1-1-2017

Assessment of Volatile Metabolites for In Situ Detection of Fungal Decay of Wood

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Assessment of volatile metabolites for *in situ* detection of fungal decay of wood

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Sustainable Bioproducts
in the Department of Sustainable Bioproducts

Mississippi State, Mississippi

August 2017

Assessment of volatile metabolites for *in situ* detection of fungal decay of wood

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Although incipient fungal decay of wood may be difficult to detect early, it causes a significant decrease in wood strength. Developing a reliable method of decay identification to overcome wood replacement costs by non-destructive methods is necessary. This study investigates a possibility of identifying fungal volatile organic compounds (VOCs) as means of fungal detection using solid phase micro-extraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS).

Volatile emissions from two brown rot (*Gloeophyllum trabeum* and *Postia placenta*) and two white rot (*Trametes versicolor* and *Irpex lacteus)* fungi on pine and aspen and their profiles related to wood mechanical strength and mass loss were investigated over 12 weeks. Principal component analysis of VOCs spectra differentiated volatiles from decayed and sound wood. Volatiles from two fungal species revealed distinct patterns of early and late degradation stages. SPME combined with GC-MS showed promissing results for non-destructive identification of incipient decay in wood structures.

ACKNOWLEDGEMENTS

First, I would like to thank my research adviser, Dr. Dragica Jeremic Nikolic, for providing me the opportunity to conduct research at the Department of Sustainable Bioproducts. Her profound knowledge, constant encouragement, and infinite patience have successfully guided me through these years. I learned a lot from her about research, how to tackle problems and develop techniques. I also gratefully acknowledge the advice and support I got from my committee members Dr. Barnes, Dr. Zhang, and especially Dr. Ingram.

I would especially like to thank Dr. Rubin Shmulsky for his great support as the department head.

I would like to thank my parents, and my brothers, for making me who I am today. Also, I would always appreciate the endless support from my husband Ashkan being with me and helping me in many ways during my studies. This dissertation is dedicated to all of you.

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CHAPTER I

INTRODUCTION

1.1 Wood

Wood is a remarkable material used by humans from ancient times. As a natural resource, it has been used as one of the major construction materials and has become very important in many other industries (Hunt and Garratt 1953). The list of wood applications is very long. High strength per unit weight, workability, relatively low cost, high energy content, aesthetic value, and sustainability make it suitable for many products (Panshin and Zeeuw 1964, Zabel and Morrell 1992). Lumber is used for building construction and furniture manufacturing, timber for mine supports and bridge construction, railroad ties, utility poles, and other products. In addition to solid wood, wood-engineered composites are also of major importance in building industry, and have found broad application for other products, as well. For example, wood is fabricated into veneer and then glued to produce plywood that is used for boat keels, aircraft carrier decking, and helicopter propellers. Wood serves as a raw material for many industries with paper and paperboard production being one of the largest. However, wood has a disadvantage that limits its use. It is degradable under certain conditions. Although weathering, insects, and marine organisms can also cause deterioration of wood, fungi are the major wood decomposers (Hunt and Garratt 1953). In spite of the fact that wood decaying fungi are essential for

natural cycling of matter in the forests (Lonsdale *et al.* 2008), they pose significant problems for construction material. Restoration and decay prevention of wooden structures require a great amount of time and money every year (Hunt and Garratt 1953, Lloyd and Singh 1994). About 10% of the timber cut in the United States is used for replacement of decayed wood annually. An early and reliable identification of decay by a non-destructive method is important for reducing maintenance and replacement costs (Hunt and Garratt 1953).

1.1.1 Wood classification

Trees are classified into two broad classes based on the botanical and anatomical charactristics: hardwoods and softwoods. The names may be misleading since some softwoods are harder than some hardwoods and vice versa.

Botanically, hardwoods are angiosperms, meaning plants having seeds in a closed carpel. Anatomically, hardwoods have a larger variety of cells, mainly tracheid and vessel elements which transport water or nutrients through the stem. They have broad leaves that fall in autumn and winter.

Botanically, softwoods are gymnosperms or conifers, meaning the seeds are not enclosed in a carpel, but they develop either on the surface of scales or leaves. Anatomically, softwoods have more uniform cells, mainly tracheids. Most have evergreen leaves, but there are some exceptions, such as bald cypress and larches that lose their needles during fall or winter. Douglas firs, pines, cedars, and hemlocks are some of the examples of softwoods and ash, aspen, elm and cottonwood are examples of hardwood trees (Hoadley 1990, Wiedenhoeft 2010).

1.1.2 Structure of wood

On a macroscopic level, features visible on a cross section of a tree include 1) bark, 2) cambium, 3) sapwood, 4) heartwood, and 5) pith.

A basic unit of the wood structure is a wood cell. Wood contains various kinds of cells that are produced in the cambium by cell division. While most of the cells in wood are elongated in a direction parallel to the tree growth, there is a small number of cells, less than 10% of the volume of wood, which are positioned perpendicularly to the tree growth. Living cells have protoplasm inside a plasma membrane. Newly divided cells are different in sizes and shape, and serve various functions in the tree. Parenchyma cells stay alive for years fulfilling metabolic functions such as carbohydrate storage in the growing stem of the tree. However, most wood cells in a tree die and lose their protoplasm. These non-living cells provide mechanical support for the tree (Hoadley 1990).

1.1.3 Chemical composition of wood

Wood material consists of cellulose and hemicelluloses (carbohydrates), and lignin as major structural polymers, with small amounts of minor organic i.e. extractives, and inorganic components, ash. Lignin makes up 18–35% and carbohydrates 65–75% of the wood structure. Extractives and ash usually account for 4–10% of wood constituents. On average, wood has an elemental composition of about 50% carbon, 6% hydrogen, 44% oxygen, and trace amounts of several metal ions (Pettersen 1984). The amount of lignin in angiosperms is commonly lower than gymnosperms.

There are enormous differences in chemical composition of various wood species. Table 1.1 shows the amounts of lignin and major monosaccharides of cellulose, and most abundant hemicelluloses - glucuronoxylan in hardwoods and galactoglucomannans in

softwoods (Eriksson *et al.* 1990). In addition, different types of cells have different composition among wood species. Hardwoods and softwoods also contain different types of lignin (Saka and Goring 1985). Hardwood lignin is composed of syringyl and guaiacyl units, while softwoods contain mainly guaiacyl lignin.

Table 1.1 Percent lignin and wood sugar residues in sapwood of various tree species

Species	Lignin	Glucose	Xylose	Mannose
Angiosperms				
Acacia koa	27.3	45.6	18.6	1.7
Acer saccharum	24.4	46.2	17.2	2.4
Alnus rubra	24.6	47.0	17.6	0.4
Betula papyrifera	19.0	44.9	24.3	2.1
B.verrucosa	21.7	35.1	20.7	0.9
Populous tremuloides	22.0	46.2	18.9	1.6
Quercus rubra	24.5	41.6	23.5	3.0
Tilia Americana	21.5	43.9	16.1	0.3
Ulmus americana	23.6	55.8	16.0	3.1
Gymnosperms				
Abies balsamea	29.1	46.6	5.6	11.7
Picea abies	27.1	41.6	5.2	13.6
P. mariana	26.6	49.0	7.3	13.8
Pinus banksiana	29.9	44.6	8.4	10.0
P. resinosa	27.9	44.9	8.4	12.3
P. strobus	28.1	48.2	6.0	15.6
P. sylvestris	30.0	38.3	6.5	11.1
Tsuga canadensis	32.3	47.9	4.2	13.8

Adopted from Eriksson *et al.* (1990)

1.1.4 Mechanical properties of wood

Mechanical properties of wood characterize its ability to resist applied external forces. These properties define the use of wood for structural applications. As an orthotropic material, wood has different mechanical properties in longitudinal, radial and tangential direction. The longitudinal axis (L) is parallel to the fiber direction (grain); the radial axis (R) is in the bark-to-pith direction, and the tangential axis (T) is tangential to the growth rings. The most important mechanical properties of wood are elasticity, compressive and crushing strength, tensile strength, shearing strength, and bending strength. Mechanical strength gradually decreases with increasing moisture content until the cell wall

is saturated. Mechanical properties of wood do not change significantly above the fiber saturation point (Siau 1984, Viitanen and Ritschkoff 1991).

1.1.4.1 Fundamental considerations and definitions

The behavior of a material under external forces is explained through the relationship of stress and strain. Stress is a distributed force causing a deformation and distortion on the material, known as strain.

A unit stress is calculated as force over the unit of area of the sample, as per Equation 1.1.

$$\sigma = \frac{P}{A} \tag{1. 1}$$

Where:

σ – Unit stress, Pa

P – Force, N

A – Area, m^2

A unit strain is a strain per unit of length of the sample.

$$\varepsilon = \frac{\Delta l}{l} \tag{1. 2}$$

Where:

ε – unit strain – unit-less

Δl – change in length, deformation (m)

l – initial length (m)

Elasticity is an intrinsic property of a material to recover its size or shape after a load is removed. Wood is not fully elastic, but residual deformations usually recover over

time. **Elastic properties** of wood are characterized by measurements of modulus of elasticity (MOE), modulus of rigidity (MOR) and Poisson's ratio (μ).

Modulus of elasticity (MOE), also known as Young's modulus, is tested in longitudinal, radial, and tangential directions, and it is usually calculated from compression and bending strength tests.

$$MOE = \frac{\sigma}{\varepsilon} \tag{1.3}$$

Elasticity is related to stiffness, a property dependent upon physical dimensions of a component.

Poisson's ratio (μ), unlike the modulus of elasticity that measures deformations in the same direction of the applied load, expresses ratio of deformations normal to each other. The deformation perpendicular to the direction of the load is proportional to the deformation parallel to the direction of the load. Six Poisson's ratios are used for wood, for each combination of L, R, and T directions (μ_{LR}, μ_{RL}, μ_{LT}, μ_{TL}, μ_{RT}, and μ_{TR}).

$$\mu_{LR} = \frac{\Delta l_L}{\Delta l_R} \tag{1.4}$$

Where:

Δl_L – deformation in longitudinal direction

Δl_R – deformation in radial direction.

Modulus of rigidity (MOR) is also known as modulus of shear. Three moduli of rigidity are measured in wood for each LR, LT, and RT planes. Modulus of rigidity MOR_{LR}, for example, presents a ratio of a shear deformation in the *LR* plane caused by shear stresses in the *LT* and *RT* planes.

$$MOR_{LR} = \frac{\sigma_{LT}}{\varepsilon_{LR}} \quad (1.5)$$

Where:

σ_{LT} – unit shear stress in LT plane (N/m^2)

ε_{LR} – unit shear strain in LR plane, unitless, $\Delta l_L/l_R$

1.1.4.2 Strength properties

Tensile strength is a resistance of the material to stretching or elongation in the plane of two forces acting in the opposite direction. This action is the opposite of compression.

Compressive or crushing strength: When the force is applied across the grain, initially fibers with thinner walls collapse. Compression parallel to the grain is an important property in wood applications for columns and posts.

Shearing strength: Shear happens when wood cells slide upon the forces acting in two adjacent planes. Shear perpendicular to the grain is usually not measured, as compression perpendicular to the grain usually occurs before perpendicular cell walls break. For most applications, the most important is shear in longitudinal direction (Anonymous 1992).

Bending strength: During bending, one surface of the material is in tension while the opposite surface compresses. The tension side elongates and compressed side shortens. Between the two surfaces, there is a line or region of zero stress called the neutral axis.

Toughness**:** It is a less commonly measured property in wood characterization. Toughness is defined as energy in a single-blow impact test needed to cause complete failure of centrally loaded bending specimen.

1.2 Wood decay fungi

The decay of wooden structures causes billions of dollars in losses each year. Although the decay fungi can cause huge economic losses, they can be used in a diversity of new technologies. These include biotechnological processes for extraction of ethanol from wood, bioprotection of agricultural plants from diseases, bioremediation of soil, and various pharmaceutical applications such as the production of enzymes and vitamins. Biotechnology, i.e. the use of living organisms allows for reduced usage of chemicals and results in lower pollution of the environment and improved life quality (Eriksson *et al.* 1990).

Wood decay fungi are heterotrophic organisms which degrade wood by secreting extracellular enzymes capable of depolymerizing wood cell wall and absorbing monosugars into the hyphae (Zabel and Morrell 1992). Wood-decaying fungi need favorable conditions for growth: 1) temperature, 2) oxygen, 3) sufficient amount of moisture, and 4) suitable food supply (Hunt and Garratt 1953, Jellison and Jasalavich 2000).

Based on the ability of fungi to degrade lignin, and physical and chemical changes that occur in wood, decay fungi are classified into brown rots, white rots, and soft rots. Brown rot and white rot fungi are basidiomycetes, while soft rots mainly belong to Ascomycota phylum. In Europe and North America, most of the structural damage to the buildings occurs by brown rot fungi which usually degrade coniferous wood; white rot fungi, preferentially attacking hardwoods, are less common (Schmidt 2007). For research

purposes, biological, biochemical and chemical methods have been developed for detection and determination of the type of decay (Eaton and Hale 1993). Molecular techniques are time-consuming and labor-intensive and they cannot be used as a preliminary screening tool for processing a large number of samples due to the time involved in the process (Sankaran *et al.* 2010). The most important limitation of these methods is that it is difficult, if not impossible, to determine the extent of the damage due to decay.

1.2.1 Classification of wood decay fungi

1.2.1.1 White rot

White rot fungi transform wood into a fibroid whitish structure and they can attack both softwoods and hardwoods (Hunt and Garratt 1953). They are capable of degrading all cell walls containing lignin, in addition to their ability to degrade cellulose. Among the microorganism, the ability of white rot fungi to metabolize a large amount of lignin in wood is unique. Some species preferably degrade lignin from wood and leave pockets of white, degraded cells' cellulose, while others metabolize lignin and cellulose simultaneously (Eriksson *et al.* 1990). These fungi have been, therefore classified into selective and simultaneous white rot, respectively.

1.2.1.2 Brown rot

Brown rot fungi preferentially attack softwoods' holocellulose leaving behind a brownish cubical wood residue of lignin (Hunt and Garratt 1953). The brownish residuals often crack into cubical pieces when dry. This type of fungi depolymerizes cellulose quickly within incipient stages of wood decay (Eriksson *et al.* 1990). Mostly before decay could be visually identified, significant losses in wood strength happen in the early

stage of decay. Brown rot fungi often cause degradation and decay in living trees, timber, and wood used in structures and buildings. Since this decay causes significant strength losses in wood in service, this decay can be hazardous.

1.2.1.3 Soft rot

Soft rots are taxonomically classified in the phylum of Ascomycota. The term was coined by Savory (1954), for differentiation from Basidiomycota wood decaying fungi. In older literature, it could be found that soft rots belong to both Ascomycota and Deuteromycota subdivisions, but according to the new classification based on phylogenetic analysis, Deuteromycota fungi are placed into Ascomycota phylum. Soft rot initially appears as a soft decayed surface of the extremely wet wood. However, soft rot can establish in dry environments and in many aspects may be macroscopically similar to brown rot. Two definite types of soft rot can be distinguished on a microscopic level. Type 1 decay is characterized by longitudinal cavities formed within the secondary wall of wood cells, and Type 2 shows erosion of the entire secondary cell wall. With soft rot attack, significant losses in wood strength can occur (Eriksson *et al.* 1990).

1.2.2 Environment for wood decay development

1.2.2.1 Wood moisture and fungi relationship

Moisture in wood exists in two forms: as bound water inside the wood cell walls and as free water (in liquid form) in the lumens of the wood. Hydrophilicity, i.e. wood affinity to adsorb moisture, is a result of the presence of the hydroxyl groups mostly on the cellulose and hemicelluloses (Siau 1984, Viitanen and Ritschkoff 1991). The bound wa-

ter content in the wood is in direct correlation with the relative humidity of the surrounding air. At the relative humidity of 0%, the wood moisture content is zero. With the increase of relative humidity of surrounding air to 100%, the equilibrium moisture content gradually increases until the cell walls are saturated. This moisture content level is known as fiber saturation point (FSP), and on average, it equals to about 30%.

Fungi can develop and survive in a wide range of moisture contents (Table 1.2). Hunt and Garratt (1953) indicated that wood decay fungi propagate above wood fiber saturation point, but their development stops below 20% moisture content. Similar statements are found in other reports (Panshin and Zeeuw 1964, Findlay 1967, Scheffer and Verrall 1973, Zabel and Morrell 1992, Simpson and TenWolde 1999, Schmidt 2007). With that, kiln or air-dried wood is resistant to decay unless wetted to moisture content above the point required by fungi (Stienen *et al.* 2014). Since most fungi can propagate above FSP, the presence of free water in wood greatly increases decay susceptibility.

Table 1.2 Cardinal parameters in terms of wood moisture content (MC, %) of some indoor wood-decay fungi with respect to the colonization and decay of wood

Species	**Minimum for colonization**	**Minimum for decay**	**Optimum for decay**	**Maximum for decay**
Serpula lacrymans	21	26	45-140	240
Leucogyrophana pinastri	30	37	44-151	184
Coniophora puteana	18	22	36-210	262
Antrodia vaillantii	22	29	52-150	209
Donkioporia expansa	21	26	34-126	256
Gloeophyllum abietinum	20	22	40-208	256
Gloeophyllum sepiarium	28	30	46-207	225
Gloeophyllum trabeum	25	31	46-179	191

Huckfeldt and Schmidt (2006)

1.2.2.2 Effect of temperature on decay fungi

Wood decay fungi are able to propagate over a wide range of temperatures, but the most rapid growth is observed during the warmer and moister periods of the year. The optimum temperatures vary with the species but mostly occur between 23°C and 32°C (Hunt and Garratt 1953, Schmidt 2007). Humphrey and Siggers (1933) found that fungi could not grow below 12°C and above 40°C except water-conducting fungi, which grow best at low temperatures and cause decay in crawl spaces of houses. Moreover, very cold temperatures are not fatal to decay fungi; the fungi recover when temperature rises to levels suitable for their growth (Hunt and Garratt 1953, Zabel and Morrell 1992). In contrast, high temperatures are lethal for fungi, and their effects are related to length of exposure, and moisture content (Hubert 1924, Lindgren 1933, Hunt and Garratt 1953).

1.3 Methods of detecting and measuring wood decay

1.3.1 Laboratory testing of wood decay

1.3.1.1 Mass loss measurements

The ability of a fungus to degrade certain wood species or progression of the decay is the best described by mass loss. AWPA E10 is a standard method that describes the procedure for measuring the mass loss of wood due to fungal degradation in a soil block test (AWPA 2014).

Although a common method for decay evaluation, this method is generally limited to laboratory work because the initial, non-decayed oven-dry weight of the sample is needed. The accuracy of mass loss estimation is affected by moisture content variation, loss of preservatives, and weight of biomass grown inside the wood (Zabel and Morrell 1992, Nicholas and Crawford 2003). To overcome some of the errors due to the moisture

content variability, oven-dry weights of samples or equilibrium moisture content (EMC) of the samples before and after inoculation are used for mass loss calculation.

Mass loss is the best indicator of wood decay above 10% weight loss (Hartley 1958, Wilcox 1978). In the early stages of decay, mass loss cannot accurately determine the extent of degradation because mass losses of 2-3% represent the variation of the data introduced by the methodology.

1.3.1.2 Wood strength tests as a measurement of wood decay

Due to the changes in chemical composition of wood, there is a noticeable reduction in strength during the early stages of decay, i.e. incipient decay (Wilcox 1978, Winandy and Morrell 1993, Kim *et al.* 1996). Even small mass losses are associated with major losses of strength, especially when wood is subjected to brown rot fungi (Wilcox 1978, Winandy and Morrell 1993).

Not all wood mechanical properties are affected to the same extent during incipient decay. According to Kretschmann (2012), toughness, impact bending, and work to maximum load in bending are the most affected properties of wood during decay. Richards (1954) found that toughness decreases about 50% by the time of 1% weight loss of softwoods by both brown rot and white rot fungi. At the same study, 60-85% decrease in toughness values were reached at 10% weight loss for all tested samples (brown and white rot decay of softwoods and hardwoods). Scheffer (1936) found that in hardwoods white rot reduced static bending by 35% at 2% weight loss, about 50% at 5% weight loss, and nearly 60% at 10% weight loss. Curling *et al.* (2001) reported a 4:1 ratio of bending strength loss to weight loss during incipient stage of wood decay by a brown rot fungus. Among all mechanical properties, compression test measurements are used as a standard

test method for evaluation of extent of incipient decay (AWPA E22). Janzen (2001) indicated that with 2-3% mass loss, the compressive strength of wood was reduced by at least 20%. In another study, 10% reduction in the compressive strength was measured in wood that lost only 2% of weight (Mizumoto 1966). The advantage of a compression strength test to the mass loss measurements is also seen in a shorter test time.

It appears that incipient and the later stages of decay involve degradation of different hemicelluloses. Arabinan and galactan are lost during the initial decay stages, with progression on loss of modulus of rigidity. At the later stages, mainly mannan and xylan components are being lost (Winandy and Morrell 1993, Curling *et al.* 2002).

1.3.2 In-service wood decay inspection

Unlike laboratory test methods, in-service wood decay inspection methods are usually non-destructive. Although they are very fast, they are not as accurate as the laboratory tests. A major issue, however, is that all of the properties measured are greatly influenced by the moisture content of wood, and not necessarily the presence of decay. Detecting and monitoring decay in wood products and wooden structures remain a problem when wood is in contact with the ground or subjected to moisture.

1.3.2.1 Wood surface decay assessment

The presence of any fruiting bodies or softening in the wood surface are the first points of inspection. Natural durability rating, history of the timber, wood species, and type of preservatives also provide useful information in decay detection. This type of inspection is mostly performed by a screwdriver or another blunt tool, by pushing it into the

surface of the decayed wood and then twisting. In sound wood, unscathed, long wood fibers are lifted up. However, in decayed wood, short cells are revealed. Another method for assessment of decay is the use of "pilodyn", a hand-held, spring-loaded instrument, with a gauge that measures the depth of penetration of the pin inserted into wood (Eaton and Hale 1993).

1.3.2.2 Detection of internal decay by strength methods

Besides a visual inspection of the wood surface for the presence of decay, a range of tools and methods are available for assessment of wood decay, many of which are based on mechanical properties of wood. In the field, wood decay is usually detected by assessing the resistance of wood to drilling force. This method is mostly used for detecting decay in utility poles (Graham and Helsing 1979). The electric hand drills have been used for many years by experienced inspectors. A development of the drilling method resulted in the use of air-driven, automatic feed drills that detect areas of internal weakness by measuring changes in drill speed (Eaton and Johnstone 1989). Another method based on strength measurements relies on stress wave analysis. Acoustic techniques, electrical resistance or conductivity, and radiation methods are also used.

1.4 Volatile organic compounds

Volatile organic compounds (VOCs) include a various group of carbon-based organic compounds, generally with a molecular weight in the range of 50-200 Daltons, and a high vapor pressure at room temperatures (Pagans *et al.* 2006, Rowan 2011). A huge amount of volatile compounds exists in nature. Because of their high vapor pressure and

low molecular weight, they can readily diffuse in the gas phase through biological systems. Therefore, they may act as signaling molecules passing information within or between organisms. VOCs can serve to identify food, find mates, detect rivals and predators, or to find proper habitats (Pichersky and Gershenzon 2002, Pichersky *et al.* 2006). Due to role of VOCs in communication, they are called infochemicals or semiochemicals (Dicke and Sabelis 1988, Campos *et al.* 2010). In plants, for example, volatiles have numerous roles. Volatiles emitted from plant roots, leaves, fruits and flowers act as defensive signaling systems in systemic acquired resistance (SAR) against pests and diseases (Frost *et al.* 2008, Heil and Ton 2008, Dicke *et al.* 2009). Plant volatiles also act in defense mechanism against herbivores or in communication with other plants (Pichersky and Gershenzon 2002, Dudareva and Pichersky 2008, Dicke *et al.* 2009). Volatile production in living systems is completely dependent on interactions with environment including responses to light, stress, pollination or predation (Dicke *et al.* 2009, Schaub *et al.* 2010).

Volatiles are produced by a range of biosynthetic routes, such as amino and fatty acids, and terpenes biosynthetic pathways (Pichersky *et al.* 2006). They comprise of a broad group of chemical classes (hydrocarbons, alcohols, aldehydes, esters, aromatics, acids, amines and thiols), with different physical properties and molecular sizes (Lundström *et al.* 2003) .

1.4.1 Fungi and volatile organic compounds

Fungal VOCs are produced both during primary and secondary metabolism (Korpi *et al.* 2009, Brakhage and Schroeckh 2011). Production of volatile organic compounds by microbial species depends on specific conditions (Zechman and Labows Jr

1985, Giudici *et al.* 1990). Nearly 250 VOCs have been identified from fungi as mixtures of simple hydrocarbons, cyclohexanes, heterocycles, aldehydes, ketones, alcohols, monoterpenes, sesquiterpenes, esters, furans, phenols and other benzene derivatives, sulfur-containing compounds (thioalcohols, thioesters and their derivatives), and nitrogen-containing compounds (Splivallo *et al.* 2007, Korpi *et al.* 2009, Ortíz-Castro *et al.* 2009, Campos *et al.* 2010, Kramer and Abraham 2012).

Higher fungi also produce VOCs for protection and communication with other organisms (Rohlfs and Churchill 2011). Fungi produce VOCs for competition between species or during mating process, but many fungal VOCs are also emitted to attract or repel insects and other invertebrates (Steinke *et al.* 2002, Rowan 2011).

In the food industry, the fungal VOCs are used as a biological control against post-harvest fungal growth, in what is called "mycofumigation" (Morath *et al.* 2012). Mycofumigation is the use of volatiles produced by fungi such as *Muscodor albus* and *Muscodor roseus* for the control of other microorganisms (Zidack *et al.* 2001).

Most of the VOCs have unique odors, which humans can smell. The moldy odor of fungal VOCs are easily recognized in damp, moldy buildings and have negative effects on human health, so called "sick building syndrome" (Morey *et al.* 1997). Possible health problems caused by fungi are irritations of eyes and mucous membranes, and respiratory health problems (Gravesen 1979, Bernstein *et al.* 1983, Strachan *et al.* 1990, Yang *et al.* 1997). Eight carbon compounds such as 1-octen-3-ol (octenol) and 2-octen-1-ol are responsible for musty odor (Wessén and Schoeps 1996, Bjurman *et al.* 1997, Morey *et al.* 1997, Pasanen *et al.* 1997, Elke *et al.* 1999, Wilkins *et al.* 2000).

Many studies have indicated that the VOCs' patterns can be useful as ecological indicators or biomarkers, and used to characterize different fungal groups/species (Nilsson *et al.* 1996, Pasanen *et al.* 1997, McNeal and Herbert 2009, Polizzi *et al.* 2012, Müller *et al.* 2013). McNeal and Herbert (2009) successfully characterized the shift in microbial activity and identified the microbial groups by using VOCs patterns as bi-omarkers of a mixed community of bacteria and fungi.

In the past, little effort has been made in studying the volatile metabolites of wood-rotting basidiomycetes. However, linoleic acid, octenol (mushroom alcohol), and some ketones, such as 2-nonanone, and different fatty acids were discovered (Tressl *et al.* 1982, Rösecke *et al.* 2000, Jones *et al.* 2004, Konuma *et al.* 2015). Drimenol, linalool, citronella, geraniol are among the identified volatile terpenoid constituents from *Gloe-ophyllum odoratum* (Kahlos *et al.* 1994, Rösecke *et al.* 2000). Table 1.3 summarizes some of the VOCs derived from wood-decaying fungi.

Table 1.3 Summary of some volatile organic compounds emitted from wood-rotting basidiomycetes

Type	Compound	Fungus	Reference
alcohol	1-octen-3-ol (octenol)	*S. lacrymans* *P. sulfureus* *P. betulinus* *G. odoratum* *F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000 Wu *et al.* 2005 Jones *et al.* 2004
	(Z)-1,5-octadien-3-ol	*P. betulinus* *T. suaveolens*	Rösecke *et al.* 2000
	(Z)-2-octen-1-ol	*P. betulinus* *F. pinicola* *S. lacrimans* *C. puteana*	Rösecke *et al.* 2000 Wu *et al.* 2005 Jones *et al.* 2004
	1-octanol	*P. betulinus* *F. pinicola*	Rösecke et al. 2000 Wu et al. 2005
	octan-3-ol	*P. betulinus* *F. pinicola*	Rösecke *et al.* 2000
	2-methyl-1-propanol	*P. sulfureus*	Wu *et al.* 2005
	phenylethanol	*P. sulfureus* *S. lacrimans* *C. puteana*	Wu *et al.* 2005 Rösecke *et al.* 2000 Jones *et al.* 2004
	1-butanol	*P. sulfureus*	Wu *et al.* 2005

Table 1.3 (Continued)

Type	Compound	Fungus	Reference
ester	hexadecanoic acid methyl ester	*S. lacrimans*	Jones *et al.* 2004
	(Z)-9-hexadecenoic acid methyl ester	*C. puteana*	
	(Z)-9-octadecenoic acid methyl ester	*P. sulfureus*	Wu *et al.* 2005
	(Z,Z)-9,12-octadecadienoic acid methyl ester pentadecanoic acid methyl ester 2-methylbutanoic acid methyl ester methylbenzoate isobutyl acetate	*C. puteana*	Jones *et al.* 2004
ketone	1-octen-3-one	*P. sulfureus*	Jones et al. 2004
	3-decanone 3-undecanone 3-dodecanone	*F. pinicola*	Rösecke *et al.* 2000
	2-butanone	*S. lacrimans*	Jones *et al.* 2004
	1-octanone	*C. puteana*	Konuma *et al.* 2015
	3-methyl-2-butanone	*F. palustris*	
alkane	hexadecane	*F. palustris*	Konuma *et al.* 2015
	heptadecane	*P. sulfureus*	Wu *et al.* 2005
	2-methylbutane	*F. pinicola*	Rösecke *et al.* 2000
	(Z)-biformene	*F. palustris*	Konuma *et al.* 2015
	abietatriene		
	7,13-abietadiene	*F. pinicola*	Rösecke *et al.* 2000

Table 1.3 (Continued)

Type	Compound	Fungus	Reference
Terpene	3-thujene	*F. palustris*	Konuma *et al.* 2015
	o-cymene	*F. pinicola*	Rösecke *et al.* 2000
	6-protoilludene	*F. pinicola*	Rösecke *et al.* 2000 Jones *et al.* 2004
	sativene		
	longifolene		
	α-barbatene		
	β-caryophyllene		
	β-copaene		
	β-barbatene		
	(E)-β-farnesene		
	γ-muurolene		
	α-pinene		
	camphene		
	camphor		
	α-cubebene		
	α-longipinene		
	α-ylangene		
	α-copaene		
	geranial	*G. odoratum*	Rösecke *et al.* 2000
	daucene	*P. betulinus*	
	trans-nerolidol drimenol	*S. lacrymans*	
	isobazzanene	*F. pinicola*	Rösecke et al. 2000
	(+)-β-barbatene		
	cadina-1(6),4-diene		
	β-chamigrene		
	selina-4, 11-diene		
	α-cuprenene		
	α-chamigrene		
	β-bazzanene		
	pentalenene		
	α-cubebene		
	(S)-(y)-daucene		
	β-cubene		
	β-elemene		
	thujopsene		
	(+)α-barbatene		
	D-cadinene	*P. betulinus*	Rösecke *et al.* 2000
		F. pinicola	Zeringue *et al.* 1993

Table 1.3 (Continued)

Type	Compound	Fungus	Reference
Terpene	linalool	*P. betulinus*	Rösecke *et al.* 2000
	α-terpineol	*G. odoratum*	
	limonene	*F. palustris* *F. pinicola*	Rösecke *et al.* 2000 Konuma *et al.* 2015
	phelandren	*F. palustris*	Konuma *et al.* 2015
	S-α-pinene	*P. sylvestris*	Jones *et al.* 2004
	3-carene	*P. betulinus*	Rösecke *et al.* 2000
	trans-calamenene	*F. pinicola*	Rösecke *et al.* 2000
	β-calacorene	*F. pinicola*	Jones *et al.* 2004
	trans-nerolidol	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	1-epi-cubenol	*F. pinicola*	Rösecke *et al.* 2000
	α-cadinol	*F. pinicola*	*Rösecke et al. 2000*
	cambrene α	*F. pinicola*	*Rösecke et al. 2000*
		T. suaveolens	
aldehyde	n-Heptanal	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	Benzaldehyde	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	Phenylacetaldehyde	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	hexanal	*P. sulfureus*	Wu *et al.* 2005
	octanal	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	nonanal	*F. pinicola* *T. suaveolens*	Rösecke *et al.* 2000
	phenylacetaldehyde 2-methylbutanal 3-methylbutanal	*P. sulfureus* *S. lacrymans* *C. puteana* *P. sulfureus*	Wu *et al.* 2005 Jones *et al.* 2004

1.4.2 Volatile terpenes and terpenoids from fungi

Terpenes are a large and diverse class of organic compounds with a broad structural diversity, produced by fungi, plants, and by some insects. Their chemical formula is $(C_5H_8)_n$, indicating that their building unit is isoprene (C_5H_8), which makes them also known as isoprenes.

Monoterpenes ($C_{10}H_{16}$) consist of two isoprene units, and they can appear in a linear or cyclic form. Alloocimene is an example of an aliphatic monoterpene, while limonene, α-pinene, and β-pinene are all examples of aromatic monoterpenes, as the two-isoprene units can be arranged differently.

Sesquiterpenes contain three isoprene units ($C_{15}H_{24}$). Diterpenes, sesterterpenes, and triterpenes have four, five, and six isoprene units, respectively, and polyterpenes more units.

Oxygenated forms of terpenes, with a methyl group substituted with O are called terpenoids. They have many applications in the medical field or in soap and perfume formulations (Merfort 2002).

Volatile sesquiterpenes from wood decaying fungi in the subkingdom Dikarya (higher fungi), have been reported (Abraham 2001, Hibbett *et al.* 2007). Polizzi *et al.* (2012) proved that emitted sesquiterpenes could be used as biomarkers of specific fungi in water-damaged buildings. In comparison to other organic compounds, sesquiterpenes are not very volatile and they are highly reactive. Sesquiterpenes can be lost or artificially enhanced in case of inappropriate collection methods. New analytical methods can identify and recognize many of them in the environment (Komenda *et al.* 2001, Merfort 2002, Helmig *et al.* 2003, Helmig *et al.* 2004, Pollmann *et al.* 2005, Tholl *et al.* 2005, Duhl

2008). Volatile terpenes from fungi were for the first time studied in 1963 (Sprecher 1963).

1.4.3 Fungal VOCs collection and detection

Recently, the focus of decay detection has shifted to an examination of volatile compounds produced during the degradation of materials. Utilization of VOCs pattern and presence is widely used in some fields. For example, one of the biological control strategies in agriculture to prevent the plant pathogen growth is the detection of fungal VOCs (Morath *et al.* 2012).

Since VOCs vary in chemical structures, polarities, and physical and chemical properties, the extraction efficiency of these volatiles differs and their detection is highly dependent on the sampling methods. There is no single analytical technique that gives a complete profile of all of the volatiles, and it seems that a combination of analytical methods is the best option.

The critical step is the capture of the various VOCs. There are two primary approaches used to collect and concentrate volatile metabolites from a sample: direct extraction of volatiles from the surrounding air around the sample (headspace), or solvent extraction of the volatiles from the samples with further purification of volatiles from non-volatile materials.

Solvent extraction methods include liquid-liquid extraction (LLE), steam distillation (SD), simultaneous distillation extraction (SDE), and supercritical fluid extraction (SFE). Direct extraction of volatiles is performed by the purge and trap (P&T) methods, and solid phase micro-extraction (SPME). Purge and trap methods involve use of a solid

adsorbent, such as graphite, Tenax® carbon fiber or silica gel. Among all of these methods, LLE, SD and SDE are conventional methods that involve long extraction times, use of huge amount of solvents, complicated procedures, and multiple steps. An additional disadvantage of thermal extraction is that some of the unstable volatiles such as alkenes, esters, and some unsaturated VOCs may be thermally decomposed (Kimbaris *et al.* 2006).

Headspace sampling, *i.e*. purge and trap methods, and solid phase micro-extraction (SPME) are of interest since they are simpler and environmentally friendly sampling techniques for assessment of VOCs.

1.4.3.1 Headspace methods for volatile collection

Universally used methods to collect or concentrate volatiles from the headspace of samples include static headspace sampling and dynamic (*i.e*. P&T) techniques (Qualley and Dudareva 2009). In the static headspace analysis, the volatiles of the sample equilibrate with the surrounding air in an airtight container. Then, a specified volume of air is collected in a gas-tight syringe, and directly injected into a gas chromatograph. In the dynamic (P&T) headspace sampling, purified air is passed over the sample and the collected volatiles are concentrated onto a solid adsorbent material. The volatiles are then removed from the adsorbent with organic solvents (typically diethyl ether) or directly transferred to a gas chromatograph (GC) injection port at high temperature to release the volatile for thermal desorption. Thermal desorption provides higher sensitivity than desorption by an organic solvent. Headspace methods are used in a variety of studies of biological samples such as plants tissues and human skin or breath gasses (Vuckovic *et al.* 2010, Zhang and Li 2010).

1.4.3.2 Solid phase micro-extraction (SPME)

The concentrations of VOCs produced in biological samples are relatively low, therefore the first step to achieve detectable levels of volatiles is a pre-concentration. However, pre-concentration consists of multiple steps which introduce errors, and decrease the reliability and accuracy of the analysis (Kataoka and Saito 2011). In recent years, SPME has become very popular because it decreases preparation time by combining collection, concentration, and elution into one step while increasing sensitivity (Zhang and Li 2010). Besides simple use and shorter time requirements, SPME provides a high extraction capacity, and a high selectivity.

SMPE was developed by Arthur and Pawliszyn in 1990 as a rapid, solvent-less alternative to the conventional sample extraction techniques. This method was proven to be one of the cleverest inventions of sample preparation in recent years. SPME was initially used for analysis of volatiles in pharmacology, disease markers, volatile analgesic gases, and various volatiles from solid and liquid materials (Arthur and Pawliszyn 1990).

A short-fused silica fiber coated with a polymeric organic materials acts as the stationary phase of SPME (Pawliszyn 1997, Stoppacher *et al.* 2010). A syringe needle protects the fiber when inserted into the jar space above the sample, or into the injector of a gas chromatograph. The fiber is pushed out of the needle so that VOCs from the headspace above the sample are adsorbed. Afterwards the VOCs are thermally desorbed in the GC injector, as shown in Figure 1.1.

The headspace-SPME technique can be used for direct and non-invasive extraction of fungal volatiles. Consequently, it is used for monitoring fungal VOC profiles over

time and for different growth conditions (Stoppacher *et al.* 2010). Separation and detection of the collected fungal volatiles are mainly performed using gas chromatography-mass spectrometry (GC-MS), as it is a highly sensitive and robust separation technique (Rösecke *et al.* 2000, Matysik *et al.* 2009, Roze *et al.* 2012, Dickschat 2014). Once eluted off the GC column, molecules are ionized into fragments, which are then detected using their mass-to-charge ratio. Compounds are further identified using a database, where the fragment patterns are compared to these of standards contained in the library. Nonetheless, one drawback of GC-MS is a limitation in the identification of novel compounds. Another limitation is that it is not portable and albeit it is fast, it requires laboratory equipment.

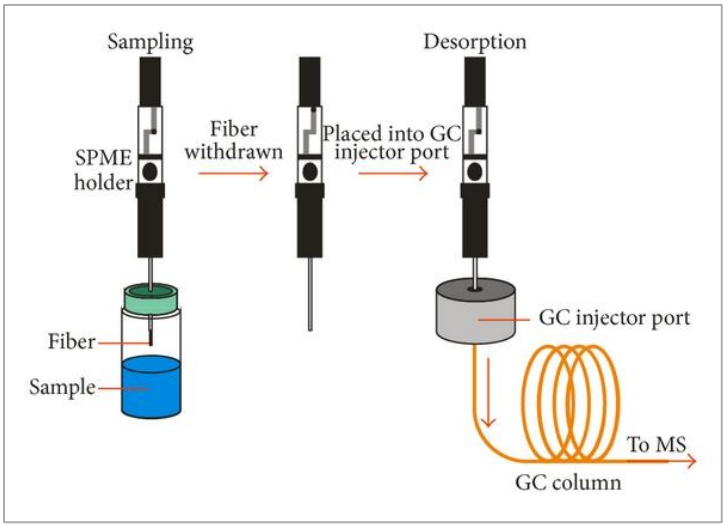


Figure 1.1 Diagram of analysis with solid phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS)

Adopted from Schmidt and Podmore (2015)

1.4.3.2.1 Principle of SPME sampling

The SPME technique includes three steps. First, the liquid or solid sample is placed inside the sampling vial and the cap of the vial is tightly closed. The sample is incubated for some time at a constant temperature. Then, the SMPE needle is injected into the vial through the septum of the cap and VOCs are adsorbed onto the fiber. VOCs can be collected by direct insertion of the SPME into the sample, or from the air above the sample (Pawliszyn 2011). Finally, the VOCs are desorbed from the fiber in a hot injector port of a GC.

1.4.3.2.2 SPME fiber type

There are four types of polymers used as SPME stationary phases: divinylbenzene (DVB), polydimethylsiloxane (PDMS), polyacrylate (PA) and polyethylene glycol (PEG). They are available in different thicknesses and used on their own or in combination with carboxen (CAR). The stationary phases differ in polarity (polar, bipolar, and non-polar) and extraction mechanism (absorbent or adsorbent), as shown in Table 1.4. For appropriate selection of the fiber coating, the polarity of the compounds and their molecular weight should be considered.

Table 1.4 Types of commercially available SPME fiber coatings and their target analytes

Analyte type	Recommended fiber
Gases and low molecular weight compounds (MW 30-225)	75/85 µm Carboxen/Polydimethylsiloxane
Volatiles (MW 60-275)	100 µm Polydimethylsiloxane
Volatiles, amines and nitro-aromatic compounds (MW 50-300)	65 µm Polydimethylsiloxane/Divinylbenzene
Polar semi-volatiles (MW 80-300)	85 µm Polyacrylate (PA)
Non-polar high molecular weight compounds (MW 125-600)	7 µm Polydimethylsiloxane
Non-polar semi-volatiles (MW 80-500)	30 µm Polydimethylsiloxane
Alcohols and polar compounds (MW 40-275)	60 µm PEG (Carbowax)
Flavor compounds: volatiles and semi-volatiles, C3-C20 (MW 40-275)	50 µm/30 µm DVB/CAR/PDMS on a StableFlex fiber
Trace compound analysis (MW 40-275)	50 µm/30 µm DVB/CAR/PDMS on a 2 cm StableFlex fiber
Amines and polar compounds (HPLC use only)	60 µm Polydimethylsilox /Divinylbenzene

CAR: Carboxen; PDMS: Polydimethylsiloxane; DVB: Divinylbenzene; HPLC: High-performance liquid chromatography; PA: Polyacrylate; PEG: polyethylene glycol (Sigma-Aldrich 2002-2006)

During sampling, analytes diffuse in and out of the stationary phase. Retention of compounds mainly depends on the polarity and thickness of the stationary phase. Smaller molecules are retained over a shorter time. Hence, thicker phases are more appropriate as they provide longer retentions (Pawliszyn 2011).

1.4.3.2.3 Extraction time and temperature

The important factors for optimization of SPME methods are extraction time and temperature. The time might be shortened by increasing temperature or by agitating aqueous solutions. High temperatures result in the higher release of compounds from the sample (Pawliszyn 2011), but, increased temperatures do not necessarily yield detection of

the new peaks, so it is important to find a balance between the temperatures and extraction times used (Cai *et al.* 2001, Wady *et al.* 2003).

1.5 Statistical analysis used in this work

For this work, two statistical methods are used: for compression strength and mass loss comparisons among samples, analysis of variance **(**ANOVA); and principal component analysis (PCA) for the spectral patterns of VOCs.

ANOVA is a statistical technique used to analyze the differences among group means and data variation of sample groups.

PCA is one of the most commonly used multivariate analysis methods for comparison of spectra patterns. PCA uses a mathematical dimension reduction method to produce new variables and replaces them with the original variables (Abdi and Williams 2010). The technique finds any hidden relationships between samples or groups, and summarizes the pattern of correlations among the observed variables. This method is used to elucidate important information from the data set, and represent them as a set of new orthogonal variables called principal components. The first principal component PC1, describes the largest difference among the samples, followed by the second, PC2, and so on. The samples on the opposite PC sides (negative and positive) of score scatter plots are recognized as having different characteristics and their difference are explained by loadings plots. Samples on the positive side of PC1, for example, are characterized by most prominent peaks on the positive side of PC1 loadings plot.

PCA has been used for VOCs spectral analysis in earlier studies. Similarly in this study, PCA was used for analyzing the volatile constituents in conifers and conifer-related wood-decaying fungi (Fäldt 2000). Wilson *et al.* (2004) used PCA to distinguish

headspace volatiles produced from plant diseases pathogenic fungi on oak sapwood. PCA has been used in the measurement of VOCs released from paper decay in libraries (Canhoto *et al.* 2004, Gibson *et al.* 2012).

1.6 Study objectives

The hypothesis of this research is that volatiles can be used as indicators of the decaying process *in situ* and the type of volatiles being produced can indicate type and stage of decay. This hypothesis is based on findings that fungal metabolites have a direct effect on the volatiles that fungi produce. Moreover, the production of VOCs is affected by the change in a substrate, type of nutrient, time of incubation, temperature, and other environmental parameters (Pasanen *et al.* 1997, Nilsson *et al.* 2004). The profiles of volatiles in the headspace of brown rot (*Fomitopsis palustris*) and white rot (*Trametes versicolor*) decayed Japanese beech have been recently published (Konuma *et al.* 2015) showing that fungi produce different volatiles depending on the type of decay, as well as on the decay stage (14-56 days). This study takes a step further to investigate whether the types of volatiles produced by decaying both softwood and hardwood species can be associated with the stage of the decay. This is done by comparing the volatiles patterns from two representative brown and two white rot fungi with the mechanical strength and mass loss in the wood.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials and instrumentation

2.1.1 Wood samples

Approximately 550 end-matched spruce pine (*Pinus glabra*) and 550 end-matched aspen (*Populus tremuloides*) sapwood wafers of 19 × 19 × 5 mm^3 (R × T × L) were sawn from four side-matched wood sticks of each species. The scheme of end-matched samples cutting pattern is shown in Figure 2.1. Samples without apparent defect were labeled, randomized as suggested in AWPA E22 standard, oven-dried at 50°C to a constant mass, and weighed. Upon drying, the samples were autoclaved and kept sterile until used. A few samples were used to estimate the average MC of the samples before inoculation with fungi, which was found to be ca. 18%.

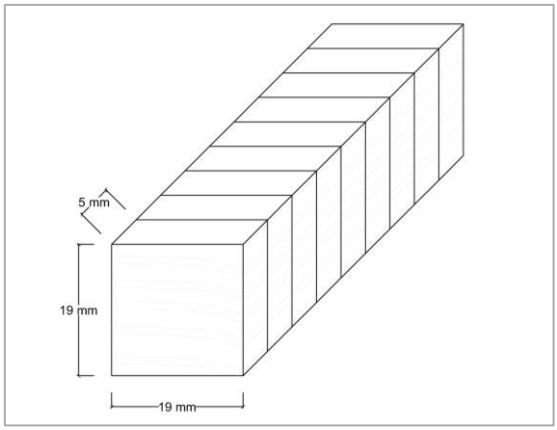


Figure 2.1 Diagram of cutting end-matched samples, 19 × 19 × 5 mm^3 (rad × tang × long)

A total of, 864 samples were exposed to fungi (4 fungi × 2 wood species × 12 weeks × 9 replicates = 864 samples). In addition, 180 pine samples were used as compression strength controls (9 replicates per fungus for each one of 5 weeks of the test). Due to insufficient amount of material, only 45 samples of aspen (9 replicates per week for 5 weeks of the test) were used as compression strength controls. While one pine stick yielded replicates for decayed and control samples for one fungus test, one control set of aspen served for all of the four fungi. Aspen control replicates were comprised of samples cut from all four sticks.

In addition, nine control replicates of each species were also used for VOCs collection.

2.1.2 Fungi

The following fungi from American Type Culture Collection were used:

Gloeophyllum trabeum (brown rot) ATCC # 11539

Postia placenta (brown rot) ATCC # 11538

Trametes versicolor (white rot) ATCC # 12679

Irpex lacteus (white rot) ATCC # 12679

2.1.3 Materials and instrumentation for VOC analysis

Pre-cleaned EPA approved jars and lids with PTFE-coated silicone septa were purchased from Cole-Parmer (Vernon Hills, IL, United States). Polycarbonate clear color tubing (1 1/4" ID× 1 1/2" OD× 1/8" wall, 72" L) was purchased from Amazon. Aluminum mesh (New York Wire Saint Gobain ADFORS Screen Wire) was purchased from Lowe's. A solid phase SPME fiber coated with 75-µm thick bi-polar Carboxen®/polydimethylsiloxane (CAR/PDMS), with needle size of 23 Ga, and a manual holder were obtained from Supelco (Bellefonte, PA, USA).

VOCs were analyzed by a Hewlett Packard (HP 5890) GC-MS system equipped with an HP 5971 mass selective detector (Hewlett Packard, Palo Alto, CA). VOCs were separated by a nonpolar Agilent DB-5MS capillary column (30m × 0.320mm, 1.0 µm film thickness) (Agilent Technologies). NIST 2005 mass spectral library was used for compound identification.

Compression strength measurements were performed by a compression instrument designed and manufactured at Mississippi State's Forest Products Laboratory.

2.2 Methods

2.2.1 Culturing fungi

Fungal medium was made by mixing 4.5 g agar, 6.0 g malt extract, 0.6 g yeast extract with 300 ml DI water (Sigma-Aldrich). Twelve flasks were autoclaved for 30 minutes and 3600 ml of media poured into 90 Petri dishes (150mm×20mm). Fungi were incubated at 27°C and 80% RH for 10 days.

According to AWPA E10-12 standard, the fungi used in the test should yield wood mass loss higher than 40% when grown on control samples. Hence, a preliminary

test was conducted to demonstrate and document the ability of the decay fungi prior to using them in the study. The results showed higher than 50% average mass loss for both wood species during a 4-week exposure for each tested fungus.

2.2.2 Inoculation of wood blocks

Inoculation of wood blocks was performed by two methods. In the first inoculation method wood samples were dipped into the solution of fungal mycelium dispersed in the mineral broth. The mycelial mass was grown on liquid media (glucose, 10.00 g; yeast extract, 5.00 g; peptone, 5.00 g; $MgSO_4$, 0.25 g; KH_2PO_4, 0.25 g; vitamin B_1, 0.05 g) in room temperature (20° C) under the light. Twenty g of mycelia was homogenized in a Waring blender set at the highest speed. Approximately 50 ml mineral salt (KH_2PO_4, 0.70 g; $MgSO_4$ $7H_2O$, 0.70 g; NH_4NO_3, 1.00 g; NaCl, 0.005 g; $FeSO_4$ $7H_2O$, 0.002 g; $ZnSO_4$ $7H_2O$, 0.002 g; $MnSO_4$ H_2O, 0.001 g; K_2HPO_4, 0.70 g / 1 liter distilled water) was added during the blending. The resulting homogenous suspension was filtered through cheesecloth. The wood blocks were inoculated in fungal suspensions overnight on a shaker (200 rpm). The inoculated wood blocks were transferred into the jars on the following day.

The second inoculation method involved placing wood samples directly onto fungal mycelia. The sterile wood blocks (108 samples) of the same species were placed in a total of nine Petri dishes containing the same fungus, 12 wood blocks per dish (Figure 2.2.). The closed Petri dishes were put in a conditioning chamber at a temperature of 28°C and relative humidity of 85% for two weeks to let mycelia cover the wood blocks.

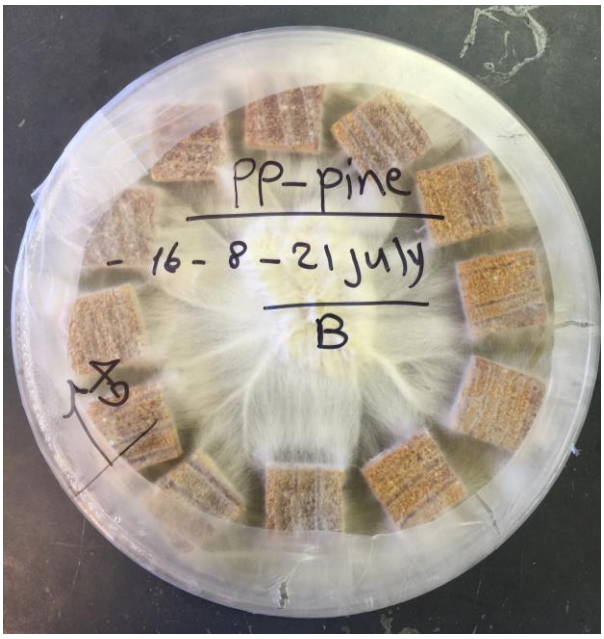


Figure 2.2 Twelve pine samples inoculated with *P. placenta* on the same plate

2.2.3 Experimental setup

The jars containing polycarbonate rings, mesh, and water were sterilized in an autoclave (Fig. 2.3). Water at the bottom of the jars provided relative necessary humidity for fungi to survive. The sterile control wood samples were placed on the mesh. Three wood samples of the same species, inoculated with the same fungus were randomly selected from different Petri dishes, cleaned of agar and transferred into one jar (Fig. 2.4). There was a total 36 jars per fungus per species. The closed jars were kept in a conditioning chamber at a temperature of 27°C and RH of about 85%. Each week for 12 weeks, volatiles were collected from 24 replicate wood-decayed jars, (four fungi, two wood species, three replicate jars), and six jars of control wood (two wood species, three replicate jars). Following the VOC examination, fungal mycelia were cleaned off the

wood samples, which were then saturated with water until compression tests were performed. The collection of the volatiles was carried out for 12 weeks.

Figure 2.3 VOC experimental setup showing aluminum mesh positioned on the polycarbonate tube ring supporting three wood replicates above sterile water

Figure 2.4 Transfer of inoculated wood blocks to the sterile jar

2.2.4 VOCs collection and analysis

The VOCs collection begun two weeks after the wood samples had been transferred into the jars to allow for a sufficient volatiles accumulation. The SPME fiber was cleaned and conditioned at 300°C for 1 hour, before use, as suggested by the manufacturer.

The jars were pre-equilibrated at 70°C in a water bath for at least 30 min before headspace volatiles were collected (Fig. 2.5). These conditions were chosen according to the preliminary results showing that at this temperature and this collection time adsorbed volatiles were well above the detection limit of the GC-MS. The collected VOCs were thermally desorbed in the injection port of the GC for 10 minutes at 250°C (Fig. 2.6). The injector temperature was maintained at 250°C. Ultrapure helium (99.999%) was used as a carrier gas at a flow rate of 1.25 ml/min. The initial oven temperature was kept at 50°C for 2 minutes and then increased to 170°C at 6°C /min. The 170°C temperature was maintained for 35 min of the analysis time. The thermally separated volatile metabolites were fragmented with an electron ionization source (70 eV), and ion masses detected.

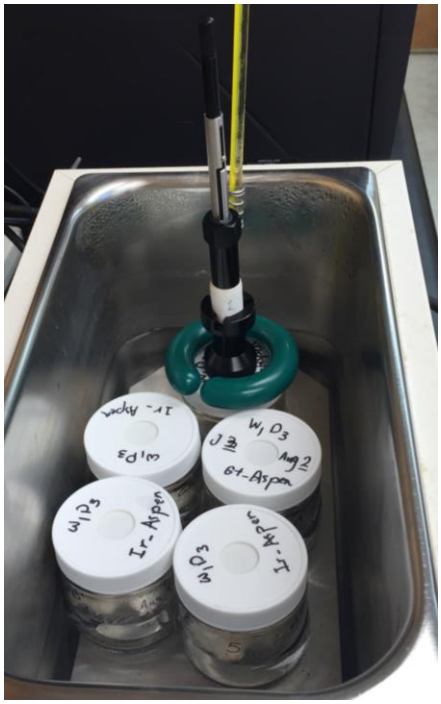


Figure 2.5 Pre-equilibration of jars at 70°C for 30 min during the VOCs collection

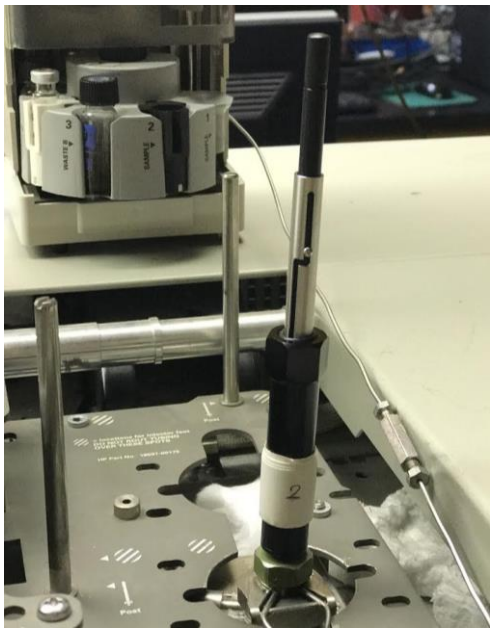


Figure 2.6 SPME inserted into the injection port of the GC-MS

2.2.5 Compression strength

As stated previously, decayed wood wafers were removed from the jars following VOCs collection, cleaned and submerged in DI water under vacuum (Fig. 2.7). Control samples were also water saturated in the same manner. It has been shown that compression strength does not change above the fiber saturation point (Fig.2.8). Before compression tests were conducted on the following day, all three dimensions of wafers were measured by an automated micrometer. A spring-loaded clamp held the samples directly under the crosshead of the compression strength instrument (Fig. 2.9).

Softwood wafers were compressed in the radial direction and hardwood wafers in the tangential direction. The surface area (mm^2) was calculated from the longitudinal and tangential, *i.e*. radial sample dimensions for softwoods and hardwoods, respectively. The wafers were compressed to 5% of the wafer dimension in the loading direction.

The compression strength device used a load cell of 45.36 kg. The compression rate was 0.5 mm/min. The crosshead movement was monitored by a linear transducer, and it was pushed against the sample until the wafer reached 5% deformation. The machine measured the load based on the amount of energy used to compress the sample. This energy was converted to grams, a stress-strain curve was automatically created and compression strength reported in g/mm^2. Once the predefined threshold (5% deformation), the transducer starts to retract in order to complete a cycle.

The effect of decay was determined by comparing compression strength of the fungi-decayed to control samples at each exposure period.

Figure 2.7 Water treatment of wood samples under vacuum before crushing tests

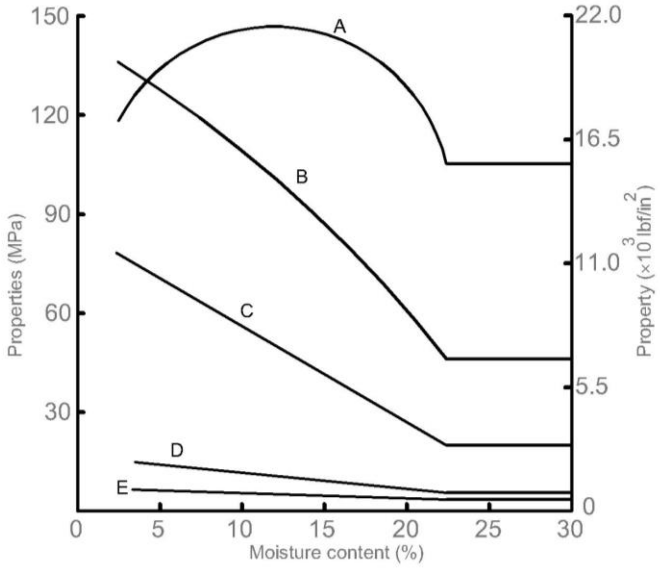


Figure 2.8 Effect of moisture content on wood strength properties. A, tension parallel to grain; B, bending; C, compression parallel to grain; D, compression perpendicular to grain; and E, tension perpendicular to the grain

(Kretschmann 2010)

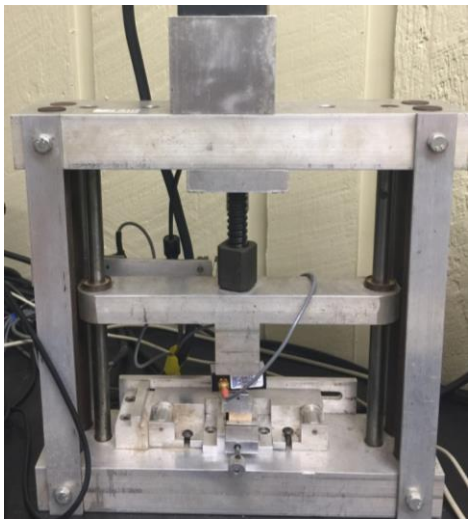


Figure 2.9 The compression strength testing instrument, showing the screw driven cross head and load plate with a specimen in place for testing

Compression strength loss was calculated for each set of samples per Eq. 2.1:

$$\Delta MOE\ (\%) = \left(\frac{MOE_1 - MOE_2}{MOE_1}\right) \times 100 \qquad (2.1)$$

MOE_1= Average MOE of control replicates measured for each week (g/mm^2)

MOE_2= Average MOE of decayed replicates measured for each week (g/mm^2)

2.2.6 Mass loss

After compression strength measurements, each wood sample was carefully brushed to remove the surface mycelium, oven dried at 50°C to constant mass and weighed. In the case of very decayed, fragile samples, some fungal biomass could not be totally removed. Average mass loss for each sample group was calculated per Eq. 2.2:

$$Mass\ loss\ (\%) = \text{avg}\left[\left(\frac{m_1 - m_2}{m_1}\right) \times 100\right] \qquad (2.2)$$

m_1= weight of oven dry sample before exposure to fungi (g)

m_2= weight of oven dry sample after exposure to fungi (g)

2.2.7 Data analysis

PCA was used to distinguish between volatiles of decayed vs control samples, and among volatiles of different fungi. In order to perform PCA, GC-MS spectra of four fungi grown on the same wood species and their controls had to be manually integrated. The GC peaks eluted from the column in a narrow time frame (± 3s), were given the same nominal retention value. All the intensities were organized based on these retention times.

PCA of volatile compounds spectra was performed using Unscrambler v.9.7 software (CAMO AS, Trondheim, Norway). PCA was used to lower the number of variables and to remove redundant information. The spectra were area normalized before the PCA was performed on mean-centered data.

Mass loss and compression strength differences between the decayed and control wood samples over time, and among different fungi were determined by ANOVA or Welch's ANOVA in case of unequal variance of data using SAS 9.4 (by SAS Institute Inc., Cary, NC, USA). Pairwise comparison was performed either by Tukey, in case of equal data variances or Games-Howell method, in case of non-homogeneous variances.

CHAPTER III

RESULTS AND DISCUSSION

3.1 General observations on fungal growth

Two method were used to obtain uniform fungal infection of wood samples: 1) blending of fungal mycelia with the mineral broth and dipping wood blocks into the solution, or 2) by placing wood samples directly onto fungal mycelia grown on plates. The former method allowed for controlled concentration of mycelia in the mineral broth, but the mineral broth negatively affected mechanical properties of control wood samples (A1, Table A.1, and Fig. A1). Despite the similar mycelial concentration solutions, the rate of fungal growth was not uniform among different fungi. *Gloeophyllum trabeum* and *Trametes versicolor* needed shorter time to initiate growth, while *Irpex lacteus* and *Postia placenta* were in general difficult to establish. The latter method showed relatively uniform growth of fungi over the wood samples (Fig. 3.1), and since the wood has not been exposed to mineral salts, this method was selected for further study. The growth of the fungi on wood upon the transfer of samples into the jars was slower than growth of the fungi grown on wood placed on soil. (A2, Table A.2).

Figure 3.1 Growth of four fungi on MYA media on pine and aspen.

Brown rots, *G. trabeum* (top, left) and *P. placenta* on (top, right) and white rots *T. versi-color* (bottom, left) and *I. lacteus* (bottom, right)

3.2 Mass loss

3.2.1 Pine

Differences in the mass loss for pine samples decayed by four types of fungi were tested by ANOVA as shown in Table 3.1. Besides significant difference in mass loss due to length of decay exposure and type of the fungi, there was a significant difference in mass loss of samples as an interaction of week and fungus effects.

Table 3.1 ANOVA results for mass loss of pine samples decayed with four types of fungi over 12 weeks

Source	Mean Square	F Value	Pr>F
Exposure (week)	2540.83524	30.38	<0.0001
fungus	719.26151	8.60	<0.0001
exposure*fun-gus	212.97824	2.55	<0.0001

Tukey's multiple comparison (Table 3.2) indicated that all fungi except *T. versicolor* showed significantly higher mass loss after eight weeks. *T. versicolor* needed 11 weeks to show significant mass losses. This observation is in agreement with the other studies showing that mass loss is not a reliable method for decay monitoring at initial stages (Wilcox 1978, Winandy and Morrell 1993, Kim *et al.* 1996). It is important to keep in mind that the mass losses above 20% in the first week of the study are actually showing results of the third week decay, as the samples were exposed to fungi in the plates for 2 weeks before transferring them into the jars. Overall, the mass loss higher than 40% at the end of the study show that experimental setup of wood decay in absence of soil is still a valid method for assessing the decaying capacity of fungi on wood samples. There was not a consistently higher or lower mass loss of one fungus in comparison to the others when compared during the same week (Figure 3.2).

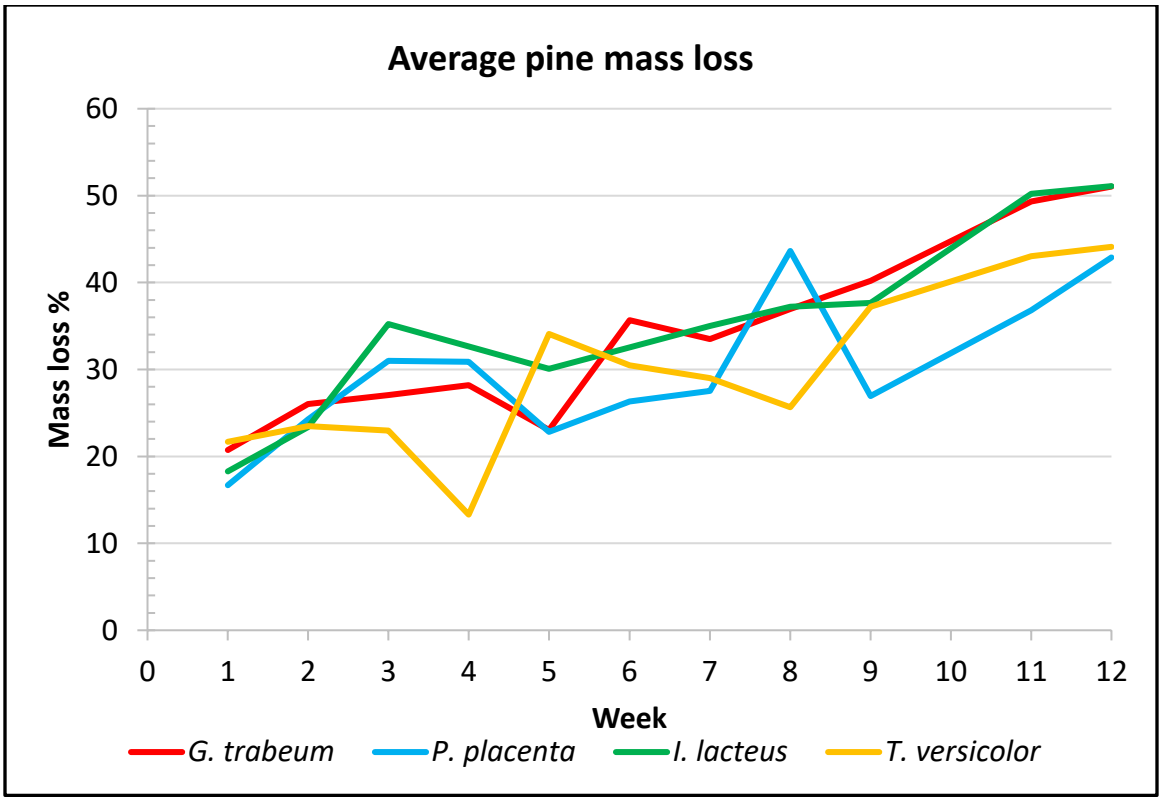


Figure 3.2 Interaction plot for mass loss of pine samples decayed with four fungi over 12 weeks

Table 3.2 Tukey pairwise comparison of pine mass loss data as affected by time and type of fungi

Expo-sure	Fungus	LSMEAN Number	Mass loss (%)		COV	Tukey
			Mean	STD		
1	*G. trabeum*	1	20.7	4.3	0.2	AB
1	*P. placenta*	2	16.7	7.1	0.4	AB
1	*I. lacteus*	3	18.3	7.6	0.4	AB
1	*T. versicolor*	4	21.7	5.5	0.3	AB
2	*G. trabeum*	5	26.0	6.0	0.2	AB
2	*P. placenta*	6	24.3	11.3	0.5	AB
2	*I. lacteus*	7	23.4	9.9	0.4	AB
2	*T. versicolor*	8	23.5	5.4	0.2	AB
3	*G. trabeum*	9	27.1	9.6	0.4	AB
3	*P. placenta*	10	31.0	6.1	0.2	B
3	*I. lacteus*	11	35.2	8.9	0.3	BCD
3	*T. versicolor*	12	23.0	9.4	0.4	AB
4	*G. trabeum*	13	28.2	11.4	0.4	AB
4	*P. placenta*	14	30.9	10.7	0.3	B
4	*I. lacteus*	15	32.7	9.1	0.3	BC
4	*T. versicolor*	16	13.3	3.0	0.2	A
5	*G. trabeum*	17	23.0	7.2	0.3	AB
5	*P. placenta*	18	22.8	8.6	0.4	AB
5	*I. lacteus*	19	30.1	9.2	0.3	AB
5	*T. versicolor*	20	34.1	8.4	0.2	BCD
6	*G. trabeum*	21	35.7	8.3	0.2	BCD
6	*P. placenta*	22	26.3	6.5	0.2	AB
6	*I. lacteus*	23	32.5	6.5	0.2	BC
6	*T. versicolor*	24	30.5	5.1	0.2	B
7	*G. trabeum*	25	33.5	8.9	0.3	BC
7	*P. placenta*	26	27.5	7.2	0.3	AB
7	*I. lacteus*	27	35.0	10.7	0.3	BCD
7	*T. versicolor*	28	29.0	11.3	0.4	AB
8	*G. trabeum*	29	37.0	5.7	0.2	BCD
8	*P. placenta*	30	43.6	10.5	0.2	BCD
8	*I. lacteus*	31	37.2	9.9	0.3	BCD
8	*T. versicolor*	32	25.7	9.2	0.4	AB
9	*G. trabeum*	33	40.2	5.1	0.1	BCD
9	*P. placenta*	34	26.9	11.5	0.4	AB
9	*I. lacteus*	35	37.6	6.6	0.2	BCD
9	*T. versicolor*	36	37.2	6.0	0.2	BCD

Table 3.2 (Continued)

Expo-sure	Fungus	LSMEAN Number	Mass loss (%)		COV	Tukey
11	*G. trabeum*	37	49.3	14.2	0.3	CD
11	*P. placenta*	38	36.8	14.2	0.4	BCD
11	*I. lacteus*	39	50.2	10.1	0.2	D
11	*T. versicolor*	40	43.1	7.2	0.2	BCD
12	*G. trabeum*	41	51.0	13.9	0.3	D
12	*P. placenta*	42	42.9	9.3	0.2	BCD
12	*I. lacteus*	43	51.1	18.2	0.4	D
12	*T. versicolor*	44	44.1	7.7	0.2	BCD

3.2.2 Aspen

Overall results from mass loss of aspen samples were similar to those of the pine samples with significant differences among fungi types, exposure time, and the interaction of the two (Table 3.3).

Table 3.3 ANOVA results for mass loss of aspen samples decayed with four types of fungi over 12 weeks

Source	Mean Square	F Value	Pr>F
exposure	3391.2	48.80	<0.0001
fungus	1271.5	18.30	<0.0001
exposure*fungus	1124.6	16.18	<0.0001

Overall, there was no consistent pattern of mass loss among the fungi when comparisons were made on a weekly basis (Fig.3.3, Table 3.4). The only noteworthy observation is that *P. placenta* caused significant increase in mass loss in comparison to *G. trabeum* and *I. lacteus* during the last two weeks of the study (Fig.3.3).

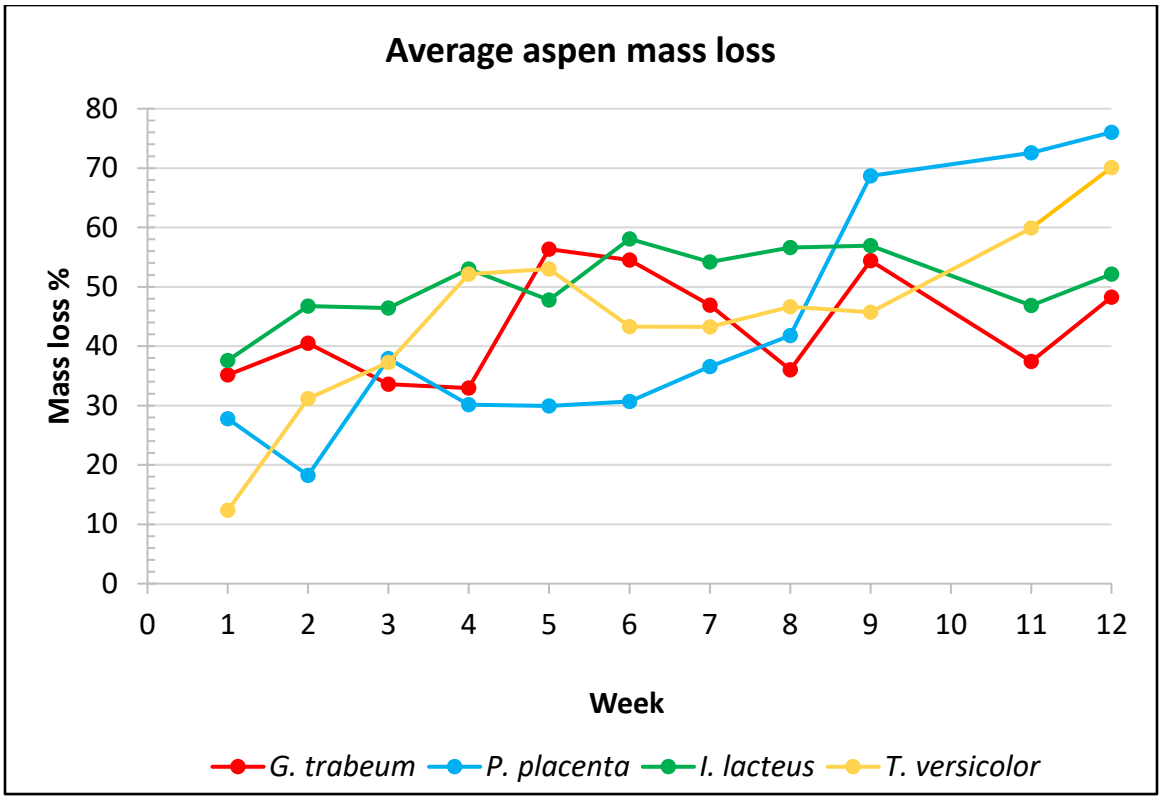


Figure 3.3 Interaction plot for mass loss of aspen samples decayed with four fungi over 12 weeks

Table 3.4 Tukey pairwise comparison of aspen mass loss data as affected by time and type of fungi

week	fungus	LSMEAN - number	massloss			TUKEY
			Mean	Std Dev	COV	
1	*G.trabeum*	1	35.2	3.9	0.11	BC
1	*P.placenta*	2	27.8	6.9	0.25	AB
1	*I. lacteus*	3	37.6	3.9	0.10	BC
1	*T.versicolor*	4	12.3	2.3	0.19	A
2	*G.trabeum*	5	40.5	4.4	0.11	BC
2	*P.placenta*	6	18.2	8.7	0.48	AB
2	*I. lacteus*	7	46.7	8.9	0.19	C
2	*T.versicolor*	8	31.2	16.3	0.52	BC
3	*G.trabeum*	9	33.6	7.7	0.23	BC
3	*P.placenta*	10	37.9	10.6	0.28	BC
3	*I. lacteus*	11	46.4	5.6	0.12	C
3	*T.versicolor*	12	37.3	11.3	0.30	BC
4	*G.trabeum*	13	32.9	6.0	0.18	BC
4	*P.placenta*	14	30.2	5.3	0.18	BC
4	*I. lacteus*	15	53.0	2.8	0.05	C
4	*T.versicolor*	16	52.2	7.1	0.14	C
5	*G.trabeum*	17	56.3	4.2	0.08	CDE
5	*P.placenta*	18	29.9	7.2	0.24	B
5	*I. lacteus*	19	47.8	3.0	0.06	C
5	*T.versicolor*	20	53.0	7.6	0.14	C
6	*G.trabeum*	21	54.5	1.8	0.03	CD
6	*P.placenta*	22	30.7	9.0	0.29	BC
6	*I. lacteus*	23	58.1	6.3	0.11	CDEF
6	*T.versicolor*	24	43.3	11.5	0.27	BC
7	*G.trabeum*	25	46.9	8.8	0.19	C
7	*P.placenta*	26	36.6	8.0	0.22	BC
7	*I. lacteus*	27	54.2	7.7	0.14	CD
7	*T.versicolor*	28	43.2	9.4	0.22	BC
8	*G.trabeum*	29	36.0	16.8	0.47	BC
8	*P.placenta*	30	41.8	5.7	0.14	BC
8	*I. lacteus*	31	56.6	8.1	0.14	CDE
8	*T.versicolor*	32	46.6	5.8	0.12	C
9	*G.trabeum*	33	54.4	6.3	0.12	CD
9	*P.placenta*	34	68.7	5.9	0.09	DEFG
9	*I. lacteus*	35	56.9	9.1	0.16	CDE
9	*T.versicolor*	36	45.7	4.5	0.10	C

Table 3.4 (Continued)

Exposure	Fungus	LSMEAN NUMBER	Mass loss			Tukey
11	*G.trabeum*	37	37.4	12.5	0.33	BC
11	*P.placenta*	38	72.6	2.3	0.03	FG
11	*I. lacteus*	39	46.9	7.8	0.17	C
11	*T.versicolor*	40	59.9	14.5	0.24	CDEF
12	*G.trabeum*	41	48.2	12.7	0.26	C
12	*P.placenta*	42	76.0	1.1	0.01	G
12	*I. lacteus*	43	52.2	12.5	0.24	C
12	*T.versicolor*	44	70.1	7.8	0.11	EFG

3.3 Compression test

3.3.1 Spruce pine

Compression test results showed unequal variances, therefore the ANOVA was performed according to Welch's method, and Games-Howell's pairwise comparisons were performed. According to Welch's ANOVA (Table 3.5), pine control samples had average 5-week MOE to 5% deformation significantly higher than the decayed samples. The average MOE value of control samples was 124.5±37.9 g/mm^2 (1.22 MPa) vs 26.8±21.5 g/mm^2 (0.26 MPa) for decayed samples (Fig. 3.4).

Table 3.5 ANOVA results for MOE values of pine samples

Source	DF	Mean Square	F Value	Pr > F
exposure	4	1602.85	2.35	0.0539
treatment	1	810393.88	1190.30	<.0001
fungus	3	1754.10	2.58	0.0539
exposure*treatment	4	2825.50	4.15	0.0027
exposure *fungus	12	1480.66	2.17	0.0128
treatment*fungus	3	15798.44	23.20	<.0001
exposure *treatment*fungus	12	2724.08	4.00	<.0001

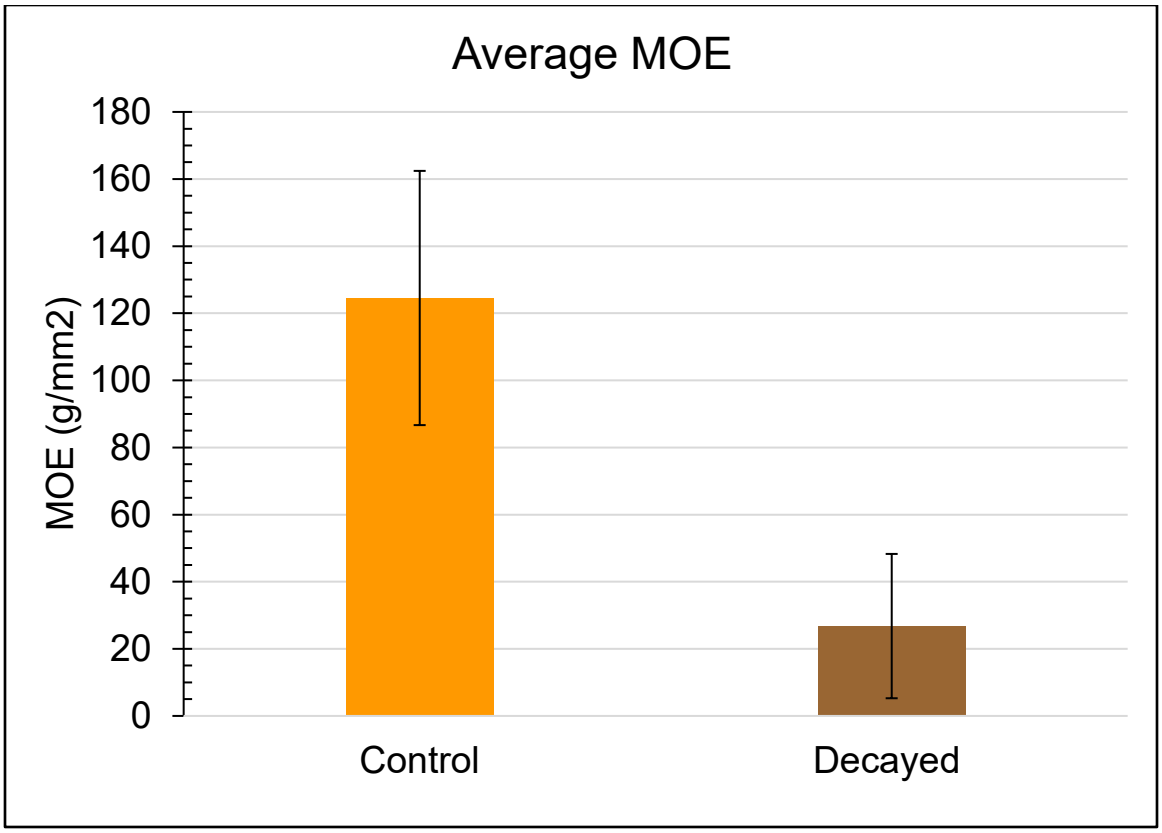


Figure 3.4 Average 5-week MOE values for all control and all decayed pine wood samples

There was no consistently significant difference among MOE values of the samples decayed by the same fungus when comparisons were made among the five weeks (Fig. 3.5, Table 3.6). It was only that *T. versicolor* and *I. lacteus* showed some differences in week four in comparison to the other weeks. In addition, there was no consistent pattern of differences in MOE of samples decayed by different fungi during the same week. These results indicate that brown rots did not decay softwood samples faster than white rots, and that no progression in compression strength loss could be observed over the five-week period.

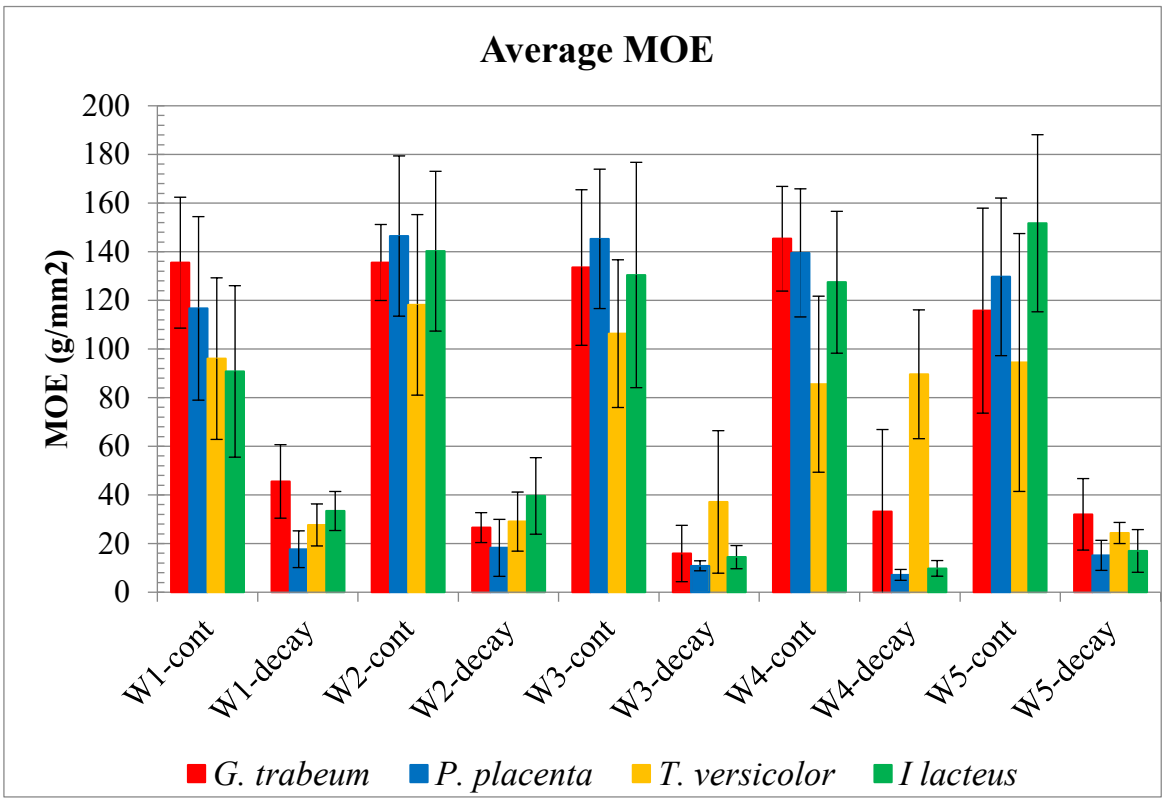


Figure 3.5 Average MOE values based on treatment, fungi and exposure (week) for pine samples

Note: W1-W5: weeks 1-5: cont: control samples; decay-decayed samples.

Table 3.6 Games-Howell pairwise comparison of pine MOE data as affected by time and type of fungi

Expo-	Treatment	Fungus	MOE LSMEAN	STD	COV	Games-Howell
1	decayed	*G. trabeum*	45.6	5.0	0.1	BC
1	decayed	*I. lacteus*	33.4	2.7	0.1	BC
1	decayed	*P. placenta*	17.7	2.5	0.1	AB
1	decayed	*T. versicolor*	27.6	2.9	0.1	BC
2	decayed	*G. trabeum*	26.6	2.0	0.1	BC
2	decayed	*I. lacteus*	39.6	5.2	0.1	BC
2	decayed	*P. placenta*	18.2	3.9	0.2	AB
2	decayed	*T. versicolor*	29.1	4.1	0.1	BC
3	decayed	*G. trabeum*	15.9	3.9	0.2	AB
3	decayed	*I. lacteus*	14.4	1.6	0.1	AB
3	decayed	*P. placenta*	10.9	0.7	0.1	A
3	decayed	*T. versicolor*	37.1	10.4	0.3	BC
4	decayed	*G. trabeum*	33.2	15.1	0.5	BC
4	decayed	*I. lacteus*	9.8	1.1	0.1	A
4	decayed	*P. placenta*	7.1	0.8	0.1	A
4	decayed	*T. versicolor*	89.6	9.4	0.1	C
5	decayed	*G. trabeum*	32.0	4.9	0.2	BC
5	decayed	*I. lacteus*	17.0	2.9	0.2	AB
5	decayed	*P. placenta*	15.2	2.1	0.1	AB
5	decayed	*T. versicolor*	24.4	1.4	0.1	B
1	cont	*G. trabeum*	135.5	9.5	0.1	C
1	cont	*I. lacteus*	90.8	12.5	0.1	C
1	cont	*P. placenta*	116.7	12.6	0.1	C
1	cont	*T. versicolor*	96.0	11.1	0.1	C
2	cont	*G. trabeum*	135.6	5.2	0.0	C
2	cont	*I. lacteus*	140.2	10.9	0.1	C
2	cont	*P. placenta*	146.4	11.0	0.1	C
2	cont	*T. versicolor*	118.1	13.1	0.1	C
3	cont	*G. trabeum*	133.5	11.3	0.1	C
3	cont	*I. lacteus*	130.4	15.4	0.1	C
3	cont	*P. placenta*	145.3	9.6	0.1	C
3	cont	*T. versicolor*	106.3	10.1	0.1	C
4	cont	*G. trabeum*	145.3	7.2	0.0	C
4	cont	*I. lacteus*	127.4	9.7	0.1	C
4	cont	*P. placenta*	139.5	8.8	0.1	C
4	cont	*T. versicolor*	85.5	12.8	0.1	C
5	cont	*G. trabeum*	115.8	14.0	0.1	C
5	cont	*I. lacteus*	151.7	12.1	0.1	C
5	cont	*P. placenta*	129.7	10.8	0.1	C
5	cont	*T. versicolor*	94.5	17.7	0.2	C

3.3.2 Aspen

Some unexpectedly high MOE results were obtained for *T. versicolor* in week one, possibly due to wrong orientation of wafers during MOE measurements, so these results were excluded from the analysis. No consistent differences in terms of MOE due to fungi type were observed (Figure 3.6, Table 3.8). Control samples showed higher MOE values in comparison to *G. trabeum* and *P. placenta* decayed aspen samples.

When MOE values of each fungus were compared from a week to a week, it was only that *G. trabeum* showed significantly lower MOE values in the last (fifth) week. Otherwise, the values of MOE did not significantly change over the weeks (Fig. 3.6, Table 3.8).

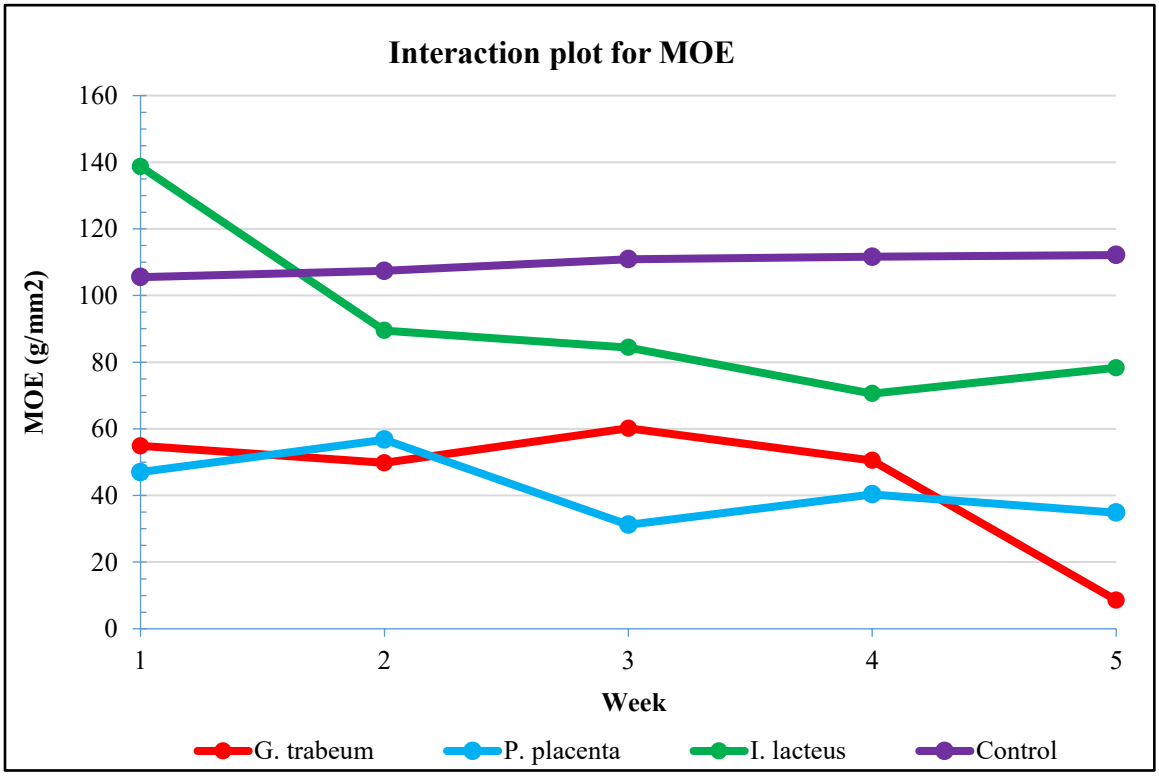


Figure 3.6 Interaction plot for MOE values of aspen samples over 5 weeks

Table 3.7 ANOVA results for MOE values of aspen samples

Source	DF	Mean Square	F Value	Pr > F
exposure	4	3135.55	18.02	<.0001
fungus	3	49617.15	285.22	<.0001
exposure*fungus	12	1802.20	10.36	<.0001

Table 3.8 Games-Howell pairwise comparison of aspen MOE data as affected by time and type of fungi

Effect	**Expo-sure**	**Fungus**	**Estimate**	**SDV**	**COV**	**Games How-ell**
block	5	*G. trabeum*	8.6	3.4	0.4	A
block	3	*P. placenta*	31.2	11.9	0.4	B
block	5	*P. placenta*	34.9	6.2	0.2	B
block	4	*P. placenta*	40.3	8.8	0.2	BC
block	1	*P. placenta*	47.0	6.6	0.1	BC
block	2	*G. trabeum*	49.8	15.0	0.3	BC
block	4	*G. trabeum*	50.5	8.3	0.2	C
block	1	*G. trabeum*	54.9	11.5	0.2	C
block	2	*P. placenta*	56.7	9.6	0.2	C
block	3	*G. trabeum*	60.2	16.5	0.3	C
block	4	*I. lacteus*	70.6	10.0	0.1	C
block	5	*I. lacteus*	78.3	21.8	0.3	CD
block	3	*I. lacteus*	84.4	26.5	0.3	CD
block	2	*I. lacteus*	89.5	27.5	0.3	CD
block	1	cont	105.5	7.4	0.1	DE
block	2	cont	107.4	8.4	0.1	DE
block	3	cont	110.9	7.9	0.1	DE
block	4	cont	111.6	5.8	0.1	DE
block	5	cont	112.1	6.8	0.1	DE
block	1	*I. lacteus*	138.7	6.6	0.0	E

3.4 Comparison of mass loss and compression strength loss

Figure 3.7 compares mass loss and compression strength loss for pine samples decayed with the four fungi over time. The loss in strengths due to brown rots were approximately 60-90% at mass loss decrease of 20-30% over the five weeks for *G. trabeum*, and 85-95% strength loss accompanied with 17-30% of 5-week weight loss for *P. placenta*. The white rots showed approximately 60-90% strength and 18-30% mass loss for *I. lacteus,* and 85-95% strength loss with 24-34% weight loss for *T. versicolor*.

From the Figure 3.7, it is obvious that the shape of mass loss follows the shape of the strength loss. The figure also shows some unexpected decreases in both the mass loss and compression strength loss for *T. versicolor* at week 4. These results indicate that there was a variability in fungal growth from jar to jar, despite the three jar replicates. This is something that should be taken into consideration for the future studies.

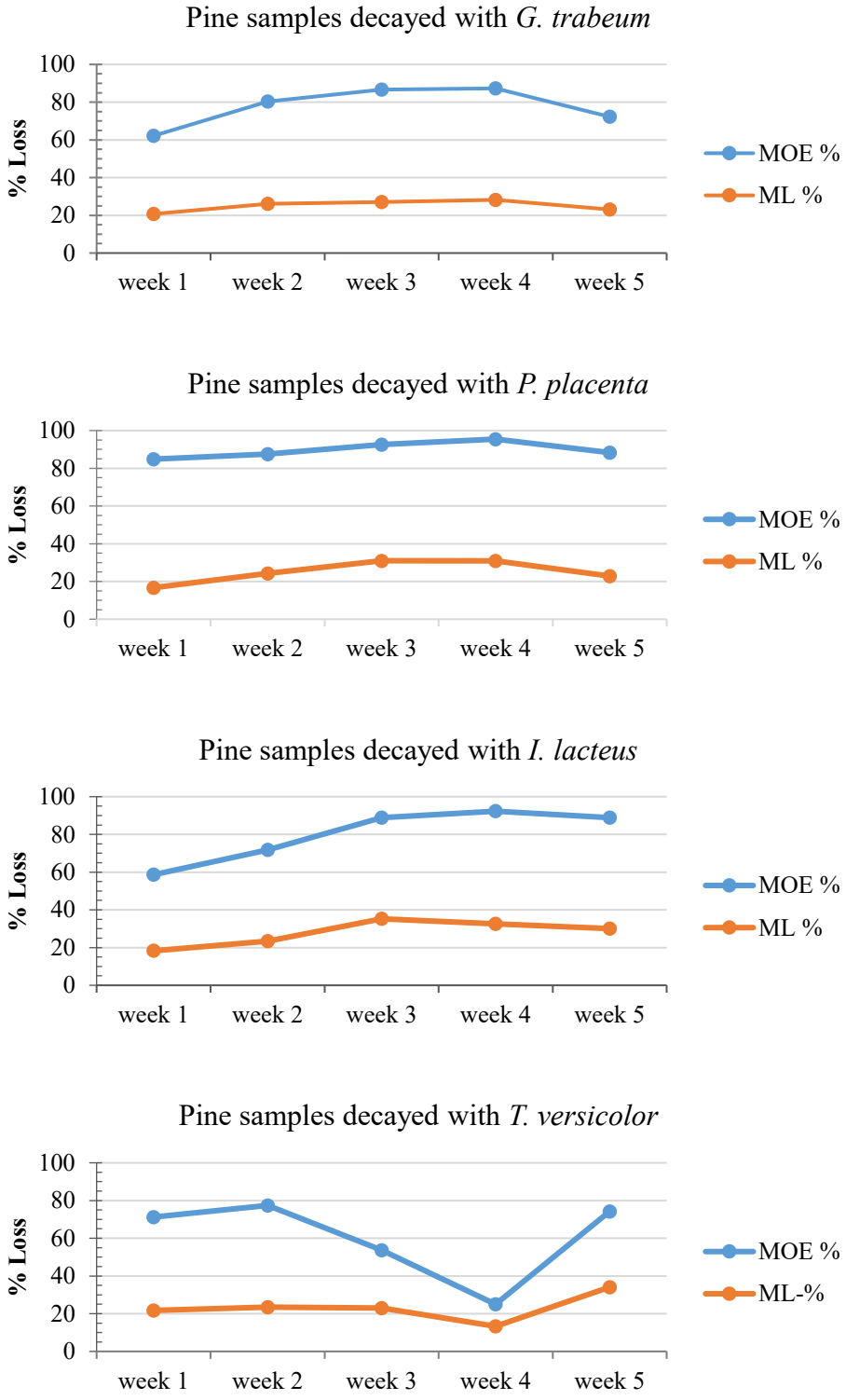


Figure 3.7 Relationships between average mass and average compression strength loss of pine samples decayed by four fungi over 5 weeks

Figure 3.8 shows the progression of mass and compression strength loss for aspen samples decayed by *G. trabeum* and *P. placenta* fungi. *T. versicolor* and *I. lacteus* samples are not shown as the control samples had lower MOE values than did the decayed samples. In case of aspen, as stated in the Materials section (2.1.1), only one control sample set was used for all fungi – the samples originating from all 4 wood sticks. It is plausible that the differences are caused by different days of MOE measurements of control and decayed samples, which seems to be a very important factor when performing compression strength tests according to AWPA E22 standard. The MOE loss of approximately 50-90% was accompanied by 35-60% mass loss in case of *G. trabeum*, while P. placenta showed MOE loss of 50-70% and mass loss of 20-40% during the first 5 weeks of the study.

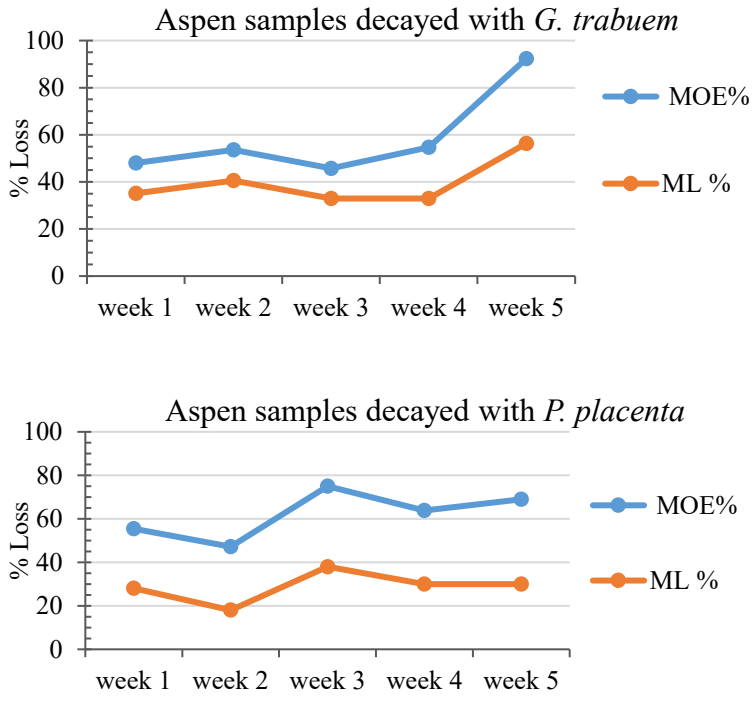


Figure 3.8 Relationships between average mass and average compression strength loss for brown rots on aspen samples.

The comparison between mass loss and compression strength loss implied a direct relationship between weight and strength loss for both brown and white rot fungi, as the shapes of the curves were similar to each other. Wilcox (1978) reported results of Tool's which showed that stress reduction at 5% compression had the highest correlation with weight loss. In this study, strength loss having 2-3 times higher percent values than mass loss, showed to be a more sensitive decay detection method. For example, strength losses of 80% (*G. trabeum* and *I. lacteus*) or 90% (*P. placenta*) in pine occurred at 25% weight loss. Many studies showed similar results. Curling *et al.* (2002) reported strength loss of 30% (*T. versicolor*) to 60% (*G. trabeum*) at 10% weight loss for pine samples during 6 weeks.

3.5 Preliminary data for selection of temperature settings during VOCs collection

Profiles of VOCs spectra were examined after collection of VOCs for 30 minutes at different temperatures: 22, 30, 50, 70 and 80°C. As reported earlier, the temperature increase yielded higher amounts of volatile compounds (Jeleń 2003, Wady *et al.* 2003). At 70°C, the peaks of GC-MS spectra were well above the noise level. At temperatures above 70°C, no significant difference was seen in the profiles of the volatiles (Figure 3.9).

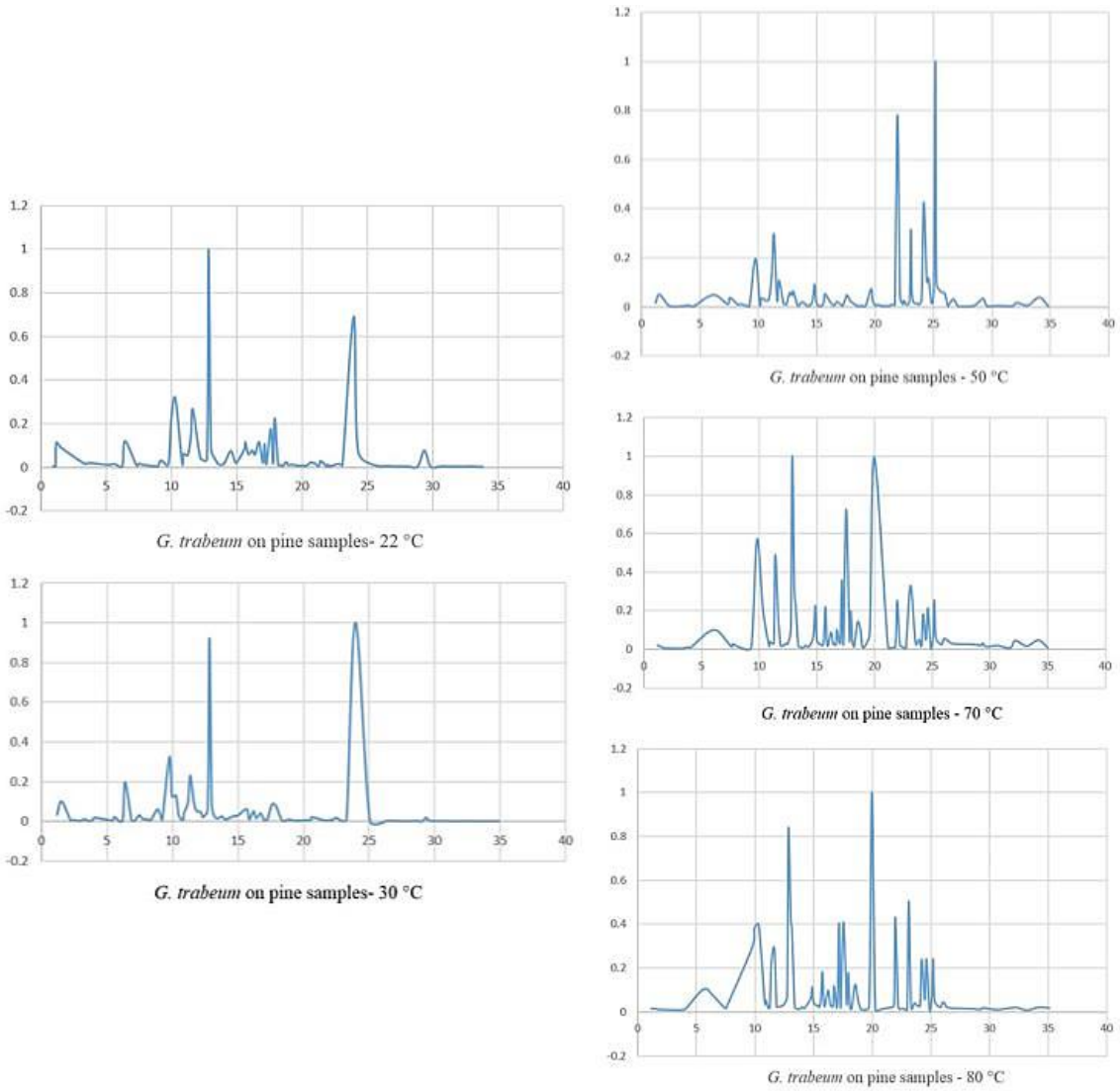


Figure 3.9 Chromatograms of volatile compounds isolated from *G. trabeum* on pine samples for 30 minutes at different temperatures.

3.6 VOCs profiles as indicators of decay

3.6.1 Spruce pine

To evaluate the capability of HS-SPME/GC-MS method for distinguishing the VOCs decay pattern, multivariate analysis was used to discriminate the differences between GC-MS spectra of fungi-decayed and control samples collected over the 12 weeks.

In the following PCA results figures, the score scatters plot (A) for the first two principal components (PC1 and PC2) represent the grouping of the samples in two most distanced directions, and corresponding loading plots (B, C), establish the relative importance of volatile compounds for the sample grouping. The list of most distinguished volatiles is given in Appendix A3.

Even though the variation of data in this biological study was large, the VOCs produced by four types of fungi and control samples were successfully differentiated in pine (Fig. 3.10). *Postia placenta* decayed pine samples showed the most distinctive pattern of GC-MS volatiles, as these spectra were differentiated from the others by PC1. The peak with retention of 22:50 min was identified as sativene. Sativene is a terpenoid reported in many fungi, such as in the fruiting body of *Fomitopsis pinicola* (Rösecke *et al.* 2000).

Pine control samples, on the other hand, were differentiated from the samples decayed by fungi by PC2. The compound most prevalent in the control samples, with retention time of 17:51 min, was identified as α- terpineol. Alpha-terpineol is a monoterpenol which is a major component of pine oils and it appears in small quantity in some other essential oils (Gomes-Carneiro *et al.* 1998).

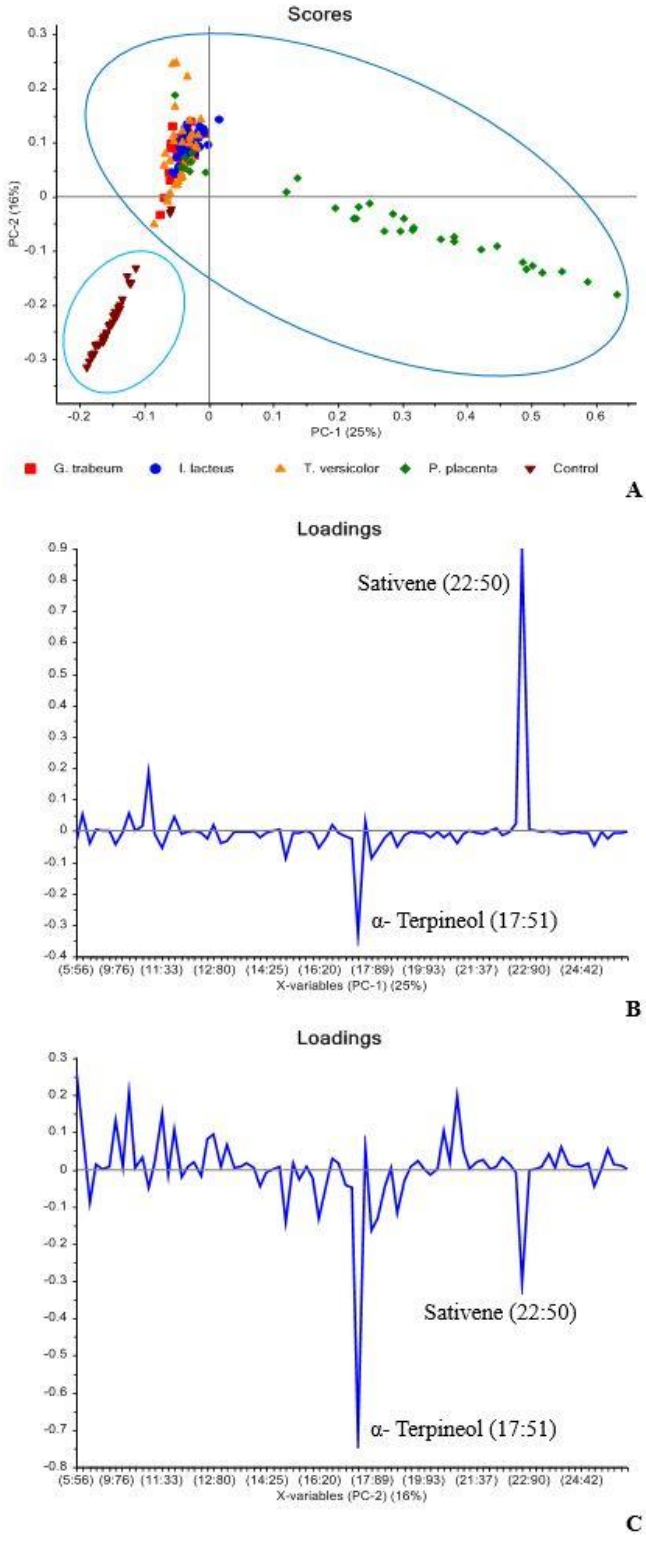


Figure 3.10 PCA score plot (A) and loading plots (B and C) of VOCs patterns of decayed and control samples of pine.

When control samples are removed from the PCA analysis (Fig.3.11), in order to enhance the differences between *P. placenta* and other fungi, then an additional peak with retention time at 11:15 min, identified as benzaldehyde also emerges as characteristic for *P. placenta* samples. This compound has been reported both in brown rot, *Piptoporus betulinus*, *Fomitopsis pinicola*, and white rot *Trametes suaveolens* fungi (Rösecke *et al.* 2000). In case of basidiomycetes *Agaricus smithii*, a leaf-litter decayer, benzaldehyde was found to be a major compound in VOCs responsible for the odor (Wood and Largent 1999).

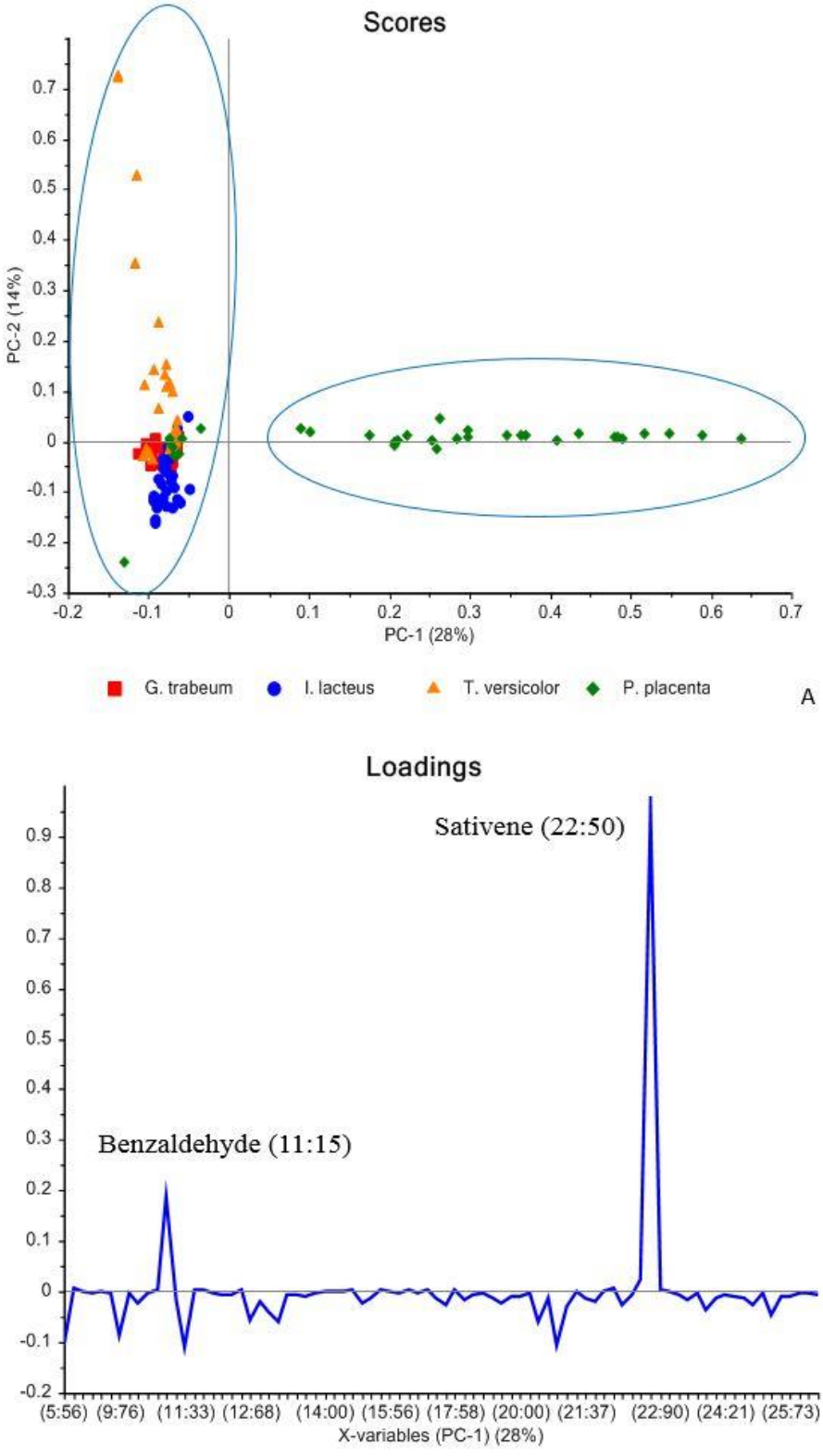
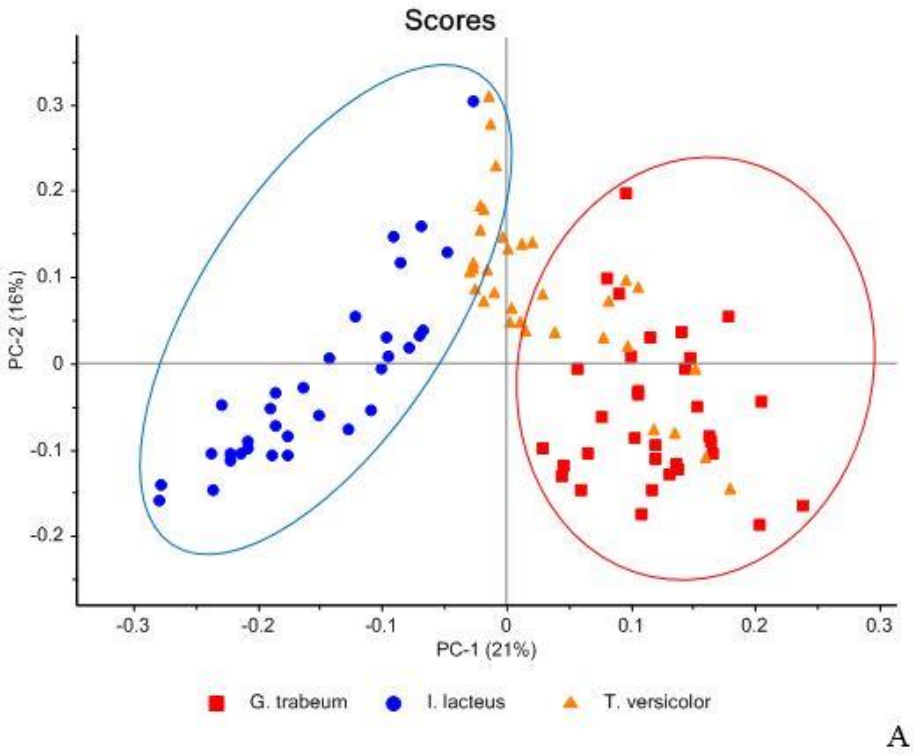


Figure 3.11 PCA score plot (A) and loading plot (B) of VOCs patterns of decayed samples of pine with four fungi

The major differences between VOCs produced on pine by *Irpex lacteus* as a white rot and *Gloeophyllum trabeum* as a brown rot fungus was the presence of cyperene with retention time at 21:15 min in *I. lacteus*. Also, several characteristic compounds for *G. trabeum* are 2-furoic hydrazide, methyl-2-furoate, 2-ethyl-1-hexanol, 2,6-di-tert-butyl-p-benzoquinone, and β-cadinene, with retention times at 9:76, 11:33, 12:80, 23:97, and 25:17 min, respectively (Fig 3.12).

Konuma *et al.* (2015) reported methyl-2-furoate production from *Fomitopsis palustris* (brown rot) and *Trametes versicolor* (white rot) in different culture conditions. In a different study, 2-ethyl-1-hexanol was identified from *Aspergillus versicolor*, *A. fumigatus*, *Penicillium chrysogenum*, and *Cladosporium cladosporoides* mold cultures (Matysik *et al.* 2009). The compound β-cadinene, was isolated from *Aspergillus flavus* mold grown on different media (Sun *et al.* 2014).



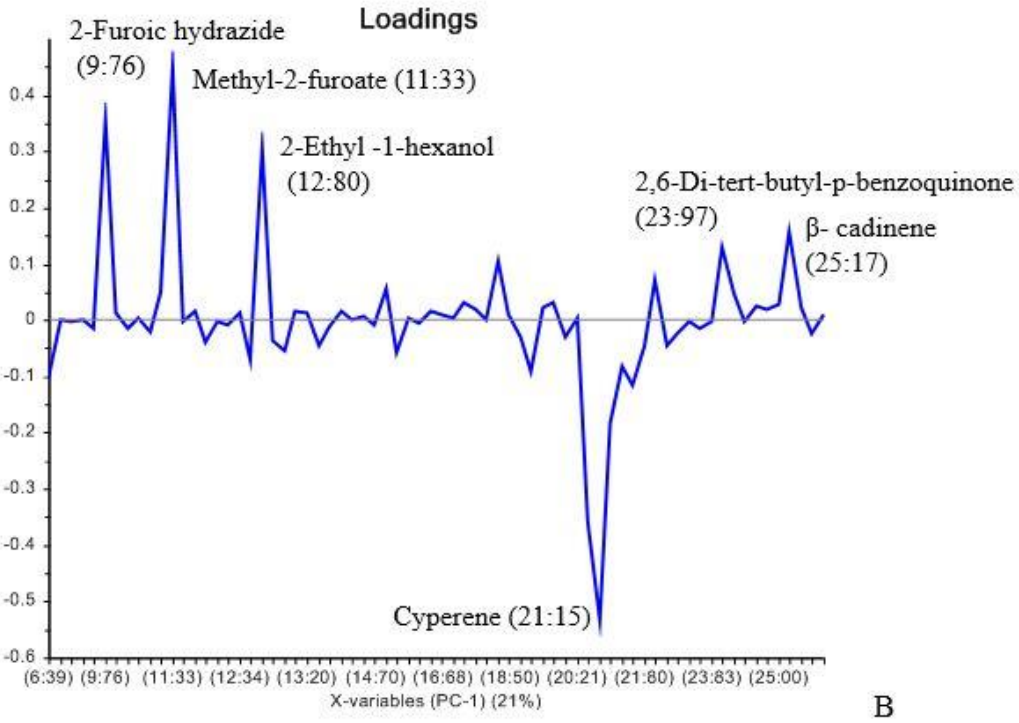


Figure 3.12 PCA score plot (A) and loading plot (B) of VOCs patterns of decayed samples of pine

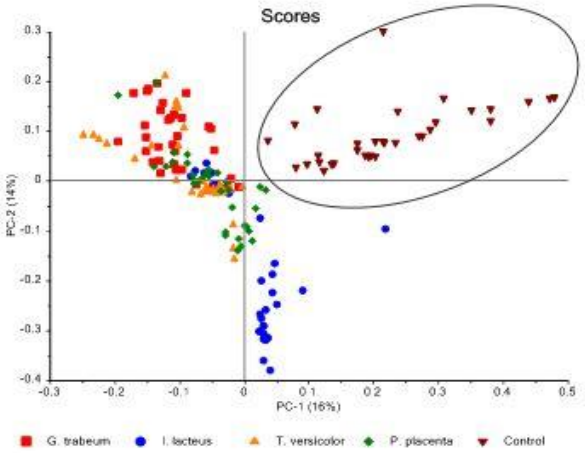
3.6.2 Aspen

The GC-MS spectra of volatiles collected on decayed and control aspen samples were clearly separated by PCA (Fig. 3.13). The control group was characterized by 2,4-bis(1,1-dimethylethyl phenol) with retention time of 25:00 min.

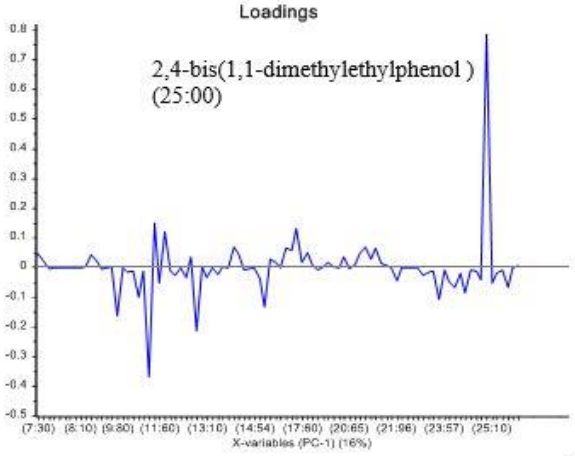
PC2 of the score plot in Figure 3.13 separated *I. lacteus* from the rest of the samples, so an analysis of only decayed samples was performed after excluding the control samples. Figure 3.14 shows that *I. lacteus* is characterized by α-gurjunene and cyperene with retention times at 20:45 and 21:15 min, respectively. The pine samples also showed prevalence of cyperene when decayed by *Irpex lacteus*.

Looking only for the difference between *G. trabeum* and *P. placenta* (Figure 3.15), it was found that *G. trabeum* is characterized by 2-ethyl-1-hexanol, α-himachalene and an unidentified compound with retention times at 12:80, 23:96, and 24:10 min respectively. *P. placenta* was characterized by the higher amounts of α-gurjunene and α-cadinene at 20:45 and 25:70 min retention times.

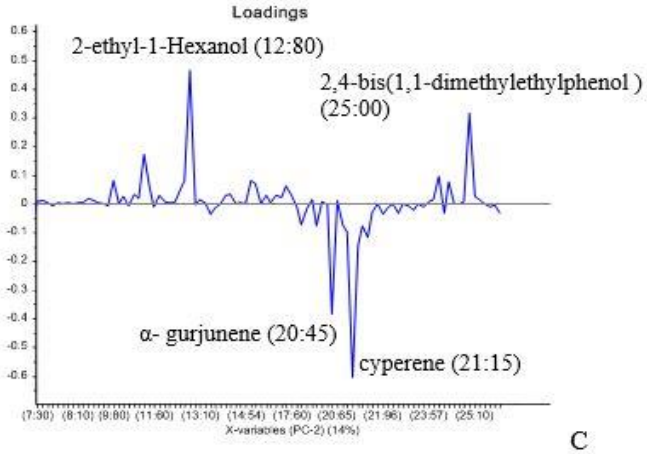
Zeringue *et al.* (1993) reported that aflatoxigenic strains of *Aspergillus flavus* mold produced several sesquiterpene compounds (e.g., α-gurjunene) which peaked in 3-day cultures and were not present in earlier (1- and 2-day) or later (8- and 10-day) cultures.



A

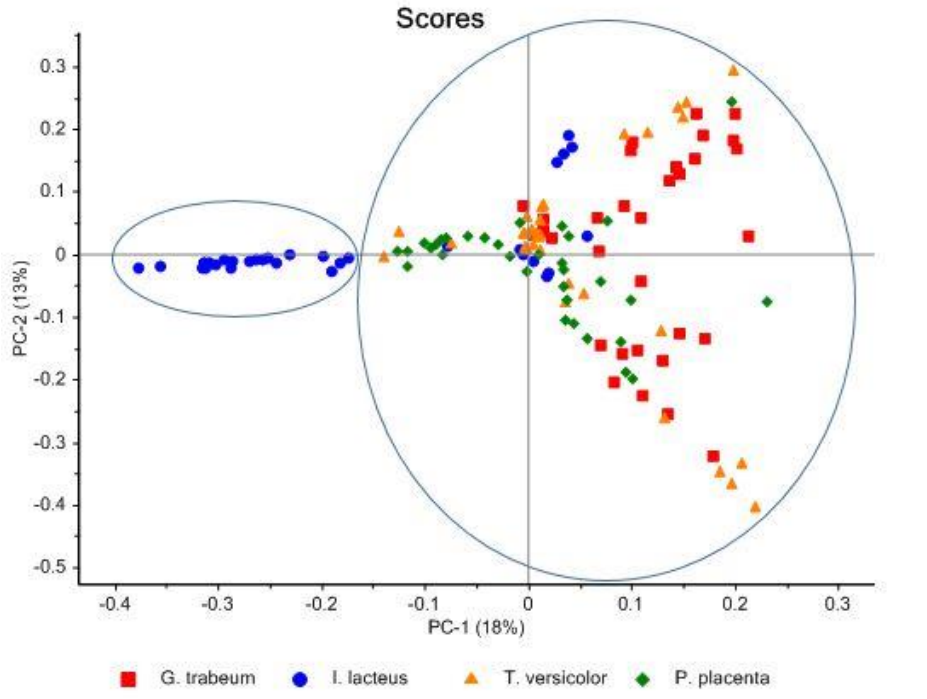


B

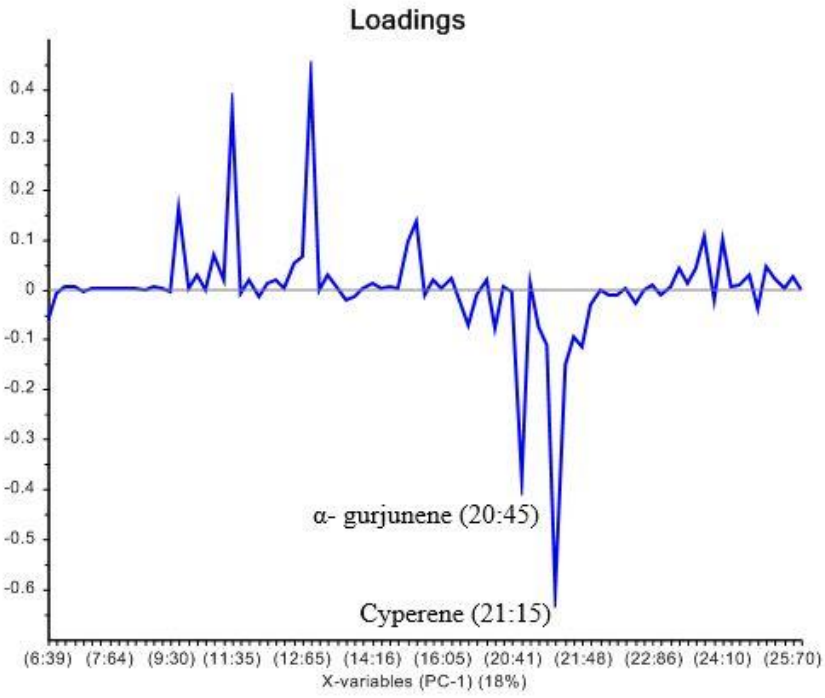


C

Figure 3.13 PCA score plot (A) and loading plot (B and C) of VOCs patterns of decayed and control samples of aspen

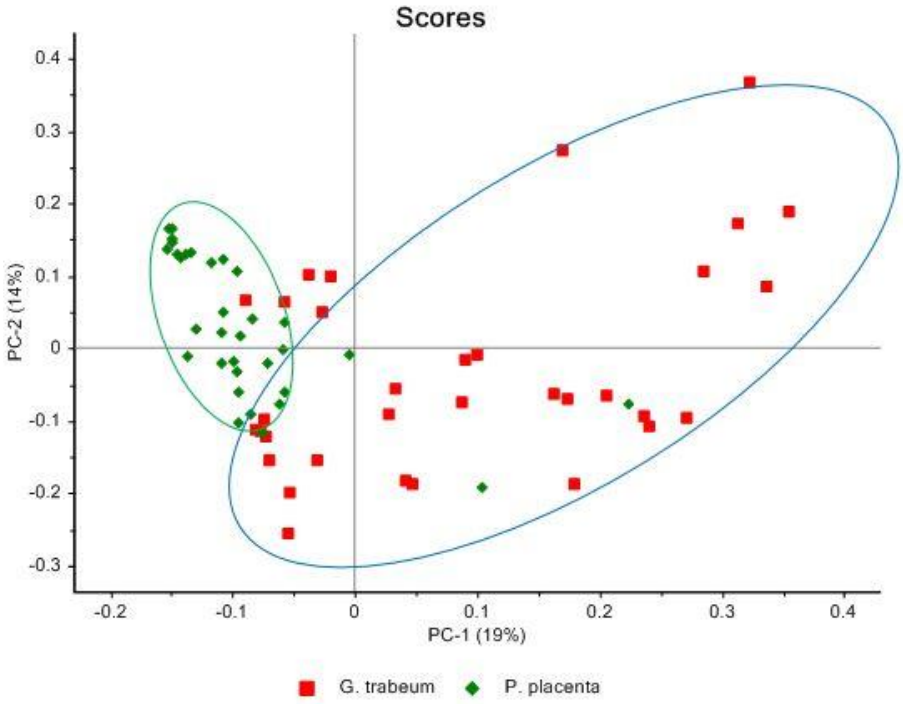


A

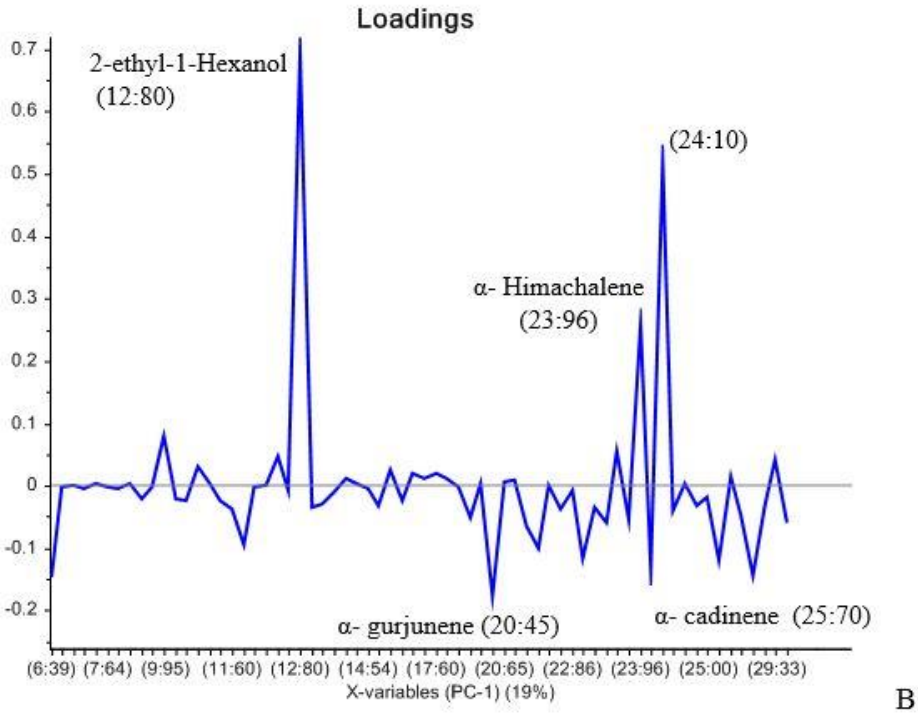


B

Figure 3.14 PCA score plot (A) and loading plot (B) of VOCs patterns decayed aspen



A



B

Figure 3.15 PCA score plot (A) and loading plot (B) of VOCs patterns of decayed aspen samples by *G. trabeum* and *P. placenta*

3.6.3 Summary

Many VOCs detected during the wood decay process by the four fungi were distinctively different from VOCs of control samples. This is an indication that volatile emissions from decayed wood are different from naturally occurring wood volatiles. Each fungus had a distinct pattern of emitted VOCs for each wood species (Figure 3.16), which can be used as a fingerprint for each fungus. Some volatiles were common and prevalent in different fungi. For example, α-gurjunene was produced by *I. lacteus* and *P. placenta* on both wood species, and 2-ethyl-1-hexanol was produced by *G. trabeum* on both wood species. Konuma *et al.* (2015) reported that 23 organic compounds were common in both *T. versicolor* and *F. palustris* grown on Japanese beech. In conclusion, the results supported the study hypothesis that fungal decay is associated with the type of volatiles fungi produce. These results indicate that VOCs can be used as a marker for the presence of wood decay fungi and for *in-situ,* non-destructive wood decay assessment.

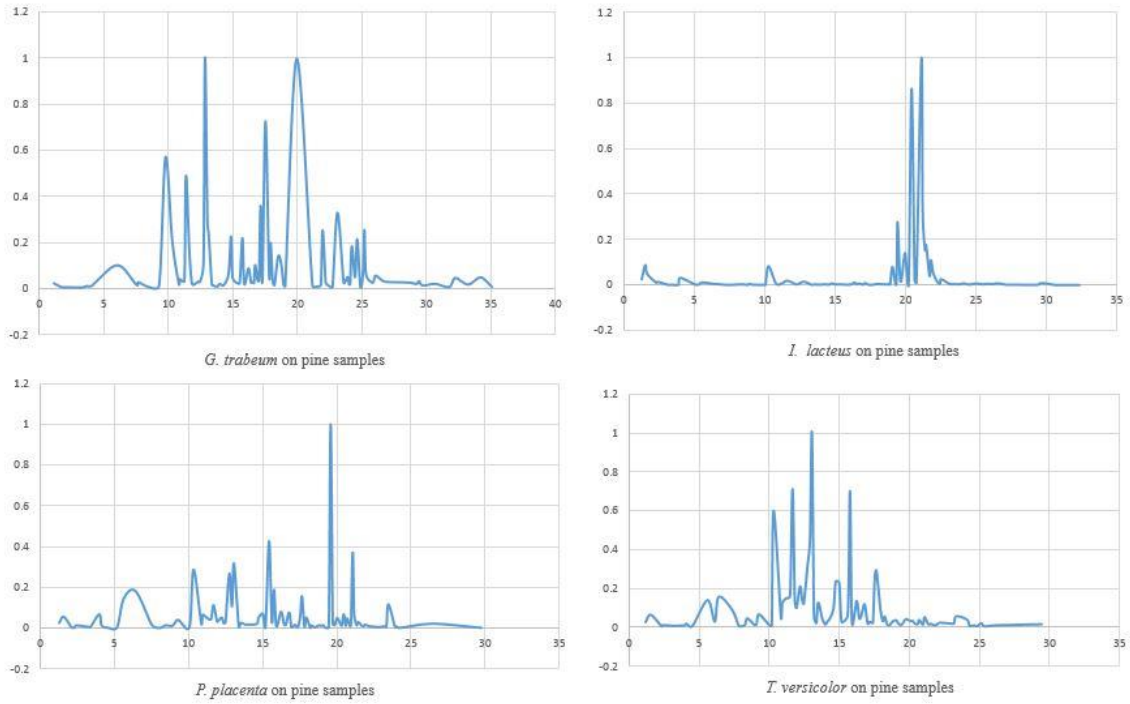


Figure 3.16 Chromatograms of volatile compounds collected during pine decay by four different fungi

3.7 Change in VOCs profiles over time (stage of decay)

3.7.1 Brown rots on pine

One of the goals of this study was to test if the fungal volatiles can be associated with the stage of decay. For this, PCA was performed on GC-MS data of each fungus separately over the period of 12 weeks of study.

PCA score plot in Figure 3. 17 shows separation of pine samples decayed by *G. trabeum* during the first five weeks (early stage of decay) and the remaining samples, decayed for the additional seven weeks (late stage). Early degradation was characterized by α-pinene, 2-ethyl-1-hexanol and 2,6-di-tert-butyl-p-benzoquinone with retention times at 10:26, 12:80, and 23:97 min, respectively. Late degradation was characterized by methyl-

2-furoate, α-cubebene, and β-cadinene at 11:33, 21:92 and 25:17 min retention times, respectively. These results indicate that the volatile production of *G. trabeum* on pine was in general associated with the stage of decay. The compound α-pinene is the most widely encountered terpenoid in nature (Noma and Asakawa 2010) and it appears in conifers and numerous other plants (Russo 2011). Breheret *et al.* (1997) reported that α-pinene was one of the most abundant compounds identified from many fresh wild mushrooms (basidiomycetes) such as *T. caligatum*, *G. glutirosus*, *A. oviodea*, and *B. aestivalis.* Rösecke *et al.* (2000) reported α-cubebene from fresh fruiting body of *P. betulinus* and *F. pinicola*.

Pine samples decayed with *P. placenta* (Figure 3. 18), on the other hand, did not show any particular VOC pattern association with time during 12 weeks of decay.

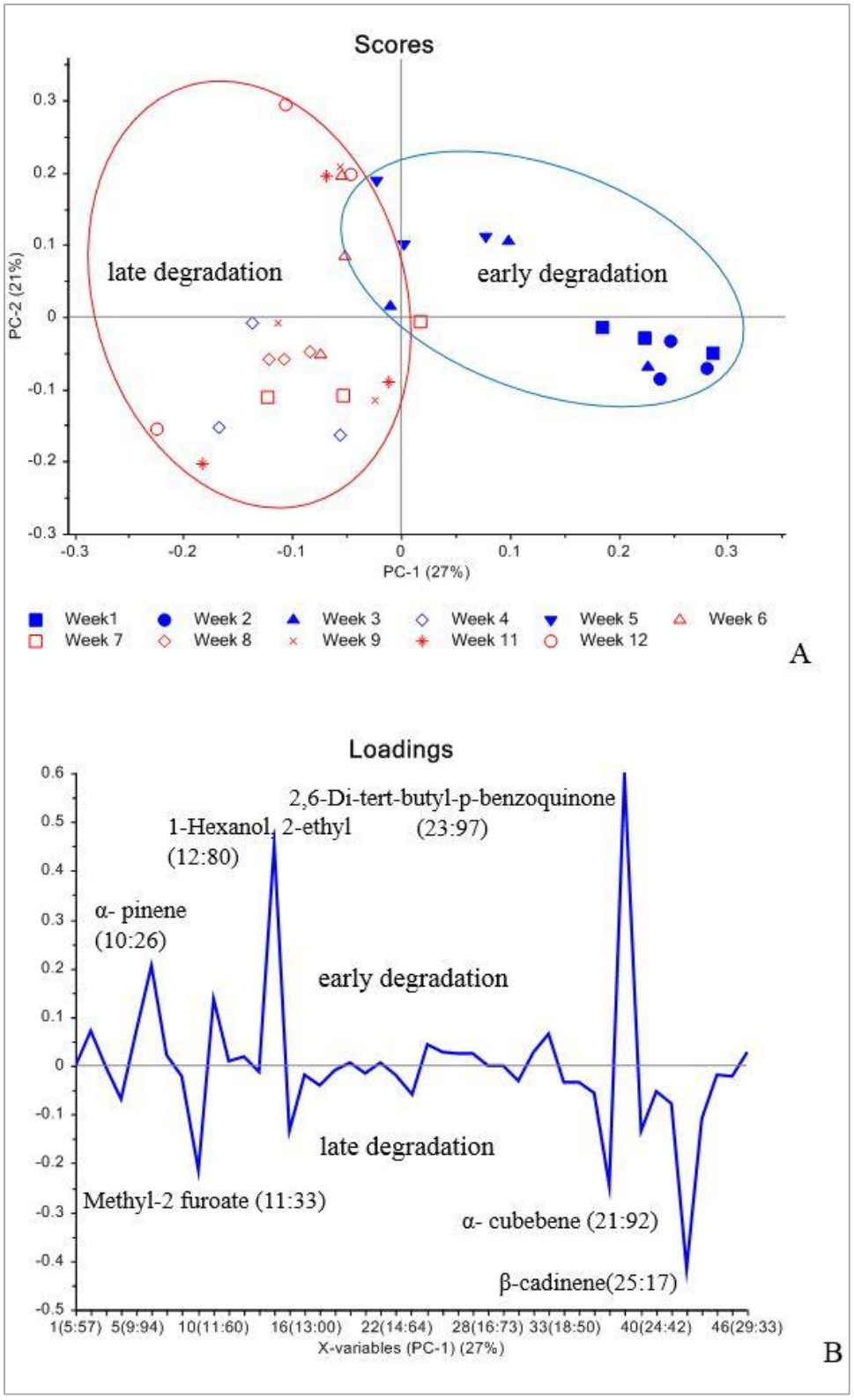


Figure 3.17 PCA score plot (A) and loading plot (B) of VOCs patterns of *G. trabeum* grown on pine for 12 weeks

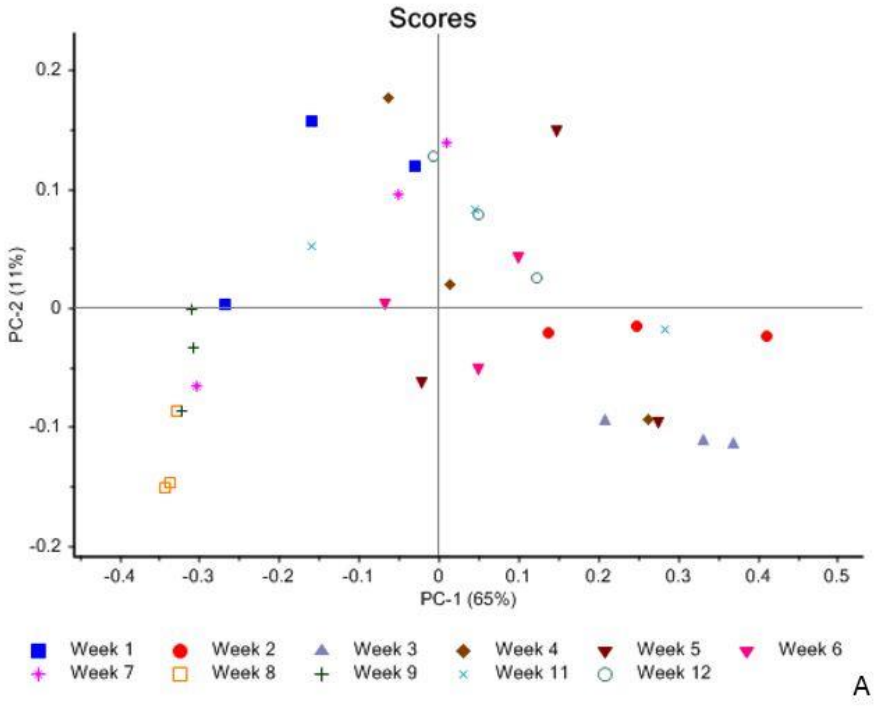


Figure 3.18 PCA score plot (A) of VOCs pattern of *P. placenta* grown on pine for 12 weeks

3.7.2 White rots on pine

PCA score plot in Figure 3.19 shows separation of pine samples between first 5 weeks (early stage of decay) and the remaining 7 weeks (late stage) of decay by *I. lacteus*. Early degradation showed prevalence of α-pinene, β-pinene, and α-gurjunene with retention times of 10:26, 11:60, and 20:45 min, respectively. Late degradation was characterized by limonene and nonanal with13:00 and 14:97 min retention times, respectively.

Pine samples decayed by *T. versicolor* did not show any meaningful separation due to the decay stage during the 12 weeks of the study (Figure 3.20).

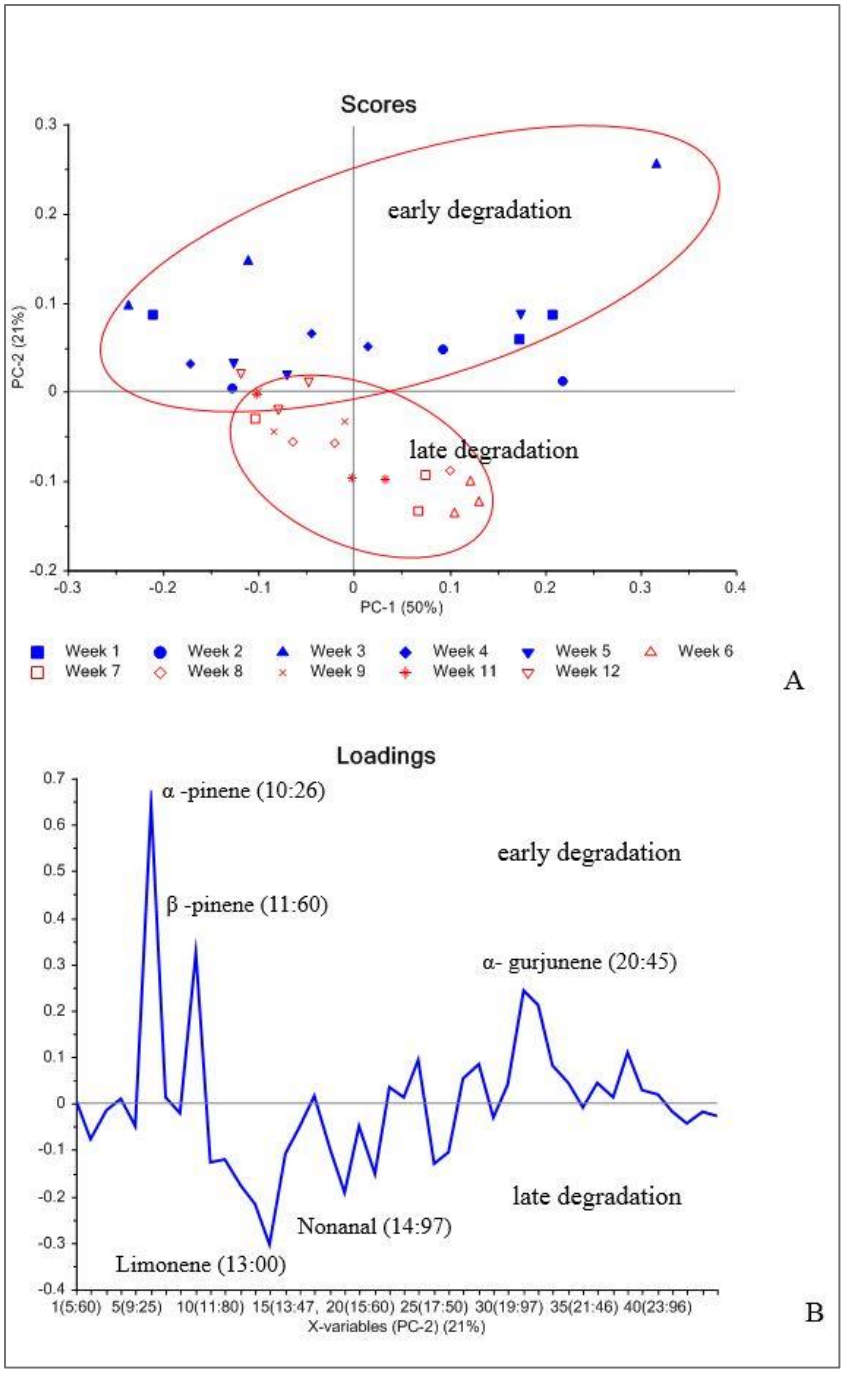


Figure 3.19 PCA score plot (A) and loading plot (B) of VOCs pattern of *I. lacteus* grown on pine for 12 weeks

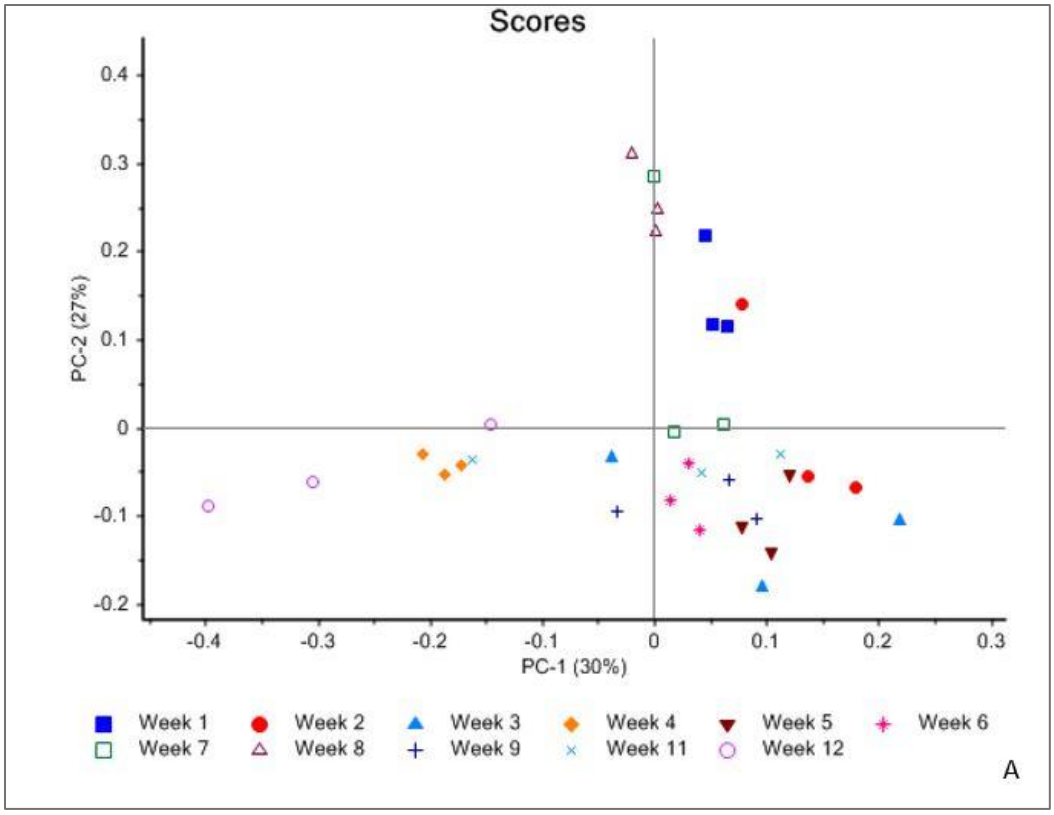


Figure 3.20 PCA score plot (A) of VOCs pattern of *T. versicolor* decayed pine for 12 weeks

3.7.3 Brown rots on aspen

PCA score plot in Figure 3.21 shows separation of aspen samples decayed during the first five weeks (early stage of decay) from the samples decayed in the later seven weeks (late stage) when exposed to *G. trabeum*. Early degradation was characterized by 2-ethyl-1-hexanol and α-himachalene with retention times at 12:80 and 23:96 min, respectively and late degradation was characterized by an unindefied compound with retention time at 24:10 min.

P. placenta decayed samples of aspen, similarly to pine, did not show any groupings with progression of decay (Fig. 3. 22).

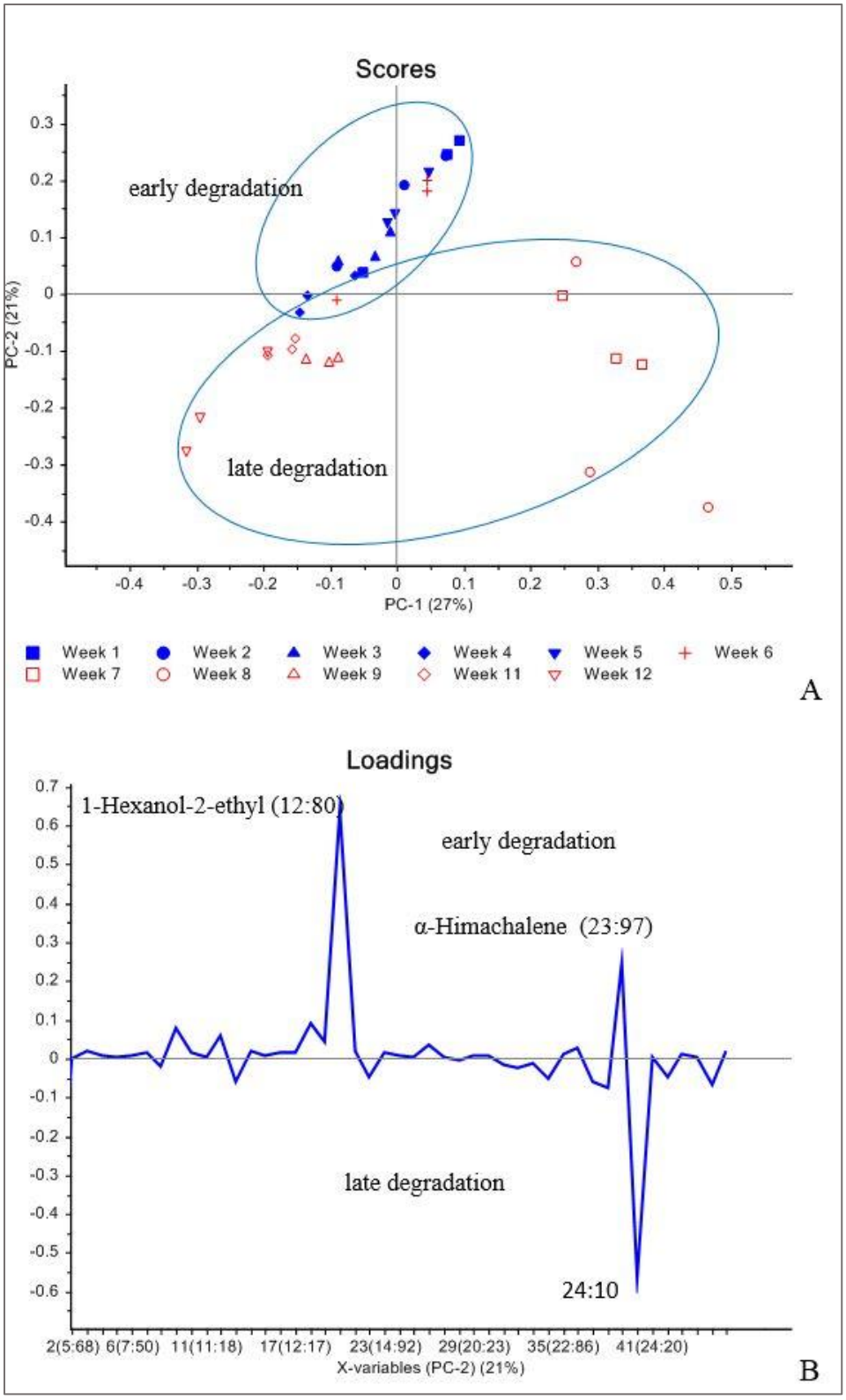


Figure 3.21 PCA score plot (A) and loading plot (B) of VOCs pattern of *G. trabeum* on aspen during 12 weeks

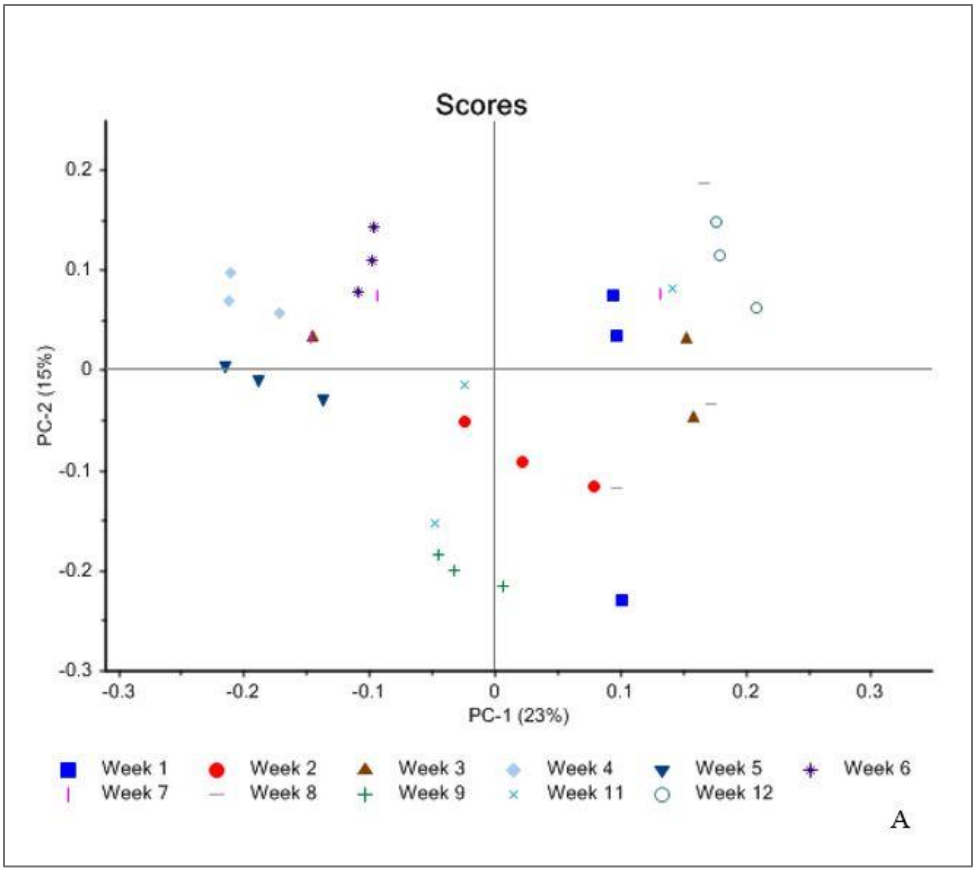


Figure 3.22 PCA score plot (A) of VOCs pattern of *P. placenta* decayed aspen for 12 weeks

3.7.4 White rots on aspen

Aspen samples decayed by *Irpex lacteus* showed good separation between 5-week (early stage of decay) and samples decayed at the later stage (Fig. 3.23). Early degradation was characterized by benzaldehyde, α-gurjunene, and cyperene with retention times at 11:15, 20:45 and 21:15 min, respectively, and late degradation showed prevalence of benzeneacetaldehyde and 2-undecanone with retention times at 13:47, and 19:70 min, respectively.

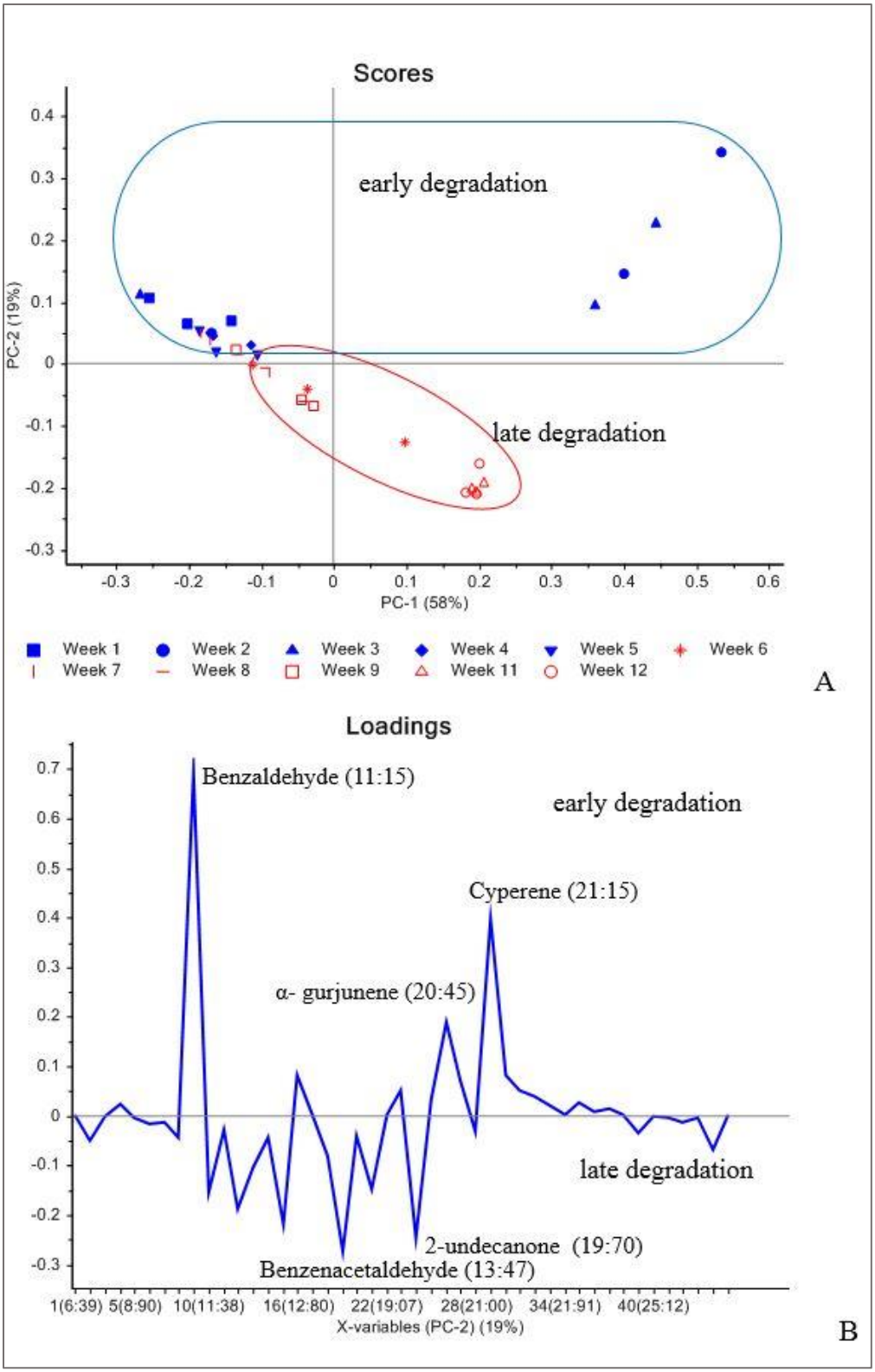


Figure 3.23 PCA score plot (A) and loading plot (B) of VOCs pattern of *I. lacteus* on aspen during 12 weeks

Similarly, to pine samples, aspen samples decayed with *T. versicolor* did not show any meaningful separation with the decay stage during the 12 weeks of the study (Fig. 3.24).

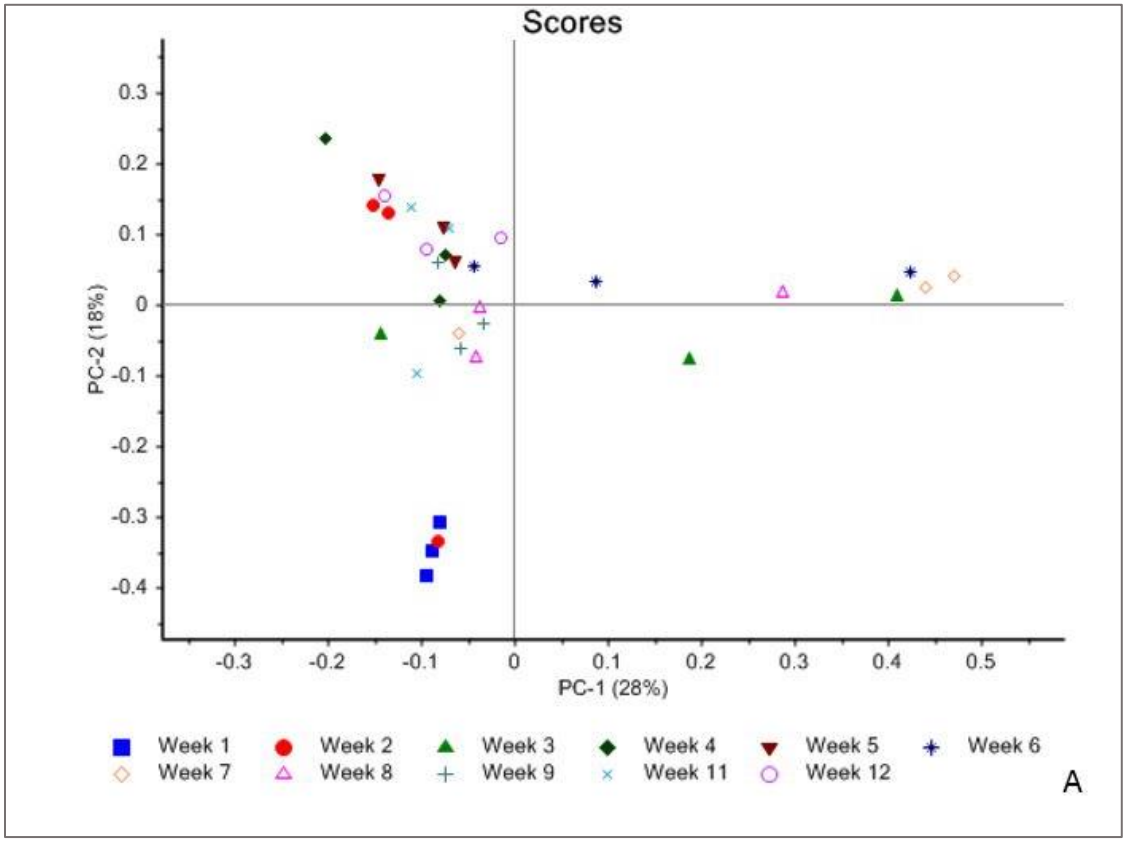


Figure 3.24 PCA score plot (A) and loading plot (B) of VOCs pattern of *T. versicolor* on aspen during 12 weeks

3.7.5 Summary

Analysis of the volatiles production of different fungi over time, showed a distinct difference of decay between early and late degradation by brown rot *G. trabeum* during the 12 weeks of study on both pine and aspen species. The other brown rot, *P. placenta,* did not show any time pattern associated with decay stage on either of the wood species.

I. lacteus white rot also showed a clear separation between the early and late stage of decay on both of the species. The volatile production by the other white rot, *T. versicolor,* was not associated with the stage of decay on either of the species.

Although in our case, *T. versicolor*'s stage of decay could not be associated with the VOCs produced, Konuma *et al.* (2015) reported that in case of Japanese beech decay, both *T. versicolor* and *F. palustris* produced distinct compounds in different stages of decay. Wu *et al.* (2005) also indicated that *P. sulphureus* produced different VOCs from young and aged fruiting bodies.

These results indicate that the stage of decay could not be associated specifically with a white or a brown rot grown on pine and aspen, but that given fungal species, in this case *G. trabeum* and *I. lacteus* produce volatiles that could indicate the stage of decay.

CHAPTER IV

CONCLUSIONS

Early detection of decay plays an important role in maintaining the health of wood structures. The primary decomposers of wood are basidiomycete decay fungi, which can degrade the wood in service. Each year in the United States, many costs incurred for raw materials, labors, and liability to replace wood in service that has decayed (Zabel and Morrell 1992).

Wood decay fungi can drastically reduce wood strength early in the decay process, as confirmed in this study. The results indicated that there was an extremely high loss of compression strength of pine and aspen wood species during the decay process, and that the compression strength test was more sensitive for decay detection than the mass loss. Brown rot fungi, *G. trabeum* and *P. placenta*, caused 60-90% and 85-90% compression strength loss in pine samples, respectively, while the samples showed only 20-30% and 17-30% of the weight loss during the same period. White rot fungi, *I. lacteus* and *T. versicolor*, caused 60-90% and 25-75% compression strength loss in pine, while 18-35% and 15-35% mass loss was measured, respectively. In case of aspen, the compression strength loss of approximately 50-90% was accompanied by 35-60% mass loss in case of *G. trabeum*, while *P. placenta* showed compression strength loss of 50-70% and mass loss of 20-40% during the first 5 weeks of the study.

Assessment of volatile organic compounds as a detection method of wood decay holds promise since the VOCs emitted from decayed wood could be easily differentiated from the volatiles emitted from sound wood. In that sense, VOC showed to be comparable to the compression strength's early differentiation between control and decayed samples. Most distinctive volatile pattern from decayed pine samples was discovered during *P. placenta* decay, while in case of aspen, the most different was *I. lacteus* VOC pattern. *I. lacteus* also caused greater mass and compression strength loss of aspen in comparison to the other fungi. *T. versicolor* showed the least different volatiles' pattern on both wood species, and this fungus needed longer time (11 weeks) to show significant mass losses.

G. trabeum (brown rot) and *I. lacteus* (white rot) produced different compounds at early and late stages of decay, which indicated that volatiles of these two fungi could be associated with the stages of pine and aspen decay. In addition, these fungi showed higher mass losses during the 12-week decay in comparison to the other two fungi on pine, while only *I. lacteus* showed higher mass losses in case of aspen decay.

While VOC detection method showed differences between decay of some fungi, MOE results could not. When comparing abilities of VOC and MOE methods for decay stage, we could conclude that VOC method also has an advantage over the MOE in some cases. VOCs showed differences in pattern after five weeks in case of *I. lacteus* and *G. trabeum* on both pine and aspen. However, MOE tests could not be performed after five weeks as the samples became too fragile. On the other hand, mass loss of *I. lacteus* and *G. trabeum* decayed pine samples and *I. lacteus* decayed aspen were also higher than the mass loss caused by other fungi.

CHAPTER V

RECOMMENDATIONS FOR FUTURE WORK

Considering the results of the current study, the following considerations for further research are recommended:

Since mass loss of 20% was measured at the beginning of the testing period, it would be beneficial to perform the first measurements before the samples are transferred into the jars, and not two weeks later.

As high variability among compression strength data was noted despite the use of nine replicates, it is assumed that the increased number of replicates would be beneficial to overcome the variability among the samples.

Due to limited number of aspen samples, one set of control samples was used for all fungi, meaning that some of the MOE tests of decayed samples had to be performed on different days than the controls. In the future, either an incresed number of control samples, or MOE measurements of all samples on the same day is recommended.

There is a possibility that using more than one SPME fiber type would result in capturing broader range of VOCs from the headspace of samples.

Since current study provided promising results for differentiation of VOCs of control and decayed wood, as well as differentiation of incipient and advanced wood decay for some fungal species, it would be beneficial to further test feasibility of using

herein identified fungal VOCs for on-site wood decay detection. For that purpose, assessment of possible use of electronic noses would be a logical step forward. E-nose technologies have been successfully applied in a variety of fields, from industry control process to environmental monitoring. With the calibration of e-noses for herein identified VOCs, there is a possibility that this technology could be applied for detection of fungal decay in wood.

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APPENDIX A

SUPPLEMENTAL MATERIAL

A.1 Effect of mineral salt on mechanical properties of wood samples

Table A.1 ANOVA results for MOE values of pine mineral-treated and control samples after 3 weeks exposure

Source	DF	Mean square	F value	Pr>F
Treatment	1	4977.65	31.52	<.0001
Exposure	2	209.18	1.32	0.2821

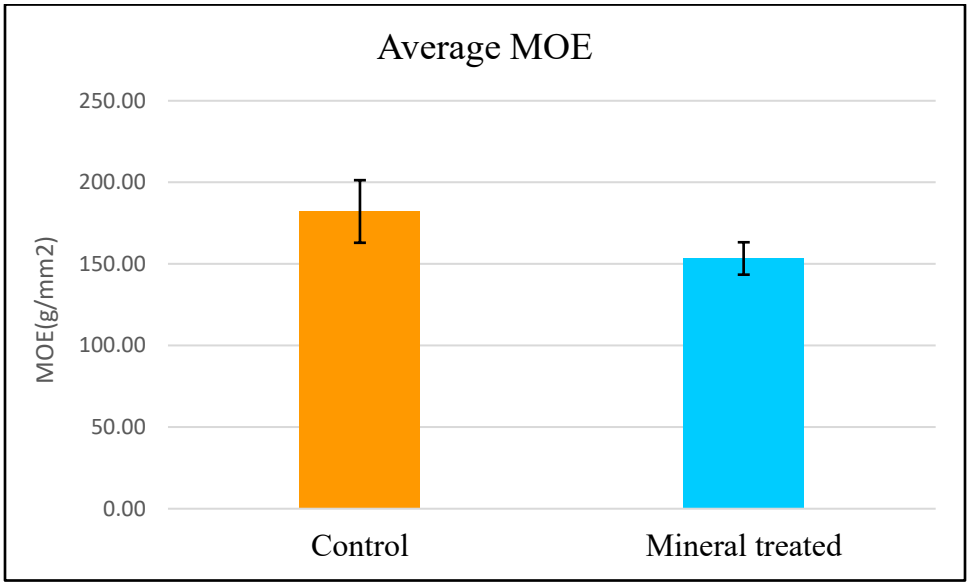

Figure A.1 MOE values for pine control and mineral-treated samples after 3 weeks exposure

A.2 Soil block test – viability of fungi

Table A.2 Mass loss (ML) of pine and aspen samples decayed by different fungi exhibited in a laboratory soil block test (AWPA E10)

Type of fungus	Fungus	% ML-aspen	%ML-pine
White rot	*T. versicolor*	67	47
White rot	*T. versicolor*	67	53
White rot	*T. versicolor*	62	56
White rot	*T. versicolor*	71	60
White rot	*T. versicolor*	76	64
White rot	*T. versicolor*	76	61
White rot	*T. versicolor*	56	51
White rot	*I. lacteus*	72	59
White rot	*I. lacteus*	70	54
White rot	*I. lacteus*	67	58
White rot	*I. lacteus*	69	55
White rot	*I. lacteus*	66	64
Brown rot	*I. lacteus*	65	63
Brown rot	*G. trabeum*	66	71
Brown rot	*G. trabeum*	68	74
Brown rot	*G. trabeum*	60	71
Brown rot	*G. trabeum*	59	65
Brown rot	*G. trabeum*	63	70
Brown rot	*G. trabeum*	62	69
Brown rot	*P. placenta*	58	65
Brown rot	*P. placenta*	67	73
Brown rot	*P. placenta*	65	66
Brown rot	*P. placenta*	71	76
Brown rot	*P. placenta*	64	76
Brown rot	*P. placenta*	68	68
Average		67	64

A.3 Identified volatiles

Table A.3 List and characteristics of volatiles distinguished by PCA

Common Name	Empirical formula	Molecular weight	Elution time (min)
2,4-bis(1,1-dimethylethyl)phenol	$C_{14}H_{22}O$	206.17	25:00
2,6-di-tert-butyl-p-benzoquinone	$C_{14}H_{20}O_2$	220.15	23:97
2-ethyl-1-hexanol	$C_8H_{18}O$	130.14	12:80
2-Furoic hydrazide	$C_5H_6N_2O_2$	126.04	9:76
2-undecanone	$C_{11}H_{22}O$	170.17	19:70
α-cubebene	$C_{15}H_{24}$	204.19	21:92
cyperene	$C_{15}H_{24}$	204.19	21:15
α-himachalene	$C_{15}H_{24}$	204.19	23:96
α-pinene	$C_{10}H_{16}$	136.13	10:26
α-terpineol	$C_{10}H_{18}O$	154.14	17:50
α-gurjunene	$C_{15}H_{24}$	204.19	20:45
α-cadenene	$C_{15}H_{24}$	204.19	25:70
β-cadinene	$C_{15}H_{24}$	204.19	25:17
benzaldehyde	C_7H_6O	106	11:15
benzeneacetaldehyde	C_8H_8O	120.06	13:47
β-pinene	$C_{10}H_{16}$	136	11:60
limonene	$C_{10}H_{16}$	136	13:00
methyl-2-furoate	$C_6H_6O_3$	126	11:33
nonanal	$C_9H_{18}O$	142	14:97
sativene	$C_{15}H_{24}$	204.19	22:50

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