DEVELOPMENT OF A NOVEL ANTISAPSTAIN PRODUCT

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Abstract

As a biodegradable product, wood is vulnerable to attack by microorganisms that can degrade it and cause loss in value. To prevent their colonization, wood can be protected by the application of chemical preservatives. Environmental and economic pressure on currently used chemicals has led to a situation where considerable effort is now directed toward the development of new preservatives. Among the most promising products are products that contain alkylammonium compounds (AACs). Based on laboratory tests, didecyl dimethyl ammonium chloride (DDAC) has been described as one of the most effective AAC used as wood preservatives. However, there were situations in the practice, where AACs had problems. Despite investigations into the reasons for these problems, no clarification was accomplished. This thesis describes the process of the development of a new wood preservative that would, while improving the characteristics of AACs, meet the objectives of efficient control of fungal growth, low toxicity, less potential for environmental impact, and easier disposal of treated products withdrawn from service. The project was divided into five general phases.

The experiments of the first phase were designed to test several potential fungicides against moulds and stains in order to eliminate less promising formulations. In Phase 2, the research was extended to other groups of fungi, such as soft rot, brown rot, white rot and DDAC tolerant species. In both phases tests were done on agar plates.

In Phase 3, the most promising formulations from Phase 1 and Phase 2 were tested on a wood substrate to determine the minimum amount of preservative that would be effective against sapstain fungi and moulds. In Phase 4, active components and additives were combined together in larger volumes and product formulation stability was investigated. The potential product solution was then submitted for toxicological testing. In the last (fifth) phase, the relation between efficacy and the required coverage and retention were examined by a simulation of real spraying conditions in wood treatment pilot plant. Samples of lumber were sprayed in the pilot plant with the test product and then stored outside in the yard to see if moulds, fungi or sapstain would develop.

The results of this thesis relate to a synergistic wood preservative composition comprising a quaternary ammonium compound and an additional second active component. The laboratory work on agar plates and the wood substrate, as well as field tests after pilot-plant spraying, have shown that the new product works. It has proved its capability for controlling a large spectrum of wood destroying microorganisms and counteracting the phenomena of the development of fungal resistance toward the applied active components.

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Abbreviations

AACs	Alkylammonium compounds
CCA	Chromium Cooper Arsenate
CRC	Carcinogenicity Peer Review Committee
Cu-8	Copper-8- quinolinolate
D. Fir	Douglas Fir
DDAC	Didecyl-dimethyl-ammonium chloride
EPA	Environmental Protection Agency
H.Fir	Western Hemlock or Hem Fir
HPLC	High Pressure Liquid Chromatography
IPBC	3-iodo-3-propanyl butyl carbamate
NP-1	Commercial Antisapstain Product
PCDD	Polychlorinated-p-dioxins
PCDF	Polychlorinated dibenzofurans,
PCP Number	Pest Control Products Registration Number
PCPs	Polychlorophenols
PMRA	Pest Management Regulatory Agency (PMRA) of Health Canada
S.I.	Synergy Index
TBTO	Tributyltin oxide
TCMTB	2-thiocianomethylthio benzothiazole
TCP	Tetra Chlorophenol

Acknowledgement

Chapter 1: Introduction

Wood is well known to be one of the most useful raw materials for a large diversity of applications. It is a cellular, fibrous biopolymer, made up of cellulose, hemicellulose, and lignin that is stable and maintains its main characteristics over long time duration. Because of its unique properties, availability in large quantities, its renewability, and its adaptability to modification treatments, wood is a material of ever increasing interest.

Today the national and international markets for high quality lumber are greater than ever. Meeting the demand for quality lumber requires attention to a number of key factors. One of these is the need to deliver lumber with the clean, clear appearance of freshly cut timber. In fact, marketplace demand is so strong and the competition so intense for this attractive lumber product, that even one shipment of discolored lumber can result in a major loss of sales.

During years of development, ways have been found to make wood more dimensionally stable, harder and stronger to meet specific needs. However, being of organic origin, wood components are readily degraded by microorganisms, insects, termites, and marine animals that attack wood for food, shelter, or both.

Perhaps the most serious threat to wood's high quality appearance is sapstain discoloration caused by moulds and fungi. Within a day of being cut, moulds and fungi that produce a mottled black discoloration can threaten the appearance of quality lumber.

Between cutting and delivery, these fast growing moulds and fungi often have both the time and the necessary environmental conditions to develop to disastrous proportions. Sapstain fungi feed and grow under moist, humid conditions. They can transform high quality lumber into spoiled, discolored shipments before they reach their destination. High demand fir and spruce are among the most susceptible species to such sapstain attack, especially when being transported through tropical conditions such as en-route to overseas markets via the Panama Canal, or during the lengthy Trans-Pacific ocean delivery process.

The history of sapstain protection via application of anti-sapstain chemicals is reviewed below. For many years sapstain was effectively controlled by application of polychlorophenols (PCPs). However the use of these has been abandoned because of their adverse environmental and health risks. This abandonment instigated a search for new, less risky anti-sapstain formulations. None of these new products has been able to meet all of the requirements for being completely safe and effective. Each has had its own specific problems. Some, to varying degrees, are toxic to fish or to humans. Others have failed to adequately protect the wood to which they were applied and some have even resulted in premature deterioration and downgrading of the lumber they were developed to protect. This thesis documents a research and development project aimed at discovering and proving the effectiveness of such a new anti-sapstain formulation.

1.1 The Lumber Industry and its Markets

The lumber industry includes the various businesses that convert trees, or timber, into lumber products. Lumber is produced from both hardwood and softwood. Wood from broad-leaved trees is called hardwood, and wood from cone-bearing trees is called softwood, regardless of its actual hardness. Many softwoods are actually harder than some of the so-called hardwoods. Most of the lumber harvested in the United States and Canada is softwood, including such species as southern yellow pine, Douglas fir, ponderosa pine, western red cedar, and the true firs. Most hardwood lumber is cut from species such as oak, gum, yellow poplar, maple, and ash, and is used for miscellaneous industrial applications including wood pallets.

The lumber industry is of significant economic importance. It is heavily dependent upon the health of the residential construction and household furniture industries. The sawn lumber softwood markets in 1998 and 1999 directly followed economic developments in North America, Europe, the former USSR and Asia. In North America consumption rose strongly and production reached record levels in 1998, but world trade was also active with a notable exception of a sharp decrease in export to Asia. European exports were also constrained by the Asian economic crisis but as European consumption remained high, exports rose (UN-ECE/FAO 1998/99).

According to the COFI Fact Book (1998), in 1996 the total world roundwood (including softwood and hardwood) harvest totaled 3.2 billion cubic meters. Industrial

production of hardwood and softwood, in 1996, totaled 1.5 billion cubic meters, accounting for 46.9 percent of the world total.

The Canadian lumber industry is based on an extensive, renewable resource. Canada's annual softwood timber harvest represents 14.5 per cent of the world total and ranks third after the United States and Europe. In 1996 British Columbia's portion of the world's softwood timber harvest was 6.7 %.

Canada is the world's largest exporter of softwood lumber. In 1996 Canada accounted for 51.7 per cent of the world's total export volume of 95.9 million cubic meters, see Figure 1. British Columbia is the largest contributor (57.6%) to Canada's softwood lumber exports representing 30% of the world total. As shown in Table 1, British Columbia plays a major role in Canadian softwood and hardwood production. Table 1 presents Canadian lumber production, in million board feet, of softwood and hardwood from 1988 until 1997:

Most of the United States' wood product industry is concentrated in the Pacific Northwest and the Southeast. Approximately one-third of the U.S. is forested. Of this forested area, two-thirds contain at least 20 cubic feet of commercially usable wood per year per acre. In 1998, United States production of softwood lumber rose to an eight-year high of 120 million cubic meters, while exports declined, primarily due to the Asian economic crisis. See Figures 2 and 3.
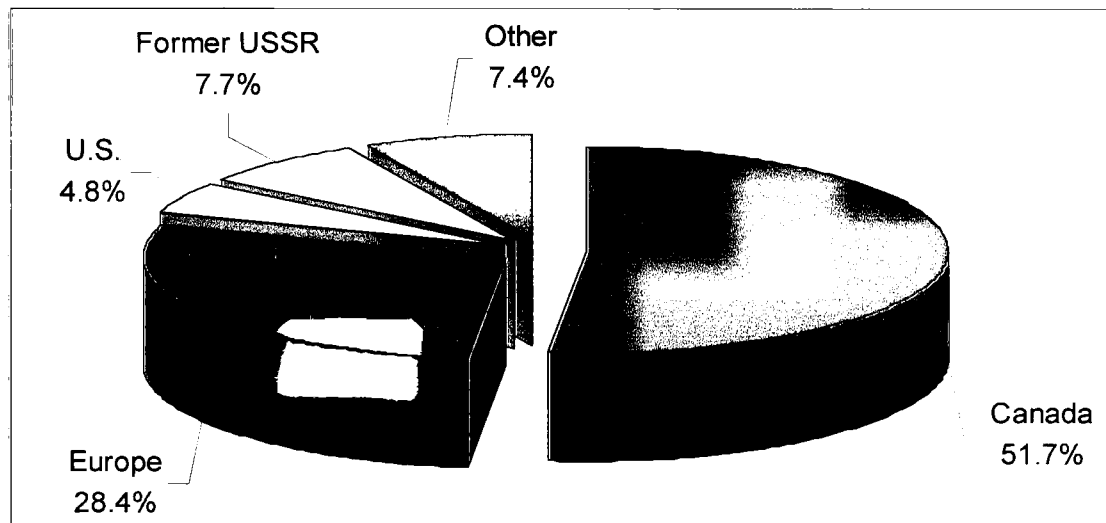
Figure 1. World Exports of Softwood Lumber: 1996

(Total for 1996: 95.9 million cubic meters)

Table 1. Canada Lumber Production - Softwood and Hardwood

Canada Lumber Production Softwood and Hardwood: 1988-1997 (Million Board Feet)											
Year	British Columbia			Alberta	Sask. &	Ontario	Quebec	New	Nova	Total	Total	Total
	Coast	Interior	Total		Mani-Toba			Brunsw.	Scotia	Soft-wood	Hard-wood	Canada
1988	4583	10989	15572	1584	333	2266	4470	727	215	25167	574	25741
1989	4140	11094	15234	1634	369	2083	4336	643	213	24512	523	25035
1990	3798	10400	14198	1618	321	1817	3879	621	218	22673	442	23115
1991	3465	9843	13308	1750	288	1719	3633	572	191	21461	413	21874
1992	3516	10625	14141	1815	273	1851	4197	669	210	23156	470	23626
1993	3585	10796	14381	1851	333	2117	5023	815	249	24769	469	25238
1994	3681	10588	14269	2041	350	2297	5741	937	312	25947	500	26447
1995	3313	10506	13819	2325	375	2367	5842	1040	325	26093	425	26518
1996	3387	10458	13845	2392	384	2363	6323	907	374	26587	437	27024
1997	3032	10344	13376	2553	389	2549	6603	1200	423	27092	354	27446

Figure 2. United States Annual Lumber Production of Softwood and Hardwood, from 1900 to 1998, (in 1,000,000 m^3):
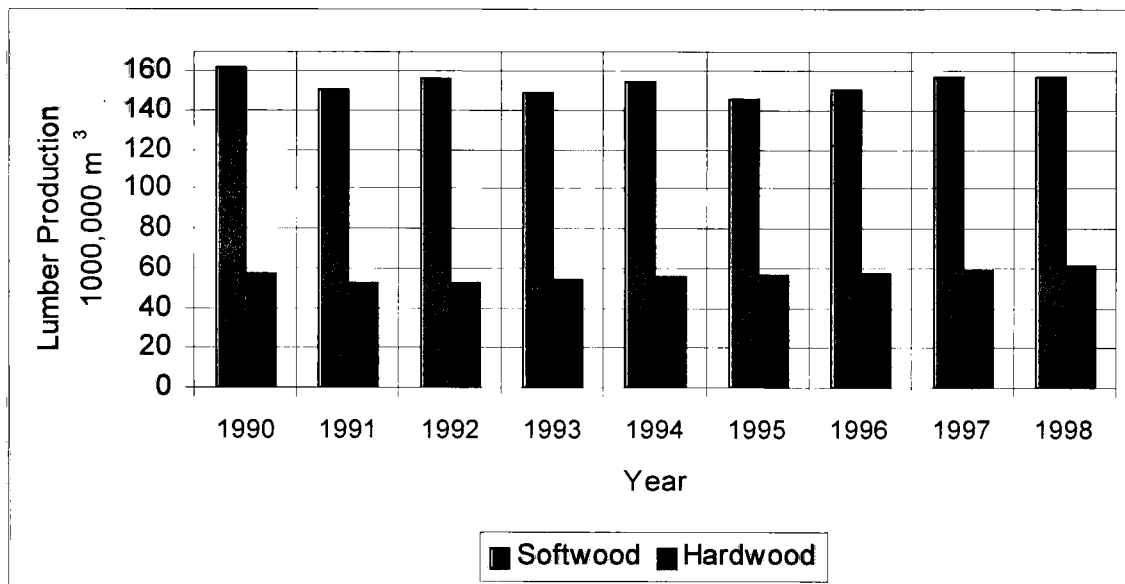
Figure 3. United States Annual Lumber Production of Softwood and Hardwood, from 1990 to 1998, (in 1,000,000 m^3):

According to FAO's, Forest Product Annual Market Review, 1998-1999, in the first four months of 1999 North American exports to Asia were recovering. For example, United States exports to the Pacific Rim moved up 5.1% in the first quarter of 1999 compared to 1998. The greatest increase appears with China, up to 19%. Table 2 shows U.S. softwood export by destination as it was in October 1999.

Table 2. US Softwood Lumber Exports by Volume (1,000 m³) - October 1999

Softwood Exports By Destination	Year to Date	Last Year to Date	Percentage Change
Western Hemisphere	2,969	2,373	25.1 %
Europe	866	822	5.5 %
Asia	1,461	1,397	4.6 %
Oceania	161	165	-2.5 %
Africa	12	10	14.7%
Total Softwood Exports	**5,469**	**4,766**	**14.17%**

The data in this section indicate the huge magnitude of the amount of lumber produced. Much of this needs to be protected against sapstain and other microbially induced deterioration. Thus there is a large market for protective chemicals.

1.2 The Wood Preservation Industry

Wood preservation extends the service life of timber products by providing effective long-term resistance to attack by fungi, bacteria, insects, and marine borers.

Furthermore, it reduces the need for harvest of already stressed forestry resources, reduces operating costs in industries such as utilities, railroads and construction, and promotes safe working conditions where timbers are used as support structures.

The successful preservation of wood by chemicals was first achieved on a commercial scale about a hundred twenty years ago, and since that time increasingly large quantities of timber have been treated with various wood preservatives (Cartwright et al., 1958).

There are two general classes of wood preservatives: oil and waterborne salts. The effectiveness of the preservatives varies greatly and can depend not only on their composition, but also upon the quantity applied to the wood, the depth of penetration, and the conditions to which the treated material is exposed in service. A great variety of substances have been suggested for use as wood preservatives. However, there can be no universally ideal preservative for all classes of timber, since the timbers to be treated and purposes for which treated wood is required, vary.

Through the sawmilling process, logs are cut into various forms of dimensioned lumber. Freshly cut green lumber, high in moisture content, sugars and starch levels provides an ideal environment for infestation by moulds, blue staining fungi, and/or wood destroying fungi. In industry terms, the sapwood of freshly cut, green lumber of most wood species is subject to fungal discoloration commonly known as "sapstain".

While these sapstain organisms usually do not cause considerable strength loss, they can sometimes reduce wood's structural value. Stained wood is generally not

recommended for structural purposes where strength is critical. In addition, conditions favorable for stain development are also conducive to decay initiation. Stained wood may also provide an ideal inoculum source for paint-disfiguring fungi (Cartwright 1958).

The individual hyphae of sapstain causing fungi are usually brown in color, however diffraction of light, to which they are exposed, results in an appearance often referred as "blue staining". Staining of this nature results in significant losses. Figure 4 illustrates some sapstained lumber; Figure 5 shows typical outdoor storage of fresh cut lumber.
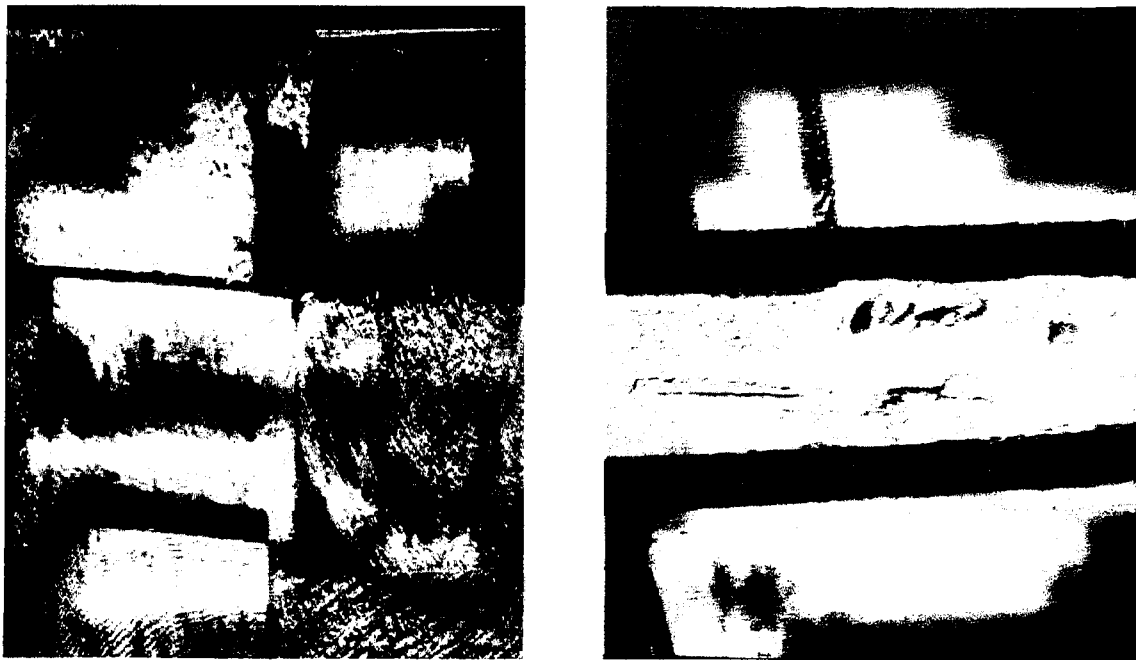
Figure 4. Examples of Stain and Mould Infected Lumbers

Figure 5. Fresh Cut Lumber (Typical settings at a lumber yard)

Sapstain organisms also substantially reduce the lumber's esthetic value and therefore its commercial value. The natural, clean unspoiled beauty of fresh sawn lumber is one of the biggest attributes in maximizing its commercial value and price. As a result, heavy emphasis has been placed on control of sapstain in freshly sawn lumber at the mill

Sawmills perform surface protection operations to protect the lumber against sapstaining that could occur during temporary storage and transit. All green lumber destined for export is protected. There are two principal fungal control processes that have been used in the North American sawmill industry to treat lumber for the control of the fungal sapstain growth. These two processes are drying processes and chemical treatments as described in the following:

Kiln and Air Drying - Since high humidity is a favorable condition for fungal growth, protection against it can be by drying the lumber to a moisture content of less than 20%. Kiln drying is utilized in some areas and air-drying in others, but these processes are not always practical or economical and can still result in some staining. Heavy rainfall during staging or high humidity conditions in the hold of a ship during transport may still cause kiln-dried lumber to become susceptible to stain and mould growth. Additionally, the cost differential between kiln drying lumber ($50-100.00/MFBM) and the use of the anti-sapstain formulations ($4-8.00/MFBM) strongly supports chemical anti-sapstain protection as the prevalent method of choice (Kanashevich, 1994).

Chemical Treatment - The most effective and economical way of protecting freshly sawn lumber against mold and fungus decay and discoloration is by the immediate treatment upon sawing with an effective anti-sapstain chemical applied to the wood's surface. Furthermore, for extra protection even kiln-dried lumber is sometimes treated with a chemical anti-sapstain treatment prior to shipping to foreign markets, especially if shipped under high humidity conditions in the hold of a ship.

1.3 Governmental Regulations and Registrations

All anti-sapstain pesticide products used for commercial purposes in both the United States and Canada must be registered with and by the appropriate governmental pesticide control agency in that country and be issued a Pest Control Products registration number (PCP Number). Each country has its own unique registration process and

operating regulations with which each applicant product must comply. A brief outline is as follows:

1. United States Procedure – In the United States all anti-sapstain products must be registered under the Environmental Protection Agency (EPA). The time frame for product acceptance and registration approval varies from three to nine months.

2. Canadian Procedure – In Canada all anti-sapstain products must be registered with the Pest Management Regulatory Agency (PMRA) of Health Canada. In Canada the registration process usually results in a significant cost. Application fees reflect the amount of work to be performed and the actual cost to conduct the examination that an application entails, and can run into the high tens of thousands of dollars. The minimum time frame for product acceptance and registration approval is one year.

1.4 Application Methods for Chemical Protection against Sapstain

A general description of anti-sapstain chemical application is illustrated in Figure 6.
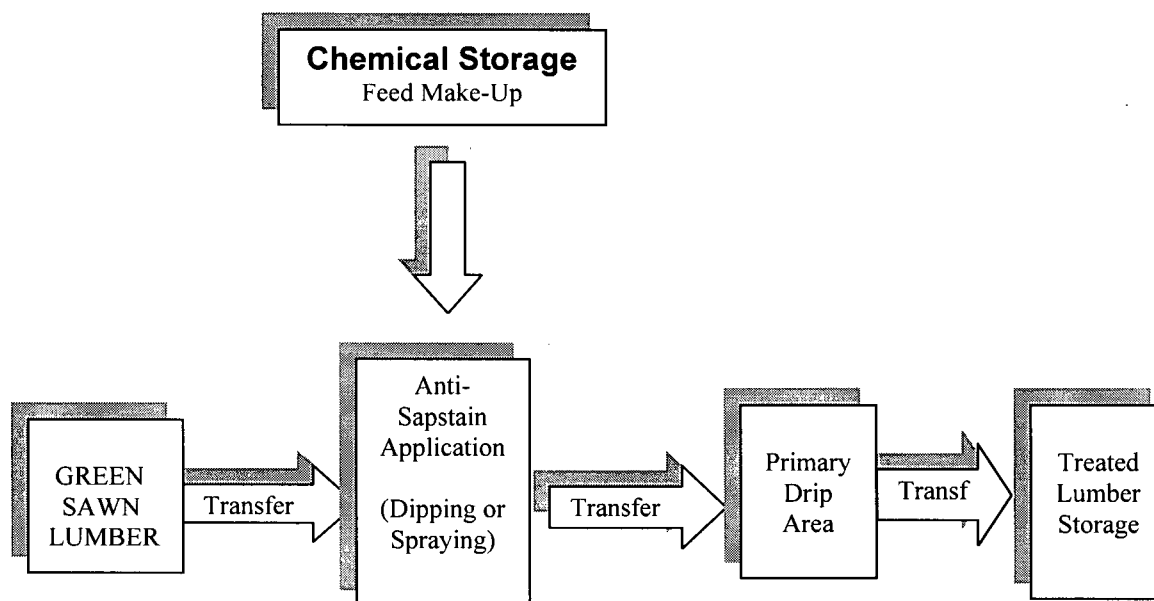
Figure 6. Anti-sapstain Process Steps for the Treatment of Fresh Sawn Lumber

According to information from Konashewich, (1994) the types and relative percentages of specific application systems that are in use in North America are shown in Figure 7 (c).

Most of the sawmills treat lumber by using Lineal Spray and Cross Chain spray systems for the application of anti-sapstain chemicals.

Variations in equipment types can be found in existing facilities, and are functions of:

- Site requirements

- Date of installation

- Recommendation of the chemical supplier, and

- Regulatory requirements

(a)   (b)

Figure 7(a & b). (a): Typical Spray Box; (b): Lumber Coming out from the Spray Box
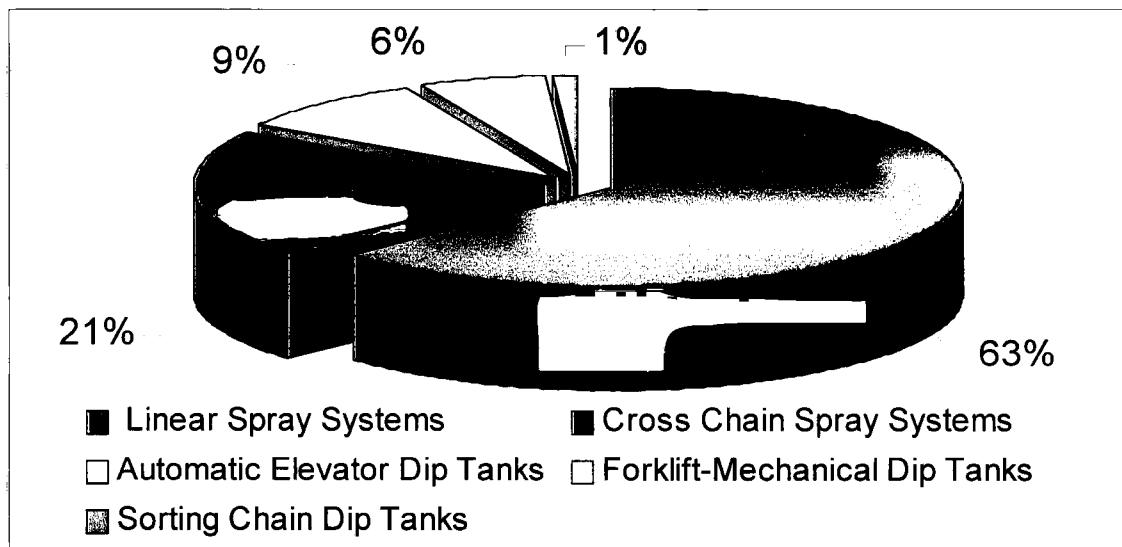
Figure 7 (c). Percentage Use of the Various Application Systems

Generally in spray systems, the lumber is moved continuously through a spray chamber or tunnel by a chain, roller, or conveyor belt system. Two types of spray box arrangements are commonly used: linear boxes where the direction of travel is parallel to the longest dimension of the piece of lumber, and the cross chains systems where the direction of travel is perpendicular to the longest dimension.

Spray systems have also been categorized according to the range of pressure applied to the spray nozzles:

- High - pressure systems (72 to 290 psi)

- Low -pressure systems (22 to 58 psi)

Chapter 2: Sapstain and Wood Rot

2.1 Factors That Cause Staining and Discoloration of Timber

When an abnormal discoloration appears on a timber, it could be due to conditions of growth, contact with chemicals or the action of microorganisms. Many timbers exhibit a wide natural variation in color, but abnormal discoloration is usually due to one of the following four main causes:

1. *Oxidation or chemical changes of cell contents* .

   The cell sap of most plants contains oxidizing enzymes, and their action in the presence of oxygen, gives rise to colored compounds which discolor the wood either superficially or to some depth.

2. *Contact with chemicals, especially iron*

   The most important of chemical stains is that caused by contact of timbers containing tannin with iron or salts of iron. This results in the formation of iron tannate, a blue-black compound, which is the principal constituent of ordinary ink. Woods that are naturally rich in tannins (oak and chestnut) are especially liable to develop ink stains when brought into contact with iron or iron rust while in moist condition. Many other timbers, including some softwood species, such as Douglas fir, contain sufficient tannin to react that way

3. *Incipient decay caused by a wood-rotting fungus*

   Under favorable environmental conditions many fungi can cause the decay of wood. In most conditions, the white rot and brown rot fungi, which belong to the group of **Basydiomicetes**, are the most important destroyers of the wood. These

fungi produce different types of enzymes, which can cause the decomposition of woody substances. White rot describes a situation in which the lignin component of the wood is attacked by the fungi, leaving the white cellulose. Brown rot occurs when the fungi consume cellulose, leaving behind the brown coloured lignin. The fungal deterioration of wood in contact with water on the ground is mainly due to the attack of soft rot fungi. Their hyphae grow inside the cell wall, forming long tunnels by consuming cellulose. If the timber is moist, its surface becomes soft. These fungi belong to *Ascomycetes*.

4. *Growth on the surface of, or throughout, the timber of sap-staining fungi or moulds.*

Moulds are able to grow mostly on freshly sawn timber, producing masses of colored hyphae and spores. The pigmented hyphae of blue stain fungi can penetrate into sapwood, consuming the carbohydrates located there. As a result of the discoloration, the quality of the timber is diminished. They do not attack the wood so as to affect its strength, but there is a loss of quality as a result of the discoloration. The spores also may be harmful to health. Such moulds belong either to *Ascomycetes* or to *Deuteromycetes.*

While there are many individual differences in the physiological requirements of the various species of fungi that cause staining, they react, on the whole, in a similar way to the following involved factors:

- *Temperature*

Staining fungi develop much more rapidly under warm rather than under cool conditions. However, the optimum temperature for the growth of most sapstain

species lies somewhat lower than those of the majority of wood-rotting fungi. The optimum and maximum temperatures for growth vary with species. The temperature relations could be classified into three main groups:

1. Low temperature group growing best at 24° C or below.

2. Intermediate temperature group growing best between 24° C and 32° C.

3. High temperature group growing best above 32° C.

- *Moisture and Oxygen Requirements*

   These two factors should be considered together, since they are closely interrelated. Wood destroying microorganisms, like other organisms obtain their energy by a process of respiration. Complex organic substances are broken down and energy is thereby released. The process can be illustrated by considering the oxidation of a cellulose unit:

   $C_6H_{10}O_5 + 6O_2 = 5H_2O + 6CO_2$
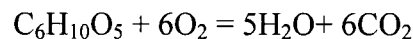
   Oxygen may be taken from the atmosphere (aerobic respiration) or energy obtained by intermolecular changes (anaerobic respiration). Wood destroying microorganisms are essentially aerobic organisms, but it is probable that a certain amount of growth can take place under relatively anaerobic conditions in which case the end products are organic substances such as alcohol, oxalic acid, etc., instead of carbon dioxide. Considerable quantities of water are produced by the respiration of certain fungi when in active growth. That means when an outbreak of rot is established, the fungus can continue to develop and spread independently of any external source of moisture. Wood destroying fungi can tolerate a very low partial pressure of oxygen. The optimum moisture content for the growth of any

particular fungus cannot be exactly defined, without reference to the particular kind of timber. The minimum moisture content for the growth of most blue-stain fungi is about 25-28 per cent. Some moulds can develop at a lower moisture content than the blue stain fungi. For example *Aspergillus glaucus,* can grow in wood containing only 19-20 percent moisture. For practical purposes, it can be taken that the minimum moisture in wood for the growth of most wood - rotting fungi is somewhere in the region of 22-24 per cent and that timber containing less than 20 percent moisture is safe from attack by any of the common wood-rotting *Basidiomicetes (*Cartwright,1958*)*.

- *Nutritional Requirements*

  Wood destroying microorganisms can derive energy from a wide range of polysaccharides. Glucose, which is formed in the largest amount when cellulose is broken down, is probably the sugar that is most favorable for their growth. They can also utilize maltose, sucrose and many other sugars, but there has been a report that they cannot utilize lactose (La Fuze, 1937). Starch is readily attacked, as are many of the hemicellulose compounds. Pentoses are also capable of acting as sources of energy, but in general are less favorable media for growth than hexoses. It has also been found that the addition of nitrogen and adequate amounts of minerals, stimulate the decomposition of wood by fungi. The nutritive substances on which the fungi feed occur only in the sapwood.

- *Types of Wood Attacked*

    The specificity of many microorganisms in relation to the hosts on which they

    grow, can be explained because they find, in the tissue of the particular host,

    special conditions, which are necessary for their development.

Chapter 3: Anti-Sapstain Protection

The use of anti-sapstain chemicals has been an integral component of lumber protection in Canada and other lumber producing nations since before the 1930's. The use of anti-sapstain chemicals to control sapstain and mould on lumber was adopted by the forest industry's essential need to satisfy markets that placed a high premium on quality lumber. Lumber producers aim to deliver lumber, which is clear of sapstain, moulds and discoloration, because the presence of any defect can essentially decrease the value of the lumber. The efficacy of anti-sapstain chemicals depends upon their direct toxicity to sapstain and mould fungi and their ability to create an unfriendly environment, which can discourage fungal growth. An important characteristic of these products is that they, to a degree, could also hinder the growth of wood decay fungi.

The amount of anti-sapstain chemicals used to protect lumber is difficult to determine, but within Canada, it has been calculated that the amount is about 1,100 tons per year. Worldwide, the amount is more than 5,000 tons per year (Byrne, 1990, Konashewich, 1994).

British Columbia's annual consumption of anti-sapstains (in terms of active ingredients) in 1987 was estimated at 300,000 to 400,000 kg per year. By 1996, the number of anti-sapstain application facilities had decreased from 100 to 51. However, consumption of fungicide had increased to approximately 846,000 kg, The lower toxicity of currently used anti-sapstains compared to PCPs has resulted in the average antisapstain

average antisapstain facility now applying four times the quantity of active ingredient as they did in 1987 (Krahn, 2000).

Prior to the 1930's, the most common chemicals used for lumber protection were sodium carbonate and bicarbonate solutions. Since the 1930's and until 1987, the protection of lumber was heavily dependent on polychlorophenols (PCP's). They provided excellent treatment and protection against sapstains and moulds as exemplified by their acceptance and use for over 50 years. In British Columbia alone more than 700 tons of polychlorophenols per year were used to treat lumber. PCP's were applied to lumber by either dipping lumber in tanks or spraying. Both application techniques produced a thick, toxic, waste sludge that created major disposal problems. PCPs came under increasing scrutiny because of their environmental and health side effects. Environment Canada (Konashevich, 1994) reported that levels of dioxin were abnormally high in workers handling PCP treated lumber. The environmental and health side effects of PCP's, resulted in their ultimate abandonment in the Canadian forest industry. The shift from PCP's to relatively new chemicals has been rapid. The following is a historical review of anti-sapstain agents used in the Fraser River Basin prior to the latest generation of anti-sapstain chemicals (Krahn, 2000: Konashevich, 1994):

- **PCP and TCP** (chlorophenols)

  The use of chlorophenols as anti-sapstains was banned on December 31, 1990. The regulated limits in stormwater runoff are 6 parts per billion (ppb). These compounds are no longer in use in any mills in BC.

- **TCMTB** (2-thiocianomethylthio benzothiazole)

  TCMTB is an active ingredient used in commercial formulations Busan 1030 and Busan 30 WB. This chemical was initially used in a significant percentage of BC mills as a replacement for chlorophenols. However, problems with fish toxicity and in worker handling resulted in a rapid phaseout at most facilities. It is currently in very limited use in BC.

- **Cu-8** (Copper-8- quinolinolate)

  Cu-8 is the active ingredient used in commercial formulations called Nytec GD and PQ-8. This chemical was used in several mill trials immediately following the banning of chlorophenates, however now it has been phased out in favor of less toxic chemicals.

- **"Borax-sodium carbonate"** formulations

  "Borax" formulations are used individually or in combination with other active ingredients. These compounds have low fish toxicity but they have proven unsatisfactory for such reasons as poor efficacy in wet climates, and rapid leaching due to their high solubility in water.

Because of still growing pressures from environmental groups and government regulators there has been increasing interest in the development of new systems that would present less potential for adverse environmental impact and would allow for easier disposal of treated products. A number of alternative anti-sapstain products that use quaternary ammonium compounds (AACs) as their active component have been developed to meet these objectives. Recently, extensive research has been reported on the

effectiveness of these alkylammonium compounds (AACs) as anti-sapstain wood preservatives (Butcher, 1979: Butcher et al., 1979: Cassens et al. 1982). The results have determined that AACs are effective against several wood-destroying fungi and have suggested that the most effective of the AACs is didecyl-dimethyl-ammonium chloride (DDAC). The new generation of anti-sapstain chemicals that use DDAC as an active component was introduced in New Zealand in 1978 (Lir, 1978). By mid-1993, 80% of the 53 B.C. lumber operations using anti-sapstain chemicals converted to the use of didecyl-dimethyl-ammonium chloride (DDAC) formulations (Konashevich, 1994).

3.1 Shortcomings of Traditional Chemical Products

To improve wood's appearance and commercial value, the lumber industry commonly treats freshly cut green lumber with several active chemical ingredients. The efficacy of these products depends upon their direct toxicity to sapstain and mould fungi thus they have an ability to create an unfriendly environment that will discourage fungal growth.

Among the traditional wood protecting chemicals, the most commonly used for years were fixing salt types based on inorganic arsenic, chromium, cooper, boron and fluorine, and organics such as creosote, pentachlorophenol (PCP's) and its sodium salt (PCP-Na), tributyltin oxide (TBTO) and other TBT formulations. These components were very effective, however they were also highly toxic to the environment. As a result, they have been taken out of use.

There have been fluctuations in the effectiveness of some of the chemicals used to control sapstain over the years when applied on lumber at their recommended levels. After extensive research, wood preservatives based on the use of DDAC as the active ingredient were introduced into commercial use. DDAC has shown a strong performance against a large number of staining and wood destroying fungi. Unfortunately, in commercial use, in products where it was the only active ingredient, a number of protection failures occurred. Various reasons for these failures (see Chapter 4) have been determined such as substandard treatment practices, poor distribution of the preservative (coverage of lumber), misuse in service, depletion of the active ingredient, and attack by DDAC tolerant organisms.

It is to be expected that, like many other living organisms, fungi may be able to develop a resistance to a toxicant. Fungicide-resistance may be defined as a stable, inheritable adjustment by a fungus to a fungicide, resulting in a less than normal sensitivity to that fungicide.

The information mentioned above suggests that problems and possible reasons for the failure of the current commercial fungicides can be ascribed to several possible causes. Despite several investigations into possible reasons, no clear, universally applicable explanations as to why some anti-sapstain and anti-fungal formulations failed to protect, have been obtained (Lyr, 1995).

To be able to avoid the development of fungicide failures in the future, it is important that a high level of efficacy be available, so that flexibility exists in the design

of counter measures. Several reports from the literature (Ward, 1988, Hsu, 1990) mention synergistic action between two fungicides. This phenomenon might also appear to have value for the avoidance of resistance if the second fungicide interferes with the fungi's resistance mechanism towards the first fungicide.

3.2 Possible Problem Solution

On account of the problems that may occur with the majority of commercial anti-sapstain products that use DDAC as an active component, the possible solution of the problem appears to be the use of a second active component for improving long-term performance and broadening the spectrum of activity. Generally, the expected response of a mixture of two chemicals is the sum of the effects of the components separately. However there is the possibility that these two chemicals can react synergistically against certain microorganisms which would result in achieving the additional advantages of the mixture which might enhance the efficiency of the individual components. At the same time, the amount of active ingredients would be reduced.

The industry resolved this situation through the addition of a second active component into formulations that had DDAC as the primary active ingredient. This represented a major improvement and broadening of the spectrum of activity. This generation of products captured the market due to their effectiveness. However there still remains a concern regarding the toxicity levels of the secondary active ingredients. As an example, the anti-sapstain product that currently retains the dominant North American

market share position with approximately 90% of the market is NP-1; NP-1 uses DDAC as its primary active ingredient and 3-iodo-3-propanyl butyl carbamate (IPBC) as its secondary active component. However as previously mentioned, there is a degree of uncertainty about IPBC and its toxicity levels to fish. The challenge has been to find a second active component that, when combined in solution with DDAC, would increase the effectiveness and lower the toxicity level of the product

Chapter 4: Fungicides - Literature Review

Under favorable environmental conditions, many fungi can cause decay and/or discoloration of wood. In most conditions white rot and brown rot, which belong to the *Basidiomycetes*, are the most important destroyers of wood. These fungi produce different types of enzymes, which can cause decomposition either of all the wood substances, with the cellulose part remaining (white rot), or vice versa (brown rot) with the lignin remaining. The fungal deterioration of wood in contact with the ground or water is mainly attack by soft rot fungi, whose hyphae form long tunnels by consuming cellulose.

Apart from these wood-destroying fungi, there are two other groups, which can cause discoloration of timber to a greater or lesser degree: blue stain fungi and moulds. The blue stain fungi develop on the surface of timber, and their pigmented hyphae can penetrate into the sapwood, consuming the carbohydrates located there. Moulds are able to grow mostly on freshly sawn timber, producing masses of colored hyphae and spores. They do not attack the wood, but there is a loss of quality as a result of the discoloration.

Many uses of wood, which has no or insufficient resistance to fungal attack, dictate that it must be protected by wood preservatives. This protection may be temporary on freshly sawn timber in order to prevent the growth of moulds and blue stain fungi (anti-sapstain protection), or to prevent the growth of wood-rotting (wood destroying) fungi (Lyr 1995).

The successful preservation of wood by chemicals was first accomplished on a commercial scale about a hundred twenty years ago (Cartwright, 1958). Since then hundreds of chemicals in various combinations have been proposed but of all of them, comparatively few have been found to be practicable and effective and at the same time devoid of objectionable properties. There can be no universally ideal preservative for all classes of timber, since the timbers to be treated and the purposes for which treated wood is required, vary (Cartwright 1958). Nevertheless it's important to underline the significance of wood degradation caused by sapstaining fungi and moulds. For example, it has been estimated that in New Zealand export losses of radiata pine are connected to an annual loss in revenue of more than 100 million NZ$ (Wakeling 1996)

A wood preservative, to be appropriate for commercial use, should be toxic to wood destroyers, permanent, penetrative, safe to handle and use, harmless to wood and metal, unlimited in availability and economic in cost. Furthermore, for the treatment of building lumber or for other special purposes it may also need to be clean, colorless, odorless, paintable or have certain combinations of these properties.

4.1 Pentachlorophenol

Production of chlorinated phenols in the United States for wood preserving experiments did not begin until about 1930. The first field tests started with tetrachlorophenol in 1931. A year later pentachlorophenol was produced in limited quantities. The penta form appeared to have better properties than the tetra form and,

since its introduction, has been the principal chlorinated phenol used in wood preservation. In 1965, a total of more than 20 million pounds was reported to be used by the organized wood-preservative industry (Cartwright, 1958)

Pentachlorophenol is a crystalline chemical compound (C_6Cl_5OH), which is sufficiently soluble, for wood-preserving purposes, in heavy petroleum oils. The corresponding structural formula is:
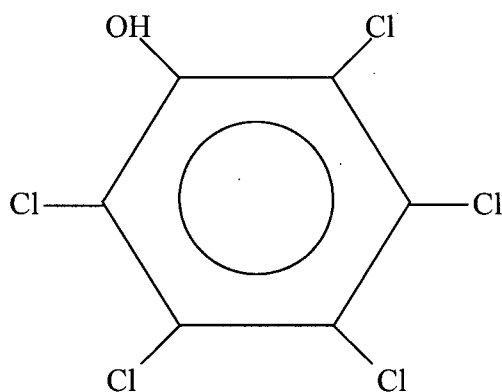
The character of solvent used with chlorinated phenols may have some effect on the performance of the preservative. Actually, changing the solvent and also the concentration of the dissolved toxic chemical can vary the preservative value of pentachlorophenol solutions.

Pentachlorophenol (PCP) is of environmental concern because of its immediate toxicity to aquatic organisms (Konashevich, 1994) Associated with PCP preservatives are such chemical impurities as polychlorinated-p-dioxins and polychlorinated dibenzofurans, which are toxic, accumulate in aquatic organisms and persist in the

environment with long-term consequences. The effects of occupational exposure to PCP and its PCDD and PCDF contamination have become very important topics in many recent research investigations. There are papers that discuss potential occupational exposures of workers. For example Hryhorczuk et al. (1998) in their study concluded that occupational exposure to PCP is associated with biochemical abnormalities which may persist years after exposure. Treated wood has been even hypothesized as an important source of PCP in milk and beef (Fries et al., 1999). A survey of reported data relating exposure to PCP and its derivatives in occupational settings has indicated that exposure via the food chain is judged to be the most significant intake route for PCP, PCDD, and PCDF (Eduljee, 1999). Investigation of the adverse neurobehavioral effects of long-term exposure to wood preserving chemicals containing PCP showed that long-term low-dose exposure could be related to subtle alterations of neurobehavioral performance in women and also might cause mutations in living cells, and might damage a developing fetus (Peper 1999). Short-term exposure to elevated levels of pentachlorophenol can lead to poisoning that is rapidly fatal.

Increasing environmental concerns initiated research towards developing alternatives that were more environmentally acceptable than those once used like pentaclorophenol and chromated copper arsenate.

4.2 Alkyl Ammonium Compounds (Quaternary Ammonium Compounds)

In 1890 Menschutkin reported the first preparation of quaternary ammonium compounds by the treatment of an alkyl halide with a tertiary amine. Following Oertel's publication in 1965, quaternaries were used as wood preservatives (Pernak et al, 1998). Wood preservatives based on alkyl ammonium compounds (AAC) were introduced into commercial use in New Zealand in 1978 (Butcher, 1977 and Drysdale 1978). Due to their generally low toxicity to mammals and fish, as well as for economical reasons, they are extensively used in the wood preserving industry.

There are two basic types of AACs that are of interest to wood preservation: quaternary ammonium compounds and tertiary amine salts. All of these compounds can be solubilized in water, and for most applications aqueous solutions would be used. However, some of the AACs could be formulated as organic solvent based systems if desired. Alkyl chain length and type of anion are two major factors that appear to effect the efficacy of the AACs against wood decay fungi (Nicholas and Preston, 1980).

AACs are synthesized by step-wise alkalization of ammonia to give an intermediate tertiary amine, which is further reacted to the end products. The hydrophobic carbon chain (s) is (are) derived from either natural fatty acids (tallow, soya, coconut) or synthetically from ethylene to give a wide range of compounds, depending on the type and length of the alkyl chain. Butcher et al. (1977) suggested that

didecyldimethyl ammonium chloride was the most effective of the AACs against *Basidiomycotina*, being equivalent to chromated-copper-arsenate wood preservatives.

AAC solutions are stable and can be used in presently operating treatment plants without modification of the equipment. Because of the AACs surfactant characteristics some foaming problems may be experienced, but experience has shown that these are readily corrected by the use of suitable anti-foams (Nicholas and Preston 1980).

Didecyldimethyl ammonium chloride (DDAC) belongs to the group of quaternary ammonium compounds. It is currently being used as a replacement for polychlorophenols. Its structural formula is:

$$\left[\begin{array}{c} CH_3 \\ | \\ (CH_2)_9 \\ | \\ CH_3 - N - CH_3 \\ | \\ (CH_2)_9 \\ | \\ CH_3 \end{array} \right]^+ \quad Cl^-$$
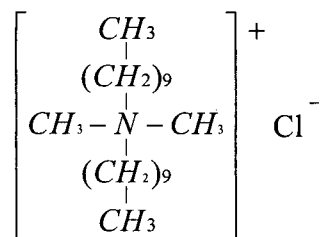
The application of quaternary ammonium compounds has led to a demand by their users for effective methods of control. The American Wood-Preservative Association recommends standards A16-93 (Standard for HPLC Method For Didecyldimethylammonium Chloride Determination in Treated Wood), A17-93 (Standard for Determination of Quaternary Ammonium Compounds in ACQ Solutions), and A18-93 (Standard for Determination of Quaternary Ammonium Compounds in Wood by 2-Phase Titration).

Biotransformation is expected to be the main route of disintegration of DDAC in the environment. Earlier studies had indicated that rapid and complete degradation of DDAC occurred when low concentrations were exposed to mixed bacterial cultures (Resuggan, 1951).

Alkyl ammonium compounds belong to a group of disinfectants having almost the same degree of effectiveness against bacteria, yeasts, moulds, fungi and protozoa. It is very likely that when they are adsorbed onto the cell wall, they form un-ionized complexes, which are responsible for the death of the cell, however, there are also many things as yet undecided about their exact mode of action (Resuggan, 1951).

Rosen (1975) outlined a mechanism for improved preservative efficacy on wood substrates by variation of the treating solution's acidity. At low levels of acid addition to the treating solutions, the added protons competed with alkylammonium cations for fixation sites. Thus they prevented the preferential adsorption of the alkylammonium cations by the first cell walls contacted during the liquid uptake phase of preservative treatment. This resulted in a uniform distribution of alkylammonium compound throughout the wood and improved effectiveness against *Basidiomicetes*. As the addition of acid to the treating solution increased, more protons were present to compete for fixation sites. This resulted in a decrease in cation exchange in the cell wall layers adjacent to the cell lumens, and a greater penetration of cell walls as "free" alkylammonium cations were adsorbed on the less readily available fixation sites within the S2 layer. This explanation was supported by biological evidence, for soft-rot control

improved in all timbers as the acid levels in the treating solutions increased. At the same time, control of *Basidiomicete* attack was reduced. These results support the general view that cell wall loading of a toxicant is most efficacious against soft-rot fungi, and lumen surface deposits are best against *Basidiomycetes*.

The probable effect of adding alkali to treating solutions is the removal of protons from the wood substance, creating negative sites, which would aid adsorption of alkylammonium cations. The result of treating with such solutions was the preferential uptake and fixation of quaternary ammonium cations in peripheral zones of the wood. This led to increased losses of wood substance during fungal exposure as all the test wood blocks were first cross-cut sawn to expose the central (untreated) zones.

Nicholas and Preston (1980) also examined the modification of the effects of alkylammonium compounds on wood protection by the addition of acid or alkali at various levels of acid (HCl) or alkali (Na_2CO_3). The effect was determined through an increase or decrease in decay after treated wood was incubated with brown rot, white-rot, and soft-rot organisms. Addition of low levels of acid (0.025-0.1%W/W HCL) improved performance against *Basidiomycetes*, and to a lesser extent soft-rot fungi. Addition of alkali to treating solutions generally led to increased loss of wood substance when treated wood was exposed to fungal attack. Observations during the preliminary work indicated that treatment with acidic solutions resulted in an even distribution of alkylammonium compounds through the wood, whereas treatment with alkaline solutions resulted in preferential adsorption in the surface layers of wood samples. The importance of this

work has been to show how simple modification of treating solutions may influence the distribution of AAC in wood, with consequent increases and decreases in preservative efficacy. Such simple modifications are not possible with CCA preservatives.

While laboratory tests have consistently provided positive results, field testing of unmodified AACs has failed to confirm their efficacy. Rudick (1986) in his paper considered two possible causes for declining AAC activity. These were leaching of AAC from the wood after application and the influence of non-wood destroying fungi. Since his results eliminated leaching as a primary cause, further study was conducted to determine whether the colonization of the treated wood stakes by staining fungi, could cause a reduction in AAC effectiveness. The results suggested that degradation of the AAC was taking place rather than rupture of the AAC-wood substrate bonding. Lee et al. (1992) suggested that wood cell wall components might react with fungicides under direct contact situations to deactivate their fungicidal properties.

There is also the possibility that uneven protection of different parts of the wood. may result in unsatisfactory fungicidal performance . Since the effect of solution pH on the adsorption of quaternary ammonium compounds (for example DDAC), onto cellulose and lignin may effect uneven protection of different parts of the wood, adsorption was found to be predominantly onto lignin with much lower adsorption onto cellulose. Consequently, DDAC by itself provides little protection against cellulose degradation by brown rot fungi (Jin and Preston, 1991).

Didecyldimethylammonum chloride has a proven history as a successful antisapstain chemical, but performance failures mentioned in the research works initiated the idea to broaden its spectrum of activities by adding a second active component.

4.3 Component 2 (C2)

Since the results of this research are the basis for a patent currently pending, the fungicide chosen to be a second active component, in a preservative combination with DDAC, in this study will be called Component 2 (C2).

C2 is widely known as an industrial biocide which is strongly antagonized by exogenous thiol-containing agents and which interacts oxidatively with accessible thiols, such as glutathione, within the cell.

This study examined the possibility of adding C2 in combination with DDAC for wood protection. The C2 used was a 3:1 mixture of two components that belong to the same group of chemicals.

This mixture is non foaming and stays effective over a broad pH range. It is easy to disperse it into formulations in the water phase, where the microorganisms live. Since C2 has lower adsorptivity than anionic or cationic materials, it stays available for use (Manufacturer of C2). When diluted below the concentrations in which they are used in industrial applications, according to a manufacturer study that used radioassay studies to

follow the biodegradation of the active ingredient in natural river water, C2 is biodegradable.

The mode of action of C2 biocides is based on a reaction with nucleophilic entities such as enzymes, proteins and amino acids. According to manufacturer's information the C2 used in this study has shown outstanding antimicrobial characteristics in the process of killing prokaryotic (bacteria) and eukaryotic (algae and fungi) microbes.

Table 3 gives the minimum levels of active ingredient that inhibited growth of various microorganisms in test tube cultures. These data demonstrate the broad-spectrum of activity of C2 and are useful tools for screening antimicrobial materials (Manufacturer of C2).

Table 3. Effectiveness of C2 Against Various Microorganisms

Organism	Type	Active Ingredient (ppm)
Aspergillus niger	Fungi	9
Aspergillus foetidus	Fungi	8
Aspergillus oryzae	Fungi	5
Chaetomium globosum	Fungi	9
Gliocladium fimbriatum	Fungi	9
Penicillium funiculosum	Fungi	5
Candida albicans (yeast)	Fungi	5
Lentinus lepideus	Fungi	4
Bacillus subtilis	Bacteria Gram-Positive	2
Steptococcus pyogenes	Bacteria Gram-Positive	9
Eschericia coli	Bacteria Gram-Negative	9
Salmonella typhosa	Bacteria Gram-Negative	5

Since this study emphasizes the importance of adding a second active component to DDAC it is important to note that the literature reports that the C2 is very active in the control of wood destroying fungi.

The analytical techniques recommended for the chemical analysis of aqueous C2 solutions are High Pressure Liquid Chromatography (HPLC) and Ultraviolet Spectroscopy (Manufacturer of C2).

Chapter 5: Development of a Novel Antisapstain Formulation

Antisapstain chemicals are products that are supposed to be toxic to wood destroying microorganisms, but at the same time, they must be environmentally responsible and safe for the people that are in contact with them. A review of literature and consideration of all the known products that are currently registered for the Canadian market gave rise to an idea for improving these products.

Since 90% of the Canadian market uses a combination of DDAC and IPBC, we analyzed the advantages and disadvantages of those formulations and tried to identify possible reasons for customers' dissatisfaction. Even though DDAC already had a proven history in the industry, the possibility of developing resistance by some microorganisms and DDAC's greater affinity for lignin than for cellulose opened the door to considering the possibility of the addition of a different (than IPBC) second active component which could counteract these problems.

On the other hand, the second active component of many major use commercial products, IPBC, does not show favorable fish toxicity characteristics (Konashevich, 1994). There are also some studies that show IPBC's weaknesses against some wood destroying microorganisms. Plackett (1982), claimed that a 1% IPBC based treatment provided protection of hem-fir timber comparable to that of a treatment containing 0.65% PCP. He also showed that a 0.5% level of IPBC was ineffective. On the contrary, the manufacturer of C2 (1989), stated that the efficacy of 0.3% to 0.4% of C2 was superior to

that of 4% PCP. It also showed complete protection of *Pinus sylvestris* panels that were exposed for eighteen (18) months to heavy rainfalls.

One compound that came to mind as a potential addition to DDAC for wood protection was C2. It is less toxic to fish than IPBC; see below.

From a health and safety point of view, DDAC and C2 are both classified in: Group D - Not Classifiable As To Human Carcinogenicity. By contrast, there are certain questions and controversies as to the carcinogenicity of IPBC. The Carcinogenicity Peer Review Committee (CRC) classified (June 16,1993) IPBC as a Group C - "Possible Human Carcinogen". On September 18, 1996, the CRC, at the request of a registrant, concluded that the additional evidence provided by the registrant supported reclassification of IPBC as "not likely" to be carcinogenic. Concern over IPBC however still remains.

Keeping in mind that currently the most used preservative on the market uses DDAC and IPBC as its active components, the toxicological characteristics of C2 are compared with those two chemicals. The charts below compare the concentration of effluent in dilution water for IPBC, and C2 that causes mortality to 50 percent of a Rainbow Trout test population (96 hour LC_{50}). Each of these components is also compared with DDAC, the primary active ingredient used with both IBPC and C2 .(Konashevich, 1994, and Manufacturer of C2)

LC50 (96 hours) Rainbow Trout (ppm)
All Active Ingredient Comparison

LC50 (96 hours) Rainbow Trout (ppm)

Second Active Component Comparison
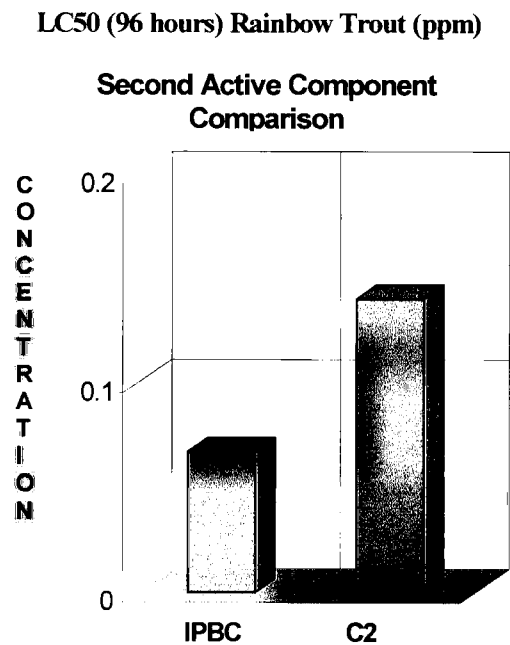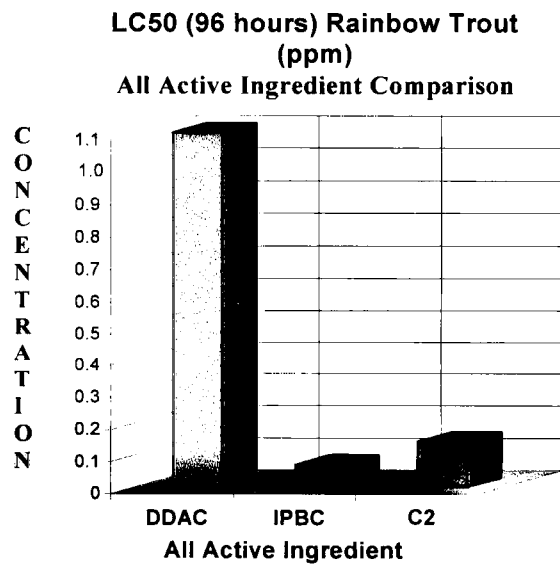
Figure 8. Comparison of Fish Toxicity of Each Active Component

LC50 is the concentration of fungicide, in dilution in water that causes mortality of 50 percent of the fish in a test population. The higher the LC50 number is the less toxic the fungicide. Thus the amount of the chemical in the water that it takes to cause mortality in 50 percent of the test population is also higher.

As can be seen from Figure 8, C2 has a toxicological advantage over IPBC.

In the summer of 1995, a questionnaire designed to determine the use pattern of antisapstain chemicals was mailed to all producing members (sawmills) of the Western Woods Products Association. In the questionnaire, the respondents were asked to provide their level of agreement with a series of statements regarding their selection of anti-sapstain chemicals. The opinions expressed were neither strongly positive nor negative, indicating that users are not totally convinced of the effectiveness of existing treatments. This convinced us that it would be desirable to conduct research toward developing a new anti-sapstain product that would satisfy consumers' desire for:

- efficient control of fungi,

- low toxicity,

- less potential for environmental impact (low leachability), and

- easier disposal of treated products withdrawn from service.

Chapter 6: Objectives of the Research

A lot of information is required to fully evaluate a chemical as a wood preservative. In general, the basic areas that must be addressed are:

- Chemical properties

- Biocidal effectiveness

- Treating solution characteristics

- Properties of treated wood

- Toxicological properties

The objectives for this research are to address the above mentioned areas and to provide results adequate to support the development of new anti-sapstains formulations that would meet the objectives of efficient control of fungal growth, low fish toxicity, less potential for environmental impact (low leachability), and easier disposal of treated products (e.g. discarded lumber treated by anti-sapstains) withdrawn from service. The research was separated into several phases:

Phase 1

Preliminary Efficacy Evaluation of Potential Fungicides Against Molds and Stains

The experiments of the first phase were designed to test several potential fungicides against moulds and stains in order to provide results that would eliminate less promising formulations and help focus our research for a new, multi-active agent,

chemical formulation. A number of commercial fungicides, with toxicological characteristics that would satisfy the safe handling and environmental requirements, were mixed in different ratios with DDAC and water and submitted to preliminary microbiological testing. The bioassays were carried out to estimate the relative effectiveness of biocides against the moulds and staining fungi and to indicate the possible resistance to such chemicals by these organisms. The organisms were grown on an artificial substrate, agar, and exposed to various potential fungicides.

Phase 2

Efficacy Evaluation of the Most Promising Fungicidal Combinations

In Phase 2, the research was extended to other groups of fungi, such as soft rot, brown rot, white rot and DDAC tolerant species. Again the tests were done on agar plates.

Phase 3

Efficacy Evaluation of the Most Promising Fungicidal Combinations on Wood Substrate

The objective of Phase 3 was to test the most promising formulations discovered in Phase 1 and Phase 2 by using the U.S. Standard for Testing Fungicides for Controlling Sapstain and Mould on Unseasoned Lumber (D445). The results narrowed the search for appropriate chemicals and suggested a range of possible concentration ratios between the

active components. They also determined the minimum amount of preservative that would be effective against sapstain fungi and moulds on different kinds of wood (obtained from the different sawmills in British Columbia). In Phase 3 the various microorganisms were grown on wood samples rather than on agar plates.

Phase 4

Determination of product stability and ecotoxicological characteristics

In Phase 4, active components and additives were combined together in larger volumes and product formulation stability was investigated. After ensuring physical and chemical stability, in order to decide whether the product met the levels accepted by Environment Canada, the potential product solution was submitted for toxicological testing to a Vancouver based laboratory BCRI.

Phase 5

Pilot-Plant Spray System Trial (Forintek Laboratory)

Wood protection can only be ensured if the required coverage and retention are achieved on the wood. Prior to going to a mill trial, the product was examined by a simulation of real spraying conditions in the Forintek's wood treatment pilot plant. In Phase 5

commercial samples of lumber were sprayed in the pilot plant with the test product and then stored to see if moulds, fungi or sapstain would develop.

Chapter 7: Methods

7.1 Microbiological Screening Tests on Agar Plates

All organisms have the potential to grow or increase in mass by cell division, cell enlargement, or both. In simple organisms such as nonmycelial fungi, cell enlargement, accompanied by nuclear division and synthesis of cytoplasm is primarily responsible for growth. Mycelial fungi grow through a combination of cell division and enlargement. The simplest method of assessing fungal growth is by linear measurement. The change in the radius of a developing colony on agar is observed over a period of time. This method is very simple, but is of much value in making rough estimates of growth. It is a non-destructive method, which allows repeated observations of the same mycelium (Moore-Landecker, 1982).

The selection of a suitable substratum is very important, since it should simulate fungal growth in nature. A disadvantage of natural media is that they can never precisely duplicated as they are of an unknown composition in each separate case (Moore-Landecker, 1982). Alternatively, a completely synthetic medium, such as malt agar that is used in this experiment, can be precisely duplicated.

Use of microorganisms to determine an amount of biocide that is necessary to suppress fungal growth, is called a *bioassay*. The chosen organisms should be sensitive enough to the presence of the test substance to show a growth response to dosage increases. These would be plotted in linear fashion over some concentration range.

Prior to use as inoculum, the fungi were grown on a medium which provided adequate amounts of all factors needed (malt agar). The amount of inoculum used must be carefully standardized to give repeatable results, see Figure 9:

(A)   (B)
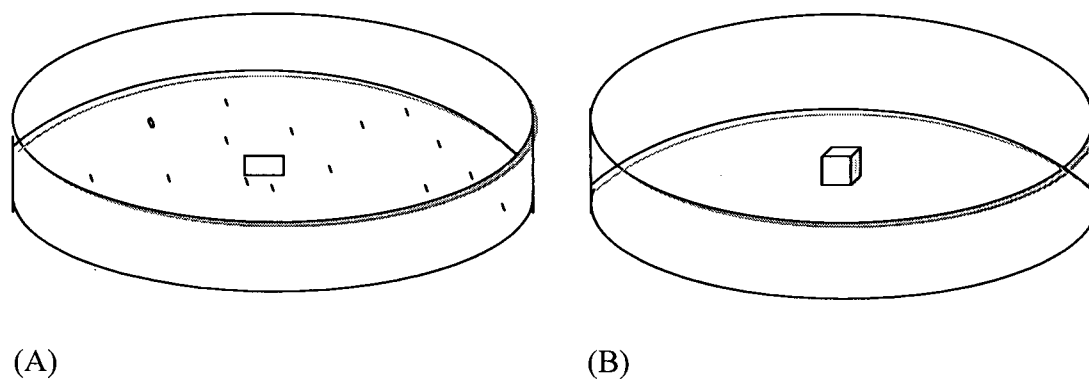
Figure 9. Steps in Inoculation Technique:

        (A) Small piece of colony is removed from Petri dish

        (B) Small piece of colony transferred to a new dish of agar

In general, myceliar growth may be qualitatively divided into the following (Moore-Landecker, 1982):

Stage 1 (lag phase) with no growth

Stage 2 (linear phase) with rapid and approximately linear growth

Stage 3 (decline phase) in which there no growth occurs

In order to examine the biocidal toxic effects of a test preservative, solutions of 4% malt agar were prepared and sterilized at 121°C for 20 minutes (at 103 KPa). Once the

malt agar cooled to "hand hot" in which state it was still liquid, the previously prepared biocidal solutions were added in to obtain the range of different concentrations. For example: in order to produce a concentration of 500 ppm DDAC in the media, 7.5ml of the 0.4 % stock solution of DDAC was added to 112.5 ml of 4% agar. Ten ml of each solution was transferred into petri plates and allowed to solidify.

Specific fungi from pure culture plates were placed in the center of each plate. Fungal cultures were obtained from Forintek Canada, see Appendix 1. Fungal growth was evaluated by monitoring the diameter of a developing colony on agar over a period of time and then calculating the percent coverage of the agar plate surface by the colony. Three replicas of every fungicide were prepared and measured. The measurements were converted to express the percentage of the plate's surface.

The above procedure was repeated for the toxicological evaluation of each concentration of each component or mixture of components. Generally, the expected response of a mixture of two chemicals is the sum of the effects of the components separately. Additional advantages of the mixture could be obtained due to synergistic interactions by which the efficiency of the individual components is increased and at the same time the amount of active ingredients is reduced. In this case, the effectiveness of the mixture cannot be computed from that of the individual ingredients.

To show more graphically any possible synergy in the fungicidal mixtures, the results from the agar-plate test were used to calculate a Synergy Index (S.I.). The S.I. used,

shares similarities with the Synergy Index used by Hsu (1988). However, it was modified for our particular research, since the activities of wood destroying microorganisms were being considered.

In order to reflect the long-term effects of a potential preservative, the 22nd day of the experiment was chosen to define a reference point (RP). The minimum biocidal concentration of the potential wood preservative (mixtures of DDAC and ITA) were analyzed using the following equation:

$$1/2 \ (MCA'/MCA) + 1/2 \ (MCB'/MCB) = SI \qquad (1)$$

$$1/2 \ SI_A + 1/2 \ SI_B = SI \qquad (2)$$

$$wherein: \ (MCA'/MCA) = SI_A \qquad (3)$$

$$and \quad (MCB'/MCB) = SI_B \qquad (4)$$

MCA = Concentration of compound A in parts per million, acting alone, which

       prevents fungal growth at the reference point

MCA' = Concentration of compound A in parts per million, in the mixture, which

       prevents fungal growth at the reference point

MCB = Concentration of compound B in parts per million, acting alone, which

       prevents fungal growth at the reference point

MCB' = Concentration of compound B in parts per million, in the mixture, which

       prevents fungal growth at the reference point

When the sum of ratios 1/2 (MCA'/MCA) = SI_A and 1/2 (MCB'/MCB) = SI_B is greater than one, antagonism is indicated. When the sum of SI_A and SI_B is equal to one, compatibility is demonstrated. When the sum of SI_A and SI_B is less than one, synergy is demonstrated. The smaller the sum, the higher the synergistic effect. The synergy index is represented by the formula:

$$S.I.= Log (SI) \hspace{4cm} (5)$$

Since in the case of additivity $SI=1$, than S.I. = Log (SI) = 0. Consequently, $SI>1$ (antagonism) give positive value for S.I. and values $SI<1$ (synergism) give negative values for S.I.

Synergism and antagonism are phenomena that attract attention from a practical point of view. The directed use of synergists is useful not only because they lower the use-level of biocide, but also because it can be very favorable for increasing the antifungal spectrum, or decreasing the danger of resistance formation in the fungal population. This is especially useful in situations where one compound does not achieve the best results due to weak activity against certain organisms.

7.2 Microbiological Screening Test Using Agar Block Tests

Modified Standard method ASTM D445 was used for determining the minimum concentration of our formulation that was effective in preventing biodeterioration by sapstain fungi and moulds in selected samples of wood. The wood specimens were treated by immersing them in solutions of a fungicide formulation prepared at five concentration levels. The toxicity to fungicides was tested against various spore

suspensions. The intensity of surface fungal growth was estimated after incubation and the results were used to determine the chemical treatment concentration that gives zero growth.

Apparatus:

- Incubation Cabinet, Maintained at a temperature of 25+/- 1°C and high humidity,

- Petri dishes and aluminum pans with aluminum foil cover

Wood Species:

- Locally available commercial species selected on the basis of their susceptibility to staining fungi (Douglas fir and Western Hemlock) .

- The dimension of the specimens were 7 by 20 mm in cross section and 7 cm long.

Culture Media:

- Malt Agar Substrate consisting of 2% malt extract and 2% agar

Preparation of Inoculum:

- The inoculum was prepared from cultures grown on petri dishes. For the preparation of a spore suspension, 10 ml of sterile water was added to each petri dish. Then the spores were loosened from the malt agar culture using a blunt glass rod. After that they were combined with other similarly collected spores.

Preparation of Test Chambers

- All specimens were autoclaved before treatment at 121°C, at 1 MPa for 20 min. then two samples of wood were placed in a petri dish. One of them was treated, the other was just immersed in water and served as a control for comparison. The temperature

was set at 25°C. To maintain high humidity during the test period; wet absorbent paper was placed on the bottom of each dish.

Inoculation

- Inoculation of the microorganisms and arrangement of test species was performed in two ways:

1. Test microorganisms that were previously grown on a nutrient medium consisting of 2% malt agar were placed on the absorbing paper in the bottom of the petri dish used as the test chamber. Then the two specimens, treated and untreated were placed on the top of them. These two specimens were adjacent, almost attached, to each other (see Figure 10).
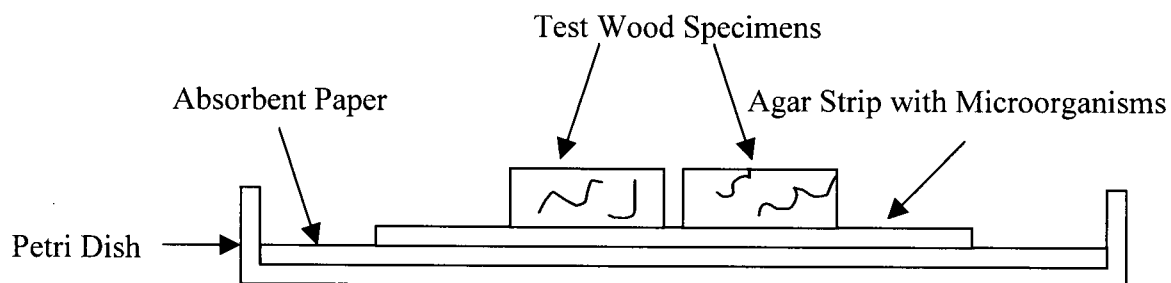
Figure 10. Arrangement of Wood Specimens on Agar Strip Within Petri Dishes

2. Two adjacent wood samples were placed on the absorbent paper. Then, about 1ml of spore suspension was streaked along the length of one flat side of each sample (see Figure 11).
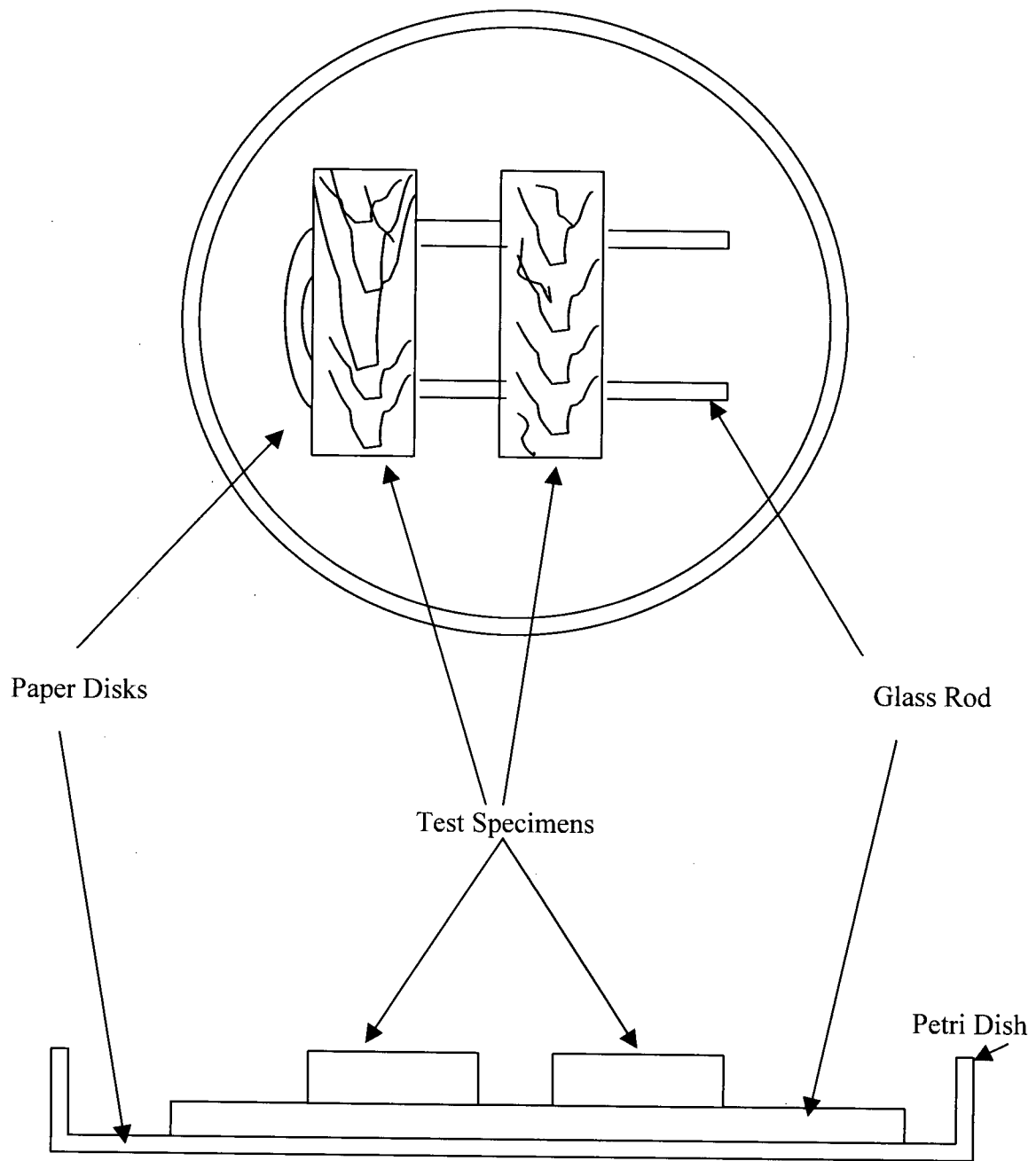
Figure 11. Arrangement of Treated Wood Samples Within the Petri Dishes

Test Evaluation

Spores of fungi germinate more or less readily, according to the particular species involved, when they come into contact with a moist substratum. The spore germinates by bursting the external cell wall and pushing out a germ tube which soon branches to form a mycelium, which is possible to see on the wood surface about 3-4 weeks after germination (Moore-Landecker, 1982).

The intensity of the surface fungal growth was estimated after incubation and the results used to determine the chemical treatment concentration giving zero growth. Evaluations were made visually, using a scale from 0 to 5. Five (5) indicates a sample covered 80-100 % with mycelium, one (1) indicates a sample covered 10-20% with mycelium etc. Values between 0.1 and 0.9 indicate that it was not possible to identify mycelium on the wood surface, but it was possible to identify *germ tubes* by using a microscope.

The estimate was based on observations of the intensity of growth and discoloration.

Data Analysis

In most of the tests reported in this thesis the value of the reported results lies in a comparison of situations in which no growth of fungi occurred compared to situations were growth did occur. Thus if no growth occurred the result was assigned a non-zero value depending on the measure of growth used as described above. No attempt has been

made to compare relative amounts of fungal growth when it did occur. The comparisons,

as said are in terms of no growth (no, 0) or growth (yes, positive number).

7.3 Field Efficacy Test by Using Pilot-Plant Linear Spraying System (Phase 5)

Objective:

To define the efficacy of fungicide depending on different chemical retention.

Wood Samples Used for Testing

220 pieces of long (2x4 green, rough-cut untreated), hem-fir and Douglas fir lumber,

were supplied by a local mill.

130 pieces were planed in Forintek to dimensions of: 96 mm X 46 mm, and

90 pieces were used unplaned (rough cut).

Wood Species:

110 pieces of Douglas fir and 110 pieces of Western Hemlock were used.

By visual estimate, the approximate breakdown of Western Hemlock and amabilis fir in

the hem-fir lumber supplied appeared to be about 80% hemlock and 20% amabilis fir

Each piece was labeled with a treatment code (Table 5) and numbered 1-20.

Antisapstain Chemicals Used in Test

- Concentrate was prepared to have 55% DDAC (Source Bardac 2280)

- Prior to treatment the concentrate was analyzed for its DDAC content as was a sample of the parent DDAC source Bardac 2280 using an HPLC method. HPLC analysis showed that the actual concentration of DDAC was 49.5% DDAC w/w. The lower than anticipated level of DDAC was attributable to

the fact that the Bardac 2280 contained 72.4% DDAC and not the 80% DDAC marked on the label.

- Samples of each diluted control product were also analyzed to check that they were correct.

Figure 12. Chemical Feed System

The concentrated chemical was diluted to concentrations shown in Table 5, and applied to the wood using Forintek's linear spray system. Figure 12 shows chemical feed passing trough the screen to be filtered and defomed. The feed speed of lumber through the spray was adjusted to a level, which combined with the specific chemical concentration would achieve the target chemical retention. Figure 13 displays steps from the spraying experiment that was done in Forintek.
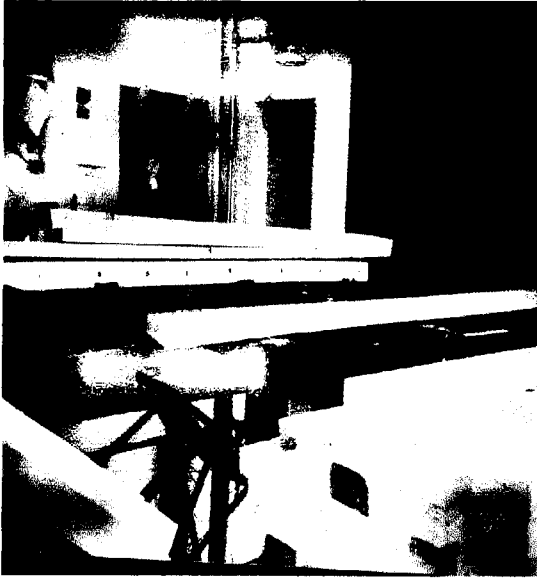
(a)
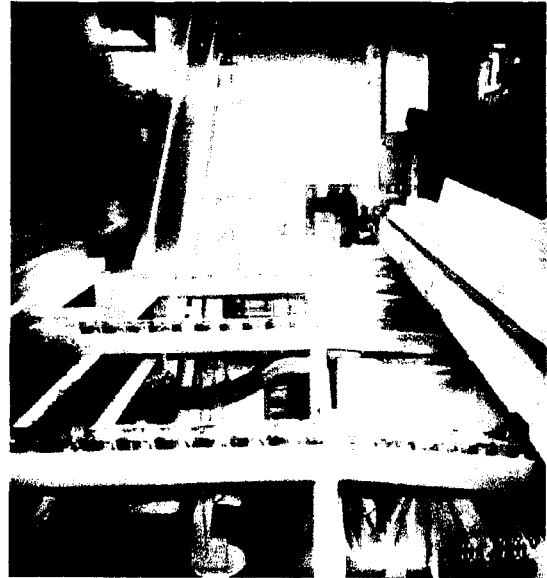
(b)
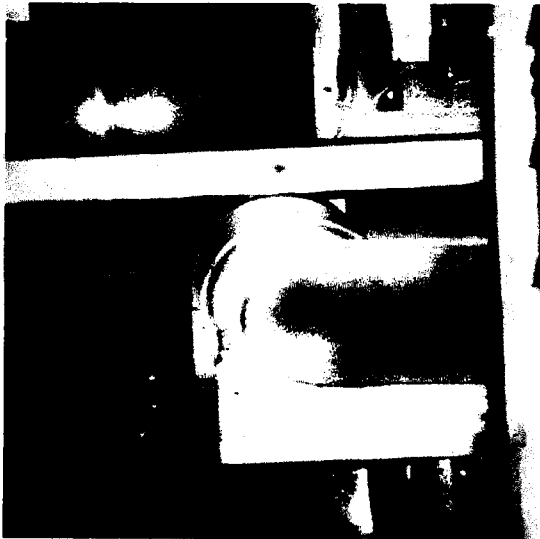
(c)
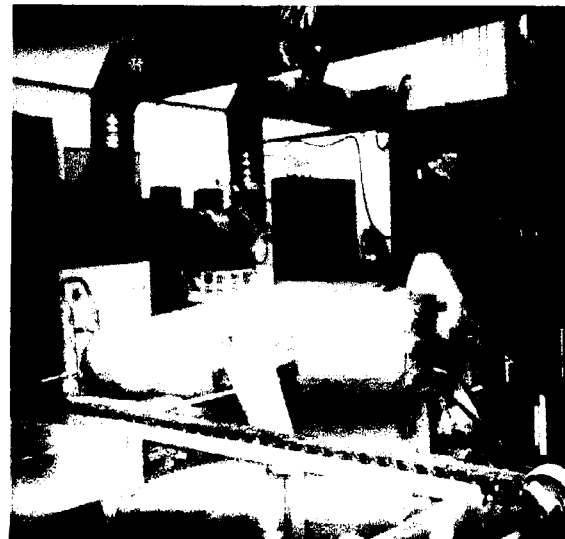
(d)

Figure 13. Forintek's Linear Spray Process System: (a) Lumber is coming into Linear Spray System; (b) System Inlet; (c) Lumber Coming out of Spray Box; (d) System Outlet

Following treatment, five 6.4 cm^2 wood surface samples were taken with a specially designed punch (Figure 14 a) from each species-target retention combination and stored in petri dishes (Figures 14 b and c). These samples were individually extracted with acetonitrile containing an internal standard and the extract was analyzed using the same HPLC analytical method.

(a)
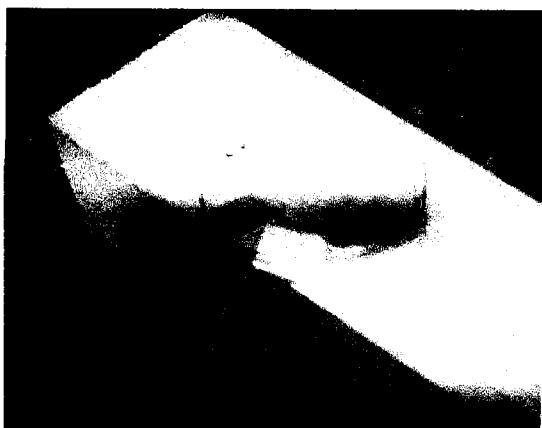
(b)
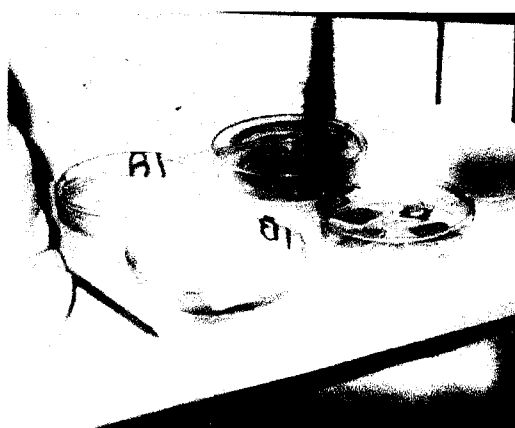
(c)

Figure 14. Surface Sampling

(a) Wood Surface Samples Taken by a Specially Designed Punch
(b) Five Samples Taken from the One Lumber Piece
(c) Samples Taken from the Four Different Species-Target Groups

7.3.1 Test Parameters and Test Evaluation

A summary of the test parameters is shown in Table 4. Samples were labeled by a code, from 1 to 5 (A or B), for each species-target retention combination, while control samples were coded as 6 for Douglas fir, and 7 for Hem-fir. From this table it is also obvious that target retention and actual retention sometimes differed from each other. Since this experiment was done for the first time using this spraying system, it was not unexpected that lumber speed and chemical concentration could not exactly predict the resulting retention.

Table 4. Summary of Test Parameters

CODE	Wood Species	Wood Surface	# of Boards	Speed (ft/min)	Spray Solution Target (and Analysed) Concentration (%DDAC w/w)	Target Retention ($\mu g/cm^2$)	Actual Retention ($\mu g/cm^2$)	Mean
7	Hem-fir	Rough	9	0	0 (0)	0	0	
6	Douglas fir	Rough	9	0	0 (0)	0	0	
5A	Hem-fir	Planed	20	400	2.2 (2.17)	60	70	57
5B	Douglas fir	Planed	20	400	2.2 (2.17)	60	44	
4B	Hem-fir	Planed	20	240	2.2 (2.11)	100	86	70
4A	Douglas fir	Planed	20	240	2.2 (2.11)	100	54	
3A	Hem-fir	Rough	20	467	5.2 (4.99)	120	130	128
3B	Douglas fir	Rough	20	467	5.2 (4.99)	120	127	
2A	Hem-fir	Planed	20	400	5.2 (4.77)	140	118	125
2B	Douglas fir	Planed	20	400	5.2 (4.77)	140	131	
1B	Hem-fir	Rough	20	400	5.9 (5.96)	160	154	166
1A	Douglas fir	Rough	20	400	5.9 (5.96)	160	177	

Wood Samples sprayed by using Forintek's spray system were stored in piles (4X5), and placed outside. Every pile had 20 samples that belonged to the same species, and had been sprayed with same concentration of the preservative (same species-target retention

combination). All together there were 20 piles with 20 samples and two piles with nine control samples.

Sources of Microorganisms:

The yard were the samples were stored was near to a forest and also near a waste water plant, so that there was high probability of infestation by different kinds of moulds and stains.

Test Evaluation

The top, bottom, left and right sides of every piece were examined weekly and the percentage of fungal growth coverage was scaled from 1 to 10, where 1 means 10% and 10 means 100%.

Chapter 8: Results and Conclusions

8.1 Results of Phase 1

Objective:

To develop guidance in making decisions about which chemicals had potential to be used in combination with DDAC against moulds and stains.

The results related to:

- microbiological testing (bioassay) of potential components

- preliminary microbiological testing of different component combinations comprising DDAC and one or more additional biocides.

Since the results of this phase will be used by Enviro-Quest Technologies Inc. in the future and represent the basis for other product patents, the information will stay confidential and all other components (not relevant for this particular study) will be called C2, Component 3, Component 4, and Component 5.

8.1.1 Microbiological Testing (Bioassay) of Potential Components

Each fungal group was represented by two species:

Mold Fungi: *Aspergillus niger* and *Gliocladium roseum* and

Sapstain Fungi: *Aereobasidium pullulans* and *Ceratocystis adiopsa*

Component 4 (an adhesive) and Component 5 (a water repellent) did not prevent fungal growth in the range of concentrations in which they were tested, but since they can improve product performance they could be used in the future, as potential additives in some new products. The bioassay results of the fungicidal components: DDAC, C2 and Component 3 are presented in Figures 15-26.
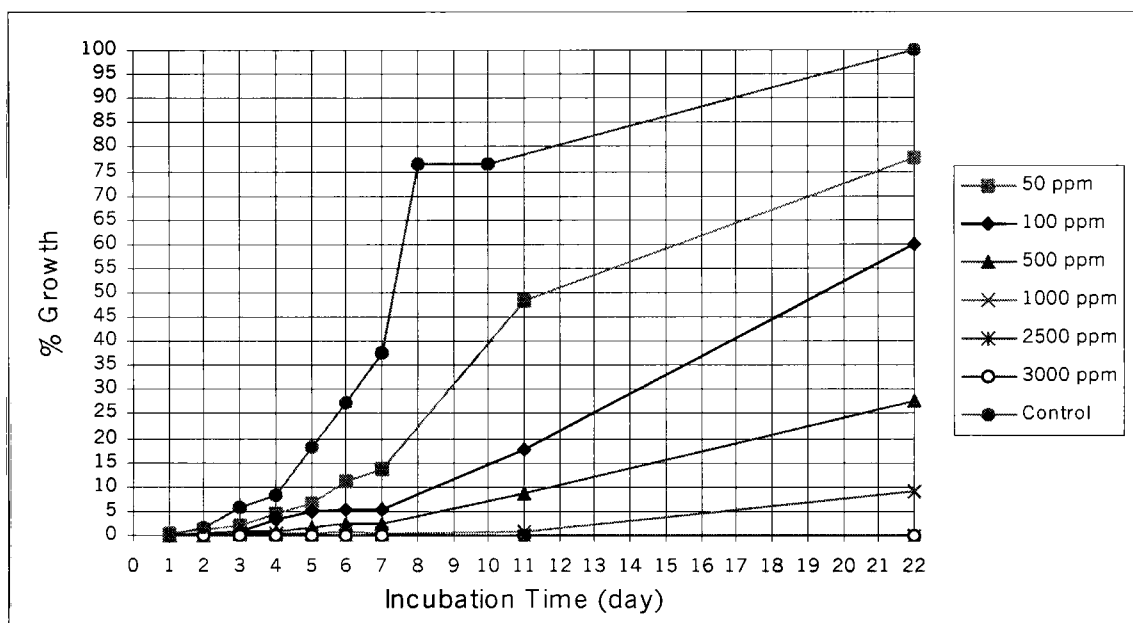
Figure 15. The growth of *G. roseum* on media containing DDAC
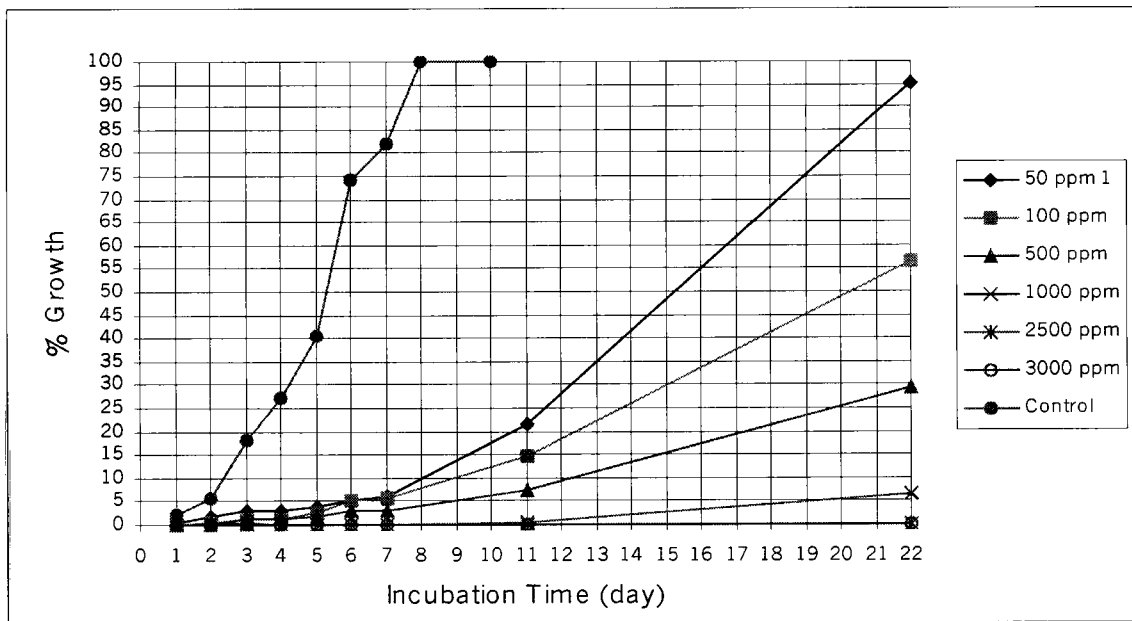
Figure 16. The growth of *A. niger* on media containing DDAC
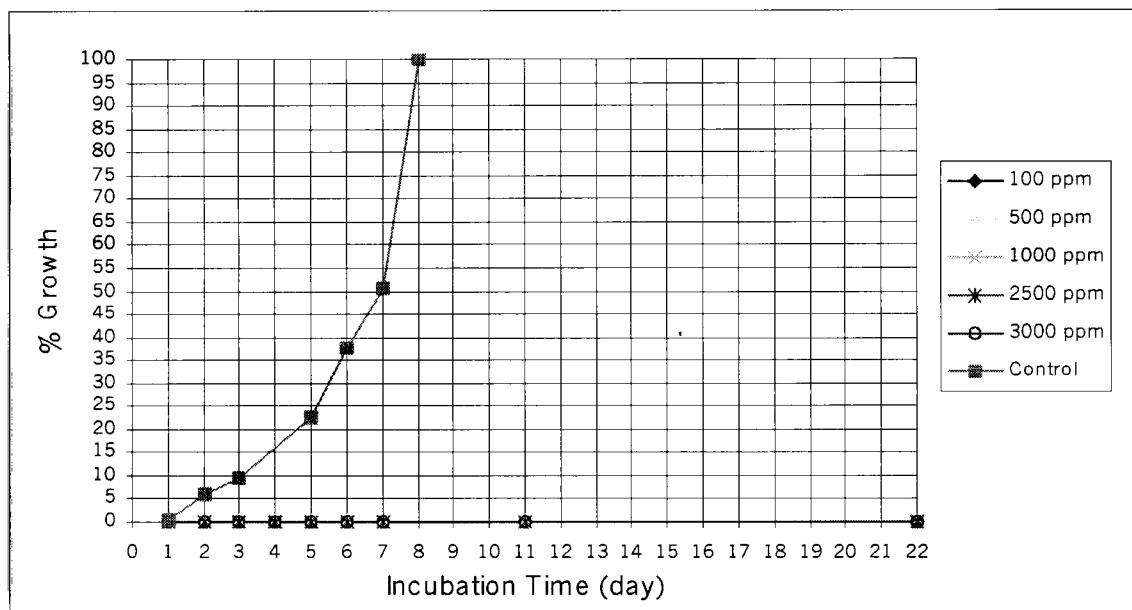
Figure 17. The growth of *A. pullulans.* on media containing DDAC
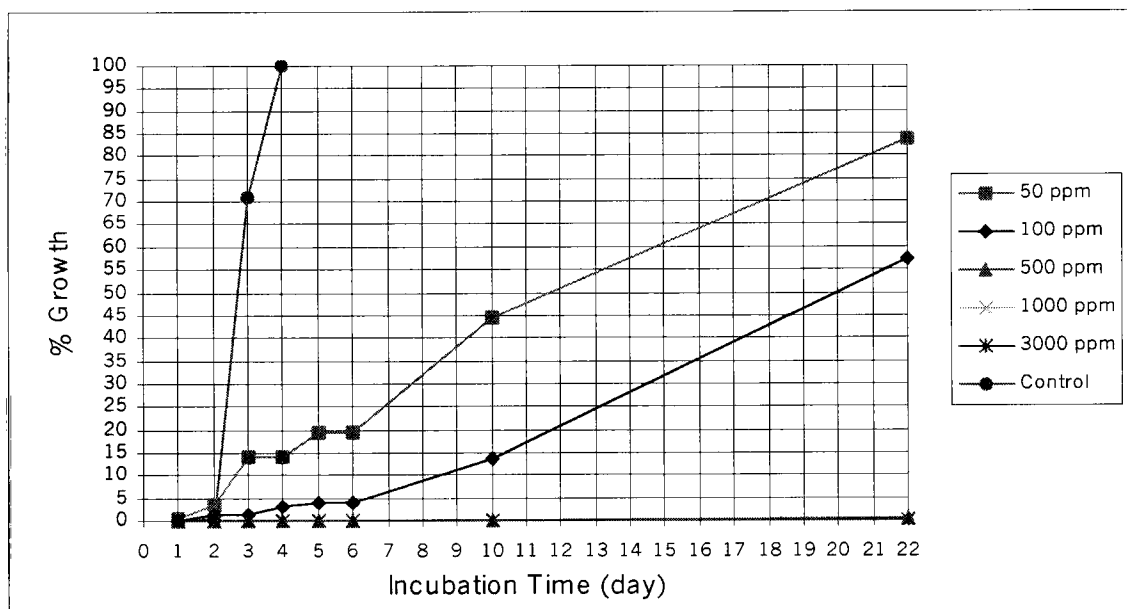
Figure 18. The growth of *C. adiopsa* on media containing DDAC
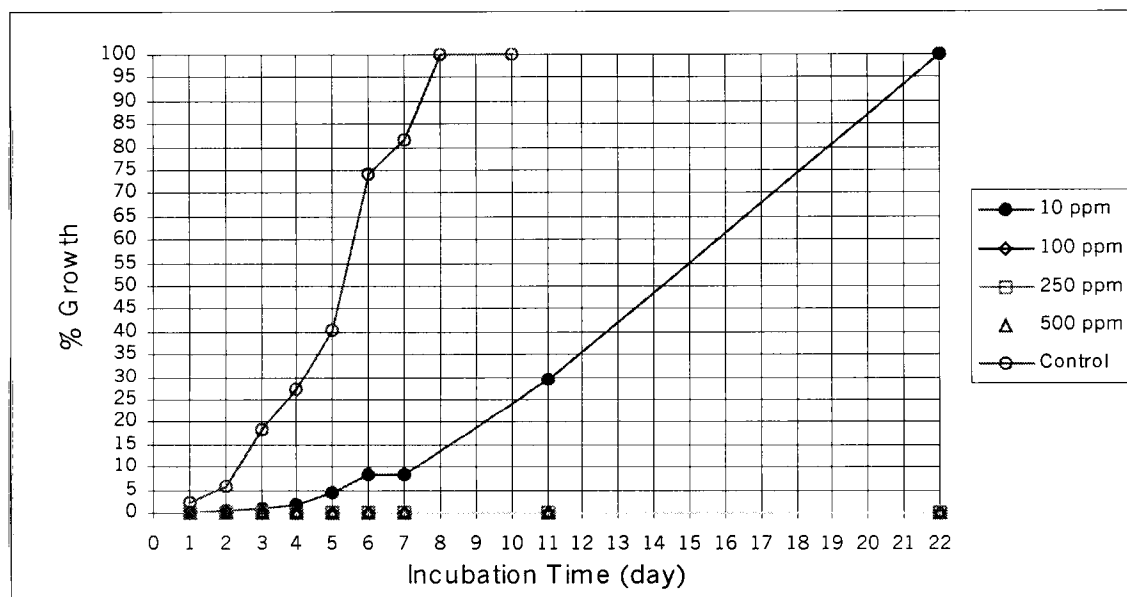
Figure 19. The growth of *A. niger* on media containing C2
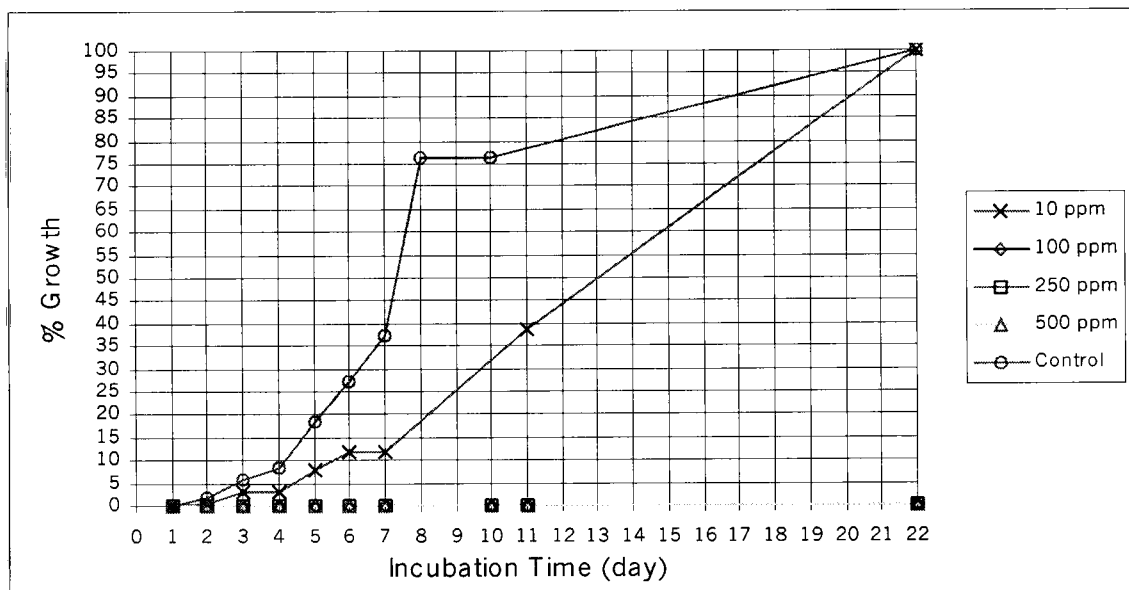
Figure 20. The growth of *G. roseum* media containing C2
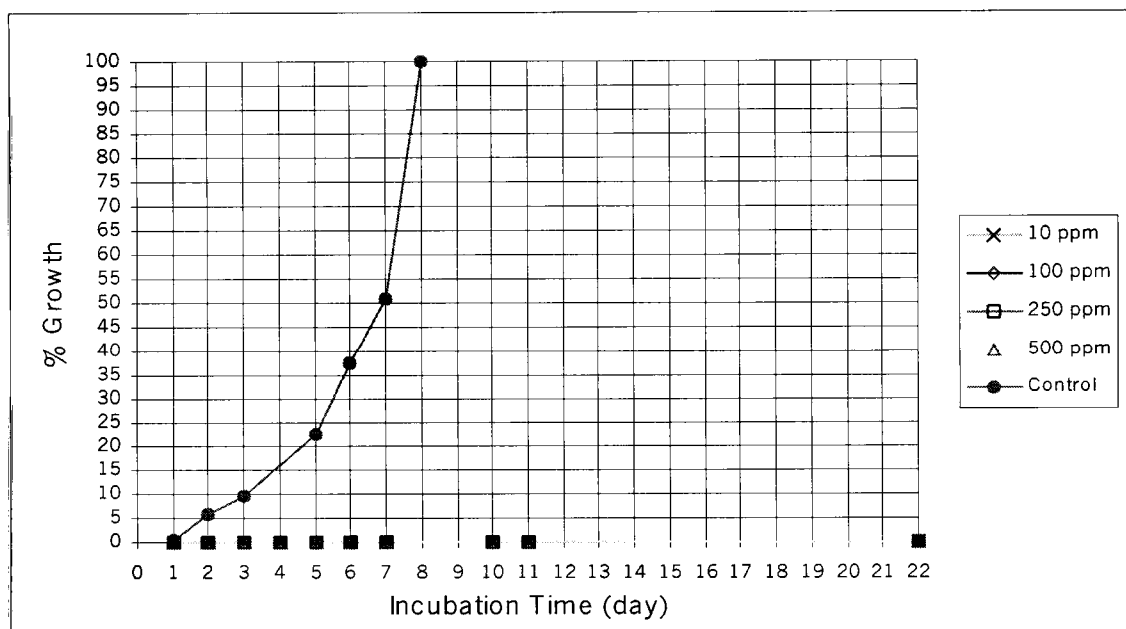
Figure 21. The growth of *A. pullulans* on media containing C2
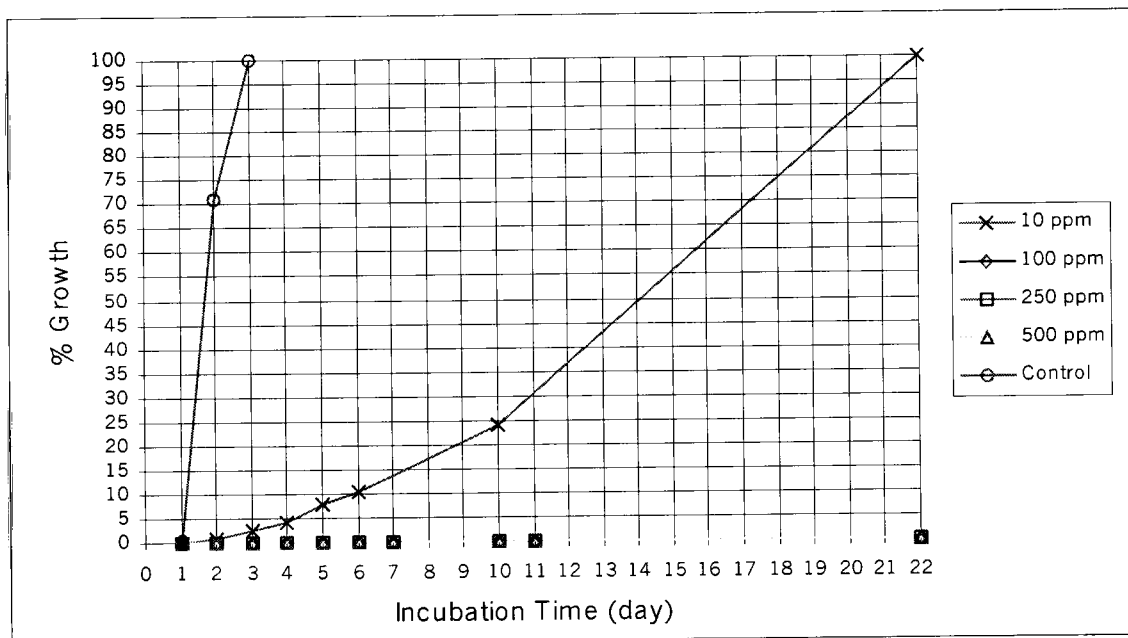
Figure 22. The growth of *C. adiopsa* on media containing C2
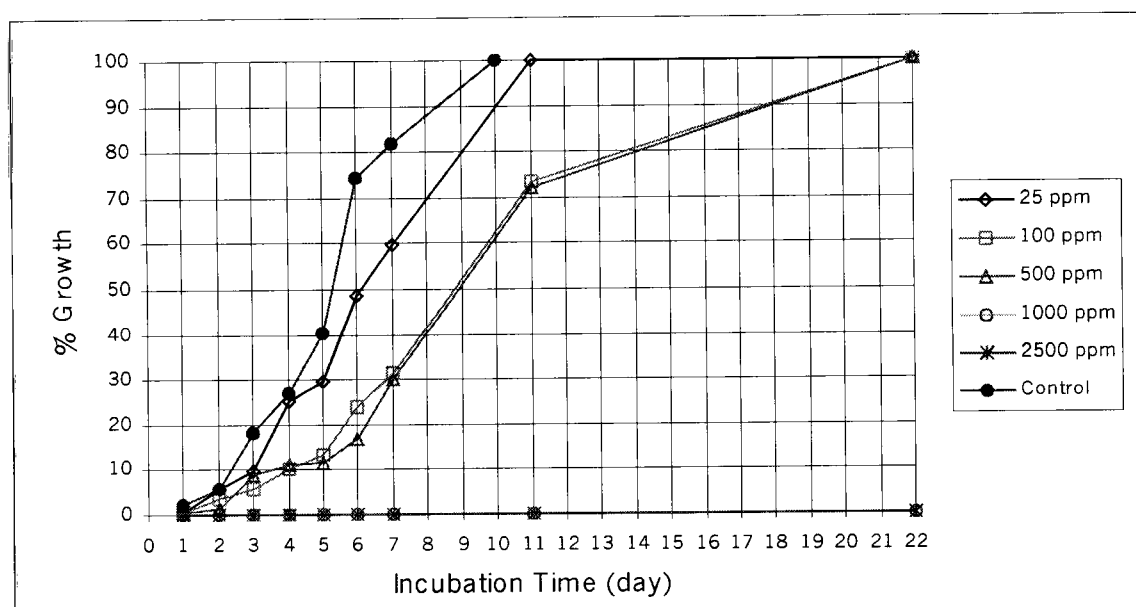
Figure 23. The growth of *A. niger* on media containing Component 3
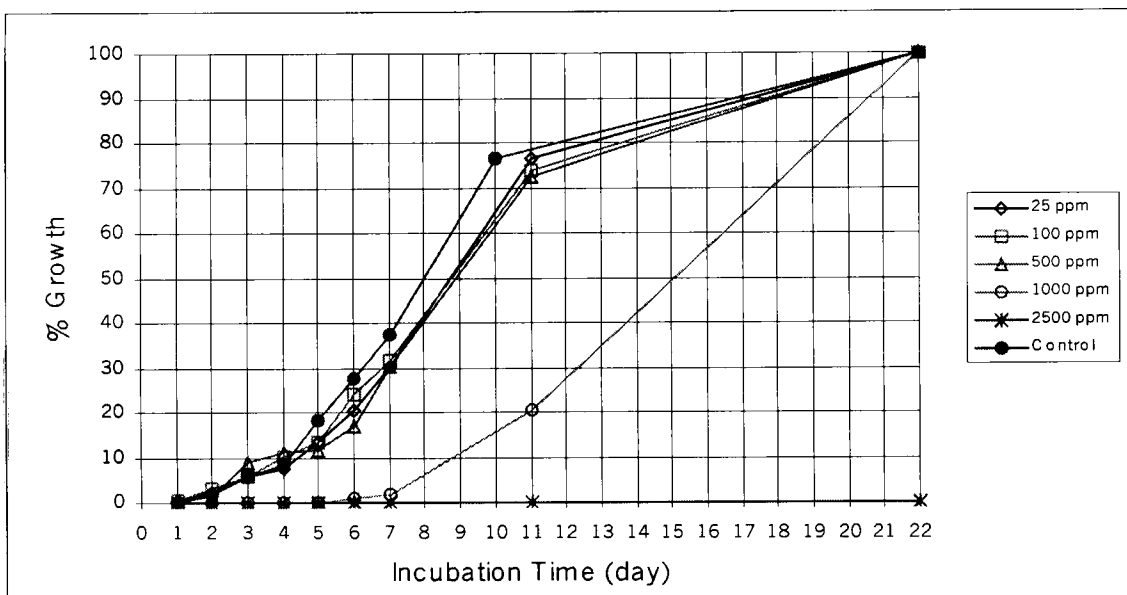
Figure 24. The growth of *G. roseum* media containing Component 3
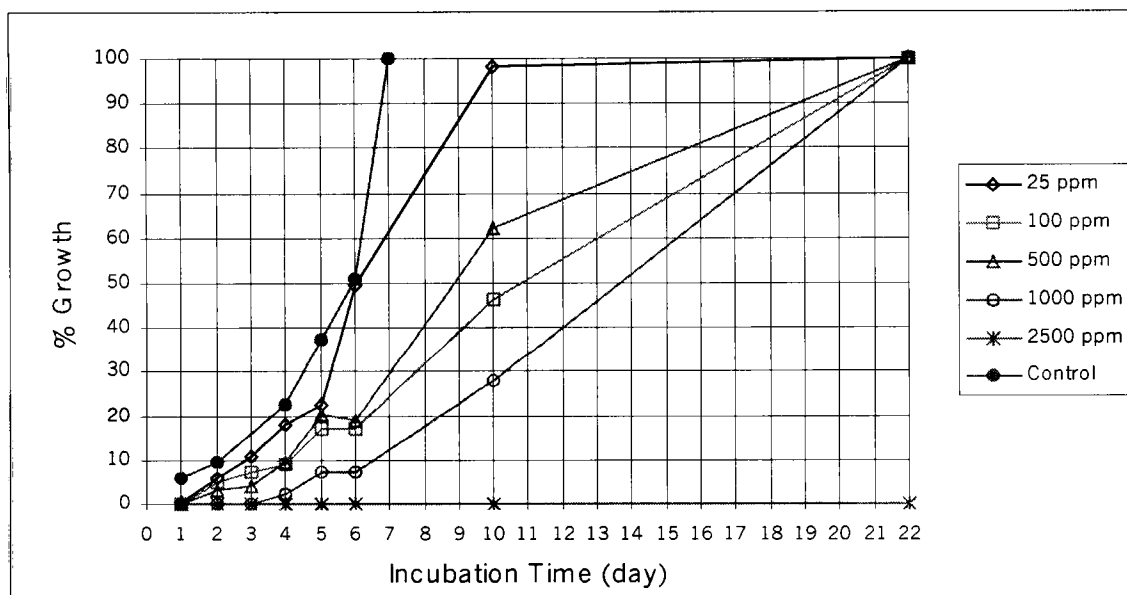
Figure 25. The growth of *A. pullulans* on media containing Component 3
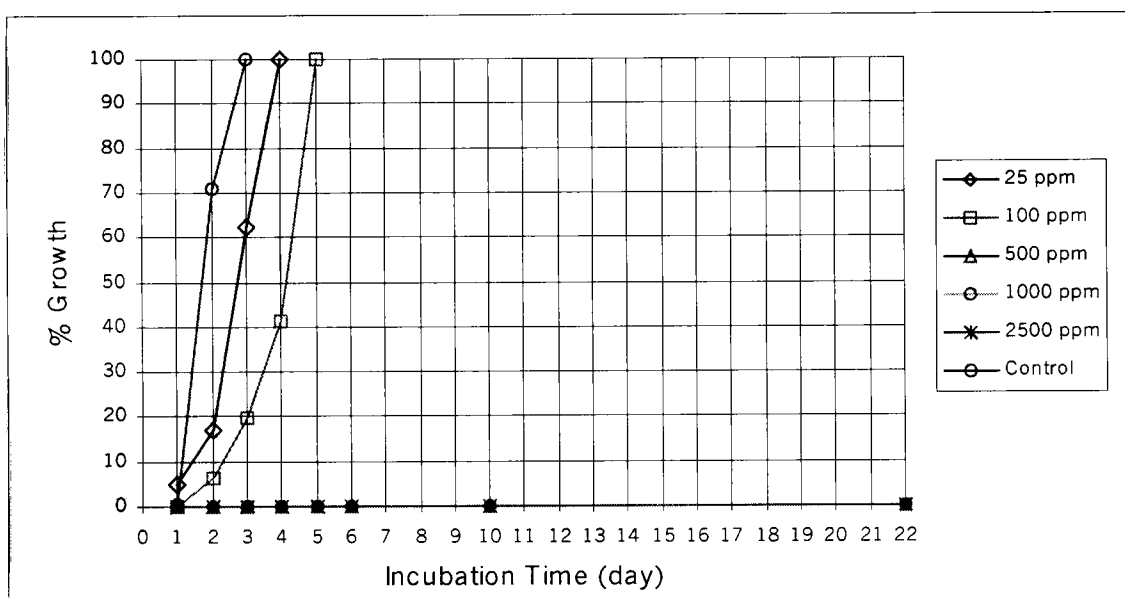
Figure 26. The growth of *C. adiopsa* on media containing Component 3

As it can be seen from Figures 15-26, C2 completely inhibited growth of all four test fungi at a concentration of 100 ppm. A second experiment was designed to determine C2 activity in the following range of concentrations: 10 ppm, 15 ppm, 25 ppm, and 50ppm. The results are presented in Table 5:

Table 5. The growth of *Aspergillus niger (1)*, *Gliocladium roseum (2)*, *Aureobasidium pullulans (3)*, and *Ceratocistis adiopsa (4)*, in the agar media containing selected concentrations of C2.

Concentration (ppm)	Fungi (#)	Incubation Period (days)								
		1	2	3	4	5	6	7	11	22
		Growth (%)								
10	1	0	1.2	3.01	7.23	9.64	9.64	16.9	43.4	86.7
15	1	0	0	0	0	0	0	0	0	0
25	1	0	0	0	0	0	0	0	0	0
50	1	0	0	0	0	0	0	0	0	0
10	2	0	0	4.22	13.3	18.1	24.1	28.9	69.9	100
15	2	0	0	4.82	9.64	12	16.9	24.1	48.2	62.7
25	2	0	0	0.8	1.61	3.21	4.02	6.43	12.9	20.9
50	2	0	0	0	0	0	0	0	0	0
10	3	0	0	0	0	0	0	0	7.23	22.5
15	3	0	0	0	0	0	0	0	0	0
25	3	0	0	0	0	0	0	0	0	0
50	3	0	0	0	0	0	0	0	0	0
10	4	0	0	0	0	0	0	0	0	0
15	4	0	0	0	0	0	0	0	0	0
25	4	0	0	0	0	0	0	0	0	0
50	4	0	0	0	0	0	0	0	0	0

8.1.2 Conclusions and Comments

8..1.2.1 Efficacy Against Moulds

Aspergillus niger and *Gliocladium roseum* were the moulds utilized in this study. *Aspergillus niger* is commonly used in bioassays. It can be seen from the results that as the concentrations of DDAC, C2, and Component 3, increased, colony quantity and size were effectively reduced. However, the control plates showed normal growth and were totally covered after 10 days. This indicated that the inoculum used was viable and the lack of growth on the treated plates can only be attributed to the presence of biocides. The extensive sporulation, observed visually, at the lower concentrations could be an

indication of the fungus being under stress due to the presence of biocides. As the concentration increased, either the spore dispersal became inhibited or the germination of spores was inhibited, this resulted in fewer colonies. It was also observed that as concentration of the fungicides changed, the morphology of the colony also changed. That is often associated with the interaction of a fungus and its surrounding medium. From results obtained in this bioassay (graphs 16, 19 and 23) *Aspergillus niger* was completely controlled by:

DDAC	2500 ppm
C2	100 ppm
Component 3	1000 ppm

Gliocladium roseum is the mould recommended by ASTM D 4445. Doyle and Rudick (1993) also suggested this fungus because it had the capability of degrading Alkyl Ammonium Compounds in a wood matrix. The control plates indicated that the inoculum used in this test was viable. The results (Graphs 15, 20 and 23) show that as the concentrations of DDAC, C2, and Component 3 were increased, colony sizes were effectively reduced.

Gliocladium. roseum was completely controlled by:

DDAC	2500 ppm
C2	100 ppm
Component 3	2500 ppm

8.1.2.2 Efficacy Against Sapstain Fungi

The sapstain fungi utilized in this study were *Aerobasidium pullulans* and *Ceratocistis adiopsa*. It was observed that most of the biocides used in the bioassay appeared to be very effective against them. The results (Graphs 17, 21, 25, 18, 22 and 26) show that *Aerobasidium pullulans* and *Ceratocistis adiopsa* were completely controlled by:

	Aerobasidium pullulans	*Ceratocistis adiopsa*
DDAC	500 ppm	500 ppm
C2	10 ppm	100 ppm
Component 3	2500 ppm	500 ppm

8.1.2.3 C2 Efficacy

- C2 shows excellent fungicidal characteristics. In the first run we tested it in the range of concentrations of 10 ppm, 100 ppm, 250 ppm and 500 ppm. Protection was complete at 100 ppm so we repeated the test over the range of lower concentrations: 10ppm, 15ppm, 25 ppm, and 50 ppm. The results show complete protection at the level of 15-25 ppm for all fungi except *Gliocladium roseum*, which was protected against by 50 ppm. Incorporation of this component in very low concentrations could result in the definition of a cost-effective and more environmentally acceptable antisapstain product.

- Since C2 showed excellent protection against moulds and stains, the conclusion at this stage was that it should be considered as a possible second active component in combination with DDAC.

8.2 Results of Phase 2

- **Objective**:

    To estimate the relative effectiveness of DDAC and C2 against,

    Soft rot, Brown rot, White rot and DDAC tolerant organisms, and to

    examine how they functioned in combination.

The results related to:

- microbiological testing (bioassay) of potential components
- preliminary microbiological testing of different component combinations comprising of a DDAC and C2.

Each fungal group was represented by one fungi:

- **Soft rot** fungus: *Chaetomium globosum*
- **Brown rot** fungus: *Coniphora puteana* (this is also a DDAC tolerant organism)
- **White rot** fungus: *Coriolus versicolor*

To evaluate the viability of each inoculum, the control plates were examined in each test for each microorganism. As seen in Figure 27, they showed normal growth, and they indicate that the inoculum used was viable. Consequently, any lack of growth on the treated plates can only be attributed to the presence of fungicides.
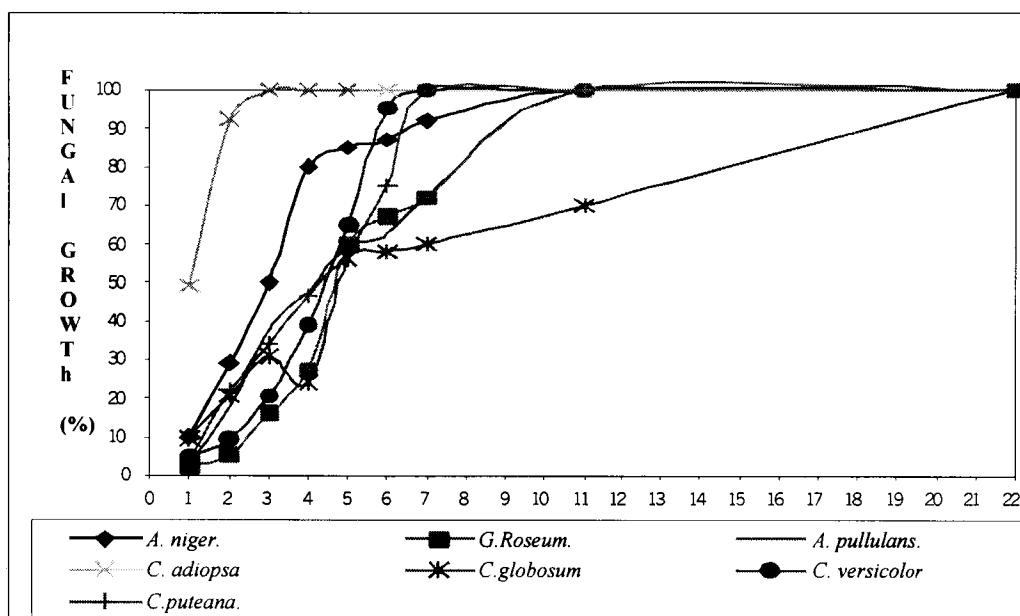
Figure 27. Fungal Growth on Control Plates

8.2.1 DDAC Efficacy

Efficacy results against *Aspergillus niger (1)*, *Gliocladium roseum (2)*, *Aureobasidium pullulans (3)*, *Ceratocistis adiopsa (4)*, *Chaetomium globosum (5)*, *Coriolus Versicolor (6)*, and *Coniophora Puteana (7)* in the agar media containing selected concentrations of DDAC are presented in Table 6. All of these tests were run at concentrations of 250, 500, 750, 1000, 2000, 2250 and 2500 ppm. At some level in each

case there was no growth. While tests above this no growth level were done, they are not

included in Table 6, since they merely continue to show no growth.

Table 6. DDAC Efficacy Against Moulds, Stains, Soft , White and Brown Rot

Concentration (ppm)	Fungi (#)	Incubation Period (days)								
		1	2	3	4	5	6	7	11	22
		Growth (%)								
250	1	0.24	1.2	2.41	3.61	6.02	9.64	11.2	20.9	45.8
500	1	0.24	0.48	1.2	2.17	3.21	5.22	7.23	16.9	35.7
750	1	0.24	0.48	0.64	0.88	1.2	2.41	3.61	9.24	23.7
1000	1	0.24	0.32	0.32	0.4	0.72	1.2	2.41	28.1	16.9
2000	1	0	0	0	0	0	0	0	0	0
2500	1	0	0	0	0	0	0	0	0	0
250	2	0.24	0.48	2.01	7.63	10.4	13.7	17.3	16.1	49.8
500	2	0.24	0.24	1.12	6.02	6.83	7.63	11.6	11.2	36.9
750	2	0.24	0.24	2.01	4.42	5.22	6.02	8.03	10	27.9
1000	2	0	0.24	1.2	2.81	3.61	4.82	6.83	10	24.3
2000	2	0	0	0	0	0	0	0	0	0
2500	2	0	0	0	0	0	0	0	0	0
250	3	0	0	0	0	0	0	0	0	4.82
500	3	0	0	0	0	0	0	0	0	2.41
750	3	0	0	0	0	0	0	0	0	0
2500	3	0	0	0	0	0	0	0	0	0
250	4	0	0	0	0	0	0	0	0	0
1000	4	0	0	0	0	0	0	0	0	0
2500	4	0	0	0	0	0	0	0	0	0
250	5	4.82	5.62	5.62	6.43	7.23	8.03	10	10	13.3
500	5	0.8	0.8	1.2	2.41	2.81	2.81	2.41	4.82	6.83
750	5	0.8	0.8	2.01	2.01	2.81	3.61	4.42	4.42	4.82
1000	5	0	0	0.8	0.8	1.2	1.2	1.2	1.2	2.81
2000	5	0	0	0	0	0	0	0	0	0
2500	5	0	0	0	0	0	0	0	0	0
250	6	1.2	1.2	3.61	6.83	9.24	10.4	11.2	15.3	36.9
500	6	0	1.2	1.2	1.2	2.41	4.02	4.82	8.84	18.7
750	6	0	0	0	0	0	0.8	0.8	0.8	4.82
1000	6	0	0	0	0	0	0.4	0.4	1.2	1.2
2000	6	0	0	0	0	0	0	0	0	0
2500	6	0	0	0	0	0	0	0	0	0
250	7	0	0	1.2	1.2	1.2	1.2	1.2	1.2	1.2
500	7	0	0	0.8	1.2	1.2	1.2	6.02	7.23	8.84
750	7	0	0	1.61	2.41	2.01	3.21	3.21	5.22	12.9
1000	7	0	0	0	0	0.4	1.61	3.21	6.43	25.7
1250	7	0	0	0	0	4.82	9.64	14.5	19.3	38.6
1500	7	0	0	0	0	1.2	4.82	9.64	16.9	27.7
2000	7	0	0	0	0	1.2	2.41	4.82	9.64	12
2250	7	0	0	0	0	0	0	1.2	2.41	4.82
2500	7	0	0	0	0	0	0	0	0	0

Test plates that had 2500 ppm of DDAC in the agar solutions did not show growth of any of seven tested microorganisms. The most resistant fungus to DDAC alone was *Coniphora puteana.*

8.2.2 C2 Efficacy

Table 7. C2 Efficacy Against Moulds, Stains, Soft, White and Brown Rot

Concentration (ppm)	Fungi (#)	Incubation Period (days)								
		1	2	3	4	5	6	7	11	22
		Growth Rate (%)								
10	1	0	1.2	3.01	7.23	9.64	9.64	16.9	43.4	86.7
15	1	0	0	0	0	0	0	0	0	0
25	1	0	0	0	0	0	0	0	0	0
50	1	0	0	0	0	0	0	0	0	0
10	2	0	0	4.22	13.3	18.1	24.1	28.9	69.9	100
15	2	0	0	4.82	9.64	12	16.9	24.1	48.2	62.7
25	2	0	0	0.8	1.61	3.21	4.02	6.43	12.9	20.9
50	2	0	0	0	0	0	0	0	0	0
10	3	0	0	0	0	0	0	0	7.23	22.5
15	3	0	0	0	0	0	0	0	0	0
25	3	0	0	0	0	0	0	0	0	0
50	3	0	0	0	0	0	0	0	0	0
10	4	0	0	0	0.	0	0	0	0	0
15	4	0	0	0	0	0	0	0	0	0
25	4	0	0	0	0	0	0	0	0	0
50	4	0	0	0	0	0	0	0	0	0
10	5	0	0.6	1.2	2.41	2.41	3.01	3.01	8.43	13.3
15	5	0	0	0	0	0	0	0	0	0
25	5	0	0	0	0	0	0	0	0	0
50	5	0	0	0	0	0	0	0	0	0
10	6	0	0	0	0	0	0	8.03	17.7	100
15	6	0	0	0	0	0	0	1.61	8.03	33.3
25	6	0	0	0	0	0	0	0	0	0
50	6	0	0	0	0	0	0	0	0	0
10	7	0	0	0	0	0	0	0	0	0
15	7	0	0	0	0	0	0	0	0	0
25	7	0	0	0	0	0	0	0	0	0
50	7	0	0	0	0	0	0	0	0	0

Table 7 show the growth of *Aspergillus niger (1), Gliocladium roseum (2), Aureobasidium pullulans (3), Ceratocistis adiopsa (4), Chaetomium globosum (5),*

Coriolus versicolor (6), and Coniphora puteana (7) on agar media containing selected concentrations of C2 . 50 ppm inhibited all growth of all the fungi tested. *Gliocladium roseum* was the most resistant. For all the other fungi, 25 ppm inhibited all growth.

8.2.3 Resistance to DDAC

According to the results from Table 6, *Coniophora puteana* showed increasing growth as the concentration of DDAC increased, implying that either DDAC was ineffective or that *Conoiphora puteana* could metabolize it. This phenomena is graphically presented in Figure 28:
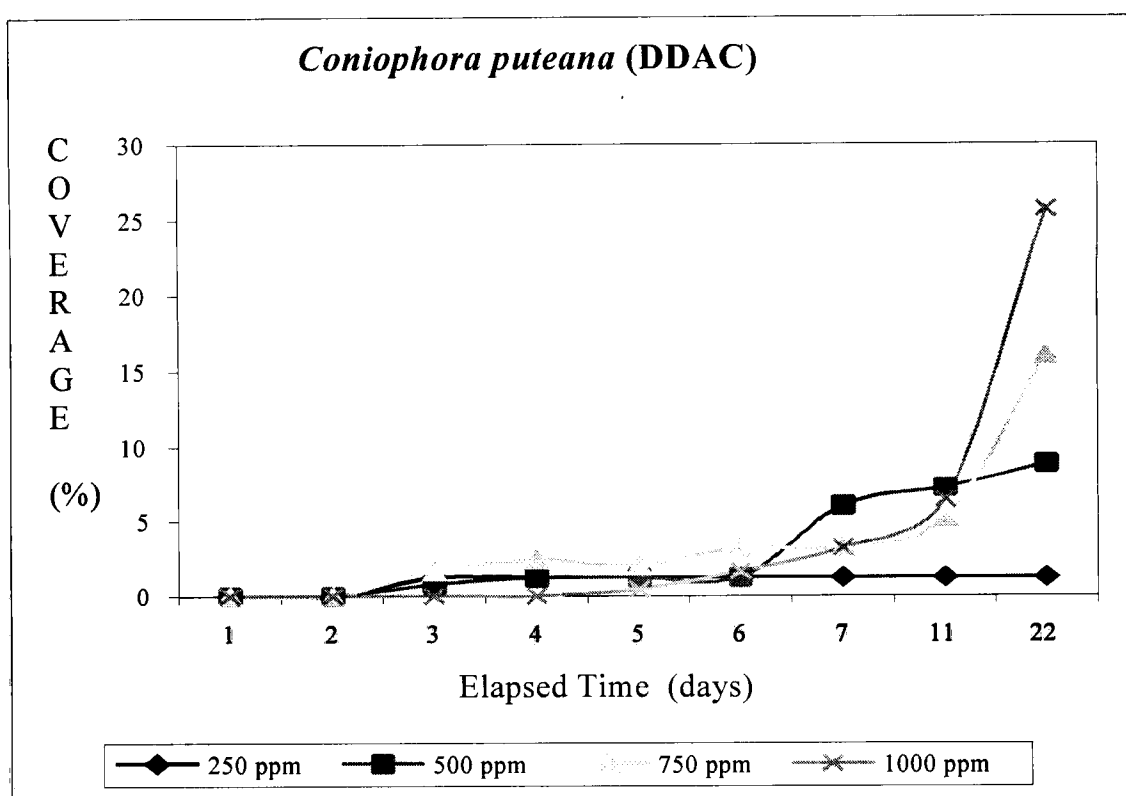
Figure 28. *Conoiphora puteana* **growth on the agar media containing various concentrations of DDAC**

Since according to Table 7, C2 showed excellent protection against *Coniophora puteana* at very low concentrations, the next step was to try to counteract this resistance to DDAC and broaden the activity spectrum of the preservative by adding C2. A selected amount of C2 was mixed with DDAC. The results, which represent the growth of *Coniophora puteana* on the agar media containing combinations of selected concentrations of DDAC and C2 are presented in Table 8.

These results indicate that low concentrations of C2 (5 and 7.5 ppm) were ineffective even after combined with a DDAC concentration of 1000 ppm. 10 ppm of C2 with 250 ppm of DDAC was also ineffective but all other combinations were completely inhibitory to the growth of *Coniophora puteana*. The amount of C2 required to inhibit growth was relatively low compared to the amount of DDAC used. Thus C2 was able to broaden the spectrum of the antifungal action of DDAC.

Table 8. The Growth Rate of *Coniophora puteana* on the Media Containing Selected Combinations of DDAC and C2

Mixture Concentration		Incubation Period (days)								
DDAC (ppm)	C2 (ppm)	1	2	3	4	5	6	7	11	22
		Growth (%)								
2500	150	0	0	0	0	0	0	0	0	0
750	10	0	0	0	0	0	0	0	0	0
750	15	0	0	0	0	0	0	0	0	0
750	25	0	0	0	0	0	0	0	0	0
500	10	0	0	0	0	0	0	0	0	0
500	15	0	0	0	0	0	0	0	0	0
500	25	0	0	0	0	0	0	0	0	0
1000	10	0	0	0	0	0	0	0	0	0
1000	15	0	0	0	0	0	0	0	0	0
1000	25	0	0	0	0	0	0	0	0	0
250	10	0	0	0.8	1.61	2.41	2.41	3.21	6.43	16.1
250	15	0	0	0	0	0	0	0	0	0
250	25	0	0	0	0	0	0	0	0	0
100	25	0	0	0	0	0	0	0	0	0
1000	5	0	0	0	2.41	4.02	4.82	9.24	18.1	39
100	15	0	0	0	0	0	0	0	0	0
750	7.5	0	0	0	0.4	0.8	3.21	6.02	10.4	22.5
1000	7.5	0	0	0	0	0.4	2.41	3.21	7.23	17.7
500	7.5	0	0	0	0	2.41	4.02	4.82	6.43	27.1

8.2.4 Synergism in Mixtures of DDAC and C2

The next experiment was designed to examine how different combinations of DDAC and C2 functioned together in mixtures to explore their possible synergistic effects.

The data presented in Tables 9.1 to 9.7 are examples of combinations of DDAC and C2 that anticipated a synergistic effect. They indicate that relatively low concentrations of C2 enhanced the fungicidal activity of DDAC and that this enhancement extended over a wide range of concentrations.

Table 9-1. Synergistic anti-*Aspergilus niger* activity of combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	15	33.33	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	75	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	10	100	1010	99	0.99	0.4	0.2	0.6	-0.22
1000	15	66.66	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-2. Synergistic anti-*Gliocladium roseum* activity of various combinations of DDAC(A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	15	16.667	265	94.3	5.66	0.1	0.3	0.4	-0.4
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-3. Synergistic anti-*Aurobasidium pullulant*s activity of various combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	15	6.6667	115	87	13	0.04	0.3	0.34	-0.47
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	15	16.667	265	94.3	5.66	0.1	0.3	0.4	-0.4
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	10	50	510	98	1.96	0.2	0.2	0.4	-0.4
500	15	33.333	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	75	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	10	100	1010	99	0.99	0.4	0.2	0.6	-0.22
1000	15	66.667	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-4. Synergistic anti-*Ceratocistis adiopsa* activity of various combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	15	6.6667	115	87	13	0.04	0.3	0.34	-0.47
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	10	50	510	98	1.96	0.2	0.2	0.4	-0.4
500	15	33.333	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	75	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	10	100	1010	99	0.99	0.4	0.2	0.6	-0.22
1000	15	66.667	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-5. Synergistic anti-*Chaetomium globosum* activity of various combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	15	33.333	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	75	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	15	66.667	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-6. Synergistic anti-*Coriolus versicolor* activity of various combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	15	6.6667	115	87	13	0.04	0.3	0.34	-0.47
100	25	4	125	80	20	0.04	0.5	0.54	-0.27
250	15	16.667	265	94.3	5.66	0.1	0.3	0.4	-0.4
250	25	10	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	10	50	510	98	1.96	0.2	0.2	0.4	-0.4
500	15	33.333	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	20	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	75	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	50	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	30	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	10	100	1010	99	0.99	0.4	0.2	0.6	-0.22
1000	15	66.667	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	40	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

Table 9-7. Synergistic anti-*Coniophora puteana* activity of various combinations of DDAC (A) and C2 (B)

Quantity producing end points				Mixture %		Ratios			Synergy Index
QA (ppm)	QB (ppm)	Ratio	Total	A	B	QA/Qa	QB/Qb	SUM	
0	50		**50**	0	100	0	1	1	0
100	15	100/15	115	87	13	0.04	0.3	0.34	-0.47
100	25	100/25	125	80	20	0.04	0.5	0.54	-0.27
250	15	250/25	265	94.3	5.66	0.1	0.3	0.4	-0.4
250	25	250/25	275	90.9	9.09	0.1	0.5	0.6	-0.22
500	10	500/10	510	98	1.96	0.2	0.2	0.4	-0.4
500	15	500/15	515	97.1	2.91	0.2	0.3	0.5	-0.3
500	25	500/25	525	95.2	4.76	0.2	0.5	0.7	-0.15
750	10	750/10	760	98.7	1.32	0.3	0.2	0.5	-0.3
750	15	750/15	765	98	1.96	0.3	0.3	0.6	-0.22
750	25	750/25	775	96.8	3.23	0.3	0.5	0.8	-0.1
1000	10	1000/10	1010	99	0.99	0.4	0.2	0.6	-0.22
1000	15	1000/15	1015	98.5	1.48	0.4	0.3	0.7	-0.15
1000	25	1000/25	1025	97.6	2.44	0.4	0.5	0.9	-0.05
2500	0	0	**2500**	100	0	1	0	1	0

8.2.5 Conclusions and Comments

Table 9.1 shows that all of the combinations of C2 and DDAC used displayed synergistic effects against *Aspergilus niger*, since all of the values of the synergy index (see Section 7.1 for definition) were negative.

Table 9.2 shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Gliocladium roseum* since all of the values of the synergy index were negative.

Table 9.3 shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Aurobasidium pullulans* since all of the values of the synergy index were negative.

Table 9.4 shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Ceratocistis adiopsa* since all of the values of the synergy index were negative.

Table 9.5 shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Chaetomium globosum* since all of the values of the synergy index were negative.

Table 9.6 shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Coriolus versicolor* since all of the values of the synergy index were negative.

Table 9.7 *Coniophora puteana* shows that all of the combinations of C2 and DDAC displayed synergistic effects against *Aurobasidium pullulans* since all of the values of the synergy index were negative.

In the summary all of the presented combinations of C2 and DDAC resulted in synergy. Thus the combinations were more effective than the individual components at the same concentrations as used in the mixtures.

8.3 Results of Phase 3

Objective:

To define the product dilution (minimum preservative concentration) that would be effective against sapstain fungi and moulds on wood.

The results were obtained by using:

- A modified ASTM D445 test on commercial wood (Douglas Fir) obtained

  from a local saw-mill

- The microorganisms tested in these experiments ar listed in table 10 :

Table 10. Microorganisms used in the experiments

#	MICROORGANISM
	Moulds
1	Aspergillus niger
2	Gliocladium roseum
	Sapstains
3	Aerobasidium pullulans
4	Ceratocistis addiopsa.
	Soft rot
5	Chaetomium globosum
	White rot
6	Coriolus versicolor
	Brown rot (DDAC tolerant microorganism)
7	Conoiphora puteana

8.3.1 Microbiological Screening Test Using Agar Blocks

8.31.1 Efficacy of the Preservative Containing 20.1% Active Ingredient

- Ten specimens per concentration of formulation for each fungus tested were used.

- Ten untreated control specimens were used for each fungus tested.

- The lowest concentration of formulation was selected to be the concentration of the

  product diluted in the ratio 1:80 (it was expected that the concentration of active

  component when the product was diluted 80 times would not provide satisfactory

fungal protection). Each of the following concentrations was twice the following concentration (1:10, 1:20, 1:40, 1:80).

- The concentrations of active ingredient for the tests on wood samples were about ten times higher than the concentrations of active ingredient when the experiments were done on agar plates.

- The wood samples were immersed into a prepared solution of the preservative for 15 sec. Similarly, untreated control specimens were treated with water. (ASTM D445)

8.3.1.1.1 Inoculated Agar Strips Placed on Absorbent Paper

Tables 11.1 to 11.5 show fungal growths on wood samples treated with various concentrations of preservative. There were two wood samples in every petri dish (see Figure 10, section 7.2). Samples that were just immersed in water and placed near samples treated with preservative were called "blank"; samples that were immersed in various preservative dilutions were called 'treated". For every microorganisms group that was examined, there were three replicas of "control" samples. Two control samples were immersed in water, infested with certain microorganisms, placed in a petri dish and examined in the same manner as all of the other samples.

Table 11.1. Fungal Growth on Wood Samples Treated with 10 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:10/Samples placed on agar strips					
Active ingredient after dilution: 20,100 ppm					
Type of Fungi	Sample Type in Petri Dish	Blank (% Growth)	Treated (% Growth)	Control (% Growth)	No. of Replicas
2 Moulds	Blank +Treated	0	0	4.2	3
2 Stains	Blank +Treated	0	0	4.8	3
2 Moulds	2 Treated	N/A	0	4.75	3
2 Stains	2Treated	N/A	0	4.5	3

Table 11.2. Fungal Growth on Wood Samples Treated with 15 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:15/Samples placed on agar strips					
Active ingredient after dilution: 13,400 ppm					
Type of Fungi	Sample Type in Petri Dish	Blank (% Growth)	Treated (% Growth)	Control (% Growth)	No. of Replicas
2 Molds	Blank +Treated	0	0	4.2	3
2 Stains	Blank +Treated	0	0	4.8	3
2 Molds	2 Treated	N/A	0	4.75	3
2 Stains	2 Treated	N/A	0	4.5	3

Table 11.3. Fungal Growth on Wood Samples Treated with 20 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:20/Samples placed on agar strips					
Active ingredient after dilution: 10,500 ppm					
Type of Fungi	Sample Type in Petri Dish	Blank (% Growth)	Treated (% Growth)	Control (% Growth)	No. of Replicas
2 Molds	Blank +Treated	0	0	4.2	3
2 Stains	Blank +Treated	0	0	4.8	3
2 Molds	2 Treated	N/A	0	4.75	3
2 Stains	2 Treated	N/A	0	4.5	3

Table 11.4. Fungal Growth on Wood Samples Treated with 40 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:40/Samples placed on agar strips Active ingredient after dilution: 5,250 ppm					
Type of Fungi	Sample Type in Petri Dish	Blank (% Growth)	Treated (% Growth)	Control (% Growth)	No. of Replicas
2 Molds	Blank +Treated	0	0	4.2	3
2 Stains	Blank +Treated	0	0	4.8	3
2 Molds	2Treated	N/A	0	4.75	3
2 Stains	2Treated	N/A	0.3	4.5	3

Table 11.5. Fungal Growth on Wood Samples Treated with 80 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:10/Samples placed on agar strips Active ingredient after dilution: 2,625 ppm					
Type of Fungi	Sample Type in Petri Dish	Blank (% Growth)	Treated (% Growth)	Control (% Growth)	No. of Replicas
2 Molds	Blank +Treated	3.5	2	4.2	3
2 Stains	Blank +Treated	4	3.5	4.8	3
2 Molds	2 Treated	N/A	1.75	4.75	3
2 Stains	2 Treated	N/A	0.3	4.5	3

Tables 11.1~ 11.5 show that the growth of the test organisms was completely inhibited at active ingredient concentrations greater than 5250 ppm. At 2625 ppm growth occurred on the treated samples

8.3.1.1.2 Samples inoculated by suspension of spores

Tables 12.1 ~ 12.5 present the results of test done of wood block specimens using inoculation with spore suspension (see Section 7.2, Figure 11). The results show that

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complete protection against all of the organisms tested occurred at active ingredient concentrations of 13,400 ppm or greater.

Table 12.1. Fungal growth on Wood Samples Treated with 10 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:10/Active ingredient: 20100 ppm					
Type of Fungi.	Sample Type in Petri Dish	Average Growth on Blank	Average Growth on Treated	Average Growth on Control	No. of Replicas
2 Moulds	Blank +Treated	0	0	4	3
2 Stains	Blank +Treated	0	0	4.5	3
2 Moulds +2 Stains	Blank +Treated	N/A	0	4	3
All from Table 10	2 Treated	N/A	0	4.5	3

Table 12.2. Fungal growth on Wood Samples Treated with 15 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:15/Active ingredient: 13400 ppm					
Type of Fungi.	Sample Type in Petri Dish	Average Growth on Blank	Average Growth on Treated	Average Growth on Control	No. of Replicas
2 Moulds	Blank +Treated	0	0	4	3
2 Stains	Blank +Treated	0	0	4.5	3
2 Moulds +2 Stains	Blank +Treated	N/A	0	4	3
All Fungi from Table 10	2 Treated	N/A	0	4.5	3

Table 12.3. Fungal growth on Wood Samples Treated with 20 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:20/ Active ingredient: **10500 ppm**					
Type of Fungi.	Sample Type in Petri Dish	Average Growth on Blank	Average Growth on Treated	Average Growth on Control	No. of Replicas
2 Moulds	Blank +Treated	0	0	4	3
2 Stains	Blank +Treated	0	0	4.5	3
2 Moulds +2 Stains	Blank +Treated	N/A	0	4	3
All Fungi from Table 10	2 Treated	N/A	0.2	4.5	3

Table 12.4. Fungal growth on Wood Samples Treated with 40 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:40/ Active ingredient: 5250 ppm					
Type of Fungi.	Sample Type in Petri Dish	Average Growth on Blank	Average Growth on Treated	Average Growth on Control	No. of Replicas
2 Moulds	Blank +Treated	4.2	2.5	4	3
2 Stains	Blank +Treated	4.5	3	4.5	3
2 Moulds +2 Stains	Blank +Treated	2	1.7	4	3
All Fungi from Table 10	2 Treated	N/A	0.2	4.5	3

Table 12.5. Fungal growth on Wood Samples Treated with 80 Times Diluted Preservative

Concentrated 20.1% /Dillution:1:80/ Active ingredient: 2625 ppm					
Type of Fungi.	Sample Type In Petri Dish	Average Growth on Blank	Average Growth on Treated	Average Growth on Control	No. of Replicas
2 Moulds	Blank +Treated	3.9	3.5	4	3
2 Stains	Blank +Treated	4.2	3.9	4.5	3
2 Moulds +2 Stains	Blank +Treated	2	1.7	4	3
All Fungi from Table 10	2 Treated	N/A	1.9	4.5	3

8.3.1.2 Efficacy of 57% Active Ingredient Freshly Prepared Preservative

Inoculation: by suspension of spores along the length of one flat side of each sample.

- Ten specimens per concentration of formulation for each fungus tested were used.

- Ten untreated control specimens were used for each fungus tested.

- The lowest concentration of formulation was selected to be the concentration of the product diluted in the ratio 1:640 (it was expected that the concentration of active component when the product was diluted 640 times would not provide satisfactory fungal protection).

- Each of the following concentrations was twice the following concentration (1:20, 1:40, 1:80, 1:160, 1:320, 1:640,).

- Microorganisms used in this test were the same as presented in Table 10.

Tables 13.1 ~ 13.6 present the results of test done of wood block specimens immersed in the various dilutions of 57% concentrated preservative.

Table 13.1. Fungal Growth on Wood Samples Treated with 20 Times Diluted Preservative

Concentrated 57% /Dillution:1:20/Active ingredient: 28500 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 13.2. Fungal Growth on Wood Samples Treated with 40 Times Diluted Preservative

Concentrated 57% /Dillution:1:40/Active ingredient: 14250 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 13.3. Fungal Growth on Wood Samples Treated with 80 Times Diluted Preservative

Concentrated 57% /Dillution:1:80/Active ingredient: 7125 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 13.4. Fungal Growth on Wood Samples Treated with 160 Times Diluted Preservative

Concentrated 57% /Dillution:1:160/Active ingredient: 3562.5 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0.4	5
Stains	10	0.1	5
All Fungi from Table 10	10	1.05	5

Table 13.5. Fungal Growth on Wood Samples Treated with 320 Times Diluted Preservative

Concentrated 57% /Dillution:1:320Active ingredient: 1781.25 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	1.1	5
Stains	10	0.25	5
All Fungi From Table 10	10	0.5	5

Table 13.6. Fungal Growth on Wood Samples Treated with 640 Times Diluted Preservative

Concentrated 57% /Dillution:1:640/Active ingredient: 890.63ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	1.1	5
Stains	10	0.45	5
All Fungi From Table 10	10	0.7	5

8.3.2 Conclusions and Comments

The results show that complete protection against all of the organisms tested occurred at active ingredient concentrations of 7125 ppm or greater.

8.4 Results of Phase 4

8.4.1 Shelf Life

Objective: To confirm that the biological activity of fungicidal combinations did not deteriorate after various elapsed times

8.4.1.1 Results of Microbiological Screening Test on Agar Plates

Since the results from the previous research phases suggested that a concentration of 2500 ppm of active ingredient completely prevented fungal growth on agar plates, a number of samples (prepared in different time frames) were diluted until 2500 ppm concentration was reached in the agar solution. They were tested against the three most resistant fungi used in previous tests. Results are presented in Table 14:

Table 14. Efficacy Determination of Preservatives Prepared in Different Ranges of Elapsed Time

#	A.I. (%)	Time Elapsed from Preparation Date to Test	*Gliocladium roseum* (2500 ppm) Fungal Growth	*Aurobasidium pullulants* (2500 ppm) Fungal Growth	*Coniophora puteana* (2500 ppm) Fungal Growth	Color Change
1	20.1	27 months	0%	0%	0%	No
2	26.7	15 months	0%	0%	0%	Yes
3	20.4	14 months	0%	0%	0%	Yes
4	20.1	13 months	0%	0%	0%	No
5	20.1	10 months	0%	0%	0%	No
6	20.1	10 months	0%	0%	0%	No
7	25.1	10 months	0%	0%	0%	Yes
8	20.1	9 months	0%	0%	0%	No
9	70.3	9 months	0%	0%	0%	No
10	54.9	7 months	0%	0%	0%	Yes
11	57	6 months	0%	0%	0%	No
12	57	5 months	0%	0%	0%	No
13	57	4 days	0%	0%	0%	No
14	57	4 days	0%	0%	0%	No

8.4.1.2 Conclusions and Comments

From the results presented in Table 14 it can be seen that the products stayed biologically active for all samples even though they were used at different lengths of time after their preparation. Activity was retained for periods of storage up to more than two years. The difference was that some samples of the preservatives, after 1-3 weeks, slightly changed color.

After contacting the manufacturer of C2, they suggested adding copper nitrate (1% of the amount of second active component) to ensure its stability. After additions of copper nitrate (samples: #11, #12, #13, and #14), there was no change in color. Since a very

small amount of copper nitrate was recommended, it did not actually effect any other product characteristics (for example, did not effect the fish toxicity).

8.4.2 Results of Microbiological Screening Test on Wood Samples

The purpose of this test was to ensure that the 57% concentrated product, after three months of storage, could protect wood against fungi. A three month old chemical composition, containing 57% active ingredient, was diluted in a same way that was done with freshly prepared product (see section 8.3.1.2). The results are presented in Tables 15.1~ 15.6:

Table 15.1. Fungal Growth on Wood Samples Treated with 20 Times Diluted Preservative

Concentrated 57% /Dilution:1:20/Active ingredient: 28500 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 15.2. Fungal Growth on Wood Samples Treated with 40 Times Diluted Preservative

Concentrated 57% /Dilution:1:40/Active ingredient: 14250 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 15.3. Fungal Growth on Wood Samples Treated with 80 Times Diluted Preservative

Concentrated 57% /Dilution:1:80/Active ingredient: **7125 ppm**			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	0	5
Stains	10	0	5
All Fungi from Table 10	10	0	5

Table 15.4. Fungal Growth on Wood Samples Treated with 160 Times Diluted Preservative

Concentrated 57% /Dilution:1:160/Active ingredient: 3562.5 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	1	5
Stains	10	0.15	5
All Fungi from Table 10	10	0.85	5

Table 15.5. Fungal Growth on Wood Samples Treated with 320 Times Diluted Preservative

Concentrated 57% /Dilution:1:320 Active ingredient: 1781.25 ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	1.1	5
Stains	10	0.25	5
All Fungi from Table 10	10	1.1	5

Table 15.6. Fungal Growth on Wood Samples Treated with 640 Times Diluted Preservative

Concentrated 57% /Dilution:1:640/Active ingredient: 890.63ppm			
Microorganisms Type	Number of Treated and Control Samples	Average Fungal Growth on Treated Samples	Average Fungal Growth on Control Samples
Molds	10	1.2	5
Stains	10	0.35	5
All Fungi from Table 10	10	1.7	5

8.4.2.2 Conclusions and Comments

Comparison of the results from Tables 14.1 - 14.6 and 15.1 ~ 15.6 show that the product did not lose its ability to protect wood samples against all of the microorganisms tested, even though the set of results presented in Tables 15.1~ 15.6 present the fungal protection ability of a 3 months old preservative. These results also show that complete protection against all of the organisms tested occurred at active ingredient concentrations of 7,125 ppm or greater (same as the fresh product).

8.4.3 Definition of DDAC/C2 ratio

Objective: To have both active components in the same liquid phase, and to incorporate as much as possible of the second active component. Thus increasing amounts of a solution of C2 were added to see if a two-phase mixture was created.

8.4.3.1 Titrations

A range of DDAC solutions in water were titrated with 0.007 % active C2. The results are presented in Tables 16, 17 and 18.

Table 16. Titration of DDAC solution (70g Bardac 2280 and 30g water), with 0.007% water solution of C2

C2 Increnented (ml)	Total Volume (ml)	% of C2	% of DDAC	Mixing Time (sec)	Ratio DDAC/ C2	Mixture Appearance
25	125	0.140	44.8		320	Clear
10	135	0.181	41.5		229	Clear
4	139	0.196	40.3		205	Clear
4	143	0.210	39.2		186	Clear
1	144	0.214	38.9	10 sec	182	Cleared after a while
0.5	144.5	0.216	38.8		180	Milky

From the results presented in Table 16 it can be seen that the mixture stayed in the same liquid phase (clear liquid) if the addition of the second active component was less than 0.214 %, or if the DDAC:C2 ratio was higher than 186.

Table 17. Titration of DDAC solution (30g Bardac 2280 and 70g water), with 0.007% water solution of C2

C2 Increnented (ml)	Total Volume (ml)	% of C2	% of DDAC	Mixing Time (sec)	Ratio DDAC/ C2	Appearance of the Mixture
4	104	0.043	23.1		536	Clear
10.8	114.8	0.144	20.9		145	Clear
3.2	118	0.171	20.3		119	Clear
1	119	0.179	20.2		113	Clear
0.5	119.5	0.183	20.1	10 sec	110	Cleared after a while
0.5	120	0.187	20.0		107	Milky

From the results presented in Table 17 it can be seen that the mixture stayed in the same liquid phase (clear liquid) if the addition of the second active component was less than 0.179%, or if the DDAC: C2 ratio was higher than 112.

Table 18. Titration of DDAC solution (20g Bardac 2280 and 80g water, with 0.007% water solution of C2)

C2 Incremented (ml)	Total Volume (ml)	% of C2	% of DDAC	Mixing Time (sec)	Ratio DDAC/ C2	Appearance of the Mixture
3	103	0.033	15.5		476	Clear
6.5	109.5	0.097	14.6		150	Clear
4.5	114	0.138	14.0		102	Clear
1.5	115.5	0.150	13.9		92	Clear
1	116.5	0.159	13.7	20 sec	87	Cleared after a while
0.5	117	0.163	13.7		84	Milky

From the results presented in Table 18 it can be seen that the mixture stayed in the same liquid phase (clear liquid) if the addition of the second active component was less than 0.159%, or if the DDAC: C2 ratio was higher than 87.5.

8.4.3.2 Conclusions and Comments

According to Tables 16, 17 and 18 it can be concluded that it was possible to incorporate more C2 when DDAC/Water solution was more concentrated, before creating an emulsion. For example, the results show that in the case where the most concentrated DDAC solution (20g Bardac 2280 and 80g water) was titrated, it was possible to add to the mixture the highest amount of C2, while mixture still remained clear.

8.4.4 Results of Toxicity Experiments

LC50 is the concentration of fungicide in dilution in water that causes mortality of 50 % of the fish or daphnia in the test population after a 96 hour exposure. If the LC50 number is high, it means that the amount of the chemical in the water that it takes to cause mortality in 50 percent of the test population is also high. In other words, the higher the LC50 number the less toxic the chemical is.

8.4.4.1 96-h LC50 Rainbow Trout Bioassay on 57.5% Active Product

Method :ASTM STP 634.1077

96-h LC50 mg/L: 1.41

Table 19. Fish Toxicity Test (Rainbow Trout); BCRI Sample #20001089

	Test Concentration (mg/l)	Percent Survival 24 h	Percent Survival 48 h	Percent Survival 72 h	Percent Survival 96 h
Control	0	100	100	100	100
	5	0	0	0	0
	2	0	0	0	0
	1	100	100	100	100
	0.5	100	100	100	100
	0.1	100	100	100	100

8.4.4.2   48-h LC50 Bioassay for Daphnia Magna on 57.5% Active Product

Method : EPS 1/RM/14

48-h LC50 mg/L: 0.13

Table 20. Daphnia Magna Toxicity Test; BCRI Sample #20001089

	Test Concentration (mg/L)	Percent Survival 24 h	Percent Survival 48h
Control	0	100	100
	2	0	0
	1	0	0
	0.5	0	0
	0.25	10	0
	0.1	100	100
	0.05	100	100
	0.025	100	100

The LC_{50} for a 57.5% active ingredient was 1.41 mg/l for fish and 0.13 mg/l for daphnia (an aqutic insect). The fish toxicity results show that this formulation was less toxic than IPBC and C2 alone (0.049 mg/lit and 0.12 mg/l respectively; see section 5, Figure 8).

8.5   Results of Phase 5

8.5.1 Field Efficacy Test by Using Pilot-Plant Linear Spraying System

Objective: To find the optimal chemical coverage capable of protecting wood samples under real outside conditions.

Wood samples were transported from a mill one week after cutting, and they stayed another week in the Forintek's yard before being treated. Prior to spraying, all samples were inspected. Most of the samples were free from fungi. All samples numbered as 3B were exceptionally dirty. Furthermore, prior to spraying, samples 3B/3,

3B/13 and 3b/15 were also probably infected with fungi (see Figure 29). These three samples were monitored weekly in order to determine whether microorganisms continued to grow or not.

Samples were carefully inspected and the results presented as percentage of fungal growth on the top, bottom, left and right sides of the test specimens. In Tables 21 to 32 the abbreviations T, B, L and R represent top, bottom, left and right side of the wood sample, respectively.

Figure 29. Rough Wood Samples Ready for Spraying (It is obvious that two samples were infected)

Tables 21 and 22 present the results of samples coded as 6 (Rough Douglas Fir) and 7 (Rough Hem Fir) in Table 4 on page 61. There were nine samples of each species group, and they were coded as A.B,C, D, E, F ,G, H, and I. Tables 23~32 present fungal growth

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on the wood species sprayed over a range of preservative chemical retention . The test

parameters and conditions are also shown in Table 4 on page 61.

Table 21. Fungal Growth on Untreated Rough Douglas Fir Samples

Species: D. Fir		Rough		Retention: 0		Speed: 0		Concentration: N/A		Code:	Control 6			
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
Code	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
A	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
B	0	0	0	0	0	0	0	0	0	2	0	0	0	4	7	0.1
C	5	0	0	0	6	0	1	0	9	0	5	1	9	0	5	1
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0.1	0	0	0	0.2	2	0	0
F	0	0	0	0	0	0	0	0	1.5	0	0	0	3	0	0.2	0.2
G	6	0	0	0	7	0	0	0	8.5	0	0	0	9	0	0	0
H	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
I	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 22. Fungal Growth on Untreated Rough Western Hemlock Samples

Species: H. Fir		Rough		Retention: 0		Speed: 0		Concentration: N/A		Code:	Control 7			
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
A	0	0	0	0	0	0	0	0	1	3	0	0	1	6	2	0.4
B	6	0	0	0	8	0	0	0	9	0	2	3	9	0	2	3
C	0	0	0	0	0	0	0	0	1	0	0	0	2.5	0	0	1.5
D	0	0	2	2	0.5	2	5	5	1	4	8	7	2	5	8	7
E	0	5	0	2	0	6	1	4	1	10	2	6	2	10	2	7
F	0	0	0	0	0	0	0	0	2	0	0	1	4	0	0	2
G	0	0	0	0	0	0	0	0	2	0	4	0	2	0	6	0
H	0	0	0	0	0	0	0	0	2	0	6	0	4	0	8	0
I	0	0	0	0	0	0	0	0	0	0	6	4	0	0	6	4

Table 23. Fungal Growth on Planed Western Hemlock Samples, Treated with 70 ug/cm^2

Species: H. Fir		Planed		Retention:70		Speed: 400		Concentration: 2.17		CODE : 5A					
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 24. Fungal Growth on Planed Douglas Fir Samples, Treated with 44 ug/cm^2

Species: D. Fir		Planed		Retention:44		Speed: 400		Concentration: 2.17		CODE : 5B					
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0.5	1	0.5	0	4	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	2	1	0	0	3	1	0	0
7	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0.2	0	0.5	0.5	0.2	0	0.5	0.5
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0.2	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 25. Fungal Growth on Planed Western Hemlock Samples, Treated with 86 ug/cm^2

Species: H. Fir			Planed		Retention:86		Speed: 240		Concentration: 2.11			CODE : 4B			
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 26. Fungal Growth on Planed Douglas Fir Samples, Treated with 54 ug/cm^2

Species: D. Fir			Planed		Retention:54		Speed: 240		Concentration: 2.11			CODE : 4A			
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 27. Fungal Growth on Rough Western Hemlock Samples, Treated with 130 ug/cm^2

Species: H. Fir		Rough		Retention:130	Speed: 467		Concentration: 4.99		CODE : 3A						
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 28. Fungal Growth on Rough Douglas Fir Samples, Treated with 127 ug/cm^2

Species: D. Fir		Rough		Retention:127	Speed:467		Concentration: 4.99		CODE : 3B						
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	3	1	1	0	3	1	1	3	0	1	1	0	3	1	1
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	2	0	5	0.5	2	0	5	0.5	2	0	5	0.5	2	0	6	0.5
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0.2	0	0.1	0	0.2	0	0.1	0	0.2	0	0.1	0	0.2	0	0.1	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 29. Fungal Growth on Planed Western Hemlock Samples, Treated with 118 ug/cm^2

Species: H. Fir		Planed		Retention:118		Speed:400		Concentration: 4.77			CODE : 2A			
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	.0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 30. Fungal Growth on Planed Douglas Fir Samples, Treated with 131 ug/cm^2

Species: D. Fir		Planed		Retention:131		Speed:400		Concentration: 4.77			CODE : 3A			
Sample	Checked after 1 week			Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 31. Fungal Growth on Rough Western Hemlock Samples, Treated with 154 ug/cm^2

Species: H. Fir			Rough		Retention:154		Speed:400		Concentration: 5.96			CODE : 1B			
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 32. Fungal Growth of Rough Douglas Fir Samples, Treated with 177 ug/cm^2

Species: D. Fir			Rough		Retention:177		Speed:400		Concentration: 5.96			CODE : 1A			
Sample	Checked after 1 week				Checked after 3 weeks				Checked after 5 weeks				Checked after 6 weeks			
#	T	B	L	R	T	B	L	R	T	B	L	R	T	B	L	R
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

8.5.2 Comments and Conclusions

The results of these field tests contributed towards the answering of important questions that could not be answered under laboratory conditions. There were several elements, which contributed to the complexity of this test:

<u>Wood Species</u>

The two most common wood species from B.C. mills were used. This was done to determine the amount of preservative necessary for application and to compare which species better absorbs the preservative. Table 33, a summary of data provided from Forintek's pilot-plant tests, demonstrates that for a planed surface of the wood, and lower concentrations of chemical (~2 %), Western Hemlock absorbed the preservative more readily. Since rough samples have much larger and varying surface areas, it was not possible to come to a conclusion about which species absorbed chemical in the rough condition easier.

Table 33. The Relation Between Preservative Concentration and Achieved Retention For Douglas Fir and Western Hemlock

Concentration (%)	D. Fir Retention (ug/cm2)	W.H. Retention (ug/cm2)	Surface Type
2.17	44	70	Planed
2.11	54	86	Planed
4.99	127	130	Rough
4.77	131	118	Planed
5.96	177	154	Rough

Number and Positions of Samples

All samples of the same species that were sprayed with the same concentration were stored in piles. There were all together 20 piles with 20 pieces (4X5) plus two piles with nine wood samples (control samples). All sprayed and control piles were placed close to natural fungal sources (forest and wastewater treatment plant), and exposed to natural weather conditions.

During the experiment, samples were carefully examined from all sides (top, bottom, left and right). To ensure that every sample had a chance to be reached by fungal spores, after examination, their position in the pile was randomly changed.

Due to natural weather and field conditions, every sample had a unique opportunity to interact with fungal infestation. This is the reason why the results are not presented as an average. If fungal growth was detected in any of twenty samples from the same pile, it could mean that after a change in position or weather conditions other samples could also become infected.

For example in Table 24, the preservative retention on Douglas Fir samples was undesirably two times lower than was targeted. The targeted retention of 60 ug/cm^2 was not obtained, instead the actual retention was 44 ug/cm^2. Consequently, samples #1, #7, #14 and #16 showed some fungal growth. In Table 27, rough Douglas Fir samples #3, #13 and #15 were sprayed with 127 ug/cm2, very close to the targeted retention (120

ug/cm^2), but still fungal growth appeared. It is important to emphasize that these samples were very dirty before spraying and also might have been infested before spraying. As shown in Tables 21 and 22, there was some infestation on both control groups, so the absence of fungal growth on all other samples could be attributed to the presence of the right amounts of preservative.

Active Ingredient Concentration

The recommended active ingredient concentration for most commercial antisapstain products is about ~100 ug/cm2 (Konashhevich, 1994). The results of this field test open the door to the possibility of applying a lower amount of chemical (60-90 ug/cm2) while still providing satisfactory fungal protection. Since in comparison with other commercial products, the fish toxicity of this preservative has had encouraging results, using lower amounts of chemicals would contribute to better compliance with environmental regulations.

Chapter 9: Economic Aspects

The primary strategy in marketing a new antisapstain product is to emphasize the features, benefits and advantages as compared to the competitors' product(s).

The specific product features and benefits are the following:

- Broader spectrum of activity

- Combating resistant fungi

- Lower fish toxicity

- Environmentally and user friendly

- Cost Effective

The first four aspects are illustrated in details in previous sections of this thesis. The final consideration, which will decide whether or not the potential new formulation can be competitive on the market, is an economical analysis and cost effectiveness.

9.1 Product Cost

According to information from the literature (Konashevich, 1994), the required fungicide coverage, for the lumber protected by DDAC is 100 micrograms per square centimeter (100 ug/cm^2) of active ingredients. This coverage concentration is the critical element for

both effective protection and the cost of chemicals to the mill. Therefore competitive price comparisons were done on this basis.

Tables 34~35 show the difference in the cost of active chemicals between the New Developed Product (NDP) and the Current Major Product (CMP). The prices of chemicals were obtained from the manufacturers of the active components. As the Table shows, there is a difference of approximately 20.7% in the cost of chemicals per kilogram of product.

Table 34. Chemical Cost Comparison (per kilogram) of the New Developed Product (NDP) and the Current Major Product (CMP)

Product:	Product Cost: ($/kg)
CMP	CDN 8.55
NDP	CDN 6.78

Table 35. Chemical Cost Comparison (per liter) of NDP (*Specific Gravity: 0.96*) and CMP *(Specific Gravity: 0.93)*

Product:	Product Cost: ($/liter)
CMP	CDN 9.19
NDP	CDN 7.06

Table 36 shows the calculated difference in price when compared on the basis of chemical coverage per 1,000 board feet ("FBM"). This difference is 5%, a definite price advantage for the New Developed Product (NDP) due primarily to a lower chemical cost of goods per unit volume of product.

Table 36. Cost Comparison (per amount of active ingredient) of the New Developed Product (NDP) and the Current Major Product (CMP), per 1,000 FBM.

Chemical Cost per 1,000 FBM (in Canadian Funds)			
Desired Coverage	100 ug/cm^2	80 ug/cm^2	120 ug/cm^2
CMP NDP	6.43 6.11	5.15 4.89	7.72 7.34
NDP's Advantage:	5.0%	5.0%	5.0%

The cost calculation comparisons presented in Table 36, are based on coverage costs on 2" x 4" dimensional lumber with desired coverage ratios of 80, 100, and 120 micrograms per square centimeter (ug/cm2) of active ingredients. These calculations are presented on a per 1,000 foot board measurement (FBM or fbm) basis.

Table 37 shows the summary of Toxicological Characteristics of New Developed Product (NDP) and the Current Major Product (CMP) and their calculated chemical costs. While maintaining better fish toxicity characteristics, NDP has an important advantage in the chemical cost.

Table 37. Toxicity comparison of the New Developed Product (NDP) and the Current Major Product (CMP)

Product	Chemical Cost Per 1Kg	LC50 (mg/l) (Rainbow Trout)	Recommended Dilutions in Water
CMP	8.55	0.68-1.3	40-50
NDP	6.78	1.41	40-50

** Konashevich, 1994 provides these two values for LC$_{50}$ Rainbow Trout

Chapter 10: General Conclusions and Recommendations for

Future Work

10.1 Conclusions

The process of the development of a new antisapstain product based on the screening of potential fungicides suitable to be incorporated as a second active component in combination with DDAC, was investigated in the present study. The main observations and conclusions from this research are:

- The research resulted in the successful development of a new antisapstain product, which is capable of protecting freshly cut wood from moulds, stains, and white, soft and brown rot fungi.

- The additional biocide, which has been combined with DDAC, showed a synergistic effect with DDAC and proved that it could significantly broaden the spectrum of DDAC's fungicidal activity.

- The combination of DDAC and C2 demonstrated the capability of counteracting any resistance of tolerant microorganisms toward DDAC.

- The new antisapstain product was designed as a highly dispersible combination, which allows DDAC to serve as a vehicle, enabling full C2 coverage of, and a strong adherence to, the wood's surface.

- The combination of active components resulted in a formulation that is environmentally friendly (less toxic and damaging to the environment than the leading competitor products).

- Extremely low concentrations of the second active component C2 not only enhance the fungicidal activity of DDAC, but due to its effectiveness, reduced the amount of DDAC required, which also improves the toxicological characteristics of the composition when DDAC and C2 are combined.

- While maintaining better fish toxicity characteristics, the New Developed Product is more cost effective than the leading product in the B.C. market.

- A patent for this process has been applied for and subject to some revisions, it appears that it will be granted.

10.2 Recommendations for Future Work

The main recommendations for future work are:

- To continue field-tests through the spring, when intensive fungal growth is expected at higher humidity and elevated temperatures.

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Appendix 1

List of Fungal Cultures

List of Fungal Cultures Obtained from FORINTEK CANADA CORP.

1. Aspergillus niger

2. Coniophora puteana 9B

3. Coriolus versicolor 105E

4. Aureobasidium pullulans 132B

5. Chaetomium globosum 172B

6. Gliocladium roseum 321F

7. Ceratocystis adiopsa

Appendix 2

Bardac 2250 & Bardac 2280 Manufacturer Specifications

LONZA

LONZA INC.
Corporate Headquarters
17-17 Route 208
Fair Lawn. N.J. 07410
(201) 794-2400

Specialty Chemicals

BARDAC® 2250
BARDAC® 2280

INTRODUCTION

Bardac 2250/2280, didecyl dimethyl ammonium chloride represents a class of germicidally active quaternary ammonium compounds that is radically different from the traditional alkyl benzyl type of quaternary. The Bardac "twin chain" type of structure has produced quaternary germicides with greatly improved performance characteristics over quaternary products with a benzene ring structure. Made by Lonza patented process technology, the Bardac products, with their superior performance, have had significant impact on the germicide industry.

The Bardac quaternary products are dialkyl dimethyl ammonium chlorides in which two alkyl groups in the C_8 to C_{10} range are attached directly to the nitrogen atom. These alkyl chains were found to maximize germicidal performance. Bardac 2250/2280 has the chemical structure shown to the right.
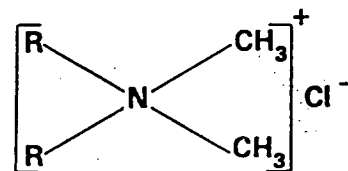
Where R = n-decyl

Chemical Composition - Typical

Active Ingredients	Bardac 2250	Bardac 2280
Didecyl dimethyl ammonium chloride	50.0%	80.0%
Inert Ingredients:	50.0%	20.0%

Specifications

	Bardac 2250	Bardac 2280
% Quaternary @ MW=361	50 - 52	80 - 82
Color (APHA)	200 Max.	250 Max.
pH (10% Active Solution)	6.5 - 9.0	6.5 - 9.0

Specifications are based upon Lonza's Analytical Test Methods, copies of which are available upon request.

Physical Properties

	Bardac 2250	Bardac 2280
Physical State	Liquid	
Flash Point (Seta Flash)	109°F	107°F
Specific Gravity at 25°C	0.927	0.891
Density (lbs./gallon)	7.73	7.43
EPA Registration Number	6836-51	6836-53
CAS No.	7173-51-5	

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GERMICIDAL ACTIVITY

The germicidal activity of Bardac 2250/2280 is substantiated by an extensive series of generally recognized microbiological tests including those required for EPA registration.

Standard laboratory evaluations indicate that Bardac 2250/2280 has superior over-all germicidal activity when compared to quaternaries with other structures. This activity is also exhibited under use conditions once considered detrimental to the performance of quaternaries. The following summarizes the advantages of Bardac 2250/2280.

. . . Broad spectrum biocidal activity against both gram positive and gram negative organisms.

. . . Better disinfectant performance at lower use concentrations.

. . . Greater hard water tolerance for sanitizing activity at lower use concentrations.

. . . Superior fungicidal performance.

. . . Substantial organic soil tolerance.

. . . Greater tolerance for anionic contaminants than previously possible.

Disinfectant Activity Determined by AOAC Use-Dilution Tests

The minimum concentration of Bardac 2250/2280 required for effective disinfection is determined by the AOAC, commonly known as the Use-Dilution Test.

Test Organism	ATCC Strain No.	Quaternary concentration on 100% active basis
Staphylococcus aureus	6538	300 ppm
Salmonella choleraesuis	10708	300 ppm
Pseudomonas aeruginosa (PRD-10)	15442	500 ppm

Organic Matter Tolerance and Residual Anionic Tolerance Determined by the AOAC Use-Dilution Test

Organic matter normally interferes with the biocidal activity of quaternary compounds. When compared to other quaternary compounds, Bardac 2250/2280 maintains an unusually high level of activity in the presence of proteinaceous soil. This is substantiated by the fact that Bardac 2250/2280 at 400 ppm active concentration in the presence of 5% blood serum is effective against Staphylococcus aureus according to the AOAC Use-Dilution Test.

Test Organism	ATCC Strain No.	Contaminent	Quaternary concentration on 100% active basis
Staphylococcus aureus	6538	Blood Serum 5%	400 ppm

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Quaternaries, which are cationic surface active agents, are deactivated by anionic surface active agents with the formation of an insoluble cationic-anionic complex. The ability of Bardac 2250/2280 to maintain bactericidal activity in the presence of anionics is important because residual amounts of anionics are often present in hard surface disinfection applications. When Bardac 2250/2280 is evaluated at a concentration of 400 ppm active against Staphylococcus aureus in the presence of 300 ppm sodium lauryl sulfate, it passes the AOAC Use-Dilution test whereas other quaternaries fail.

Test Organism	Strain No.	Contaminent	Quaternary concentration on 100% active basis
Staphylococcus aureus	6538	300 ppm sodium lauryl sulfate	400 ppm

Sanitizing Activity Determined by the AOAC Germicidal and Detergent Sanitizer Method

The germicidal activity of quaternary products decreases in the presence of hard water. The hard water tolerance of Bardac 2250/2280 is measured by the AOAC Germicidal and Detergent Sanitizer Method, commonly called the Hard Water Tolerance test. Exposure of 100 million organisms of Escherichia Coli (#11229) to 150 ppm of Bardac 2250/2280 for 30 seconds at 25°C in *1,000 ppm of water hardness* results in the required reduction of 99.999% of the bacteria.

Calcium and magnesium salts are typical hard water components; however, other electrolytes may be present during actual field applications. The unusually high hard water tolerance of Bardac 2250/2280 affords users a margin of safety over a range of quaternary concentrations.

Test Organism	Concentration of Bardac 2250/2280 Required for 99.999% Reduction	Hard Water Ceiling
Escherichia Coli #11229	50 ppm	250 ppm hard water
	100 ppm	600 ppm hard water
	150 ppm	1,000 ppm hard water
	200 ppm	1,500 ppm hard water

Fungicidal Performance as Determined by the AOAC Fungicidal Test

Possessing superior fungicidal activity, Bardac 2250/2280 effectively passes the AOAC Fungicidal test at one-fifth to one-seventh the concentration required for alkyl benzyl quaternaries.

Bardac 2250/2280	Ten Minute Killing Dilution (100% Active)		
	P. ovale #12098	T. mentagrophytes CDC #-X-32	C. albicans #14053
Ten Minute Killing Dilution	1:4845	1:7350	1:7350
In Use Dilution =	200 ppm	150 ppm	150 ppm

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Phenol Coefficient as Determined by the AOAC Phenol Coefficient Test

The phenol coefficient of a germicide is measured by its relative effectiveness in comparison to phenol. The bactericidal activity of Bardac 2250/2280 produces phenol coefficient results that surpass the alkyl benzyl quaternaries. The test protocol is AOAC.

Test Organism	ATCC Strain No.	Phenol Coefficient 100% active quaternary
Staphylococcus aureus	6538	1050
Salmonella typhosa	6539	1050

Bactericidal Efficiency as Determined by the Minimum Inhibitory Concentration Test

This tube-dilution test determines the minimum concentration of Bardac 2250/2280 which will reduce the bacterial count of a suspension by at least 99.999% from a level of 100 million organisms per ml. in 30 seconds at 25°C. The test method is a broth dilution test with 18 hour immersion at 37°C. Minimum inhibitory concentration test results indicate that Bardac 2250/2280 offers superior performance.

Test Organism	ATCC Strain No. (unless otherwise noted)	Gram Stain	Minimum Inhibitory Concentration (100% active)
Staphylococcus aureus	6538	+	0.5 ppm
Escherichia Coli	11229	-	5.0 ppm
Pseudomonas aeruginosa	15442	-	50.0 ppm
Proteus vulgaris	9920	-	20.0 ppm
Fungi			
A. niger	16404		5.0 ppm
T. mentagrophytes	(SWRI) Enmons		5.0 ppm
C. albicans	10231		1.5 ppm

Summary of Germicidal Activity

The test data presented substantiates the over-all superior germicidal performance of Bardac 2250/2280.

APPLICATIONS

The unique performance characteristics of Bardac 2250/2280 have been used to develop a series of disinfectants, sanitizers, and cleaners that are in commercial use in homes, hospitals, and institutions. These systems range from simple dilutions to more complex blends of quaternaries, surfactants and detergent builders.

Summary of Applications and Recommended Use Levels

Application Areas	Recommended Use-Levels on 100% Active Basis
General-Hospital Disinfection	300-500 ppm
Sanitizing	150 ppm
Water Treatment/Cooling Towers	5-20 ppm
Water Treatment/Secondary Oil Recovery	5-20 ppm
Laundry Mildew Preventative/Sanitizer	630 ppm
Bacteriostat/Preservative/Fungicide	5-1000 ppm

Wood Preservation

Bardac 2250/2280 will effectively control the decay of wood caused by a broad spectrum of fungi when utilized in exterior above ground situations such as fence and porch rails, decks, roofing, siding, window and door frames, etc. To achieve decay control, laboratory studies utilizing a vacuum impregnation method of application indicate that retention levels of 0.06 to 0.4 lbs. per cubic foot (1.0 to 6.4 Kg/m³) are required to control all of the test fungi.

Solutions of Bardac 2250/2280 are stable and can be used in presently available treatment plants without equipment modifications.

Lumber treated with solutions of Bardac 2250/2280 show no color change after treatment. Light colored paints and coatings also show no discoloration on treated surfaces.

Operators handling freshly treated wet lumber should wear rubber gloves, but dry treated lumber can be handled without any dermal protection.

In developing your final product and label based on Bardac 2250/2280, you should specify methods of application which will provide sufficient retention levels (as noted above) to prevent decay. If brush or soak applications are recommended, the thoroughness of such applications required to obtain the desired retention level must be indicated (i.e., two flowing brush coats or 30 minutes soak, etc.).

GENERAL INFORMATION

Product Registration

Biocides and products with biocidal claims require registration by the Environmental Protection Agency under the Federal Insecticide, Fungicide and Rodenticide Act. In addition, most state authorities require separate registration. The EPA NUMBERS FOR BARDAC 2250 AND BARDAC 2280 are 6836-51 and 6836-53 respectively, and may be referred to by consumers of this product. As a technical service, LONZA INC. provides advice on the registration of Bardac 2250/2280 based products.

Safety and Handling

The toxicity of Bardac 2250/2280 is of the same order of magnitude as other commercial quaternaries. Bardac 2250/2280 at an "as-is" concentration may be considered a primary skin and eye irritant. The data below summarizes the acute oral and dermal toxicity of Bardac 2250/2280 on a 80% activity basis as determined in mice and rabbits respectively.

Oral LD_{50} = 450 mg/kg

Acute Dermal LD_{50} = 3342 mg/kg

For detailed handling information consult the Bardac 2250/2280 Material Safety Data Sheet which is available upon request

Packaging

Bardac 2250 is available in 425 lb. net weight drums.
Bardac 2280 is available in 410 lb. net weight drums.

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General and Hospital Disinfection

A general purpose Disinfectant-Sanitizer-Fungicide-Deodorizer product may be produced by diluting Bardac 2250/2280 to 7.5% active level. This product will afford effective disinfection and/or sanitizing in hospitals, schools, homes, dairy, farm and industrial areas when used at appropriate use dilutions. A sample label for a Disinfectant-Sanitizer-Fungicide-Deodorizer based on Bardac 2250/2280 is attached. It may be used as a guide in registering your product with the Environmental Protection Agency.

Sanitizing

Bardac 2250/2280 has been cleared by the FDA, Title 21, Code of Federal Regulations, Section 178.1010, Food Additives, for sanitizing at a concentration of *150 ppm without the requirement of a potable water rinse*. In addition, the use of this sanitizing solution is consistent with the current practices of the *Grade "A" Pasteurized Milk Ordinance, 1978 Recommendations* of the United States Public Health Service. The high hard water tolerance of Bardac 2250/2280 allows the use of a lower concentration (150 ppm use-level) at a hard water ceiling of 1,000 ppm. In contrast, the alkyl benzyl quaternaries when used as sanitizers must be used at 200 ppm. This lower use concentration for Bardac 2250/2280 results in a material saving of 25%.

Water Treatment Microbiocide/Cooling Tower

Bardac 2250/2280 provides the formulator with a superior microbiocide for building and industrial cooling towers at use levels of 5 - 20 ppm.

Water Treatment Microbiocide/Secondary Oil Recovery

Many water floods in the secondary recovery of oil contain bacteria including the sulfate reducing bacteria Desulfovibrio desulfuricans. This micro-organism produces corrosive degradation products. Bardac 2250/2280 will inhibit the growth of D. desulfuricans, and thus reduce the corrosive nature of the water flood. Where oil field flood waters and salt water disposal systems require the use of a microbiocide, Bardac 2250/2280 may be added to the system at a level of 5.0 to 20.0 ppm.

Bardac 2250/2280 is registered with the Environmental Protection Agency (file #6836-32) for both cooling tower and secondary oil recovery applications of water treatment use; a sample label is available for these applications.

Laundry Mildew Preventative/Sanitizer

Soiled commercial linens are prone to mold and mildew attack causing permanent staining. Bardac 2250/2280 at use level 630 ppm based on the dry weight of the fabric is recommended in the final rinse of the laundry cycle to reduce the loss of linens due to staining. At this level, Bardac 2250/2280 provides residual bacteriostatic and self sanitizing protection. For these sanitizing process purposes, Bardac 2250/2280 is EPA registered as a Lonza laundry mildew preventative (file #6836-30); a sample label is available as a technical service.

Bacteriostat/Preservative/Fungicide

Bardac 2250/2280 is a highly effective broad spectrum bacteriostat for a variety of industrial applications. For situations where compatibility with a cationic material has been established, the actual use levels for the quaternary should be determined for each application.

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IMPORTANT — PLEASE READ CAREFULLY!

Production Formula and Procedure of Preparation for
7.5% Active Bardac 2250/2280 Dilution

The formula you are manufacturing is EPA registered, thus specifying by law the correct amounts of active ingredients to be present in your finished product. Therefore, the amounts and directions for the proper production of the formula given below must be followed explicitly.

The greatest accuracy in preparing this formulation is achieved when all ingredients are added by weight. If this is not possible, measure the liquids and weigh the solids.

The amounts given are for a production batch of 1,000 lbs. In order to produce larger or smaller batches, merely add multiples or fractions of the amounts listed.

The specific gravity/density of the ingredients required to correctly produce a 7.5% active Bardac 2250/2280 dilution have been taken into account in the amounts given below:

Bardac 2250/2280 /7.5% Dilution
1,000 lb. batch

Ingredients	Density lb./gal.	% wt/wt 2250	% wt/wt 2280	If Addition is by Weight, add in lbs. 2250	If Addition is by Weight, add in lbs. 2280	If Addition is by Volume, add in gallons 2250	If Addition is by Volume, add in gallons 2280
Bardac 2250	7.73	15.0	—	150.0	—	19.4	—
Bardac 2280	7.43	—	9.38	—	93.8	—	12.62
Water	8.34	85.0	90.62	850.0	906.2	101.92	108.66
Total	—	100.0	100.00	1,000.0	1,000.0	123.32	121.28

- Specific Gravity at room temperature of Bardac 2250/2280 - 7.5% active dilution = 0.974

- Density at room temperature of Bardac 2250/2280 - 7.5% active dilution = 8.12 lbs./gal.

Production Procedure for Bardac 2250/2280 - 7.5% Active Dilution

In a suitable blending vessel add together the water and Bardac 2250/2280. Mix for 15 minutes, making sure a clear, uniform solution has been achieved. Package.

SAMPLE LABEL

DISINFECTANT-SANITIZER FUNGICIDE DEODORIZER

Disinfectant-Sanitizer-Fungicide
Deodorizer for Hospital, Institutional,
Industrial, School, Dairy and Other
Farm Use

Active Ingredients

Didecyl dimethyl ammonium chloride 7.5%

Inert Ingredients 92.5%
100.0%

KEEP OUT OF REACH OF CHILDREN

DANGER

STATEMENT OF PRACTICAL TREATMENT

In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. For eyes, call a physician. Remove and wash contaminated clothing before reuse.

If swallowed, drink promptly a large quantity of milk, egg whites, gelatin solution; or if these are not available, drink large quantities of water. Avoid alcohol. Call a physician immediately.

NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage. Measures against circulatory shock, respiratory depression, and convulsion may be needed.

SEE LEFT PANEL FOR ADDITIONAL PRECAUTIONARY STATEMENTS

EPA Registration No. _____

EPA Establishment No. _____

Net Contents _____

MANUFACTURED BY:

LONZA INC., 17-17 Route 208, Fair Lawn, N.J. 07410

For Sale For Use And Storage By Maintenance Personnel Only

GENERAL CLASSIFICATION

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

Apply _____ with a cloth, mop or mechanical spray device. When applied with a mechanical spray device, surface must be sprayed until thoroughly wetted. Treated surfaces must remain wet for 10 minutes. Fresh solution should be prepared daily or when the use solution becomes visibly dirty.

Disinfection In Hospitals, Nursing Homes and Other Health Care Institutions.

For disinfecting floors, walls, countertops, bathing areas, lavatories, bedframes, tables, chairs, garbage pails and other hard surfaces.

Add 3½ oz. _____ to 4 gallons water. Apply to previously cleaned hard surface with mop or cloth. At this use-level, _____ is effective against *Pseudomonas aeruginosa.*

Disinfectant in Institutions, Industry, and Schools

For disinfecting floors, walls, bedframes, countertops, tables, chairs, garbage pails, bathroom fixtures and other hard surfaces.

Add 2 oz. of _____ to 4 gallons of water. Apply to previously cleaned hard surface with mop or cloth. At 2 oz./4 gallon use-level _____ is effective in 4 gallons of water with a mop, cloth or brush. against Staphylococcus aureus. Salmonella choleraesuis and Trichophyton interdigi'ale (the athlete's foot fungus).

Disinfection of Barber Tools

Precleaned barber tools (such as combs, brushes, razors, and scissors) can be disinfected by immersing in a ½ oz./gallon solution of _____

Disinfection of Poultry Equipment, Animal Quarters and Kennels.

Poultry brooders, watering founts, feeding equipment and other animal quarters (such as stalls and kennel areas) can be disinfected after thorough cleaning by applying a solution of 2 oz. _____. Small utensils should be immersed in this solution.

Prior to disinfection, all poultry, other animals and their feeds must be removed from the premises. This includes emptying all troughs, racks and other feeding and watering appliances. Remove all litter and droppings from floors, walls and other surfaces occupied or traversed by poultry or other animals.

After disinfection, ventilate buildings, coops and other closed spaces. Do not house poultry, or other animals, or employ equipment until treatment has been absorbed, set or dried. All treated equipment that will contact feed or drinking water must be rinsed with potable water before reuse.

LD-4   7/84

Sanitizing of Food Processing Equipment and other Hard Surfaces in Food Contact Locations.

For sanitizing food processing equipment, dairy equipment, food utensils, dishes, silverware, glasses, sink tops, countertops, refrigerated storage and display equipment and other hard surfaces. No potable water rinse is required. Wash and rinse all articles thoroughly, then apply a solution of 1 oz. _____ in 4 gallons of water. (150 ppm active). Surfaces should remain wet for at least one minute followed by adequate draining and air drying. Fresh solution should be prepared daily or when use solution becomes visibly dirty. For mechanical application, use solution may not be reused for sanitizing applications.

Apply to sink tops, countertops, refrigerated storage and display equipment and other stationary hard surfaces by cloth or brush. No potable water rinse is required.

Dishes, silverware, glasses, cooking utensils and other similar size food processing equipment can be sanitized by Immersion in a 1 oz./4 gallon dilution of _____. No potable water rinse is required.

At 1 oz./4 gallons, _____ fulfills the criteria of appendix F of the Grade "A" Pasteurized Milk Ordinances 1978 Recommendations of the U.S. Public Health Services in waters up to 1000 ppm of hardness calculated as CaCO₃ when evaluated by the AOAC Germicidal and Detergent Sanitizer Method against Escherichia coli and Staphylococcus aureus.

The udders, flanks, and teats of dairy cows can be sanitized by washing with a solution of 1 oz. _____ in 4 gallons of warm water. No potable water rinse is required.

Use a fresh towel for each cow. Avoid contamination of sanitizing solution by dirt and soil. Do not dip used towel back into sanitizing solution. When solution becomes visibly dirty, discard and provide fresh solution.

Precautionary Statements

Hazardous to Humans and Domestic Animals

DANGER

Keep Out of Reach of Children. Corrosive. Causes eye damage and skin irritation. Do not get in eyes, on skin, or on clothing. Protect eyes and skin when handling. Harmful if swallowed. Avoid contamination of food.

Storage and Disposal

—Do not contaminate water, food, or feed by storage disposal.
—Do not store on side.
—Avoid creasing or impacting of side walls.

PESTICIDE DISPOSAL

Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray or mixture of rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

CONTAINER DISPOSAL

Triple rinse (or equivalent). Then offer for recycling or reconditioning or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities by burning. If burned, stay out of smoke.

Metal Container: Triple rinse (or equivalent), then offer for recycling or reconditioning, or dispose of in a sanitary landfill, or by other procedures approved by state and local authorities. (If container is 1 gallon or less, use this container disposal statement.)

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