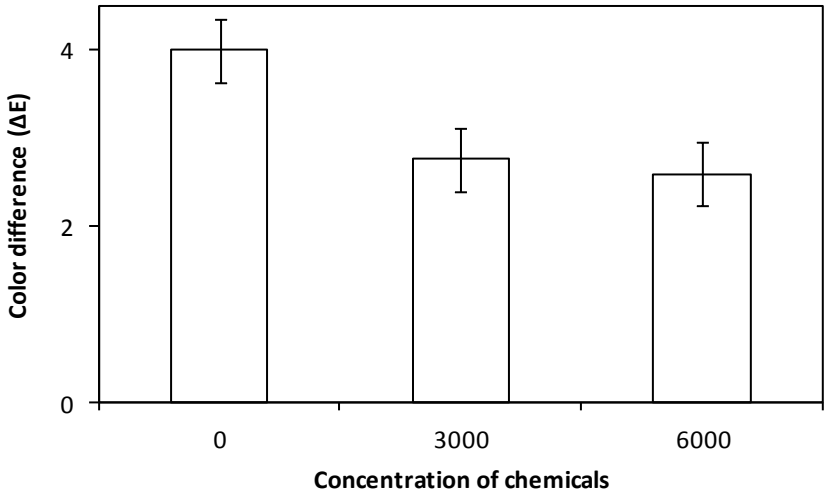


Figure 7.20: Effects of chemical treatment on color differences (ΔE) of spruce veneers impregnated with either carpropamid or quinoxyfen and inoculated with spores of *A. pullulans* v. spruce veneers sections impregnated with either carpropamid or quinoxyfen and not inoculated with the fungus, after 5 days of exposure to either UV or visible light. Results averaged across veneer sections treated with different chemicals and exposed to UV or visible light. Error bars correspond to ±SED

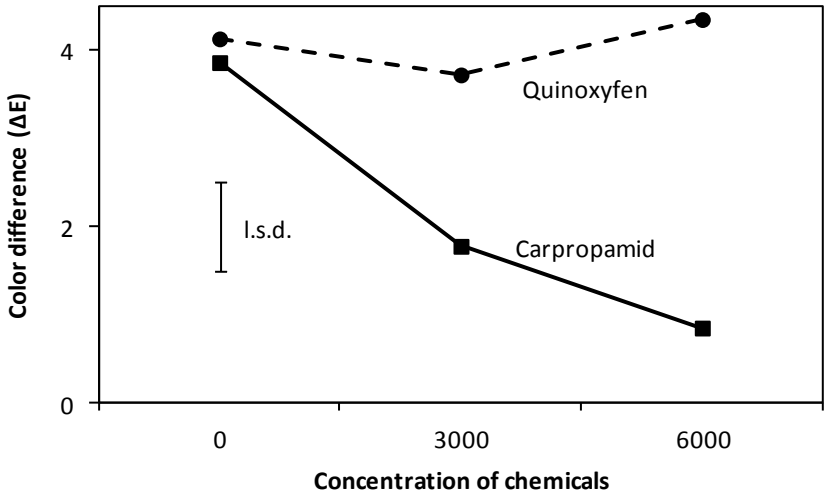


Figure 7.21: Effects of chemical treatments and concentrations on color differences (ΔE) of spruce veneers impregnated with carpropamid or quinoxyfen and inoculated with spores of *A. pullulans* v. spruce veneers impregnated with carpropamid or quinoxyfen and not inoculated with the fungus, after 5 days of exposure to either UV or visible light. Results averaged across veneer sections exposed to UV or visible light. L.s.d. bar is shown for comparison of means

7.3.2.4. Effect of the treatment on the natural color of wood

There was no significant effect of experimental factors on the staining and color of uninoculated veneer sections. Veneers sections that were not inoculated with spores of *A. pullulans* but treated with carpropamid and quinoxyfen were 'cleaner' (less discolored) after exposure to UV radiation or visible light, and showed no color differences in comparison with control veneers impregnated with acetone (Figure 7.22 and Figure 7.23). As expected veneer surfaces 'yellowed' due to exposure to UV radiation. Determination of these color differences was relevant to verify whether the chemical treatments had an effect on the natural color of wood surfaces. In addition, light microscopy confirmed that these veneer sections were not colonized by *A. pullulans* (Figure 7.24 and Figure 7.25).

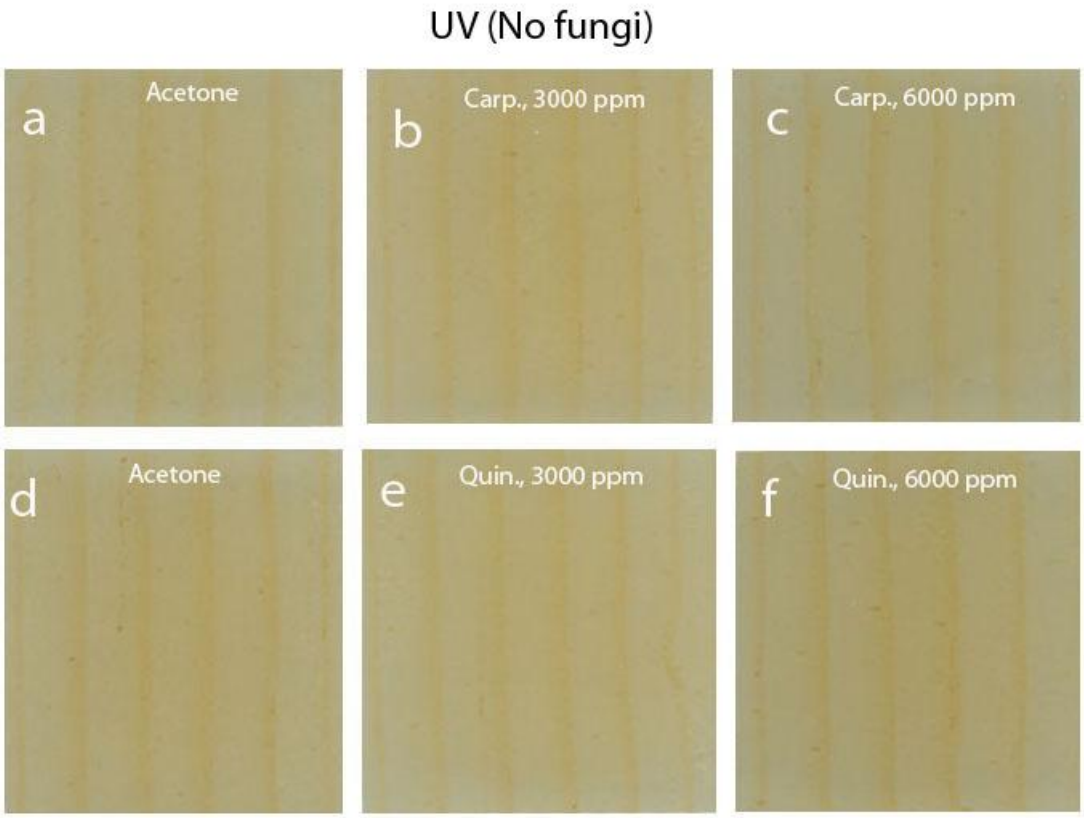


Figure 7.22: Appearance of spruce control (not inoculated) veneer sections impregnated with carpropamid or quinoxyfen and exposed to UV radiation for 5 days: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm

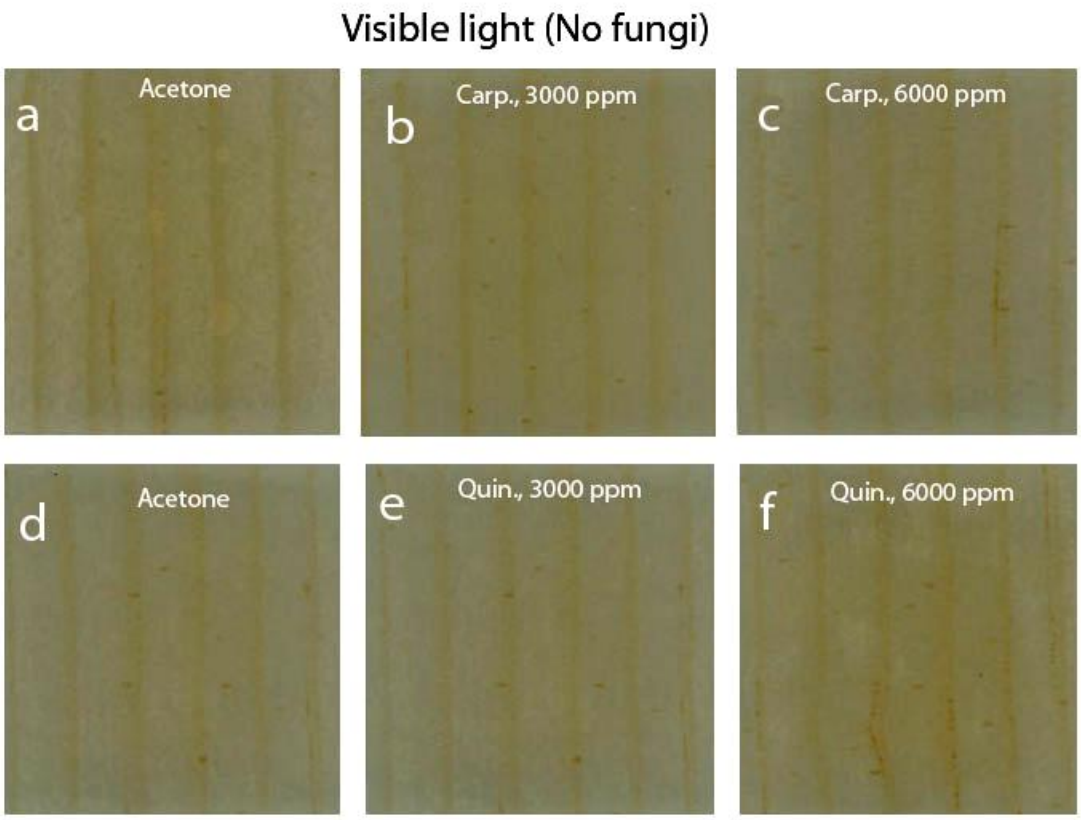


Figure 7.23: Appearance of spruce veneer control (not inoculated) sections not inoculated and impregnated with carpropamid or quinoxyfen and exposed to visible light for 5 days: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm

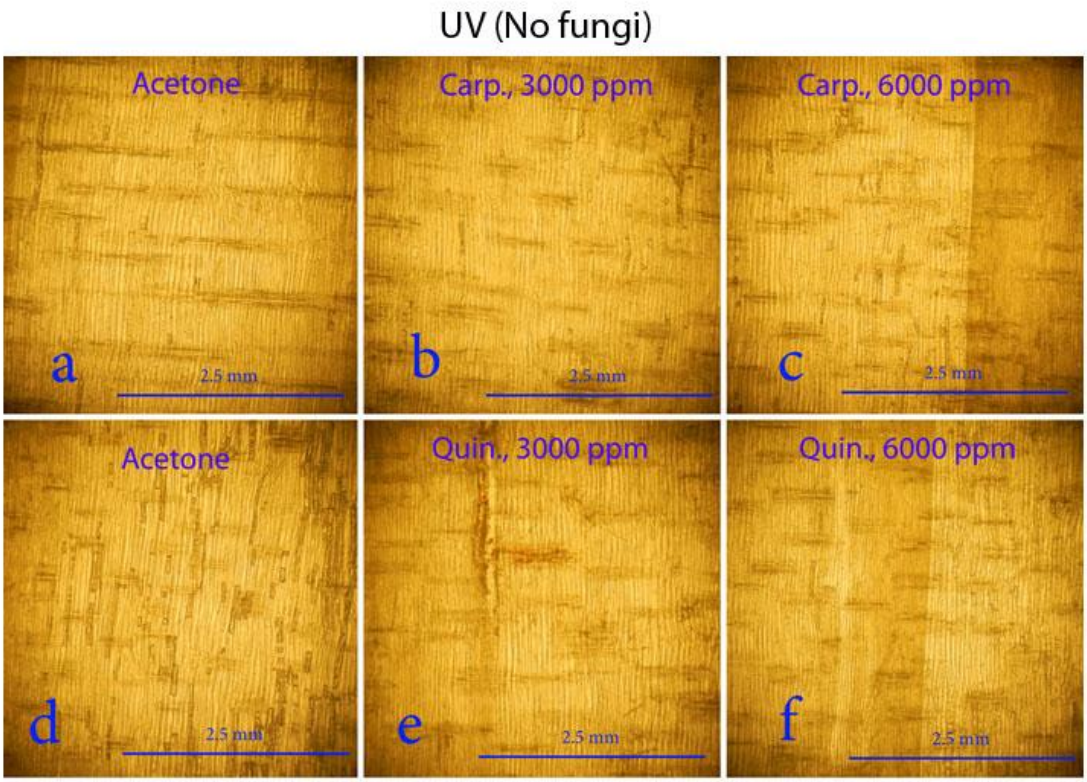


Figure 7.24: Magnified appearance of spruce veneer control (not inoculated) sections impregnated with carpropamid or quinoxyfen and exposed to UV radiation for 5 days: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (f) veneer impregnated with quinoxyfen at 6000 ppm. Veneers were not stained by *A. pullulans*, as expected

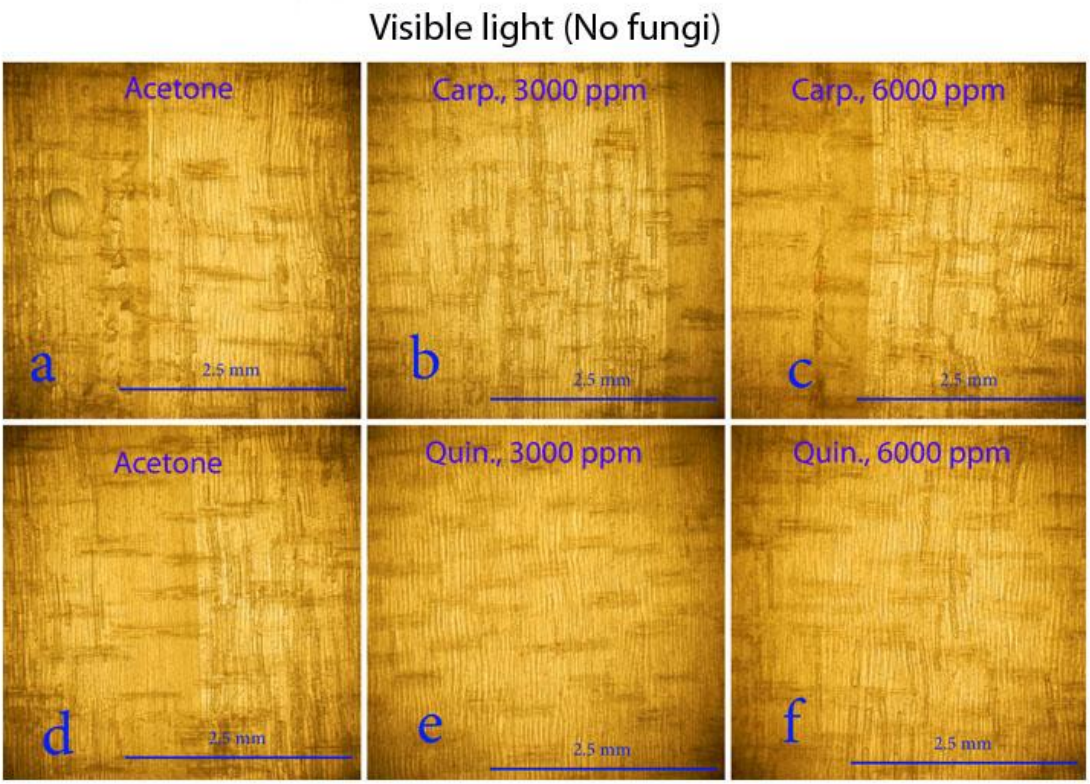


Figure 7.25: Magnified appearance of spruce veneer control (not inoculated) sections impregnated with carpropamid or quinoxyfen and exposed to visible light for 5 days: (a) carpropamid control veneer (impregnated with acetone); (b) veneer impregnated with carpropamid at 3000 ppm; (c) veneer impregnated with carpropamid at 6000 ppm; (d) quinoxyfen control veneer (impregnated with acetone); (e) veneer impregnated with quinoxyfen at 3000 ppm; and (c) veneer impregnated with quinoxyfen at 6000 ppm. Veneers were not stained by *A. pullulans*, as expected

7.4. Discussion

My results support the hypothesis that melanin biosynthesis inhibitors (MBIs) can inhibit the germination of spores, and the filamentous growth of two highly melanized fungi (*A. pullulans* and *C. cladosporioides*). Inhibition of growth and pigmentation of different blue-sapstaining fungi was reported by Fleet and Breuil (2002). They showed that both the growth and melanin biosynthesis pathway of such fungi were affected by the melanin biosynthesis inhibitors (MBIs) cerulenin, carpropamid and tricyclazole. My hypothesis that melanin-inhibited-fungi would be more susceptible to UV radiation was confirmed by my first experiment with *A. pullulans* and *C. cladosporioides* grown on MEA supplemented with the different MBIs and exposed to UV radiation. Melanin confers protection against different environmental factors, including UV radiation (Fogarty and Tobin 1996; Butler and Day 1998; Henson et al. 1999). Inhibition of enzymes in the dihydroxynaphthalene (DHN) melanin biosynthetic pathway by MBIs evidently decreased the tolerance of *A. pullulans* and *C. cladosporioides* to artificial UV radiation (340 nm). Differences in the effectiveness of the MBIs at inhibiting growth of fungi on plates exposed to UV light may be related to the different modes of action of cerulenin, tricyclazole and carpropamid, and the amount and nature of the intermediate products generated from the inhibition of steps in the DHN melanin biosynthetic pathway. For example, carpropamid is an inhibitor of scytalone dehydratase, an enzyme responsible for converting scytalone to 1,3,8 – trihydroxynaphthalene by water elimination. The compound also inhibits a second dehydration step in the conversion of vermelone to 1,8-DHN. Cultures supplemented with carpropamid show accumulation of scytalone, as reported by Tsuji et al. (1997) for the

fungus *Colletotrichum lagenarium*. Tricyclazole inhibits two reductase enzymes (1,3,8-trihydroxynpahthalene reductase and 1,3,6,8-tetrahydroxynaphthalene reductase) which control the transformation of 1,3,6,8- tetrahydroxynaphthalene into scytalone and 1,3,8-trihydroxynpahthalene into vermelone (Wheeler and Greenblatt 1988). There is evidence indicating that flaviolin and 2-hydroxyjuglone are the intermediate melanin products produced by the action of tricyclazole in DHN melanin-producing fungi. Accumulation of flaviolin and 2-hydroxyjuglone has been reported by several authors for cultures of *W. dermatitidis*, *H. werneckii*, *P. triangularis*, and *T. salinum* supplemented with tricyclazole (Wheeler and Stipanovic 1985; Kogej et al. 2004). The intermediate melanin products generated by the action of carpropamid and tricyclazole have different photo-chromatic properties that might explain the differences in their effectiveness at inhibiting germination of spores of *A. pullulans* and *C. cladosporioides* exposed to UV radiation. For example, intermediate melanin products absorb limited amounts of radiation around 340 nm, but they have a peak absorption at 280 nm (Romero-Martinez et al., 2000). On the other hand, cerulenin affects the early stages of melanin production by inhibiting the enzyme polyketide synthase (Kubo et al. 1986), but it can also inhibit fatty acid synthase which is critical for physiological processes in many fungi (Kubo et al., 1986). Since the inhibition of DHN melanin pathway by cerulenin occurs at an early stage there is no accumulation of intermediate melanin product, which could explain the differences in its effectiveness compared to other MBIs (Fleet and Breuil, 2002). However, unlike carpropamid and tricyclazole, cerulenin only interferes with one enzymatic reaction in the DHN melanin pathway. This might limit its inhibitory properties at low concentrations, such as the 10 ppm

concentration used in my experiment. Amongst the three MBIs inhibitors tested, carpropamid was the most effective at decreasing the survival of fungal spores during exposure to UV radiation. Hence, the inhibition of scytalone dehydratase may be the most effective target in the melanin biosynthesis pathway of *A. pullulans* and *C. cladosporioides*, which may increase the susceptibility of these fungi to UV radiation and consequently inhibit their growth under UV-rich environments such as wood surfaces exposed outdoors. Carpropamid was able to reduce the staining of wood veneers by *A. pullulans*, but there was no synergistic effect of carpropamid and UV exposure (unlike the results from in-vitro tests). This discrepancy may be related to the higher concentrations of carpropamid used to treat veneers. The concentration of carpropamid was deliberately increased when treating spruce veneers to ensure that sufficient chemical was available that could restrict the germination of *A. pullulans* spores. The amount of chemical applied to veneers was not optimized to find a lower dose that would act in combination with UV radiation to restrict staining of veneers by *A. pullulans*.

In addition to the melanin biosynthesis inhibitors (MBIs) tested here, the fungicide quinoxyfen was also tested in artificial media and on wood veneers. Quinoxyfen was developed to act against powdery mildew fungi in different crops (Coghlan et al., 1991). The active compound in this fungicide appears to be different from those of other biocides. However, tests performed in *Blumeria graminis* showed that, in the same way as MBIs, quinoxyfen can affect the appressorium development, which requires the presence of a high concentration of melanin (Wheeler et al. 2003). Hence, my interest in testing quinoxyfen here. Quinoxyfen was slightly more effective than carpropamid at inhibiting the

growth of *A. pullulans* and *C. cladosporioides* in agar plates exposed to UV or visible light. Quinoxyfen interferes with fungal growth by promoting the production of tubular cells instead of appressoria (Wheeler et al. 2003). These tubular cells might be susceptible to UV radiation? In contrast, quinoxyfen was less effective when used to treat wood veneers, and *A. pullulans* responded to its presence by becoming darker, possibly because the concentration of quinoxyfen was too low for it to have a fungicidal effect.

Today there is increasing concern about the toxicity of chemicals used as wood preservatives and great interest in new more environmentally friendly preservatives with lower mammalian toxicity (Evans, 2003). The use of MBIs in combination with UV radiation to restrict fungal staining of wood surfaces is a new approach to 'preserving wood'. However, further research needs to be done to find the concentration of MBIs that can work synergistically with UV radiation to control fungal staining. Other areas that require attention are, for example, testing of MBIs together or using them in combination with biocides that are currently used to control staining fungi. The mode of action of the three MBIs tested here differs from each other. Therefore, it would be interesting to examine any possible additive effects among them. In addition, other fungi need to be tested to confirm that MBIs can restrict fungal staining at wood surfaces exposed outdoors. MBIs are organic molecules, which are photo-sensitive and they might be photo-degraded at exposed wood surfaces. Therefore, consideration needs to be given to protecting them against this effect. It is also possible that they could be leached from wood surfaces as suggested by results in Chapter 5. Therefore ways of grafting or binding them to wood surfaces may need to be

developed. MBIs may be better suited under a coating or used in combination with hydrophobic additives such as oils and waxes.

7.5. Conclusions

My experimental results show that melanin biosynthesis inhibitors (MBIs) are able to inhibit the growth of two of the most common fungi isolated from weathered wood surfaces (*A. pullulans* and *C. cladosporioides*). There was evidence that the fungi's ability to withstand the deleterious effects of UV radiation was reduced when they were grown on media containing MBIs. In addition, carpropamid (one of the MBIs tested) reduced the staining of wood surfaces inoculated with spores of *A. pullulans* exposed to artificial UV radiation *in-vitro*. This outcome is very interesting because, if reproduced at wood surfaces exposed outdoors, it may reduce the amount of chemical needed to prevent fungal staining, which might decrease the environmental impacts and cost of the preservative treatment. To my knowledge this is the first attempt to use this approach to control staining by the black moulds that colonize weathered wood surfaces. Therefore, I conclude that the use of MBIs as anti-stain agents appears sufficiently promising to do further full-scale tests and outdoor trials. Such trials should seek to optimize the concentration of MBIs to produce a synergistic effect with solar UV radiation, and possibly use MBIs as mixed formulations and with additives to prevent their photodegradation and leaching from exposed wood surfaces.

Chapter 8: General discussion, conclusions and suggestions for further research

8.1. General discussion

In this thesis I hypothesized that the graying of wood surfaces exposed outdoors was due to the presence of melanized fungi with resistance to UV radiation. The experimental results in Chapters 3, 5 and 6 support this hypothesis. The results in Chapter 4 also provide new information on the role that such fungi have on the micro-structural properties of wood. Chapter 7 suggests that the staining of wood surfaces exposed outdoors by melanized fungi can be decreased or eliminated by inhibiting melanin production, thereby increasing the susceptibility of the fungi to the deleterious effects of solar radiation.

The interactive effects of UV radiation and colonization of wood surfaces by fungi has received little attention with the exception of studies that have shown that photodegradation of lignin provides a source of carbon for some of the fungi that colonize weathered wood. It is clear from my findings that the interactive effects of UV radiation and fungi play a more significant role in the surface degradation of wood exposed outdoors.

Examination of the colonization of wood exposed outdoors by fungi revealed that changes in the appearance of wood surfaces were clearly driven by the interactive effect of solar radiation and fungal colonization. During the first four to eight weeks of outdoor exposure, yellowing of wood surfaces occurred due to photodegradation of lignin. These findings accord with those of other researchers (Gellerstendt and Gierer, 1975; Feist and Hon, 1984; Feist, 1990). Thereafter surfaces became darker, bluer and greener and finally acquired a grey color, again as others have observed (Duncan, 1963; Feist, 1990). The fungi isolated

from exposed surfaces were mainly from the ascomycota phylum. *A. pullulans* and *H. dematioides* were frequently isolated. These two species are recognized wood stainers, have dark mycelia and spores and have been widely documented as colonizing wood surfaces exposed outdoors (Seifert, 1964; Dickinson, 1971; Amburgey, 1974; Schmidt and French, 1976; Bardage and Bjurman, 1998; Held et al. 2006). Other fungi that were frequently isolated were *E. nigrum* and species of *Phoma,* which do not possess black hyphae, but produce aggregations of black spores and dark protective structures (sporodochia and pycnidia, respectively) (Barnett and Hunter, 1998; Rotem and Aust, 1991). Other fungi (*Cladosporium* spp. and *Alternaria* spp.) were isolated less frequently. All the aforementioned observations confirm the results of previous studies of the colonization of weathered wood by fungi. New information was generated by my experiment that examined the colonization of wood exposed under polymethylmethacrylate filters. This experiment showed that when wood samples were exposed to the full solar spectrum under a filter they were colonized by the same organisms that colonize fully exposed surfaces. However, when energetic radiation (UV and visible light) was blocked from reaching the surface, the black fungi (*A. pullulans* and *H. dematioides*) were isolated less frequently and less melanized fungi became more common. This finding hinted at the comparative advantage given by black pigmentation to fungi colonizing wood surfaces exposed to UV and visible light. A subsequent experiment showed that *A. pullulans* increased its melanin production when exposed to UV radiation, in accord with the findings of previous studies that have shown that melanized fungi are better able to survive exposure to UV radiation than hyaline (white) fungi (Wang and Casadevall, 1994). However,

not all black fungi increased their melanin production when exposed to UV radiation. For example, a fungus from the genera *Cladosporium* was naturally melanized irrespectively of the radiation it was exposed to. The interactive effects of UV radiation and fungal colonization had an effect on the color of weathered wood. For example, wood samples exposed under filters that transmitted UV and visible radiation tended to be darker, in accords with their frequent colonization by black fungi and the ability of such fungi to increase their melanin production in the presence of UV and visible light. On the other hand, samples exposed to less energetic radiation (IR and no light) showed less pronounced darkening and they tended to be greener. As stated above, these results point to a complex relationship between the surface photodegradation of wood, UV radiation and colonization of wood surfaces by fungi. Based on my experimental results and information available in literature the relationship between photodegradation of wood and fungal colonization can be summarized as follows: wood surfaces exposed outdoors are rapidly photodegraded by solar radiation which produces the first color changes seen at weathered wood surfaces (Gellerstendt and Gierer, 1975; Feist and Hon, 1984). Wood photodegradation products that accumulate at wood surfaces provide a carbon source for fungal spores that alight on wood surfaces (Schoeman and Dickinson, 1997). Days after exposure and subject to the availability of water, colonization of wood surfaces by fungi begins as small black colonies (spots) at the wood surface. Subsequently these colonies spread and produce darkening (graying) of the wood surface due to the presence of melanized fungi (Chedgy, 2006). Black fungi are common colonizers of wood surfaces exposed to the full solar spectrum. These fungi use the melanin contained in their cells to protect themselves against the damaging

effects of UV radiation and visible light. However, UV radiation can also promote increased melanin production in some of these black fungi, which may lead to darker wood surfaces.

The number of hyaline (white) fungi discovered colonizing wood surfaces exposed to weathering was surprising in view of my findings and those of other researchers that melanin protects fungi exposed to solar radiation. These fungi do not produce high amounts of melanin. Therefore, it is valid to question how they survive the conditions found at wood surfaces exposed to the weather? Their survival may be explained in part by their reproductive strategies, such as those described above for *Phoma* and *Epicoccum* species. A second explanation is that they are protected by association with fungi that possess melanin. Melanin can confer protection against UV radiation, extreme temperatures and desiccation, conditions all found at wood surfaces exposed outdoors (Fogarty and Tobin 1996; Henson et al. 1999; Butler and Day 1998; 2001; Dadachova et al. 2007). Thus, black fungi growing at the outer wood surface may confer protection to hyaline fungi growing immediately below the exposed surface layers. If this occurs then it is possible that a symbiotic relationship could exist between both types of fungi. This suggestion is based on results obtained in Chapter 4, which showed that some hyaline fungi could decay woody tissues. After the photodegradation of wood tissues has reached an advanced stage, carbon sources from such degradation may become limited. Decay of the remaining tissue by hyaline fungi may provide carbon sources for black fungi, which protect the hyaline fungi growing in the sub-surface layers?

The ability of certain fungi isolated from weathered wood to breakdown wood tissues (Chapter 4 of this thesis) has a number of important implications that are worthy of

discussion. Firstly, my finding that species such as *Cladosporium* sp., *C. ligniaria*, *E. nigrum*, *L. infectoria*, *M. minutella* and *Phialocephala* sp. can significantly reduce the mechanical properties of thin wood veneers helps to change the idea that fungi at weathered wood surfaces simply alter the appearance of wood. Secondly, my findings raise a number of questions about the damage that these fungi produce *in-vivo* and how much they contribute to the erosion of wood surfaces exposed outdoors. Erosion of wood surfaces during weathering is thought to be caused only by the combined action of UV radiation and water. However, my results suggest that microbial degradation could contribute to surface erosion. The occurrence of conditions that favor decay are possibly a limiting factor, but it certainly seems possible that fungi accelerate the erosion of weathered wood in situations where the moisture content at wood surfaces favor microbial colonization.

Fungal staining during weathering affects the appearance of wood, which decreases its value as a construction material. Furthermore, the maintenance and replacement of weathered wooden cladding is costly (Amburgey and Ragon, 2008). Therefore, protection of wood against this type of damage is commercially important. Wood can be protected against photodegradation by using coatings and various additives, for example UV absorbers and hindered amine stabilizers. Protection of wood against fungal colonization relies on the use of biocides, which are indiscriminate and do not specifically target the organisms that cause weathered wood to become grey. My experimental results (Chapters 5, 6 and 7) suggest that in principle it is possible to use an alternative approach to decrease and possibly eliminate fungal stains at wood surfaces exposed outdoors. This approach involves preventing staining fungi from synthesizing melanin using chemicals applied at low

concentrations. Fungi prevented from synthesizing melanin were lighter and also appeared to be more susceptible to UV radiation. An experiment showed that two of the common fungi isolated from weathered wood *A. pullulans* and *C. cladosporioides,* could be prevented from growing by the combination of the melanin biosynthesis inhibitor (MBI) carpropamid and UV radiation. The approach when tested with wood veneers did not produce a statistically significant interaction between carpropamid and UV radiation on fungal growth, but positive effects were achieved with the MBI on its own. It is possible that lower doses of MBIs in wood might achieve the desired synergistic effect with UV radiation, similar to that seen in artificial media. This might decrease the toxicity of treated samples, with obvious environmental benefits. A possible drawback of the treatment is the relatively high cost of the MBIs tested. However, in the near future the demand for more environmentally friendly and less toxic preservative treatments may justify their use.

8.2. Conclusions

My results demonstrated that melanized fungi are responsible for the graying of wood surfaces exposed outdoors. However, initial color changes at wood surfaces exposed to weathering were due to photodegradation of lignin. As anticipated solar radiation affected the colonization of wood surfaces by fungi. Solar radiation interfered with the ecology of wood surfaces by encouraging colonization of the wood by melanized fungi (*A. pullulans* and *H. dematioides*). Furthermore, in the presence of UV radiation *A. pullulans* increased its production of melanin, apparently as an adaptive measure. Such an adaptation probably

gives the fungus a competitive advantage when colonizing wood surfaces exposed to the weather. However, as a result the wood surface became darker. Therefore, I conclude that UV radiation and staining fungi interact to influence the color of wood surfaces exposed outdoors.

Fungi colonizing weathered wood surfaces consist of a diverse group of ascomycetes. Among them, black ascomycetes with relatively high resistance to UV radiation, were found colonizing weathered wood surfaces in association with a number of less melanized fungi. Some of these fungi were able to reduce the strength properties of thin wood veneers (*in-vitro*). The type of degradation produced by one of these fungi appeared to be different from that of soft-rot decay (Type 1 or 2). Therefore I conclude that some fungi colonizing weathered wood surfaces can degrade woody tissues, but the extent of degradation probably depends on wood species and presence of conditions at wood surfaces that favor microbial decay.

The use of melanin biosynthesis inhibitors (MBIs) and UV radiation to decrease or eliminate fungal stains in wood surfaces subjected to artificial weathering was explored. MBIs were able to block melanin production in fungi isolated from weathered wood making the fungi more susceptible to UV radiation. At low doses, MBIs tested against fungi in artificial media, acted synergistically with artificial UV radiation (at wavelengths within the solar spectrum) to inhibit the development of fungi from fungal spores. However, such a synergistic effect was not reproduced with treated wood veneers, but the MBI carpropamid was able to decrease fungal staining irrespective of the presence of UV or visible light. I conclude that the use of MBIs on their own or in combination with UV radiation is a promising approach

to controlling the fungi responsible for the graying of weathered wood surfaces, but further research is required to optimize the system and test it against a much greater range of fungi.

The research in this thesis provides some new insights into the role played by the fungi that colonize weathered wood surfaces. However, as normally occurs in science, new knowledge is also accompanied by new questions. Therefore, I suggest further research that is needed to more fully explore some of my findings and to develop new treatments to reduce fungal staining of weathered wood surfaces.

8.3. Suggestion for further research

This thesis described a number of experiments that were performed to better understand the role of non-decay fungi on the weathering of wood. My findings produced a number of new research questions, which could not be answered here. One important question concerns the ecological relationship between the different fungi colonizing weathered wood surfaces. For example, melanized and hyaline (white) fungi were found growing together at weathered wood surfaces. I speculated on a possible synergistic relationship between the two types of fungi. However, a better understanding of their ecological interaction is needed and could be achieved by isolating fungi in different layers from the surface to the sub-surface of weathered wood samples. Using this approach it should be possible to confirm or reject the hypothesis that black fungi protect hyaline fungi in weathered wood from exposure to UV radiation. A second area that would benefit from

further research is the extent to which fungi degrade weathered wood *in-vivo*. It is important to find out whether conditions conductive for such decay exist at wood surfaces exposed outdoors and if so whether they occur sporadically or seasonally. Also, it is important to establish whether fungi colonizing weathered wood increase the erosion of wood surfaces outdoors or reduce erosion by shielding the wood from UV radiation. A number of experiments could be performed to answer these questions. These experiments could include the use of chemicals (biocides) to restrict fungal colonization of wood surfaces and measurements of erosion of treated and untreated wood surfaces exposed to the weather.

Future research should also focus on finding the optimum concentration of the MBI carpropamid to treat wood samples and achieve synergy with UV radiation in controlling fungal growth in wood. My research did not use mixes of different MBIs to control fungal staining. The MBIs tested here possess different modes of action that could have additive effects against a broader spectrum of fungi. Future large scale outdoor trials to test whether MBIs can restrict the fungal staining of wood should be carried out. However, this brings other problems to consider such as, protecting MBIs which are organic compounds, from photodegradation. Another issue that should be addressed is the development of methods to prevent the leaching of MBIs from wood. This might be achieved by grafting the MBIs to wood or incorporating them in or under a hydrophobic polymer matrix.

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Appendices

Appendixes can be found in the DVD attached to this thesis.

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Appendix 1: Statistical analysis Chapter 4

Analysis of variance tensile stress ratio

Variate: Tensile_stress_ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	10		1.30944	0.13094	3.43	
Block.Dish stratum						
Fungi	17		20.63514	1.21383	31.76	<.001
Residual	148	(22)	5.65690	0.03822	0.82	
Block.Dish.Area stratum						
W_specie	1		2.30182	2.30182	49.17	<.001
Fungi.W_specie	17		7.38402	0.43435	9.28	<.001
Residual	158	(22)	7.39683	0.04682		
Total	351	(44)	40.76775			

Message: the following units have large residuals.

Block 5	0.120	s.e. 0.058
Block 6	-0.118	s.e. 0.058
Block 1 Dish 4	0.378	s.e. 0.120
Block 1 Dish 18	0.479	s.e. 0.120
Block 8 Dish 5	0.358	s.e. 0.120
Block 9 Dish 18	-0.429	s.e. 0.120
Block 1 Dish 18 Area 1	-0.402	s.e. 0.137
Block 1 Dish 18 Area 2	0.402	s.e. 0.137
Block 2 Dish 2 Area 1	0.376	s.e. 0.137
Block 2 Dish 2 Area 2	-0.376	s.e. 0.137
Block 9 Dish 3 Area 1	-0.401	s.e. 0.137
Block 9 Dish 3 Area 2	0.401	s.e. 0.137
Block 9 Dish 13 Area 1	-0.390	s.e. 0.137
Block 9 Dish 13 Area 2	0.390	s.e. 0.137

Tables of means

Variate: Tensile_stress_ratio
Grand mean 0.849

Fungi	A. pull (B)	A. pull (W)	Alt	Botry	Chaet glob
	0.920	0.983	0.907	1.034	0.431

Fungi	Clad	Con put	Conioch	Control	Epicoc
	0.339	1.115	0.391	1.000	0.903

Fungi	Hormonema	Lecyth	Lewia	Mollisia	Phialocephala
	1.051	0.983	0.908	0.850	0.808

Fungi	Phialophora	Phoma	Trichaptum
	1.005	0.979	0.674

W_specie	Lime	Spruce
	0.773	0.925

Fungi	W_specie	Lime	Spruce
A. pull (B)		0.999	0.841
A. pull (W)		0.985	0.981
Alt		0.847	0.967
Botry		1.098	0.970
Chaet glob		0.101	0.760
Clad		-0.004	0.682
Con put		0.979	1.251
Conioch		0.137	0.646
Control		1.000	1.000
Epicoc		0.846	0.959
Hormonema		1.044	1.058
Lecyth		1.073	0.894
Lewia		0.874	0.941
Mollisia		0.788	0.913
Phialocephala		0.537	1.080
Phialophora		1.097	0.913
Phoma		0.970	0.988
Trichaptum		0.540	0.808

Standard errors of differences of means

Table	Fungi	W_specie	Fungi	
				W_specie
rep.		22	198	11
s.e.d.		0.0589	0.0217	0.0879
d.f.		148	158	304.57
Except when comparing means with the same level(s) of				
Fungi				0.0923
d.f.				158

(Not adjusted for missing values)

Analysis of variance modulus of elasticity (MOE) ratio

Variate: MOE_ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	10		3.70210	0.37021	13.93	
Block.Dish stratum						
Fungi	17		11.02075	0.64828	24.39	<.001
Residual	148	(22)	3.93356	0.02658	0.66	
Block.Dish.Area stratum						
W_specie	1		3.13308	3.13308	77.64	<.001
Fungi.W_specie	17		8.93710	0.52571	13.03	<.001
Residual	158	(22)	6.37573	0.04035		
Total	351	(44)	33.76693			

Message: the following units have large residuals.

Block 7	0.203	s.e. 0.097
Block 1 Dish 18	0.293	s.e. 0.100
Block 8 Dish 5	0.278	s.e. 0.100
Block 9 Dish 18	-0.336	s.e. 0.100
Block 1 Dish 3 Area 1	0.368	s.e. 0.127
Block 1 Dish 3 Area 2	-0.368	s.e. 0.127
Block 1 Dish 13 Area 1	0.449	s.e. 0.127
Block 1 Dish 13 Area 2	-0.449	s.e. 0.127
Block 7 Dish 4 Area 1	0.384	s.e. 0.127
Block 7 Dish 4 Area 2	-0.384	s.e. 0.127
Block 8 Dish 5 Area 1	-0.519	s.e. 0.127
Block 8 Dish 5 Area 2	0.519	s.e. 0.127

Tables of means

Variate: MOE_ratio

Grand mean 0.894

Fungi	A. pull (B)	A. pull (W)	Alt	Botry	Chaet glob
	0.926	0.953	0.959	1.023	0.567

Fungi	Clad	Con put	Conioch	Control	Epicoc
	0.511	1.047	0.615	1.000	0.987

Fungi	Hormonema	Lecyth	Lewia	Mollisia	Phialocephala
	1.006	0.988	1.004	1.020	0.742

Fungi	Phialophora	Phoma	Trichaptum
	1.020	0.933	0.791

W_specie	Lime	Spruce
	0.805	0.983

Fungi	W_specie	Lime	Spruce
A. pull (B)		0.933	0.920
A. pull (W)		0.911	0.995
Alt		1.009	0.910
Botry		1.009	1.036
Chaet glob		0.164	0.970
Clad		-0.013	1.035
Con put		0.999	1.096
Conioch		0.404	0.825
Control		1.000	1.000
Epicoc		0.939	1.035
Hormonema		0.963	1.049
Lecyth		1.042	0.934
Lewia		0.945	1.063
Mollisia		0.942	1.098
Phialocephala		0.539	0.944
Phialophora		1.002	1.038
Phoma		0.957	0.909
Trichaptum		0.746	0.835

Standard errors of differences of means

Table	Fungi	W_specie	Fungi	W_specie
rep.		22	198	11
s.e.d.		0.0492	0.0202	0.0780
d.f.		148	158	297.09
Except when comparing means with the same level(s) of Fungi				0.0857
d.f.				158

(Not adjusted for missing values)

Analysis of variance peak stiffness ratio

Variate: Peak_stiffness_ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	10		3.85027	0.38503	10.83	
Block.Dish stratum						
Fungi	17		13.67544	0.80444	22.62	<.001
Residual	148	(22)	5.26328	0.03556	0.71	
Block.Dish.Area stratum						
W_specie	1		3.59532	3.59532	72.09	<.001
Fungi.W_specie	17		6.97775	0.41046	8.23	<.001
Residual	158	(22)	7.87991	0.04987		
Total	351	(44)	38.62168			

Message: the following units have large residuals.

Block 1 Dish 5	0.334	s.e. 0.115
Block 7 Dish 5	-0.362	s.e. 0.115
Block 8 Dish 5	0.313	s.e. 0.115
Block 8 Dish 7	-0.322	s.e. 0.115
Block 9 Dish 18	-0.325	s.e. 0.115
Block 1 Dish 3 Area 1	0.448	s.e. 0.141
Block 1 Dish 3 Area 2	-0.448	s.e. 0.141
Block 1 Dish 13 Area 1	0.536	s.e. 0.141
Block 1 Dish 13 Area 2	-0.536	s.e. 0.141
Block 8 Dish 5 Area 1	-0.421	s.e. 0.141
Block 8 Dish 5 Area 2	0.421	s.e. 0.141

Tables of means

Variate: Peak_stiffness_ratio

Grand mean 0.921

Fungi	A. pull (B)	A. pull (W)	Alt	Botry	Chaet glob
	0.891	1.026	1.008	1.007	0.536

Fungi	Clad	Con put	Conioch	Control	Epicoc
	0.474	1.044	0.581	1.000	1.035

Fungi	Hormonema	Lecyth	Lewia	Mollisia	Phialocephala
	1.045	1.029	1.052	1.049	0.952

Fungi	Phialophora	Phoma	Trichaptum
	1.066	0.969	0.812

W_specie	Lime	Spruce
	0.826	1.016

Fungi	W_specie	Lime	Spruce
A. pull (B)		0.928	0.854
A. pull (W)		0.950	1.102
Alt		1.027	0.989
Botry		1.028	0.985
Chaet glob		0.166	0.905
Clad		-0.012	0.961
Con put		0.948	1.140
Conioch		0.372	0.790
Control		1.000	1.000
Epicoc		0.956	1.113
Hormonema		0.991	1.100
Lecyth		0.994	1.064
Lewia		0.960	1.143
Mollisia		0.977	1.120
Phialocephala		0.814	1.090
Phialophora		1.017	1.115
Phoma		0.970	0.968
Trichaptum		0.773	0.850

Standard errors of differences of means

Table	Fungi	W_specie	Fungi W_specie
rep.	22	198	11
s.e.d.	0.0569	0.0224	0.0881
d.f.	148	158	300.53
Except when comparing means with the same level(s) of			
Fungi			0.0952
d.f.			158

(Not adjusted for missing values)

Analysis of variance peak toughness (work) ratio

Variate: Peak_work_ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	10		5.2188	0.5219	4.51	
Block.Dish stratum						
Fungi	17		33.0956	1.9468	16.82	<.001
Residual	148	(22)	17.1253	0.1157	0.61	
Block.Dish.Area stratum						
W_specie	1		2.3098	2.3098	12.12	<.001
Fungi.W_specie	17		9.8291	0.5782	3.03	<.001
Residual	158	(22)	30.1147	0.1906		
Total	351	(44)	90.4632			

Message: the following units have large residuals.

Block 1 Dish 4	0.750	s.e. 0.208
Block 1 Dish 18	0.636	s.e. 0.208
Block 1 Dish 18 Area 1	-0.756	s.e. 0.276
Block 1 Dish 18 Area 2	0.756	s.e. 0.276
Block 2 Dish 2 Area 1	1.058	s.e. 0.276
Block 2 Dish 2 Area 2	-1.058	s.e. 0.276

Tables of means

Variate: Peak_work_ratio

Grand mean 0.874

Fungi	A. pull (B)	A. pull (W)	Alt	Botry	Chaet glob
	0.991	1.076	0.911	1.123	0.370

Fungi	Clad	Con put	Conioch	Control	Epicoc
	0.261	1.270	0.294	1.000	0.859

Fungi	Hormonema	Lecyth	Lewia	Mollisia	Phialocephala
	1.155	1.049	0.859	0.795	0.898

Fungi	Phialophora	Phoma	Trichaptum
	1.047	1.111	0.663

W_specie	Lime	Spruce
	0.798	0.950

Fungi	W_specie	Lime	Spruce
A. pull (B)		1.111	0.870
A. pull (W)		1.107	1.045
Alt		0.745	1.078
Botry		1.248	0.998
Chaet glob		0.069	0.671
Clad		0.001	0.521
Con put		1.047	1.493
Conioch		0.050	0.538
Control		1.000	1.000
Epicoc		0.782	0.936
Hormonema		1.168	1.141
Lecyth		1.155	0.943
Lewia		0.847	0.871
Mollisia		0.729	0.861
Phialocephala		0.554	1.242
Phialophora		1.244	0.850
Phoma		1.041	1.181
Trichaptum		0.459	0.867

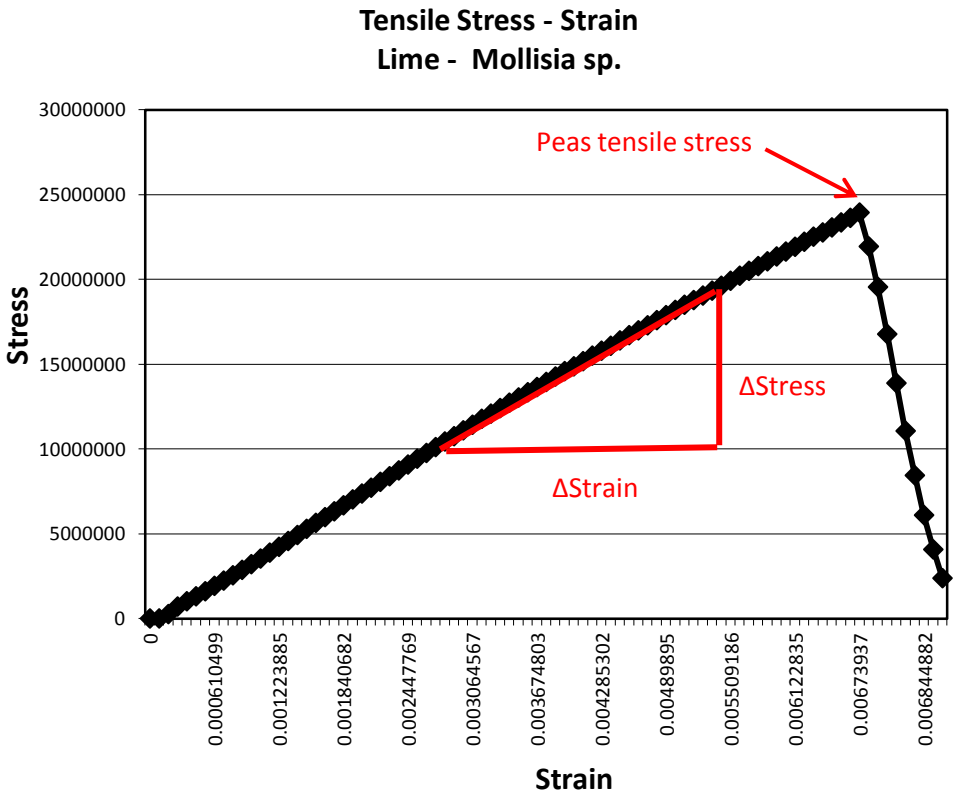
Standard errors of differences of means

Table	Fungi	W_specie	Fungi W_specie
rep.	22	198	11
s.e.d.	0.1026	0.0439	0.1669
d.f.	148	158	292.85
Except when comparing means with the same level(s) of Fungi			0.1862
d.f.			158

(Not adjusted for missing values)

Appendix 2: Graphic determination of modulus of elasticity, example of calculation

Figure A2.1: Tensile stress vs strain of lime wood veneer (block 1) incubated with *Mollisia* sp. red triangle used to calculate the modulus of elasticity directly from the figure



Modulus of elasticity (MOE) = (Δ stress / Δ strain)
MOE = (25285173.1 - 15456205.1) / (0.011158 - 0.008097)
MOE = 3210593954 N/m2

Peak tensile stress = 23941131.7 N/m2

Appendix 3: Statistical analysis Chapter 5

Analysis of variance frequency of isolation of fungi

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	0.00000	0.00000	0.00	
block.*Units* stratum					
filter	4	0.00000	0.00000	0.00	1.000
fungi	6	1.06571	0.17762	12.19	<.001
filter.fungi	24	0.63406	0.02642	1.81	0.018
Residual	136	1.98119	0.01457		
Total	174	3.68096			

Message: the following units have large residuals.

block 2 *units* 28	-0.2857	s.e.	0.1064
block 3 *units* 7	0.3056	s.e.	0.1064

Tables of means

Variate: freq

Grand mean 0.1429

filter	1	2	3	4	5
	0.1429	0.1429	0.1429	0.1429	0.1429

fungi	Alternaria sp.	Aureobasidium pullulans
	0.0574	0.3054

fungi	Cladosporium sp.	Epicoccum sp.
	0.1211	0.1509

fungi	Hormonema dematioides	Others
	0.1372	0.1718

fungi	Phoma sp.
	0.0562

filter	fungi	Alternaria sp.	Aureobasidium pullulans
1		0.0500	0.3111
2		0.0400	0.3500
3		0.0900	0.4171
4		0.0000	0.2386
5		0.1071	0.2100

filter	fungi	Cladosporium sp.	Epicoccum sp.
1		0.0500	0.2389
2		0.1300	0.0800
3		0.0400	0.0686
4		0.1586	0.1586
5		0.2271	0.2086

filter	fungi	Hormonema dematioides	Others
1		0.1889	0.1389
2		0.2200	0.1400
3		0.1186	0.1371
4		0.0686	0.2857
5		0.0900	0.1571

filter	fungi	Phoma sp.
1		0.0222
2		0.0400
3		0.1286
4		0.0900
5		0.0000

Standard errors of differences of means

Table	filter	fungi	filter fungi
rep.	35	25	5
d.f.	136	136	136
s.e.d.	0.02885	0.03414	0.07633

Least significant differences of means (5% level)

Table	filter	fungi	filter fungi
rep.	35	25	5
d.f.	136	136	136
l.s.d.	0.05706	0.06751	0.15096

Analysis of variance fungal stains 0 to 40 weeks

Variate: W1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	0.00045807	0.00011452	1.97	
rack.sample stratum					
Exposure	6	0.00020982	0.00003497	0.60	0.727
Residual	24	0.00139711	0.00005821	0.88	
rack.sample.area stratum					
treatment	3	0.00013568	0.00004523	0.68	0.565
Exposure.treatment	18	0.00125571	0.00006976	1.05	0.413
Residual	84	0.00556556	0.00006626	1.00	
rack.sample.area.strip stratum					
chem_charg	3	0.00012939	0.00004313	0.65	0.583
Exposure.chem_charg	18	0.00126200	0.00007011	1.06	0.394
treatment.chem_charg	9	0.00054106	0.00006012	0.91	0.519
Exposure.treatment.chem_charg	54	0.00363311	0.00006728	1.02	0.451
Residual	336	0.02226224	0.00006626		
Total	559	0.03684976			

Message: the following units have large residuals.

rack 5 sample 2	0.00572	s.e. 0.00158
rack 3 sample 1 area 3	0.01431	s.e. 0.00315
rack 3 sample 3 area 3	0.01371	s.e. 0.00315
rack 3 sample 4 area 3	0.01011	s.e. 0.00315
rack 5 sample 2 area 1	0.01843	s.e. 0.00315
rack 3 sample 1 area 3 strip 1	-0.01909	s.e. 0.00631
rack 3 sample 1 area 3 strip 2	-0.01909	s.e. 0.00631
rack 3 sample 1 area 3 strip 3	-0.01909	s.e. 0.00631
rack 3 sample 1 area 3 strip 4	0.05726	s.e. 0.00631
rack 3 sample 3 area 3 strip 4	0.05486	s.e. 0.00631
rack 3 sample 4 area 3 strip 4	0.04046	s.e. 0.00631
rack 5 sample 2 area 1 strip 1	-0.02457	s.e. 0.00631
rack 5 sample 2 area 1 strip 2	0.07371	s.e. 0.00631
rack 5 sample 2 area 1 strip 3	-0.02457	s.e. 0.00631
rack 5 sample 2 area 1 strip 4	-0.02457	s.e. 0.00631

Tables of means

Variate: W1

Grand mean 0.00067

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	0.00114	0.00119	0.00084	0.00000	0.00000	0.00154	0.00000

treatment	acetic acid	carpropamid	tinuvin	water
	0.00048	0.00088	0.00133	0.00000

chem_charg	1	2	3	4
	0.00068	0.00000	0.00065	0.00136

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		0.00000	0.00000	0.00457	0.00000
filter 2		0.00000	0.00000	0.00477	0.00000
filter 3		0.00337	0.00000	0.00000	0.00000
filter 4		0.00000	0.00000	0.00000	0.00000
filter 5		0.00000	0.00000	0.00000	0.00000
full		0.00000	0.00614	0.00000	0.00000
None		0.00000	0.00000	0.00000	0.00000

Exposure	chem_charg	1	2	3	4
filter 1		0.00000	0.00000	0.00457	0.00000
filter 2		0.00477	0.00000	0.00000	0.00000
filter 3		0.00000	0.00000	0.00000	0.00337
filter 4		0.00000	0.00000	0.00000	0.00000
filter 5		0.00000	0.00000	0.00000	0.00000
full		0.00000	0.00000	0.00000	0.00614
None		0.00000	0.00000	0.00000	0.00000

treatment	chem_charg	1	2	3	4
acetic acid		0.00000	0.00000	0.00000	0.00193
carpropamid		0.00000	0.00000	0.00000	0.00351
tinuvin		0.00273	0.00000	0.00261	0.00000
water		0.00000	0.00000	0.00000	0.00000

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.00000	0.00000	0.01829	0.00000
	water		0.00000	0.00000	0.00000	0.00000
filter 2	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.01909	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000
filter 3	acetic acid		0.00000	0.00000	0.00000	0.01349
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.00000	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000
filter 4	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.00000	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000
filter 5	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.00000	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000
full	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.02457
	tinuvin		0.00000	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000
None	acetic acid		0.00000	0.00000	0.00000	0.00000
	carpropamid		0.00000	0.00000	0.00000	0.00000
	tinuvin		0.00000	0.00000	0.00000	0.00000
	water		0.00000	0.00000	0.00000	0.00000

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	0.001206	0.000973	0.000973	0.002535
d.f.	24	84	336	107.99
Except when comparing means with the same level(s) of				
Exposure				0.002574
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	0.002535	0.001946	0.005129
d.f.	255.19	413.54	443.99
Except when comparing means with the same level(s) of			
Exposure	0.002574		0.005148
d.f.	336		413.54
treatment		0.001946	
d.f.		336	
Exposure.treatment			
			0.005148
d.f.			336
Exposure.chem_charg			
			0.005148
d.f.			413.54

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	0.002490	0.001935	0.001914	0.005024
d.f.	24	84	336	107.99
Except when comparing means with the same level(s) of				
Exposure				0.005119
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	0.004992	0.003825	0.010079
d.f.	255.19	413.54	443.99
Except when comparing means with the same level(s) of			
Exposure	0.005063		0.010120
d.f.	336		413.54
treatment		0.003827	
d.f.		336	
Exposure.treatment			0.010127
d.f.			336
Exposure.chem_charg			0.010120
d.f.			413.54

Analysis of variance

Variate: W2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	0.032655	0.008164	0.21	
rack.sample stratum					
Exposure	6	12.509154	2.084859	52.38	<.001
Residual	24	0.955231	0.039801	5.04	
rack.sample.area stratum					
treatment	3	0.012641	0.004214	0.53	0.661
Exposure.treatment	18	0.054858	0.003048	0.39	0.987
Residual	84	0.663816	0.007903	0.85	
rack.sample.area.strip stratum					
chem_charg	3	0.027087	0.009029	0.98	0.405
Exposure.chem_charg	18	0.107357	0.005964	0.64	0.864
treatment.chem_charg	9	0.110122	0.012236	1.32	0.224
Exposure.treatment.chem_charg	54	0.437373	0.008100	0.87	0.720
Residual	336	3.110674	0.009258		
Total	559	18.020967			

Message: the following units have large residuals.

rack 1 sample 1	0.1363	s.e. 0.0413
rack 2 sample 3	-0.1296	s.e. 0.0413
rack 2 sample 6	0.0932	s.e. 0.0413
rack 1 sample 1 area 1	-0.1216	s.e. 0.0344
rack 1 sample 1 area 3	0.0972	s.e. 0.0344
rack 2 sample 3 area 2	-0.1111	s.e. 0.0344
rack 2 sample 3 area 3	0.1444	s.e. 0.0344
rack 3 sample 5 area 3	0.0989	s.e. 0.0344
rack 3 sample 5 area 4	-0.1166	s.e. 0.0344
rack 4 sample 5 area 2	0.1290	s.e. 0.0344
rack 4 sample 5 area 4	-0.1047	s.e. 0.0344
rack 5 sample 2 area 1	0.1010	s.e. 0.0344
rack 5 sample 2 area 4	-0.0938	s.e. 0.0344
rack 1 sample 1 area 1 strip 1	-0.2741	s.e. 0.0745
rack 1 sample 1 area 1 strip 3	0.5086	s.e. 0.0745
rack 1 sample 1 area 2 strip 2	0.4340	s.e. 0.0745
rack 1 sample 1 area 2 strip 3	-0.2479	s.e. 0.0745
rack 1 sample 1 area 2 strip 4	-0.3020	s.e. 0.0745
rack 1 sample 1 area 3 strip 1	-0.5247	s.e. 0.0745
rack 1 sample 1 area 3 strip 3	0.5299	s.e. 0.0745
rack 2 sample 3 area 1 strip 2	-0.2462	s.e. 0.0745
rack 2 sample 3 area 1 strip 3	0.2564	s.e. 0.0745
rack 2 sample 3 area 3 strip 3	0.2231	s.e. 0.0745
rack 2 sample 3 area 4 strip 1	-0.3201	s.e. 0.0745
rack 2 sample 3 area 4 strip 3	0.3710	s.e. 0.0745
rack 3 sample 5 area 3 strip 3	0.2738	s.e. 0.0745
rack 3 sample 5 area 4 strip 1	-0.2451	s.e. 0.0745
rack 4 sample 5 area 1 strip 3	0.3098	s.e. 0.0745
rack 4 sample 5 area 1 strip 4	-0.3125	s.e. 0.0745
rack 5 sample 2 area 1 strip 2	0.3834	s.e. 0.0745
rack 5 sample 2 area 1 strip 3	-0.2537	s.e. 0.0745

Tables of means

Variate: W2

Grand mean 0.0703

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	0.0406	0.0077	0.0065	0.0012	0.0012	0.4350	0.0000

treatment	acetic acid	carpropamid	tinuvin	water
	0.0774	0.0710	0.0685	0.0643

chem_charg	1	2	3	4
	0.0759	0.0584	0.0739	0.0730

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		0.0391	0.0409	0.0403	0.0420
filter 2		0.0024	0.0095	0.0095	0.0095
filter 3		0.0167	0.0047	0.0024	0.0024
filter 4		0.0048	0.0000	0.0000	0.0000
filter 5		0.0047	0.0000	0.0000	0.0000
full		0.4741	0.4422	0.4277	0.3960
None		0.0000	0.0000	0.0000	0.0000

Exposure	chem_charg	1	2	3	4
filter 1		0.0405	0.0238	0.0540	0.0440
filter 2		0.0048	0.0048	0.0167	0.0047
filter 3		0.0000	0.0024	0.0047	0.0190
filter 4		0.0000	0.0000	0.0000	0.0048
filter 5		0.0000	0.0000	0.0024	0.0024
full		0.4862	0.3779	0.4398	0.4360
None		0.0000	0.0000	0.0000	0.0000

treatment	chem_charg	1	2	3	4
acetic acid		0.0756	0.0572	0.0924	0.0844
carpropamid		0.0890	0.0671	0.0483	0.0798
tinuvin		0.0832	0.0636	0.0543	0.0731
water		0.0559	0.0458	0.1008	0.0547

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		0.0191	0.0191	0.0477	0.0706
	carpropamid		0.0667	0.0190	0.0294	0.0485
	tinuvin		0.0571	0.0381	0.0469	0.0190
	water		0.0190	0.0191	0.0920	0.0381
filter 2	acetic acid		0.0000	0.0000	0.0095	0.0000
	carpropamid		0.0000	0.0191	0.0095	0.0095
	tinuvin		0.0191	0.0000	0.0095	0.0095
	water		0.0000	0.0000	0.0382	0.0000
filter 3	acetic acid		0.0000	0.0000	0.0000	0.0666
	carpropamid		0.0000	0.0095	0.0000	0.0095
	tinuvin		0.0000	0.0000	0.0095	0.0000
	water		0.0000	0.0000	0.0095	0.0000
filter 4	acetic acid		0.0000	0.0000	0.0000	0.0191
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000
filter 5	acetic acid		0.0000	0.0000	0.0095	0.0095
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000
full	acetic acid		0.5102	0.3810	0.5801	0.4252
	carpropamid		0.5563	0.4220	0.2992	0.4912
	tinuvin		0.5063	0.4071	0.3143	0.4832
	water		0.3720	0.3016	0.5658	0.3446
None	acetic acid		0.0000	0.0000	0.0000	0.0000
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	0.03154	0.01063	0.01150	0.03985
d.f.	24	84	336	55.48
Except when comparing means with the same level(s) of				
Exposure				0.02811
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	0.04110	0.02258	0.06607
d.f.	66.86	418.79	278.60
Except when comparing means with the same level(s) of			
Exposure	0.03043		0.05973
d.f.	336		418.79
treatment		0.02300	
d.f.		336	
Exposure.treatment			
			0.06085
d.f.			336
Exposure.chem_charg			
			0.05973
d.f.			418.79

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	0.06510	0.02113	0.02262	0.07984
d.f.	24	84	336	55.48
Except when comparing means with the same level(s) of				
Exposure				0.05590
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	0.08204	0.04438	0.13006
d.f.	66.86	418.79	278.60
Except when comparing means with the same level(s) of			
Exposure	0.05985		0.11741
d.f.	336		418.79
treatment		0.04524	
d.f.		336	
Exposure.treatment			0.11970
d.f.			336
Exposure.chem_charg			0.11741
d.f.			418.79

Analysis of variance

Variate: W3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	0.053170	0.013293	0.34	
rack.sample stratum					
Exposure	6	13.159952	2.193325	55.80	<.001
Residual	24	0.943390	0.039308	4.74	
rack.sample.area stratum					
treatment	3	0.012742	0.004247	0.51	0.675
Exposure.treatment	18	0.046322	0.002573	0.31	0.997
Residual	84	0.697054	0.008298	0.87	
rack.sample.area.strip stratum					
chem_charg	3	0.026898	0.008966	0.94	0.422
Exposure.chem_charg	18	0.158559	0.008809	0.92	0.551
treatment.chem_charg	9	0.102381	0.011376	1.19	0.299
Exposure.treatment.chem_charg	54	0.414593	0.007678	0.80	0.834
Residual	336	3.207419	0.009546		
Total	559	18.822481			

Message: the following units have large residuals.

rack 1 sample 1	0.1326	s.e. 0.0410
rack 2 sample 3	-0.1211	s.e. 0.0410
rack 2 sample 6	0.0929	s.e. 0.0410
rack 1 sample 1 area 1	-0.1150	s.e. 0.0353
rack 1 sample 1 area 3	0.0918	s.e. 0.0353
rack 2 sample 3 area 2	-0.1039	s.e. 0.0353
rack 2 sample 3 area 3	0.1657	s.e. 0.0353
rack 3 sample 5 area 3	0.0971	s.e. 0.0353
rack 3 sample 5 area 4	-0.1159	s.e. 0.0353
rack 4 sample 5 area 2	0.1236	s.e. 0.0353
rack 4 sample 5 area 4	-0.0982	s.e. 0.0353
rack 5 sample 2 area 4	-0.1169	s.e. 0.0353
rack 1 sample 1 area 1 strip 1	-0.2718	s.e. 0.0757
rack 1 sample 1 area 1 strip 2	-0.2282	s.e. 0.0757
rack 1 sample 1 area 1 strip 3	0.5015	s.e. 0.0757
rack 1 sample 1 area 2 strip 2	0.4292	s.e. 0.0757
rack 1 sample 1 area 2 strip 3	-0.2527	s.e. 0.0757
rack 1 sample 1 area 2 strip 4	-0.2591	s.e. 0.0757
rack 1 sample 1 area 3 strip 1	-0.5200	s.e. 0.0757
rack 1 sample 1 area 3 strip 3	0.5251	s.e. 0.0757
rack 2 sample 3 area 1 strip 2	-0.2674	s.e. 0.0757
rack 2 sample 3 area 4 strip 1	-0.3367	s.e. 0.0757
rack 2 sample 3 area 4 strip 3	0.3638	s.e. 0.0757
rack 3 sample 5 area 3 strip 3	0.2810	s.e. 0.0757
rack 3 sample 5 area 4 strip 1	-0.2499	s.e. 0.0757
rack 3 sample 5 area 4 strip 3	0.2274	s.e. 0.0757
rack 4 sample 5 area 1 strip 3	0.2885	s.e. 0.0757
rack 4 sample 5 area 1 strip 4	-0.2959	s.e. 0.0757
rack 5 sample 2 area 1 strip 2	0.3906	s.e. 0.0757
rack 5 sample 2 area 1 strip 3	-0.2466	s.e. 0.0757

Tables of means

Variate: W3

Grand mean 0.0757

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	0.0489	0.0172	0.0119	0.0018	0.0012	0.4493	0.0000

treatment	acetic acid	carpropamid	tinuvin	water
	0.0828	0.0755	0.0753	0.0694

chem_charg	1	2	3	4
	0.0790	0.0638	0.0790	0.0811

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		0.0533	0.0433	0.0545	0.0444
filter 2		0.0095	0.0262	0.0143	0.0190
filter 3		0.0285	0.0095	0.0024	0.0071
filter 4		0.0048	0.0000	0.0000	0.0024
filter 5		0.0047	0.0000	0.0000	0.0000
full		0.4789	0.4493	0.4562	0.4126
None		0.0000	0.0000	0.0000	0.0000

Exposure	chem_charg	1	2	3	4
filter 1		0.0452	0.0309	0.0611	0.0583
filter 2		0.0048	0.0262	0.0262	0.0119
filter 3		0.0000	0.0047	0.0047	0.0381
filter 4		0.0000	0.0024	0.0000	0.0048
filter 5		0.0000	0.0000	0.0024	0.0024
full		0.5028	0.3827	0.4589	0.4526
None		0.0000	0.0000	0.0000	0.0000

treatment	chem_charg	1	2	3	4
acetic acid		0.0756	0.0626	0.0965	0.0966
carpropamid		0.0917	0.0766	0.0496	0.0839
tinuvin		0.0873	0.0649	0.0679	0.0812
water		0.0613	0.0512	0.1021	0.0628

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		0.0191	0.0286	0.0571	0.1086
	carpropamid		0.0667	0.0285	0.0294	0.0485
	tinuvin		0.0761	0.0381	0.0658	0.0381
	water		0.0190	0.0286	0.0920	0.0381
filter 2	acetic acid		0.0000	0.0190	0.0190	0.0000
	carpropamid		0.0000	0.0762	0.0095	0.0190
	tinuvin		0.0191	0.0000	0.0286	0.0095
	water		0.0000	0.0095	0.0477	0.0190
filter 3	acetic acid		0.0000	0.0095	0.0000	0.1046
	carpropamid		0.0000	0.0095	0.0000	0.0286
	tinuvin		0.0000	0.0000	0.0095	0.0000
	water		0.0000	0.0000	0.0095	0.0191
filter 4	acetic acid		0.0000	0.0000	0.0000	0.0191
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0095	0.0000	0.0000
filter 5	acetic acid		0.0000	0.0000	0.0095	0.0095
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000
full	acetic acid		0.5102	0.3810	0.5896	0.4347
	carpropamid		0.5754	0.4220	0.3087	0.4912
	tinuvin		0.5158	0.4166	0.3715	0.5211
	water		0.4099	0.3111	0.5658	0.3635
None	acetic acid		0.0000	0.0000	0.0000	0.0000
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	0.03135	0.01089	0.01168	0.04006
d.f.	24	84	336	57.44
Except when comparing means with the same level(s) of				
Exposure				0.02881
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	0.04121	0.02297	0.06685
d.f.	69.09	418.41	288.35
Except when comparing means with the same level(s) of			
Exposure	0.03090		0.06077
d.f.	336		418.41
treatment		0.02336	
d.f.		336	
Exposure.treatment			
			0.06179
d.f.			336
Exposure.chem_charg			
			0.06077
d.f.			418.41

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	0.06470	0.02165	0.02297	0.08021
d.f.	24	84	336	57.44
Except when comparing means with the same level(s) of				
Exposure				0.05729
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	0.08222	0.04515	0.13157
d.f.	69.09	418.41	288.35
Except when comparing means with the same level(s) of			
Exposure	0.06077		0.11946
d.f.	336		418.41
treatment		0.04594	
d.f.		336	
Exposure.treatment			0.12155
d.f.			336
Exposure.chem_charg			0.11946
d.f.			418.41

Analysis of variance

Variate: W4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	0.06681	0.01670	0.35	
rack.sample stratum					
Exposure	6	15.26489	2.54415	53.04	<.001
Residual	24	1.15120	0.04797	5.51	
rack.sample.area stratum					
treatment	3	0.00785	0.00262	0.30	0.825
Exposure.treatment	18	0.05030	0.00279	0.32	0.996
Residual	84	0.73090	0.00870	0.84	
rack.sample.area.strip stratum					
chem_charg	3	0.03362	0.01121	1.08	0.357
Exposure.chem_charg	18	0.20165	0.01120	1.08	0.370
treatment.chem_charg	9	0.08335	0.00926	0.89	0.531
Exposure.treatment.chem_charg	54	0.41719	0.00773	0.75	0.906
Residual	336	3.48143	0.01036		
Total	559	21.48919			

Message: the following units have large residuals.

rack 1 sample 1	0.1489	s.e. 0.0453
rack 2 sample 3	-0.1052	s.e. 0.0453
rack 2 sample 6	0.1200	s.e. 0.0453
rack 1 sample 1 area 1	-0.1125	s.e. 0.0361
rack 1 sample 1 area 3	0.1464	s.e. 0.0361
rack 2 sample 3 area 1	-0.1187	s.e. 0.0361
rack 2 sample 3 area 3	0.1591	s.e. 0.0361
rack 4 sample 5 area 2	0.1254	s.e. 0.0361
rack 4 sample 5 area 4	-0.0940	s.e. 0.0361
rack 5 sample 2 area 4	-0.1028	s.e. 0.0361
rack 1 sample 1 area 1 strip 1	-0.3100	s.e. 0.0788
rack 1 sample 1 area 1 strip 3	0.4823	s.e. 0.0788
rack 1 sample 1 area 2 strip 2	0.4007	s.e. 0.0788
rack 1 sample 1 area 2 strip 3	-0.2906	s.e. 0.0788
rack 1 sample 1 area 3 strip 1	-0.5244	s.e. 0.0788
rack 1 sample 1 area 3 strip 2	-0.2630	s.e. 0.0788
rack 1 sample 1 area 3 strip 3	0.4824	s.e. 0.0788
rack 1 sample 1 area 3 strip 4	0.3050	s.e. 0.0788
rack 2 sample 3 area 2 strip 3	0.2585	s.e. 0.0788
rack 2 sample 3 area 4 strip 1	-0.3082	s.e. 0.0788
rack 2 sample 3 area 4 strip 3	0.3828	s.e. 0.0788
rack 3 sample 5 area 3 strip 3	0.2929	s.e. 0.0788
rack 3 sample 5 area 4 strip 1	-0.3140	s.e. 0.0788
rack 4 sample 5 area 1 strip 3	0.2505	s.e. 0.0788
rack 4 sample 5 area 1 strip 4	-0.2674	s.e. 0.0788
rack 5 sample 2 area 1 strip 2	0.4572	s.e. 0.0788
rack 5 sample 2 area 1 strip 3	-0.2466	s.e. 0.0788
rack 5 sample 2 area 4 strip 1	0.2383	s.e. 0.0788

Tables of means

Variate: W4

Grand mean 0.0860

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	0.0744	0.0238	0.0148	0.0018	0.0012	0.4861	0.0000

treatment	acetic acid	carpropamid	tinuvin	water
	0.0899	0.0877	0.0865	0.0799

chem_charg	1	2	3	4
	0.0908	0.0727	0.0892	0.0913

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		0.0628	0.0789	0.0854	0.0705
filter 2		0.0142	0.0381	0.0214	0.0214
filter 3		0.0285	0.0143	0.0047	0.0119
filter 4		0.0048	0.0000	0.0000	0.0024
filter 5		0.0047	0.0000	0.0000	0.0000
full		0.5145	0.4826	0.4942	0.4530
None		0.0000	0.0000	0.0000	0.0000

Exposure	chem_charg	1	2	3	4
filter 1		0.0761	0.0499	0.0943	0.0773
filter 2		0.0095	0.0333	0.0381	0.0142
filter 3		0.0024	0.0095	0.0095	0.0381
filter 4		0.0000	0.0024	0.0000	0.0048
filter 5		0.0000	0.0000	0.0024	0.0024
full		0.5480	0.4136	0.4802	0.5026
None		0.0000	0.0000	0.0000	0.0000

treatment	chem_charg	1	2	3	4
acetic acid		0.0824	0.0694	0.1005	0.1075
carpropamid		0.1066	0.0847	0.0659	0.0934
tinuvin		0.0981	0.0744	0.0815	0.0921
water		0.0762	0.0621	0.1089	0.0723

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		0.0286	0.0381	0.0571	0.1275
	carpropamid		0.1142	0.0474	0.0863	0.0675
	tinuvin		0.1235	0.0666	0.1038	0.0475
	water		0.0379	0.0475	0.1301	0.0666
filter 2	acetic acid		0.0000	0.0285	0.0285	0.0000
	carpropamid		0.0000	0.0952	0.0286	0.0285
	tinuvin		0.0286	0.0000	0.0477	0.0095
	water		0.0095	0.0095	0.0477	0.0190
filter 3	acetic acid		0.0000	0.0095	0.0000	0.1046
	carpropamid		0.0000	0.0190	0.0095	0.0286
	tinuvin		0.0000	0.0095	0.0095	0.0000
	water		0.0095	0.0000	0.0190	0.0191
filter 4	acetic acid		0.0000	0.0000	0.0000	0.0191
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0095	0.0000	0.0000
filter 5	acetic acid		0.0000	0.0000	0.0095	0.0095
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000
full	acetic acid		0.5482	0.4096	0.6086	0.4916
	carpropamid		0.6323	0.4315	0.3371	0.5294
	tinuvin		0.5348	0.4450	0.4095	0.5876
	water		0.4764	0.3681	0.5658	0.4016
None	acetic acid		0.0000	0.0000	0.0000	0.0000
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	0.03463	0.01115	0.01217	0.04303
d.f.	24	84	336	52.76
Except when comparing means with the same level(s) of				
Exposure				0.02950
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	0.04446	0.02384	0.07043
d.f.	63.29	419.08	262.45
Except when comparing means with the same level(s) of			
Exposure	0.03219		0.06308
d.f.	336		419.08
treatment		0.02433	
d.f.		336	
Exposure.treatment			
			0.06438
d.f.			336
Exposure.chem_charg			
			0.06308
d.f.			419.08

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	0.07147	0.02217	0.02393	0.08632
d.f.	24	84	336	52.76
Except when comparing means with the same level(s) of				
Exposure				0.05866
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	0.08883	0.04686	0.13868
d.f.	63.29	419.08	262.45
Except when comparing means with the same level(s) of			
Exposure	0.06332		0.12398
d.f.	336		419.08
treatment		0.04786	
d.f.		336	
Exposure.treatment			0.12664
d.f.			336
Exposure.chem_charg			0.12398
d.f.			419.08

Analysis of variance

Variate: W6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	0.03918	0.00980	0.21	
rack.sample stratum					
Exposure	6	16.88594	2.81432	59.67	<.001
Residual	24	1.13197	0.04717	4.61	
rack.sample.area stratum					
treatment	3	0.00807	0.00269	0.26	0.852
Exposure.treatment	18	0.14473	0.00804	0.79	0.711
Residual	84	0.85984	0.01024	0.82	
rack.sample.area.strip stratum					
chem_charg	3	0.03842	0.01281	1.02	0.382
Exposure.chem_charg	18	0.28069	0.01559	1.25	0.221
treatment.chem_charg	9	0.10613	0.01179	0.94	0.487
Exposure.treatment.chem_charg	54	0.52708	0.00976	0.78	0.865
Residual	336	4.19905	0.01250		
Total	559	24.22110			

Message: the following units have large residuals.

rack 1 sample 1	0.1396	s.e. 0.0450
rack 2 sample 3	-0.1043	s.e. 0.0450
rack 2 sample 6	0.1304	s.e. 0.0450
rack 1 sample 1 area 1	-0.1203	s.e. 0.0392
rack 1 sample 1 area 3	0.1647	s.e. 0.0392
rack 2 sample 3 area 1	-0.1248	s.e. 0.0392
rack 2 sample 3 area 3	0.1627	s.e. 0.0392
rack 4 sample 5 area 2	0.1431	s.e. 0.0392
rack 1 sample 1 area 1 strip 1	-0.3029	s.e. 0.0866
rack 1 sample 1 area 1 strip 3	0.4894	s.e. 0.0866
rack 1 sample 1 area 2 strip 2	0.3720	s.e. 0.0866
rack 1 sample 1 area 2 strip 3	-0.2716	s.e. 0.0866
rack 1 sample 1 area 3 strip 1	-0.5530	s.e. 0.0866
rack 1 sample 1 area 3 strip 2	-0.2820	s.e. 0.0866
rack 1 sample 1 area 3 strip 3	0.4918	s.e. 0.0866
rack 1 sample 1 area 3 strip 4	0.3431	s.e. 0.0866
rack 2 sample 3 area 4 strip 1	-0.2749	s.e. 0.0866
rack 2 sample 3 area 4 strip 3	0.3780	s.e. 0.0866
rack 3 sample 3 area 2 strip 2	0.3353	s.e. 0.0866
rack 3 sample 3 area 3 strip 4	0.2824	s.e. 0.0866
rack 3 sample 5 area 3 strip 3	0.2709	s.e. 0.0866
rack 3 sample 5 area 4 strip 1	-0.3306	s.e. 0.0866
rack 4 sample 5 area 1 strip 3	0.2577	s.e. 0.0866
rack 4 sample 5 area 1 strip 4	-0.2602	s.e. 0.0866
rack 5 sample 2 area 1 strip 2	0.4211	s.e. 0.0866

Tables of means

Variate: W6

Grand mean 0.1106

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	0.1243	0.0654	0.0398	0.0113	0.0089	0.5246	0.0000

treatment	acetic acid	carpropamid	tinuvin	water
	0.1113	0.1124	0.1144	0.1043

chem_charg	1	2	3	4
	0.1156	0.0964	0.1136	0.1168

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		0.0961	0.1240	0.1566	0.1204
filter 2		0.0499	0.0903	0.0500	0.0713
filter 3		0.0547	0.0333	0.0214	0.0499
filter 4		0.0119	0.0119	0.0190	0.0024
filter 5		0.0167	0.0119	0.0000	0.0071
full		0.5502	0.5154	0.5537	0.4791
None		0.0000	0.0000	0.0000	0.0000

Exposure	chem_charg	1	2	3	4
filter 1		0.1307	0.1164	0.1466	0.1035
filter 2		0.0571	0.0784	0.0714	0.0546
filter 3		0.0190	0.0308	0.0309	0.0785
filter 4		0.0143	0.0071	0.0190	0.0048
filter 5		0.0071	0.0047	0.0095	0.0143
full		0.5813	0.4374	0.5178	0.5620
None		0.0000	0.0000	0.0000	0.0000

treatment	chem_charg	1	2	3	4
acetic acid		0.1082	0.0802	0.1236	0.1333
carpropamid		0.1297	0.1064	0.0874	0.1260
tinuvin		0.1321	0.0948	0.1182	0.1125
water		0.0925	0.1041	0.1252	0.0954

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		0.0665	0.0666	0.0762	0.1751
	carpropamid		0.1902	0.1139	0.1053	0.0866
	tinuvin		0.1995	0.1235	0.2273	0.0761
	water		0.0665	0.1614	0.1775	0.0761
filter 2	acetic acid		0.0760	0.0379	0.0570	0.0286
	carpropamid		0.0381	0.1426	0.0665	0.1138
	tinuvin		0.0571	0.0285	0.0858	0.0285
	water		0.0570	0.1045	0.0762	0.0475
filter 3	acetic acid		0.0191	0.0190	0.0286	0.1521
	carpropamid		0.0000	0.0285	0.0381	0.0666
	tinuvin		0.0286	0.0190	0.0190	0.0190
	water		0.0285	0.0569	0.0379	0.0761
filter 4	acetic acid		0.0095	0.0000	0.0190	0.0191
	carpropamid		0.0191	0.0000	0.0286	0.0000
	tinuvin		0.0286	0.0190	0.0285	0.0000
	water		0.0000	0.0095	0.0000	0.0000
filter 5	acetic acid		0.0191	0.0000	0.0381	0.0095
	carpropamid		0.0095	0.0095	0.0000	0.0285
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0095	0.0000	0.0191
full	acetic acid		0.5672	0.4382	0.6465	0.5489
	carpropamid		0.6513	0.4506	0.3732	0.5864
	tinuvin		0.6112	0.4736	0.4665	0.6636
	water		0.4954	0.3871	0.5848	0.4491
None	acetic acid		0.0000	0.0000	0.0000	0.0000
	carpropamid		0.0000	0.0000	0.0000	0.0000
	tinuvin		0.0000	0.0000	0.0000	0.0000
	water		0.0000	0.0000	0.0000	0.0000

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	0.03434	0.01209	0.01336	0.04412
d.f.	24	84	336	58.36
Except when comparing means with the same level(s) of				
Exposure				0.03199
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	0.04600	0.02611	0.07547
d.f.	73.98	419.45	303.84
Except when comparing means with the same level(s) of			
Exposure	0.03535		0.06909
d.f.	336		419.45
treatment		0.02672	
d.f.		336	
Exposure.treatment			
			0.07070
d.f.			336
Exposure.chem_charg			
			0.06909
d.f.			419.45

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	0.07087	0.02405	0.02628	0.08831
d.f.	24	84	336	58.36
Except when comparing means with the same level(s) of				
Exposure				0.06362
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	0.09167	0.05133	0.14851
d.f.	73.98	419.45	303.84
Except when comparing means with the same level(s) of			
Exposure	0.06954		0.13580
d.f.	336		419.45
treatment		0.05257	
d.f.		336	
Exposure.treatment			0.13908
d.f.			336
Exposure.chem_charg			0.13580
d.f.			419.45

Analysis of variance

Variate: W8

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	18999.31	4749.83	0.92	
rack.sample stratum					
Exposure	6	168632.35	28105.39	5.47	0.001
Residual	24	123263.41	5135.98	48.06	
rack.sample.area stratum					
treatment	3	433.69	144.56	1.35	0.263
Exposure.treatment	18	4261.70	236.76	2.22	0.008
Residual	84	8976.56	106.86	1.79	
rack.sample.area.strip stratum					
chem_charg	3	52.56	17.52	0.29	0.830
Exposure.chem_charg	18	603.47	33.53	0.56	0.925
treatment.chem_charg	9	394.28	43.81	0.73	0.678
Exposure.treatment.chem_charg	54	2869.02	53.13	0.89	0.693
Residual	336	20061.61	59.71		
Total	559	348547.96			

Message: the following units have large residuals.

rack 1 sample 1	43.31	s.e. 14.84
rack 2 sample 3	-32.18	s.e. 14.84
rack 3 sample 5	-35.55	s.e. 14.84
rack 5 sample 2	43.30	s.e. 14.84
rack 1 sample 1 area 2	-11.56	s.e. 4.00
rack 4 sample 1 area 2	14.73	s.e. 4.00
rack 4 sample 1 area 4	-11.25	s.e. 4.00
rack 4 sample 5 area 2	22.81	s.e. 4.00
rack 4 sample 5 area 3	-20.32	s.e. 4.00
rack 5 sample 2 area 2	-11.56	s.e. 4.00
rack 2 sample 3 area 1 strip 3	20.83	s.e. 5.99
rack 2 sample 3 area 4 strip 2	-17.86	s.e. 5.99
rack 3 sample 5 area 4 strip 3	26.68	s.e. 5.99
rack 4 sample 1 area 2 strip 4	-18.93	s.e. 5.99
rack 4 sample 4 area 4 strip 1	18.33	s.e. 5.99
rack 4 sample 5 area 1 strip 2	47.80	s.e. 5.99
rack 4 sample 5 area 1 strip 3	-19.82	s.e. 5.99
rack 4 sample 5 area 1 strip 4	-21.38	s.e. 5.99
rack 4 sample 5 area 2 strip 1	-60.86	s.e. 5.99
rack 4 sample 5 area 2 strip 2	28.41	s.e. 5.99
rack 4 sample 5 area 2 strip 3	21.21	s.e. 5.99
rack 4 sample 5 area 4 strip 2	56.43	s.e. 5.99
rack 4 sample 5 area 4 strip 3	-18.71	s.e. 5.99
rack 4 sample 5 area 4 strip 4	-20.78	s.e. 5.99

Tables of means

Variate: W8

Grand mean 8.35

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	3.42	0.05	0.04	1.39	2.82	50.74	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	7.70	7.42	9.67	8.61

chem_charg	1	2	3	4
	8.88	8.13	8.24	8.16

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		3.64	4.37	4.00	1.68
filter 2		0.03	0.06	0.05	0.07
filter 3		0.06	0.02	0.02	0.05
filter 4		1.31	0.85	0.16	3.24
filter 5		0.01	3.60	1.16	6.50
full		48.86	43.04	62.30	48.76
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		4.70	2.96	3.90	2.13
filter 2		0.03	0.06	0.06	0.07
filter 3		0.04	0.03	0.04	0.04
filter 4		1.40	1.54	1.07	1.55
filter 5		3.20	1.85	5.28	0.96
full		52.78	50.46	47.36	52.36
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		10.12	6.88	7.25	6.56
carpropamid		7.34	7.79	7.83	6.72
tinuvin		9.85	10.31	9.12	9.41
water		8.20	7.54	8.77	9.95

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		8.24	2.39	3.84	0.07
	carpropamid		3.95	5.21	4.17	4.13
	tinuvin		3.80	3.95	4.02	4.23
	water		2.81	0.29	3.56	0.07
filter 2	acetic acid		0.01	0.04	0.01	0.07
	carpropamid		0.06	0.08	0.09	0.03
	tinuvin		0.03	0.07	0.03	0.06
	water		0.02	0.05	0.10	0.12
filter 3	acetic acid		0.05	0.07	0.07	0.05
	carpropamid		0.02	0.02	0.03	0.02
	tinuvin		0.03	0.00	0.02	0.04
	water		0.05	0.04	0.05	0.06
filter 4	acetic acid		0.00	1.43	0.54	3.26
	carpropamid		2.93	0.03	0.42	0.02
	tinuvin		0.64	0.00	0.00	0.00
	water		2.03	4.69	3.31	2.94
filter 5	acetic acid		0.03	0.00	0.02	0.01
	carpropamid		2.46	5.56	6.39	0.00
	tinuvin		0.00	0.07	4.13	0.45
	water		10.29	1.77	10.57	3.36
full	acetic acid		62.48	44.22	46.29	42.45
	carpropamid		41.96	43.65	43.72	42.81
	tinuvin		64.43	68.06	55.63	61.09
	water		42.24	45.93	43.80	63.09
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	11.331	1.236	0.924	11.680
d.f.	24	84	336	27.06
Except when comparing means with the same level(s) of				
Exposure				3.269
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	11.527	2.021	12.423
d.f.	25.70	353.39	34.58
Except when comparing means with the same level(s) of			
Exposure	2.444		5.348
d.f.	336		353.39
treatment		1.847	
d.f.		336	
Exposure.treatment			
			4.887
d.f.			336
Exposure.chem_charg			
			5.348
d.f.			353.39

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	23.387	2.457	1.817	23.962
d.f.	24	84	336	27.06
Except when comparing means with the same level(s) of				
Exposure				6.501
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	23.708	3.975	25.230
d.f.	25.70	353.39	34.58
Except when comparing means with the same level(s) of			
Exposure	4.806		10.517
d.f.	336		353.39
treatment		3.633	
d.f.		336	
Exposure.treatment			9.613
d.f.			336
Exposure.chem_charg			10.517
d.f.			353.39

Analysis of variance

Variate: W10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	42703.7	10675.9	1.81	
rack.sample stratum					
Exposure	6	473741.2	78956.9	13.36	<.001
Residual	24	141872.3	5911.3	9.00	
rack.sample.area stratum					
treatment	3	2054.6	684.9	1.04	0.378
Exposure.treatment	18	8288.4	460.5	0.70	0.800
Residual	84	55144.8	656.5	3.37	
rack.sample.area.strip stratum					
chem_charg	3	76.0	25.3	0.13	0.942
Exposure.chem_charg	18	2688.1	149.3	0.77	0.738
treatment.chem_charg	9	2950.9	327.9	1.68	0.091
Exposure.treatment.chem_charg	54	8347.3	154.6	0.79	0.848
Residual	336	65393.0	194.6		
Total	559	803260.4			

Message: the following units have large residuals.

rack 3 sample 2	-36.60	s.e. 15.92
rack 3 sample 6	33.72	s.e. 15.92
rack 4 sample 7	37.96	s.e. 15.92
rack 5 sample 3	36.66	s.e. 15.92
rack 1 sample 4 area 3	-28.82	s.e. 9.92
rack 1 sample 7 area 1	-28.22	s.e. 9.92
rack 1 sample 7 area 3	27.44	s.e. 9.92
rack 2 sample 4 area 4	-27.37	s.e. 9.92
rack 3 sample 6 area 4	-34.26	s.e. 9.92
rack 4 sample 2 area 1	-29.47	s.e. 9.92
rack 5 sample 3 area 1	-27.08	s.e. 9.92
rack 1 sample 4 area 1 strip 3	54.24	s.e. 10.81
rack 1 sample 4 area 1 strip 4	-34.97	s.e. 10.81
rack 1 sample 4 area 2 strip 1	-32.45	s.e. 10.81
rack 1 sample 4 area 2 strip 4	55.85	s.e. 10.81
rack 1 sample 4 area 4 strip 2	34.79	s.e. 10.81
rack 1 sample 7 area 2 strip 1	-33.50	s.e. 10.81
rack 1 sample 7 area 2 strip 4	44.80	s.e. 10.81
rack 2 sample 4 area 1 strip 1	-34.20	s.e. 10.81
rack 2 sample 4 area 1 strip 3	35.98	s.e. 10.81
rack 4 sample 2 area 4 strip 4	-45.80	s.e. 10.81
rack 4 sample 4 area 1 strip 4	37.61	s.e. 10.81
rack 4 sample 7 area 1 strip 1	-51.91	s.e. 10.81
rack 5 sample 3 area 1 strip 1	-44.33	s.e. 10.81
rack 5 sample 3 area 1 strip 3	35.33	s.e. 10.81
rack 5 sample 3 area 1 strip 4	39.16	s.e. 10.81
rack 5 sample 6 area 3 strip 3	32.81	s.e. 10.81
rack 5 sample 6 area 3 strip 4	-44.20	s.e. 10.81

Tables of means

Variate: W10

Grand mean 36.43

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	21.58	22.16	28.01	43.14	40.11	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	36.34	38.33	33.35	37.70

chem_charg	1	2	3	4
	35.87	36.58	36.88	36.39

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		20.57	25.50	21.12	19.15
filter 2		32.44	21.99	15.01	19.21
filter 3		28.84	31.83	23.34	28.03
filter 4		40.99	48.39	37.38	45.82
filter 5		31.52	40.60	36.64	51.66
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		24.08	18.60	25.24	18.42
filter 2		23.57	22.29	19.86	22.92
filter 3		27.70	30.22	30.19	23.93
filter 4		37.82	44.42	41.95	48.38
filter 5		37.90	40.55	40.90	41.08
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		39.20	37.38	35.56	33.20
carpropamid		32.74	40.59	40.56	39.43
tinuvin		33.79	32.93	30.99	35.71
water		37.74	35.43	40.41	37.21

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		25.68	20.23	22.20	14.18
	carpropamid		24.59	22.84	26.02	28.53
	tinuvin		25.04	19.10	22.72	17.60
	water		21.01	12.22	30.01	13.36
filter 2	acetic acid		46.27	25.37	25.83	32.29
	carpropamid		11.00	32.60	21.00	23.38
	tinuvin		16.67	17.45	12.82	13.08
	water		20.36	13.73	19.79	22.94
filter 3	acetic acid		32.54	35.42	29.20	18.22
	carpropamid		30.26	33.26	35.07	28.72
	tinuvin		13.04	28.79	28.46	23.07
	water		34.95	23.42	28.04	25.73
filter 4	acetic acid		40.86	40.69	42.28	40.11
	carpropamid		34.34	56.72	50.00	52.49
	tinuvin		41.30	28.98	27.82	51.40
	water		34.79	51.29	47.71	49.50
filter 5	acetic acid		29.05	39.97	29.42	27.62
	carpropamid		29.02	38.70	51.80	42.90
	tinuvin		40.44	36.18	25.10	44.85
	water		53.08	47.33	57.29	48.93
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	12.157	3.062	1.667	14.036
d.f.	24	84	336	41.34
Except when comparing means with the same level(s) of				
Exposure				8.102
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	12.743	4.209	15.981
d.f.	28.95	250.35	68.74
Except when comparing means with the same level(s) of			
Exposure	4.412		11.137
d.f.	336		250.35
treatment		3.335	
d.f.		336	
Exposure.treatment			
			8.823
d.f.			336
Exposure.chem_charg			
			11.137
d.f.			250.35

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	25.090	6.090	3.280	28.340
d.f.	24	84	336	41.34
Except when comparing means with the same level(s) of				
Exposure				16.112
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	26.064	8.290	31.884
d.f.	28.95	250.35	68.74
Except when comparing means with the same level(s) of			
Exposure	8.678		21.934
d.f.	336		250.35
treatment		6.560	
d.f.		336	
Exposure.treatment			17.356
d.f.			336
Exposure.chem_charg			21.934
d.f.			250.35

Analysis of variance

Variate: W12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	54957.3	13739.3	2.36	
rack.sample stratum					
Exposure	6	526440.6	87740.1	15.09	<.001
Residual	24	139584.1	5816.0	10.15	
rack.sample.area stratum					
treatment	3	3824.6	1274.9	2.22	0.091
Exposure.treatment	18	6752.2	375.1	0.65	0.845
Residual	84	48155.1	573.3	3.49	
rack.sample.area.strip stratum					
chem_charg	3	1063.1	354.4	2.16	0.093
Exposure.chem_charg	18	2641.8	146.8	0.89	0.587
treatment.chem_charg	9	1070.5	118.9	0.72	0.687
Exposure.treatment.chem_charg	54	8491.1	157.2	0.96	0.563
Residual	336	55197.4	164.3		
Total	559	848177.9			

Message: the following units have large residuals.

rack 2 sample 5	-35.60	s.e. 15.79
rack 1 sample 7 area 1	-38.39	s.e. 9.27
rack 1 sample 7 area 4	28.27	s.e. 9.27
rack 3 sample 3 area 2	23.79	s.e. 9.27
rack 4 sample 3 area 1	-39.22	s.e. 9.27
rack 4 sample 3 area 3	33.42	s.e. 9.27
rack 1 sample 2 area 1 strip 1	-50.32	s.e. 9.93
rack 1 sample 2 area 1 strip 3	42.08	s.e. 9.93
rack 1 sample 2 area 1 strip 4	29.57	s.e. 9.93
rack 1 sample 2 area 2 strip 4	-33.30	s.e. 9.93
rack 1 sample 2 area 4 strip 4	-39.50	s.e. 9.93
rack 1 sample 5 area 3 strip 1	40.69	s.e. 9.93
rack 1 sample 5 area 3 strip 3	-31.20	s.e. 9.93
rack 1 sample 7 area 2 strip 1	-33.57	s.e. 9.93
rack 2 sample 4 area 4 strip 4	-30.10	s.e. 9.93
rack 3 sample 2 area 4 strip 1	-32.14	s.e. 9.93
rack 3 sample 4 area 1 strip 1	-35.58	s.e. 9.93
rack 4 sample 4 area 1 strip 1	-41.94	s.e. 9.93
rack 5 sample 4 area 1 strip 1	-46.74	s.e. 9.93
rack 5 sample 4 area 4 strip 4	-37.21	s.e. 9.93
rack 5 sample 7 area 1 strip 1	-35.09	s.e. 9.93
rack 5 sample 7 area 1 strip 3	33.32	s.e. 9.93
rack 5 sample 7 area 4 strip 4	-30.89	s.e. 9.93

Tables of means

Variate: W12

Grand mean 69.97

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	70.38	71.16	69.36	86.24	92.69	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	73.62	69.85	66.24	70.20

chem_charg	1	2	3	4
	69.24	68.13	71.66	70.88

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		73.58	71.26	67.13	69.53
filter 2		86.14	70.60	61.14	66.75
filter 3		76.92	64.44	64.08	71.99
filter 4		85.66	87.72	82.68	88.91
filter 5		93.04	94.89	88.62	94.20
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		71.27	71.27	70.26	68.70
filter 2		71.26	68.72	72.61	72.04
filter 3		67.56	65.59	75.66	68.63
filter 4		81.25	84.66	86.98	92.08
filter 5		93.32	86.66	96.08	94.69
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		74.41	71.41	74.23	74.42
carpropamid		66.95	67.21	74.21	71.02
tinuvin		67.29	64.91	65.19	67.55
water		68.30	68.98	73.00	70.52

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		74.85	76.93	69.21	73.32
	carpropamid		74.96	64.30	71.45	74.34
	tinuvin		67.21	71.10	66.19	64.03
	water		68.08	72.74	74.19	63.12
filter 2	acetic acid		86.52	87.12	85.92	84.98
	carpropamid		59.05	76.12	75.96	71.28
	tinuvin		67.65	57.36	57.89	61.67
	water		71.83	54.27	70.66	70.24
filter 3	acetic acid		71.39	76.24	83.21	76.82
	carpropamid		55.42	57.28	82.32	62.75
	tinuvin		67.13	63.58	64.07	61.56
	water		76.31	65.24	73.06	73.36
filter 4	acetic acid		89.57	73.46	83.04	96.57
	carpropamid		84.66	87.73	89.72	88.78
	tinuvin		81.57	82.30	81.26	85.59
	water		69.21	95.14	93.91	97.38
filter 5	acetic acid		98.57	86.13	98.24	89.22
	carpropamid		94.55	85.02	100.00	100.00
	tinuvin		87.50	80.03	86.94	100.00
	water		92.66	95.48	99.15	89.52
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	12.058	2.862	1.532	13.726
d.f.	24	84	336	39.31
Except when comparing means with the same level(s) of				
Exposure				7.571
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	12.559	3.903	15.417
d.f.	28.23	245.20	62.07
Except when comparing means with the same level(s) of			
Exposure	4.053		10.325
d.f.	336		245.20
treatment		3.064	
d.f.		336	
Exposure.treatment			
			8.106
d.f.			336
Exposure.chem_charg			
			10.325
d.f.			245.20

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	24.887	5.691	3.013	27.756
d.f.	24	84	336	39.31
Except when comparing means with the same level(s) of				
Exposure				15.057
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	25.716	7.687	30.817
d.f.	28.23	245.20	62.07
Except when comparing means with the same level(s) of			
Exposure	7.973		20.338
d.f.	336		245.20
treatment		6.027	
d.f.		336	
Exposure.treatment			15.945
d.f.			336
Exposure.chem_charg			20.338
d.f.			245.20

Analysis of variance

Variate: W14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	22266.7	5566.7	1.67	
rack.sample stratum					
Exposure	6	568970.9	94828.5	28.48	<.001
Residual	24	79903.4	3329.3	9.68	
rack.sample.area stratum					
treatment	3	3072.6	1024.2	2.98	0.036
Exposure.treatment	18	5659.0	314.4	0.91	0.564
Residual	84	28885.8	343.9	2.38	
rack.sample.area.strip stratum					
chem_charg	3	606.0	202.0	1.40	0.243
Exposure.chem_charg	18	3045.2	169.2	1.17	0.282
treatment.chem_charg	9	1165.3	129.5	0.90	0.528
Exposure.treatment.chem_charg	54	7863.6	145.6	1.01	0.464
Residual	336	48508.7	144.4		
Total	559	769947.1			

Message: the following units have large residuals.

rack 1 sample 3	-32.52	s.e. 11.95
rack 2 sample 5	-37.58	s.e. 11.95
rack 1 sample 5 area 1	-21.40	s.e. 7.18
rack 1 sample 7 area 4	19.56	s.e. 7.18
rack 3 sample 1 area 1	-19.18	s.e. 7.18
rack 3 sample 1 area 4	23.14	s.e. 7.18
rack 4 sample 3 area 1	-34.83	s.e. 7.18
rack 1 sample 2 area 1 strip 1	-53.01	s.e. 9.31
rack 1 sample 2 area 1 strip 3	37.78	s.e. 9.31
rack 1 sample 2 area 2 strip 4	-31.18	s.e. 9.31
rack 1 sample 2 area 4 strip 4	-39.15	s.e. 9.31
rack 1 sample 5 area 1 strip 1	-34.96	s.e. 9.31
rack 1 sample 5 area 1 strip 4	32.48	s.e. 9.31
rack 1 sample 5 area 3 strip 1	27.96	s.e. 9.31
rack 1 sample 5 area 4 strip 4	-37.16	s.e. 9.31
rack 1 sample 7 area 1 strip 1	-35.61	s.e. 9.31
rack 1 sample 7 area 2 strip 1	-39.32	s.e. 9.31
rack 3 sample 3 area 1 strip 1	-28.75	s.e. 9.31
rack 4 sample 4 area 1 strip 1	-36.47	s.e. 9.31
rack 5 sample 4 area 4 strip 4	-30.79	s.e. 9.31
rack 5 sample 5 area 1 strip 1	-28.12	s.e. 9.31

Tables of means

Variate: W14

Grand mean 75.26

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	75.92	84.73	77.16	95.15	93.88	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	77.25	75.36	71.41	77.03

chem_charg	1	2	3	4
	75.29	74.04	76.89	74.84

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		79.02	78.09	73.83	72.74
filter 2		89.87	86.93	70.53	91.58
filter 3		82.18	71.82	73.45	81.21
filter 4		94.29	95.39	92.16	98.75
filter 5		95.39	95.32	89.88	94.94
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		76.59	79.99	76.11	70.99
filter 2		88.04	82.45	85.80	82.62
filter 3		73.25	76.16	81.86	77.39
filter 4		94.12	92.82	97.40	96.25
filter 5		95.02	86.85	97.06	96.60
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		77.78	74.98	78.34	77.89
carpropamid		72.54	75.01	77.61	76.30
tinuvin		73.27	69.54	71.32	71.51
water		77.57	76.62	80.29	73.64

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		76.46	82.76	76.76	80.09
	carpropamid		84.34	78.77	70.55	78.69
	tinuvin		72.51	78.12	76.80	67.90
	water		73.03	80.31	80.34	57.28
filter 2	acetic acid		92.10	89.90	89.20	88.26
	carpropamid		76.04	90.90	90.57	90.23
	tinuvin		88.59	60.77	66.77	66.00
	water		95.42	88.23	96.66	86.00
filter 3	acetic acid		79.10	83.45	85.63	80.53
	carpropamid		58.11	73.87	85.66	69.63
	tinuvin		71.47	75.15	70.28	76.89
	water		84.31	72.15	85.88	82.51
filter 4	acetic acid		96.79	82.60	98.09	99.68
	carpropamid		93.52	95.97	96.51	95.58
	tinuvin		91.17	92.73	94.99	89.76
	water		94.99	100.00	100.00	100.00
filter 5	acetic acid		100.00	86.17	98.70	96.70
	carpropamid		95.74	85.53	100.00	100.00
	tinuvin		89.11	80.03	90.36	100.00
	water		95.23	95.66	99.16	89.71
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	9.123	2.216	1.436	10.441
d.f.	24	84	336	40.08
Except when comparing means with the same level(s) of				
Exposure				5.864
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	9.698	3.332	12.342
d.f.	30.61	307.07	76.80
Except when comparing means with the same level(s) of			
Exposure	3.800		8.815
d.f.	336		307.07
treatment		2.872	
d.f.		336	
Exposure.treatment			
			7.599
d.f.			336
Exposure.chem_charg			
			8.815
d.f.			307.07

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	18.829	4.408	2.825	21.102
d.f.	24	84	336	40.08
Except when comparing means with the same level(s) of				
Exposure				11.661
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	19.790	6.556	24.578
d.f.	30.61	307.07	76.80
Except when comparing means with the same level(s) of			
Exposure	7.474		17.345
d.f.	336		307.07
treatment		5.650	
d.f.		336	
Exposure.treatment			14.948
d.f.			336
Exposure.chem_charg			17.345
d.f.			307.07

Analysis of variance

Variate: W16

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	21168.8	5292.2	2.14	
rack.sample stratum					
Exposure	6	589554.1	98259.0	39.68	<.001
Residual	24	59435.4	2476.5	9.75	
rack.sample.area stratum					
treatment	3	1564.6	521.5	2.05	0.113
Exposure.treatment	18	2341.0	130.1	0.51	0.945
Residual	84	21337.6	254.0	2.10	
rack.sample.area.strip stratum					
chem_charg	3	625.1	208.4	1.72	0.162
Exposure.chem_charg	18	2615.9	145.3	1.20	0.258
treatment.chem_charg	9	638.1	70.9	0.59	0.809
Exposure.treatment.chem_charg	54	6234.3	115.5	0.95	0.571
Residual	336	40679.7	121.1		
Total	559	746194.6			

Message: the following units have large residuals.

rack 1 sample 3	-34.84	s.e. 10.30
rack 2 sample 5	-25.98	s.e. 10.30
rack 1 sample 5 area 1	-22.33	s.e. 6.17
rack 3 sample 1 area 1	-20.96	s.e. 6.17
rack 3 sample 1 area 4	19.35	s.e. 6.17
rack 1 sample 2 area 1 strip 1	-51.76	s.e. 8.52
rack 1 sample 2 area 1 strip 3	34.13	s.e. 8.52
rack 1 sample 2 area 1 strip 4	27.92	s.e. 8.52
rack 1 sample 2 area 4 strip 4	-36.88	s.e. 8.52
rack 1 sample 5 area 1 strip 1	-30.08	s.e. 8.52
rack 1 sample 5 area 1 strip 2	-28.61	s.e. 8.52
rack 1 sample 5 area 1 strip 4	33.41	s.e. 8.52
rack 1 sample 5 area 3 strip 1	27.14	s.e. 8.52
rack 1 sample 5 area 4 strip 4	-31.70	s.e. 8.52
rack 1 sample 7 area 1 strip 1	-36.05	s.e. 8.52
rack 1 sample 7 area 2 strip 1	-37.36	s.e. 8.52
rack 2 sample 1 area 4 strip 1	25.84	s.e. 8.52
rack 2 sample 1 area 4 strip 4	-28.20	s.e. 8.52
rack 2 sample 4 area 4 strip 4	-28.57	s.e. 8.52
rack 3 sample 3 area 1 strip 1	-26.05	s.e. 8.52
rack 4 sample 3 area 1 strip 1	-45.58	s.e. 8.52
rack 5 sample 4 area 4 strip 4	-37.10	s.e. 8.52

Tables of means

Variate: W16

Grand mean 77.80

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	80.34	89.89	82.81	96.32	95.21	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	79.70	76.90	75.53	79.05

chem_charg	1	2	3	4
	77.93	77.11	79.45	76.69

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		82.26	79.72	80.06	79.33
filter 2		90.31	89.23	84.78	95.22
filter 3		90.49	78.24	79.76	82.76
filter 4		97.52	95.54	93.01	99.22
filter 5		97.35	95.56	91.08	96.83
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		80.42	83.55	80.54	76.86
filter 2		91.00	91.42	92.58	84.54
filter 3		81.17	81.71	87.50	80.88
filter 4		95.77	94.83	98.15	96.55
filter 5		97.17	88.27	97.41	97.97
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		80.30	78.47	80.16	79.89
carpropamid		74.55	76.67	79.61	76.78
tinuvin		76.85	74.95	76.57	73.74
water		80.03	78.36	81.47	76.34

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		80.12	85.84	80.80	82.28
	carpropamid		85.02	80.90	73.01	79.96
	tinuvin		75.57	86.23	84.08	74.34
	water		80.97	81.26	84.25	70.85
filter 2	acetic acid		92.10	90.66	89.77	88.72
	carpropamid		77.27	93.99	93.64	92.04
	tinuvin		98.38	83.95	86.91	69.88
	water		96.26	97.10	100.00	87.52
filter 3	acetic acid		89.87	91.54	90.55	90.01
	carpropamid		69.82	79.25	94.10	69.80
	tinuvin		77.85	81.66	78.48	81.04
	water		87.11	74.38	86.88	82.67
filter 4	acetic acid		100.00	90.37	100.00	99.69
	carpropamid		93.83	96.20	96.51	95.63
	tinuvin		92.35	92.73	96.07	90.88
	water		96.89	100.00	100.00	100.00
filter 5	acetic acid		100.00	90.89	100.00	98.53
	carpropamid		95.89	86.34	100.00	100.00
	tinuvin		93.79	80.08	90.45	100.00
	water		99.01	95.77	99.19	93.36
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	7.868	1.905	1.315	8.998
d.f.	24	84	336	39.96
Except when comparing means with the same level(s) of				
Exposure				5.040
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	8.426	2.969	10.830
d.f.	31.51	328.20	81.90
Except when comparing means with the same level(s) of			
Exposure	3.480		7.856
d.f.	336		328.20
treatment		2.630	
d.f.		336	
Exposure.treatment			
			6.959
d.f.			336
Exposure.chem_charg			
			7.856
d.f.			328.20

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	16.240	3.788	2.587	18.186
d.f.	24	84	336	39.96
Except when comparing means with the same level(s) of				
Exposure				10.023
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	17.173	5.842	21.544
d.f.	31.51	328.20	81.90
Except when comparing means with the same level(s) of			
Exposure	6.844		15.455
d.f.	336		328.20
treatment		5.174	
d.f.		336	
Exposure.treatment			
			13.689
d.f.			336
Exposure.chem_charg			
			15.455
d.f.			328.20

Analysis of variance

Variate: W16

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	21168.8	5292.2	2.14	
rack.sample stratum					
Exposure	6	589554.1	98259.0	39.68	<.001
Residual	24	59435.4	2476.5	9.75	
rack.sample.area stratum					
treatment	3	1564.6	521.5	2.05	0.113
Exposure.treatment	18	2341.0	130.1	0.51	0.945
Residual	84	21337.6	254.0	2.10	
rack.sample.area.strip stratum					
chem_charg	3	625.1	208.4	1.72	0.162
Exposure.chem_charg	18	2615.9	145.3	1.20	0.258
treatment.chem_charg	9	638.1	70.9	0.59	0.809
Exposure.treatment.chem_charg					
	54	6234.3	115.5	0.95	0.571
Residual	336	40679.7	121.1		
Total	559	746194.6			

Message: the following units have large residuals.

rack 1 sample 3	-34.84	s.e. 10.30
rack 2 sample 5	-25.98	s.e. 10.30
rack 1 sample 5 area 1	-22.33	s.e. 6.17
rack 3 sample 1 area 1	-20.96	s.e. 6.17
rack 3 sample 1 area 4	19.35	s.e. 6.17
rack 1 sample 2 area 1 strip 1	-51.76	s.e. 8.52
rack 1 sample 2 area 1 strip 3	34.13	s.e. 8.52
rack 1 sample 2 area 1 strip 4	27.92	s.e. 8.52
rack 1 sample 2 area 4 strip 4	-36.88	s.e. 8.52
rack 1 sample 5 area 1 strip 1	-30.08	s.e. 8.52
rack 1 sample 5 area 1 strip 2	-28.61	s.e. 8.52
rack 1 sample 5 area 1 strip 4	33.41	s.e. 8.52
rack 1 sample 5 area 3 strip 1	27.14	s.e. 8.52
rack 1 sample 5 area 4 strip 4	-31.70	s.e. 8.52
rack 1 sample 7 area 1 strip 1	-36.05	s.e. 8.52
rack 1 sample 7 area 2 strip 1	-37.36	s.e. 8.52
rack 2 sample 1 area 4 strip 1	25.84	s.e. 8.52
rack 2 sample 1 area 4 strip 4	-28.20	s.e. 8.52
rack 2 sample 4 area 4 strip 4	-28.57	s.e. 8.52
rack 3 sample 3 area 1 strip 1	-26.05	s.e. 8.52
rack 4 sample 3 area 1 strip 1	-45.58	s.e. 8.52
rack 5 sample 4 area 4 strip 4	-37.10	s.e. 8.52

Tables of means

Variate: W16

Grand mean 77.80

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	80.34	89.89	82.81	96.32	95.21	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	79.70	76.90	75.53	79.05

chem_charg	1	2	3	4
	77.93	77.11	79.45	76.69

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		82.26	79.72	80.06	79.33
filter 2		90.31	89.23	84.78	95.22
filter 3		90.49	78.24	79.76	82.76
filter 4		97.52	95.54	93.01	99.22
filter 5		97.35	95.56	91.08	96.83
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		80.42	83.55	80.54	76.86
filter 2		91.00	91.42	92.58	84.54
filter 3		81.17	81.71	87.50	80.88
filter 4		95.77	94.83	98.15	96.55
filter 5		97.17	88.27	97.41	97.97
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		80.30	78.47	80.16	79.89
carpropamid		74.55	76.67	79.61	76.78
tinuvin		76.85	74.95	76.57	73.74
water		80.03	78.36	81.47	76.34

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		80.12	85.84	80.80	82.28
	carpropamid		85.02	80.90	73.01	79.96
	tinuvin		75.57	86.23	84.08	74.34
	water		80.97	81.26	84.25	70.85
filter 2	acetic acid		92.10	90.66	89.77	88.72
	carpropamid		77.27	93.99	93.64	92.04
	tinuvin		98.38	83.95	86.91	69.88
	water		96.26	97.10	100.00	87.52
filter 3	acetic acid		89.87	91.54	90.55	90.01
	carpropamid		69.82	79.25	94.10	69.80
	tinuvin		77.85	81.66	78.48	81.04
	water		87.11	74.38	86.88	82.67
filter 4	acetic acid		100.00	90.37	100.00	99.69
	carpropamid		93.83	96.20	96.51	95.63
	tinuvin		92.35	92.73	96.07	90.88
	water		96.89	100.00	100.00	100.00
filter 5	acetic acid		100.00	90.89	100.00	98.53
	carpropamid		95.89	86.34	100.00	100.00
	tinuvin		93.79	80.08	90.45	100.00
	water		99.01	95.77	99.19	93.36
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	7.868	1.905	1.315	8.998
d.f.	24	84	336	39.96
Except when comparing means with the same level(s) of				
Exposure				5.040
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	8.426	2.969	10.830
d.f.	31.51	328.20	81.90
Except when comparing means with the same level(s) of			
Exposure	3.480		7.856
d.f.	336		328.20
treatment		2.630	
d.f.		336	
Exposure.treatment			
			6.959
d.f.			336
Exposure.chem_charg			
			7.856
d.f.			328.20

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	16.240	3.788	2.587	18.186
d.f.	24	84	336	39.96
Except when comparing means with the same level(s) of				
Exposure				10.023
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	17.173	5.842	21.544
d.f.	31.51	328.20	81.90
Except when comparing means with the same level(s) of			
Exposure	6.844		15.455
d.f.	336		328.20
treatment		5.174	
d.f.		336	
Exposure.treatment			13.689
d.f.			336
Exposure.chem_charg			15.455
d.f.			328.20

Analysis of variance

Variate: W18

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	11058.43	2764.61	2.41	
rack.sample stratum					
Exposure	6	628804.85	104800.81	91.41	<.001
Residual	24	27516.86	1146.54	8.44	
rack.sample.area stratum					
treatment	3	586.96	195.65	1.44	0.237
Exposure.treatment	18	3074.65	170.81	1.26	0.238
Residual	84	11414.88	135.89	1.74	
rack.sample.area.strip stratum					
chem_charg	3	474.53	158.18	2.03	0.110
Exposure.chem_charg	18	1868.60	103.81	1.33	0.165
treatment.chem_charg	9	411.25	45.69	0.59	0.809
Exposure.treatment.chem_charg	54	3750.95	69.46	0.89	0.691
Residual	336	26204.62	77.99		
Total	559	715166.62			

Message: the following units have large residuals.

rack 1 sample 3	-24.30	s.e. 7.01
rack 1 sample 5	-15.27	s.e. 7.01
rack 1 sample 3 area 1	-12.81	s.e. 4.51
rack 1 sample 5 area 1	-17.97	s.e. 4.51
rack 1 sample 5 area 2	11.79	s.e. 4.51
rack 3 sample 1 area 1	-24.22	s.e. 4.51
rack 3 sample 1 area 4	12.90	s.e. 4.51
rack 1 sample 2 area 1 strip 1	-49.28	s.e. 6.84
rack 1 sample 2 area 4 strip 4	-29.17	s.e. 6.84
rack 1 sample 3 area 2 strip 4	-21.48	s.e. 6.84
rack 1 sample 5 area 1 strip 1	-26.46	s.e. 6.84
rack 1 sample 5 area 1 strip 2	-30.73	s.e. 6.84
rack 1 sample 5 area 1 strip 3	23.55	s.e. 6.84
rack 1 sample 5 area 1 strip 4	33.65	s.e. 6.84
rack 1 sample 5 area 3 strip 1	23.91	s.e. 6.84
rack 1 sample 5 area 4 strip 4	-32.23	s.e. 6.84
rack 2 sample 5 area 4 strip 1	26.14	s.e. 6.84
rack 2 sample 6 area 1 strip 1	-23.41	s.e. 6.84
rack 3 sample 1 area 2 strip 1	-24.04	s.e. 6.84
rack 3 sample 3 area 1 strip 1	-26.40	s.e. 6.84
rack 4 sample 3 area 1 strip 1	-26.16	s.e. 6.84
rack 5 sample 4 area 4 strip 4	-30.19	s.e. 6.84

Tables of means

Variate: W18

Grand mean 81.51

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	88.80	94.01	90.78	99.37	97.64	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	81.99	80.59	80.50	82.96

chem_charg	1	2	3	4
	81.72	80.77	82.93	80.63

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		89.33	88.78	88.19	88.89
filter 2		90.41	96.59	90.95	98.08
filter 3		96.52	81.88	90.54	94.18
filter 4		99.74	99.34	98.83	99.57
filter 5		97.96	97.57	95.02	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		89.12	91.20	87.76	87.10
filter 2		95.05	93.38	96.96	90.64
filter 3		89.95	89.87	95.86	87.44
filter 4		98.81	99.19	100.00	99.47
filter 5		99.12	91.76	99.93	99.75
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		81.80	81.69	82.09	82.39
carpropamid		79.72	80.91	83.13	78.61
tinuvin		81.99	78.58	81.90	79.54
water		83.38	81.90	84.61	81.96

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		86.03	91.60	86.91	92.79
	carpropamid		92.46	93.08	84.66	84.92
	tinuvin		89.47	90.47	86.61	86.19
	water		88.54	89.66	92.87	84.51
filter 2	acetic acid		92.28	90.75	89.83	88.78
	carpropamid		90.59	98.48	99.82	97.49
	tinuvin		98.65	87.01	98.19	79.94
	water		98.70	97.27	100.00	96.35
filter 3	acetic acid		94.30	97.66	97.90	96.21
	carpropamid		75.52	84.56	97.44	69.99
	tinuvin		91.85	90.88	88.74	90.67
	water		98.13	86.36	99.36	92.87
filter 4	acetic acid		100.00	98.94	100.00	100.00
	carpropamid		99.47	100.00	100.00	97.89
	tinuvin		97.50	97.82	100.00	100.00
	water		98.29	100.00	100.00	100.00
filter 5	acetic acid		100.00	92.87	100.00	98.99
	carpropamid		100.00	90.28	100.00	100.00
	tinuvin		96.48	83.89	99.72	100.00
	water		100.00	100.00	100.00	100.00
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	5.354	1.393	1.056	6.233
d.f.	24	84	336	42.56
Except when comparing means with the same level(s) of				
Exposure				3.686
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	5.875	2.299	7.890
d.f.	34.69	357.40	104.46
Except when comparing means with the same level(s) of			
Exposure	2.793		6.082
d.f.	336		357.40
treatment		2.111	
d.f.		336	
Exposure.treatment			
			5.585
d.f.			336
Exposure.chem_charg			
			6.082
d.f.			357.40

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	11.050	2.771	2.076	12.575
d.f.	24	84	336	42.56
Except when comparing means with the same level(s) of				
Exposure				7.331
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	11.930	4.521	15.645
d.f.	34.69	357.40	104.46
Except when comparing means with the same level(s) of			
Exposure	5.493		11.960
d.f.	336		357.40
treatment		4.153	
d.f.		336	
Exposure.treatment			
			10.987
d.f.			336
Exposure.chem_charg			
			11.960
d.f.			357.40

Analysis of variance

Variate: W20

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	4129.59	1032.40	1.97	
rack.sample stratum					
Exposure	6	652197.44	108699.57	207.06	<.001
Residual	24	12599.11	524.96	5.17	
rack.sample.area stratum					
treatment	3	345.59	115.20	1.13	0.340
Exposure.treatment	18	1887.64	104.87	1.03	0.433
Residual	84	8527.32	101.52	2.37	
rack.sample.area.strip stratum					
chem_charg	3	265.12	88.37	2.06	0.105
Exposure.chem_charg	18	1001.33	55.63	1.30	0.187
treatment.chem_charg	9	415.67	46.19	1.08	0.380
Exposure.treatment.chem_charg					
	54	2884.64	53.42	1.24	0.129
Residual	336	14421.69	42.92		
Total	559	698675.15			

Message: the following units have large residuals.

rack 1 sample 3	-19.75	s.e. 4.74
rack 3 sample 1	-10.39	s.e. 4.74
rack 1 sample 3 area 2	10.47	s.e. 3.90
rack 1 sample 5 area 1	-16.37	s.e. 3.90
rack 3 sample 1 area 1	-21.15	s.e. 3.90
rack 3 sample 1 area 4	12.06	s.e. 3.90
rack 3 sample 4 area 1	9.84	s.e. 3.90
rack 3 sample 4 area 4	-11.59	s.e. 3.90
rack 1 sample 2 area 1 strip 1	-42.72	s.e. 5.07
rack 1 sample 2 area 4 strip 4	-28.56	s.e. 5.07
rack 1 sample 3 area 1 strip 1	-19.27	s.e. 5.07
rack 1 sample 3 area 4 strip 4	-18.65	s.e. 5.07
rack 1 sample 5 area 1 strip 1	-42.32	s.e. 5.07
rack 1 sample 5 area 1 strip 3	22.73	s.e. 5.07
rack 1 sample 5 area 1 strip 4	23.52	s.e. 5.07
rack 1 sample 5 area 4 strip 4	-22.52	s.e. 5.07
rack 2 sample 5 area 4 strip 1	15.15	s.e. 5.07
rack 2 sample 6 area 1 strip 1	-16.75	s.e. 5.07
rack 3 sample 1 area 2 strip 1	-21.34	s.e. 5.07
rack 3 sample 4 area 1 strip 4	18.76	s.e. 5.07
rack 4 sample 7 area 2 strip 1	17.92	s.e. 5.07
rack 5 sample 7 area 1 strip 2	18.74	s.e. 5.07

Tables of means

Variate: W20

Grand mean 83.40

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	92.78	96.58	96.07	99.88	98.48	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	83.56	82.27	83.29	84.48

chem_charg	1	2	3	4
	83.63	82.82	84.42	82.73

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		93.08	91.87	91.77	94.38
filter 2		91.95	97.23	98.17	98.99
filter 3		100.00	89.18	97.14	97.96
filter 4		100.00	100.00	99.53	100.00
filter 5		99.88	97.62	96.44	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		92.57	93.02	94.03	91.48
filter 2		96.62	96.28	97.52	95.91
filter 3		96.70	96.47	99.41	91.70
filter 4		99.53	100.00	100.00	100.00
filter 5		100.00	93.94	100.00	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		83.35	83.38	83.53	83.97
carpropamid		82.49	82.42	84.78	79.40
tinuvin		83.89	81.94	84.27	83.07
water		84.79	83.52	85.11	84.48

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		88.87	93.14	94.12	96.19
	carpropamid		93.19	93.71	93.84	86.75
	tinuvin		94.66	92.69	92.35	87.38
	water		93.56	92.56	95.80	95.60
filter 2	acetic acid		94.59	91.04	90.60	91.57
	carpropamid		91.89	99.43	99.82	97.77
	tinuvin		100.00	95.16	99.65	97.84
	water		100.00	99.51	100.00	96.45
filter 3	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		92.35	93.32	99.78	71.26
	tinuvin		94.43	100.00	97.86	96.27
	water		100.00	92.55	100.00	99.28
filter 4	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		98.11	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
filter 5	acetic acid		100.00	99.50	100.00	100.00
	carpropamid		100.00	90.48	100.00	100.00
	tinuvin		100.00	85.76	100.00	100.00
	water		100.00	100.00	100.00	100.00
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	3.623	1.204	0.783	4.554
d.f.	24	84	336	54.67
Except when comparing means with the same level(s) of				
Exposure				3.186
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	4.043	1.814	5.798
d.f.	37.06	308.26	135.15
Except when comparing means with the same level(s) of			
Exposure	2.072		4.799
d.f.	336		308.26
treatment		1.566	
d.f.		336	
Exposure.treatment			
			4.144
d.f.			336
Exposure.chem_charg			
			4.799
d.f.			308.26

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	7.477	2.395	1.540	9.127
d.f.	24	84	336	54.67
Except when comparing means with the same level(s) of				
Exposure				6.336
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	8.191	3.569	11.466
d.f.	37.06	308.26	135.15
Except when comparing means with the same level(s) of			
Exposure	4.075		9.442
d.f.	336		308.26
treatment		3.081	
d.f.		336	
Exposure.treatment			
			8.150
d.f.			336
Exposure.chem_charg			
			9.442
d.f.			308.26

Analysis of variance

Variate: W24

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	4063.56	1015.89	1.95	
rack.sample stratum					
Exposure	6	652508.29	108751.38	208.78	<.001
Residual	24	12501.48	520.89	5.17	
rack.sample.area stratum					
treatment	3	338.92	112.97	1.12	0.345
Exposure.treatment	18	1857.59	103.20	1.02	0.443
Residual	84	8467.51	100.80	2.37	
rack.sample.area.strip stratum					
chem_charg	3	254.30	84.77	1.99	0.115
Exposure.chem_charg	18	995.70	55.32	1.30	0.186
treatment.chem_charg	9	401.65	44.63	1.05	0.402
Exposure.treatment.chem_charg					
	54	2843.21	52.65	1.24	0.136
Residual	336	14312.43	42.60		
Total	559	698544.64			

Message: the following units have large residuals.

rack 1 sample 3	-19.67	s.e. 4.72
rack 3 sample 1	-10.35	s.e. 4.72
rack 1 sample 3 area 2	10.50	s.e. 3.89
rack 1 sample 5 area 1	-16.41	s.e. 3.89
rack 1 sample 5 area 3	9.78	s.e. 3.89
rack 3 sample 1 area 1	-21.10	s.e. 3.89
rack 3 sample 1 area 4	11.91	s.e. 3.89
rack 3 sample 4 area 1	9.80	s.e. 3.89
rack 3 sample 4 area 4	-11.57	s.e. 3.89
rack 1 sample 2 area 1 strip 1	-42.72	s.e. 5.06
rack 1 sample 2 area 4 strip 4	-28.56	s.e. 5.06
rack 1 sample 3 area 1 strip 1	-19.06	s.e. 5.06
rack 1 sample 3 area 4 strip 4	-18.82	s.e. 5.06
rack 1 sample 5 area 1 strip 1	-42.33	s.e. 5.06
rack 1 sample 5 area 1 strip 3	22.66	s.e. 5.06
rack 1 sample 5 area 1 strip 4	23.56	s.e. 5.06
rack 1 sample 5 area 4 strip 4	-22.38	s.e. 5.06
rack 2 sample 6 area 1 strip 1	-16.34	s.e. 5.06
rack 3 sample 1 area 2 strip 1	-21.11	s.e. 5.06
rack 3 sample 4 area 1 strip 4	18.76	s.e. 5.06
rack 4 sample 7 area 2 strip 1	17.92	s.e. 5.06
rack 5 sample 7 area 1 strip 2	18.72	s.e. 5.06

Tables of means

Variate: W24

Grand mean 83.42

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	92.88	96.55	96.08	99.95	98.48	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	83.57	82.31	83.31	84.50

chem_charg	1	2	3	4
	83.64	82.84	84.43	82.78

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		93.12	92.08	91.84	94.48
filter 2		91.99	97.25	97.94	99.01
filter 3		100.00	89.23	97.14	97.97
filter 4		100.00	100.00	99.81	100.00
filter 5		99.88	97.62	96.44	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		92.59	93.12	94.07	91.75
filter 2		96.40	96.31	97.52	95.97
filter 3		96.70	96.52	99.41	91.71
filter 4		99.81	100.00	100.00	100.00
filter 5		100.00	93.94	100.00	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		83.35	83.39	83.54	84.00
carpropamid		82.50	82.45	84.78	79.51
tinuvin		83.92	81.97	84.27	83.08
water		84.79	83.55	85.13	84.51

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		88.87	93.16	94.19	96.27
	carpropamid		93.20	93.76	93.85	87.51
	tinuvin		94.73	92.82	92.35	87.48
	water		93.56	92.73	95.89	95.76
filter 2	acetic acid		94.59	91.07	90.60	91.71
	carpropamid		91.97	99.43	99.82	97.79
	tinuvin		99.05	95.21	99.66	97.85
	water		100.00	99.52	100.00	96.54
filter 3	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		92.35	93.47	99.78	71.30
	tinuvin		94.43	100.00	97.87	96.27
	water		100.00	92.60	100.00	99.28
filter 4	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		99.22	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
filter 5	acetic acid		100.00	99.51	100.00	100.00
	carpropamid		100.00	90.48	100.00	100.00
	tinuvin		100.00	85.76	100.00	100.00
	water		100.00	100.00	100.00	100.00
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	3.609	1.200	0.780	4.537
d.f.	24	84	336	54.69
Except when comparing means with the same level(s) of				
Exposure				3.175
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	4.027	1.807	5.776
d.f.	37.06	308.16	135.20
Except when comparing means with the same level(s) of			
Exposure	2.064		4.781
d.f.	336		308.16
treatment		1.560	
d.f.		336	
Exposure.treatment			
			4.128
d.f.			336
Exposure.chem_charg			
			4.781
d.f.			308.16

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	7.448	2.386	1.534	9.093
d.f.	24	84	336	54.69
Except when comparing means with the same level(s) of				
Exposure				6.314
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	8.159	3.556	11.423
d.f.	37.06	308.16	135.20
Except when comparing means with the same level(s) of			
Exposure	4.060		9.408
d.f.	336		308.16
treatment		3.069	
d.f.		336	
Exposure.treatment			
			8.120
d.f.			336
Exposure.chem_charg			
			9.408
d.f.			308.16

Analysis of variance

Variate: W32

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	3073.36	768.34	1.43	
rack.sample stratum					
Exposure	6	659370.22	109895.04	203.97	<.001
Residual	24	12930.70	538.78	8.35	
rack.sample.area stratum					
treatment	3	220.62	73.54	1.14	0.338
Exposure.treatment	18	1104.01	61.33	0.95	0.522
Residual	84	5418.19	64.50	2.63	
rack.sample.area.strip stratum					
chem_charg	3	97.70	32.57	1.33	0.265
Exposure.chem_charg	18	585.87	32.55	1.33	0.168
treatment.chem_charg	9	226.74	25.19	1.03	0.418
Exposure.treatment.chem_charg					
	54	1861.58	34.47	1.41	0.039
Residual	336	8240.80	24.53		
Total	559	693129.80			

Message: the following units have large residuals.

rack 1 sample 3	-20.93	s.e. 4.81
rack 3 sample 1	-10.53	s.e. 4.81
rack 1 sample 3 area 2	9.92	s.e. 3.11
rack 3 sample 1 area 1	-20.36	s.e. 3.11
rack 3 sample 1 area 3	8.41	s.e. 3.11
rack 3 sample 1 area 4	11.41	s.e. 3.11
rack 3 sample 4 area 4	-9.99	s.e. 3.11
rack 1 sample 2 area 1 strip 1	-24.75	s.e. 3.84
rack 1 sample 2 area 4 strip 4	-24.43	s.e. 3.84
rack 1 sample 3 area 1 strip 1	-18.92	s.e. 3.84
rack 1 sample 3 area 3 strip 1	-15.26	s.e. 3.84
rack 1 sample 3 area 4 strip 4	-18.37	s.e. 3.84
rack 1 sample 5 area 1 strip 1	-33.39	s.e. 3.84
rack 1 sample 5 area 1 strip 3	12.04	s.e. 3.84
rack 1 sample 5 area 4 strip 4	-17.26	s.e. 3.84
rack 3 sample 1 area 2 strip 1	-20.83	s.e. 3.84
rack 3 sample 4 area 1 strip 4	14.89	s.e. 3.84
rack 4 sample 7 area 2 strip 1	14.11	s.e. 3.84
rack 5 sample 7 area 1 strip 2	14.11	s.e. 3.84

Tables of means

Variate: W32

Grand mean 83.91

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	93.68	97.12	97.57	100.00	98.98	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	83.66	83.24	83.80	84.93

chem_charg	1	2	3	4
	83.96	83.75	84.54	83.38

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		93.50	92.86	92.26	96.11
filter 2		92.09	97.32	99.19	99.87
filter 3		100.00	94.54	97.18	98.56
filter 4		100.00	100.00	100.00	100.00
filter 5		100.00	97.96	97.94	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		92.63	94.81	94.79	92.49
filter 2		96.68	97.22	97.54	97.03
filter 3		98.38	98.30	99.43	94.18
filter 4		100.00	100.00	100.00	100.00
filter 5		100.00	95.90	100.00	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		83.36	83.47	83.76	84.03
carpropamid		83.47	83.48	84.95	81.06
tinuvin		84.19	83.51	84.29	83.19
water		84.80	84.53	85.14	85.26

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		88.90	93.16	95.65	96.27
	carpropamid		93.20	94.12	95.07	89.04
	tinuvin		94.81	94.25	92.44	87.53
	water		93.62	97.69	96.01	97.10
filter 2	acetic acid		94.61	91.14	90.66	91.95
	carpropamid		92.11	99.43	99.82	97.91
	tinuvin		100.00	98.56	99.67	98.54
	water		100.00	99.77	100.00	99.72
filter 3	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		99.00	98.95	99.78	80.44
	tinuvin		94.50	100.00	97.95	96.27
	water		100.00	94.25	100.00	100.00
filter 4	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
filter 5	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	91.86	100.00	100.00
	tinuvin		100.00	91.75	100.00	100.00
	water		100.00	100.00	100.00	100.00
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	3.670	0.960	0.592	4.279
d.f.	24	84	336	42.76
Except when comparing means with the same level(s) of				
Exposure				2.540
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	3.913	1.404	5.066
d.f.	30.96	290.46	82.34
Except when comparing means with the same level(s) of			
Exposure	1.566		3.716
d.f.	336		290.46
treatment		1.184	
d.f.		336	
Exposure.treatment			
			3.132
d.f.			336
Exposure.chem_charg			
			3.716
d.f.			290.46

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	7.575	1.909	1.164	8.630
d.f.	24	84	336	42.76
Except when comparing means with the same level(s) of				
Exposure				5.051
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	7.980	2.764	10.077
d.f.	30.96	290.46	82.34
Except when comparing means with the same level(s) of			
Exposure	3.081		7.314
d.f.	336		290.46
treatment		2.329	
d.f.		336	
Exposure.treatment			6.161
d.f.			336
Exposure.chem_charg			7.314
d.f.			290.46

Analysis of variance

Variate: W40

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rack stratum	4	3073.36	768.34	1.43	
rack.sample stratum					
Exposure	6	659370.22	109895.04	203.97	<.001
Residual	24	12930.70	538.78	8.35	
rack.sample.area stratum					
treatment	3	220.62	73.54	1.14	0.338
Exposure.treatment	18	1104.01	61.33	0.95	0.522
Residual	84	5418.19	64.50	2.63	
rack.sample.area.strip stratum					
chem_charg	3	97.70	32.57	1.33	0.265
Exposure.chem_charg	18	585.87	32.55	1.33	0.168
treatment.chem_charg	9	226.74	25.19	1.03	0.418
Exposure.treatment.chem_charg	54	1861.58	34.47	1.41	0.039
Residual	336	8240.80	24.53		
Total	559	693129.80			

Message: the following units have large residuals.

rack 1 sample 3	-20.93	s.e. 4.81
rack 3 sample 1	-10.53	s.e. 4.81
rack 1 sample 3 area 2	9.92	s.e. 3.11
rack 3 sample 1 area 1	-20.36	s.e. 3.11
rack 3 sample 1 area 3	8.41	s.e. 3.11
rack 3 sample 1 area 4	11.41	s.e. 3.11
rack 3 sample 4 area 4	-9.99	s.e. 3.11
rack 1 sample 2 area 1 strip 1	-24.75	s.e. 3.84
rack 1 sample 2 area 4 strip 4	-24.43	s.e. 3.84
rack 1 sample 3 area 1 strip 1	-18.92	s.e. 3.84
rack 1 sample 3 area 3 strip 1	-15.26	s.e. 3.84
rack 1 sample 3 area 4 strip 4	-18.37	s.e. 3.84
rack 1 sample 5 area 1 strip 1	-33.39	s.e. 3.84
rack 1 sample 5 area 1 strip 3	12.04	s.e. 3.84
rack 1 sample 5 area 4 strip 4	-17.26	s.e. 3.84
rack 3 sample 1 area 2 strip 1	-20.83	s.e. 3.84
rack 3 sample 4 area 1 strip 4	14.89	s.e. 3.84
rack 4 sample 7 area 2 strip 1	14.11	s.e. 3.84
rack 5 sample 7 area 1 strip 2	14.11	s.e. 3.84

Tables of means

Variate: W40

Grand mean 83.91

Exposure	filter 1	filter 2	filter 3	filter 4	filter 5	full	None
	93.68	97.12	97.57	100.00	98.98	100.00	0.00

treatment	acetic acid	carpropamid	tinuvin	water
	83.66	83.24	83.80	84.93

chem_charg	1	2	3	4
	83.96	83.75	84.54	83.38

Exposure	treatment	acetic acid	carpropamid	tinuvin	water
filter 1		93.50	92.86	92.26	96.11
filter 2		92.09	97.32	99.19	99.87
filter 3		100.00	94.54	97.18	98.56
filter 4		100.00	100.00	100.00	100.00
filter 5		100.00	97.96	97.94	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

Exposure	chem_charg	1	2	3	4
filter 1		92.63	94.81	94.79	92.49
filter 2		96.68	97.22	97.54	97.03
filter 3		98.38	98.30	99.43	94.18
filter 4		100.00	100.00	100.00	100.00
filter 5		100.00	95.90	100.00	100.00
full		100.00	100.00	100.00	100.00
None		0.00	0.00	0.00	0.00

treatment	chem_charg	1	2	3	4
acetic acid		83.36	83.47	83.76	84.03
carpropamid		83.47	83.48	84.95	81.06
tinuvin		84.19	83.51	84.29	83.19
water		84.80	84.53	85.14	85.26

Exposure	treatment	chem_charg	1	2	3	4
filter 1	acetic acid		88.90	93.16	95.65	96.27
	carpropamid		93.20	94.12	95.07	89.04
	tinuvin		94.81	94.25	92.44	87.53
	water		93.62	97.69	96.01	97.10
filter 2	acetic acid		94.61	91.14	90.66	91.95
	carpropamid		92.11	99.43	99.82	97.91
	tinuvin		100.00	98.56	99.67	98.54
	water		100.00	99.77	100.00	99.72
filter 3	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		99.00	98.95	99.78	80.44
	tinuvin		94.50	100.00	97.95	96.27
	water		100.00	94.25	100.00	100.00
filter 4	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
filter 5	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	91.86	100.00	100.00
	tinuvin		100.00	91.75	100.00	100.00
	water		100.00	100.00	100.00	100.00
full	acetic acid		100.00	100.00	100.00	100.00
	carpropamid		100.00	100.00	100.00	100.00
	tinuvin		100.00	100.00	100.00	100.00
	water		100.00	100.00	100.00	100.00
None	acetic acid		0.00	0.00	0.00	0.00
	carpropamid		0.00	0.00	0.00	0.00
	tinuvin		0.00	0.00	0.00	0.00
	water		0.00	0.00	0.00	0.00

Standard errors of differences of means

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
s.e.d.	3.670	0.960	0.592	4.279
d.f.	24	84	336	42.76
Except when comparing means with the same level(s) of				
Exposure				2.540
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
s.e.d.	3.913	1.404	5.066
d.f.	30.96	290.46	82.34
Except when comparing means with the same level(s) of			
Exposure	1.566		3.716
d.f.	336		290.46
treatment		1.184	
d.f.		336	
Exposure.treatment			
			3.132
d.f.			336
Exposure.chem_charg			
			3.716
d.f.			290.46

Least significant differences of means (5% level)

Table	Exposure	treatment	chem_charg	Exposure treatment
rep.	80	140	140	20
l.s.d.	7.575	1.909	1.164	8.630
d.f.	24	84	336	42.76
Except when comparing means with the same level(s) of				
Exposure				5.051
d.f.				84

Table	Exposure chem_charg	treatment chem_charg	Exposure treatment chem_charg
rep.	20	35	5
l.s.d.	7.980	2.764	10.077
d.f.	30.96	290.46	82.34
Except when comparing means with the same level(s) of			
Exposure	3.081		7.314
d.f.	336		290.46
treatment		2.329	
d.f.		336	
Exposure.treatment			
			6.161
d.f.			336
Exposure.chem_charg			
			7.314
d.f.			290.46

Analysis of variance color of wood surfaces 0 to 40 weeks

Analysis of variance week 1
Variate: L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		203.640	50.910	1.62	
block.board stratum						
exposure	5		3247.592	649.518	20.70	<.001
Residual	20		627.563	31.378	1.13	
block.board.area stratum						
treatment	3		114.951	38.317	1.38	0.255
exposure.treatment	15		167.494	11.166	0.40	0.974
Residual	72		1995.746	27.719	3.61	
block.board.area.Strip stratum						
Chem_Charge	3		51.084	17.028	2.22	0.086
exposure.Chem_Charge	15		245.000	16.333	2.13	0.009
treatment.Chem_Charge	9		85.084	9.454	1.23	0.275
exposure.treatment.Chem_Charge						
	45		353.529	7.856	1.02	0.436
Residual	286	(2)	2193.402	7.669		
Total	477	(2)	9188.195			

Message: the following units have large residuals.

block 3 board 4	2.34	s.e. 1.14
block 3 board 5 area 3	-5.55	s.e. 2.04
block 5 board 3 area 1 Strip 2	6.26	s.e. 2.14

Tables of means

Variate: L

Grand mean 74.64

exposure	full	I.R.	none	UVA	UVB	Vis light
	69.90	74.48	76.79	75.86	73.09	77.73

treatment	acetic acid	carpropamid	tinuvin	water
	73.87	74.88	75.20	74.61

Chem_Charge	high	low	medium	Very high
	75.18	74.40	74.36	74.63

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		68.72	70.01	71.08	69.77
I.R.		73.28	74.83	75.80	73.99
none		75.31	77.21	77.22	77.41
UVA		75.19	75.63	76.98	75.66
UVB		73.70	72.59	72.81	73.25
Vis light		77.05	79.00	77.30	77.56

exposure	Chem_Charge	high	low	medium	Very high
full		70.23	70.43	68.86	70.06
I.R.		75.01	75.37	74.40	73.11
none		76.69	75.17	77.62	77.67
UVA		76.77	75.80	75.38	75.51
UVB		73.91	73.35	71.89	73.20
Vis light		78.45	76.29	77.98	78.20

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		74.36	73.79	73.99	73.36
carpropamid		75.48	73.77	74.88	75.39
tinuvin		75.96	75.33	74.06	75.45
water		74.90	74.72	74.49	74.31

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		70.13	67.63	67.97	69.13
	carpropamid		70.18	70.39	69.38	70.11
	tinuvin		70.18	72.87	69.28	72.01
	water		70.45	70.83	68.83	68.98
I.R.	acetic acid		73.48	75.91	73.63	70.11
	carpropamid		76.60	73.56	75.00	74.15
	tinuvin		76.11	76.88	74.49	75.70
	water		73.85	75.14	74.49	72.49
none	acetic acid		75.58	73.46	77.05	75.13
	carpropamid		77.54	76.36	76.31	78.64
	tinuvin		76.93	76.12	77.85	77.97
	water		76.69	74.74	79.26	78.95
UVA	acetic acid		75.49	74.22	75.72	75.32
	carpropamid		75.28	74.22	76.64	76.39
	tinuvin		79.46	78.01	74.73	75.73
	water		76.86	76.74	74.43	74.60
UVB	acetic acid		73.87	75.67	71.71	73.56
	carpropamid		74.48	70.00	72.72	73.16
	tinuvin		74.73	73.41	69.77	73.34
	water		72.56	74.34	73.37	72.74
Vis light	acetic acid		77.60	75.84	77.89	76.88
	carpropamid		78.80	78.11	79.20	79.89
	tinuvin		78.37	74.67	78.23	77.94
	water		79.01	76.54	76.60	78.11

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.626	0.481	0.253	1.197
d.f.	20	72	286	90.30
Except when comparing means with the same level(s) of exposure				1.177
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.825	0.650	1.607
d.f.	57.91	205.49	243.98
Except when comparing means with the same level(s) of			
exposure	0.619		1.593
d.f.	286		205.49
treatment		0.506	
d.f.		286	
exposure.treatment			
			1.238
d.f.			286
exposure.Chem_Charge			
			1.593
d.f.			205.49

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.886	0.680	0.358	1.692
d.f.	20	72	286	90.30
Except when comparing means with the same level(s) of				
exposure				1.665
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.166	0.919	2.272
d.f.	57.91	205.49	243.98
Except when comparing means with the same level(s) of			
exposure	0.876		2.252
d.f.	286		205.49
treatment		0.715	
d.f.		286	
exposure.treatment			
			1.751
d.f.			286
exposure.Chem_Charge			
			2.252
d.f.			205.49

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.848	1.355	0.704	3.362
d.f.	20	72	286	90.30
Except when comparing means with the same level(s) of				
exposure				3.319
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.334	1.813	4.476
d.f.	57.91	205.49	243.98
Except when comparing means with the same level(s) of			
exposure	1.724		4.440
d.f.	286		205.49
treatment		1.407	
d.f.		286	
exposure.treatment			3.447
d.f.			286
exposure.Chem_Charge			4.440
d.f.			205.49

(Not adjusted for missing values)

Analysis of variance

Variate: a

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		52.758	13.190	2.22	
block.board stratum						
exposure	5		661.387	132.277	22.26	<.001
Residual	20		118.855	5.943	1.54	
block.board.area stratum						
treatment	3		14.674	4.891	1.26	0.293
exposure.treatment	15		32.193	2.146	0.55	0.899
Residual	72		278.444	3.867	2.81	

block.board.area.Strip stratum						
Chem_Charge	3		6.770	2.257	1.64	0.180
exposure.Chem_Charge	15		23.106	1.540	1.12	0.339
treatment.Chem_Charge	9		16.868	1.874	1.36	0.205
exposure.treatment.Chem_Charge	45		78.789	1.751	1.27	0.126
Residual	287	(1)	395.129	1.377		
Total	478	(1)	1666.813			

Message: the following units have large residuals.

block 3 board 1	1.076	s.e.	0.498
block 1 board 3 area 1	2.173	s.e.	0.762
block 2 board 5 area 2	2.072	s.e.	0.762
block 1 board 5 area 3 Strip 3	-2.720	s.e.	0.907
block 1 board 5 area 3 Strip 4	3.519	s.e.	0.907
block 2 board 5 area 1 Strip 3	3.005	s.e.	0.907
block 3 board 5 area 3 Strip 4	-3.165	s.e.	0.907
block 5 board 6 area 2 Strip 3	2.693	s.e.	0.907

Tables of means

Variate: a

Grand mean 5.984

exposure	full	I.R.	none	UVA	UVB	Vis light
	7.844	6.131	5.552	5.127	6.970	4.284

treatment	acetic acid	carpropamid	tinuvin	water
	6.223	5.833	5.802	6.080

Chem_Charge	high	low	medium	Very high
	5.903	6.143	6.051	5.842

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		7.935	7.997	7.207	8.237
I.R.		6.609	5.605	6.150	6.159
none		6.161	5.442	5.223	5.380
UVA		5.322	5.252	4.678	5.254
UVB		6.825	6.940	6.969	7.144
Vis light		4.483	3.763	4.582	4.306

exposure	Chem_Charge	high	low	medium	Very high
full		7.812	7.737	8.148	7.680
I.R.		6.165	6.110	6.104	6.144
none		5.729	6.125	5.175	5.177
UVA		4.743	5.153	5.449	5.160
UVB		6.934	6.935	7.222	6.787
Vis light		4.032	4.795	4.204	4.102

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		6.280	6.226	6.136	6.248
carpropamid		5.736	6.298	5.738	5.561
tinuvin		5.498	5.963	6.284	5.462
water		6.096	6.085	6.044	6.097

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		7.660	7.972	8.556	7.554
	carpropamid		7.984	7.904	8.226	7.874
	tinuvin		7.490	6.790	7.658	6.892
	water		8.114	8.282	8.152	8.402
I.R.	acetic acid		7.210	5.630	7.048	6.550
	carpropamid		5.314	6.824	4.498	5.784
	tinuvin		5.962	6.232	6.846	5.560
	water		6.174	5.756	6.022	6.684
none	acetic acid		5.840	7.296	5.180	6.328
	carpropamid		5.298	5.568	5.858	5.044
	tinuvin		5.430	5.852	4.884	4.726
	water		6.348	5.786	4.778	4.610
UVA	acetic acid		5.260	5.602	5.094	5.332
	carpropamid		5.316	5.822	5.150	4.720
	tinuvin		4.006	4.060	5.820	4.826
	water		4.390	5.130	5.734	5.764
UVB	acetic acid		7.190	5.934	7.020	7.156
	carpropamid		6.804	7.618	6.766	6.572
	tinuvin		6.066	6.986	8.300	6.524
	water		7.676	7.202	6.804	6.896
Vis light	acetic acid		4.522	4.920	3.920	4.570
	carpropamid		3.702	4.050	3.930	3.370
	tinuvin		4.034	5.858	4.194	4.242
	water		3.872	4.352	4.774	4.228

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.2726	0.1795	0.1071	0.4683
d.f.	20	72	287	84.67
Except when comparing means with the same level(s) of				
exposure				0.4397
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.3548	0.2582	0.6525
d.f.	55.59	239.41	253.03
Except when comparing means with the same level(s) of			
exposure	0.2624		0.6324
d.f.	287		239.41
treatment		0.2142	
d.f.		287	
exposure.treatment			
			0.5247
d.f.			287
exposure.Chem_Charge			
			0.6324
d.f.			239.41

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.3854	0.2539	0.1515	0.6623
d.f.	20	72	287	84.67
Except when comparing means with the same level(s) of				
exposure				0.6219
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.5018	0.3651	0.9228
d.f.	55.59	239.41	253.03
Except when comparing means with the same level(s) of			
exposure	0.3710		0.8943
d.f.	287		239.41
treatment		0.3030	
d.f.		287	
exposure.treatment			
			0.7421
d.f.			287
exposure.Chem_Charge			
			0.8943
d.f.			239.41

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	0.8040	0.5061	0.2982	1.3169
d.f.	20	72	287	84.67
Except when comparing means with the same level(s) of				
exposure				1.2397
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.0054	0.7192	1.8174
d.f.	55.59	239.41	253.03
Except when comparing means with the same level(s) of			
exposure	0.7303		1.7617
d.f.	287		239.41
treatment		0.5963	
d.f.		287	
exposure.treatment			1.4606
d.f.			287
exposure.Chem_Charge			1.7617
d.f.			239.41

(Not adjusted for missing values)

Analysis of variance

Variate: b

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		193.012	48.253	3.90	
block.board stratum						
exposure	5		7183.577	1436.715	116.25	<.001
Residual	20		247.177	12.359	1.34	
block.board.area stratum						
treatment	3		18.869	6.290	0.68	0.565
exposure.treatment	15		144.988	9.666	1.05	0.417
Residual	72		662.691	9.204	3.12	

block.board.area.Strip stratum						
Chem_Charge	3		27.156	9.052	3.07	0.028
exposure.Chem_Charge	15		42.079	2.805	0.95	0.507
treatment.Chem_Charge	9		42.809	4.757	1.61	0.111
exposure.treatment.Chem_Charge	45		216.364	4.808	1.63	0.010
Residual	287	(1)	846.301	2.949		
Total	478	(1)	9593.434			

Message: the following units have large residuals.

block 4 board 6	-2.102	s.e. 0.718
block 1 board 3 area 1	3.414	s.e. 1.175
block 5 board 6 area 4	-3.117	s.e. 1.175
block 1 board 4 area 2 Strip 1	4.481	s.e. 1.328
block 1 board 4 area 2 Strip 4	-4.505	s.e. 1.328
block 1 board 5 area 2 Strip 4	-4.611	s.e. 1.328
block 1 board 5 area 3 Strip 3	-4.167	s.e. 1.328
block 5 board 6 area 2 Strip 3	4.623	s.e. 1.328
block 5 board 6 area 2 Strip 4	-4.292	s.e. 1.328

Tables of means

Variate: b

Grand mean 26.625

exposure	full	I.R.	none	UVA	UVB	Vis light
	32.156	25.095	24.795	24.102	31.654	21.950

treatment	acetic acid	carpropamid	tinuvin	water
	26.910	26.366	26.548	26.677

Chem_Charge	high	low	medium	Very high
	26.715	26.897	26.645	26.246

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		31.857	32.529	31.422	32.816
I.R.		25.409	24.159	26.179	24.634
none		25.292	24.812	24.254	24.820
UVA		24.663	24.451	23.062	24.230
UVB		31.950	31.502	31.502	31.661
Vis light		22.291	20.742	22.866	21.901

exposure	Chem_Charge	high	low	medium	Very high
full		32.207	32.408	32.247	31.763
I.R.		25.466	25.176	24.906	24.833
none		25.066	25.420	24.429	24.264
UVA		23.565	24.094	24.658	24.090
UVB		32.189	31.567	31.755	31.104
Vis light		21.793	22.716	21.872	21.419

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		27.092	27.076	26.616	26.859
carpropamid		26.486	26.972	26.351	25.656
tinuvin		26.118	27.037	27.043	25.993
water		27.163	26.503	26.568	26.474

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		32.418	32.090	32.166	30.754
	carpropamid		32.220	32.708	32.880	32.310
	tinuvin		31.214	31.776	31.594	31.106
	water		32.976	33.058	32.348	32.882
I.R.	acetic acid		25.824	23.904	26.224	25.684
	carpropamid		24.202	26.752	21.822	23.860
	tinuvin		26.456	26.216	27.248	24.798
	water		25.384	23.832	24.328	24.992
none	acetic acid		24.694	26.960	23.934	25.582
	carpropamid		24.516	25.050	25.470	24.214
	tinuvin		24.352	25.128	23.982	23.556
	water		26.704	24.542	24.332	23.704
UVA	acetic acid		24.408	25.074	24.936	24.236
	carpropamid		24.792	25.002	24.762	23.250
	tinuvin		22.056	21.868	24.822	23.502
	water		23.004	24.434	24.112	25.372
UVB	acetic acid		32.608	31.682	30.956	32.554
	carpropamid		32.498	31.274	31.836	30.400
	tinuvin		30.716	32.126	32.514	30.652
	water		32.936	31.186	31.714	30.810
Vis light	acetic acid		22.598	22.744	21.478	22.344
	carpropamid		20.688	21.044	21.334	19.902
	tinuvin		21.912	25.108	22.100	22.344
	water		21.974	21.968	22.576	21.086

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.3930	0.2769	0.1568	0.7069
d.f.	20	72	287	87.66
Except when comparing means with the same level(s) of				
exposure				0.6784
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.5148	0.3878	0.9705
d.f.	56.85	224.82	251.39
Except when comparing means with the same level(s) of			
exposure	0.3840		0.9500
d.f.	287		224.82
treatment		0.3135	
d.f.		287	
exposure.treatment			
			0.7680
d.f.			287
exposure.Chem_Charge			
			0.9500
d.f.			224.82

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.5559	0.3917	0.2217	0.9996
d.f.	20	72	287	87.66
Except when comparing means with the same level(s) of exposure				0.9594
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7281	0.5485	1.3726
d.f.	56.85	224.82	251.39
Except when comparing means with the same level(s) of exposure	0.5430		1.3435
d.f.	287		224.82
treatment		0.4434	
d.f.		287	
exposure.treatment			1.0861
d.f.			287
exposure.Chem_Charge			1.3435
d.f.			224.82

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.1595	0.7808	0.4363	1.9867
d.f.	20	72	287	87.66
Except when comparing means with the same level(s) of				
exposure				1.9125
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.4581	1.0808	2.7032
d.f.	56.85	224.82	251.39
Except when comparing means with the same level(s) of			
exposure	1.0688		2.6475
d.f.	287		224.82
treatment		0.8727	
d.f.		287	
exposure.treatment			
			2.1376
d.f.			287
exposure.Chem_Charge			
			2.6475
d.f.			224.82

(Not adjusted for missing values)

Analysis of variance week 2

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	183.691	45.923	0.33	
block.board stratum					
exposure	5	2917.945	583.589	4.23	0.009
Residual	20	2761.097	138.055	5.33	
block.board.area stratum					
treatment	3	164.561	54.854	2.12	0.106
exposure.treatment	15	251.601	16.773	0.65	0.826
Residual	72	1866.594	25.925	2.95	

block.board.area.Strip stratum					
Chem_Charge	3	19.789	6.596	0.75	0.523
exposure.Chem_Charge	15	96.547	6.436	0.73	0.751
treatment.Chem_Charge	9	118.107	13.123	1.49	0.150
exposure.treatment.Chem_Charge	45	238.213	5.294	0.60	0.979
Residual	288	2530.019	8.785		
Total	479	11148.164			

Message: the following units have large residuals.

block 4 board 1	5.24	s.e. 2.40
block 5 board 4	-5.29	s.e. 2.40
block 4 board 3 area 1 Strip 3	-7.61	s.e. 2.30
block 5 board 2 area 3 Strip 1	-7.04	s.e. 2.30

Tables of means

Variate: L

Grand mean 74.13

exposure	full	I.R.	none	UVA	UVB	Vis light
	70.84	74.77	76.10	73.38	71.70	77.98

treatment	acetic acid	carpropamid	tinuvin	water
	73.64	74.24	75.04	73.59

Chem_Charge	high	low	medium	Very high
	74.38	74.27	73.88	73.99

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		70.34	69.93	71.89	71.19
I.R.		72.41	75.94	76.48	74.25
none		75.64	76.64	77.19	74.94
UVA		73.59	73.45	73.43	73.04
UVB		72.22	70.77	72.24	71.57
Vis light		77.66	78.72	79.01	76.55

exposure	Chem_Charge	high	low	medium	Very high
full		70.74	70.79	69.89	71.92
I.R.		74.92	74.77	74.52	74.87
none		76.05	76.43	76.58	75.34
UVA		73.57	73.53	73.16	73.25
UVB		72.63	71.63	71.03	71.50
Vis light		78.33	78.48	78.07	77.05

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		74.08	73.34	73.84	73.31
carpropamid		74.49	73.97	74.37	74.12
tinuvin		75.05	75.13	74.07	75.91
water		73.87	74.65	73.22	72.61

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		70.66	69.55	69.99	71.16
	carpropamid		70.21	69.50	68.89	71.10
	tinuvin		70.20	71.98	70.40	74.99
	water		71.87	72.15	70.29	70.44
I.R.	acetic acid		73.17	71.93	71.95	72.60
	carpropamid		75.94	75.74	75.50	76.56
	tinuvin		77.46	76.03	75.77	76.65
	water		73.12	75.35	74.87	73.66
none	acetic acid		75.10	76.41	76.23	74.82
	carpropamid		77.66	75.35	77.68	75.85
	tinuvin		76.63	78.41	76.35	77.38
	water		74.83	75.56	76.05	73.32
UVA	acetic acid		74.21	73.37	74.60	72.16
	carpropamid		72.94	72.48	74.02	74.34
	tinuvin		73.22	73.87	73.06	73.57
	water		73.90	74.38	70.95	72.94
UVB	acetic acid		72.56	72.12	72.21	71.99
	carpropamid		71.71	71.14	70.44	69.81
	tinuvin		74.90	70.68	69.72	73.66
	water		71.38	72.59	71.77	70.53
Vis light	acetic acid		78.80	76.65	78.06	77.13
	carpropamid		78.50	79.61	79.71	77.06
	tinuvin		77.91	79.81	79.11	79.22
	water		78.12	77.85	75.41	74.80

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	1.314	0.465	0.271	1.643
d.f.	20	72	288	44.92
Except when comparing means with the same level(s) of exposure				1.139
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	1.434	0.660	2.004
d.f.	28.29	232.68	95.95
Except when comparing means with the same level(s) of			
exposure	0.663		1.617
d.f.	288		232.68
treatment		0.541	
d.f.		288	
exposure.treatment			
			1.326
d.f.			288
exposure.Chem_Charge			
			1.617
d.f.			232.68

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.858	0.657	0.383	2.323
d.f.	20	72	288	44.92
Except when comparing means with the same level(s) of				
exposure				1.610
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.027	0.933	2.834
d.f.	28.29	232.68	95.95
Except when comparing means with the same level(s) of			
exposure	0.937		2.286
d.f.	288		232.68
treatment		0.765	
d.f.		288	
exposure.treatment			
			1.875
d.f.			288
exposure.Chem_Charge			
			2.286
d.f.			232.68

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	3.875	1.310	0.753	4.679
d.f.	20	72	288	44.92
Except when comparing means with the same level(s) of				
exposure				3.210
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	4.151	1.839	5.625
d.f.	28.29	232.68	95.95
Except when comparing means with the same level(s) of			
exposure	1.845		4.505
d.f.	288		232.68
treatment		1.506	
d.f.		288	
exposure.treatment			
			3.690
d.f.			288
exposure.Chem_Charge			
			4.505
d.f.			232.68

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	109.664	27.416	1.09	
block.board stratum					
exposure	5	906.451	181.290	7.24	<.001
Residual	20	501.068	25.053	5.36	
block.board.area stratum					
treatment	3	13.666	4.555	0.97	0.410
exposure.treatment	15	33.972	2.265	0.48	0.941
Residual	72	336.610	4.675	2.71	
block.board.area.Strip stratum					
Chem_Charge	3	1.390	0.463	0.27	0.848
exposure.Chem_Charge	15	28.593	1.906	1.11	0.350
treatment.Chem_Charge	9	18.186	2.021	1.17	0.312
exposure.treatment.Chem_Charge	45	75.967	1.688	0.98	0.514
Residual	288	496.247	1.723		
Total	479	2521.813			

Message: the following units have large residuals.

block 4 board 1	-2.442	s.e. 1.022
block 3 board 5 area 1	2.311	s.e. 0.837
block 1 board 5 area 3 Strip 2	3.023	s.e. 1.017
block 1 board 5 area 4 Strip 2	3.619	s.e. 1.017
block 4 board 4 area 4 Strip 3	3.567	s.e. 1.017
block 5 board 2 area 3 Strip 1	3.208	s.e. 1.017

Tables of means

Variate: a

Grand mean 6.975

exposure	full	I.R.	none	UVA	UVB	Vis light
	8.578	6.288	5.905	7.289	8.770	5.019

treatment	acetic acid	carpropamid	tinuvin	water
	7.038	7.014	6.696	7.150

Chem_Charge	high	low	medium	Very high
	6.911	6.944	7.055	6.989

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		8.559	8.748	8.430	8.574
I.R.		7.177	5.812	5.927	6.237
none		5.994	5.770	5.531	6.325
UVA		7.069	7.579	7.135	7.373
UVB		8.417	9.176	8.420	9.066
Vis light		5.014	4.999	4.736	5.329

exposure	Chem_Charge	high	low	medium	Very high
full		8.799	8.526	8.888	8.099
I.R.		6.180	6.374	6.654	5.945
none		5.966	6.010	5.571	6.074
UVA		6.979	7.308	7.480	7.389
UVB		8.503	8.816	8.881	8.880
Vis light		5.043	4.630	4.858	5.546

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		6.932	6.960	7.011	7.251
carpropamid		6.996	7.171	6.945	6.943
tinuvin		6.621	6.782	7.105	6.276
water		7.097	6.862	7.158	7.484

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		8.072	8.486	9.272	8.408
	carpropamid		8.744	8.960	9.276	8.010
	tinuvin		9.610	7.738	8.878	7.494
	water		8.770	8.918	8.124	8.482
I.R.	acetic acid		6.964	7.260	7.754	6.728
	carpropamid		5.528	6.030	6.488	5.200
	tinuvin		5.460	6.306	6.236	5.704
	water		6.766	5.898	6.136	6.146
none	acetic acid		6.222	6.004	5.332	6.418
	carpropamid		5.384	6.818	4.986	5.890
	tinuvin		5.740	5.276	5.816	5.292
	water		6.516	5.940	6.148	6.694
UVA	acetic acid		6.724	6.980	7.094	7.478
	carpropamid		7.672	8.076	7.422	7.144
	tinuvin		6.788	6.940	7.752	7.058
	water		6.730	7.234	7.650	7.876
UVB	acetic acid		8.572	8.006	8.142	8.948
	carpropamid		8.904	8.788	9.068	9.944
	tinuvin		7.172	9.726	9.346	7.436
	water		9.364	8.742	8.966	9.190
Vis light	acetic acid		5.036	5.022	4.470	5.526
	carpropamid		5.742	4.352	4.430	5.472
	tinuvin		4.958	4.708	4.604	4.672
	water		4.436	4.438	5.926	6.514

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.5596	0.1974	0.1198	0.6989
d.f.	20	72	288	44.76
Except when comparing means with the same level(s) of exposure				0.4835
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.6146	0.2864	0.8643
d.f.	29.02	244.51	100.30
Except when comparing means with the same level(s) of			
exposure	0.2935		0.7016
d.f.	288		244.51
treatment		0.2397	
d.f.		288	
exposure.treatment			
			0.5870
d.f.			288
exposure.Chem_Charge			
			0.7016
d.f.			244.51

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.7914	0.2791	0.1695	0.9884
d.f.	20	72	288	44.76
Except when comparing means with the same level(s) of				
exposure				0.6837
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.8692	0.4051	1.2222
d.f.	29.02	244.51	100.30
Except when comparing means with the same level(s) of			
exposure	0.4151		0.9922
d.f.	288		244.51
treatment		0.3389	
d.f.		288	
exposure.treatment			
			0.8302
d.f.			288
exposure.Chem_Charge			
			0.9922
d.f.			244.51

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.6509	0.5565	0.3335	1.9911
d.f.	20	72	288	44.76
Except when comparing means with the same level(s) of				
exposure				1.3630
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.7777	0.7979	2.4248
d.f.	29.02	244.51	100.30
Except when comparing means with the same level(s) of			
exposure	0.8170		1.9543
d.f.	288		244.51
treatment		0.6671	
d.f.		288	
exposure.treatment			1.6340
d.f.			288
exposure.Chem_Charge			1.9543
d.f.			244.51

Analysis of variance

Variate: b

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		201.926	50.481	0.28	
block.board stratum						
exposure	5		4442.499	888.500	4.89	0.004
Residual	20		3631.580	181.579	21.58	
block.board.area stratum						
treatment	3		16.419	5.473	0.65	0.585
exposure.treatment	15		36.286	2.419	0.29	0.995
Residual	72		605.803	8.414	2.49	
block.board.area.Strip stratum						
Chem_Charge	3		3.699	1.233	0.36	0.779
exposure.Chem_Charge	15		51.625	3.442	1.02	0.436
treatment.Chem_Charge	9		15.720	1.747	0.52	0.862
exposure.treatment.Chem_Charge	45		180.694	4.015	1.19	0.204
Residual	286	(2)	967.006	3.381		
Total	477	(2)	9973.390			

Message: the following units have large residuals.

block 4 board 1	-5.605	s.e. 2.751
block 5 board 2	-6.176	s.e. 2.751
block 5 board 3	6.592	s.e. 2.751
block 4 board 4 area 4	3.008	s.e. 1.123
block 1 board 5 area 3 Strip 1	4.589	s.e. 1.419
block 1 board 5 area 3 Strip 4	-4.431	s.e. 1.419
block 4 board 4 area 4 Strip 3	5.017	s.e. 1.419
block 5 board 2 area 2 Strip 1	4.340	s.e. 1.419

Tables of means

Variate: b

Grand mean 28.377

exposure	full	I.R.	none	UVA	UVB	Vis light
	30.119	26.524	25.598	30.865	32.764	24.389

treatment	acetic acid	carpropamid	tinuvin	water
	28.144	28.479	28.262	28.620

Chem_Charge	high	low	medium	Very high
	28.419	28.463	28.393	28.231

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		29.534	30.146	30.460	30.336
I.R.		26.925	26.123	26.319	26.729
none		24.995	25.891	25.497	26.009
UVA		30.559	31.165	30.471	31.263
UVB		32.339	32.973	32.524	33.219
Vis light		24.512	24.576	24.301	24.164

exposure	Chem_Charge	high	low	medium	Very high
full		30.339	30.276	30.345	29.517
I.R.		26.389	26.830	26.827	26.051
none		25.843	25.853	25.417	25.280
UVA		30.158	30.987	31.040	31.273
UVB		33.062	32.979	32.547	32.467
Vis light		24.724	23.851	24.182	24.796

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		28.247	28.058	28.099	28.173
carpropamid		28.575	28.635	28.637	28.071
tinuvin		28.082	28.555	28.489	27.924
water		28.774	28.604	28.347	28.756

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		29.192	29.412	30.042	29.492
	carpropamid		29.824	30.730	31.158	28.874
	tinuvin		31.622	29.614	30.690	29.916
	water		30.720	31.348	29.492	29.786
I.R.	acetic acid		26.604	27.424	27.570	26.104
	carpropamid		25.396	26.536	27.340	25.220
	tinuvin		25.670	26.914	26.074	26.620
	water		27.886	26.446	26.324	26.260
none	acetic acid		25.684	25.210	24.222	24.866
	carpropamid		25.072	27.426	25.738	25.330
	tinuvin		25.818	25.302	25.924	24.946
	water		26.798	25.474	25.786	25.978
UVA	acetic acid		30.189	30.208	31.324	30.514
	carpropamid		31.132	31.484	31.372	30.674
	tinuvin		28.888	30.568	31.494	30.934
	water		30.422	31.688	29.972	32.971
UVB	acetic acid		32.888	32.196	31.718	32.556
	carpropamid		33.682	32.208	32.548	33.454
	tinuvin		32.094	34.094	32.590	31.320
	water		33.586	33.420	33.332	32.538
Vis light	acetic acid		24.926	23.898	23.720	25.506
	carpropamid		26.342	23.424	23.668	24.872
	tinuvin		24.398	24.838	24.164	23.806
	water		23.232	23.246	25.178	25.000

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	1.5066	0.2648	0.1679	1.6079
d.f.	20	72	286	25.81
Except when comparing means with the same level(s) of exposure				0.6486
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	1.5481	0.3932	1.7585
d.f.	22.29	256.42	36.80
Except when comparing means with the same level(s) of			
exposure	0.4112		0.9633
d.f.	286		256.42
treatment		0.3357	
d.f.		286	
exposure.treatment			
			0.8223
d.f.			286
exposure.Chem_Charge			
			0.9633
d.f.			256.42

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.1306	0.3745	0.2374	2.2739
d.f.	20	72	286	25.81
Except when comparing means with the same level(s) of				
exposure				0.9173
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.1893	0.5561	2.4869
d.f.	22.29	256.42	36.80
Except when comparing means with the same level(s) of			
exposure	0.5815		1.3623
d.f.	286		256.42
treatment		0.4748	
d.f.		286	
exposure.treatment			
			1.1630
d.f.			286
exposure.Chem_Charge			
			1.3623
d.f.			256.42

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.4444	0.7465	0.4672	4.6757
d.f.	20	72	286	25.81
Except when comparing means with the same level(s) of				
exposure				1.8286
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	4.5369	1.0952	5.0399
d.f.	22.29	256.42	36.80
Except when comparing means with the same level(s) of			
exposure	1.1445		2.6826
d.f.	286		256.42
treatment		0.9345	
d.f.		286	
exposure.treatment			2.2890
d.f.			286
exposure.Chem_Charge			2.6826
d.f.			256.42

(Not adjusted for missing values)

Analysis of variance week 3

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	223.759	55.940	1.96	
block.board stratum					
exposure	5	4873.958	974.792	34.17	<.001
Residual	20	570.569	28.528	1.39	
block.board.area stratum					
treatment	3	69.557	23.186	1.13	0.342
exposure.treatment	15	135.898	9.060	0.44	0.960
Residual	72	1474.141	20.474	2.90	

block.board.area.Strip stratum					
Chem_Charge	3	25.185	8.395	1.19	0.314
exposure.Chem_Charge	15	113.108	7.541	1.07	0.385
treatment.Chem_Charge	9	100.597	11.177	1.58	0.119
exposure.treatment.Chem_Charge	45	361.153	8.026	1.14	0.263
Residual	288	2031.166	7.053		
Total	479	9979.092			

Message: the following units have large residuals.

block 2 board 4	2.90	s.e. 1.09
block 5 board 1 area 4	4.79	s.e. 1.75
block 1 board 4 area 2 Strip 3	-7.13	s.e. 2.06
block 1 board 5 area 4 Strip 2	-6.55	s.e. 2.06
block 4 board 5 area 3 Strip 3	7.02	s.e. 2.06

Tables of means

Variate: L

Grand mean 73.07

exposure	full	I.R.	none	UVA	UVB	Vis light
	67.47	74.70	76.79	73.45	70.45	75.54

treatment	acetic acid	carpropamid	tinuvin	water
	72.49	73.09	73.56	73.13

Chem_Charge	high	low	medium	Very high
	73.42	73.10	72.93	72.81

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		66.99	66.67	68.50	67.71
I.R.		73.79	74.69	75.94	74.36
none		75.94	76.95	76.62	77.63
UVA		72.49	73.46	74.73	73.14
UVB		70.99	70.70	69.89	70.23
Vis light		74.73	76.05	75.68	75.69

exposure	Chem_Charge	high	low	medium	Very high
full		67.67	67.94	66.88	67.38
I.R.		75.21	75.70	74.18	73.69
none		76.68	75.61	77.61	77.25
UVA		74.02	73.64	73.30	72.85
UVB		71.00	70.41	70.04	70.35
Vis light		75.93	75.32	75.55	75.34

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		72.91	72.80	73.00	71.24
carpropamid		73.34	72.56	72.95	73.49
tinuvin		73.86	73.89	72.63	73.86
water		73.56	73.17	73.13	72.65

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		68.51	67.17	67.85	64.43
	carpropamid		67.00	66.27	65.84	67.59
	tinuvin		66.29	69.66	68.34	69.70
	water		68.89	68.66	65.50	67.80
I.R.	acetic acid		74.07	75.31	73.02	72.77
	carpropamid		75.64	74.33	74.41	74.37
	tinuvin		76.72	77.37	74.73	74.95
	water		74.40	75.81	74.57	72.68
none	acetic acid		77.05	74.21	77.70	74.81
	carpropamid		77.42	76.40	76.08	77.89
	tinuvin		75.59	76.12	77.59	77.18
	water		76.65	75.71	79.06	79.12
UVA	acetic acid		72.02	72.31	73.25	72.37
	carpropamid		72.94	73.50	73.67	73.72
	tinuvin		76.79	75.65	72.97	73.49
	water		74.33	73.11	73.33	71.81
UVB	acetic acid		70.09	73.54	70.78	69.55
	carpropamid		72.11	68.51	71.59	70.59
	tinuvin		71.74	69.58	66.88	71.35
	water		70.07	70.00	70.92	69.92
Vis light	acetic acid		75.75	74.26	75.43	73.50
	carpropamid		74.96	76.36	76.11	76.78
	tinuvin		76.02	74.93	75.26	76.50
	water		77.00	75.74	75.42	74.59

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.597	0.413	0.242	1.060
d.f.	20	72	288	86.92
Except when comparing means with the same level(s) of				
exposure				1.012
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.788	0.589	1.477
d.f.	58.43	234.97	258.38
Except when comparing means with the same level(s) of			
exposure	0.594		1.443
d.f.	288		234.97
treatment		0.485	
d.f.		288	
exposure.treatment			
			1.188
d.f.			288
exposure.Chem_Charge			
			1.443
d.f.			234.97

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.845	0.584	0.343	1.500
d.f.	20	72	288	86.92
Except when comparing means with the same level(s) of				
exposure				1.431
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.115	0.833	2.089
d.f.	58.43	234.97	258.38
Except when comparing means with the same level(s) of			
exposure	0.840		2.040
d.f.	288		234.97
treatment		0.686	
d.f.		288	
exposure.treatment			
			1.680
d.f.			288
exposure.Chem_Charge			
			2.040
d.f.			234.97

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.762	1.164	0.675	2.981
d.f.	20	72	288	86.92
Except when comparing means with the same level(s) of				
exposure				2.852
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.231	1.641	4.114
d.f.	58.43	234.97	258.38
Except when comparing means with the same level(s) of			
exposure	1.653		4.020
d.f.	288		234.97
treatment		1.350	
d.f.		288	
exposure.treatment			
			3.306
d.f.			288
exposure.Chem_Charge			
			4.020
d.f.			234.97

Analysis of variance

Variate: a

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		45.592	11.398	3.43	
block.board stratum						
exposure	5		1667.440	333.488	100.49	<.001
Residual	20		66.375	3.319	0.98	
block.board.area stratum						
treatment	3		10.706	3.569	1.06	0.372
exposure.treatment	15		42.379	2.825	0.84	0.633
Residual	72		242.610	3.370	2.91	
block.board.area.Strip stratum						
Chem_Charge	3		0.533	0.178	0.15	0.927
exposure.Chem_Charge	15		17.233	1.149	0.99	0.463
treatment.Chem_Charge	9		21.491	2.388	2.06	0.033
exposure.treatment.Chem_Charge	45		80.316	1.785	1.54	0.020
Residual	286	(2)	331.019	1.157		
Total	477	(2)	2503.762			

Message: the following units have large residuals.

block 2 board 4	-0.870	s.e. 0.372
block 3 board 1	0.780	s.e. 0.372
block 3 board 2	-0.755	s.e. 0.372
block 1 board 3 area 1	1.948	s.e. 0.711
block 1 board 5 area 3	2.015	s.e. 0.711
block 2 board 5 area 2	1.989	s.e. 0.711
block 1 board 5 area 3 Strip 1	-2.548	s.e. 0.830
block 1 board 5 area 3 Strip 2	2.472	s.e. 0.830
block 2 board 5 area 1 Strip 3	2.545	s.e. 0.830
block 4 board 3 area 2 Strip 3	2.924	s.e. 0.830
block 5 board 6 area 2 Strip 3	2.775	s.e. 0.830

Tables of means

Variate: a

Grand mean 7.209

exposure	full	I.R.	none	UVA	UVB	Vis light
	9.825	6.036	5.335	6.894	9.672	5.492

treatment	acetic acid	carpropamid	tinuvin	water
	7.453	7.126	7.059	7.198

Chem_Charge	high	low	medium	Very high
	7.267	7.186	7.192	7.190

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		9.542	10.348	9.483	9.925
I.R.		6.810	5.582	5.711	6.042
none		5.624	5.522	5.320	4.873
UVA		7.242	7.003	6.447	6.882
UVB		9.654	9.290	9.817	9.925
Vis light		5.846	5.009	5.572	5.539

exposure	Chem_Charge	high	low	medium	Very high
full		9.895	9.790	9.925	9.688
I.R.		6.320	5.745	5.928	6.151
none		5.371	5.855	4.944	5.169
UVA		6.779	6.870	6.885	7.041
UVB		9.845	9.422	9.872	9.548
Vis light		5.389	5.435	5.598	5.544

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		7.703	7.192	7.155	7.763
carpropamid		7.215	7.424	7.081	6.783
tinuvin		7.029	7.076	7.321	6.810
water		7.119	7.054	7.212	7.407

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		9.260	9.406	9.326	10.178
	carpropamid		10.516	10.608	10.536	9.732
	tinuvin		10.172	9.216	9.448	9.098
	water		9.632	9.932	10.392	9.746
I.R.	acetic acid		8.306	5.402	7.052	6.478
	carpropamid		5.512	6.496	4.826	5.494
	tinuvin		5.540	5.420	5.902	5.984
	water		5.922	5.664	5.932	6.650
none	acetic acid		5.040	6.604	4.724	6.130
	carpropamid		5.338	5.856	5.728	5.168
	tinuvin		5.638	5.804	4.846	4.994
	water		5.468	5.158	4.480	4.386
UVA	acetic acid		7.412	7.326	6.848	7.382
	carpropamid		7.160	7.070	7.018	6.766
	tinuvin		6.450	5.810	6.990	6.538
	water		6.094	7.274	6.684	7.478
UVB	acetic acid		10.412	8.698	9.302	10.204
	carpropamid		9.234	9.610	9.236	9.082
	tinuvin		9.030	10.158	11.154	8.926
	water		10.704	9.222	9.796	9.980
Vis light	acetic acid		5.786	5.716	5.678	6.204
	carpropamid		5.532	4.904	5.144	4.456
	tinuvin		5.342	6.046	5.584	5.318
	water		4.896	5.074	5.988	6.200

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.2037	0.1676	0.0982	0.4097
d.f.	20	72	286	91.52
Except when comparing means with the same level(s) of exposure				0.4105
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.2914	0.2388	0.5843
d.f.	77.79	234.23	282.17
Except when comparing means with the same level(s) of			
exposure	0.2406		0.5849
d.f.	286		234.23
treatment		0.1964	
d.f.		286	
exposure.treatment			
			0.4811
d.f.			286
exposure.Chem_Charge			
			0.5849
d.f.			234.23

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.2880	0.2370	0.1389	0.5794
d.f.	20	72	286	91.52
Except when comparing means with the same level(s) of				
exposure				0.5805
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.4120	0.3377	0.8264
d.f.	77.79	234.23	282.17
Except when comparing means with the same level(s) of			
exposure	0.3402		0.8272
d.f.	286		234.23
treatment		0.2778	
d.f.		286	
exposure.treatment			
			0.6804
d.f.			286
exposure.Chem_Charge			
			0.8272
d.f.			234.23

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	0.6008	0.4724	0.2734	1.1508
d.f.	20	72	286	91.52
Except when comparing means with the same level(s) of				
exposure				1.1572
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	0.8203	0.6653	1.6267
d.f.	77.79	234.23	282.17
Except when comparing means with the same level(s) of			
exposure	0.6696		1.6296
d.f.	286		234.23
treatment		0.5467	
d.f.		286	
exposure.treatment			1.3393
d.f.			286
exposure.Chem_Charge			1.6296
d.f.			234.23

(Not adjusted for missing values)

Analysis of variance

Variate: b

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		88.173	22.043	1.38	
block.board stratum						
exposure	5		8606.887	1721.377	107.47	<.001
Residual	20		320.337	16.017	2.38	
block.board.area stratum						
treatment	3		4.003	1.334	0.20	0.897
exposure.treatment	15		110.708	7.381	1.10	0.376
Residual	72		485.073	6.737	3.08	

block.board.area.Strip stratum						
Chem_Charge	3		9.754	3.251	1.48	0.219
exposure.Chem_Charge	15		33.599	2.240	1.02	0.432
treatment.Chem_Charge	9		24.747	2.750	1.26	0.261
exposure.treatment.Chem_Charge	45		106.004	2.356	1.08	0.353
Residual	285	(3)	624.300	2.191		
Total	476	(3)	10288.922			

Message: the following units have large residuals.

block 1 board 1	-1.733	s.e. 0.817
block 1 board 6	1.763	s.e. 0.817
block 4 board 6	-1.725	s.e. 0.817
block 1 board 1 area 4 Strip 4	3.445	s.e. 1.140
block 1 board 5 area 3 Strip 2	4.988	s.e. 1.140
block 1 board 5 area 3 Strip 3	-4.762	s.e. 1.140
block 1 board 6 area 1 Strip 3	4.193	s.e. 1.140
block 1 board 6 area 4 Strip 1	3.874	s.e. 1.140
block 2 board 6 area 1 Strip 1	3.728	s.e. 1.140

Tables of means

Variate: b

Grand mean 28.663

exposure	full	I.R.	none	UVA	UVB	Vis light
	32.811	24.776	24.569	28.865	35.572	25.386

treatment	acetic acid	carpropamid	tinuvin	water
	28.693	28.508	28.742	28.710

Chem_Charge	high	low	medium	Very high
	28.874	28.655	28.653	28.471

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		31.830	33.419	33.124	32.872
I.R.		24.981	24.163	24.861	25.097
none		24.460	25.154	24.503	24.159
UVA		29.381	29.138	28.088	28.852
UVB		35.717	34.845	36.020	35.705
Vis light		25.787	24.329	25.857	25.571

exposure	Chem_Charge	high	low	medium	Very high
full		32.938	32.806	32.904	32.595
I.R.		25.260	24.666	24.469	24.708
none		24.652	25.013	24.247	24.364
UVA		28.641	28.959	28.828	29.031
UVB		36.341	35.159	35.632	35.154
Vis light		25.409	25.327	25.833	24.975

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		29.132	28.593	28.403	28.643
carpropamid		28.861	28.818	28.514	27.839
tinuvin		28.636	28.626	29.069	28.639
water		28.866	28.584	28.624	28.764

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		31.946	31.934	31.692	31.748
	carpropamid		33.890	33.098	33.724	32.964
	tinuvin		33.278	32.896	33.412	32.910
	water		32.640	33.298	32.790	32.760
I.R.	acetic acid		26.176	23.766	25.466	24.518
	carpropamid		24.432	25.942	22.512	23.766
	tinuvin		24.668	24.434	25.114	25.230
	water		25.766	24.522	24.784	25.318
none	acetic acid		23.754	25.508	23.724	24.854
	carpropamid		24.684	26.052	25.134	24.746
	tinuvin		25.024	24.200	24.430	24.360
	water		25.146	24.294	23.700	23.496
UVA	acetic acid		29.596	29.398	29.098	29.432
	carpropamid		29.280	29.226	29.328	28.720
	tinuvin		27.524	27.542	28.826	28.462
	water		28.166	29.670	28.062	29.512
UVB	acetic acid		37.116	35.388	34.500	35.864
	carpropamid		35.874	34.326	35.398	33.782
	tinuvin		36.022	36.248	36.398	35.412
	water		36.352	34.676	36.234	35.558
Vis light	acetic acid		26.204	25.564	25.940	25.442
	carpropamid		25.008	24.262	24.990	23.056
	tinuvin		25.298	26.436	26.232	25.462
	water		25.126	25.046	26.172	25.942

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.4474	0.2369	0.1351	0.6729
d.f.	20	72	285	70.94
Except when comparing means with the same level(s) of				
exposure				0.5804
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.5314	0.3330	0.8840
d.f.	39.31	226.52	186.77
Except when comparing means with the same level(s) of			
exposure	0.3309		0.8157
d.f.	285		226.52
treatment		0.2702	
d.f.		285	
exposure.treatment			
			0.6619
d.f.			285
exposure.Chem_Charge			
			0.8157
d.f.			226.52

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6328	0.3351	0.1911	0.9517
d.f.	20	72	285	70.94
Except when comparing means with the same level(s) of				
exposure				0.8208
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7515	0.4710	1.2501
d.f.	39.31	226.52	186.77
Except when comparing means with the same level(s) of			
exposure	0.4680		1.1536
d.f.	285		226.52
treatment		0.3821	
d.f.		285	
exposure.treatment			
			0.9361
d.f.			285
exposure.Chem_Charge			
			1.1536
d.f.			226.52

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.3200	0.6680	0.3761	1.8976
d.f.	20	72	285	70.94
Except when comparing means with the same level(s) of				
exposure				1.6362
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.5196	0.9280	2.4662
d.f.	39.31	226.52	186.77
Except when comparing means with the same level(s) of			
exposure	0.9212		2.2732
d.f.	285		226.52
treatment		0.7522	
d.f.		285	
exposure.treatment			1.8425
d.f.			285
exposure.Chem_Charge			2.2732
d.f.			226.52

(Not adjusted for missing values)

Analysis of variance week 4

Variate: L4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	206.440	51.610	1.86	
block.board stratum					
exposure	5	4924.346	984.869	35.55	<.001
Residual	20	554.127	27.706	1.32	
block.board.area stratum					
treatment	3	70.698	23.566	1.12	0.345
exposure.treatment	15	187.691	12.513	0.60	0.868
Residual	72	1508.248	20.948	2.86	
block.board.area.strip stratum					
Chem_Charge	3	22.726	7.575	1.03	0.378
exposure.Chem_Charge	15	93.926	6.262	0.85	0.617
treatment.Chem_Charge	9	66.939	7.438	1.01	0.428
exposure.treatment.Chem_Charge	45	336.982	7.488	1.02	0.441
Residual	288	2111.840	7.333		
Total	479	10083.961			

Message: the following units have large residuals.

block 2 board 5	2.48	s.e. 1.07
block 5 board 1 area 4	4.84	s.e. 1.77
block 1 board 4 area 4 strip 2	-6.74	s.e. 2.10
block 1 board 5 area 2 strip 3	-6.24	s.e. 2.10
block 5 board 3 area 1 strip 2	6.44	s.e. 2.10

Tables of means

Variate: L4

Grand mean 72.37

exposure	full	I.R.	none	UVA	UVB	Vis. light
	67.07	75.15	76.37	72.60	69.49	73.53

treatment	acetic acid	carpropamid	tinuvin	water
	71.79	72.65	72.77	72.25

Chem_Charge	high	low	medium	very high
	72.71	72.14	72.23	72.39

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		66.42	66.69	68.26	66.89
I.R.		74.63	75.93	74.70	75.33
none		75.89	76.46	76.35	76.77
UVA		71.85	72.46	73.81	72.30
UVB		70.22	69.73	68.80	69.20
Vis. light		71.75	74.66	74.71	73.00

exposure	Chem_Charge	high	low	medium	very high
full		67.29	67.04	66.31	67.63
I.R.		75.56	74.74	75.46	74.84
none		75.80	75.63	77.18	76.87
UVA		73.44	72.47	72.53	71.97
UVB		70.05	69.37	69.20	69.33
Vis. light		74.13	73.56	72.73	73.70

treatment	Chem_Charge	high	low	medium	very high
acetic acid		72.04	71.87	72.10	71.16
carpropamid		73.02	71.96	72.63	73.01
tinuvin		73.28	72.64	71.81	73.35
water		72.50	72.06	72.39	72.04

exposure	treatment	Chem_Charge	high	low	medium	very high
full	acetic acid		67.02	65.66	66.02	66.98
	carpropamid		67.72	66.16	66.25	66.65
	tinuvin		66.92	68.29	67.54	70.28
	water		67.48	68.05	65.44	66.59
I.R.	acetic acid		75.06	74.54	75.87	73.05
	carpropamid		75.01	75.92	76.07	76.71
	tinuvin		75.18	73.42	74.51	75.70
	water		76.98	75.08	75.37	73.90
none	acetic acid		75.42	74.89	77.71	75.54
	carpropamid		76.44	75.95	76.12	77.33
	tinuvin		75.59	75.61	77.30	76.89
	water		75.74	76.04	77.58	77.74
UVA	acetic acid		72.28	71.24	73.11	70.75
	carpropamid		71.86	71.99	73.06	72.93
	tinuvin		75.37	74.72	72.32	72.82
	water		74.25	71.94	71.61	71.38
UVB	acetic acid		69.59	72.76	70.32	68.20
	carpropamid		71.27	67.73	70.04	69.86
	tinuvin		71.43	68.02	66.15	69.58
	water		67.89	68.97	70.29	69.66
Vis. light	acetic acid		72.84	72.14	69.59	72.42
	carpropamid		75.80	74.03	74.22	74.59
	tinuvin		75.17	75.78	73.07	74.81
	water		72.69	72.30	74.04	72.98

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure	treatment
rep.		80	120	120	20
e.s.e.		0.588	0.418	0.247	1.064
d.f.		20	72	288	87.94
Except when comparing means with the same level(s) of					
exposure					1.023
d.f.					72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.788	0.598	1.494
d.f.	61.67	237.22	265.39
Except when comparing means with the same level(s) of			
exposure	0.606		1.465
d.f.	288		237.22
treatment		0.494	
d.f.		288	
exposure.treatment			
			1.211
d.f.			288
exposure.Chem_Charge			
			1.465
d.f.			237.22

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.832	0.591	0.350	1.505
d.f.	20	72	288	87.94
Except when comparing means with the same level(s) of exposure				1.447
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.115	0.846	2.113
d.f.	61.67	237.22	265.39
Except when comparing means with the same level(s) of exposure	0.856		2.072
d.f.	288		237.22
treatment		0.699	
d.f.		288	
exposure.treatment			1.713
d.f.			288
exposure.Chem_Charge			2.072
d.f.			237.22

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.736	1.178	0.688	2.990
d.f.	20	72	288	87.94
Except when comparing means with the same level(s) of exposure				2.885
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.229	1.667	4.160
d.f.	61.67	237.22	265.39
Except when comparing means with the same level(s) of			
exposure	1.685		4.083
d.f.	288		237.22
treatment		1.376	
d.f.		288	
exposure.treatment			3.371
d.f.			288
exposure.Chem_Charge			4.083
d.f.			237.22

Analysis of variance

Variate: a_4

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		52.178	13.044	3.01	
block.board stratum						
exposure	5		1903.812	380.762	87.97	<.001
Residual	20		86.571	4.329	1.20	
block.board.area stratum						
treatment	3		8.538	2.846	0.79	0.503
exposure.treatment	15		51.071	3.405	0.95	0.518
Residual	72		258.856	3.595	2.74	
block.board.area.strip stratum						
Chem_Charge	3		5.731	1.910	1.46	0.227
exposure.Chem_Charge	15		22.106	1.474	1.12	0.334
treatment.Chem_Charge	9		21.041	2.338	1.78	0.071
exposure.treatment.Chem_Charge	45		78.753	1.750	1.33	0.085
Residual	287	(1)	376.406	1.312		
Total	478	(1)	2840.669			

Message: the following units have large residuals.

block 3 board 1	1.326	s.e. 0.425
block 1 board 3 area 1	1.851	s.e. 0.734
block 3 board 4 area 2	-1.854	s.e. 0.734
block 1 board 4 area 3 strip 3	-2.838	s.e. 0.886
block 3 board 2 area 4 strip 4	2.725	s.e. 0.886
block 5 board 6 area 2 strip 3	2.954	s.e. 0.886

Tables of means

Variate: a_4

Grand mean 7.585

exposure	full	I.R.	none	UVA	UVB	Vis. light
	10.295	5.835	5.316	7.440	10.205	6.420

treatment	acetic acid	carpropamid	tinuvin	water
	7.759	7.440	7.472	7.670

Chem_Charge	high	low	medium	very high
	7.575	7.752	7.568	7.445

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		9.948	10.617	9.881	10.733
I.R.		6.118	5.316	6.010	5.894
none		5.522	5.492	5.246	5.005
UVA		7.737	7.681	6.859	7.484
UVB		10.080	9.888	10.449	10.402
Vis. light		7.148	5.647	6.385	6.498

exposure	Chem_Charge	high	low	medium	very high
full		10.287	10.451	10.546	9.896
I.R.		5.706	5.957	5.763	5.912
none		5.630	5.641	4.898	5.097
UVA		7.050	7.523	7.460	7.728
UVB		10.242	10.143	10.310	10.124
Vis. light		6.537	6.796	6.430	5.915

treatment	Chem_Charge	high	low	medium	very high
acetic acid		7.888	7.760	7.525	7.862
carpropamid		7.454	7.914	7.320	7.073
tinuvin		7.290	7.585	7.922	7.089
water		7.669	7.748	7.504	7.757

exposure	treatment	Chem_Charge	high	low	medium	very high
full	acetic acid		9.702	10.290	10.198	9.602
	carpropamid		10.692	11.156	10.752	9.870
	tinuvin		10.352	9.538	10.222	9.414
	water		10.402	10.820	11.012	10.698
I.R.	acetic acid		6.316	6.064	5.492	6.600
	carpropamid		5.636	5.414	5.426	4.790
	tinuvin		5.554	6.806	5.962	5.718
	water		5.318	5.546	6.172	6.540
none	acetic acid		5.690	6.252	4.658	5.488
	carpropamid		5.338	5.880	5.450	5.298
	tinuvin		5.682	5.382	4.942	4.980
	water		5.808	5.050	4.542	4.622
UVA	acetic acid		7.438	7.848	7.190	8.472
	carpropamid		7.814	7.958	7.628	7.324
	tinuvin		6.408	6.256	7.692	7.080
	water		6.540	8.030	7.332	8.036
UVB	acetic acid		10.858	9.110	9.540	10.812
	carpropamid		9.618	10.122	10.078	9.736
	tinuvin		9.294	11.162	11.724	9.616
	water		11.198	10.178	9.900	10.334
Vis. light	acetic acid		7.324	6.998	8.074	6.198
	carpropamid		5.624	6.956	4.588	5.420
	tinuvin		6.452	6.368	6.992	5.728
	water		6.750	6.864	6.066	6.314

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.2326	0.1731	0.1045	0.4347
d.f.	20	72	287	89.50
Except when comparing means with the same level(s) of exposure				0.4240
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.3214	0.2505	0.6210
d.f.	68.92	242.86	278.69
Except when comparing means with the same level(s) of			
exposure	0.2561		0.6136
d.f.	287		242.86
treatment		0.2091	
d.f.		287	
exposure.treatment			
			0.5122
d.f.			287
exposure.Chem_Charge			
			0.6136
d.f.			242.86

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.3290	0.2448	0.1478	0.6147
d.f.	20	72	287	89.50
Except when comparing means with the same level(s) of				
exposure				0.5996
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.4545	0.3543	0.8782
d.f.	68.92	242.86	278.69
Except when comparing means with the same level(s) of			
exposure	0.3621		0.8677
d.f.	287		242.86
treatment		0.2957	
d.f.		287	
exposure.treatment			
			0.7243
d.f.			287
exposure.Chem_Charge			
			0.8677
d.f.			242.86

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	0.6862	0.4880	0.2910	1.2213
d.f.	20	72	287	89.50
Except when comparing means with the same level(s) of				
exposure				1.1953
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	0.9067	0.6978	1.7288
d.f.	68.92	242.86	278.69
Except when comparing means with the same level(s) of			
exposure	0.7128		1.7093
d.f.	287		242.86
treatment		0.5820	
d.f.		287	
exposure.treatment			1.4256
d.f.			287
exposure.Chem_Charge			1.7093
d.f.			242.86

(Not adjusted for missing values)

Analysis of variance

Variate: b_4

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		140.897	35.224	1.67	
block.board stratum						
exposure	5		7529.344	1505.869	71.50	<.001
Residual	20		421.207	21.060	3.11	
block.board.area stratum						
treatment	3		22.932	7.644	1.13	0.343
exposure.treatment	15		121.155	8.077	1.19	0.297
Residual	72		487.453	6.770	2.72	

block.board.area.strip stratum						
Chem_Charge	3		27.929	9.310	3.74	0.012
exposure.Chem_Charge	15		51.769	3.451	1.39	0.153
treatment.Chem_Charge	9		22.259	2.473	0.99	0.446
exposure.treatment.Chem_Charge	45		127.222	2.827	1.13	0.267
Residual	287	(1)	714.918	2.491		
Total	478	(1)	9630.811			

Message: the following units have large residuals.

block 1 board 1	-1.983	s.e. 0.937
block 3 board 1	2.038	s.e. 0.937
block 2 board 1 area 2	2.700	s.e. 1.008
block 4 board 1 area 2	-2.647	s.e. 1.008
block 1 board 4 area 3 strip 1	4.267	s.e. 1.220
block 3 board 2 area 4 strip 4	3.827	s.e. 1.220
block 4 board 4 area 3 strip 4	3.710	s.e. 1.220
block 5 board 2 area 1 strip 1	-3.701	s.e. 1.220

Tables of means

Variate: b_4

Grand mean 29.156

exposure	full	I.R.	none	UVA	UVB	Vis. light
	32.869	26.467	24.735	29.888	35.461	25.514

treatment	acetic acid	carpropamid	tinuvin	water
	28.993	28.897	29.432	29.302

Chem_Charge	high	low	medium	very high
	29.369	29.384	29.072	28.798

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		31.703	32.990	33.303	33.481
I.R.		26.895	25.362	27.058	26.552
none		24.494	25.356	24.651	24.442
UVA		30.035	30.206	29.202	30.108
UVB		35.168	34.758	36.343	35.576
Vis. light		25.662	24.709	26.033	25.652

exposure	Chem_Charge	high	low	medium	very high
full		32.850	33.222	33.117	32.289
I.R.		26.426	26.689	26.561	26.191
none		25.202	24.965	24.288	24.488
UVA		29.548	29.930	29.836	30.236
UVB		36.272	35.237	35.365	34.972
Vis. light		25.918	26.261	25.266	24.611

treatment	Chem_Charge	high	low	medium	very high
acetic acid		29.274	29.275	28.704	28.717
carpropamid		29.086	29.514	28.770	28.216
tinuvin		29.502	29.345	29.789	29.090
water		29.614	29.400	29.025	29.167

exposure	treatment	Chem_Charge	high	low	medium	very high
full	acetic acid		31.242	32.430	31.808	31.332
	carpropamid		33.564	33.786	33.234	31.374
	tinuvin		33.644	32.396	33.982	33.190
	water		32.948	34.274	33.442	33.260
I.R.	acetic acid		27.222	26.906	26.576	26.876
	carpropamid		25.738	25.532	25.700	24.478
	tinuvin		26.320	28.122	26.892	26.896
	water		26.424	26.196	27.074	26.514
none	acetic acid		24.728	25.396	23.826	24.024
	carpropamid		24.630	26.082	24.860	25.850
	tinuvin		25.322	24.262	24.690	24.330
	water		26.126	24.118	23.776	23.746
UVA	acetic acid		29.746	29.982	29.518	30.892
	carpropamid		30.284	30.312	30.696	29.532
	tinuvin		28.808	28.548	29.920	29.532
	water		29.354	30.878	29.210	30.988
UVB	acetic acid		36.664	34.822	33.980	35.206
	carpropamid		35.404	34.414	35.216	33.998
	tinuvin		36.436	36.594	36.866	35.476
	water		36.582	35.116	35.398	35.206
Vis. light	acetic acid		26.044	26.112	26.518	23.972
	carpropamid		24.896	26.960	22.916	24.064
	tinuvin		26.484	26.150	26.382	25.116
	water		26.248	25.820	25.250	25.290

Standard errors of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
e.s.e.	0.5131	0.2375	0.1441	0.7191
d.f.	20	72	287	61.33
Except when comparing means with the same level(s) of				
exposure				0.5818
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
e.s.e.	0.5972	0.3445	0.9438
d.f.	36.39	244.07	163.72
Except when comparing means with the same level(s) of			
exposure	0.3529		0.8439
d.f.	287		244.07
treatment		0.2882	
d.f.		287	
exposure.treatment			
			0.7058
d.f.			287
exposure.Chem_Charge			
			0.8439
d.f.			244.07

(Not adjusted for missing values)

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.7256	0.3359	0.2038	1.0170
d.f.	20	72	287	61.33
Except when comparing means with the same level(s) of				
exposure				0.8228
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.8446	0.4872	1.3348
d.f.	36.39	244.07	163.72
Except when comparing means with the same level(s) of			
exposure	0.4991		1.1934
d.f.	287		244.07
treatment		0.4075	
d.f.		287	
exposure.treatment			
			0.9982
d.f.			287
exposure.Chem_Charge			
			1.1934
d.f.			244.07

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.5136	0.6696	0.4010	2.0334
d.f.	20	72	287	61.33
Except when comparing means with the same level(s) of				
exposure				1.6402
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.7123	0.9597	2.6356
d.f.	36.39	244.07	163.72
Except when comparing means with the same level(s) of			
exposure	0.9824		2.3508
d.f.	287		244.07
treatment		0.8021	
d.f.		287	
exposure.treatment			
			1.9647
d.f.			287
exposure.Chem_Charge			
			2.3508
d.f.			244.07

(Not adjusted for missing values)

Analysis of variance week 6

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	205.531	51.383	1.62	
block.board stratum					
exposure	5	5110.868	1022.174	32.14	<.001
Residual	20	636.106	31.805	1.50	
block.board.area stratum					
treatment	3	66.188	22.063	1.04	0.381
exposure.treatment	15	172.792	11.519	0.54	0.908
Residual	72	1531.343	21.269	2.82	
block.board.area.Strip stratum					
Chem_Charge	3	21.694	7.231	0.96	0.413
exposure.Chem_Charge	15	113.209	7.547	1.00	0.455
treatment.Chem_Charge	9	94.317	10.480	1.39	0.192
exposure.treatment.Chem_Charge	45	395.825	8.796	1.17	0.228
Residual	288	2172.563	7.544		
Total	479	10520.435			

Message: the following units have large residuals.

block 2 board 4	2.33	s.e. 1.15
block 5 board 1 area 4	4.88	s.e. 1.79
block 5 board 3 area 1 Strip 2	7.77	s.e. 2.13

Tables of means

Variate: L

Grand mean 71.34

exposure	full	I.R.	none	UVA	UVB	Vis light
	66.35	73.42	75.89	70.78	68.23	73.35

treatment	acetic acid	carpropamid	tinuvin	water
	70.95	71.64	71.77	70.98

Chem_Charge	high	low	medium	Very high
	71.66	71.09	71.22	71.38

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		65.56	66.03	67.93	65.89
I.R.		72.62	74.39	74.45	72.21
none		75.30	75.91	76.08	76.25
UVA		70.01	70.95	71.79	70.37
UVB		69.00	68.57	67.37	67.98
Vis light		73.23	73.97	73.00	73.21

exposure	Chem_Charge	high	low	medium	Very high
full		67.00	66.23	65.75	66.43
I.R.		73.64	74.13	72.76	73.13
none		75.22	75.16	76.82	76.35
UVA		71.82	70.05	70.33	70.91
UVB		68.66	67.76	67.95	68.55
Vis light		73.59	73.19	73.70	72.93

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		71.05	71.28	70.95	70.53
carpropamid		72.03	70.40	71.65	72.48
tinuvin		72.52	71.74	71.06	71.76
water		71.03	70.92	71.22	70.77

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		66.26	64.84	64.91	66.22
	carpropamid		66.89	64.92	65.59	66.73
	tinuvin		67.05	68.45	67.82	68.41
	water		67.81	66.71	64.69	64.34
I.R.	acetic acid		73.01	74.28	70.51	72.66
	carpropamid		75.37	73.89	73.89	74.42
	tinuvin		75.54	75.23	72.79	74.22
	water		70.64	73.12	73.87	71.23
none	acetic acid		74.67	74.40	77.37	74.78
	carpropamid		76.37	74.32	75.81	77.16
	tinuvin		75.09	76.18	77.21	75.85
	water		74.74	75.75	76.88	77.62
UVA	acetic acid		69.76	69.62	70.39	70.27
	carpropamid		70.70	69.32	71.30	72.49
	tinuvin		74.05	71.51	70.59	71.02
	water		72.78	69.75	69.06	69.88
UVB	acetic acid		68.43	71.17	68.73	67.68
	carpropamid		70.54	66.04	68.89	68.82
	tinuvin		69.68	66.92	64.80	68.07
	water		66.00	66.90	69.39	69.63
Vis light	acetic acid		74.18	73.37	73.80	71.56
	carpropamid		72.29	73.91	74.41	75.28
	tinuvin		73.69	72.17	73.16	72.97
	water		74.19	73.33	73.43	71.89

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.892	0.595	0.355	1.546
d.f.	20	72	288	85.34
Except when comparing means with the same level(s) of exposure				1.458
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.167	0.855	2.157
d.f.	56.60	239.07	255.55
Except when comparing means with the same level(s) of exposure	0.869		2.095
d.f.	288		239.07
treatment		0.709	
d.f.		288	
exposure.treatment			1.737
d.f.			288
exposure.Chem_Charge			2.095
d.f.			239.07

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.860	1.187	0.698	3.074
d.f.	20	72	288	85.34
Except when comparing means with the same level(s) of exposure				2.907
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.336	1.685	4.248
d.f.	56.60	239.07	255.55
Except when comparing means with the same level(s) of			
exposure	1.709		4.127
d.f.	288		239.07
treatment		1.396	
d.f.		288	
exposure.treatment			3.419
d.f.			288
exposure.Chem_Charge			4.127
d.f.			239.07

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	61.548	15.387	2.88	
block.board stratum					
exposure	5	1848.915	369.783	69.15	<.001
Residual	20	106.952	5.348	1.63	
block.board.area stratum					
treatment	3	12.257	4.086	1.25	0.298
exposure.treatment	15	35.428	2.362	0.72	0.755
Residual	72	235.552	3.272	2.29	
block.board.area.Strip stratum					
Chem_Charge	3	5.929	1.976	1.38	0.249
exposure.Chem_Charge	15	18.526	1.235	0.86	0.606
treatment.Chem_Charge	9	31.965	3.552	2.48	0.010
exposure.treatment.Chem_Charge	45	99.745	2.217	1.55	0.019
Residual	288	412.130	1.431		
Total	479	2868.948			

Message: the following units have large residuals.

block 3 board 1	1.320	s.e. 0.472
block 3 board 5 area 1	2.029	s.e. 0.701
block 1 board 5 area 4 Strip 2	3.904	s.e. 0.927
block 5 board 3 area 1 Strip 1	3.037	s.e. 0.927
block 5 board 6 area 2 Strip 3	3.151	s.e. 0.927

Tables of means

Variate: a

Grand mean 8.005

exposure	full	I.R.	none	UVA	UVB	Vis light
	10.082	6.437	5.492	8.423	10.871	6.726

treatment	acetic acid	carpropamid	tinuvin	water
	8.109	7.850	7.849	8.212

Chem_Charge	high	low	medium	Very high
	7.913	8.129	8.101	7.878

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		9.921	10.322	9.554	10.532
I.R.		6.909	5.890	6.087	6.862
none		5.639	5.650	5.253	5.428
UVA		8.562	8.508	7.997	8.623
UVB		10.749	10.490	11.218	11.026
Vis light		6.876	6.239	6.987	6.801

exposure	Chem_Charge	high	low	medium	Very high
full		9.825	10.213	10.311	9.980
I.R.		6.499	6.380	6.818	6.052
none		5.736	5.757	5.299	5.177
UVA		7.980	8.716	8.622	8.373
UVB		10.859	10.943	10.999	10.682
Vis light		6.577	6.769	6.556	7.001

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		8.142	7.883	8.268	8.144
carpropamid		7.736	8.530	7.872	7.261
tinuvin		7.441	7.995	8.184	7.777
water		8.332	8.109	8.079	8.328

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		9.594	9.940	10.780	9.370
	carpropamid		10.128	11.114	10.302	9.742
	tinuvin		9.786	9.292	9.506	9.634
	water		9.792	10.504	10.658	11.176
I.R.	acetic acid		7.072	6.276	8.264	6.024
	carpropamid		5.538	6.568	6.200	5.252
	tinuvin		5.644	6.254	6.690	5.760
	water		7.742	6.420	6.116	7.170
none	acetic acid		5.610	6.270	4.932	5.742
	carpropamid		5.430	6.382	5.620	5.166
	tinuvin		5.646	5.286	4.998	5.080
	water		6.258	5.088	5.644	4.720
UVA	acetic acid		8.408	8.620	8.602	8.620
	carpropamid		8.642	9.324	8.544	7.522
	tinuvin		7.300	7.786	8.580	8.322
	water		7.570	9.134	8.762	9.028
UVB	acetic acid		11.298	9.544	10.592	11.560
	carpropamid		9.950	11.418	10.356	10.236
	tinuvin		9.802	11.840	12.602	10.626
	water		12.384	10.968	10.446	10.306
Vis light	acetic acid		6.868	6.648	6.440	7.548
	carpropamid		6.726	6.374	6.208	5.648
	tinuvin		6.468	7.514	6.728	7.240
	water		6.248	6.540	6.848	7.568

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.3656	0.2335	0.1544	0.6157
d.f.	20	72	288	83.06
Except when comparing means with the same level(s) of exposure				0.5720
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.4909	0.3551	0.8991
d.f.	62.22	269.10	275.74
Except when comparing means with the same level(s) of			
exposure	0.3783		0.8697
d.f.	288		269.10
treatment		0.3089	
d.f.		288	
exposure.treatment			
			0.7566
d.f.			288
exposure.Chem_Charge			
			0.8697
d.f.			269.10

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	0.7627	0.4655	0.3040	1.2245
d.f.	20	72	288	83.06
Except when comparing means with the same level(s) of				
exposure				1.1402
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	0.9813	0.6991	1.7699
d.f.	62.22	269.10	275.74
Except when comparing means with the same level(s) of			
exposure	0.7446		1.7124
d.f.	288		269.10
treatment		0.6079	
d.f.		288	
exposure.treatment			
			1.4891
d.f.			288
exposure.Chem_Charge			
			1.7124
d.f.			269.10

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	92.598	23.149	0.89	
block.board stratum					
exposure	5	4477.607	895.521	34.44	<.001
Residual	20	520.072	26.004	3.31	
block.board.area stratum					
treatment	3	37.018	12.339	1.57	0.204
exposure.treatment	15	102.226	6.815	0.87	0.602
Residual	72	565.765	7.858	2.49	
block.board.area.Strip stratum					
Chem_Charge	3	21.034	7.011	2.22	0.086
exposure.Chem_Charge	15	32.221	2.148	0.68	0.803
treatment.Chem_Charge	9	88.312	9.812	3.11	0.001
exposure.treatment.Chem_Charge	45	207.015	4.600	1.46	0.037
Residual	288	908.560	3.155		
Total	479	7052.427			

Message: the following units have large residuals.

block 1 board 1	-2.780	s.e. 1.041
block 1 board 6	2.152	s.e. 1.041
block 3 board 1	2.377	s.e. 1.041
block 2 board 1 area 2	2.753	s.e. 1.086
block 5 board 1 area 1	3.711	s.e. 1.086
block 5 board 1 area 4	-3.219	s.e. 1.086
block 1 board 2 area 1 Strip 1	4.369	s.e. 1.376
block 1 board 5 area 4 Strip 2	4.618	s.e. 1.376
block 1 board 6 area 4 Strip 1	4.032	s.e. 1.376
block 3 board 2 area 4 Strip 4	4.714	s.e. 1.376
block 4 board 1 area 1 Strip 3	-4.475	s.e. 1.376
block 5 board 1 area 3 Strip 4	4.247	s.e. 1.376
block 5 board 6 area 2 Strip 3	4.075	s.e. 1.376

Tables of means

Variate: b

Grand mean 28.707

exposure	full	I.R.	none	UVA	UVB	Vis light
	29.410	25.647	25.091	30.448	34.067	27.581

treatment	acetic acid	carpropamid	tinuvin	water
	28.497	28.370	28.993	28.970

Chem_Charge	high	low	medium	Very high
	28.793	28.869	28.819	28.348

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		28.829	29.164	29.503	30.146
I.R.		25.936	25.139	25.343	26.171
none		24.707	25.612	24.809	25.236
UVA		29.972	30.441	30.470	30.910
UVB		33.716	33.169	35.422	33.964
Vis light		27.824	26.696	28.413	27.392

exposure	Chem_Charge	high	low	medium	Very high
full		29.044	29.657	29.853	29.088
I.R.		25.873	25.921	25.813	24.981
none		25.561	25.158	25.120	24.524
UVA		30.021	30.769	30.567	30.436
UVB		34.728	33.895	33.962	33.685
Vis light		27.534	27.815	27.602	27.374

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		28.865	28.289	28.749	28.085
carpropamid		28.297	29.519	28.458	27.205
tinuvin		28.599	28.947	29.261	29.165
water		29.412	28.720	28.809	28.937

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		28.418	28.964	29.962	27.972
	carpropamid		28.928	30.910	29.862	26.954
	tinuvin		30.222	28.180	29.314	30.294
	water		28.606	30.572	30.274	31.132
I.R.	acetic acid		26.250	26.022	27.190	24.280
	carpropamid		24.782	26.608	25.164	24.002
	tinuvin		24.798	25.844	25.660	25.068
	water		27.662	25.210	25.238	26.574
none	acetic acid		25.052	24.982	24.562	24.230
	carpropamid		25.210	26.682	25.304	25.250
	tinuvin		25.198	24.698	24.810	24.528
	water		26.784	24.268	25.802	24.088
UVA	acetic acid		29.710	29.860	30.274	30.044
	carpropamid		30.558	31.582	30.598	29.024
	tinuvin		29.828	29.954	31.038	31.060
	water		29.988	31.680	30.356	31.614
UVB	acetic acid		35.554	32.148	32.682	34.478
	carpropamid		33.214	34.266	32.910	32.284
	tinuvin		33.752	35.976	36.726	35.232
	water		36.390	33.190	33.530	32.746
Vis light	acetic acid		28.206	27.760	27.824	27.504
	carpropamid		27.090	27.066	26.910	25.718
	tinuvin		27.796	29.032	28.016	28.806
	water		27.042	27.402	27.656	27.466

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.8063	0.3619	0.2293	1.1133
d.f.	20	72	288	59.19
Except when comparing means with the same level(s) of				
exposure				0.8864
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.9416	0.5373	1.4785
d.f.	36.87	256.76	164.39
Except when comparing means with the same level(s) of			
exposure	0.5617		1.3161
d.f.	288		256.76
treatment		0.4586	
d.f.		288	
exposure.treatment			
			1.1233
d.f.			288
exposure.Chem_Charge			
			1.3161
d.f.			256.76

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.6819	0.7214	0.4513	2.2276
d.f.	20	72	288	59.19
Except when comparing means with the same level(s) of				
exposure				1.7671
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.9082	1.0581	2.9192
d.f.	36.87	256.76	164.39
Except when comparing means with the same level(s) of			
exposure	1.1055		2.5918
d.f.	288		256.76
treatment		0.9026	
d.f.		288	
exposure.treatment			2.2110
d.f.			288
exposure.Chem_Charge			2.5918
d.f.			256.76

Analysis of variance week 8

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	604.751	151.188	2.96	
block.board stratum					
exposure	5	6238.411	1247.682	24.45	<.001
Residual	20	1020.494	51.025	1.99	
block.board.area stratum					
treatment	3	39.023	13.008	0.51	0.679
exposure.treatment	15	155.713	10.381	0.40	0.974
Residual	72	1850.658	25.704	3.09	
block.board.area.Strip stratum					
Chem_Charge	3	4.310	1.437	0.17	0.915
exposure.Chem_Charge	15	173.374	11.558	1.39	0.152
treatment.Chem_Charge	9	41.208	4.579	0.55	0.837
exposure.treatment.Chem_Charge	45	459.497	10.211	1.23	0.165
Residual	288	2398.378	8.328		
Total	479	12985.816			

Message: the following units have large residuals.

block 2 board 1	3.30	s.e. 1.46
block 1 board 1 area 3	-5.32	s.e. 1.96
block 1 board 1 area 4	5.15	s.e. 1.96
block 4 board 2 area 4 Strip 1	-7.91	s.e. 2.24

Tables of means

Variate: L

Grand mean 69.97

exposure	full	I.R.	none	UVA	UVB	Vis light
	64.22	72.75	75.35	69.18	67.38	70.96

treatment	acetic acid	carpropamid	tinuvin	water
	69.50	70.23	70.17	70.00

Chem_Charge	high	low	medium	Very high
	70.00	70.11	69.85	69.93

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		63.12	63.59	64.83	65.35
I.R.		72.18	73.31	73.64	71.85
none		74.72	75.59	75.54	75.55
UVA		68.89	69.12	70.02	68.70
UVB		67.47	68.38	66.22	67.47
Vis light		70.63	71.37	70.74	71.08

exposure	Chem_Charge	high	low	medium	Very high
full		63.60	65.01	64.08	64.19
I.R.		73.08	73.95	72.14	71.81
none		74.93	74.51	76.07	75.88
UVA		70.09	69.15	68.75	68.75
UVB		66.98	68.07	66.95	67.54
Vis light		71.29	69.98	71.14	71.42

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		69.21	69.78	69.80	69.21
carpropamid		70.31	70.05	69.81	70.73
tinuvin		70.34	70.34	69.67	70.31
water		70.12	70.27	70.13	69.47

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		63.51	62.24	63.84	62.89
	carpropamid		63.39	64.68	61.96	64.31
	tinuvin		61.92	65.90	66.19	65.30
	water		65.60	67.24	64.32	64.24
I.R.	acetic acid		72.08	73.82	71.46	71.36
	carpropamid		73.87	73.53	73.02	72.81
	tinuvin		74.65	74.85	71.55	73.53
	water		71.71	73.61	72.53	69.55
none	acetic acid		74.98	72.71	76.45	74.73
	carpropamid		75.81	74.97	74.92	76.65
	tinuvin		74.12	75.68	76.92	75.45
	water		74.82	74.68	76.00	76.68
UVA	acetic acid		68.73	68.67	70.06	68.07
	carpropamid		68.75	68.63	69.09	70.03
	tinuvin		72.12	70.94	67.90	69.11
	water		70.76	68.34	67.93	67.77
UVB	acetic acid		64.59	70.94	66.26	68.08
	carpropamid		69.85	67.05	68.33	68.29
	tinuvin		67.91	65.98	63.98	67.03
	water		65.56	68.31	69.24	66.76
Vis light	acetic acid		71.36	70.32	70.76	70.10
	carpropamid		70.21	71.47	71.52	72.28
	tinuvin		71.31	68.68	71.50	71.47
	water		72.28	69.45	70.77	71.83

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.129	0.655	0.373	1.790
d.f.	20	72	288	77.17
Except when comparing means with the same level(s) of exposure				1.603
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.378	0.919	2.388
d.f.	43.65	226.49	210.22
Except when comparing means with the same level(s) of			
exposure	0.913		2.251
d.f.	288		226.49
treatment		0.745	
d.f.		288	
exposure.treatment			
			1.825
d.f.			288
exposure.Chem_Charge			
			2.251
d.f.			226.49

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.356	1.305	0.733	3.564
d.f.	20	72	288	77.17
Except when comparing means with the same level(s) of				
exposure				3.196
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.779	1.811	4.707
d.f.	43.65	226.49	210.22
Except when comparing means with the same level(s) of			
exposure	1.796		4.436
d.f.	288		226.49
treatment		1.467	
d.f.		288	
exposure.treatment			
			3.592
d.f.			288
exposure.Chem_Charge			
			4.436
d.f.			226.49

Analysis of variance

Variate: a

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		65.085	16.271	1.21	
block.board stratum						
exposure	5		1517.003	303.401	22.59	<.001
Residual	20		268.570	13.429	2.50	
block.board.area stratum						
treatment	3		4.462	1.487	0.28	0.842
exposure.treatment	15		86.348	5.757	1.07	0.396
Residual	72		386.064	5.362	2.94	
block.board.area.Strip stratum						
Chem_Charge	3		2.106	0.702	0.39	0.764
exposure.Chem_Charge	15		22.437	1.496	0.82	0.654
treatment.Chem_Charge	9		19.403	2.156	1.18	0.305
exposure.treatment.Chem_Charge	45		139.945	3.110	1.71	0.005
Residual	286	(2)	520.835	1.821		
Total	477	(2)	2994.412			

Message: the following units have large residuals.

block 1 board 1	-1.892	s.e. 0.748
block 3 board 1	1.908	s.e. 0.748
block 3 board 2	-1.782	s.e. 0.748
block 4 board 1 area 3	2.424	s.e. 0.897
block 5 board 1 area 1	3.107	s.e. 0.897
block 5 board 1 area 4	-2.374	s.e. 0.897
block 1 board 1 area 3 Strip 1	4.114	s.e. 1.042
block 2 board 5 area 2 Strip 2	-3.450	s.e. 1.042
block 3 board 2 area 4 Strip 4	4.043	s.e. 1.042

Tables of means

Variate: a

Grand mean 8.186

exposure	full	I.R.	none	UVA	UVB	Vis light
	8.129	6.680	5.665	9.453	11.138	8.053

treatment	acetic acid	carpropamid	tinuvin	water
	8.347	8.115	8.109	8.174

Chem_Charge	high	low	medium	Very high
	8.243	8.260	8.136	8.107

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		7.921	9.406	7.669	7.520
I.R.		7.213	6.104	6.567	6.838
none		6.015	5.664	5.391	5.588
UVA		9.484	9.540	9.043	9.743
UVB		11.195	10.430	11.642	11.283
Vis light		8.256	7.543	8.342	8.072

exposure	Chem_Charge	high	low	medium	Very high
full		8.329	8.243	7.768	8.176
I.R.		6.911	6.451	6.679	6.680
none		5.782	6.069	5.499	5.309
UVA		9.058	9.494	9.683	9.575
UVB		11.427	10.911	11.045	11.167
Vis light		7.951	8.389	8.141	7.731

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		8.374	8.441	8.179	8.396
carpropamid		8.362	8.336	8.137	7.623
tinuvin		7.978	8.107	8.396	7.957
water		8.260	8.155	7.831	8.451

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		7.036	9.260	7.744	7.644
	carpropamid		10.128	9.160	10.148	8.190
	tinuvin		8.546	6.658	7.116	8.358
	water		7.608	7.894	6.065	8.514
I.R.	acetic acid		7.716	6.436	7.834	6.866
	carpropamid		6.496	6.762	5.187	5.970
	tinuvin		6.294	6.522	7.052	6.400
	water		7.140	6.086	6.642	7.484
none	acetic acid		5.690	7.266	5.378	5.726
	carpropamid		5.490	6.164	5.694	5.308
	tinuvin		5.878	5.352	5.190	5.146
	water		6.070	5.492	5.732	5.056
UVA	acetic acid		9.390	9.526	9.154	9.868
	carpropamid		9.910	9.858	9.628	8.766
	tinuvin		8.366	8.390	10.104	9.312
	water		8.568	10.204	9.846	10.356
UVB	acetic acid		12.092	9.950	10.826	11.912
	carpropamid		10.192	10.476	10.362	10.690
	tinuvin		10.938	12.230	12.868	10.532
	water		12.488	10.988	10.124	11.534
Vis light	acetic acid		8.320	8.206	8.140	8.358
	carpropamid		7.956	7.596	7.806	6.814
	tinuvin		7.844	9.488	8.044	7.994
	water		7.686	8.268	8.576	7.760

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.5794	0.2989	0.1742	0.8590
d.f.	20	72	286	69.08
Except when comparing means with the same level(s) of				
exposure				0.7323
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.6872	0.4248	1.1332
d.f.	39.13	232.66	184.78
Except when comparing means with the same level(s) of			
exposure	0.4267		1.0404
d.f.	286		232.66
treatment		0.3484	
d.f.		286	
exposure.treatment			
			0.8535
d.f.			286
exposure.Chem_Charge			
			1.0404
d.f.			232.66

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.2086	0.5959	0.3429	1.7136
d.f.	20	72	286	69.08
Except when comparing means with the same level(s) of				
exposure				1.4597
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.3899	0.8369	2.2357
d.f.	39.13	232.66	184.78
Except when comparing means with the same level(s) of			
exposure	0.8400		2.0499
d.f.	286		232.66
treatment		0.6858	
d.f.		286	
exposure.treatment			1.6799
d.f.			286
exposure.Chem_Charge			2.0499
d.f.			232.66

(Not adjusted for missing values)

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	88.335	22.084	0.34	
block.board stratum					
exposure	5	3886.552	777.310	12.10	<.001
Residual	20	1284.675	64.234	5.30	
block.board.area stratum					
treatment	3	19.512	6.504	0.54	0.659
exposure.treatment	15	197.737	13.182	1.09	0.383
Residual	72	872.297	12.115	2.57	
block.board.area.Strip stratum					
Chem_Charge	3	7.013	2.338	0.50	0.685
exposure.Chem_Charge	15	39.080	2.605	0.55	0.908
treatment.Chem_Charge	9	27.982	3.109	0.66	0.744
exposure.treatment.Chem_Charge	45	291.794	6.484	1.38	0.064
Residual	288	1355.383	4.706		
Total	479	8070.359			

Message: the following units have large residuals.

block 1 board 1	-4.031	s.e. 1.636
block 4 board 1	3.873	s.e. 1.636
block 2 board 1 area 2	3.524	s.e. 1.348
block 5 board 1 area 1	5.106	s.e. 1.348
block 5 board 1 area 4	-3.713	s.e. 1.348
block 1 board 1 area 3 Strip 1	6.881	s.e. 1.680
block 1 board 5 area 4 Strip 2	7.062	s.e. 1.680
block 2 board 2 area 1 Strip 1	-4.955	s.e. 1.680
block 2 board 2 area 3 Strip 3	5.296	s.e. 1.680
block 3 board 2 area 4 Strip 4	5.534	s.e. 1.680
block 4 board 1 area 1 Strip 2	8.528	s.e. 1.680
block 4 board 1 area 1 Strip 3	-5.571	s.e. 1.680
block 5 board 1 area 1 Strip 2	-5.306	s.e. 1.680

Tables of means

Variate: b

Grand mean 27.395

exposure	full	I.R.	none	UVA	UVB	Vis light
	23.512	25.941	25.160	30.092	31.736	27.929

treatment	acetic acid	carpropamid	tinuvin	water
	27.263	27.320	27.742	27.255

Chem_Charge	high	low	medium	Very high
	27.544	27.435	27.393	27.209

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		22.660	25.106	23.509	22.775
I.R.		25.906	25.602	26.331	25.926
none		25.099	25.738	24.704	25.101
UVA		29.732	30.119	29.993	30.522
UVB		31.923	30.431	33.278	31.313
Vis light		28.261	26.926	28.635	27.895

exposure	Chem_Charge	high	low	medium	Very high
full		23.530	23.528	23.339	23.652
I.R.		26.101	25.876	26.316	25.473
none		25.204	25.377	25.105	24.957
UVA		29.847	30.057	30.247	30.216
UVB		32.561	31.532	31.160	31.691
Vis light		28.022	28.239	28.190	27.265

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		27.474	27.484	27.090	27.005
carpropamid		27.512	27.618	27.518	26.632
tinuvin		27.707	27.616	27.979	27.664
water		27.483	27.020	26.983	27.534

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		21.210	25.076	22.170	22.184
	carpropamid		26.092	24.730	26.472	23.128
	tinuvin		24.760	21.494	22.478	25.302
	water		22.056	22.812	22.236	23.994
I.R.	acetic acid		26.574	25.384	27.282	24.384
	carpropamid		25.282	26.904	25.320	24.902
	tinuvin		25.734	26.546	27.278	25.766
	water		26.814	24.668	25.384	26.838
none	acetic acid		24.796	26.022	24.796	24.782
	carpropamid		24.916	26.404	25.028	26.602
	tinuvin		25.058	24.588	24.970	24.200
	water		26.044	24.494	25.624	24.242
UVA	acetic acid		29.722	29.508	29.698	30.000
	carpropamid		30.548	30.664	30.198	29.066
	tinuvin		29.696	28.982	30.850	30.444
	water		29.420	31.072	30.242	31.354
UVB	acetic acid		33.766	30.736	30.480	32.708
	carpropamid		30.928	29.890	30.372	30.532
	tinuvin		32.786	34.450	33.866	32.008
	water		32.764	31.052	29.920	31.514
Vis light	acetic acid		28.776	28.178	28.116	27.972
	carpropamid		27.306	27.118	27.716	25.562
	tinuvin		28.208	29.636	28.434	28.262
	water		27.798	28.024	28.492	27.264

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.2672	0.4494	0.2801	1.5857
d.f.	20	72	288	45.03
Except when comparing means with the same level(s) of exposure				1.1007
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.3996	0.6612	1.9815
d.f.	29.66	252.03	104.64
Except when comparing means with the same level(s) of			
exposure	0.6860		1.6197
d.f.	288		252.03
treatment		0.5601	
d.f.		288	
exposure.treatment			
			1.3720
d.f.			288
exposure.Chem_Charge			
			1.6197
d.f.			252.03

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.6434	0.8958	0.5512	3.1937
d.f.	20	72	288	45.03
Except when comparing means with the same level(s) of				
exposure				2.1942
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.8597	1.3022	3.9291
d.f.	29.66	252.03	104.64
Except when comparing means with the same level(s) of			
exposure	1.3502		3.1898
d.f.	288		252.03
treatment		1.1025	
d.f.		288	
exposure.treatment			
			2.7005
d.f.			288
exposure.Chem_Charge			
			3.1898
d.f.			252.03

Analysis of variance week 10

Variate: L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		1599.351	399.838	2.58	
block.board stratum						
exposure	5		13901.431	2780.286	17.97	<.001
Residual	20		3093.703	154.685	4.99	
block.board.area stratum						
treatment	3		165.881	55.294	1.78	0.158
exposure.treatment	15		334.734	22.316	0.72	0.756
Residual	72		2231.307	30.990	3.11	
block.board.area.Strip stratum						
Chem_Charge	3		110.679	36.893	3.71	0.012
exposure.Chem_Charge	15		131.662	8.777	0.88	0.585
treatment.Chem_Charge	9		89.308	9.923	1.00	0.442
exposure.treatment.Chem_Charge	45		390.873	8.686	0.87	0.703
Residual	287	(1)	2855.697	9.950		
Total	478	(1)	24381.991			

Message: the following units have large residuals.

block 1 board 1	-5.33	s.e. 2.54
block 3 board 6	-6.72	s.e. 2.54
block 2 board 1 area 3	5.73	s.e. 2.16
block 4 board 5 area 1 Strip 1	-7.37	s.e. 2.44

Tables of means

Variate: L

Grand mean 65.87

exposure	full	I.R.	none	UVA	UVB	Vis light
	54.95	70.48	71.60	65.77	65.94	66.50

treatment	acetic acid	carpropamid	tinuvin	water
	66.08	66.05	64.90	66.47

Chem_Charge	high	low	medium	Very high
	66.04	65.43	65.44	66.59

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		54.96	54.25	52.82	57.76
I.R.		71.09	70.27	70.44	70.11
none		70.99	72.14	71.96	71.32
UVA		67.07	65.69	64.17	66.14
UVB		65.96	66.68	64.05	67.09
Vis light		66.39	67.25	65.94	66.41

exposure	Chem_Charge	high	low	medium	Very high
full		55.17	54.46	54.57	55.60
I.R.		70.74	70.63	70.41	70.13
none		70.86	71.89	71.12	72.54
UVA		66.30	64.86	65.16	66.75
UVB		65.79	66.03	65.20	66.76
Vis light		67.38	64.72	66.16	67.74

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		66.49	65.59	65.55	66.69
carpropamid		66.12	65.82	65.12	67.13
tinuvin		65.13	65.17	64.26	65.03
water		66.42	65.14	66.82	67.50

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		56.29	52.27	55.03	56.26
	carpropamid		53.29	55.33	54.18	54.22
	tinuvin		52.31	54.19	50.54	54.24
	water		58.78	56.02	58.55	57.67
I.R.	acetic acid		70.57	71.17	72.65	69.97
	carpropamid		70.90	71.42	68.88	69.87
	tinuvin		71.97	70.78	69.96	69.05
	water		69.54	69.14	70.13	71.62
none	acetic acid		69.03	71.29	70.08	73.57
	carpropamid		70.99	72.46	71.48	73.62
	tinuvin		72.52	72.79	72.06	70.46
	water		70.89	71.02	70.87	72.51
UVA	acetic acid		69.15	65.69	65.50	67.95
	carpropamid		65.72	64.48	64.64	67.91
	tinuvin		64.86	63.70	64.32	63.80
	water		65.48	65.58	66.19	67.33
UVB	acetic acid		66.16	67.36	65.72	64.60
	carpropamid		67.35	65.10	65.41	68.85
	tinuvin		62.65	65.25	62.29	66.01
	water		67.01	66.39	67.36	67.58
Vis light	acetic acid		67.73	65.73	64.31	67.77
	carpropamid		68.49	66.11	66.11	68.31
	tinuvin		66.46	64.34	66.38	66.60
	water		66.84	62.69	67.85	68.28

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.967	0.719	0.407	2.488
d.f.	20	72	287	46.59
Except when comparing means with the same level(s) of exposure				1.760
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.148	1.007	3.029
d.f.	28.39	225.11	98.63
Except when comparing means with the same level(s) of exposure	0.998		2.467
d.f.	287		225.11
treatment		0.814	
d.f.		287	
exposure.treatment			1.995
d.f.			287
exposure.Chem_Charge			2.467
d.f.			225.11

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.102	1.433	0.802	5.007
d.f.	20	72	287	46.59
Except when comparing means with the same level(s) of exposure				3.509
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	4.397	1.984	6.011
d.f.	28.39	225.11	98.63
Except when comparing means with the same level(s) of			
exposure	1.963		4.861
d.f.	287		225.11
treatment		1.603	
d.f.		287	
exposure.treatment			3.927
d.f.			287
exposure.Chem_Charge			4.861
d.f.			225.11

(Not adjusted for missing values)

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	93.043	23.261	2.02	
block.board stratum					
exposure	5	2221.254	444.251	38.60	<.001
Residual	20	230.156	11.508	1.76	
block.board.area stratum					
treatment	3	66.787	22.262	3.40	0.022
exposure.treatment	15	77.459	5.164	0.79	0.685
Residual	72	470.941	6.541	2.76	
block.board.area.Strip stratum					
Chem_Charge	3	5.338	1.779	0.75	0.523
exposure.Chem_Charge	15	29.486	1.966	0.83	0.646
treatment.Chem_Charge	9	26.700	2.967	1.25	0.264
exposure.treatment.Chem_Charge	45	82.429	1.832	0.77	0.853
Residual	288	683.439	2.373		
Total	479	3987.031			

Message: the following units have large residuals.

block 4 board 1	1.566	s.e. 0.692
block 5 board 1	-1.431	s.e. 0.692
block 1 board 3 area 1	2.442	s.e. 0.991
block 2 board 1 area 2	2.489	s.e. 0.991
block 4 board 2 area 3	2.987	s.e. 0.991
block 1 board 1 area 1 Strip 1	3.729	s.e. 1.193
block 1 board 2 area 1 Strip 3	3.865	s.e. 1.193
block 4 board 1 area 1 Strip 2	3.959	s.e. 1.193

Tables of means

Variate: a

Grand mean 7.667

exposure	full	I.R.	none	UVA	UVB	Vis light
	4.713	6.149	6.135	9.597	10.792	8.617

treatment	acetic acid	carpropamid	tinuvin	water
	7.673	7.431	8.269	7.295

Chem_Charge	high	low	medium	Very high
	7.716	7.650	7.795	7.508

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		5.114	4.560	5.256	3.923
I.R.		6.500	5.257	6.667	6.173
none		6.547	6.326	5.924	5.741
UVA		8.572	9.758	10.625	9.433
UVB		10.648	10.485	11.869	10.163
Vis light		8.658	8.198	9.274	8.338

exposure	Chem_Charge	high	low	medium	Very high
full		4.553	4.656	5.119	4.525
I.R.		6.616	5.894	5.698	6.389
none		6.019	6.321	6.226	5.973
UVA		9.509	9.811	9.735	9.333
UVB		11.022	10.458	11.361	10.324
Vis light		8.575	8.759	8.633	8.502

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		7.909	7.171	7.853	7.761
carpropamid		7.513	7.890	7.459	6.861
tinuvin		8.248	8.251	8.240	8.337
water		7.194	7.288	7.628	7.072

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		5.254	5.156	5.398	4.648
	carpropamid		4.746	5.028	4.422	4.044
	tinuvin		5.218	4.718	5.516	5.574
	water		2.996	3.722	5.140	3.836
I.R.	acetic acid		7.242	5.690	6.198	6.872
	carpropamid		5.898	5.204	4.220	5.706
	tinuvin		6.564	6.946	6.498	6.660
	water		6.762	5.736	5.876	6.318
none	acetic acid		7.360	6.688	6.026	6.116
	carpropamid		6.418	7.198	6.265	5.424
	tinuvin		4.984	5.572	6.186	6.954
	water		5.314	5.828	6.426	5.398
UVA	acetic acid		8.098	7.998	9.392	8.802
	carpropamid		9.552	10.700	9.564	9.216
	tinuvin		10.584	10.692	10.460	10.764
	water		9.804	9.856	9.524	8.550
UVB	acetic acid		10.916	9.728	11.070	10.880
	carpropamid		10.602	10.720	11.544	9.076
	tinuvin		12.786	11.206	12.566	10.918
	water		9.786	10.178	10.266	10.424
Vis light	acetic acid		8.582	7.766	9.036	9.248
	carpropamid		7.860	8.490	8.742	7.700
	tinuvin		9.354	10.372	8.216	9.154
	water		8.504	8.406	8.536	7.906

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.5364	0.3302	0.1989	0.8822
d.f.	20	72	288	80.97
Except when comparing means with the same level(s) of exposure				0.8088
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.6824	0.4771	1.2207
d.f.	51.04	242.27	240.31
Except when comparing means with the same level(s) of exposure	0.4871		1.1688
d.f.	288		242.27
treatment		0.3977	
d.f.		288	
exposure.treatment			0.9743
d.f.			288
exposure.Chem_Charge			1.1688
d.f.			242.27

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.1189	0.6582	0.3914	1.7553
d.f.	20	72	288	80.97
Except when comparing means with the same level(s) of exposure				1.6122
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.3700	0.9399	2.4047
d.f.	51.04	242.27	240.31
Except when comparing means with the same level(s) of			
exposure	0.9588		2.3022
d.f.	288		242.27
treatment		0.7829	
d.f.		288	
exposure.treatment			1.9176
d.f.			288
exposure.Chem_Charge			2.3022
d.f.			242.27

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
block stratum		4	72.034	18.008	0.40	
block.board stratum						
exposure		5	8093.225	1618.645	35.85	<.001
Residual		20	902.954	45.148	2.59	
block.board.area stratum						
treatment		3	280.390	93.463	5.36	0.002
exposure.treatment		15	284.302	18.953	1.09	0.383
Residual		72	1255.187	17.433	2.57	
block.board.area.Strip stratum						
Chem_Charge		3	7.471	2.490	0.37	0.777
exposure.Chem_Charge		15	76.946	5.130	0.75	0.727
treatment.Chem_Charge		9	105.982	11.776	1.73	0.081
exposure.treatment.Chem_Charge		45	222.243	4.939	0.73	0.902
Residual		288	1957.093	6.795		
Total		479	13257.826			

Message: the following units have large residuals.

block 4 board 1	3.65	s.e. 1.37
block 2 board 1 area 2	5.66	s.e. 1.62
block 4 board 1 area 2	-4.15	s.e. 1.62
block 4 board 1 area 4	4.45	s.e. 1.62
block 4 board 2 area 3	5.47	s.e. 1.62
block 1 board 1 area 1 Strip 1	7.87	s.e. 2.02
block 1 board 1 area 1 Strip 2	-8.19	s.e. 2.02
block 1 board 1 area 2 Strip 3	5.96	s.e. 2.02
block 2 board 2 area 1 Strip 3	-6.11	s.e. 2.02
block 4 board 1 area 1 Strip 2	9.63	s.e. 2.02

Tables of means

Variate: b

Grand mean 25.17

exposure	full	I.R.	none	UVA	UVB	Vis light
	16.56	25.02	25.49	27.86	29.29	26.81

treatment	acetic acid	carpropamid	tinuvin	water
	25.13	24.65	26.43	24.47

Chem_Charge	high	low	medium	Very high
	25.37	25.03	25.16	25.13

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		17.06	16.01	18.27	14.90
I.R.		25.72	23.26	25.96	25.14
none		25.97	25.88	25.10	25.01
UVA		26.39	27.87	29.29	27.88
UVB		28.89	28.54	32.03	27.69
Vis light		26.77	26.38	27.91	26.19

exposure	Chem_Charge	high	low	medium	Very high
full		16.41	16.54	17.22	16.05
I.R.		25.80	24.82	24.15	25.31
none		25.33	25.77	25.27	25.59
UVA		27.95	27.98	27.67	27.83
UVB		29.80	28.67	29.95	28.73
Vis light		26.92	26.39	26.67	27.26

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		25.58	24.31	25.10	25.53
carpropamid		25.25	25.41	24.13	23.83
tinuvin		26.42	26.17	26.26	26.86
water		24.22	24.22	25.13	24.30

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		17.48	17.57	17.33	15.84
	carpropamid		16.76	17.29	15.70	14.28
	tinuvin		18.31	16.82	18.91	19.03
	water		13.10	14.47	16.94	15.07
I.R.	acetic acid		26.28	24.72	25.65	26.21
	carpropamid		25.06	23.26	20.83	23.90
	tinuvin		26.02	26.37	25.42	26.03
	water		25.84	24.93	24.70	25.10
none	acetic acid		27.14	25.57	24.49	26.68
	carpropamid		26.21	27.39	25.03	24.87
	tinuvin		23.41	25.03	25.40	26.56
	water		24.57	25.07	26.17	24.23
UVA	acetic acid		26.36	25.36	26.80	27.06
	carpropamid		27.72	29.40	26.79	27.55
	tinuvin		29.22	29.02	29.16	29.76
	water		28.50	28.12	27.93	26.97
UVB	acetic acid		29.28	27.56	29.61	29.09
	carpropamid		29.61	28.67	29.80	26.07
	tinuvin		33.85	31.06	32.14	31.07
	water		26.46	27.38	28.23	28.69
Vis light	acetic acid		26.96	25.08	26.72	28.32
	carpropamid		26.15	26.42	26.63	26.30
	tinuvin		27.70	28.69	26.55	28.70
	water		26.87	25.37	26.79	25.71

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.062	0.539	0.337	1.561
d.f.	20	72	288	67.87
Except when comparing means with the same level(s) of exposure				1.320
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.280	0.794	2.115
d.f.	41.55	252.52	196.56
Except when comparing means with the same level(s) of exposure	0.824		1.945
d.f.	288		252.52
treatment		0.673	
d.f.		288	
exposure.treatment			1.649
d.f.			288
exposure.Chem_Charge			1.945
d.f.			252.52

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.216	1.075	0.662	3.115
d.f.	20	72	288	67.87
Except when comparing means with the same level(s) of exposure				2.632
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.584	1.564	4.172
d.f.	41.55	252.52	196.56
Except when comparing means with the same level(s) of			
exposure	1.623		3.830
d.f.	288		252.52
treatment		1.325	
d.f.		288	
exposure.treatment			3.245
d.f.			288
exposure.Chem_Charge			3.830
d.f.			252.52

Analysis of variance week 12

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3366.18	841.54	4.99	
block.board stratum					
exposure	5	11796.16	2359.23	14.00	<.001
Residual	20	3370.98	168.55	4.72	
block.board.area stratum					
treatment	3	51.43	17.14	0.48	0.697
exposure.treatment	15	259.11	17.27	0.48	0.941
Residual	72	2568.72	35.68	2.36	
block.board.area.Strip stratum					
Chem_Charge	3	0.33	0.11	0.01	0.999
exposure.Chem_Charge	15	355.45	23.70	1.56	0.083
treatment.Chem_Charge	9	131.81	14.65	0.97	0.468
exposure.treatment.Chem_Charge	45	761.35	16.92	1.12	0.291
Residual	288	4361.95	15.15		
Total	479	27023.47			

Message: the following units have large residuals.

block 2 board 4	5.94	s.e. 2.65
block 4 board 4	-6.15	s.e. 2.65
block 2 board 3 area 3	5.97	s.e. 2.31
block 1 board 1 area 4 Strip 3	12.49	s.e. 3.01
block 4 board 6 area 1 Strip 4	-9.20	s.e. 3.01
block 5 board 3 area 1 Strip 2	9.63	s.e. 3.01

Tables of means

Variate: L

Grand mean 62.38

exposure	full	I.R.	none	UVA	UVB	Vis light
	52.10	65.70	67.83	62.50	62.93	63.21

treatment	acetic acid	carpropamid	tinuvin	water
	62.23	62.33	62.92	62.04

Chem_Charge	high	low	medium	Very high
	62.40	62.41	62.35	62.36

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		52.61	49.85	53.16	52.78
I.R.		65.62	65.83	66.31	65.02
none		67.71	67.37	68.93	67.32
UVA		61.78	63.47	63.58	61.18
UVB		62.90	63.82	62.20	62.83
Vis light		62.77	63.61	63.33	63.13

exposure	Chem_Charge	high	low	medium	Very high
full		52.69	52.75	50.65	52.30
I.R.		66.65	66.41	65.19	64.55
none		65.80	67.49	69.64	68.40
UVA		63.77	61.65	62.67	61.91
UVB		63.06	62.85	62.41	63.42
Vis light		62.45	63.29	63.53	63.56

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		61.44	62.88	62.85	61.76
carpropamid		62.95	61.85	62.25	62.26
tinuvin		63.59	63.07	61.84	63.18
water		61.63	61.85	62.46	62.24

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		53.34	52.41	52.08	52.61
	carpropamid		52.81	50.59	47.60	48.42
	tinuvin		51.60	54.43	51.12	55.48
	water		53.02	53.59	51.81	52.70
I.R.	acetic acid		66.44	67.27	65.39	63.40
	carpropamid		66.88	65.82	63.78	66.84
	tinuvin		68.14	67.29	65.86	63.97
	water		65.12	65.25	65.74	63.98
none	acetic acid		65.60	66.75	70.48	68.02
	carpropamid		66.90	66.19	69.57	66.82
	tinuvin		65.99	70.49	69.93	69.33
	water		64.71	66.55	68.59	69.44
UVA	acetic acid		61.01	61.94	62.92	61.24
	carpropamid		62.96	64.01	63.11	63.81
	tinuvin		67.31	61.77	61.97	63.26
	water		63.80	58.89	62.69	59.34
UVB	acetic acid		61.09	65.93	62.86	61.71
	carpropamid		67.62	60.43	64.28	62.94
	tinuvin		64.75	60.62	59.33	64.09
	water		58.79	64.43	63.15	64.94
Vis light	acetic acid		61.17	62.95	63.37	63.56
	carpropamid		60.54	64.04	65.13	64.72
	tinuvin		63.77	63.81	62.84	62.92
	water		64.32	62.36	62.78	63.06

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.053	0.771	0.502	2.625
d.f.	20	72	288	48.08
Except when comparing means with the same level(s) of				
exposure				1.889
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.313	1.163	3.381
d.f.	32.07	264.80	123.45
Except when comparing means with the same level(s) of			
exposure	1.231		2.848
d.f.	288		264.80
treatment		1.005	
d.f.		288	
exposure.treatment			
			2.461
d.f.			288
exposure.Chem_Charge			
			2.848
d.f.			264.80

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.282	1.537	0.989	5.277
d.f.	20	72	288	48.08
Except when comparing means with the same level(s) of				
exposure				3.765
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	4.711	2.289	6.693
d.f.	32.07	264.80	123.45
Except when comparing means with the same level(s) of			
exposure	2.422		5.608
d.f.	288		264.80
treatment		1.978	
d.f.		288	
exposure.treatment			4.845
d.f.			288
exposure.Chem_Charge			5.608
d.f.			264.80

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	295.715	73.929	3.86	
block.board stratum					
exposure	5	2305.684	461.137	24.07	<.001
Residual	20	383.150	19.158	3.73	
block.board.area stratum					
treatment	3	12.897	4.299	0.84	0.478
exposure.treatment	15	95.710	6.381	1.24	0.261
Residual	72	369.422	5.131	2.32	
block.board.area.Strip stratum					
Chem_Charge	3	10.855	3.618	1.63	0.182
exposure.Chem_Charge	15	28.341	1.889	0.85	0.617
treatment.Chem_Charge	9	24.195	2.688	1.21	0.286
exposure.treatment.Chem_Charge	45	123.594	2.747	1.24	0.152
Residual	288	637.596	2.214		
Total	479	4287.158			

Message: the following units have large residuals.

block 4 board 4	-1.860	s.e. 0.893
block 1 board 3 area 1	3.499	s.e. 0.877
block 1 board 5 area 1 Strip 1	3.689	s.e. 1.153
block 2 board 3 area 3 Strip 2	3.674	s.e. 1.153
block 3 board 2 area 4 Strip 4	4.047	s.e. 1.153
block 4 board 2 area 1 Strip 3	-3.449	s.e. 1.153
block 4 board 2 area 4 Strip 1	3.752	s.e. 1.153
block 4 board 4 area 3 Strip 4	3.581	s.e. 1.153
block 4 board 5 area 4 Strip 2	-3.417	s.e. 1.153

Tables of means

Variate: a

Grand mean 5.874

exposure	full	I.R.	none	UVA	UVB	Vis light
	3.215	4.492	3.619	8.016	8.828	7.076

treatment	acetic acid	carpropamid	tinuvin	water
	6.059	5.618	5.862	5.960

Chem_Charge	high	low	medium	Very high
	5.789	5.735	6.126	5.848

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		3.064	3.743	2.752	3.302
I.R.		5.266	3.970	3.864	4.870
none		4.173	3.539	3.447	3.319
UVA		7.969	8.019	7.851	8.226
UVB		8.517	7.706	9.756	9.334
Vis light		7.365	6.729	7.501	6.710

exposure	Chem_Charge	high	low	medium	Very high
full		3.125	2.902	3.711	3.124
I.R.		4.776	4.105	4.748	4.341
none		3.395	4.007	3.627	3.449
UVA		7.774	7.826	8.382	8.083
UVB		8.964	8.226	9.269	8.854
Vis light		6.702	7.345	7.018	7.240

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		5.839	5.731	6.180	6.485
carpropamid		5.654	5.892	5.726	5.198
tinuvin		5.698	5.772	6.299	5.678
water		5.966	5.544	6.297	6.033

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		2.216	2.834	3.810	3.396
	carpropamid		3.588	3.940	4.062	3.382
	tinuvin		3.334	2.288	2.544	2.842
	water		3.360	2.546	4.426	2.874
I.R.	acetic acid		5.440	4.472	6.500	4.652
	carpropamid		4.090	4.428	3.424	3.938
	tinuvin		3.806	3.592	4.468	3.590
	water		5.766	3.928	4.600	5.184
none	acetic acid		3.848	5.198	3.650	3.996
	carpropamid		3.408	3.488	4.232	3.026
	tinuvin		3.300	3.646	3.292	3.548
	water		3.022	3.696	3.332	3.226
UVA	acetic acid		7.378	7.956	7.884	8.656
	carpropamid		8.558	8.810	7.712	6.996
	tinuvin		7.866	6.542	8.756	8.238
	water		7.294	7.994	9.174	8.442
UVB	acetic acid		9.230	6.956	8.376	9.504
	carpropamid		7.864	7.934	7.638	7.386
	tinuvin		8.772	9.640	11.812	8.798
	water		9.988	8.372	9.248	9.728
Vis light	acetic acid		6.920	6.972	6.860	8.706
	carpropamid		6.414	6.754	7.288	6.460
	tinuvin		7.108	8.924	6.922	7.050
	water		6.366	6.730	7.002	6.742

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6921	0.2924	0.1921	0.9294
d.f.	20	72	288	55.16
Except when comparing means with the same level(s) of				
exposure				0.7163
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.8031	0.4430	1.2361
d.f.	35.97	267.14	155.04
Except when comparing means with the same level(s) of			
exposure	0.4705		1.0850
d.f.	288		267.14
treatment		0.3842	
d.f.		288	
exposure.treatment			
			0.9410
d.f.			288
exposure.Chem_Charge			
			1.0850
d.f.			267.14

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.4436	0.5829	0.3781	1.8624
d.f.	20	72	288	55.16
Except when comparing means with the same level(s) of				
exposure				1.4279
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.6288	0.8721	2.4417
d.f.	35.97	267.14	155.04
Except when comparing means with the same level(s) of			
exposure	0.9261		2.1363
d.f.	288		267.14
treatment		0.7561	
d.f.		288	
exposure.treatment			1.8522
d.f.			288
exposure.Chem_Charge			2.1363
d.f.			267.14

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	663.050	165.762	2.46	
block.board stratum					
exposure	5	7830.401	1566.080	23.22	<.001
Residual	20	1348.633	67.432	4.67	
block.board.area stratum					
treatment	3	40.053	13.351	0.92	0.433
exposure.treatment	15	273.387	18.226	1.26	0.248
Residual	72	1039.288	14.435	2.23	
block.board.area.Strip stratum					
Chem_Charge	3	42.893	14.298	2.21	0.087
exposure.Chem_Charge	15	77.772	5.185	0.80	0.676
treatment.Chem_Charge	9	53.484	5.943	0.92	0.509
exposure.treatment.Chem_Charge	45	329.979	7.333	1.13	0.270
Residual	288	1863.679	6.471		
Total	479	13562.620			

Message: the following units have large residuals.

block 4 board 4	-3.60	s.e. 1.68
block 1 board 3 area 1	4.85	s.e. 1.47
block 1 board 5 area 1 Strip 1	6.40	s.e. 1.97
block 3 board 2 area 4 Strip 4	5.86	s.e. 1.97
block 4 board 1 area 1 Strip 2	5.95	s.e. 1.97
block 4 board 4 area 3 Strip 4	6.00	s.e. 1.97
block 4 board 5 area 4 Strip 2	-6.46	s.e. 1.97

Tables of means

Variate: b

Grand mean 21.47

exposure	full	I.R.	none	UVA	UVB	Vis light
	12.93	21.75	21.18	24.69	24.39	23.89

treatment	acetic acid	carpropamid	tinuvin	water
	21.56	21.01	21.80	21.50

Chem_Charge	high	low	medium	Very high
	21.44	21.15	21.95	21.33

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		12.27	13.89	12.47	13.07
I.R.		22.87	20.82	20.99	22.30
none		21.70	21.04	21.15	20.81
UVA		24.48	24.57	24.93	24.77
UVB		23.84	22.40	26.56	24.75
Vis light		24.21	23.33	24.72	23.31

exposure	Chem_Charge	high	low	medium	Very high
full		12.64	12.34	13.94	12.79
I.R.		22.43	21.29	21.97	21.29
none		20.62	21.46	21.71	20.91
UVA		24.61	24.21	25.23	24.69
UVB		25.02	23.30	24.92	24.32
Vis light		23.33	24.28	23.96	24.00

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		21.22	21.15	21.98	21.90
carpropamid		21.04	21.43	21.21	20.35
tinuvin		21.87	21.43	22.28	21.63
water		21.64	20.57	22.34	21.46

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		10.29	11.98	13.60	13.23
	carpropamid		13.55	14.73	14.46	12.80
	tinuvin		13.75	11.12	12.18	12.84
	water		12.96	11.52	15.51	12.31
I.R.	acetic acid		23.23	22.37	24.88	21.00
	carpropamid		21.11	21.68	19.39	21.11
	tinuvin		21.51	20.63	21.66	20.16
	water		23.89	20.49	21.95	22.89
none	acetic acid		20.84	22.76	21.85	21.37
	carpropamid		20.71	20.55	22.62	20.28
	tinuvin		20.69	21.63	20.87	21.40
	water		20.26	20.89	21.48	20.60
UVA	acetic acid		23.61	24.72	24.54	25.06
	carpropamid		25.21	25.73	24.07	23.25
	tinuvin		25.71	22.32	26.03	25.65
	water		23.90	24.09	26.28	24.81
UVB	acetic acid		25.84	21.56	23.20	24.78
	carpropamid		23.13	22.45	22.29	21.74
	tinuvin		25.37	26.28	29.04	25.56
	water		25.75	22.90	25.15	25.18
Vis light	acetic acid		23.52	23.54	23.84	25.94
	carpropamid		22.55	23.44	24.43	22.92
	tinuvin		24.19	26.62	23.89	24.17
	water		23.05	23.53	23.67	22.97

Standard errors of differences of means

Tableexposure	treatment	Chem_Charge	exposure	treatment
rep.	80	120	120	20
s.e.d.	1.298	0.490	0.328	1.664
d.f.	20	72	288	48.39
Except when comparing means with the same level(s) of				
exposure				1.201
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.473	0.751	2.170
d.f.	32.98	272.62	129.37
Except when comparing means with the same level(s) of			
exposure	0.804		1.840
d.f.	288		272.62
treatment		0.657	
d.f.		288	
exposure.treatment			
			1.609
d.f.			288
exposure.Chem_Charge			
			1.840
d.f.			272.62

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.708	0.978	0.646	3.345
d.f.	20	72	288	48.39
Except when comparing means with the same level(s) of				
exposure				2.395
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	2.998	1.479	4.294
d.f.	32.98	272.62	129.37
Except when comparing means with the same level(s) of			
exposure	1.583		3.622
d.f.	288		272.62
treatment		1.293	
d.f.		288	
exposure.treatment			
			3.167
d.f.			288
exposure.Chem_Charge			
			3.622
d.f.			272.62

Analysis of variance week 14

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3383.04	845.76	3.41	
block.board stratum					
exposure	5	12653.41	2530.68	10.19	<.001
Residual	20	4966.51	248.33	5.65	
block.board.area stratum					
treatment	3	314.43	104.81	2.38	0.076
exposure.treatment	15	485.25	32.35	0.74	0.741
Residual	72	3166.01	43.97	2.07	

block.board.area.Strip stratum					
Chem_Charge	3	2.82	0.94	0.04	0.988
exposure.Chem_Charge	15	624.44	41.63	1.96	0.018
treatment.Chem_Charge	9	144.15	16.02	0.76	0.658
exposure.treatment.Chem_Charge	45	1023.51	22.74	1.07	0.357
Residual	288	6107.76	21.21		
Total	479	32871.33			

Message: the following units have large residuals.

block 2 board 3	-6.99	s.e. 3.22
block 4 board 4	-7.02	s.e. 3.22
block 2 board 2 area 4	-6.49	s.e. 2.57
block 2 board 3 area 3	6.91	s.e. 2.57
block 3 board 1 area 1	-6.49	s.e. 2.57
block 1 board 1 area 4 Strip 3	12.63	s.e. 3.57
block 1 board 6 area 4 Strip 2	12.54	s.e. 3.57
block 2 board 1 area 4 Strip 1	13.77	s.e. 3.57
block 5 board 3 area 1 Strip 2	10.59	s.e. 3.57

Tables of means

Variate: L

Grand mean 60.36

exposure	full	I.R.	none	UVA	UVB	Vis light
	49.46	63.80	65.32	60.61	61.94	61.01

treatment	acetic acid	carpropamid	tinuvin	water
	60.00	60.54	61.55	59.34

Chem_Charge	high	low	medium	Very high
	60.44	60.26	60.30	60.43

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		49.65	48.33	50.22	49.65
I.R.		62.96	64.91	65.84	61.48
none		66.22	64.14	67.25	63.68
UVA		60.07	60.39	63.10	58.87
UVB		62.05	62.79	61.23	61.68
Vis light		59.08	62.67	61.64	60.67

exposure	Chem_Charge	high	low	medium	Very high
full		50.01	50.42	48.20	49.22
I.R.		65.69	64.06	62.07	63.37
none		62.18	64.83	67.93	66.35
UVA		61.37	59.52	61.31	60.24
UVB		62.61	61.83	61.12	62.19
Vis light		60.76	60.91	61.18	61.20

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		59.49	60.01	60.81	59.71
carpropamid		61.04	60.34	60.59	60.18
tinuvin		62.47	61.70	60.68	61.34
water		58.76	59.00	59.11	60.48

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		50.29	46.96	50.61	50.74
	carpropamid		50.26	52.65	45.86	44.54
	tinuvin		49.11	51.66	48.18	51.94
	water		50.38	50.39	48.15	49.67
I.R.	acetic acid		66.18	63.66	60.34	61.66
	carpropamid		65.62	65.36	62.15	66.49
	tinuvin		67.91	66.94	64.29	64.25
	water		63.06	60.27	61.49	61.08
none	acetic acid		62.93	64.44	70.08	67.43
	carpropamid		63.00	62.33	67.60	63.63
	tinuvin		63.75	69.09	69.72	66.45
	water		59.04	63.47	64.32	67.88
UVA	acetic acid		58.29	60.66	62.07	59.27
	carpropamid		60.32	60.15	60.35	60.73
	tinuvin		67.05	60.29	62.70	62.38
	water		59.83	56.98	60.11	58.57
UVB	acetic acid		60.81	64.80	61.85	60.72
	carpropamid		66.03	59.19	63.83	62.12
	tinuvin		65.13	59.51	58.03	62.26
	water		58.49	63.83	60.76	63.63
Vis light	acetic acid		58.41	59.54	59.94	58.42
	carpropamid		60.99	62.34	63.77	63.58
	tinuvin		61.88	62.71	61.20	60.77
	water		61.74	59.06	59.82	62.04

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.492	0.856	0.595	3.083
d.f.	20	72	288	43.48
Except when comparing means with the same level(s) of exposure				2.097
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.793	1.339	3.984
d.f.	31.42	282.98	113.49
Except when comparing means with the same level(s) of exposure	1.456		3.280
d.f.	288		282.98
treatment		1.189	
d.f.		288	
exposure.treatment			2.913
d.f.			288
exposure.Chem_Charge			3.280
d.f.			282.98

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	5.197	1.707	1.170	6.216
d.f.	20	72	288	43.48
Except when comparing means with the same level(s) of exposure				4.180
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.693	2.636	7.892
d.f.	31.42	282.98	113.49
Except when comparing means with the same level(s) of			
exposure	2.866		6.457
d.f.	288		282.98
treatment		2.340	
d.f.		288	
exposure.treatment			5.733
d.f.			288
exposure.Chem_Charge			6.457
d.f.			282.98

Analysis of variance

Variate: a

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		236.968	59.242	2.17	
block.board stratum						
exposure	5		2314.830	462.966	16.95	<.001
Residual	20		546.418	27.321	4.51	
block.board.area stratum						
treatment	3		44.234	14.745	2.43	0.072
exposure.treatment	15		128.586	8.572	1.41	0.164
Residual	72		436.453	6.062	3.00	
block.board.area.Strip stratum						
Chem_Charge	3		6.819	2.273	1.13	0.339
exposure.Chem_Charge	15		23.236	1.549	0.77	0.713
treatment.Chem_Charge	9		56.138	6.238	3.09	0.001
exposure.treatment.Chem_Charge	45		145.102	3.224	1.60	0.013
Residual	286	(2)	577.236	2.018		
Total	477	(2)	4485.939			

Message: the following units have large residuals.

block 1 board 2	2.458	s.e. 1.067
block 1 board 3 area 1	3.465	s.e. 0.954
block 2 board 2 area 2	2.775	s.e. 0.954
block 4 board 2 area 1 Strip 3	-3.582	s.e. 1.097
block 4 board 4 area 3 Strip 4	3.748	s.e. 1.097
block 5 board 2 area 1 Strip 1	-3.550	s.e. 1.097

Tables of means

Variate: a

Grand mean 5.376

exposure	full	I.R.	none	UVA	UVB	Vis light
	2.468	4.168	3.170	7.356	8.178	6.918

treatment	acetic acid	carpropamid	tinuvin	water
	5.498	4.920	5.755	5.333

Chem_Charge	high	low	medium	Very high
	5.322	5.211	5.450	5.522

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		2.843	2.792	1.963	2.274
I.R.		4.678	3.584	4.049	4.362
none		3.475	3.050	3.332	2.821
UVA		7.225	7.221	7.763	7.216
UVB		7.748	6.456	9.652	8.857
Vis light		7.022	6.414	7.768	6.465

exposure	Chem_Charge	high	low	medium	Very high
full		2.298	2.516	2.622	2.437
I.R.		4.473	3.940	4.190	4.069
none		2.884	3.400	3.347	3.047
UVA		7.333	6.830	7.398	7.864
UVB		8.204	7.631	8.352	8.525
Vis light		6.740	6.951	6.788	7.190

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		5.311	5.293	5.143	6.247
carpropamid		4.999	5.219	5.064	4.397
tinuvin		5.602	5.672	6.150	5.594
water		5.377	4.661	5.441	5.851

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		2.212	3.090	2.954	3.114
	carpropamid		2.608	2.994	2.882	2.682
	tinuvin		2.372	1.932	1.516	2.034
	water		1.998	2.046	3.134	1.918
I.R.	acetic acid		4.638	4.392	5.204	4.476
	carpropamid		3.568	4.422	2.916	3.428
	tinuvin		4.178	3.852	4.576	3.588
	water		5.508	3.093	4.064	4.784
none	acetic acid		2.998	4.104	3.424	3.374
	carpropamid		2.624	2.914	3.930	2.732
	tinuvin		3.050	3.866	3.298	3.116
	water		2.866	2.716	2.736	2.968
UVA	acetic acid		6.968	7.492	5.807	8.632
	carpropamid		7.914	7.574	7.122	6.274
	tinuvin		8.074	5.684	9.222	8.072
	water		6.376	6.570	7.442	8.476
UVB	acetic acid		8.402	6.206	7.144	9.240
	carpropamid		6.762	6.822	6.648	5.594
	tinuvin		8.226	9.858	11.272	9.252
	water		9.426	7.640	8.346	10.016
Vis light	acetic acid		6.646	6.472	6.326	8.644
	carpropamid		6.516	6.586	6.884	5.672
	tinuvin		7.712	8.842	7.018	7.502
	water		6.088	5.904	6.926	6.944

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.8265	0.3179	0.1834	1.0666
d.f.	20	72	286	49.41
Except when comparing means with the same level(s) of				
exposure				0.7786
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.9135	0.4494	1.3203
d.f.	29.75	229.92	110.58
Except when comparing means with the same level(s) of			
exposure	0.4493		1.1008
d.f.	286		229.92
treatment		0.3668	
d.f.		286	
exposure.treatment			
			0.8985
d.f.			286
exposure.Chem_Charge			
			1.1008
d.f.			229.92

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.7239	0.6336	0.3610	2.1430
d.f.	20	72	286	49.41
Except when comparing means with the same level(s) of				
exposure				1.5521
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.8662	0.8854	2.6163
d.f.	29.75	229.92	110.58
Except when comparing means with the same level(s) of			
exposure	0.8843		2.1689
d.f.	286		229.92
treatment		0.7220	
d.f.		286	
exposure.treatment			1.7685
d.f.			286
exposure.Chem_Charge			2.1689
d.f.			229.92

(Not adjusted for missing values)

Analysis of variance

Variate: b

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		513.312	128.328	1.32	
block.board stratum						
exposure	5		9896.041	1979.208	20.40	<.001
Residual	20		1940.548	97.027	6.31	
block.board.area stratum						
treatment	3		194.994	64.998	4.23	0.008
exposure.treatment	15		366.835	24.456	1.59	0.098
Residual	72		1106.978	15.375	2.19	
block.board.area.Strip stratum						
Chem_Charge	3		18.965	6.322	0.90	0.442
exposure.Chem_Charge	15		94.886	6.326	0.90	0.564
treatment.Chem_Charge	9		119.720	13.302	1.89	0.053
exposure.treatment.Chem_Charge	45		376.011	8.356	1.19	0.202
Residual	287	(1)	2016.661	7.027		
Total	478	(1)	16600.051			

Message: the following units have large residuals.

block 1 board 2	4.06	s.e. 2.01
block 4 board 4	-4.31	s.e. 2.01
block 1 board 3 area 1	5.07	s.e. 1.52
block 1 board 3 area 3	-3.88	s.e. 1.52
block 2 board 2 area 2	4.17	s.e. 1.52
block 4 board 4 area 3 Strip 4	6.28	s.e. 2.05
block 4 board 5 area 4 Strip 2	-6.37	s.e. 2.05
block 5 board 2 area 1 Strip 1	-6.45	s.e. 2.05

Tables of means

Variate: b

Grand mean 20.09

exposure	full	I.R.	none	UVA	UVB	Vis light
	10.39	20.65	19.89	23.66	22.70	23.22

treatment	acetic acid	carpropamid	tinuvin	water
	20.24	19.31	21.03	19.75

Chem_Charge	high	low	medium	Very high
	20.06	19.78	20.30	20.21

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		11.18	11.00	9.55	9.84
I.R.		21.06	19.90	21.07	20.59
none		20.37	19.46	20.45	19.30
UVA		23.53	23.23	24.70	23.16
UVB		22.03	19.69	25.81	23.27
Vis light		23.30	22.61	24.61	22.36

exposure	Chem_Charge	high	low	medium	Very high
full		9.96	10.55	10.62	10.45
I.R.		21.56	20.40	20.30	20.35
none		18.94	20.05	20.74	19.84
UVA		23.68	22.65	24.18	24.11
UVB		23.08	21.88	22.97	22.87
Vis light		23.11	23.12	22.98	23.66

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		19.75	19.97	20.13	21.13
carpropamid		19.42	19.79	19.58	18.47
tinuvin		21.07	20.82	21.46	20.77
water		19.99	18.52	20.03	20.48

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		9.68	12.11	11.07	11.86
	carpropamid		10.79	11.69	11.06	10.48
	tinuvin		10.23	9.04	8.76	10.16
	water		9.13	9.36	11.58	9.29
I.R.	acetic acid		21.33	21.40	21.45	20.04
	carpropamid		19.94	21.61	18.08	19.95
	tinuvin		21.79	21.13	21.38	19.99
	water		23.16	17.48	20.29	21.43
none	acetic acid		18.60	20.90	21.46	20.51
	carpropamid		18.37	18.91	21.52	19.03
	tinuvin		19.80	21.23	20.73	20.03
	water		19.00	19.16	19.23	19.81
UVA	acetic acid		22.27	23.51	23.66	24.67
	carpropamid		24.12	23.76	23.25	21.80
	tinuvin		25.91	21.24	26.56	25.08
	water		22.42	22.09	23.25	24.88
UVB	acetic acid		23.48	19.71	20.95	24.00
	carpropamid		20.57	20.27	20.15	17.77
	tinuvin		24.20	26.08	27.82	25.14
	water		24.08	21.45	22.98	24.55
Vis light	acetic acid		23.12	22.19	22.17	25.71
	carpropamid		22.71	22.52	23.44	21.78
	tinuvin		24.49	26.21	23.48	24.25
	water		22.13	21.55	22.83	22.91

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.557	0.506	0.342	1.892
d.f.	20	72	287	40.96
Except when comparing means with the same level(s) of exposure				1.240
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.718	0.779	2.385
d.f.	29.54	275.07	98.55
Except when comparing means with the same level(s) of exposure	0.838		1.909
d.f.	287		275.07
treatment		0.684	
d.f.		287	
exposure.treatment			1.677
d.f.			287
exposure.Chem_Charge			1.909
d.f.			275.07

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	3.249	1.009	0.674	3.821
d.f.	20	72	287	40.96
Except when comparing means with the same level(s) of exposure				2.472
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.512	1.534	4.732
d.f.	29.54	275.07	98.55
Except when comparing means with the same level(s) of			
exposure	1.650		3.759
d.f.	287		275.07
treatment		1.347	
d.f.		287	
exposure.treatment			3.300
d.f.			287
exposure.Chem_Charge			3.759
d.f.			275.07

(Not adjusted for missing values)

Analysis of variance week 16

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3444.14	861.03	3.99	
block.board stratum					
exposure	5	12408.31	2481.66	11.49	<.001
Residual	20	4321.14	216.06	4.99	
block.board.area stratum					
treatment	3	216.92	72.31	1.67	0.181
exposure.treatment	15	466.23	31.08	0.72	0.758
Residual	72	3116.25	43.28	2.41	
block.board.area.Strip stratum					
Chem_Charge	3	4.55	1.52	0.08	0.968
exposure.Chem_Charge	15	319.56	21.30	1.19	0.280
treatment.Chem_Charge	9	139.26	15.47	0.86	0.559
exposure.treatment.Chem_Charge	45	800.77	17.79	0.99	0.491
Residual	288	5163.58	17.93		
Total	479	30400.70			

Message: the following units have large residuals.

block 2 board 4	7.29	s.e. 3.00
block 2 board 2 area 4	-6.96	s.e. 2.55
block 2 board 3 area 3	6.53	s.e. 2.55
block 5 board 1 area 4	7.07	s.e. 2.55
block 1 board 1 area 4 Strip 3	10.67	s.e. 3.28
block 1 board 6 area 1 Strip 3	-13.90	s.e. 3.28
block 1 board 6 area 4 Strip 2	11.98	s.e. 3.28

Tables of means

Variate: L

Grand mean 58.95

exposure	full	I.R.	none	UVA	UVB	Vis light
	48.32	62.54	64.13	58.72	60.27	59.71

treatment	acetic acid	carpropamid	tinuvin	water
	58.55	58.47	60.11	58.66

Chem_Charge	high	low	medium	Very high
	59.06	58.81	59.00	58.91

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		48.96	45.67	49.79	48.84
I.R.		61.74	63.02	64.31	61.09
none		64.15	63.32	66.07	62.96
UVA		58.30	58.72	60.52	57.34
UVB		59.91	59.21	60.19	61.76
Vis light		58.24	60.87	59.75	59.97

exposure	Chem_Charge	high	low	medium	Very high
full		48.94	48.02	47.97	48.33
I.R.		63.93	63.20	61.89	61.14
none		61.93	63.83	66.01	64.72
UVA		59.50	57.90	58.88	58.61
UVB		60.09	60.28	59.85	60.84
Vis light		59.98	59.60	59.41	59.84

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		58.32	58.63	59.06	58.18
carpropamid		59.03	57.62	58.52	58.72
tinuvin		61.13	60.38	59.37	59.54
water		57.77	58.59	59.07	59.21

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		49.39	46.85	50.32	49.31
	carpropamid		47.65	45.12	45.16	44.75
	tinuvin		48.80	51.37	47.88	51.11
	water		49.94	48.74	48.53	48.17
I.R.	acetic acid		64.22	62.75	60.85	59.13
	carpropamid		63.81	63.58	60.78	63.93
	tinuvin		65.59	65.36	64.64	61.68
	water		62.12	61.12	61.29	59.82
none	acetic acid		63.87	62.95	66.43	63.33
	carpropamid		62.51	61.73	65.88	63.16
	tinuvin		63.25	67.69	68.06	65.27
	water		58.10	62.97	63.68	67.11
UVA	acetic acid		57.04	58.72	59.37	58.08
	carpropamid		58.57	57.95	58.71	59.66
	tinuvin		64.79	57.92	59.61	59.78
	water		57.59	57.00	57.85	56.93
UVB	acetic acid		56.55	61.57	60.70	60.82
	carpropamid		62.34	56.68	58.82	59.02
	tinuvin		63.30	59.26	56.86	61.31
	water		58.18	63.61	63.02	62.22
Vis light	acetic acid		58.89	58.94	56.71	58.42
	carpropamid		59.28	60.68	61.77	61.77
	tinuvin		61.04	60.69	59.15	58.11
	water		60.71	58.08	60.03	61.05

Standard errors of differences of means

Tableexposure	treatment	Chem_Charge	exposure		
					treatment
rep.		80	120	120	20
s.e.d.		2.324	0.849	0.547	2.941
d.f.		20	72	288	46.59
Except when comparing means with the same level(s) of					
exposure					2.080
d.f.					72

Table	exposure	treatment	exposure
	Chem_Charge	Chem_Charge	treatment
			Chem_Charge
rep.	20	30	5
s.e.d.	2.597	1.272	3.745
d.f.	31.06	261.27	115.35
Except when comparing means with the same level(s) of			
exposure	1.339		3.116
d.f.	288		261.27
treatment		1.093	
d.f.		288	
exposure.treatment			
			2.678
d.f.			288
exposure.Chem_Charge			
			3.116
d.f.			261.27

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.848	1.693	1.076	5.917
d.f.	20	72	288	46.59
Except when comparing means with the same level(s) of				
exposure				4.147
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.297	2.505	7.418
d.f.	31.06	261.27	115.35
Except when comparing means with the same level(s) of			
exposure	2.635		6.135
d.f.	288		261.27
treatment		2.152	
d.f.		288	
exposure.treatment			
			5.271
d.f.			288
exposure.Chem_Charge			
			6.135
d.f.			261.27

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	191.028	47.757	2.15	
block.board stratum					
exposure	5	1991.722	398.344	17.90	<.001
Residual	20	445.171	22.259	3.45	
block.board.area stratum					
treatment	3	26.781	8.927	1.38	0.255
exposure.treatment	15	101.698	6.780	1.05	0.416
Residual	72	464.537	6.452	3.16	

block.board.area.Strip stratum					
Chem_Charge	3	6.360	2.120	1.04	0.375
exposure.Chem_Charge	15	26.278	1.752	0.86	0.611
treatment.Chem_Charge	9	43.923	4.880	2.39	0.013
exposure.treatment.Chem_Charge	45	122.493	2.722	1.33	0.085
Residual	288	587.291	2.039		
Total	479	4007.282			

Message: the following units have large residuals.

block 1 board 2	2.333	s.e. 0.963
block 4 board 4	-2.000	s.e. 0.963
block 1 board 3 area 1	3.979	s.e. 0.984
block 1 board 3 area 3	-2.821	s.e. 0.984
block 2 board 2 area 2	3.217	s.e. 0.984
block 2 board 3 area 3 Strip 2	3.466	s.e. 1.106
block 3 board 2 area 4 Strip 3	-3.696	s.e. 1.106
block 3 board 2 area 4 Strip 4	3.565	s.e. 1.106
block 5 board 2 area 2 Strip 1	3.631	s.e. 1.106

Tables of means

Variate: a

Grand mean 5.223

exposure	full	I.R.	none	UVA	UVB	Vis light
	2.546	4.102	3.142	7.134	7.763	6.651

treatment	acetic acid	carpropamid	tinuvin	water
	5.302	4.878	5.533	5.181

Chem_Charge	high	low	medium	Very high
	5.060	5.178	5.362	5.293

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		2.816	2.636	2.223	2.509
I.R.		4.388	3.825	3.723	4.472
none		3.302	3.106	3.255	2.905
UVA		7.634	6.994	7.149	6.760
UVB		7.254	6.528	9.212	8.056
Vis light		6.414	6.177	7.634	6.380

exposure	Chem_Charge	high	low	medium	Very high
full		2.483	2.480	2.695	2.526
I.R.		4.366	4.055	4.051	3.936
none		2.812	3.430	3.456	2.869
UVA		6.736	6.996	7.587	7.218
UVB		7.518	7.323	7.975	8.234
Vis light		6.441	6.782	6.408	6.973

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		5.081	5.103	5.433	5.589
carpropamid		4.809	5.445	4.945	4.313
tinuvin		5.247	5.439	5.951	5.495
water		5.102	4.725	5.121	5.775

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		2.330	3.048	2.830	3.058
	carpropamid		2.508	2.912	3.010	2.116
	tinuvin		2.686	1.756	1.894	2.556
	water		2.410	2.204	3.048	2.374
I.R.	acetic acid		4.344	3.782	5.304	4.124
	carpropamid		4.192	4.610	2.842	3.656
	tinuvin		3.540	3.674	4.390	3.290
	water		5.388	4.156	3.670	4.674
none	acetic acid		2.890	3.942	3.514	2.862
	carpropamid		2.524	3.082	4.030	2.788
	tinuvin		2.916	3.870	3.308	2.926
	water		2.920	2.828	2.974	2.900
UVA	acetic acid		7.140	7.728	7.996	7.674
	carpropamid		7.294	7.940	7.040	5.704
	tinuvin		7.062	5.626	8.484	7.424
	water		5.450	6.690	6.830	8.070
UVB	acetic acid		7.696	5.766	7.266	8.290
	carpropamid		6.166	7.468	6.392	6.086
	tinuvin		7.718	9.116	10.832	9.184
	water		8.494	6.944	7.412	9.376
Vis light	acetic acid		6.088	6.354	5.688	7.526
	carpropamid		6.168	6.660	6.354	5.526
	tinuvin		7.560	8.590	6.800	7.588
	water		5.950	5.526	6.792	7.254

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.7460	0.3279	0.1844	1.0200
d.f.	20	72	288	57.77
Except when comparing means with the same level(s) of exposure				0.8032
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.8423	0.4577	1.2853
d.f.	32.33	223.12	136.24
Except when comparing means with the same level(s) of exposure	0.4516		1.1211
d.f.	288		223.12
treatment		0.3687	
d.f.		288	
exposure.treatment			0.9032
d.f.			288
exposure.Chem_Charge			1.1211
d.f.			223.12

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.5561	0.6537	0.3629	2.0419
d.f.	20	72	288	57.77
Except when comparing means with the same level(s) of exposure				1.6012
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.7149	0.9020	2.5418
d.f.	32.33	223.12	136.24
Except when comparing means with the same level(s) of			
exposure	0.8888		2.2094
d.f.	288		223.12
treatment		0.7257	
d.f.		288	
exposure.treatment			1.7776
d.f.			288
exposure.Chem_Charge			2.2094
d.f.			223.12

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	517.811	129.453	1.57	
block.board stratum					
exposure	5	8579.239	1715.848	20.75	<.001
Residual	20	1653.538	82.677	5.08	
block.board.area stratum					
treatment	3	166.528	55.509	3.41	0.022
exposure.treatment	15	278.724	18.582	1.14	0.337
Residual	72	1171.336	16.269	2.31	
block.board.area.Strip stratum					
Chem_Charge	3	13.262	4.421	0.63	0.597
exposure.Chem_Charge	15	95.812	6.387	0.91	0.556
treatment.Chem_Charge	9	101.273	11.253	1.60	0.115
exposure.treatment.Chem_Charge	45	345.714	7.683	1.09	0.327
Residual	288	2025.951	7.035		
Total	479	14949.188			

Message: the following units have large residuals.

block 1 board 2	3.78	s.e. 1.86
block 4 board 4	-4.03	s.e. 1.86
block 1 board 3 area 1	5.50	s.e. 1.56
block 1 board 3 area 3	-4.30	s.e. 1.56
block 2 board 2 area 2	4.71	s.e. 1.56
block 1 board 5 area 1 Strip 1	7.23	s.e. 2.05
block 1 board 6 area 1 Strip 3	-6.82	s.e. 2.05
block 3 board 2 area 4 Strip 4	6.35	s.e. 2.05

Tables of means

Variate: b

Grand mean 19.46

exposure	full	I.R.	none	UVA	UVB	Vis light
	10.26	20.40	19.72	22.49	21.59	22.28

treatment	acetic acid	carpropamid	tinuvin	water
	19.37	18.79	20.40	19.27

Chem_Charge	high	low	medium	Very high
	19.24	19.41	19.70	19.49

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		10.75	10.18	9.85	10.27
I.R.		20.51	19.83	20.45	20.81
none		19.90	19.53	20.30	19.15
UVA		22.63	22.13	23.33	21.88
UVB		20.73	19.19	24.74	21.70
Vis light		21.69	21.87	23.76	21.81

exposure	Chem_Charge	high	low	medium	Very high
full		10.07	10.12	10.55	10.31
I.R.		21.10	20.57	20.13	19.79
none		18.70	20.03	20.85	19.30
UVA		22.02	22.35	22.96	22.64
UVB		21.51	20.91	21.80	22.14
Vis light		22.00	22.46	21.90	22.75

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		19.06	19.34	19.52	19.56
carpropamid		18.65	19.71	18.91	17.89
tinuvin		20.23	20.15	20.91	20.33
water		19.01	18.44	19.46	20.18

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		9.72	11.41	10.40	11.46
	carpropamid		10.25	10.89	10.91	8.69
	tinuvin		10.86	8.51	9.23	10.79
	water		9.47	9.66	11.66	10.29
I.R.	acetic acid		20.76	20.36	21.72	19.17
	carpropamid		20.05	21.68	17.82	19.77
	tinuvin		20.87	20.37	21.31	19.23
	water		22.72	19.88	19.65	20.99
none	acetic acid		18.97	20.93	21.19	18.53
	carpropamid		18.32	19.03	21.58	19.19
	tinuvin		19.34	21.36	20.76	19.74
	water		18.18	18.82	19.87	19.74
UVA	acetic acid		21.62	23.25	22.77	22.88
	carpropamid		22.48	23.63	22.00	20.40
	tinuvin		24.09	20.69	24.96	23.60
	water		19.91	21.82	22.10	23.68
UVB	acetic acid		21.89	18.42	20.58	22.03
	carpropamid		19.07	20.69	18.84	18.16
	tinuvin		22.67	24.62	26.66	25.02
	water		22.43	19.92	21.10	23.34
Vis light	acetic acid		21.38	21.68	20.44	23.24
	carpropamid		21.73	22.33	22.30	21.13
	tinuvin		23.56	25.32	22.53	23.62
	water		21.32	20.52	22.35	23.02

Standard errors of differences of means

Tableexposure	treatment	Chem_Charge	exposure	treatment
rep.	80	120	120	20
s.e.d.	1.438	0.521	0.342	1.813
d.f.	20	72	288	46.12
Except when comparing means with the same level(s) of				
exposure				1.275
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.611	0.789	2.323
d.f.	31.37	267.44	116.65
Except when comparing means with the same level(s) of			
exposure	0.839		1.933
d.f.	288		267.44
treatment		0.685	
d.f.		288	
exposure.treatment			
			1.677
d.f.			288
exposure.Chem_Charge			
			1.933
d.f.			267.44

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.999	1.038	0.674	3.649
d.f.	20	72	288	46.12
Except when comparing means with the same level(s) of				
exposure				2.543
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.284	1.554	4.601
d.f.	31.37	267.44	116.65
Except when comparing means with the same level(s) of			
exposure	1.651		3.806
d.f.	288		267.44
treatment		1.348	
d.f.		288	
exposure.treatment			
			3.302
d.f.			288
exposure.Chem_Charge			
			3.806
d.f.			267.44

Analysis of variance week 18

Variate: L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	4		4018.45	1004.61	5.20	
block.board stratum						
exposure	5		10259.75	2051.95	10.62	<.001
Residual	20		3863.08	193.15	5.14	
block.board.area stratum						
treatment	3		139.81	46.60	1.24	0.302
exposure.treatment	15		416.26	27.75	0.74	0.738
Residual	72		2706.75	37.59	2.02	

block.board.area.Strip stratum						
Chem_Charge	3		60.46	20.15	1.08	0.357
exposure.Chem_Charge	15		263.56	17.57	0.94	0.516
treatment.Chem_Charge	9		110.14	12.24	0.66	0.747
exposure.treatment.Chem_Charge	45		764.38	16.99	0.91	0.635
Residual	286	(2)	5325.80	18.62		
Total	477	(2)	27825.87			

Message: the following units have large residuals.

block 2 board 3	-5.80	s.e. 2.84
block 2 board 4	6.43	s.e. 2.84
block 2 board 3 area 3	6.44	s.e. 2.37
block 5 board 1 area 4	5.97	s.e. 2.37
block 1 board 5 area 3 Strip 1	10.02	s.e. 3.33
block 1 board 6 area 4 Strip 2	11.76	s.e. 3.33

Tables of means

Variate: L

Grand mean 58.12

exposure	full	I.R.	none	UVA	UVB	Vis light
	48.67	61.48	63.23	57.62	59.11	58.57

treatment	acetic acid	carpropamid	tinuvin	water
	57.83	57.62	59.02	57.99

Chem_Charge	high	low	medium	Very high
	58.65	57.65	58.09	58.07

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		49.62	46.61	49.73	48.71
I.R.		60.50	62.40	63.01	60.03
none		63.15	61.62	65.27	62.88
UVA		57.28	57.52	58.91	56.79
UVB		58.74	58.06	58.84	60.82
Vis light		57.70	59.50	58.37	58.70

exposure	Chem_Charge	high	low	medium	Very high
full		49.90	48.18	47.92	48.67
I.R.		63.07	61.59	60.70	60.57
none		61.85	62.27	64.95	63.87
UVA		58.80	56.70	57.87	57.13
UVB		59.69	58.59	58.56	59.62
Vis light		58.59	58.58	58.57	58.54

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		57.61	57.96	58.17	57.58
carpropamid		58.57	56.43	57.88	57.60
tinuvin		60.45	58.56	58.34	58.73
water		57.97	57.65	57.98	58.35

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		50.53	48.28	49.83	49.83
	carpropamid		49.92	45.89	45.29	45.35
	tinuvin		49.11	49.93	48.26	51.61
	water		50.06	48.62	48.28	47.89
I.R.	acetic acid		62.66	60.65	59.44	59.25
	carpropamid		63.87	61.93	60.47	63.32
	tinuvin		64.74	62.99	63.16	61.16
	water		61.02	60.80	59.73	58.56
none	acetic acid		62.18	61.69	64.16	64.59
	carpropamid		60.62	59.58	65.53	60.77
	tinuvin		64.04	65.78	67.15	64.13
	water		60.55	62.01	62.95	66.00
UVA	acetic acid		55.49	57.57	59.67	56.38
	carpropamid		57.44	57.01	57.83	57.81
	tinuvin		62.91	55.90	57.66	59.18
	water		59.36	56.31	56.32	55.17
UVB	acetic acid		57.08	61.40	58.71	57.75
	carpropamid		62.08	54.29	58.09	57.79
	tinuvin		61.54	58.29	55.41	60.10
	water		58.04	60.39	62.04	62.82
Vis light	acetic acid		57.69	58.20	57.20	57.71
	carpropamid		57.50	59.89	60.07	60.56
	tinuvin		60.39	58.48	58.43	56.20
	water		58.78	57.77	58.58	59.68

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.197	0.792	0.557	2.766
d.f.	20	72	286	45.83
Except when comparing means with the same level(s) of exposure				1.939
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.495	1.248	3.638
d.f.	33.05	285.99	126.43
Except when comparing means with the same level(s) of exposure	1.365		3.057
d.f.	286		285.99
treatment		1.114	
d.f.		286	
exposure.treatment			2.729
d.f.			286
exposure.Chem_Charge			3.057
d.f.			285.99

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.584	1.578	1.097	5.567
d.f.	20	72	286	45.83
Except when comparing means with the same level(s) of exposure				3.865
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.076	2.457	7.199
d.f.	33.05	285.99	126.43
Except when comparing means with the same level(s) of			
exposure	2.686		6.017
d.f.	286		285.99
treatment		2.193	
d.f.		286	
exposure.treatment			5.372
d.f.			286
exposure.Chem_Charge			6.017
d.f.			285.99

(Not adjusted for missing values)

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	115.697	28.924	1.71	
block.board stratum					
exposure	5	1740.227	348.045	20.62	<.001
Residual	20	337.576	16.879	3.11	
block.board.area stratum					
treatment	3	13.883	4.628	0.85	0.469
exposure.treatment	15	83.259	5.551	1.02	0.442
Residual	72	390.416	5.422	3.11	
block.board.area.Strip stratum					
Chem_Charge	3	4.360	1.453	0.83	0.477
exposure.Chem_Charge	15	11.031	0.735	0.42	0.972
treatment.Chem_Charge	9	37.250	4.139	2.37	0.013
exposure.treatment.Chem_Charge	45	106.705	2.371	1.36	0.073
Residual	288	502.795	1.746		
Total	479	3343.197			

Message: the following units have large residuals.

block 1 board 2	2.234	s.e. 0.839
block 4 board 4	-1.854	s.e. 0.839
block 1 board 3 area 1	3.333	s.e. 0.902
block 1 board 3 area 3	-2.346	s.e. 0.902
block 2 board 2 area 2	2.943	s.e. 0.902
block 2 board 2 area 3	-2.404	s.e. 0.902
block 1 board 3 area 1 Strip 2	-3.155	s.e. 1.023
block 1 board 3 area 1 Strip 4	3.696	s.e. 1.023
block 2 board 3 area 3 Strip 2	3.744	s.e. 1.023
block 3 board 2 area 1 Strip 2	3.046	s.e. 1.023
block 3 board 2 area 4 Strip 3	-3.181	s.e. 1.023

Tables of means

Variate: a

Grand mean 4.896

exposure	full	I.R.	none	UVA	UVB	Vis light
	2.396	3.782	3.006	6.587	7.335	6.268

treatment	acetic acid	carpropamid	tinuvin	water
	4.990	4.632	5.087	4.874

Chem_Charge	high	low	medium	Very high
	4.814	4.896	5.051	4.822

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		2.497	2.578	1.918	2.593
I.R.		4.495	3.459	3.497	3.677
none		2.945	3.035	3.167	2.879
UVA		6.927	6.543	6.685	6.193
UVB		7.030	6.076	8.474	7.761
Vis light		6.048	6.101	6.783	6.140

exposure	Chem_Charge	high	low	medium	Very high
full		2.304	2.330	2.505	2.447
I.R.		4.006	3.690	3.877	3.553
none		2.765	3.265	3.174	2.822
UVA		6.307	6.573	6.708	6.761
UVB		7.462	7.162	7.668	7.049
Vis light		6.040	6.354	6.376	6.301

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		4.860	4.784	5.091	5.225
carpropamid		4.400	5.211	4.784	4.132
tinuvin		5.027	5.105	5.504	4.713
water		4.969	4.482	4.826	5.218

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		2.066	2.588	2.518	2.814
	carpropamid		2.456	2.760	2.844	2.250
	tinuvin		2.284	1.578	1.726	2.084
	water		2.410	2.392	2.932	2.638
I.R.	acetic acid		4.598	4.034	5.192	4.154
	carpropamid		3.426	4.330	2.732	3.348
	tinuvin		3.622	3.374	3.896	3.094
	water		4.380	3.024	3.690	3.616
none	acetic acid		2.654	3.356	2.798	2.972
	carpropamid		2.448	3.178	3.796	2.716
	tinuvin		2.992	3.622	3.254	2.800
	water		2.966	2.902	2.848	2.798
UVA	acetic acid		6.546	7.234	7.026	6.902
	carpropamid		6.490	7.424	6.456	5.802
	tinuvin		6.594	5.548	7.592	7.006
	water		5.596	6.086	5.756	7.334
UVB	acetic acid		7.694	5.312	7.398	7.718
	carpropamid		5.860	6.914	6.302	5.230
	tinuvin		7.328	9.136	10.142	7.290
	water		8.966	7.286	6.832	7.960
Vis light	acetic acid		5.602	6.182	5.614	6.792
	carpropamid		5.718	6.660	6.576	5.448
	tinuvin		7.344	7.370	6.412	6.004
	water		5.494	5.204	6.900	6.960

Standard errors of differences of means

Tableexposure	treatment	Chem_Charge	exposure	treatment
rep.	80	120	120	20
s.e.d.	0.6496	0.3006	0.1706	0.9103
d.f.	20	72	288	61.31
Except when comparing means with the same level(s) of exposure				0.7364
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7436	0.4215	1.1629
d.f.	34.11	225.63	150.50
Except when comparing means with the same level(s) of exposure	0.4178		1.0325
d.f.	288		225.63
treatment		0.3412	
d.f.		288	
exposure.treatment			0.8357
d.f.			288
exposure.Chem_Charge			1.0325
d.f.			225.63

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.3550	0.5993	0.3357	1.8201
d.f.	20	72	288	61.31
Except when comparing means with the same level(s) of exposure				1.4679
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.5110	0.8306	2.2978
d.f.	34.11	225.63	150.50
Except when comparing means with the same level(s) of exposure	0.8224		2.0345
d.f.	288		225.63
treatment		0.6715	
d.f.		288	
exposure.treatment			1.6448
d.f.			288
exposure.Chem_Charge			2.0345
d.f.			225.63

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	394.740	98.685	1.41	
block.board stratum					
exposure	5	8528.227	1705.645	24.31	<.001
Residual	20	1403.458	70.173	4.50	
block.board.area stratum					
treatment	3	104.567	34.856	2.23	0.092
exposure.treatment	15	282.581	18.839	1.21	0.287
Residual	72	1123.477	15.604	2.56	
block.board.area.Strip stratum					
Chem_Charge	3	10.114	3.371	0.55	0.647
exposure.Chem_Charge	15	73.753	4.917	0.81	0.670
treatment.Chem_Charge	9	96.364	10.707	1.76	0.076
exposure.treatment.Chem_Charge	45	297.242	6.605	1.08	0.340
Residual	288	1755.910	6.097		
Total	479	14070.433			

Message: the following units have large residuals.

block 1 board 2	3.51	s.e. 1.71
block 4 board 4	-3.98	s.e. 1.71
block 1 board 3 area 1	4.69	s.e. 1.53
block 1 board 3 area 3	-3.86	s.e. 1.53
block 2 board 2 area 2	4.31	s.e. 1.53
block 5 board 1 area 1	4.16	s.e. 1.53
block 1 board 3 area 4 Strip 1	5.92	s.e. 1.91
block 1 board 5 area 1 Strip 1	6.12	s.e. 1.91
block 1 board 6 area 1 Strip 3	-5.62	s.e. 1.91
block 3 board 2 area 1 Strip 2	5.90	s.e. 1.91
block 4 board 5 area 4 Strip 2	-5.62	s.e. 1.91

Tables of means

Variate: b

Grand mean 18.57

exposure	full	I.R.	none	UVA	UVB	Vis light
	9.34	19.64	19.14	21.41	20.47	21.43

treatment	acetic acid	carpropamid	tinuvin	water
	18.53	18.00	19.30	18.46

Chem_Charge	high	low	medium	Very high
	18.49	18.58	18.80	18.41

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		9.31	9.56	8.57	9.91
I.R.		20.37	19.12	19.57	19.50
none		19.00	18.90	19.96	18.72
UVA		21.52	21.26	22.28	20.58
UVB		20.06	17.83	23.03	20.94
Vis light		20.93	21.31	22.36	21.14

exposure	Chem_Charge	high	low	medium	Very high
full		8.94	9.28	9.56	9.57
I.R.		20.49	19.73	19.37	18.97
none		18.27	19.44	19.91	18.95
UVA		21.15	21.44	21.45	21.58
UVB		21.05	20.04	20.81	19.97
Vis light		21.07	21.56	21.70	21.41

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		18.36	18.32	18.67	18.78
carpropamid		17.56	19.00	18.27	17.16
tinuvin		19.48	19.17	19.85	18.69
water		18.58	17.85	18.41	19.02

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		8.33	9.60	9.13	10.16
	carpropamid		9.17	10.24	10.26	8.57
	tinuvin		9.25	7.69	8.12	9.23
	water		8.99	9.57	10.72	10.35
I.R.	acetic acid		21.05	20.15	21.22	19.07
	carpropamid		18.97	21.01	17.37	19.12
	tinuvin		20.31	19.40	19.91	18.67
	water		21.61	18.38	18.97	19.04
none	acetic acid		18.14	19.45	19.14	19.26
	carpropamid		17.62	18.94	20.90	18.13
	tinuvin		19.79	20.64	20.35	19.07
	water		17.55	18.73	19.25	19.34
UVA	acetic acid		20.63	22.22	21.84	21.38
	carpropamid		21.04	22.95	20.87	20.16
	tinuvin		22.96	19.98	23.36	22.81
	water		19.98	20.61	19.75	21.98
UVB	acetic acid		21.61	17.29	20.28	21.05
	carpropamid		17.90	18.92	18.11	16.40
	tinuvin		21.64	23.91	25.29	21.29
	water		23.05	20.03	19.55	21.15
Vis light	acetic acid		20.41	21.18	20.39	21.74
	carpropamid		20.67	21.92	22.08	20.57
	tinuvin		22.92	23.39	22.07	21.07
	water		20.27	19.77	22.25	22.25

Standard errors of differences of means

Tableexposure	treatment	Chem_Charge	exposure	treatment
rep.	80	120	120	20
s.e.d.	1.325	0.510	0.319	1.710
d.f.	20	72	288	49.47
Except when comparing means with the same level(s) of				
exposure				1.249
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.487	0.752	2.180
d.f.	31.64	252.87	122.47
Except when comparing means with the same level(s) of			
exposure	0.781		1.841
d.f.	288		252.87
treatment		0.638	
d.f.		288	
exposure.treatment			
			1.562
d.f.			288
exposure.Chem_Charge			
			1.841
d.f.			252.87

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.763	1.017	0.627	3.436
d.f.	20	72	288	49.47
Except when comparing means with the same level(s) of				
exposure				2.490
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.031	1.480	4.316
d.f.	31.64	252.87	122.47
Except when comparing means with the same level(s) of			
exposure	1.537		3.626
d.f.	288		252.87
treatment		1.255	
d.f.		288	
exposure.treatment			
			3.074
d.f.			288
exposure.Chem_Charge			
			3.626
d.f.			252.87

Analysis of variance week 20

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3810.63	952.66	5.30	
block.board stratum					
exposure	5	11414.69	2282.94	12.70	<.001
Residual	20	3595.57	179.78	4.38	
block.board.area stratum					
treatment	3	62.43	20.81	0.51	0.679
exposure.treatment	15	466.57	31.10	0.76	0.719
Residual	72	2958.15	41.09	2.14	

block.board.area.Strip stratum					
Chem_Charge	3	22.09	7.36	0.38	0.765
exposure.Chem_Charge	15	412.96	27.53	1.43	0.130
treatment.Chem_Charge	9	217.58	24.18	1.26	0.258
exposure.treatment.Chem_Charge	45	846.24	18.81	0.98	0.513
Residual	288	5525.28	19.18		
Total	479	29332.19			

Message: the following units have large residuals.

block 2 board 3	-5.49	s.e. 2.74
block 2 board 4	6.20	s.e. 2.74
block 2 board 2 area 4	-6.23	s.e. 2.48
block 2 board 3 area 3	7.34	s.e. 2.48
block 5 board 1 area 4	6.13	s.e. 2.48
block 1 board 1 area 4 Strip 3	10.35	s.e. 3.39
block 1 board 5 area 2 Strip 3	10.80	s.e. 3.39
block 1 board 6 area 1 Strip 3	-12.10	s.e. 3.39
block 1 board 6 area 4 Strip 2	13.14	s.e. 3.39

Tables of means

Variate: L

Grand mean 57.47

exposure	full	I.R.	none	UVA	UVB	Vis light
	47.31	60.93	62.39	56.96	59.07	58.17

treatment	acetic acid	carpropamid	tinuvin	water
	57.39	57.25	58.08	57.17

Chem_Charge	high	low	medium	Very high
	57.55	57.14	57.73	57.47

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		48.62	45.20	47.42	47.99
I.R.		60.42	61.65	62.44	59.22
none		62.57	61.25	64.12	61.61
UVA		56.64	57.13	58.56	55.53
UVB		58.58	59.06	58.31	60.33
Vis light		57.51	59.18	57.64	58.34

exposure	Chem_Charge	high	low	medium	Very high
full		48.42	47.06	46.29	47.47
I.R.		62.36	61.03	60.29	60.05
none		59.96	61.72	64.65	63.22
UVA		57.52	55.66	58.15	56.53
UVB		59.08	59.16	58.64	59.40
Vis light		57.95	58.22	58.37	58.13

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		57.25	57.35	58.01	56.95
carpropamid		57.75	56.33	57.55	57.36
tinuvin		59.19	58.36	56.98	57.80
water		56.01	56.52	58.39	57.75

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		49.67	47.27	48.90	48.65
	carpropamid		48.12	44.95	43.74	44.01
	tinuvin		46.55	48.22	44.95	49.96
	water		49.35	47.79	47.56	47.26
I.R.	acetic acid		63.40	60.38	59.37	58.54
	carpropamid		62.17	61.43	60.22	62.77
	tinuvin		64.16	63.19	61.79	60.60
	water		59.71	59.10	59.77	58.28
none	acetic acid		61.58	60.64	64.84	63.19
	carpropamid		59.54	59.99	64.18	61.30
	tinuvin		61.84	65.67	65.30	63.66
	water		56.89	60.55	64.26	64.74
UVA	acetic acid		54.81	55.77	60.01	55.96
	carpropamid		57.05	55.71	57.68	58.06
	tinuvin		61.87	56.96	57.06	58.35
	water		56.34	54.18	57.84	53.76
UVB	acetic acid		56.62	60.97	58.30	58.42
	carpropamid		63.29	56.02	58.90	58.05
	tinuvin		61.17	58.44	54.91	58.70
	water		55.25	61.21	62.46	62.40
Vis light	acetic acid		57.39	59.07	56.62	56.96
	carpropamid		56.30	59.86	60.56	59.99
	tinuvin		59.55	57.66	57.84	55.53
	water		58.55	56.28	58.47	60.05

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.120	0.827	0.565	2.752
d.f.	20	72	288	50.26
Except when comparing means with the same level(s) of exposure				2.027
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.436	1.282	3.651
d.f.	34.61	278.42	141.40
Except when comparing means with the same level(s) of exposure	1.385		3.141
d.f.	288		278.42
treatment		1.131	
d.f.		288	
exposure.treatment			2.770
d.f.			288
exposure.Chem_Charge			3.141
d.f.			278.42

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.422	1.650	1.113	5.528
d.f.	20	72	288	50.26
Except when comparing means with the same level(s) of exposure				4.041
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	4.947	2.524	7.218
d.f.	34.61	278.42	141.40
Except when comparing means with the same level(s) of			
exposure	2.726		6.183
d.f.	288		278.42
treatment		2.226	
d.f.		288	
exposure.treatment			5.452
d.f.			288
exposure.Chem_Charge			6.183
d.f.			278.42

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	131.962	32.990	1.75	
block.board stratum					
exposure	5	1493.529	298.706	15.82	<.001
Residual	20	377.611	18.881	3.76	
block.board.area stratum					
treatment	3	11.515	3.838	0.77	0.517
exposure.treatment	15	88.784	5.919	1.18	0.307
Residual	72	361.073	5.015	2.85	
block.board.area.Strip stratum					
Chem_Charge	3	1.966	0.655	0.37	0.773
exposure.Chem_Charge	15	14.333	0.956	0.54	0.915
treatment.Chem_Charge	9	42.120	4.680	2.66	0.006
exposure.treatment.Chem_Charge	45	122.833	2.730	1.55	0.019
Residual	288	507.448	1.762		
Total	479	3153.174			

Message: the following units have large residuals.

block 1 board 2	2.407	s.e. 0.887
block 4 board 4	-1.936	s.e. 0.887
block 1 board 2 area 3	2.367	s.e. 0.867
block 1 board 3 area 1	3.494	s.e. 0.867
block 1 board 3 area 3	-2.401	s.e. 0.867
block 2 board 2 area 2	2.543	s.e. 0.867
block 3 board 5 area 2	-2.151	s.e. 0.867
block 1 board 3 area 1 Strip 2	-3.268	s.e. 1.028
block 1 board 3 area 1 Strip 4	3.237	s.e. 1.028
block 2 board 3 area 3 Strip 2	3.820	s.e. 1.028
block 3 board 2 area 4 Strip 4	4.371	s.e. 1.028
block 4 board 2 area 1 Strip 3	-3.738	s.e. 1.028
block 4 board 2 area 4 Strip 1	3.030	s.e. 1.028

Tables of means

Variate: a

Grand mean 4.769

exposure	full	I.R.	none	UVA	UVB	Vis light
	2.286	3.984	2.990	6.400	6.806	6.151

treatment	acetic acid	carpropamid	tinuvin	water
	4.886	4.508	4.885	4.799

Chem_Charge	high	low	medium	Very high
	4.743	4.704	4.875	4.755

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		2.332	2.436	2.042	2.334
I.R.		4.569	3.466	3.535	4.366
none		3.118	2.996	2.892	2.953
UVA		6.755	6.592	6.351	5.905
UVB		6.435	5.534	7.958	7.298
Vis light		6.106	6.027	6.532	5.938

exposure	Chem_Charge	high	low	medium	Very high
full		2.282	2.215	2.416	2.231
I.R.		4.129	4.095	3.869	3.844
none		2.864	3.121	3.175	2.800
UVA		6.116	6.179	6.649	6.658
UVB		7.115	6.361	6.932	6.816
Vis light		5.956	6.252	6.211	6.185

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		4.702	4.676	4.972	5.193
carpropamid		4.301	5.071	4.537	4.123
tinuvin		4.781	4.797	5.318	4.643
water		5.189	4.271	4.673	5.062

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		1.934	2.348	2.528	2.518
	carpropamid		2.454	2.512	2.654	2.122
	tinuvin		2.460	1.862	1.670	2.174
	water		2.278	2.138	2.812	2.108
I.R.	acetic acid		4.212	4.286	5.072	4.704
	carpropamid		3.560	4.148	2.840	3.316
	tinuvin		3.278	4.094	3.840	2.928
	water		5.464	3.850	3.724	4.426
none	acetic acid		2.778	3.440	3.182	3.072
	carpropamid		2.382	3.302	3.568	2.730
	tinuvin		2.704	3.114	3.104	2.646
	water		3.590	2.628	2.844	2.750
UVA	acetic acid		6.312	6.768	6.890	7.050
	carpropamid		6.490	7.682	6.548	5.646
	tinuvin		6.488	4.562	7.478	6.874
	water		5.174	5.704	5.678	7.062
UVB	acetic acid		7.216	4.840	6.682	7.002
	carpropamid		5.308	6.364	5.012	5.450
	tinuvin		6.782	7.990	9.722	7.336
	water		9.154	6.250	6.312	7.476
Vis light	acetic acid		5.760	6.374	5.478	6.812
	carpropamid		5.614	6.418	6.602	5.474
	tinuvin		6.972	7.160	6.094	5.902
	water		5.476	5.054	6.670	6.552

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6870	0.2891	0.1714	0.9209
d.f.	20	72	288	54.89
Except when comparing means with the same level(s) of exposure				0.7082
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7773	0.4143	1.1733
d.f.	32.59	237.74	134.66
Except when comparing means with the same level(s) of exposure	0.4198		1.0149
d.f.	288		237.74
treatment		0.3427	
d.f.		288	
exposure.treatment			0.8395
d.f.			288
exposure.Chem_Charge			1.0149
d.f.			237.74

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.4331	0.5763	0.3373	1.8457
d.f.	20	72	288	54.89
Except when comparing means with the same level(s) of exposure				1.4117
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.5821	0.8163	2.3206
d.f.	32.59	237.74	134.66
Except when comparing means with the same level(s) of			
exposure	0.8262		1.9994
d.f.	288		237.74
treatment		0.6746	
d.f.		288	
exposure.treatment			1.6524
d.f.			288
exposure.Chem_Charge			1.9994
d.f.			237.74

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	433.112	108.278	1.48	
block.board stratum					
exposure	5	8458.490	1691.698	23.18	<.001
Residual	20	1459.843	72.992	4.76	
block.board.area stratum					
treatment	3	69.942	23.314	1.52	0.216
exposure.treatment	15	298.505	19.900	1.30	0.226
Residual	72	1103.630	15.328	2.42	
block.board.area.Strip stratum					
Chem_Charge	3	9.438	3.146	0.50	0.685
exposure.Chem_Charge	15	75.739	5.049	0.80	0.680
treatment.Chem_Charge	9	92.774	10.308	1.63	0.107
exposure.treatment.Chem_Charge	45	308.978	6.866	1.08	0.339
Residual	288	1823.109	6.330		
Total	479	14133.558			

Message: the following units have large residuals.

block 1 board 2	4.04	s.e. 1.74
block 4 board 4	-4.15	s.e. 1.74
block 1 board 3 area 1	5.10	s.e. 1.52
block 1 board 3 area 3	-4.24	s.e. 1.52
block 2 board 2 area 2	4.06	s.e. 1.52
block 5 board 1 area 1	4.18	s.e. 1.52
block 1 board 3 area 1 Strip 2	-6.06	s.e. 1.95
block 1 board 5 area 1 Strip 1	5.76	s.e. 1.95
block 2 board 2 area 4 Strip 4	-6.17	s.e. 1.95
block 3 board 2 area 4 Strip 4	7.42	s.e. 1.95
block 4 board 2 area 1 Strip 3	-6.12	s.e. 1.95
block 4 board 5 area 4 Strip 2	-5.78	s.e. 1.95

Tables of means

Variate: b

Grand mean 18.23

exposure	full	I.R.	none	UVA	UVB	Vis light
	9.00	19.81	19.00	21.04	19.43	21.08

treatment	acetic acid	carpropamid	tinuvin	water
	18.34	17.76	18.78	18.02

Chem_Charge	high	low	medium	Very high
	18.22	18.09	18.46	18.14

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		9.01	9.14	8.81	9.04
I.R.		20.66	19.20	19.25	20.15
none		19.35	18.92	19.16	18.55
UVA		21.31	21.29	21.59	19.95
UVB		18.82	16.92	22.09	19.92
Vis light		20.89	21.10	21.80	20.52

exposure	Chem_Charge	high	low	medium	Very high
full		8.95	8.87	9.21	8.97
I.R.		20.37	20.08	19.40	19.40
none		18.17	19.08	19.84	18.89
UVA		20.76	20.68	21.39	21.31
UVB		20.27	18.58	19.63	19.27
Vis light		20.78	21.25	21.27	21.01

treatment	Chem_Charge	high	low	medium	Very high	
acetic acid		18.10	18.11	18.62	18.53	
carpropamid		17.36	18.61	17.88	17.19	
tinuvin		18.80	18.61	19.32	18.41	
water		18.61	17.03	18.00	18.44	

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		8.28	9.17	9.17	9.41
	carpropamid		9.27	9.36	9.60	8.34
	tinuvin		9.60	8.33	7.97	9.34
	water		8.65	8.63	10.08	8.79
I.R.	acetic acid		20.49	20.91	21.16	20.06
	carpropamid		19.10	20.77	17.70	19.23
	tinuvin		19.74	19.60	19.50	18.18
	water		22.16	19.07	19.24	20.12
none	acetic acid		18.26	19.52	20.39	19.23
	carpropamid		17.50	19.19	20.42	18.56
	tinuvin		18.46	19.72	19.68	18.80
	water		18.47	17.88	18.86	18.99
UVA	acetic acid		20.46	21.34	21.84	21.61
	carpropamid		21.14	23.00	21.01	20.03
	tinuvin		22.41	18.78	22.86	22.32
	water		19.05	19.59	19.88	21.28
UVB	acetic acid		20.65	16.08	19.13	19.42
	carpropamid		16.88	17.86	16.35	16.57
	tinuvin		20.32	22.23	24.56	21.24
	water		23.23	18.13	18.47	19.84
Vis light	acetic acid		20.48	21.65	20.00	21.44
	carpropamid		20.29	21.49	22.23	20.41
	tinuvin		22.25	23.00	21.37	20.58
	water		20.11	18.87	21.49	21.61

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.351	0.505	0.325	1.725
d.f.	20	72	288	47.86
Except when comparing means with the same level(s) of exposure				1.238
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.516	0.756	2.208
d.f.	31.61	260.83	120.33
Except when comparing means with the same level(s) of exposure	0.796		1.853
d.f.	288		260.83
treatment		0.650	
d.f.		288	
exposure.treatment			1.591
d.f.			288
exposure.Chem_Charge			1.853
d.f.			260.83

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.818	1.008	0.639	3.468
d.f.	20	72	288	47.86
Except when comparing means with the same level(s) of exposure				2.468
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.090	1.489	4.371
d.f.	31.61	260.83	120.33
Except when comparing means with the same level(s) of			
exposure	1.566		3.648
d.f.	288		260.83
treatment		1.279	
d.f.		288	
exposure.treatment			3.132
d.f.			288
exposure.Chem_Charge			3.648
d.f.			260.83

Analysis of variance week 24

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3894.60	973.65	4.56	
block.board stratum					
exposure	5	7941.62	1588.32	7.44	<.001
Residual	20	4271.26	213.56	5.23	
block.board.area stratum					
treatment	3	210.35	70.12	1.72	0.171
exposure.treatment	15	389.00	25.93	0.64	0.836
Residual	72	2938.99	40.82	2.68	
block.board.area.Strip stratum					
Chem_Charge	3	12.60	4.20	0.28	0.843
exposure.Chem_Charge	15	260.58	17.37	1.14	0.319
treatment.Chem_Charge	9	144.25	16.03	1.05	0.398
exposure.treatment.Chem_Charge	45	829.55	18.43	1.21	0.180
Residual	288	4385.50	15.23		
Total	479	25278.32			

Message: the following units have large residuals.

block 2 board 3	-6.29	s.e. 2.98
block 2 board 4	6.62	s.e. 2.98
block 2 board 3 area 3	6.98	s.e. 2.47
block 4 board 3 area 3	-6.18	s.e. 2.47
block 1 board 6 area 1 Strip 3	-8.98	s.e. 3.02
block 1 board 6 area 4 Strip 2	10.65	s.e. 3.02
block 5 board 3 area 1 Strip 2	9.16	s.e. 3.02

Tables of means

Variate: L

Grand mean 56.86

exposure	full	I.R.	none	UVA	UVB	Vis light
	48.70	59.93	61.57	56.22	57.66	57.08

treatment	acetic acid	carpropamid	tinuvin	water
	56.55	56.31	57.99	56.60

Chem_Charge	high	low	medium	Very high
	57.10	56.74	56.69	56.91

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		48.65	46.73	50.44	48.99
I.R.		58.88	60.86	61.57	58.43
none		61.81	59.85	64.02	60.60
UVA		55.72	56.14	57.64	55.41
UVB		57.75	56.61	57.52	58.76
Vis light		56.49	57.67	56.78	57.40

exposure	Chem_Charge	high	low	medium	Very high
full		49.66	48.90	47.70	48.54
I.R.		61.14	60.34	58.68	59.57
none		60.22	60.57	62.95	62.55
UVA		57.29	55.68	56.07	55.87
UVB		57.21	57.60	57.47	58.35
Vis light		57.11	57.37	57.27	56.58

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		56.55	56.69	56.75	56.20
carpropamid		57.04	55.53	56.07	56.60
tinuvin		59.17	58.12	57.13	57.54
water		55.66	56.62	56.81	57.30

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		49.64	47.46	48.91	48.59
	carpropamid		49.87	46.50	45.02	45.54
	tinuvin		49.76	51.85	48.62	51.51
	water		49.37	49.77	48.26	48.54
I.R.	acetic acid		61.46	58.45	57.79	57.81
	carpropamid		61.11	61.86	57.57	62.88
	tinuvin		63.10	61.79	61.77	59.61
	water		58.87	59.27	57.60	57.96
none	acetic acid		62.10	60.54	62.47	62.15
	carpropamid		59.54	57.28	63.03	59.54
	tinuvin		63.00	65.03	64.75	63.29
	water		56.23	59.43	61.55	65.21
UVA	acetic acid		54.45	56.53	56.48	55.41
	carpropamid		56.63	55.27	56.30	56.35
	tinuvin		60.54	56.21	55.94	57.84
	water		57.52	54.69	55.55	53.86
UVB	acetic acid		55.36	59.77	58.20	57.66
	carpropamid		59.59	53.68	56.21	56.96
	tinuvin		59.81	57.01	54.18	59.08
	water		54.10	59.94	61.28	59.71
Vis light	acetic acid		56.28	57.41	56.67	55.60
	carpropamid		55.46	58.59	58.28	58.34
	tinuvin		58.83	56.84	57.54	53.90
	water		57.86	56.64	56.60	58.50

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.311	0.825	0.504	2.898
d.f.	20	72	288	45.37
Except when comparing means with the same level(s) of exposure				2.020
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.546	1.201	3.601
d.f.	29.38	246.23	103.32
Except when comparing means with the same level(s) of exposure	1.234		2.941
d.f.	288		246.23
treatment		1.008	
d.f.		288	
exposure.treatment			2.468
d.f.			288
exposure.Chem_Charge			2.941
d.f.			246.23

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.820	1.644	0.992	5.836
d.f.	20	72	288	45.37
Except when comparing means with the same level(s) of exposure				4.028
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.204	2.365	7.142
d.f.	29.38	246.23	103.32
Except when comparing means with the same level(s) of			
exposure	2.429		5.793
d.f.	288		246.23
treatment		1.983	
d.f.		288	
exposure.treatment			4.858
d.f.			288
exposure.Chem_Charge			5.793
d.f.			246.23

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	110.494	27.623	1.56	
block.board stratum					
exposure	5	1402.918	280.584	15.81	<.001
Residual	20	354.842	17.742	3.96	
block.board.area stratum					
treatment	3	11.560	3.853	0.86	0.466
exposure.treatment	15	83.596	5.573	1.24	0.261
Residual	72	322.622	4.481	2.70	
block.board.area.Strip stratum					
Chem_Charge	3	0.637	0.212	0.13	0.943
exposure.Chem_Charge	15	17.343	1.156	0.70	0.786
treatment.Chem_Charge	9	32.613	3.624	2.19	0.023
exposure.treatment.Chem_Charge	45	107.965	2.399	1.45	0.039
Residual	288	477.212	1.657		
Total	479	2921.803			

Message: the following units have large residuals.

block 1 board 2	2.315	s.e. 0.860
block 1 board 3 area 1	2.950	s.e. 0.820
block 1 board 3 area 3	-2.128	s.e. 0.820
block 2 board 2 area 2	2.582	s.e. 0.820
block 3 board 5 area 1	2.147	s.e. 0.820
block 3 board 5 area 2	-2.153	s.e. 0.820
block 1 board 1 area 1 Strip 4	3.169	s.e. 0.997
block 1 board 2 area 2 Strip 4	-2.990	s.e. 0.997
block 1 board 3 area 1 Strip 4	3.211	s.e. 0.997
block 3 board 2 area 4 Strip 3	-3.881	s.e. 0.997
block 3 board 2 area 4 Strip 4	3.496	s.e. 0.997
block 5 board 2 area 4 Strip 3	4.891	s.e. 0.997

Tables of means

Variate: a

Grand mean 4.664

exposure	full	I.R.	none	UVA	UVB	Vis light
	2.248	3.865	2.980	6.121	6.682	6.089

treatment	acetic acid	carpropamid	tinuvin	water
	4.697	4.414	4.704	4.841

Chem_Charge	high	low	medium	Very high
	4.624	4.638	4.677	4.717

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		2.393	2.389	1.633	2.576
I.R.		4.480	3.301	3.395	4.282
none		3.004	2.927	2.961	3.029
UVA		6.364	6.126	6.149	5.843
UVB		6.139	5.614	7.652	7.321
Vis light		5.802	6.128	6.433	5.993

exposure	Chem_Charge	high	low	medium	Very high
full		2.020	2.158	2.365	2.448
I.R.		3.985	3.964	3.825	3.685
none		2.955	3.144	3.085	2.737
UVA		5.893	5.921	6.364	6.304
UVB		7.066	6.446	6.379	6.834
Vis light		5.823	6.195	6.043	6.295

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		4.577	4.567	4.686	4.958
carpropamid		4.242	4.900	4.383	4.133
tinuvin		4.566	4.765	4.960	4.525
water		5.111	4.320	4.679	5.254

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		2.056	2.494	2.470	2.552
	carpropamid		2.118	2.466	2.640	2.330
	tinuvin		1.834	1.492	1.358	1.848
	water		2.072	2.178	2.992	3.062
I.R.	acetic acid		4.124	4.404	4.866	4.526
	carpropamid		3.178	4.058	2.764	3.206
	tinuvin		3.302	3.668	3.746	2.864
	water		5.336	3.726	3.924	4.144
none	acetic acid		2.866	3.496	2.990	2.664
	carpropamid		2.354	2.942	3.438	2.976
	tinuvin		3.102	3.362	2.920	2.462
	water		3.500	2.776	2.992	2.848
UVA	acetic acid		6.268	6.148	6.470	6.572
	carpropamid		5.976	7.186	6.098	5.244
	tinuvin		6.078	4.828	7.060	6.632
	water		5.250	5.524	5.830	6.770
UVB	acetic acid		7.012	4.760	5.910	6.874
	carpropamid		5.858	6.320	5.038	5.240
	tinuvin		6.214	8.408	8.748	7.240
	water		9.182	6.298	5.820	7.984
Vis light	acetic acid		5.138	6.100	5.410	6.560
	carpropamid		5.966	6.430	6.318	5.800
	tinuvin		6.864	6.834	5.930	6.104
	water		5.326	5.416	6.514	6.718

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6660	0.2733	0.1662	0.8830
d.f.	20	72	288	53.29
Except when comparing means with the same level(s) of				
exposure				0.6694
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7535	0.3969	1.1299
d.f.	32.60	244.98	132.91
Except when comparing means with the same level(s) of			
exposure	0.4071		0.9722
d.f.	288		244.98
treatment		0.3324	
d.f.		288	
exposure.treatment			
			0.8141
d.f.			288
exposure.Chem_Charge			
			0.9722
d.f.			244.98

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.3892	0.5448	0.3271	1.7708
d.f.	20	72	288	53.29
Except when comparing means with the same level(s) of				
exposure				1.3344
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.5338	0.7818	2.2349
d.f.	32.60	244.98	132.91
Except when comparing means with the same level(s) of			
exposure	0.8012		1.9149
d.f.	288		244.98
treatment		0.6542	
d.f.		288	
exposure.treatment			1.6024
d.f.			288
exposure.Chem_Charge			1.9149
d.f.			244.98

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	471.081	117.770	1.65	
block.board stratum					
exposure	5	8648.067	1729.613	24.19	<.001
Residual	20	1429.840	71.492	5.27	
block.board.area stratum					
treatment	3	67.167	22.389	1.65	0.185
exposure.treatment	15	285.940	19.063	1.41	0.168
Residual	72	976.059	13.556	2.37	
block.board.area.Strip stratum					
Chem_Charge	3	0.145	0.048	0.01	0.999
exposure.Chem_Charge	15	76.705	5.114	0.89	0.573
treatment.Chem_Charge	9	77.452	8.606	1.50	0.146
exposure.treatment.Chem_Charge	45	315.358	7.008	1.22	0.167
Residual	288	1649.174	5.726		
Total	479	13996.990			

Message: the following units have large residuals.

block 1 board 2	3.70	s.e. 1.73
block 4 board 4	-3.95	s.e. 1.73
block 1 board 3 area 1	4.40	s.e. 1.43
block 1 board 3 area 3	-3.65	s.e. 1.43
block 2 board 2 area 2	4.04	s.e. 1.43
block 5 board 1 area 1	3.93	s.e. 1.43
block 1 board 2 area 1 Strip 1	5.88	s.e. 1.85
block 3 board 2 area 4 Strip 3	-6.66	s.e. 1.85
block 3 board 2 area 4 Strip 4	5.73	s.e. 1.85
block 5 board 2 area 4 Strip 3	8.55	s.e. 1.85

Tables of means

Variate: b

Grand mean 17.52

exposure	full	I.R.	none	UVA	UVB	Vis light
	8.16	19.20	18.56	20.04	18.67	20.50

treatment	acetic acid	carpropamid	tinuvin	water
	17.48	16.98	18.03	17.58

Chem_Charge	high	low	medium	Very high
	17.52	17.53	17.49	17.54

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		8.45	8.51	7.19	8.47
I.R.		19.97	18.39	18.84	19.60
none		18.61	18.11	19.17	18.33
UVA		20.14	19.94	20.82	19.28
UVB		17.76	16.38	21.01	19.53
Vis light		19.98	20.55	21.17	20.29

exposure	Chem_Charge	high	low	medium	Very high
full		7.69	8.04	8.52	8.37
I.R.		19.64	19.73	18.66	18.76
none		18.24	18.63	19.05	18.31
UVA		19.91	19.81	20.26	20.19
UVB		19.57	18.15	17.98	18.98
Vis light		20.09	20.82	20.46	20.61

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		17.41	17.32	17.48	17.71
carpropamid		16.63	17.89	16.87	16.54
tinuvin		18.01	18.12	18.25	17.75
water		18.04	16.79	17.36	18.15

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		7.87	8.67	8.41	8.85
	carpropamid		7.81	8.77	9.11	8.37
	tinuvin		7.46	6.63	6.63	8.04
	water		7.63	8.10	9.94	8.21
I.R.	acetic acid		19.77	20.40	20.42	19.27
	carpropamid		18.04	20.31	16.51	18.69
	tinuvin		19.13	19.47	19.09	17.66
	water		21.61	18.75	18.63	19.41
none	acetic acid		18.34	19.06	18.95	18.08
	carpropamid		17.05	17.83	19.71	17.86
	tinuvin		19.60	19.88	19.03	18.19
	water		17.96	17.76	18.50	19.10
UVA	acetic acid		19.65	20.04	20.43	20.42
	carpropamid		19.76	21.95	19.53	18.51
	tinuvin		21.34	18.54	21.91	21.48
	water		18.86	18.71	19.18	20.36
UVB	acetic acid		19.70	15.34	17.14	18.86
	carpropamid		16.91	17.35	15.44	15.83
	tinuvin		18.85	22.15	22.24	20.79
	water		22.80	17.75	17.11	20.45
Vis light	acetic acid		19.14	20.43	19.54	20.80
	carpropamid		20.23	21.12	20.90	19.95
	tinuvin		21.64	22.08	20.62	20.33
	water		19.35	19.64	20.80	21.36

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.337	0.475	0.309	1.675
d.f.	20	72	288	45.17
Except when comparing means with the same level(s) of exposure				1.164
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.489	0.716	2.126
d.f.	30.64	264.08	110.93
Except when comparing means with the same level(s) of exposure	0.757		1.753
d.f.	288		264.08
treatment		0.618	
d.f.		288	
exposure.treatment			1.513
d.f.			288
exposure.Chem_Charge			1.753
d.f.			264.08

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.789	0.948	0.608	3.372
d.f.	20	72	288	45.17
Except when comparing means with the same level(s) of exposure				2.321
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.038	1.409	4.214
d.f.	30.64	264.08	110.93
Except when comparing means with the same level(s) of			
exposure	1.489		3.452
d.f.	288		264.08
treatment		1.216	
d.f.		288	
exposure.treatment			2.979
d.f.			288
exposure.Chem_Charge			3.452
d.f.			264.08

Analysis of variance week 32

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	4801.66	1200.41	5.19	
block.board stratum					
exposure	5	8825.57	1765.11	7.63	<.001
Residual	20	4626.46	231.32	5.92	
block.board.area stratum					
treatment	3	154.91	51.64	1.32	0.274
exposure.treatment	15	328.81	21.92	0.56	0.895
Residual	72	2814.78	39.09	2.42	
block.board.area.Strip stratum					
Chem_Charge	3	19.26	6.42	0.40	0.755
exposure.Chem_Charge	15	350.99	23.40	1.45	0.123
treatment.Chem_Charge	9	172.91	19.21	1.19	0.300
exposure.treatment.Chem_Charge	45	832.61	18.50	1.15	0.252
Residual	288	4646.64	16.13		
Total	479	27574.61			

Message: the following units have large residuals.

block 2 board 4	7.05	s.e. 3.10
block 2 board 2 area 4	-5.99	s.e. 2.42
block 2 board 3 area 3	6.04	s.e. 2.42
block 4 board 2 area 3	-7.26	s.e. 2.42
block 5 board 1 area 4	6.09	s.e. 2.42
block 1 board 1 area 4 Strip 3	9.55	s.e. 3.11
block 1 board 5 area 2 Strip 3	10.52	s.e. 3.11
block 1 board 6 area 1 Strip 3	-10.40	s.e. 3.11
block 1 board 6 area 4 Strip 2	11.39	s.e. 3.11
block 2 board 2 area 1 Strip 3	9.31	s.e. 3.11
block 5 board 3 area 1 Strip 2	9.57	s.e. 3.11

Tables of means

Variate: L

Grand mean 56.25

exposure	full	I.R.	none	UVA	UVB	Vis light
	47.47	59.19	60.82	55.64	58.07	56.31

treatment	acetic acid	carpropamid	tinuvin	water
	55.85	56.22	57.18	55.74

Chem_Charge	high	low	medium	Very high
	56.59	56.08	56.13	56.21

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		48.13	45.63	48.52	47.60
I.R.		58.58	59.89	60.34	57.97
none		60.55	59.80	63.36	59.57
UVA		55.12	56.27	56.79	54.37
UVB		57.21	58.48	57.89	58.70
Vis light		55.53	57.26	56.21	56.24

exposure	Chem_Charge	high	low	medium	Very high
full		48.57	46.96	46.97	47.38
I.R.		60.71	59.95	58.22	57.90
none		58.77	60.34	62.23	61.93
UVA		56.55	54.84	56.00	55.15
UVB		58.92	57.78	57.28	58.30
Vis light		56.00	56.59	56.07	56.57

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		55.84	56.33	56.42	54.82
carpropamid		57.10	55.36	56.15	56.28
tinuvin		58.23	57.03	55.99	57.49
water		55.18	55.59	55.96	56.23

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		49.25	45.40	49.49	48.39
	carpropamid		48.80	44.80	44.92	44.00
	tinuvin		47.66	49.91	46.16	50.37
	water		48.58	47.74	47.30	46.78
I.R.	acetic acid		60.90	60.33	57.57	55.52
	carpropamid		60.90	60.82	57.20	60.64
	tinuvin		62.38	59.88	60.17	58.93
	water		58.64	58.77	57.93	56.53
none	acetic acid		60.46	58.51	61.99	61.24
	carpropamid		59.53	58.40	61.68	59.61
	tinuvin		60.57	64.67	64.89	63.30
	water		54.52	59.79	60.37	63.60
UVA	acetic acid		54.52	55.41	57.47	53.06
	carpropamid		55.41	55.94	56.49	57.22
	tinuvin		60.08	55.68	54.51	56.89
	water		56.20	52.31	55.52	53.45
UVB	acetic acid		55.16	60.90	56.94	55.85
	carpropamid		62.23	54.84	58.58	58.29
	tinuvin		60.77	56.18	54.50	60.11
	water		57.53	59.23	59.09	58.94
Vis light	acetic acid		54.76	57.42	55.03	54.89
	carpropamid		55.73	57.33	58.04	57.94
	tinuvin		57.93	55.88	55.68	55.35
	water		55.59	55.73	55.54	58.10

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.405	0.807	0.519	2.952
d.f.	20	72	288	42.39
Except when comparing means with the same level(s) of exposure				1.977
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.644	1.208	3.682
d.f.	29.16	260.73	98.11
Except when comparing means with the same level(s) of exposure	1.270		2.958
d.f.	288		260.73
treatment		1.037	
d.f.		288	
exposure.treatment			2.540
d.f.			288
exposure.Chem_Charge			2.958
d.f.			260.73

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	5.016	1.609	1.021	5.956
d.f.	20	72	288	42.39
Except when comparing means with the same level(s) of exposure				3.942
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.407	2.378	7.306
d.f.	29.16	260.73	98.11
Except when comparing means with the same level(s) of			
exposure	2.500		5.825
d.f.	288		260.73
treatment		2.041	
d.f.		288	
exposure.treatment			5.000
d.f.			288
exposure.Chem_Charge			5.825
d.f.			260.73

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	88.399	22.100	1.25	
block.board stratum					
exposure	5	1219.149	243.830	13.78	<.001
Residual	20	353.978	17.699	5.89	
block.board.area stratum					
treatment	3	19.400	6.467	2.15	0.101
exposure.treatment	15	79.938	5.329	1.77	0.056
Residual	72	216.440	3.006	2.30	
block.board.area.Strip stratum					
Chem_Charge	3	6.000	2.000	1.53	0.207
exposure.Chem_Charge	15	13.202	0.880	0.67	0.811
treatment.Chem_Charge	9	24.663	2.740	2.09	0.030
exposure.treatment.Chem_Charge	45	95.029	2.112	1.61	0.011
Residual	288	377.065	1.309		
Total	479	2493.262			

Message: the following units have large residuals.

block 1 board 2	2.631	s.e. 0.859
block 1 board 2 area 3	1.708	s.e. 0.672
block 1 board 3 area 1	2.978	s.e. 0.672
block 2 board 2 area 2	1.717	s.e. 0.672
block 1 board 2 area 1 Strip 3	3.108	s.e. 0.886
block 1 board 2 area 2 Strip 4	-2.599	s.e. 0.886
block 2 board 3 area 3 Strip 2	3.700	s.e. 0.886
block 2 board 5 area 1 Strip 3	2.655	s.e. 0.886
block 4 board 2 area 2 Strip 1	-3.085	s.e. 0.886
block 5 board 2 area 1 Strip 2	-2.690	s.e. 0.886

Tables of means

Variate: a

Grand mean 4.405

exposure	full	I.R.	none	UVA	UVB	Vis light
	1.785	3.918	3.140	5.923	5.547	6.116

treatment	acetic acid	carpropamid	tinuvin	water
	4.527	4.061	4.559	4.472

Chem_Charge	high	low	medium	Very high
	4.278	4.311	4.534	4.497

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		1.886	1.850	1.553	1.851
I.R.		4.606	3.385	3.474	4.208
none		3.170	3.149	3.052	3.192
UVA		5.979	5.934	6.012	5.766
UVB		5.323	4.177	6.678	6.012
Vis light		6.200	5.871	6.589	5.806

exposure	Chem_Charge	high	low	medium	Very high
full		1.714	1.790	1.851	1.785
I.R.		3.926	3.778	3.961	4.009
none		3.077	3.296	3.235	2.954
UVA		5.729	5.441	6.349	6.172
UVB		5.463	5.370	5.754	5.604
Vis light		5.763	6.189	6.055	6.460

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		4.364	4.348	4.461	4.936
carpropamid		4.062	4.350	4.055	3.776
tinuvin		4.277	4.434	5.070	4.457
water		4.410	4.111	4.550	4.818

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		1.464	2.146	1.862	2.070
	carpropamid		1.790	1.946	1.938	1.726
	tinuvin		1.936	1.310	1.494	1.470
	water		1.664	1.758	2.108	1.874
I.R.	acetic acid		4.390	4.288	5.128	4.618
	carpropamid		3.446	3.558	2.770	3.766
	tinuvin		3.196	3.082	4.212	3.406
	water		4.670	4.184	3.732	4.244
none	acetic acid		3.034	3.488	3.006	3.150
	carpropamid		2.786	3.270	3.722	2.816
	tinuvin		2.990	3.104	3.170	2.942
	water		3.496	3.322	3.040	2.908
UVA	acetic acid		5.664	5.774	6.056	6.420
	carpropamid		6.408	6.324	5.960	5.044
	tinuvin		6.066	4.502	6.948	6.532
	water		4.778	5.164	6.432	6.690
UVB	acetic acid		6.026	4.050	5.270	5.946
	carpropamid		4.164	4.854	3.854	3.836
	tinuvin		5.176	7.522	8.326	5.686
	water		6.484	5.054	5.564	6.946
Vis light	acetic acid		5.604	6.340	5.442	7.414
	carpropamid		5.780	6.150	6.084	5.470
	tinuvin		6.298	7.082	6.268	6.708
	water		5.370	5.182	6.424	6.246

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6652	0.2238	0.1477	0.8173
d.f.	20	72	288	42.51
Except when comparing means with the same level(s) of				
exposure				0.5483
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.7353	0.3399	1.0299
d.f.	29.76	268.48	102.00
Except when comparing means with the same level(s) of			
exposure	0.3618		0.8327
d.f.	288		268.48
treatment		0.2954	
d.f.		288	
exposure.treatment			
			0.7237
d.f.			288
exposure.Chem_Charge			
			0.8327
d.f.			268.48

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.3876	0.4462	0.2907	1.6487
d.f.	20	72	288	42.51
Except when comparing means with the same level(s) of				
exposure				1.0930
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.5022	0.6693	2.0428
d.f.	29.76	268.48	102.00
Except when comparing means with the same level(s) of			
exposure	0.7122		1.6394
d.f.	288		268.48
treatment		0.5815	
d.f.		288	
exposure.treatment			1.4244
d.f.			288
exposure.Chem_Charge			1.6394
d.f.			268.48

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	549.088	137.272	1.69	
block.board stratum					
exposure	5	9791.420	1958.284	24.10	<.001
Residual	20	1625.091	81.255	8.23	
block.board.area stratum					
treatment	3	121.507	40.502	4.10	0.010
exposure.treatment	15	290.766	19.384	1.96	0.030
Residual	72	710.443	9.867	1.84	
block.board.area.Strip stratum					
Chem_Charge	3	12.599	4.200	0.78	0.504
exposure.Chem_Charge	15	63.313	4.221	0.79	0.692
treatment.Chem_Charge	9	53.239	5.915	1.10	0.360
exposure.treatment.Chem_Charge	45	291.265	6.473	1.21	0.183
Residual	288	1543.885	5.361		
Total	479	15052.616			

Message: the following units have large residuals.

block 1 board 2	4.71	s.e. 1.84
block 1 board 2 area 3	3.14	s.e. 1.22
block 1 board 3 area 1	5.13	s.e. 1.22
block 1 board 3 area 3	-3.09	s.e. 1.22
block 1 board 2 area 1 Strip 3	5.68	s.e. 1.79
block 1 board 2 area 1 Strip 4	-5.40	s.e. 1.79
block 1 board 5 area 1 Strip 1	5.96	s.e. 1.79
block 2 board 3 area 3 Strip 2	6.03	s.e. 1.79
block 4 board 2 area 2 Strip 1	-6.43	s.e. 1.79
block 5 board 2 area 1 Strip 4	5.55	s.e. 1.79
block 5 board 2 area 2 Strip 1	5.37	s.e. 1.79

Tables of means

Variate: b

Grand mean 16.36

exposure	full	I.R.	none	UVA	UVB	Vis light
	6.64	18.64	18.24	18.94	15.83	19.87

treatment	acetic acid	carpropamid	tinuvin	water
	16.39	15.66	17.08	16.31

Chem_Charge	high	low	medium	Very high
	16.16	16.24	16.54	16.49

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		6.83	6.66	6.32	6.76
I.R.		19.49	17.89	18.37	18.82
none		18.22	18.06	18.82	17.86
UVA		18.78	18.84	19.60	18.54
UVB		15.23	12.97	18.61	16.51
Vis light		19.79	19.56	20.79	19.35

exposure	Chem_Charge	high	low	medium	Very high
full		6.45	6.60	6.72	6.81
I.R.		19.10	18.76	18.30	18.39
none		17.59	18.53	18.70	18.14
UVA		18.79	18.10	19.71	19.15
UVB		15.83	15.40	16.12	15.97
Vis light		19.19	20.09	19.71	20.49

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		16.20	16.25	16.33	16.77
carpropamid		15.61	16.18	15.66	15.20
tinuvin		16.85	16.83	17.69	16.96
water		15.97	15.72	16.50	17.03

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		6.02	7.39	6.54	7.38
	carpropamid		6.56	6.85	6.91	6.31
	tinuvin		7.13	5.58	6.01	6.57
	water		6.09	6.59	7.40	6.97
I.R.	acetic acid		19.64	19.65	20.26	18.40
	carpropamid		18.00	18.82	16.05	18.70
	tinuvin		18.73	17.58	19.31	17.85
	water		20.05	19.00	17.60	18.63
none	acetic acid		17.86	18.45	18.29	18.29
	carpropamid		17.20	18.28	19.50	17.25
	tinuvin		18.15	19.23	19.36	18.55
	water		17.16	18.15	17.66	18.47
UVA	acetic acid		18.07	18.73	19.40	18.91
	carpropamid		19.50	19.42	18.87	17.57
	tinuvin		20.44	16.92	20.57	20.46
	water		17.15	17.31	20.01	19.67
UVB	acetic acid		16.93	12.88	14.95	16.15
	carpropamid		13.31	13.79	12.46	12.32
	tinuvin		16.16	20.05	20.90	17.32
	water		16.93	14.86	16.18	18.07
Vis light	acetic acid		18.69	20.41	18.56	21.50
	carpropamid		19.09	19.89	20.19	19.06
	tinuvin		20.50	21.64	19.97	21.04
	water		18.47	18.41	20.13	20.39

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.425	0.406	0.299	1.665
d.f.	20	72	288	35.90
Except when comparing means with the same level(s) of exposure				0.993
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.560	0.658	2.093
d.f.	28.62	299.24	86.05
Except when comparing means with the same level(s) of exposure	0.732		1.611
d.f.	288		299.24
treatment		0.598	
d.f.		288	
exposure.treatment			1.464
d.f.			288
exposure.Chem_Charge			1.611
d.f.			299.24

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	2.973	0.808	0.588	3.377
d.f.	20	72	288	35.90
Except when comparing means with the same level(s) of exposure				1.980
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.192	1.294	4.160
d.f.	28.62	299.24	86.05
Except when comparing means with the same level(s) of			
exposure	1.441		3.170
d.f.	288		299.24
treatment		1.177	
d.f.		288	
exposure.treatment			2.882
d.f.			288
exposure.Chem_Charge			3.170
d.f.			299.24

Analysis of variance week 40

Variate: L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	6832.59	1708.15	7.64	
block.board stratum					
exposure	5	9144.39	1828.88	8.18	<.001
Residual	20	4472.72	223.64	7.05	
block.board.area stratum					
treatment	3	100.59	33.53	1.06	0.373
exposure.treatment	15	367.88	24.53	0.77	0.702
Residual	72	2283.97	31.72	2.44	
block.board.area.Strip stratum					
Chem_Charge	3	33.42	11.14	0.86	0.464
exposure.Chem_Charge	15	290.32	19.35	1.49	0.109
treatment.Chem_Charge	9	125.30	13.92	1.07	0.385
exposure.treatment.Chem_Charge	45	801.50	17.81	1.37	0.068
Residual	288	3748.45	13.02		
Total	479	28201.14			

Message: the following units have large residuals.

block 2 board 3	-6.26	s.e. 3.05
block 2 board 4	6.69	s.e. 3.05
block 2 board 3 area 3	5.57	s.e. 2.18
block 4 board 3 area 3	-5.62	s.e. 2.18
block 5 board 1 area 4	6.40	s.e. 2.18
block 1 board 5 area 2 Strip 3	9.37	s.e. 2.79
block 1 board 6 area 4 Strip 2	10.49	s.e. 2.79
block 5 board 3 area 3 Strip 3	-8.48	s.e. 2.79

Tables of means

Variate: L

Grand mean 54.64

exposure	full	I.R.	none	UVA	UVB	Vis light
	45.18	56.39	58.00	54.62	57.53	56.10

treatment	acetic acid	carpropamid	tinuvin	water
	54.15	54.52	55.39	54.49

Chem_Charge	high	low	medium	Very high
	54.89	54.23	54.58	54.84

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		45.20	44.33	45.41	45.78
I.R.		55.63	57.28	57.81	54.84
none		58.02	57.29	59.89	56.78
UVA		53.10	54.98	56.35	54.04
UVB		57.23	57.13	56.40	59.37
Vis light		55.73	56.09	56.48	56.10

exposure	Chem_Charge	high	low	medium	Very high
full		45.94	44.94	44.28	45.56
I.R.		57.95	56.65	55.37	55.59
none		56.34	57.16	59.92	58.56
UVA		55.04	54.14	54.82	54.46
UVB		58.05	56.56	57.03	58.50
Vis light		56.03	55.91	56.08	56.39

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		54.14	54.41	54.12	53.95
carpropamid		54.87	53.58	54.57	55.06
tinuvin		56.30	55.55	54.47	55.24
water		54.27	53.38	55.17	55.12

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		46.99	43.22	45.02	45.58
	carpropamid		46.43	43.53	43.52	43.85
	tinuvin		43.99	47.63	42.73	47.30
	water		46.36	45.39	45.86	45.51
I.R.	acetic acid		58.02	55.80	54.65	54.05
	carpropamid		58.31	58.05	54.52	58.24
	tinuvin		60.06	57.63	57.47	56.07
	water		55.40	55.11	54.84	54.02
none	acetic acid		57.35	56.79	59.80	58.15
	carpropamid		56.04	55.11	60.46	57.56
	tinuvin		58.17	61.13	61.84	58.44
	water		53.82	55.61	57.59	60.11
UVA	acetic acid		52.15	54.20	53.34	52.73
	carpropamid		52.56	55.16	55.31	56.90
	tinuvin		60.62	55.40	53.93	55.44
	water		54.85	51.82	56.69	52.79
UVB	acetic acid		55.02	59.83	57.00	57.08
	carpropamid		61.34	53.12	57.09	56.98
	tinuvin		57.51	54.76	54.05	59.27
	water		58.31	58.54	59.98	60.65
Vis light	acetic acid		55.29	56.60	54.91	56.13
	carpropamid		54.52	56.48	56.50	56.84
	tinuvin		57.46	56.76	56.82	54.90
	water		56.86	53.80	56.07	57.68

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	2.365	0.727	0.466	2.823
d.f.	20	72	288	38.70
Except when comparing means with the same level(s) of exposure				1.781
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	2.563	1.086	3.446
d.f.	27.54	259.90	83.22
Except when comparing means with the same level(s) of exposure	1.141		2.660
d.f.	288		259.90
treatment		0.932	
d.f.		288	
exposure.treatment			2.282
d.f.			288
exposure.Chem_Charge			2.660
d.f.			259.90

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	4.932	1.449	0.917	5.712
d.f.	20	72	288	38.70
Except when comparing means with the same level(s) of exposure				3.550
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	5.253	2.139	6.854
d.f.	27.54	259.90	83.22
Except when comparing means with the same level(s) of			
exposure	2.245		5.238
d.f.	288		259.90
treatment		1.833	
d.f.		288	
exposure.treatment			4.491
d.f.			288
exposure.Chem_Charge			5.238
d.f.			259.90

Analysis of variance

Variate: a

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	23.3843	5.8461	0.37	
block.board stratum					
exposure	5	865.9996	173.1999	11.04	<.001
Residual	20	313.6738	15.6837	8.19	
block.board.area stratum					
treatment	3	8.4121	2.8040	1.46	0.231
exposure.treatment	15	27.4774	1.8318	0.96	0.508
Residual	72	137.8261	1.9143	2.23	
block.board.area.Strip stratum					
Chem_Charge	3	4.1611	1.3870	1.61	0.186
exposure.Chem_Charge	15	10.9747	0.7316	0.85	0.619
treatment.Chem_Charge	9	12.6021	1.4002	1.63	0.106
exposure.treatment.Chem_Charge	45	53.5583	1.1902	1.39	0.061
Residual	288	247.4463	0.8592		
Total	479	1705.5157			

Message: the following units have large residuals.

block 1 board 2	2.450	s.e. 0.808
block 1 board 3 area 1	2.058	s.e. 0.536
block 1 board 3 area 3	-1.464	s.e. 0.536
block 3 board 5 area 2	-1.424	s.e. 0.536
block 4 board 3 area 3	1.531	s.e. 0.536
block 1 board 2 area 1 Strip 3	2.935	s.e. 0.718
block 1 board 2 area 2 Strip 2	2.254	s.e. 0.718
block 1 board 3 area 1 Strip 2	-2.429	s.e. 0.718
block 1 board 3 area 1 Strip 4	2.733	s.e. 0.718
block 2 board 3 area 3 Strip 2	2.661	s.e. 0.718
block 2 board 5 area 1 Strip 3	2.119	s.e. 0.718
block 4 board 2 area 4 Strip 1	2.128	s.e. 0.718
block 5 board 2 area 1 Strip 4	2.227	s.e. 0.718

Tables of means

Variate: a

Grand mean 3.751

exposure	full	I.R.	none	UVA	UVB	Vis light
	1.202	3.851	3.108	5.255	4.106	4.983

treatment	acetic acid	carpropamid	tinuvin	water
	3.836	3.540	3.886	3.741

Chem_Charge	high	low	medium	Very high
	3.591	3.790	3.819	3.804

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		1.213	1.242	1.189	1.165
I.R.		4.316	3.610	3.415	4.064
none		3.268	3.035	3.046	3.085
UVA		5.257	4.992	5.601	5.170
UVB		4.202	3.417	4.652	4.150
Vis light		4.759	4.944	5.414	4.816

exposure	Chem_Charge	high	low	medium	Very high
full		1.075	1.197	1.312	1.226
I.R.		3.934	3.732	3.890	3.848
none		2.825	3.481	3.191	2.936
UVA		4.960	5.120	5.419	5.521
UVB		4.008	3.925	4.231	4.258
Vis light		4.742	5.287	4.871	5.034

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		3.613	3.724	3.943	4.063
carpropamid		3.505	3.783	3.541	3.331
tinuvin		3.695	3.860	4.222	3.769
water		3.550	3.794	3.570	4.052

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		1.038	1.188	1.312	1.312
	carpropamid		1.254	1.252	1.360	1.102
	tinuvin		1.250	0.908	1.324	1.274
	water		0.756	1.438	1.252	1.214
I.R.	acetic acid		3.966	4.158	4.934	4.206
	carpropamid		3.704	3.772	3.146	3.818
	tinuvin		3.388	3.246	3.806	3.220
	water		4.680	3.754	3.674	4.148
none	acetic acid		2.982	3.748	3.184	3.156
	carpropamid		2.744	3.134	3.434	2.830
	tinuvin		2.778	3.348	3.134	2.924
	water		2.798	3.696	3.012	2.834
UVA	acetic acid		4.962	5.006	5.616	5.444
	carpropamid		5.106	5.726	5.010	4.124
	tinuvin		5.426	4.468	6.322	6.188
	water		4.346	5.278	4.728	6.326
UVB	acetic acid		4.290	3.306	4.358	4.856
	carpropamid		3.394	3.782	3.244	3.248
	tinuvin		4.008	4.716	5.704	4.182
	water		4.338	3.898	3.618	4.746
Vis light	acetic acid		4.442	4.938	4.252	5.406
	carpropamid		4.826	5.034	5.054	4.862
	tinuvin		5.318	6.474	5.040	4.824
	water		4.380	4.700	5.136	5.046

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	0.6262	0.1786	0.1197	0.7319
d.f.	20	72	288	35.99
Except when comparing means with the same level(s) of exposure				0.4375
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	0.6757	0.2736	0.8907
d.f.	27.06	272.79	76.73
Except when comparing means with the same level(s) of exposure	0.2931		0.6702
d.f.	288		272.79
treatment		0.2393	
d.f.		288	
exposure.treatment			0.5862
d.f.			288
exposure.Chem_Charge			0.6702
d.f.			272.79

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	1.3062	0.3561	0.2355	1.4844
d.f.	20	72	288	35.99
Except when comparing means with the same level(s) of exposure				0.8722
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	1.3862	0.5387	1.7738
d.f.	27.06	272.79	76.73
Except when comparing means with the same level(s) of			
exposure	0.5769		1.3194
d.f.	288		272.79
treatment		0.4711	
d.f.		288	
exposure.treatment			1.1539
d.f.			288
exposure.Chem_Charge			1.3194
d.f.			272.79

Analysis of variance

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	301.434	75.358	0.84	
block.board stratum					
exposure	5	12379.447	2475.889	27.53	<.001
Residual	20	1798.434	89.922	11.87	
block.board.area stratum					
treatment	3	93.004	31.001	4.09	0.010
exposure.treatment	15	127.254	8.484	1.12	0.355
Residual	72	545.474	7.576	2.00	
block.board.area.Strip stratum					
Chem_Charge	3	8.948	2.983	0.79	0.502
exposure.Chem_Charge	15	61.849	4.123	1.09	0.367
treatment.Chem_Charge	9	35.524	3.947	1.04	0.407
exposure.treatment.Chem_Charge	45	181.090	4.024	1.06	0.374
Residual	288	1091.413	3.790		
Total	479	16623.870			

Message: the following units have large residuals.

block 1 board 2	5.284	s.e. 1.936
block 1 board 3 area 1	4.031	s.e. 1.066
block 1 board 3 area 3	-3.094	s.e. 1.066
block 2 board 2 area 3	-2.654	s.e. 1.066
block 2 board 5 area 3	2.757	s.e. 1.066
block 1 board 2 area 1 Strip 3	6.143	s.e. 1.508
block 1 board 2 area 1 Strip 4	-4.583	s.e. 1.508
block 1 board 3 area 1 Strip 2	-4.805	s.e. 1.508
block 1 board 5 area 1 Strip 1	5.331	s.e. 1.508
block 1 board 6 area 4 Strip 2	4.445	s.e. 1.508
block 2 board 3 area 3 Strip 2	4.990	s.e. 1.508
block 5 board 2 area 1 Strip 4	5.425	s.e. 1.508

Tables of means

Variate: b

Grand mean 14.978

exposure	full	I.R.	none	UVA	UVB	Vis light
	4.577	18.063	17.453	18.003	12.715	19.057

treatment	acetic acid	carpropamid	tinuvin	water
	14.948	14.461	15.674	14.830

Chem_Charge	high	low	medium	Very high
	14.750	14.998	15.102	15.061

exposure	treatment	acetic acid	carpropamid	tinuvin	water
full		4.441	4.654	4.602	4.609
I.R.		18.701	17.768	17.668	18.116
none		17.765	17.025	17.784	17.239
UVA		17.377	17.296	19.557	17.784
UVB		12.782	11.164	14.351	12.562
Vis light		18.620	18.857	20.081	18.667

exposure	Chem_Charge	high	low	medium	Very high
full		4.498	4.450	4.745	4.614
I.R.		18.558	18.095	17.733	17.868
none		16.575	17.908	18.062	17.268
UVA		17.535	17.683	18.435	18.360
UVB		12.725	12.203	12.720	13.213
Vis light		18.612	19.649	18.920	19.045

treatment	Chem_Charge	high	low	medium	Very high
acetic acid		14.637	14.765	15.155	15.233
carpropamid		14.268	14.859	14.550	14.166
tinuvin		15.602	15.584	16.143	15.367
water		14.495	14.784	14.560	15.479

exposure	treatment	Chem_Charge	high	low	medium	Very high
full	acetic acid		4.086	4.440	4.574	4.662
	carpropamid		4.794	4.448	5.060	4.314
	tinuvin		4.894	3.624	4.854	5.038
	water		4.218	5.288	4.490	4.442
I.R.	acetic acid		18.432	18.704	19.452	18.216
	carpropamid		17.904	18.534	16.226	18.408
	tinuvin		18.450	17.430	17.978	16.814
	water		19.446	17.710	17.274	18.034
none	acetic acid		17.034	18.420	18.162	17.442
	carpropamid		16.094	16.662	18.600	16.744
	tinuvin		17.048	18.610	18.338	17.140
	water		16.124	17.938	17.146	17.748
UVA	acetic acid		16.524	16.970	18.336	17.676
	carpropamid		17.032	18.800	17.330	16.020
	tinuvin		19.994	17.456	20.352	20.428
	water		16.590	17.508	17.722	19.316
UVB	acetic acid		13.618	10.854	12.698	13.960
	carpropamid		11.436	11.556	10.746	10.920
	tinuvin		13.214	14.488	15.770	13.933
	water		12.632	11.914	11.666	14.038
Vis light	acetic acid		18.128	19.200	17.710	19.444
	carpropamid		18.346	19.156	19.340	18.588
	tinuvin		20.014	21.894	19.568	18.850
	water		17.960	18.348	19.064	19.298

Standard errors of differences of means

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
s.e.d.	1.4993	0.3553	0.2513	1.6782
d.f.	20	72	288	30.84
Except when comparing means with the same level(s) of				
exposure				0.8704
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
s.e.d.	1.5913	0.5619	1.9882
d.f.	25.35	288.06	59.72
Except when comparing means with the same level(s) of			
exposure	0.6156		1.3764
d.f.	288		288.06
treatment		0.5026	
d.f.		288	
exposure.treatment			
			1.2312
d.f.			288
exposure.Chem_Charge			
			1.3764
d.f.			288.06

Least significant differences of means (5% level)

Table	exposure	treatment	Chem_Charge	exposure treatment
rep.	80	120	120	20
l.s.d.	3.1276	0.7084	0.4947	3.4234
d.f.	20	72	288	30.84
Except when comparing means with the same level(s) of				
exposure				1.7351
d.f.				72

Table	exposure Chem_Charge	treatment Chem_Charge	exposure treatment Chem_Charge
rep.	20	30	5
l.s.d.	3.2751	1.1060	3.9775
d.f.	25.35	288.06	59.72
Except when comparing means with the same level(s) of			
exposure	1.2116		2.7091
d.f.	288		288.06
treatment		0.9893	
d.f.		288	
exposure.treatment			
			2.4233
d.f.			288
exposure.Chem_Charge			
			2.7091
d.f.			288.06

Appendix 4: Images of fungal colonization evolution in southern pine samples exposed under filter transmitting different wavelengths of solar radiation (Chapter 5)

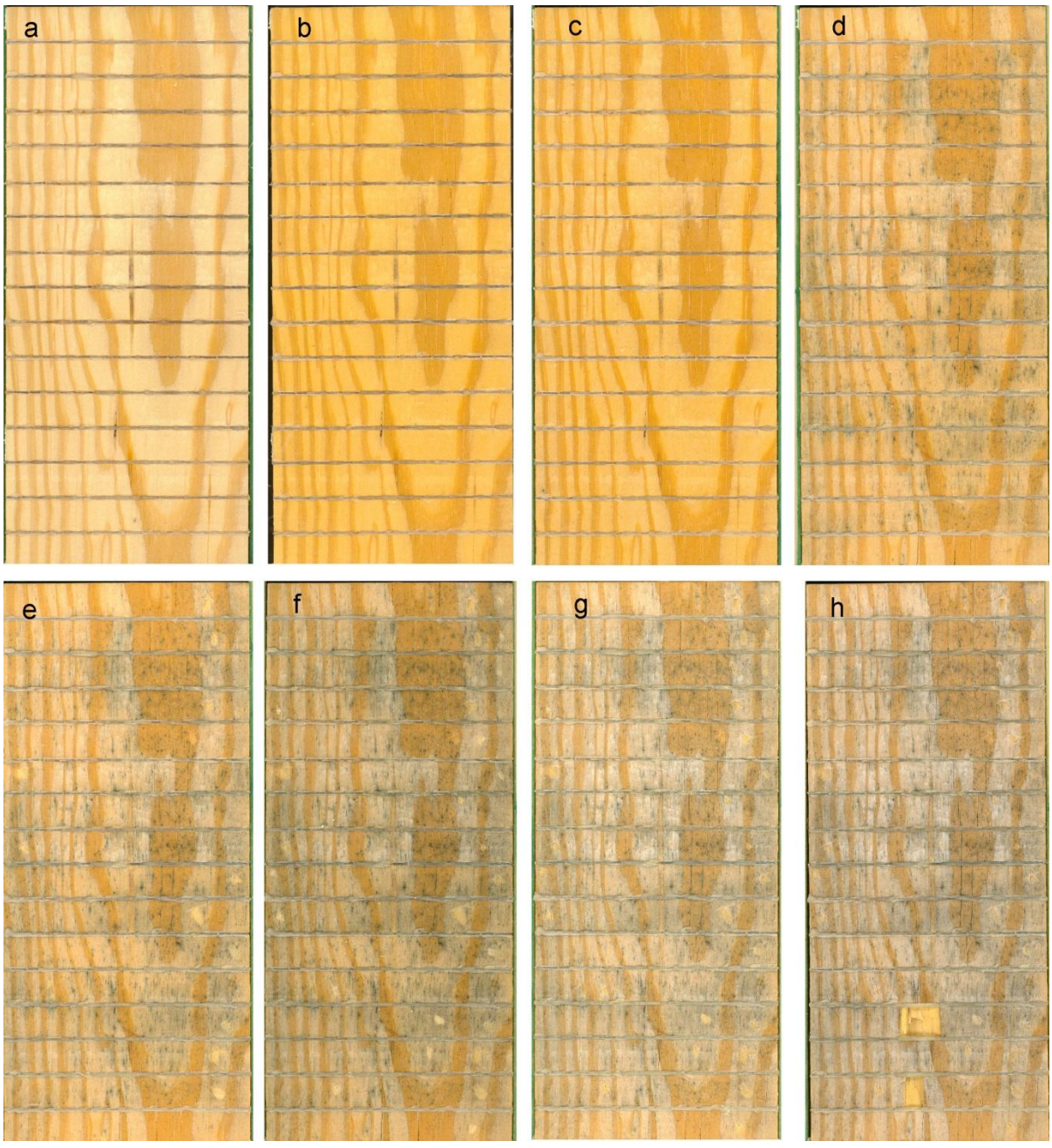


Figure A4.1: Appearance of southern pine wood samples exposed to weather for 40 in Vancouver, Canada, under a polymethylmethacrylate filter transmitting UVB+UVA+Vis.light+IR (Filter 1). (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

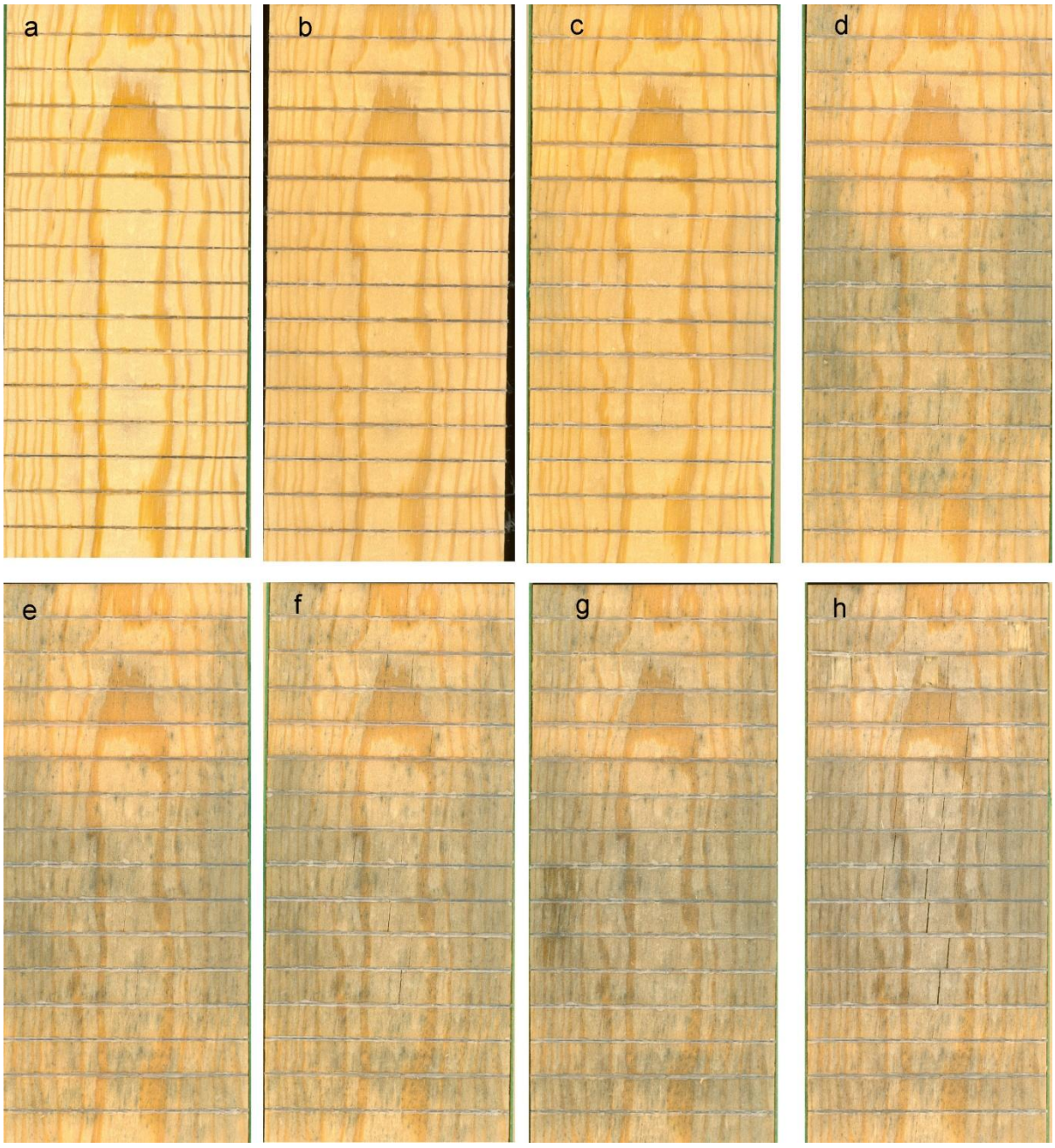


Figure A4.2: Appearance of southern pine wood samples exposed to weather for 40 in Vancouver, Canada, under a polymethylmethacrylate filter transmitting UVA+Vis.light+IR (Filter 2). (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

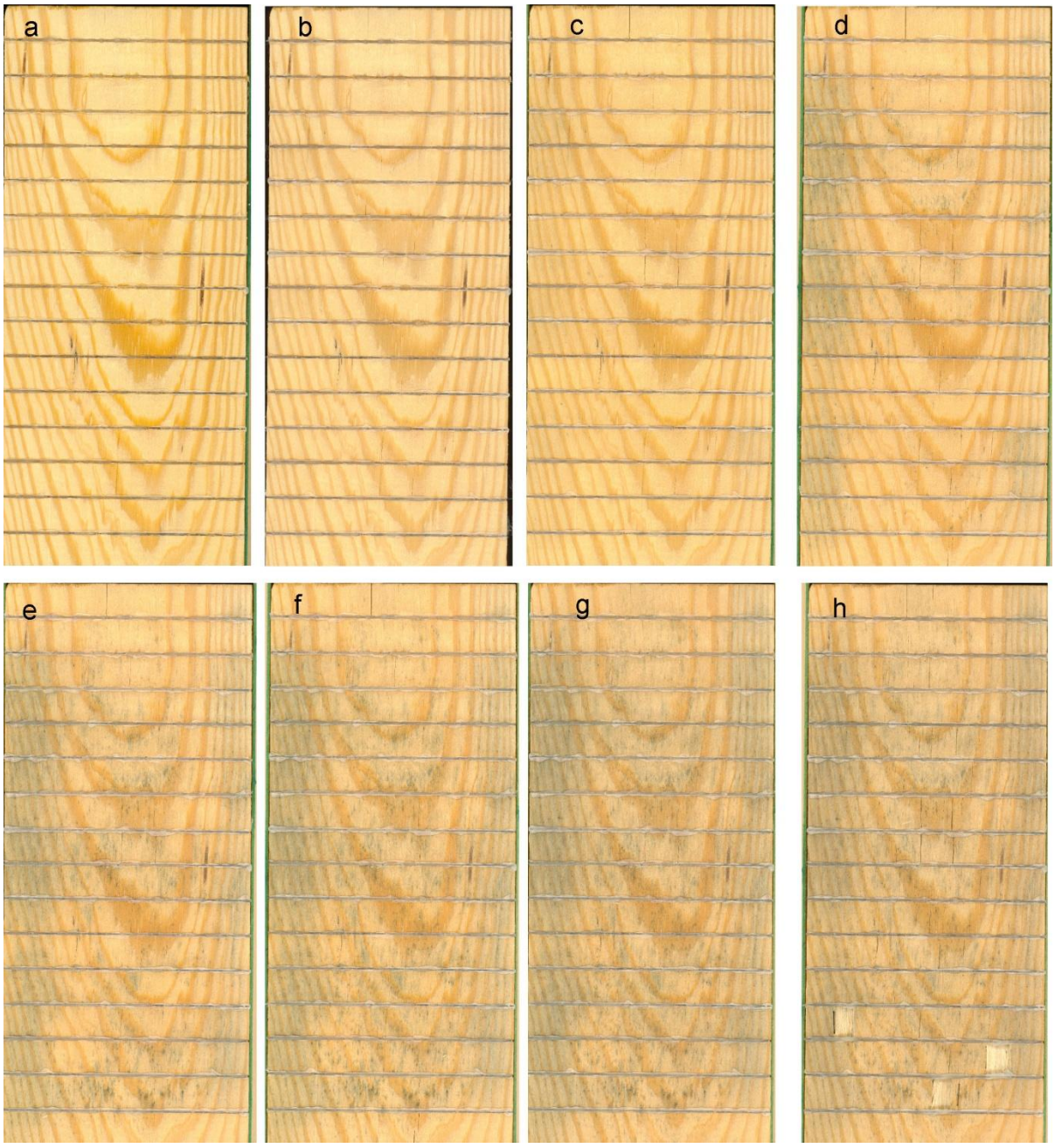


Figure A4.3: Appearance of southern pine wood samples exposed to weather for 40 in Vancouver, Canada, under a polymethylmethacrylate filter transmitting Vis.light+IR (Filter 3). (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

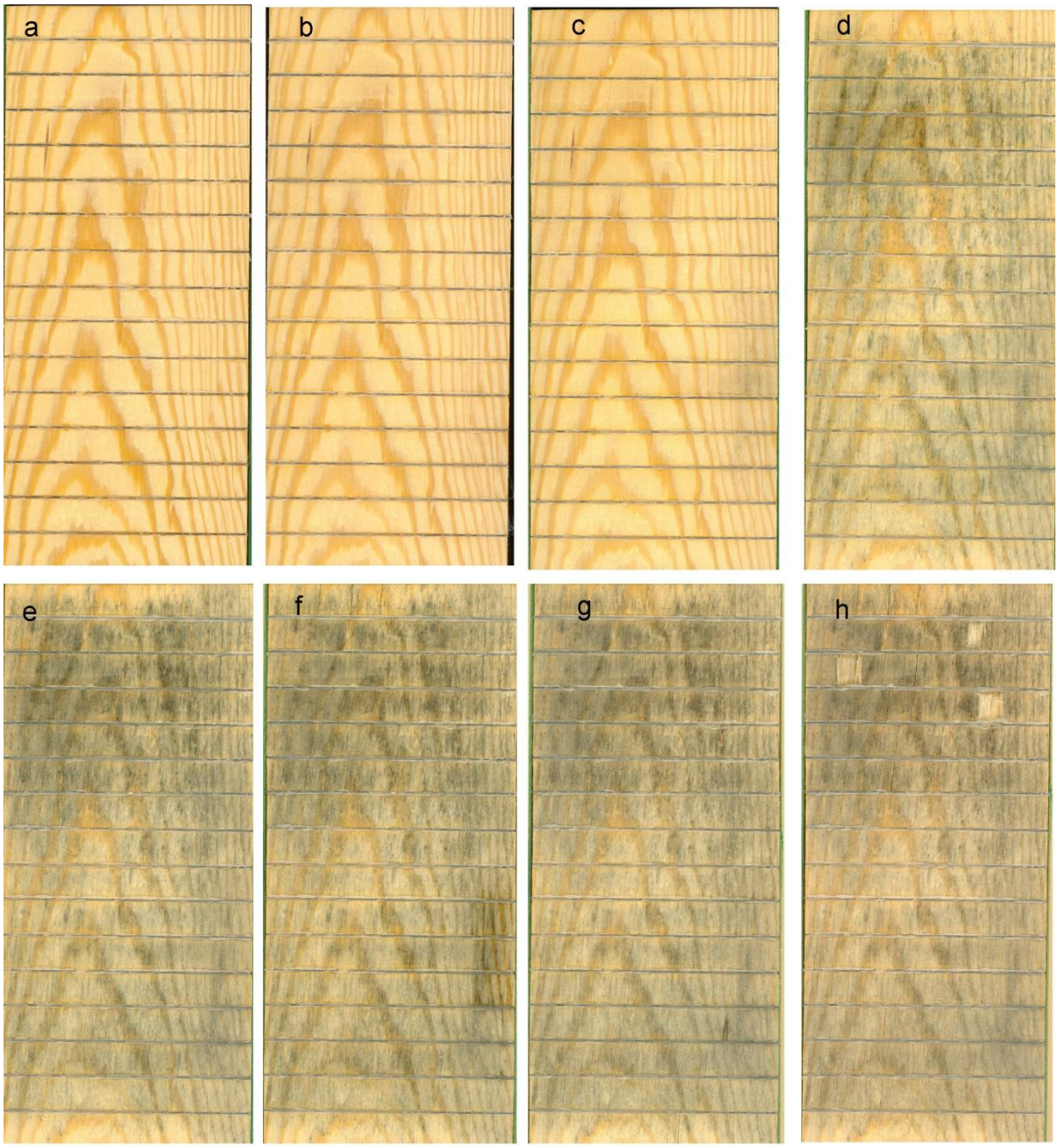


Figure A4.4: Appearance of southern pine wood samples exposed to weather for 40 in Vancouver, Canada, under a polymethylmethacrylate filter transmitting IR (Filter 4). (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

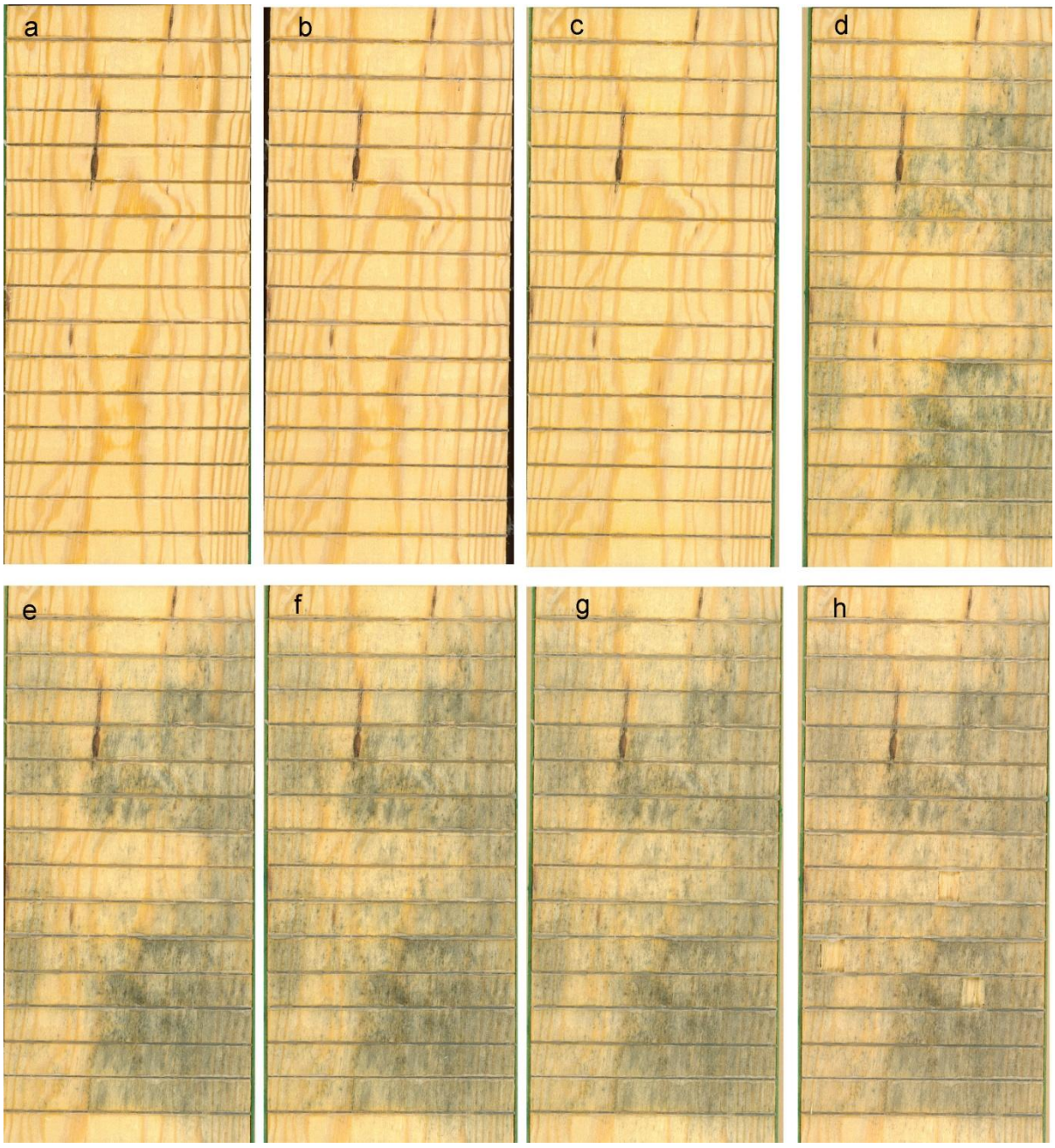


Figure A4.5: Appearance of southern pine wood samples exposed to weather for 40 in Vancouver, Canada, under a polymethylmethacrylate filter blocking all wavelengths of solar radiation (Filter 5). (a) week 0, (b) week 4, (c) week 8, (d) week 12, (e) week 16, (f) week 20, (g) week 32, (h) week 40

Appendix 5: Result for reciprocal Simpson index (Chapter 5)

Table A5.1: reciprocal diversity Simpson index for fungi isolated from weathered southern pine samples exposed outdoors under different filters for 40 weeks

	Simpson index				
Rack	UVB+UVA+Vis.light+IR transmitted	UVA+Vis.light+IR transmitted	Vis. light+IR transmitted	IR transmitted	No light transmitted
1	4	5	4	5	4
2	4	5	5	4	4
3	9	4	5	5	5
4	4	5	4	7	2.3
5	3	4	7	5	2.3
Ave.	3	4.8	4.6	5	5.2
SD	1.6	2.4	0.5	1.2	1.1

Appendix 6: Statistical analysis Chapter 6

Analysis of variance fungal biomass

Variate: grams of biomass

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	5		2.851E-05	5.701E-06	7.34	
block.*Units* stratum						
exposure	2		1.607E-04	8.035E-05	103.38	<.001
fungi	5		1.754E-04	3.508E-05	45.14	<.001
exposure.fungi	10		1.460E-04	1.460E-05	18.79	<.001
Residual	83	(2)	6.451E-05	7.772E-07		
Total	105	(2)	5.724E-04			

Message: the following units have large residuals.

block 2 *units* 5	0.002343	s.e.	0.000773
block 2 *units* 14	0.001943	s.e.	0.000773
block 4 *units* 15	0.002004	s.e.	0.000773

Tables of means

Variate: grams

Grand mean 0.003421

exposure	1	2	3
	0.001781	0.003778	0.004704

fungi	1	2	3	4	5	6
	0.003985	0.005117	0.002556	0.001466	0.004656	0.002744

exposure	fungi	1	2	3	4	5	6
1		0.003350	0.004050	0.000000	0.001000	0.002283	0.000000
2		0.003850	0.007417	0.003250	0.001416	0.003717	0.003017
3		0.004756	0.003883	0.004417	0.001983	0.007967	0.005217

Standard errors of differences of means

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	83	83	83
s.e.d.	0.0002078	0.0002939	0.0005090

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	83	83	83
l.s.d.	0.0004133	0.0005845	0.0010124

(Not adjusted for missing values)

Analysis of variance lightness fungal mycelia

Variate: L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	5		270.36	54.07	2.19	
block.*Units* stratum						
exposure	2		4078.05	2039.03	82.41	<.001
fungi	5		3346.93	669.39	27.05	<.001
exposure.fungi	8	(2)	1893.98	236.75	9.57	<.001
Residual	75	(10)	1855.79	24.74		
Total	95	(12)	11331.83			

Message: the following units have large residuals.

block 1 *units* 13	14.46	s.e. 4.15
block 2 *units* 13	-10.48	s.e. 4.15
block 3 *units* 13	-14.48	s.e. 4.15

Tables of means

Variate: L

Grand mean 20.70

exposure	1	2	3
	12.86	21.39	27.86

fungi	1	2	3	4	5	6
	17.89	13.39	25.32	17.67	19.61	30.34

exposure	fungi	1	2	3	4	5	6
1		13.83	9.00	17.47	7.50	6.83	22.51
2		18.50	6.83	33.00	16.67	19.00	34.33
3		21.33	24.33	25.50	28.83	33.00	34.17

Standard errors of differences of means

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	75	75	75
s.e.d.	1.172	1.658	2.872

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	75	75	75
l.s.d.	2.336	3.303	5.721

(Not adjusted for missing values)

	Mean				
fungi	1	2	3	4	5
exposure					
1	13.83	9.00	*	7.50	6.83
2	18.50	6.83	33.00	16.67	19.00
3	21.33	24.33	25.50	28.83	33.00

fungi	6
exposure	
1	*
2	34.33
3	34.17

Analysis of variance melanin concentration

Variate: mg_melanin_mg_biomass_new

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	5		0.087432	0.017486	3.81	
block.*Units* stratum						
exposure	2		0.033081	0.016540	3.60	0.032
fungi	5		0.320606	0.064121	13.96	<.001
exposure.fungi	10		0.123496	0.012350	2.69	0.007
Residual	81	(4)	0.372158	0.004595		
Total	103	(4)	0.901307			

Message: the following units have large residuals.

block 2 *units* 10	-0.1708	s.e. 0.0587
block 2 *units* 15	0.1633	s.e. 0.0587

Tables of means

Variate: mg_melanin_mg_biomass_new

Grand mean 0.0826

exposure	1	2	3
	0.0983	0.0913	0.0581

fungi	1	2	3	4	5	6
	0.0861	0.0961	0.0447	0.1900	0.0606	0.0180

exposure	fungi	1	2	3	4	5	6
1		0.1089	0.1455	0.0000	0.2067	0.1285	0.0000
2		0.0906	0.1070	0.0373	0.2325	0.0456	0.0346
3		0.0586	0.0357	0.0967	0.1307	0.0077	0.0194

Standard errors of differences of means

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	81	81	81
s.e.d.	0.01598	0.02259	0.03913

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	81	81	81
l.s.d.	0.03179	0.04496	0.07787

(Not adjusted for missing values)

Analysis of variance radial growth

Variate: ln_mm (natural logarithm radial growth (mm))

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	5	0.86397	0.17279	2.75	
block.*Units* stratum					
exposure	2	23.72496	11.86248	188.65	<.001
fungi	5	2.34376	0.46875	7.45	<.001
exposure.fungi	10	4.76523	0.47652	7.58	<.001
Residual	85	5.34476	0.06288		
Total	107	37.04268			

Message: the following units have large residuals.

block 2 *units* 1	0.687	s.e.	0.222
block 5 *units* 5	0.723	s.e.	0.222
block 6 *units* 13	-0.708	s.e.	0.222

Tables of means

Variate: ln_mm

Grand mean 1.004

exposure	1	2	3
	0.344	1.385	1.283

fungi	1	2	3	4	5	6
	0.972	0.819	1.045	1.288	0.892	1.008

exposure	fungi	1	2	3	4	5	6
1		0.359	0.548	0.000	0.877	0.278	0.000
2		1.289	0.898	1.566	1.587	1.358	1.609
3		1.270	1.009	1.569	1.399	1.039	1.415

Standard errors of differences of means

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	85	85	85
s.e.d.	0.0591	0.0836	0.1448

Least significant differences of means (5% level)

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	85	85	85
l.s.d.	0.1175	0.1662	0.2879

Analysis of variance spore concentration

Variate: ln_spore (natural ogarithm spore concentration)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum	5		5.5762	1.1152	4.57	
block.*Units* stratum						
exposure	2		73.4410	36.7205	150.62	<.001
fungi	5		107.6042	21.5208	88.27	<.001
exposure.fungi	10		79.3314	7.9331	32.54	<.001
Residual	84	(1)	20.4787	0.2438		
Total	106	(1)	286.2279			

Tables of means

Variate: ln_spore

Grand mean 3.432

exposure	1	2	3
	2.281	4.170	3.845

fungi	1	2	3	4	5	6
	2.987	2.834	2.390	5.204	4.346	2.830

exposure	fungi	1	2	3	4	5	6
1		2.543	2.305	0.000	4.869	3.968	0.000
2		3.153	2.251	4.140	5.411	5.307	4.758
3		3.265	3.946	3.030	5.332	3.762	3.734

Standard errors of differences of means

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	84	84	84
s.e.d.	0.1164	0.1646	0.2851

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	exposure	fungi	exposure fungi
rep.	36	18	6
d.f.	84	84	84
l.s.d.	0.2314	0.3273	0.5669

(Not adjusted for missing values)

Appendix 7: Calibration curves for calculation of fungal melanin concentration (Chapter 6)

Table A7.1: UV-VIS light absorbance and concentration of fungal melanin produced by *C. cladiosporiodes* [R2F33]

Fungi 1	Clad. [R2F33]	
Concentration	ABS	mg/g
0.0004	6.3073	0.063419
(1:1)	0.1495	0.063419
(1:2)	0.081467	0.031709
(1:5)	0.031133	0.012684
(1:10)	0.015	0.006342
(1:20)	0.007233	0.003171
(1:50)	0.004667	0.001268
(1:70)	0.003667	0.000906
(1:100)	0.0016	0.000634
(1:200)	0.0009	0.000317

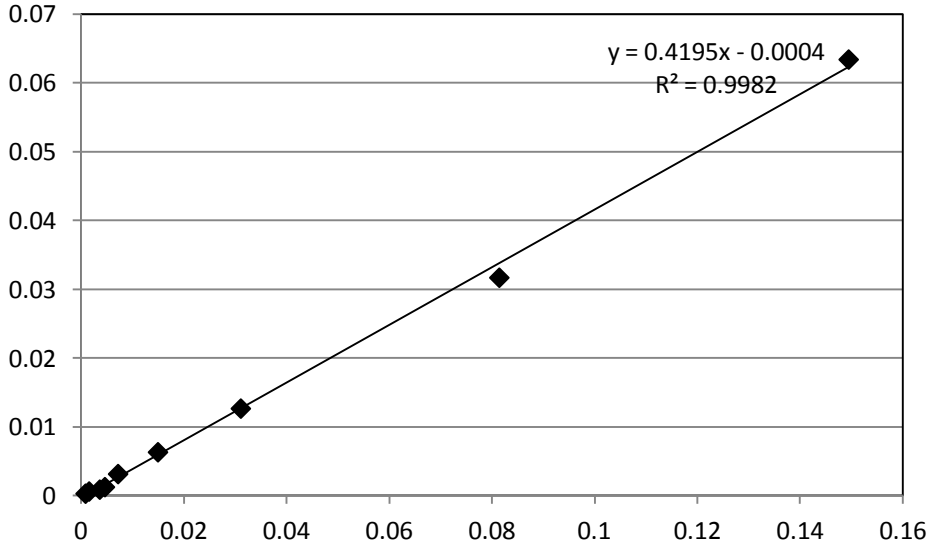


Figure A7.1: Calibration curve absorbance vs concentration *C. cladiosporioides*

Table A7.2: UV-VIS light absorbance and concentration of fungal melanin produced by *A. pullulans* [R2F32.2]

Fungi 2	A. pull. [R2F32.2]	
Concentration	ABS	mg/g
0.0004	6.3073	0.063419
(1:1)	0.098667	0.063419
(1:2)	0.048167	0.031709
(1:5)	0.0199	0.012684
(1:10)	0.012067	0.006342
(1:20)	0.005967	0.003171
(1:50)	0.002867	0.001268
(1:70)	0.001833	0.000906
(1:100)	0.001233	0.000634
(1:200)	0.0009	0.000317

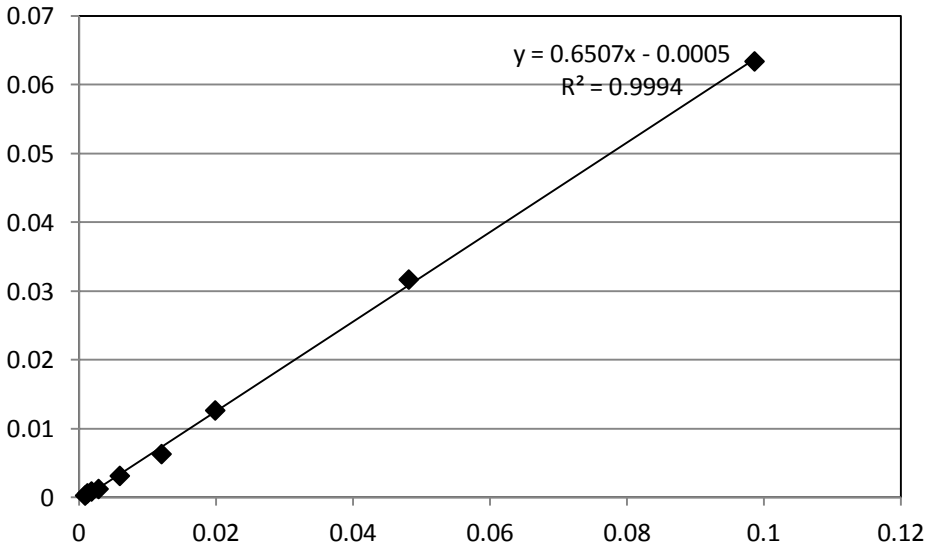


Figure A7.2: Calibration curve absorbance vs concentration *A. pullulans* [R2F32.2]

Table A7.3: UV-VIS light absorbance and concentration of fungal melanin produced by *O. piliferum* [TAB28]

Fungi 3	O. pilif. [TAB28]	
Concentration	ABS	mg/g
0.0002	6.3073	0.031709
(1:1)	0.028433	0.031709
(1:2)	0.011667	0.015855
(1:5)	0.006	0.006342
(1:10)	0.003167	0.003171
(1:20)	0.0009	0.001585
(1:50)	0.000167	0.000634
(1:70)	6.67E-05	0.000453
(1:100)	0.000667	0.000317
(1:200)	0.0004	0.000159

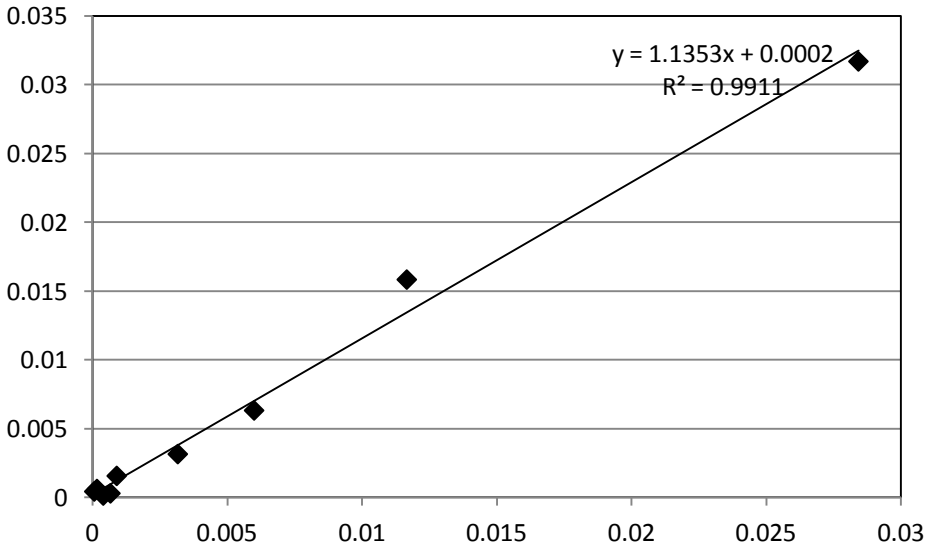


Figure A7.3: Calibration curve absorbance vs concentration *O. piliferum* [TAB28]

Table A7.4: UV-VIS light absorbance and concentration of fungal melanin produced by *A. pullulans* [ATCC 42371]

Fungi 4	A. pull. [ATCC 42371]	
Concentration	ABS	mg/g
0.0003	6.3073	0.047564
(1:1)	0.067767	0.047564
(1:2)	0.0341	0.023782
(1:5)	0.0153	0.009513
(1:10)	0.009033	0.004756
(1:20)	0.0058	0.002378
(1:50)	0.003933	0.000951
(1:70)	0.002933	0.000679
(1:100)	0.003233	0.000476
(1:200)	0.002133	0.000238

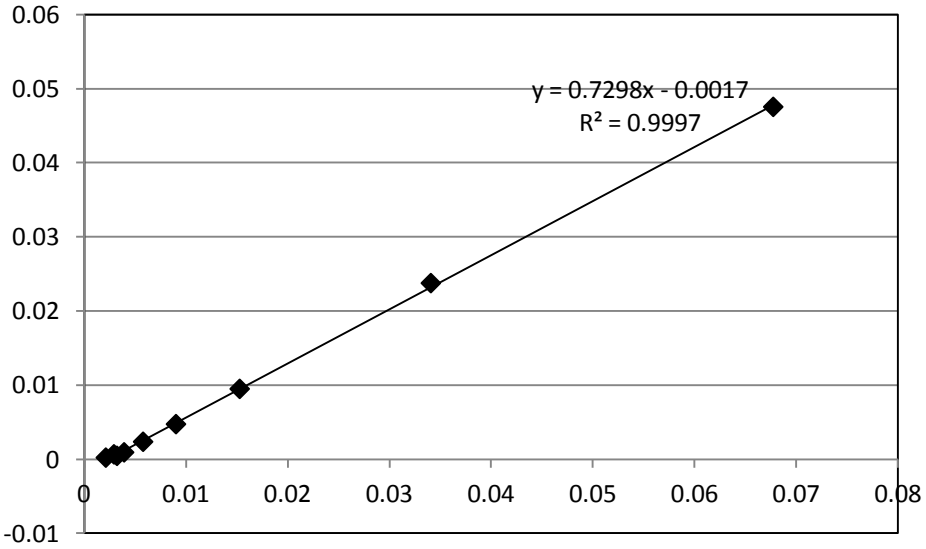


Figure A7.4: Calibration curve absorbance vs concentration *A. pullulans* [ATCC 42371]

Table A7.5: UV-VIS light absorbance and concentration of fungal melanin produced by *A. pullulans* [R1F22W]

Fungi 5	A. pull. [R1F22W]	
Concentration	ABS	mg/g
0.0008	6.3073	0.126837
(1:1)	0.474367	0.126837
(1:2)	0.239033	0.063419
(1:5)	0.0966	0.025367
(1:10)	0.048633	0.012684
(1:20)	0.027267	0.006342
(1:50)	0.012433	0.002537
(1:70)	0.008133	0.001812
(1:100)	0.0059	0.001268
(1:200)	0.0034	0.000634

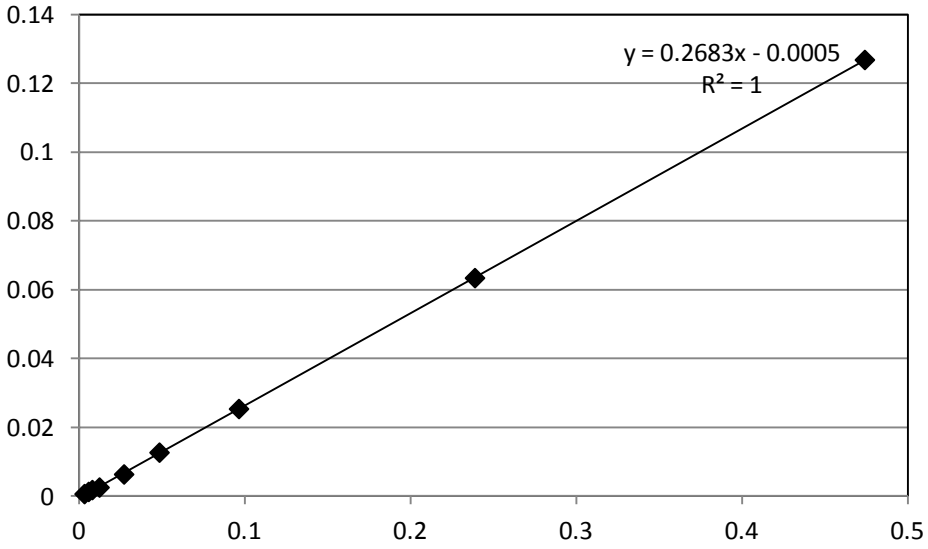


Figure A7.5: Calibration curve absorbance vs concentration *A. pullulans* [R1F22W]

Table A7.6: UV-VIS light absorbance and concentration of fungal melanin produced by *O. piliferum* [Cartapip97]

Fungi 6	O. pilif. [Cartapip97]	
Concentration	ABS	mg/g
0.0002	6.3073	0.031709
(1:1)	0.0157	0.031709
(1:2)	0.009	0.015855
(1:5)	0.0047	0.006342
(1:10)	0.0026	0.003171
(1:20)	0.0026	0.001585
(1:50)	0.002333	0.000634
(1:70)	0.0003	0.000453
(1:100)	0.0002	0.000317
(1:200)	0.002	0.000159

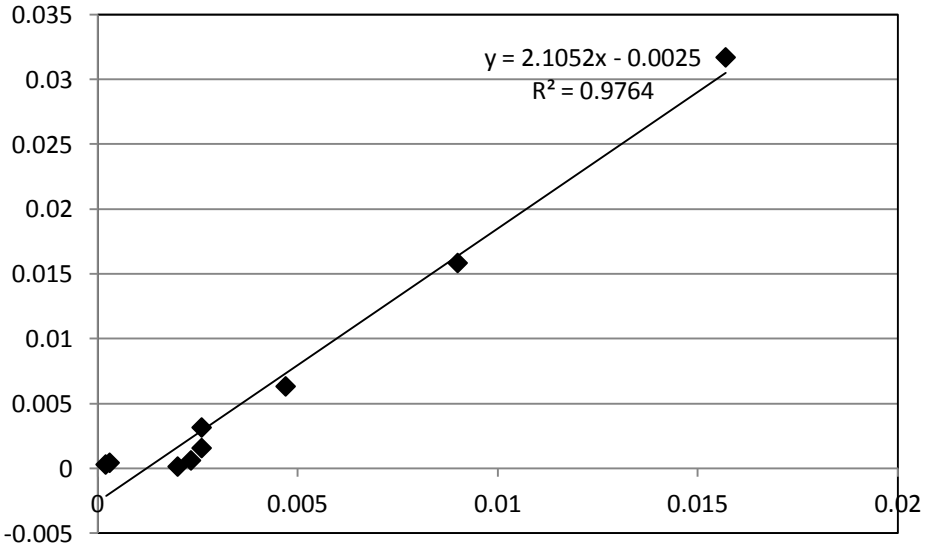


Figure A7.6: Calibration curve absorbance vs concentration *O. piliferum* [Cartapip97]

Appendix 8: Statistical analysis melanin biosynthesis inhibitors tested in artificial media (Chapter 7)

Analysis of variance fungal colonies in plates after exposure artificial media

Variate: Number of colonies

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	4		80919.	20230.	9.86	
Block.*Units* stratum						
Fungi	1		116459.	116459.	56.77	<.001
Exposure	1		71344.	71344.	34.78	<.001
chemical	4		106128.	26532.	12.93	<.001
Fungi.Exposure	1		32781.	32781.	15.98	<.001
Fungi.chemical	4		17082.	4270.	2.08	0.092
Exposure.chemical	4		54044.	13511.	6.59	<.001
Fungi.Exposure.chemical	4		12783.	3196.	1.56	0.195
Residual	72	(4)	147692.	2051.		
Total	95	(4)	609273.			

Message: the following units have large residuals.

Block 1 *units* 1	96.1	s.e. 38.4
Block 1 *units* 7	99.7	s.e. 38.4
Block 1 *units* 12	98.3	s.e. 38.4
Block 5 *units* 5	102.3	s.e. 38.4

Tables of means

Variate: Colonies

Grand mean 118.2

Fungi	A pull	Clad
	84.1	152.3

Exposure	uv	v
	91.5	144.9

chemical	Carp	Cer	Control	Quin	Tricy
	98.4	106.9	157.7	74.1	154.0

Fungi	Exposure	uv	v
A pull		75.5	92.7
Clad		107.5	197.2

Fungi	chemical	Carp	Cer	Control	Quin	Tricy
A pull		66.8	87.1	99.9	48.7	117.9
Clad		130.0	126.6	215.5	99.5	190.1

Exposure	chemical	Carp	Cer	Control	Quin	Tricy
uv		53.3	87.7	160.0	12.8	143.7
v		143.5	126.0	155.4	135.4	164.3

Fungi	Exposure	chemical	Carp	Cer	Control	Quin	Tricy
A pull	uv		50.4	95.0	110.4	13.0	108.6
	v		83.2	79.2	89.4	84.4	127.2
Clad	uv		56.2	80.4	209.6	12.6	178.8
	v		203.8	172.8	221.4	186.4	201.4

Standard errors of differences of means

Table	Fungi	Exposure	chemical	Fungi Exposure
rep.	50	50	20	25
d.f.	72	72	72	72
s.e.d.	9.06	9.06	14.32	12.81

Table	Fungi chemical	Exposure chemical	Fungi Exposure chemical
rep.	10	10	5
d.f.	72	72	72
s.e.d.	20.25	20.25	28.64

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Fungi	Exposure	chemical	Fungi Exposure
rep.	50	50	20	25
d.f.	72	72	72	72
l.s.d.	18.06	18.06	28.55	25.54

Table	Fungi chemical	Exposure chemical	Fungi Exposure chemical
rep.	10	10	5
d.f.	72	72	72
l.s.d.	40.38	40.38	57.10

(Not adjusted for missing values)

Appendix 9: Statistical analysis melanin biosynthesis inhibitors tested in wood veneers (Chapter 7)

Analysis of variance color differences veneers inoculated

Variate: Color difference (Delta E) fungi inoculated veneers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	4	10.134	2.534	2.35	
Block.*Units* stratum					
exposure	1	2.243	2.243	2.08	0.160
Chemical	1	16.814	16.814	15.59	<.001
Concentration	1	1.379	1.379	1.28	0.268
exposure.Chemical	1	0.490	0.490	0.45	0.506
exposure.Concentration	1	0.975	0.975	0.90	0.350
Chemical.Concentration	1	0.174	0.174	0.16	0.691
exposure.Chemical.Concentration	1	0.013	0.013	0.01	0.913
Residual	28	30.192	1.078		
Total	39	62.414			

Message: the following units have large residuals.

Block 1 *units* 8	-1.97	s.e. 0.87
Block 2 *units* 6	-1.85	s.e. 0.87

Tables of means

Variate: Delta_E_F

Grand mean 2.10

exposure	UV	V
	1.87	2.34

Chemical	Carp	Qui
	2.75	1.45

Concentration	3000	6000
	1.92	2.29

exposure	Chemical	Carp	Qui
UV		2.40	1.33
V		3.10	1.58

exposure	Concentration	3000	6000
UV		1.52	2.21
V		2.31	2.37

Chemical	Concentration	3000	6000
Carp		2.50	3.00
Qui		1.34	1.57

	Chemical	Carp		Qui	
exposure	Concentration	3000	6000	3000	6000
UV		1.98	2.83	1.07	1.59
V		3.02	3.18	1.60	1.56

Standard errors of differences of means

Table	exposure	Chemical	Concentration	exposure Chemical
rep.	20	20	20	10
d.f.	28	28	28	28
s.e.d.	0.328	0.328	0.328	0.464

Table	exposure Concentration	Chemical Concentration	exposure Chemical Concentration
rep.	10	10	5
d.f.	28	28	28
s.e.d.	0.464	0.464	0.657

Analysis of variance color differences veneers inoculated vs not inoculated

Variate: Color differences (Delta E) veneers inoculated vs not inoculated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	4	30.194	7.548	6.09	
Block.*Units* stratum					
exposure	1	1.619	1.619	1.31	0.259
Chemical	1	54.507	54.507	43.96	<.001
Concentration	2	23.633	11.817	9.53	<.001
exposure.Chemical	1	0.688	0.688	0.55	0.460
exposure.Concentration	2	5.527	2.764	2.23	0.120
Chemical.Concentration	2	26.156	13.078	10.55	<.001
exposure.Chemical.Concentration	2	1.410	0.705	0.57	0.570
Residual	44	54.560	1.240		
Total	59	198.295			

Tables of means

Variate: Delta_E_F_vs_NF

Grand mean 3.12

exposure	UV	V
	3.29	2.96

Chemical	Carp	Qui
	2.17	4.07

Concentration	0	3000	6000
	4.00	2.76	2.60

exposure	Chemical	Carp	Qui
UV		2.23	4.35
V		2.11	3.80

exposure	Concentration	0	3000	6000
UV		3.98	3.35	2.53
V		4.03	2.16	2.68

Chemical	Concentration	0	3000	6000
Carp		3.87	1.78	0.85
Qui		4.14	3.73	4.36

	Chemical	Carp			Qui		
exposure	Concentration	0	3000	6000	0	3000	6000
UV		3.63	2.16	0.88	4.33	4.53	4.17
V		4.11	1.41	0.82	3.95	2.92	4.54

Standard errors of differences of means

Table	exposure	Chemical	Concentration	exposure Chemical
rep.	30	30	20	15
d.f.	44	44	44	44
s.e.d.	0.288	0.288	0.352	0.407

Table	exposure Concentration	Chemical Concentration	exposure Chemical Concentration
rep.	10	10	5
d.f.	44	44	44
s.e.d.	0.498	0.498	0.704

Least significant differences of means (5% level)

Table	exposure	Chemical	Concentration	exposure Chemical
rep.	30	30	20	15
d.f.	44	44	44	44
l.s.d.	0.579	0.579	0.710	0.819

Table	exposure Concentration	Chemical Concentration	exposure Chemical Concentration
rep.	10	10	5
d.f.	44	44	44
l.s.d.	1.004	1.004	1.419

Analysis of variance color differences veneers not inoculated

Variate: Color differences (delta E) veneer not inoculated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	4	1.0189	0.2547	0.33	
Block.*Units* stratum					
exposure	1	0.2412	0.2412	0.31	0.583
Chemical	1	0.0147	0.0147	0.02	0.892
Concentration	1	0.2225	0.2225	0.28	0.598
exposure.Chemical	1	0.0231	0.0231	0.03	0.865
exposure.Concentration	1	0.0914	0.0914	0.12	0.735
Chemical.Concentration	1	0.0470	0.0470	0.06	0.808
exposure.Chemical.Concentration	1	0.0081	0.0081	0.01	0.920
Residual	28	21.9259	0.7831		
Total	39	23.5928			

Message: the following units have large residuals.

Block 1 *units* 2	-1.63	s.e. 0.74
Block 1 *units* 5	1.87	s.e. 0.74
Block 1 *units* 7	1.82	s.e. 0.74

Tables of means

Variate: Delta_E_NF

Grand mean 1.33

exposure	UV	V
	1.41	1.26

Chemical	Carp	Qui
	1.35	1.31

Concentration	3000	6000
	1.26	1.41

exposure	Chemical	Carp	Qui
UV		1.41	1.42
V		1.30	1.21

exposure	Concentration	3000	6000
UV		1.29	1.53
V		1.23	1.28

Chemical	Concentration	3000	6000
Carp		1.31	1.39
Qui		1.20	1.42

	Chemical	Carp		Qui	
exposure	Concentration	3000	6000	3000	6000
UV		1.33	1.48	1.24	1.59
V		1.29	1.30	1.17	1.26

Standard errors of differences of means

Table	exposure	Chemical	Concentration	exposure Chemical
rep.	20	20	20	10
d.f.	28	28	28	28
s.e.d.	0.280	0.280	0.280	0.396

Table	exposure Concentration	Chemical Concentration	exposure Chemical Concentration
rep.	10	10	5
d.f.	28	28	28
s.e.d.	0.396	0.396	0.560

Analysis of variance fungal stain ratio

Variate: natural logarithm ratio of fungal stains (lnrat)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	4		0.81069	0.20267	4.11	
Block.*Units* stratum						
exposure	1		0.03632	0.03632	0.74	0.398
Chemical	1		7.97230	7.97230	161.75	<.001
Concentration_ppm	1		0.02397	0.02397	0.49	0.492
exposure.Chemical	1		0.13816	0.13816	2.80	0.106
exposure.Concentration_ppm	1		0.01718	0.01718	0.35	0.560
Chemical.Concentration_ppm	1		0.09800	0.09800	1.99	0.170
exposure.Chemical.Concentration_ppm	1		0.00936	0.00936	0.19	0.666
Residual	27	(1)	1.33073	0.04929		
Total	38	(1)	10.23561			

Message: the following units have large residuals.

Block 2 *units* 8	0.398	s.e.	0.182
Block 4 *units* 7	-0.513	s.e.	0.182

Tables of means

Variate: lnrat

Grand mean 0.597

exposure	UV	V
	0.627	0.567

Chemical	Carp	Qui
	0.151	1.043

Concentration_ppm	3000	6000
	0.572	0.621

exposure	Chemical	Carp	Qui
UV		0.239	1.015
V		0.062	1.072

exposure	Concentration_ppm	3000	6000
UV		0.623	0.631
V		0.522	0.612

Chemical	Concentration_ppm	3000	6000
Carp		0.176	0.125
Qui		0.969	1.117

	Chemical	Carp		Qui	
exposure	Concentration_ppm	3000	6000	3000	6000
UV		0.270	0.209	0.977	1.053
V		0.081	0.042	0.962	1.182

Standard errors of differences of means

Table	exposure	ChemicalConcentration_ppm		exposure Chemical
rep.	20	20	20	10
d.f.	27	27	27	27
s.e.d.	0.0702	0.0702	0.0702	0.0993

Table	exposure Concentration_ppm	Chemical Concentration_ppm	exposure Chemical Concentration_ppm
rep.	10	10	5
d.f.	27	27	27
s.e.d.	0.0993	0.0993	0.1404

(Not adjusted for missing values)