Pilot study investigating the potential of filamentous fungi for textile bioremediation

This thesis is submitted by Rachel HARPER in partial fulfilment of the requirements of the University for the MSc by Research.

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Abstract

Synthetic petroleum-derived textiles shed microfibres that contribute to microplastic pollution and tonnes of textiles are disposed of in landfills every year. There is a need for sustainable methods to safely dispose of textile waste, and bioremediation may be the key. This is a pilot study to assess whether wood decay fungi show any ability to bioremediate mixed-fibre textiles, under controlled conditions that may then be scaled up for industry use.

Microcosms were established with two wood decay fungi species, *Hypholoma fasciculare* and *Serpula himantioides*, grown on mixed-fibre semi-synthetic fabric cut from pre-washed garments. Three types of fabric were used, each with a cotton/bamboo viscose base, but with varying percentages of elastane. Microcosms were then incubated at 25 °C, and data sets (light microscopy and scanning electron microscopy, dye loss and volatile production via GCMS) were collected at 3, 5 and 8 months.

This is a new field of study and as such new protocols are required to establish the nature of the fungal interaction and potential degradation of the textiles. The samples showed significant loss of dye from the fabric, as determined by paired two-tailed t-tests (p-values<0.05 for all fabric types with *Hypholoma fasciculare*), accompanied by changes in volatile production over time. Scanning electron microscopy showed damage to textile fibres and the increasing presence of crystal metabolites. This is the first study of its kind to have recorded bioremediation of dye directly from fabric using filamentous fungi. Overall, initial results look promising and core methods have been established that can be used for comparison with other studies.

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1. Introduction

1.1 An overview of sustainability in the fashion industry

The acceleration of climate change has led to closer attention being paid to the environmental impact of items being produced and sold, including products within the fashion industry. A combination of unrelenting fashion cycles and the availability of 'fast fashion' has led to some sections of the fashion industry "promoting obsolescence" with disregard for the disposal of unwanted items of clothing (Lewis, 2015). Equally, sustainable practices are on the rise, as some large companies like H&M have widespread sustainability initiatives – from funding companies to explore various options for thread made from waste products to the implementation of in-store garment recycling machines (Hennes & Mauritz AB, 2021). They have also restricted the chemicals used in the process of making clothing items, reduced packaging, and set up their Garment Collecting program to reuse or recycle unwanted garments from any brand (Hennes & Mauritz AB, 2021). While this is an effective multi-faceted approach to reducing total environmental impact, it does rely on consumer interaction in terms of their choices when purchasing clothing and using the Garment Collecting program, and ultimately does not cover what happens when people choose to throw away their clothes rather than make use of such programs.

As more consumers become environmentally aware, more purchases are being made with sustainability in mind, with trends suggesting that they prefer companies with active sustainability campaigns (Neumann *et al*, 2021; Zhang *et al*, 2021). However, companies with 'eco-friendly' campaigns may not always fully deliver on what is promised. The term used to describe such acts is 'greenwashing', coined by Jay Westerveld in an essay in 1986 about the hotel industry (cited in Orange and Cohen, 2010), and was used to point out that companies produced more advertisements promoting how 'green' the company was than effort put into actually making products more environmentally friendly (Orange and Cohen, 2010). The effects of both fast fashion and greenwashing within the current state of the industry are devastating, as they impede progress towards sustainability, and oftentimes the environmental damage is done in low and middle income countries (LMICs) that produce the garments, as pollution is less likely to be controlled or monitored as strictly (Papamichael *et al*, 2022). End of life fabrics and unwanted garments are also exported to LMICs as waste or an attempt at recycling – in 2021 alone, 60000 tons of textiles and clothes exported from EU and USA to Chile were dumped in the Atacama Desert (Papamichael *et al*, 2022).

Data modelling studies have identified that the most damage done to the environment within the life cycle of a garment occurs before the product even gets to the consumer (Peters *et al*, 2021). The entire life cycle of an item of clothing has impact on the environment – acquisition of materials for

fabric and processing into thread require large volumes of water and the use of fossil fuels to power machinery (Wang *et al*, 2017). Dyeing the fabric uses large volumes of water and often the wastewater is discarded into sewer systems that can leach into freshwater sources (Stone *et al*, 2020). Shipping the finished garments requires fossil fuels, which increases carbon emissions (Niinimäki *et al*, 2020). Once purchased, washing clothes requires a significant amount of water and microfibres from synthetic textiles are shed each time they are cleaned (De Falco *et al*, 2017). Disposal of an unwanted or end of life garment leads to more waste in landfills, and microfibres from synthetic textiles can leach into marine and freshwater ecosystems via the groundwater (Niinimäki *et al*, 2020). Even fibres with a natural origin such as cotton have significant impact on the environment from the sheer volume of water used to grow and process the cotton into thread and can therefore not be considered fully sustainable in the current industry (Shen *et al*, 2015).

The disposal of garments is the least investigated part of the life cycle of a garment in terms of the potential to mitigate negative environmental impact. In America alone, clothing occupied nearly 5% of landfill space as of 2018 (Bick *et al*, 2018). Whether it be from consumers or companies, disposal is a neglected part of the life cycle of garments, and the volume of waste continues to build in landfills as it was estimated in 2017 that “every second, the equivalent of one garbage truck’s worth of textiles is landfilled or burned” (Ellen MacArthur Foundation, 2017). With an estimated 62 million tonnes of apparel purchased every year, now more than ever, a solution for end-of-life garment disposal is needed (Niinimäki *et al*, 2020). Alternative methods to landfill or incineration need to be developed to safely dispose of used clothing.

1.2 Current trends in the sustainable fashion industry

Consumer awareness of ethical and environmental issues and how the fashion industry is linked to them has led to increased sales for ethical clothing brands over time (Figure 1).

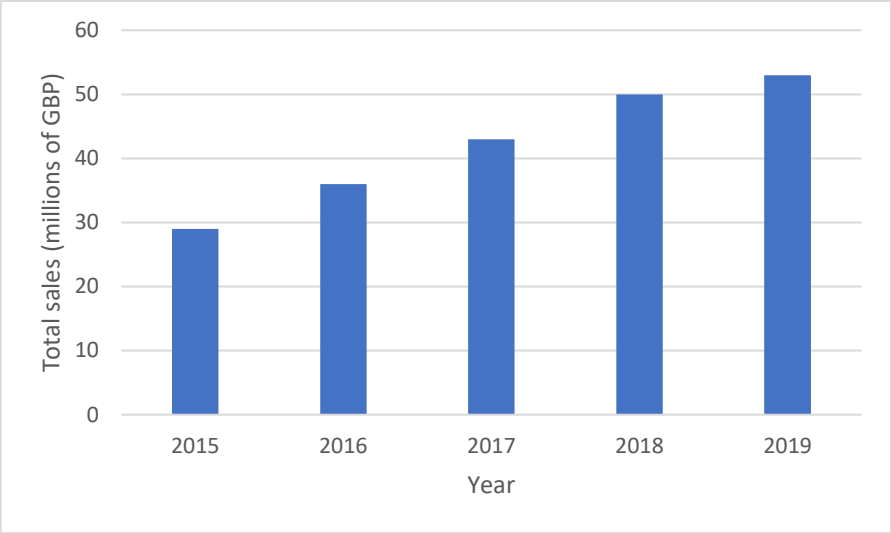

Figure 1: The total ethical clothing sales revenue in the UK 2015-2019 (Ref: Ethical Consumer Market Reports 2016-2020).

Smaller companies pursuing sustainability goals do not have the same resources as larger companies, and as such, must either rapidly develop sustainability initiatives or be environmentally-minded from the beginning. For the latter, one such company is Bamboo Clothing. The company uses bamboo viscose mixed with cotton in their products as a more sustainable alternative to petroleum-based textiles like polyester, and is investigating multiple methods of reducing the environmental impact of their products, including the disposal of end of life or waste garments (Bamboo Clothing Ltd, 2021). For some companies, unsold merchandise is among multiple items routinely destroyed rather than recycled – for example, Burberry destroyed £28.6 million worth of unsold products, including garments, in 2017 (Burberry PLC, 2019). However even when recycling, there is a substantial amount of waste material being generated, with 1.1 million tonnes of textile material lost during collection and processing of a total 6.4 million tonnes (Niinimäki *et al*, 2020). Garments generated by 'fast fashion' companies deteriorate after ten washes due to poor quality of material (Joy *et al*, 2015), making them a difficult challenge for recycling or reuse and likely to end up in landfill or incinerated, which is unsustainable and reduces local air quality.

1.3 Current challenges in textile waste disposal

Disposal of textile waste is a challenge as currently the assumption is made that textiles will all easily degrade naturally over time, yet approximately two-thirds of all textile items contain synthetic fibres

with the most common types being petroleum-based synthetic polymers, which are not readily biodegradable (Henry *et al*, 2019; Szostak-Kotowa, 2004). The different fibre types within mixed-fibre textiles have different timeframes for naturally degrading, so the natural fibres degrade first, leading to more microfibre release within landfills as the synthetic fibres are left to degrade over a longer period of time (Haslinger *et al*, 2019; Liu *et al*, 2020). Additionally, many garments use fastenings or "trims" like buttons, that decrease the sustainability of garments significantly as they may also contain or are comprised of plastics which are inert and will not naturally degrade on their own (Fletcher and Grose, 2011). The challenge is to find sustainable methods of safely breaking down all fibres within an end-of-life garment within a similar timeframe, and one such method may be through bioremediation.

1.4 The potential for fungi to bioremediate textiles

Bioremediation has been defined as the elimination, attenuation, or transformation of polluting substances through the use of biological processes (Lynch and Moffat, 2005) and as part of this, bacteria and fungi are currently being tested for use in mitigating and removing traces of hazardous chemical spills, radioactivity, and both micro- and macro- plastics, including microfibers from clothing (Chatterjee *et al,* 2017; Noman *et al,* 2019; Treu and Falandysz, 2017; Singh *et al,* 2020; Viswanath *et al,* 2014). Certain fungi are potentially good candidates for bioremediation of textiles as their decay mechanisms may be able to degrade everything in fabric offering the potential for complete mineralisation of waste fabric (Baldrian and Valaskova, 2008). As some fungi are filamentous, they can grow on and through fabrics by branching out thin hyphae, making it easier for potential degradation to occur through widespread coverage of the textile. Fungi have been proven to be capable of xenobiotic degradation in previous studies into degradation of microplastics, and so they may also prove to be able to do the same with fabrics (Balasubramanian et al, 2014) - however the species tested so far are soil-based Ascomycetes mainly, such as *Aspergillus sp*. and *Fusarium sp.* (Sakhalkar and Mishra, 2013).

Wood decay fungi in particular are known to naturally degrade and metabolise cellulose (and other sugars used structurally in plant matter, such as hemicellulose), making it likely that they will at least be able to degrade the cotton, and possibly the bamboo viscose within the fabric, as viscose is also a cellulose-based polymer (Figure 1). Wood decay fungi are generally split into three categories based on the type of degradation they cause to the wood – white rot or brown rot being the main distinction, and soft rot being an additional category (Carlile *et al*, 1994; Bari *et al*, 2020), although white vs brown rot is recognised as being ends of a spectrum for categorizing fungi and not a true dichotomy (Riley et al, 2014). Species that cause white rot are mainly Basidiomycetes, with few

Ascomycetes such as *Xylaria hypoxylon* and *Daldinia concentrica* now being classified as soft rot species but previously being considered white rot species (Nilsson, 1985; Nilsson, 1988; Anagost, 1998); whilst species that cause brown rot are exclusively Basidiomycetes. The degradation mechanisms for both white and brown rot fungi are extracellular, meaning they can degrade large polymers such as lignocellulose and transport broken-down products into cells to be utilised by the fungi (Li *et al*, 2022).

White rot fungi are able to completely mineralise lignin, a complex and irregular aromatic polymer known to be recalcitrant to breakdown, as the method of degradation they possess has low substrate specificity. They produce a wide variety of extracellular enzymes, including peroxidases that use hydrogen peroxide for activity and then oxidise lignin bonds (ten Have and Teunissen, 2001). Two main types of peroxidases are produced, Manganese peroxidases (MnPs) and Lignin peroxidases (LiPs), and along with laccase, these comprise the main enzymatic arsenal for lignin degradation in white rot species (Arora and Gill, 2000; Reyes et al, 2021). White rot fungi also produce enzymes such as cellulases and hemicellulases, and are thus able to fully break down the components of a lignocellulosic material, such as wood or paper (Pathak et al, 2017; Reyes et al, 2021). The ability to degrade lignin can be linked to the ability to degrade synthetic polymers, providing they have similar chemical structures for the enzymes to attack, such as aromatic rings.

Brown rot fungi are unable to degrade lignin completely, but the mechanism of decay they use is also non-specific, as it uses oxidation via Fenton chemistry, hydrogen peroxide and an Fe^{2+} ion to generate hydroxyl radicals, which then react with the cellulose to break bonds (Arantes *et al*, 2012; Eastwood *et al*, 2011). This non-specificity, along with the extracellular decomposition mechanisms, are why fungi are used and often successful when used in bioremediation (Saraswat *et al*, 2021). As textile waste is solid, it is likely that any filamentous fungi will be able to grow through the material to increase the surface area with which the extracellular degradation processes can occur, making it easier to set up as minimal processing of the fabric would be necessary. Filamentous fungi are known to extend hyphae in this way while growing to forage, maximising surface area reached by the fungus and its enzymes (Moore and Robson, 2011). This is unlike bacterial bioremediation which would require the fabric to be physically broken down and placed into a liquid medium to ferment.

There is a distinct lack of studies regarding the bioremediation of textiles using a methodology that could be scaled up for industrial use and most studies focus on microfibers, textile effluent, and dyes (Bhattacharya *et al,* 2011; Przystaś and Zablocka-Godłewska, 2017; Winardi *et al,* 2019). Studies directly involving textiles either focus on naturally occurring species degrading textiles over time or experimental systems utilising laboratory grade nylon and agar plates (Table 1). While the studies do

not necessarily focus on exactly the same topic as this research, it is important to note that white-rot fungi species are more often reported as having successfully degraded the synthetic fibre nylon, and that the ability of fungi to degrade textile materials is increased in the absence of Nitrogen or Carbon sources (Deguchi *et al*, 1997; Friedrich *et al*, 2007). This indicates that a white rot fungus species in a Carbon- or Nitrogen-poor environment may be the most likely to cause the most degradation to a textile containing synthetic fibres. In this microcosm setup, the only available resources for the fungi to obtain Carbon and Nitrogen from are the originally inoculated wood chip and the fabric samples, but wood contains relatively low concentrations of Nitrogen (Cowling and Merrill, 1966).

The lack of studies can partially be attributed to the assumption that natural fibres will naturally deteriorate quickly without intervention, but with the rise of plastics in textiles and the large volume of waste fast fashion and a growing population generates, this large gap in knowledge must be explored and addressed.

Table 1: Studies with a focus on fungal colonization and degradation of textile materials.

Reference (by year published)	Substrate	Fungi species	Study outcome
Smith, G. (1928)	Cotton	*Aspergillus spp.*	Initial study and identification of Aspergillus spp. isolated from cotton bales and cloth.
Marsh, P.B., Bollenbacher, K. (1949)	Cotton, flax, hemp, jute	*Alternaria sp., Cladosporium herbarum, Fusarium moniliforme, Phoma sp., Leptosphaeria sp., Pullularia pullulans, Aspergillus spp., Penicillium sp., Stachybotrys sp., Chaetomium sp., Aspergillus fumigatus, Halo phiobolus*	Multiple observations of species growing on cotton before harvest, cotton fabric, flax, hemp, and jute in a bale. Mentions different moisture and weather conditions and their impact on fungal growth.
White *et al*. (1950)	Wool	*Fusarium moniliforme*	Studies based on observation of *Fusarium moniliforme* on woolen clothing in the military in 1945, testing multiple species on wool fabric autoclaved in tubes with liquid media and incubated with the fungi.
Siu, R.G.H, and Reese, E.T. (1953); Fisher *et al*. (2020)	Cotton	*Trichoderma reesei*	First observation in the field of this species on cotton tents.
Deguchi *et al*. (1997)	Nylon-66	Unidentified white rot fungus (IZU-154)	When nylon-66 membranes were incubated with the fungus on agar plates with differing nutrient content, and the nylon was degraded more when the fungus was starved of Carbon or Nitrogen.
Friedrich *et al*. (2007)	Nylon-6	*Bjerkandera adusta* and *Phanerochaete chrysosporium*	Out of 58 fungal species, these two degraded the polyamide nylon-6 the most within liquid medium, with no additional Nitrogen sources present.
Blyskal, B. (2014)	Wool	*Gymnoascus arxii*	Raw wool on agar plates with no additional nutrients was degraded the most, but some natural and synthetic dyed samples were also degraded, dependent on chemical structure of the dye.

This project seeks to build evidence for this sector-wide knowledge gap. In collaboration with Bamboo Clothing Ltd, the potential for fungal bioremediation of fabrics will be tested as a potential means of providing a sustainable method for fabric disposal. The products investigated were made primarily of bamboo viscose, a naturally sourced semi-synthetic fibre, mixed with cotton. Mixed fibre textiles, where synthetic fibres are mixed with either semi-synthetic or natural fibres, are representative of the norms of the fashion industry, as mixes like cotton and polyester are some of the most prevalent in garments today.

Bamboo viscose is primarily composed of cellulose from bamboo in polymer form, which would have a structure similar to that of a cellulose polymer, pictured below (Figure 2, middle image). As wood decay fungi are already known to be capable of metabolising cellulose (Baldrian and Valaskova, 2008), the expectation is that the same can be done with the viscose and cotton fibres within the fabric. As white rot fungi can mineralise lignocellulose as well as degrade cellulose, it is likely that they can do the same with elastane, as it is a polymer with multiple reactive groups that could be used to catalyse the process of bond breaking. Additionally, white rot fungi in particular are competent at breaking down complex aromatic compounds into smaller components, like the rings seen in elastane - aromatic diisocyanates (Figure 2, bottom image; Jiang *et al*, 2018).

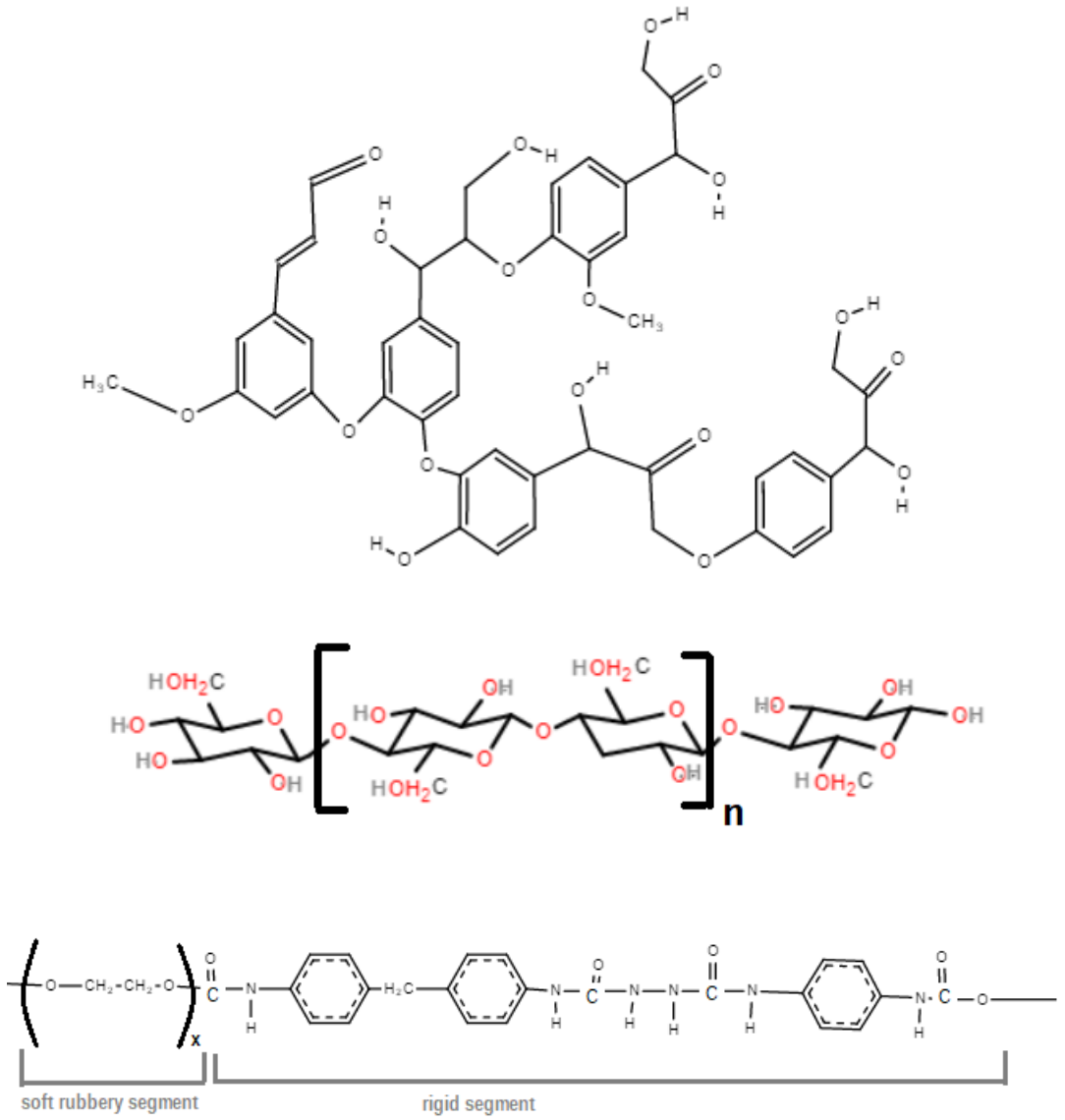

Figure 2: Chemical structure images generated using Chemspider and Microsoft Paint of lignin (top image, inspired by an image found in Mahmood *et al*, 2018), cellulose polymer (middle image, inspired by an image found in Meng *et al*, 2013), and elastane (bottom image, inspired by an image found in Reisch, 1999)

As previous studies show that fungi can bioremediate textile dyes from wastewater from the dying process, it is possible that the fabric does not need to be pre-treated for dye removal before incubation with fungi, as certain fungi may able to metabolise both fabric and dye (Bhattacharya *et al* 2011; Yesilada *et al*, 2018). Both species used in this project are wood decaying basidiomycetes, the same as the previously identified *Bjerkandera adusta* and *Phanerochaete chrysoporium*. The chosen species were a white rot fungus, *Hypholoma fasciculare*, and a brown rot fungus, *Serpula himantioides*. These species were chosen for this pilot study as their different methods of decay

would give an indication of which biological processes may be important for bioremediation and inform choices of fungi for future studies.

The disposal of end of life or unwanted garments is of extreme environmental concern, and bioremediation may be the key to safe textile disposal. However, the knowledge gap is huge and more research must be done into potential bioremediation methods, and the industry is clearly in need of such methods to be developed as consumers look for involvement in sustainability before purchasing products. As such, the project is a timely and informative pilot study.

This pilot study aims to assess the mycoremediation potential of *H. fasciculare* and *S. himantioides* for textiles waste. Key objectives include:

1. To develop an agar-independent microcosm for assessment of growth of trial fungi on textiles.
2. Assess growth of study fungi on fabrics with a range of synthetic textile content, using microscopy techniques.
3. Identify the volatile metabolome released during degradation, including the impact of increasing synthetic content.
4. To develop a protocol for quantification of bioremediation of the dye under the different conditions.

2. Materials and Methods

2.1 Culturing fungi

The strains used in this study were *Serpula himantioides* (MUCL38935, from Professor Inger Skrede at the University of Oslo, Norway) and *Hypholoma fasciculare* (HfGTWV2, from Professor Lynne Boddy at Cardiff University, Wales). Fungal strains were maintained on 2% malt extract agar (henceforth referred to as MEA: comprised of malt extract from Oxoid, UK and agar from Thermo Fisher Scientific, USA), 2% MEA plates inoculated with the fungi were incubated at 25 °C in the dark (LMS Cooled Incubator Model 600, LMS, UK).

2.2 Woodchip colonization setup

There were two different species of wood used to accommodate for what each species of fungi would normally grow on and therefore encourage growth, Scots Pine (*Pinus sylvestris,* for *Serpula himantioides)* and European Beech (*Fagus sylvatica,* for *Hypholoma fasciculare*). The wood was

provided as 2 cm^3 blocks of Pine (sourced from Portswood Timber Supplies, UK) and Beech (sourced from John Harrison, UK) that were then chipped using a hammer and chisel into chips that on average weighed 0.3442 g for Beech and 0.2754 g for Pine. The wood chips were autoclaved twice (15 min at 121 °C, two separate runs) then soaked in sterile dH_2O overnight before being drained and autoclaved for a third time. At least ten of these wood chips were added to 0.5% MEA in an autoclave sterilised 250 ml polypropylene pot (sourced from Cater4you Ltd, UK), and inoculated with the appropriate fungus. These pots were incubated at 25 °C for 4 weeks in the dark, to allow the fungi to fully colonize the wood chips, before they were added to the microcosm setup.

2.3 Microcosm setup

A layer of sterile perlite (sourced from Westland Horticulture Ltd, UK) was placed in the bottom of each sterile 250 ml polypropylene pot, and approximately 15 ml of sterile dH_2O was added. Using a sterile needle, 5 holes were poked in the side of each pot and were then covered with micropore tape (sourced from Wilko, UK) to allow airflow.

The fabric (in the form of three different clothing items provided by Bamboo Clothing Ltd) was washed in a domestic washing machine using a non-biological washing powder 5 times at 30 °C with 1000 rpm spin cycle. The fabric was then line dried outside before use.

To each pot, three pieces of 3 cm x 3 cm fabric were added, and a total of 90 microcosms were set up, with 45 per fungal species as shown below (Table 2).

Table 2: Visual aid for explaining microcosm type allocation and replicates that were set up.

	3 months	**5 months**	**8 months**	**Subtotal**
0% elastane	5	5	5	15
4% elastane	5	5	5	15
12% elastane	5	5	5	15
				Total: 45 per species

Pre-colonised wood chips were scraped to remove agar, then placed on top of the fabric, in the centre of the pot. All pots were sealed, labelled, and incubated in the dark at 25 °C.

2.4 Gas Chromatography/Mass Spectrometry (GCMS) of volatiles

A StableFlex™ 2 cm Solid-phase microextraction (SPME) fiber (Supelco, USA) was attached to the inside of a bell jar and used to capture volatiles released from an opened microcosm for one hour.

Three microcosms were chosen per fabric type to be sampled, for a total of nine samples per timepoint (3 months). The SPME fibers, once retrieved, were placed into a manual SPME holder (Supelco, USA), and manually injected into an Agilent 6890N GC with 5973N Inert MSD and 7683 Injector (Agilent, USA). Method run with a starting temperature of 80 °C up to a maximum of 320 °C with the temperature increasing at a rate of 10 °C per minute – for a total run time of 29 minutes. The 3 min solvent delay was overridden as the quantity of any volatiles captured on the SPME fibre was unlikely to require extra dilution before being sampled. At least 3 minutes were left between each subsequent run to allow the machine to cool down to its starting temperature of 80 °C. Peak data was collected from Total Ion Chromatograms (TICs) generated using Chemstation (Agilent, USA), and potential compound matches were collected from the NIST 2.0 database (National Institute of Standards and Technology, USA) for each peak. Quality cutoff point was 60, and only compounds that were at or above 60 were recorded. Compounds with a NIST quality match (qual) of 60 or higher were then manually curated using the following databases to identify any known functions or health hazards associated with them: MetaCyc Metabolic Pathway Database version 20 (Caspi *et al*, 2020); PhytoHub version 1.4 (Bento da Silva *et al*, 2016); the Yeast Metabolome Database version 2.0 (Jewison *et al*, 2012; Ramirez-Guana *et al*, 2017); Golm Metabolome Database (Hummel *et al*, 2007); the Metabolomics Workbench (https://www.metabolomicsworkbench.org/search/index.php); and PubChem (Kim *et al*, 2019) for hazards and toxicity.

Control samples were run in the same way, but instead of fungi on the samples, fabric squares with the relevant wood chip type (Beech or Pine) were added to a microcosm pot setup with perlite and distilled water and volatile capture was done. One sample was done for each combination of fabric type and wood type, for 6 total control samples, with an additional sample done with just the SPME fibre inserted into the manual injection port of the GC/MS. Any compounds listed in the control samples were then removed from the lists for each sample to generate the final tables.

2.5 Scanning electron microscopy (SEM) of fabrics

A 1cm x 1cm square of fabric was cut from each sample and affixed to individual titanium stubs using a double-sided adhesive carbon disk, and then placed into a sputter coater (Polaron SC7640 Sputter Coater, Quorum Technologies, UK). One side of the fabric sample was coated with gold palladium, with the machine set to 2 kV for 2 min at 20 mA, with Argon pumped into the chamber and maintained at a pressure of 10^{-7} mbar/PA. Coated samples were kept and could be imaged for up to 6 months following this process. Each sample was placed into the chamber of the SEM (Zeiss Evo 50, Zeiss, Germany), with the beam set to 5 kV. Images were taken at various magnifications,

ranging from 35 to 2500x. Samples used were from microcosms at the different time points, and controls were taken from the original fabric provided.

2.6 Light microscopy

A 1 cm x 1 cm square of fabric was cut from each sample at the appropriate time points and placed into individual Petri dishes. These samples were then imaged using a Nikon SMZ1500 (Nikon, Japan) microscope. Scale bars were then added to each image and saved. Objective lenses used ranged from 1x to 2x, but generally 1x was used.

2.7 Thermogravimetric analysis of fabric to determine dye content

Samples from each of the three fabric types was cut into 1 cm x 1 cm pieces, and 10 mg for each fabric type was weighed into an alumina crucible for loading into a Thermogravimetric Analyzer (Mettler Toledo, US). The starting temperature was set to 25 ^{o}C, and machine was programmed to increase to 1000 ^{o}C in increments of 10 ^{o}C per minute. Results were recorded as graphs recording mass change over time in the STARe software (Mettler Toledo, US).

2.8 Acid digestion of fabric

The ability to fully digest fabric samples was tested using two different acids, in order to assess the dye content of a 3 cm x 3 cm square of fabric. Both concentrated and diluted Hydrochloric acid and Sulfuric acid were used (both from Fisher Chemical, UK), for a total of 4 acid mixes tested. As an initial test, 0% elastane fabric samples were used only to determine if the acid mixes were capable of total fabric digestion. One 3 cm x 3 cm square of prewashed 0% elastane fabric was placed into each volumetric flask, and 20ml of each acid (Sulfuric acid 95%, Hydrochloric acid 37%) was added to one of two flasks. For the remaining two flasks, the total volume was 40 ml, as 20 ml of each acid was mixed with 20 ml of distilled water to create 50% dilutions of each acid. All flasks were sonicated for 1 min in a 45°C water bath to ensure complete absorption of the acid into the fabric. After 1 hour, a 1:1000 dilution of each acid and fabric mix was taken to measure absorbance at 597 nm on a spectrophotometer (Cary UV-Vis Compact, Agilent, USA). The dye used in the garments was reactive black 5 (RB5), a very common black dye in the textile industry. RB5 in powder form was obtained from Sigma (Sigma-Aldrich, USA) to act as a control, and the stated λ_{max} of RB5 is 597 nm.

2.9 Dye extraction assay

This protocol was based on the method developed by Home and Dudley (Home and Dudley, 1981). A 1 cm x 1 cm square of fabric from each sample was rolled up and placed into Eppendorf 1.5 ml microcentrifuge tubes, with approximately 1 ml 1.5% (v/v) aqueous Sodium hydroxide (VWR Chemicals, USA) added to each tube. Each tube was incubated on a heat block (Techne® Dri-block® heater Model DB-2A, Cole-Palmer, UK) at 95 ^{o}C for 3 min. Liquid was removed from Eppendorf tubes using pipette, and added to cuvettes for measurement of absorbance via use of a spectrophotometer (Unicam Helios Epsilon, Thermo Fisher, USA). Fabric samples without fungi were used as a control.

RB5 dye was used to make standards, with a range from 1 mg/L to 10 mg/L. Prior to taking absorbance measurements, the samples were all diluted 1:4 in 1.5% (v/v) aqueous Sodium hydroxide, as the undiluted samples exceeded an absorbance reading of 1. The same was done for the controls. 1 ml of 1.5% (v/v) aqueous Sodium hydroxide was used as a blank in a spectrophotometer set to a wavelength of 597 nm, and absorbance of the samples was then checked at the same wavelength.

2.10 Statistical analysis of dye extraction data

Statistical analysis of the absorbance data was done using t-tests in Microsoft Excel. Paired t-tests were done when comparing the samples at 8 months to the controls, independent t-tests were done for comparing samples from the two species, and all tests were two-tailed. These statistics can be found in Appendix 2 and 3.

2.11 Bioinformatics for each of the fungal species used

Both *Hypholoma fasciculare* and *Serpula himantioides* were searched for their genome in the JGI mycocosm database (Grigoriev *et al*, 2014). There is no full genome listed for *Hypholoma fasciculare,* so *Hypholoma sublaterium* was used instead. Searches were done for laccases, peroxidases, and cellulases for each species and only the search results that had relevant annotations (e.g. laccase specified in the annotation for the search result) were counted. Search results without annotations or with irrelevant annotations were not counted, as well as those with no E.C. number listed. The E.C. number, gene type, annotation, and protein ID were compiled into tables and graphs.

3. Results

This study used three types of fabric to assess mycoremediation with an increasing synthetic content in the textiles. The fabrics were a 100% natural fibre mixture of cotton and bamboo viscose (0% elastane), the same natural fibre mixture with 4% elastane, and the same natural fibre mixture with 12% elastane. The fabrics were pre-washed and line dried to simulate regular garment usage and bioremediation of end-of-life textile waste. A range of imaging techniques were used to give a baseline and to document changes over time in the different fabrics.

3.1 Imaging uncolonized fabric

An initial step was to document the impact of use, simulated via washing the fabric. Light microscopy was used to image both the washed 'used' and the unwashed 'off-the-shelf' fabrics. There are little to no differences in surface-level appearance between washed and unwashed samples, suggesting that washing the fabric did not cause that much damage (Figure 3). The different fibres within the fabrics were not visualised using light microscopy, but were visible and identified within the fabric using SEM comparisons to individual fibre samples (Figure 4). The damage to the fabric caused by washing was only visible in SEM images (Figure 4).

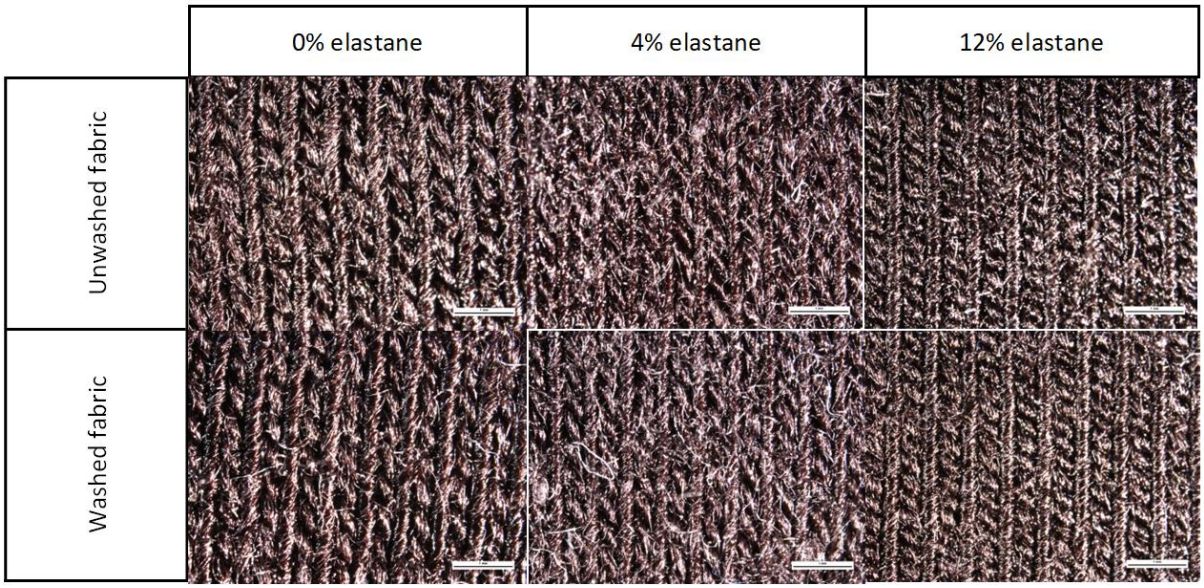

Figure 3: Light microscopy images of the unwashed and washed fabric samples of differing elastane content at 1x magnification. These are representative images for the samples, from several that were taken.

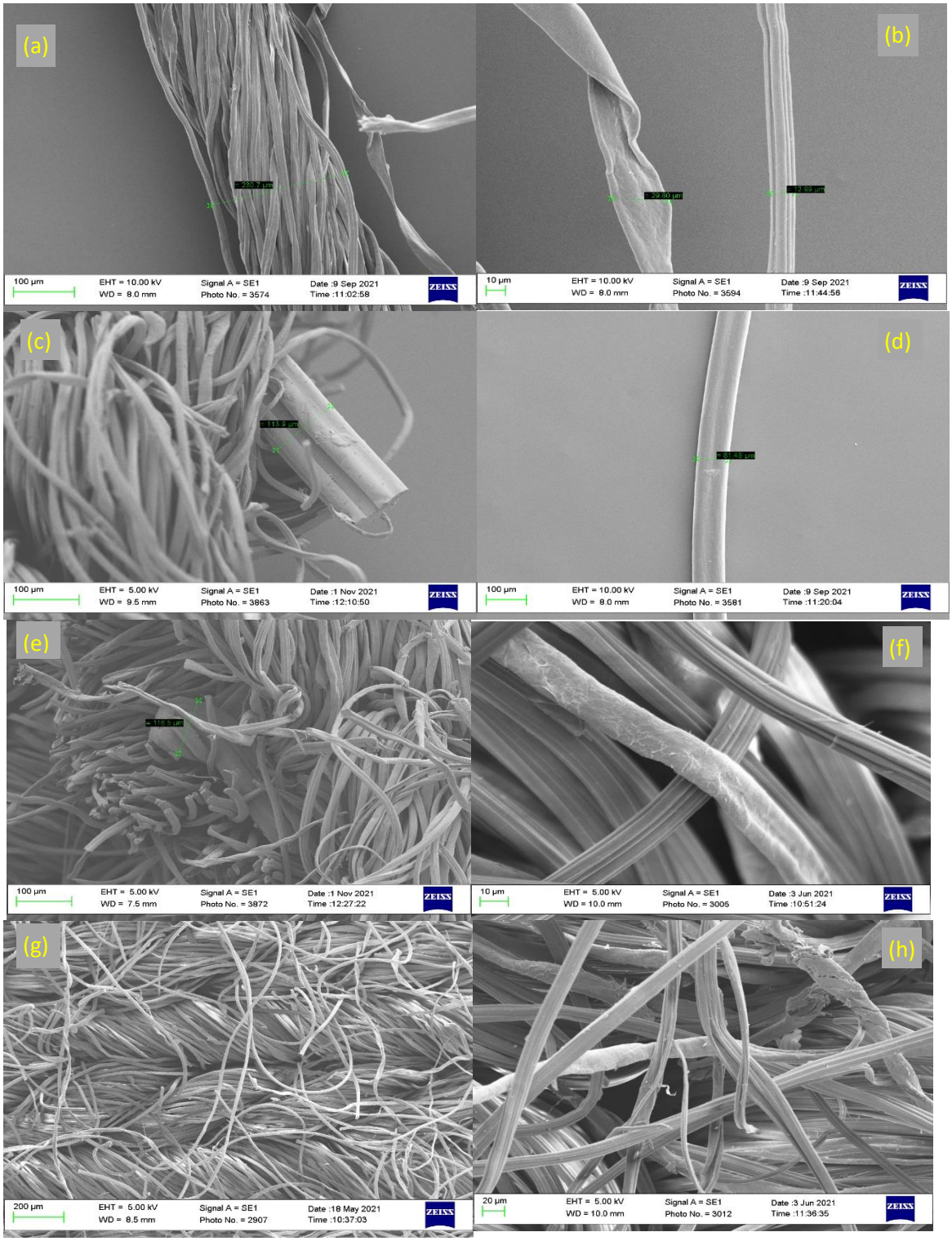

Figure 4: SEM images of individual fibres to identify fibre types within the fabric. (a) shows a single woven thread of the bamboo viscose and cotton mix, with a total width of 230.7 µm. (b) shows individual fibres of cotton (left) and bamboo viscose (right) from the thread, with widths of 29.8 µm and 12.99 µm respectively. (c) and (e) show elastane fibres identified within the woven fabric structure seen in (g). (f) shows a clearer example of cotton vs bamboo viscose fibres in the fabric, the

bamboo viscose having striations and the cotton being less uniform. (h) shows some of the damage to the individual fibres seen in the washed fabric. These are representative images for the washed fabric controls, from several that were taken.

3.2 Image timelines of growth in microcosms

The microcosms were incubated for a total of 8 months and photographed regularly throughout to document growth. Within three months, both species appeared to have grown across the surface of the fabric, but the *H. fasciculare* microcosms exhibited a colour change as the dye appeared to be removed from the fabric. Dye removal was almost complete by 8 months with the fabric returning to close to a natural undyed colour (Figure 5). This process was impacted by the elastane content, as the colour change was most obvious in the fabric with no elastane (Figures 5, 8). On visual examination, *S. himantioides* did not produce the same colour reduction (Figures 6, 9).

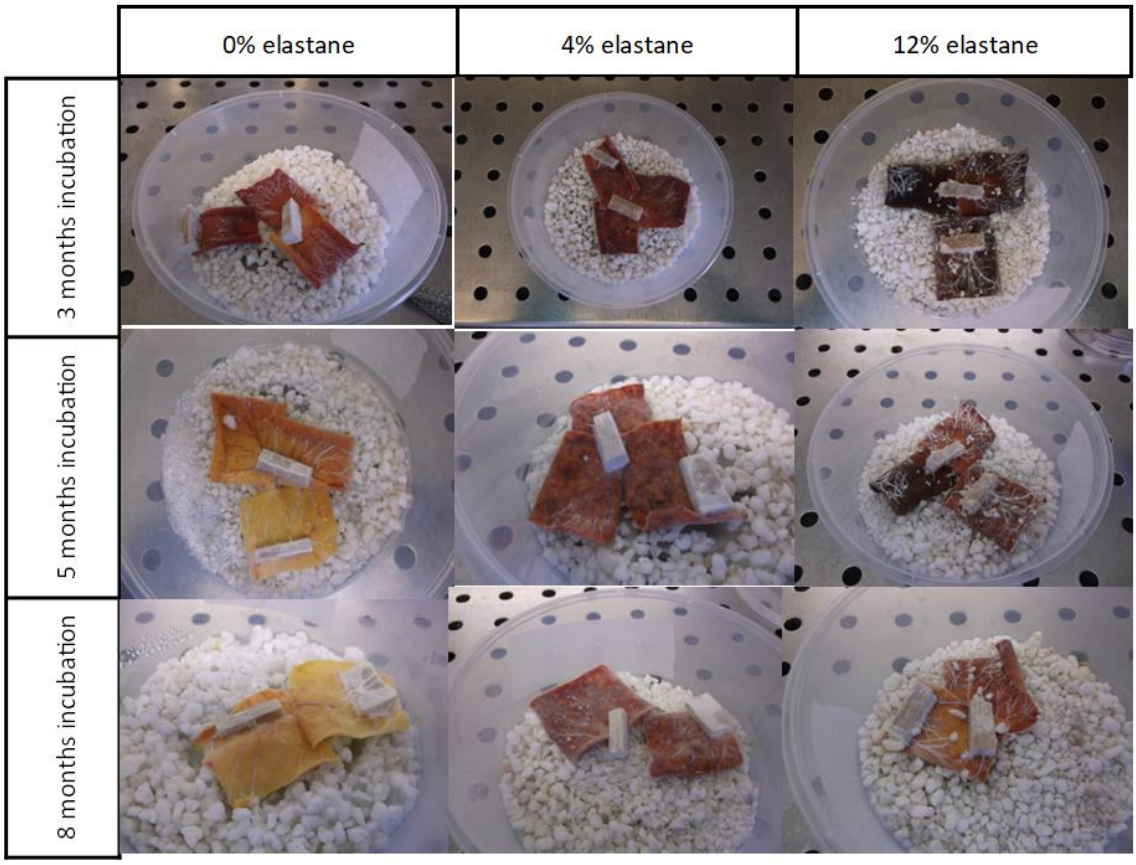

Figure 5: Photos of microcosms inoculated with woodchips containing *H. fasciculare*, showing the progression of colonisation and dye removal over time in each of the different fabric types. These are representative images for the samples, from several that were taken.

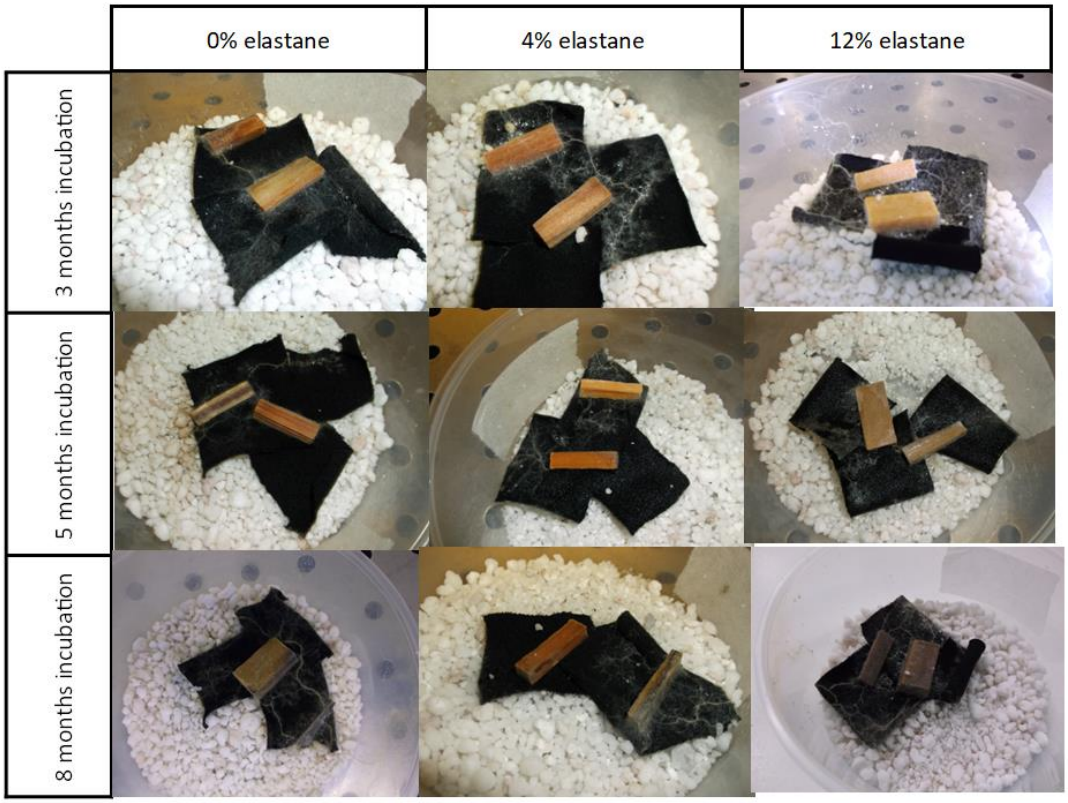

Figure 6: Photos of microcosms inoculated with woodchips containing *S. himantioides*, showing the progression of the fungal growth on the different fabric types. These are representative images for the samples, from several that were taken.

The fabrics were examined by light microscopy and SEM to identify any physical damage caused by the fungus over time. Upon closer examination using light microscopy, *S. himantioides* had clearly just spread across the surface of the fabric (Figure 7), whereas the *H. fasciculare* had hyphae growing through the fabric, and focal colour change had occurred (Figure 7).

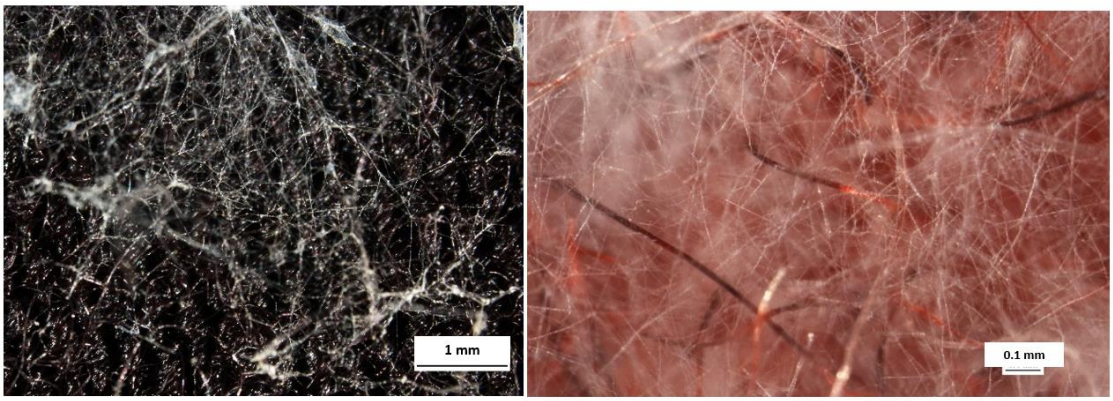

Figure 7: Light microscopy images of fungal hyphae after 3 months incubation with the fabric. Left is *S. himantioides* on 4% elastane fabric, right is *H. fasciculare* on 4% elastane fabric. These are representative images for the samples, from several that were taken.

For *H. fasciculare*, the dye removal was most rapid in the 0% and 4% elastane samples that were incubated for 3 months, but by 5 months the 0% elastane samples were paler than the 4% elastane samples (Figure 8). At 8 months, the 0% elastane samples are much paler than the others, indicating more dye was removed. In the 12% elastane samples, the dye removal at 3 months was obvious but not as fast as the other two fabric types, and by 8 months, was comparable to the 4% elastane samples.

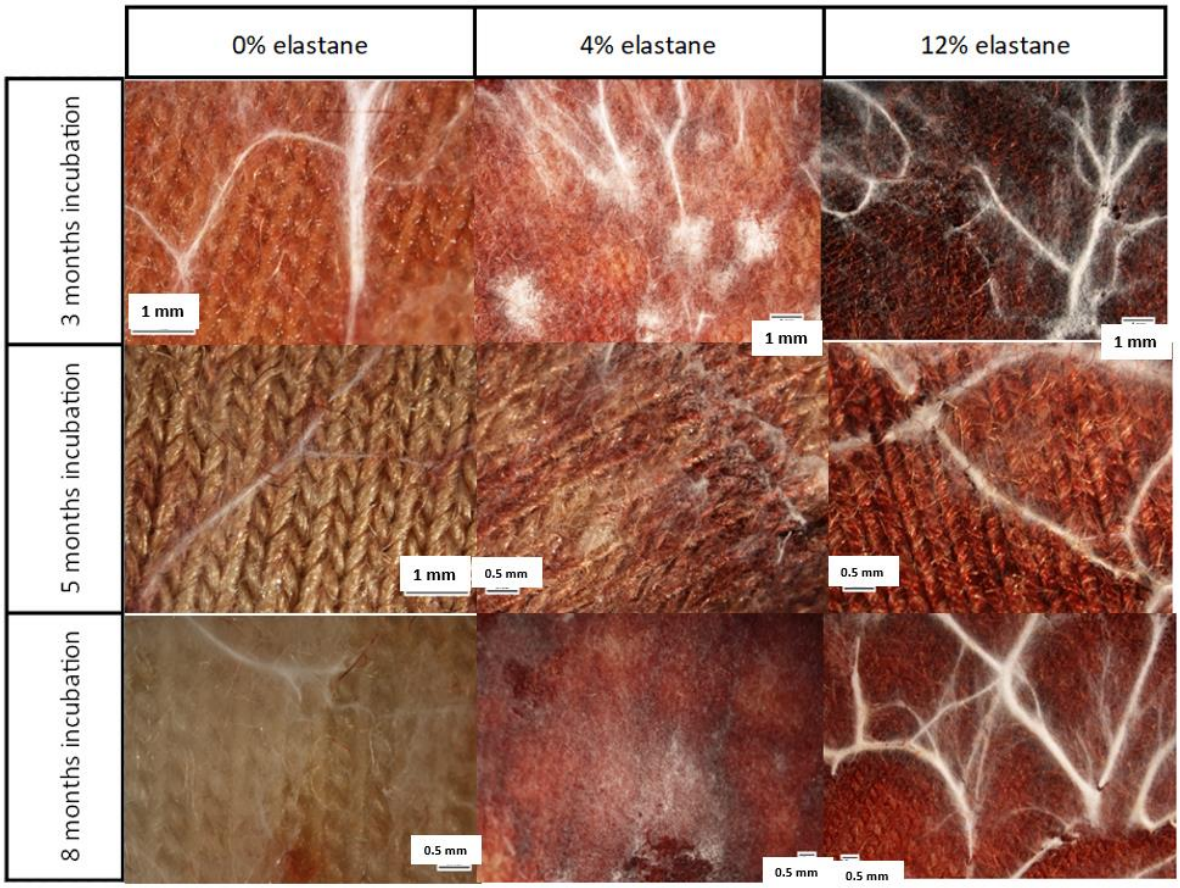

Figure 8: Light microscopy images of 1 cmx1 cm samples cut from fabric inoculated with *H. fasciculare* at various timepoints and on fabric with varying elastane content. These are representative images for the samples, from several that were taken.

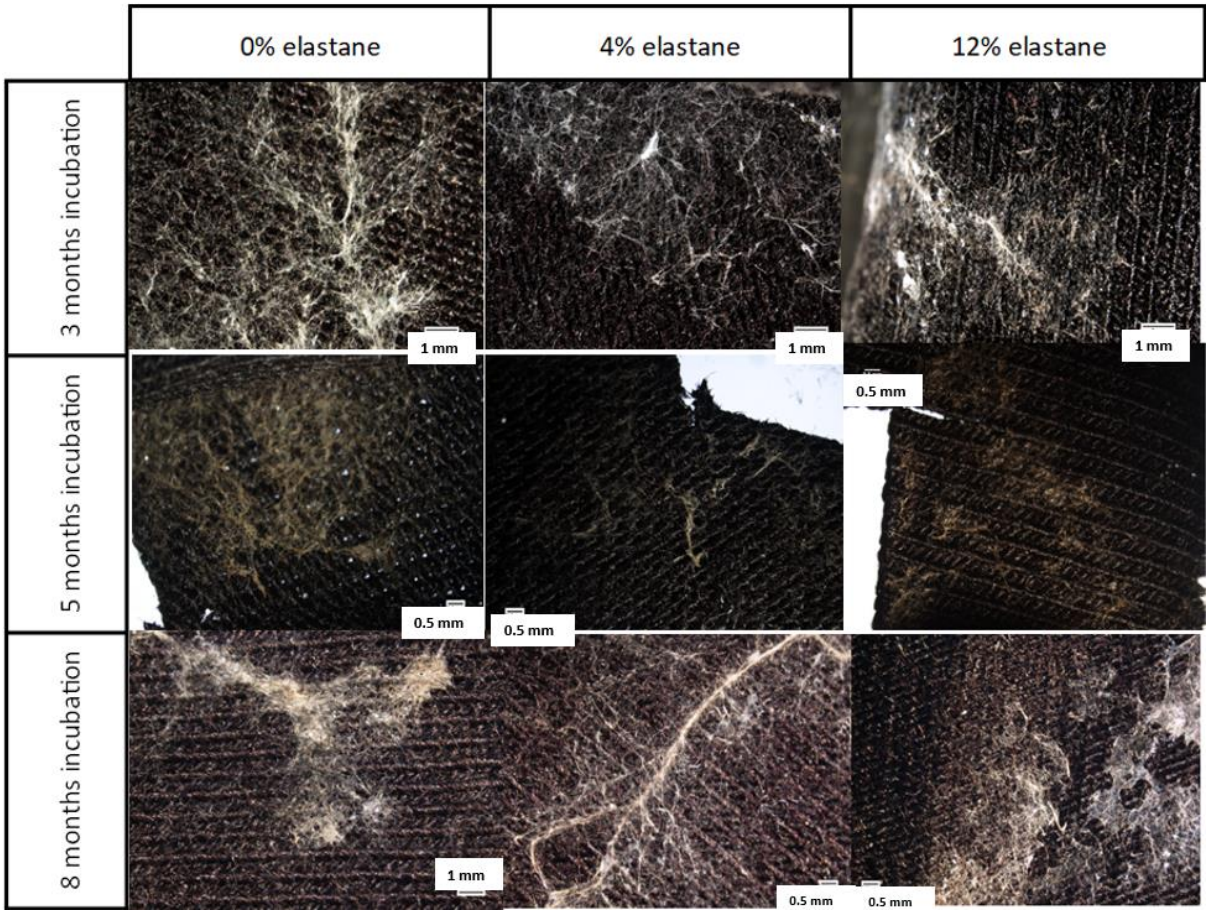

Figure 9: Light microscopy images of 1 cmx1 cm samples cut from fabric inoculated with *S. himantioides* at various timepoints and on fabric with varying elastane content. These are representative images for the samples, from several that were taken.

In comparison with *H.fasciculare*, there appeared to be no major differences in the speed of growth or any particular changes to the fabric across the fabric types for *S. himantioides*.

As any damage to the fabric itself was not immediately visible via light microscopy, SEM was used to image the samples to see if any damage to the fibres by the fungi was observable (Figure 10).

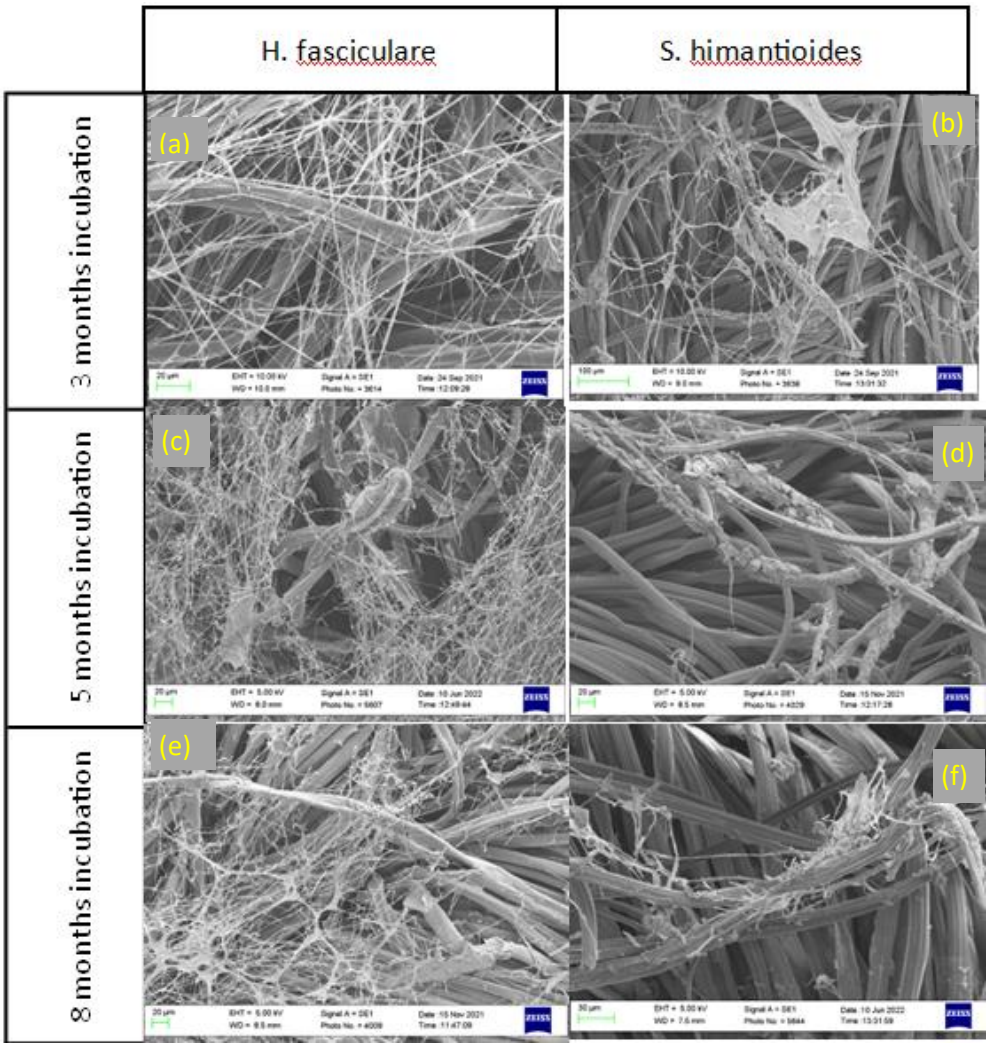

Figure 10: SEM images of *H. fasciculare* and *S. himantioides* samples to observe hyphal growth and potential fibre damage by the fungi. (a) is a 0% elastane fabric sample, (d) and (e) were 4% elastane fabric samples, and (b), (c) and (f) were 12% elastane fabric samples. These are representative images for the samples, from several that were taken.

Any fibre damage that was seen was mainly to cotton fibres ((a), Figure 10), but hyphae appeared to grow and attach to all the different fibres for each species.

Crystalline structures were seen in the samples at 3 months and 5 months for both species. For *S. himantioides*, they were found on the 4% and 12% elastane samples at 3 months, and on the 0% elastane sample at 5 months (Figure 11). For *H. fasciculare*, they were found on all three types of fabric at 3 months, and on 0% and 4% elastane at 5 months.

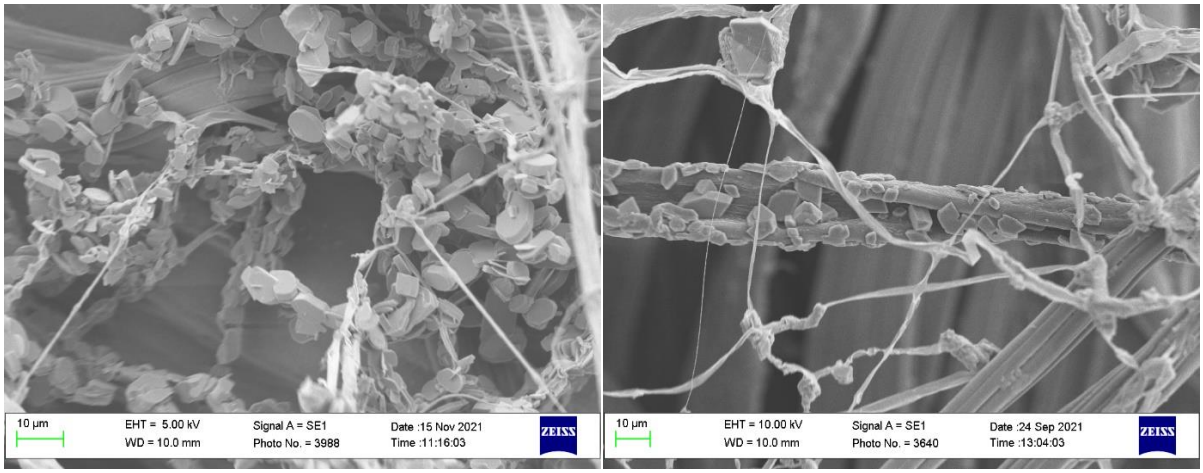

Figure 11: SEM image of crystalline structures on the samples. Left is *H. fasciculare* on 0% elastane at 5 months, and right is *S. himantioides* on 12% elastane at 3 months.

Following the visible change in the colour of the fabric in samples inoculated with *H. fasciculare* seen in photos of the microcosms (Figure 5), suggesting metabolism of the dye by the fungi, methods were investigated to quantify this change over the timeframe of the study.

3.3 Thermogravimetric Analysis

Thermogravimetric analysis was considered as an alternative method of quantifying the dye loss in the samples, and potentially any decrease in elastane content. The samples were weighed into crucibles and the percentage mass change was recorded as the temperature of the furnace increased, at a rate of approximately 10 °C per minute.

The assumption was that each fibre type within the fabric would have a different thermal decomposition point and that the dye would also have a significantly different thermal decomposition point that would be noticeable on a mass change graph over time.

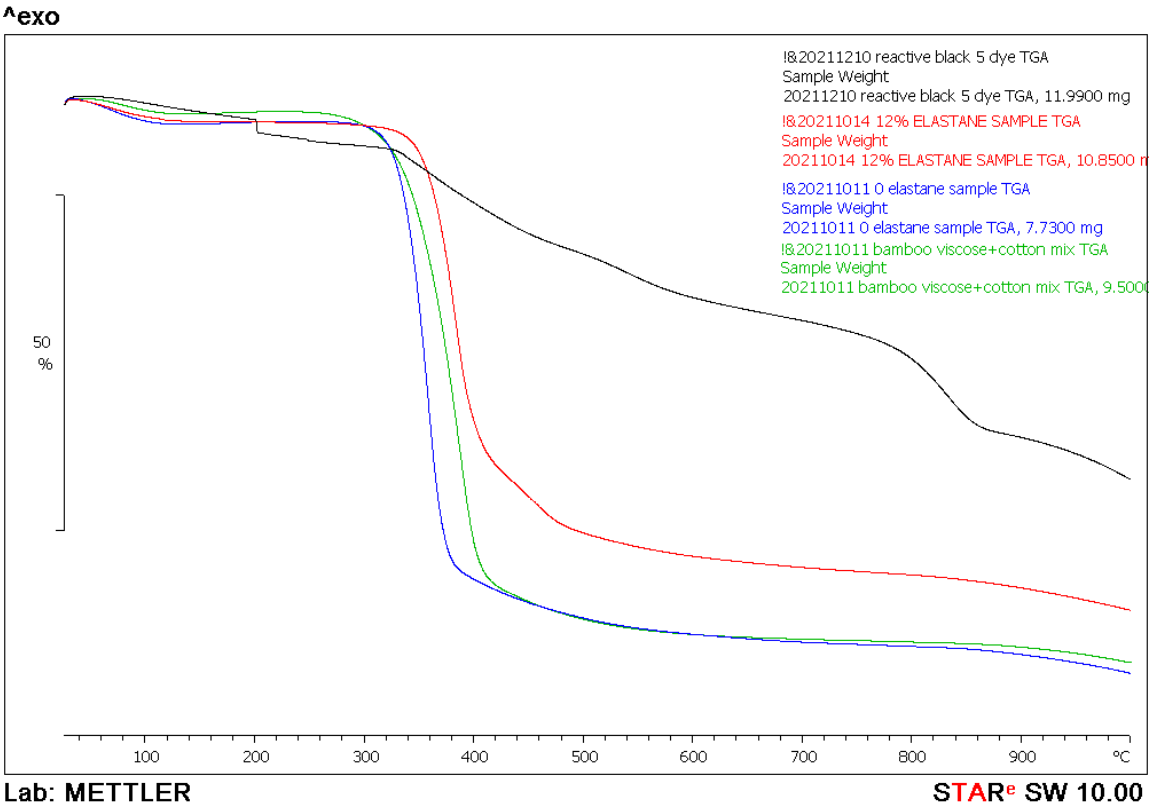

Figure 12: Aggregate graph of thermogravimetric analysis (TGA) curves plotting percentage of initial mass against temperature in °C for RB5 dye (black line), fabric with 12% elastane (red line), fabric with no elastane content (blue line) and the undyed bamboo viscose-cotton fibres (green line).

However, no obvious difference in the decomposition of the 0% elastane fabric sample and the undyed bamboo viscose and cotton mixture was noted, making it difficult to determine any dye loss via this method (Figure 12, blue and green lines). A sample of pure RB5 powder was analysed in the same way, and rather than showing a clear 'step' where a large mass loss occurred due to decomposition, it was more of a gradual loss over time. This confirmed that it would have been difficult to quantify dye loss via TGA (Figure 12, black line). When a 12% elastane sample was used to test whether elastane content could be quantified this way instead, there was only a slight difference in decomposition over time compared to the 0% elastane sample, ruling it out as a possibility (Figure 12, red and blue lines). The possibility of using TGA to determine elastane loss in the fabric was also tested, using a sample of pure elastane threads as a control to compare both the 0% elastane and 12% elastane samples to compare to, but this was similarly unsuccessful (Appendix 4).

3.4 Acid digest data

Acid digest was a possible solution to the challenge of quantifying RB5 dye in the fabric. The aim was to digest the entire sample and use the lambda max of RB5 (597 nm), to compare the incubated

samples to a baseline of untreated fabric digested in the same way. As a control, 100 mg of RB5 was dissolved in each of the acids and their 50% diluted versions, as well as in water to see if the absorbance could be detected for a pure dye sample in acids. Table 3 shows that the absorbances are higher for RB5 in the acids than the acid digested fabric samples.

Table 3: Absorbance at 597 nm for acid digested 0% elastane fabric samples and 0.02 µM RB5 samples, with acids at full concentration and 50% concentration. All samples were diluted 1:1000 in distilled water before measurement with a UV-Vis spectrophotometer, as the absorbance data for the undiluted samples and samples that were less diluted was too high to be measured properly – i.e. above 1 (Cary UV-Vis Compact, Agilent, USA).

	Absorbance at 597 nm	
	0% elastane fabric samples	**RB5 samples**
dH_2O	-	0.751
Concentrated Hydrochloric acid	0.134	0.514
50% Hydrochloric acid	0.004	0.598
Concentrated Sulfuric acid	0.038	0.430
50% Sulfuric acid	0.096	0.569

3.5 Dye extraction data

A protocol based on a paper by Home and Dudley (1981) was used. This gave reliable results and was used to analyse test and control fabric samples.

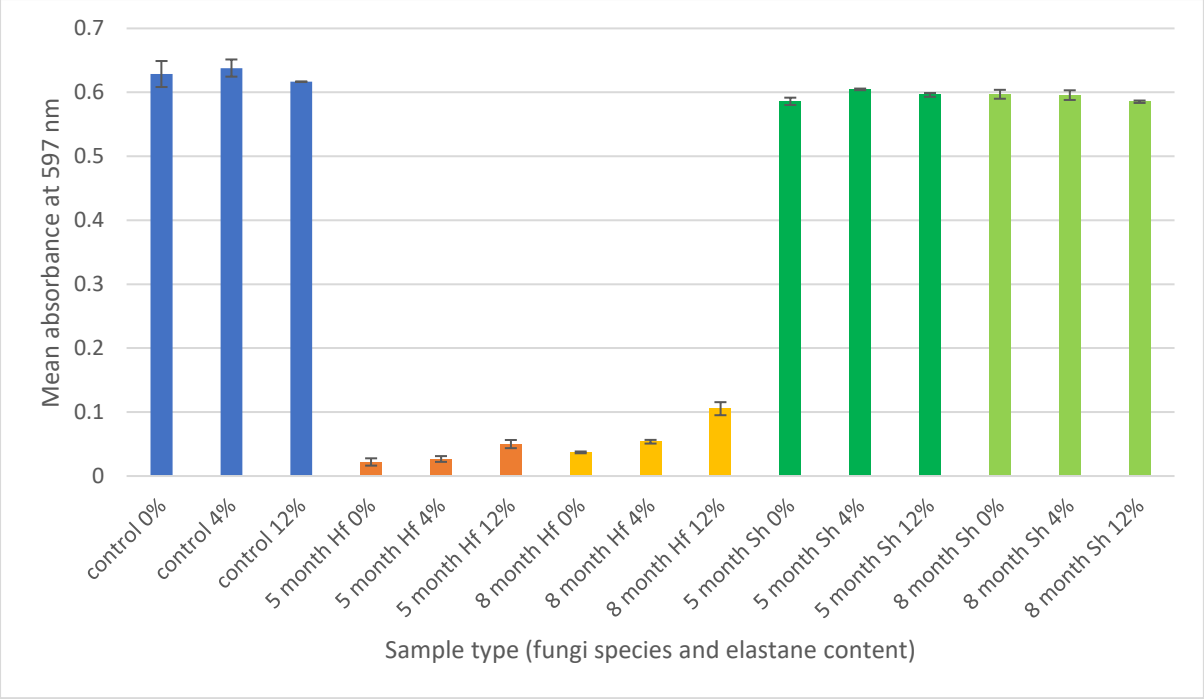

Figure 13: Mean absorbance at 597 nm for controls of each fabric type, and samples incubated with each species of fungi (Hf being *H.fasciculare*, Sh being *S. himantioides*) for 5 months and 8 months. (n=3, per fabric type per species; standard error measurement bars used.)

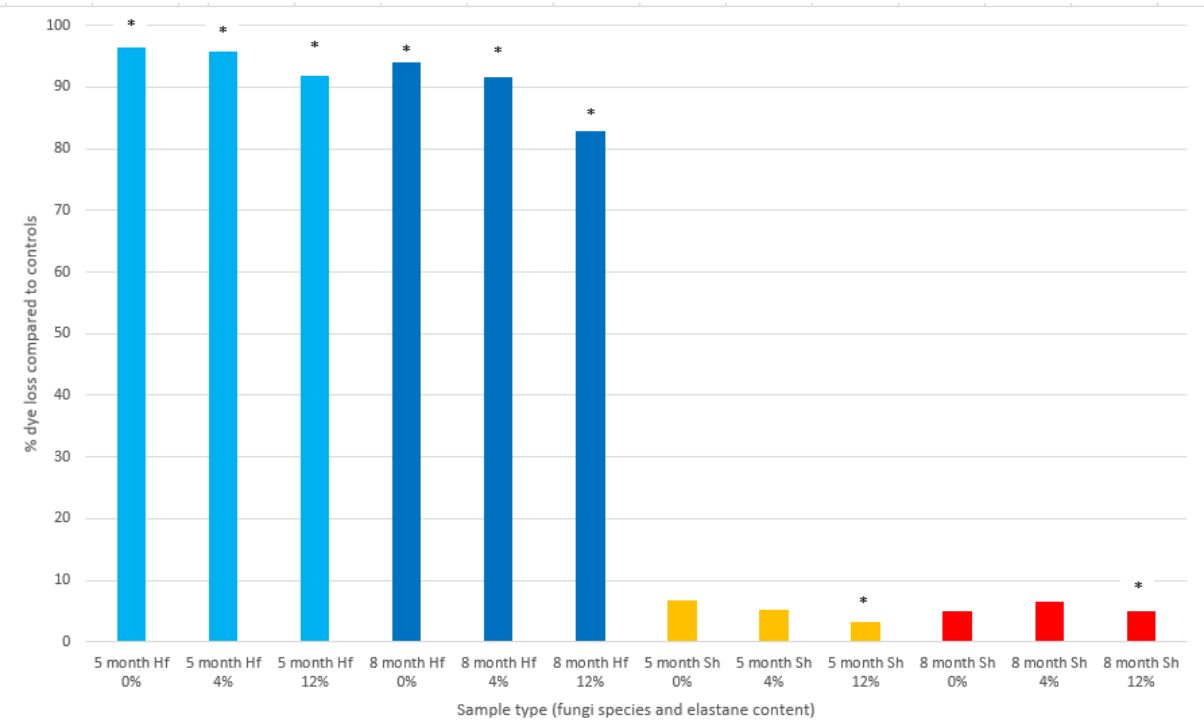

Figure 14: Percentage dye loss based on absorbance data from controls and samples from each species at 5 months and 8 months. Samples with an asterisk on top are significantly different to controls, as determined by paired two-tail t-test (See Appendix 2 and 3).

Dye was extracted from the incubated samples and untreated control fabric samples to quantify this possible dye reduction. The λmax for RB5 is 597 nm – so the extracted dye samples all had their absorbance measured at that wavelength. As seen in Figure 13, the mean absorbances for *H. fasciculare* samples were much lower than that of the controls, while the *S. himantioides* samples had similar absorbances to the controls. The mean absorbance values for dye extracted from the control samples and from the 8 month incubated *H. fasciculare* samples were significantly different for the 0% elastane fabric, with a p-value of 0.000546. This was also true for the 4% elastane and 12% elastane fabrics inoculated with *H. fasciculare*, with p-values of 0.000282 and 0.000199 respectively. The 8 month incubated *S. himantioides* samples were not significantly different compared to the controls for the 0% elastane and 4% elastane fabrics, but were for the 12% elastane samples, with a p-value of 0.001468, despite there being little visible change to the fabric. In terms of percentage dye loss, it can clearly be seen in Figure 14 that the *H. fasciculare* samples have a much higher percentage dye loss than the *S.himantioides* samples. The raw absorbance values can be found in Appendix 1, and the statistical analyses can be found in Appendix 2 and 3.

3.6 GCMS analysis of the volatile metabolome

For *H. fasciculare*, over time the number of unique compounds identified from the GCMS data generally peaked at 5 months, except for in the 12% elastane samples, where they peaked at 3 months and decreased for the remaining months (Table 4). There were more compounds identified in the controls for *H. fasciculare*, which were beech chips on the relevant fabric types, than for *S.himantioides*, which used pine chips on the relevant fabric types. These compounds found in the controls were removed from sample data. All TICs collected can be found in Appendix 5.

Table 4: Total numbers of unique compounds identified in GCMS analysis of *Hypholoma fasciculare* samples. Compound abundance peaks were sampled from TICs and run through the NIST 2.0 database with a NIST quality match cutoff of 60.

Total number of unique compounds in *Hypholoma fasciculare* samples			
Timepoint sample was collected at	**0% elastane**	**4% elastane**	**12% elastane**
3 months	3	12	10
5 months	33	20	7
8 months	5	7	3

The *S. himantioides* samples showed a similar increase in the number of unique compounds identified, again peaking at 5 months (Table 5).

For both species, all sample types showed a sharp decrease in the number of unique compounds identified at 8 months compared to at 5 months (Tables 4 and 5).

Table 5: Total numbers of unique compounds identified in GCMS analysis of *Serpula himantioides* samples. Compound abundance peaks were sampled from TICs and run through the NIST 2.0 database with a NIST quality match cutoff of 60.

Total number of unique compounds in *Serpula himantioides* samples			
Timepoint sample was collected at	**0% elastane**	**4% elastane**	**12% elastane**
3 months	19	11	13
5 months	28	22	28
8 months	6	1	3

Table 6: Peak heights (relative abundance) of compounds that appeared in *H. fasciculare* samples at multiple timepoints, as well as which fabric type they were identified from.

Peak heights of compounds that appeared in samples at multiple timepoints				
Compound name	3 months	5 months	8 months	Fabric type (elastane percentage)
10-Methylnonadecane	81764	260673	-	0%
Octacosane	71403	68690	-	4%
Decane, 3,8-dimethyl-	81244	41796	-	4%
6-[1-[4-Fluorophenyl]ethyl]-1,3-benzodioxol-5-ol	-	44832	90050	0%

Of the unique compounds found in *H. fasciculare* samples, there are few that are seen multiple times in the same fabric types. Both 10-methylnonadecane and 6-[1-[4-Fluorophenyl]ethyl]-1,3-benzodioxol-5-ol show an increase in relative abundance within the 0% elastane samples over time (Table 6). Searching the databases listed in section 2.4 yielded no specific functions known for these compounds within fungal metabolic pathways, nor were hazard symbols associated with the volatiles identified (Table 7). At this time, the compounds cannot be assigned a role or process within the microcosm and further work is needed to identify their source. The remaining two unique compounds identified in Table 6 – both Octacosane and Decane, 3,8-dimethyl- decreased over time

within the 4% elastane samples. Octacosane was identified as being part of a metabolic pathway for cuticular wax biosynthesis, but nothing was identified for the Decane, 3,8-dimethyl- (Table 7).

Table 7: Functions and hazards associated with the unique compounds identified from *H. fasciculare* samples.

Compound name	Function	Hazards
10-Methylnonadecane	none found	N/A
Octacosane	cuticular wax biosynthesis	N/A
Decane, 3,8-dimethyl-	none found	N/A
6-[1-[4-Fluorophenyl]ethyl]-1,3-benzodioxol-5-ol	none found	N/A

Table 8: Peak heights (relative abundance) of compounds that appeared in *S. himantioides* samples at multiple timepoints, as well as which fabric type they were identified from.

Peak heights of compounds that appeared in *Serpula* samples at multiple timepoints			
Compound name	**3 months**	**5 months**	**Fabric type (elastane percentage)**
10-Methylnonadecane	54203	132103	0%
10-Methylnonadecane	74072	106327, 110675	4%
Hexadecane, 2,6,10,14-tetramethyl-	97577	78128, 48232	0%
3-Carene	65206	144858	0%
Octadecane	39378	72295	0%
Dodecane	48672, 71363, 41252, 125826	191346	4%
Dodecane	112482	188704, 45241	12%
Undecane	53820, 50717	73200, 82582	4%
Undecane	59715	52807	12%
Dodecane, 4,6-dimethyl-	121171	49227	4%
Octacosane	50034	41435	4%
Heneicosane	77709	54281, 122313	12%
Cyclopentane, 1-butyl-2-propyl-	91205	164816	12%

More unique compounds were found in multiple timepoints for *S.himantioides* than in *H. fasciculare*, but none of these compounds were seen again in the 8 month samples, only at the 3 month and 5 month timepoints (Table 8). Generally there is an increase in relative abundance over time for these compounds, with the exception of Hexadecane, 2,6,10,14-tetramethyl-, Dodecane, Undecane, Octacosane, and Heneicosane – these compounds showed a decrease in relative abundance over

time instead. The compounds that showed a decrease in relative abundance over time for *S. himantioides* were found to be associated with metabolic pathways for cuticular wax synthesis, with the exception of Hexadecane, 2,6,10,14-tetramethyl-, which was identified as possibly being linked to chlorophyll or petroleum (Table 9).

Table 9: Functions and hazards associated with the unique compounds identified from *S.himantioides* samples.

Compound name	Function	Hazards
10-Methylnonadecane	none found	N/A
Hexadecane, 2,6,10,14-tetramethyl-	aka phytane - can be from chlorophyll or petroleum	irritant
3-Carene	from pine resin	flammable, irritant, health hazard
Octadecane	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Dodecane	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Undecane	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Dodecane, 4,6-dimethyl-	none found	N/A
Octacosane	cuticular wax biosynthesis	N/A
Heneicosane	cuticular wax biosynthesis	N/A
Cyclopentane, 1-butyl-2-propyl-	none found	N/A

Within the identified compounds, only a few were found to be associated with metabolic pathways. Many compounds were found to have hazard warnings associated with them, including: flammable, irritant, and health hazard (Appendix 6). This is something to be aware of in future studies – if these results can be replicated, the amounts of these compounds must be taken note of, to check if they might present a danger if the process were scaled up for industry.

4. Discussion

The results presented here indicate that *H. fasciculare* colonises the fabric faster than *S. himantioides* and, based on the SEM imaging showing fibre damage, is more able to break down elements of the fabric (Figure 10). *Hypholoma fasciculare* also demonstrated a higher degree of bioremediating the dye from the fabric than *S. himantioides* (Figure 8). These differences may be due to the different methods of degradation each species uses, and it is possible that *S. himantioides*

lacks the array of enzymes that *H. fasciculare* uses to enable degradation of the fabric and bioremediation of the dye. *Hypholoma fasciculare* is a more promising prospect for further method development and research.

Both species were visualized as having grown on the fabric samples, with hyphae seen connecting to the individual fibres and penetrating through the structure of the textiles in SEM imaging, and were still alive and growing after 8 months. These results suggest fungal bioremediation of textiles is a promising avenue for further exploration.

4.1 Differences in dye bioremediation ability between species

One of the key novel findings of this project was the ability of *H. fasciculare* to remove the dye from the fabric (Figure 8). Although *H. fasciculare* has previously shown an ability to decolourise dyes used in the textile industry, like remazol Brilliant Blue R (Przystaś and Zablocka-Godłewska, 2017), this is the first time that remediation from fabric has been reported. White rot fungi in general have demonstrated this ability with many other textile dyes such as Congo red, and the importance of peroxidase and laccase enzymes in this process has previously been confirmed in other studies (Jayasinghe *et al*, 2008; Novotný *et al*, 2004; Yesilada *et al*, 2018). Only one dye, Reactive Black 5, was used as a control as the information provided by Bamboo Clothing Ltd identified it as the only dye used in the sample garments they provided. In future studies, fabric samples with different dyes should be tested, as different dyes have different chemical structures and may bond differently to the fabric.

As a trial dye, RB5 presented a significant challenge as it is an azo dye, a class of dyes that contain toxic and mutagenic dyes, and are generally recalcitrant to removal from the environment (Bafana *et al*, 2011). Thus, it is of note that incubation with *H. fasciculare* appears to have removed the dye from the fabric. Further testing must be done to both confirm this and attempt to identify the fungal processes used and any metabolites produced.

Of the two methods tested, the dye extraction method based on Home and Dudley's method was favoured over the acid digest method. While having a total dye extraction may be desirable, the absorbance measurements at 597 nm seen with the acid digest method were too small to be able to compare the baseline to any changes in dye content. It is likely that the fabric does not have a high enough dye content to be quantified using this method.

In order to investigate the enzymatic reasons for the difference in dye bioremediation ability between *H. fasciculare* and *S. himantioides*, the MycoCosm subsection of the Joint Genomes

Initiative (JGI) Genome portal was used to identify the number of peroxidases and laccases in both species (Grigoriev *et al*, 2012; Nordberg *et al*, 2014).

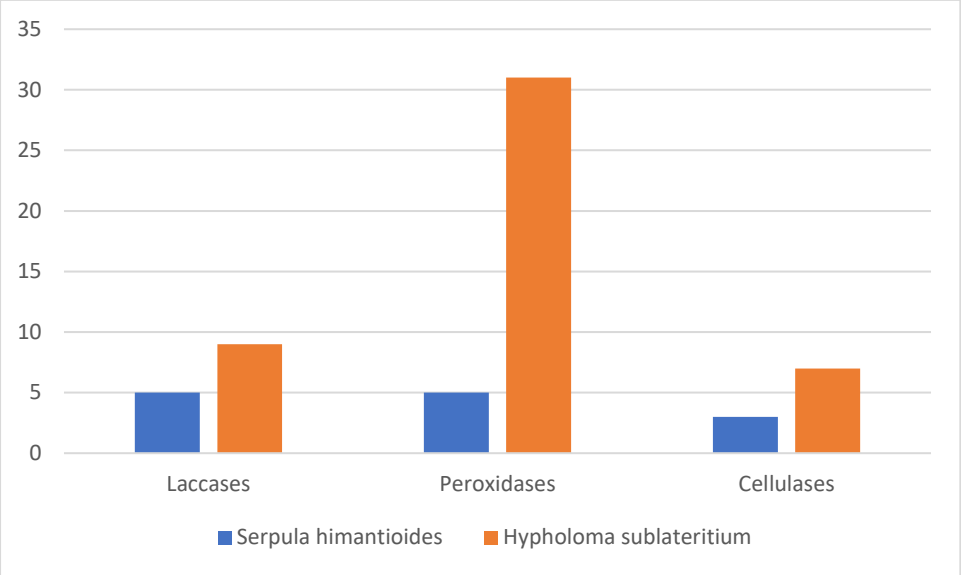

Figure 15: Summarised numbers of laccases, peroxidases, and cellulases listed for the species *S. himantioides* and *H. sublaterium* found in curated search results on JGI Mycocosm. Full tables can be found in Appendix 7.

Hypholoma fasciculare does not have its full genome listed, so a closely related species, *Hypholoma sublateritium* was investigated in its place – it has 31 peroxidase genes and 9 laccase genes listed (Figure 15). In comparison, *S. himantioides* only has 5 peroxidase genes and 5 laccase genes listed. The species related to *H. sublaterium* has almost double the number of peroxidases and laccases compared to the *S. himantioides*. As for cellulases, *H. sublaterium* has 7 listed, while *S. himantioides* has 3 listed. Overall, it appears that on an enzymatic level, the *Hypholoma* species has more degradation ability than *S. himantioides* based on the number of enzymes it can produce. Identification of the enzymes potentially important in the bioremediation process will enable future research to use bioinformatic screening prior to experimental work and more intelligent selection of fungal candidates.

4.2 Difficulty interpreting volatile metabolome

The GCMS analysis showed a change in volatile profile between the fungi, between the different fabrics and over time. The majority of peaks were not assigned a specific compound, or were compounds with no previously published connection to fungal metabolomics, which made interpretation challenging. Of those assigned to functional pathways, the dominant ones were

associated with plant cell wall metabolism, which was likely from the natural fibres in the fabric being biodegraded, and the fungal isoprenoid pathway.

Table 10: Number of identified compounds shared within two or more replicates of each species of fungus on each condition at the 5 month timepoint.

	Number of compounds shared within two or more replicates	
	H. fasciculare	*S. himantioides*
0% elastane	7	7
4% elastane	7	13
12% elastane	7	11

At 3 months, only 5 different compounds were found to be present within two or more replicates, over all fabric types, for both species. At 5 months, there were more compounds shared within two or more replicates at each condition for each species (Table 10). At 8 months, only one compound was found in two replicates of the *H. fasciculare* on 0% elastane fabric – there were no shared compounds within replicates in any of the other microcosm sets. This brings into question the replicability of this method to identify volatiles, aside from at the 5 month timepoint. Overall, the number of both unique and shared compounds identified was highest at 5 months for both species, which could indicate that the highest activity of the fungi on the fabric was then (Appendix 6).

Table 11: Number of identified compounds present in controls and in the samples incubated with both species of fungi at each time point.

	Number of compounds present in both the controls and samples	
	H. fasciculare	*S. himantioides*
3 month timepoint	3	7
5 month timepoint	13	11
8 month timepoint	3	3

The number of identified compounds found in both the control samples and the samples incubated with each species of fungus is highest at the 5 month timepoint (Table 11). This high amount of

compound overlap between the controls and the samples suggests that the overall processes of the fungi growing on the fabric do not produce many volatiles, and that these are instead coming from the wood or fabric itself.

Table 12: Number of identified compounds found in both *H. fasciculare* and *S. himantioides* at each timepoint from GCMS data.

	Number of compounds found in both species
3 month timepoint	12
5 month timepoint	36
8 month timepoint	0

The highest number of identified compounds shared between species was at the 5 month timepoint (Table 12). This could indicate similar processes occurring in both species at that time in terms of metabolism, or that the number was only raised due to the sheer number of volatiles released at that timepoint in comparison to the other two timepoints. At 8 months there was an average of 7 unique compounds identified per set for *H. fasciculare*, and an average of 4 unique compounds identified per set for *S. himantioides*, the least of the three timepoints for each species. No shared compounds were found between the species. For all of the above tables, the raw data used can be found in Appendix 6.

The same pathways (plant cell wall and fungal isoprenoid pathway) were identified in both fungi, however farnesane appeared as an identified compound more often in *S. himantioides* samples, across all time points, and particularly in 12% elastane samples.
Some compounds were identified as being linked to petroleum or gasoline, these were Dodecane, Butylated Hydroxytoluene, Undecane, and Hexadecane, 2,6,10,14-tetramethyl-. These compounds being linked to petroleum is interesting as elastane is a petroleum-based product, which could imply that these are being produced from the degradation of elastane, however there is no other evidence for this.

4.3 Unidentified crystalline structures

Further efforts were made to identify the crystalline structures seen in Figure 11 as they could potentially reveal which metabolic processes were occurring or whether they were a hazard that

needed mitigating if the process was scaled up for industry. Visually, they appeared similar to calcium oxalate crystals seen on hyphae previously (Figures 16, 17, 18). An attempt was made to quantify any oxalates, using a modified version of a protocol by Clausen *et al* (2008), but was unsuccessful. Modifications to the protocol were made with relevant information from papers by Clausen and Green, 2003; García-Esquivel *et al*, 2021; and Ngo and Lenhoff, 1980.

The reason the crystalline structures were thought to be oxalates is due to the importance of oxalic acid/oxalates to wood decay fungi. In brown rot fungi, oxalic acid is produced and binds to Fe^{3+} ions, making an Fe-oxalate complex that is then reduced to Fe^{2+} ions, which are needed for the Fenton reaction to produce hydroxyl radicals to disrupt lignocellulose (Andlar *et al*, 2018). In white rot fungi, oxalates are broken down by peroxidases to produce hydrogen peroxidase, which is also needed for its own degradation mechanism (Mäkelä *et al*, 2014). The presence of oxalate crystals could be indicative of degradation of the fabric occurring.

The assumption that these structures are oxalates was also influenced by several SEM images from papers, including: calcium oxalate crystals on hyphae of *Perenniporia meridionalis* on MEA, calcium oxalate crystal formations from a sample of *S. himantioides* on rock phosphate, and Calcium oxalate crystals from a sample of *S. himantioides* on MEA with 0.5% gypsum (Girometta *et al*, 2017; Gadd et al, 2014; Gharieb *et al*, 1998). These images showed structures similar to those found in the microcosm samples.

4.4 Future considerations for further research

Further research into this topic should ideally start with repeat trials for these species and trialling of additional species, including some Ascomycete species, to see if they have any effect on the fabric comparable to that of *H. fasciculare* in this study. This can be guided by bioinformatic interrogation of the growing number of fungal genomes available to ensure enzymatic capacity is considered. A method for quantifying any degradation of the fabric is necessary to determine whether this has definitely occurred in any samples, since the samples being heterogenous makes it incredibly difficult to quantify mass loss, as fungal biomass cannot be completely removed without also disturbing the fabric sample itself. Additionally, the imaging that was done on the samples was qualitative, and an assay has not yet been developed for analysing cellulose or elastane content in the fabric, although an attempt was made with thermogravimetric analysis for the latter.

Enzyme assays should proceed in order to identify the enzymes involved and the quantities of these enzymes produced by the fungi. If any enzymes are identified that are likely involved in the degradation of textiles, the genes involved in the production of these enzymes can be investigated,

and if identified, there is potential for genetic engineering of the fungi to overproduce these enzymes and harvest them for industrial use on a larger scale, or for using genetically modified fungi in the microcosms to speed up the process. However, this process was being developed as a low-tech method using widely available non-modified fungi in order to make it accessible to many different companies and LMICs – using genetically modified fungi would cause this to become much more expensive and less accessible. More research needs to be done to understand the enzymatic activity of the fungi, and potentially look into ways of improving conditions to more organically induce overproduction of the relevant enzymes without the use of biotech, if this accessible process is the ultimate goal.

As it is currently, the microcosms could be used as a model for how to maintain conditions for fungi to grow on textiles in larger scale sterilized fabric piles for industry use in bioremediating textile waste.

5. Conclusion

As a preliminary study, methods were tested and developed based on the aims and objectives laid out in the introduction. During this project, an agar-independent microcosm culture was successfully trialled and developed for medium term (8 month incubation) cultures, showing that longer term incubation for complete bioremediation may be possible. Two different fungal species have been successfully trialled as microcosms, and the same method may be used in future as an easy lab-based method to assess other species of fungi for their ability to degrade textiles. Microscopy techniques were used to assess fungal growth on the fabrics, and structures were identified that require further analysis to understand their relevance. Analysis of volatile compounds emitted from the microcosms identified some relevant functional pathways but compounds were left mostly unidentified. This was possibly due to the age of the NIST database available. Quantification of dye loss via bioremediation was a key challenge and several methods were trialled. The dye extraction protocol presented here was sufficiently sensitive and accurate to enable quantification in this system.

While this project provides a much-needed starting point for this field of research, some challenges remain beyond the scope of this work. The textile industry uses metal or plastic zips, buttons, and fastenings. As a result, if the rest of the garment is metabolised by the fungi, these ‘trims’ and fastenings are likely to remain as potential pollutants, as sustainable alternatives for them all are not readily available. Additionally, the chemical processes that bamboo viscose goes through during

production may hinder the fungi from treating it as a carbon source that would normally be metabolised.

There is much more to be explored in terms of the ability of fungi to bioremediate fabrics, and as a result of this project, protocols have been successfully developed to enable those future studies.

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Appendices

Appendix 1: Table of raw and mean absorbance data recorded at 597nm for dye extracted from fabric controls and fabric incubated at 25°C with both species of fungi, *H. fasciculare* and *S. himantioides*, for 5 months and 8 months respectively.

				5 months incubation at 25°C				8 months incubation at 25°C			
		Untreated fabric (no fungi)		*Hypholoma fasciculare* HfGTWV2		*Serpula himantioides* MUCL38935		*Hypholoma fasciculare* HfGTWV2		*Serpula himantioides* MUCL38935	
Fabric type	Sample No.	Absorbance at 597nm	Mean absorbance at 597nm	Absorbance at 597nm	Mean absorbance at 597nm	Absorbance at 597nm	Mean absorbance at 597nm	Absorbance at 597nm	Mean absorbance at 597nm	Absorbance at 597nm	Mean absorbance at 597nm
0% elastane	1	0.6	0.629	0.029	0.022	0.594	0.586	0.036	0.037	0.598	0.597
	2	0.645		0.015		0.581		0.039		0.588	
	3	0.641		0.022		0.583		0.036		0.605	
4% elastane	1	0.648	0.638	0.027	0.027	0.605	0.605	0.057	0.054	0.601	0.596
	2	0.647		0.032		0.606		0.05		0.585	
	3	0.619		0.021		0.603		0.054		0.601	
12% elastane	1	0.617	0.617	0.055	0.05	0.593	0.596	0.093	0.105	0.588	0.585
	2	0.616		0.041		0.595		0.105		0.584	
	3	0.617		0.054		0.6		0.118		0.584	

Appendix 2: t-Tests for absorbance measurements at 597nm from extracted dye, based on raw data from Appendix 1. Data from samples incubated for 5 months for each condition (fabric type and fungi species) was compared to the dye control, and then data from samples with the same fabric type was compared across the two fungi species.

0% elastane 5 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.022	0.628666667
Variance	0.000049	0.000620333
Observations	3	3
Pearson Correlation	-0.90338	
Hypothesized Mean Difference	0	
df	2	
t Stat	-33.4919	
P(T<=t) one-tail	0.000445	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.00089	
t Critical two-tail	4.302653	

4% elastane 5 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.026667	0.638
Variance	3.03E-05	0.000271
Observations	3	3
Pearson Correlation	0.876844	
Hypothesized Mean Difference	0	
df	2	
t Stat	-88.7535	
P(T<=t) one-tail	6.35E-05	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000127	
t Critical two-tail	4.302653	

12% elastane 5 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.05	0.616666667
Variance	6.1E-05	3.33333E-07
Observations	3	3
Pearson Correlation	0.997949	
Hypothesized Mean Difference	0	
df	2	
t Stat	-135.675	
P(T<=t) one-tail	2.72E-05	
t Critical one-tail	2.919986	
P(T<=t) two-tail	5.43E-05	
t Critical two-tail	4.302653	

0% elastane 5 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.586	0.628666667
Variance	4.9E-05	0.000620333
Observations	3	3
Pearson Correlation	-0.99802	
Hypothesized Mean Difference	0	
df	2	
t Stat	-2.31696	
P(T<=t) one-tail	0.07322	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.146439	
t Critical two-tail	4.302653	

4% elastane 5 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.604667	0.638
Variance	2.33E-06	0.000271
Observations	3	3
Pearson Correlation	0.934533	
Hypothesized Mean Difference	0	
df	2	
t Stat	-3.83765	
P(T<=t) one-tail	0.030842	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.061684	
t Critical two-tail	4.302653	

12% elastane 5 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.596	0.616666667
Variance	0.000013	3.33333E-07
Observations	3	3
Pearson Correlation	0.240192	
Hypothesized Mean Difference	0	
df	2	
t Stat	-10.1927	
P(T<=t) one-tail	0.004744	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.009489	
t Critical two-tail	4.302653	

0% elastane 5 month Hf and Sh		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.022	0.586
Variance	0.000049	4.9E-05
Observations	3	3
Pearson Correlation	0.928571	
Hypothesized Mean Difference	0	
df	2	
t Stat	-369.225	
P(T<=t) one-tail	3.67E-06	
t Critical one-tail	2.919986	
P(T<=t) two-tail	7.34E-06	
t Critical two-tail	4.302653	

4% elastane 5 month Hf and Sh		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.026667	0.604666667
Variance	3.03E-05	2.33333E-06
Observations	3	3
Pearson Correlation	0.990536	
Hypothesized Mean Difference	0	
df	2	
t Stat	-250.281	
P(T<=t) one-tail	7.98E-06	
t Critical one-tail	2.919986	
P(T<=t) two-tail	1.6E-05	
t Critical two-tail	4.302653	

12% elastane 5 month Hf and Sh		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.05	0.596
Variance	6.1E-05	0.000013
Observations	3	3
Pearson Correlation	0.177555	
Hypothesized Mean Difference	0	
df	2	
t Stat	-118.212	
P(T<=t) one-tail	3.58E-05	
t Critical one-tail	2.919986	
P(T<=t) two-tail	7.16E-05	
t Critical two-tail	4.302653	

Appendix 3: t-Tests for absorbance measurements at 597nm from extracted dye, based on raw data from Appendix 1. Data from samples incubated for 8 months for each condition (fabric type and fungi species) was compared to the dye control, and then data from samples with the same fabric type was compared across the two fungi species.

0% elastane 8 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.628667	0.037
Variance	0.00062	3E-06
Observations	3	3
Pearson Correlation	0.567927	
Hypothesized Mean Difference	0	
df	2	
t Stat	42.76177	
P(T<=t) one-tail	0.000273	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000546	
t Critical two-tail	4.302653	

4% elastane 8 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.638	0.053666667
Variance	0.000271	1.23333E-05
Observations	3	3
Pearson Correlation	-0.05189	
Hypothesized Mean Difference	0	
df	2	
t Stat	59.5007	
P(T<=t) one-tail	0.000141	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000282	
t Critical two-tail	4.302653	

12% elastane 8 month Hf and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.616667	0.105333333
Variance	3.33E-07	0.000156333
Observations	3	3
Pearson Correlation	0.023088	
Hypothesized Mean Difference	0	
df	2	
t Stat	70.83354	
P(T<=t) one-tail	9.96E-05	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000199	
t Critical two-tail	4.302653	

0% elastane 8 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.628667	0.597
Variance	0.00062	7.3E-05
Observations	3	3
Pearson Correlation	-0.18092	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.976167	
P(T<=t) one-tail	0.093393	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.186786	
t Critical two-tail	4.302653	

4% elastane 8 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.638	0.595667
Variance	0.000271	8.53E-05
Observations	3	3
Pearson Correlation	-0.47347	
Hypothesized Mean Difference	0	
df	2	
t Stat	3.278033	
P(T<=t) one-tail	0.040903	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.081805	
t Critical two-tail	4.302653	

12% elastane 8 month Sh and controls		
t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.616667	0.585333
Variance	3.33E-07	5.33E-06
Observations	3	3
Pearson Correlation	0.5	
Hypothesized Mean Difference	0	
df	2	
t Stat	26.07091	
P(T<=t) one-tail	0.000734	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.001468	
t Critical two-tail	4.302653	

0% elastane 8 month Hf and Sh		
t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	0.037	0.597
Variance	3E-06	7.3E-05
Observations	3	3
Pooled Variance	3.8E-05	
Hypothesized Mean Difference	0	
df	4	
t Stat	-111.261	
P(T<=t) one-tail	1.96E-08	
t Critical one-tail	2.131847	
P(T<=t) two-tail	3.91E-08	
t Critical two-tail	2.776445	

4% elastane 8 month Hf and Sh		
t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	0.053667	0.595667
Variance	1.23E-05	8.53E-05
Observations	3	3
Pooled Variance	4.88E-05	
Hypothesized Mean Difference	0	
df	4	
t Stat	-94.9919	
P(T<=t) one-tail	3.68E-08	
t Critical one-tail	2.131847	
P(T<=t) two-tail	7.36E-08	
t Critical two-tail	2.776445	

12% elastane 8 month Hf and Sh		
t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	0.105333	0.585333
Variance	0.000156	5.33E-06
Observations	3	3
Pooled Variance	8.08E-05	
Hypothesized Mean Difference	0	
df	4	
t Stat	-65.387	
P(T<=t) one-tail	1.64E-07	
t Critical one-tail	2.131847	
P(T<=t) two-tail	3.28E-07	
t Critical two-tail	2.776445	

Appendix 4: TGA curves plotting percentage of initial mass against temperature in °C for RB5 dye, fabric with 12% elastane content, fabric with no elastane content, pure elastane threads, and the undyed bamboo viscose-cotton fibres.

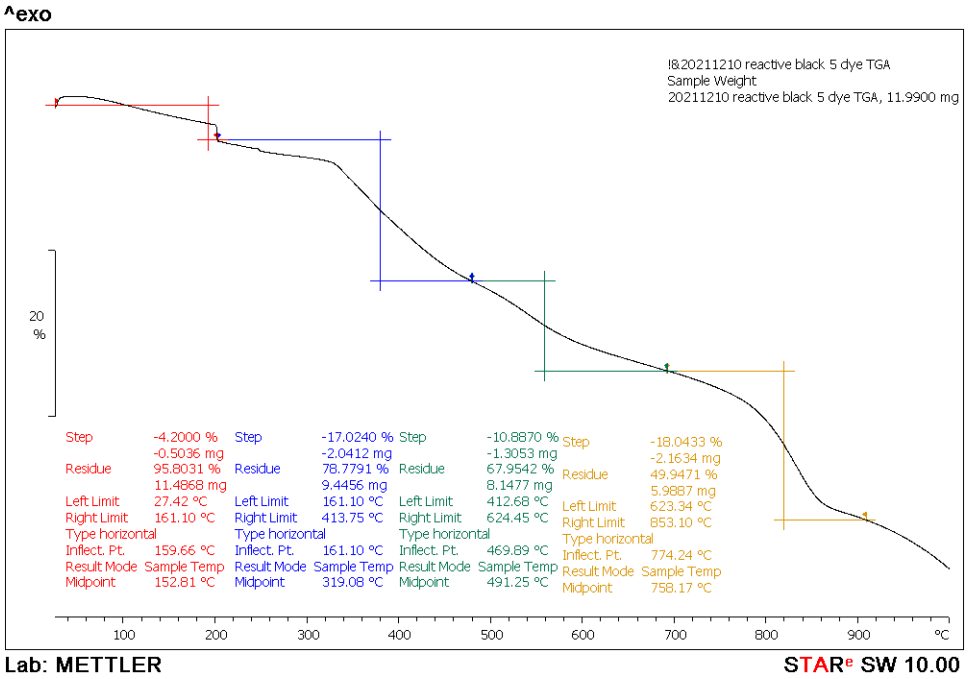

Figure 1: TGA curve for pure RB5 dye sample, with potential mass loss 'steps' labelled in an attempt to find an identifying 'step' that can be used when checking for dye loss in fabric samples. No identifiable 'steps' were found.

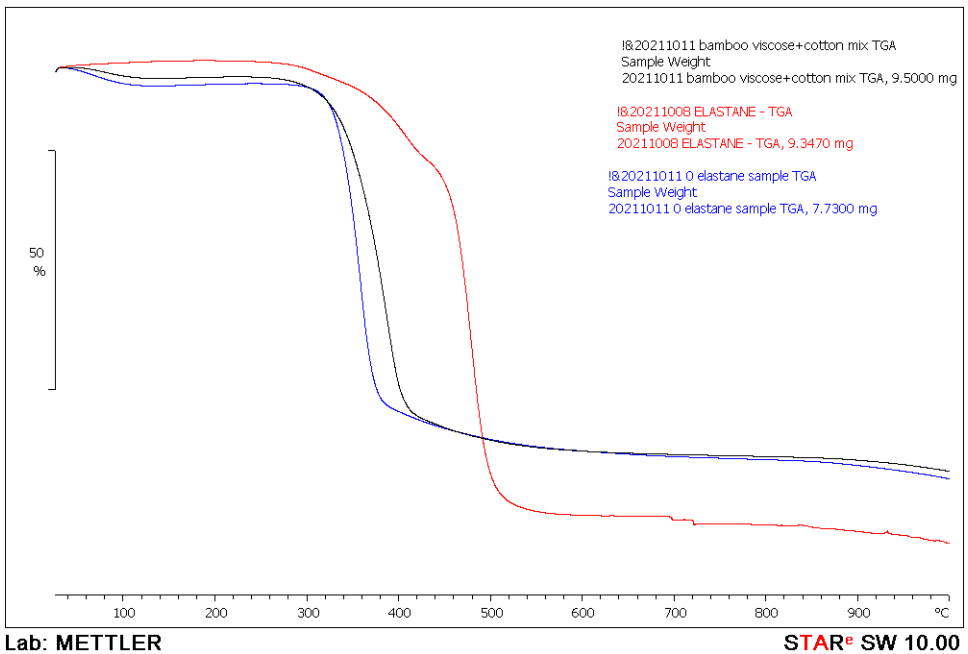

Figure 2: Aggregate graph of TGA curves for elastane (red line), bamboo viscose+cotton thread (black line), and 0% elastane sample (blue line). This was an attempt to find an identifying 'step' that can be used when checking for elastane loss in fabric samples, but there was no such thing to be found.

Appendix 5: All TICs recorded for all samples and replicates at each time point. X axis for all TICs is retention time in minutes, y axis is relative abundance.

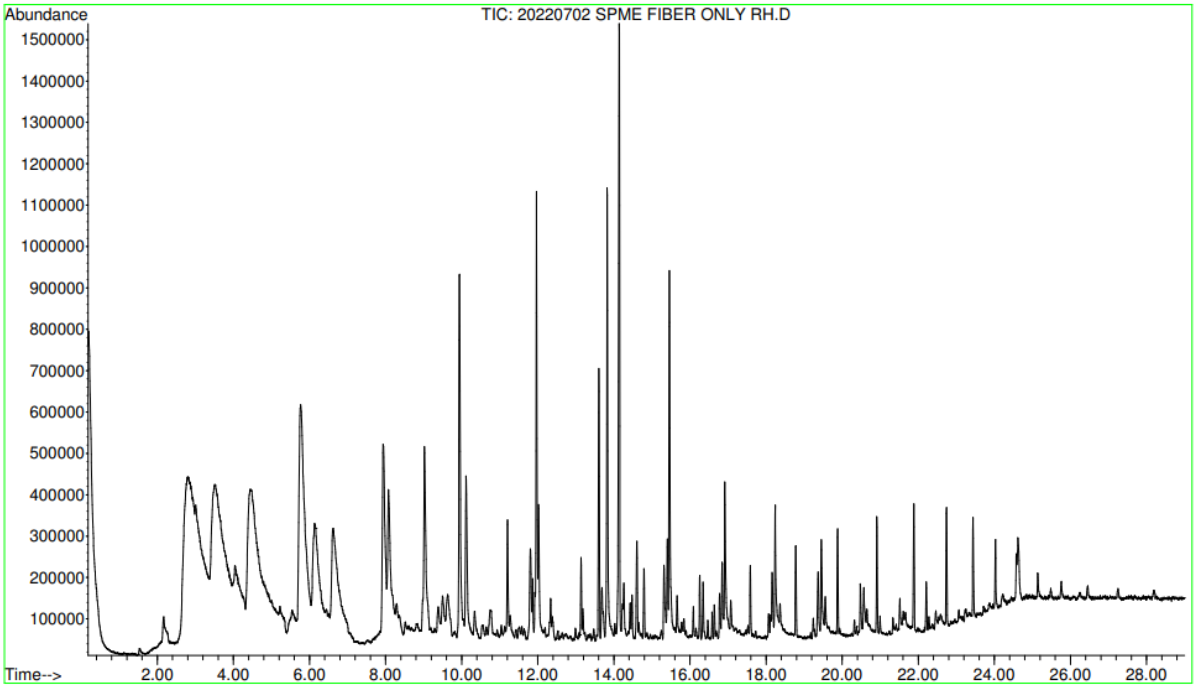

Figure 1: TIC for the SPME fiber itself with no exposure to the samples, as a background control.

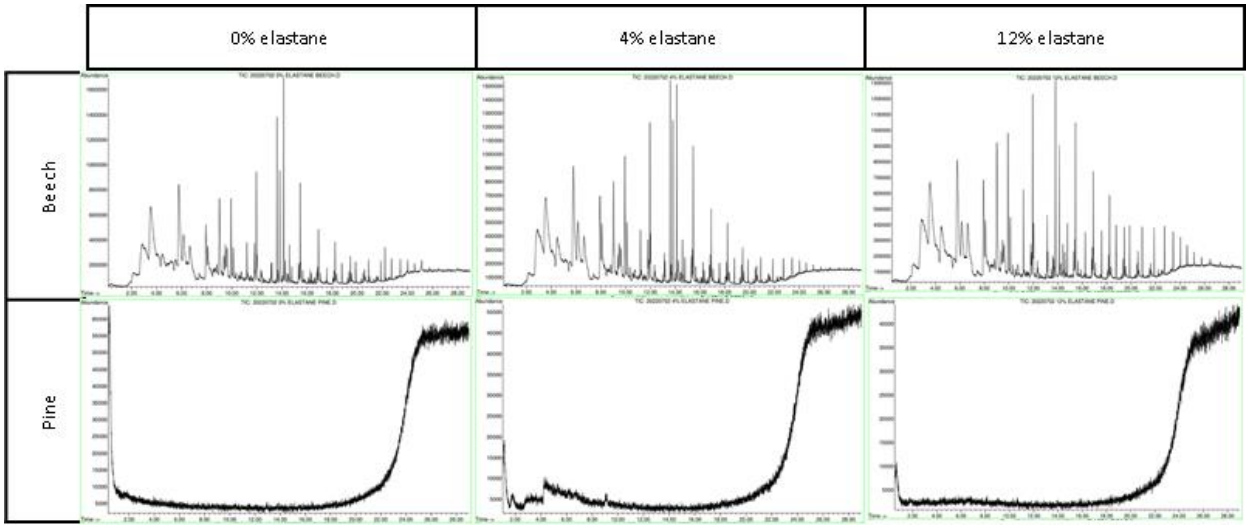

Figure 2: Aggregate image of TICs for all controls – wood chips on each fabric type.

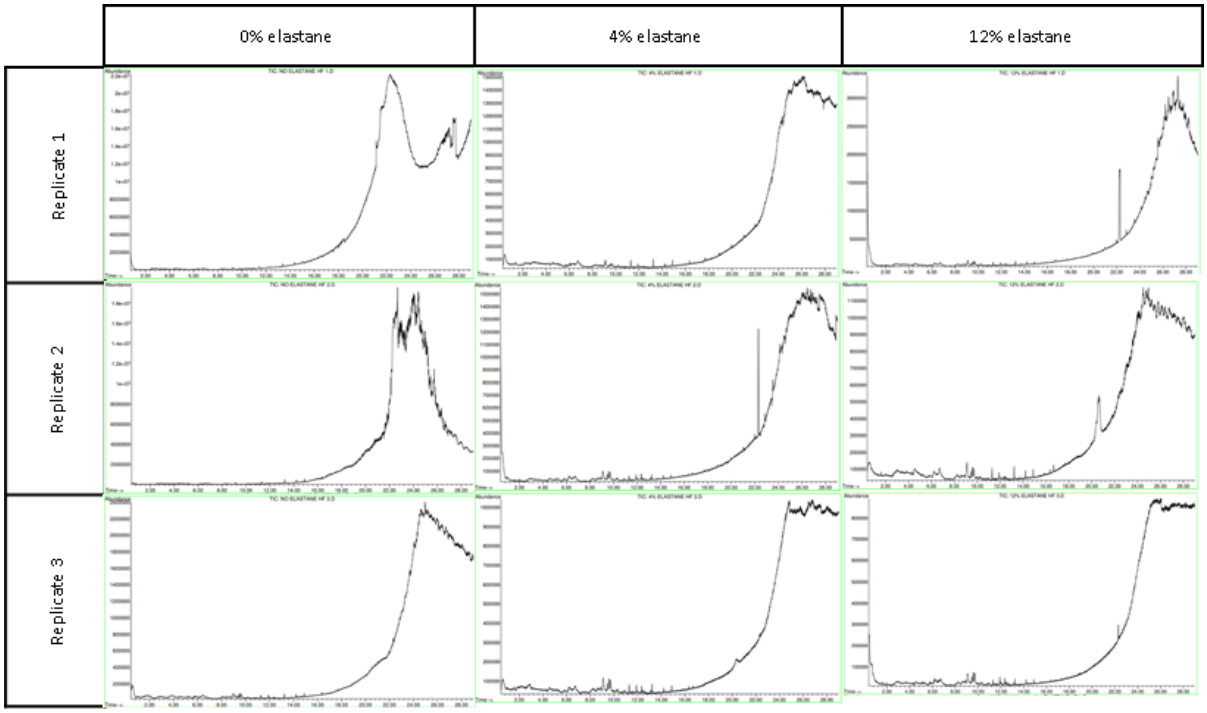

Figure 3: Aggregate image of TICs for all replicates of *H. fasciculare* on each fabric type at 3 months.

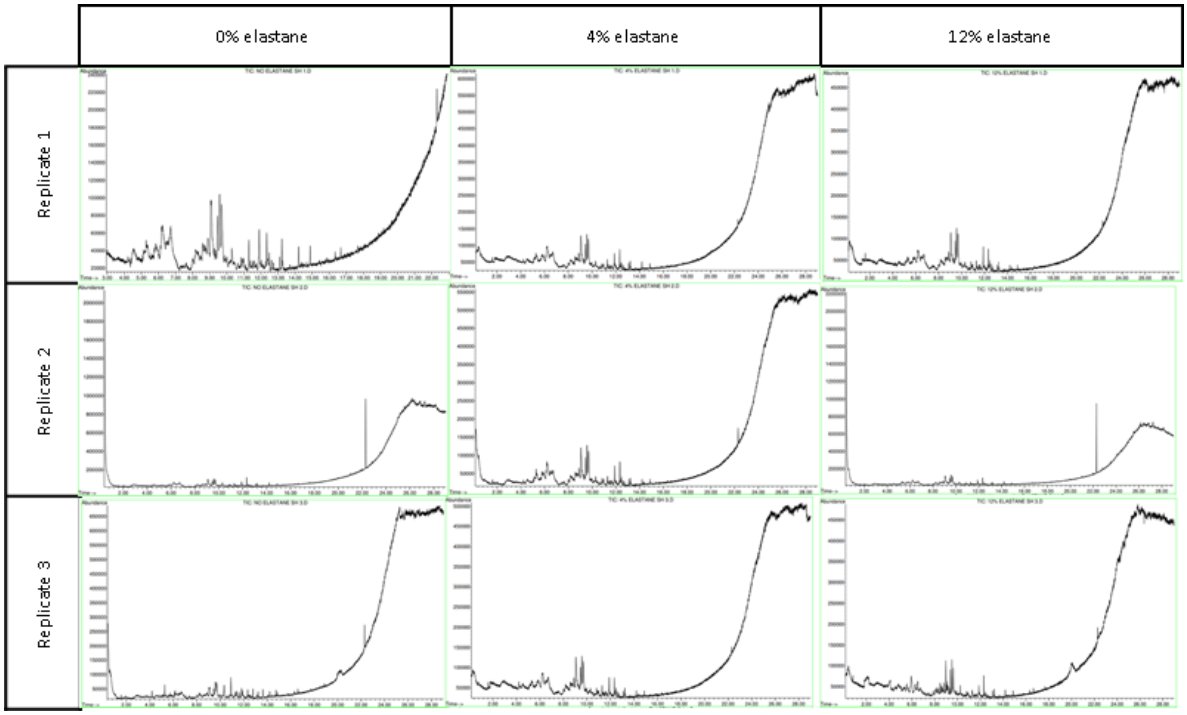

Figure 4: Aggregate image of TICs for all replicates of *S. himantioides* on each fabric type at 3 months.

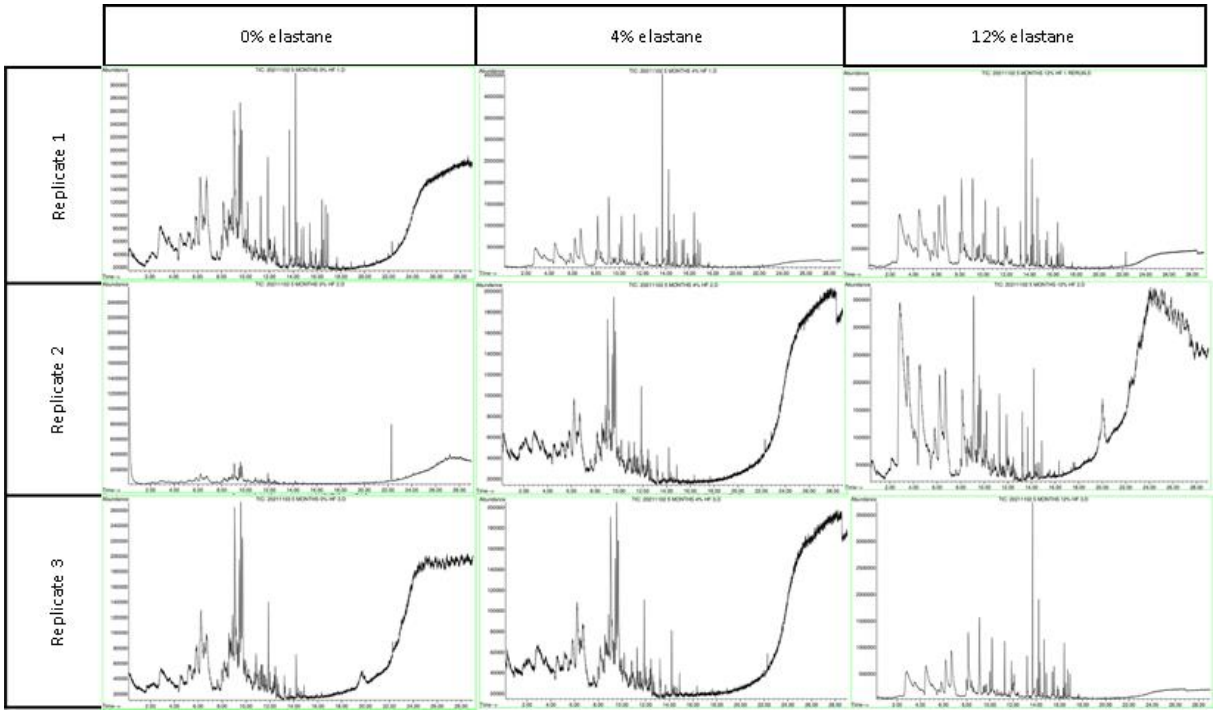

Figure 5: Aggregate image of TICs for all replicates of *H. fasciculare* on each fabric type at 5 months.

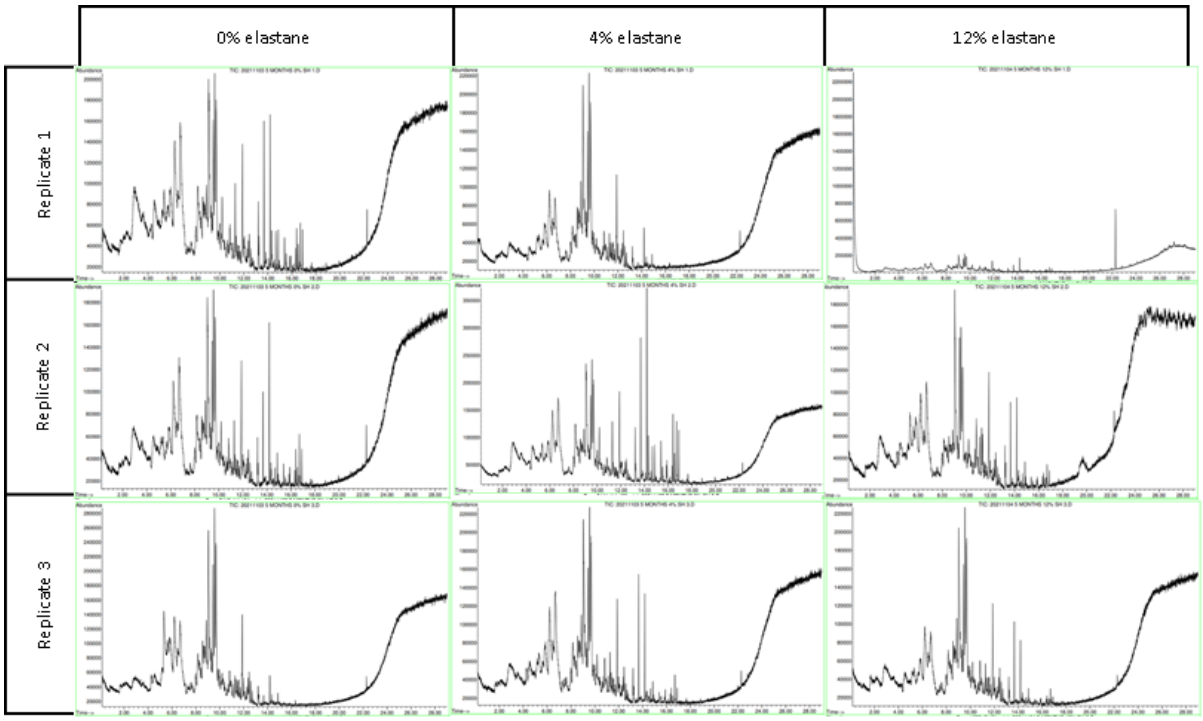

Figure 6: Aggregate image of TICs for all replicates of *S. himantioides* on each fabric type at 5 months.

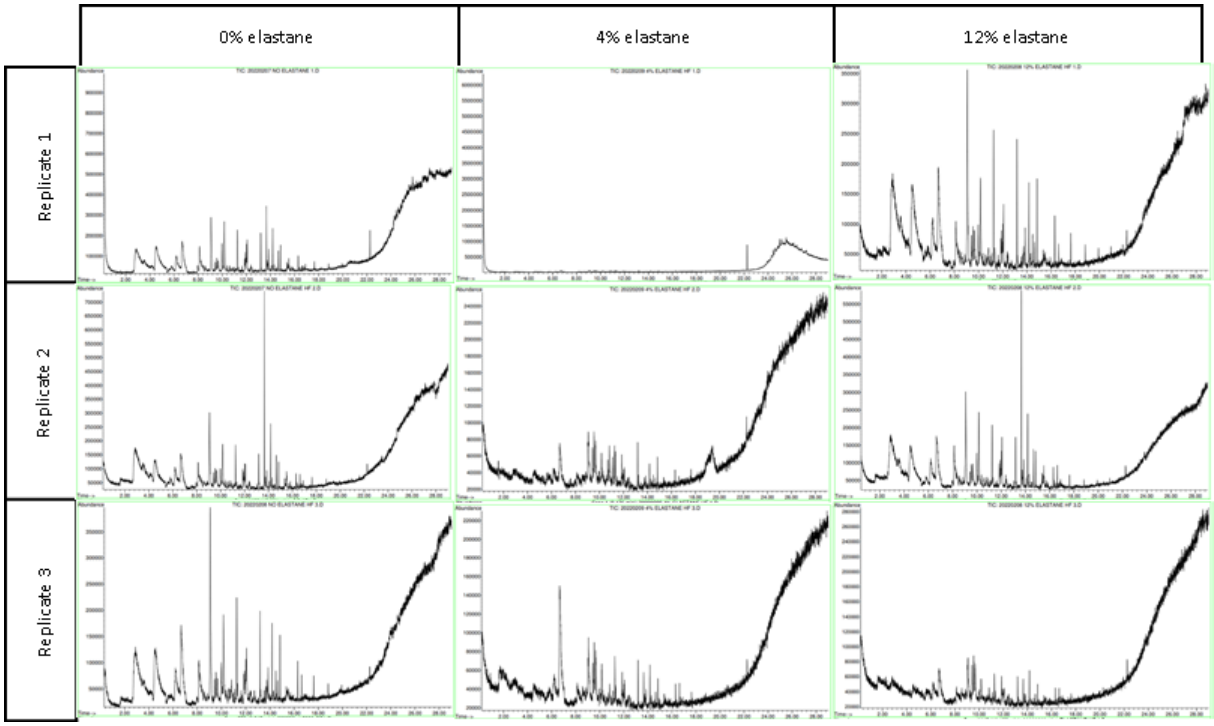

Figure 7: Aggregate image of TICs for all replicates of *H. fasciculare* on each fabric type at 8 months.

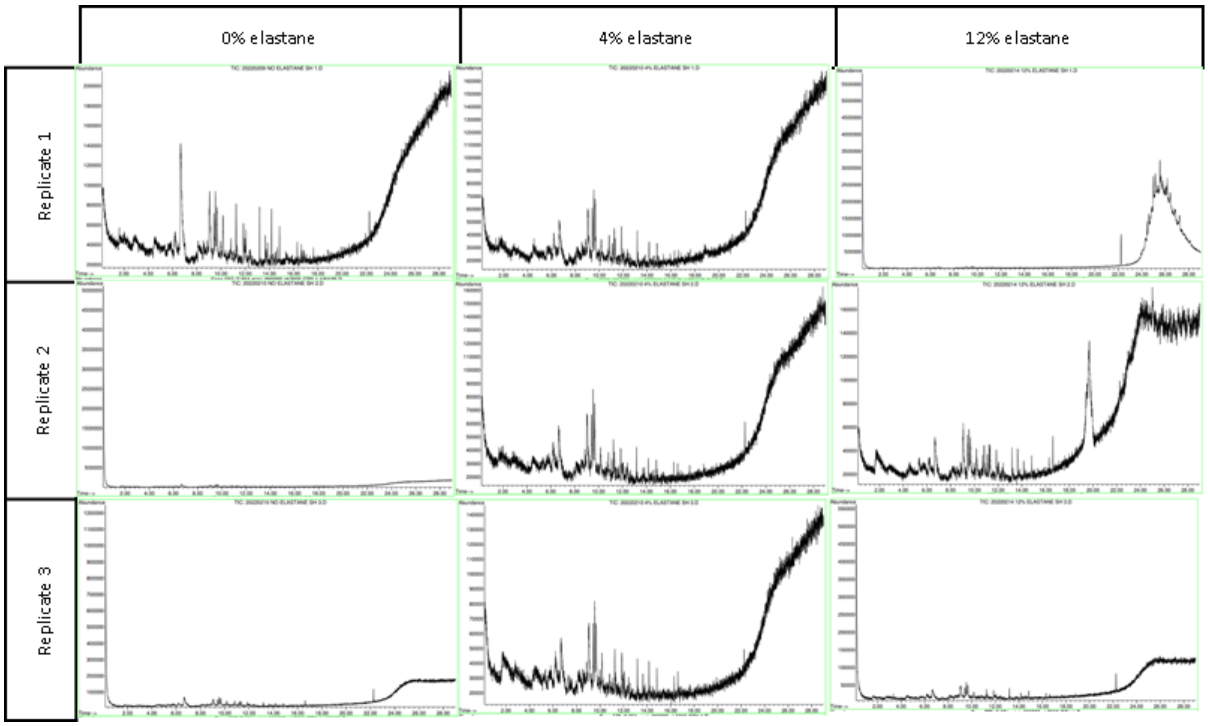

Figure 8: Aggregate image of TICs for all replicates of *S. himantioides* on each fabric type at 8 months.

Appendix 6: Tables of compounds identified from TICs for all replicates using Chemstation and NIST 2.0, with retention time, molecular formula, associated metabolic pathways listed (where applicable), and hazard warnings.

Table 1: List of identified compounds from control run of the SPME fiber with no sample on it.

SPME FIBER ONLY				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 584 (3.512 min)	Oxime-, methoxy-phenyl-_	C8H9NO2	none found	
Scan 745 (4.433 min)	7H-Dibenzo[b,g]carbazole, 7-methyl-	C21H15N	none found	
Scan 1653 (9.629 min)	1-Bromodocosane	C22H45Br	none found	irritant
Scan 1849 (10.750 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 2033 (11.803 min)	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2348 (13.605 min)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2463 (14.263 min)	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 2879 (16.644 min)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 3355 (19.367 min)	Eseroline, 7-bromo-, methylcarbamate(ester)	C15H20BrN3O2	none found	
Scan 3852 (22.211 min)	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
Scan 4273 (24.620 min)	1,4,7,10,13,16-Hexaoxacyclooctadecane	C12H24O6	none found	irritant

Table 2: List of identified compounds from control run of Beech wood chips on 0% elastane fabric samples.

0% ELASTANE BEECH				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 592 (3.543 min)	Oxime-, methoxy-phenyl-_	C8H9NO2	none found	
Scan 1458 (8.499 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1476 (8.602 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1487 (8.665 min)	Decane, 2-methyl-	C11H24	none found	flammable, health hazard
Scan 1512 (8.808 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1655 (9.626 min)	Eicosane, 9-octyl-	C28H58	none found	
Scan 2035 (11.800 min)	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2276 (13.179 min)	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	C16H30O4	none found	
Scan 2350 (13.603 min)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2464 (14.255 min)	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 3446 (19.874 min)	Thiocyanic acid, 5.alpha.-cholestan-3.beta.-yl ester	C28H47NS	none found	
Scan 3627 (20.910 min)	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 3854 (22.208 min)	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard

Table 3: List of identified compounds from control run of Beech wood chips on 4% elastane fabric samples.

4% ELASTANE BEECH				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 589 (3.547 min)	Oxime-, methoxy-phenyl-_	C8H9NO2	none found	
Scan 1509 (8.812 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1607 (9.372 min)	2-Isopropyl-5-methyl-1-heptanol	C11H24O	none found	
Scan 1651 (9.624 min)	Tridecane, 7-propyl-	C16H34	none found	
Scan 2272 (13.177 min)	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	C16H30O4	none found	
Scan 2346 (13.601 min)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2520 (14.597 min)	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	

Table 4: List of identified compounds from control run of Beech wood chips on 12% elastane fabric samples, along with retention time, molecular formula, associated metabolic pathways, and hazard warnings.

12% ELASTANE BEECH				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 545 (3.569 min)	Oxime-, methoxy-phenyl-_	C8H9NO2	none found	
Scan 707 (4.496 min):	7H-Dibenzo[b,g]carbazole, 7-methyl-	C21H15N	none found	
Scan 1462 (8.816 min)	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1604 (9.629 min)	Decane, 3,6-dimethyl-	C12H26	none found	
Scan 1984 (11.803 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2225 (13.182 min)	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	C16H30O4	none found	
Scan 2298 (13.600 min)	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2994 (17.582 min)	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 3395 (19.877 min)	Thiocyanic acid, 5.alpha.-cholestan-3.beta.-yl ester	C28H47NS	none found	
Scan 3745 (21.879 min)	Cholestane, 3-thiocyanato-, (3.alpha.,5.alpha.)-	C28H47NS	none found	

Table 5: List of identified compounds from control run of Pine wood chips on 4% elastane fabric samples. No compounds were identified for pine on 0% elastane and 12% elastane fabric samples.

4% ELASTANE PINE				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 570 (4.285 min)	1R-.alpha.-Pinene	C10H16	bicyclic monoterpene in conifer resin (i.e. pine)	flammable, corrosive, irritant, health hazard, environmental hazard

Table 6: List of identified compounds from *H. fasciculare* on 0% elastane fabric samples at 3 months. No compounds were identified for the first replicate.

0% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 3886 (22.669 min)	erythro-9,10-Dibromopentacosane	C25H50Br2	none found	
0% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1489 (8.971 min)	10-Methylnonadecane	C20H42	none found	
Scan 2000 (11.895 min)	Pentadecane	C15H32	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 3821 (22.314 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	

Table 7: List of identified compounds from *H. fasciculare* on 4% elastane fabric samples at 3 months.

4% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 192 (1.395 min)	17-Pentatriacontene	C35H70	none found	
Scan 942 (5.687 min):	Tetracosane	C24H50	cuticular wax biosynthesis	
Scan 944 (5.698 min):	Hexadecane, 1-chloro-	C16H33Cl	none found	irritant
Scan 1007 (6.059 min)	5-Ethyl-1-nonene	C11H22	none found	
Scan 1527 (9.034 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1645 (9.709 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
4% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1516 (9.073 min)	Tridecane, 1-iodo-	C13H27I	none found	
Scan 1585 (9.468 min):	2-Decene, 7-methyl-, (Z)-	C11H22	none found	
Scan 2012 (11.911 min):	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 2088 (12.346 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 3603 (21.014 min)	1-Bromo-11-iodoundecane	C11H22BrI	none found	
Scan 3828 (22.302 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
4% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2002 (11.905 min	Decane, 3,8-dimethyl-	C12H26	none found	
Scan 2078 (12.340 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2836 (16.677 min)	Acetamide, 2-chloro-N-t-butyl-	C6H12ClNO	none found	irritant

Table 8: List of identified compounds from *H. fasciculare* on 12% elastane fabric samples at 3 months.

12% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1608 (9.582 min)	4-Isopropyl-1,3-cyclohexanedione	C9H14O2	none found	
Scan 2015 (11.911 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2848 (16.678 min):	Hexadecanal, 2-methyl-	C17H34O	none found	
Scan 3819 (22.234 min)	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
Scan 4701 (27.280 min):	Hexacosane	C26H54	cuticular wax biosynthesis	irritant
12% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1584 (9.458 min)	5-Methyl-Z-5-docosene	C23H46	none found	
Scan 1628 (9.710 min)	Disulfide, di-tert-dodecyl	C24H50S2	none found	health hazard
Scan 2011 (11.901 min):	Triacontane, 1-bromo-	C30H61Br	none found	
Scan 2788 (16.347 min):	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	C13H10F3N3O	none found	
Scan 2845 (16.673 min):	1-Propanone, 1-(1-adamantyl)-3-dimethylamino-	C15H25NO	none found	
Scan 4211 (24.489 min):	erythro-9,10-Dibromopentacosane	C25H50Br2	none found	
Scan 4293 (24.959 min):	17-Pentatriacontene	C35H70	none found	
12% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1513 (9.059 min)	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 1603 (9.574 min)	4-Isopropyl-1,3-cyclohexanedione	C9H14O2	none found	
Scan 2011 (11.909 min):	Nonadecane, 2-methyl-	C20H42	none found	
Scan 2085 (12.332 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard

Table 9: List of identified compounds from *S. himantioides* on 0% elastane fabric samples at 3 months.

0% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1431 (8.588 min)	Nonacosane	C29H60	cuticular wax biosynthesis	irritant
Scan 1485 (8.897 min):	10-Methylnonadecane	C20H42	none found	
Scan 1515 (9.069 min)	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll or petroleum	irritant
Scan 1605 (9.584 min)	4-Isopropyl-1,3-cyclohexanedione	C9H14O2	none found	
Scan 2010 (11.901 min)	Tridecane, 3-methyl-	C14H30	none found	
Scan 2086 (12.336 min)	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2219 (13.097 min)	Bicyclo[3.1.0]hexan-3-one	C6H8O	none found	flammable, irritant
Scan 2844 (16.673 min)	Hexadecanal, 2-methyl-	C17H34O	none found	
0% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1513 (9.062 min)	Tridecane, 1-iodo-	C13H27I	none found	
Scan 2009 (11.900 min)	Nonadecane	C19H40	cuticular wax biosynthesis	irritant
Scan 2086 (12.340 min	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2219 (13.101 min	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	C15H24	none found	corrosive
Scan 3825 (22.291 min)	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
0% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 853 (5.262 min)	3-Carene	C10H16	from pine resin	flammable, irritant, health hazard
Scan 936 (5.737 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced), also used in plasticizers	health hazard
Scan 1584 (9.445 min)	tert-Hexadecanethiol	C16H34S	none found	
Scan 1739 (10.332 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1839 (10.904 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 1926 (11.402 min)	Pentadecane, 2,6,10-trimethyl-	C18H38	none found	

Scan 1963 (11.614 min):	Octadecane	C18H38	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1997 (11.808 min):	5-Hexenal, 4-methylene-	C7H10O	none found	
Scan 2012 (11.894 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2088 (12.329 min):	Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	C15H24O	none found	irritant
Scan 2836 (16.609 min):	N-Acetyl-N-methyl-2-methoxyamphetamine	C13H19NO2	none found	
Scan 3830 (22.297 min):	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C28H25NO7	none found	

Table 10: List of identified compounds from *S. himantioides* on 4% elastane fabric samples at 3 months.

4% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 883 (5.283 min)	13-Methylhentriacontane	C32H66	none found	
Scan 1460 (8.585 min)	Octane, 5-ethyl-2-methyl-	C11H24	none found	
Scan 1513 (8.888 min):	Nonadecane, 9-methyl-	C20H42	none found	
Scan 1542 (9.054 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2114 (12.327 min)	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2140 (12.476 min)	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
4% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 979 (5.760 min)	Undecane, 5-methyl-	C12H26	none found	health hazard
Scan 1473 (8.586 min)	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1488 (8.672 min):	Tricosane	C23H48	cuticular wax biosynthesis	
Scan 1502 (8.752 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1526 (8.890 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1555 (9.055 min)	Dodecane, 4,6-dimethyl-	C14H30	none found	
Scan 1864 (10.824 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2052 (11.899 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2128 (12.334 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2152 (12.471 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2262 (13.101 min)	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	C15H24	none found	corrosive
4% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 251 (1.608 min	5-Methyl-Z-5-docosene	C23H46	none found	

Scan 708 (4.223 min):	1R-.alpha.-Pinene	C10H16	bicyclic monoterpene in conifer resin (i.e. pine)	flammable, corrosive, irritant, health hazard, environmental hazard
Scan 1522 (8.881 min)	10-Methylnonadecane	C20H42	none found	
Scan 1553 (9.058 min)	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 1862 (10.826 min):	Tricosane	C23H48	cuticular wax biosynthesis	
Scan 2048 (11.890 min)	Tetratriacontane	C34H70	none found	
Scan 2125 (12.331 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2149 (12.468 min)	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 2258 (13.092 min)	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	C15H24	none found	corrosive

Table 11: List of identified compounds from *S. himantioides* on 12% elastane fabric samples at 3 months.

12% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 257 (1.612 min)	Distearyl sulfide	C36H74S	none found	
Scan 1559 (9.062 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1868 (10.830 min)	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 2054 (11.895 min):	Hentriacontane	C31H64	cuticular wax biosynthesis	
Scan 2130 (12.330 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2155 (12.473 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2200 (12.730 min):	3-Methyl-2-(2-oxopropyl)furan	C8H10O2	none found	
Scan 2263 (13.091 min):	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-, (S)-	C10H14O	limonene degradation, perillyl aldehyde biosynthesis	irritant
12% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1519 (9.057 min)	Heptadecane, 8-methyl-	C18H38	none found	
Scan 2015 (11.895 min):	Heneicosane	C21H44	cuticular wax biosynthesis	
Scan 2091 (12.330 min)	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 3830 (22.281 min)	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
12% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 116 (0.836 min):	Pentatriacontane	C35H72	none found	
Scan 943 (5.568 min)	Heptacosane	C27H56	cuticular wax biosynthesis	
Scan 1074 (6.317 min):	Nonane, 4,5-dimethyl-	C11H24	none found	
Scan 1452 (8.480 min):	Nonadecane, 9-methyl-	C20H42	none found	
Scan 1470 (8.583 min):	Decane, 2-methyl-	C11H24	none found	flammable, health hazard
Scan 1486 (8.675 min):	Dodecane, 2,6,10-trimethyl-	C15H32	aka farnesane; farnesane biosynthesis	health hazard

Scan 1508 (8.801 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1539 (8.978 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 1594 (9.293 min):	Dodecane, 2,6,10-trimethyl-	C15H32	aka farnesane; farnesane biosynthesis	health hazard
Scan 1609 (9.379 min):	Cyclopentane, 1-butyl-2-propyl-	C12H24	none found	
Scan 1854 (10.781 min):	Hexadecane, 1-chloro-	C16H33Cl	none found	irritant
Scan 2044 (11.868 min):	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 2121 (12.308 min):	Butylated Hydroxytoluene	C15H24O	found in soft necked garlic, antioxidant, used in petroleum products	environmental hazard
Scan 2881 (16.657 min):	Hexadecanal, 2-methyl-	C17H34O	none found	
Scan 3058 (17.670 min):	Cyclotriacontane	C30H60	none found	

Table 12: List of identified compounds from *H. fasciculare* on 0% elastane fabric samples at 5 months.

0% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1297 (7.611 min):	Hexadecane, 1-chloro-	C16H33Cl	none found	irritant
Scan 1366 (8.006 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1466 (8.578 min):	Heptacosane	C27H56	cuticular wax biosynthesis	
Scan 1484 (8.681 min)	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	none found	
Scan 1496 (8.750 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1519 (8.881 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1549 (9.053 min):	10-Methylnonadecane	C20H42	none found	
Scan 1662 (9.699 min):	Nonane, 4,5-dimethyl-	C11H24	none found	
Scan 2357 (13.676 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2472 (14.334 min):	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 2683 (15.542 min):	6-[1-[4-Fluorophenyl]ethyl]-1,3-benzodioxol-5-ol	C15H13FO3	none found	
Scan 2747 (15.908 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2887 (16.709 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2921 (16.903 min):	Phosphoric acid, 3-(1,1-dimethylethyl)-4-hydroxyphenyl dimethyl ester	C12H19O5P	none found	
Scan 3805 (21.962 min):	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	C11H13NO3	none found	
0% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1471 (8.571 min)	Undecane, 3,8-dimethyl-	C13H28	none found	
Scan 1488 (8.669 min):	Undecane, 3,8-dimethyl-	C13H28	none found	
Scan 1501 (8.743 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1526 (8.886 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1554 (9.046 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1588 (9.241 min):	Methoxyacetic acid, 3-pentadecyl ester	C18H36O3	none found	

Scan 1607 (9.350 min):	Undecane, 4,7-dimethyl-	C13H28	none found	
Scan 1644 (9.561 min):	Cyclopentane, 1-butyl-2-propyl-	C12H24	none found	
Scan 1668 (9.699 min):	Nonane, 4,5-dimethyl-	C11H24	none found	
Scan 1868 (10.843 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1915 (11.112 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1956 (11.347 min):	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	C15H24	none found	
Scan 1980 (11.484 min):	Heptadecane	C17H36	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis, heptadecane biosynthesis (produced)	health hazard
Scan 2050 (11.884 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2150 (12.457 min):	Tridecane, 3-methyl-	C14H30	none found	
Scan 2293 (13.275 min):	Phenol, 2,4,6-tris(1,1-dimethylethyl)-	C18H30O	antioxidant in hydrocarbonfuels	irritant, environmental hazard
Scan 3865 (22.270 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
Scan 4726 (27.196 min):	Acetamide, N-[2-(acetyloxy)-2-[3,4-bis(acetyloxy)phenyl]ethyl]-N-methyl-, (R)-	C17H21NO7	none found	
0% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1103 (6.488 min):	Nonane, 4,5-dimethyl-	C11H24	none found	
Scan 1297 (7.598 min	Decane	C10H22	cuticular wax biosynthesis	flammable, health hazard
Scan 1366 (7.993 min)	Docosane, 7-butyl-	C26H54	none found	
Scan 1444 (8.439 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed),component of gasoline	health hazard
Scan 1467 (8.571 min):	Decane, 5-propyl-	C13H28	none found	
Scan 1484 (8.668 min)	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1497 (8.743 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed),component of gasoline	health hazard
Scan 1550 (9.046 min)	Tridecanol, 2-ethyl-2-methyl-	C16H34O	none found	

Scan 1585 (9.246 min):	Methoxyacetic acid, 3-pentadecyl ester	C18H36O3	none found	
Scan 1604 (9.355 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1640 (9.561 min):	4-Isopropyl-1,3-cyclohexanedione	C9H14O2	none found	
Scan 1663 (9.693 min):	Dodecane, 2,6,11-trimethyl-	C15H32	none found	
Scan 1710 (9.961 min):	Heptadecane, 8-methyl-	C18H38	none found	
Scan 1912 (11.117 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1952 (11.346 min):	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	C15H24	aka aromadendrene	
Scan 1975 (11.478 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1993 (11.581 min):	Nonadecane, 9-methyl-	C20H42	none found	
Scan 2045 (11.878 min):	Tridecane	C13H28	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2066 (11.999 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2145 (12.451 min):	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	none found	
Scan 2359 (13.675 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 3413 (19.706 min):	6-Chloro-4-phenyl-2-(3-pyridyl)quinoline	C20H13ClN2	none found	

Table 13: List of identified compounds from *H. fasciculare* on 4% elastane fabric samples at 5 months.

4% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1858 (10.810 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2043 (11.868 min):	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2361 (13.688 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2432 (14.094 min):	Oxymetazoline	C16H24N2O	none found	corrosive, acute toxic
Scan 2474 (14.334 min):	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 2510 (14.540 min):	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	C18H26O	none found	
Scan 2684 (15.536 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2749 (15.908 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2823 (16.331 min):	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 2859 (16.537 min):	3-(3-Nitrophenyl)-5-(trifluoromethyl)-1,2,4-oxadiazole	C9H4F3N3O3	none found	
Scan 2889 (16.709 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2923 (16.904 min):	Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	C17H26N2O	none found	irritant, environmental hazard
Scan 3862 (22.276 min):	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	none found	health hazard
4% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1466 (8.564 min):	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll or petroleum	irritant
Scan 1485 (8.673 min):	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 1497 (8.741 min):	Dodecane, 2,6,10-trimethyl-	C15H32	aka farnesane; farnesane biosynthesis	health hazard
Scan 1521 (8.879 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1549 (9.039 min):	Heptadecane, 8-methyl-	C18H38	none found	
Scan 1619 (9.439 min):	Cyclopentane, 1-butyl-2-propyl-	C12H24	none found	
Scan 1662 (9.685 min):	2-Isopropyl-5-methyl-1-heptanol	C11H24O	none found	
Scan 1712 (9.972 min):	Octane, 5-ethyl-2-methyl-	C11H24	none found	

Scan 1912 (11.116 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1976 (11.482 min):	10-Methylnonadecane	C20H42	none found	
Scan 2046 (11.883 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2146 (12.455 min):	Pentacosane	C25H52	cuticular wax biosynthesis	health hazard
Scan 2359 (13.674 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
4% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 988 (5.828 min)	Decane, 3,6-dimethyl-	C12H26	none found	
Scan 1365 (7.986 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1444 (8.438 min):	Nonacosane	C29H60	cuticular wax biosynthesis	irritant
Scan 1467 (8.569 min):	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll or petroleum	irritant
Scan 1483 (8.661 min):	Decane, 2,3,5,8-tetramethyl-	C14H30	none found	
Scan 1497 (8.741 min):	Nonacosane	C29H60	cuticular wax biosynthesis	irritant
Scan 1521 (8.878 min):	Heneicosane	C21H44	cuticular wax biosynthesis	
Scan 1550 (9.044 min):	Heptadecane, 8-methyl-	C18H38	none found	
Scan 1711 (9.965 min):	Heptacosane	C27H56	cuticular wax biosynthesis	
Scan 1861 (10.824 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1911 (11.110 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1953 (11.350 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1976 (11.482 min):	Docosane	C22H46	none found	irritant
Scan 1991 (11.568 min):	Decane, 3,8-dimethyl-	C12H26	none found	
Scan 2045 (11.877 min):	Heptacosane	C27H56	cuticular wax biosynthesis	
Scan 2066 (11.997 min):	Methoxyacetic acid, 4-tetradecyl ester	C17H34O3	none found	
Scan 2146 (12.454 min):	Pentadecane, 4-methyl-	C16H34	none found	
Scan 2359 (13.673 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard

Scan 3863 (22.279 min):	Adamantane, 1-isothiocyanato-3-methyl-	C12H17NS	none found	

Table 14: List of identified compounds from *H. fasciculare* on 12% elastane fabric samples at 5 months.

12% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 755 (4.490 min):	7H-Dibenzo[b,g]carbazole, 7-methyl-	C21H15N	none found	
Scan 1665 (9.697 min):	Tetradecane, 4-ethyl-	C16H34	none found	
Scan 2044 (11.866 min):	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2361 (13.679 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2433 (14.091 min):	Oxymetazoline	C16H24N2O	none found	corrosive, acute toxic
Scan 2475 (14.332 min):	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 2660 (15.390 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2685 (15.533 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2824 (16.329 min):	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 2860 (16.535 min):	Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	C17H26N2O	none found	irritant, environmental hazard
Scan 2890 (16.706 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2924 (16.901 min):	Phosphoric acid, 3-(1,1-dimethylethyl)-4-hydroxyphenyl dimethyl ester	C12H19O5P	none found	
Scan 3863 (22.274 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
12% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1489 (8.882 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1632 (9.700 min):	Dodecane, 2,6,11-trimethyl-	C15H32	none found	
Scan 1826 (10.810 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 2014 (11.886 min):	Pentacosane	C25H52	cuticular wax biosynthesis	health hazard

Scan 2327 (13.677 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2441 (14.329 min):	1,4-Dimethyl-1,4-dihydro-2,3-quinoxalinedithione	C10H10N2S2	none found	
Scan 2791 (16.332 min):	.pi.-Cyclopentadienyl-dicarbonyl-ethylisonitril-trichlorgermyl-tungsten	C10H10Cl3GeNO2W	none found	
12% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1631 (9.559 min)	Trichloroacetic acid, pentadecyl ester	C17H31Cl3O2	none found	
Scan 1850 (10.813 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 2035 (11.871 min):	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2352 (13.685 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2423 (14.091 min):	Piperonal, 6-(4-methoxy-1-cyclohexen-1-yl)-	C15H16O4	none found	
Scan 2465 (14.332 min):	5,6,7-Trimethoxy-1-indanone	C12H14O4	none found	
Scan 2502 (14.543 min):	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	C18H26O	none found	
Scan 2676 (15.539 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2740 (15.905 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2815 (16.334 min):	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 2881 (16.712 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2914 (16.901 min):	Naphthalene, 2-methyl-1-(3,4-dimethylbenzoyl)-	C20H18O	none found	

Table 15: List of identified compounds from *S. himantioides* on 0% elastane fabric samples at 5 months.

0% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1367 (7.998 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1468 (8.575 min):	Tridecane, 2-methyl-	C14H30	none found	
Scan 1522 (8.884 min):	Nonadecane, 9-methyl-	C20H42	none found	
Scan 1551 (9.050 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2045 (11.877 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2359 (13.674 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2473 (14.326 min):	Thiourea, N-(3-methoxyphenyl)-N'-(2-propenyl)-	C11H14N2OS	none found	
Scan 2533 (14.669 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2659 (15.390 min):	4-Aminodiphenylsulfone	C12H11NO2S	none found	
Scan 2684 (15.533 min):	Naphthalene, 2-methyl-1-(4-methylbenzoyl)-	C19H16O	none found	
Scan 2748 (15.900 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2858 (16.529 min):	Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	C17H26N2O	none found	irritant, environmental hazard
Scan 2889 (16.706 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2922 (16.895 min):	Naphthalene, 2-methyl-1-(3,4-dimethylbenzoyl)-	C20H18O	none found	
Scan 3862 (22.274 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
0% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1359 (8.005 min)	Heptacosane, 1-chloro-	C27H55Cl	none found	
Scan 1459 (8.577 min):	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll or petroleum	irritant
Scan 1511 (8.874 min):	Decane, 3,8-dimethyl-	C12H26	none found	
Scan 1541 (9.046 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1576 (9.246 min):	Octadecane	C18H38	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard

Scan 1595 (9.355 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1702 (9.967 min):	Heptacosane	C27H56	cuticular wax biosynthesis	
Scan 1850 (10.814 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1967 (11.484 min):	Tetradecane, 2,6,10-trimethyl-	C17H36	none found	
Scan 2036 (11.878 min):	Octadecane, 1-iodo-	C18H37I	none found	irritant
Scan 2136 (12.451 min):	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll, or petroleum	irritant
Scan 2350 (13.675 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2739 (15.901 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2850 (16.536 min):	Phosphoric acid, 3-(1,1-dimethylethyl)-4-hydroxyphenyl dimethyl ester	C12H19O5P	none found	
Scan 2879 (16.702 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2913 (16.897 min):	Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	C17H26N2O	none found	irritant, environmental hazard
Scan 3853 (22.275 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
0% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 905 (5.334 min):	3-Carene	C10H16	from pine resin	flammable, irritant, health hazard
Scan 957 (5.632 min):	1R-.alpha.-Pinene	C10H16	bicyclic monoterpene in conifer resin (i.e. pine)	flammable, corrosive, irritant, health hazard, environmental hazard
Scan 1449 (8.447 min):	Octane, 5-ethyl-2-methyl-	C11H24	none found	
Scan 1470 (8.567 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1524 (8.876 min):	10-Methylnonadecane	C20H42	none found	
Scan 1865 (10.828 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1955 (11.342 min):	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	C15H24	aka aromadendrene	
Scan 1979 (11.480 min):	Heneicosane	C21H44	cuticular wax biosynthesis	

Scan 2049 (11.880 min):	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 2135 (12.372 min):	6-Tridecene, 7-methyl-	C14H28	none found	
Scan 2149 (12.453 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2173 (12.590 min):	Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl-	C18H36	none found	
Scan 2362 (13.671 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2483 (14.364 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	

Table 16: List of identified compounds from *S. himantioides* on 4% elastane fabric samples at 5 months.

4% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1366 (7.988 min)	Docosane, 7-butyl-	C26H54	none found	
Scan 1425 (8.326 min):	Undecane, 4,8-dimethyl-	C13H28	none found	
Scan 1446 (8.446 min):	Dodecane, 2,6,10-trimethyl-	C15H32	aka farnesane; farnesane biosynthesis	health hazard
Scan 1468 (8.572 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1486 (8.675 min):	Decane, 2,3,5,8-tetramethyl-	C14H30	none found	
Scan 1499 (8.749 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1522 (8.881 min):	10-Methylnonadecane	C20H42	none found	
Scan 1551 (9.047 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1665 (9.699 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 1712 (9.968 min):	Pentadecane	C15H32	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1861 (10.821 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1913 (11.118 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1977 (11.484 min):	Dodecane, 2-methyl-	C13H28	none found	health hazard
Scan 1995 (11.587 min):	Dodecane, 2-methyl-	C11H24	none found	health hazard
Scan 2047 (11.885 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2133 (12.377 min):	Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl-	C18H36	none found	
Scan 2147 (12.457 min):	Octadecane	C18H38	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2482 (14.374 min):	Pentacosane	C25H52	cuticular wax biosynthesis	health hazard
4% SH 2				

Retention Time (min)	Compound name	Molecular formula	Function/Activity	Hazard warnings
Scan 992 (5.852 min):	Decane, 3,6-dimethyl-	C12H26	none found	
Scan 1366 (7.992 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1467 (8.570 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1484 (8.668 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1497 (8.742 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1520 (8.874 min):	Dodecane, 3-methyl-	C13H28	none found	
Scan 1550 (9.045 min):	Decane, 3,7-dimethyl-	C12H26	none found	
Scan 1584 (9.240 min):	Methoxyacetic acid, 2-tridecyl ester	C16H32O3	none found	
Scan 1604 (9.354 min):	Decane, 3,6-dimethyl-	C12H26	none found	
Scan 1663 (9.692 min):	Dodecane, 5-methyl-	C13H28	none found	
Scan 1860 (10.819 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 2044 (11.872 min):	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	none found	irritant
Scan 2359 (13.674 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2532 (14.664 min):	Stannane, (4-fluorophenyl)trimethyl-	C9H13FSn	none found	
Scan 2684 (15.534 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2748 (15.900 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2888 (16.701 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2922 (16.896 min):	Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	C17H26N2O	none found	irritant, environmental hazard
Scan 3056 (17.663 min):	3-(4-N,N-Dimethylaminophenyl)propenoic acid, 2-(diethoxyphosphinyl)-, ethyl ester	C17H26NO5P	none found	
4% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 993 (5.872 min):	Decane, 3,6-dimethyl-	C12H26	none found	

Scan 1244 (7.308 min):	17-Pentatriacontene	C35H70	none found	
Scan 1274 (7.480 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1298 (7.617 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1362 (7.983 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1441 (8.435 min):	Heptane, 2,6-dimethyl-	C9H20	none found	
Scan 1464 (8.567 min):	Octadecane, 2,6-dimethyl-	C20H42	none found	
Scan 1481 (8.664 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed), component of gasoline	health hazard
Scan 1495 (8.744 min):	Triacontane	C30H62	cuticular wax biosynthesis	
Scan 1519 (8.882 min):	10-Methylnonadecane	C20H42	none found	
Scan 1547 (9.042 min):	Pentadecane	C15H32	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1583 (9.248 min):	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	none found	
Scan 1600 (9.345 min):	Decane, 3,7-dimethyl-	C12H26	none found	
Scan 1658 (9.677 min)	4-Isopropyl-1,3-cyclohexanedione	C9H14O2	none found	
Scan 1859 (10.827 min):	Heptacosane, 1-chloro-	C27H55Cl	none found	
Scan 1909 (11.113 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1973 (11.479 min):	Tetradecane, 2,6,10-trimethyl-	C17H36	none found	
Scan 1987 (11.560 min):	Octacosane	C28H58	cuticular wax biosynthesis	
Scan 2013 (11.708 min):	Heptacosane, 1-chloro-	C27H55Cl	none found	
Scan 2042 (11.874 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 2143 (12.452 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 2356 (13.671 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2746 (15.903 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2885 (16.698 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2919 (16.892 min):	1,3-Diethyl-2-hydroxybenzothiazolo[3,2-a]pyrimidinium-4(1H)-one, inner salt	C14H14N2O2S	none found	

Table 17: List of identified compounds from *S. himantioides* on 12% elastane fabric samples at 5 months.

12% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1468 (8.571 min)	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1487 (8.680 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1499 (8.748 min):	Octane, 5-ethyl-2-methyl-	C11H24	none found	
Scan 1523 (8.886 min):	Decane, 2,4,6-trimethyl-	C13H28	none found	
Scan 1551 (9.046 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1666 (9.704 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 1864 (10.837 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1913 (11.117 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1953 (11.346 min)	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]-	C15H24	oleoresin sesquiterpene volatiles biosynthesis	irritant, health hazard, environmental hazard
Scan 1978 (11.489 min	Dodecane, 2-methyl-	C13H28	none found	health hazard
Scan 2046 (11.878 min):	Octadecane, 1-iodo-	C18H37I	none found	irritant
Scan 2147 (12.456 min):	Dodecane, 2-methyl-	C13H28	none found	health hazard
Scan 2360 (13.675 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2750 (15.907 min):	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2889 (16.702 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2923 (16.896 min):	Naphthalene, 2-methyl-1-(3,4-dimethylbenzoyl)-	C20H18O	none found	
Scan 3862 (22.269 min):	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	none found	health hazard
Scan 4721 (27.185 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
12% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 975 (5.803 min	Decane, 3,6-dimethyl-	C12H26	none found	

Scan 1359 (8.000 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1456 (8.556 min)	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	aka phytane - can be from chlorophyll or petroleum	irritant
Scan 1474 (8.659 min):	Undecane	C11H24	cuticular wax biosynthesis (consumed),component of gasoline	health hazard
Scan 1486 (8.727 min)	Tridecane, 4-methyl-	C14H30	none found	
Scan 1511 (8.870 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1526 (8.956 min):	Heneicosane	C21H44	cuticular wax biosynthesis	
Scan 1540 (9.036 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1577 (9.248 min):	Methoxyacetic acid, 4-tetradecyl ester	C17H34O3	none found	
Scan 1594 (9.345 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1655 (9.694 min):	Dodecane, 2,6,11-trimethyl-	C15H32	none found	
Scan 1854 (10.833 min):	1,2,4-Methenoazulene, decahydro-1,5,5,8a-tetramethyl-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]-	C15H24	aka Longicyclene	environmental hazard
Scan 1902 (11.108 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1941 (11.331 min):	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	C15H24	none found	
Scan 1967 (11.479 min):	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	none found	
Scan 1985 (11.582 min):	Tetradecane, 2,6,10-trimethyl-	C17H36	none found	
Scan 2036 (11.874 min):	Pentacosane	C25H52	cuticular wax biosynthesis	health hazard
Scan 2057 (11.994 min):	Methoxyacetic acid, 3-pentadecyl ester	C18H36O3	none found	
Scan 2136 (12.446 min):	Dodecane	C12H26	cuticular wax biosynthesis (consumed); component of gasoline	irritant, health hazard
Scan 2199 (12.807 min):	Benzene, 1-(5,5-dimethyl-1-cyclopenten-1-yl)-2-methoxy-	C14H18O	none found	
Scan 2350 (13.671 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2523 (14.661 min):	1,3,5-Triazin-2-amine, 4,6-dimethoxy-N-(2,6-dimethylphenyl)-	C13H16N4O2	none found	
Scan 2570 (14.930 min):	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	C18H20	none found	irritant
Scan 2649 (15.382 min):	2,2':6',2"-Terpyridine	C15H11N3	none found	corrosive, acute toxic, irritant

Scan 2739 (15.897 min)	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	none found	irritant
Scan 2849 (16.526 min)	Naphthalene, 2-methyl-1-(3,4-dimethylbenzoyl)-	C20H18O	none found	
Scan 2879 (16.698 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2913 (16.892 min):	1-(4-Undecylphenyl)ethanone	C19H30O	none found	
12% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 985 (5.806 min):	Decane, 3,8-dimethyl-	C12H26	none found	
Scan 1297 (7.592 min):	Octadecane, 1-chloro-	C18H37Cl	none found	irritant
Scan 1369 (8.004 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 1466 (8.559 min)	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	none found	
Scan 1486 (8.673 min):	Decane, 2,3,5,8-tetramethyl-	C14H30	none found	
Scan 1497 (8.736 min):	Dodecane, 4,6-dimethyl-	C14H30	none found	
Scan 1520 (8.868 min):	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 1550 (9.039 min):	10-Methylnonadecane	C20H42	none found	
Scan 1585 (9.239 min):	Disulfide, di-tert-dodecyl	C24H50S2	none found	health hazard
Scan 1605 (9.354 min):	Methoxyacetic acid, 2-tridecyl ester	C16H32O3	none found	
Scan 1619 (9.434 min):	Cyclopentane, 1-butyl-2-propyl-	C12H24	none found	
Scan 1664 (9.692 min):	1-Octanol, 2-butyl-	C12H26O	none found	environmental hazard
Scan 1711 (9.960 min):	Hexadecane, 4-methyl-	C17H36	none found	
Scan 1751 (10.189 min):	Thiocolchicine	C22H25NO5S	none found	corrosive, acute toxic, health hazard
Scan 1859 (10.807 min):	Tetradecane	C14H30	none found	health hazard if inhaled or ingested
Scan 1883 (10.945 min):	Octadecane, 1-chloro-	C18H37Cl	none found	irritant
Scan 1911 (11.105 min):	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	C15H24	none found	
Scan 1953 (11.345 min):	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	C15H24	none found	
Scan 1976 (11.477 min):	Dodecane, 2-methyl-	C13H28	none found	health hazard
Scan 1991 (11.563 min):	Decane, 3,8-dimethyl-	C12H26	none found	
Scan 2017 (11.711 min):	2,6-Dimethyldecane	C12H26	none found	

Scan 2046 (11.877 min):	Heneicosane	C21H44	cuticular wax biosynthesis	
Scan 2146 (12.450 min):	Hexadecane, 7,9-dimethyl-	C18H38	none found	
Scan 2359 (13.668 min):	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C18H30O	none found	irritant, environmental hazard
Scan 2481 (14.366 min):	Tetrapentacontane	C54H110	none found	
Scan 2889 (16.701 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2923 (16.895 min):	Pyridine-3-carbonitrile, 1,2-dihydro-5-acetyl-6-methyl-4-(2-thienyl)-2-thioxo-	C13H10N2OS2	none found	

Table 18: List of identified compounds from *H. fasciculare* on 0% elastane fabric samples at 8 months.

0% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 759 (4.530 min)	4-Nitro-4'-chlorodiphenylsulfoxide	C12H8ClNO3S	none found	
Scan 2355 (13.663 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 3258 (18.830 min):	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 4473 (25.782 min):	Cyclodocosane, ethyl-	C24H48	none found	
0% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2356 (13.658 min)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2681 (15.518 min):	6-[1-[4-Fluorophenyl]ethyl]-1,3-benzodioxol-5-ol	C15H13FO3	none found	
0% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2851 (16.621 min)	N,N-Dimethyl-3-benzyloxypropylamine	C12H19NO	none found	
Scan 3835 (22.252 min)	Diethyl Phthalate	C12H14O4	none found	irritant

Table 19: List of identified compounds from *H. fasciculare* on 4% elastane fabric samples at 8 months.

4% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 4239 (24.426 min):	Eicosane	C20H42	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	
Scan 4266 (24.580 min):	Oxirane, tetradecyl-	C16H32O	none found	irritant, health hazard
Scan 4331 (24.952 min):	2-Piperidinone, N-[4-bromo-n-butyl]-	C9H16BrNO	none found	
Scan 4362 (25.130 min):	2-Piperidinone, N-[4-bromo-n-butyl]-	C9H16BrNO	none found	
Scan 4429 (25.513 min):	Heneicosane, 11-cyclopentyl-	C26H52	none found	
4% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1941 (11.333 min):	Cycloheptane, 1,3,5-tris(methylene)-	C10H14	none found	
Scan 2490 (14.474 min):	3-Methylpyridazine	C5H6N2	none found	irritant
4% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2043 (11.861 min)	Hexacosane	C26H54	cuticular wax biosynthesis	irritant
Scan 2356 (13.652 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2876 (16.628 min):	Cyclohexanone, 2-dimethylaminomethyl-4-(1,1-dimethylethyl)-	C13H25NO	none found	

Table 20: List of identified compounds from *H. fasciculare* on 12% elastane fabric samples at 8 months.

12% HF 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2818 (16.300 min)	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
Scan 3050 (17.628 min):	1,3-Xylyl-15-crown-4, 2,3-.pinanedioxyboryl-	C24H35BO6	none found	
12% HF 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 428 (2.903 min):	Benzo[h]quinoline, 2,4-dimethyl-	C15H13N	none found	
Scan 2307 (13.655 min):	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	none found	
Scan 2826 (16.625 min):	Pentanal, 2,3-dimethyl-	C7H14O	none found	flammable, irritantz
Scan 3810 (22.255 min)	Diethyl Phthalate	C12H14O4	none found	irritant
12% HF 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2875 (16.626 min)	2-Decanone	C10H20O	cuticular wax biosynthesis	environmental hazard

Table 21: List of identified compounds from *S. himantioides* on 0% elastane fabric samples at 8 months.

0% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2040 (11.860 min):	Heptadecane	C17H36	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis, heptadecane biosynthesis (produced)	health hazard
Scan 2872 (16.620 min)	Undecanal, 2-methyl-	C12H24O	none found	irritant, environmental hazard
0% SH 2				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1945 (11.318 min	1,5-Heptadiyne	C7H8	none found	
0% SH 3				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2045 (11.860 min)	Decane, 5-propyl-	C13H28	none found	

Scan 2878 (16.626 min):	9-Azabicyclo[6.1.0]non-4-en-9-amine, (1.alpha.,4Z,8.alpha.)-	C8H14N2	none found	
Scan 3862 (22.256 min):	2-(2-Carboxyvinyl)pyridine, trans	C8H7NO2	none found	irritant

Table 22: List of identified compounds from *S. himantioides* on 4% elastane fabric samples at 8 months. No compounds were identified for replicates 2 and 3.

4% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 1743 (10.161 min):	Thiocolchicine	C22H25NO5S	none found	corrosive, acute toxic, health hazard

Table 23: List of identified compounds from *S. himantioides* on 12% elastane fabric samples at 8 months. No compounds were identified for replicates 2 and 3.

12% SH 1				
Retention Time (min)	**Compound name**	**Molecular formula**	**Function/Activity**	**Hazard warnings**
Scan 2040 (11.868 min)	Nonadecane	C19H40	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 4237 (24.439 min):	Hexadecane	C16H34	cuticular wax biosynthesis, alkane oxidation (consumed); alkane biosynthesis (produced)	health hazard
Scan 4331 (24.977 min):	Heneicosane, 11-cyclopentyl-	C26H52	none found	
Scan 4361 (25.148 min):	Heneicosane, 11-cyclopentyl-	C26H52	none found	
Scan 4430 (25.543 min):	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	C20H40	none found	
Scan 4540 (26.172 min):	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	C23H30N2O5	none found	

Appendix 7: Tables of genes found in search results of JGI Mycocosm for the fungal species *Hypholoma sublaterium* and *Serpula himantioides.*

Table 1: Genes listed on JGI Mycocosm database as coding for laccases in *Serpula himantioides*

Gene type	Protein Id	E.C. Number	Annotation
Laccase	20432	1.10.3.2	multicopper oxidase/laccase
Laccase	13040	1.10.3.2	multicopper oxidase/laccase
Laccase	16926	1.10.3.2	multicopper oxidase/laccase
Laccase	16927	1.10.3.2	multicopper oxidase/laccase
Laccase	30351	1.10.3.2	multicopper oxidase/laccase
Total number of laccase genes:		5	

Table 2: Genes listed on JGI Mycocosm database as coding for laccases in *Hypholoma sublateritium*

Gene type	Protein Id	E.C. Number	Annotation
Laccase	41407	1.10.3.2	multicopper oxidase/laccase
Laccase	46791	1.10.3.2	multicopper oxidase/laccase
Laccase	91331	1.10.3.2	multicopper oxidase/laccase
Laccase	139684	1.10.3.2	multicopper oxidase/laccase
Laccase	164160	1.10.3.2	multicopper oxidase/laccase
Laccase	186693	1.10.3.2	multicopper oxidase/laccase
Laccase	201837	1.10.3.2	multicopper oxidase/laccase
Laccase	201849	1.10.3.2	multicopper oxidase/laccase
Laccase	202502	1.10.3.2	multicopper oxidase/laccase
Total number of laccase genes:		9	

Table 3: Genes listed on JGI Mycocosm database as coding for cellulases in *Serpula himantioides*

Gene type	Protein Id	E.C. Number	Annotation
Cellulase	37274	3.2.1.4	Cellulase
Total number of cellulase genes:			1

Table 4: Genes listed on JGI Mycocosm database as coding for cellulases in *Hypholoma sublateritium*

Gene type	Protein Id	E.C. Number	Annotation
Cellulase	209533	3.2.1.4	Cellulase
Cellulase	103441	3.2.1.4	Cellulase
Cellulase	126694	3.2.1.4	Cellulase
Total number of cellulase genes:			3

Table 5: Genes listed on JGI Mycocosm database as coding for peroxidases in *Serpula himantioides*

Gene type	Protein Id	E.C. Number	Annotation
Peroxidase	40707	1.11.1.5	cytochrome-c peroxidase
Peroxidase	15277	1.11.1.9	glutathione peroxidase
Peroxidase	32974		peroxidase, family 2
Peroxidase	36405		peroxidase, family 2
Peroxidase	118144		peroxidase, family 2
Total number of peroxidase genes:			5

Table 6: Genes listed on JGI Mycocosm database as coding for peroxidases in *Hypholoma sublateritium*

Gene type	Protein Id	E.C. Number	Annotation
Peroxidase	46322	1.11.1.5	cytochrome-c peroxidase
Peroxidase	52546	1.11.1.9	glutathione peroxidase
Peroxidase	43989	1.11.1.11	L-ascorbate peroxidase
Peroxidase	47465	1.11.1.11	L-ascorbate peroxidase
Peroxidase	47473	1.11.1.11	L-ascorbate peroxidase
Peroxidase	47480	1.11.1.11	L-ascorbate peroxidase
Peroxidase	47481	1.11.1.11	L-ascorbate peroxidase
Peroxidase	48449	1.11.1.11	L-ascorbate peroxidase
Peroxidase	86525	1.11.1.11	L-ascorbate peroxidase
Peroxidase	132620	1.11.1.11	L-ascorbate peroxidase
Peroxidase	167926	1.11.1.11	L-ascorbate peroxidase
Peroxidase	207174	1.11.1.11	L-ascorbate peroxidase
Peroxidase	207400	1.11.1.11	L-ascorbate peroxidase
Peroxidase	220069	1.11.1.11	L-ascorbate peroxidase
Peroxidase	47826		peroxidase
Peroxidase	48818		peroxidase
Peroxidase	122627		peroxidase
Peroxidase	202342		peroxidase
Peroxidase	39413		peroxidase,family 2
Peroxidase	48125		peroxidase,family 2
Peroxidase	58650		peroxidase,family 2
Peroxidase	71872		peroxidase,family 2
Peroxidase	151803		peroxidase,family 2
Peroxidase	161601		peroxidase,family 2
Peroxidase	172280		peroxidase,family 2
Peroxidase	642519		peroxidase,family 2
Peroxidase	642555		peroxidase,family 2

Peroxidase	184750		peroxidase
Peroxidase	296328		peroxidase
Peroxidase	72108		peroxidase
Peroxidase	68515		peroxidase/oxygenase
Total number of peroxidase genes:			31