RECENT RESEARCH ON CARBON-BASED PRESERVATIVES

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Summary

To facilitate the introduction of carbon-based preservative-treated wood into the Canadian market, FPInnovations identified five key knowledge gaps and initiated research to address them. These were:How do we predict performance of preservative treated wood above ground? Which fungi limit the performance of wood treated with carbon-based biocides? How do we monitor change in biocide concentration, re-distribution and depletion with time? Will carbon-based preservatives be effective as shell treatments on Canadian species? Can wood treated with carbon-based biocides be recycled without contamination? This paper primarily describes the work completed to address the second and fifth of these.

After three to four years in a field setting, decks treated with carbon-based preservatives were colonized by a range of fungal species that could potentially be predictive of future decay. Some of these species may be of importance due to their potential ability to degrade the preservatives, while other species may be indicators of decay or weaknesses in a formulation.

In combustion experiments, significant quantities of triazoles were detected in the filtered smoke. After one year of exposure in a small-scale composter, triazoles showed no signs of depletion, while the concentration of DDAcarbonate was reduced.

1. Introduction

Given sustainable harvesting, wood products have a very favourable environmental profile in uses where their service life can match or exceed the harvest rotation period. In uses with conditions conducive to weathering, decay, and termites, the attributes of wood products must be enhanced to extend their service life, reduce maintenance requirements, and improve aesthetics.

Wood preservative manufacturers have developed a first generation of carbon-based (organic in the chemical sense) preservative for above-ground uses that do not contain copper or other metals (copper will still be used for ground contact and products with high consequences of failure). The omission of copper, which is capable of moving onto and protecting untreated wood exposed in checks and cuts, requires that other means are found to ensure performance in Canadian species, which cannot be treated all the way through. The omission of copper, which is also a UV absorber, requires some other method to control weathering. The major potential benefit of the omission of copper is

that it may be possible to recycle or recover value from the treated wood with no detectable contaminants. Currently, end of service life issues are the major Achilles heal of treated wood products. Industry's ability to confidently transition to these new preservatives and improved consumer perception of wood that can be readily recycled would help ensure that wood retains and expands markets currently under threat from non-renewable materials with considerably higher energy consumption.

Five key knowledge gaps were identified and are summarized below. This paper focuses primarily on II and V.

I. How do we predict performance of preservative treated wood above ground?

An Accelerated Field Simulator (AFS), designed and installed at FPInnovations' Vancouver laboratory, will facilitate the prediction of treated wood performance above ground. This chamber has controlled temperature and relative humidity, programmable water spray, leachate collection, and holders for dispersing basidiospores from Petri plates.

II. Which fungi limit the performance of wood treated with carbon-based biocides?

One of the major issues from a Canadian perspective is that there is a very limited number of biocides registered by Canada's Pest Management Regulatory Agency for use on wood. This limits the permutations and combinations of biocides that can be formulated together to provide cross-protection against biodegradation. These new preservatives have so far seen limited use in North America, but knowledge of the characteristics of these biocides enables the prediction of anticipated advantages and disadvantages of these formulations. The biodegradability of the active ingredients is both an advantage and a disadvantage. It may be a disadvantage for depletion in service and reduction in durability but it may be an advantage for disposal, or value recovery at the end of the service life. It is therefore important to study the organisms that can colonize wood treated with carbon-based preservatives, particularly those that have the potential to degrade xenobiotic compounds.

FPInnovations has had decking material treated with carbon-based preservatives in test for a range of contract clients for 3 to 4 years. With the permission of two of our contract clients, the opportunity was taken to isolate organisms present close to the surface of some of this material. At the request of these clients, the identity of the preservative formulations will remain confidential. Molecular methods were used to identify these organisms in order to establish a profile of species that may have the capability to break down carbon-based preservatives or decay wood in the presence of carbon-based preservatives.

III. How do we monitor change in biocide concentration, re-distribution and depletion with time?

Liquid Chromatography/Mass Spectrometry (LC/MS) is our primary tool for measuring the concentration, re-distribution, and depletion of carbon-based actives. Methods for analysis of very low concentrations of triazoles and DDAC have been developed (Stirling *et al*., 2010, Woo *et al*., 2010).

IV. Will carbon-based preservatives be effective as shell treatments on Canadian species?

This work has been completed by a UBC Masters student (Chelsea Woo) with whom we have worked closely. Her analysis on the efficacy of shell treatments is summarized in her thesis (Woo, 2010).

V. Can wood treated with carbon-based biocides be recycled without contamination?

When wood treated with metal-based preservatives reaches the end of its service life disposal options are limited if it cannot be re-used (Morrell, 2003), or converted into a composite wood product (Felton and DeGroot, 1996; Vick *et al.*, 1996). Such wood is either placed in a landfill or incinerated, depending on the jurisdiction.

Other than re-using treated wood, kraft pulping would be the best use of treated wood at the end of its service life because it makes a new product that itself can be recycled. This was investigated in this project and is described by Stirling *et al*. (2010) who found residues of triazoles and quaternary ammonium compounds in both the pulp fibres and in the black liquor. This indicated that ordinary kraft pulping would not be a suitable process to recycle wood treated with carbon-based preservatives.

The composting of wood treated with metal-based preservatives is not recommended, as the metals may contaminate the resulting soil. As a result, little research has been conducted on composting treated wood. The composting of wood treated with carbon-based preservatives would not suffer these problems provided that the preservatives are broken down by the composting process. Several bacteria (Ghisalba and Küenzi, 1983; Cook *et al*., 2002; Wallace and Dickinson, 2006) and fungi (Lee *et al*., 1992; DuBois and Ruddick, 1998; Obanda and Shupe, 2008) have been found to break down carbon-based preservatives. Since wood is very high in carbon and low in nitrogen, an additional source of nitrogen is needed for composting. Chicken manure is an ideal source, as it is a waste product from the poultry industry and has very high nitrogen content. Wood waste and chicken manure have been successfully used to make compost (Borazjani *et al*., 2004). This methodology was investigated in the present study to assess whether composting could be used to break down carbon-based actives in treated wood.

The primary concern with combustion of wood treated with carbon-based preservatives is the potential for environmental contamination by resistant preservatives, and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) formed by the combustion process. While most carbon-based preservatives should be broken down in a combustion reaction, this process may not be complete, especially if the process is poorly controlled. For example, between one and ten percent of pesticides are believed to survive an uncontrolled fire (Nelson, 2000), and significant quantities of imidacloprid, a carbon-based insecticide, have been shown to survive combustion of tobacco (Clark *et al*., 1998). Tame *et al*. (2007) found that combustion of tebuconazole- and permethrin-treated wood produced PCDD/F concentrations three orders of magnitude greater than untreated wood. However, it may be possible to limit the formation of dioxins through strict process controls. Salthammer *et al*. (1994) found PCDD/F emissions could be controlled to acceptable levels through process optimization for a range of different preservatives. The present research evaluates the survival of carbon-based actives in treated wood exposed to simple combustion.

2. Methodology

2.1 Microorganisms Associated with Carbon-Based Preservative-Treated Wood

Three different carbon-based preservatives were investigated in this study and will be referred to as P1, P5, and P6. These formulation codes are part of a larger group of carbon-based preservatives under investigation by FPInnovations and its contract clients. Details on the treatment parameters and field set up of the decking material are confidential to these contract clients. The sampled decks were situated in the Maple Ridge test site located within the University of BC's Malcolm Knapp Research Forest. This site has an oceanic climate with rainfall of over 2150 mm per year, an average yearly temperature of 9.6°C with mean daily maximum and minimum temperatures of 6°C and 1°C in January, and 23°C and 12°C in July. It has an updated Scheffer climate index of 55 (Morris and Wang 2008) and falls within the moderate decay hazard zone for outdoor above-ground exposure. This zone includes most of the major population centers of North America.

For two of the treatments (P1 and P6) eight Pacific Silver Fir decks showing signs of fungal colonization were selected and sampled and for the third treatment (P5) three Lodgepole pine decks were sampled. Boards from decks treated with P5 had been brought to FPInnovations for chemical analysis and stored at room temperature prior to sampling for this study.

Five boards (uncoated) were sampled from each deck with preference placed upon locations showing possible signs of fungal colonization. A sterile chisel was used to remove the surface layer of wood on the tops of the boards to expose a clean area. A small chip of wood was chiseled out and placed in a sterile 1.5 ml Eppendorf tube. The exposed surface was sealed with an epoxy seal (Intergard 740, International Paint).

Wood chips were aseptically plated onto acidified media and onto 1.5% malt agar and incubated at 25°C until substantial growth was present. Subcultures were made onto 1.5% malt agar for each different morphology type (based on visual observations). Subcultures were incubated at 25°C. When growth was substantial, cultures were grouped according to morphology and representatives of each group were selected for molecular work.

Tissue (10-30 mg) was scraped from each culture and placed into a sterile 1.5mL Eppendorf tube. DNA was extracted using the methods of Al-Samarrai and Schmid (2000) with the following modifications: RNAse was not added, and DNA was precipitated with NaCl in a final concentration of 0.2 M. For fungal isolates, the internal transcribed spacer region (ITS 1 and ITS 2) of the ribosomal DNA was amplified using the primers ITS 1 and ITS 4 (White *et al*. 1990). Total reaction volume was 50 µL and contained 1X Go*Taq* PCR buffer (Promega Corporation, USA), 2.0 mM magnesium chloride, 0.2 mM dNTPs, 0.5 µM of each primer, 1.25 units of Go*Taq* polymerase (Promega Corporation, USA) and 1 µL of DNA ranging from 20 to 200 ng/µL of DNA. Reactions were run with the following thermal cycler conditions: 95°C for 2 minutes followed by 6 cycles of 94°C for 60 seconds, 58°C for 60 seconds deceasing by 1°C each cycle, 72°C for 90 seconds and 35 cycles of 94°C for 60 seconds, 55°C for 90 seconds, 72°C for 90 seconds, and a 10 minute extension step at 72°C. DNA was electrophoresed through a 1% agarose gel stained with GelRed (Biotium Inc. USA) and visualized under UV light. PCR products were sent to the Plateforme de Séquençage et de Génotypage des genomes, Centre de Recherche du Centre Hospitalier Université Laval (Quebec, Canada) where they were purified using glass fibre filtration on a Biomek Fx (Beckman Coulter, USA) and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Species identities were obtained by comparing the unidentified ITS sequences with sequence data in Genbank using BLAST searches (Altschul *et al*. 1990).

2.2 Recycling Wood Treated with Carbon-Based Preservatives

Full size 2 x 6 inch (51 x 152 mm) Pacific silver fir was obtained from Western Forest Products (Vancouver Island). Proprietary carbon-based preservatives (P1, P2 and P3) were provided by three wood preservative chemical suppliers. P1 and P2 contained quaternary ammonium compounds and triazoles. P3 contained triazoles as well as other biocides not measured in this experiment. The wood was cut into either 1 meter or 30 cm sections and treated to retentions that would likely be used for above-ground applications. The wood was not subjected to any weathering or ageing prior to further processing.

Three pieces were set aside from each treatment group for the combustion experiment and two for future consideration. The remainder of the wood was chipped using a 36 inch CM&E 10-knife disc chipper. The chips were air-dried and screened using a Wennberg chip classifier to yield a 2 to 6 mm thickness fraction and a less than 2 mm thickness fraction.

2.2.1 Combustion

Three samples from each treatment group (19 x 19 x 38 mm) were cut from the outer edge of three boards and sent to Intertek (Montreal, QC) for combustion. Adjacent samples were cut for analysis of initial preservative content. Each sample was burned using a Bunsen burner under controlled conditions. Samples of ash, particulates and smoke were collected and returned to FPInnovations for LC/MS analysis for carbon-based actives.

2.2.2 Composting

Chicken manure was obtained from Agriculture Canada's Pacific Agri-Food Research Centre in Agassiz, BC. Soil was obtained from the FPInnovations' test site at the University of British Columbia's Malcolm Knapp Research Forest in Maple Ridge, BC. The wood chips used were enriched with smaller fractions left over from chip screening for other experiments (Stirling *et al.* 2010). The treated wood chips from the material described above were comprised of between 12 and 25% material that passed through a 2 mm slotted screen. Analysis of wood, soil and manure for total carbon and total nitrogen was completed by Exova (Surrey, BC). The wood, soil and manure were mixed to target a C:N ratio between 35 and 40, a bulk density below 640 kg/m^3, and a moisture content between 50 and 60% (based on wet weight). The compost mixture was prepared by thoroughly mixing the wood chips, chicken manure and soil in an approximately 5:5:1 dry weight ratio on a piece of lumber wrap. This mixture was added to composters made from 25L heat resistant, non-leaching food-grade plastic buckets. Duplicate composters were set up for each treatment. The silicone used was aquarium-grade and contained no preservatives. Holes in the sides of the composter at the top and bottom, a perforated false bottom, and perforated tubes through the compost mixture were designed to maximize airflow. The composters were stored in an unheated shed at FPInnovations' Vancouver laboratory. Internal and ambient temperature and moisture content were measured every three hours using thermocouples and a HOBO data logger.

Composters were inspected monthly and weighed to determine moisture loss. The compost mixture was aerated by mixing with shovels on a clean piece of lumber wrap. To maintain sufficient moisture content distilled water was added to each bucket on a couple of occasions. After ten months in test the composters were moved into a temperature and humidity controlled chamber set at 20°C and 90% relative humidity to facilitate activity over the winter months.

After six months and one year of exposure, compost mixtures were analyzed for preservative content. Samples were also taken for moisture content determination by oven drying, and for total carbon and total nitrogen analysis.

When fungal growth was observed on the composting mixtures, mycelia were collected, and using a pipette tip, a small sub-sample was taken and mixed in a sterile 1.5 mL tube containing 1000 µL of sterile water. One in ten dilutions were made and 20 µL of the

diluted solution was spread on 1% Malt Extract Agar (MEA) amended with Benomyl and Chloramphenicol, and on 1% MEA amended with Chloramphenicol. In addition, fungal mycelium was placed directly on plates containing the same media. Plates were incubated at 25°C. When necessary, sub-cultures were made to obtain pure samples. Fungal morphology was observed under the light microscope on tissue collected from plates, as well as on tissue collected by sticky transparent tape directly from compost. Identification was attempted based on this information.

DNA was extracted from mycelia grown on agar, following the procedures of Lim *et al*. (2005) with the following modifications: tissue was ground with micropestles instead of glass beads; DNA extraction was done with 24:24:1 phenol:chloroform:isoamyl alcohol followed by one to two cleanings with an equal volume of chloroform; during precipitation with isopropanol, sodium chloride was added in a final concentration of 0.2 M. DNA was then stored in 50 μL of TE-8.

The internal transcribed spacer region of the ribosomal DNA was amplified using the primers ITS 1, ITS 3 and ITS4 (White *et al*. 1990). PCR amplification and product sequencing was as described above.

3. Results and Discussion

3.1 Microorganisms Associated with Carbon-Based Preservative-Treated Wood

The fungal species found on wood treated with carbon-based preservatives are listed in Table 1. There were a total of 20 different Ascomycetes found, including two unidentified species. Four of these were recognized as soft-rot fungi, and two others were possible wood-rotting species (Table 1). There were also three black-stain species and a secondary mould. Several of the Ascomycetes found are species that are commonly associated with grasses and sedges. These species may not have actually established on the treated wood, rather their presence may have been due to the abundance of grass at the test site.

Eight Basidiomycetes were found, two of which were the white-rot species, *Phlebia radiata* and *Phanerochaete sordida*. The other species included the secondary invader *Sistotrema brinkmannii* and another *Sistotrema* species as well as three different *Tremella* species. These secondary invaders can be pathogenic to decay fungi and may be an indicator that decay is already underway in some of the decks. One species was found that could be, or is closely related to, a *Rhodotorula* sp. which is a Basidiomyceteous yeast. Species of *Rhodotorula* have been reported as being able to break down chlorophenols (Walker 1973, from Gupta *et al*. 1986) as well as vanillic and ferulic acid (Cain *et al*. 1968, from Gupta *et al*. 1986) which can be generated during the breakdown of lignin by decay fungi and are also present on exposed wood surfaces as a result of UV breakdown of lignin. These fungi would be candidates as potential biodegraders of carbon-based preservatives and may require further investigation into their capabilities in the presence of such preservatives.

Table 1: Identity of fungi found on treated wood using the ITS rDNA.

Fungus	***Family***	***Genbank match***	***Decay type/Ecological niche***
Ascomycetes			
Cadophora melinii (Phialophora melinii)	Helotiales; Incertae sedis	99%	Soft-rot
Coniochaeta ligniaria or *Lecythophora hoffmannii*	Coniochaetaceae	99%	Soft-rot
Lecythophora sp.	Coniochaetaceae	100%	Soft-rot
Phialocephala dimorphospora	Vibrisseaceae	98%	Soft-rot
Mollisia cinerea	Dermateaceae	99%	Possible wood-rotting species (cup fungus)
Mollisia melaleuca	Dermateaceae	99%	Possible wood-rotting species (cup fungus)
Epicoccum nigrum	Pleosporaceae	100%	Black-stain
Aureobasidium pullulans	Dothioraceae	100%	Black-stain
Cladosporium sp.	Davidiellaceae	100%	Black-stain
Hypocrea lixii/ Trichoderma harzianum	Hypocreaceae	98%	Secondary mould
Dothideomycetes sp	Pleosporaceae	92%	Possibly a plant pathogen on grasses
Drechslera dematioidea	Pleosporaceae	100%	Plant pathogen of grasses
Drechslera triseptata	Pleosporaceae	99%	Plant pathogen of grasses
Nectria punicea	Nectriaceae	98%	Plant pathogen on broadleaf trees
Nectria sp.	Nectriaceae	99%	Plant pathogen on broadleaf trees
Paraphaeosphaeria michotii	Montagnulaceae	99%	Plant pathogen on rushes
Arthrinium sp.	Apiosporaceae, Incertae sedis	99%	Saprobe on dead plant material
Sordariomycete sp.		97%	Possibly a plant pathogen on grasses
Basidiomycetes			

Fungus	***Family***	***Genbank match***	***Decay type/Ecological niche***
Phanerochaete sordida	Phanerochaetaceae	99%	White-rot
Phlebia radiata	Meruliaceae	99%	White-rot
Rhodotorula sp.	Sporidiobolales; Incertae sedis	96%	Surficial basidiomycete yeast
Sistotrema brinkmannii	Hydnaceae	99%	Non wood rotting; parasitize wood decay fungi
Sistotrema sp.	Hydnaceae	98%	Non wood rotting; parasitize wood decay fungi
Tremella aurantia or *T. microspora*	Tremellaceae	99%	Non wood rotting; parasitize wood decay fungi
Tremella encephala	Tremellaceae	99%	Non wood rotting; parasitize wood decay fungi
Tremella sp.	Tremellaceae	98%	Non wood rotting; parasitize wood decay fungi

* Results based on the ITS region using primers ITS1 and ITS4

Table 2 shows the frequency of fungal isolation and the treated wood from which they were isolated. The most commonly isolated species were the grass pathogen *Drechslera dematoidea* and the black-stain fungus *Epicoccum nigrum*, both of which were common to all treatments. The two white-rots were found on P6 (Table 2) on the medium-high retention treatment. The soft-rot fungi were found slightly more frequently on P6 than on P1, and the secondary Basidiomycetes were found only on P6. Conditions on this decking material would not be conducive for soft-rot and their rate of decay would be extremely slow (Morris *et al.* 2008), consequently the presence of soft-rot fungi may be of little consequence. The *Rhodotorula* species were found on P1 and P5. More work is needed to determine the importance of this species on these treatments.

Table 2: Frequency of fungi isolated from each treatment

Species	***Total Isolates***	***P1***		***P6***		***P5***	
		Boards	**Decks**	**Boards**	**Decks**	**Boards**	**Decks**
Species causing or possibly causing wood rot							
Phanerochaete sordida	1			1	1		
Phlebia radiata	1			1	1		
Coniochaeta ligniaria or *Lecythophora hoffmannii*	3	1	1	2	1		
Lecythophora sp.	3			3	2		
Coniochaeta sp.	2			2	2		

Phialocephala dimorphospora	12	2	2	5	4	5	3
*Cadophora melinii (Phialophora melinii)**	4	3	3	1	1		
*Mollisia cinerea**	6	3	3			3	2
Mollisia melaleuca or *Mollisia cinerea**	1					1	1
Staining and mould species							
*Aureobasidium pullulans**	6	4	3			2	1
Cladosporium sp.	2					2	2
Epicoccum nigrum	18	7	3	9	3	2	2
Hypocrea lixii/Trichoderma	5	3	2	1	1	1	1
Surface and/or secondary species							
Rhodotorula sp.	4	3	1			1	1
Sistotrema brinkmannii	1			1	1		
Sistotrema sp.	1			1	1		
Tremella aurantia	1			1	1		
Tremella encephala	1			1	1		
Tremella sp.	1			1	1		
Other species							
Arthrinium sp.	1			1	1		
*Dothideomycetes sp.**	1			1	1		
Drechslera dematioidea	20	9	4	9	3	2	2
Drechslera triseptata	1					1	1
Nectria punicea	1	1	1				
Nectria sp.	1	1	1				
Paraphaeosphaeria michotii	1					1	1
Phoma herbarum	1			1	1		
Sordariomycete sp.	3	2	2	1	1		
Unidentified species							
unidentified ascomycete	3	2	2			1	1
unidentified yeast like fungi	20	7	3	3	2	10	3

* grouped morphologically with the yeast like fungi

There was a large number of isolates that grouped together morphologically and appeared to resemble black yeast fungi. Representatives of this group turned out to belong to five different species (noted in Table 2), some of which could potentially be wood rot fungi. These species occurred more frequently on P1 and P5. The two *Mollisia* sp. from this group, which could possibly be wood rotting species, were also found on P1 and P5.

3.2 Recycling Wood Treated with Carbon-Based Preservatives

3.2.1 Combustion

Data from the LC/MS analysis of triazole and DDAcarbonate concentration in matched wood samples, and filtered smoke and ash samples are shown in Table 3. There were no triazoles detected in any of the ash samples. Low concentrations of DDAcarbonate were detected in all of the ash samples, including the untreated control. Ash is a very active matrix than can bind cationic surfactants. Evidence of this was seen in the 10-fold reduction in internal standard area.

There were significant quantities of triazoles detected in the particulate filtered from the smoke produced during sample combustion (Table 3). None was detected in the particulate from the untreated wood. There was no DDAcarbonate detected in any of the samples. The survival of the triazoles suggests that simple uncontrolled burning of wood treated with these preservatives might not be advisable. However, strict process control or filtration technology may be able to reduce or eliminate these emissions. Moreover, the small scale of this combustion experiment may represent overly mild conditions. Larger, more intense fires may destroy the azoles. Boscak (date unknown) reports ignition temperatures of 430°C and 468°C for propiconazole and tebuconazole respectively, and suggests that both propiconazole and tebuconazole would be destroyed in a commercial furnace. It is also important to note that these tests only evaluated carbon-based preservatives. It would be inappropriate to assume that metal-cobiocide systems would behave in the same manner.

Table 3: Concentrations of Actives in Wood Samples Prior to and After Combustion

Treatment	Triazoles (ppm)			DDAcarbonate (ppm)		
	Wood	Smoke	Ash	Wood	Smoke	Ash
Untreated	BLOQ*	BLOQ	BLOQ	1.5	BLOQ	4.9
P1	8.3×10^2	7.3×10^2	BLOQ	9.2×10^2	BLOQ	12
P2	1.9×10^3	2.1×10^3	BLOQ	9.7×10^3	BLOQ	14
P3	78	3.4×10^2	BLOQ	9.4	BLOQ	11**

* BLOQ = Below Limit of Quantitation

** Not present in treating solution

3.2.2 Composting

Moisture content, carbon to nitrogen ratio, and initial bulk density are summarized in Table 4. Initial mixtures that met the target C:N ratio of between 35 and 40 were found to have bulk densities greater than 640 kg/m^3. To reduce the bulk density more wood chips were added, which increased the initial C:N ratio. After six months the C:N ratio had dropped significantly to within or close to the optimum 35 to 40 range. After one year the C:N ratio was steady around 40 for the mixtures containing untreated wood and P1 and P2. However, the C:N ratio for P3 had dropped to 22.

The composter temperatures largely followed the ambient temperature for most of the experiment. However, the composters containing wood chips that were untreated or treated with P1 or P2 had temperature spikes at various times.

After one year the untreated control and P1 and P2 had a much more homogenous colour and less offensive odour. P3 retained its original appearance of discrete wood chips and manure, as well as its original odour. None of the samples appeared to have completed composting.

The concentration of triazoles appears to have increased after six months and one year of exposure, relative to initial concentrations, though this was likely due to error introduced by limited sample numbers (Figure 1). However, in the same samples concentration of DDAcarbonate was greatly reduced in P1 and P2 within six months of exposure (P3 did not contain any DDAcarbonate initially). This suggests that while the triazoles are relatively stable, the DDAcarbonate may be vulnerable to degradation under these conditions.

Table 4: Compost Mixture Parameters

Parameter		Untreated	P1	P2	P3
Initial Conditions	Moisture content (%, wet wt.)	59	62	63	60
	C:N ratio	55	54	52	56
	Bulk density (kg/m^3)	586	588	628	523
6 months	Moisture content (%, wet wt.)	42	48	56	50
	C:N ratio	42	44	31	37
12 months	Moisture content (%, wet wt.)	41	48	51	52
	C:N ratio	42	42	41	22

Identification of organisms was based on morphological and molecular techniques (Table 5). The most frequent isolation was of *Scopulariopsis* sp. This fungus was found on both untreated and treated wood chips. *Scopulariopsis* spp. have been reported on wood

treated with copper-based preservatives and some are known to have oxidase and cellulase activity (Bridžiuvienė and Levinskaitė, 2007).

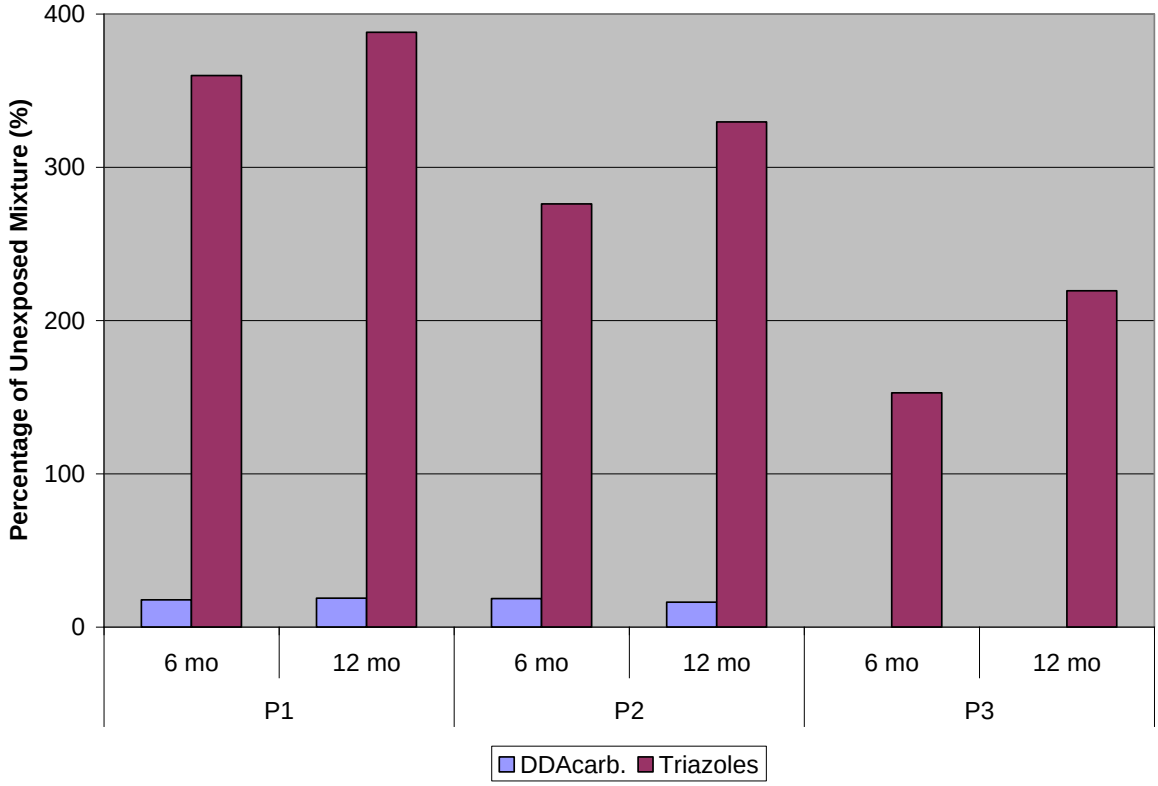

Figure 1: Concentration of Carbon-Based Preservatives in Composting Mixtures

One month after being moved into the temperature and relative humidity controlled room the untreated controls were heavily colonized by a *Scopulariopsis* sp., while mixtures of treated chips showed little or no growth.

Table 5: Fungi Isolated from Compost Trials

Isolation			Morphological ID		Molecular ID
#	Date	Source	Description	Identification	Closest BLAST (% Homology)
AU 315	15/04/09	U*	White flat powdery, medium fast growth, hyaline-white on reverse	*Scopulariopsis* sp.	*Scopulariopsis brevicaulis* (97%)
AU 315-1	15/04/09	U	White flat powdery, medium fast growth, hyaline-white on reverse	*Scopulariopsis* sp.	*Scopulariopsis brevicaulis* (97%)
AU 315-2	15/04/09	P2	Slow growth in dark, brown granular texture with white margin, white on reverse	*Scopulariopsis* sp.	*Scopulariopsis brevicaulis* (97%)
AU	15/04/09	P2	White flat powdery, medium	*Scopulariopsis*	*Scopulariopsis*

315-3			fast growth, hyaline-white on reverse	sp.	*brevicaulis* (96%)
AU 315-4	21/05/09	U	White flat powdery, medium fast growth, hyaline-white on reverse	*Scopulariopsis* sp.	*N/A*
AU 315-5	21/05/09	P2	White flat powdery, medium fast growth, hyaline-white on reverse	*Scopulariopsis* sp.	*Scopulariopsis brevicaulis* (97%)
AU 315-6	21/05/09	P2	Slow growth in dark, brown granular texture with white margin, white on reverse	*Scopulariopsis* sp.	*Scopulariopsis brevicaulis* (83%)
AU 315-7	07/01/10	U	Flat white, fast growth	*Scopulariopsis* sp.	N/A
AU 315-8	18/02/10	P3	Green, flat, fast growing	*Penicillium* sp.	*Penicillium solitum* (98%) *Penicillium echinulatum* (98%)

* U = Untreated wood chips

4. Conclusions

After 3 to 4 years in a field setting, decks treated with carbon-based preservatives were colonized by a range of fungal species that could potentially indicate weaknesses in certain preservative formulations. Some of these species may be of importance due to their potential ability to degrade the preservatives, while other species may be indicators of decay or weaknesses in a formulation.

In addition to developing a profile of fungi with the potential to break down carbon-based preservatives or to decay wood in the presence of carbon-based preservatives, this study may provide an early indication of formulations with a weakness against particular groups of fungi.

No triazoles were found in the ash produced from combustion, but significant quantities were detected in the filtered smoke. DDAcarbonate was not detected in the filtered smoke. Analysis of DDAcarbonate in ash was inconclusive.

After one year of exposure in a small-scale composter, triazoles showed no signs of depletion, while the concentration of DDAcarbonate was reduced.

5. Literature

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