EFFICACY OF TEBUCONAZOLE AND DDAC IN SHELL-TREATED WOOD

by

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ABSTRACT

In 2008, the Canadian standard for decking installed above ground was revised, and the penetration requirement was eliminated. This decision was based on field test data and fundamental work on mobility of copper in the preservative formulations that dominated the market at the time. Recently the wood protection industry has shown interest in shifting towards carbon-based preservatives. Thus, it is important to test the efficacy of carbon-based preservative formulations as shell-treatments on Canadian wood species. In this study, samples of spruce heartwood were treated with a formulation containing either tebuconazole or didecyldimethylammonium carbonate (DDAC). The treated wood was exposed outdoors for one year and the leachate from these samples was collected. Tebuconazole and DDAC were detected in the leachate collected, and this indicates that these active ingredients were mobile in the wood after treatment. DDAC was detected in very low concentrations on wood surfaces that were untreated before exposure: 0.03 mg DDAC/g of wood and 0.02 mg of DDAC/g wood were measured for the high and low retentions respectively. The concentration of tebuconazole detected was not different from the control samples. This suggests that mobile DDAC may be able to re-deposit in the wood, but tebuconazole does not re-deposit once it is dislodged from the wood. Furthermore, results showed that spores of Gloeophyllum sepiarium and Oligoporus *placentus* were able to germinate on untreated check surfaces within 2 weeks on samples collected from exposed, treated wood. This indicates that the re-deposited carbon-based active ingredients were not able to protect the untreated check surfaces against germination of basidiospores of some common fungi isolated from above-ground decking in Canada.

PREFACE

This research was conducted in collaboration with FPInnovations–Forintek division. The original research question was identified by Dr. Paul Morris. The design of the research program was broadly adapted from previous work by Dr. Sungmee Choi, otherwise experiment design and troubleshooting was performed by the author. I performed all portions of this research except for development of the LC/MS methods for detecting tebuconazole and DDAC. This method development work was performed by Bob Daniels.

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LIST OF ABBREVIATIONS

LC/MS	Liquid Chromatography Mass Spectrometry
DDAC	Didecyldimethylammonium Carbonate
ESI	Electrospray Ionization
SIM	Selective ion mode
PMT	Photomultiplier tube
CSA	Canadian Standards Association
CCA	Copper Chromium Arsenate
ACQ	Ammonium Quaternary Compound
SPF	Spruce-Pine-Fir
MIC	Minimum Inhibitory Concentration
UV	Ultraviolet
LOQ	Limit of Quantitation
ACN	Acetonitrile
DoDAB	Didecyldimethyl ammonium bromide
TMAC	Trimethylammonium Chloride
HPLC	High Performance Liquid Chromatography
BPB	Bromophenol Blue
AWPA	American Wood Preservers Association

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DEDICATION

I dedicate this work to Byron Thiessen and Mitzi

My precious Byron, you enabled me to see that life is a series of compromises, and that you have to "let the small things slide" to remain peacefully in the nook. | love you.

CHAPTER 1: BACKGROUND AND INTRODUCTION

1.1 GENERAL INTRODUCTION

The aim of this research was to understand the mode of protection for wood provided by two preservative formulations containing carbon-based active ingredients, didecyldimethylammonium carbonate (DDAC) and tebuconazole (Fig. 1.1). Specifically, the emphasis of this thesis was to determine whether the selected carbon-based¹ preservatives were mobile in the wood after treatment when exposed outdoors and above ground.

The first chapter of this thesis describes the importance of wood protection and the main issues facing the wood preservation industry in Canada. The introduction focuses on the mobility of copper ions in CCA-treated wood, and how this mechanism contributes to the protection of CCA-treated lumber exposed outdoors. This segues into a discussion about carbon-based preservatives and the possible industry transition from metal-based wood preservatives, to metal-free preservation systems. Chapter 2 investigates the mobility of carbon-based active ingredients. Chapter 3 assesses the redistribution of carbon-based active ingredients within the wood. Chapter 4 provides a basic biological perspective for the quantitative results obtained from previous chapters. Chapter 5 summarizes the implications of this work and recommends future research.



Figure 1.1 Chemical structures of didecyldimethylammonium carbonate (left) and tebuconazole (right)

¹ These active ingredients are organic molecules as defined by chemical terminology, but the word "organic" is often interpreted as pesticide free to the general public. As a result, the term carbon-based preservatives will be used in this document to describe a molecule that is primarily composed of carbon, hydrogen, nitrogen, oxygen, chlorine and sulfur.

DDAC is a non-volatile, photolytically stable salt that is highly soluble in water. DDAC has a half-life of 3 years in soil. FIM-1 has a specific gravity of 1.0 at 20°C. Tebuconazole has an average half-life of 600 days in soil and a much lower solubility in water, 32 mg/L of water. The tebuconazole formulation used in this study has a specific gravity of 1.12 at 20°C.

1.2 THE IMPORTANCE OF WOOD PROTECTION

Organisms such as bacteria, fungi, and insects inhabit wood materials to obtain sources of energy and carbon that enable them to grow. During this process, the organisms degrade wood into low molecular weight materials. This contributes to the breakdown of the strength and structural integrity of the wood.

The deterioration of wood involves a succession of micro-organisms that move through wood based on a number of factors including moisture content, nutritional status of the wood, and competition between micro-organisms (Eaton and Hale, 1993). There are four stages of microbial succession: (1) bacteria; (2) moulds and staining fungi; (3) brown-rot and soft-rot fungi or white-rot fungi; and (4) scavenging mould fungi. This particular succession of microorganisms in wood is mainly described in the colonization of sapwood; however, it is possible that under some circumstances colonization by decay fungi could occur, particularly in the heartwood, without prior colonization of wood by bacteria or ascomycetes. At the stage when wood decay fungi begin to infect the wood, either brown-rot and soft-rot fungi or white-rot fungi will be present. Brown-rot fungi chemically modify lignin by hydroxylation and can penetrate the tracheids and fibres and deplete carbohydrates in the wood cell wall. The action of brown-rot fungi leaves the wood cracked and crumbled. White-rot fungi degrade lignin through an oxidative radical mechanism involving a lignin peroxidase (ligninase) and hydrogen peroxide. White rot fungi can also degrade hemicelluloses and cellulose. To degrade cellulose and hemicelluloses, fungi release enzymes that breakdown polysaccharides. These enzymes include oxidases, such as cellobiose oxidase and glucose oxidase, and hydrolases, such cellobiohydrolases and endoglucanases. Although both oxidases and hydrolayses are involved in degradation of cellulose and hemicelluloses, it is the hydrolayses that play the major role. The degradation of these components compromises the strength of the wood and can lead to failure of the wood structure.

Wood protection aims to extend the service life of wood products. Treating wood prolongs the available timber supply and allows wood to be marketed as a sustainable, renewable building material. Over 3.4 million cubic meters of wood was treated with preservatives in 1999 (Stephens, 2001). This figure represents the most recent data available for the volume of treated wood that has been installed in Canada. To put this into perspective, Stephens *et al.* (1994) have indicated that extending the service life of wood products otherwise susceptible to decay may be equated to saving 66 million trees in Canada annually. This represents a significant contribution to extending the available timber supply in Canada.

1.3 CANADIAN WOOD SPECIES AND CHEMICAL PENETRATION

The major wood species in Canadian forests belong to the spruce-pine-fir species group (SPF). SPF mixtures are the main softwoods used for treated consumer products. In 1999, the volume of preservative-treated lumber and plywood was 1,873,063 m³ (Stephens *et al.*, 2001). In Western Canada, the four dominant species in the SPF group are: White Spruce (Picea glauca (Moench) Voss.), Engelmann Spruce (Picea engelmannii Parry), Lodgepole Pine (Pinus contorta Dougl.) and Alpine Fir (Abies lasiocarpa (Hook.) Nutt.). In Eastern Canada, SPF is predominantly composed of White Spruce (picea glauca), Red Spruce (Picea rubens), Jack Pine (Pinus banksiana Lamb.), and Balsam Fir (Abies balsamea (L.) Mill.). White Spruce (Picea glauca) was used in this study because it is the most dominant species in the SPF mix and it grows across Canada from East coast to West coast. The wood species in the SPF group often have narrow sapwood, non-durable heartwoods and they are impermeable to chemical treatment. Wood species that have low permeability and resist chemical treatment are termed refractory wood species. The poor treatability of Canadian species has been, and continues to be, one of the biggest challenges facing the Canadian wood industry (Ruddick, 1980; Morris et al., 1994; Morris et al., 2002). A number of innovative solutions have been developed to improve the treatability of refractory wood species. These methods include: toothed-roller incisors, needle, laser, drill, and high-pressure water-jet incisors, biological incising and pre-treatment conditioning (Ruddick, 1986; Ruddick, 1987; Morris et al., 1994; Lebow et al., 1996; Morrell and Morris, 2002; Rhatigan et al., 2003; Cooper and Morris, 2007). Toothed-roller incising is

currently used by the Canadian wood industry to improve chemical penetration and satisfy the Canadian Standards Association's chemical retention and uniformity requirements for treated lumber.

The Canadian Decking Standard is important because decking boards are one of the most popular consumer wood products for residential applications. In the 1930's, a penetration requirement of 10 mm was included in the standard CSA O80.2-97 which originated from requirements set up by the AWPA. However, wood that was treated and un-incised was sold to consumers without meeting the penetration requirements suggested by the CSA. Treated wood products with shallow penetration were expected to fail prematurely in service. Instead, excellent long-term field performance of sawn refractory softwoods treated with chromated copper arsenate (CCA) was reported (Richards and McNamara, 1997; Ingram and Morris, 2000). In 1997, the standard was revised based on a limited amount of field and laboratory test data provided by Ruddick and Doyle (1990). The 10 mm penetration, which could not be achieved when treating un-incised Canadian wood species, was replaced by a 5 mm penetration requirement for decking lumber (CSA O80.32-97). In 2005, the Canadian Decking Standard was revised again, and the penetration requirement was eliminated (CSA O80.1-08) based on field test data and fundamental work conducted by Choi (2004) which investigated the good performance of shell Chromated Copper Arsenate (CCA)-treated wood.

Before the unexpectedly good performance of shell CCA-treated wood is discussed, it is important to understand why shell-treated wood products exposed outdoors were expected to fail prematurely. The expectation was based on the understanding that environmental factors such as solar radiation, water, and heat cause wood weathering (Feist, 1983). The most well-known features of weathered wood are the grey coloration, surface roughness, and the formation of cracks and checks (Sell, 1968; Chang *et al.*, 1982). A penetration requirement was implemented because it was hypothesized that checks extending beyond the depth of chemical penetration would reveal untreated surfaces highly susceptible to fungal spore germination, fungal colonization, and subsequent decay.

1.4 FORMATION OF CHECKS IN DECKING

An explanation of how checks form in wooden decking boards exposed outdoors is essential for understanding the discussions that follow Chapter 1 of this thesis. Checks form naturally in wood exposed outdoors due to environmental factors such as solar radiation, water, and heat. Since wood is a hygroscopic material, it is affected by the moisture content and relative humidity of the surrounding environment (Eaton and Hale, 1993). Wooden decking boards exposed outdoors adjust to the equilibrium moisture content of the surrounding environment. The outer shell of the wood responds quickly to environmental changes, while the inner core of the wood is largely unaffected (Ratu, 2009). In addition, pronounced environmental differences are present between the top and bottom surfaces of decking boards. By nature of design, decking boards are installed so that one surface (top) is fully exposed to weathering while the other surface (bottom) does not experience the same severity of weathering (Urban and Evans, 2005). The bottom surface is relatively protected from direct rain and solar radiation, and this affects the moisture distribution in the wood. When rain strikes the top surface of the board, the water is absorbed and the moisture eventually penetrates the wood core. When solar radiation heats the surface area of the board, the moisture escapes from the outer layers of the wood and the surface dries. Under direct sunlight, the top surface reaches higher temperatures compared to the bottom surface and this causes the top surface to dry faster than the bottom surface. The top surface shrinks, due to the removal of moisture, but the inner core remains wet. The expanded wet inner core prevents the surface from shrinking and at the same time fasteners also restrain the surface from cupping and warping (Evans, 2003). This cycle of wetting and drying is accompanied by these differential rates of swelling and shrinkage in the surface and sub-surface zones of the board (Schniewind, 1963; Stamm, 1965). In the winter, water can freeze in checks. When water freezes in a check, it expands and forces the wood cells apart. If the temperature increases, and the ice thaws back into water, then the wood cells move back to their original position. The freeze-thaw cycles in winter can also contribute to the formation of checks. Eventually, the tension stress at the surface of the wood exceeds the tensile strength of the surface cells perpendicular to the grain, and this causes the wood to check (Stamm, 1965; Evans, 2008).

Checks are differentiated from cracks based on depth of penetration; checks are strictly defined as cracks in the wood that do not penetrate greater than 75 % of the depth of the board. When a

check forms in shell-treated wood, the check can extend deeper than the zone of chemical penetration (Fig 1.2). This results in a check surface with a treated upper surface, in the zone of chemical penetration, and an untreated lower check surface, below the zone of chemical penetration. The check surface is exposed to weathering, detritus, and potential sources of fungal and bacterial inocula. The exposed check surface, treated or untreated, becomes discolored during exposure due to weathering and detritus. The width of a check changes depending on the moisture content of the wood. When the board is wet due to precipitation then the wood swells at the surface and causes the check to look "closed" because the check width is small. When the board is dry, the wood shrinks at the surface and this causes the check to look "open" because the check width is large (Fig 1.2).



Figure 1.2 Conceptual diagram of an open check in a shell-treated board exposed with the sapwood face oriented as the top and the heartwood face as the bottom. The red line represents the zone of chemical penetration and illustrates that a check surface is exposed to the outdoor environment when a check is open. The check recess is the deepest point of penetration into the wood.

1.5 MECHANISM OF PROTECTION IN SHELL CCA-TREATED WOOD

As previously mentioned, shell CCA-treated wood decking performed extremely well in field tests, showing no signs of decay after more than ten years (*inter alia*, Ingram and Morris, 2000). The mode of protection occurring in shell CCA-treated wood has been fully described by Choi (2004) and reinforced by Morris *et al.* (2004). Choi *et al.* (2001a) showed that the components of CCA: copper, chromium, and arsenic were mobile in the wood after treatment when exposed outdoors and above ground. All the components in CCA were mobile, but the copper ions were identified as providing protection to the untreated check surfaces. Copper was extracted from the untreated check surfaces in the highest concentration: 0.26-0.27 mg copper/g of wood. The copper ions were redistributed by the movement of water from the treated wood surface to the untreated check surfaces during rain events (Choi *et al.*, 2004).

On the assumption that spores are the primary mode of infection in wood installed above ground, Choi *et al.* (2003) tested the concentration of copper recovered from the check surfaces against spore germination of *Gloeophyllum sepiarium* (Wulfen) P. Karst. and *O. placentus*, a known copper tolerant fungus. The spores did not germinate at 0.26-0.27 mg copper/g of wood (Choi *et al.*, 2003). Recently, a similar concentration of copper was shown to prevent spore germination of *Fomitopsis palustris* (Berk. et. Curt), another known copper-tolerant fungus (Woo and Morris, 2010). These studies show that fungi deemed to be copper tolerant based on their mycelium, do not produce spores that are also copper tolerant. Since spores are sensitive to the concentrations of redistributed copper ions on the untreated check surfaces, they are unable to germinate.

For the past 44 years, scientists have been investigating basidiospores as a source of infection in wood installed outdoors and above ground (Morton and French, 1966; O'Toole, 1971; Schmidt and French, 1978 (a,b); Carey, 1981; Fougerousse, 1984; Bjurman, 1985; Cymorek and Hegarty, 1986; Highley 1992; Eaton and Hale, 1993; Morrell, 1996; and Morris *et al.*, 2009). A strong consensus supports the hypothesis that spore dispersal by wind, insects, and rain-splash is the primary mode of infection above ground. It is possible that some of the infected wood above ground is initiated by insects, such as mites and beetles, carrying mycelial fragments (Eaton and Hale, 1993), or dispersal of mycelial fragments by rain-splash (Ashton and Macauley, 1972).

However, the main source of infection above ground occurs as a result of spore dispersal; and there are no scientific arguments that suggest another possibility.

When spores are prevented from germinating on wood above ground, the dominant source of fungal infection is removed. The mobility of copper ions in CCA-treated wood protects the untreated check surfaces from fungal spore germination. This provides an understanding of one mechanism that imparts good protection to CCA-treated wood (Choi *et al.*, 2002). The study conducted by Choi *et al.* (2004), along with accumulated field test data, resulted in the elimination of the heartwood penetration requirements for residential deck surface boards and small dimension above ground residential treated products (CSA O80.1-08). There is evidence from a study in Canada that elimination of the penetration requirement for wood products treated with CCA, or other copper-containing formulations, does not appear to present a problem due to the mobility of the copper ion. However, if solely carbon-based preservative formulations are used in shell-treatments, then it is necessary to evaluate these preservatives and determine if the active ingredients are mobile in the wood after treatment.

1.6 TRANSITION TO CARBON-BASED PRESERVATIVES

Carbon-based preservatives are described as being "metal-free" or "non-metal biocides". As the term suggests, the active ingredient is a molecule that is largely composed of carbon atoms, but may also include other atoms found in organic material, such as nitrogen, oxygen, hydrogen, sulfur, and chlorine atoms. Some of the earliest preservatives still used today would fit into the broad category of carbon-based preservatives, including creosote and pentachlorophenol (PCP). These preservatives were heavily used in the mid to late 1900's for treating railroad ties, marine structures, and some utility poles. The use of creosote was limited due to its obnoxious odor, oily appearance, and inability to be used indoors. Due to creosote shortages during World War II in North America, PCP-treated wood took over and has retained part of the market for creosote-treated products. Although PCP was effective, the toxic dioxins that were produced as a by-product during manufacturing and handling and the environmental concerns resulted in the banning of PCP's in many countries. Work to reduce dioxin contamination and further studies on the toxicity of the specific dioxins in PCP has allowed continued use of this preservative in Canada.

The rise of the residential treated wood market required products with a non-oily surface that could be painted and stained and chromated copper arsenate met consumer demands. CCA was the dominant preservative in the residential treated wood market in the 1970s, 80s and 90s.

However, a notable change occurred in 2003 when CCA was voluntarily removed from most residential applications by U.S. wood suppliers. And in the same year, Canadian wood preservative suppliers and the Pest Management Regulatory Agency (PMRA) decided to voluntarily withdraw CCA-treated lumber from most residential uses as well. In January 2004, the use of CCA was restricted to certain applications such as utility poles, wood foundations, shingles, fence posts and plywood. Increased consumer interest in environmentally benign products, coupled with Environment Canada's stricter regulations for toxic chemicals, has presented an atmosphere of change in the wood preservation industry. Environment Canada has short-listed a number of toxic industrial chemicals with the potential of causing harm to humans and the environment, and is more closely regulating their use in industry operations. Among the chemicals under increased scrutiny are PCP, creosote, and CCA, all of which are currently in use by the wood preservation industry.

Since 2004, most of the residential consumer lumber has been treated with either alkaline copper quaternary (ACQ) or alkaline copper azole (CA) preservatives. Ruddick (2008) showed that the copper in ACQ is mobile on the surface of the wood after treatment. Therefore, ACQ-treated wood is protected by a mechanism similar to CCA-treated wood when placed outdoors. Although ACQ provides sufficient protection for consumer products, wood protection scientists are increasingly interested in designing wood preservative formulations that are completely metal-free (Helmer, 2009). The desire for metal-free formulations is motivated by two important factors: (1) the possibility that more stringent regulations may be implemented in the future of the wood preservation industry and (2) the difficulty of recycling metal-containing wood at the end-of-its service life (Janin *et al.*, 2008).

Modern carbon-based preservatives were originally developed for use on wood as anti sap-stain fungicides in the late 1980's. Next, they were used on millwork treatments, and then on fence-

treatments in Europe. Eventually, carbon-based preservatives were used as mouldicides for framing lumber and now they are finally being used to treat residential products. In the early 1980s residential wood treated with various alkyl ammonium compounds (AAC) was commercially available in New Zealand. The AAC's were discontinued soon after they were introduced in New Zealand due to instances of poor performance in field exposed material after 8-9 years (Eaton and Hale, 1993).

There are several different classes of carbon-based active ingredients being used for wood protection in the United States, Canada, and Europe. Many of the carbon-based preservatives are only effective towards a specific class of organism such as moulds, sap-stains, or decay fungi; in other words, they do not have a wide spectrum of activity. In some cases, as with the triazoles, synergism was reported when more than one active compound was used (Buchhus and Valke, 1995). As a result, compounds from different classes are frequently combined into one formulation to achieve a wider spectrum of protection.

Formulations containing solely carbon-based preservatives are already in use in the USA, Canada and Europe for above ground applications (Helmer, 2009). The active ingredients in preservatives used in above ground applications include active ingredients such as: propiconazole, tebuconazole and quaternary ammonium compounds (QAC's). Additional carbon-based biocides currently used in formulations in North America and/or in Europe include: IPBC (3-iodo-2-propynyl butyl carbamate), MBT (2-mercaptobenzothiazole), chlorothalonil (tetrachloroisophthalonitrile), DCOI (4,5- dichloro-2-N-octyl-4-isothiazoln-3-one), PXTS (an oligomeric mixture of alkylphenol polysulfides), TCMTB (1,3-benzothiazol-2-ylsulfanylmethyl thiocyanate), and thiabendazole (a benzimidazole). Many of these biocides were identified first by the agricultural industry and used by the wood preservation industry to avoid the costs associated with research, development, health and safety testing, and registration of new active ingredients. Sometimes these active ingredients are combined together to try and isolate a synergistic formulation that inhibits microbial attack (Helmer, 2009).

Given that the protection mechanism of CCA in some Canadian wood species was dependent on the chemical mobility of a metal ion, it is prudent to test non-metal based preservatives for chemical mobility too. So far, this testing has not been conducted; perhaps because metal-free systems are relatively new to Canada where shell-treatments are common.

Carbon-based preservatives are often included in co-biocide systems to prevent degradation by copper-tolerant fungal species. If copper, which is known to redistribute and protect untreated surfaces, is present then it would not be necessary to know whether the carbon-based preservatives are also mobile. However, the development and use of formulated products composed of only carbon-based preservatives warrants a study to determine if the carbon-based preservatives are mobile.

Both tebuconazole and DDAC are known to leach out of treated wood during rain events (Ruddick and Sam, 1982; Kennedy and Collins, 2001; Hwang *et al.*, 2006; Li *et al.*, 2009). Most of the leaching information on carbon-based preservatives has been obtained from accelerated laboratory leaching tests. These studies provide insight into the behavior of carbon-based preservatives, but they have limitations, and are primarily used for estimating the relative leaching performance of preservative systems. In this study, laboratory leaching was not conducted; instead, an outdoor field test was set up so that the leaching data was more comparable to the real-life application of treated decking installed outdoors.

Rates of chemical leaching are dependent on a number of different variables. In an in-house laboratory leaching test, 11-21% of DDAC was leached in a static cycle over a two day period where treated blocks were vacuum impregnated with distilled water (Ruddick and Sam, 1982). In this case, the amount of DDAC leached from the treated blocks was different depending on the species of wood used. In another leaching test, wood materials treated with copper azole formulations had tebuconazole and propiconazole leaching rates of 7.7-37.3 % and 7.5-29.6 % respectively, when tested using the E11-06 (AWPA) leaching method (Li *et al.*, 2009). Furthermore, Li *et al.* (2009) showed that the presence of copper or ammonia had an effect on the observed leaching of tebuconazole. This evidence, along with the depletion study by Jin and Preston (1993), suggests that the leaching of preservative components depends on the properties of the chemical formulation used in treatment. Therefore, to obtain meaningful results that can be applied to the wood protection industry, studies must be conducted with real formulated

products. In this study, formulated products were supplied by two major companies involved in wood preservation (Table 1.1).

Table 1.1 Formulated products and then relevant chemical properties					
Code	Active Ingredient	Company Name	Formulation	Solvent	РН
P4	Tebuconazole	Arch Wood Protection	1279-23	Waterborne Emulsion	8.5
Р5	DDAC	Timber Specialties Co.	FIM-1	Waterborne Emulsion	9.3

Table 1.1 Formulated products and their relevant chemical properties

The exact chemical composition of these formulations is proprietary and is not disclosed in this report, but the important chemical properties are listed in Table 1.1. The formulated products used in this study will be identified by the code listed in Table 1.1 throughout this report. The P4 and P5 products each contained a single carbon-based biocide and an amine oxide adjuvant. P4 contained the active ingredient tebuconazole and P5 contained DDAC (fig 1.1). The interactions between the chemical components within the product and between the product and the wood made each formulation unique. These chemical interactions are difficult to tease apart, but they were necessary to accurately represent the behavior of the product in terms of leaching and performance.

Research conducted on DDAC and tebuconazole, among other carbon-based active ingredients, has enabled companies in the wood preservation industry to prepare metal-free wood treatment formulations. The unique chemical properties of some carbon-based preservatives have the potential to re-define the use and re-use of treated wood products. One of the most significant properties of carbon-based preservatives is the possibility that they may be able to biodegrade at the end of the service life of the treated wood. There is evidence that tebuconazole can be degraded by some fungal strains of *Trichoderma harzianum* (Obanda *et al.*, 2008) and *Fomitopsis palustris* (Woo *et al.*, 2010). In addition, Dubois and Ruddick (1998) showed that DDAC is significantly degraded by the mould *Gliocladium roseum*. Moreover, it has been shown that triazoles can be degraded through hydrolysis reactions, UV and high temperatures (Cabras, 1997; Bromilow, 1999; Calza *et al.*, 2002; Garland, 2004; Kahle, 2008). The potential for carbon-based preservatives to biodegrade alludes to the possibility that treated wood could be more easily recyclable at the end of its service life. On the other hand, the ease with which

carbon-based active ingredients degrade may also limit its use in the protection of wood from micro-organisms in the surrounding environment.

Other methods of wood protection, purported to be more environmentally benign such as chemical wood modification, are currently being researched as an alternative to carbon-based wood preservatives (Hill, 2006). A variety of wood modification options are being considered as possible methods of providing wood protection: acetylation and furfurylation; thermal modification; surface treatments with hydrophobic wood preservatives and/or UV stabilizers; and impregnation using resin treatments and silicon-containing compounds are a few of the modification options. Some of these potential alternatives, such as the use of hydrophobic chemicals and UV stabilizers, may be very useful in conjunction with carbon-based preservatives (Christy, 2005).

As the feasibility of a potential shift towards non-metal based wood preservative systems is considered, it is necessary to ensure that the level of wood protection is comparable to that of CCA or ACQ. Metal-free formulations with carbon-based active ingredients are currently being used by consumers in a limited number of residential applications. The growing interest and current use of carbon-based systems means that it is important to fully understand the limitations of these systems when used as shell-treatments. For shell-treatments the limitations can be understood by determining whether the carbon-based active ingredients are mobile in the wood after treatment. If these actives are not mobile and do not deposit in checks, then the untreated check surfaces that are exposed during the service life of a residential deck are potential points of decay initiation which could lead to possible failure of the wood material.

1.7 OBJECTIVES

The primary objectives of this work were: (1) to determine if the carbon-based active ingredients, DDAC and tebuconazole, were mobile in the wood after treatment when exposed outdoors and above ground; and (2) to assess whether the quantity of carbon-based active ingredients found on the untreated check surfaces was sufficient to prevent basidiospore germination of some common wood degrading fungi.

CHAPTER 2: PRESSURE TREATMENT AND LEACHING OF CARBON-BASED PRESERVATIVES IN SPRUCE HEARTWOOD

2.1 INTRODUCTION

One direct method used to assess the mobility of formulation components in wood after treatment is by analyzing rain water that interacts with treated wood surfaces (Choi *et al.*, 2004). Precipitation that comes in contact with treated wood can interact with chemicals that are impregnated in the wood. The water may solubilize chemicals in the wood and then drip from the wood surfaces. The water that drips off the surfaces of the wood is called leachate because it leaches chemicals from the treated wood. If active ingredients are detected in the leachate, then the chemical components must be mobile. To simultaneously investigate the mobility and the possibility that the carbon-based active ingredients redistribute, it was necessary to prepare wood samples with two specific properties: (1) a very shallow chemical penetration and (2) a high tendency for check formation during exterior exposure. These sample conditions allowed checks to penetrate the wood beyond the depth of chemical treatment during outdoor exposure. When checks formed in shell-treated material after outdoor exposure, the area below the limit of penetration and above the lowest recess in the check is an untreated surface (Fig. 1.2). The untreated surfaces were sampled and analyzed to determine whether active ingredient had redistributed from other treated surfaces of the wood.

The heartwood of a refractory Canadian wood species, white spruce (*Picea glauca* (Moench) Voss.), was used in this study because it is commonly used in the construction of decking and treatment of this species results in a thin shell of chemical treatment (1-2 mm). Flat-grained boards were preferentially chosen since they are present in commercial lumber and are known to be more susceptible to check formation (Sandberg, 1999). Spruce heartwood was treated with formulated products P4 and P5.

Simulated decking samples were prepared and scheduled to be exposed outdoors for one year. While this is usually enough time for checks to form, the samples in this study were installed outdoors in the fall instead of the typical spring or summer deck construction. Since wood maintains high, and relatively constant, moisture content throughout the fall season, the wood does not experience the cyclic wetting and drying that is needed to promote checking. In this study, the boards were prepared in the lab to simulate the condition of the wood that one might expect from a wooden deck constructed in summer. To do this, micro-checks were created in the wood to expedite the formation of checks when the material was placed outdoors. When checks formed, the check surfaces could interact with water and any solubilized components in the rain water in the first 3-4 months of outdoor exposure, as if it were a summer installation.

The use of an accelerated checking device to expedite check formation (Ratu and Evans, 2008) was considered for this study, but was rejected due to the limited capacity of the device. Decking board constraints, such as nails, screws, and other fasteners, were not used in the experimental set-up. Although this does not simulate the real life application of decking boards, this set-up was chosen to prevent contamination by any metal ions derived from the fasteners. In some cases metal ions, such as iron and zinc, can have biocidal effects. Thus, to achieve the objectives of the study, alternate methods of encouraging check formation were considered.

Carbon-based wood preservatives leach from wood when exposed to water (Ruddick and Sam, 1982; Kennedy and Collins, 2001; Hwang *et al.*, 2006; Li *et al.*, 2009). The methods used to collect leachate have a substantial impact on the results. Accelerated laboratory leaching tests consistently over-estimate the amount of preservative that is leached by naturally exposed decking (Yamamoto *et al.*, 1999; Hingston *et al.*, 2001; Kennedy and Collins, 2001; Cooper and Ung, 2009). This is mainly due to the inverse relationship between chemical depletion and sample size. It has been shown that more alkylammonium compounds are leached as the surface area to volume ratio of the sample increases (Ruddick and Sam, 1982). Hingston *et al.* (2001) and Cooper and Ung (2009) provide a comprehensive analysis of the merits and limitations of different leaching methods so these are not discussed in detail here. Due to the different formulation components and variable methods of leaching, it was not possible to estimate the amount of mobile active ingredient in P4 and P5 material by using leaching rates of DDAC and tebuconazole found in the literature. It was necessary to analyze the leachate from wood treated with P4 and P5 formulations directly to obtain an estimate of mobile chemical on the surface of boards.

If the active ingredients in P4 and P5 are present in the leachate, then this suggests that the chemicals are mobile in the wood after pressure treatment when exposed outdoors and above ground.

2.2 MATERIALS AND METHODS

2.2.1 Wood Sourcing and Transport

White spruce wood material for this experiment was obtained from Tolko High Level in Alberta. The spruce was J-grade, and was received as 2 x 6", 20 ft. boards. Wood that is J-graded is named after the top-grade class of lumber which is often exported to Japan. It is premium wood, free of stains and holes with aesthetically pleasing knots. The material arrived kiln dried and enveloped in lumber wrap. The wood remained wrapped and was stored in an open shed until ready for use.

2.2.2 Sample Pre-screening and Preparation for Chemical Treatments

To optimize conditions necessary for check development, twenty-six flat-grained boards were selected and pre-screened for their tendency to form checks. Ten centimeter pieces of 2 x 6'' wood were cut from each end of the selected 20 ft. boards. These pieces were end-sealed using a two-part epoxy resin (International: Interguard 740) to minimize moisture loss from the end-grain and prevent end-grain checking. The samples were dried in order to promote the formation of checks. The end of each 20 ft. board selected for treatment was screened to prepare end-matched samples for outdoor exposure. Checks that were present in samples before drying were identified by circling them with permanent ink. Samples for pre-screening were dried in a 3 ft. kiln. The drying schedule was designed to simulate the temperature that a decking surface may encounter during a dry summer season in a severe climate. The schedule consisted of a 50°C dry bulb temperature (such as might be attained via solar heating of decking) and a high forced airflow (90% fan speed) through the oven. To maximize the moisture gradient, the samples were arranged so that the airflow and heat were focused on the sapwood faces of the samples (Fig. 2.1). Sets of two samples were placed heartwood faces together with a piece of black lumber wrap between the heartwood faces of the two samples and stickers between the sapwood faces.

The lumber wrap was used as a divider between samples to prevent chemical migration between any two treated wood samples. Additional pairs of samples divided by black lumber wrap were separated by metal stickers to allow airflow and heat to penetrate the sapwood face of the next tier of samples (Fig. 2.1). The drying regime was applied for 24 hours. After drying, the samples were inspected for formation of new checks. If checks formed as a result of this process, then these boards were selected for chemical treatment and subsequent outdoor exposure.



Figure 2.1 Sample-layering and positioning during kiln-drying of wood samples. The sapwood faces of each sample were exposed to high airflow and direct heat, while the heartwood faces were separated only by lumber wrap to prevent chemical redistribution between samples during drying.

Fifteen pre-screened 20 ft. boards were chosen for outdoor exposure. To prepare these boards for the leaching experiment, each one was cut into twelve 32 cm long pieces. Two corners from each sample were removed to enable the two samples to fit into the test basin and maximize the exposed surface area of wood. The resulting samples were end-sealed in preparation for pressure treatment. The samples were end-matched and pressure-treated with P4 and P5. The concentrated solutions of the formulated products were diluted to working solution strengths and these were used for pressure-treatments. The chemical loadings were suggested by the suppliers of the formulated products. The P5 formulation, containing DDAC, was treated with solution strengths

of 1% a.i. for the low retention and 2% a.i. for the high retention. The P4 formulation, containing tebuconazole, was treated with solution strengths of 0.4% a.i. for the low retention and 0.6% a.i. for the high retention. The samples were end-matched and divided between the four treatments, with representative boards being placed aside as controls. The control samples, which were untreated and exposed outdoors, were accompanied by additional reference samples that were treated but not exposed outdoors.

2.2.3 Pressure Treatment of Spruce Heartwood

For P4 and P5 formulations, the lower concentration solution was prepared first and used in treating the low retention batch. In the subsequent treatment, the higher retention solution was prepared by adding concentrated formulated product to the more dilute solution. This solution was then used in treating the higher retention batch to minimize waste and simulate the treating practices in an industrial plant. After the solutions were prepared, a sample was collected and analysed by mass spectrometry to determine the actual concentration of active ingredient in the treating solution.

Treatments for both formulations at the high and low retentions were conducted using the same treating schedules. The pressure schedule consisted of a 30-minute vacuum at a minimum 25 kPa followed by a 120-minute press at 1035 kPa and a final vacuum for 15 minutes at a minimum of 25 kPa (absolute pressure). Samples were weighed before and after treatment. The weight of the solution uptake and the concentration of the treating solution were used to determine active ingredient uptake. Treated samples were loosely covered in plastic following the treatment. After 48 hours the plastic covering was removed and the samples were air dried to equilibrium moisture content under ambient lab conditions for three days. After the treated samples were air dried, they were stacked in a small kiln, as shown in Figure 2.1, to induce checking. All samples were heated in the kiln for 24 hours with the following programmed parameters: an initial 2 hour dry bulb temperature ramp to a target of 50 °C with 80 % fan speed. And for the remaining 22 hours, the dry bulb was maintained at 50 °C, and the fan speed was increased to 90 %. A relatively low kiln temperature was used to avoid any potential loss of biocide activity due to

heat degradation. After kiln drying, the samples had an average moisture content of 10.1% before being placed outdoors.

After kiln drying, a slice of treated wood 1.5 inches thick was removed from the end of each sample. The wood wafers were individually stored in plastic bags and retained for chemical penetration analysis. The epoxy coatings were re-applied to cut ends before the samples were exposed outdoors.

2.2.4 Outdoor Exposure of Pressure-treated Spruce Heartwood

The treated samples were exposed outdoors in a test site at FPInnovations–Forintek division, located on the University of British Columbia Campus in Point Grey, Vancouver. Vancouver is in a zone of medium above-ground decay potential, as calculated by Setliff (1986) using Scheffer's (1971) climate index. Scheffer's climate index value is predictor of decay that is based on the mean monthly average temperature and the number of days per month with 0.25 mm or more of precipitation. Morris and Wang (2008) recently updated the index value for Vancouver airport to 50 and the test site is close to the airport. Temperatures annually average 10°C. The average temperature in December is 3°C, and in July the average is 17°C. The site receives about approximately 1250 mm of precipitation per year, with an average 34 mm of rain in July and 140 mm of rain in December.

The site was prepared by covering the grass with black landscape fabric, to minimize vegetative growth around the samples during exposure. Eleven untreated wooden flats were placed on the landscape fabric. On each flat, 5-6 RubbermaidTM plastic basins with dimensions of 30 x 35 cm and volume of 10.7 L, each containing two samples, were arranged by active ingredient. All samples were labeled on top and bottom faces and placed in a numbered basin. The samples were arranged with the growth rings oriented convex to the exposed surface to produce more severe checking (Urban and Evans, 2005). This occurs because more shrinkage occurs in the tangential plane compared to the radial plane, which results in more checking on the tangential surface compared to the radial surface (Sandberg, 1999). Treated samples were paired in basins so that both samples were treated with the same active ingredient and solution strength. At least

1 cm of space was left between samples to allow for lateral expansion of the wood during exterior exposure.

The leaching set-up used in this study was designed based on the methods used by Choi (2001b) and the draft "Standard Method for Determining the Leachability of Wood Preservatives from Decking" (AWPAXX-09). Plastic RubbermaidTM basins were modified in this study to prevent water from splashing out of the basin, and to minimize evaporation of the leachate. With this arrangement two 2 x 6' samples could rest just inside the basin, flush with the rim. Four holes were drilled into the sides of each plastic basin. Fitted plastic supports were cut to length and forced through the holes to create a level resting place for the wood specimens. With this set-up, it is possible that some of the rain water in contact with the treated surface of the samples could have splashed from the surface and not been collected in the basin.



Figure 2.2 Wood samples sit inside the basin to minimize the leachate evaporation and splashing. Two corners from each sample in the basin were removed to maximize the exposed surface area (left). Field test set up at FPInnovations–Forintek division (right). Samples with the same active ingredient and chemical retention were grouped together on the wooden pallets.

2.2.5 Collection of Leachate from Exposed Treated Wood Samples

Leachate samples were collected after rain or snow events and removed from the RubbermaidTM basins once per month, or when the water level was close to the bottom of the samples. Rainfall at the site was estimated based on the volume of leachate measured in the basins each month. The volume of leachate in each basin was determined by weighing the leachate. The volume was recorded; a twenty-five milliliter sample of leachate was taken and retained for chemical

analysis; and then the leachate was discarded onto nearby soil where adsorption to clays and biodegradation was expected to mitigate the low level of contamination.

If snow was present on the samples, or the leachate was frozen, then the leachate was not collected. Snow was not removed from the boards during exposure; instead, it was simply given time to melt while outside. In the event of snow, the leachate collections were made after the snow melted, and there was no ice left in the leachate.

2.2.6 LC/MS Analysis of Leachate for Tebuconazole and DDAC

The chemical analyses of tebuconazole and DDAC from the leachate were performed using Liquid Chromatography Mass Spectrometry (LC/MS). Chemical analysis of the leachate samples was performed to quantify the active ingredient (DDAC or tebuconazole). For each active ingredient, a unique leachate sample preparation was required before analysis. Similarly, an LC/MS method specific for DDAC or tebuconazole was used in quantification.

Both LC/MS methods used to analyze leachate containing DDAC or tebuconazole used ESI (electrospray ionization) to initiate ionization of the active ingredients. Selective ion mode (SIM) was used to isolate the single ion of interest in the ion-trap and ions were detected by a photomultiplier tube (PMT). Three molecular ions were simultaneously monitored in SIM mode, but individually scanned at a narrow range unique to the molecular mass of the ion. Data were obtained from one scan, approximately 3 minutes in duration, for each molecular ion of interest. A Thermo-Fisher Accela UHPLC with a Thermo LTQ XL mass spectrometer detector was used for all analyses.

To analyse tebuconazole, 5 μ L of each sample was injected onto a C18 reverse phase Gemini column (150 x 3 mm, Phenomenex) with a particle size of 3 μ m. The mobile phase consisted of water / 0.1% formic acid (A) and acetonitrile / 0.1% formic acid (B). Gradient elution was performed starting at 50% A / 50% B over 10 mins ending at 35% A / 65% B with a flow rate of 500 μ l min⁻¹. The column temperature was 50°C. Quantification of tebuconazole was calculated using azaconazole as the internal standard. Retention times were as follows: azaconazole at 3.60

mins, tebuconazole at 6.53 mins. The column was flushed at the end of each analysis with 95% B at a flow rate of 1000 μ l min⁻¹. A five-level calibration curve was constructed from 0.05 to 20 μ g ml⁻¹. All analyses were run twice. The limit of quantitation (LOQ) was 0.05 μ g ml⁻¹ for tebuconazole.

To analyse DDAC, 5 μ L of each sample was injected onto a C18 reverse phase Gemini column (150 x 3 mm, Phenomenex) with a particle size of 3 μ m. The solvent gradient conditions are described by Stirling *et al.* (2010). A 10-point calibration curve was prepared to quantify samples from 0.01 to 25 μ g ml⁻¹. The LOQ for DDAC was 0.01 μ g ml⁻¹ for DDAC. For both tebuconazole and DDAC, the limit of detection (LOD) was 1 ng ml⁻¹. An in-house external standard method was used for the analysis of the leachate, the same instrumental conditions described in the internal standard method were used in this method. The only difference from the method described above is that the calibration standards were diluted in the same manner as the leachate samples (50:50, H₂O:ACN). The calibration curve was linear from concentrations of 0-20 ppm, and began to plateau at concentrations higher than 30 ppm. DDAC concentration was calculated using the peak height, instead of peak area, due to a more consistent correlation with the calibration standards.

2.2.6.1 Sample Preparation for Analysis by LC/MS

Leachate samples collected from DDAC treated wood specimens were mixed 50:50 with an extraction solvent composed of acetonitrile (ACN) containing 50 μ g ml⁻¹ TMAC (trimethylammonium chloride) and 10 μ g ml⁻¹ DoDAB (didodecyldimethylammonium bromide). Leachate samples collected from tebuconazole treated wood specimens were mixed 50:50 with an extraction solvent composed of methanol with 5 μ g ml⁻¹ azaconazole: the internal standard. After the appropriate solvent was added to the leachate samples in the HPLC vials, they were mixed by vortexing.

2.2.6.2 Active Ingredient Leached from Exposed Surface Area

There were two active ingredients each with two treatment conditions, for a total of four treatment variables not including the exposed control samples. For each treatment variable, 17

basins were exposed with 2 treated samples in each basin. The leachate in each basin was analyzed to determine the concentration of active ingredient in mg ml⁻¹. An estimate of the total weight (mg) of active ingredient present in each basin was obtained by multiplying the concentration and the volume of leachate recorded for that particular basin. The amount of active ingredient leached from the upper surface area of each board was calculated by dividing the total weight of the active ingredient found in the basin by two and dividing by the upper surface area of the board to get units expressed in mg m⁻².

The average active ingredient uptake after pressure treatment is shown for all treatment solution strengths (Table 2.2) and the chemical retention is calculated based on the estimated volume of treated wood.

Active Ingredient	Treatment	Active Ingredient Uptake (g)	Estimated volume of Wood Treated (m ³)	Chemical Retention (kg/m ³)
Tabuconazola	0.4%	0.4 ± 0.15	0.00018 ± 0.00011	2.4 ± 0.8
Tebuconazore	0.6%	0.7 ± 0.4	0.00018 ± 0.00011	4.1 ± 2.1
	1.%	1.4 ± 0.6	0.00026 + 0.00022	7.4 ± 3.3
	2.%	1.4 ± 1.0	0.00020 ± 0.00025	11.3 ± 5.1

Table 2.2 Chemical retention and active ingredient uptake for treated wood samples

Active ingredient uptake = weight of solution taken up during treatment (g) x solution concentration (% a.i.)

Estimated volume of wood treated is calculated by using the chemical penetration of each sample to determine the volume of wood that was actually treated (length x width x height). The two corners of each sample that were removed are subtracted from the volume of treated wood. Chemical retention is calculated based on the volume of the wood that was treated and the amount of active ingredient that was taken up during the treatment

Chemical retention= active ingredient uptake / Estimated volume of wood treated (kg m⁻³)

2.3 RESULTS

2.3.1 Chemical Depletion of Tebuconazole for Samples Installed in DecemberFigure 2.3 shows the depletion of tebuconazole from the sample material installed in December,2009. In the first 4 months of exposure, wood treated with P4 at the high retention lost 91% ofthe total tebuconazole that leached in 1 year. Similarly, at the low retention, 88% of the totaltebuconazole that was leached was lost in the first 4 months.



Figure 2.3 The line graph illustrates the depletion of tebuconazole leached from spruce samples treated with P4. The average volume of leachate measured in each basin is plotted on the secondary Y-axis and is indicated by the purple bar graph. The yellow arrow on the x-axis indicates the point where deep checks were open in the exposed material.

The yellow arrows on the x-axis in Figures 2.3 and 2.4 indicate the time when large, deep checks were open in the exposed material. Between March and April, large checks were present in the wood. This allowed check surfaces, both treated and untreated, to interact with rain water and any water soluble compounds. As seen in Figure 2.3, the available mobile chemical decreased quickly with increased exposure time. For example, checks that formed in March on low retention material were exposed to a cumulative 761 mg m⁻² of tebuconazole. At the higher retention, 1163 mg m⁻² of cumulative tebuconazole was available from March until December (Fig. 2.4).

Due to the high volume of precipitation received in October and November, the exposed upper surface of the material was constantly wet. On November 20th, 2009 all the samples were moved indoors and placed in a mildly heated pilot plant for three days of drying. The samples were moved indoors to artificially create a wet and dry cycle necessary to induce checking. After drying, the checks opened to reveal check surfaces, and the samples were placed outside again. The subsequent leachate collections, following drying of the samples in late November, showed an increase in the concentration of the active ingredient present in the leachate collected in December. This observation is consistent with results that show there was an increase in copper concentration after summer rains for CCA-treated wood (Choi et al., 2004; Chung and Ruddick 2004a).

Samples treated with 0.6% P4 showed an increase in the amount of tebuconazole leached from 25 mg m⁻² in November to 104 mg m⁻² in December. Similarly, samples treated with 0.4% P4 showed an increase in the amount of tebuconazole leached from 16 mg m⁻² in November to 76 mg m⁻² in December (Fig 2.3). Samples treated with 2% P5 showed a decrease in the amount of DDAC in the leachate from 43 mg m⁻² in November to 30 mg m⁻² in December (Fig 2.5). However, the samples treated with 1% DDAC showed an increase in the concentration of DDAC in the leachate from 4 mg m⁻² in November to 30 mg m⁻² in December. Analysis of the leachate from August, September and October indicates that there was a very small amount of chemical in found in the leachate from the treated material: an average of 0.16-0.17 mg m⁻² of tebuconazole and 13.6-39.5 mg m⁻² of DDAC was present in the low and high retention material, respectively.



Exposure outdoors (months)

Figure 2.4 The cumulative amount of tebuconazole leached from boards treated with P4 in short-term field exposure. The line graphs show the cumulative amount of tebuconazole leached from samples treated at two different retentions: 0.4% solution strength (pink) and 0.6% solution strength (blue). The higher treatment retention shows a corresponding higher loss of tebuconazel throughout the year. The arrow on the x-axis indicates the point where deep checks were open in the exposed material.

Figure 2.4 indicates that the majority of tebuconazole was leached from the boards within the first 5 months of exposure. After 5 months, the cumulative loss of tebuconazole maintained approximately the same level of cumulative loss for the rest of the year. The high and low retentions showed similar leaching patterns. The loss of tebuconazole occurred more quickly in the material treated at a higher retention, as indicated by the steepness of the cumulative loss of chemical from January to March. Although similar at the lower retention, the loss of chemical did not occur as rapidly in the first 3 months of exposure. After initial leaching of tebuconazole from the surface of the boards, the curves plateau. The plateau regions showed that a very small amount of chemical was lost from the treated material after the initial leaching in the first 3
months. The ratio of cumulative loss between the high and the low retention for tebuconazole was 1.8 and this was not the same as the solution concentration ratio which was 1.5. This suggests that the loss of tebuconazole from the treated material was not proportional to the initial treatment retention. Moreover, some of the leachate samples collected from the basins were shown to have a tebuconazole concentration that was greater than the solubility of tebuconazole in water. These observations suggest that tebuconazole was not solubilized in water and then leached from the wood. Instead, these observations indicate that it may be possible that tebuconazole was physically dislodged from the surface of the wood during rain events; this implies that tebuconazole was physically deposited on the surface of the wood as solution dried after treatment and was not bonded to the wood. If this was occurring, then one would expect that the initial rain events would result in higher chemical concentrations in the leachate due to the presence of more chemical on the surface of the wood at the beginning of exposure. However, after a few rain events the dislodged surface chemical would start to deplete and the concentration of chemical in the leachate would also level off. This was observed for both the high and the low retentions. The higher ratio of cumulative loss between the high and low retention for tebuconazole can also be explained by the rationalization that there was more surface chemical to be dislodged at the higher retention compared to the lower retention.

2.3.2 Chemical Depletion of DDAC for Samples Installed in December

In Figure 2.5, the profile of DDAC depletion from the treated wood showed a similar leaching profile to the loss of tebuconazole from wood (Fig 2.3). In the first 4 months of exposure, wood treated with P5 at the high retention lost 87% of the total DDAC that was leached from the samples in 1 year. Similarly, at the low retention, 86% of the DDAC leached was lost in the first 4 months. The arrows on the x-axis in Figures 2.5 and 2.6 indicate the point where deep checks formed and fresh surfaces were available to interact with water containing solubilized chemicals. At the low treatment retention, 400 mg m⁻² of cumulative DDAC was available from March to December. At the higher retention, 1046 mg m⁻² of cumulative DDAC was available from March to December (Fig 2.6). The ratio of cumulative loss between the high and the low retention for DDAC was 2.7, and this was different from the solution concentration ratio which was 2.0. As was discussed for tebuconazole treated material, the relative loss of DDAC from the two sets of treated material was not proportional to the initial treatment level.



Exposure Time (months)

Figure 2.5 The line graph illustrates the depletion of DDAC leached from spruce samples treated with P5. The monthly precipitation is plotted on the secondary Y-axis. The purple bar graph provides an estimation of the precipitation at the site based on the average volume of leachate that was measured in each basin per month. The yellow arrow on the x-axis indicates the point where deep checks were open in the exposed material.

Fig 2.6 shows that the 86-87% of DDAC that was lost due to leaching occurred within the first 4-5 months for the high retention, and within the first 3 months for the low retention. For both retentions, the amount of DDAC found in the leachate was low for the rest of the year after the initial period of heavy leaching. The absolute rate of chemical loss of DDAC in the first three months was higher in the material treated at a higher retention.



Figure 2.6 The cumulative amount of DDAC leached from boards treated with P4 in 1-year field exposure. The lines graphs show the amount leached from samples treated at two different retentions: 1 % and 2 % solution strength. The yellow arrow on the x-axis indicates the point where deep checks were open in the exposed material.

2.3.3 The Percent of Active Ingredient Leached from P4 and P5 Treated Wood

Wood samples arranged outdoors as simulated decking leached very small quantities of active ingredient during the first year of exposure. The percent of active ingredient leached from the treated wood for each treatment was calculated in Table 2.3 for the samples exposed for 7 and 12 months. The active ingredient in each basin (mg/basin) was calculated based on the leachate samples taken from the basin each month. For each treatment, the average amount of active ingredient in the basin was calculated. For each basin, the pair of treated wood samples being leached was used to determine the total active ingredient in the pair of boards based on chemical

uptake. To estimate the percent of the biocide that was dislodged from the pair of treated boards, the ratio of the leached biocide to the biocide initially present in the pair of boards was determined. This was a conservative estimate of the percent biocide that could have leached from the boards because not all of the treated surfaces were exposed to rain, for example the bottom surface. For each active ingredient and solution strength, 16 replicates (basins) were analyzed. In each basin, 2 treated samples were leached together. The average active ingredient uptake was calculated based on the 16 basins containing 2 samples, for a total of 32 samples treated at each solution concentration.

The average cumulative DDAC measured in the leachate was 114.4 and 117.7 mg/basin for the low retention and 353.4 and 370.5 mg/basin for the high rentention after 7 and 12 months respectively. The average cumulative tebuconazole measured in the leachate was 214.1 and 254.4 mg/basin at the low retention and 391.7 and 419.7 mg/basin at the high retention after 7 and 12 months respectively. For both DDAC and tebuconazole, the ratio of the average active ingredient found in the leachate at the high and low retentions was not proportional to the ratio of the solution strengths. After one year, 4.5% of DDAC at the low retention and 7.7% of DDAC at the high retention was leached from the treated boards. For tebuconazole, the low retention samples leached 30.3% and the high retention samples leached 22.5%. The percent of active ingredient lost from P4 treated boards. The difference in the amount of active ingredient leached from the boards suggests that the biocides have very different interactions with the wood. The active ingredient with fewer interactions, if any at all, would be removed more readily from the wood; and the active ingredient with chemical interactions with the wood would not leach as much.

	Average cumulative AI* in the leachate (mg/basin)		Average AI* in a pair of exposed boards	Biocide Leached from Boards (%)			
Treatment	7 months	12 months	after treatment (mg)	7 months	12 months		
DDAC 1%	114.4	117.7	2634.2	4.3	4.5		
DDAC 2%	353.4	370.5	4812.9	7.3	7.7		
Tebuconazole 0.4%	214.1	254.4	839.5	25.5	30.3		
Tebuconazole 0.6%	391.7	419.7	1867.4	21.0	22.5		

Table 2.3 Leaching of DDAC and Tebuconazole from the Surfaces of Exposed Boards

*AI=Active Ingredient

Average cumulative AI in the leachate is calculated from each basin at 7 months (1st sample collection) and 12 months (2nd sample collection) and then averaged for the different treatments Average AI in a pair of exposed boards after treatment is calculated by determining the AI(mg) in each board directly after treatment based on chemical uptake (Table 2.2). The total mg of AI for the pair of boards is the sum of the AI (mg) in each sample.

Biocide leached from Boards (%) = cumulative biocide in the leachate (mg/basin) / total biocide in a pair of boards in the basin (mg) *100.

Instead of considering the averages for the values of active ingredient in the leachate and the pairs of treated samples (Table 2.3), the 16 individual basins for each treatment can be compared to determine if there are any trends based on chemical retention. Table 2.4 and Table 2.5 show the total active ingredient in a pair of treated boards before exposure as well as the concentration of active ingredient in the leachate after the very first collection in January (1 of 2 collections) and the amount of cumulative active ingredient collected after 7 months. Regression analysis of the data, specifically the chemical retention and the amount of active ingredient recovered in the leachate, did not show any trends within a specific treatment level. The data are sorted in descending order for all of the variables in Table 2.4 and 2.5. Basins with pairs of samples treated with 2% DDAC have a range of initial chemical retentions, but samples with a high amount of active ingredient did not necessarily leach the most chemical. When the DDAC in the cumulative leachate was compared between treatment levels, there was more DDAC in the high compared to the low (t=0.00001; α =0.05). The same trend existed for the high and low treatment levels for tebuconazole (t=0.0002; α =0.05).

Biocide	Level	Basin	AI* in a pair of treated boards (mg)	January: 1 st Leachate Collection (µg/mL)	Al in 7 months of Cumulative Leachate (mg/basin)
		25	6402	23.6	385.7
		42	6242	25.5	452.8
		57	5850	16.3	331.7
		50	5580	47.7	641.9
		12	5508	30.1	402.4
		51	5348	22.3	294.3
		53	5322	33.5	471.0
	High	41	5276	21.2	377.1
	(2%)	8	5016	20.2	282.2
		3	4458	16.7	371.6
		52	4300	30.1	359.7
		44	4262	20.7	259.7
C		14	3750	12.8	259.6
		19	3622	26.1	142.0
		5	3234	16.8	233.8
DA		20	2836	24.7	389.4
D		33	3796	6.6	158.8
		32	3667	7.7	110.6
		28	3469	6.7	146.5
		4	3183	9.4	76.7
		35	3173	7.5	147.1
	Low	16	2915	7.0	139.5
		7	2828	6.1	143.6
	(1%)	17	2810	2.8	105.0
	(1)0)	37	2755	6.6	124.7
		49	2603	5.6	118.1
		13	2135	3.6	100.2
		1	1870	6.1	87.5
		27	1635	7.1	125.2
		62	1426	2.7	27.7
		26	1248	5.6	104.5

 Table 2.4 Leachate Data from Individual basins holding DDAC-treated samples

Biocide	Level	Basin	AI* in a pair of treated boards (mg)	January: 1 st Leachate Collection* (µg/mL)	Al in 7 months of Cumulative Leachate (mg/basin)		
		36	7382	<mark>42.8</mark>	613.9		
		24	3743	16.6	301.3		
		47	2771	<mark>37.0</mark>	413.6		
		48	2590	14.6	287.6		
		23	1592	27.4	500.9		
		61	1383	18.6	386.2		
	High	58	1336	<mark>33.4</mark>	519.9		
	(0.6%)	30	1059	24.4	362.4		
	(0.070)	60	1038	<mark>50.2</mark>	543.1		
		59	1025	17.2	325.3		
		29	992	13.8	288.8		
		46	959	30.8	420.5		
Ľ		54	836	21.4	325.7		
IAZO		9	724	22.8	320.6		
		22	577	15.8	265.4		
ð		6	1392	3.4	93.6		
2		2	1349	15.0	322.3		
TEBL		10	1200	9.2	198.6		
		40	949	12.0	247.3		
		31	902	14.0	311.1		
		21	817	7.8	187.9		
		39	790	11.8	242.1		
	Low	38	785	14.2	283.4		
	(0.4%)	56	778	10.8	211.7		
		34	756	10.6	186.6		
		18	745	8.2	211.2		
		43	706	12.8	235.1		
		55	639	9.4	192.0		
		45	633	10.2	189.4		
		11	599	12.6	222.1		
		63	388	4.4	91.2		

Table 2.5 Leachate Data from Individual basins holding Tebuconazole-treated samples

AI*= Active Ingredient *1st Leachate Collection: The highlighted values indicate that the concentration of tebuconazole in the basin was greater than the solubility of tebcuonazole in water.

2.3.4 Pre-screening Boards for Checking

Pre-screening 20 ft. lengths of board for their tendency to check based on two samples, one from each end of the board was not very successful. This is likely due to a high degree of natural variation in the wood structure of a length of board that is 20 ft. long. In anticipation of the unpredictable factors related to checking, 3 extra samples were treated for each condition and exposed so that the number of experimental replicates would not diminish.

2.4 DISCUSSION

2.4.1 Mobility of Carbon-based Preservatives after Pressure Treatment

The presence of active ingredient in the leachate suggests that DDAC and tebuconazole were mobile in the wood after treatment. Samples treated at a higher chemical retention leached more active ingredient compared to matched samples treated at a lower chemical retention, but the loss was not proportional to the ratio of the treating solution concentrations. The disproportionate loss suggests that these active ingredients may not have been leached from the wood. Instead, the chemicals may have been physically dislodged from the surfaces of the boards by rain water.

If the solubility of tebuconazole in water is compared to the concentration of active ingredient found in the very first leachate collection in Janurary (Table 2.5), we find further evidence that suggests tebuconazole was dislodged from the surface of the wood. The solubility of tebuconazole in water at 25 °C is 0.032g/L ($32\mu g m L^{-1}$). If the concentration of tebuconazole in the leachate collection is greater than 32 ppm, then the tebuconazole could not have been entirely solubilized by the water and then carried into the leachate. Table 2.5 shows that there were 4 leachate collections from basins at the high treatment level that measured greater than 32 ppm of tebuconazole. Unlike tebuconazole, DDAC has a very high solubility in water. Therefore, it is possible that rain water solubilized DDAC on the surface of the wood and then leached into the basin. Based on the results, it is likely a combination of DDAC solubilized in water as well as a physical dislodging of DDAC from the surface of the wood. Li *et al.* (2009) reported tebuconazole leaching rates of 7.7 to 37.3% using a laboratory leaching method (AWPA E11-06) with various copper-azole formulations, as well as with a tebuconazole emulsion without copper. The copper-azole formulations had leaching rates ranging from 7.7 to 28.3% with tebuconazole solution concentrations from 0.006 to 0.028%. Higher leaching rates were observed for formulations that did not contain copper, such as the tebuconazole emulsion with leaching rates of 30.4 to37.3% and tebuconazole concentrations of 0.052 to 0.026% respectively. The results from the current study showed that the addition of copper to a solution containing tebuconazole reduced the leaching rate of tebuconazole. The high copper fixation rate of wood treated with the copper-azole formulations and favorable reactions between metals and triazoles is likely responsible for the reduced leaching of tebuconazole. The effect of metals on the fixation and complex formation of formulations containing propiconazole and tebuconazole has been investigated by Evans *et al.* (2007). Evans *et al.* (2007) showed that metal-triazole complexes, such as the reaction between divalent copper and tebuconazole, are easily formed under mild laboratory conditions.

In the current study, our tebuconazole formulation was prepared with 0.4 and 0.6% tebuconazole solutions as waterborne emulsions (Table 1.1) and leaching rates were calculated to be 30.3 and 22.5% respectively. In the current study, as well as the one conducted by Li *et al.* (2009), the lower tebuconazole concentration solution resulted in a higher leaching rate by approximately 7%. The reason for this trend is unknown at this time, but it does support the hypothesis that the tebuconazole active ingredient is not fixed in the wood, but is physically dislodged from the surface of the wood by rain water. For example, consider the equation used to determine what percent of the active ingredient is dislodged from the surface:

Leaching rate (%) =
$$\underline{\text{mg of tebuconazole/basin}}_{\text{mg of tebuconazole in treated samples}}$$
 [Equation 1]

Table 2.3 shows that the cumulative active ingredient in the leachate is different for the high and low retentions, but not proportional to the concentration of the treating solutions. If the tebuconazole molecules are more densely packed on the surface of the wood at a high solution concentration compared to a low solution concentration, then exposure to the same amount of rain may dislodge more tebuconazole into the leachate at a high retention. However, even if the

numerator in Equation 1 increases because there is more tebuconazole on the surface, the initial amount of tebuconazole in the treated samples is higher, and this would decrease the leaching rate.

For P5-treated material a DDAC leaching rate of 4.5 and 7.7 % was measured after 365 days of outdoor exposure for solution strengths of 1% and 2% respectively. The percent of DDAC lost in the leachate was lower than the percent of tebuconazole lost in the leachate. In a water-leaching study with 19 mm square wafers of southern pine and DDAC retention levels from 6.3 to 7.2 kg m⁻³, Nicholas *et al.* (1991) observed very little depletion of DDAC in the wood after leaching. This suggests that DDAC has the ability to be fixed in the wood and resist leaching during exposure to water. This observation is congruent with the different leaching patterns of DDAC and tebuconazole.

The field performance of both DDAC and tebuconazole was recently studied in a seven-year field test using lap joints with triazole-based formulations and DDAC-based formulations (Jiang *et al.*, 2009). In a tebuconazole, Barlox[®]12/Barlox[®]16S formulation with active ingredient retentions of 0.016, 0.032, and 0.048 kg m⁻³ field test materials performed consistently well at all retentions over the 7-year test. The analogous formulation with DDAC/ Barlox[®]16S/Barlox[®]12 did not perform as well as the tebucoanzole formulation. In the fourth year of the test, ratings started to decline for all of the retentions that were tested: 0.4, 0.8, and 1.2 kg m⁻³.

2.4.2 Movement of Carbon-based Preservatives

After 7 months of continuous leaching, there were some basins with no measurable DDAC or tebuconazole in the leachate (< 1 ng ml⁻¹). At this point, in late November, the samples were placed inside to dry. When the next batch of leachate was collected, the concentration of the active ingredients had increased from the previous analysis. This pattern of leaching has been previously discussed in the literature (Chung and Ruddick, 2004a; Chung and Ruddick, 2004b), and has been described as a migration of chemical to the surface by diffusion as the wood wets and dries. Following the drying period and the slow diffusion of chemical to the surface of the wood, it was observed in the next leachate collection that tebuconazole was present in at higher

concentrations than DDAC at the high and low retentions. The concentration of DDAC in the leachate remained the same at the low retention and increased very slightly at the high retention. The relative concentration increase of active components in the leachate may be explained by describing the interactions that exist between the active ingredient of interest and the wood. Chung and Ruddick (2004b) note that the chemical diffusion is slow, and that it is possible that "some of the mobile chemical can migrate deeper into the board, where it can be fixed to reactive sites on the wood". This suggests that the mobile chemicals can redistribute in the wood, and that not all the mobile chemical re-appears on the surface of the wood. DDAC is believed to interact with the wood through ion exchange mechanisms with acidic functionalities in lignin such as phenolic hydroxyls and carboxylic acids (Jin and Preston, 1991; Doyle and Ruddick, 1994). DDAC is also believed to adsorb to cellulosic material through electrostatic forces at both pH extremes (Cooper, 1991; Jin and Preston, 1991). The electrostatic effects are believed to be a result of the interaction between DDAC and the electrically charged interior of cellulosic materials accessed by swelling that is caused by pH extremes (Jin and Preston, 1991). Given these chemical interactions, mobile DDAC molecules in the wood encounter several possible reactive sites before potentially being washed into the leachate. Moreover, the described reactivity of DDAC allows this molecule to ion exchange to all surfaces that it interacts with, including the basin and all collection vials. This is the reason that a gentle acid wash is necessary for all surfaces in contact with DDAC. It is possible that some DDAC was not analyzed in the leachate due to loss on various surfaces.

Conversely, adsorption mechanisms between tebuconazole molecules and wood are not well described in the literature. Hingston *et al.*, (2001) hypothesizes that the triazole biocides bind to wood through ion exchange mechanisms similar to those of copper, but no evidence is given to support this claim. Furthermore, tebuconazole does not have an atom with a formal charge, so it is unlikely to participate in ion exchange mechanisms. Instead, adsorption of tebuconazole to wood structures through electrostatic interactions is a more simple explanation for the reaction mechanism with wood. Electrostatic interactions are typically weaker than ion exchange interactions, so a higher concentration of tebuconazole, compared to DDAC, would be expected to appear in the leachate due to weaker interactions with the wood and better recovery from surfaces that interact with tebuconazole.

Wood materials treated with carbon-based preservatives showed the characteristic rapid loss of active ingredient in the first 3-4 months of leaching. These results are consistent with studies that report a high initial loss of metal components from CCA-treated wood and ACQ-treated wood during the first few months of exposure (Yamamoto *et al.*, 1999; Choi *et al.*, 2004a; Chung and Ruddick, 2004 a,b; Ruddick, 2008). Approximately 80% of the carbon-based preservatives that were leached in one year were lost in the first 3 months. Similarly, Choi (2004) found that 50% of the copper and chromium that leached in 2 years was leached in the first 3 months of exposure.

2.5 SUMMARY

The carbon-based preservatives from P4 and P5 treated wood exposed outdoors for 7 and 12 months were quantified in monthly leachate collections after rain events. Small amounts of Tebuconazole and DDAC were mobile in the wood after treatment, based on the presence of these active ingredients in the leachate.

Pre-screening boards for their propensity to check using aggressive drying is a worthwhile preparative step. However, for long lengths of board, the screening must be conducted with sections from multiple locations in the board.

CHAPTER 3: DO MOBILE CARBON-BASED PRESERVATIVES RE-DEPOSIT ON UNTREATED CHECK SURFACES?

3.1 INTRODUCTION

The active ingredients from spruce shell-treated with P4 and P5 were mobile in the wood during outdoor exposure. As described in Chapter 2, some of these mobile components were washed from the surface of the board with rain water, and were found in the leachate. The aim of this section of work was to determine whether some of the mobile carbon-based preservatives were redistributed within the board.

This may be done by analyzing the check surfaces in the wood that were untreated prior to outdoor exposure. Untreated check surfaces are formed when checks extend beyond the depth of chemical penetration in shell-treated wood. To accurately sample these surfaces, it was important to have a record of the original depth of active ingredient penetration for each treated sample before outdoor exposure. With this information, the check surface beyond the depth of chemical penetration could be accurately sampled. DDAC can be visualized by using acidified bromophenol blue. However, to date, there are no known visual indicators which show the penetration of tebuconazole in wood.

In 1995, an in-house method was developed to investigate the depth of DDAC penetration in wood that was dip-treated (Weigel and Daniels, 1995). However, this method could only provide a localized estimation of the DDAC penetration due to the high variability in chemical distribution resulting from the natural variation in wood. These methods could not be used here because a detailed penetration profile for each treated sample was necessary to ensure sampling of only the untreated check surfaces. A visual indicator of cross-sectional chemical penetration was required to achieve the required degree of accuracy.

Recently, Jiang (2008) showed that amine oxide can act as a surrogate chemical to indicate the penetration of azoles in wood. Using an indicator, Jiang (2008) visualized the penetration of amine oxide in the wood; analyzed the indicated colored regions by HPLC and GC; and showed

that the quats and azoles can be carried into the wood up to the same depth that amine oxide travels in the wood.

Both P4 and P5 formulations contain an amine oxide component. Consequently, a visual indicator identifying the limit of amine oxide penetration could be used as a surrogate to identify the maximum depth of penetration of the active ingredients.

The primary objective of this chapter was to determine if the active ingredients from the surface of the wood was present on wood check surfaces that were untreated before exposure.

3.2 MATERIALS AND METHODS

After 7 months of outdoor exposure, 10 treated boards from each treatment condition were collected and moved indoors for sampling. The untreated control samples were left outdoors with the remainder of the samples scheduled for sampling after 12 months of exposure. After 1 year of exterior exposure, the remaining samples were collected and prepared for sampling. Samples collected after 7 and 12 months of exposure were sampled and analyzed using the same methods.

3.2.1 Preparation of Acidified Bromophenol Blue Indicator (BPB)

A 0.1 % w/w solution of bromophenol blue (BPB) indicator composed of 5 % w/w acetic acid, 20 % w/w ethanol, and 74.9 % w/w water was prepared. The components were weighed on a scale and the densities of acetic acid and ethanol were approximated as 1 g cm⁻³. The components were mixed until the bromophenol blue was completely dissolved giving a reddishyellow colored solution. Compressed air was forced into the flask and a fine mist of the BPB indicator was sprayed onto the treated wood wafers. Bromophenol blue is a pH indicator; the yellow color indicates a pH of 3.0, whereas the blue color indicates a pH of 4.6. The outer zones of the wafers turned blue, which indicated the presence of amine oxide or DDAC molecules. The inner zones turned yellow, which indicated the wood was untreated. Since the BPB solution is acidic with 5% acetic acid, the untreated wood (pH~5) turns yellow as the acid decreases the pH at the wood surface. The reaction of carboxylic acid groups in wood with amine oxide increases the pH of the amine oxide-reacted areas to a less acidic pH (pH≈4.6). Digital images of the wafers were taken immediately after spraying and stored for reference use during the sampling of checks (Appendix I and II).

3.2.2 Penetration of the Carbon-Based Preservatives

Before checks were sampled, the chemical penetration profile of each board was visualized with acidified BPB indicator (Figure 3.1). Cross sectional wafers from each board were removed after pressure-treatment and oven drying, but before outdoor exposure. The wafers provided a visual record of chemical penetration for each sample before it was exposed outdoors. The penetration profile was used to determine the best check-sampling location on the board; to prevent well-treated sapwood corners from being sampled; and to ensure that a reasonable buffer zone was

left between the treated and untreated areas of the check surface. During sampling, the location of the check on the board; the depth of the check; and the sampling depth were recorded.



Figure 3.1 Penetration profile slices from two different boards treated with tebuconazole and sprayed with an acidified BPB indicator. The presence of amine oxide penetration is indicated in blue. The above samples illustrate the variability of the penetration between samples. They also show the importance of visually inspecting each sample's penetration profile to ensure sampled check surfaces were originally untreated. Spruce boards primarily consisting of heartwood were selected for treatment, however, in some cases there were well-treated sapwood corners.

3.2.3 Sampling Checks from Boards Exposed Outdoors

Exposed samples showing check depths a minimum of 4 mm were chosen for sampling. Each board was split using a maul at the cut end of the board. After splitting the board in two pieces, the board was marked to indicate how far the axe entered the interior of the board (Fig. 3.2). The site where the axe penetrated the board during splitting was avoided when sampling checks because of possible contamination between samples and chemical smear. The axe was wiped clean in between samples to avoid cross contamination.



Figure 3.2 The maul is hit with a hammer to get a site-specific split in the board that exposes the check of interest. The rectangle indicates the site where check sampling was avoided after the board was split into two pieces.



Figure 3.3 After splitting the board along the check, the exposed regions of the check were obvious compared to the unexposed interior. The exposed checks were a darker brown-orange color and the untreated interior was the original color. The arrows indicate three matched check surfaces that were exposed along a single check in the board.

Before a board was sampled, the image of the penetration profile was referenced. The chemical penetration was measured with a ruler in the centre of each board. The centre of each board was chosen for penetration measurement because most of the checks formed here and a single location was necessary due to the variable depth of chemical treatment within a board. This also mirrors the sampling of treated wood for quality assurance. The penetration was measured on120 treated samples and averaged. During sampling, a 0.5 mm buffer zone was left beyond the limit of penetration, and at this depth a flame sterilized razor blade was used to score the wood. A chisel was used to remove hand-cut samples from below the razor blade line. A check that forms in a shell-treated material has two distinct check surfaces that are divided by the limit of chemical penetration (Fig. 3.4). The check surface above the limit of penetration is referred to as

the treated check surface. The region below the limit of chemical penetration is referred to as the untreated check surface; and this was the area isolated for chemical analysis of the active ingredients.



Figure 3.4 The anatomy of a check. The sample was split along the grain of the wood to reveal two matched check surfaces. This material was shell treated with a chemical that penetrated 2 mm into the wood. This is indicated by the dotted line which divides the check surface at the limit of chemical penetration. The check surface below the limit of penetration is the untreated check surface. Below the depth of the check is the untreated interior of the wood sample.

The checks were split open and the two matched surfaces were sampled below the depth of chemical penetration. Samples from the checks were chiseled by hand below the originally treated surface. Sampling near the end grain was avoided whenever possible. However, when necessary, samples were collected no closer than 5 cm from the end grain and noted. The samples were stored in sealed glass vials and then extracted and analyzed for active ingredients using LC/MS. The matched check samples were collected from 7 month and 12 month exposed materials and vacuum sealed for use in the basidiospore germination test. Matched check samples are illustrated in Figure 3.3.

3.2.4 Extraction of DDAC and Tebuconazole from Check Surfaces

Before samples were extracted, all the check samples were hand-cut using a sterile razor blade to include only the exposed check surface area. At this time, the thickness of the sample was also reduced to approximately 1-2 mm. This ensured that chemical adsorption to the untreated interior

of the wood was minimized. The surface area and weight of the check samples were measured and recorded before extraction.

DDAC was extracted from check samples using 5 mL of acetonitrile (ACN) acidified with formic acid (HPLC grade, pH=3). These samples were extracted in the ultra-sonicator for two hours. The pH of the samples was checked after sonication (pH 3-3.5). In the extraction vials, the samples were brought to dryness with a gentle stream of high purity nitrogen. The check surface was removed from the sample vial, and the DDAC was reconstituted with 1 mL of acetonitrile containing TMAC and DoDAB as an internal standard. A second extraction was performed on each check sample with a fresh 5 mL aliquot of acidified acetonitrile. The amount of DDAC extracted from the check surface is represented by the sum of the DDAC found from each extraction.

Tebuconazole was extracted from check samples using 5 mL of methanol (HPLC grade). These samples were extracted in the ultra-sonicator for 2 hours and then brought to dryness with a stream of high purity nitrogen. After solvent evaporation, the check surface was removed from the sample vial and the tebuconazole was reconstituted with 1 mL of methanol containing azaconazole as an internal standard. A second extraction was performed on each sample with a fresh 5 mL aliquot of methanol. The reported tebuconazole represents the total tebuconazole found in each extraction.

The active ingredients were quantified by LC/MS using the same methods described in Chapter 4 for DDAC and tebuconazole. For DDAC, the calibration curve was changed to reflect the sample preparation in ACN as opposed to the 50:50 sample preparations in ACN: H₂O.

3.1.1. Statistical Analysis

Statistical analysis was performed using a statistical package, SPSS version 16.0.2. The data was transformed using the natural logarithm to get a normally distributed data set before any analyses were performed. Assumptions of equal variance, independence of measurements, and normally distributed residuals were satisfied. The data set was split by active ingredient, so DDAC and tebuconazole were run as separate sets of data. An ANOVA was used to determine whether

exposure time and treatment levels were significant in the model. The Bonferroni multiple comparisons tests was used to compare the differences between the three treatment levels: control, low, and high (α =0.05).

3.2. RESULTS

3.2.1. Chemical Penetration of Carbon-based Preservatives in Spruce Heartwood The penetration of active ingredients in most samples showed an even distribution based on the penetration of DDAC or amine oxide, the surrogate chemical used to estimate the penetration of tebuconazole. Some boards had well-treated sapwood corners, or deep penetration into the side grain. When penetration was measured in the center of each board, formulation P5 showed an average penetration of $\leq 2.3 \pm 1.1$ mm. Similarly, the P4 formulation averaged a penetration of $\leq 1.6 \pm 0.6$ mm. Most untreated surfaces were sampled from checks that formed in the center of boards, where checks were the deepest.

3.2.2. Active Ingredients on Untreated Check Surfaces

DDAC was detected on the untreated check surfaces after 7 and 12 months of outdoor exposure. The amount of tebuconazole detected on the untreated check surfaces was not different from the reference samples (Table 3.1). There was no significant difference between the concentrations of the active ingredients on the check surfaces at the high and low retentions for P4 or P5 (α =0.05). Furthermore, there was no difference in the amount of chemical found on the untreated check surfaces during the two exposure periods: 7 months and 12 months (α =0.05). Subsequent averages were calculated by consolidating the data for the two periods of exposure. The data for the different treatment conditions were not consolidated.

Formulation	Solution Strengths	Average AI* on check surface (mg/g wood)
	DDAC 1%	0.02 ± 0.05
P5	DDAC 2%	0.03 ± 0.07
(FIM-1)	Control Samples	0.000 ± 0.0
	Reference Samples	0.004 ± 0.001
	Teb 0.4%	0.008 ± 0.02
P4	Teb 0.6%	0.002 ± 0.002
(1279-23)	Control Samples	0.000 ± 0.0
	Reference Samples	0 004 + 0 001

Table 3.1 Concentration of Active Ingredient on Exposed Untreated Check Surfaces

*Active Ingredient. The active ingredient on 30 check surfaces was used to calculated the average AI Control samples were untreated and exposed; no active ingredients were present on the check surfaces of these samples. Matched reference samples that were treated, but unexposed were sampled using the same methods described above. These samples were analyzed to control for the possibility that the active ingredients were mobile in the wood without being exposed outdoors. In addition, the reference samples verified that the sampling method was reliable and established a practical reference for the zero base line. The amount of active ingredient detected in the un-penetrated interior of P5 and P4 reference samples was 4 mg of active ingredient/g wood. The preservative detected in the unpenetrated wood could represent preservative that extended beyond the visual estimate due to the threshold of the indicator and the possibility that chemical may have been penetrated down rays but not spread into adjacent tracheids beyond the boundary of the visibily penetrated zone.

3.2.3. Redistribution of Carbon-Based Preservatives in Wood

An exposed check surface from a sample treated with P5 was analyzed in increments based on depth from the surface of the board. This analysis was performed to determine the vertical distribution of active ingredient on the check surface (Fig. 3.5). For analysis of the sample removed from the upper check surface, the first 0-3 mm, 9.6 mg DDAC/ g wood was present. At a depth of 3-4.5 mm from the surface of the board, 0.0357 mg DDAC/g wood was present; and at 4.5-6 mm from the surface of the board 0.0049 mg DDAC/g wood was detected. Based on the penetration profile of this particular sample, the shell-treatment penetrated 3 mm vertically through the radial surface. This is consistent with the incremental chemical depth analysis. At depths greater than 3 mm from the surface, the amount of active ingredient decreased by 3 orders

of magnitude. The sharp decrease in the active component on the check surface at depths greater than 3 mm from the surface indicated that the detected active ingredients were not from the original pressure treatment; it was redistributed to this location from elsewhere in the wood.



0-3 mm: 9.6 mg DDAC/g wood

3-4.5 mm: 0.0357 mg DDAC/g wood 4.5-6 mm: 0.0049 mg DDAC/g wood

Figure 3.5 Chemical distribution of DDAC on an exposed check surface. The material was shelltreated with a 2% solution of P5 and sampled from a board exposed outdoors for 12 months. Below a depth of 3 mm, the amount of DDAC found on the check surface drops 3 orders of magnitude. Based on the penetration profile, the DDAC found at this depth represents the redistributed chemical.

The amount of active ingredient found on untreated check surfaces was extremely low and variable as shown by the high standard deviations in Table 3.1. The percentage of mobile, or dislodged, active ingredient that re-deposited onto untreated check surfaces is calculated in Table 3.2. This is calculated by dividing the average active ingredient found on the check surfaces (mg m⁻²) by the average cumulative mobile active ingredient that was available at the upper surface of the wood for redistribution into checks (mg of active ingredient per m² of the exposed upper surface). The average cumulative amount of active ingredient collected in the leachate was assumed to be the amount of mobile chemical available at the upper surface of the wood. To get a better estimation of the cumulative active ingredient that was lost in the months of January and February. This was done because there were very few checks in the exposed material at this time. Since most of the active ingredient was lost in the first two months, and was not available to most of the checks, it was not included in the calculation of the percent that re-deposited. The active ingredient on the check surface was calculated as an average value resulting from the analysis of 30 checks for each treatment level and active ingredient.

Formulation	Solution Strengths	Average Cumulative AI* from Mar-Dec (mg m ⁻²)	Average AI* on check surfaces (mg m ⁻²)	Available AI that deposited on check surfaces (%)
P5	DDAC 1%	376	3.5	0.9
	DDAC 2%	1186	6.1	0.5
P4	Tebuconazole 0.4%	843	1.8	0.2
	Tebuconazole 0.6%	1227	0.8	0.06

Table 3.2 Mobility of DDAC and tebuconazole in Shell-Treated Wood Exposed Outdoors for one year

*Active Ingredient.

3.3 DISCUSSION

The results suggest that mobile active ingredients from the surface of treated wood can interact with untreated check surfaces that form during outdoor exposure. It is unknown whether chemical bonds were formed between tebuconazole and the untreated check surfaces. The presence of very low concentrations of DDAC, 0.02-0.03 mg/g on wood surfaces that were not treated before exposure suggests that a small amount of chemical redistribution can occur after treatment during exposure. For DDAC, between 0.5 and 1% of the mobile active ingredient at the upper surface of the wood is able to re-deposit onto a previously untreated check surface. It is difficult to extract DDAC from wood that is not ground into wood meal, even under acidic conditions favorable to ion-exchange with protons. Therefore these percentages represent a conservative estimation of the DDAC that is present on the check surfaces. Despite the fact that each check surface was extracted twice under acidic conditions, it is possible that some of the DDAC may have not been extracted from the check surfaces.

Since very low concentrations of the active ingredients were isolated from the untreated check surfaces, reference samples were analyzed to ensure that the presence of these active components was a result of redistributed chemical, and not an artifact of the sampling procedure. Treated and unexposed reference samples had an average of 0.004 mg active ingredient/g wood analyzed. This confirmed that the active ingredients were not mobile in the wood when unexposed. Moreover, this small amount of active ingredient was sufficient to verify that the sampling technique was accurate and reliable. These samples served as a reference for residual

contamination resulting from the sampling procedure, and they ensured that the penetration profiles were accurate representations of the active ingredient penetration. Furthermore, the vertical distribution of active ingredient on the check surface based on incremental depth analysis confirmed that the BPB indicator was a reliable surrogate for the presence of triazole active ingredients and DDAC, as suggested by Jiang (2008).

Tebuconazole was not found on untreated check surfaces in amounts that differed from the reference samples. This suggests that although tebuconazole is mobile in the wood after treatment, it cannot re-deposit onto untreated check surfaces. For both active ingredients, there was no difference between the high and low retentions or the 7 and 12 month exposure periods.

There was a very high variability in the amount of active ingredient recovered from untreated check surfaces for a given treatment. This may be a consequence of the micro-checks that were created on the surface of wood samples prior to outdoor exposure. Small fractures in the wood that were present in January and February would have encountered the highest concentrations of mobile active ingredient. It is possible that some of the active ingredients were deposited into micro-check structures in the first few months of outdoor exposure. This could have resulted in untreated check surfaces with high amounts of active ingredient. Similarly, checks that formed early in the 7-month or 12-month exposure had surfaces that were exposed to a higher cumulative concentration of active ingredient compared to checks that formed later during exposure. This may have contributed to the high variation of active ingredient found on the check surfaces. The untreated check surfaces were sampled very carefully to ensure that the treated upper portion of the check surface was not sampled and did not chemically contaminate the lower check surface. In one instance, a check surface that was sampled close to a knot in the wood measured a concentration of tebuconazole that was approximately 100 times larger than the average of the sampled check surfaces. Based on the analysis of the vertical distribution of active ingredient, the measured concentration suggested that this sample may have been partially treated prior to exposure; this was likely due to the knot structure in the wood. This particular sample was omitted from the data set. For each treatment, two samples were omitted from the data set based on mass spectrometry analyses and irregularities in the penetration profiles. The extreme sensitivity of mass spectrometry analysis aided in identifying anomalously high

concentrations from samples that were clearly the result of sampling a portion of a check surface that was treated prior to exposure.

The leachate was not analyzed for the presence of amine oxide. However, this information may have been useful in determining whether the amine oxide molecules were involved in the leaching behavior of the active ingredients. Since amine oxides are responsible for promoting penetration of the active ingredients into the wood, it is possible that these molecules could also be involved in the mechanism that is responsible for removing them from the wood during leaching or dislodging.

3.3.1 Re-deposition of DDAC and Tebuconazole in Exposed Wood after Treatment Based on the percent of available active ingredient that deposited on check surfaces, DDAC has a greater affinity for remaining on the surfaces of wood compared to tebuconazole (Table 3.2). This observation is consistent with the known reaction mechanisms between DDAC and the components in wood; DDAC interacts with anionic sites in wood via ion exchange, pH dependent adsorption, and electrostatic interactions (Tascioglu *et al.*, 2005; Loubinoux *et al.*, 1992; Jin and Preston, 1991). These bonding mechanisms allow the quaternary ammonium compounds to be highly effective at securing anionic sites in wood. In comparison, chemical interactions between tebuconazole and wood components are not published in literature. During the 12 month exposure, 22-30% of tebuconazole retained in the samples after treatment was lost and no tebuconazole re-deposited on the untreated check surfaces. Taken together, these results suggest that any chemical interactions between tebuconazole and wood are weak. They may solely consist of a combination of Van Der Waals forces and weak adsorption.

To determine if the concentration of active ingredient is sufficient to provide protection to untreated check surfaces, these surfaces must be tested against the primary source of infection of wood exposed outdoors and above ground–basidiospores.

3.4 SUMMARY

DDAC from wood samples treated with P5 and exposed outdoors was found to re-deposit in small amounts onto previously untreated check surfaces. However, wood samples treated with P4 and exposed outdoors did not have any tebuconazole on the untreated check surfaces. This

suggests that the mobile DDAC can re-deposit onto check surfaces, but mobile tebuconazole cannot re-deposit onto check surfaces.

CHAPTER 4: BASIDIOSPORE GERMINATION AND FUNGAL DIVERSITY ON CHECK SURFACES

4.1 INTRODUCTION

Wood that is unprotected and exposed outdoors is susceptible to decay. After a succession of microorganisms invades wood either brown-rot, soft-rot, or white-rot fungi can enter wood (Eaton and Hale, 1993). Decay fungi produce spores that germinate and form filamentous hyphae that can penetrate wood structure through pits and cell walls. The hyphae search for nutrients in the wood, and eventually form a vast network of fungal hyphae called a mycelium. Wood decay fungi belong to the phylum Basidiomycota, but they are often reported as Basidiomycetes. This phylum includes a diversity of genera and some of them can decompose cellulose, hemicelluloses, and lignin. Degradation or chemical modification of cellulose, hemicelluloses, are responsible for providing strength to the wood.

As a network of fungal hyphae continues to grow and extract nutrients from wood, the fungal mycelium begins to mature. Eventually the mycelium produce reproductive structures called fruiting bodies which emerge from the mass of mycelia. The fruiting bodies of basidiomycetes produce millions of basidiospores that are disseminated into the surrounding environment. Once they are released, the basidiospores are transported to new environments by different vectors including: wind, rain, insects and animals (Eaton and Hale, 1993).

Spores are generally accepted as the primary infection mode of wood materials exposed outdoors and above-ground (Morton and French, 1966; O'Toole, 1971; Schmidt and French, 1978; Carey, 1981; Fougerousse, 1984; Bjurman, 1985; Cymorek and Hegarty, 1986; Highley, 1992; Morrell, 1996; and Morris *et al.*, 2009). Choi *et al.* (2003) suggested that checks may be one of the main avenues for basidiospores to access the interior of wood when exposed above-ground. Since checks are a susceptible entrance point for fungal attack, it was important to test whether basidiospore germination would occur on the untreated check surfaces from exposed treated material or when additional chemicals were added to checks. In our work, the low concentrations of DDAC and the absence of tebuconazole on untreated check surfaces was not expected to prevent spore germination. To determine if low concentrations of carbon-based active ingredient would prevent spore germination, an experiment was designed to simulate a situation that might be encountered on exposed decking material. Different DDAC and tebuconazole concentrations were tested against a known amount of basidiospores from two wood decay fungi: *Oligoporus placentus* (Fr.) Gilbertson et. Ryvarden and *Gloeophyllum sepiarium* (Wulf. Ex Fr.) Karst. A series of check surfaces with varying concentrations of active ingredients was achieved by adding a known amount of biocide to recovered check surfaces.

In the second part of this chapter we examined the fungal diversity present on boards treated with DDAC and Tebuconazole. The diversity of fungi on the check surfaces was studied by collecting samples from the treated, exposed material. In order to assess the severity of the field test and the efficacy of the treatment levels and the preservative formulations, it was important to know which fungal species were interacting with samples at the field test site. The primary goal was to determine if the frequency of fungal isolation was different on the upper (treated) and lower (untreated) check surfaces (Fig 1.2). The secondary aim was to compare the fungal species isolated from the two different preservative treatments: P4 and P5.

4.2 MATERIALS AND METHODS

4.2.1 Check Surface Sample Collection for Basidiospore Germination Test Before the basidiospore germination test, 7 unsterilized exposed check samples from each treatment were collected and incubated under favorable conditions for assessing fungal growth (Fig. 4.2). The samples were monitored and aerated weekly. After 3 months of incubation the check surfaces were removed and observed by light microscopy to detect fungal growth.

Figure 4.1 illustrates how check samples were collected in pairs (matched check surfaces). One surface of the check was extracted and analyzed to determine the amount of active ingredient (tebuconazole or DDAC) on the check surface. The matching check surface was vacuum sealed to prevent surface contamination. The vacuum sealed samples were sterilized using 25 kgray of

ionizing irradiation (Iotron Technologies Corp.). From these samples, 75 sterilized check surfaces were chosen for the spore germination test. The check samples were chosen based on the amount of chemical present on the paired check sample. The concentration of active ingredient found on the check surface of 'A' serves as an estimate for the concentration present on check surface 'B'.





Check surface 'B' was sampled and used in the basidiospore germination test, it was not extracted.

Figure 4.1 'A' and 'B' represent a pair of matched check surfaces. Matched surfaces provided an approximation for the concentration of active ingredient on the un-extracted check surface used in the bio-assay. The treating limit is not shown in this picture, but was measured before sampling.

The check surfaces were spiked with known amounts of the active ingredients to achieve a range of different concentrations. Exposed check surface samples were spiked with diluted formulated product so that 1 μ g and 2 μ g of tebuconazole and 2 μ g and 4 μ g of DDAC were added to the check surfaces, in addition to the amount that was redistributed during exposure.

The neat formulated products of P4 and P5 were use to prepare diluted solutions of the active ingredients for spiking. The solutions of tebuconazole and DDAC were prepared in ethanol to achieve 200 ppm of the active ingredient. The solutions were filter-sterilized (Nalgene Disposable filter unit) through a 0.20 μ m membrane suitable for organic solvents. The concentration of active ingredient in the diluted formulated product was analyzed before and after filter-sterilization to ensure that chemicals did not adsorb to the filter membrane. The concentration of the active ingredient did not change, as indicated by LC/MS analysis.

Both formulations, P4 and P5, contain an amine oxide in the solution to improve chemical penetration, uniformity and distribution. To isolate the effect of the active ingredient alone, amine oxide was tested for its biocidal activity on spore germination. The amine oxide that was tested in the bio-assay was present in the P4 formulation. A concentrated amine oxide solution prepared in water was diluted to a 1% solution in ethanol and amended on exposed UV sterilized check surfaces obtained from control samples, where no active ingredients were present.

4.2.2 Spore Germination Chambers

Check surfaces, with a range of different active ingredient concentrations, were placed in chambers specifically designed to maintain an environment favorable to spore germination (Choi, 2004). The chambers were prepared in 50 mL Falcon tubes, as described by Choi (2004) and altered to create a high relative humidity within the tube (Fig. 4.2).



Figure 4.2 Basidiospore germination incubation chambers

The individual tubes contained cotton wrapped in cheese cloth and saturated with UV-sterilized ultrapure water. Excess water, unabsorbed by the cotton, was removed from the tubes and a polypropylene mesh screen was placed on top of the cotton. Assembled tubes were autoclaved and equilibrated overnight. The following day, the check surfaces were placed in the center of the plastic mesh screen, out of direct contact with moisture and cotton. Next, the check surfaces were inoculated with spores of either *O. placentus* or *G. sepiarium*. *G. sepiarium* was selected for this test because it is one of the most common fungi found on wood exposed above ground (Verrall, 1966; Choi, 2004). The isolate used in this study was collected in 2003 from untreated decking at an FPInnovation's field test site. *O. placentus* was selected for this test because it is also commonly isolated from wood above-ground in Canada. The isolate used in this study was obtained from a leaky wood-frame condominium in the Vancouver area. These two fungal species were tested against two formulated products and one adjuvant (amine oxide), at four

different treatment levels and replicated four times for a total of 72 samples to be assessed for basidiospore germination.

Spores of *O. placentus* and *G. sepiarium* were collected from actively sporulating cultures on MEA. Fruiting bodies were produced *in vitro* following the methods of Choi *et al.* (2001a). Sterile 20 % glycerol was used to collect spores from the Petri-dish lids of inverted fruiting cultures. Glycerol was used to increase the surface tension of the spore suspension that was applied onto the check surfaces; the increased surface tension prevented the spore solution from leaking off from the wood surface and contaminating the surrounding cotton.

A liquid suspension of basidiospores was inoculated directly onto check surfaces recovered from exposed materials and monitored for germination. Spore suspensions of *O. placentus* or *G. sepiarium* were applied onto check surfaces resting on polypropylene mesh screens inside the Falcon tubes. Ten microlitres of the spore solution was used to achieve approximately 2400 spores on each check surface. The spore suspensions were vortexed and the concentration of spores per milliliter of solution was estimated using a haemocytometer. Spore solutions were diluted with 20 % glycerol to a concentration of 2.5×10^5 spores ml⁻¹. The viability of the spores was determined by inoculating plates of MEA with 1 mL of the spore solution. The spores were considered viable when germination on the MEA occurred within 72 hours.

After inoculation with spore suspensions the tubes were incubated at 25°C and aerated in a sterile flow bench once per week. The check surfaces were monitored weekly for evidence of spore germination.

4.2.3 Check Surface Sample Collection for Fungal Diversity Study

Checks were sampled aseptically from wood samples exposed outside for one year. A ruler, microtome blade, and a chisel were used to divide the check surfaces into an upper and lower surface. The upper check surface area ranged from the surface of the board to 2-3 mm below the surface. The lower check surface was collected from the region of the check \geq 3 mm from the surface of the board. The lower check surface sample was collected beyond the zone of chemical penetration; this region of the check was not treated before exposure. The depth of penetration

was different for each sample, but 120 out of 128 samples had a shallow penetration (\leq 3 mm into the wood).

Two samples, an upper piece and a lower piece (Fig 3.5), were collected from each check surface from 25 P4-treated boards and 25 P5-treated boards. The upper and lower pieces of each check were plated on 1% MEA amended with chloramphenicol to prevent the growth of bacteria. After one week, the plates were assessed to determine how many different fungal species were present. This initial assessment was performed based on colony morphology. Unique fungal species derived from the check surfaces were sub-cultured onto new MEA medium. Fungal cultures were separated into colony morphology categories. Plates from each grouping were selected, and slides were prepared to determine spore structures using light microscopy and hyphal morphology. One culture from each group was prepared for DNA analysis. Cultures with ambiguous or unknown structures were also analyzed using DNA analysis.

A quick DNA analysis method was used to survey fungal isolates collected from the exposed treated wood after one-year. No further attempt to positively identify these cultures was pursued. Mycelial tissue was aseptically collected from pure cultures and frozen at -20°C until the DNA was extracted. Fungal DNA was extracted using method described by Al Samarrai and Schmid (2000) with two modifications: RNAase was not used in the extraction and NaCl was used to precipitate DNA, in both precipitation steps, to a final concentration of 0.2 M. The internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal DNA were amplified with fungal primers ITS1 and ITS4 (White et al. 1990). The reaction volume was a total of 50 µl. The reaction contained 1X GoTag PCR buffer (Promega Corporation, USA), 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.5 µM of each primer, 0.7 units of GoTaq polymerase (Promega Corporation, USA) and 1 μ L of DNA ranging from 20 to 200 ng/ μ L of DNA. The reactions were run in a thermal cycler programmed with the following 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. Agarose gel electrophoresis was used to separate DNA. The 1% agarose gel was stained with GelRed (Biotium Inc. USA) and visualized under UV light. The PCR products were sent to the Plateforme de Séquençage et de Génotypage des genomes, Centre de

Recherche du Centre Hospitalier Universitaire de Québec . The samples were purified by glass fibre filtration on a Biomek Fx (Beckman Coulter, USA) and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Species were identified by comparing the unidentified ITS sequences with the sequence data available in Genbank (US National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool) searches (Altschul *et al.* 1990)

4.3 RESULTS

4.3.1 Basidiospore Germination on Exposed Check Surfaces

Table 4.1 summarizes the basidiospore germination of *O. placentus* (OP) and *G. sepiarium* (GS) on the check surfaces treated with P4 (DDAC), P5 (tebuconazole) or amine oxide. The spore germination test was monitored for 5 weeks, but no further spore germination occurred after the fourth week of observation. Therefore, the results obtained in week 4 and 5 are identical.

Table 4.1 Basidiospore Germination on Exposed Check Surfaces Treated with P4 (DDAC) or P5(tebuconazole).

		Week 1		Week 2		Week 3		Week 4		Week 5		
Formulation	Treatment	Total AI* (µg/g)	OP	GS	OP	GS	OP	GS	OP	GS	OP	GS
1279-23 (Tebuconazole)	Control	0.2 ± 0.09	1/4	1/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4
	Low	5 ± 3	0/4	0/4	3/4	3/4	3/4	4/4	4/4	4/4	4/4	4/4
	Medium	53 ± 19	0/4	0/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4
	High	152 ± 39	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
FIM-1 (DDAC)	Control	0.3 ± 0.4	1/4	1/4	3/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4
	Low	57 ± 52	1/4	0/4	3/4	1/4	4/4	1/4	4/4	3/4	4/4	3/4
	Medium	160 ± 60	0/4	1/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
	High	270 ± 70	1/4	1/4	3/4	2/4	3/4	4/4	4/4	4/4	4/4	4/4
	Amine oxide Reference		2/4	0/4	4/4	3/4	4/4	3/4	4/4	4/4	4/4	4/4

OP =*Oligoporus placentus*; GS=*Gloeophyllum sepiarium*. X/Y: X= number of samples that germinated; Y=total number of replicates. *Total AI is only relevant to the medium and high treatment levels. It represents the sum of the original active ingredient on the check surface and the spiked concentration of active ingredient added to the check surface.

Samples used to represent the low concentration treatment levels for tebuconazole and DDAC were selected based on the concentration found on the matched check surface samples. Note the average total AI for these selected samples differs from the average total for the entire sample set

in Table 3.1. The low treatment levels are estimated concentrations used to indicate whether active ingredient found on un-amended (not spiked) check surfaces from pressure treated P4 and P5 material would prevent spore germination. The average concentration of chemicals extracted from samples used in the "low" treatment category was 0.005 ± 0.003 mg/g wood for tebuconazole and 0.057 ± 0.052 mg/g wood for DDAC. The medium and high treatment levels were prepared by spiking exposed check surfaces with known amounts of the active ingredient prepared from the formulated product. The total active ingredient on the medium and high check surfaces is more accurate since the biocides were spiked from a solution with a known concentration.

After 3 weeks, spore germination was complete on all control check samples. Spores of *G. sepiarium* and *O. placentus* germinated well on the low concentration check surfaces after 2 weeks. After 4 weeks, spore germination was complete on all but one replicate of the check surfaces at the low concentration.

Spores on the reference samples, amended with the 1% solution of amine oxide, were also fully germinated after 4 weeks. At a concentration of 1%, the amine oxide did not affect spore germination. In the presence of 1 % amine oxide, both *G. sepiarium* and *O. placentus* germinated in the same time frame as the control samples – between 3 and 4 weeks after inoculation.

After 4 weeks, spore germination was observed on one out-of four replicates treated with 1 μ g of tebuconazole. Spores did not germinate on samples treated with 2 μ g of tebuconazole. The toxic threshold limit for spores of *G. sepiarium* and *O. placentus* towards tebuconazole was 0.034 - 0.191 mg/g wood when applied as a solution of P4 (tebuconazole). As shown in Figure 4.3, the check surfaces spiked with P5 (DDAC) did not prevent spore germination. The medium (0.16 ± 0.06 mg/g) and high (0.27 ± 0.07 mg/g) levels of DDAC did not prevent spore germination of *G. sepiarium* or *O. placentus*. Spores on check surfaces that were spiked with medium and high concentrations of FIM-1 germinated in the same time frame as the control samples – 3 weeks. The toxic threshold limit for DDAC against spores of *G. sepiarium* or *O. placentus* is greater than 0.27 ± 0.07 mg/g. An additional test to determine the toxic threshold was not carried out

because the concentration tested was already 10 times greater than the average DDAC found on the untreated check surfaces.



Figure 4.3 Pictures of germinated *G. sepiarium* spores after 4 weeks of incubation at 25°C on DDAC treated samples. (A) Control sample (B) Low concentration $(0.057 \pm 0.052 \text{ mg/g})$ (C) Medium concentration $(0.16 \pm 0.06 \text{ mg/g})$ and (D) High concentration $(0.27 \pm 0.07 \text{ mg/g})$.

4.3.2 Fungal Diversity on Exposed Check Surfaces

The fungal species found in this study are listed in Table 4.2. There were eight different ascomycetes: Sydowia polyspora, Pestalotiopsis sp., Leptosphaerulina chartarum, Trichoderma viride, Arthrinium sp., Epicoccum nigrum, Aureobasidium pullulans, and Alternaria alternata. Most of these fungal species are commonly found on grasses, trees, or dead plant material. This is likely due to the presence of surrounding trees and grass at the test site. The fungal species isolated from the treated wood indicated that after one year the wood was not colonized by any fungi involved in wood deterioration. Choi (2004) exposed decking samples at the same test site used in this study and after one year she also reported the presence of Trichoderma viride, Arthrinium sp., Epicoccum nigrum, Aureobasidium pullulans, and Alternaria alternata. Choi (2004) also isolated some fungi that were not found in this study such as *Penicillum spp.*, Phialophora sp., Acremonium kiliense, Cladosporium cladosporioides, Curvularia harveyi, Fusarium sp., Oedocephalum sp., Ophiostoma piceae, Phialemonium dimorphospora, Phoma spp., Trametes versicolor, Sistotrema brinkmannii, and a few unidentified ascomycetes. It is possible that the different ascomycetes isolated from the treated wood in this study and in the work by Choi (2004) was because Choi treated materials with CCA instead of DDAC or tebuconazole. However, there is no evidence to support this possibility, and it is more likely that the different number of samples taken in each study, as well as the sampling locations on the boards, resulted in these observed differences. In the current study, wood samples were only

collected from the check surfaces which are less accessible to spores compared to the board surfaces that were sampled in the study conducted by Choi (2004).

Five fungal species that were identified were isolated from both P4 and P5 treated wood. Exceptions included *S. polyspora* which was isolated from P4 treated wood but not P5 treated wood, as well as *L. chartarum*, and *Arthrinium sp*. that were isolated from P5 treated wood but not P4 treated wood. The fungi selectively isolated from either P4 or P5 treated wood: *S. polyspora*, *Pestalotiopsis sp*., and *Arthrinium sp*., are all known plant pathogens. These fungi are not known to be potential wood colonizers and are unlikely to be a problem in terms of protecting the wood from deterioration.
Table 4.2 Identity and distribution of fungi found on check surfaces after 12 months exposure. The number of times each fungus was found was tallied and split according to the type of treated wood and the depth of the check surface sampling location. Upper check surfaces = 0-3 mm and lower check surfaces=3-6 mm. Twenty-five checks were sampled from each formulation, so this is the maximum number of occurrences possible.

Closest Fungal	GenBank	Potential Identity		Source	Sample Depth	Frequency of Isolation	
Match	Number	(%)	Family	Isolate*	(mm)	Teb.	DDAC
Aureobasidium				DVBPG	0-3	18	21
pullulans	GQ911532	99	Dothidiomycetes	5028	2.6	17	10
Black-stain on wood					3-0	17	18
Alternaria alternata		00	Dathidiamusatas	5009	0-3	13	13
Plant pathogen/wood colonizer	RQ115752	99	Dothidiomycetes	3000	3-6	2	5
Arthrinium sp.	ADE66292	00	Sodariamusatas		0-3	0	1
Saprobe on dead material	ADJ00205	55	Souanomycetes	111030370	3-6	0	0
Sydowia polyspora				CBS	0-3	0	0
	GQ412724	99	Dothidiomycetes	248.93			
Plant pathogen					3-6	1	0
Epicoccum nigrum					0-3	22	22
	AF455403	100	Dothidiomycetes	wb583			
Black-stain on wood					3-6	10	7
Leptosphaerulina				UBC	0-3	0	1
chartarum	DQ384571	99	Dothidiomycetes	F15184		_	_
Plant pathogen				10101	3-6	0	0
Trichoderma viride				NRRL	0-3	2	2
Mould on wood/Primary	GQ328858	99	Sodariomycetes	54021	26	1	0
					5-0	1	0
Pestalotiopsis sp.					0-3	4	/
maculiformans	EU552146	99	Sodariomycetes	CBS 119350	3-6	3	2
funera	EF055197	99					
funereoides	AY838893	99					

 * NRRL: Northern Regional Research Center, Agricultural Research Service Collection DBVPG: Collezione del Lieviti Industriali, Dipartimento di Biologia Vegetale Universita degli Studi di Perugia SC: Squibb Institute for Medical Research, New Brunswick, New Jersey, USA
Wb: Department of Bacteriology, University of Wisconsin, Madison, WI, USA
CBS: Central bureau voor schimmelcultures, uppsalalaan 8, 3584 CT, The Netherlands
UBC: University of British Columbia Herbarium

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

*Tebuconazole treatement is formulation 1279-23 and DDAC treatment is formulation FIM-1

Fungal isolates that were sub-cultured from wood treated with P4 and P5 expressed different fungal morphologies when grown on MEA. This occurred most often with isolates of *E. nigrum* which was most frequently isolated from the upper check surfaces. Despite morphological differences, these isolates were shown to be the same based on a closest match DNA comparison using a BLAST search. *E. nigrum* was isolated from 22 out of 25 upper check surfaces sampled from both P4 and P5 treated wood. The second most frequently isolated fungus was *A. pullulans*; this black stain fungus was isolated from both the upper and the lower check surfaces with almost the same frequency. On P4 treated wood, *A. pullulans* was found on 18 of the upper and 17 of the lower check surfaces. Wood colonized by *A. pullulans* leaves a distinct black stain that is visually unpleasant to consumers. The black stain only affects the physical appearance of the wood; it does not destroy any of the structural properties of the wood so it is not a concern in terms of wood decay. After one year of outdoor exposure there were no basidiomycetes isolated from any of the check surfaces.

Some ascomycetes, such as *Trichoderma viride* have been shown to detoxify and degrade carbon-based preservatives such as tebuconazole (Obanda *et al.*, 2008). This is important because ascomycetes with the ability to detoxify treatments may reduce the efficacy of the carbon-based preservative treatments and leave the wood susceptible to premature attack by wood decay fungi such as basidiomycetes.

More fungi were isolated from the upper treated check surfaces (0-3 mm) compared to the lower untreated check surfaces (3-6 mm) based on the frequency of isolation. Seven out of the eight fungal species that were isolated in this study were found more frequently on the upper (0-3 mm) check surface compared to the lower check surface (3-6 mm). The one exception to this trend was *S. polyspora*, which was isolated only once from the lower check surface of a P4 treated board. The black-stain fungus, *A. pullulans*, was dominant on P4 and P5 treated material on both the upper and lower check surfaces. Higher isolation of fungal species from the upper check surfaces than on the lower check surface indicates that the lower check surface may not be as accessible to spores as the upper check surface. Since the lower check surfaces are very

minimally treated in the case of carbon-based preservative treatments, the fungal frequency on the lower check surface is important. Since spores of ascomycetes were found on the lower check surfaces, a longer exposure time may result in spores from basidiomycetes accessing the lower check surfaces as biological succession continues. Given time, the combination of the lack of preservative treatment on the lower check surfaces and the accessibility of spores to these surfaces could eventually lead to pre-mature failure of the wood.

4.3.3 Visual Examination of Exposed Wood Surfaces

The condition of the treated boards was inspected monthly for signs of stains, moulds, and fungal attack. After 7 months of outdoor exposure there were no signs of any micro-organisms on wood treated with P4 or P5. However it is very difficult to detect fungi when growth is not abundant; and intensive sectioning and staining for fungi was not performed. Figure 4.3 (A, B) illustrates the appearance of matched P4 treated wood samples exposed for 7 and 12 months. After 7 months of exposure, the wood surfaces appeared bleached and showed no signs of staining discoloration. The bleaching effect was likely due to sunlight exposure, since the top surface was bleached, but the bottom surface was not. After 12 months of exposure, the surface of the treated boards turned a grayish-silver color indicative of weathering due to delignification of the wood. Figure 4.3 (C) shows a P4 treated board with exposed check surfaces. The surfaces of the checks are discolored due to detritus, and the inner wood that was never exposed to weathering has not changed color. It is possible that the discoloration of the check surfaces is a result of bacterial agents, but the check surfaces were not analyzed for bacteria so this possibility cannot be ruled out.



Figure 4.4 Visual inspection of the exposed treated wood did not show any indication of stains, moulds, or fungal attack. Board A was collected after 7 months of outdoor exposure and sample B was collected after 12 months of outdoor exposure. Sample B is a grayish-silver color as a result of weathering. The difference between exposed and unexposed wood is illustrated in C.

4.4 DISCUSSION

The results from the bio-assay show that the redistributed active ingredients found on the check surfaces of P4 and P5 treated wood were not effective at preventing spore germination of two common decay fungi isolated from decking installed out-of-doors and above-ground in Canada. Spores of *G.sepiarium* and *O. placentus* germinated on un-amended (not spiked) check surfaces within 2 weeks on samples collected from pressure treated P4 and P5 material exposed outdoors for 12 months. After 4 weeks, all of the un-amended check surfaces were covered with fungal hyphae, indicating that spore germination was successful. The toxic threshold for tebuconazole was found to be 0.034 - 0.191 mg/g wood when applied as a solution of P4. This is approximately 10-100 times greater than the average concentration that was found on check surfaces of treated P4 wood are not protected against spore germination of some common fungi isolated from decking above-ground. The redistributed active ingredient from P5 treated material was unable to protect the untreated check surfaces from spore germination. The toxic threshold limit for DDAC against spores of *G. sepiarium* or *O. placentus* is greater than 0.27 ± 0.07 mg/g. Even with 0.27 mg DDAC/g of wood, the spores were unaffected.

Since higher initial solution concentrations did not change the amount of redistributed active ingredient recovered from the check surfaces, it is unlikely that further increases to the initial treatment solution concentrations would provide protection of the untreated check surfaces. At a concentration of 1%, the amine oxide solution did not affect spore germination. The amine oxide reference samples produced spore germination in the same time as the control samples. Since the concentration and proportion of amine oxide in the formulation was not disclosed during experimentation, it was not possible to test exactly the same concentration of amine oxide that was used in the formulations. On the other hand, it would be beneficial to test higher concentrations of amine oxide against spores, in case 1 % is below the toxic threshold for spores. This study was intended to simulate a "realistic" inoculation of check surfaces with spores, and since there was no literature available on the number of spores that land in checks, an approximation was made. Choi (2004) used 1×10^6 spores in an investigation of spore

germination on copper amended agar. Although millions of spores are released from each fruiting body, the geometry of a check makes it improbable that one million spores would be present. Instead, each check surface was inoculated with 2400 spores in a liquid suspension.

Ascomycetes isolated from the check surfaces of treated P4 and P5 materials are believed to have originated from resting spores. This is supported by two observations: (1) there were no visible hyphae observed on any of the check surfaces during sampling; and (2) check surfaces from exposed wood did not reveal any hyphal growth when the surfaces were placed in high moisture incubation chambers favourable to fungal growth. If undetected hyphal fragments were already present on the sampled check surfaces then favourable germination conditions should have promoted the growth of these fragments. However, after 3 months no growth was observed on the check surfaces.

Increased fungal isolation from the upper check surfaces (0-3 mm) compared to lower check surfaces (3-6 mm) suggests that spores may have better accessibility to the upper check surfaces. In addition to accessibility, the concentration of active ingredient on check surfaces, moisture content, availability of nutrients, water movement in checks, and presence of detritus may have also affected the frequency of fungal isolation from the check surfaces.

The movement of spores into the deepest part of the check was most likely mediated by rain water. Spores from the surface of the board, or the upper portion of the check, were probably dispersed onto lower check surfaces by water during rain events. Since water is easily trapped in the deepest portion of the check, spores that are washed into a check are likely to remain there until the water is evaporated or diffuses into the wood. Spores that get trapped in a check may begin to germinate when conditions are favourable. If spores germinate in a check and fungal hyphae become established in a poorly protected part of the wood, such as a check, then this puts the treated wood at risk of decay.

The movement of spores from the upper check surface to the lower surface is supported by the matching identities of the fungal species found at both sampling depths. The fungi in this study were isolated by simply placing the sampled check surfaces onto MEA. Therefore, this study reports the identity of fungal spores that were able to enter the checks and remain on these surfaces. This study does not report on which fungi were found on the interior of the wood. A

study where the fungi are cultured from beneath the wood surface would indicate whether fungi were able to penetrate the surface layer and gain access to the sub-surface layers. This type of sampling is useful when biological succession has proceeded to the stage where wood-decaying basidiomycetes may be present in the wood. After only 12 months of outdoor exposure, mainly early colonizers are present on the wood. However, some of these early colonizers, including black-stain fungi such as *Epicoccum purpurascens*, are able to reduce the concentration of carbon-based preservatives, including propiconazole and tebuconazole, in treated wood (Stirling and Morris, 2010). An important implication of the preservative degradation by early colonizers is the potential for more rapid colonization of carbon-based preservative-treated wood by decay fungi. If a longer field test was performed, then sub-surface sampling would have been appropriate.

A similar wood decking study involving two commercial carbon-based preservative formulations (Dale and Morris, 2010a) investigated the fungal colonization of decking exposed for 3-4 years at a field test site at the University of BC's Malcolm Knapp Research Forest. Dale and Morris (2010a) sampled wood in the field from boards showing early signs of decay. In addition, the wood samples were collected from just beneath the surface of the boards. This method of sampling was chosen to minimize surface contaminants, including resting spores, and ensure that only fungi in the interior of the wood were sampled. Not surprisingly, five species that were identified in the inner wood by Dale and Morris (2010a) were also present on the check surfaces: *E. nigrum, A. pullulans, T. harzianum, Arthrinium sp.* and *Sordariomycete sp.* The presence of these fungal isolates on the surface of checks in the first year of this study, and in the inner wood 2-3 years later in a similar study suggests that checks are likely avenues for spores to access the interior of treated wood, as previously suggested by Choi (2004).

4.5 SUMMARY

The redistributed DDAC and tebuconazole on exposed check surfaces of spruce shell treated with concentrations of active ingredients recommended by the suppliers was not effective in preventing spore germination of *O. placentus* or *G. sepiarium*. The toxic threshold limit for DDAC against spores of *G. sepiarium* or *O. placentus* is greater than 0.27 ± 0.07 mg/g. Check surfaces from P4-treated material did not prevent spore germination. The toxic threshold for tebuconazole was found to be 0.034 - 0.191 mg/g. In 4 weeks, all of the check surfaces from the exposed materials were covered in fungal mycelium. Only ascomycetes were isolated from the exposed check surfaces, perhaps due to the short duration of the field exposure.

CHAPTER 5: CONCLUDING REMARKS AND FUTURE WORK

5.1 GENERAL CONCLUSIONS

The primary aim of this project was to determine whether the carbon-based active ingredients in wood formulations provide protection when used in shell-treatments and exposed outdoors and above-ground. Specifically, the experiments in this thesis were designed to assess whether tebuconazole and DDAC are mobile, and if they can be redistributed and re-deposited in the wood after treatment when exposed outdoors. The issue of chemical mobility is of interest because it has been proposed that this is one one of the mechanisms by which CCA protects check surfaces in shell treated wood (Choi *et al.*, 2004).

After only one month of exposure, DDAC and tebucoanzole were detected in the leachate of the treated samples after rain events. After 12 months of exposure, 4.5-7.7% of the DDAC retained after treatment was lost in the leachate, and 22.5-30% of tebuconazole retained after treatment was lost in the leachate for low and high retentions, respectively. Both biocides were found to be mobile in wood after pressure treatment when exposed outdoors.

After 12 months of exposure, DDAC was detected on untreated check surfaces in very low concentrations: 0.02-0.03 mg/g wood. However, the amount of tebuconazole detected on untreated check surfaces was not different from the control samples: 0.002-0.008 mg/g wood. The amount of active ingredient found on the check surfaces did not differ between the high and the low retentions for P4 or P5. Furthermore, there was no significant difference in the amount of chemical found on check surfaces for the two exposure periods: 7 months and 12 months.

A bio-assay was used to test whether the untreated check surfaces could be expected to protect against spore germination. The check surfaces were inoculated with spores from two fungi that commonly occur on wood above-ground: *O. placentus* and *G. sepiarium*. The study showed that the originally untreated check surfaces, from treated material exposed for 12 months, were susceptible to spore germination of *O. placentus* or *G. sepiarium*. Within 2 weeks, visible spore germination was observed on untreated check surfaces from P4 and P5 exposed material. After 4

weeks, the spore germination was complete on all but one replicate. The toxic threshold limit of DDAC for preventing spore germination of *G. sepiarium* or *O. placentus* is greater than 0.27 mg of DDAC/g wood and the toxic threshold limit of tebuonazole for preventing spore germination was found to be 0.034 - 0.191 mg tebuconazole/g wood.

In order to determine which local fungi might pose a threat to the untreated check surfaces, the fungal spores on the exposed check surfaces were identified through culturing. Eight different ascomycetes were identified from the upper and lower check surfaces. Most of the same ascomycetes were isolated from the upper check surfaces and the lower check surfaces and more fungi were isolated from the upper surfaces than the lower surfaces. Despite the presence of spores, and the susceptibility of the untreated check surfaces, the wood treated with P4 and P5 did not show any visual signs of stains, moulds, or fungal colonization over the course of the 12-month field test.

5.2 IMPLICATIONS

Currently, there is no penetration requirement for the treatment of sawn wood used in above ground applications. This means that shell-treated wood will be exposed outdoors with variable chemical penetration. In some of these materials, checks will undoubtedly form in the wood and extend beyond the limit of chemical penetration. If the wood is shell-treated with a formulation containing one of the carbon-based biocides: tebuconazole or DDAC at the concentrations used in this study, then the untreated surfaces exposed by checking will not be protected from spore germination. Spore germination is the most common form of infection above-ground, and has been shown to cause infection in wood (Morrell, 1996; Morris *et al.*, 2009). The untreated check surfaces in material shell-treated with a single biocide (tebuconazole or DDAC based) will be susceptible to fungal infection via spore germination. After spore germination on the untreated check surfaces, it is likely that fungal hyphae will begin to penetrate in the interior of the wood if given enough time.

Results for DDAC and Tebuconazole formulated in commercially produced emulsions suggests suggests that the wood protection industry needs to amend the carbon-based preservative formulations to ensure that treated wood surfaces are protected when exposed outdoors.

5.3 POSSIBLE SOLUTIONS

Wood treated with solely carbon-based preservative formulations used as thin-shell treatments in above-ground applications may be susceptible fungal colonization and early failure in service. If the industry intends to move towards carbon-based preservatives, then it is important to understand the issues that affect existing carbon-based formulations.

It is unlikely that the toxic thresholds necessary to achieve protection of the check surfaces can be reached by simply increasing the chemical retentions of the treated wood at least for spruce where the penetrated zone is extremely thin. However, higher initial solution concentrations than those used in this study should be tested to determine whether the amount of chemical redeposition onto check surfaces is increased.

One possible solution, which slightly deviates from an entirely non-metal system, is to add a moderate amount of copper to the carbon-based preservative systems. Copper has been shown to redistribute in the wood after treatment, during exposure, for three different formulations: CCA, ACQ, and MCQ (Choi, 2004; Chung and Ruddick, 2004; Ruddick, 2009; Stirling and Morris, 2010). On shell-treated CCA material, 0.35 mg Cu/g wood was present on check surfaces after one year (Choi, 2004). Ruddick (2008) found that shell-treated ACQ material averaged 0.49 mg Cu/g wood after six months; and most recently, Stirling and Morris (2010) showed that 0.99 mg Cu/g wood from a micronized copper system (MCQ) redistributed onto check surfaces after 1 year. The concentrations of copper found on the check surfaces from the copper-containing formulations are above the threshold (0.27 mg copper/g of wood) required to inhibit germination of *O. placentus* and *G. sepiarium* basidiospores (Choi, 2004). Copper has a broad spectrum of activity, a low mammalian toxicity, and it is an important micronutrient found in trace amounts in soil. If low concentrations of copper were added to carbon-based formulations as a co-biocide, then untreated check surfaces may be protected by the mobile copper ions which have a high affinity for re-attachment to reactive sites in wood. The addition of copper at low

concentrations may still allow the material to be recycled through chemical remediation using sulphuric acid leaching which extracts 99% of the copper from wood (Janin *et al.*, 2008).

If solely carbon-based formulations are going to be used in the industry, then a multi-component biocide system may be necessary. The ideal formulation would combine a chemical that is effective in inhibiting spore germination with other carbon-based biocides. Combining carbon-based biocides to take advantage of possible synergistic effects is one possible solution that is currently being used (Buschhaus and Valcke, 1995; Clausen and Yang, 2007). However, more than synergistic effects are needed to overcome the issue that is associated with the low redeposition of carbon-based preservatives on untreated check surfaces.

Preventing checks from developing in wood in the first place would eliminate the issue surrounding the mobility and re-attachment of carbon-based preservatives. Fundamental work to prevent the physical formation of checks in wood exposed outdoors has been actively studied by Evans *et al.* (2003, 2008). To prevent photodegradation of wood components by UV and visible light during exterior exposure, photostabilizers are being used to absorb or reflect UV to help prevent the wood from checking. In addition, incorporating surface water repellents, such as oils or waxes, into formulations can minimize moisture gradients and reduce the number of checks that form. Surface profiling has also been found to reduce checking (McFarling and Morris 2005, Morris and McFarling 2008).

The industry must continue to develop innovative and practical solutions to deal with the refractory nature of Canadian wood species. One option is to select the more treatable species for treated wood products. Incising is proven to increase chemical penetration, and this may be one of the possible solutions for protecting wood shell-treated with carbon-based formulations. Some consumers dislike the aesthetics associated with wood that has been through a toothed-roller. Thus, the industry may have to turn to methods of incising that do not visibly perforate the surface of the wood – such as bio-incising. Studies have shown that certain fungal isolates can increase heartwood permeability to preservative treatments by preferentially degrading the pit membranes in the heartwood (Rosner *et al.*, 1998; Schwarze *et al.*, 2006). Additional work by Schwarze and Schubert (2009) includes a European Patent (05027812.6) for methods which use the white-rot fungus, *Physisporinus vitreus*, to improve permeability of refractory wood species.

Increasing European momentum in favor of bio-incising, as well as interests and efforts from Canada (Dale and Morris, 2010b) suggest that the use of bio-incising as an industrial process may be possible.

5.4 PROJECT LIMITATIONS AND SCOPE

This study must be considered with certain experimental limitations in mind. These limitations define the scope of this study, and are discussed here to put the results in context of their real-life application. When the leaching study was conducted, the material was placed outdoors in December. The timing of the outdoor exposure for these decking boards was not ideal. Chemical depletion from the boards occurred for approximately 2-3 months before sufficiently deep checks extended into the wood surfaces. The ideal situation would have been to install the decking in spring or summer, as is common practice. This would have allowed 3-4 months for checks to form in wood before chemical leaching from the surface started. If the checks were open during the full duration of chemical leaching, then this may have increased the concentration of active ingredients recovered from the check surfaces. Alternatively, there could be a limited number of reactive sites on the wood available to react with tebuconazole or DDAC, which could also explain the low concentrations detected on the surfaces.

In addition, where conclusions or discussions regarding "carbon-based active ingredients" are mentioned, the intent is not to generalize all the carbon-based active ingredients that exist. Instead, these remarks only refer to the active ingredients that were tested here: tebuconazole and DDACarbonate. The fungal species that were used in this study were chosen to highlight the practical implications of chemical redistribution in treated wood out-of-doors above ground. Spores from other fungal species need to be tested against the concentrations of active ingredient found on the check surfaces to determine if they are able to germinate. Without performing these tests, it is uncertain whether spores from other fungal species could pose a threat to the untreated exposed wood surfaces. Moreover, the conclusions provided in this document are limited to the particular chemical loadings that were originally applied to wood, as suggested by the chemical suppliers. In this study, the higher chemical retentions did not result in more redistributed

chemical on the check surfaces. However, to ensure that higher chemical loadings do not have an impact on redistributed active ingredient, more chemical retentions must be tested.

5.5 FUTURE WORK

Future work related to this area of research include the following investigations: (1) a study which determines the minimum amount of copper or other carbon-based preservative that must be added to a carbon-based formulation to protect untreated check surfaces; (2) a study which determines the chemical mobility and redistribution of propiconazole used in shell-treatments; and (3) a study which looks at the role that amine oxide plays in the leaching of the carbon-based preservatives. This can be done by monitoring the concentration of amine oxide in the leachate over the course of the exposure and correlating the loss with the loss of active ingredient.

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APPENDIX I.

PENETRATION OF DDAC VISUALIZED USING BROMOPHENOL BLUE INDICATOR









APPENDIX II.

PENETRATION OF TEBUCONAZOLE VISUALIZED USING BROMOPHENOL BLUE INDICATOR









